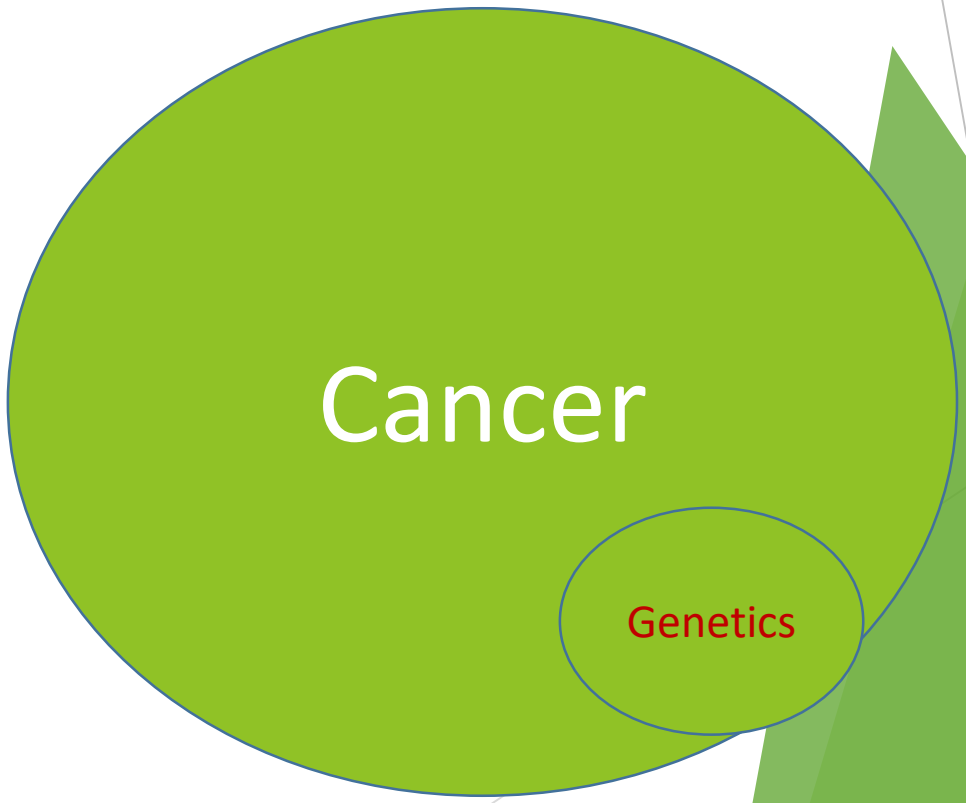
GENETICS AND GENOMICS IN CANCER PREVENTION AND TREATMENT

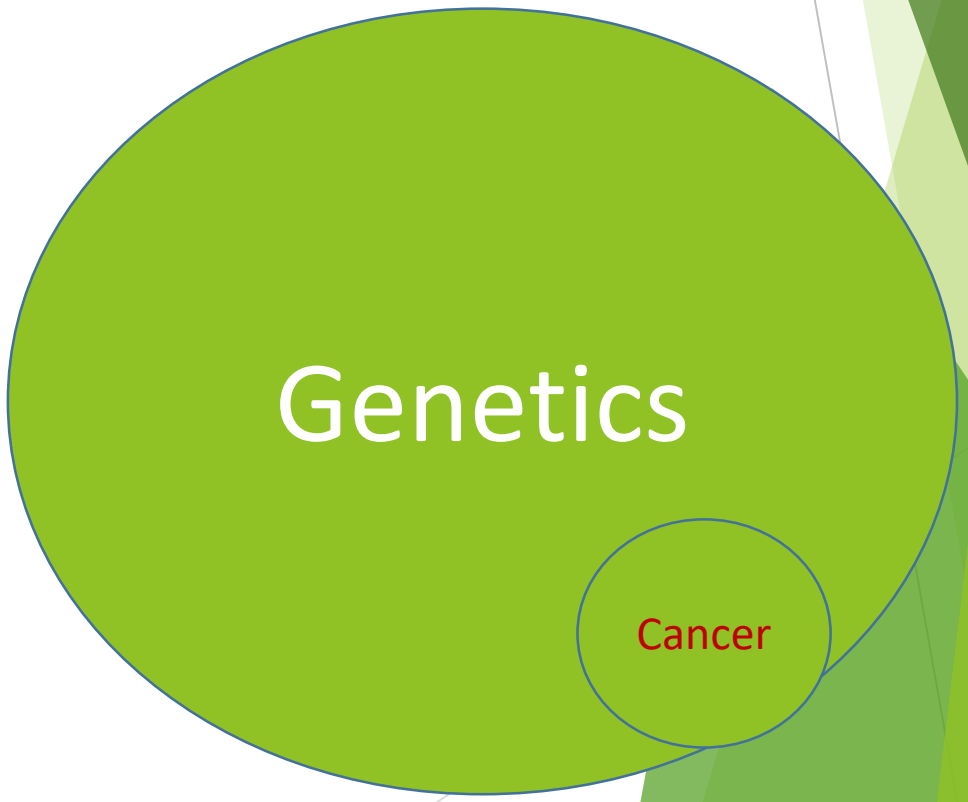
Robert Nathan Slotnick MD PhD

Director, Medical Genetics and Genomics

The Medical/Surgical/Radiation Oncologist's View of Genetics



The Medical Geneticist's View of Cancer



Cancer and the Medical Geneticist

- LR is a 52 year old with a recent diagnosis of advanced stage breast cancer (invasive ductal), triple negative (ER, PR, Her2neu); after surgery, her therapeutic options appeared limited
- In an effort to determine the most reasonable management plan, a sample of archived cancer tissue was sent for analysis to a laboratory in Boston
- At the laboratory, 315 cancer related genes and 28 cancer related DNA rearrangements were queried; **tumor genotyping suggested a BRCA2 genomic alteration**
- The oncology team were unsure of the germline (familial) implications of the result and queried Foundation; I was asked to contact the clinicians
- Of the 6663 BRCA2 variants reported in the literature, the variant detected in this patient's tumor had been reported as **likely pathologic**
- Pedigree analysis/germline testing identified **3 immediate family members** at risk for cancer development; surveillance was begun; **an early cancer was found**

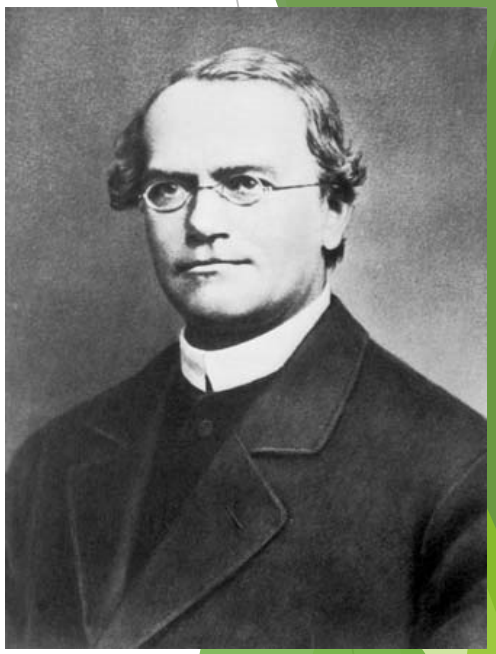
Cancer and the Medical Geneticist

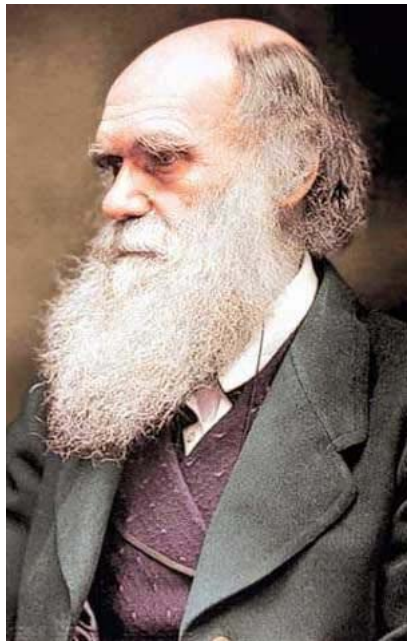
Introduction

- Personal disclaimer/disclosure
  - Personal history: academics and teaching, research, private practice
  - What I am not
  - What I am
- Watson's fly
- Commitment/apologies to audience
  - "Splitters" and "Lumpers"
  - Importance of details and danger of esoterica
  - So… limited esoterica
- Poor understanding of medical genetics in clinical community
  - **"…although 98% of physicians know that patient genetic information will influence therapy-less than 10% believe that they are adequately informed about the use of genetic/genomic testing information to apply it in practice"…** AMA 2016

A LITTLE HISTORY

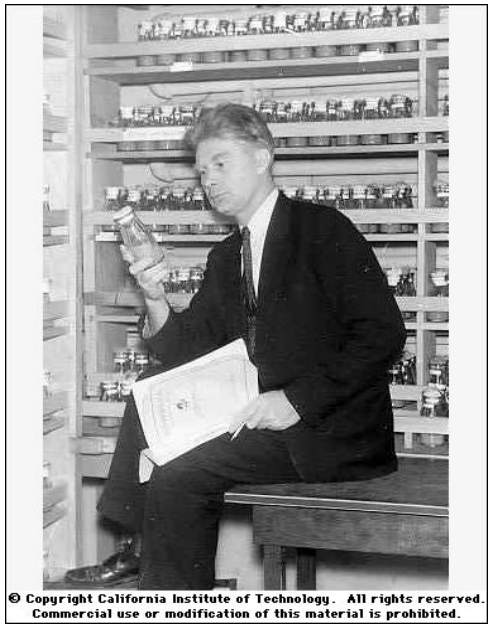
- **Darwin and Mendel**
  - Natural selection
  - Independent assortment of factors
  - Patterns of inheritance





