Mechanisms of Resistance and Neuroendocrine Differentiation in Prostate Cancer

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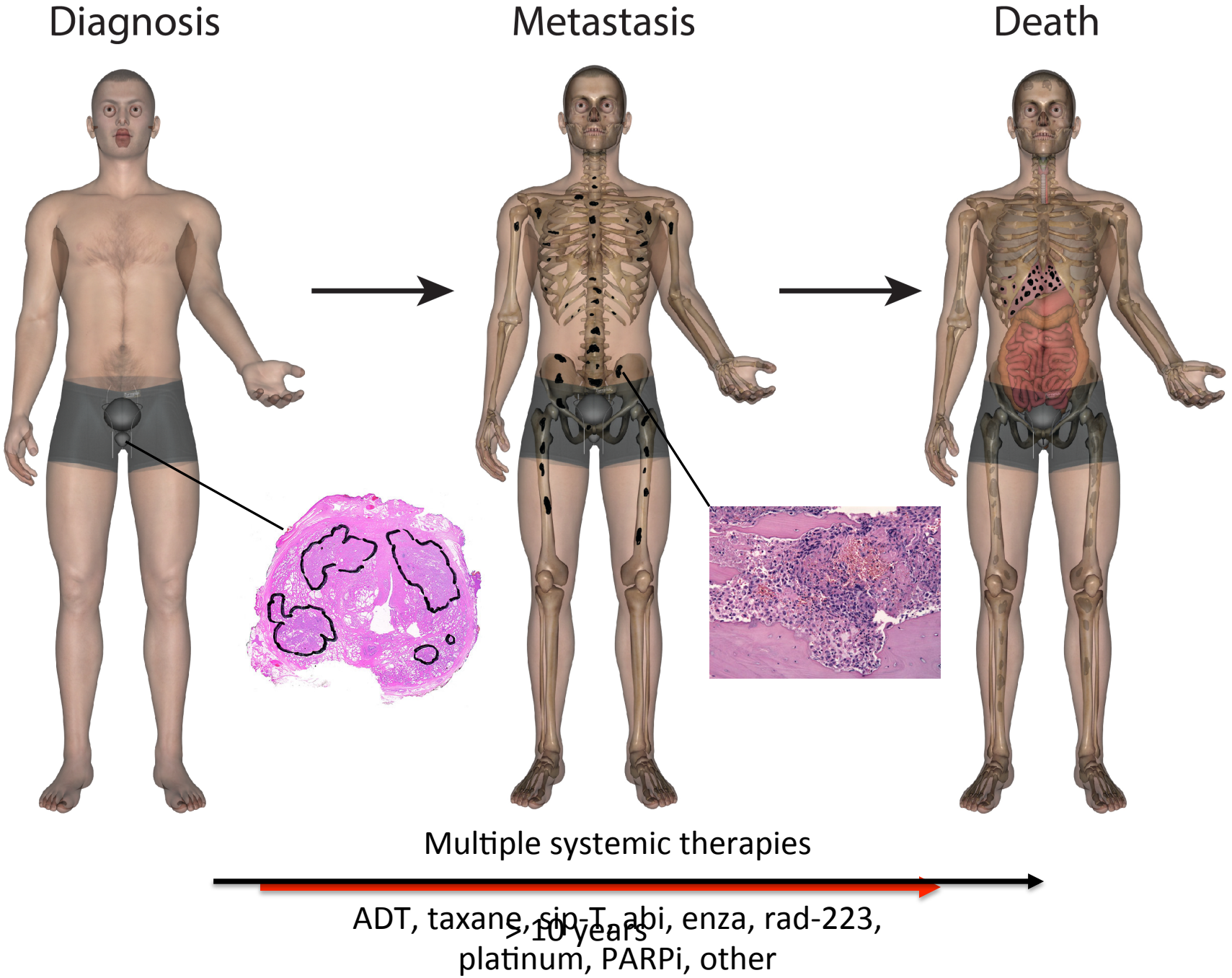
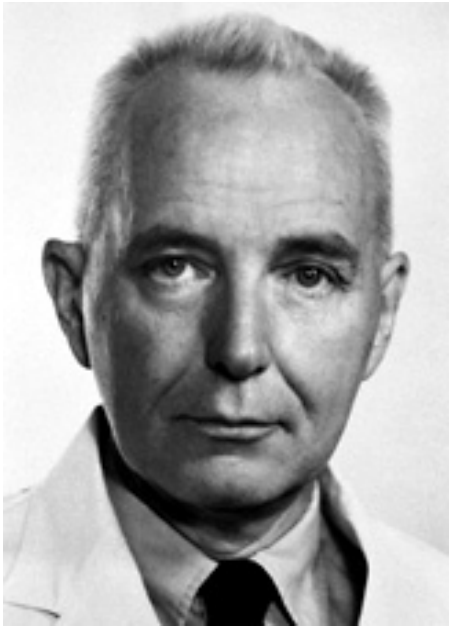

Diagnosis
Metastasis
Death
Multiple systemic therapies
ADT, taxane, sip-T, abi, enza, rad-223,
platinum, PARPi, other
>10 years



Charles Huggins and Clarence Hogdes recognized as an androgen -sensitive disease in 1941

21 patients metastatic prostate cancer-> orchiectomy or administration of phenolic estrogens (stibestrol) resulted in regression in prostate cancer, extended life span, and decreased man-pain hours

Huggins et al, Cancer Res 1941
Huggins et al, Arch Surg 1941

AR still active in the castration resistant setting

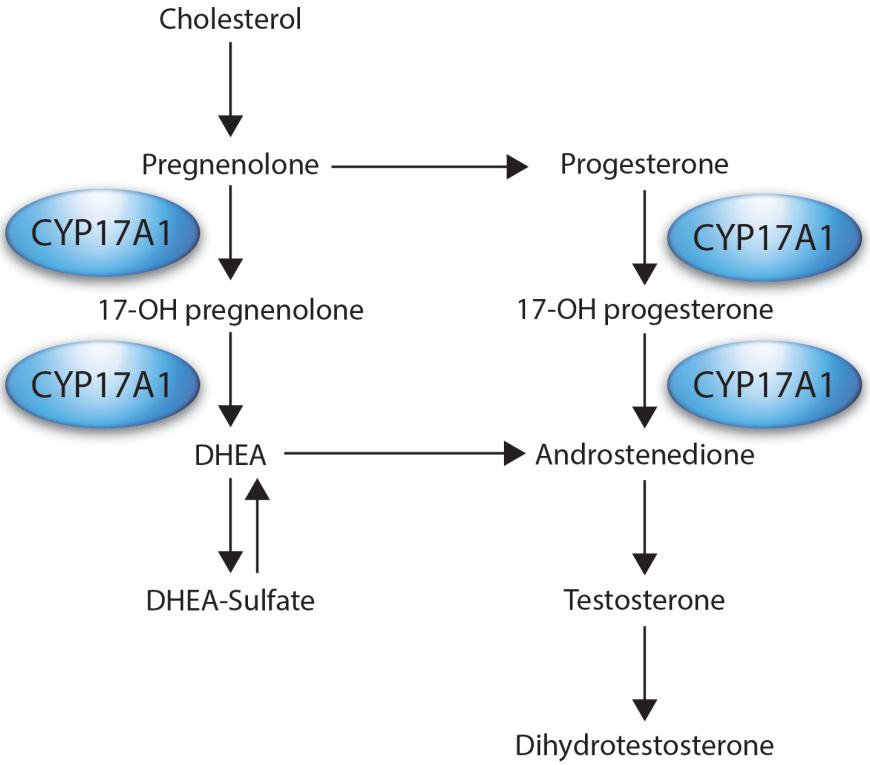
- Androgen receptor expression persists
- AR signaling drives tumor growth and treatment resistance

Castration resistant prostate cancers are not truly "hormone refractory"

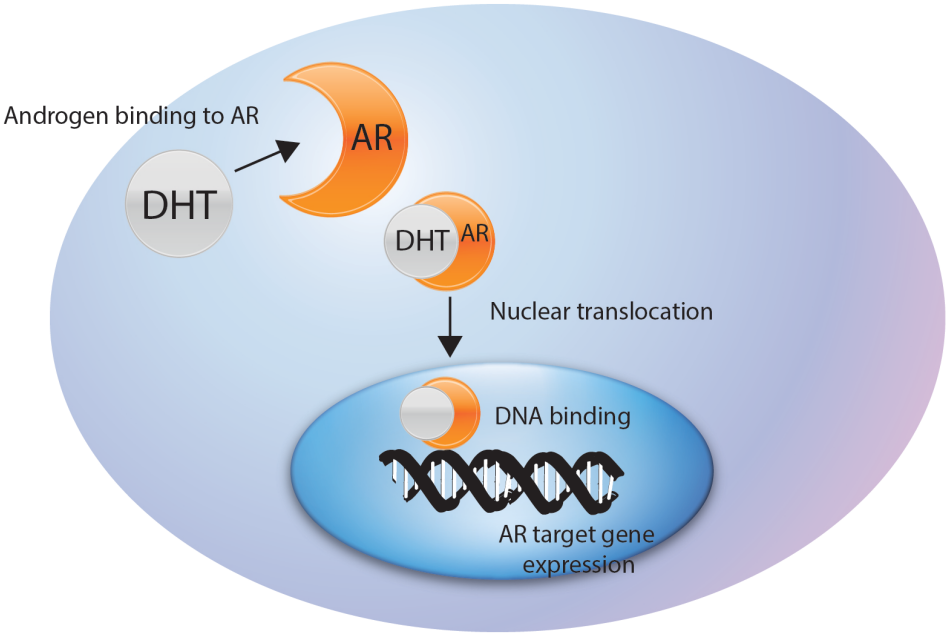
Chen and Sawyers, Nature Medicine 2004

Therefore the mainstay of therapy for CRPC= agents that target the AR

Abiraterone inhibits androgen synthesis



Enzalutamide is a potent AR antagonist



What does resistant prostate cancer look like?

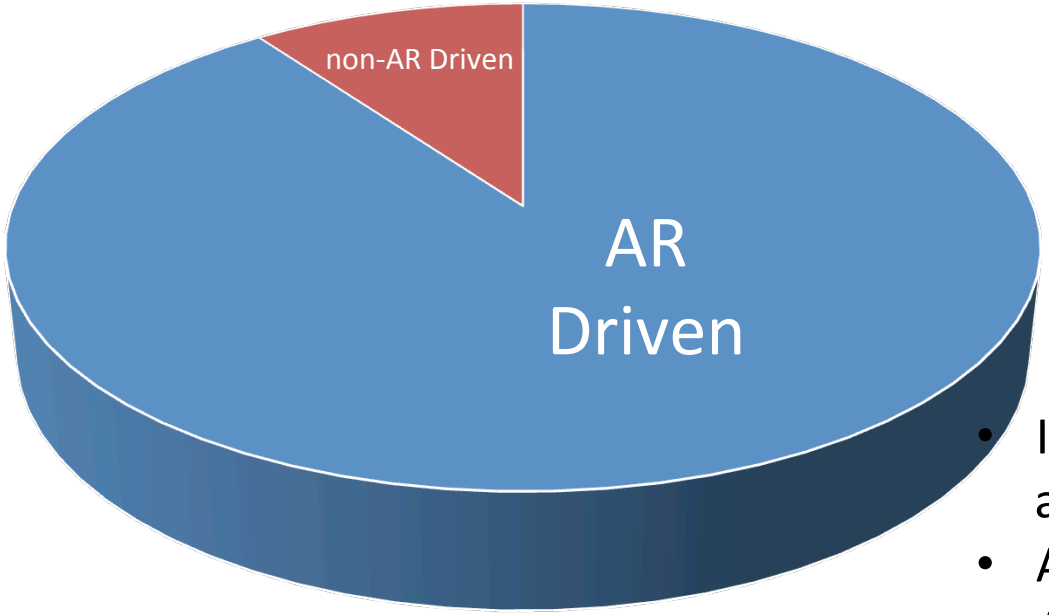
Group 1:

- Gradually progressive: bone, LN mets, rising PSA
- Initially responsive to potent ADT

Group 2:

- Resistant/refractory
- Rapidly progressive: visceral mets, low or non-rising PSA

Treatment resistance after primary and secondary hormonal therapies involves re-activation of the androgen receptor

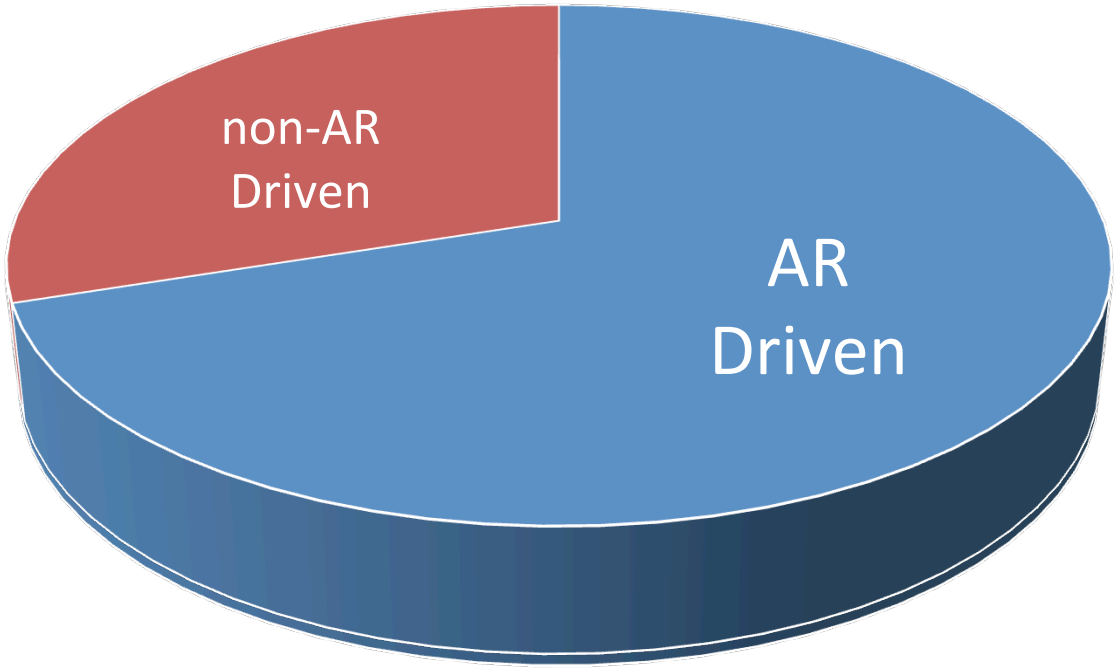


- Intratumoral production of androgens
- AR overexpression
- AR amplification
- AR point mutations
- AR splice variants
- AR pathway crosstalk, bypass
- AR co-factors

.. *even more potent* therapeutic approaches

- **Novel AR drugs targeting the AR (NTD or ARv)**
  - eg., Epi- 506, ODM-201, niclosamide, ISIS-ARRx
- **Combination strategies**
  - eg., abiratone +enzalutamide
- **AR co-targeting**
  - Abiraterone + PI3K or Akt inhibitor
  - Abiraterone + CDK4/6 inhibitor
  - Abiraterone + PARP inhibitor
  - Abiraterone + OGX-427
- **Earlier intervention** (before CRPC)

Will the proportions on this pie chart change as we more effectively target the AR?

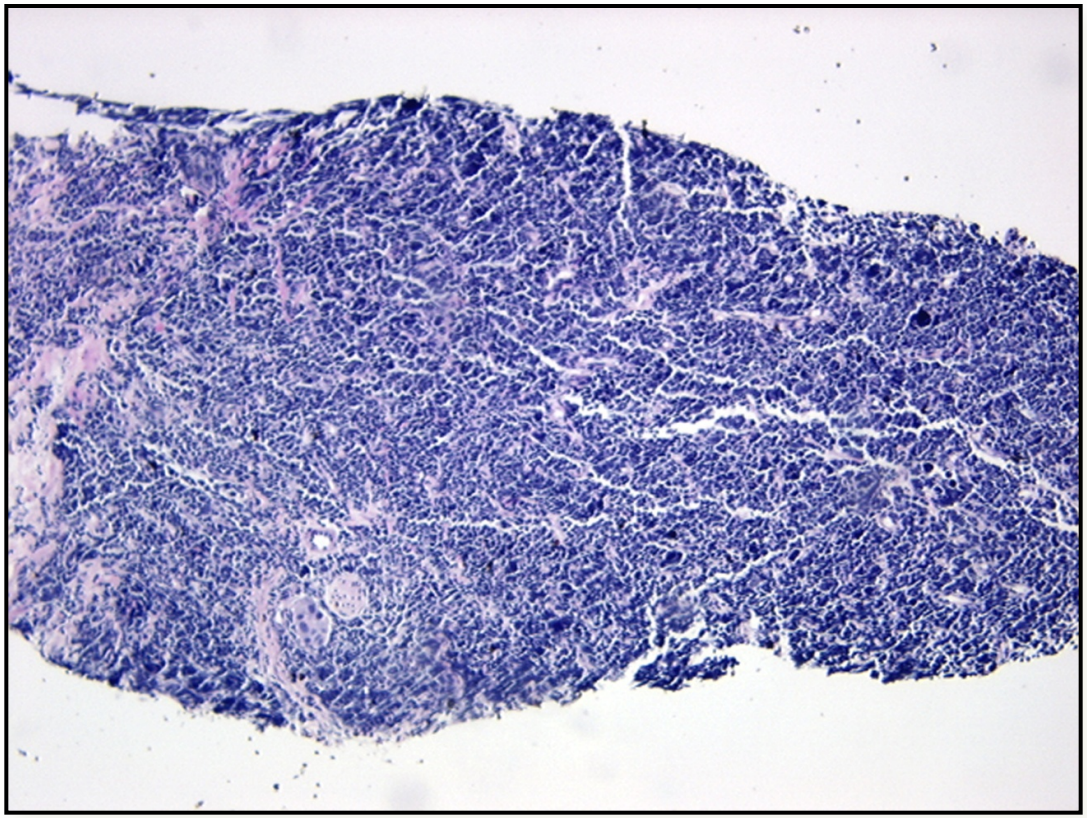


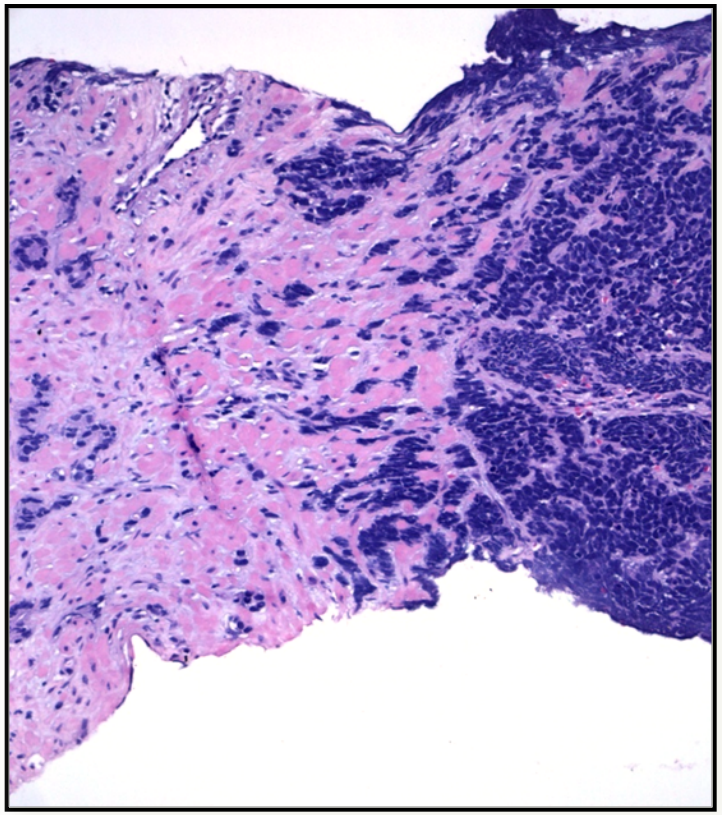
What are the mechanisms underlying non-AR driven or ‘AR indifferent’ CRPC?

What are the mechanisms underlying non-AR driven or 'AR indifferent' CRPC?

