L6 – **KB-0742:** A Phase 2 clinical candidate discovered by SMMs
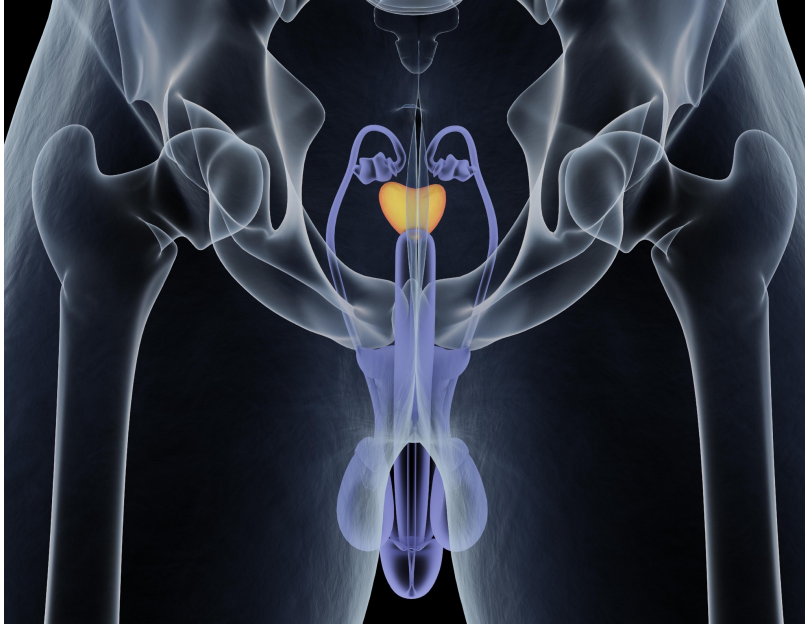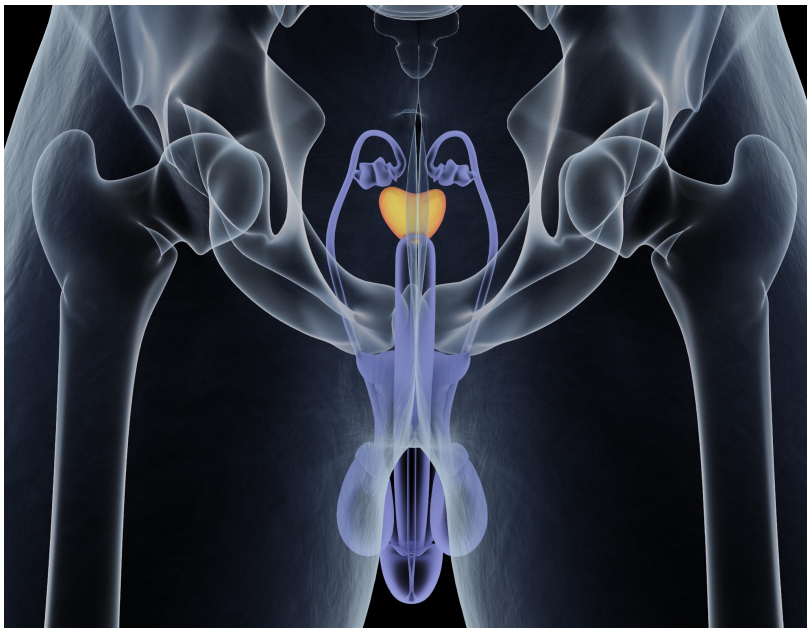
March 2nd, 2023

Prostate Cancer

Other than skin cancer, prostate cancer is the most common cancer in men

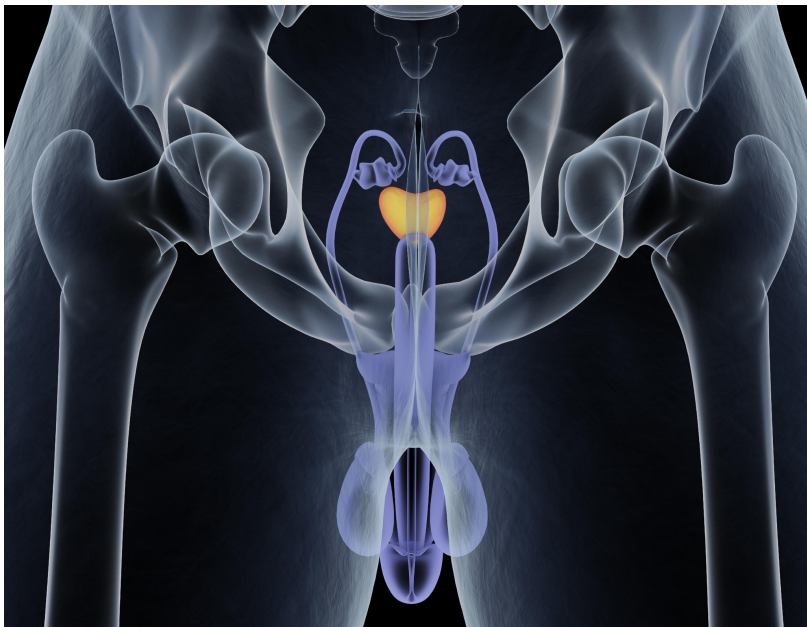# Prostate Cancer

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2023 US estimates: 288,300 new cases
                       34,700 deaths

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1 out of 8 men will be diagnosed in their lifetime

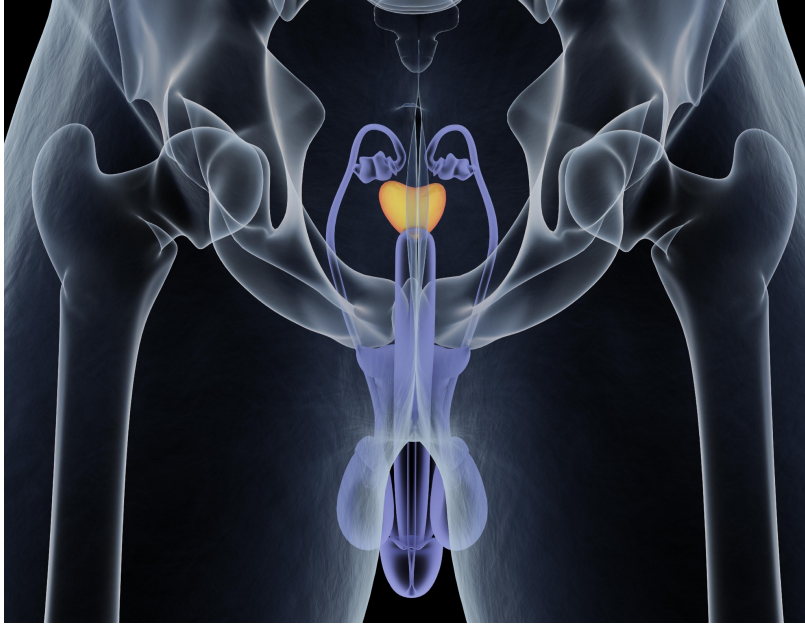# Prostate Cancer

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Prostate Cancer
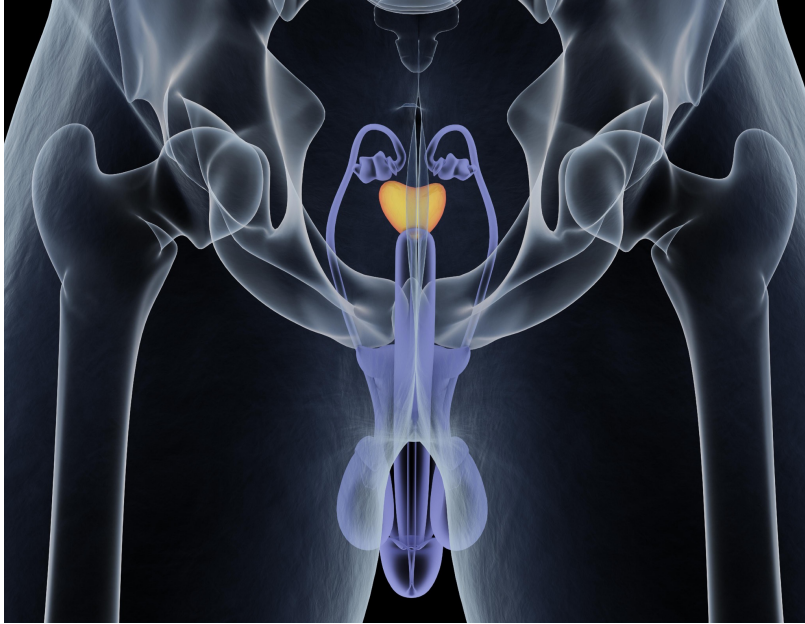

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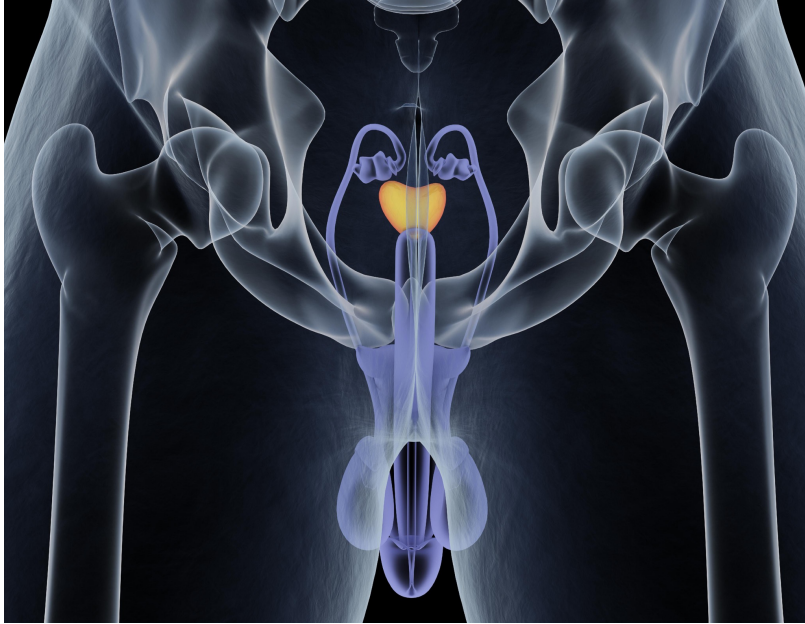
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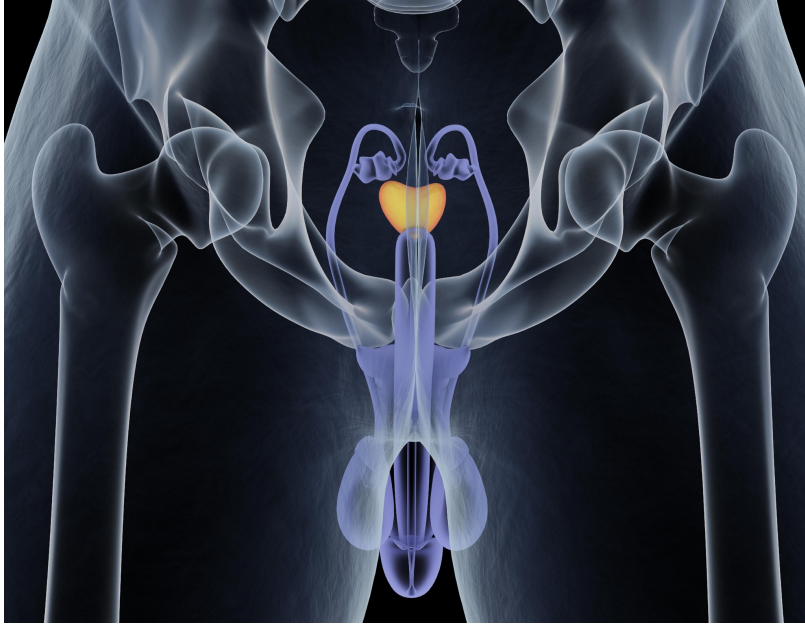   34,700 deaths

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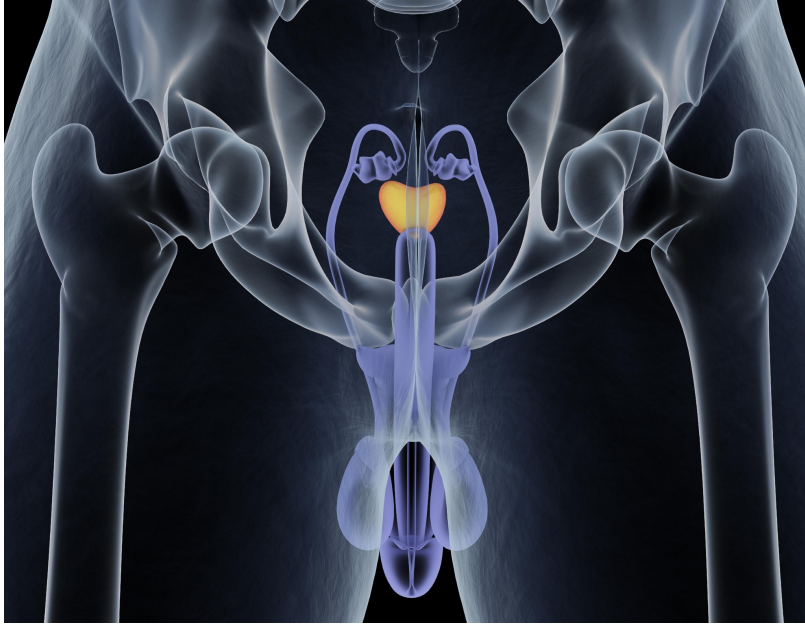Avg. age of first diagnosis is 66-yo

