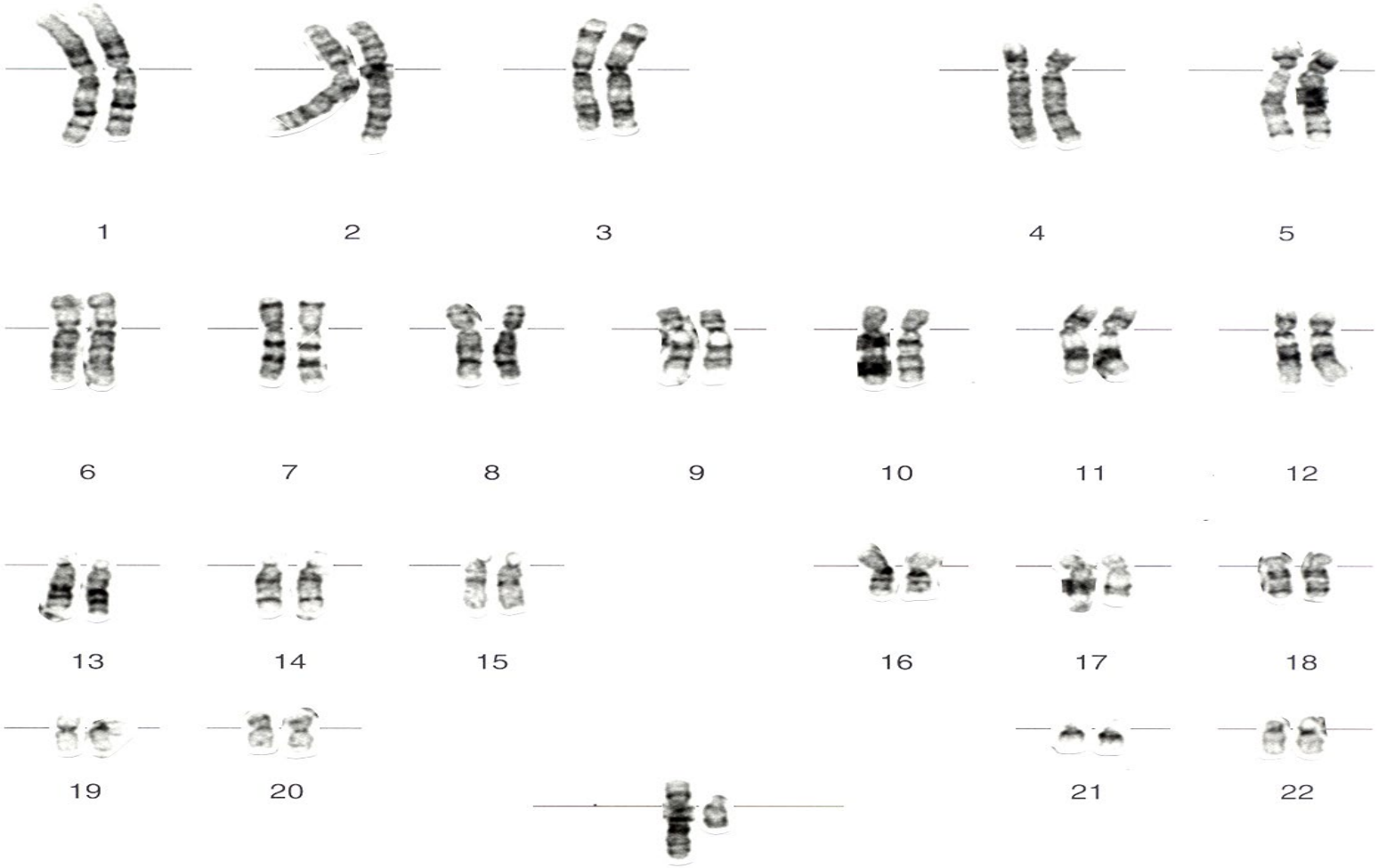
Genetics/Genomics 101

Heather Hampel, MS, LGC
Associate Director, Division of Human Genetics
Professor, Department of Internal Medicine
@HHampel1

The James

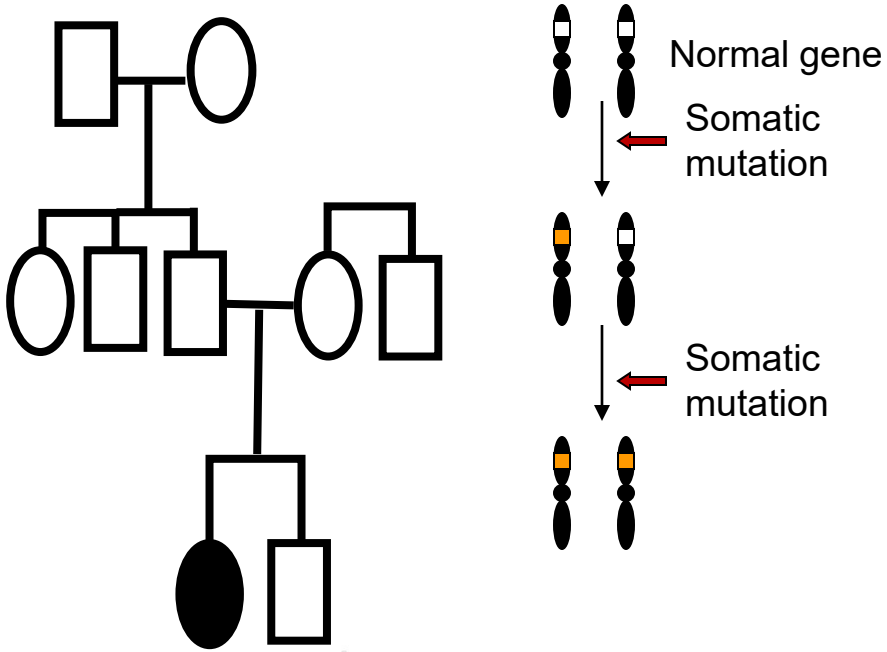
THE OHIO STATE UNIVERSITY
COMPREHENSIVE CANCER CENTER

