Constellation PHARMACEUTICALS

Transcriptomic analysis identifies EZH2 inhibition as a therapeutic strategy in metastatic castration-resistant prostate cancer

Andy Conery, Ph.D.

Omicsoft/IPA Users Group Meeting

September 2019

Stellar Science, Breakthrough Medicine

Constellation's Approach to Cancer Therapeutics

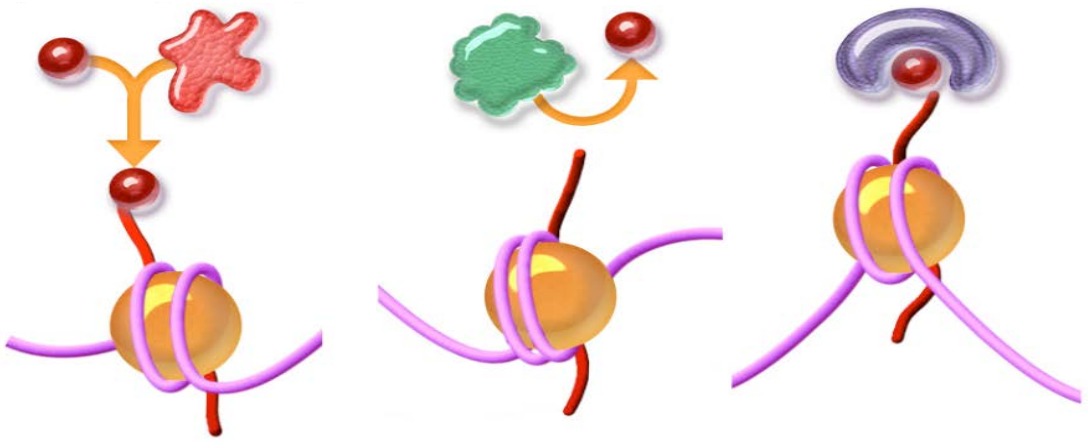
Manipulation of Transcriptional Programs in Tumor Cells and Immune Cells

Target Chromatin Regulatory Cues with Small Molecules

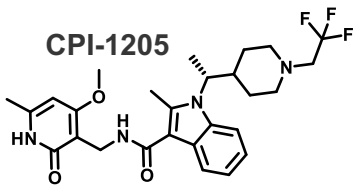
Writers

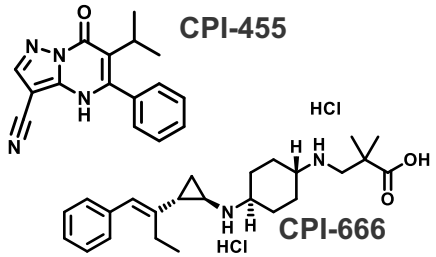
Erasers

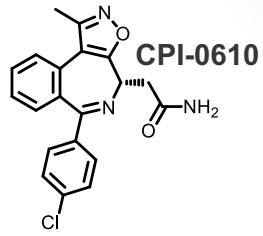
Readers



Turn Genes On or Off



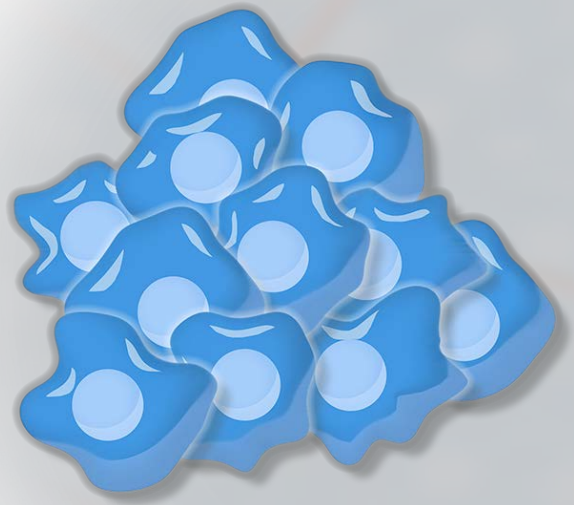




+ 10 probe compounds targeting other mechanisms

Oncology Applications

Tumor Cells



T Cell

NK Cell

MDSC

Target Transcriptional Programs That Result in Cell Death

Re-program Immune Cells to Promote Tumor Immunity

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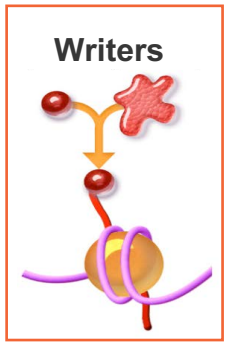
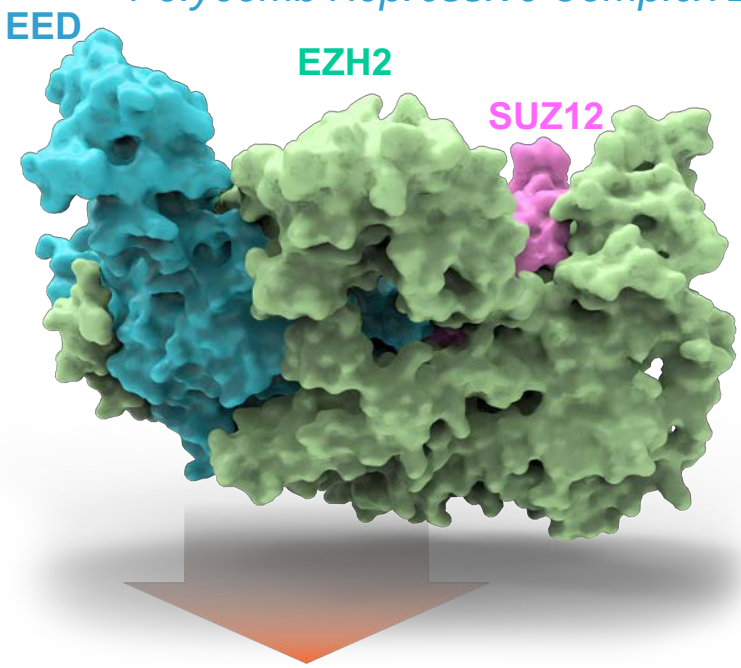
EZH2 Inhibition Offers Broad Therapeutic Potential

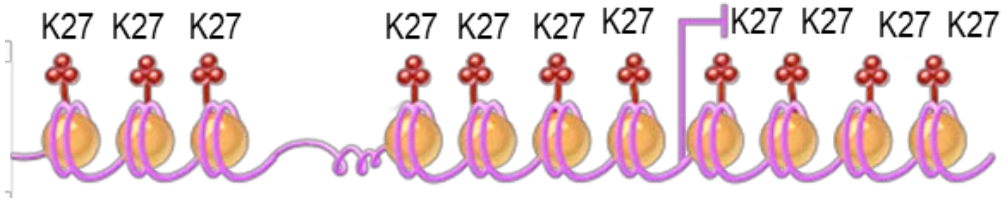
EZH2 "Writer" Activity Suppresses Gene Transcription



Polycomb Repressive Complex 2 (PRC2)



… Methylates Histone H3 at Lysine 27 (K27)



SUPPRESSED TRANSCRIPTION

Disease Progression and Acquired Drug Resistance:

- EZH2 mediates gene silencing that alters cell state and diminishes response to existing therapies

Cancer Genetics:

- Mutations in genes which create a functional dependency on EZH2

Regulation of Immune Cells:

- EZH2 reprograms T cells to suppress an anti-tumor immune response

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Disease progression and treatment landscape of prostate cancer

Progression to mCRPC is driven by genomic and transcriptomic changes

Pre-metastatic prostate cancer

Metastatic Castration-Resistant Prostate Cancer

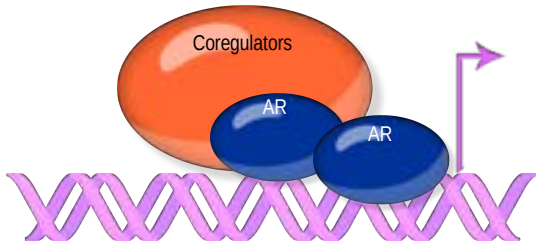
Local Therapy

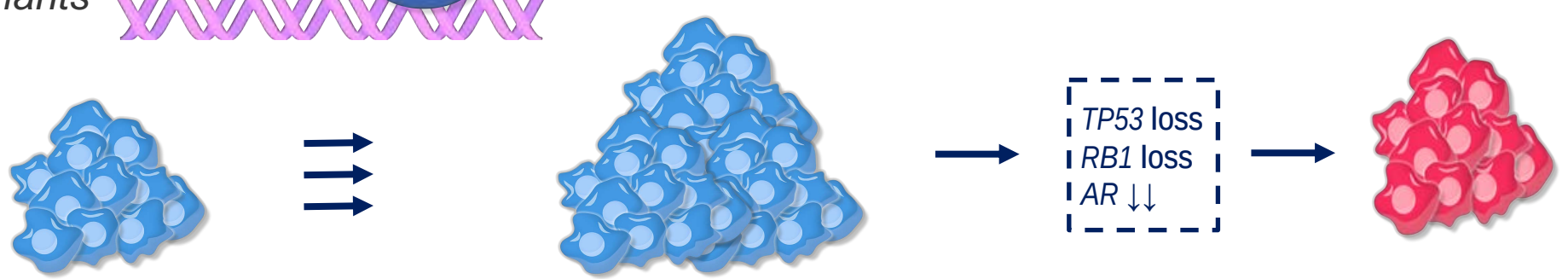
Androgen Deprivation

1st Line: enzalutamide or abiraterone	75% Response Rate 9-15 month PFS
2nd Line: abiraterone or enzalutamide	
3rd Line: chemo or palliative care	

Transcriptional rewiring is key to disease progression and development of resistance

AR amplification
AR mutation
AR splice variants





AR signaling-dependent
- Luminal "fixed" lineage, maintained by AR
- Become resistant to ARS inhibitors

AR signaling-independent
- Lineage plastic, loss of luminal identity
- Resistant to ARS inhibitors

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Integrated data processing

Alignment and expression quantification with Omicsoft

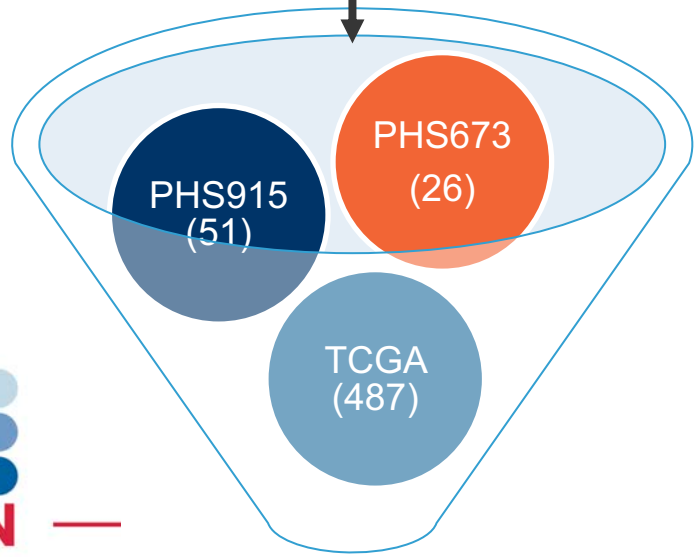
Cell
Resource
Integrative Clinical Genomics of Advanced Prostate Cancer

Robinson et al. 2015/PHS000915

ARTICLE
Integrative clinical genomics of metastatic cancer

Robinson et al. 2017/PHS000673

THE CANCER GENOME ATLAS



OmicSoft Corporation
Now a QIAGEN company

QIAGEN

Counts matrix (gene and transcript-level)

- Stratify mCRPC patients through integration of clinical and molecular features with transcriptional profiling
- Identify transcriptional programs specific to metastatic disease relative to primary prostate cancer
- Integrate transcriptional impacts of EZH2 inhibition in prostate cancer cell models with transcriptional programs specific to mCRPC