- Activation of Alternate /bypass pathways
- Genomic instability
- Selection of resistant clones
- Epithelial plasticity, neuroendocrine phenotype

One Extreme Resistance Phenotype: Small cell/Neuroendocrine Prostate Cancer





Clinically aggressive, treated like small cell lung cancer

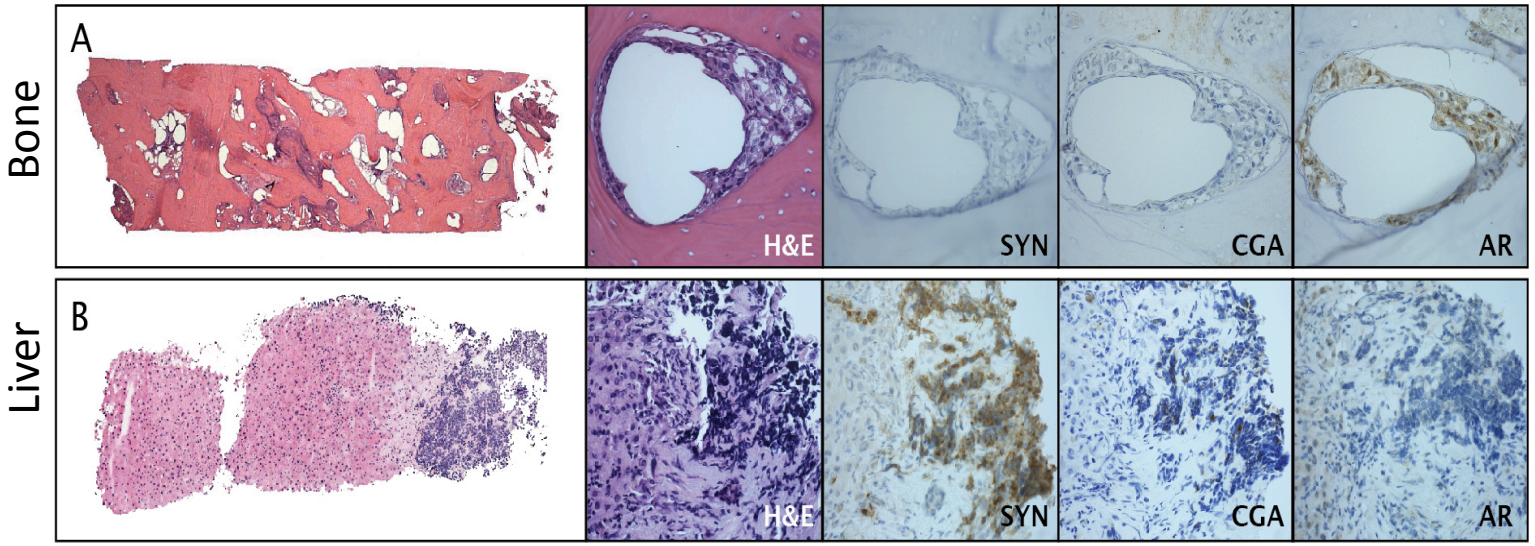
Clinical Case

65 yo M with metastatic CRPC s/p multiple lines of therapy, developed progressive liver metastases while on abiraterone, stable/non-rising PSA 26 ng/ml, NSE 45 ul/mg

Biopsy before abiraterone- adenocarcinoma

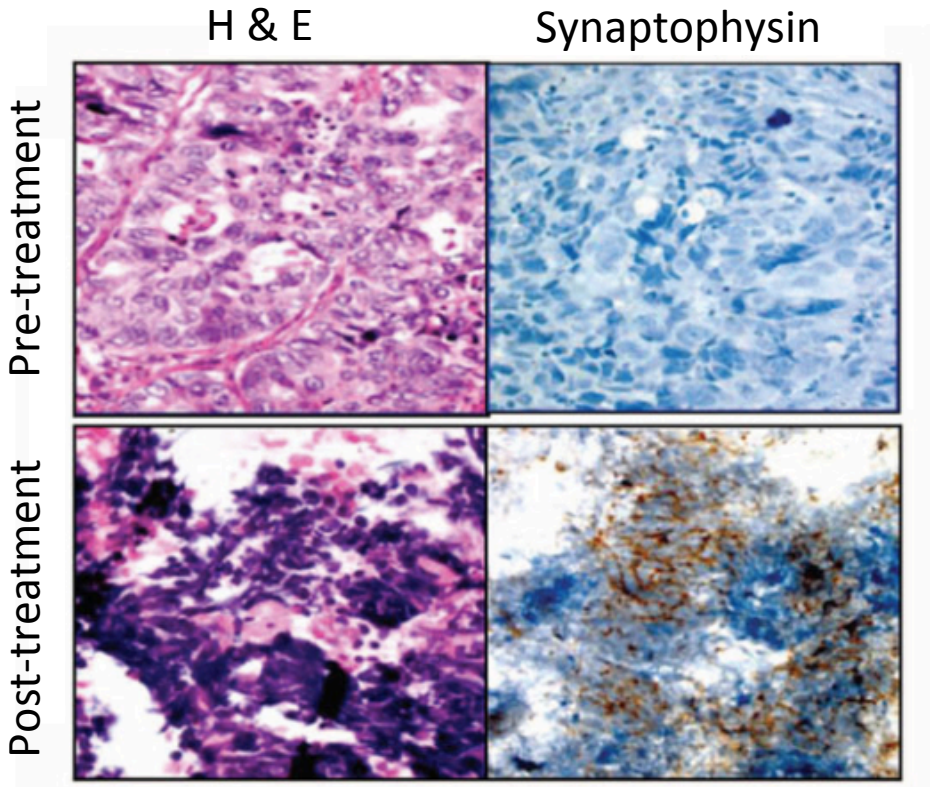
Biopsy at progression = small cell NEPC

Patient died 3 months later



Selective pressures to targeted therapy in lung cancer

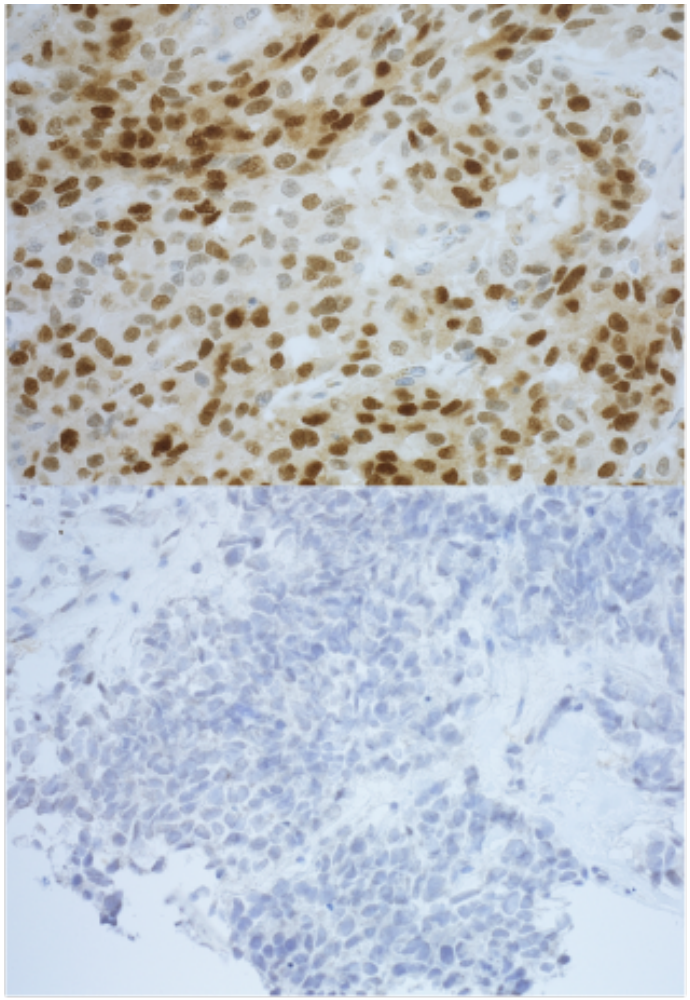
15-20% of lung adenocarcinomas transform to small cell lung cancer at time of resistance to EGFR inhibitor therapy



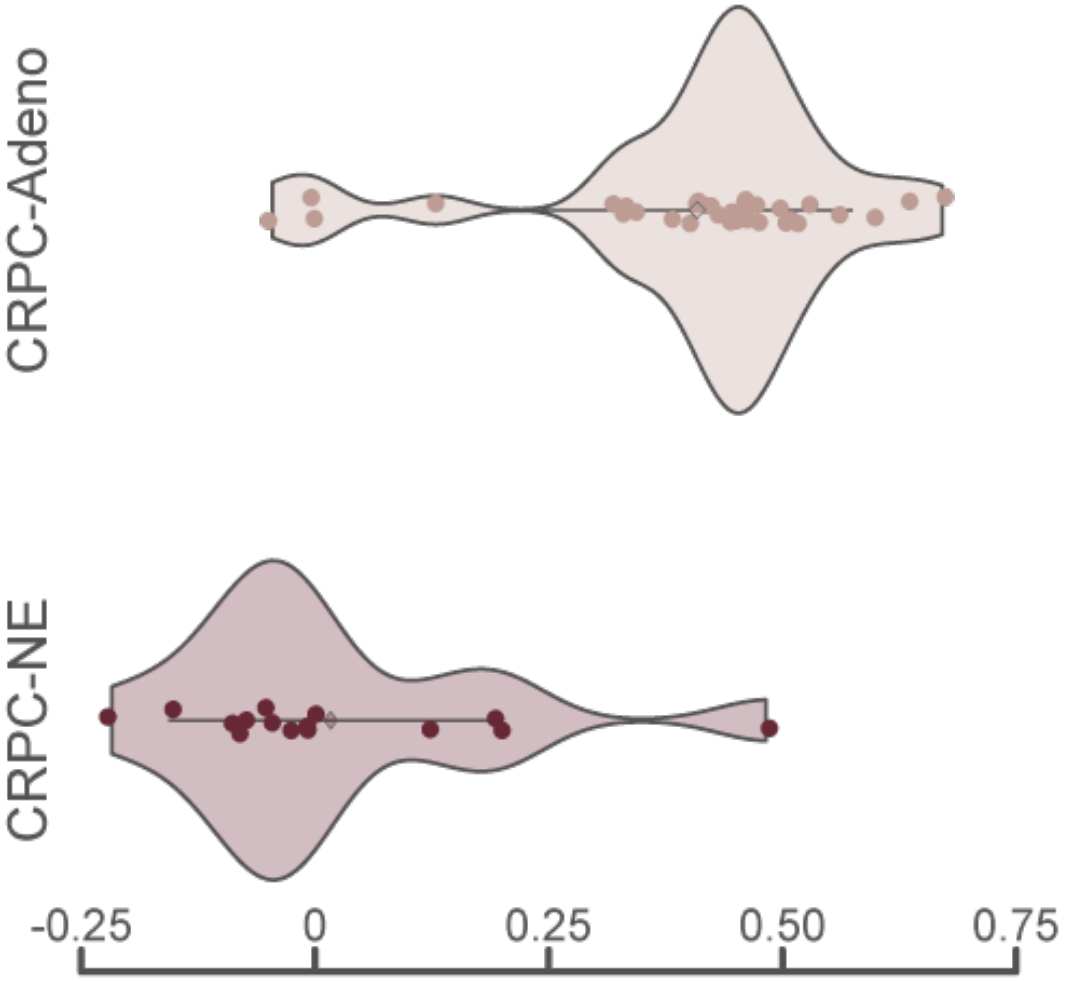
EGFR mutation retained

Sequist et al, Sci Transl Med, 2011

Lower AR expression and signaling in Castration Resistant Neuroendocrine Prostate Cancer (CRPC-NE)



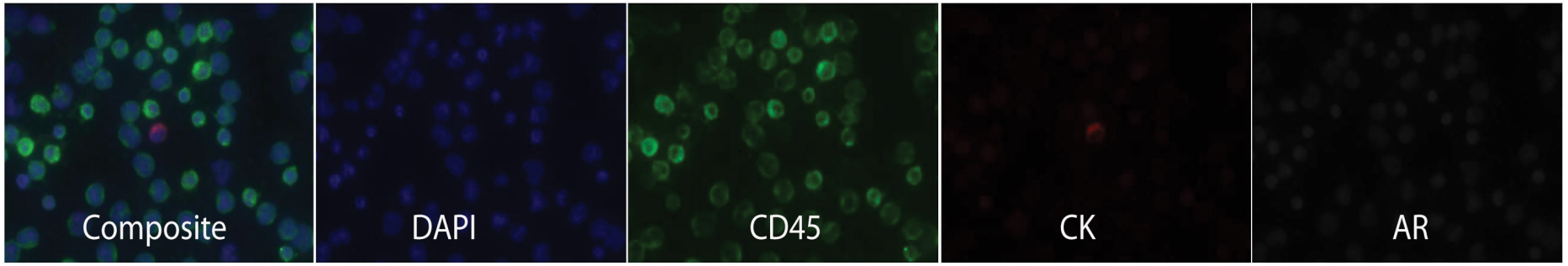
AR Expression



AR Signaling

Beltran et al, Nat Med 2016

Circulating tumor cells in NEPC



Smaller cells, Low AR, EMT changes

159 patients with CRPC treated with abiraterone or enzalutamide (MSKCC)– NEPC +CTC populations present in 10%, associated with poor prognostic features including visceral metastases and higher CTC count

Beltran et al, Clinical Cancer Research 2016

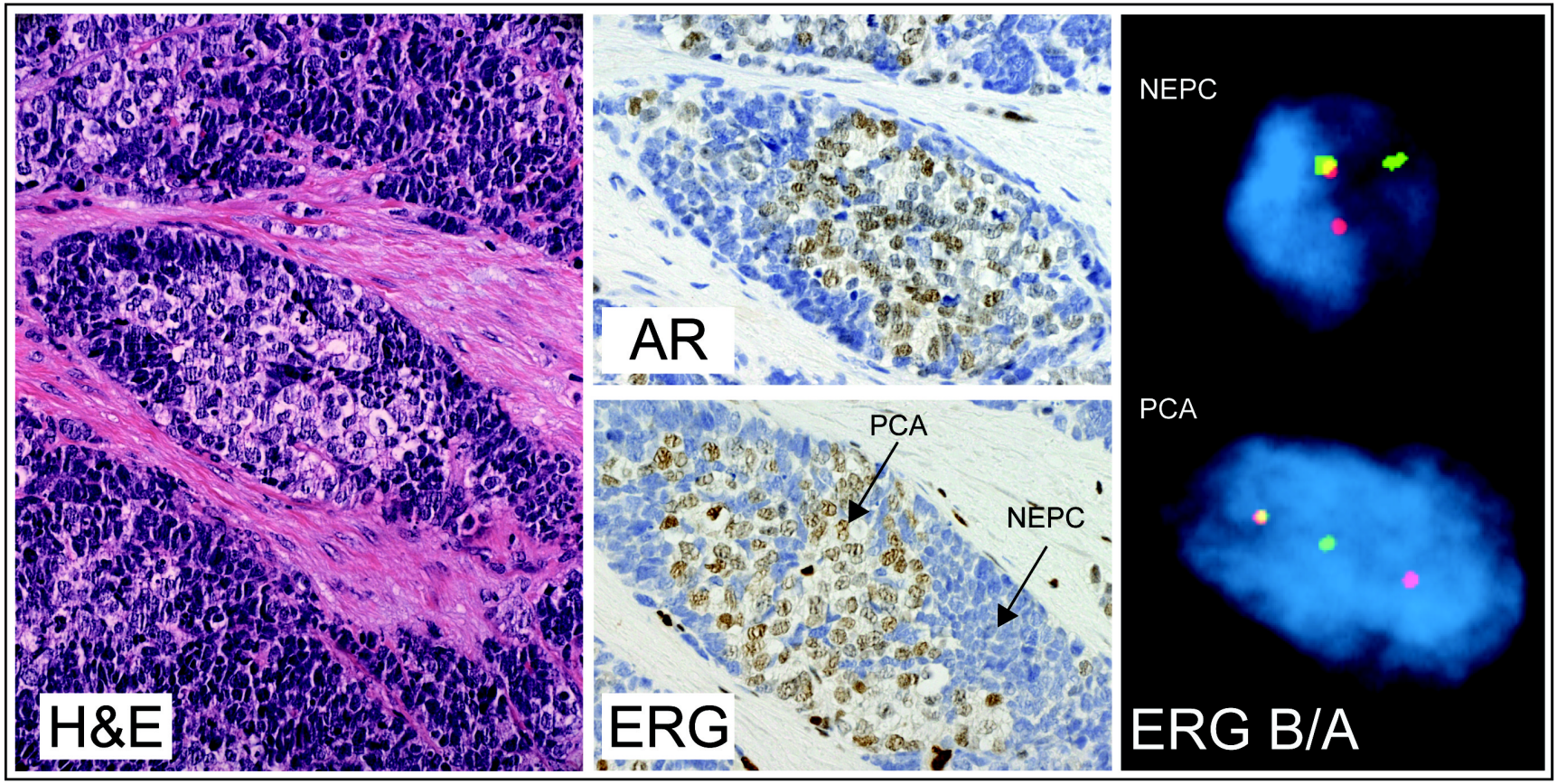
NEPC Clinical Features

- De novo presentation rare (<1% new diagnoses)
- Metastatic disease, including unusual sites of metastases
- Low or modestly rising PSA
- Paraneoplastic syndromes (uncommon)
- Elevated CEA or serum neuroendocrine markers (chromogranin, neuron specific enolase) can support the diagnosis
- Often treated like small cell lung cancer

Clinical Challenges

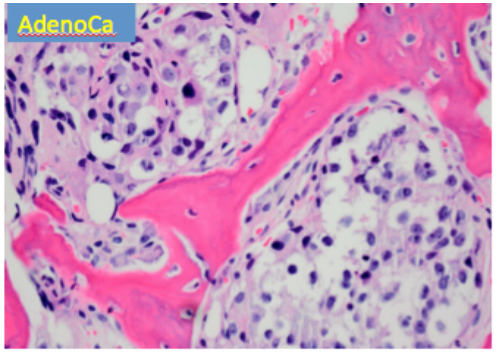
1) Pure NEPC is uncommon. More commonly, tumors show mixed features (adenocarcinoma +NEPC)

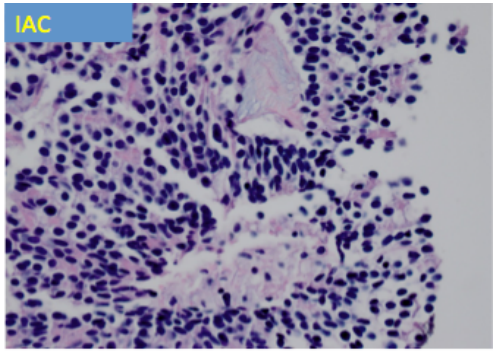
Metastatic Biopsy from Patient with CRPC: Mixed NEPC – Adenocarcinoma

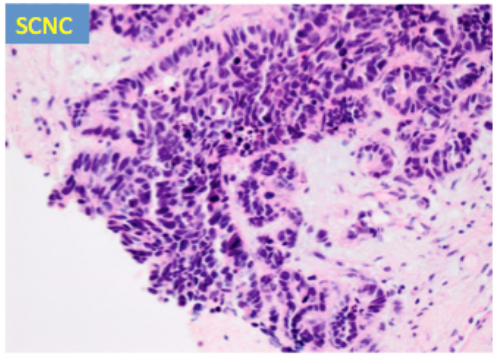


Beltran et al, Cancer Discovery 2011

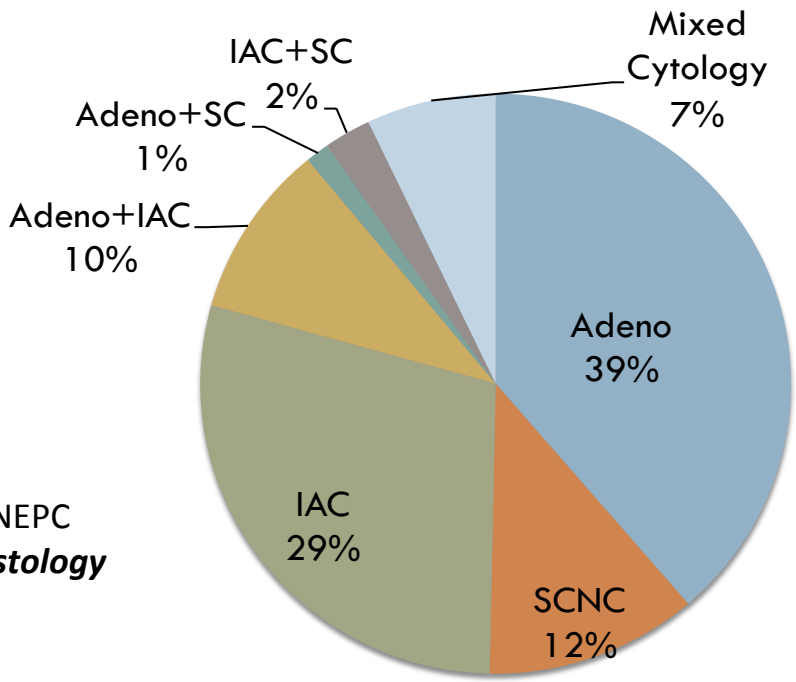
West Coast SU2C-PCF Dream Team:
Post-abiraterone and Post-enzalutamide Tumor Biopsies
Wide spectrum of morphologies







39% Adenocarcinoma
12% Small cell NEPC
20% Mixed Adenocarcinoma/NEPC
29% Intermediate Atypical Histology



West Coast SU2C-PCF dream team

Courtesy of Eric J. Small, Presented ASCO 2015

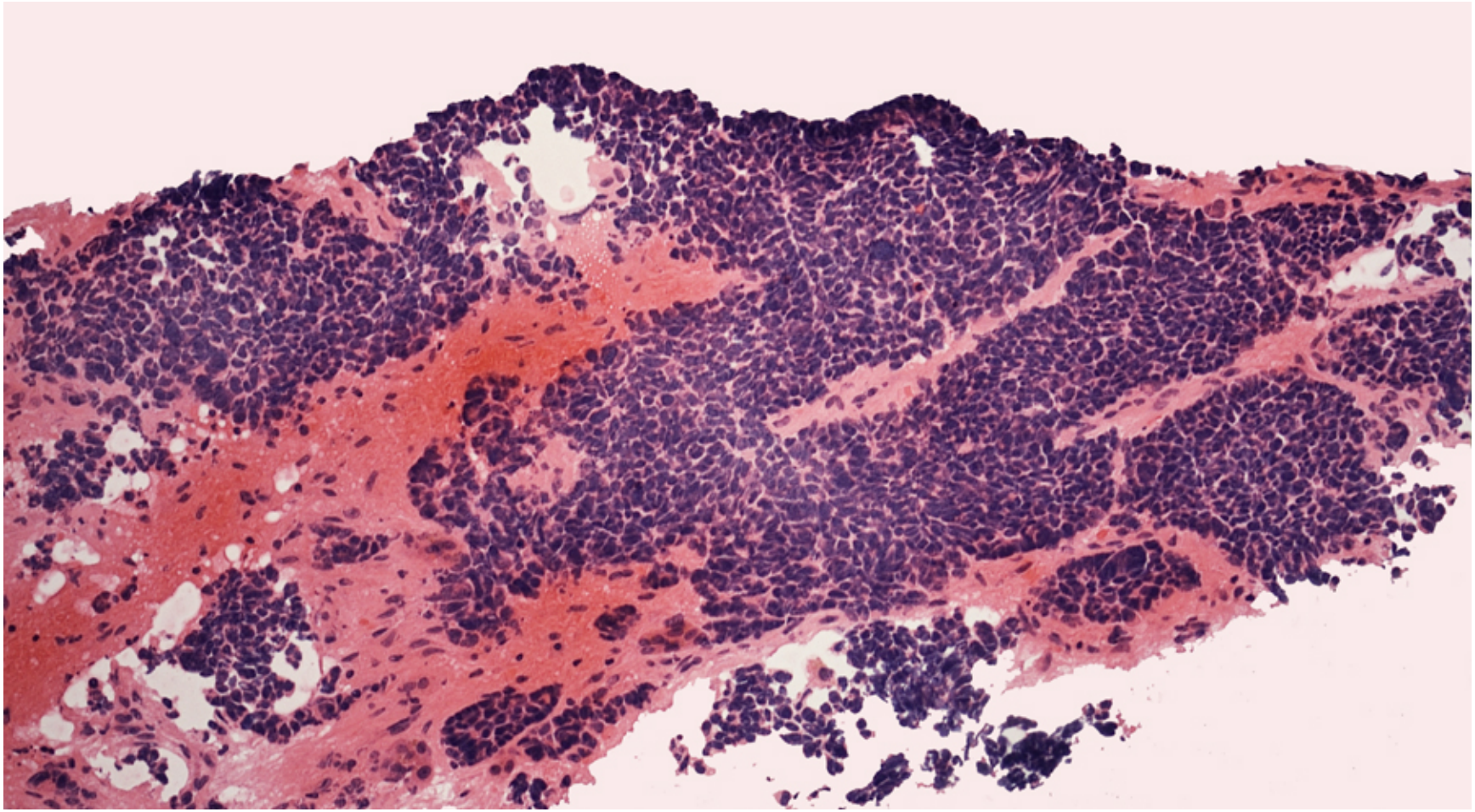
Clinical Challenges

1) Pure NEPC is uncommon. More commonly, tumors show mixed features (adenocarcinoma +NEPC), variable AR and NE marker expression by IHC, varied clinical response to AR therapies
2) **Not all patients with clinical features suggestive of AR independence demonstrate NEPC on biopsy**

Two Aggressive CRPC Cases

Case 1: Patient developed new liver and lung metastases while on abiraterone in the absence of significant PSA progression (PSA 234, CgA 17340).

