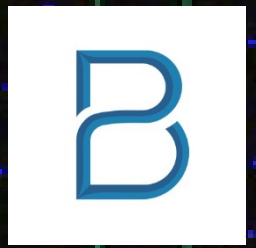

Emerging NGS Applications at the Intersection Germline and Somatic Cancer Genetics

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ARUP Conference Park City

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Disclosure Information

Nothing to disclose

Learning Objectives

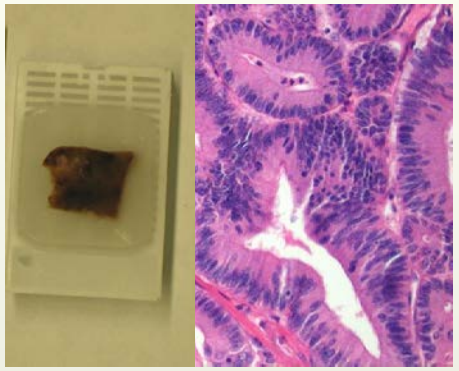
1 Understand when and how testing for inherited mutations in *BRCA1, BRCA2*, and other homologous recombination DNA repair genes is used to guide cancer treatment.

2. Describe the clinical scenario and utility of tumor sequencing of mismatch DNA repair genes as part of a Lynch syndrome workup.

3. List at least two types of tumor findings that increase the probability that a germline variant in a cancer predisposition gene is pathogenic.

Interplay

Tumor



Germline

Targeted Therapy

Cancer Syndrome Screening

Variant Interpretation

Outline

- DNA repair gene mutations and cancer treatment
  - Background
  - Testing Approaches
- Tumor sequencing in a Lynch workup
- How tumors can help with variant classification
- Case vignette

DNA Repair Genes Guide Cancer Treatment

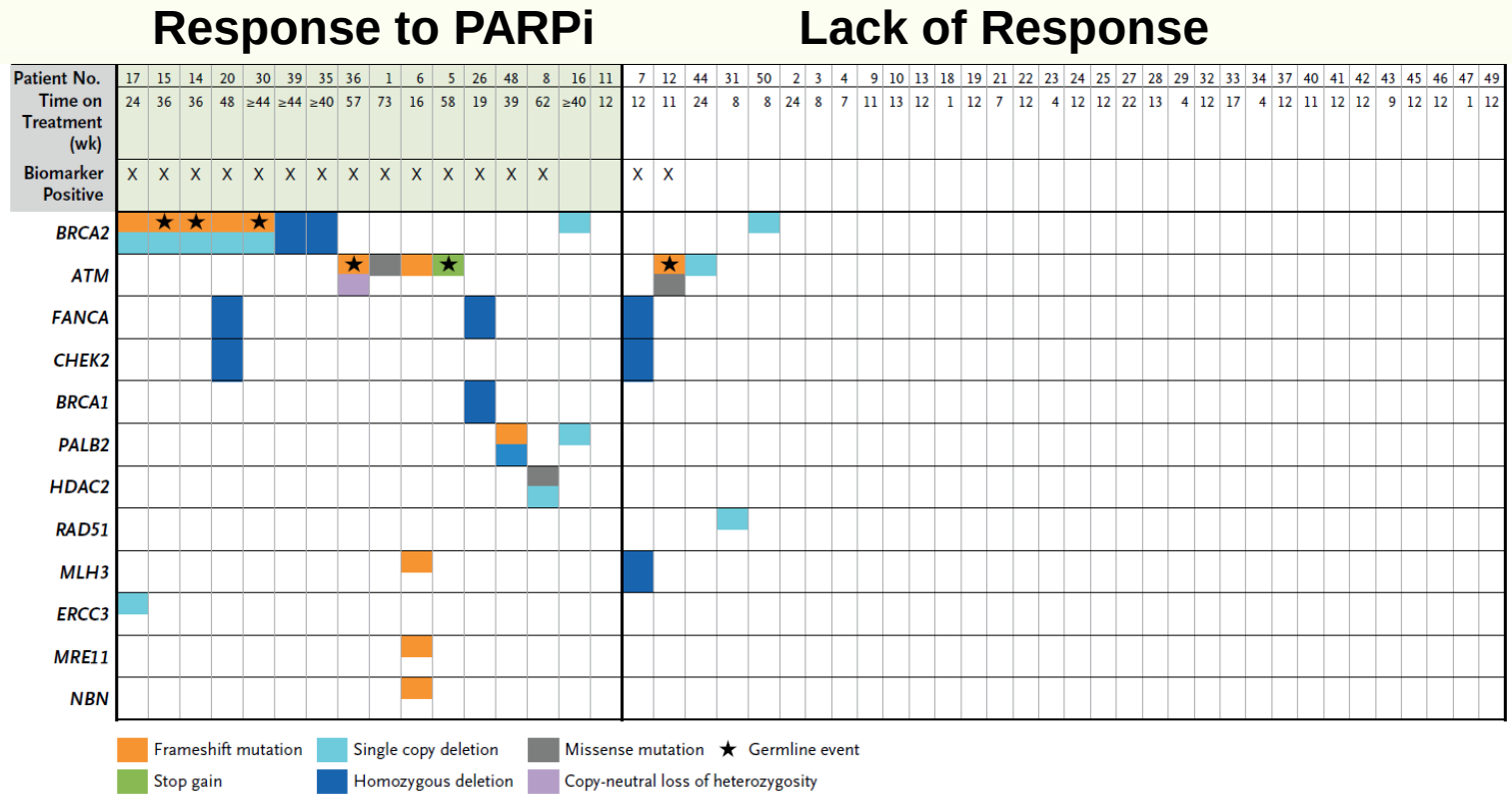
DNA Repair Pathway	Example Genes	Germline Syndrome	Treatment Implications
Homologous Recombination Repair (HR)	*BRCA1, BRCA2*	Hereditary breast/ovarian/ prostate	PARPi, platinum
Mismatch Repair (MMR)	*MSH2, MLH1*	Lynch	PD1/PDL-1 inhibitors

PARPi= poly(ADP) ribose polymerase inhibitor
PD1/PDL-1= programmed cell death 1/ligand 1

FDA Approves PARPi for Ovarian and Breast

- Three PARPi approved, 2014 (ovary), 2018 (breast)
- Two drugs based on *BRCA1/2* mutation status
- Germline + somatic *BRCA1/2* testing now standard
- Other cancers close behind: prostate, pancreatic

