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Biomarcadores en Cáncer de Próstata Resistente a la Castración: Adquisición, Valor Pronóstico-Predictivo y Patrones de Uso en la Práctica Clínica

TESIS DOCTORAL

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CERTIFICAN QUE:

La presente tesis doctoral

"Biomarcadores en Cáncer de Próstata Resistente a la Castración: Adquisición, Valor Pronóstico-Predictivo y Patrones de Uso en la Práctica Clínica"

ha sido realizada por D. David Lorente Estellés bajo su dirección, y reúne todos los requisitos para su depósito y lectura.

Y para que así conste, firman la presente el 8 de marzo de 2020

D. David Olmos Hidalgo

D. Óscar José Juan Vidal

D. Pedro Luis Roldán Badía

Não sou nada.
Nunca serei nada.
Não posso querer ser nada.
À parte isso, tenho em mim todos os sonhos do mundo

Álvaro de Campos (Fernando Pessoa)

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List of Abbreviations

95%CI:	95% confidence interval
ADC:	Apparent diffusion coefficient
ADT:	Androgen-deprivation therapy
ALP:	Alkaline phosphatase
APC:	Adenomatous Polyposis Coli gene
AR-V7:	Androgen receptor splice variant 7
AR:	Androgen receptor
AR:	Full-length androgen receptor
ARSI:	Androgen receptor signaling inhibitor
ATM:	Ataxia telangiectasia mutated gene
AUC:	Area under the curve
AURKA:	Aurora-kinase A gene
BCL2:	B-cell lymphoma 2 gene
BEAMing:	Beads, emulsion, amplification, magnetics digital PCR method
BLNLR:	Baseline neutrophil-to-lymphocyte ratio
BRAF:	Murine sarcoma viral oncogene homolog B1 gene
BRCA:	Breast cancer gene
BSI:	Bone scan index
C-index:	Concordance index
CALGB:	Cancer and Leukemia Group B
CDK:	Cyclin-dependent kinase
CDKN1B:	Cyclin-dependent kinase Inhibitor 2A
CGH:	Comparative genomic hybridization
CHD1:	Chromodomain-helicase-DNA-binding protein gene
CK:	Cytokeratin
CNA:	Copy number alterations
CpG:	Cytosine-Guanine DNA sites
CT:	Computed Tomography
CTC:	Circulating tumor cells
cfDNA:	Cell-free circulating DNA
ctDNA:	Circulating tumor DNA
CYP17:	Cytochrome P-17
CTLA4:	Cytotoxic T-lymphocyte associated antigen 4
DBD:	DNA-binding domain
ddPCR:	Digital droplet polymerase chain reaction
DDR:	DNA damage repair
DFS:	Disease-free survival
DNA:	DeoxyriboNucleic Acid
DWI:	Diffusion-weighted imaging
ECOG:	Eastern Cooperative Oncology Group
EGFR:	Epidermal growth factor receptor
EORTC:	European Organization for Research and Treatment of Cancer
EPCAM:	Epithelial cell adhesion molecules

ERG:	ETS-related gene
ETS:	E26 transformation-specific gene
FACTP:	Functional assessment of cancer therapy – prostate scale
FANCA:	Fanconi anemia gene
FDA:	Food and Drug Administration
FFPE:	Formalin-fixed, paraffin embedded
FISH:	Fluorescence in situ hybridization
FOXA1:	Forkhead box protein A1
GABA:	gamma aminobutyric acid
γ-H2AX:	Gamma H2A histone family member X
GNAS:	Stimulatory guanine nucleotide-binding protein gene
GS:	Gleason score
HER2:	Human epidermal growth factor receptor 2
HR:	Hazard ratio
H-Score:	Histological score
HRQoL:	Health-related quality of life
IDH:	Isocitrate dehydrogenase
IDMC:	Independent data monitoring committee
IGFR:	Insulin growth factor receptor
LBD:	Ligand-binding domain
LDH:	Lactate Dehydrogenase
LHRH:	Luteinizing Hormone Releasing Hormone
LLN:	Lower limit of normality
MAPK:	Mitogen-Activated Protein Kinase
mCRPC:	Metastatic, castration-resistant prostate cancer
mHSPC:	Metastatic, hormone-sensitive prostate cancer
mRNA:	Messenger ribonucleic acid
M0:	Non-metastatic
M1:	Metastatic
MGMT:	Methyl-guanine methyl-transferase
MLH1:	MutL homolog 1
mm:	millimeters
MMR:	Mismatch repair
MRI:	Magnetic resonance imaging
MSI:	Microsatellite instability
MSH2:	MutS homolog 2
mTOR:	Mammalian target of rapamycin
N:	Number of patients
NCoA:	Nuclear receptor coactivator
NCoR:	Nuclear receptor corepressor
NE:	Neuroendocrine
NEPC:	Neuroendocrine prostate cancer
NHEJ:	Non-homologous end join
ng/dL:	Nanograms per deciliter
NGS:	Next-generation sequencing
NLR:	Neutrophil-to-lymphocyte ratio
nmCRPC:	Non-metastatic, castration-resistant prostate cancer
NTD:	Amino-terminal domain

OS:	Overall survival
PALB2:	Partner and localizer of BRCA2
PARP:	Poly ADP ribose polymerase
PARPi:	Poly ADP ribose polymerase inhibitor
PCWG:	Prostate Cancer Working Group
PD:	Progressive disease
PD:	Pharmacodynamic
PD-1:	Programmed-death 1 receptor
PDL1:	Programmed-death 1 ligand
PET:	Positron emission tomography
PFS:	Progression-free survival
PFS2:	Progression-free survival to second-line therapy
PI3K:	Phosphoinositide 3-kinase
PIK3C:	Phosphatidylinositol 3-Kinase Catalytic Subunit
PR:	Partial response
PROs:	Patient-reported outcomes
PSA:	Prostate-specific antigen
PSMA:	Prostate specific membrane antigen
PTEN:	Phosphatase and tensin homologue
PWB:	Physical well-being
qRT-PCR:	quantitative reverse-transcriptase polymerase chain reaction
RB1:	Retinoblastoma 1 gene
RECIST:	Response Evaluation Criteria in Solid Tumors
Resp:	Response
RNA:	Ribonucleic acid
ROC:	Receiver operating curve
rPFS:	Radiographic progression-free survival
RT-PCR:	reverse-transcriptase polymerase chain reaction
SD:	stable disease
SPINK:	Serine peptidase inhibitor
SPOP:	Speckle Type BTB/POZ Protein
SWOG:	South-western oncology group
TCGA:	Tumor Cancer Genome Atlas
TMPRSS2:	Transmembrane protease, serine 2
TP53:	Tumor Protein 53
TPE:	Treatment proportion estimate
ULN:	Upper limit of normality
USA:	United States of America
WES:	Whole exome sequencing
WNT:	Wingless/Integrated

Resumen

1. Introducción

El cáncer de próstata representa el segundo cáncer más frecuente en hombres, con un 15% de los casos de cáncer en varones, y es la quinta causa de mortalidad en varones (6,6% del número total de muertes).[1] El cáncer de próstata se caracteriza por ser una enfermedad con una importante heterogeneidad clínica, con un número de estados clínicos observados a lo largo de su historia natural, que se expanden desde la enfermedad localizable, curable mediante cirugía o radioterapia hasta la fase de resistencia a la castración metastásica, la fase final y letal de la enfermedad.

Los objetivos del tratamiento varían de forma significativa en los diferentes estadios clínicos; también lo hacen, por tanto, las preguntas que es necesario contestar para mejorar los resultados del tratamiento. ¿Por qué algunos pacientes desarrollan una enfermedad indolente mientras otros presentan metástasis ya al diagnóstico? ¿Podemos mejorar la estimación del pronóstico para prevenir la morbilidad del sobre tratamiento en pacientes con enfermedad indolente, e intensificar el tratamiento para maximizar las opciones de curación o respuesta en pacientes con enfermedad agresiva? Los clínicos dependen del uso de biomarcadores para estimar variables clínicamente relevantes que puedan ayudar en la toma de decisiones clínicas. Ejemplos en cáncer de próstata localizado son el grado de Gleason, estadiaje local y valor de PSA, que orientan las decisiones sobre el tratamiento local y adyuvante.

El cáncer de próstata avanzado engloba diferentes estadios clínicos, principalmente el cáncer de próstata resistente a la castración metastásico y el cáncer de próstata metastásico hormono-sensible. Éstos se definen por la presencia de enfermedad metastásica (mediante la realización de una gammagrafía ósea o tomografía computarizada) y la presencia o ausencia de castración (definida por un nivel de testosterona < 50 ng/dL). En estos pacientes, los objetivos del tratamiento son la paliación de síntomas presentes (dolor) además de la prevención de la aparición de nueva sintomatología o de complicaciones (eventos esqueléticos) y la prolongación de la supervivencia.

En los últimos años, el avance en el diseño de fármacos y en el conocimiento de la biología molecular del cáncer de próstata avanzado ha permitido un tratamiento más eficiente y preciso mediante el uso de biomarcadores predictivos y de respuesta o resistencia al tratamiento. Sin embargo, continúa habiendo cuestiones pendientes de resolución como son las siguientes:

(1) ¿Podemos mejorar la estimación del pronóstico, para comprender cuándo se necesita iniciar un tratamiento, o cuándo es esperable esperar y ahorrar toxicidad?
(2) ¿Podemos seleccionar tratamientos basados en el perfil molecular individual de cada paciente?
(3) ¿Podemos mejorar la evaluación de la eficacia del tratamiento? ¿Podemos identificar la resistencia al tratamiento antes de que haya progresión clínica?
(4) ¿Podemos definir objetivos alternativos a la supervivencia global, para acelerar la incorporación de nuevos fármacos al armamentario terapéutico?

2. Hipótesis

El desarrollo de biomarcadores mejorados para la estimación del riesgo de muerte (biomarcadores con valor pronóstico) de la posibilidad de respuesta de cada una de las opciones terapéuticas específicas (valor predictivo) o de la determinación de respuesta o resistencia al tratamiento (biomarcadores indicadores de respuesta) es uno de los retos a los que se enfrenta la investigación clínica en el cáncer de próstata avanzado en el momento actual. Este proyecto desarrolla hipótesis específicas alrededor de tres aspectos relevantes en el estudio de biomarcadores en cáncer de próstata avanzado:

(1) Biomarcadores tisulares
(2) Biomarcadores circulantes
(3) El uso de los biomarcadores disponibles por parte de los especialistas que tratan el cáncer de próstata avanzado

En base a estos aspectos generales, se desarrolla las siguientes hipótesis específicas:

(1) Biomarcadores tisulares:

El uso de técnicas de imagen puede mejorar el rendimiento de las biopsias de médula ósea con material tumoral suficiente para el análisis genómico de las muestras.

(2) Biomarcadores circulantes:

Los nuevos biomarcadores circulantes (células tumorales circulantes, índice neutrófilo- linfocitario) pueden mejorar la estimación pronóstica y la evaluación de respuesta al tratamiento (respuesta o progresión) en comparación con las herramientas clínicas disponibles en la actualidad.

(3) Utilización clínica de los biomarcadores disponibles:

El conocimiento y utilización de las recomendaciones de consenso para la práctica clínica por parte de los expertos en el tratamiento del cáncer de próstata avanzado es insuficiente e inapropiado.

3. Objetivos

En base a las hipótesis previamente expuestas, se plantea los siguientes objetivos:

Objetivos principales:

(1) Biomarcadores tisulares:

Determinación de los factores asociados a la positividad en la biopsia de médula ósea en pacientes con adenocarcinoma de próstata avanzado con enfermedad metastásica ósea.

(2) Biomarcadores circulantes:

a. Células tumorales circulantes: determinación de el valor pronóstico y como indicador de respuesta al tratamiento de la enumeración de células tumorales circulantes en cáncer de próstata avanzado tratados con abiraterona en el ensayo fase III COU-AA-301.

b. Ratio linfocito-neutrófilo: determinación del valor pronóstico y como indicador de respuesta al tratamiento de la ratio linfocito-neutrófilo en cáncer de próstata avanzado tratados con cabazitaxel o mitoxantrona en el ensayo fase III TROPIC.

(3) Utilización clínica de biomarcadores:

Descripción de los patrones de utilización de biomarcadores en la práctica clínica, y su adaptación a las recomendaciones de guías clínicas actuales (guías PCWG).

Objetivos específicos:

(1) Biomarcadores tisulares

- Asociación de biomarcadores clínicos, serológicos y radiológicos con un resultado positivo (presencia de cualquier número de células tumorales, o la presencia de > 50 células tumorales) en biopsias de médula ósea.
- Desarrollo de un "score" basado en parámetros radiológicos y serológicos para la identificación de pacientes con mayor posibilidad de obtención de una biopsia positiva.
- Validación del "score" en un grupo de validación independiente.

(2) Biomarcadores circulantes

a. Células tumorales circulantes
    - Determinación de la asociación del recuento basal de células tumorales con la supervivencia global, supervivencia libre de progresión y tasa de respuesta de los pacientes tratados en el ensayo COU-AA-301.
    - Comparación del valor pronóstico del recuento basal de células tumorales circulantes con otros biomarcadores disponibles en la clínica diaria.
    - Determinación de la asociación de la disminución en el recuento de CTCs 4, 8 y 12 semanas tras la iniciación de tratamiento y la supervivencia global y supervivencia libre de progresión en pacientes con recuentos de CTCs basales ≥ 5 células / 7,5 mL.
    - Determinación de la asociación del aumento en el recuento de CTCs 4, 8 y 12 semanas tras la iniciación de tratamiento y la supervivencia global y supervivencia libre de progresión en pacientes con recuentos de CTCs basales < 5 células / 7,5 mL.

b. Ratio linfocito-neutrófilo
    - Determinación de la asociación de la ratio linfocito-neutrófilo basal y otras variables clínicas basales.
    - Determinación de la asociación de la ratio linfocito-neutrófilo y la supervivencia global, supervivencia libre de progresión y tasa de respuesta al tratamiento. Asociación de los cambios en la ratio linfocito-neutrófilo tras 12 semanas de tratamiento y supervivencia global, supervivencia libre de progresión y tasa de respuesta.

(3) Utilización clínica de biomarcadores:

- Evaluación de las preferencias de uso de biomarcadores en la práctica clínica por parte de especialistas en el tratamiento del cáncer de próstata avanzado.

- Evaluación del patrón de decisiones en diferentes escenarios clínicos en cáncer de próstata avanzado.
- Determinación de la proporción de pacientes familiarizados con las recomendaciones resumidas en las guías de la PCWG-2.

4. Métodos

a. **BIOMARCADORES TISULARES**

Análisis retrospectivo de pacientes con CPRCm a los que se realizó una biopsia de médula ósea (BMO) entre octubre de 2011 y noviembre de 2014 en el Royal Marsden NHS Foundation Trust de Sutton, Reino Unido. Se incluyó pacientes ≥ 18 años, con evidencia de metástasis óseas pélvicas a partir de estudios de imagen (TAC, rastreo óseo, resonancia magnética).

Se recogió tejido a partir de una biopsia ósea de la cresta ilíaca posterior, derecha izquierda. No se utilizó guía de imagen para el procedimiento. Las muestras de biopsia se sellaron en un contenedor con una solución parafinada al 10% y fijadas a temperatura ambiente durante 24-30 horas. Tras la fijación, se aclararon brevemente en agua destilada, colocadas en un contenedor de EDTA (ácido etilenodiaminotetraacético (EDTA), sellados e incubados durante aproximadamente 48 horas a 37ºC. Un patólogo, ciego a los datos clínicos y de imagen, tiñó entonces con hematoxilina-eosina las secciones, de aproximadamente 2 mm y analizó las muestras. Los casos se consideraron negativos cuando no se pudo identificar células tumorales intactas. Los casos positivos, con células intactas identificables, se clasificaron como aquéllas con < 50 y ≥ 50 células.

Se excluyó a aquellos pacientes con una TAC de pelvis realizada más allá de 6 semanas previas a la biopsia. Las imágenes fueron analizadas por una radióloga experta en el cáncer de próstata. Se evaluó un área con un diámetro de 0,8-1 cm, en la cresta ilíaca posterior, representativa del área biopsiada; dicha zona fue equivalente en todos los pacientes. La media de unidades de Hounsfield (UH) se determinó en tres cortes sucesivos (grosor 5 mm). Se revisó los rastreos óseos para estimar la carga global de enfermedad ósea, clasificada como < 5, 5-20 y >20 metástasis óseas.

Se realizó un análisis descriptivo de características clínicas y radiológicas. Se utilizó algoritmos de asignación aleatoria para determinar las biopsias a utilizar en el grupo de derivación y el de validación. El grupo de derivación se utilizó para desarrollar el modelo para la predicción de la positividad de las biopsias de médula ósea. La variable dependiente del modelo (positividad de la biopsia) se definió como la presencia de células en la biopsia. Se comparó la media de valores de parámetros basales entre los grupos mediante el test de t-Student.

Se realizó una serie de modelos de regresión logística univariable, para seleccionar las variables para su inclusión en el modelo multivariable (aquéllas con p <0,05 en el modelo univariable). La validez interna del modelo multivariable se realizó mediante la determinación de curvas ROC en el modelo de derivación. La validez externa se determinó mediante la construcción de curvas ROC en el modelo de validación; se determinó asimismo el valor predictivo positivo y negativo en ambos grupos. La tasa de biopsias positivas en la cohorte global se utilizó como la prevalencia para el cálculo de los valores predictivos. El "score" desarrollado se evaluó entonces para determinar su asociación con la presencia de ≥ 50 células. Todos los análisis estadísticos se realizaron con SPSS, versión 20.

b. BIOMARCADORES CIRCULANTES

Valor pronóstico y como biomarcador de repuesta del recuento de células tumorales circulantes.
Análisis retrospectivo de pacientes tratados en los ensayos clínicos COU-AA-301 e IMMC-38. En el ensayo COU-AA-301, pacientes con CPRCm previamente tratados con quimioterapia fueron aleatorizados a recibir tratamiento con abiraterona y prednisona o placebo y prednisona.[2] El ensayo IMMC-38 es un ensayo prospectivo, abierto en pacientes con CPRC metastásico que recibieron tratamiento con quimioterapia. En ambos estudios, se determinó el recuento de células tumorales circulantes basales y periódicamente durante el tratamiento. En el ensayo COU-AA-301, se determinó el recuento de CTCs en el ciclo 2 día 1 (semana 4-5), ciclo 3 día 1 (semana 8-9) y ciclo 4 día 1 (semana 12-13). En el ensayo IMMC-38, se determinó el recuento de CTCs a las 2-3 semanas (mediana: 4 semanas), 6-8 semanas (mediana: 7 semanas) y semana 9-12 (mediana: semana 11,9). El recuento de CTCs se realizó mediante el método CellSearch. Se evaluó también la hemoglobina, fosfatasa alcalina, albúmina y lactato deshidrogenasa basales, además del ECOG PS previo al inicio de tratamiento, como co-variables del modelo multivariante. Los niveles de PSA se evaluaron cada 4 semanas en IMMC-38 y cada 12 semanas en COU-AA-301.

El método de Kaplan Meyer se utilizó para estimar la supervivencia: La asociación entre el biomarcador de respuesta/progresión y supervivencia se determinó mediante modelos de regresión de Cox uni- y multivariable. Para la asociación con la respuesta por PSA, se calculó las odds ratio (OR) mediante modelos de regresión logística.

Se definió los criterios de respuesta / progresión como:

- Respuesta por CTCs: disminución ≥ 30% del nivel de CTCs en pacientes con CTCs ≥ 5 / 7,5 mL
- Conversión de CTCs (respuesta): cambio de recuento desfavorable (CTCs ≥ 5 / 7,5 mL) a recuento favorable (CTCs < 5 / 7,5 mL), en pacientes con recuento basal CTCs ≥ 5 / 7,5 mL.
- Progresión por CTCs: cualquier aumento de CTCs respecto al recuento basal (en pacientes con recuento basal de CTCs < 5 / 7,5 mL).

- Conversión de CTCs (progresión): cambio de recuento favorable (CTCs < 5 / 7,5 mL) a desfavorable (CTCs ≥ 5 / 7,5 mL) en pacientes con recuento basal < 5 CTCs / 7,5 mL.

Se utilizó un *landmark analysis* para explorar la asociación entre la respuesta o progresión por CTCs y la supervivencia, mediante el establecimiento de poblaciones específicas de pacientes con recuentos basales y a las 4, 8 y 12 semanas, respectivamente. Se aplicó la corrección de Bonferroni para corregir el test múltiple, por lo que la significación estadística se fijó en un p-valor ≤ 0,0167. Se realizó una transformación logarítmica de la LDH, FA, PSA y CTC basales. Se calculó modelos de supervivencia con variables clínicas, variables clínicas más recuentos de CTC basales y variables clínicas más recuentos CTC basales más las medidas de respuesta o progresión por CTCs, y se comparó su asociación con la supervivencia global. El rendimiento global de los modelos de supervivencia se evaluó mediante curvas ROC a 6 y 11 meses de supervivencia, y mediante el cálculo del índice de concordancia (c-index) según el método de Uno y colaboradores.[3]

Los análisis fueron realizados utilizando los programas SPSS v21 (SPSS Inc., Chicago, IL, USA) y el paquete estadístico R v3.2.1 (R Foundation, Vienna, Austria).

Valor pronóstico y como biomarcador de repuesta del índice neutrófilo-linfocitario.

Análisis retrospectivo, no planificado, de pacientes tratados en el ensayo TROPIC, un ensayo fase III aleatorizado que evaluó la eficacia de cabazitaxel frente a mitoxantrona en pacientes con CPRCm previamente tratados con docetaxel.[4] Para el diseño y consecución de este estudio, se utilizó las recomendaciones y criterios *Tumor Marker Prognostic Studies* (REMARK) en la medida de lo posible.

Las características clínicas recogidas al inicio del estudio incluyeron: número y duración de líneas previas de tratamiento, localización de metástasis, edad, ECOG PS, uso de corticoides, y parámetros de bioquímica y hemograma en analíticas de sangre. Se realizó hemogramas de forma semanal, determinaciones de bioquímica con frecuencia trisemanal, y estudios de imagen (TAC, rastreo óseo) cada 12 semanas.

Se definió la ratio linfocito-neutrófilo (NLR) como el cociente entre el recuento neutrófilo absoluto y el recuento linfocitario absoluto (ambos: nº células/mm3). Se utilizó los recuentos en ciclo 1 día 1 como recuentos basales. Se evaluó la asociación del NLR basal (BLNLR) como variable continua y dicotómica con la supervivencia global y supervivencia libre de progresión mediante modelos de regresión de Cox uni- y multivariables. Para evaluar la asociación de BLNLR como variable continua, se recurrió a modelos de regresión lineal. Como covariables, se utilizó las variables recogidas en el modelo pronóstico de Halabi y colaboradores, desarrollado a partir de el mismo ensayo TROPIC.[5] Entre estas covariables, se incluyó la presencia de dolor, presencia de enfermedad medible, ECOG PS, progresión en los 6 meses posteriores a la finalización de docetaxel, enfermedad visceral, duración de tratamiento hormonal

previo y valores basales de PSA, hemoglobina y fosfatasa alcalina, al que se añadió el brazo de tratamiento y el uso de corticoesteroides basal.

El punto de corte óptimo para definir la variable NLR dicotómica se estableció mediante la comparación de diferentes puntos de corte pre-establecidos (NLR 2, 3 o 5 – el primer, segundo y tercer cuartiles de la muestra) mediante el índice de concordancia (c-index) y las curvas ROC. Se aplicó una corrección de Bonferroni para definir la significación estadística en $p \leq 0,0167$. Para evaluar la asociación con la respuesta radiológica o por PSA, se utilizó un único punto de corte (NLR 3) por lo que el límite de significación estadística se fijó en $p \leq 0,05$. Se consideró elegible a todo paciente con valor de BLNLR válido y al menos un valor de NLR post-basal.

Se consideró elegibles para evaluación de la asociación con respuesta por PSA a todos aquellos pacientes con al menos PSA basal ≥ 20 ng/mL y al menos una determinación de PSA post-basal. Se definió la respuesta por PSA (disminución ≥ 50% respecto al valor basal) y la disminución máxima de PSA. De manera similar, la "conversión" por NLR se analizó en pacientes con BLNLR y al menos un valor post-basal. Sólo se evaluó la respuesta radiológica en pacientes con enfermedad medible por RECIST.

Los análisis fueron realizados utilizando los programas SPSS v21 (SPSS Inc., Chicago, IL, USA) y el paquete estadístico R v3.2.1 (R Foundation, Vienna, Austria).

c. UTILIZACIÓN CLÍNICA DE BIOMARCADORES

Se redactó un cuestionario de 23 preguntas para la evaluación de especialistas en el tratamiento de cáncer de próstata avanzado. El objetivo fue la evaluación de el conocimiento de los criterios de progresión PCWG2, opinión y tendencias en el uso de biomarcadores en la práctica clínica diaria, y el conocimiento del valor del recuento de células tumorales circulantes en el manejo del cáncer de próstata avanzado.

Los cuestionarios incluyeron:

1. Preguntas generales acerca de su práctica clínica diaria
2. Evaluación de la familiaridad con los criterios de progresión de los diferentes biomarcadores en cáncer de próstata avanzado
3. CTCs y su evaluación en pacientes con cáncer de próstata avanzado
4. Decisiones clínicas basadas en biomarcadores de respuesta en cáncer de próstata avanzado.

Se envío e-mails para invitar a la participación a 485 investigadores en el Reino Unido con participación en ensayos clínicos en cáncer urológico, 29 médicos pertenecientes al Grupo Suizo de Investigación en Cáncer Urológico y 20 especialistas en cáncer de próstata de Australia y Nueva Zelanda, con un enlace a la encuesta online, creada mediante *Survey Monkey.*

Se realizó un análisis descriptivo de la muestra, incluyendo la proporción (%) de participantes que respondieron a cada una de las preguntas. Se clasificó a los participantes de acuerdo al número de pacientes tratados (≥ 50 vs < 50 pacientes/año) o incluidos en ensayos clínicos (≥ 25% vs < 25%) y el número de ciclos de docetaxel generalmente utilizados (4, 5-6, ≥ 7 ciclos). Se comparó las proporciones utilizando un test de Ji-cuadrado, o un test exacto de Fisher en caso de que la frecuencia esperada fuera < 5. Se definió el límite de la significación estadística en $p < 0,05$. No se realizó ningún ajuste por comparaciones múltiples. Los análisis fueron realizados utilizando el programa SPSS v21 (SPSS Inc., Chicago, IL, USA).

5. Resultados

a. BIOMARCADORES TISULARES

Realizamos un total de 115 biopsias de médula ósea en 101 pacientes. 75 biopsias (65,2%) dieron resultado positivo, una tasa que concuerda con lo publicado en series de biopsias de médula ósea no dirigidas por imagen previamente publicadas.[6–8] En 20 biopsias (26,7%) se obtuvo < 50 células mientras que en 55 casos (73,3%) se obtuvo ≥50 células. Se dividió la población del estudio (115 biopsias) en un grupo de derivación (57 biopsias) y un grupo de validación (58 biopsias). En el grupo de derivación, 35 (61,4%) biopsias dieron resultado positivo, mientras que fueron 40 (69%) las biopsias positivas en el grupo de validación; no se observó una diferencia estadísticamente significativa en la tasa de positividad entre los dos grupos (p = 0,395). El grupo de derivación y validación presentaron características de laboratorio y radiológicas basales similares.

En el grupo de derivación, una nivel reducido de hemoglobina (≥ 11.5 g/dL vs. < 11.5 g/dL; p=0.019), un nivel alto de lactato-deshidrogenasa (≥ 225 IU/L vs. < 225 IU/L; p=0.003), nivel elevado de PSA (≥ 225 vs. < 225 ng/mL; p=0 .005), un nivel elevado de fosfatasa alcalina (≥100 vs. < 100 IU/L; p=0.025) y una alta HU media en la TAC (≥ 125HU vs.< 125 HU; p=0.004) mostraron una asociación estadísticamente significativa con un resultado positivo de la biopsia de médula ósea en análisis univariante, y se utilizaron en el modelo multivariante inicial.

Por otro lado, en el modelo multivariante, únicamente una HU media en la TAC ≥ 125 (odds ratio [OR], 3.85; intervalo de confianza 95% [IC95%], 1.06- 13.94; p=0.036) y una LDH ≥ 225 UI/L (OR 8.7; IC95%, 1.68-45.11; p=0.003) mostraron una asociación estadísticamente significativa con un resultado positivo en la biopsia. Estos resultados llevaron al desarrollo de una “score” de biopsia de médula ósea (“Bone Marrow Biopsy Score” [BMB Score]), al asignar un punto en caso de alteración de cada uno de los parámetros (0 puntos si HU no es ≥ 125 ni LDH ≥ 200 UI/L; un punto si HU ≥ 125 o LDH ≥ 200 UI/L, y dos puntos si HU ≥ 125 y LDH ≥ 200 UI/L).

En el grupo de derivación, sólo un 23,5% de las biopsias con un score de 0 fueron positivas, en comparación con un 77,5% de las biopsias con un score de 1-2 ($p < 0.001$). Al analizar los resultados en el grupo de validación, se observó unos resultados similares: únicamente un 21,4% de las biopsias con un score 0 fueron positivas, en contraposición a un 81,4% de las biopsias con un score 1-2 ($p < 0.001$). Se evaluó el rendimiento global del BMB Score mediante el cálculo del área bajo la curva (AUC) ROC ("Receiver Operating Characteristic"); el AUC del BMB Score fue de 0,79 (IC95%: 0,67-0,91; $p <0.001$) en el grupo de derivación y de 0,77 (IC95%: 0,59-0,88; $p < 0,001$) en el grupo de validación.

Nuestro estudio, similar a otros publicados previamente, establece asociaciones entre los parámetros clínicos, analíticos y radiológicos con el rendimiento de las biopsias de médula ósea. Es el primer estudio en establecer el valor de parámetros analíticos y de la TAC ampliamente utilizados en práctica clínica, para el desarrollo de un "score" con aplicabilidad directa en la práctica clínica habitual, que se valida en un grupo independiente de pacientes. Demostramos el potencial predictivo de un score sencillo que puede asistir en la selección de pacientes en los que el procedimiento de biopsia de médula ósea tiene una alta probabilidad de éxito, para proporcionar tejido para la secuenciación exómica y transcriptómica.

La BMB Score presenta una alta sensibilidad (88,6% en el test de derivación, 92,5% en el test de validación) con una menor especificidad (59,1% en el test de derivación, 61,1% en el test de validación). Esta alta sensibilidad apoya su uso para la identificación de pacientes con una baja posibilidad de resultado positivo de la biopsia. De acuerdo con nuestros resultados, no se debería realizar una biopsia a pacientes con un score de 0 (pacientes en los que la densidad ósea de la cresta ilíaca no es ≥ 125 HU, con niveles de LDH < 225 UI/L), ya que el valor predictivo negativo (probabilidad de obtener un resultado negativo) es de 78-79%. Al extrapolar los resultados al grupo de validación, se concluye que no realizar una biopsia a pacientes con un score de 0 habría "ahorrado" una biopsia negativa a 11 (18,9%) pacientes, y únicamente habría "perdido" 3 (5,2%) biopsias positivas, lo que habría incrementado el rendimiento de biopsias positivas de 69% a 84,1%.

Finalmente, también evaluamos la asociación del BMB Score con la obtención en la biopsia de al menos 50 células, que es el mínimo de tejido necesario para, por ejemplo, evaluar la presencia o ausencia de proteína PTEN en estudios traslacionales.[9,10] En nuestro estudio, 23 (40,4%) biopsias en el grupo de derivación y 32 (55,2%) en el grupo de validación presentaron al menos 50 células. Se observó una una asociación estadísticamente significativa entre el BMB Score y la presencia de ≥ 50 células tanto en el grupo de derivación (OR, 3.1; IC95%, 1.41-6.84; p=0.005) como en el de validación (OR, 3.7; IC95%, 1.6-8.4; p= 0.002). El ROC AUC de la BMB Score fue de 0,72 () en el grupo de derivación y 0,73 () en el grupo de validación. En el grupo de validación, se obtuvo ≥ 50 células en únicamente en 2 biopsias (14,3%) con un score de 0, frente a 30 (68,2%) en aquellos pacientes con un score 1-2.

b. BIOMARCADORES CIRCULANTES

Disminución de Células Tumorales Circulantes en Pacientes con CTC basales ≥ 5 / 7,5 mL

Inicialmente, se realizó un análisis para determinar el punto de corte más apropiado para definir la respuesta. Para ello, comparamos la sensibilidad, especificidad y rendimiento medido como área bajo la curva (AUC) de la curva ROC. Observamos diferencias estadísticamente significativas a favor de un descenso del 30% (en comparación a un descenso del 50%) únicamente al analizar el cambio a las 4 semanas de tratamiento (AUC 0.68 vs 0.65; p=0.006), pero no a las 8 o a las 12 semanas. Como era esperable, observamos una mayor sensibilidad en la respuesta 30% (4 semanas: 71.5% vs 61%; 8 semanas: 71.7% vs 66.6%; 12 semanas: 68.2% vs 63.5%), mientras que observamos una mayor especificidad en la respuesta 50% (4 semanas: 65.1% vs 68.6%; 8 semanas: 76.5% vs 78.4%; 12 semanas: 72.7% vs 78.8%). Finalmente, se eligió un punto de corte de 30% para definir la respuesta ya que se consideró que el coste de un falso negativo (clasificación de un respondedor como no respondedor) es superior al coste de un falso positivo (clasificación de un no respondedor como respondedor), lo que lleva a valorar la sensibilidad de la prueba sobre la especificidad.

Globalmente, se observó una disminución en el contaje de CTCs ≥ 30% en 283 (64,3%), 248 (65,3%) y 226 (64,4%) pacientes a las 4, 8 y 12 semanas, respectivamente. Se observó un beneficio estadísticamente significativo en la población referencia de las 4 (HR 0.45; p<0.001), 8 (HR: 0.41; p <0.001) y 12 (HR 0.39; p<0.001) semanas. Asimismo, se observó un impacto similar al evaluar la población global (ensayos IMMC-38 y COU-AA-301 conjuntamente) y al analizar las poblaciones de los diferentes ensayos, por separado. En modelos multivariantes, se observó que la asociación entre la disminución de CTCs y la supervivencia global es independiente de otras covariables como los niveles basales de PSA, LDH, albúmina, hemoglobina, fosfatasa alcalina y el ECOG PS.

También se analizó cómo el nivel basal de CTCs y la respuesta por CTCs podían mejorar el rendimiento de modelos pronóstico disponibles en la actualidad. Se construyó tres modelos de regresión multivariable de Cox: un primer modelo “clínico” con PSA, LDH, albúmina, fosfatasa alcalina y ECOG PS como covariables; un segundo modelo “CTC basal”, al que se añade el recuento basal de CTCs al modelo clínico, y un modelo de “respuesta CTCs”, al añadir la disminución ≥ 30% de CTCs al modelo “CTC basal”. Se comparó el rendimiento de los tres modelos mediante la comparación del índice de concordancia (c-index).

En la población referencia de 12 semanas, se observó un c-index de 0,646 para el modelo “clínico”, que aumentó marginalmente a 0,656 en el modelo “CTC basal”. Al añadir la respuesta por CTCs (modelo “respuesta CTCs”, sin embargo, se observa un incremento más marcado del c-index, a 0,710. Se observó un aumento no estadísticamente significativo al comparar la ROC AUC de los modelos “clínico” y “CTC

basal" (AUC 0,669 vs 0,684), mientras que la ROC AUC del modelo de "respuesta por CTCs" (AUC 0,772) fue significativamente superior tanto al modelo "clínico" ($p < 0,001$) y al modelo "CTC basal" ($p < 0,001$).

Seguidamente, comparamos la capacidad pronóstica de la reducción de CTCs 30% y la conversión de CTCs (cambio de ≥ 5 CTCs a < 5 CTCs), al comparar la ROC AUC para la supervivencia a 6 meses en pacientes con CTCs basales ≥ 10 céls/7,5 mL y ≥ 30 céls/7,5 mL, respectivamente. Aunque se observó valores de AUC superiores en la disminución 30% de CTCs, sólo se observó diferencias estadísticamente significativas en la población de pacientes con CTCs ≥ 10 a las 4 semanas de tratamiento (AUC 0,701 vs 0,624; $p = 0.008$).

También evaluamos el impacto del brazo de tratamiento en la respuesta 30% por CTCs. En la población con CTCs basales ≥ 5 / 7,5 mL, se observó que abiraterona mantiene un beneficio significativo en supervivencia en comparación con placebo (HR 0,75; p=0,02) en todas las poblaciones evaluadas. Los pacientes en el brazo de abiraterona presentaron una tasa de respuesta 30% significativamente superior (73,3% vs 43,3%) a aquéllos tratados en el brazo placebo. El test de interacción entre brazo de tratamiento y respuesta por CTCs fue no significativo, lo que indica un beneficio equivalente para los pacientes que experimentaron una respuesta por CTCs en el brazo de abiraterona y placebo.

Finalmente, se evaluó si la "estabilidad" en el recuento de CTCs (es decir, la ausencia de tanto una disminución como de un aumento de CTCs con tratamiento) confiere un valor pronóstico diferente a la respuesta o al aumento de CTCs. Sólo 57 (13%), 43 (11.3%), y 42 (12%) pacientes cumplieron los criterios de "estabilidad" (aumento de CTCs no superior a 30%, y disminución de CTCs no superior a 30%) a las 4, 8 y 12 semanas, respectivamente. Se observó un beneficio estadísticamente significativo de la disminución ≥ 30% en CTCs en comparación a los recuentos "estables", pero no se observó diferencias significativas al comparar la "progresión" (aumento ≥ 30% en el recuento de CTCs) y la "estabilidad" de CTCs. Ante la ausencia de diferencias entre pacientes con recuentos "estables" y en "progresión", se concluyó que la ausencia de respuesta ≥ 30% debe considerarse un factor de mal pronóstico, independientemente de si los recuentos se mantienen estables o aumentan, que debería llevar a la valoración de cambio de tratamiento.

Aumento de Células Tumorales Circulantes en Pacientes con CTC basales < 5 / 7,5 mL

En el mismo set de datos que el apartado anterior (pacientes tratados en los ensayos COU-AA-301 e IMMC-38) seleccionamos los pacientes con un recuento basal < 5 CTCs /7,5 mL, para determinar el valor de la "progresión" por CTCs las 4, 8 y 12 semanas. Se definió los criterios de progresión como:

(a) Cualquier aumento del recuento de CTCs (progresión)
(b) Conversión de < 5 CTCs a ≥ 5 CTCs (conversión)

Se incluyó un total de 511 pacientes, 421 (82,4%) del ensayo COU-301 y 90 (17,6%) del ensayo IMMC-38. Observamos una mayor supervivencia global en pacientes con recuento basal < 5 CTCs / 7,5 mL frente a pacientes con ≥ 5 CTCs. Evaluamos entonces el valor pronóstico del recuento de CTCs dentro del subgrupo con < 5 CTCs: el recuento de CTCs, como variable continua con transformación logarítmica, se asoció con la supervivencia global (HR 1,65; p < 0,001). Los pacientes con un recuento basal de 0 tuvieron una supervivencia global (27,1 meses) significativamente mejor que aquéllos con 1-2 CTCs/7,5 mL (21,6 meses) o 3-4 CTCs/7,5 mL (15,1 meses) basales, con un p-valor de tendencia lineal significativo (p = 0,001).

Se observó una progresión por CTCs en 213 (41,7%) de pacientes en las primeras 12 semanas de tratamiento; 184 (43,7%) en COU-AA-301 y 29 (32,2%) en IMMC-38. 117 (25,8%), 103 (23.8%) y 124 (24,4%) pacientes experimentaron progresión de enfermedad en las primeras 4, 8 y 12 semanas, respectivamente. Globalmente, 90 (17,7%) pacientes presentaron una conversión a recuentos desfavorables (≥ 5 CTCs/7,5 mL en las primeras 12 semanas; 76 (18,1%) en COU-AA-301 y 14 (15,6%) en IMMC-38.

De manera similar a lo realizado en pacientes con CTCs basales ≥ 5/7,5 mL, realizamos una comparativa de los modelos pronósticos con y sin CTCs. Observamos una mayor c-índice en el modelo incluyendo progresión por CTCs que en el modelo incluyendo la conversión por CTCs (0,750 vs 0,705; delta c-índice: 0,045 [IC95% 0,019-0,071]). También observamos una mayor área bajo la curva (AUC) ROC en el modelo incluyendo la progresión por CTCs (0,77 vs 0,69; IC95%: 0,61-0,76). En base a estos resultados, se decidió utilizar la progresión por CTCs en los análisis posteriores. La progresión por CTCs se asoció a una peor supervivencia global (27.1 versus 15.1 meses; HR: 3.4; p < 0.001), con un impacto similar en pacientes tratados en el ensayo COU-301 e IMMC-38. 128 (28,2%) pacientes en COU-301 y 42 (51,9%) pacientes en IMMC-38 obtuvieron una respuesta (disminución ≥ 50%) por PSA, con una menor tasa de respuesta en pacientes con progresión por CTCs (11.4% versus 47.1%; OR: 0.14 (95% CI 0.09–0.23), p < 0.001).

De manera similar a lo realizado en pacientes con recuentos basales desfavorables, posteriormente se evaluó cómo la progresión por CTCs mejora el rendimiento de los modelos pronóstico disponibles. Para ello, se construyó un modelo de regresión de Cox con variables clínicas (PSA, LDH, albúmina, hemoglobina, fosfatasa alcalina, ECOG PS) basales (“modelo clínico”), un modelo al que se añadió el recuento CTC basal (“modelo CTC-basal”) y un modelo al que se añadió, al modelo clínico, el recuento CTC basal y la progresión por CTCs (“modelo progresión”). El AUC-ROC del modelo basal fue de 0,66 (IC95% 0,59-0,74). Se observó un incremento no estadísticamente significativo a 0,67 (IC95% 0,59-0,75) al añadir el recuento basal de CTCs en el “modelo CTC-basal”. Al añadir la progresión por CTCs (“modelo progresión”) la AUC-ROC aumentó de forma sustancial (AUC 0,77; IC95% 0,70-0,84), con una diferencia

estadísticamente significativa frente al "modelo CTC basal" (p<0,001). El índice-c fue de 0,682 en el "modelo clínico", 0,694 en el "modelo basal" y de 0,748 en el "modelo progresión"). Se observó una diferencia estadísticamente significativa entre el "modelo basal" y el "modelo progresión" (delta índice-c: 0,056; IC95% 0,025-0,087).

Finalmente, se evaluó el impacto de el brazo de tratamiento del ensayo COU-AA-301 en la progresión por CTCs. No se observó un beneficio significativo en supervivencia de abiraterona sobre prednisona en pacientes con recuentos de CTC basal < 5 CTCs / 7,5 mL (HR 0,86; IC95% 0,63-1,17; p=0,330). La progresión por CTCs se observó más frecuentemente en pacientes tratados con placebo (n=68, 51,9%) que con abiraterona (68 [51,9%] vs 115 [39,9%] pacientes, OR: 0,6; p=0,022). El impacto de la progresión por CTCs en supervivencia observado fue similar en pacientes tratados en el brazo de abiraterona (24,1 vs 15,1 meses; HR 3,76; p<0,001) y en el brazo de placebo (no alcanzado vs 13,8 meses; HR 3,23; p < 0,001). El test de interacción entre brazo de tratamiento y progresión por CTCs fue no significativo (p=0,952), lo que se interpretó como un similar impacto en supervivencia en ambos brazos de tratamiento.

Valor Pronóstico y como indicador de respuesta al tratamiento del índice neutrófilo-linfocitario

Realizamos un análisis retrospectivo de pacientes tratados en el ensayo clínico TROPIC, en el que pacientes con adenocarcinoma de próstata resistente a la castración metastásico en progresión a tratamiento con docetaxel, a recibir tratamiento con cabazitaxel o mitoxantrona,[4] para evaluar la asociación del índice neutrófilo-linfocitario basal (BLNLR) con la supervivencia global y la tasa de respuesta radiológica y por PSA, además de una evaluación de su valor como indicador de respuesta al tratamiento, a través de la "conversión" (cambio de NLR elevado a disminuido y viceversa) tras el inicio de tratamiento. Un total de 755 pacientes fueron elegibles para el análisis. 377 (49,9%) pacientes fueron aleatorizados a recibir mitoxantrona y prednisona, frente a 378 (50,1%) pacientes aleatorizados a recibir cabazitaxel y prednisona.

Observamos una asociación del BLNLR con otras variables de reconocido valor pronóstico como la presencia de metástasis viscerales (p=0,019), presencia de dolor (p=0,007), hemoglobina baja (p=0,002) y fosfatasa alcalina elevada (p=0,012). Además, un ECOG PS bajo (p < 0,001) y el uso de corticoesteroides basales (p = 0,026) también estuvo asociado a un BLNLR elevado.

Inicialmente, evaluamos el valor pronóstico de BLNLR como una variable continua. Observamos un asociación estadísticamente significativa de BLNLR con supervivencia en el modelo univariante (HR 2.89; IC95%: 2.12–3.94; p<0.001) y en el modelo multivariante (HR 1.91; 95%CI 1.31– 2.79; p = 0.001) con modelos incluidos en el nomograma de Halabi[5] (presencia de dolor, enfermedad medible, ECOG performance status, progresión dentro de los 6 meses tras finalizar docetaxel, enfermedad visceral,

duración del tratamiento hormonal previo, valor basal de PSA, hemoglobina y fosfatasa alcalina), además del brazo de tratamiento y el uso basal de corticoesteroides.

Evaluamos también cómo la adición del BLNLR podría mejorar los nomogramas pronósticos establecidos. Para ello, comparamos el índice de concordancia (c-index) del modelo de regresión de Cox multivariante derivado del nomograma de Halabi con y sin la adición de BLNLR como covariable. El c-index del modelo derivado del nomograma de Halabi fue de 0,728 (CI95% 0,699–0,757); al añadir BLNLR al modelo, se observó un incremento del c-index a 0,736 (IC95% 0,707–0,765). Dicho incremento en c-index no fue estadísticamente significativo (diferencia c-index: 0,008; IC95%: -0,005 a 0,020). Por tanto, a pesar de la asociación con la supervivencia, no pudimos concluir que el NLR aumentara de forma significativa la capacidad pronóstica de los modelos disponibles.

Utilizando NLR = 3 como criterio para diferenciar NLR “disminuido” (BLNLR < 3) de NLR “elevado”, evaluamos entonces la asociación de la variable dicotómica con la supervivencia global, supervivencia libre de progresión radiológica y supervivencia libre de progresión por PSA. Los pacientes con BLNLR < 3 tuvieron una supervivencia global (15,9 vs 12,6 meses, HR 1,55 (IC95% 1,3– 1,84), p < 0,001), una supervivencia libre de progresión por PSA (5,3 vs 3,1 meses; HR 1,35 (IC95% 1,12–1,62); p=0,002) y una supervivencia libre de progresión radiológica (9,3 vs 5,7 meses; HR 1,42 (IC95% 1,15–1,76); p = 0,001) superiores a los pacientes con BLNLR ≥ 3. El beneficio observado fue independiente del brazo de tratamiento (cabazitaxel o mitoxantrona). Al realizar una comparación estratificada de ambos brazos de tratamiento en pacientes con NLR elevada o disminuida, se observó que cabazitaxel mantenía el beneficio en supervivencia global sobre mitoxantrona, por lo que concluimos que el BLNLR no tiene valor predictivo para la selección de pacientes tratados con cabazitaxel.

Seguidamente, evaluamos si el NLR podría identificar pacientes con mayor probabilidad de obtener una respuesta radiológica o por PSA. Se evaluó la respuesta radiológica únicamente en pacientes con enfermedad medible por RECIST (405 pacientes, un 53,6% del total). Se observó respuesta radiológica en un 15,6% (32/147) de pacientes con BLNLR bajo, frente a un 7,7% (14/183) de pacientes con BLNLR elevado (p = 0,022). Observamos resultados similares al evaluar la respuesta por PSA, que se definió como una disminución de PSA ≥ 50% a las 12 semanas de tratamiento, con un total de 654 pacientes definidos como evaluables. La tasa de respuesta por PSA fue superior en pacientes con BLNLR < 3 (35,7% vs 22,1%; p < 0,001). Las diferencias observadas en tasa de respuesta radiológica y tasa de respuesta por PSA fueron ambas independientes del brazo de tratamiento administrado. Estos hallazgos, sin embargo, no implican un valor predictivo del NLR; a lo largo de diferentes estudios, se ha observado cómo los pacientes con mejor perfil pronóstico tienden a presentar mejores tasas de respuesta al tratamiento, por lo que nuestra interpretación es que la diferencia en tasas de respuesta a favor de pacientes con NLR bajos está relacionada con el perfil pronóstico favorable de éstos.

Los corticoesteroides son comúnmente utilizados en cáncer de próstata, tanto como medicación concomitante del tratamiento antineoplásico (tratamiento con abiraterona o taxanos) como para el tratamiento específico del dolor, la astenia o la anorexia, por ejemplo. Además, los corticoesteroides tienen un efecto reconocido sobre el sistema inmune, con propiedades inmunosupresoras, y el potencial para incrementar el recuento de neutrófilos y disminuir el recuento linfocitario. Ninguno de los estudios previos había investigado el potencial efecto como factor de confusión del tratamiento concomitante con corticoesteroides al evaluar el impacto de NLR en la supervivencia. In nuestro estudio, 342 (45%) pacientes recibían corticoesteroides en la visita basal. Observamos una mayor mediana de NLR (3,9 vs 2,9; p < 0,001) y una mayor proporción de pacientes con BLNLR ≥ 3 entre aquéllos que recibían corticoesteroides (49,6% vs 40,6%; p = 0,016). Estos hallazgos pueden estar relacionados con el efecto directo del tratamiento corticoideo sobre los recuentos de neutrófilos y linfocitarios, pero también con el hecho de que los pacientes con peor ECOG PS y más dolor, características que están asociadas a un peor pronóstico (y a un mayor NLR basal) reciben con más frecuencia tratamiento corticoesteroideo. En cualquier caso, al incluir el tratamiento corticoideo basal en el modelo pronóstico, se mantuvo la asociación estadísticamente significativa de BLNR y supervivencia global. Además, realizamos un test de interacción entre uso de corticoides y NLR basal, que resultó no estadísticamente significativo (p=0,82), lo que interpretamos como que el NLR basal tiene un valor pronóstico que es independiente de la toma de corticoides por parte del paciente.

Finalmente, evaluamos si los cambios en NLR podían ser indicativos de respuesta al tratamiento, al estudiar el valor de la "conversión" de NLR "favorable" (NLR < 3) a "desfavorable" (NLR ≥ 3) durante las primeras 12 semanas de tratamiento. En total, 345 pacientes con NLR basal desfavorable tenían valor de NLR y al menos un valor de seguimiento en las primeras 12 semanas; de estos, 141 (44%) experimentaron una "conversión" durante el tratamiento. Pacientes con una conversión de NLR "desfavorable" a "favorable" experimentaron una mayor supervivencia global (14,5 versus 11,7 meses; HR 0,76; p = 0,032) y tasa de respuesta por PSA (30,4% vs 18,6%; p = 0,016). La diferencia en supervivencia global fue independiente del brazo de tratamiento y de los otros factores pronóstico en el análisis multivariante. Por otro lado, 326 pacientes tenían una NLR basal "favorable"; de ellos, 201 (62%) experimentaron una conversión a recuentos "desfavorables". La conversión a un recuento desfavorable (progresión), sin embargo, no se vio asociada de forma estadísticamente significativa a la peor supervivencia (15,7 vs 16,5 meses; HR 1,1; p = 0,4) o a una menor tasa de respuesta por PSA (35,9% vs 39,3%; p = 0,56).

c. UTILIZACIÓN CLÍNICA DE BIOMARCADORES

Realizamos una encuesta *online* de facultativos especialistas en el tratamiento del adenocarcinoma de próstata resistente a la castración metastásico, para valorar los patrones de uso habitual de biomarcadores en la práctica clínica, las preferencias y el conocimiento general de los criterios

reflejados en las guías de la *Prostate Cancer Working Group* (PCWG2).[11] Se envió e-mails con una invitación para participar a 485 especialistas del Reino Unido que participaban activamente en ensayos clínicos en cáncer de próstata, 29 especialistas miembros del Grupo de Investigación de Tumores Genitourinarios de Suiza y 20 especialistas en el tratamiento del cáncer de próstata en Australia y Nueva Zelanda. 118 (22,1%) especialistas respondieron el cuestionario.

I. Opinión sobre el valor de los biomarcadores y conocimiento general de los criterios de progresión
Evaluamos la opinión de los participantes sobre los biomarcadores disponibles en la actualidad (PSA, rastreo óseo [RO], tomografía computarizada [TC] y células tumorales circulantes [CTCs]) para la monitorización de la respuesta al tratamiento. 79 participantes (74,5%) opinaron que éstos eran útiles (71,7%) o muy útiles (2,8%). Sólo 39,6% utilizaban los criterios PCWG2 la mayoría del tiempo, y 27,3% lo utilizaban raramente o nunca. Un total de 59 participantes (55,7%) consideraban el PSA como un biomarcador útil o muy útil para monitorizar la respuesta al tratamiento.

A pesar de la opinión generalmente favorable respecto a los biomarcadores disponibles, observamos que únicamente una tercera parte de los participantes los utilizaban en su práctica clínica habitual. También observamos un conocimiento insuficiente de los criterios actuales (PCWG2). Al utilizar diferentes ejemplos gráficos sobre la progresión por PSA, observamos que únicamente un 41,4% de participantes fueron capaces de identificar que se requiere al menos 12 semanas para definir la progresión por PSA. Un 84% de los participantes fue capaz de reconocer que la progresión se define por un aumento de al menos un 25% sobre el valor nadir, que debe confirmarse al menos 3 semanas después. Un 91% de los participantes fue incapaz de reconocer que existe progresión por PSA si el segundo valor de confirmación es inferior al primer valor de progresión, siempre que ambos sean un 25% superiores al valor nadir.

Evaluamos además el conocimiento de los criterios de progresión por rastreo óseo. Se pidió a los participantes que eligieran de entre varias posibles definiciones (existía la posibilidad de elegir más de una). Únicamente un 39,4% de los pacientes contestaron la respuesta correcta (según criterios PCWG2) y descartaron las respuestas incorrectas, lo que se indica variabilidad en la interpretación de los resultados del rastreo óseo.

Seguidamente, se preguntó a los participantes sobre su conocimiento del valor de las CTCs. Un 98% de los participantes respondió estar familiarizado con el concepto de las CTCs, pero únicamente un 53,1% fue capaz de reconocer que presentan un valor pronóstico. Asimismo, sólo un 50% y un 54,1% de los participantes eran conscientes de la asociación con supervivencia global de una disminución del recuento de CTCs en pacientes tratados con abiraterona y quimioterapia, respectivamente.

II. Decisiones Clínicas en CPRCm

Preguntamos a los participantes sobre los motivos de discontinuación del tratamiento en su práctica clínica habitual. Casi todos los participantes (90,5%) consideraron la progresión clínica como la más importante para suspender un tratamiento e iniciar una nueva línea. Un 71,6% y un 47,7% de los participantes consideraron la progresión por RECIST (TC) y RO, respectivamente, como importante; únicamente un 23,2% y un 21,1%, por otro lado, consideraron que el PSA y las CTCs, respectivamente, eran importantes a la hora de considerar un cambio de tratamiento. A pesar de que un 74,5% de los participantes consideraban el PSA como útil o muy útil para guiar el tratamiento , únicamente un 21,1% lo consideró importante para la toma de decisiones clínicas. Además, sólo un 30% de los participantes que consideraron el PSA como importante o muy importante para el cambio de tratamiento habían reconocido que se necesita al menos 12 semanas para definir la progresión por PSA.

Se solicitó también a los participantes que indicaran cuál sería su actitud clínica ante cuatro escenarios clínicos diferentes con datos contradictorios de respuesta/progresión en los diferentes biomarcadores, en los que se asumía que el paciente presentaba enfermedad metastásica ósea exclusivamente, y que no existía un empeoramiento clínico. En un escenario clínico con una conversión (respuesta) por CTCs, sin cambios en el rastreo óseo, pero progresión por PSA, una gran mayoría de participantes (92%) respondió que no cambiaría el tratamiento. La proporción de participantes que continuarían tratamiento en un escenario similar al anterior, pero con un aumento de la captación del rastreo óseo (no considerado progresión de enfermedad por criterios PCWG2) cayó a un 69%; 13% refirieron que suspenderían el tratamiento, y 18% no estaban seguros de la decisión. En pacientes con una respuesta por PSA pero un aumento de CTCs, sin cambios en el rastreo óseo, un 11% de participantes respondió que cambarían de línea de tratamiento, mientras que aproximadamente un 75% continuarían. Finalmente, en un escenario clínico con respuesta por PSA y CTCs pero un rastreo con nuevas lesiones (progresión no confirmada), un 10% respondieron que cambiarían de tratamiento, mientras que un 71% continuaría el tratamiento.

En pacientes con progresión por PSA y disminución de CTCs, en ausencia de progresión radiológica, la mayoría de participantes, la mayoría de participantes respondieron que no suspenderían / cambiarían el tratamiento de quimioterapia (83,2%) o de abiraterona/enzalutamida (90,5%), reconociendo por tanto el valor de las CTCs pero también en concordancia por lo recogido en las guías clínicas de la PCWG2. En la misma línea, únicamente un 33,7% de los participantes contestaron estar preparados para utilizar los cambios en CTCs únicamente, independientemente del PSA o rastreo óseo, para guiar el cambio o suspensión de tratamiento en pacientes con enfermedad metastásica ósea exclusivamente. Entre aquéllos que no estaban dispuestos a cambiar el tratamiento en base a cambios en CTCs únicamente, un 57,6% refirió la incertidumbre sobre el valor como indicador de respuesta como un reto para su uso. Un 52,5% y un 42,4%, respectivamente, refirieron además la incertidumbre sobre su valor pronóstico y la dificultada para la interpretación de los cambios en CTCs con el tratamiento.

Reconocimos, como limitación del estudio, la baja tasa de respuesta (22,1%), sin que todos los participantes completaran la encuesta. Las razones para no completar la encuesta son desconocidas, aunque podría estar en relación con la ausencia de una compensación económica para ello. Además, no realizamos distinciones entre centros académicos y no académicos, y tampoco se realizó una distinción entre participantes dentro y fuera del Reino Unido. Para maximizar el rendimiento de la información, incluimos únicamente tres preguntas sobre criterios de biomarcadores, lo que ha podido resultar insuficiente para evaluar de forma exhaustiva el conocimiento de los participantes.

Introduction

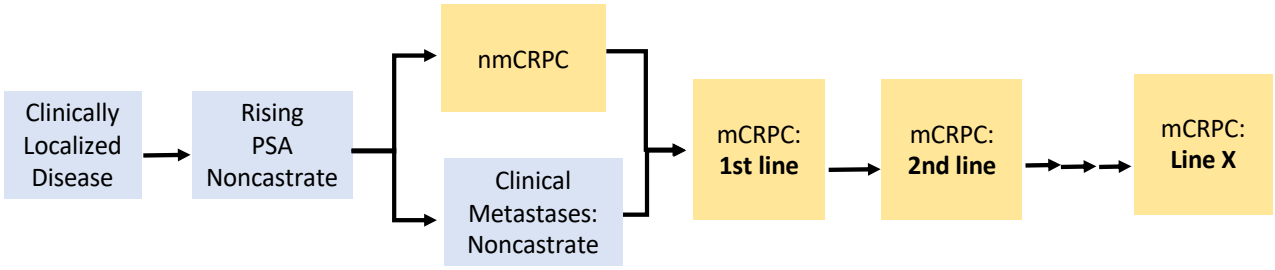
1. What is a Biomarker? Concepts in Biomarker Development

1.1 Why are Biomarkers Important in Prostate Cancer?

Prostate cancer is currently the second most common cancer in men, accounting for 15% of male cancer cases, the fifth leading cause of death in men worldwide (6.6% of total deaths) and a major cause of morbidity.[1] Prostate cancer is characterized by a clinically heterogeneous course, with a number of distinct clinical states observed across its natural history that have different diagnosis, prognosis and treatment options (Figure 1).

For instance, in intermediate or favorable-risk localized prostate cancer, where active surveillance, radical prostatectomy or radiotherapy are established treatment options, has an estimated 10-year survival of 95%.[12] This is in stark contrast with metastatic castration-resistant prostate cancer, the final stage of the disease, which is invariably lethal, resulting in death after a median of approximately 32-34 months.[13,14] There is no continuum in disease states, and patients can be diagnosed with clinically localized disease, with varying options of cure, or with metastatic disease, where no curative options exist. In fact, current reports of patients dying from prostate cancer estimate an approximate 50% of patients that are diagnosed with localized disease, while the other 50% results from patients that present with metastases at diagnosis.[15]

Figure 1 Clinical States in Prostate Cancer
(adapted from Scher et al.)[16]



The objectives of treatment will therefore vary in each of the different clinical states: while in healthy individuals the main objective is the prevention and early detection of disease, objectives in clinically localized disease will be to maximize options of cure while minimizing morbidity, and the delay or preventing the development of metastases will be the main goal of treatment in patients with a PSA relapse after localized therapy.

As the objectives of treatment vary across the different clinical states, so do the relevant questions that need answering in order to improve outcomes. Why do some patients prevent with clinically indolent disease, while others are diagnosed with aggressive, metastatic disease? Can we improve risk estimation in order to prevent treatment morbidity in indolent disease, and intensify treatment in order to

maximize the options of cure in patients with more aggressive disease? Clinicians rely on biomarkers to estimate clinically relevant endpoints that may assist in decision-making. In localized prostate cancer, for example, clinical characteristics such as the Gleason Grade, PSA at diagnosis or local extent of the disease are used to estimate the risk of relapse, and decide between the different treatment options.[17]

Advanced prostate cancer is a concept that encompasses several disease states, namely metastatic castration-resistant prostate cancer (mCRPC) and metastatic, hormone-sensitive prostate cancer (mHSPC). These are defined by the presence of metastatic disease (determined by bone scintigraphy or CT scan) and the presence or absence of a castrate state (defined by testosterone levels below 50 ng/dL). In these patients, the main goals of treatment will be to relieve disease manifestations (pain), prevent the development of new symptoms or disease manifestations (skeletal related events) and to prolong survival.

In recent years, advances in drug development and molecular studies for advanced prostate cancer have enabled a more efficient and precise patient care through the use of predictive, response, and resistance biomarkers. However, clinical unmet needs remain to be answered such as:

(1) Can we improve our prognostic assessment, in order to understand when treatment is needed, and when a "watch and wait" approach is acceptable?
(2) Can we select therapies based on the underlying biology of the disease?
(3) Can we improve our assessment of the effect of treatment? Can we identify disease progression before clinical deterioration develops?
(4) Can we define alternative endpoints to overall survival, in order to accelerate the incorporation of effective drugs into the therapeutic armamentarium?

1.2 Definition and Types of Biomarkers

A biomarker is defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes, or of pharmacologic responses to a therapeutic intervention".[18]
In prostate cancer, a number of different clinical (extent of metastatic disease, ECOG performance status or age) and laboratory (Alkaline Phosphatase, Lactate Dehydrogenase or PSA) parameters have been used as biomarkers. Many novel imaging, genetic and molecular biomarkers are also currently under development.

Depending on their clinical use, biomarkers can be classified according to their clinical application into diagnostic, monitoring, pharmacodynamic, prognostic, predictive or safety biomarkers. Clinical applications of a biomarker are not mutually exclusive. For example, biomarkers can exhibit both prognostic and predictive value depending on the context of use. Conceptual distinction between these

types must be made in order to correctly establish the clinical potential and applicability of a biomarker. According to the FDA-NIH Biomarker Working Group,[19] biomarkers can be classified as (Table 1):

Table 1. Types of Biomarkers[19]

Diagnostic biomarker	Biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease
Monitoring biomarker	Biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent.
Pharmacodynamic biomarker	Biomarker used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent
Prognostic biomarker	Biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest
Predictive biomarker	Biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favourable or unfavourable effect from exposure to a medical product or an environmental agent.
Safety biomarker	Biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect
Surrogate endpoint	Endpoint supported by a clear mechanistic rationale and clinical data providing strong evidence that an effect on the surrogate endpoint predicts a specific clinical benefit

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Prognostic and predictive biomarkers both include pre-treatment characteristics, that is, clinical or molecular characteristics that are present before treatment is initiated. It is relatively common, however, to misrepresent prognostic biomarkers as predictive due to methodologically flawed studies. While, on one hand, prognostic biomarkers provide information on the likelihood of a clinical event (death, disease progression), predictive biomarkers, on the other hand, provide information on the likelihood that biomarker positive patients receive benefit from a specific treatment, in comparison to biomarker negative patients. For this reason, the predictive potential of a biomarker can generally not be established in single arm cohorts; treatment benefit (comparison of outcome in treated vs control patients) in biomarker positive must be evident, while absent in biomarker negative patients. A statistical test of the interaction between treatment arm and biomarker status may help to establish the statistical significance of the difference in benefit. An exception to this rule can be made if there is such an evident benefit from biomarker positive patients in comparison to historical controls, that clinical trials are only ethically acceptable if performed as single-cohort trials in biomarker-enriched populations. As an example, the BRAF inhibitor vemurafenib was approved in August 2011 for advanced melanoma patients with BRAF V600E mutations as a predictive biomarker without a randomized trial.[20]

Prognostic biomarkers are useful for the assessment of the individual risk a patient has of experiencing a clinically relevant endpoint (in advanced prostate cancer, examples include disease progression, a skeletal-related event or death). Prognostic biomarkers are also important when interpreting phase III trial data, to minimize the risk of selection bias by allocating patients with equivalent prognosis into each arm of the trial. In prostate cancer, a number of clinical parameters have shown prognostic value, and have been incorporated into a number of different nomograms for the estimation of individual risk in each of the stages of the disease.

Predictive biomarkers, on the other hand, may help select treatment options based on molecular or clinical characteristics of the individual patient. Improved insights into the molecular biology of the disease have identified a number of molecular aberrations (PTEN loss, ERG rearrangements, AR amplifications/mutations, DNA-repair defects) that may be associated with increased response or resistance to different treatment options in mCRPC. Despite the numerous candidate biomarkers being evaluated, most of these have not yet completed a satisfactory analytical validation (i.e. the development of an assay with a consistent mechanism of acquisition and interpretation across laboratories). Only DNA-repair aberrations have undergone preliminary clinical validation, that has led to FDA accelerated approval for PARP inhibitor in biomarker-positive populations.

Pharmacodynamic biomarkers (treatment-response biomarkers) are post-treatment characteristics that may provide information on whether the patient is benefitting from a specific treatment. An example of treatment-response biomarker are RECIST criteria, which evaluate benefit based on the difference in size of target lesions obtained in CT or MRI scans.[21] In prostate cancer, implementation has been difficult since RECIST does not include criteria to evaluate changes in bone metastases (up to 50% of patients with advanced prostate cancer exhibit metastases exclusively to the bone) or changes in PSA levels.[22] Treatment-response biomarkers are important for the identification of patients that are not benefitting from treatment as early as possible, in order to be able to change to an alternative treatment option before clinical deterioration ensues.

Surrogate biomarkers are those intended to serve as a substitute for a clinically meaningful endpoint (in advanced prostate cancer, generally overall survival) which are expected to predict the effect of a therapeutic intervention.[23] If accepted by regulatory agencies, a trial that proves a significant benefit in a surrogate endpoint can be approved for use. This can increase the efficiency of clinical trial design, by being able to improve the design of (less costly in time and resources) clinical trials that can enable the incorporation of active agents into the clinic more efficiently. In colorectal cancer, for instance, 3-year disease free survival has been accepted as a surrogate of 5-year overall survival, accepted for drug approval in the adjuvant setting.[24] In prostate cancer, metastasis-free survival (localized prostate cancer), radiographic progression-free survival (mCRPC), PSA declines and a CTC-LDH biomarker panel (mCRPC) have all been evaluated as candidate surrogates of survival.[25,26]

1.3 Analytical and Clinical Qualification

Before being incorporated into clinical practice, the candidate biomarker must undergo a thorough process of analytical and clinical validation. Validation is defined as "a process to establish that the performance of a test, tool, or instrument is acceptable for its intended purpose"[19] Validation refers to the assurance that the biomarker measures what is intended (analytical validation), and that information is relevant for improving outcomes in daily clinical practice (clinical validation).

Data generated during the analytical validation process are those that define the how accurate and reproducible the measurement of the biomarker is, including parameters such as sensitivity, specificity, accuracy or precision, defined when following a detailed protocol of use. Analytical validation can be subdivided into pre-analytical assessments (acquisition of the biomarker, storage, reproducibility of the measurement, storage) and post-analytical assessments (interpretation and reporting of the biomarker).[27] Especially important is ensuring the consistency of measurements when performed in different laboratories, which is a key issue in order to ensure the assessment of the biomarker is generalizable. This is usually performed by evaluating the degree of correlation (correlation coefficient, if continuous variables) or agreement (Kappa index, if categorical values) of measurements by the same assay in independent centres. Analytical validation therefore ensures that what the biomarker is measuring is reliably informing of a biological process, but does not provide any proof that the biological process is at all related to any relevant clinical endpoint.

To be successful, clinical validation can only be pursued after analytical validation has been established. By clinically validating a biomarker, one evaluates to what degree the use of the biomarker will provide additional or improved information on a clinically relevant biologic process that can help make better clinical decisions. For example, whether the biomarker improves our estimation of the risk a patient has of experiencing disease progression or pain (prognostic biomarkers), of the likelihood of experiencing clinical benefit from a given therapeutic option (predictive biomarkers) or the benefit (or lack thereof) a patient may be experiencing from the current treatment, and whether it should be continued or stopped. Retrospective analyses of prospective clinical trials are generally used to explore the clinical utility of candidate biomarkers, but prospective validation in well-designed clinical trials or prospective cohorts is required. Clinical qualification represents the final step in the development of a biomarker, with regulatory implications, if the use of the biomarker is shown to improve a clinically relevant endpoint; thus, a clinically qualified biomarker is one for which sufficient evidence has been generated for acceptance for use in regulatory submissions.[27]

For a biomarker to prove its value as a surrogate, the following criteria defined by Prentice and collaborators must be met.[28] For a proposed surrogate of overall survival, for example:

(1) Treatment must have a significant effect on a clinical endpoint (meaning only trials with a significant improvement in, for instance, overall survival are adequate for analysis.
(2) Treatment must have a significant effect on the proposed biomarker (there must be a significant difference in the proposed surrogate biomarker on both arms of the trial).
(3) The biomarker must have a significant impact on the clinical end-point (there must be a significant association between the proposed surrogate and overall survival. This is generally evaluated by the treatment proportion estimate (TPE) or concordance index [c-index])
(4) The full effect of treatment on the clinical end-point must be captured by the biomarker (meaning that, after accounting for the surrogate, the treatment has no significant effect on survival).

Additionally, these criteria must be met not only in one clinical trial (individual level surrogacy), but on a multitude of clinical trials, evaluated through a meta-analytical approach (trial level surrogacy),[29] required for acceptance by regulatory authorities. For example, 3-year DFS required a meta-analysis of 18 randomized phase III trials including 20,898 patients.[24] In prostate cancer, no biomarker has been accepted to date as a surrogate of overall survival by regulatory authorities.

2. Defining the Optimal Treatment Sequence in Advanced Prostate Cancer

2.1 The Evolving Therapeutic Landscape of Advanced Prostate Cancer

In the past years, the successful development of novel therapeutic agents such as cabazitaxel, abiraterone, enzalutamide and Radium-223 has dramatically improved the outcome of metastatic castration-resistant prostate cancer. More recently, the use of some of these agents in earlier settings of advanced prostate cancer, as is the case with abiraterone or docetaxel in metastatic, hormone-sensitive disease (mHSPC)[30–32] or of enzalutamide, apalutamide and darolutamide in non-metastatic CRPC (nmCRPC)[33–35] have also shown to significantly improve outcome. The rapid incorporation of such a high number of effective drugs into the therapeutic armamentarium has dramatically changed the therapeutic landscape of the disease (Figure 3)

Before 2004, there were no systemic agents that had proven survival benefit in patients with mCRPC, after progression on androgen deprivation; patients who were fit enough to continue on treatment would be subject to secondary hormonal therapies (antiandrogen switch, antiandrogen withdrawal, treatment with corticosteroids, stylboestrol or estramustine), neither of which prolonged survival.[36] The chemotherapeutic agent mitoxantrone was also used in symptomatic patients, on the basis of symptom improvement in randomized trials.[37]

After 2004, with the publication of the landmark trials demonstrating a survival benefit of docetaxel in mCRPC,[38,39] docetaxel was approved and became the standard of care as first-line therapy for symptomatic patients for the following years. It was not until 2010-2012, with the publication of clinical trials evaluating cabazitaxel[4], abiraterone[2] and enzalutamide[40] in patients that had previously progressed on chemotherapy, that these agents were approved and incorporated into second-line therapy. In 2012, the treatment sequence therefore included docetaxel as first-line treatment option, and abiraterone, enzalutamide and cabazitaxel as second or third-line options.

Subsequent trials (COU-AA-302, PREVAIL)[13,41] then proved the benefit of both abiraterone and enzalutamide over placebo in chemotherapy-naïve, minimally symptomatic patients, led to their approval as first-line treatment options. Results of the ALSYMPCA trial[42] with Ra-223 in patients with symptomatic bone metastases, which included both patients after progression on docetaxel and docetaxel-naïve patients that were unfit for docetaxel, incorporated Ra-223 generally as a third-line option, but also as a first-line or second-line option for select cases.

The incorporation of all these agents into the first-line of treatment (now including options such as docetaxel, abiraterone, enzalutamide and Ra-223 for specific cases), exposure to docetaxel was used as

a spurious classifier in order to allocate the subsequently approved agents into the pre-docetaxel (generally less symptomatic, with a better prognosis) and the post-docetaxel (generally more symptomatic, with a worse prognosis) space.[43] This categorization has been now largely abandoned in favor of a "clinical states model" as proposed by Scher and colleagues, with treatment line (first, second, third, etc.…) listed for each of the disease states (localized, PSA relapse, mHSPC, nmCRPC or mCRPC).[44]

After 2015, with the successful outcomes of trials evaluating docetaxel in the metastatic-castration naïve setting,[30,45] followed more recently by trials establishing the value of abiraterone, enzalutamide and apalutamide in a similar patient population,[31,32] the landscape has been further complicated by the fact that a proportion of patients receiving first-line treatment for mCRPC may already be abiraterone or docetaxel-refractory. Similarly, recently presented phase III trials evaluating the role of apalutamide, enzalutamide and darolutamide in non-metastatic, castration-resistant disease[33–35] which will likely lead to the approval of these agents will add further complexity in the following years.