More common in non-Hispanic Black men:
1.7x diagnoses
2.1x deaths

Prostate Cancer

Second-leading cause of death in American men, behind only lung cancer
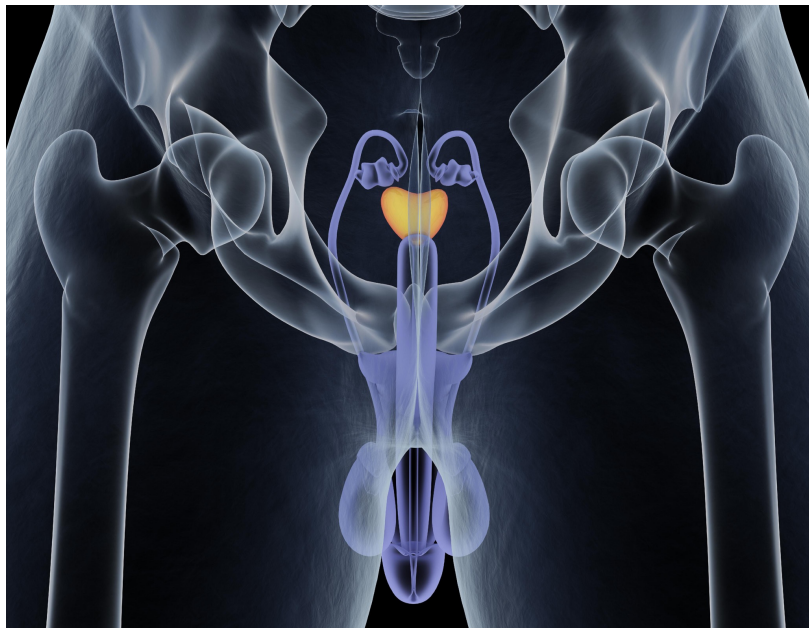
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1 out of 41 men will die of prostate cancer

Prostate Cancer

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Most men survive and do not die from this cancer. >3.1 million diagnosed men in the US are alive today
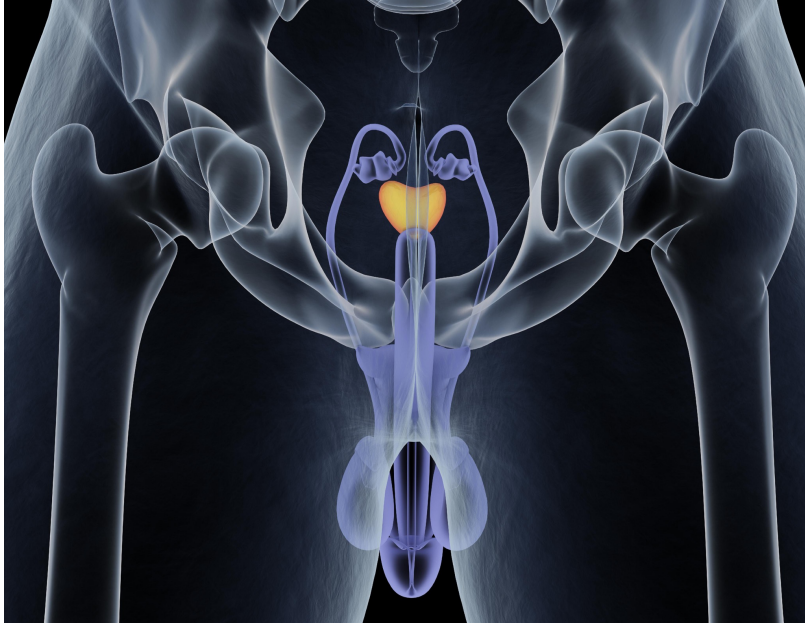
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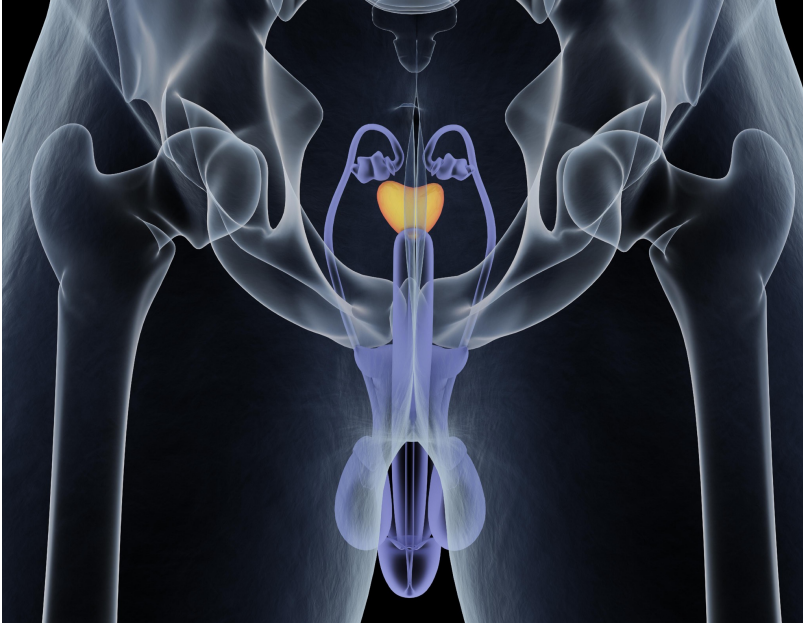
Prostate Cancer

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1993-2013: death rate declined by half, likely due to earlier detection and advances in treatment

2013 onward: pace of decline slowed, likely reflecting the rise in cancers found at an **advanced stage with resistance to therapies**

Increasing disease burden following primary prostate cancer therapy

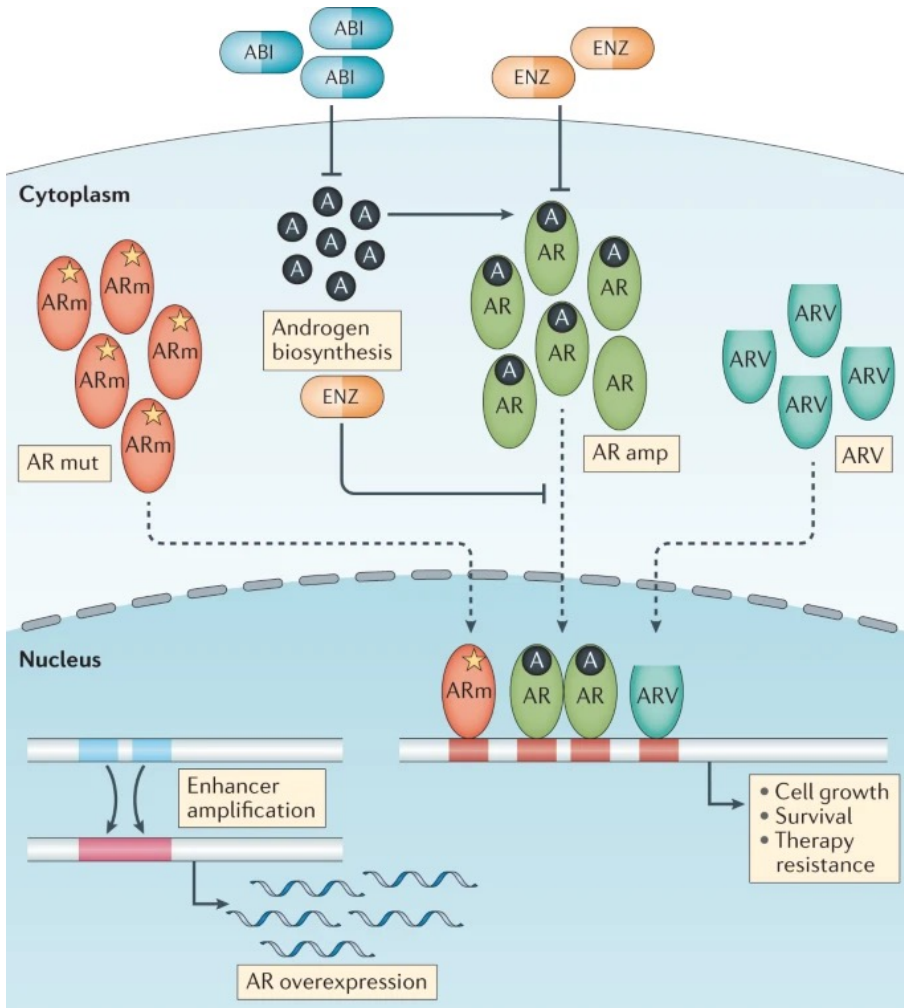Watson, Arora, Sawyers, *Nature Reviews Cancer*, 15, 701-711 (2015)
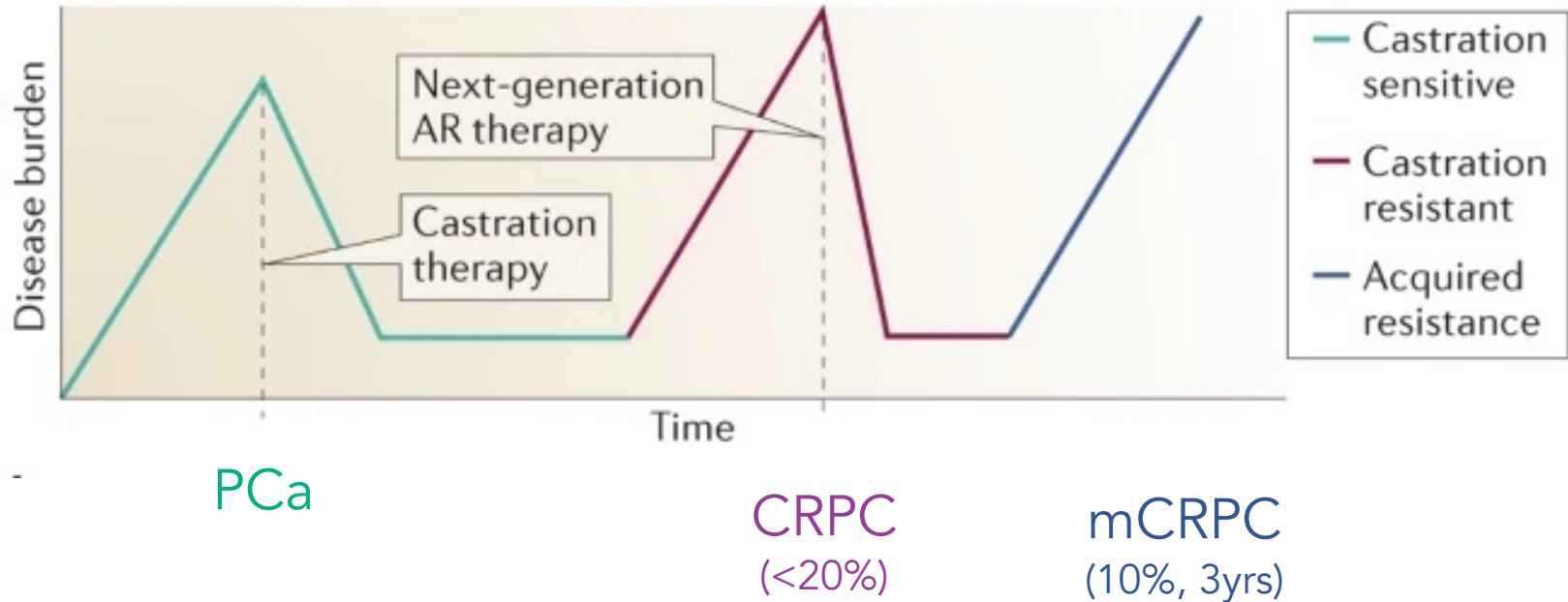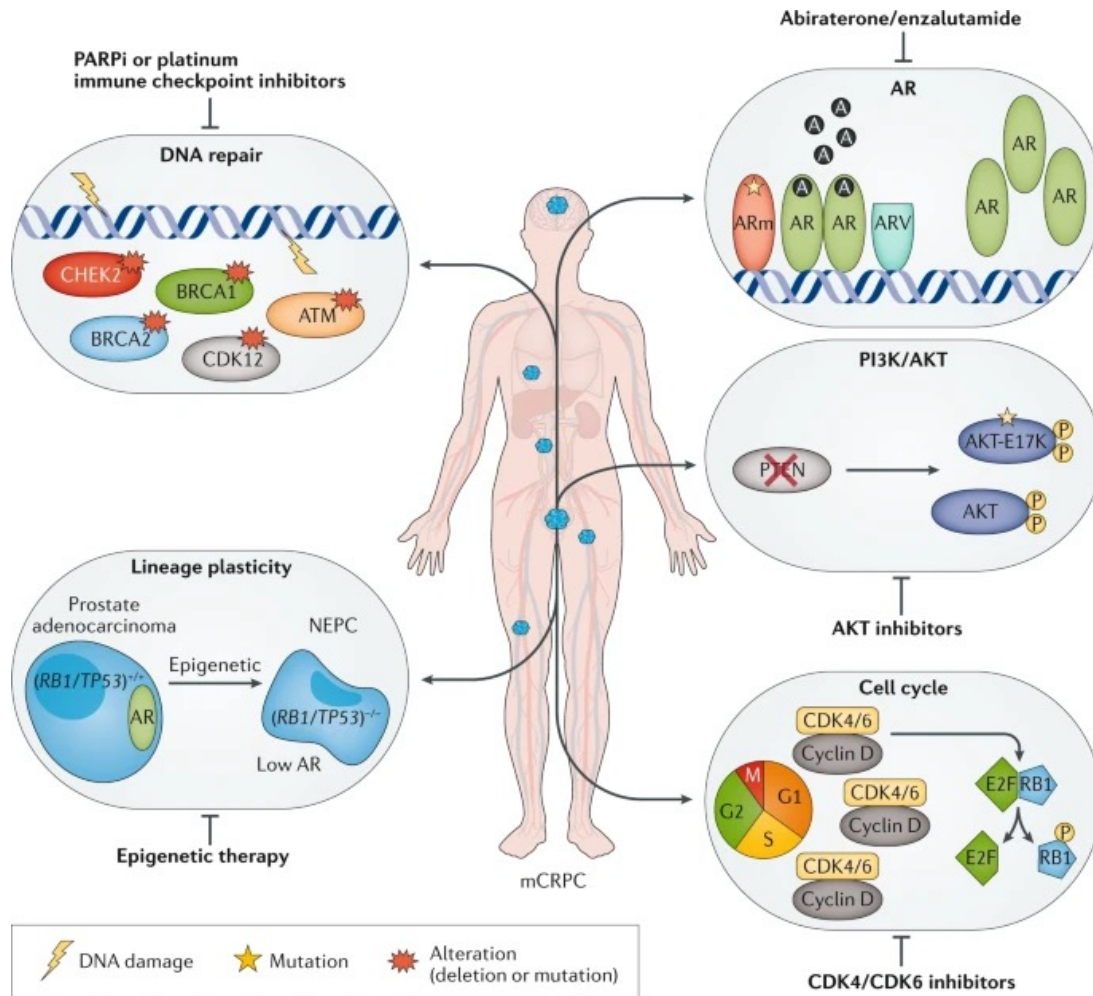
Molecular landscape of advanced disease

Genomic alterations are heterogenous across patients with metastatic castration-resistant prostate cancer (mCRPC)

By understanding the genes or pathways altered in any given individual, precision medicine has the potential to improve clinical outcomes.