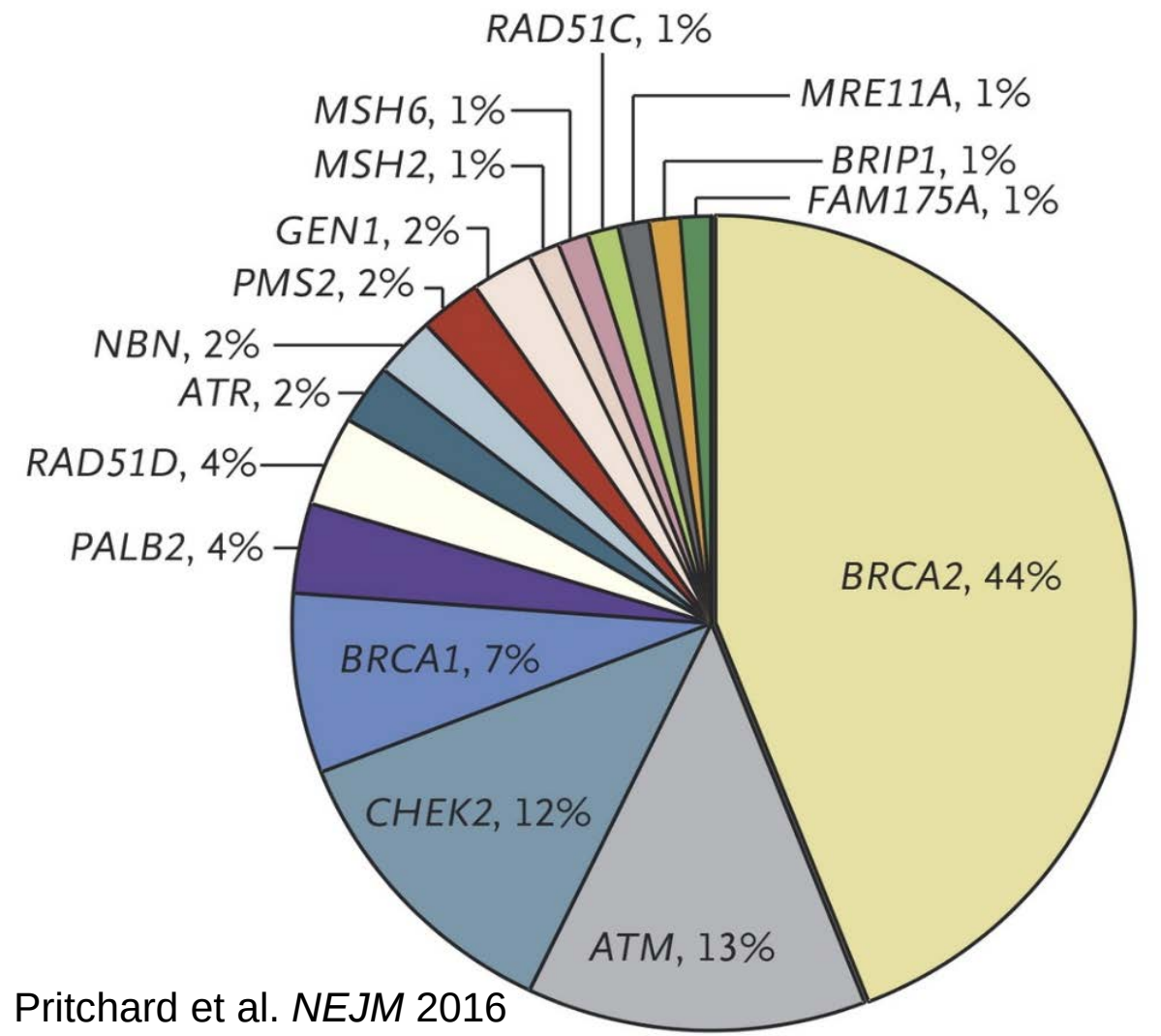
Extraordinary PARP Inhibitor Responses in DNA Repair-Mutated Prostate Cancer



Mateo et al. *NEJM* (2015)

14/16 (88%) with bi-allelic DNA repair defects responded
2/33 (6%) without bi-allelic DNA repair defects responded

Germline DNA Repair Mutations Are Common in Metastatic Prostate Cancer



Pritchard et al. *NEJM* 2016

12% (82/692) with deleterious germline mutations in 16 DNA repair genes

59% (36/61) with avail. tumors had second allele loss-of-function mutation

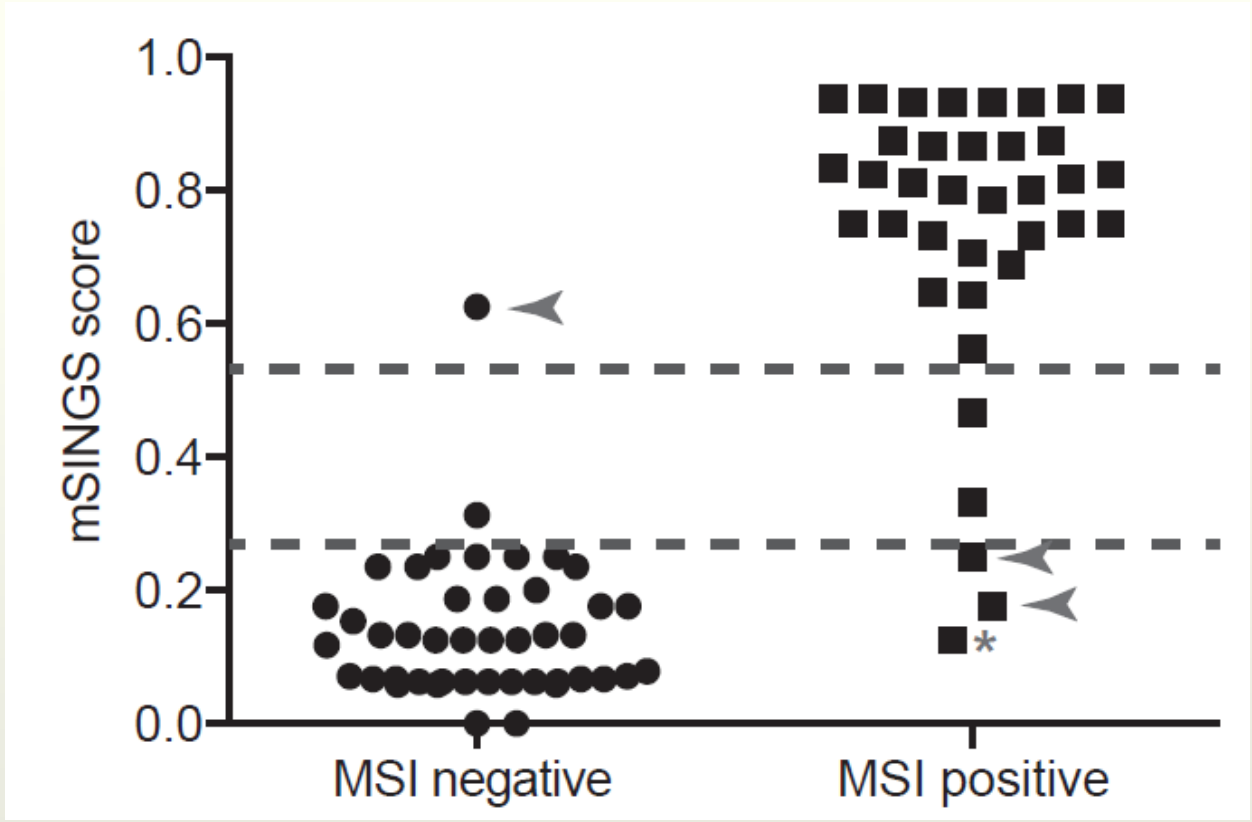
FDA Approves PD-1 Inhibitor for *Any* MMR-Deficient Cancer