More recently, PARP inhibitors (Olaparib) have been approved as a treatment option in patients with DNA-repair deficiencies (mainly, although not restricted to BRCA and ATM mutations), after results from the single arm, biomarker-driven phase II TOPARP trial,[46,47] which led to the first drug approval based on a specific molecular biomarker in prostate cancer. Olaparib has now proven superiority over abiraterone or enzalutamide in third-line (after a previous AR signaling inhibitor and docetaxel) in the phase III PROFOUND trial.[48] However, future trials may establish PARP inhibitors in earlier states of the disease.

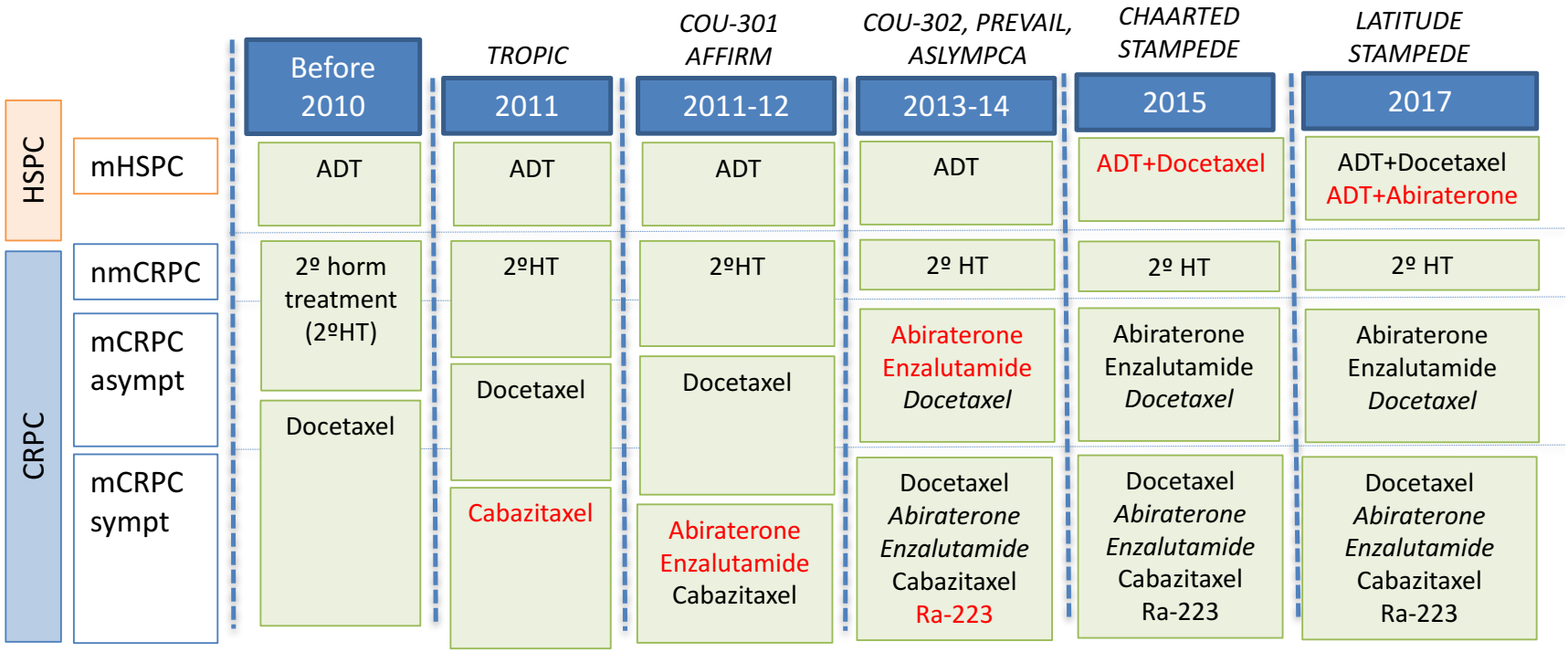
At the moment, little is known of the optimal treatment sequencing; data derived from clinical trials is unable to elucidate the issue due to a number of reasons:

(1) There are virtually no face-to-face comparisons of available agents in first-line mCRPC, mHSPC or nmCRPC.
(2) None of the control arms of the landmark trials is a currently accepted standard of care.
(3) Due to differences in post-progression treatment options, the direct comparison of data from landmark clinical trials is a biased approach.
(4) There is a lack of validated clinical and molecular biomarkers that may help tailor treatment selection based on individual patient characteristics.

We are therefore left with indirect data from clinical trials and from data mainly from prospective and retrospective cohort studies in order to make relevant decisions on treatment selection.

Figure 2. The evolving landscape of advanced prostate cancer treatment.

The years in the blue boxes refer to dates of EU regulatory approval.



2.2 Choice of First-Line Agent

The choice of a first-line agent based on clinical activity or survival is limited by the lack of face-to-face comparison between approved agents, which has led to the absence of prospective data on treatment efficacy in first-line. Interpretation of clinical trial data is further complicated by the fact that, due to the particular timeline when these agents underwent clinical evaluation in pivotal clinical trials, none of the control arms of the trials consisted in what could be considered standard of care. For example, control arms of the abiraterone trials (COU-AA-301 and COU-AA-302) consisted in placebo + prednisone, while control arms of the enzalutamide (AFFIRM, PREVAIL) or Ra-223 (ALSYMPCA) consisted in placebo (at a time when docetaxel was an established first-line treatment option). In the only randomized trial (FIRSTANA) where two life-prolonging agents were directly compared, there were no significant differences in overall or progression-free survival between docetaxel and cabazitaxel when used as first-line agents for mCRPC.[49]

Table 2 summarizes the results from pivotal phase III clinical trials. When comparing clinical trials, the heterogeneity in treatment populations must be taken into account. For instance, asymptomatic or minimally symptomatic mCRPC patients were evaluated in trials of abiraterone (COU-302) enzalutamide (PREVAIL), with overall survival in the control (placebo) arms of approximately 30 months, which is in stark contrast to the only 16.5 months survival observed in the first-line clinical trial evaluating docetaxel (TAX-327),[39] closer to the survival in the control arms of the TROPIC, COU-301, AFFIRM or ALSYMPCA trials (ranging between 11.2 and 16.5 months), where patients with a more advanced disease were included. Despite differences in median overall survival, a similar reduction in the risk of death observed in trials with asymptomatic and symptomatic patients, with HRs 0.63-0.76 (Table 2).

These differences in the survival of control arms of the trials are likely related to two factors:

(1) Patients treated in the TAX-327 trial had worse prognostic factors (higher burden of disease, pain, other prognostic factors).
(2) A significant proportion of patients treated in the placebo arm of the COU-AA-302 or PREVAIL studies received life-prolonging agents (abiraterone, enzalutamide, docetaxel, cabazitaxel) which were not available for patients treated in the TAX-327 trial 10 years earlier.

Only cabazitaxel was compared to a different chemotherapy (mitoxantrone) in the TROPIC trial, although there is no evidence of a survival benefit with this treatment option. The fact that prednisone, used as a control arm in the abiraterone (COU-AA-301 and COU-AA-302) but not in the enzalutamide (AFFIRM and PREVAIL) trials, is an active agent in monotherapy (PSA response of 24% , 5.1-month time to PSA progression in a randomized, phase II trial)[50] implies that, despite the seemingly equivalent patient populations, a direct comparison of these trials may lead to biased conclusions.

What agent should, therefore, be used as the first-line agent of choice? Despite the differences in clinical trial design, docetaxel, abiraterone and enzalutamide have proven a survival benefit in the first-line mCRPC setting, with similar reductions in the risk of death (19-29%) and no head-to-head comparisons. Treatment choices are therefore not based on the superiority of one agent over another, but rather on a combination of factors including patient characteristics (extrapolation from trial populations), cost, toxicity of patient/physician preference.[51] In real-world practice, differences in toxicity and more convenient oral administration have made abiraterone and enzalutamide the preferred first-line option over taxanes despite a lack of evidence of superior activity.[52]

Table 2. Summary of Pivotal Phase III Trials in mCRPC

Trial	Line	N	OS exper	OS control	OS diff	OS HR (95%CI)	RECIST resp	PSA resp
Symptomatic, 1st – 3rd line mCRPC								
TAX-327[39] (docetaxel)	1st	1006	18.9 m	16.5 m	2.4 m	0.76 (0.62-0.94)	12% vs 7% (p=0.11)	45% vs 32% (p<0.001)
TROPIC[4] (cabazitaxel)	2nd	755	15.1 m	12.7 m	2.4 m	0.70 (0.59-0.83)	14.4% vs 4% (p<0.001)	39% vs 18% (p<0.001)
COU-301[2] (abiraterone)	2nd	1195	15.8 m	11.2 m	3.4 m	0.74 (0.59-0.83)	14.8% vs 3.3% (p<0.001)	29.5% vs 6% (p<0.001)
AFFIRM[40] (enzalutamide)	2nd	1199	18.4 m	13.6 m	4.8 m	0.63 (0.53-0.75)	29% vs 4% (p<0.001)	54% vs 2% (p<0.001)
ALSYMPCA[42] (Ra-223)	1st-2nd	921	14.9 m	11.3 m	3.6 m	0.70 (0.58-0.83)	-	16% vs 6% (p<0.001)
Minimally symptomatic or asymptomatic, 1st line mCRPC								
COU-302[13] (abiraterone)	1st	1088	34.7 m	30.3 m	4.4 m	0.81 (0.70-0.93)	36% vs 16% (p<0.001)	62% vs 24% (p<0.001)
PREVAIL[41] (enzalutamide)	1st	1717	32.4 m	30.2 m	2.2 m	0.71 (0.60-0.84)	59% vs 5% (p<0.001)	78% vs 3% (p<0.001)
IMPACT[53] (sipuleucel-T)	1st	512	25.8 m	21.7 m	4.1 m	0.78 (0.61-0.98)	-	2.6% vs 1.3% (p=NS)

Toxicity profiles clearly benefit hormonal agents (abiraterone, enzalutamide) over chemotherapy. Taxane chemotherapeutics such as docetaxel and especially cabazitaxel carry a significant risk of development of neutropenia, which may be a serious complication if febrile neutropenia ensues, a potentially fatal event that generally warrants an in-hospital admission. Peripheral neuropathy, on the other hand, is a potential dose-limiting toxicity of docetaxel, present in up to 32% of patients in the treatment arm of TAX-327. Other toxicities associated with taxane chemotherapy include risk of asthenia, alopecia, nail toxicity, mucositis or diarrhea.[54] Toxicities associated with abiraterone and enzalutamide are considered, on the other hand, mild and manageable for the majority of patients (fluid retention, hypokalemia and hypertension in the COU-AA-302 trial, and fatigue, diarrhea, hot flashes and hypertension in the PREVAIL trial).[43]

2.3 What should be the first-line hormonal agent of choice?

As discussed earlier, hormonal agents are usually the first-line agents of choice in mCRPC. Both agents were explored in equivalent scenarios, have similar efficacy, and are well tolerated.[51] Due to their similar activity and favorable toxicity profiles, choosing between agents based on objective data is often difficult. Furthermore, differences in the control arms of the clinical trials (prednisone in abiraterone trials, placebo in enzalutamide trials) make comparison of clinical trials impractical.

Indirect data from one prospective clinical trial is available to compare outcome from both agents: abiraterone and enzalutamide were compared in a randomized phase II trial including 202 mCRPC patients receiving first-line therapy, with an aim to evaluate the optimal sequence (abiraterone → enzalutamide vs enzalutamide → abiraterone). The trial, which was not powered to detect overall survival differences, reported a significantly higher rate of PSA response for first-line enzalutamide over abiraterone (82% v vs 68%; p=0.023), but no significant differences in time to PSA progression (11.2 vs 10.2 months; p=0.78) or overall survival (28.8 vs 24.7 months; p=0.23).[55]

Differences in trial design or specific toxicities have helped choose between agents in selected cases. For instance, the presence of visceral metastases was an exclusion factor in the COU-AA-302 trial (abiraterone) but not in the PREVAIL (enzalutamide) trial; although one may extrapolate data from the COU-AA-301 trial to infer that abiraterone is active in this patient population, enzalutamide is generally the preferred option in this case. A post-hoc analysis of the PREVAIL trial confirmed the activity of first-line enzalutamide in this subgroup of patients.[56] Enzalutamide, on the other hand, is known to increase the risk of seizures. Patients with a prior history of seizures were excluded from participation in the AFFIRM and PREVAIL trials, and a recommendation against the use enzalutamide in these patients has been issued.[57] This may, however, not be the case, as a recent phase IV trial reported an incidence of seizures of only 1.9% in mCRPC patients at risk (patients receiving medications that lowered the seizure threshold or with a history of brain injury, cerebrovascular accident or transient ischemic attack), a similar rate to patients not receiving enzalutamide.[58]

The use of concomitant prednisone with abiraterone has led some authors to suggest that patients with immune deficiencies, or diabetes mellitus should receive enzalutamide instead of abiraterone. A pooled analysis of the COU-AA-301 and COU-AA-302 trials, however, reported on a low incidence of corticosteroid-associated adverse events, with hyperglycemia (7%) and weight gain (4.8%) being the most common, and a 0.5% corticosteroid-related discontinuation rate.[59] Enzalutamide, on the other hand, is also an inhibitor ofγ-aminobutyric acid (GABA)-gated chloride channels, an off-target effect that may be responsible for the hypertension and seizures reported in clinical trials.[60] Fatigue, falls or cognitive decline have also been observed in enzalutamide treated patients.[61]

Although both COU-AA-302 and PREVAIL incorporated QoL endpoints as secondary objectives, and both were found to exert a significant delay to the worsening of quality of life,[62,63] some studies have compared cognitive function and overall HRQoL in abiraterone and enzalutamide-treated patients, with contradictory results. In the previously discussed phase II crossover trial, a higher rate of significant worsening for the physical well-being (PWB) subscale of the FACT-P questionnaire in enzalutamide patients was observed, with no difference in the other subscales.[64] A greater number of enzalutamide-treated patients have reported worsening on questions related to energy, appetite, and psychomotor symptoms.[65] Other studies have reported no differences in the proportion of patients with clinically meaningful symptom improvement or worsening.[66] The AQUARIUS study, a two-cohort, prospective, observational, non-randomized, multicenter, phase IV study assessing the effects of abiraterone and enzalutamide on PROs, reported a statistically significant difference in fatigue favoring abiraterone over enzalutamide on an initial 105 patients.[67] Results are, however, still preliminary and no specific recommendations on the choice of initial therapy are currently made based on baseline cognitive function or on quality of life outcomes.[68]

2.4 Choice of second- and third-line agents. Cross-resistance.

As discussed previously, the rapidly changing therapeutic landscape in mCRPC over the past years has led to a paucity of prospective data that is clinically relevant to present-day patients. Abiraterone and enzalutamide were both evaluated initially as second-line options in the COU-AA-301 and AFFIRM clinical trials and their activity in docetaxel-refractory patients is well documented (Table 3); however, their shift towards first-line has made results of these trials obsolete. Similarly, cabazitaxel was approved as second-line therapy at a time when neither abiraterone nor enzalutamide were available.

Cross-resistance between anticancer agents would imply that the choice of first-line agents could potentially affect the efficacy of further lines of treatment and determine the optimal treatment sequence. This notion is supported by preclinical studies showing impaired efficacy of docetaxel, cabazitaxel and enzalutamide in abiraterone-resistant cell lines, and of docetaxel, cabazitaxel and abiraterone in enzalutamide-resistant cell lines.[69] Preclinical data also suggest that docetaxel could exert its mechanism of action by inhibiting tubulin and blocking AR translocation into the nucleus;[70] an association between AR cytoplasmic sequestration and clinical response to taxanes has been reported.[71] Other studies have suggested cabazitaxel could overcome this cross-resistance: studies in mouse models have shown that docetaxel was able to inhibit tumor growth and AR signaling in enzalutamide-naïve but not in enzalutamide-resistant mice, while cabazitaxel was able to inhibit growth both in enzalutamide-naïve and enzalutamide-resistant mice.[72]

No adequately powered trials that have prospectively evaluated the abiraterone/enzalutamide → docetaxel vs docetaxel → abiraterone/enzalutamide sequence in mCRPC. To complicate things further, there is no prospective data on the clinical activity of abiraterone or docetaxel as first-line options in mCRPC when either of them was administered as treatment of metastatic, castration-sensitive disease. Most available data to date is limited to retrospective series, which are generally underpowered and carry an implicit selection bias, and of reports of response to subsequent lines of treatment in patients treated in first-line clinical trials.

Table 3 summarizes data on retrospective series evaluating potential cross-resistance between agents. Overall, data in retrospective series evaluating docetaxel after abiraterone/enzalutamide and in patients treated with docetaxel after abiraterone in the COU-302 trial suggest a lower rate of PSA response (26%), PSA progression-free survival (4.6 months) and overall survival are lower than those reported in the TAX-327 trial.[73] However, a significantly worse outcome has been reported when comparing TAX-327-treated patients with non-trial docetaxel treated patients in a single institution study, suggesting the TAX-327 population is likely highly selected and possibly not representative of the real-world activity of docetaxel.[74] On the other hand, retrospective series evaluating post-abiraterone/enzalutamide and post-docetaxel patients treated with cabazitaxel show a similar activity rate to that in the TROPIC trial (27-41% PSA response, 15% radiographic response, median OS 10.9-20.3 months, median PFS 4.4-5.5 months).[75–78] Data from the PROSELICA trial in evaluating cabazitaxel 25 mg/m^2 vs cabazitaxel 20 mg/m^2, where 302 patients (25,7%) had received abiraterone or enzalutamide previously[79] could provide valuable information, if analyzed, on the potential cross resistance between agents.

Clinical data, mostly retrospective series, also indicate a significant degree of cross-resistance between abiraterone and enzalutamide (with or without docetaxel incorporated into the treatment sequence).[80–84] There is also a significant body of prospective data emerging that recommend against the use of these agents in sequence. A prospective, phase IV study evaluating enzalutamide in patients progressing on abiraterone reported a median rPFS of 8.1 months, a median time to PSA progression of 5.7 months and a 12% objective response rate. These were, however, highly selected patients with hormone-sensitive biology, since only patients that had received at least 24 weeks of abiraterone were included.[85] In the randomized PLATO study, patients received enzalutamide plus abiraterone or placebo plus abiraterone at PSA progression on enzalutamide, with a PSA response rate of only 1% in the control arm (receiving enzalutamide followed by abiraterone).[86] Similarly, in a phase II crossover study, patients that received first-line enzalutamide had a PSA response rate of 4% and a time to PSA progression of only 1.7 months on second-line abiraterone, while patients receiving first-line abiraterone experienced a PSA response rate of 36% and a time to PSA progression of 3.6 months on second-line enzalutamide.[55] Prospective evidence therefore clearly indicates lower activity than that observed in the second-line COU-AA-301 and AFFIRM trials for post-enzalutamide abiraterone and post-abiraterone enzalutamide, respectively.

Some authors have suggested that treatment with taxane chemotherapy could re-sensitize patients to hormonal agents,[87] based on reports suggesting clearance of AR-V7 clones that could drive progression with taxane chemotherapy between first- and third-line hormonal agents.[88] Data from retrospective studies is conflicting, with reports suggesting treatment with cabazitaxel before or after an AR-targeted agent was associated with greater OS than with two subsequent AR-targeting agents.[89]

Table 3. Results from retrospective studies evaluating cross-resistance

	N	PSA response	RX Response	Survival
DOCETAXEL POST ABIRATERONE				
Mezinsky et al.[90]	35	- 30% PSA decline: 13/35 (37%) - 50% PSA decline: (26%)	Partial response: 4/24 (16.7%)	- OS: 12·5 m (95%CI 10·6-19·4) - PSA PFS: 4·6 m (95%CI 4·2-5·9)
Schweizer et al[91]	24	- 30% PSA decline: 13/24 (54·2%) - 50% PSA decline 9/24 (38%)	NR	- OS: NR - PSA PFS: 4·1 m (2·8-5·8)
Aggarwal et al.[92]	23	- 30% PSA decline 15/23 (65%) - 50% PSA decline 11/23 (48%)	NR	- OS: 12·4 (95%CI 8·2-19·6)
TAX-327[39]	1006	- 30% PSA decline: 52.4%	12%	- OS: 19.2 m
ABIRATERONE POST-ENZALUTAMIDE				
Loriot et al.[80]	38	- 30% PSA decline 7/38 (18%) - 50% PSA decline 3/38 (8%)	Partial response: 1/12 (8%)	- OS: 7.2 m (95%CI 5 – NR) - PFS: 2.7 m (2.3-4.1)
Noonan et al.[81]	30	- 30% PSA decline: 3/27 (11%) - 50% PSA decline: 1/27 (3%)	Partial response: 0%	- OS: 11.6m (95%CI 6.5–16.6) - PFS: 3.6 m (2.5-4.7)
COU-AA-302[13]	1088	- 50% PSA decline: 62%	Partial response: 36%	- OS: 34.7 m - rPFS: 16.5 months
ENZALUTAMIDE POST-ABIRATERONE				
Schrader et al.[82]	35	- 30% PSA decline: NR - 50% PSA decline: 10/35 (28.5%)	Partial response: 1/17 (5.9%)	- OS: 7.1 m (95%CI 6.2-8.1)* - PFS: Not reported
Bianchini et al.[93]	39	- 30% PSA decline: 16/39 (41%) - 50% PSA decline: 5/39 (12.8%)	Partial response: 1/23 (4.3%)	- Overall survival: median OS NR - PFS: 2.8 m (95%CI 2-3.6)
Thomsen et al.[83]	24	- 30% PSA decline: 11/24 (46%) - 50% PSA decline: 4/24 (16.7%)	Not reported	- OS: 4.8 m (95%CI 3-8.4) - PFS: Not reported
Badrising et al.[84]	61	- 30% PSA decline: 28/61 (46%) - 50% PSA decline: 13/61 (21%)	Not reported	- OS: 7.3 m (95%CI 6.6 – NR) - PFS: 2.8 m (2.6-3.7) - PSA PFS: 4 m (95%CI 3.7 – NR)
PREVAIL[14]	1717	- 50% PSA decline: 78%	Partial response: 59%	- OS 32.4 m - PFS: not reached
CABAZITAXEL POST-ABIRATERONE/ENZALUTAMIDE				
Pezaro et al.[75]	37	- 30% PSA decline: 21/37 (56·8%) - 50% PSA decline: 15/37 (41%)	Partial response: 3/20 (15%)	- OS: 20·3 m (95%CI 14-26·6) - PFS: 5·5 m (95%CI 4.2-6·8)
Sella et al.[77]	24	- 50% PSA decline: 6/19 (31.5%)	Partial response: 2/13 (15.3%)	- OS: 8·2 m (3·3 – 13·1)
Wissing et al.[94]	69	- 50% PSA decline: 18/69 (26.8%)	-	- PFS: 3·2 m (2·5 – 3·8)
Al Nakouzi et al.[78]	79	- 30% PSA decline: 48/79 (62%) - 50% PSA decline: 28/79 (35%)	NR	- OS: 10·9 m (95%CI 8-14) - PFS: 4·4 m (95%CI 4·6-8·7)
TROPIC[4]	755	- 50% PSA decline: 39.2%	Partial response: 14.4%	- OS: 15.1 m - PFS: 2.8 m

Recently, two randomized phase III studies have finally provided level 1 evidence to help clarify the optimal treatment sequence. The CARD study is a phase III randomized study that allocated 255 mCRPC patients progressing after treatment with docetaxel and an AR-signaling inhibitor (abiraterone or enzalutamide) to the other AR-signaling inhibitor or cabazitaxel. Patients treated in the cabazitaxel arm experienced significantly higher PSA response rates (35.7% vs 13.5%; p<0.001), longer rPFS (8 vs 3.7 months, HR: 0.54; p<0.001) and overall survival (13.6 vs 11.1 months; HR: 0.64; p=0.008), establishing cabazitaxel as the standard third-line therapy option. Of note, cabazitaxel was superior to the sequence of abiraterone and enzalutamide also in patients that had received docetaxel between the two hormonal agents.[95] Similarly, in a population of patients harboring BRCA or ATM mutations, the PARP inhibitor Olaparib was compared with the investigator's choice of abiraterone or enzalutamide as second or third line in the randomized phase III PROFOUND study. Both rPFS (7.4 vs 3.6 months; HR 0.35; p<0.001) and OS (18.5 vs 15.1 months; HR: 0.64; p=0.017) were significantly longer in the Olaparib arm. Of note, the control arm had a response rate of only 2.3%, highlighting the ineffectiveness of a second AR signaling inhibitor after progression on an initial one.[48]

In summary, evidence indicates significant cross-resistance between hormonal agents. Despite it being a widespread practice among many practitioners, it is clearly inferior to treatment with second-line chemotherapy or PARP inhibition, and should be considered detrimental for patients that are fit to receive standard therapy.

2.5 Choice of treatment in mHSPC

2.5.1 Choice of First-Line Therapy in mHSPC

In recent years, several phase III trials have reported a significant improvement with the addition of docetaxel, abiraterone, enzalutamide and apalutamide to androgen deprivation therapy in metastatic, hormone-sensitive prostate cancer (Table 4). Initial evidence came from trials evaluating the addition of docetaxel to ADT (GETUG-AFU-15, CHAARTED, STAMPEDE),[30,45,96] followed by trials evaluating ADT + abiraterone (LATITUDE, STAMPEDE)[31,32] and, more recently, trials evaluating apalutamide (TITAN)[97] and enzalutamide (ENZAMET, ARCHES).[98,99]

As is the with mCRPC, evidence is limited by the fact that trials evaluating abiraterone, apalutamide or enzalutamide completed accrual before the combination of ADT +Docetaxel became standard of care. Therefore, all trials were compared with ADT only, and no randomized evidence of superior efficacy is available for neither of the treatment options. As an exception, 15% of patients in the ENZAMET trial received docetaxel; evidence from subgroup analysis suggested that the addition of enzalutamide in patients already receiving docetaxel showed no survival benefit.[98]

When performing cross-trial data comparisons, differences in trial populations must be taken into account. For instance, in the STAMPEDE trial, both metastatic and high-risk, non-metastatic patients were eligible, in contrast to the CHAARTED, ENZAMET and TITAN trials, with only metastatic patients, and the LATITUDE trial, with only high-risk metastatic patients were included. This is reflected in the clearly higher survival rates of control groups in STAMPEDE (81 months) than in CHAARTED (44 months) or LATITUDE (34.7 months).

Table 4. Summary of Pivotal Phase III trials in mHSPC

	N	Trial population	OS (exp)	OS (control)	ΔOS	HR (IC95%)
Docetaxel						
CHAARTED[30]	790	M1	57,6 m	44 m	13,6 m	0,61 (0,47-0,8)
CHAARTED – HV[100]	513	High volume M1	51,2 m	34,4 m	16,8 m	0.6 (0,45-0,81)
CHAARTED – LV[100]	277	Low volume M1	63,5 m	NR	-	
STAMPEDE[45]	1776	M1 / High risk M0**	81 m	71 m	10 m	0,78 (0,66-0,93)
Abiraterone						
LATITUDE[32]	1199	High-risk M1 (GS>=8, >=3 bone mets, visceral mets)	NR	34,7 m	-	0,62 (0.51 to 0.76)
STAMPEDE[31]	1917	M1 / High risk M0**	-	-	-	0,63 (0.52 to 0.76)
STAMPEDE – HV[101]	402	High volume M1	-	-	-	0,60 (0,46-0,78)
STAMPEDE – LV[101]	499	Low volume M1	83%***	77%***	6%	0,64 (0,42-0,97)
Apalutamide						
TITAN[102]	1052	M1	82,4%****	73,5%****	8,9%	0,67 (0,51-0,89)
Enzalutamide						
ARCHES[99]	1150	M1	NR	NR	-	0,81 (0,53-1,25)
ENZAMET[98]	1125	M1	80%***	72%***	8%	0,67 (0,52-0,86)

**High risk M1*
***High risk M0: Gleason Score ≥ 8, PSA ≥ 40 ng/mL, T3-T4 disease*
****3-year OS rates*
*****2-year OS rates*

Additionally, not all patients with mHSPC carry the same prognosis or benefit from treatment in the same manner. The CHAARTED study, for instance, stratified patients into “high risk” (presence of visceral metastases or ≥4 bone lesions with ≥1 beyond the vertebral bodies and pelvis) and “low risk” patients.[30] While there is strong evidence that docetaxel does significantly impact survival in high-risk patients, longer follow-up has revealed a lack of significant benefit in patients with low risk disease. In a pooled analysis of the CHAARTED and GETUG-AFU-15 clinical trials, pooled HR for docetaxel in patients with high-volume criteria showed a significant survival benefit (HR 0.68; 95%CI:0.56-0.82) that was not present in patients with low-volume criteria (HR: 1.03; 95%CI: 0.77-1.38).[100] The LATITUDE study, on the other hand, included only patients with adverse risk features, at least two of the three following: a Gleason score ≥8, at least three bone lesions, and the presence of measurable visceral metastasis.[32] The

survival benefit of radiotherapy to the prostate, on the other hand, has been established in low-volume patients exclusively.[103]

Data from post-hoc analyses of the STAMPEDE trial seem to contradict this notion. Investigators reported no significant heterogeneity of the effect of treatment on survival according to risk criteria (as defined) in the abiraterone or the docetaxel arms of the trial, suggesting both options could increase survival in high as well as in low-risk patients.[101,104] Trials evaluating apalutamide and enzalutamide included all metastatic patients, and showed survival benefit in patients irrespective of risk or volume criteria.

No trial to date has results on direct comparison between docetaxel and abiraterone in mHSPC. The PEACE-1 trial is a randomized, factorial 2x2 clinical trial (NCT01957436) with aims to evaluate the role of local radiotherapy and abiraterone in first-line mHSPC; this trial will incorporate a control arm of patients receiving docetaxel, and will provide the first directly randomized data on treatment efficacy.[105] The population of the LATITUDE study may be considered to overlap the high-volume population of the CHAARTED study, providing therefore some basis for indirect comparison only with the high-risk CHAARTED population. Most published indirect cross-trial comparisons favor abiraterone over docetaxel;[106–108] however, due to previously mentioned differences in trial design, these comparisons carry an inherent risk of bias. The most solid evidence available to date on the comparable efficacy of abiraterone and docetaxel in the mHSPC setting comes from an indirect comparison of contemporarily-treated patients in the STAMPEDE trial. 566 patients (189 receiving docetaxel and 377 receiving abiraterone) were randomized into different arms of the STAMPEDE trial between November 2011 and March 2013, of which 60% were metastatic. Although there was a significantly higher progression-free survival in patients treated with abiraterone (HR: 0.65 [95%CI:0.48-0.88]), there was no difference in clinically significant outcomes such as time to symptomatic skeletal events (HR: 0.83 [95%CI: 0.55-1.25]), overall survival or cancer-specific survival.[109] Mirroring treatment options in mCRPC, no agent is favored as first-line therapy in mHSPC on the basis of an increased antitumor efficacy.

As is the case with mCRPC trials, the toxicity burden in trials evaluating docetaxel and abiraterone is markedly different. In the GETUG-AFU-15, CHAARTED or STAMPEDE trials, patients receiving docetaxel had a significantly higher rate of neutropenia (12-32%), febrile neutropenia (6-15%) and fatigue (4-7%) than those in the control arm;[110] rates of clinically significant (grade 3-4) sensory neuropathy were however low (0.5-3%).[30,45,96] In trials evaluating abiraterone (LATITUDE, STAMPEDE), a recent metaanalysis reported a significant increase (three-fold) in liver or cardiac grade 3-4 AEs, and a two-fold increase in vascular events, although the absolute number of patients experiencing these toxicities was low, and hypertension was manageable with medication adjustments.[111] The duration of treatment is also a major difference between treatment strategies; while 6 cycles of docetaxel are now accepted as standard, trials evaluating abiraterone mandated treatment until progression, with a median 33 months

radiographic progression-free survival in the LATITUDE trial. This has led some authors to advocate treatment with docetaxel as more intense but short-lived toxicity with docetaxel as opposed to a more insidious, long-lasting, low intensity toxicity for abiraterone-treated patients.[105] So called "financial toxicity", with a huge increase in treatment related costs with the introduction of abiraterone in mHSPC, must also be taken into account.[112]

2.5.2 Impact of First-Line mHSPC in further mCRPC treatment

Can the choice of initial therapy impair the efficacy of further lines of treatment in the mCRPC stage of the disease? If previously discussed data on cross-resistance between agents in mCRPC is extrapolated, a certain degree of cross-resistance, and therefore a diminished efficacy, should be expected. On the other hand, since docetaxel is not continued until disease progression, it could be argued that relapse should not be docetaxel-refractory. Again, data are limited and clinical trials comparing treatment options in patients that have progressed after docetaxel for mHSPC are lacking.

In a recently published analysis of patients treated with docetaxel + ADT or ADT alone in the GETUG-AFU-15 trial, rates of PSA response after treatment with docetaxel as first-line therapy for mCRPC were higher in patients that had received ADT than those that had received ADT + docetaxel for mHSPC (38 vs 20%; p=0.14); time to PSA progression was also lower in patients that had received docetaxel at mHSPC (6 vs 4.1 months). Although only 19 patients had received subsequent abiraterone or enzalutamide, a PSA response was observed in 10 (53%) of them. Authors concluded that docetaxel retreatment seemed to offer limited efficacy in mCRPC when previously used as treatment in mHSPC.[113] No data are currently available on further lines of treatment received by patients treated in the CHAARTED, LATITUDE or STAMPEDE trials, likely because of the short time elapsed since accrual was completed.

2.6 Combinations of Agents

Although the standard approach to the treatment of mCRPC is use of as many approved agents as possible in a sequential manner, strategies combining some of these agents may improve outcome and delay the development of resistance.[114] Data on combination trials has been disappointing to date: none of the chemotherapy combinations evaluated in nine randomized phase 3 trials enrolling more than 10,000 patients was able to prove superiority over single-agent docetaxel.[115] Novel combinations of both docetaxel and cabazitaxel with novel hormonal agents and other targeted therapeutics are ongoing.

Due to its seemingly non-overlapping mechanism of action (short range radiation to bone metastases) and the low toxicity rates observed in the ALSYMPCA trial, the radiopharmaceutical Ra-223 has been a robust candidate for combinations with either chemotherapy or hormonal agents. Retrospective data

analyzing the expanded-access Ra-223 programme in the USA found no added toxicity Ra-223 was combined with abiraterone or enzalutmide, compared to Ra-223 monotherapy.[116] This led to the planification of several phase III studies evaluating the safety and efficacy of the combination of Ra-223 with abiraterone and enzalutamide. The ERA-223 study (NCT02043678) is a phase III trial evaluating that randomized mCRPC patients to receive abiraterone + Ra-223 or placebo. After the accrual of 806 patients, the IDMC recommended the early unblinding of the trial due to the observation of a higher number of osteoporotic fractures and deaths in patients receiving the combination.[117] This situation highlights how, despite theoretical synergy between agents and a lack of apparent toxicity in retrospective studies, well designed prospective trials are still needed to prove the efficacy and safety of treatment combinations in mCRPC. Another currently ongoing phase III trial (PEACE-3; EUDRACT 2014-001787-36) will evaluate the safety and activity of the combination of enzalutamide and Ra-223 in mildly symptomatic patients.

Although both enzalutamide and abiraterone both act through targeting the AR, their different mechanism of action has led to the hypothesis that the combination of both agents could provide a higher efficacy than each of the agents separately. Recent clinical data have, however, not confirmed this hypothesis. In a clinical trial evaluating neoadjuvant (prior to surgery) treatment for localized prostate cancer, the combination of enzalutamide, abiraterone, and leuprolide acetate (an LHRH antagonist widely used as primary androgen deprivation therapy) showed a lower rate of tumor downstaging than abiraterone (without enzalutamide) and leuprolide acetate, suggesting that the combination could be detrimental.[118] In the recently published phase IV PLATO trial, patients received enzalutamide as first-line mCRPC therapy. At the time of PSA progression, patients were randomized to receive the combination of enzalutamide + abiraterone or to placebo + abiraterone. After the enrolment of 509 patients, no significant differences in PFS (5.7 vs 5.6 months; HR: 0.83 [95%CI: 0.61-1.12]; p=0.22), time to PSA progression (2.8 vs 2.8 months; HR: 0.87; [95%CI: 0.62-1.24]; p=0.45) or PSA responses (0.8% vs 2.5%; p=0.31) were observed.[119] The low rates of PSA progression and PSA response led to authors to confirm the lack of activity of the combination of abiraterone and enzalutamide. Similarly, in the phase III ALLIANCE A031301 trial randomizing 1,311 patients to treatment with the combination of abiraterone + enzalutamide or abiraterone alone as first-line therapy in mCRPC, no survival benefit was observed from the combination (34.2 vs 32.5 months; HR: 0.9; p=0.19).[120] After results from these two trials, the hormone-agent combination approach in mCRPC has been largely abandoned due to its lack of efficacy.

3. Clinical Biomarkers in Advanced Prostate Cancer

As discussed previously, there is no established therapy sequence based on clinical trial data in advanced prostate cancer, and clinicians are left with the challenge of how to optimize outcome with the available therapeutic agents. A major problem related with this approach is that currently available biomarkers only indirectly inform the biology of the disease, and provide no information on the molecular mechanisms responsible for tumor progression or of which treatment options are most likely to provide benefit. To date, clinicians must rely on clinical biomarkers to estimate prognosis, decide among different therapy options and determine response and progression to treatment.

3.1 Prognostic Biomarkers

The estimation of individual prognosis is a crucial step in the initial evaluation of a patient with advanced prostate cancer. Clinical heterogeneity is high in prostate cancer, with patients that experience long progression-free survival intervals on successive lines of hormone therapy, and other patients that suffer rapid progression despite different lines of hormone therapy, chemotherapy and radiopharmaceuticals. Therefore, differentiating patients with an indolent, non-aggressive disease from patients in whom rapid progression into clinical deterioration is expected is essential in order to, on one hand, adequately inform patients of the expected survival and, on the other hand, to effectively design therapeutic strategies that may maximize therapeutic benefit. Failure to identify patients likely to progress rapidly may mean missing a 'window of opportunity' to initiate treatment while the performance status is adequate, thereby reducing the likelihood of clinical benefit.

Clinical Biomarkers – Prognostic Nomograms

A number of different clinical biomarkers with prognostic value have been evaluated in advanced prostate cancer. Different clinical variables have been identified as conferring a worse prognosis, and may help identify patients with aggressive disease characteristics. The presence of visceral metastases, for instance, has been associated with lower progression-free and overall survival time in a pooled analysis of clinical trials.[121] Other factors, such as a shorter time on androgen deprivation therapy prior to the development of castration-resistance or lower testosterone levels may indicate a less androgen-driven biology, and have also been associated with adverse outcome.[122,123] Laboratory values, such as a lower hemoglobin, higher LDH and higher Alkaline-Phosphatase levels have also been associated with a higher burden of disease and worse outcome.[124,125] Finally, clinical factors suggesting a worse general condition, such as the presence of pain, a worse ECOG Performance Status or the use of corticosteroids, usually given for palliation of pain or asthenia, are also associated with worse outcomes.[126,127] On the other hand, age in itself is not a validated prognostic factor; in chemotherapy treated patients, those

older than 75 years with a good performance status have been shown to derive the same benefit as younger patients.[128]

Rather than using them individually, clinical biomarkers have been incorporated into prognostic models or "nomograms" for the estimation of individual patient benefit. These nomograms allow for individual estimation of patient prognosis, which can be performed through online tools that calculate 1- or 2-year mortality risk from the input of clinical variables. These models have been developed from retrospective analyses from the different phase III trials that led to the approval of different agents; however, as novel agents have been incorporated into first-line mCRPC or mHSPC, estimations developed from some of the older nomograms may not provide accurate prognostic information. This was illustrated by a study by Omlin and colleagues, where actual outcomes of 442 mCRPC men treated with abiraterone as first- or second-line mCRPC therapy was compared with predicted outcomes by the Halabi (derived from a pooled analysis of patients treated in 6 CALGB trials)[129] and Smalletz (derived from a pooled analysis of different trials)[130] nomograms. Both nomograms were developed from trials conducted before the incorporation of abiraterone, enzalutamide, novel taxanes or radiopharmaceuticals into the therapeutic landscape of mCRPC. Survival of abiraterone-treated patients was of 30.6 months, in contrast with a predicted 21 and 18 months predicted by the Halabi and Smaletz nomograms, respectively, highlighting how modifications in the therapeutic landscape may render older nomograms obsolete.[131] Different novel prognostic nomograms have been developed over the past years, based on data from the TAX-327, TROPIC, CALGB-90401, or COU-AA-301 trials.[5,132–135] All these, however, are prior to the incorporation of abiraterone or enzalutamide as first-line therapy options in mCRPC.

More recently, two groups have published prognostic models based on data from the COU-AA-302 and PREVAIL trials, which may reflect more accurately the current therapeutic landscape. In the first one, Ryan and colleagues developed a prognostic model to estimate the risk of radiographic progression based on data from the COU-AA-302 trial, in treatment-naïve mCRPC patients. The presence of lymph node metastases, LDH > ULN, >10 bone metastases, hemoglobin < LLN and a PSA value > 39.5 ng/mL were selected from multivariable regression analysis, and were able to classify patients with good (median 27.6 month rPFS), intermediate (median 16.6 month rPFS) and poor prognosis.[136] A second nomogram, developed by Armstrong and colleagues, was developed based on similar patient data treated in the first-line PREVAIL study, based on overall survival data, with a derivation and a test set. Up to 11 different clinical variables (albumin, alkaline phosphatase, number of bone metastases, hemoglobin, LDH, NLR, presence of pain, presence of liver metastases, PSA, time from diagnosis, treatment arm) were selected from the training set (n=1159 patients). In the testing set, grouping of the variables into low (median survival not reached), intermediate (median 34.2 months OS) and high (median 21.1 months) was significantly associated with overall survival.[137]

The Neutrophil-to-Lymphocyte Ratio

Cancer-related inflammation has been recognized as one of the hallmarks of cancer with an essential role in the modulation of the tumor microenvironment.[138] The neutrophil–lymphocyte ratio (NLR), a measure of the proportion of systemic neutrophils and lymphocytes, has been proposed as an indicator of cancer-related inflammation, and has been shown to have prognostic relevance across a large variety of tumor types.[139] In prostate cancer, a number of retrospective studies have evaluated the prognostic significance of baseline NLR (BLNLR)[140–142] The prognostic significance of baseline NLR has also been extensively documented in post-hoc studies of the TAX-327 (first-line docetaxel), TROPIC (second-line cabazitaxel), VENICE (first-line docetaxel and aflibercept) or SUN-1120 (second-line sunitinib), confirming the favorable outcome associated to low baseline NLR levels.[141,143,144]

Baseline NLR has also been associated with PSA response to different anticancer agents. In a retrospective review of 353 abiraterone-treated patients (a derivation cohort with 108 patients treated at the Princess Margaret Hospital, and a validation cohort with 245 patients treated in the Royal Marsden Hospital), a combined score of NLR and disease extent was significantly associated with increased PSA response rates (p=0.003).[145] This was confirmed in a meta-analysis performed in six studies comprising a total 3194 patients treated with different agents, where a lower NLR was associated with higher response rates (OR 1.69; 95%CI 1.4-1.98).[146] Similar outcomes have been observed in mCRPC treated with corticosteroids, despite concerns for their potential tumor-promoting and immunosuppressive impact.[147]

Despite the large body of evidence supporting its prognostic impact, the NLR has not been incorporated into routine clinical practice. This is due, on one hand, to the fact that different studies have evaluated different thresholds to define high and low NLR counts, which limits the applicability of the biomarker in the clinic; it is likely that the optimal cut-off may be slightly different in different populations. On the other hand, the NLR has not been shown to be useful in selecting one treatment option over another; results are consistent throughout different studies with different therapy options. Finally, NLR has not been shown to outperform PSA, a widely used biomarker, in determining response. Further evidence from clinical trials, if conducted, is needed to elucidate the exact role of NLR in advanced prostate cancer care.

3.2 Response and Follow-Up Biomarkers

Prostate-Specific Antigen (PSA)

PSA is a serine protease that is synthesized in healthy prostate tissue, benign prostatic hypertrophy and in prostate cancer. Its use has been widely implemented in screening, diagnosis and monitoring of response to prostate cancer. PSA levels are used for risk stratification in localized disease, in the

surveillance of patients after localized therapy, and to determine the frequency of imaging in the follow-up of non-metastatic prostate cancer patients.[68,148] In advanced, metastatic prostate cancer, PSA is generally used for determining response and progression to systemic treatment.

The transcription of PSA is regulated by androgen response elements in the promoter of the KLK3 gene.[149] Its close relationship to the AR-signaling pathway make it a more reliable indicator of response and progression in hormone-naïve stages, where a more clear impact of the AR pathway is observed, than in the castration-resistant setting, when additional signaling pathways may be preponderant. The association of changes in PSA levels on treatment and overall survival have been extensively studied in a number of clinical trials, showing that declines in PSA levels from baseline greater than 30% or 50% are associated with an improved outcome. A PSA response at any time point has been shown to be significantly associated with improved survival across a number of different clinical trials, evaluating both hormonal agents and chemotherapy.[150–152] Clinical guidelines, however, recommend not evaluating PSA changes before at least 12 weeks of treatment, due to the potential for "PSA flares", that is, rises in PSA that do not correspond with progressive disease. Early evidence of PSA flares comes from an analysis of docetaxel or mitoxantrone-treated patients in the TAX-327 trial, where 83 patients were found to have an initial surge in PSA levels, with subsequent declines that qualified as PSA response.[126] Depending on the definition, incidences of between 8-30% PSA flares have been reported in cohorts of abiraterone, docetaxel and cabazitaxel-treated mCRPC patients.[153–155] PSA was not determined before 12 weeks in the COU-AA-302 or PREVAIL trials; therefore, no estimation on the incidence of PSA flares can be made from trial datasets. Despite the evidence for flare, other studies suggest early PSA response could be reliable indicator of benefit to treatment, with a strong and significant association with PSA response at 12 weeks.[156]

PSA progression, defined as a 25% increase over baseline values, has also been studied as a biomarker of disease progression. Time to the development of PSA progression or death (PSA progression-free survival) has also been included as a secondary endpoint in all of the landmark phase III trials in mCRPC to date. PSA progression at 9 months in metastatic, hormone-naïve prostate cancer patients treated with androgen deprivation in the SWOG-9346 trial was found to be significantly associated with adverse outcome (HR 4.4; $p<0.001$). Similar results were observed when evaluating PSA progression at 3 months in chemotherapy-treated mCRPC patients in the SWOG-9916 trial (HR 2.1; $p<0.001$). The larger hazard ratios observed for mHSPC than for mCRPC suggest PSA progression may be a more reliable indicator at earlier stages of the disease.

Additionally, some studies have evaluated the value of PSA declines as a surrogate biomarker for overall survival, that is, whether PSA response rates could substitute overall survival as a valid endpoint in clinical trials. In the SWOG 99-16 phase III trial (first-line docetaxel and estramustine or mitoxantrone for mCRPC), authors reported that a 30% decline in PSA after 3 months, but not a 50% decline, fulfilled the

Prentice Criteria for surrogacy.[150] Unfortunately, further analyses have not confirmed these findings. In a similar analysis performed on the phase III TAX 327 study PSA declines showed a modest proportion of treatment effect (TPE – an indicator of the strength of surrogacy) of only 0.66, suggesting an only moderate survival surrogacy at best. Similar results from analyses performed in patients treated in the TROPIC (cabazitaxel in previous docetaxel-treated patients) and AFFIRM (enzalutamide in previous docetaxel-treated patients) failed to confirm the surrogate value of PSA declines.[151,152] In the TROPIC trial, a meta-analytical approach was performed to explore surrogacy at the trial level, and these confirmed that PSA is not a surrogate for overall survival.[151]

Taken together, these data indicate that PSA is not a valid biomarker for the evaluation of treatment benefit in clinical trials in CRPC. PSA, a marker of androgen receptor activity, is not always representative of the disease, especially in late stages when other molecular mechanisms may be driving disease progression. Furthermore, PSA fluctuations during the first 12 weeks of treatment of CRPC are not indicative of treatment failure.[157] Newer strategies that do not target the AR such as immunotherapy or radiopharmaceuticals have shown significant discrepancies between PSA response and PFS or OS outcomes.[42,53]

Imaging Biomarkers in Advanced Prostate Cancer

Standard imaging assessment in advanced prostate cancer is performed by serial computed tomography (CT) scans and bone scintigraphy. Novel imaging techniques such as prostate-specific membrane antigen positron emission tomography (PSMA-PET) or magnetic resonance imaging (MRI) scans may offer additional benefit, although these remain still investigational currently.

In the vast majority of tumor types, the assessment of response is performed through CT scans, with the widely accepted Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Tumor burden is assessed by summing the products of bi-dimensional lesion measurements; response to therapy is evaluated by calculating the change from baseline while on treatment.[158] Revised RECIST 1.1 guidelines define measurable lesions as those with a diameter at least 10 mm in longest axis (for soft-tissue and visceral lesions) and at least 15 mm in short axis (for lymph node disease); for assessment in clinical trials, a number of up to 10 target lesions (up to 5 per organ) to be followed-up must be selected. Lesions that do not fulfill evaluability criteria are termed non-target lesions. Response is defined as either a disappearance of all target lesions, with all lymph nodes reduced to < 10 mm (complete response), or a ≥30% decrease in the sum of diameters of target lesions. Progressive disease, on the other hand, is defined as a ≥20% increase in the sum of diameters of target lesions with an absolute increase of at least 5 mm in size. A non-equivocal progression of non-target lesions, or the appearance of new lesions, is also considered progressive disease. Finally, stable disease is defined when neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD is observed.[21]

In prostate cancer, however, assessment of response to therapy is limited by the fact that most patients develop bone metastatic disease as their main site of metastases. Up to 90% of patients with late-stage disease mCRPC present with bone metastases; up to 50% present with bone metastases as the only site of disease. In one study evaluating RECIST-evaluable disease in advanced prostate cancer patients, only 43.5% with castrate metastatic and 16% of non-castrate metastatic disease had measurable target lesions >2 cm in size.[159] Overall, 84.4% of the target lesions were lymph nodes, of which 67.7% were ≥2 cm in the long axis. Changes in bone metastatic disease are not assessable by standard RECIST 1.1 criteria; therefore, a significant proportion of prostate cancer patients are not evaluable for radiographic response, if only CT scans are taken into account.[22]

We rely on bone scintigraphy as the standard procedure for the follow-up and response assessment of bone metastatic disease. As the nature of bone metastasis is osteoblastic, bone scintigraphy can visualize abnormalities as hot spots or localized accumulation. One of the major drawbacks of bone scintigraphy, however, is the lack of utility in defining response to treatment, due to lack of spatial resolution from the technique. There is no validated method for the quantification of contrast uptake, and it is currently unclear whether a reduction in uptake of previously existing lesions is associated with improved outcome. The sole utility of bone scintigraphy in the follow-up of advanced prostate cancer is, therefore, to identify new lesions. At least two new bone lesions must be identified, with further confirmation in a subsequent bone scan being necessary to qualify as progression.[11] Additionally, new lesions in early bone scans (before 12 weeks) must not be interpreted as progressive disease due to potential spurious flare reactions. A 'flare reaction' is defined as the spurious appearance of bone lesions in early scans after treatment initiation, that are not related to progressive disease but rather to inflammatory changes in previously non-visualized lesions induced by treatment. This was illustrated in a phase II study that evaluated 33 abiraterone-treated patients, of which 23 had evaluable bone scans and 13 qualified as progression. 12 of 13 patients had bone scans showing progression; of these, 11 patients showed improvement or stability. Taken together, 11 of 23 (48%) patients with evaluable scans showed a bone scan flare.[160] A recently published study evaluated the significance of new, unconfirmed lesions on follow-up bone scans of patients in the PREVAIL and AFFIRM clinical trials of enzalutamide in docetaxel-naïve and docetaxel-treated patients, respectively. The presence of new, unconfirmed bone lesions in patients with otherwise stable (soft-tissue lesions, PSA) disease was not associated with rPFS or quality of life. Although there was no difference in OS in patients treated in the PREVAIL trial, investigators did find a significantly lower OS in patients with new unconfirmed bone lesions treated in the AFFIRM trial.[161] Taken together, these data support the need for a confirmatory bone scan, which makes disease progression by bone scan impossible before at least 16-18 weeks have elapsed from treatment initiation.

Several automated methods have been developed in order to quantify bone scintigraphy uptake and standardize measures of disease response in bone metastases. The bone scan index (BSI) is calculated from bone scintigraphic images after a process of whole-body image segmentation, standardization and the detection, quantitation and classification of "hot spots" (regions of abnormal uptake identified as bone metastases).[162] The bone scan index has been evaluated mainly in patients with bone metastases from prostate and breast cancer. In prostate cancer, an association between baseline extent of bone metastatic disease and outcome (overall or progression-free survival) has been reported in a number of studies.[163–165] Changes in BSI after treatment have also been associated with survival, with reductions in the BSI being indicative of treatment benefit in a number of cohorts.[166,167] The utility of the bone scan index has, however, not been prospectively validated to date; for this reason it is not currently used in daily clinical practice.

Other promising imaging techniques, such as whole-body magnetic resonance imaging (WB-MRI) or prostate-specific membrane antigen-directed positron-emission tomography (PET-PSMA) may offer higher sensitivity and specificity than currently used imaging techniques. Diffusion-weighted imaging (DWI) is a functional MRI technique used to study the motion of water molecules within tissue. By measuring the apparent diffusion coefficient (ADC), cellularity in bone metastases can be estimated, providing a direct measure of the functional activity of the different metastatic sites. Both baseline ADC and post-treatment changes in ADC are associated with overall survival in mCRPC, highlighting its potential role in the follow-up of bone metastatic disease.[168,169] Additionally, new PET tracers such as PSMA have significantly improved the rate of detection of metastases in prostate cancer, and may be of improved utility in localized disease, biochemical relapse and non-metastatic CRPC.[170] In mCRPC, despite the higher sensitivity and specificity of PET scans over bone scintigraphy for the assessment of bone metastases, it is unclear whether the use of PET-CT can lead to improved outcome.[171] Therefore, until prospective studies prove a significant clinical benefit derived from the use of DW-MRI or PSMA-PET scans in mCRPC, CT and bone scans remain the current standard imaging techniques in mCRPC.

3.3 The Prostate Cancer Working Group Criteria

As highlighted previously, limitations in currently available biomarkers in advanced prostate cancer pose significant challenges, including the difficulty of determining the baseline extent and clinical heterogeneity of the disease, and the lack of a standardized treatment assessment to adequately quantify the benefit from systemic treatment.[172] The Prostate Cancer Clinical Trials Working Group (PCWG) is an international working group of clinical and translational experts in prostate cancer that have designed guidelines for clinical trial endpoints and assessments. Treatment objectives are based on either the control/relief of disease manifestations in symptomatic patients (induction of response) or the delay/prevention of manifestations of disease progression in otherwise asymptomatic or minimally symptomatic patients.

PCWG3 guidelines state a number of baseline assessments that must be evaluated at baseline, but also recommendations on the follow-up and the definitions of disease progression in prostate cancer, which are summarized in Table 5. Interestingly, PCWG3 now incorporate the enumeration of circulating tumor cells (CTCs) as a baseline assessment for correct patient stratification, and also as an outcome measure by defining categories of responders (those who convert to < 5 CTCs/7.5 mL) and non-responders (those who remain with ≥ 5 CTCs/7.5 mL).[44]

Table 5. Suggested outcome measures by PCWG3 criteria[44]

PSA	Monitor PSA by cycle but plan to continue through early rises for a minimum of 12 weeks unless other evidence of progression. Ignore early rises (before 12 weeks) in determining PSA response. **CONTROL/RELIEVE ENDPOINTS (RESPONSE):** - Record the percent change from baseline (rise or fall) at 12 weeks, and separately, the maximal change (rise or fall) at any time using a waterfall plot **DELAY/PREVENT ENDPOINTS (PROGRESSION):** - After decline from baseline: record time from start of therapy to first PSA increase that is ≥ 25% and ≥ 2 ng/mL above the nadir, and which is confirmed by a second value ≥ 3 weeks later (i.e., a confirmed rising trend) - No decline from baseline: PSA progression ≥ 25% increase and ≥ 2 ng/mL increase from baseline beyond 12 weeks
CTCs	Enumerate at the start of treatment: Record as favorable (≤ 4cells per 7.5 mL) or unfavorable (≥5 cells per 7.5 mL). If unfavorable, monitor for post-treatment changes. **CONTROL/RELIEVE ENDPOINTS (RESPONSE):** Report as change from unfavorable (five or more cells per 7.5 mL of blood) to favorable (four or fewer cells per 7.5 mL) and separately, the percent change from baseline using a waterfall plot **DELAY/PREVENT ENDPOINTS (PROGRESSION):** No validated definition (however, rising CTC counts associated with poor prognosis)
CT scans	**CONTROL/RELIEVE ENDPOINTS (RESPONSE):** Use RECIST 1.1 with caveats: - Record changes in size using waterfall plot - Confirm favorable change with second scan - Only report changes in lymph nodes that were ≥1.5 cm in the short axis - Record changes in pelvic (regional) nodes v extra-pelvic (distant/metastatic) nodes separately **DELAY/PREVENT ENDPOINTS (PROGRESSION):** Use RECIST 1.1 but clearly record type of progression (growth of existing lesions v development of new lesions) separately by site - Report proportion who have not progressed at fixed time points (6 or 12 months) - Previously normal (< 1.0-cm) lymph nodes must have grown by ≥ 5 mm in the short axis from baseline or nadir and be ≥ 1.0 cm in the short axis for progression - Nodes that have progressed to 1.0 to less than 1.5 cm are pathologic and non-measurable - For existing pathologic adenopathy (≥1.5 cm), progression defined per RECIST 1.1

Bone scans	**CONTROL/RELIEVE ENDPOINTS (RESPONSE):** Record changes as improved or stable (no new lesions) or worse (new lesions) Changes in intensity of uptake alone do not constitute progression or regression No new lesions: continue therapy in absence of other signs of progression **DELAY/PREVENT ENDPOINTS (PROGRESSION):** - Exclude pseudo progression in the absence of symptoms or other signs of progression - At least two new lesions **on first post-treatment scan**, with at least two additional lesions on the next scan (2+2 rule). If at least two additional new lesions are seen on the confirmatory scan, date of progression is the date of the first post-treatment scan (when first two new lesions documented) - For scans **after the first post-treatment** scan, at least two new lesions relative to the first post-treatment scan confirmed on a subsequent scan. Date of progression is the date of the scan that first documents the second lesion.
Patient-Reported Outcomes	Pain palliation assessment requires a patient population with clinically meaningful pain at baseline (e.g., ≥ 4 on a 10-point pain intensity scale) and response defined as a clinically meaningful score improvement at a subsequent time point (e.g., a 30% relative or 2-point absolute improvement from baseline at 12 weeks, confirmed at least 2 weeks later, without an overall increase in opiate use) **CONTROL/RELIEVE ENDPOINTS (RESPONSE):** - Perform serial assessments of global changes in HRQoL, urinary or bowel compromise, pain management, additional anticancer therapy - Ignore early changes (≥ 12 weeks) in pain or HRQoL in absence of compelling evidence of disease progression **DELAY/PREVENT ENDPOINTS (PROGRESSION):** - Patients with any level of baseline pain, including no pain, are eligible; those without pain are followed for development of pain, whereas those with baseline pain are followed for progression (e.g., a 2-point increase without an overall decrease in opiate use) - Confirm response or progression of pain or HRQoL end points ≥ 3 weeks later - Time to deterioration of physical function and/or HRQoL scores should also be included, with a priori thresholds defining clinically meaningful deterioration score changes that are based on prior published data for the selected questionnaire

Measures of progression-free survival (that is, the time from treatment initiation to progression or death) as defined by Prostate Cancer Working Group criteria have been incorporated as endpoints in phase II and phase III clinical trials in mCRPC. Although overall survival is a necessary endpoint for drug approval from large phase III trials, PFS measures, especially radiographic PFS (rPFS) has been used as a primary endpoint in multiple phase II trials. For instance, the combination of abiraterone and ipatasertib was shown to improve rPFS in patients harboring a PTEN deletion;[173] on the basis these results, a large phase III trial (IPATential150 trial - NCT03072238) is now evaluating the combination, with overall survival as its primary endpoint. In metastatic, asymptomatic or minimally symptomatic mCRPC patients, abiraterone was approved on the basis of a combined improvement in overall survival (which did not meet pre-specified protocol criteria) and a significant improvement in rPFS, the two co-primary endpoints of the trial.[13]

The association of rPFS and OS has been evaluated in retrospective studies from several phase III trials. PCWG2-defined rPFS was found to be significantly correlated with OS in the COU-AA-302 trial, with a Spearman's correlation coefficient of 0.72 ($p<0.001$); no relevant differences were found when comparing investigator-assessed and centrally reviewed radiographic progression.[25] A similar analysis performed in the PREVAIL trial showed a Spearman's correlation coefficient of 0.72 (95%CI: 0.67-0.76; $p<0.001$) when evaluating all randomized patients, which increased to 0.89 (95%CI: 0.86-0.92; $p<0.001$) when evaluating only enzalutamide-treated patients.[174] The correlation of other measures of progression-free survival such as PSA-PFS, have not been evaluated, and extrapolation of data from rPFS must therefore not be performed. This is relevant since, in mHSPC, an indirect comparison between patients treated with ADT + abiraterone and ADT + docetaxel in different arms of the STAMPEDE trial showed a significant benefit in failure-free survival (mainly driven by PSA progression) for abiraterone over docetaxel, but no significant difference in metastatic (radiographic) progression-free survival or overall survival.[109] Based on these results, despite a difference in PSA PFS, one cannot conclude that a benefit of abiraterone over docetaxel exists in mHSPC.

Other measures of progression-free survival have been proposed for the evaluation of advanced prostate cancer patients. Progression-free survival to subsequent therapy (PFS2), defined as the time from initiation of first-line therapy to progression on second-line therapy or death, has been proposed as an alternative endpoint to overall survival to evaluate the benefit of agents in sequence. In the phase III SPARTAN trial, Apalutamide was shown to significantly increase PFS2 in comparison to placebo in non-metastatic, castration-resistant prostate cancer patients.[175] Data from other clinical trials in the same disease setting, such as the ARAMIS or PROSPER trials, evaluating darolutamide and enzalutamide in nmCRPC, respectively, will be needed to evaluate the value of PFS2 and its correlation with OS. In localized prostate cancer, on the other hand, a strong correlation between metastasis-free survival and OS (Kendall's correlation coefficient: 0.91) has been documented in an analysis of 28 clinical trials with over 28,000 patients, suggesting its value as an intermediate endpoint for trials in this setting.[176]

3.4 Decision-Making in Standard Clinical Practice

The particularities of prostate cancer management discussed above and the rapid changes the field has experienced have significantly complicated the management of prostate cancer patients in recent years. Clinicians are now challenged with multiple treatment options without clear data on the best treatment sequencing and the adequate follow-up and use of biomarkers, which makes clinical decision-making more complex. Clinical surveys performed to both urologists and medical oncologists who treat patients with mCRPC have revealed significant inconsistencies in the understanding and use of guidelines for treatment, and management of patients with mCRPC.[177] Guidelines from the different medical societies, and follow-up guidelines by the PCWG are not consistently followed-up by a majority of doctors treating advanced prostate cancer.

Physicians generally agree that patients should be involved in clinical decision-making, although many patients, especially older ones, are found to take a passive role in decision-making and relegate decisions to their doctors.[178] Other surveys have found that patients, in contrast to doctors, are more worried about reduced quality of life from side effects of treatment than from extension of survival, a finding that could affect shared decision-making, especially in second and subsequent lines of disease. Fatigue and the delay of symptomatic bone events have been found to be highly valued by prostate cancer patients.[179] Patient with higher education have been found to be more skeptical about the value of certain drugs in advanced prostate cancer, and many would value adequate end-of-life care than certain costly treatment options.[180]

In conclusion, more education for physicians and higher evidence on the clinical benefit of the use of biomarkers is necessary to optimize treatment benefit by improving the assessment of the disease. Additionally, certain new biomarkers such as CTCs will need widespread implementation outside high-quality academic centres in order to impact the widespread community of prostate cancer patients.

4. The Molecular Biology of Prostate Cancer

Due to its particular natural history, and the selective pressure exerted by androgen deprivation therapy, molecular alterations driving the disease may be fundamentally different in the primary, localized, treatment-naïve and the advanced, metastatic, castration-resistant stage of the disease.[181] For this reason, primary prostate cancer tissue from primary surgery specimens may be sometimes inadequate for the study of therapeutic targets in the advanced disease setting.

In recent years, advances in the technology for the study of the cancer genome have allowed for an increased understanding of the genomic landscape of the disease. Comparing the genomic landscape of primary and advanced cancers has also allowed a better understanding of the pathogenesis of the disease and has enabled the development of novel anticancer agents. For example, identifying the continued role of the androgen receptor in driving tumor growth despite androgen deprivation therapy, which eventually led to the successful development of abiraterone and enzalutamide as anticancer agents,[2,40] or the identification of DNA-repair abnormalities in approximately 15-20% of patients with advanced disease and the development of PARP inhibitors as specific anticancer agents targeting these abnormalities.[46]

Combined efforts from a number of research groups with whole-exome (WES) or whole-genome (WGS) sequencing has enabled the identification of molecular subtypes of the disease with distinct genomic hallmarks.[182,183] Despite the advances, however, tumor heterogeneity (both intra- and inter-patient) continues to represent a formidable hurdle for the development of personalized medicine approaches in cancer, due to the coexistence of multiple clones with potentially different driver events in different metastatic sites of the same patient.[184] Liquid-biopsy approaches have been proposed as a potential solution to overcome the problem, by identifying and targeting the predominant clones in the blood-stream of patients at each specific time-point.

4.1 Overview of Genomic Aberrations in Prostate Cancer

Molecular studies have identified potentially relevant genomic alterations in human prostate cancer, some of which are associated to key regulatory genes.[185] Chromosomal deletions, for instance, have been found to accumulate early in prostate carcinogenesis, inactivating tumor suppressor genes such as phosphatase and tensin homologue (*PTEN)*, *TP53*, or *CDKN1B*. Recurrent DNA copy number alterations (amplifications or deletions) that dysregulate genes in a number of key cellular processes have been identified in localized prostate cancer. A higher amount of copy number alterations has been associated with increased clinical aggressiveness, both in primary and metastatic prostate cancers.[186]

Some of the most frequent alterations include loss of chromosome 8p (NKX3.1 gene), which is the most

frequent alteration in the prostate onco-genome; deletion of PTEN on 10q23.31; deletion of the Retinoblastoma (RB1) tumor gene on 13q14.2; deletion of the TP53 gene on 17p31.1; or an interstitial 21q22.2-3 deletion spanning ERG and TMPRSS2.[187] Retinoblastoma tumor (RB1) suppressor gene pathway aberrations have been identified in 34% of primary and 74% of metastatic prostate cancers.[186] Deletions in the CHD1 gene (5q21) have been identified in 10–17% of prostate tumor samples, possibly second only to PTEN loss as the most frequently homozygously deleted gene in the prostate cancer genome, increasing invasiveness in prostate cancer cell lines. CHD1 deleted tumors appear to have a high frequency of intra-chromosomal rearrangements and may confer a worse prognosis.[188,189]

Gene fusions are present in approximately half of patients with localized prostate cancer. In most cases, androgen-regulated promoters are fused to androgen-driven promoter elements to transcription factors of the ETS family. Among these, fusions of TMPRSS2 to the oncogenic ETS transcription factor ERG occur most frequently, in approximately 40-50% of patients.[190] Epigenomic alterations, namely chromatin remodeling and/or DNA methylation at specific CpG sites, have also been related to disease progression in localized prostate cancer. An enrichment of mutations in epigenetic regulators and chromatin remodelers in ETS-fusion negative tumors has been reported.[191] Point mutations, on the other hand, are found at significantly lower rate than CNAs or translocations, and at a lower frequency than other epithelial cancer types. SPOP mutations are the most frequently found mutations in prostate cancer, found in approximately 10% of localized cases;

4.2 The Molecular Taxonomy of Localized Prostate Cancer

Localized prostate cancer is characterized by a number of genomic aberrations gene deletions or amplifications, gene fusions and DNA copy number alterations (CNAs)[186,190,192,193]. Sequencing studies have identified molecular subgroups, generally based on the presence or absence of gene fusion involving the ETS family of transcription factors. These alterations, which are mutually exclusive with a number of other molecular aberrations in prostate cancer, have enabled the characterization of ETS fusion positive (ETS+) and ETS fusion negative (ETS-) prostate cancers. Serine peptidase inhibitor (SPINK1) overexpression,[194] SPOP gene point mutations,[195] CHD1 gene mutations[196] and fusions involving the RAS/RAF family genes are generally considered mutually exclusive from ETS fusion genes, and characterize the subgroup of ETS fusion negative tumors. On the other hand, in the group of ETS fusion positive tumors, fusions involving the ERG gene represent approximately 90%, with non-ERG gene fusions representing the remaining 10%.[181]

In 2015, The Cancer Genome Atlas (TCGA) published the results of a comprehensive molecular analysis of 333 primary prostate carcinomas revealing seven genomically distinct subtypes, defined by ERG fusions (present in 46%), other gene fusions (ETV1, ETV4, FLI1 – 13%) and SPOP (11%), FOXA1 (3%) or

IDH1 (1%) mutations.[182] Androgen receptor activity was found to vary widely between cancer subtypes, with the highest level of activity found in the SPOP and FOXA1 mutant tumor subtypes. Additionally, PI3K or MAPK pathway alterations were observed in 25% of cases, and DNA repair genes were altered in 19% of cases. Approximately 26% of tumors, however, were not classified into any of the categories, suggesting further insight may be required in order to appropriately molecularly categorize the disease.

4.3 The Molecular Landscape of Advanced Prostate Cancer

Advanced prostate cancer presents with molecularly distinct characteristics in comparison with localized prostate cancer. The reason for this difference is (1) the adaptive pressure and treatment selection imposed by primary, androgen deprivation therapy which, for example, induces an increase in AR copy number as a general feature of CRPC and (2) an enrichment for features indicating disease aggressiveness and metastatic potential.

Some genomic alterations, SPOP mutations or ERG rearrangements, appear at similar rates in localized and metastatic prostate cancer, suggesting these lesions develop early, before metastatic disease develops.[197] On the other hand, alterations in the androgen receptor pathway, such as AR amplification or AR mutations are significantly more frequent in mCRPC than in localized disease. As is observed in localized prostate cancer, the mutation rate of metastatic, CRPC is relatively low, at approximately 2 mutations per megabase.[198] A small proportion of mCRPC patients, however, have been shown to harbor significantly higher mutation rates (nearly 50 mutations / megabase), a situation that has been associated with alterations in the mismatch repair genes MLH1 and MSH2.[183]

In 2015, Robinson and colleagues reported data from a study where integrative next-generation sequencing (whole-exome, matched germline, and transcriptome data) were performed in metastatic biopsies from 150 individuals with mCRPC.[183] As previously described, copy number alterations were the most frequently observed aberrations, with focal amplification found in the AR (8q) gene locus but also in regions harboring the CCND1, PIK3CA and PIK3CB genes, and focal deletions in the CHD1, PTEN, RB1 and TP53 gene loci. Somatic TP53 mutations were the most selectively mutated in mCRPC, followed by mutations in the AR, MKT2D, APC, BRCA and GNAS genes; focal amplifications in the AR were the most frequent copy number alterations selectively found in mCRPC. Aberrations in DNA-repair genes (BRCA2, BRCA1, ATM) were also observed at higher frequencies in mCRPC (19.3%) than in primary prostate cancer.

Alterations in the AR pathway were the most frequently observed (71.3% of cases), most of which were direct AR mutations or amplifications. Hotspot mutations in the AR were observed at locations known to confer agonistic activity to first- and second-generation AR antagonists (flutamide, bicalutamide) and glucocorticoids. AR splice variants were also detected at varying levels in primary prostate cancer and

benign tissue. AR co-factor alterations was also shown, with alterations in the NCOR1, NCOR2 and FOXA1 genes. Alterations in the PI3K pathway was also observed in up to 49% of individuals. The most frequently observed alteration of the pathway was biallelic PTEN loss, although other alterations such as PIK3CA mutations, amplifications or gene fusions, and AKT1 mutations, inducing pathway activation, were also observed. Alterations in the WNT (18%), with activating mutation in the CTNNB1 and aberrations in the APC gene, and cell-cycle pathways were also observed in a significant number of cases. RB1 loss was detected in 21% of cases, with additional focal amplification events in CCND1, and less common alterations in the CDKN2A/B, CDKN1B and CDK4 genes. Finally, alterations in the DNA-repair pathway were found in 22.7% of cases; mainly BRCA2 loss (mainly biallelic) in 12.7% of cases but also loss in ATM and other less-frequently altered genes (BRCA1, CDK12, FANCA, RAD51B, RAD51C). Altogether, authors reported that up to 89% of patients had a potential clinically actionable aberration, highlighting the potential for NGS in the selection of treatment options in advanced prostate cancer.[183]

The genomic and transcriptomic landscape of mCRPC described by Robinson and colleagues was largely reproduced in a second large cohort of 429 mCRPC patients, with whole-exome sequencing and RNA-sequencing performed in tissue from lymph node (37%), bone (36%) or liver (14%) metastases. Authors reported mutual exclusivity between ETS-family alterations and between alterations in ERG and SPOP or FOXA1, which are known distinct genomic subsets of prostate cancer. Alterations in ERG and PTEN were found to be co-occurring, as were alterations in CHD1 and SPOP mutations, and in CDK12 (implicated in the control of genomic stability) and cell-cycle genes such as CCND1 and CDK4. Co-occurrence between TP53 and Rb1 alterations was also reported, associated with an adverse outcome and the presence of neuroendocrine features.[199]

Despite the fact that the overall incidence of mutations in advanced prostate cancer is low, studies have shown that the incidence of mutated genes follows a long-tail distribution, with many genes mutated in less than 3% of cases. A number of significantly mutated genes at low frequencies with potential functional and clinical relevance have been identified. These alterations, which may be clinically significant if considered as an aggregate group, have only been identified after large cohorts of over 900 samples (needed to achieve the required statistical power to establish significance), have been sequenced.[191]

Recent studies performing whole genome sequencing of advanced prostate cancer have also revealed the role structural variations of the genome, some of them located in introns and therefore missed by whole exome sequencing, may have over specific oncogenes or tumor suppressor genes. Some structural variations were found to inactivate key genes in the development of prostate cancer such as TP53 or PTEN.[200] Furthermore, a novel region of amplification 66.94 MB upstream of the AR, present in up to 81% of patients was found to be associated with increased AR expression, suggesting a novel

mechanism of castration resistance.[200,201] Different structural variations in the genome appeared to be associated with distinct DNA repair alterations.