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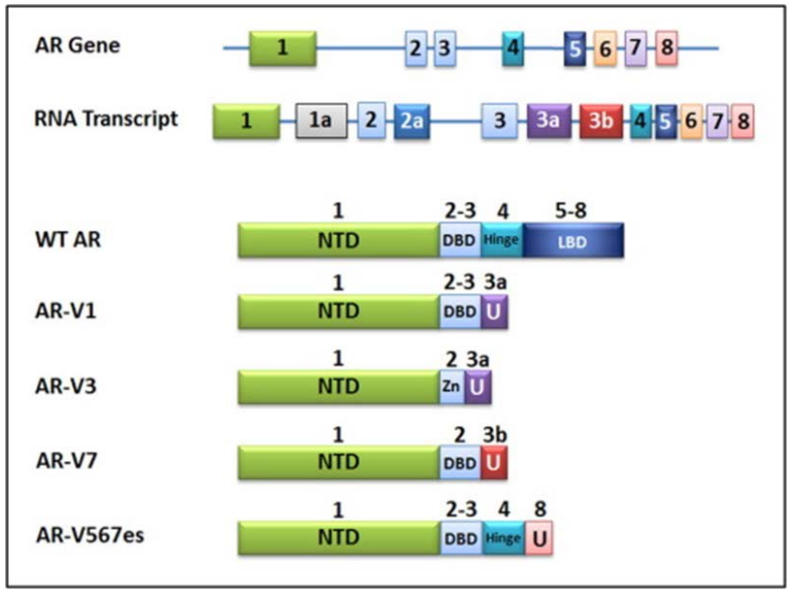
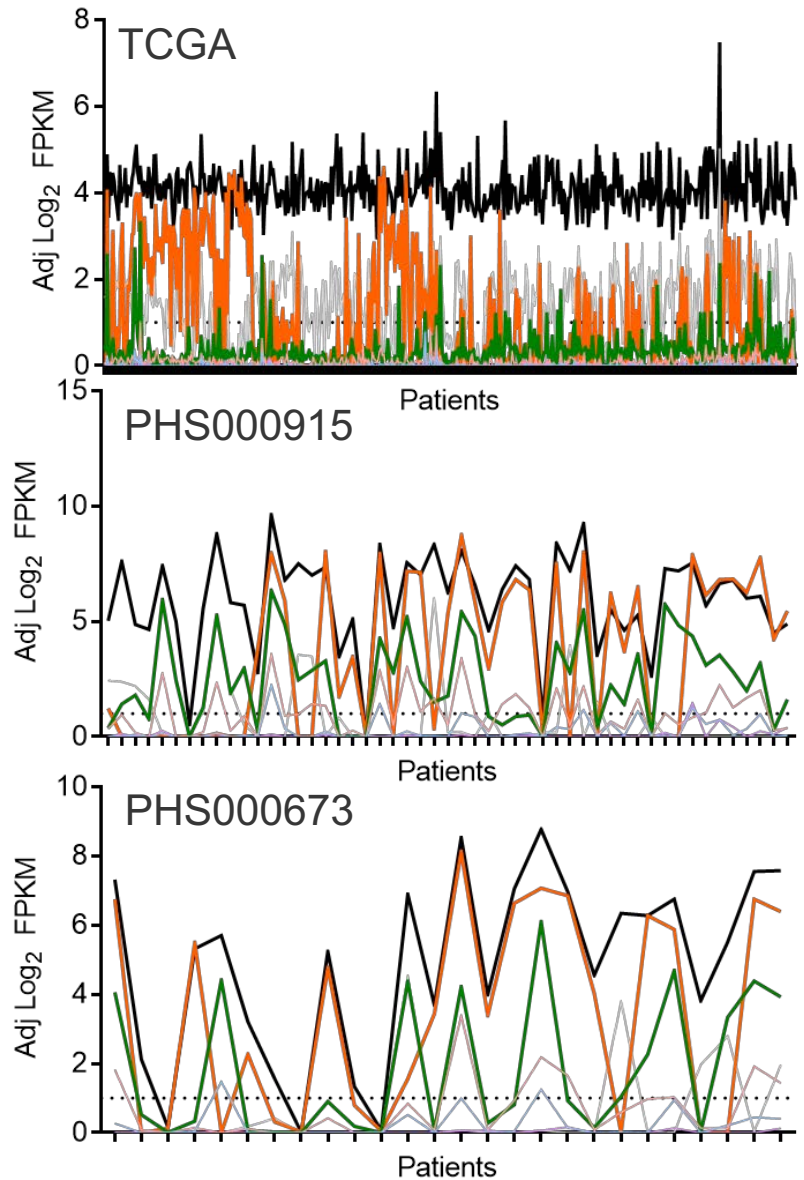
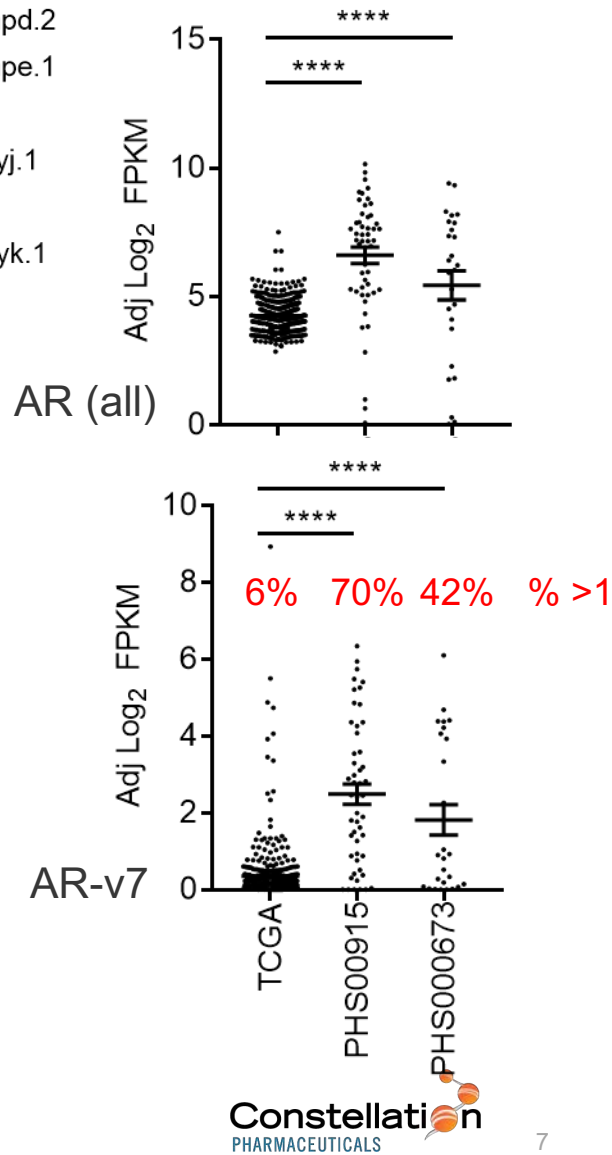
Linking mCRPC transcriptional programs to molecular and clinical features

AR and AR-v7 expression

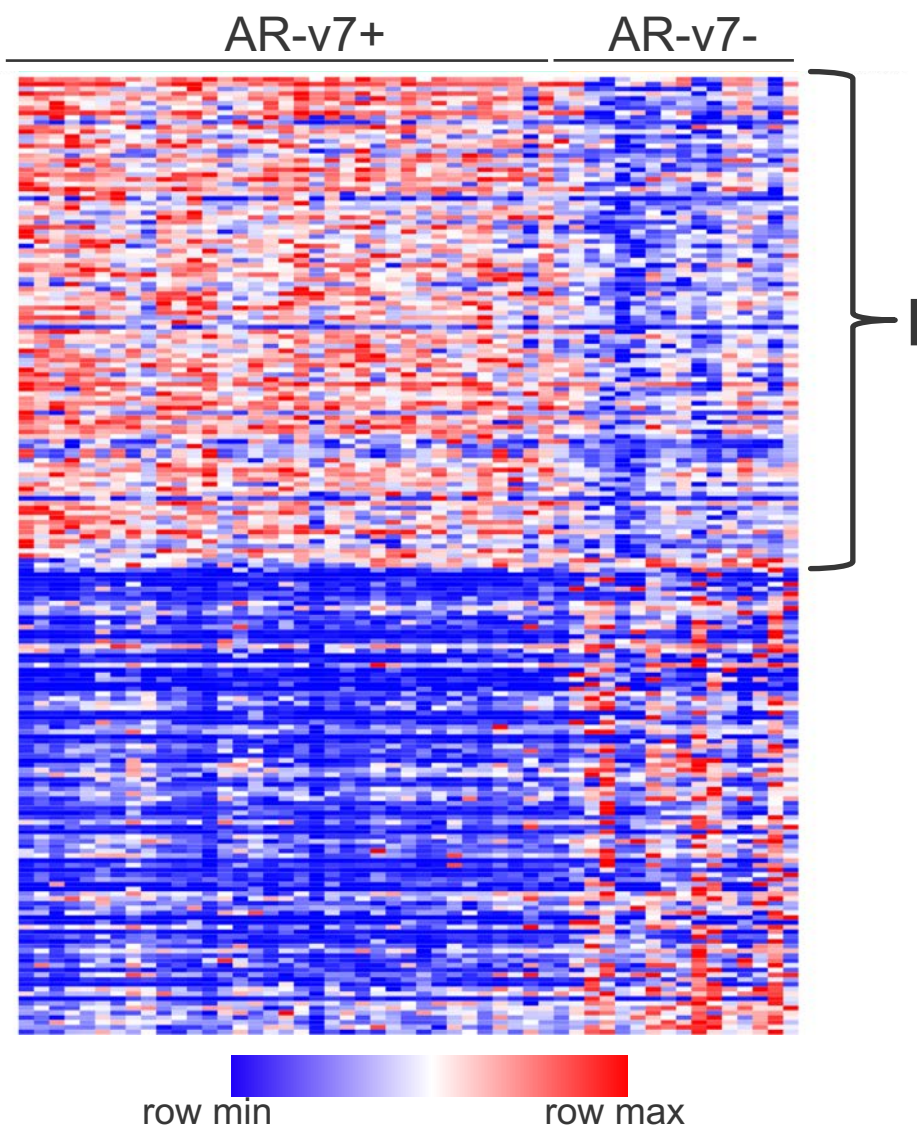


- AR is highly expressed in mCRPC, and splice variants are frequently co-expressed
- AR-V7 expression is a frequent event in mCRPC, and is associated with poor prognosis and resistance to ARSi



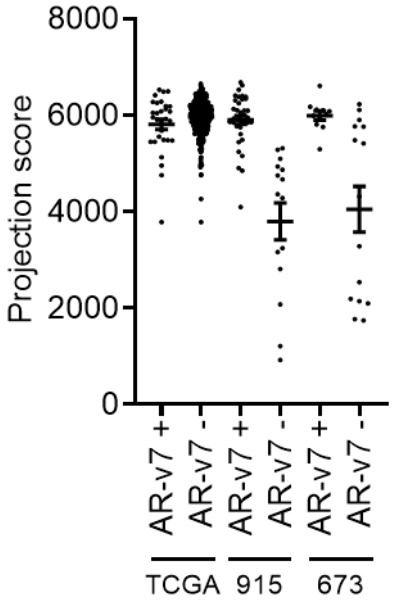


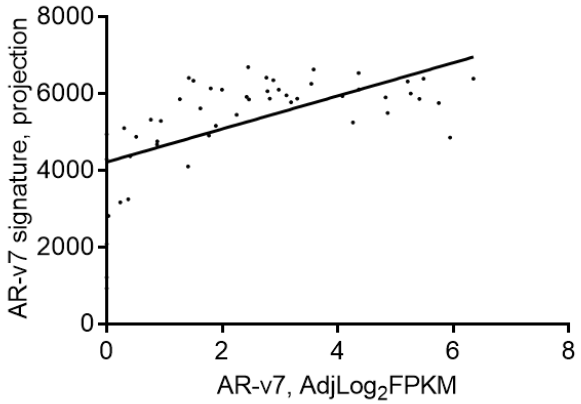
Identification of an AR-v7 expression signature

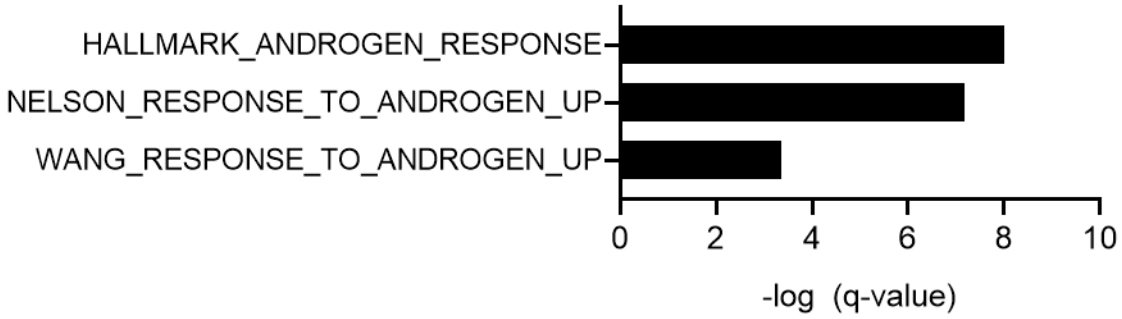


Gene set
(915 cohort)

ssGSEA
(all cohorts)







