Genomics of Prostate Cancer Disparities
FLASCO Puerto Rico
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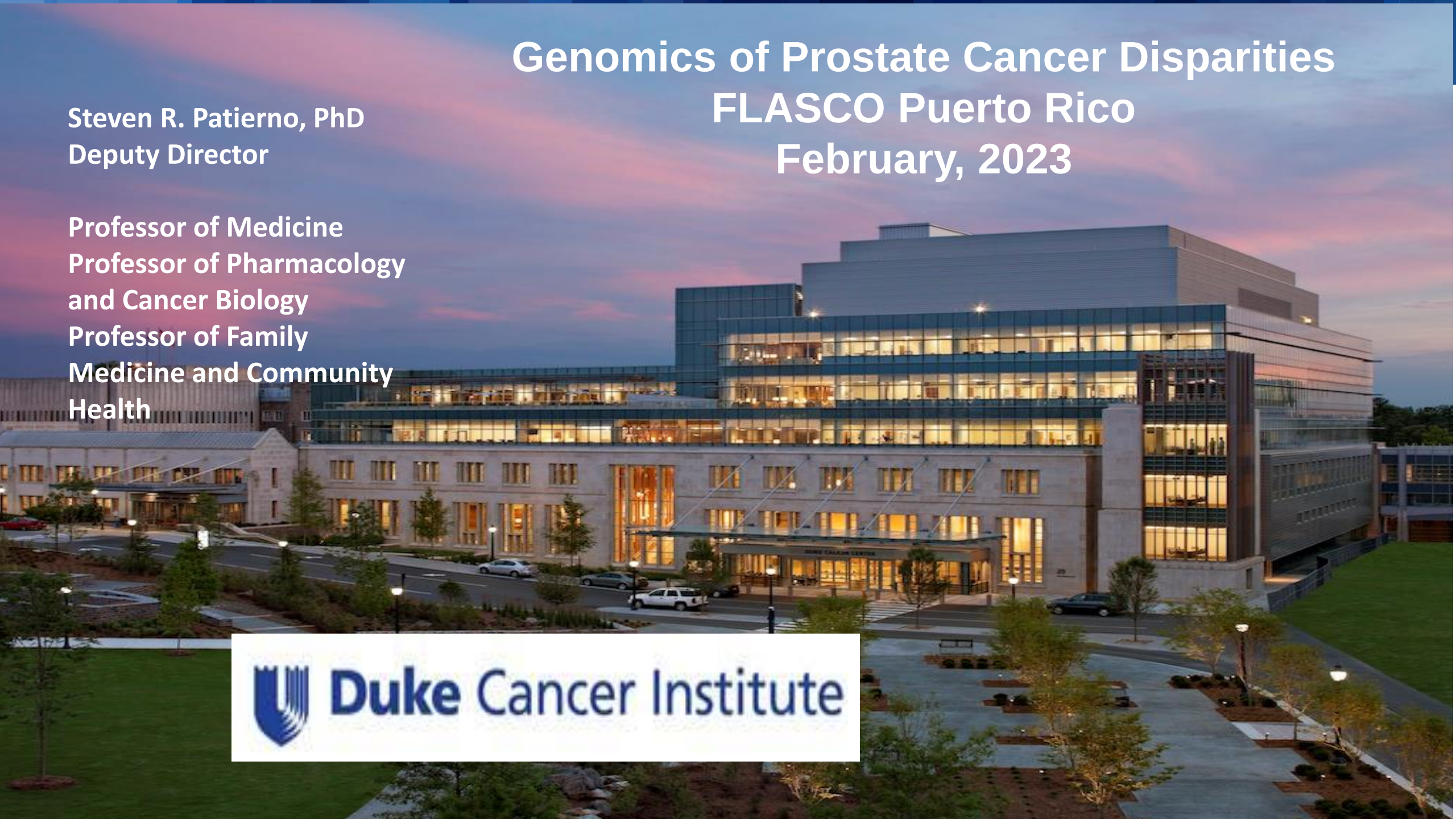
Steven R. Patierno, PhD
Deputy Director

Professor of Medicine
Professor of Pharmacology and Cancer Biology
Professor of Family Medicine and Community Health



Duke Cancer Institute

Disclosures

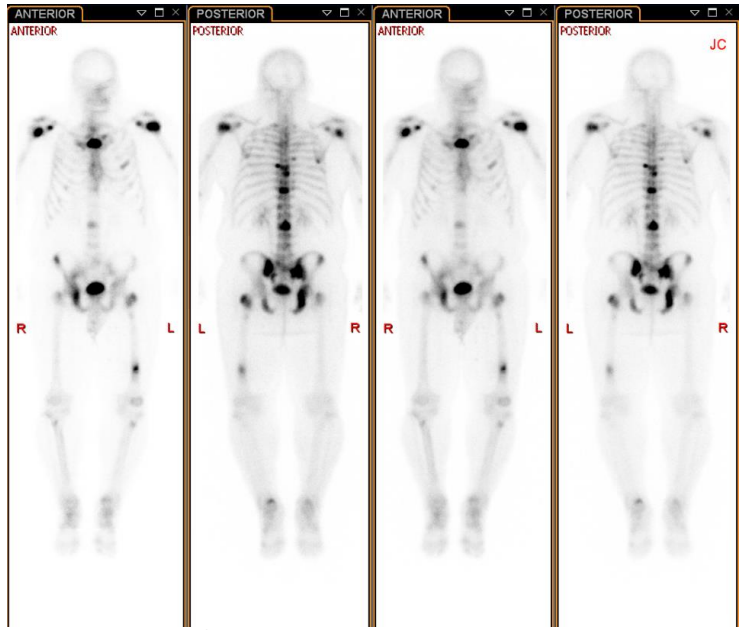
- Funding sources: National Cancer Institute grants (R-01, U01, P20 PACHE, P20 SPORE), Department of Defense, Prostate Cancer Foundation, V-Foundation, Movember Foundation, Susan G. Komen Foundation
- U.S. patents issued and pending, licensing in progress. TheraSplice Inc recently founded, not funded yet.
- Consulting and stock relationship with several companies, unrelated to any aspect of this research

Learning objectives

- Understand the multi-level contributors to cancer disparities.
- Explain how SEOH-related and/or ancestry-related biology may contribute to cancer disparities.
- Recognize the importance of precision oncology approaches to achieving cancer health equity.

Case Presentation

- MB is a 55 yo AAM with tobacco-related COPD
- Has no health insurance - Does not own a car – takes public transportation
- Seen in a clinic for the underserved with inconsistent healthcare
- Had not been seen in clinic for 16 months
- After addressing the dyspnea, provider noticed he had lost 25 lbs since last visit 1.5 years prior
- Asked if he had ever discussed cancer screening at previous visits including colonoscopy and PSA testing
- Patient noted “nobody in my family has cancer so I don’t need that”
- Also stated “I don’t want to be experimented on”
- Declined lab work and colonoscopy
- **3 months later – presents to the ED with back pain and leg weakness, found to have spinal cord compression and PSA 1300**



Specimen: A. LEFT ILIUM BIOPSY

DIAGNOSIS AND INTERPRETATION:

"LEFT ILIUM BIOPSY":
- METASTATIC POORLY-DIFFERENTIATED CARCINOMA; CONSISTENT WITH PROSTATIC PRIMARY.

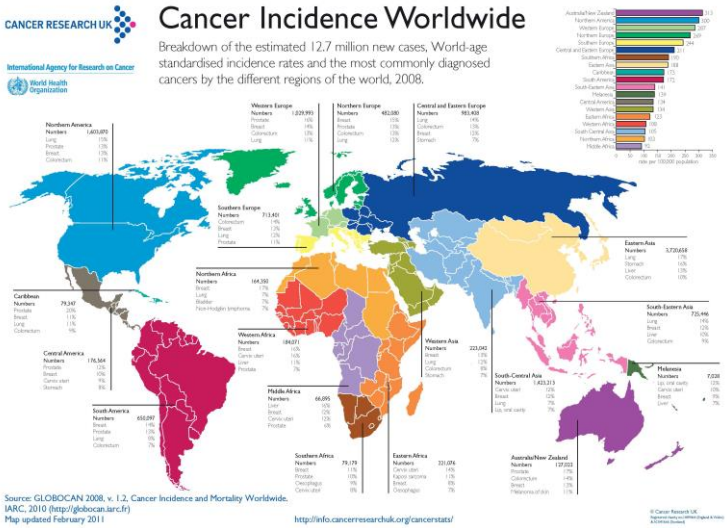
88307, 88342, 88341X7, 88311

COMMENT: The carcinoma involving the bone is very poorly differentiated, so a panel of immunistains is performed to identify a possible primary site. The tumor cells are positive for pancytokeratin and PSA and are negative CK7, CK20, Gata3, S100, CD45, and synaptophysin. This pattern is consistent with a prostatic primary. An intradepartmental review is performed on this case prior to sign out.

What Are Cancer Health Disparities?

- **The unequal burden of cancer among minoritized and marginalized persons/populations and the medically vulnerable and underserved**

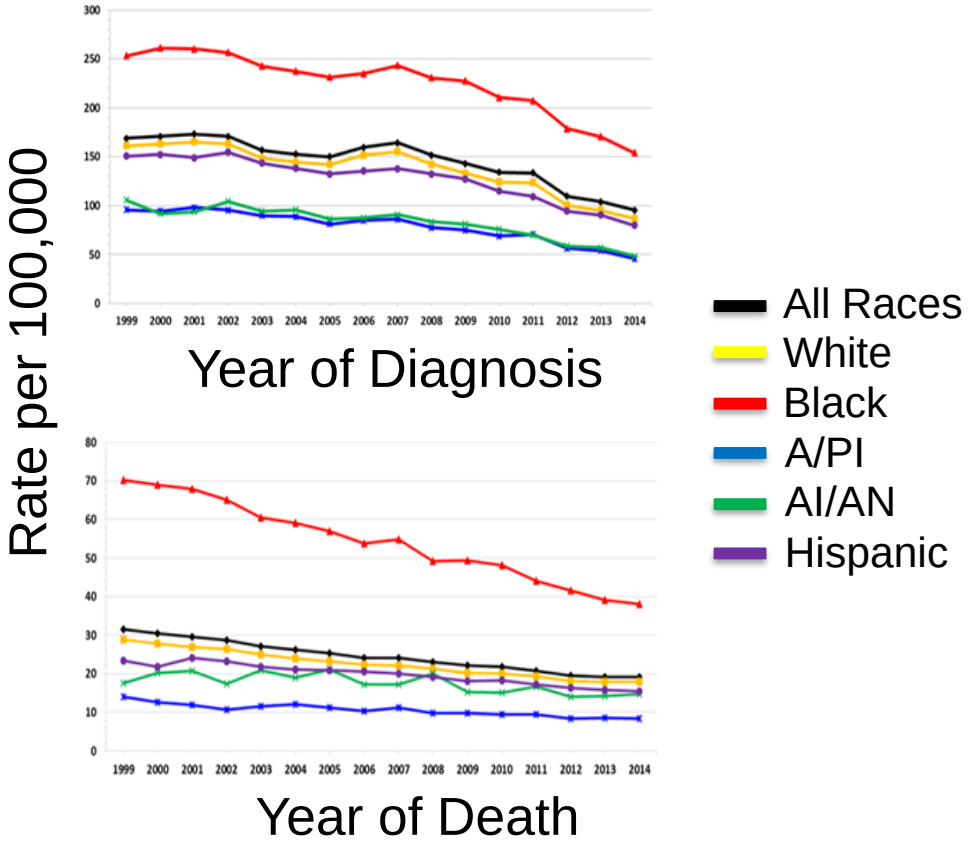
- **This problem is local, regional, national, global, urban and rural**



Prostate Cancer Disparities Among Racial Groups

Incidence Rates by Race and Ethnicity US, 1999-2014

Death Rates by Race and Ethnicity US, 1999-2014



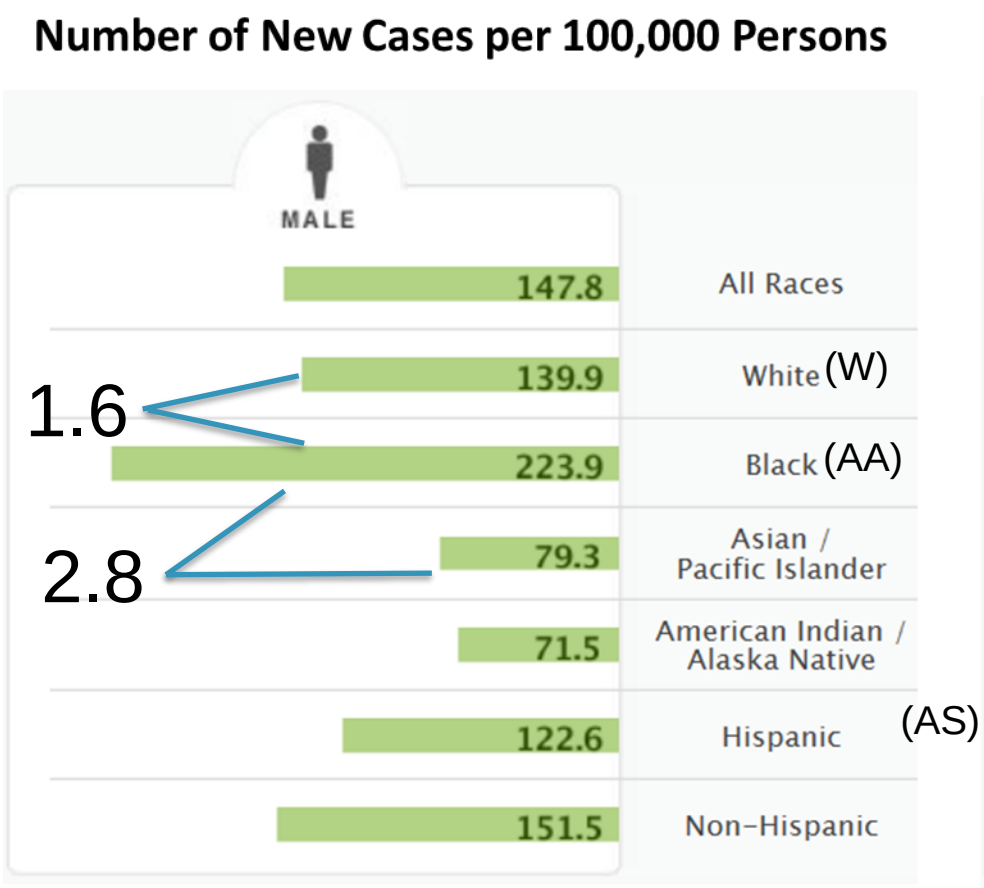
Disparities in Prostate Cancer Aggressiveness

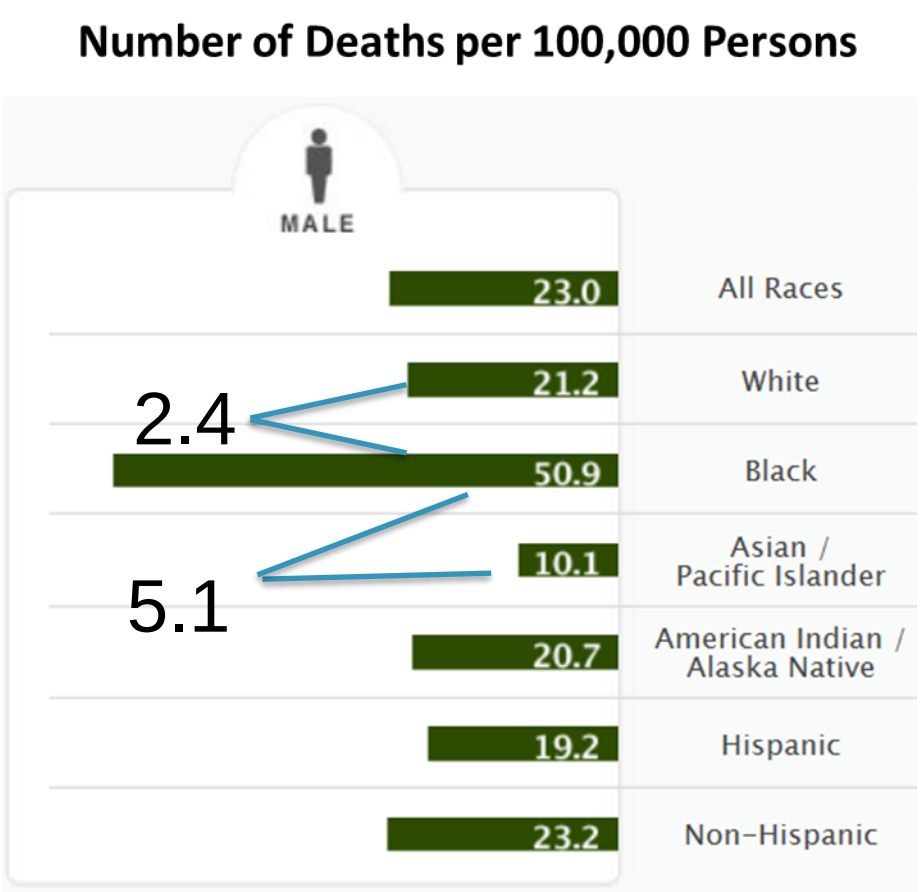
- Earlier mean age at diagnosis
- Shorter mean survival
- More common advanced stage diagnosis
- Greater risk of early recurrence
- More common metastasis

- **Much the same in equal access health systems**

- **Exacerbated by the dramatic drop in screening after 2012 – increased # and % of men being diagnosed with late stage disease**