Normal Male Chromosomes



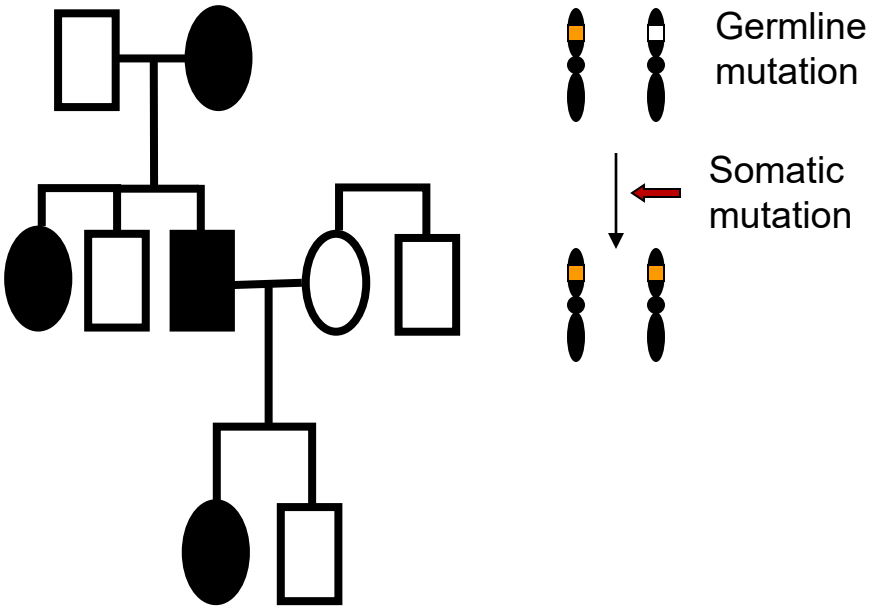
Sex chromosomes

Sporadic (somatic)



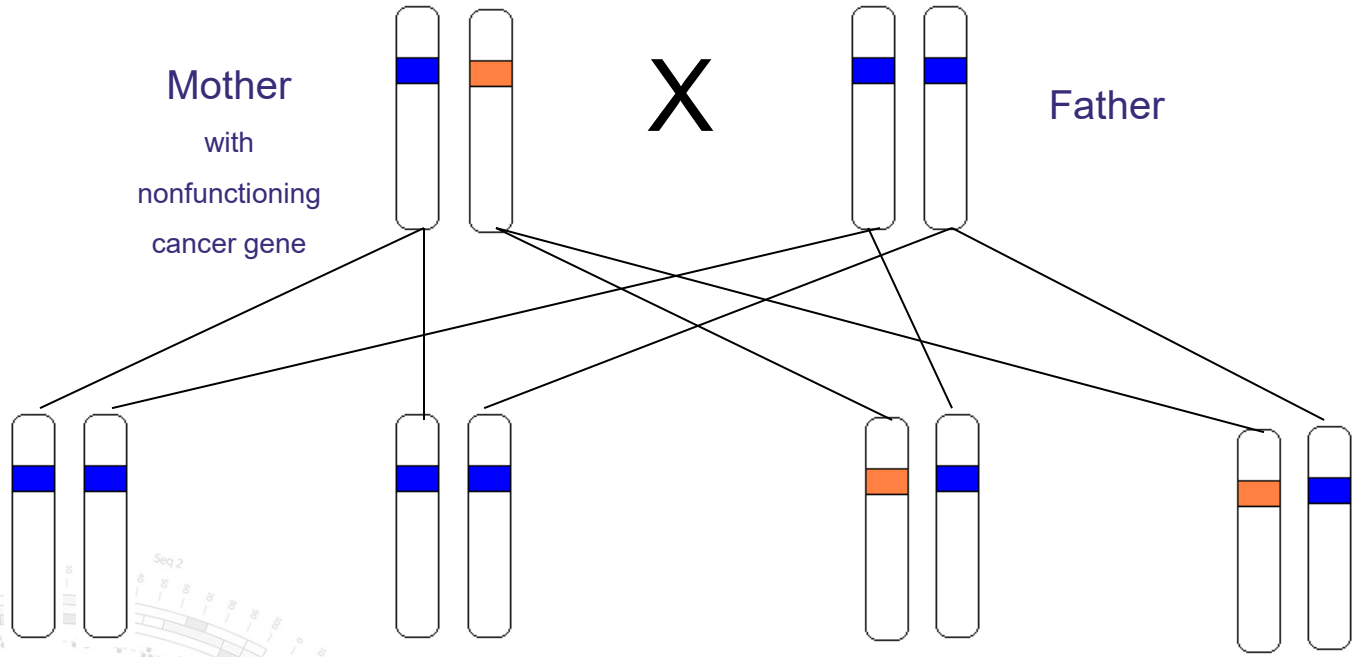
- Later age at onset (>60)
- Little or no family history of cancer
- Single or unilateral tumors

Inherited (germline)



- Early age at onset (<50)
- Multiple generations with cancer
- Bilateral or multiple primary cancers
- Clustering of certain cancers (i.e. breast/ovarian)