Alterations in Androgen Receptor (AR) signaling are the most prevalent events in metastatic castration-resistant prostate cancer leading to *persistent AR activation*

AR amplifications (AR amp)
AR mutations (AR mut)
AR splice variants (ARVs)
Intratumoral androgen biosynthesis
AR enhancer amplification

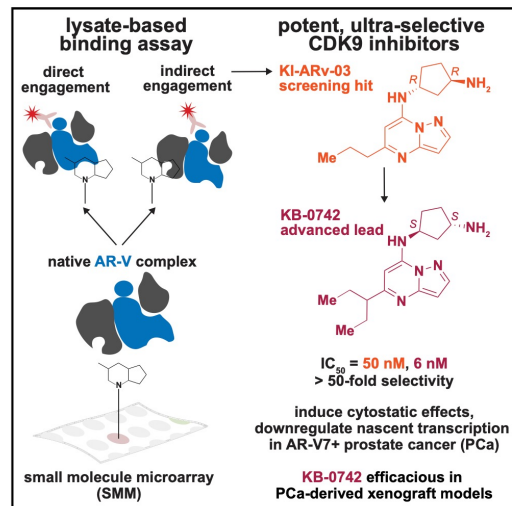Ku, Gleaves, and Beltran., *Nature Reviews Urology*, 16, 645-654 (2019)

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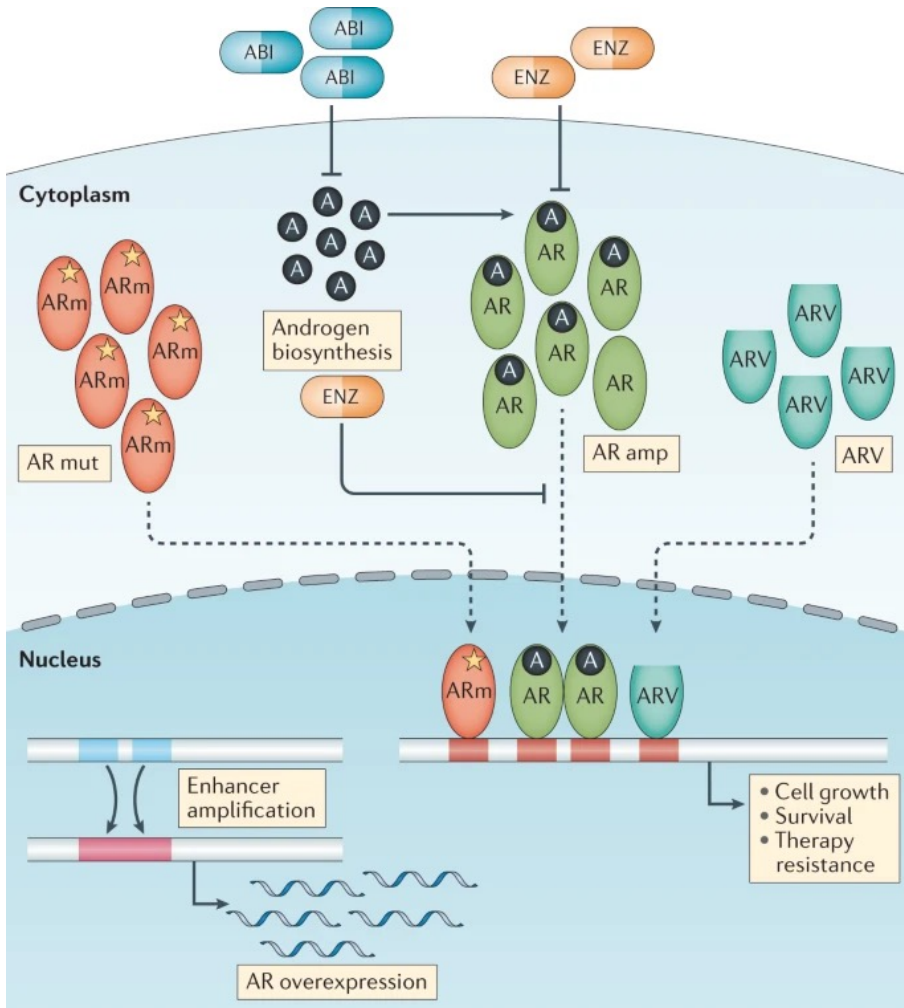
Cell Chemical Biology

Modulating Androgen Receptor-Driven Transcription in Prostate Cancer with Selective CDK9 Inhibitors

Graphical Abstract

lysate-based binding assay

potent, ultra-selective CDK9 inhibitors

direct engagement

indirect engagement

KI-ARv-03 screening hit

native AR-V complex

KB-0742 advanced lead

IC_{50} = 50 nM, 6 nM
> 50-fold selectivity

induce cytostatic effects, downregulate nascent transcription in AR-V7+ prostate cancer (PCa)

small molecule microarray (SMM)

KB-0742 efficacious in PCa-derived xenograft models

Authors
André Richters, Shelby K. Doyle, David B. Freeman, ..., Charles Y. Lin, Marius S. Pop, Angela N. Koehler

Correspondence
koehler@mit.edu

In Brief
In the pursuit of hormone receptor modulators in prostate cancer, a potent, ultraselective CDK9 inhibitor is discovered. This study describes the most selective inhibitors of CDK9 known to date and provides compelling preclinical *in vitro* and *in vivo* support for CDK9 as a therapeutic target.
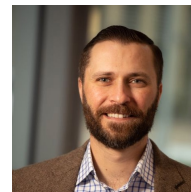
Highlights

- KI-ARv-03 reduces AR protein levels and AR-driven transcription

- KI-ARv-03 is deduced to be a potent, ultraselective inhibitor of CDK9

- Optimization led to the orally bioavailable and selective CDK9 inhibitor KB-0742

- KB-0742 displays potent anti-tumor activity in cancer models *in vitro* and *in vivo*
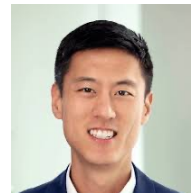
Andre Richters   Shelby Doyle   Becky Leifer

David Freeman   Marius Pop   Nick Struntz

Charles Lin   Stefan Knapp   Steven Balk

janssen   THE BRIDGE PROJECT

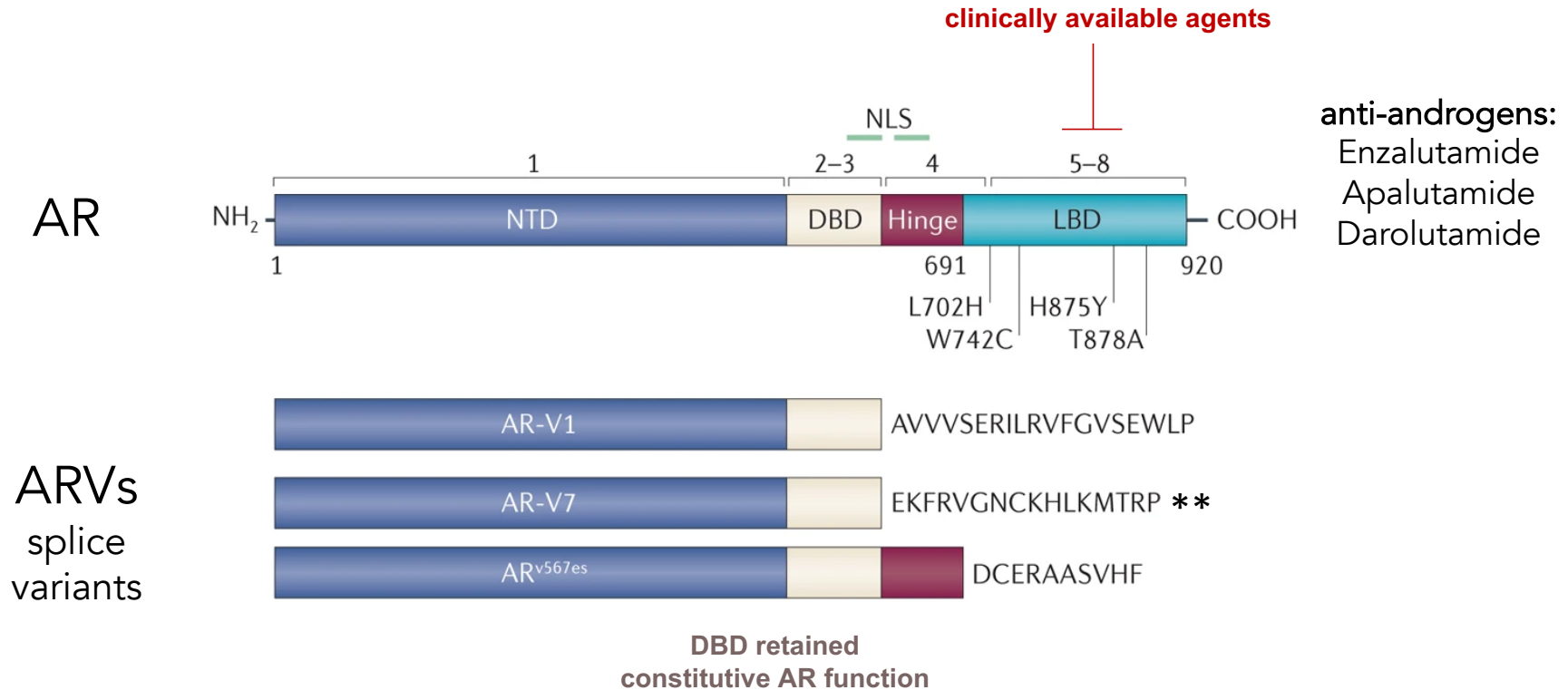# Domain structure of AR, cancer-associated mutations, and splice variants

