



Case Study: il tumore alla prostata

*Dalla caratterizzazione molecolare allo sviluppo di
un test clinico non-invasivo
per l'identificazione di una forma letale*

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Surveillance, Epidemiology, and End Results Program

Turning Cancer Data Into Discovery

Common Types of Cancer	Estimated New Cases 2015	Estimated Deaths 2015
1. Breast Cancer (Female)	231,840	40,290
2. Lung and Bronchus Cancer	221,200	158,040
3. Prostate Cancer	220,800	27,540
4. Colon and Rectum Cancer	132,700	49,700
5. Bladder Cancer	74,000	16,000
6. Melanoma of the Skin	73,870	9,940
7. Non-Hodgkin Lymphoma	71,850	19,790
8. Thyroid Cancer	62,450	1,950
9. Kidney and Renal Pelvis Cancer	61,560	14,080
10. Endometrial Cancer	54,870	10,170

Prostate cancer represents 13.3% of all new cancer cases in the U.S.



Prostate Cancer, long follow-up

5-year relative survival, 2005-2011

For the most common cancers

Among cases diagnosed from 2005 to 2011, followed through 2012

Prostate



Melanoma of the skin



Breast (female)



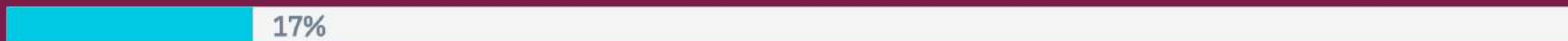
Urinary bladder



Colorectum



Lung and bronchus

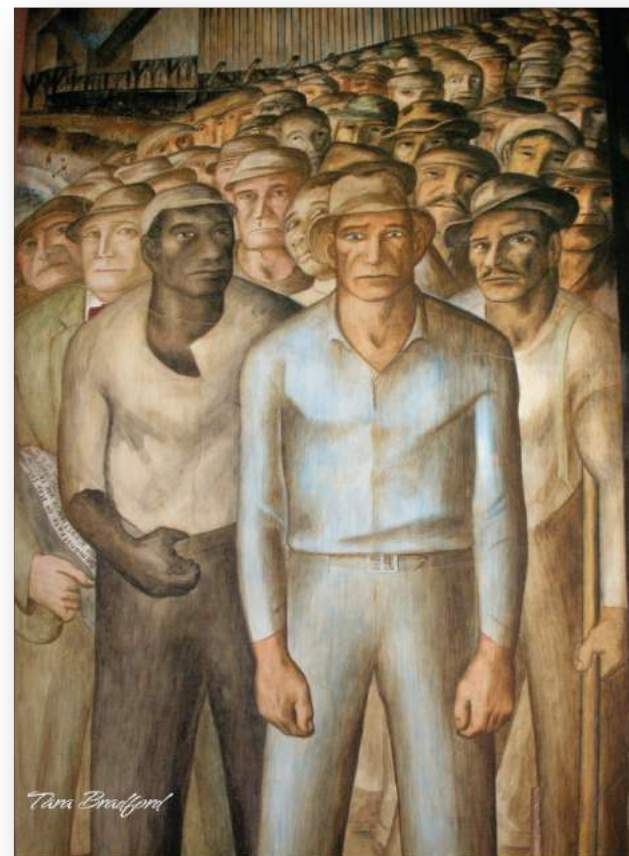


Prostate Cancer

-1 in 7 lifetime risk diagnosis*

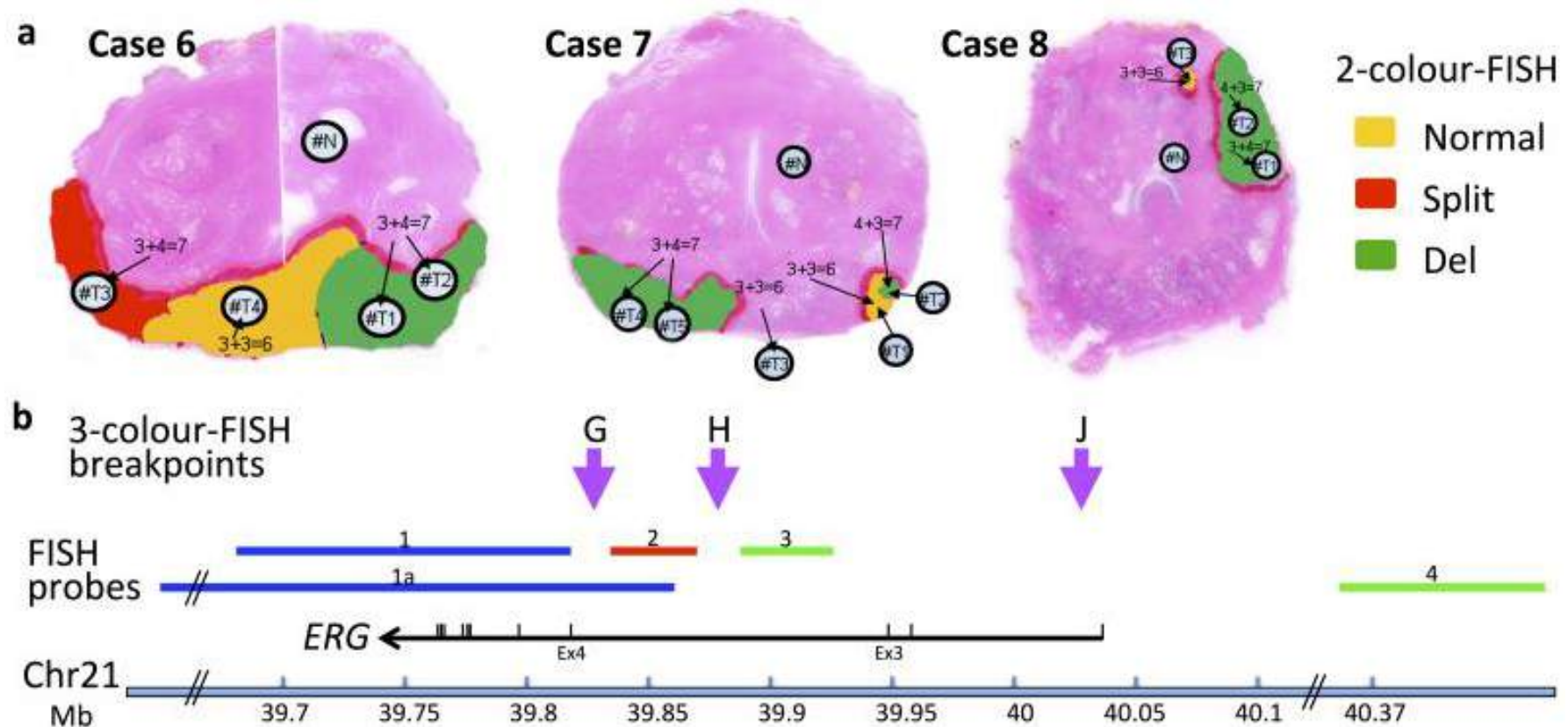
-1 in 39 lifetime risk of dying*

Understanding the transition
from indolent to aggressive
disease is critical.



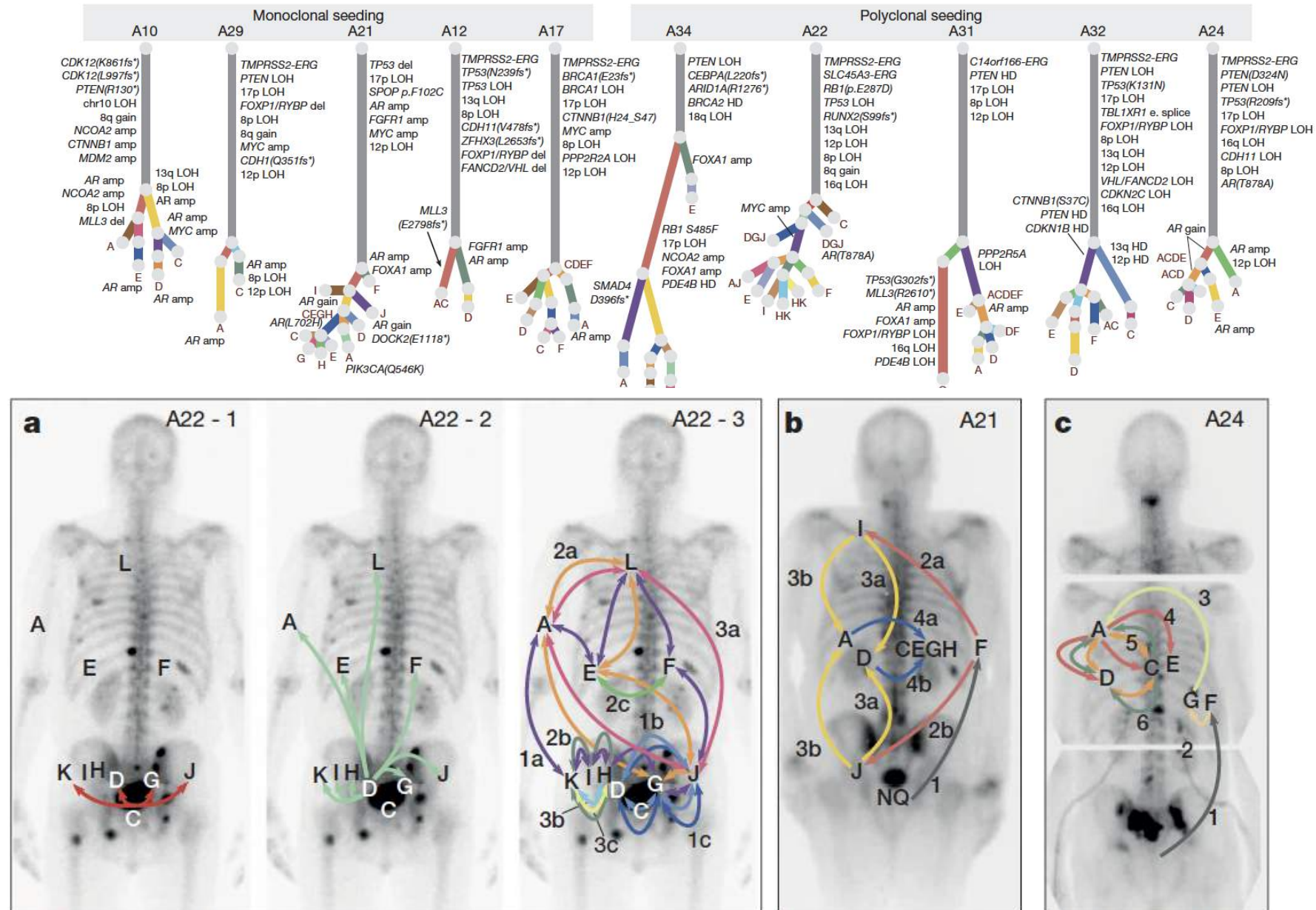
*US National Cancer Institute's Surveillance Epidemiology and End Results (SEER) Database, and is based on incidence and mortality data for the United States from 2010 through 2012 (most recent data)

Prostate Cancer is a multi-focal disease



Metastasis-to-metastasis seeding occurs either by a linear or by a branching pattern of spread

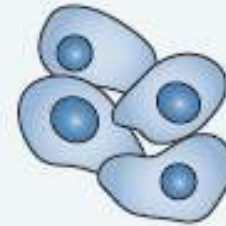
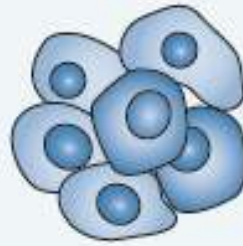
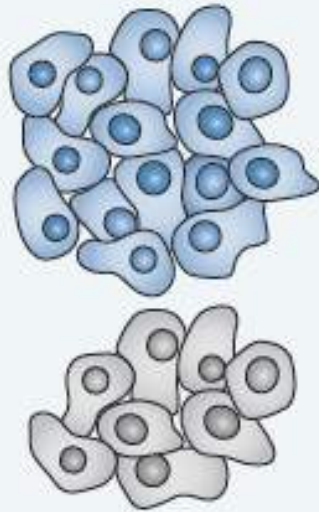
Gundem et al, Nature 2015



Multifocal prostate cancer

Metastases

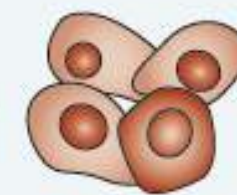
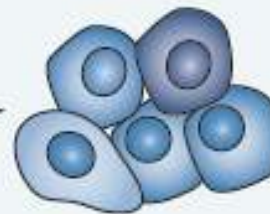
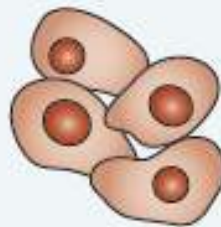
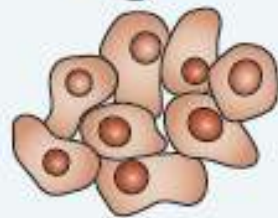
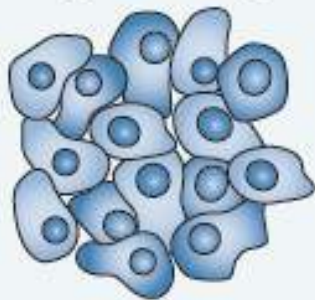
a Monoclonal origin



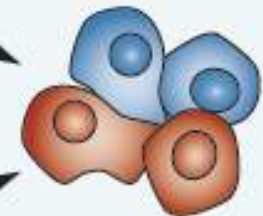
Metastatic cells share lesions from founding clone



