Genetic Testing and Prostate Cancer: Recoupling Molecular Biology and Clinical Expertise

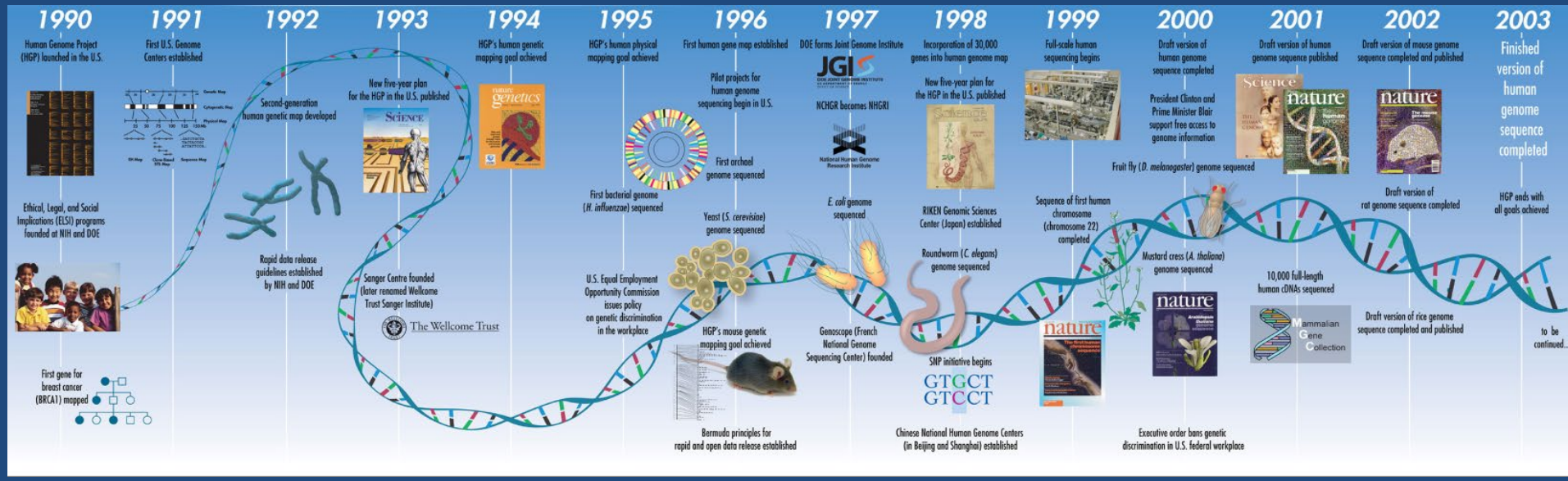
Raoul S. Concepcion, MD, FACS
Chief Science Officer
U.S. Urology Partners
Nashville TN

Disclosures

- Consultant: GoPath, ImmunityBio, AZ, Astellas, Janssen, Minomic, Pfizer, Accord Pharma, Sun Pharma
- Speaker's Bureau: Astellas, Amgen, Pfizer, Sun Pharma
- Thanks to Drs. Leonard Gomella (PA), Emmanuel Antonarakis (MN) and City of Hope for slides

Human Genome Project

1990-2003



3.2 billion base pairs

https://www.mun.ca/biology/scarr/Human_Genome_Project_timeline.html

Spring 2013: Everything Changed

May 13, 2013

SUPREME COURT OF THE UNITED STATES

No. 12–398

ASSOCIATION FOR MOLECULAR PATHOLOGY, ET AL., PETITIONERS *v.* MYRIAD GENETICS, INC., ET AL.

ON WRIT OF CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

[June 13, 2013]

JUSTICE SCALIA, concurring in part and concurring in the judgment.

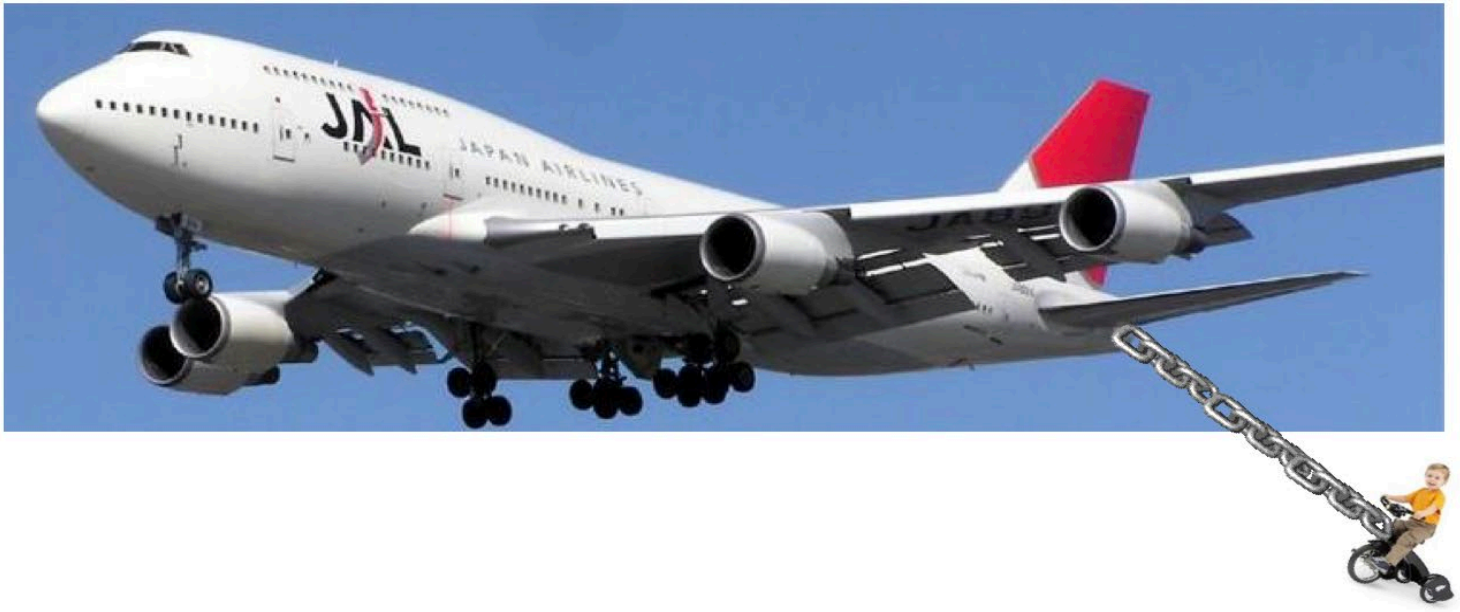
I join the judgment of the Court, and all of its opinion except Part I–A and some portions of the rest of the opinion going into fine details of molecular biology. I am unable to affirm those details on my own knowledge or even my own belief. It suffices for me to affirm, having studied the opinions below and the expert briefs presented here, that the portion of DNA isolated from its natural state sought to be patented is identical to that portion of the DNA in its natural state; and that complementary DNA (cDNA) is a synthetic creation not normally present in nature.

June 13, 2013

Next Generation Sequencing (NGS)

- Deep sequencing (takes hours/days)
- "Reads" a sequence over and over
- Gene chip technology
- Much more robust than DTC testing (23 and Me, Ancestry.com, etc)

Next-Generation Sequencing (NGS) in Clinical Cancer Genetics

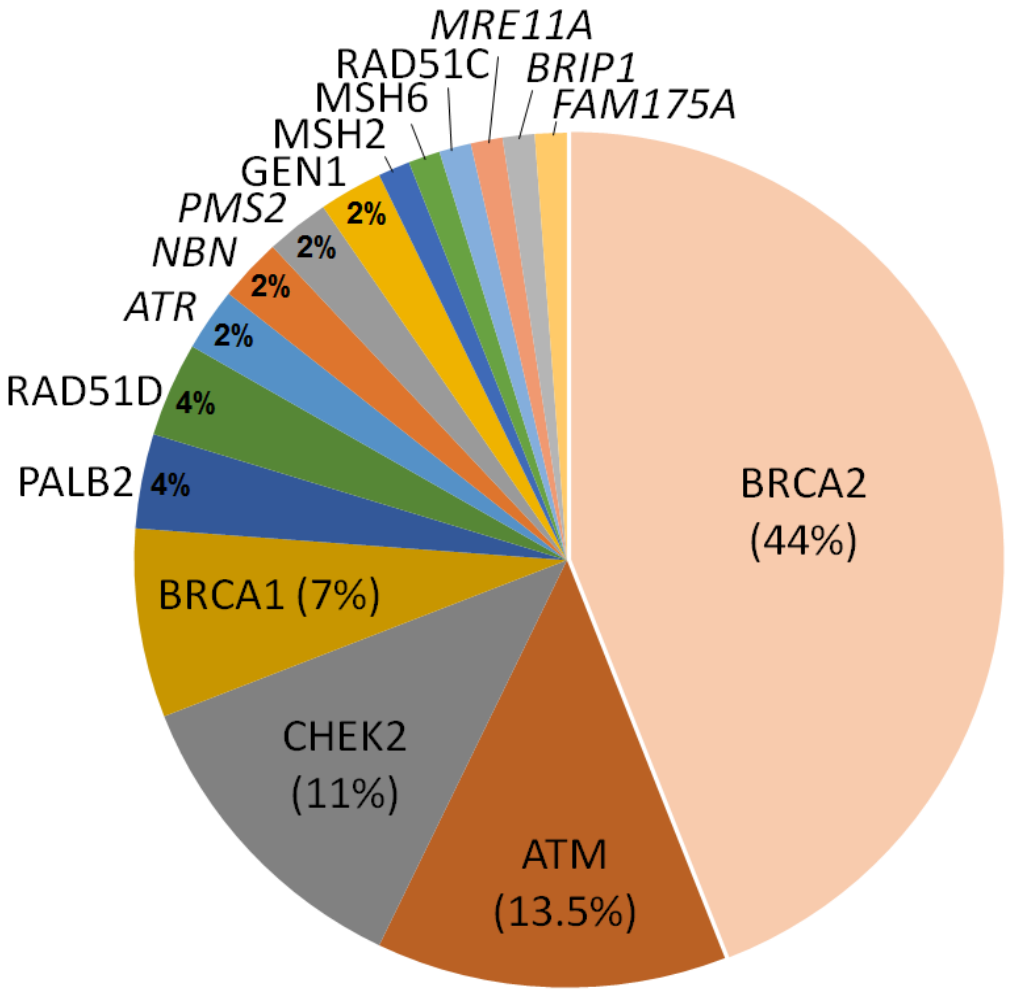


BRCA 1/2 Mutations and PCa

- DNA damage response (DDR) genes
- 2-6 fold ↑ lifetime risk (BRCA2 > BRCA1)
- 8.6-fold ↑ risk by age 65 (BRCA2)
- PCa: Likely to be aggressive: Gleason 8 or higher, node +, mets, poor survival
- ↑ self and family risk for other hereditary cancers: breast, ovarian, melanoma, pancreatic, Lynch Syndrome, colon, gastric

Germline mutations in metastatic PCa

- BRCA-2 best studied for potential screening and treatment
- PCa males with BRCA-2 have more aggressive disease
- More work is needed on the other PCa genes identified
- Germline mutations in 11.8% of metastatic vs. 4.6% localized disease



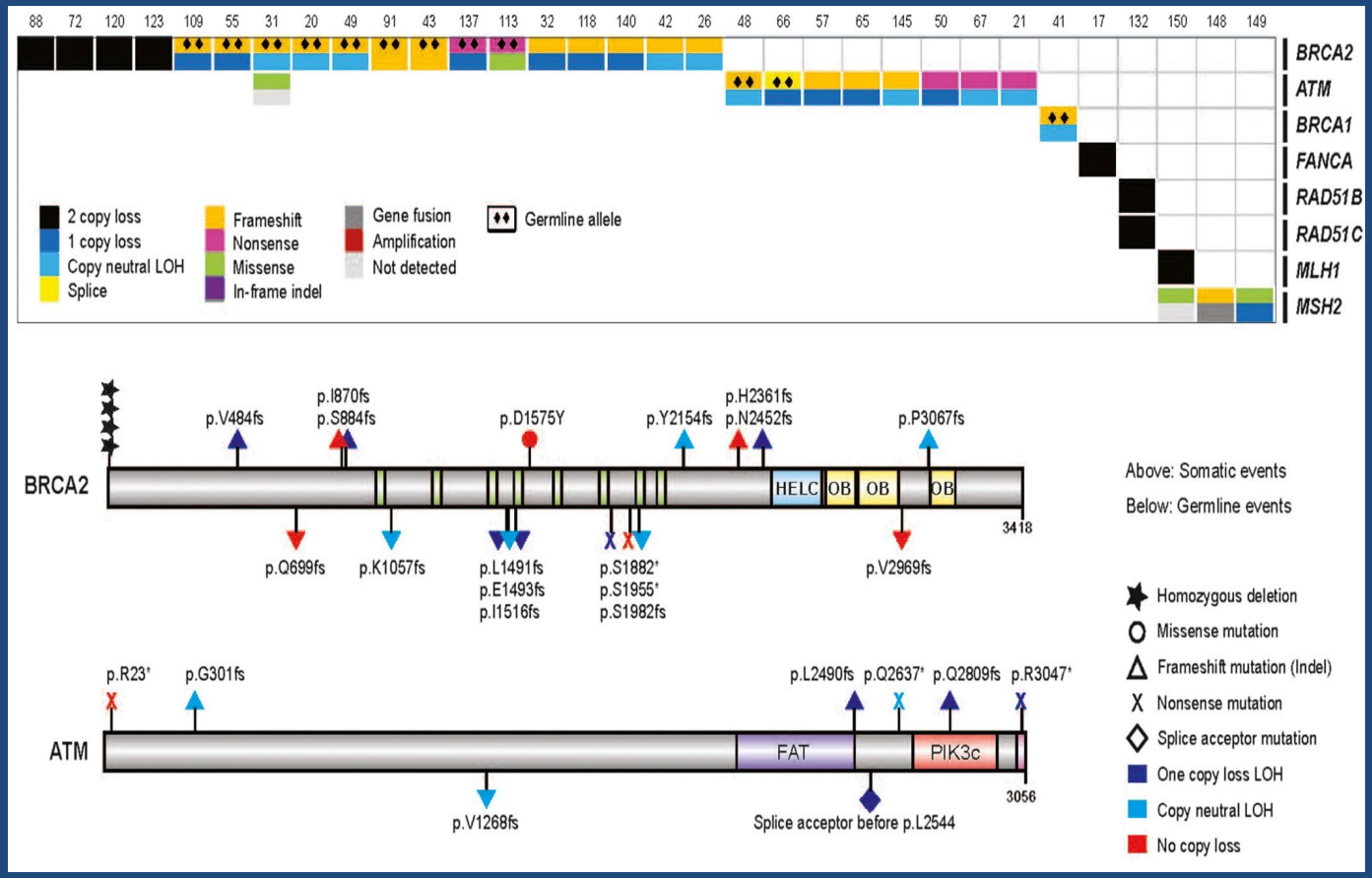
Pritchard, N Engl J Med. 2016 Aug 4;375(5):443-53

DNA Repair Defects in *Metastatic* CRPC

- **32/150 (21.3%) mCRPC pts had bi-allelic DNA repair mutations**

All DNA Repair Defects

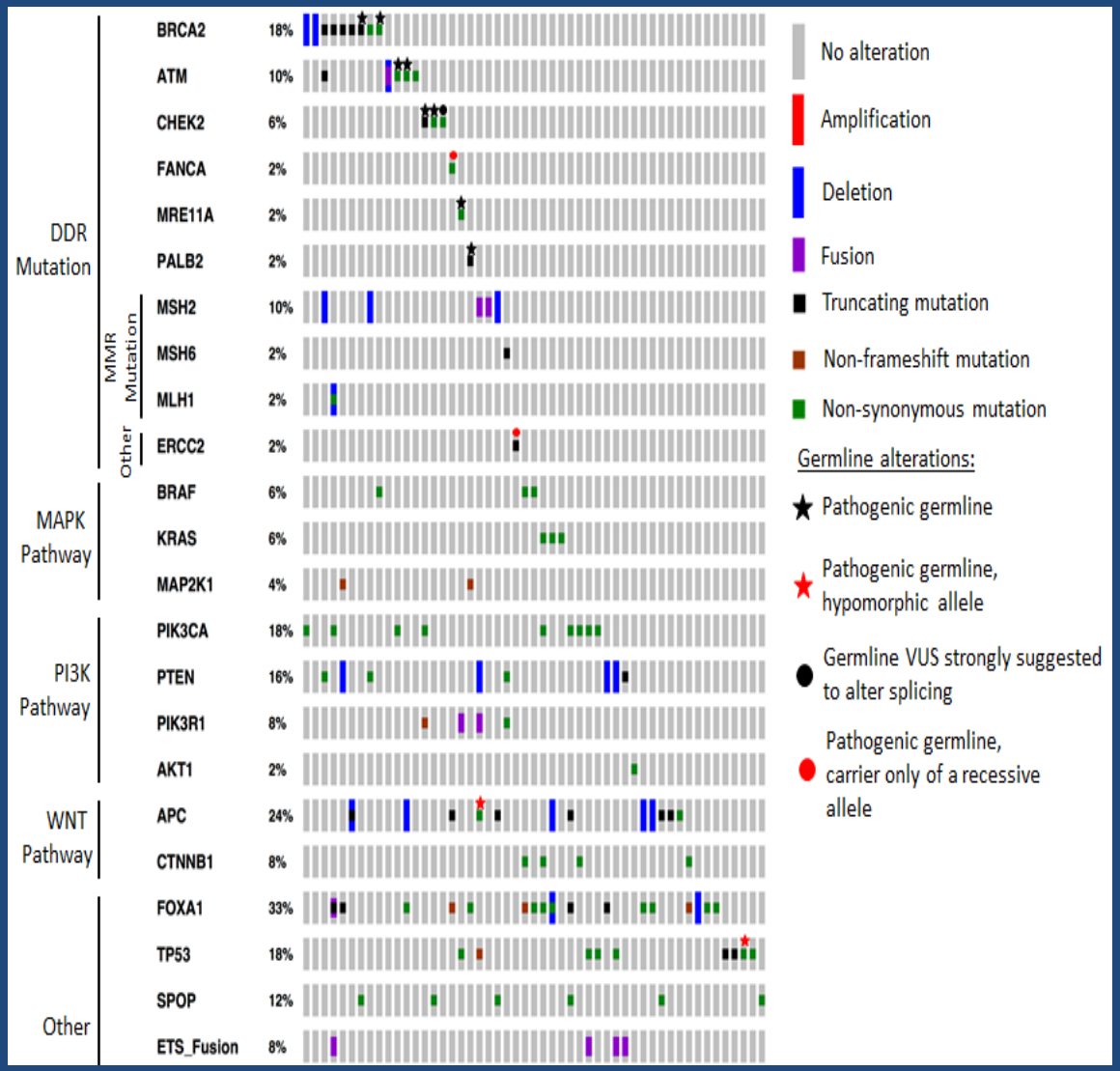
Mutations in *BRCA2, ATM*



Robinson D, et al. Cell. 2015; 5: 1215-1228

DNA Repair Defects and Prostatic Ductal Cancer

- Overall, **49%** had DNA repair gene mutations:
  - –MMR mutations 14%
  - –<u>HRD mutations</u> **31%**



Schweizer MT, et al. ASCO 2018; abstract 5030.