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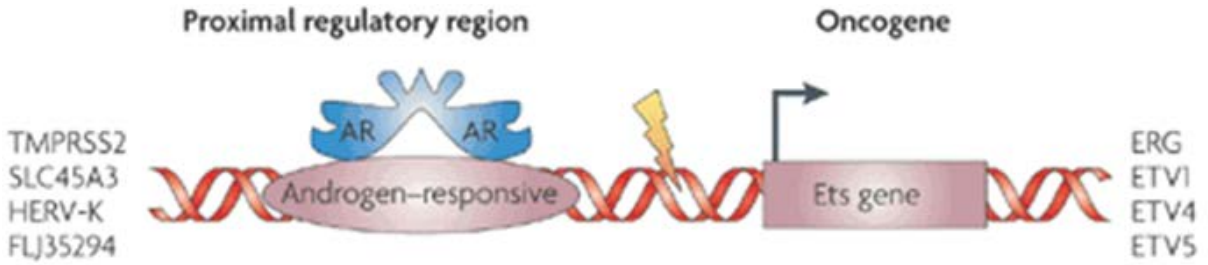
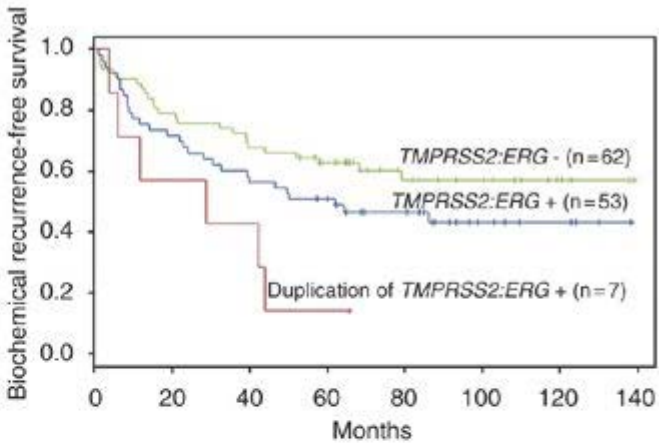
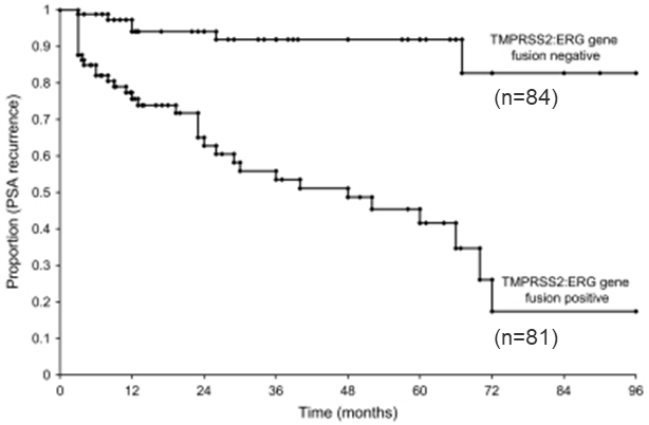
Expression of ETS fusion proteins



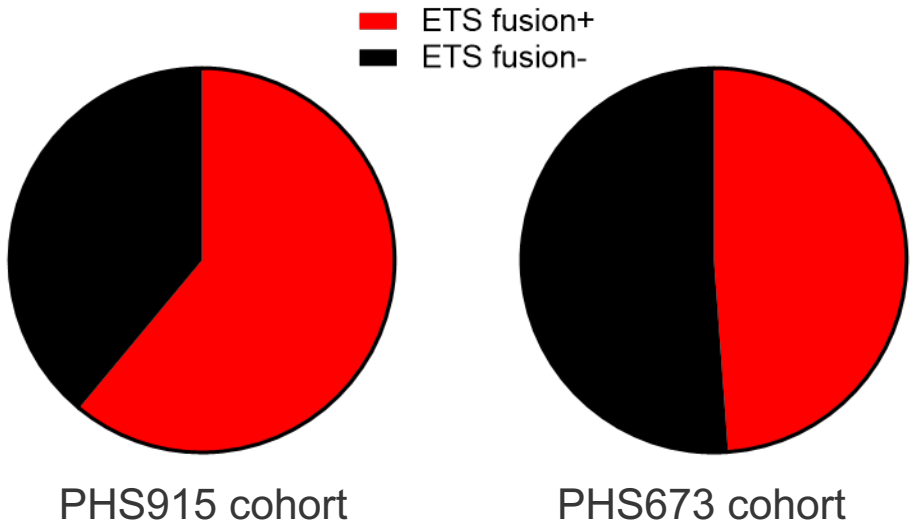
Kumar-Sinha et al. *Nat. Rev. Cancer* 2008



Yoshimoto et al. *Modern Pathology* 2008



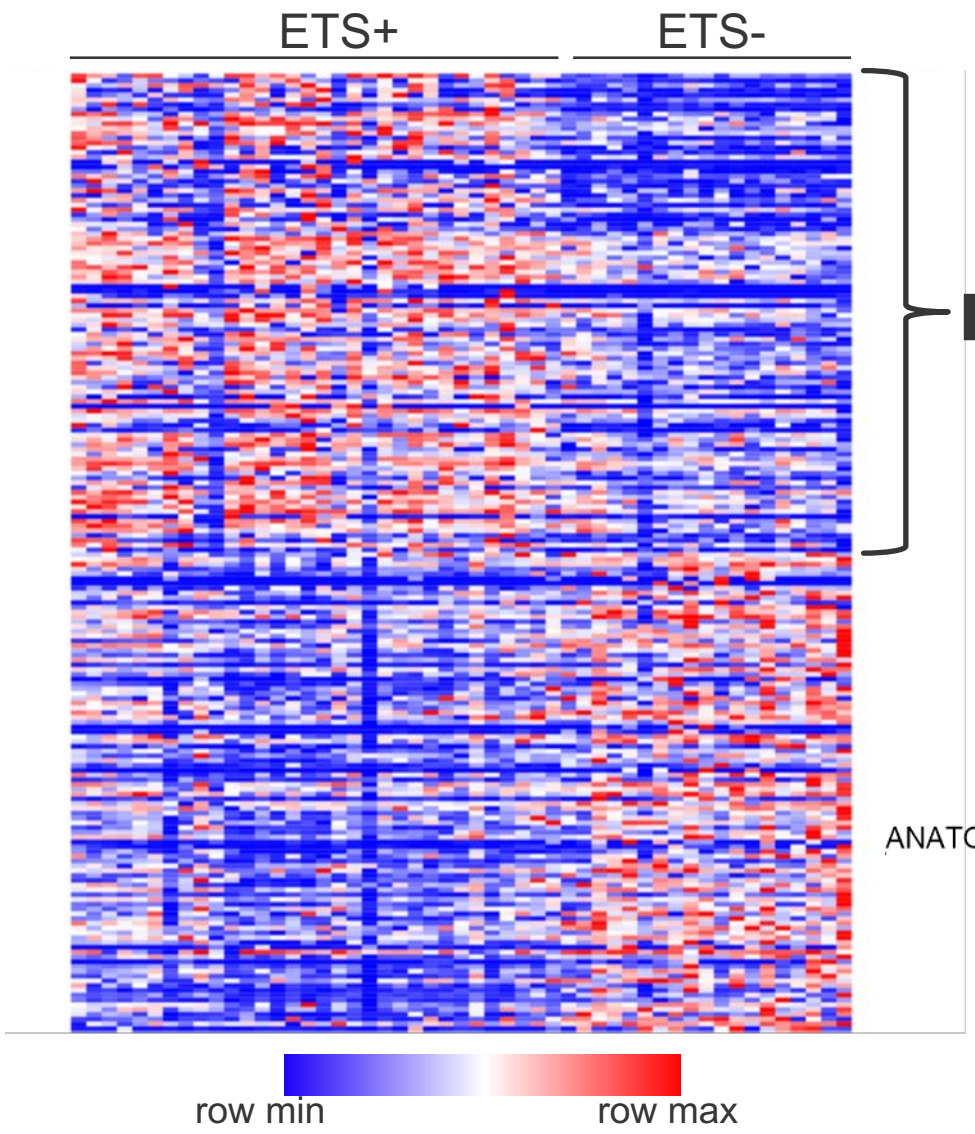
Nam et al. *Br. J. Cancer* 2007



PHS915 cohort

PHS673 cohort

Transcriptional signature of ETS fusion+ mCRPC



Gene set
(915 cohort)

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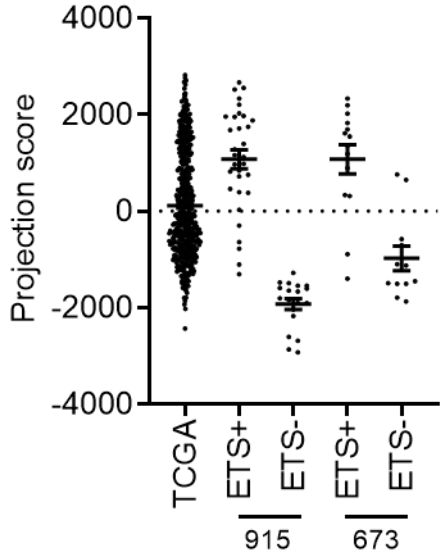

