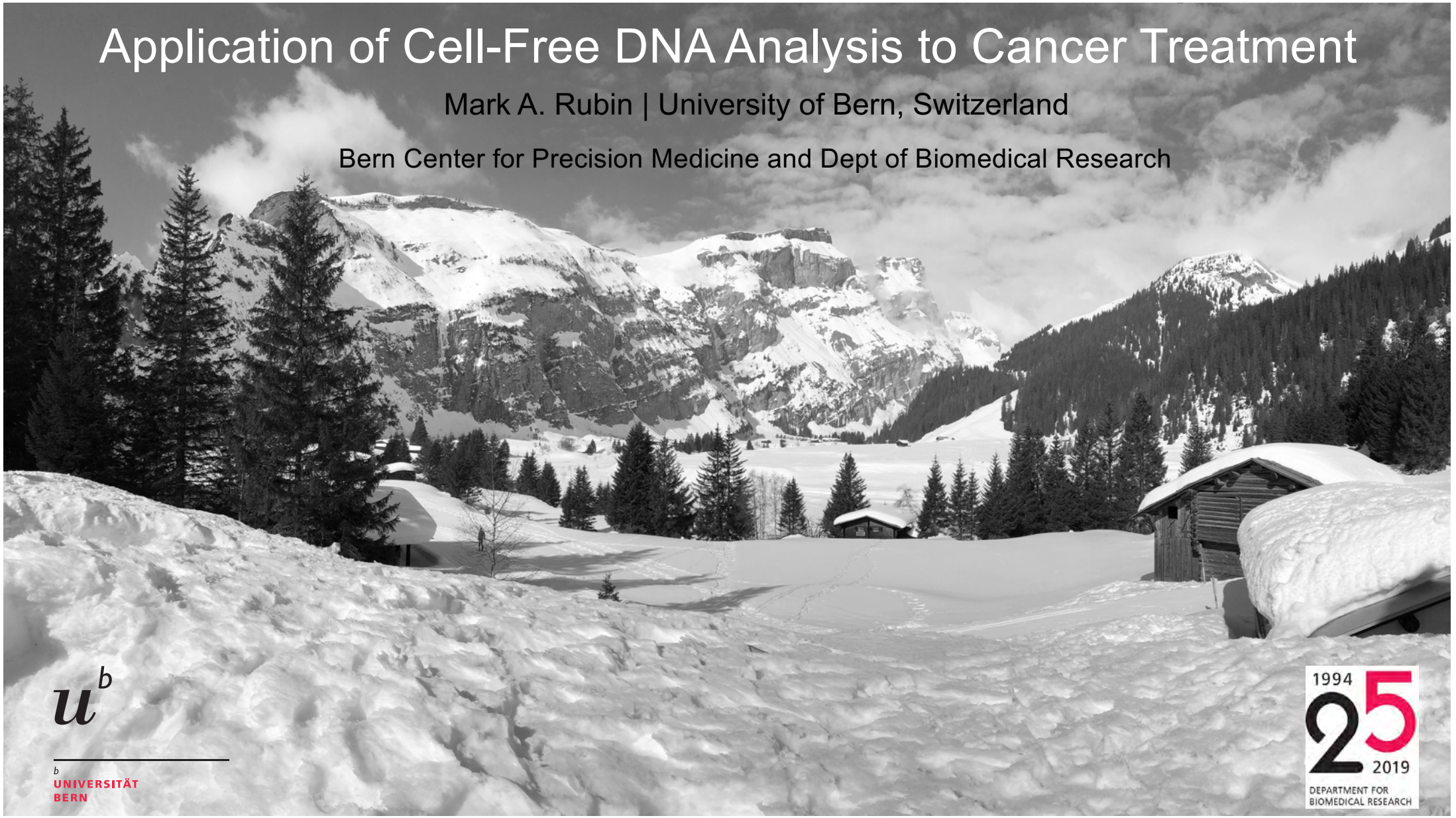
Application of Cell-Free DNA Analysis to Cancer Treatment

Mark A. Rubin | University of Bern, Switzerland

Bern Center for Precision Medicine and Dept of Biomedical Research



UNIVERSITÄT BERN

1994 25 2019 DEPARTMENT FOR BIOMEDICAL RESEARCH

DISCLOSURES

FUNDING:

NCI, EDRN,PCF, SU2C/PCF, Starr Cancer Consortium, DOD, SNF, Krebsliga, SPHN
Sanofi-Aventis, Millennium Pharma, Eli-Lilly, and Janssen

PATENTS:

Listed as co-inventor on patents in the diagnostic and treatment fields for ETS fusions (Harvard/Michigan), EZh2 (Michigan), SPOP (Cornell), and AURKA (Cornell)

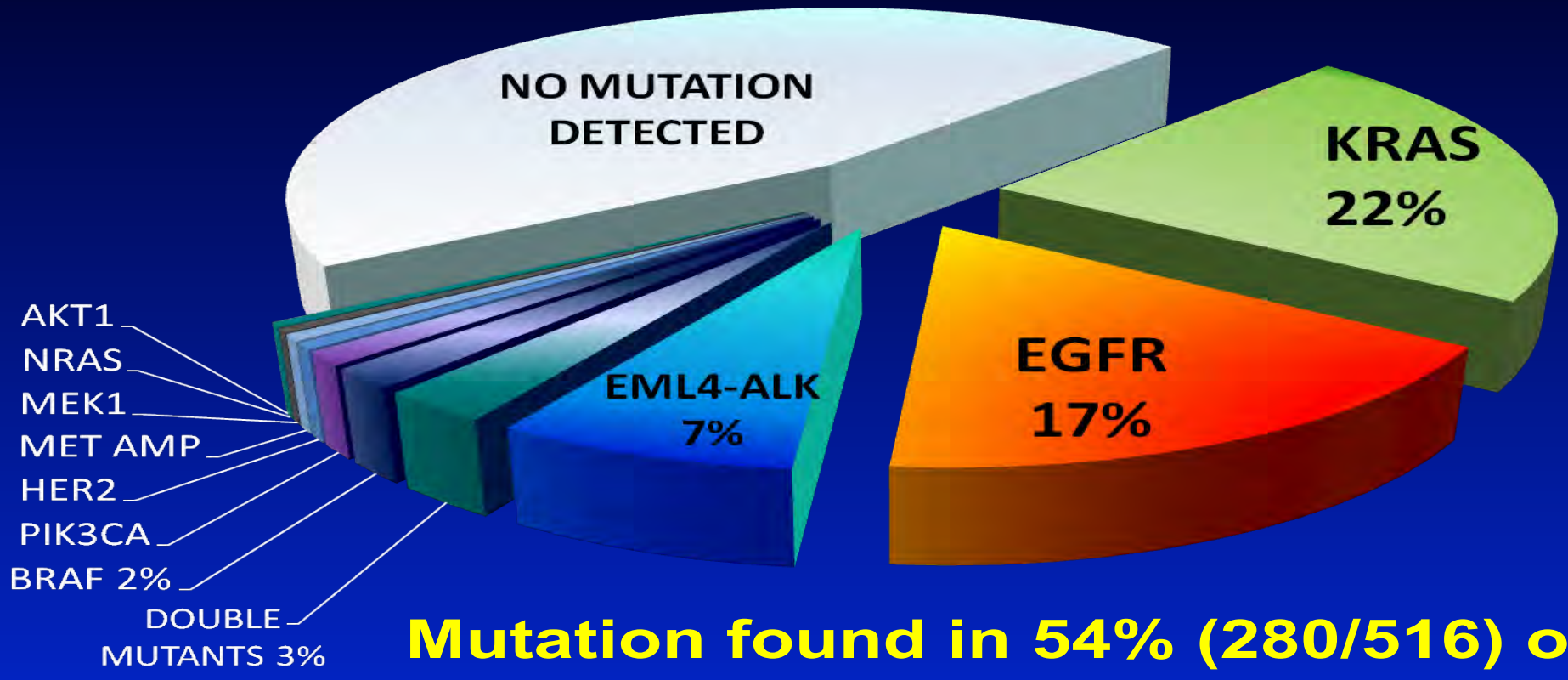
OFF-LABEL USE OF DRUGS WILL BE DISCUSSED

Co-Founder and stock holder of THUCYDX, LLC.

Lung Cancer Mutation Consortium

Incidence of Single Driver Mutations

5/13/11 data cut



Mutation found in 54% (280/516) of tumors completely tested (CI 50-59%)

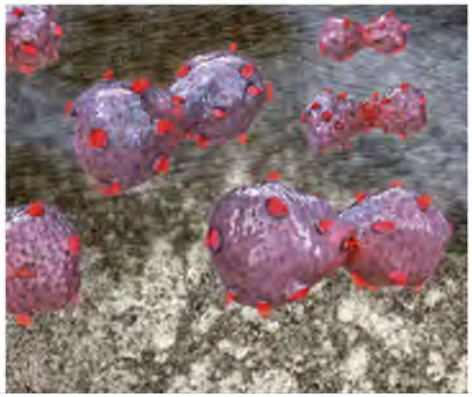
CANCER RESEARCH

The Official Blog of the American Association for Cancer Research

FDA Approves First Liquid Biopsy Test for Lung Cancer Patients

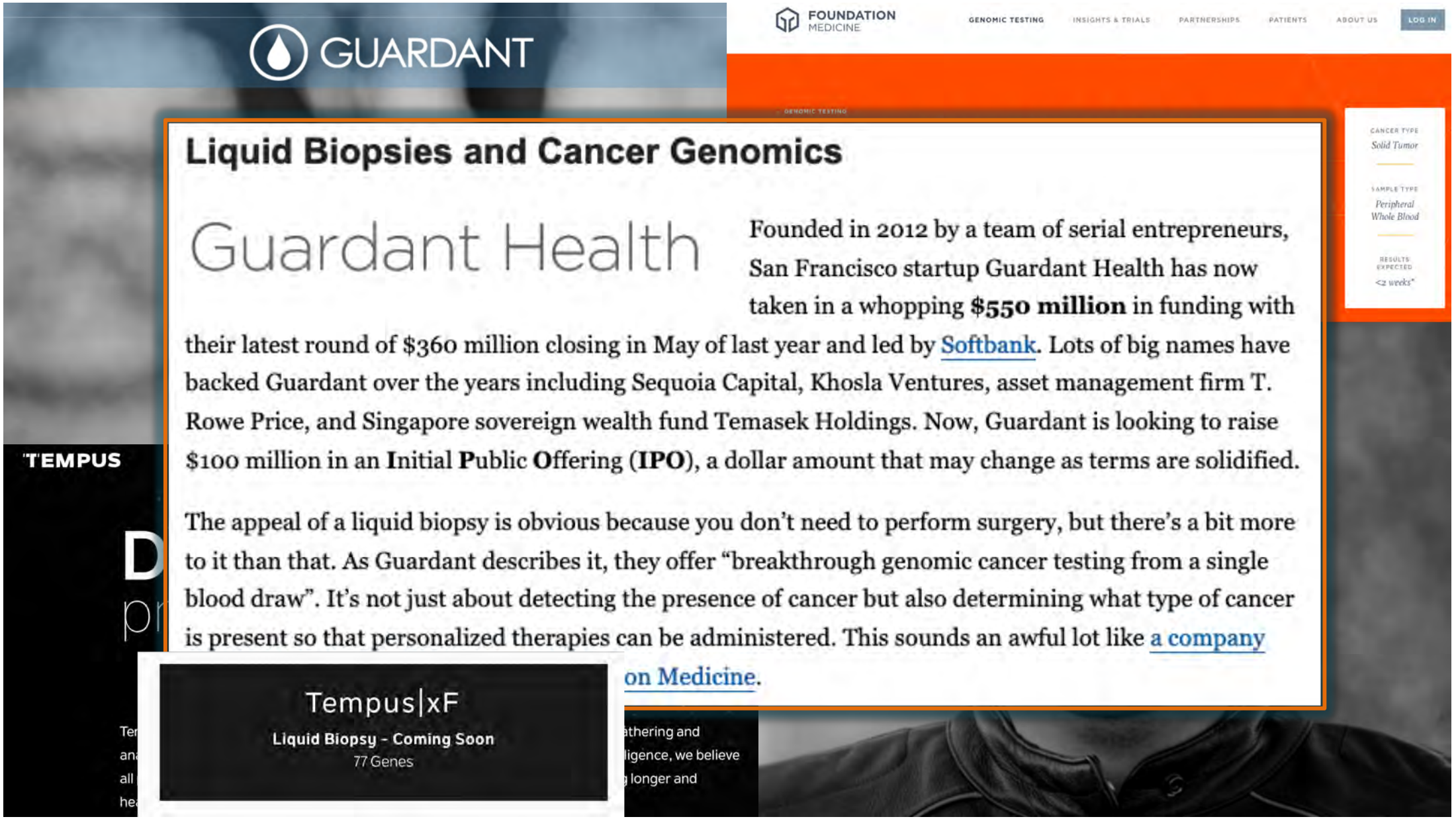
Posted on June 6, 2016 by Srivani Ravoori, PhD

On June 1, the U.S. Food and Drug Administration (FDA) approved a liquid biopsy test, a companion diagnostic test called cobas EGFR Mutation Test v2. The test uses plasma samples to identify patients with metastatic non-small cell lung cancer (NSCLC) eligible for treatment with the EGFR-targeted therapeutic erlotinib (Tarceva).



Becoming the new standard of care

In an interview to forecast cancer research and treatment advances in 2016, a precision medicine expert at Memorial Sloan Kettering Cancer Center, David Solit, MD, said, "The use of circulating free DNA collected from blood [liquid biopsy] to determine which treatment a cancer patient should receive is already a reality, and will begin to change the way we diagnose and treat patients in 2016. In 2016 and 2017, we will likely see liquid biopsies becoming a standard of care for some cancer types."

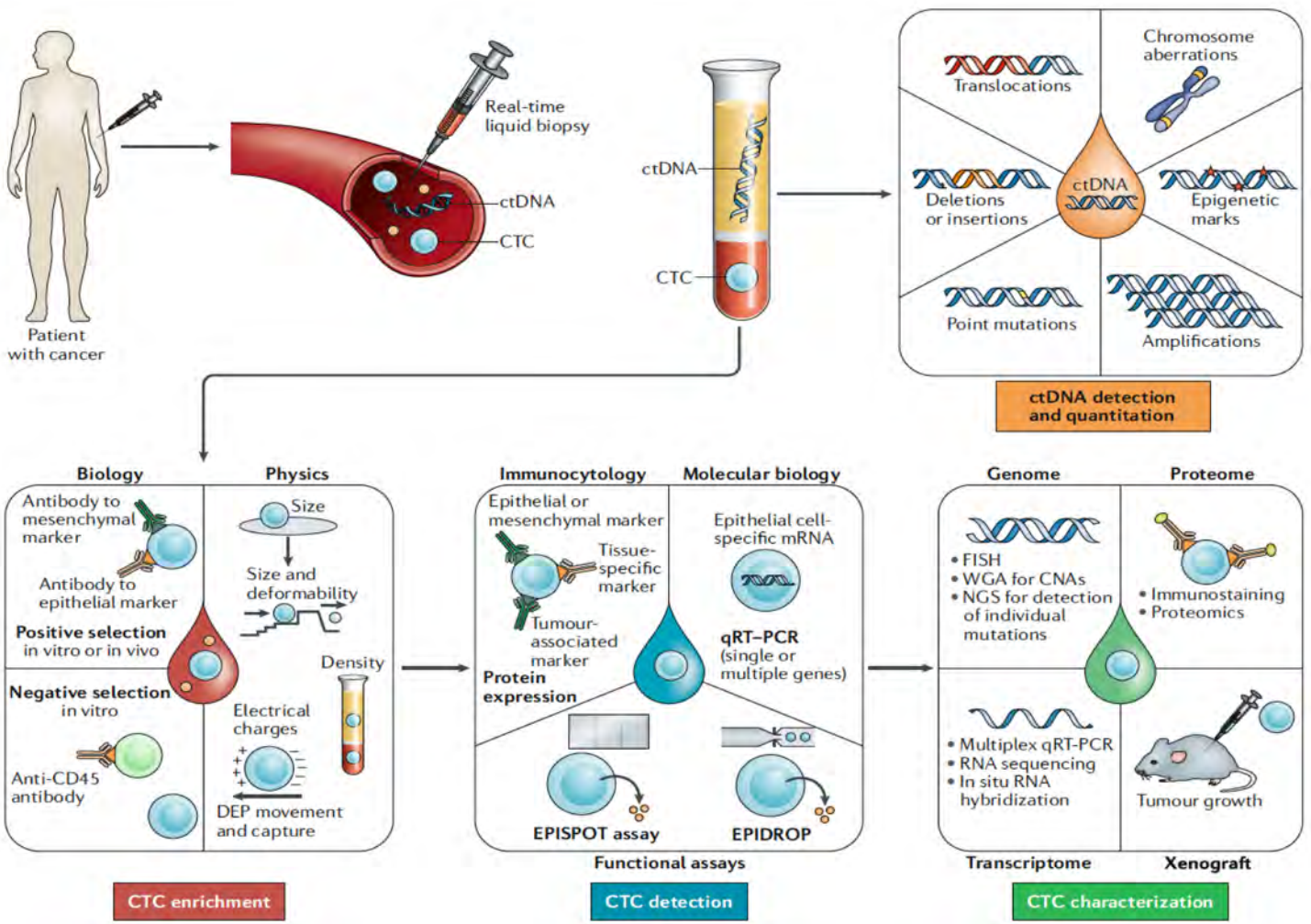


Liquid Biopsies and Cancer Genomics

Guardant Health

Founded in 2012 by a team of serial entrepreneurs, San Francisco startup Guardant Health has now taken in a whopping **$550 million** in funding with their latest round of $360 million closing in May of last year and led by Softbank. Lots of big names have backed Guardant over the years including Sequoia Capital, Khosla Ventures, asset management firm T. Rowe Price, and Singapore sovereign wealth fund Temasek Holdings. Now, Guardant is looking to raise $100 million in an **I**nitial **P**ublic **O**ffering (**IPO**), a dollar amount that may change as terms are solidified.

The appeal of a liquid biopsy is obvious because you don't need to perform surgery, but there's a bit more to it than that. As Guardant describes it, they offer "breakthrough genomic cancer testing from a single blood draw". It's not just about detecting the presence of cancer but also determining what type of cancer is present so that personalized therapies can be administered. This sounds an awful lot like a company on Medicine.



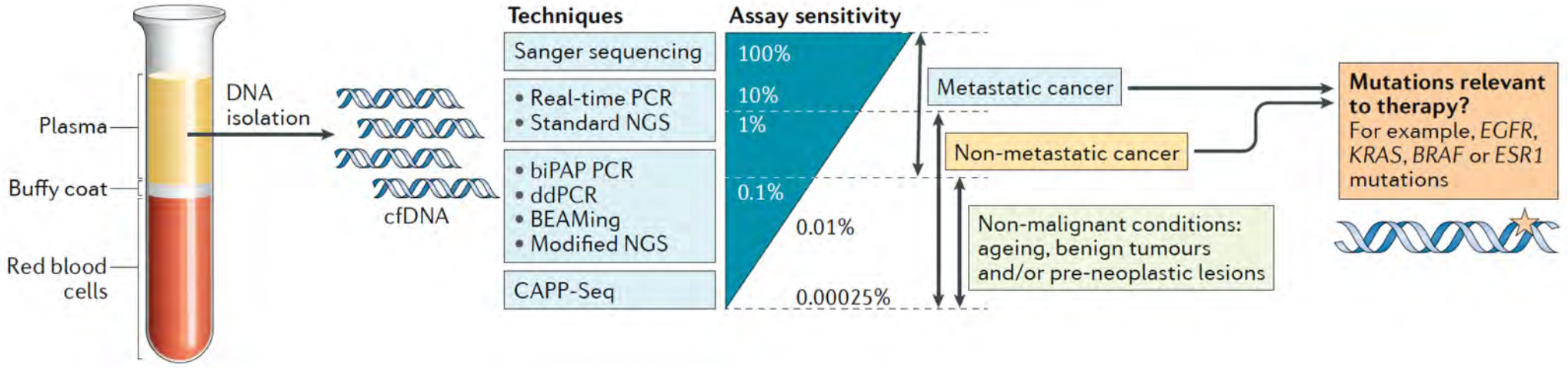
REVIEWS

Liquid biopsy and minimal residual disease — latest advances and implications for cure