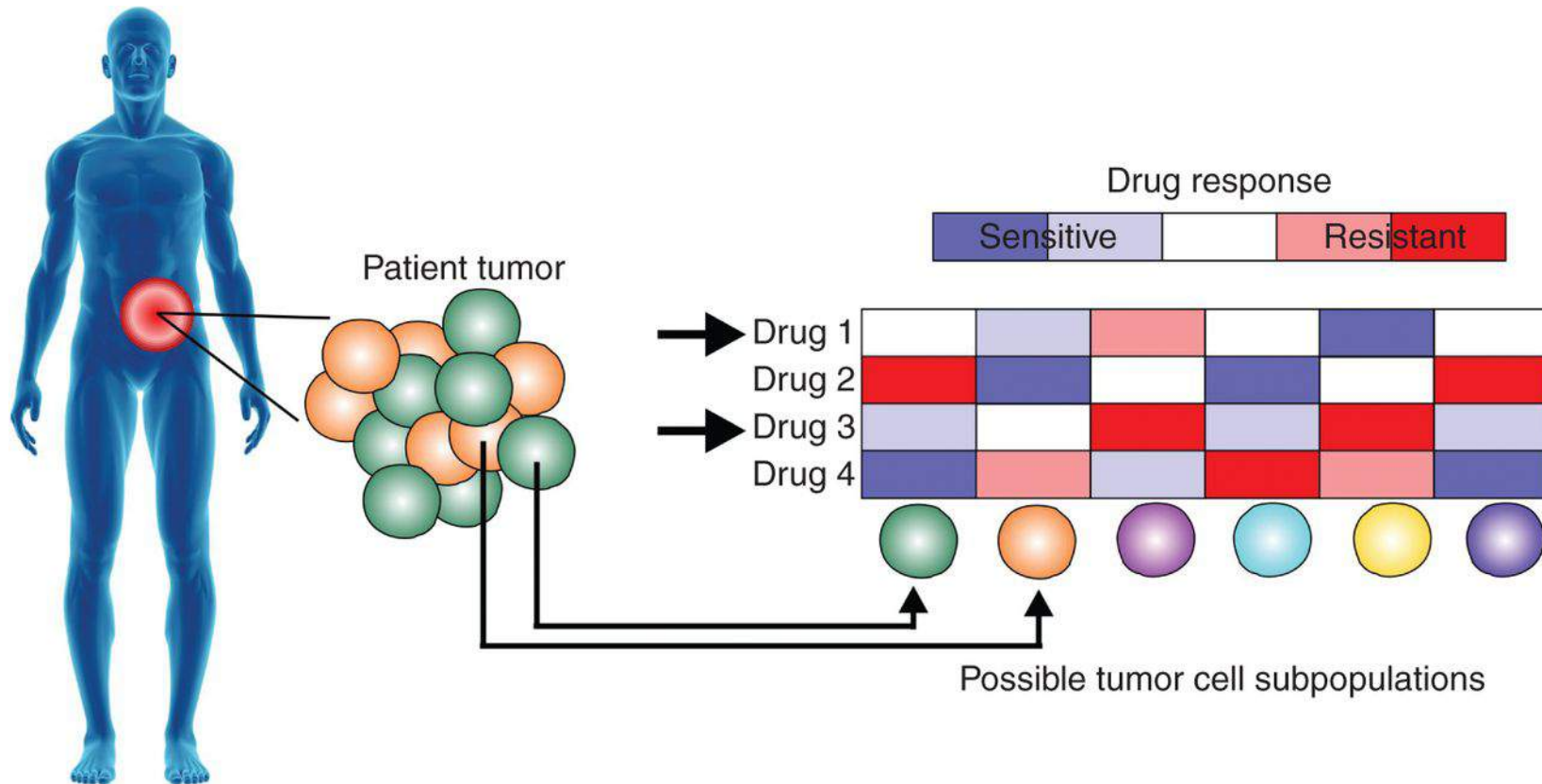
b Polyclonal origin



Higher genomic diversity in metastatic cells

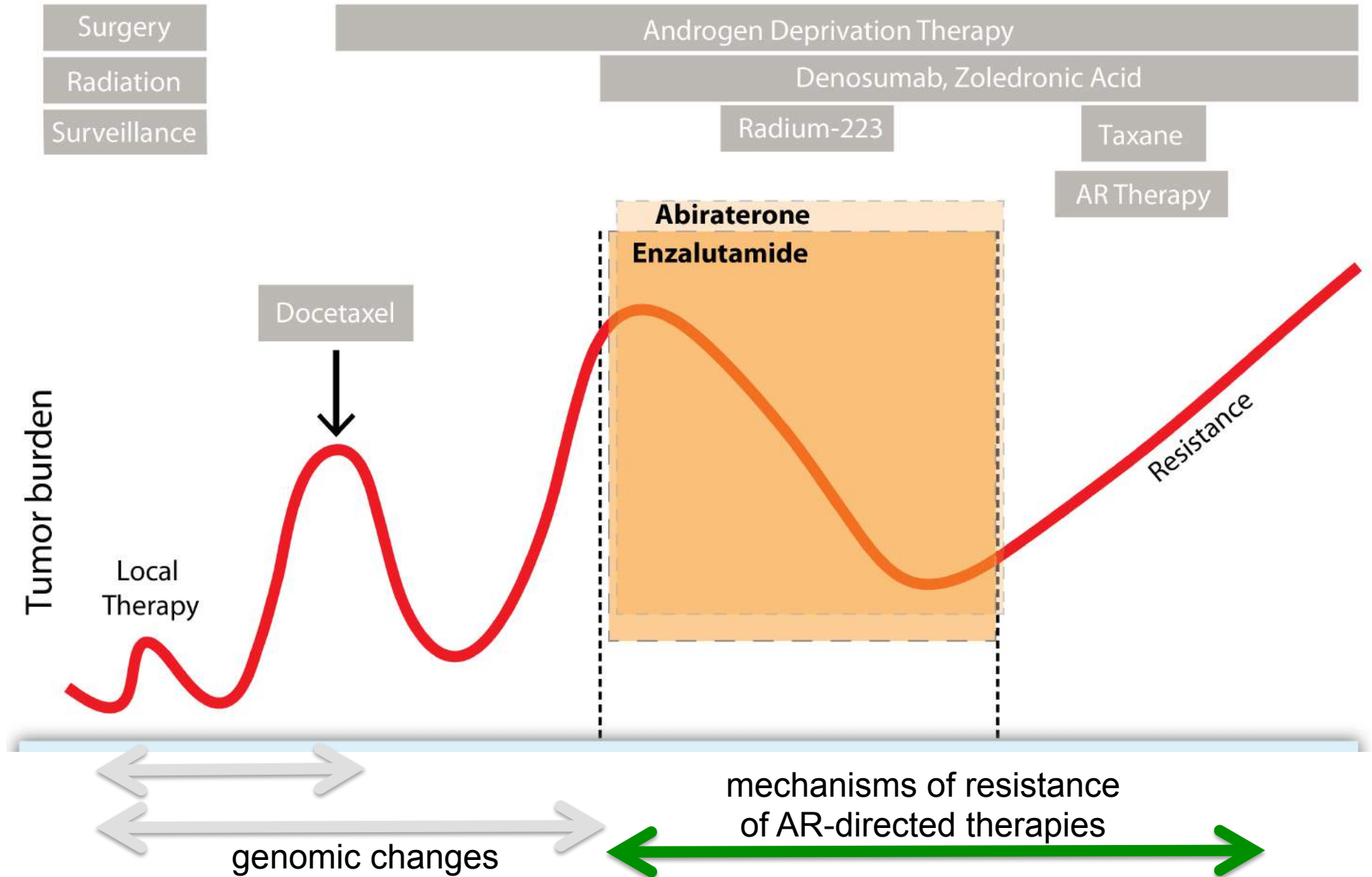


Cancer heterogeneity And Treatment response



Clare Fedele et al. *Cancer Discovery* 2014.

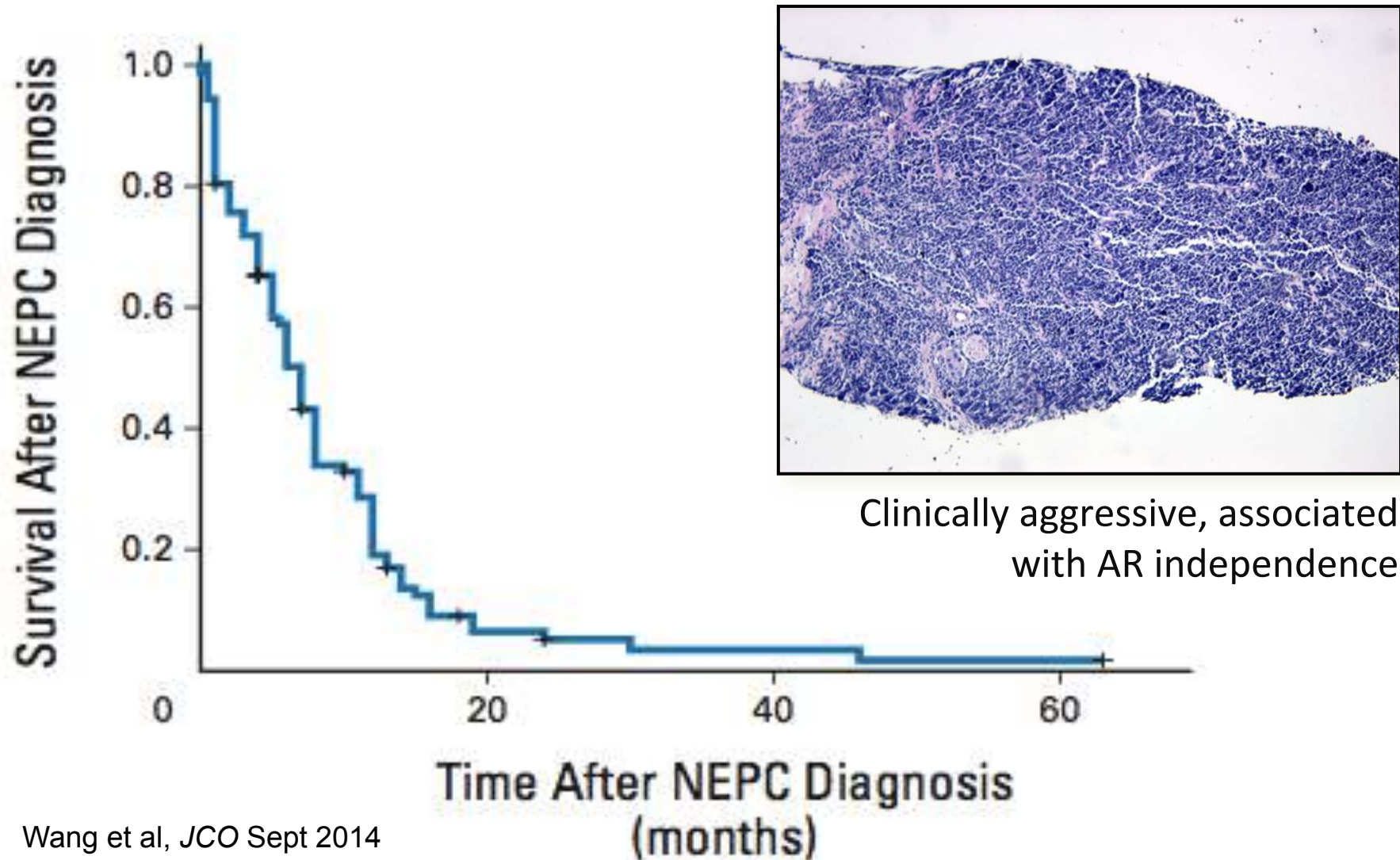
The genomics of resistance



Case study

Divergent clonal evolution of
castration-resistant neuroendocrine
prostate cancer

Small Cell/Neuroendocrine Prostate Cancer (NEPC)
Meta-analysis, 54 studies, 123 pts
Average Survival = 7 months

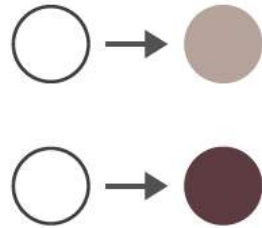


Clinical Challenges: How is “NEPC” defined?

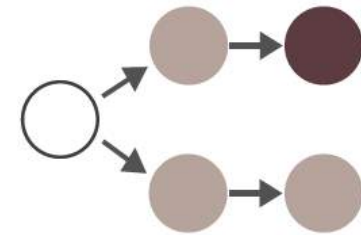
- **Histology** – there is pathologic heterogeneity within NEPC, mixed phenotypes
- **NE markers in tissue or serum-** not always positive, can also be present in adenocarcinoma
- **Clinical criteria-** not well defined, have been used in Phase 2 trials (Aparicio, MD Anderson; Beltran, WCMC)
- **Molecular criteria?**