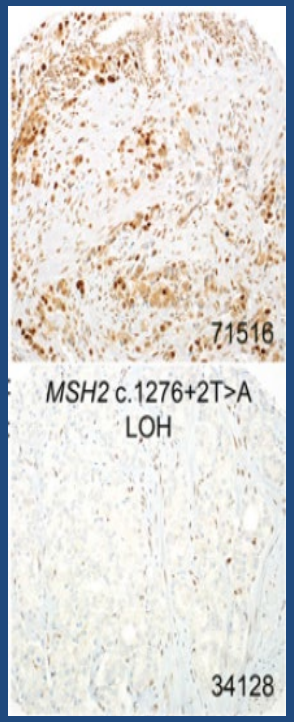
MMR Defects and Gleason grade

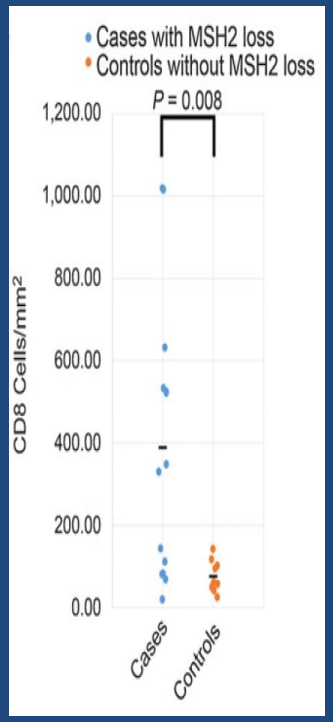
Personalized Medicine and Imaging

Clinical Cancer Research

MSH2 Loss in Primary Prostate Cancer

Liana B. Guedes[1], Emmanuel S. Antonarakis[2], Michael T. Schweizer[3], Nooshin Mirkheshti[1], Fawaz Almutairi[1], Jong Chul Park[2], Stephanie Glavaris[1], Jessica Hicks[1], Mario A. Eisenberger[2], Angelo M. De Marzo[1,2,4], Jonathan I. Epstein[1,2,4], William B. Isaacs[4], James R. Eshleman[1,2], Colin C. Pritchard[5], and Tamara L. Lotan[1,2]





- **1.2%** (14/1176) of primary PCa had MSH2 protein loss
- Pathology and MSH2 loss:
  - Primary Gleason pattern 5 enriched for MSH2 loss: **8%** (7/91) vs. <1% (5/1042), *P*<0.0001

Guedes LB, et al. Clin Cancer Res. 2017; 23: 6863-74.

Active Surveillance

Germline Mutations in *ATM* and *BRCA1/2* Are Associated with Grade Reclassification in Men on Active Surveillance for Prostate Cancer EU ★ ACME

H. Ballentine Carter[a,*], Brian Helfand[b,c], Mufaddal Mamawala[a], Yishuo Wu[b,d], Patricia Landis[a], Hongjie Yu[b], Kathleen Wiley[a], Rong Na[b,e], Zhuqing Shi[b,f], Jacqueline Petkewicz[c], Sameep Shah[b], Richard J. Fantus[b,g], Kristian Novakovic[c], Charles B. Brendler[b], S. Lilly Zheng[b], William B. Isaacs[a], Jianfeng Xu[b,d,f]

Associate Editor: James Catto

Statistical Editor: Andrew Vickers

Carter, HB et al . European Urology, Vol 75, issue 5, 743-749

Genomics and Precision Medicine

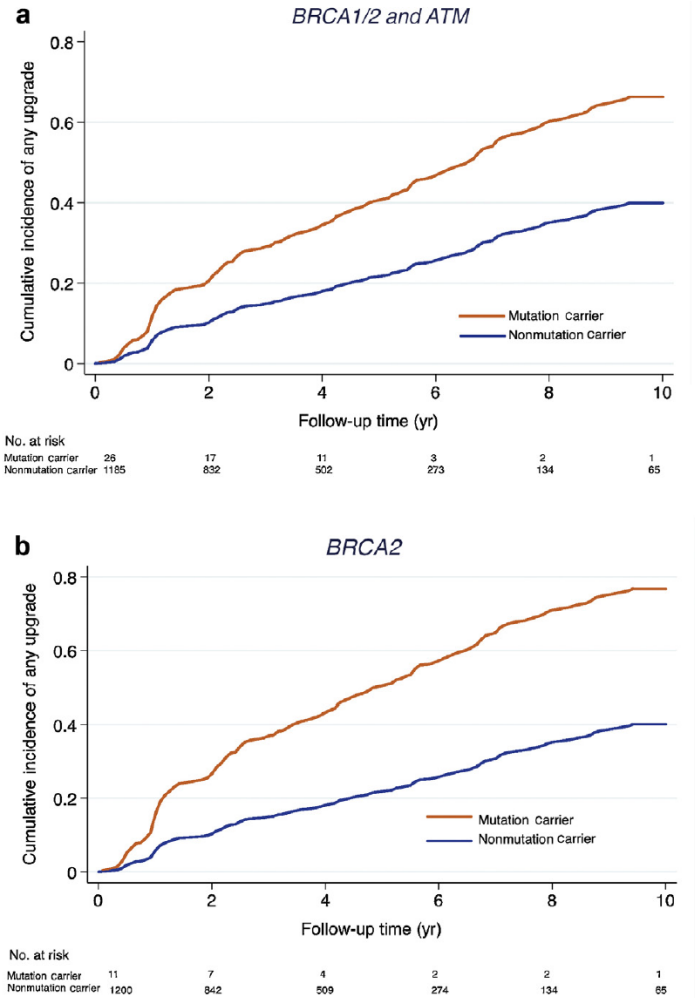


Fig. 1 – Cumulative incidence of upgrading on biopsies after the diagnostic biopsy in (A) carriers and noncarriers of mutations in *BRCA1/2* and/or *ATM*; (B) carriers and noncarriers of mutations in *BRCA2* only. Cumulative incidence based on competing risk analysis. Upgrading refers to any grade group (GG) or Gleason score higher than diagnostic biopsy GG irrespective of initial grade at biopsy.

ACTIVE SURVEILLANCE

Upgrading of biopsies of men on active surveillance for early-stage prostate cancer --> among *BRCA2* carriers

Carter et al. *Eur Urol* 2018

INITIAL RISK STRATIFICATION AND STAGING WORKUP FOR CLINICALLY LOCALIZED DISEASE

Risk Group	Clinical/Pathologic Features			Imaging[f,g]	Germline Testing[c]	Molecular/ Biomarker Analysis of Tumor[c]	Initial Therapy
Very low[d]	Has all of the following: • T1c • Grade Group 1 • PSA <10 ng/mL • Fewer than 3 prostate biopsy fragments/cores positive, ≤50% cancer in each fragment/core[e] • PSA density <0.15 ng/mL/g			Not indicated	Recommended if family history positive or intraductal/cribriform histology See PROS-1	Not indicated	See PROS-3
Low[d]	Has all of the following but does not qualify for very low risk: • T1–T2a • Grade Group 1 • PSA <10 ng/mL			Not indicated	Recommended if family history positive or intraductal/cribriform histology See PROS-1	Consider if life expectancy ≥10 y[j]	See PROS-4
Intermediate[d]	Has all of the following: • No high-risk group features • No very-high-risk group features • Has one or more intermediate risk factors (IRF): ▸ T2b–T2c ▸ Grade Group 2 or 3 ▸ PSA 10–20 ng/mL	Favorable intermediate	Has all of the following: • 1 IRF • Grade Group 1 or 2 • <50% biopsy cores positive[e]	• Bone imaging[h]: not recommended for staging • Pelvic ± abdominal imaging[i]: recommended if nomogram predicts >10% probability of pelvic lymph node involvement • If regional or distant metastases are found, see PROS-8	Recommended if family history positive or intraductal/cribriform histology See PROS-1	Consider if life expectancy ≥10 y[j]	See PROS-5
		Unfavorable intermediate	Has one or more of the following: • 2 or 3 IRFs • Grade Group 3 • ≥50% biopsy cores positive[e]	• Bone imaging[h]: recommended if T2 and PSA >10 ng/mL • Pelvic ± abdominal imaging[i]: recommended if nomogram predicts >10% probability of pelvic lymph node involvement • If regional or distant metastases are found, see PROS-8	Recommended if family history positive or intraductal/cribriform histology See PROS-1	Consider if life expectancy ≥10 y[j]	See PROS-6
High	Has no very-high-risk features and has at least one high-risk feature: • T3a OR • Grade Group 4 or Grade Group 5 OR • PSA >20 ng/mL			• Bone imaging[h]: recommended • Pelvic ± abdominal imaging[i]: recommended if nomogram predicts >10% probability of pelvic lymph node involvement • If regional or distant metastases are found, see PROS-8	Recommended	Consider if life expectancy ≥10 y[j]	See PROS-7
Very high	Has at least one of the following: • T3b–T4 • Primary Gleason pattern 5 • 2 or 3 high-risk features • >4 cores with Grade Group 4 or 5			• Bone imaging[h]: recommended • Pelvic ± abdominal imaging[i]: recommended if nomogram predicts >10% probability of pelvic lymph node involvement • If regional or distant metastases are found, see PROS-8	Recommended	Not routinely recommended	See PROS-7

See Footnotes for Initial Risk Stratification And Staging Workup For Clinically Localized Disease (PROS-2A).

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

Genomic/Genetic Testing for Prostate Cancer Risk

Some mutated genes associated with prostate cancer

Most appear to be related to defects in DNA repair mechanisms

HOXB13 is the gene linked with clearly defined inherited prostate cancer

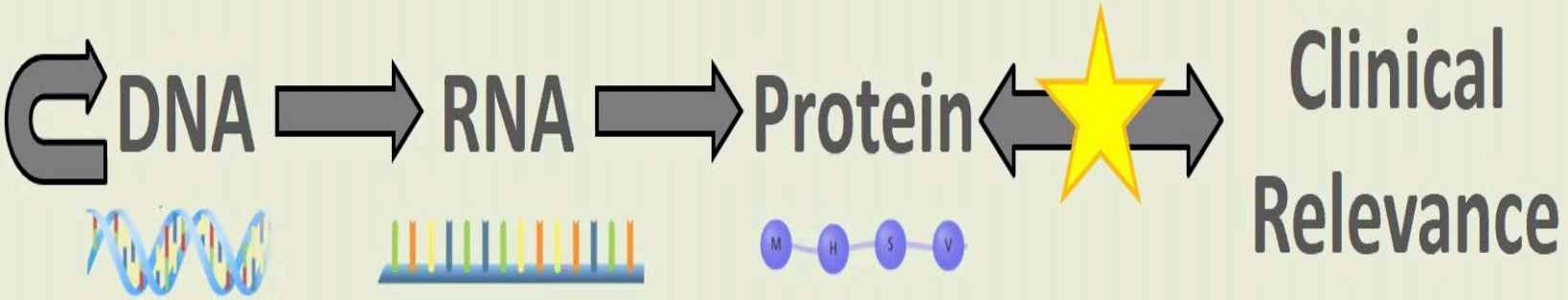
Gene	PCa Risk	Mechanism
ATM	elevated	DNA damage response
BRCA1	~ 20%	DNA damage repair
BRCA2	~ 20%	DNA damage repair
CHEK2	elevated	DNA repair through phosphorylation of BRCA2
EPCAM	up to 30%	Upregulate c-myc
HOXB13	up to 60%	AR repressor
MLH1	up to 30%	DNA repair
MSH2	up to 30%	DNA repair
MSH6	up to 30%	DNA repair
NBN	elevated	DNA repair
PMS2	up to 30%	DNA mismatch repair
TP53	unknown	Tumor suppressor
PALB2	preliminary	Tumor suppressor
RAD51D	preliminary	DNA repair

Based on data in Nicolosi, et al ASCO Abstract 5009 2017 Chicago;
https://www.ncbi.nlm.nih.gov/gene/

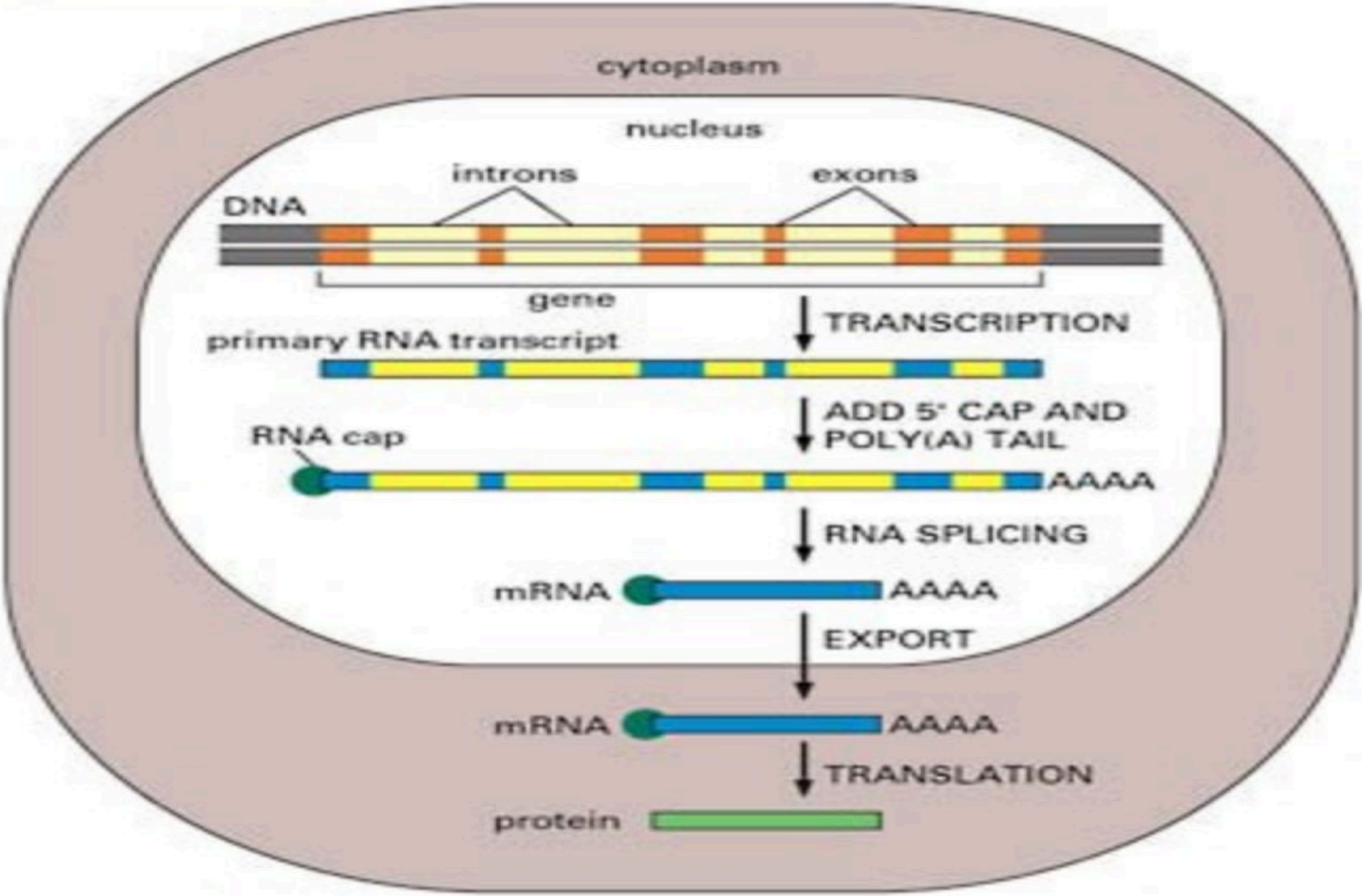
Chemical Individuality and Cancer Predisposition

Archibald Garrod (1931): "In every case of every malady there are two sets of factors at work in the formation of the morbid picture, namely internal or constitutional factors, inherent in the sufferer and usually inherited from his forebears, and external ones which fire the train"

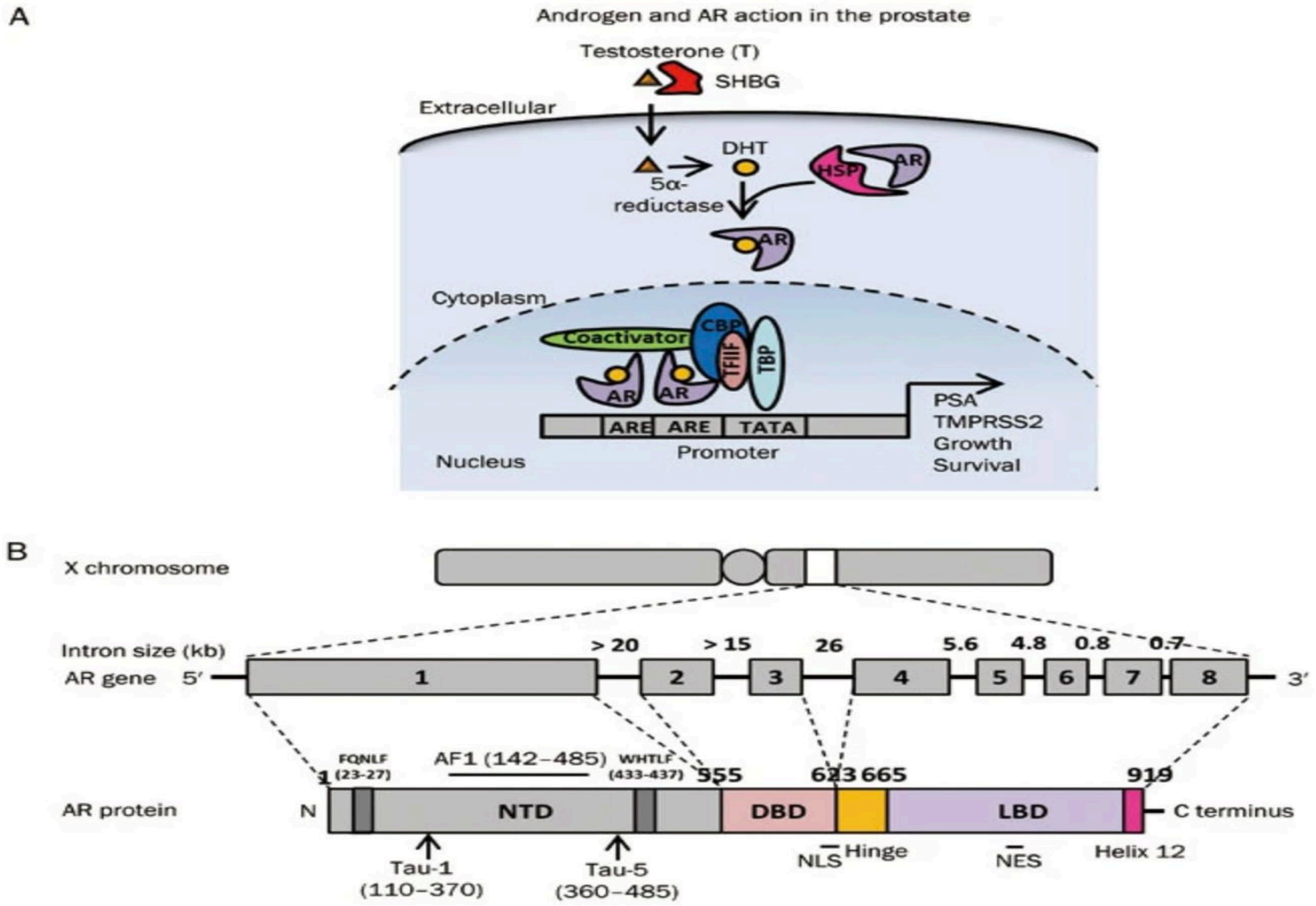
Recoupling molecular biology *AND* clinical expertise