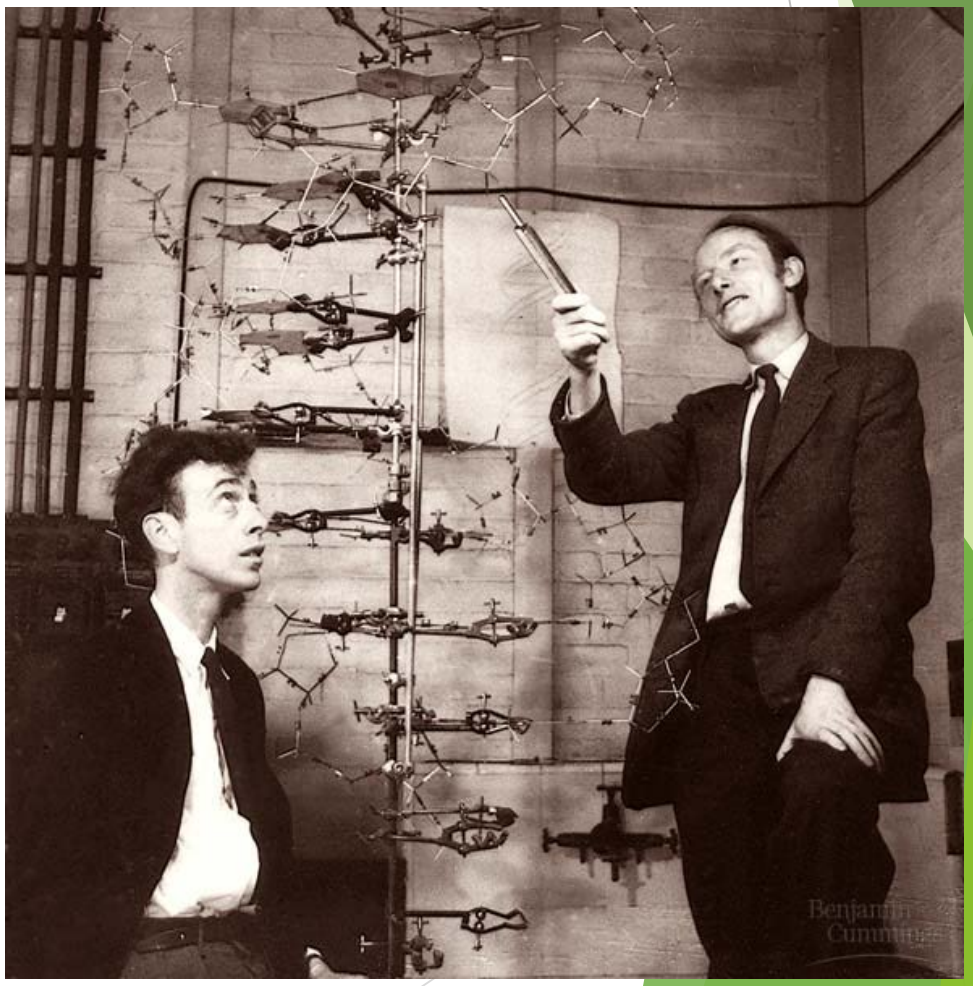
A LITTLE HISTORY

- **Columbia University Fruit Fly Lab**
  - TH Morgan
  - Calvin Bridges
  - HJ Muller
    - Genetic linkage
    - Recombination
    - Gene theory
    - Radiation and mutation



A LITTLE HISTORY

- **Watson and Crick**
  - Structure of DNA (Double Helix)



Objectives

- Review of the genetics of cancer
- Discuss the approach & management of the most common hereditary cancer syndromes
  - Hereditary Breast & Ovarian Cancer syndrome (HBOC)
  - Polyposis syndromes (FAP, AFAP, MAP, & Peutz Jeghers)
  - Hereditary Non-Polyposis Colorectal Cancer (HNPCC) aka Lynch Syndrome
- At end be able to identify the features of the above conditions, know when to initiate referral, and appreciate the changes in medical management and its purpose in early detection and/or prevention of cancer
- What's new: next generation sequencing, panels, cancer genotyping, genetic directed cancer therapeutics, cell free DNA

Genetics of Cancer

Cancer is due to an alteration in the DNA of specific genes within specific cells in the body

If the alteration occurs within one of the many "cellular control" genes, normal cellular controls are lost.

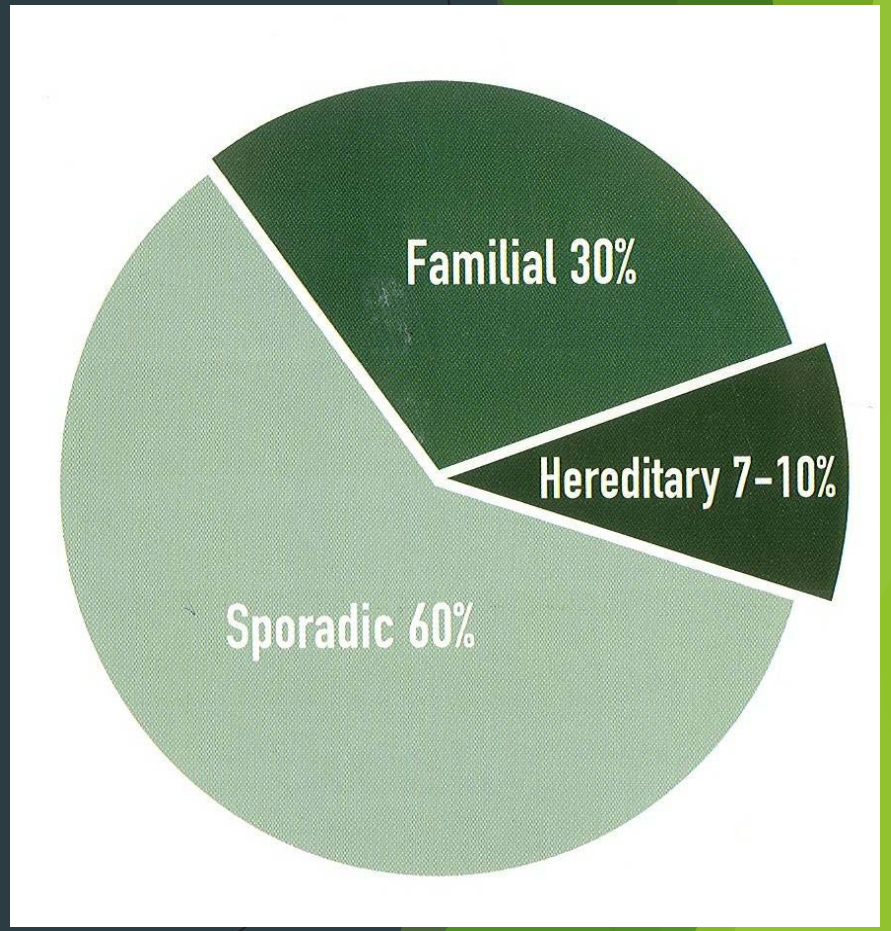
The damaged cell reproduces into more control-less cells and a tumor develops

Further loss of control results in spread away from the original cell's location (metastatic disease)

Therefore all cancers are "genetic" (DNA etiology) but not all cancers are hereditary

In every sense, then:

CANCER IS A GENETIC DISEASE



Cancer Etiologies

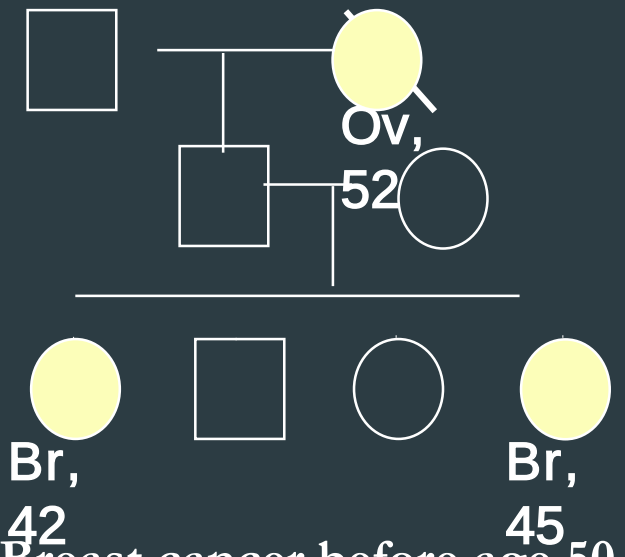
- **Sporadic cancer (60%)**
  - "By chance"
  - Environmental insults & aging damages the DNA within a given cell
- **Familial cancer (30%)**
  - Multifactorial
  - Several genes (not typically known) *in combination with* several environmental factors
  - These multiple factors will tend to be shared by family members thereby moderately increasing risk
- **Hereditary cancer (10%)**
  - Cancer that develops due to the person INHERITING a single gene that is mutated
  - The mutated gene is in *every* cell of the body & dramatically increase the risk to develop cancer over the lifetime
  - Genes have roles in certain parts of the body & therefore a mistake will increase the risk of not just any cancer but cancer in the corresponding area
  - Because the predisposition is inherited, environment & age play less of a role & cancer typically develops earlier than usual