Evolution Models towards NEPC

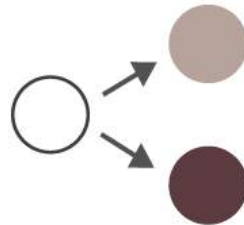
I. INDEPENDENT
from PRIMARY



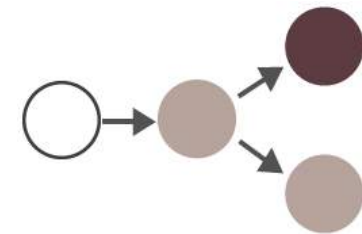
II. INDEPENDENT
from CRPC



III. DIVERGENT
from PRIMARY



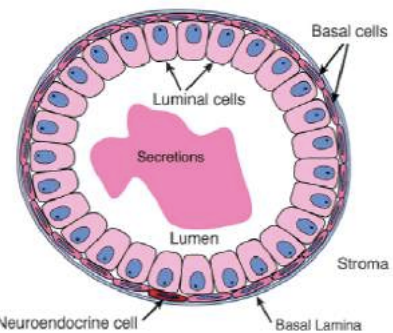
IV. DIVERGENT
from CRPC



V. LINEAR



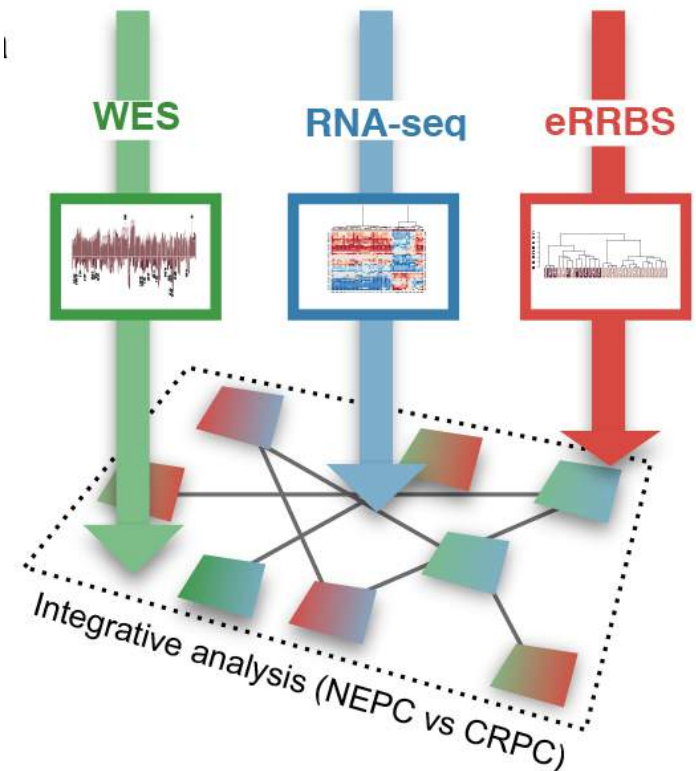
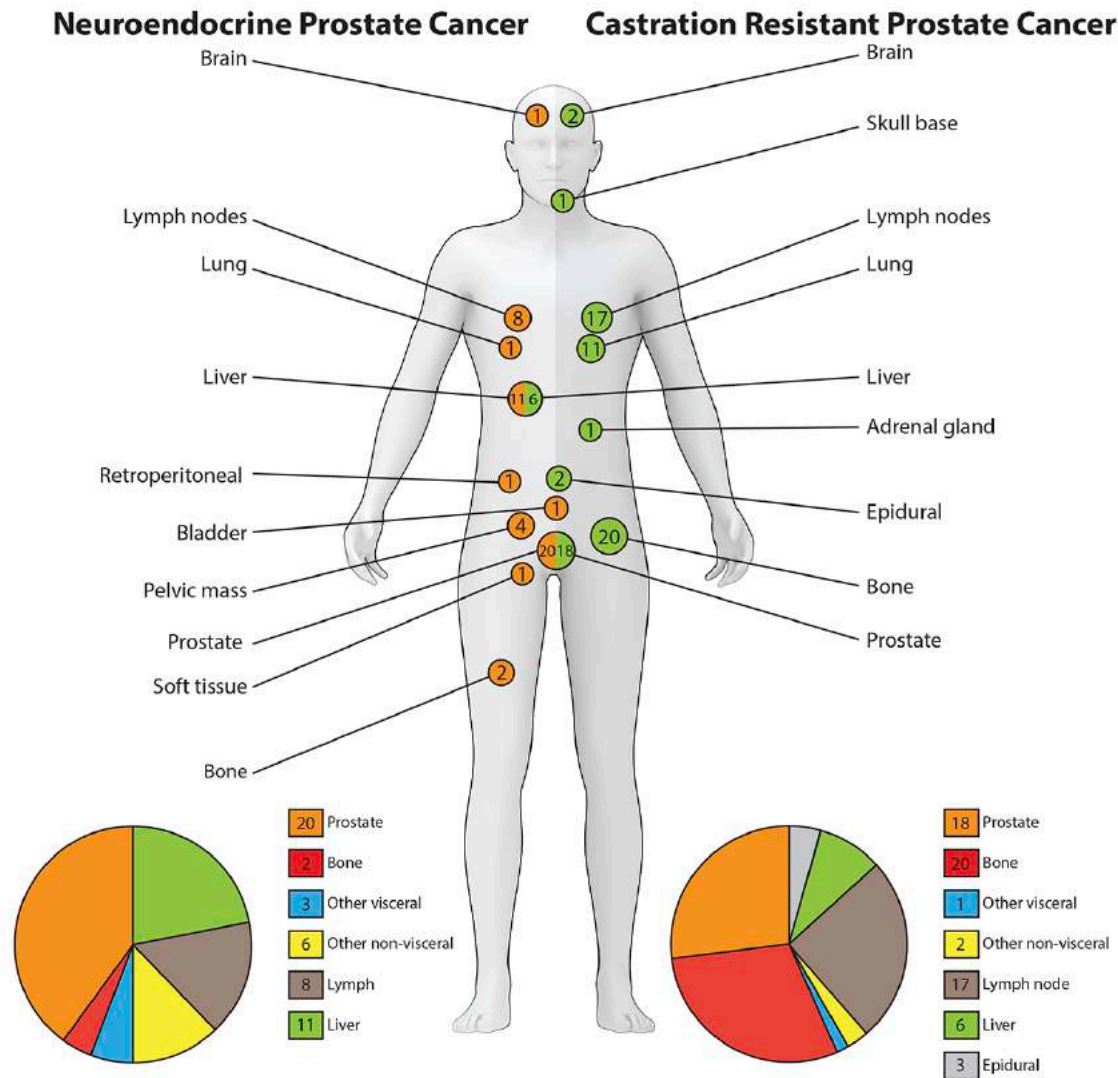
V. FROM
NEUROENDOCRINE
CELL



Abate-Shen and Shen,
Genes Dev 2000



NEPC is associated with distinct molecular features



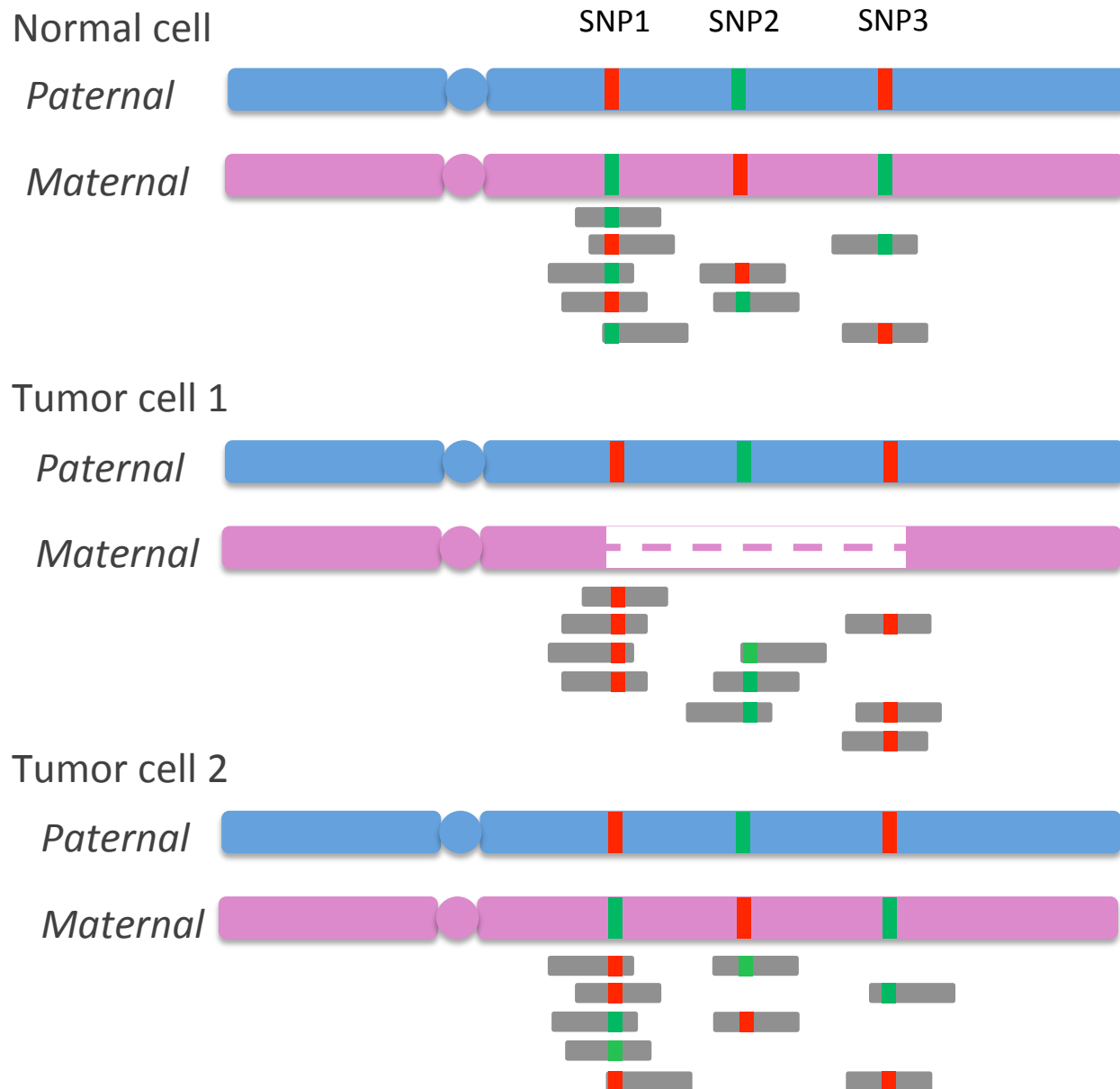
114 samples from 81 patients with metastatic CRPC (35 NEPC)

Blinded Path Review (Epstein et al, AJSP 2014)

Collaboration with H Beltran, MA Rubin (WCM) and L Garraway (Broad Institute)

Allelic Fraction (AF) Properties

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Informative SNPs

- reference base
- alternative base

Allelic Fraction

Proportion of reads supporting the reference base

Neutral Reads

Equally representing parental chromosomes

Beta

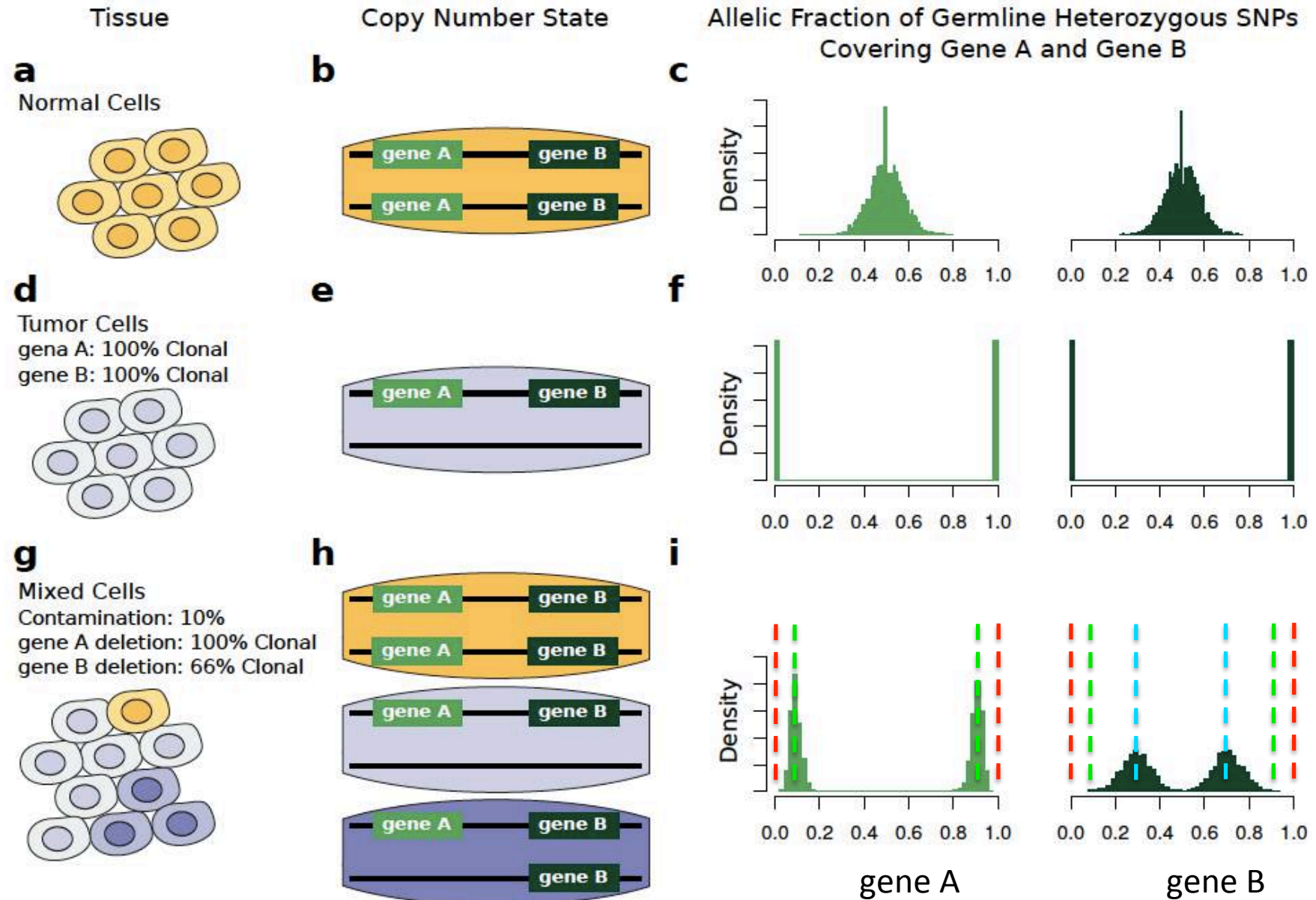
Percentage of neutral reads

Nref

Percentage of reference base in the not deleted allele

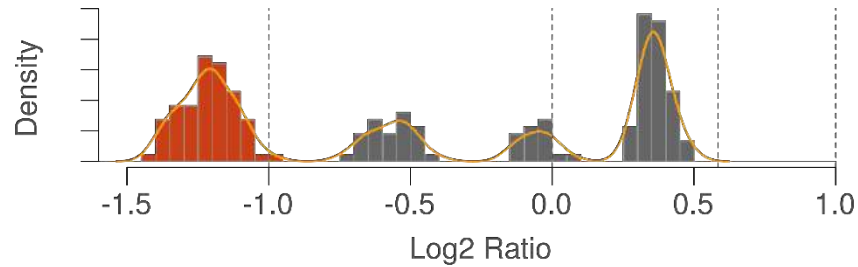
Allelic Fraction (AF) Distribution

NGS



Allele specific copy number space CLONET 2.0

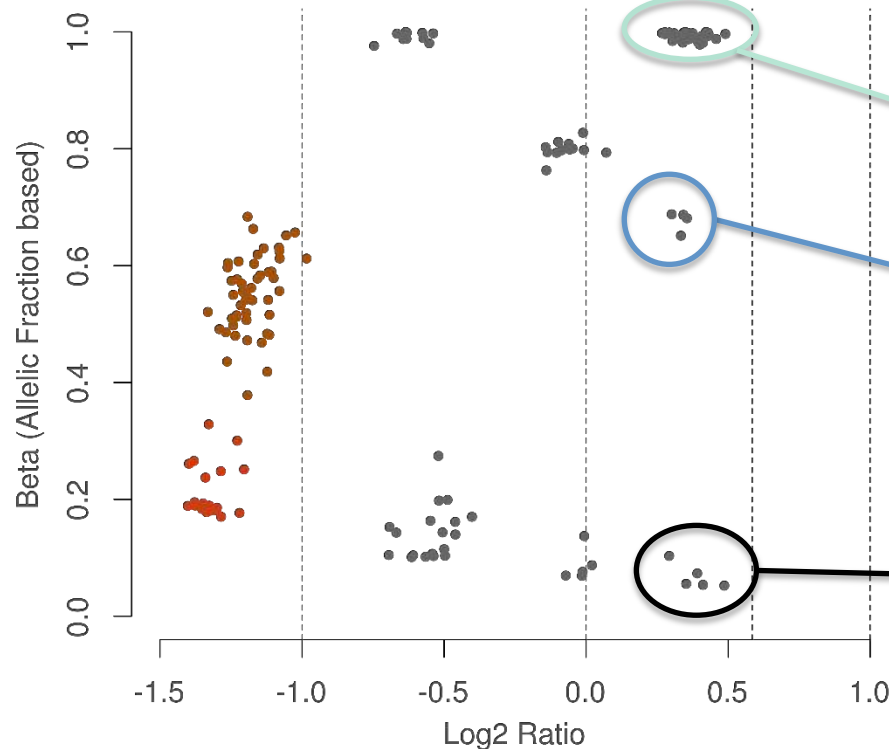
NGS



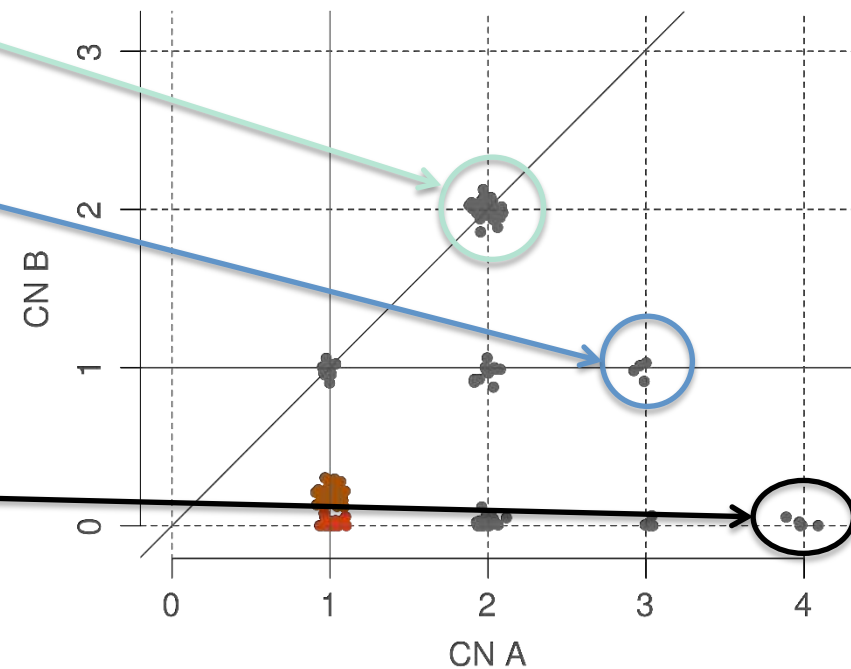
$$CN B = \frac{\beta 2^{Log2R} - G}{1 - G}$$