Dominant Inheritance of a Cancer Gene

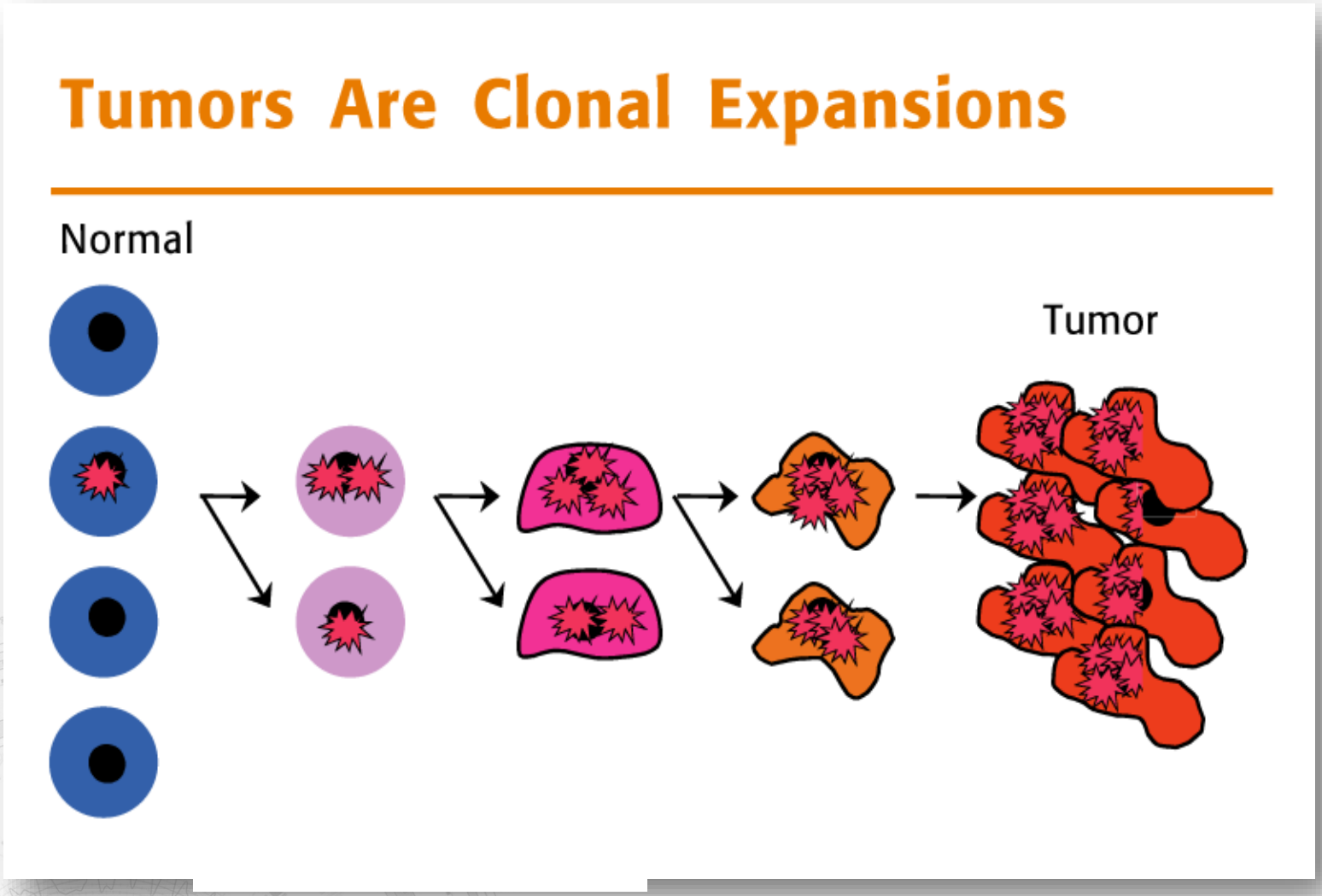


Offspring have a 50% chance of inheriting the nonfunctioning cancer gene

Key

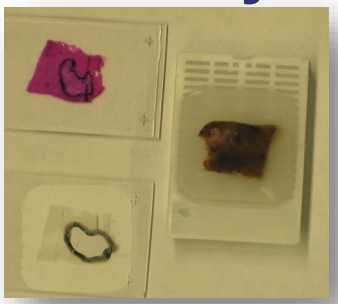
- Nonfunctioning Cancer Gene
- Functioning Cancer Gene

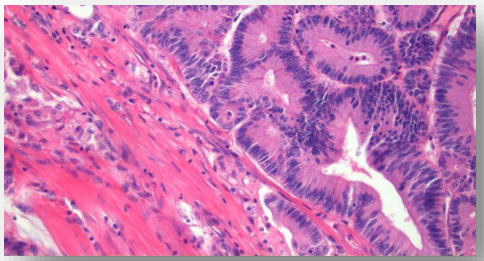
Somatic variants occur in the tumor



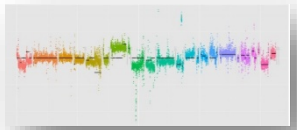
Emerging Model of Cancer Treatment

Tumor tissue routinely acquired for molecular diagnostics

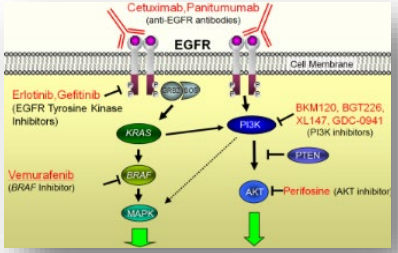




All/or a subset of actionable mutations assessed



Therapy selected based on molecular characteristics



The James

THE OHIO STATE UNIVERSITY
COMPREHENSIVE CANCER CENTER

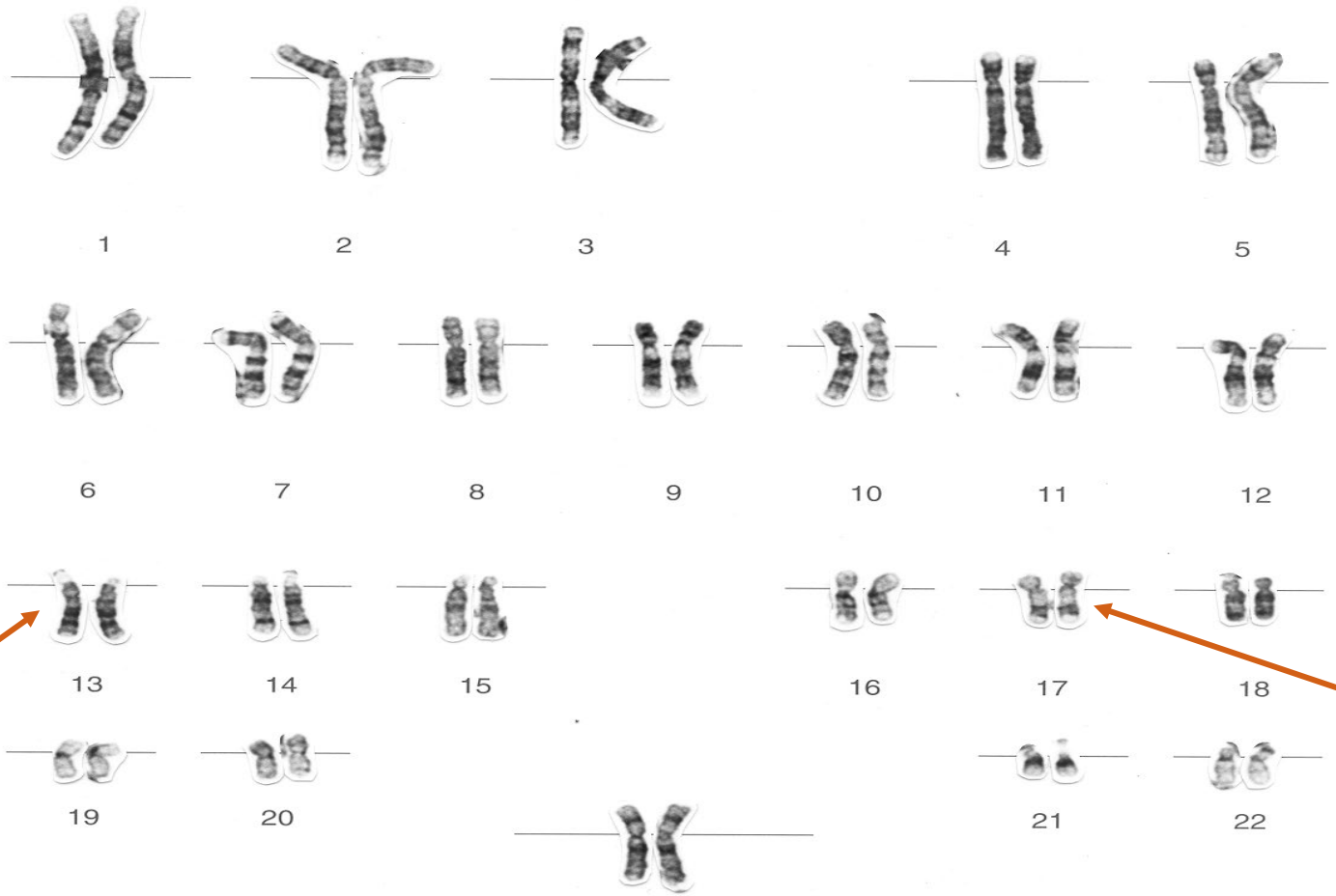
Differences between Somatic & Germline Variants

