Understanding the Application: NGS Panel Testing for Hereditary Cancer Syndromes and Cancer Targeted Therapy

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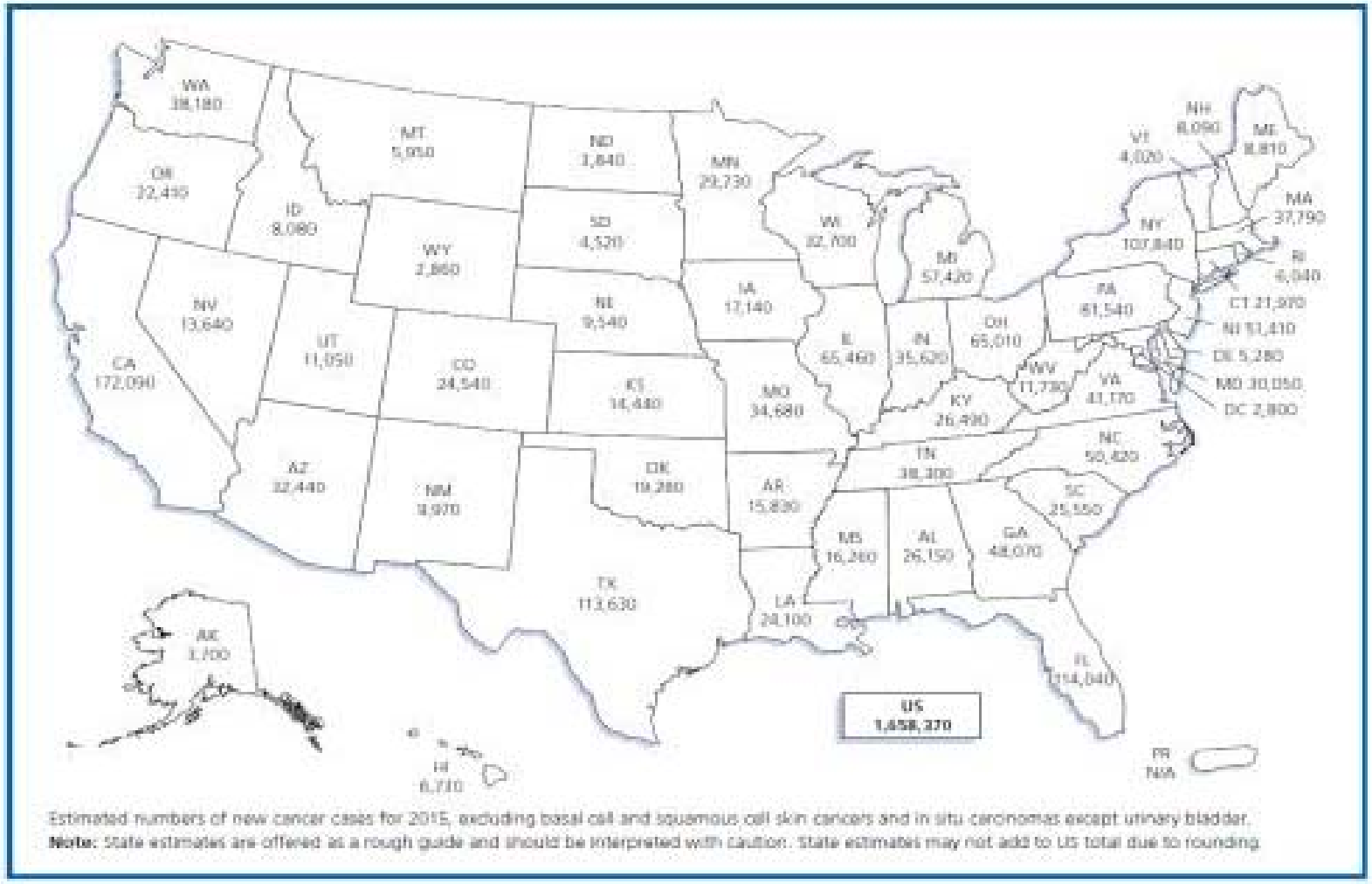
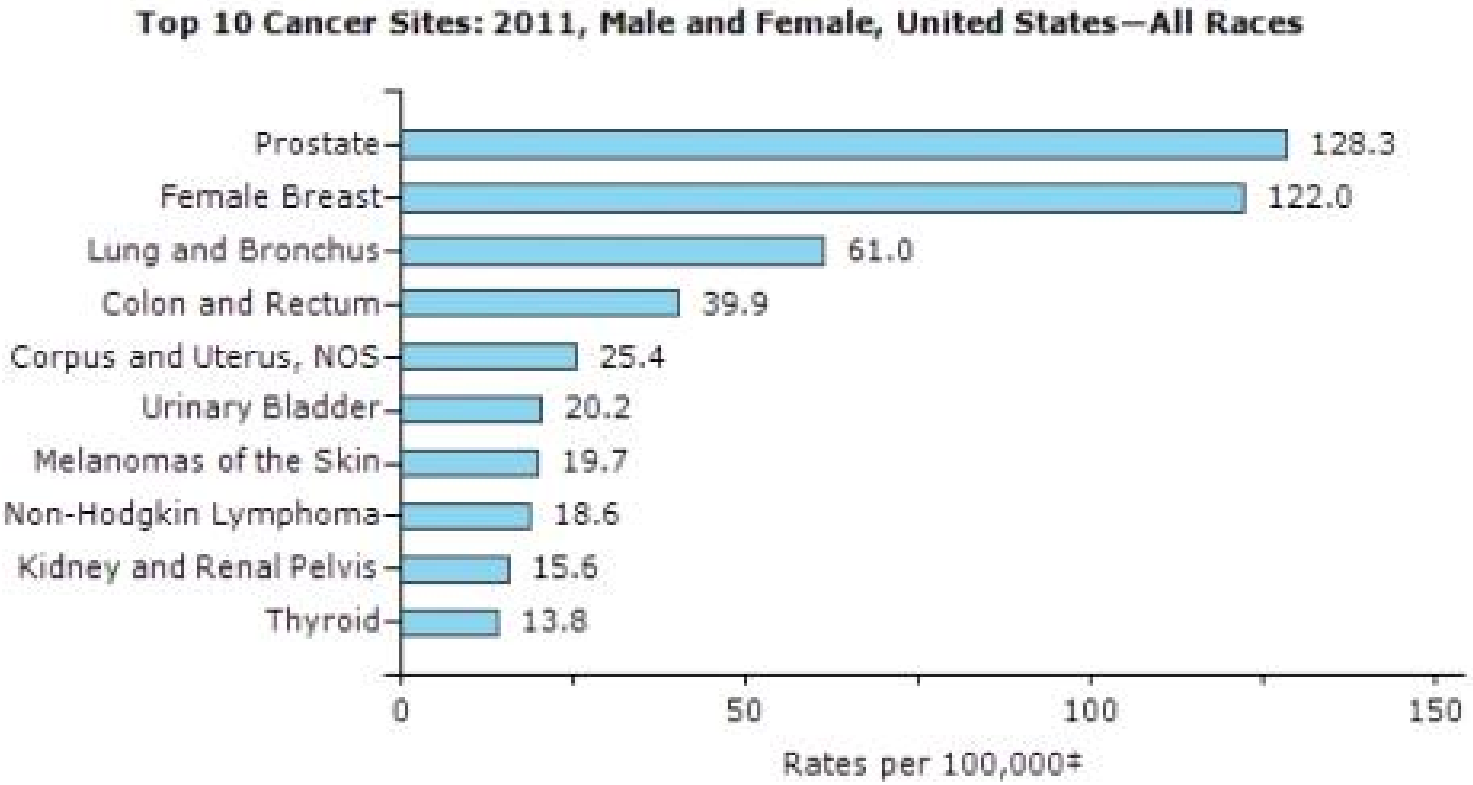
2015 Cancer Fact (American Cancer Society)



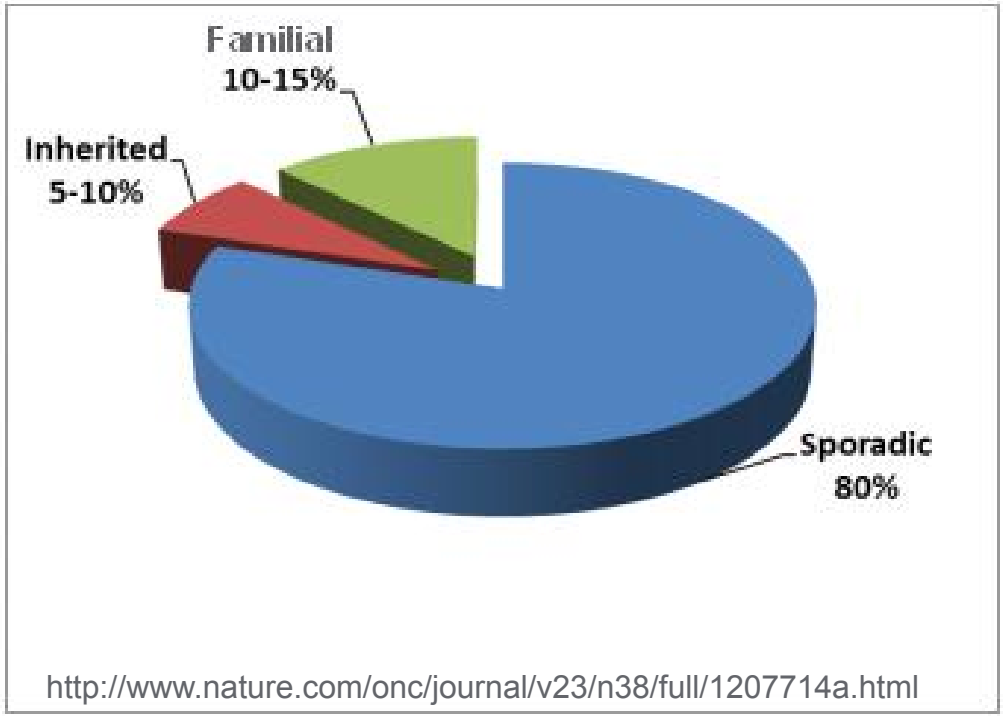
Estimated numbers of new cancer cases for 2015, excluding basal cell and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Note: State estimates are offered as a rough guide and should be interpreted with caution. State estimates may not add to US total due to rounding.

