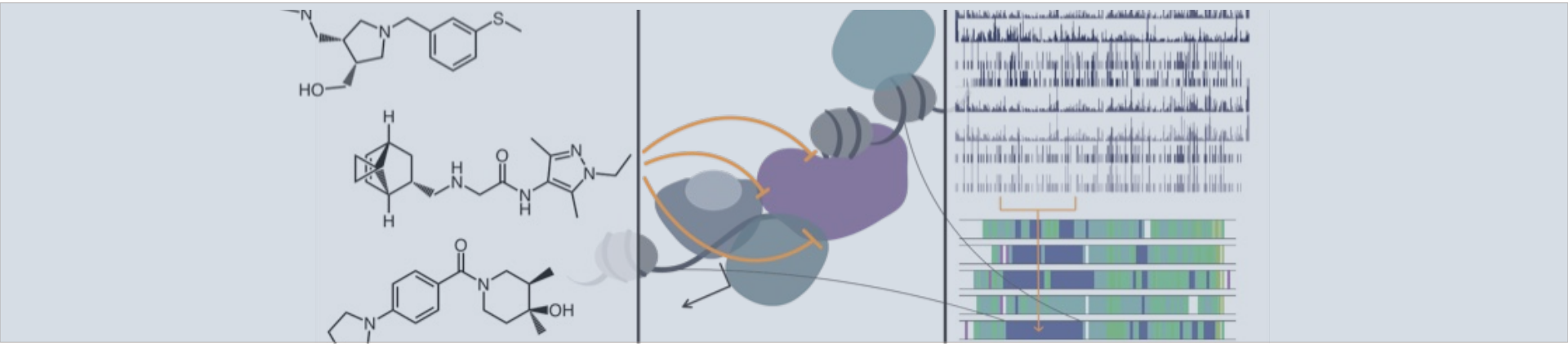
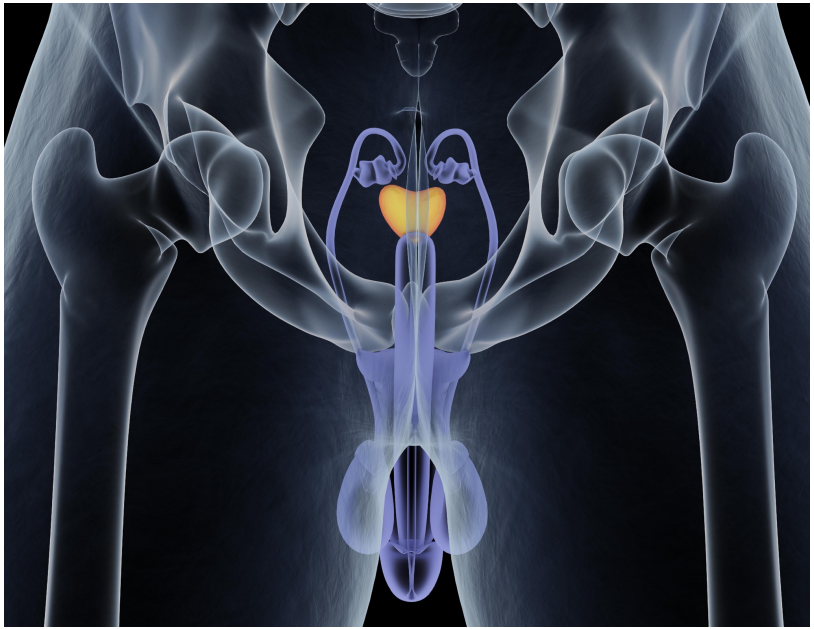


# Lecture 5 – KB-0742: A clinical candidate discovered by SMMs

# Prostate Cancer



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

2024 US estimates: 299,010 new cases
35,250 deaths

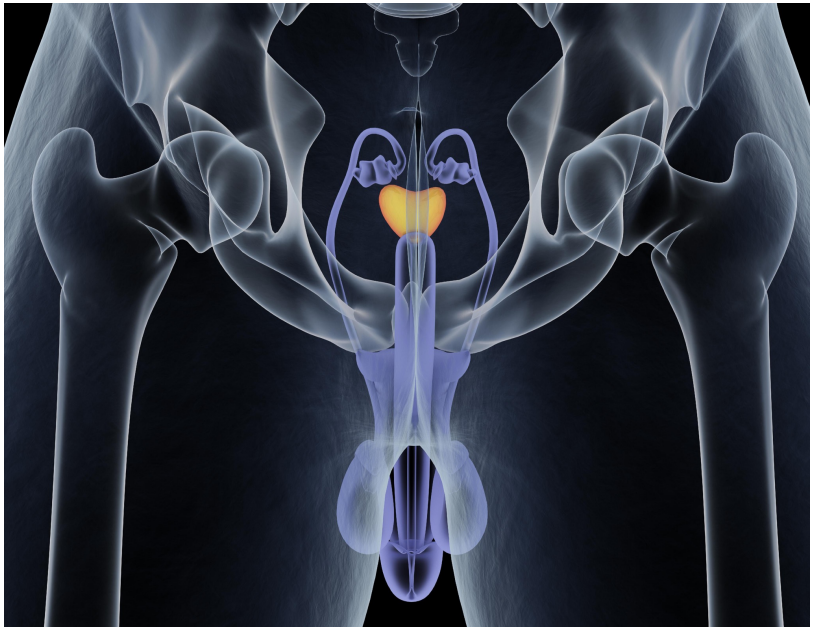
1 out of 8 men will be diagnosed in their lifetime

Develops more frequently in older men (6 out of 10 cases in men 65-yo or older), rare under 40-yo

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

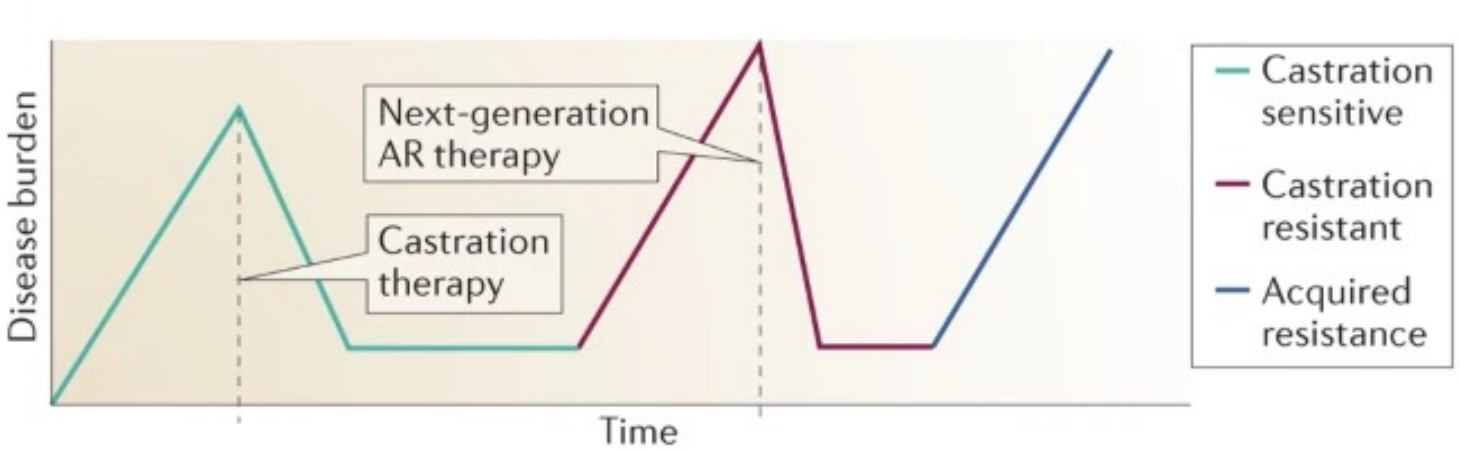
1 out of 44 men will die of prostate cancer

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

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

2013 onward: death rate stabilized, likely reflecting the rise in cancers found at an **advanced stage with resistance to therapies**

# Increasing disease burden following primary prostate cancer therapy

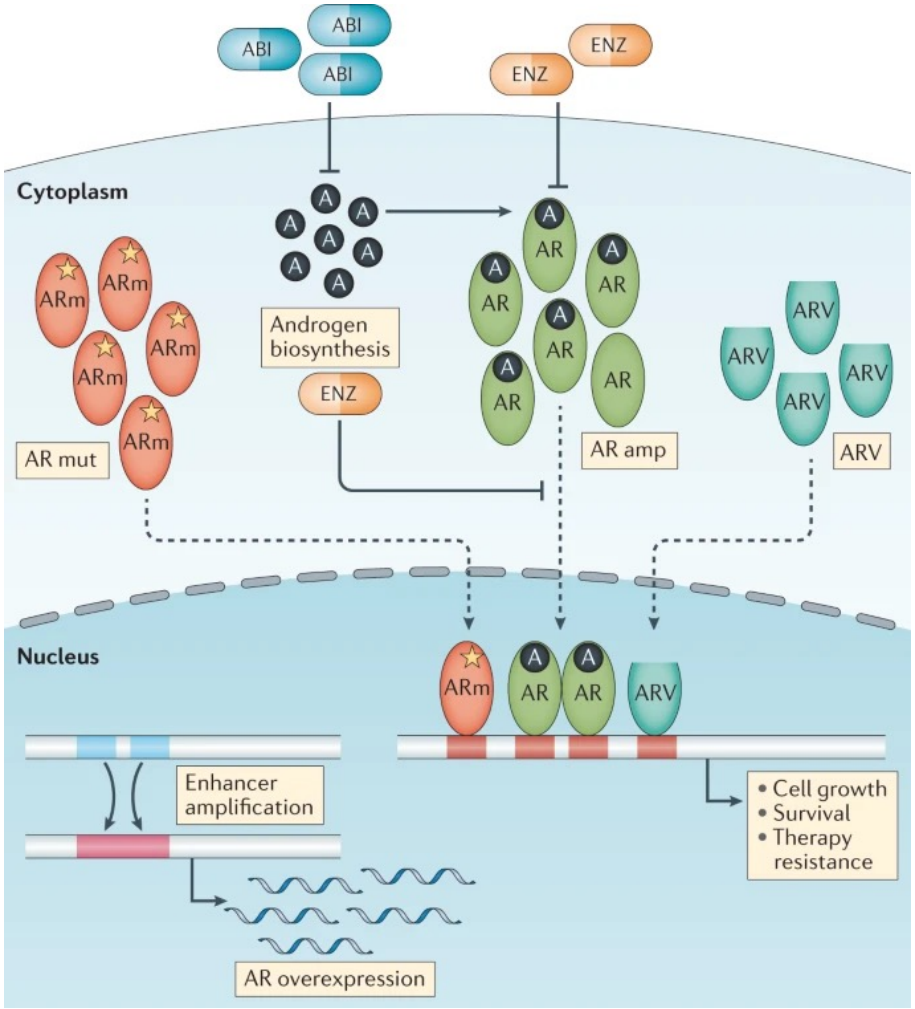



PCa

CRPC
(<20%)

mCRPC
(10%, 3yrs)