MSI by NGS (mSINGS)

Exome

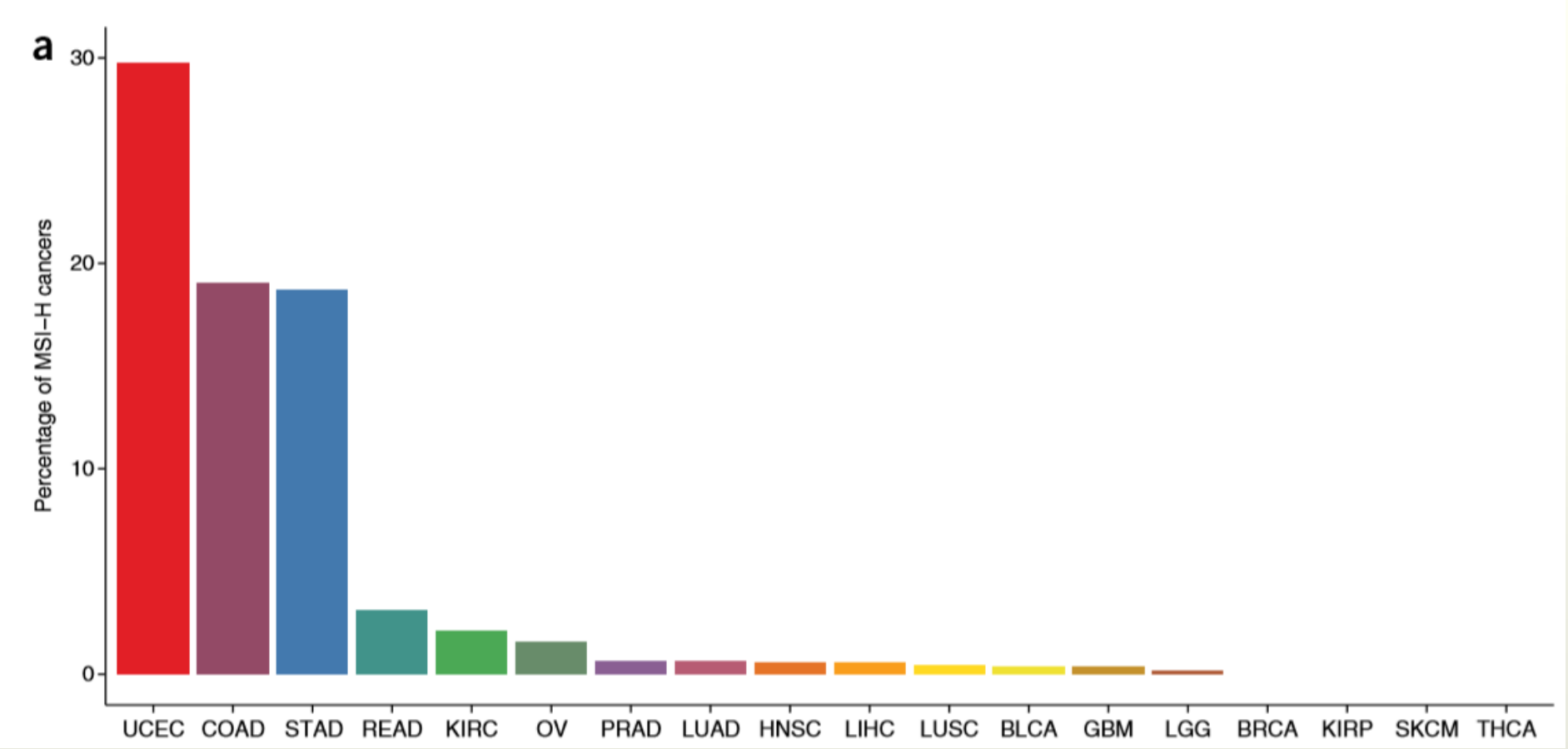
Large Panel NGS (e.g. UW-OncoPlex)

Targeted PCR (MSI-plus)



Salipante et al. 2015 *Clin. Chem*. and Hempelmann et al. 2015 *JMD.*

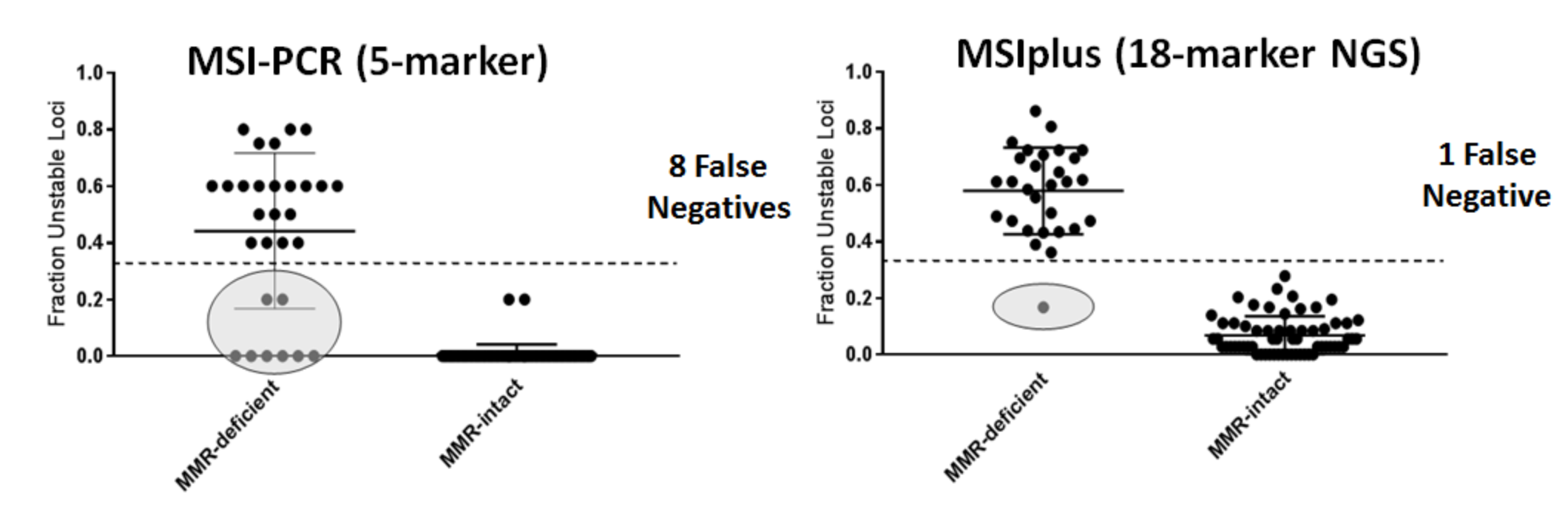
“Long Tail” of MSI Cancers Now Being Tested for MMR