4.4 Germline Aberrations in Advanced Prostate Cancer

Prostate cancer is one of the most heritable tumor types. According to some estimates, up to 60% of the risk could be attributable to genetic factors alone. Inherited mutations in several genes involved in DNA damage response and repair (DDR) have been associated with predisposition to the development of prostate cancer. Among these, the BRCA2 gene confers the highest risk, with approximately a 6.5-fold increased risk in men older than 65 years. The prevalence of germline mutations in DDR genes has been estimated at approximately 8-12% of cases of metastatic prostate cancer,[183,202,203] which is significantly higher to the rates of DDR germline mutations in localized disease or the general population.[182]

In a large, retrospective study, Pritchard and colleagues sequenced germline DNA of 692 patients with metastatic CRPC. 84 germline DNA-repair gene mutations that were presumed to be deleterious were identified in 82 men (11.8%); mutations were found in 16 genes, including BRCA2 (37 men [5.3%]), ATM (11 [1.6%]), CHEK2 (10 [1.9% of 534 men with data]), BRCA1 (6 [0.9%]), RAD51D (3 [0.4%]), and PALB2 (3 [0.4%]).[202] Interestingly, there was no difference in mutation frequency among patients with and without a family history of prostate cancer.

Patients with germline BRCA1 or BRCA2 mutations are enriched for adverse clinicopathologic features, and have a worse prognosis than non-BRCA carriers. In a large retrospective study evaluating 2,019 patients with prostate cancer, the presence of germline BRCA1 or BRCA2 mutations was associated with a higher Gleason score, T3/T4 stage, nodal involvement and metastatic disease. Carriers of germline BRCA2 mutations with localized disease were found to have a lower cancer-specific survival (HR 2.6; p=0.01) and metastasis-free survival (HR 2.7; p=0.009) than non-carriers.[204] The prognostic value of germline mutations in advanced prostate cancer is less-well established with different series reporting conflicting results; while some series have reported an improved outcome with germline BRCA mutations,[205] others have found no significant differences,[206] and others have reported a significantly worse prognosis.[207] Methodological differences between studies (most of which were retrospective) are likely responsible for these seemingly contradictory evidence.

Recently, a Spanish prospective longitudinal study has reported results on the rate of germline aberrations in patients with mCRPC. The PROREPAIR study was a multicenter prospective cohort study with the aim of evaluating the impact of germline mutations on the overall prognosis of patients, and to estimate the prevalence of germline DDR mutations. A total 419 patients were included in the study. Altogether, 16.2% of patients were carriers of mutations in DDR gene, with approximately 6.2% presenting with mutations in BRCA2, ATM or BRCA1. Ninety-six (22.9%) patients had a family history of

cancer, which was significantly more common in carriers (60,3%) than in non-carriers (16%, p<0.0001). Of all carriers, those with BRCA2 mutations had the greater association with a family history of cancer (85.7% vs 20.7%, p<0•0001).[207]

Based on results from this and other studies, clinical guidelines now recommend germline BRCA1/BRCA2 screening in patients with metastatic prostate cancer.[208] This is highly relevant, since the identification of patients with germline DNA repair defects can allow for the treatment with PARP inhibitors, which have proven a significant clinical benefit in this patient population subgroup, and are now FDA-approved in this setting.

4.5 Neuroendocrine Prostate Cancer

Neuroendocrine prostate cancer is a rare pathological variant that is clinically characterized by low prostate-specific antigen (PSA) levels, poor response to hormonal agents, visceral metastases and an aggressive clinical course. Neuroendocrine cells are defined pathologically by the NE cells are defined in current practice by immunohistochemical positivity for either synaptophysin, chromogranin, or CD56.[209] Neuroendocrine cells lack androgen receptor, unlike the vast majority of prostate adenocarcinomas, and are therefore not responsive to hormonal therapies directed at androgen suppression. Neuroendocrine differentiation has been found to increase after androgen deprivation, and in castration-resistant prostate cancer.[210] Furthermore, chromogranin expression in prostate cancer tissue and plasma has been associated with an increased risk of development of castration resistance.[211]

Neuroendocrine features may appear at diagnosis (de-novo) or may appear as a resistance mechanism to extreme androgen deprivation induced by sustained hormone therapy. In a retrospective analysis in 87 patients with metastatic NEPC, 54% of patients had *de novo* NEPC, while in 46% NEPC were therapy related. Median PSA was 1.2 ng/mL, and 65.5% presented with visceral metastases. Median survival for *de novo* metastatic disease was 16.8 months, compared with Authors reported that features such as a small cell histology had a worse survival (8.9 vs 26.1 months) than mixed histology. De novo metastatic NEPC, RB1 and TP53 loss and the presence of liver metastases were other features of adverse prognosis.[212] Circulating tumor cells from neuroendocrine prostate cancer also present with unique morphological characteristics which are distinct from those from castration-resistant adenocarcinomas,[213] which could potentially identify neuroendocrine transformation in patients progressing on therapy, especially those with discordant radiographic or clinical progression and low PSA levels.

NEPC is a distinct molecular entity, with significant differences in the biology compared with prostate adenocarcinoma. Overexpression of the N-Myc (2p24) and Aurora- kinase A (AURKA, 20q13) genes are some of the molecular hallmarks of the disease, although other known alterations include loss of TP53

and RB1, or PEG10 gene amplification. Concurrent loss of RB1 and TP53 has been found in up to 53.3% of CRPC-NE as opposed to 13.7% of CRPC-adenocarcinoma samples.[214] Overexpression and gene co-amplification of AURKA and N-MYC has been found in 40% of NEPC as opposed to 5% of non-neuroendocrine prostate tumors, with TMPRSS2-ERG rearrangements present in 50% of NEPC samples.[215] Epigenetic transformation could be responsible for the transition from adenocarcinoma to neuroendocrine histology induced by sustained AR signaling suppression; studies have shown substantial genomic overlap between castration-resistant histologically characterized as adenocarcinoma and neuroendocrine variants, suggesting a common origin. However, marked marked epigenetic differences between CRPC-NE tumors and CRPC-Adeno were observed in genome-wide methylation analyses, suggesting a role of epigenetic modifiers in the induction or transition to an AR-independent phenotype.[214]

Treatment of neuroendocrine prostate cancer remains challenging. These patients are generally resistant to standard hormone therapy and chemotherapy used in adenocarcinomas, and have traditionally been treated with platinum chemotherapy, based on experience with high-grade neuroendocrine carcinoma in other sites. Some authors have suggested the combination of cabazitaxel and carboplatin could be more effective in patients with aggressive variants of prostate cancer, a wide concept that encloses (but is not restricted to) neuroendocrine prostate cancer. Results are, however, disappointing, with overall survival of only 18.5 months.[216] Pre-clinical studies have suggested an upregulation of BCL2 in small cell prostate cancer, which could be potentially targeted by combined BCL2 and Wee1 inhibitors.[217]

5. Clinically Relevant Molecular Pathways in Advanced Prostate Cancer

5.1 AR Pathway Biomarkers

The Androgen Receptor (AR) pathway represents the most frequently altered molecular pathway in prostate cancer. Pharmacological inhibition of the AR has been the cornerstone of treatment for advanced prostate cancer since Higgins and Hodges discovered the induction of tumor regression by surgical castration.[218] The AR gene is located on chromosome X at q11–12 and is a 90 kb gene containing 8 exons. This gene is the largest and most complex of all nuclear steroid receptor genes, and encodes the AR, which consists of an amino-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region and a carboxy-terminal ligand-binding domain (LBD).[219]

Before ligand binding, the AR is located in the cytoplasm, where binding to chaperone molecules prevents its degradation. Upon binding to its ligands (most frequently testosterone and dihydrotestosterone), the AR is translocated to the nucleus, where it exerts its function as a transcriptional factor by binding to androgen-responsive elements (AREs). This interaction is modulated by coactivator (NCoA-1, NCoA-2) and co-repressor (NCoR, SMRT) proteins present in the nucleus.[220] Interaction of the AR with co-activator and co-repressor proteins is enabled by the alteration in its tridimensional protein structure induced by the receptor-ligand interaction.[219]

AR signaling suppression represents the most effective therapeutic strategy in advanced prostate cancer. Both surgical (bilateral orchiectomy) or chemical (through LHRH analogues) castration induces almost universal responses as initial therapy for advanced prostate cancer. In patients with newly diagnosed metastatic prostate cancer, despite the initial response, progression develops after a median of 11 months, and disease enters into the lethal, castration-resistant phase.[221]

Disease progression despite castrate levels of testosterone is frequently driven by alterations in the AR pathway. A rise in prostate-specific antigen (PSA), a serine protease that is widely used for the diagnosis and monitoring of patients with prostate cancer is one of the hallmarks of progression into the castration-resistant phase. Since the PSA protein is encoded by the KLK3 gene, tightly related to the activity of the androgen receptor, progression generally represents sustained activity of the AR pathway. In fact, the recognition of the sustained role of the AR pathway in castration-resistant prostate cancer led initially to the development of therapies targeting the AR pathway such as the CYP17 inhibitor abiraterone and the second-generation antiandrogen enzalutamide, and represented a breakthrough in the understanding and treatment of the disease. New, third-generation antiandrogens such as apalutamide and darolutamide have recently shown to significantly delay disease progression in CRPC patients without metastatic disease.

A number of different mechanisms have been described as responsible for the persistence of AR pathway signaling in situations of extreme depletion of circulating androgen levels. AR genomic alterations, AR mRNA splice variant expression, AR cofactor activation, alterative steroid receptor pathways (such as the glucocorticoid receptor) or AR crosstalk pathway (PI3k pathway activation) are all mechanisms that have been described to maintain AR pathway signaling in testosterone-depleted scenarios.[197,222]

AR Amplifications / Mutations

AR gene aberrations, such as increased copy gene number (amplification) or mutations are amongst the most frequent alterations that lead to the development of castration-resistance, and have also been associated to worse response to novel hormonal agents. AR amplification, which has been described in 30-50% of mCRPC biopsy samples, is the most frequently observed alteration. An increase in the number of copies of the AR gene leads to increased sensitivity to circulating and intra-tumoral androgens.

AR point mutations, on the other hand, are much less common (2-18%) and may confer resistance by causing promiscuous activation of the receptor by alternative ligands such as adrenal steroids or even first-generation AR antagonists. Different mutations may confer different sensitivities, and may induce activation by different ligands such as adrenal steroids (Gln670Arg, lle672Thr)[223], corticosteroids (Leu701His, Thr877Ala)[224,225] or first-generation antiandrogens (Trp741Cys, Trp741Cys)[226,227]. Paradoxical declines in PSA after discontinuation of first-generation antiandrogens has been described in up to 20% of patients,[228] and is explained by the cessation of a stimulus towards a mutant androgen-receptor.[36] Next-generation antiandrogens such as enzalutamide or apalutamide were developed based on maintained antagonistic activity with previously defined mutations. Novel mutations (Phe876Leu) have been, however, identified in patients progressing on these agents, with the ability to up-regulate AR expression *in vitro*.[229,230]

Both AR amplifications and mutations are known to be an adaptive mechanism to initial hormone therapy, as they are practically non-existent in hormone-treatment naïve patients. Association with worse response to novel hormonal agents has been reported in different clinical studies; interestingly, this is not observed in chemotherapy-treated patients, supporting its use as a potential predictive biomarker for the selection of therapy in advanced prostate cancer. In a prospective study of 98 men starting enzalutamide as first-line treatment with enzalutamide, AR amplification was observed in 11% of patients. These patients had a significantly worse PSA-PFS (3.6 vs 15.5 moths; HR 4.3; $p<0.001$), radiographic PFS (3.9 vs not reached; HR 8.1; $p<0.001$) and overall survival (medians not reached; HR 11.1; $p=0.004$) than those patients without AR gene amplification. The association of AR copy number

gain with worse PSA-PFS, rPFS and OS was independent of other prognostic characteristics in multivariable analysis.[231] In another study evaluating plasma AR aberrations in 163 docetaxel-treated mCRPC patients, AR amplification was associated with worse overall survival (HR 1.61; p=0.018) but not PFS or PSA responses. Furthermore, when performing an indirect comparison between patients treated with first-line abiraterone/enzalutamide and first-line docetaxel treated patients, a significant interaction between treatment type and AR copy number gain status was observed. Increased overall survival in abiraterone/enzalutamide over docetaxel-treated patients without AR amplification was observed; conversely, a trend towards improved outcome with docetaxel was observed in AR-amplified patients.[232] Similarly, as second-line therapy, patients with AR amplification treated with cabazitaxel chemotherapy have shown improved outcome compared to those treated with abiraterone/enzalutamide.[233] Taken together, the evidence suggests AR plasma status (amplification or mutation) could be used as a potential biomarker for treatment selection (hormonal versus chemotherapy); this hypothesis must be, however, adequately addressed in well designed, randomized clinical trials.

Apart from the evaluation of AR amplifications or mutations, the nuclear localization of the AR has also been suggested as a potential biomarker in chemotherapy treated patients, since both new antiandrogens such as enzalutamide and taxanes such as docetaxel and cabazitaxel are known to exert their antitumoral action by partly blocking translocation of the AR into the nucleus.[69,234] For instance, in patients receiving docetaxel or cabazitaxel in the TAXYNERGY trial, a taxane-induced reduction in the percentage AR with nuclear localization after 1 week was associated with a higher PSA response at cycle 4.[235]

AR Splice Variants

Alternative splicing of the AR messenger RNA (mRNA) has also been shown to be able to maintain persistent signaling in testosterone-depleted environments. Abnormal splicing during the transcription phase leads to the synthesis of truncated AR proteins that, despite lacking the ligand-binding domain, remain constitutively active and are therefore not targetable by drugs aimed at the ligand-receptor interaction.[236] Of the various different AR splice variants that have been identified and described, AR variant 7 (AR-V7) is the most abundant and has the most documented role in the development of resistance to hormone therapy in advanced prostate cancer.

AR-V7 expression can be detected and quantified by immunohistochemistry in both primary and metastatic prostate cancer tissue. A recent study reported that AR-V7 protein expression is found rarely in tissue from primary (treatment-naïve) biopsies (<1%), but increased significantly (75%) in tissue from patients progressing on androgen deprivation. Furthermore, patients progressing on abiraterone or enzalutamide had a significantly higher AR-V7 expression than those progressing on ADT only. AR-V7

was predominantly located in the nucleus, suggesting constitutive activation, and was correlated with AR full length (AR-FL) expression and with increased AR copy number. Authors also reported heterogeneous AR-V7 expression in different metastases within a patient. Patients with AR-V7 expression was associated with a lower rate of PSA response (100% vs 54%) and overall survival (HR 0.23; p=0.02) from hormonal treatment in chemotherapy-naïve patients.[237]

AR-V7 expression can also be determined from blood samples of prostate cancer patients, by evaluating its expression in circulating tumor cells (CTCs). Two different methods have been extensively evaluated for the assessment of AR-V7 status in CTCs: the AdnaTest,[238] which evaluates the expression of AR-V7 mRNA transcripts in CTCs by quantitative RT-PCR (qRT-PCR), and the Epic Sciences Test, which is based on automated detection of intranuclear AR-V7 protein using immunofluorescent staining of CTCs.

Initial, retrospective studies evaluating the presence of mRNA AR-V7 in CTCs (AdnaTest) from mCRPC patients reported an association between AR-V7 and worse outcome (PSA response, overall survival) in abiraterone and enzalutamide-treated patients.[239] A second, prospective study evaluated 202 mCRPC patients receiving enzalutamide and abiraterone, and classified patients into CTC negative (no detectable CTCs), CTC positive, AR-V7 negative (CTC+/ARV7-) and CTC positive, AR-V7 positive (CTC+/ARV7+). CTC+/ARV7+ patients were more likely to present with adverse prognostic features such as higher Gleason scores, metastatic disease at diagnosis, worse ECOG performance status or higher PSA. CTC+/ARV7+ was more likely in pre-treated patients; while only 12 of 124 (12.1%) first-line patients were CTC+/ARV7+, up to 21/78 (26.9%) of those treated in second-line were CTC+/ARV7+. These patients had significantly worse PSA-PFS, radiographic PFS and OS than CTC+/ARV7- and CTC negative patients. CTC negative patients, on the other hand, showed significantly better prognosis than CTC+/ARV7- patients.[240] While a number of additional studies have validated the adverse prognostic role of AR-V7 expression in CTCs of abiraterone or enzalutamide-treated patients,[88,241] this does not seem to be the case in taxane-treated patients. In another prospective study led by the Johns Hopkins group, 37 taxane-treated patients were enrolled, with 17 (46%) showing AR-V7 positive CTCs. No significant differences in PSA-PFS, PFS or OS were observed in AR-V7 positive vs negative patients.[242] When incorporating data from 62 patients from the previously cited study,[239] a significant interaction between treatment agent (abiraterone/enzalutamide vs taxanes) and outcome was observed, suggesting PSA-PFS (p=0.001) and PFS (p=0.003), though not OS (p=0.160), to be superior for taxanes over hormonal agents in AR-V7 positive patients, and viceversa.[242] In another study of 29 cabazitaxel-treated patients, outcome (PSA-PFS, PFS, OS) was likewise not different in those with AR-V7 positive and negative CTCs.[243] On the other hand, in patients treated with either docetaxel or cabazitaxel in the TAXYNERGY trial, AR-V7 and AR^{v567es} expression detected by digital droplet PCR (ddPCR) in CTCs was associated with worse outcomes with both docetaxel and cabazitaxel, suggesting a prognostic value but not a significant role for optimal taxane agent selection.[244]

These results led some authors to explore the possibility of dynamically studying the evolution of AR-V7 during treatment. In a small, prospective study, sequential CTC samples from 14 mCRPC patients were analyzed for AR-V7 status with the AdnaTest assay. Only 3 patients remained AR-V7 positive during the course of therapy; the rest of patients had different changes in AR-V7 status (changes from AR-V7 negative to AR-V7 positive status in eight patients, suggesting a mechanism of progression to hormone agents, and changes from AR-V7 positive to AR-V7 negative during chemotherapy in another six patients).[88] These results led to some authors to suggest a potential "wash-out" effect of chemotherapy between a first-line and a third-line hormonal agent could potential "clear" AR-V7 positive clones and increase efficacy.[245] This hypothesis, however, has been refuted in the recent phase III CARD trial, where cabazitaxel was superior to ARSIs regardless of the administration of docetaxel between hormonal agents.[95]

Similar results have been observed when evaluating AR-V7 protein immunofluorescence in CTCs (Epic Sciences Test). In a prospective study in 161 abiraterone/enzalutamide or taxane treated mCRPC patients, AR-V7 positivity again was found in greater frequency in heavily pre-treated patients, with only 3% in first-line and 18% in second-line, but in as many as 31% patients treated in third or subsequent lines of therapy. AR-V7 positive patients treated with abiraterone or enzalutamide exhibited significantly lower rates of PSA response, and shorter rPFS and OS than AR-V7 negative patients. On the other hand, there were no differences in PSA response or rPFS in AR-V7 positive or negative patients treated with taxanes. A multivariable Cox-regression model showed superior OS with taxanes compared with abiraterone/enzalutamide in AR-V7 positive patients (HR 0.24; p=0.035).[246] A subsequent study performed in the same cohort evaluated whether the presence of AR-V7 in the nucleus had an impact on the prognostic performance of the biomarker. Authors classified AR-V7 positive events as nuclear-specific (requiring expression of AR-V7 in the nucleus) and nuclear-agnostic (not requiring AR-V7 expression in the nucleus). While CTCs with nuclear-specific criteria were less frequent (18% vs 29%), there was a significant interaction between OS and treatment type in AR-V7 positive patients only when nuclear-specific criteria were used, suggesting the presence of AR-V7 protein in the nucleus is necessary to discriminate which patients will receive benefit from treatment with chemotherapy versus hormone therapy.[247]

Which assay should be used? Both assays have been compared in a prospective validation study (PROPHECY Study) where 118 mCRPC patients starting abiraterone or enzalutamide treatment were enrolled. AR-V7 positivity by either qRT-PCR mRNA detection (AdnaTest) or intranuclear protein immunofluorescence (Epic Sciences) was associated with shorter PFS and OS. AR-V7 positivity by the Epic Sciences assay was less frequent, but its detection had a greater impact on survival than the AdnaTest assay. The agreement between both assays was high (82%).[248]

Taken together, results suggest AR-V7 detection could represent a clinically relevant biomarker for the choice of treatment between ARSIs and taxanes in advanced prostate cancer patients. One must caution, however, that prospective validation of the predictive role of AR-V7 has not been performed. The PROPHECY study, on one hand, evaluated only patients treated with abiraterone or enzalutamide; the prognostic role was clearly validated, but no conclusions on whether AR-V7 positive patients should receive ARSIs or taxane chemotherapy can be made. Furthermore, the low prevalence of AR-V7 positivity in the first-line scenario, where a decision between chemotherapy and ARSI therapy is most relevant, is very low, a fact that can limit significantly the clinical utility of the assay. In the ARMOR3-SV trial, a randomized phase III trial comparing galeterone (a novel antiandrogen with AR degrading properties) and enzalutamide in previously untreated AR-V7 positive mCRPC patients. Unfortunately, the trial had to be stopped after only 73 (8%) of 953 screened patients had AR-V7+ CTCs.[249] These results highlight the importance of selecting biomarkers with a sufficient prevalence that can significantly impact a sufficient number of patients. In second-line, where most patients will have received either ARSIs or taxanes in first-line, choice of subsequent therapy will generally be guided by previous therapy, and not by AR-V7 status.

In order to incorporate AR-V7 detection into clinical routine practice, a trial showing that choices made based on the results of the assay lead to significant improvements in a clinically significant endpoint will be needed, ideally in a well powered, prospective, randomized trial. Such evidence is, to date, lacking.

6.2 TMPRSS2-ERG Rearrangements

Gene fusions involving members of the ETS family of transcription regulator genes (ERG, ETV1, ETV4, ETV5, FLI1) and androgen-responsive elements of a second gene are amongst the most common genomic abnormalities in advanced prostate cancer, found in approximately 50% of cases.[250] The most frequent of these translocations is the TMPRSS2-ERG gene fusion, formed by the juxtaposition of non-coding androgen-driven promoter elements of the TMPRSS2 gene to the ERG gene.[251] The presence of these gene fusions, which are develop from early stages of tumorigenesis, allow the classification of localized disease into fusion-positive and fusion-negative prostate cancer.[182] Fusion-positive prostate cancer is enriched for other relevant aberrations, such as loss of the tumor suppressor PTEN, and studies have suggested that co-operation between both abnormalities may accelerate progression from intraepithelial neoplasia (PIN) to prostate adenocarcinoma in a subgroup of tumors.[252] In localized prostate cancer, ETV1 gene fusions have been associated with higher Gleason score and PSA at diagnosis.[253] The presence of ERG/ETV1 rearrangements in localized prostate cancer, although by itself not a significant prognostic factor in multivariate analysis, has been shown to present a highly significant value when combined with PTEN loss. In a cohort of 308 localized prostate cancer patients, those with PTEN loss/non-ERG rearranged patients represented an adverse prognosis subgroup, ERG rearranged

patients (with or without PTEN loss) presented with an intermediate prognosis subgroup and PTEN normal/non-ERG rearranged patients experienced the most favorable prognosis.[254]

ETS gene fusions and, specifically, TMPRSS2-ERG rearrangements, have been extensively studied as a potential biomarker for treatment selection in advanced prostate cancer, with data suggesting its value as an indicator of treatment benefit in abiraterone-treated patients and a potential role as a biomarker of resistance in docetaxel-treated patients. Different techniques have been used to assess either the gene fusion TMPRSS2-ERG by fluorescent in situ hybridization or the consequent ERG overexpression (by RT-PCR, immunohistochemistry and immunofluorescence) in tumor tissue specimens or CTCs.[255]

In patients treated with abiraterone in early phase I/II trials, assessment of TMPRSS2-ERG gene fusion status (mRNA levels of the ERG transcript) in circulating tumor cells through quantitative-RT-PCR showed a high correlation with ERG gene status in original, treatment-naïve biopsies, suggesting gene fusion is an early event in tumor development. Furthermore, the presence of an ERG rearrangement was associated with higher PSA response rates, with 80% of ERG-rearranged vs 32% of non-ERG rearranged tumors experiencing ≥ 90% PSA declines on therapy.[256] These results were validated in a post-hoc analysis of the phase III COU-AA-302 trial, where ERG gene fusion status in tumor tissue was determined in 348 patients. ERG rearrangement was found in 35% of patients. Class 2+ Edel rearranged tumors were found to have an increased rPFS when treated with abiraterone than tumors with no rearrangement or with other types of rearrangement (HR: 0.53; p=0.002).[257]

On the other hand, ERG rearrangement has been proposed as a biomarker of treatment resistance to taxane chemotherapy. By modifying microtubule dynamics at the molecular level, ERG rearrangements may affect the drug-target interaction between taxanes and tubulin, thereby impairing their mechanism of action. Studies in ERG-overexpressed *in vitro* and *in vivo* models have shown decreased sensitivity to taxanes, with similar findings in small retrospective patient cohorts.[258] The presence of TMPRSS2-ERG gene fusions through RT-PCR in peripheral mononuclear blood cells was also associated with lower PSA response rates (13 vs 69%; p=0.005) and lower PSA (HR: 3.7, p < 0.001) and clinical/radiographic PFS (HR 6.3; p<0.001) in a cohort of 72 patients treated with docetaxel and cabazitaxel chemotherapy. Interestingly, a switch from negative to positive TMPRSS2-ERG fusion status was observed at progression in 41% of patients with no detectable gene fusion at baseline.[259]

Intriguingly, data regarding the predictive role of ERG fusion status in mHSPC, where TMPRSS2-ERG status appears to be associated with improved outcomes, seems to contradict data observed in mCRPC. In a post-hoc study evaluating tumor tissue from 334 patients treated in two phase III trials, treatment with docetaxel was associated with a significant benefit in relapse free survival in ERG+ but not in ERG- patients (interaction p-value: 0.02).[260] Similar results were observed in an independent, retrospective

cohort of 55 mHSPC patients treated with docetaxel + ADT, with an association of ERG positivity with improved relapse-free survival (26 vs 11.4 months; p=0.003).[261]

6.3 THE PI3K-Akt-mTOR Pathway

The PI3k-Akt-mTOR is the second most frequently altered pathway in advanced prostate cancer, second only to AR pathway alterations. The PI3K pathway is a critical regulator of proliferation, survival, metabolism, angiogenesis, and immune function.[181] Loss of PTEN is the most frequent aberration, present in approximately 40% of individuals with mCRPC.[183] PTEN functions as a negative regulator of PI3k. Its inactivation leads to activation of the AKT and mTOR signaling cascades, that result in dysregulation of key cellular processes such as apoptosis, cell cycle progression, cell proliferation, metabolism and invasion.[262] PTEN loss is an early event in prostate tumorigenesis, and has been linked to aggressive prostate tumor types, either alone or in combination with other aberrations such as ERG-family rearrangements, p53 inactivation or Rb1 loss.[263,264] Other aberrations, such as amplifications or activating fusions in PIK3CA, or activating mutations of PIK3CA, AKT1 or PIK3CB are less common (~10%) in advanced prostate cancer, although these may be therapeutically relevant in specific cases.[183]

Hyperactivation of the PI3k pathway, generally through PTEN loss, has been associated with resistance to both hormone therapy and chemotherapy in mCRPC, mediated by signaling crosstalk between the PI3k and the AR pathways. In patients with PTEN deletion, AR transcriptional output is generally decreased; PI3k pathway inhibition may activate AR signaling by reducing this feedback inhibition. On the other hand, inhibiting AR may activate Akt signaling by reducing levels of Akt phosphatases that normally exert an inhibitory effect on the AR pathway.[265]

The main current techniques to determine the status of the pathway are immunohistochemistry and immunofluorescence. Immunofluorescence, which has been traditionally considered the gold standard, is based on detection of loss of the PTEN gene at the genomic level.[266] This has been shown to be feasible in tumor tissue, but also on liquid biopsies in circulating tumor cells.[267] It may, however, miss other alterations in the pathway beyond genomic loss, such as mutation or epigenetic silencing. Immunohistochemistry, on the other hand, is based on the localization of pathway proteins intracellularly, is cheaper and easier to perform. It is, however, a semiquantitative approach at best, and standardized cut-offs to define positive and negative samples are generally lacking.[266] An H-Score for PTEN IHC has been proposed based on the percentage of strongly, moderately and weakly staining cells, and has been associated with outcome in abiraterone and docetaxel-treated patients.[9,10]

Several studies have assessed the impact of PTEN and other PI3k pathway aberrations in the outcome of patients treated for prostate cancer. In patients with localized disease, the co-occurrence of PTEN loss (assessed through FISH) and ERG/ETV1 gene fusions identified a patient populations with significantly

shorter cancer-specific survival.[254] In a cohort study of 144 mCRPC patients receiving second-line abiraterone after progression on docetaxel, PTEN loss (assessed by immunohistochemistry) was present in approximately 40% of patients. Loss of PTEN expression was associated with shorter overall survival (14 vs 21 months; HR 1.75; p = 0.004) and duration of abiraterone treatment (24 vs 28 weeks; HR: 1.6; p=0.009).[9] In a subsequent study in 215 patients treated with docetaxel for mCRPC, PTEN loss was again associated with shorter overall survival (25.4 vs 34.7 months; HR 1.66; p=0.001) although no significant differences in PFS (8 vs 9.1 months; HR 1.2; p=0.28) or PSA response rates (53.4% vs 50.6%; p=0.74) were observed. In this study, ERG positivity was not associated with OS or PFS.[10]

Attempts to develop effective drugs targeting the PI3K pathway in prostate cancer have, until recently, been disappointing due to a lack of efficacy, unacceptable toxicity, or both.[268,269] Newer approaches focus on combination with androgen-receptor targeting agents to overcome feedback loop mechanisms of resistance. However, pharmacological interactions between agents and overlapping toxicities such as hyperglycemia, stomatitis, infection, pneumonitis and diarrhea have limited early combinations of PI3k-pathway agents.[270,271] Recently, a randomized phase II trial evaluated the combination of abiraterone and ipatasertib (GDC-0068), a small-molecule AKT inhibitor, in 253 mCRPC patients. PTEN loss was associated through immunohistochemistry, FISH and next-generation sequencing (NGS), with adequate concordance between assays. There was no significant benefit in rPFS (the primary endpoint of the trial) in the intention-to-treat population for the combination over abiraterone alone (HR: 0.75; p=0.17). In the subgroup of PTEN-loss patients, however, the combination of abiraterone and ipatasertib did confer a significant benefit in rPFS in combination with abiraterone alone (11.5 vs 4.6 months; HR 0.39; 90%CI: 0.22-0.70). There were no significant differences in OS, time to PSA progression or PSA response rates.[173] The randomized phase III IPATential 150 trial (NCT03072238) is currently ongoing to evaluate the combination in first-line mCRPC.

6.4 DNA Repair Defects

DNA damage repair (DDR) in normal and tumoral cells is a very complex process, with several different mechanisms taking place for the repair of specific lesions occurring in DNA. For instance, single strand breaks are repaired by base excision repair (BER), bulky adducts are repaired by nuclear excision repair (NER), base mismatches via mismatch repair (MMR), and repair of direct damage to bases is generally repaired by the methyl-guanine methyl-transferase (MGMT) enzyme. Double-strand breaks, on the other hand, are dealt with by two different pathways, namely homologous recombination (HR) and the non-homologous end-join (NHEJ) repair pathway.[272]

BRCA1 and BRCA2 are two key tumor suppressor proteins involved in double-strand DNA break repair by homologous recombination. Tumor cells lacking BRCA1 or BRCA2 have been found to be sensitive to pharmacological inhibition of the PARP family of DNA repair enzymes, involved in response to DNA

damage through base excision repair, by a mechanism of "synthetic lethality".[273] By this mechanism, inhibition of PARP causes an increase in DNA single-strand breaks with a subsequent accumulation of double strand breaks at replication forks, which are repaired by the BRCA1 and BRCA2 proteins in normal cells.[274] In cells with inactivating BRCA mutations, lacking a proficient mechanism to repair these double strand breaks leads to cell death, which is selective only in cells without an adequate BRCA protein function.

In prostate cancer, the prevalence of inactivating mutations in DDR genes is of 19% in primary and up to 23% in metastatic prostate cancers.[182,183] These alterations may be present in germline DNA and be inheritable, or as somatic mutations emerging during tumorigenesis. Somatic mutations are present in 20% of mCRCP and 13% of primary prostate cancer; mutational inactivation of the BRCA2 gene, for instance, occurs in 13% of advanced and only 3% of primary tumors. The presence of germline BRCA mutations, especially BRCA2, is associated with a higher risk of prostate cancer, with younger age at diagnosis and a higher proportion of clinically significant tumors.[275]

DDR in prostate tumor tissue has been shown to be a predictive biomarker for the treatment with PARP inhibitors. In the phase II TOPARP-A trial, 50 heavily pre-treated mCRPC patients had their tumor tissue and blood evaluated for alterations in DDR, and treated with the PARP inhibitor Olaparib. Of 49 evaluable patients, 16 (33%) showed a response. 16 patients (33%) had alterations in DDR genes (BRCA1/2, ATM, Fanconi's anemia genes, CHEK2); of these patients, 14 (88%) showed a response to olaparib and prolonged overall survival (13.8 vs. 7.5 months; p=0.05).[46] This led to the breakthrough designation of Olaparib for BRCA1/2 or ATM-mutated mCRPC after treatment with ARSIs and docetaxel. Recently, data from a second phase of the TOPARP trial (TOPARP-B) were presented, in which 98 patients with DDR aberrations were recruited and randomized to two doses of Olaparib (300 mg vs 400 mg twice daily). Response was achieved in 54.3% of 46 evaluable patients in the 400 mg cohort, and 39.1% of 46 evaluable patients in the 300 mg cohort, confirming the significant antitumor activity of PARP inhibitors in patients with DDR alterations.[47] Olaparib has also recently shown superiority over physician's choice treatment in second and third-line mCRPC patients with DDR alterations. In the recently communicated phase III PROFOUND trial, patients with DDR alterations (cohort 1: BRCA 1/2, ATM; cohort 2: other alterations) were compared with the choice of abiraterone or enzalutamide in 387 patients previously treated with an ARSI +/- taxanes. Radiographic PFS, the primary endpoint of the trial, was significantly longer in Olaparib-treated patients (7.4 vs 3.6 months; HR 0.34; p<0.001), as was overall survival (18.5 vs 15.1 months; HR 0.64; p=0.017) and response rate (33.3 vs 2.3%).[48] A number of other PARP inhibitors (Niraparib, Talazoparib, Rucaparib) are currently being evaluated in different clinical trials.

Data from the TOPARP and PROFOUND trials suggest, however, that not all DDR aberrations have the same impact on activity of PARP-inhibitors. In the TOPARP-B trial, while BRCA1/2 and PALB2 mutations

showed response rates of 83.3% and 57.1%, respectively, response rates in ATM and CDK12 mutated-patients were only 36.8% and 25%.[47] In an exploratory analysis of the PROFOUND trial, while a relevant rPFS benefit was observed with olaparib in BRCA2 (10.8 vs 3.5 months) or CDK12 (5.1 vs 2.2 months) mutated patients, no clear benefit was observed in ATM-mutated patients (5.4 vs 4.7 months).[48] These results are in line with previous reports from patient cohorts suggesting ATM-mutated patients may derive a significantly lower benefit from PARP inhibitors.

The presence of aberrations in DNA repair mechanisms has also been proposed as a biomarker of sensitivity to platinum chemotherapy, in particular carboplatin. Other platinum salts, the oral platinum agent satraplatin showed significant antitumor activity, with PSA response in 33% of patients, despite failing to prolong survival in metastatic castration-resistant prostate cancer in a randomized phase III trial.[276] Retrospective next-generation sequencing of patients exhibiting exceptional responses to carboplatin has identified germline and somatic homozygous BRCA2 mutations or copy losses;[277] other case reports have also identified germline ATM mutations in exceptional responders.[278] In a single-institution series, pathogenic germline BRCA2 variants were observed in 8 (5.7%) of 141 patients. Of these eight patients, 6 (75%) experienced a 50% PSA response, compared with 23 (17%) non-carriers ($p < 0.001$).[279]

Finally, germline and somatic DNA repair aberrations could also provide information on the likelihood of response to standard therapy such as hormonal agents (abiraterone, enzalutamide) or chemotherapy. In a recently reported Spanish prospective longitudinal study, where patients received standard therapy, BRCA2 carriers treated with the taxane → abiraterone/enzalutamide treatment sequence had significantly worse cancer-specific survival (28.4 v 10.7 months; HR: 4.16; $p<0.001$) and progression-free survival to second-line therapy (PFS2: 17.1 v 8.6 months; HR, 8.16 $p<0.001$) than noncarriers. There were no differences between outcome between BRCA2 carriers and non-carriers treated with the abiraterone/enzalutamide → taxane sequence.[207] These data suggest first-line abiraterone or enzalutamide could be superior to taxanes as first-line therapy for germline BRCA2 carriers.

6.5 Immune Response Biomarkers

Although prostate cancer was one of the first diseases where an immunotherapeutic agents was approved (Sipuleucel-T),[53] results with novel immune checkpoint inhibitors have been disappointing. In a recently published phase II study, mCRPC patients previously treated with docetaxel and ARSIs reported an objective rate of only 5% in RECIST-measurable patients with PDL1 positive disease, and of 3% in PDL1 negative disease.[280] The combination of anti-PD1 and anti-CTLA4 agents, although modestly increasing response rates, is still significantly lower than other approved agents in that setting. In the recently presented CheckMate 650 trial, the combination of Nivolumab and Ipilimumab was able to induce PSA responses in 17.6% of ARSI-treated, chemotherapy naïve patients and in only 10% of

chemotherapy treated patients.[281] Similarly, data from the IND-232 trial evaluating the anti-PDL1 agent durvalumab alone or in combination with the anti-CTLA4 agent Tremelimumab reported no objective or PSA responses in patients receiving single-agent durvalumab, and a 16.6% PSA response rate in patients receiving the combination.[282] These data suggest an "all comers" approach to immunotherapy will not be successful in advanced prostate cancer, and that biomarkers must be developed to adequately identify patients with a higher likelihood of response.

Mismatch repair alterations have become the most promising biomarkers for immune therapy selection in advanced prostate cancer. In 2015, the FDA issued a tissue-agnostic approval for pembrolizumab in patients with mismatch-repair defects or microsatellite instability based on a study involving 86 patients with MMR deficiencies across 12 different tumor types, including prostate, with an overall radiographic response rate of 53%.[283] In prostate cancer, mismatch repair deficiency has been estimated in up to 8% of advanced tumors, and is associated with higher T-cell infiltration, PDL1 protein expression and immune-cell associated transcripts.[284] MSH2 and MSH6 mutations, associated with Lynch's syndrome, have been associated with hypermutated microsatellite unstable prostate cancers.[285] Although mismatch repair gene mutations have been also associated with aggressive clinical and pathological features, these have also been shown to be sensitive to standard and novel hormonal therapies.[286] In the greatest published cohort of MSI-high/dMMR mCPRC patients, 11 patients were treated with anti-PD1 or anti-PDL1 therapy, with an objetvice response rate of 54.5%; most of these responses were long-lasting and still ongoing.[287] These results suggest the determination of microsatellite stability or mutations in the mismatch-repair pathway could identify the subgroup of approximately 3-10% mCRPC patients that derive a significant clinical benefit from immune checkpoint inhibitors.

Recently, biallelic cyclin-dependent kinase 12 (CDK12)-loss has been shown to define a subgroup of prostate cancers that are associated with an elevated neoantigen burden and increased T-cell infiltration and clonal expansion, which some have suggested could increase the likelihood of response to immune therapy.[288] CDK12 biallelic loss or inactivating mutations have been estimated at around 3-7% of all prostate cancers, and have been associated with a shorter time to metastasis, castration-resistant disease and shorter time to PSA progression on first-line ARSIs.[289] Data on the response to checkpoint inhibitor therapy in these patients is, to date, still lacking.

6. Challenges for the Evaluation of Tissue-Based Biomarkers

The concept of precision oncology has emerged as the promise that the molecular assessment of tumor material will be able to inform on the biology of the disease, providing critical information for the selection of the most appropriate treatment option, or "personalized therapy". This approach has enabled the introduction of specific anticancer agents in other tumor types, such as EGFR tyrosine-kinase inhibitors for EGFR mutant non-small cell lung cancer[290] or monoclonal antibodies targeting HER2 in HER2-amplified breast cancer, which has significantly improved the outcomes of these patients.[291]

In the specific landscape of prostate cancer, analyzing archival tissue samples poses significant challenges:

(1) On one hand, prostate cancer has, in many cases, a long natural history, meaning that tumor material is generally years old when the evaluation of molecular biomarkers may impact outcome. Differences in the processing and storage of FFPE samples may have technical implications for the analysis of these samples in later timepoints.

(2) Treatment received throughout the natural history of prostate cancer, mainly androgen deprivation therapy, exerts selective pressure on prostate cancer cells, which acquire specific molecular alterations when developing resistance. Androgen receptor molecular alterations, for example, are almost non-existing in patients with androgen-deprivation therapy naïve prostate cancer, but may appear in up to 60% of patients with castration-resistant disease.[292] This has significant implications when interpreting archival tumor tissue, since tissue at diagnosis does not present with alterations that will develop as tumors progress on different lines of therapy.

(3) Since not all localized prostate cancers progress into metastatic-castration disease, analyzing molecular alterations in localized prostate cancer specimens may not adequately represent the molecular alterations of the aggressive variants that ultimate progress into the advanced, lethal stages of the disease.

This was illustrated by a recently published report of 61 paired diagnostic and mCRPC metastatic tissue biopsies. The most common finding in mCRPC tissue in compared with diagnostic tissue was an increase in the prevalence of AR mutations and amplifications. Mutations in TP53 in four patients and RB1 in another four patients were detected in mCRPC samples that had not been observed in diagnostic or hormone-sensitive biopsies. On the other hand, alterations in DNA repair genes were not significantly changed in diagnostic vs castration-resistant tissue. Authors concluded that, although diagnostic tissue could be adequate for selection for trials evaluating DNA-repair targeting agents, trials evaluating drugs targeting the TP53/RB1 pathways would probably need evaluation from metastatic tissue.[293]

6.1 Tissue Acquisition from Metastases in Advanced Prostate Cancer

Tissue acquisition in more advanced stages of the disease is also complicated by the fact that most patients develop bone metastatic disease as their main site of metastases. Up to 90% of patients with late-stage disease mCRPC present with bone metastases; up to 50% present with bone metastases as the only site of disease. Furthermore, visceral disease Is known to develop late in the disease, at stages where biomarker evaluation may have a lower impact on potential decision-making, due to the lack of subsequent therapy options. In one study, visceral involvement in patients 24 months prior to death was of only 14%, but increased to 32% and 49% in patients in scans 3-6 and <3 months prior to death, respectively.[294]

Tissue acquisition from bone biopsies in advanced prostate cancer is limited by the following factors:

(1) Low yield of acceptable tumor tissue for molecular analyses
(2) Limitations of technical processing of tissue from sclerotic bone biopsies
(3) Potential morbidity of the procedure

Bone Marrow Biopsy Procedure

Bone marrow biopsies are performed to obtain tumor material for diagnosis and for the assessment of molecular biomarkers, generally within clinical research protocols. The posterior iliac spine is the usual site, although the anterior iliac spine can also be used. A trephine specimen is obtained by inserting the biopsy needle into the bone and using a to-and-fro rotation to obtain a core of tissue.[295] Bone marrow biopsy is generally a safe procedure, with serious adverse events in fewer than 0.05% of procedures.[296] The most common complication is bleeding, which can lead to significant morbidity, such as “gluteal compartment syndrome,” and very rarely death.[297] Bleeding is more often related to impairment of platelet function than to thrombocytopenia or a coagulation factor defect. Despite the potential risk of complications, patient anxiety in relation to the performance of metastatic biopsies for clinical research has been shown to be lower than that anticipated by medical oncologists. A risk of major biopsy complication was reported to be acceptable by as many as 22% of patients.[298]

6.2 Challenges for Tissue Acquisition and Analysis from Bone Marrow Biopsies

Can the output of tissue from bone biopsies be increased, in order to increase the yield of the procedure? When bone marrow biopsies are performed without imaging guidance (that is, “blinded” biopsies), a low yield of bone marrow positivity can be expected. For instance, in a retrospective analysis of the CALGB Study 9663, unguided bone marrow biopsies of the posterior iliac crest performed in an office based setting from 184 patients were positive in only 47 (25.5%).[6] In these patients, clinical parameters such as lower hemoglobin, greater alkaline phosphatase, and greater lactate dehydrogenase (LDH) levels were able to identify patients with an increased probability of a positive biopsy.[6]

Imaging guidance, with *a priori* detection of bone metastases either from bone scintigraphy, CT or MRI scans, has been shown to increase the rate of bone marrow positivity. In a retrospective cohort of 57 abiraterone-treated patients that underwent trans-iliac bone marrow biopsies in areas that had shown bone metastases by bone scintigraphy, 27 (47%) of patients had a positive procedure.[7] In another study in 54 patients, CT-guidance was able to increase the yield of positive bone marrow biopsies (evaluated with hematoxylin-eosin staining) in up to 67% of patients. In this study, differences in bone density parameters on pelvic CT scans (Hounsfield units [HUs]), indicating sclerotic bone reaction associated with malignant infiltration, was associated with a higher positive yield. [299] Imaging by MRI could also be useful to increase bone biopsy yield. In a report by Perez-Lopez and colleagues, forty-three bone marrow biopsies from 33 metastatic CRPC (mCRPC) patients with multiparametric MRI and documented bone metastases were evaluated. Additionally, 10 patients with no bone metastatic disease were also evaluated. 31 (72.1%) of 43 biopsies from metastatic patients had detectable cancer cells. Different MRI parameters were associated with biopsy positivity; apparent diffusion coefficient (ADC) was significantly lower and median normalized diffusion-weighted imaging (nDWI) signal was significantly higher in biopsies with tumor cells versus nondetectable tumor cells. When analyzing tumor cellularity an inverse correlation with ADC and a positive correlation with nDWI signal ($p < 0.001$) was observed, highlighting the potential role of functional MR imaging to guide biopsies to sites with the highest cellular density.[300]

In addition to the intrinsic difficulty of obtaining tumor tissue from sclerotic bone metastases, processing of the obtained tissue has traditionally been challenging due to the scarce tumor material obtained and the need for specific processing protocols, such as decalcification, making the tissue often inadequate for molecular analyses.[301] For instance, biopsies for adequate RNA sequencing require a high tumor content with low contamination from stromal cell content for a high yield of cancer RNA.[302] In recent years, technological advances in the processing of tissue from bone biopsies has enabled the performance as a valid approach for molecular biomarker analysis in these patients.[298] Whole exome sequencing has been reported to be feasible in patients from bone biopsies, with mean target sequencing coverage was 145-fold and 88% of territory covered at ≥ 30-fold, results that meet standard quality criteria.[303] In larger series, tissue processing protocols consisting in formalin fixation with a decalcifying agent for diagnosis, followed by freezing of bone marrow and blood clots, cutting of frozen slides and tissue macrodissection for DNA and RNA extraction after tumor purity have been described. Using these protocols, as many as 81.7% positive bone biopsies were able to produce successful whole-exome sequencing procedures; although only 33.3% were adequate for additional RNA sequencing. Taken together, 70% and 28.6% of all biopsies performed were suitable for WES and RNA sequencing studies, respectively.[304]

These advances have enabled the design of large, prospective biomarker studies for the discovery of molecular biomarkers based on metastatic tissue analysis from prostate cancer patients;[183] this has led,

among others, to the discovery of DNA-repair deficiencies as predictive biomarkers of PARP inhibition efficacy.[46,47] In the Stand Up To Cancer prospective, multicenter collaboration study, metastatic tissue from 187 mCRPC patients was prospectively collected form complete integrative clinical sequencing (whole-exome, matched germline, and transcriptome data); sequencing was feasible in 150 patients (80.2%) with biopsies with >20% tumor content. Of the analyzed tissue, approximately 29% biopsies were obtained from metastatic bone.[183]

Table 6. Summary of Studies Evaluating the Yield of Bone Marrow Biopsies

Study	N	Imaging	% positive biopsies	Comments
McKay et al.[305]	39	CT-guided	30 (77%)	-
Spritzer et al.[299]	54	CT-assessed*	36 (67%)	Only 39% with adequate tissue for RNA profiling
Ross et al.[6]	184	No guidance	47 (25,5%)	-
Efstathiou et al.[7]	57	Not reported	27 (47%)	25 (44%) patients with ≥5% tumor infiltration in biopsies
Lorente et al.[306]	115	CT-assessed*	75 (62.5%)	55 (47.8%) biopsies with ≥ 50 cells

7 Liquid Biopsies in Advanced Prostate Cancer

7.1 What are liquid biopsies?

As discussed previously, the molecular characterization of prostate cancer has traditionally been performed using nucleic acid material (DNA or RNA) from tissue obtained from either tumor material from an original prostate biopsy, or a biopsy from a metastatic site. Recent advances in drug development suggest data from next-generation sequencing of tissue can be relevant in the decision, for instance, on whether a patient will be a candidate to receive treatment with PARP inhibitors, or potentially receive a combination of abiraterone and an Akt inhibitor.[173]

In recent years, awareness has grown on the presence of tumor-related material, either in the form of circulating tumor cells, circulating nucleic acids (DNA or RNA) and tumor vesicles (exosomes), that coexist with similar material from our normal, non-tumoral cells in the blood stream. Due to the rapid growth and turnover of tumor cells, there is a constant source of tumor genomic material entering the bloodstream. It is now feasible to detect and isolate both tumor cells and tumor nucleic acids for genomic analysis, a possibility that has enabled the repeated molecular assessment of tumor material in a wide range of cancers. The term “liquid biopsy” therefore refers to the possibility of evaluating the molecular landscape of solid tumors via analysis of blood samples.[307]

In prostate cancer, the isolation of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) from patients with prostate cancer has enabled a better understanding of the mechanisms driving disease progression and resistance to treatment.[308] Although tissue biopsies generally enable a much higher content of tumor material, which may be relevant for some of the molecular techniques used, liquid biopsies may provide a much more general picture of the molecular tumor landscape, taking into account the molecular heterogeneity of the disease. This is due to the assumption that, although different metastatic sites may present different genomic alterations, those that are driving disease progression and dissemination will be represented in the bloodstream and will be detected by liquid biopsies, and will be more relevant for choosing the right therapeutic agent.

Additionally, liquid biopsies offer other advantages over tissue biopsies, namely:

(1) Liquid biopsies enable serial assessments, and enable the dynamic changes in the molecular landscape that emerge as tumor cells adapt to the different therapeutic agents.
(2) Liquid biopsies are non-invasive, with less morbidity than tissue biopsies
(3) Drawing blood is faster and less resource-consuming than tissue biopsies.

The generalization of liquid biopsies in academic research has provided invaluable insight on the mechanisms of disease progression for the development of novel targeted agents. Additionally, these

may also provide an indirect measurement of tumor burden, and changes after treatment may indicate response or progression. In advanced prostate cancer as discussed below, the assessment of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) have been extensively studied in recent years.

7.2 Circulating Tumor Cells (CTCs)

CTCs are extremely rare cells present in the blood in an estimated frequency of one in a few million blood cells, originating from shedding from the original tumor. CTCs can be isolated from prostate cancer patients in order to obtain tumor material for the determination of prognostic or predictive molecular biomarkers, or may be used as a measure of tumor burden for the follow-up of patients on treatment and the determination of response and progression to treatment.

CTC detection and isolation

The most common approach for isolation or enrichment of CTC is based on immune-magnetic systems; samples are incubated with specific antibodies to select certain cell populations and are afterwards separated by magnetic means. This may be performed through positive selection (by conjugation with antibodies against epithelial cell adhesion molecules [EpCAM], expressed in most CTC and not in other blood cells) or negative selection (using antibodies against leukocyte-expressed antigens such as CD45). The CellSearch System (Menarini Biosystems) is, to date, the only FDA-cleared technology for quantification of CTCs, with demonstrated clinical relevance in breast, prostate, and colorectal cancer, and has become the standard comparator for any novel platform in development.[309]

The CellSearch System is based on an automated immuno-magnetic enrichment and staining system: anti-EpCAM and anti-creatinine kinase (CK) antibodies are used for positive selection, complemented by counterstaining with anti-CD45 antibodies to discard leukocytes. The final step of any quantitative analysis also requires an image-based system with the input from a human operator to identify CTCs among the selected cells. Widely accepted characteristics to define a CTC include:

- Round to oval morphology
- Size greater than 5 μm
- Visible nucleus (4,6-diamidino-2-phenylindole positive)
- Positive staining for cytokeratin 8, 18, and/or 19 (phycoerythrin)
- Negative staining for CD45 (allophycocyanin)

Alternative methods have been established for the isolation and characterization of CTCs. Although CellSearch is the only FDA cleared method for CTC enumeration, isolation by other methods is still valid for molecular characterization. Other platforms, such as the EPIC HD-CTC platform, avoid the enrichment step; all nucleated cells are retained, and the selection process is performed on all cells.[310] Other platforms, such as the AdnaTest, widely used for splice variant analysis, is based on the detection

of prostate cancer-associated RNA transcripts through reverse-transcriptase polymerase chain reaction (RT-PCR).[311] Functional assays, such as the EPISPOT assay are based on the detection of proteins secreted by CTCs, and can potentially specifically differentiate viable cells from apoptotic ones.[312] It is important to note that CTCs that are detected by different methodologies may have completely different clinical and biological significance. This was illustrated by a recently published study, where 202 mCRPC patients had CTCs detected by the CellSearch and AdnaTest assays. Although AR-V7 positivity (AdnaTest) was associated with a higher CTC count (CellSearch) there were cases with undetectable CTCs by CellSearch with positive AR-V7, and vice versa. There was only a modest correlation between CTC enumeration by the CellSearch and AdnaTest assays (Pearson's coefficient: 0.52 [95%CI: 0.32-0.68]; $p<0.001$).[313]

Molecular Characterization of Circulating Tumor Cells

The qualitative assessment of CTCs at genomic and proteomic levels can provide insight into the biology of the disease, with applications in diagnosis, staging, biomarker discovery, and individualization of treatment. There is a wide variety of genome- and protein-based assays that can be performed on CTCs, including immunohistochemistry, immunofluorescence, gene copy number analysis via comparative genomic hybridization (aCGH), genome sequencing analysis, and epigenetic studies.

Different molecular biomarkers with prognostic value have been assessed in prostate cancer patients. In one study, Attard and collaborators isolated CTCs with the CellSearch system and performed FISH to determine the presence of ERG rearrangements, PTEN loss and AR status, and compared results with FISH performed in diagnostic tissue. Of 31 patients with both blood and tissue available, there was coincidence in ERG status in the CTC and diagnostic tissue of all patients, which contrasted with the heterogeneity observed in PTEN loss and AR copy number gain. Authors also reported a significant association between ERG status in CTCs and the magnitude of PSA decline on treatment with abiraterone.[314] PTEN status (determined by FISH) in CTCs (EPIC Platform) and archival and fresh tissue has also been compared in a cohort of 48 mCRPC patients. Authors reported a higher concordance between CTCs and fresh (metastatic) biopsies (84%) than with archival tissue (62%), suggesting PTEN loss may be a later event, that may be not captured when analyzing archival tissue.[267]

Additionally, whole exome sequencing (WES) of single CTCs is now feasible, and may have different applications. Low coverage WES may be useful for an easier identification of copy number alterations and chromosomal abnormalities across the genome,[315] while higher depth of coverage, while more expensive and time-consuming, may identify structural variations in CTC genomes that are highly concordant with matched tissue.[316] Furthermore, RNA sequencing[317] has also been reported from CTCs as well as methylome analysis,[318] emphasizing the potential of CTCs as the source of tissue for a comprehensive analysis of molecular alterations potentially driving tumor growth and resistance.[319]

Arguably, the most extensively studied biomarker in CTCs has been the androgen receptor. The detection of splice variants of the androgen receptor, as discussed extensively in Section 5.1, has been evaluated in multiple cohorts with different methodologies, and is arguably one of the most promising biomarkers in advanced prostate cancer clinical development nowadays. Other AR-related biomarkers, however, have been detected in CTCs, with potential prognostic and predictive values. One of the advantages of molecular characterization of CTCs over other techniques such as circulating tumor nucleic acids is the fact that nuclear localization of the AR in CTCs may indicate functionally active protein. In a cohort of 48 patients progressing on abiraterone and enzalutamide, CTCs were analyzed to determine AR expression; nuclear AR expression was maintained in both CTCs and metastatic tissue biopsies, confirming the maintained functionality of the AR in progressing patients.[320] In prospective clinical trial evaluating 63 taxane-treated patients, decrease in the percentage of nuclear localization of the AR after 1 week of treatment was associated with higher PSA response rates at cycle 4.[235]

The molecular characterization of CTCs may also be applied in early drug development as a pharmacodynamic (PD) biomarker. This can be performed by characterizing drug effects on CTC membrane antigens, or the selective reduction of genetically distinct subpopulations of CTCs.[321] For example, the detection of γ-H2AX, a biomarker of nuclear DNA double-strand break, has been incorporated into early phase clinical trials evaluating combinations of PARP inhibitors and chemotherapy.[322] As these agents increase DNA damage, an increase in the percentage of γ-H2AX-positive CTCs may be considered an indicator of drug effect, and may be used to determine the optimal biologically effective dose.[323] Other markers such as RAD51, phosphorylated histone H3, insulin-growth factor (IGF)-IR expression in CTCs have also been used as PD biomarkers in early phase clinical trials with prostate cancer patients.[324,325]

CTC Enumeration as a Prognostic, Response and Surrogate Biomarker

The value of CTCs as a prognostic and predictive biomarker has been validated in studies across multiple cancer types. The initial clinical validation of CTC enumeration was based on parallel studies evaluating advanced colorectal, breast and prostate cancer patients. In all three studies, a high level of CTCs as determined by the CellSearch system was associated with worse outcome, although the threshold for defining an “adverse” CTC count was different for each of the tumor types.[326]

The IMMC38 study was the first to evaluate the prognostic and predictive role of CTC enumeration using the CellSearch System in CRPC patients. In this study, 164 patients starting a new cytotoxic chemotherapy regimen were eligible and CTC counts were determined in 3- to 4-weekly intervals. An un-favorable pretreatment count (defined as ≥ 5 CTC in 7.5 mL of blood) predicted a worse overall

survival (OS) than a favorable count (< 5 CTC in 7.5mL of blood) after adjusting for known prognostic factors in multivariate analysis.[327] Subsequently, analyses from large, randomized phase III trials such as the COU-AA-301, AFFIRM, MAINSAIL or SWOG S0421 trials have confirmed the prognostic value of baseline CTCs in mCRPC patients.[29,328–330] Alternatives to the favorable/unfavorable cut-off points have also been explored. In one single center study evaluating 119 mCRPC patients, a significant difference in OS was found in patients with CTCs < 5 / 7.5mL, CTCs 5-50 / 7.5mL and patients with CTCs > 50 / 7.5 mL.[331] The absolute baseline CTC count, evaluated as a continuous variable, has also been found to be significantly associated with overall survival.[332] Current PCWG3 guidelines now recommend the assessment of baseline CTC counts, categorized as favorable or unfavorable, in the design of clinical trials for mCRPC.[44]

The association of the change in CTC counts and outcome, and how these compare to currently used biomarkers, have also been extensively reported. Initial studies on the IMMC-38 trial reported how the "conversion" from an unfavorable to a favorable count was associated with improved outcome and, conversely, how a conversion from favorable to unfavorable counts was associated with worse overall survival. Patients that maintained a favorable count at all draws had the longest OS (26 months), followed by those that converted from unfavorable to favorable (21.3 months), those that converted from favorable to unfavorable (9.3 months), and those that maintained an unfavorable count (6.8 months).[327] In that same trial, the log-change in CTCs was also found to be associated with survival.[332] In comparison with PSA response, CTC counts showed a significantly higher receiver operating curve (ROC) area under the curve (AUC) (81.5 vs 67.5%).[327] These data led to the incorporation of CTC conversions as an endpoint in phase II clinical trials. For instance, CTC conversion was part of the composite response definition in the TOPARP-A trial, and was crucial for meeting pre-specified criteria of response that were met, leading to the accelerated approval of Olaparib after the association of response with DNA repair defects was established.[46]

In most studies evaluating CTC enumeration as a response biomarker, analysis has been restricted to the value of a conversion from unfavorable to favorable counts. This approach has the disadvantage of rendering patients with baseline favorable CTC counts non-evaluable. CTC counts are known to increase with subsequent lines of therapy, which means a significant number of patients may be excluded from response assessment in first-line mCRPC trials. In the ELM-PC4 trial, evaluating orteronel as first-line mCRPC therapy, only 39% of patients presented with unfavorable baseline CTC counts and were therefore evaluable for CTC response assessment.[333] For this reason, alternative thresholds for response have been evaluated. In early studies, a 30% decline in CTC counts was associated with increased survival; this increased survival could be observed as early as 4 weeks post-treatment initiation, with the potential for earlier identification of responders than PSA, which requires at least 12 weeks of treatment before results are evaluable.[331] A recent study evaluated different CTC-based response endpoints in a pooled analysis of five prospective randomized trials with a total of 6,081 patients. CTC conversions,

CTC0 (conversion from ≥ 1 CTC to 0 CTCs) and 30%, 50% and 70% CTC declines were evaluated; 30%, 50% and 70% PSA declines were also evaluated. CTC-based endpoints were found to be consistently superior to PSA response endpoints; of the CTC-based endpoints, CTC0 and CTC conversion were found to have the highest discriminatory power for overall survival. Using the CTC0 endpoint, 75% of patients were eligible for response, as compared with only 51% with the CTC conversion endpoint.[333]

The value of CTCs as a surrogate of overall survival in mCRPC has also been reported. If approved by regulatory agencies, a surrogate biomarker may substitute overall survival as an endpoint that is accepted for regulatory drug approval. As discussed previously, the candidate biomarker must meet the stringent Prentice Criteria, not only in one dataset but in a number of large prospective trials, and a meta-analytic approach must prove surrogacy at the trial level as well as at individual level.[28] In an analysis of the COU-AA-301 trial, a biomarker model composed of CTCs (≥ 5 CTC vs. < 5 CTCs in 7.5 mL of blood) and LDH (normal vs. abnormal) at the 12-week landmark time point was reported to fulfill all Prentice Criteria at the individual level. The model exhibited a high concordance index (0.81), with 2-year survival probability of 46% in low risk (CTCs < 5), 10% in intermediate risk (CTCs ≥ 5 and LDH ≤ 250 U/L) and 2% in high (CTCs ≥ 5 and LDH ≥ 250 U/L) risk patients, respectively.[29] Proof of surrogacy at the trial level has, unfortunately, not been proven to date. If future analysis of ongoing clinical trials confirm these results drug approval could be based on CTC endpoints, increasing the efficiency and reducing the costs of developing therapeutic agents, and eliminating the bias in OS from subsequent therapy.

7.3 Circulating Tumor DNA (ctDNA)

Cell-free circulating nucleic acids have been known to be present in our blood stream for the past 70 years,[334] in the form of fragments of varying size. Cell-free nucleic acids originating from non-cancer cells are found in low concentrations in healthy individuals, and can increase after, for example, exercise or traumatisms.[335] One of its most widespread and early applications is the detection of fetal aneuploidy for the screening of trisomy 21 in pregnant women at risk.[336] In patients with cancer, circulating DNA is present in higher concentrations, a proportion of which is of tumoral origin; the proportion of circulating tumor DNA (ctDNA) to total cell-free DNA (cfDNA) is known as the tumor DNA fraction.

Cell-free nucleic acids are known to enter the circulation through several mechanisms, although the process is not completely understood to date. A large proportion originate from the digestion of apoptotic cells by phagocytes and macrophages, remains of which are shed into the blood. Apoptosis is known to be the main source of circulating DNA from both normal and tumor cells; however, other processes such as necrosis has also been shown to contribute to the shedding of tumor DNA into the circulation.[337] Fragments of tumor derived cell-free DNA have been found to be smaller in size than those fragments originating from normal cells. In melanoma patients, for instance, the BRAF V600E

mutant allele occurred more commonly at a shorter fragment length than the fragment length of the wild-type allele in healthy volunteers (132-145 vs. 165 base pairs).[338]

Fragments of tumor-derived cell-free DNA (ctDNA) can be isolated for molecular characterization, providing a means to profile the molecular characteristics of a tumor, with broad potential clinical applications. For example, EGFR mutation detection in plasma ctDNA has received regulatory approval for the selection of patients with advanced non-small cell lung cancer harboring EGFR mutations for the treatment of EGFR tyrosine-kinase inhibitors in clinical practice.[307] In prostate cancer, however, no assay has undergone the analytical and clinical validation required for regulatory approval, although a number of different studies are underway. Circulating tumor DNA analysis in advanced prostate cancer, despite its widespread potential applications, remains therefore investigational to date.

Sequencing of Circulating Tumor DNA in Advanced Prostate Cancer

Circulating tumor DNA can be analyzed by different sequencing methods in order to extract relevant information on tumor biology. Despite the technical advances, challenges still remain. For instance, in comparison with the sequencing of tissue samples, the low quantity of tumor DNA (usually, 10-20 ng/mL) and the variable tumor DNA fraction, which can range from < 1% to > 90%, may limit detection accuracy. The quantity of ctDNA is associated with certain clinical characteristics such as the overall metastatic burden, sites of metastases and tumor biology; certain tumor types (and certain histopathologic subtypes within tumor types) maybe more likely to “spill” nucleic acids into the circulation. This is relevant when, for instance, designing assays for the early detection of relapse, where low ctDNA fractions will require highly sensitive assays,[339] which may not be necessary in late stages of advanced disease, with higher ctDNA fractions. Other limitations, such as the difficulties for determining sub-clonal architecture (i.e. whether ctDNA originates from one or another metastatic site) or the variable contribution of normal and tumoral circulating DNA, which may induce significant intra-patient variability. In prostate cancer, the lack of recurrent hot-spot mutations represents an additional challenge for the estimation of tumor fraction; in other tumor types, the allelic frequency of a common and recurrent hot-spot point mutation is generally used to establish the proportion of tumor DNA.[340] Prostate cancer, however, is characterized by copy number alterations (amplifications, deletions), but only a limited number of mutations. Quantification of a panel of early genomic changes, such as ETS rearrangements or NKX3.1 deletions, the use of mutation calls from broad next-generation sequencing, or the use of genome-wide copy number aberrations are strategies that have been proposed to estimate the tumor fraction in prostate cancer.[341]

Different technologies have been used for the analysis of ctDNA. Most current tests rely on specific, pre-defined targets such as limited NGS panels restricted to certain hotspot mutations or targeted approaches, such as BEAMing or droplet digital PCR (ddPCR),[341] for the determination of mutations or

copy number aberrations or mutations of specific genes. The number of alterations that can be determined by these methods are, however, limited. On the other hand, whole exome or genome next-generation sequencing approaches may provide information on the general genomic landscape, but are less sensitive than targeted approaches, as well as a higher cost. Analysis of epigenetic modifications are also feasible; recently, methylation of the SGTPI gene has been shown to be prognostic in advanced prostate cancer.[342]

Molecular Characterization of Circulating Tumor DNA

Circulating tumor DNA is an invaluable source for genomic material to interrogate the molecular alterations driving resistance in prostate cancer patients. In several studies, an acceptable concordance between alterations in ctDNA and metastatic tumor tissue has been reported. In one study, whole-exome sequencing of cfDNA was feasible in advanced breast or prostate cancer patients with a cfDNA fraction ≥10%; authors reported good concordance of somatic mutations, number alterations and mutational signatures with matched tumor biopsies. WES was feasible, however in only 34% of 520 screened patients.[343] In another study, a targeted sequencing approach of patients with a cfDNA fraction > 2% enabled evaluation of 75.6% of 42 mCRPC patients. All somatic mutations were found to be present in matched metastatic tissue, with an 88.9% concordance in clinically-actionable copy number alterations. Tumor DNA sequencing, on the other hand, was able to identify alterations in the AR, WNT or PI3k pathways not present in matched tumor biopsies, highlighting how liquid biopsies may overcome limitations due to tumor heterogeneity.[344] In a subsequent analysis of a randomized clinical trial, evaluating first-line abiraterone vs enzalutamide, both approaches (a targeted 72-gene panel and a whole exome sequencing approach) were performed; all somatic mutations detected with the panel were confirmed by exome sequencing, with highly concordant allele fractions with both approaches.[345]

AR gene aberrations are amongst the most studied alterations in cell-free DNA. The presence of AR copy number gain or AR mutations has been associated with adverse prognosis in mCRPC patients, although other aberrations such as TP53 mutations or Rb1 deletions have been shown to provide greater efficacy in identifying patients with an adverse prognosis.[345,346] Additionally, data suggest the presence of AR gene aberrations could provide information as to the likelihood of response to novel hormonal agents vs taxanes as first-line therapy. In the PREMIERE trial evaluating 94 mCRPC patients treated with first-line enzalutamide, AR amplification detected by ddPCR was associated with a significantly worse PSA-PFS (HR 4.3; p<0.001), rPFS (HR: 8.1; p<0.001) and OS (HR 11.1; p<0.001).[231] In a subsequent study evaluating plasma AR status in 163 docetaxel-treated mCRPC patients using the same methodology, AR amplification was associated with worse OR (HR 1.6; p=0.018) but no differences in PFS (HR: 1.04; p=0.8) or PSA response (OR: 1.14; p=0.7). When incorporating data from first-line abiraterone/enzalutamide-treated patients, a significant interaction for AR-plasma status in first-line mCRPC was observed, favoring abiraterone/enzalutamide in AR-normal patients (HR: 1.93; p=0.008) and docetaxel in patients

with AR amplification (HR: 0.53; p=0.11).[232] Similar results have been observed in patients treated with second, or third-line cabazitaxel, favoring cabazitaxel in patients with AR amplification.[233] CfDNA analysis has also been shown to be able to identify patients with somatic hypermutation, associated with mismatch repair deficiency, microsatellite instability and deletions in the MSH2, MSH6, or MLH1 genes.[347] In this small (3.7%) group of adverse prognosis-patients, treatment with the programmed-death-1 (PD1) inhibitor pembrolizumab has been associated with durable tumor responses, and is now FDA-approved.[348,349]

Sequential analysis of cell-free DNA alterations may also provide insight into mechanisms of resistance to therapy, and potentially identify new targets that can guide treatment selection in subsequent lines. In a study of 80 abiraterone-treated patients, the emergence of new T878A or L702H AR mutations were found in up to 13% of patients;[350] these mutations are associated with promiscuous activation by cortisone which is given concomitantly with abiraterone,[36] providing a potential mechanistic explanation for responses observed after a switch from prednisone to dexamethasone in patients progressing on abiraterone.[351] An AR F876L mutant variant, which has been shown to be activated by the AR antagonists enzalutamide and apalutamide, has been identified as the driver of progression in cfDNA of patients treated with these agents.[229,230] Molecular characterization of cfDNA of patients progressing on the PARP inhibitor Olaparib have identified novel reversion mutations in the BRCA-gene, which restores the function of the BRCA protein and confers resistance to Olaparib and Talazoparib.[352,353] CfDNA WES was able to identify multiple clones with different previously undetected mutations all resulting in reversion of the BRCA2 reading frame to normal, highlighting the multiclonality of PARPi resistance mechanisms.[353]

Circulating Tumor DNA Quantification as a Measure of Tumor Burden

In a similar manner to CTC enumeration, the quantification of ctDNA, either as an absolute value or as the circulating tumor fraction, has been associated with overall tumor burden and evaluated as a prognostic factor in mCRPC. Circulating tumor fraction has been associated with known clinical biomarkers of adverse prognosis, such as the number of bone metastases, alkaline phosphatase, hemoglobin, but not with baseline PSA.[354] The ctDNA fraction has been described as an adverse prognostic factor, as a continuous variable but also when classified into < 10%, 10-30% and ≥ 30% categories;[345,346] similarly, an absolute ctDNA concentration ≥ 2 ng/mL is also an indicator of adverse prognosis. For this reason, the potential bias associated with baseline ctDNA fraction and total ctDNA concentration (which are associated with a higher likelihood of detection) must be always taken into account when assessing the value of a molecular biomarker.

Changes in cfDNA concentration after treatment may also be used as indicator of response or progression to treatment. In a combined analysis of 571 patients treated with taxanes as first-

(FIRSTANA trial) or second- (PROSELICA trial) line therapy, absolute decline in cfDNA concentration was associated with PSA response.[355] In the TOPARP trial, a 50% decline in cell-free DNA as early as 8 weeks after treatment was associated with improved rPFS and OS. Furthermore, serial follow-up of certain clones during treatment were reported to anticipate disease progression, with some alterations increasing their allele frequency in parallel to an increase in total cfDNA concentrations, indicating the development of specific mechanisms of resistance.[353] The rapid decline in cfDNA levels after treatment may also have important implications, especially in metastatic hormone-sensitive disease, where initial response to androgen deprivation is almost universal; median ctDNA fractions have been reported to drop to a median 1% as early as 22 days after ADT, which may limit the applicability of the test if not determined prior to therapy initiation.[356]

7.4 Which should be the test of choice?

In summary, the enumeration and characterization of CTCs and the genomic profiling of cfDNA can provide invaluable information on the biology and burden of the disease, that can be used to estimate prognosis, determine predictive biomarkers for therapy selection, evaluate response and identify mechanisms of resistance to therapy. Each of the approaches has advantages and drawbacks that must be taken into account. Analysis of circulating tumor DNA may be cheaper to analyze, especially with targeted approaches such as hotspot panels or ddPCR; furthermore, sufficient material may be available for analysis (tumor fractions > 2%) even in patients with undetectable CTCs. On the other hand, CTCs may yield additional information (for example, specific protein or RNA assays, nuclear localization, cell culture) and may represent the “true” disseminating cells, in contrast with cfDNA, which may be shed by dying tumor cells.[319] CTCs, however, are more expensive to capture, and it is unclear whether benefit will overcome the potential cost associated to its use in the everyday clinical setting, while cfDNA assays are current state-of-the art in other malignancies such as advanced non-small cell lung cancer.

Some authors have argued that combining both techniques may yield complementary information on the biology of the disease. For instance, splice variants could result from AR amplified cases where an increased transcriptional activity could lead to the increase of truncated variants from defective protein translation. In a study that evaluated both WGS and targeted sequencing of the AR in ctDNA, and AR splice variants in CTCs, authors were able to identify additional aberrations of the AR in poor responders that were AR-V7 negative.[357]

In conclusion, additional studies are necessary to define the exact role of liquid biopsies in advanced prostate cancer. Assay validity, with well-designed studies that reflect adequate pre-analytical and analytical validity, correctly reflect the biology of the disease and produce clinically relevant information that leads to improved outcomes will be necessary. To date, only CTC enumeration with the CellSearch

platform has received FDA clearance for CTC enumeration; other assays for the molecular characterization of CTCs or cfDNA will require further evidence before they are “ready for prime time”.