Watson, Arora, Sawyers, *Nature Reviews Cancer*, 15, 701-711 (2015)

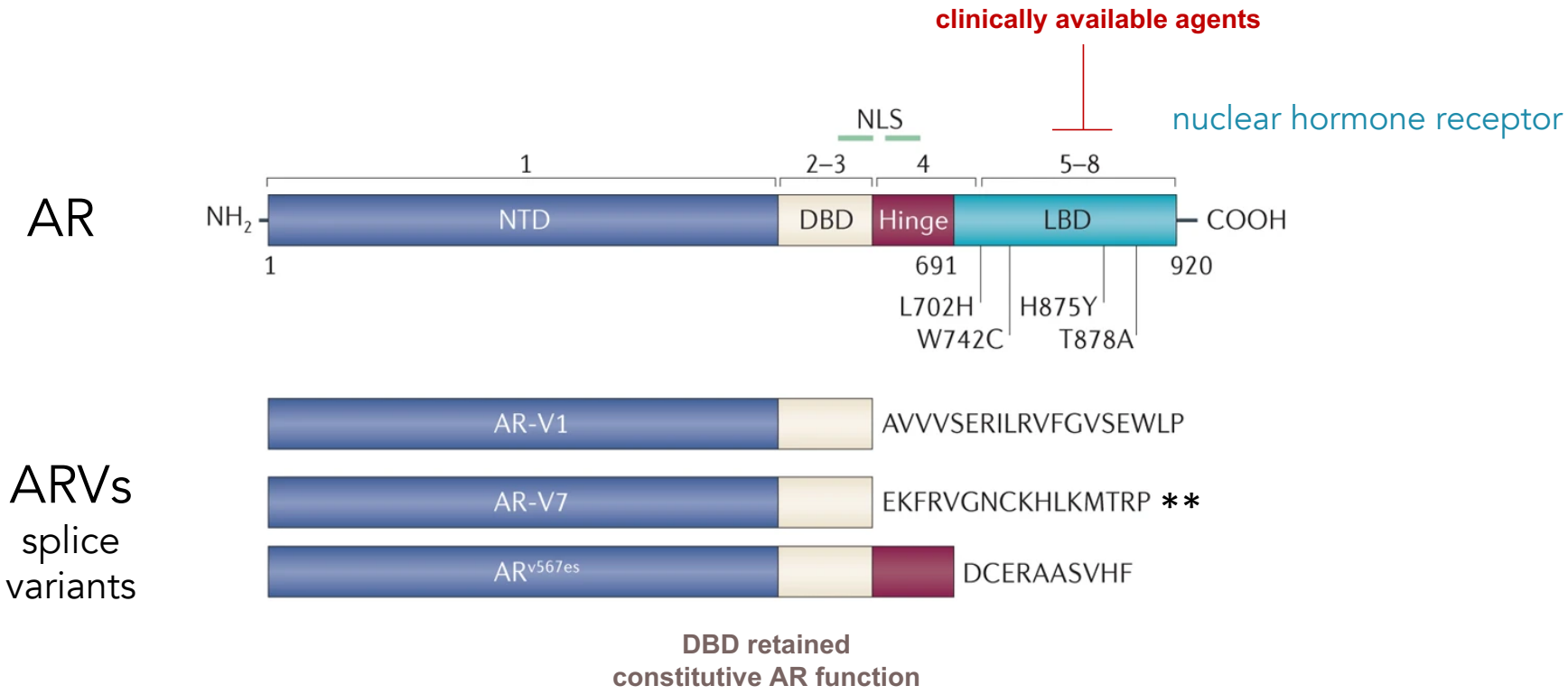



Alterations in Androgen Receptor (AR) signaling are the most prevalent events in metastatic castration-resistant prostate cancer leading to *persistent AR activation*

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

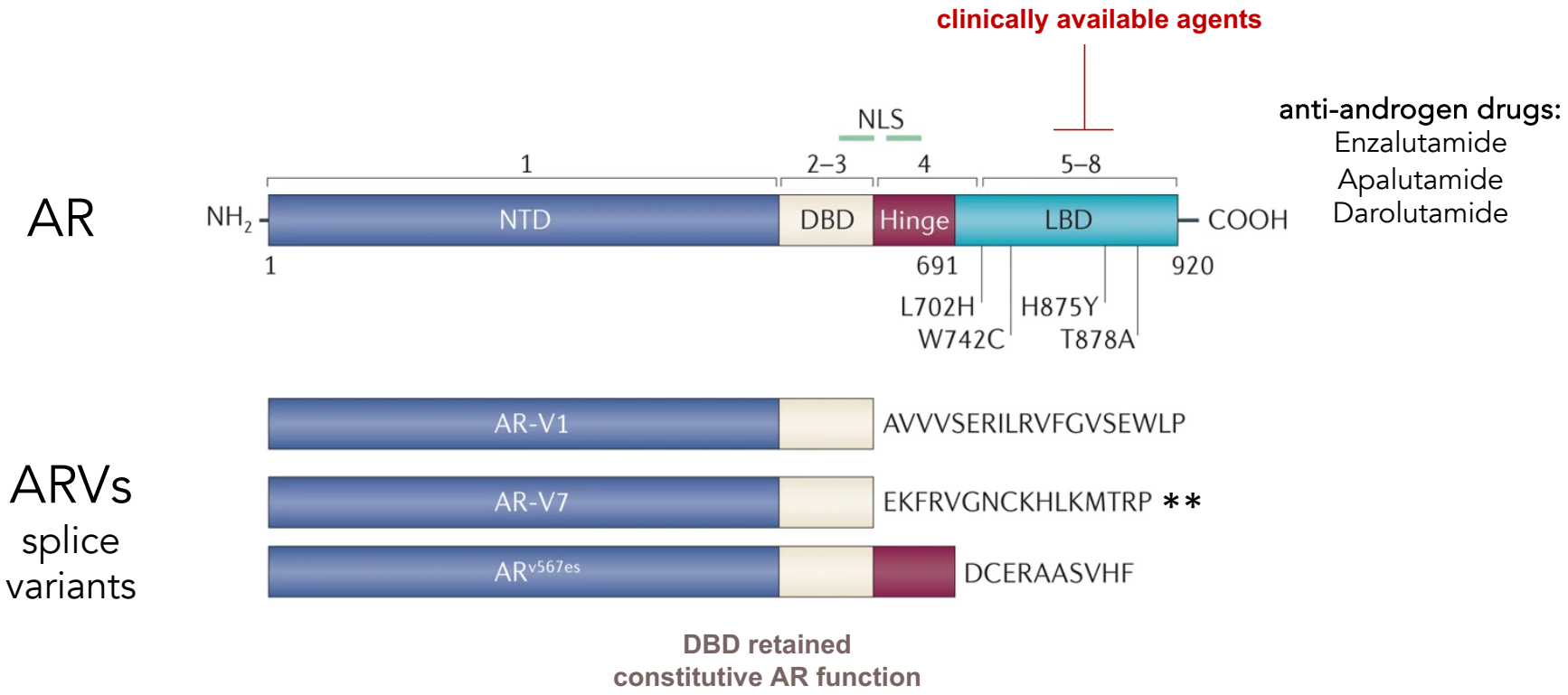
Ku, Gleaves, and Beltran., *Nature Reviews Urology*, *16*, 645-654 (2019)

# Domain structure of AR: mutations and splice variants




Watson, Arora, Sawyers, *Nature Reviews Cancer*, 15, 701-711 (2015)

# Domain structure of AR: mutations and splice variants

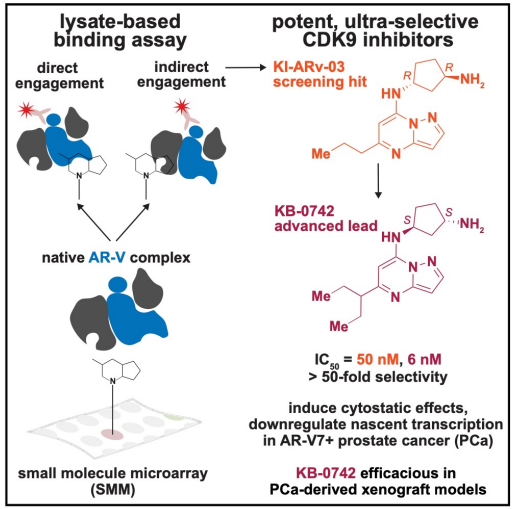



Watson, Arora, Sawyers, *Nature Reviews Cancer*, 15, 701-711 (2015)

