NARAYANA
MEDICAL COLLEGE

Narayana Translational Research Centre

Invites you for an International Webinar on

"PKN1 - Alternative Target in Advanced Prostate Cancer"

Thursday, 7th January, 2021; 10 am to 11 am

By

Dr. Varadha Balaji Venkadakrishnan, Ph.D.

Department of Medical Oncology,

Dana-Farber Cancer Institute and Research Fellow at

Harvard Medical School, Boston, Massachusetts, USA

Patron

Dr. Surya Prakasa Rao, MD
Professor and Dean,
Narayana Medical College,
Andhra Pradesh, India.

Convenor

Dr. Sivakumar Vijayaraghavalu, Ph.D.
Professor and Head, NTRC,
Mobile - +91 8925744196;
drvshiva@narayanamedicalcollege.com

Registrants Profile – In a total of 190 registrants – 93% were from India and 7% from foreign countries such as USA, Saudi Arabia, Bahrain, Japan and Sweden. Indian nationals were mostly from the state of Andhra Pradesh (88%) and rest 12% are from the following states – Tamil Nadu, Karnataka, Sikkim and Kerala.

The international webinar started with greeting notes from Convenor – Dr. Sivakumar Vijayaraghavalu to all the online participants and invited them on behalf of Narayana Medical College, Nellore, India and honorable dean – Dr. Surya Prakasa Rao. He then started to introduce the speaker as follows - Today's speaker is the youngest of all the speaker we had so far in our forum. We are privileged and honored to have a young scientist – Dr. Varadha Balaji Venkadakrishnan (Balaji) from world renowned cancer center and medical school- *Dana-Farber Cancer Institute and Harvard Medical School,* Boston, Massachusetts, USA. For some of our students, would like to highlight that Dana-Farber Cancer Institute is one of the world very best cancer hospitals by Newsweek in its new World's Best Specialized Hospitals in 2020-2021 rankings and ranks 3rd in the world. Then he told that Harvard Medical School ranks number ONE among

medical schools in the world, University of Oxford and University of Cambridge from United Kingdom are at 2nd and 3rd positions.

Further, he continued the speaker introduction saying that he knows Dr. Balaji from the time he was doing PhD in department of Cancer Biology, Cleveland Clinic Foundation (CCF), Cleveland, Ohio, USA. CCF is also one of the world renowned and top most hospitals. Dr. Balaji was well nurtured in science by great physicians and scientists like Dr. Nima Sharifi – US presidential awardee in the field of prostate cancer from dept. of cancer biology, CCF; as well by other great scientists such as - Dr. Warren Heston, inventor of PSMA (diagnostic test for prostate cancer/prostitis), George Stark (western blot technique inventor) and Robert Silverman (well-known interferon researcher)– are the pioneers in the field. He was under the sphere of influence

of all these great scientists. He by himself is an intellect and has fire in his belly to achieve something great, we use to have science café at the end of each day for an hour discussing the day to day experiments and data. I was astonished by the knowledge, meticulous thinking and approach of Dr. Balaji towards science. I do not want to take much of the time. I will brief his CV with few sentences and then I will request Dr. Balaji to take over the session.

As mentioned earlier – Dr. Balaji obtained his PhD from Dept. of Cancer Biology, CCF. He is currently a post-doctoral fellow at Dana-Farber Cancer Institute and Research fellow in Harvard Medical School. He did his engineering in Biotechnology from Anna University, a prestigious university in India. He also worked as project assistant in – Indian Institute of Technology, Bombay, India. Advanced Centre for Treatment, Research and Education in Cancer, Bombay, India. For doing his

PhD he got graduate student research award and his PhD thesis publication was one of the best and he received the Graduate Student Award for Excellence. He won first place in three-minute thesis competition where he has to summarize his research to a lay audience crisply in three minutes. This shows his ability to communicate a complicated topic like prostate cancer to common public comprehensively. He represented his school at the regional level and finished second in the regional competition. His ability to get educated in the indicated world's top institutions and working in the most prestigious institutions shows his competency. We believe that his quest for finding answers to the problems pertaining to the prostate cancer will benefit the mankind; further the knowhow knowledge from his research outcome could be helpful to understand the underlying mechanism in other cancer(s). The convenor once again told that their institution is honored to have Dr. Balaji as one of the speakers in their forum and requested him to take over the session. Dr. Balaji started his talk by thanking the convenor for the introduction,

participants for their interest in his talk and the organization for giving him an opportunity. He started his presentation with describing the anatomy and physiology of prostate gland in brief and then talked about the global cancer incidence and mortality. He also shown the projected incidence of cancer statistics in India of the year 2020. The Indian map with comparison of cancer incidence in 1990 vs. 2020 was shown and explained the steep raise in number of cancer cases in almost all the states of India.

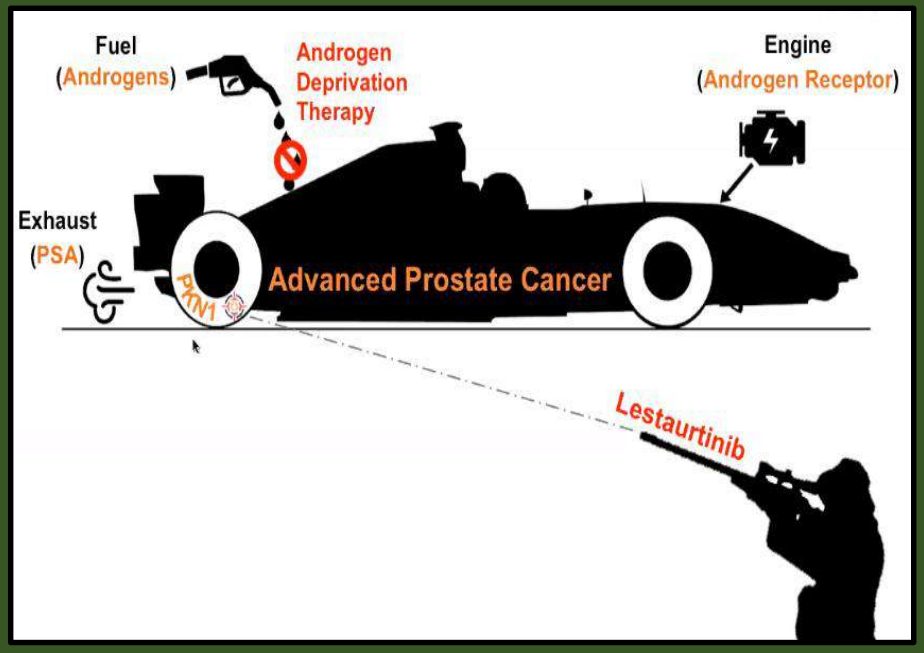
An interesting observation was that the Indian states with high literacy rate shown higher incidence on comparison with states with poor literacy rate (figures below). For example, the crude cancer incidence rate in Kerala is 135.3/100,000 inhabitants and it ranks top in the table,

interestingly it is also second most literate state (96%) in India. Similarly, Mizoram ranks second in cancer incidence (121.7/100,000 inhabitants) and third in the literacy rate (91%) in India.

Many other states in India with high literacy rates show similar trend. However, Tripura is the exception, an Indian state with highest literacy (97%) and low cancer incidence (69/100,000 inhabitants) on comparison with other Indian states that tops the literacy table.

Of all the states in India Bihar has the lowest cancer incidence with 53.9/100,000 inhabitants and has the lower literacy rate (71%) on comparison with the Indian states mentioned above. Another example is Manipur, which occupies second last position in the cancer incidence table with 64.3/100,000 inhabitants and with the literacy rate of 77%. Jharkhand another Indian state with literacy rate of 68% also shares the same rank in cancer incidence with Manipur. These statistics

pose a challenge – to find a correlation between the cancer incidence and literacy rate among the Indian population. He then spoke about the incidence and prevalence of prostate cancer and therapeutic strategies to address it with an illustration presented below. He told that prostate cancer responds initially to Androgen Deprivation Therapy (ADT), with time cancer resistance builds and eventually develops into castration resistant prostate cancer (CRPC). He then started to explain the molecular mechanism and the need for new targeted therapies to address the castration – sensitive or –resistant prostate cancers. Prostate – specific antigen (PSA), an AR target gene is a biomarker used to monitor disease progression, because it plays a crucial role in disease advancement. He used this figure



as analogy to explain the disease progression easily to the audience and it received high appreciation from them.

In this illustration, if one considers advanced prostate cancer as the race car moving at high speed, the fuel for its progression is androgens and the car engine propelling it is Androgen Receptor (AR). The tire of the car is - protein kinase N1 (PKN1), which belongs to the PKC superfamily; it is activated by Rho family of small G proteins and may mediate the Rho-dependent signaling pathway. PKN1 is involved in various processes such as actin cytoskeletal organization, cell migration, tumor cell invasion and transcriptional regulation. It also acts as a key coactivator of androgen receptor (ANDR) –dependent transcription and mediates Thr-11 phosphorylation which in-turn serves as tag to cellular machinery, to demethylate histone H3 'Lys-9'. Since the Rho cannot be targeted which is like axel of the car, PKN1 is an indirect target to slow down or stopping the car using the drug –lestaurtinib (like a bullet from the gun). He then explained about the

mechanism in which Rho is mediating the cancer progression and the way and means he used to find the effector molecules that can be targeted for therapeutic purpose. From various molecular experiments the molecule chosen was PKN1. He found that androgens enhance the PKN1-SRF interaction and advances the cancer. The PKN1 is overexpressed in CRPCs and lead to poorer survival of the organism. In contrast, inhibition of PKN1 decreases the cancer cell viability, thus making it as suitable candidate for targeted chemotherapy. The speaker concluded his talk by thanking the participants for patiently listening to him, a lively Q & A session followed it. Then the convenor ended the webinar by thanking the speaker, participants, organization and the dean.

The screen shots of selected PPT slides from his presentation is given below.

PKN1 – An alternative therapeutic target in advanced prostate cancer

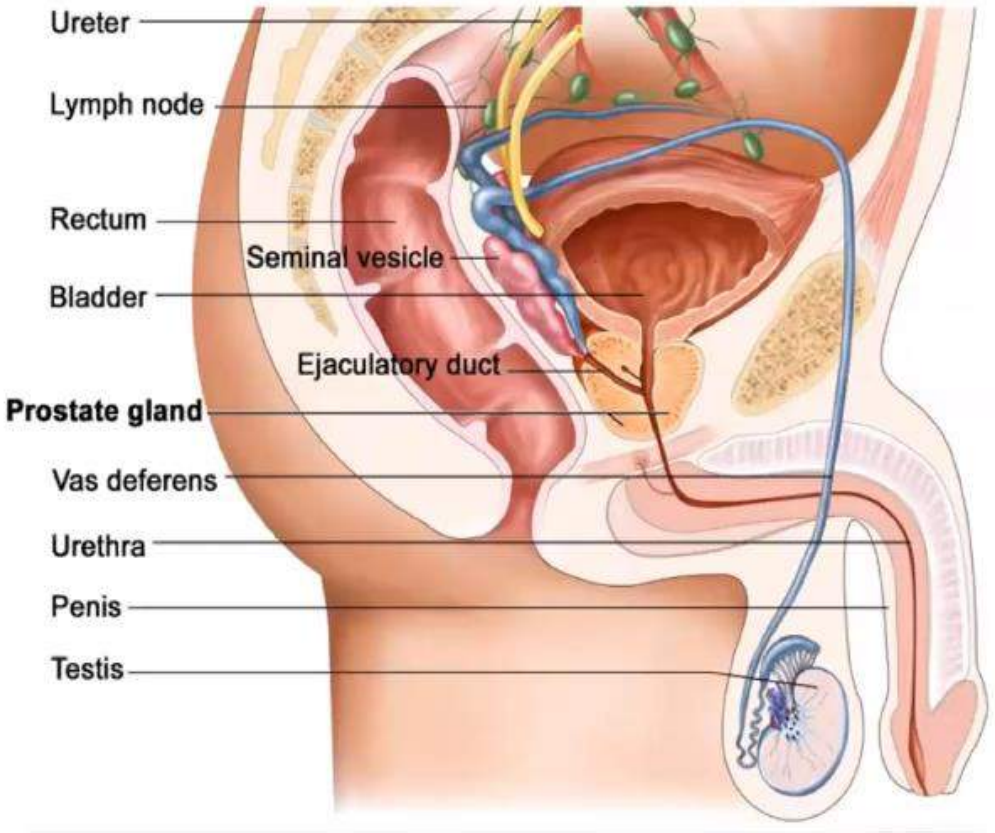
Varadha Balaji Venkadakrishnan

Graduate Student
Department of Biological Geological and Environmental Sciences
Cleveland State University

Dr. Hannelore Heemers' Lab
Department of Cancer Biology
Lerner Research Institute, Cleveland Clinic

Prostate

- A walnut-sized gland part of the male genitourinary system.
- Found below the bladder, surrounds upper urethra
- Contributes up to 30% of the volume of semen.
- Prostate is composed of stromal and epithelial tissue. Three major epithelial cell types: luminal, basal, and neuroendocrine.
- Diseases include inflammation, infection, and cancer.