Hypothesis and Objectives

Hypothesis

The development of improved biomarkers for an improved prediction of the risk of death (prognostic biomarkers), of the likelihood of clinical benefit from each specific therapeutic option (predictive biomarkers) or the assessment of response or progression to treatment (treatment-response biomarkers) is one of the greatest challenges faced by clinicians treating advanced prostate cancer today. We have developed specific hypothesis around three relevant aspects of the study of biomarkers in advanced prostate cancer:

(1) Tissue-based biomarkers
(2) Circulating biomarkers
(3) The use of available biomarkers by specialists treating advanced prostate cancer.

We have developed specific hypotheses for each of the aforementioned aspects:

(1) Tissue based biomarkers:

    We hypothesize that the use of imaging techniques may improve the yield of bone marrow biopsies with sufficient tumor material for genomic analyses in patients with advanced prostate cancer and bone metastatic disease.

(2) Circulating biomarkers:

    We hypothesize that novel circulating biomarkers (circulating tumor cells, the neutrophil-lymphocyte ratio) may improve the estimation of prognosis and the assessment of benefit (response or progression) to treatment, in comparison to current clinical biomarkers.

(3) Clinical use of available biomarkers:

    We hypothesize that awareness and adoption of currently available consensus recommendations in daily clinical practice by physicians specialized in advanced prostate cancer care is insufficient and inadequate.

Objectives

Based on the central concepts of the project, and the general hypotheses previously described, the general and specific objectives of the project include:

Main Objectives

(1) Tissue based biomarkers: to determine which factors are associated with a positive bone marrow biopsy in advanced prostate patients with bone metastatic disease.

(2) Circulating biomarkers:

   a. Circulating tumor cells: to determine the prognostic value and role as a treatment response biomarker of circulating tumor cell enumeration in advanced prostate cancer patients treated with abiraterone in the COU-AA-301 phase III clinical trial.

   b. Lymphocyte-to-neutrophil ratio: to determine the prognostic value and role as a treatment response biomarker of the neutrophil-to-lymphocyte ratio in advanced prostate cancer patients treated with cabazitaxel or mitoxantrone in the TROPIC phase III clinical trial.

(3) Use of Clinical Biomarkers: to determine patterns of use of biomarkers in clinical practice, and adaptation of clinical practice to available clinical guidelines (PCWG guidelines).

Specific Objectives

(1) Tissue based biomarkers

- Association of clinical, blood and radiographic biomarkers with a positive result (presence of any number of tumor cells, or presence of > 50 tumor cells) of a bone marrow biopsy.
- To develop a “score” based on parameters based on blood tests and radiographic imaging, to identify patients at a higher likelihood of obtaining a positive result.
- Validation of the score in an independent dataset of mCRPC patients with bone metastatic disease.

(2) Circulating biomarkers

   a. Circulating Tumor Cells

      - To determine the association of baseline CTC counts with overall survival, progression-free survival and response rates in patients treated in the COU-AA-301 trial
      - To compare the prognostic performance of baseline CTC counts with other clinically available clinical biomarkers.

- To determine the association between the decline in CTC counts after treatment initiation after 4, 8 or 12 weeks of treatment initiation with overall survival, progression-free survival in patients with baseline CTCs ≥ 5 cells/7.5 mL.
- To determine the association between an increase in CTC counts after treatment initiation after 4, 8 or 12 weeks of treatment initiation with overall survival, progression-free survival in patients with baseline CTCs < 5 cells/7.5 mL.

b. Neutrophil-to-Lymphocyte ratio

- To determine the association of baseline NLR with other clinical variables.
- To determine the association between baseline NLR with overall survival, progression-free survival and response rates to determine the association between changes in NLR after 12 weeks of treatment with overall survival, progression-free survival and response rates

(3) Use of Clinical Biomarkers

- To evaluate the physician preferences on the use of clinical available biomarkers in contemporary prostate cancer care.
- To evaluate pattern of clinical decisions in different clinical scenarios in advanced prostate cancer.
- To determine the proportion of physicians that are familiar with recommendations summarized in the PCWG2 guidelines.

Material & Methods

Objective I. Tissue based biomarkers

To determine factors associated with a positive bone marrow biopsy in advanced prostate patients with bone metastatic disease.

Patient Population

Patients with mCRPC who undergone a bone marrow biopsy (BMB) from October 2011 to November 2014 at the Royal Marsden National Health Services Foundation Trust (Sutton, UK) were retrospectively identified. The criteria for inclusion in the present study were CRPC, age ≥ 18 years, and evidence from imaging studies (CT, bone scan, or magnetic resonance imaging) of bone metastases from prostate cancer. Patients with a CT scan of the pelvis performed > 6 weeks before the biopsy were excluded. The clinical and imaging parameters were retrospectively collected from the electronic patient records. All patients provided informed consent before undergoing biopsy. The method for image acquisition (CT scanner) remained consistent throughout the study.

Tissue Acquisition and Analysis

Tissue was collected using a bone trephine biopsy from the right or left posterior iliac crest. No image guidance was used for tissue acquisition. Biopsies were performed using 8-gauge (3.05-mm) needles. The biopsy specimens were sealed in a container with a 10% parafilm solution and fixed at room temperature for 24 to 30 hours with agitation. After fixing the samples, they were briefly rinsed in distilled water, placed in a container of ethylenediaminetetraacetic acid (EDTA) solution, sealed, and incubated for about 48 hours at 37 C. The EDTA solution was prepared by (1) dissolving 50 g of sodium hydroxide in 3500 mL of distilled water; (2) adding EDTA; and (3) stirring until the solution cleared. The pH of the solution was checked and adjusted to 7.0 each day the solution was used. Next, 2-mm-thick sections were stained with hematoxylin-eosin (Figure 1) and analyzed by 1 pathologist (D.N.R.), who was un- aware of the clinical and imaging data. Cases were considered negative when no intact tumor cells could be identified. Positive cases, with intact tumor cells identified, were classified into those showing < 50 cells and those showing ≥ 50 cells.

Imaging Studies

Patients with a CT scan of the pelvis performed > 6 weeks before the biopsy were excluded from the analyses. The images were analyzed by an experienced radiologist specializing in the field of prostate cancer. An area with a diameter of 0.8 to 1 cm (depending on the patient's anatomy) was drawn in the posterior aspect of the iliac crest in a region thought to be representative of the biopsied area; the location was equivalent for all patients. The mean HU of the biopsy site (left or right) was determined in 3 consecutive slices (5 mm thickness), and the average value was used in the analyses. The bone scans were reviewed for the presence of metastatic disease in the iliac crests and to estimate the bone tumor

burden, classified as < 5 bony sites, 5 to 20 bone metastases, or > 20 metastases, indicating widespread disease.

Statistical Analysis

A descriptive analysis of the baseline laboratory and imaging features was performed, and the median and interquartile range (IQR) are reported. Random assignment algorithms were used to allocate biopsies to the test or the validation group. The test group was used to obtain a model for the prediction of positivity in BMBs. The dependent variable of the model (bone marrow positivity) was defined as the presence of tumor in the processed tissue. The cutoff values for dichotomous variables were established from the test set. Those that presented with greater receiver operating characteristic (ROC) area under the curve (AUC) values were selected for development of the predictive model, which was validated in the second, validation group. The mean values of the baseline parameters between the groups were compared using the Student t test.

Univariable analyses were performed using logistic regression models with only 1 covariate. Variables with a statistically significant association to the dependent variable ($P < .05$) were selected for inclusion in a multivariable logistic regression model, with bone marrow positivity as the dependent variable. Internal validity of the model was tested by establishing the ROC AUC in the test set. External validity was established by determining the ROC AUC in the validation set. Statistical significance was determined by testing the obtained AUCs against a null hypothesis of 0.5. The sensitivity, specificity, and positive and negative predictive values of the model were determined in the test and validation sets. The observed positivity rate of the biopsy specimens in the whole cohort was used as the prevalence value for the calculation of the predictive values. The score was then tested for its association with bone marrow positivity, defined as biopsy specimens yielding ≥ 50 tumor cells using logistic regression modeling. All statistical procedures were performed using SPSS Statistics, version 20 (IBM Corp., Armonk, NY).

Objective IIa. Circulating biomarkers – Circulating Tumor Cells.

To determine the prognostic value and role as a treatment response biomarker of circulating tumor cell enumeration.

Study population and procedures

We performed a post hoc analysis of the COU-AA-301 and IMMC- 38 trials. COU-AA-301 was a phase 3 trial in which post-chemotherapy patients with metastatic CRPC were randomly assigned to abiraterone and prednisone or placebo and prednisone. IMMC-38 was a prospective, open-label study in patients with metastatic CRPC undergoing treatment with chemotherapy. Details of the methodology and the final results for both trials have been published. Both studies were approved by local institutional boards. All patients provided written informed consent before participation. CTC counts were measured at baseline and on day 1 of cycle 2 (weeks 4–5), day 1 of cycle 3 (weeks 8–9), and day 1 of cycle 4 (weeks 12–13) in the COU-AA-301 trial. In the IMMC-38 trial, CTC counts were measured in weeks 2–5 (median 4 wk), weeks 6–8 (median 7 wk), and weeks 9–12 (median 11.9 wk). All CTC counts were measured using the CellSearch assay. Hemoglobin (Hb), alkaline phosphatase (ALP), albumin (ALB), and LDH concentrations were measured at baseline and at each study visit. Eastern Cooperative Oncology Group performance status (ECOG-PS) was recorded at baseline. PSA levels were measured every 4 wk in IMMC-38 and every 12 wk in COU-AA-301.

Statistical analysis

Kaplan-Meier analysis was used to estimate survival. Univariable and multivariable Cox proportional hazards models were used to test the association between the response biomarker and survival. Logistic regression models were used to calculate odds ratios (ORs) to evaluate the association with PSA response.

Post-treatment criteria were defined as follows:

- CTC response: 30% decline from baseline (in patients with baseline CTCs ≥ 5 / 7.5 mL)
- CTC conversion (response): a change from unfavorable (CTCs ≥ 5/7.5 mL) to favorable (CTCs < 5/7.5 mL) CTC counts.
- CTC progression: any increase in CTCs from baseline (in patients with baseline CTCs < 5 / 7.5 mL)
- CTC conversion (progression): a change from favorable (CTCs < 5/7.5 mL) to unfavorable (CTCs ≥ 5/7.5 mL) CTC counts.

A landmark analysis was used to explore the association between CTC response/progression measures and survival, and specific 4-, 8- and 12-week populations were defined.

A Bonferroni correction was applied to account for multiple testing at three different time points; p values were considered statistically significant if $p < 0.0167$. Baseline LDH, ALP, PSA, and CTC data were log- transformed because of positively skewed distributions.

Different survival models were constructed, to evaluate the performance of the different biomarkers: in a first, clinical model, only clinical covariates (ECOG PS, baseline blood parameters) were included; in a second model, baseline CTC counts were added to the clinical model. Finally, a third model added CTC response or progression measures to the previous model. The overall performance of the survival models was evaluated by calculating receiver operating characteristic (ROC) curves for 6- and 11-mo survival endpoints (approx. the median and third survival quartile of the data set) and the c-index for each model using the method proposed by Uno et al.[358] The area under the ROC curve (AUC) was compared by calculating the U statistic (nonparametric).[3] Bootstrapping techniques were used to calculate the 95% confidence interval (CI) of the difference between c-indices.

Analyses were performed using SPSS v21 (SPSS Inc., Chicago, IL, USA) and the R statistics package v3.2.1 (R Foundation, Vienna, Austria).

Objective IIb. Circulating biomarkers – The Neutrophil-to-Lymphocyte Ratio. To determine the prognostic value and role as a treatment response biomarker of the neutrophil-to-lymphocyte ratio.

Study population and procedures

We carried out an unplanned analysis of patients enrolled in the TROPIC trial, a randomized, open-label phase III trial comparing the efficacy of 3- weekly cabazitaxel (25 mg/m2) versus 3-weekly mitoxantrone (12 mg/m2), both in combination with prednisone 10 mg daily, in men with mCRPC who had received prior docetaxel-containing chemotherapy. Details of the eligibility criteria have been previously reported.[4] Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria[359] were followed when possible for the design and analysis of the study.

Patient characteristics collected at trial entry included number and duration of prior lines of treatment, sites of metastases, age, Eastern Cooperative Group (ECOG) performance status, steroid use, full blood count (including absolute neutrophil and lymphocyte counts) and biochemistry. Full blood counts were carried out on a weekly basis (day 1, 8 and 15 of each of each 21-day cycle) and biochemistry was carried out every 3 weeks (day 1 of each cycle). Imaging studies (computed tomography and bone scintigraphy) were carried out every 12 weeks.

Statistical analysis

The NLR was defined as the quotient of baseline absolute peripheral neutrophil count lymphocyte count (cells/mm3) by absolute peripheral baseline lymphocyte count (cells/mm3). For BLNLR values, counts from day 1 of the first cycle were used. OS was defined as the time from randomization to date of death from any cause with censoring at date of last contact for patients alive at the cut-off date. Progression-free survival was defined as the time from randomization to the date of progression by clinical, radiological or PSA criteria.[11]

The association of BLNLR as a continuous and dichotomous variable with survival was evaluated in univariable and multivariable Cox regression models. The association of BLNLR (as a continuous variable) and other prognostic factors were evaluated with linear regression models. Predictors for OS considered in uni- and multivariable analyses were selected based on the prognostic model developed by Halabi et al. from the TROPIC trial dataset.[5] These covariates included: the presence of pain (defined as a present pain intensity scale score ≥2 of and an analgesic score ≥10, the presence of measurable disease before initiation of treatment, ECOG performance status, disease progression within 6 months of docetaxel-based therapy, presence of visceral disease (defined as metastases in the liver, lungs or adrenal glands), duration of previous hormonal treatment and baseline values of PSA, hemoglobin and alkaline phosphatase. Additionally, treatment arm (cabazitaxel versus mitoxantrone) and the use of

corticosteroids at baseline (yes versus no) were included in the analysis. Baseline PSA, BLNLR and baseline alkaline phosphatase were log-transformed due to their skewed distribution. Concordance-index (c-index) values of different pre-specified cut-off points (NLR 2, 3 or 5, corresponding approximately with the first, second and third quartiles) were determined for the selection of the most appropriate cut-off for the dichotomous NLR (Tables 1 and 2). To account for multiple testing, a Bonferroni correction was applied for the association of dichotomous NLR at each of the selected cut-offs and OS, with a significant P value set at 0.0167. To evaluate the association of BLNLR with PSA and radiographic response, a single NLR threshold (NLR equal or greater than or less than 3) was selected based on the survival analysis, and the significant P value was set at 0.05. C-index values were calculated with the method proposed by Uno et al.[358]

Patients with at least one follow-up PSA reading and a baseline PSA value ≥20 ng/ml were eligible for PSA response analysis. PSA response was defined as a 50% decrease in PSA relative to baseline, confirmed with a second reading at least 3 weeks later. Maximum PSA decline was defined as the percentage decrease of the nadir PSA relative to baseline PSA; in cases where no PSA decline was observed, the first rising PSA value was used. Similarly, NLR conversion was only analyzed in patients with a baseline and at least one follow-up NLR reading. NLR conversion was defined as a change from NLR ≥ 3 to < 3 (response) or a change from NLR < 3 to ≥ 3 (progression); the association of both conversion types with overall survival, radiographic and PSA progression-free survival, and radiographic and PSA response rates was evaluated. Radiographic response was only considered in patients with measurable disease by RECIST criteria at baseline.

SPSS Statistics version 20.0 (IBM, Inc.) and RStudio Version 0.98.501 (RStudio, Inc.) were used for the statistical analyses.

Objective III. Use of Clinical Biomarkers

To evaluate patterns of use of biomarkers in clinical practice, and adaptation of clinical practice to available clinical guidelines (PCWG).

A 23-part online questionnaire, divided in four sections as outlined below, was compiled by the authors for completion by specialists in the treatment of prostate cancer in order to evaluate awareness on PCWG2 progression criteria, trends and opinion on use of biomarkers in daily clinical practice, and the awareness on the value of circulating tumor cells in advanced prostate cancer.

Questionnaires included:

1. General questions on clinical practice.
2. Familiarity with progression criteria for currently established biomarkers.
3. CTCs and their assessment in patients with advanced prostate cancer.
4. Clinical decision-making using response indicators.

E-mails inviting participation in the survey were sent to 485 UK investigators participating in urologic cancer clinical trials, 29 physician members of the GU Group of the Swiss Group for Clinical Cancer Research, and 20 practicing prostate cancer physicians in Australia and New Zealand. A link to the web-based survey (created with Survey- Monkey) was included.

Statistical analyses

Descriptive statistics were used; the proportion (%) of physicians responding to each option is presented. Physicians were classified according to the number of patients they treated (50 vs <50 patients/ year) or recruited to clinical trials (25% vs <25%), and the number of cycles of docetaxel/cabazitaxel prescribed (4, 5–6, 7 cycles). No pre- existing evidence was used in choosing classification cutoff values. Proportions were compared using a Chi-square x2 test or Fisher's exact test (for cell frequencies 5). A p value of 0.05 was set as the limit for statistical significance. No adjustment for multiple testing was performed. SPSS version 21 (IBM, Armonk, NY, USA) was used.

Summary of Results and Discussion

Objective I. Tissue based biomarkers

To determine factors associated with a positive bone marrow biopsy in advanced prostate patients with bone metastatic disease.

With the advent of novel agents for the treatment of CRPC and the improved understanding of the molecular biology mechanisms driving disease progression beyond castration, the improvement of mechanisms for tissue acquisition and molecular analysis has become of paramount importance. Up to 89% of patients with mCRPC might harbor clinically actionable genomic aberrations.[183] Assessing single metastasis through soft tissue biopsies or BMBs could therefore provide a reasonable assessment of the oncogenic landscape and prove informative for treatment selection.[360]

The acquisition of tissue for the assessment of molecular biomarkers in advanced prostate cancer poses significant challenges, as exposed in the **Introduction, Section 6**. In brief, prostate cancer has a long natural history, and tissue from the original diagnosis may not be suitable for analysis at the moment when metastatic castration-resistant prostate cancer ensues. On the other hand, selective pressure derived from treatment may force tumor cells to adapt and develop specific changes that contribute to disease progression and are relevant as biomarkers in the advanced setting, but may be absent from the original tissue. When analyzing molecular aberrations from large datasets in order to understand the molecular landscape of advanced disease, analyzing large datasets of localized tumors may oversee the fact that only aggressive subtypes progress into the advanced, lethal phases of the disease.

The propensity to spread to the bones (in many cases, the only metastatic site) is a distinct characteristic of prostate cancer. Thus, a large proportion of patients do not have soft tissue metastases amenable for biopsy. A number of studies published in the past decade have reported variable rates of positive BMBs ranging from 25% to 50% for non-imaging-guided biopsies[6–8] and increasing to 67% to 77% when performed under direct CT guidance.[299,305]

With this aim, we performed a retrospective analysis of patients of patients with mCRPC who had undergone a bone marrow biopsy from October 2011 to November 2014 at the Royal Marsden National Health Services Foundation Trust. Criteria for inclusion in the present study were CRPC, age ≥ 18 years, and evidence from imaging studies (CT, bone scan, or magnetic resonance imaging) of bone metastases from prostate cancer. Images from the pelvic CT scan images were analyzed by an experienced radiologist specializing in the field of prostate cancer; the mean Hounsfield Units (HU) of the biopsy site (left or right) was determined in 3 consecutive slices (5 mm thickness), and the average value was used.

The main objective of our study was to develop a score for the prediction of bone marrow biopsy positivity. Our dataset was divided into a test set, to develop a score based on clinical and imaging

parameters intended to predict the positivity of a bone marrow biopsy, and a validation set to confirm the utility of the score. A total of 115 biopsies in 101 patients were performed. Overall, 75 biopsies (65.2%) were positive; this biopsy positivity rate is consistent with previous findings of biopsies performed without CT guidance.[6–8] Of these, 20 biopsies (26.7%) yielded < 50 cells and 55 biopsies (73.3%) ≥ 50 cells. Of the 115 biopsy specimens, 57 were included in the test set and 58 were included in the validation set. Of the 57 biopsy specimens in the test set and 58 in the validation set, 35 (61.4%) in the test set and 40 (69%) in the validation set were positive; with no significant differences between the 2 groups (p = 0.395). The test and validation cohorts had similar prognostic baseline laboratory and CT parameter distributions.

In the test set, low hemoglobin levels (≥ 11.5 g/dL vs. < 11.5 g/dL; p=0.019), high LDH levels (≥ 225 IU/L vs. < 225 IU/L; p=0.003), PSA levels (≥ 225 vs. < 225 ng/mL; p=0 .005), high alkaline phosphate levels (≥100 vs. < 100 IU/L; p=0.025), and high mean HU on CT (≥ 125HU vs.< 125 HU; p=0.004) were significantly associated with a positive BMB and were selected for multivariable analysis. On multivariable analysis, only mean HUs ≥ 125 (odds ratio [OR], 3.85; 95% confidence interval [CI], 1.06-13.94; p=0.036) and elevated LDH ≥ 225 IU/L (OR 8.7; 95% CI, 1.68-45.11; p=0.003) were significantly associated with BMB positivity. This led to the development of a Bone Marrow Biopsy Score (BMB Score), by assigning 1 point to each of the parameters (0 points if neither the HUs were ≥ 125 nor the LDH was ≥ 200; 1 point if either the HU was ≥ 125 or LDH was ≥ 200; and 2 points if both the HUs were ≥ 125 and the LDH was ≥ 200).

Table 7. Tissue Based Biomarkers: Results from the MV Logistic Regression Analysis on the Test Set

Table 5 Multivariate Analysis[a] (Test Set) Results

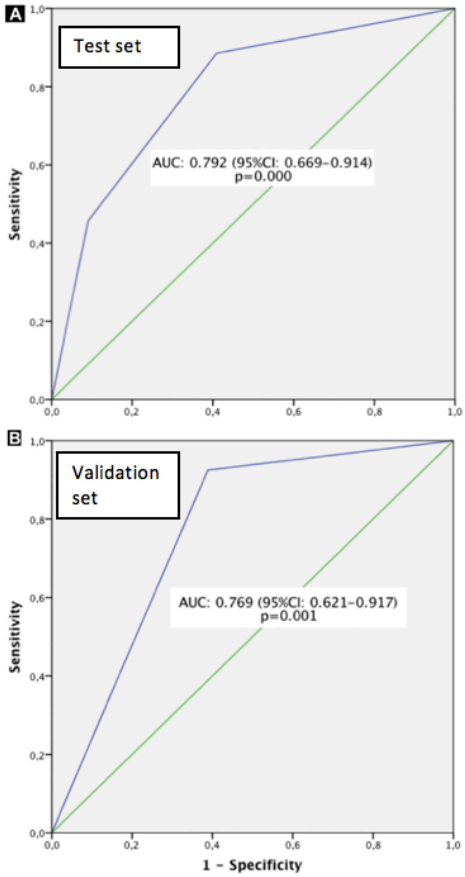
Variable	OR (95% CI)	*P* Value[a]
Hemoglobin	0.68 (0.15-3.02)	.610
LDH	8.7 (1.68-45.11)	.003[b]
ALP	2.06 (0.47-9.03)	.336
PSA	2.79 (0.7-11.12)	.144
Mean HU	3.85 (1.06-13.94)	.036[b]

In the test set, only 23.5% of the biopsies with a score of 0 were positive compared with 77.5% of the biopsies with a score of 1 or 2 (p < 0.001). We then analyzed the performance of the score in the validation set, where similar results were obtained: only 21.4% of the biopsies with a score of 0 were positive for tumor content compared with 84.1% of biopsies with a score of 1 to 2 (p < .001). We evaluated the overall performance of the scores by calculating the Receiver Operating Characteristic (ROC) curve Area Under the Curve (AUC). The AUC of the BMB score was 0.79 (95% CI, 0.67-0.91; P < .001) in the test and 0.77 (95% CI, 0.59- 0.88; P < .001) in the validation set.

Figure 3. Tissue Based Biomarkers: Sensitivity and ROC Curve Analysis
Left: Sensitivity, Specificity and Predictive Values. Right: ROC Curves for the BMB Score in the Test (upper right) and validation (lower right) datasets

Table 8 Sensitivity, Specificity, and Predictive Values

Variable	Estimate (95% CI)	
	Test Set	Validation Set
BMB score (0 vs. 1-2)		
Sensitivity (%)	88.6 (74-95.5)	92.5 (80.1-97.4)
Specificity (%)	59.1 (38.7-76.7)	61.1 (38.6-79.7)
Positive predictive value (%)	78.3 (68.3-85.8)	79.9 (68.8-82.8)
Negative predictive value (%)	75.6 (53.7-89.3)	83 (60.8-93.9)
Mean HU ≥ 125		
Sensitivity (%)	80 (64.1-90)	87.5 (73.9-94.5)
Specificity (%)	59.1 (38.7-76.7)	66.7 (43.7-83.7)
Positive predictive value (%)	75.7 (59.8-86.6)	85.4 (71.6-93.2)
Negative predictive value (%)	65 (43.3-81.8)	70.6 (46.9-86.7)
LDH ≥ 225 IU/L		
Sensitivity (%)	54.3 (38.2-69.5)	45 (30.7-60.2)
Specificity (%)	90.1 (72.2-97.5)	77.8 (54.8-91)
Positive predictive value (%)	90.5 (71.1-97.4)	81.8 (61.5-92.7)
Negative predictive value (%)	55.6 (39.6-70.5)	38.9 (24.8-55.1)



Our study, similar as others published previously, established associations among the clinical, analytical, and CT parameters with BMB positivity. It is, however, the first study to establish the value of the widely used CT and analytical parameters and develop a score with direct applicability in the clinical setting, with validation of these results in a separate control group. We prove the predictive potential of a simple score that can help select patients likely to provide enough tissue for molecular analyses such as exome and transcriptome next-generation sequencing.

The high sensitivity of the BMB score supports its use for the identification of patients with a low likelihood of a positive result. According to our results, patients with a score of 0 (i.e., if the bone density of the iliac crest does not exceed a HU of 125 and the LDH levels are < 225 IU/L) should not have a bone marrow biopsy performed, since the negative predictive value (the probability of achieving a negative result) is about 76-83%. On the other hand, patients with a score of 0 to 1 have a positive predictive value (probability of achieving a positive result) of 78-79%, with is a higher rate than that reported in most studies. Extrapolating our findings to the validation set, excluding patients with a score of 0 would have “saved” 11 patients (18.9%) from undergoing biopsy with negative results and would have only “missed” 3 (5.2%) biopsies with positive results, increasing the positive yield from 69% to 84.1%.

We also evaluate the association of our Bone Marrow Biopsy Score with the likelihood of obtaining at least 50 viable cells; this is due to the fact that a positive bone marrow biopsy (the presence of any number of cells) does not necessarily mean that the sample should be adequate for translational studies. The choice of this cut-off derives from previous studies evaluating the role of PTEN loss in mCRPC biopsies.[9,10] In this study, samples were considered adequate for immunohistochemistry if at least 50 viable cells were positive. In our study, 23 biopsy specimens (40.4%) in the test set and 32 (55.2%) in the validation set contained ≥ 50 cells. The BMB score was associated with positivity (≥ 50 cells) in both the test (OR, 3.1; 95% CI, 1.41-6.84; p=0.005) and the validation (OR, 3.7; 95% CI, 1.6-8.4; p= 0.002) sets. The AUC of the BMP score was 0.72 (95% CI, 0.58-0.85) in the test set and 0.73 (95% CI, 0.59-0.86) in the validation set. In the validation set, only 2 biopsy specimens (14.3%) with a score of 0 had ≥ 50 cells but 30 (68.2%) of those with a score of 1 to 2 were positive.

Translational Impact

In summary, in this study we prove the feasibility of a serial bone marrow biopsy approach in prostate cancer patients. We develop a score that may identify patients with a higher likelihood of obtaining a positive bone marrow biopsy, proving how the use of imaging and laboratory parameters can help select patients and increase the rate of positive biopsy specimens. The extraction of tumor genomic material for molecular analysis may help identify patients with specific aberrations that may help guide treatment decisions or inclusion in clinical trials.

Objective IIa. Circulating biomarkers – Circulating Tumor Cells.

To determine the prognostic value and role as a treatment response biomarker of circulating tumor cell enumeration.

As discussed in the **Introduction, Section 3 and Section 7**, one of the greatest challenges in the current management of CRPC is adequate assessment of response to treatment. A significant proportion of patients present with disease exclusively in bone, which is not amenable to evaluation by the commonly used Response Evaluation Criteria in Solid Tumors (RECIST). PCWG2 criteria rely on bone scintigraphy and changes in prostate-specific antigen (PSA) levels to evaluate response to treatment in these patients.[11] Progression according to bone scintigraphy is not evaluable before 16 weeks because of the possibility of spurious flare reactions,[160] so a confirmatory scan is required after a first scan indicating progression. Likewise, evaluation of PSA values for progression is not recommended before 12 weeks of treatment. Most studies evaluating PSA declines as a surrogate of survival have yielded negative results[26,150,151] and treatment discontinuation based solely on rising PSA values is not recommended.[11,44] Recent studies have reported a stronger association between radiological progression-free survival (rPFS) and overall survival (OS); however, a definition of progression according to rPFS cannot currently be acquired before at least 12–16 wk of treatment, and is difficult to evaluate in men with widespread bone involvement.[25,174] Improved biomarkers to identify patients not benefitting from anticancer treatment are urgently needed.

Enumeration of the circulating tumor cell count has emerged as a powerful biomarker for evaluating prognosis and treatment response in CRPC. The CellSearch assay has proven utility in classifying counts into unfavorable (≥ 5 cells/7.5ml) and favorable (≤ 4 cells/7.5ml) prognostic groups in prospective trials including IMMC-38, COU-AA-301, AFFIRM, and SWOG-S0421.[327,329,331–333,361] Association between post-treatment CTC changes and CRPC survival has been reported, and has been incorporated into the most recent PCWG3 guidelines.[44] In the PCWG3 guidelines, baseline CTC enumeration is recommended to stratify patients in to favorable and unfavorable prognostic groups. When evaluation of response to treatment is considered, only patients with baseline unfavorable CTC counts are considered eligible for assessment of a "conversion" to favorable CTC counts; patients with favorable baseline counts, which can amount to over 50% of patients especially in first-line mCRPC, are considered inevaluable.[44] Additionally, a CTC conversion ascribes value to a post-treatment CTC count < 5 that is independent of the baseline counts, thereby assigning the same value to a decline from 6 to 4 CTCs as a decline from 1000 to 2 CTCs.

We therefore conducted an integral analysis of CTC enumeration in patients participating in the COU-AA-301 and IMMC-38 clinical trials (evaluating patients treated with both chemotherapy and abiraterone), with an aim to evaluate the prognostic role of baseline CTC counts, and the value of a 30%

decline in CTCs (in patients with baseline unfavorable counts) but also of an increase in CTCs in patients with baseline favorable counts, a strategy that would render all patients eligible for response. In the COU-AA-301 trial, CTC counts were measured at baseline and on day 1 of cycle 2 (weeks 4–5), day 1 of cycle 3 (weeks 8–9), and day 1 of cycle 4 (weeks 12–13). In the IMMC-38 trial, CTC counts were measured in weeks 2–5 (median 4 wk), weeks 6–8 (median 7 wk), and weeks 9–12 (median 11.9 wk). In the pooled analysis, these counts were classified as baseline, 4-week, 8-week and 12-week CTC counts. Consistent with previous findings, an unfavorable baseline CTC count was associated with a worse overall survival in the combined dataset (22 vs 11.1 months; HR 2.87; p<0.001).

A. 30% DECLINE IN CTC COUNTS IN PATIENT WITH BASELINE UNFAVORABLE CTCs (≥ 5 CTCs/7.5 mL)

We carried out a post hoc analysis of data for patients in the prospective IMMC-38 (chemotherapy) and COU-AA-301 (abiraterone) trials with baseline CTC 5 cells/7.5ml, evaluating the value of a 30% CTC decline from baseline at 4, 8, and 12 wk as a biomarker of response to treatment. Overall, 486 patients with baseline CTC 5 cells/7.5 ml participating in the IMMC-38 (n = 122) and COU-AA-301 (n = 364) trials were included in the analysis.

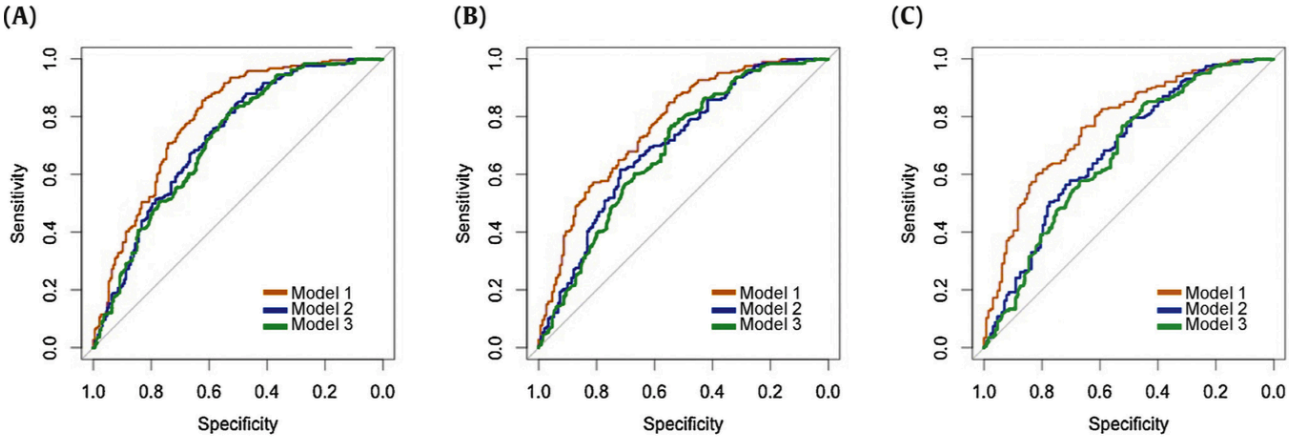
We initially sought to define the most appropriate response cutoff. To achieve this, we compared the performance of 30% and 50% CTC declines based on sensitivity, specificity and receiving operating curve (ROC) area under the curve (AUC) values. We observed a significant difference in ROC AUC only in the 4-week landmark survival population (AUC 0.68 vs 0.65; p=0.006). As expected, sensitivity was higher for the 30% cut-off (4-weeks: 71.5% vs 61%; 8-weeks: 71.7% vs 66.6%; 12-weeks: 68.2% vs 63.5%), while specificity was higher for the 50% cut-off (4-weeks: 65.1% vs 68.6%; 8-weeks: 76.5% vs 78.4%; 12-weeks: 72.7% vs 78.8%). Since we considered that the cost of a false negative (classifying a responder as a non-responder) is higher than that of a false positive (classifying a non-responder as a responder), sensitivity was valued over specificity and the 30% cut-off was chosen.

Overall, 283 (64.3%), 248 (65.3%), and 226 (64.4%) patients experienced a 30% decline in CTC count at 4, 8, and 12 wk, respectively, with a statistically significant benefit in overall survival in the 4-week (HR 0.45; p<0.001), 8-week (HR: 0.41; p <0.001) and 12-week (HR 0.39; p<0.001) landmark survival populations, with a similar impact when evaluated the pooled population and the different trial populations separately. The association was independent of other covariates such as PSA, LDH, ALB, Hb, ALP, and ECOG PS in multivariable analyses.

We analyzed how baseline CTCs and a CTC response improved readily available survival biomarkers, by constructing three multivariable Cox-regression models and comparing their c-indices. Model 1 was a “clinical” model, constructed with PSA, LDH, ALB, Hb, ALP, and ECOG PS as covariates. Model 2 was a “baseline CTC model”, constructed with variables in the “clinical model” and baseline CTC counts. Model

3 comprised variables in the “baseline CTC model” and 30% CTC declines. In the 12-week landmark models, Model 1 (clinical) had a c-index of 0.646, which increased marginally, to 0.656, with the addition of baseline CTCs (baseline CTC model). Model 3, with the addition of 30% CTC declines to baseline CTC and clinical variables, markedly increased the c-index (0.710). Similarly, there was a non-significant increase in ROC curve AUC with the addition of 30% CTC declines in comparison to both the clinical and baseline CTC models, highlighting how the incorporation of CTC declines can improve outcome prediction.

Figure 4. CTC Decline – ROC Curve Analysis of the Different Cox-regression models
ROC Curves for the Model 1 (Clinical variables), Model 2 (Clinical variables + Baseline CTCs) and Model 3 (Clinical variables + baseline CTCs + 30% CTC declines) in the 4-week, 8-week and 12-week landmark survival populations



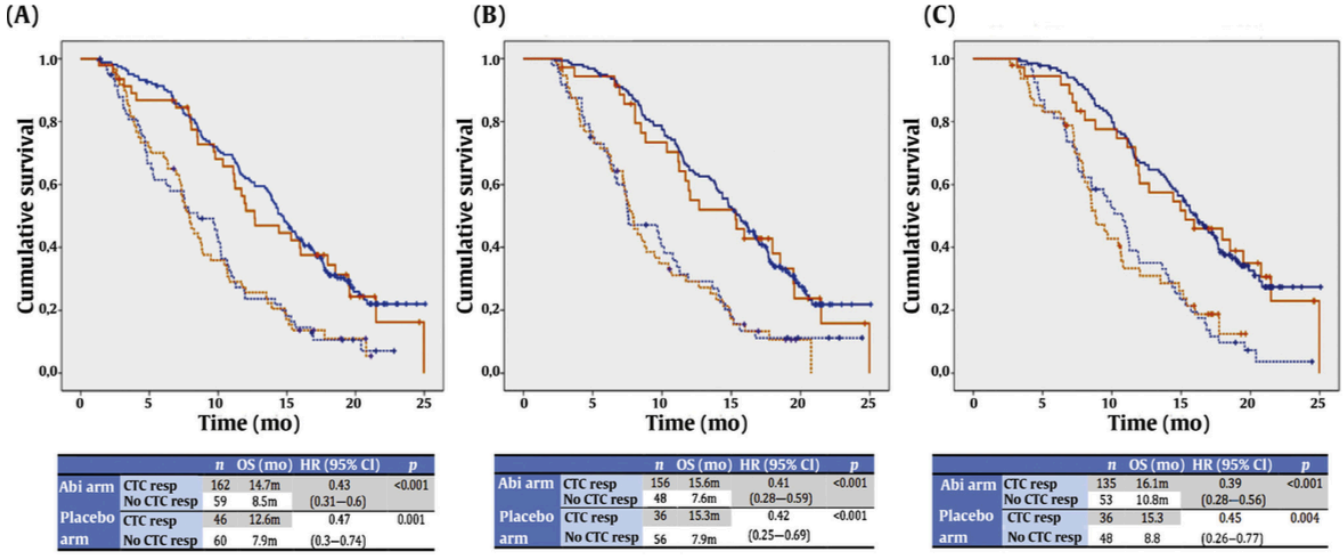
	4 wk			8 wk			12 wk		
	c-index	AUC*	*p***	c-index	AUC*	*p***	c-index	AUC*	*p***
Model 1	0.720	0.790	–	0.710	0.770	–	0.710	0.772	–
Model 2	0.681	0.726	<0.001	0.658	0.700	0.001	0.656	0.684	<0.001
Model 3	0.673	0.717	<0.001	0.651	0.686	<0.001	0.646	0.669	<0.001

We compared the prognostic ability of 30% CTC declines to CTC conversion in patients with baseline unfavorable CTCs. We compared AUC values for CTC conversion and 30% CTC response (6-month OS) among all patients and among patients with baseline CTC 10 and 30 cells/7.5 ml. Although the AUC was consistently higher for a 30% CTC decline than for CTC conversion, no significant differences were found except for patients with high baseline CTC (10 cells/7.5 ml) at 4 wk (AUC 0.701 vs 0.624; p = 0.008).

We also evaluated the impact of treatment arm on 30% CTC declines. In the sub-population with baseline unfavorable CTCs, abiraterone maintained a significant overall survival benefit (HR 0.75; p=0.02) in all landmark survival populations. Patients on abiraterone had a significantly higher 30% CTC decline rates than prednisone (73.3% vs 43.4%). Furthermore, interaction tests between treatment arm and a 30% CTC decline were not significant (p = 0.758), suggesting an equivalent survival benefit for

abiraterone and prednisone or prednisone alone in post-chemotherapy patients who achieved a 30% CTC decline (Figure).

Figure 5. Prognostic value of 30% CTC declines
Patients in the abiraterone and prednisone arms of the COU-AA-301 trial in the 4-week (a), 8-week (b) and 12-week (c) landmark survival populations.



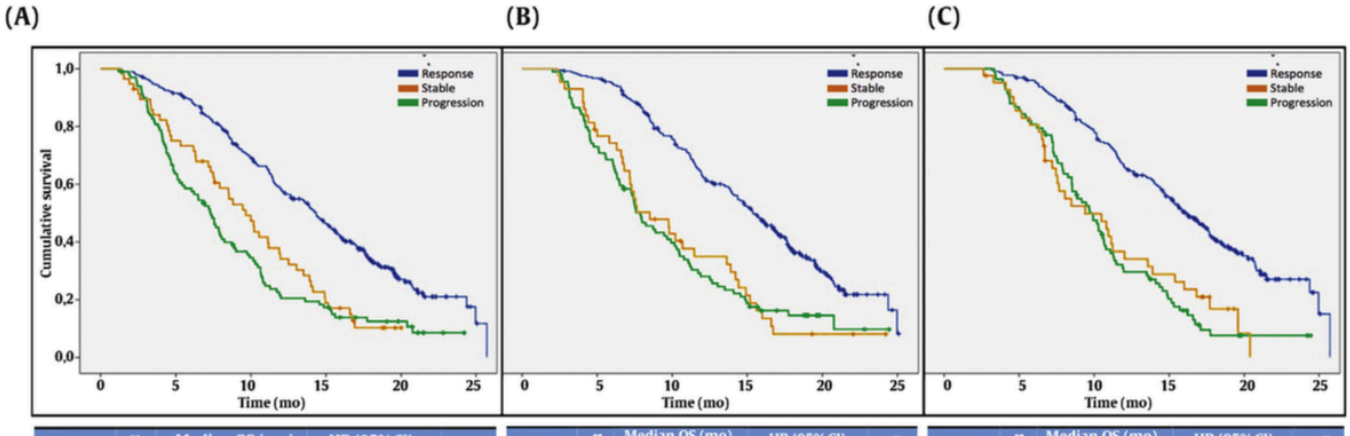
		n	OS (mo)	HR (95% CI)	p
Abi arm	CTC resp	162	14.7m	0.43 (0.31–0.6)	<0.001
	No CTC resp	59	8.5m		
Placebo arm	CTC resp	46	12.6m	0.47 (0.3–0.74)	0.001
	No CTC resp	60	7.9m		

		n	OS (mo)	HR (95% CI)	p
Abi arm	CTC resp	156	15.6m	0.41 (0.28–0.59)	<0.001
	No CTC resp	48	7.6m		
Placebo arm	CTC resp	36	15.3m	0.42 (0.25–0.69)	<0.001
	No CTC resp	56	7.9m		

		n	OS (mo)	HR (95% CI)	p
Abi arm	CTC resp	135	16.1m	0.39 (0.28–0.56)	<0.001
	No CTC resp	53	10.8m		
Placebo arm	CTC resp	36	15.3	0.45 (0.26–0.77)	0.004
	No CTC resp	48	8.8		

We finally evaluated whether CTCs that do not change on treatment represent a distinct prognostic subgroup. We defined “stable CTC counts” as a change from baseline that did not exceed a 30% decline or a 30% increase. Only 57 (13%), 43 (11.3%), and 42 (12%) patients experienced a stable CTC count at 4, 8 and 12 weeks, respectively. A 30% CTC decline showed a significant OS benefit when compared to a stable CTC count at all time points, but no difference was observed when comparing stable and progressive (>30% increase) CTC counts. Since there was no significant difference between patients experiencing “stable” or “rising” CTCs, we concluded that a failure to achieve a 30% CTC decline should be considered an adverse prognostic feature that should prompt evaluation on whether a change of treatment should be performed (Figure).

Figure 6. Overall survival according to 30% CTC declines, Stable CTCs or Rising CTCs
In the 4-week (a), 8-week (b) and 12-week (c) landmark survival populations.



	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	283	14.4 (12.8–15.9)	0.5 (0.37–0.69)[a]	<0.001
Stable	57	9.7 (7.9–11.4)	–	
Progression	100	7.3 (6.1–8.5)	1.19 (0.84–1.7)[b]	0.330

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	248	15.4 (13.9–16.8)	0.42 (0.29–0.6)[a]	<0.001
Stable	43	8.5 (5.6–11.4)	–	
Progression	89	7.9 (6.6–9.1)	1.02 (0.69–1.51)[b]	0.913

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	226	16.1 (14.6–17.7)	0.42 (0.29–0.62)[a]	<0.001
Stable	42	9.4 (5.3–13.5)	–	
Progression	83	9.9 (8.6–11.3)	1.12 (0.75–1.69)[b]	0.576

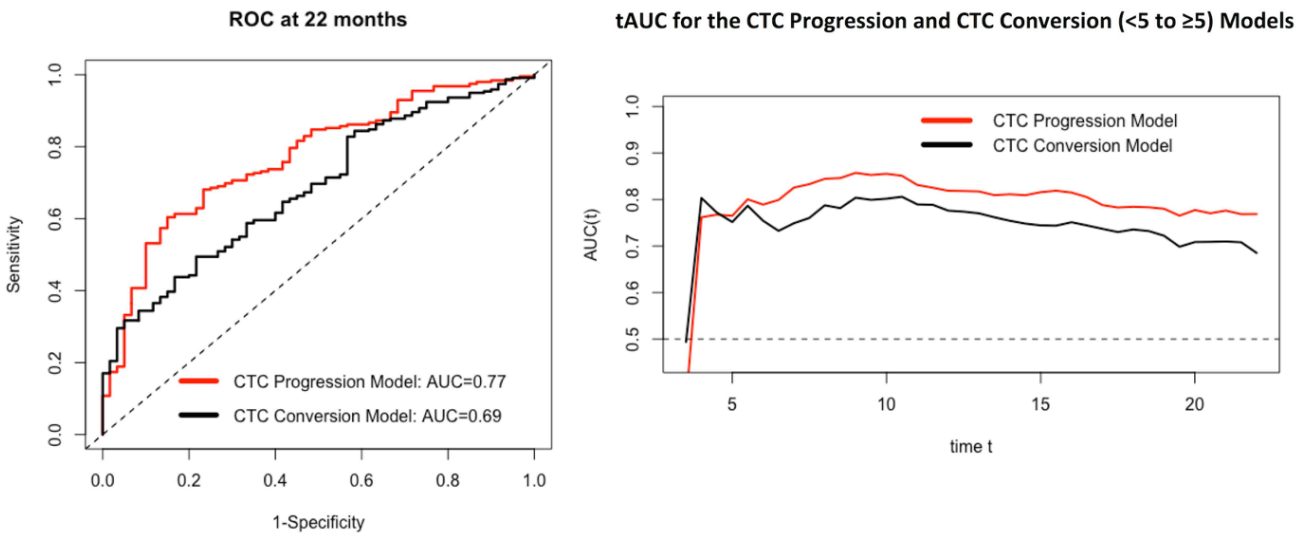
B. CTC COUNT INCREASE IN PATIENTS WITH BASELINE FAVORABLE CTCs (< 5 CTCs/7.5 mL)

We carried out a post hoc analysis of data for patients in the prospective IMMC-38 (chemotherapy) and COU-AA-301 (abiraterone) trials with baseline CTC < 5 cells/7.5ml, evaluating the value of a "CTC progression", defined as either any increase in CTC counts or as a conversion to unfavorable counts, at the 4-week, 8-week and 12-week timepoints. Overall, a total of 511 patients participating in the COU-AA-301 (n = 421; 82.4%) and IMMC-38 (n = 90; 17.6%) clinical trials met the selection criteria with baseline CTC counts<5 cells/ 7.5 ml and were included in the analysis.

As previously reported, patients with a baseline favorable count had a significantly improved overall survival compared with patients with baseline unfavorable counts. We evaluated whether different prognostic subgroups could be established within patients with a favorable prognosis. Baseline CTC count, as a log10-transformed continuous variable, was associated with survival in these patients overall (HR 1.65; p < 0.001]. Patients with 0 baseline CTCs had a significantly better median overall survival (27.1 months) than those with 1-2 baseline CTCs (21.6 months) or patients with 3-4 baseline CTCs (15.1 months); a significant p-value for the linear trend was observed (p = 0.001).

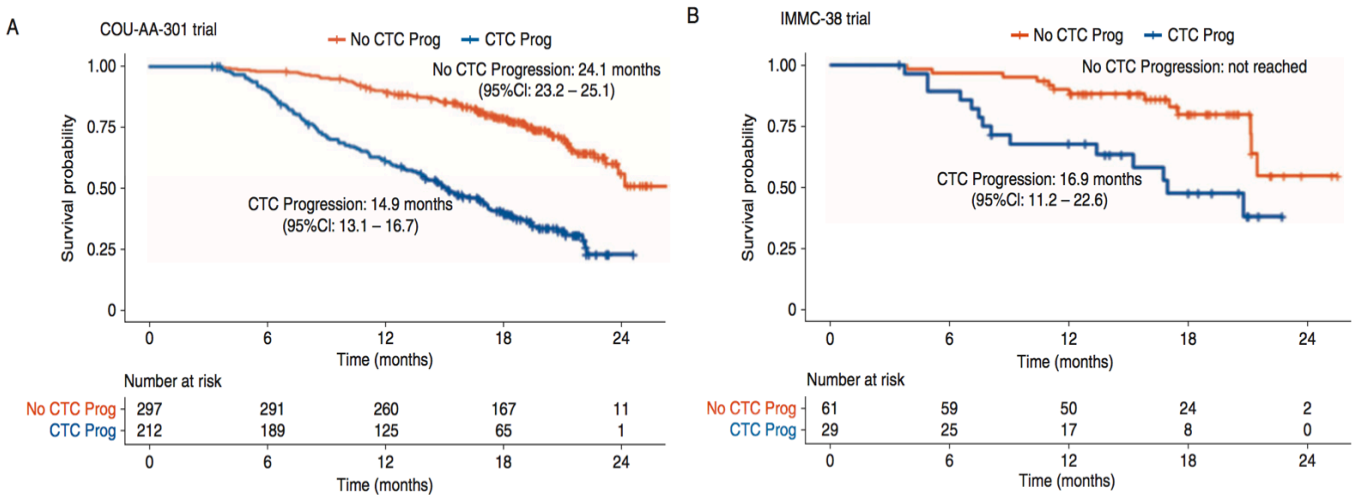
Overall, 213 (41.7%) patients experienced CTC progression in the first 12 weeks of treatment; 184 (43.7%) in COU-AA-301 and 29 (32.2%) in IMMC-38; 117 (25.8%), 103 (23.8%) and 124 (24.4%) patients experienced CTC progression at 4, 8 and 12 weeks, respectively. Overall, 90 patients (17.7%) experienced a conversion to unfavorable (≥ 5 CTCs/7.5 mL) counts during the first 12 weeks of treatment; 76 (18.1%) in the COU-AA-301 ad 14 (15.6%) in the IMMC-38 trial. We compared the prognostic ability of CTC progression vs CTC progression by calculating ROC curve AUCs (at the 22-month OS time-point), of time-dependent AUCs and of concordance indices in univariable Cox-regression models (Figure).

Figure 7. ROC and tAUC curves for CTC progression vs CTC conversion models



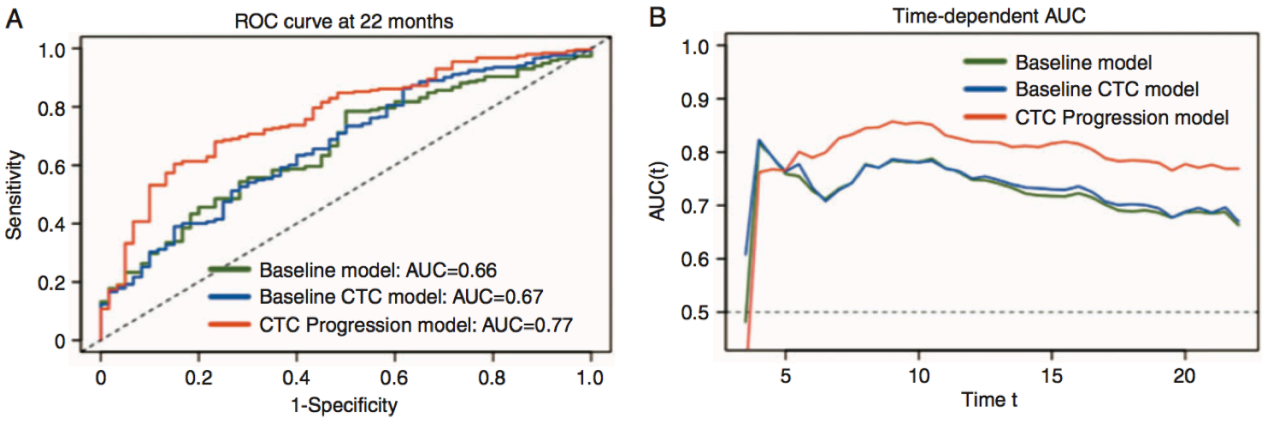
The weighted c-index of the model including CTC progression was significantly higher than that of the model including CTC conversion (0.750 vs 0.705; delta c-index: 0.045 [95%CI: 0.019- 0.071]). The ROC curve AUC index was also significantly higher for CTC progression than for CTC conversions (0.77 vs 0.69; 95% CI: 0.61-0.76; p < 0.001). Based on these results, we favored CTC progression over CTC conversion in all subsequent analyses. CTC progression during the first 12 weeks was associated with a significantly worse OS (27.1 versus 15.1 m; HR: 3.4; p < 0.001); the impact was similar in COU-AA-301 and IMMC-38 datasets (Figure 8). Similarly, a significant association with PSA response (defined as a 50% decline from baseline) was observed, with lower rates of PSA response in patients with CTC progression. PSA response, was observed in 118 (28.2%) patients from COU-AA-301 and 42 (51.9%) patients from IMMC-38. Patients with CTC progression had a significantly lower rate of PSA response than those without CTC progression [11.4% versus 47.1%; OR: 0.14 (95% CI 0.09–0.23), p < 0.001).

Figure 8. Kaplan-Meier curves (CTC progression vs no CTC progression) in the COU-AA-301 and IMMC-38 datasets



As previously described in the unfavorable baseline CTC population, we then analyzed how baseline CTCs and a CTC progression improved readily available survival biomarkers, by constructing three multivariable Cox-regression models and comparing their c-indices. We constructed a “Baseline model” including PSA, LDH, Albumin, Hemoglobin, Alkaline Phosphatase, and ECOG PS as covariates. A second model, named “Baseline CTC Model” included covariates included in the “Baseline model” and baseline CTC counts and a third model, the “CTC Progression Model” by adding CTC progression (within the first 12 weeks) to the “Baseline CTC Progression Model”. The ROC curve AUC for the baseline model was 0.66 (95% CI 0.59–0.74). A non-significant increase to an AUC of 0.67 (95% CI 0. 59–0.75) was observed when adding baseline CTC counts to this baseline CTC model (p = 0.63). Adding CTC progression to the model substantially increased the ROC AUC value (AUC 0.77; 95% CI 0.70–0.84) when compared with the baseline CTC model (P < 0.001). Similarly, the weighted c-index of the baseline model (0.682; SE: 0.023) increased to 0.694 (SE: 0.026) after including baseline CTCs. Inclusion of CTC progression in the model increased the weighted c-index to 0.748 (SE: 0.019) (delta c-index: 0.056; 95% CI 0.025–0.087).

Figure 9. ROC Curve and time-dependent AUC models of the Baseline, Baseline CTC and CTC Progression Cox-regression models



We then evaluated the impact of treatment arm on CTC progression. There was no significant benefit in survival of abiraterone over prednisone in patients with baseline favorable counts (HR 0.86; 95% CI 0.63–1.17; p = 0.330). CTC progression was more frequent in the placebo (68 patients, 51.9%) arm than in the abiraterone (115 patients, 39.9%) arm (OR: 0.6; p = 0.022). The survival decrease in patients experiencing CTC progression was similar in the abiraterone (24.1 versus 15.1 months; HR 3.76; p<0.001) and placebo arms (NR versus 13.8 months; HR 3.23; P < 0.001). The interaction test between treatment arm and CTC progression was not significant (p=0.952), indicating that the impact of CTC progression on survival was similar for patients in both trial arms.

C. CONCLUSIONS

The following conclusions can be drawn from these analyses.

(a) We confirm the prognostic value of baseline CTC counts, with a risk 2.87 times greater of death in patients with baseline unfavorable counts. This is similar to other series, such as that by Olmos and colleagues (HR 3.25),[331] or the SWOG S0421 trial (HR 2.74).[329] As high baseline CTC counts are associated with other adverse prognostic factors, the impact of baseline CTC counts in multivariable models tends to be diminished. Differences in hazard ratios may be related to the differences in patient populations.

(b) In patients with low (< 5 CTCs) baseline CTCs, we prove that further prognostic stratification based on CTCs (0 vs 1-2 vs 3-4), a feature that had not previously been reported.

(c) In patients with baseline unfavorable counts, we prove that a 30% decline is an adequate measure of treatment benefit. We argue that a 30% decline could be more adequate than a CTC conversion, especially in patients with high CTC counts (>10), where a 30% CTC decline has a higher AUC than a CTC conversion. In a recently published pooled analysis of 5 clinical trials comparing different CTC endpoints, CTC0 (mean c-index: 0.81) and CTC Conversion (mean c-index: 0.79) had higher c-indices than a 30% CTC decline (mean c-index: 0.72).[333] Low baseline CTC counts could have driven these

results and, in this study, patients without valid CTC counts at 12 weeks were considered non-responders, a potential bias to the results.

(d) We also prove that a failure to achieve a significant decline in CTC counts is equivalent to an increase, and therefore "stable CTC counts" have no intrinsic prognostic value.

(e) In patients with baseline favorable counts, we prove that an increase in CTCs is a more powerful biomarker than a conversion to unfavorable counts. This is likely due to the fact that CTC conversion is highly specific, but it "misses out" patients in whom a rise without conversion criteria is also associated with a worse prognosis.

(f) Both a CTC decline in patients with baseline unfavorable count, and a CTC increase in patients with a baseline favorable count significantly improve outcome prediction in comparison with baseline clinical variables and baseline CTC counts; this supports performing follow-up CTCs, at least until week 12 of treatment.

(g) We prove our results are applicable to patients receiving both hormonal treatment and chemotherapy, with no significant differences in the impact of baseline or response/progression by CTCs.

(h) In contrast to CTC conversion or a CTC0 endpoint, which classify a significant proportion of patients as inevaluable, we propose a double approach based on CTCs. Our results suggest patients could be stratified in clinical trials according to the presence of unfavorable or favorable CTC counts, and efficacy could be based on the proportion of patients with no progression (in favorable baseline counts) and the proportion of patients that achieve response (in unfavorable baseline counts). With this approach, we could ensure that all patients with evaluable baseline CTC counts could be eligible for response assessment.

We acknowledge the following limitations, mainly arising from the unplanned post hoc nature of both studies. Furthermore, only 858/1195 (71.8%) patients enrolled in the COU-AA-301 trial could be evaluated for CTCs. Although CTCs were investigated until progression in the IMMC-38 study, these were only determined at 4, 8, and 12 weeks in the COU-AA-301 study. The value of a stable CTC count was not investigated in the COU-AA-301 and IMMC-38 data sets independently owing to a lack of sufficient events. Finally, although both median OS and baseline characteristics were similar in the data sets for both trials, approximately three times as many patients were treated with abiraterone (COU-AA-301) than with chemotherapy (IMMC-38). Also, LDH kinetics were not incorporated into the analyses.

Translational Impact

In summary, we prove that a post-treatment decline in CTCs and a post-treatment increase in CTCs may be an indicator of treatment benefit (decline) or lack thereof (increase) in patients with baseline unfavorable and favorable CTC counts, respectively. This can be used to determine response or progression earlier and more accurately than with currently available biomarkers, and may allow to

switch treatment before clinical progression appears, when patients may have a higher likelihood of responding to subsequent therapy due to a good performance status.

Objective IIb. Circulating biomarkers – The Neutrophil-to-Lymphocyte Ratio. To determine the prognostic value and role as a treatment response biomarker of the neutrophil-to-lymphocyte ratio.

As discussed in the **Introduction, Section 3**, finding the optimal treatment sequence in order to maximize the therapeutic benefit obtained from the different treatment options is a major challenged faced by physicians treating advanced prostate cancer. Due to the scarcity of molecular biomarkers, we are left with clinical biomarkers such as patient characteristics, response to prior treatments, extent or burden of disease, or parameters based on routine blood tests. These have been generally grouped into prognostic models or nomograms, which are limited by the trial populations they were derived from initially, and PSA as the only blood-based accepted response biomarker in prostate cancer.

Cancer-related inflammation has been recognized as one of the hallmarks of cancer[138] with an essential role in the modulation of the tumor microenvironment. The neutrophil–lymphocyte ratio (NLR), a measure of the proportion of systemic neutrophils and lymphocytes, has been proposed as an indicator of cancer-related inflammation, and has been shown to have prognostic relevance across a large variety of tumor types.[139] In prostate cancer, a number of retrospective studies have evaluated the prognostic significance of baseline NLR (BLNLR)[140–142] and its association with PSA response.[145] To date, the optimal cut-off for the clinical application of BLNLR as a binary variable has not been established, with some of these studies favoring an NLR cut-off of 3 and other studies evaluating a cut-off of 5.

With this aim, we performed a retrospective analysis of patients of patients treated in the TROPIC trial, which randomized mCRPC patients that had progressed on previous treatment with docetaxel to receive cabazitaxel or mitoxantrone,[4] to evaluate the impact of BLNRL on overall survival, but also on PSA and radiographic response. We also investigated the value of changes in NLR (NLR 'conversion' from low to high or high to low counts) with treatment, to investigate its role as a response indicator. Overall, 755 patients were randomized in the trial and were eligible for analysis. 377 (49.9%) patients were randomized to receive mitoxantrone plus prednisone and 378 (50.1%) to receive cabazitaxel plus prednisone.

Consistent with a pro-inflammatory effect observed in advanced cancers,[138] we observed an association of baseline NLR with other prognostic features associated with high tumor burden such as the presence of visceral metastases (p=0.019), pain (p=0.007), low hemoglobin (p=0.002) and high alkaline-phosphatase (p=0.012). Additionally, ECOG PS score (p <0.001) and use of steroids at baseline (p=0.026) was also associated with a higher baseline NLR.

We initially evaluated the prognostic value of BLNLR as a continuous variable. BLNLR was significantly associated with survival in univariate (HR 2.89; 95%CI: 2.12–3.94; p<0.001) and multivariate (HR 1.91; 95%CI 1.31– 2.79; p = 0.001) models including covariates that formed the Halabi nomogram[5] derived from the TROPIC trial (the presence of pain, the presence of measurable disease before initiation of treatment, ECOG performance status, disease progression within 6 months of docetaxel-based therapy, presence of visceral disease, duration of previous hormonal treatment and baseline values of PSA, hemoglobin and alkaline phosphatase) as well as treatment arm and the use of corticosteroids at baseline.

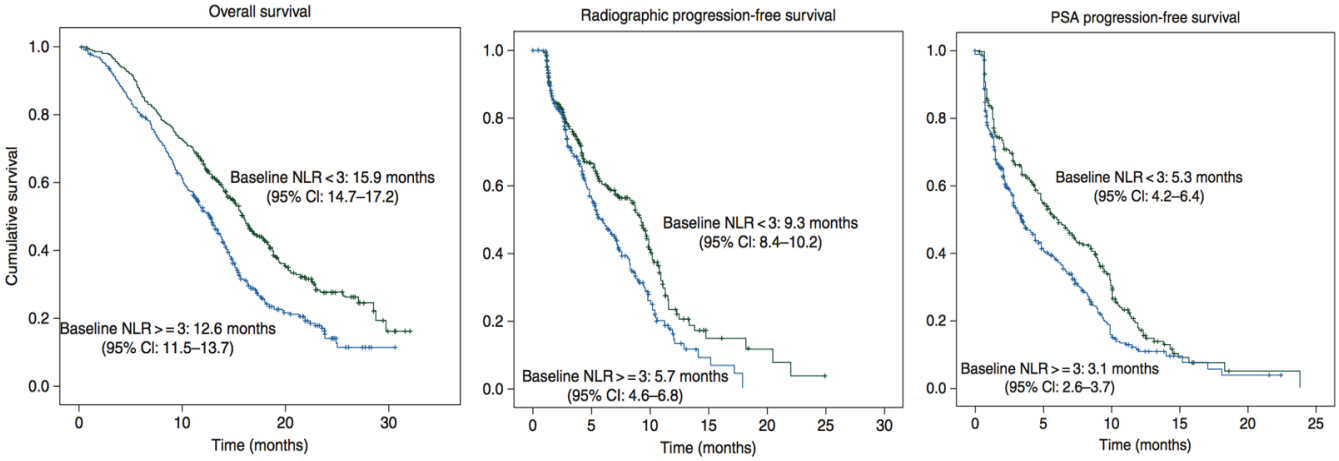
We then evaluated whether the addition of baseline NLR could improve the established prognostic nomogram; we calculated the concordance-index (c-index) of the multivariable Cox Regression Model derived from the Halabi nomogram with and without baseline NLR, and compared the outcomes. The c-index of the model with factors included in the Halabi nomogram was 0.728 (95% CI 0.699–0.757); when adding BLNLR to the model, the c-index increased to 0.736 (95% CI 0.707–0.765). The difference in c-index with and without BLNLR was however not statistically significant (difference in c-index: 0.008; 95% CI: -0.005 to 0.020). Therefore, despite the important association with overall survival, we could not conclude that NLR improved significantly the prognostic ability of models that are readily available.

There is no consensus regarding the optimal cut-off point when considering criteria for “high” and “low” NLR values. Different studies have considered 2,[143] 3[140] or 5[145] as the cut-off to classify NLR. The difference in choice has been traditionally related to the distribution of NLR, as most studies choose NLR values close to the median of their dataset. In our study, we selected the optimal cut-off point for a dichotomous NLR and survival by comparing c-statistic values of NLR in univariate survival models; we selected values representing approximately the median (NLR3) and first (NLR2) and third quartiles (NLR5). All three cut-off points met the pre-specified criteria for statistical significance (NLR2: P = 0.016; NLR3: p<0.001; NLR5: p<0.001). A cut-off of 3 had a higher c-index (c-index NLR3: 0.544; 95% CI 0.522–0.566) than a cut-off of 2 (c-index NLR2: 0.524; 95% CI 0.499–0.549) or a cut-off of 5 (c-index NLR5: 0.539: 95% CI 0.520–0.558) and was therefore selected as the optimal cut-off threshold for further analyses.

Using a 3 as the threshold to define “low” (BLNLR < 3) and “high” (BLNLR ≥ 3) counts, we then evaluated the association with overall survival, radiographic progression-free survival and PSA progression-free survival. Patients with BLNLR <3 had a statistically significant higher median OS (15.9 vs 12.6 months, HR 1.55 (95% CI 1.3– 1.84), p < 0.001), PSA progression-free survival (5.3 vs 3.1 months; HR 1.35 (95% CI 1.12–1.62); p=0.002) and radiographic progression-free survival (9.3 versus 5.7 months; HR 1.42 (95% CI 1.15–1.76); p = 0.001). The observed benefit was independent of treatment arm (cabazitaxel or mitoxantrone). When performing a stratified comparison of both arms in NLR high and NLR low

patients, cabazitaxel maintained its overall survival advantage over mitoxantrone. We therefore conclude that baseline NLR is not a predictive biomarker for the selection of treatment with cabazitaxel.

Figure 10. Overall Survival, Radiographic Progression-Free Survival and PSA Progression-Free Survival in low vs high baseline NLR patients



We also evaluated whether NLR would be able to identify patients with a higher likelihood of achieving a radiographic or PSA response. Radiographic responses were evaluated using RECIST criteria, only in the 405 patients (53.6%) with RECIST measurable disease at baseline. 15.6% (32/147) of patients with baseline low NLR levels (< 3) versus 7.7% (14/183) of those with high BLNLR counts (≥ 3) achieved a radiographic response (p = 0.022). Similarly, a PSA response was defined as a >50% decline from baseline after at least 12 weeks; 654 patients were assessable for PSA response. PSA response rates were higher patients with BLNLR <3 (35.7% versus 22.1%; p < 0.001). Both radiographic response and PSA response rate were independent of treatment arm. This, however, does not imply that baseline NLR can be considered to entail predictive abilities; across multiple studies, patients with favorable prognoses have been observed to achieve higher response rate on anticancer agents. Our interpretation of these results is that the favorable prognostic value of low BLNLR counts is responsible for these findings.

Corticosteroids are widely used in prostate cancer, both as an adjunct to antitumor therapy (in patients receiving abiraterone or taxanes, for instance) or as treatment of pain, asthenia or anorexia, for instance. Corticosteroids have a known effect on the immune system, with immunosuppressive properties, and the potential to increase neutrophil and decrease lymphocyte counts. None of the previous studies, however, had evaluated the potential confounding effect of concomitant corticosteroid therapy when evaluating the impact of NLR on survival. In our dataset, 342 (45%) patients were receiving systemic corticosteroids at baseline, before initiating trial treatment. We observed a higher median NLR in patients receiving corticosteroids at baseline (3.9 versus 2.9; P < 0.001); a higher proportion of patients with BLNLR counts ≥3 were receiving treatment with corticosteroids before study entry (49.6% versus 40.6%; p = 0.016). This may be related to the direct effect of corticosteroid therapy

on the neutrophil and lymphocyte counts, but also to the fact that patients with worse performance status and more pain, features that are associated with a worse prognosis (and a higher baseline NLR) are more frequently receiving corticosteroid therapy. In any case, when including baseline corticosteroid use in the prognostic model, BLNLR remained significant. Additionally, we performed an interaction test between corticosteroid use and NLR in their association with overall survival, which was not statistically significant (p=0.82). Taken together, these findings indicate that NLR is prognostic independently of whether a patient is receiving steroid treatment at baseline.

Finally, we evaluated whether changes in NLR on could be indicative of a response to treatment, by evaluating the significance of conversions between 'favorable NLR' (NLR <3) and 'unfavorable NLR' (NLR ≥3) during the first 12 weeks of treatment (cycle 1 day 1 to cycle 5 day 1). Overall 345 patients presented with baseline unfavorable NLR and at least one follow-up NLR; of these, 151 (44%) experienced a 'conversion' during treatment. Patients with a conversion from 'unfavorable' to 'favorable' NLR had a significantly longer OS (14.5 versus 11.7 months; HR 0.76; p = 0.032) and a higher PSA response rate (30.4% versus 18.6%; p = 0.016). The difference in survival was independent of treatment arm and all other prognostic factors in multivariable analyses. On the other hand, 326 patients presented with a baseline favorable NLR, 201 (62%) of whom experienced a conversion to an unfavorable NLR. A conversion to unfavorable NLR (progression) was, however, not significantly associated with a worse survival (15.7 versus 16.5 months; HR 1.1; p = 0.4) or a lower rate of PSA response (35.9% versus 39.3%; p = 0.56).

Figure 11. NLR conversion from high to low (response) or low to high (progression) on treatment

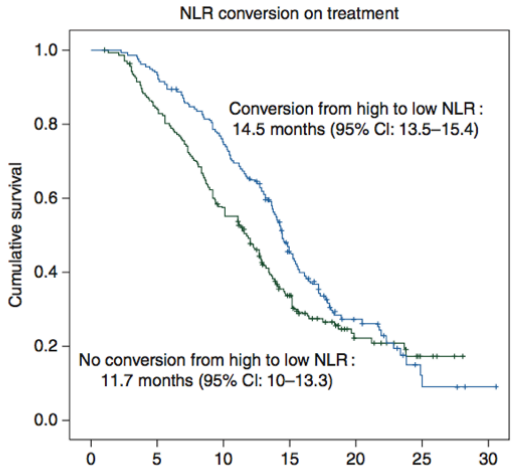


Table 2. Association of NLR conversion and survival

Baseline NLR	NLR conversion	*N*	Survival (months)	HR (95% CI) *P* value
High (*n* = 345)	Yes (responder)	151	14.5 (13.5–15.4)	0.76 (0.59–0.98)
	No (non-responder)	194	11.7 (10–13.3)	*P* = 0.032
Low (*n* = 326)	No (non-progression)	125	16.5 (14–19)	1.12 (0.84–1.5)
	Yes (progression)	201	15.7 (14.3–17.2)	*P* = 0.432

In this report, we perform a complete analysis of the value of the NLR as a biomarker in men with CRPC treated with second-line chemotherapy. From this study, we were able to draw the following conclusions:

(a) In line with previously published studies, we prove a significant association of baseline NLR counts with outcome. We observe a significant association with PSA and radiographic responses, probably related with the favorable biology (prognostic value) rather than due to a potential predictive value.

(b) We prove that the prognostic value of NLR is independent of baseline corticosteroid use, which is a potential confounding factor that had not been previously reported, and points towards a broader applicability of the biomarker.

(c) Despite its proven prognostic value, we were not able to prove that the addition of baseline NLR could increase the prognostic value of currently established nomograms in advanced prostate cancer.[5] This therefore calls into question whether including baseline NLR into the baseline assessments would provide any benefit at all in patient care. Consistent with these findings, nomograms that initially considered NLR as a potential covariate in multivariable Cox-regression models were finally not included due to its lack of additional prognostic value.[362]

(d) In contrast to previously reported studies, we perform a formal assessment of the value of different potential cut-offs for the definition of "high" and "low" NLR counts. Although our analyses point towards a higher performance of the NLR3 cutoff, this should not be considered sufficient evidence to recommend its widespread use. Differences in performance between NLR3 and NLR5 were small. Other datasets, with different (higher or lower) median NLR values could yield different "optimal" cut-offs.

(e) We observe a significant association with a conversion ("response") from high to low NLR on treatment, that is independent of other prognostic variables, but not of a conversion ("progression") from low to high NLR. This suggests a "response" measure based on NLR could be used to assess the benefit of treatment.

(f) Our results do not suggest baseline NLR does not have value as a predictive biomarker for treatment selection, since cabazitaxel was superior to mitoxantrone in both NLR high and low subsets, with a non-significant interaction term.

Translational Impact

In our study, we perform an analysis of the prognostic and predictive value of baseline NLR counts in chemotherapy-treated patients. Despite a significant association with overall survival and response, it is unclear whether the use of NLR counts can add benefit in the treatment of mCRPC patients. Our results also suggest NLR conversions could be used as a response measure, although comparison with currently established response measures was not performed, and therefore potential clinical utility cannot be established from these data.

Objective III. Use of Clinical Biomarkers

To evaluate patterns of use of biomarkers in clinical practice, and adaptation of clinical practice to available clinical guidelines (PCWG).

As discussed in the **Introduction, Section 4.3**, the particularities of prostate cancer management discussed above and the rapid changes the field has experienced have significantly complicated the management of prostate cancer patients in recent years. Clinicians are now challenged with multiple treatment options without clear data on the best treatment sequencing and the adequate follow-up and use of biomarkers, which makes clinical decision-making more complex.