Case 1 liver Biopsy: Small cell prostate cancer

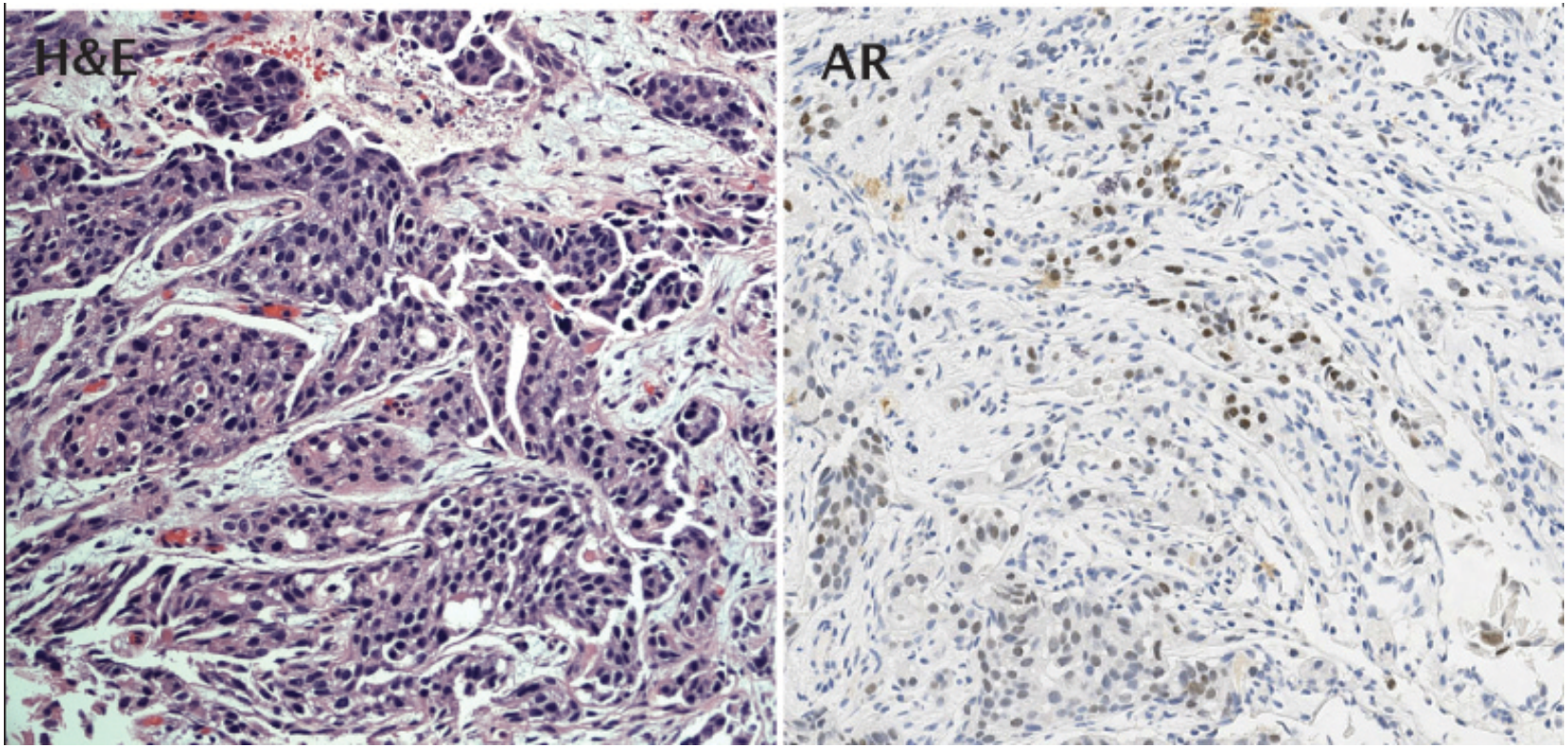


Two Aggressive CRPC Cases

Case 1: Patient developed new liver and lung metastases while on abiraterone in the absence of significant PSA progression (PSA 234, CgA 17340).

Case 2: Patient developed new liver and adrenal metastases after abiraterone, radium-223, docetaxel in absence of PSA progression (PSA 14, serum NE markers normal).

Case 2 TUR and Bone biopsy: Prostate Adenocarcinoma



How do we define NEPC?

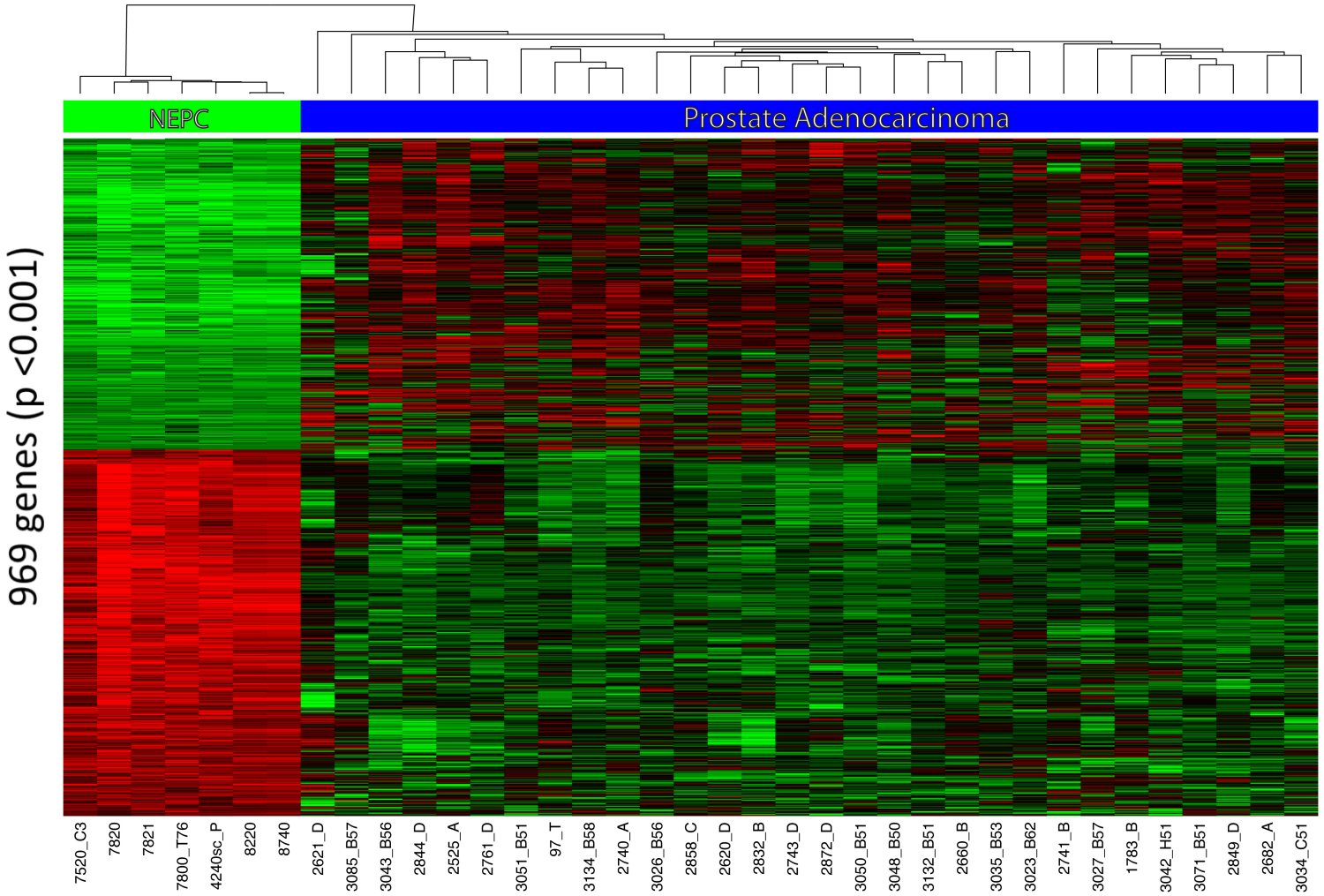
- **Histology**
- **NE markers** (CgA, NSE, SYP, CD56, other): tissue, serum
- **Clinical criteria**
  - Anaplastic/aggressive variant prostate cancer (Aparicio, MD Anderson)
- **Molecular criteria**
  - AR status?
  - Can we define a NEPC resistance signature?

Implications:

- Select AR targeted therapies only for patients that are *still* AR driven

- Select patients for NEPC-directed therapies

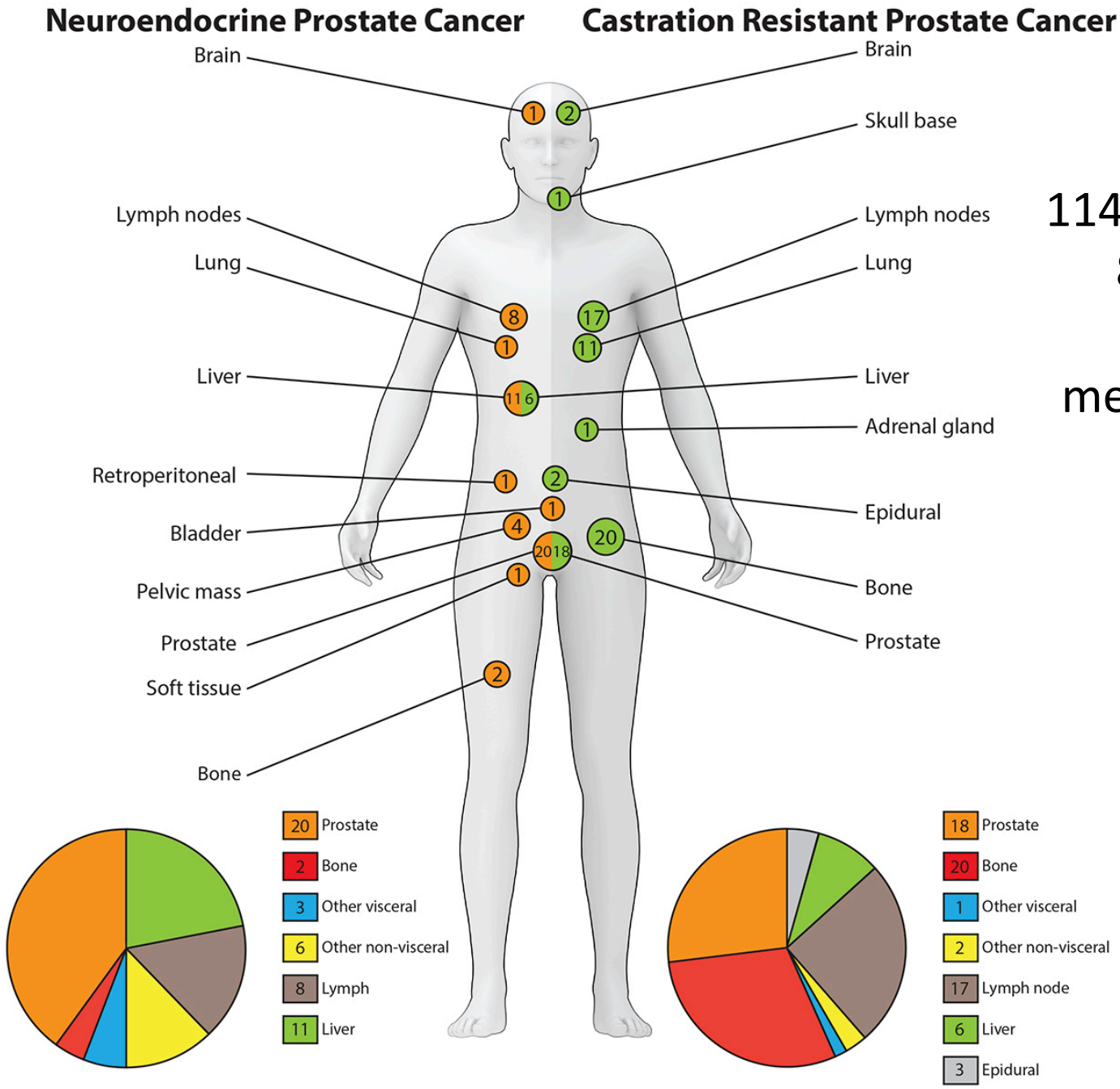
Transcriptome Analysis: NEPC is molecularly distinct



Red= High Expression

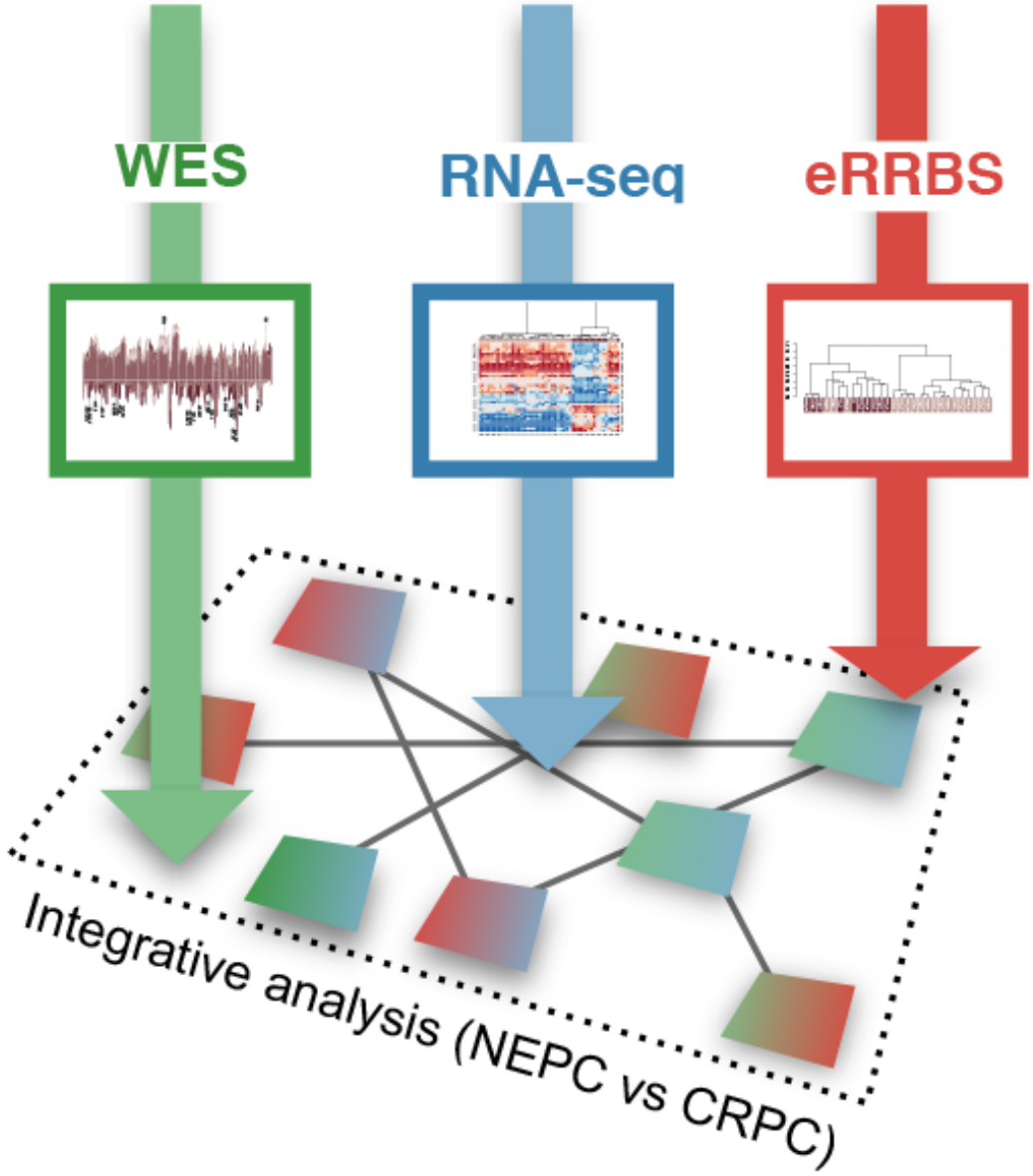
Green= Low Expression

Beltran et al, Cancer Discovery 2011



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

Beltran et al, Nature Med 2016

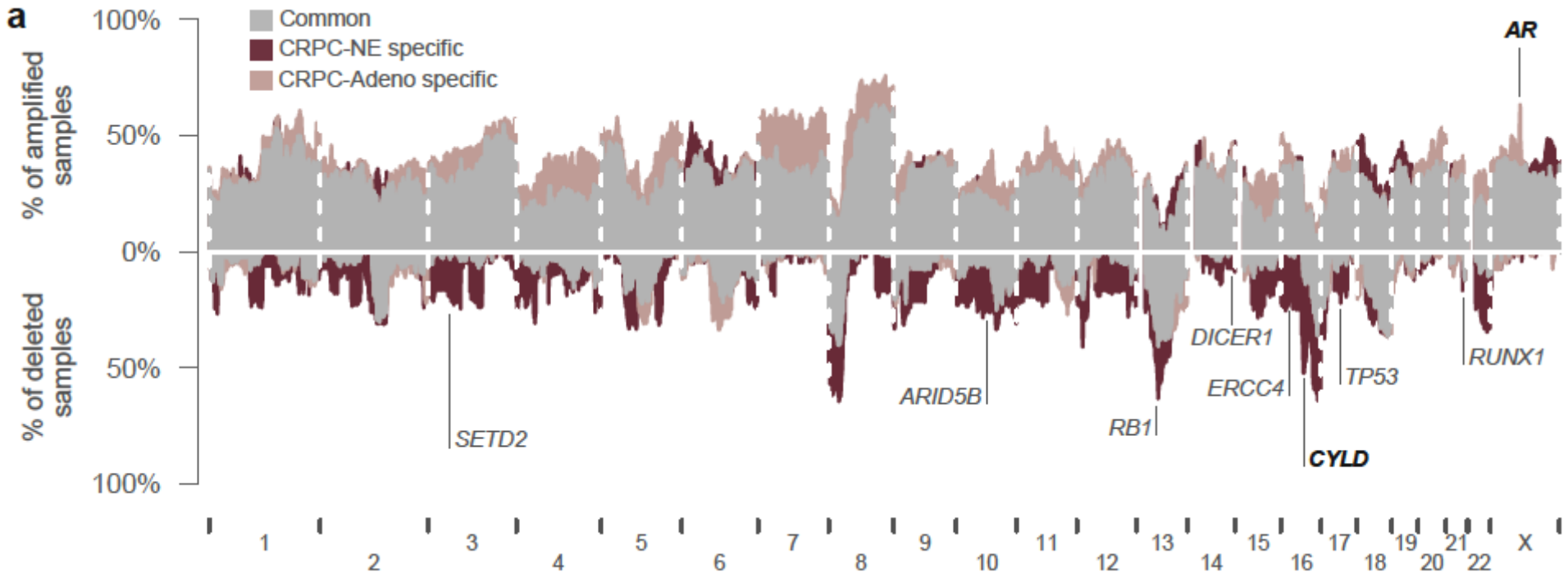


Hypothesis:

NEPC (extreme phenotype) is associated with distinct molecular features

Collaboration with MA Rubin (WCM), F Demichelis (Trento) and L Garraway (Broad Institute)

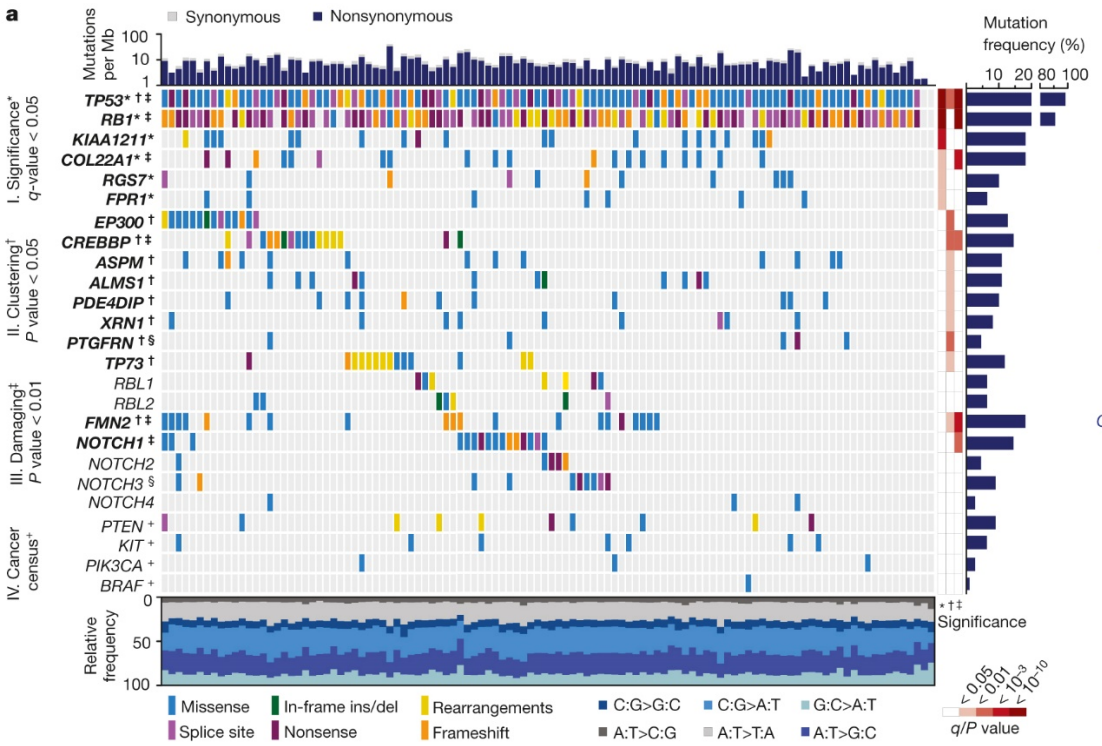
CRPC (Adeno) and NEPC genomic profiles show significant overlap



- *RB1* loss (70% CRPC-NE, 32% CRPC-Adeno, p=0.003)
- *TP53* mutation or deletion (66.6% NEPC, 31.4% CRPC, p=0.04)

Beltran et al, Nature Med 2016

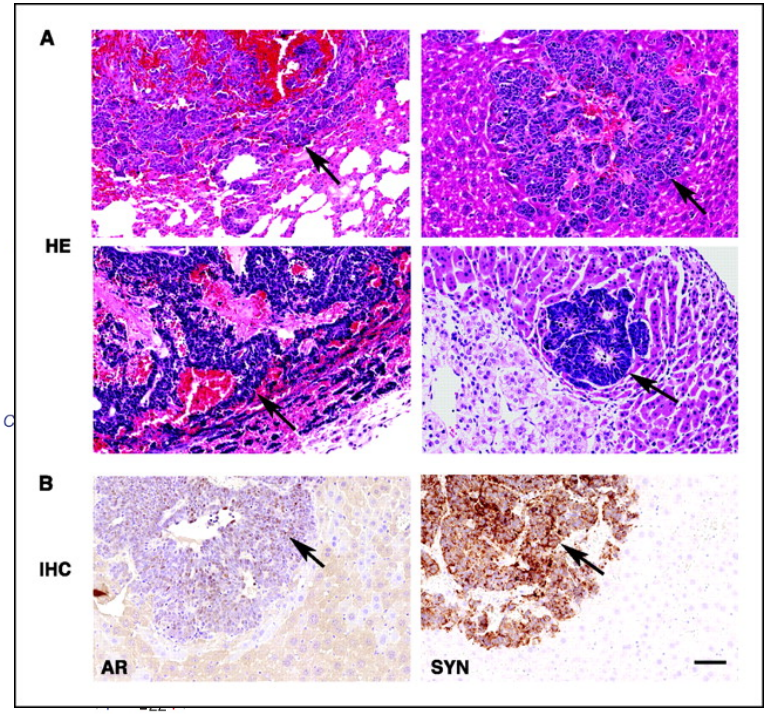
Loss of RB1 and TP53 universal in SCLC



N=120, Inactivating events included mutations, translocations, homozygous deletions, hemizygous losses, copy-neutral losses of heterozygosity (LOH) and LOH at higher ploidy