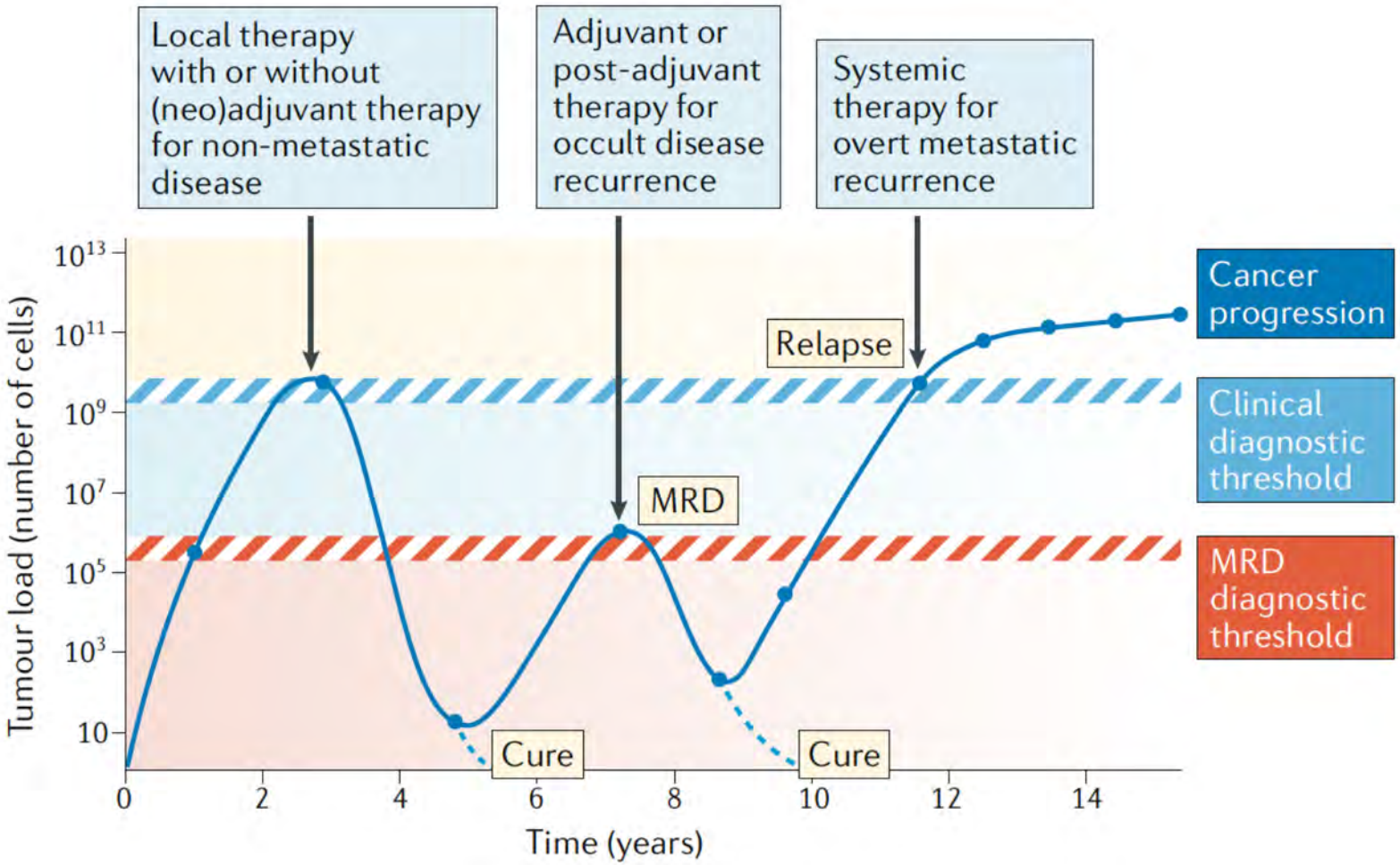
*Klaus Pantel[1] * and Catherine Alix-Panabières[2]*

Abstract | Liquid biopsy has been introduced as a new diagnostic concept predicated on the analysis of circulating tumour cells (CTCs) or circulating tumour-derived factors, in particular, cell-free tumour DNA (ctDNA). Highly sensitive liquid biopsy assays have been developed that can now be applied to detect and characterize minimal residual disease (MRD), which reflects the presence of tumour cells disseminated from the primary lesion to distant organs in patients who lack any clinical or radiological signs of metastasis or residual tumour cells left behind after local therapy that eventually lead to local recurrence. This application is the new frontier of liquid biopsy analyses, which are challenged by the very low concentrations of CTCs and ctDNA in blood samples. In this Review, we discuss the key technologies that can be used to detect and characterize CTCs in surveillance of MRD and provide a brief overview of similar roles of ctDNA analyses. We then focus on the current clinical data on the use of CTCs and ctDNA in the detection and monitoring of MRD and in obtaining information on therapeutic targets and resistance mechanisms relevant to the management of individual patients with cancer.

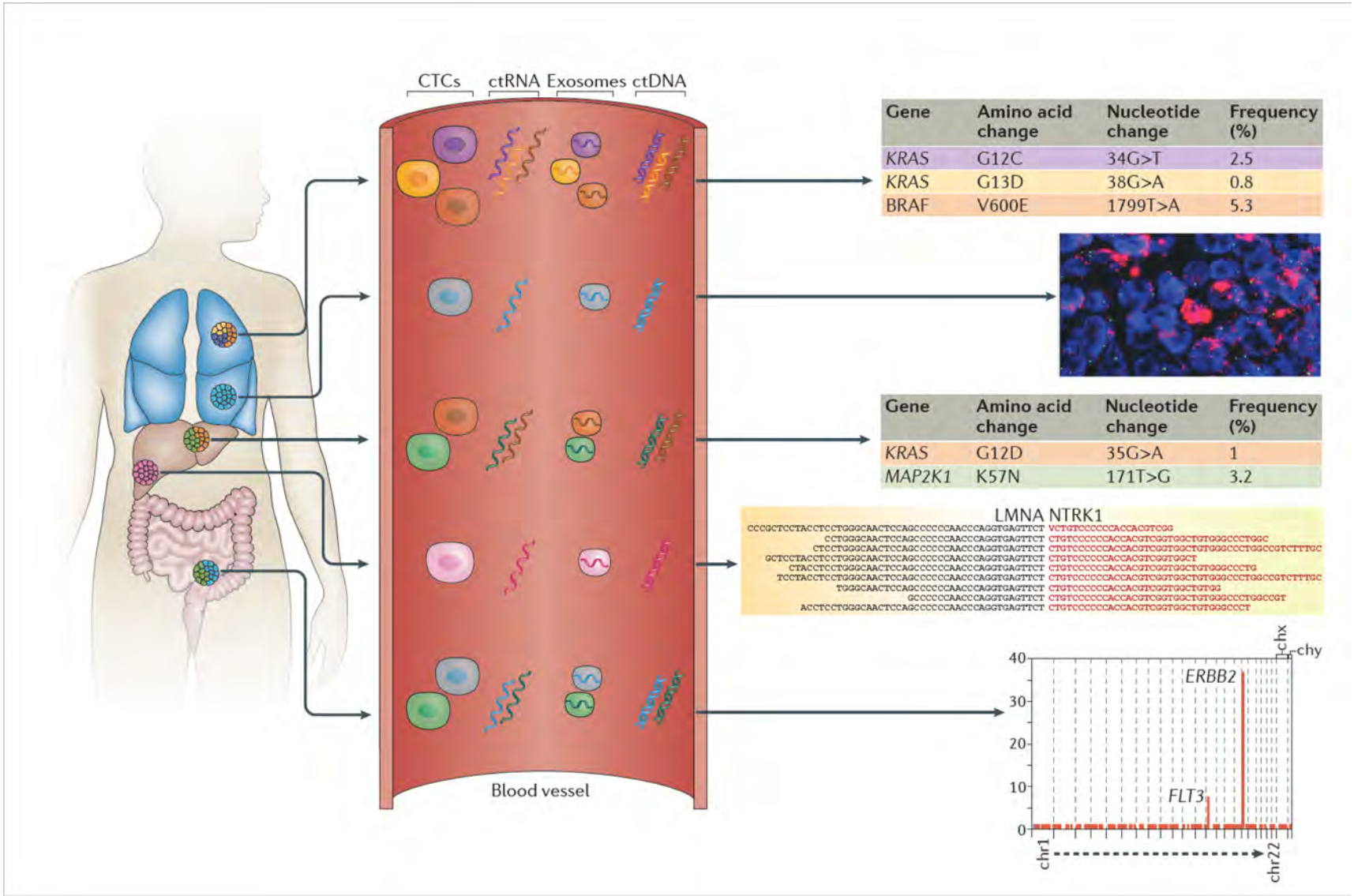
Pantel et al., Nature Rev Clin Onc, 2019



Pantel et al., Nature Rev Clin Onc, 2019



Pantel et al., Nature Rev Clin Onc, 2019



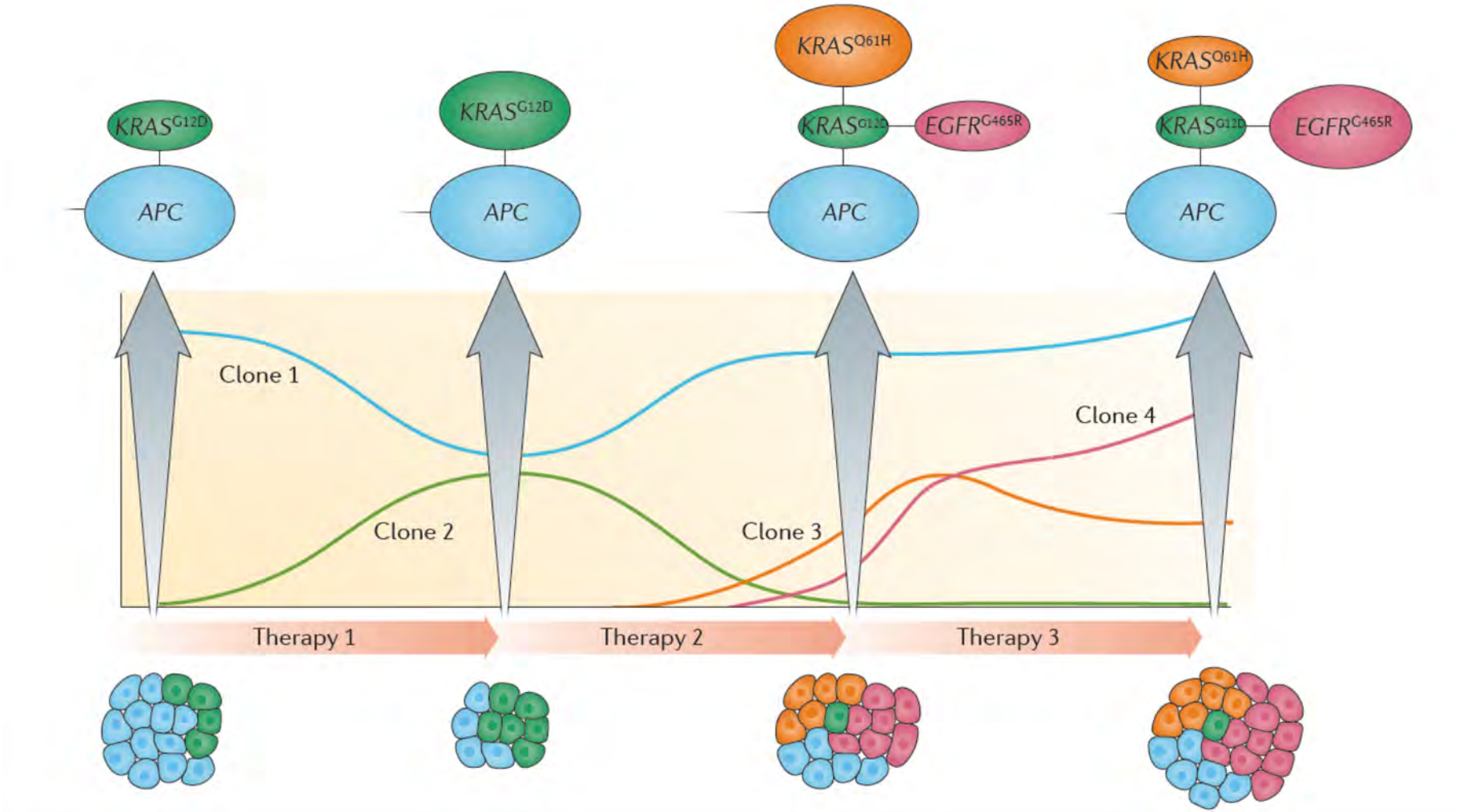
Siravegna et al., Nature Rev Clin Onc, 2017

Table 1 | **Comparison between the applications of ctDNA, CTCs, and exosomes**

	ctDNA/RNA	CTCs	Exosomes
Potential to fully recapitulate spatial and temporal tumour heterogeneity	Yes[3,4,164]	No	No
Assesment of pre/post-analytical variability	Yes[12,68]	Yes[201]	Yes[35]
Detection of somatic mutations, InDels, copy-number alterations and gene-fusions	Yes[1–3,7,10,11,60,64,71, 72,75,92,125,129–132,148, 149,151–156,158,189,202]	Yes[13,19,21]	Yes[203,204]
Evaluation of methylation patterns	Yes[137–142,145,146]	Yes[205]	Yes[206]
Analysis of mRNA/miRNA/lncRNA/RNA splice variants	Yes[45,49]	Yes[20]	Yes[40,43,46,51]
Analysis of RNA expression	No	Yes[19,207]	Yes[50,86]
Cell morphology and functional studies *ex vivo*	No	Yes[26–34]	No
Demonstration of signal colocalization	No	Yes[121]	No
Proteomics analysis	No	Yes[116–118]	Yes[50]

'Yes' indicates that the approach is feasible, possible, and/or published studies are available; 'No' indicates that the application is not feasible and/or no studies are available. CTCs, circulating tumour cells; ctDNA, circulating tumour DNA; InDels, DNA insertions and/or deletions; lncRNA, long noncoding RNA; mRNA, messenger RNA; miRNA, microRNA.

Siravegna et al., Nature Rev Clin Onc, 2017



Siravegna et al., Nature Rev Clin Onc, 2017

Liquid Biopsies: General Observations

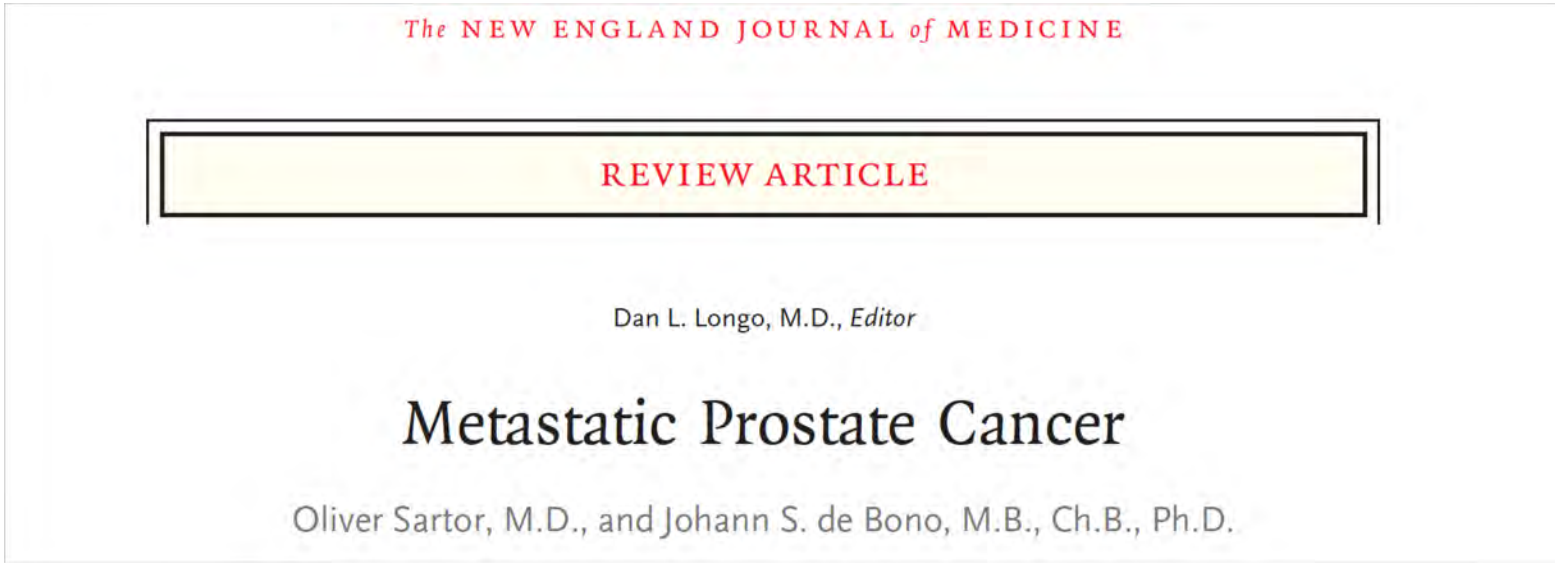
-Considered the future by many because of **feasibility** and **cost**
    Non-invasive
    Does not require metastatic biopsy
-Appealing for companies as this is a **recurring** test
-Works well to detect **point mutations** less well for clonality and copy number alterations
-Requires considerations for **analysis** that have not been standardized
-Few tests have been **FDA approved**

cfDNA applications in solid cancers is broad. This talk will focus on prostate cancer as a specific example

Focus on advanced prostate cancer

Will not cover molecular imagining (e.g., PSMA)

Slides available @ Rubinlab.unibe.ch *or*
@MarkARubin1



"The use of advanced genomic analysis is now feasible to a greater extent than ever before. Whether its use improves treatment decisions is not yet clear…advanced genetics and immunology, two major drivers of progress in oncology, are not routinely incorporated into the care of patients with prostate cancer."

NEJM 378;7 February 15, 2018 647

Advanced Prostate Cancer

5%, 10%, and 20%

5% have MSI or MMR alterations

Immunotherapy FDA

10% have germline DRM (e.g. BRCA)

PARPi or Platinum-based Tx/ Family implications

20% have DRM somatic-germline

PARPi or Platinum-based Tx

Definitions: What we count

Genetic Testing- counting germline sequence
Genomic Testing-counting tumor (somatic) seq context germline
Molecular Imagining-measuring protein expression

Numerous types of tests available for localized prostate cancer (e.g., Genomic Health, Myriad-CCP, Decipher, PCA3). These are usually predicting some outcome or assessing risk of disease progression.

Focus today will be on assessing advanced prostate cancer prognosis, and/or prediction

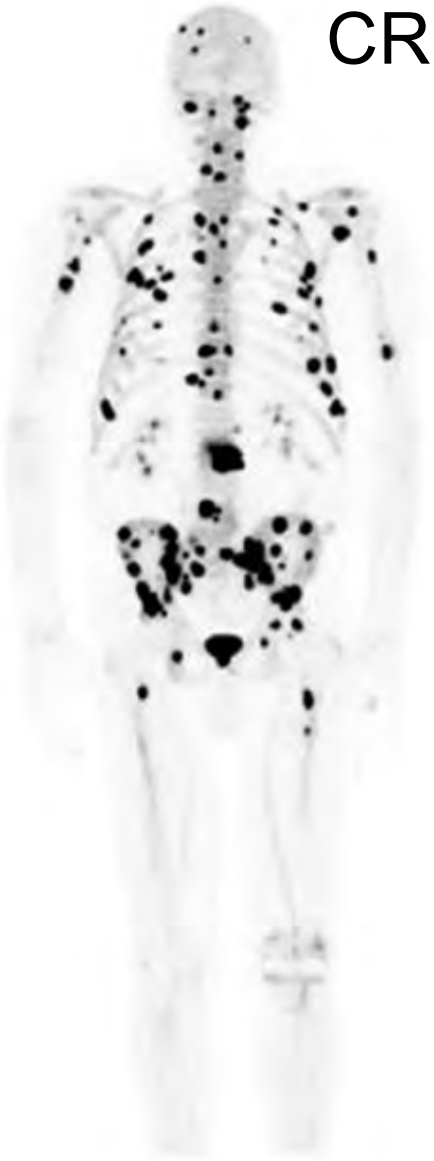
Definitions

A **prognostic biomarker** is one that indicates an increased (or decreased) likelihood of a future clinical event, disease recurrence or progression in an identified population. Prognostic biomarkers are measured at a defined baseline, which may include a background treatment

A **predictive biomarker** is used to identify individuals who are more likely to respond to exposure to a particular medical product or environmental agent. The response could be a symptomatic benefit, improved survival, or an adverse effect.

Given for lab tests (CLIA/CLEP):
Accuracy
Reproducibility
Sensitivity
Specificity

FDA-NIH **Biomarker** Working Group.
Silver Spring (MD): Food and Drug Administration (US);
Bethesda (MD): National Institutes of Health (US); 2016

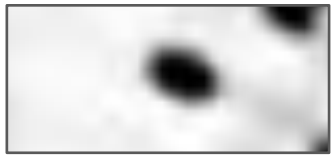


CRPC Patient and acquisition of samples for testing

Buccal sample

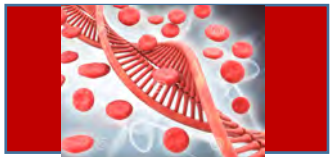
Germline DNA
Genetic testing (e.g., BRCA1/2)
Control normal sample for genomics

Tumor sample



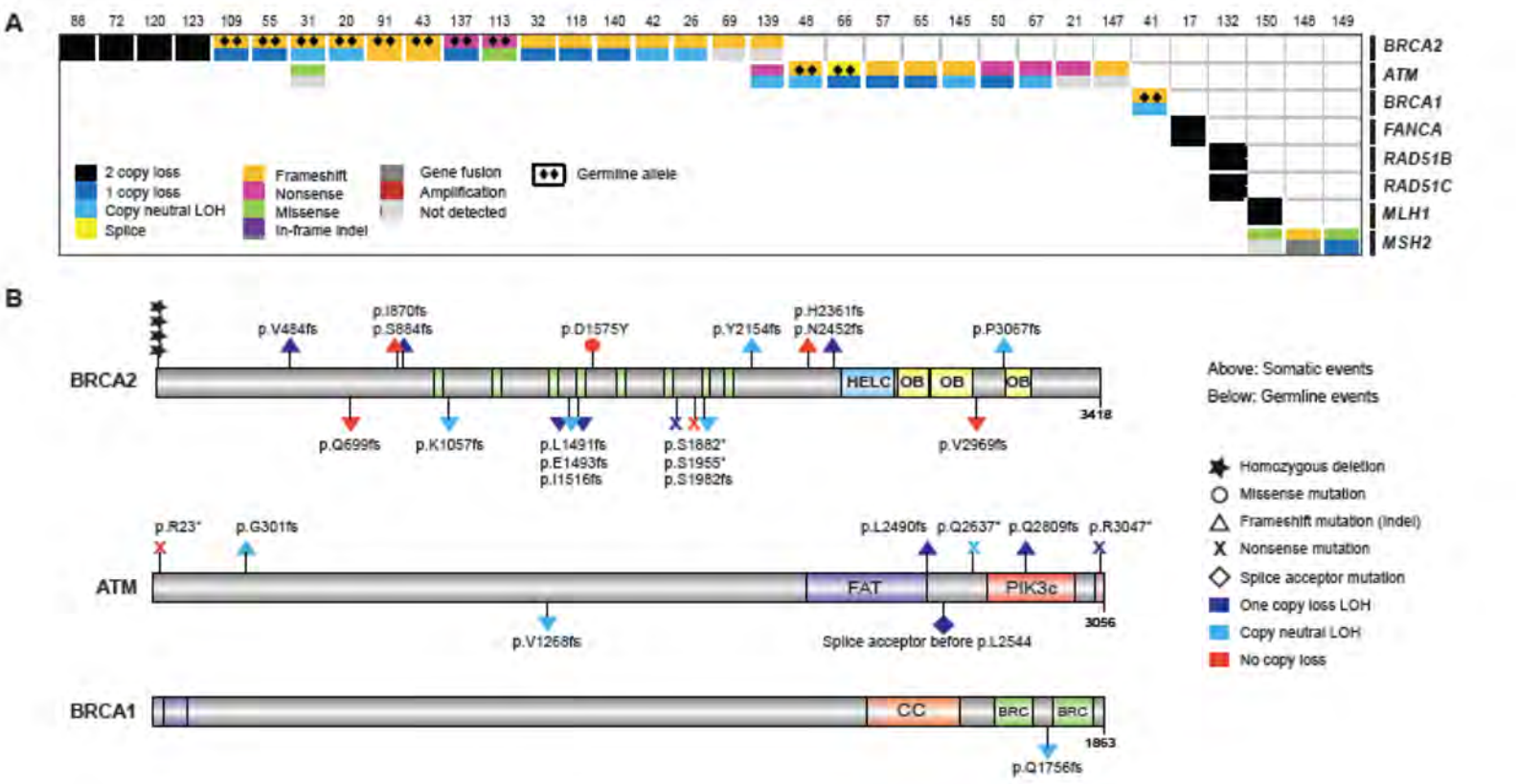
Tumor DNA/RNA/Protein
For genomic sequencing, transcriptomic sequencing, etc.