http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf

Incidence Rates:10 Primary Cancer Sites



Distribution by Cancer Type



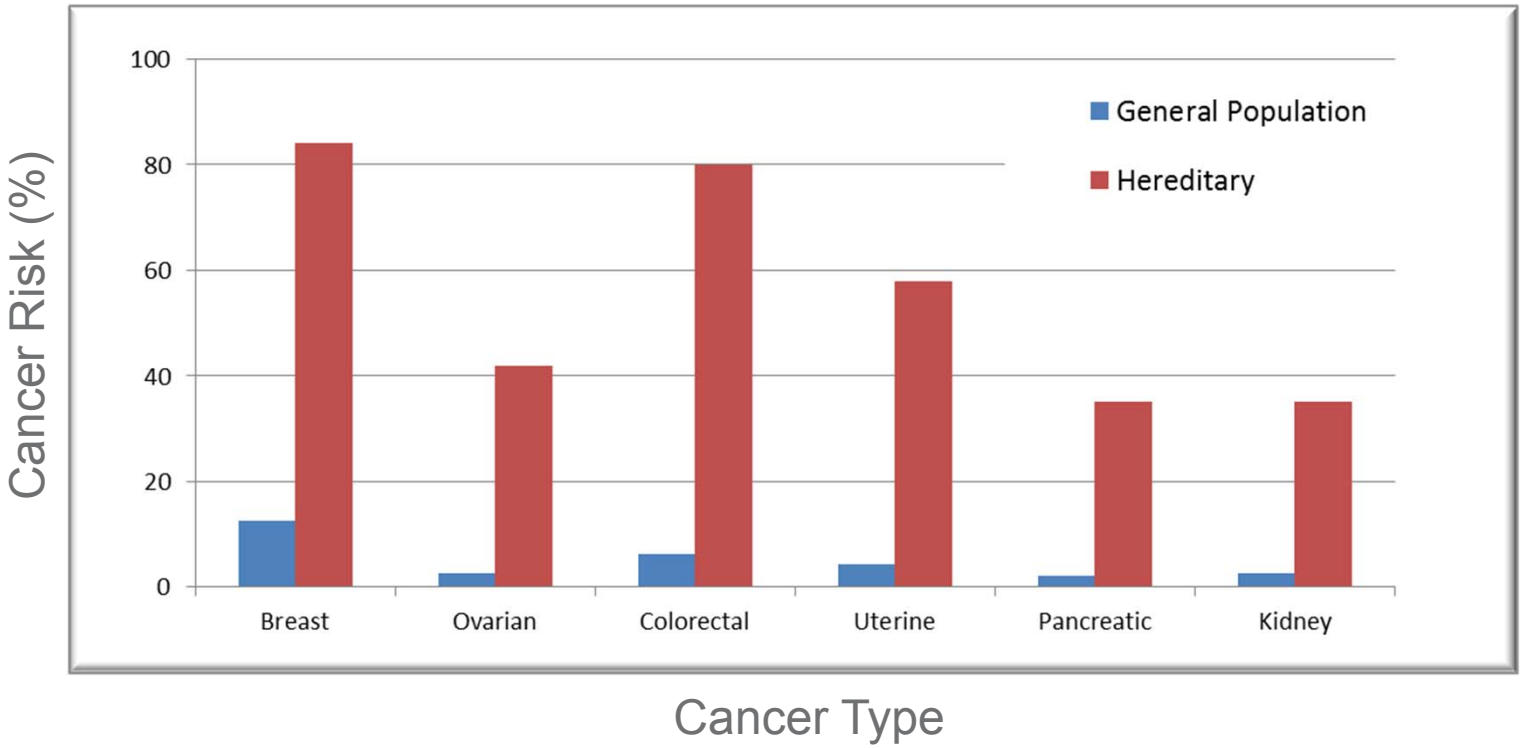
http://www.nature.com/onc/journal/v23/n38/full/1207714a.html

Inherited cancers arise due to highly penetrant germ-line mutations.
Familial cancers may be caused by the interaction of low-penetrance genes, gene–environment interactions, or both.

Hereditary Cancers

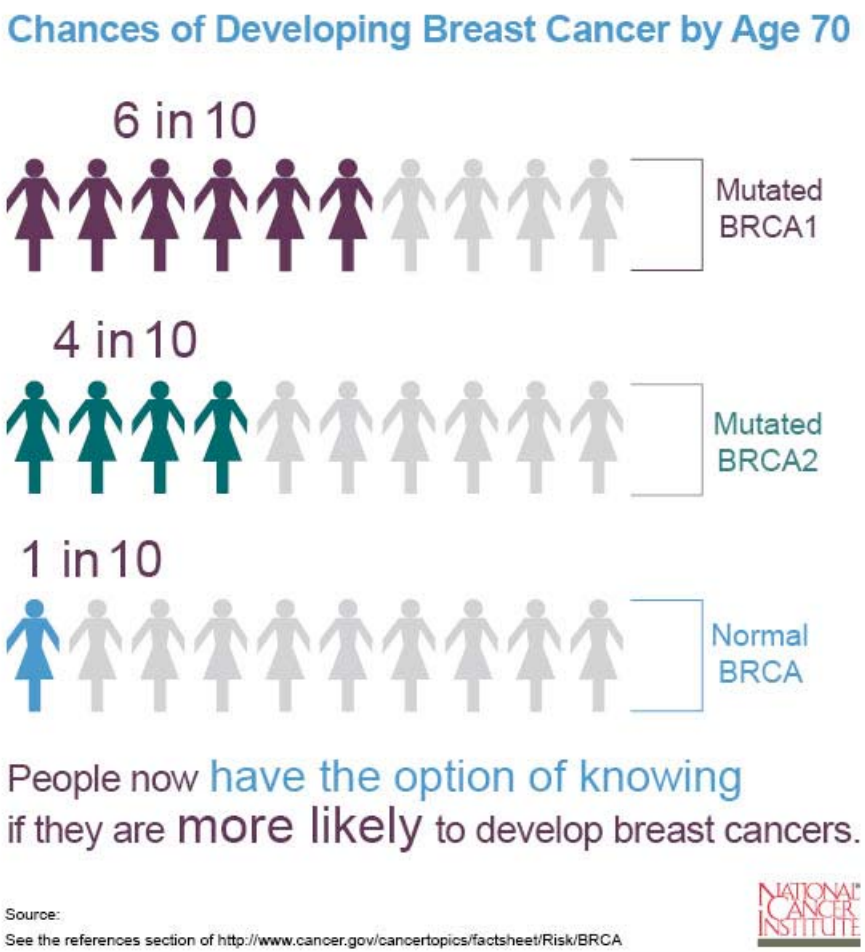
- About 5% to 10% of all cancers
- Inheriting a gene mutation or pathogenic variant does not necessarily mean that a person will develop cancer, but it increases his/her risk
- Most common hereditary cancers are:
  - Breast cancer
  - Ovarian cancer
  - Colorectal cancer
  - Prostate cancer
- Understanding if cancer is due to an inherited pathogenic variant/mutation can help clarify future risks of developing cancer and help determine options for cancer screening and prevention, possibly therapy

Lifetime Cancer Risks for Common Cancers



http://seer.cancer.gov/

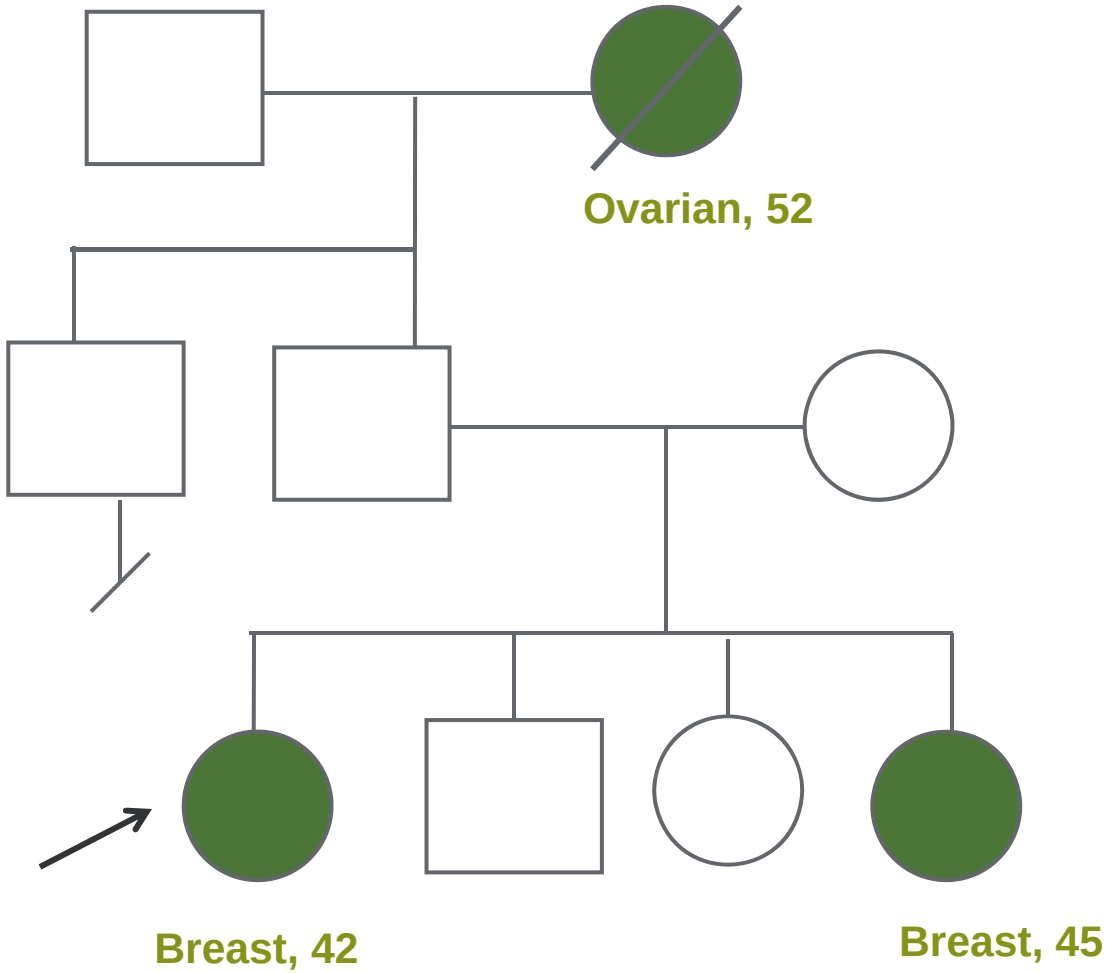
Lifetime Risks: Breast Cancer



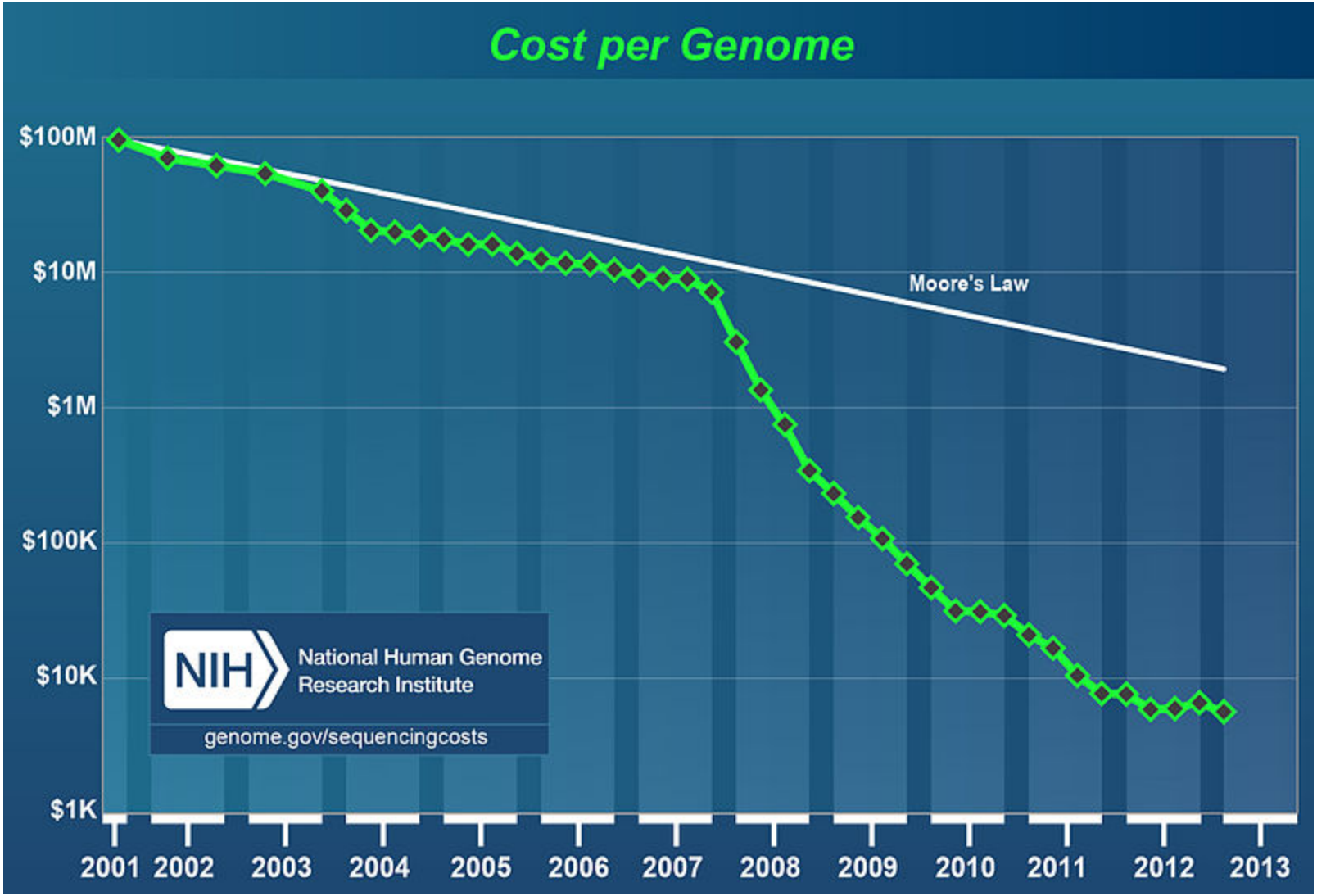
“Red Flags” for Inherited Susceptibility to Cancer