Cancer Statistics

Estimated New Cases

Males

Prostate	191,930	21%
Lung & bronchus	116,300	13%
Colon & rectum	78,300	9%
Urinary bladder	62,100	7%
Melanoma of the skin	60,190	7%
Kidney & renal pelvis	45,520	5%
Non-Hodgkin lymphoma	42,380	5%
Oral cavity & pharynx	38,380	4%
Leukemia	35,470	4%
Pancreas	30,400	3%
All Sites	**893,660**	**100%**

Estimated Deaths

Males

Lung & bronchus	72,500	23%
Prostate	33,330	10%
Colon & rectum	28,630	9%
Pancreas	24,640	8%
Liver & intrahepatic bile duct	20,020	6%
Leukemia	13,420	4%
Esophagus	13,100	4%
Urinary bladder	13,050	4%
Non-Hodgkin lymphoma	11,460	4%
Brain & other nervous system	10,190	3%
All Sites	**321,160**	**100%**

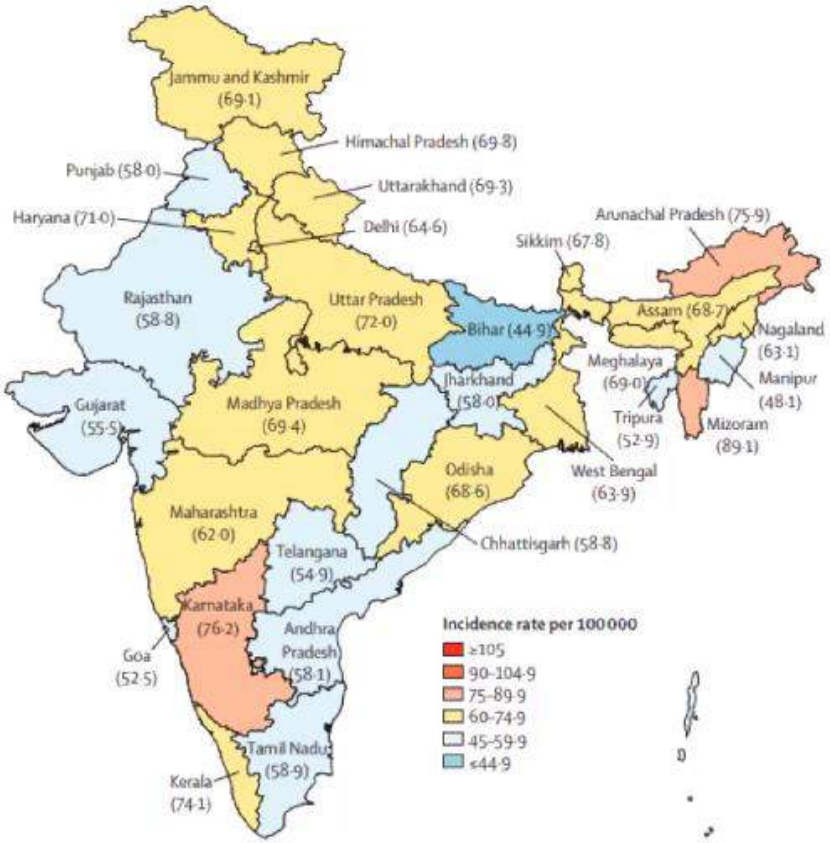
Projected cancer incidence for 2020 in India

TABLE 2. Projected Incidence of Cancer Statistics in India, 2020

Site	Male			Female		
	Patients	CR	Cum Risk	Patients	CR	Cum Risk
Genital system	51,994	7.2	1 in 105	155,630	22.6	1 in 36
Uterine cervix	—	—	—	75,209	10.9	1 in 75
Uterine corpus	—	—	—	26,514	3.9	1 in 190
Ovary	—	—	—	43,886	6.4	1 in 133
Vulva	—	—	—	2,138	0.3	1 in 2,459
Vagina and other genital, female	—	—	—	7,570	1.1	1 in 745
Placenta	—	—	—	313	0.0	1 in 30,912
Prostate	41,532	5.7	1 in 125	—	—	—
Testis	4,352	0.6	1 in 2,095	—	—	—
Penis and other genital, male	6,110	0.8	1 in 916	—	—	—

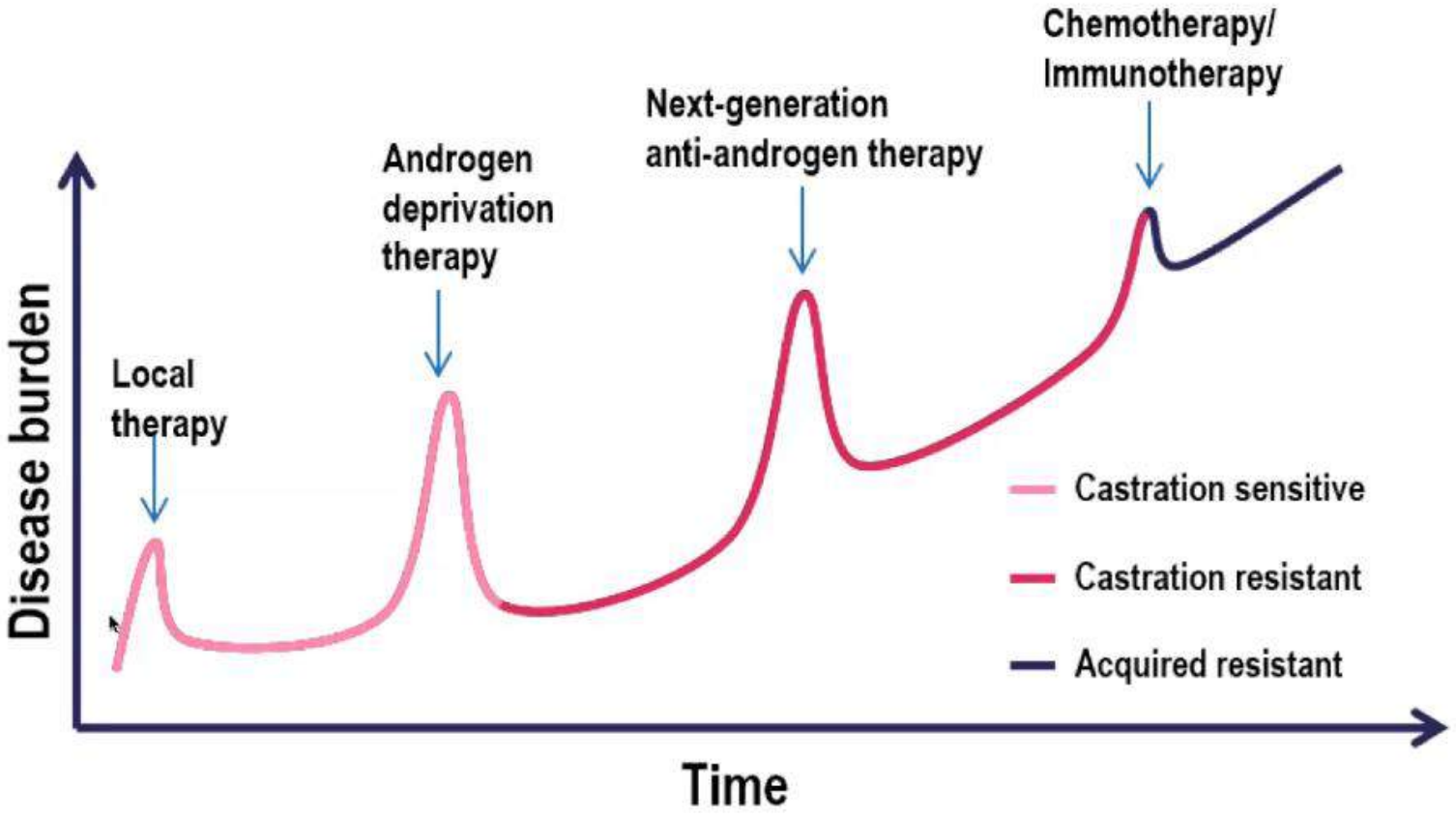
Projected cancer incidence for 2020 in India

1990



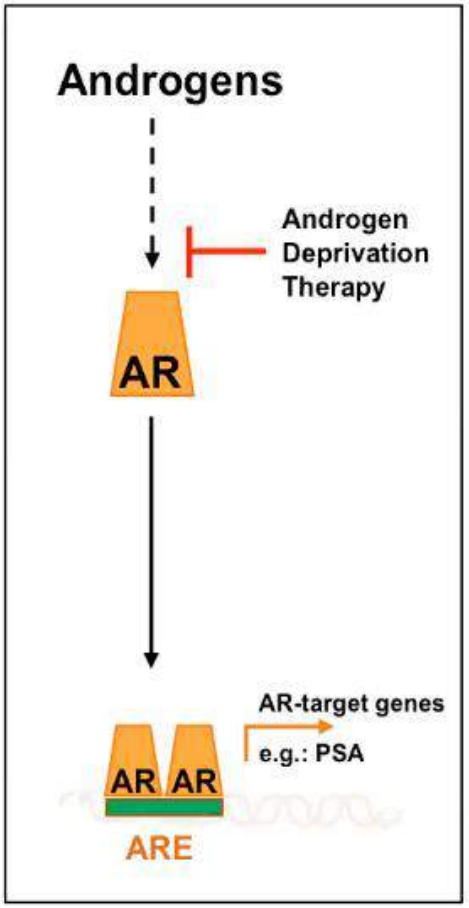
2016

Conventional treatment plan of an average patient



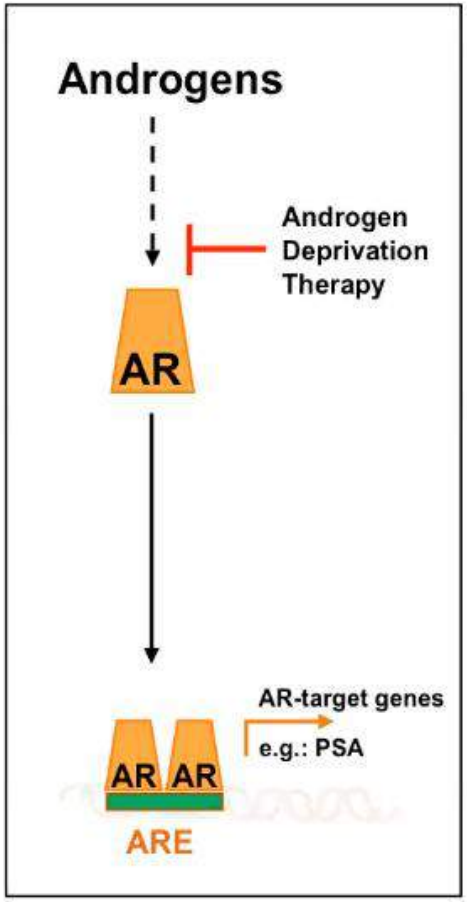
After initial responses from ADT, treatment eventually fails giving rise to castration-resistant prostate cancer (CRPC). Most CRPC still relies on androgen for progression.

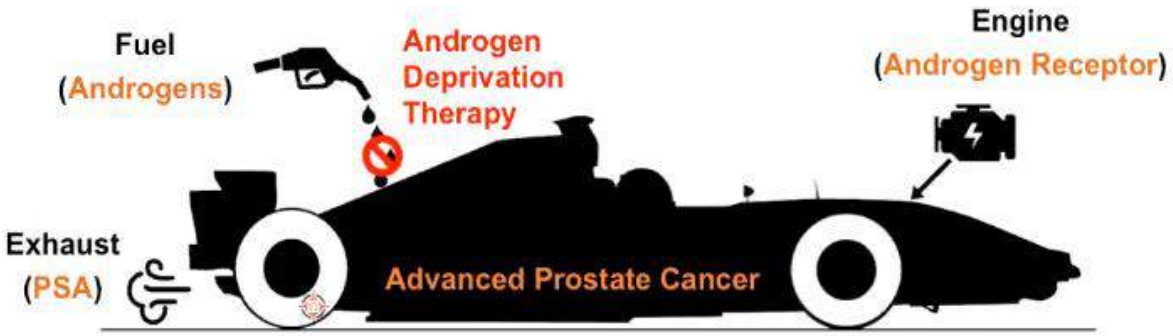
Canonical Androgen Receptor Signaling



- A ligand-regulated transcription factor.
- PSA, an AR-target gene is a biomarker used to monitor disease progression.
- Plays a central role in disease progression