Cell Chemical Biology

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

## Graphical Abstract





## 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

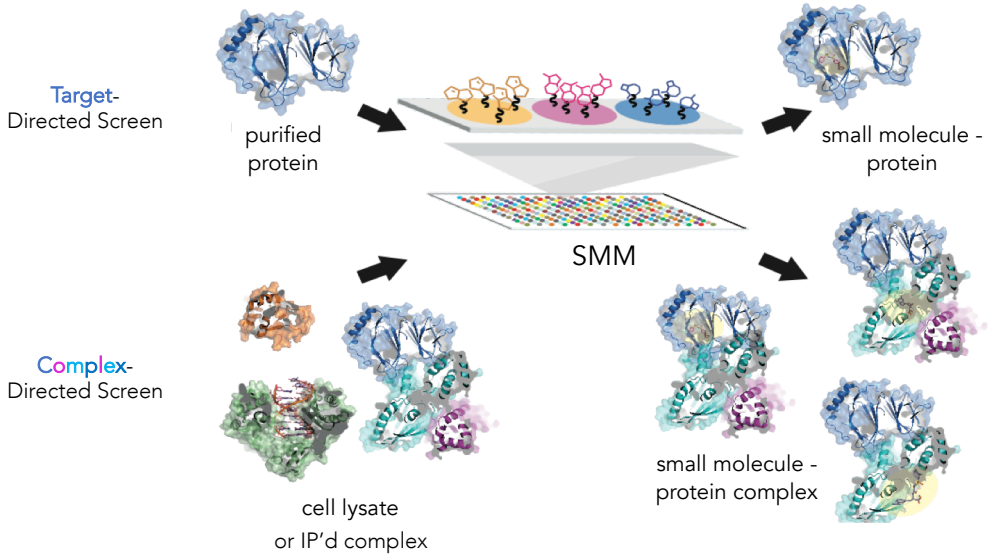
Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

## Rationale:

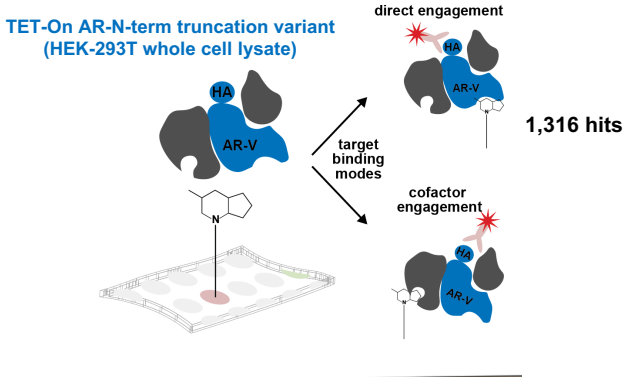
(1) AR splice variants lack ligand binding domain (LBD), contributing to resistance associated with AR antagonists in CRPC,

(2) screening ARv-containing complexes in cell lysates avoids purification,

(3) reflects more relevant state, and casts a net for targeting co-factors.



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




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

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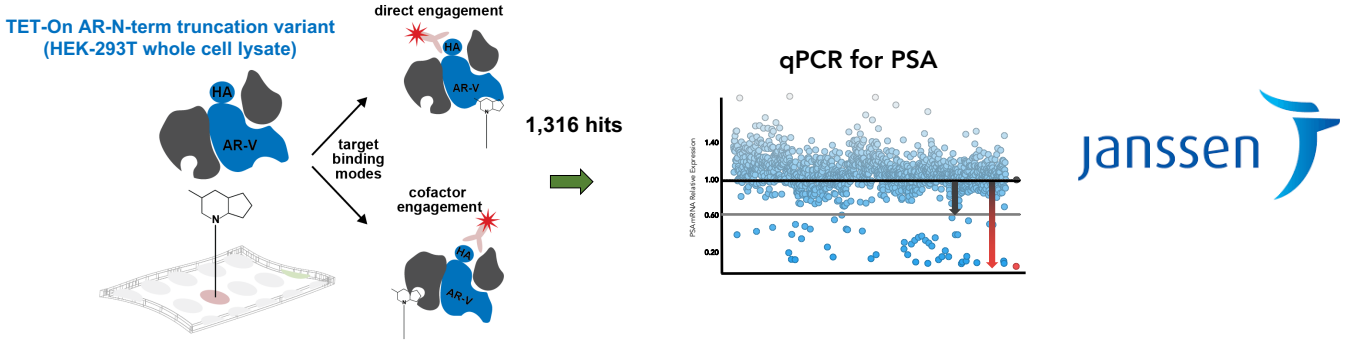
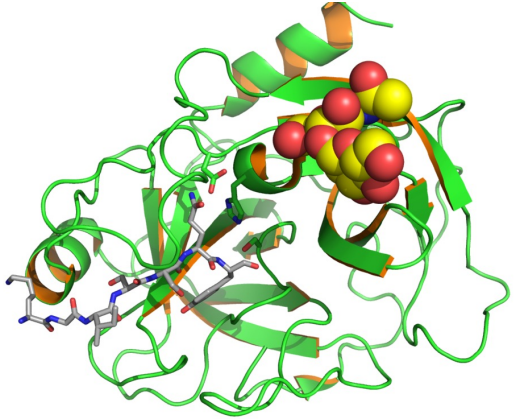
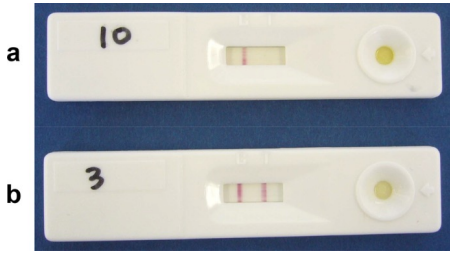



Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

# What is PSA?



*Human prostate specific antigen (PSA) with bound substrate from complex with antibody (PDB id: 2ZCK)*




BMC Urology **21**, Article number: 135 (2021)

Prostate-specific antigen (PSA) is a glycoprotein and peptidase secreted by epithelial cells in the prostate gland.

PSA enables sperm to swim freely and it dissolves cervical mucus, enabling entry of sperm into the uterus.

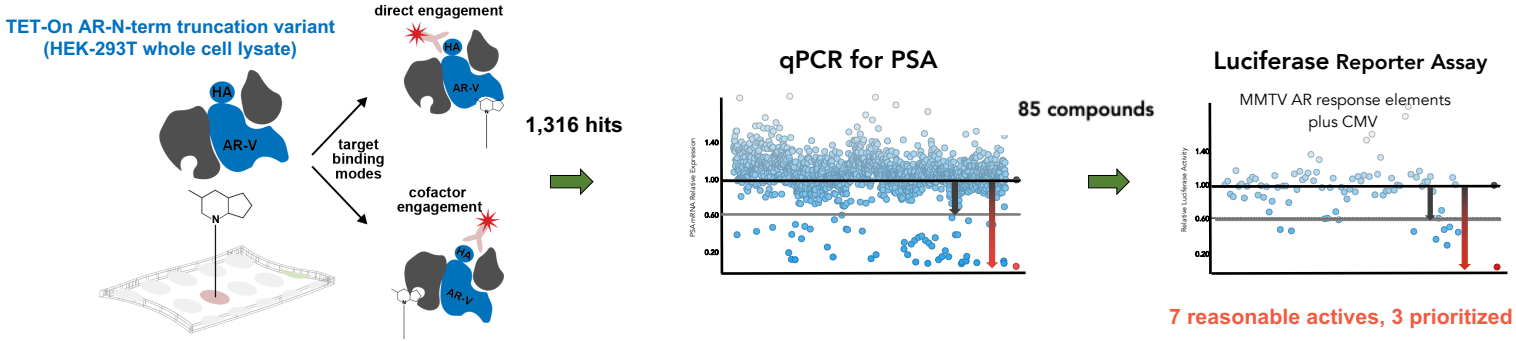
PSA is present in low levels in the serum of individuals with healthy prostates.

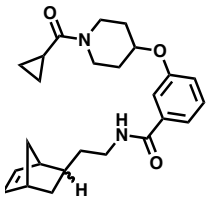
PSA is elevated in prostate cancer and other prostate disorders.

PSA is a pivotal downstream target gene of AR.

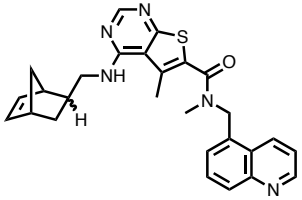
PSA is a useful serum biomarker to monitor cancer progression.

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

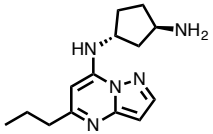


**KI-ARv-01**
MW = 408.54
cLogP = 3.27
$IC_{50}$ = 5.43 μM



**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

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

# KI-ARv compounds impacts AR-V7 levels in an enzalutamide-resistant prostate cancer cellular model

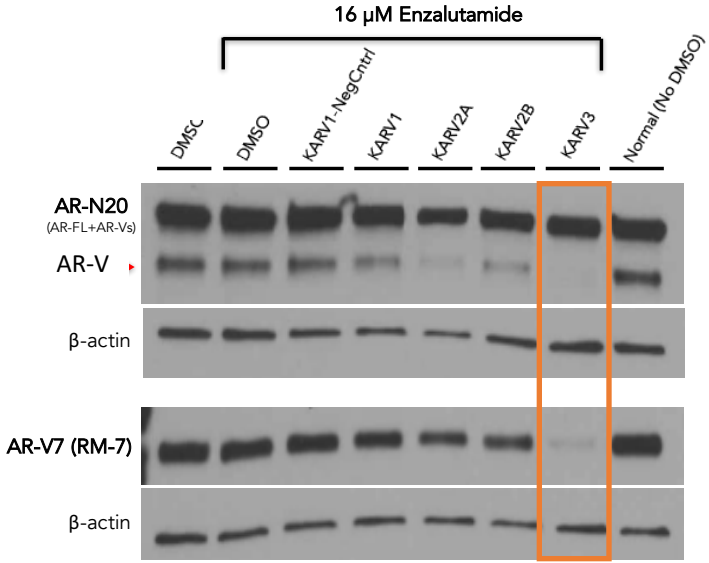
THE BRIDGE PROJECT

Dr. Joshua Russo

Dr. Steven Balk

## VCaP-16 cells

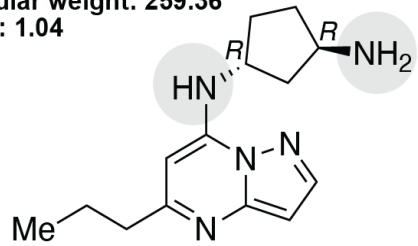
enzalutamide-resistant
increased expression of AR-v7
5 µM compound, 24-hour exposure