- Germline variants are in EVERY cell in your body.
- Somatic variants are ONLY in tumor cells.
- Germline variants are in 50% of your DNA.
- Somatic variants are usually in <35% of the DNA in your tumor.
- Germline testing uses blood, saliva, or skin.
- Somatic testing uses tumor tissue acquired through biopsy, surgery, or circulating cell free DNA in blood.

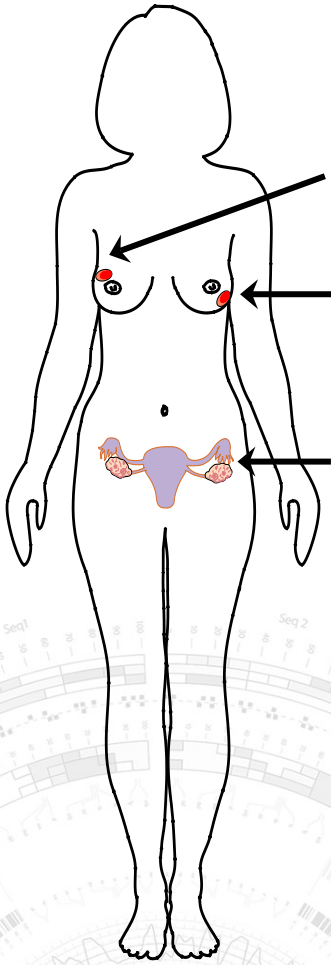
The Most Common Hereditary Cancer Syndromes

- Hereditary Breast-Ovarian Cancer Syndrome
  - Due to mutations in the BRCA1 and BRCA2 genes
- Lynch Syndrome
  - Due to mutations in MLH1, MSH2, MSH6, PMS2, and EPCAM genes
- Considered Tier One Genetic Diseases by CDC along with Familial Hypercholesterolemia
  - Common
  - Easy to test for
  - Actionable
- Geisinger MyCode assessed for Tier 1 conditions in 50,000 health plan participants
  - 1.32% (1 in 76 individuals) had one of these conditions
  - Compare to the 1 in 800 positive rate in newborn screening programs

Hereditary Breast-Ovarian Cancer Syndrome (HBOC)



BRCA1-Associated Cancers: Risk by age 70



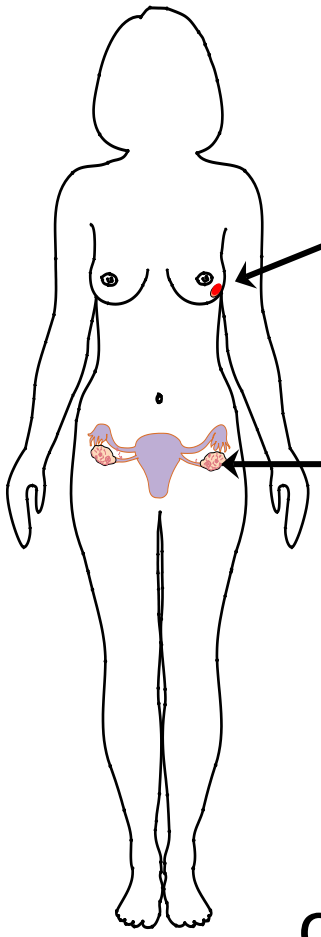
Breast cancer 50-85% (often early age at onset)

Second primary breast cancer 40%-60%

Ovarian cancer 15-45%

Possible increased risk of other cancers (eg, prostate)

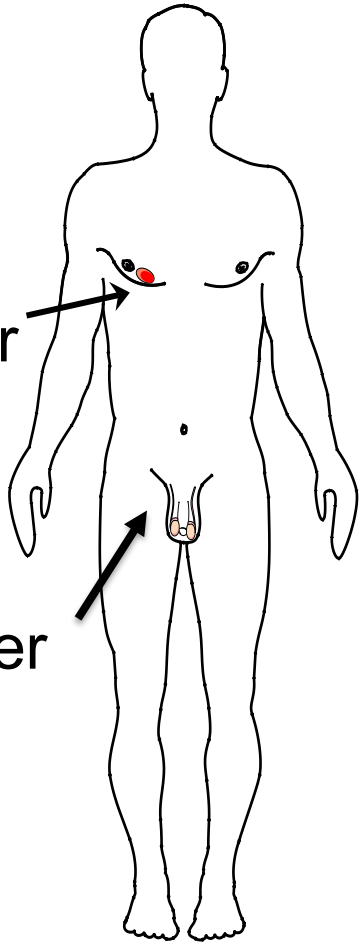
BRCA2-Associated Cancers: Risk by age 70



breast cancer
(50-85%)

ovarian cancer
(10-20%)

male breast cancer
(6%)



prostate cancer
(30%)

Increased risk of pancreatic cancer (~7%) and melanoma

HBOC Breast Cancer Management

NCCN Guidelines v1.2020

- Breast awareness starting at age 18 y.
- Clinical breast exam, every 6–12 months starting at age 25 y.
- Age 25–29 y, annual breast MRI screening with contrast (or mammogram with consideration of tomosynthesis, only if MRI is unavailable) or individualized based on family history if a breast cancer diagnosis before age 30 is present.
- Age 30–75 y, annual mammogram with consideration of tomosynthesis and breast MRI screening with contrast.
- Age >75 y, management should be considered on an individual basis.
- Discuss option of risk-reducing mastectomy
  - Counseling should include a discussion regarding degree of protection, reconstruction options, and risks.
  - Prophylactic mastectomy has been shown to reduce the risk for developing breast cancer by about 90%
- Discuss options for risk reduction agents (e.g. chemoprevention with Tamoxifen) including risks and benefits of each medication