- Cancer in 2 or more closely related relatives
- Multiple generations affected
- Early age at diagnosis
- Multiple primary tumors
- Bilateral or rare cancers
- Constellation of tumors consistent with a specific cancer syndrome
- Certain ethnic backgrounds (e.g. Ashkenazi Jewish ancestry)

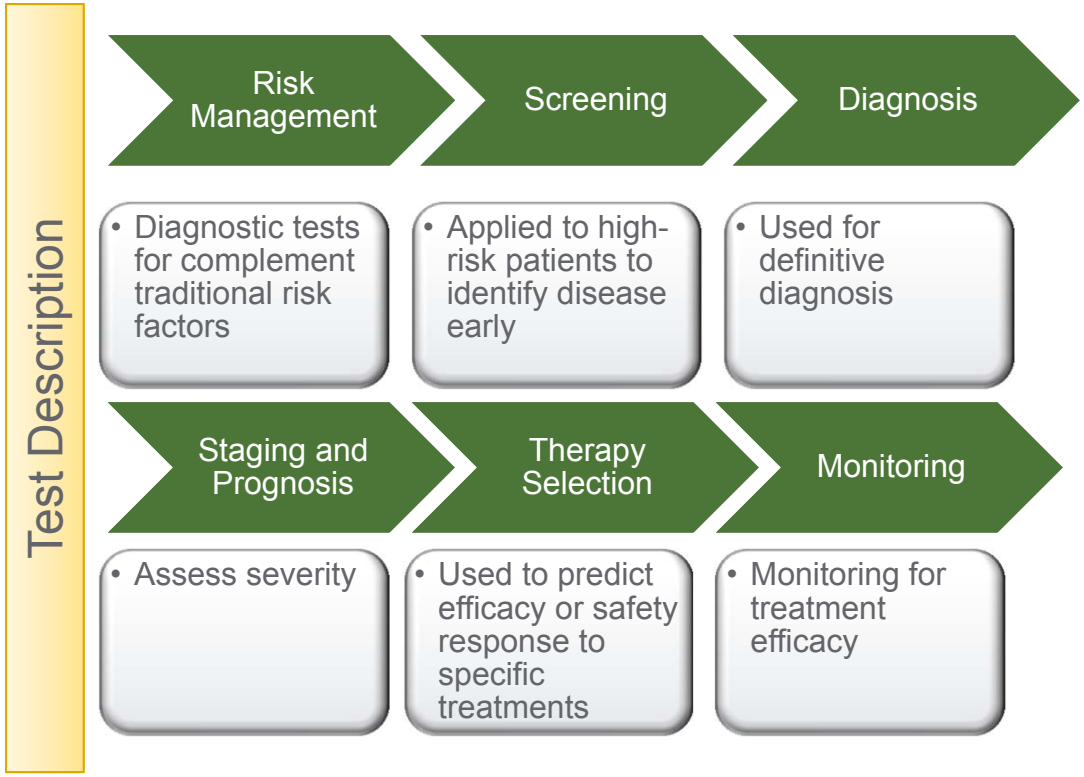
Assessing Patient's Family History



Cost per Genome Decreasing Dramatically



Diagnostic Applications of Sequencing



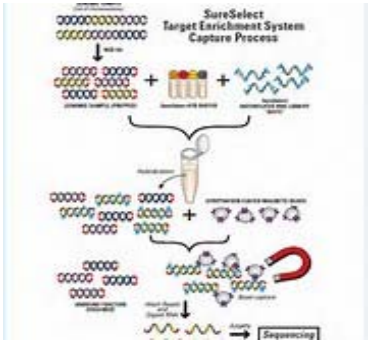
Testing Workflow for Cancer Gene Panel

Blood →
FFPE →
cfDNA →

DNA Extraction

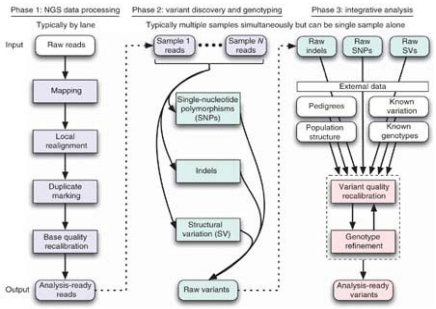
Library prep

Target enrichment

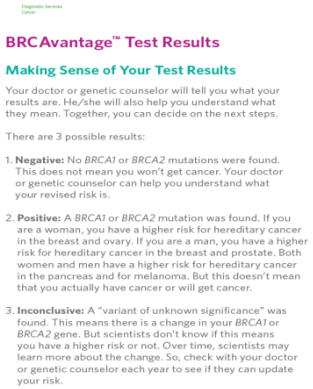


Sequencing

Informatics



Report



BRCAvantage™ Test Results

Making Sense of Your Test Results

Your doctor or genetic counselor will tell you what your results are. He/she will also help you understand what they mean. Together, you can decide on the next steps.

There are 3 possible results:

1. **Negative:** No *BRCA1* or *BRCA2* mutations were found. This does not mean you won't get cancer. Your doctor or genetic counselor can help you understand what your revised risk is.
2. **Positive:** A *BRCA1* or *BRCA2* mutation was found. If you are a woman, you have a higher risk for hereditary cancer in the breast and ovary. If you are a man, you have a higher risk for hereditary cancer in the breast and prostate. Both women and men have a higher risk for hereditary cancer in the pancreas and for melanoma. But this doesn't mean that you actually have cancer or will get cancer.
3. **Inconclusive:** A "variant of unknown significance" was found. This means there is a change in your *BRCA1* or *BRCA2* gene. But scientists don't know if this means you have a higher risk or not. Over time, scientists may learn more about the change. So, check with your doctor or genetic counselor each year to see if they can update your risk.

BRCA1 and *BRCA2* Review

- Hereditary breast and/or ovarian cancer (HBOC) syndrome - most common high-risk breast cancer susceptibility syndrome
- Mutations occur in 1:300 to 1:800 people
  - 1:40 in Ashkenazi Jewish individuals
- Cancer risks by age 70 y.o. for *BRCA1* and *BRCA2* mutation carriers without a personal history of cancer:

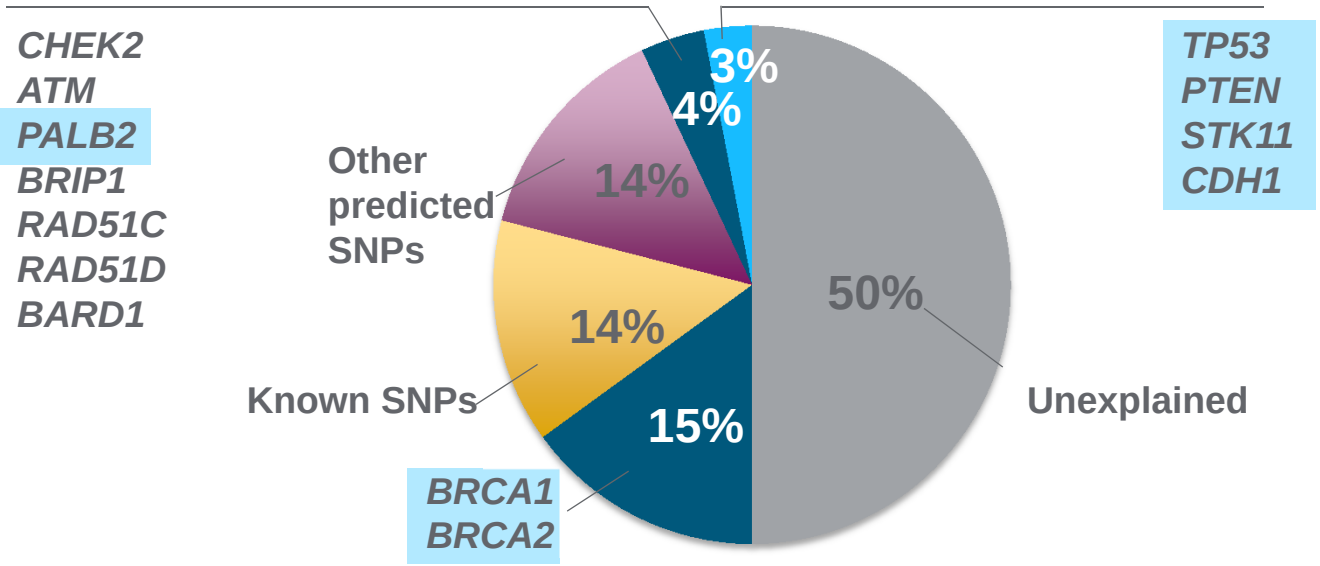
Condition	*BRCA1*	*BRCA2*
Female Breast Cancer	55% to 65%	45% to 47%
Ovarian Cancer	39%	11% to 17%
Male Breast Cancer	1.2%	6.8%

Mavaddat N *et.al.*, Cancer Risks for *BRCA1* and *BRCA2* Mutation Carriers: Results from Prospective Analysis of EMBRACE. *J Natl Cancer Inst.* 105:812-822, 2013

Other Hereditary Breast Cancer Genes

- Every year, more than 200,000 women in the U.S. will be diagnosed with breast cancer
- Hereditary breast cancer accounts for about 5% to 10% of female breast cancer and 4% to 40% of male breast cancer

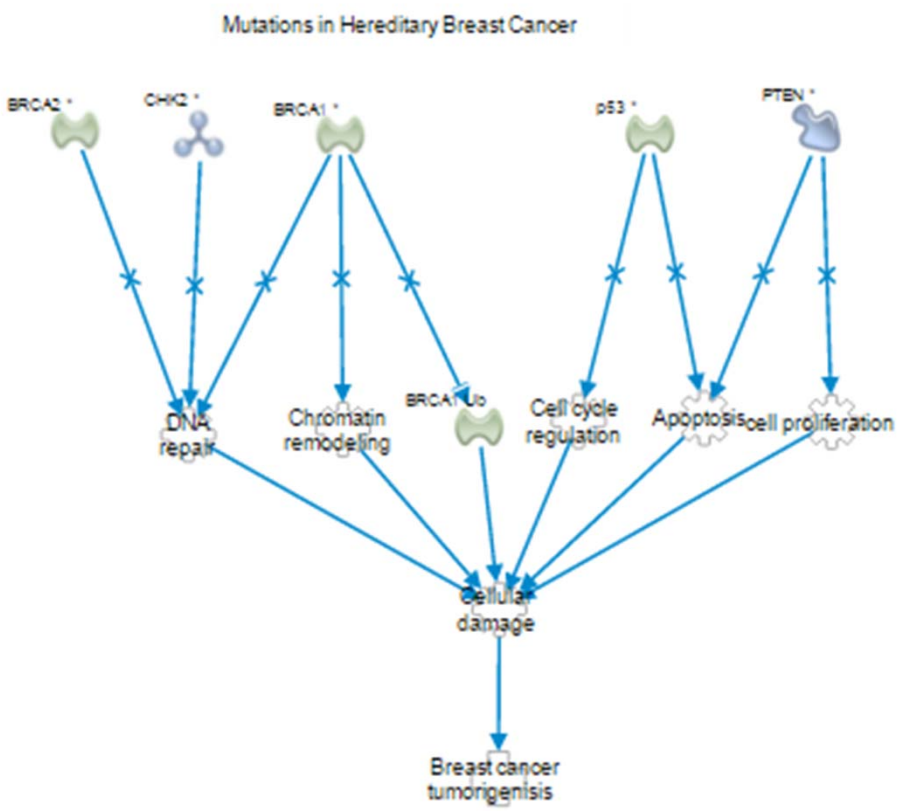
Genes with highest increased risk for breast cancer (highlighted):