$$CN A = \frac{(2 - \beta)(\beta 2^{Log2R} - G) + 2G(1 - \beta)}{(1 - G)\beta}$$

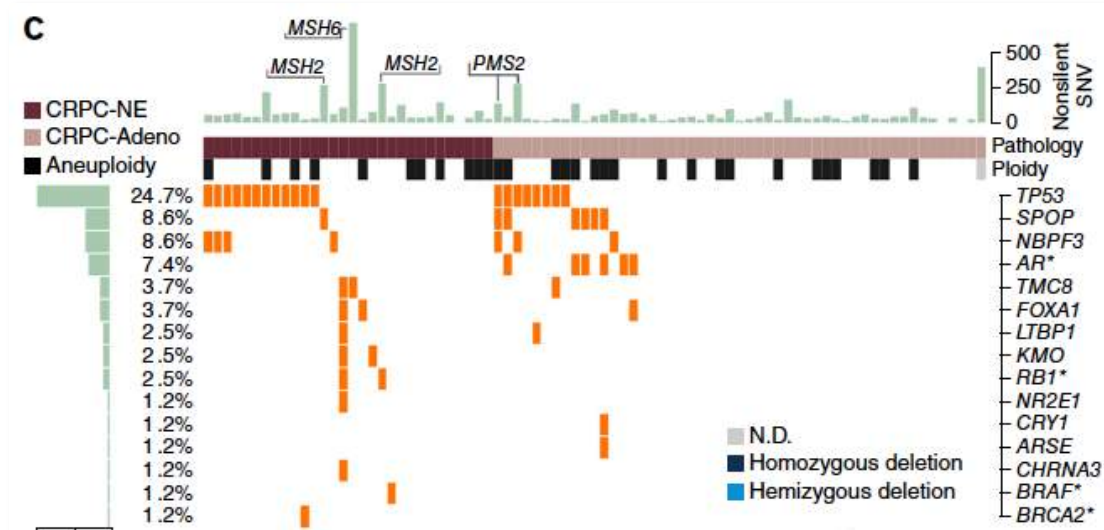
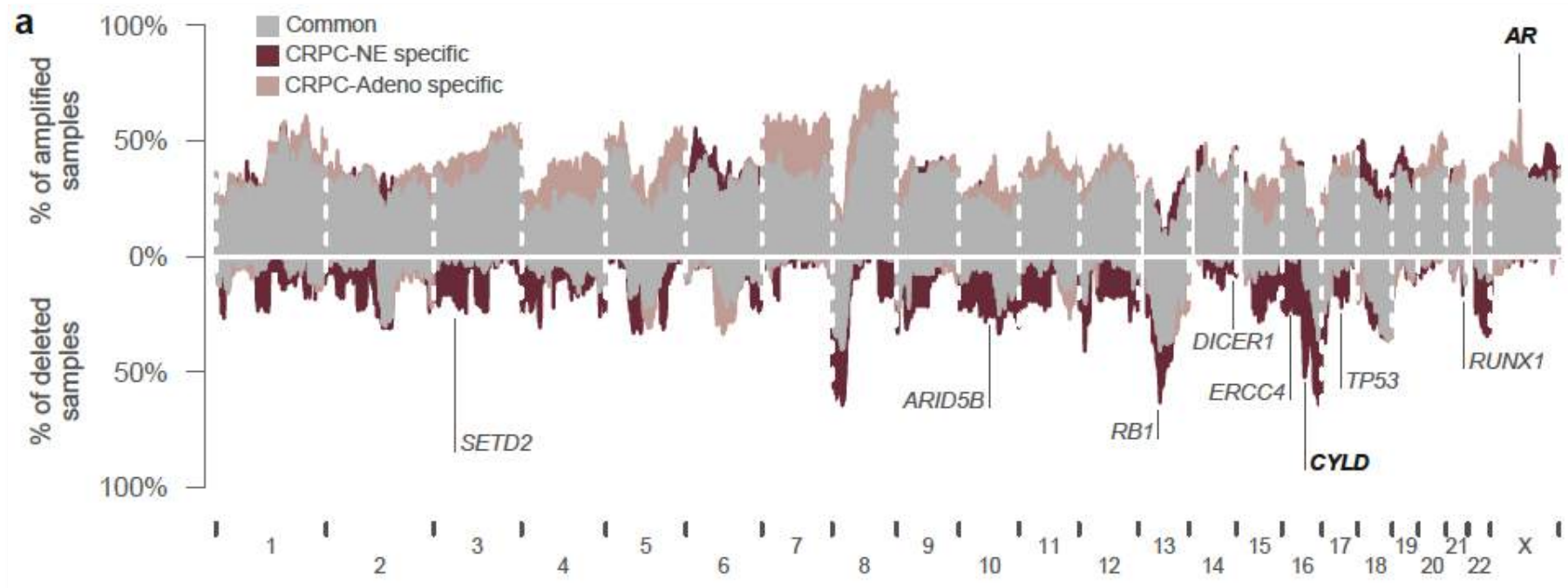
Estimated
CNA+CNB



where: $G = 1 - \text{Purity (CLONET)}$
 $CN A \geq CN B$, arbitrarily



CRPC-Adeno and NEPC profiles show extended overlap

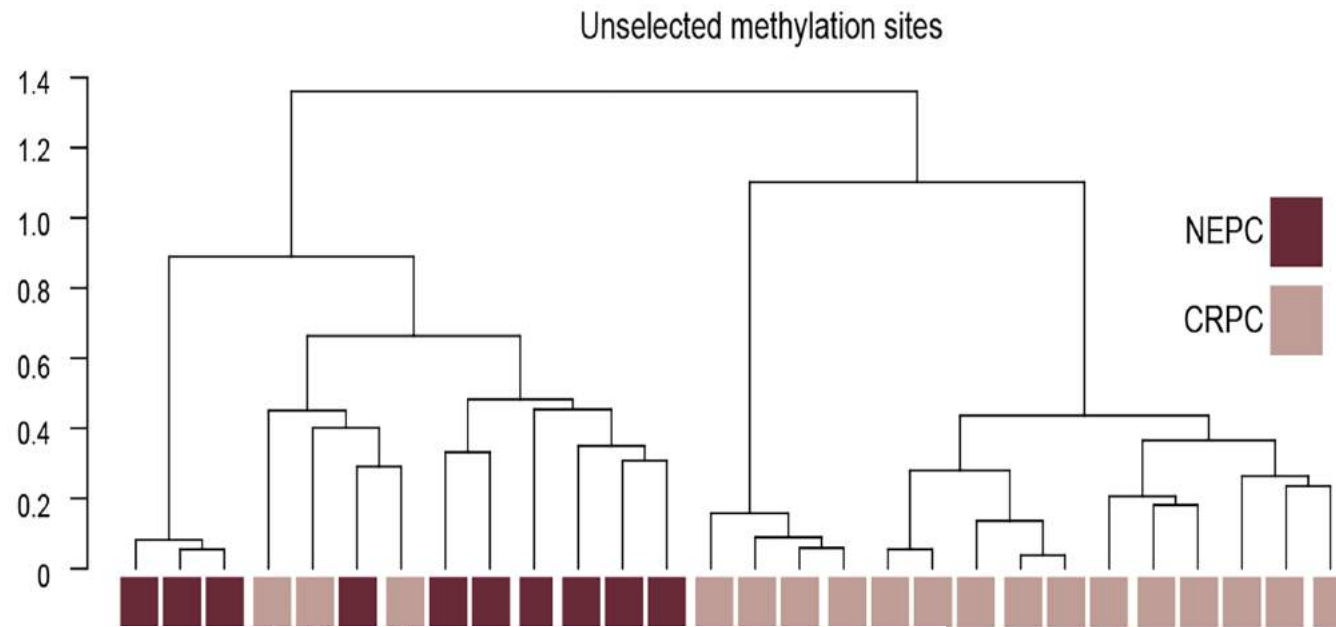


Mut. rates are no significant different

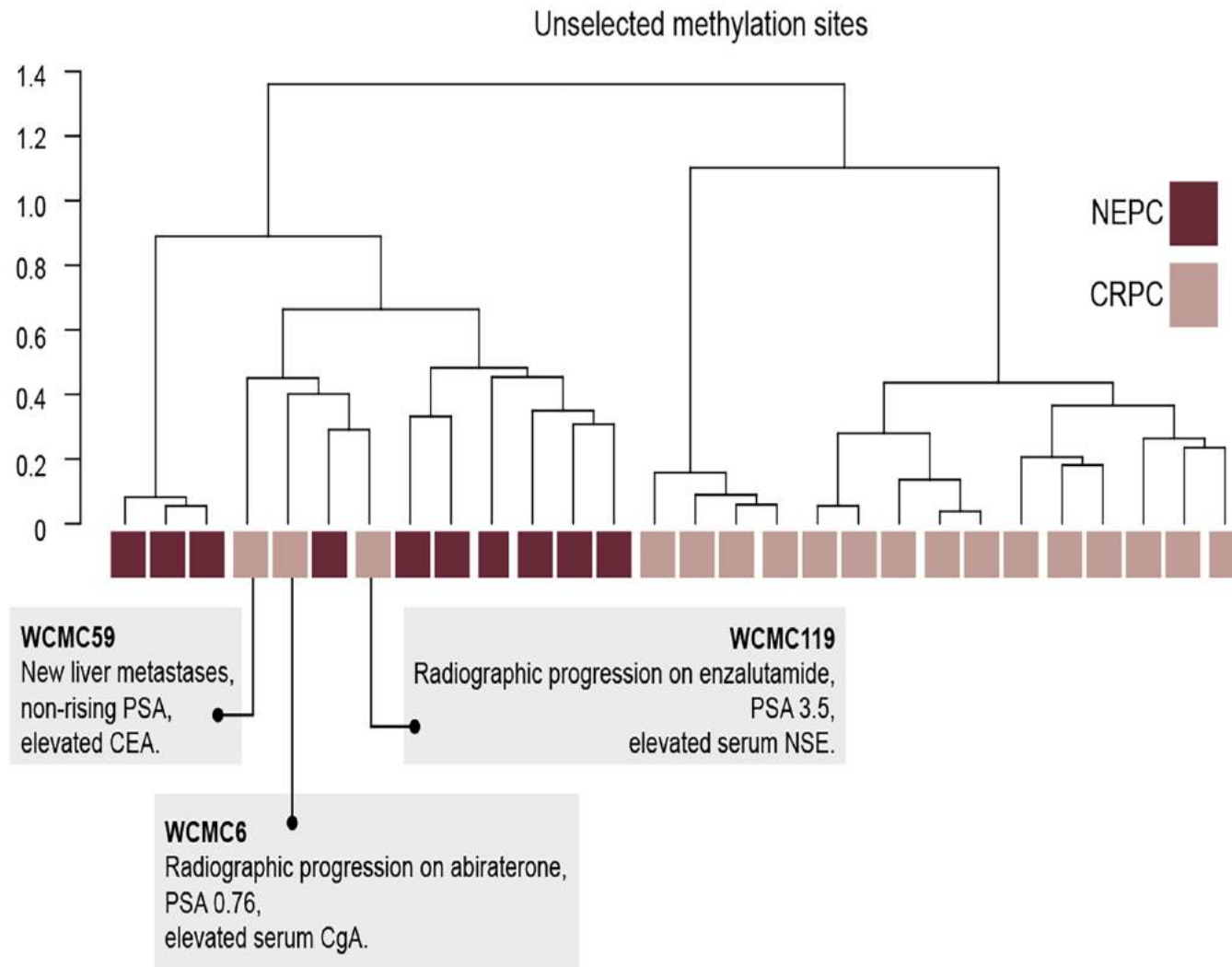
RB1 del: 70% CRPC-NE, 32% CRPC-Adeno, $p=0.003$

TP53 mut/del: 66.6% NEPC, 31.4% CRPC, $p=0.04$

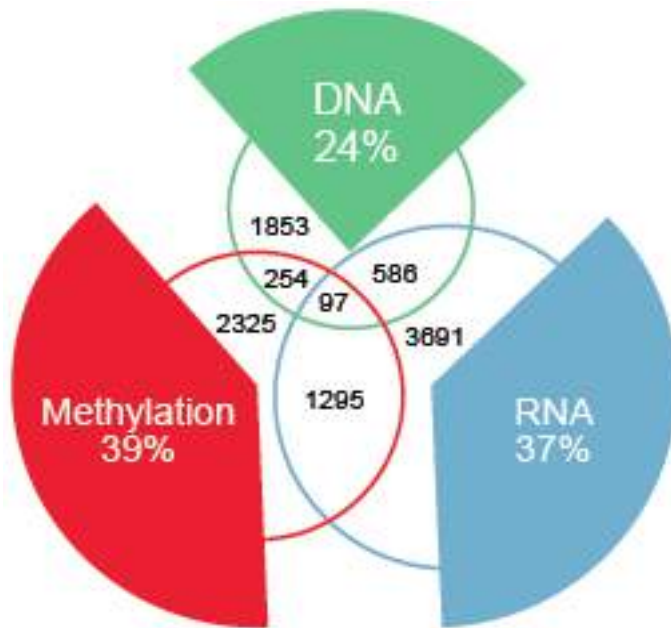
Unsupervised Analysis of Genome-wide CpG Methylation