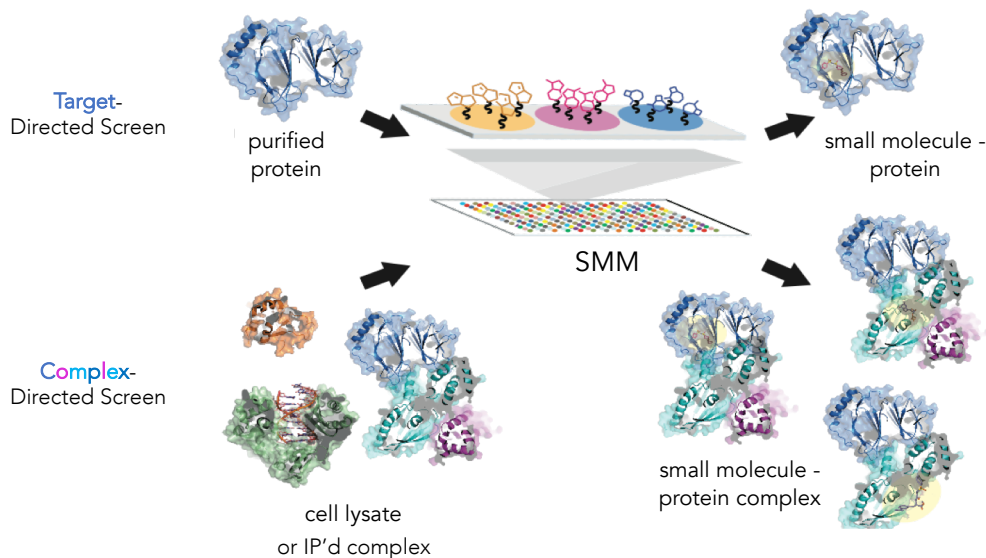**clinically available agents**

AR
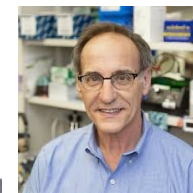
ARVs
splice variants
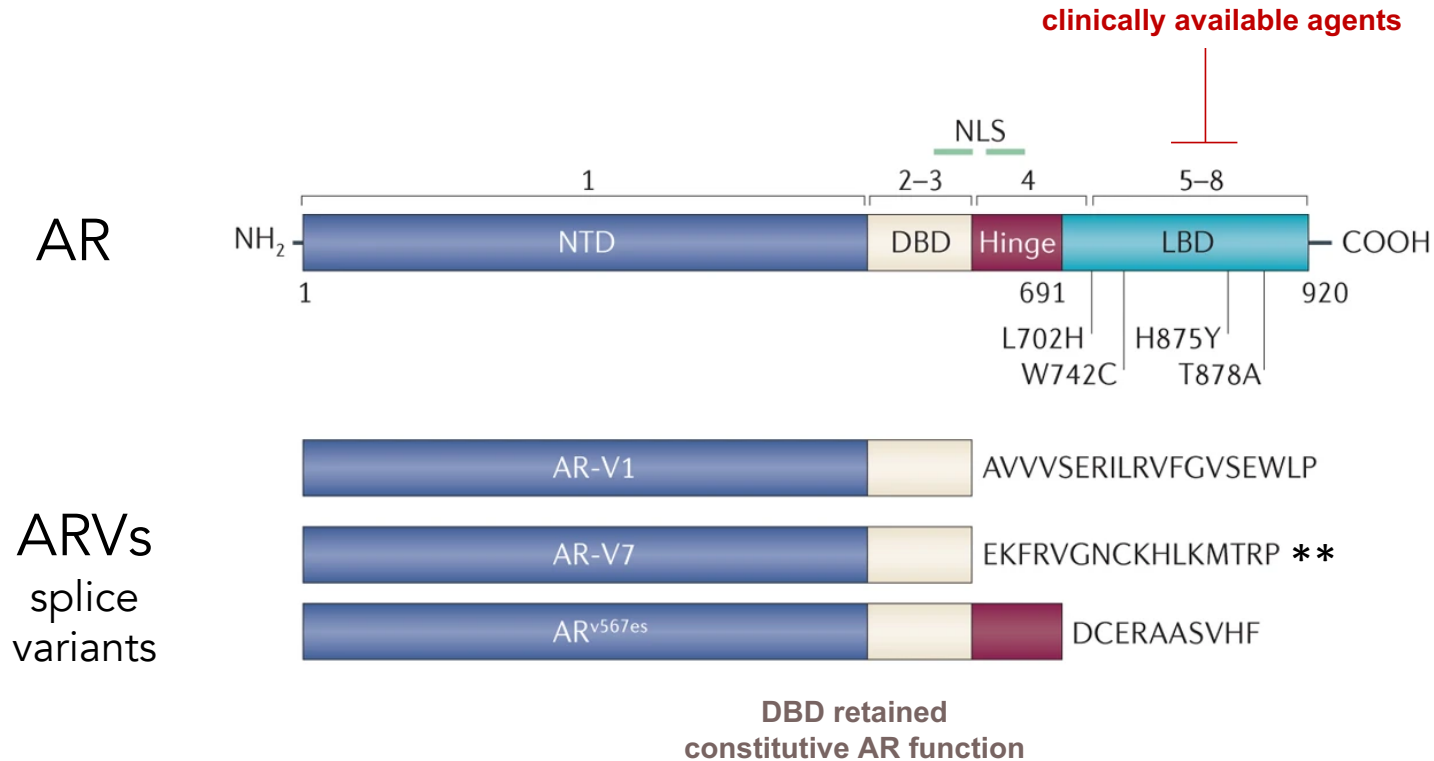
# Domain structure of AR, cancer-associated mutations, and splice variants

Rationale:

(1) AR splice variants lack ligand binding domain (LBD), contributing to resistance associated with AR antagonists in CRPC,

(2) screening ARv-containing complexes in cell lysates avoids purification, (3) reflects more relevant state, and casts a net for targeting co-factors.

Target-Directed Screen

purified protein

small molecule - protein

SMM

Complex-Directed Screen

cell lysate or IP'd complex

small molecule - protein complex

Active compounds prioritized in prostate-specific antigen (PSA) expression and AR reporter assays

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Active compounds prioritized in prostate-specific antigen (PSA) expression and AR reporter assays

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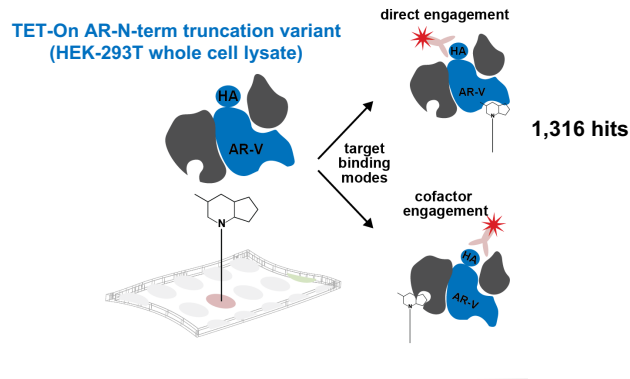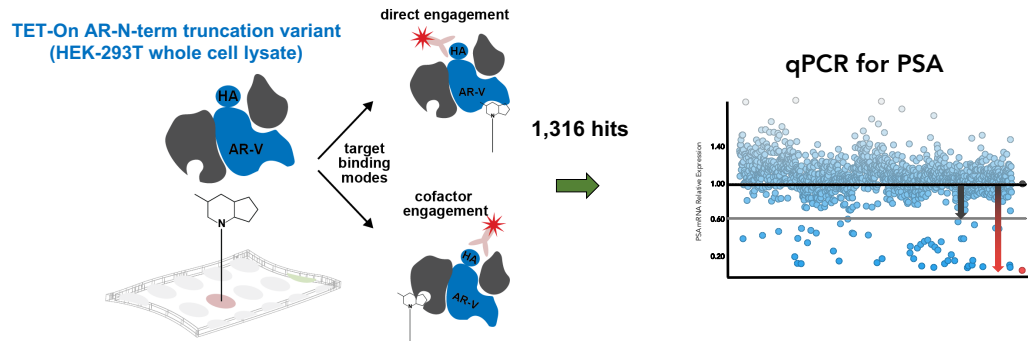
Active compounds prioritized in prostate-specific antigen (PSA) expression and AR reporter assays

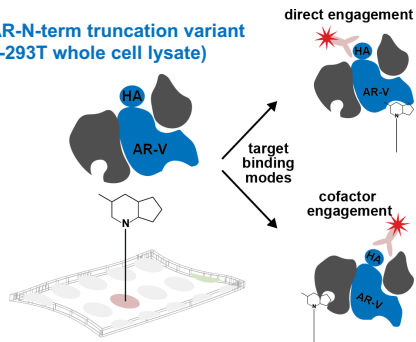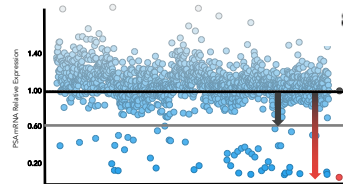**TET-On AR-N-term truncation variant (HEK-293T whole cell lysate)**
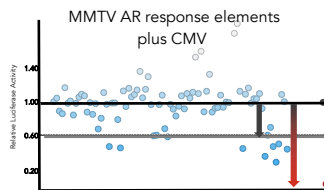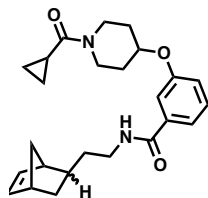
direct engagement

target binding modes

cofactor engagement

1,316 hits

qPCR for PSA

85 compounds

Luciferase Reporter Assay

MMTV AR response elements plus CMV
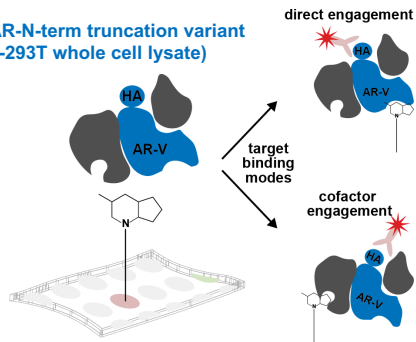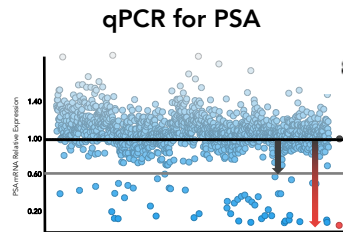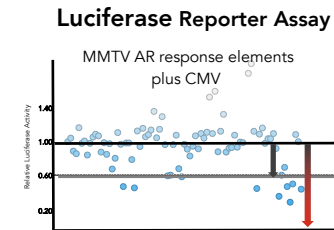
7 reasonable actives, 3 prioritized

janssen

KI-ARv-01
MW = 408.54
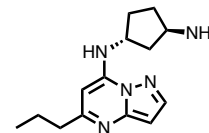cLogP = 3.27
IC$_{50}$ = 5.43 μM
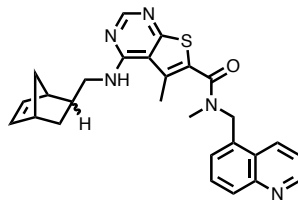
KI-ARv-02
MW = 469.61
cLogP = 5.48
IC$_{50}$ = 6.86 μM

KI-ARv-03
MW = 259.36
cLogP = 2.55
IC$_{50}$ = 7.61 μM

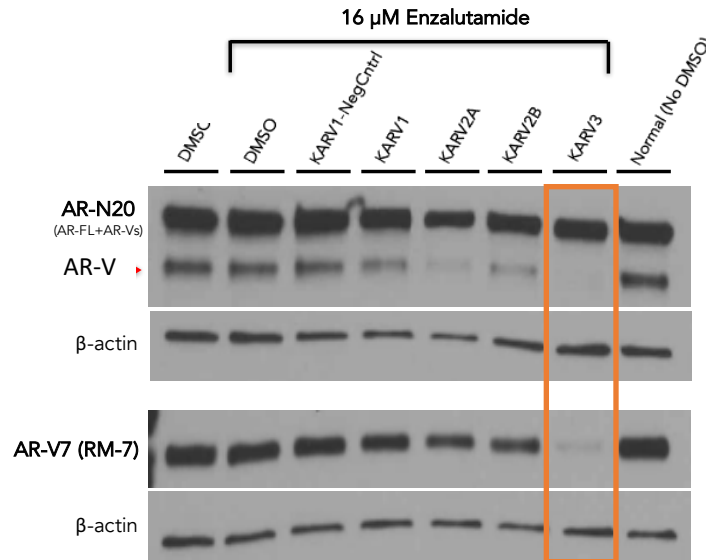# KI-ARv-03 impacts AR-V7 levels in an enzalutamide-resistant prostate cancer cellular model
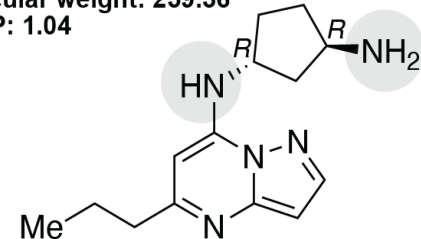
Dr. Joshua Russo

Dr. Steven Balk

VCaP-16 cells
enzalutamide-resistant
increased expression of AR-v7
5 µM compound, 24-hour exposure

16 µM Enzalutamide

DMSO
DMSO
KARV1-NegCntrl
KARV1
KARV2A
KARV2B
KARV3
Normal (No DMSO)

AR-N20
(AR-FL+AR-Vs)

AR-V ►

β-actin

AR-V7 (RM-7)

β-actin

KI-ARv-03
molecular weight: 259.36
cLogP: 1.04

assay	cell line	IC$_{50}$ (µM)	
ARv driven PSA reporter 24h	LNCaP	7.6	mutAR+
Cell viability cell-titer glo 72h	LNCaP	7.7	mutAR+
	VCaP	7.9	AR+
	DU145	9.3	AR-
	PC3	17	AR-

nM inhibitor in MYC-driven reporter assay?