Western blot to measure protein levels

## KI-ARv-03

**molecular weight: 259.36**
**cLogP: 1.04**



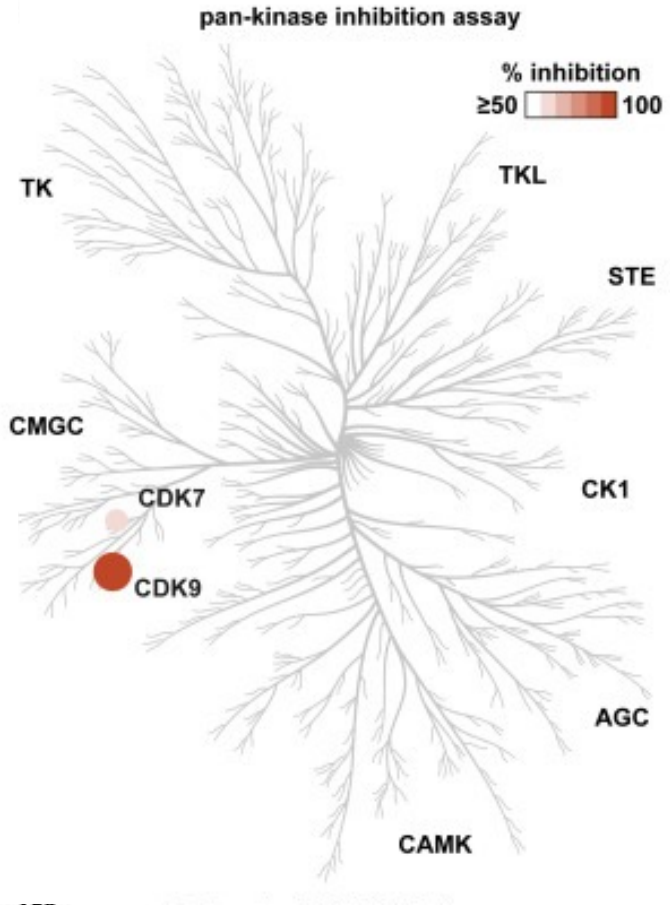

| assay | cell line | $IC_{50}$ (µM) | |
|---|---|---|---|
| ARv driven PSA reporter 24h | LNCaP | 7.6 | mutAR+ |
| Cell viability cell-titer glo 72h | LNCaP | 7.7 | mutAR+ |
| | VCaP | 7.9 | AR+ |
| | DU145 | 9.3 | AR- |
| | PC3 | 17 | AR- |

*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

Enzyme activity,
not binding




% 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)

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10 µM KI-ARv-03
[ATP] -/+ 15 µM
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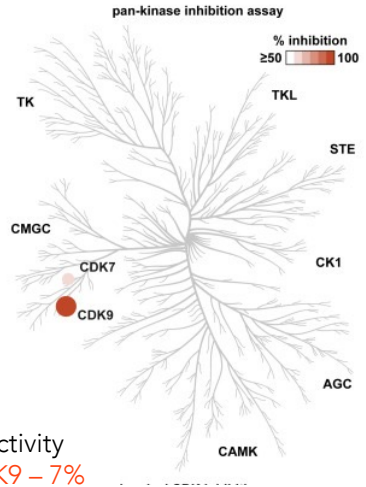
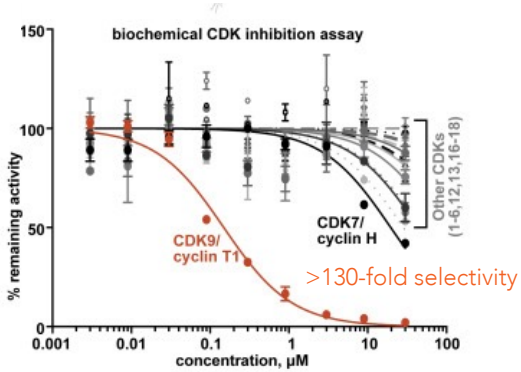



% activity
CDK9 – 7%
CDK7 – 47%

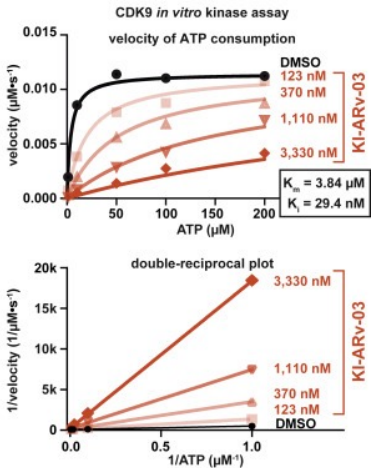
13 cyclin-dependent kinases (CDKs) in 10-pt dose



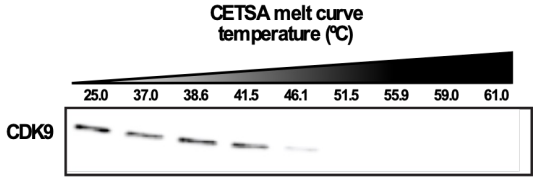

>130-fold selectivity

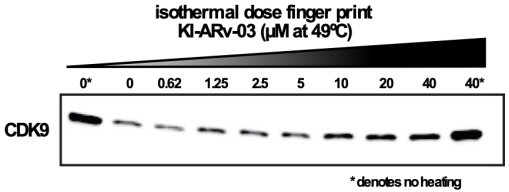
CDK9 $IC_{50}$ = 0.15 µM
CDK7 $IC_{50}$ = 20.1 µM

Michaelis-Menten and Lineweaver-Burk




ATP competitive



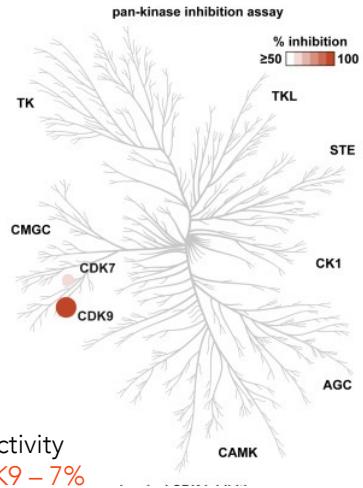




stabilization in live 22RV1 cells

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

# Kinase selectivity profiling suggests CDK9 as a target

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

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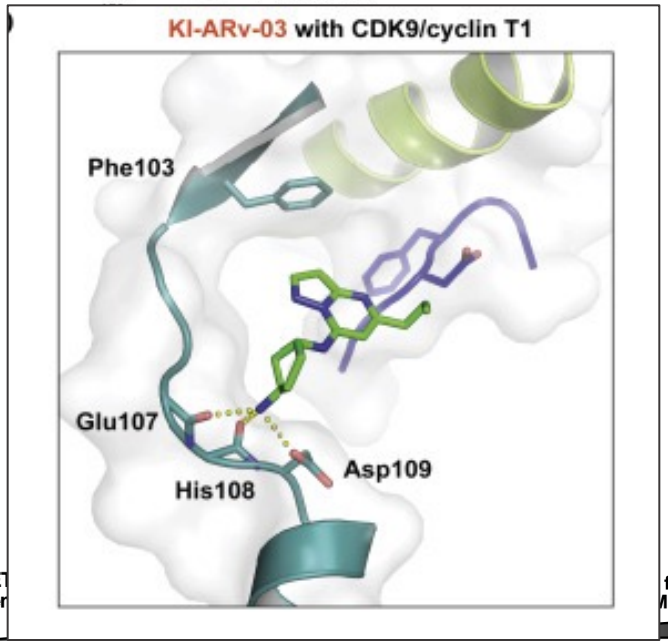
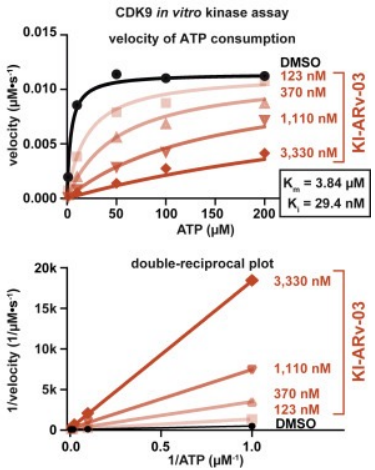
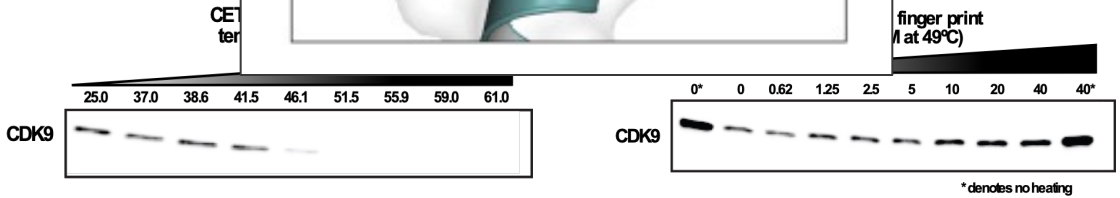



Michaelis-Menten and Lineweaver-Burk




ATP competitive

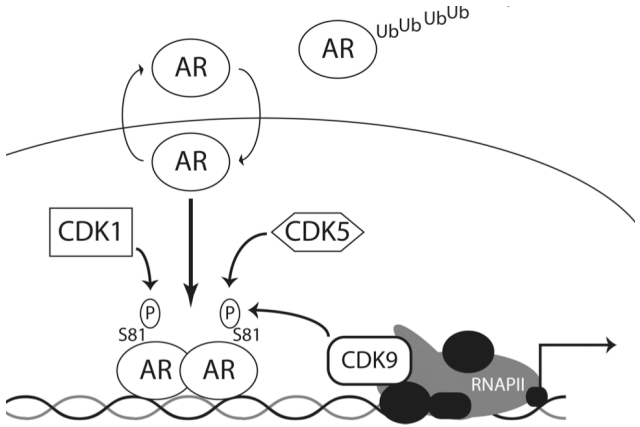



stabilization in live 22RV1 cells

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

# CDK9 is a known regulator of AR/ARV species activity

CDK9 regulates AR and ARV activity, stability through N-terminal S81



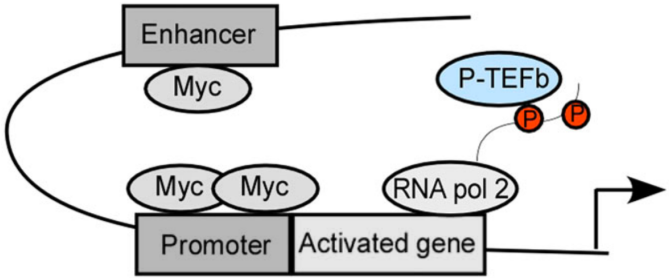

Koryakina, Y., Ta, H. Q., and Gioeli, D. (2014) Endocr. Relat. Cancer 21, T131–45.