Hereditary Breast Cancer Syndromes

- **Hereditary Breast & Ovarian Cancer Syndrome (HBOC)**
  - Breast <50, ovarian, male breast, pancreatic, melanoma & prostate <50
- Cowden Syndrome—PTEN gene
  - Both malignant and benign tumors of the breasts, uterus, thyroid, and skin; benign macrocephaly
- Li Fraumeni—p53 gene
  - Young breast cancer (20s), childhood adrenocortical tumors, sarcomas, leukemia, etc

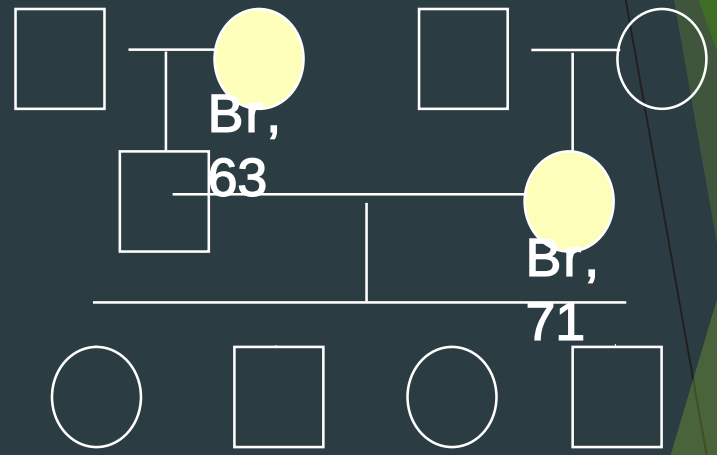
Family History of HBOC

Hereditary



- Breast cancer before age 50
- Ovarian cancer at any age
- Male Breast Caner
- Bilateral Breast Cancer
- Ashkenazi ancestry

Sporadic



- None of the breast cancer is diagnosed before age 50
- No ovarian cancer
- No clear pattern on one side of family or other

New Engl J Med 2000;342:564-571

BRCA1/2 Mutations Increase the Risk of Early-Onset Breast Cancer

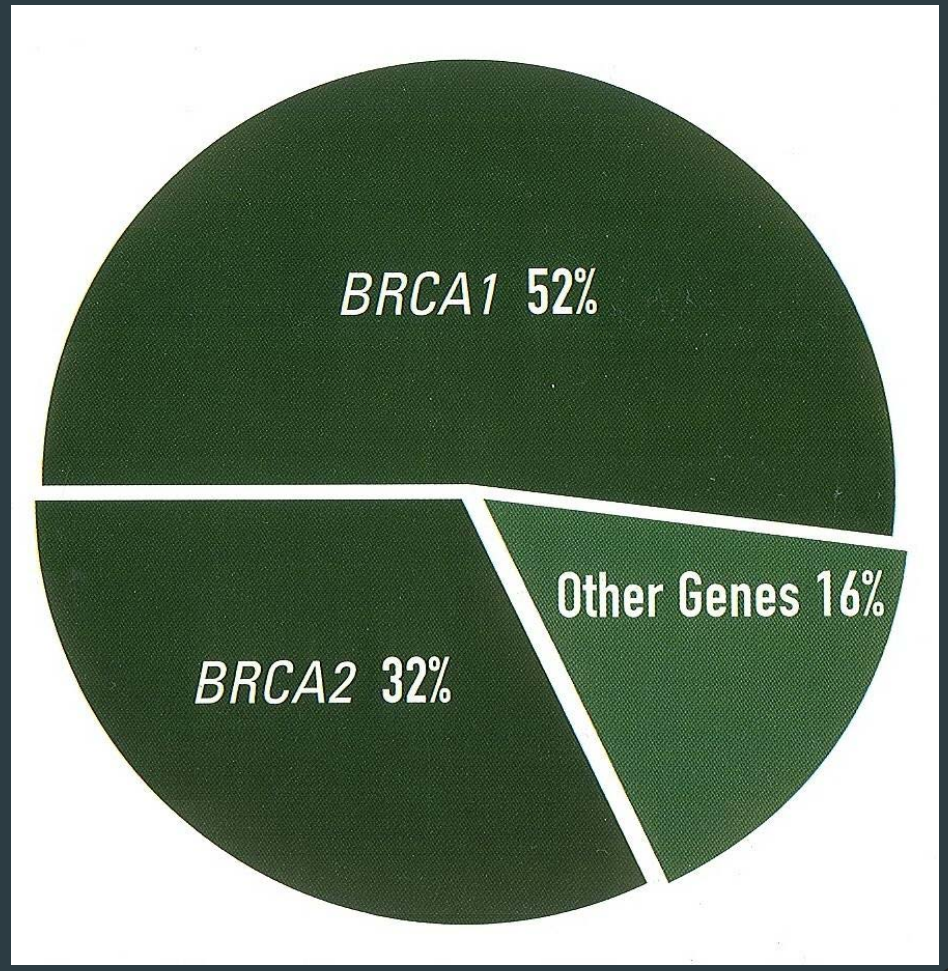
	By age 40	By age 50	Lifetime
			
Population Risk	**0.5%**	**2%**	**12%**
Hereditary Risk	**10%-20%**	**33%-50%**	**56%-87%**

BRCA1/2 Mutations Increase the Risk of Ovarian Cancer

Lifetime

Population Risk	1.8%
Hereditary Risk	**~44% (*BRCA1*)** **27% (*BRCA2*)**

Hereditary Breast & Ovarian Cancer Syndrome



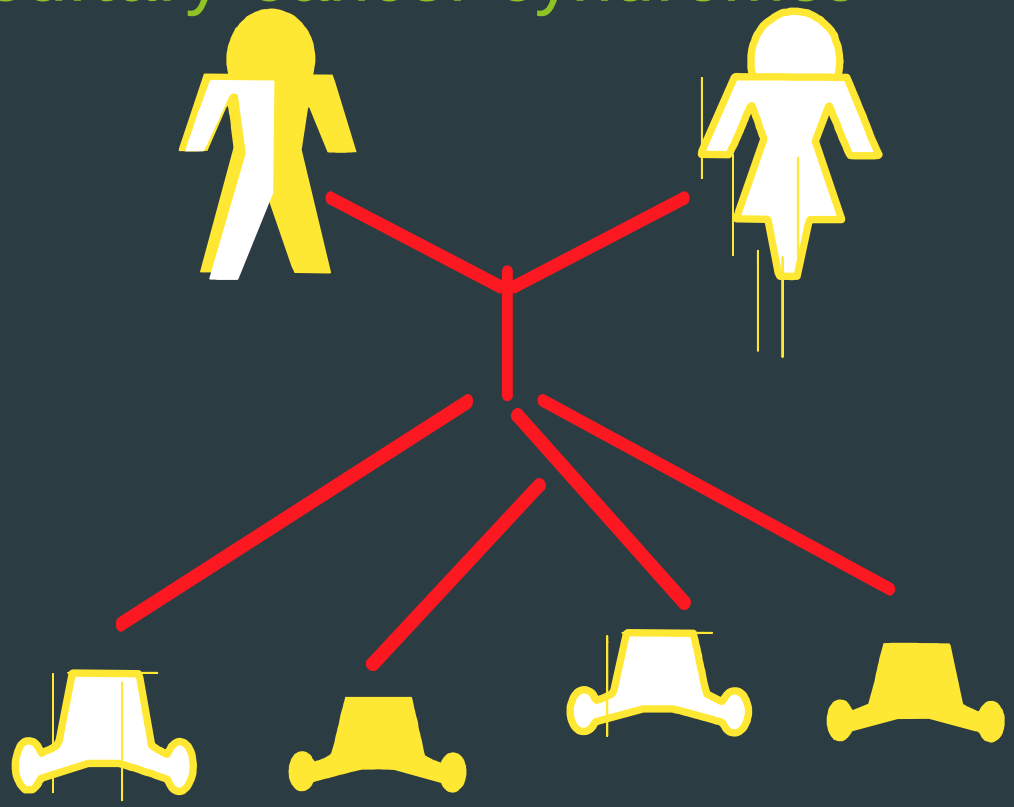
BRCA1/2 Mutations Increase the Risk of a Second Primary Breast Cancer

	First 5 yrs from dx	Lifetime
		
Population Risk	**5%**	**11%**
Hereditary Risk	**12%-20%**	**40%-62%**

Beyond Females, Breasts, & Ovaries

Cancer Site	General Pop Risk	BRCA Risk
Male Breast	<1%	7%
Prostate	15%	20%
Melanoma	1%	2-4%
Pancreatic	1%	2-4%