Hause et al. (2016) *Nat. Med*. PMID:27694933

MMR= mismatch DNA repair

MSI by NGS Outperforms Traditional Methods in New Cancer Types



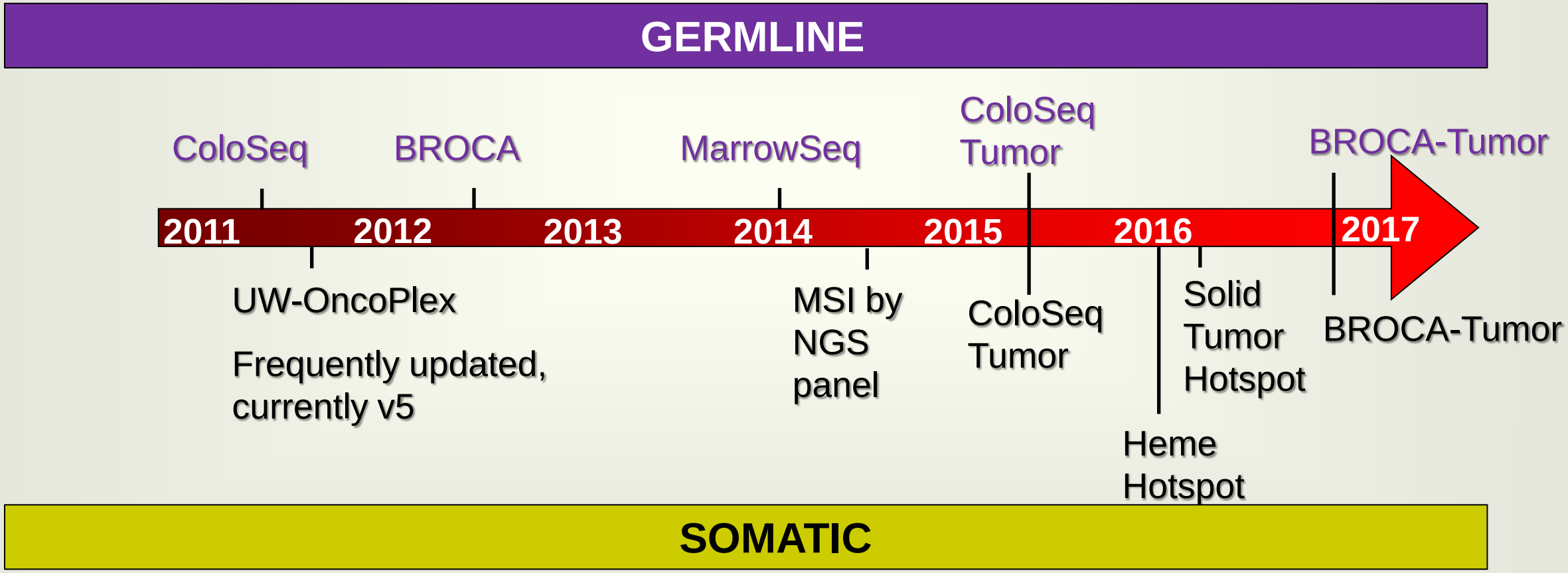
Adapted from Hempelmann et al. (2018) *JITC*. PMID:29665853

Types of Tests

DNA Repair Pathway	Functional	Mutation-Based
MMR	IHC, MSI	NGS Panel (e.g. ColoSeq), Total Mutation Burden
HR	LOH Burden	NGS panel (e.g. BROCA), HRD Mutation Signature

MMR= mismatch repair, HR= homologous recombination, HRD= homologous recombination deficiency
IHC= immunohistochemistry, MSI= microsatellite instability, LOH= loss of heterozygosity

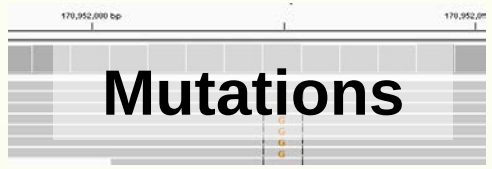
Implementation of Cancer NGS Testing: *Not* One-Size Fits All

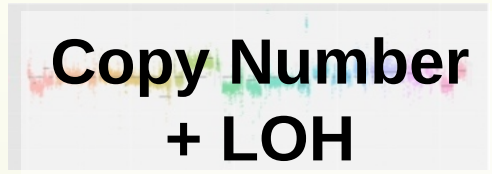


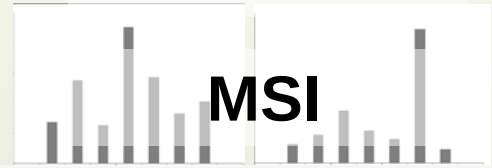
Approach at UW/BBI/SCCA

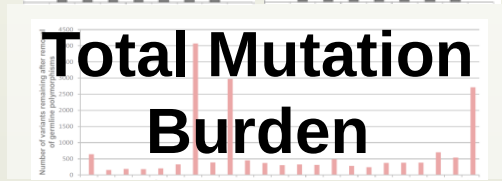
- Upfront **germline and tumor** paired sequencing

- DNA repair-focused NGS panels – **exons *AND* introns**
  - e.g. BROCA-tumor



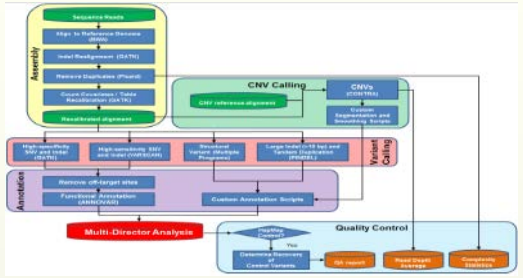






Patient-Tailored
Expert Interpretation

Practice of Genomic Medicine: *Patient-Tailored* Expert Review



Pipeline Output

Multiple Director Review

Multi-Disciplinary Review

Report

SCCA Prostate Cancer Genetic Care Clinic (launched 2016, Heather Cheng)

Two-Part Consultation

1. Medical Oncology
   - Discuss genetics (somatic and germline) for treatment planning
   - Discuss trial/research options

2. Genetic Counselor
   - Risk assessment, pre- and post-test counseling, as relevant
   - Education and guidance on discussing with family

Outline

- DNA repair gene mutations and cancer treatment
- **Tumor sequencing in a Lynch workup**
  - **After germline testing**
  - **As first-line screening**
- How tumors can help with variant classification
- Case vignette