Prostate-specific antigen (PSA), bone scans, and Response Evaluation Criteria in Solid Tumors (RECIST) criteria are commonly utilized to evaluate responses and are recommended as outcome measures by the Prostate Cancer Working Group (PCWG2) for clinical trials.[11] However, these biomarkers have significant limitations. In particular, PSA and bone scans do not allow early response assessment, and none of the biomarkers provide patient-level surrogates of clinical benefit.[363,364] This challenge is compounded by the lack of RECIST-evaluable disease in a substantial proportion of patients.[159] For daily clinical practice, existing guidelines do not recommend specific treatment monitoring, an issue addressed by the Advanced Prostate Cancer Consensus conference.[52,365]

The lack of adequate biomarkers may impact the dose intensity of chemotherapy and other anticancer (hormonal, radiopharmaceutical) agents administered in daily clinical practice. The fact that determining disease progression in the absence of clear clinical deterioration is impossible before 12 weeks (owing to the possibility of an early PSA or bone scan ‘‘flare reaction’’) in patients with no RECIST-evaluable disease may contribute to both the administration of more chemotherapy cycles to patients with bone-only disease (overtreatment) and a higher reliance on PSA changes for early treatment discontinuation (undertreatment).

With this aim, we conducted an online survey of physicians treating mCRPC. E-mails were sent to 485 UK investigators participating in urologic cancer clinical trials, 29 physician members of the GU Group of the Swiss Group for Clinical Cancer Research, and 20 practicing prostate cancer physicians in Australia and New Zealand. 118 practicing prostate cancer physicians (22.1%) replied.

The survey focused on how physicians make treatment switch decisions, opinion on response indicators, utilization of PCWG2 criteria in routine practice, and the value of CTC counts to guide treatment switch decisions. The survey consisted of 23 questions, divided in the following four sections:

1. General questions on clinical practice.
2. Familiarity with progression criteria for currently established

biomarkers.

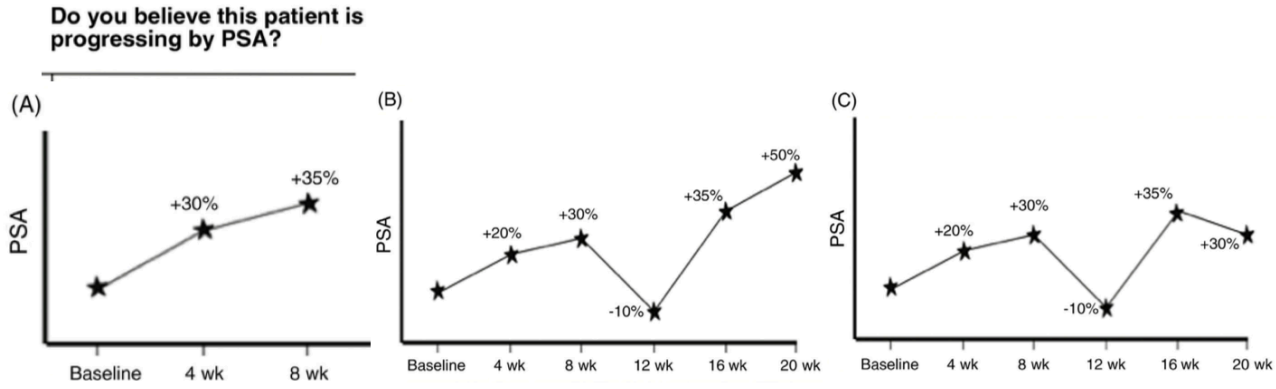
3. CTCs and their assessment in patients with advanced prostate cancer. 4. Clinical decision-making using response indicators.

I. Opinion on Biomarkers and Familiarity with Progression Criteria

We evaluated the opinion of physicians on currently available biomarkers (PSA, bone scan, and CTCs) for monitoring response. 79 respondents (74.5%) rated these as useful (71.7%) or very useful (2.8%). Only 39.6% reported using PCWG2 criteria most or all of the time, and 27.3% reported rarely or never using the criteria. A total of 59 respondents (55.7%) reported that PSA was a useful/very useful biomarker for monitoring response to treatment.

Despite the favorable opinion of the clinically available biomarkers, we therefore found that only approximately one third of physicians used them regularly in clinical practice. We also found an insufficient awareness on the actual PCWG2 criteria in prostate cancer. We asked, for instance, to identify PSA progression in graphical examples (Figure 12). In this example, the first graph (a) represents an increase before 12 weeks have elapsed, and therefore does not correspond with progression. The second (b) graph represents a 25% increase over nadir, confirmed by a second progression, and therefore does constitute progression and (c) represents a 25% increase over nadir, confirmed by a second read despite the fact that the second progression value is slightly lower than the first progression value, which also represents progression by PCWG2. Only 41.4% of physicians correctly recognized that at least 12 weeks are required to define PSA progression (graph a). Eighty-four percent correctly identified that a 25% increase from the nadir value (confirmed by a second value at least 3 weeks later) constituted progression (graph b); however, 91% failed to recognize that PSA progression holds even if the confirmatory second value is lower than the first, providing both values show a 25% increase from the nadir (graph c).

Figure 12. Questionnaire - PSA Progression



We then evaluated the awareness on bone scintigraphy criteria for progression. Participants were asked to choose from a number of definitions. Only 39.4% answered the correct option (as per PCWG2) and discarded the incorrect options, indicating diversity in bone scan interpretation.

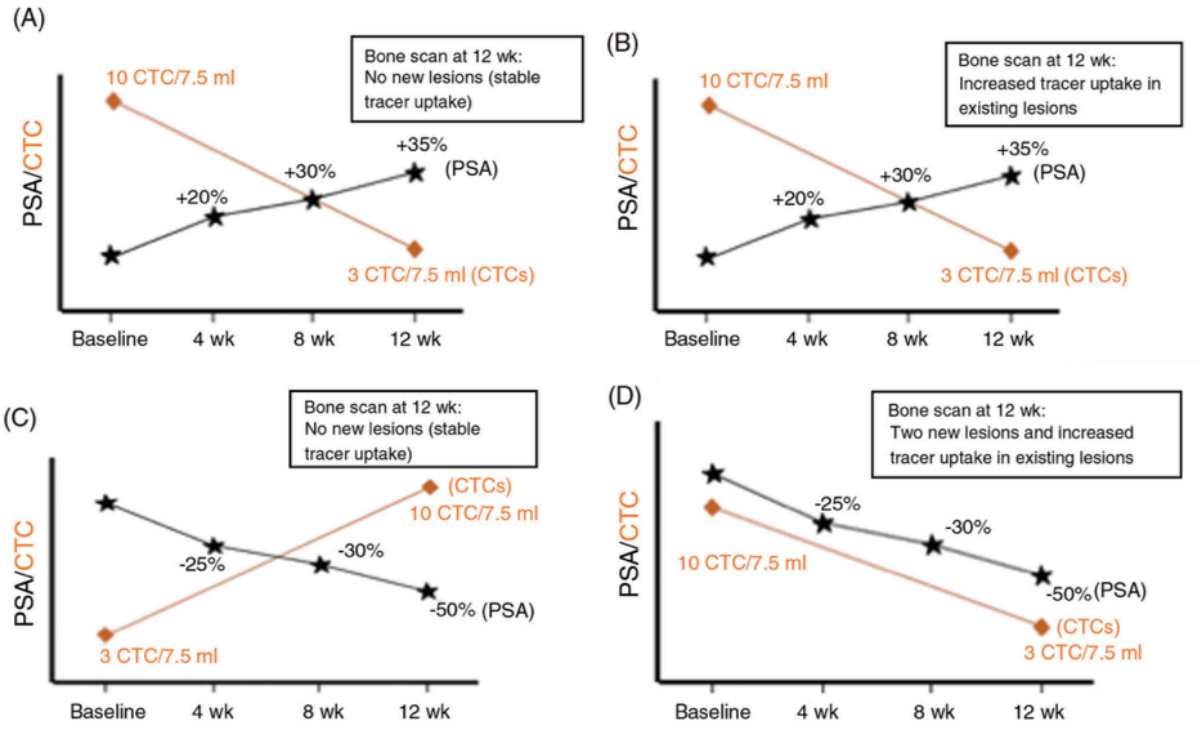
We also interrogated participants on their awareness of the value of CTCs. As discussed in the Introduction, CTC enumeration (divided into high [≥5 CTCs/7.5 mL] and low [<5 CTCs/7.5 mL]) has a proven prognostic role, validated in a number of different clinical trials.[29,327,329,332] Different studies have established an association with CTC declines in patients with baseline unfavorable CTCs, and of an increase in CTCs in patients with baseline favorable CTCs.[327,333,366,367] Ninety-eight percent of respondents were familiar with the concept of CTCs, but only 53.1% recognized that baseline CTCs have prognostic value. Similarly, only 50.0% and 54.1% respondents were aware that a post-treatment change in CTCs was associated with outcome in patients treated with abiraterone and chemotherapy, respectively.

II. Clinical Decision-Making in CRPC

We asked participants on the reasons for treatment discontinuation in their daily clinical practice. Almost all physicians (90.5%) considered clinical progression to be important for driving discontinuation, and an initiation of a new line of treatment. 71.6% and 47.7% felt RECIST and bone scan progression to be important, and only 23.2% and 21.1% felt CTC and PSA progression to be important, respectively. Despite the fact that 74,5% considered PSA useful/very useful in guiding therapy, only 21.1% considered it important for decision-making. Furthermore, only 30% of physicians who considered PSA important/very important in guiding a change of treatment recognized that at least 12 weeks are needed to define PSA progression.

We also asked participants to indicate what their attitude would be considering four different clinical scenarios assuming bone-only disease and the absence of clinical worsening, with conflicting biomarker results (Figure 13). In the first scenario (a), a situation compatible with a CTC response, with progression by PSA and no change in bone scans was shown. The second (b) scenario again showed a CTC response, with progression by PSA and increased tracer uptake in the bone scans (which does not qualify as disease progression by PCWG2 criteria). The third (c) scenario showed an opposite situation to the previous ones, with an increase in CTCs but a response by PSA, and no changes in bone scans. Finally, the fourth (d) scenario depicted a decline in both CTCs and PSA, but an increased uptake and new bone lesions in the week 12 bone scan (which requires, by PCWG2 criteria, a second bone scan to confirm progressive disease). In the same scenarios, we interrogated whether the response would be the same in patients receiving abiraterone/enzalutamide or chemotherapy.

Figure 13. Questionnaire - Clinical Scenarios



In patients with a CTC conversion and unchanged bone scans, but PSA progression (scenario a), an overwhelming majority (92%) would not switch therapy. No physician would change therapy in this scenario. When asked about a patient in this same scenario but with a bone scan showing increased uptake (scenario b), not consistent with progression by bone criteria, however, the proportion of physicians that would continue therapy was reduced to 69%; 13% would stop therapy, and up to 18% reported to be unsure. In patients with a PSA response and a CTC increase and no change in bone scan (scenario c), again approximately 11% of physicians would change treatment, while approximately 57% would continue treatment. Finally, with PSA and CTC declines but a bone scan with an unconfirmed progression, 10% of physicians would change therapy while 71% would continue.

In patients with PSA progression and CTC decline, in the absence of radiographic progression, most physicians were unlikely to switch/stop chemotherapy (83.2%) or abiraterone/enzalutamide (90.5%), recognizing therefore the value of CTC declines but also following guidelines by the PCWG. Similarly, only 33.7% of respondents were ready to use CTC changes alone, independently of PSA or bone scans, to guide switching/stopping therapy in patients with bone-only disease. Among those who were unlikely/unwilling to switch on CTC changes alone, 57.6% cited uncertainty over predictive information on treatment response as a challenge in use of CTCs, with 52.5% and 42.4% citing uncertainty over prognostic significance and difficulty in interpreting CTC changes, respectively.

Limitations to our study include a survey return rate was 22.1%, with not all physicians completed the entire survey. Reasons for not completing the survey are unknown, although this could be related to the lack of compensation offered. Furthermore, no distinction was made between academic and nonacademic centres, and no comparison was made between UK-based and non–UK- based physicians. To maximize the yield of information and study participants, the size of the questionnaire included only three questions on biomarker criteria, which may be insufficient to fully evaluate physician knowledge.

From this study, we were able to draw the following conclusions:

(a) Awareness of the PCWG2 criteria is suboptimal, despite most physicians considering currently available biomarkers (74.5%), and PSA in particular (55.6%), to be useful for monitoring disease. In particular, many physicians were unable to acknowledge the possibility of a PSA flare in evaluating PSA progression. Furthermore, Only 39.4% of respondents followed the PCWG2 definition of bone scan progression, despite recent studies indicating an association between radiographic progression-free survival (combining a bone scan and RECIST) and survival in the COU-AA-302 and PREVAIL trials.[25,174] There was no significant association between the perception of the importance of biomarkers and the ability to correctly interpret progression.

(b) Despite the fact that clinical progression is challenging to define and liable to subjective interpretation it was, by far, the most important feature to change treatment in advanced prostate cancer. This underscores the need to develop biomarkers, since the goal of treatment is to prevent worsening of symptoms and prolong survival, which is exactly what occurs when clinical progression ensues. There is also evidence that patients with clinical progression (patients with pain or ECOG PS status deterioration) respond worse to treatment and have a worse prognosis.[26] One can argue, therefore, that continuing treatment until clinical progression is not an adequate strategy, and that improved biomarkers are necessary to change treatment before clinical deterioration ensues.

(c) When confronted with contradictory biomarker results (PSA response and CTC progression, CTC conversion and PSA progression), physicians tended to assume a conservative attitude and continue treatment if the patient was clinically stable. Only approximately 10-15% of physicians would interrupt treatment. An increased uptake in bone metastases, despite not being an indicator of progression, did increase slightly the proportion of patients that would stop treatment.

(d) Despite the well documented value as a prognostic and treatment-response biomarker, only half of the responding physicians were familiar with available CTC data, with very few prepared to stop abiraterone (9.5%) or chemotherapy (16.8%) on the basis of CTC progression. Nonetheless, physicians cognizant of available CTC data were more willing to guide treatment according to CTC changes. Cost was reported as a major caveat to the routine use of CTCs, although most of this could be easily recouped by earlier discontinuation of ineffective

treatment. This underscores the need improve physician education on the value of CTC enumeration, and to design clinical trials where the use of CTCs leads to improved outcomes, evidence which is, to date, lacking.

Translational Impact

In this study, we underscore the gap between academic evidence from clinical studies and everyday practice. We highlight the need not only for improved biomarkers, but also for improved physician education on evidence-based measures of response, that may enable changes in treatment before clinical deterioration appears. We also define some of the hurdles that the development of CTCs as a clinical biomarker may face before it is incorporated as a biomarker in advanced prostate cancer.

Conclusions

Our work highlights the relevance of clinical and molecular biomarkers in the treatment of advanced prostate cancer, which bring forth the promise of molecular oncology for the personalized treatment of cancer. We suggest methods to improve tissue acquisition, evaluate the role of circulating biomarkers and assess the challenges in the translation and implementation of these biomarkers into daily clinical practice.

1. We develop and validate a score based on CT imaging (Hounsfield Units) and blood (Lactate Dehydrogenase) parameters for the prediction of bone marrow biopsy positivity, which can help clinicians select the adequate patients for the procedure, and may help generalize this biopsy procedure beyond the research setting in highly specialized academic centers where it is currently performed.
2. We define the prognostic value of circulating tumor cells in chemotherapy and hormone-treated metastatic, castration-resistant prostate cancer patients, and prove its added value to currently established prognostic biomarkers.
3. We affirm the value of post-therapy changes in circulating tumor cells, both as a 30% decline in patients with high baseline counts, and as a CTC rise in patients with low baseline counts. Using this strategy, we are able to classify all patients are eligible for response assessment, and overcome one of the major pitfalls of post-therapy CTC enumeration assessment which is the potentially high rate of non-evaluable patients.
4. We propose NLR-3 as an optimal cut-off point based on statistical grounds, which would now need validation in prospective studies.
5. We establish the prognostic role of baseline NLR in patients treated with second-line chemotherapy, although the lack of significant addition in the prognostic ability compared with currently available biomarkers may call into question the exact clinical utility.
6. We prove that the prognostic value of NLR counts is independent of baseline use of corticosteroids.
7. We set forth the potential role of “NLR responses” (high to low NLR counts after therapy) as a potential response, pending formal comparison with currently established response biomarkers such as PSA.
8. We prove that, despite a favorable general opinion of current biomarkers by physicians treating prostate cancer, general knowledge on the specific criteria for response or progression are deficient, suggesting a lack of generalized use or a systematic misuse of currently available biomarkers.
9. We show that, although a number of biomarkers are available for clinical decision-making, physicians continue to rely on clinical progression as the main factor for a treatment change in advanced prostate cancer. This suggests that better biomarkers are needed, since avoiding clinical progression is one of the main goals for non-curative treatment options as those available in mCRPC.

10. We describe how, in the face of contradictory biomarker results, physicians tend to be conservative and continue treatment, highlighting the lack of preference of one specific biomarker over another.
11. We highlight some of the difficulties for implementation of circulating tumor cells in general practice, such as cost, lack of confidence and insufficient background on the established value of the biomarker.

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Publications

Castration-Resistant Prostate Cancer Tissue Acquisition From Bone Metastases for Molecular Analyses. Lorente et al. Clinical Genitourinary Cancer 2016;14(6):485-93.

Original Study

Castration-Resistant Prostate Cancer Tissue Acquisition From Bone Metastases for Molecular Analyses

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Abstract

We analyzed 115 iliac crest bone marrow biopsy specimens from 101 patients with metastatic castration-resistant prostate cancer, divided into a test (n = 57) and a validation (n = 58) set. We developed a score based on computed tomography Hounsfield units and lactate dehydrogenase levels, which were associated with a positive biopsy result. The score can be used to select patients for whom a bone marrow biopsy will provide tissue for molecular characterization.

Background: The urgent need for castration-resistant prostate cancer molecular characterization to guide treatment has been constrained by the disease's predilection to metastasize primarily to bone. We hypothesized that the use of clinical and imaging criteria could maximize tissue acquisition from bone marrow biopsies (BMBs). We aimed to develop a score for the selection of patients undergoing BMB. **Materials and Methods:** A total of 115 BMBs were performed in 101 patients: 57 were included in a derivation set and 58 were used as the validation set. The clinical and laboratory data and prebiopsy computed tomography parameters (Hounsfield units [HUs]) were determined. A score for the prediction of biopsy positivity was developed from logistic regression analysis of the derivation set and tested in the validation set. **Results:** Of the 115 biopsy specimens, 75 (62.5%) were positive; 35 (61.4%) in the test set and 40 (69%) in the validation set. On univariable analysis, hemoglobin ($P = .019$), lactate dehydrogenase ($P = .003$), prostate-specific antigen ($P = .005$), and mean HUs ($P = .004$) were selected. A score based on the LDH level ($\geq$ 225 IU/L) and mean HUs ($\geq$ 125) was developed in multivariate analysis and was associated with BMB positivity in the validation set (odds ratio, 5.1; 95% confidence interval, 1.9%-13.4%; $P = .001$). The area under the curve of the score was 0.79 in the test set and 0.77 in the validation set. **Conclusion:** BMB of the iliac crest is a feasible technique for obtaining tumor tissue for genomic analysis in patients with castration-resistant prostate cancer metastatic to the bone. A signature based on the mean HUs and LDH level can predict a positive yield with acceptable internal validity. Prospective studies of independent cohorts are needed to establish the external validity of the score.

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Introduction

Prostate cancer is currently the second most common cancer in men, accounting for 15% of male cancer cases. Prostate cancer is the fifth leading cause of death in men worldwide (6.6% of total deaths) and is a major cause of morbidity.[1] Death from this disease follows the development of metastatic castration-resistant prostate cancer (mCRPC), for which no validated predictive molecular biomarkers to aid treatment selection are available to date. The low cost and high throughput evaluation of tumor genomes and transcriptomes is, nevertheless, rapidly enabling unprecedented opportunities to pursue the study of putative predictive tumor biomarkers. This is especially critical as the intra- and interpatient heterogeneity of the prostate cancer genome is described.[2,3]

We have previously described how the optimal evaluation of novel agents for the treatment of mCRPC requires the pursuit of a pharmacologic audit trail.[4-6] The pharmacologic audit trail involves the study of putative predictive biomarkers for patient selection, the evaluation of pre- and post-treatment normal tissue, and tumor biopsy evaluation of target modulation by medication, and reanalysis of the tumor at disease progression after a response to determine the mechanisms of resistance. Critical to this is access to tumor tissue, although it is hoped that the molecular characterization of circulating biomarkers such as messenger RNA,[7] circulating tumor DNA,[8-10] and/or circulating tumor cells[11-13] will also have clinical utility.

Up to 90% of patients with advanced prostate cancer will have disease metastatic to the bone, with most having disease involving the pelvis. Assessment of disease in the bone, which is commonly performed by bone scintigraphy, is, at best, suboptimal. Scintigraphy currently provides no qualitative information on the activity of the lesions, and progression is determined exclusively by the appearance of new tracer uptake. Technological advances in the processing of tissue from bone biopsies has enabled the performance as a valid approach for tissue acquisition from these patients.[14] Moreover, DNA and RNA sequencing from bone biopsy specimens is now technically feasible.[15] Such biopsies are being increasingly undertaken and even mandated in clinical trials. We hypothesized that the yield of CRPC tissue from bone biopsies could be increased by routine and inexpensive, nonsimultaneous imaging guidance using computed tomography (CT) and clinical parameters. A previous report on iliac crest CRPC bone biopsies yielded 25% positive samples without imaging guidance, with lower hemoglobin, greater alkaline phosphatase, and greater lactate dehydrogenase (LDH) levels associatinzg with increased yield.[16] A more recent report evaluating the effect of abiraterone acetate on androgen signaling in bone metastases had a positive yield in 47% of bone biopsies undertaken.[17] Studies evaluating bone biopsies performed under simultaneous CT guidance reported a positive yield of $\leq$ 67%.[15] Differences in bone density parameters on pelvic CT scans (Hounsfield units [HUs]), indicating sclerotic bone reaction associated with malignant infiltration, have also been reported.[15]

In the present study, we evaluated the association of clinical and radiologic factors with bone marrow biopsy (BMB) positivity. We propose a model that can predict the success rate and maximize tumor tissue acquisition for biomarker evaluation and molecular characterization in developmental therapeutic agents for CRPC.

Materials and Methods

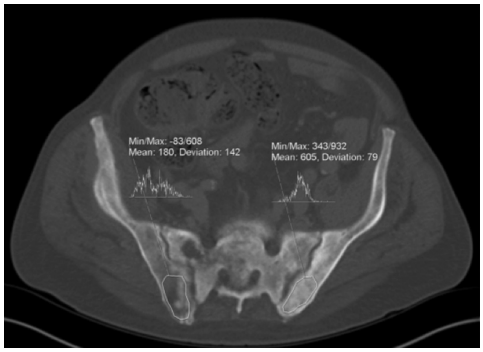
Patient Population

Patients with mCRPC who undergone a BMB from October 2011 to November 2014 at the Royal Marsden National Health Services Foundation Trust (Sutton, UK) were retrospectively identified. The criteria for inclusion in the present study were CRPC, age $\geq$ 18 years, and evidence from imaging studies (CT, bone scan, or magnetic resonance imaging) of bone metastases from prostate cancer. Patients with a CT scan of the pelvis performed $>$ 6 weeks before the biopsy were excluded. The clinical and imaging parameters were retrospectively collected from the electronic patient records. All patients provided informed consent before undergoing biopsy. The method for image acquisition (CT scanner) remained consistent throughout the study.

Tissue Acquisition and Analysis

Tissue was collected using a bone trephine biopsy from the right or left posterior iliac crest. No image guidance was used for tissue acquisition. Biopsies were performed using 8-gauge (3.05-mm) needles. The biopsy specimens were sealed in a container with a 10% parafilm solution and fixed at room temperature for 24 to 30 hours with agitation. After fixing the samples, they were briefly rinsed in distilled water, placed in a container of ethylenediaminetetraacetic acid (EDTA) solution, sealed, and incubated for about 48 hours at 37°C. The EDTA solution was prepared by (1) dissolving 50 g of sodium hydroxide in 3500 mL of distilled water; (2) adding EDTA; and (3) stirring until the solution cleared. The pH of the solution was checked and adjusted to 7.0 each day the solution was used. Next, 2-µm-thick sections were stained with hematoxylin-eosin (Figure 1) and analyzed by 1 pathologist (D.N.R.), who was unaware of the clinical and imaging data. Cases were considered negative when no intact tumor cells could be identified. Positive cases, with intact tumor cells identified, were classified into those showing $<$ 50 cells and those showing $\geq$ 50 cells.

Figure 1 Computed Tomography Parameters in the Posterior Iliac Crest



Imaging Studies

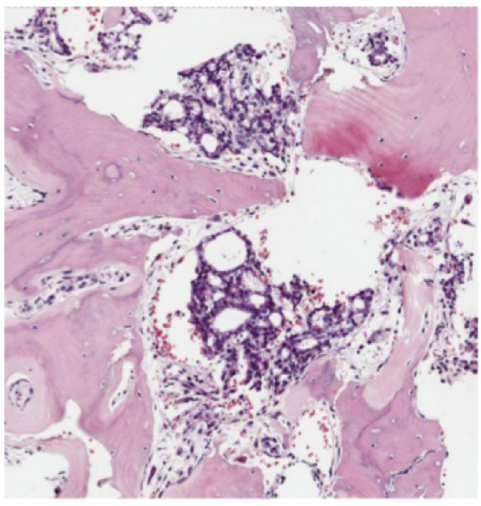
Patients with a CT scan of the pelvis performed > 6 weeks before the biopsy were excluded from the analyses. The images were analyzed by an experienced radiologist (N.T.) specializing in the field of prostate cancer. An area with a diameter of 0.8 to 1 cm (depending on the patient's anatomy) was drawn in the posterior aspect of the iliac crest in a region thought to be representative of the biopsied area; the location was equivalent for all patients. The mean HU of the biopsy site (left or right) was determined in 3 consecutive slices (5 mm thickness), and the average value was used in the analyses (Figure 2). The bone scans were reviewed for the presence of metastatic disease in the iliac crests and to estimate the bone tumor burden, classified as < 5 bony sites, 5 to 20 bone metastases, or > 20 metastases, indicating widespread disease.

Statistical Analysis

A descriptive analysis of the baseline laboratory and imaging features was performed, and the median and interquartile range (IQR) are reported. Random assignment algorithms were used to allocate biopsies to the test or the validation group. The test group was used to obtain a model for the prediction of positivity in BMBs. The dependent variable of the model (bone marrow positivity) was defined as the presence of tumor in the processed tissue. The cutoff values for dichotomous variables were established from the test set. Those that presented with greater receiver operating characteristic (ROC) area under the curve (AUC) values were selected for development of the predictive model, which was validated in the second, validation group. The mean values of the baseline parameters between the groups were compared using the Student *t* test.

Univariable analyses were performed using logistic regression models with only 1 covariate. Variables with a statistically significant association to the dependent variable ($P < .05$) were selected for inclusion in a multivariable logistic regression model, with bone marrow positivity as the dependent variable. Internal validity of the model was tested by establishing the ROC AUC in the test set (Figure 3). External validity was established by determining the ROC AUC in the validation set (Figure 3). Statistical significance was determined by testing the obtained AUCs against a null hypothesis of 0.5. The sensitivity, specificity, and positive and negative predictive values of the model were determined in the test and validation sets. The observed positivity rate of the biopsy specimens in the whole cohort was used as the prevalence value for the calculation of the predictive values. The score was then tested for its association with bone marrow positivity, defined as biopsy specimens yielding ≥ 50 tumor cells using logistic regression modeling. All statistical procedures were performed using SPSS Statistics, version 20 (IBM Corp., Armonk, NY).

Figure 2 Hematoxylin and Eosin Staining of a Positive Bone Marrow Biopsy



Results

Samples and Patient Characteristics

A total of 115 biopsies in 101 patients were performed from October 19, 2011 to November 11, 2014. Overall, 75 biopsies (65.2%) were positive. Of these, 20 biopsies (26.7%) yielded < 50 cells and 55 biopsies (73.3%) > 50 cells. The biopsy cores had a median length of 17 mm (IQR, 12-22 mm). Of the 115 biopsies, 67 (58.3%) were acquired from the right pelvis and 48 (41.7%) from the left pelvis. The median interval from the CT scan to the performance of the biopsy was 14 days (IQR, 4-28 days). Of the 101 patients, 83 (72.2%) had received previous docetaxel and 80 (69.6%) had received previous abiraterone. Details of the last treatment before the biopsy are summarized in Table 1. In 34 biopsies (29.6%), the patients had undergone previous radiotherapy to the pelvis, and in 33 biopsies (28.7%), the patients had received previous bone targeting agents (Table 1). In total, 27 patients (23.5%) were using opioids for the treatment of bone metastatic pain at biopsy and 70.3% of patients had been revealed to have > 20 bone metastases on the bone scan.

Of the 115 biopsy specimens, 57 were included in the test set and 58 were included in the validation set. The baseline laboratory and CT (mean HU) parameters in the test and validation sets are listed in Table 2. Of the 57 biopsy specimens in the test set and 58 in the validation set, 35 (61.4%) in the test set and 40 (69%) in the validation set were positive; with no significant differences between the 2 groups ($P = .395$). The test and validation cohorts had similar prognostic baseline laboratory and CT parameter distributions, with no statistically significant differences.

Uni- and Multivariable Analysis (Test Set)

Of the 57 biopsy specimens in the test set, 35 (61.4%) were classified as positive for tumor content. The variables were first tested as continuous variables (Table 3). Only the baseline LDH ($P = .006$) and baseline prostate-specific antigen ($P = .006$) levels were significantly associated with positive biopsy results. Continuous variables were dichotomized and tested in univariable logistic regression models (Table 4). The type of previous anticancer treatment ($P = .705$), use of previous pelvic radiotherapy ($P = .120$), and previous bisphosphonate use ($P = .975$) were not associated with biopsy positivity. Low hemoglobin levels (≥ 11.5 g/dL vs. < 11.5 g/dL; $P = .019$), high LDH levels (≥ 225 IU/L vs. < 225 IU/L;

Figure 3 Receiver Operating Characteristic Curve Analysis of the Test and Validation Sets

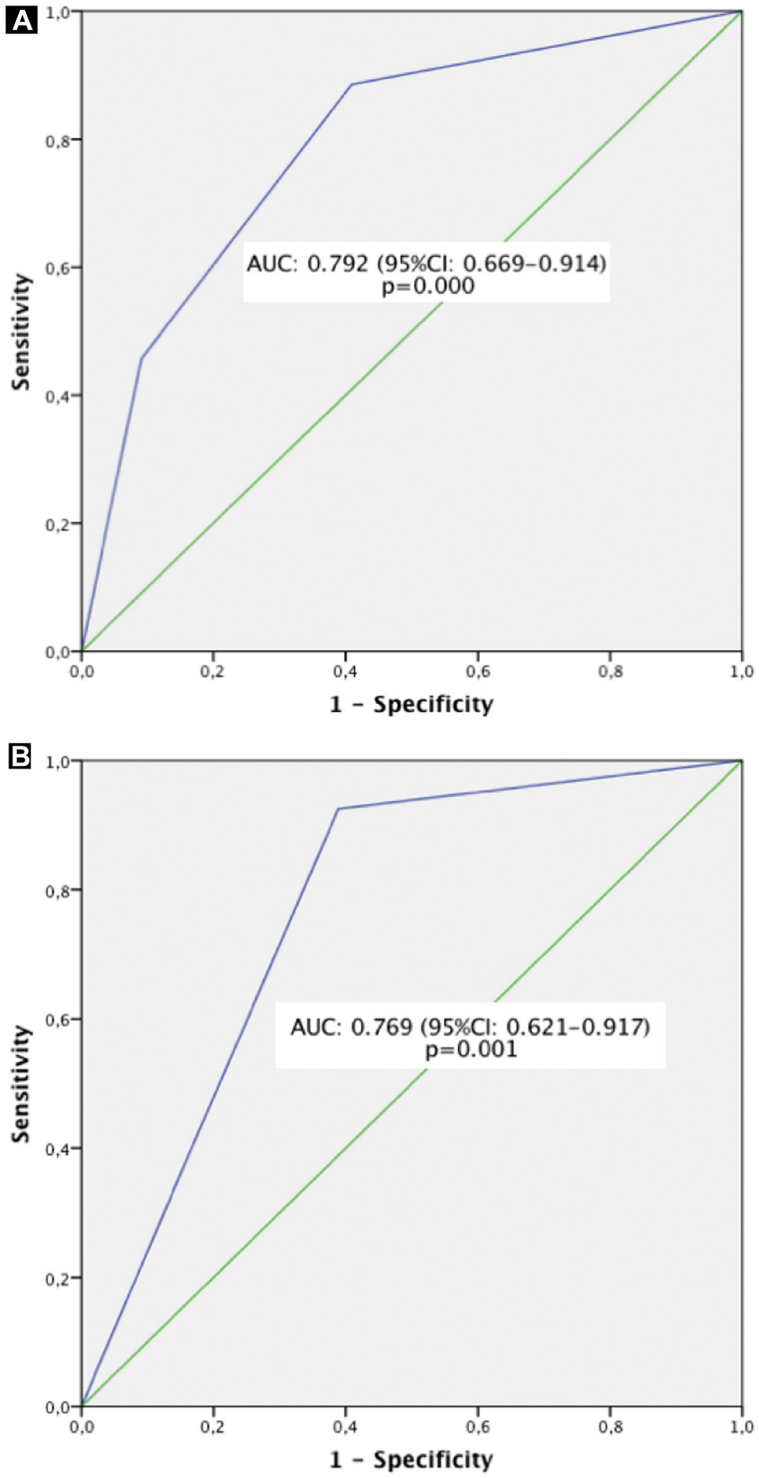


Table 1 Clinical Characteristics

Characteristic	n (%)
Total patients	115 (100)
Last treatment before BMB	
Hormonal agents[a]	70 (60.9)
Chemotherapy[b]	28 (24.3)
Other (investigational agents; phase I/II clinical trials)	17 (14.8)
Previous bone targeting agents	
None	82 (71.3)
Bisphosphonates	27 (23.5)
Radium-223	1 (0.9)
Strontium	3 (3.6)
Cabozantinib	1 (0.9)
Samarium	1 (0.9)
Previous RT to pelvis	
Yes	35 (30.4)
No	80 (69.6)
Pain requiring opioids	
Yes	27 (23.5)
No	88 (76.5)

Abbreviation: RT = radiotherapy.
[a]Abiraterone, enzalutamide, bicalutamide, goserelin, and dexamethasone.
[b]Docetaxel, cabazitaxel.

$P = .003$), PSA levels ($\geq$ 225 vs. $<$ 225 ng/mL; $P = .005$), high alkaline phosphate levels ($\geq$ 100 vs. $<$ 100 IU/L; $P = .025$), and high mean HUs on CT ($\geq$ 125 HU vs. $<$ 125 HU; $P = .004$) were significantly associated with a positive BMB and were selected for multivariable analysis. On multivariable analysis, only mean HUs $\geq$ 125 (odds ratio [OR], 3.85; 95% confidence interval [CI], 1.06-13.94; $P = .036$) and elevated LDH $\geq$ 225 IU/L (OR, 8.7; 95% CI, 1.68-45.11; $P = .003$) were significantly associated with BMB positivity (Table 5).

Predictive Score: Performance in Test and Validation Sets

From the results of the multivariable analysis in the test set, a score (BMB score) was developed by assigning 1 point to each of the parameters (0 points if neither the HUs were $\geq$ 125 nor the LDH was $\geq$ 200; 1 point if either the HU was $\geq$ 125 or LDH was $\geq$ 200; and 2 points if both the HUs were $\geq$ 125 and the LDH was $\geq$ 200). The score was significantly associated with bone marrow positivity in both the test (OR, 5.4; 95% CI, 2.1-13.7; $P < .001$) and validation (OR, 5.1; 95% CI, 1.9-13.4; $P = .001$) sets. In the validation set, the score was associated with a positive result, independent of other parameters (Tables 6 and 7). In the test set, only 23.5% of the biopsies with a score of 0 were positive compared with 77.5% of the biopsies with a score of 1 to 2 ($P < .001$). Similarly, in the validation set, only 21.4% of the biopsies with a score of 0 were positive for tumor content compared with 84.1% of biopsies with a score of 1 to 2 ($P < .001$). The AUC of the BMB score was 0.79 (95% CI, 0.67-0.91; $P < .001$) in the test and 0.77 (95% CI, 0.59-0.88; $P < .001$) in the validation set.

Table 3 Univariate Analysis (Test Set) Results: Continuous Variables

Variable	HR (95% CI)	*P* Value
Hemoglobin	0.53 (0.14-1.95)	.340
Platelets	0.75 (0.2-2.75)	.663
Neutrophils	2.44 (0.57-10.5)	.231
Lymphocytes	1.06 (0.36-3.12)	.922
NLR	1.3 (0.52-3.2)	.575
LDH	32.4 (2.69-391.6)	.006[a]
ALP	1.52 (0.77-3.02)	.231
Albumin	0.89 (0.76-1.03)	.113
PSA	1.92 (1.2-3.04)	.006[a]
Mean HU	1.01 (0.57-2.11)	.78

Hemoglobin, platelets, neutrophils, lymphocytes, NLR, LDH, ALP, PSA, and mean HUs were log-transformed.
Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HR = hazard ratio; HUs = Hounsfield units; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; PSA = prostate-specific antigen.
[a]Statistically significant.

Sensitivity, Specificity, and Predictive Values

We established the sensitivity, specificity, and predictive values of each of the parameters in the model. The global positivity rate (65.2%) was used to calculate positive and negative

Table 2 Baseline Laboratory and Computed Tomography Parameters

Variable	All Biopsies (n = 115)	Test Set (n = 57)	Validation Set (n = 58)	*P* Value[a]
Hemoglobin (g/L)	11.3 (10.7-12.8)	11.6 (10.8-12.8)	11.3 (10.6-12.8)	.868
Platelets	220 (176-270)	220 (169-276)	220 (181-269)	.911
Neutrophils	3.8 (3-5.1)	3.8 (3-5.1)	3.8 (2.9-5.2)	.906
Lymphocytes	1.1 (0.8-1.4)	1.1 (0.8-1.5)	1.1 (0.8-1.4)	.817
NLR	3.6 (2.4-6.1)	3.6 (2.1-6.3)	3.2 (2.4-6.1)	.685
ALP (IU/L)	172 (96-423)	205 (95-345)	167 (105-450)	.546
Albumin (g/L)	36 (33-38)	36 (33-38)	36 (33-37)	.268
LDH (IU/L)	196 (166-255)	198 (165.5-265.5)	195.5 (168-252)	.310
PSA (ng/mL)	212 (94-500)	212 (96.5-609)	205 (85-455)	.215
Mean HU	136.5 (27.5-235.8)	144 (42-241)	114 (5-230.5)	.282

Data presented as mean (range).
Abbreviations: ALP = alkaline phosphatase; HUs = Hounsfield units; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; PSA = prostate-specific antigen.
[a]Student *t* test for equivalence of mean values.

Table 4 Univariate Analysis (Test Set) Results: Categorical Variables (Cutoff Values)

Variable	Positive (%)	OR (95% CI)	*P* Value
Hemoglobin		0.25 (0.08-0.8)	.019[a]
<11.5	77.8 (21/27)		
≥11.5	46.7 (14/30)		
Platelets		0.97 (0.32-2.93)	.953
<200	61.9 (13/21)		
≥200	61.1 (22/36)		
Neutrophils		2.03 (0.69-6)	.200
<3.5	52 (13/25)		
≥3.5	68.8 (22/32)		
Lymphocytes		1.41 (0.48-4.17)	.534
<1	56.5 (13/23)		
≥1	64.7 (22/34)		
NLR		2.08 (0.68-6.35)	.197
<3	50 (10/20)		
≥3	67.6 (25/37)		
LDH		11.3 (2.27-56)	.003[a]
<225	44.4 (16/36)		
≥225	90.5 (19/21)		
PSA		5.75 (1.72-19.3)	.005[a]
<225	43.3 (13/30)		
≥225	81.5 (22/27)		
ALP		4.03 (1.2-13.6)	.025[a]
<100	37.5 (6/16)		
≥100	70.7 (29/41)		
Albumin		0.44 (0.13-1.47)	.441
<34	73.7 (14/19)		
≥34	55.3 (21/38)		
Mean HU		5.78 (1.76-18.93)	.004[a]
<125	35 (7/20)		
≥125	75.7 (28/37)		
Treatment before biopsy		0.87 (0.42-1.81)	.705
Hormonal	62.5 (20/32)		
Chemotherapy	64.7 (11/17)		
Other	50 (4/8)		
Previous pelvic RT		0.4 (0.12-1.27)	.120
Yes	47.1 (8/17)		
No	67.5 (27/40)		
Bisphosphonates		0.98 (0.31-3.1)	.975
Yes	61.5 (24/39)		
No	61.1 (11/18)		
Strong opioids		1.29 (0.27-6.16)	.751
Yes	66.7 (7/12)		
No	57.8 (26/45)		

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen; RT = radiotherapy.
[a]Statistically significant.

Table 5 Multivariate Analysis (Test Set) Results

Variable	OR (95% CI)	*P* Value[a]
Hemoglobin	0.68 (0.15-3.02)	.610
LDH	8.7 (1.68-45.11)	.003[b]
ALP	2.06 (0.47-9.03)	.336
PSA	2.79 (0.7-11.12)	.144
Mean HU	3.85 (1.06-13.94)	.036[b]

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen.
[a]Backward stepwise logistic regression, with *P* values calculated according to change in log-likelihood.
[b]Statistically significant.

predictive values. The mean HU number had greater sensitivity (0.80 in the test set; 0.88 in the validation set) and the LDH level had greater specificity (0.90 in the test and 0.78 in the validation set). The BMB score (0 vs. 1-2) showed a high sensitivity (0.89 in the test and 0.93 in the validation sets), with relatively low specificity (0.59 in the test set and 0.61 in the validation set; Table 8).

Ability of the BMB Score to Predict Biopsy Yield of ≥ 50 Cells

The biopsy specimens were further classified into those yielding ≥ 50 cells and < 50 cells, because of previous reports of

Table 6 Bone Marrow Biopsy Score: Uni- and Multivariable Analysis Results

Variable	OR (95% CI)	*P* Value
Univariate analysis (validation set)		
BMB score	5.07 (1.9-13.4)	.001[a]
Hemoglobin	0.34 (0.11-1.08)	.068
Platelets	0.93 (0.29-3)	.900
Neutrophils	1.20 (0.39-3.69)	.751
Lymphocytes	0.47 (0.14-1.57)	.470
NLR	1.53 (0.5-4.68)	.458
PSA	3.18 (0.95-10.6)	.060
ALP	1.54 (0.42-5.59)	.513
Albumin	0.42 (0.1-1.69)	.220
Previous pelvic RT	1.63 (0.44-5.98)	.465
Bisphosphonates	1.45 (0.34-6.16)	.613
Strong opioids	1.43 (0.38-5.48)	.598
Multivariate analysis (validation set)		
BMB score	4.18 (1.55-11.25)	.005
Hemoglobin	0.55 (0.14-2.06)	.372
ALP	1.17 (0.25-5.39)	.844
PSA	2.05 (0.53-7.99)	.300

Abbreviations: ALP = alkaline phosphatase; BMB = bone marrow biopsy; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen; RT = radiotherapy.
[a]Statistically significant.

Table 7 BMB Score: Categorical Analysis Results for Test and Validation Sets

BMB Results	Test Set			Validation Set		
	Positive BM (%)	OR (95% CI)[a]	*P* Value	Positive BM (%)	OR (95% CI)[a]	*P* Value
Any positive cells						
0	4/17 (23.5)	—	—	3/14 (21.4)	—	—
1	15/22 (68.2)	7 (1.7-171.2)	.008	21/25 (84)	19.3 (3.6-101.7)	< .001
2	16/18 (88.9)	20 (4.1-165.1)	.001	16/19 (84.2)	19.6 (3.3-115.4)	.001
Total	35/57 (61.4)	—	—	40/58 (69)	—	—
≥50 Cells						
0	1/17 (5.9)	—	—	2/14 (14.3)	—	—
1	12/22 (54.5)	19.2 (2.15-171.5)	.008	16/25 (64)	10.7 (1.9-58.7)	.007
2	10/18 (55.6)	20 (2.16-184.9)	.008	14/19 (73.7)	16.8 (2.7-102.9)	.002
Total	23/57 (40.4)			26/58 (55.2)		

Abbreviations: BM = bone marrow; BMB = bone marrow biopsy; CI = confidence interval; OR = odds ratio.
[a]BMB score of 0 used as a reference for logistic regression analysis.

phosphatase and tensin homolog status and survival in CRPC BMB samples. In those studies, biomarker status had only been considered in those biopsy specimens containing ≥ 50 cells.[18] In our studies, 23 biopsy specimens (40.4%) in the test set and 32 (55.2%) in the validation set contained ≥ 50 cells. The BMB score was associated with positivity (≥ 50 cells) in both the test (OR, 3.1; 95% CI, 1.41-6.84; $P = .005$) and the validation (OR, 3.7; 95% CI, 1.6-8.4; $P = .002$) sets. The AUC of the BMP score was 0.72 (95% CI, 0.58-0.85) in the test set and 0.73 (95% CI, 0.59-0.86) in the validation set. In the validation set, only 2 biopsy specimens (14.3%) with a score of 0 had ≥ 50 cells but 30 (68.2%) of those with a score of 1 to 2 were positive.

Table 8 Sensitivity, Specificity, and Predictive Values

Variable	Estimate (95% CI)	
	Test Set	Validation Set
BMB score (0 vs. 1-2)		
Sensitivity (%)	88.6 (74-95.5)	92.5 (80.1-97.4)
Specificity (%)	59.1 (38.7-76.7)	61.1 (38.6-79.7)
Positive predictive value (%)	78.3 (68.3-85.8)	79.9 (68.8-82.8)
Negative predictive value (%)	75.6 (53.7-89.3)	83 (60.8-93.9)
Mean HU ≥ 125		
Sensitivity (%)	80 (64.1-90)	87.5 (73.9-94.5)
Specificity (%)	59.1 (38.7-76.7)	66.7 (43.7-83.7)
Positive predictive value (%)	75.7 (59.8-86.6)	85.4 (71.6-93.2)
Negative predictive value (%)	65 (43.3-81.8)	70.6 (46.9-86.7)
LDH ≥ 225 IU/L		
Sensitivity (%)	54.3 (38.2-69.5)	45 (30.7-60.2)
Specificity (%)	90.1 (72.2-97.5)	77.8 (54.8-91)
Positive predictive value (%)	90.5 (71.1-97.4)	81.8 (61.5-92.7)
Negative predictive value (%)	55.6 (39.6-70.5)	38.9 (24.8-55.1)

Abbreviations: BMB = bone marrow biopsy; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase.

Discussion

With the advent of novel agents for the treatment of CRPC and the improved understanding of the molecular biology mechanisms driving disease progression beyond castration, the improvement of mechanisms for tissue acquisition and molecular analysis has become of paramount importance. Up to 89% of patients with mCRPC might harbor clinically actionable genomic aberrations.[19] Furthermore, despite significant interpatient heterogeneity, the alterations in known oncogenic drivers have been highly concordant within the individual's metastatic sites. Assessing single metastasis through soft tissue biopsies or BMBs could therefore provide a reasonable assessment of the oncogenic landscape and prove informative for treatment selection.[3]

The propensity to spread to the bones (in many cases, the only metastatic site) is a distinct characteristic of prostate cancer. Thus, a large proportion of patients do not have soft tissue metastases amenable for biopsy. A number of studies published in the past decade have reported variable rates of positive BMBs ranging from 25% to 50% for nonimaging-guided biopsies[16,17,20] and increasing to 67% to 77% when performed under direct CT guidance.[15,21] Our cohort, with biopsies performed without direct CT guidance, had a bone biopsy positivity rate of 62.5%, consistent with the findings from previous reports.

Previous studies have established associations among the clinical, analytical, and CT parameters with BMB positivity.[16,21] The present study, however, is the first study to establish the value of the widely used CT and analytical parameters and develop a score with direct applicability in the clinical setting, with validation of these results in a separate control group. We have proved the predictive potential of a simple score that can help select patients likely to provide enough tissue for molecular analyses such as exome and transcriptome next-generation sequencing, which is now becoming embedded in many of our therapeutic trials in CRPC. In a recently published multi-institutional CRPC genomic sequencing project,[19]

29% of all sequenced tissue was from bone metastases, highlighting the importance of adequate patient selection for the performance of BMBs.

The high sensitivity of the BMB score supports its use for the identification of patients with a low likelihood of a positive result. We would therefore recommend not performing the procedure in patients with a score of 0 (ie, if the bone density of the iliac crest does not exceed a HU of 125 and the LDH levels are < 225 IU/L). In such cases, the probability of achieving a negative result (negative predictive value) is about 76% to 83% compared with a 78% to 79% chance of a positive result (positive predictive value) if the score is > 0. Extrapolating our findings to the validation set, excluding patients with a score of 0 would have "saved" 11 patients (18.9%) from undergoing biopsy with negative results and would have only "missed" 3 (5.2%) biopsies with positive results, increasing the positive yield from 69% to 84.1%. The model presents high internal validity, as determined by the AUC model obtained when testing the ROC AUC in the test and validation sets, which had very similar AUC values.

Our study had a number of limitations. The variety of treatments received by the patients could have made our data set less homogeneous than that of other cohorts of biopsies performed in the setting of clinical trials.[16] Furthermore, our patient population represented patients with advanced, CRPC and a high burden of bone metastases. It remains unclear whether our BMB score would be valid for patients with earlier disease stages. Finally, because all biopsies were performed in a single center, validation of the score is needed in independent centers for external validity of the score to be established. The high consistency of the results between the test and validation sets does, nevertheless, suggest the potential applicability in other centers that regularly perform BMBs.

Our BMB score was developed by defining positive BMBs as those with any evidence of tumor cells found after hematoxylin-eosin staining. The heterogeneity of the data set, which included patients participating in different studies over several years, precluded the association of the score with the successful determination of specific molecular biomarkers. However, previous studies reporting an association of phosphatase and tensin homolog status (determined in soft tissue biopsies and BMBs) and survival had restricted evaluable samples to those with ≥ 50 tumor cells.[18] We have shown that our score is capable of discriminating those patients likely to yield > 50 cells. In the validation set, the exclusion of patients with a score of 0 would have increased the positivity yield from 55.2% to 68.2%.

Conclusion

Performing serial BMBs in patients with mCRPC is a feasible and valid approach for the acquisition of cancer tissue for molecular analysis. We have presented a BMB score that demonstrates how the use of imaging and laboratory parameters can help select patients and increase the rate of positive biopsy specimens.

Clinical Practice Points

- Up to 90% of patients with advanced prostate cancer have disease metastatic to the bone, which is, in many cases, the only site of metastatic disease.
- The development of circulating and tissue-based predictive biomarkers such as AR-V7 splice variants or genomic aberrations of DNA repair genes has been proposed for treatment selection in advanced prostate cancer.
- Previous reports have established the yield of non–image-guided positive BMB specimens in 25% to 47% of cases.
- Using a score based on the CT HUs (mean HU > 125) and LDH level (> 225 IU/L) can help select patients with an increased likelihood of having a positive BMB specimen from the iliac crest.
- Patients with a score of 0 (mean HUs < 125 and LDH < 225 IU/L) will have a very low BMB yield and should not be selected for the procedure.
- Optimization of the methods for patient selection for a fresh biopsy procedure could help in molecular stratification and adequate treatment selection for patients with mCRPC.

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Disclosure

A. Omlin reports travel grants from Bayer and an advisory role with AstraZeneca, Janssen, Pfizer, and Astellas. G. Attard reports personal fees from Janssen-Cilag, Veridex, Novartis, Millennium Pharmaceuticals, Takeda, and Sanofi-Aventis; personal fees and nonfinancial support from Roche/Ventana, Astellas, Medivation, and Abbott Laboratories; grants, personal fees, and nonfinancial support from Janssen; and grants from AstraZeneca. J. de Bono reports personal fees from Astellas, AstraZeneca, Johnson & Johnson, and from Medivation. The remaining authors declare that they have no competing interests.

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Platinum Priority – Prostate Cancer
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Decline in Circulating Tumor Cell Count and Treatment Outcome in Advanced Prostate Cancer

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Abstract

Background: Treatment response biomarkers are urgently needed for castration-resistant prostate cancer (CRPC). Baseline and post-treatment circulating tumor cell (CTC) counts of ≥5 cells/7.5 ml are associated with poor CRPC outcome.
Objective: To determine the value of a ≥30% CTC decline as a treatment response indicator.
Design, setting, and participants: We identified patients with a baseline CTC count ≥5 cells/7.5 ml and evaluable post-treatment CTC counts in two prospective trials.
Intervention: Patients were treated in the COU-AA-301 (abiraterone after chemotherapy) and IMMC-38 (chemotherapy) trials.
Outcome measures and statistical analysis: The association between a ≥30% CTC decline after treatment and survival was evaluated using univariable and multivariable Cox regression models at three landmark time points (4, 8, and 12 wk). Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC) and c-indices.
Results: Overall 486 patients (122 in IMMC-38 and 364 in COU-AA-301) had a CTC count ≥5 cells/7.5 ml at baseline, with 440, 380, and 351 patients evaluable at 4, 8, and 12 wk, respectively. A 30% CTC decline was associated with increased survival at 4 wk (hazard ratio [HR] 0.45, 95% confidence interval [CI] 0.36–0.56; $p < 0.001$), 8 wk (HR 0.41, 95% CI 0.33–0.53; $p < 0.001$), and 12 wk (HR 0.39, 95% CI 0.3–0.5; $p < 0.001$) in univariable and multivariable analyses. Stable CTC count (<30% fall or <30% increase) was not associated with a survival benefit when compared with increased CTC count. The association between a 30% CTC decline after treatment and survival was independent of baseline CTC count. CTC declines significantly improved the AUC at all time-points. Finally, in the COU-AA-301 trial, patients with CTC ≥5 cells/7.5 ml and a 30% CTC decline had similar overall survival in both arms.

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Conclusions: A 30% CTC decline after treatment from an initial count ≥5 cells/7.5 ml is independently associated with CRPC overall survival following abiraterone and chemotherapy, improving the performance of a multivariable model as early as 4 wk after treatment. This potential surrogate must now be prospectively evaluated.
Patient summary: Circulating tumor cells (CTCs) are cancer cells that can be detected in the blood of prostate cancer patients. We analyzed changes in CTCs after treatment with abiraterone and chemotherapy in two large clinical trials, and found that patients who have a decline in CTC count have a better survival outcome.

1. Introduction

Prostate cancer is the second most common malignancy in men, and the fifth leading cause of death from cancer worldwide [1]. Although initially responsive to androgen deprivation, lethal castration-resistant prostate cancer (CRPC) ultimately develops. In recent years, unprecedented advances in drug development for CRPC have been observed with the approval of abiraterone, enzalutamide, cabazitaxel, and radium [2–7].

One of the greatest challenges in the current management of CRPC is adequate assessment of response to treatment. A significant proportion of patients present with disease exclusively in bone, which is not amenable to evaluation by the commonly used Response Evaluation Criteria in Solid Tumors (RECIST). Consensus Prostate Cancer Working Group 2 (PCWG2) criteria [8] rely on bone scintigraphy and changes in prostate-specific antigen (PSA) levels to evaluate response to treatment in these patients. Progression according to bone scintigraphy is not evaluable before 16 wk because of the possibility of spurious flare reactions [9], so a confirmatory scan is required after a first scan indicating progression. Likewise, evaluation of prostate-specific antigen (PSA) values for progression is not recommended before 12 wk of treatment. Most studies evaluating PSA declines as a surrogate of survival have yielded negative results [10–12] and treatment discontinuation based solely on rising PSA values is not recommended [8]. Recent studies have reported a stronger association between radiological progression-free survival (rPFS) and overall survival (OS); however, a definition of progression according to rPFS cannot currently be acquired before at least 12–16 wk of treatment, and is difficult to evaluate in men with widespread bone involvement [13]. Improved biomarkers to identify patients not benefitting from anticancer treatment are urgently needed.

Enumeration of the circulating tumor cell (CTC) count has emerged as a powerful biomarker for evaluating prognosis and treatment response in CRPC. The utility of the CellSearch assay (Janssen Diagnostics, Raritan, NJ, USA) in classifying counts into unfavorable (≥5 cells/7.5 ml) and favorable (≤4 cells/7.5 ml) prognostic groups has been proven in prospective trials including IMMC-38, COU-AA-301, AFFIRM, and SWOG-S0421 [14–19]. Association between post-treatment CTC changes and CRPC survival has been reported in terms of CTC conversion (change from unfavorable at baseline to favorable or vice versa) [14], fold-change in CTC [17], and a 30% CTC decline from baseline [16], and it has been shown that CTC count has superior performance to other circulating biomarkers including PSA. CTCs have also been evaluated as a surrogate endpoint in several prospective trials. In the COU-AA-301 trial, a composite biomarker panel comprising CTC and lactate dehydrogenase (LDH) at 12 wk after treatment satisfied the Prentice criteria for surrogacy at the individual patient level [20]. It is envisaged that validation of these results in further prospective clinical trials could contribute to testing trial-level surrogacy so that CTC counts could become a clinical trial endpoint to accelerate drug approval for advanced CRPC.

We carried out a post hoc analysis of data for patients in the prospective IMMC-38 (chemotherapy) and COU-AA-301 (abiraterone) trials with baseline CTC ≥5 cells/7.5 ml, evaluating the value of a 30% CTC decline from baseline at 4, 8, and 12 wk as a biomarker of response to treatment.

2. Patients and methods

2.1. Study population and procedures

We performed a post hoc analysis of the COU-AA-301 and IMMC-38 trials. COU-AA-301 was a phase 3 trial in which postchemotherapy patients with metastatic CRPC were randomly assigned to abiraterone and prednisone or placebo and prednisone. IMMC-38 was a prospective, open-label study in patients with metastatic CRPC undergoing treatment with chemotherapy. Details of the methodology and the final results for both trials have been published elsewhere [2,14,21]. Both studies were approved by local institutional boards. All patients provided written informed consent before participation. CTC counts were measured at baseline and on day 1 of cycle 2 (weeks 4–5), day 1 of cycle 3 (weeks 8–9), and day 1 of cycle 4 (weeks 12–13) in the COU-AA-301 trial. In the IMMC-38 trial, CTC counts were measured in weeks 2–5 (median 4 wk), weeks 6–8 (median 7 wk), and weeks 9–12 (median 11.9 wk). All CTC counts were measured using the CellSearch assay [22]. Hemoglobin (Hb), alkaline phosphatase (ALP), albumin (ALB), and LDH concentrations were measured at baseline and at each study visit. Eastern Cooperative Oncology Group performance status (ECOG-PS) was recorded at baseline. PSA levels were measured every 4 wk in IMMC-38 and every 12 wk in COU-AA-301.

2.2. Statistical analysis

Kaplan-Meier analysis was used to estimate survival. Univariable and multivariable Cox proportional hazards models were used to test the association between the response biomarker and survival. Logistic regression models were used to calculate odds ratios (ORs). Post-treatment CTC response was defined as a 30% decline from baseline at 4, 8, and 12 wk from treatment initiation. A landmark analysis was used to explore the association between CTC response and survival, and specific 4-, 8- and 12-week populations were defined (Supplementary Fig. 1).

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Table 1 – Baseline characteristics for the whole trial population

	All patients	COU-AA-301	IMMC-38
Patients (*n*)	486	364	122
CTC count (cells/7.5 ml)	19.5 (9–43.8)	18 (9–38.5)	24 (10–97)
PSA (ng/ml)	214.4 (69–579)	197.3 (64.8–570)	244 (90–604)
ALP (U/l)	216 (121–385.5)	205.5 (116–401.5)	231 (129.8–363.8)
LDH (U/l)	263 (199.3–389.5)	267 (199.5–384.8)	250 (199.3–404.8)
Hemoglobin (g/dl)	11.4 (10.3–12.5)	11.2 (10.2–12.4)	11.8 (10.8–12.9)
Albumin (g/dl)	3.9 (3.6–4.2)	4 (3.7–4.2)	3.7 (3.4–4)
ECOG PS, *n* (%)[a]			
0–1	419 (87.3)	315 (86.5)	104 (89.7)
2	61 (12.7)	49 (13.5)	12 (10.3)

CTC = circulating tumor cell; PSA = prostate-specific antigen; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; ECOG PS = Eastern Cooperative Oncology Group performance status.
[a] Six missing baseline ECOG PS values in the IMMC-38 data set.

Bonferroni correction was applied to account for multiple testing at three different time points; *p* values were considered statistically significant if $p < 0.0167$. Baseline LDH, ALP, PSA, and CTC data were log-transformed because of positively skewed distributions. The overall performance of the survival models was evaluated by calculating receiver operating characteristic (ROC) curves for 6- and 11-mo survival endpoints (approx. the median and third survival quartile of the data set) and the c-index for each model using the method proposed by Uno et al [23]. The area under the ROC curve (AUC) was compared by calculating the *U* statistic (nonparametric) [24]. Bootstrapping techniques were used to calculate the 95% confidence interval (CI) of the difference between c-indices. Analyses were performed using SPSS v21 (SPSS Inc., Chicago, IL, USA) and the R statistics package v3.2.1 (R Foundation, Vienna, Austria).

3. Results

Overall, 486 patients with baseline CTC ≥5 cells/7.5 ml participating in the IMMC-38 ($n = 122$) and COU-AA-301 ($n = 364$) trials were included in the analysis. The patient inclusion criteria are presented in a CONSORT diagram in Supplementary Figure 1**)**. An analysis of patients with baseline CTC <5 cells/7.5 ml, who had significantly better outcome compared to patients with CTC ≥5 cells/7.5 ml **(**Supplementary Fig. 2**)**, will be published separately. The median follow-up was 11.2 mo (10.2 mo in IMMC-38; 11.3 mo in COU-AA-301). At the time of analysis, 360 (74.1%) patients had died, with median OS of 11.6 mo (95% CI 10.3–12.8). The median OS for patients with baseline CTC ≥5 cells/7.5 ml was comparable between IMMC-38 (11.5 mo, 95% CI 9.8–13.2) and COU-AA-301 (11.7 mo, 95% CI 10.3–13.1). The median baseline CTC was 19.5 cells/7.5 ml (24 in IMMC-38 and 18 in COU-AA-301). Other baseline characteristics are summarized in Table 1 and Supplementary Table 1.

To define the most appropriate response cutoff, we initially compared the performance of 30% and 50% CTC declines. A 30% cutoff was chosen because of its higher sensitivity in comparison to a 50% CTC decline (Supplementary Tables 2 and 3).

3.1. A 30% CTC response is associated with survival benefit

Overall, 283 (64.3%), 248 (65.3%), and 226 (64.4%) patients experienced a 30% decline in CTC count at 4, 8, and 12 wk, respectively (Table 2). A 30% CTC decline was associated with better survival at 4 wk (14.4 vs 7.9 mo; HR 0.45, 95% CI 0.36–0.56; $p < 0.001$), 8 wk (15.4 vs 7.9 mo; HR 0.41, 95% CI 0.33–0.53; $p < 0.001$), and 12 wk (16.1 vs 9.7 mo; HR 0.39, 95% CI 0.3–0.5; $p < 0.001$). The association was consistent in both the COU-AA-301 and IMMC-38 data sets (Table 2). A 30% CTC decline was associated with survival in multivariable analysis. In addition to a 30% CTC decline, baseline CTC count, and baseline LDH were associated with survival across all three landmark populations (Supplementary Table 4).

Addition of a 30% CTC decline to multivariable survival models significantly enhanced the AUC and c-indices. Addition of baseline CTC count to a multivariable model comprising baseline PSA, LDH, ALB, Hb, ALP, and ECOG PS increased the c-index marginally (0.681 at 4 wk, 0.658 at 8 wk, and 0.669 at 12 wk). Addition of a 30% CTC decline to the model caused a more pronounced increase in the c-index to 0.72 at 4 wk and 0.71 at 8 and 12 wk. Likewise the ROC curves (6- and 11-mo mortality endpoints) showed a significant increase in AUC when a 30% CTC decline was added to the models (Fig. 1).

Some 113/486 patients (23.1%) achieved a confirmed 50% PSA response. PSA response was significantly associated with a 30% CTC decline at 4 wk (OR 14.8; $p < 0.001$), 8 wk (OR 18; $p < 0.001$), and 12 wk (OR 13.6; $p < 0.001$) in both the COU-AA-301 and IMMC-38 populations (Supplementary Table 5).

3.2. CTC response and treatment arm in the COU-AA-301 trial

Of the 364 COU-AA-301 trial participants in the analysis, 245 (67.3%) received abiraterone + prednisone and 119 (32.7%) received placebo + prednisone; the abiraterone cohort had better OS (13.8 vs 9.5 mo; HR 0.75, 95% CI 0.58–0.96; $p = 0.02$). This benefit was maintained across all three landmark survival populations (Fig. 2), confirming that abiraterone provided a significant survival benefit in patients with baseline CTC ≥5 cells/7.5 ml. Overall, 162 (73.3%) patients receiving abiraterone + prednisone and 46 (43.4%) patients receiving prednisone + placebo had a 30% CTC decline, confirming the intrinsic antitumor activity of prednisone. Treatment arm was not significantly

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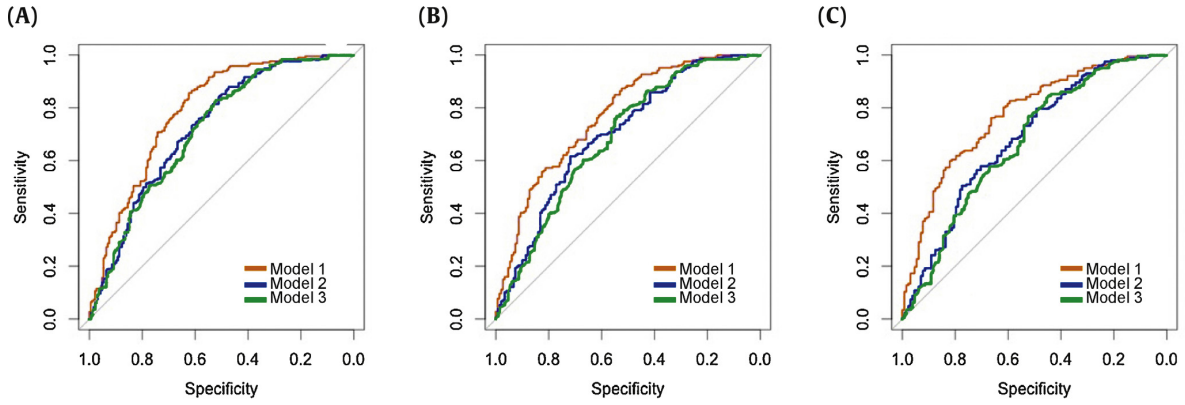
Table 2 – Association between survival and CTC response[a]

	n (%)	Median OS, mo (95% CI)	HR (95% CI)[b]	*p* value[b]
Week 4				
All patients	440	11.4 (10.5–12.4)		
Response	283 (64.3)	14.4 (12.8–15.9)	0.45 (0.36–0.56)	<0.001
Non-response	157 (35.7)	7.9 (6.9–8.9)		
IMMC-38	113	11.2 (9.7–12.6)		
Response	75 (66.4)	12.3 (8.2–16.3)	0.46 (0.29–0.74)	0.001
Non-response	38 (33.6)	6.8 (4.4–9.2)		
COU-AA-301	327	11.7 (10.3-13.1)		
Response	208 (63.6)	14.4 (13.2–15.5)	0.44 (0.34–0.57)	<0.001
Non-response	119 (36.4)	7.9 (6.9–9)		
Week 8				
All patients	380	12.5 (11.1–13.9)		
Response	248 (65.3)	15.4 (13.9–16.8)	0.41 (0.33–0.53)	<0.001
Non-response	132 (34.7)	7.9 (15.4–12.5)		
IMMC-38	84	12.3 (9.4–15.1)		
Response	56 (66.7)	17.2 (9.7–24.6)	0.42 (0.24–0.74)	0.003
Non-response	28 (33.3)	10.2 (5.5–14.9)		
COU-AA-301	296	12.6 (11.1–14.2)		
Response	192 (64.9)	15.4 (14.1–16.7)	0.4 (0.31–0.53)	<0.001
Non-response	104 (35.1)	7.7 (6.7–8.5)		
Week 12				
All patients	351	13.8 (12.3–15.3)		
Response	226 (64.4)	16.1 (14.6–17.7)	0.39 (0.3–0.5)	<0.001
Non-response	125 (35.6)	9.7 (8.3–11.1)		
IMMC-38	79	13.6 (10.6–16.6)		
Response	55 (69.6)	18.2 (11.7–24.7)	0.35 (0.19-0.63)	<0.001
Non-response	24 (30.4)	13.6 (10.6–16.6)		
COU-AA-301	272	13.9 (12.2–15.6)		
Response	171 (62.9)	15.9 (14.5–17.4)	0.41 (0.3–0.54)	<0.001
Non-response	101 (37.1)	9.7 (7.7–11.7)		

CTC = circulating tumor cell; OS = overall survival; HR = hazard ratio; CI = confidence interval.
[a] Response was defined as a 30% decline in CTC count relative to baseline at each of the landmark time points.
[b] Univariable Cox regression.



	4 wk			8 wk			12 wk		
	c-index	AUC*	*p***	c-index	AUC*	*p***	c-index	AUC*	*p***
Model 1	0.720	0.790	–	0.710	0.770	–	0.710	0.772	–
Model 2	0.681	0.726	<0.001	0.658	0.700	0.001	0.656	0.684	<0.001
Model 3	0.673	0.717	<0.001	0.651	0.686	<0.001	0.646	0.669	<0.001

Fig. 1 – Receiver operating characteristic (ROC) curves for three models at (A) 4 wk, (B) 8 wk, and (C) 12 wk. Model 1 comprised CTC response, baseline CTC (log-transformed), baseline LDH (log-transformed), and baseline ECOG status at 4 wk; and CTC response, baseline CTC (log-transformed), and baseline LDH (log-transformed) at 8 and 12 wk. Model 2 comprised baseline CTC (log-transformed), baseline LDH (log-transformed), and baseline ECOG status at 4 wk; and baseline CTC (log-transformed) and baseline LDH (log-transformed) at 8 and 12 wk. Model 3 comprised baseline LDH (log-transformed) and baseline ECOG status at 4 wk; and baseline LDH (log-transformed) at 8 and 12 wk. CTC = circulating tumor cell; LDH = lactate dehydrogenase; ECOG = Eastern Cooperative Oncology Group; AUC = area under the ROC curve.
*** Status variable: survival at 11 mo (yes vs no).**
**** Comparison of two correlated ROC curves (De Long's rest) with model 1 as the reference model.**

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Table 3 – Effect of treatment arm on multivariable models with and without CTC response in the COU-301 trial

	Model without CTC response[a]		Model with CTC response[b]	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Week 4	0.65 (0.49–0.84)	0.001	0.87 (0.65–1.17)	0.352
Week 8	0.65 (0.49–0.86)	0.003	0.9 (0.66–1.24)	0.529
Week 12	0.73 (0.53–0.98)	0.041	0.86 (0.63–1.18)	0.360

CTC = circulating tumor cell; HR = hazard ratio for treatment arm (abiraterone vs placebo); CI = confidence interval.
[a] Model includes: treatment arm; baseline CTC count (log-transformed); lactate dehydrogenase (log-transformed); albumin; alkaline phosphatase (log-transformed); hemoglobin; prostate-specific antigen (log-transformed); and Eastern Cooperative Oncology Group performance status.
[b] Model includes: 30% CTC response at 4, 8, or 12 wk; treatment arm; baseline CTC count (log-transformed); lactate dehydrogenase (log-transformed); albumin; alkaline phosphatase (log-transformed); hemoglobin; prostate-specific antigen (log-transformed); and Eastern Cooperative Oncology Group performance status.

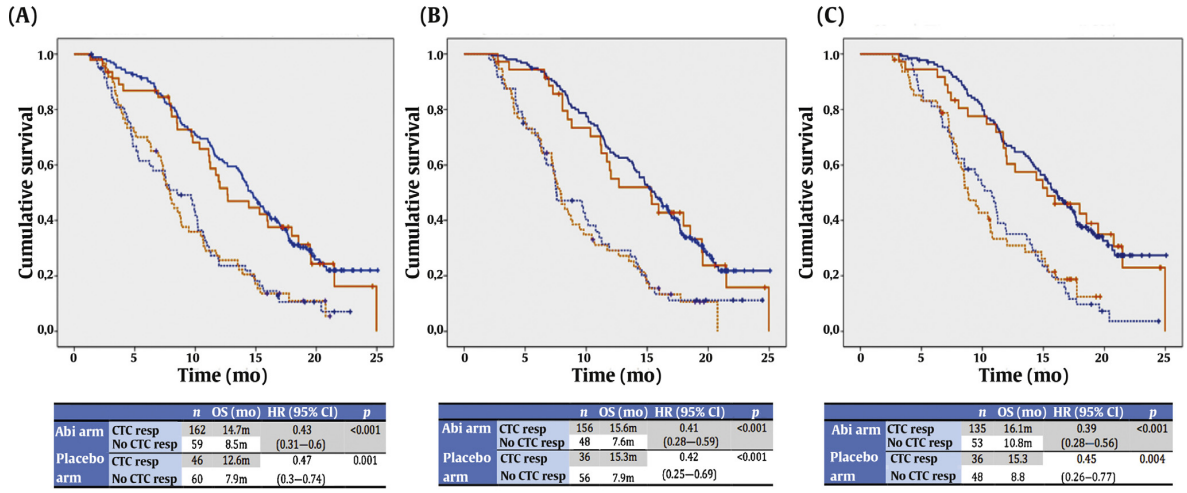

Fig. 2 – Survival in COU-AA-301 according to treatment arm and CTC response at (A) 4 wk, (B) 8 wk, and (C) 12 wk. Blue lines denote data for patients who received abiraterone + prednisone and red lines patients who received placebo + prednisone. Continuous lines indicate patients with a CTC response and dotted lines patients with no CTC response. CTC = circulating tumor cell; OS = overall survival; CI = confidence interval; Abi = abiraterone; resp = response.

associated with survival when a 30% CTC decline was included in the model. Furthermore, interaction tests between treatment arm and a 30% CTC decline were not significant ($p = 0.758$), suggesting an equivalent survival benefit for abiraterone and prednisone or prednisone alone in post-chemotherapy patients who achieved a 30% CTC decline (Table 3).

3.3. *Stable CTC count and CTC conversion*

We investigated the utility of a stable CTC count, defined as a change from baseline that did not exceed a 30% decline or a 30% increase, at each of the prespecified time points. Overall, 57 (13%), 43 (11.3%), and 42 (12%) patients experienced a stable CTC count at 4, 8 and 12 wk, respectively. A 30% CTC decline showed a significant OS benefit when compared to a stable CTC count at all time points, but no difference was observed when comparing stable and progressive (>30% increase) CTC counts (Fig. 3).