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 + cyclinT1 = P-TEFb**




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

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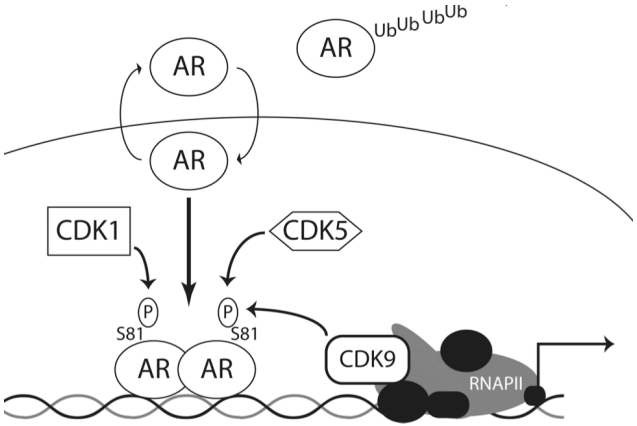
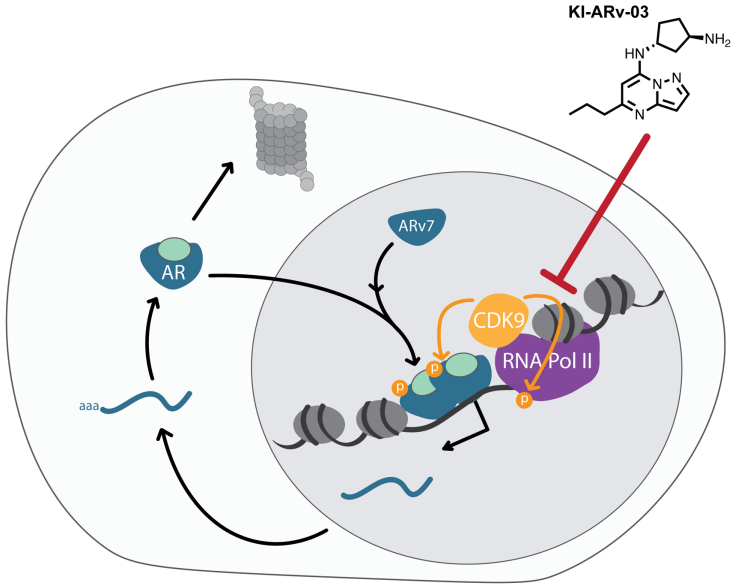


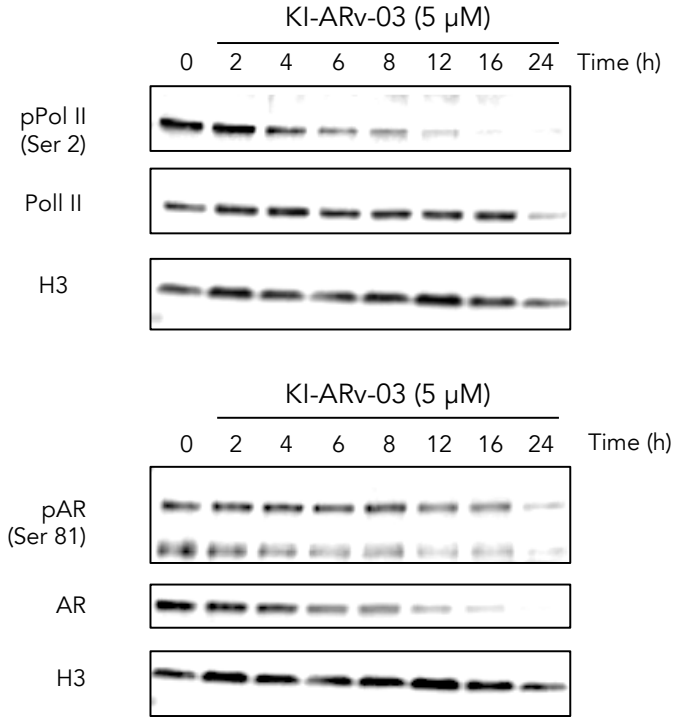

Koryakina, Y., Ta, H. Q., and Gioeli, D. (2014) Endocr. Relat. Cancer 21, T131–45

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



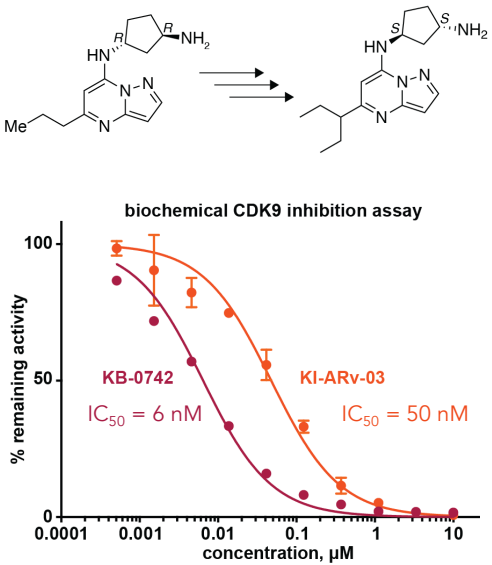




Richters et al., *Cell Chem Biol*, 28, 134-147 (2021)

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

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

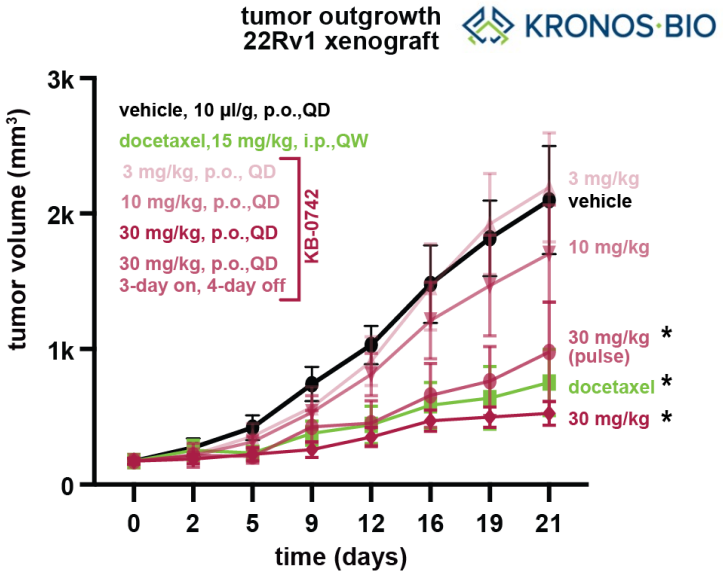
**KB-0742**
molecular weight: 287.4
cLogP: 1.97




| | KB-0742 | KI-ARv-03 |
|---|---|---|
| CDK9 | $IC_{50}$ = 6 nM | $IC_{50}$ = 50 nM |
| 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 |

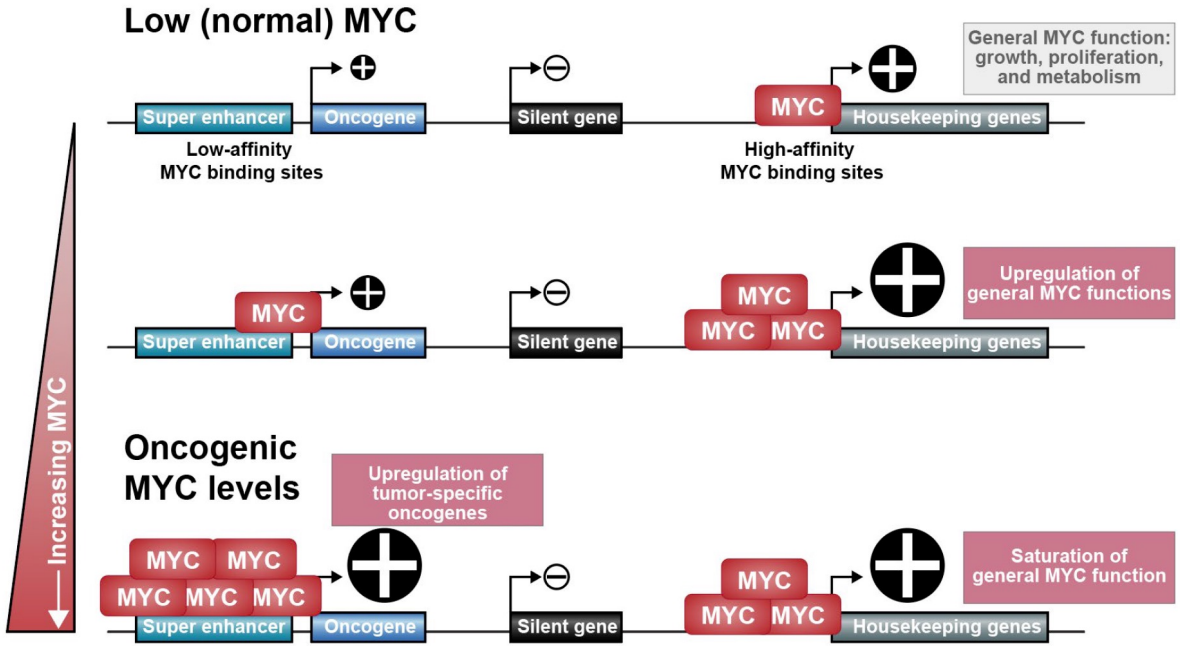
Comparison of relative biochemical $IC_{50}$