Universal Lynch Syndrome Screening: Colorectal Cancer

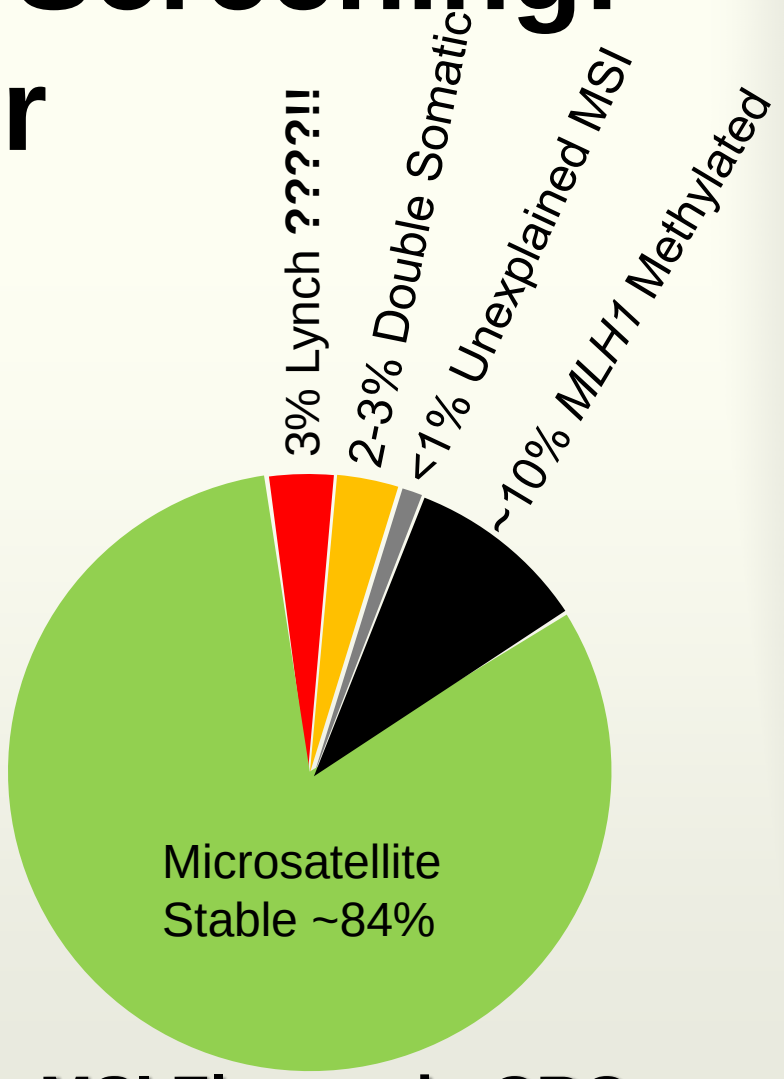
Tumor MSI/IHC **POS**

⇩

BRAF V600E/*MLH1* methylation **NEG**

⇩

Germline testing **NEG**
Lynch excluded??



MSI Flavors in CRC

MSI= microsatellite instability, CRC= colorectal cancer

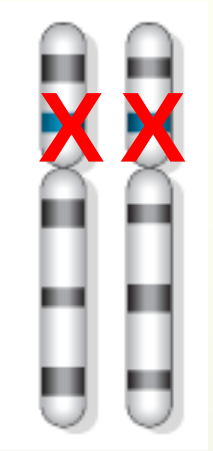
After Germline Testing is Unexpectedly Negative: Sometimes Called “Lynch Like”

- Lynch syndrome in ~3% of colorectal cancer

- “Lynch-like” also in ~2-3% of colorectal cancer
  - Positive tumor screening and no germline mutation
  - **Increasingly clinically important with universal screening**

Double Somatic MMR Mutations Common

- Explain most “Lynch-like” cases (up to 75%)
- About as common as Lynch syndrome
- Positive screening results explained by somatic mutation
- Patients unlikely to have Lynch syndrome (“undiagnosed”)



MMR= mismatch repair genes

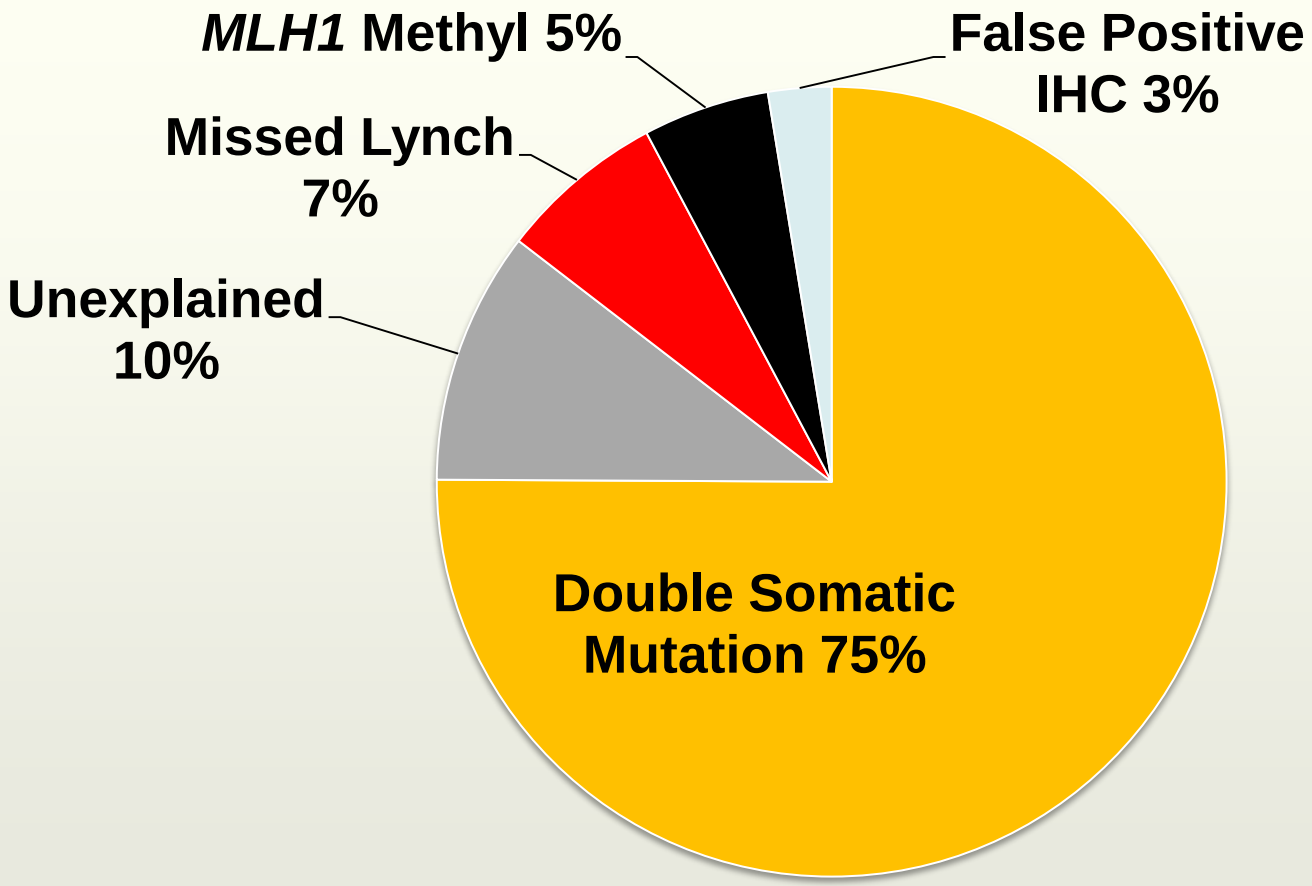
National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2015 Updates
Genetic/Familial High-Risk Assessment: Colorectal