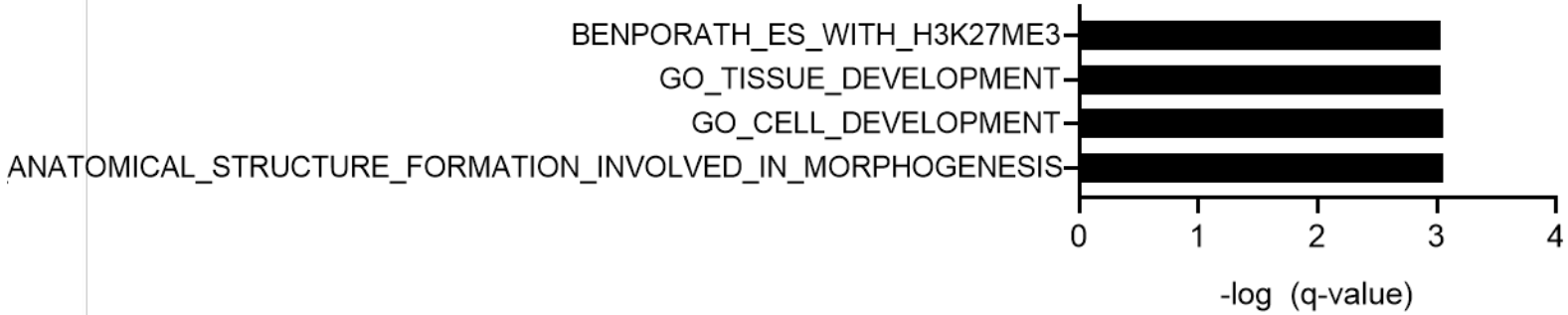

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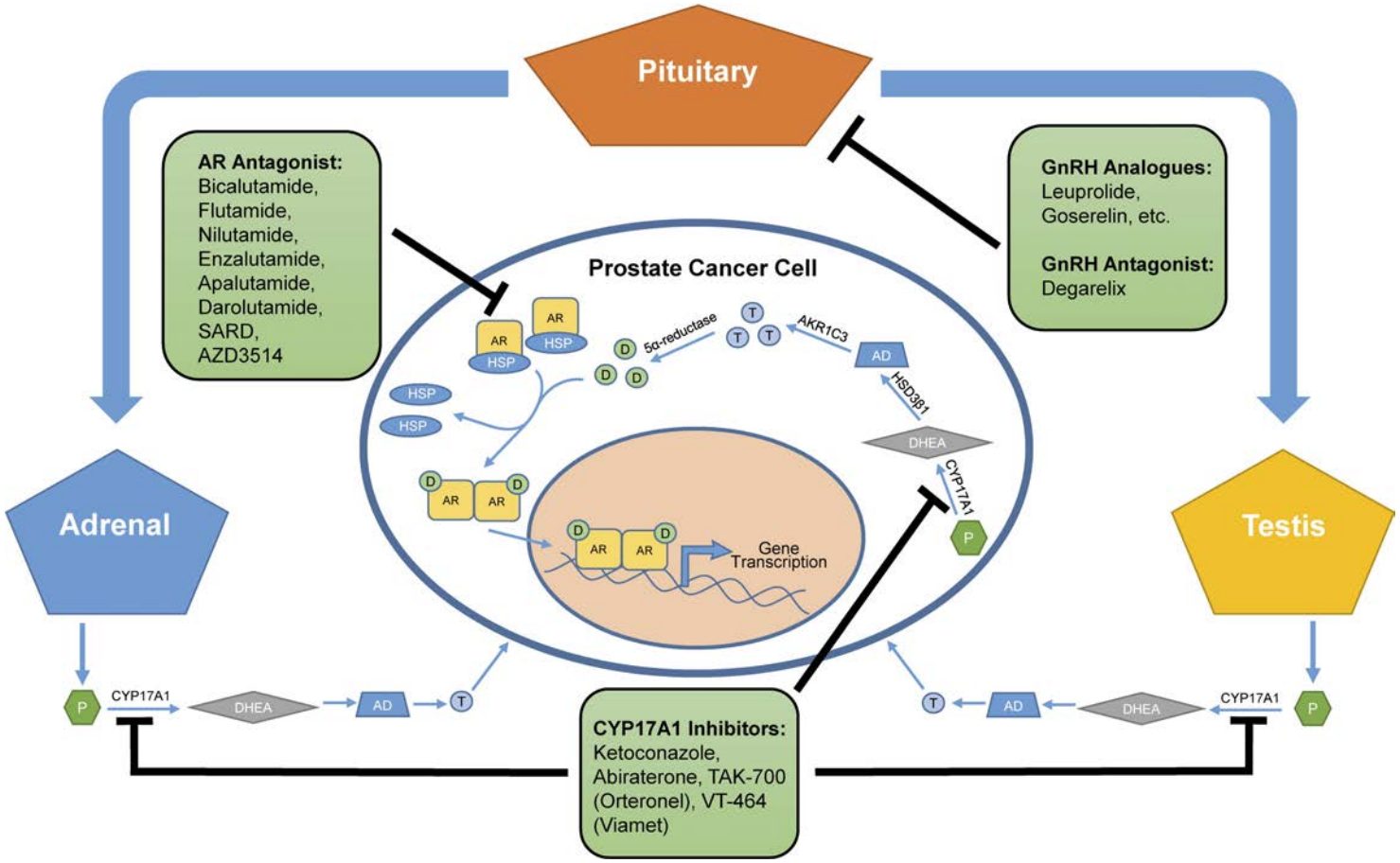
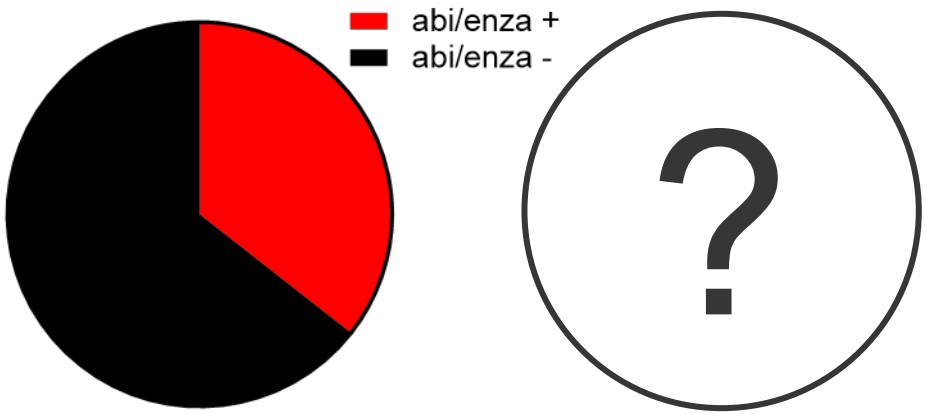
Androgen receptor signaling inhibitors (ARSi)





PHS915 cohort

PHS673 cohort

Hahn et al. *Am. Soc. Clin. Oncol. Educ. Book* 2018

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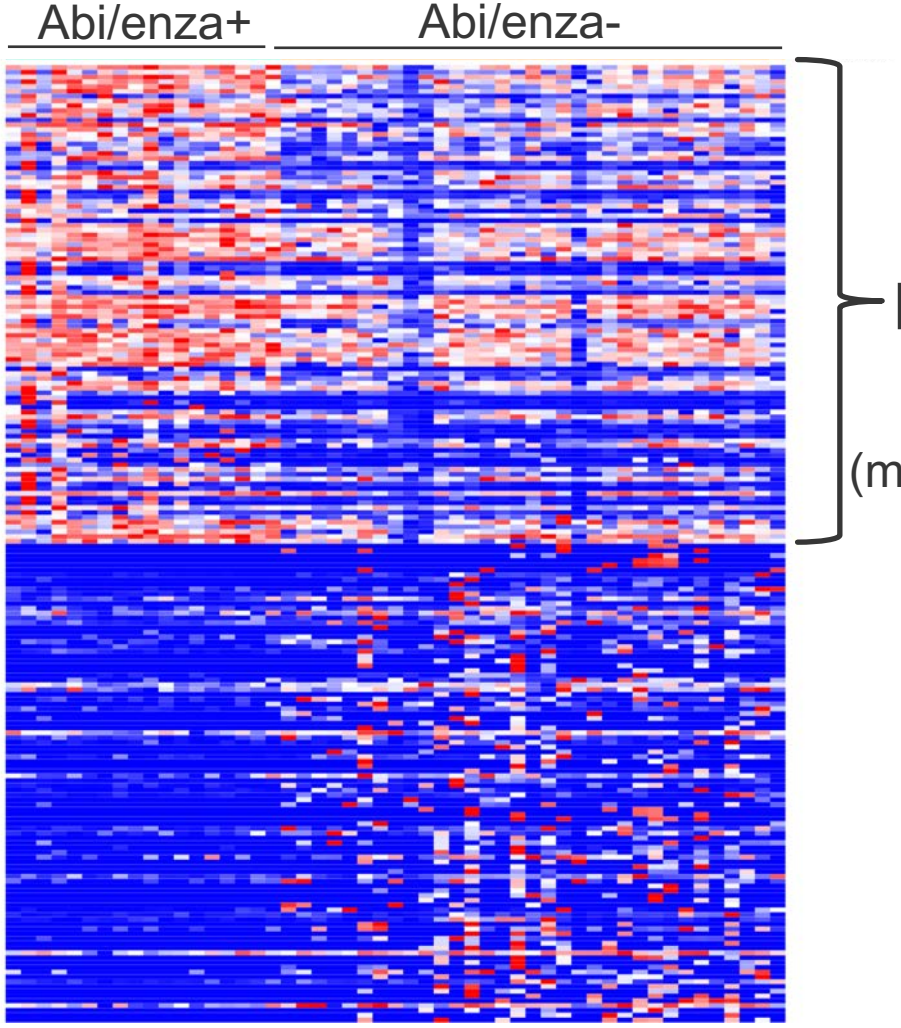
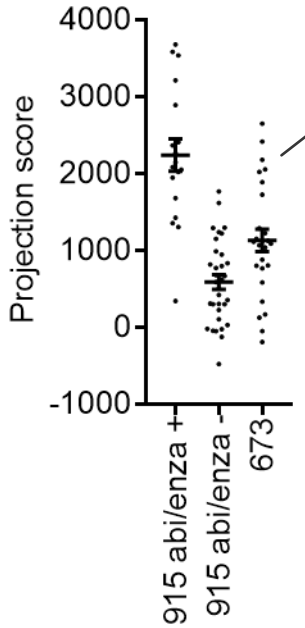
Transcriptional signature of ARS inhibition

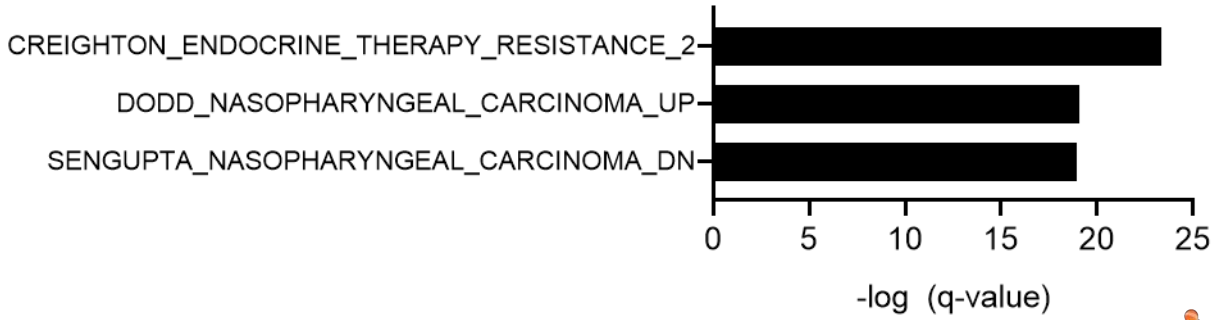


Gene set
(915 cohort)

ssGSEA
(mCRPC cohorts)



Suggests that ~10 patients in 673 cohort have seen abi/enza



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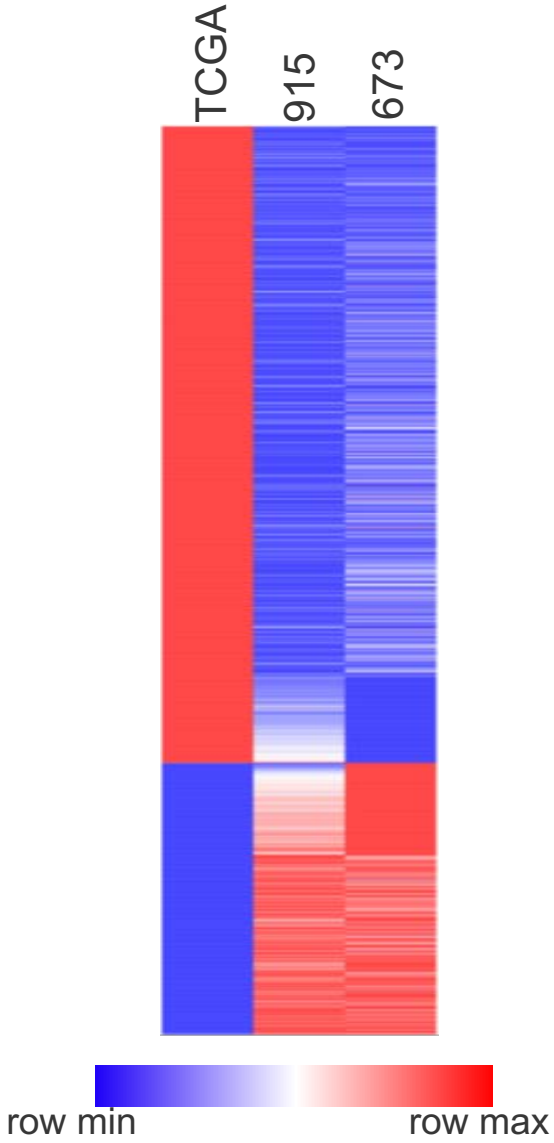
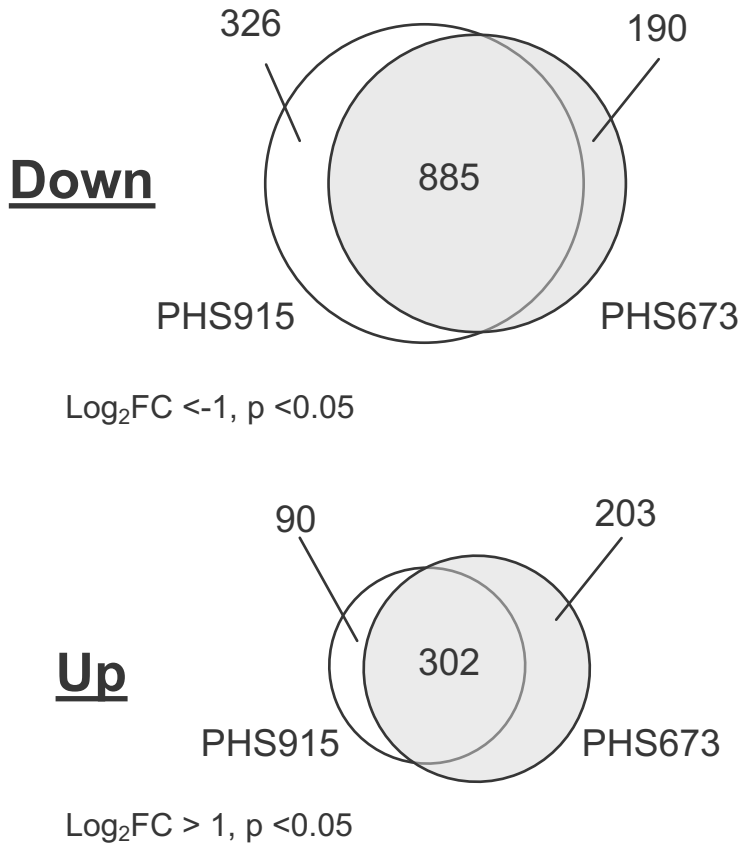
12