Unsupervised Analysis of Genome-wide CpG Methylation

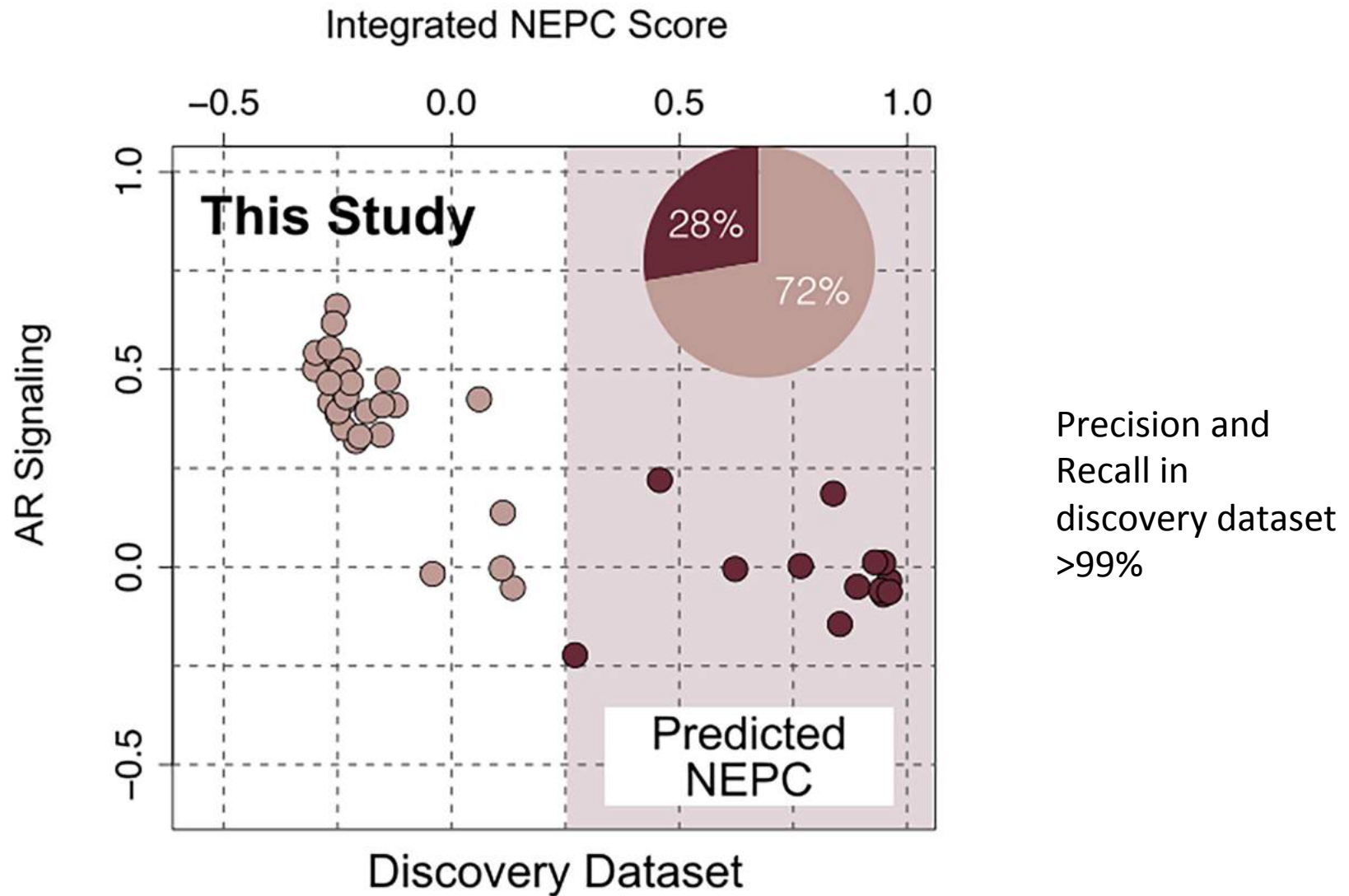


Development of a Molecular Classifier

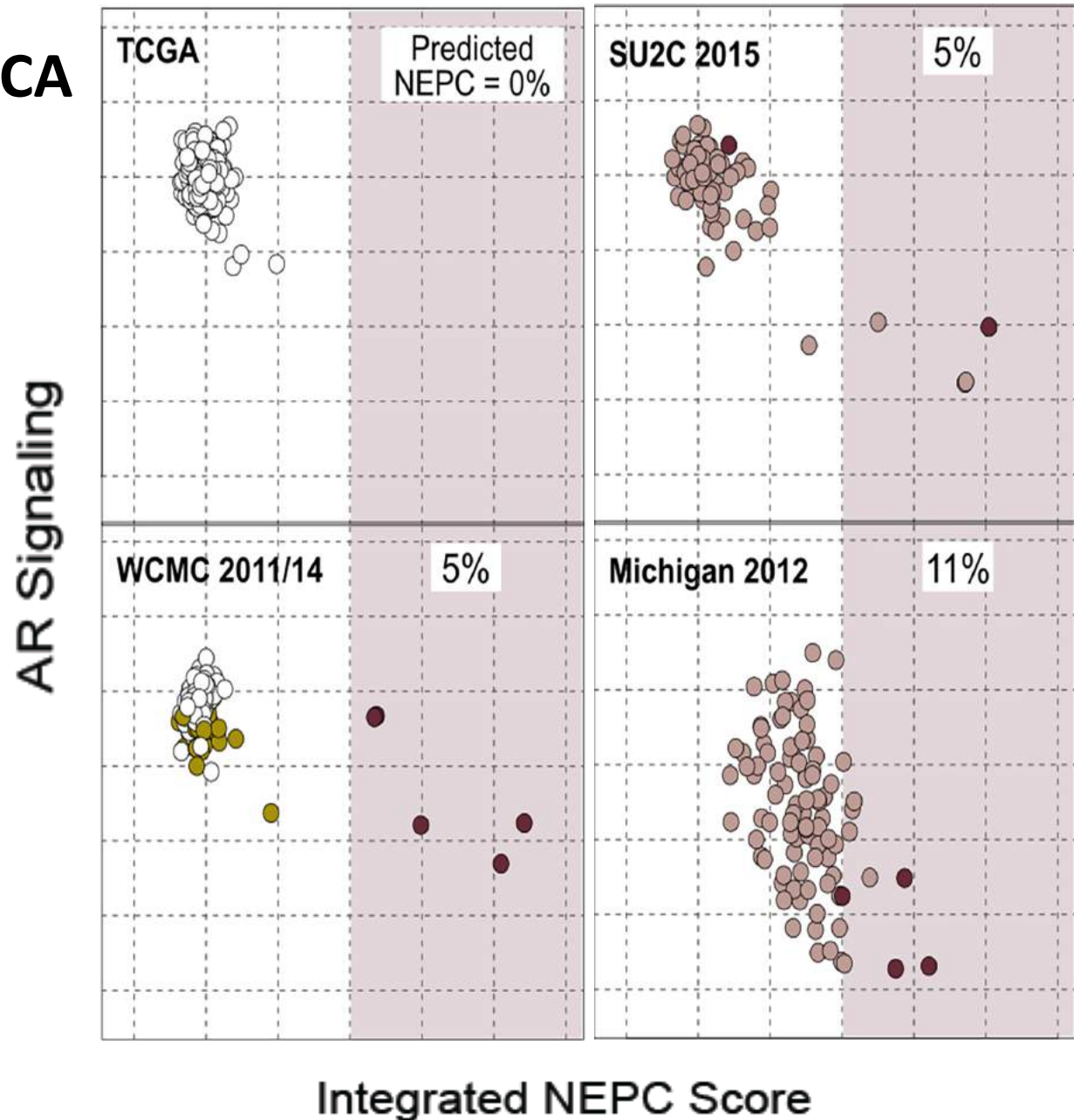


- By integration of key distinguishing features at DNA, methylation, mRNA level, we identified a 70 gene classifier of NEPC
- Validation cohort: TCGA, Michigan, Weill Cornell (WCMC), SU2C datasets

Development of a NEPC Classifier



Application of Classifier to other PCA Datasets (500 samples)



TCGA-PRAD, Cell 2015

SU2C, Robinson D, Van Allen E, Cell 2015

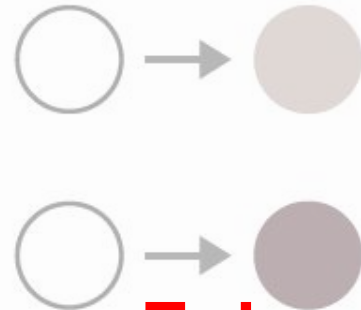
WCMC, Cancer Discovery 2011/Nat Comm 2014

Michigan, Grasso C et al, Nature 2012

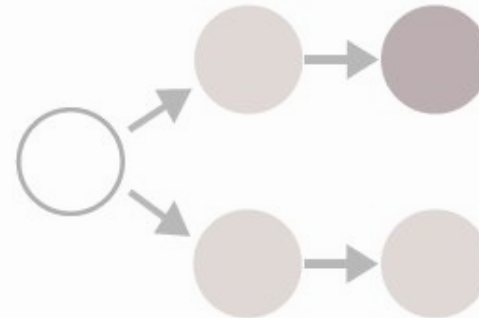
Evolution from CRPC-Adeno to CRPC-NE



I. INDEPENDENT
from PRIMARY

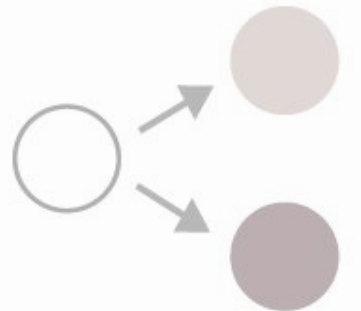


II. INDEPENDENT
from CRPC



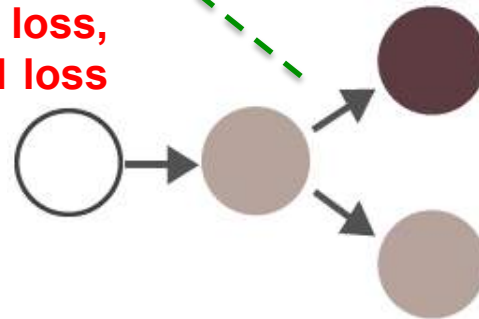
Epigenetic reprogramming

III. DIVERGENT
from PRIMARY



AR-wild-type,
TP53 loss,
RB1 loss

IV. DIVERGENT
from CRPC



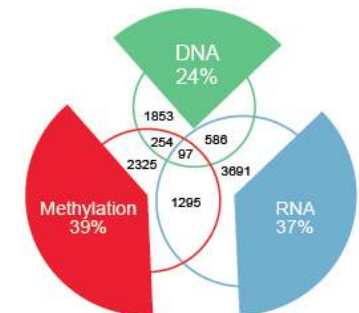
V. LINEAR

Overall, our data supports **Divergent Clonal Evolution** rather than linear progression or independent clonal evolution

Conclusions

NGS

- emerging subclass of advanced prostate cancer that undergoes neuroendocrine reprogramming during AR targeted therapy (“class switch” from CRPC to NEPC). **Cell Plasticity**
- clinical evaluation of the NEPC classifier should be tested as potential biomarker for early detection of AR independence and patient selection for co-targeting approaches in the advanced cancer setting. **Co-targeting**
- Further testing of the reversibility of the NEPC state with early intervention or genetic/epigenetic modifiers possibly with EZH2 inhibitors. **Epigenetic modifiers**
- larger prospective clinical evaluation to verify whether this classifier could be useful as **Non-Invasive potential prognostic or predictive biomarker** (associated with lack of response to AR therapies).



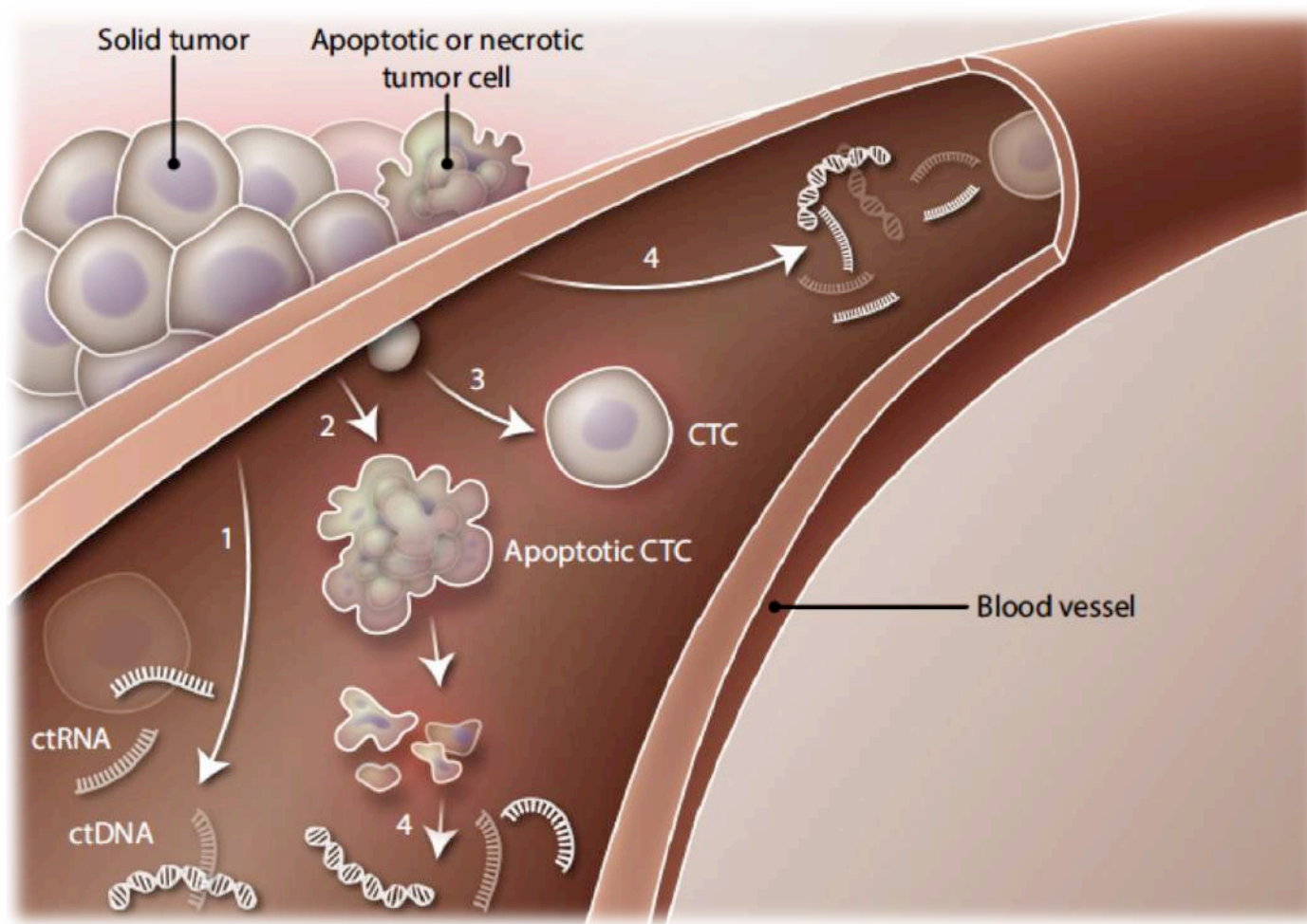
What is next

- **Early detection of trans-differentiation from circulating material (genomics + epigenetics)**
- **Non invasive test (LIQUID BIOPSY)**