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Kinase selectivity profiling suggests CDK9 as a target

pan-kinase inhibition assay

% inhibition
≥50 [____] 100

% activity
CDK9 – 7%
CDK7 – 47%

10 µM KI-ARv-03
[ATP] -/+ 15 µM
apparent Km
for each kinase

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Kinase selectivity profiling suggests CDK9 as a target

pan-kinase inhibition assay

% inhibition
≥50 ☐ 100

TK
TKL
STE
CMGC
CDK7
CDK9
CK1
AGC
CAMK

10 µM KI-ARv-03
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for each kinase

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CDK7 – 47%

13 CDKs in 10-pt dose

biochemical CDK inhibition assay

CDK9/
cyclin T1

CDK7/
cyclin H

Other CDKs
(1-6,12,13,16-18)

>130-fold selectivity

CDK9 IC$_{50}$ = 0.15 µM (45 µM ATP)
CDK7 IC$_{50}$ = 20.1 µM

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

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Michaelis-Menten and Lineweaver-Burk

CDK9 in vitro kinase assay
velocity of ATP consumption

DMSO
123 nM
370 nM
1,110 nM
3,330 nM

KI-ARv-03

K_m = 3.84 µM
K_i = 29.4 nM

double-reciprocal plot

3,330 nM
1,110 nM
370 nM
123 nM
DMSO

KI-ARv-03

ATP competitive

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Kinase selectivity profiling suggests CDK9 as a target

pan-kinase inhibition assay

10 μM KI-ARv-03 [ATP] -/+ 15 μM apparent Km for each kinase

% activity
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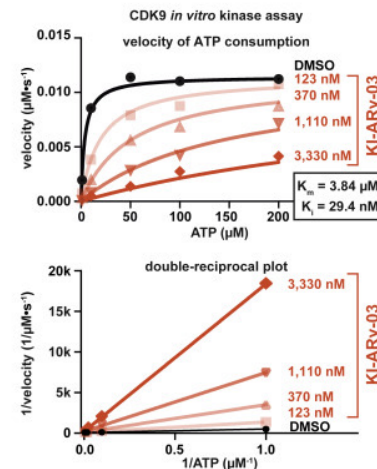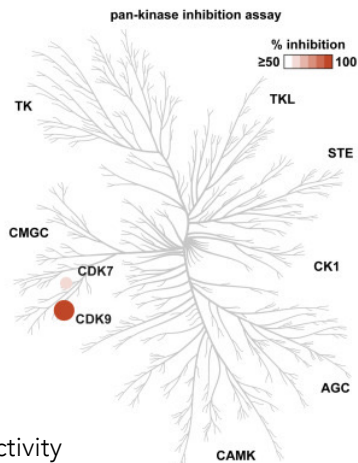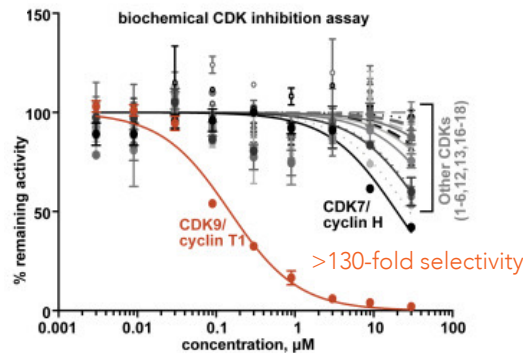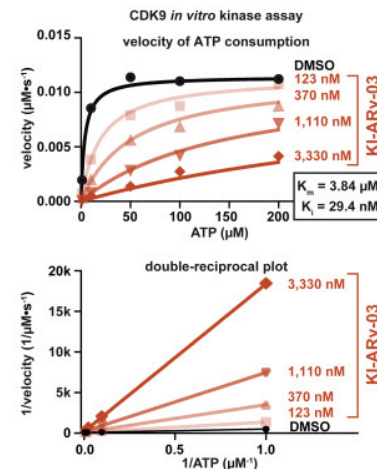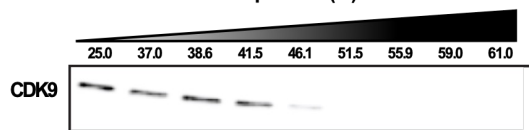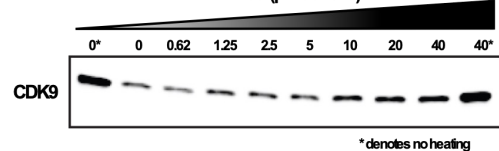CDK7 IC$_{50}$ = 20.1 μM

Michaelis-Menten and Lineweaver-Burk

K_m = 3.84 μM
K_i = 29.4 nM

ATP competitive

CETSA melt curve temperature (°C)

isothermal dose finger print KI-ARv-03 (μM at 49°C)

*denotes no heating

stabilization in live 22RV1 cells

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Kinase selectivity profiling suggests CDK9 as a target

pan-kinase inhibition assay

% inhibition
≥50 � 100

TK
TKL
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CK1
CMGC
CDK7
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CAMK

10 μM KI-ARv-03
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apparent Km
for each kinase

% activity
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13 CDKs in 10-pt dose

KI-ARv-03 with CDK9/cyclin T1

Phe103

Glu107

His108

Asp109

CET
ter

| 25.0 | 37.0 | 38.6 | 41.5 | 46.1 | 51.5 | 55.9 | 59.0 | 61.0 |

CDK9

finger print
(M at 49°C)

| 0* | 0 | 0.62 | 1.25 | 2.5 | 5 | 10 | 20 | 40 | 40* |

CDK9

*denotes no heating

stabilization in live 22RV1 cells

Michaelis-Menten and Lineweaver-Burk

CDK9 in vitro kinase assay
velocity of ATP consumption

DMSO
123 nM
370 nM
1,110 nM
3,330 nM

$K_m = 3.84$ μM
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double-reciprocal plot

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ATP
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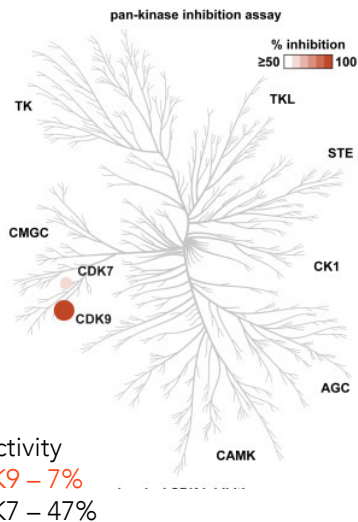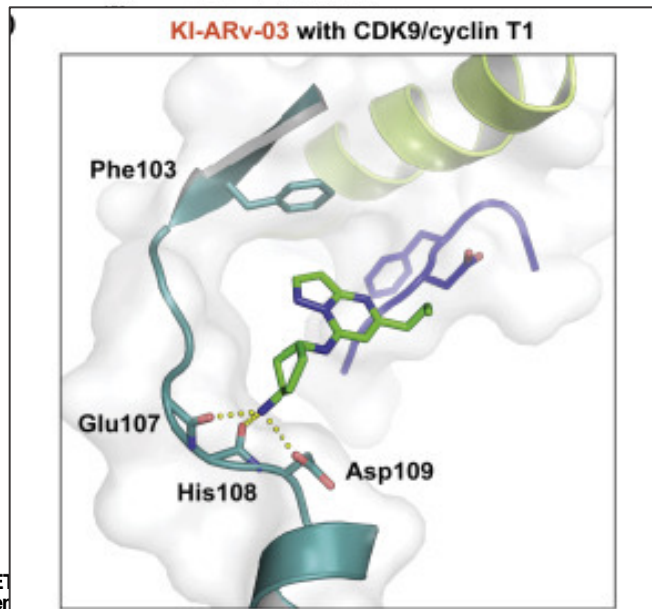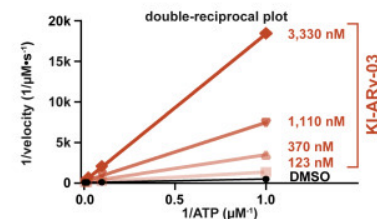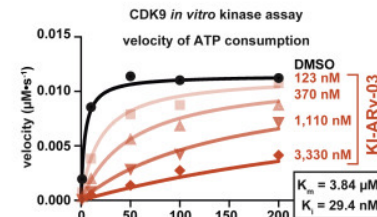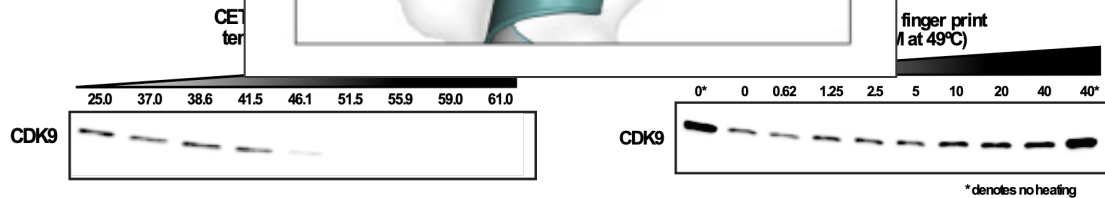
CDK9 is a known regulator of AR/ARV species activity
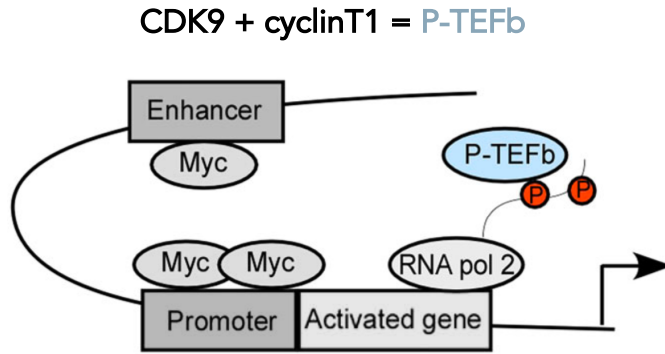
CDK9 regulates AR and ARV
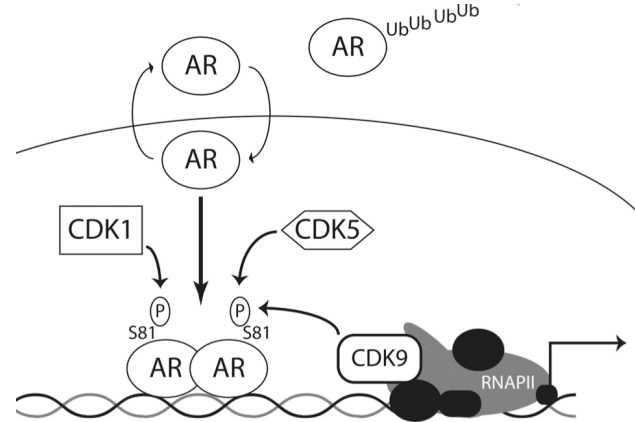
Koryakina, Y., Ta, H. Q., and Gioeli, D. (2014) Endocr. Relat. Cancer 21, T131–45.
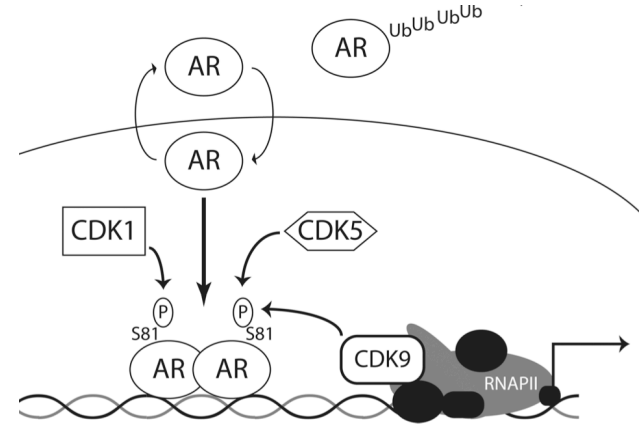
ARVs (and AR) physically interact with CDK9 in cells

CDK9 is a known regulator of AR/ARV species activity

transcriptional oncogene activity frequently reliant on CDK9 via elongation factor P-TEFb

CDK9 regulates AR and ARV

Chen, H., Liu, H., and Qing, G. (2018)]*Sig Transduct Target Ther 3*, 635–7
Bai et al., (2019) *Oncogene* 38, 4977-4989
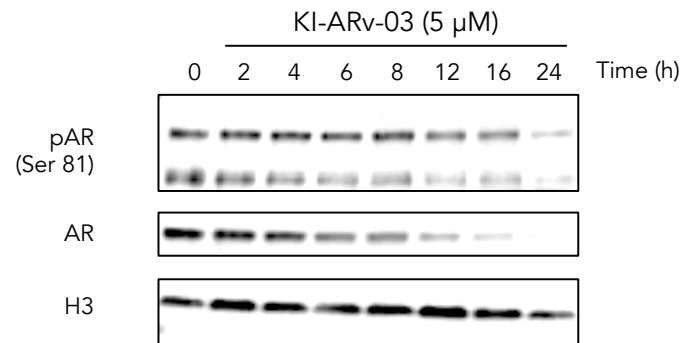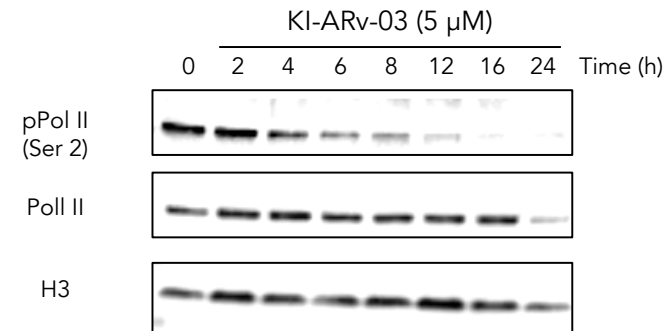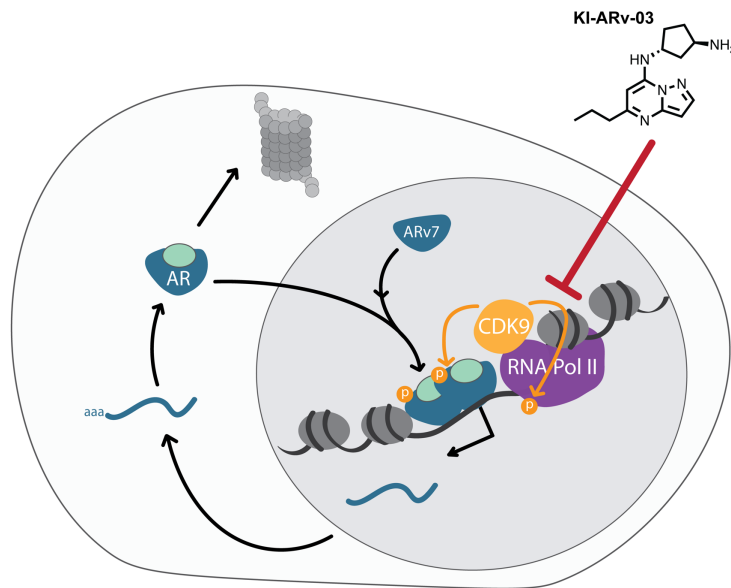Huang et al. (2014) Genes Dev 28, 1800-1814

Koryakina, Y., Ta, H. Q., and Gioeli, D. (2014) Endocr. Relat. Cancer 21, T131–45

MYC regulates expression of AR and ARVs in PCa
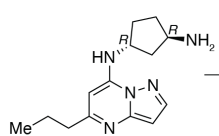
KI-ARv-03 impairs phosphorylation of known CDK9 targets

Pol II and AR monitored in 22RV1 cells

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

Advanced lead KB-0742 shows improved potency while retaining selectivity with activity in preclinical model of prostate cancer

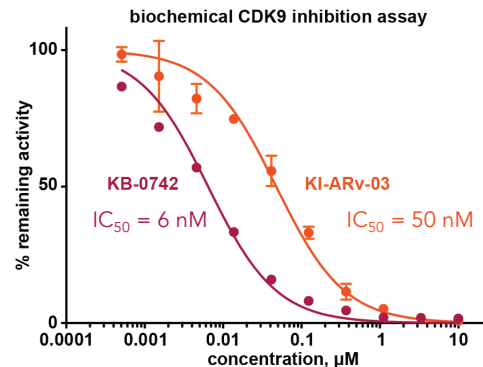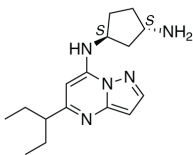|	KI-ARv-03	KB-0742
	molecular weight: 259.36	molecular weight: 287.4
	KB-0742 IC_{50} = 6 nM	**KI-ARv-03** IC_{50} = 50 nM
CDK9		
CDK13	62x	91x
CDK2	66x	200x
CDK12	98x	124x
CDK18	>200x	>200x
CDK3	>200x	>200x
CDK7	>200x	176x
CDK16	>200x	>200x
CDK5	>200x	>200x
CDK17	>200x	>200x
CDK1	>200x	>200x
CDK4	>200x	>200x
CDK6	>200x	>200x
CDK14	>200x	>200x
CDK8	>200x	>200x
CDK19	>200x	>200x

CDK9 fold selectivity vs.