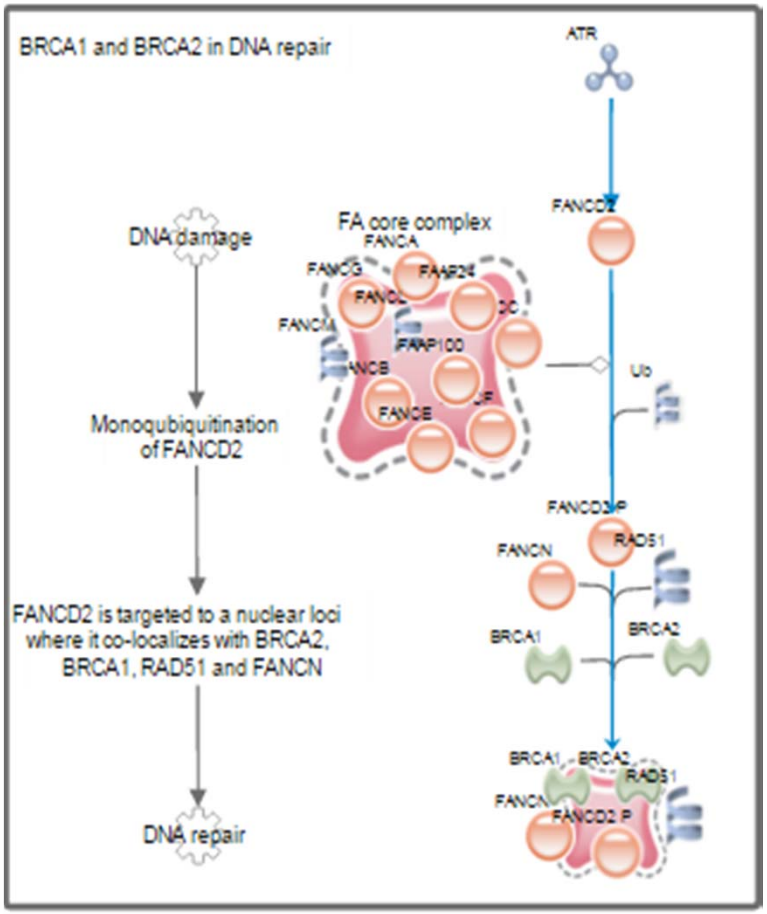
Fergus Couch *et al* 2014 *Science* 344, 1466

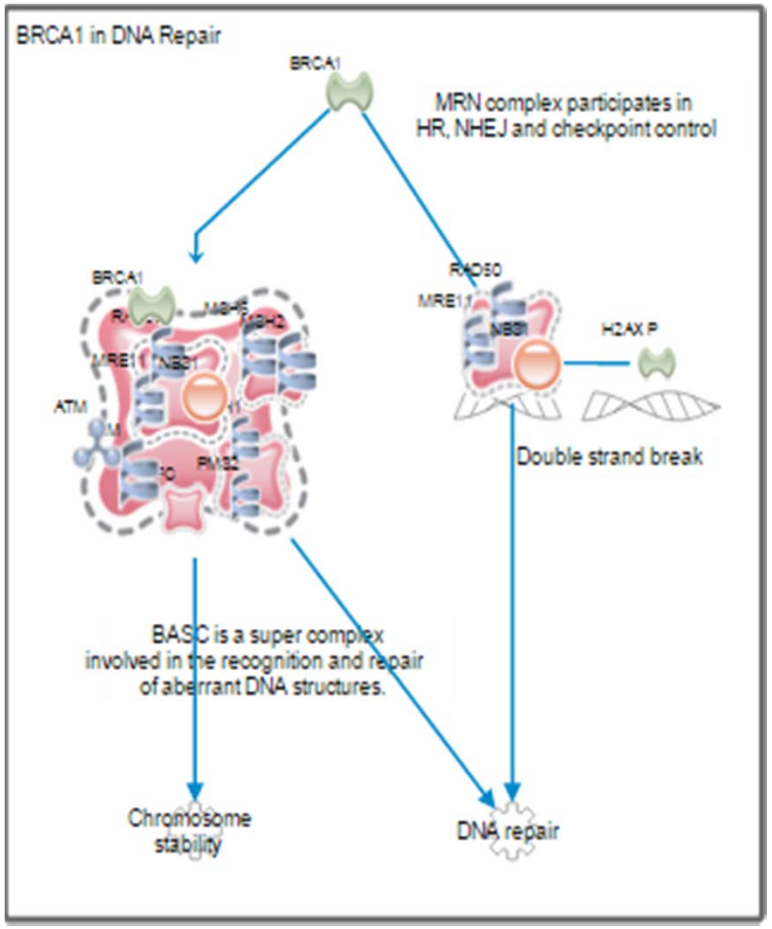
Breast Cancer Tumorigenesis

- DNA repair – BRCA1/2, CHK2
- Chromatin remodeling – BRCA1
- Protein Ubiquitination - BRCA1
- Cell cycle regulation – p53
- Apoptosis – PTEN
- Cell proliferation - PTEN



DNA Repair: *BRCA1* and *BRCA2*





Genes and Associated Syndromes

Gene	Condition
BRCA1 and *BRCA2*	Hereditary breast and/or ovarian (HBOC) syndrome
TP53	Li-Fraumeni syndrome (LFS)
PTEN	*PTEN* Hamartoma Tumor syndrome (PHTS), which includes Cowden syndrome (CS)
CDH1	Hereditary diffuse gastric cancer (HDGC)
STK11	Peutz-Jeghers syndrome (PJS)
PALB2	*PALB2*-associated breast cancer

Lifetime Risk of Breast Cancer

***TP53*: breast cancer relative risk of 6.4x**

***PTEN* : breast cancer risk of 85% by approximately age 70 y.o.**

***CDH1l* hereditary diffuse gastric cancer : lobular breast cancer risk of 39% to 52% by age 80 y.o.**

***STK11* : breast cancer risk of 45% by age 70 y.o.**

***PALB2*: breast cancer risk of 35% by age 70 y.o.**

Ruijs M *et al* 2010 *J Med Genet* 47: 421-248
Bubien V *et al* 2013 *J Med Genet* 50: 255-63
Schrader KA *et al* 2008 *Fam Cancer* 7(1): 73-82
Antoniou AC *et al* 2014 *N Engl J Med* 371(6): 497-506
Hearle N et al *et al 2006 Clin Can Res* 12;3209

Lifetime Risk of Other Key Cancers

Compared to the general population, *TP53* relative risks of cancer:

- Bone (107)
- Connective tissue (61)
- Brain (35)
- Pancreas (7.3)
- Colon (2.8)
- Liver (1.8)

Cowden syndrome and a mutation in *PTEN* risksof cancer by age approximately age 70:

- Thyroid (35%)
- Endometrial (28%)
- Renal (34%)
- Colorectal (9%)
- Melanoma (6%)

Hereditary diffuse gastric cancer and a mutation in *CDH1* gastric cancer risk of 40% to 67% in males and 63% to 83% in females

Peutz-Jeghers syndrome –gastrointestinal cancer risk of 57% (includes pancreatic); pancreatic cancer risk of 11% by age 70.

McBride KA *et a*l 2014 *Nature Reviews Clin Oncology* 11, 260-271
Bubien V *et al* 2013 *J Med Genet* 50: 255-63
Caldas C et al. 1999 *J Med Genet* 36(12):873-80
Kaurah P et al. 2007 *JAMA* 6;297(21): 236(
Hearle N et al et al *2006 Clin Can Res* 12;

Genetic Testing Criteria: HBOC

Family History of Breast Cancer

- Relative with a previously identified *BRCA1* or *BRCA2* mutation
- 1st/2nd-degree blood relative who meets any criteria in the Personal History sections
- 3rd-degree relative with breast[a] and/or ovarian[b] cancer and ≥2 close blood relatives[c] with breast and/or ovarian[b] cancer

Personal History of Other (Nonbreast) Cancers

- Epithelial ovarian cancer[b]
- Pancreatic or prostate cancer with ≥2[d] close blood relatives[c] diagnosed with breast, ovarian,[b] pancreatic, or prostate cancer (Gleason score ≥7)

Personal History of Breast Cancer[a]

Age at Diagnosis	Additional Criteria Only 1 of the following is necessary.
≤45 y	• No additional criteria necessary
≤50 y	• ≥2 primary breast tumors[e] • ≥1 close blood relative[c] with breast cancer • Limited family history
≤60 y	• Breast cancer that is negative for ER, PR, and HER2 (triple negative)
Any age	• Patient is male • ≥1 close blood relative[c] with breast cancer diagnosed by age 50 or with epithelial ovarian[b] cancer diagnosed at any age • ≥2 close blood relatives[c] with breast cancer • ≥2 close blood relatives[c] with prostate cancer (Gleason score ≥7) or pancreatic cancer • ≥1 close male blood relative[c] with breast cancer • Ethnicity (eg, Ashkenazi Jewish) associated with higher mutation frequency

National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Genetic/familial high-risk assessment: breast and ovarian. V2.2014.

BRCA1 or *BRCA2* (Hereditary Breast and/or Ovarian Cancer Syndrome)

NCCN Genetic Testing Criteria*

- Individual from a family with a known deleterious *BRCA1* or *BRCA2* mutation
- Personal history of breast cancer diagnosed at age ≤45 y**
- Personal history of breast cancer with additional criteria**
- Personal history of epithelial ovarian cancer
- Personal history of male breast cancer
- Personal history of pancreatic cancer or prostate cancer with additional criteria
- Additional criteria for patients without a personal history of cancer, with a family history of HBOC-related cancers

NCCN Management Guidelines*

- Women
  - Clinical breast exam: every 6 to 12 months, starting at age 25 y
  - Age 25 to 29 y: annual breast MRI
  - Age >30 to 75 y: annual breast MRI and mammogram
  - Discuss risk reducing mastectomy
  - Recommend risk-reducing salpingo-oophorectomy
  - Consider chemoprevention options
- Men
  - Clinical breast exam: every 6 to 12 months, starting at age 35 y
  - Consider baseline mammogram at 40 y
  - Prostate cancer screening starting at age 40 y
- Additional recommendations

* NCCN clinical practice guidelines in oncology. Genetic/familial high-risk assessment: breast and ovarian. V2.2014.