Central Dogma



AR Pathway


A
Androgen and AR action in the prostate
Testosterone (T)
SHBG
Extracellular
DHT
5α-
reductase
HSP
AR
AR
Cytoplasm
Coactivator
CBP
TFIIF
TBP
AR
AR
ARE
ARE
TATA
Promoter
Nucleus
PSA
TMPRSS2
Growth
Survival
B
X chromosome
Intron size (kb)
> 20
> 15
26
5.6
4.8
0.8
0.7
AR gene 5′
1
2
3
4
5
6
7
8
3′
FQNLF
(23-27)
AF1 (142–485)
WHTLF
(433-437)
1
555
623
665
919
AR protein
N
NTD
DBD
LBD
C terminus
Tau-1
(110–370)
Tau-5
(360–485)
NLS
Hinge
NES
Helix 12

Definitions

Basics

- **Genetics**: The evaluation of a patient's inherited genetic make-up (Mendelian traits)

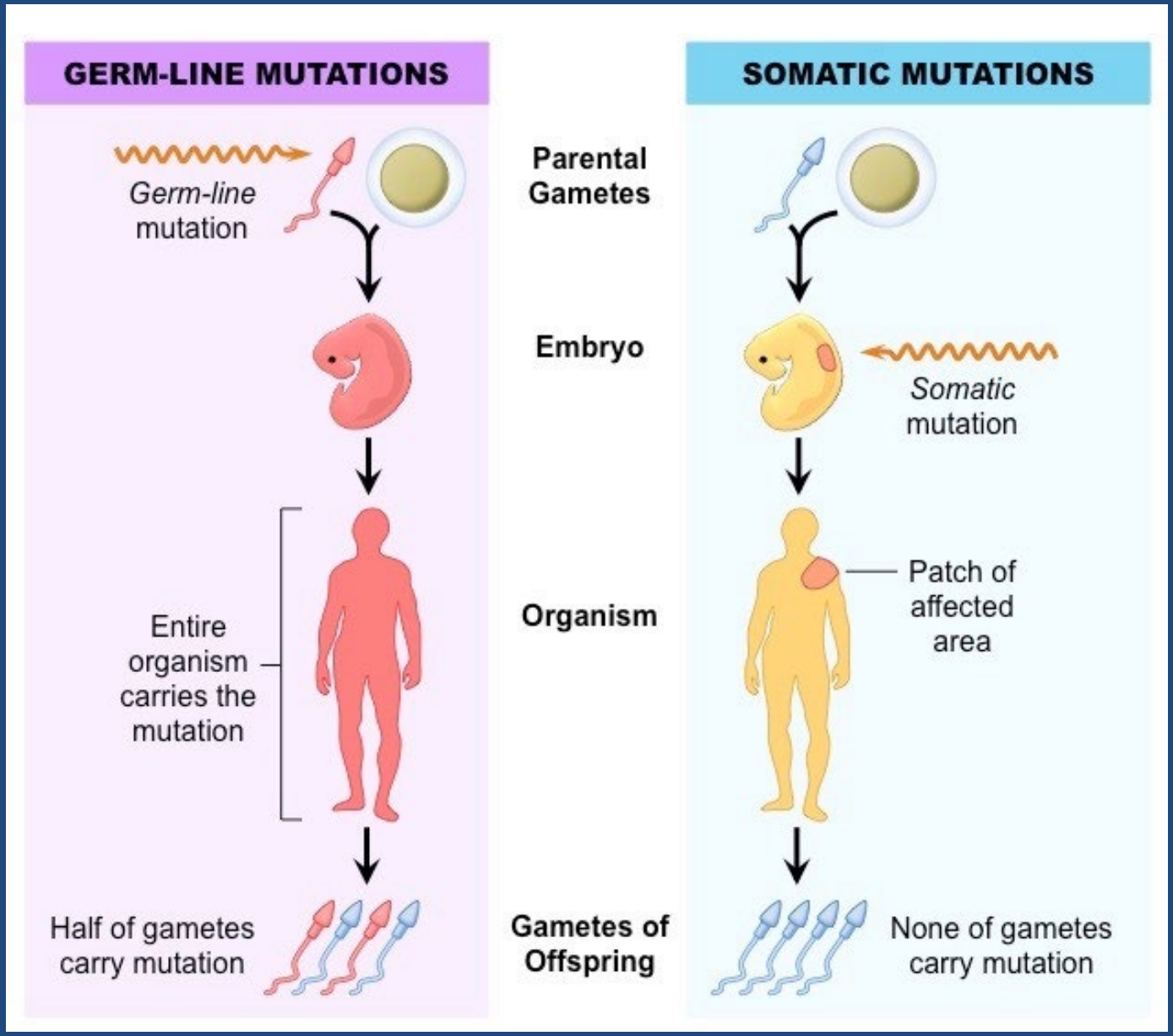
- **Genome**: Organism's complete DNA set
  - Exons/Introns/Codons

- **Genomics**: The complex analysis of the patient's genes, their interaction with other genes as well as the environment that may result in unregulated cell growth.

Testing Basics

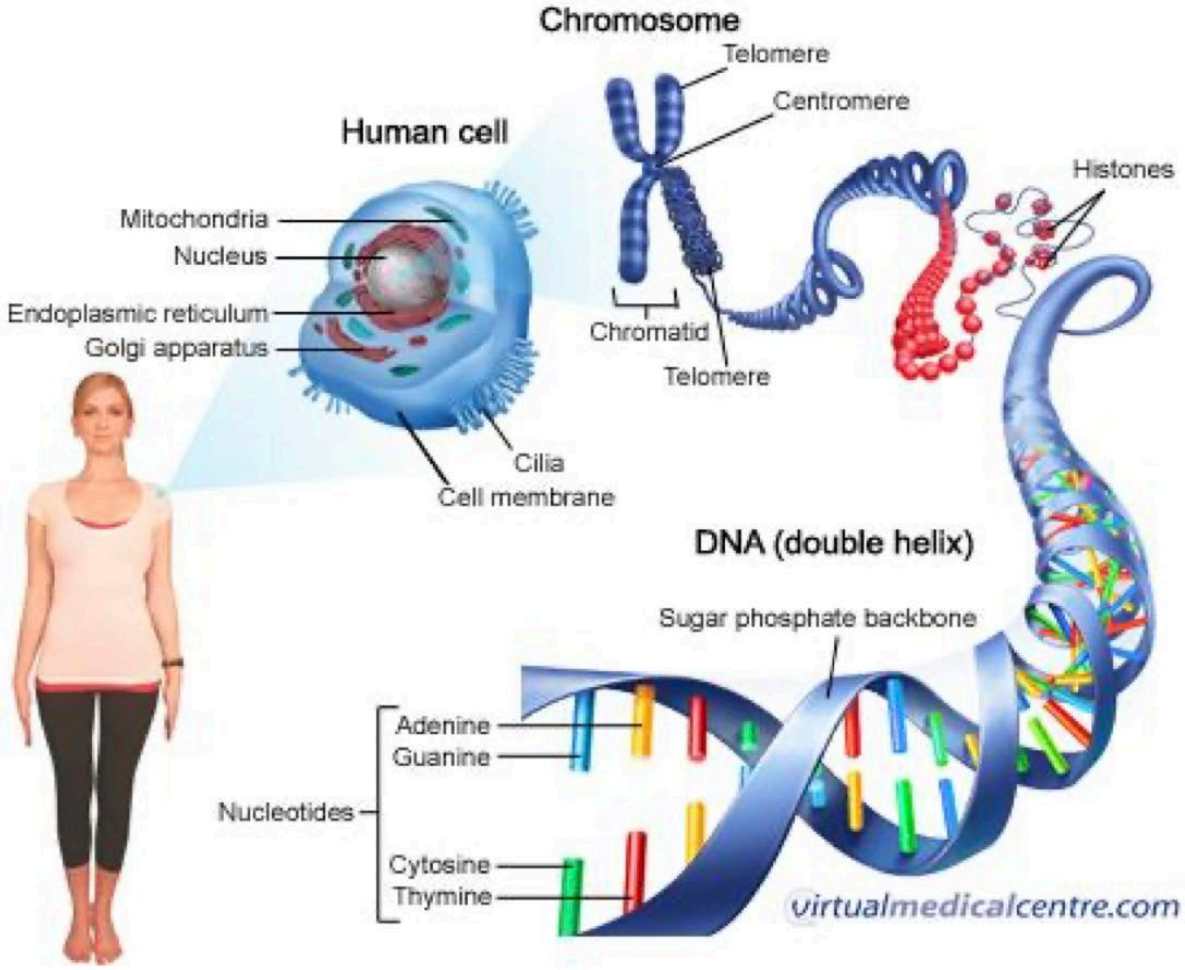
- **Germline/Inherited**: Changes/mutations in DNA that are present in the reproductive cells of the patient (sperm or ovum) and passed from generation to generation. Thus, can be identified in EVERY cell of the body (saliva).

- **Somatic/Acquired**: Mutations can be defined as any alteration in DNA that occurs after conception (tumor).

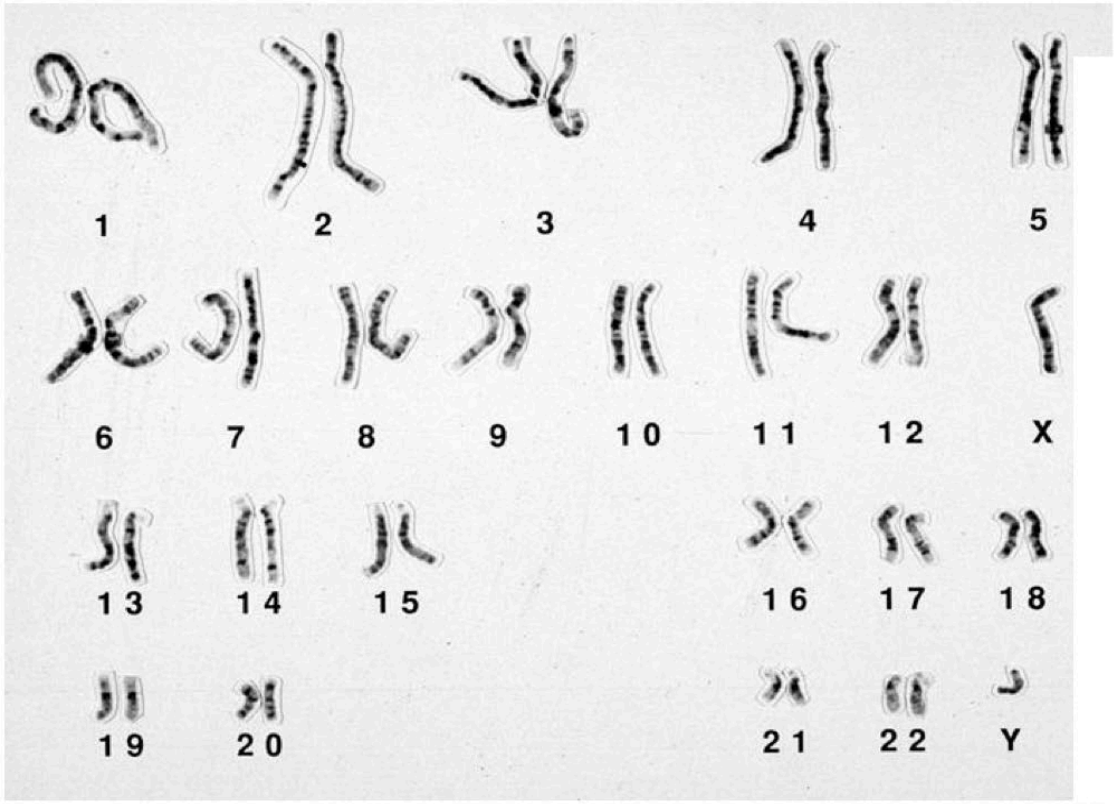


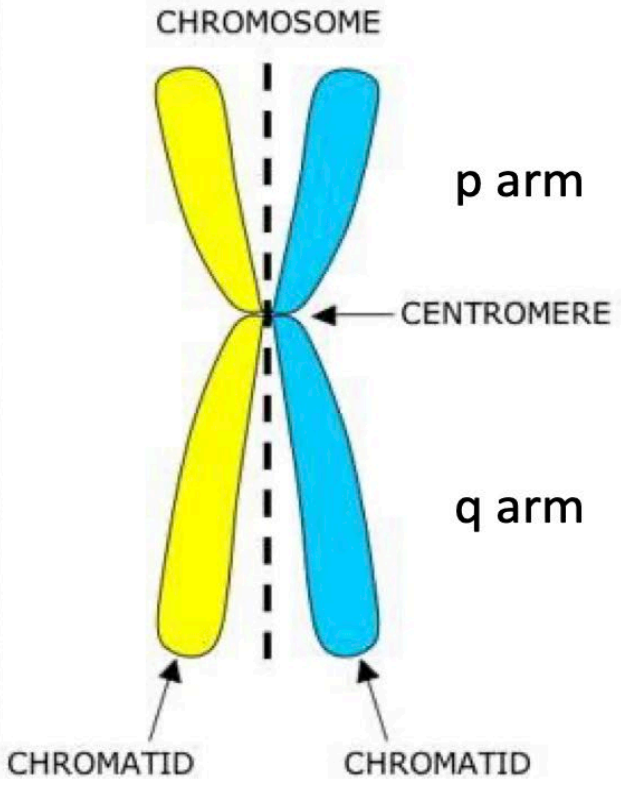
http://ib.bioninja.com.au/

Chromosomes, DNA, and Genes

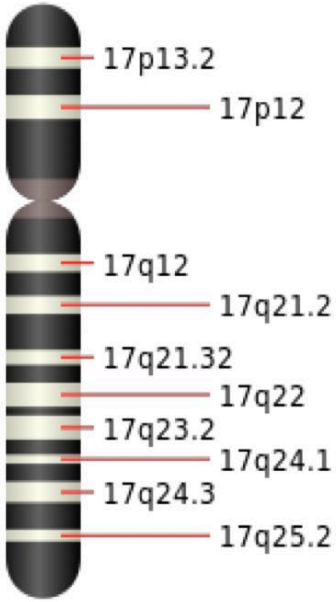


Human Genome: Normal Karyotype





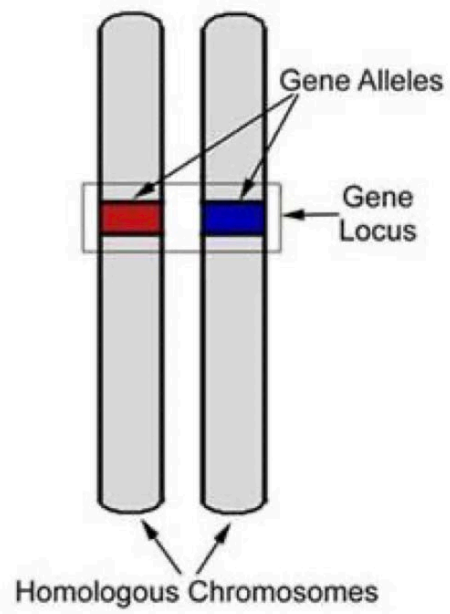
Chromosome Structure



- Bands are given numbers
- The combination of band number and arm = coordinate = LOCUS
- Ex. BRCA1 is on 17q21, BRCA2 13q23

http://upload.wikimedia.org/wikipedia/commons/thumb/7/75/Chromosome_17.svg/175px-Chromosome_17.svg.png

Gene Allele vs. Gene Locus



- **Locus**: the position occupied by a gene on a chromosome
- **Allele**: is an alternative version of a gene or DNA sequence at a specific locus
- Different **alleles** may occupy the same **locus**

http://www.nwcreation.net/articles/images/genelocus.JP

Central Dogma

- DNA → RNA → Protein
  - Transcription
  - Translation

- Base Pairs/Nucleotide Pairing (purines/pyrimidines)
  - DNA (Adenine-Thymine, Guanine-Cytosine)
  - RNA (Adenine-Uracil, Guanine-Cytosine)
  - Genes/Chromosomes
    - 25,000 genes (coding regions/exons, 10% of entire genome)
    - Non-coding regions/introns

Amino Acids

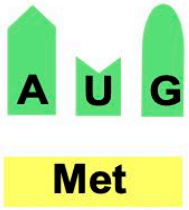
20 Primary Amino Acids in the Genetic Code

Amino Acid	ABBREVIATION 3-Letter	1-Letter	Amino Acid	ABBREVIATION 3-Letter	1-Letter
Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamic acid	Glu	E	Serine	Ser	S
Glutamine	Gln	Q	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V

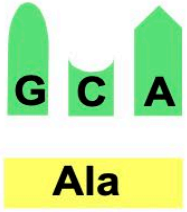
Genetic Code

A **codon** is made of 3 bases
4 DNA bases (A,C,T,G)
4 RNA bases (A,C,G,U)
4^3 = 64 codons total