HBOC Ovarian Cancer Management
NCCN Guidelines v1.2020

- Risk-reducing bilateral salpingo-oophorectomy between the ages of 35-40, or after child bearing is complete. Because ovarian cancer in women with *BRCA2* mutations occurs later than in *BRCA1*, it is reasonable to delay risk-reducing BSO until age 40-45 unless family history warrants earlier age of prophylactic surgery
- Some evidence of slight increased risk for serous uterine cancer among BRCA1 mutation carriers – discuss consideration of hysterectomy with BSO
- If delaying BSO: transvaginal ultrasound with color Doppler imaging at age 30-35 with concurrent serum CA-125 - not been shown to be sufficiently sensitive to support a positive NCCN recommendation
- Consider oral contraceptives – discussion of risk/benefit

Cancer Screening in Males

NCCN Guidelines v1.2020

- Breast self-examination training and education beginning at age 35.
- Clinical breast examination every 12 months beginning at age 35.
- (BRCA2) Recommend prostate cancer screening including annual digital rectal examination and PSA test beginning at age 40.
- (BRCA1) Consider prostate cancer screening including annual digital rectal examination and PSA test beginning at age 40.

Screening for other cancers

- Melanoma: No specific screening guidelines fbut general melanoma risk management is appropriate, such as annual full-body skin exaination and minimizing UV exposure.
- Pancreatic cancer: Individuals with *BRCA1/2, ATM, PALB2, TP53*, or Lynch genes (except *PMS2*) with a FDR or SDR with pancreatic cancer:
  - Consider pancreatic cancer screening beginning at age 50 or 10 years younger than the earliest dx in family.
  - Annual contrast-enhanced MRI/MRCP and/or EUS with consideration of shorter screening intervals for individuals found to have worrisome abnormalities on screening.
  - Most small cystic lesions found on screening will not warrant biopsy, surgical resection, or any intervention.
- Follow American Cancer Society guidelines for other cancer surveillance

BRCA1/2 testing has changed over the years

- 1999-2002: BRCA1 and BRCA2 genes were only sequenced
- 2002-2006: BRCA1 and BRCA2 genes were sequenced and the 5 most common large rearrangements (all in BRCA1) were also tested
- 2006-2013: BRCA1 and BRCA2 genes were sequenced and the 5 most common large rearrangements were tested AND patients were offered a $750 follow-up test called BART that tested for any large rearrangement in BRCA1 or BRCA2
- 2013-present: BRCA1 and BRCA2 usually included in panel gene tests with multiple breast/ovarian & other genes
- What does this mean? Women whose test did not include BART could still have a BRCA1 or BRCA2 mutation

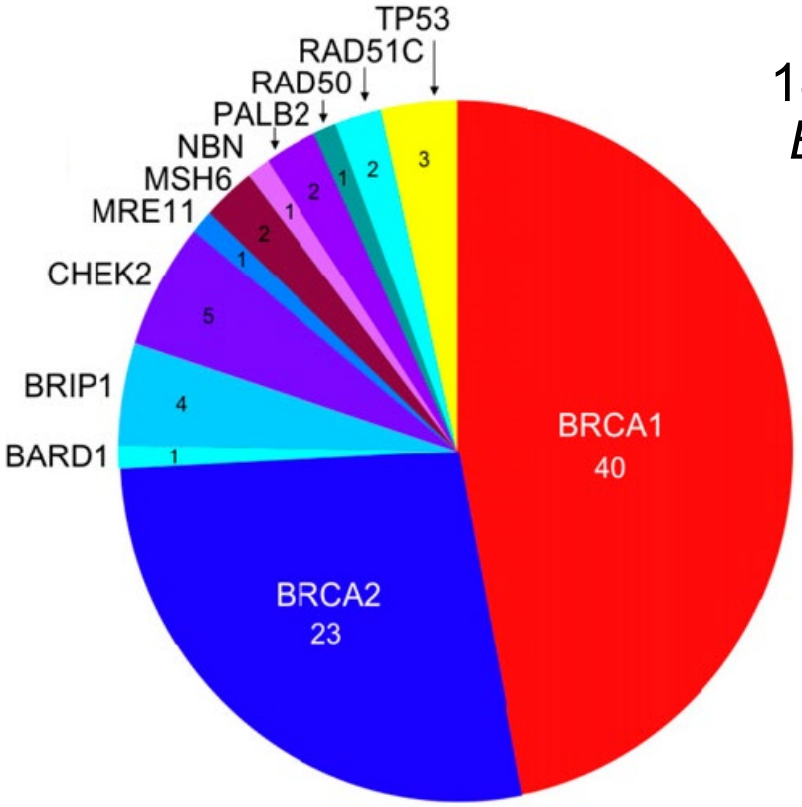
Panel Results: 35,049 Breast Cancer Patients

- 9.3% had a pathogenic mutation in one of 25 cancer genes
  - Half were in BRCA1/2
  - Half were in other genes including CHEK2, ATM, & PALB2
- Women dx <40 were more likely to test positive
- Women diagnosed >59 were less likely to test positive
- Women diagnosed between 40-59 had an 8-9% chance of testing positive

Buys, S, et al. Cancer. May 15, 2017:1721-30.