Funded through Challenge Award with Misha Beltran (WCM) and Gert Attard (ICR)

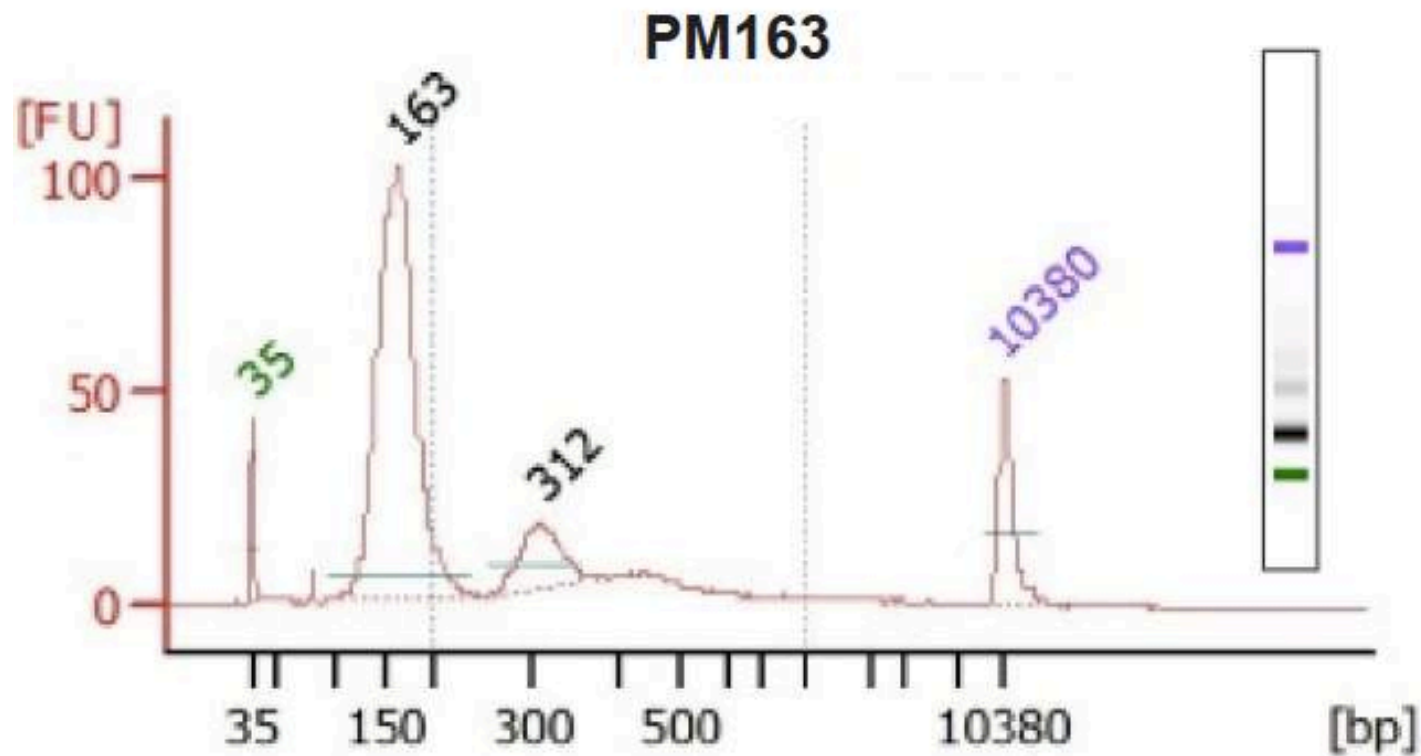


What is next



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

Bioanalyzer of plasma DNA from CRPC patient



DNA amount ranges between 5 and 300ng (per ml)

Jenny Xiang WCM

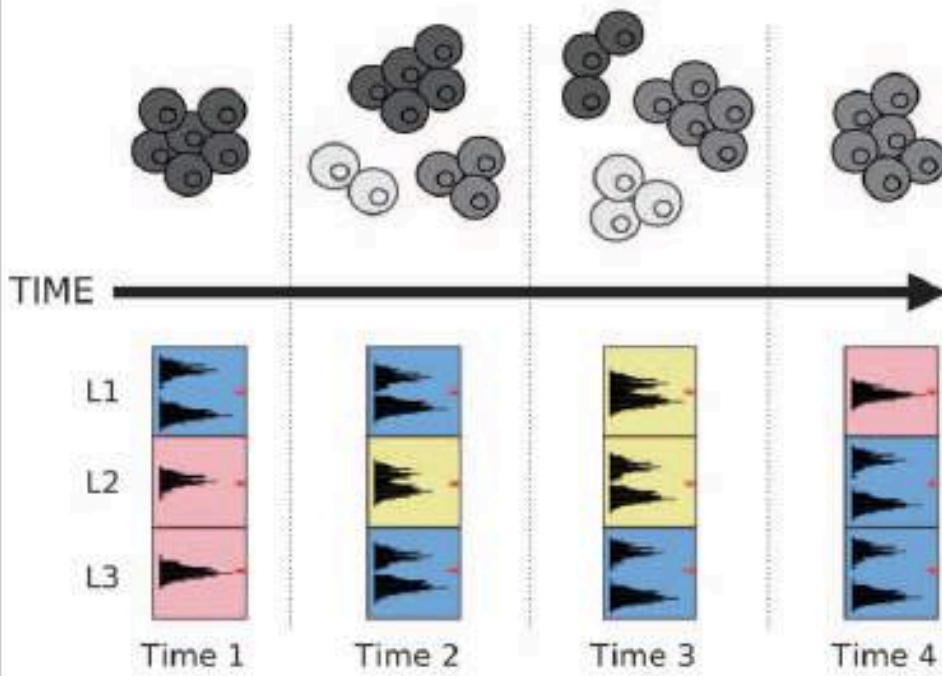


Different tumor clones releasing DNA in plasma

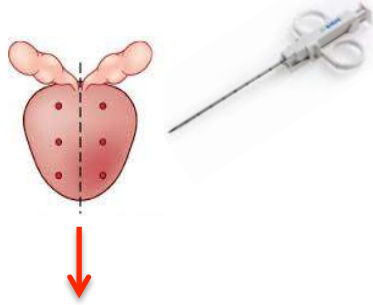
● L1.del/L2.no.del/L3.no.del

● L1.no.del,L2.del,L3.del

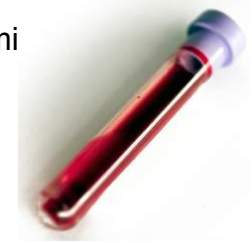
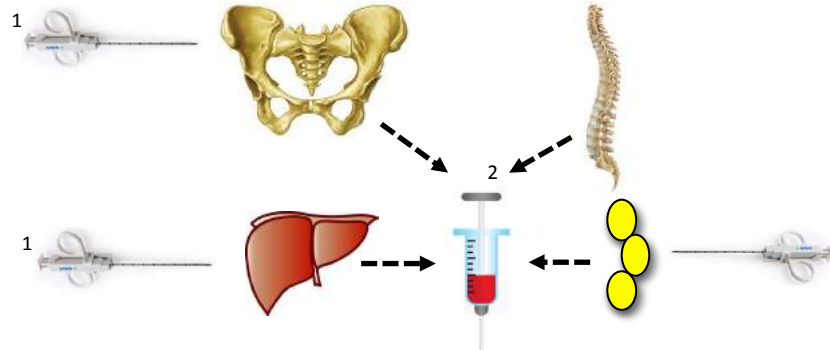
○ L1.no.del,L2.no.del,L3.del



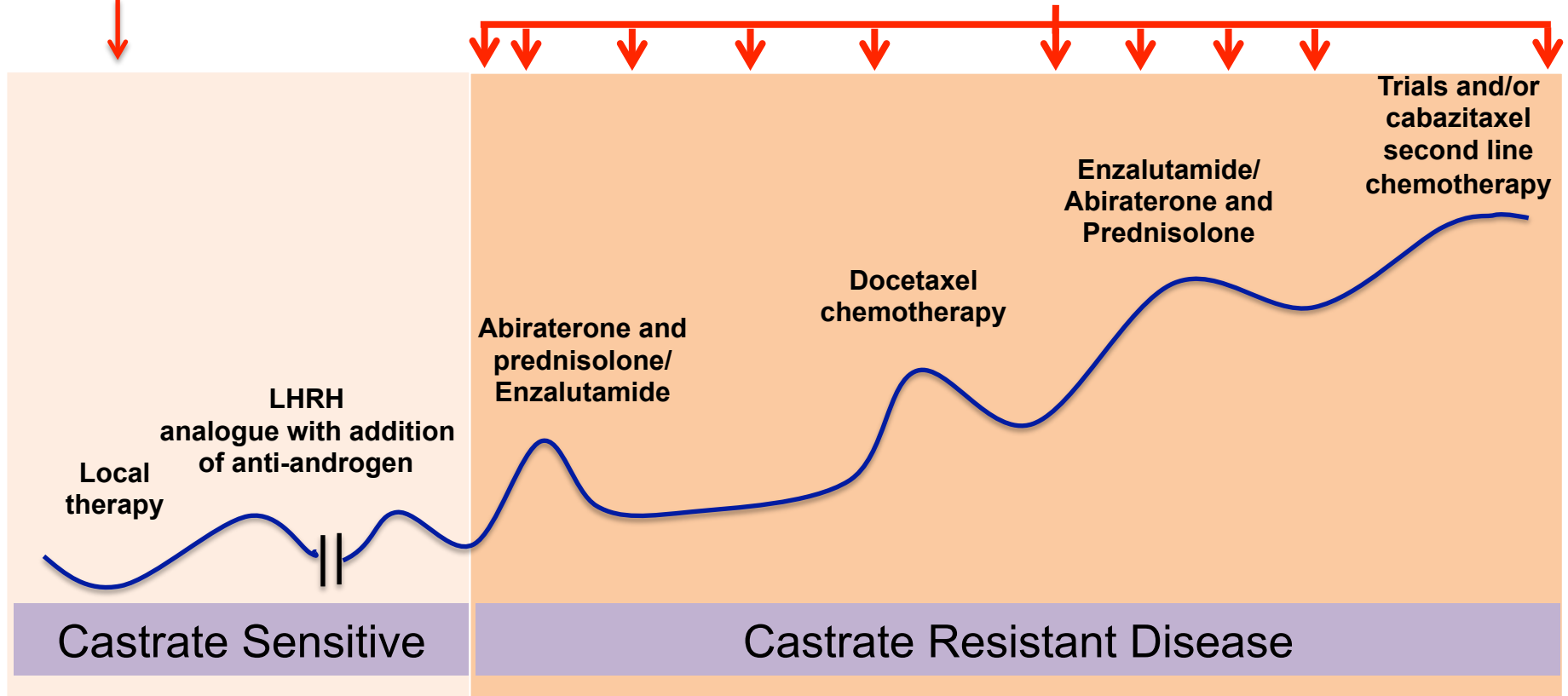
Pre-Castration samples
(TRBP or cores from prostatectomy samples)



CRPC samples (Tumor biopsies or plasma containing genomic material from multiple metastases)



Tumour Volume and Activity

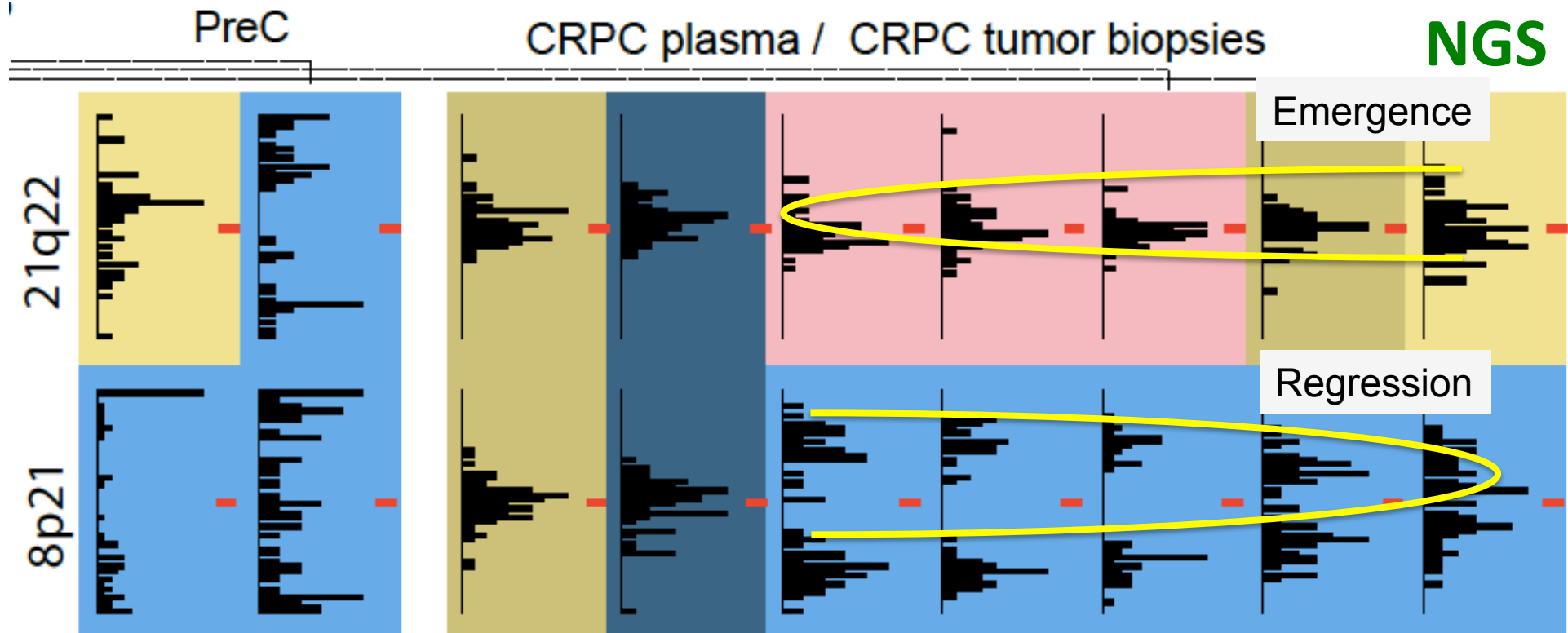


Castrate Sensitive

Castrate Resistant Disease

Time

Following copy number aberrations over time



NGS

- Clonal DNA loss with AF bimodality
- Clonal DNA loss without AF bimodality
- Subclonal DNA loss with AF bimodality
- Subclonal DNA loss without AF bimodality
- No evidence of lesion
- ▲ CRPC tumor biopsy