1x — >200x

concentration, µM: 0.0001 0.001 0.01 0.1 1 10

caspase-3/7 positive cells 22Rv1 (72h)

concentration (10 µM top, 3.16-fold dilution)

compound	GR50 (µM)	GRmax	AOC
KI-ARv-03	3.26	0.0826	0.0745
BAY-1143572	1.25	0.105	-0.136
KB-0742	0.183	-0.0275	0.344
NVP-2	0.00336	0.0261	0.856
AZD4573	0.00109	-0.0221	0.951

caspase-3/7 positive cells 22Rv1 (72h)

tumor outgrowth 22Rv1 xenograft

KRONOS·BIO

vehicle, 10 µl/g, p.o.,QD
docetaxel,15 mg/kg, i.p.,QW
3 mg/kg, p.o., QD
10 mg/kg, p.o.,QD
30 mg/kg, p.o.,QD
30 mg/kg, p.o.,QD 3-day on, 4-day off } KB-0742

3 mg/kg
vehicle
10 mg/kg
30 mg/kg (pulse) *
docetaxel *
30 mg/kg *

time (days): 0 2 5 9 12 16 19 21

	NVP-2	0.00336	0.0261	0.856
	AZD4573	0.00109	-0.0221	0.951

Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)
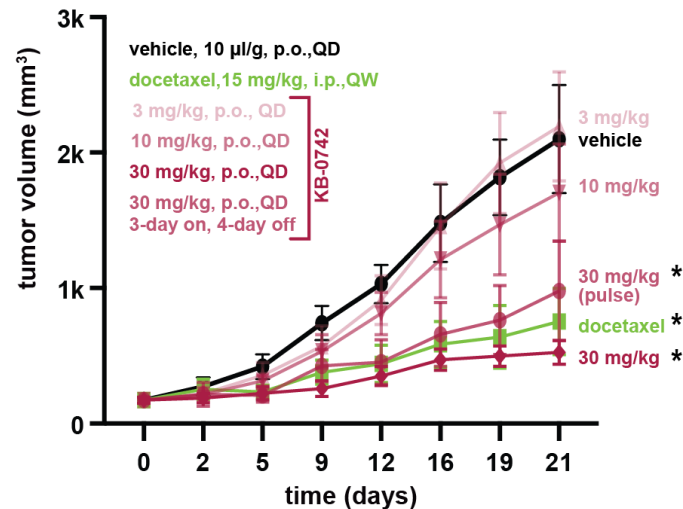
# From L3: Cancers dysregulate MYC by increasing its expression
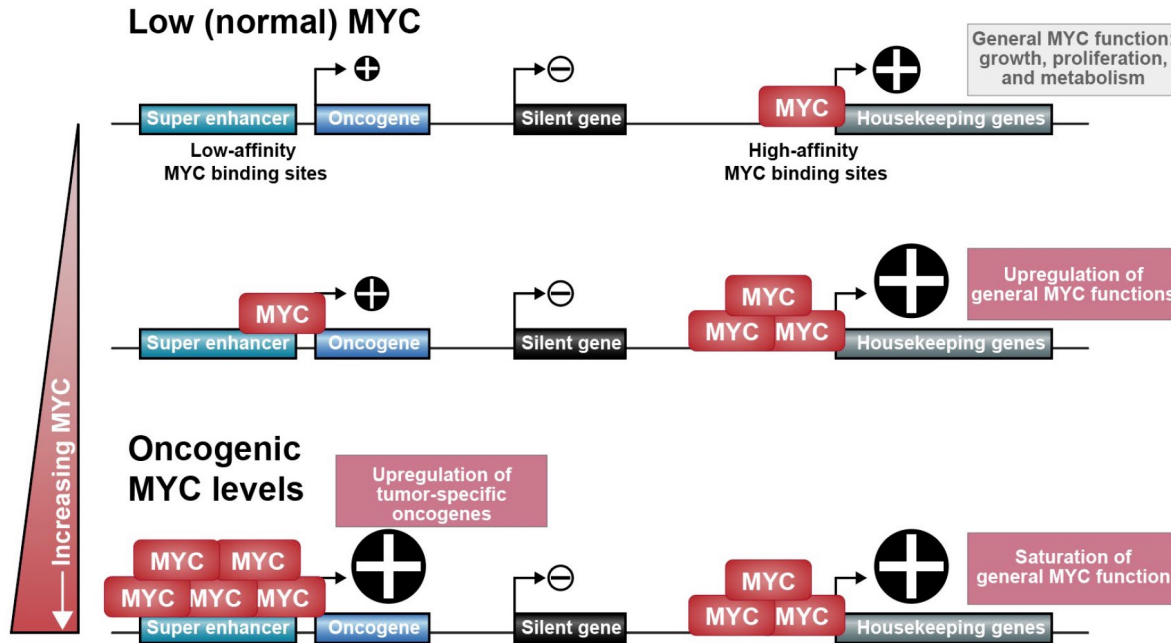
in typical cells, steady state *MYC* levels regulate general housekeeping functions

MYC can be transiently upregulated in typical cells (e.g. during wound healing)

tumor cells need persistently upregulated *MYC* at super physiologic levels to drive tumor-specific oncogenes

Dependence on persistently high MYC expression creates a vulnerability to CDK9 inhibition
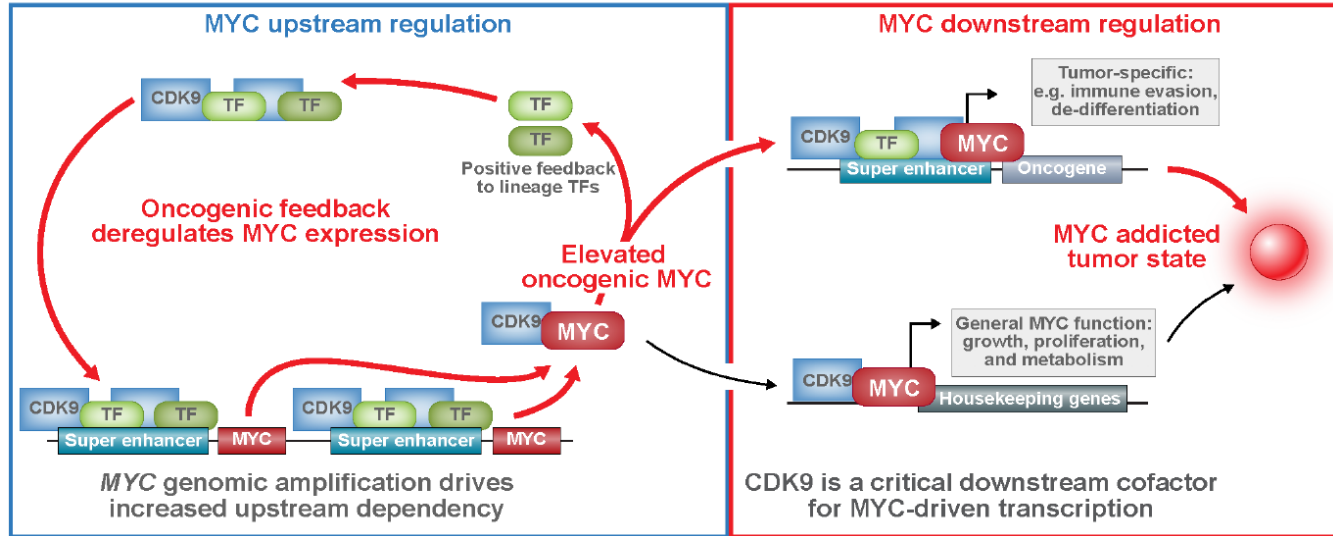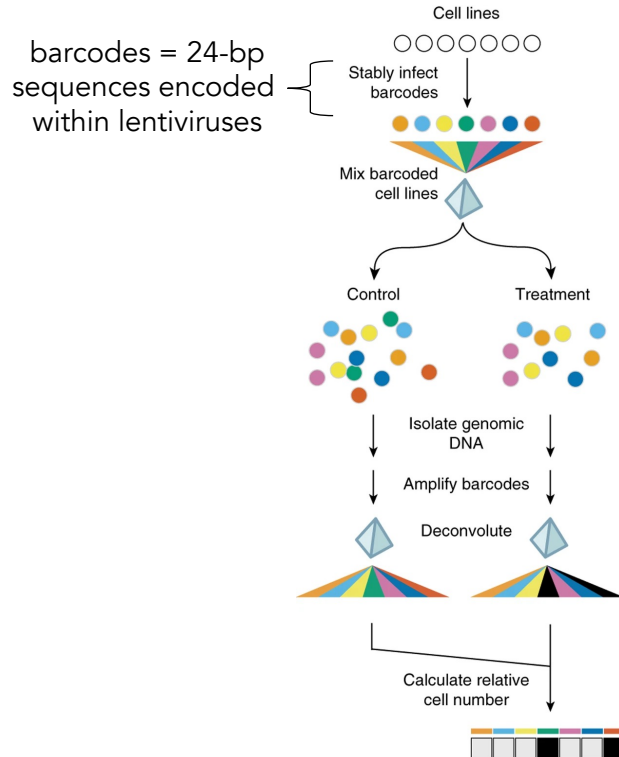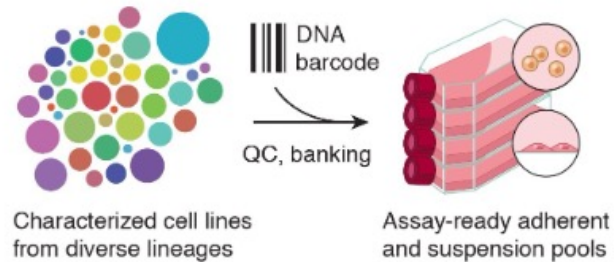

Therapeutic hypothesis:
intermittent/partial inhibition of CDK9 may be sufficient to disrupt the oncogenic MYC network

Multiplexed Cell Line Viability Profiling

PRISM is a powerful approach to rapidly screen drugs across hundreds of cancer cell lines


barcodes = 24-bp sequences encoded within lentiviruses

Generation of Barcoded Models for PRISM Screens

Characterized cell lines from diverse lineages

DNA barcode

QC, banking

Assay-ready adherent and suspension pools

Over 930 genomically characterized CCLE cell lines have been barcoded with a DNA barcode. All cell lines are tested for mycoplasma, verified with SNP fingerprinting, and the barcode identity is confirmed. Cell lines are then mixed together in assay ready pools according to doubling time.