Panel Results – 360 Unselected Ovarian Cancer Patients

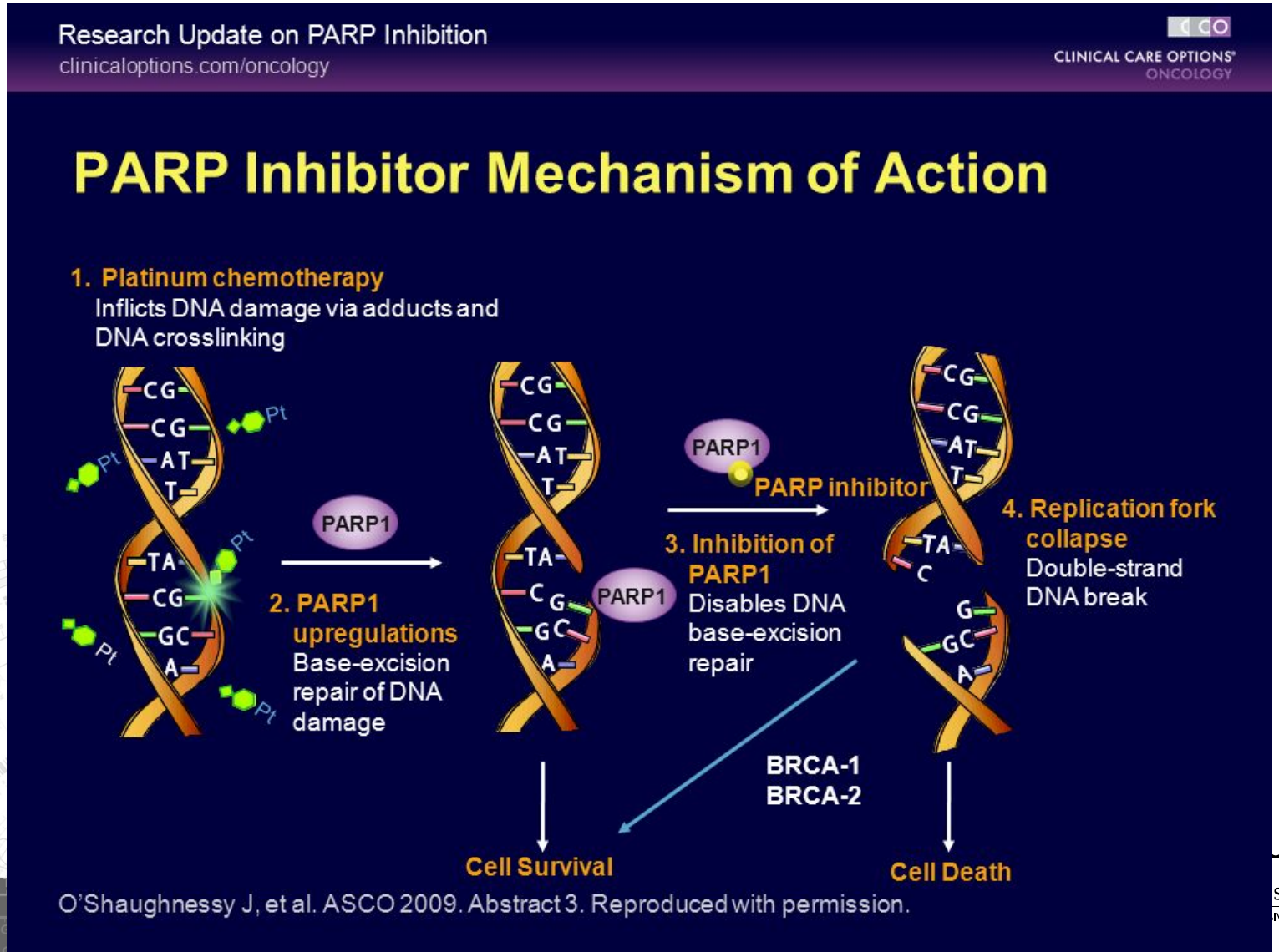
6% had mutations in other genes



18% had mutations in *BRCA1* and *BRCA2*

Walsh T et al. PNAS 2011;108:18032-7.