1 codon (AUG) encodes methionine *and* starts translation of all proteins

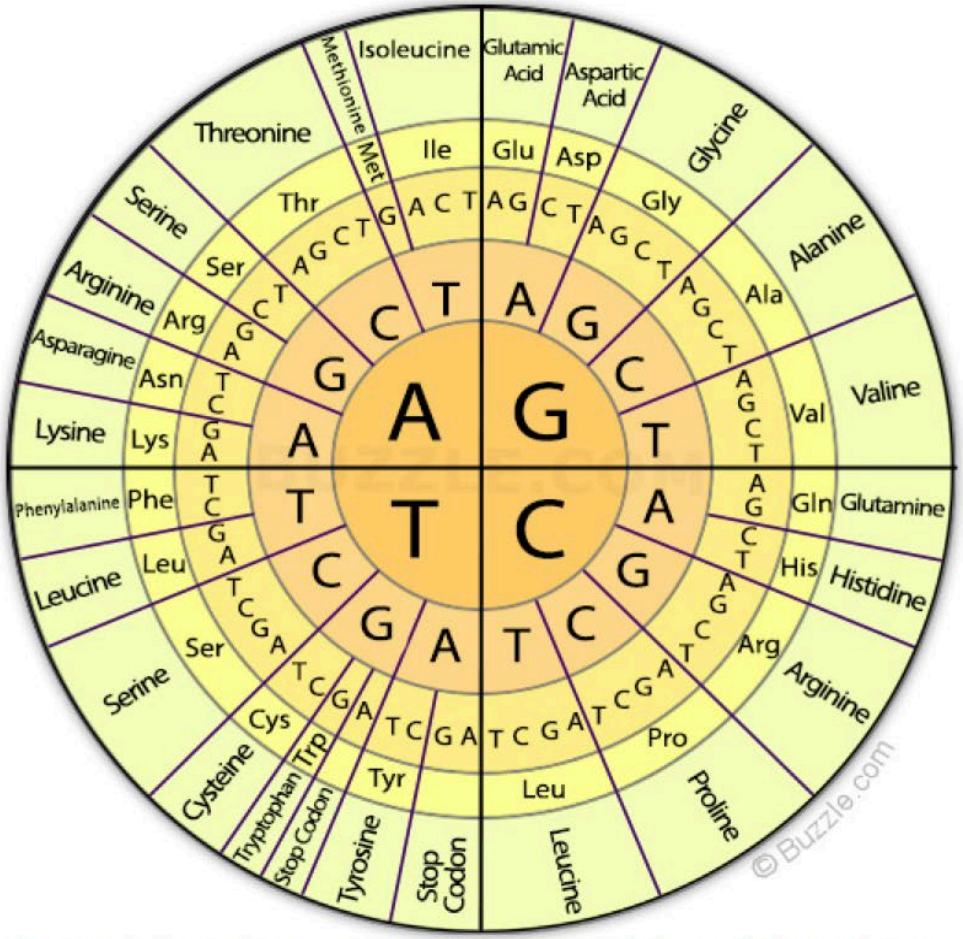


61 codons encode 20 amino acids (redundant code)



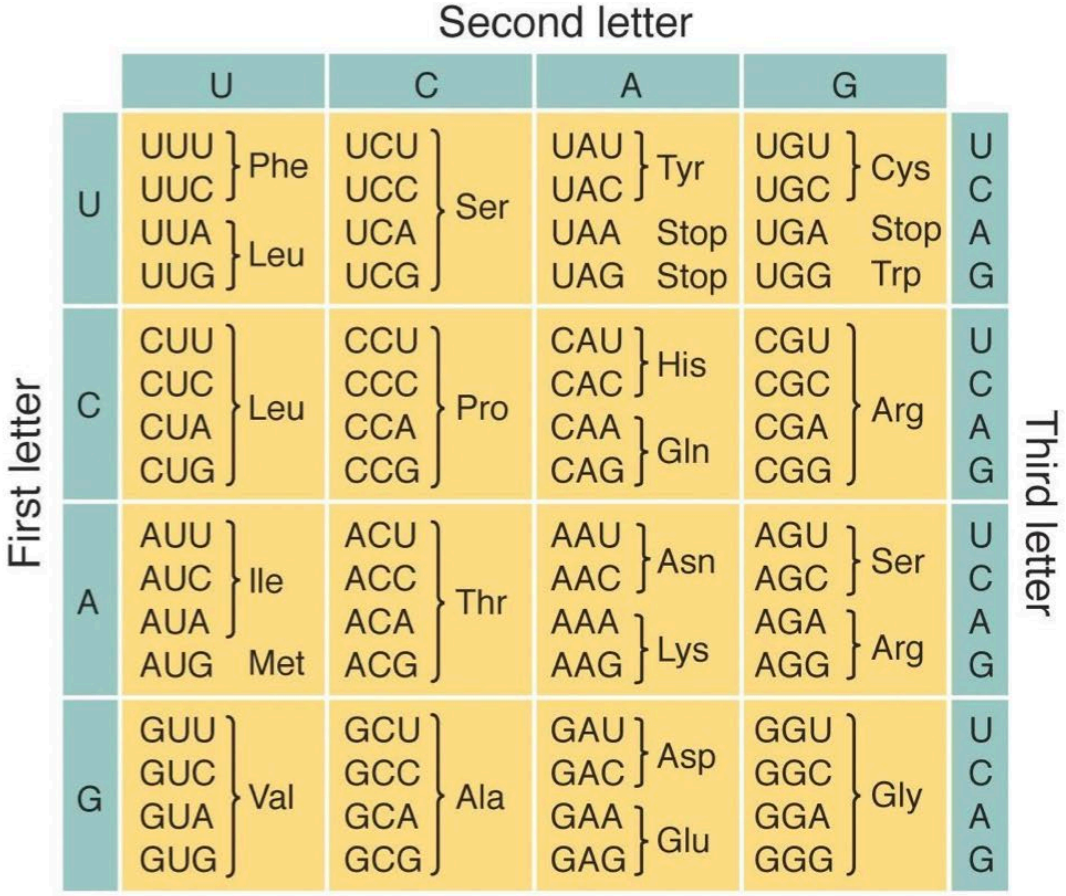
3 codons stop protein translation

The Degenerate Genetic Code



To decode the codon, move from the center circle towards the periphery.

Genetic Code



START CODON:

AUG

STOP CODONS:

UAA (U Are Away)

UGA (U Go Away)

UAG (U Are Gone)

http://www.lucasbrouwers.nl/blog/wp-content/uploads/2010/04/genetic-code.jpg

INVITAE

Name DOB

Patient Name	DOB	Sex	MRN	Invitae #
		Female		
Clinical Team	**Report Date**	**Sample Type**	**Sample Collection Date**	**Sample Accession Date**
		Blood		

Test Performed
Sequence analysis and deletion/duplication testing of the 9 genes listed in the results section below.

- Add-on CHEK2 Gene
- Invitae Breast Cancer STAT Panel
- Add-on ATM Gene

Reason for Testing
Diagnostic test for a personal and family history of disease

Summary

Positive result. Pathogenic variant identified in BRCA2.

Clinical Summary

- A Pathogenic variant, c.4631delA (p.Asn1544Thrfs*24), was identified in BRCA2.
  - The BRCA2 gene is associated with autosomal dominant hereditary breast and ovarian cancer (HBOC) syndrome (MedGen UID: 151793) and autosomal recessive Fanconi anemia, type D1 (FA-D1) (MedGen UID: 325420).
  - This result is consistent with a predisposition to, or diagnosis of, autosomal dominant BRCA2-related conditions.
  - The lifetime risk for female breast cancer in individuals with a pathogenic BRCA2 sequence variant is 40-85%. The risk for contralateral breast cancer in these individuals is 23% within 5 years of the primary breast cancer (PMID: 10498392, 14576434, 15197194). The lifetime risk for ovarian, fallopian tube, or peritoneal cancer is 16-27% (PMID: 9145676, 9497246). The risk for male breast cancer in individuals with a pathogenic BRCA2 sequence variant is 7-8% (PMID: 20587410). There are also increased risks for melanoma, prostate cancer (20%), and pancreatic cancer (2-3%) (PMID: 10433620). Clinical management guidelines for HBOC syndrome can be found at www.nccn.org.
  - Close relatives (children, siblings, and each parent) have up to a 50% chance of being a carrier of this variant. More distant relatives may also be carriers. Carriers are at increased risk of developing autosomal dominant BRCA2-related conditions and may have reproductive risks related to autosomal recessive BRCA2-related conditions as well. Testing for this variant is available.

- Universal nomenclature
  - c. for coding DNA
  - p. for Protein
  - g. for genomic DNA
  - r. for RNA
  - m. for mitochondrial DNA

HGVS
HUMAN GENOME VARIATION SOCIETY

CLINICAL CANCER GENOMICS COMMUNITY OF PRACTICE
City of Hope

Specific Codes Used

+, -,	intronic
*, X	stop codon
_	range
del	deletion
dup	duplication
ins	insertion
inv	inversion
con	conversion
ext	extension
fsX	frame shift
o	opposite strand
t	translocation
?	Cannot determine breakpoint

HGVS
HUMAN GENOME VARIATION SOCIETY

CLINICAL CANCER GENOMICS COMMUNITY OF PRACTICE
City of Hope

INVITAE

Name DOB

Patient Name	DOB	Sex	MRN	Invitae #
		Female		
Clinical Team	**Report Date**	**Sample Type**	**Sample Collection Date**	**Sample Accession Date**
		Blood		

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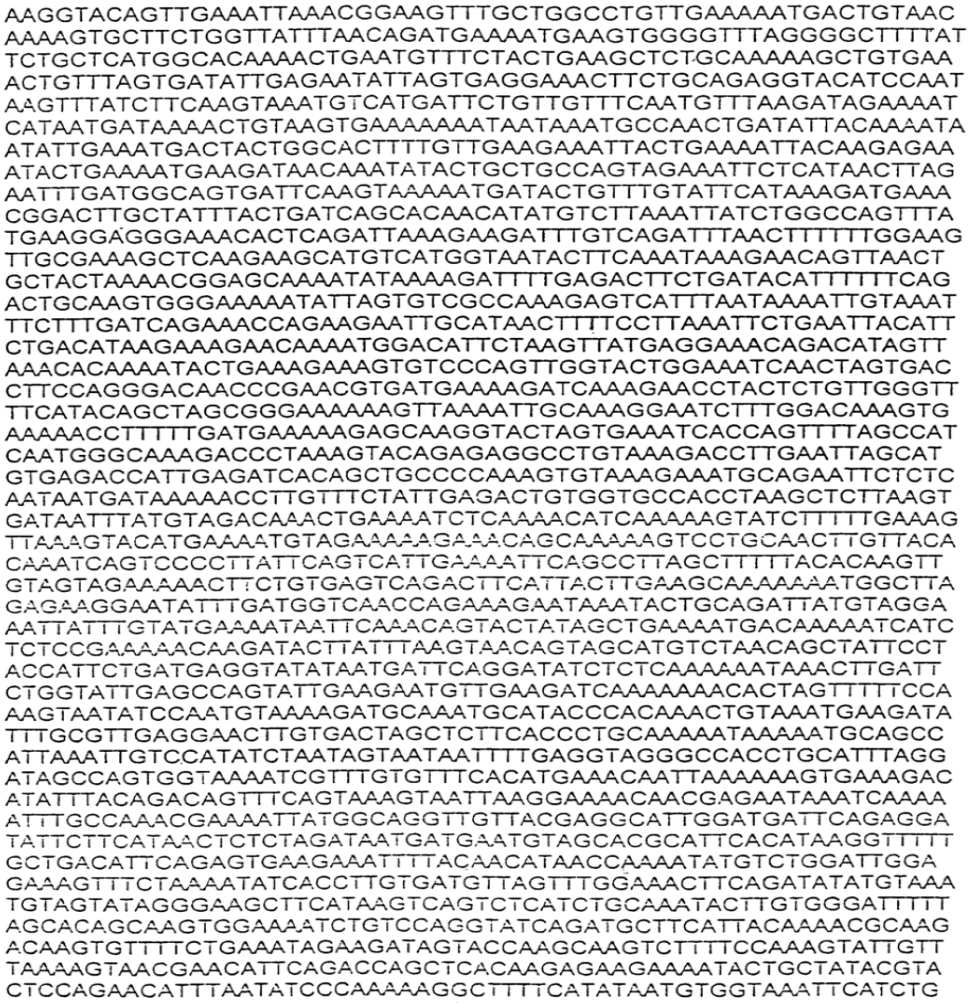
  - Close relatives (children, siblings, and each parent) have up to a 50% chance of being a carrier of this variant. More distant relatives may also be carriers. Carriers are at increased risk of developing autosomal dominant BRCA2-related conditions and may have reproductive risks related to autosomal recessive BRCA2-related conditions as well. Testing for this variant is available.

Our use of genomics relies on computational biology support



Example:
BRCA2 gene

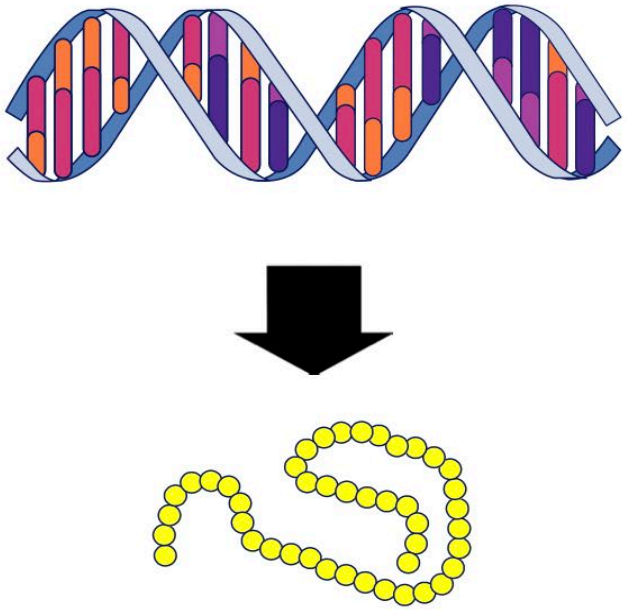
- 27 exons
- > 3,000 a.a.

-10,433 base pairs
or > 20K nucleotides
-image is small portion of exon 11
-12 pages long

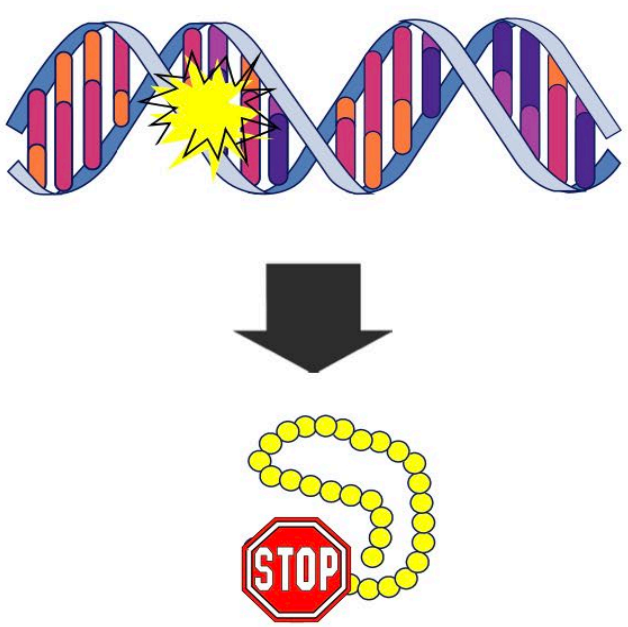
Patent Novel coding sequence haplotypes of the human BRCA2 gene US 20060154272 A1

Point Mutations Can Alter Protein Function

Point mutation: a change in a single base pair



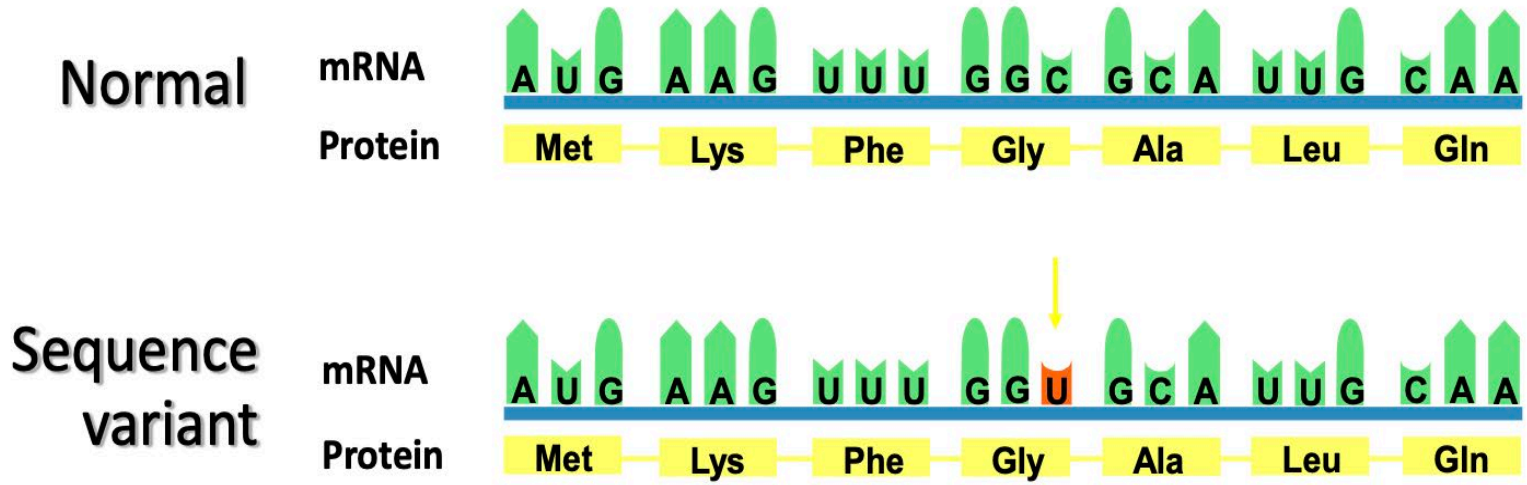
Functional protein



Nonfunctional or missing protein

Silent Mutations

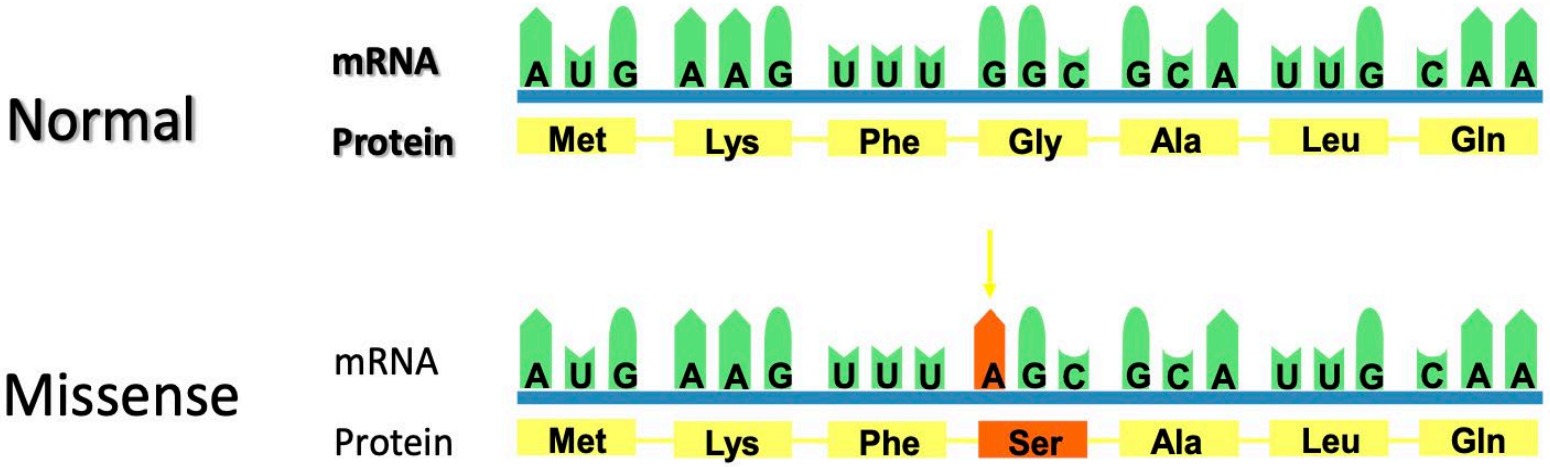
A base pair change that does not change the amino acid sequence (a type of polymorphism)