Recommendation to **consider somatic MMR testing** in some scenarios when germline testing is negative was added to the 2015 NCCN guidelines

MMR= mismatch repair genes

Tumor NGS Can Explain MMR Deficiency After Germline is Unexpectedly Negative



Jacobson et al. (2018), manuscript in preparation

Outline

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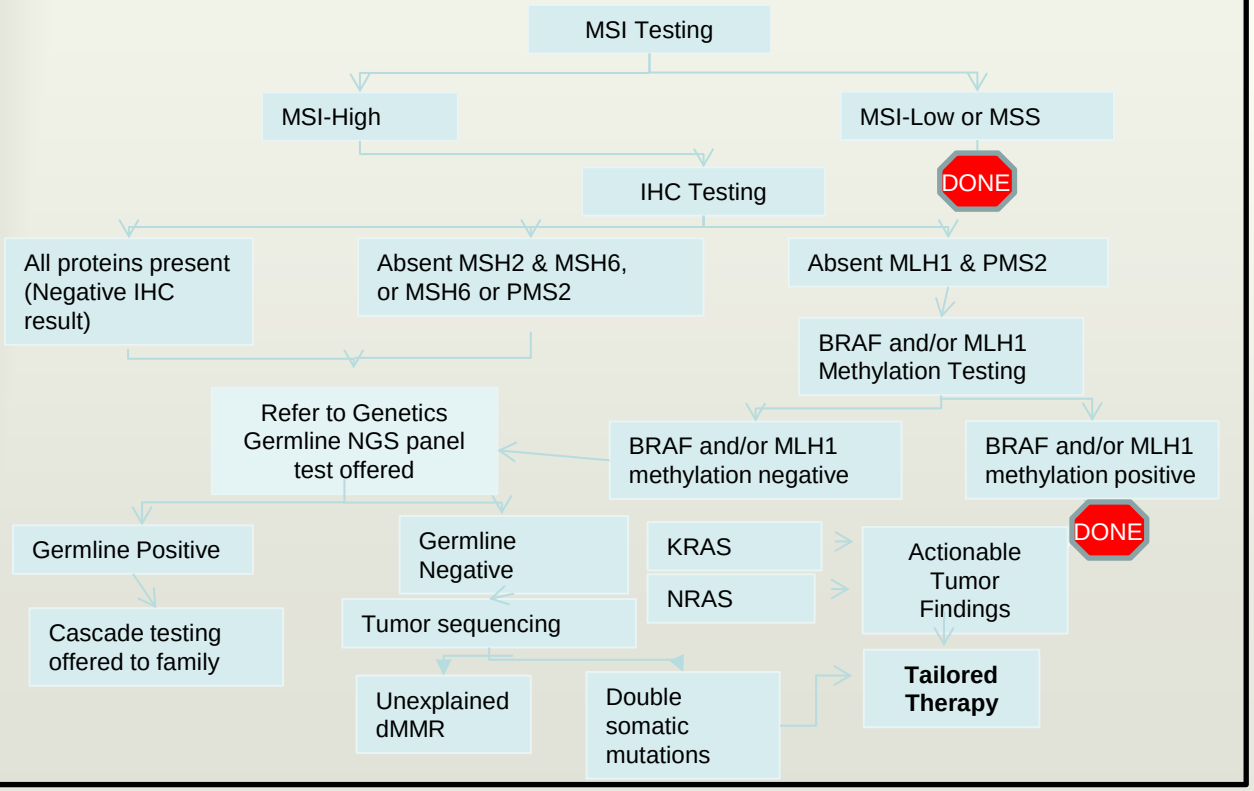
Tumor Sequencing as First-Line Lynch Screening Can Simplify Testing

TRADITIONAL SCREENING



TUMOR NGS SCREENING

One Test: MSI, MMR mutation status + *BRAF, KRAS, NRAS*

POS

Germline

NEG

DONE

Study Design

- 419 OSU prospective cases with known MMR IHC, MSI, *BRAF*, *MLH1*-methylation status by conventional assays
- Tumor-only NGS at UW, blinded expert review
- MMR genes, MSI by NGS, *BRAF, KRAS, NRAS*

Tumor NGS as First-Line Lynch Screening Performs Better Than Traditional Screening

	Tumor NGS	MSI + BRAF	IHC + BRAF
Sensitivity	100% (94-100)	91% (81-97)	90% (79-96)
Specificity	95% (93-97)	95% (92-97)	95% (92-97)
PPV	40% (30-51)	34% (25-45)	33% (24-44)
NPV	>99% (99-100)	>99% (98-100)	>99% (98-100)
Lynch Cases Missed	**0 missed**	**5 missed**	**6 missed**

PPV= positive predictive value;
NPV= negative predictive value
(95% confidence intervals)

Hampel et al. *JAMA Oncology* 2018 PMID: 29887214

Outline

- DNA repair gene mutations and cancer treatment
- Tumor sequencing in a Lynch workup
- **How tumors can help with variant classification**
  - **In patients with germline VUS**
  - **Incorporating somatic data into classification**
- Case vignette

Tumor Data Is Being Used To Clarify Germline Variants

- Increasing tumor testing to clarify “Lynch-like” cases
- Same tests used in patients with germline MMR VUS

VUS= variant of uncertain significance
MMR= mismatch repair genes
“Lynch-like”= patients with positive Lynch screening tests, but negative germline testing