Canonical Androgen Receptor Signaling





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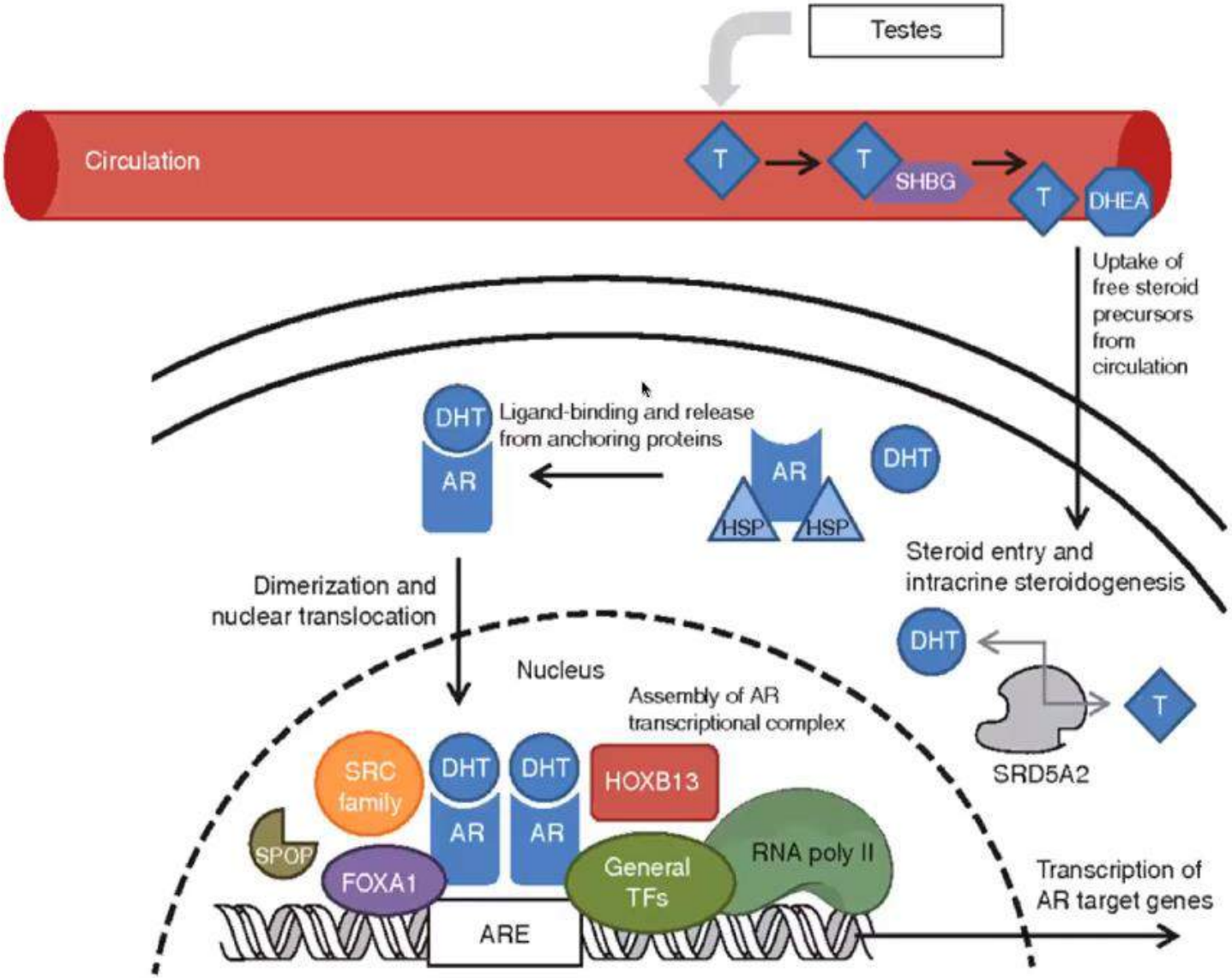
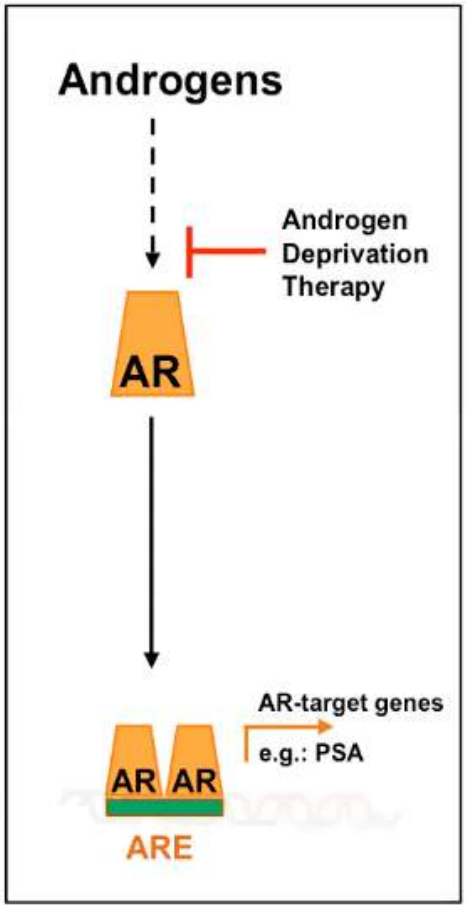
Canonical Androgen Receptor Signaling



Mechanisms of CRPC

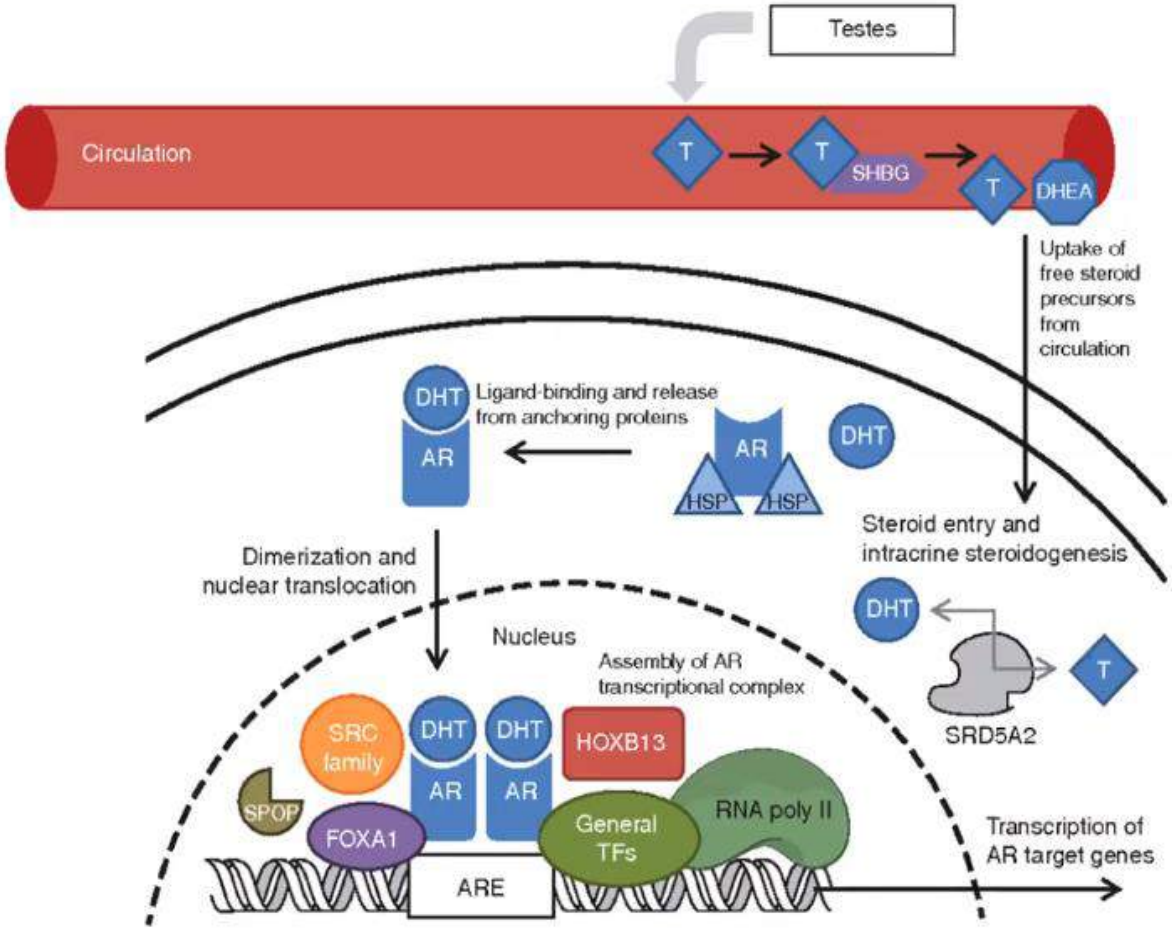


- AR Amplification
- AR Mutations
- AR variants
- Intratumoral androgen biosynthesis
- GR upregulation
- Dysregulation AR co-regulator action

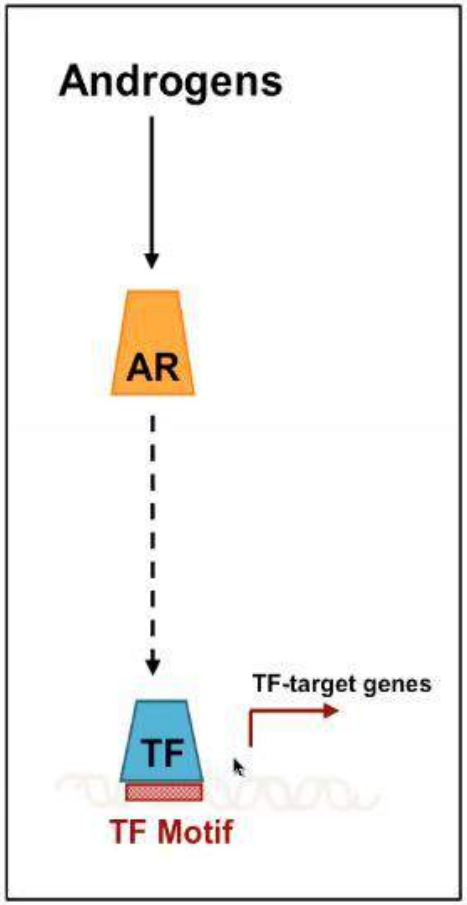
Non-canonical mechanisms of AR action

Novel mechanisms of AR signaling

Canonical AR action

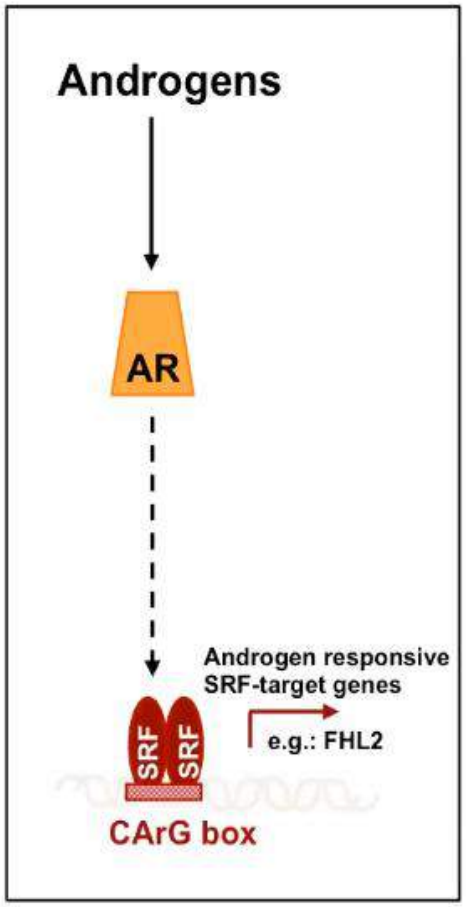


Non-canonical AR action



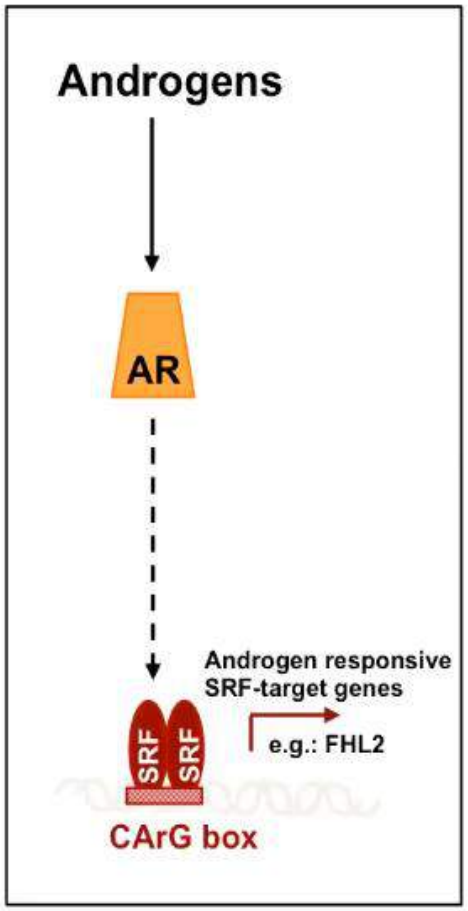
AR can directly or indirectly control other transcription factors

AR-SRF signaling



- Serum response factor (SRF) is an essential transcription factor
- Regulates immediate early response and cytoskeletal organization.
- Nuclear and constitutively bound to its consensus motif.