Blood sample



Tumor and normal DNA/RNA/Protein *fraction*
cfDNA, CTC, metabolites, etc.

Significant alterations in DNA repair genes



Robinson et al, Cell 2015

ELSEVIER

Check for updates

UROLOGIC ONCOLOGY

Urologic Oncology: Seminars and Original Investigations 36 (2018) 385–388

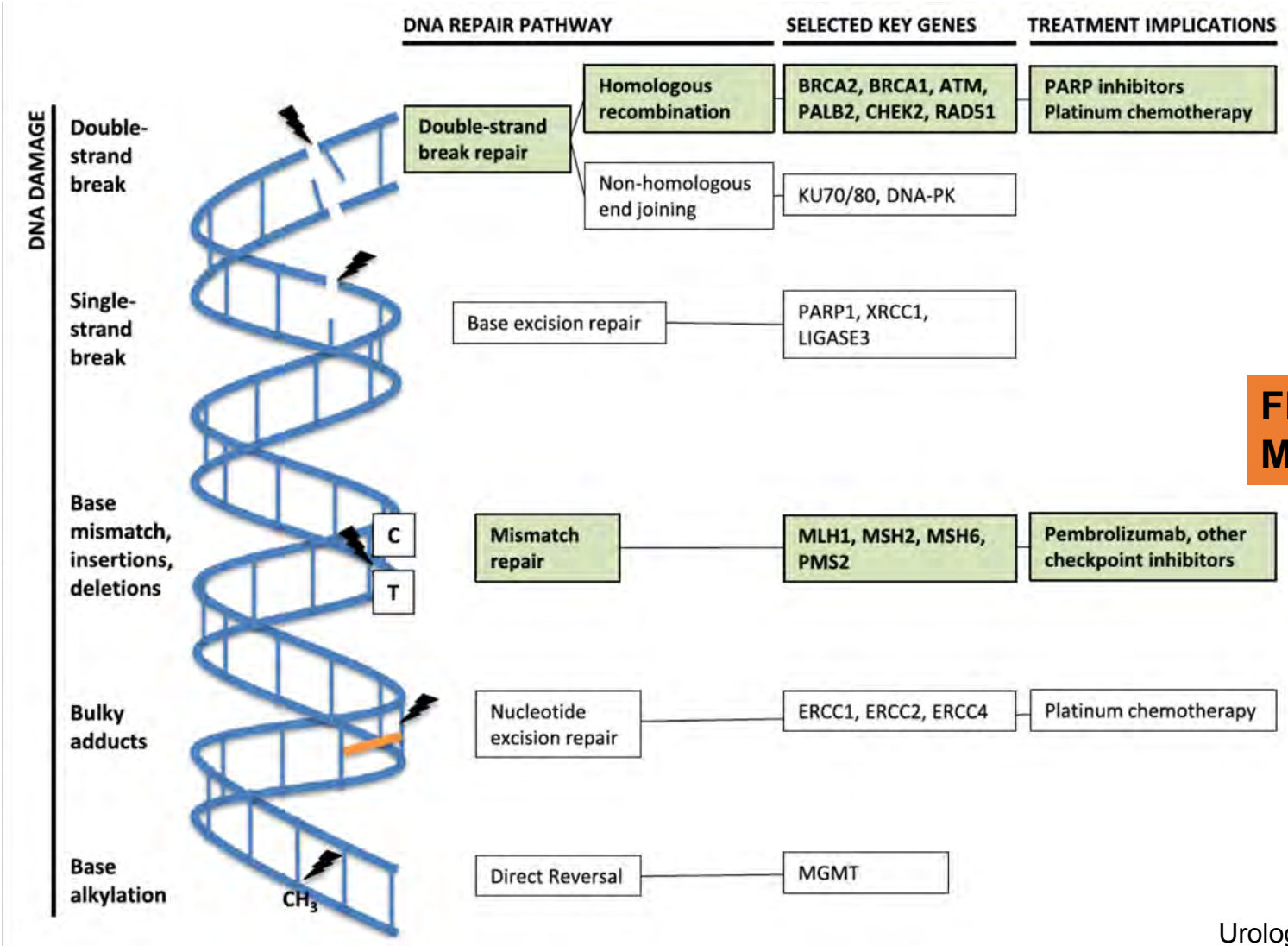
Seminars article

The resounding effect of DNA repair deficiency in prostate cancer

Heather H. Cheng, M.D., Ph.D.[a,b,*]

[a] *Division of Medical Oncology, University of Washington, Seattle, WA*
[b] *Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA*

Urologic Oncology: Sem. and Orig. Invest.
36(**2018**)385–388



20%

FDA (May 2017) approval for MSI and MMR deficiency

5%

Urologic Oncology: Sem. and Orig. Invest. 36(2018)385–388

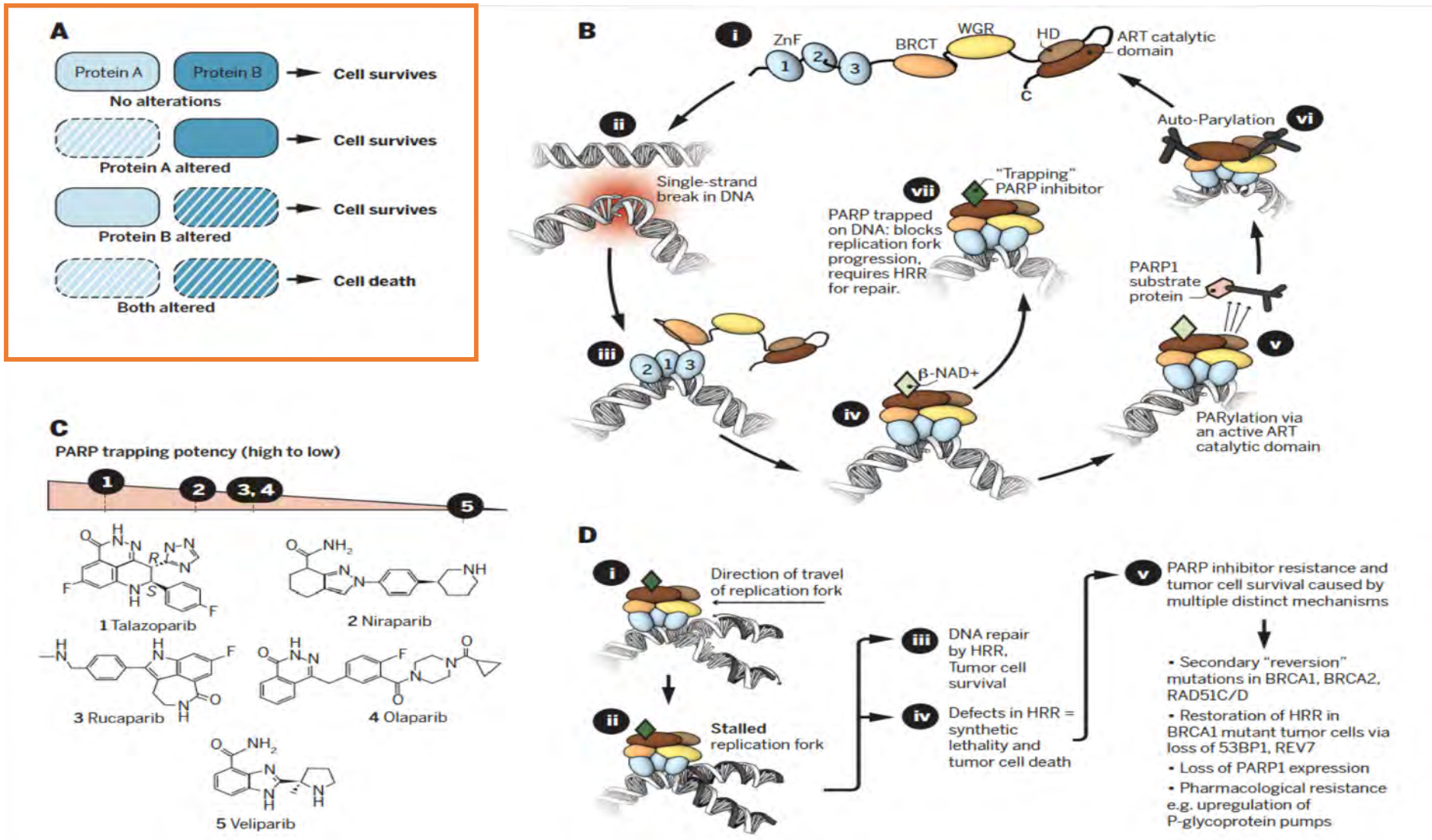
Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy

Hannah Farmer[1,2*], Nuala McCabe[1,2*], Christopher J. Lord[2*], Andrew N. J. Tutt[2,3], Damian A. Johnson[2], Tobias B. Richardson[2], Manuela Santarosa[2†], Krystyna J. Dillon[4], Ian Hickson[4], Charlotte Knights[4], Niall M. B. Martin[4], Stephen P. Jackson[4,5], Graeme C. M. Smith[4] & Alan Ashworth[1,2]

[1]*Cancer Research UK Gene Function and Regulation Group and* [2]*The Breakthrough Breast Cancer Research Centre Institute of Cancer Research, Fulham Road, London SW3 6JB, UK*
[3]*Guy's Hospital, St Thomas' Street, London SE1 9RT, UK*
[4]*KuDOS Pharmaceuticals Ltd, Cambridge Science Park, Cambridge CB4 0WG, UK*
[5]*Wellcome Trust and Cancer Research UK, Gurdon Institute of Cancer and Developmental Biology, and Department of Zoology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK*

* These authors contributed equally to this work
† Present address: Division of Experimental Oncology1, CRO-IRCCS, Aviano 33081 PN, Italy

NATURE |VOL 434 | 14 APRIL 2005



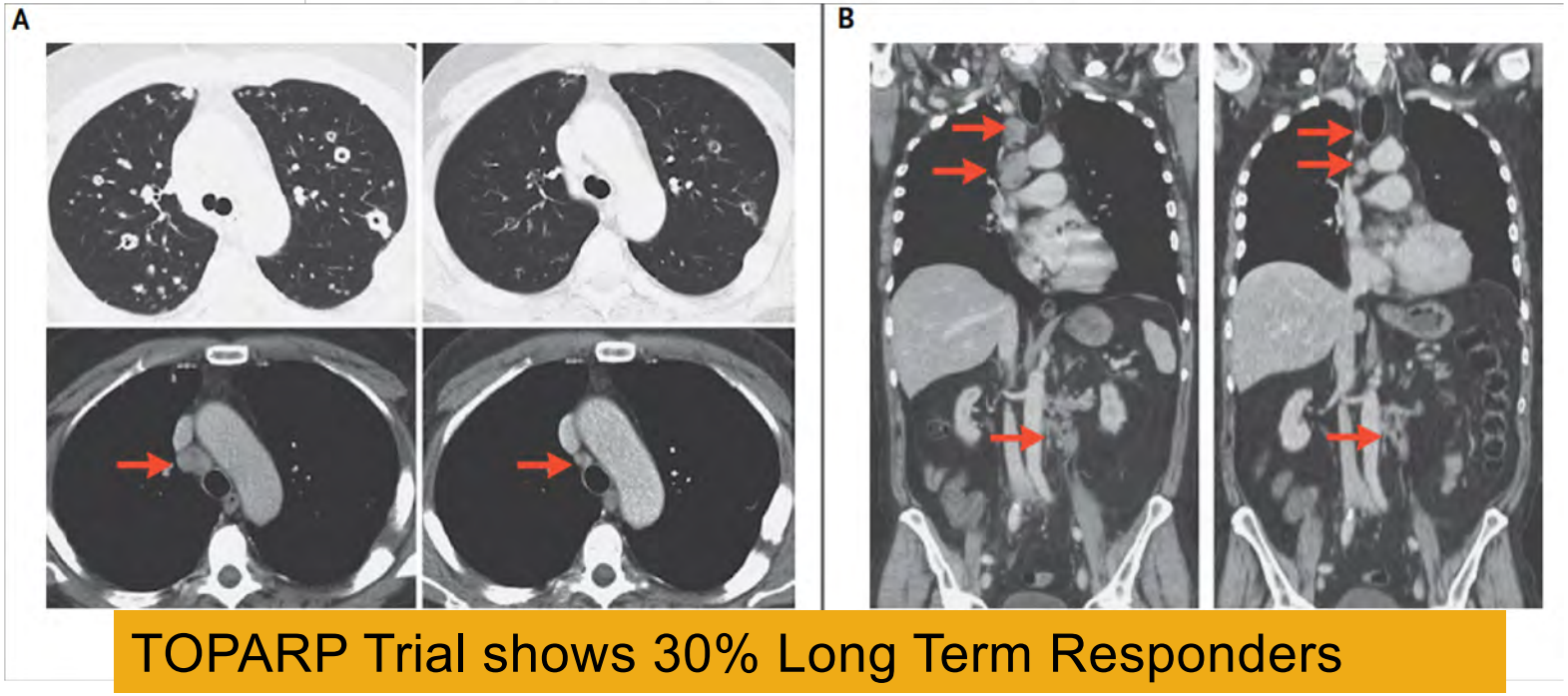
Lord and Ashworth, Science 355, 1152–1158 (2017)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 29, 2015 VOL. 373 NO. 18

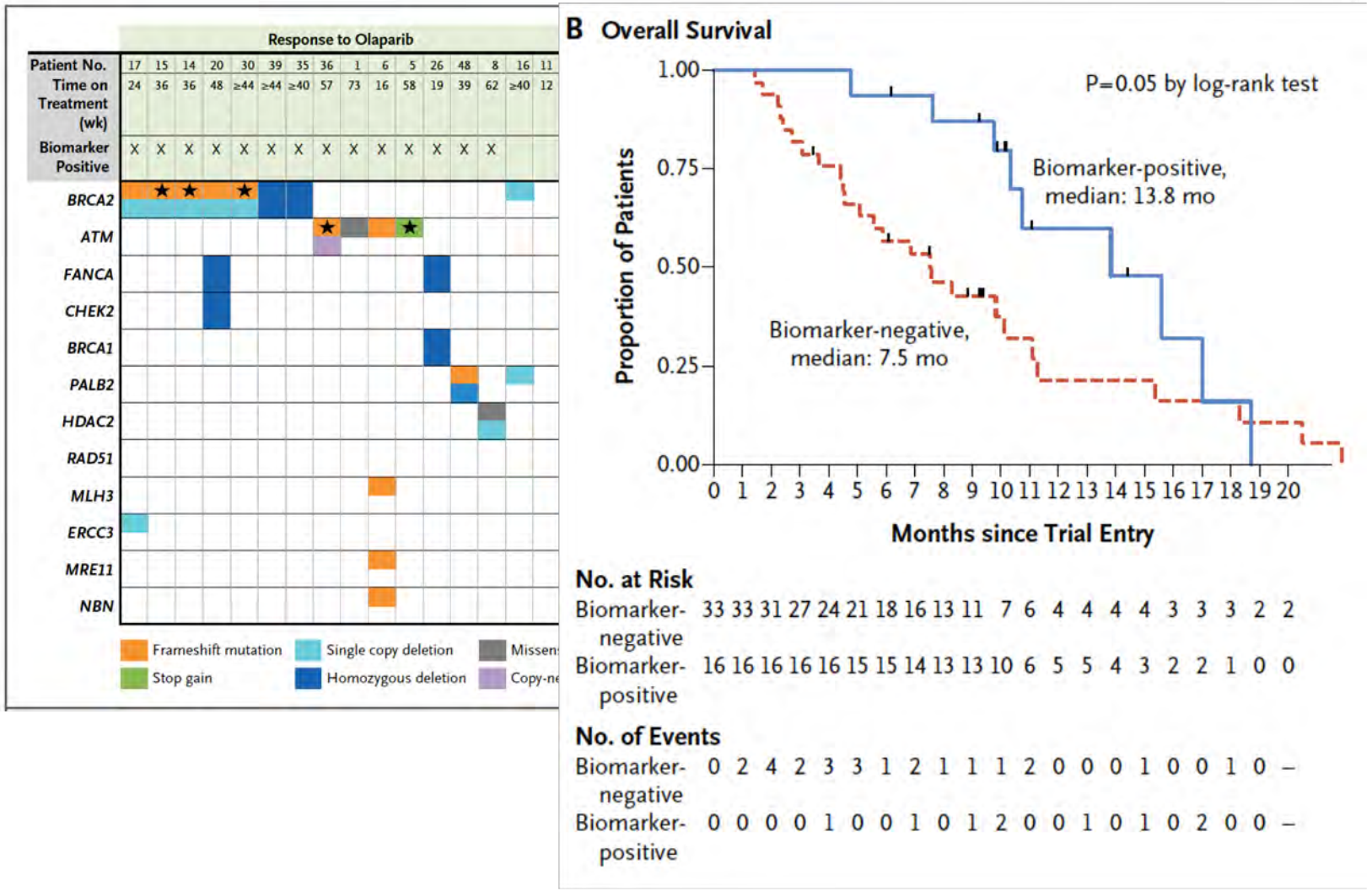
DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer

J. Mateo, S. Carreira, S. Sandhu, S. Miranda, H. Mossop, R. Perez-Lopez, D. Nava Rodrigues, D. Robinson, A. Omlin, N. Tunariu, G. Boysen, N. Porta, P. Flohr, A. Gillman, I. Figueiredo, C. Paulding, G. Seed, S. Jain, C. Ralph, A. Protheroe, S. Hussain, R. Jones, T. Elliott, U. McGovern, D. Bianchini, J. Goodall, Z. Zafeiriou, C.T. Williamson, R. Ferraldeschi, R. Riisnaes, B. Ebbs, G. Fowler, D. Roda, W. Yuan, Y.-M. Wu, X. Cao, R. Brough, H. Pemberton, R. A'Hern, A. Swain, L.P. Kunju, R. Eeles, G. Attard, C.J. Lord, A. Ashworth, M.A. Rubin, K.E. Knudsen, F.Y. Feng, A.M. Chinnaiyan, E. Hall, and J.S. de Bono



TOPARP Trial shows 30% Long Term Responders

NEJM, Oct 29 2015


Response to Olaparib
Patient No. 17 15 14 20 30 39 35 36 1 6 5 26 48 8 16 11
Time on Treatment (wk) 24 36 36 48 ≥44 ≥44 ≥40 57 73 16 58 19 39 62 ≥40 12
Biomarker Positive X X X X X X X X X X X X X X
BRCA2
ATM
FANCA
CHEK2
BRCA1
PALB2
HDAC2
RAD51
MLH3
ERCC3
MRE11
NBN
Frameshift mutation
Stop gain
Single copy deletion
Homozygous deletion
B Overall Survival
P=0.05 by log-rank test
Biomarker-positive, median: 13.8 mo
Biomarker-negative, median: 7.5 mo
Proportion of Patients
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Months since Trial Entry
No. at Risk
Biomarker-negative 33 33 31 27 24 21 18 16 13 11 7 6 4 4 4 4 3 3 3 2 2
Biomarker-positive 16 16 16 16 16 15 15 14 13 13 10 6 5 5 4 3 2 2 1 0 0
No. of Events
Biomarker-negative 0 2 4 2 3 3 1 2 1 1 1 2 0 0 0 1 0 0 1 0 –
Biomarker-positive 0 0 0 0 1 0 0 1 0 1 2 0 0 1 0 1 0 2 0 0 –

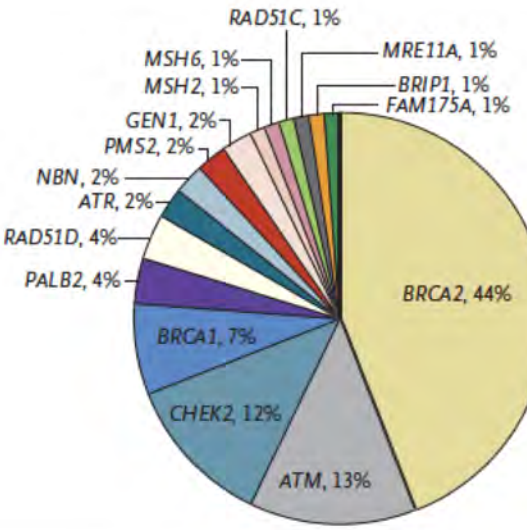
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer

Table 2. Germline Mutations in Metastatic Cases as Compared with the General Population and Primary Cases.