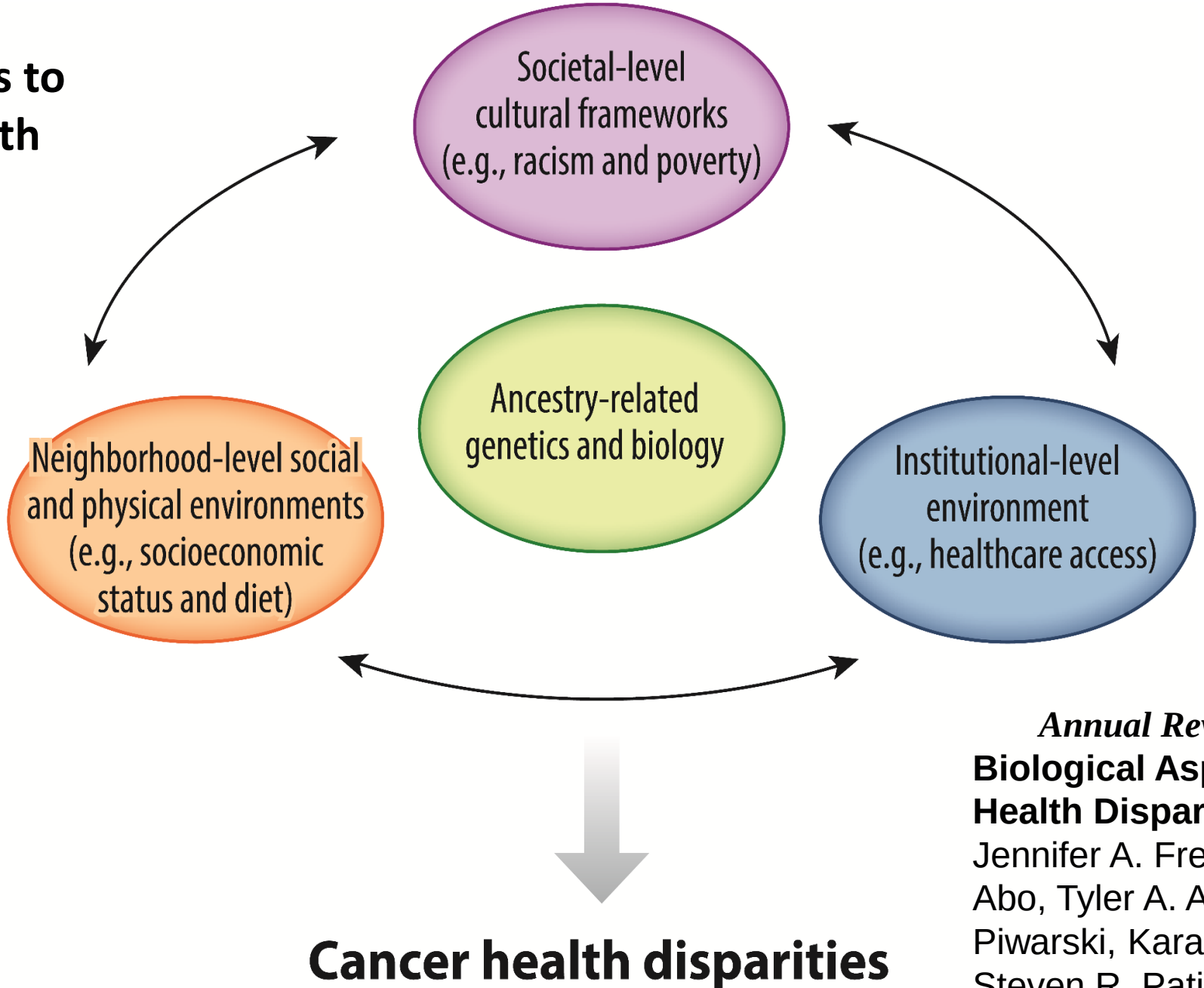
Prostate Cancer Disparities Among Racial Groups





SEER Stat Fact Sheets: Prostate. Available from: http://seer.cancer.gov/statfacts/html/prost.html

Multi-level Contributors to Cancer Health Disparities



Annual Review of Medicine

Biological Aspects of Cancer Health Disparities

Jennifer A. Freedman, Muthana Al Abo, Tyler A. Allen, Sean A. Piwarski, Kara Wegermann, Steven R. Patierno

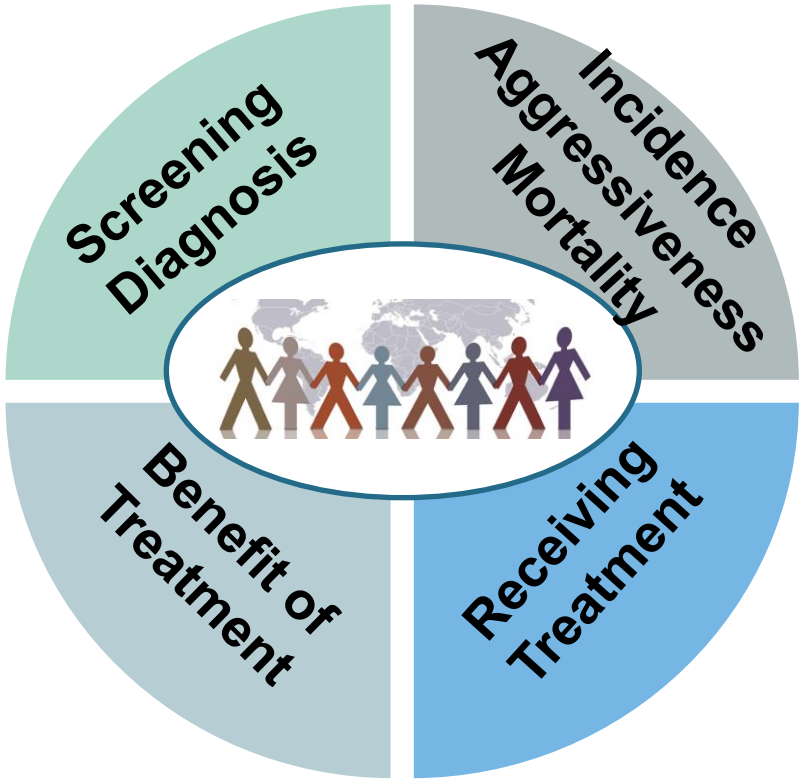
Intersecting Contributors to Cancer Disparities

Social & Environmental

Personal Health Beliefs
Individual Responsibility
Socioeconomic Status
Environmental Exposures

Biological

Germline/Somatic
Genome
Epigenome
Transcriptome
Proteome
Metabolome



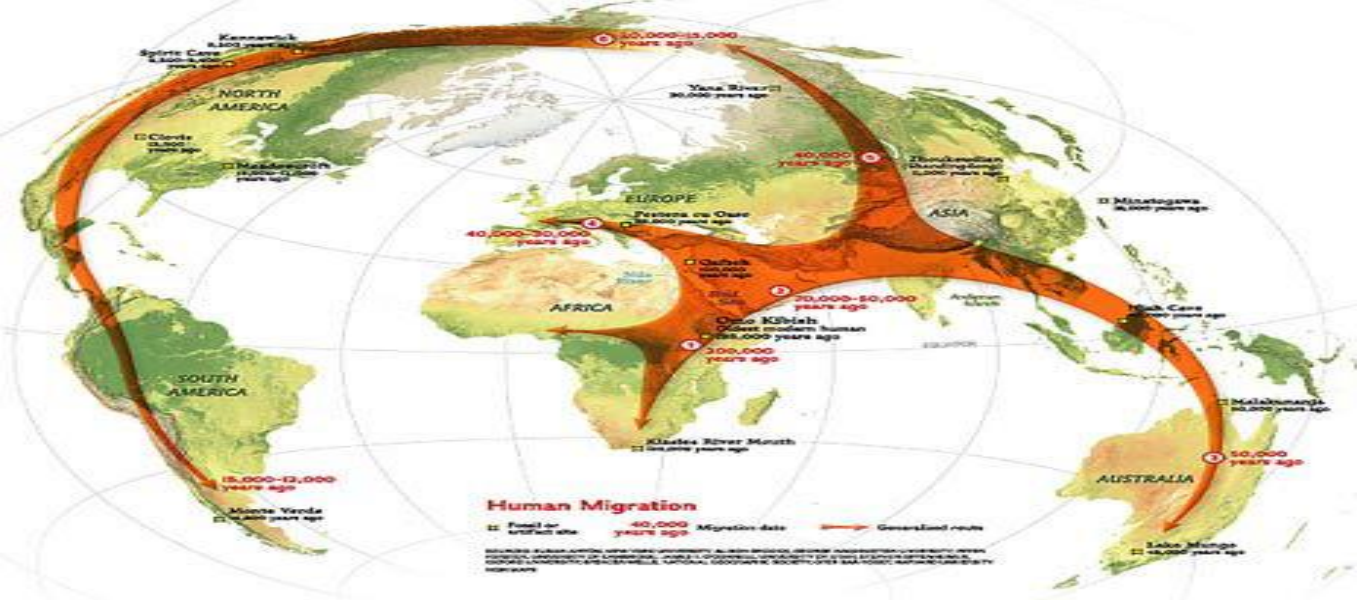
Illustrates the need for new methodologies in "convergence science"

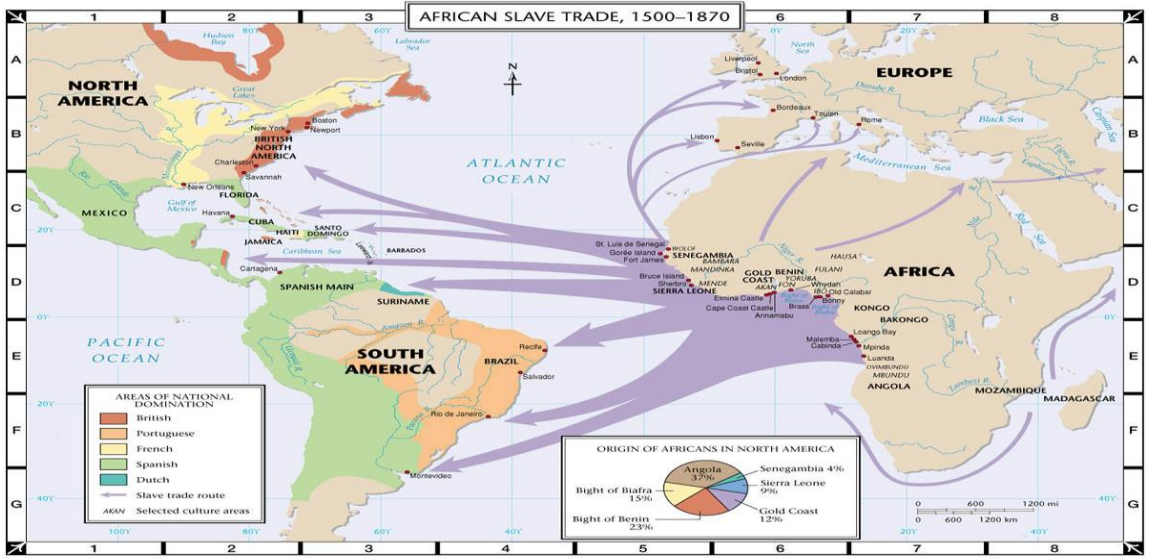
Lifestyle

Smoking Status
Diet
Exercise
Obesity
Comorbidities
Physical Environment

Structural

Health System
Access To Care
Policy
Physical Environment



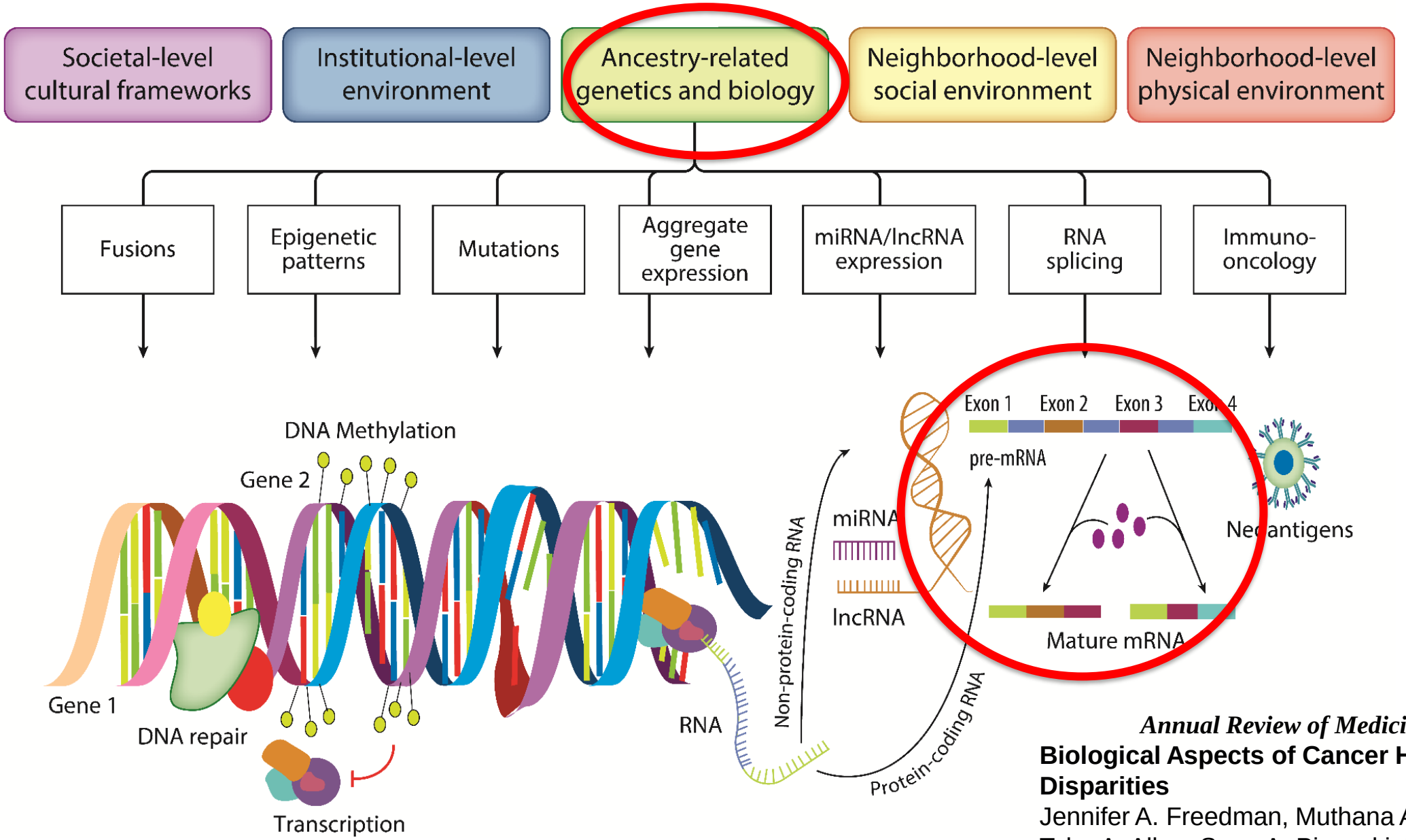


RACE IS NOT A BIOLOGICAL CONSTRUCT

RACE/ETHNICITY ARE SOCIO-CULTURAL CONSTRUCTS

HOWEVER, RACIAL ANCESTRY, AS A FUNCTION OF THE HUMAN DIASPORA (including FORCED), AFFECTS PHENOTYPIC AND GENETIC DIVERSITY AND THEREFORE DISEASE RISK, BIOLOGY, AND OUTCOMES

RACE AND ETHNIICTY ALSO INTERSECT WITH MULTI-LEVEL SOCIAL ELEMENTS OF HEALTH THAT IMPACT PHYSIOLOGY AND PATHOLOGY AND THEREFORE DISEASE RISK, BIOLOGY, AND OUTCOMES



Annual Review of Medicine

Biological Aspects of Cancer Health Disparities

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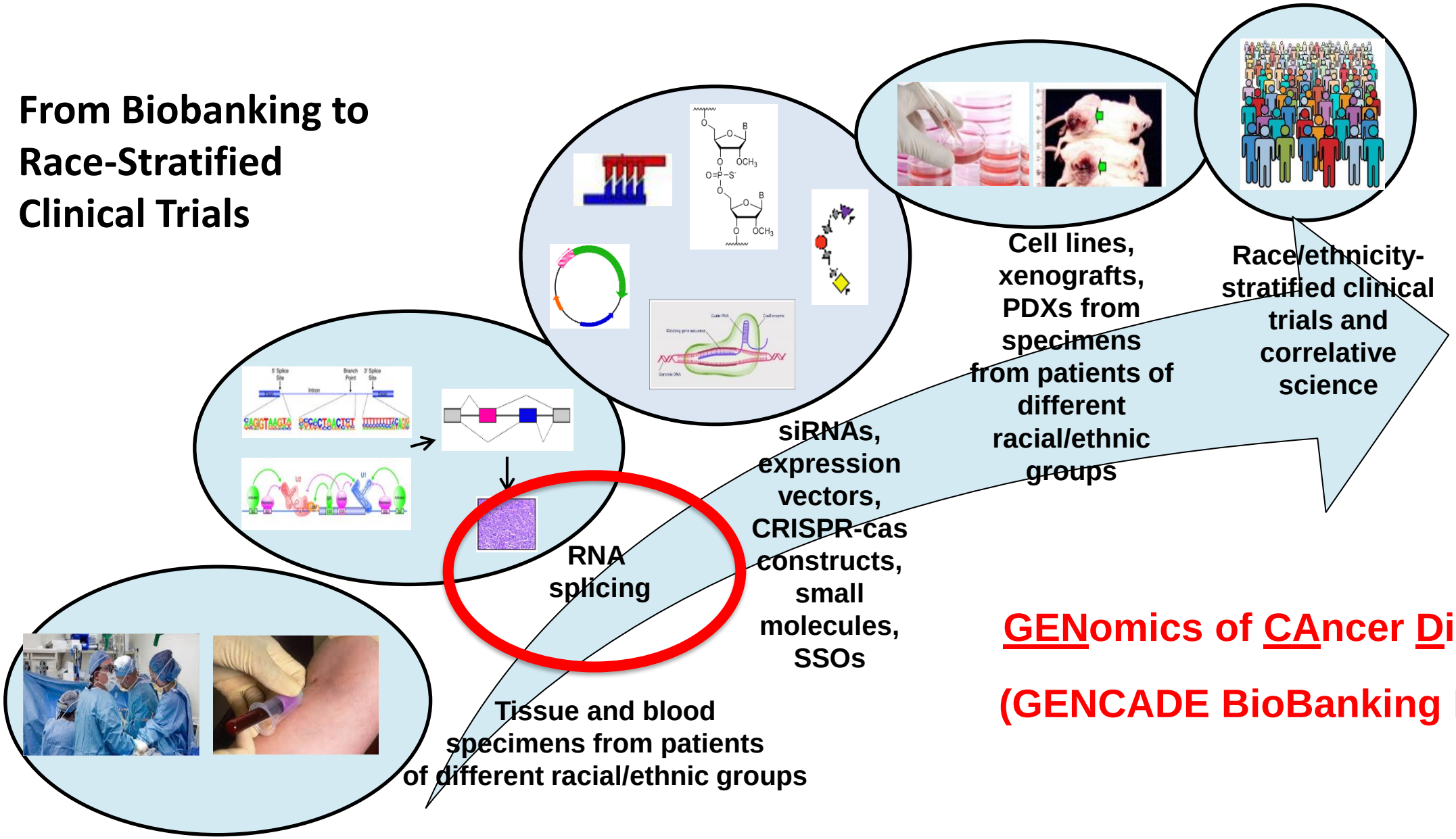
Translational Cancer Disparities Laboratory Research