Overall, 165 (37.5%), 193 (44.3%), and 154 (43.9%) patients achieved conversion to a favorable CTC count of <5 cells/7.5 ml at 4, 8, and 12 wk, respectively. Patients achieving such CTC conversion also had a significant OS benefit at all time points studied (Supplementary Table 6). We compared AUC values for CTC conversion and 30% CTC response (6-mo OS) among all patients and among patients with baseline CTC ≥10 and ≥30 cells/7.5 ml (Supplementary Table 7). Although the AUC was consistently higher for a 30% CTC decline than for CTC conversion, no significant differences were found except for patients with high baseline CTC (≥10 cells/7.5 ml) at 4 wk (AUC 0.701 vs 0.624; $p = 0.008$).

4. Discussion

The prognostic value of baseline CTC has been evaluated in a number of studies in which patients received chemotherapy [14,17,18] and androgen receptor (AR) signaling inhibitors [19,20]. The value of a post-treatment change, defined as the percentage change from baseline in the manner for other established treatment response biomarkers such as PSA decline or a change in diameter of target lesions (RECIST), has been suggested by our group in a report on a large single-centre series [16] but has not been explored in a clinical trial data set to date. This is the first report to exclusively study patients whose CTC response

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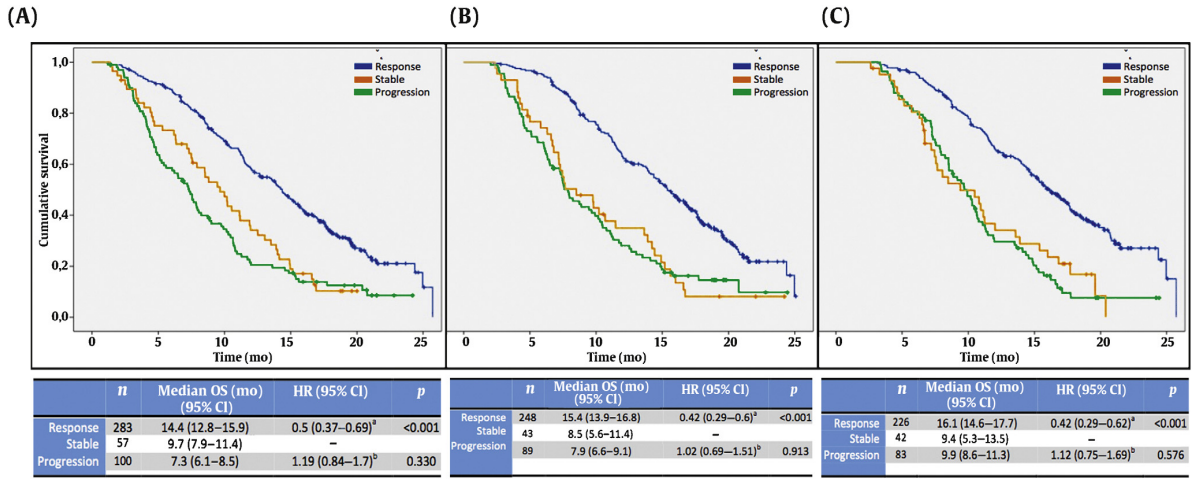

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	283	14.4 (12.8–15.9)	0.5 (0.37–0.69)[a]	<0.001
Stable	57	9.7 (7.9–11.4)	–	
Progression	100	7.3 (6.1–8.5)	1.19 (0.84–1.7)[b]	0.330

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	248	15.4 (13.9–16.8)	0.42 (0.29–0.6)[a]	<0.001
Stable	43	8.5 (5.6–11.4)	–	
Progression	89	7.9 (6.6–9.1)	1.02 (0.69–1.51)[b]	0.913

	n	Median OS (mo) (95% CI)	HR (95% CI)	p
Response	226	16.1 (14.6–17.7)	0.42 (0.29–0.62)[a]	<0.001
Stable	42	9.4 (5.3–13.5)	–	
Progression	83	9.9 (8.6–11.3)	1.12 (0.75–1.69)[b]	0.576

Fig. 3 – Overall survival (OS) according to circulating tumor cell (CTC) response at (A) 4 wk, (B) 8 wk, and (C) 12 wk. The hazard ratio (HR) and 95% confidence interval (CI) were determines using Cox regression with CTC response as the categorical variable and stable disease as the reference covariable.
[a] **Stable versus response.**
[b] **Stable versus progression.**

could be evaluated (ie, with baseline CTC ≥5 cells/5.7 ml), amounting to approximately 50% of patients with advanced CRPC (47.2% in COU-AA-301 and 57.9% in IMMC-38). An analysis of patients with baseline CTC <5 cells/7.5 ml will be published separately.

This pooled post hoc analysis for two prospective clinical trials shows that a 30% CTC decline as early as 4 wk after treatment initiation can effectively distinguish between patients benefiting from improved OS and patients not benefiting from treatment who may require a switch to an alternative therapeutic regimen.

We previously reported separate data showing that a 30% CTC decline was associated with improved OS in a smaller cohort [16]. Using larger prospective series, we now report that a post-treatment 30% CTC decline is associated with longer OS in patients treated with abiraterone + prednisone, corticosteroids alone, and chemotherapy. We considered the choice of a 30% cutoff for a number of reasons. When compared with a 50% CTC decline, although global AUC and c-index values did not differ significantly, a 30% CTC decline was a more sensitive biomarker; a test for early identification of nonresponders should value sensitivity over specificity to minimize the risk of false negatives and unnecessary discontinuation of potentially effective treatments. Likewise, establishing a percentage decline criterion for response is more sensitive than a conversion from ≥5 to <5 cells/7.5 ml. Critically, it is difficult to consider a patient whose CTC count falls from 100 to 5 cells/7.5 ml after three cycles as a "nonresponder" while considering a patient whose CTC count falls from 5 to 4 cells/7.5 ml as a "responder". The CTC threshold of ≥5 cells/7.5 ml, initially chosen to differentiate patients with and without cancer (false-positive cells identified incorrectly as CTCs by detection platforms), has limitations when estimating disease response. We also found that patients in whom CTCs do not decrease following treatment have similar OS to those whose CTCs rise following treatment, suggesting that a treatment switch may need to be considered in both groups.

Importantly, we found that the effect of a post-treatment CTC decline was equivalent in patients treated with chemotherapy and AR signaling inhibitors. HR values for responders participating in the IMMC-38 (chemotherapy) and COU-AA-301 (abiraterone after chemotherapy) trials were very similar, which supports the validity of CTC count as a response biomarker in both treatment groups. The similar median OS and baseline characteristics of both populations support the suitability of pooled analysis.

Addition of a 30% CTC post-treatment decline to multivariable models can provide independent and additional information on outcome to that provided by baseline CTC. Addition of a 30% CTC decline to the multivariable models significantly increased AUC values at all time points studied.

When analyzing the COU-AA-301 data set separately, CTC response was able to identify patients with longer survival in both the abiraterone and prednisone arms of the study. Although the frequency of a 30% CTC decline was significantly lower in the prednisone than in the abiraterone arm of COU-AA-301, patients experiencing a 30% CTC decline on prednisone had median OS comparable to that for participants experiencing a CTC response in the abiraterone arm, and higher than that for nonresponders who received abiraterone, suggesting that corticosteroids had antitumor activity in these patients.

Our study has a number of limitations. Although this is the largest analysis of patients with baseline CTC ≥5 cells/7.5 ml, limitations arising from its unplanned post hoc nature must be acknowledged. Furthermore, only 858/1195 (71.8%) patients enrolled in the COU-AA-301 trial could be evaluated for CTCs. Although CTCs were investigated until progression in the IMMC-38 study, these were only

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determined at 4, 8, and 12 wk in the COU-AA-301 study. Moreover, the value of a stable CTC count was not investigated in the COU-AA-301 and IMMC-38 data sets independently owing to a lack of sufficient events. Finally, although both median OS and baseline characteristics were similar in the data sets for both trials, approximately three times as many patients were treated with abiraterone (COU-AA-301) than with chemotherapy (IMMC-38).

5. Conclusions

In conclusion, we believe that changes in CTCs as early as 4 wk after treatment can identify patients not benefiting from treatment. Clinical trials are now under way to explore the benefit of a treatment switch in nonresponding patients. Further prospective phase 3 trials are needed to confirm the surrogate value of CTC and the CTC-LDH panel already reported for the COU-AA-301 trial [20]. We envisage that the clinical qualification of CTC count as a intermediate endpoint biomarker of OS in advanced prostate cancer may be close to a positive conclusion.

Author contributions: David Lorente had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Lorente, Olmos, Mateo, McCormack, de Bono.
Acquisition of data: Lorente, Olmos, Mateo, Bianchini, Seed, Flohr, Crespo, Figueiredo, Miranda, Baeten, Molina, Kheoh, McCormack, Terstappen, Scher, de Bono.
Analysis and interpretation of data: Lorente, Olmos, Mateo, de Bono.
Drafting of the manuscript: Lorente, Olmos, Mateo, de Bono.
Critical revision of the manuscript for important intellectual content: Lorente, Olmos, Mateo, Bianchini, Seed, Flohr, Crespo, Figueiredo, Miranda, Baeten, Molina, Kheoh, McCormack, Terstappen, Scher, de Bono.
Statistical analysis: Lorente, Olmos, de Bono.
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Administrative, technical, or material support: None.
Supervision: None.
Other: None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2016.05.023.

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Circulating Tumour Cell Increase as a Biomarker of Disease Progression in Metastatic Castration-Resistant Prostate Cancer with Low Baseline CTC Counts. Lorente et al. Annals of Oncology 2018;29(7):1554-60

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ORIGINAL ARTICLE

Circulating tumour cell increase as a biomarker of disease progression in metastatic castration-resistant prostate cancer patients with low baseline CTC counts

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Background: The development of treatment response and surrogate biomarkers for advanced prostate cancer care is an unmet clinical need. Patients with baseline circulating tumour cell (BLCTCs) counts <5/7.5 mL represent a good prognosis subgroup but are non-evaluable for response assessment (decrease in CTCs). The aim of the study is to determine the value of any increase in CTCs (CTC progression) as an indicator of progression in prostate cancer patients with low pre-treatment CTCs (<5).

Patients and methods: We carried out a post hoc analysis of patients with BLCTCs < 5 treated in the COU-AA-301 (abiraterone or placebo + prednisone) and IMMC-38 (chemotherapy) trials. The association of CTC progression (increase in CTCs at 4, 8 or 12 weeks) with overall survival (OS) was evaluated in multi-variable Cox regression models. Performance of survival models with and without CTC progression was evaluated by calculating ROC curve area under the curves (AUCs) and weighted c-indices.

Results: Overall, 511 patients with CTCs < 5 (421 in COU-AA-301 and 90 in IMMC-38) were selected; 212 (41.7%) had CTC progression at 4, 8 or 12 weeks after treatment initiation. CTC progression was associated with significantly worse OS [27.1 versus 15.1 m; hazard ratio (HR) 3.4 (95% confidence interval [CI] 2.5–4.5; $P < 0.001$)], independent of baseline CTCs and established clinical variables. Adding CTC progression to the OS model significantly improved ROC AUC (0.77 versus 0.66; $P < 0.001$). Models including CTC progression had superior ROC AUC (0.77 versus 0.69; $P < 0.001$) and weighted c-index [0.750 versus 0.705; delta c-index: 0.045 (95% CI 0.019–0.071)] values than those including CTC conversion (increase to CTCs ≥ 5). In COU-AA-301, the impact of CTC progression was independent of treatment arm.

Conclusions: Increasing CTCs during the first 12 weeks of treatment are independently associated with worse OS from advanced prostate cancer in patients with baseline CTCs < 5 treated with abiraterone or chemotherapy and improve models with established prognostic variables. These findings must be prospectively validated.

Key words: castration-resistant prostate cancer, treatment outcome, progression, circulating tumour cells, abiraterone, chemotherapy

Introduction

Advanced prostate cancer is a major cause of cancer morbidity and mortality. In the past decade, several drug development breakthroughs have greatly increased the therapeutic armamentarium, improving outcomes from this lethal disease [1]. Despite this, resistance eventually occurs and the prognosis remains, in fit

patients, approximately 34 and 58 months, respectively, for metastatic castration-resistant and metastatic non-castrate disease [1].

Determining response to treatment continues to represent one of the greatest challenges in advanced prostate cancer care. Prostate Cancer Working Group (PCWG) 3 guidelines, which summarize recommendations for outcome assessment of patients treated within clinical trials, have incorporated circulating tumour cell (CTC) enumeration as an end point in clinical trials [2]. Outside clinical trials, however, treatment response assessment continues to rely on prostate-specific antigen (PSA), bone scintigraphy (BS) and computed tomography, which have important limitations. Neither PSA nor bone scans allow early evaluation of disease progression. For instance, PCWG3 recommend that rising PSA values before 12 weeks not be considered progression [2]; similarly, progression by bone scintigraphy cannot be determined before at least 12–16 weeks of treatment due to the potential for spurious, 'flare reactions' [2, 3]. Furthermore, neither BS nor PSA response are established surrogates of survival [4].

A significant number of patients have exclusively bone disease for much of their disease course, which is not amenable to evaluation by RECIST [5]. Furthermore, currently available biomarkers for advanced prostate cancer treatment response assessment are not consistently utilized in daily clinical practice, with many physicians continuing to rely on highly subjective 'clinical progression' to discontinue treatment [6]. Delays in identifying progressive disease lead to overtreatment with ineffective agents, and arguably to more patients experiencing clinical deterioration on progression.

The enumeration of circulating tumour cell counts (CTCs) has emerged as a powerful biomarker for the assessment of prognosis and response to treatment. A baseline CTC count $\geq$ 5/7.5 ml has been consistently associated with worse outcome across large, randomized clinical trials [7–9]. Furthermore, the assessment of a composite biomarker [CTCs and lactate dehydrogenase (LDH)] after 12 weeks of treatment has been shown to be a surrogate of survival at the individual-patient level [9].

A number of studies have also evaluated the value of CTC enumeration as a response biomarker, that is, the association of changes in CTCs during treatment with outcome. In patients with unfavourable ($\geq$5 CTCs/7.5 ml) counts, a decline in CTCs has been associated with improved outcomes and response to treatment in patients treated with both chemotherapy and hormone therapy [10]. Furthermore, CTC enumeration has proven to be a more powerful biomarker than PSA [11]. PCWG3 recommendations now include CTC enumeration for the assessment of patients in clinical trials.

Patients with favourable (<5 CTCs/7.5 mL) baseline counts represent a subgroup of patients with a significantly better prognosis. These patients, especially those with undetectable CTCs at baseline, are not evaluable for response. Monitoring CTC counts in these patients can enable the detection of 'CTC progression', which has been evaluated as either a 'conversion' to unfavourable CTC counts [12, 13] or as any increase in CTC numbers.

We have previously reported the association of 30% CTC falls with improved outcome in patients with unfavourable ($\geq$5 CTCs/7.5 ml) baseline CTCs [10]. In the present study, we aimed to analyse the value of CTC increases in patients with low (< 5 CTCs/7.5 ml) baseline CTCs participating in the prospective COU-AA-301 and IMMC-38 trials.

Methods

Study population and procedures

We report an unplanned post hoc analysis of the COU-AA-301 and IMMC-38 trials, both of which have been published previously [12, 14]. The phase III COU-AA-301 trial compared abiraterone and prednisone with placebo with prednisone in metastatic castration-resistant prostate cancer (mCRPC) patients previously treated with chemotherapy. IMMC-38 was a prospective, open-label study in patients with mCRPC undergoing treatment with chemotherapy (70% of patients receiving docetaxel) as first, second or third line [12]. CTCs were collected at baseline, cycle 2 day 1 (weeks 4–5), cycle 3 day 1 (weeks 8–9) and cycle 4 day 1 (weeks 12–13) in COU-AA-301. In IMMC-38, CTCs were evaluated at weeks 2–5 (median: 4 weeks), weeks 6–8 (median: 7 weeks) and weeks 9–12 (median: 11.9 weeks). CTCs were determined with the CellSearch™ (Menarini Silicon Biosystems) assay. Haemoglobin (Hb), alkaline phosphatase (ALP), albumin and LDH concentrations were obtained at baseline and at each study visit. Eastern Cooperative Oncology Group (ECOG)-PS was obtained at baseline. PSA values were obtained every 4 weeks in IMMC-38 and every 12 weeks in COU-AA-301. Both studies were approved by local institutional boards. All patients provided written informed consent.

Statistical analysis

Kaplan–Meier analysis was used to estimate overall survival. CTC progression was defined as any increase in CTC count relative to baseline at either 4, 8 or 12 weeks after treatment initiation. Uni- and multi-variable Cox proportional hazards models were used to explore the association of baseline CTC counts, CTC progression and CTC conversion (defined as increase in CTCs from <5 to $\geq$5), with survival. Baseline LDH, ALP, PSA and CTCs, included as continuous variables, were $\log_{10}$-transformed due to their positively skewed distribution. In order to include patients with no detectable CTCs in the baseline count in the survival analyses, which required $\log_{10}$ transformation, 0.1 was added to all the baseline CTC counts. Logistic regression models were used to compare differences in PSA response and treatment arm by CTC progression and CTC conversion status.

Cox-regression models constructed including a 'Baseline' model (which included established clinical prognostic biomarkers: ECOG-PS, LDH, PSA, Hb, ALP and albumin); a 'Baseline CTC model' (adding baseline CTC counts to the 'baseline model') and a 'CTC progression Model' (adding CTC progression to the 'baseline CTC model'). A test of proportionality based on the Schoenefeld residuals was applied to evaluate the proportional hazards assumption (supplementary Figure S2, available at *Annals of Oncology* online). The value of baseline CTCs and of CTC progression was assessed by calculating Uno's inverse-probability weighted c-index and time-dependent incident dynamic ROC area under the curve (AUC) values (with a 22-month survival end point, which represents the median survival of the dataset) of each of the models, according the method proposed by Blanche et al. [15]. Bootstrapping was used to calculate the 95% confidence interval (CI) and the difference (delta) between c-indices of each of the models [16]. Analyses were carried out with SPSS v23 (SPSS Inc, IBM Corporation, Armonk, New York, US) and the R statistics package v3.4.0 (R Foundation).

Table 1. Baseline patient characteristics

	All patients	COU-301 Subset	IMMC-38 Subset
N	511	421	90
BLCTC			
0	259 (50.7%)	212 (50.4%)	47 (52.2%)
1–2	175 (34.3%)	146 (33.7%)	29 (32.2%)
3–4	77 (15.2%)	63 (16%)	14 (15.6%)
LDH (IU/L)	197.5 (167–233)	196 (167–230.8)	203 (167.8–247.3)
PSA (ng/mL)	71.6 (23.5–211.6)	69.6 (23–214.4)	79 (26.1–214.3)
Hb (g/dL)	12.5 (11.4–13.4)	12.4 (11.3–13.1)	13.2 (12.1–13.8)
ALP (IU/L)	87 (68–130)	86 (67–127.8)	96 (76–142)
Albumin (g/dL)	4.1 (3.8–4.3)	4.1 (3.9–4.4)	3.9 (3.6–4.3)
ECOG-PS			
0–1	485 (95.3%)	401 (95.2%)	84 (93.3%)
2	24 (4.7%)	20 (4.8%)	4 (4.4%)
Abiraterone	—	289 (68.6%)	—
Placebo		132 (31.4%)	

BLCTC, baseline circulating tumour cell; LDH, lactate dehydrogenase; PSA, prostate-specific antigen; Hb, haemoglobin; ALP, alkaline phosphatase; ECOG, Eastern Cooperative Oncology Group.

Results

Patient characteristics

Overall, a total of 511 patients participating in the COU-AA-301 ($n = 421$; 82.4%) and IMMC-38 ($n = 90$; 17.6%) clinical trials met the selection criteria with baseline CTC counts < 5 cells/7.5 ml and were included in the analysis. Supplementary Figure S1, available at *Annals of Oncology* online represents the Consort Diagram with details of patients excluded from the analysis. An analysis of patients with baseline CTC counts ≥5 cells/7.5 ml has been published previously [10]. No major differences in baseline patient characteristics were observed between IMMC-38 and COU-AA-301 participants (Table 1). Median follow-up was 17.4 months (range: 3.2–27.1 months); 217 patients (43.6%) had died at the time of analysis, 190 (45.3%) in COU-AA-301 and 27 (30%) in IMMC-38. Median overall survival was 21.98 (95% CI 20.7–23.3) months; there were no significant differences in survival between patients in the COU-AA-301 and IMMC-38 trials (22.0 and 21.4 months, respectively; $P = 0.146$).

Baseline CTC count and survival

Median baseline CTC count was 0 cells/7.5 ml (0 cells/7.5 ml in both COU-AA-301 and IMMC-38). 259 patients (50.7%) had 0 CTCs at baseline; 212 (50.4%) in COU-301 and 47 (52.2%) in IMMC-38. Baseline CTC count, as a $\log_{10}$-transformed continuous variable, was associated with survival in these patients overall [hazard ratio (HR) 1.65; 95% CI 1.32–2.05; $P < 0.001$], and when analysing patients from COU-AA-301 (HR 1.57; 95% CI: 1.25–1.96; $P < 0.001$) and IMMC-38 (1.98; 95% CI 1.09–3.61; $P = 0.026$) separately. There was a significant linear trend in survival when comparing patients with 0 (median 27.1 months; 95% CI NR–NR), 1–2 (median 21.6 months; 95% CI 19.7–23.5) and 3–4 (median 15.1 months; 95% CI 12.4–17.8) baseline CTCs (P-value for linear trend = 0.001) (Figure 1).

CTC progression is associated with adverse outcome

Overall, 213 (41.7%) patients experienced CTC progression in the first 12 weeks of treatment; 184 (43.7%) in COU-AA-301 and 29 (32.2%) in IMMC-38; 117 (25.8%), 103 (23.8%) and 124 (24.4%) patients experienced CTC progression at 4, 8 and 12 weeks, respectively. Patients experiencing CTC progression at 4 weeks [23.8 versus 14.8 months; HR 2.8 (95% CI 2.1–3.7); $P < 0.001$], 8 weeks [24.1 versus 14.7 months; HR 3.0 (95% CI 2.2–4); $P < 0.001$] and 12 weeks [27.1 versus 13.6 months; HR 3.9 (95% CI 2.9–5.2); $P < 0.001$] had significantly reduced survival compared with those not experiencing CTC progression. At any of the time-points, the association of CTC progression with reduced survival was independent of other known prognostic baseline characteristics. The impact of CTC progression was similar for both COU-AA-301 and IMMC-38 cohorts (Figure 2; supplementary Table S1, available at *Annals of Oncology* online). Similarly, the impact of CTC progression in multi-variable analysis (Table 2) was similar among patients with undetectable [baseline CTC (BLCTC) = 0: HR 2.9 (95% CI 1.8–4.7); $P < 0.001$] and detectable [BLCTC ≥1: HR 3.5 (95% CI 2.4–5.1); $P < 0.001$] counts (interaction test: $P = 0.734$).

To evaluate the added value of incorporating CTC Progression for predicting survival, we constructed a survival model incorporating baseline CTC counts and other prognostic clinical variables and determined the survival models' receiver operating characteristic (ROC) curve AUC and c-index. The ROC curve AUC for the baseline model was 0.66 (95% CI 0.59–0.74). A non-significant increase to an AUC of 0.67 (95% CI 0. 59–0.75) was observed when adding baseline CTC counts to this baseline CTC model ($P = 0.63$). Adding CTC

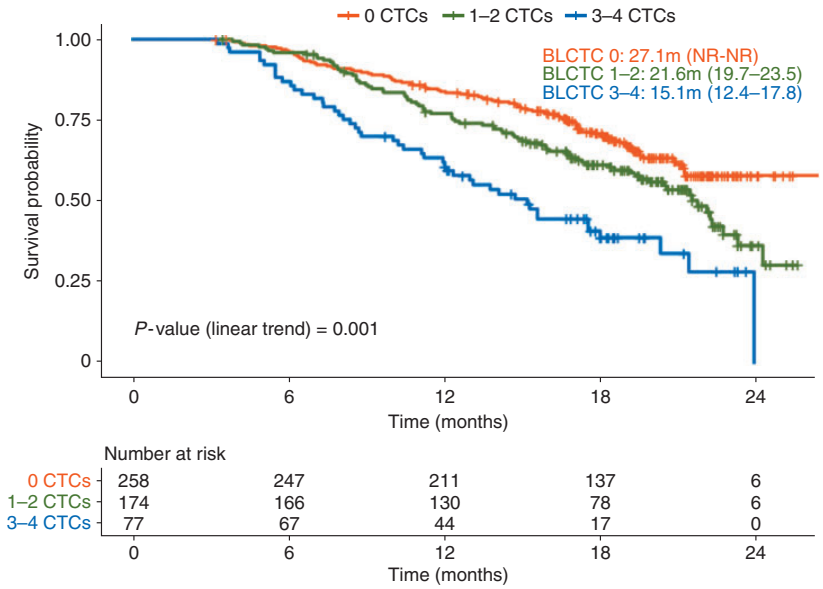

Figure 1. Kaplan–Meier survival curves in patients with 0, 1–2 or 3–4 circulating tumour cells at baseline (BLCTC).

progression to the model substantially increased the ROC AUC value (AUC 0.77; 95% CI 0.70–0.84) when compared with the baseline CTC model ($P < 0.001$) (Figure 3). The weighted c-index of the baseline model (0.682; SE: 0.023) increased to 0.694 (SE: 0.026) after including baseline CTCs. Inclusion of CTC progression in the model increased the weighted c-index to 0.748 (SE: 0.019) (delta c-index = 0.056; 95% CI 0.025–0.087).

Overall, furthermore, 500 patients (98.2%) had data on PSA response. PSA response, defined as a 50% decline from baseline, was observed in 118 (28.2%) patients from COU-AA-301 and 42 (51.9%) patients from IMMC-38. Patients with CTC progression had a significantly lower rate of PSA response than those without CTC progression [11.4% versus 47.1%; odds ratio (OR) 0.14 (95% CI 0.09–0.23), $P < 0.001$]; similar associations were observed in the COU-AA-301 [OR 0.14 (95% CI 0.08–0.24); $P < 0.001$] and IMMC-38 [OR 0.17 (95% CI 0.06–0.48); $P = 0.001$] patient subsets (supplementary Table S2, available at *Annals of Oncology* online).

Comparing CTC progression and CTC conversion

Overall, 90 patients (17.7%) experienced a conversion to unfavourable (≥5 CTCs/7.5 mL) counts during the first 12-weeks of treatment; 76 (18.1%) in the COU-AA-301 and 14 (15.6%) in the IMMC-38 trials. A CTC conversion was associated with a worse outcome (23.8 vs 10 months; HR: 3.78 [95%CI: 2.82-5.06]; $p < 0.001$) in both uni- and multi-variable Cox-regression models (supplementary Table S3, available at *Annals of Oncology* online), as well as a reduced PSA response rate (OR 0.08 [95%CI: 0.03-0.2]; $p < 0.001$); only 4 (4.4%) patients with a CTC conversion experienced a PSA response (supplementary Table S2, available at *Annals of Oncology* online).

The weighted c-index of the model including CTC progression was significantly higher than that of the model including CTC conversion (0.750 vs 0.705; delta c-index: 0.045 [95%CI: 0.019-0.071]). The ROC curve AUC index was also significantly higher for CTC progression than for CTC conversions (0.77 vs 0.69; 95% CI: 0.61-0.76; $p < 0.001$) (supplementary Figure S3, available at Annals of Oncology online).

CTC progression in COU-AA-301: Interaction with treatment arm

Overall, 419 patients participating in the COU-AA-301 trial were included in this analysis, 288 (68.7%) receiving abiraterone + prednisone and 131 (31.3%) placebo + prednisone. There was no significant difference in survival between these cohorts (HR 0.86; 95% CI 0.63–1.17; $P = 0.330$). CTC progression was more frequent in the placebo (68 patients, 51.9%) arm than in the abiraterone (115 patients, 39.9%) arm (OR 0.6; $P = 0.022$). The survival decrease in patients experiencing CTC progression was similar in the abiraterone (24.1 versus 15.1 months; HR 3.76; $P < 0.001$) and placebo arms (NR versus 13.8 months; HR 3.23; $P < 0.001$). The interaction test between treatment arm and CTC progression was not significant ($P = 0.952$), indicating that the impact of CTC progression on survival was similar for patients in both trial arms.

Discussion

Improvements in the development of predictive biomarkers for advanced prostate cancer care including AR splice variants and AR genomic aberrations for novel hormonal agents; DNA repair aberrations for PARP inhibitors and PTEN loss for agents targeting the PI3K/AKT pathway are anticipated in the future [17]. The

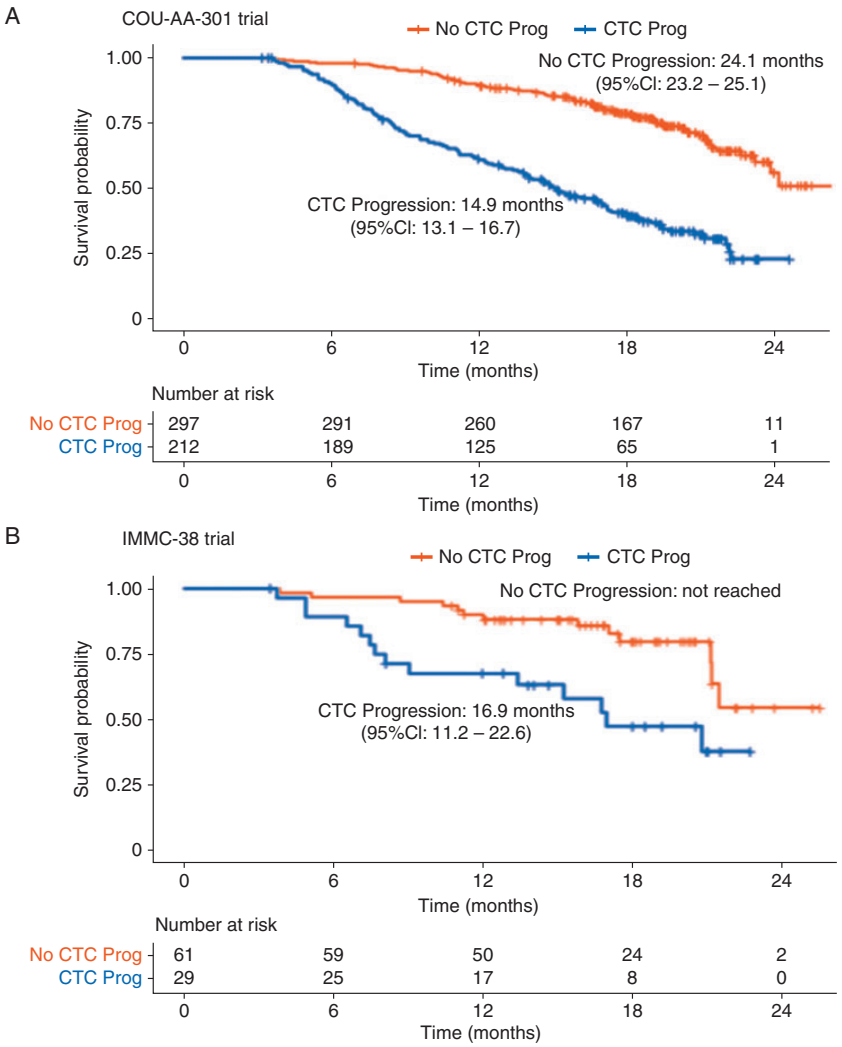

Figure 2. Kaplan–Meier survival curves in patients with and without circulating tumour cells (CTC) progression at 4, 8, or 12 weeks in the COU-AA-301 (abiraterone or placebo + prednisone) subset (A) and IMMC-38 (chemotherapy) subset (B).

development of response biomarkers to rapidly identify resistant disease and guide early treatment switches remains, however, an unmet clinical need. The value of circulating tumour cells as a prognostic indicator for advanced prostate cancer care has been well described [8, 9, 13]. Because of regulatory concerns about assay performance when CTCs are low, patients have been categorized into unfavourable (CTCs $\geq$ 5/7.5 ml) and favourable CTC count groups, which have distinct prognoses. The value of CTCs as an indicator of clinical activity has also been reported: post-treatment CTC declines, either as a fold-decline, 30% decline or conversion to favourable counts have all been associated with improved survival in the subgroup of patients with unfavourable baseline CTC counts [7, 10, 12]. PCWG3 now recommends the use of CTCs as an end point for activity in patients with unfavourable counts at baseline in the setting of clinical trials [2]. This approach, however, captures only approximately 50% of patients (with unfavourable baseline counts) as assessable and classifies those with favourable baseline counts as non-assessable for response.

The role of increasing CTC counts as an indicator of disease progression has been less well studied. We present what is, to our knowledge, the largest dataset analysing the role of increasing CTCs as a biomarker of progression analysing exclusively patients with low (<5) baseline CTC counts at baseline, treated with AR targeting agents (COU-AA-301) and chemotherapy (IMMC-38) in each of the prospective clinical trials. In our study, CTC progression (defined as any increase in CTC counts) and 'CTC conversions' (defined as an increase to at least 5 CTCs/7.5 ml) during the first 12 weeks of treatment are associated with a worse outcome in patients treated with either abiraterone or chemotherapy. Furthermore, CTC progression increased the power of the survival model that included key clinical variables and baseline

Table 2. Association of CTC progression and survival (multivariable cox-regression model – CTC progression model)

	HR	95% CI	p-value
CTC progression[a]	3.33	2.50–4.44	<0.001
Baseline CTC count[b]	1.48	1.19–1.85	<0.001
Baseline PSA[b]	0.98	0.79–1.21	0.85
Baseline ALP[b]	1.56	0.95–2.58	0.08
Baseline LDH[b]	15.33	6.04–38.95	<0.001
Baseline albumin	0.65	0.44–0.95	0.03
Baseline haemoglobin	0.94	0.84–1.04	0.24
ECOG performance status	3.01	1.84–4.92	<0.001

[a]CTC progression at 4, 8 or 12 weeks.
[b]Baseline CTC count, PSA, ALP and LDH were log-transformed.
CTC, circulating tumour cell; HR, hazard ratio; CI, confidence interval; PSA, prostate-specific antigen; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group.

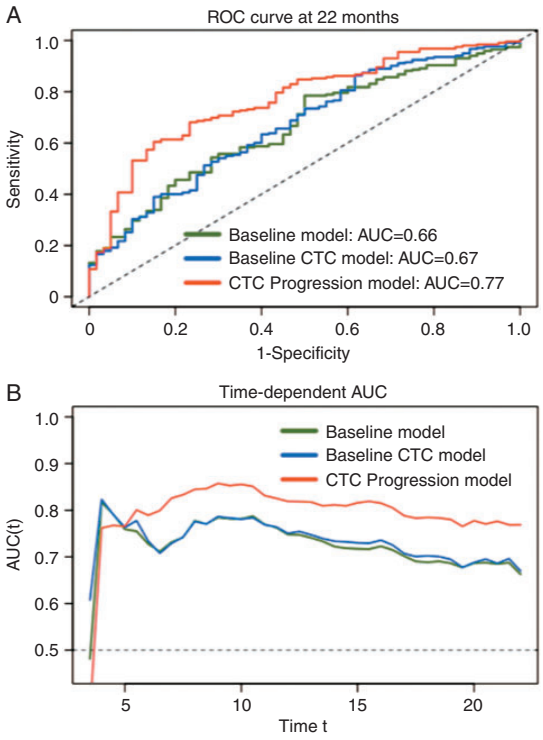

Figure 3. Area under the curves (AUC) of the baseline, baseline circulating tumour cell (CTC) and CTC progression survival models.

CTCs. We show that CTC progression is superior to CTC conversion as a biomarker of poor overall survival with superior model performance as defined by ROC AUC values and c-indices. This is in line with our previous conclusions in patients with unfavourable CTC counts, where failure to effectively reduce CTCs ('stable' CTC counts) had a similar adverse impact to primary 'progressing' CTC counts [10]. Recently, Heller et al. [18] presented a pooled analysis of five randomized mCRPC trials, where both a CTC conversion (≥5 CTCs to < 5CTCs) and a CTC0 end point (>1 CTCs to 0 CTCs) had a higher discriminatory value (c-index) than commonly used PSA end points. CTC0 end points were able to evaluate a significantly higher number of patients than CTC conversion end points. In patients with treatment naive mCRPC (ELM-PC-4 trial), however, as many as 33% and 61% of patients were non-assessable for CTC0 (due to baseline 0 CTC) and CTC conversion (due to baseline < 5 CTCs), respectively [18]. An approach incorporating CTC increase end points for patients with low baseline CTC counts could therefore render all patients assessable for CTC efficacy end points.

A number of limitations to our study should, however, be acknowledged: (i) its unplanned, post hoc nature (ii) not all patients enrolled in COU-AA-301 had CTCs (CTCs were collected in 858 of 1195 [71.8%] patients), which could have led to a selection bias; (iii) the unavailability of CTC counts beyond 12 weeks in COU-AA-301, with our results therefore not being applicable to CTC counts beyond that time-point; (iv) the fact that patients treated in COU-AA-301 were over fourfold more numerous than those in IMMC-38 and (v) LDH kinetics were not incorporated into the analyses.

In conclusion, these data indicate that CTC progression in the first 12 weeks of chemotherapy or endocrine therapy can identify patients with low baseline CTC counts (<5) not benefiting from treatment. These data have significant clinical and health economic implications and could guide the response assessment of patients during the first 12 weeks of treatment, identifying early disease progression, and could be used as efficacy biomarkers in clinical trials. Prospective phase III trials are now needed to validate these findings, and confirm the clinical utility of CTCs [9].

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Disclosure

DD, DB, GS, PF, MC, IF, SM, and JSdB are employees of the Institute of Cancer Research, which has a commercial interest in abiraterone. All remaining authors have declared no conflicts of interest.

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Molecular Characterization and Clinical Utility of Circulating Tumor Cells in the Treatment of Prostate Cancer. Lorente et al. American Society of Clinical Oncology Educational Book 2014;34:e197-e203

Molecular Characterization and Clinical Utility of Circulating Tumor Cells in the Treatment of Prostate Cancer

David Lorente, MD, Joaquin Mateo, MD, and Johann S. de Bono, MB ChB, FRCP, MSc, PhD

OVERVIEW

Circulating tumor cells (CTCs) are rare cancer cells that can be detected in the blood of patients with solid malignancies. The Veridex CellSearch Assay was analytically and clinically validated, and has received U.S. Food and Drug Administration (FDA) clearance for the enumeration of CTCs in breast, colorectal, and prostate cancer. A number of alternative assays, with potential advantages, are currently undergoing clinical and/or analytic validation before their routine use can be established. In prostate cancer, high pretreatment CTC counts have been associated with worse survival, and changes in CTC counts in response to treatment have been established as indicators of response to treatment. Additional analyses are ongoing to establish the value of CTC counts as a surrogate of survival in prospective, phase III trials, which could influence the process of drug development and regulatory approval. Additionally, CTCs have a potential role in the molecular characterization of prostate cancer, serving as "liquid biopsies" to determine the molecular characteristics of the disease. The study of androgen receptor (AR) mutations or amplification, chromosomal rearrangements, or the determination of DNA repair biomarkers has been evaluated in clinical trials. CTCs have a wide range of potential applications, from their prognostic use in stratification of patients in clinical trials or the assessment of response to treatment, to the pharmacodynamic evaluation of novel agents, or the discovery and use of predictive biomarkers that can aid in the development of personalized medicine.

Prostate cancer currently represents an exciting area of clinical research, with substantial improvements in our understanding of the molecular biology of the disease that have led to the approval of several new agents in recent years. However, these improvements have stressed the importance of developing adequate biomarkers for patient selection and the assessment of response.

Current recommendations for the assessment of outcome and the design of endpoints in clinical trials were developed by the Prostate Cancer Working Group (PCWG) and are summarized in the PCWG2 Criteria.[1] These are based on a composite endpoint that takes into account imaging (CT scans and bone scans), prostate-specific antigen (PSA) levels, and clinical outcomes. These criteria have important limitations, especially in the large proportion of patients with metastatic castration-resistant prostate cancer (CRPC) that present with exclusively bone metastatic disease not measurable by RECIST criteria. PSA has been shown to correlate weakly with survival, is not adequate to guide treatment in the first 12 weeks,[2] and could be less reliable in more advanced, potentially less AR-driven stages of the disease. Confirmatory bone scans are required at least 6 weeks after the appearance of new lesions to exclude a "flare reaction" in response to treatment.

In addition to these limitations in the evaluation of response, the high prevalence of bone-exclusive disease has traditionally hindered our ability to obtain tissue for molecular analysis. Current histologic analyses are usually performed in the original diagnostic biopsies, and have not taken into account clonal selection and acquired resistance mechanisms. Bone marrow biopsies, although feasible in the setting of specialized research units, are not routinely used in daily practice.

CTCs are extremely rare cells present in the blood in an estimated frequency of one in a few million blood cells, originating from shedding from the original tumor. An FDA-cleared assay, the Veridex CellSearch System,[3] was approved based on studies performed in breast, colorectal, and prostate cancers and is available for the enumeration of CTCs.

The use of CTCs as prognostic and treatment-response biomarkers has been proposed in the CRPC setting; their potential use as a surrogate of survival could also accelerate the development of active agents. Their use as a "liquid biopsy" for molecular characterization may assist in the devel-

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opment of precision medicine by assessing the molecular biology of the disease in real-time and personalizing treatment on a patient-to-patient basis.

METHODS FOR ISOLATION AND QUANTIFICATION OF CTC

Assays for quantitative analysis of CTC from blood samples typically requires an initial preparatory step (centrifugation, washing, and addition of cellular preservatives to delay CTC entering apoptosis for up to 72 to 96 hours), a second enrichment or isolation step (which can be based on immunoaffinity or on the particular physical properties of CTCs), and last a semi-automated image-based approach for identification.

The most common approach for isolation or enrichment of CTC is based on immune-magnetic systems; samples are incubated with specific antibodies to select certain cell populations and are afterwards separated by magnetic means. This may be performed through positive selection (by conjugation with antibodies against epithelial cell adhesion molecules [EpCAM], expressed in most CTC and not in other blood cells) or negative selection (using antibodies against leukocyte-expressed antigens such as CD45). Selection based on antibody binding to specific prostate cancer proteins, such as prostate-specific membrane antigen (PSMA), can also be utilized.

Alternatively, technologies have been developed to isolate CTC from blood samples based on their different size, deformability capacities, or electrical properties of CTC compared with other blood cells, mainly leukocytes. The principal limitation of these approaches is a lower discriminatory capacity as a result of some overlap in physical properties, and the dependence of the results on the blood flow rate, which results in lower sensibility and specificity. Although, these techniques allow for isolation at a lower cost when compared with antibody-based approaches.

Recently, enrichment-free systems for study of CTCs have emerged. Fiberoptic scanning enables high-throughput assaying of the entire population of cells in blood without requiring any protein-based selection but only erythrocyte lysis, being therefore less vulnerable to loss of cells. One of the reasons for developing these approaches is the direct study of clusters of circulating cells rather than individual CTCs.[4]

Evolution of isolation techniques has led to the development of systems for composite selection, where cells are first targeted by conjugation with EpCAM antibodies with selection being based on their size through a multiobstacle architectural design.[5]

The CellSearch System (Janssen Diagnostics, LLC, Raritan, NJ)[3] is however, to date, the only FDA-cleared technology for quantification of CTCs, with demonstrated clinical relevance in breast, prostate, and colorectal cancer, and has become the standard comparator for any novel platform in development. The CellSearch System is based on an automated immunomagnetic enrichment and staining system: anti-EpCAM and anti-creatinine kinase (CK) antibodies are used for positive selection, complemented by counterstaining with anti-CD45 antibodies to discard leukocytes.

A current challenge for CTC enumeration is to address the effect of tumor heterogeneity in CTC. Systems based on positive selection for epithelial markers may be missing those CTCs that have undergone epithelial-mesenchyme transition (ETM), which arguably representatives of the more aggressive clones of disease and also have limited applicability in nonepithelial malignancies (e.g., sarcomas or melanoma). Platforms using several concomitant antibodies for a composite selection (including markers that are not repressed during EMT) would help in addressing this pitfall, although this would asffect the monetary cost of the assay.

The final step of any quantitative analysis also requires an image-based system with the input from a human operator to identify CTCs among the selected cells. Algorithms for completely automated counting of CTCs are being optimized.[6]

Widely accepted characteristics to define a CTC include: round to oval morphology, size greater than 5 μm, a visible nucleus (4#,6-diamidino-2-phenylindole positive), positive staining for cytokeratins 8, 18, and/or 19 (phycoerythrin), and negative staining for CD45 (allophycocyanin). Modifications of this definition may increase the numbers of isolated cells, but may weaken the prognostic value of the enumeration.[7]

To assess the validity of the CellSearch System, Kraan et al analyzed aliquots of the same six blood samples drawn from patients with metastatic cancer in 14 different laboratories. Interestingly, inconsistency in scoring was mainly derived from the manual interpretation by a trained operator of the events identified by the semiautomatic system, especially in those samples containing a high number of dead or apoptotic cells.[8]

Overall, among the different approaches for isolation,

KEY POINTS

- CTCs are extremely rare cells of malignant origin that can be isolated from the blood of patients with cancer for enumeration and molecular analysis.
- The Veridex CellSearch Platform is the only FDA-approved platform for CTC enumeration, based on immunomagnetic selection of EPCAM positive cells and the negativity of the CD-45 receptor, size, and nuclear staining.
- Pretreatment CTC counts are validated prognostic biomarkers in prostate cancer, and changes in CTC counts in response to treatment have been proposed as surrogate biomarkers of overall survival.
- CTC analysis can be incorporated in drug development by patient selection and pharmacodynamic evaluation of novel agents.
- The molecular characterization of CTCs has potential applications in diagnostic, staging, biomarker discovery, and individualization of treatment by serving as "liquid biopsies."

there is probably not a single ideal method, but selection of the appropriate method would depend on the intended downstream application.

CTC COUNTS AS PROGNOSTIC AND TREATMENT-RESPONSE BIOMARKERS

The value of CTCs as a prognostic and predictive biomarker has been validated in studies across multiple cancer types. As a result of potential variability in the determination of CTCs, threshold values have generally been proposed to distinguish "favorable" from "unfavorable" counts.

The IMMC38 study was the first to evaluate the prognostic and predictive role of CTC enumeration using the CellSearch System in CRPC patients. In the study, 164 patients starting a new cytotoxic chemotherapy regimen were eligible and CTC counts were determined in 3- to 4-weekly intervals. An unfavorable pretreatment count ($\geq$ 5 CTC in 7.5 mL of blood) predicted a worse overall survival (OS) than a favorable count ($<$5 CTC in 7.5 mL of blood) after adjusting for known prognostic values (Eastern Cooperative Oncology Group [ECOG] status, hemoglobin, lactate dehydrogenase [LDH], and alkaline phosphatase) in multivariate analysis. The predictive value of a CTC conversion from an unfavorable to a favorable count at different time points was also explored. Patients that maintained a favorable count at all draws had the longest OS (26 months), followed by those that converted from unfavorable to favorable (21.3 months), those that converted from favorable to unfavorable (9.3 months), and those that maintained an unfavorable count (6.8 months). CTC count was superior to PSA declines in predicting survival, especially in earlier time points. At 12 weeks, receiver operating characteristic (ROC) curve analysis showed a statistically significant superiority of CTC counts over 30% PSA declines in predicting death at 12 months (area under the curve [AUC] 81.5 vs 67.5%; $p = 0.022$).[9] A second analysis of the same study evaluated the prognostic value of baseline CTCs as a continuous variable, before treatment initiation and at different time points. After incorporating CTC counts in the multivariate model, only CTC counts and LDH retained clinical significance, which was lost for all other variables, including PSA.[10]

Alternative cut-off points have also been proposed in evaluating the prognostic and predictive role of CTCs. In a cohort of 99 metastatic CRPC patients treated at the Memorial Sloan-Kettering Cancer Center (MSKCC), there was a strong correlation between baseline CTC number and survival, without a threshold effect. Baseline CTC counts were modestly correlated with other indicators of disease burden, such as baseline PSA values and bone scan index.[11] Another single-center study performed in the Royal Marsden evaluated 119 CRPC patients undergoing CTC enumeration, and reported a statistically significant difference in OS in patients with a CTC count less than 5/7.5 mL, 5 to 50/7.5 mL, and greater than 50/7.5 mL at baseline and after the first and second cycle of treatment. Additionally, a decline of 30% in CTC was also associated with improved survival in patients with a baseline unfavorable CTC count. Baseline CTC counts were associated with other known baseline prognostic factors, such as high alkaline phosphatase levels, low hemoglobin, high PSA, prior cytotoxic chemotherapy, or the presence of bone metastases.[12]

The prognostic and predictive value of CTCs using the 5 CTC/7.5 mL cutoff proposed in the IMMC38 study was prospectively validated in the phase III COU-AA-301 trial, evaluating abiraterone against placebo in the postchemotherapy setting. CTC conversion was associated with improved OS as early as 4 weeks after commencing treatment. The inclusion of CTC count changes in the multivariate model significantly reduced the treatment effect at all post-treatment time points, modifying the hazard ratio (HR) for OS in the abiraterone versus placebo groups from 0.74 in the model without CTC count changes to 0.94 in the model that included CTC count changes.[13]

CHANGES IN CTC COUNTS AS SURROGATE OF SURVIVAL IN CRPC

Based on the results of the COU-301 study, a model for assessing CTC response has been developed and evaluated for surrogacy. The degree on which a response biomarker captures the effect of treatment on survival, and can therefore be used in regulatory submissions for novel agents, has been tested using the Prentice Criteria. These criteria require that the biomarker is evaluated in therapies that provide survival benefit, that the treatment has an effect on the proposed biomarker, that the biomarker has an effect on the clinical endpoint, and that the full effect of treatment on the endpoint is captured by the biomarker.[14] To qualify as an accepted surrogate for regulatory drug approval, these criteria must be met in a number of large prospective trials, and a meta-analytic approach must prove surrogacy at the trial level as well as at individual level.

A model including CTCs ($\geq$ 5 CTC vs. $<$5 CTC in 7.5 mL of blood) and LDH (normal vs. abnormal) at the 12-week landmark time point fulfilled all Prentice Criteria at the individual level. However, proof of surrogacy at trial level requires that these results be reproduced in several large trials.[15] If a number of ongoing clinical trials confirm the results of the COU-301 analysis, we may be able to validate the role of CTCs as a response-indicator biomarker, potentially with drug approval based on CTC endpoints. This would increase the efficiency and reduce the costs of the development of novel active agents, and eliminate the bias in OS that would be introduced by treatment in post-trial settings.

MOLECULAR CHARACTERIZATION OF CTC

Qualitative assessment of CTC at genomic and proteomic levels provides an insight into biologic processes of the disease and has applications in diagnostic, staging, biomarker discovery, and individualization of treatment. There is great interest in obtaining molecular information from CTCs, as they may constitute a read-out for the cancer molecular un-

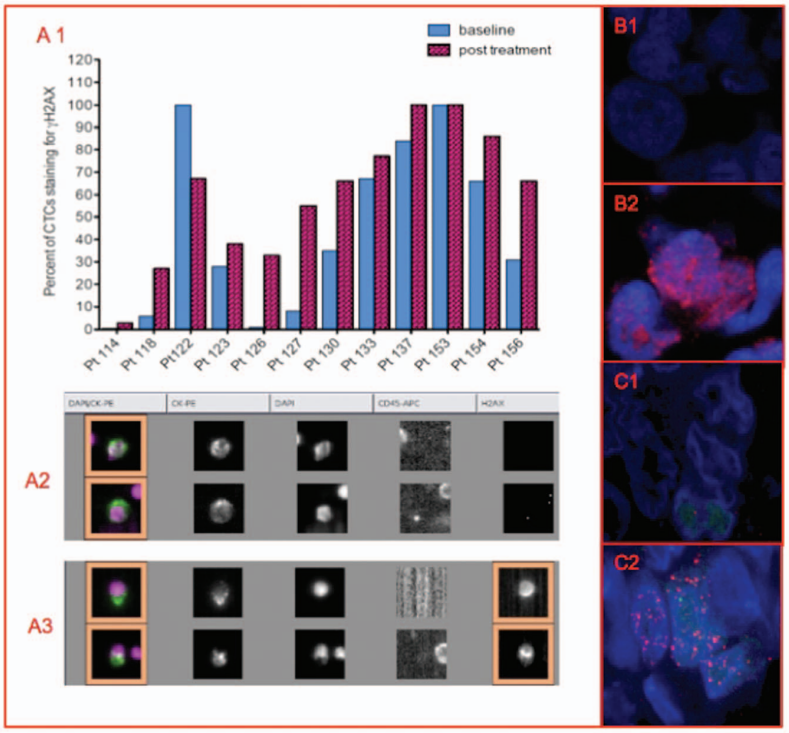

FIG 1. Nuclear γH2AX staining in CTCs as a PD biomarker in the phase I trial evaluating the PARP inhibitor niraparib.

(A1) Pretreatment and maximal post treatment increase in proportion of CTCs staining positive for nuclear γH2AX in patients with castration-resistant prostate cancer (Part B) with baseline CTC counts of > 3 cells/7.5 mL of blood. **(A2)** CTCs at baseline from a patient on study with no nuclear staining for γH2AX and the panel. **(A3)** CTCs from the same patient during treatment with positive nuclear staining for γH2AX. **(B1-2)** Fresh tumor tissue collected at baseline (B1) and in cycle 2 (B2) stained for γH2AX immunofluorescence (red), showing an increase in the level of γH2AX induction in the post treatment tumor biopsy.

Reprinted from The Lancet Oncology, Volume 14, Issue 9, S Sandhu et al "The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase I dose-escalation trial." Pages 882-892, Copyright 2013, with permission from Elsevier.

derpinnings without requiring the invasiveness of tumor biopsies, and permit longitudinal analyses by collecting sequential samples over time to assess the effect of treatments in tumor evolution. In the field of prostate cancer, molecular characterization of CTCs in parallel to new drug development should bring advances in the current lack of biomarker-driven individualization of treatment.

A wide variety of genome- and protein-based assays can be performed on CTCs, including immunohistochemistry, immunofluorescence, gene copy number analysis via comparative genomic hybridization (aCGH), genome sequencing analysis, and epigenetic studies.

Immunophenotyping of CTCs is the basis of the most implemented assays for enumeration, but also can be used toward their molecular characterization. Multiplexed characterization by immunophenotyping is partially limited by the overlap with antibodies necessary to identify CTCs.

Cytogenetic studies based on fluorescence in situ hybridization (FISH) allow the study in CTCs of the presence or loss (heterozygous or homozygous) of phosphatase and tensin homolog (*PTEN*), to assess the number of copies of the *AR* gene, or the presence of erythroblast transformation-specific–related gene (*ERG*)-based translocations. Multicolored fluorescence permits simultaneous study of these genes, offering a comprehensive profiling of prostate cancer cells, with prognostic value and associated to response to abiraterone acetate in retrospective series.[16]

Assessment of the *AR* gene by FISH is complemented by detection of mutations in DNA or altered gene copy number from CTCs.[17] Detection of hotspot mutations by targeting sequencing in phosphoinositide 3-kinase (*PI3K*)/*AKT* genes in this circulating genomic material would complement the assessment of PTEN function in scrutinizing this highly relevant signaling pathway.[18] On of the key interests in assessment of CTCs is the opportunity for both longitudinal assessment (assessing tumor evolution over time as results to treatment-induced selective pressure) and studying intratumor clonal heterogeneity. As an example, detection of emerging mutations in epidermal growth factor receptor (EGFR) in CTCs has served for the study of resistance mechanisms to EGFR inhibitors.

With the rapid technical development of next generation sequencing techniques from circulating nucleic acids[19] and the ability to perform single cell whole genome amplifications,[20] it is envisioned that CTCs could serve as an easy and economic source for whole genome and transcriptome anal-

ysis for diagnostic, predictive, and monitoring for treatment purposes. Lastly, DNA extracted from CTCs would also serve as a source for epigenetic studies, including methylation analysis.[21]

IMPLEMENTATION OF CTC ANALYSIS IN DRUG DEVELOPMENT

Phase I trials of novel targeted compounds demands biomarker-driven patient selection and markers of antitumor effect with early read-out to optimize drug development programs. The easy access to CTCs offer unique platform for pharmacodynamics (PDs) studies, overcoming the restricted anatomic accessibility to soft tissue or visceral metastases for fresh biopsies in patients with prostate cancer and the limited success rate of bone marrow biopsies in obtaining tumor tissue.

PD assessments in circulating biomarkers allows for monitoring the effect of a drug in tumor cells repeatedly and at different dose levels to determine pharmacokinetic/PD correlations.

Sequential CTC counts to detect early CTC decreases as a surrogate marker of antitumor activity were incorporated in the early phase trials of abiraterone acetate in prostate cancer[22] and are now commonly implemented as a PD read-out in many first-in-human trials of drugs in development for prostate cancer. In the case of early trials of figitumumab (CP751,781), a monoclonal antibody targeting insulin-like growth factor 1 receptor (IGFR-IR), a mixed quantitative/qualitative approach was attempted, by selectively monitoring CTCs expressing IGF-IR.[23]

One of the most successful examples of implementation of CTCs for PD studies in early clinical trials is the monitoring of induction of gamma H2A histone family member X (γH2AX) foci in CTC after exposure to DNA targeting agents (Fig. 1) and has contributed to development of several trials of poly ADP ribose polymerase (PARP) inhibitors including patients with prostate cancer.[24]

CONCLUSION

CTCs have emerged as an important biomarker in current drug development in prostate cancer. Applications in the research and clinical settings are multifold: (1) its prognostic value will be important in the stratification of patients in clinical trials, (2) its value as a surrogate of survival could accelerate drug approval, (3) the rational understanding of the molecular biology can aid in our understanding of prostate cancer and the development of novel agents, and (4) its use as PD biomarkers in early drug development can aid in the selection of biologically active treatment regimens. The application of CTCs in the assessment of response as a tool in clinical decision-making, mirroring its development in breast cancer, has also been proposed. The potential of CTCs for identifying nonresponders earlier than the currently established response biomarkers (CT scans, bone scans or PSA) could be useful in avoiding the administration of toxic and often costly therapeutic options, and receiving subsequent therapy in a more favorable condition, with a better general condition that could increase the likelihood of achieving benefit. See Fig. 2 for a summary of this approach.

Several challenges in the development of CTCs lie ahead. The currently approved CellSearch System, based on the EpCAM positivity of CTCs, could be missing EpCAM negative malignant cells undergoing epithelial-mesenchymal transition, which are potentially relevant in metastatic dissemination. The dependency on a human operator for the counting of CTCs in the CellSearch system has been pointed out as a potential for bias. Novel automated methods in the enrichment and characterization of CTCs may help overcome these issues; however, thorough analytic and clinical validation will be required before their routine clinical use is cleared.

In conclusion, CTCs are among the most promising biomarkers in development in prostate cancer; they are easily accessible and provide material for the assessment of prognosis, response to treatment, and molecular characterization. Further research

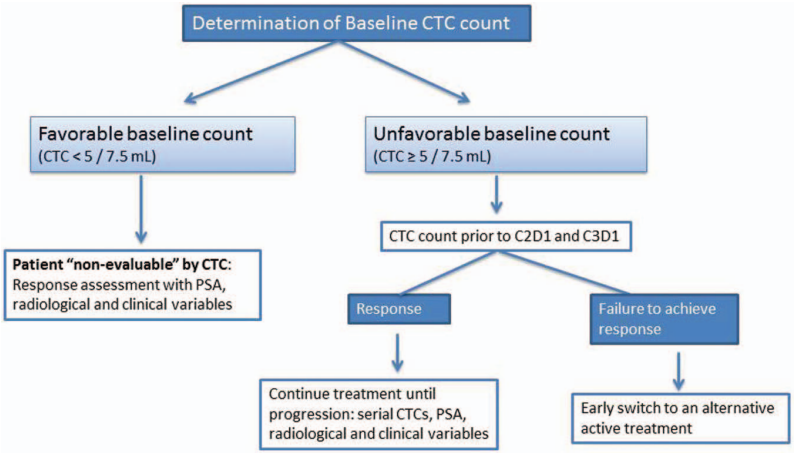

FIG 2. Clinical decision making based on CTC results.

will improve the detection and enrichment of CTCs, and may establish their role as surrogates of survival.

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Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked "L" indicate leadership positions. Relationships marked "I" are those held by an immediate family member; those marked "B" are held by the author and an immediate family member. Relationships marked "U" are uncompensated.

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Baseline neutrophil–lymphocyte ratio (NLR) is associated with survival and response to treatment with second-line chemotherapy for advanced prostate cancer independent of baseline steroid use

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Background: The neutrophil–lymphocyte ratio (NLR), proposed as an indicator of cancer-related inflammation, has known prognostic value in prostate cancer. We examine its association with survival (OS) and response in patients treated with second-line chemotherapy.

Methods: We analysed patients with metastatic castration-resistant prostate cancer (mCRPC) treated in the TROPIC trial, evaluating cabazitaxel versus mitoxantrone. Cox regression models were used to investigate the association of baseline NLR (BLNLR) with OS and the significance of a change in NLR count with treatment. Logistic regression models were used to determine the association of BLNLR counts with prostate specific antigen (PSA) and RECIST responses. The optimal NLR cut-off was established based on the concordance index of different values.

Results: Data from 755, 654 and 405 patients was available for OS, PSA and RECIST response analysis respectively. Median OS was 14.0 months [95% confidence interval (CI) 13.2–14.8]. Median NLR was 2.9 (IQR: 1.9–5.1). BLNLR was associated with survival (HR 1.5, 95% CI 1.1–2.1, $P = 0.011$) in multivariable analysis (MVA) independently of variables included in the Halabi nomogram, treatment arm and corticosteroid use. The optimal cut-off for a dichotomous NLR was selected at 3.0 based on its higher c-index related to survival. BLNLR ≥3.0 was associated with lower PSA response (40.1% versus 59.9%; $P < 0.001$) and RECIST response (7.7% versus 15.6%, $P = 0.022$) in MVA. Conversion from high (≥3) to low (<3) NLR was associated with improved survival (HR 0.66; 95% CI 0.51–0.85; $P = 0.001$) and higher PSA response rates (66.4% versus 33.6%; $P = 0.000$). Use of corticosteroids at baseline did not modify the association between NLR and survival.

Conclusions: NLR is a valid prognostic biomarker in CRPC and is associated with survival, PSA and RECIST responses in patients treated with second-line chemotherapy. Changes in NLR counts with treatment may indicate benefit. NLR prognostic value is independent of prior use of corticosteroids.

ClinicalTrials.gov: NCT00417079.

Key words: neutrophil–lymphocyte ratio, castration-resistant prostate cancer, cabazitaxel, steroids, prognostic biomarker, treatment response

introduction

The past 5 years have seen an unprecedented advance in therapy for castration-resistant prostate cancer (CRPC), with the approval of the chemotherapeutic agent cabazitaxel, the hormonal agents abiraterone and enzalutamide, the radiopharmaceutical alpharadin and the immunotherapeutic agent sipuleucel-T emerging as therapeutic options [1]. With this plethora of novel agents available, a pressing need to develop improved prognostic and predictive biomarkers to assist in the selection of treatment and sequencing of agents has emerged. Day-to-day clinical decisions are based on prostate specific antigen (PSA), a marker of AR signalling activity and imaging techniques such as CT or bone scans [2]. The widely used Response Evaluation Criteria in

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Solid Tumors (RECIST) are not applicable in a large proportion of CRPC patients due to the presence of bone metastatic disease exclusively, which is inevaluable by RECIST [3].

To assist in the risk stratification of patients, prognostic nomograms based on retrospective analyses of pre-treatment baseline characteristics in large trials have been developed [4]. Additionally, the characterization and enumeration of circulating tumour cells has been formulated as a prognostic biomarker as well as an indicator of treatment response [5].

Cancer-related inflammation has been recognized as one of the hallmarks of cancer [6] with an essential role in the modulation of the tumour microenvironment. The neutrophil–lymphocyte ratio (NLR), a measure of the proportion of systemic neutrophils and lymphocytes, has been proposed as an indicator of cancer-related inflammation, and has been shown to have prognostic relevance across a large variety of tumour types [7]. In prostate cancer, a number of retrospective studies have evaluated the prognostic significance of baseline NLR (BLNLR) [8–10] and its association with PSA response [11]. To date, the optimal cut-off for the clinical application of BLNLR as a binary variable has not been established, with some of these studies favouring an NLR cut-off of 3 and other studies evaluating a cut-off of 5. None of these studies has evaluated the impact of corticosteroids, widely used drugs with known immunosuppressive effects, on BLNLR.

In this study, we carried out a retrospective analysis evaluating the impact of BLNLR on overall survival (OS), but also PSA and radiological response in the phase III TROPIC study, which led to the approval of cabazitaxel as second-line chemotherapy in mCRPC [12]. We hypothesized that BLNLR counts would have prognostic significance but also an association with PSA and radiological response, and evaluated the role of baseline corticosteroid use in modulating these effects. We also investigated the value of changes in NLR (NLR 'conversion' from low to high or high to low counts) with treatment, to investigate its role as a response indicator.

methods

patients

We carried out an unplanned analysis of patients enrolled in the TROPIC trial, a randomized, open-label phase III trial comparing the efficacy of 3-weekly cabazitaxel (25 mg/m^2) versus 3-weekly mitoxantrone (12 mg/m^2), both in combination with prednisone 10 mg daily, in men with mCRPC who had received prior docetaxel-containing chemotherapy. Details of the eligibility criteria have been previously reported [12]. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria [13] were followed when possible for the design and analysis of the study.

Patient characteristics collected at trial entry included number and duration of prior lines of treatment, sites of metastases, age, Eastern Cooperative Group (ECOG) performance status, steroid use, full blood count (including absolute neutrophil and lymphocyte counts) and biochemistry. Full blood counts were carried out on a weekly basis (day 1, 8 and 15 of each of each 21-day cycle) and biochemistry was carried out every 3 weeks (day 1 of each cycle). Imaging studies (computed tomography and bone scintigraphy) were carried out every 12 weeks.

statistical analysis

The NLR was defined as the quotient of baseline absolute peripheral neutrophil count lymphocyte count (cells/mm^3) by absolute peripheral baseline lymphocyte count (cells/mm^3). For BLNLR values, counts from day 1 of the first cycle were used. OS was defined as the time from randomization to date of death from any cause with censoring at date of last contact for patients alive at the cut-off date. Progression-free survival was defined as the time from randomization to the date of progression by clinical, radiological or PSA criteria [2].

The association of BLNLR as a continuous and dichotomous variable with survival was evaluated in univariable and multivariable Cox regression models. The association of BLNLR (as a continuous variable) and other prognostic factors were evaluated with linear regression models. Predictors for OS considered in uni- and multivariable analyses were selected based on the prognostic model developed by Halabi et al. from the TROPIC trial dataset [4]. These covariates included: the presence of pain (defined as a present pain intensity scale score ≥2 of and an analgesic score ≥10, the presence of measurable disease before initiation of treatment, ECOG performance status, disease progression within 6 months of docetaxel-based therapy, presence of visceral disease (defined as metastases in the liver, lungs or adrenal glands), duration of previous hormonal treatment and baseline values of PSA, haemoglobin and alkaline phosphatase. Additionally, treatment arm (cabazitaxel versus mitoxantrone) and the use of corticosteroids at baseline (yes versus no) were included in the analysis. Baseline PSA, BLNLR and baseline alkaline phosphatase were log-transformed due to their skewed distribution.

Concordance-index (c-index) values of different pre-specified cut-off points (NLR 2, 3 or 5, corresponding approximately with the first, second and third quartiles) were determined for the selection of the most appropriate cut-off for the dichotomous NLR (Tables 1 and 2). To account for multiple testing, a Bonferroni correction was applied for the association of dichotomous NLR at each of the selected cut-offs and OS, with a significant P value set at 0.0167. To evaluate the association of BLNLR with PSA and

Table 1. Association of baseline NLR (cut-off: 3) with survival, PSA response and RECIST response

Survival					
	N	NLR <3	NLR ≥3	HR (95% CI)	*P* value
Overall survival (months)	755	15.9 months	12.6 months	1.55 (1.30–1.84)	<0.001
PSA PFS (months)	654	5.3 months	3.1 months	1.35 (1.12–1.62)	0.002
rPFS (months)	687	9.3 months	5.7 months	1.42 (1.15–1.76)	0.001
Response					
	N	NLR ≥3	NLR <3	OR (95% CI)	*P* value
PSA response (%)	654	22.1%	35.7%	0.51 (0.36–0.72)	<0.001
Radiographic response (%)	330	7.7%	15.6%	0.45 (0.22–0.90)	0.025

Table 2. Association of NLR conversion and survival

Baseline NLR	NLR conversion	*N*	Survival (months)	HR (95% CI) *P* value
High ($n = 345$)	Yes (responder)	151	14.5 (13.5–15.4)	0.76 (0.59–0.98)
	No (non-responder)	194	11.7 (10–13.3)	$P = 0.032$
Low ($n = 326$)	No (non-progression)	125	16.5 (14–19)	1.12 (0.84–1.5)
	Yes (progression)	201	15.7 (14.3–17.2)	$P = 0.432$

radiographic response, a single NLR threshold (NLR equal or greater than or less than 3) was selected based on the survival analysis, and the significant *P* value was set at 0.05. C-index values were calculated with the method proposed by Uno et al. [14].

Patients with at least one follow-up PSA reading and a baseline PSA value ≥20 ng/ml were eligible for PSA response analysis. PSA response was defined as a 50% decrease in PSA relative to baseline, confirmed with a second reading at least 3 weeks later. Maximum PSA decline was defined as the percentage decrease of the nadir PSA relative to baseline PSA; in cases where no PSA decline was observed, the first rising PSA value was used. Similarly, NLR conversion was only analysed in patients with a baseline and at least one follow-up NLR reading. Radiographic response was only considered in patients with measurable disease by RECIST criteria at baseline.

SPSS Statistics version 20.0 (IBM, Inc.) and RStudio Version 0.98.501 (RStudio, Inc.) were used for the statistical analyses.

results

patient characteristics

Overall 755 patients were randomized in the trial and were eligible for analysis; 377 (49.9%) patients were randomized to receive mitoxantrone plus prednisone and 378 (50.1%) to receive cabazitaxel plus prednisone. Median age was 67 years [interquartile range (IQR): 62–73]. A total of 405 (53.6%) patients presented with measurable disease by RECIST criteria, and 181 (24%) had visceral metastases at study entry. Median BLNLR was 3.1 (IQR: 1.9–5.1). Other clinical characteristics at baseline are summarized in supplementary Table S1, available at *Annals of Oncology* online. Median OS was 14.0 months [95% confidence interval (95% CI) 13.2–14.8] with 486 (67.4%) events with a median follow-up of 12.8 months (IQR: 7.8–16.9). Patients receiving cabazitaxel had a median OS of 15.2 months (95% CI 14.1–16.3) and patients treated with mitoxantrone had a median OS of 12.7 months (95% CI 11.6–13.7).

BLNLR was significantly associated with other established prognostic factors such as ECOG PS Score ($P < 0.001$), visceral metastases at study entry ($P = 0.019$), presence of pain at baseline ($P = 0.007$), haemoglobin ($P = 0.002$), alkaline phosphatase ($P = 0.012$) and use of steroids at baseline ($P = 0.026$) (supplementary Table S2, available at *Annals of Oncology* online).

baseline NLR is associated with overall survival

BLNLR, treated as a continuous variable, was associated with OS in univariable analysis (HR 2.89; 95% CI 2.12–3.94; $P < 0.001$), and in multivariable analysis (HR 1.91; 95% CI 1.31–2.79; $P = 0.001$), when including prognostic factors from the Halabi nomogram, treatment arm and corticosteroid use at baseline (supplementary Table S3, available at *Annals of Oncology* online). To investigate the potential value of adding BLNLR to the variables identified by the Halabi nomogram in predicting OS, we calculated the concordance index (c-index) values for Halabi's prognostic score with and without BLNLR. The c-index of the model with factors included in the Halabi nomogram was 0.728 (95% CI 0.699–0.757); when adding BLNLR to the model, the c-index increased to 0.736 (95% CI 0.707–0.765). The difference in c-index with and without BLNLR was however not statistically significant (difference in c-index: 0.008; 95% CI −0.005–0.020).

Different reports have suggested different cut-off values when analysing BLNLR and OS. We attempted first to establish the optimal cut-off point for the analysis of a dichotomous NLR and survival by comparing c-statistic values of NLR values representing approximately the median (NLR3) and first (NLR2) and third quartiles (NLR5). All three cut-off points met the pre-specified criteria for statistical significance (NLR2: $P = 0.016$; NLR3: $P = 0.000$; NLR5: $P < 0.001$). A cut-off of 3 had a higher c-index (c-index NLR3: 0.544; 95% CI 0.522–0.566) than a cut-off of 2 (c-index NLR2: 0.524; 95% CI 0.499–0.549) or a cut-off of 5 (c-index NLR5: 0.539: 95% CI 0.520–0.558) and was therefore selected as the optimal cut-off threshold for further analyses.