** Includes invasive and ductal carcinoma in situ breast cancers.

Genetic Testing Criteria and Management Guidelines

Gene	Genetic Testing Criteria	Management Guidelines
BRCA1 and *BRCA2*	NCCN: Genetic/Familial High-Risk Assessment: Breast and Ovarian	
TP53		
PTEN		
CDH1	International Gastric Cancer Linkage Consortium consensus guidelines	
STK11	**None**	NCCN: Genetic/Familial High-Risk Assessment: Colorectal
PALB2	**None**	ACS recommends screening with MRI for women with at least 20% to 25% lifetime risk of breast cancer

Shiovitz & Korde LA, 2015 *Ann Oncol*

Breast Cancer Expanded Menu

- *BRCA1* and *BRCA2* may explain 15% to 20% of hereditary breast cancer cases
- *TP53*, *PTEN*, *CDH1*, *STK11*, and *PALB2*: breast cancer susceptibility genes together explain an additional 3% to 4.5% of hereditary breast cancers
- Focused risk-assessment options exist for guideline-supported and emerging genes
  - Guideline supported: *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *CDH1*, *STK11*
  - Emerging: *PALB2* *(Antoniou et al 2014 N Engl J Med 371(6): 497-506)*

When to Consider Multi-Gene Testing

Society of Gynecologic Oncology (SGO) Clinical Practice Statement: Next Generation Cancer Gene Panels Versus Gene by Gene Testing[2] (March 2014)

- Advantages: decreased cost and improved efficiency of cancer genetic testing by decreasing the time involved, number of patient visits, and number of tests sent
- Primary disadvantage: increased complexity of results
- Genetic counselors or knowledgeable medical professionals should carefully discuss the pros and cons with patients
- Additional recommendations

National Comprehensive Cancer Network (NCCN) Guidelines V2.2014 Genetic/Familial High-Risk Assessment: Breast and Ovarian, GENE-1 (September 2014)

- The decision to use multi-gene testing for patient care should be no different than the rationale for testing a single gene known to be associated with the development of a specific type of cancer
- Multi-gene testing may be more cost-effective and time-effective in certain cases than sequentially testing more than 2 to 3 single genes associated with a phenotype
- *BRCA1*/*BRCA2*, *TP53,* and *PTEN*: Consider multi-gene testing, if appropriate
- Additional recommendations

Hereditary Breast Cancer Panel Test Options: Part 1

Comprehensive	Includes sequencing and large rearrangement analysis of all coding exons in *BRCA1* and *BRCA2*
Ashkenazi Jewish Screen	Includes detection of the 3 HBOC founder mutations (187delAG, 5385insC, 6174delT)
Ashkenazi Jewish Screen with Reflex Comprehensive	Ashkenazi Jewish screen test that reflexes to the comprehensive test when Ashkenazi test is negative
Single Site	Specific mutation testing for a known familial mutation
Rearrangements	*BRCA1* and *BRCA2* complete rearrangement testing not performed

Hereditary Breast Cancer Panel Test Options: Part 2

Test Name	Clinical Application
***BRCA* Expanded Panel** Includes point mutations, deletions, and duplications in the *BRCA1, BRCA2, TP53, PTEN, CDH1, STK11* and *PALB2* genes	• **Assess hereditary breast cancer risk when there is no known familial mutation and the patient has a personal or family history consistent with more than 1 condition related to hereditary breast cancer** • **Simultaneous analyses of relevant genes**
***BRCA* w/ Reflex to Breast Plus Panel** Includes test code 91863; test code 92586 added with additional charge and CPT code, if no *BRCA1* or *BRCA2* mutations detected	• **Assess hereditary breast cancer risk when no known familial mutation and the patient has a personal or family history consistent with more than 1 condition related to hereditary breast cancer** • **Two-step analysis begins with testing for mutations in *BRCA1* and *BRCA2*, the most common causes of hereditary breast cancer; 5 additional genes analyzed if pathogenic or likely pathogenic mutations not detected in the first step**
BRCA* Expanded Panel w/o *BRCA 1/2 Includes point mutations, deletions, and duplications in the *TP53, PTEN, CDH1, STK11, PALB2* genes	• **Second-tier test to assess hereditary breast cancer risk in people negative for *BRCA1* and *BRCA2* point mutations, deletions, and duplications**

Cancer Predisposition Panel: 34 Genes

34 genes		Breast	Ovarian	GYN	Colon	Pancreatic	Renal
7G	**BRCA1**	BRCA1	BRCA1	BRCA1		BRCA1	
	BRCA2	BRCA2	BRCA2	BRCA2		BRCA2	
	STK11	STK11	STK11		STK11	STK11	
	CDH1	CDH1	CDH1		CDH1		
	PTEN	PTEN	PTEN	PTEN	PTEN		PTEN
	TP53	TP53	TP53	TP53	TP53	TP53	TP53
	PALB2	PALB2	PALB2			PALB2	
27G	BARD1	BARD1	BARD1				
	BRIP1	BRIP1	BRIP1				
	NBN	NBN	NBN				
	NF1	NF1	NF1				
	RAD51C	RAD51C	RAD51C				
	RAD51D	RAD51D	RAD51D				
	ATM	ATM	ATM			ATM	
	MUTYH	MUTYH	MUTYH		MUTYH		
	CHEK2	CHEK2	CHEK2		CHEK2		
	RET	RET			RET		
	MEN1	MEN1					
	MLH1		MLH1	MLH1	MLH1	MLH1	MLH1
	MSH2		MSH2	MSH2	MSH2	MSH2	MSH2
	MSH6		MSH6	MSH6	MSH6	MSH6	MSH6
	PMS2		PMS2	PMS2	PMS2	PMS2	PMS2
	EPCAM		EPCAM	EPCAM	EPCAM	EPCAM	EPCAM
	APC				APC	APC	
	BMPR1A				BMPR1A		
	SMAD4				SMAD4		
	POLD1				POLD1		
	POLE				POLE		
	CDKN2A					CDKN2A	
	CDK4						
	SDHB						SDHB
	SDHC						SDHC
	SDHD						SDHD
	VHL						VHL

Clinical Actionability: HBOC Risk Assessment

	Desmond, 2015	LAB A	LAB B	LAB C
# Genes	***29***	***25***	***34***	***34***
		BRCA1	*BRCA1*	*BRCA1*
		BRCA2	*BRCA2*	*BRCA2*
High risk BR and OV CA	*TP53*	*TP53*	*TP53*	*TP53*
	PTEN	*PTEN*	*PTEN*	*PTEN*
	STK11	*STK11*	*STK11*	*STK11*
	CDH1	*CDH1*	*CDH1*	*CDH1*
Low-mod risk BR and OV CA	*BARD1*	*BARD1*	*BARD1*	*BARD1*
	CHEK2	*CHEK2*	*CHEK2*	*CHEK2*
	PALB2	*PALB2*	*PALB2*	*PALB2 (FANCN)*
	ATM	*ATM*	*ATM*	*ATM*
	BRIP1	*BRIP1*	*BRIP1*	*BRIP1*
	RAD51C	*RAD51C*	*RAD51C*	*RAD51C*
	RAD51D	*RAD51D*		*RAD51D*
	NBN	*NBN*	*NBN*	*NBN (NBS1),*
Lynch syndrome	*MLH1*	*MLH1*	*MLH1*	*MLH1*
	MSH2	*MSH2*	*MSH2*	*MSH2*
	MSH6	*MSH6*	*MSH6*	*MSH6*
	PMS2	*PMS2*	*PMS2*	*PMS2*
	EPCAM	*EPCAM*	*EPCAM*	*EPCAM*
Other Familial	*APC*	*APC*	*APC*	*APC*
	BMPR1A	*BMPR1A*	*BMPR1A*	*BMPR1A*
	SMAD4	*SMAD4*	*SMAD4*	*SMAD4*
	CDK4	*CDK4*	*CDK4*	*CDK4*
	CDKN2A	*CDKN2A (p16, p14)*	*CDKN2A*	*CDKN2A (p16, p14)*
	MUTYH Biallelic	*MUTYH*	*MUTYH*	*MUTYH (MYH)*
	MET		*MET*	
	MEN1		*MEN1*	*MEN1*
	RET		*RET*	*RET*
	PTCH1		*PTCH1*	
	VHL		*VHL*	*VHL*
			FANCC	
			NF1	*NF1*
			RAD50	
				SDHB
				SDHC
				SDHD
				POLD1
				POLE
	PALLD			

Desmond et al., 2015 JAMA Oncol

Analytical Performance Review

- Assay validation
  - Dictated by assay design, panel content, gene structure (i.e. *CHEK2* & *PMS2* pseudogenes)
  - Accuracy, sensitivity, specificity, limits of detection
  - Efforts in reducing sequencing errors, increase capture
  - Limitations of platform used – mosaicism, low copy number variants (somatic)
  - Type of mutation and rearrangements – triplet repeats, CNVs, large rearrangement (inversion/translocation)
- Sequencing performance/Quality metrics
- Alignment software for accurate allele identification
  - Detection ability - ≥10bp deletions and insertions

Variant Annotation & Classification

- Variant classification using the American College of Medical Genetics (ACMG) 5-tier classification system
- Multiple data sources
  - ClinVar archive
  - Breast Cancer Information Core (BIC)
  - Universal Mutation Database (UMD)
  - Leiden Open Variation Database (LOVD)
  - Prediction tools such as PolyPhen-2, SIFT, Align GVGD, and MutationTaster
- Final interpretation performed by board-certified directors
- Multiple reviews for VUS and pathogenic cases
- Co-segregation family study program to help with VUS reclassification
- Reclassified variants will be communicated to the ordering provider when a patient result is amended

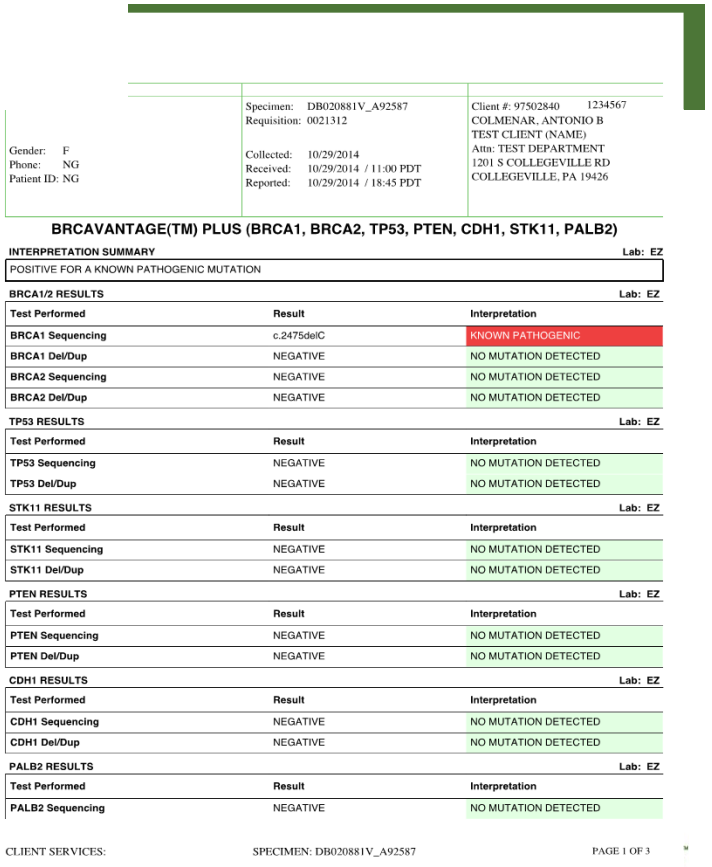
Breast Cancer: Report

Turnaround time (TAT)

- Up to 14 days (upon receipt of complete requisition)
- Reflex test option up to 21 days (upon receipt of complete requisition)

Content

- Interpretation Summary
- Color-Coded 5-Tier Classification
  - Known Pathogenic (**RED**)
  - Likely Pathogenic (**RED**)
- VUS (**YELLOW**)
- Likely Benign Polymorphism (**GREEN**)
- No Mutation Detected (**GREEN**)
- Comprehensive Interpretation
- ACMG Guidelines



Gender: F
Phone: NG
Patient ID: NG

Specimen: DB020881V_A92587
Requisition: 0021312

Collected: 10/29/2014
Received: 10/29/2014 / 11:00 PDT
Reported: 10/29/2014 / 18:45 PDT

Client #: 97502840 1234567
COLMENAR, ANTONIO B
TEST CLIENT (NAME)
Attn: TEST DEPARTMENT
1201 S COLLEGEVILLE RD
COLLEGEVILLE, PA 19426

BRCAVANTAGE(TM) PLUS (BRCA1, BRCA2, TP53, PTEN, CDH1, STK11, PALB2)