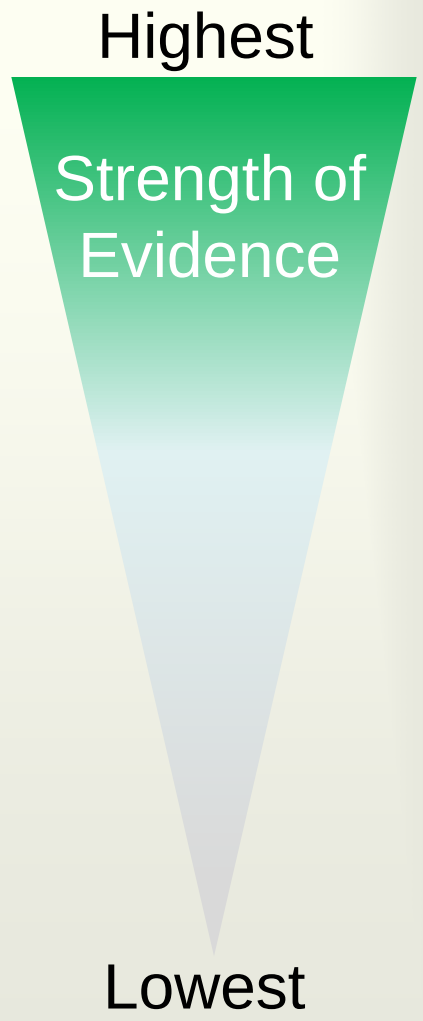
When Tumor Testing *Might* Help

“High” germline VUS, close to likely pathogenic

OR

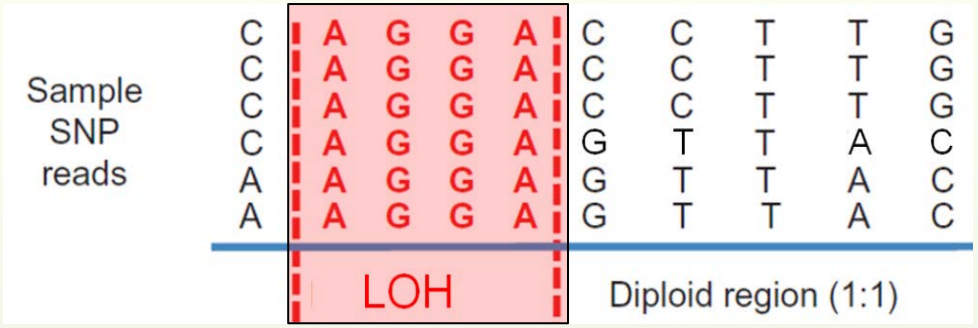
“Low” germline VUS, close to likely benign

VUS= variant of uncertain significance



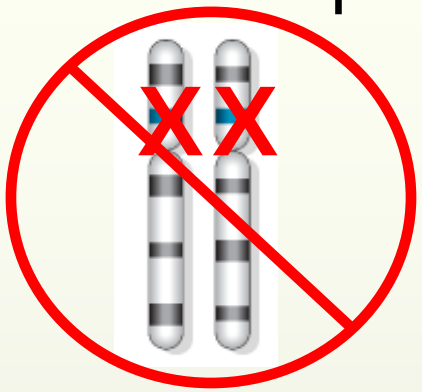
Clues From Tumor: Germline VUS Pathogenic

Loss of Heterozygosity (LOH)



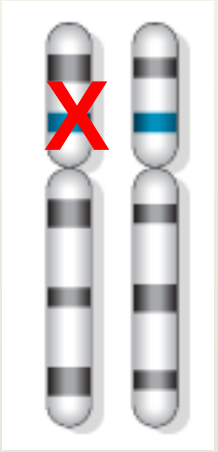
+

No Somatic Explanation



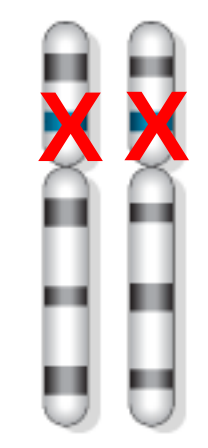
OR

Single Somatic Mutation
Consistent With 2nd Hit



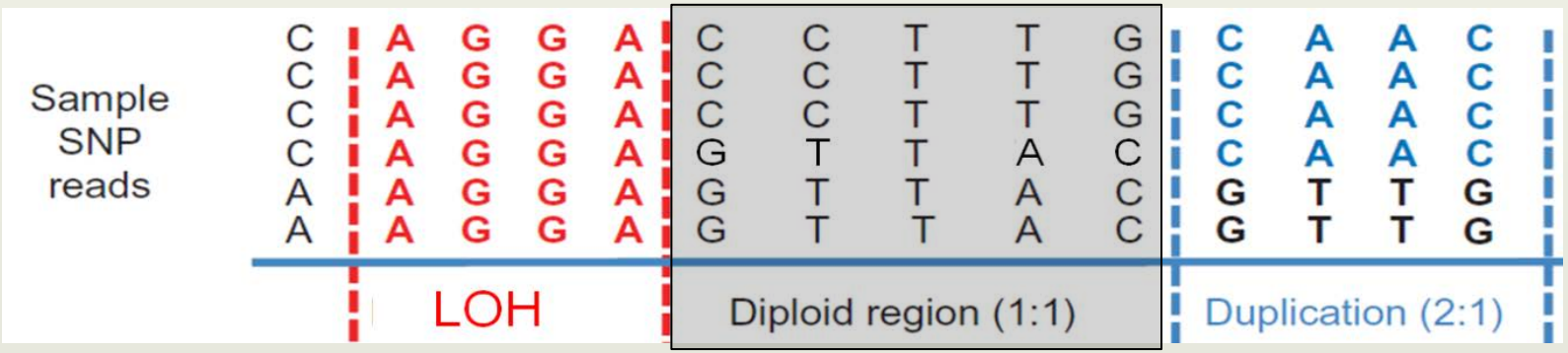
Clues From Tumor: Germline VUS Benign

Double somatic mutation



AND

Absence of LOH



LOH

Diploid region (1:1)

Duplication (2:1)

Pitfalls

- Missed germline or somatic mutation
- Double somatic mutations are *in cis*
- Multiple clones

Tumor NGS Enables Variant Re-Classification

- 40 patients with germline MMR VUS

- 5 re-classified based in part on tumor sequencing
  - 4 to likely pathogenic
  - 1 to likely benign

MMR= mismatch repair gene
VUS= variant of uncertain significance

Outline

- DNA repair gene mutations and cancer treatment
- Tumor sequencing in a Lynch workup
- **How tumors can help with variant classification**
  - **In patients with germline VUS**
  - **Incorporating somatic data into classification**
- Case vignette