Adapted from Campbell NA (ed). *Biology*, 2nd ed, 1990

Missense Mutations

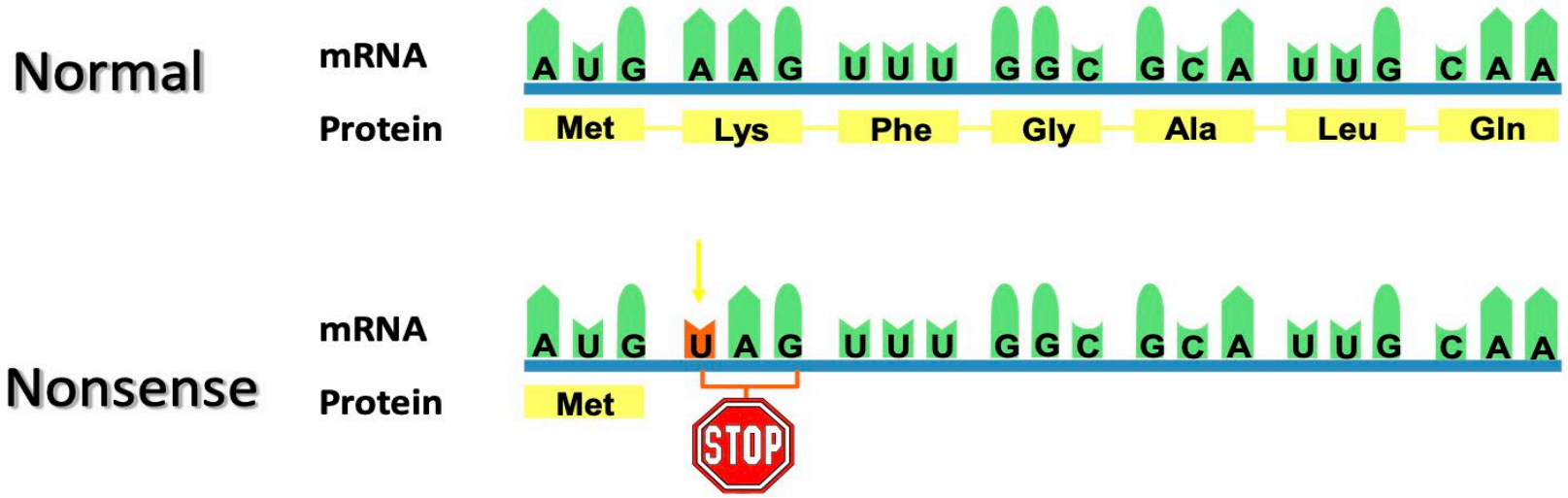
Changes to a codon for another amino acid - can be a harmful mutation or a neutral polymorphism



Adapted from Campbell NA (ed). *Biology*, 2nd ed, 1990

Nonsense Mutations

Change from an amino acid codon to a stop codon, producing a truncated protein



Adapted from Campbell NA (ed). *Biology*, 2nd ed, 1990

Frameshifts: Insertions, Deletions and Duplications

- Insertions
  - designated by “ins”
  - “_” indicates range
  - give inserted sequence
  - Ex c.2040_2041insA
- Deletions
  - designated by “del”
  - “_” indicates range
  - give deleted sequence
  - Ex c.2252_2253delT
- Duplications
  - designated by “dup”
  - “_” indicates range
  - Ex c.592_595dup

CLINICAL CANCER GENOMICS COMMUNITY OF PRACTICE

City of Hope

Frame Shift Mutations

Frame shift (deletion)	THE B I GR EDD OGR ANO UT.
Frame shift (insertion)	THE BIG RED Z ZDO GRA NOU.

Point Mutations

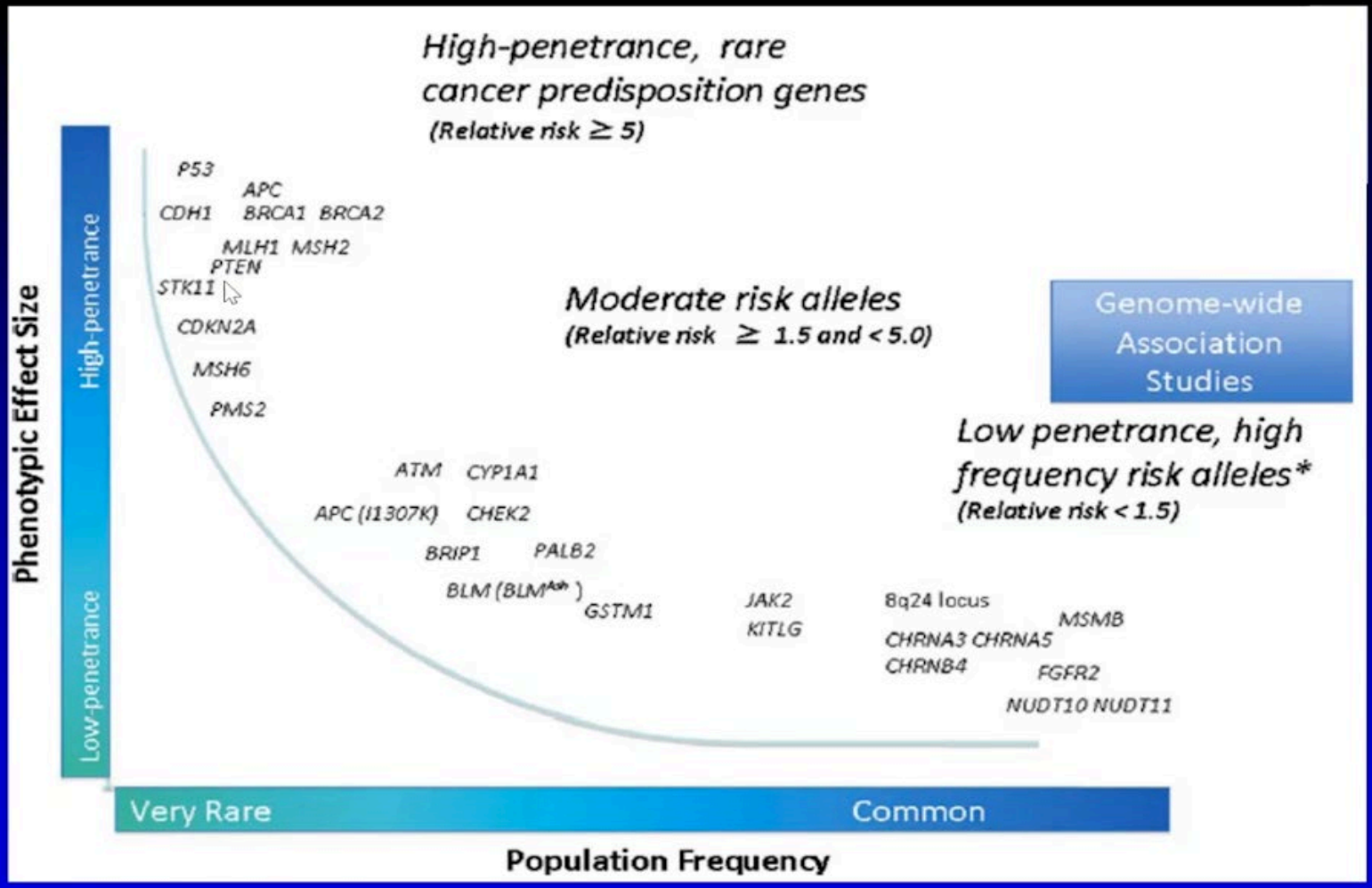
Normal	THE BIG RED DOG RAN OUT.
Missense	THE BIG RAD DOG RAN OUT.
Nonsense	THE BIG RED.

CLINICAL CANCER GENOMICS COMMUNITY OF PRACTICE

City of Hope

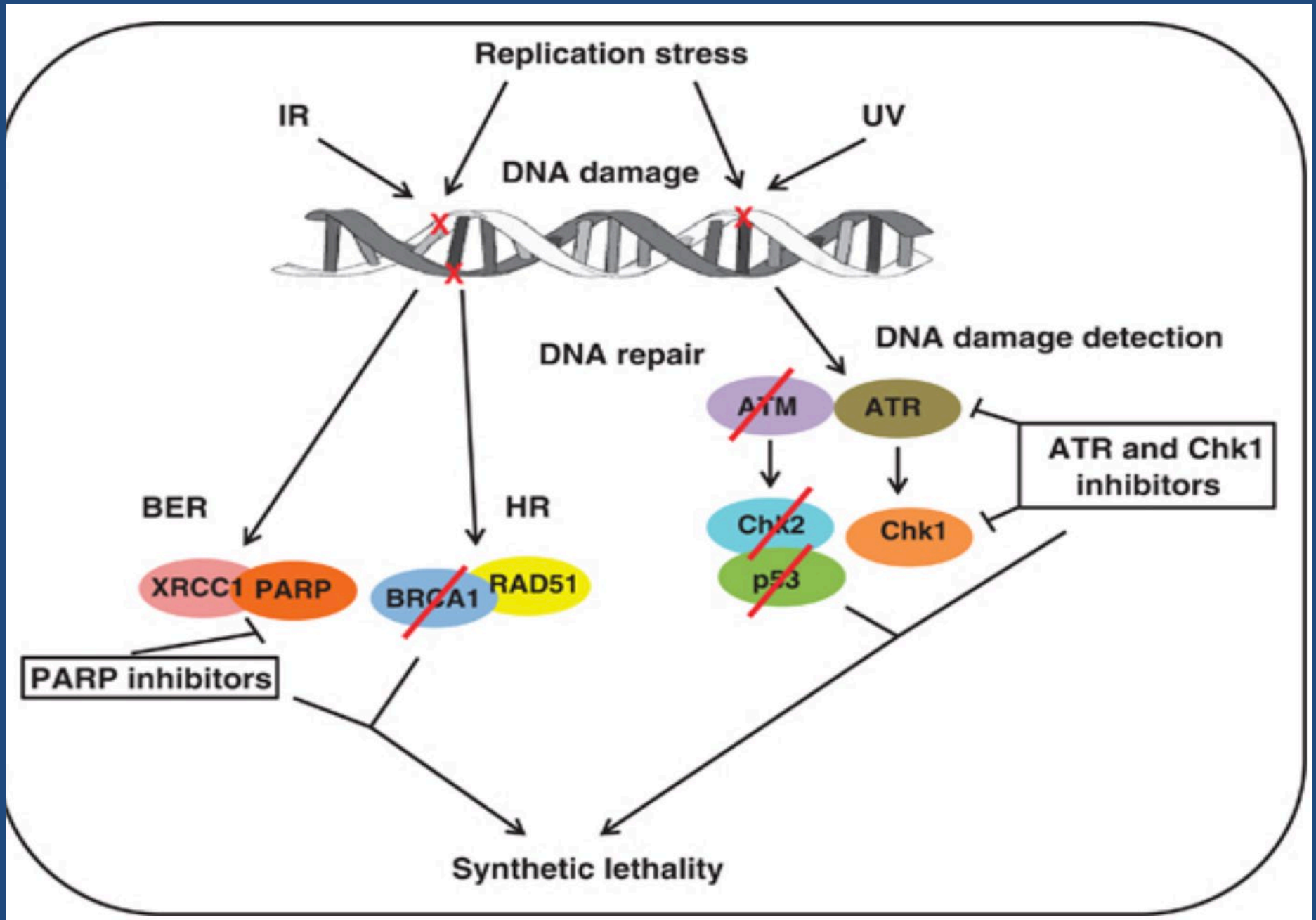
Mutations

Normal	THE BIG RED DOG RAN OUT.
Missense	THE BIG RAD DOG RAN OUT.
Nonsense	THE BIG RED...
Frameshift (deletion)	THE BRE DDO GRA.
Frameshift (insertion)	THE BIG RED ZDO GRA.



Weitzel et al. Genetics, Genomics, and Cancer Risk Assessment: State of the Art and Future Directions in the Era of Personalized Medicine. CA Cancer J Clin 2011;61:327–359

DNA Damage and Repair



DNA Damage Response (DDR) Genes

- Identification/Excision
  - Poly (ADP-ribose) polymerase (PARP)
- Repair pathways
  - Single Strand Break (SSB) repair
  - Double Strand Break (DSB) repair

DNA Damage Response (DDR)

Single Stranded Break (SSB) Repair Pathways

- Mismatch repair (MMR)
  - Base errors from DNA replication and recombination
  - Microsatellite Instability (MSI)
  - *MSH2, MSH6, MLH1, PMS2*
- Nucleotide excision repair (NER)
  - DNA damage from UV light, polycyclic aromatic hydrocarbons
  - *XPA-G, ERCC1-8, CSA/B, RPA, RAD23A/B*
- Base excision repair (BER)
  - DNA damage from alkylation, oxidation/ROS, deamination
  - *PARP1/2/3, POLβ, MUTYH, XRCC1, MBD4, NTHL1*

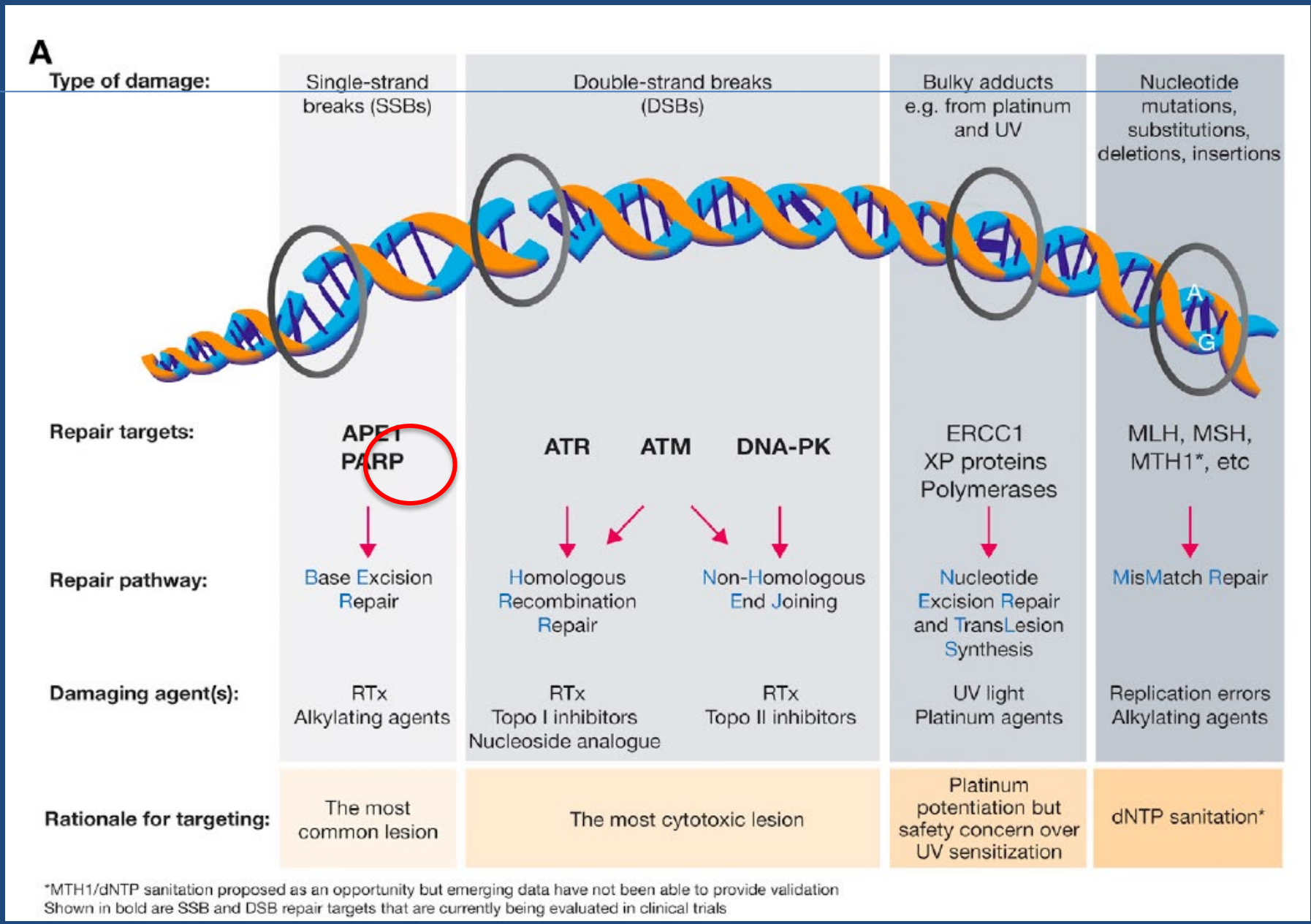
Microsatellite Instability (MSI) and Immunohistochemistry

- Screening tools for Lynch Syndrome
- MSI instability
  - ~10 markers to test for instability/replication error in tumor
- Immunohistochemistry: Presence or Absence of Mismatch Repair Genes
  - MLH1, MSH2, PMS2, MSH6
  - Positive/No Loss of Expression-MMR genes present
  - Negative/Loss of Expression-MMR genes absent in tumor
    - Possible Genetic Mutation