CDK9 fold selectivity vs.
1x >200x




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

# From L3: Cancers dysregulate MYC by increasing its expression



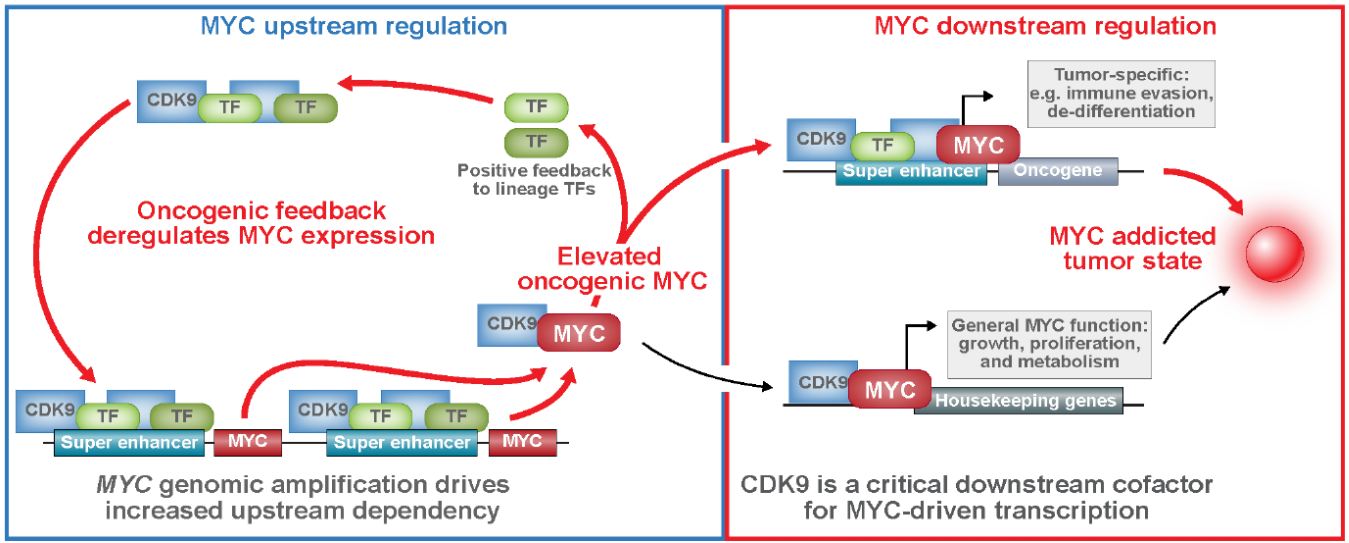

in typical cells, steady state *MYC* levels regulate general housekeeping functions

*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

Adapted from Wolf et al., *Trends Cell Biol*, 25, 241-248 (2015)

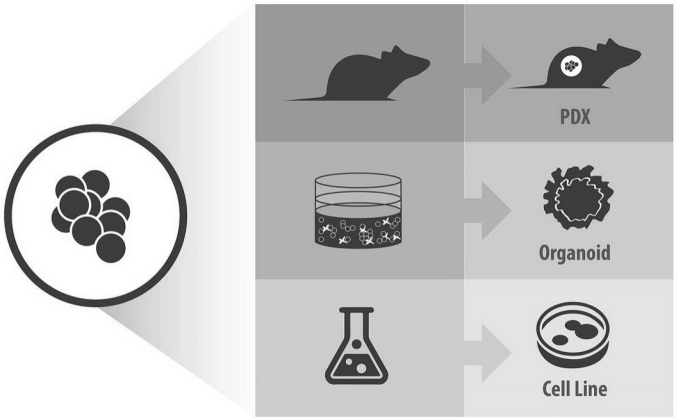
# Dependence on persistently high MYC expression creates a vulnerability to CDK9 inhibition




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

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

## organoid model description

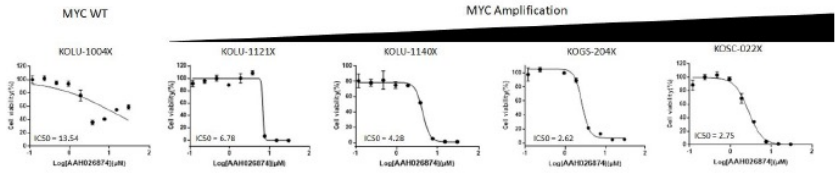
| Model Number | Indication | Treatment history | MYC TPM |
|---|---|---|---|
| KOLU-045 | Small Cell Lung Cancer | Naïve | 70 |
| KOLU-299 | | Naïve | 30 |
| KOLU-448 | | Lobaplatin+Etoposide | 30 |
| KOLU-775H | | Cisplatin | 20 |
| KOLU-545H | | VP16+Lobaplatin | 68 |
| KOLU-643H | | VP16 + Lobaplatin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

## 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 | | | 31.56 | 59.99 | 100.00 |
| KOBR-472 | | | No effect | 15.06 | 89.00 |

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




$IC_{50}$ = 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

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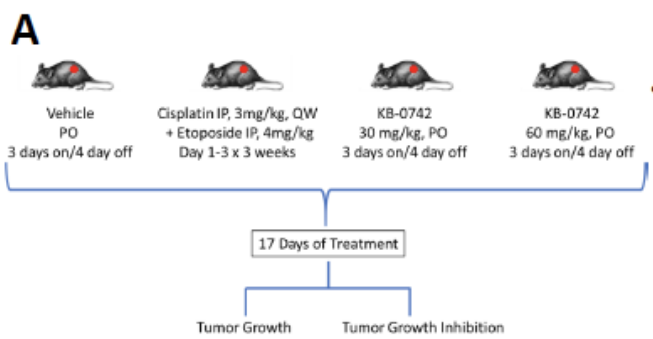
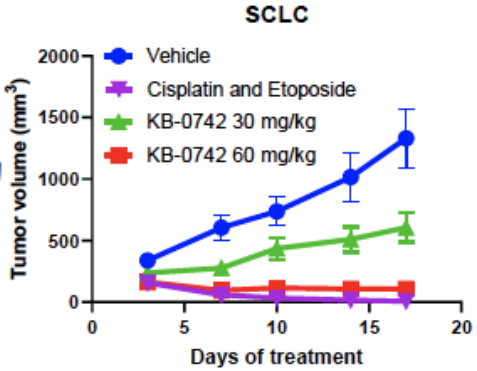
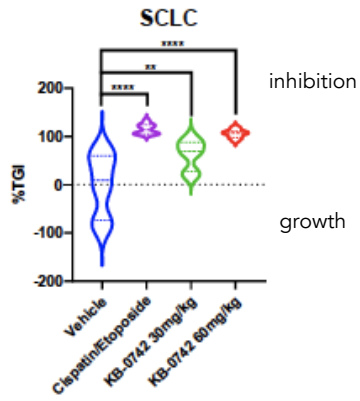
# KB-0742 shows anti-tumor activity in patient-derived xenografts (PDX)

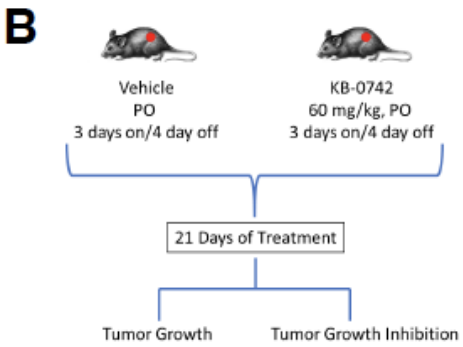
Intermittent dosing in 'MYC high' expressing murine PDX models

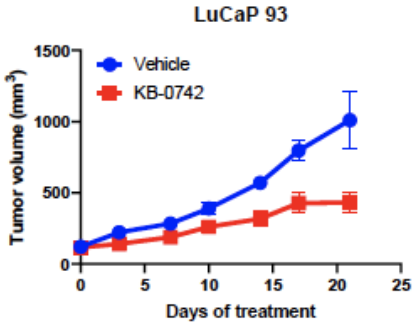
subcutaneous engraftment of tumor cells + treatment