AR-SRF signaling



Tumor and Stem Cell Biology

Cancer Research

Identification of a Clinically Relevant Androgen-Dependent Gene Signature in Prostate Cancer

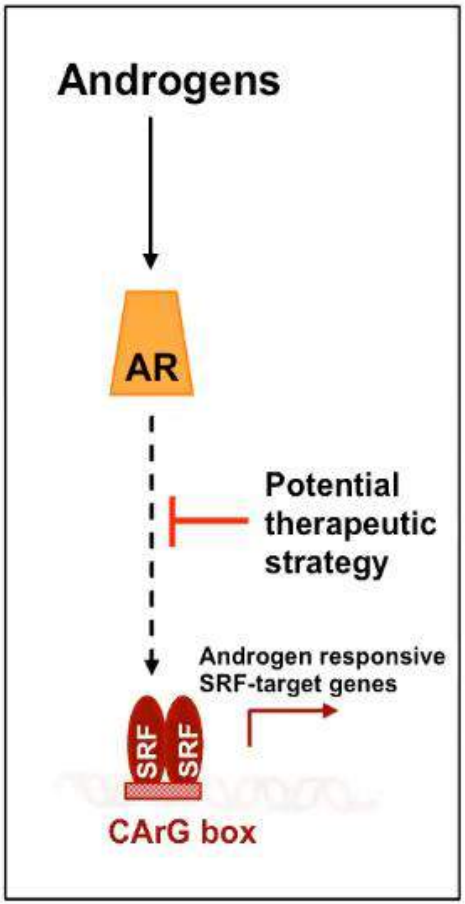
Hannelore V. Heemers[1], Lucy J. Schmidt[3], Zhifu Sun[4], Kevin M. Regan[3], S. Keith Anderson[5], Kelly Duncan[1], Dan Wang[2], Song Liu[2], Karla V. Ballman[5], and Donald J. Tindall[3]

- **AR-SRF gene signature was enriched in prostate cancer as compared to benign prostate.**
- **Correlated with biochemical recurrence.**
- **Pathway analysis revealed an associated with hallmarks of cancer**

Clinical significance of SRF signaling

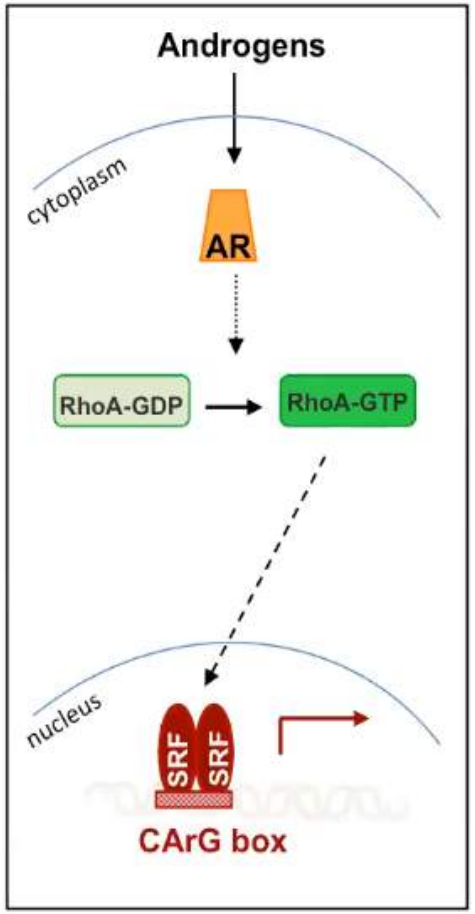
- **Prostate cancer**
  - Androgen response of SRF was verified.
  - Higher expression of SRF was associated with shorter biochemical recurrence and poorer survival.
  - In docetaxel-resistant prostate cancer models, SRF was isolated as a critical transcription factor mediating resistance.
- **Other cancers**
  - Implicated in breast cancer stemness
  - Implicated in melanoma therapy resistance.

AR-SRF signaling



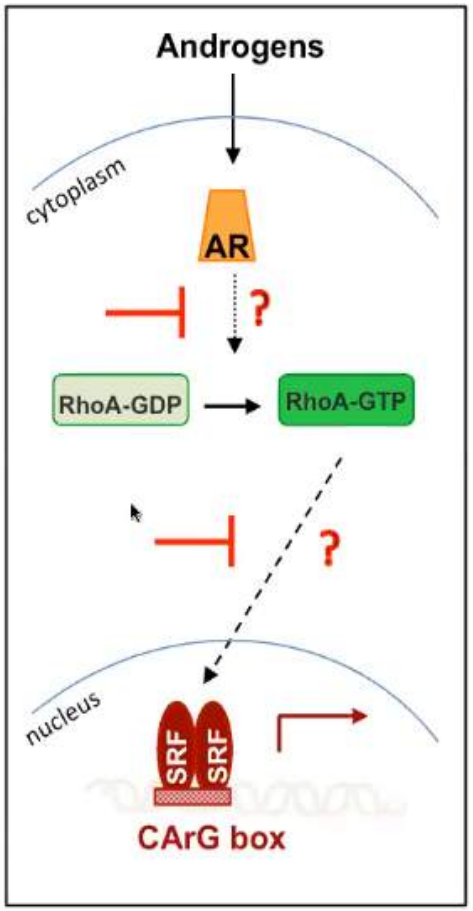
Targeting a critical fraction of AR action maybe an alternative therapeutic strategy

RhoA mediates AR-SRF signaling



- RhoA over-expression associated with poorer disease free-survival

RhoA mediates AR-SRF signaling



- RhoA is an essential regulator of several cellular processes
- Cannot be pharmacologically targeted.
- RhoA mediates its function via its effectors.

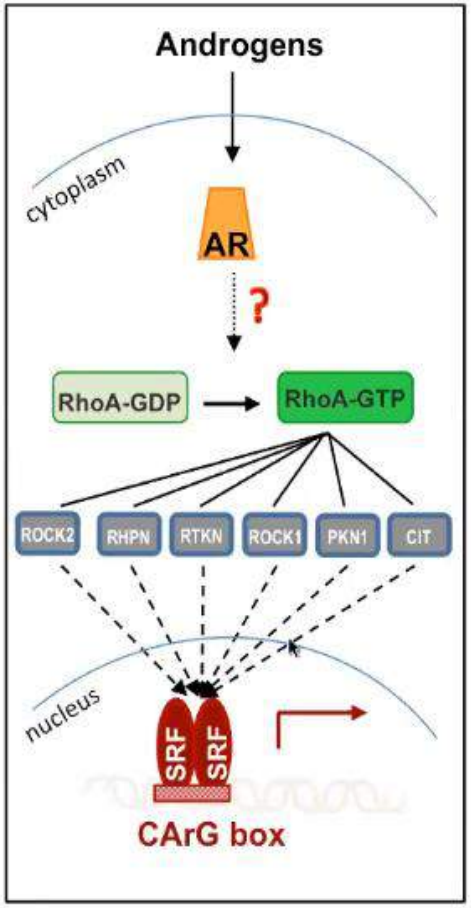
Hypothesis

The RhoA effector that confers AR-SRF signaling is a novel therapeutic target to block SRF-dependent AR action in advanced prostate cancer

1. **Mechanism of AR-RhoA-SRF signaling**

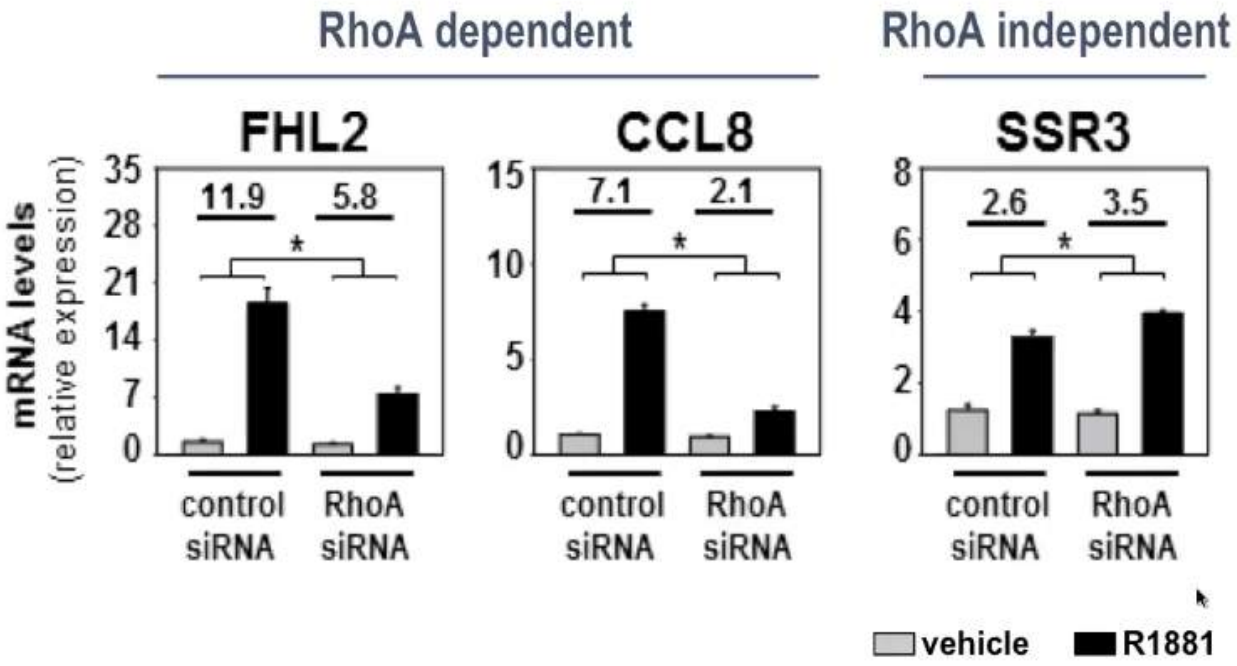
2. **Therapeutic targeting of AR-RhoA-SRF signaling**

Screening for RhoA-effector

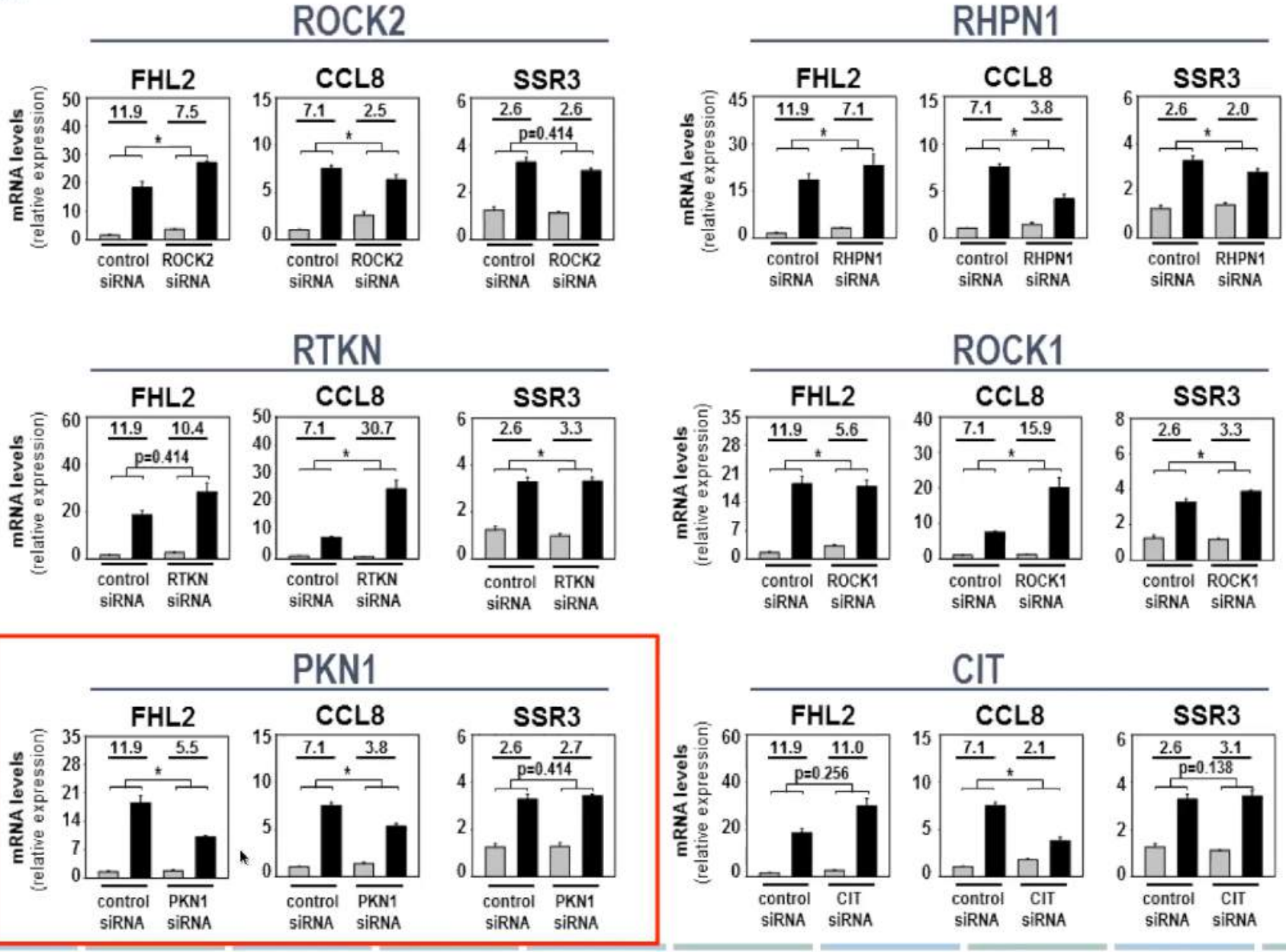


RhoA effector mediating AR-SRF signaling

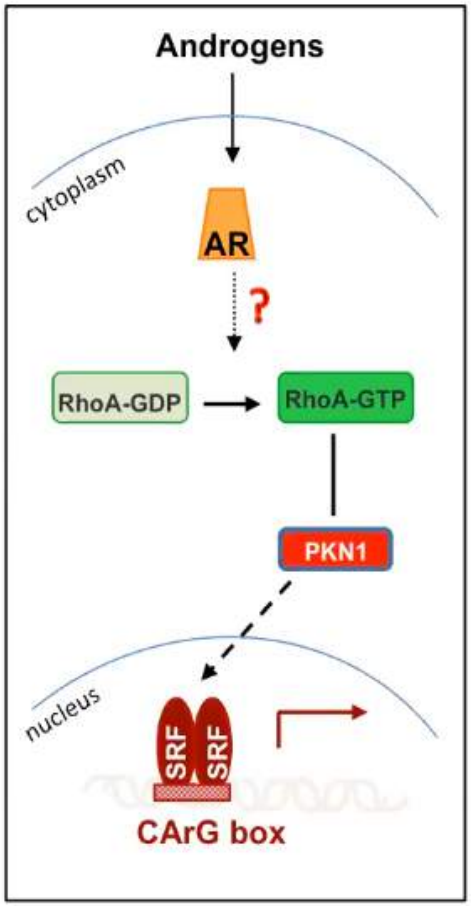
- **Individual knockdown and evaluate androgen responsiveness of 3 candidate SRF dependent-genes.**