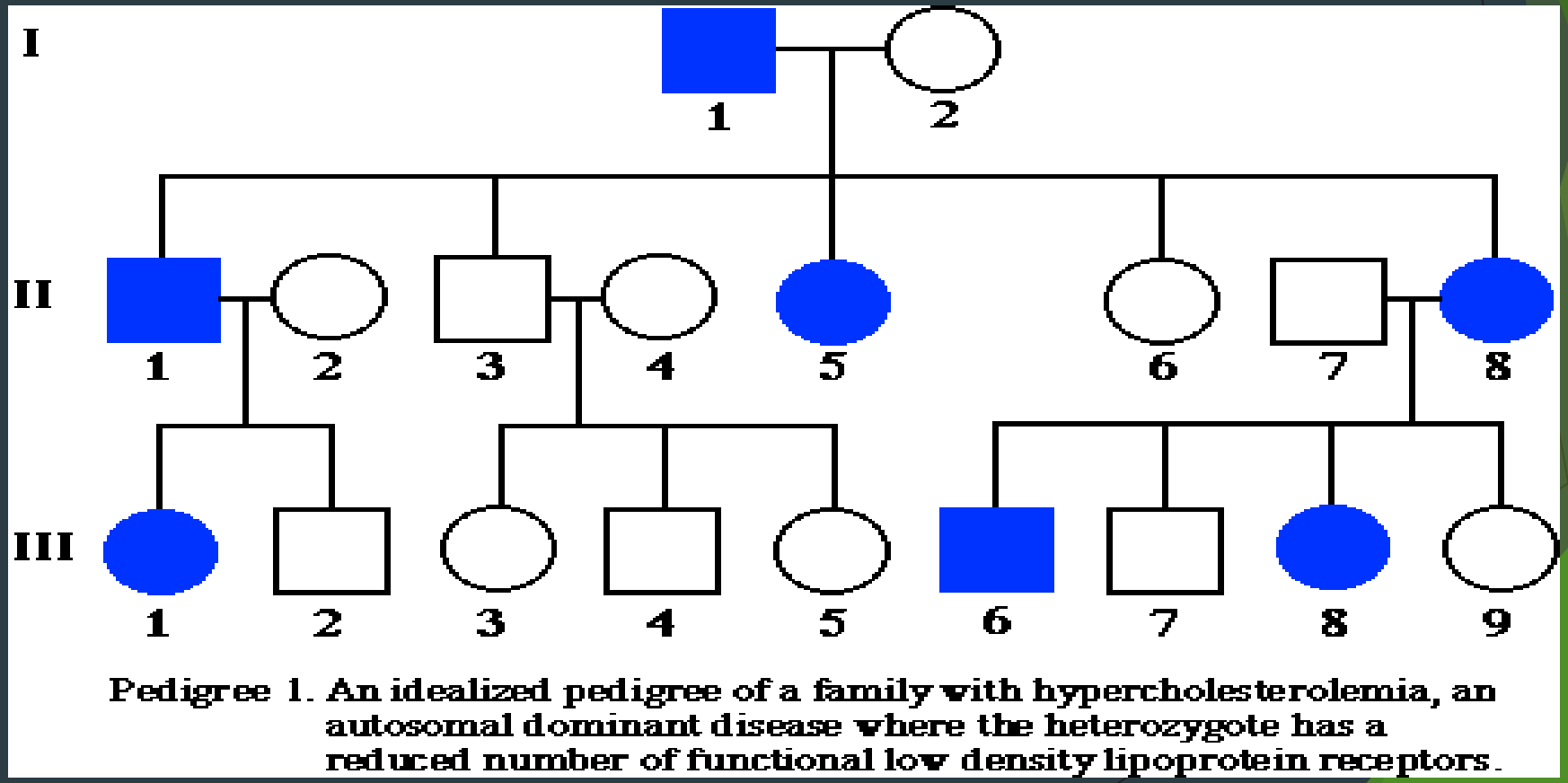
Inheritance of Hereditary Cancer Syndromes



Each child has a ½ or 50% chance of inheriting the same mutation

Inheritance…Who is at risk?

- But it's not just about the patient's children…..



Pedigree 1. An idealized pedigree of a family with hypercholesterolemia, an autosomal dominant disease where the heterozygote has a reduced number of functional low density lipoprotein receptors.

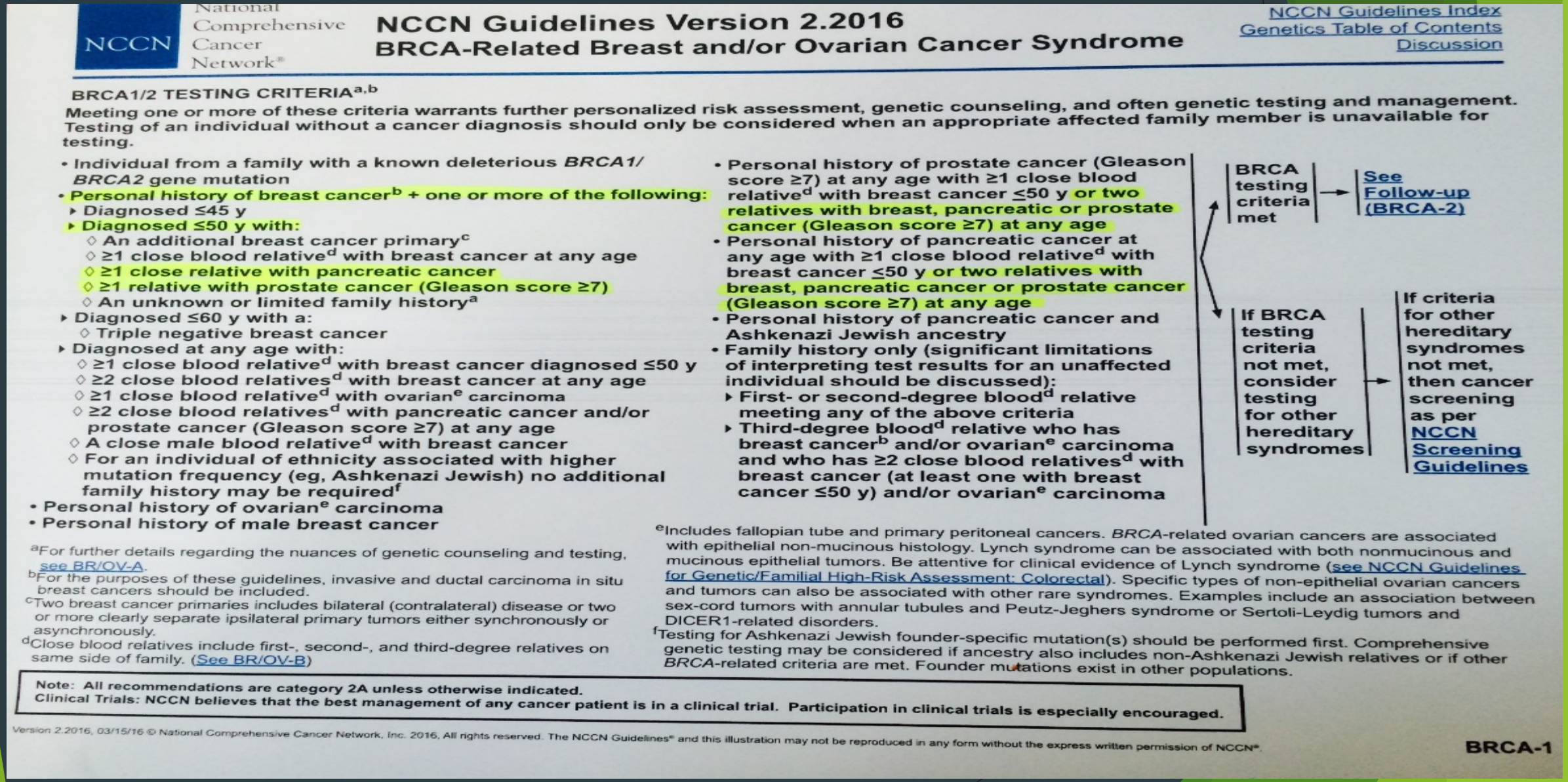
NCCN Guidelines for Medical Management- Breast & Ovarian Cancer Chemoprevention

Cancer	Chemoprevention	Age to Begin	Goal
Breast	Tamoxifen	Variable	Reduces risk by 50%
Ovarian	Oral contraceptives	Variable	Reduces risk by 60%

NCCN Guidelines for Medical Management Breast & Ovarian Cancer Surgical Prevention

Cancer	Surgery	Age to Begin	Goal
Breast	Bilateral Mastectomy	Variable	Reduces risk by 90+%
Breast	Bilateral Salpingo-Oophorectomy	35-40	Reduces risk by 50%
Ovarian	Bilateral Salpingo-Oophorectomy	35-40	Reduces risk by 96+%

BRCA1/2 Testing Criteria-Summer 2016



National Comprehensive Cancer Network®

NCCN Guidelines Version 2.2016
BRCA-Related Breast and/or Ovarian Cancer Syndrome

NCCN Guidelines Index
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Discussion

BRCA1/2 TESTING CRITERIA[a,b]

Meeting one or more of these criteria warrants further personalized risk assessment, genetic counseling, and often genetic testing and management. Testing of an individual without a cancer diagnosis should only be considered when an appropriate affected family member is unavailable for testing.

- Individual from a family with a known deleterious *BRCA1/BRCA2* gene mutation
- Personal history of breast cancer[b] + one or more of the following:
  - Diagnosed ≤45 y
  - Diagnosed ≤50 y with:
    - An additional breast cancer primary[c]
    - ≥1 close blood relative[d] with breast cancer at any age
    - ≥1 close relative with pancreatic cancer
    - ≥1 relative with prostate cancer (Gleason score ≥7)
    - An unknown or limited family history[a]
  - Diagnosed ≤60 y with a:
    - Triple negative breast cancer
  - Diagnosed at any age with:
    - ≥1 close blood relative[d] with breast cancer diagnosed ≤50 y
    - ≥2 close blood relatives[d] with breast cancer at any age
    - ≥1 close blood relative[d] with ovarian[e] carcinoma
    - ≥2 close blood relatives[d] with pancreatic cancer and/or prostate cancer (Gleason score ≥7) at any age
    - A close male blood relative[d] with breast cancer
    - For an individual of ethnicity associated with higher mutation frequency (eg, Ashkenazi Jewish) no additional family history may be required[f]
- Personal history of ovarian[e] carcinoma
- Personal history of male breast cancer
- Personal history of prostate cancer (Gleason score ≥7) at any age with ≥1 close blood relative[d] with breast cancer ≤50 y or two relatives with breast, pancreatic or prostate cancer (Gleason score ≥7) at any age
- Personal history of pancreatic cancer at any age with ≥1 close blood relative[d] with breast cancer ≤50 y or two relatives with breast, pancreatic cancer or prostate cancer (Gleason score ≥7) at any age
- Personal history of pancreatic cancer and Ashkenazi Jewish ancestry
- Family history only (significant limitations of interpreting test results for an unaffected individual should be discussed):
  - First- or second-degree blood[d] relative meeting any of the above criteria
  - Third-degree blood[d] relative who has breast cancer[b] and/or ovarian[e] carcinoma and who has ≥2 close blood relatives[d] with breast cancer (at least one with breast cancer ≤50 y) and/or ovarian[e] carcinoma

BRCA testing criteria met → See Follow-up (BRCA-2)

If BRCA testing criteria not met, consider testing for other hereditary syndromes → If criteria for other hereditary syndromes not met, then cancer screening as per NCCN Screening Guidelines

[a] For further details regarding the nuances of genetic counseling and testing, see BR/OV-A.