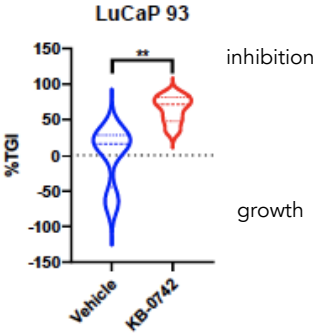




tumor volume changes




tumor growth inhibition

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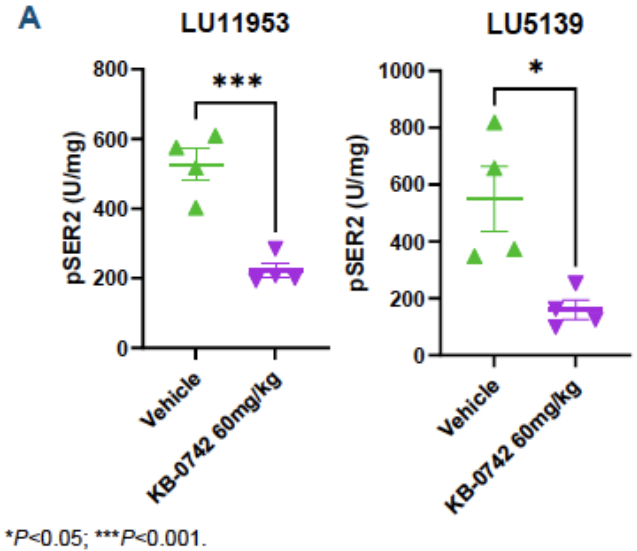
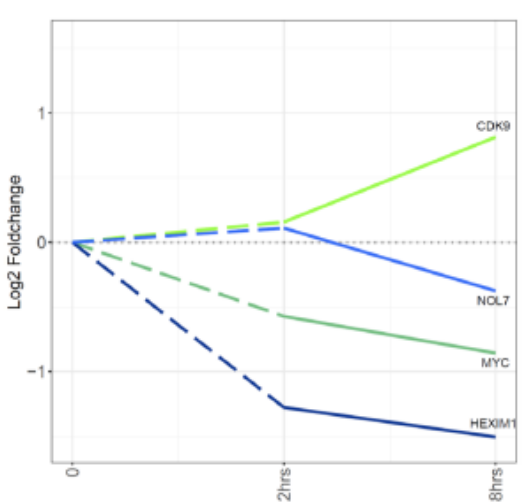
# Modulating a biomarker in vivo – small cell lung cancer PDX models

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*

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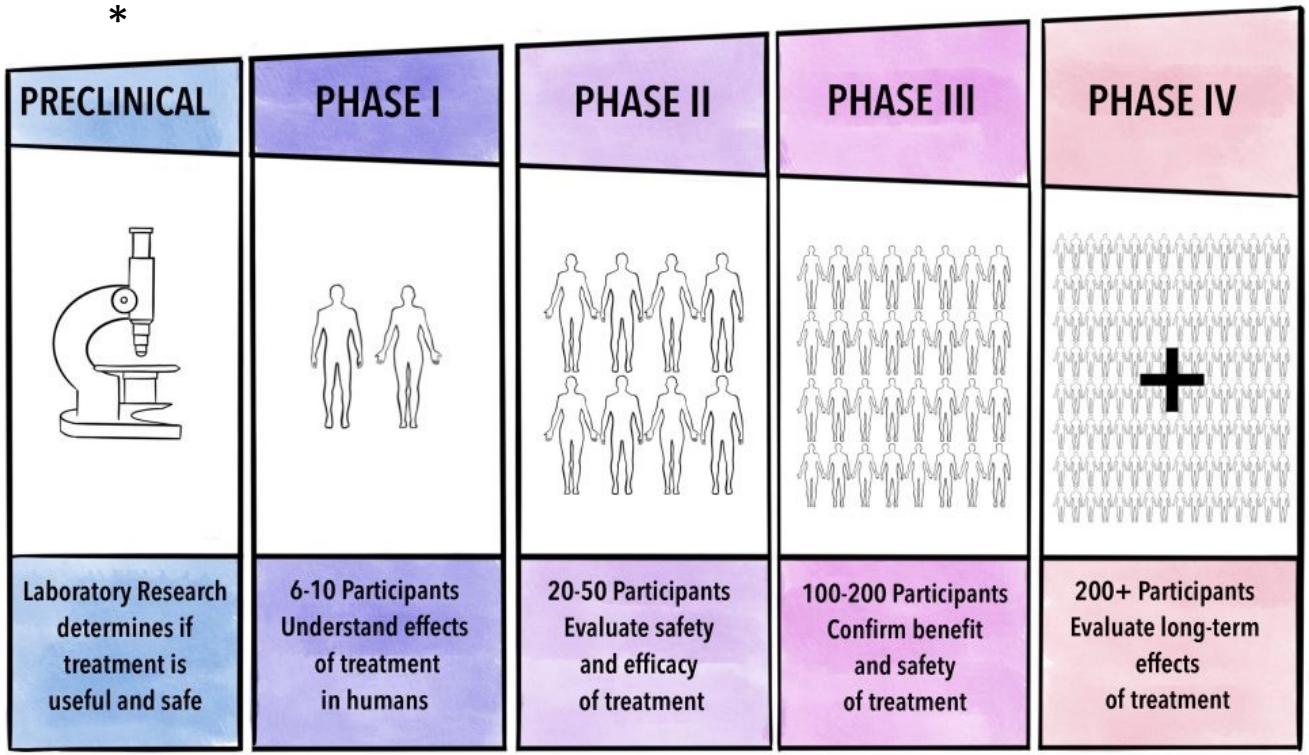
target engagement also observed in ovarian and TNBC models

# Clinical Trials

*



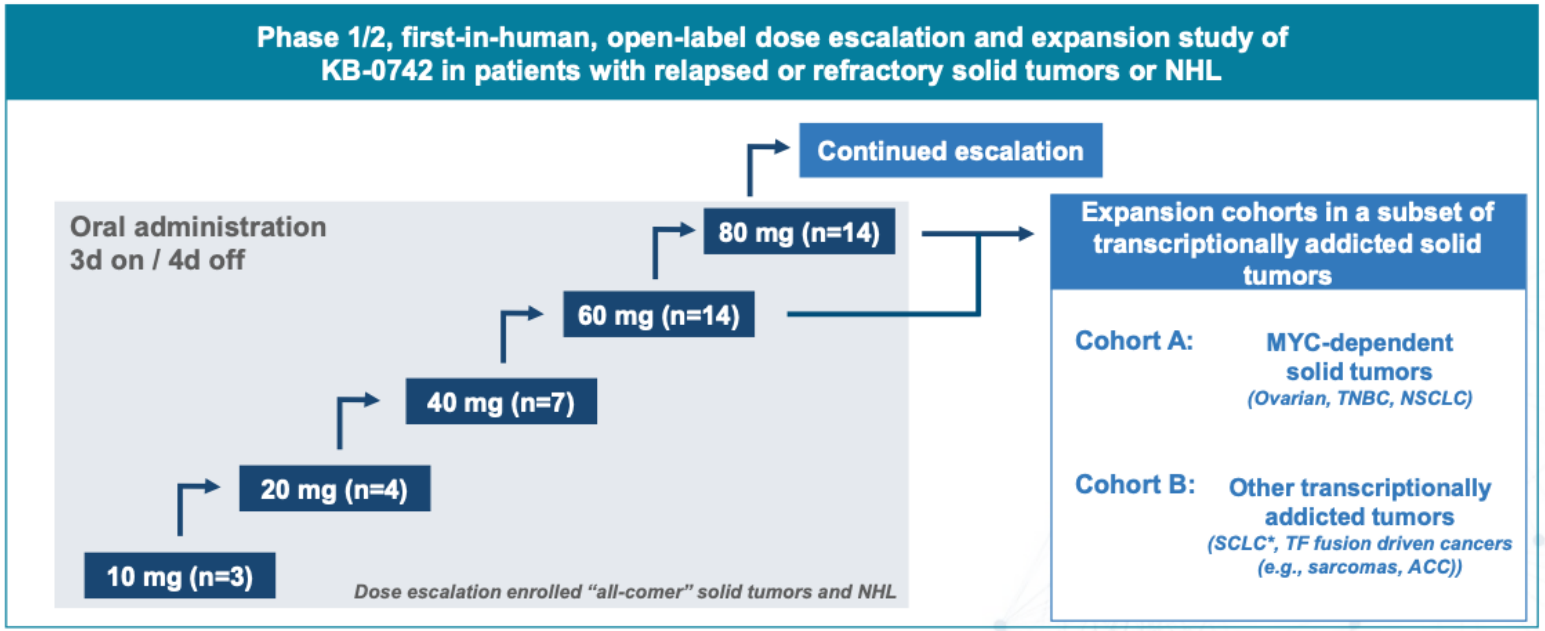

Is it safe?
Best way to dose?

Do patients have a response?
Still safe in larger cohort?

Better than a standard of treatment?
Survival? Side effects?

Side effects over time?
Contraindications?

# KB-0742 Clinical Trial Design- Combined Phase 1 & 2



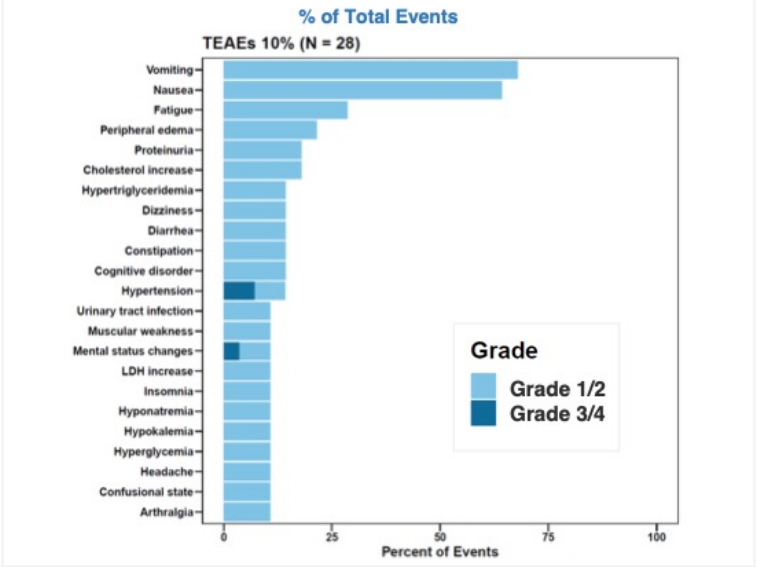

ACC: adenoid cystic carcinoma. NHL: Non-Hodgkin lymphoma; NSCLC: non-small cell lung cancer. SCLC: small cell lung cancer. TF: transcription factor. TNBC: triple negative breast cancer.