Patients with BLNLR <3 had a statistically significant higher median OS [15.9 versus 12.6 months, HR 1.55 (95% CI 1.3–1.84), $P < 0.001$], PSA progression-free survival [5.3 versus 3.1 months; HR 1.35 (95% CI 1.12–1.62); $P = 0.002$] and radiographic progression-free survival [9.3 versus 5.7 months; HR 1.42 (95% CI 1.15–1.76); $P = 0.001$] than patients with BLNLR ≥3 (Figure 1, supplementary Table S4, available at *Annals of Oncology* online). The observed benefit was independent of treatment arm, as BLNLR remained significant when including treatment arm in the model. The survival benefit of cabazitaxel over mitoxantrone was consistent in patients with high (≥3) BLNLR [14.1 versus 11.6 months; HR 0.72 (95% CI 0.56–0.91)] and patients with low (<3) BLNLR (16.7 versus 14.8 months; HR 0.67 (95% CI 0.51–0.87)] (supplementary Figure S1, available at *Annals of Oncology* online).

baseline NLR and baseline corticosteroid use

Overall 342 (45%) patients were receiving systemic corticosteroids at baseline, before initiating trial treatment; 171 (50%) patients were receiving prednisone, 80 (23%) prednisolone, 48 (14%) hydrocortisone, 32 (10%) dexamethasone and 11 (3%) were receiving other systemic corticosteroids (betamethasone, cortisone, methylprednisolone or triamcinolone). Median NLR was higher in patients receiving corticosteroids at baseline than in patients not receiving corticosteroids at baseline (3.9 versus 2.9; $P < 0.001$). Conversely, a higher proportion of patients with

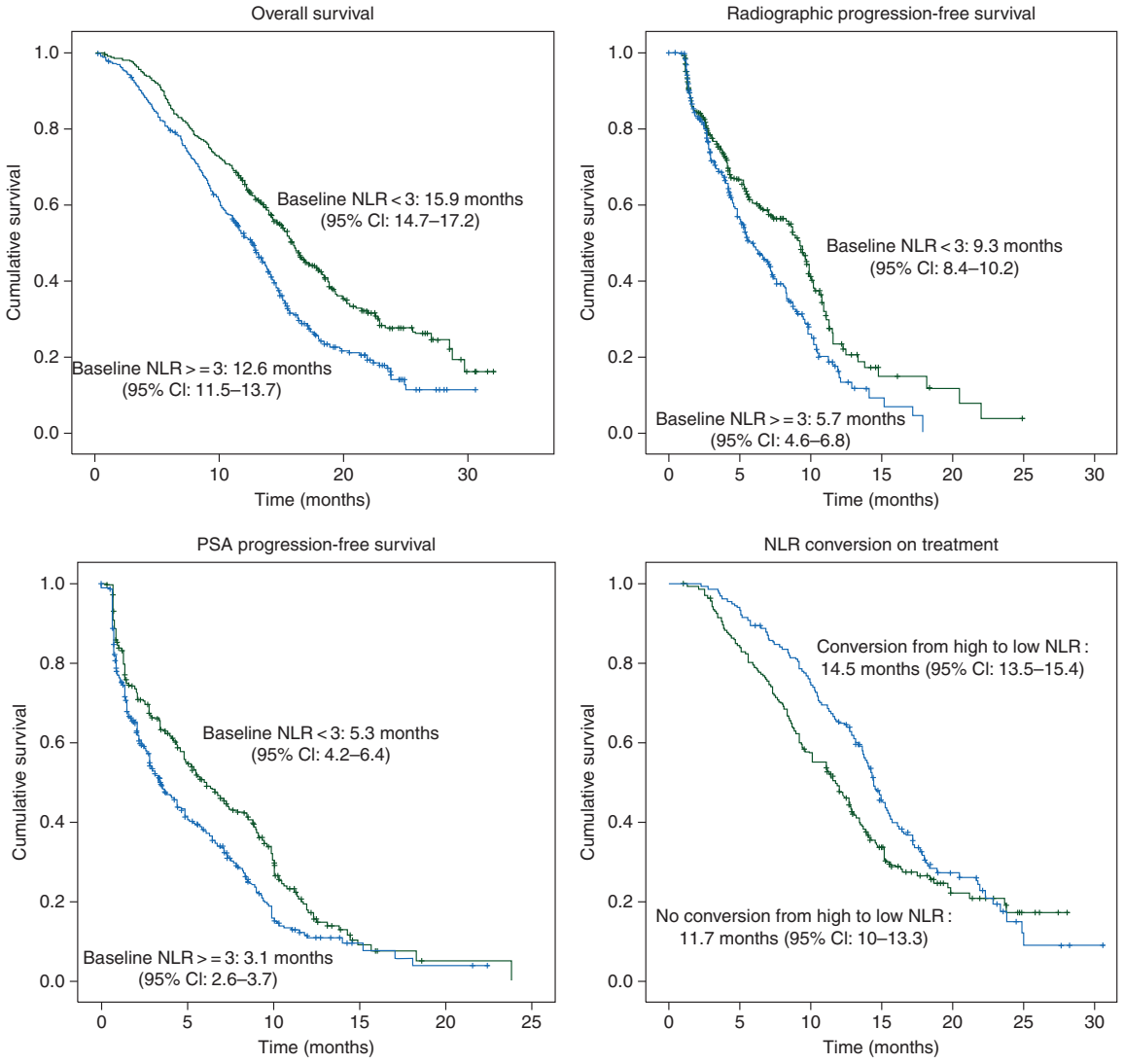

Figure 1. Association of baseline NLR (≥3 versus <3) with overall survival (top left), radiographic progression-free survival (top right) and PSA progression-free survival (bottom left). Bottom right: association of a conversion of NLR from high (≥3) to low (<3) counts during the first 12 weeks of treatment and overall survival.

BLNLR counts ≥3 were receiving treatment with corticosteroids before study entry (49.6% versus 40.6%; $P = 0.016$). When including baseline corticosteroid use in the prognostic model, BLNLR remained significant, therefore indicating that the prognostic value of BLNLR is independent of baseline corticosteroid use (supplementary Table S3, available at *Annals of Oncology* online). The use of corticosteroids at baseline did not modify the association between NLR and survival, as indicated by a non-significant interaction test ($P = 0.82$).

baseline NLR and response to treatment

baseline NLR and radiological response by RECIST criteria. A total of 405 patients (53.6%) had measurable disease at baseline; of these, 330 patients had at least one follow-up scan and thus were eligible for radiographic response analysis. We conducted an initial univariable analysis of factors associated with a radiographic response of which only BLNLR levels (equal or greater versus less than 3) and treatment arm were significant. 15.6% (32/147) of patients with baseline low NLR levels versus 7.7% (14/183) of those with high BLNLR counts achieved a radiological response ($P = 0.022$). The association of BLNLR and radiological response was independent of treatment arm (supplementary Table S5, available at *Annals of Oncology* online).

baseline NLR and PSA response. We carried out an analysis of the association of BLNLR and PSA response. Overall 17 patients did not have PSA data at baseline and 85 patients with values

below 20 ng/ml were deemed unassessable. PSA response was analysed in the remaining 654 patients. Median PSA decline was greater in patients with NLR <3 (41.3% versus 15.4%, $P < 0.001$). Supplementary Figure S2, available at *Annals of Oncology* online, shows waterfall plots depicting maximum PSA declines in each of the groups. PSA response was defined as a 50% decline from baseline confirmed by a second reading at least 3 weeks later. A total of 187 patients (24.8%) achieved a PSA response. PSA response was greater in patients receiving cabazitaxel (39.2% versus 17.8%; $P < 0.001$), in patients not receiving steroids at baseline (32.7% versus 23.6%; $P = 0.004$), in patients with BLNLR <3 (35.7% versus 22.1%; $P < 0.001$) and in patients that had progressed on docetaxel more than 6 months before study entry (50.8% versus 26.4%; $P < 0.001$). Time on hormonal treatment and baseline alkaline phosphatase levels were associated with PSA response in univariable, but not in multivariable analyses. The association between BLNLR and PSA response remained statistically significant in multivariable analyses, and was independent of the use of corticosteroids at baseline (supplementary Table S6, available at *Annals of Oncology* online) There was no interaction between BLNLR levels and steroid use at baseline ($P = 0.364$), indicating that steroid use did not modify the association between NLR and PSA response.

NLR conversion on treatment. We studied the value of NLR as an indicator of response to treatment by evaluating the significance of conversions between 'favourable NLR' (NLR <3) and 'unfavourable NLR' (NLR ≥3) during the first 12 weeks of treatment (cycle 1 day 1 to cycle 5 day 1) in response to treatment. Overall 345 patients presented with baseline unfavourable NLR and at least one follow-up NLR; of these, 151 (44%) experienced a 'conversion' during treatment. Patients with a conversion from 'unfavourable' to 'favourable' NLR had a significantly longer OS (14.5 versus 11.7 months; HR 0.76, 95% CI 0.59–0.98; $P = 0.032$) (Figure 1); the difference in survival was independent of treatment arm and all other prognostic factors in multivariable analyses (supplementary Table S7, available at *Annals of Oncology* online). A conversion from unfavourable to favourable counts was also associated with a higher 50% PSA response rate (30.4% versus 18.6%; $P = 0.016$). Conversely, 326 patients presented with a baseline favourable NLR and were eligible for analysis; of these, 201 (62%) experienced a conversion to an unfavourable NLR. This was, however, not significantly associated with a worse survival (15.7 versus 16.5 months; HR 1.12, 95% CI 0.84–1.5; $P = 0.4$) or a lower rate of PSA response (35.9% versus 39.3%; $P = 0.56$).

discussion

In this report, we perform a complete analysis of the value of the NLR as a biomarker in men with CRPC treated with second-line chemotherapy. We validate the prognostic value of BLNLR, which had been previously reported in the post-docetaxel setting [10]. The prognostic value of BLNLR was independent of other factors incorporated into a clinically validated nomogram [4].

The potential detrimental effect of concomitant corticosteroids in prostate cancer treatment is currently a matter of debate. Immunosuppression is a well-known side-effect of steroid treatment; therefore, treatment with corticosteroids is a potentially confounding factor that has not been studied in previous reports. In our dataset, patients receiving corticosteroids at baseline had a significantly higher NLR. However, the association of NLR counts and both survival and response to treatment was independent of treatment with corticosteroids at baseline, and steroid use at baseline did not modify the association of NLR with survival or response.

To investigate the applicability of BLNLR in the daily clinical setting, we identified the optimal cut-off as a dichotomous variable. A cut-off of 3.0, classifying patients with a BLNLR of 3 or more as having 'high' NLR and those with >3 with 'low' NLR conferred the highest prognostic value. Using this cut-off, we validated the strong association of BLNLR counts and PSA response, previously reported in patients treated with abiraterone [11] but reported for the first time in patients treated with chemotherapy. BLNLR count was also associated with response by RECIST criteria, this being independent of treatment arm. The value of NLR counts as an indicator of response to treatment was examined by evaluating the value of a conversion from low to high baseline counts. We found that a conversion from NLR 'high' to 'low' in the first 12 weeks of treatment is associated with an improved survival as well as higher rates of PSA response.

We acknowledge a number of limitations in our study. As a *post hoc* and unplanned analysis, these results need to be validated in prospective studies. Moreover, LDH was not collected in patients treated in the TROPIC trial, although other studies have reported an association of NLR with OS independent of baseline LDH. The NLR cut-off of 3, which was selected based on its prognostic value in our dataset, will also have to be validated in prospective studies. It is noteworthy, however, that an NLR cut-off of 3 corresponds with the median NLR in our dataset; NLR counts dichotomised around the median value of each study were associated with survival in a meta-analysis of different tumour types [7].

The biologic changes generating the results observed in our study remain unclear, although they underscore the importance of the host immune system against prostate cancer. Cancer-related inflammation has long been recognized as one of the hallmarks of cancer [6], and the successful development of immune-checkpoint targeting agents has opened a promising avenue for anticancer drug development. In advanced prostate cancer, the immunotherapeutic agent sipuleucel-T has shown survival benefit in asymptomatic or mildly symptomatic CRPC [15], and is approved by the FDA for this indication.

We hypothesize that BLNLR count is an indicator of the host immune response to cancer. It is, however, unclear whether the value of NLR count is driven by the relative lymphopenia or an increase in myeloid cells.

Studies evaluating the prognostic value of mRNA signatures in patients with advanced prostate cancer identified the down-regulation of genes associated with T-cell function [16] as strongly associated with a worse prognosis. On the other hand, the presence of intra-tumoral Gr1+ myeloid precursors has been reported to protect PTEN null prostate transgenic cells from chemotherapy-induced senescence [17], fuelling their proliferation; whether a high relative neutrophil count reflects such an increase in myeloid infiltration of tumour tissue remains unknown. Nonetheless, these emerging data now indicate that targeting neutrophils/granulocytes, potentially by leukapheresis,

splenectomy, radiotherapy to the spleen or specific granulocyte-targeting agents may have anti-tumour activity against CRPC.

In conclusion, baseline NLR has emerged as an important biomarker in castration-resistant prostate cancer due to its association with OS but also to response to anticancer treatment. The ubiquitious availability of the NLR makes this an important tool in risk assessment with immediate clinical applicability.

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disclosure

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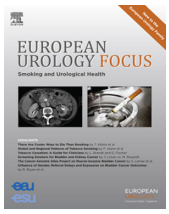

Prostate Cancer

Interrogating Metastatic Prostate Cancer Treatment Switch Decisions: A Multi-institutional Survey

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Abstract

Background: Evaluation of responses to treatment for metastatic castration-resistant prostate cancer (mCRPC) remains challenging. Consensus criteria based on prostate-specific antigen (PSA) and clinical and radiologic biomarkers are inconsistently utilized. Circulating tumor cell (CTC) counts can inform prognosis and response, but are not routinely used.
Objective: To evaluate the use of biomarkers and trends in clinical decision-making in current mCRPC treatment.
Design, setting, and participants: A 23-part online questionnaire was completed by physicians treating mCRPC.
Outcome measures and statistical analysis: Results are presented as the proportion (%) of physicians responding to each of the options. We used χ^2 and Fisher's tests to compare differences.
Results and limitations: A total of 118 physicians (22.1%) responded. Of these, 69.4% treated ≥50 mCRPC patients/year. More physicians administered four or fewer courses of cabazitaxel (27.9%) than for docetaxel (10.4%), with no significant difference in the number of courses between bone-only disease and Response Evaluation Criteria in Solid Tumours (RECIST)–evaluable disease. Some 74.5% of respondents considered current biomarkers useful for monitoring disease, but only 39.6% used the Prostate Cancer Working Group (PCWG2) criteria in clinical practice. PSA was considered an important biomarker by 55.7%, but only 41.4% discarded changes in PSA before 12 wk, and only 39.4% were able to identify bone-scan progression according to PCWG2. The vast majority of physicians (90.5%) considered clinical progression to be important for switching treatment. The proportion considering biomarkers important was 71.6% for RECIST, 47.4% for bone scans, 23.2% for CTCs, and 21.1% for PSA. Although 53.1% acknowledged that baseline CTC counts are prognostic, only 33.7% would use CTC changes alone to switch treatment in patients with bone-only disease. The main challenges in using CTC counts were access to CTC technology (84.7%), cost (74.5%), and uncertainty over utility as a response indicator (58.2%).

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Conclusions: A significant proportion of physicians discontinue treatment for mCRPC before 12 wk, raising concerns about inadequate response assessment. Many physicians find current biomarkers useful, but most rely on symptoms to drive treatment switch decisions, suggesting there is a need for more precise biomarkers.
Patient summary: In this report we analyse the results of a questionnaire evaluating tools for clinical decision-making completed by 118 prostate cancer specialists. We found that most physicians favour clinical progression over prostate-specific antigen or imaging, and that criteria established by the Prostate Cancer Working Group are not widely used.

1. Introduction

The past decade has seen an increase in the therapeutic armamentarium against metastatic prostate cancer, with agents proving survival benefit both in the castrate-resistant (mCRPC) [1–7] and castration-naïve stages [8,9] of the disease. This increased availability of treatment options necessitates improved biomarkers to determine treatment responses more rapidly and facilitate optimised decisions on therapeutic sequencing [10].

Prostate-specific antigen (PSA), bone scans, and Response Evaluation Criteria in Solid Tumours (RECIST) criteria are commonly utilized to evaluate responses and are recommended as outcome measures by the Prostate Cancer Working Group (PCWG2) for clinical trials [11]. However, these biomarkers have significant limitations. In particular, PSA and bone scans do not allow early response assessment, and none of the biomarkers provide patient-level surrogates of clinical benefit [12,13]. This challenge is compounded by the lack of RECIST-evaluable disease in a substantial proportion of patients [14]. For daily clinical practice, existing guidelines do not recommend specific treatment monitoring, an issue addressed by the Advanced Prostate Cancer Consensus conference [15].

The lack of adequate biomarkers may impact the dose intensity of chemotherapy and other anticancer (hormonal, radiopharmaceutical) agents administered in daily clinical practice. The fact that determining disease progression in the absence of clear clinical deterioration is impossible before 12 wk (owing to the possibility of an early PSA or bone scan "flare reaction") in patients with no RECIST-evaluable disease may contribute to both the administration of more chemotherapy cycles to patients with bone-only disease (overtreatment) and a higher reliance on PSA changes for early treatment discontinuation (undertreatment).

Circulating tumour cell (CTC) counts are prognostic and are associated with treatment response in mCRPC patients, with recent studies indicating value as a patient-level surrogate of survival [16,17]. Increasing evidence suggests that CTCs could be utilised to monitor disease progression in mCRPC [18]. However, CTC use is largely limited to academic centres in the setting of clinical trials.

We conducted an online survey of physicians treating mCRPC. The survey focused on how physicians make treatment switch decisions, opinion on response indicators, utilisation of PCWG2 criteria in routine practice, and the value of CTC counts to guide treatment switch decisions. The results will help to inform the design of an international trial and health economic evaluation to improve treatment switch decisions for mCRPC patients to improve outcomes, decrease overtreatment, and maximise resource utilisation.

2. Materials and methods

A 23-part online questionnaire, divided in four sections as outlined below, was compiled by the authors (Supplementary Fig. 1):

1. General questions on clinical practice.
2. Familiarity with progression criteria for currently established biomarkers.
3. CTCs and their assessment in patients with advanced prostate cancer.
4. Clinical decision-making using response indicators.

E-mails inviting participation in the survey were sent to 485 UK investigators participating in urologic cancer clinical trials, 29 physician members of the GU Group of the Swiss Group for Clinical Cancer Research, and 20 practising prostate cancer physicians in Australia and New Zealand. A link to the web-based survey (created with SurveyMonkey) was included.

2.1. Statistical analyses

Descriptive statistics were used; the proportion (%) of physicians responding to each option is presented. Physicians were classified according to the number of patients they treated ($\geq$50 vs $<$50 patients/year) or recruited to clinical trials ($\geq$25% vs $<$25%), and the number of cycles of docetaxel/cabazitaxel prescribed ($\leq$4, 5–6, $\geq$7 cycles). No pre-existing evidence was used in choosing classification cutoff values. Proportions were compared using a χ^2 test or Fisher's exact test (for cell frequencies $\leq$5). A p value of 0.05 was set as the limit for statistical significance. No adjustment for multiple testing was performed. SPSS version 21 (IBM IBM, Armonk, NY, USA) was used.

3. Results

3.1. Participant characteristics and their clinical practice

Between November 21, 2014 and December 18, 2014, 118 practising prostate cancer physicians (22.1%) replied. Sections 1, 2, 3, and 4 were completed by, 111, 106, 98, and 89 physicians, respectively. Most respondents (77.1%) practised in the UK. Nearly 70% treated $\geq$50 mCRPC patients/year (Table 1). Most reported prescribing 7–10 courses of docetaxel and 5–6 cycles of cabazitaxel (Fig. 1); there was no difference in the number of courses of either docetaxel ($p(\chi^2_2) = 0.519$) or cabazitaxel ($p(\chi^2_2) = 0.814$) administered to patients with RECIST-evaluable disease compared to patients with bone-only disease. Physicians

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Table 1 – Participant characteristics

Question (number of responses)	*n* (%)
Q1: Specialty (*n* = 118)	
Oncologist	100 (84.7)
Urologist	17 (14.4)
Other	1 (0.8)
Q2: Practice location (*n* = 118)	
UK	91 (77.1)
Europe (non-UK)	16 (13.6
Australia/New Zealand	11 (9.3)
Q3: Number of mCRPC patients treated per year (*n* = 111)	
<10	3 (2.7)
10–49	31 (27.9)
50–99	48 (43.2)
≥100	29 (26.1)
Q4: Percentage of mCRPC patients entered into clinical trials (*n* = 111)	
None	6 (5.4)
<25%	53 (47.7)
25–49%	38 (34.2)
50–74%	12 (10.8)
≥75%	2 (1.8)

mCRPC = metastatic castration-resistant prostate cancer.

reported giving more courses of docetaxel than cabazitaxel in patients with both RECIST-evaluable and bone-only disease ($p(\chi^2_2) < 0.001$). Physicians with larger patient practices prescribed more courses of chemotherapy (Supplementary Table 1).

3.2. *Evaluation of currently available response biomarkers*

Current guidelines provide little instruction on the evaluation of response to treatment in mCRPC; this is particularly challenging in patients with only bone metastases and no other measurable disease [15,19]. PCWG2 progression criteria (Supplementary Table 2) are mainly used among patients treated within clinical trials. We evaluated the opinion of physicians on currently available biomarkers (PSA, bone scan, and CTCs) for monitoring response. Some 79 respondents (74.5%) rated these as useful (71.7%) or very useful (2.8%). Only 39.6% reported using PCWG2 criteria most or all of the time, and 27.3% reported rarely or never using the criteria (Table 2). Physicians recruiting more patients to trials were more likely to use PCWG2 frequently (56% vs 25%; $p(\chi^2_2) = 0.001$) = 0.001; Supplementary Table 3).

3.2.1. *PSA*

A total of 59 respondents (55.7%) reported that PSA was a useful/very useful biomarker for monitoring response to treatment (Table 2). We asked participants to identify PSA progression in graphical examples showing consecutive PSA values to evaluate their ability to utilize PCWG2 criteria. Only 41.4% of physicians correctly recognised that at least 12 wk are required to define PSA progression (Fig. 2A). Most physicians (84.8%) correctly identified that a 25% increase from the nadir value (confirmed by a second value at least 3 wk later) constituted progression (Fig. 2B). Some 90.9% failed to recognise that PSA progression holds even if the confirmatory second value is lower than the first, providing both values show a 25% increase from the nadir (Fig. 2C). Only two physicians (2.0%) answered all three questions correctly.

3.2.2. *Bone scintigraphy*

PCWG2 criteria define bone scan progression as a minimum of two new lesions, with new lesions observed at the first 12-wk reassessment requiring a confirmatory scan (Supplementary Table 2). When respondents were asked to choose from a number of definitions of bone scan progression (selecting more than one was permitted), only 39.4% answered the correct option (as per PCWG2) and discarded the incorrect options, indicating diversity in bone scan interpretation.

3.2.3. *CTCs*

Some 98% of respondents were familiar with the concept of CTCs, but only 53.1% recognised that baseline CTCs have

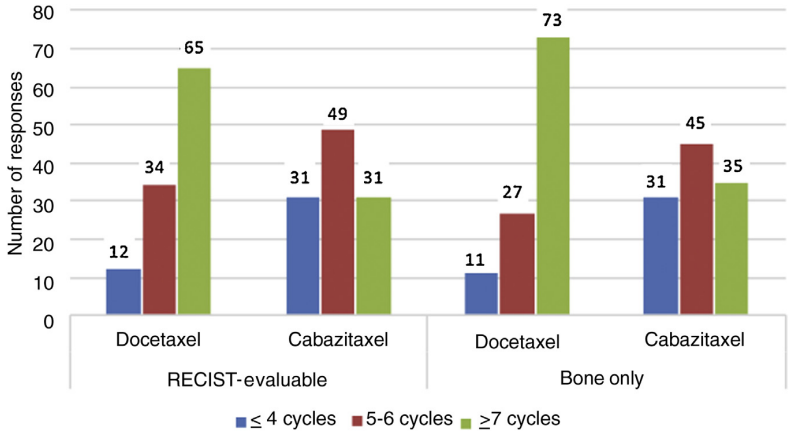

Fig. 1 – Number of cycles of chemotherapy administered to patients with Response Evaluation Criteria in Solid Tumours (RECIST)–evaluable disease and bone-only metastatic castration-resistant prostate cancer (mCRPC). The figure summarises replies for Questions 5–8 ("How many cycles of docetaxel/cabazitaxel do you prescribe, on average, to mCRPC patients with RECIST-evaluable/bone only disease?").

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Table 2 – Evaluation of currently available biomarkers, CTCs and use of Prostate Cancer Working Group (PCWG2) criteria in mCRPC

Question (number of responses)	*n* (%)
Q9: Suitability of currently available biomarkers (PSA, bone scans, CTCs) in monitoring disease in mCRPC (*n* = 106)	
Very useful	3 (2.8)
Useful	76 (71.7)
Not very useful	25 (23.6)
Poor	2 (1.9)
Q11: Suitability of PSA as a chemotherapy response marker in mCRPC (*n* = 106)	
Very useful	3 (2.8)
Useful	56 (52.8)
Not very useful	44 (41.5)
Poor	3 (2.8
Q10: Use of PCWG2 criteria for decision-making when treating patients with mCRPC (*n* = 106)	
Always	3 (2.8)
Mostly	39 (36.8)
Sometimes	35 (33)
Rarely	12 (11.3)
Never	17 (16)
Q14: Familiar with the concept of CTCs (*n* = 98)	
Yes	96 (98)
No	2 (2)
Q15: Baseline number of CTCs at start of chemotherapy is prognostic for overall survival in mCRPC (*n* = 98)	
Yes	52 (53.1)
No	0 (0)
Unsure	46 (46.9)
Q16–17: Change in number of CTCs is associated with response in mCRPC during (*n* = 98): Chemotherapy	
Yes	53 (54.1)
No	0 (0)
Unsure	45 (45.9)
Abiraterone	
Yes	49 (50)
No	0 (0)
Unsure	49 (50)
Q18: Challenges associated with use of CTCs in prostate cancer (*n* = 98)	
Cost	73 (74.5)
Lack of/uncertainty about prognostic significance	43 (43.9)
Lack of/uncertainty about predictive information on treatment response	57 (58.2)
Difficulty in interpreting changes in CTC number	41 (41.8)
Poor access to CTC enumeration technology	83 (84.7)
Other	4 (4.1)
Q20: Likelihood of switching or stopping chemotherapy in an asymptomatic mCRPC patient with PSA increase at 12 wk and no radiologic progression (*n* = 95)	
Definitely	0 (0)
Likely	16 (16.8)
Unlikely	70 (73.7)
Definitely not	9 (9.5)
Q21: Likelihood of switching or stopping abiraterone or enzalutamide in an asymptomatic mCRPC patient with PSA increase at 12 wk and no radiologic progression (*n* = 95)	
Definitely	0 (0)
Likely	9 (9.5)
Unlikely	68 (71.6)
Definitely not	18 (18.9)
Q23: Likelihood of using CTC changes alone, independently of PSA or bone scan findings, in guiding decision-making to switch or stop therapy in an mCRPC patient with bone-only disease (*n* = 89)	
Definitely	1 (1.1)
Likely	29 (32.6)
Unlikely	55 (61.8)
Definitely not	4 (4.5)

CTC = circulating tumour cell; mCRPC = metastatic castration-resistant prostate cancer; PSA = prostate-specific antigen.

prognostic value. Similarly, only 50.0% and 54.1% respondents were aware that a post-treatment change in CTCs was associated with outcome in patients treated with abiraterone and chemotherapy, respectively (Table 2).

Major challenges identified by respondents as currently limiting the use of CTCs in prostate cancer were assay cost (74.5%), poor access to CTC enumeration tests (84.7%), and uncertainty over their clinical utility in response assessment (58.2%; Table 2).

3.3. *Clinical decision-making in CRPC*

According to PCWG2, clinical progression is defined as worsening pain and analgesic use, deteriorating quality of life, urinary or bowel compromise, or a need for new anticancer therapy. Of these, only worsening pain is associated with outcome in prospective clinical trials [20]. Almost all physicians (90.5%) considered clinical progression to be important for driving treatment switches.

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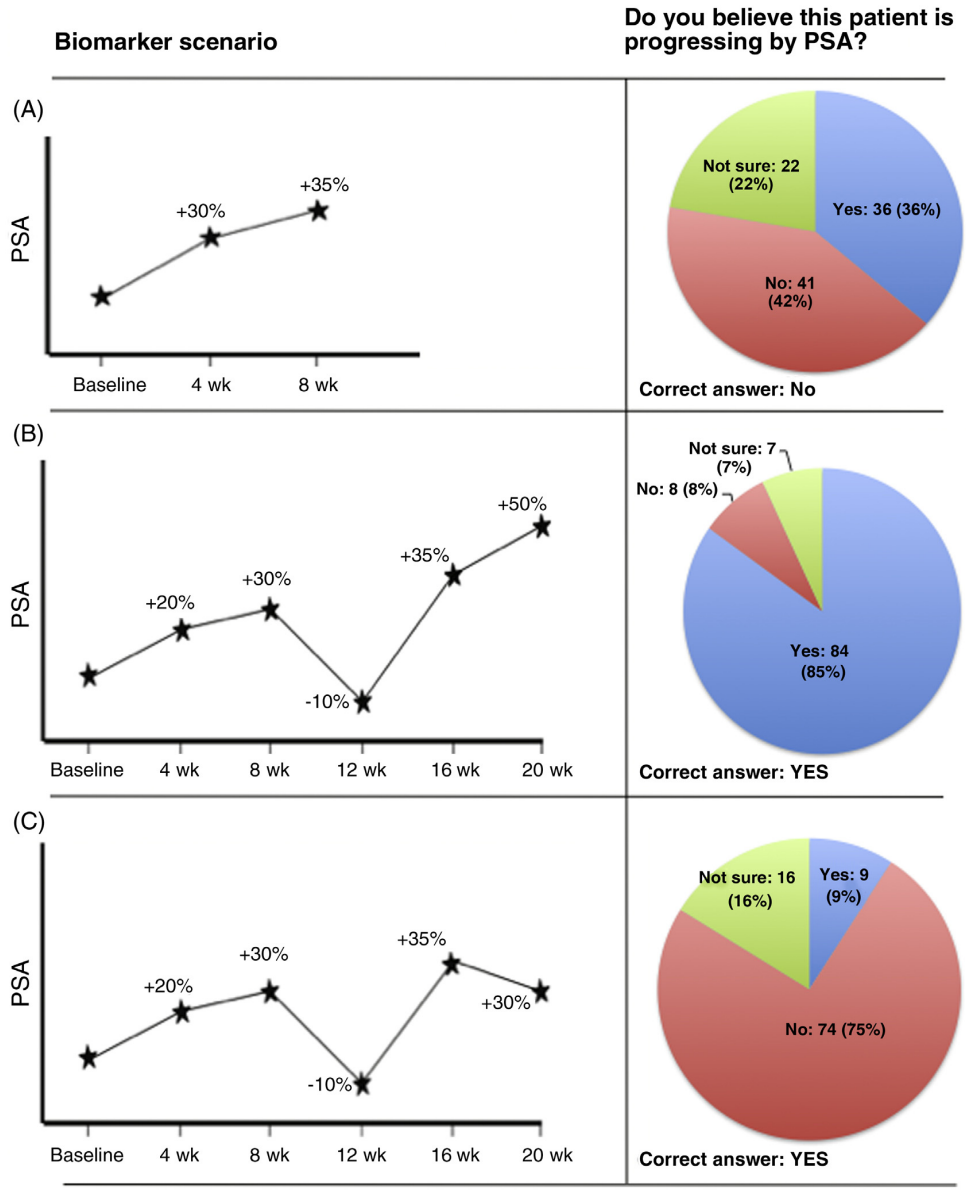

Fig. 2 – Evaluation of prostate-specific antigen (PSA) progression criteria. The figure summarises replies for Question 12. Participants were shown three different PSA biomarker scenarios for patients with bone-only disease. The percentage of participants who believed the scenario corresponded to PSA progression is shown in the pie charts. Correct response: (A) No; (B) Yes; (C) Yes.

Some 71.6% and 47.7% felt RECIST and bone scan progression to be important, and only 23.2% and 21.1% felt CTC and PSA progression to be important, respectively.

Overall, 55.7% considered PSA useful/very useful in guiding therapy, but only 21.1% considered it important for decision-making (Fig. 3). Physicians who considered PSA and bone scans important/very important for decision-making did not have a better understanding of response criteria (Supplementary Table 4). Only 30% of physicians who considered PSA important/very important in guiding treatment switches acknowledged that at least 12 wk is needed to define PSA progression (Supplementary Table 4).

In the case of an asymptomatic mCRPC patient with a rising PSA at 12 wk but no evidence of radiologic progression, most physicians were unlikely to switch/stop chemotherapy (83.2%) or abiraterone/enzalutamide (90.5%). Only 33.7% of respondents were ready to use CTC changes alone, independently of PSA or bone scans, to guide switching/stopping therapy in patients with bone-only disease; among those who acknowledged the value of

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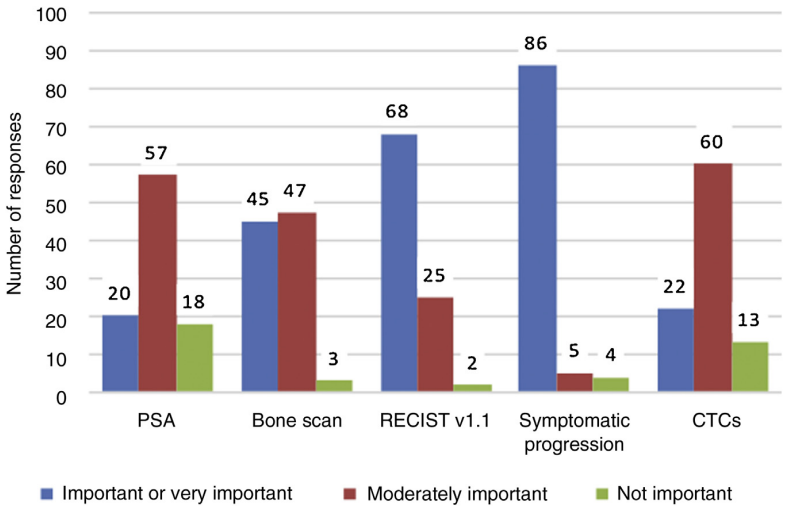

Fig. 3 – Importance of different biomarkers in clinical decision-making (stopping therapy) in metastatic castration-resistant prostate cancer. The figure summarises replies for Question 19. Participants were asked to rank each of the different types of disease progression listed from 1 (extremely important) to 6 (not at all important) in their clinical decisions to switch or stop therapy. RECIST = Response Evaluation Criteria in Solid Tumours; PSA = prostate-specific antigen; CTCs = circulating tumour cells.

CTCs as a response-biomarker, the proportion was 43.5%. Of those who were likely/very likely to switch on CTC changes alone independently of PSA or bone scans, a larger proportion were physicians who felt that currently available biomarkers are not very useful/poor in monitoring disease ($p = 0.03$; Supplementary Table 5). Among those who were unlikely/unwilling to switch on CTC changes alone, 57.6% cited uncertainty over predictive information on treatment response as a challenge in use of CTCs, with 52.5% and 42.4% citing uncertainty over prognostic significance and difficulty in interpreting CTC changes, respectively.

3.4. Treatment switches in mCRPC

The final part of the questionnaire asked respondents to consider scenarios involving clinically stable mCRPC patients with bone-only disease. For a >25% PSA rise but a CTC decline to <5 cells/7.5 ml ("favourable" CTC conversion) and a stable bone scan at 12 wk, 92.1% of respondents would not switch/stop therapy (Fig. 4A). The proportion fell to 68.5% if the bone scan showed increased tracer uptake but no new lesions (Fig. 4B). For a 50% fall in PSA but a CTC rise to ≥5 cells/7.5 ml ("unfavourable" CTC conversion) at 12 wk and stable disease according to a bone scan, only 11.2% would switch/stop therapy (Fig. 4C). For a 50% PSA decline and CTC conversion from "unfavourable" to "favourable" count at 12 wk, but two new lesions on a bone scan, most respondents (70.8%) reported they would not switch/stop therapy (Fig. 4D).

Respondents who believed that post-treatment CTC changes were associated with treatment response were more likely to switch/stop therapy on CTC progression as in Figure 4C ($p = 0.023$), and were more likely to continue treatment with CTC response as in Figure 4B ($p = 0.003$) and Figure 4D ($p = 0.005$; Supplementary Table 3).

4. Discussion

It is imperative that more precise response biomarkers that can guide more rapid identification of drug resistance and treatment termination are developed to minimise the overtreatment of patients with ineffective therapies, decrease the toxicity of ineffective treatment, and maximize the utilisation of resources. We conducted this survey to evaluate current practice in clinical decision-making by physicians specialised in the treatment of CRPC. Our results highlight difficulties in the application of current biomarkers in the treatment of advanced prostate cancer in daily clinical practice.

Are physicians giving too much chemotherapy, or too little? The optimum number of chemotherapy courses is unclear. In the TROPIC trial, although a maximum number of ten cycles of chemotherapy was allowed, a median of six courses was reported, and 28% of patients completed ten courses [7]. This is similar to numbers reported in expanded-access programmes [21,22]. In TAX-327, in which the number of cycles of docetaxel was not limited to ten, the median number of cycles in the three-weekly docetaxel arm was 9.5 [23]. Our survey, however, indicates that a significant number of physicians discontinue treatment before four courses (12 wk) of treatment; this is especially true for cabazitaxel. According to our survey, early discontinuation does not appear to be related to radiologic disease progression, since no difference in the number of chemotherapy courses between RECIST-evaluable and bone-only disease was reported.

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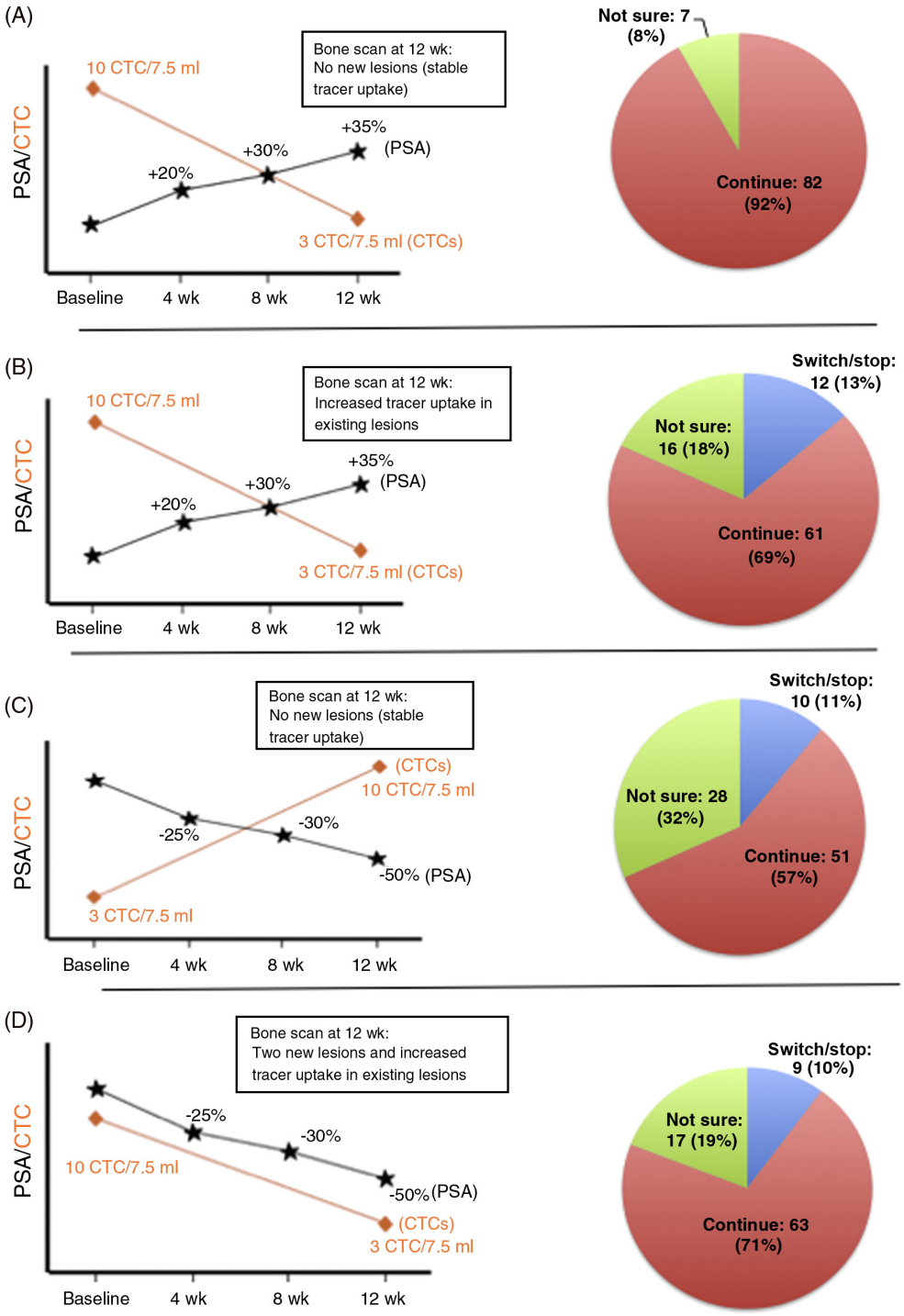

Fig. 4 – Decision-making for different biomarker scenarios. The figure summarises replies for Question 22. Participants were shown four different biomarker scenarios combining prostate-specific antigen (PSA), circulating tumor cells (CTCs), and bone scan findings for clinically stable patients. The proportion of participants who would switch or stop therapy at 12 wk is shown in the pie charts.

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How familiar are physicians with consensus response criteria? In our survey, although most physicians considering currently available biomarkers (74.5%), and PSA in particular (55.6%), to be useful for monitoring disease, knowledge of the specific PCWG2 criteria is suboptimal. PCWG2 requires a confirmatory value at least 3 wk after a first progressing PSA, and recommends discarding any early (before 12 wk) PSA increase owing to the possibility of PSA "flare", reported in 16.7% of patients in TAX-327 [24]. In our survey, many physicians failed to acknowledge the possibility of a PSA flare in evaluating PSA progression.

Concerns regarding the interpretation of bone-scan imaging were also identified. Only around 40–60% of mCRPC patients are evaluable according to RECIST, with many patients having bone-only disease [14]. PCWG2 criteria indicate that bone scans can only be used for the assessment of progression and not response. New lesions at the first 12-wk assessment require a confirmatory scan, since early bone-scan "flare" is not uncommon [25]. Only 39.4% of respondents followed the PCWG2 definition of bone scan progression, despite recent studies indicating an association between radiographic progression-free survival (combining a bone scan and RECIST) and survival in the COU-302 phase 3 trial [26].

These findings suggest that decisions to switch treatment are challenging for physicians treating advanced prostate cancer. PCWG2 guidelines acknowledge difficulties in assessing progression according to clinical symptoms alone because of "subjectivity" [11]; however, this was overwhelmingly acknowledged as the most important determinant of disease progression in routine practice. RECIST criteria ranked second in importance, despite being useful for only some patients. Interestingly, only 39.6% commonly use PCWG2 criteria for clinical-decision making. When confronting physicians with clinical scenarios based on CTC, PSA and bone scan information no significant predominance of one biomarker was found. Physicians generally continued treatment in the face of "contradictory" biomarker information (ie, rising CTCs with falling PSA; falling CTCs with rising PSA; or falling CTCs and PSA with new lesions on bone scan), for which current European Association of Urology and European Society for Medical Oncology guidelines do not offer clear recommendations on optimal decision-making. Importantly, we observed no significant differences in the familiarity with PSA or bone scan progression criteria (questions 12 and 13), the importance of each of the biomarkers in the decision to switch or stop therapy (question 19), or the likelihood of switching or stopping in the face of the different proposed biomarker scenarios (question 20) between physicians treating in high-volume centres ($\geq$50 patients/yr) and those in low-volume centres ($<$50 patients/yr). These data suggest a need for more precise biomarkers to report on response and progression, since patients today appear to continue receiving treatment despite biomarkers indicating a lack of response.

CTC count holds promise as a response biomarker, with well-established prognostic utility that has been validated prospectively with chemotherapy [16,27], abiraterone [17], and enzalutamide [28]. A combination of lactate dehydrogenase and CTCs is a patient-level surrogate of survival [17], and post-treatment changes are robustly associated with outcome [29,30]. Moreover, CTC counts have greater sensitivity and specificity and inform on outcome earlier than changes in PSA do [30,31]. However, only half of the responding physicians were familiar with available CTC data, with very few prepared to stop abiraterone (9.5%) or chemotherapy (16.8%) on the basis of CTC progression. Nonetheless, physicians cognisant of available CTC data were more willing to guide treatment according to CTC changes. Cost was reported as a major caveat to the routine use of CTCs, although most of this could be easily recouped by earlier discontinuation of ineffective treatment.

We acknowledge a number of limitations to our study. The return rate was 22.1%, and not all physicians completed the entire survey. Reasons for not completing the survey are unknown, although this could be related to the lack of compensation offered. Furthermore, no distinction was made between academic and nonacademic centres, and no comparison was made between UK-based and non–UK-based physicians. To maximise the yield of information and study participants, the size of the questionnaire included only three questions on biomarker criteria, which may be insufficient to fully evaluate physician knowledge.

5. Conclusions

In conclusion, our data indicate that more precise response biomarkers and physician education are needed to interrogate outcome in daily clinical practice in mCRPC, and that it is likely that many patients are being over- and under-treated. Many physicians rely on the highly subjective reporting of symptoms for treatment switch decisions. Physician education on these challenges, and established working group criteria, are needed, as are prospective trials to clinically qualify biomarker utility, improve treatment switch decisions and patient outcome as well as change clinical practice.

Author contributions: David Lorente had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Lorente, Ravi, de Bono.
Acquisition of data: All authors.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Lorente, Gilman, Miranda, Porta, Hall, de Bono.
Obtaining funding: None.
Administrative, technical, or material support: None.
Supervision: None.
Other: None.

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stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Heather Payne has attended and received honoraria for advisory boards, travel expenses to medical meetings, and served as a consultant for AstraZeneca, Astellas, Janssen, Sanofi Aventis, Takeda, Amgen, Ipsen, Ferring, Sandoz, Roche, and Novartis. Leon Terstappen is listed as an inventor on US patents related to the CellSearch system, the rights of which are assigned to Johnson & Johnson, and is the chairman of the Department of Medical Cell BioPhysics at the University of Twente, which has received research funding related to the CellSearch system from Johnson & Johnson. Aurelius Omlin has served on advisory boards for AstraZeneca, Astellas, Bayer, Janssen, Pfizer and Sanofi Aventis, and has received research funding from TEVA and Janssen and travel support from Astellas, Bayer, and Sanofi Aventis. David Lorente has served on advisory boards for Bayer and has received travel support from Janssen. Diletta Bianchini has received travel grants from Janssen. The remaining authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euf.2016.09.005.

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Sequencing of agents in castration-resistant prostate cancer

David Lorente, Joaquin Mateo, Raquel Perez-Lopez, Johann S de Bono, Gerhardt Attard

Until 2010, docetaxel was the only agent with proven survival benefit for castration-resistant prostate cancer. The development of cabazitaxel, abiraterone acetate, enzalutamide, radium-223, and sipuleucel-T has increased the number of treatment options. Because these agents were developed concurrently within a short period of time, prospective data on their sequential use efficacy are scarce. The challenge now is to reach a consensus on the best way to sequence effective treatments, ideally by the use of an approach specific to patient subgroups. However, the absence of robust surrogates of survival and the lack of predictive biomarkers makes data for the sequential use of these agents difficult to obtain and interpret.

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Introduction

Despite progress in the management of advanced prostate cancer during the past decade, metastatic prostate cancer remains a disease that causes substantial morbidity and mortality worldwide.[1] Since Huggins and Hodges[2] showed the effectiveness of hormonal manipulations more than 70 years ago, androgen deprivation therapy has been the mainstay of treatment for advanced prostate cancer. However, despite pronounced—and sometimes complete—remission with chemical or surgical castration, most patients with metastatic prostate cancer will eventually relapse, despite castrate levels of serum androgens. This condition is known as castration-resistant prostate cancer. Figure 1 shows the most common natural history of metastatic prostate cancer. The disease evolves from an initial asymptomatic phase with an absence or very low volume of metastatic disease, through to the development of metastatic disease in a minimally symptomatic condition, then to a symptomatic state with a larger burden of disease. A subgroup of patients present with, or rapidly progress to, bulky, symptomatic metastases, which could be indicative of late presentation or different biology. These patients were selected for investigation in the CHAARTED trial[3] (NCT00309985) that compared docetaxel plus androgen deprivation therapy with androgen deprivation therapy alone. A press release and subsequent presentations have reported substantial improvement in overall survival for early use of docetaxel at the start of androgen deprivation therapy in patients with bulky bone metastases, although the potential benefit in patients with low volume metastatic disease will need more data.

Until 2010, docetaxel was the only agent with survival benefit in randomised trials.[4,5] Subsequently, an improved understanding of the biology underlying castration-resistant prostate cancer led to the development and approval of agents targeting androgen synthesis (abiraterone acetate), the androgen receptor (enzalutamide), microtubules (cabazitaxel), the immune system (sipuleucel-T), and active osteoblasts at sites of bone metastases (radium-223; table 1). Abiraterone acetate and enzalutamide were tested in clinical trials within 24 months of each other. All patients treated were initially naive to the other agent, and no prospectively collected data on the efficacy of these agents exists when given sequentially. Both drugs showed efficacy prechemotherapy and are increasingly used in this setting for asymptomatic patients. Similarly, phase 3 trials of docetaxel and cabazitaxel also recruited patients naive to both these agents. Data from small retrospective series suggest the possibility of cross-resistance between abiraterone acetate, enzalutamide, and docetaxel, and reduced activity when agents are used in sequence. However, the inherent bias of retrospective ad-hoc analyses makes these data difficult to interpret and has led to variations in practice. Robust data on the best sequencing strategy are therefore needed. However, the absence of surrogate measures for survival in castration-resistant prostate cancer makes it difficult to conduct sequencing trials. Consensus guidelines to assist physicians to optimise treatment selection and pursue a rational and efficient treatment sequence might now be helpful. Table 2 summarises ongoing clinical trials addressing sequencing issues in castration-resistant prostate cancer.

No agent for castration-resistant prostate cancer has yet been developed with a companion predictive biomarker. Treatment stratification for prostate cancer based on biological predictive markers will probably be crucial to the successful development of therapeutic agents for castration-resistant prostate cancer. Trials of novel agents in unselected populations are unlikely to succeed. To identify a population with a uniformly limited life expectancy and thus minimise time to demonstrate a benefit in overall survival, accrual in several large clinical trials has been restricted to populations defined by previous chemotherapy exposure. However, assessment of chemotherapy-treated patients might be more challenging since such patients are less fit, and have disease, with greater intrapatient heterogeneity secondary to resistance to many different therapeutic strategies. In this Review, we summarise the evidence on the activity of approved agents for the treatment of the different phases of the disease, the sequence and combination of agents, and the development of biomarkers for patient selection and measurement of response to therapy.

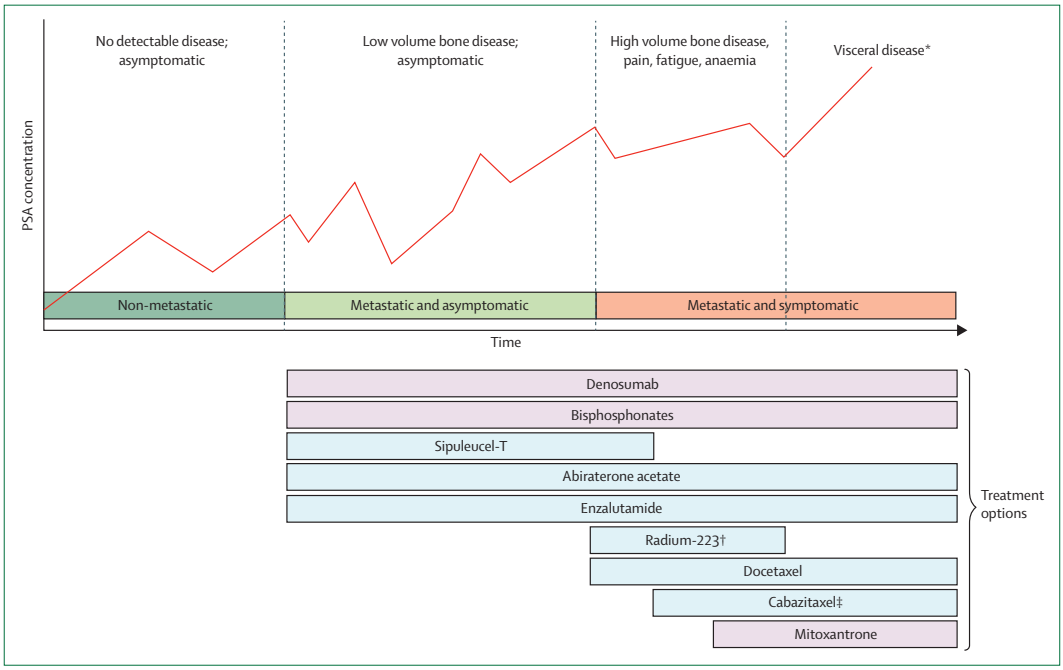

Figure 1: **Typical progression of metastatic castration-resistant prostate cancer**
Treatments licensed for use in the indicated stages as of 2014. PSA=Prostate-specific androgen. The agents in the purple bars have shown no proven survival benefit in randomised clinical trials, whereas those in the blue bars have shown a proven survival benefit in randomised phase 3 trials. *Visceral metastases can also present in the absence of bone metastases. †Ra-223 only in patients with no visceral metastases. ‡Cabazitaxel second-line chemotherapy after progression on docetaxel.

Symptomatic metastatic disease

Chemotherapy

Two large randomised studies published in 2004[4,5] established the survival benefit of docetaxel. The TAX-327 trial[4] reported a 2·9 month benefit in median overall survival with docetaxel every 3 weeks over mitoxantrone (hazard ratio [HR] 0·76, 95% CI 0·62–0·94) and the SWOG 99–16 trial[5] reported a 1·9 month benefit in median overall survival for the combination of docetaxel every 3 weeks and estramustine 3 times per day (HR 0·8, 95% CI 0·67–0·97) versus mitoxantrone. Most patients included in these trials had symptomatic disease (table 3), with 277 (36%) of 770 patients in SWOG 99–16 and 464 (46%) of 1006 patients in TAX-327 reporting substantial pain at baseline.[16] Although quality of life was not significantly improved in the docetaxel group of the SWOG 99–16 trial,[17] there were significant reductions in pain and improvement in quality of life in the TAX-327 trial.[6] Docetaxel in combination with prednisone at 5 mg twice per day was established as the standard of care because of the toxic effects and absence of additional efficacy of the combination with estramustine, with neurotoxic effects identified as a potentially limiting factor of the cumulative docetaxel dose.

In 2010, cabazitaxel, a new taxane and second chemotherapeutic agent, was approved. Cabazitaxel was developed in part on the basis of its preclinical resistance to the P-glycoprotein family and its activity in docetaxel-resistant cell lines.[18] In the TROPIC trial,[19] patients who had previously received docetaxel were randomly assigned to cabazitaxel versus mitoxantrone. Most of the patients had substantial symptoms. Patients given cabazitaxel had a 2·4 month benefit in median overall survival compared with mitoxantrone (HR 0·7, 95% CI 0·59–0·83) although pain palliation was not better in the cabazitaxel group. Grade 3–4 neutropenia, febrile neutropenia, and diarrhoea were more common with cabazitaxel. Neurotoxic effects were reported less than with docetaxel.[19] Despite a clear improvement across most subgroups, concern over toxic effects might be limiting the widespread use of cabazitaxel, especially in less fit patients. Appropriate monitoring and expert oncology input could, however, mitigate the risk of most side-effects. In view of these side-effects, the 25 mg/m² dose used in the TROPIC trial, which was higher than the recommended dose established in phase 1 studies,[20] is being compared in a randomised trial with a lower 20 mg/m² dose (NCT01308580, PROSELICA; table 2).

Novel endocrine agents

Abiraterone acetate and enzalutamide are approved for chemotherapy-naive and docetaxel-treated patients with

	Experimental group	Control group	Primary endpoint	Secondary endpoints	Comments
TAX-327 (n=1006)[6]	Docetaxel 75 mg/m² every 3 weeks (D75)	Mitoxantrone 12 mg/m² every 3 weeks (M) Docetaxel 30 mg/m² per week (D30)	Overall survival D75: 18·9 months M: 16·5 months D30: 17·4 months	PSA response rate Pain response Quality of life (FACT-P)	45% of patients with pain at baseline D75 superior to D30 and M D30 not superior to M
SWOG 99–16 (n=674)[5]	Docetaxel 60 mg/m² (day 1) plus estramustine 260 mg (days 1–5) every 3 weeks	Mitoxantrone 12 mg/m² every 3 weeks	Overall survival 17·5 months *vs* 15·6 months; HR 0·8, p=0·02	PSA response Radiographic response rate	33% of patients with pain at baseline Significant toxicity associated with estramustine
TROPIC (n=755)[7]	Cabazitaxel 25 mg/m² every 3 weeks (C)	Mitoxantrone 12 mg/m² every 3 weeks	Overall survival 15·1 months *vs* 12·7 months; HR 0·7, p=0·0001	PFS PSA response rate Radiographic response rate Pain response	Post docetaxel Significant haematologic toxicity with cabazitaxel No difference in pain response
COU-AA-301 (n=1195)[8]	Abiraterone acetate 1000 mg once a day plus prednisone 5 mg twice a day	Prednisone 5 mg twice a day plus placebo	Overall survival 14·8 months *vs* 10·9 months; HR 0·65, p<0·001	Time to PSA progression PSA response rate Radiographic PFS	Post docetaxel
COU-AA-302 (n=1088)[9]	Abiraterone acetate 1000 mg once a day plus prednisone 5 mg twice a day	Prednisone 5 mg twice a day plus placebo	Radiographic (PCWG2) PFS 16·5 months *vs* 8·3 months; HR 0·53 (p<0·001) Overall survival NR *vs* 27·2 months; HR 0·75, p=0·01	Time to opiate use Time to initiation of cytotoxic chemotherapy Time to ECOG performance status decrease PSA response rate Radiographic response rate Quality of life	Coprimary endpoint: overall survival plus radiographic PFS Chemotherapy naive patients No visceral metastases included Overall survival did not meet prespecified significance criteria
AFFIRM (n=1199)[10]	Enzalutamide 160 mg once a day	Placebo	Overall survival 18·4 months *vs* 13·6 months; HR 0·63, p<0·001	PSA response rate Pain response rate Quality of life (EQ-5D) PSA PFS Radiographic PFS Time to first SRE	Post docetaxel population Patients with risk factors for seizures excluded
PREVAIL (n=1715)[11]	Enzalutamide 160 mg once a day	Placebo	32·4 months *vs* 30·2 months; HR 0·7, p<0·0001	Time to initiation of cytotoxic chemotherapy Time to first SRE	Chemotherapy naive patients 11% of patients had visceral disease Patients with risk factors for seizure were excluded
Small et al[12]	Sipuleucel-T every 2 weeks (total of 3 infusions)	Placebo	PFS 11·7 weeks *vs* 10·8 weeks; HR 1·45, p=0·052	Overall survival 25·9 months *vs* 21·4 months; HR 2·1, p=0·01	Primary endpoint PFS not met Visceral metastases excluded
D9901 and D9902A (n=225)[13]	Sipuleucel-T every 2 weeks (total of 3 infusions)	Placebo	PFS 11·1 weeks *vs* 9·7 weeks; HR 1·26, p=0·111	Overall survival 23·2 months *vs* 18·9 months; HR 1·5, p=0·011	Integrated analysis of two identical small phase 3 trials Primary endpoint not met At least 3 month chemotherapy-free interval Visceral metastases and pain from bone metastases excluded
IMPACT (n=512)[14]	Sipuleucel-T every 2 weeks (total of 3 infusions)	Placebo	Overall survival 25·8 months *vs* 21·7 months; HR 0·78, p=0·03	Time to objective disease progression	No differences in time to objective disease progression Visceral metastases excluded Absent or minimal pain 19·6% post chemotherapy
223-Radium ALSYMPCA (n=809)[15]	223-Ra 50 kBq intravenous every 4 weeks (total of 6 injections)	Placebo	Overall survival 14·0 months *vs* 11·2 months; HR 0·7, p=0·002	Time to increase in alkaline phosphatase, alkaline phosphatase response and normalisation rate Time to first SRE Time to PSA progression	Symptomatic bone metastases 57% of patients were post-docetaxel Docetaxel-naive patients unfit for docetaxel were included Visceral metastases were excluded

PSA=prostate-specific antigen. PFS=progression-free survival. SRE=skeletal-related event HR=hazard ratio. NR=not reported. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Phase 3 trials of approved agents in castration-resistant prostate cancer**

castration-resistant prostate cancer. Abiraterone acetate is a potent and irreversible inhibitor of cytochrome p450 17A1 that suppresses androgen synthesis and might also act as an androgen receptor antagonist.[21] Enzalutamide is a second-generation anti-androgen that was developed on the basis of activity in bicalutamide-resistant preclinical models that overexpressed androgen receptor or harboured an androgen receptor point mutation.[22]

	Phase	Description
NCT01308567 (FIRSTANA)	3	Cabazitaxel *vs* docetaxel both with prednisone in patients with metastatic castration-resistant prostate cancer
NCT02254785	2	Cabazitaxel *vs* abiraterone or enzalutamide in patients with poor prognosis metastatic castration-resistant prostate cancer
NCT01995513 (PLATO)	4	Continued enzalutamide with abiraterone beyond progression on abiraterone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer
NCT02125357	2	Sequencing of abiraterone and enzalutamide in metastatic castration-resistant prostate cancer
NCT01576029	2	Continued treatment with docetaxel *vs* switch to cabazitaxel after minor PSA response to docetaxel in patients with castration-resistant metastatic prostate cancer
NCT01718353	2	Early switch from first-line docetaxel/prednisone to cabazitaxel/prednisone and the opposite sequence, exploring molecular markers in men with metastatic castration-resistant prostate cancer
NCT01487863	2	Concurrent *vs* sequential treatment with sipuleucel-T and abiraterone in men with metastatic castration-resistant prostate cancer
NCT01934790	1/2	Re-treatment safety of radium-223 dichloride in castration-resistant prostate cancer with bone metastases
NCT01949337 (ALLIANCE)	3	Enzalutamide with or without abiraterone acetate and prednisone in treating patients with castration-resistant metastatic prostate cancer
NCT02043678	3	Radium-223 dichloride and abiraterone acetate compared to placebo and abiraterone acetate for men with treatment-naive, asymptomatic or mildly symptomatic castration-resistant prostate cancer with bone metastases.
NCT01650194	2	Safety and tolerability of enzalutamide in combination with abiraterone acetate in bone metastatic castration-resistant prostate cancer patients
NCT02036060	2	Abiraterone acetate in combination with docetaxel after disease progression to abiraterone in metastatic castration-resistant prostate cancer
NCT01487863	2	Concurrent vs sequential treatment with sipuleucel-T and abiraterone in men with metastatic castration-resistant prostate cancer
NCT01981122	2	Sipuleucel-T with administration of enzalutamide in men with metastatic castration-resistant prostate cancer
NCT01845792	1/2	Abiraterone acetate and prednisone in combination with cabazitaxel in patients with prostate cancer in astration-resistant prostate cancer
NCT01511536	1	Cabazitaxel and abiraterone acetate in patients with metastatic castration-resistant prostate cancer
NCT01400555	1	Safety of abiraterone acetate administered in combination with docetaxel in metastatic castration-resistant prostate cancer
NCT01565928	1	Safety and tolerability of MDV3100 in combination with docetaxel in men with advanced prostate cancer
NCT01308580 (PROSELICA)	3	Cabazitaxel 20 mg/m² *vs* 25 mg/m² with prednisone for the treatment of metastatic castration-resistant prostate cancer
NCT01867710	2	Abiraterone with different steroid regimens for adverse event related to mineralocorticoid excess prevention in chemotherapy-naive and metastatic castration-resistant prostate cancer
NCT02025010	2	Abiraterone acetate without exogenous glucocorticoids in men with castration-resistant prostate cancer with correlative assessment of hormone intermediates
NCT01558219	2	Safety of biweekly cabazitaxel in metastatic castration-resistant prostate cancer patients previously treated with a docetaxel-containing regimen
NCT01518283	2	Weekly cabazitaxel for advanced prostate cancer
NCT01541007	2	Conventional 3 weekly schedule of cabazitaxel *vs* weekly regimen in patients with metastatic castration-resistant prostate cancer
NCT02023697	2	Standard dose *vs* high dose *vs* extended standard dose radium-223 dichloride in castration-resistant prostate cancer metastatic to the bone
NCT01637402	2	Increased-dose abiraterone acetate in patients with castration-resistant prostate cancer

Table 2: **Clinical trials examining issues of treatment sequencing in castration-resistant prostate cancer**

Both agents were first studied in two large randomised clinical trials, COU-AA-301 (abiraterone acetate)[8] and AFFIRM (enzalutamide),[10] in symptomatic patients with increasing prostate-specific antigen (PSA) concentrations after one or two lines of chemotherapy, which included at least one docetaxel-based regimen. Both trials mandated for treatment discontinuation, documented disease progression by a composite marker with at least two of three requisites: PSA progression, radiological progression, or clinical progression. The COU-AA-301 trial established the survival benefit of abiraterone acetate in the post-chemotherapy setting and led to regulatory approval in 2011. A significant improvement in overall survival for the combination of abiraterone acetate and prednisone versus prednisone alone was noted (median 15·8 months *vs* 11·2 months; HR 0·74, 95% CI 0·64–0·86).[8] Similarly, a 4·8 month benefit in median overall survival over placebo (HR 0·63, 95% CI 0·53–0·75) was reported for enzalutamide in the phase 3 AFFIRM trial,[10] leading to the approval of enzalutamide for patients previously treated with docetaxel in 2012 (table 1). Treatment was well tolerated, with fatigue as the most common grade 3 side-effect in only 6% of patients. Seizures reported at doses greater than 240 mg daily in the phase 1–2 trial[23] led to the use of 160 mg once a day in the AFFIRM trial, and the exclusion of patients with risk factors for seizures. Both trials reported significant improvement for secondary endpoints including delay to skeletal events, improved pain palliation, improved quality of life, and increased PSA progression-free survival and radiographic progression-free survival.[8,10]

Radiopharmaceuticals

Treatment with radium-223 is an option for patients with symptomatic bone metastases as the main site of disease and no visceral metastases. Radium-223 dichloride is a targeted α-emitter that is selectively taken up at areas of increased bone turnover, emitting high-energy α particles of short range (<100 μm) to induce double-strand DNA breaks in targeted areas. The ALSYMPCA trial[15] in patients with progressing castration-resistant prostate

	Median age (years)	Overall survival in control group	Previous treatment	Sites of disease	Pain	Blood measurements*	Comments
TAX-327 (n=1006)[16]	68	16·5 months	Estramustine: 19% 23% >2 hormonal manipulations	Bone: 91% Visceral: 23%	BPI >2 or analgesic score >10: 45%	PSA: 115	Karnofsky 70% in 14% 20% ≤75 years of age
SWOG 99–16 (n=674)[5]	70	15·6 months	Not reported	Bone: 86% Lymph nodes: 25% Lung: 9% Liver: 10%	Grade ≥2 pain: 36%	PSA: 87	SWOG PS 2–3 in 10% 18% rising PSA as only evidence of progression
TROPIC (n=755)[7]	68	12·7 months	Docetaxel: 100% 2 or more previous chemotherapy lines: 30%	Bone: 84% Visceral: 25%	Pain intensity scale >2 analgesic score >10: 45%	PSA: 136	ECOG PS 0–1 in 92%
COU-AA-301 (n=1195)[8]	69	10·9 months	Docetaxel: 100% 2 or more previous chemotherapy lines: 30%	Bone: 89% Lymph nodes: 44% Liver: 10%	BPI-SF median score: 3	PSA: 132	ECOG PS 0–1 in 90% 28% ≥75 years old 67% had radiographic evidence of progression at inclusion
COU-AA-302 (n=1088)[9]	71	27·2 months	Docetaxel: 0% ADT: 100%	Bone only: 50% Lymph nodes/soft tissue: 50% Visceral: 0%	BPI-SF median score: 0 BPI-SF 2–3: 32% BPI-SF ≥4: 2%	PSA: 40 LDH: 186 ALP: 92	32% ≥75 years old Patients with visceral metastases were excluded
AFFIRM (n=1199)[10]	69	13·6 months	Docetaxel: 100% 2 or more previous chemotherapy lines: 27%	Bone: 92% Lymph nodes: 55% Lung: 15% Liver: 11%	BPI-SF ≥4: 28%	PSA: 115	ECOG PS 0–1 in 92% 59% had radiographic evidence of progression at inclusion
PREVAIL (n=1715)[11]	72	30·2 months	Docetaxel: 0% Two or more previous hormonal agents: 21%	Bone: 83% Lymph nodes: 51% Visceral: 12% (Lung: 8% Liver: 4%)	BPI-SF 2–3: 32% BPI-SF ≥4: 2%	PSA: 50 LDH: 185 ALP: 91	ECOG PS 0–1: 100% 35% ≥75 years of age 4% received steroids
Small et al[12]	72	21·4 months	Chemotherapy: 6%	Bone only: 37% Bone and soft tissue: 56% Soft tissue only: 7% Visceral: 0%	Not reported	PSA: 47 LDH: 173 ALP: 99	ECOG PS 0–1: 100% Patients with cancer-related bone pain, visceral disease, or receiving steroids were excluded
D9901 and D9902A pooled data (n=225)[13]	72	18·9 months	Chemotherapy: 8% (at least 6 months from previous chemotherapy)	Bone only: 39% Bone and soft tissue: 51% Soft tissue only: 10% Visceral: 0%	Not reported	PSA: 49 LDH: 181 ALP: 108	ECOG PS 0: 77% Peroxidase anti-peroxidase plus immunohistochemistry required for inclusion Patients with cancer-related bone pain, visceral disease, or receiving steroids were excluded
IMPACT (n=512)[14]	71	21·7 months	Chemotherapy: 17% (docetaxel: 14%) CAB: 82%	Bone only: 47% Bone and soft tissue: 45% Soft tissue only: 8% Visceral: 0%	BPI 0: 52%	PSA: 50 LDH: 194 ALP: 102	Only asymptomatic patients were enrolled ECOG PS 0: 82% Patients receiving steroids were excluded
ALSYMPCA (n=809)[15]	71	11·2 months	Docetaxel: 57%	At least 2 bone lesions required for inclusion >20 bone metastases: 40% Visceral: 0%	WHO pain ladder: 1: 43% 2: 26% 3: 31%	PSA: 155 LDH: 322 ALP: 215	ECOG PS ≥2: 13% Only patients with symptomatic disease (pain) or previous palliative radiotherapy for pain were included Patients with visceral metastases were excluded

ALP=alkaline phosphatase. BPI-SF=Brief Pain Inventory Short Form. ADT=androgen deprivation therapy. CAB=combined androgen blockade. ECOG=Eastern Cooperative Oncology Group. LDH=lactate dehydrogenase. PS=performance status. PSA=prostate-specific antigen. SWOG=South-west Oncology Group. *Median values at baseline (PSA: ng/mL; LDH: IU/L; ALP: IU/L).

Table 3: **Treatment populations in phase 3 trials of approved agents in castration-resistant prostate cancer**

cancer with symptomatic bone metastases (excluding patients with visceral disease, soft tissue disease >2 cm or fewer than two bone metastases) reported a 5·1 month benefit in median overall survival (HR 0·70, 95% CI 0·55–0·83) and significant delay in time to first skeletal-related event for patients given six cycles of intravenous radium-223 at 50 kBq/kg. Adverse effects were more common in the placebo group versus the radium-223 group, with no reported late secondary malignancies or post-radium-223 myelosuppression with subsequent treatments.

Asymptomatic or minimally symptomatic metastatic disease

Hormonal agents

Enzalutamide and abiraterone acetate were studied in two large, randomised, placebo-controlled trials in mainly asymptomatic, chemotherapy-naive patients. The COU-AA-302 trial assigned 1088 patients randomly to receive either abiraterone acetate and prednisone, or placebo and prednisone.[9] Although the trial did not meet pre-specified criteria of significance for overall survival at the first interim analysis (median not reached *vs*

27·2 months; HR 0·75, p=0·01), extension of the marketing licence was granted on the basis of the significant improvement in the coprimary endpoint of radiographic progression-free survival, a consistent improvement in all secondary endpoints, and the overall survival benefit achieved in the COU-AA-301 trial. The final analysis,[24] after a median of 49·4 months follow-up, reported a significant improvement in overall survival (median 34·7 *vs* 30·3 months; HR 0·8, 95% CI 0·69–0·83). 236 (44%) of 542 patients receiving placebo who participated in this trial received subsequent abiraterone acetate, suggesting improved survival for earlier compared with later use of these agents. Enzalutamide was similarly compared with placebo in the prechemotherapy setting in the phase 3 PREVAIL trial, in which 1717 patients were randomly assigned equally to receive enzalutamide or placebo. The protocol was amended to postpone the first interim analysis until more deaths had been reported. A significant benefit in both overall survival (median 32·4 *vs* 30·2 months; HR 0·7, 95% CI 0·59–0·83) and radiographic progression-free survival (median not reached *vs* 3·9 months, respectively; HR 0·19, 95% CI 0·15–0·23) was reported.[11]

Immunotherapy

Sipuleucel-T is an immunotherapeutic agent that consists of activated antigen-presenting cells derived from autologous peripheral blood mononuclear cells that are stimulated ex vivo with a recombinant fusion protein (prostate antigen, prostatic acid phosphatase, and granulocyte stimulating factors) before being reinfused. Sipuleucel-T was assessed in several randomised trials, none of which induced meaningful improvements in progression-free survival but all of which achieved significant benefits in overall survival.[12,13] The largest of these trials, the phase 3 IMPACT trial,[14] reported a benefit in median overall survival of 4·1 months (HR 0·78, 95% CI 0·61–0·98) for sipuleucel-T versus placebo in patients with bone or lymph node metastases and a chemotherapy-free interval of at least 3 months. The IMPACT trial was highly selected, with more than 80% of patients chemotherapy naive, 75% with a Gleason score of 7 or less, 53% of whom were pain free, and 43% of whom had low-volume bone metastases only.

Possible sequences

Sipuleucel-T and radium-223

The timing of sipuleucel-T seems most appropriate in early stage castration-resistant prostate cancer with a low disease burden, and is lent support by subanalyses showing greater benefit in patients with a lower PSA and more significant changes in indices of immune modulation in the neoadjuvant setting compared with castration-resistant prostate cancer.[25] The failure of the anti-CTLA-4 antibody ipilimumab in a large randomised trial[26] of patients with symptomatic bone metastatic disease treated with docetaxel reinforces the idea that immunotherapy is probably most beneficial in patients with a low burden of disease. Results from a trial assessing ipilimumab in chemotherapy-naive patients are awaited (NCT01057810). Moreover, the excellent tolerability of sipuleucel-T, its different mechanism of action, the finite treatment regimen, and often absence of an effect on PSA and imaging likewise favours its use early on at development of metastatic castration-resistant prostate cancer before use of novel endocrine treatments or chemotherapy. However, the optimum timing for sipuleucel-T has not been proved so far, and evidence suggesting an early use of sipuleucel-T should be deemed preliminary. Furthermore, despite the proven survival benefit, a great amount of logistics involved with sipuleucel-T have restricted its availability to the USA. The absence of predictive biomarkers and the high cost of treatment could likewise limit its widespread use.

The non-overlapping mechanism of action and toxicity profile of radium-223 would potentially allow its use irrespective of the use of novel endocrine agents or taxanes. However, although potentially effective with any bone metastases burden, the benefit in patients with a low burden of disease or asymptomatic metastases is unproven. Despite no patients with visceral disease being allowed in the ALSYMPCA trial,[15] the median overall survival of the control group (11·4 months) was more similar to the TROPIC or COU-AA-301 trials than to the PREVAIL or COU-AA-302 studies (table 2). This may therefore favour the use of radium-223 after enzalutamide or abiraterone acetate. Overall, however, due to potential cross-resistance, the main challenge for physicians lies in deciding when and for which subgroup of patients abiraterone, enzalutamide, docetaxel, or cabazitaxel (table 4) should be used.