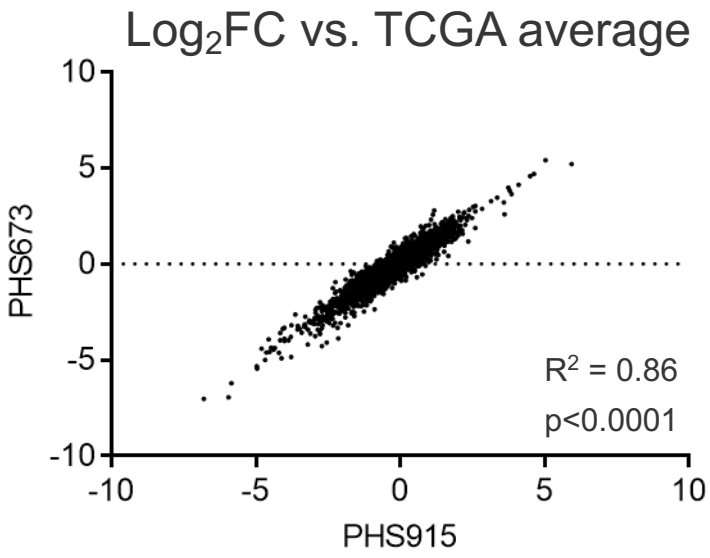
Engagement of mCRPC transcriptional programs through EZH2 inhibition

mCRPC transcriptional programs

Comparing TCGA with mCRPC cohorts

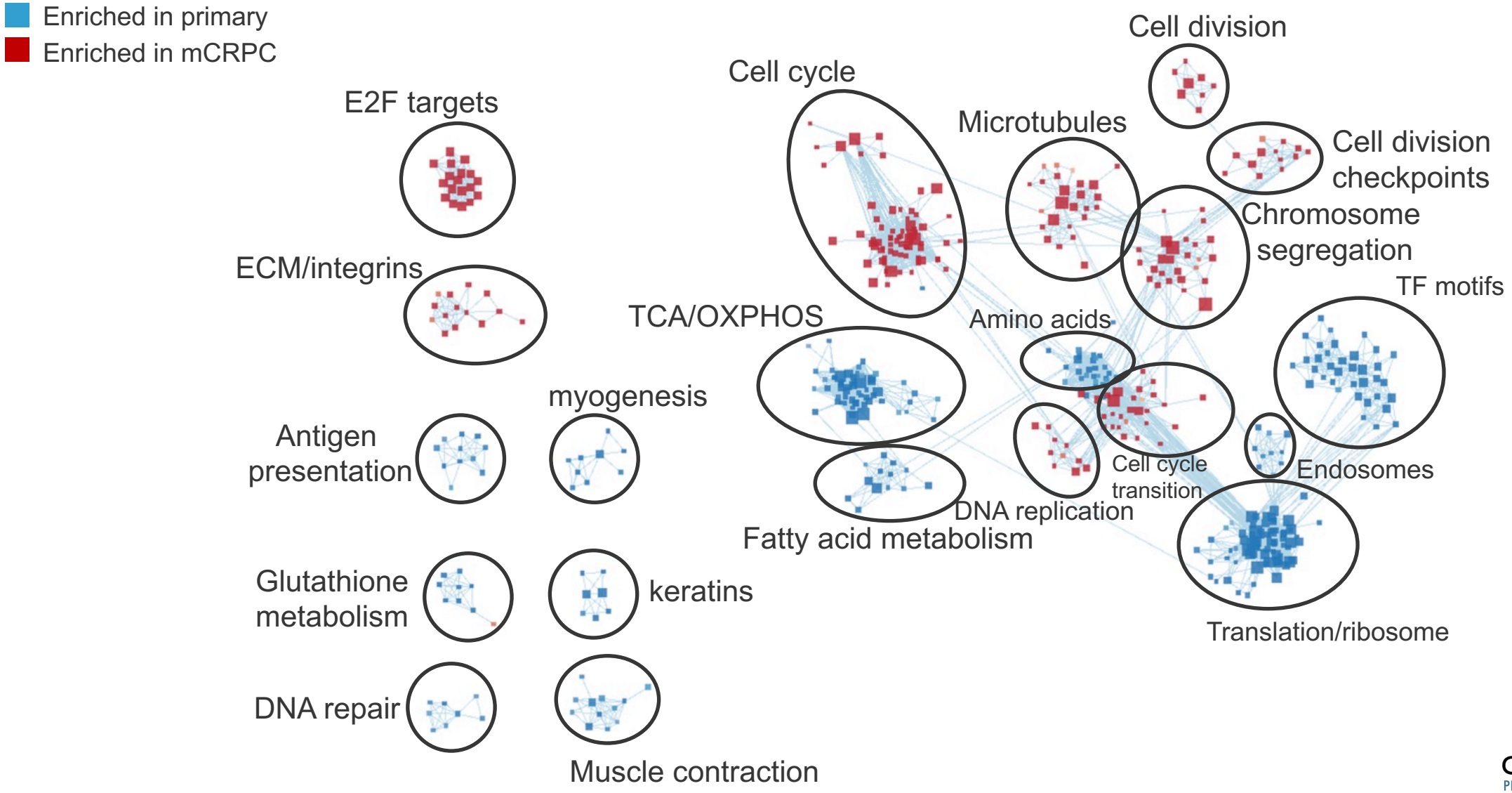






Primary vs. mCRPC

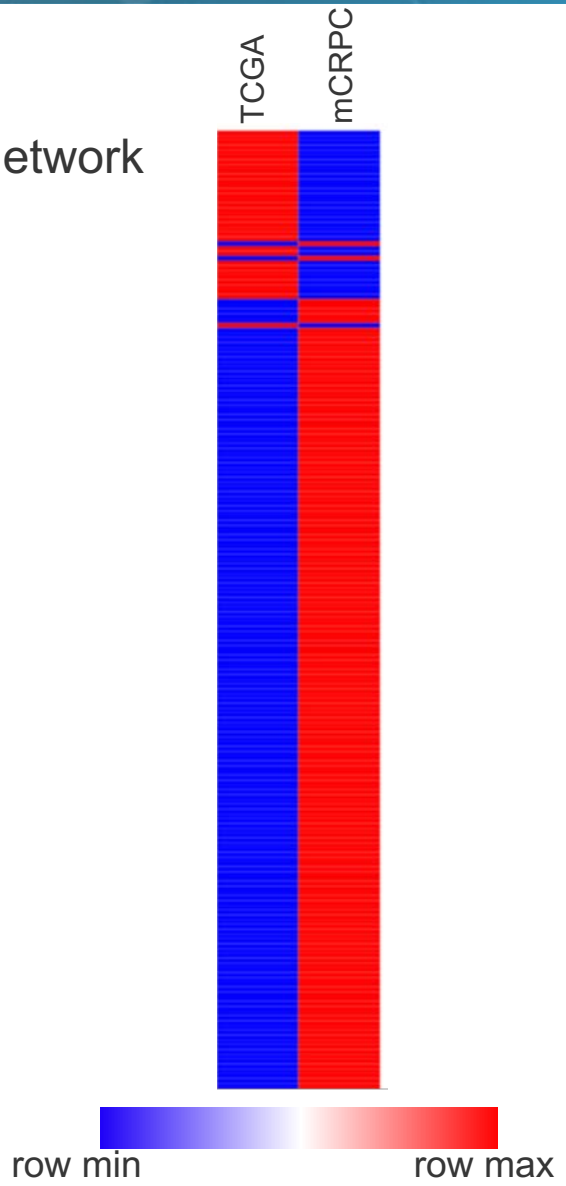
Network analysis: GSEA

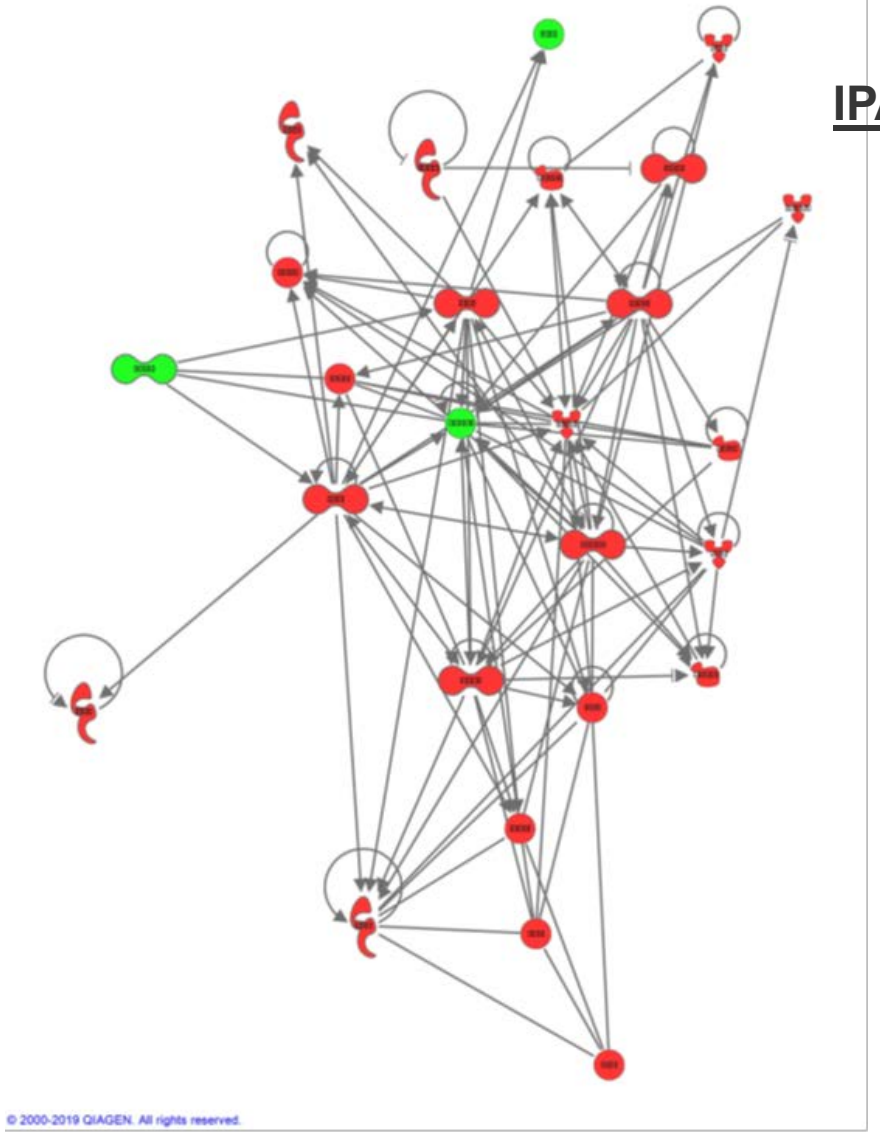


Primary vs. mCRPC

Global transcriptional remodeling is associated with disease progression

Hallmark_TF X Network





IPA Network for TF X

- Up in mCRPC
- Up in TCGA

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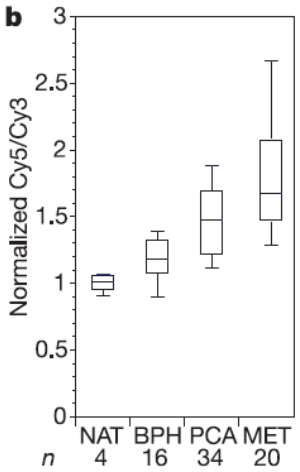
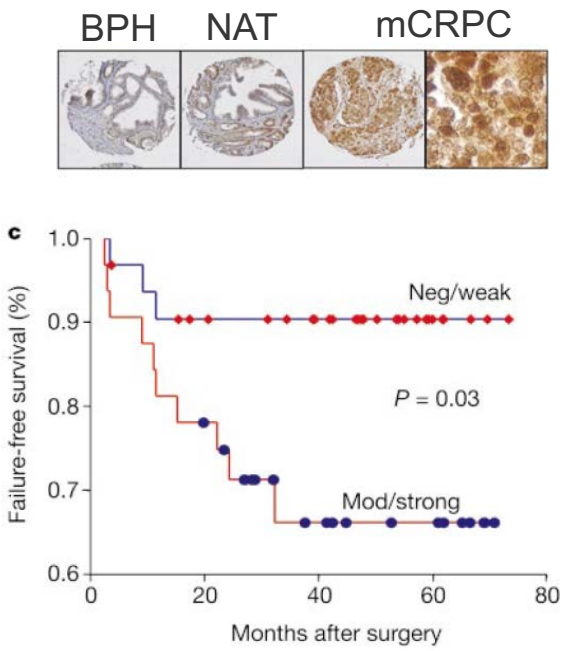
EZH2 expression and prostate cancer