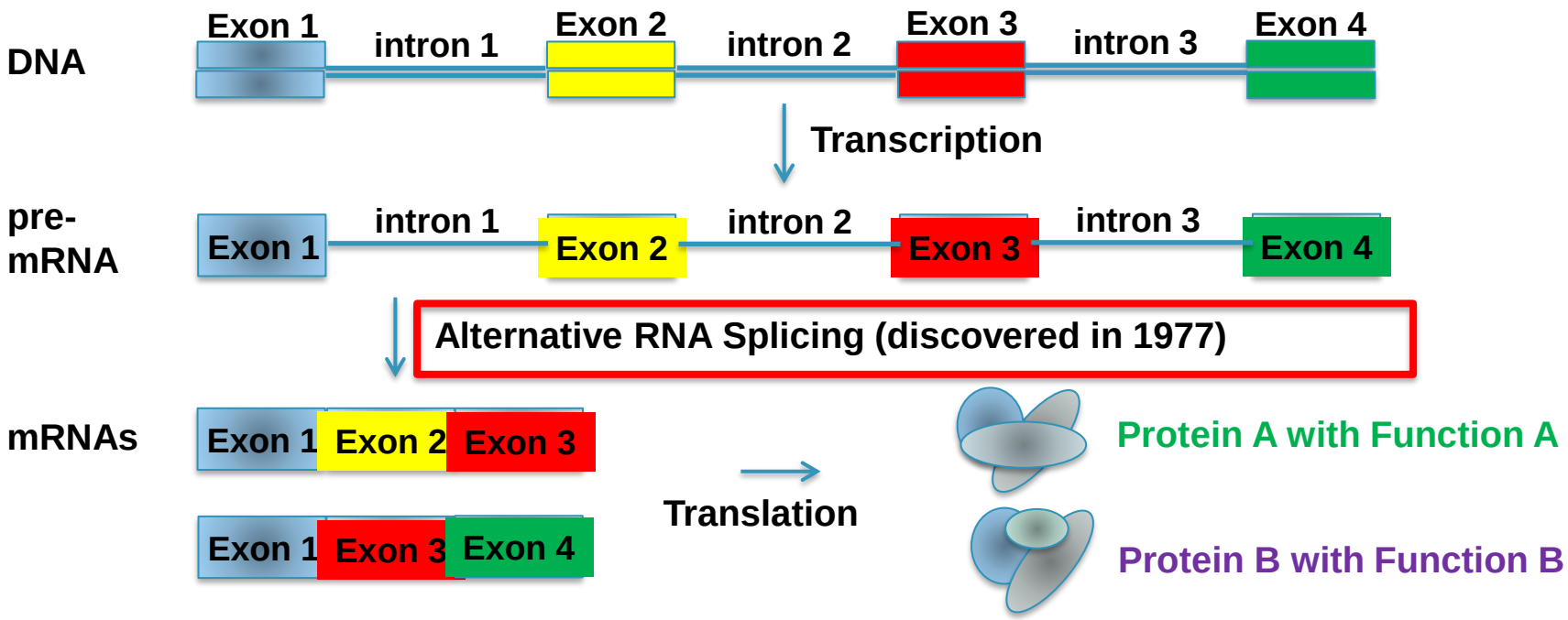
From Biobanking to Race-Stratified Clinical Trials



GENomics of CAncer DisparitiEs

(GENCADE BioBanking Protocol)

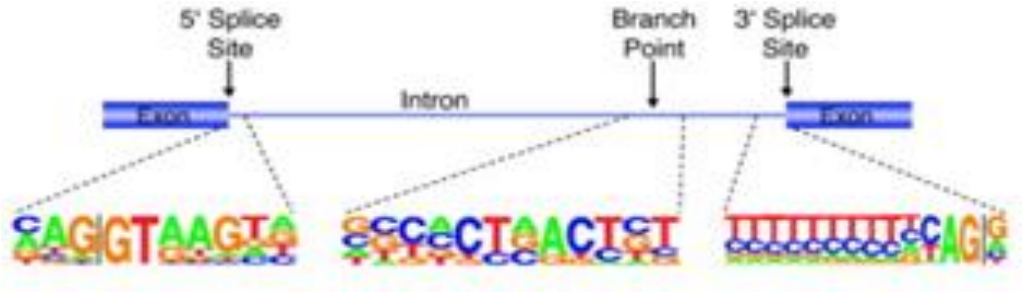
Central Dogma of Biology: Splicing 101

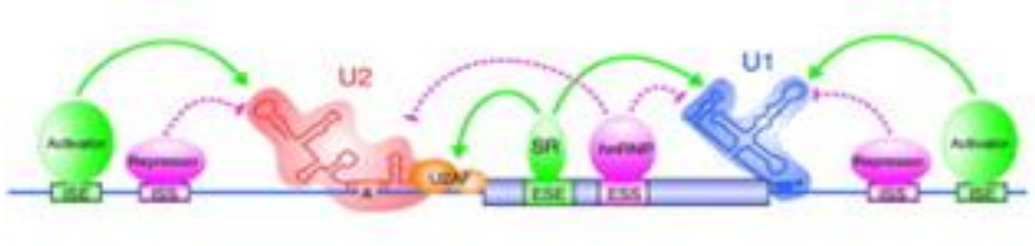


- Drives evolutionary biological diversity, increasingly recognized as a principle driver of tumor biology
- "Functions of proteins produced from RNA splice variants from a given gene (proteoforms) can be as different as proteins encoded by entirely different genes" Yang et al., Cell, 2016

- **Alternative RNA Splicing Impacts Every Hallmark of Cancer**

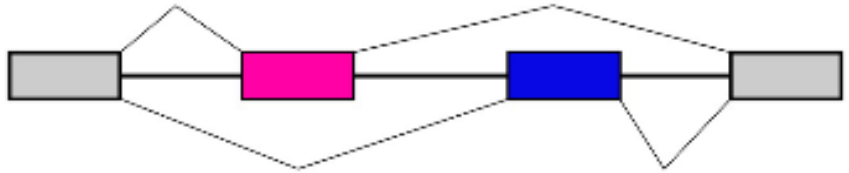
What regulates Alternative RNA Splicing?





***cis*-acting splicing elements**

***trans*-acting splicing factors**

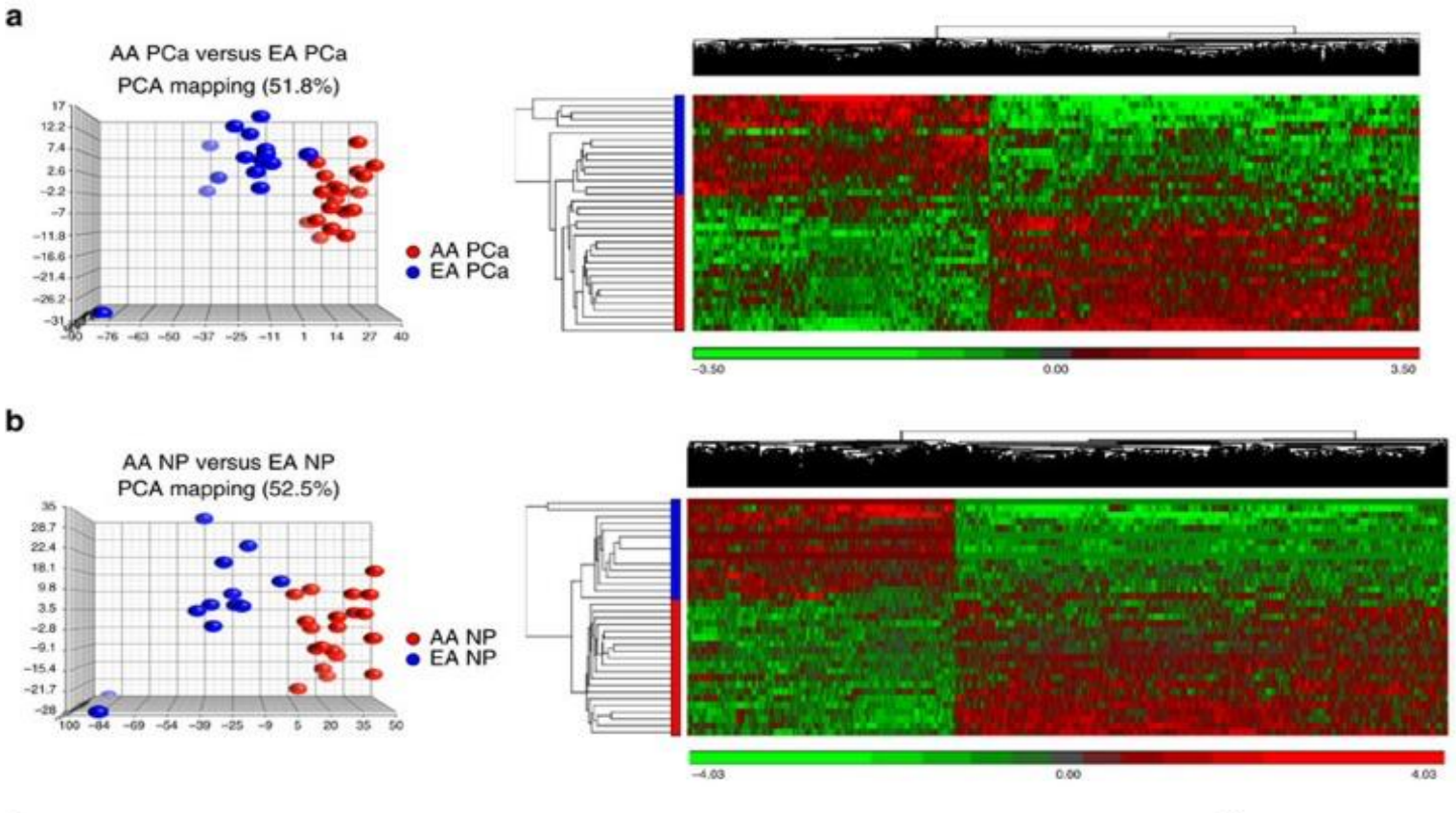


Does Alternative RNA Splicing differ by race, ethnicity or ancestry?
How does it affect tumor biology?
Can ARS help predict risk or survival?
Is it clinically meaningful and targetable?

ARS in Prostate Cancer by Exon Array

nature COMMUNICATIONS

Bi-dar Wang, PhD GWU, now at
U. MD Eastern Shore



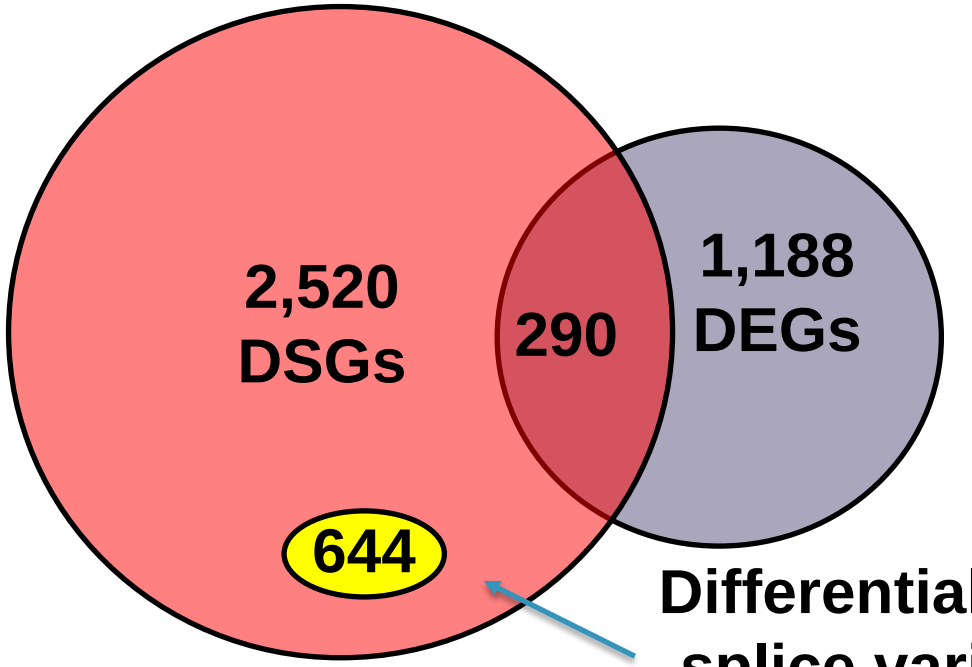
Norman Lee, PhD GW Cancer Center

Alternative splicing promotes tumour aggressiveness and drug resistance in African American prostate cancer

Bi-Dar Wang, Kristin Ceniccola, SuJin Hwang, Ramez Andrawis, Anelia Horvath, Jennifer A. Freedman, Jacqueline Olender, Stefan Knapp, Travers Ching, Lana Garmire, Vyomesh Patel, Mariano A. Garcia-Blanco, Steven R. Patierno, Norman H. Lee. Nat Comm 2017

Race-related ARS in Prostate Cancer by Exon Arrays

Prostate Cancer Biopsy Samples
Self-identified AA vs W Patients
Analyzed by exon array



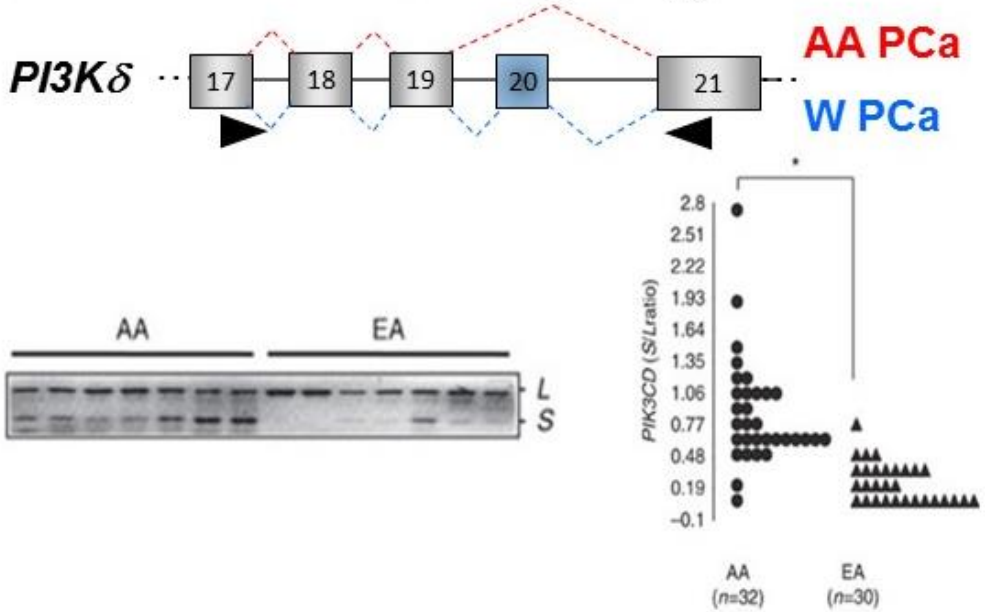
Differential RNA splice variants also present in the patient's adjacent normal prostate tissue

72% of these DSG were previously implicated in cancer

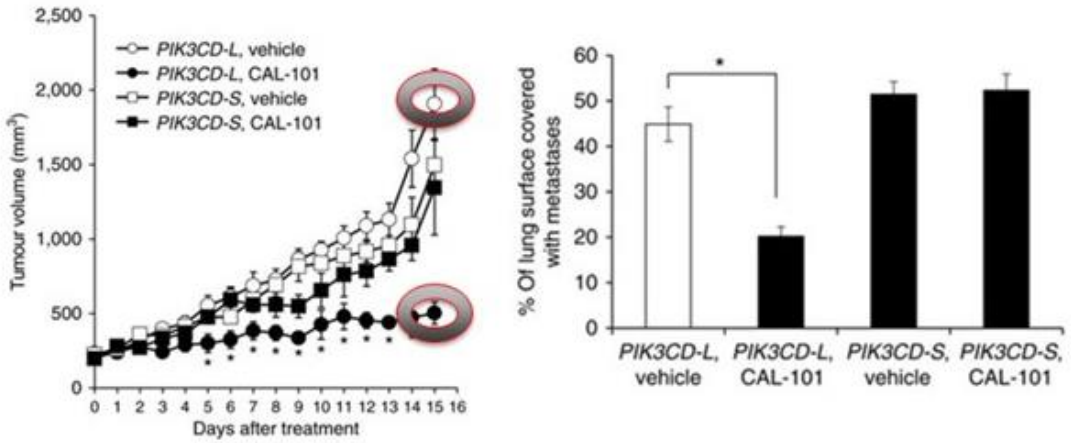
Functional Impact of Race-related *PI3Kδ* ARS

nature COMMUNICATIONS

A. *PI3Kδ* differential RNA splicing, PCa

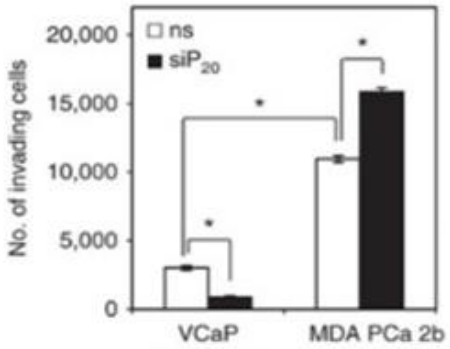


C. *PI3Kδ-S* is resistant to PI3Kδ inhibition



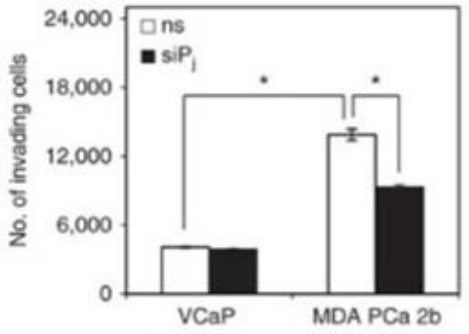
B. *PI3Kδ-S* drives race-related PCa aggressiveness

Knockdown Long Variant

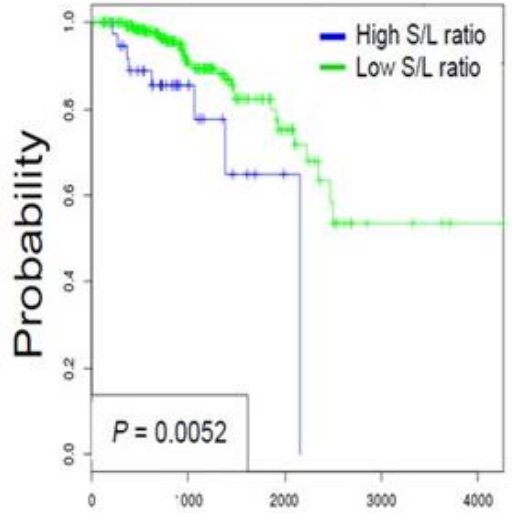


(Increase Ratio Short:Long In AA PCa)