PARP Inhibitors work for tumors with BRCA variants

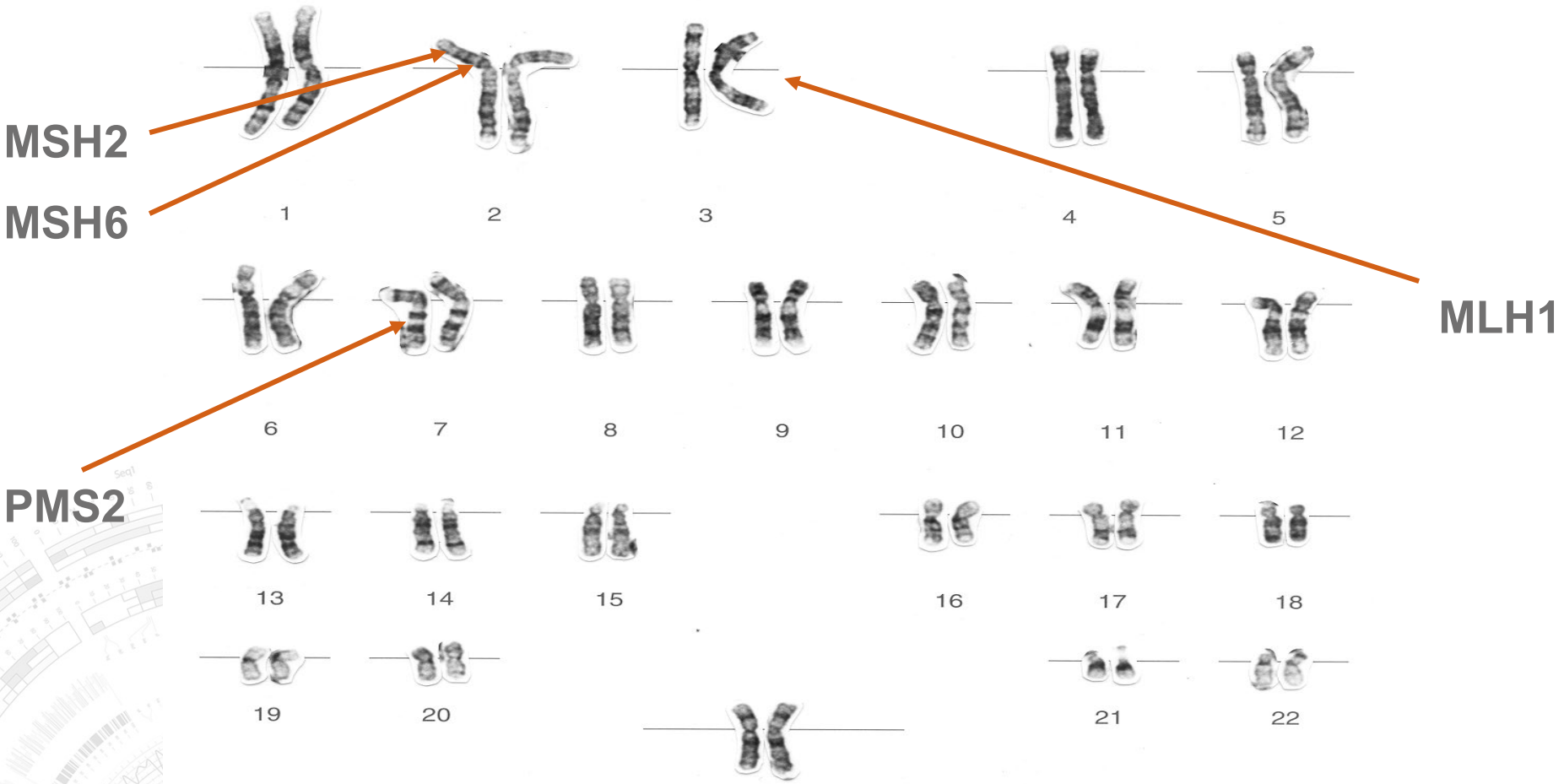


James

State University

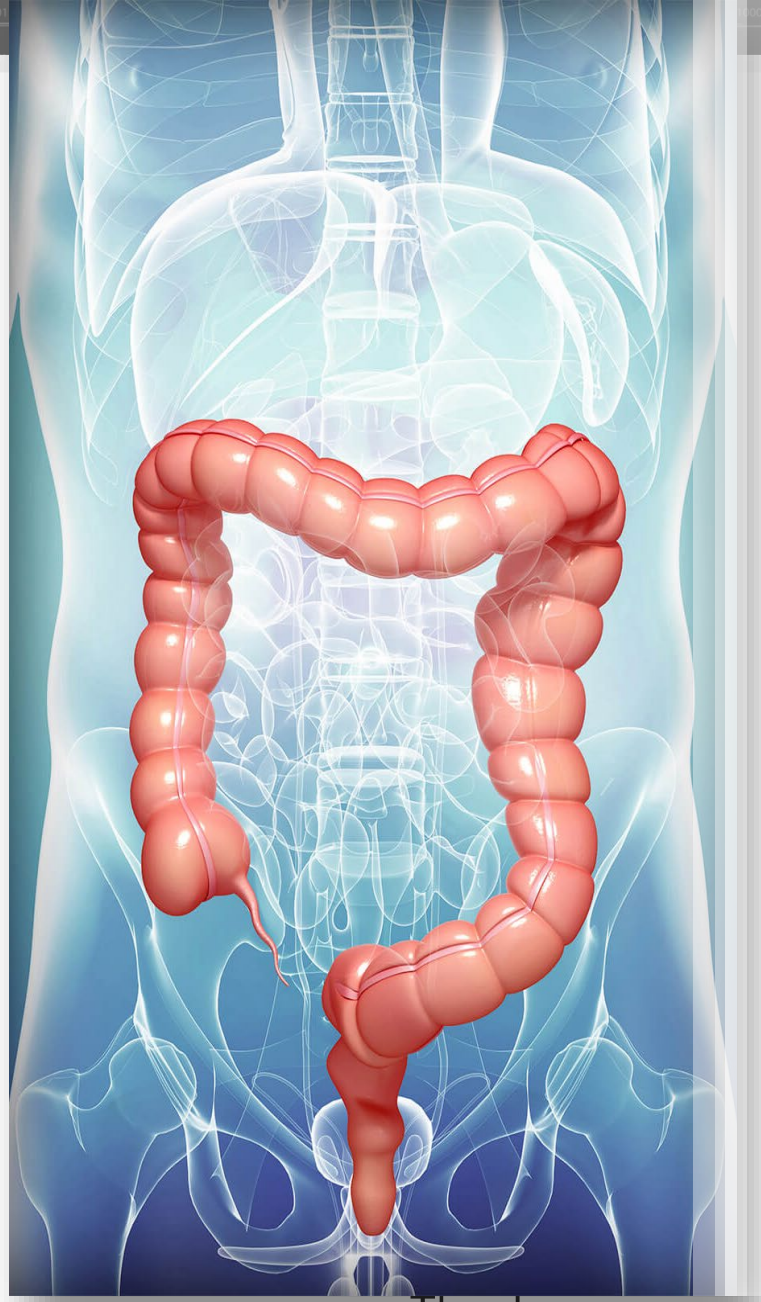
ive Cancer Center

Lynch Syndrome



Lynch Syndrome

- Over 1.2 million individuals in the United States have Lynch syndrome
- Inherited condition that causes high risks for colorectal cancer, endometrial cancer, and other cancers
- Preventable cancers with early and more frequent screening
- 95% of affected individuals do not know they have Lynch syndrome



Lynch Syndrome Cancer Risks (to 70)

Cancer Type	MLH1 and MSH2	MSH6	PMS2	General Public
Colon cancer	40%-80%	10%-22%	15%-20%	4.5%
Endometrial cancer	25%-60%	16%-26%	15%	2.7%
Stomach	1%-13%	≤ 3%	< 6%	< 1%
Ovarian	4%-24%	1%-11%	< 6%	1.6 %

National Comprehensive Cancer Network Guidelines for Colorectal Cancer Screening and Prevention v1.2019; Bonadona V, et al. *JAMA* 2011;305:2304-10; Senter L, et al. *Gastroenterology* 2008;135:419-48.

Lynch Syndrome Surveillance Options NCCN v1.2020

Intervention	Recommendation
Colon Cancer	MLH1 & MSH2: Colonoscopy every 1-2 y beginning at age 20-25 (or 2-5 years younger than earliest diagnosis if <25 MSH6 & PMS2: Colonoscopy every 1-2 y beginning at age 30-35 (or 2-5 years younger than earliest diagnosis if <25
Endometrial Cancer	Education regarding symptoms Consideration of hysterectomy after childbearing Endometrial biopsy every 1-2 y beginning at age 30-35 can be considered
Ovarian Cancer	Education regarding symptoms TVUS and CA-125 surveillance could be considered by no evidence of efficacy BSO can be considered after childbearing
Gastric & Small Bowel Cancer	Risk factors: male sex, older age, MLH1 or MSH2 pathogenic variants, FDR with gastric cancer, Asian ethnicity, chronic autoimmune gastritis, gastric intestinal metaplasia and gastric adenomas. Consider EGD with random biopsy of the proximal and distal stomach for H.pylori, autoimmune gastritis, and intestinal metasplasia beginning at age 40 and surveillance EGD every 3-5 y in those with the above risk factors.

Lynch Syndrome Surveillance Options
NCCN v1.2020

Intervention	Recommendation
Urothelial cancer	No clear evidence to support. Consider in select individuals with a family history of urothelial cancer and individuals with *MSH2* pathogenic variants (especially males). Annual urinalysis starting at age 30-35
Pancreatic Cancer	Consider pancreatic cancer screening beginning at age 50 or 10 years younger than the earliest dx in family. Annual contrast-enhanced MRI/MRCP and/or EUS with consideration of shorter screening intervals for individuals found to have worrisome abnormalities on screening. Most small cystic lesions found on screening will not warrant biopsy, surgical resection, or any intervention.
Prostate Cancer	General population screening
Breast Cancer	General population screening
Brain Cancer	Annual physical/neurologic examination starting at age 25-30y
Reproductive Risks	Advise about prenatal diagnosis and assisted reproduction including preimplantation genetic testing Advise about risk of rare recessive syndrome called CMMR deficiency if both partners are carriers of pathogenic variants in the same MMR gene