George et al, *Nature* 2015

Rb and p53 in prostate cancer

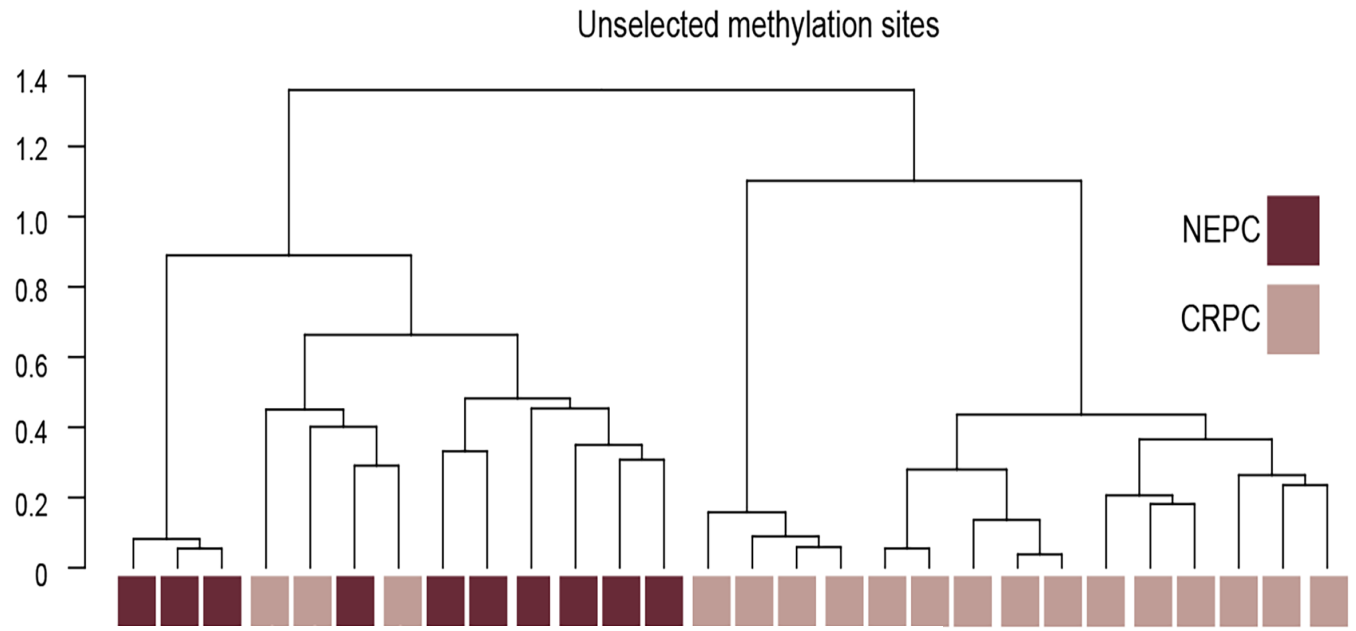


Zhou et al, *Cancer Research* 2006

Conditional knockout p53 and Rb-> NEPC, metastasis

TRAMP model- expression SV40 large T antigen-> loss of p53 and Rb

Unsupervised Analysis of Genome-wide CpG Methylation



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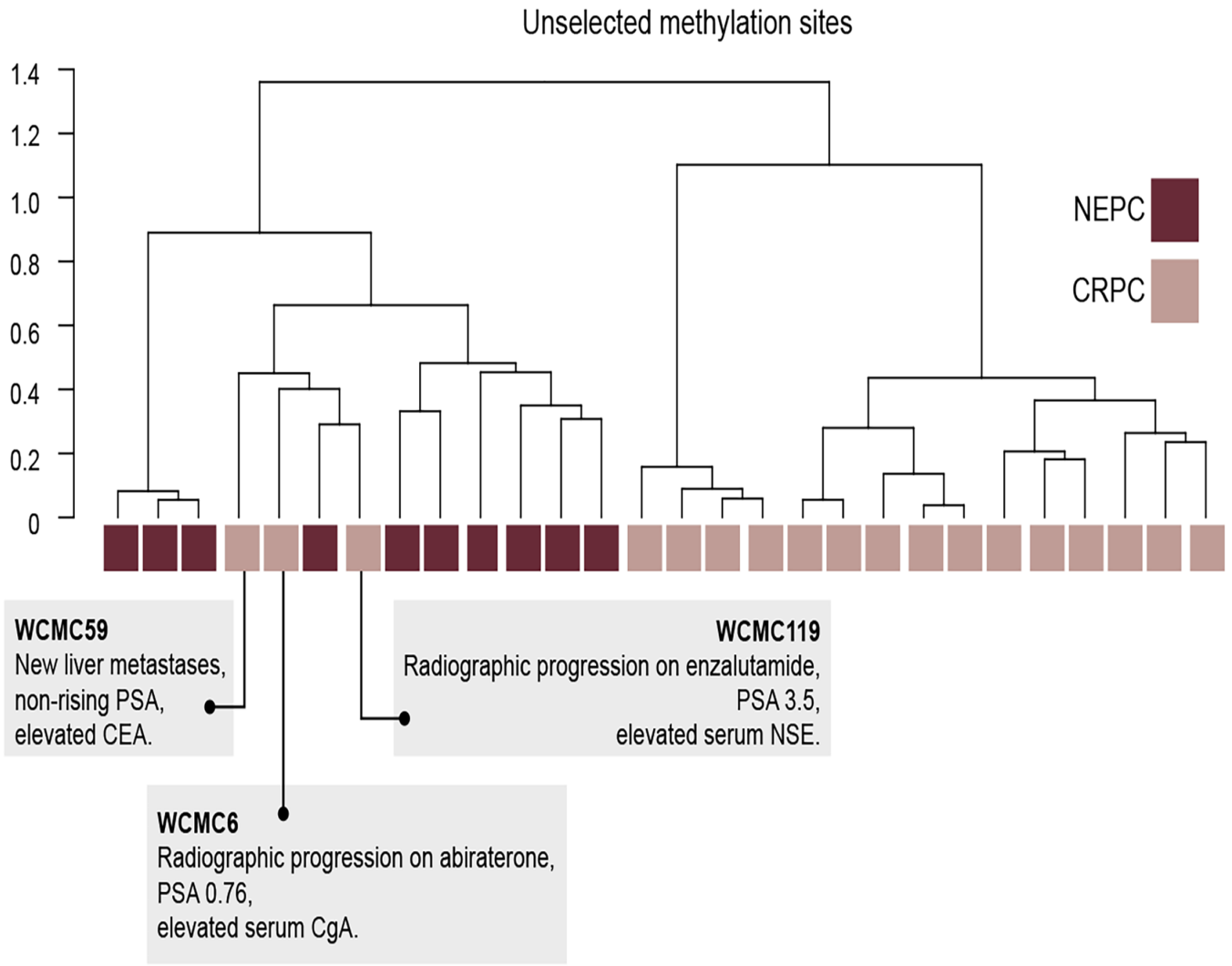


Beltran et al, Nature Med 2016

Epigenetically dysregulated pathways in NEPC

Pathway	P value
Cell- Cell Adhesion	2.24E-09
Epithelial Mesenchymal Transition	3.76E-13
Neuron Differentiation	3.60E-05
Synapse	3.89E-07
Homeobox	3.766E-13
Organ Morphogenesis	1.24E-06

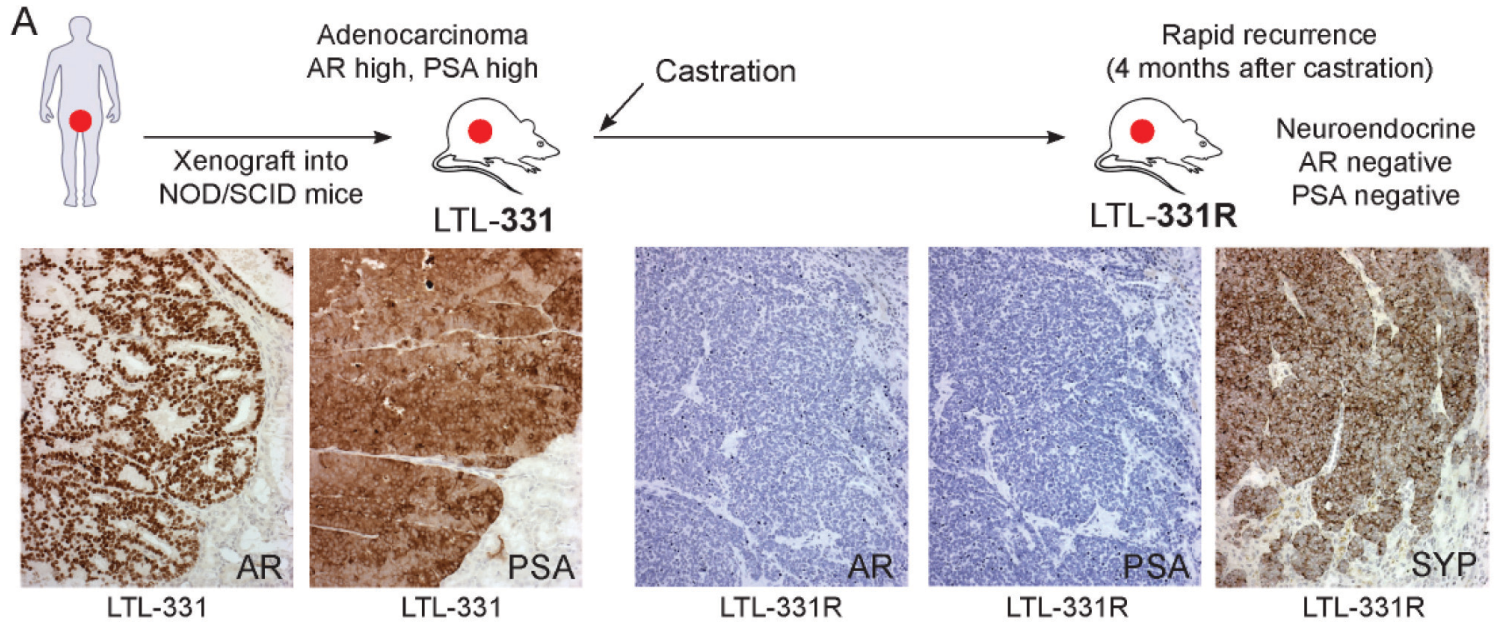
Does this represent EMT or similar state (reversible), de-differentiation to stem-like state, 2 primaries (parallel evolution), or transformation to new cancer?

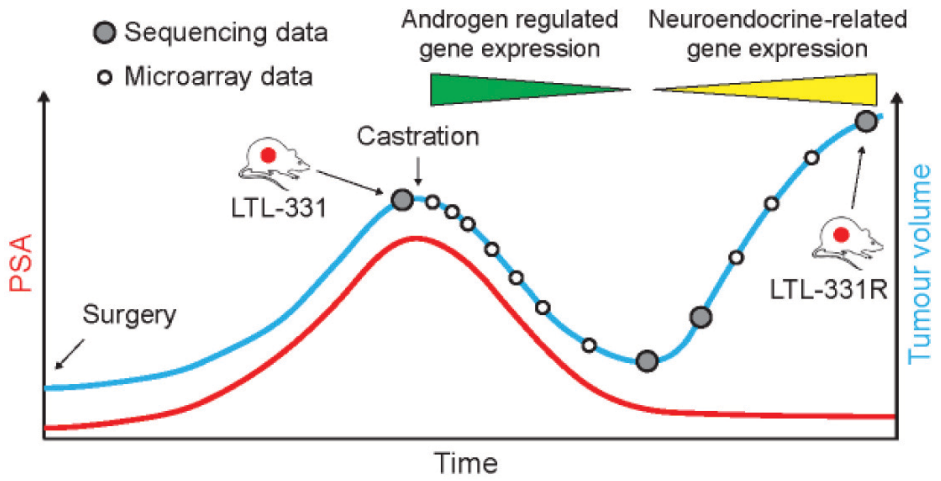
Does this represent EMT or similar state (reversible), de-differentiation to stem-like state, 2 primaries (parallel evolution), or transformation to new cancer?

Implications:

- Opportunities for early diagnosis? Co-targeting? Reversal?

Trans-differentiation Patient Derived Xenograft Model



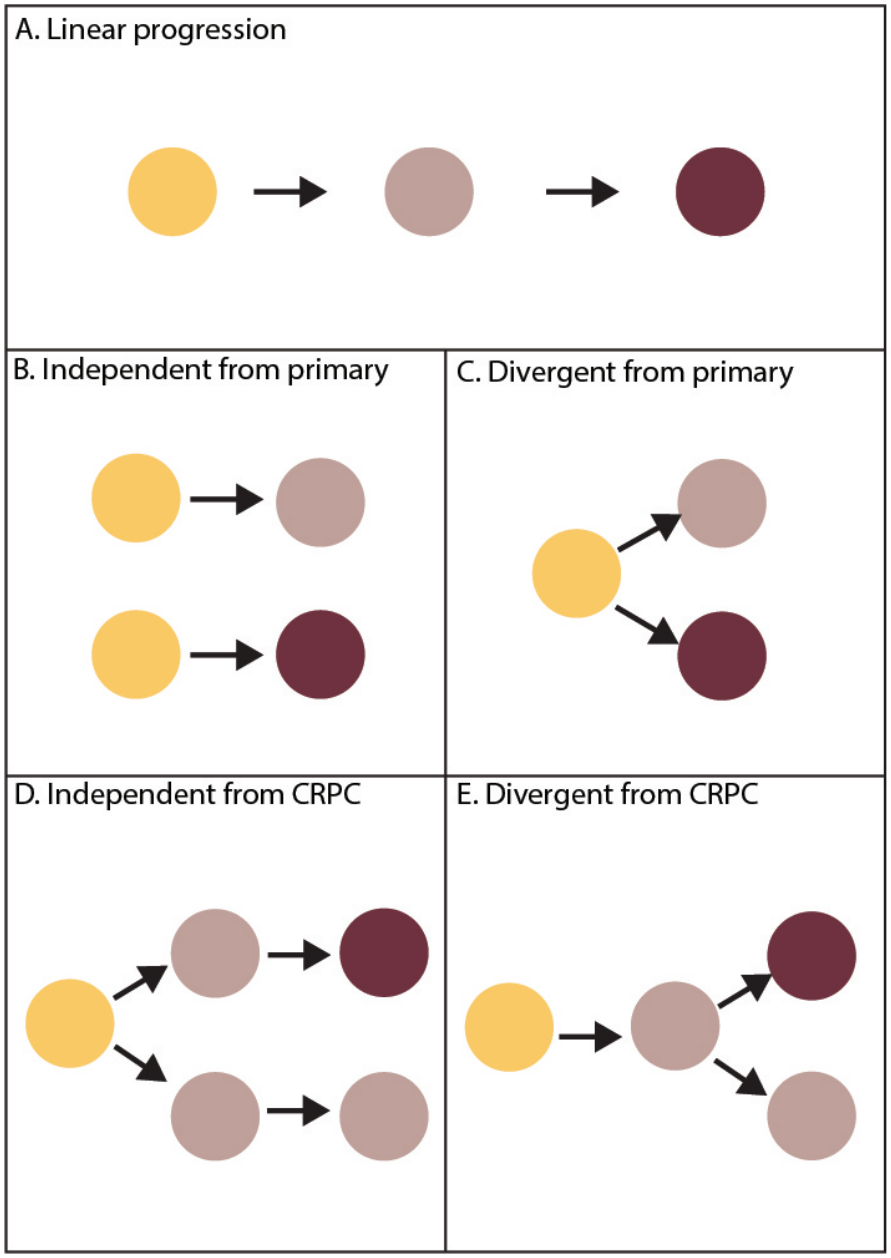


Vancouver CoastalHealth Research Institute
Healthier lives through discovery

UBC

Lin et al, Cancer Research 2013

How/when does the NE resistant phenotype arise?

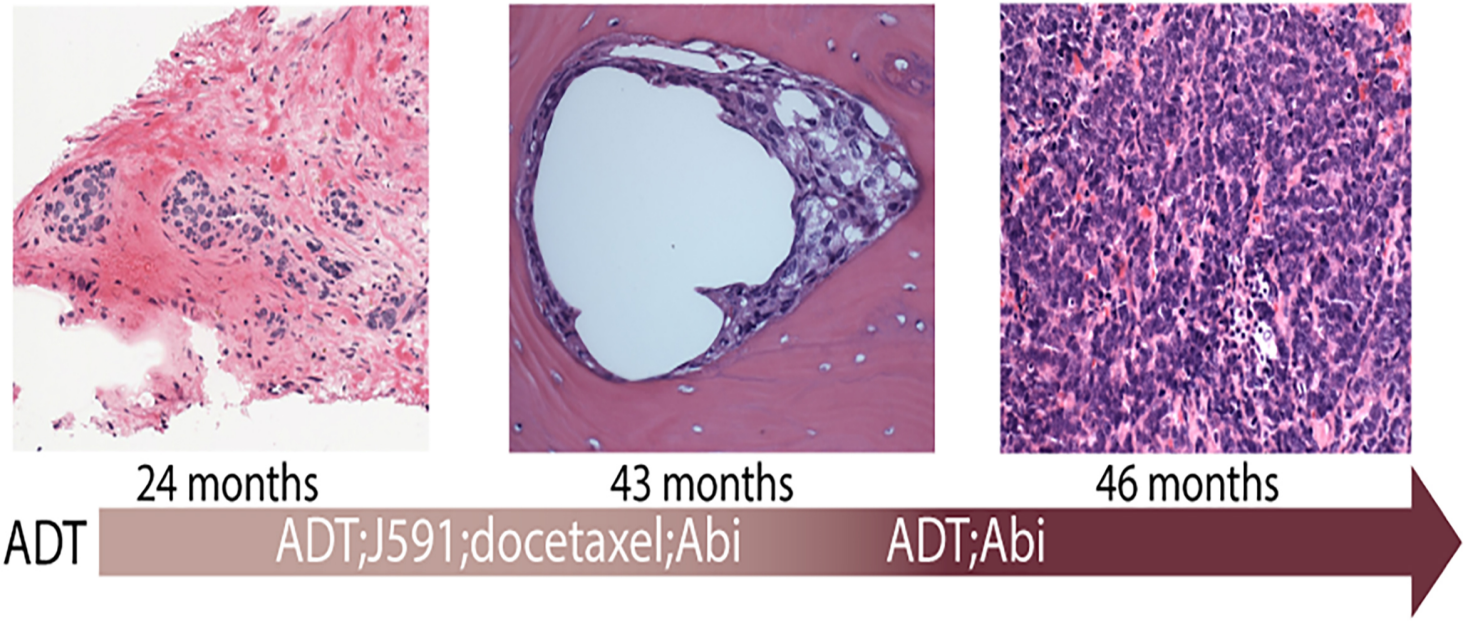


Analysis of serial biopsies during progression provides insight into tumor evolution

CRPC
Lymph Node Biopsy

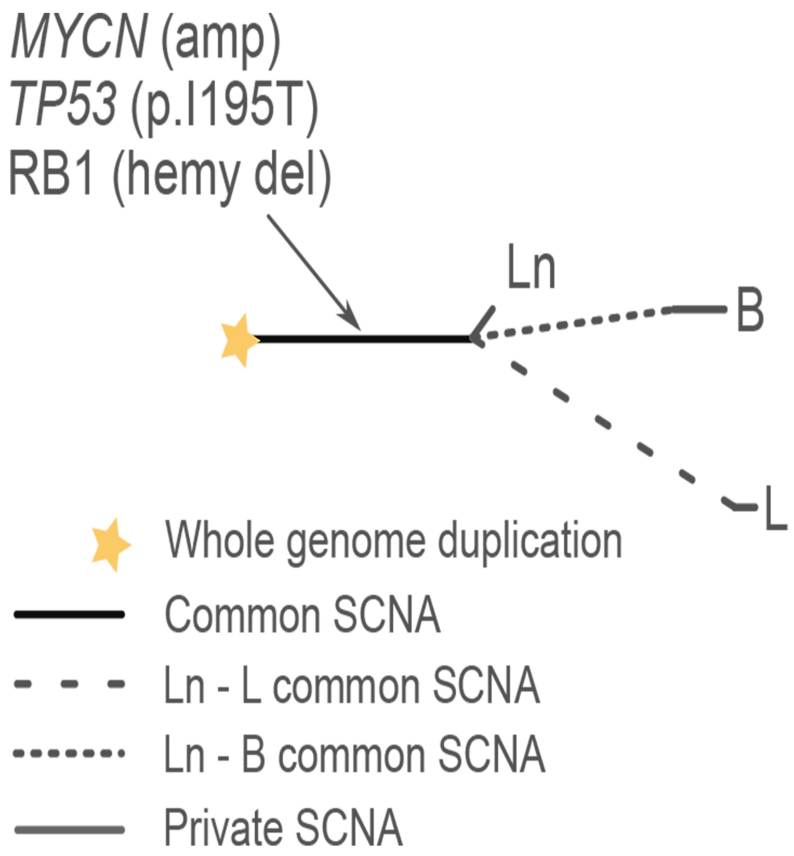
CRPC
Bone biopsy

NEPC
Liver biopsy



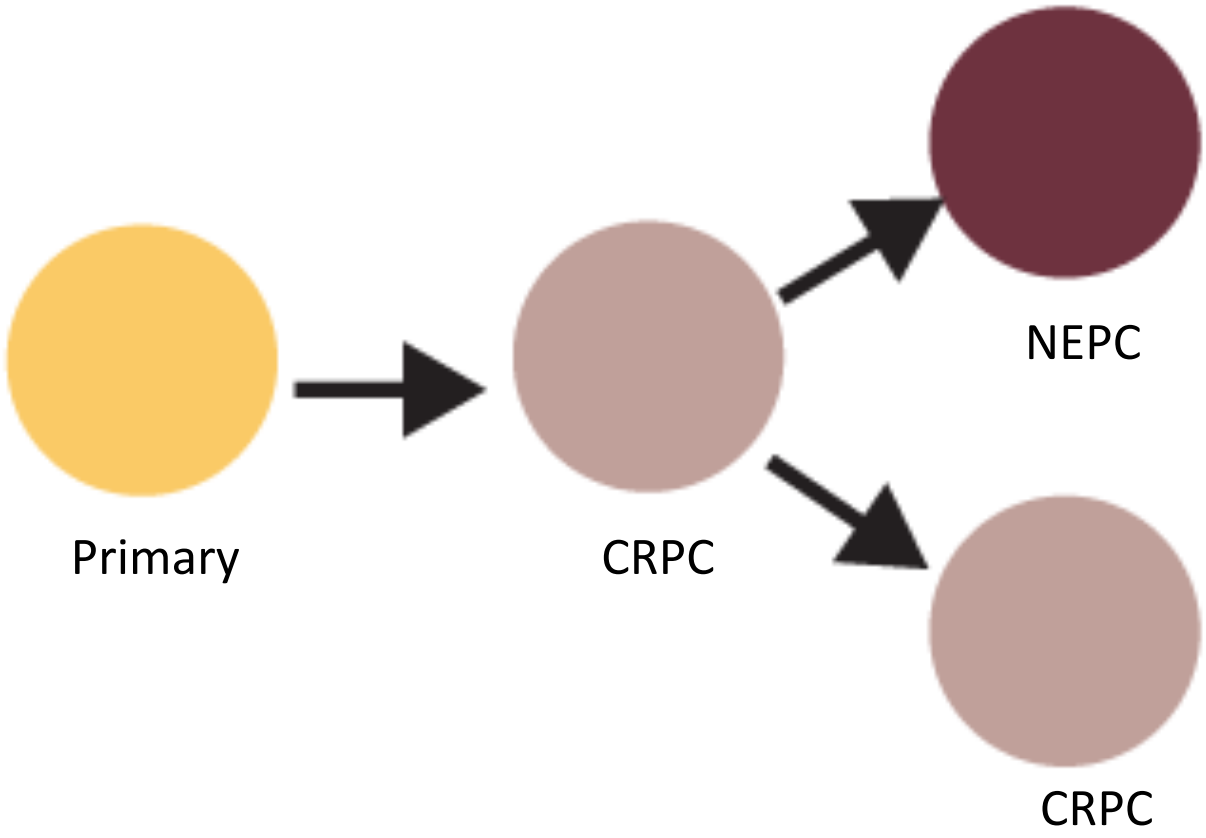
65 yo M with metastatic CRPC s/p multiple lines of therapy, developed progressive liver mets while on abiraterone, stable PSA 26 ng/ml. Liver bx= small cell NEPC. Patient died 3 months later.