Knockdown Short Variant



D. Lower DFS, PRAD patients high *PI3Kδ-S/PI3Kδ-L* ratio



CanSplice® visualizes TCGA data and compares ARS, aggregate gene expression, and survival in cancers of patients of different races

Select tumor type

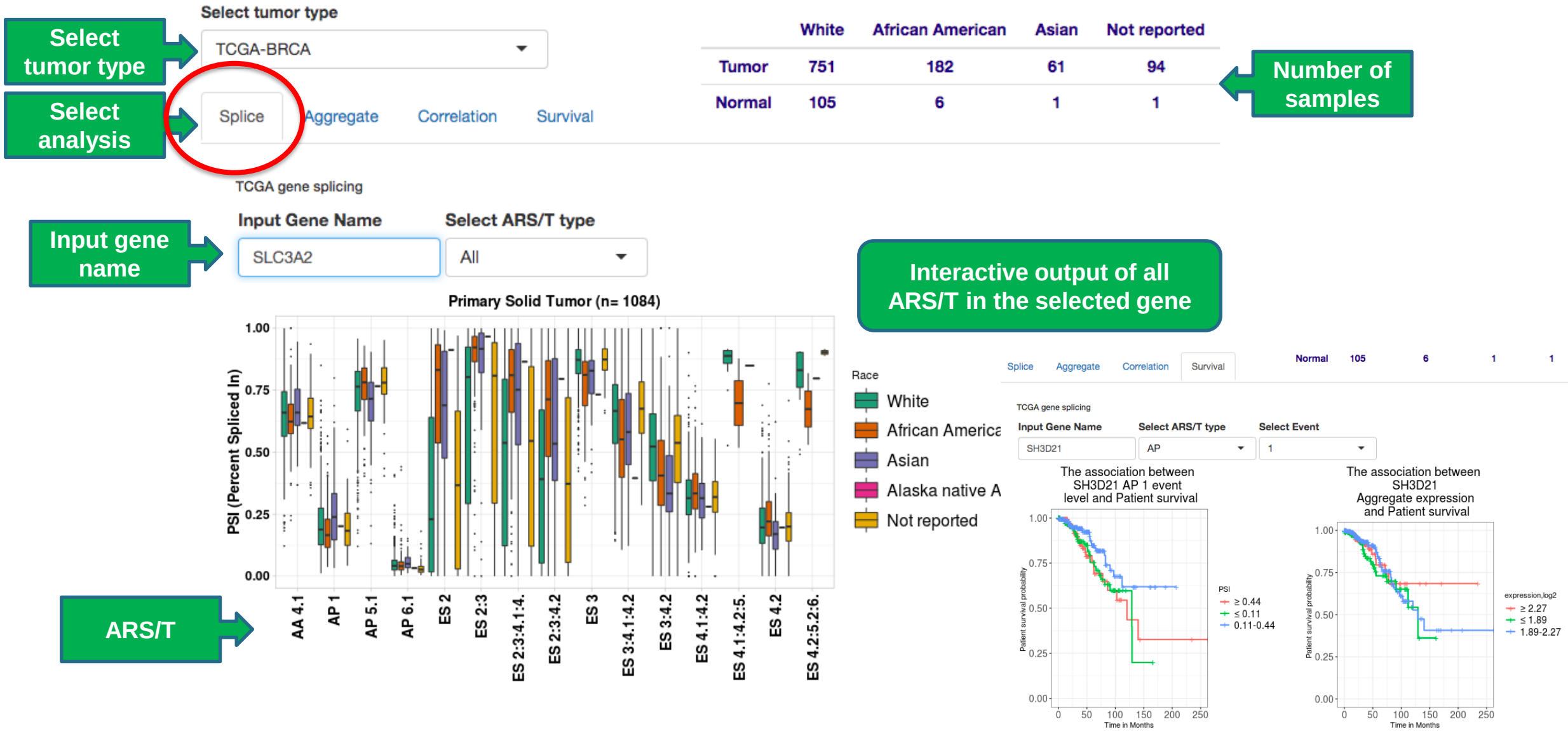
Select analysis

Number of samples

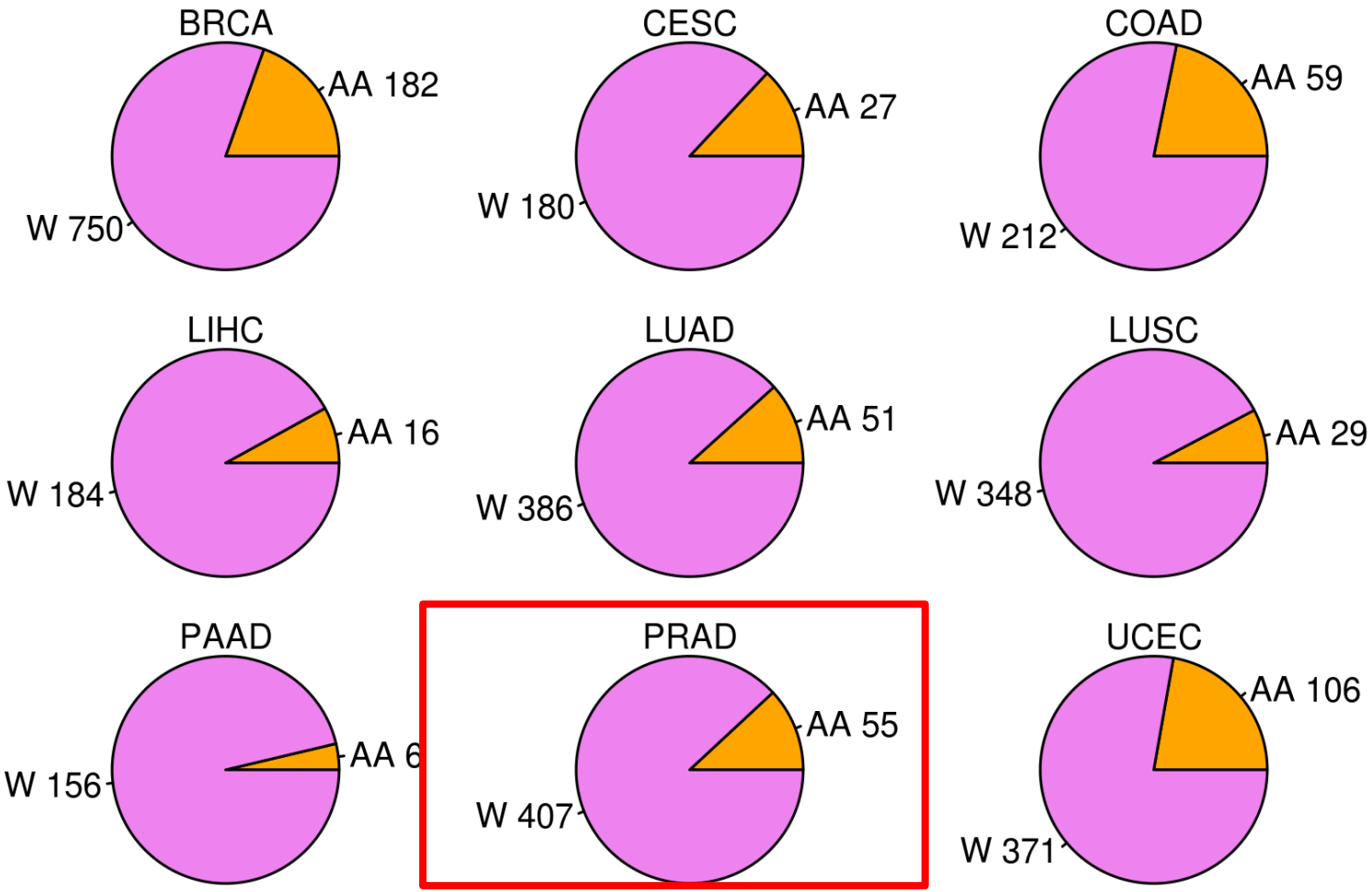
Input gene name

Interactive output of all ARS/T in the selected gene

ARS/T



TCGA samples included in our analysis

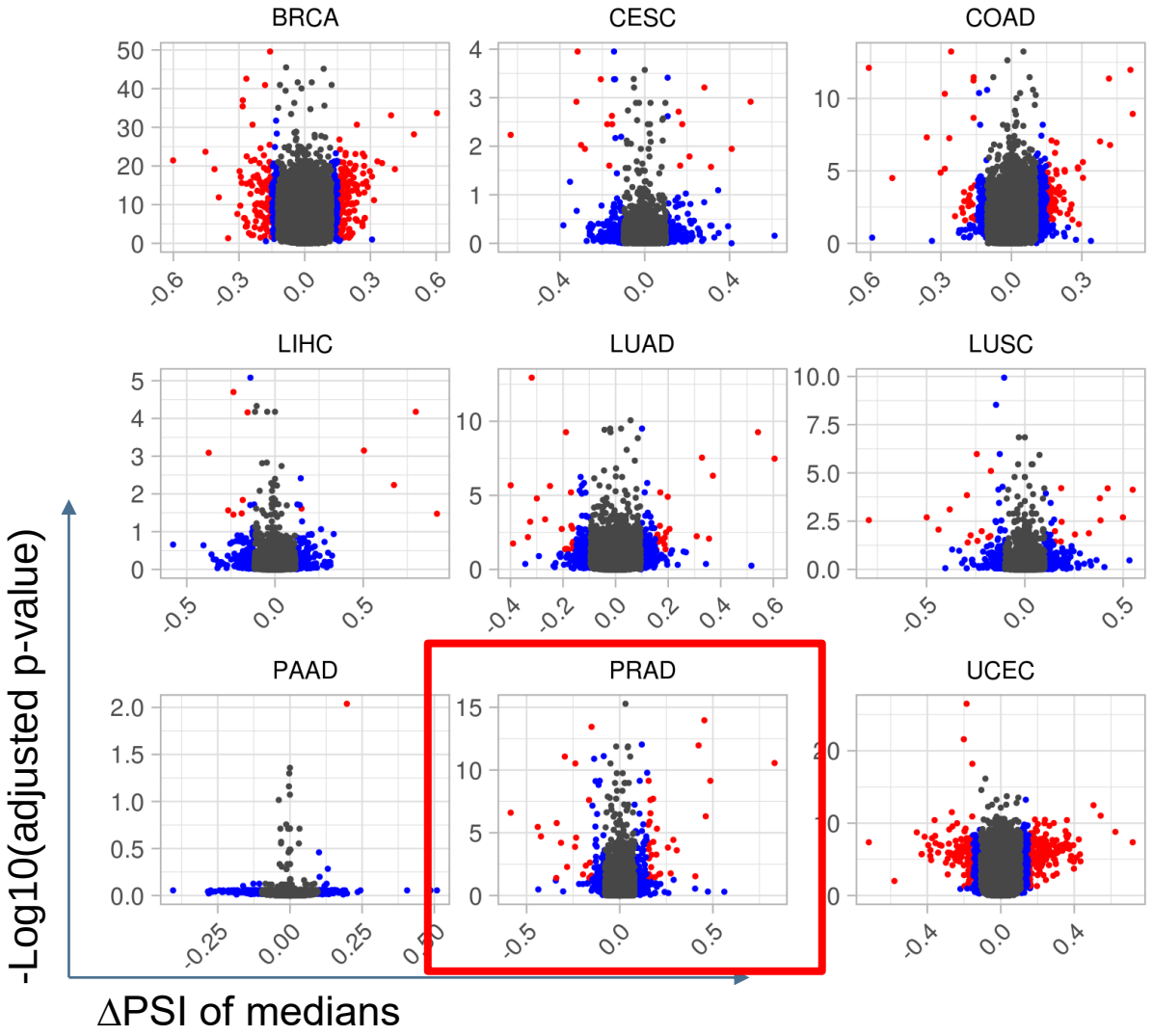


AA: African American
W: White

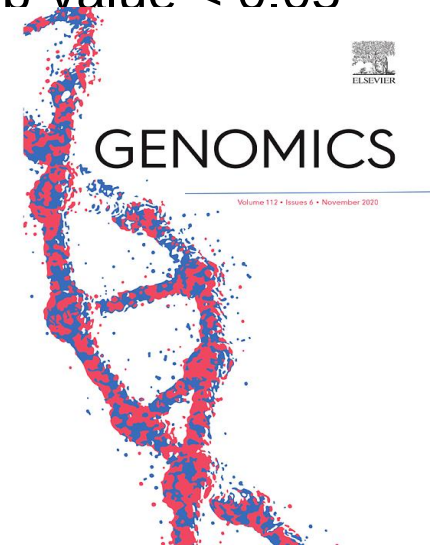
Cancer	TCGA Study Abbreviation
Breast	BRCA
Cervical Cancer	CESC
Colon	COAD
Liver	LIHC
Lung adeno	LUAD
Lung squamous	LUSC
Pancreatic	PAAD
Prostate	PRAD
Uterine	UCEC

Al Abo et al., 2021

Identification of race-related ARS in TCGA

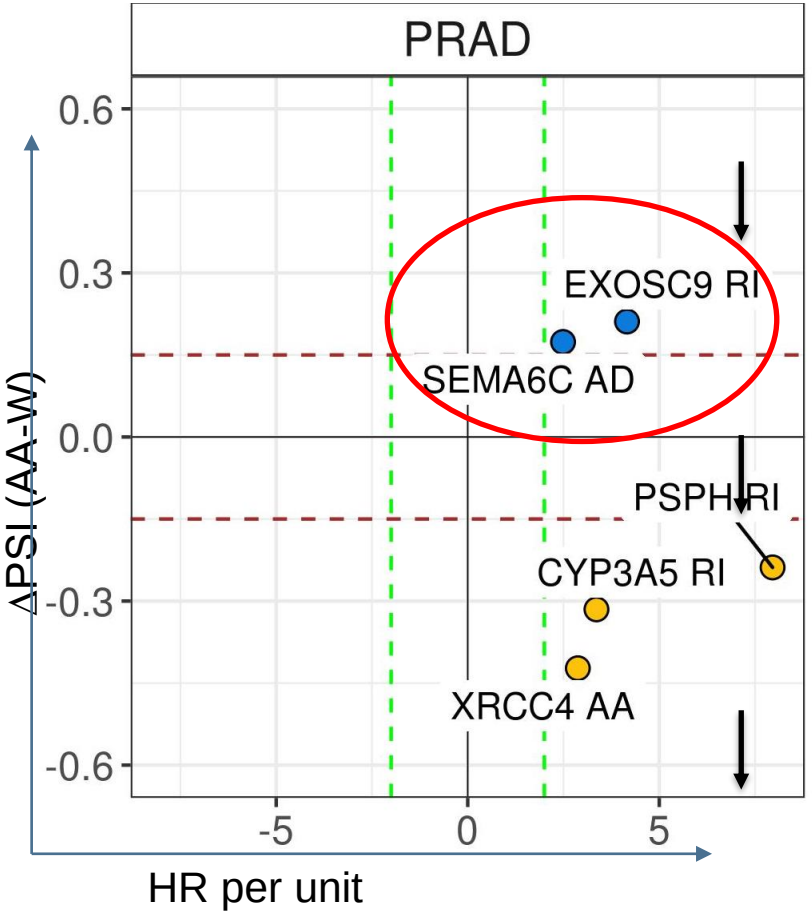


- **Top-ARS**: top 500 D-ARS ranked by absolute ΔPSI between AA and W
- **D-ARS:** top D-ARS exhibiting absolute ΔPSI > 15% between AA and W and adjusted p-value < 0.05



Al Abo et al., 2021

Race-related ARS associated with prostate cancer patient disease-free survival probability



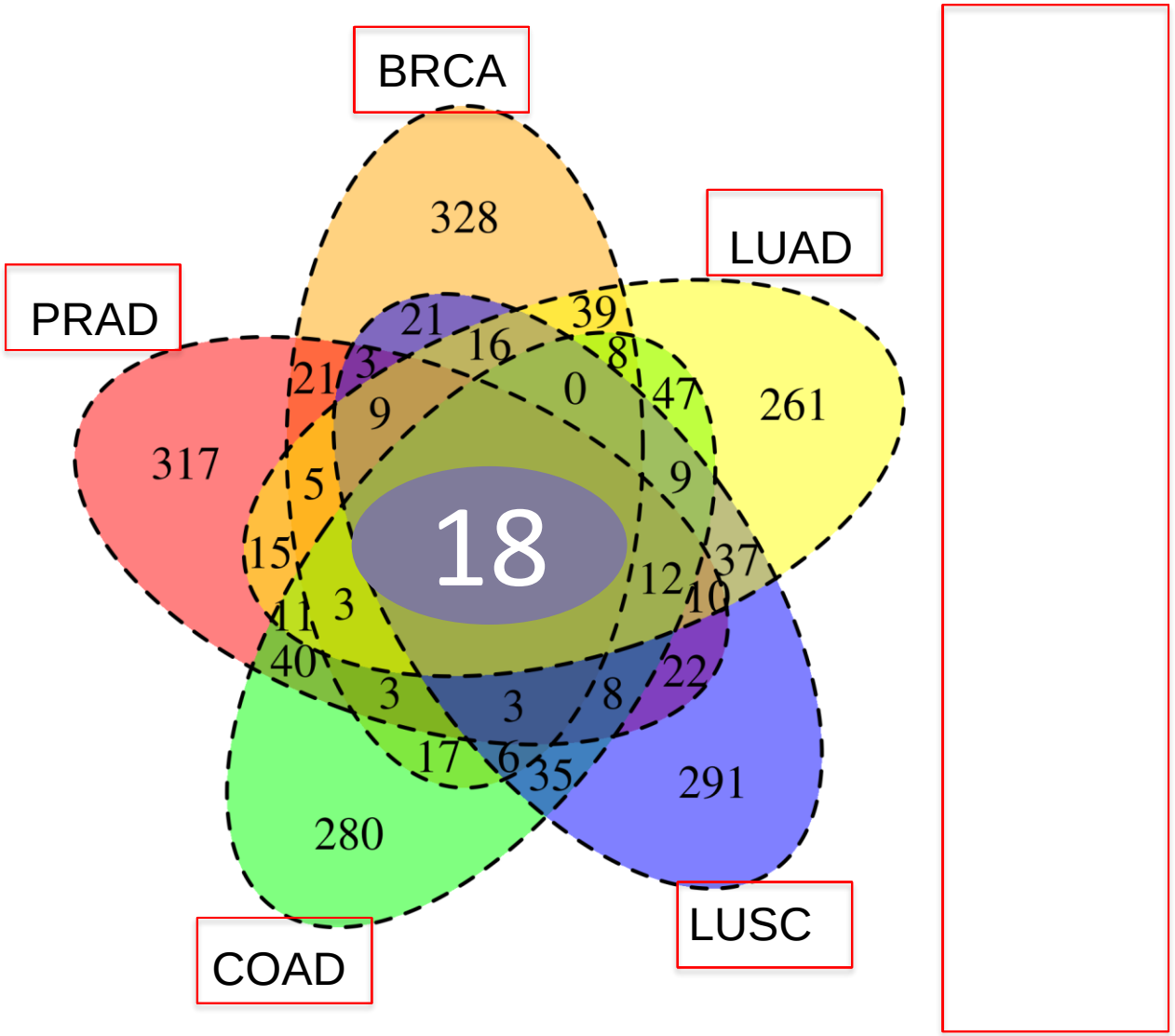
PSI: Percent Spliced In
ΔPSI: difference in splicing level