Gene	Metastatic Prostate Cancer (N=692)*	Exome Aggregation Consortium (N=53,105)†	TCGA Cohort with Primary Prostate Cancer (N=499)	Metastatic Prostate Cancer vs. Exome Aggregation Consortium		Metastatic Prostate Cancer vs. TCGA Cohort	
	No. of Mutations (% of Men)			Relative Risk (95% CI)	P Value	Relative Risk (95% CI)	P Value
ATM	11 (1.59)	133 (0.25)	5 (1.00)	6.3 (3.2–11.3)	<0.001	1.6 (0.8–2.8)	0.12
ATR	2 (0.29)	43 (0.08)	0	3.6 (0.4–12.8)	0.11	—	—
BAP1‡	0	1	0	—	—	—	—
BARD1‡	0	38 (0.07)	1 (0.20)	—	—	—	—
BRCA1	6 (0.87)	104 (0.22)	3 (0.60)	3.9 (1.4–8.5)	0.005	1.4 (0.5–3.1)	0.32
BRCA2	37 (5.35)	153 (0.29)	1 (0.20)	18.6 (13.2–25.3)	<0.001	26.7 (18.9–36.4)	<0.001
BRIP1‡	1 (0.18)	100 (0.19)	1 (0.20)	0.9 (0.02–5.3)	1.0	0.9 (0.0–4.9)	1.0
CHEK2‡	10 (1.87)	314 (0.61)	2 (0.40)	3.1 (1.5–5.6)	0.002	4.7 (2.2–8.5)	<0.001
FAM175A‡	1 (0.18)	52 (0.10)	0	1.8 (0.05–10.1)	0.42	—	—
GEN1‡	2 (0.46)	42 (0.08)	0	5.8 (0.7–20.8)	0.048	—	—
MLH1	0	11 (0.02)	0	—	—	—	—
MRE11A	1 (0.14)	36 (0.07)	1 (0.20)	2.1 (0.1–11.8)	0.38	0.7 (0.0–4.0)	1.0
MSH2	1 (0.14)	23 (0.04)	1 (0.20)	3.3 (0.1–18.5)	0.26	0.7 (0.0–4.0)	1.0
MSH6	1 (0.14)	41 (0.08)	1 (0.20)	1.9 (0.05–10.4)	0.41	0.7 (0.0–4.0)	1.0
NBN	2 (0.29)	61 (0.11)	1 (0.20)	2.5 (0.3–9.1)	0.19	1.4 (0.2–5.2)	0.40
PALB2	3 (0.43)	65 (0.12)	2 (0.40)	3.5 (0.7–10.3)	0.05	1.1 (0.2–3.1)	0.76
PMS2	2 (0.29)	56 (0.11)	1 (0.20)	2.7 (0.3–9.8)	0.17	1.4 (0.2–5.2)	0.40
RAD51C	1 (0.14)	59 (0.11)	2 (0.40)	1.3 (0.03–7.2)	0.54	0.4 (0.0–2.0)	0.54
RAD51D	3 (0.43)	40 (0.08)	1 (0.20)	5.7 (1.2–16.7)	0.02	2.2 (0.4–6.3)	0.16
XRCC2	0	23 (0.04)	0	—	—	—	—



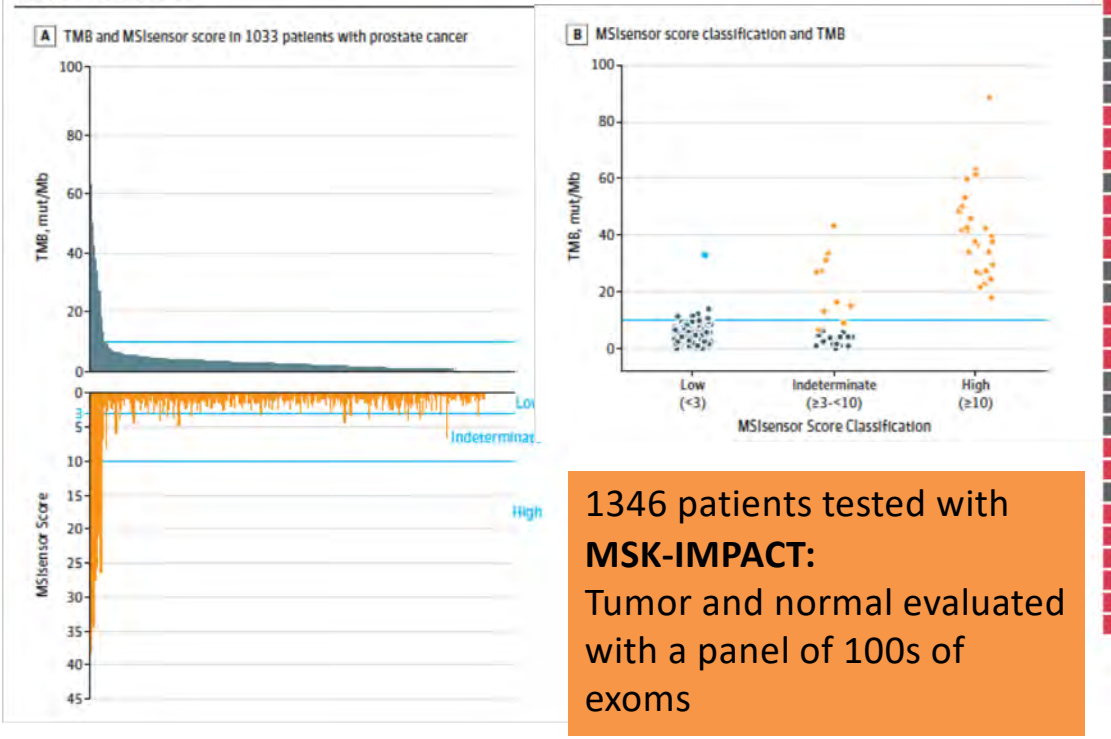
Selected *DNA repair germline mutations from targeted panel and WES reveal 10-20% frequency* (Pritchard and Nelson, 2016)

JAMA Oncology | Original Investigation

Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade

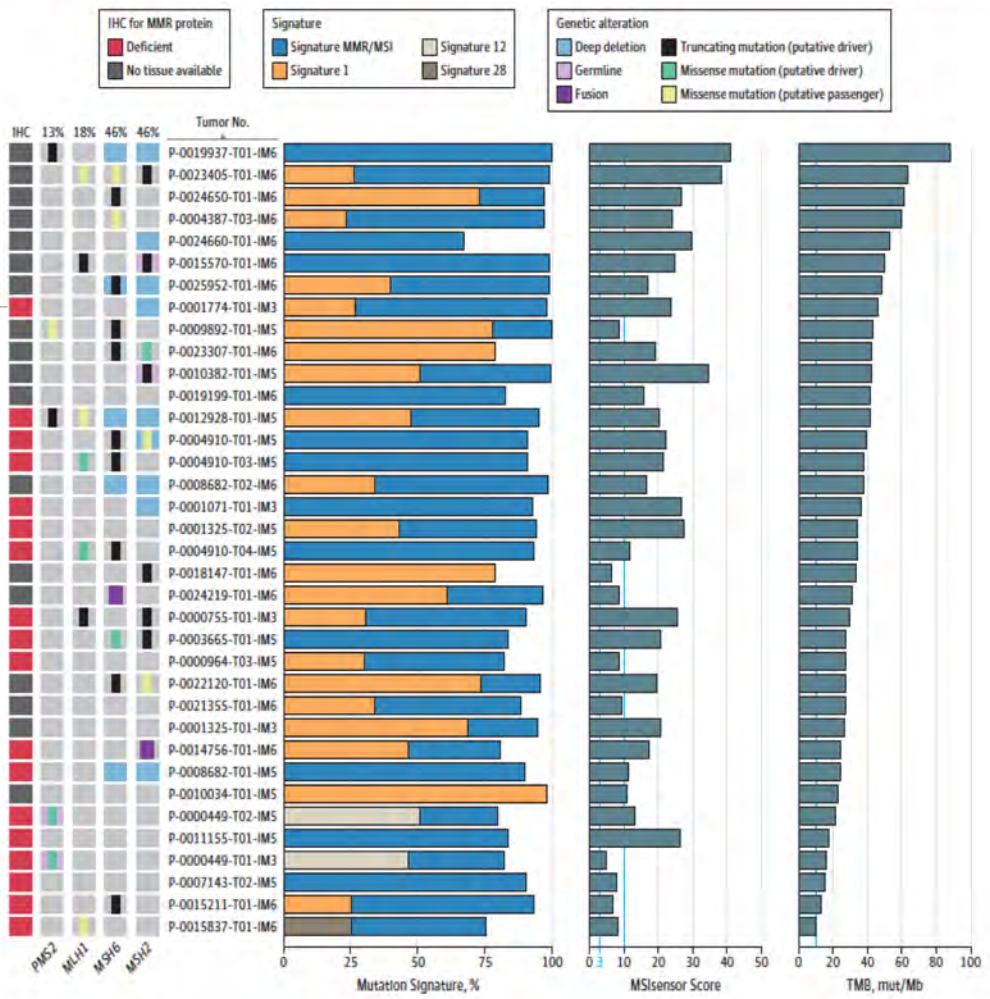
Wassim Abida, MD, PhD; Michael L. Cheng, MD; Joshua Armenia, PhD; Sumit Middha, PhD; Karen A. Autio, MD; Hebert Alberto Vargas, MD; Dana Rathkopf, MD; Michael J. Morris, MD; Daniel C. Danila, MD; Susan F. Slovin, MD, PhD; Emily Carbone, BA; Ethan S. Barnett, MS; Melanie Hullings, BA; Jaclyn F. Hechtman, MD; Ahmet Zehir, PhD; Jinru Shia, MD; Philip Jonsson, PhD; Zsofia K. Stadler, MD; Preethi Srinivasan, BA; Vincent P. Laudone, MD; Victor Reuter, MD; Jedd D. Wolchok, MD, PhD; Nicholas D. Socci, PhD; Barry S. Taylor, PhD; Michael F. Berger, PhD; Philip W. Kantoff, MD; Charles L. Sawyers, MD; Nikolaus Schultz, PhD; David B. Solit, MD; Anuradha Gopalan, MD; Howard I. Scher, MD

Figure 1. Tumor Mutation Burden (TMB) and Microsatellite Instability (MSI) in Prostate Cancer



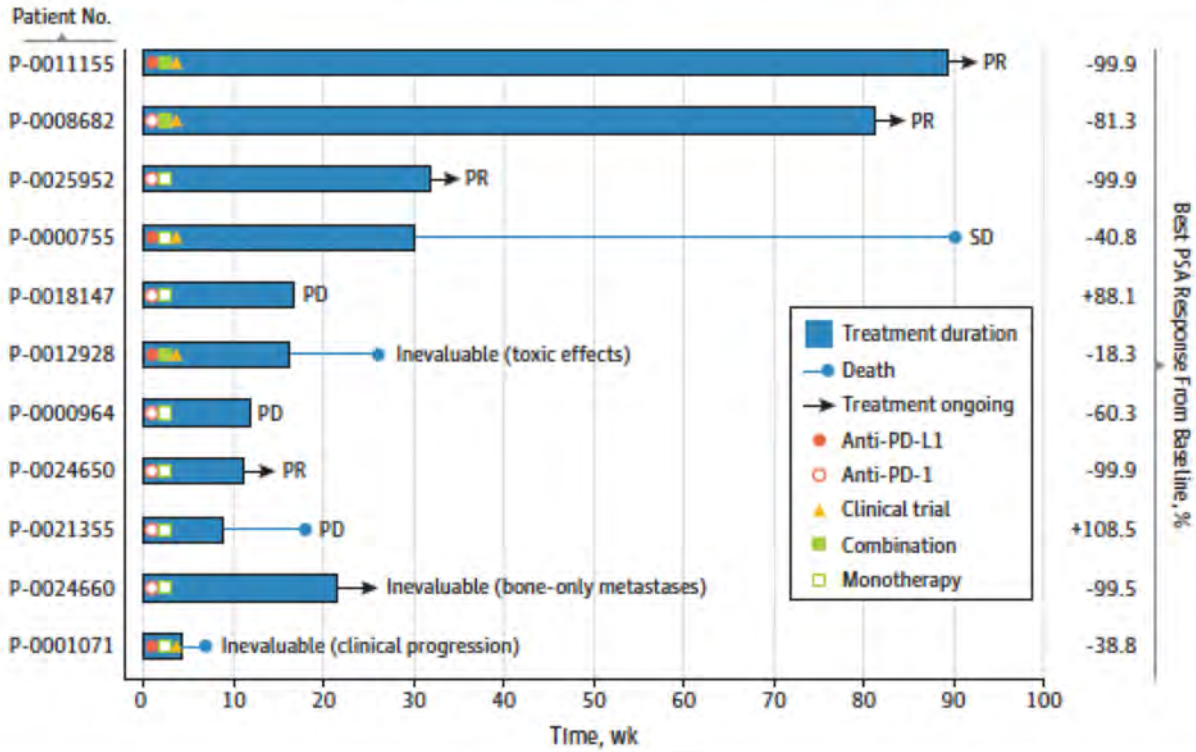
1346 patients tested with **MSK-IMPACT:**
Tumor and normal evaluated with a panel of 100s of exoms

Figure 2. Integrative Analysis of Microsatellite Instability (MSI), Tumor Mutation Burden (TMB), Mutational Signature Decomposition, and Mismatch Repair (MMR) Gene and Protein Status

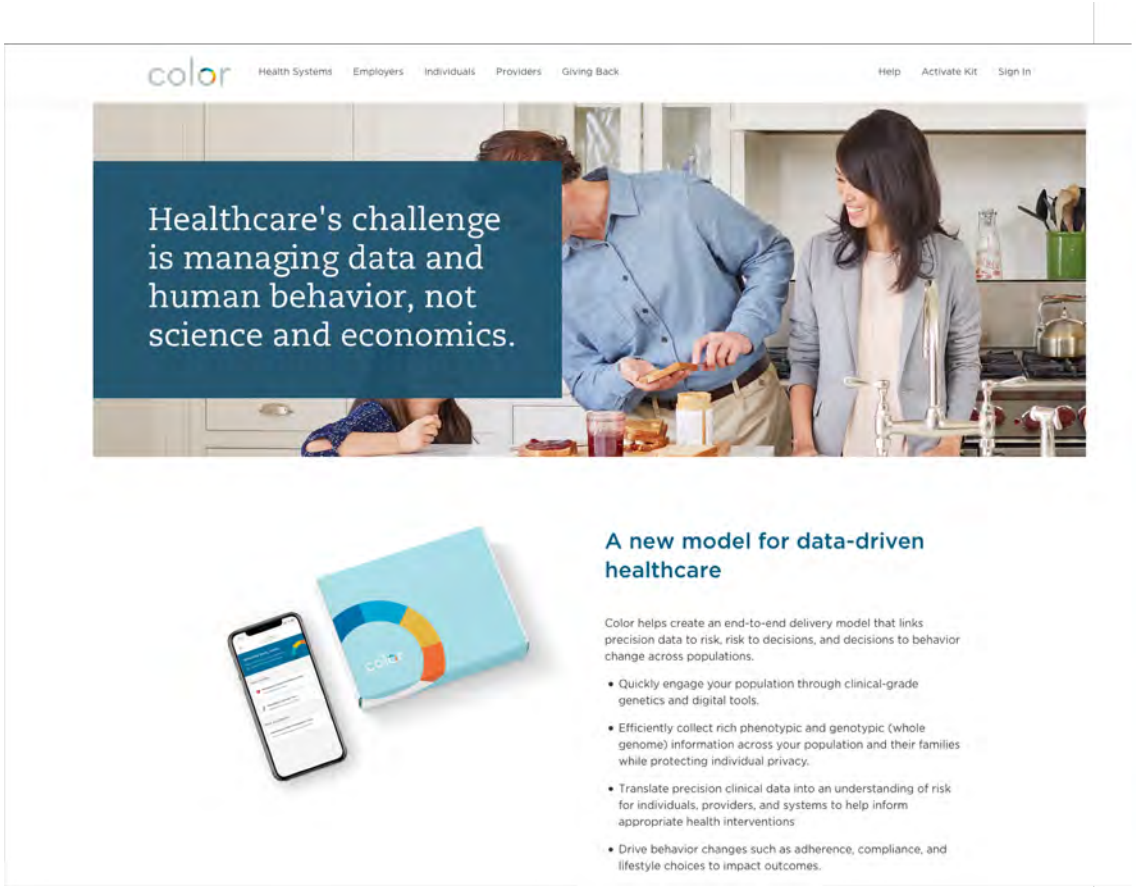


JAMA Oncology Published online December 27, 2018

Figure 4. Responses to Immune Checkpoint Blockade in Microsatellite Instability–High and Mismatch Repair Deficient (MSI-H/dMMR) Prostate Cancer



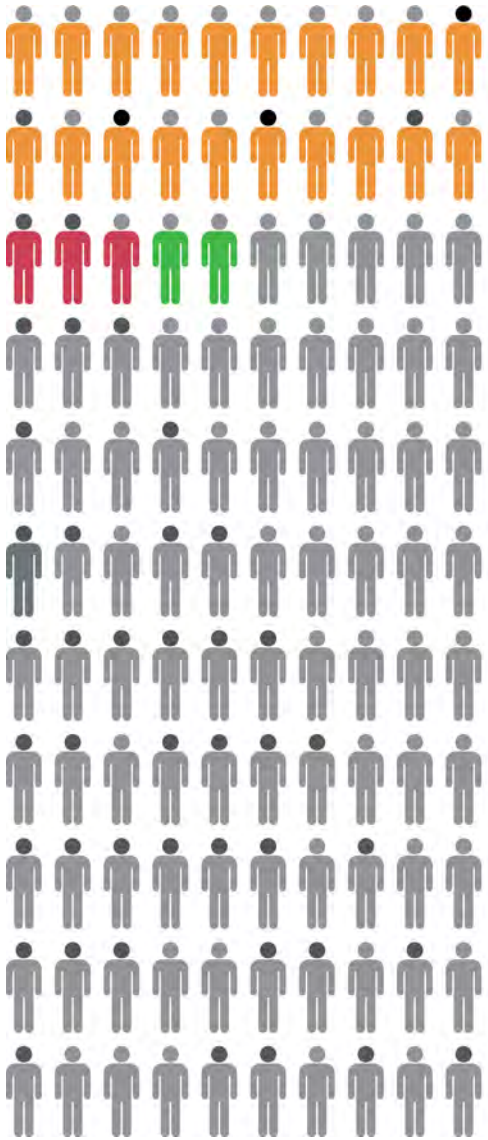
JAMA Oncology Published online December 27, 2018



Color Extended: The most relevant genes for common hereditary cancers

Gene	Breast	Ovarian	Uterine	Colorectal	Melanoma	Pancreatic	Stomach†	Prostate*
BRCA1	•	•				•		•
BRCA2	•	•			•	•		•
MLH1		•	•	•		•	•	•
MSH2		•	•	•		•	•	•
MSH6		•	•	•			•	•
PMS2***		•	•	•				•
EPCAM**		•	•	•		•	•	•
APC				•		•	•	
MUTYH				•				
MITF**					•			
BAP1					•			
CDKN2A					•	•		
CDK4**					•			
TP53	•	•	•	•	•	•	•	•
PTEN	•		•	•	•			
STK11	•	•	•	•		•	•	
CDH1	•						•	
BMPR1A				•		•	•	
SMAD4				•		•	•	
GREM1**				•				
POLD1**				•				
POLE**				•				
PALB2	•	•				•		
CHEK2	•			•				•
ATM	•					•		•
NBN	•							•
BARD1	•							
BRIP1	•	•						
RAD51C		•						
RAD51D		•						

Many tests available – need test that is designed to address clinically relevant alterations. For advanced PCa, combining somatic and germline will be critical



DNA Repair (BRCA1/2, ATM, etc.) 20%

MMR /MSI 5%

The remaining 75%

Overview of Tests that are Ready/Promising*

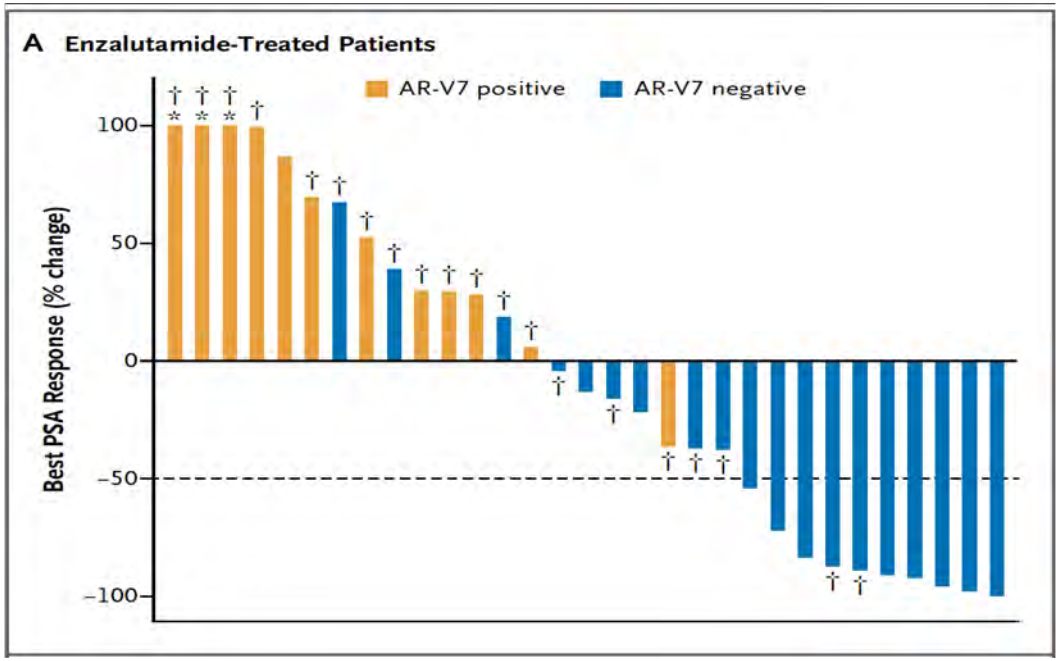
a. **MSI testing**
b. **DNA repair status** ("BRCAness"-assay for BRCA1/2/ATM,PALB2) for mutation/loss or HR signature useful for for platinum therapy or PARPi
c. **Loss of AR** lack of response to AR therapy (AR-V7, mutations)
d. **cfDNA** amount associated with prognosis
e. **PTEN loss** - possibly response to AKT inhibitor (de Bono CCR 2018)
f. **CDK12 loss** - possibly response to checkpoint blockade
g. **Loss of TP53/RB1** - short duration of response to AR-therapy--possibly predictive response to platinum
h. **CTC heterogeneity** ("clusters") response to docetaxel vs AR therapy
i. **Pathology** phenotype for NEPC response to platinum
j. **Double negative (AR- and NE-)** response to FGFRi
k. **PSMA expression response** to PSMA-drug therapies
l. **DLL3** expression response to chemoconjugate