Analysis of serial biopsies during progression provides insight into tumor evolution



Ln= Lymph node (CRPC- Adeno)
B= Bone (CRPC- Adeno)
L= Liver (CRPC- NE)

Beltran et al, Nature Med 2016



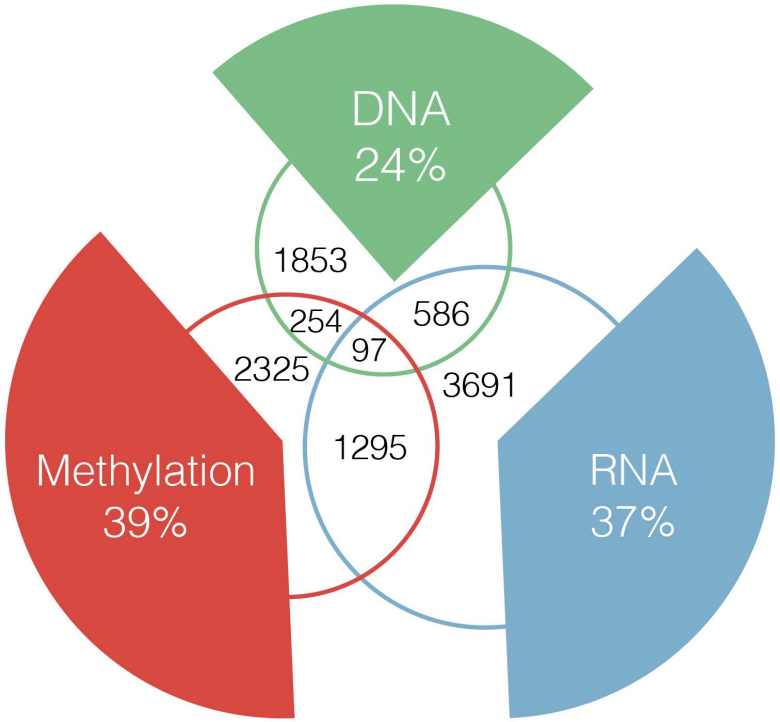
Supports *Divergent Clonal Evolution* rather than linear progression or independent clonal evolution

Can we identify patients prior to progressing to NEPC?

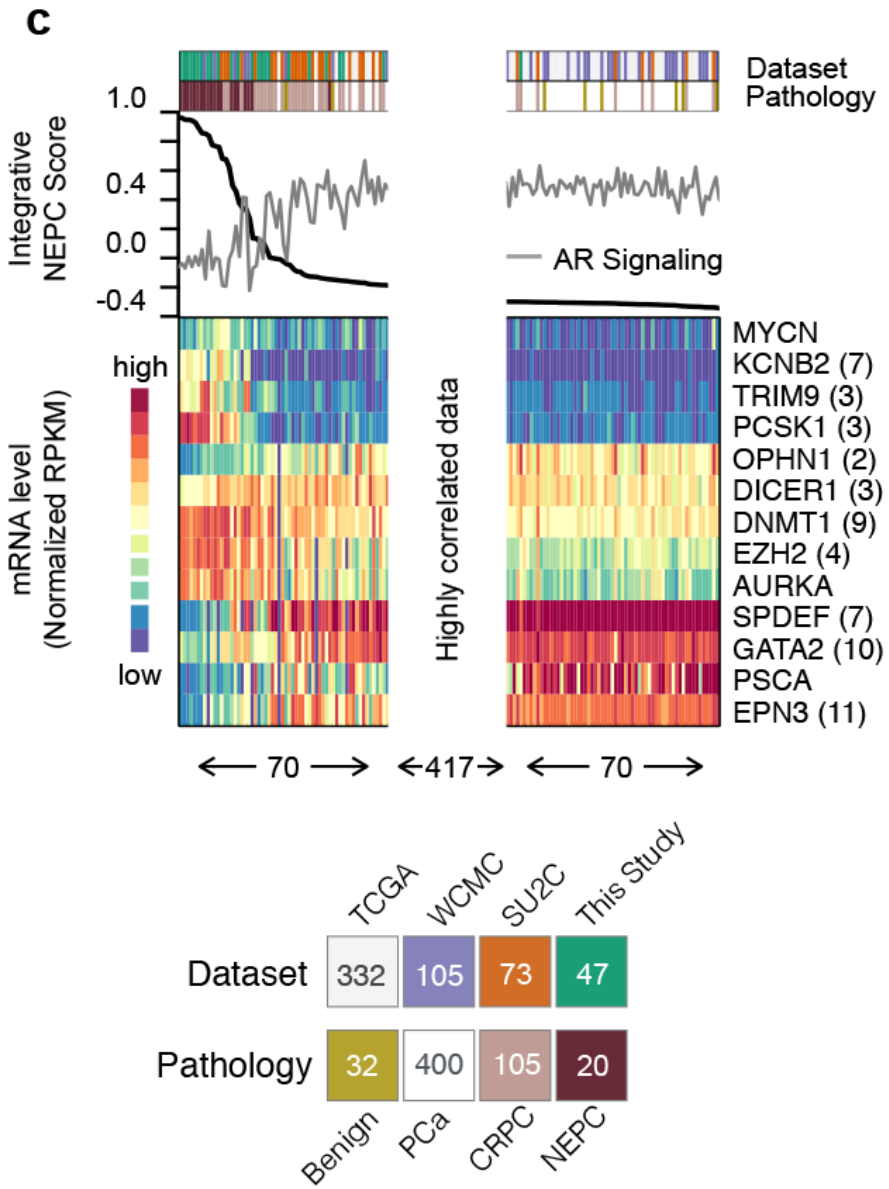
Implications:

- early detection
- early intervention

Development of a Molecular Classifier

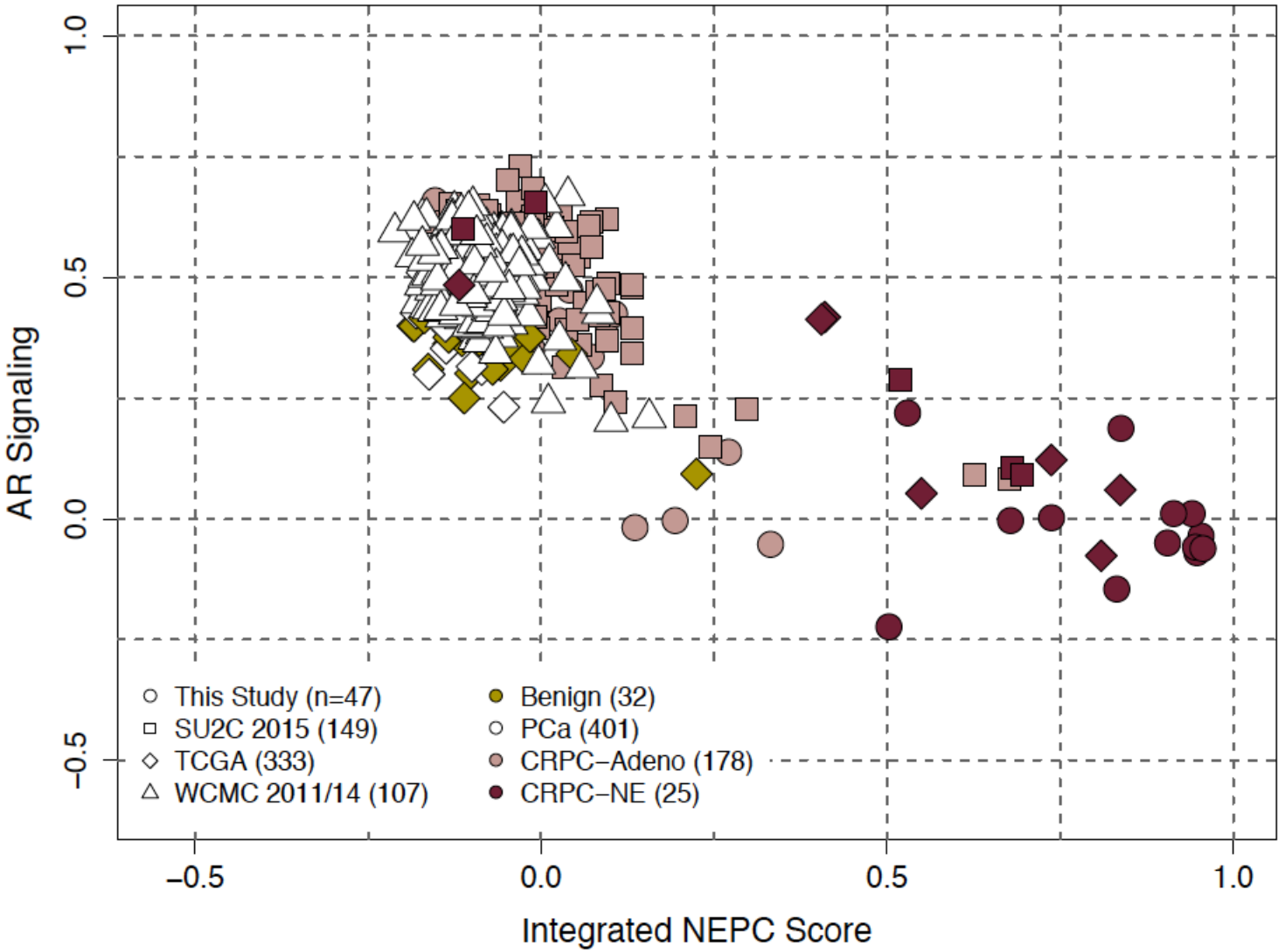


Evaluation of datasets (n=523)



Beltran, Prandi et al, Nature Med 2016

NEPC classifier



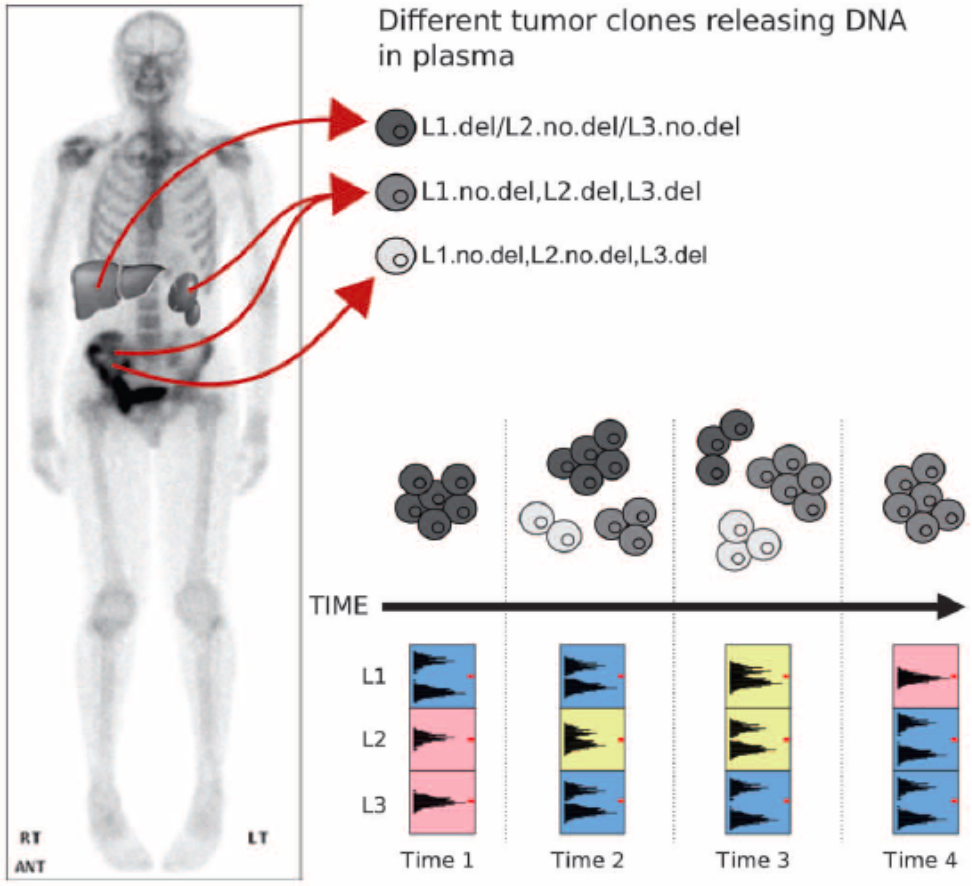
Next steps: Liquid Biopsies

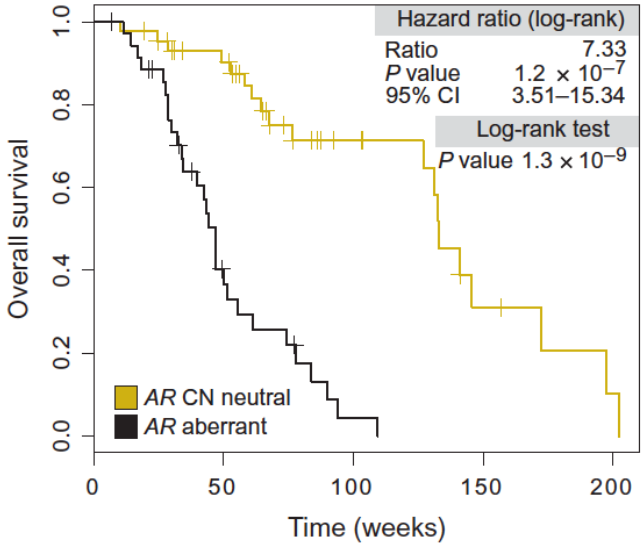
- Circulating tumor DNA from CRPC patients for emergence of NEPC molecular features

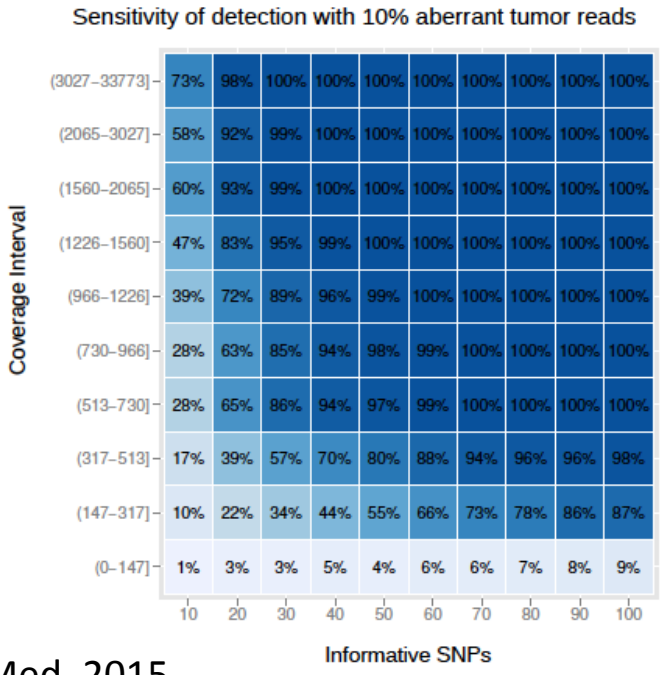
- Correlation with response to AR therapies and outcomes

PCF Challenge Award
(H Beltran, F Demichelis, G Attard)

Approach for detecting lesions from circulating DNA



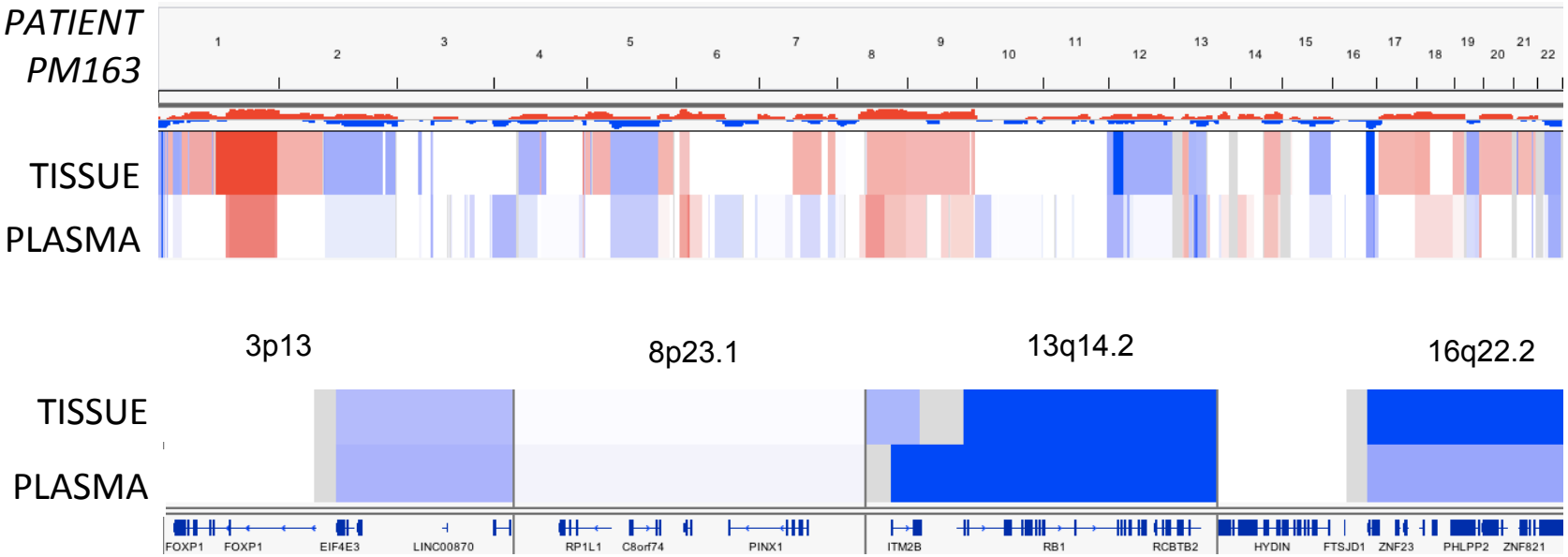




Carreira et al, Sci Transl Med. 2014; Romanel et al, Sci Transl Med. 2015

Detection of NEPC Genomic Changes using Circulating Genomic Signatures

Whole exome sequencing of matched plasma/tumor tissue



Beltran and Demichelis, unpublished

in development: plasma DNA methylation

PROSTATE CANCER FOUNDATION
Accelerating the world's most promising research

Development and qualification of the PCF SELECT (Specific Evaluation in Liquid biopsies of Established prostate Cancer Targets) plasma DNA assay

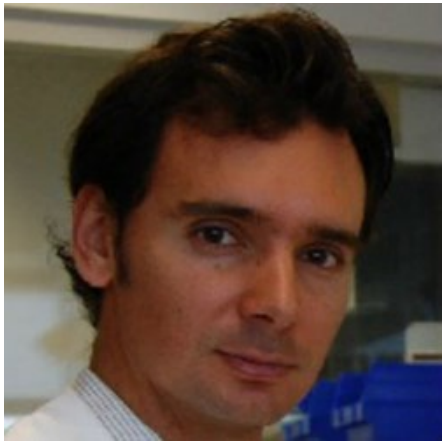
PCF Challenge Award 2016

(Beltran, Attard, Chi, Wyatt, Demichelis, Van Allen, Rubin, Maher)

@PCF_select

PROSTATE CANCER FOUNDATION
Accelerating the world's most promising research

G Attard



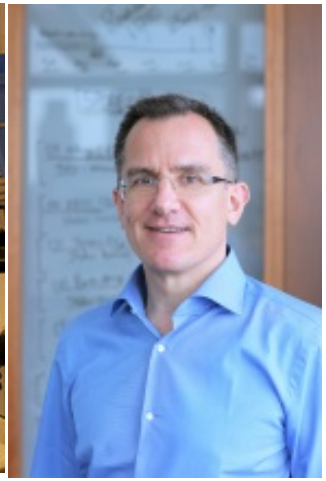
F Demichelis

H Beltran

A Wyatt

K Chi

M Rubin



E Van Allen

C Maher

A Armstrong

@PCF_select

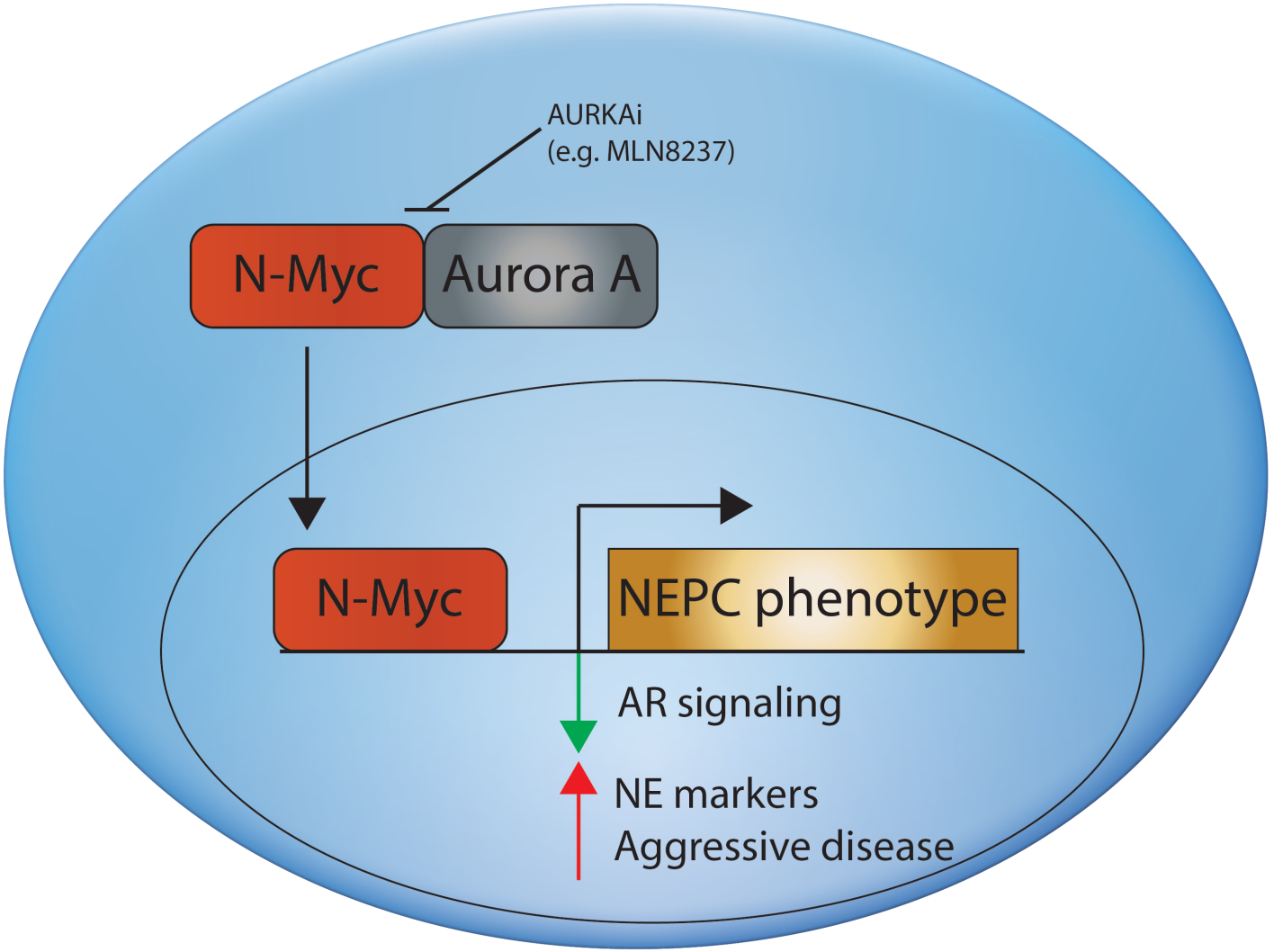
How can we better treat or co-target NEPC?