PKN1 mediates AR-RhoA-SRF signaling

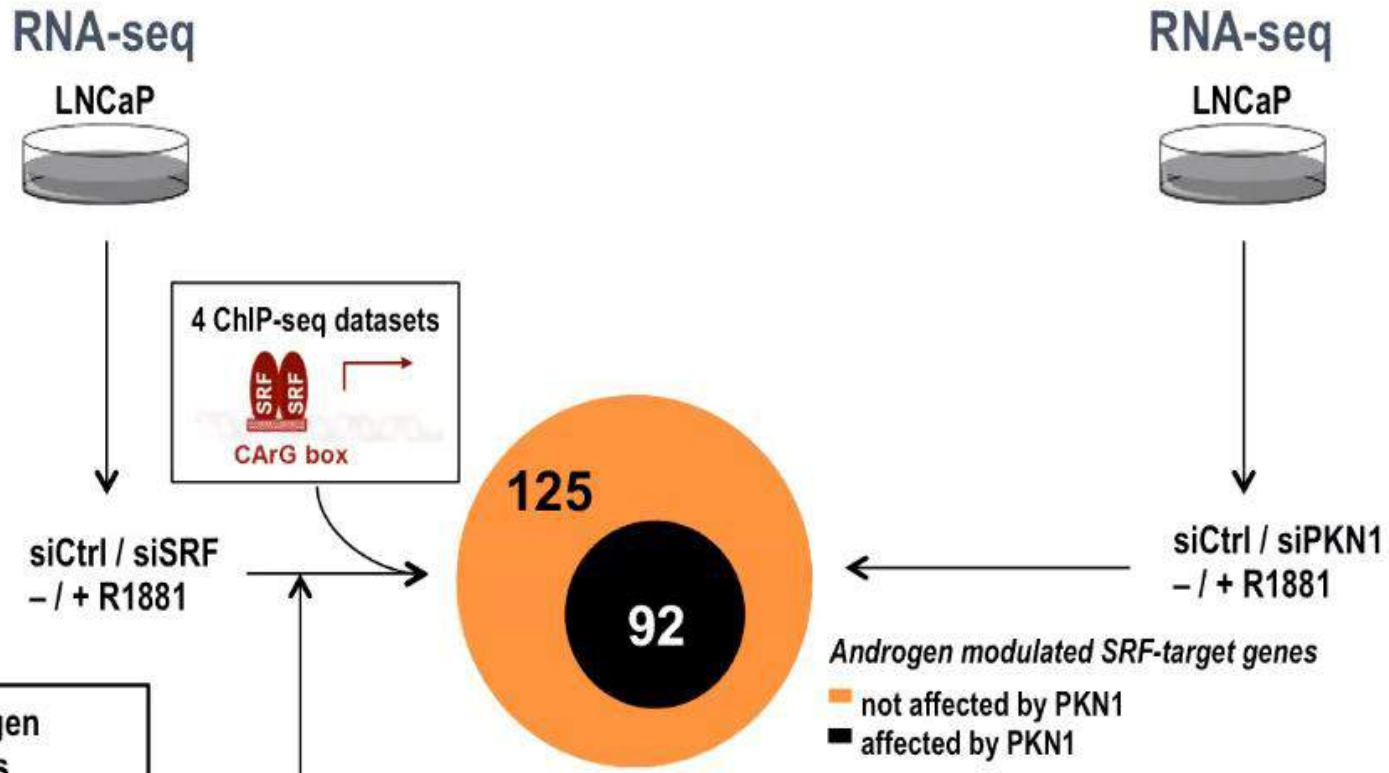


PKN1 mediates AR-SRF signaling



- Protein kinase N1 is serine/threonine kinase.
- Belongs to the AGC family of kinases and is related to protein kinase C.
- Involved in actin cytoskeletal organization, cell migration and transcriptional regulation.
- Been reported as an AR-coregulator for a few canonical AR-target genes.

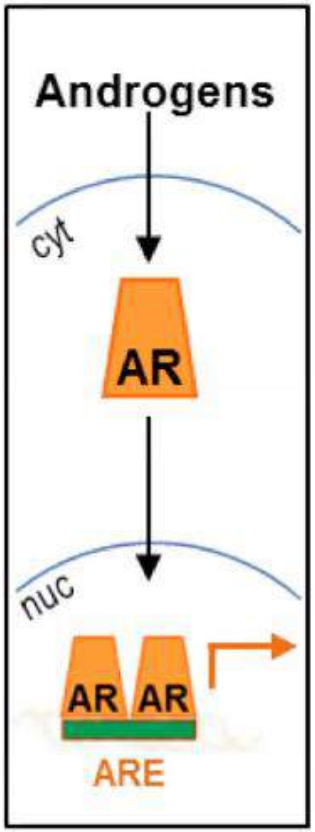
PKN1 in AR-SRF signaling



>40% of SRF-target genes are affected by PKN1

PKN1 delivers androgen responsiveness to a significant fraction of SRF-target genes

PKN1 – an AR coregulator?



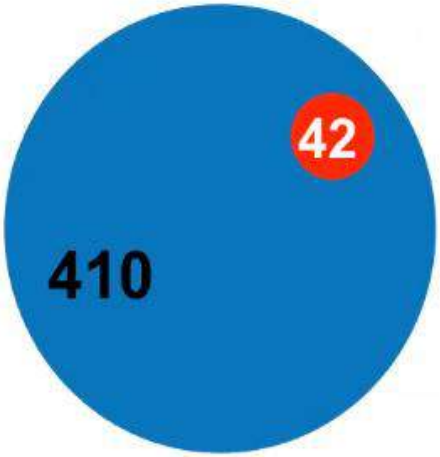
RNA-seq

LNCaP

siCtrl / siPKN1
– / + R1881

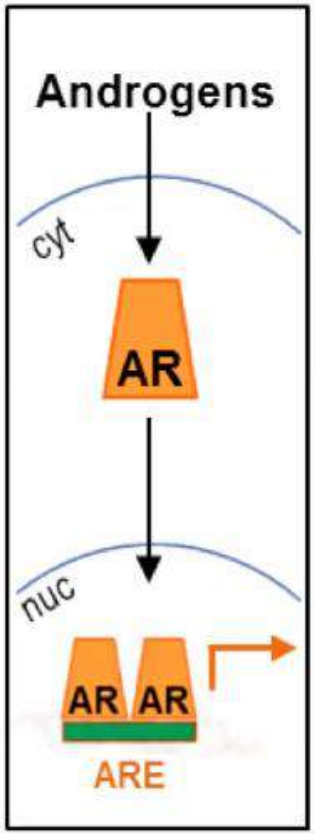
AR-target genes

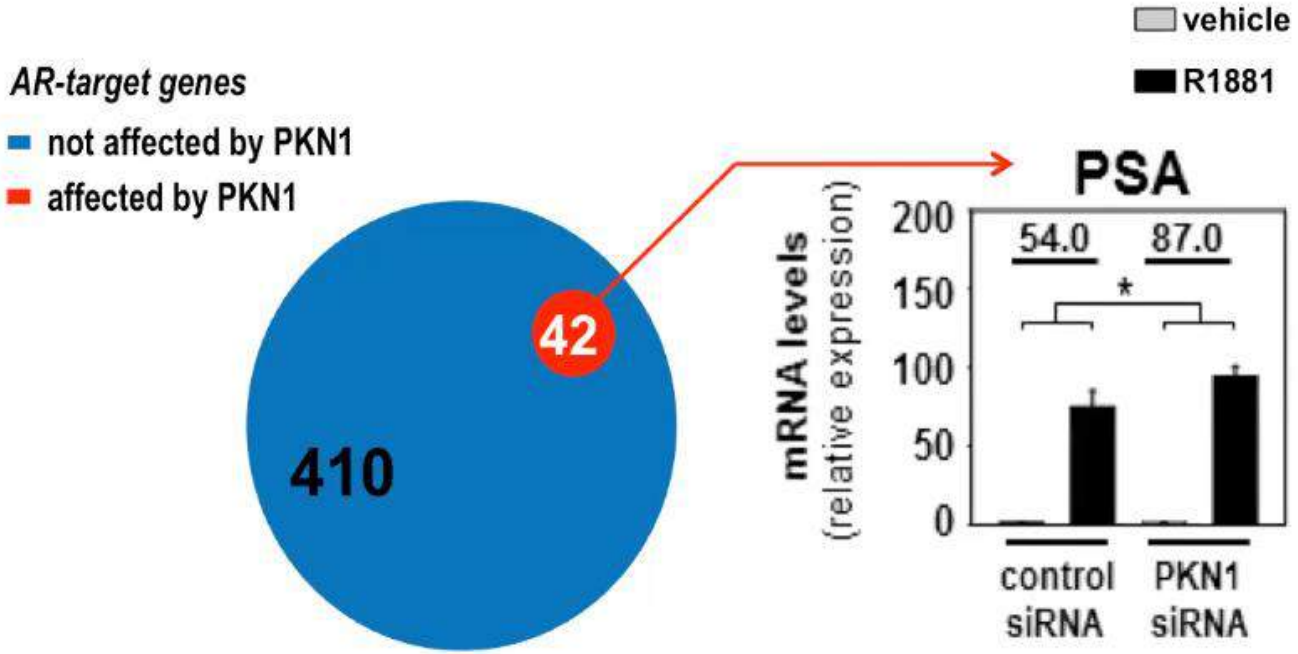
- not affected by PKN1
- affected by PKN1



<10% of AR-target genes are affected by PKN1

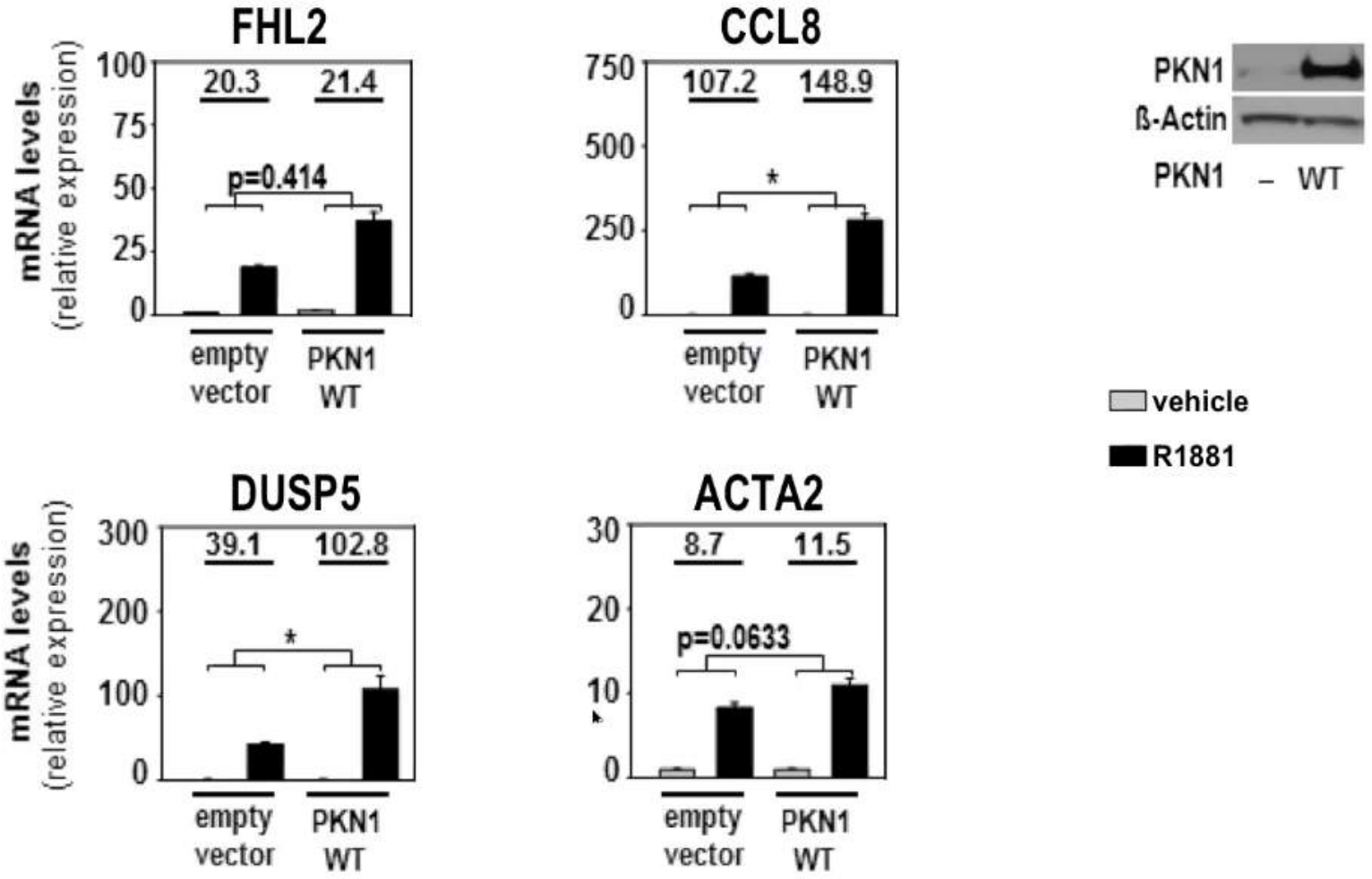
PKN1 – an AR coregulator?