High EZH2 expression correlates with poor outcome

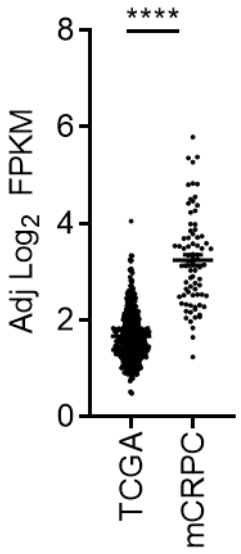
***EZH2* RNA**



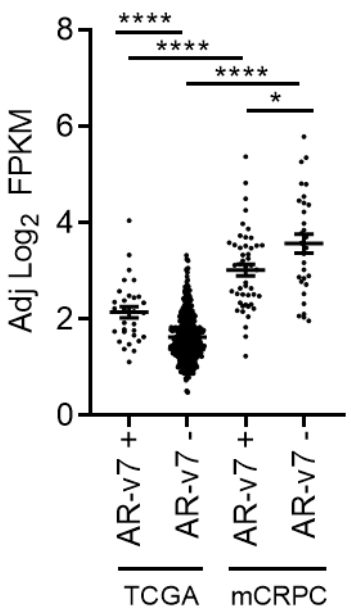
EZH2 IHC



EZH2



EZH2



Varambally et al. (2002) Nature; Yu et al. (2007) Cancer Res

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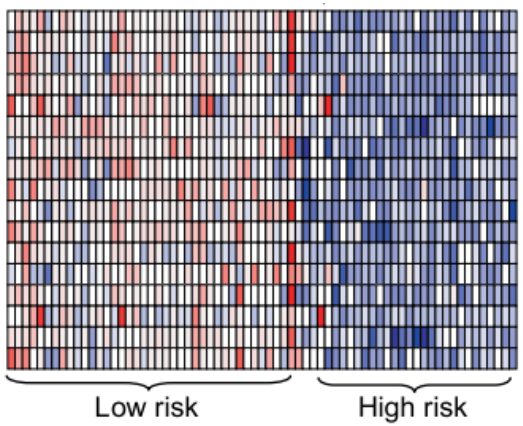
EZH2 expression and the Polycomb Repression Signature

EZH2 gene signature repression correlates with poor outcome

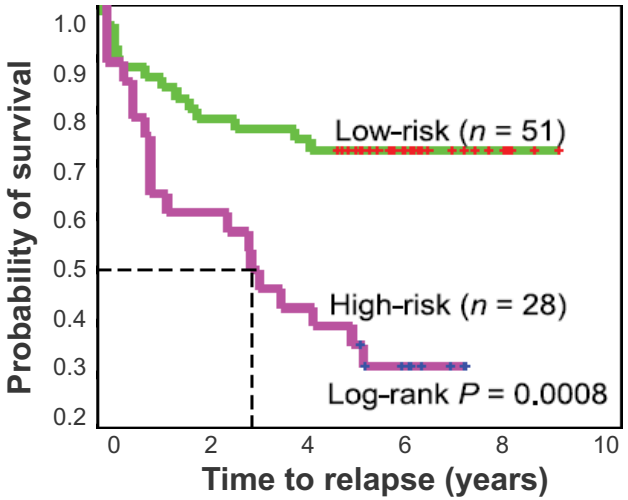
H3K27me3 promoter occupancy

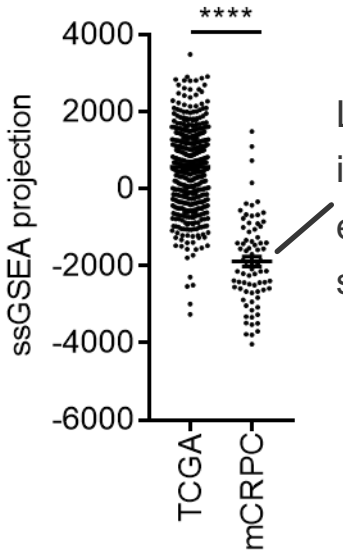
Genes down-regulated in mPC

→ **Polycomb repression signature**

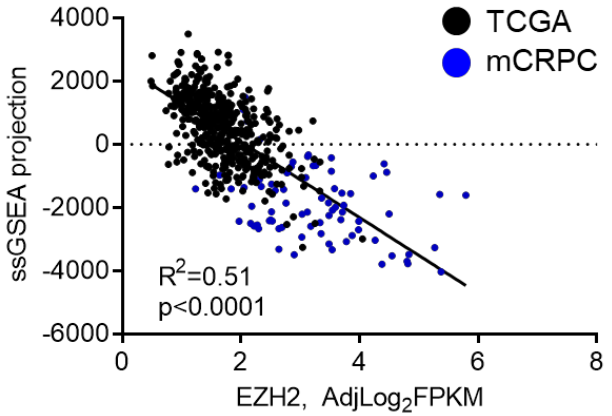


Cancer Polycomb repression signature





Lower value indicates lower expression of the signature in mCRPC



Varambally et al. (2002) Nature; Yu et al. (2007) Cancer Res

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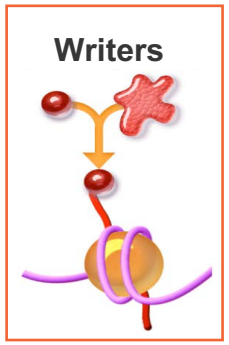
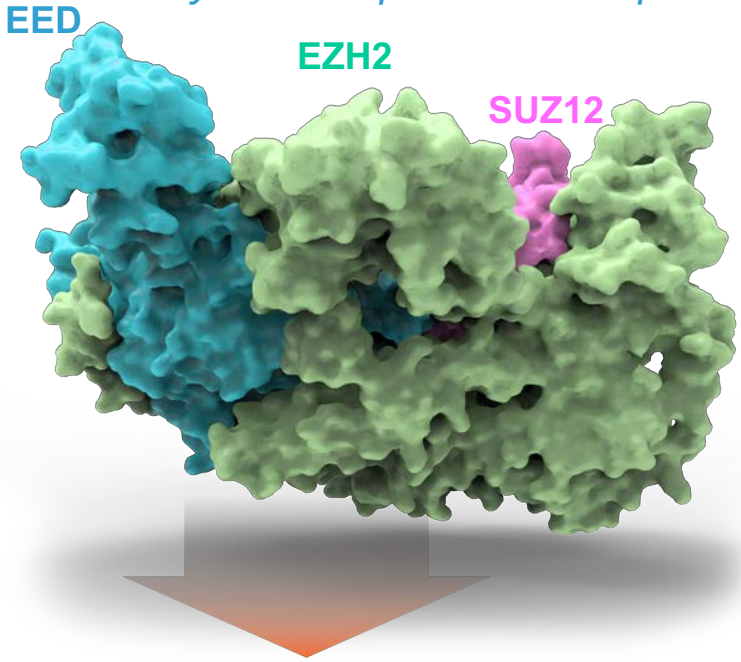
EZH2 Inhibition in models of mCRPC

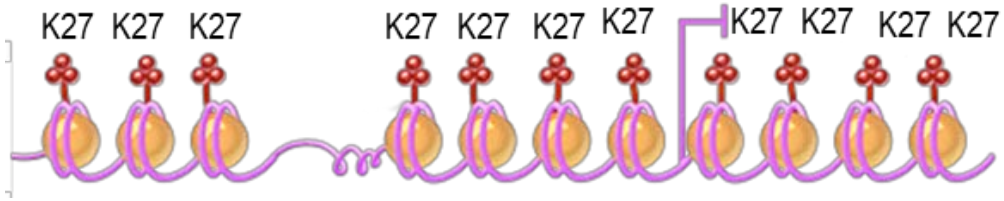
Cell line models share transcriptional programs with mCRPC tumors



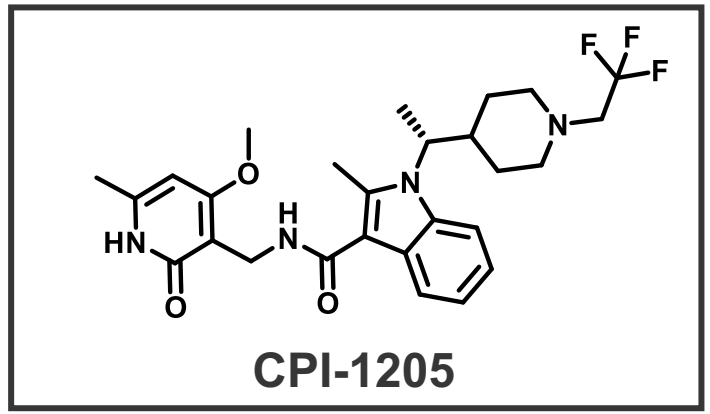
Polycomb Repressive Complex 2 (PRC2)



… Methylates Histone H3 at Lysine 27 (K27)

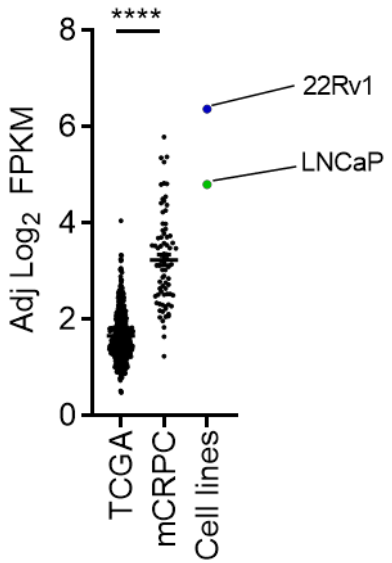


SUPPRESSED TRANSCRIPTION



CPI-1205

EZH2



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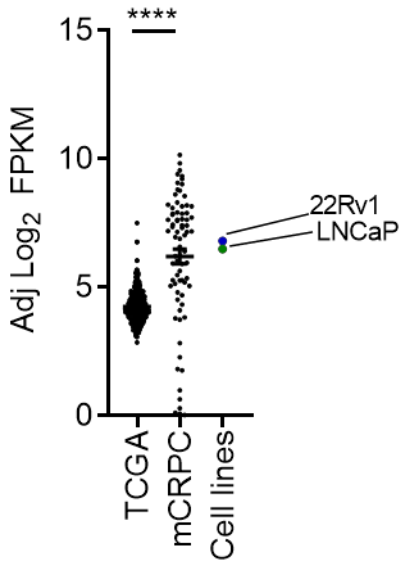

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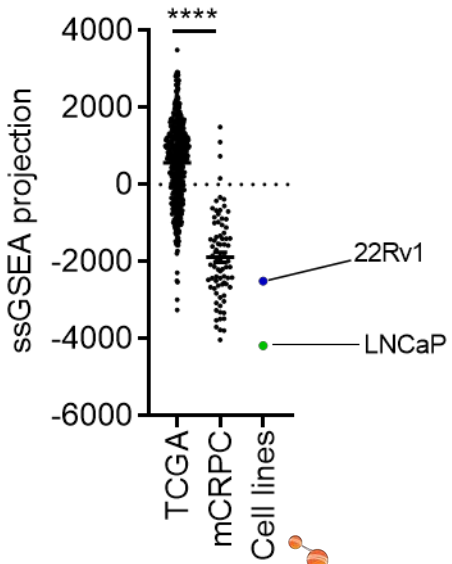