D-ARS associated with poor survival probability in AA (better in W)

D-ARS associated with poor survival probabilitv in W (better in AA)

Al Abo et al., 2021

Overlapping race-related ARS among prostate, colon, lung and breast cancers

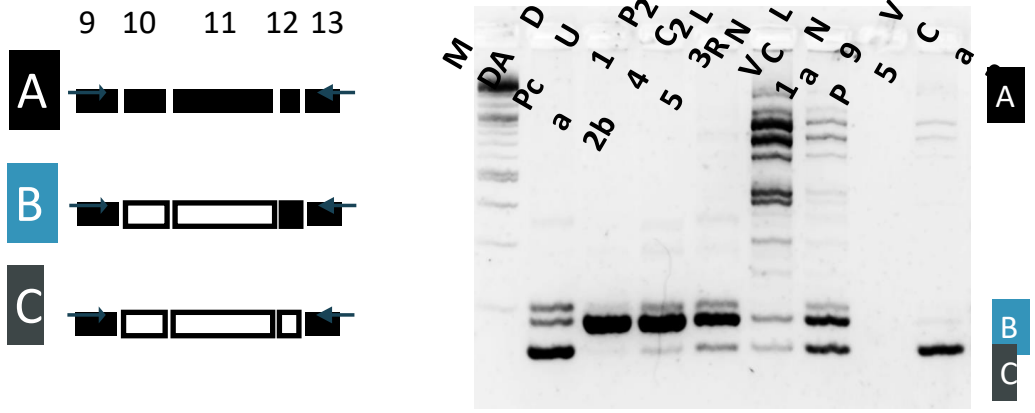


Al Abo et al., Genomics 2021

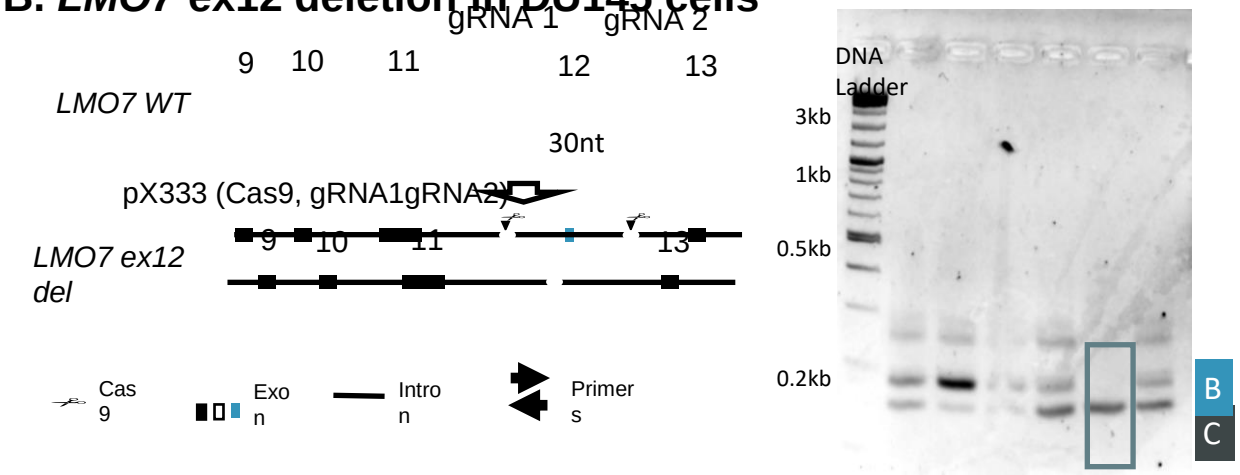
Targets in Common to PRAD, BRCA, LUAD, LIHC	Gene Description	Alternative Splicing Event
INSR	Insulin receptor	Skip of exon 11
CD44	Receptor for hyaluronic acid	Skip of exon v1-v10
ITGA6	Integrin, alpha 6	Skip of exon 5
RELN	Extracellular matrix serine protease	Skip of last exon
ABLIM3	Actin binding LIM protein family, 3	Skip of exon 14
BPTF	Bromodomain PHD finger transcription factor	Skip of exon 5
COL6A3	Collagen, type VI, alpha 3	Skip of exon 4
EHBP1	EH domain binding protein 1	Skip of exon 15
FN1	Fibronectin 1	Skip of exon 40
LMO7	LIM domain 7 – modulates actin signaling	Skip of exon 12

Functional Impact of Race-related *LMO7* ARS – exon 12

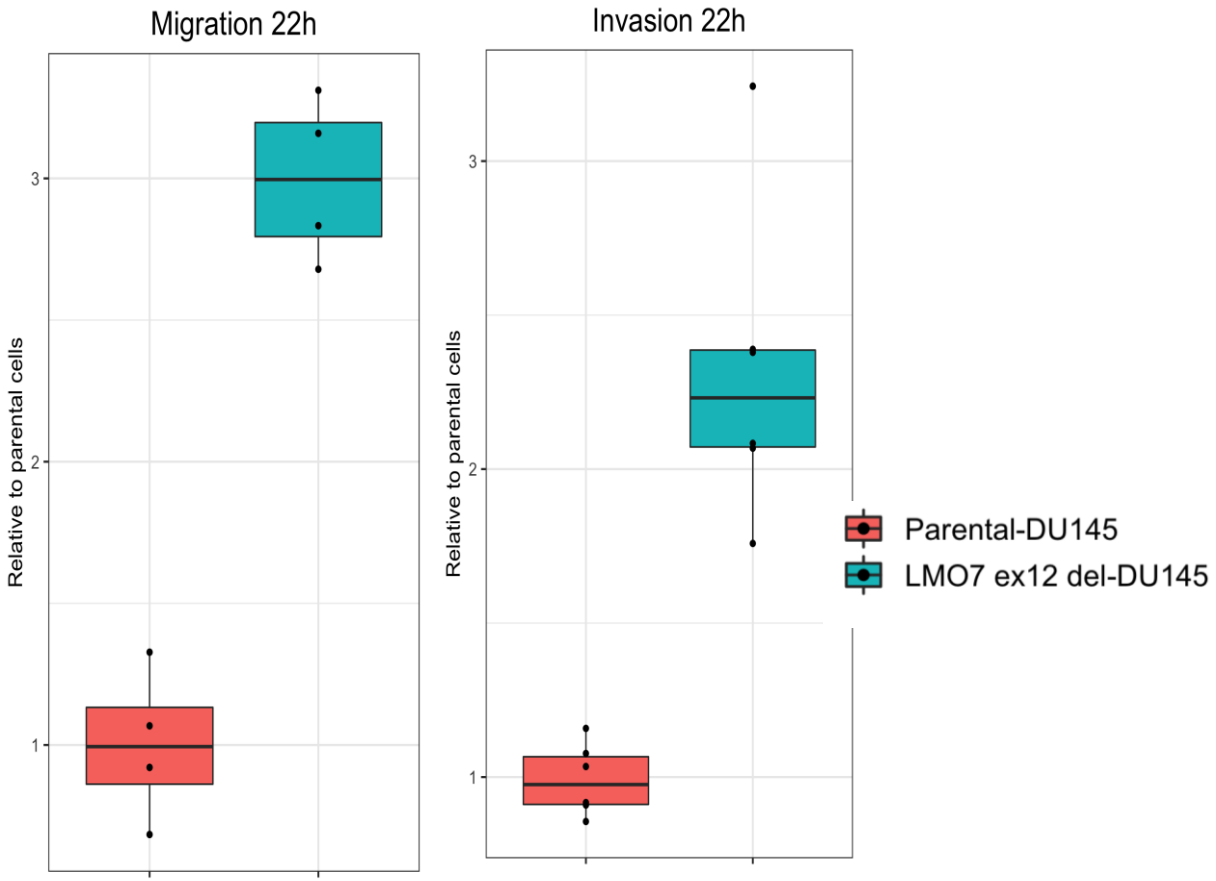
A. *LMO7* differential RNA splicing, prostate cancer cells



B. *LMO7* ex12 deletion in DU145 cells



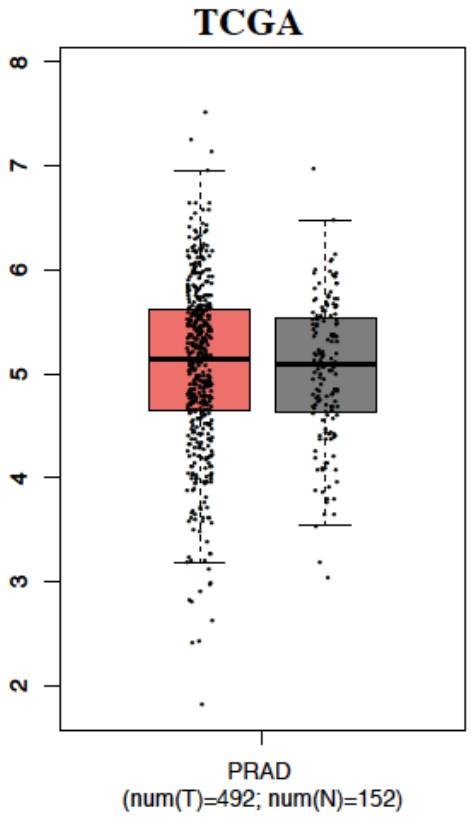
C. Increased migration and invasion in LMO7 ex12 del cells

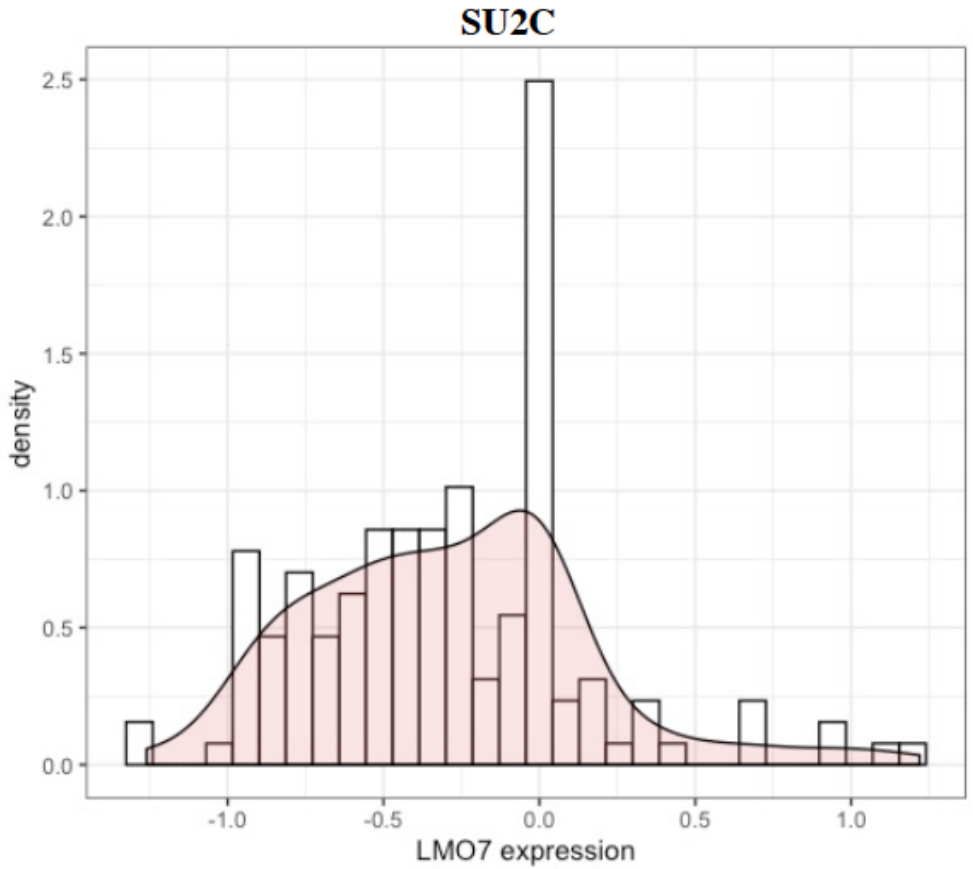


LMO7 in primary vs metastatic PC

TCGA (primary cancer): No difference in aggregate expression of LMO7 in Normal vs Tumor in PC

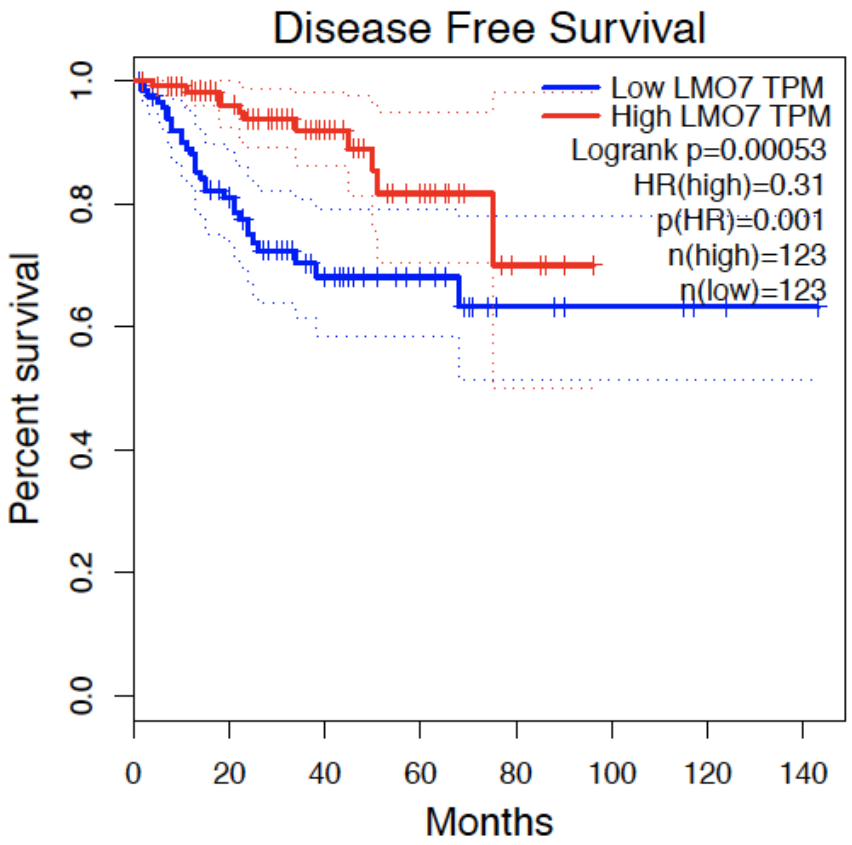
SU2C (metastatic): Big difference in frequency of loss of expression of LMO7

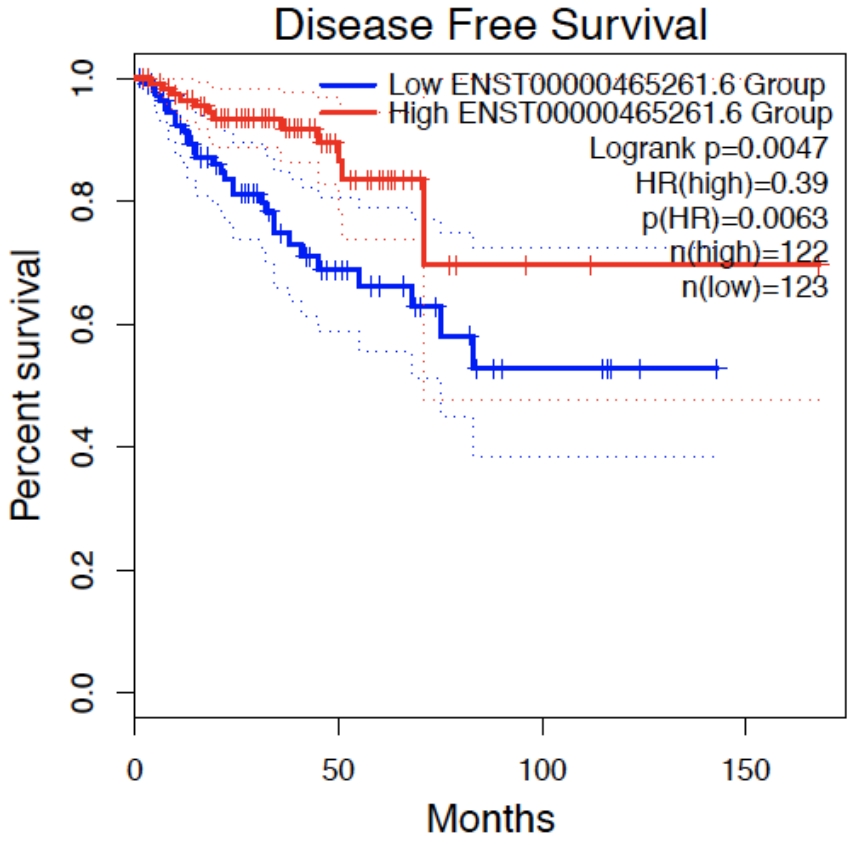




Clinical Impact of Race-related *LMO7* ARS in PC

Association between *LMO7* low expression or ex12 skipping and disease free survival in PCa