[b] For the purposes of these guidelines, invasive and ductal carcinoma in situ breast cancers should be included.

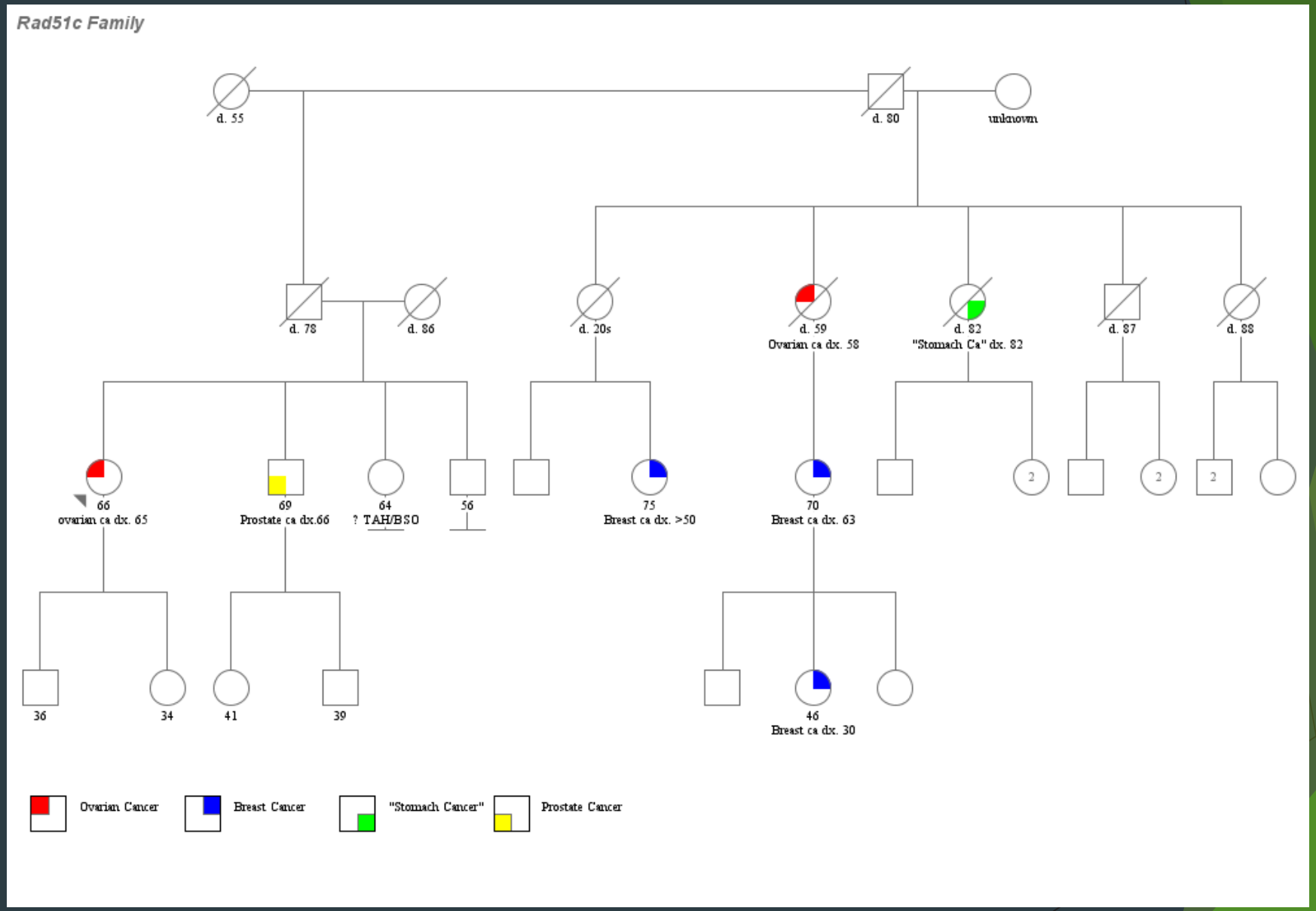
[c] Two breast cancer primaries includes bilateral (contralateral) disease or two or more clearly separate ipsilateral primary tumors either synchronously or asynchronously.

[d] Close blood relatives include first-, second-, and third-degree relatives on same side of family. (See BR/OV-B)

[e] Includes fallopian tube and primary peritoneal cancers. *BRCA*-related ovarian cancers are associated with epithelial non-mucinous histology. Lynch syndrome can be associated with both nonmucinous and mucinous epithelial tumors. Be attentive for clinical evidence of Lynch syndrome (see NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal). Specific types of non-epithelial ovarian cancers and tumors can also be associated with other rare syndromes. Examples include an association between sex-cord tumors with annular tubules and Peutz-Jeghers syndrome or Sertoli-Leydig tumors and DICER1-related disorders.

[f] Testing for Ashkenazi Jewish founder-specific mutation(s) should be performed first. Comprehensive genetic testing may be considered if ancestry also includes non-Ashkenazi Jewish relatives or if other *BRCA*-related criteria are met. Founder mutations exist in other populations.

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


Rad51c Family
d. 55
d. 80
unknown
d. 78
d. 86
d. 20s
d. 59
Ovarian ca dx. 58
d. 82
"Stomach Ca" dx. 82
d. 87
d. 88
66
ovarian ca dx. 65
69
Prostate ca dx.66
64
? TAH/BSO
56
75
Breast ca dx. >50
70
Breast ca dx. 63
2
2
2
36
34
41
39
46
Breast ca dx. 30
Ovarian Cancer
Breast Cancer
"Stomach Cancer"
Prostate Cancer

History

- Patient had genetic counseling and based on the Penn II model, the patient had a 59% chance to have a mutation in either BRCA1 or BRCA2
  - Patient elected for full sequencing and rearrangement studies of BRCA1 and BRCA2 which were negative, NO MUTATION DETECTED
- Based on a significant risk for a hereditary breast and ovarian cancer syndrome in this family, patient elected the multigene panel testing