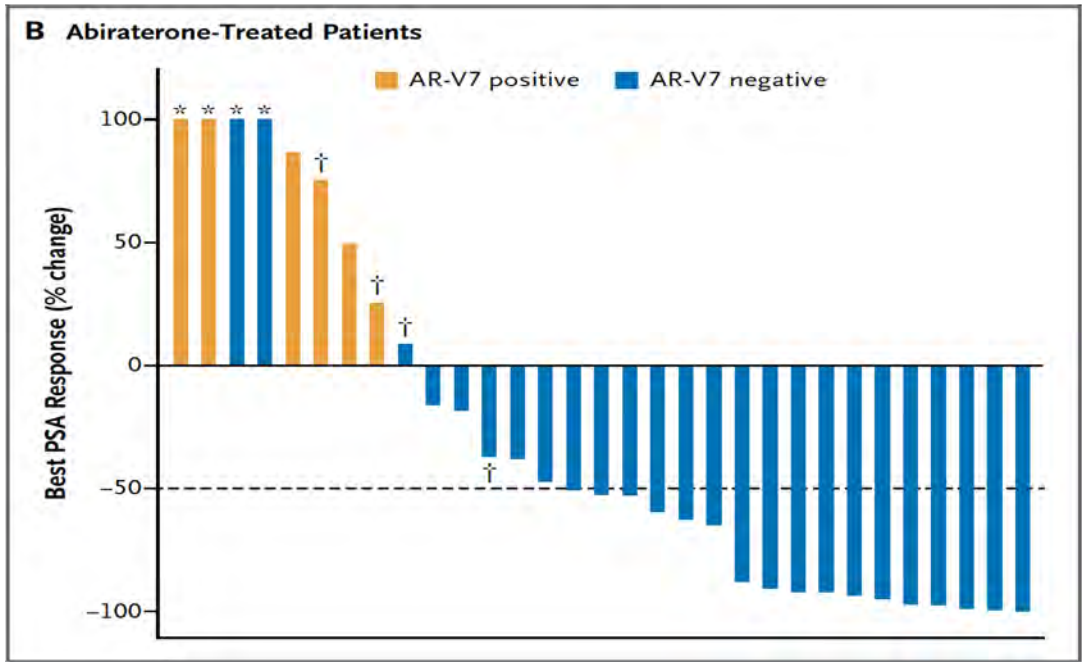
*Thanks Pete Nelson
Always comprehensive!

The NEW ENGLAND JOURNAL of MEDICINE

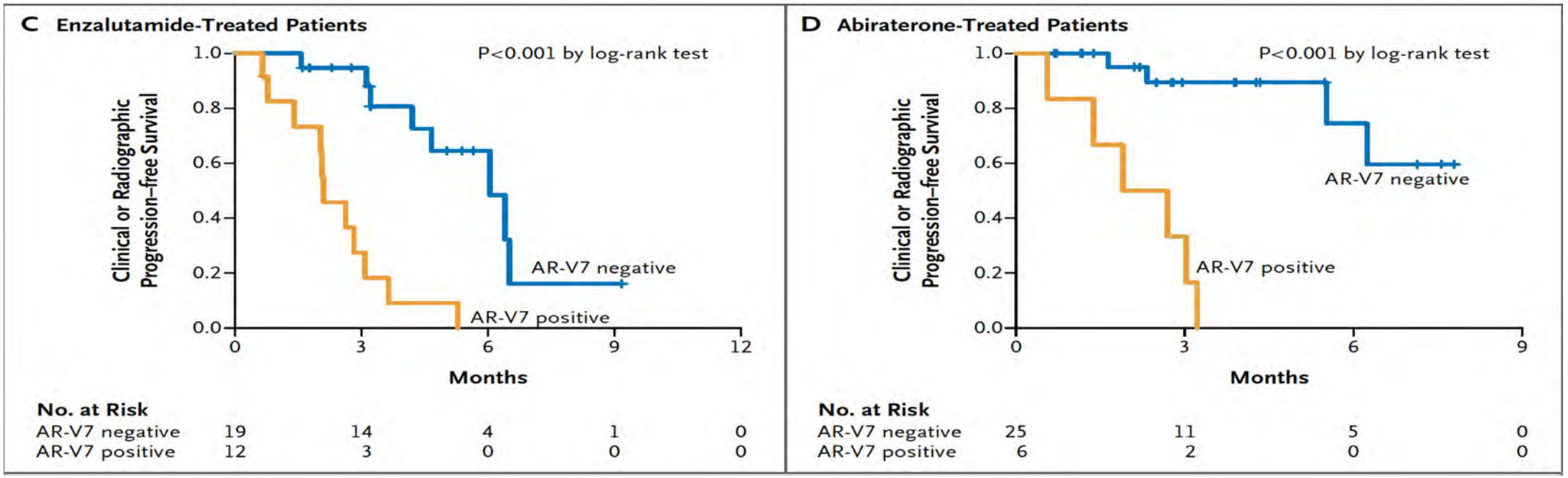
ORIGINAL ARTICLE

AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer

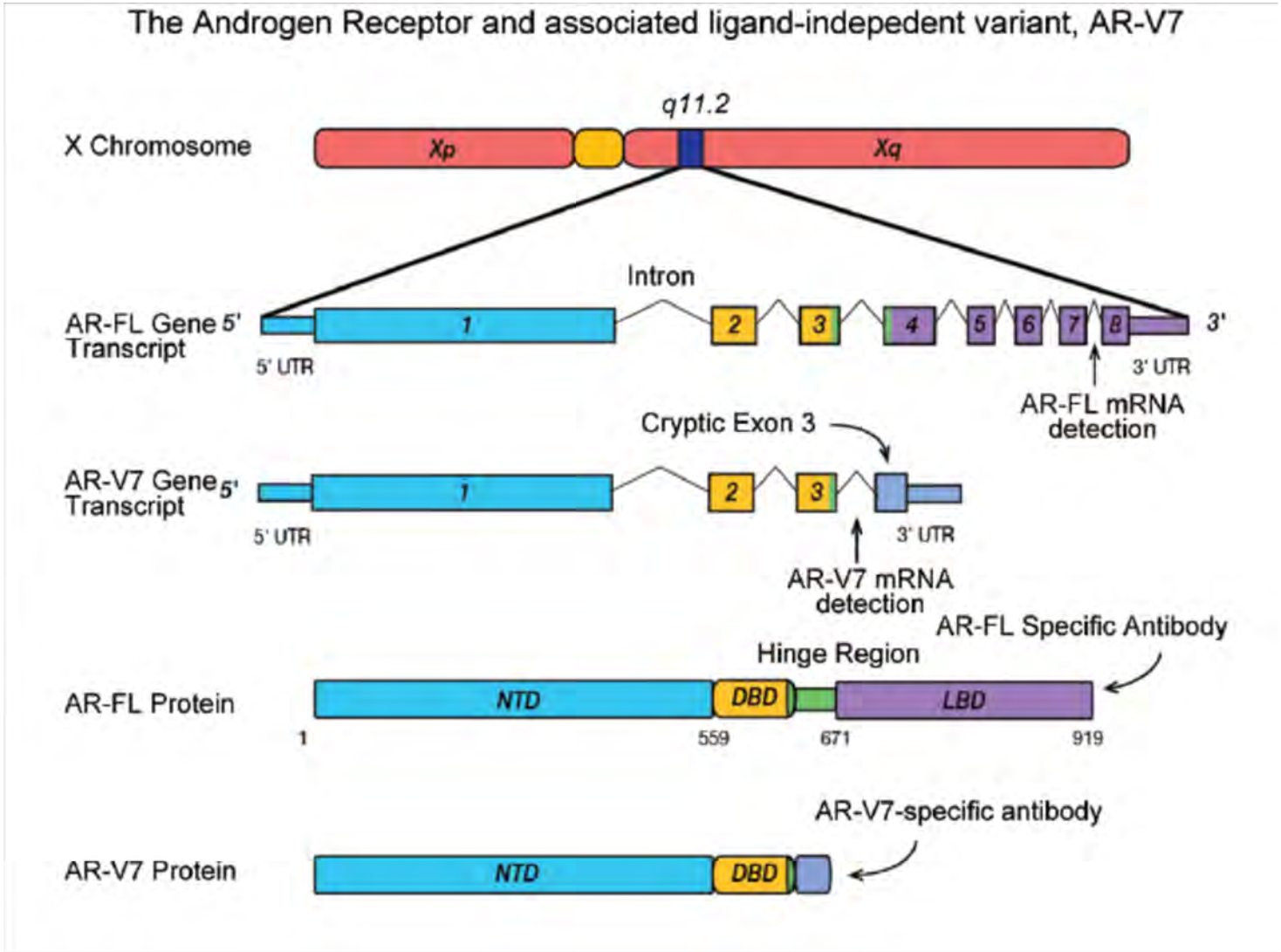




Antonarakis ES et al, NEJM 2014



Antonarakis ES et al, NEJM 2014

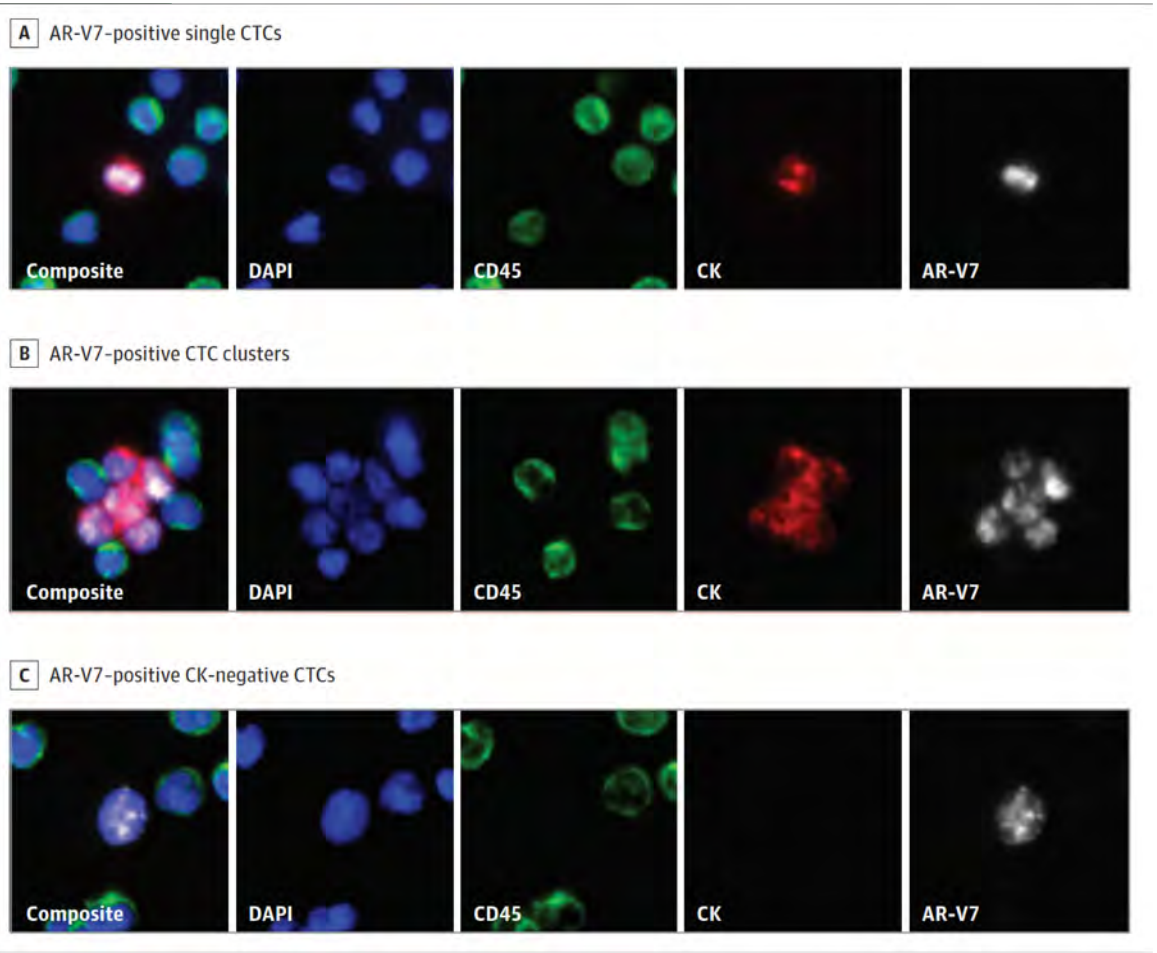

The Androgen Receptor and associated ligand-indepedent variant, AR-V7
q11.2
X Chromosome
Xp
Xq
Intron
AR-FL Gene Transcript
5'
3'
1
2
3
4
5
6
7
8
5' UTR
3' UTR
AR-FL mRNA detection
Cryptic Exon 3
AR-V7 Gene Transcript
5'
1
2
3
5' UTR
3' UTR
AR-V7 mRNA detection
AR-FL Specific Antibody
Hinge Region
AR-FL Protein
NTD
DBD
LBD
1
559
671
919
AR-V7-specific antibody
AR-V7 Protein
NTD
DBD

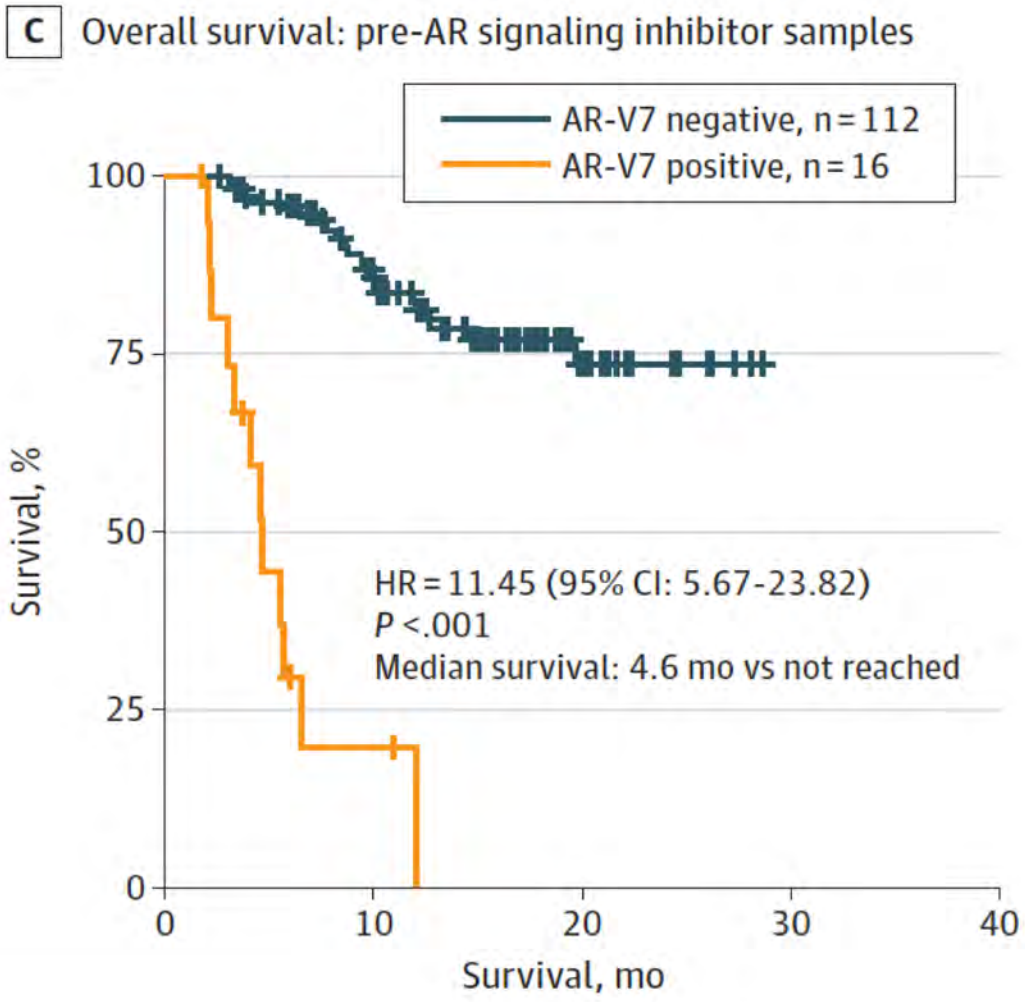
Research

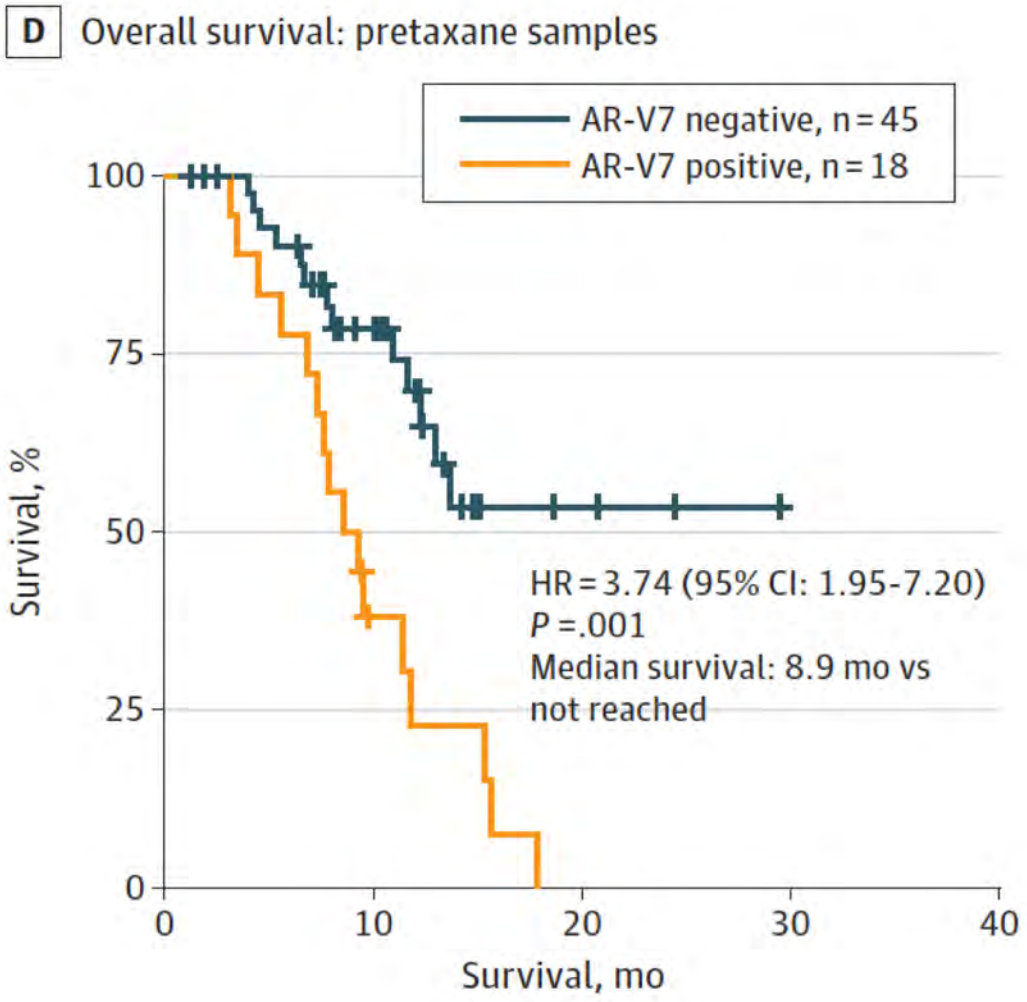
JAMA Oncology | Original Investigation

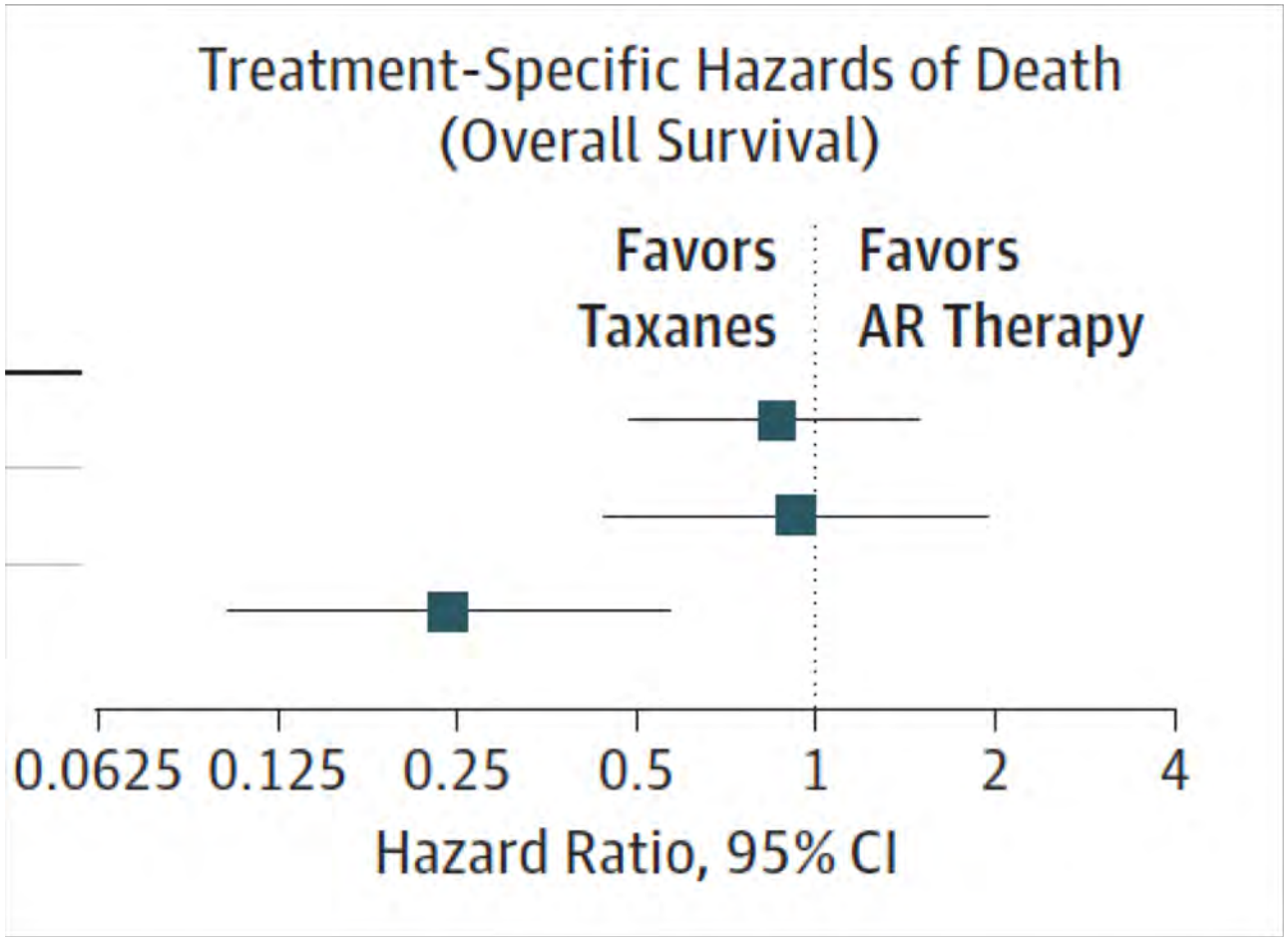
Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer