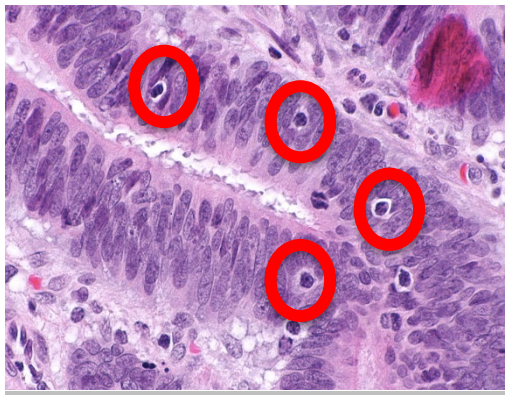
Panel Results: 1,058 Colorectal Cancer Patients

- 9.9% had a pathogenic mutation in one of 25 cancer genes
- 3.1% had Lynch syndrome
- 7% had non-Lynch syndrome gene mutations including:
  - 2.2% had mutations high-penetrance genes (5 *APC*, 3 biallelic *MUTYH*, 11 *BRCA1/2*, 2 *PALB2*, 1 *CDKN2A* and 1 *TP53*)
  - 3.6% had mutations in moderate-penetrance CRC risk genes (19 *MUTYH* heterozygotes, 17 *APC* I1307K, and 2 *CHEK2*)
- Age at dx, family history of CRC, nor personal history of other cancers significantly predicted the presence of mutations in non-Lynch syndrome genes

Yurgelun M, et al. J Clin Oncol. 2017;35:1086-95.

Why are mismatch repair (MMR) deficient tumors responsive to immunotherapies?

- MMR deficient tumors are more immunogenic than other CRCs
  - More tumor infiltrating lymphocytes
  - Higher mutational burden
  - Greater production of protein products that are truncated or incorrectly coded—therefore seen as foreign to the body (FSP or frameshift proteins)
- Studies have shown association of mutational burden, microsatellite instability and TILs to immunotherapy response



Tumor infiltrating lymphocytes

- Yoshinaga O et al. Gastroenterology 149(5); 2015. Carethers JM et al. World J Gastroenterology 21(31); 2015.Goyal G Fam Cancer 15; 2016; Wesstdorp H et al. Cancer Immunol Immunotherapy 65(10) 2016.

Mismatch repair deficiency predicts response to anti-PD1 and PD-LI immunotherapy

- Le DT et al NEJM 2015
- Responses in non-CRC MMR deficient GI cancers also reported (GI ASCO 2016)
  - CRs in gastric, ampullary, and cholangiocarcinoma
- FDA has recently approved pembrolizumab for MMR deficient solid tumors

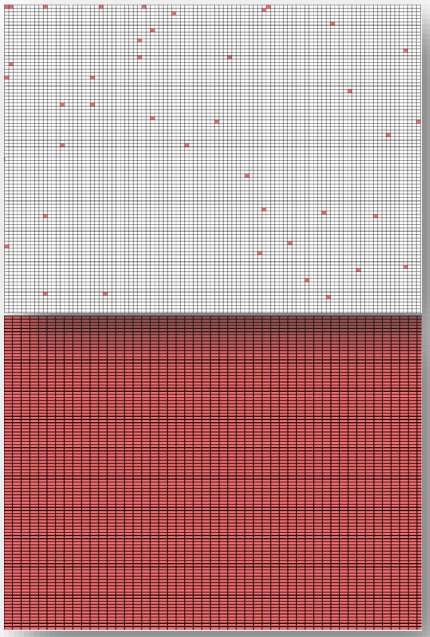
Objective responses by RECIST Criteria

Response	MMR deficient CRC N=10	MMR proficient CRC N=18	MMR deficient non-CRC* N=7
CR	0	0	1 (14)
PR	4 (40)	0	4 (57)
SD	5 (50)	2 (11)	0
PD/NE	1 (10)	15 (89)	2 (29)
OR	40 (12-47)	0	71 (29-96)
DCR	90 (55-100)	11 (1-35)	71 (29-96)

- Le DT et al NEJM 372 (2015); Le et al GI ASCO Abstract 195 2016

Tumor Testing Options

- **Hotspot Panels (using tumor tissue)**
  - Look at specific, common mutations (e.g. V600E variant in BRAF)
  - Do not sequence the entire gene
- **Comprehensive Sequencing Panels (using tumor tissue)**
  - Completely sequence multiple genes + testing for common gene fusions and other actionable targets for therapy
  - Vary from lab to lab in terms of numbers of genes
  - FDA approved therapies for some genes
  - Other therapies only available through clinical trials
  - Some labs test ONLY tumor or report only tumor mutations; others include germline testing
- **Liquid Biopsy**
  - Blood test looking for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood
  - It may also be used to help plan treatment or to find out how well treatment is working or if cancer has come back.
  - Being able to take multiple samples of blood over time may also help doctors understand what kind of molecular changes are taking place in a tumor.
  - A liquid biopsy may be used to help find cancer at an early stage



Variant Allele Fraction (VAF)

- What percentage of the cells tested have the mutation in them?
- Germline mutations often ~50%
  - Can be higher if loss of heterozygosity
  - Can be lower for indel mutations
- Somatic mutations often <35%
- Most next-generation sequencing somatic panels produce accurate VAF (have to request them – usually not included on the report)
- VAF can be used in some cases to assess the probability a mutation is germline
- Does not always work
  - Affected by tumor percentage, ploidy, type of mutation, and loss of heterozygosity

Variant Allele Fraction (VAF)

Example

Two *MSH2* loss of function mutations in a colorectal tumor with ~50% tumor cells

- Mutation 1 VAF is 25%
- Mutation 2 VAF is 50%

Q. Does this result suggest one mutation is germline?

A. Yes, mutation 2 may be germline based on 50% VAF. Heterozygous somatic mutations in tumor are expected to be at ~25% VAF because only half the sample is tumor cells.

Take Home Messages

- All cancer is genetic (contains somatic gene variants), but NOT all cancer is hereditary (due to a germline gene variant)
- Germline testing is designed to detect any mutation in a cancer susceptibility gene
  - Unlikely to detect a tumor mutation unless there is **a lot** of tumor circulating in the blood
- Tumor testing is designed to look for actionable therapeutic targets
  - Can detect germline variants but can also miss them
- Therapy effective whether variants are in tumor or germline
- Patients may need BOTH types of tests
- Liquid biopsy is exciting new way to test tumors which may lead to new tumor screening option


The James
THE OHIO STATE UNIVERSITY
COMPREHENSIVE CANCER CENTER
A CANCER-FREE WORLD
BEGINS HERE