INTERPRETATION SUMMARY Lab: EZ

POSITIVE FOR A KNOWN PATHOGENIC MUTATION

BRCA1/2 RESULTS Lab: EZ

Test Performed	Result	Interpretation
BRCA1 Sequencing	c.2475delC	KNOWN PATHOGENIC
BRCA1 Del/Dup	NEGATIVE	NO MUTATION DETECTED
BRCA2 Sequencing	NEGATIVE	NO MUTATION DETECTED
BRCA2 Del/Dup	NEGATIVE	NO MUTATION DETECTED

TP53 RESULTS Lab: EZ

Test Performed	Result	Interpretation
TP53 Sequencing	NEGATIVE	NO MUTATION DETECTED
TP53 Del/Dup	NEGATIVE	NO MUTATION DETECTED

STK11 RESULTS Lab: EZ

Test Performed	Result	Interpretation
STK11 Sequencing	NEGATIVE	NO MUTATION DETECTED
STK11 Del/Dup	NEGATIVE	NO MUTATION DETECTED

PTEN RESULTS Lab: EZ

Test Performed	Result	Interpretation
PTEN Sequencing	NEGATIVE	NO MUTATION DETECTED
PTEN Del/Dup	NEGATIVE	NO MUTATION DETECTED

CDH1 RESULTS Lab: EZ

Test Performed	Result	Interpretation
CDH1 Sequencing	NEGATIVE	NO MUTATION DETECTED
CDH1 Del/Dup	NEGATIVE	NO MUTATION DETECTED

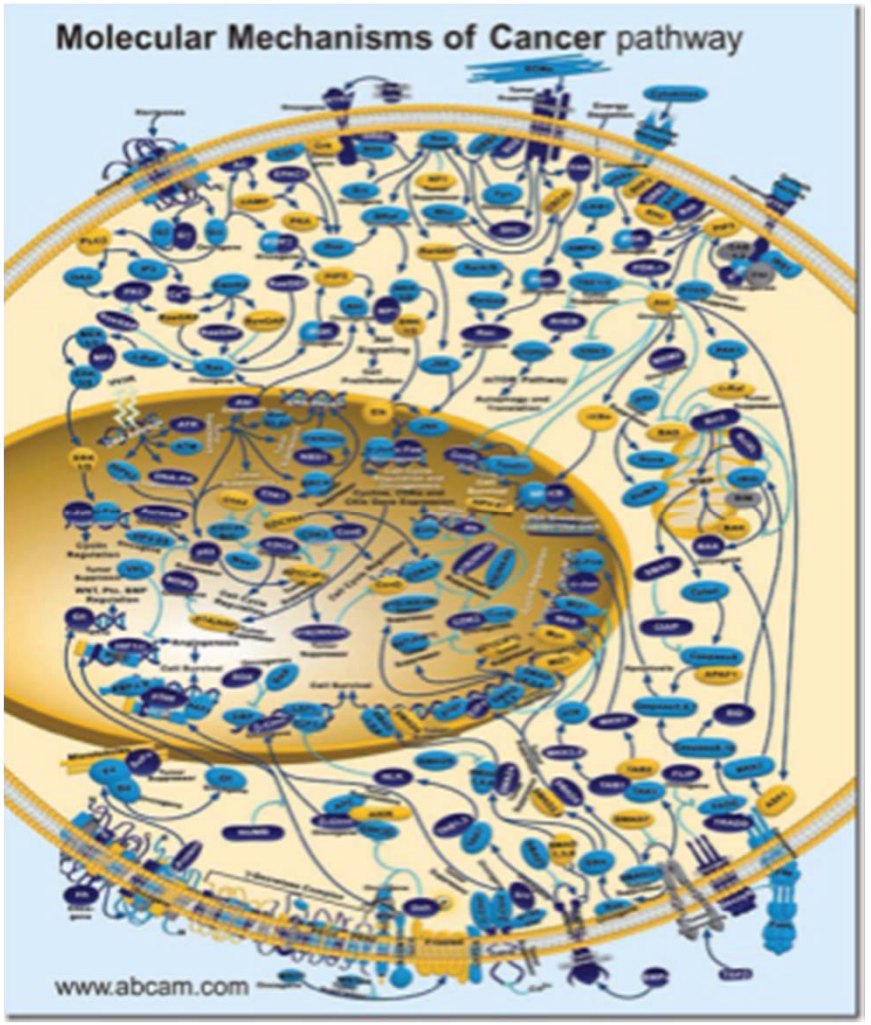
PALB2 RESULTS Lab: EZ

Test Performed	Result	Interpretation
PALB2 Sequencing	NEGATIVE	NO MUTATION DETECTED

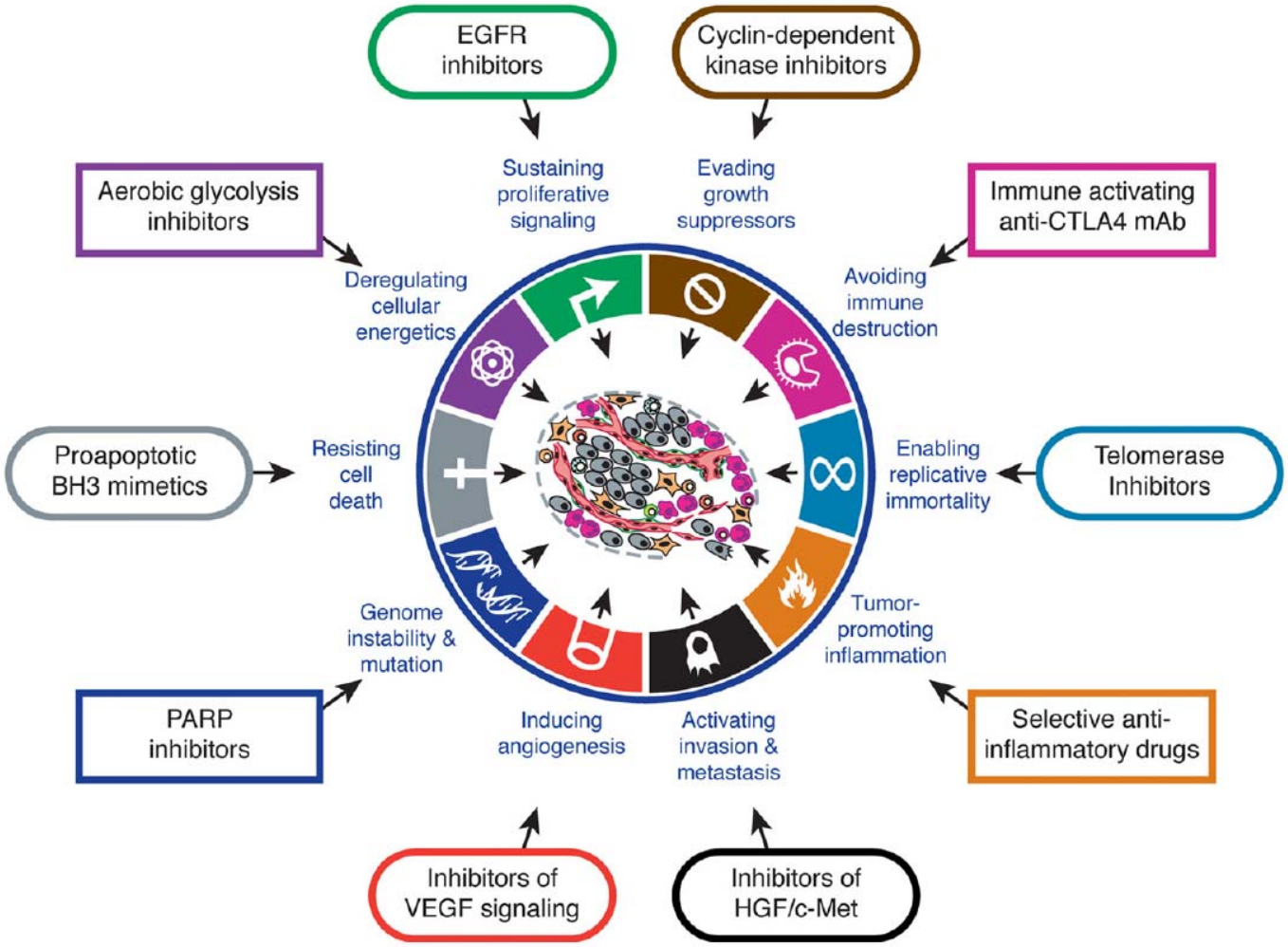
CLIENT SERVICES: SPECIMEN: DB020881V_A92587 PAGE 1 OF 3

Solid Tumors

- Multiple genes and pathways altered
- Tumor suppressor genes and oncogenes
- Targeted therapies available
- Clinical annotation and clinical utility must be established