Computational framework for plasma DNA based assays



Progression on Abiraterone



Start Cabazitaxel

Prostate cancer specific targeted assay to study dynamics across serial plasma samples



NGS

- **Design:** Prostate Cancer *ad hoc* design; Adequate coverage **>1,000X**; Capability to quantify presence of aberrations;
- **Test:** Assessment of detection performance as function of lesion allele frequency, sequencing depth, and lesion genomic size;
- **Samples:** ~ **350 plasma samples** from ~ **110 CRPC patients**

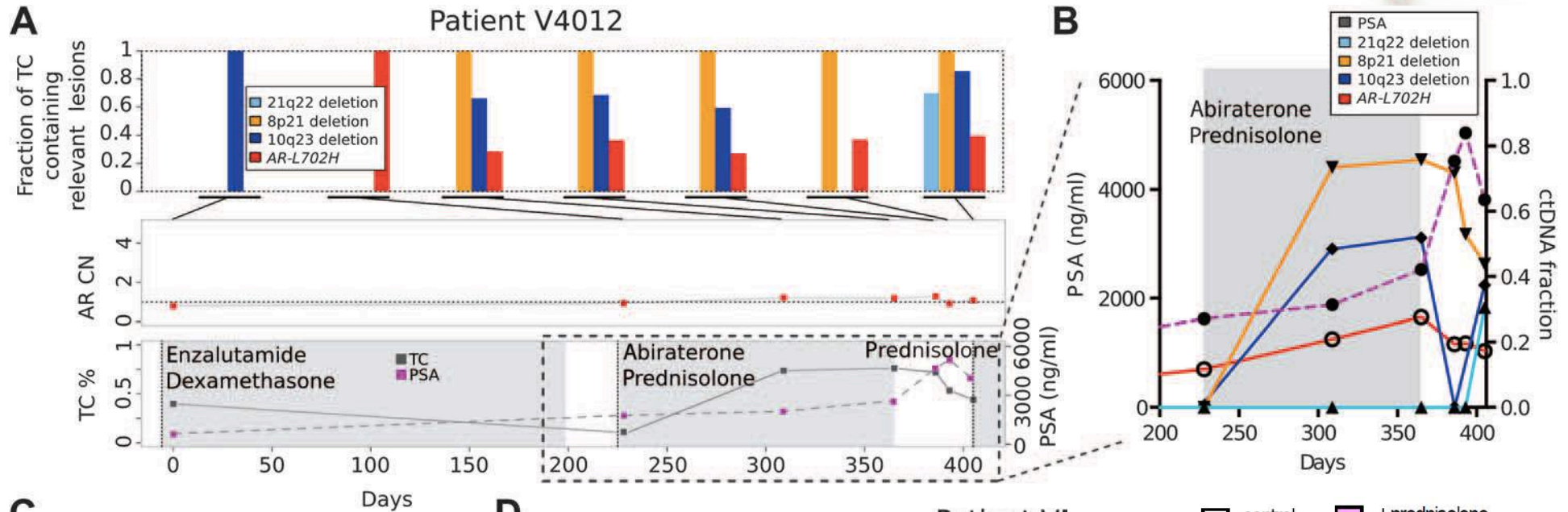
Baca S *et al*, Cell 2013

Prandi D *et al*, Genome Biology 2014

TCGA-PRAD, Cell 2015

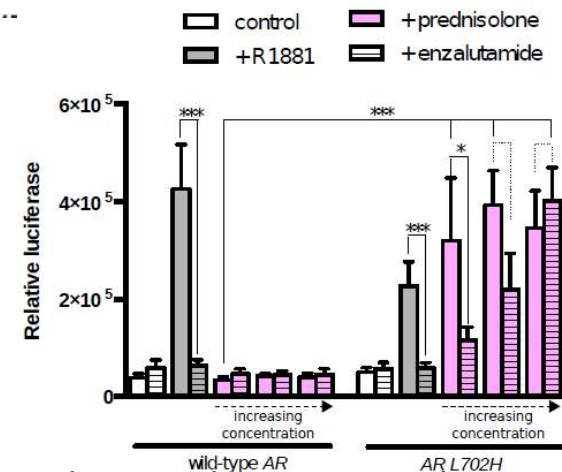
Collaboration with Gert Attard
Royal Marsden (London)

Result 1: Emergence of AR-L702H on Abiraterone



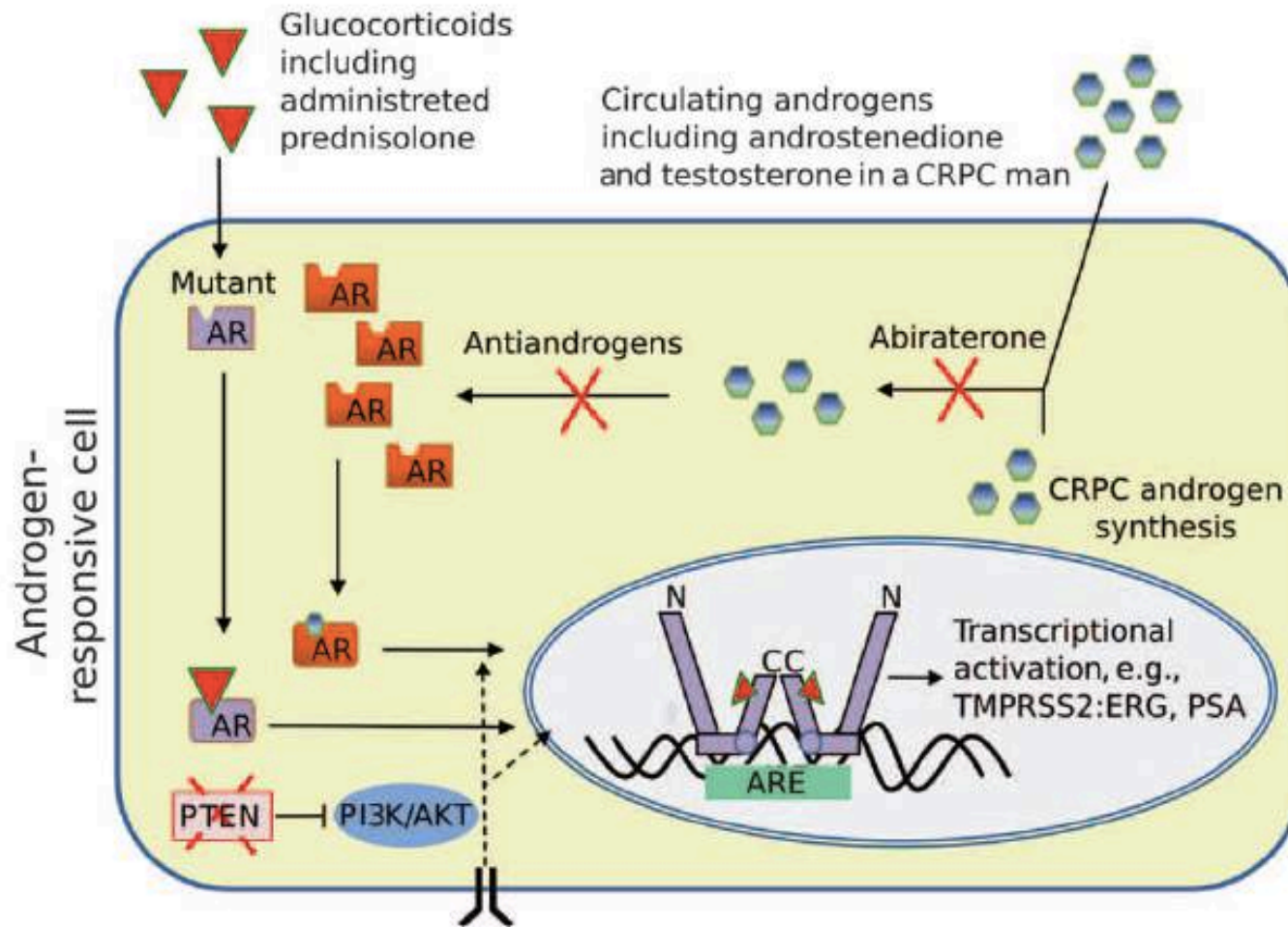
L702H mutation was first detected on treatment with glucocorticoids combined with **enzalutamide**

Subsequent treatment was associated with **primary resistance** and progressive increase of AR-L702H



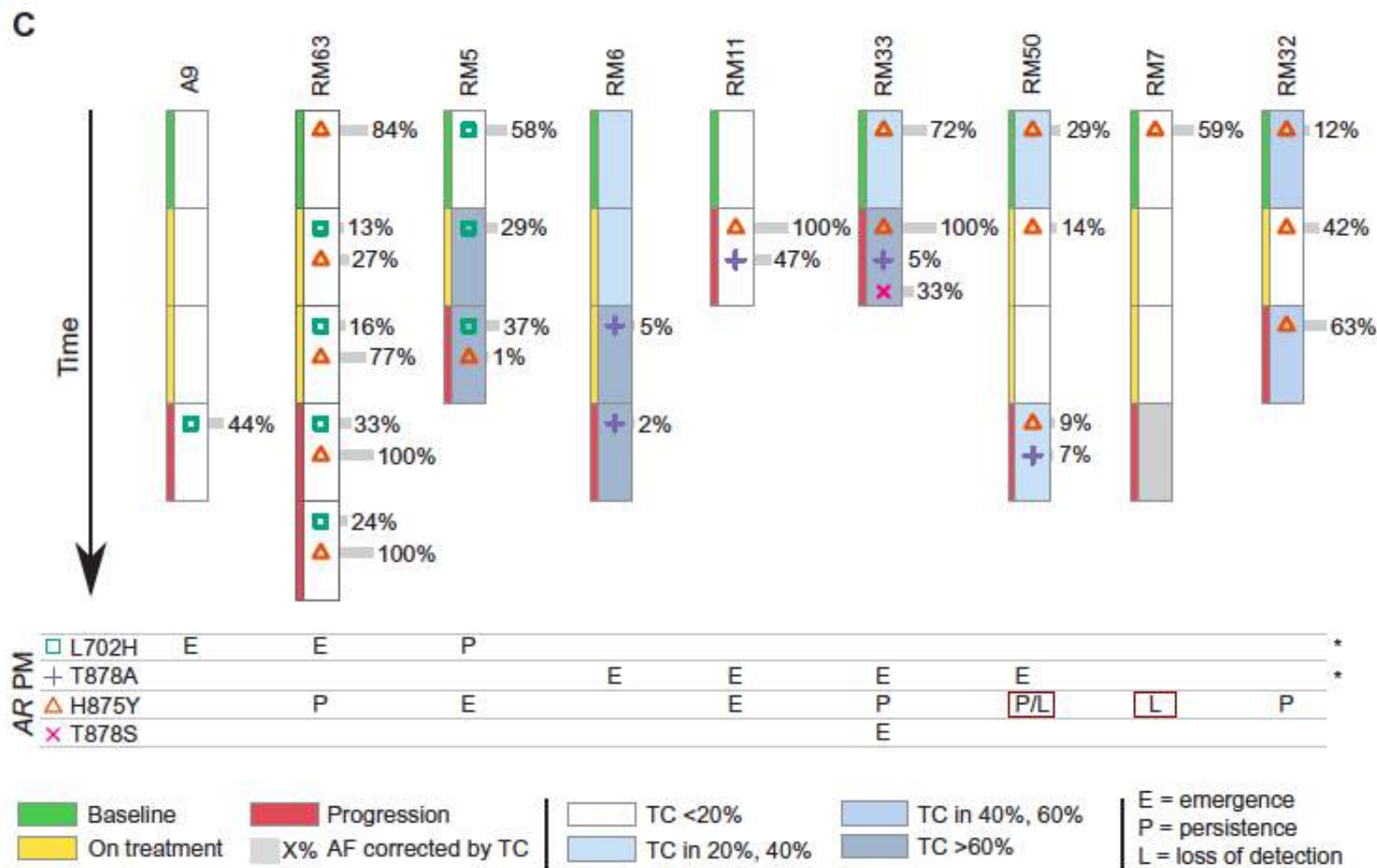
Zhao et al, Nature Med 2000
Richards et al, Cancer Res 2012