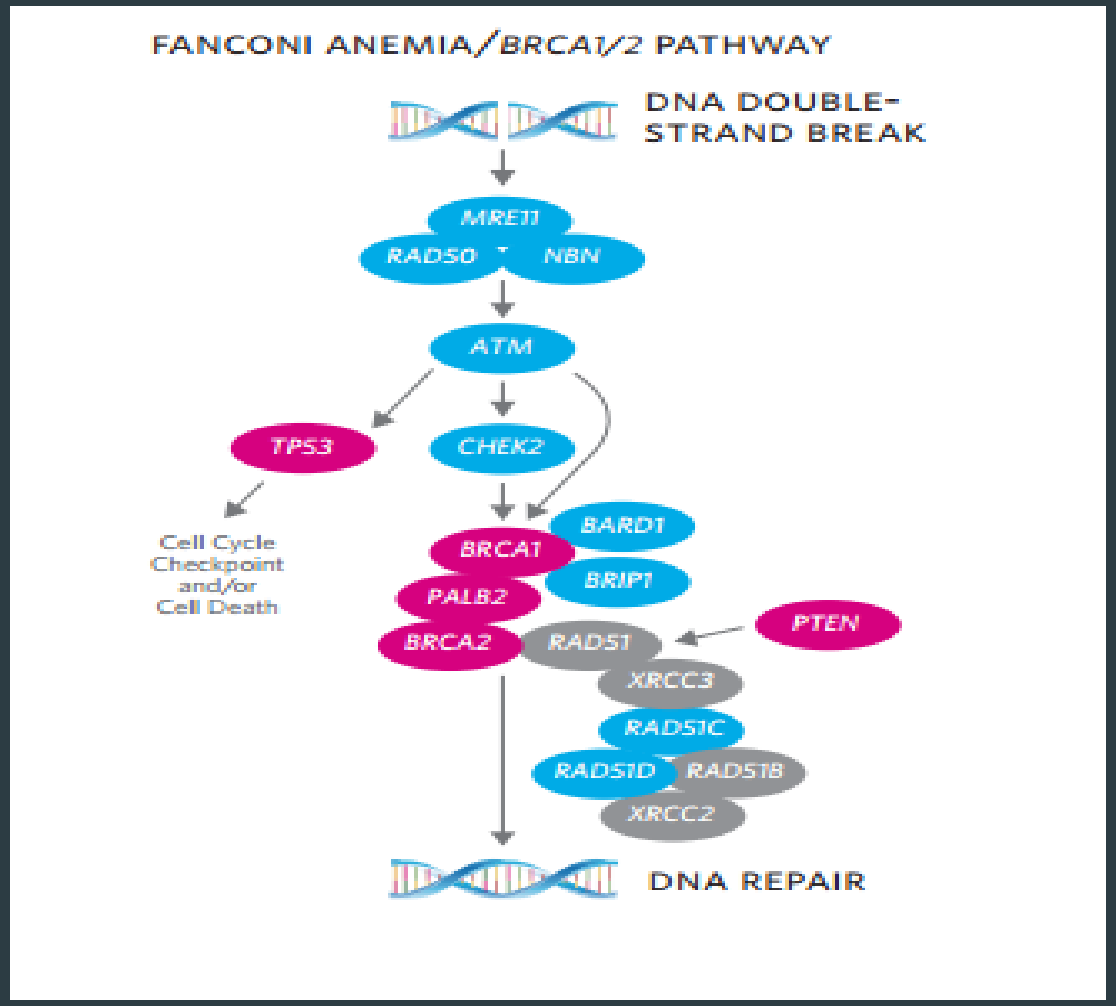
“OvaNext” Panel

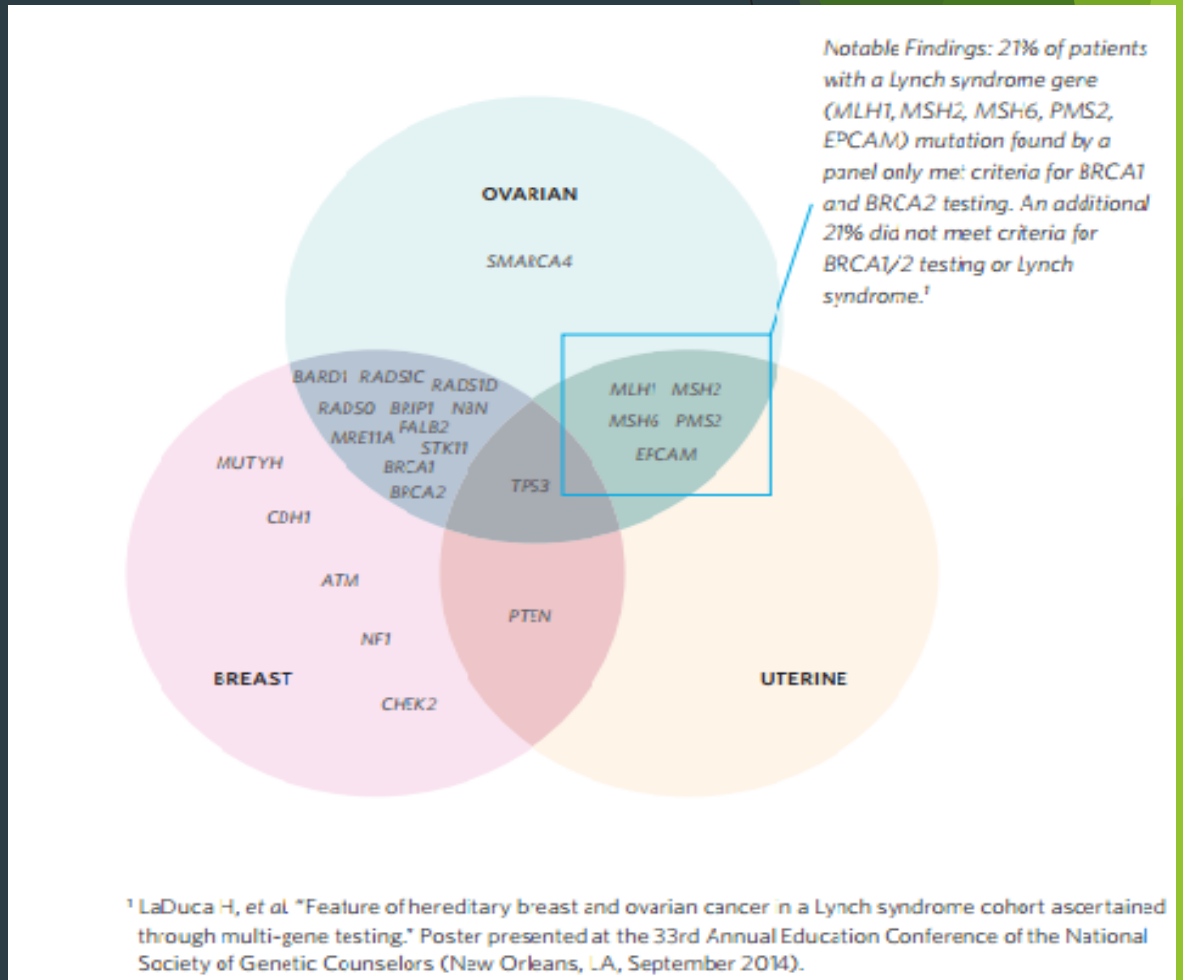
- The test employed is a next generation sequencing panel that simultaneously analyzes 19 genes that contribute to increased risk for breast, ovarian, and/or uterine cancers
  - Genes include:
    - *ATM, BARD1, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, STK11,* and *TP53*
- There are dozens of different panels which contain different combinations of genes with different TATs and costs; most are underwriter approved

Results from panel test (Ovanext)

- The patient was found to have a germline deleterious mutation in the RAD51C gene!!!
- RAD51C:
  - *RAD51C* along with *BARD1, BRIP1, MRE11A, NBN, and RAD50* are genes involved in the Fanconi anemia (FA)–BRCA pathway, which is critical for DNA repair by homologous recombination and interact in vivo with *BRCA1* and/or *BRCA2*
  - Mutations in RAD51C is expected to increase the risk of breast cancer 3 fold with a lifetime risk of breast cancer of ~36% and a 5.88 RR with an up to an 11% risk of ovarian cancer

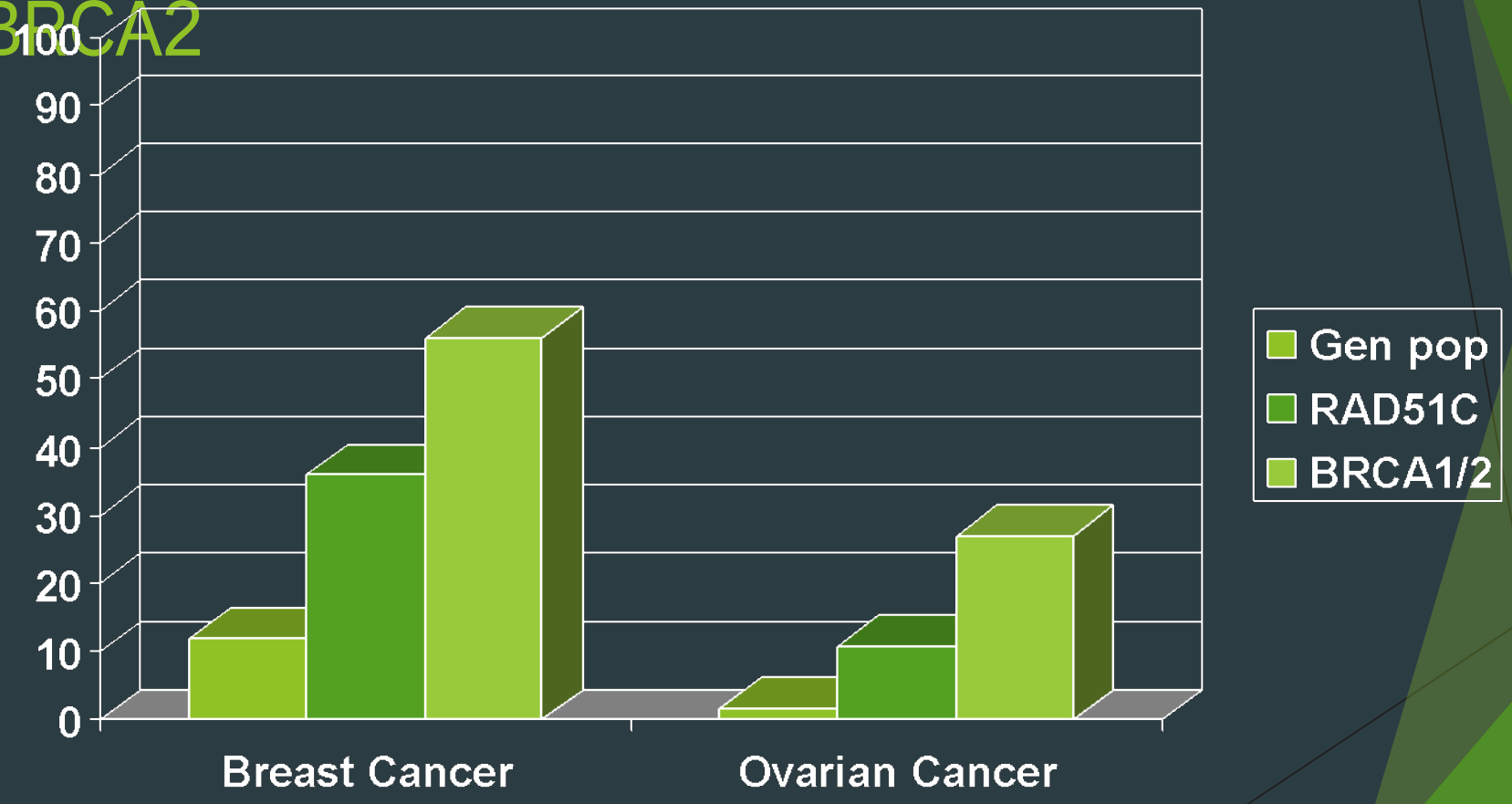
Multi-gene panel testing for hereditary cancer