DNA Damage Response (DDR)

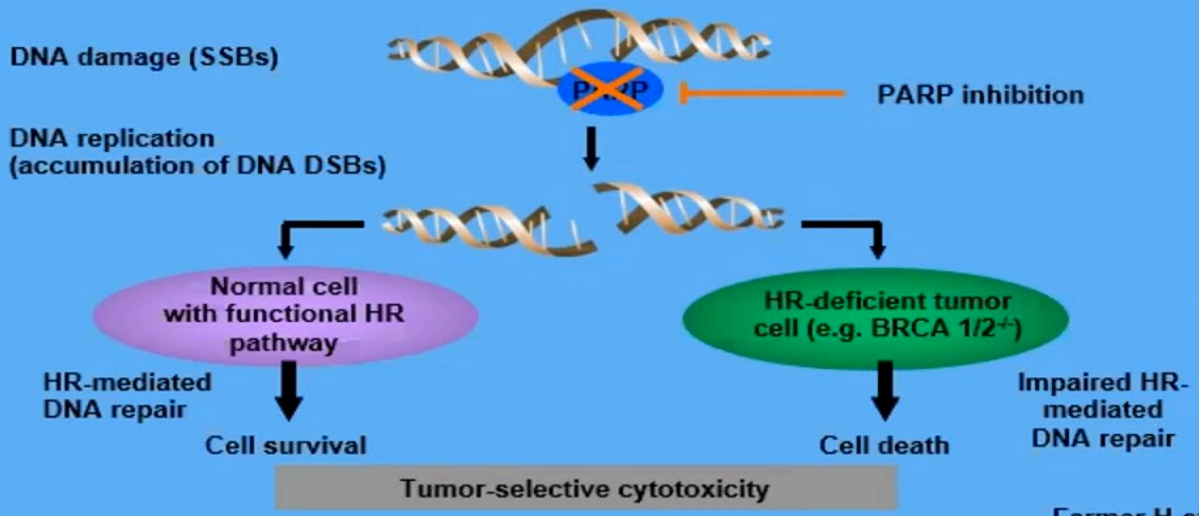
Double Stranded Repair (DSB) Pathway Repairs

- Homologous recombination (HR)
  - DNA damage from ionizing radiation or other dsDNA injury
  - *FANC* genes, *BRCA1/2*, *ATM*, *PALB2*, *RAD50*, *RAD51*, *NBN*, *MRE11*, *BLM*, *ATR*
- Non-homologous end joining (NHEJ)
  - DNA damage from ionizing radiation or other dsDNA injury
  - *XRCC4/5/6*, *LIG4*, *DCLRE1C*, *PRKDC*, *NHEJ1*, *POLL/M*
- Trans-lesion DNA synthesis (TLS)
  - Error-prone recovery mechanism when no DNA template
  - *POLH*, *POLI*, *POLK*, *PCNA*, *REV1/3* (error-prone DNA polymerases)



O'Connor MJ, *Molecular Cell*, 2015

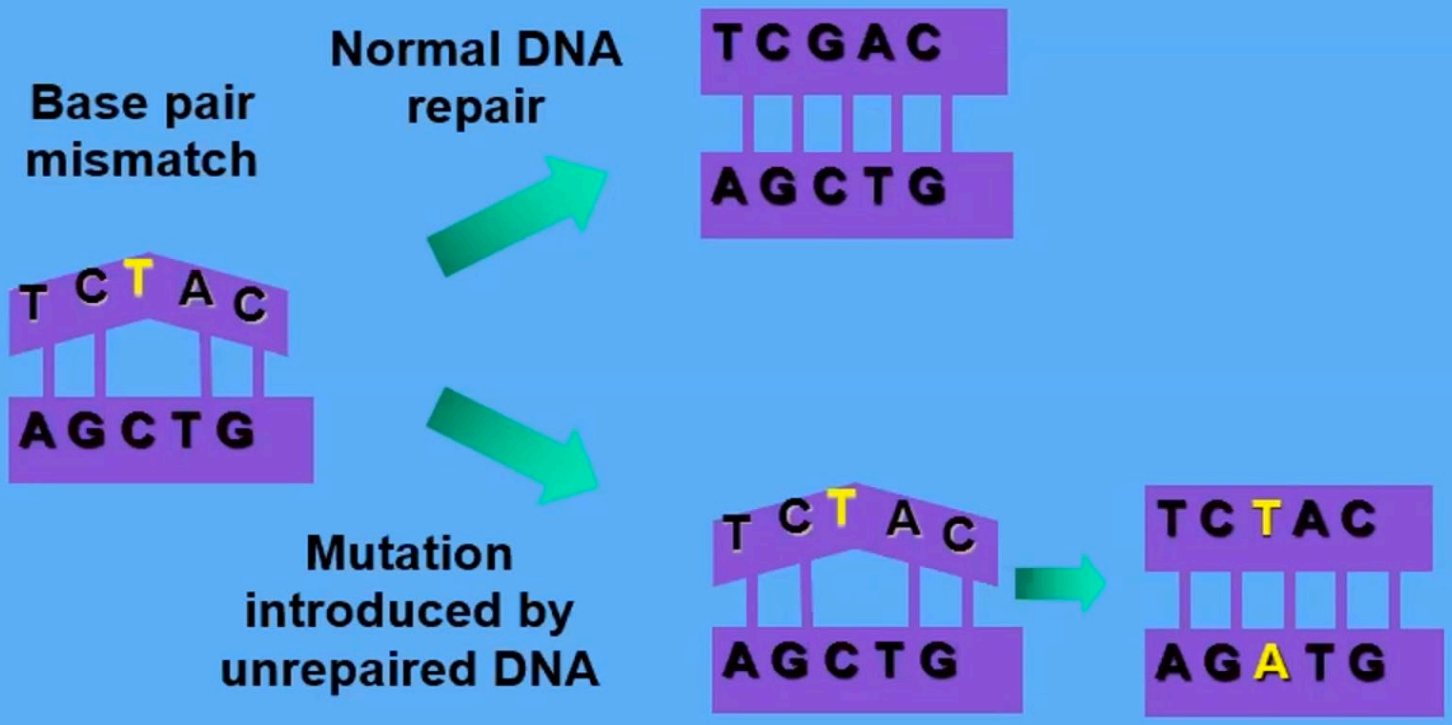
PARP Inhibition and Tumor-Selective Synthetic Lethality



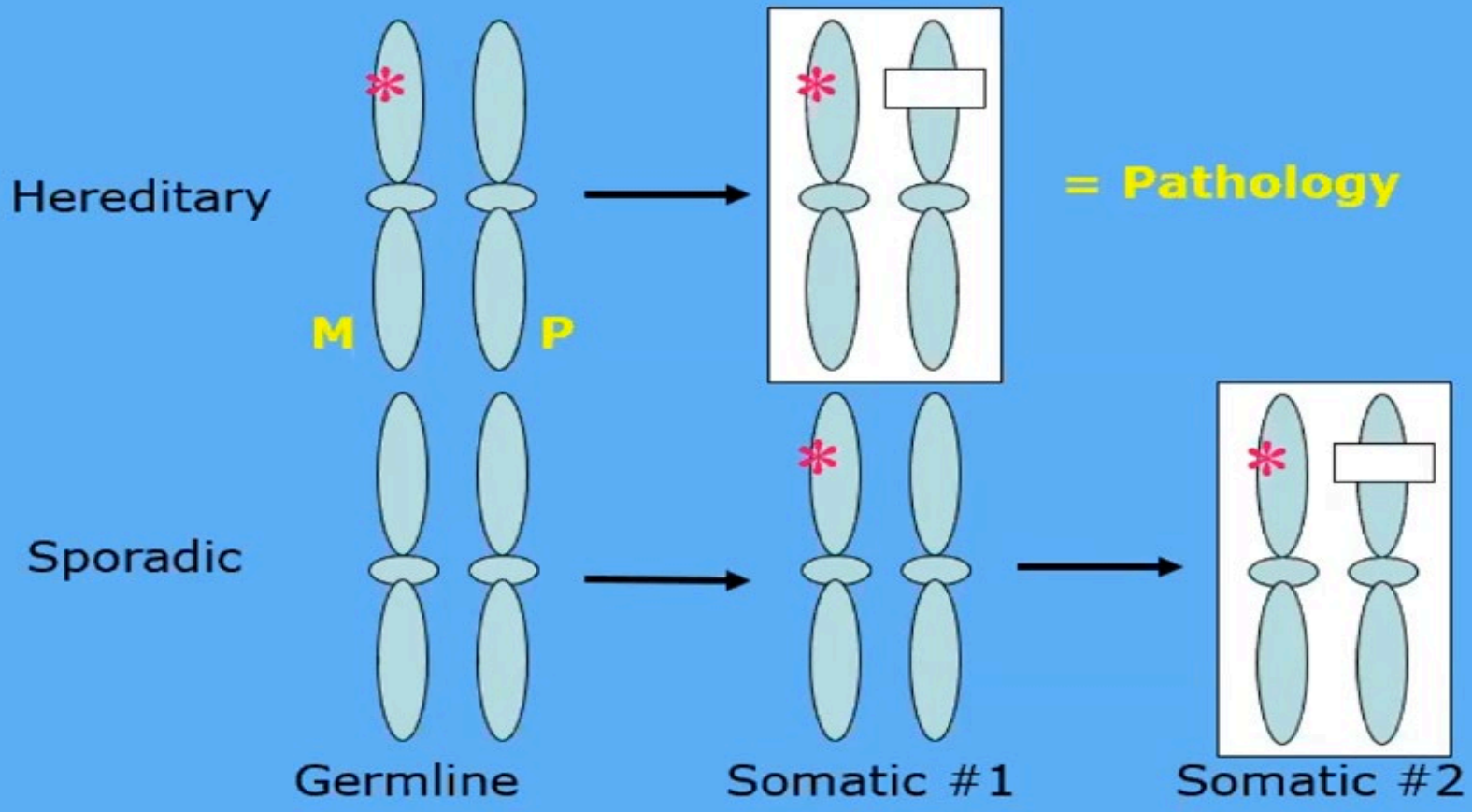
DSB, double-strand break; HR, homologous recombination
SSB, single-strand break

Farmer H *et al. Nature* 2005;434:917–921
Bryant HE *et al. Nature* 2005;434:913–917
McCabe N *et al. Cancer Res* 2006;66:8109–8115

DNA Mismatch Repair



Knudson 2-HIT Model

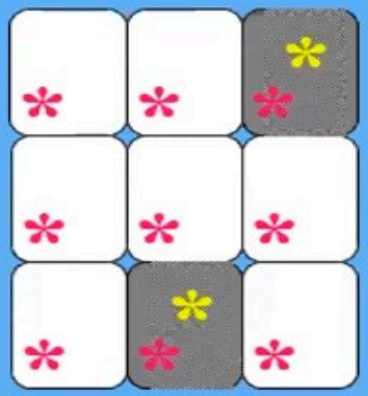


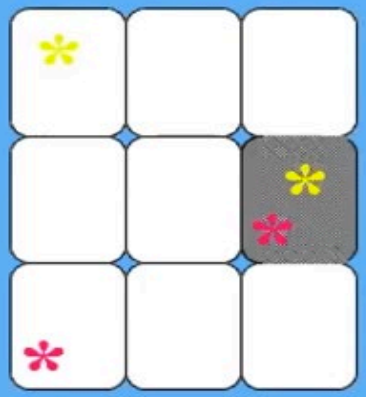
The Human Genome and Cancer

- All cancers arise from genetic alterations
- Tumorigenesis is a multi-step process
- About 5% to 10% of cancer is hereditary
- The Human Genome Project is catalyzing discovery of cancer genes and development of:
  - predictive tests to identify genetic predisposition
  - diagnostic tests to detect cancer in its earliest stages
  - therapies that target gene abnormalities in cancer cell
- A basic understanding of genetic principles, cancer genetics, and the complexities of risk assessment is necessary for responsible translation of these advances into clinical practice

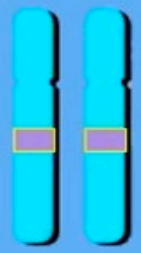
Knudson 2-HIT Model

* First Hit
* Second Hit

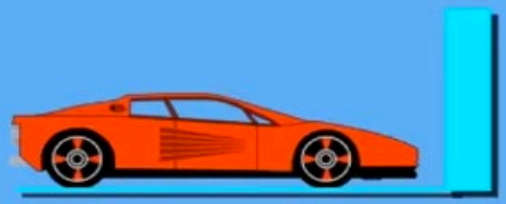


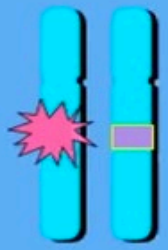


Tumor Suppressor Genes

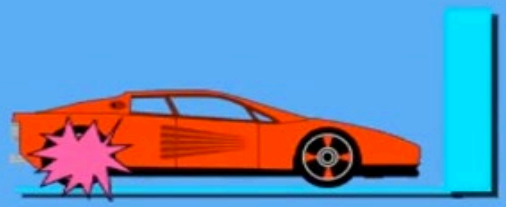


Normal genes
(prevent cancer)





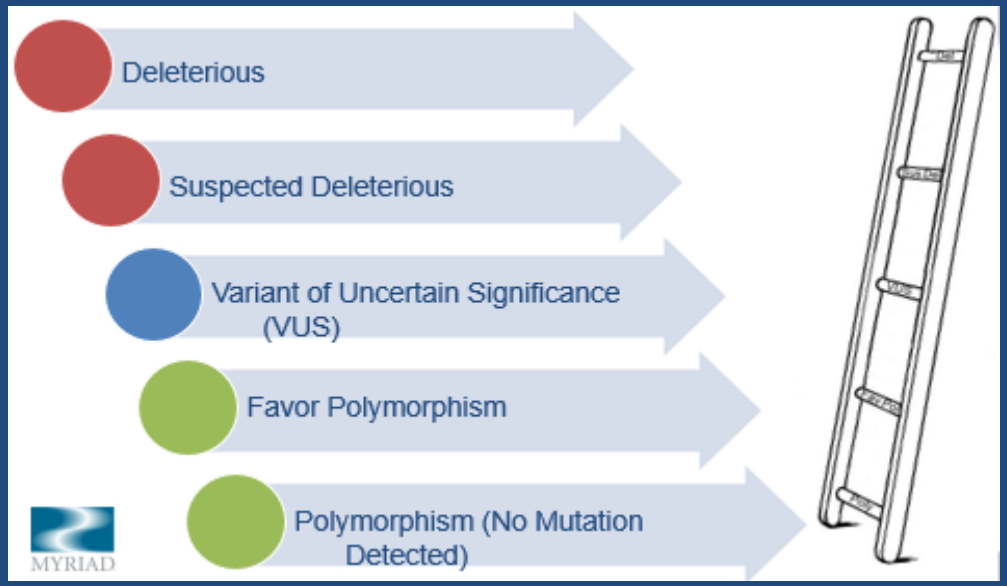
1st mutation
(susceptible cell)



2nd mutation or loss
(leads to cancer)

ACMG Classification System

- Pathogenic- Sequence change directly contributes to disease development
- Likely Pathogenic->90% likelihood the change is disease causing
- Variant of Uncertain Significance (VUS)- There isn't enough information to support a definitive classification
- Likely Benign->90% likelihood that change is not disease causing
- Benign- Sequence change is not disease causing.

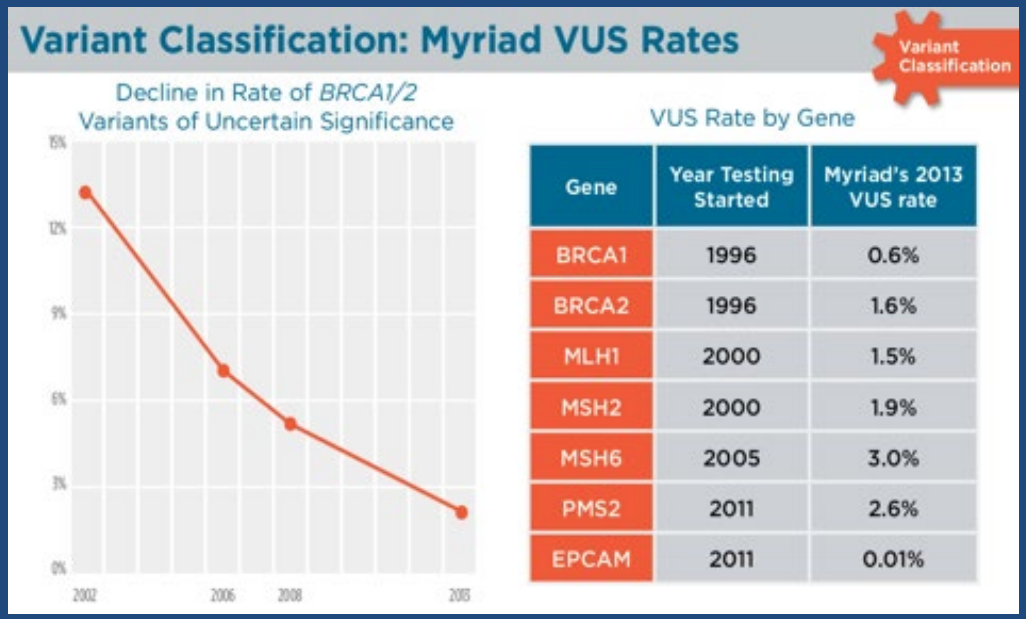


Genetic Variant of Uncertain Significance (VUS)

Can be reported on a patients genetic analysis

Definition: inconclusive Interpretation: a genetic change that is different from normal control. Most VUS results are ultimately given definitive classifications.