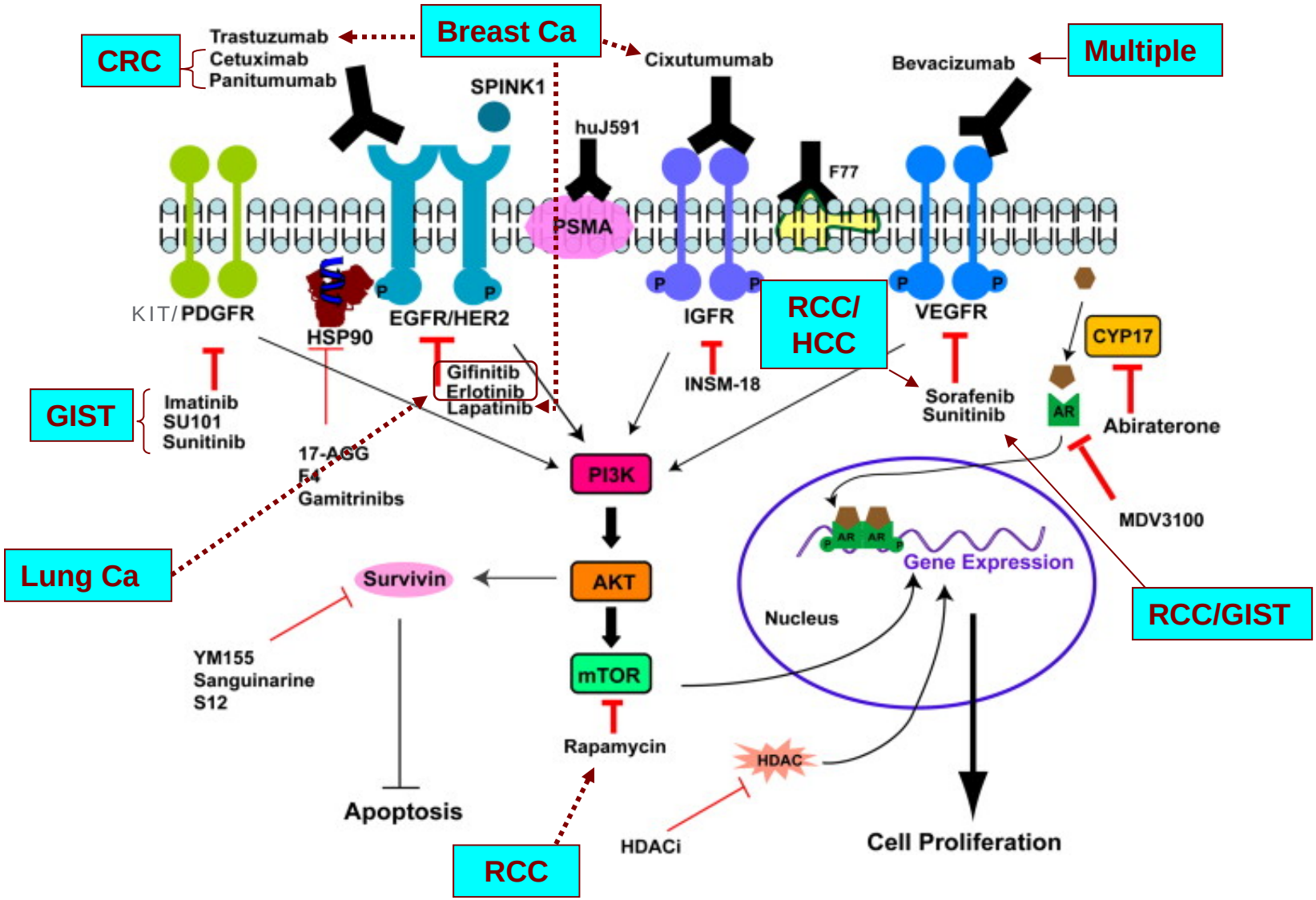


Clinical View of Cancer

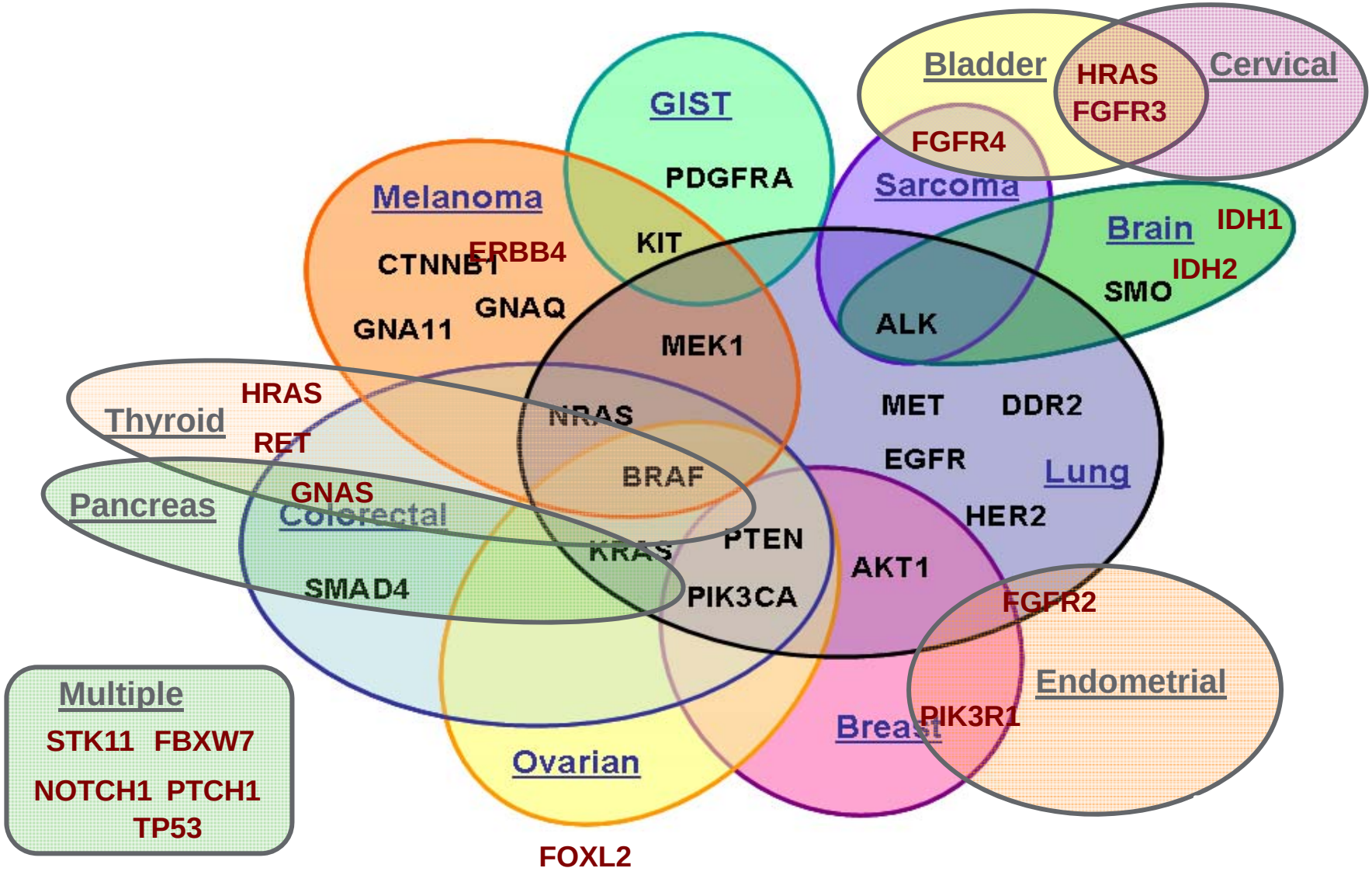


Hanahan & Weinberg *Cell* 144, 643 (2011).

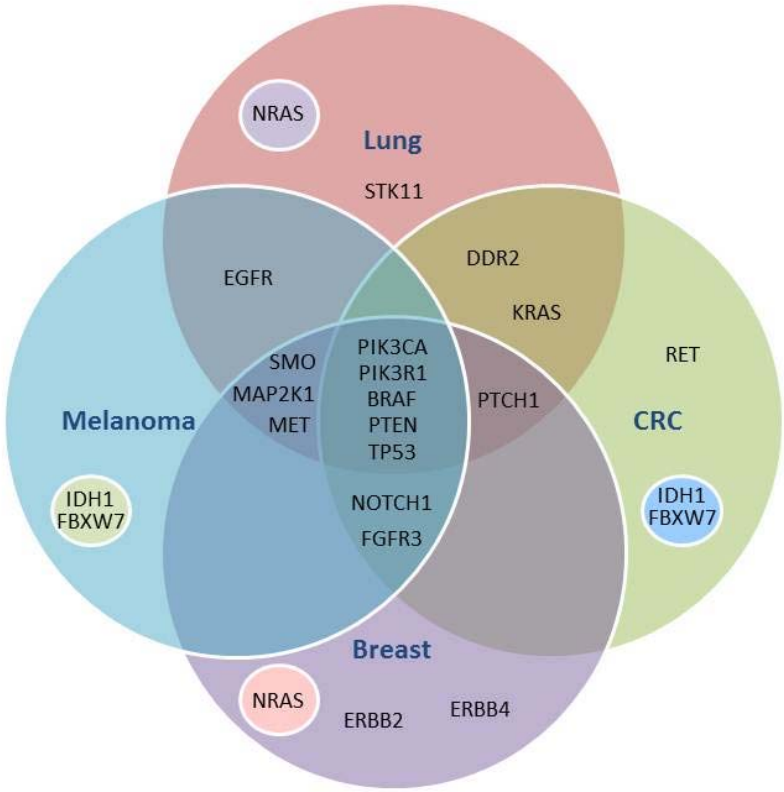
Cancer Pathways and Targeted Treatments



Solid Tumor Gene Mutations Related to Therapy



More Common Solid Tumor Genes



Solid Tumor by NGS

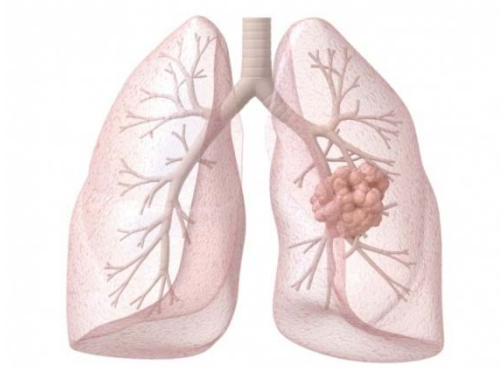
- Targeted actionable genes
- 34-gene panel with broad mutation representation
- Panel applicable to all solid tumor types
- Annotation directed at FDA-approved drugs in selected tumor types and clinical trial availability
- FFPE tissue, small biopsies, FNAs

Lung Cancer

NCCN Guidelines Version 3.2014
Non-Small Cell Lung Cancer

TARGETED AGENTS FOR PATIENTS WITH GENETIC ALTERATIONS

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
EGFR mutations	erlotinib,[1] gefitinib,[2] afatinib[3]
ALK rearrangements	crizotinib[4]
HER2 mutations	trastuzumab,[5] afatinib[6]
BRAF mutations	vemurafenib,[7] dabrafenib[8]
MET amplification	crizotinib[9]
ROS1 rearrangements	crizotinib[10]
RET rearrangements	cabozantinib[11]

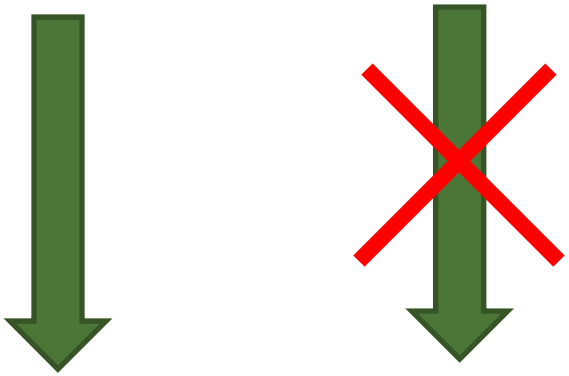


Treatment Options Based on Molecular Profile

EGFR exon 18, 19 or 21 mutation

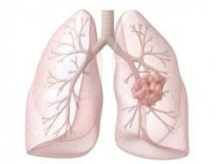
EGFR T790M

KRAS codon 12, 13, 61, etc.



Tarceva® (erlotinib)

Current Tumor Panels

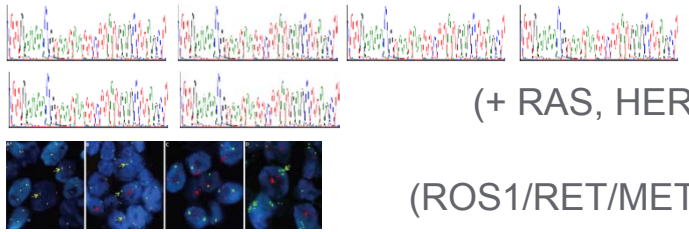


Lung Panel

EGFR

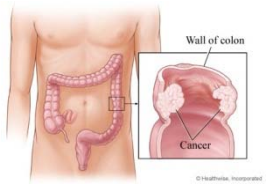
BRAF

ALK



(+ RAS, HER2 mutation)

(ROS1/RET/MET FISH)



Colorectal Panel

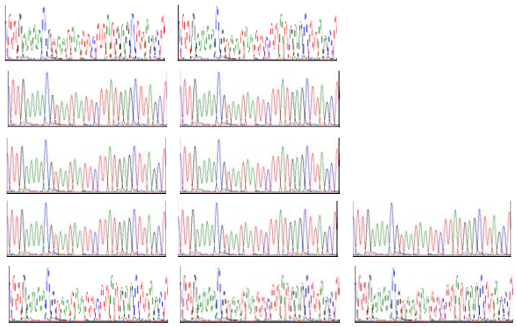
KRAS

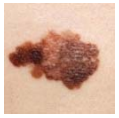
NRAS

HRAS

PIK3CA

BRAF

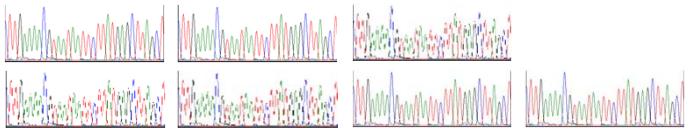


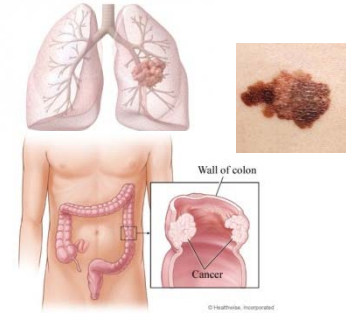


Melanoma Panel

BRAF

KIT





34 Gene
NGS Panel

1 Assay
(34 genes; >230 amplicons)

Level 1 Associations Between Genes and FDA-Approved Therapies

Gene Mutation	Drugs	Tumor Type	Association
BRAF	Vemurafenib	Melanoma	Sensitive
EGFR	Cetuximab	Head and Neck	Sensitive
EGFR	Gefitinib, Erlotinib, Afatinib, Cetuximab	Lung	Sensitive or resistant depending on mutation
HRAS	Vemurafenib	Cutaneous Squamous Cell Carcinoma	Resistant
KIT	Imatinib, Sunitinib, Regorafenib, Sorafenib	Gastrointestinal Stromal Tumor (GIST)	Sensitive
RET	Vandetanib, Cabozantinib,	Thyroid	Sensitive
SMO	Vismodegib	Basal Cell Carcinoma (skin)	Sensitive
KRAS	Cetuximab, Panitumumab	Colorectal	Resistant
NRAS	Cetuximab, Panitumumab	Colorectal	Resistant