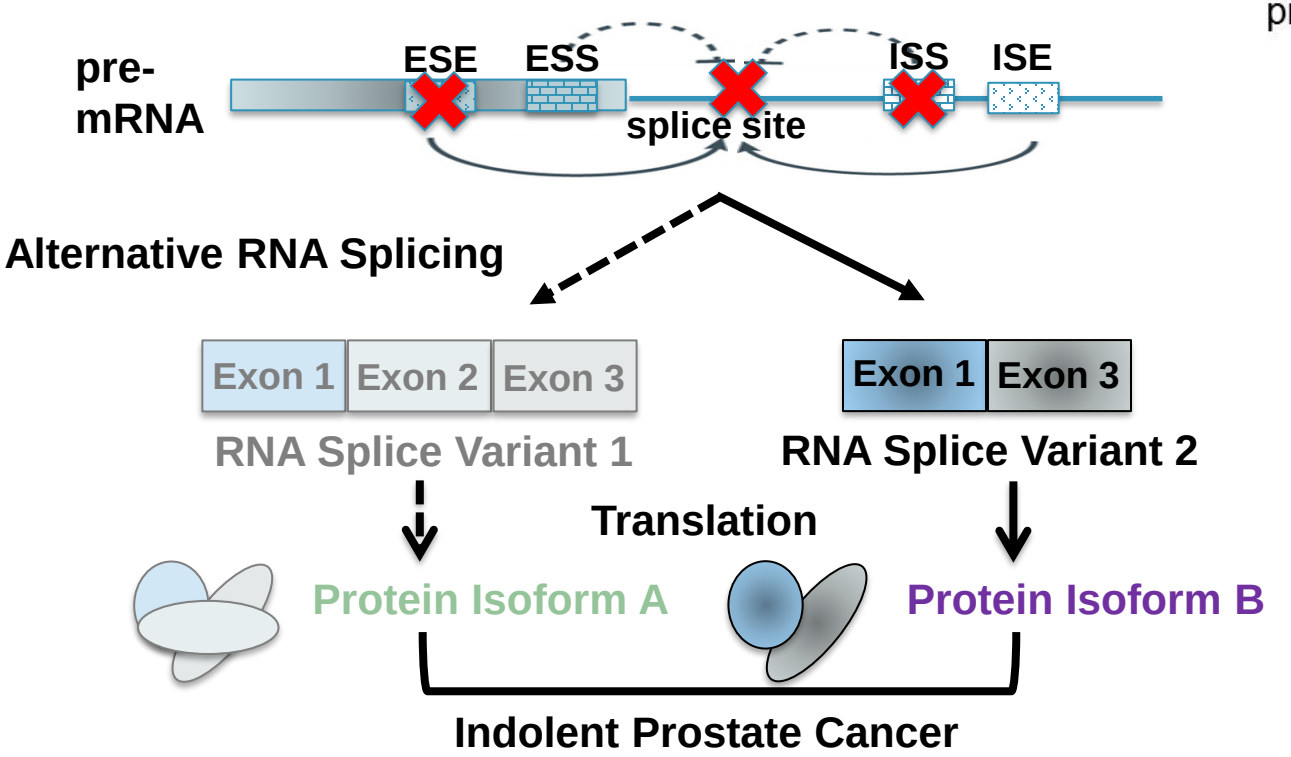




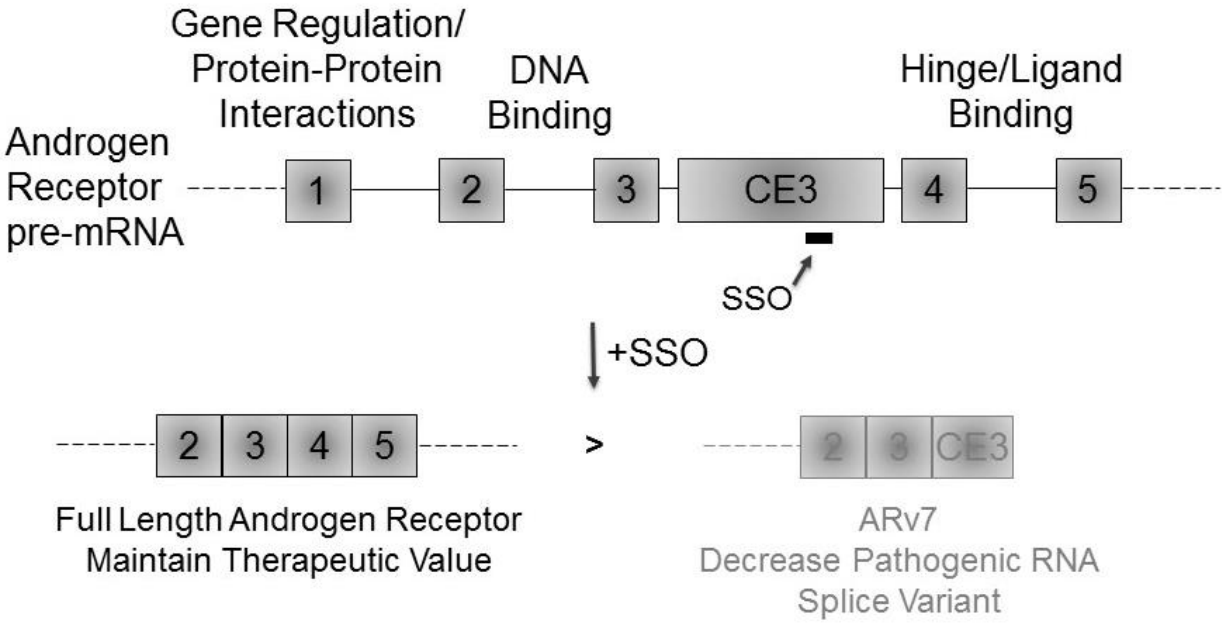
Therapeutic Targeting of Alternative RNA Splicing

Splicing regulatory regions can be targeted with **Splice-Switching Oligonucleotides SSOs**

Simultaneously limit pathogenic RNA splice variants and maintain/induce RNA splice variants with therapeutic value

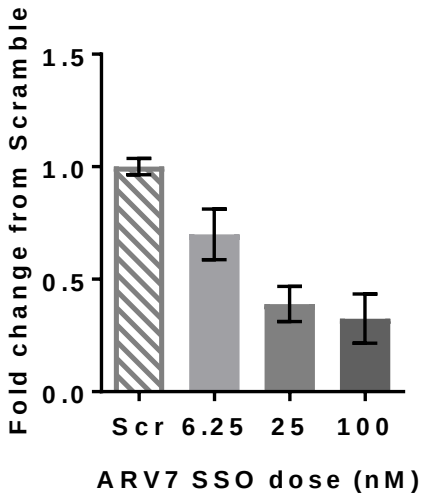


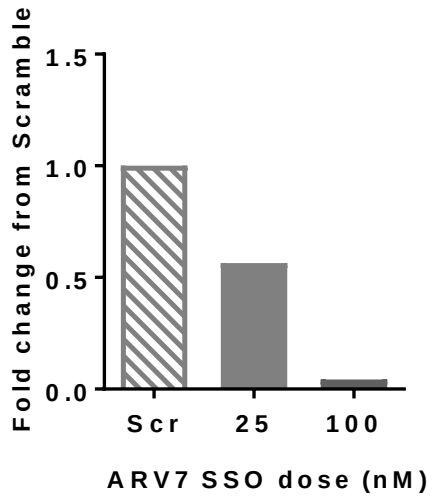
SSOs against the AR-V7 variant of the Androgen Receptor



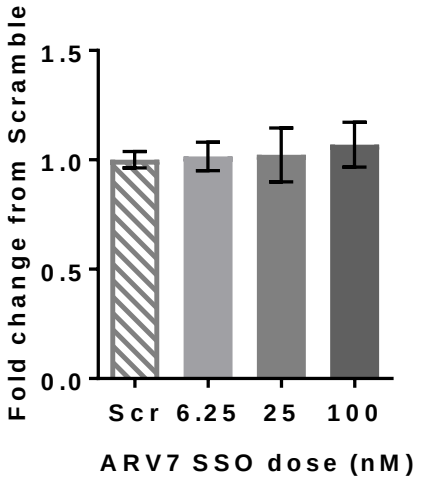
Targeting ARS: Splice-Switching Oligonucleotides (SSO)

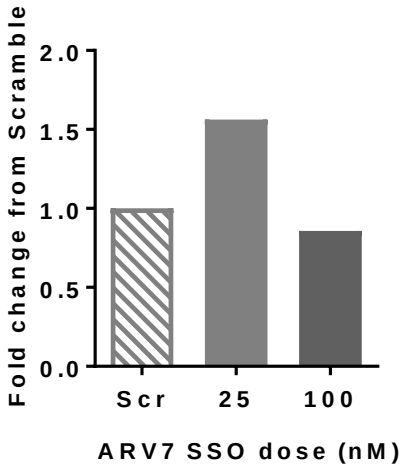
ARv7 SSO decreases ARv7 RNA and protein



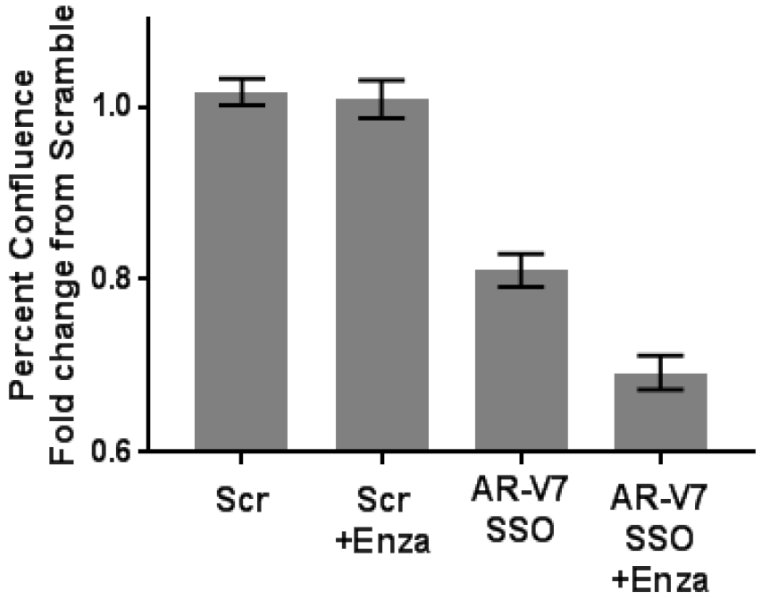


ARv7 SSO maintains full length AR RNA and protein





ARv7 SSO decreases proliferation, restores sensitivity to enzalutamide



LaCroix *et al*.,

Deep RNAseq of GENCADE

72 patients (Blacks and Whites)

Fresh frozen – tumor and adjacent

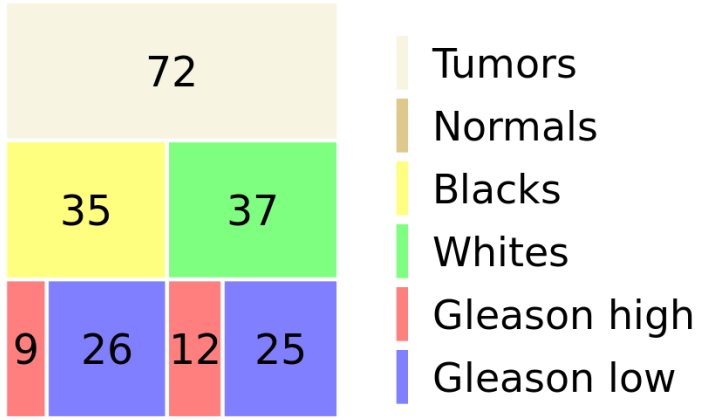
Extensive MR data available

DNA for ancestry

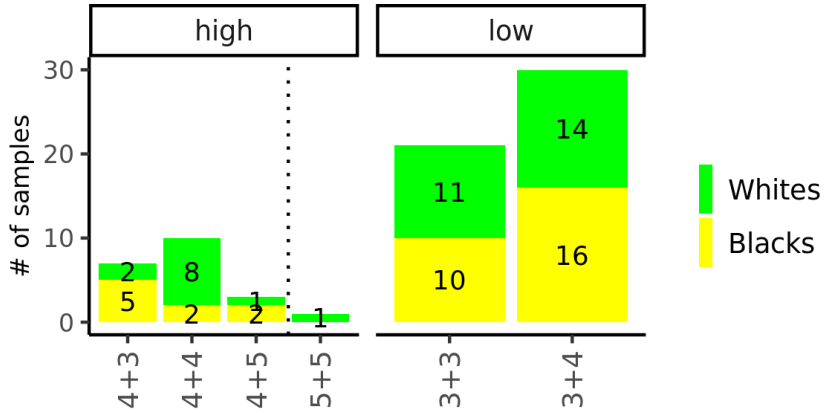
RNA for deep RNAseq – 150 length reads
And >100 million read per samples

Aggregate gene expression by STAR2 pass and htseq

Splicing by SpliceSeq



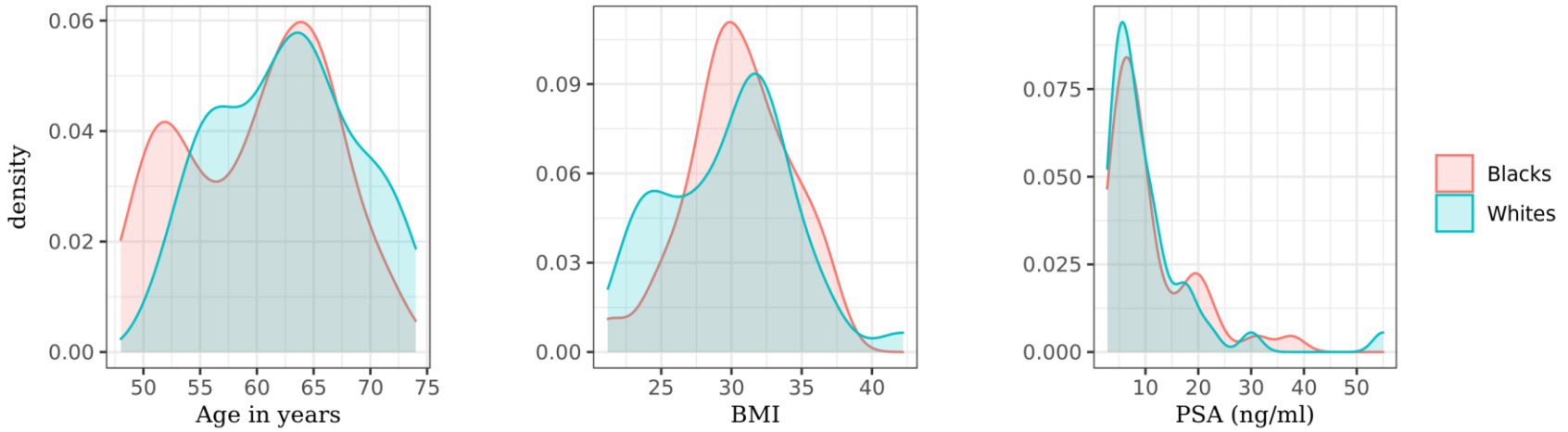
Tumor samples have matching adjacent normal samples



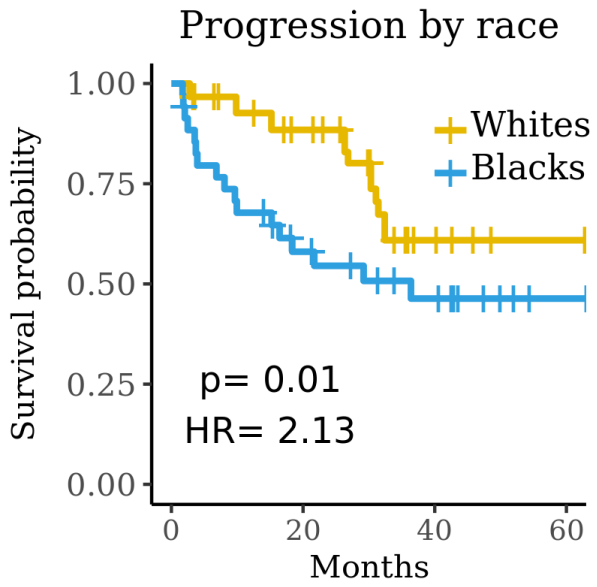
Breakdown of the samples by Gleason scores and race.

DO NOT POST

Deep RNAseq of GENCADE



Density plots showing age, BMI and PSA levels in Black and White GENCADE prostate cancer patients



Survival analysis of GENCADE prostate cancer patients shows significant increase in progression in Black patients compared to White patients

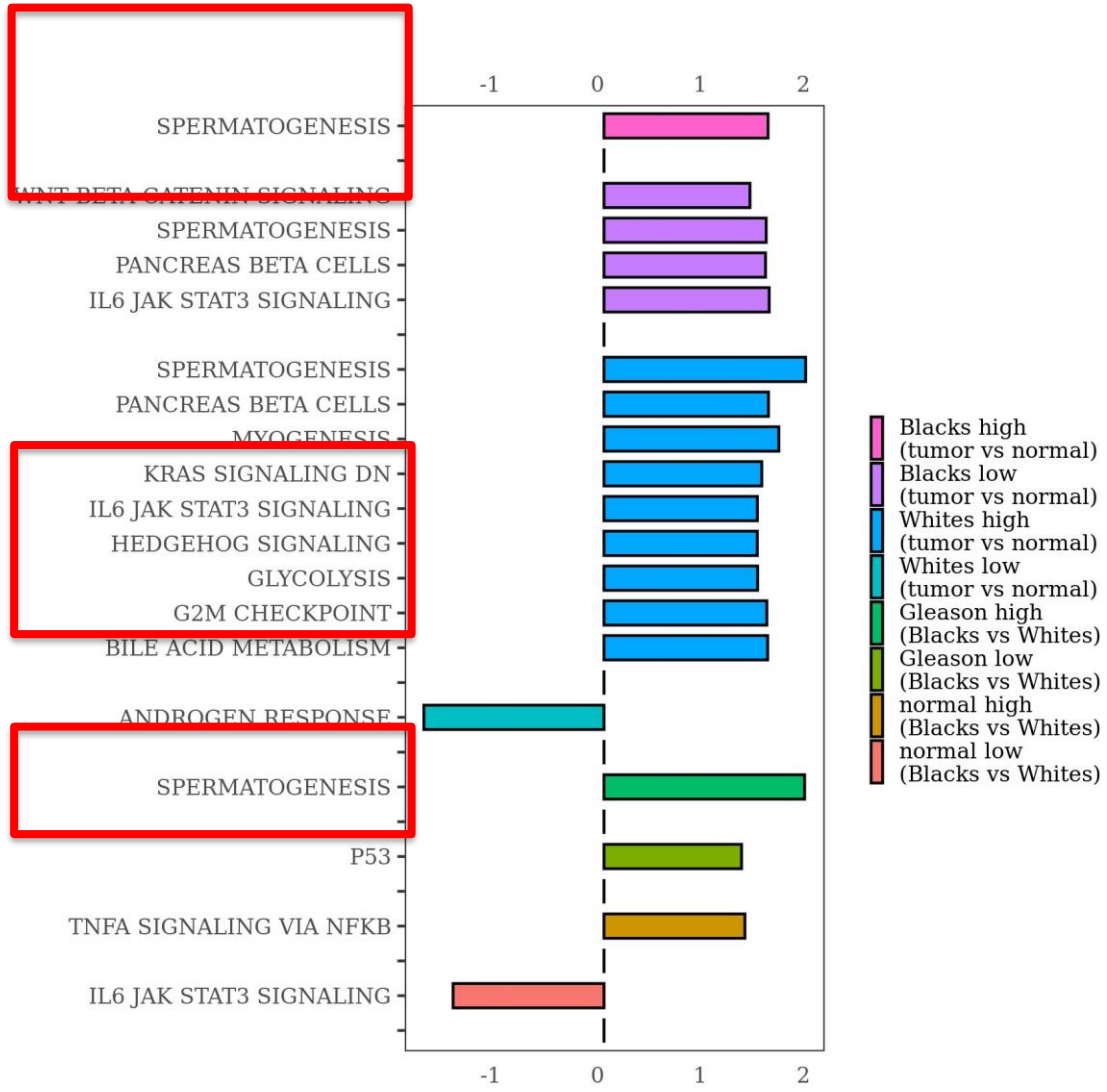
DO NOT POST

Gene Set Enrichment Analysis (Hallmark 50 pathways)

The gene set enrichment analysis for the identified race-related ARSTs in tumors or adjacent normal tissue and between tumors and adjacent normal tissue within Blacks and Whites exhibit enrichment in cancer-related pathways.