Yu et al., *Nature Biotechnology*, 34, 419-423 (2016)

Multiplexed Cell Line Viability Profiling

PRISM is a powerful approach to rapidly screen drugs across hundreds of cancer cell lines


PRISM Viability Assay
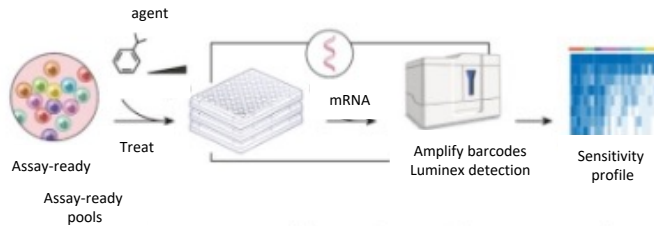
Pools of cells are treated for 5 days with compounds, then cells are lysed and mRNA is isolated. The barcode sequences are then amplified by PCR and detected by a Luminex scanner. The quantity of each barcode remaining after treatment serves as a readout to generate cell line sensitivity signatures for each compound.
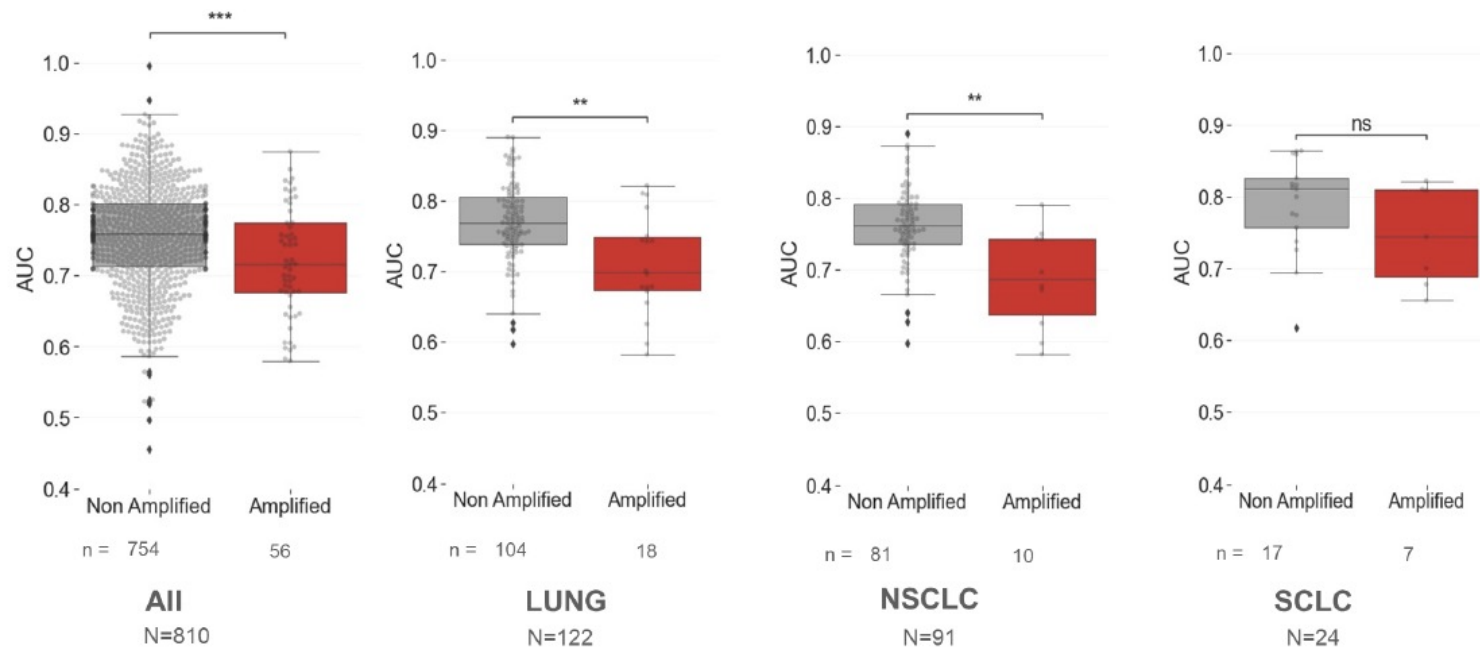
~$7,000/compound for >900 cell lines

Predictive Modeling
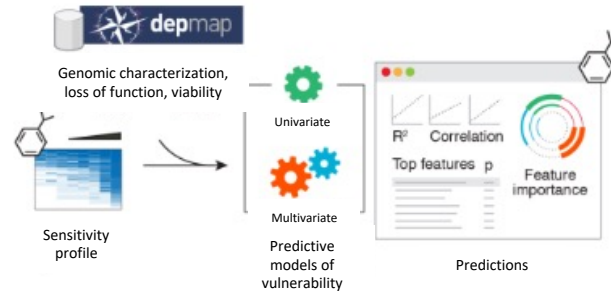
Sensitivity signatures from PRISM data are run through predictive modeling algorithms, such as random forest in order to identify biomarkers using CCLE genomic characterization data, Repurposing drug viability data, and Dependency Map loss-of-function genetic perturbation data.
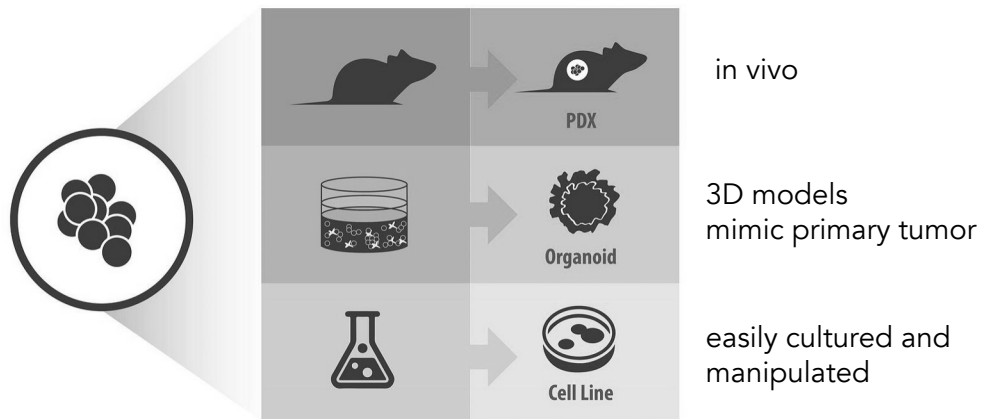
Yu et al., *Nature Biotechnology*, 34, 419-423 (2016)

Cancer cell lines with MYC genomic copy number amplification are more sensitive to KB-0742 than non-MYC-amplified lines

Jorge DiMartino, Charles Lin, Doug Saffran, Kronos Bio

KB-0742 is active in patient-derived organoids that express MYC

model types

in vivo

3D models
mimic primary tumor

easily cultured and
manipulated

organoid model description

Model Number	Indication	Treatment history	MYC TPM
KOLU-045	Small Cell Lung Cancer	Naïve	70
KOLU-299		Naïve	30
KOLU-448		Lobaplatin+Etoposide	30
KOLU-775H		Cisplatin	20
KOLU-545H		VP16+Lobaplatin	68
KOLU-643H		VP16 + Lobaplatin	88
KOBR-011	Triple Negative Breast Cancer	TNBC: EPI + PTX 6 cycle	UNK
KOBR-472		TNBC: PTX + CBP 4 cycle	UNK

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drug activity profiles

Maximum % Inhibition

	Cisplatin	Pemetrexed	Paclitaxel	Gemcitabine	KB-0742
KOLU-045	10.52	12.83	44.81	53.02	99.99
KOLU-299	10.00	10.00	48.42	57.21	94.19
KOLU-448	10.00	18.97	21.28	34.95	99.02
KOLU-775H	10.00	10.00	49.61	71.74	94.69
KOLU-545H	11.57	4.79	17.50	25.06	95.88
KOLU-643H	No effect		16.29	No effect	70.65
KOBR-011	No effect		31.56	59.99	100.00
KOBR-472	No effect		No effect	15.06	89.00

KB-0742 is more potent than standard of care agents (chemo)

MYC WT — KOLU-1004X

MYC Amplification — KOLU-1121X, KOLU-1140X, KOG5-204X, KOSC-022X

IC_{50} = 13.54 μM *unusual fit

IC_{50} = 6.78 μM

IC_{50} = 4.28 μM

IC_{50} = 2.62 μM

IC_{50} = 2.75 μM

Jorge DiMartino, Charles Lin, Doug Saffran, Kronos Bio
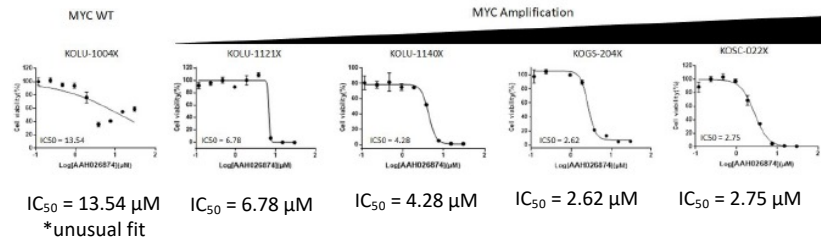
KB-0742 shows anti-tumor activity in patient-derived xenografts (PDX)

Intermittent dosing in 'MYC high' expressing murine PDX models

subcutaneous engraftment of tumor cells + treatment

tumor volume changes

tumor growth inhibition

Jorge DiMartino, Charles Lin, Doug Saffran, Kronos Bio
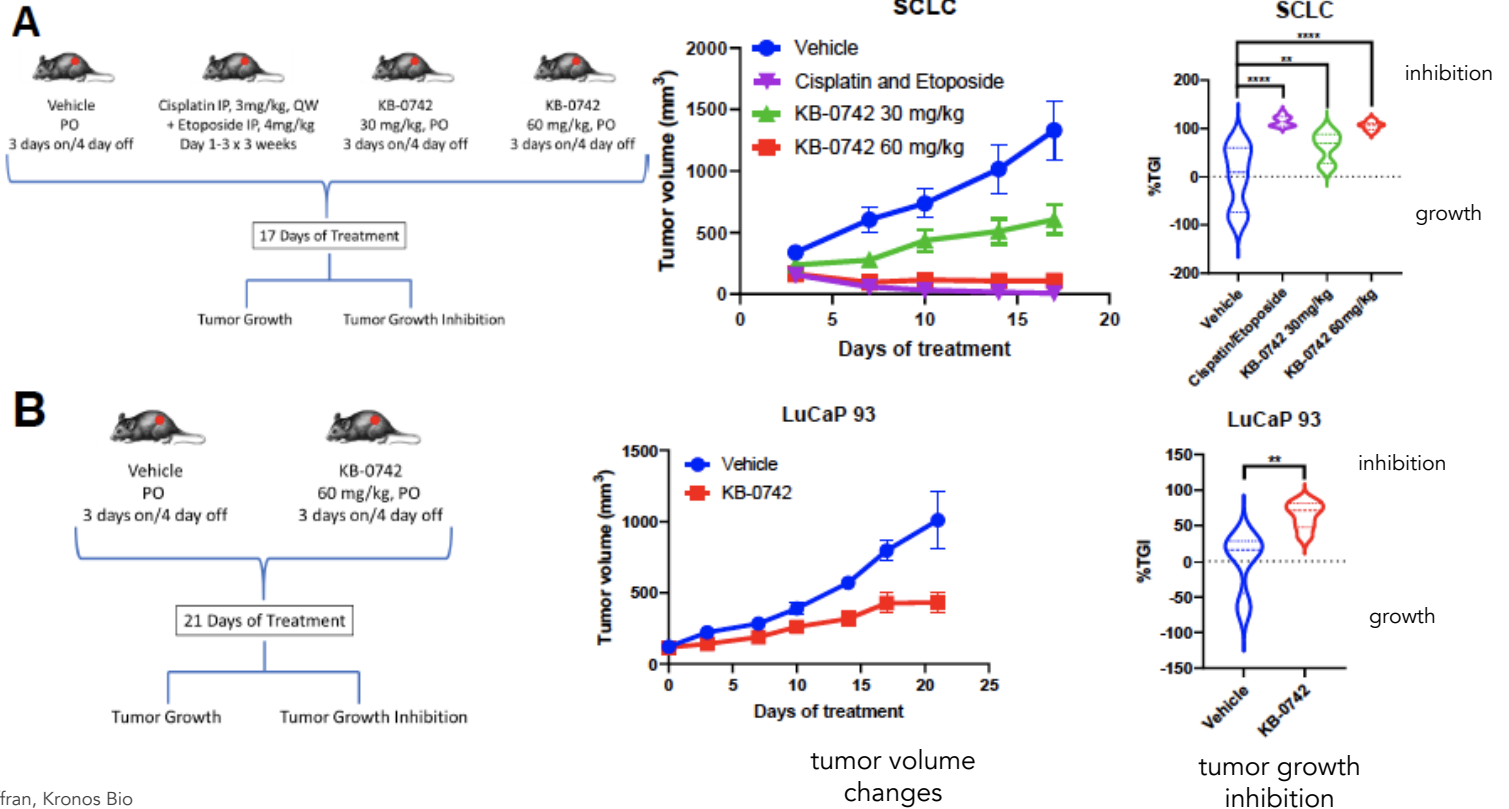
Target engagement in vivo – small cell lung cancer PDX models
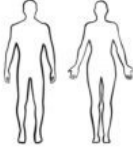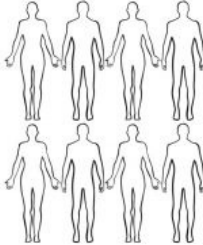## KB-0742 reduces phosphorylation of RNA Pol II (pSER2)
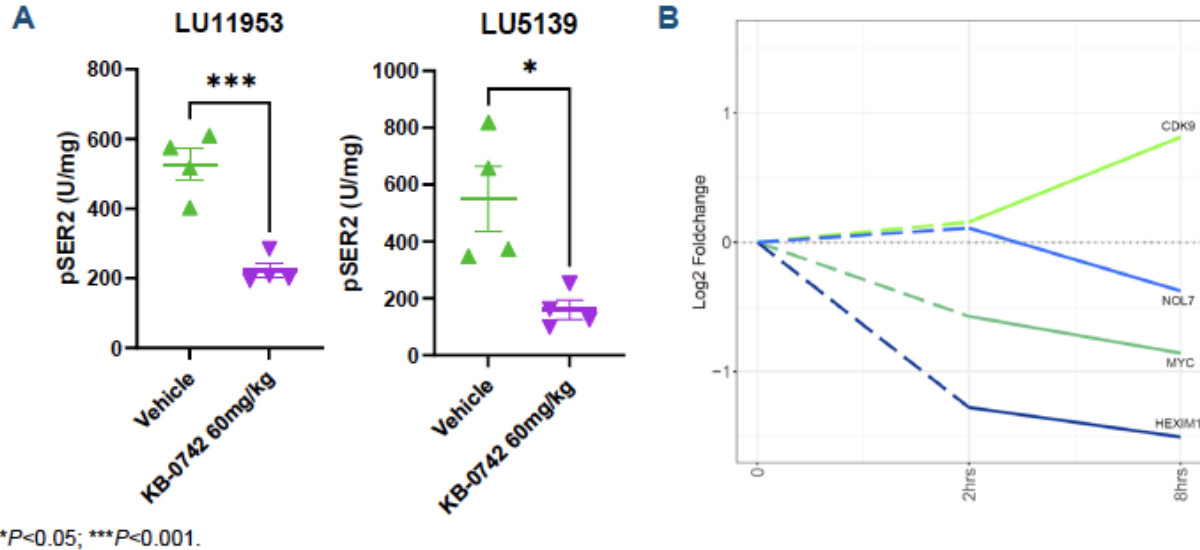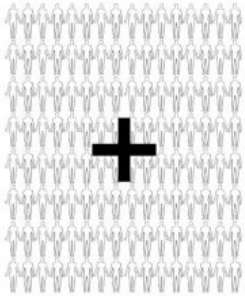
60 mg/kg dose resulted in a 50% or greater reduction in pSer2 after 3 days of dosing