Overall survival is less than one year

There are no approved therapies for NEPC

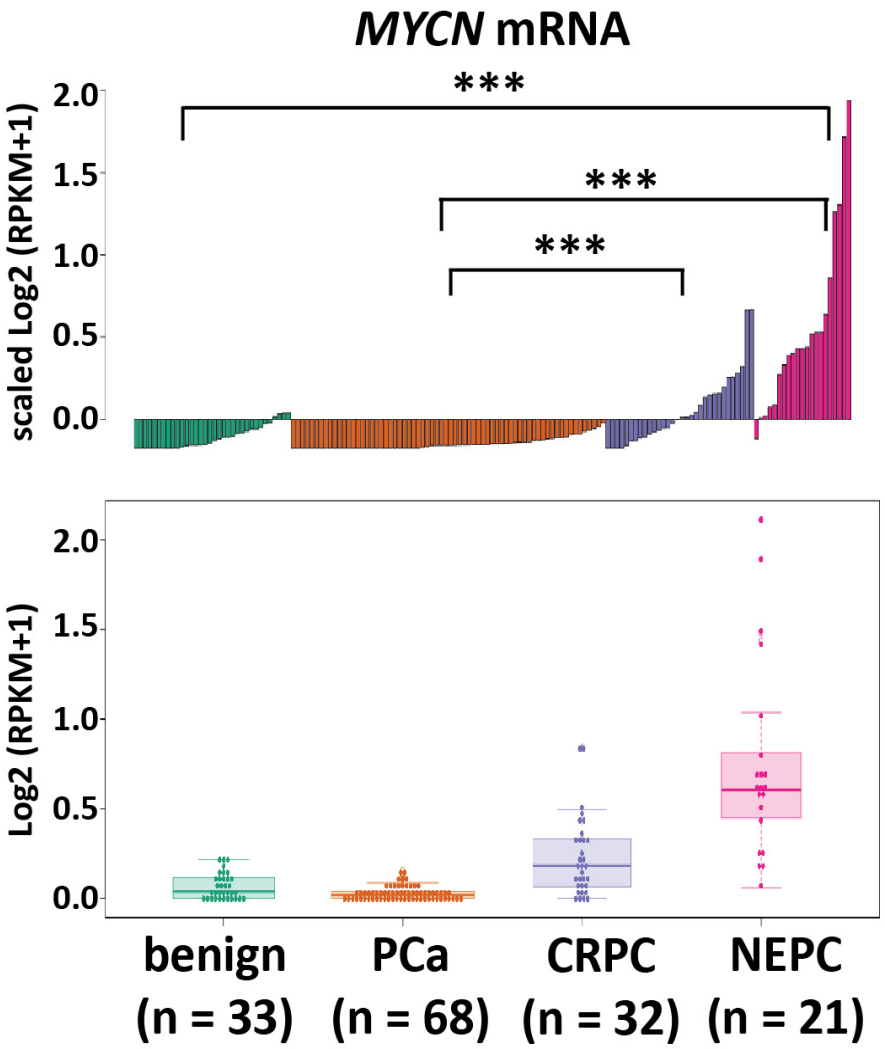
- Platinum chemotherapy
- Targeted therapies
- Co-targeting AR and non-AR pathways
- Immunotherapies?
- Other approaches?

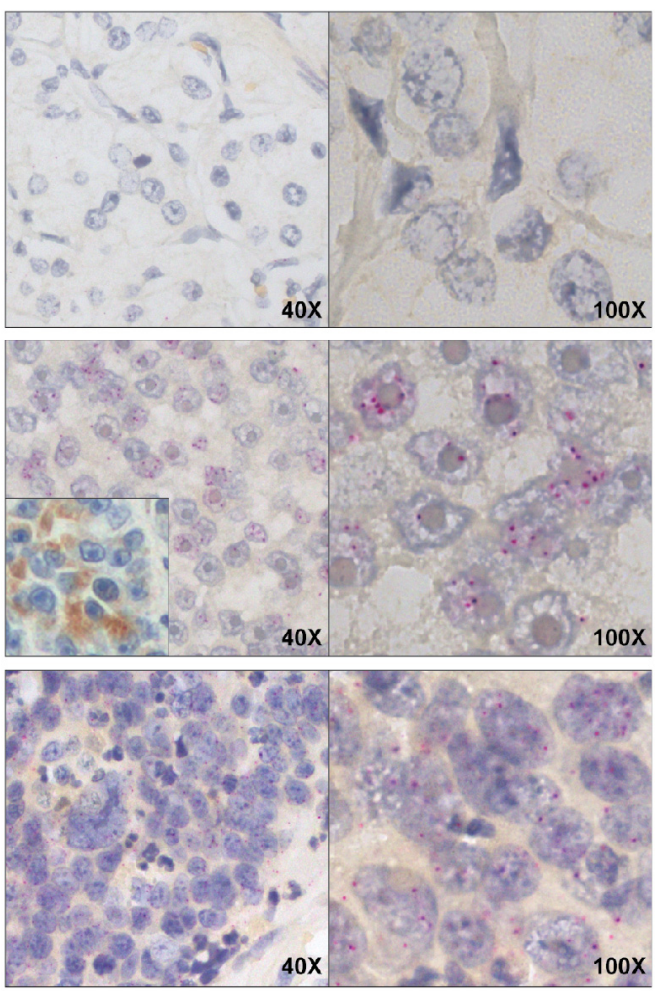
N-Myc and Aurora A are overexpressed and amplified and can drive the NEPC phenotype



Beltran et al, Cancer Discovery 2011

N-Myc is over-expressed during NEPC progression



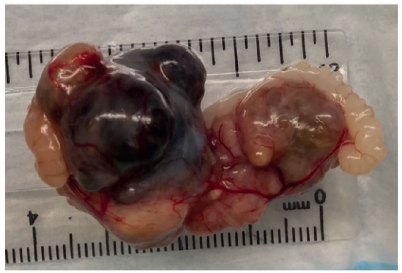


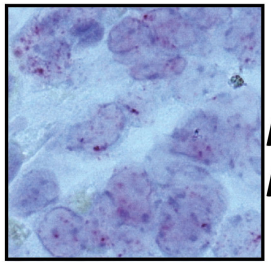
**** p value < 0.00004 (pairwise Wilcoxon)*

N-Myc drives NEPC phenotype in pre-clinical models

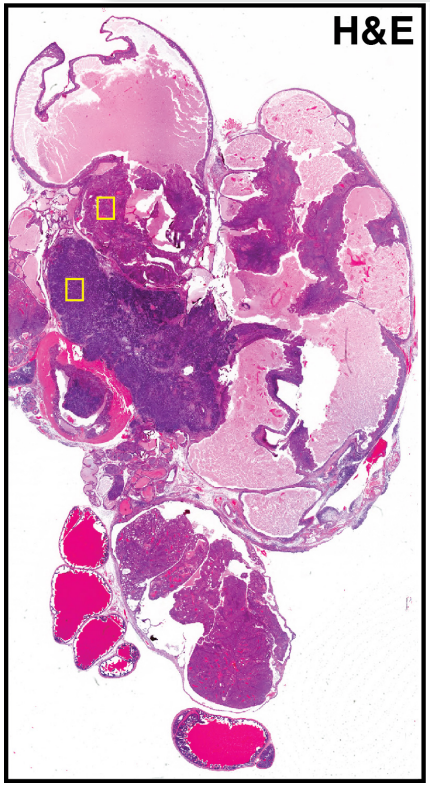
Tmprss2-CreERT2 or *Pb-Cre*
Pten$^{f/f}$
Rosa26 - LSL-MYCN

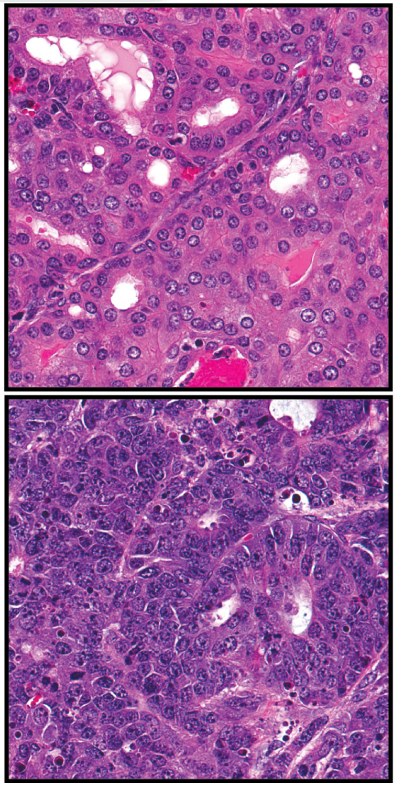
Pb-Cre$^{+/-}$; *Pten*$^{f/f}$; *LSL-MYCN*$^{+/+}$

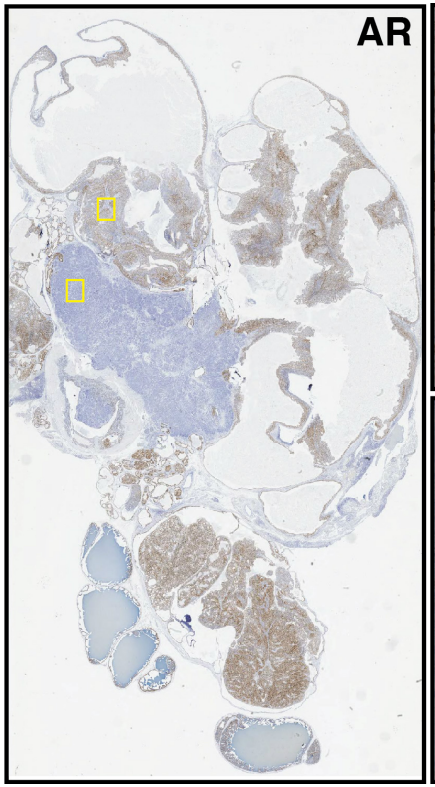


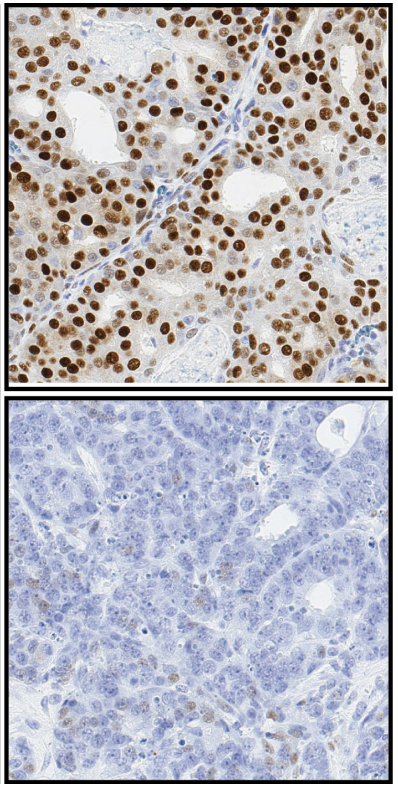


MYCN RNAish



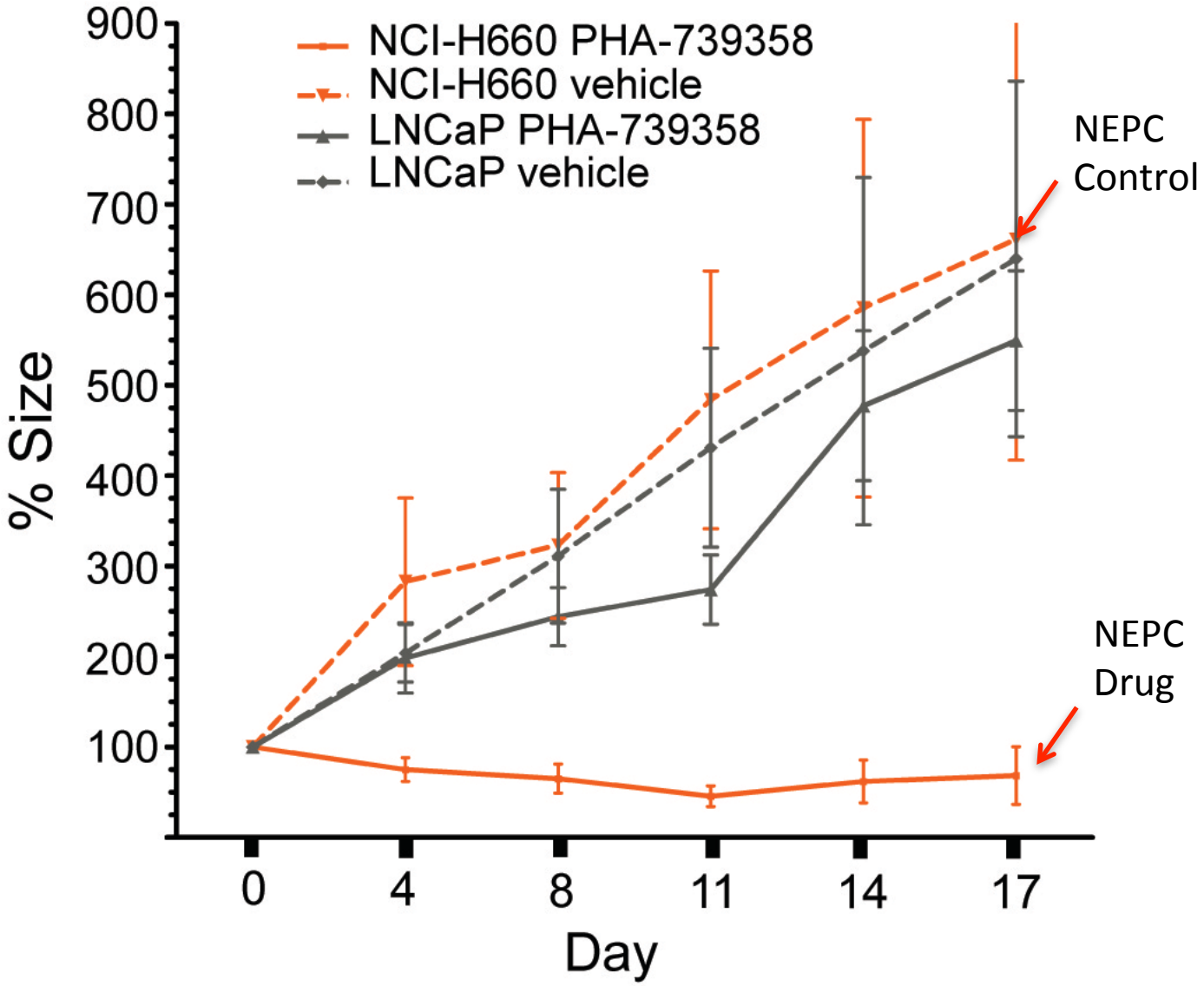






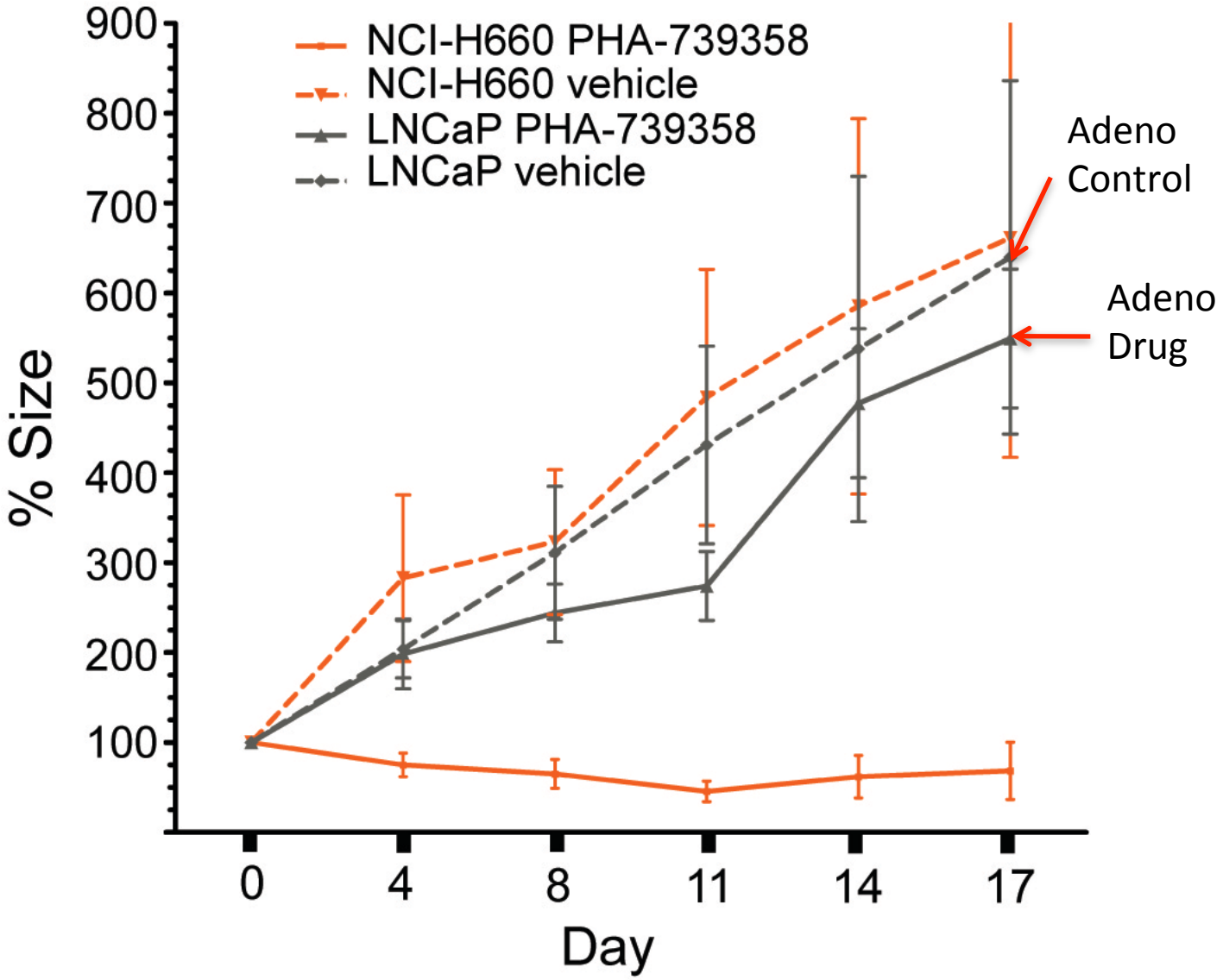
Dardenne, Beltran et al, Cancer Cell 2016

NEPC demonstrates enhanced *in vivo* sensitivity to PHA-739358 vs PCA in xenografts