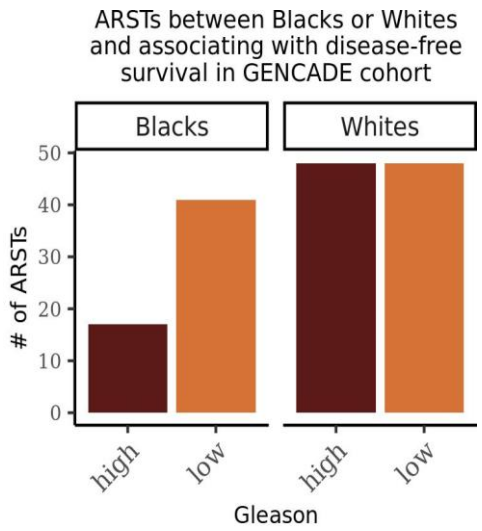
Cancer-related “spermatogenesis” genes showing race-related differential RNA splicing:

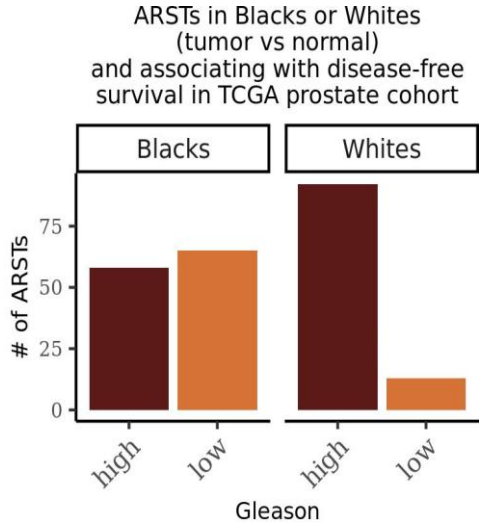
NF2, MTOR, AURKA, RADS17, BRCA, CDK1, PARP2, HOXB1



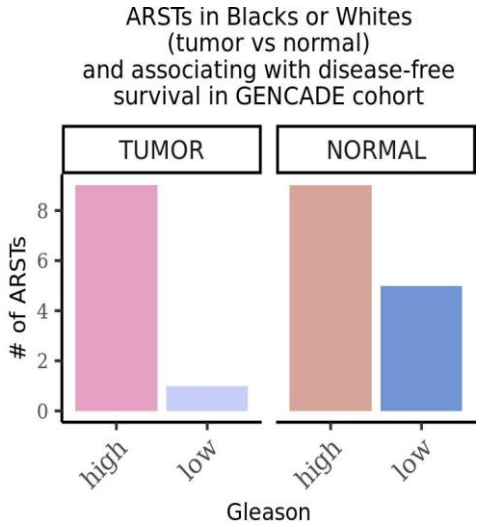
DO NOT POST

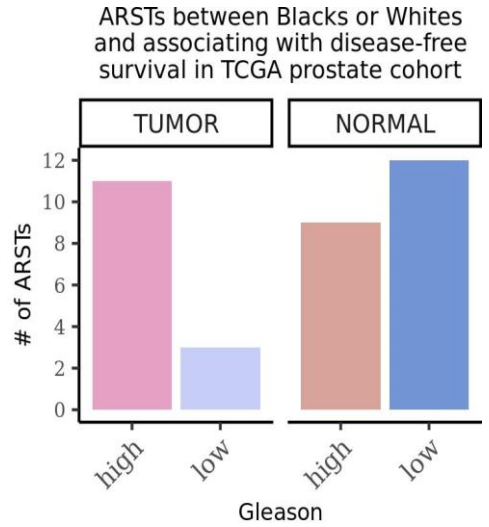
Number of race-related differentially spliced genes associated with disease-free survival - Hazard ratio > 2 and p-value < 0.05





Race-related ARSTs identified in adjacent normal tissue are also associated with survival. This implicates differences in the tumor microenvironment that may play a critical role in differential cancer progression.





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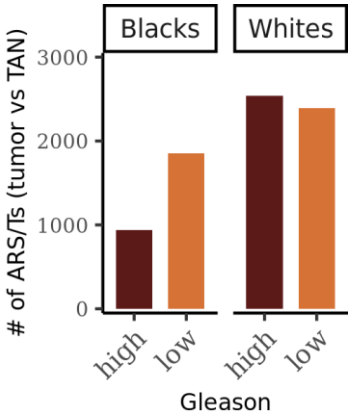
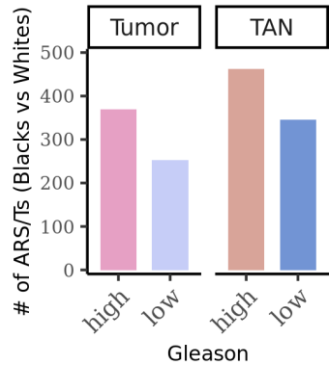
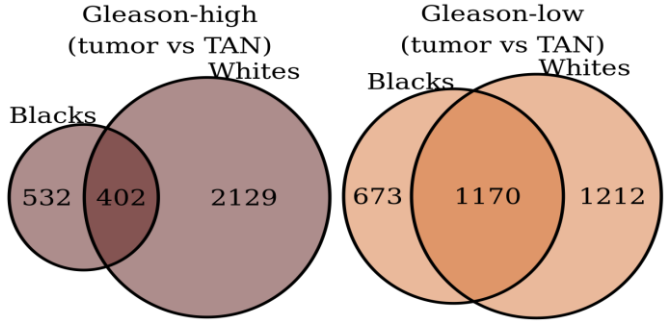
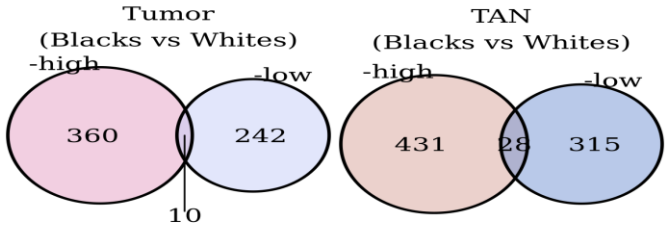

Blacks
Whites
# of ARS/Ts (tumor vs TAN)
3000
2000
1000
0
high
low
high
low
Gleason

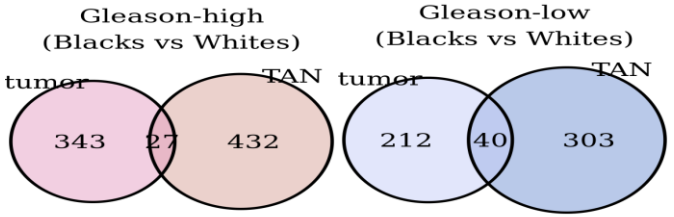

Tumor
TAN
# of ARS/Ts (Blacks vs Whites)
500
400
300
200
100
0
high
low
high
low
Gleason


Gleason-high
(tumor vs TAN)
Blacks
Whites
532
402
2129

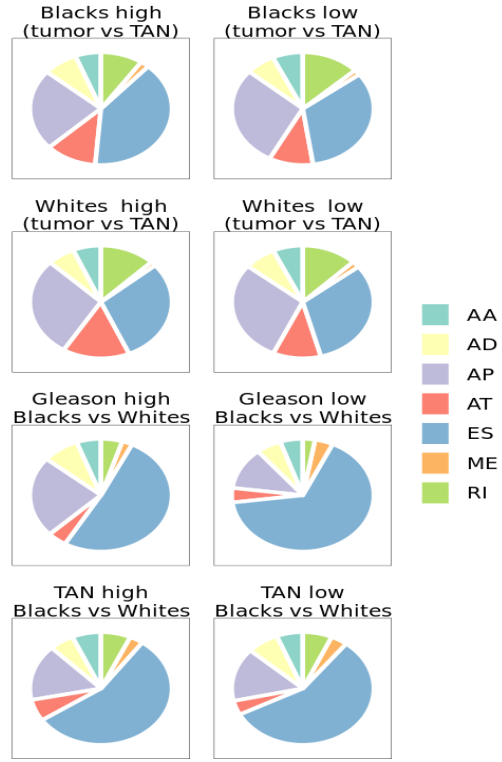
Gleason-low
(tumor vs TAN)
Blacks
Whites
673
1170
1212

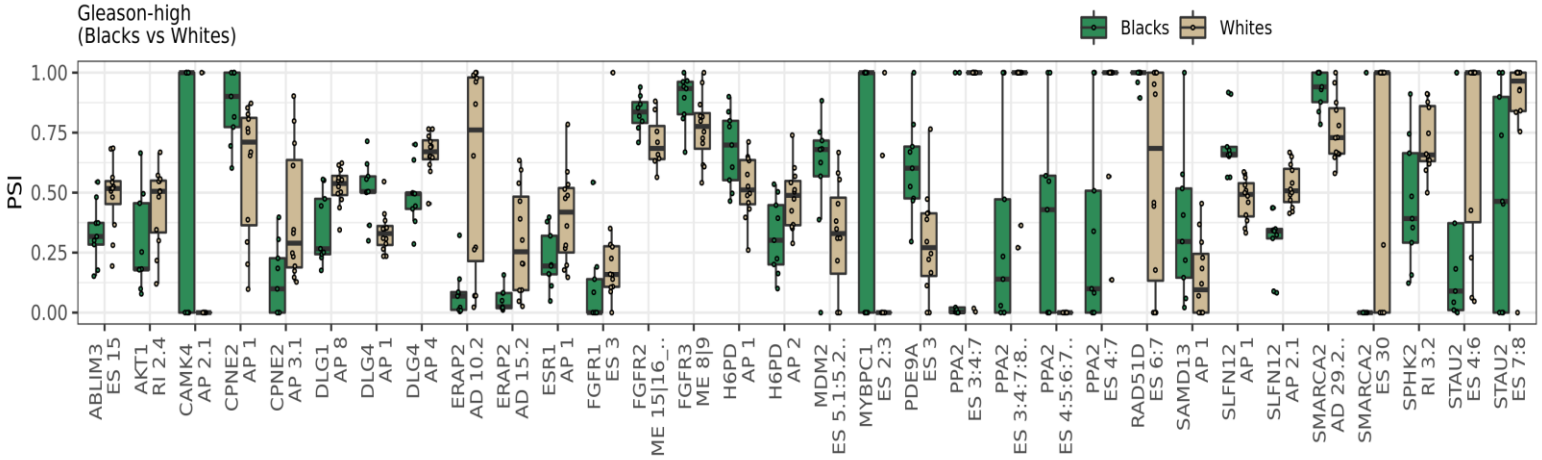

Tumor
(Blacks vs Whites)
-high
-low
360
242
10

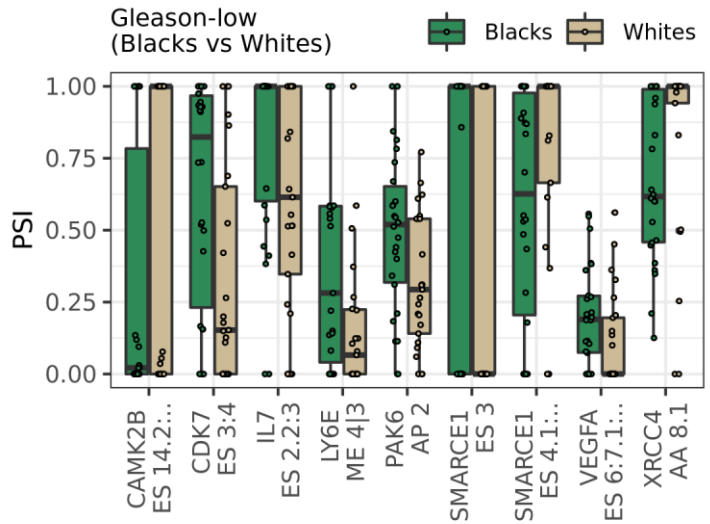
TAN
(Blacks vs Whites)
-high
-low
431
28
315


Gleason-high
(Blacks vs Whites)
tumor
TAN
343
27
432

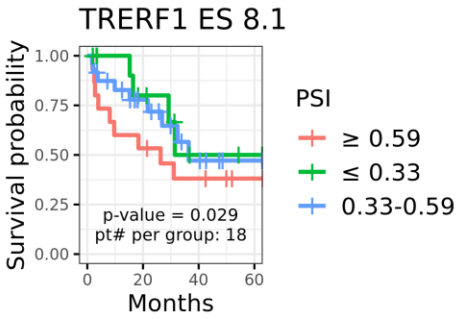
Gleason-low
(Blacks vs Whites)
tumor
TAN
212
40
303

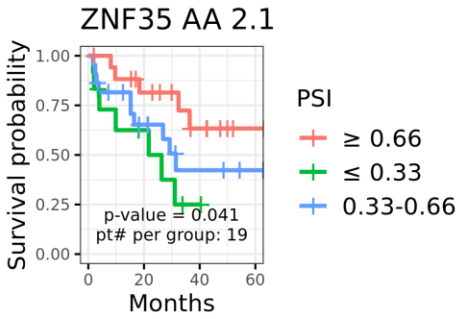

Blacks high
(tumor vs TAN)
Blacks low
(tumor vs TAN)
Whites high
(tumor vs TAN)
Whites low
(tumor vs TAN)
Gleason high
Blacks vs Whites
Gleason low
Blacks vs Whites
TAN high
Blacks vs Whites
TAN low
Blacks vs Whites
AA
AD
AP
AT
ES
ME
RI

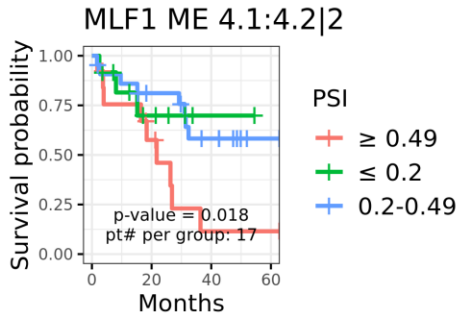

Gleason-high
(Blacks vs Whites)
Blacks
Whites
PSI
1.00
0.75
0.50
0.25
0.00
ABLIM3 ES 15
AKT1 RI 2.4
CAMK4 AP 2.1
CPNE2 AP 1
CPNE2 AP 3.1
DLG1 AP 8
DLG4 AP 1
DLG4 AP 4
ERAP2 AD 10.2
ERAP2 AD 15.2
ESR1 AP 1
FGFR1 ES 3
FGFR2 ME 15|16_..
FGFR3 ME 8|9
H6PD AP 1
H6PD AP 2
MDM2 ES 5.1:5.2..
MYBPC1 ES 2:3
PDE9A ES 3
PPA2 ES 3:4:7
PPA2 ES 3:4:7:8..
PPA2 ES 4:5:6:7..
PPA2 ES 4:7
RAD51D ES 6:7
SAMD13 AP 1
SLFN12 AP 1
SLFN12 AP 2.1
SMARCA2 AD 29.2..
SMARCA2 ES 30
SPHK2 RI 3.2
STAU2 ES 4:6
STAU2 ES 7:8

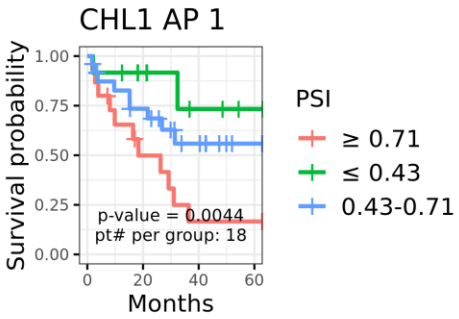

Gleason-low
(Blacks vs Whites)
Blacks
Whites
PSI
1.00
0.75
0.50
0.25
0.00
CAMK2B ES 14.2:...
CDK7 ES 3:4
IL7 ES 2.2:3
LY6E ME 4|3
PAK6 AP 2
SMARCE1 ES 3
SMARCE1 ES 4.1:...
VEGFA ES 6:7.1:...
XRCC4 AA 8.1

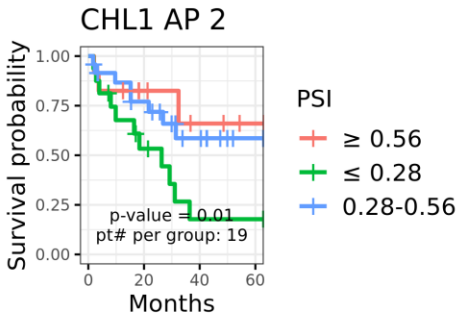
Disease-free survival Analysis: association between race-related differentially spliced genes in High or Low Gleason tumors (GENCADE)