RNA sequencing of LU11953 tumors showed altered gene expression of key genes, including *MYC*

Jorge DiMartino, Charles Lin, Doug Saffran, Kronos Bio

Clinical Trials

PRECLINICAL	PHASE I	PHASE II	PHASE III	PHASE IV
*				
Laboratory Research determines if treatment is useful and safe	6-10 Participants Understand effects of treatment in humans	20-50 Participants Evaluate safety and efficacy of treatment	100-200 Participants Confirm benefit and safety of treatment	200+ Participants Evaluate long-term effects of treatment

KB-0742 Phase 1/2 trial design

STAGE 1: DOSE ESCALATION

Dose Level N

Dose Level 4

RP2D

40 mg

20 mg

10 mg

3d on/ 4d off QW

completed in late 2022

- Relapsed/refractory solid tumor population not selected for *MYC* amplification
- Understand safety, PK and PD in PBMC
- Refine dosing schedule to maximize therapeutic window

STAGE 2: EXPANSION COHORTS

Cohort A: MYC-Dependent Solid Tumors

NSCLC, SCLC, TNBC, Ovarian
N=40

Cohort B: Other Transcriptionally Addicted Tumors

Sarcoma, Chordoma
N=30

Path to Registrational Trial

- Biomarker selected patients most likely to benefit from CDK9 inhibition
- Confirm safety and PD in tumor tissue
- Anti-tumor activity in specific tumor types

RP2D (recommended Phase 2 dose):
60 mg/kg (oral, 3 day on/4 day off) → ~50% reduction in levels of pSer2 on Pol II

Stage 2 Cohort B will evaluate the anti-tumor activity of KB-0742 in a basket of transcriptionally addicted tumors
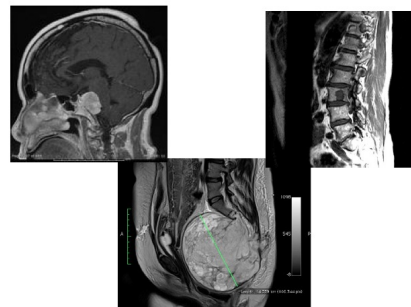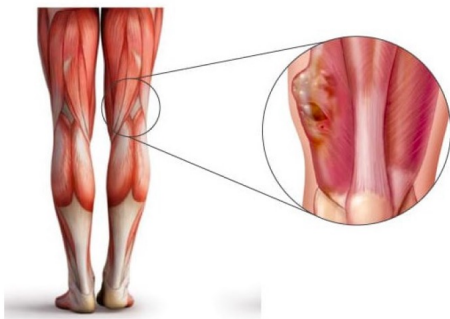
Soft-tissue sarcomas with transcription factor fusions as driver mutations, for example:
- Ewing sarcoma (EWS-FLI1)
- Rhabdomyosarcoma (PAX-FOXO1)
- Myxoid liposarcoma (FUS-CHOP)
- Clear cell sarcoma (EWSR1-ATF1)
- Desmoplastic round cell tumor (EWSR1-WT1)

Chordoma: dependent on brachyury transcription factor
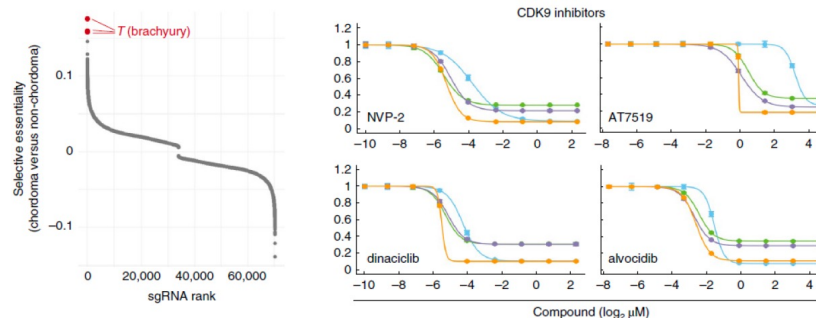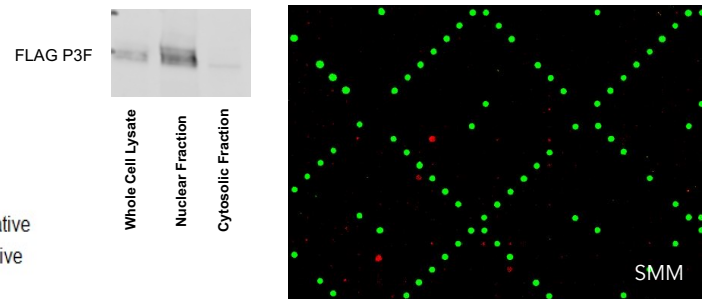

Sharafinia et al 2019 Nature Med 295:292-300.

Pathognomonic PAX3-FOXO1 fusion -
a highly disordered protein
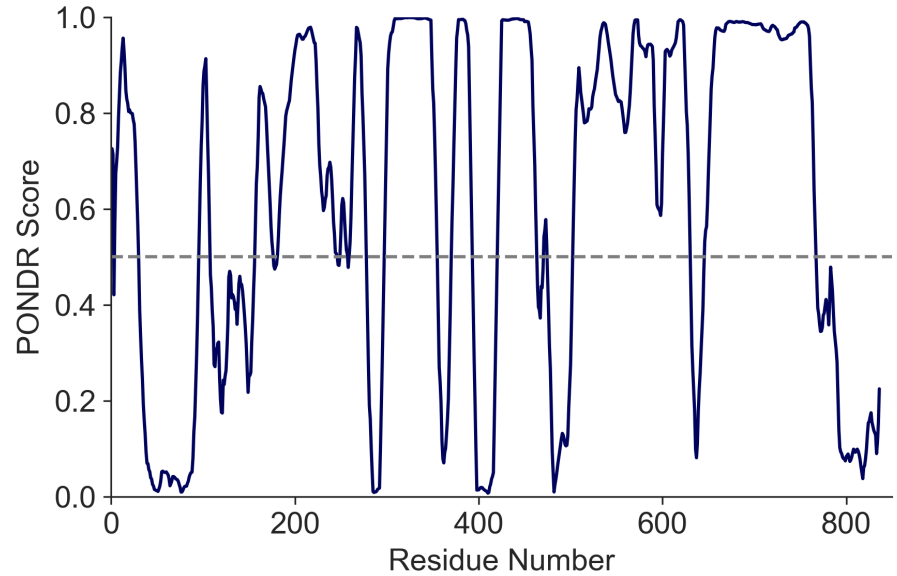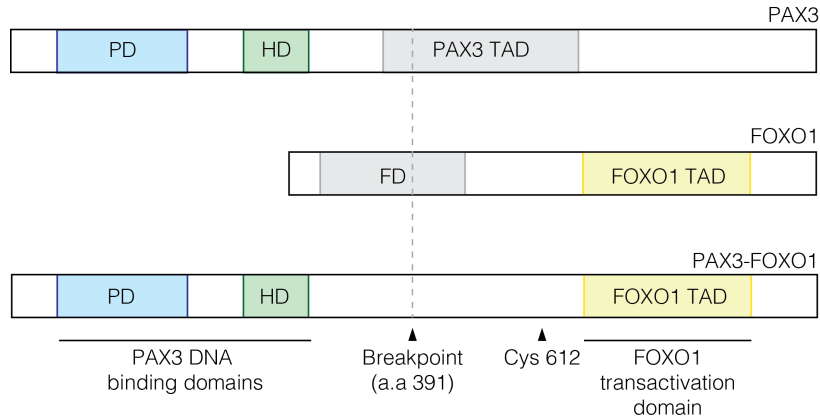
SMM screens for PAX3-FOXO1

SMM screening data for PAX3-FOXO1 from
Rh4 RMS cell lysates

Screen : ~65,000 small molecules

~60 assay positives

0.09% hit rate

Morgan Stilgenbauer, Maddy Henley, Bonnie Su

KI-P3F-032 as a starting point for targeted protein degradation (PROTACs)

1. Establish gross SAR

(e.g., CETSA)

2. Establish readouts for P3F degradation
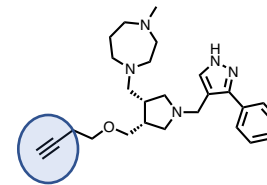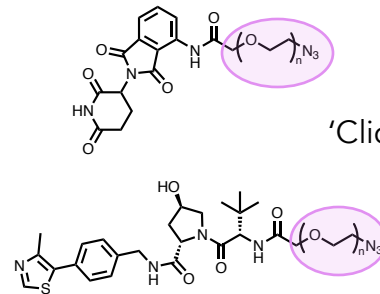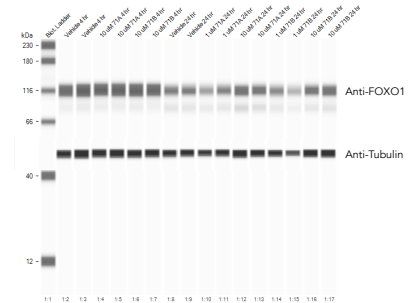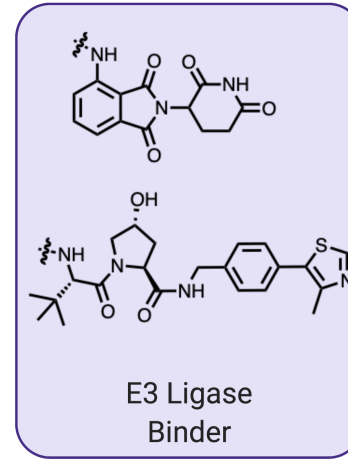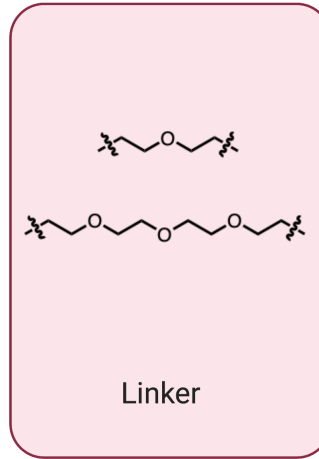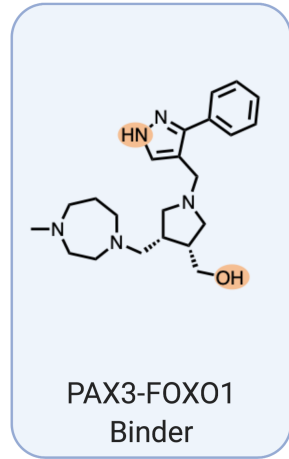
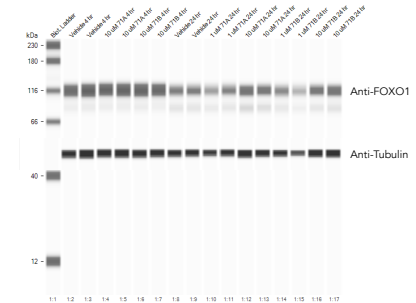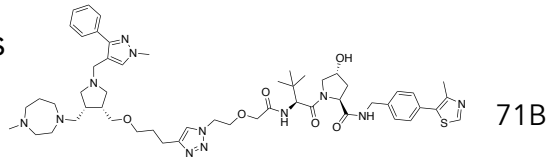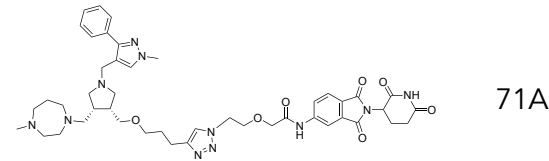(e.g., HT-western endogenous HiBiT cellular assays)

PAX3-FOXO1 Binder

Linker

E3 Ligase Binder

3. Synthesize variants of KI-P3F-032 for coupling

4. Assemble linker library

5. Assembled E3 ligands

6. PROTAC synthesis

'Click' chemistry

US application 63/280,091

Sean Quinnell
Maddy Henley

KI-P3F-032 as a starting point for targeted protein degradation (PROTACs)

1. **Establish gross SAR**

   (e.g., CETSA)

2. **Establish readouts for P3F degradation**

   (e.g., HT-western endogenous HiBiT cellular assays)

PAX3-FOXO1 Binder

Linker

E3 Ligase Binder

3. **Synthesize variants of KI-P3F-032 for coupling**

4. **Assemble linker library**

5. **Assembled E3 ligands**

6. **PROTAC synthesis**

Anti-FOXO1

Anti-Tubulin

PoC degraders candidates for optimization reduce to 80% original P3F levels

71A

71B

Sean Quinnell
Maddy Henley

Upcoming Lectures