PHA-739358 30 mg/kg IP BID x 5 days

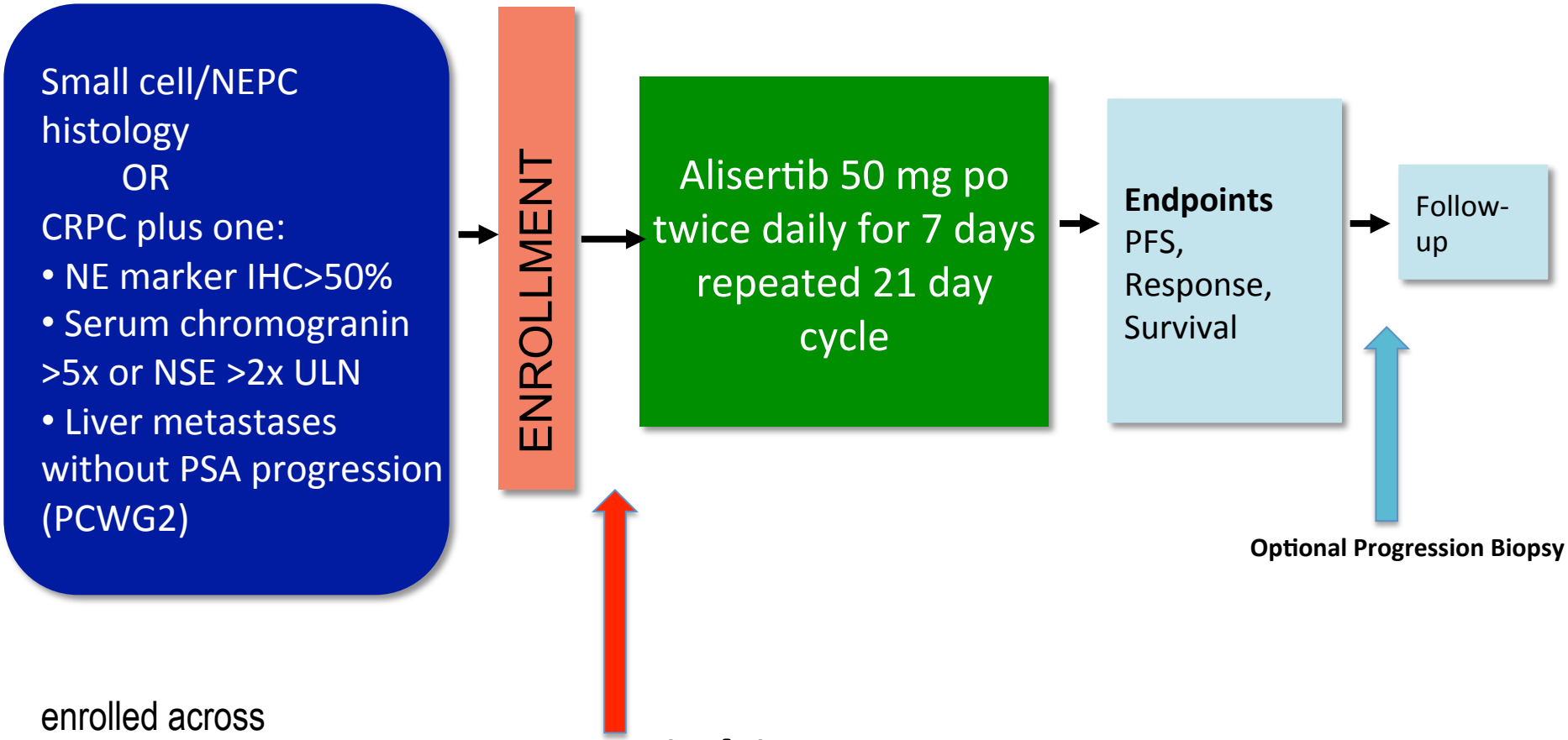
NEPC demonstrates enhanced *in vivo* sensitivity to PHA-739358 vs PCA in xenografts



PHA-739358 30 mg/kg IP BID x 5 days

Beltran et al, Cancer Discovery 2011

A Phase II Trial of The Aurora kinase A inhibitor Alisertib for Patients with Metastatic Castration Resistant and Neuroendocrine Prostate Cancer



enrolled across
9 US Centers

N=60

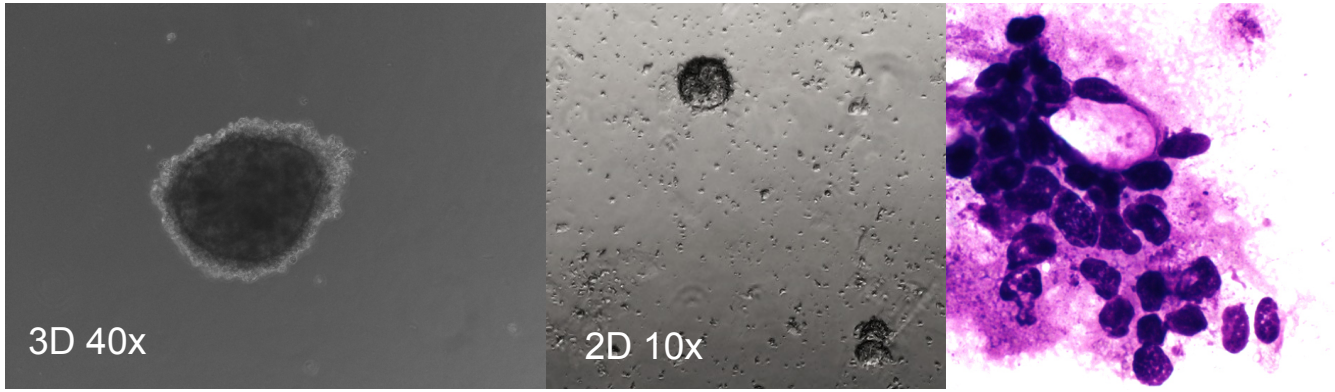
Weill Cornell Medicine

Patient derived organoids from patients treated with MLN8237

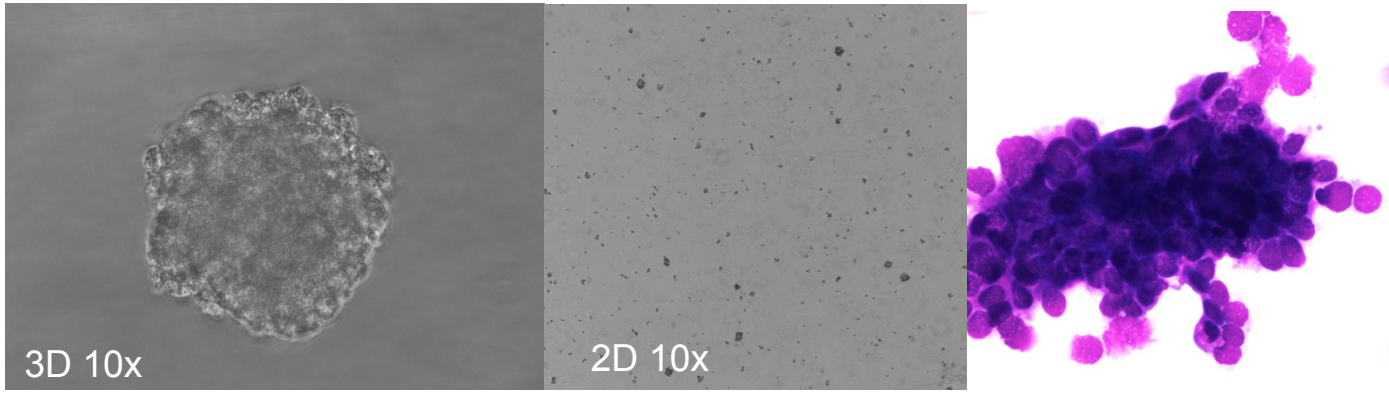
PM154	PM155
NEPC	NEPC
liver , bone, and lung metastasis	liver, bone and lung metastasis
Treated with ADT, cisplatin, etoposide x 3	Treated with ADT
No response to MLN8237	Exceptional response to MLN8237
Exome and transcriptome seq (bone met biopsy)	Exome and transcriptome seq (liver met biopsy)

Organoids for Co-Clinical Trials: MLN8237 Trial

Patient Organoid PM155 (Exceptional Responder)

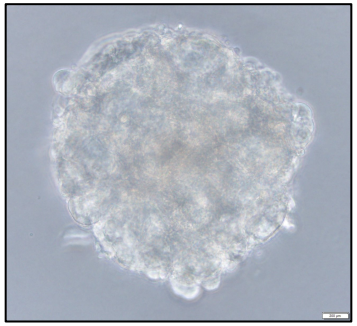


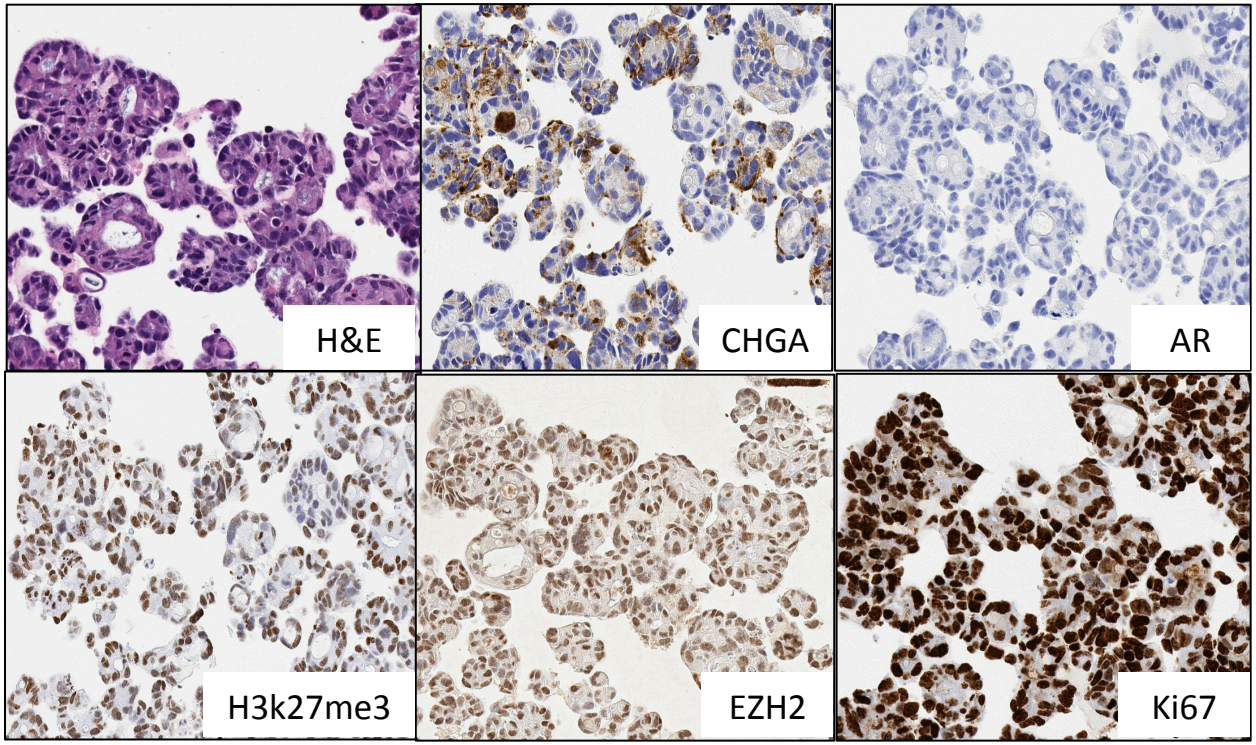
Patient Organoid PM154 (Non-responder)



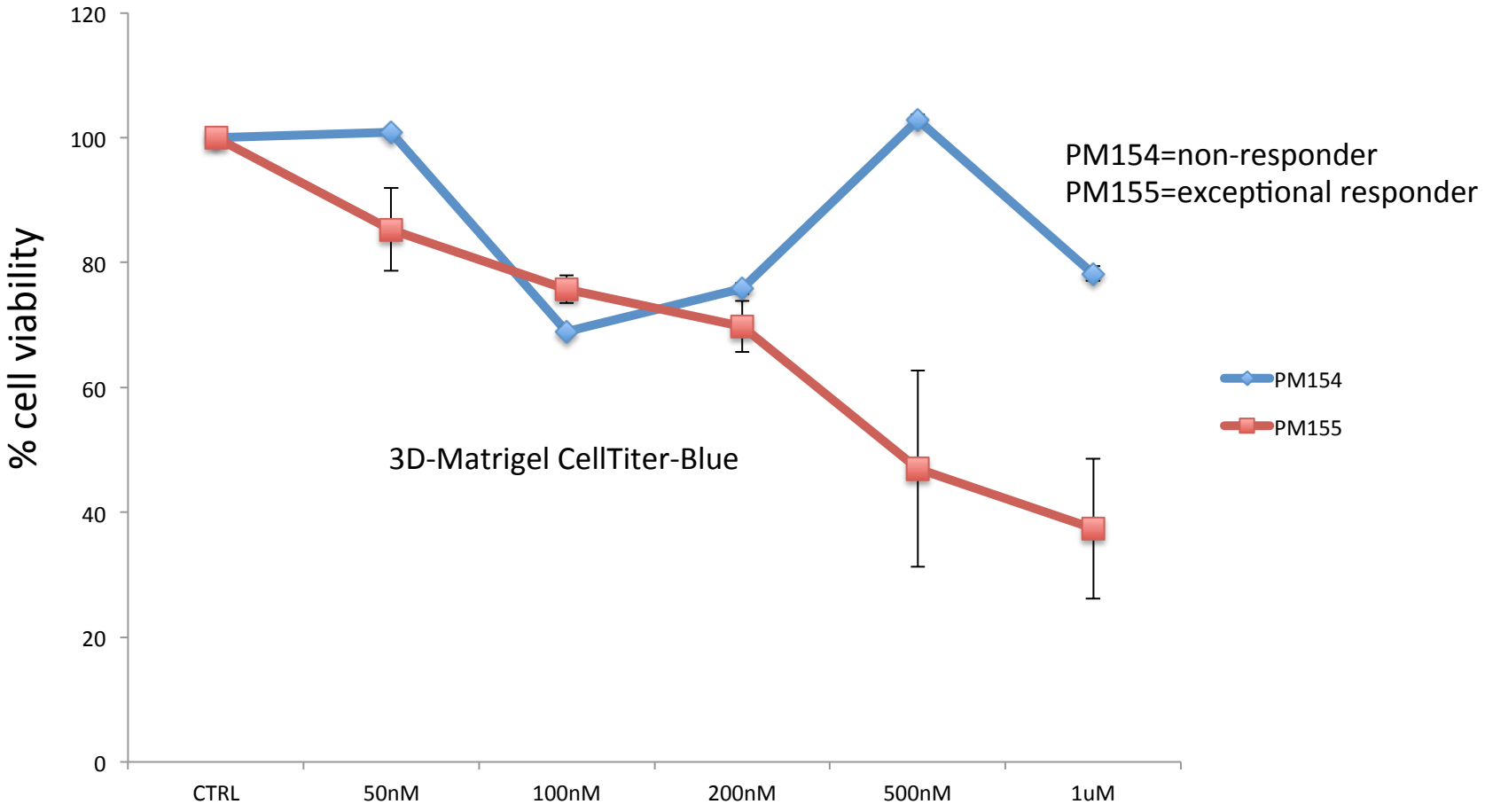
Organoid protocol adapted from Gao et al, Cell 2014

PM154 Organoid from MLN8237 Trial



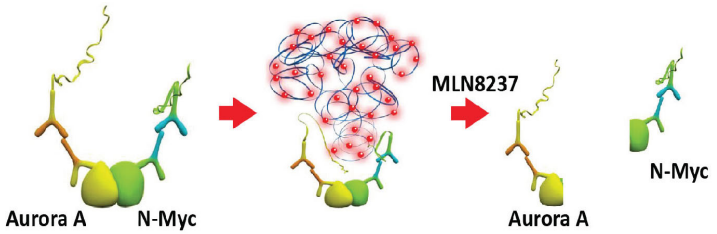


MLN8237 Treatment of Patient Organoids derived from Trial Patients

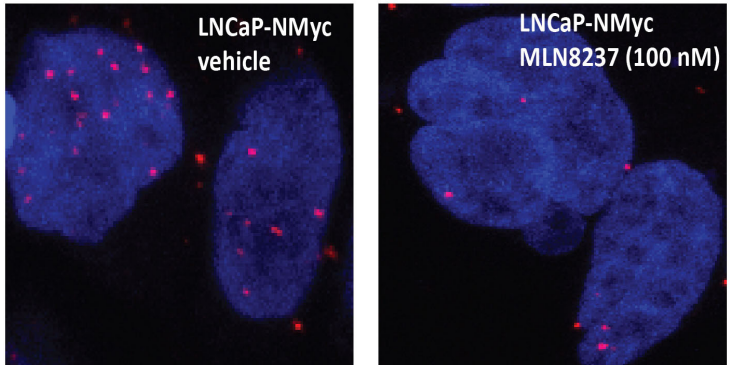


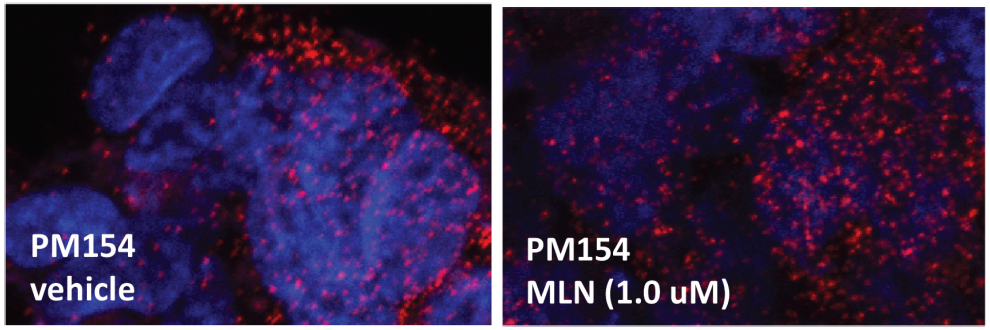
Predictive assay development

to measure on target effect and disruption Aurora-N-myc complex

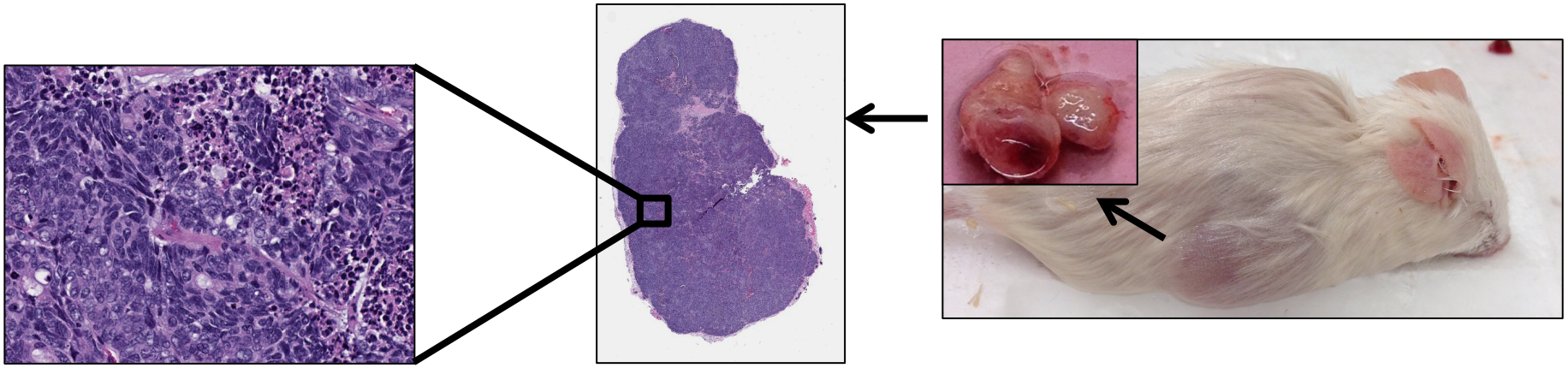


non-responder patient organoid expresses high levels of Aurora-N-myc complex, not disrupted with alisertib