Howard I. Scher, MD; David Lu, PhD; Nicole A. Schreiber, BA; Jessica Louw, BS; Ryon P. Graf, PhD; Hebert A. Vargas, MD; Ann Johnson, MS; Adam Jendrisak, MBA; Richard Bambury, MB, BCh, BAO; Daniel Danila, MD; Brigit McLaughlin, BS; Justin Wahl, BS; Stephanie B. Greene, PhD; Glenn Heller, PhD; Dena Marrinucci, PhD; Martin Fleisher, PhD; Ryan Dittamore, MBA




C
Overall survival: pre-AR signaling inhibitor samples
AR-V7 negative, n = 112
AR-V7 positive, n = 16
Survival, %
100
75
50
25
0
0
10
20
30
40
Survival, mo
HR = 11.45 (95% CI: 5.67-23.82)
P <.001
Median survival: 4.6 mo vs not reached


D
Overall survival: pretaxane samples
AR-V7 negative, n = 45
AR-V7 positive, n = 18
Survival, %
100
75
50
25
0
0
10
20
30
40
Survival, mo
HR = 3.74 (95% CI: 1.95-7.20)
P =.001
Median survival: 8.9 mo vs not reached



JAMA Oncology November 2016 Volume 2, Number 11

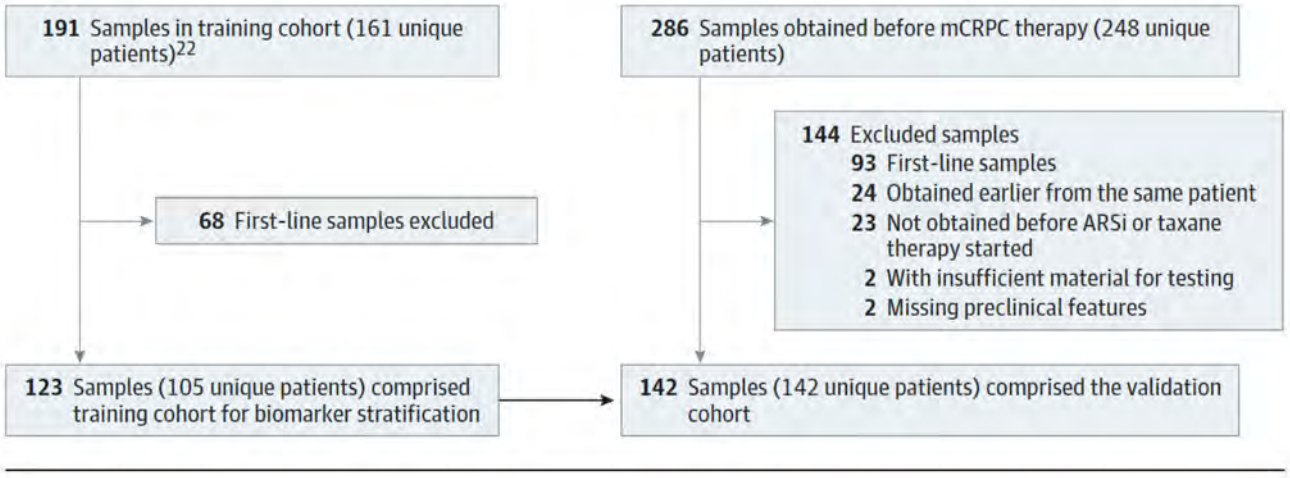
Research

JAMA Oncology | Original Investigation

Assessment of the Validity of Nuclear-Localized Androgen Receptor Splice Variant 7 in Circulating Tumor Cells as a Predictive Biomarker for Castration-Resistant Prostate Cancer

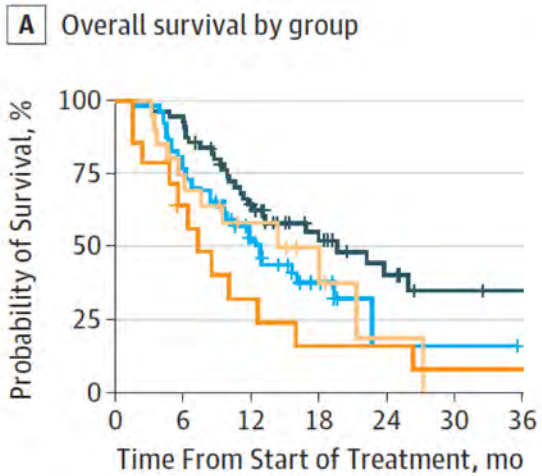
Howard I. Scher, MD; Ryon P. Graf, PhD; Nicole A. Schreiber, BA; Anuradha Jayaram, MB, BCh; Eric Winquist, MD; Brigit McLaughlin, BS; David Lu, PhD; Martin Fleisher, PhD; Sarah Orr, MS; Lori Lowes, PhD; Amanda Anderson, PhD; Yipeng Wang, MD, PhD; Ryan Dittamore, MBA; Alison L. Allan, PhD; Gerhardt Attard, MD, PhD; Glenn Heller, PhD

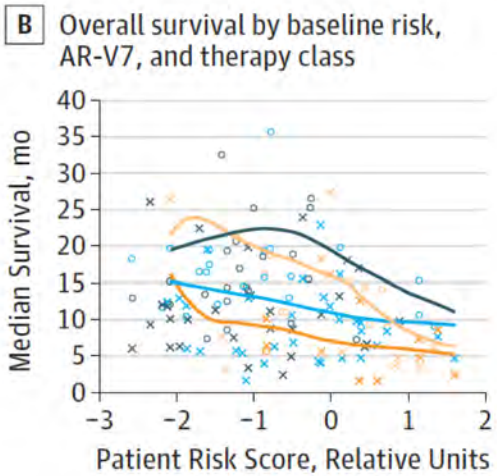
Figure 1. Distribution of Patient Samples in the Training Cohort and Validation Cohort

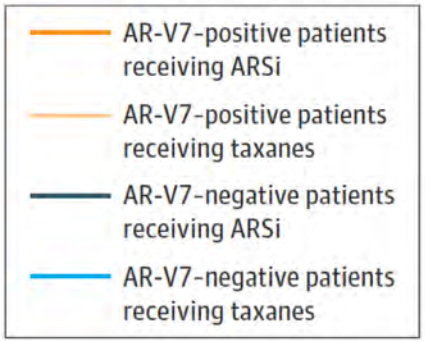


JAMA Oncology September 2018 Volume 4, Number 9

Figure 2. Association Between Patient Risk, Androgen Receptor Splice Variant 7 (AR-V7) Status, and Therapy

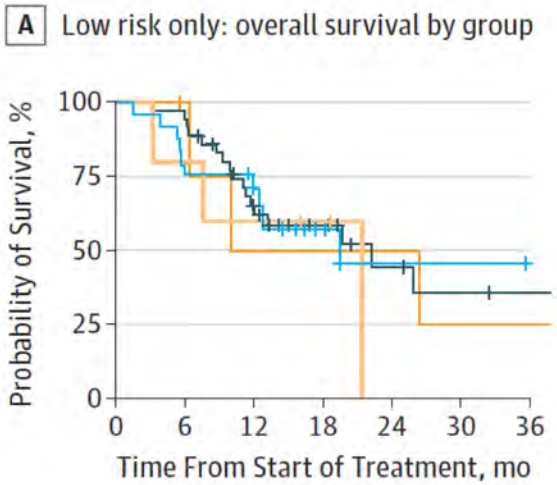


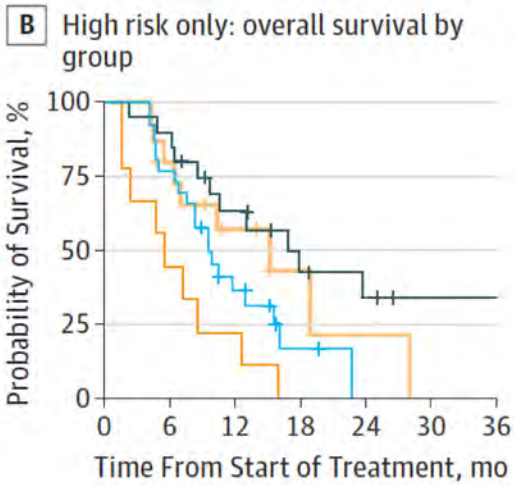


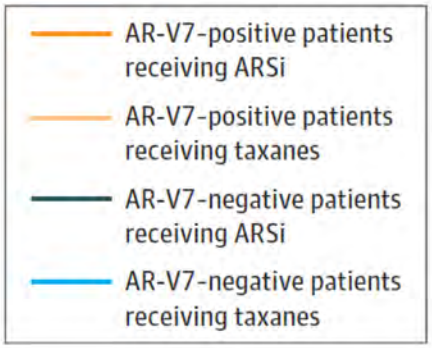


No. at risk

AR-V7-positive receiving ARSi	14	8	4	2	2	1	1
AR-V7-positive receiving taxanes	20	14	8	4	1	0	0
AR-V7-negative receiving ARSi	56	53	33	18	10	6	5
AR-V7-negative receiving taxanes	52	40	23	9	1	1	0



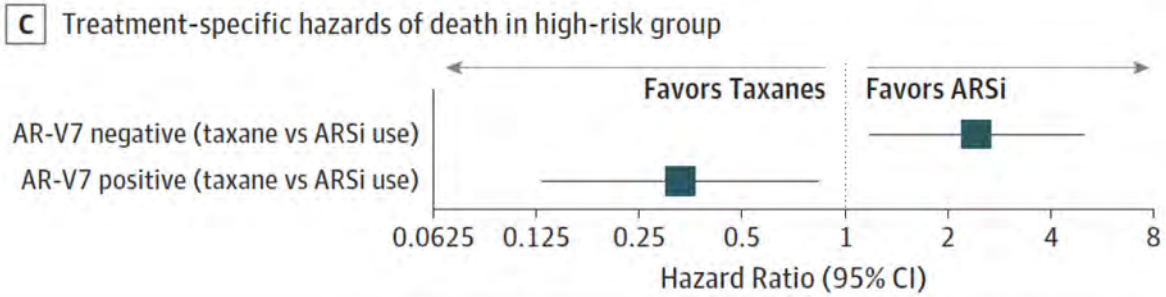




No. at risk

AR-V7-positive receiving ARSi	5	4	2	2	2	1	1
AR-V7-positive receiving taxanes	5	4	3	2	0	0	0
AR-V7-negative receiving ARSi	36	35	22	11	6	4	3
AR-V7-negative receiving taxanes	26	20	15	7	1	1	0

	9	4	2	0	0	0	0
	15	10	5	2	1	0	0
	20	18	11	7	4	2	2
	26	20	8	2	0	0	0



Invited Commentary

Nuclear Circulating Tumor Cell Androgen Receptor Variant 7 in Castration-Resistant Prostate Cancer

The Devil Is in the Detail

Stephen R. Plymate, MD; Adam Sharp, MD, PhD; Johann S. de Bono, MD, PhD

Concerns regarding the assay…

1) positivity not continuous but binary (only 1 positive CTC needed)
2) Total CTC counts not reported
3) False-negative rate cannot be interpreted with total CTC count
4) Anti-body to cryptic exon 3 may be non-specific leading to false positivity
5) AR-V7 may be more prognostics of overall survival

JAMA Oncology September 2018 Volume 4, Number 9

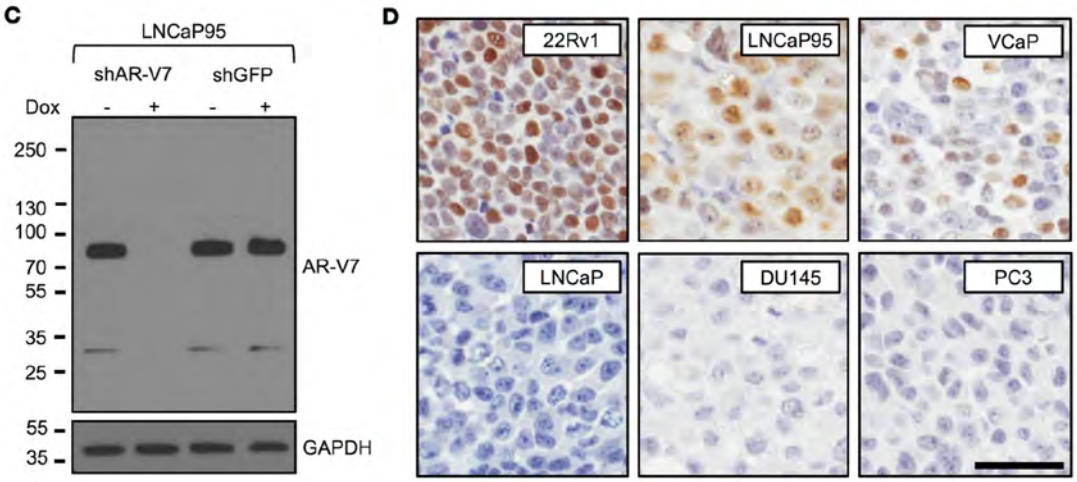
CLINICAL MEDICINE

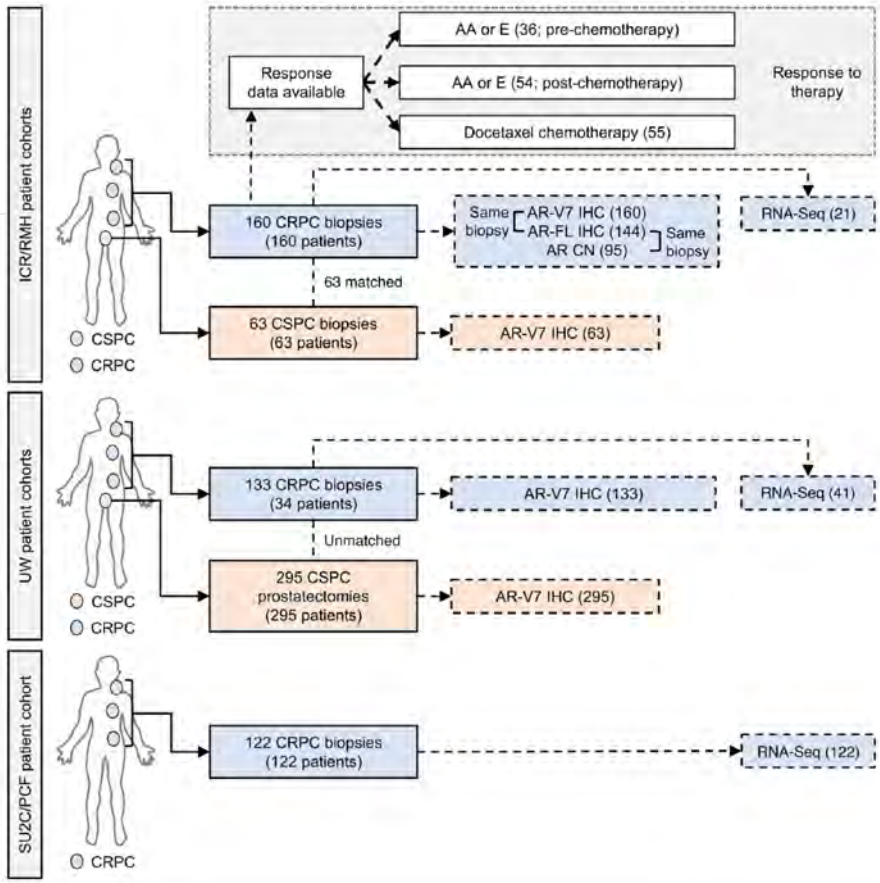
The Journal of Clinical Investigation

Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer

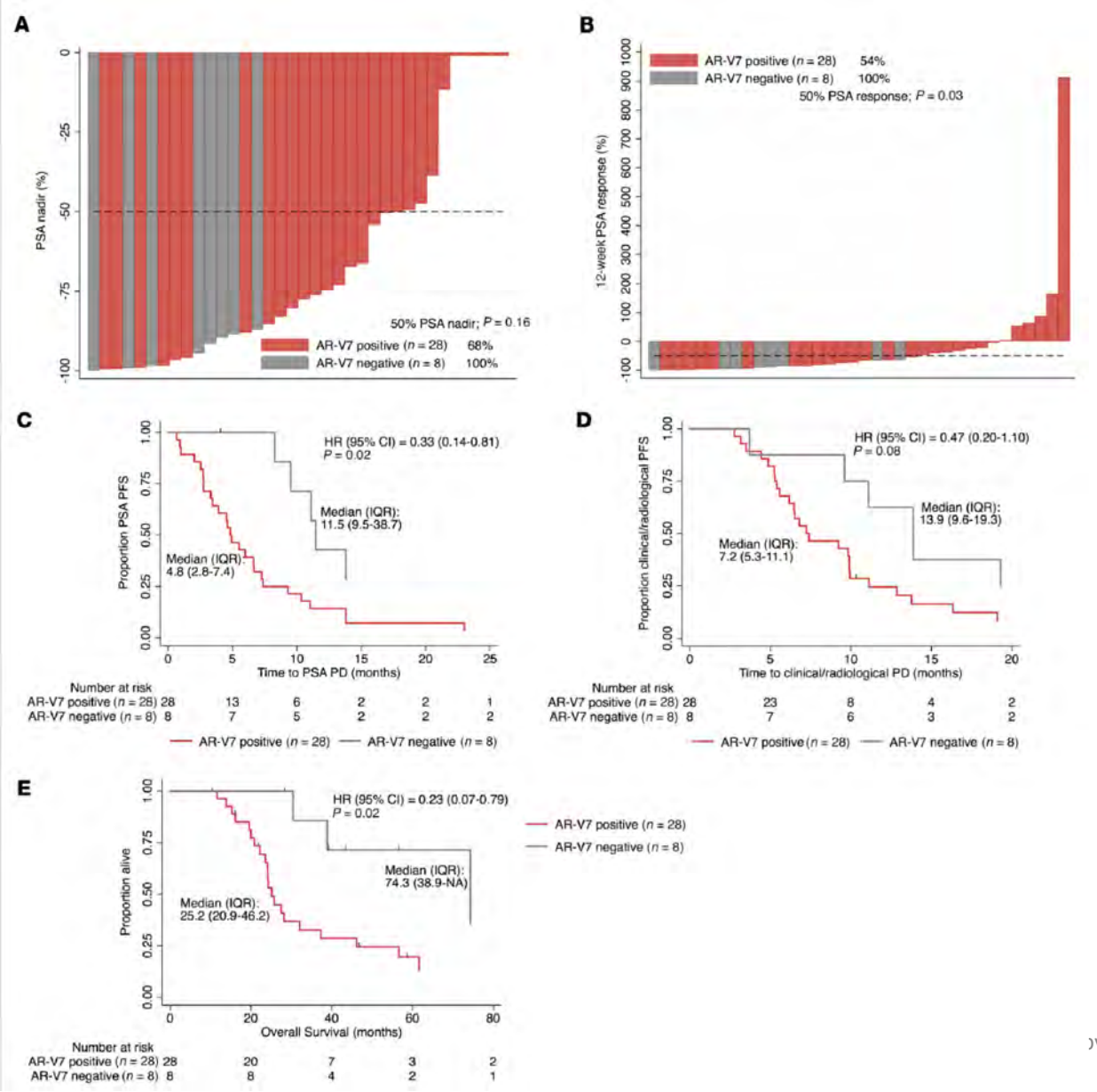
Adam Sharp,[1,2] Ilsa Coleman,[3] Wei Yuan,[1] Cynthia Sprenger,[4] David Dolling,[1] Daniel Nava Rodrigues,[1] Joshua W. Russo,[5] Ines Figueiredo,[1] Claudia Bertan,[1] George Seed,[1] Ruth Riisnaes,[1] Takuma Uo,[4] Antje Neeb,[1] Jonathan Welti,[1] Colm Morrissey,[4] Suzanne Carreira,[1] Jun Luo,[6] Peter S. Nelson,[3,4] Steven P. Balk,[5] Lawrence D. True,[4] Johann S. de Bono,[1,2] and Stephen R. Plymate[4,7]

[1]The Institute of Cancer Research, London, United Kingdom. [2]The Royal Marsden, London, United Kingdom. [3]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [4]Department of Medicine, University of Washington, Seattle, Washington, USA. [5]Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA. [6]Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [7]Puget Sound VA Health Care System, Geriatric Research Education and Clinical Center (PSVAHCS-GRECC), Seattle, Washington, USA.