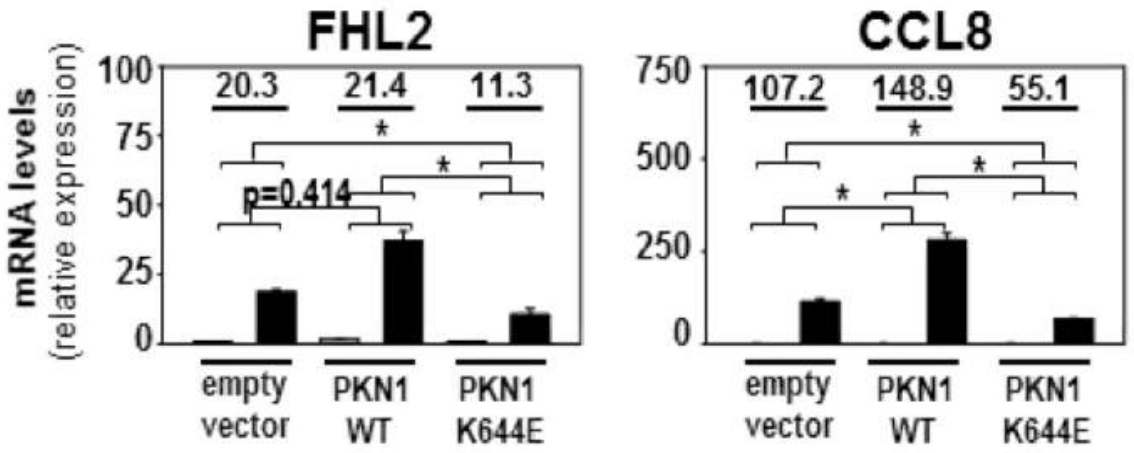


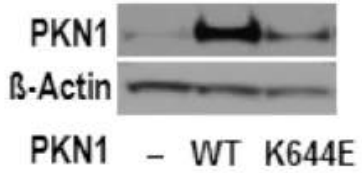
<10% of AR-target genes are affected by PKN1

PKN1 mediates AR-SRF signaling



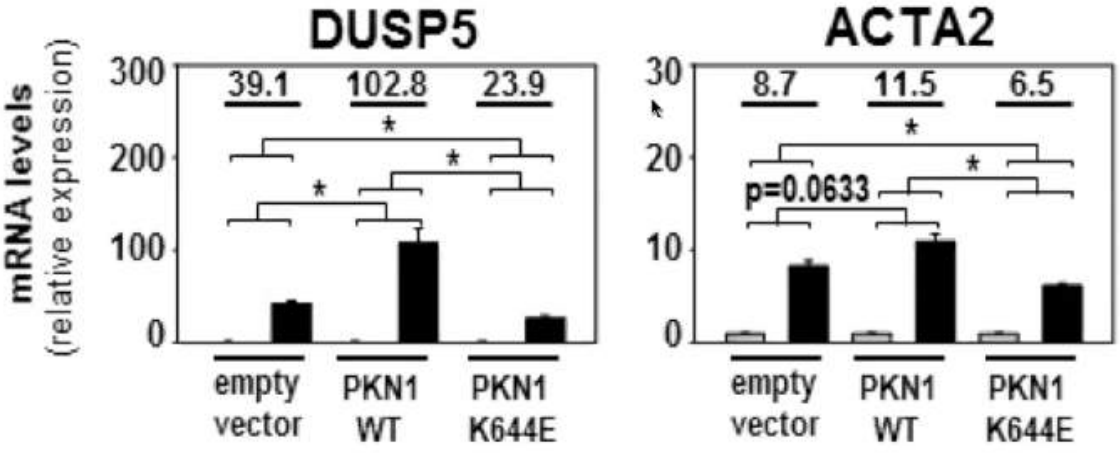
Kinase domain of PKN1 is critical





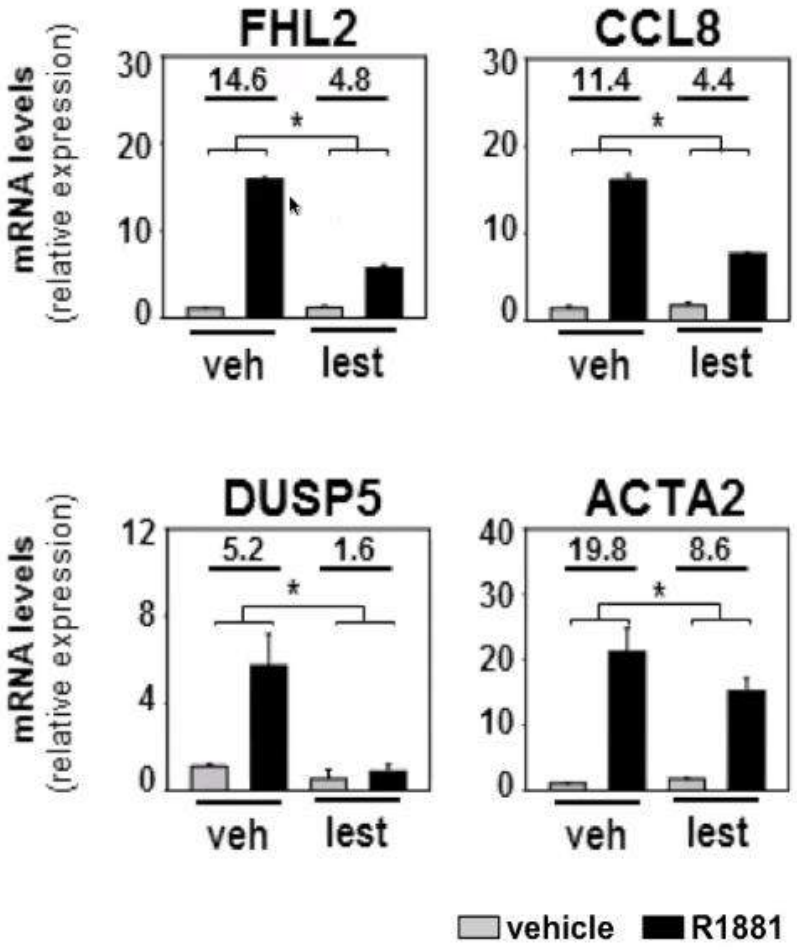
vehicle

R1881



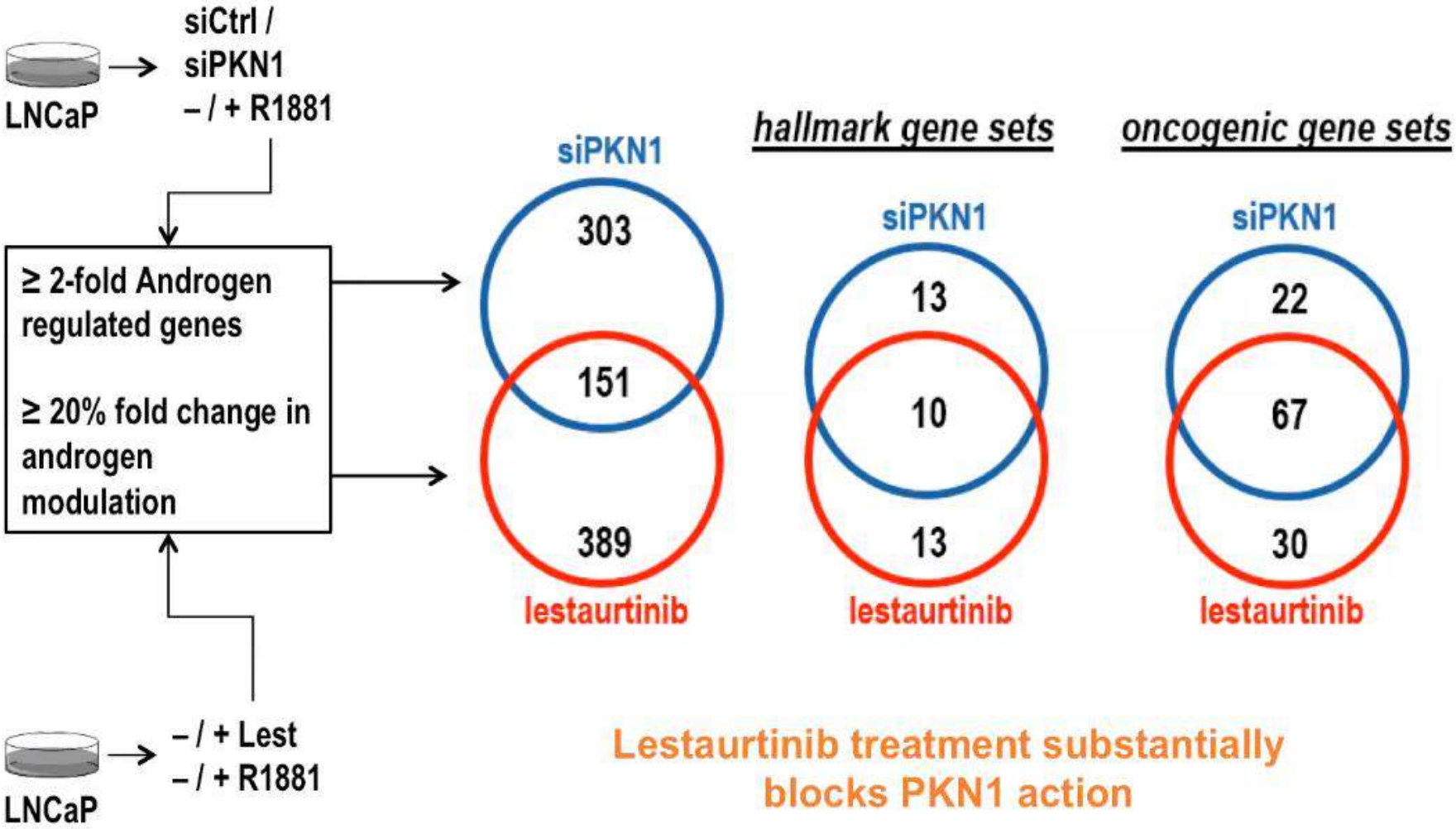
Lestaurtinib – a PKN1 inhibitor?

- Lestaurtinib has been reported to block PKN1 activity.
- It has been well-tolerated in clinical trials.
- Studied clinically for the treatment of acute myeloid leukemia and myelofibrosis



Lestaurtinib blocks PKN1 activity

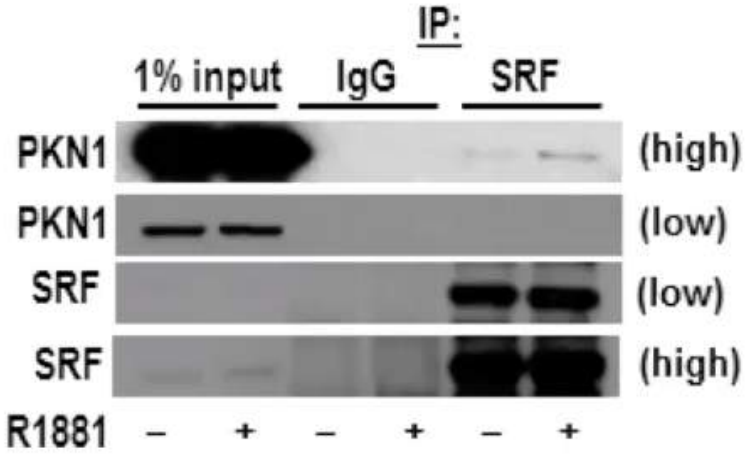
RNA-seq

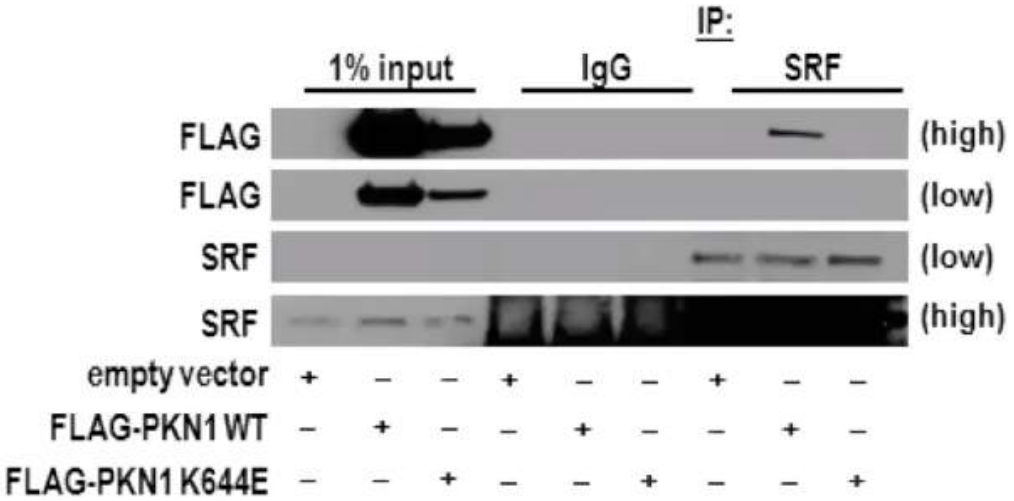


Lestaurtinib treatment substantially blocks PKN1 action

PKN1-SRF signaling

co-immunoprecipitation assays





PKN1-SRF interaction is enhanced by androgens

1. Mechanism of AR-RhoA-SRF signaling

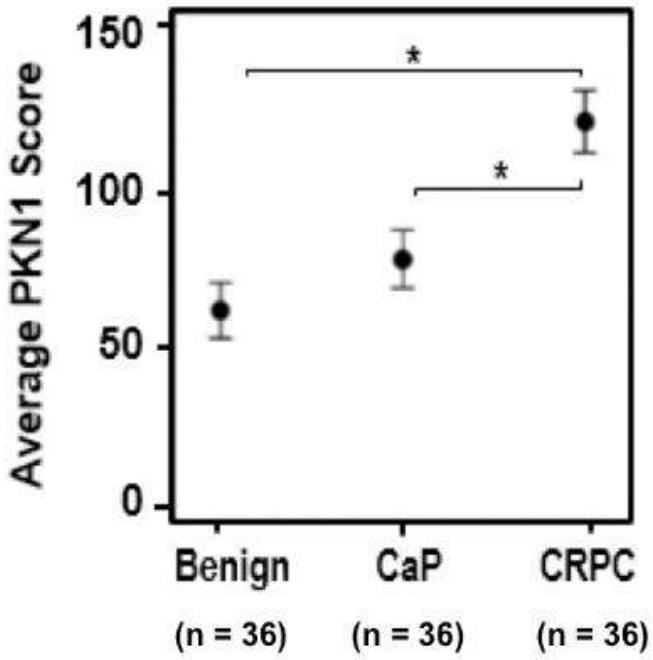
2. **Therapeutic targeting of AR-RhoA-SRF signaling**