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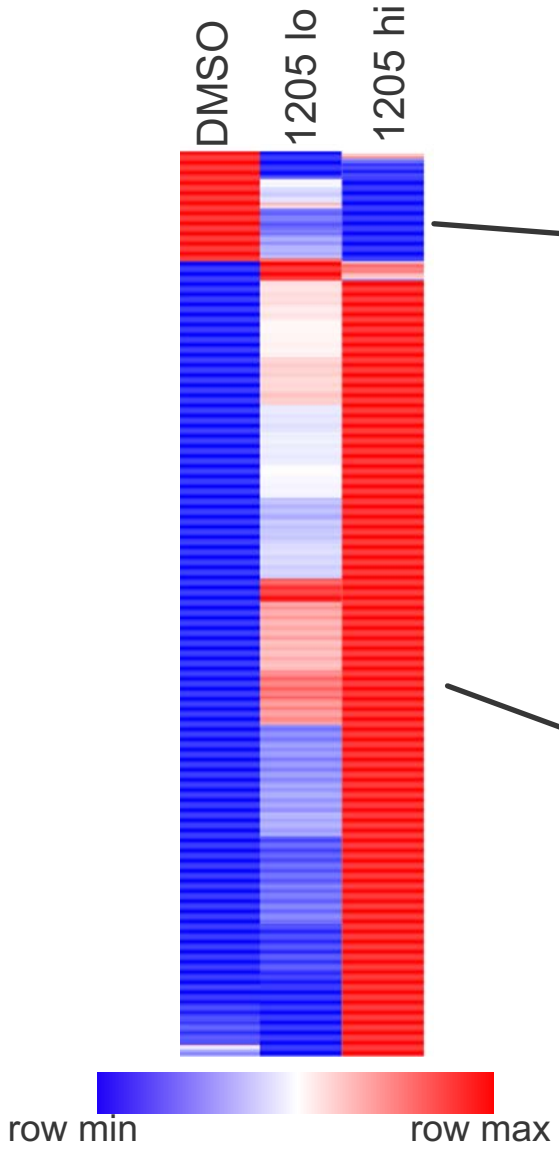
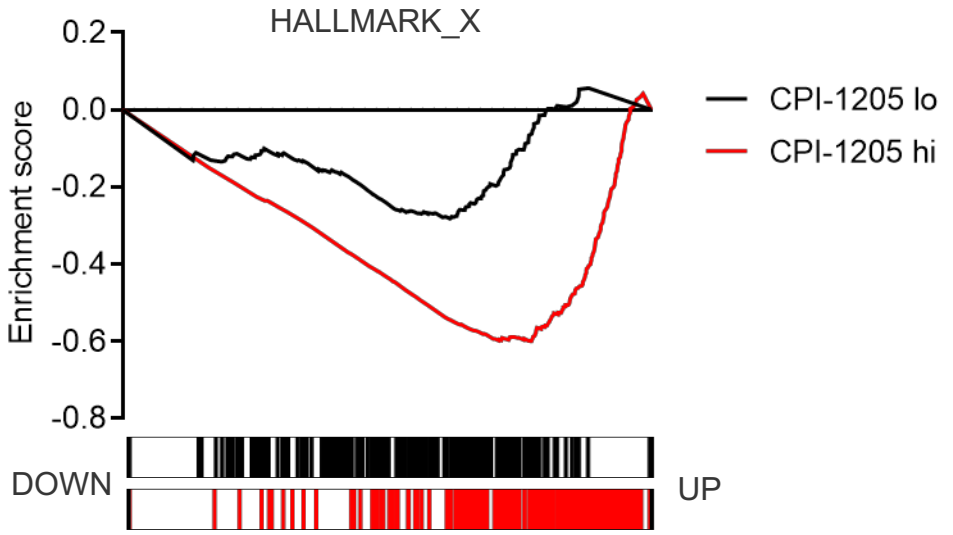
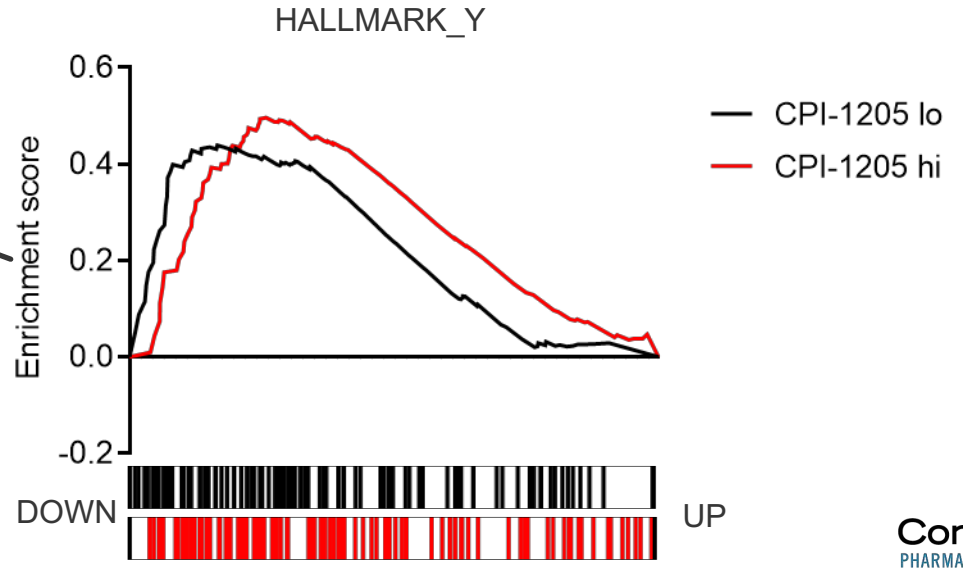
CPI-1205 engages prostate cancer transcriptional networks