Evidence for cross-resistance

Preclinical evidence has shown impaired efficacy of docetaxel in abiraterone acetate and enzalutamide-resistant cell lines, putatively related to docetaxel's activity in blocking androgen receptor nuclear translocation.[42] By contrast, the results of some studies[41] have reported maintained activity of cabazitaxel in enzalutamide-resistant and androgen receptor-negative cell lines. No studies investigating the cross-resistance between these agents and radiopharmaceuticals (eg, radium-223), or immunotherapy (eg, sipuleucel-T) have yet been reported.

Novel endocrine agents versus taxanes in symptomatic patients

Restriction of patients in the pre-docetaxel COU-AA-302 and PREVAIL trials to asymptomatic or mildly symptomatic disease, defined as an Eastern Cooperative Oncology Group performance status of 0–1 and a score of 0–3 out of 10 in the Brief Pain Inventory Short Form enabled the comparison against placebo, and not

docetaxel. The licensing approval for both abiraterone acetate and enzalutamide limits their use in the pre-chemotherapy setting to asymptomatic patients, with docetaxel remaining the licensed treatment for symptomatic patients. However, with benefits seen in the COU-AA-301 and AFFIRM trials and the better tolerability of the hormonal agents, many physicians might prefer to consider abiraterone acetate or enzalutamide before chemotherapy. This is especially true for men with contraindications to docetaxel, or poor fitness. For docetaxel-treated patients, there is no evidence comparing novel hormonal agents with cabazitaxel chemotherapy.

Novel endocrine agents versus taxanes in asymptomatic patients

No direct comparison has been made between the benefit of novel hormonal agents and docetaxel in asymptomatic or minimally symptomatic patients. Although the TAX-327 and SWOG 99–02 studies included asymptomatic patients, the better tolerability of the hormonal agents abiraterone acetate or enzalutamide could increase their use in this setting when funding is available. Although patients with visceral metastases were excluded from the COU-AA-302 trial, 204 (12%) of 1717 patients treated in the PREVAIL trial presented with visceral metastases.[11] In patients with visceral metastases, a significant benefit in radiographic progression-free survival (HR 0·28, 95% CI 0·16–0·49) was seen. Because of the few patients with visceral metastases, the benefit in overall survival in this subset was not significant (HR 0·82, 95% CI 0·61–1·23),[11] but the HR is consistent with benefit in this subgroup. Furthermore, the rate of radiographic response among patients with measurable disease in the PREVAIL trial (response evaluation criteria in solid tumors [RECIST] 1·1 criteria) favoured the enzalutamide group (54% *vs* 5%; p<0·001) and was remarkably better than the 12% radiographic response rate seen in the docetaxel group of the TAX-327 study (WHO criteria).[6] Patients should therefore not necessarily be selected for docetaxel solely on the presence of visceral metastases.

Taxanes in the post-abiraterone or post-enzalutamide setting

The activity of docetaxel after abiraterone acetate (PSA reductions ≥50% in 26%, median progression-free survival 4·6 months, and median overall survival 12·5 months), so far reported in small, retrospective studies is unclear, with conflicting reports of equivalent or lower PSA response rates compared with the TAX-327 trial (table 4). These studies could be subject to bias and, although they raise the possibility of decreased activity with docetaxel after either agent, docetaxel should remain the recommended treatment in the absence of prospective data. The use of cabazitaxel in the post-abiraterone or post-enzalutamide setting has been analysed in retrospective series, with 50% PSA reductions in 27–41% of patients,

	Cohort size	Previous treatment: % of patient population	PSA response	Radiographic response	Survival	Comments
Docetaxel after abiraterone						
Mezynski et al[27]	35	Anti-androgens: 100% Dexamethasone: 71% Diethylstilboestrol: 46%	30% PSA decrease: 13/35 (37%) 50% PSA decrease: 9/35 (26%)	Partial response: 4/24 (17%)	Overall survival: 12·5 months (95% CI 10·6–19·4) PSA PFS: 4·6 months (95% CI 4·2–5·9)	None of the abiraterone refractory patients responded to docetaxel
Schweizer et al[28]	24	Anti-androgens: 92% Ketoconazole: 25%	30% PSA decrease: 13/24 (54·2%) 50% PSA decrease: 9/24 (38%)	NR	Overall survival: NR PSA PFS: 4·1 months (95% CI 2·8–5·8)	Significantly worse outcome compared to contemporary control group of abiraterone-naive patients 39% of abiraterone refractory patients achieved PSA response on docetaxel
Aggarwal et al[29]	23	Anti-androgens: 4%* Ketoconazole: 26% Diethylstilboestrol : 4%	30% PSA decrease: 15/23 (65%) 50% PSA decrease: 11/23 (48%)	NR	Overall survival: 12·4 months (95% CI 8·2–19·6)	Similar rate of response in patients with primary and acquired resistance to abiraterone
Azad et al[30]	86	Docetaxel: 57%	50% PSA decrease: 30/86 (35%)	NR	Overall survival: 11·7 months (95% CI 9·5–13·9) PFS: 4 months (95% CI 3·1–5·0)	No association between response to abiraterone and response to docetaxel
Abiraterone after enzalutamide						
Loriot et al[31]	38	NR	30% PSA decrease: 7/38 (18%) 50% PSA decrease: 3/38 (8%)	Partial response: 1/12 (8%)	Overall survival: 7·2 months (95% CI 5–NR) PFS: 2·7 months (95% CI 2.3–4.1)	No difference in response to abiraterone in responders *vs* non-responders to previous enzalutamide
Noonan et al[32]	30	Anti-androgens: 97·4% Docetaxel: 100% Mitoxantrone: 2·6%	30% PSA decrease: 3/27 (11%) 50% PSA decrease: 1/27 (3%)	Partial response: 0%	Overall survival: 11·6 months (95% CI 6·5–16·6) PFS: 3·6 months (95% CI 2·5–4·7)	One patient (5%) with previous 30% PSA decline on enzalutamide achieved a 30% PSA decline on abiraterone

(Table 4 continues on next page)

	Cohort size	Previous treatment	PSA response	Radiographic response	Survival	Comments
(Continued from previous page)						
Enzalutamide after abiraterone						
Schrader et al[33]	35	Abiraterone: 100% Docetaxel: 100% Cabazitaxel: 2·9%	30% PSA decrease: NR 50% PSA decrease: 10/35 (29%)	Partial response: 1/17 (5.9%)	Overall survival: 7·1 months (95% CI 6·2–8·1)† PFS: Not reported	Response to previous abiraterone not predictive of response to enzalutamide
Bianchini et al[34]	39	Anti-androgens: 89.7% Abiraterone: 100% Docetaxel: 100% Cabazitaxel: 35·9%	30% PSA decrease: 16/39 (41%) 50% PSA decrease: 5/39 (13%)	Partial response: 1/23 (4.3%)	Overall survival: median OS not reached PFS: 2·8 months (95% CI 2·0–3·6)	No association between 50% PSA response on abiraterone and 50% PSA response on enzalutamide
Thomsen et al[35]	24	Abiraterone: 100% Docetaxel: 100% Cabazitaxel: 33·3%	30% PSA decrease: 11/24 (46%) 50% PSA decrease: 4/24 (17%)	NR	Overall survival: 4·8 months (95% CI 3·0–8.4) PFS: Not reported	Non-significant trend associating response to abiraterone with response to enzalutamide (p=0·05) Significantly worse PSA response in post-cabazitaxel patients (p=0·03)
Badrising et al[36]	61	Abiraterone: 100% Docetaxel: 100% Mitoxantrone: 3% Cabazitaxel: 30%	30% PSA decrease: 28/61 (46%) 50% PSA decrease: 13/61 (21%)	NR	Overall survival: 7·3 months (95% CI 6·6–NR) PFS: 2·8 months (95% CI 2·6–3·7) PSA PFS: 4 months (95% CI 3·7–NR)	No significant difference in PSA response or time on treatment between previous responders and non-responders to abiraterone
Azad et al[37]	115	Abiraterone: 100% Docetaxel: 59%	50% PSA decrease: 27/115 (24%)	NR	Overall survival: 10·6 months (95% CI NR) PFS: 5·3 months (95% CI NR)	No difference in PSA or overall survival in patients previously treated with docetaxel *vs* docetaxel naive
Cabazitaxel after abiraterone or enzalutamide						
Pezaro et al[38]	37	Abiraterone: 100% Enzalutamide: 13·5% Docetaxel: 100%	30% PSA decrease: 21/37 (57%) 50% PSA decrease: 15/37 (41%)	Partial response: 3/20 (15%)	Overall survival: 20·3 months (95% CI 14–26·6) PFS: 5·5 months (95% CI 4·2–6·8)	Results comparable to TROPIC trial Inferior activity in control group of abiraterone or enzalutamide naive patients Higher rates of 50% PSA reduction in patients with no previous PSA response to abiraterone
Sella et al[39]	24	Abiraterone: 100% Docetaxel: 100%	50% PSA decrease: 6/19 (32%)	Partial response: 2/13 (15·3%)	Overall survival: 8·2 months (95% CI 3·3–13·1)	
Wissing et al[40]	69	Abiraterone: 100% Docetaxel: 100% Enzalutamide: 1·4%	50% PSA decrease: 18/69 (27%)	NR	PFS: 3·2 months (95% CI 2·5–3·8)	Compared sequence cabazitaxel followed by abiraterone *vs* abiraterone followed by cabazitaxel
Al Nakouzi et al[41]	79	Abiraterone: 100% Docetaxel: 100%	30% PSA decrease: 48/79 (62%) 50% PSA decrease: 28/79 (35%)	NR	Overall survival: 10·9 months (95% CI 8·0–14) PFS: 4·4 months (95% CI 4·6–8·7)	No preclinical evidence of cross-resistance

NR=not reported. PSA=prostate-specific antigen. PFS=progression-free survival. *Only treatment administered between abiraterone and docetaxel was reported. †Mean overall survival reported (in all other studies, median overall survival was reported).

Table 4: **Retrospective studies of treatment sequencing in castration-resistant prostate cancer**

radiological responses in 15% of patients, and a median overall survival and progression-free survival of 10·9–20·3 and 4·4–5·5 months, respectively.[38,41] These results are similar to those reported in the TROPIC study,[7] suggesting that cabazitaxel could retain its activity in this setting. These data suggest maintained activity for cabazitaxel after docetaxel and novel endocrine agents, but do not inform on the best sequence, although the data do support the use of cabazitaxel in heavily pretreated men. Analysis of the subgroup of abiraterone acetate or enzalutamide pre-treated patients in the phase 3 PROSELICA trial might provide further important information.

The taxane of choice

The TROPIC trial recruited only patients who had received a docetaxel-containing regimen with at least 225 mg/m² cumulative docetaxel dose.[7] Data from randomised phase 3 trials comparing docetaxel with cabazitaxel as first-line chemotherapy for metastatic castration-resistant prostate cancer are yet to be reported (FIRSTANA, NCT01308567). Up to now, docetaxel remains the first agent of choice for chemotherapy. Several case series[43] have reported responses with docetaxel rechallenge in patients selected on duration and magnitude of response to first-line docetaxel. However, in view of the results of the TROPIC study,[7] treatment with cabazitaxel should be the recommended chemotherapeutic agent after disease progression with first-line docetaxel (table 5). Retrospective data suggest so-called traditional hormonal manipulations, such as oestrogen therapy in symptomatic men resistant to novel endocrine agents, are inactive.[44]

Cross-resistance between abiraterone acetate and enzalutamide

In view of the substantial clinical benefit reported by the results of landmark trials for abiraterone acetate and enzalutamide,[8–11] the question of whether the benefit will be additive when these agents are given sequentially still remains unanswered. Several retrospective series have reported clinical outcomes for abiraterone acetate in patients who had previously progressed on enzalutamide, with an 8–10% rate of PSA reduction and a median progression-free survival of 2·7–3·4 months, lower than those reported in the COU-AA-301 trial.[31,32] Conversely, several groups have reported on the activity of enzalutamide in patients progressing on abiraterone acetate. 50% PSA reductions in around 13–29% of patients and a median progression-free survival of around 2·8–5·3 months have been reported.[33–37] These outcomes are meaningfully lower than the outcome (50% PSA responses in 54% of patients and median progression-free survival of 8·3 months) reported in the AFFIRM trial.[10] No association between the magnitude of response to abiraterone acetate and response to enzalutamide was present. One of the studies did suggest previous treatment with cabazitaxel predicted failure to respond to enzalutamide.[35] Although available data seem to suggest that the activity reported when abiraterone acetate and enzalutamide are given sequentially is substantially lower than those reported in their respective landmark trials. In the absence of prospective studies, the exact effect from the sequential use of these agents has yet to be adequately defined.

Abiraterone acetate or enzalutamide

Data from prospective direct comparisons between abiraterone acetate and enzalutamide are not yet available, and no prospective head-to-head comparison is underway. Both agents were explored in equivalent scenarios, have similar efficacy, and are well tolerated. Selection is generally made on a patient-to-patient basis, based on clinical factors (contraindications to corticosteroids, risk factors for seizure), availability, cost, and patient preference. Minor differences in the respective trials including design, participating centres and countries, and non-overlapping accrual periods make subtle differences between the two agents indistinguishable. Importantly, the control groups of the trials were different, with prednisone used in all patients in the abiraterone acetate studies compared with roughly 45% of patients in the AFFIRM[10] trial and 4% in the PREVAIL[11] trial (table 3).

Glucocorticoids

Glucocorticoids such as prednisone 5 mg twice a day or dexamethasone 0·5 mg once a day induce PSA responses in castration-resistant prostate cancer.[45] In the COU-AA-302 trial,[9] about 130 (24%) of 542 patients given prednisone and placebo had a reduction in PSA of more than 50%, and 16% achieved an objective response. Emerging data suggest that glucocorticoids can become disease drivers when given in combination with androgen receptor-targeting drugs. In the presence of androgen receptor inhibition, the glucocorticoid receptor has been postulated to drive resistance to enzalutamide because of substantial overlap with androgen receptor DNA binding sites, and rescue of expression of androgen receptor-regulated genes after effective androgen receptor inhibition.[46] Furthermore, point mutations of the androgen receptor that are activated by glucocorticoids could cause resistance to abiraterone acetate or enzalutamide when given with glucocorticoids.[21,47,48] The association of baseline corticosteroid and outcome has been studied in unplanned post-hoc analyses of the AFFIRM and COU-AA-301 trials,[49,50] with reported association with worse prognosis in the AFFIRM but not in the COU-AA-301 trial. Overall, best practice recommends that glucocorticoids are not continued indiscriminately in patients with progressing disease and early discontinuation should be considered dependent on patient tolerance.

	Appropriate patients
Abiraterone acetate	
Post chemotherapy	ECOG performance status 0–2 Significant survival benefit patients with visceral metastases Symptomatic or asymptomatic No contra-indication for corticosteroid treatment*
Prechemotherapy	No visceral metastases† No contraindication for corticosteroid treatment* Asymptomatic or minimally symptomatic
Enzalutamide	
Post chemotherapy	ECOG performance status 0–2 With or without visceral metastases Symptomatic or asymptomatic Not suitable if risk factors for seizure are present
Prechemotherapy	Not suitable if risk factors for seizure are present Asymptomatic or minimally symptomatic Visceral metastases
Docetaxel	
First-line chemotherapy for CRPC‡	No significant survival benefit in patients with visceral metastases
Cabazitaxel	
Post docetaxel§	ECOG performance status 0–1 Caution with frail patients and patients at risk for febrile neutropenia No established benefit in pain palliation
Radium-223	
Prechemotherapy or post chemotherapy	Symptomatic bone metastatic disease (more than two bone metastases) No established benefit in patients with visceral metastases
Sipuleucel-T	
Prechemotherapy	Minimally symptomatic patients
Early CRPC with minimal burden of disease	Increased efficacy in patients with lower metastatic burden and lower baseline PSA levels
Post chemotherapy	At least 3 month interval since previous chemotherapy Possibly less effective with higher burden of disease

ECOG=Easter Cooperative Oncology Group. CRPC=castration-resistant prostate cancer. PSA=prostate-specific antigen. *Abiraterone acetate is currently being evaluated with alternative, lower doses of corticosteroids . †Patients with visceral metastases were excluded from the COU-AA-302 trial. ‡Docetaxel increasingly used in the post-abiraterone setting. §Predocetaxel efficacy currently being evaluated in the FIRSTANA trial.

Table 5: **Appropriate times and groups of patients for treatment with approved agents for patients with castration-resistant prostate cancer**

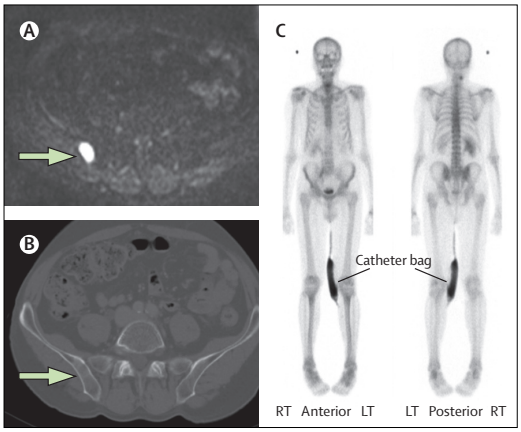

Figure 2: **Imaging of bone metastases from castration-resistant prostate cancer**
Patient with a right iliac bone metastasis from prostate cancer, detected on the diffusion-weighted sequence of an MRI (A) after prostate specific antigen rise on castration. The bone lesion was not evident on conventional CT (B), or in the bone scan (C).

Treatment of non-metastatic disease

A systematic review of studies including over 70 000 patients estimated that 84% of patients with prostate cancer had metastases detectable on CT or bone scans at the time of developing castration-resistant disease. About a third of patients with non-metastatic castration-resistant prostate cancer developed visible metastases within 2 years.[51] In most cases, labelling a patient as non-metastatic castration-resistant prostate cancer is probably indicative of the limited sensitivity of conventional imaging (CT and bone scan), and the frequency of imaging at the time when PSA rises on castration (figure 2). For example, 21% of patients providing consent for a phase 3 study assessing denosumab in non-metastatic patients were finally not enrolled after bone metastases were detected in their baseline imaging assessments.[52] Other imaging techniques such as PET-CT or diffusion-weighted MRI, not generally implemented into routine clinical practice, have been shown to have higher sensitivity than CT or bone scan for detecting bone lesions.[53] Patients with non-metastatic castration-resistant prostate cancer are mostly asymptomatic, and the heterogeneity in the pace of disease progression makes patient selection of paramount importance; in a proportion of patients, treatment-derived toxic effects could outweigh the potential benefits. In a first placebo-controlled phase 3 trial[54] investigating zoledronic acid, which was halted because of lower rates of progression than expected, only 33% of patients developed bone metastases at 2 years. Post-hoc analyses identified baseline PSA concentrations (>10 ng/mL), and PSA doubling time as factors associated with a lower metastasis-free survival and were used to select patients in subsequent trials. Different agents such as the bisphosphonates clodronate[55] and zoledronate,[54,56] the receptor activator of nuclear factor B ligand inhibitor denosumab,[52] and the endothelin antagonists atrasentan[57] and zibotentan[58] have been studied in non-metastatic castration-resistant prostate cancer. Only denosumab has shown a significant 4·2 month increase in median time to the development of skeletal events (HR 0·85, 95% CI 0·73–0·98) despite failing to show benefit in overall survival.[52] Trials studying androgen receptor-targeting drugs in this patient population (enzalutamide, PROSPER, NCT02003924; ARN-509, SPARTAN, NCT01946204) have adopted delay of onset of detectable metastatic disease as a primary endpoint. This endpoint was selected in view of the many effective agents available in later stages, the longer life expectancy, and the absence of surrogate biomarkers of survival. However, these trials might not show improved overall survival or quality of life and their overall benefit to patients could therefore remain uncertain.

Patient selection and assessment of response

Predictive biomarkers

So far, no predictive biomarkers have been clinically validated for patient selection. Table 6 summarises the evidence for proposed molecular biomarkers in advanced prostate cancer.

Clinicopathological variables

The initial docetaxel trials reported that patients achieving pain improvement and PSA responses had longer overall survival[67] than those who did not, and that patients with higher Gleason score tumours derived greater benefit from treatment.[68] Higher baseline androgen (testosterone, androstenodione, dehydroepiandrosterone sulphate) concentrations measured with an ultrasensitive assay in patients in the COU-AA-301 trial have been associated with increased survival irrespective of abiraterone acetate or placebo treatment, suggesting cancers that progress in a low androgen environment represent a more aggressive molecular subtype, and highlights its role as a stratification factor in future trials.[69] A high pretreatment lymphocyte-to-neutrophil ratio has been associated with a worse prognosis, but is also associated with lower rates of PSA and radiological response after treatment with abiraterone acetate[64] or chemotherapy.[70]

Molecular biomarkers

An analysis of the IMPACT and D9901/D9902A trials[71] reported the association of cumulative antigen-presenting cell activation (CD54 upregulation), antigen-presenting cell number and total nucleated cell numbers, and the development of antigen-specific immune reponses with survival. In the IMPACT trial, the development of a high antibody titre against PA2024 or prostatic acid phosphatase was predictive of increased survival. However, none of these proposed immunological biomarkers have been prospectively validated, and therefore remain experimental.

	Biomarker type	Biomarker trial design	Results
AR-V7 splice variants; prognostic, potentially predictive	Circulating tumour cells[59]	Prospective, 62 patients treated with abiraterone acetate or enzalutamide	AR-V7 positivity in circulating tumour cells was significantly associated with lower PSA response rates, shorter PSA and radiographic PFS, and shorter overall survival in both abiraterone acetate and enzalutamide-treated patients
ERG rearrangement; prognostic, potentially predictive	Archival tissue[60]	Prospective (COU-AA-302 trial), 497 patients treated with abiraterone acetate or placebo	2+Edel *ERG* rearrangement might have derived greater benefit from abiraterone acetate (HR 0·31, 95% CI 0·15–0·68; p=0·0033) *vs* for cancers with no *ERG* rearrangement (0·53, 95% CI 0·38–0·74, p=0·0002)
ERG rearrangement; prognostic, potentially predictive	Circulating tumour cells, archival and fresh tissue[61]	Retrospective, 77 patients treated with abiraterone acetate	*TMPRSS2-ERG* rearrangement (FISH) in tissue or circulating tumour cells associated with increased 90% PSA response rates (80% *vs* 32%, p=0·001)
ERG rearrangement; prognostic, potentially predictive	Archival tissue[62]	Retrospective, 34 patients treated with docetaxel	*TMPRSS2-ERG* rearrangements (FISH) associated with a non-significant trend towards reduced PSA response rate (45% *vs* 79%, p=0·056)
PTEN loss; prognostic	Archival and fresh tissue[63]	Retrospective, 143 patients treated with abiraterone acetate	Worse survival in patients with PTEN loss (14 *vs* 21 months, p=0·004) and non-significant trend towards a lower PSA response rate (32% *vs* 43%, p=0·2)
Serum androgens; prognostic	Blood[64]	Retrospective (COU-AA-301 trial), 1150 patients treated with abiraterone acetate or placebo	Longer survival for patients with baseline serum androgens (testosterone, androstenodione, and DHEAS) above median in both abiraterone acetate and placebo groups (testosterone: HR 0·67, p<0·001; androstenodione: HR 0·68, p<0·001; DHEAS: HR 0·69, p<0·001)
AR F876L mutation; potentially predictive	Circulating tumour DNA[65]	Retrospective, 29 patients treated with ARN-509 in phase 1 trial	Detection of the AR Phe876Leu mutation (absent at baseline) in three patients at progression on ARN-509
AR T878A mutation; potentially predictive	Fresh tissue[66]	Retrospective, 18 patients treated with abiraterone acetate or ketoconazole	Progesterone-activated AR mutation (AR Thr878Ala) present in three cases
GR mutation; potentially predictive	Fresh tissue[46] (pretreatment and post-treatment)	Prospective, 22 patients treated with enzalutamide; pretreatment and post-treatment biopsies	Proportion of GR-positive cells in post-treatment biopsies higher in poor responders (on treatment <6 months) than in good responders (on treatment >6 months) (29% *vs* 10%, p=0·02); no patients with high GR expression at baseline had a good response

AR=androgen receptor. PSA=prostate-specific antigen. PFS=progression-free survival. HR=hazard ratio. FISH=fluorescence in-situ hybridisation. PTEN=phosphatase and tensin homolog. DHEAS=dehydroepiandrosterone. GR=glucocorticoid receptor.

Table 6: **Clinical evidence for potential molecular biomarkers in patients with castration-resistant prostate cancer**

A preplanned analysis[60] of archival tumour samples on a subgroup of patients treated in the COU-AA-302 suggested that patients with a *TMPRSS2-ERG* gene fusion secondary to deletion and associated duplication of the fusion sequences (2+Edel) might have derived increased benefit from abiraterone acetate (HR 0·31, 95% CI 0·15–0·68; p=0·0033) versus cancers with no *ERG* rearrangement (0·53, 0·38–0·74; p=0·0002). These data suggest that *ERG* mutation alone is not sufficient to select patients for abiraterone acetate but could have a role as part of a multiplex biomarker panel.

Androgen receptor splice variants that lack the ligand-binding domain can cause resistance to abiraterone acetate and enzalutamide in preclinical models.[72] The detection of androgen receptor splice variants (AR-V7) in circulating tumour cells from patients starting abiraterone acetate or enzalutamide was associated with fewer incidences of PSA reduction and shortened radiographic progression-free survival and overall survival.[59] AR-V7 was most often detected after treatment rather than before treatment with either abiraterone acetate or enzalutamide and could therefore be valuable for selecting sensitive patients to be treated with one agent after development of resistance to the other. Assessment of other androgen receptor splice variants could likewise improve the sensitivity of this assay. Circulating tumour cells can be captured and characterised with fluorescence in-situ hybridisation, immunofluorescence, or sequencing strategies.[61]

The Phe876Leu point mutation in the androgen receptor that causes resistance to enzalutamide and ARN-509 has been detected in plasma from patients given these agents.[65,73] Similarly, androgen receptor point mutations that result in activation of androgen receptor signalling by glucocorticoids have been detected in plasma. Sequencing of circulating DNA identifies genomic aberrations associated with many emergent independent tumour clones, that by sequential and repeated sampling could allow selection or enrichment for the next treatment.[48]

Assessment of treatment response

In up to 80% of patients with prostate cancer, metastases are restricted to the bone, which is not measurable by standard radiological criteria (RECIST). Consensus criteria developed by the Prostate Cancer Working Group recommend assessing response to treatment according to a composite endpoint based on imaging (CT and bone scans), PSA, and clinical measures,[74] and are widely used, in trial design and reporting outcomes.

PSA, a widely used marker in the assessment of response, is a marker of androgen receptor transcriptional activity. Its association with overall survival is stronger in the hormone-sensitive setting than in later stages of the disease, with a less established dependence on androgen receptor signalling. Changes in PSA concentrations are not interpretable in the first 12 weeks of treatment, as shown by 103 (12%) of 873 responding patients in the

TAX-327 trial having an initial PSA rise.[67] Discrepancies between PSA response and radiological progression-free survival or overall survival have been seen with non-androgen receptor targeting agents.[75]

Although Prostate Cancer Working Group criteria define progression of disease in the bone on the basis of appearance of new lesions on bone scans, they do not describe how to document benefit in bone metastasis on the basis of imaging markers, which is of high importance for clinical trials with response-based endpoints. Advances in functional imaging techniques, assessing not only the anatomical features of the disease but also measuring the activity of lesions, offer the opportunity to develop new imaging biomarkers for prostate cancer. Examples of imaging biomarkers in the development of metastatic castration-resistant prostate cancer include diffusion-weighted MRI or functional PET imaging with radiotracers such as ^{11}C-labelled and ^{18}F-labelled choline.

In addition to providing information about the underlying molecular characteristics of metastatic disease, circulating tumour cells could also allow assessment of treatment response. The only platform with regulatory clearance for circulating tumour cells enumeration is CellSearch (Janssen Diagnostics, Raritan, NJ, USA) that uses immunomagnetic enrichment followed by secondary staining with cytokeratin, CD45, and a nuclear stain to distinguish circulating tumour cells from leucocytes and other circulating epithelial cell adhesion molecule-positive objects.[76] A pretreatment circulating tumour cells count assessed as unfavourable (five or more circulating tumour cells per 7·5 mL of blood) is an indicator of worse prognosis. Additionally, a conversion from unfavourable to favourable (fewer than five circulating tumour cells per 7·5 mL) as early as after 4 weeks of treatment, shows a stronger association with improved survival than reduction in PSA.[76,77] A composite panel of circulating tumour cells count and lactate dehydrogenase met the Prentice criteria as a surrogate measure for overall survival at the individual level in the COU-AA-301 trial.[78] Continuing analyses of reported trials (AFFIRM, COU-AA-302) and not yet reported trials (ARN-509) could potentially add to the prognostic value and validate surrogacy at the trial level, and obtain support from funding agencies for these assays, which could provide a means to reduce the delay and costs in the development of novel agents. Furthermore, the early identification of non-responding patients through circulating tumour cells enumeration could avoid unnecessary treatment.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed as well as the ESMO, ECCO, and ASCO annual conferences with the search terms "castration resistant prostate cancer", "metastatic", "non-metastatic", "androgen receptor", "treatment sequencing", "combination trials", "biomarkers", "docetaxel", "cabazitaxel", "abiraterone", "enzalutamide", "immunotherapy", and "radium" published from Jan, 2004 until Dec, 2014. ClinicalTrials.gov was accessed for information about ongoing clinical trials. Only reports published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this review.

Future directions

Substantial efforts are being made to identify distinct molecular subtypes in prostate cancer for focused therapeutic targeting. These studies will lead to more rational sequencing approaches. Studies of circulating biomarkers have introduced the possibility to allow real-time patient characterisation and more accurate patient selection. Until these assays are validated in prospective clinical trials, physicians will aim to use the approved treatments in a sequence that is patient-specific and adheres to the overarching principles of treatment with the best tolerability profile for men who are asymptomatic or minimally symptomatic as well as using close monitoring to ensure early change of a treatment that is ineffective.

Contributors

DL, JM, RP-L, JSdB, and GA all contributed to study design, data collection, data interpretation, and writing of this Review. DL, JM, and RP-L did the literature search and contributed to the creation of figures.

Declaration of interests

GA reports personal fees and non-financial support from Roche and Ventana, personal fees and non-financial support from Astellas, personal fees and non-financial support from Medivation, personal fees from Novartis, personal fees from Millennium Pharmaceuticals, personal fees and non-financial support from Abbott Laboratories, personal fees, and non-financial support from Janssen, personal fees from Takeda, personal fees from Sanofi-Aventis, grants from AstraZeneca and Janssen. GA is on the ICR rewards to inventors list for abiraterone. JSdB reports personal fees from Astellas, personal fees from AstraZeneca, personal fees from Johnson & Johnson, personal fees from Medivation. GA, JSdB, RP-L, JM, and DL are employees of the ICR, which has a commercial interest in the development of abiraterone. DL is supported by a 2 year grant from the Spanish Society of Medical Oncology (Beca SEOM para la Investigacion Traslacional en el Extranjero). RP-L is a doctoral candidate at Universidad Autonoma Barcelona (Department of Medicine), Spain. JM is supported by an MRC Prostate Cancer UK-Movember fellowship. GA is supported by a Cancer Research UK Clinician Scientist Fellowship.

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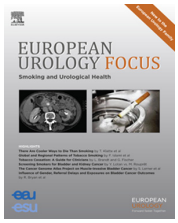

Review – Prostate Cancer

Optimal Treatment Sequence for Metastatic Castration-resistant Prostate Cancer

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Abstract

Context: Unprecedented development of therapeutics for prostate cancer in recent years has left clinicians with the challenge of adequately sequencing therapeutic agents to optimise patient benefit. No clear guidelines exist on optimal treatment sequences.
Objective: To summarise the evidence on first-line activity, cross-resistance, and potential combinations of agents approved for metastatic castration-resistant prostate cancer (mCRPC).
Evidence acquisition: A nonsystematic literature search of articles on agent sequencing in mCRPC in PubMed and relevant cancer conferences up to June 2016 was performed.
Evidence synthesis: No definitive evidence on the optimal mCRPC treatment sequence exists. Hormonal agents are preferred for first-line treatment on the basis of favourable toxicity, but no evidence of superiority over chemotherapy exists. Evidence suggests significant cross-resistance between agents in first- and second-line settings. The impact of prior chemotherapy in metastatic hormone-sensitive disease is unknown. No combinations have proven benefit to date. Molecular biomarker assessment in liquid biopsies may aid selection of treatment in the near future.
Conclusions: It is unlikely that a single sequence will be adequate for all mCRPC patients. An individualised strategy that assesses the biological mechanisms of the disease and monitors molecular drivers of progression and resistance to treatment is required to maximise benefit for each patient and bring us closer to the goal of best care.
Patient summary: In this review we summarise evidence on the optimal sequence of anticancer drugs for metastatic castration-resistant prostate cancer. No agent has proven superior to another as front-line treatment, and the exact impact of prior treatments on drug efficacy is unknown. Better biomarkers for treatment selection and evaluation of response to treatment will be needed to personalise the optimal sequence for each individual patient.

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1. Introduction

Advanced prostate cancer is a major cause of cancer morbidity and mortality worldwide. Although initially sensitive to androgen deprivation therapy (ADT), progression despite castrate levels of testosterone eventually occurs, and patients enter the lethal castration-resistant (CRPC) phase of the disease. Since its approval in 2004, docetaxel was the only agent until 2010 that had proven survival benefit in metastatic CRPC (mCRPC) [1]. After 2010, however, the

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approval of five new agents with survival benefit revolutionised the therapeutic landscape for the disease (Table 1) [2–8]. Most of the trials evaluating novel agents were developed in patient populations that had only received docetaxel, and no evidence on their clinical activity when given sequentially is available. Furthermore, trials evaluating the activity of some of these agents in earlier stages of the disease could significantly change the landscape in the near future.

Adequate evidence on how to effectively sequence and combine therapies in CRPC is necessary to optimise benefit to patients suffering from this disease. There is a need to develop predictive biomarkers for treatment selection based on disease biology, and treatment response biomarkers to assess therapeutic benefit and allow early changes in treatment for nonresponding patients.

Here we synthesise current evidence on the optimal sequence of agents in CRPC, as well as the potential role of

Table 1 – Phase 3 trials in metastatic castration-resistant prostate cancer

Trial	Experimental arm	Control arm	Primary endpoint	Secondary endpoints	Comments
Hormonal agents					
COU-301 [3] (n = 1195)	Abiraterone 1000 mg OD + prednisone 5 mg BD	Prednisone 5 mg BD + placebo	OS 14.8 vs 10.9 mo (HR 0.65; $p < 0.001$)	Time to PSA progression; PSA response rate; rPFS	Post-docetaxel
AFFIRM [5] (n = 1199)	Enzalutamide 160 mg OD	Placebo	OS 18.4 vs 13.6 mo (HR 0.63; $p < 0.001$)	PSA response rate; pain response rate; quality of life (EQ-5D); PSA-PFS; rPFS; time to first SRE	Post-docetaxel population; patients with risk factors for seizures excluded
COU-302 [4] (n = 1088)	Abiraterone 1000 mg OD + prednisone 5 mg BD	Prednisone 5 mg BD + placebo	rPFS (PCWG2) 16.5 vs 8.3 mo (HR 0.53; ($p < 0.001$) OS NR vs 27.2 mo (HR 0.75; $p = 0.01$)	Time to opiate use; time to initiation of cytotoxic chemotherapy; time to ECOG PS decline; PSA response rate; radiographic response rate; quality of life	Co-primary endpoints OS + rPFS; chemotherapy-naïve patients; no visceral metastases included; OS did not meet prespecified significance criteria
PREVAIL [6] (n = 1715)	Enzalutamide 160 mg OD	Placebo	32.4 vs 30.2 mo (HR 0.7; $p < 0.0001$)	Time to initiation of cytotoxic chemotherapy; time to first SRE	Chemotherapy-naïve patients; 11% with visceral disease; patients with risk factors for seizure were excluded
Chemotherapy					
TAX 327 [1] (n = 1006)	Docetaxel 75 mg/m^2 every 3 wk (D75)	Mtx 12 mg/m^2 every 3 wk (M) Docetaxel 30 mg/m^2 weekly (D30)	OS - D75 18.9 mo - D30: 17.4 mo - M: 16.5 mo	PSA response rate; pain response; quality of life (FACT-P)	45% with pain at baseline; D75 superior toD30 and M; D30 not superior to M
SWOG 99-16 [61] (n = 674)	Docetaxel 60 mg/m^2+ estramustine 260 mg days 1–5 every 3 wk	Mtx 12 mg/m^2 every 3 wk	OS 17.5 vs 15.6 mo ($p = 0.02$)	PSA response; radiologic response rate	33% with pain at baseline; significant toxicity associated with estramustine
TROPIC [2] (n = 755)	Cabazitaxel 25 mg/m^2 every 3 wk	Mtx 12 mg/m^2 every 3 wk	OS 15.1 vs 12.7 mo (HR 0.7; $p = 0.0001$)	PFS; PSA response rate; radiographic response rate; pain response	Post-docetaxel; significant haematologic toxicity with cabazitaxel; no difference in pain response
FIRSTANA [16] (n = 1168)	Cabazitaxel 25 mg/m^2 (C25) Cabazitaxel 20 mg/m^2 (C20)	Docetaxel 75 mg/m^2 (D75)	OS (C25 vs D75) 25.2 vs 24.3 mo (HR 0.97) OS (C20 vs D75) 24.5 vs 24.3 mo (HR 1.01)	PFS; PSA response rate; radiographic response rate; pain response; quality of life	No significant benefit of cabazitaxel over docetaxel in first-line treatment; tumor response rate higher in the C25 arm
PROSELICA [32] (n = 1200)	Cabazitaxel 20 mg/m^2 (C20)	Cabazitaxel 25 mg/m^2 (C25)	OS (noninferiority) 13.4 vs 14.5 mo (HR 1.01)	PFS; PSA response rate; radiographic response rate; pain response; quality of life	Noninferiority of C20 established; PSA and RECIST response rates higher in the C25 arm; lower toxicity rates in the C20 arm

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Table 1 (*Continued*)

Trial	Experimental arm	Control arm	Primary endpoint	Secondary endpoints	Comments
Radiopharmaceuticals					
ALSYMPCA [7] (n = 809)	223-Ra 50 kBq IV every 4 wk × 6 injections	Placebo	OS 14 vs 11.2 mo (HR 0.7; p = 0.002)	Time to increase in ALP; ALP response; ALP normalisation rate; time to first SRE; time to PSA progression	Symptomatic bone metastases; 57% of patients were post-docetaxel; docetaxel-naïve patients unfit for docetaxel were included; visceral metastases were excluded
Immunotherapy					
IMPACT [8] (n = 512)	Sipuleucel-T every 2 wk × 3 infusions	Placebo	OS 25.8 vs 21.7 mo (HR 0.78; p = 0.03)	Time to objective disease progression	No differences in time to objective disease progression; visceral metastases excluded; only absent or minimal pain; 19.6% post-chemotherapy
D9901 and D9902A [62] (n = 225)	Sipuleucel-T every 2 wk × 3 infusions	Placebo	PFS 11.1 vs 9.7 wk (HR 1.26; p = 0.111)	OS 23.2 vs 18.9 mo (HR 1.5; p = 0.011)	Integrated analysis of 2 identical small phase 3 trials; primary endpoint not met; at least 3-mo chemo-free interval; visceral metastases and pain from bone metastases excluded
Small et al. [63]	Sipuleucel-T every 2 wk × 3 infusions	Placebo	PFS 11.7 vs 10.8 wk (HR 1.45; p = 0.052)	OS 25.9 vs 21.4 mo (HR 2.1; p = 0.01)	Primary endpoint PFS not met; visceral metastases excluded

OD = once daily; BD = twice daily; IV = intravenous; OS = overall survival; HR = hazard ratio; PFS = progression-free survival; rPFS = radiographic PFS; ALP = alkaline phosphatase; SRE = skeletal-related event; PSA = prostate-specific antigen; RECIST = Response Evaluation Criteria in Solid Tumours; Mtx = mitoxantrone; NR = not reached; ECOG PS = Eastern Cooperative Oncology Group performance status.

treatment combinations and the development of novel biomarkers for patient selection and the assessment of treatment-derived benefit.

2. Evidence acquisition

References for this review were identified from PubMed and relevant conferences using the search terms *castration-resistant prostate cancer*, *metastatic*, *androgen receptor*, *sequencing*, *combination*, *docetaxel*, *cabazitaxel*, *abiraterone*, *enzalutamide*, *immunotherapy*, and *radium* in publications up to June 2016. The clinicaltrials.gov resource was accessed for ongoing clinical trials. Only papers published in English were reviewed.

3. Evidence synthesis

3.1. Treatment options in mCRPC

Prior exposure to docetaxel, traditionally used to classify agents in pre- and post-docetaxel trial populations, may have become obsolete, as docetaxel has been displaced from first-line treatment in a significant number of cases. The Prostate Cancer Working Group (PCWG3) [9] provides a framework for conceptualising CRPC according to natural disease history, presence of metastases, testosterone levels, and prior therapy (Fig. 1).

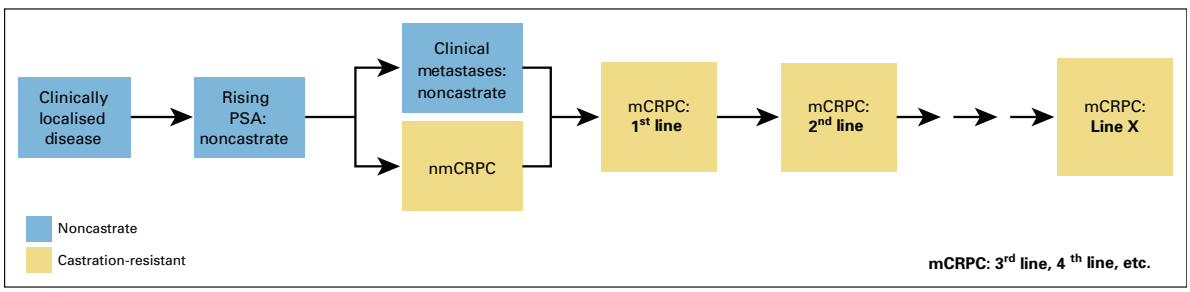

Fig. 1 – Natural history of the prostate cancer. Disease states as defined by the Prostate Cancer Clinical Trials Working Group 3 criteria. PSA = prostate-specific antigen; nmCRPC = nonmetastatic castration-resitant prostate cancer; mCRPC = metastatic CRPC. Taken from: Scher HI, Morris MJ, Stadler WM, et al. Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the Prostate Cancer Clinical Trials Working Group 3. J Clin Oncol 2016;34:1402–18 [9]. Permission requested from the *Journal of Clinical Oncology*.

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3.1.1. *Novel hormonal agents (NHAs): abiraterone and enzalutamide*

Abiraterone is a small-molecule inhibitor of the CYP17A1 enzyme and prevents the synthesis of steroid precursors that can be transformed into testosterone by cancer cells. One of its commonest metabolites is also a potent androgen receptor (AR) antagonist [10]. Enzalutamide is a second-generation AR inhibitor with 50-fold greater AR-binding capacity than bicalutamide and the ability to impair AR binding to DNA, inhibition of coactivator recruitment, and DNA transcription [11].

Abiraterone and enzalutamide were initially evaluated in patients with predominantly symptomatic disease that had progressed after docetaxel chemotherapy. The first of these trials, COU-AA-301, randomised 1195 patients to receive the combination of abiraterone and prednisone or placebo and prednisone. Patients receiving abiraterone had a 4.6-mo survival benefit (15.8vs 11.2 mo, hazard ratio [HR] 0.74; $p < 0.001$) [3]. The AFFIRM trial established a survival benefit of enzalutamide over placebo (18.4 vs 13.6 mo, HR 0.63; $p < 0.001$) in 1,199 patients. All secondary endpoints (prostate-specific antigen [PSA] response, PSA–progression-free survival [PFS], skeletal-related events, quality of life) favoured abiraterone and enzalutamide over the control arms. These results led to regulatory approval of abiraterone in 2011 and enzalutamide in 2012.

Both agents were then evaluated in a population of chemotherapy-naïve, asymptomatic or minimally symptomatic patients with a lower disease burden. Abiraterone and prednisone resulted in improved survival over prednisone alone (34.7vs 30 mo, HR 0.81; $p < 0.001$) in the COU-AA-302 trial [12]; regulatory approval was based on a significant improvement in radiographic PFS (rPFS) and all secondary endpoints, before longer follow-up confirmed a significant survival benefit [13]. With a similar design, the PREVAIL trial showed a survival benefit for enzalutamide (HR 0.71; $p < 0.001$) over placebo in a preplanned interim analysis, prompting early termination of the study and crossover of placebo patients to enzalutamide.

Toxicity rates for both agents are low (Table 2). The most characteristic adverse events are mineralocorticoid excess (hypokalaemia, hypertension, fluid retention) with abiraterone, and fatigue with enzalutamide. Patients with risk factors for the development of seizures were excluded from AFFIRM and PREVAIL.

3.1.2. *Taxanes*

Docetaxel was the first agent to increase survival in mCRPC in two trials published in 2004. Both docetaxel-estramustine (17.5 vs 15.6 mo, HR 0.8; $p = 0.02$) in the SWOG-99 trial and three-weekly docetaxel (18.9 vs 17.4 mo, HR 0.76; $p = 0.009$) in the TAX-327 trial showed superiority over mitoxantrone. Three-weekly docetaxel became the standard first-line chemotherapy owing to the risk of thrombosis associated with estramustine [14,15].

Cabazitaxel is a taxane that was developed based on preclinical screening of agents in docetaxel-resistant cell lines. Cabazitaxel 25 mg/m^2 improved survival compared to mitoxantrone (15.1 vs 12.7 mo, HR 0.7; $p < 0.001$) in the TROPIC study evaluating 755 men with mCRPC who had progressed on docetaxel [2], leading to approval in 2010 for post-docetaxel mCRPC.

Cabazitaxel has been linked to haematologic and gastrotintestinal (diarrhoea) toxicity. Recently, the PROSELICA phase 3 trial compared cabazitaxel at 25 mg/m^2 and 20 mg/m^2 doses and proved noninferiority for the 20 mg/m^2 dose, although the higher dose had greater antitumour activity. Grade 3–4 neutropenia (41.8% vs 73.3%) and diarrhoea (1.4% vs 4%) were significantly lower with

Table 2 – Frequent adverse events in phase 3 trials

	Event frequency (%) for all grades (grade 3-4 events)		
Abiraterone	COU-301 [3]	COU-302 [4]	
Liver function test abnormality	10 (3)	12 (5)	
Fluid retention	31 (2)	28 (<1)	
Hypokalaemia	17 (3)	17 (2)	
Hypertension	17 (3)	22 (4)	
Enzalutamide	AFFIRM [5]	PREVAIL [6]	
Fatigue	34 (6)	36 (2)	
Diarrhoea	21 (1)	16 (1)	
Hot flashes	20 (0)	18 (0)	
Musculoskeletal pain	12 (<1)	20 (<1)	
Docetaxel	TAX-327 [1]	FIRSTANA [16]	
Anaemia	NR (5)	99.5 (5.5)	
Thrombopenia	NR (1)	32.6 (1.6)	
Febrile neutropenia	3 (3)	8.3 (8.3)	
Diarrhoea	32 (NR)	37 (2.3)	
Stomatitis	20 (NR)	13.7 (2.3)	
Haematuria	NR	2.6 (0.3)	
Peripheral neuropathy	30 (NR)	25.1 (2.1)	
Cabazitaxel 25 mg/m^2	TROPIC	FIRSTANA [16]	PROSELICA [32]
Anaemia	97 (11)	99.7 (8.7)	99.7 (13.7)
Thrombopenia	47 (4)	45.4 (3.1)	42.5 (4.2)
Febrile neutropenia	8 (8)	12 (12)	NR (9.2)
Diarrhoea	47 (6)	49.9 (5.6)	NR (4)
Stomatitis	NR	6.6 (0.3)	NR
Haematuria	17 (2)	25.1 (3.6)	NR (4)
Peripheral neuropathy	14 (1)	12.3 (0)	NR
Cabazitaxel 20 mg/m^2	FIRSTANA [16]	PROSELICA [32]	
Anaemia	99.5 (6.5)	99.8 (9.9)	
Thrombopenia	35.3 (1.6)	35 (2.6)	
Febrile neutropenia	2.4 (2.4)	2.1 (2.1)	
Diarrhoea	32.5 (3.5)	NR (1.4)	
Stomatitis	4.9 (0)	NR	
Haematuria	20.3 (3.5)	NR (1.9)	
Peripheral neuropathy	11.7 (0.3)	NR	
Ra-223	ALSYMPCA [7]		
Anaemia	31 (13)		
Thrombopenia	12 (6)		
Neutropenia	5 (3)		
Febrile neutropenia	1 (1)		
Diarrhoea	25 (2)		
Sipuleucel-T	IMPACT [8]		
Chills	54.1 (1.2)		
Fever	29.3 (0.3)		
Headache	16 (0.3)		
Myalgia	9.8 (0.6)		

NR = not reported.

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20 mg/m^2; PSA and Response Evaluation Criteria in Solid Tumours [RECIST] responses were higher with 25 mg/m^2. Docetaxel has importantly higher rates of neurotoxicity, alopecia, and nail toxicity than cabazitaxel. Peripheral neuropathy can limit treatment with the maximum cumulative dose (Table 2).

3.1.3. *Radiopharmaceuticals and immunotherapy*

Ra-223 is an α-emitting radiopharmaceutical that binds to newly formed bone stroma of osteoblastic and sclerotic metastases. Its cytotoxic effect is mediated by short-range (<100 mcm) α-particles that induce double-stranded DNA breaks in tumor cells.

The ALSYMPCA trial compared six cycles of Ra-223 to placebo in 921 mCRPC patients with significant pain, at least two bone metastases, and no visceral metastases. A 5.1-mo survival benefit (14 vs 11.2 mo, HR 0.7; $p = 0.002$) was observed for Ra-223, as well as a benefit for all other secondary endpoints. Patients with and without prior docetaxel (contraindication or refusing docetaxel) were allowed to enter the trial. Treatment was well tolerated (Table 2), with adverse events being more frequent in the control arm.

Sipuleucel-T is an autologous active cellular immunotherapy that was approved in 2010 based on results of the 9902B (IMPACT) trial, which reported a 4.1-mo survival benefit over placebo (25.8 vs 21.7 mo, HR 0.77; $p = 0.03$) in 512 asymptomatic mCRPC patients without visceral metastases. Prior docetaxel was allowed, provided that no progression had occurred in the previous 3 mo. No benefit for PSA response or PFS was observed. Toxicity consisted mainly of mild/moderate influenza-like symptoms. Despite approval by the European Medicines Agency (EMA), sipuleucel-T is not commercially available in the EU.

3.2. *Finding the optimal treatment sequence in mCRPC*

3.2.1. *Choice of initial agent*

With the exception of the FIRSTANA trial [16], no formal comparison of life-prolonging agents in mCRPC has been made. Differences in toxicity and more convenient oral administration have made abiraterone and enzalutamide the preferred first-line option over taxanes despite a lack of evidence of superior activity. No prospective evidence is available to evaluate if a strategy with first-line taxanes followed by NHAs at progression is superior to the opposite sequence, especially in symptomatic patients, who were not represented in COU-AA-302 and PREVAIL [6,12].

Cross-comparison of clinical trials is not helpful owing to differences in trial design or patient populations. For example, control arms included chemotherapy (TAX-327, TROPIC) [2,1], corticosteroids (COU-AA-301, COU-AA-302) [12,3], or placebo (AFFIRM, PREVAIL, ALSYMPCA, IMPACT) [5,6,8,7]. Prior and subsequent treatments in most trials do not correspond to those available for current mCRPC patients, which adds difficulty to extrapolating trial-reported benefits to daily clinical practice.

Asymptomatic or minimally symptomatic mCRPC patients were evaluated in trials of abiraterone (COU-302), enzalutamide (PREVAIL), and sipuleucel-T (IMPACT). Only patients with a score of ≤3 on the Brief Pain Inventor–Short Form were included in COU-AA-302 and PREVAIL, and approximately 50% patients in IMPACT were pain-free. Survival in the control arm of these trials ranged between 22 and 34 mo (Table 1). Trial populations in TAX-327, TROPIC, COU-301, AFFIRM or ALSYMPCA comprised patients with more advanced disease and worse prognosis; survival in the control arms ranged between 11.2 and 16.5 mo. Despite differences in survival, a similar reduction in the risk of death was observed in trials with asymptomatic and symptomatic patients, with HRs between 0.63 and 0.76 (Table 1).

The presence of visceral metastases should not favour treatment with first-line chemotherapy over NHAs, since subgroup analyses favoured NHAs over placebo in this subgroup in PREVAIL. Patients with visceral metastases were excluded from ALSYMPCA and should not be considered for Ra-223 because of its bone-specific mechanism of action. In accordance with the IMPACT trial population, sipuleucel-T should be considered only in selected patients with a low disease burden.

Attempts have been made to identify patient subgroups that are less likely to respond to hormonal agents and should be considered for chemotherapy. Progression after a short duration of ADT (6–12 mo) is associated with limited NHA antitumour activity with [17], while docetaxel seems to remain active [18]. Clinical characteristics grouped into an anaplastic phenotype (Table 3) that has been related to neuroendocrine gene signatures [19] may identify aggressive variants that could possibly benefit from upfront chemotherapy rather than AR axis–targeting therapy; these data now need to be validated in multicentre prospective trials.

Table 3 – Proposed clinical features of "anaplastic" prostate carcinomas

Histologic evidence of small-cell prostate carcinoma (pure or mixed)
Exclusively visceral metastases
Radiographically predominant lytic bone metastases by plain X-ray or computed tomography scan
Bulky (≥5 cm) lymphadenopathy or bulky (≥5 cm) high-grade (Gleason ≥8) tumor mass in prostate/pelvis
Low PSA (≤10 ng/ml) at initial presentation (before ADT or at symptomatic progression in the castrate setting) plus a high volume (≥20) of bone metastases
Presence of neuroendocrine markers on histology (positive staining of chromogranin A or synaptophysin) or in serum (abnormal high serum levels of chromogranin A or GRP) at initial diagnosis or at progression
Plus any of the following in the absence of other causes:
A. Elevated serum lactate dehydrogenase (≥2 IULN)
B. Malignant hypercalcaemia
C. Elevated serum carcinoembryonic antigen (≥2 IULN)
Short interval (≤6 mo) to androgen-independent progression following initiation of hormonal therapy with or without the presence of neuroendocrine markers

PSA = prostate-specific antigen; ADT = androgen deprivation therapy; GRP = gastrin-releasing peptide; IULN = institutional upper limit of normal.
Adapted from Aparicio et al. Clin Cancer Res 2013;19:3621–30 [64].

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3.2.2. Abiraterone versus enzalutamide

Abiraterone and enzalutamide trials had very similar designs and patient populations; no head-to-head comparison is available or ongoing. Choice is based on toxicity (contraindications for steroid use, history of seizures), availability, and patient/physician preference.

Some differences in the design of the abiraterone and enzalutamide trials should be considered. Patients with visceral metastases (excluded from COU-AA-302) accounted for 12% of PREVAIL participants; survival benefit in these patients was consistent with the overall benefit of enzalutamide. Patients in the control arm received prednisone 10 mg daily in the abiraterone trials and placebo in the enzalutamide trials, although 30% of AFFIRM and 4% of PREVAIL patients were receiving steroids at baseline.

The role of steroids (given as monotherapy or with abiraterone to prevent secondary mineralocorticoid excess) in mCRPC response or progression is unclear. On one hand, PSA response rates of 29% and 3%, and PSA-PFS of 5.6 and 2.8 mo were observed in the control arms of COU-AA-302 (prednisone) and PREVAIL (placebo) respectively. Some 43% of patients with unfavourable baseline circulating tumour cell (CTC) counts experienced CTC declines in the prednisone arm of COU-AA-301 [20]. On the other hand, mutant AR variants activated by corticosteroids were observed in 13% patients progressing on abiraterone [21]. Treatment with corticosteroids before entry into COU-AA-301 appeared to be correlated with poorer outcome, although this was also associated with other adverse prognostic features [22]. Although long-term steroid toxicity was infrequent in the abiraterone trials [23], ongoing studies are evaluating lower iatrogenic corticosteroid doses, alternative steroids, and mineralocorticoid antagonists for administration with abiraterone(Table 4).

3.2.3. Choosing the right taxane

Toxicity profiles for cabazitaxel and docetaxel differ. More frequent neutropenia, diarrhoea, and haematuria occurred with cabazitaxel at 25 mg/m^2 in TROPIC, while peripheral neuropathy, oedema, alopecia, and nail disorders were associated with docetaxel in TAX-327 (Table 2).

In the FIRSTANA trial [16], 1168 patients were randomised to receive 3-weekly docetaxel 75 mg/m^2 (D75), cabazitaxel 25 mg/m^2 (C25), or cabazitaxel 20 mg/m^2 (C20). No significant differences in survival (C20 24.5 mo, C25 25.2 mo, D75 24.3 mo) were observed. The haematologic toxicity of C20 was significantly lower than for C25, and similar to D75 (Table 2). Although C20 had a similar toxicity profile to D75 in both FIRSTANA and PROSELICA, response rates were higher in the C25 arm of FIRSTANA and C25 showed higher antitumour activity in the abiraterone/enzalutamide pretreated PROSELICA subgroup.

On the basis of the TROPIC trial results, EMA and US Food and Drug Administration approvals of cabazitaxel mandated its use in docetaxel pretreated patients. Although FIRSTANA results suggested that both docetaxel and cabazitaxel could have similar efficacy and toxicity in first-line treatment, the trial was designed to test the superiority of cabazitaxel over docetaxel, and therefore noninferiority cannot be assumed. The higher cost of cabazitaxel, its documented post-docetaxel activity, and the fact cabazitaxel was designed to be less susceptible to docetaxel resistance mechanisms are other reasons to favour docetaxel as the first-line taxane of choice.

3.2.4. Cross-resistance between agents

The efficacy of taxanes when administered after NHAs, or of abiraterone or enzalutamide given after each other, has not been prospectively evaluated. None of the participants in AFFIRM or PREVAIL, studies initiated after COU-AA-301 and COU-AA-302, had previously received abiraterone. By contrast, the activity of abiraterone and enzalutamide after docetaxel is well documented in COU-AA-301 and AFFIRM (Table 1).

Preclinical evidence suggests lower antitumour activity for docetaxel and enzalutamide in abiraterone- and enzalutamide-resistant cell lines, but stable activity of cabazitaxel in enzalutamide-resistant cell lines [24]. A proposed role for taxanes as AR-targeting drugs by blocking nuclear AR translocation could potentially justify cross-resistance, although other mechanisms are probably involved [25].

Clinical evidence of cross-resistance is derived from retrospective studies with a risk of bias and should be interpreted with caution. Overall, none of the different sequences has shown clear superiority [26]. Initial single-centre studies indicated lower antitumour activity for docetaxel post-abiraterone [27]. Retrospective post hoc analyses for COU-AA-302 participants receiving docetaxel after abiraterone, limited by a >80% censoring rate, revealed a 50% PSA-response rate of 27% and a time on treatment that is lower than for TAX-327 [1] but comparable to non–clinical-trial, registry-level retrospective reviews [28]. Some series evaluating cabazitaxel in patients after NHAs reported results comparable to those from TROPIC (PSA response 27–41%, radiographic response 15%, overall survival [OS] 10.9–20.3 mo, PFS 4.4–5.5 mo) [24,29]. However, data on the use of abiraterone after prior disease progression on enzalutamide and taxanes [30], and of enzalutamide after disease progression on abiraterone and taxanes (50%-PSA response 18%; OS 8.3 mo) [31] do suggest significantly lower antitumour activity compared to COU-AA-301 and AFFIRM. Moreover, the activity of enzalutamide and abiraterone is also lower after docetaxel than before docetaxel, suggesting decreasing activity for AR-targeted therapy in later disease settings. The lower response rates and shorter survival when using agents as second or third lines of treatment could also indicate a more aggressive phenotype at the time of therapeutic resistance and should not be interpreted as definitive evidence of cross-resistance.

These results do not provide definitive evidence on the optimal treatment sequence. In the PROSELICA trial, 308 patients (25.7%) had previously received NHAs, and 809 patients (67.4%) received NHAs after progression on cabazitaxel. Data on the outcome for these subgroups is awaited for a better assessment of the activity of these agents in sequence [32].

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Table 4 – Clinical trials addressing treatment sequencing in castration-resistant prostate cancer (CRPC)

NCT number	Phase	Description
Biomarker-based patient selection		
NCT02438007 (ARMOR3-SV)	3	Study of galeterone compared to enzalutamide in men expressing androgen receptor splice variant-7 mRNA (AR-V7) metastatic CRPC
NCT02601014 (STARVE-PC)	2	Biomarker-driven therapy with nivolumab and ipilimumab in treating patients with metastatic hormone-resistant prostate cancer expressing AR-V7
NCT02621190 (CARVE)	2	Cabazitaxel in mCRPC patients with AR-V7-positive circulating tumour cells
NCT01682772 (TOPARP)	2	Trial of olaparib in patients with advanced CRPC
NCT02598895	1/2	Docetaxel and carboplatin in treating patients with metastatic, hormone-resistant prostate cancer containing inactivated genes in the BRCA 1/2 Pathway
NCT02215096	1	Dose-finding study of GSK2636771 when administered in combination with enzalutamide in male subjects with metastatic CRPC
NCT02552394	1	J591 in patients with advanced prostate cancer and unfavourable circulating tumor cell counts
NCT01692262 (PYRUS)	1b	Investigating the safety, tolerability and efficacy of AZD5363 in prostate cancer
Sequencing of approved agents		
NCT01995513 (PLATO)	4	Continued enzalutamide with abiraterone beyond progression on abiraterone in patients with chemotherapy-naïve metastatic CRPC
NCT02125357	2	Sequencing abiraterone and enzalutamide in mCRPC
NCT01576029 (SWITCH)	2	Continued treatment with docetaxel versus switch to cabazitaxel after minor prostate specific antigen response to docetaxel in patients with metastatic CRPC
NCT01718353 (TAXYNERGY)	2	Early switch from first-line docetaxel/prednisone to cabazitaxel/prednisone and the opposite sequence, exploring molecular markers in men with metastatic CRPC
NCT01487863	2	Concurrent versus sequential treatment with sipuleucel-t and abiraterone in men with metastatic CRPC
NCT01934790	1/2	Re-treatment safety of Ra-223 dichloride in CRPC with bone metastases
Combinations of approved agents		
NCT01949337 (ALLIANCE)	3	Enzalutamide with or without abiraterone acetate and prednisone in treating patients with metastatic CRPC
NCT02043678 (ERA-223)	3	Radium-223 dichloride and abiraterone acetate compared to placebo and abiraterone acetate for men with cancer of the prostate when medical or surgical castration does not work and when the cancer has spread to the bone, has not been treated with chemotherapy and is causing no or only mild symptoms
NCT02194842 (PEACE-III)	3	Trial comparing enzalutamide vs a combination of Ra223 and enzalutamide in asymptomatic or mildly symptomatic CRPC metastatic to bone
NCT01650194	2	Safety and tolerability of enzalutamide (MDV3100) in combination with abiraterone acetate in bone metastatic CRPC
NCT02036060 (ABIDO)	2	Abiraterone acetate in combination with docetaxel after disease progression to abiraterone
NCT02453009 (CHEIRON)	2	Addition of enzalutamide to first line docetaxel for CRPC
NCT01487863	2	Concurrent versus sequential treatment with sipuleucel-T and abiraterone in men with metastatic CRPC
NCT01981122	2	Study of sipuleucel-T with administration of enzalutamide in men with metastatic CRPC
NCT01845792	1/2	Study of abiraterone acetate and prednisone in combination with cabazitaxel in patients with prostate cancer
NCT01400555	1	Safety study of abiraterone acetate administered in combination with docetaxel in patients with metastatic CRPC
NCT01511536	1	Cabazitaxel and abiraterone acetate in patients with metastatic CRPC
NCT01565928	1	Safety and tolerability study of MDV3100 in combination with docetaxel in men with advanced prostate cancer
Alternative dosing and schedules for approved agents		
NCT01867710	2	Abiraterone with different steroid regimens for side effects related to mineralcorticoid excess prevention in prostate cancer prior to chemotherapy
NCT02025010	2	Trial of abiraterone acetate without exogenous glucocorticoids in men with CRPC with correlative assessment of hormone intermediates
NCT01558219 (PROSTYII)	2	Safety of biweekly cabazitaxel in metastatic CRPC patients previously treated with a docetaxel-containing regimen
NCT01518283	2	Study of weekly cabazitaxel for advanced prostate cancer
NCT01541007 (ConCab)	2	Trial comparing the conventional 3-weekly schedule of cabazitaxel with a weekly regimen in patients with metastatic CRPC
NCT02023697	2	Standard dose versus high dose and versus extended standard dose Ra-223 dichloride in CRPC metastatic to bone
NCT01637402	2	A phase 2 study of increased-dose abiraterone acetate in patients with CRPC

References to all trials were accessed on the website www.clinicaltrials.gov.

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3.2.5. Impact of treatment in earlier stages of the disease

3.2.5.1. Taxanes in metastatic hormone-sensitive disease. Docetaxel has emerged as the new standard of care for fit patients with metastatic hormone-sensitive prostate cancer after three randomised studies — GETUG-AFU-15 [33], CHAARTED [34], and STAMPEDE [35] — overall indicated a survival benefit from docetaxel in combination with ADT over ADT alone, with a pooled 9% improvement in 4-yr survival and a 23% reduction in the risk of death [36].

It will be important to define how prior docetaxel in this subgroup of patients impacts on the efficacy of later treatments in mCRPC. Treatment with subsequent docetaxel at progression in GETUG-AFU-15 was associated with lower antitumour activity in the ADT plus docetaxel arm than in the ADT alone arm (PSA-response rate 11% vs 41%, PFS 4.1 vs 7 mo). This could suggest the development of resistance to docetaxel, in marked contrast to studies evaluating the activity of docetaxel rechallenge in mCRPC, possibly indicating selection of chemosensitive cancers in the latter studies [37]. No results from the CHAARTED or STAMPEDE trials are yet available to help address this issue.

3.2.5.2. Abiraterone/enzalutamide in nonmetastatic CRPC. The IMAAGEN trial (NCT01314118) evaluating abiraterone in nonmetastatic CRPC has completed accrual and results are awaited. The recently reported STRIVE trial revealed a significant PFS benefit for enzalutamide over bicalutamide (19.4 vs 5.7 mo; $p < 0.001$) among 396 men [38]; the benefit was similar for nonmetastatic and metastatic disease. If approved for this indication, cross-resistance could be a significant issue when deciding first-line treatment in metastatic disease.

3.2.6. Combinations of agents

Potential synergistic activity between agents has led to the evaluation of multiple combinations. No combination has proved to have superior efficacy in mCRPC to date; nine randomised phase 3 trials enrolling more than 10 000 patients failed to prove the benefit of any docetaxel combination. Ongoing combination trials of approved agents are summarised in Table 4.

Owing to the nonoverlapping mechanism of action and low toxicity rates reported in ALSYMPCA, Ra-223 combinations, particularly with NHAs, have attracted interest. In an expanded-access Ra-223 programme in the USA, no differences in toxicity were reported between the combination of Ra-223 with abiraterone/enzalutamide and monotherapy [39]. The ongoing PEACE-3 trial is now evaluating the combination of enzalutamide and Ra-223 in mildly symptomatic mCRPC (EUDRACT 2014-001787-36).

However, it is not a foregone conclusion that any of these combinations will improve outcome. In a recent neoadjuvant trial, the combination of enzalutamide, abiraterone, and leuprolide acetate (a luteinising hormone–releasing hormone antagonist) showed a lower rate of tumor downstaging than abiraterone and leuprolide acetate, calling into question the synergy between these NHAs [40].

3.3. Biomarkers in prostate cancer

3.3.1. Predictive biomarkers for patient selection

A number of genomic aberrations that can be targeted with currently available agents have been reported in mCRPC [41] and could potentially serve as predictive biomarkers for patient selection.

Alterations of the DNA repair pathway are present in 20% of mCRPC samples, mainly in the *BRCA2* and *ATM* genes [42]. Higher tumor responses (88% vs 6%) and longer survival (13.8 vs 7.5 mo; $p = 0.05$) were observed among patients with DNA repair–deficient cancers treated with the PARP inhibitor olaparib [43]. On the basis of these results, breakthrough designation for a drug based on biomarker selection in mCRPC was granted for the first time. PI3K-Akt pathway alterations, although frequent (>50% of patients) [42], remain elusive targets; ongoing efforts focus on targeting the p110β subunit of PI3k, which could have a specific role in PTEN-deficient mCRPC. Poorer outcome for abiraterone in PTEN-deficient cancers has been reported [44]. *TMPRSS-ERG* gene fusions, present in 40–50% of CRPCs, have been proposed as a biomarker for sensitivity to abiraterone; *TMPRSS2:ERG* fusion variants with deletion of 21q22 and higher copy numbers of fusion sequences (class 2+ Edel) showed greater benefit from abiraterone in COU-AA-302 [45]. However, tissue acquisition in mCRPC is limited in clinical practice by the morbidity of the biopsy procedure and the frequent absence of lesions that are suitable for biopsy.

Identification of circulating biomarkers in the bloodstream of patients, a less invasive and more generalisable approach, could also have the advantage of providing a global genomic landscape of the disease. *PTEN* deletions, *TMPRSS:ERG* fusions, and other molecular biomarkers can be detected in CTCs [46]. AR splice variants (SVs) lacking the carboxy-terminal domain present an amino-terminal domain that is constitutively active, independent of ligand-binding and AR-targeting drugs. The presence of AR-V7 (the most frequent SV) in CTCs has been associated with lower response and shorter survival among patients treated with abiraterone/enzalutamide [47], but not patients treated with taxanes [48], suggesting that AR-V7 could be useful in deciding between NHA and taxane treatment. Prospective validation in clinical trials is ongoing and will be required before these biomarkers are incorporated into clinical practice. Similarly, detection of AR gene aberrations (mutations or amplifications) in circulating tumor DNA (ctDNA) is associated with lower rates of PSA decline and survival among patients treated with abiraterone/enzalutamide [21,49].

Sequential monitoring of AR-V7 and AR genomic aberrations can identify changes in AR-V7 status (“conversions”) or AR mutations that are activated by enzalutamide (F876L) or prednisone (T878A, L702H) to guide the selection of subsequent therapy [49], anticipating radiographic progression by months [21]. Baseline levels of ctDNA in FIRSTANA and PROSELICA had prognostic value [50]; functional analyses of these samples may identify biomarkers predictive of taxane efficacy.

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Clinical trials are increasingly selecting patients on the basis of molecular biomarkers. For example, the ongoing phase 3 ARMOR-SV study (NCT02438007) compares galeterone (a novel AR-targeting agent) with enzalutamide in a population of patients with AR-V7–positive mCRPC; other biomarker-directed trials are listed in Table 3.

3.3.2. *Assessing response to treatment*

Early identification of patients who do not benefit from treatment could increase the probability that patients will receive effective second-line therapy while still having a good performance status. PCWG3 provides guidance [9] for the assessment of prognosis and response in CRPC clinical trials. In daily clinical practice, response assessment relies on circulating (PSA), imaging (computed tomography [CT] scans, bone scintigraphy), and clinical (pain) biomarkers.

3.3.2.1. Circulating biomarkers. PSA is the most widely used biomarker in prostate cancer throughout all stages of the disease. As an indicator of AR transcriptional activity, PSA is less interpretable in later, less AR-driven stages of the disease; for example, neuroendocrine variants typically do not express PSA. Furthermore, PSA has limited value as a surrogate of survival [51]. Early PSA changes may not always be interpretable because of PSA flares (initial rise followed by a response) in up to 20% of docetaxel-treated patients [52] but only 9% of abiraterone-treated patients [53]. Early PSA declines among patients treated with abiraterone/enzalutamide are strongly associated with outcome [53,54]. CTCs may have more value than PSA for prognosis and response assessment. Pretreatment "unfavourable" CTC levels ($\geq$5 CTCs/7.5 ml of blood) indicate poorer prognosis, and a 12-wk post-treatment biomarker combination of CTC and lactate dehydrogenase levels has value as a surrogate of survival at the individual patient level [55]. Post-treatment CTC declines indicate benefit from treatment with abiraterone and chemotherapy [20] and have been incorporated into PCWG3 recommendations for response assessment in clinical trials [9].

3.3.2.2. Imaging biomarkers. Up to 80% of patients with mCRPC have exclusively bone metastatic disease, which is not amenable to response evaluation according to RECIST 1.1 criteria [56]. rPFS using an endpoint combining a CT scan and bone scintigraphy has been prospectively evaluated in several randomised trials, and showed a strong correlation with survival in COU-AA-302 [57]. However, bone scintigraphy has important limitations. The Tc-99m tracer is more sensitive in detecting progression than response, with no validated criteria to determine response. Initial transient worsening means that radiographic progression cannot be evaluated before 16 wk, mandating the appearance of two new lesions in two successive scans [9].

Novel imaging techniques can provide significant improvements in disease assessment and provide information on the biology of the disease. Positron emission tomography scans with a variety of different tracers may have greater sensitivity and specificity for detection of bone metastasis and could have a role in identifying sites of relapse after radical therapy in the presence of rising PSA, but their use in mCRPC is less well established [41]. Functional tracers evaluating the AR (FDHT) can potentially detect changes in activity for full-length AR, but not splice variants, after treatment with AR-targeting agents. Magnetic resonance imaging techniques such as diffusion-weighted imaging can also provide information on mCRPC bone tumor cellularity [58] and are being evaluated in randomised trials.

4. Conclusions

The unprecedented therapeutic developments in prostate cancer in recent years have left prostate cancer clinicians with the challenge of how to best sequence available therapeutic agents. Deciding on the best treatment sequence is one of the greatest challenges in advanced prostate cancer today, and recommendations by experts have recently become available via the St. Gallen Advanced Prostate Cancer Consensus Conference [59]. The actual degree of cross-resistance between drugs such as abiraterone and enzalutamide has not been fully elucidated and will require prospective evaluation. Extrapolating benefit from landmark trials to clinical decisions for current patients may therefore overestimate the benefit from agents when used in sequence. Personalising treatment on the basis of molecular stratification will hopefully be cost-saving and more profitable for the individual patient than sequencing all the active drugs one by one [60].

Finally, it is unlikely that a single sequence will be appropriate for all prostate cancer patients. Rather, an individualised approach combining frequent assessment of the biological processes involved, monitoring of molecular drivers of progression and resistance to treatment, and more accurate evaluation of treatment benefits will maximise the benefit for patients with advanced prostate cancer and bring us closer to the goal of optimal and precise prostate cancer care.

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Study concept and design: Lorente.
Acquisition of data: Lorente.
Analysis and interpretation of data: Lorente, Fizazi, Sweeney, de Bono.
Drafting of the manuscript: Lorente.
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