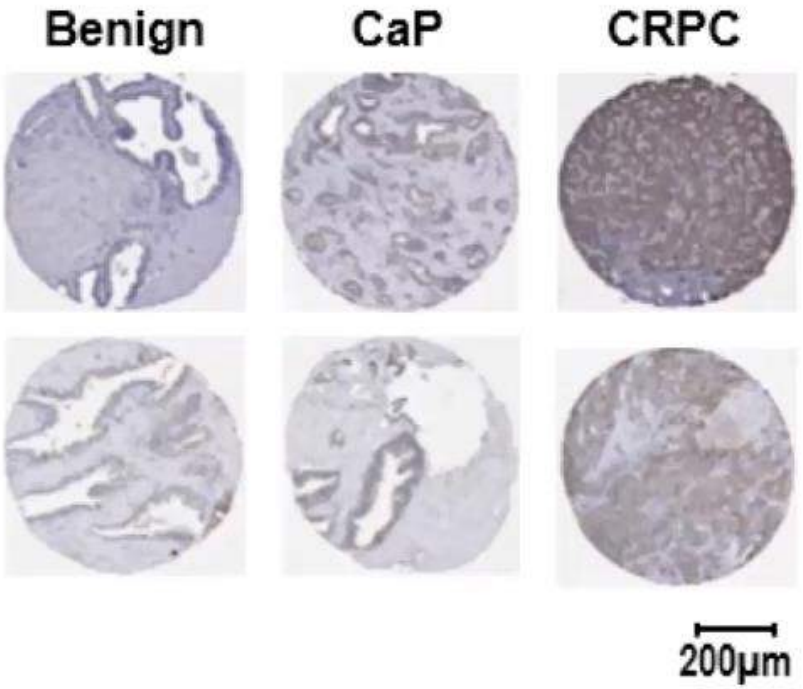
Is PKN1 a viable therapeutic target to block this pathway?

PKN1 is overexpressed in CRPC

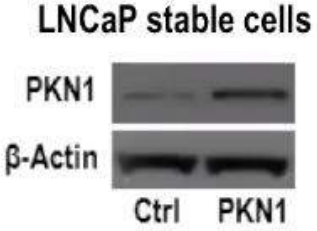
Cleveland Clinic

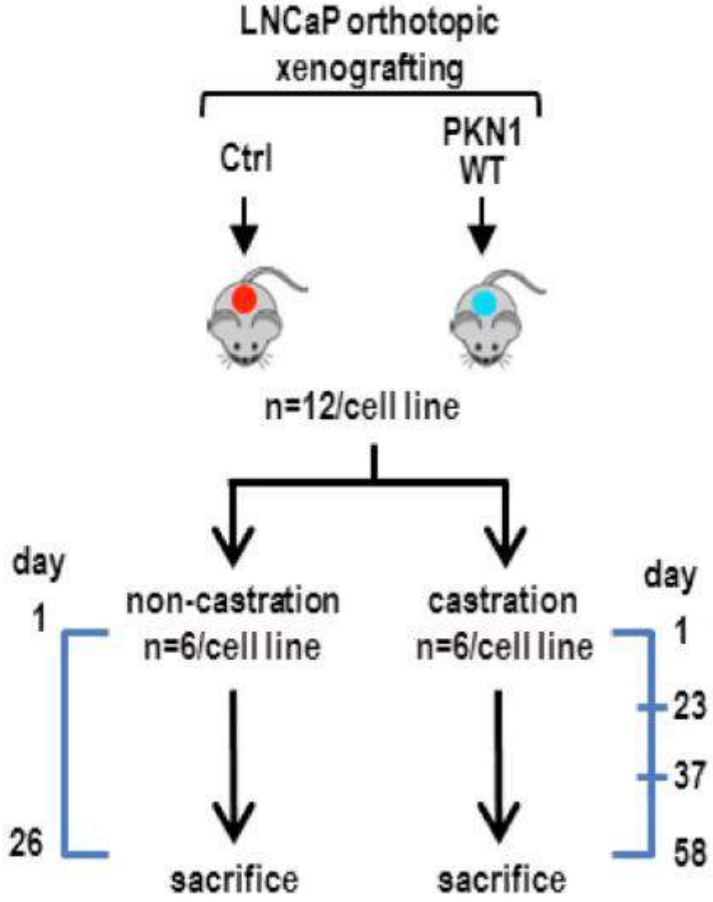
Tissue microarray

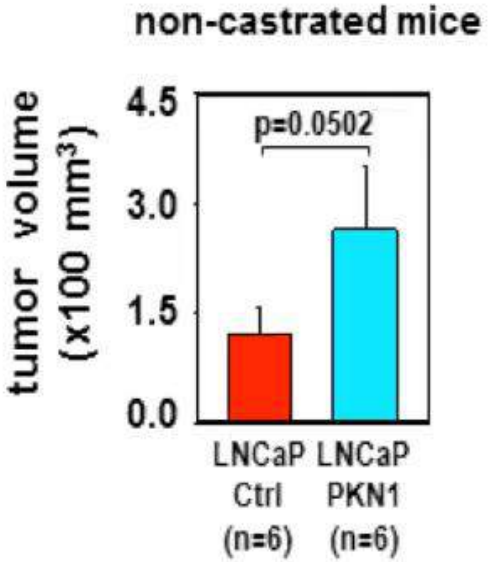


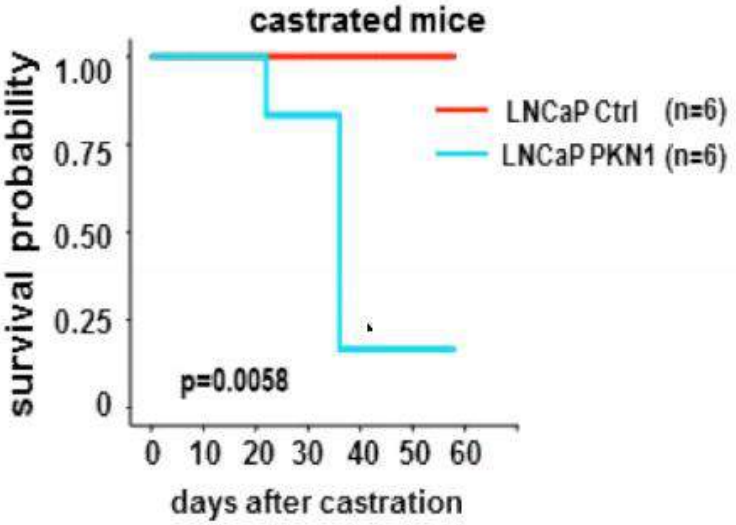


PKN1 over-expression leads to poorer survival



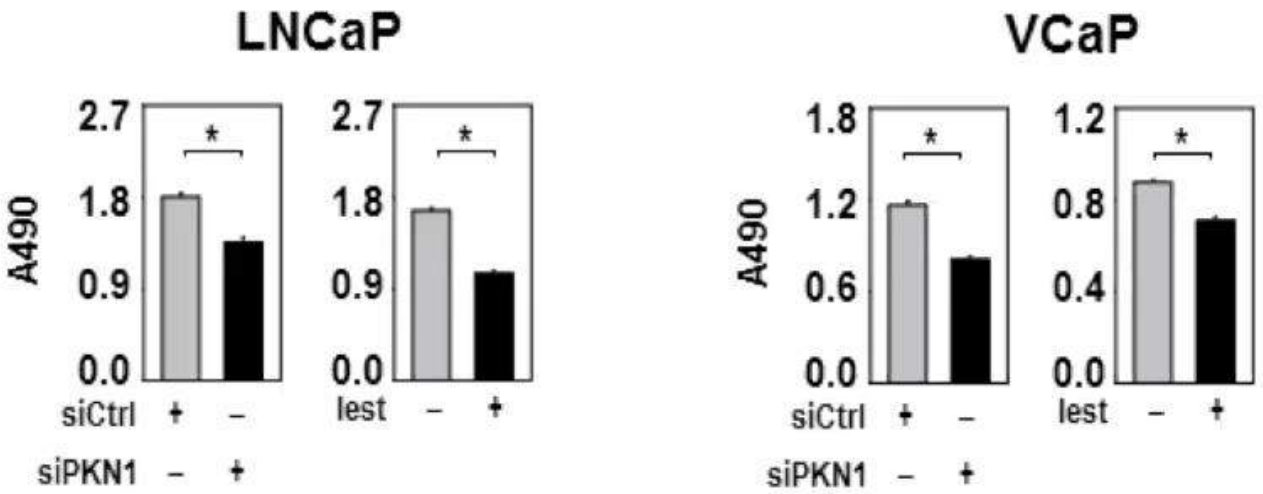




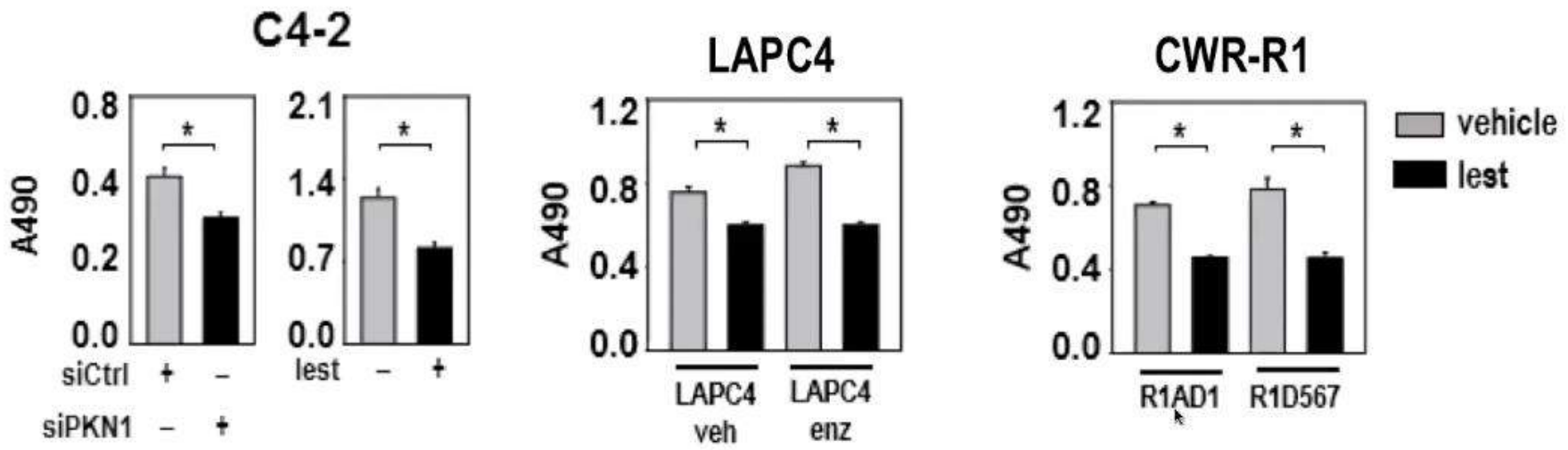


PKN1 inhibition reduces cell viability

Representative ADT-naïve cells:



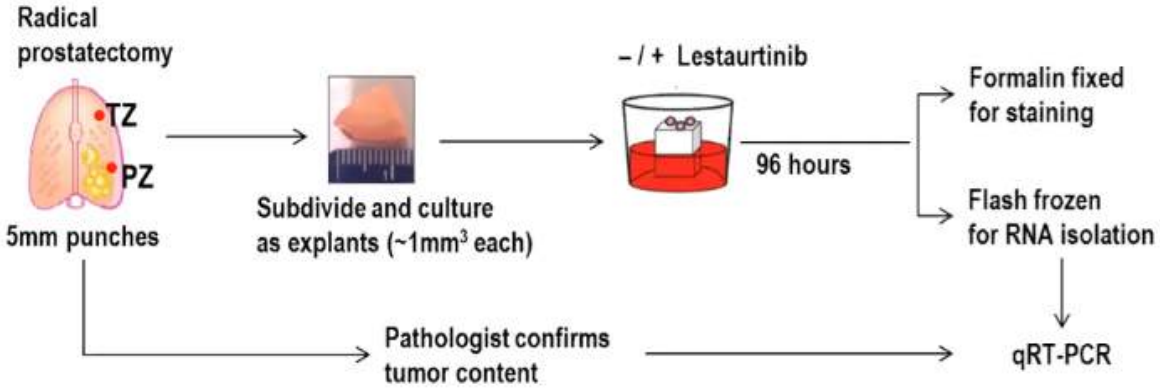
Representative CRPC cells:



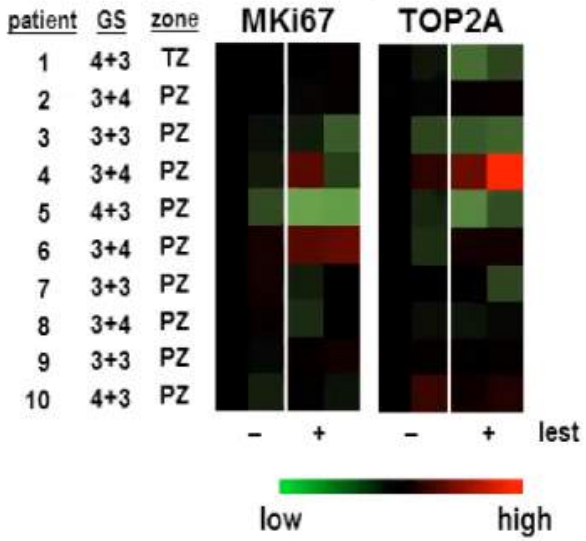
PKN1 inhibition in ADT-naïve explants

Cleveland Clinic

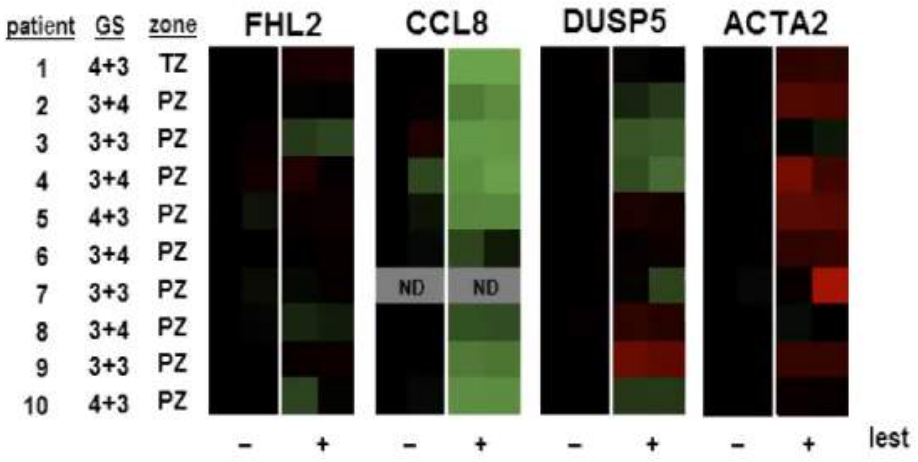
Work Flow:



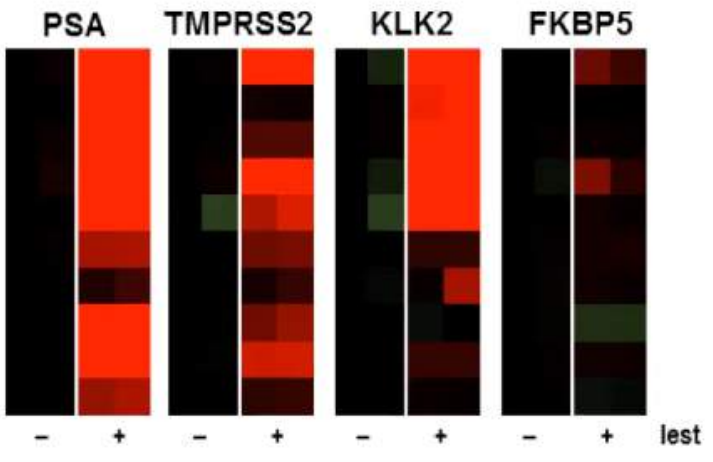
Markers of cell proliferation



SRF-dependent genes



Canonical AR-target genes



Lestaurtinib failed prostate cancer clinical trial

[Cancer Biology & Therapy 6:9, 1360-1367; September 2007]; ©2007 Landes Bioscience

Clinical Studies

Preclinical and Clinical Studies with the Multi-Kinase Inhibitor CEP-701 as Treatment for Prostate Cancer Demonstrate the Inadequacy of PSA Response as a Primary Endpoint

Connie Collins[1]
Michael A. Carducci[1,2]
Mario A. Eisenberger[1,2]
John T. Isaacs[1,2]
Alan W. Partin[1,2]
Roberto Pili[1,2]
Victoria J. Sinibaldi[1]
Janet S. Walczak[1]
Samuel R. Denmeade[1,*]

[1]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; [2]The James Buchanan Brady Urological Institute; The Johns Hopkins University School of Medicine, Baltimore, Maryland USA

*Correspondence to: Samuel R. Denmeade; Bunting Blaustein Cancer Research Building; 1650 Orleans Street; Baltimore, Maryland 21231 USA; Tel.: 410.502.3941; Fax: 410.614.8397; Email: denmesa@jhmi.edu

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ABSTRACT

Purpose: CEP-701 is a potent inhibitor of trk receptors that causes cell death in prostate cancer (PC) models. CEP-701 binds to serum proteins and a preprostatectomy study was performed to assess prostate tissue penetration and clinical response to CEP-701.

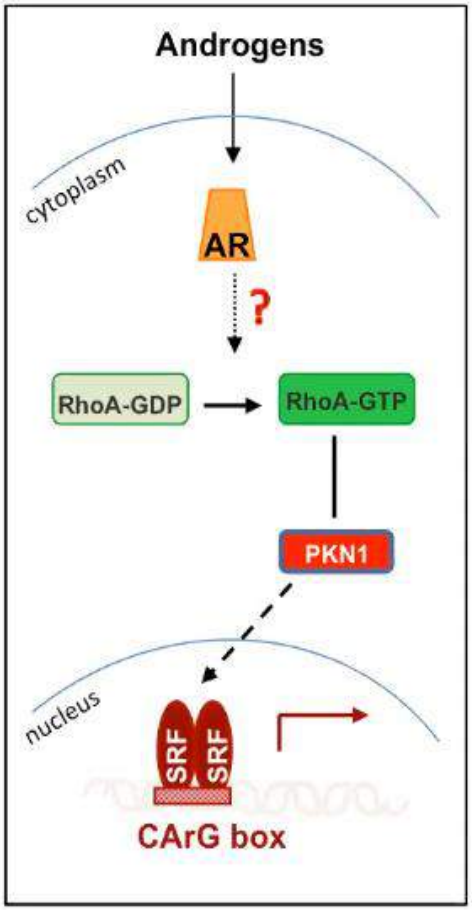
Methods: Growth assays and Western blot analyses were performed to evaluate CEP-701 kinase inhibition. In a preprostatectomy study, patients received CEP-701 for five days prior to prostatectomy and prostate tissue analyzed for CEP-701 levels. A phase II dose escalation study was performed in patients with hormone refractory PC with rising PSA and no metastases. Endpoints included PSA response and safety.

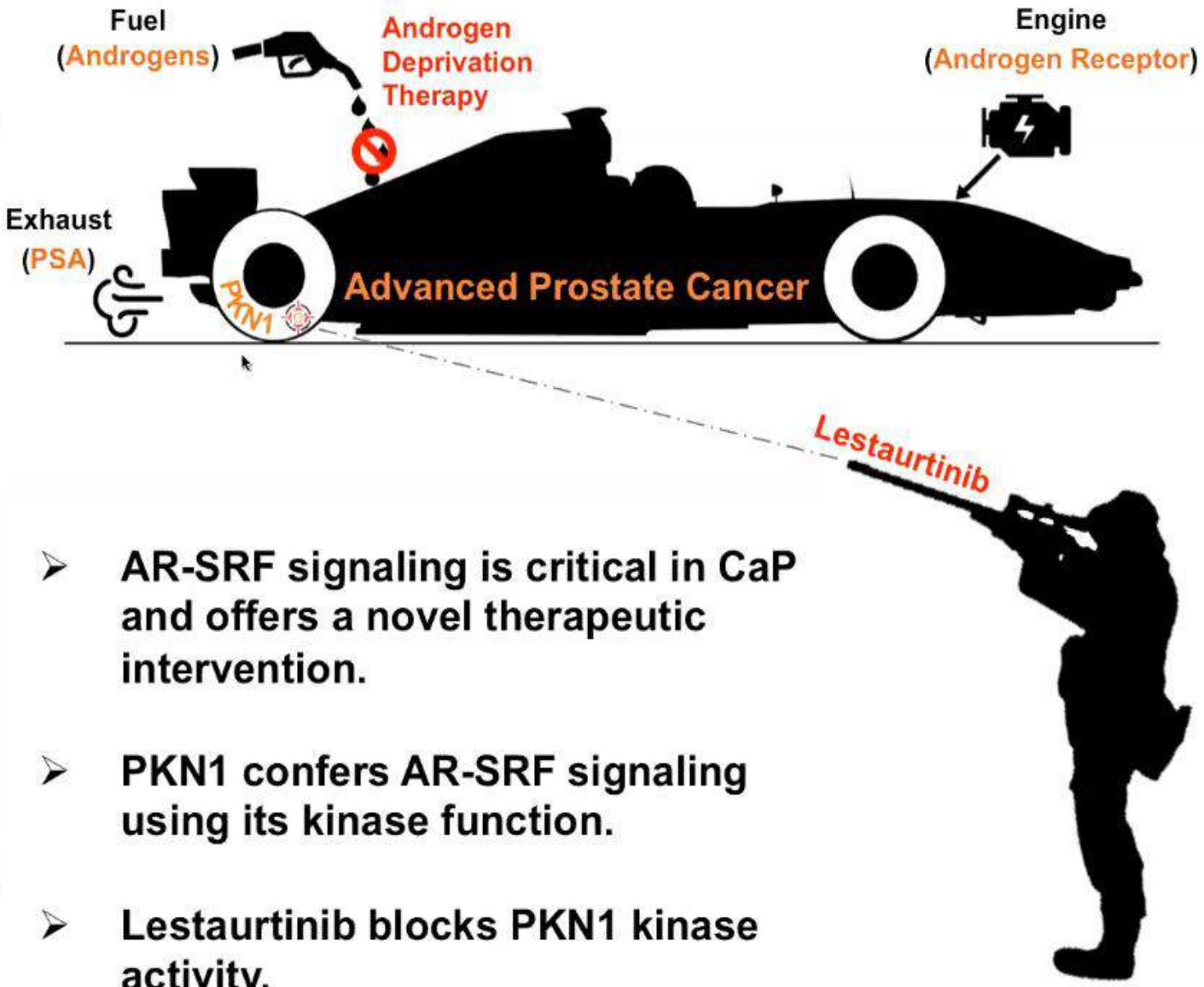
Results: CEP-701 binds to serum proteins limiting tissue penetration. An oral dose of 40 mg bid of CEP-701 for five days produced levels of 219 ± 38 nM in prostate at time of prostatectomy. No patients in the Phase II study met the primary response criteria of >50% PSA decline. Seven/9 patients had increase in PSA slope on CEP-701 compared to PSA slope prestudy. Five/9 patients had a decrease in PSA levels after stopping CEP-701. Laboratory studies showed increased PSA production by CEP-701 growth arrested human PC cells in vitro and in vivo.

Conclusions: Evaluation of PSA response is an inadequate indicator of response in CEP-701 treated PC patients. Therefore, the effectiveness of CEP-701 as treatment for prostate cancer has not been adequately tested. Based on a strong preclinical rationale, further clinical studies with CEP-701 using alternative endpoints are indicated.

PSA is an inadequate biomarker of response to PKN1 inhibition

Conclusion





- AR-SRF signaling is critical in CaP and offers a novel therapeutic intervention.
- PKN1 confers AR-SRF signaling using its kinase function.
- Lestaurtinib blocks PKN1 kinase activity.

General Implications

- Targeting critical fractions of AR action that confer aggressive prostate cancer behavior serve as selective ADT.
- Implicate a kinase as a potential therapeutic target that remain under-studied in prostate cancer.
- Rationale to employ more appropriate biomarkers for targeted therapies.
- Novel insights in regulation of SRF activity.

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COLLABORATORS

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- Dr. Eric Klein
- Dr. Nima Sharifi
- Dr. Jesse McKenney
- Dr. Christina Magi-Galluzzi
- Dr. Jesse Mckenney
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- Dr. Song Liu
- Dr. James Mohler
- Dr. Khristopher Attwood
- Dr. Gissou Azabdaftari

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THESIS COMMITTEE

- Dr. Hannelore Heemers
- Dr. Robert Silverman
- Dr. Angela Ting
- Dr. Girish Shukla
- Dr. Nima Sharifi
- Dr. Justin Lathia
- Dr. Ian Mills

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