Remodeling transcriptional networks with EZH2 inhibition in LNCaP cells



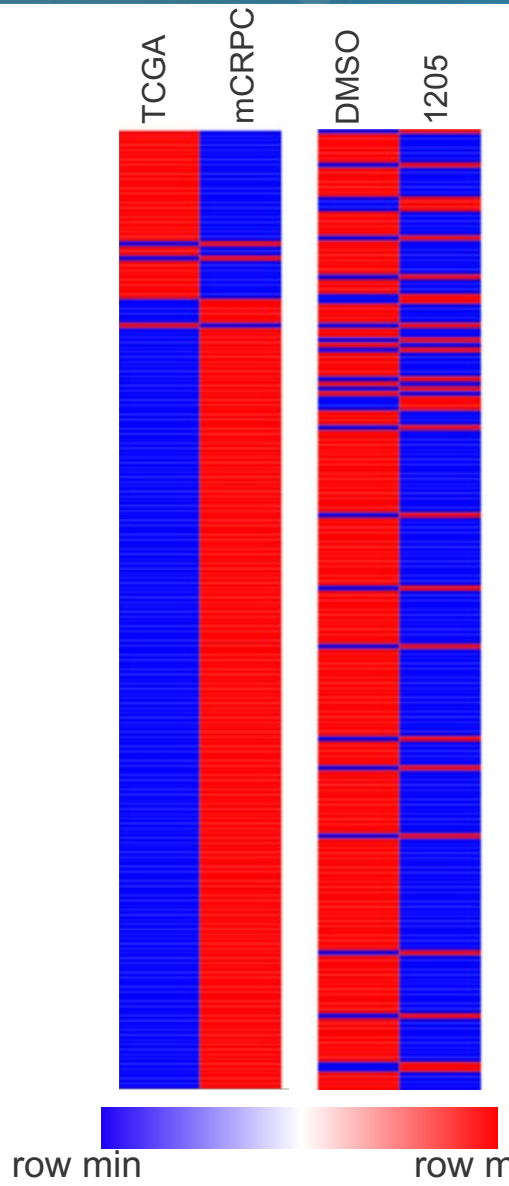




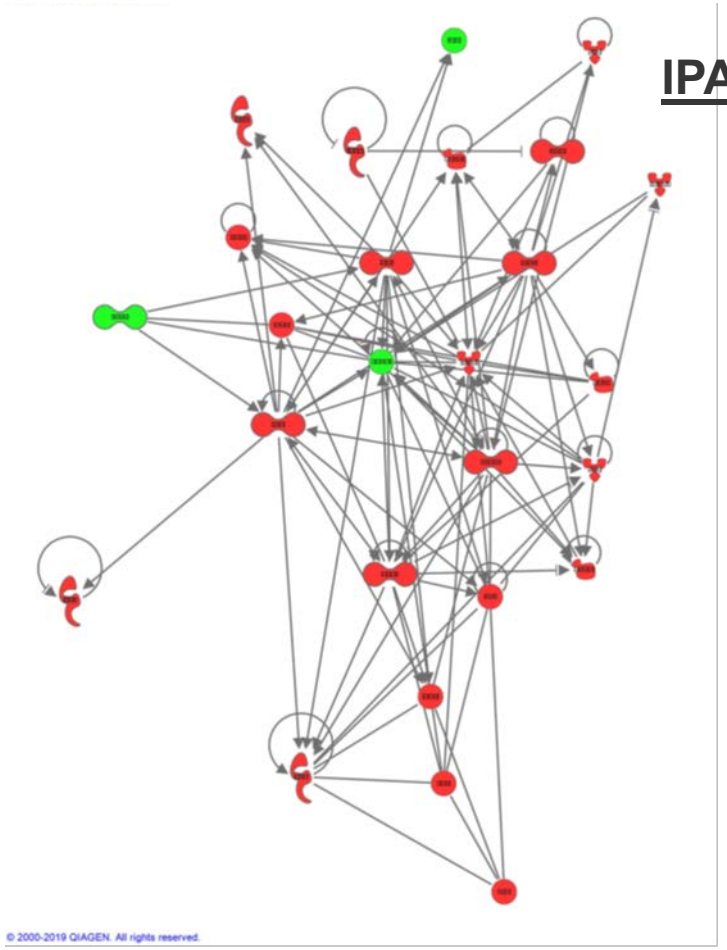
DENTIAL

EZH2 inhibition engages prostate cancer signaling networks

Modulation of components of a key transcriptional network



mCRPC vs. TCGA

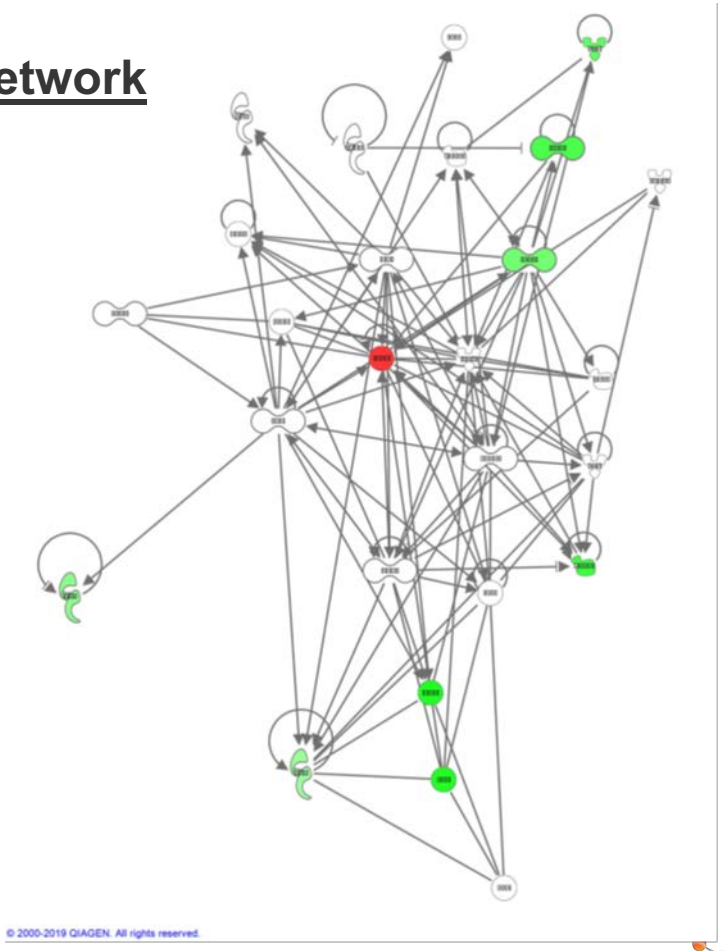


IPA TF X Network

Up

Down

LNCaP CPI-1205



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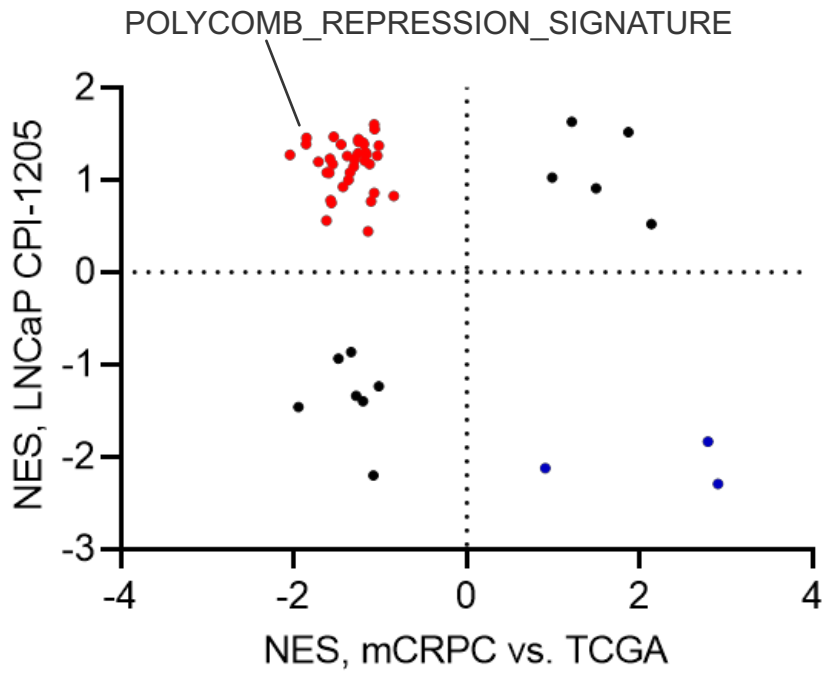
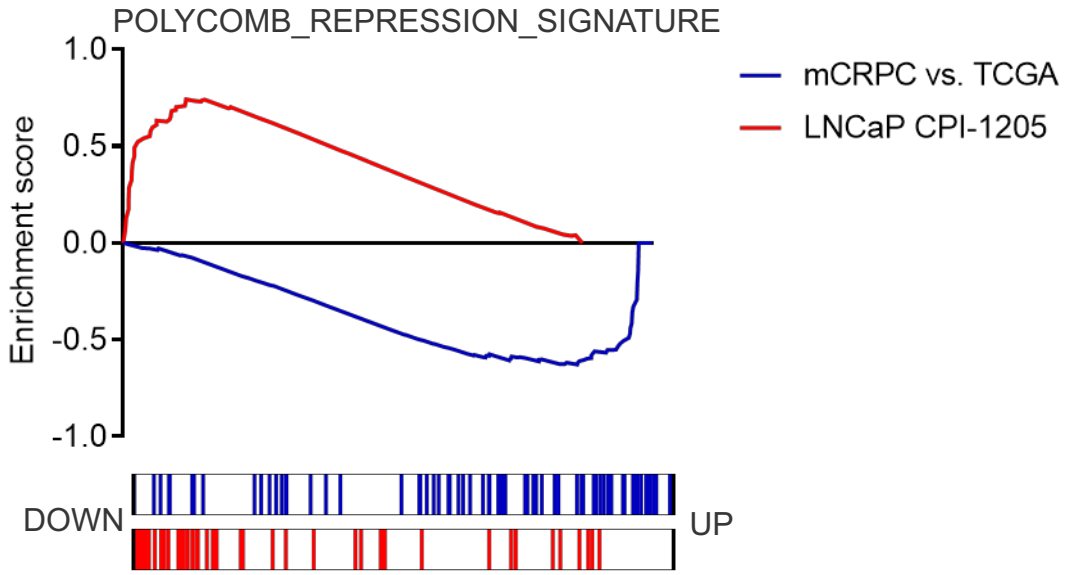
21

EZH2 inhibition antagonizes mCRPC transcriptional programs

GSEA profiling shows global de-repression of transcriptional networks





*Bradley, WD, et al., "EZH2 Inhibition as an Effective Treatment for Metastatic Castration-Resistant Prostate Cancer," presentation at 2018 EORTC/NCI/AACR meeting

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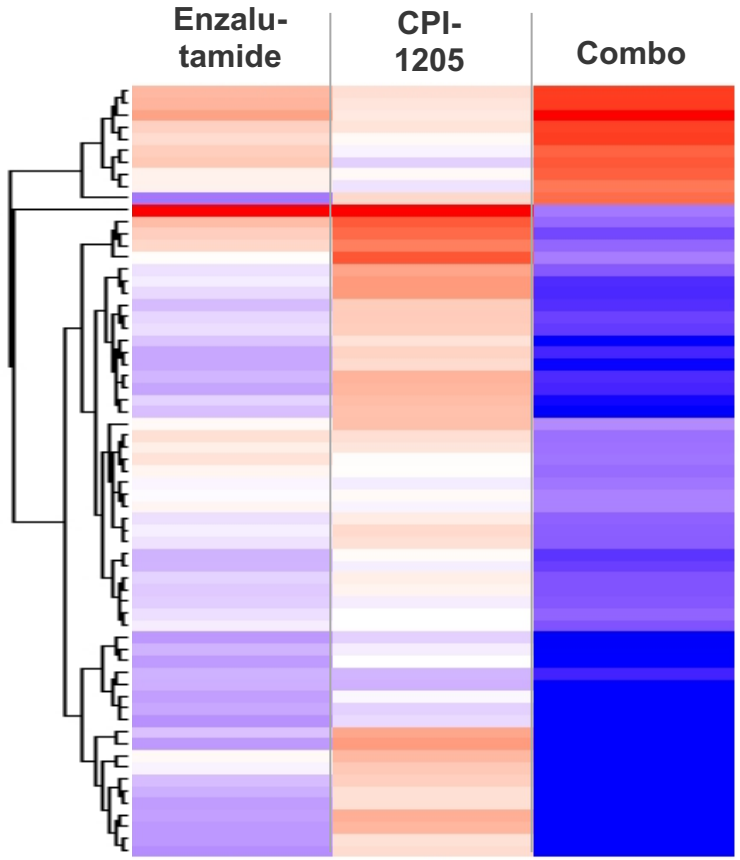
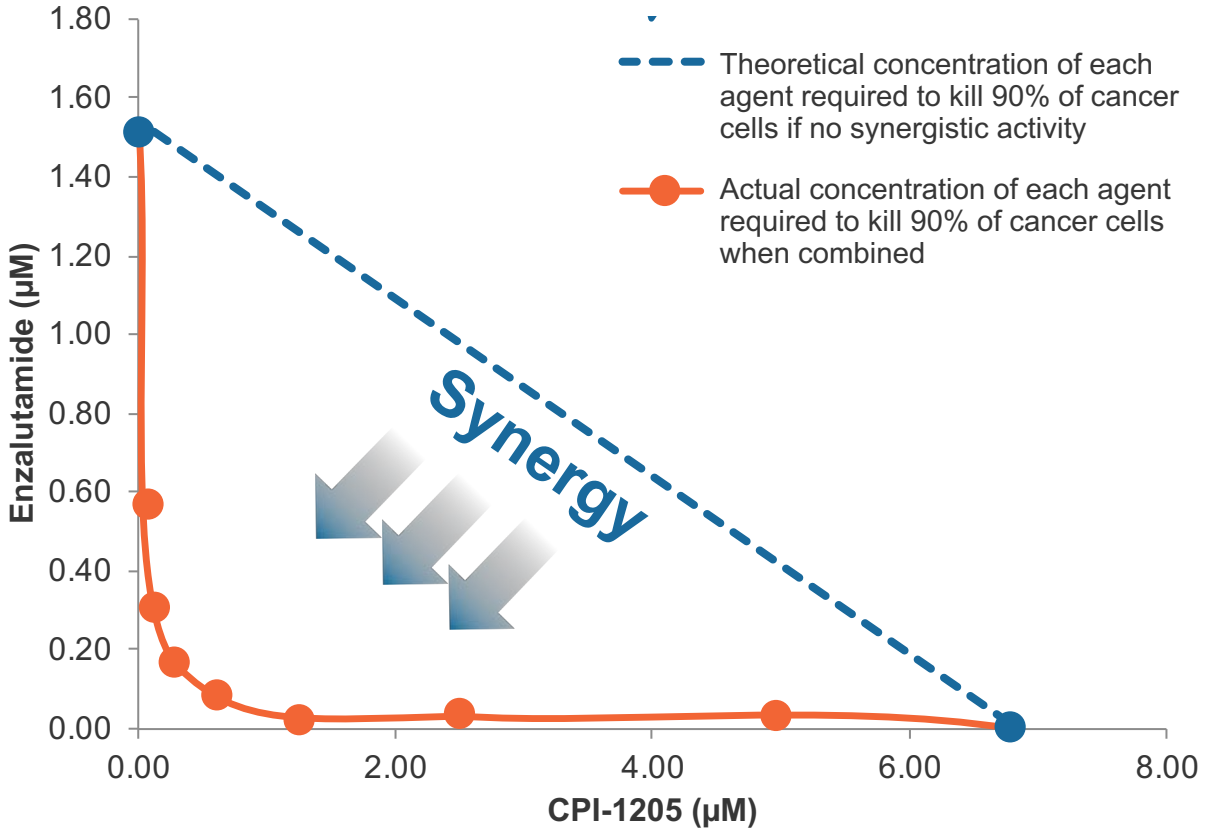
EZH2 Synergy with Androgen Receptor Signaling (ARS)

CPI-1205 Intensifies Gene Expression Effects of Enzalutamide in Prostate Cancer Cells*



CPI-1205 is Active as Monotherapy and Synergistic with Enzalutamide in Killing Prostate Cancer Cells



*Bradley, WD, et al., "EZH2 Inhibition as an Effective Treatment for Metastatic Castration-Resistant Prostate Cancer," presentation at 2018 EORTC/NCI/AACR meeting

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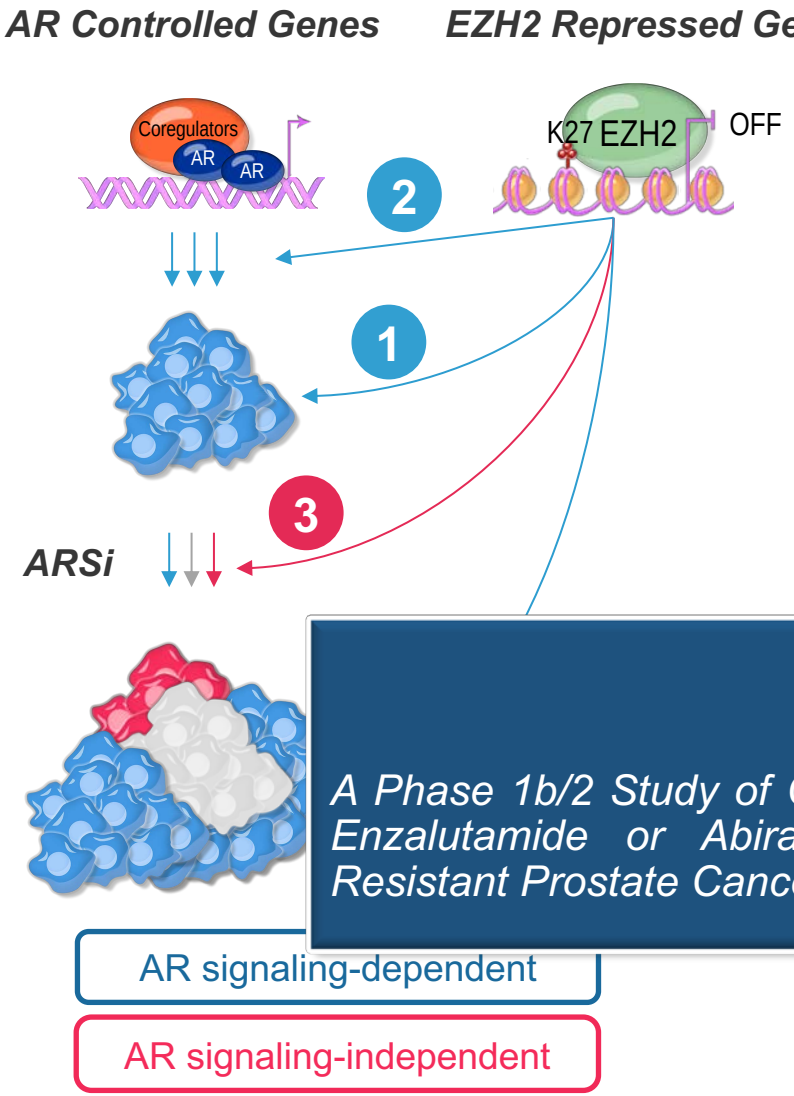
Model for the role EZH2 plays in prostate cancer

Transcriptional dependencies can be therapeutically targeted through EZH2 inhibition



EZH2 is required for ARS-dependent prostate cancer growth

1 *Single agent activity via direct gene de-repression*

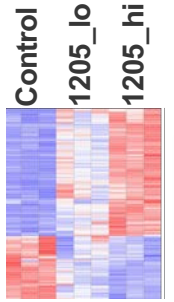
EZH2 cooperates with AR signaling directly to promote cancer cell growth

NCT03480646

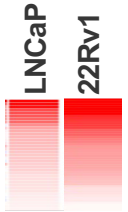
A Phase 1b/2 Study of CPI-1205, a Small Molecule Inhibitor of EZH2, Combined With Enzalutamide or Abiraterone/Prednisone in Patients With Metastatic Castration Resistant Prostate Cancer

3 *Prevention or reversion of NEPC*

RNA-seq shows evidence of MOA



CPI-1205 de-represses genes



Polycomb signature de-repression

Enhancement of enzalutamide MOA

Synergistic activity

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Acknowledgments

Functional Genomics/Bioinformatics

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- Charlie Hatton (former)

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