*SCLC is also MYC-dependent

Ongoing enrollment

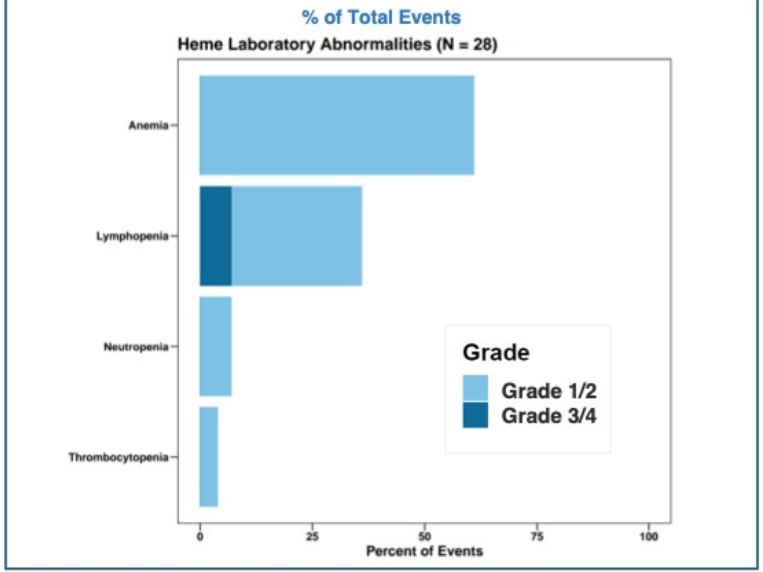
# KB-0742 Safety Profile: few adverse effects

## Most common TEAES (occurring in ≥ 10% of patients)



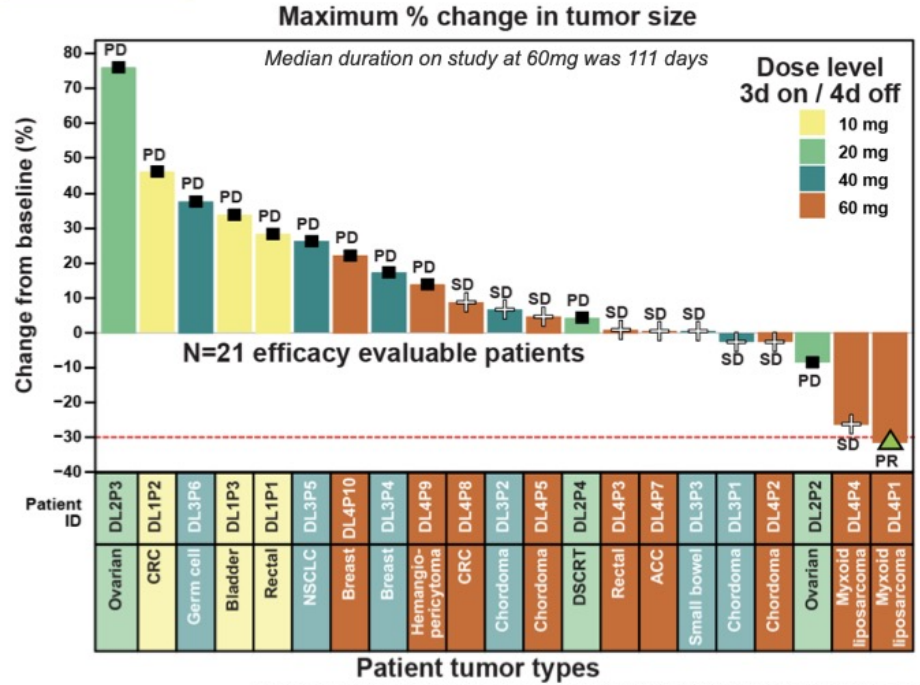

TEAEs: Treatment Emergent Adverse Events

## Hematologic laboratory abnormalities (based on NCI CTCAE grading)




Source: A First-In-Human Study of CDK9 Inhibitor KB-0742 Demonstrates Evidence of Tolerability and Clinical Activity [Poster Presentation]. 2023 EORTC-NCI-AACR (Villalona-Calero, Miguel, et al.), Boston and 2023 CTOS, Dublin (Van Tine, Brian, et al.)

# KB-0742 anti-tumor activity: objective regressions in two patients




**Tumors with TF fusions**

**Oncogenic TF** = **Fusion TF**

**TF#1** **TF#2**

| Example tumor type | TF#1 | TF#2 |
|---|---|---|
| Ewing sarcoma | EWSR1 | FLI1 |
| | FUS | ERG |
| Myoxoid liposarcoma | DDIT3 | FUS |
| Adenoid cystic carcinoma | MYB | NFIB |
| Alveolar rhabdomyosarcoma | PAX3 | FOXO1 |
| | PAX7 | FOXO1 |

- One partial response lasting 113 days in a 7th line myxoid liposarcoma patient. Second patient achieved 26% reduction in tumor diameters.
- 9 (43%) patients had stable disease (SD) as the best response.
- Overall disease control rate was 47.8% - defined as a CR (complete response), partial response (PR), or stable disease (SD).

Source: A First-In-Human Study of CDK9 Inhibitor KB-0742 Demonstrates Evidence of Tolerability and Clinical Activity [Poster Presentation]. 2023 EORTC-NCI-AACR (Villalona-Calero, Miguel, et al.), Boston and 2023 CTOS, Dublin (Van Tine, Brian, et al.)

# Case Report: Patient DL4P1 with myxoid liposarcoma achieved partial response at cycle 10 (60 mg dose)

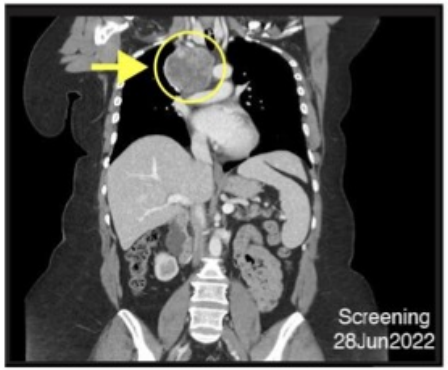
## Patient characteristics and treatment history

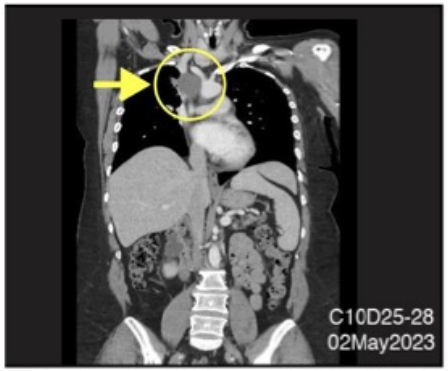
- 50-year-old female
- Diagnosed with myxoid liposarcoma in MAY 2009
- Stage 4 at enrollment
- Six prior lines of therapy and best overall response included ():
  - Adriamycin/Ifosfamide: APR-SEP 2015 (PD)
  - Atezolizumab: JUL-SEP 2016 (PD)
  - Trabectedin: DEC 2016-JAN 2017 (PD)
  - NY-ESO-1C259 T: SEP 2017-JUN 2018 (SD)
  - Atezolizumab: NOV 2018-JUN 2019 (SD)
  - Ifosfamide: DEC 2021-JAN 2022 (SD)

## KB-0742 treatment course

- KB-0742 treatment initiated in JUL 2022
- 60 mg for 398 days on treatment
- PR achieved at cycle 10 lasting 113 days

PR: partial response. SD: stable disease.




Reference: https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.3005

Brian Van Tine, M.D., Ph.D.

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Article | Open Access | Published: 13 May 2022

## MYC drives aggressive prostate cancer by disrupting transcriptional pause release at androgen receptor targets

Xintao Qiu, Nadia Boufaied, Tarek Hallal, Avery Feit, Anna de Polo, Adrienne M. Luoma, Walaa Alahmadi, Janie Larocque, Giorgia Zadra, Yingtian Xie, Shengqing Gu, Qin Tang, Yi Zhang, Sudeepa Syamala, Ji-Heui Seo, Connor Bell, Edward O'Connor, Yang Liu, Edward M. Schaeffer, R. Jeffrey Karnes, Sheila Weinmann, Elai Davicioni, Colm Morrissey, Paloma Cejas, ... David P. Labbé + Show authors

*Nature Communications* **13**, Article number: 2559 (2022) | Cite this article

**6425** Accesses | **14** Citations | **31** Altmetric | Metrics

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## Androgen receptor and MYC equilibration centralizes on developmental super-enhancer

Haiyang Guo, Yiming Wu, Mannan Nouri, Sandor Spisak, Joshua W. Russo, Adam G. Sowalsky, Mark M. Pomerantz, Zhao Wei, Keegan Korthauer, Ji-Heui Seo, Liyang Wang, Seiji Arai, Matthew L. Freedman, Housheng Hansen He, Shaoyong Chen & Steven P. Balk

*Nature Communications* **12**, Article number: 7308 (2021) | Cite this article

**5677** Accesses | **12** Citations | **15** Altmetric | Metrics

**ORIGINAL ARTICLES UROGENITAL TUMORS** | VOLUME 26, ISSUE 11, P2293-2300, NOVEMBER 2015

## High burden of copy number alterations and *c-MYC* amplification in prostate cancer from *BRCA2* germline mutation carriers

E. Castro • S. Jugurnauth-Little • Q. Karlsson • ... R. Eeles † • Z. Kote-Jarai † • on behalf of the UKGPCS, EMBRACE and IMPACT studies • Show all authors • Show footnotes

Open Archive • DOI: https://doi.org/10.1093/annonc/mdv356

Prostate Cancer Foundation

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# Upcoming Lectures

| | | |
|---|---|---|
| 2/8/24 | Lecture 1 | Intro to chemical biology: small molecules, probes, and screens |
| 2/13/24 | No Lecture | Snow Day |
| 2/15/24 | Lecture 2 | Small Molecule Microarrays |
| 2/20/24 | No Lecture | |
| 2/22/24 | Lecture 3 | Our protein target – MAX |
| 2/27/24 | Lecture 4 | Quantitative evaluation of protein-ligand interactions |
| 2/29/24 | Lecture 5 | KB-0742: A Phase 2 clinical candidate discovered by SMMs |
| 3/5/23 | Lecture 6 | Wrap up discussion for Mod 1 experiments and report |