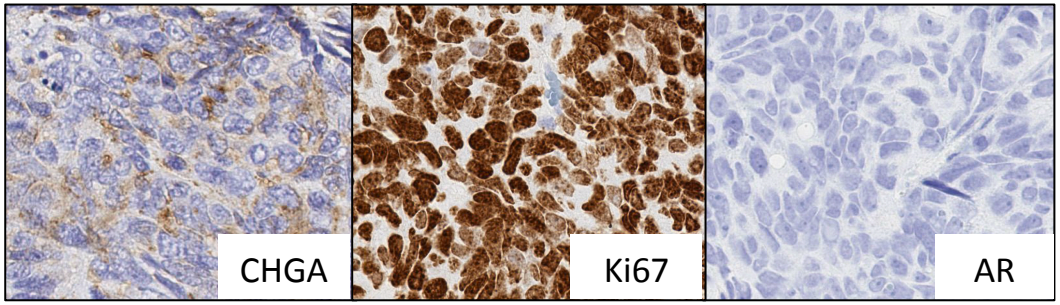


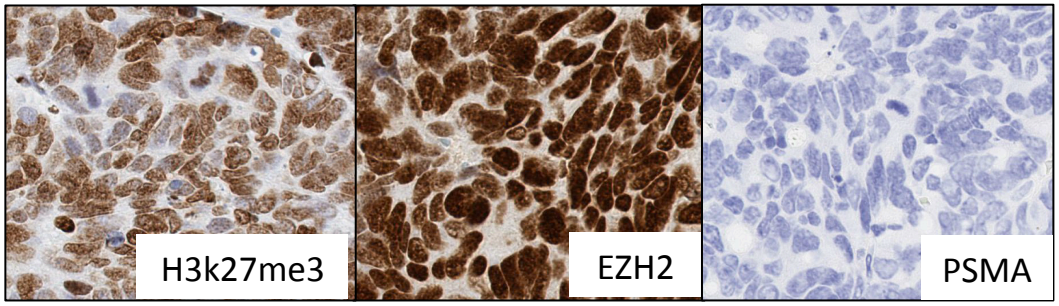


From organoid to PDX



PM154



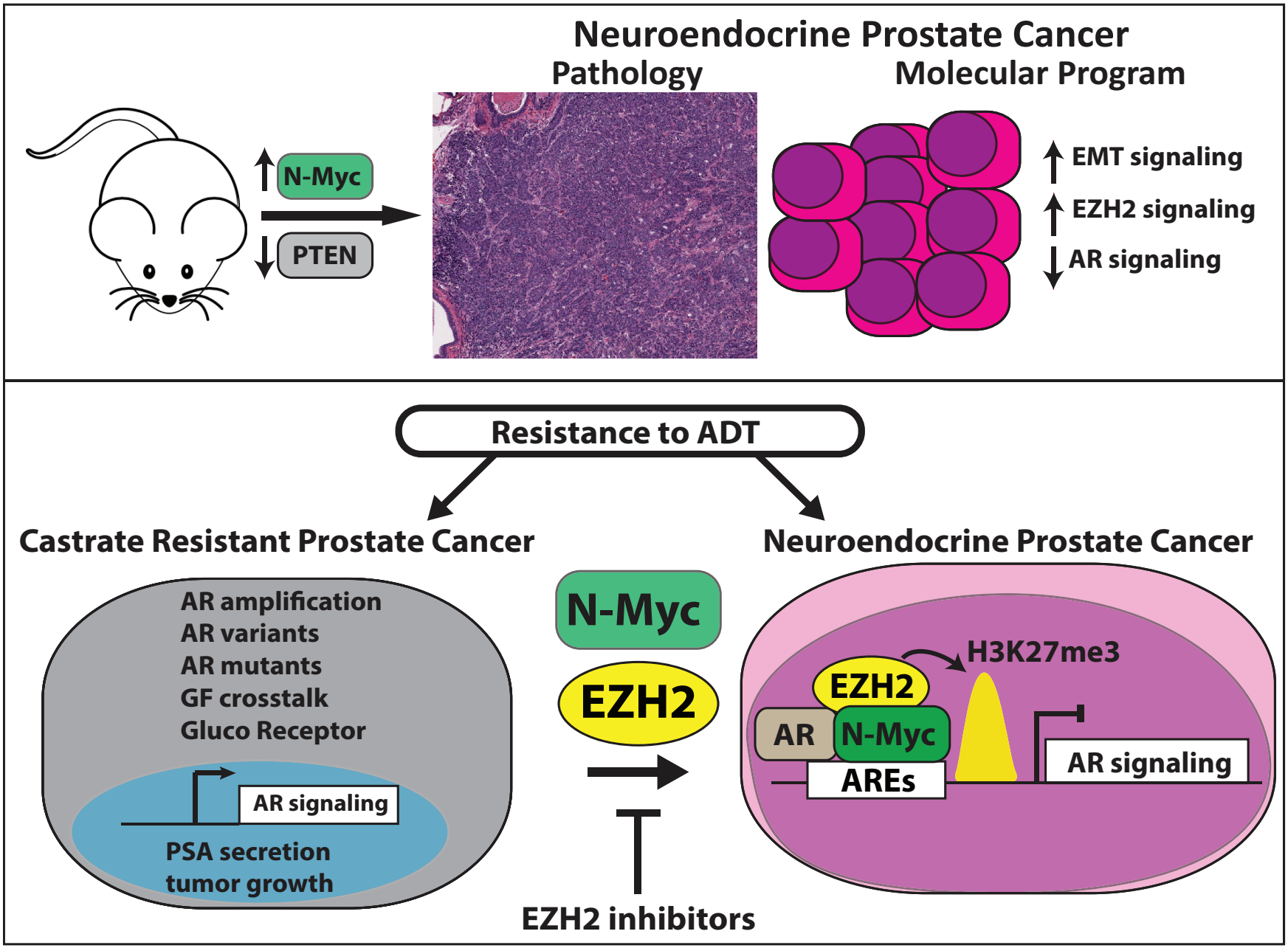


Loredana Puca

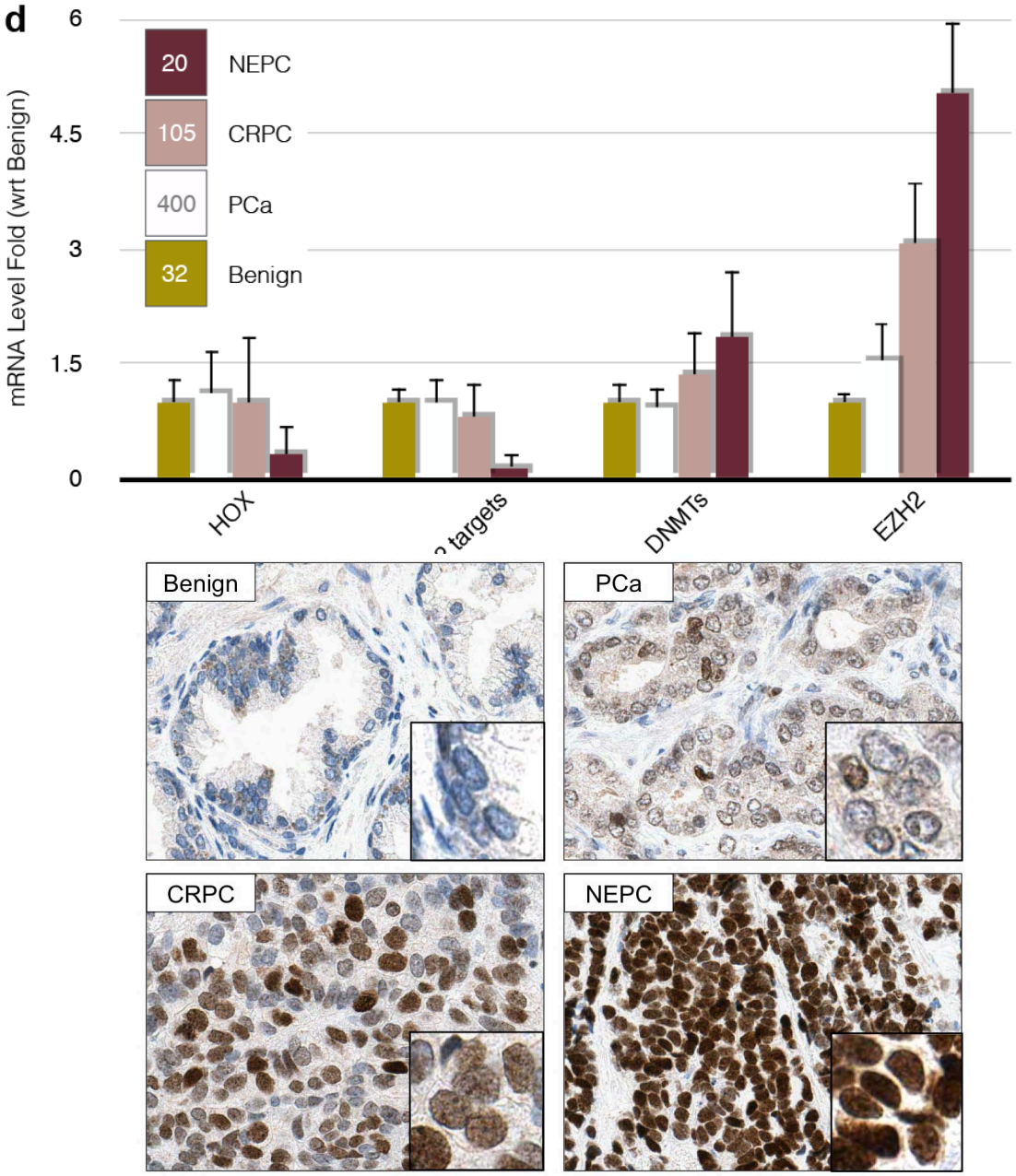
NEPC pathways

- Developmental/stem cell, differentiation, neuronal
  - PEG10 de-repression (placental gene)
  - Loss of RE1 silencing transcription factor (REST)
  - N-myc overexpression
  - Loss of Erg expression
  - BRN2 overexpression
- Epigenetic regulators
  - SRMM4 alternative splicing of REST
  - Upregulation of EZH2, DEK
- Cell cycle: AURKA, PLK, RB1

Wyatt et al, Cell Rep 2015, Lin et al, Cancer Res 2014, Lapuk et a, N Pathol 2013, Beltran et al 2011, Mouiner at al 2014, Akamatus et al 2005, Li et al 2016, Tzelpi et al,2012. Zhange et al 2015, Creia et al 2016, Tan et al, 2014, Bishop et al, Cancer Cell 2016



Dardenne, Beltran et al Cancer Cell 2016

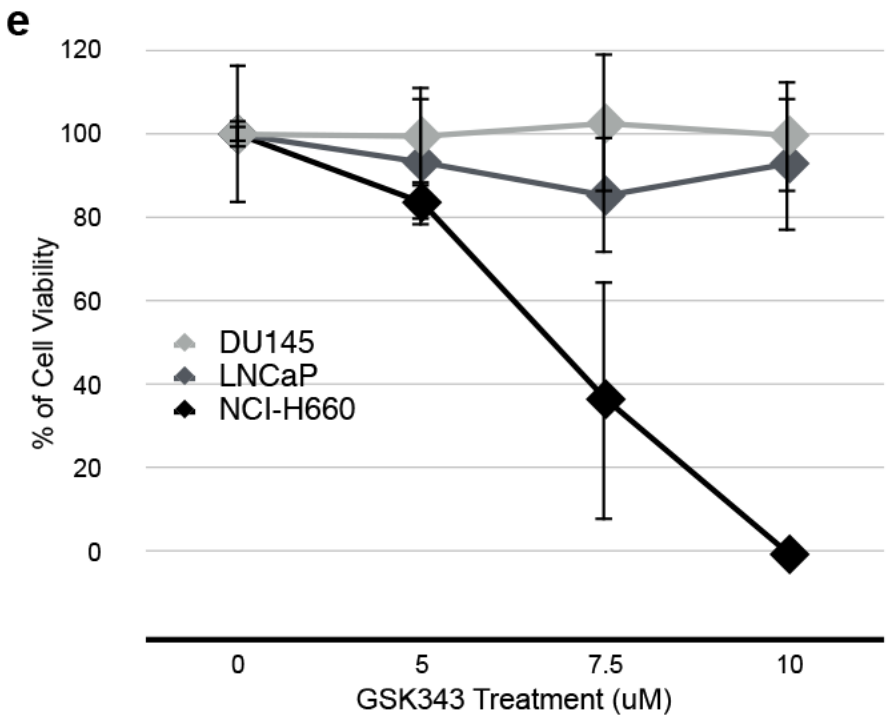


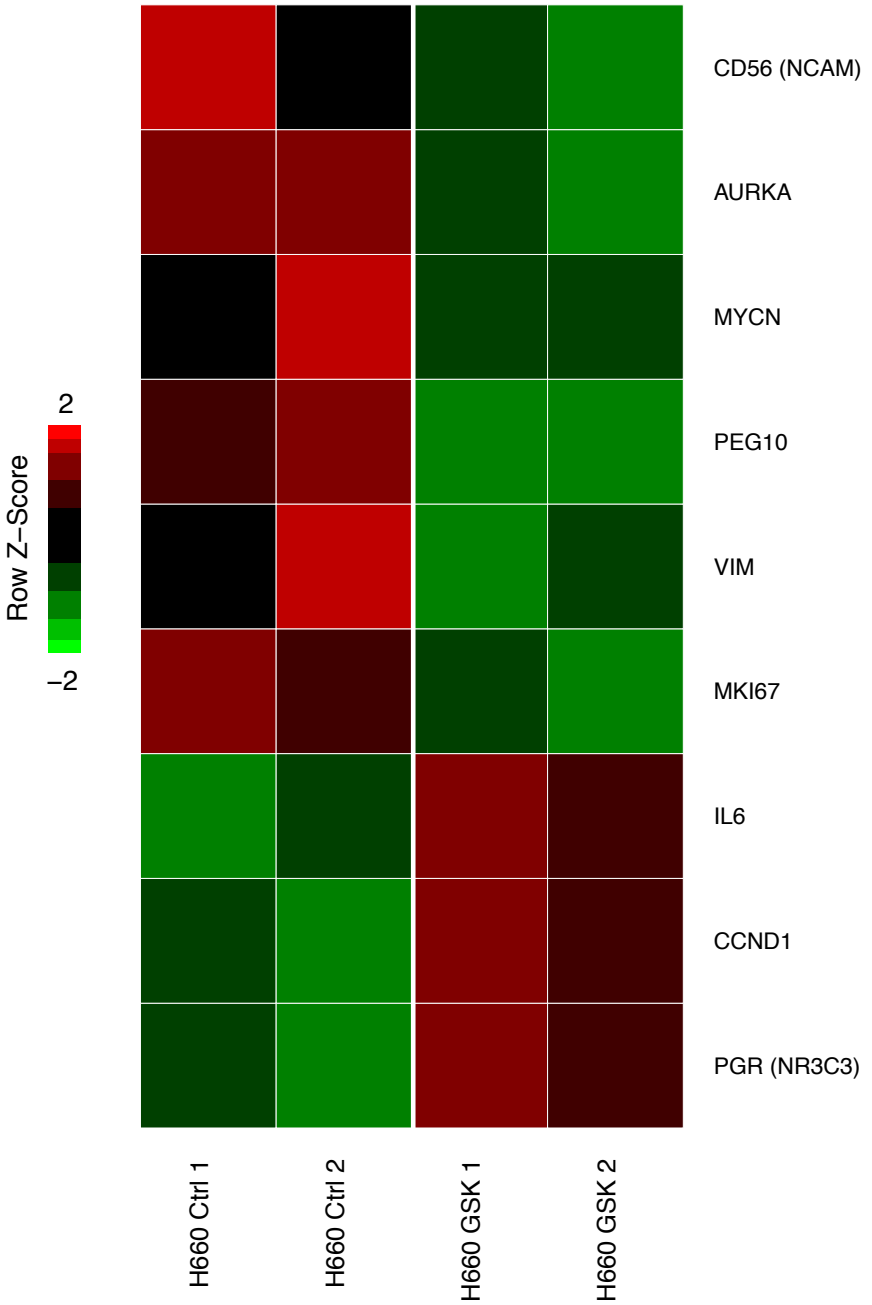
EZH2 Immunihistochemistry

DNA methyltransferase (DNMTs)
and the histone methyltransferase EZH2 are overexpressed in NEPC and target genes downregulated

Beltran et al, Nat Med 2016

GSK343 EZH2 inhibitor in cell lines

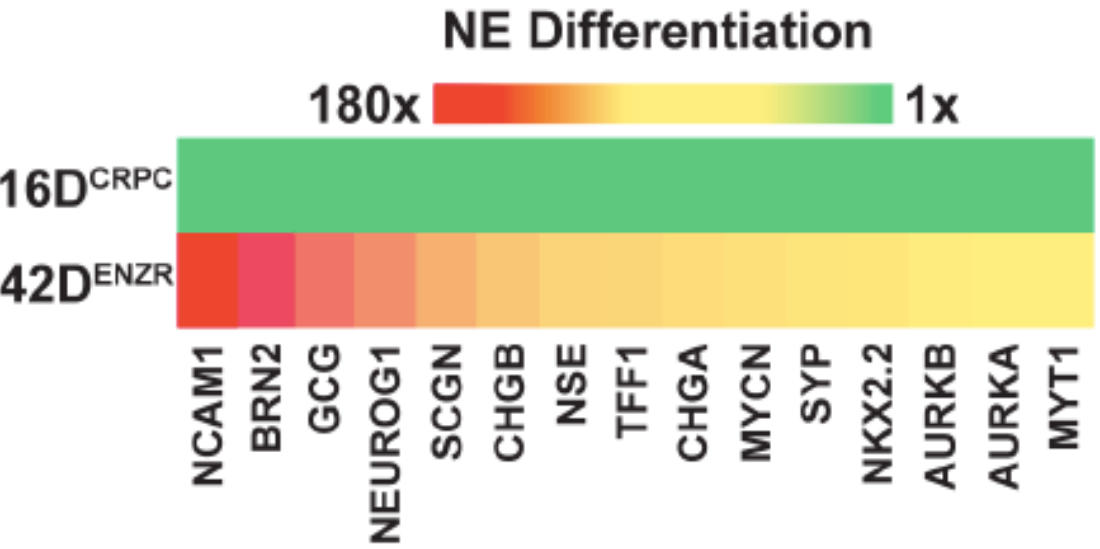


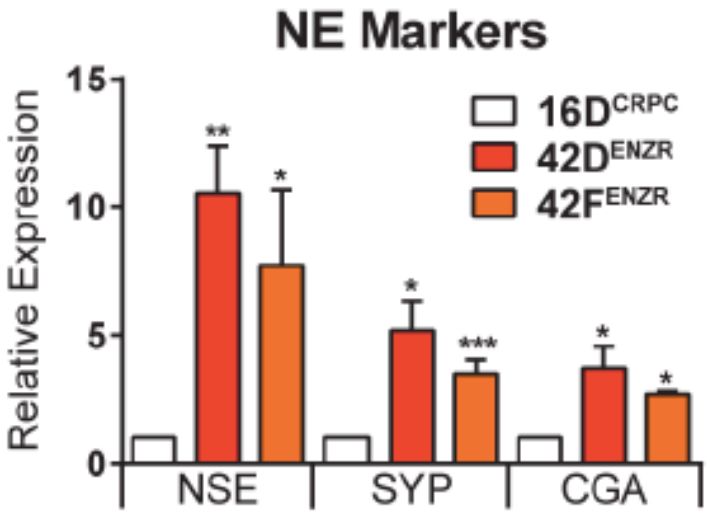


Loredana Puca

Does loss of AR signaling contribute to the NEPC phenotype?

Enza resistant, Low AR-signaling (42D) associated with BRN2 overexpression



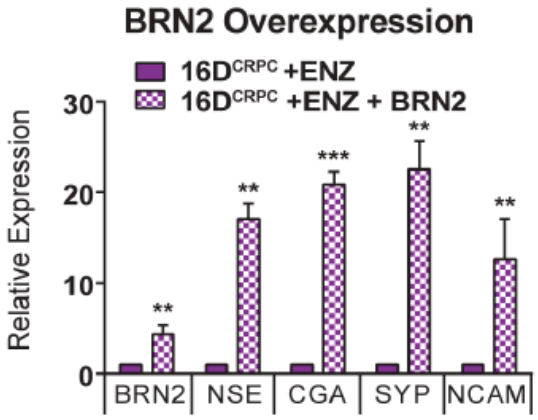


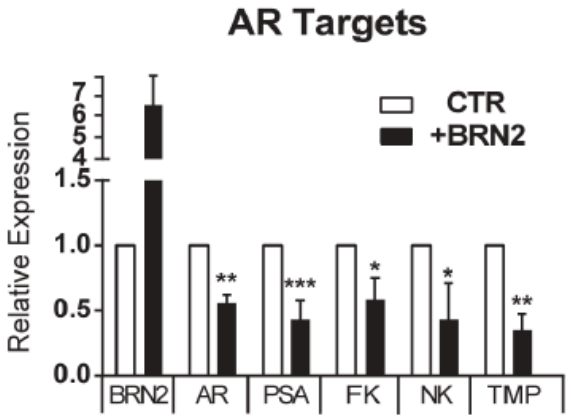
BRN2= Neuronal Transcription Factor, master regulator of neuronal differentiation

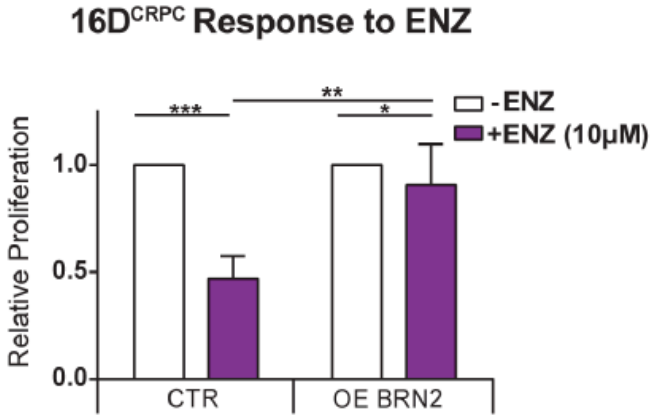
AR suppresses BRN2 transcription
BRN2 up-regulated in NEPC patient samples

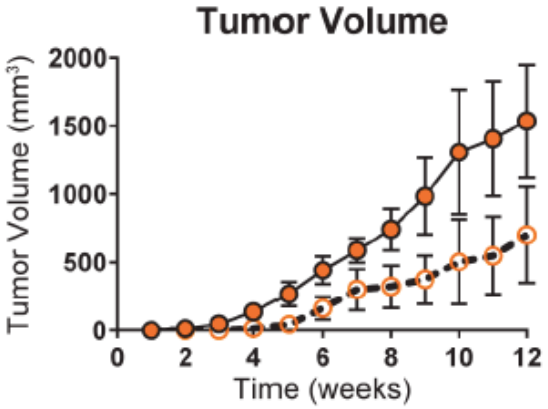
Bishop et al, Cancer Discovery 2016

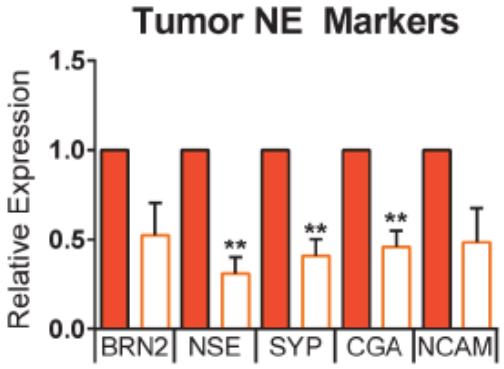
BRN2 is key regulator of NEPC











Bishop et al, Cancer Discovery 2016

Summary

- Prostate cancer including CRPC is an androgen driven disease. The mainstay of therapy focuses on targeting the AR.
- NEPC is an AR independent resistance phenotype.
  - Arises clonally from adenocarcinoma
  - Associated with distinct clinical features and molecular alterations (eg., low AR, loss of RB1/ TP53, REST, high NMYC, AURKA, EZH2, BRN2, PEG10, SRRM4, DLL3, epigenetic)

Future Directions

- The use of a combination of Pathologic, Clinical, and Molecular features to define AR independent CRPC, including NEPC

- Development of targeted treatment approaches based on molecular sub-classification of advanced prostate cancer

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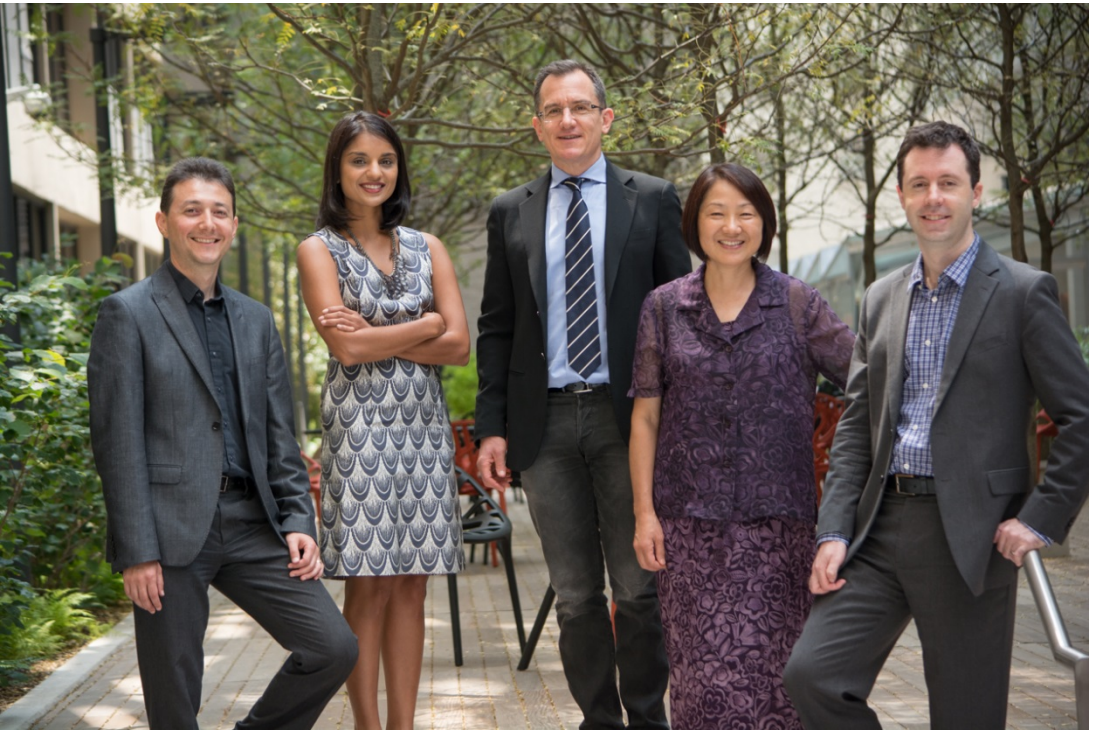
Damon Runyon Foundation

Weill Cornell Medicine

Englander Institute for Precision Medicine



From left:

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Himisha Beltran, MD, Clinical Director

Mark A. Rubin, **MD,** Director

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Olivier Elemento, PhD, Computational Leader

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Peter Martin, MD
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Theresa MacDonald
Tong Dai, MD
Tuo Zhang
Wayne Tam, MD