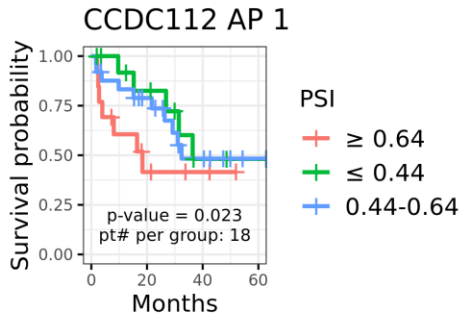


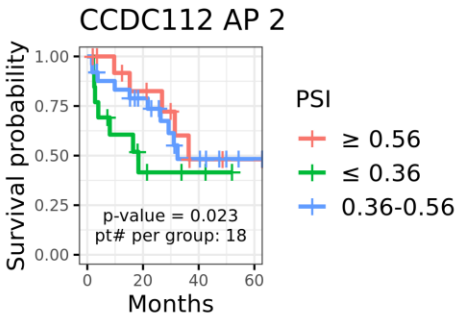


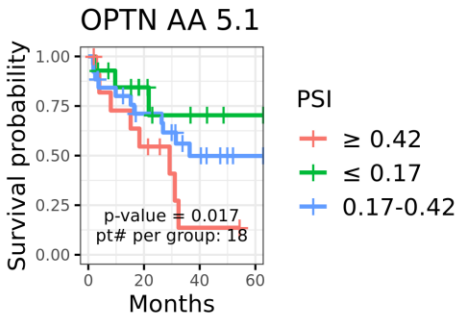


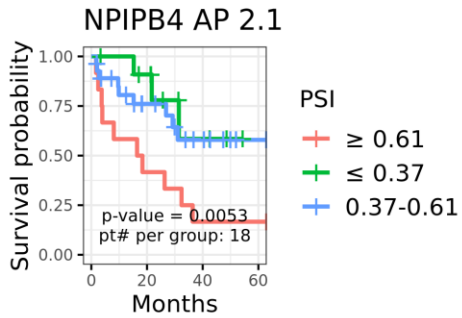




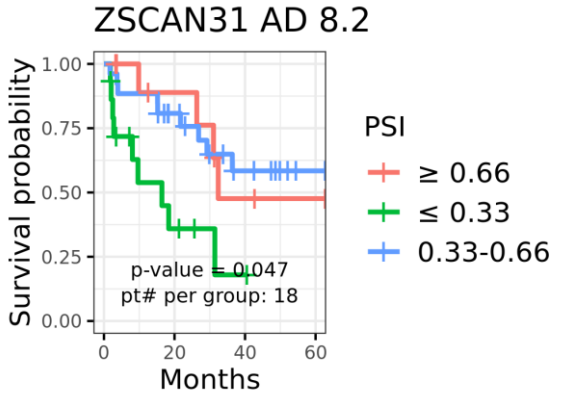








PSI = Percent Spliced In: An arbitrary measure of frequency of exon skipping in a particular gene



DO NOT POST

Rationale for Comparative Ancestral Transcriptomics (CAT) Approach

This approach is focused on patient-centered precision oncology, NOT race-based medicine

Population-level differences in RNA splicing allow us to hone in on targets driving tumor aggressiveness

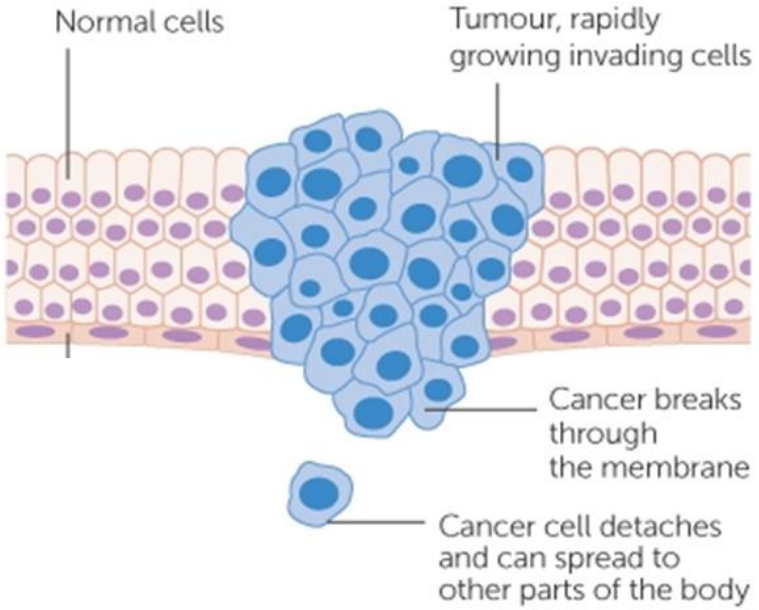
Once identified, these targets can become the basis for biomarker stratified precision clinical trials for ALL patients whose cancer expresses the pathological splice variant

https://www.nih.gov/news-events/news-releases/nci-match-precision-medicine-clinical-trial-releases-new-findings-strengthens-path-forward-targeted-cancer-therapies

Translational Impact of CAT

Thousands of novel pathological splice variants across 9 tumors that:

- **Increase cancer cell growth**
- **Decrease cancer cell death**
- **Increase cancer spread**
- **Drive resistance to therapy**
- **Correlate with worse survival**

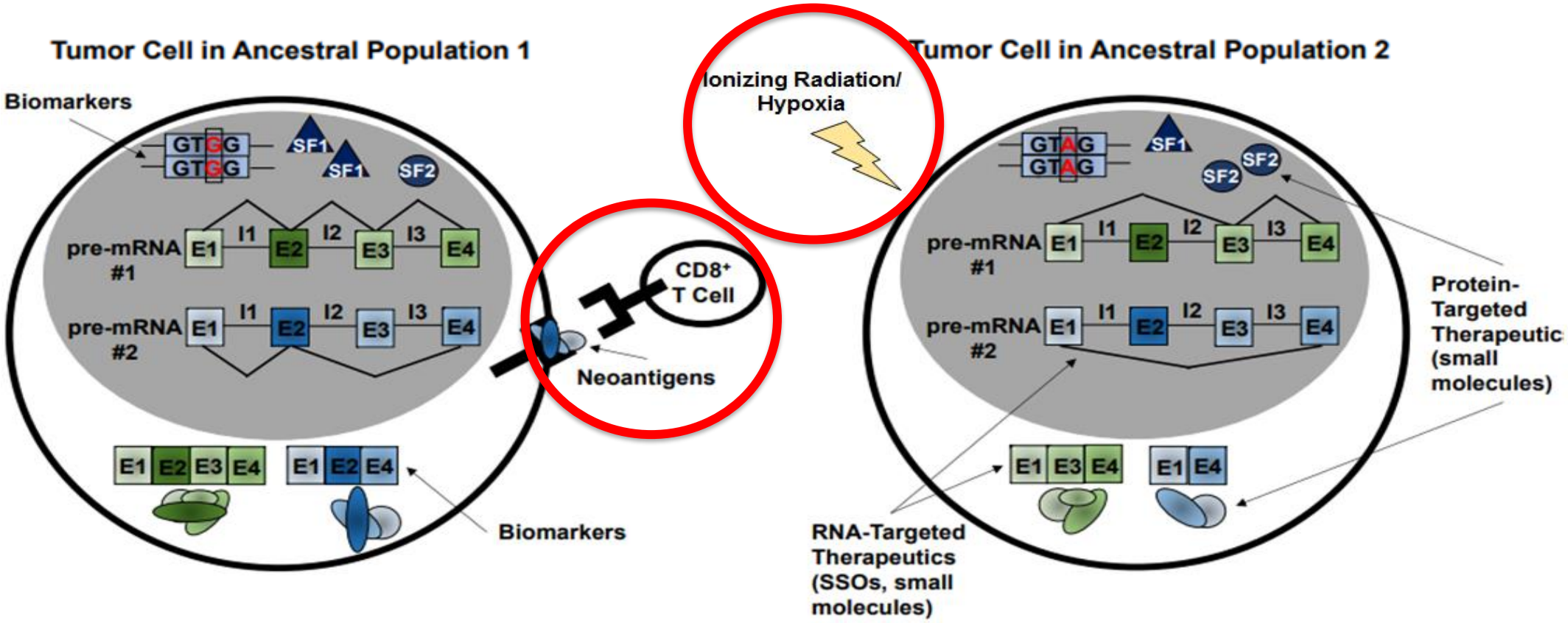


Can target the pathological RNA splicing event with Splice Switching Oligonucleotides (SSOs)

Can target the pathological splice variant proteoforms (small molecules)

ARS in Precision Oncology & Disparities

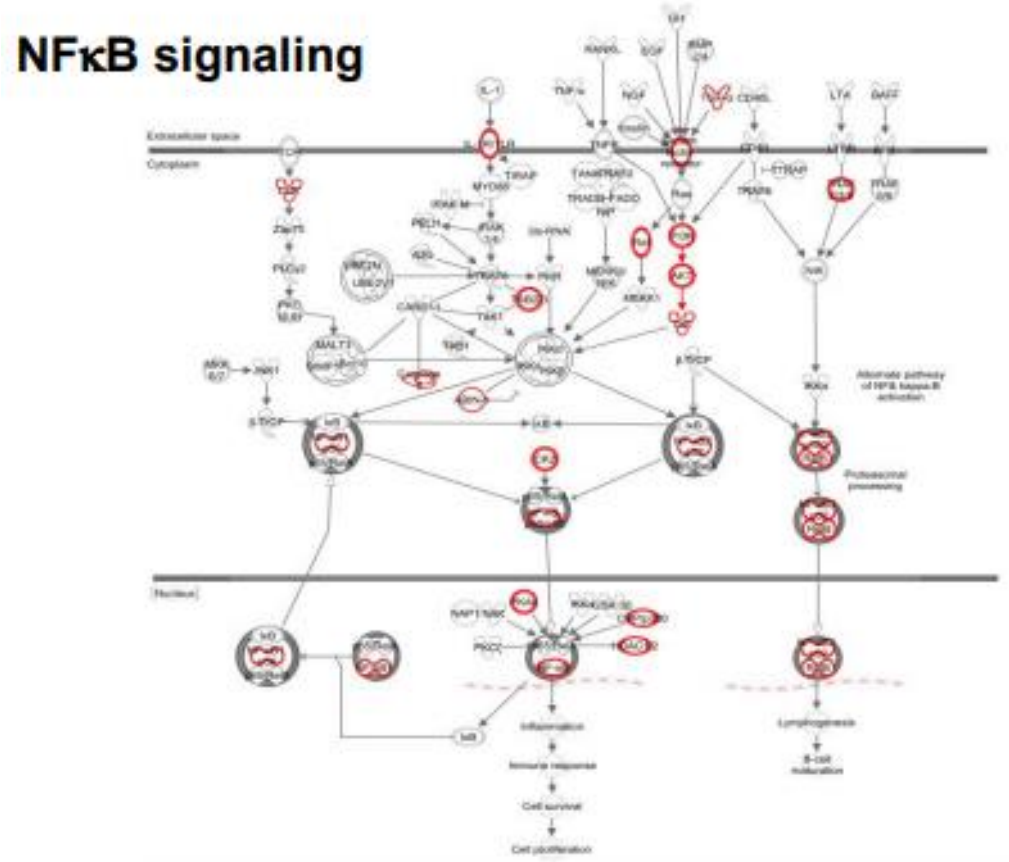
Clinical Cancer Research



Alternative RNA Splicing as a Potential Major Source of Untapped Molecular Targets in Precision Oncology and Cancer Disparities: Timothy Robinson, Jennifer Freedman, Muthana Al Abo, April Deveaux, Bonnie LaCroix, Brendon Patierno, Daniel George, Steven Patierno, 2019

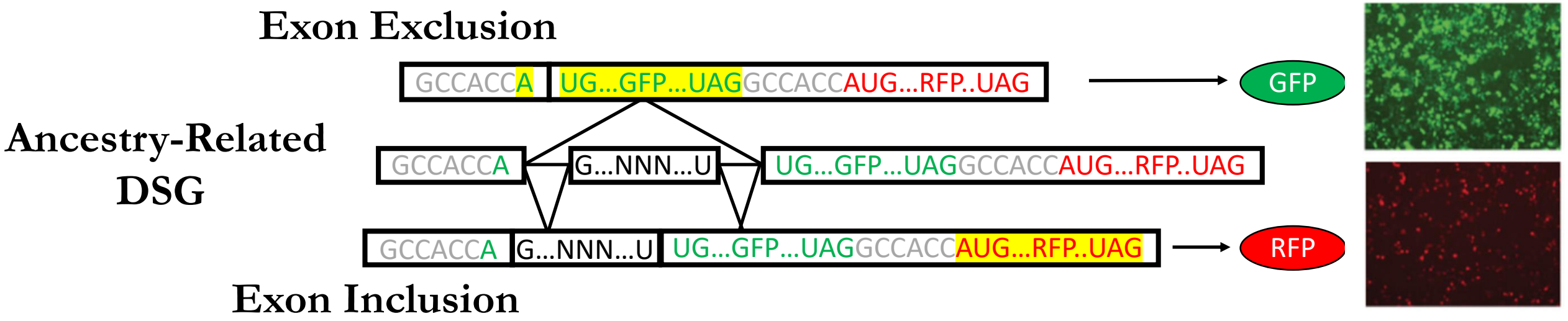
Race-related D-ARS and Immune-related Pathways

- **Race-related D-ARS in prostate cancer**
  - Adaptive immunity
  - Defense response to bacteria
  - Defense response to viruses
  - NFKB signaling
  - Innate immunity
  - Inflammasome signaling



Oncogenic signaling pathway of D-ARS over-represented in AA PC vs EA PC

Ancestry-Related Splicing Reporters for Drug Discovery

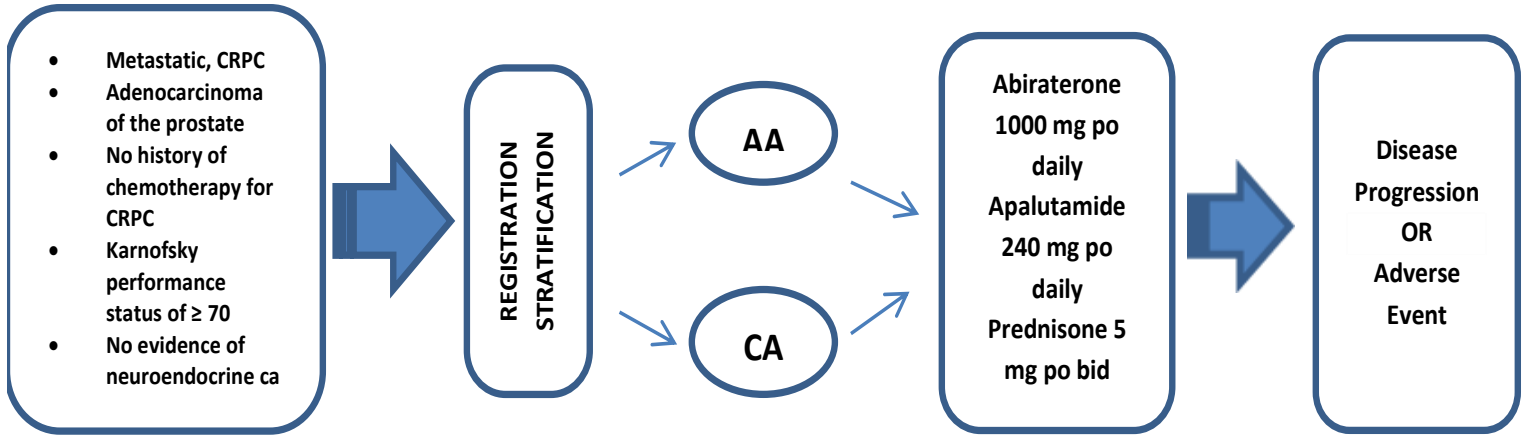


- High-throughput, screening, development and optimization of drugs targeting RNA splicing with real-time analysis and accurate quantitation
- **RNA splicing plasticity**: readout in response to drug treatment, environmental agents
- Identification of factors driving ancestry-related RNA splicing

Sean Piwarski, PhD, in preparation. Zheng, *et al.*, Genome Research, 2013, 23, p.998-1007

Extraordinary Results from Race-Stratified or Equal Representation by Race Clinical Trials

***ABI-RACE:** Abiraterone + Prednisone (EQR)
PROCEED: Sipuleucil-T stratified by race
DOCETAXEL: Docetaxel retrospective meta-analysis by race
***PANTHER:** Apalutamide, Abiraterone, Pred (EQR) (in progress)
***ASPIRE:** ADT, PARP Inhibitor in localized disease (EQR)



Improved biochemical response for African-American men with advanced prostate cancer and a stronger response to both hormone and chemotherapy therapy: Duke's Prostate & Urologic Cancer Center


Muthana Al Abo, PhD
April Deveaux, MD, PhD
Tyler Allen, PhD
Karen Wegermann, MD, PhD
Sean Piwarski, PhD
Joab Odura, PhD
Jennifer Freedman, PhD

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Laboratory Team

- **Jennifer Freedman, PhD**
- **Daniel George, MD**
- April Deveaux, MD PhD
- Muthana Al Abo, MD PhD
- Tyler Allen, PhD
- Timothy Robinson, MD PhD
- Sean Piwarski, PhD
- Kara Wegermann, MD
- Joab Odura, PhD
- Bonnie LaCroix
- Brendon Patierno

GU and Lung Clinical Partners

- Andrew Armstrong, MD
- Michael Ferrandino MD
- Brant Inman, MD
- Judd Moul, MD
- Cary Robertson, MD
- Tom Polascik, MD
- Jeffrey Clarke, MD

Duke Collaborators

- **Nadine Barrett, PhD**
- **Amanda Hargrove, PhD**
- **Terry Hyslop, PhD**
- **David Hsu, MD PhD**
- **Jiaoti Huang, MD PhD**
- **Kouros Owzar, PhD**
- Jason Somarelli, PhD
- Kathryn Ware, PhD
- Hongliang Liu, MD PhD
- Donald McDonnell, PhD
- Susan Murphy, PhD
- Smita Nair, PhD
- Andrew Nixon, PhD
- Bruce Sullenger, PhD
- Tuan Vo-Dinh, PhD
- Qingyi Wei, MD PhD
- Wen-Chi Foo, MD

Clinical Research Team

- ARPM: Julie Rasmussen
- Data: Monika Anand, PhD
- Regulatory: Rhonda Wilder
- Multi-site: Carol Winters Colleen Riggan

DCI Shared Resources

Biospecimen Repository Team

- Shannon McCall, MD

Outside Collaborators

- Norman Lee, PhD
- Bi-Dar Wang, PhD
- Zefeng Wang, PhD
- **Rick Kittles, PhD**

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NATIONAL CANCER INSTITUTE

NIH NATIONAL CANCER INSTITUTE

Center to Reduce Cancer Health Disparities

P20 PACHE: Duke-NCCU Translational Cancer Disparities Research Partnership

Duke Cancer Disparities SPORE

RO1's

Prostate Cancer Foundation
Curing Together.

MOVEMBER FOUNDATION

Congressionally Directed Medical Research Programs
CDMRP
Department of Defense

Prostate and Lung

V FOUNDATION
Victory Over Cancer

MURDOCK STUDY

Peter Michael FOUNDATION

susan g. komen.