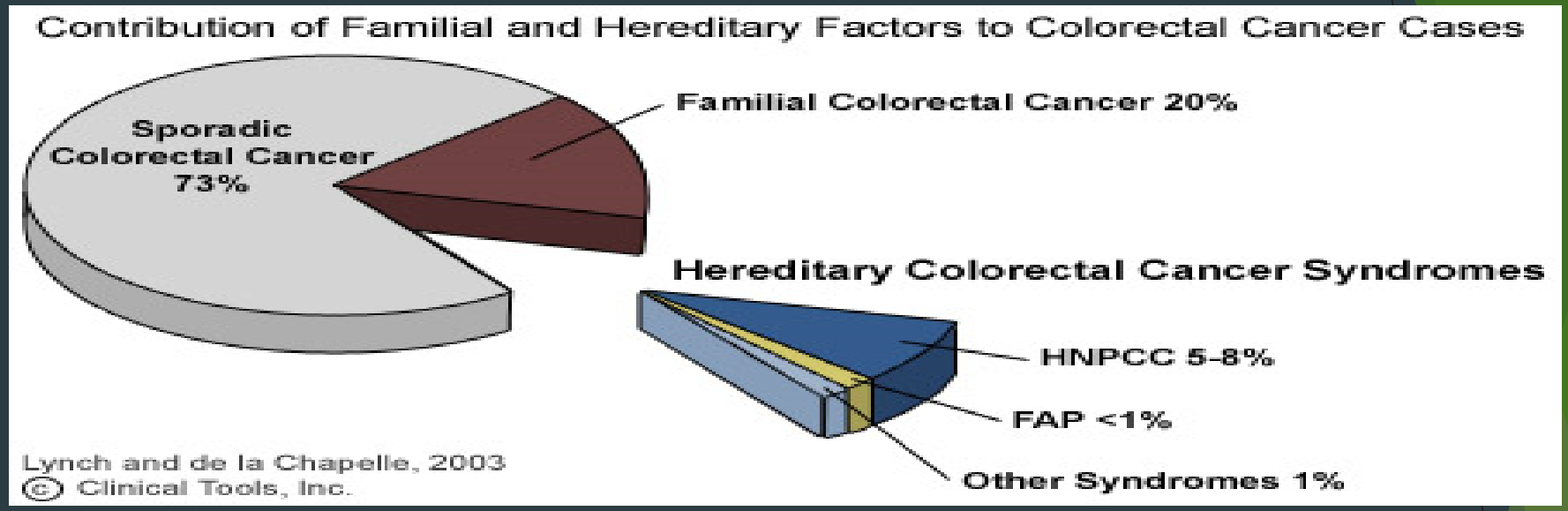


[1] LaDuca H, *et al.* "Feature of hereditary breast and ovarian cancer in a Lynch syndrome cohort ascertained through multi-gene testing." Poster presented at the 33rd Annual Education Conference of the National Society of Genetic Counselors (New Orleans, LA, September 2014).

Breast & Ovarian Cancer Risks for the General Population vs. RAD51C vs. BRCA1/BRCA2



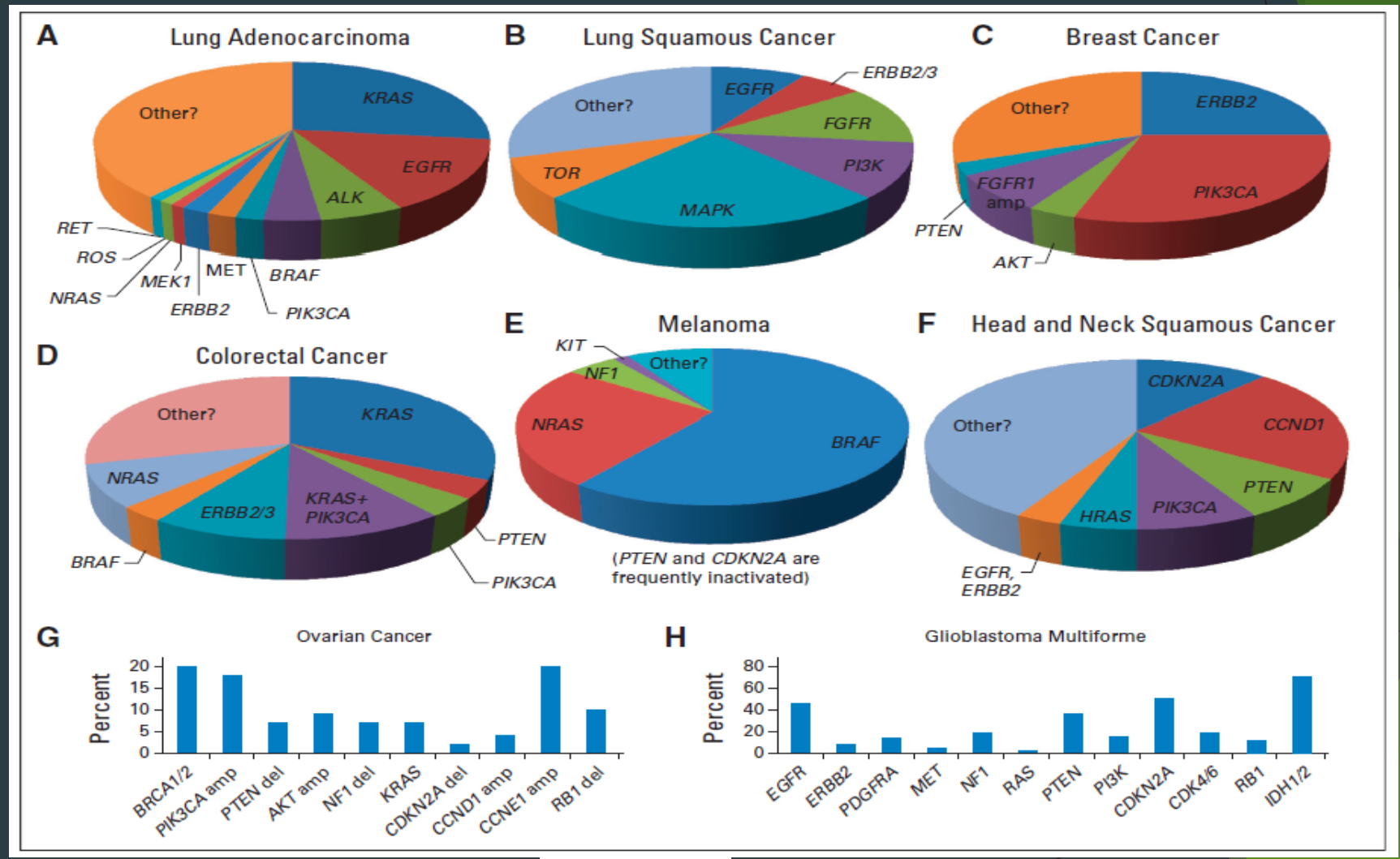
Hereditary Colorectal Cancer Syndromes



- **Polyposis**
  - **Familial Adenomatous Polyposis (FAP)**
    - Turcot & Gardner syndrome
    - Attenuated Familial Adenomatous Polyposis (AFAP)
  - Peutz Jeghers Syndrome:
    - Colon, breast, ovarian, pancreatic, sex cord tumors, gastric, mucocutaneous pigmentation
  - MYH Associated Polyposis (MAP)
    - AR polyposis (~55 polyps); increased risk of colon ca; age of onset 48-56

- ***Diagnosis relies primarily on clinical findings***

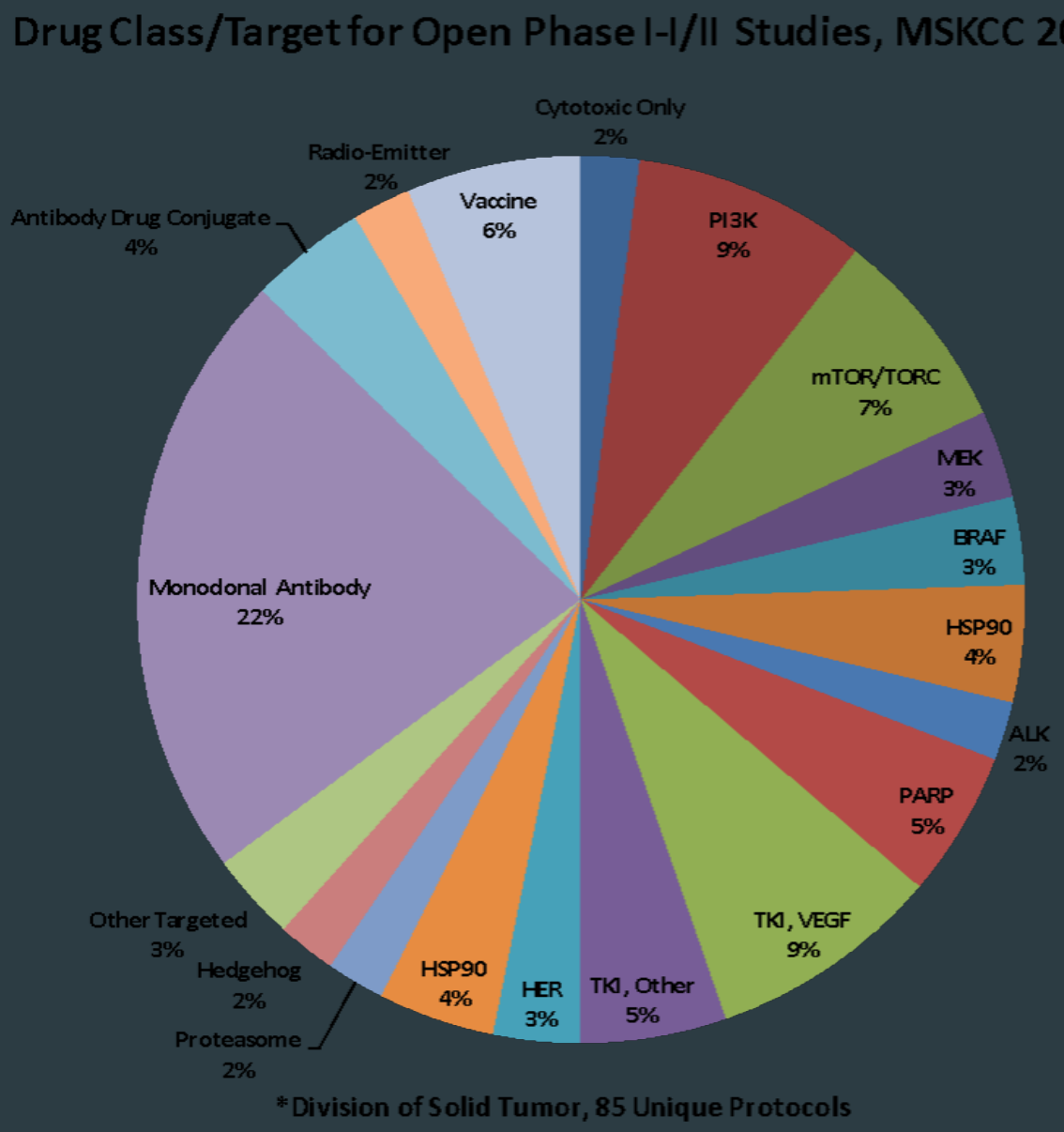
Cancer therapy is changing—bigtime!!