Result 1: AR-L702H causes resistance to abi/enza when given with prednisolone



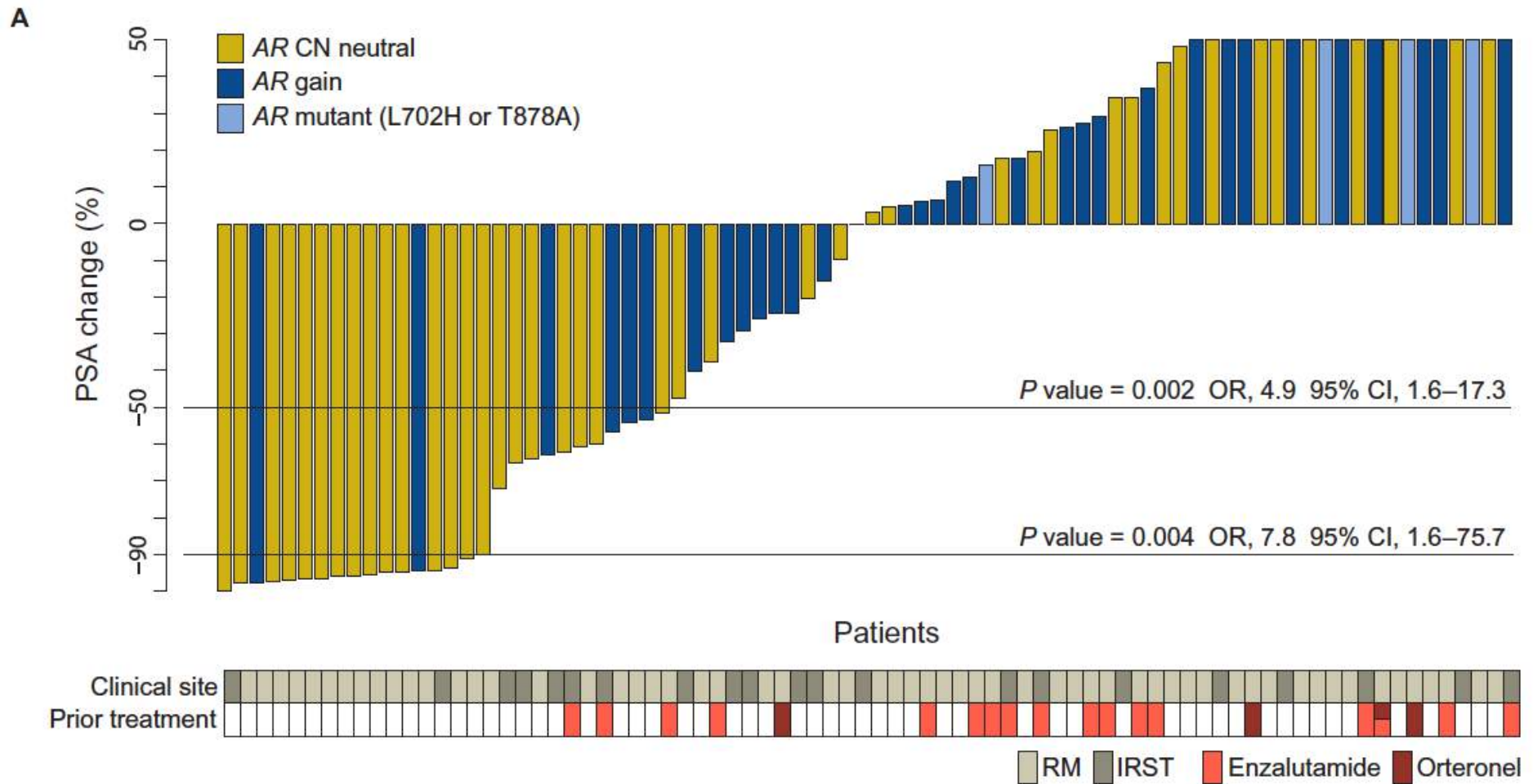
Inhibition of wild-type AR signaling by enzalutamide or abiraterone with bypass activation of mutant AR by glucocorticoids, such as exogenous prednisolone.

Result 1: AR SNVs on Abiraterone (N=59)

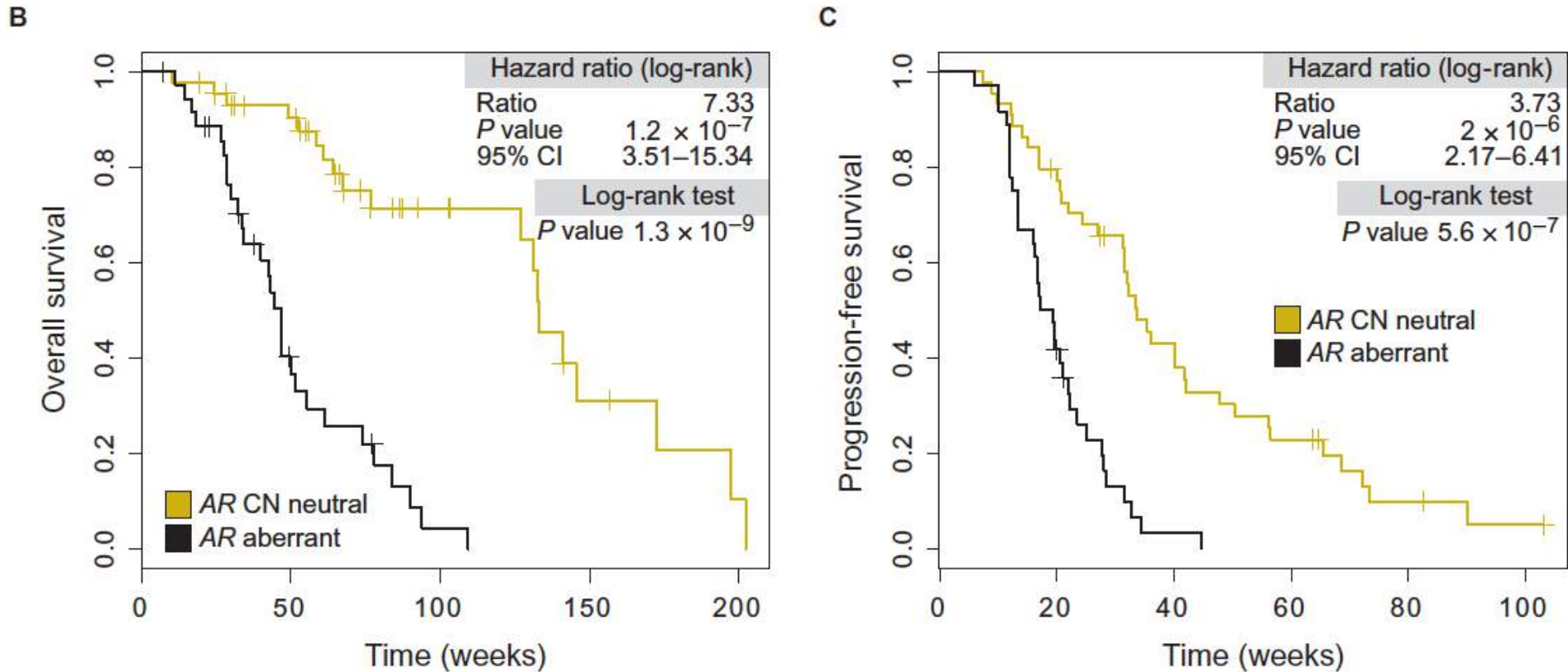


L702H or T878A show a temporal relationship with progression in 16%

Result 2: Association of AR gene status prior to Abiraterone treatment with outcome (N=80)



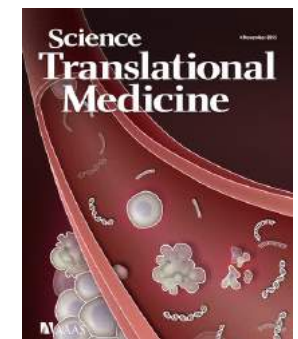
Result 2: Association of AR gene status prior to abiraterone treatment with outcome (N=80)



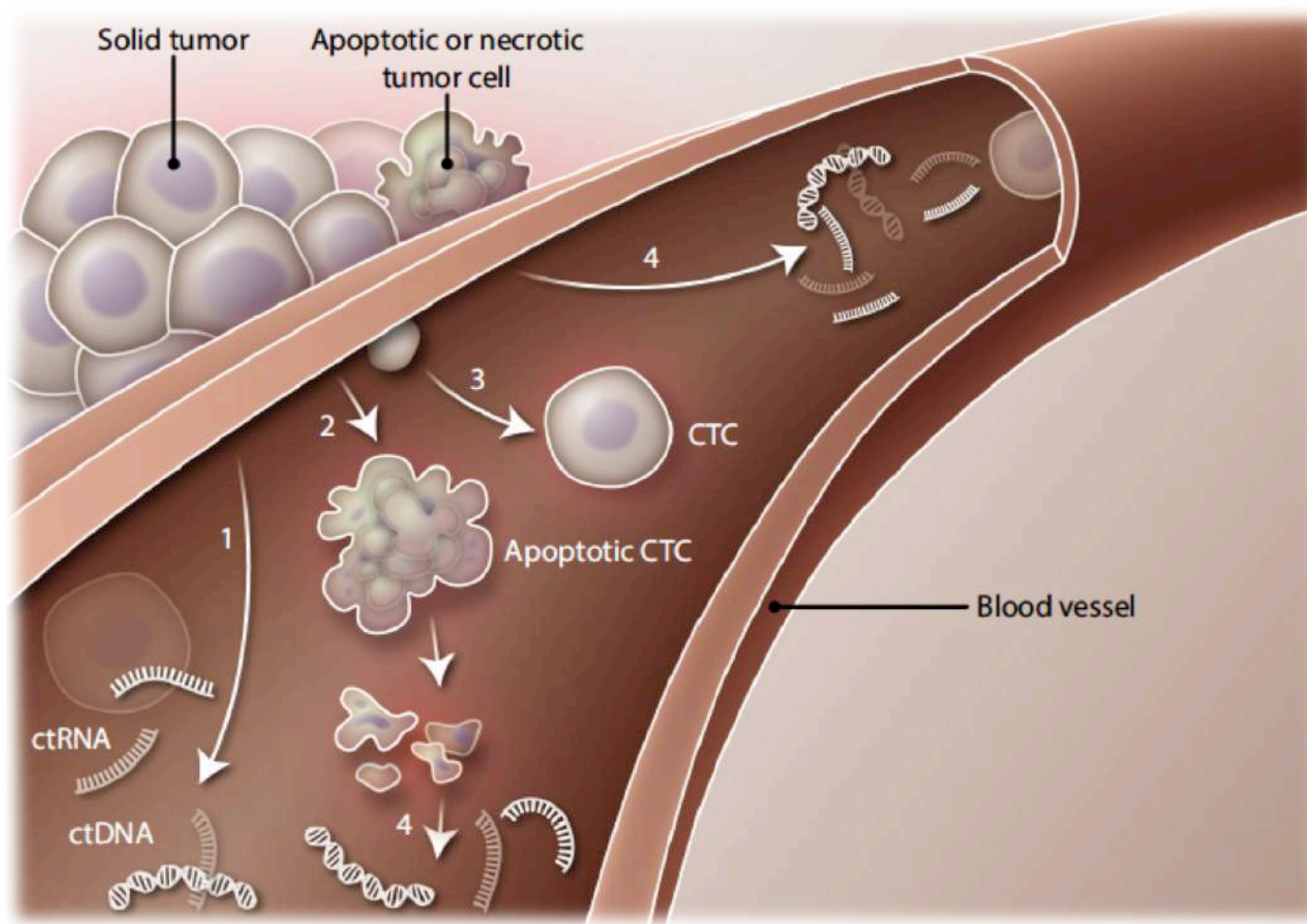
Carreira S, Romanel A, et al. *Sci Transl Med*. 2014 Sep 17;6(254):254ra125.

Romanel A, Gasi Tandefelt D, et al. *Sci Transl Med*. 2015 Nov 4;7(312):312re10.

Wyatt A, *Jama Oncology* 2016

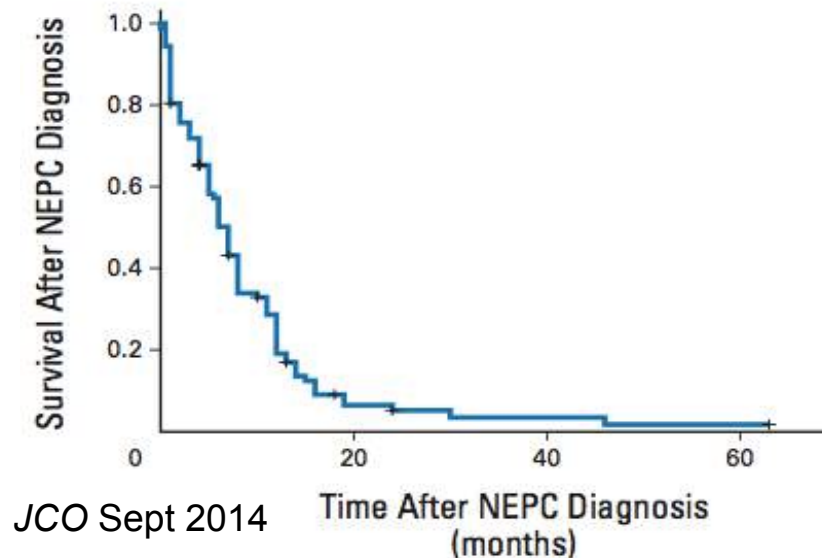


What is next



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

- Towards the development of a blood based test for advanced prostate cancer patients as part of a multi-institutional effort
- 1-10ml of blood draw during treatment to promptly detect tumor trans-differentiation



NGS

ctDNA PCF SELECT

A clinically- applicable, custom next generation sequencing plasma DNA assay optimised for treatment selection and response surrogacy in metastatic prostate cancer

Anticipated outcomes

Establishment of an academic-industry partnership to commercialise the ctDNA PCF SELECT assay and design and initiate prospective trials for clinical qualification.

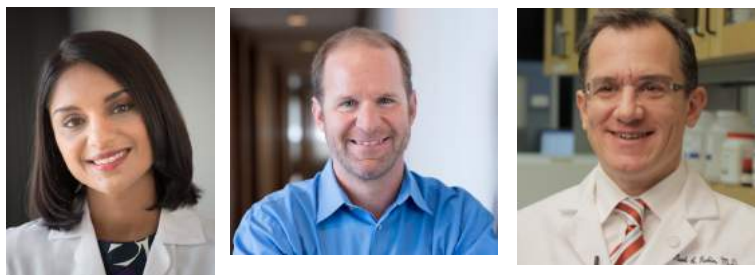
Impact of the project

The implementation of a plasma DNA test for metastatic prostate cancer into widespread clinical practice within 5 years.

Funded by Movember/PCF (Attard (The Institute of Cancer Research, London), Beltran and Rubin (Weill Cornell Medicine), Demichelis (University of Trento), Chi and Wyatt (University of British Columbia), Van Allen (Dana Farber Cancer Institute), Maher (Washington University))



Weill Cornell Medicine



LaBSSAH (UNITN)

Veronica De Sanctis
Roberto Bertorelli



ICR UK

Gert Attard
Johann De Bono

INT (Milan)

Nadia Zaffaroni
Riccardo Valdagni



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FDA News Release

FDA approves first blood test to detect gene mutation associated with non-small cell lung cancer



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PRINT

**For Immediate
Release**

June 1, 2016

Release

The U.S. Food and Drug Administration today approved the cobas EGFR Mutation Test v2, a blood-based companion diagnostic for the cancer drug Tarceva (erlotinib). This is the first FDA-approved, blood-based genetic test that can detect epidermal growth factor receptor (EGFR) gene mutations in non-small cell lung cancer patients. Such mutations are present in approximately 10-20 percent of non-small cell lung cancers (NSCLC).