Management: based on personal and family history; clinical single site testing for a VUS in relatives is not recommended



Gene	Year Testing Started	Myriad's 2013 VUS rate
BRCA1	1996	0.6%
BRCA2	1996	1.6%
MLH1	2000	1.5%
MSH2	2000	1.9%
MSH6	2005	3.0%
PMS2	2011	2.6%
EPCAM	2011	0.01%

Richards CS, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. Genet Med 2008: 10: 294;
https://www.myriadpro.com/blog/variants-of-uncertain-significance-what-you-need-to-know

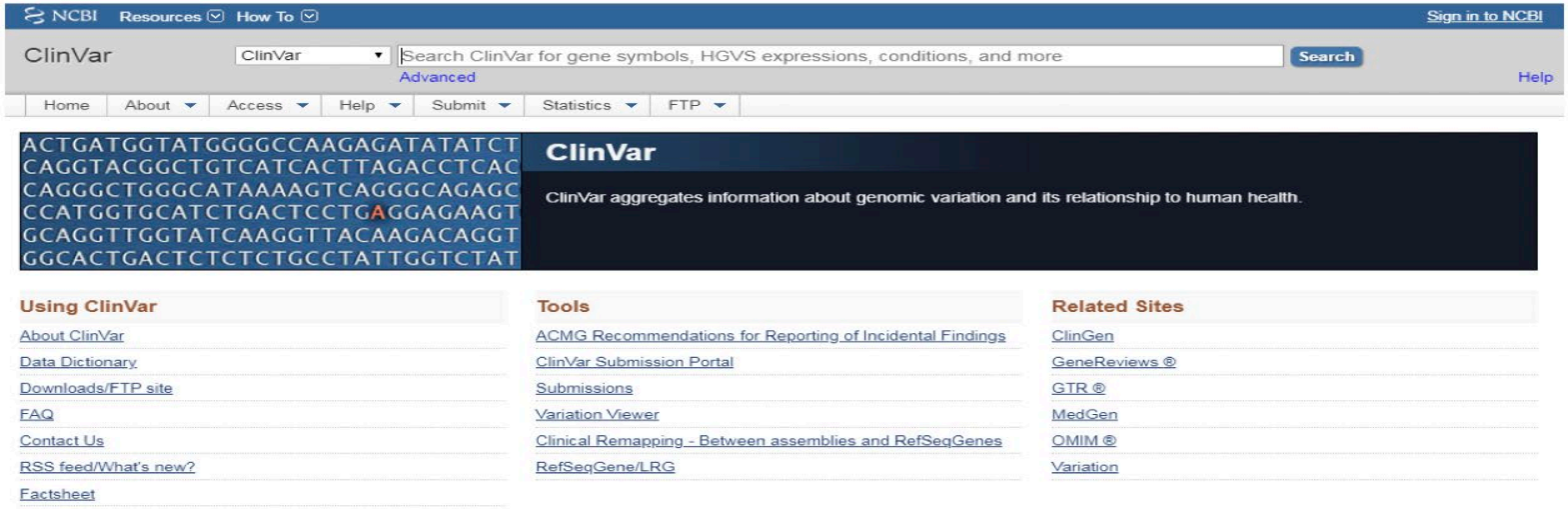
Variant reclassification considerations/recommendations

Table 2: Variant reclassification considerations/ recommendations

Variant reclassification considerations/ recommendations for those ordering genetic testing
1. Consider using laboratories that have an active variant reclassification follow-up program.
2. Develop a standard operating procedure to notify patients and update the medical record for all reclassified variants.
3. Let patients know to update their contact information with you if address changes occur.
4. Suggest all patients with variants follow-up every few (~3-5) years for new information regarding their variants or consideration for update testing.
5. If the patient is being seen as a second opinion, consider taking steps to become listed as a managing provider for their testing in case of any reclassifications.
6. Maintain an element of skepticism for variants that do, or do not, fit a particular phenotype.

NCBI Clinical Variant Database (ClinVar)

https://www.ncbi.nlm.nih.gov/clinvar/



So what is a SNP?

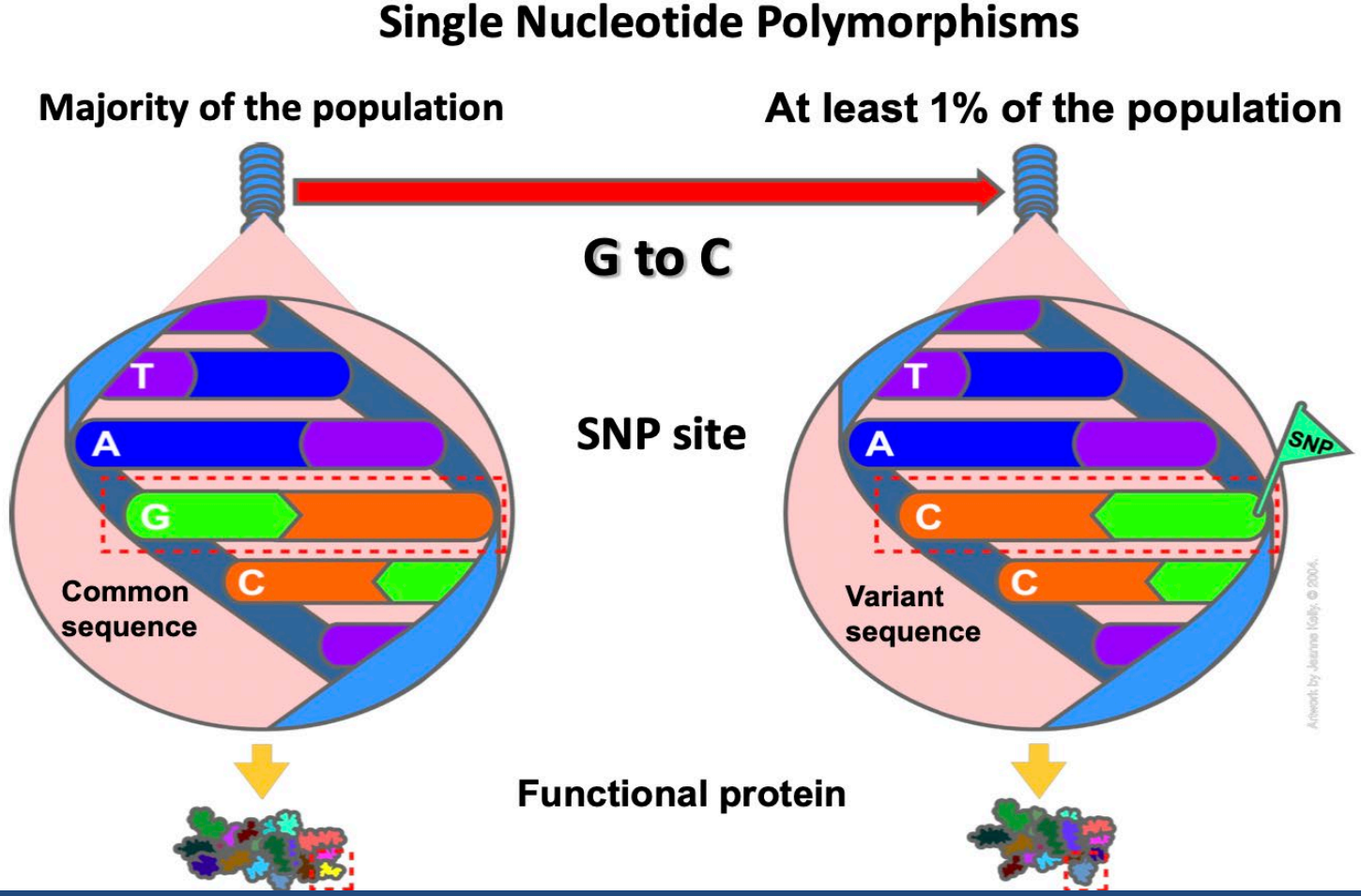
- SNP: single nucleotide polymorphism

Affecting one nucleotide of the DNA

Typically found in at least 1% of a population

- So, a SNP is common human variation
  - Generally, something common would be unlikely to have a large impact on disease risk
- In some cases, a single SNP may have a small effect on disease risk (positive or negative)
  - If combined with other SNPs, this may ultimately have enough of an effect to significantly modify disease risk

SNPs: Frequently Occurring Genetic Variants



Polygenic Risk Scores

- Combining information from multiple SNPs (protective and risky SNPs) to estimate a risk for a specific type cancer
- SNPs that affect cancer risk do not need to be located within a cancer gene
- Each SNP is inherited independently from other SNPs
  - While a person has a 50% chance of sharing a particular SNP with their first degree relative, chances are they will not share every single SNPs with them
  - This means SNP scores can be dramatically different between close relatives
    - May account for some of the variability in families

When do SNPs come up in cancer genomics?

Describing	Something common Ex: Ancestry testing is often SNP-based
Describing	Something that may influence risk only slightly Ex: Polygenic risk scores incorporate multiple SNPs to estimate cancer risk

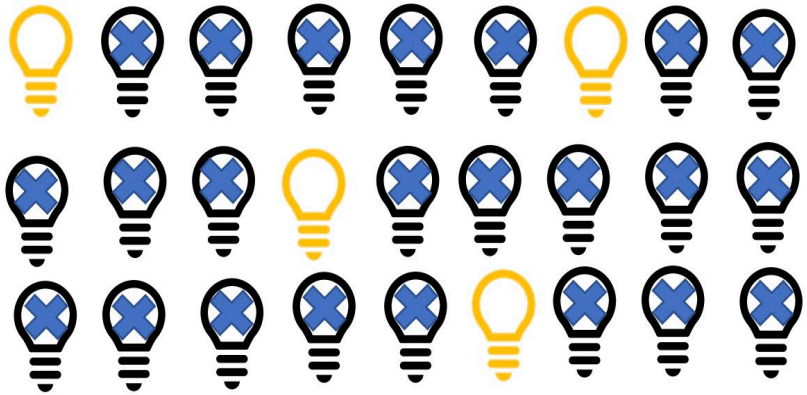
High risk gene mutation versus SNPs

Let's think of risk to develop breast cancer as the amount of light in a room: a darker room means the risk is higher.

The *BRCA1* gene would be one huge light bulb in a room

Having a burned out BRCA1 light bulb will make a big difference in how dark the room is (breast cancer risk)

Breast cancer risk SNPs would each be a tiny bulb



Having one burned out SNP light bulb will not make a significant difference in how dark the room is. BUT having many SNP light bulbs that are burned out WILL.

Emerging Role of Genetic Testing in Prostate Cancer

- Screening
  - Individual
  - Family members “cascade testing”
- Active surveillance
- Treatment decisions all stages
- Prostate biopsy confirmation

The Family History
Who in family should be offered "cascade testing"?

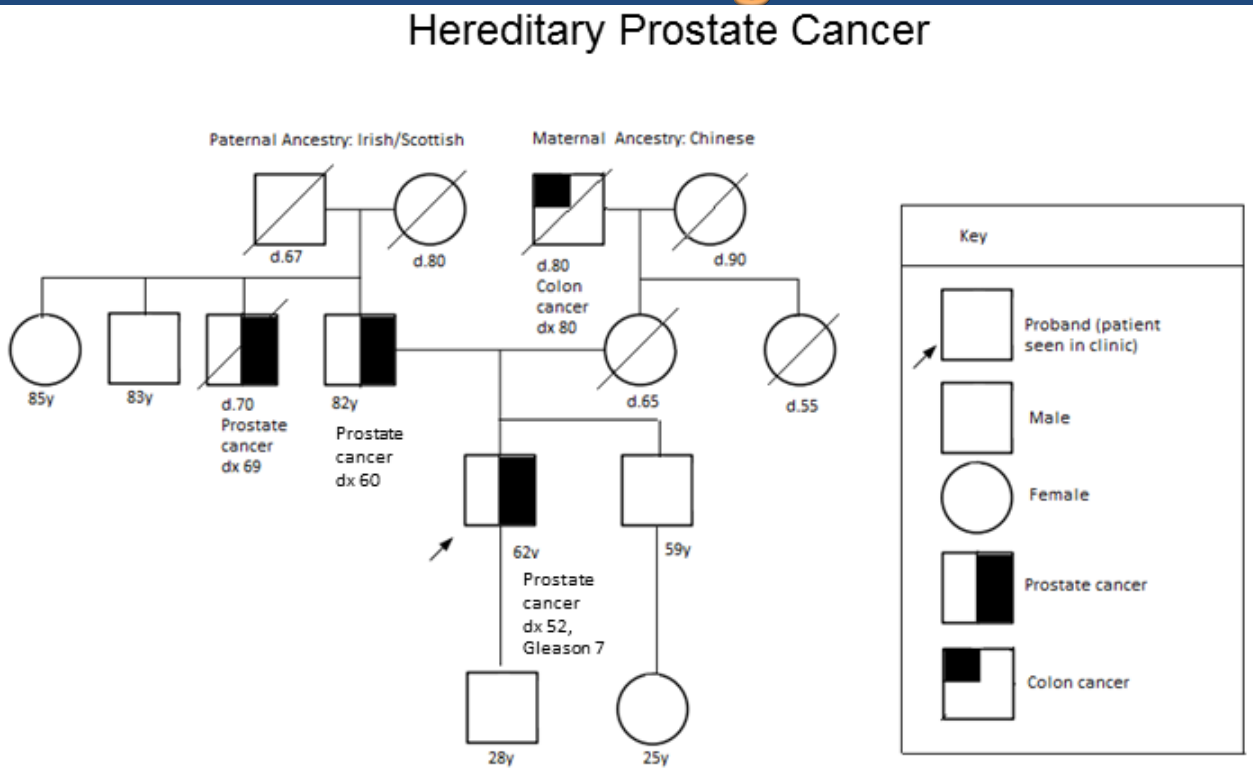


Figure 1a. Hereditary prostate cancer showing the proband (with arrow) with prostate cancer diagnosed at age 52, patient's father diagnosed with prostate cancer at age 60, and paternal uncle with prostate cancer at age 69.

"Implementation of Genetic Testing for Inherited Prostate Cancer"

2019 Consensus Conference

October 4,5 2019

Sidney Kimmel Cancer Center, Thomas Jefferson University

Sidney Kimmel Cancer Center at Jefferson
NCI – designated

Germline testing for prostate cancer: community urology perspective

Raoul S. Concepcion, MD,[1,2]

[1]Department of Urology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[2]Urology Division, Integra Connect, West Palm Beach, Florida, USA

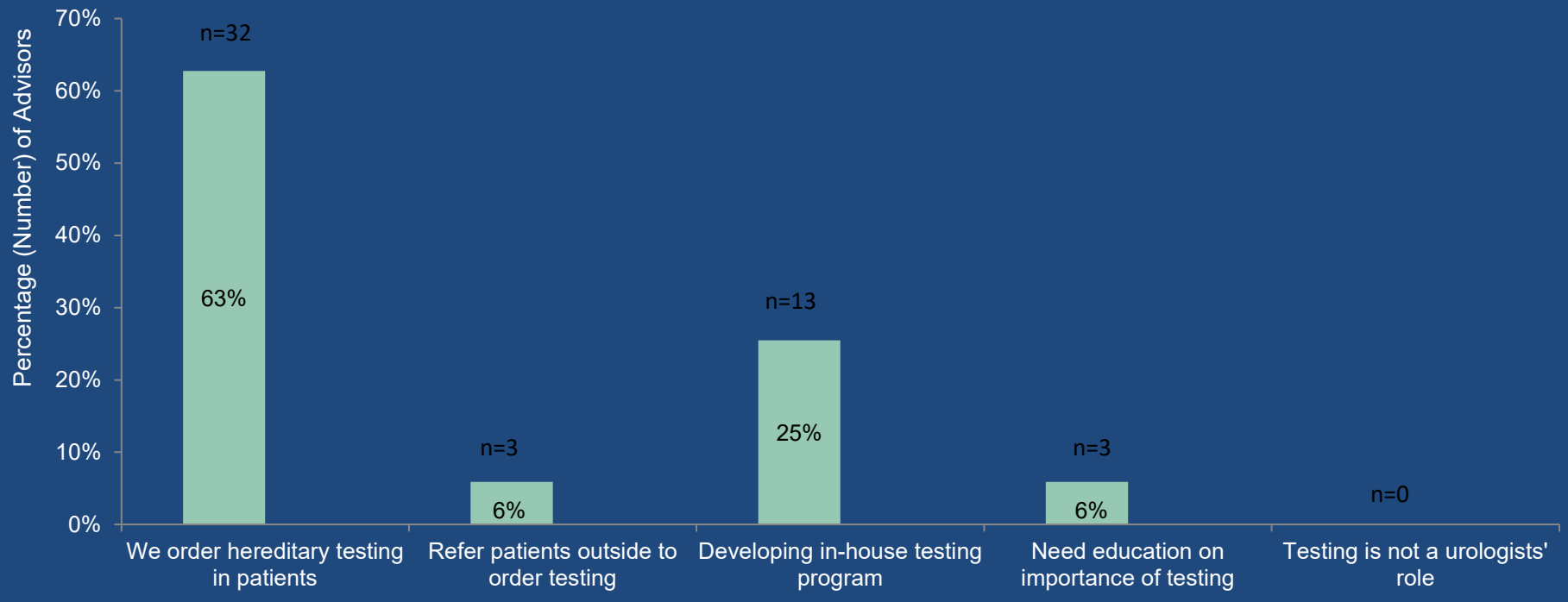
CONCEPCION RS. Germline testing for prostate cancer: community urology perspective. *Can J Urol* 2019;26(Suppl 2):50-51.

In an attempt to better understand how community urology practices would begin to incorporate hereditary testing in prostate cancer patients, we developed an eight-question on line survey to identify current testing patterns, utilization of genetic counseling and barriers that practices face. Fifty-two large community urology practices participated. A total of 32/52 (63%) of the responders were already offering testing to select patients. The big hurdles practices were concerned when initiating testing were fear of medical/legal liability (22%), concerns over reimbursement and out of pocket patient expense (20%) and the complexity, time and difficulty to enter a complete family history/pedigree into the EHR (18%).