Gene Targeting

Gene	Treatment	Marker
AKT1	PI3K/mTOR/AKT	
ALK	ALK inhibitors, incl. crizotinib, Xalkori	Gene & Resistance
BRAF	RAF inhibitors, MEK inhibitors, PI3K inhibitors	Gene
CTNNB1	mTor inhibitors	Gene
DDR2	Some TYR-Kinase inhibitors, Nilotinib	Other, Gene
EGFR	EGFR inhibitors, EGFR antibodies	Gene
ERBB2	anti HER2,ERBB2 inhibitors, ERBB2 antibodies	Gene
ERBB4	lapatinib	Gene
ESR1	Associated with resistance to anti-estrogen	Resistance
FGFR2	FGFR inhibitors, FGFR antibodies	Gene
FGFR3	FGFR inhibitors, FGFR antibodies	Gene
HRAS	RAF inhibitors, MEK inhibitors, PI3K inhibitors	Resistance
IDH1	IDH1 inhibitor	Gene & Other
KIT	imatinib/sunitinib	Gene
KRAS	RAF inhibitors, MEK inhibitors, PI3K inhibitors	Resistance
MAP2K1	RAF inhibitors, MEK inhibitors, PI3K inhibitors (eg. Mekinist)	Gene
MET	MET inhibitors, MET antibodies	Gene
NRAS	RAF inhibitors, MEK inhibitors, PI3K inhibitors	Resistance
PDGFRA	Kinase Inhibitors, Antibodies	Gene
PIK3CA	PI3K inhibitors, AKT inhibitors,mTor inhibitors	Gene & Pathway
PIK3R1	PI3K inhibitors, AKT inhibitors	Pathway
PTEN	PI3K inhibitors	Pathway
SMAD4	MEK-ERK, p38-MAPK	Pathway
SMO	observed vismodegib resistance,	Gene
VHL	VEGF inhibitors	Pathway

Clinical Applications of NGS Multigene Cancer Panel

Primarily for cancer patients with few or no standard treatment options remaining. Assist oncologist decision on potentially effective drug or clinical trial that would not have been previously considered.

- All solid tumor types may be tested
- Metastatic or locally advanced disease at presentation
- When no actionable mutations in guideline-recommended testing
- Small specimens without sufficient material for all guideline-recommended studies to be completed
- Recurrent or metastatic disease that has progressed through all standard of care options
- Tumors of unknown primary origin
- Rare tumor types where no or few standard of care options exist

Actionability: Results That Guide Decision Making

An evolving concept, varying with patient, clinician, guideline committee, and payer

- Is contextual for stage of disease (primary vs metastatic) and tumor type
- Guidelines and FDA-approved drug labels formally define accepted criteria
- Inclusion in a clinical trial may be considered actionable
- Anticipation of additional genes/mutants that may be actionable in near future is necessary
- Actionability is not binary but is best thought of as a continuum of evidence

Multigene NGS Cancer Panel

- Actionable genes: approved targeted therapies, NCCN guidelines, clinical trials, prognostic indications
- 34 genes; >230 amplicons
- Advantages
  - Multiple genes interrogated simultaneously
  - Enhanced sensitivity over Sanger sequencing
  - Multiplexing patient samples reduces cost of sequencing
  - Targeted sequencing with modifiable content and verification

Answers to guide treatment of most solid tumors, including approved or investigational targeted therapies

Solid Tumor Mutation Panel			
AKT1	*FGFR2*	*IDH2*	*PIK3R1*
ALK	*FGFR3*	*KIT*	*PTCH1*
BRAF	*FGFR4*	*KRAS*	*PTEN*
CTNNB1	*FOXL2*	*MAP2K1*	*RET*
DDR2	*GNA11*	*MET*	*SMO*
EGFR	*GNAQ*	*NOTCH1*	*STK11*
ERBB2	*GNAS*	*NRAS*	*TP53*
ERBB4	*HRAS*	*PDGFRA*	
FBXW7	*IDH1*	*PIK3CA*	

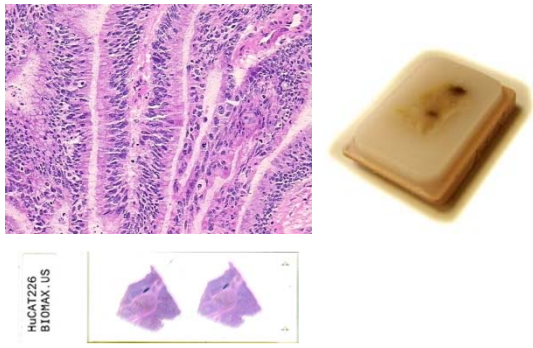
Assay Characteristics

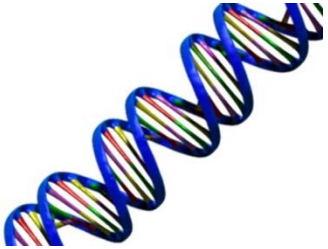
- Released March 24
- Formalin-fixed and paraffin-embedded sections
- 2-5 X 5 um X 1 cm^2 (5-20 ng)
- Sensitivity 5%*
- Verification (Sanger, PCR, MiSeq)
- Alternate Specimen Types (FNA, cells, etc.)

* 5% against wild-type background.

Specimen Flow

Validated sample types
(FFPE, FNA, cells)

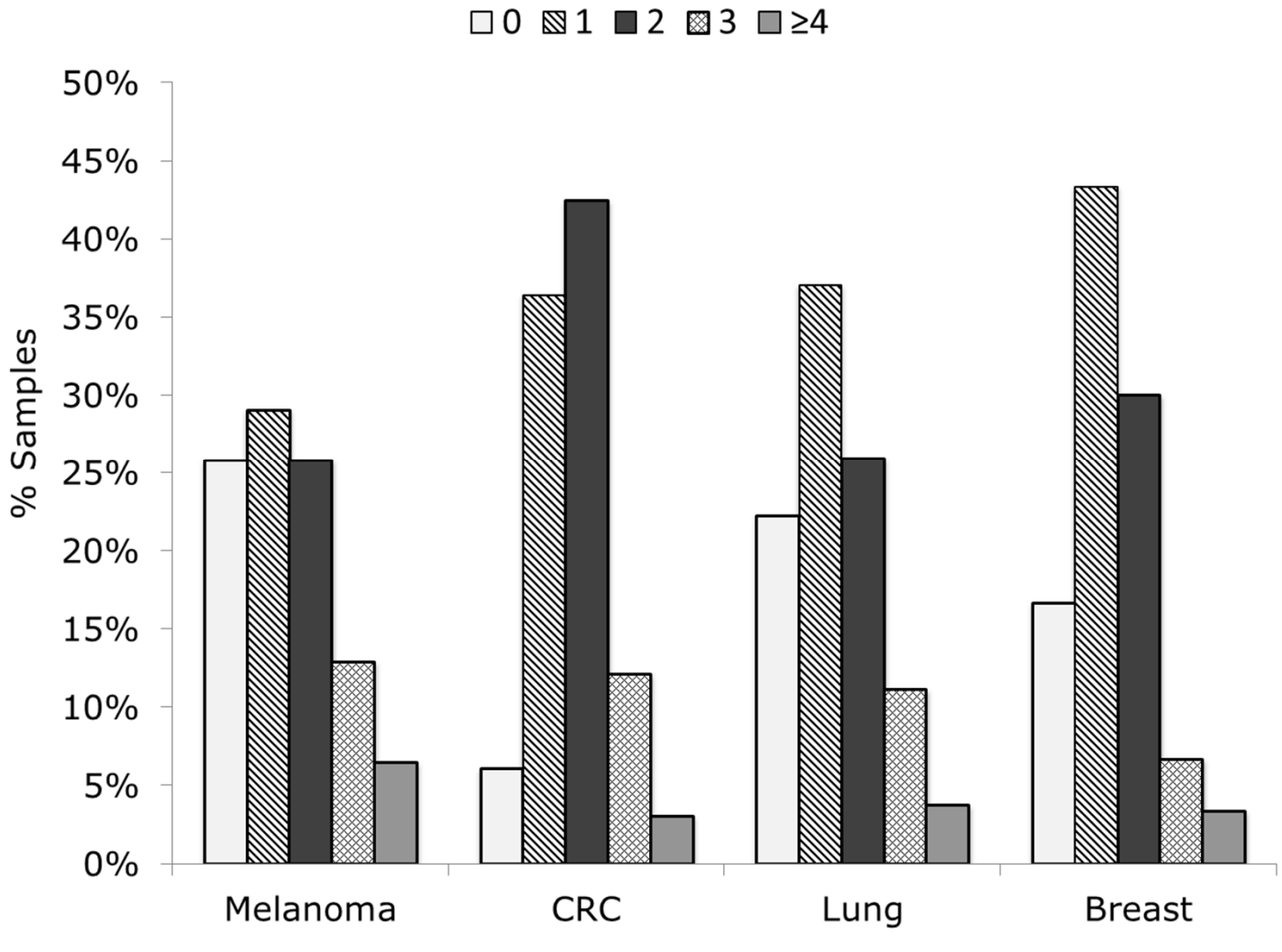




DNA Extraction and Quantitation

Sectioning, Staining, Pathologist
Review and Macrodissection

Mutation Distribution in Common Cancer Types

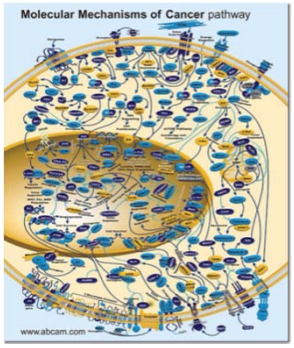


n=30 each

Quest Diagnostics internal data on file.

Annotation Goal Is to Simplify Complex Information

Complex Biology



Simple Direction

- 34 Genes
- 100s of mutations
- 1000s of scientific papers
- FDA-approved and investigational cancer drugs
- Clinical trials
- Guidelines

Multigene Tissue (Somatic) Report

- Mutations identified
- Clinical relevance
- National and international guidelines
- Treatment options: tumor type-specific and additional tumor types
- Clinical trials
- Level of evidence (e.g. publications)

It’s Not so Simple….Challenges

- Primary tumors are heterogeneous
- Metastases differ from primaries
- Tumors can “evolve” and become resistant
- Individual may need multiple tests
- Reimbursement

Other Approaches

- Larger Panels 300 – 400 genes
- Whole Genome/Whole Exome Sequencing
  - With or without comparison to germline
- Liquid Biopsy (free floating tumor DNA)
  - Hot spot (mutation) analysis
  - Sequencing
  - Can be used for primary diagnosis, drug selection, or monitor recurrence

Summary

- During recent years, access to genetic testing for cancer predisposition and solid tumor genetic profile has broadened
- Important technical advances have made it possible to perform multi-gene sequencing assays; test performance may vary according to performing laboratory test validation design and platform used
- Existing mutation databases and a newly created public/private organization are expected to enhance the clinical utility of genetic results
- Overall, the field of genetic testing for predisposition to cancer is becoming fundamentally important and proving clinical validity and utility
- Solid tumor genetic profile influence drug selection, enabling targeted therapy

Thank You for Your Attention!