JCI Volume 129 Number 1 January 2019



Major Findings

-AR-V7 found in <1% of hormone naïve PCA (therefore not likely a useful biomarker at this stage) and appears only after resistance to ADT

-Differences in prevalence of AR-V7 likely due to different antibodies used (methods)

-AR-V7 expressed in 75% progressing CRPC

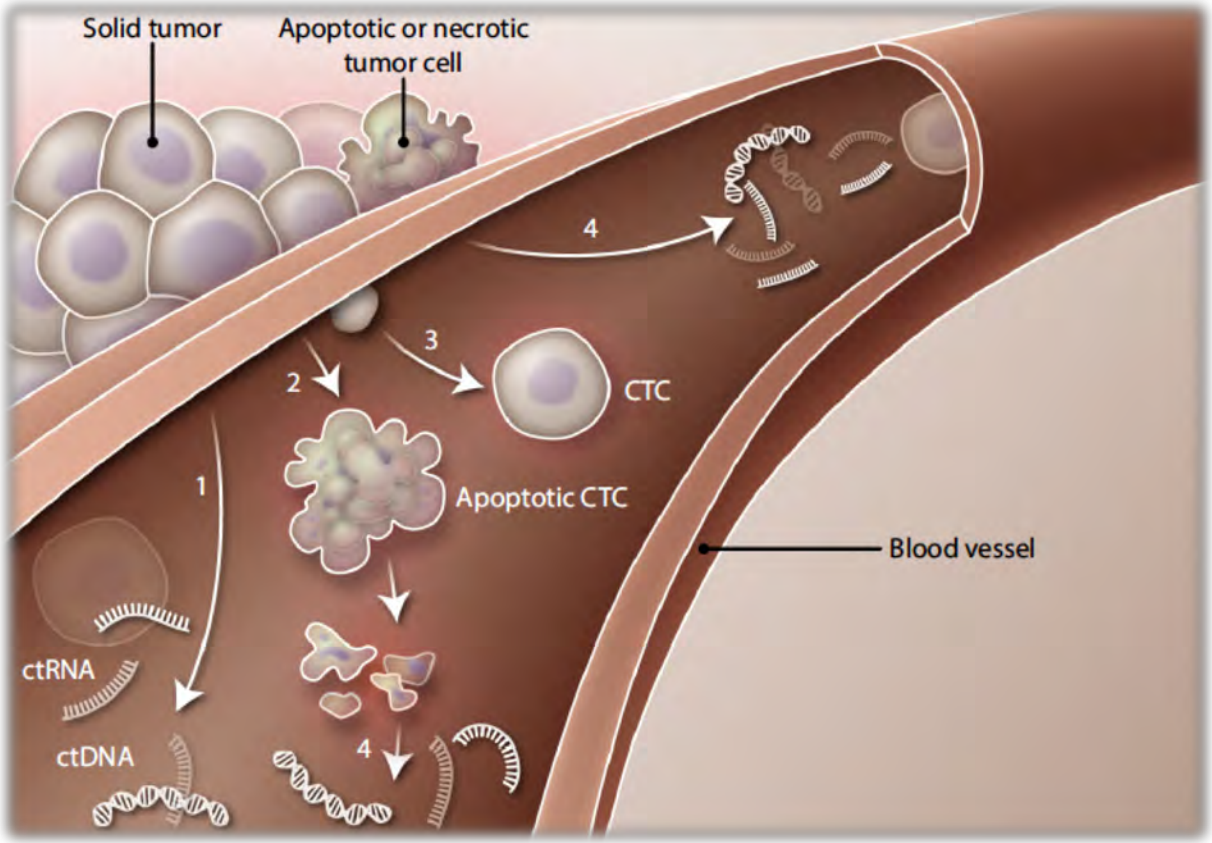
-AR-V7 higher in biopsy as compared to liquid biopsy

-Heterogeneity observed with implications for partial response if some lesions have low AR-V7

-Associated with resistance to AR targeted agents but not taxane

JCI Volume 129 Number 1 January 2019

What is next for CRPC Diagnostics



Liquid biopsy to overcome limits of multiple metastasis biopsies to capture heterogeneity and/or serial biopsies

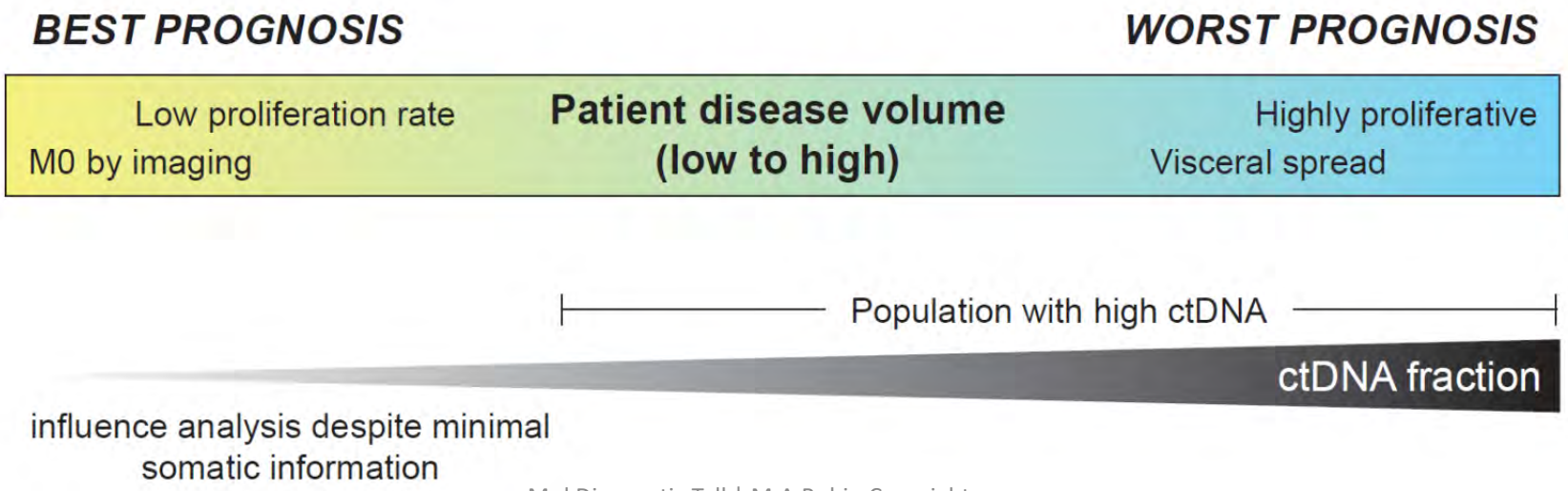
from Schweizer MT and Antonarakis ES, Sci Transl Med. 2015 Nov 4;7(312)

CIRCULATING BIOMARKERS FOR ADAVANCED PCA: Non-Invasive Approaches to Monitor PCA evolution

Assay	Pros	Cons	Example
CTC-EpCAM	FDA approved	Epithelial selection	CELLSEARCH
CTC without selection (AR-V7, PTEN, etc)	Unbiased	Not regulatory approved	Epic Sciences
Plasma cfDNA (ctDNA)	Monitor genomic alterations (NGS)	Signal/noise	Attard/Demichelis et al. Wyatt et al.
Oncosomes/Exosomes	Potential informative packets of RNA/DNA	Research grade	
RNA (lncRNA,mRNA, miRNA)	Disease/tissue specificity	Clinical and research grade	T2-ERG/PCA3/SCHLAP1/AR-v7

Plasma circulating tumour DNA (ctDNA) is abundant in progressing mCRPC patients

- Cell-free DNA (cfDNA) is shed by apoptosing normal and cancer cells
- Putative ctDNA can be identified via somatic alterations in cfDNA
- CtDNA / cfDNA 'fractions' are high in mCRPC but very variable

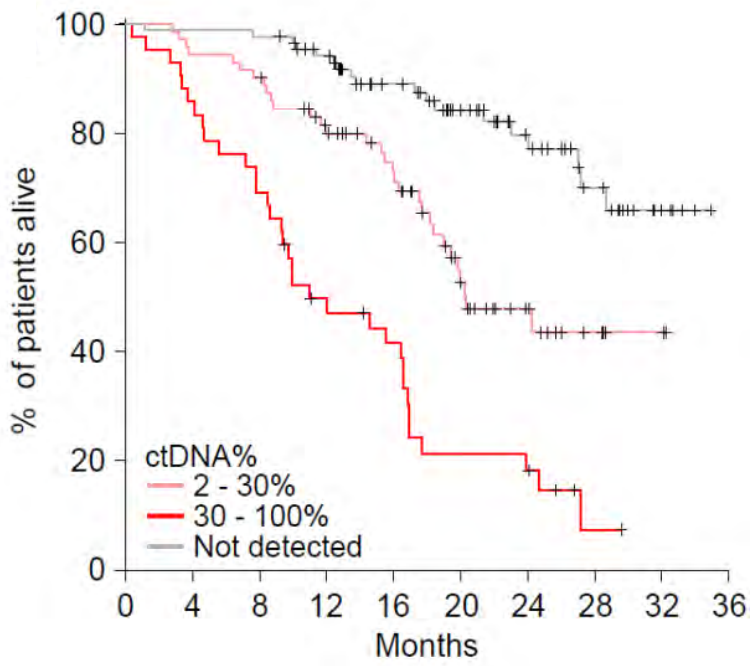


Courtesy of A. Wyatt

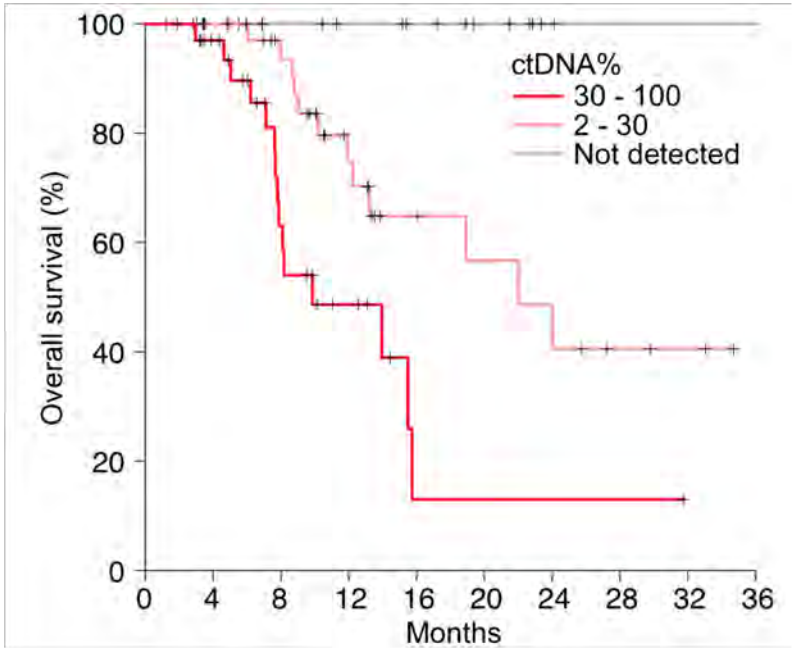
Warner *et al.*, BJUI 2018

Prognostic effect of ctDNA fraction in mCRPC

First line mCRPC general population (n = 202)
Khalaf et al., ASCO 2018



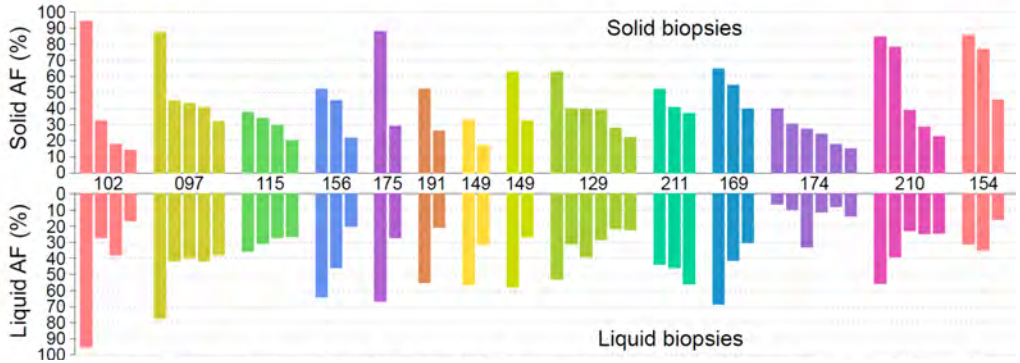
First line mCRPC poor prognosis (n = 95)
Chi et al., ESMO 2018



Courtesy of A. Wyatt

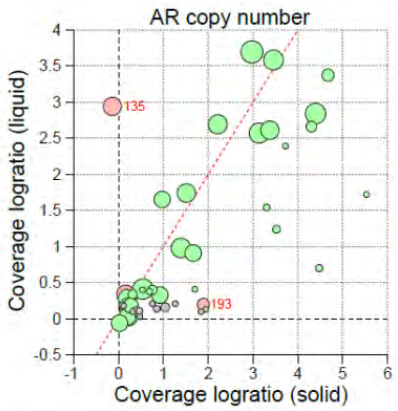
High concordance between ctDNA and matched metastatic tissue biopsy (in CRPC)

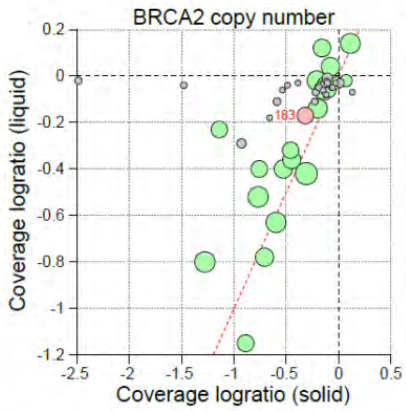
Wyatt et al studied 45 plasma samples collected at time of metastatic tissue biopsy (SU2C / PCF West Coast Dream Team, Eric Small *et al.*)



Similar mutation profiles, ctDNA vs tissue

Similar gene copy numbers, ctDNA vs tissue





See also: Hovelson, Tomlins *et al.* Oncotarget. 2017; 8(52): 89848–89866.

Wyatt, Annala, *et al.*, J Natl Cancer Inst. 2017

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Prostate Cancer

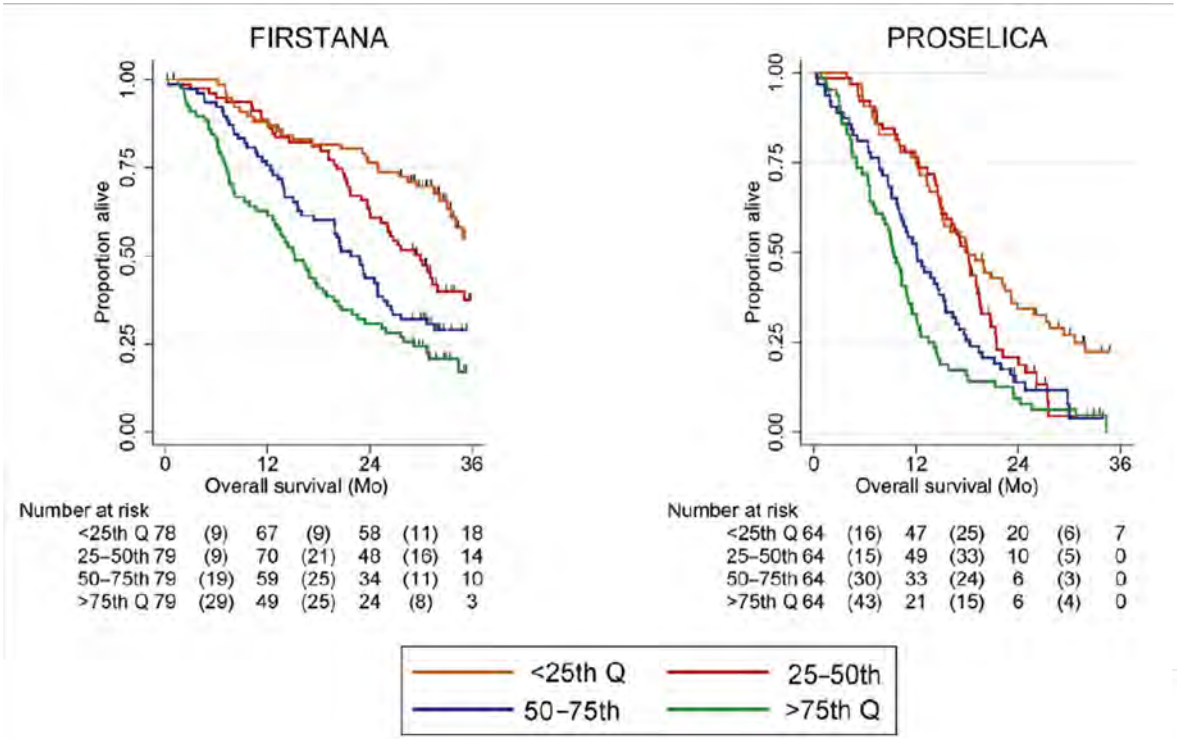
Editorial by Robert J. van Soest, Bertrand Tombal, Martijn P. Lolkema and Ronald de Wit on pp. 292–293 of this issue

Plasma Cell-free DNA Concentration and Outcomes from Taxane Therapy in Metastatic Castration-resistant Prostate Cancer from Two Phase III Trials (FIRSTANA and PROSELICA)

Niven Mehra[a], David Dolling[b], Semini Sumanasuriya[a], Rossitza Christova[c], Lorna Pope[c], Suzanne Carreira[c], George Seed[c], Wei Yuan[c], Jane Goodall[c], Emma Hall[b], Penny Flohr[c], Gunther Boysen[c], Diletta Bianchini[a], Oliver Sartor[d], Mario A. Eisenberger[e], Karim Fizazi[f], Stephane Oudard[g], Mustapha Chadjaa[h], Sandrine Macé[h], Johann S. de Bono[a,]*

Conclusions: We report that changes in cfDNA concentrations correlate with both rPFSand OS in patients receiving first- and second-line taxane therapy, and may serve as independent prognostic biomarkers of response to taxanes.

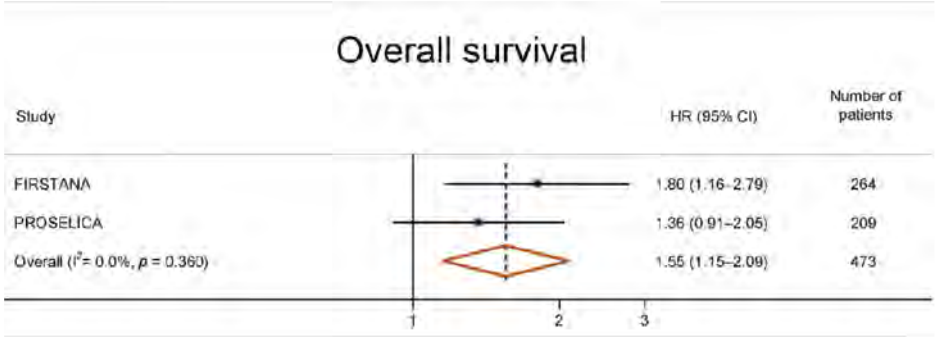
EUR Urol 74 (2 0 18) 2 8 3 – 2 9 1



"Our study identifies baseline cfDNA concentration as an independent prognostic biomarker in patients with mCRPC, with higher baseline concentrations associated with shorter rPFS and OS following taxane therapy. A decline in total cfDNA concentration during the first 9 wk of treatment was associated with response to taxane therapy."

-Two phase III clinical trials

FIRSTANA (NCT01308567) and PROSELICA(NCT01308580)
Patients received docetaxel (75 mg/m2) or cabazitaxel (20 or 25 mg/m2) as first-line chemotherapy (FIRSTANA), and cabazitaxel (20 or 25 mg/m2) as second-line chemotherapy (PROSELICA).