Key Words: germline testing, community urology practices

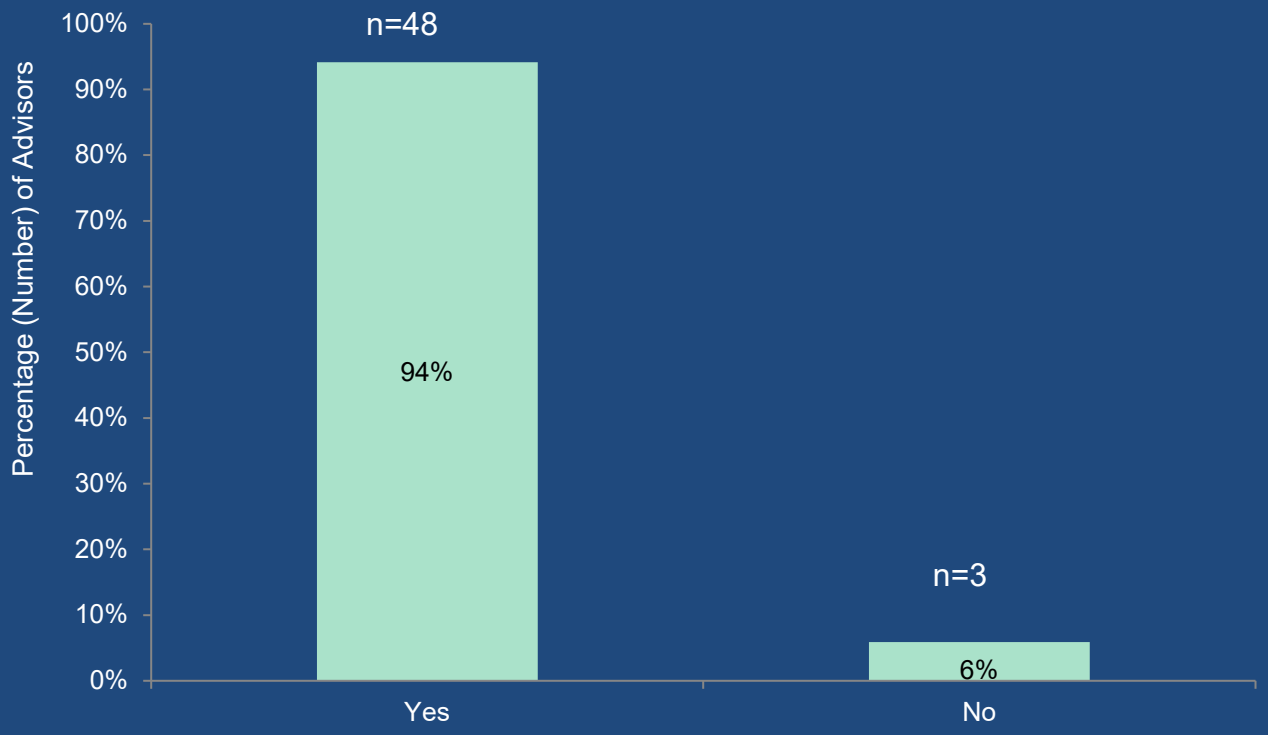
Hereditary Testing Patterns in Practices

Q6: Which of the following BEST describes your practice habits in regards to the ordering of hereditary testing in men with prostate cancer? (n=51)



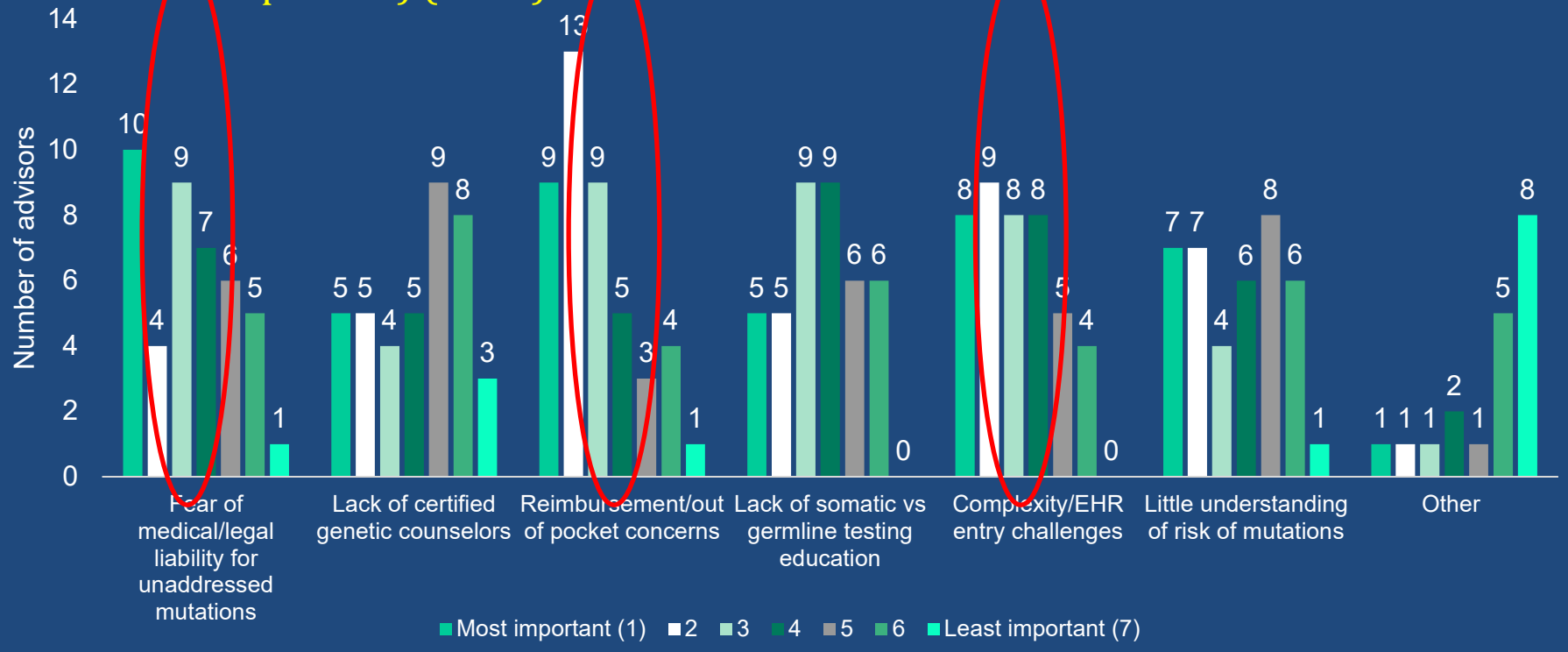
Awareness of Genetic Testing and Counseling Guidance in Prostate Cancer

Q5: Are you currently aware of the most recent statement (Dec 2018) published by SUO, supported by LUGPA and AACU, regarding the utilization of genetic testing and counseling in prostate cancer as outlined by NCCN? (n=51)



Barriers to In-House Hereditary Testing Program

Q8: Please rank the following statements in order of importance in the implementation of an in-house hereditary testing program (1 = most important, 7 = least important) (n=46)



Other: refer to oncologist/genetic counselor; where to send the blood, saliva; insurance coverage for some patients; implemented; N/A; unforeseen issues; cost of counseling is prohibitive for urologists

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Genomic Testing – Practice Launch

March 2022

Objectives of the Program

- Incorporate testing in eligible patients
- Take work off the providers and staff
- Provide treatment recommendations for the providers (Litho Link model)
- Establish a comprehensive testing program which will include pts for risk assessment
- Incorporate genomic information with existing longitudinal data that the practices already own (Histopathology, treatments, lab, therapies, etc)

Hereditary Testing Model: Flow

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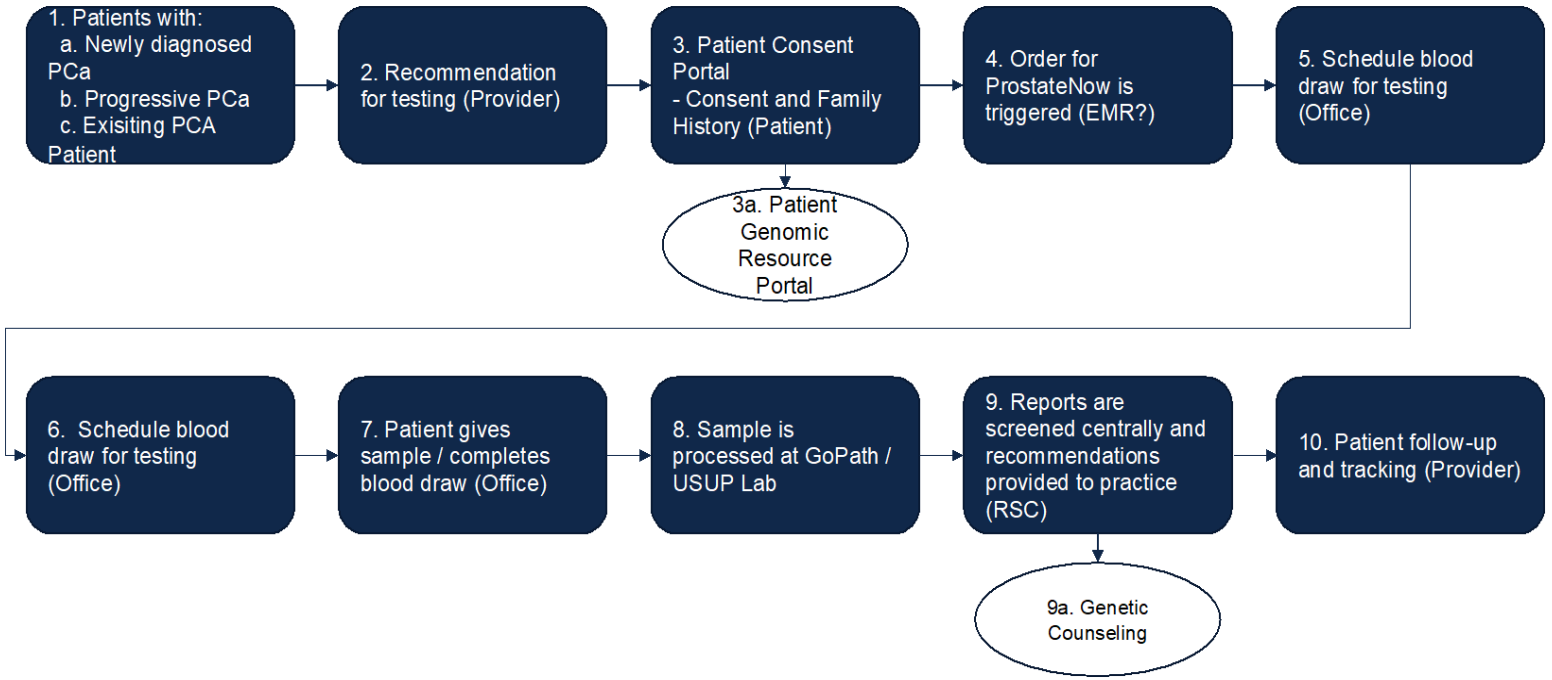
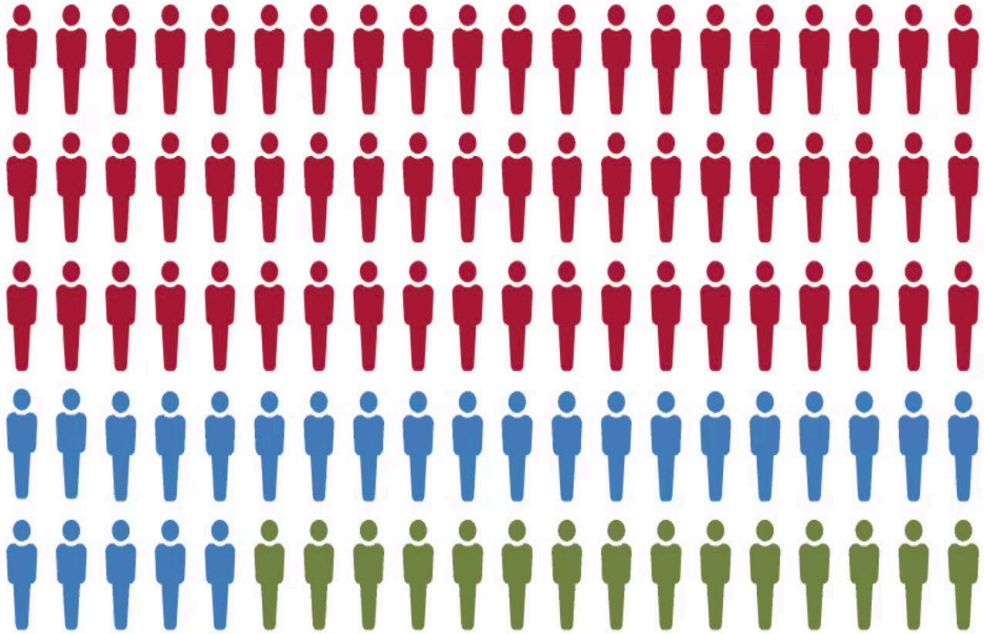
Workflow Overview



NGS Oncology Panels Are Ordered in 15% of Eligible Patients



100 U.S. Metastatic Cancer Patients

60 No Molecular Testing

25 Single-Marker Testing

15 Next Generation Sequencing Testing

Inside drugmakers' strategy to boost cancer medicines with 'Lazarus effect' – Reuters, Health News, September 5 2019 https://www.reuters.com/article/us-bayer-cancer-insight/inside-drugmakers-strategy-to-boost-cancer-medicines-with-lazarus-effect-idUSKCN1VR0EA

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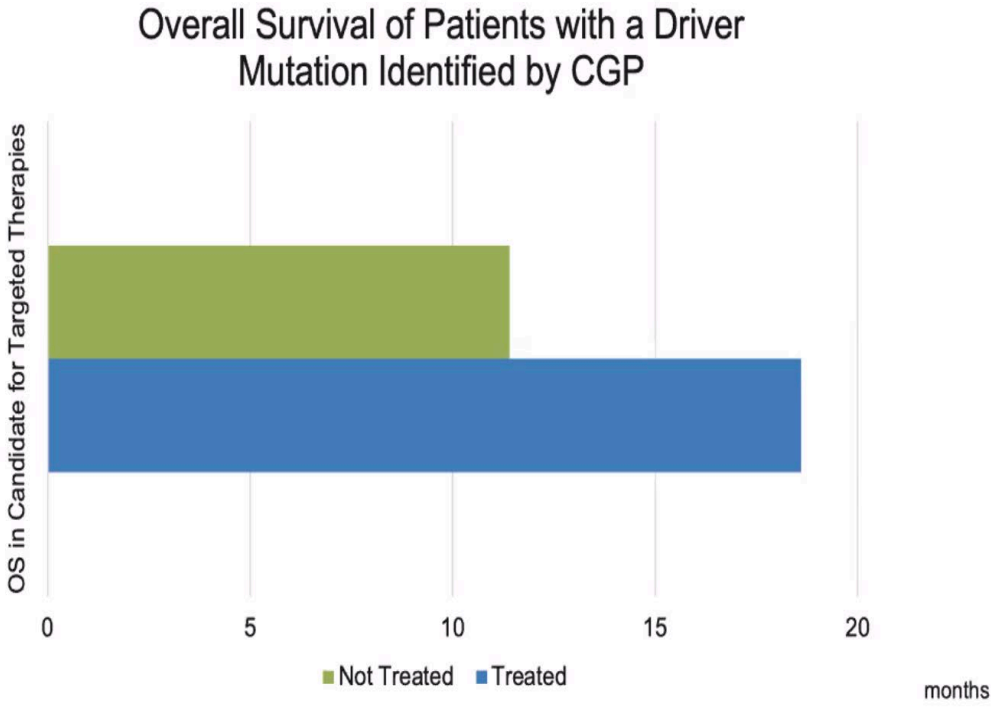
CGP Identifies Candidates for Targeted Therapies Leading to increase OS by 63%



A study involving over 4,000 NSCLC patients identified **14%** are candidates for targeted therapies using CGP

Patients treated with targeted therapies showed **63%** longer OS vs untreated patients with driver mutation

Adapted from: Singal G, Miller PG, Agarwala V, et al. Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non–Small Cell Lung Cancer Using a Clinicogenomic Database, *JAMA*. 2019: 321(14): 1391-9.

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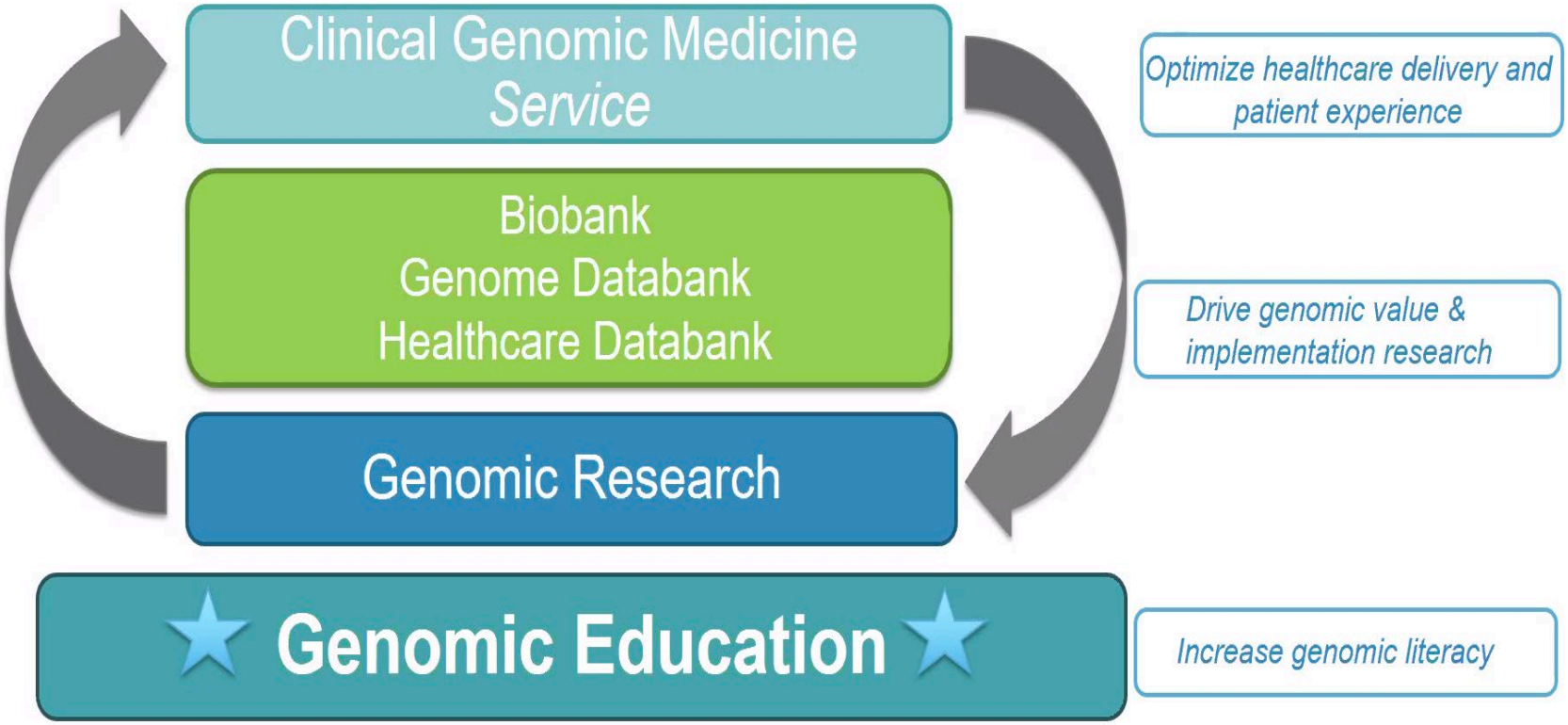
Key Concept

Genomic Literacy underlies a thriving service



Conclusions

- An understanding of modern day genomics and DNA repair is critical
- There may be enrichment for DNA repair gene mutations in high-grade PCa and in certain histological subtypes
- We have well established PCa genomic tissue testing but the recommendations for PCa genetic testing and decision making continue to evolve.
- The major concerns facing practices regarding testing are reimbursement, EHR complexity, medical legal liability

“Knowledge that does not change behavior is useless.”

Yuval Harari, Homo Deus, 2015

Thank you!

rconcepcion@us-uro.com