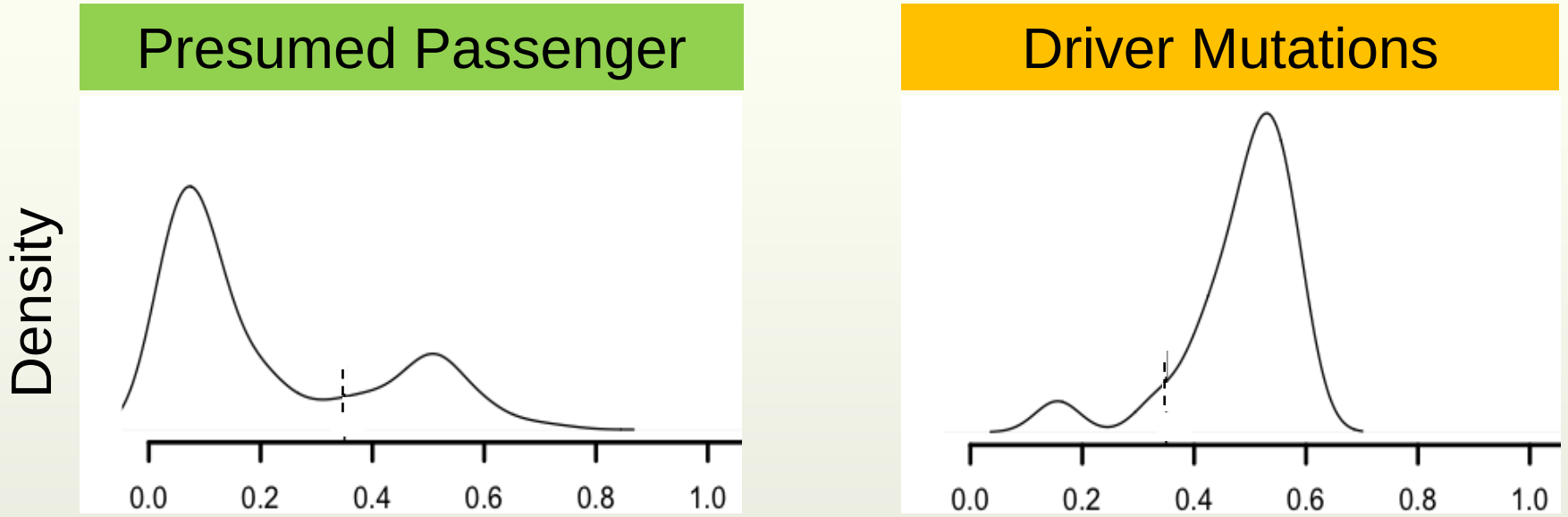
Incorporating Somatic Data into Classification: Lynch is the Perfect Model

- Highly specific tumor phenotype (e.g. MSH2 loss)
- Somatic mutations are *de novo*
- Analogous to *de novo* germline mutation in a patient with a matching phenotype -strong criteria for pathogenicity

Somatic Mutations That Explain IHC

- Somatic missense mutations often explain IHC
- Same mutations may be seen as germline VUS
- Can we use the tumor information to reclassify?
- Yes!

Tumor NGS for Variant Classification: Passenger or Driver?



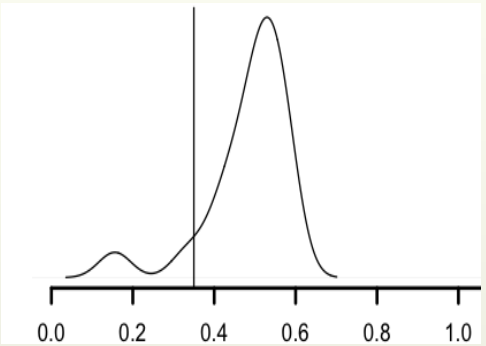
Brian Shirts

Shirts et al. (2018), *Am. J. Hum Genet.* PMID:29887214

Likelihood Modeling

- Prior probability based on InSiGHT

- Likelihood ratio (LR) from normalized variant allele fraction



- Posterior probability >95% = Likely Pathogenic

Results

- 61 somatic missense MMR mutations that fit IHC
- 20 reported as germline
  - 10/20 classified as pathogenic/likely pathogenic
  - 10/20 classified as VUS
- **4 of 10 VUS reclassified as pathogenic/likely pathogenic**

Outline

- DNA repair gene mutations and cancer treatment
- Tumor sequencing in a Lynch workup
- How tumors can help with variant classification
- **Case vignette**

Case

- 45 year old woman with colorectal cancer that loss of MSH2 and MSH6 protein by IHC
- Father had colon cancer at age 55
- Colon cancer predisposition panel testing identifies a rare deep intronic variant in *MSH2*

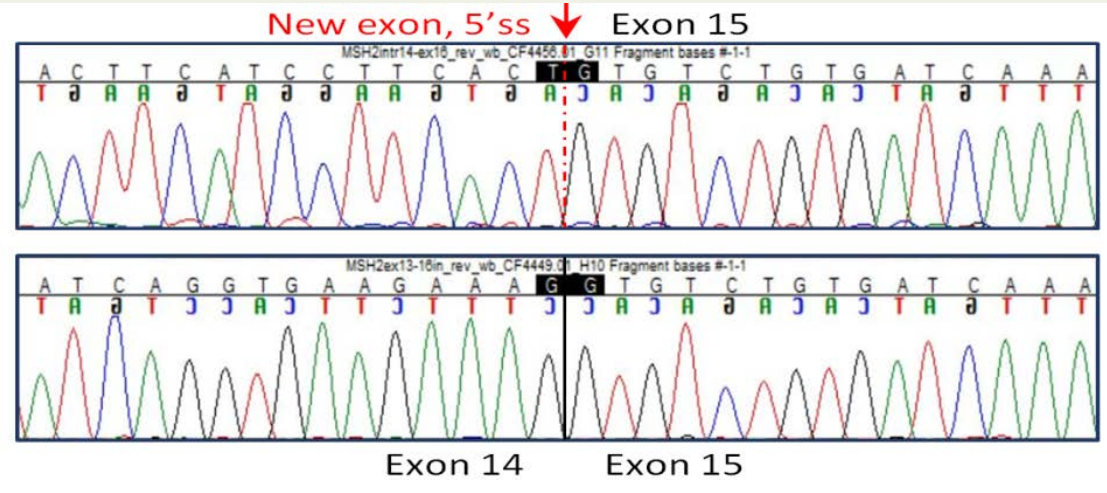
Case

- Nucleotide position is completely conserved
- Computer prediction strongly suggests the creation of a cryptic splice site
- Variant is reported as VUS with offer of RNA functional studies to follow up

Case

- Same *MSH2* deep intronic variant is observed ***twice*** as a somatic mutation in colorectal cancer that had loss of MSH2 and MSH6

- Functional RNA splicing studies confirm introduction of a cryptic exon and frameshift in ~50% of transcripts



Thanks to Silvia Casadei

Case: Final Diagnosis

Lynch syndrome caused by an inherited pathogenic deep intronic mutation in *MSH2* that results in the introduction of a cryptic exon and a premature frameshift

Summary

- **Germline and tumor** NGS testing of DNA repair genes is increasingly **needed to guide cancer treatment**
- **Tumor NGS** testing increasingly **used in a Lynch workup**
- **Tumor NGS testing assists with DNA repair gene variant classification**

Thank You!

- UW/SCCA/FHCRC/BBI Clinical
  - Genetics and Solid Tumors Lab
  - NGS Analytics Lab
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