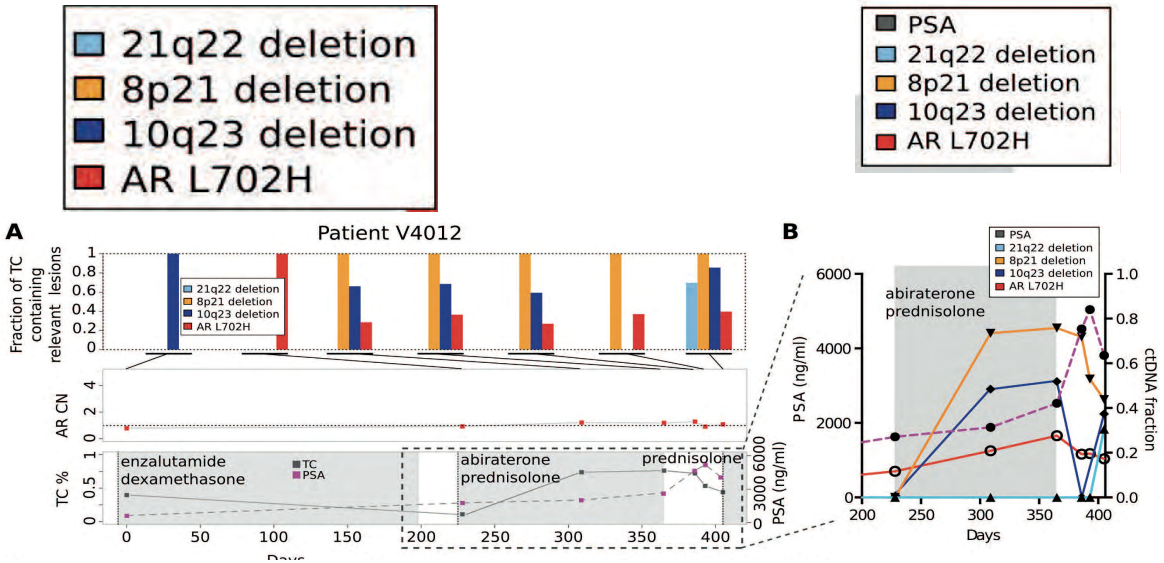
EUR Urol 74 (2 0 18) 2 8 3 – 2 9 1

RESEARCH ARTICLE

CANCER

Tumor clone dynamics in lethal prostate cancer

Suzanne Carreira,[1]* Alessandro Romanel,[2]* Jane Goodall,[1]* Emily Grist,[1,3] Roberta Ferraldeschi,[1,3] Susana Miranda,[1] Davide Prandi,[2] David Lorente,[1,3] Jean-Sebastien Frenel,[1] Carmel Pezaro,[1,3] Aurelius Omlin,[1,3] Daniel Nava Rodrigues,[1] Penelope Flohr,[1] Nina Tunariu,[1,3] Johann S. de Bono,[1,3] Francesca Demichelis,[2,4,5†‡] Gerhardt Attard[1,3†‡]



Emergence of *AR-L702H* on treatment

Sci Transl Med **6**, *254ra125 (2014)*

REPORT

CANCER

Plasma *AR* and abiraterone-resistant prostate cancer

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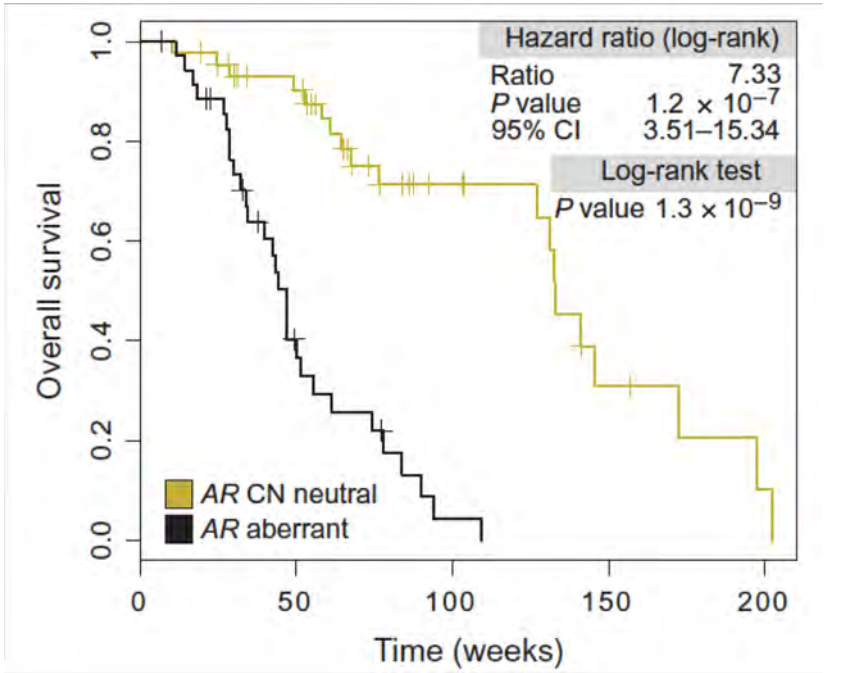
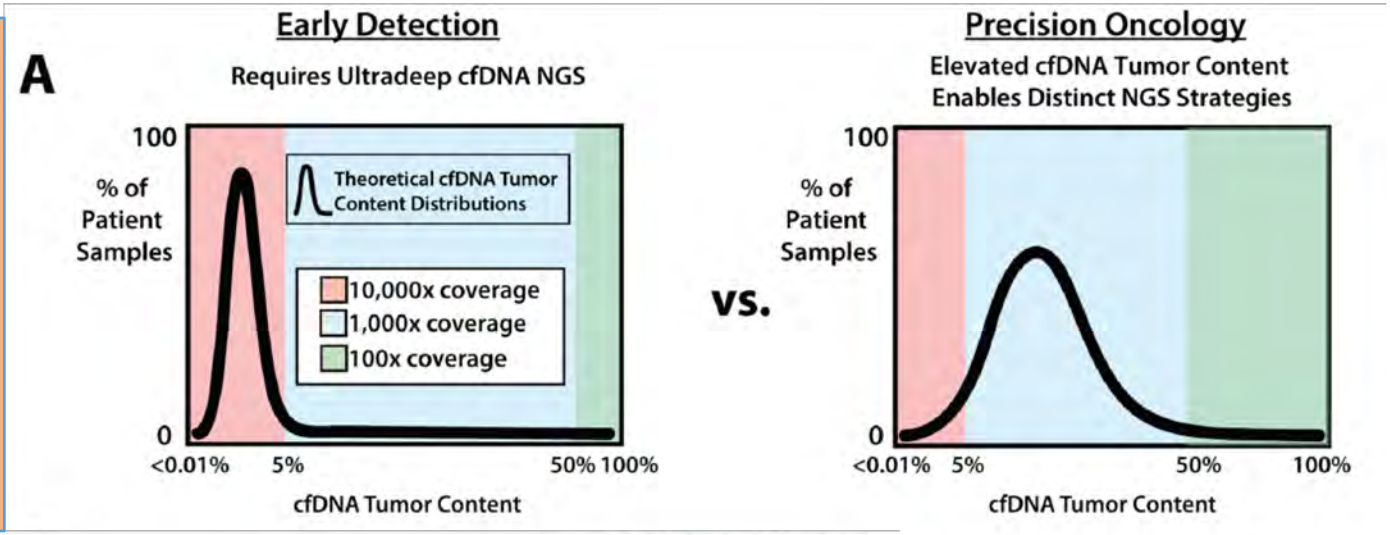


Plasma AR and abiraterone-resistant PCa

Sci Transl Med, 2015 Vol 7 Issue 312 312re10

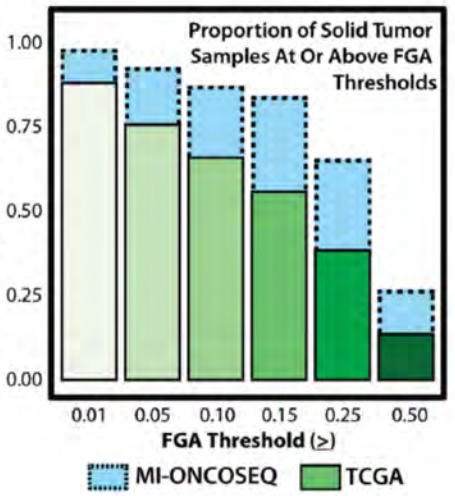
Need to address evolution as a time course with cfDNA, scSeq, molecular imaging, etc.



Research Paper

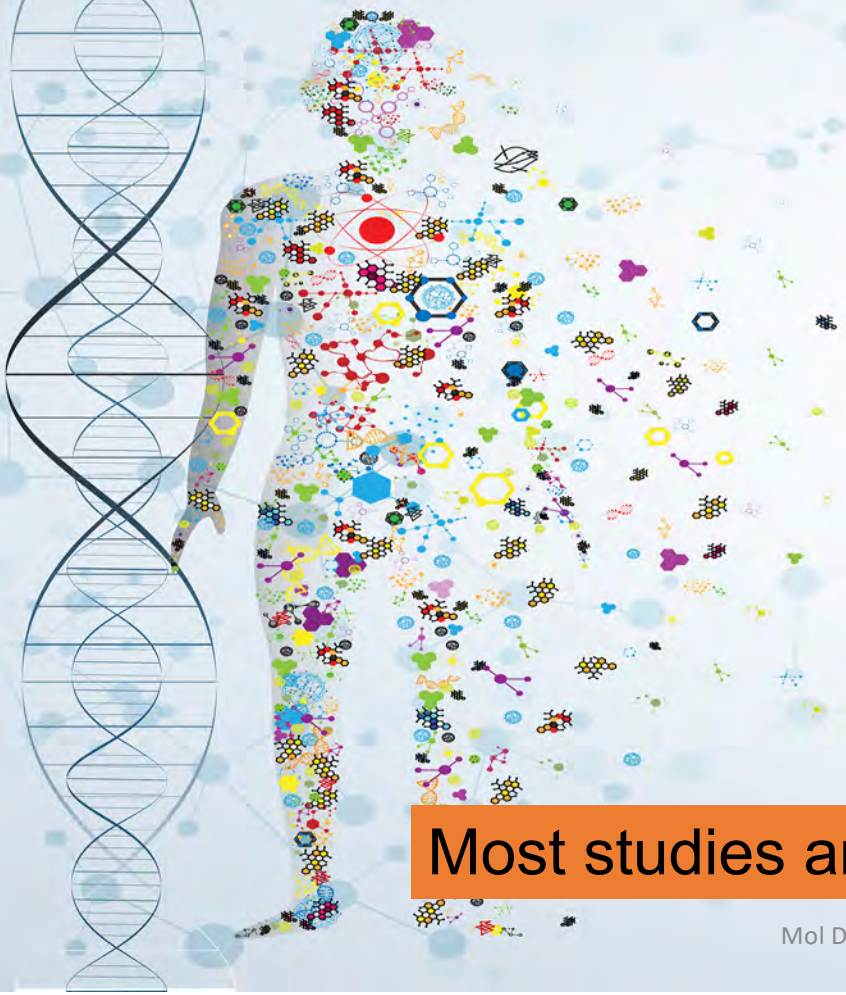
Rapid, ultra low coverage copy number profiling of cell-free DNA as a precision oncology screening strategy

Daniel H. Hovelson[1,2], Chia-Jen Liu[1,3], Yugang Wang[4], Qing Kang[5], James Henderson[4], Amy Gursky[4], Scott Brockman[1], Nithya Ramnath[5], John C. Krauss[5], Moshe Talpaz[5], Malathi Kandarpa[5], Rashmi Chugh[5], Missy Tuck[5], Kirk Herman[5], Catherine S. Grasso[10,11], Michael J. Quist[10,11], Felix Y. Feng[12], Christine Haakenson[13], John Langmore[13], Emmanuel Kamberov[13], Tim Tesmer[13], Hatim Husain[14], Robert J. Lonigro[1,3], Dan Robinson[1,3,8], David C. Smith[5,8], Ajjai S. Alva[5,8], Maha H. Hussain[5,8,15], Arul M. Chinnaiyan[1,3,8,10], Muneesh Tewari[2,5,6,7,8,9], Ryan E. Mills[2,7], Todd M. Morgan[1,4,8,*] and Scott A. Tomlins[1,3,4,8,*]



Oncotarget 2017

From the blood:
What is predictive? Prognostics? Reproducible?



cfDNA (tumor DNA)

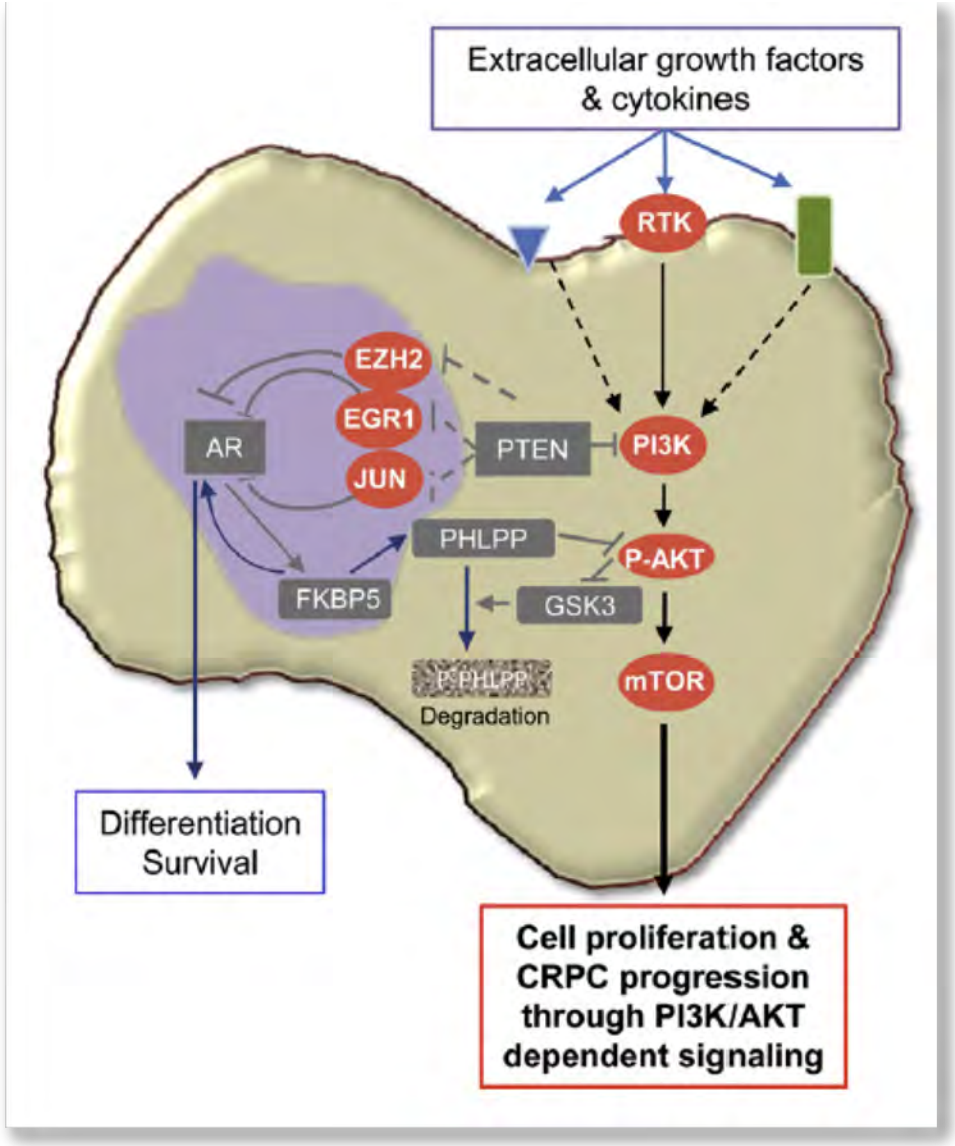
AR-V7

AR gain

AR mutations

Other (neuroendocrine differentiation)

Most studies are not exploring these parameters together



Mulholland et al., Cancer Cell 2011

PTEN loss in circulating tumour cells correlates with PTEN loss in fresh tumour tissue from castration-resistant prostate cancer patients

Elizabeth A Punnoose[1,6], Roberta Ferraldeschi[2,3,6], Edith Szafer-Glusman[1,6], Eric K Tucker[4], Sankar Mohan[5], Penelope Flohr[3], Ruth Riisnaes[3], Susana Miranda[3], Ines Figueiredo[3], Daniel Nava Rodrigues[2], Aurelius Omlin[2,3], Carmel Pezaro[2,3], Jin Zhu[1], Lukas Amler[1], Premal Patel[1], Yibing Yan[1], Natalee Bales[4], Shannon L Werner[4], Jessica Louw[4], Ajay Pandita[5], Dena Marrinucci[4], Gerhardt Attard[3] and Johann de Bono*[,3]

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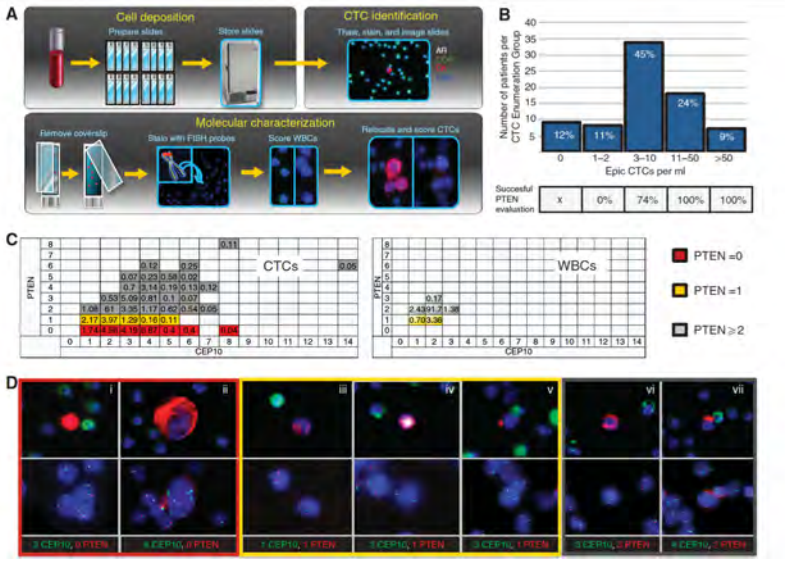
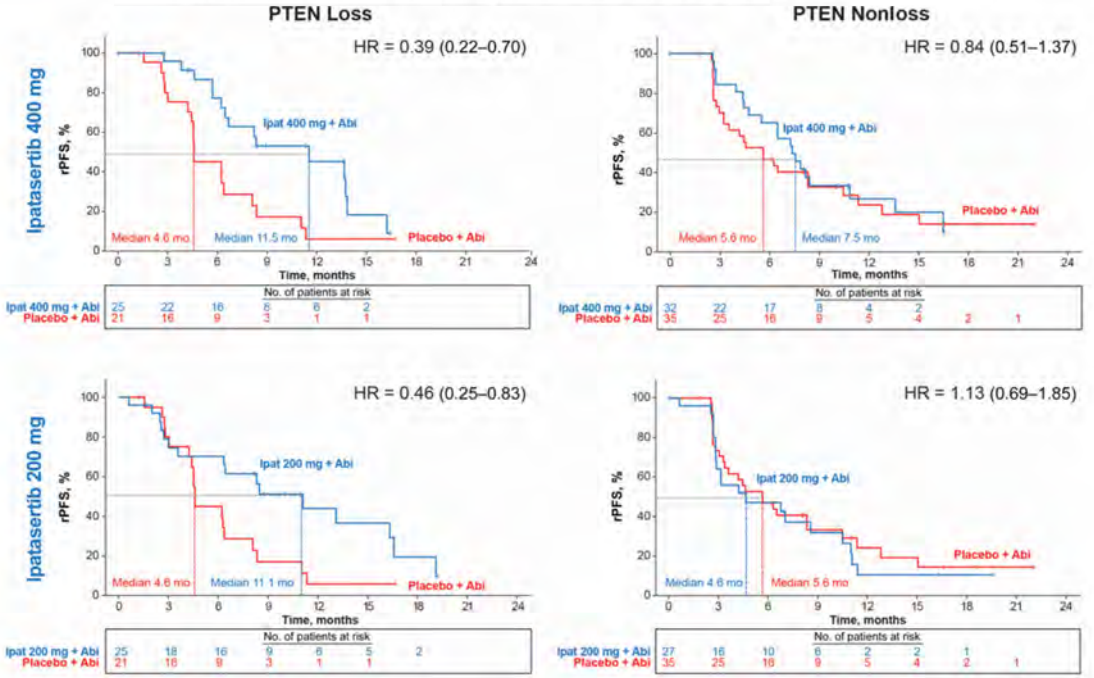


Randomized Phase II Study Evaluating Akt Blockade with Ipatasertib, in Combination with Abiraterone, in Patients with Metastatic Prostate Cancer with and without PTEN Loss

Johann S. de Bono[1], Ugo De Giorgi[2], Daniel Nava Rodrigues[1], Christophe Massard[3], Sergio Bracarda[4], Albert Font[5], Jose Angel Arranz Arija[6], Kent C. Shih[7], George Daniel Radavoi[8], Na Xu[9], Wai Y. Chan[9], Han Ma[9], Steven Gendreau[9], Ruth Riisnaes[1], Premal H. Patel[9], Daniel J. Maslyar[9], and Viorel Jinga[8]



www.bjcancer.com | DOI:10.1038/bjc.2015.332

Clin Cancer Res; 25(3) February 1, 2019

In conclusion:

What is "*actionable*" or ready for clinical use?

Need prospective validation

-Blood/biopsy/cfDNA DNA repair BRCA1/2, ATM (multiple clinical tests)
-CTC for AR v7 (Available via CTC Episciences)
-Metastatic biopsy - AR gain (multiple tests)
-cfDNA for DNA fraction, AR, others

Approved by FDA (Not Prostate Specific)

-MSI/MMR (multiple tests)-clinical ready/FDA indication broad

Liquid Biopsies: Challenges for Cancer

-Detection
-Analysis
-Setting (Indication)
-Prognosis versus Predictive
-Not one test fits all

Thanks for your input on this presentation

Alex Wyatt
Gert Attard
Pete Nelson
Johann de Bono
Colin Pritchard

All Slides available @ Rubinlab.unibe.ch *or* @MarkARubin1