Levi A. Garraway

J Clin Oncol 31:1806-1814.

Targeted therapy is here (to stay?)



- 19% PI3K/MAPK inhibitors
- 14% RTK inhibitors
- 26% monoclonal or ADCs
- Only 2% cytotoxic alone
- Many genomic targets are low-prevalence (<5%) in disease(s) of interest

Created by David Hyman, MD, MSKCC

Cancer Genomics

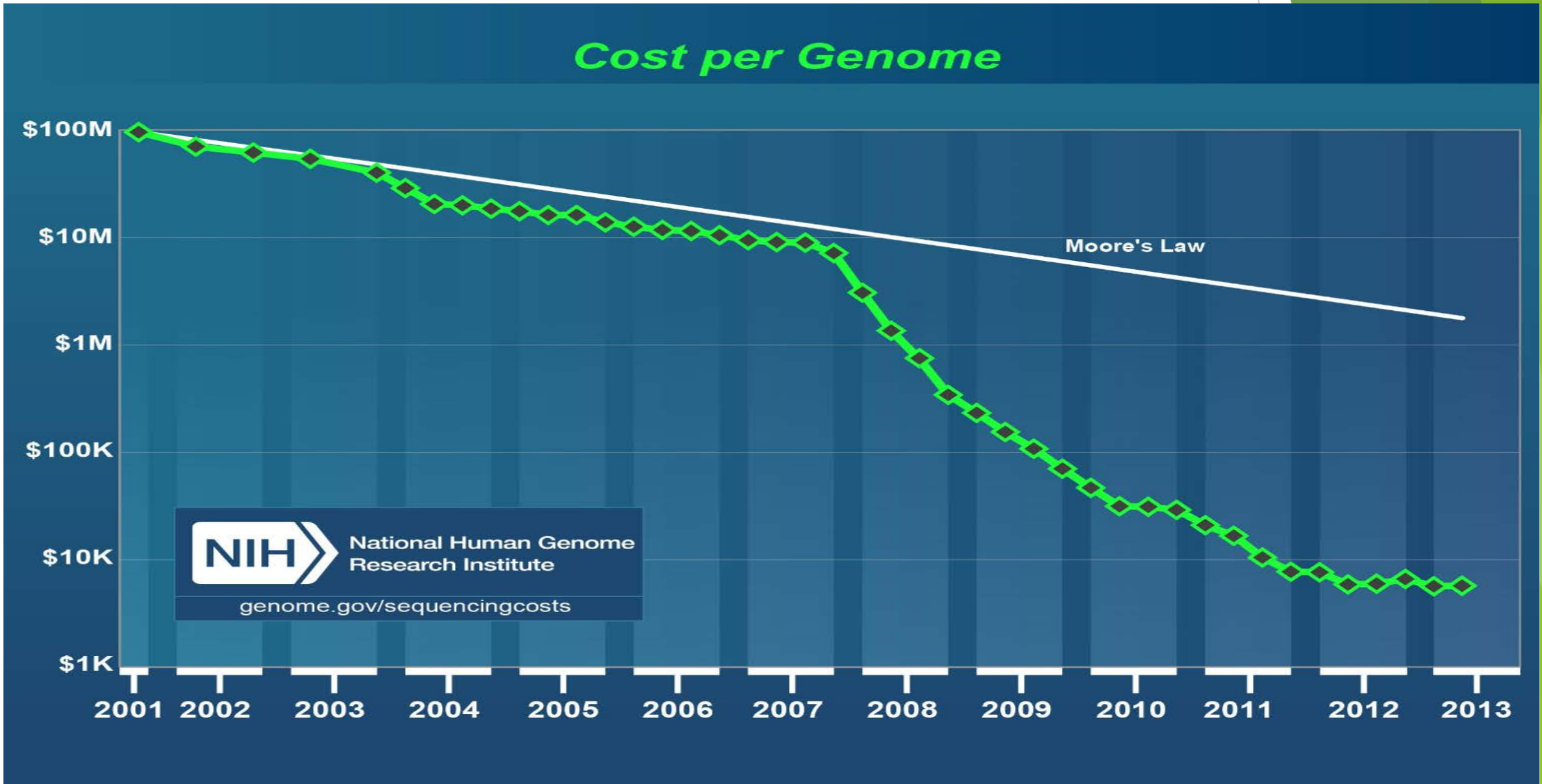
- Determining Genetically Compatible Therapies to Guide Patient Treatment
  - September 23-24 in Boston, MA
  - 250+ attendees: oncologists, surgeons, geneticists, hospital and health plan administrators, underwriters, basic researchers, information tech specialists, academic, corporate and private practice
  - 4 invited poster presentations (including ours)
  - Technologies: couple genomics with risk assessment, diagnosis, treatment/therapies and surveillance

mycancergenome.org

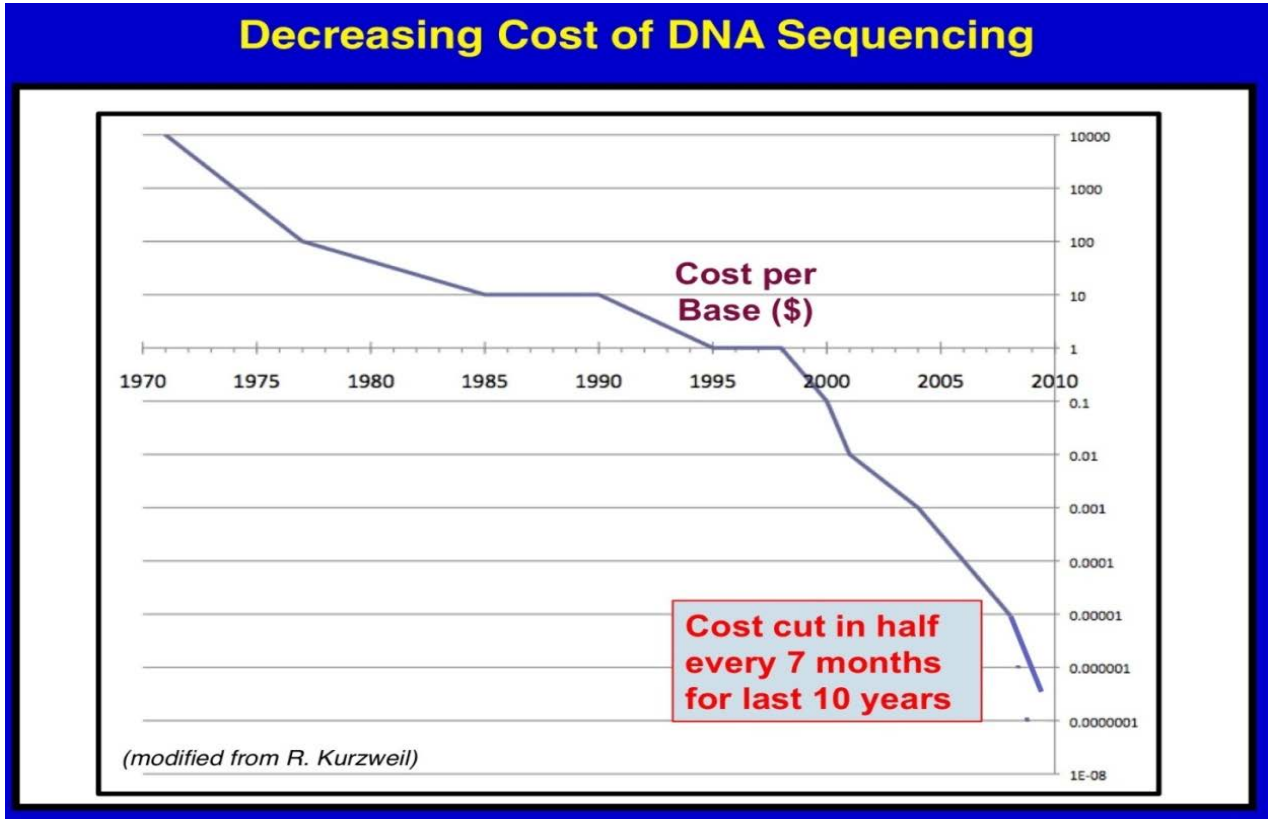
- Check out this website!!!

Cell Free DNA and its application to Cancer

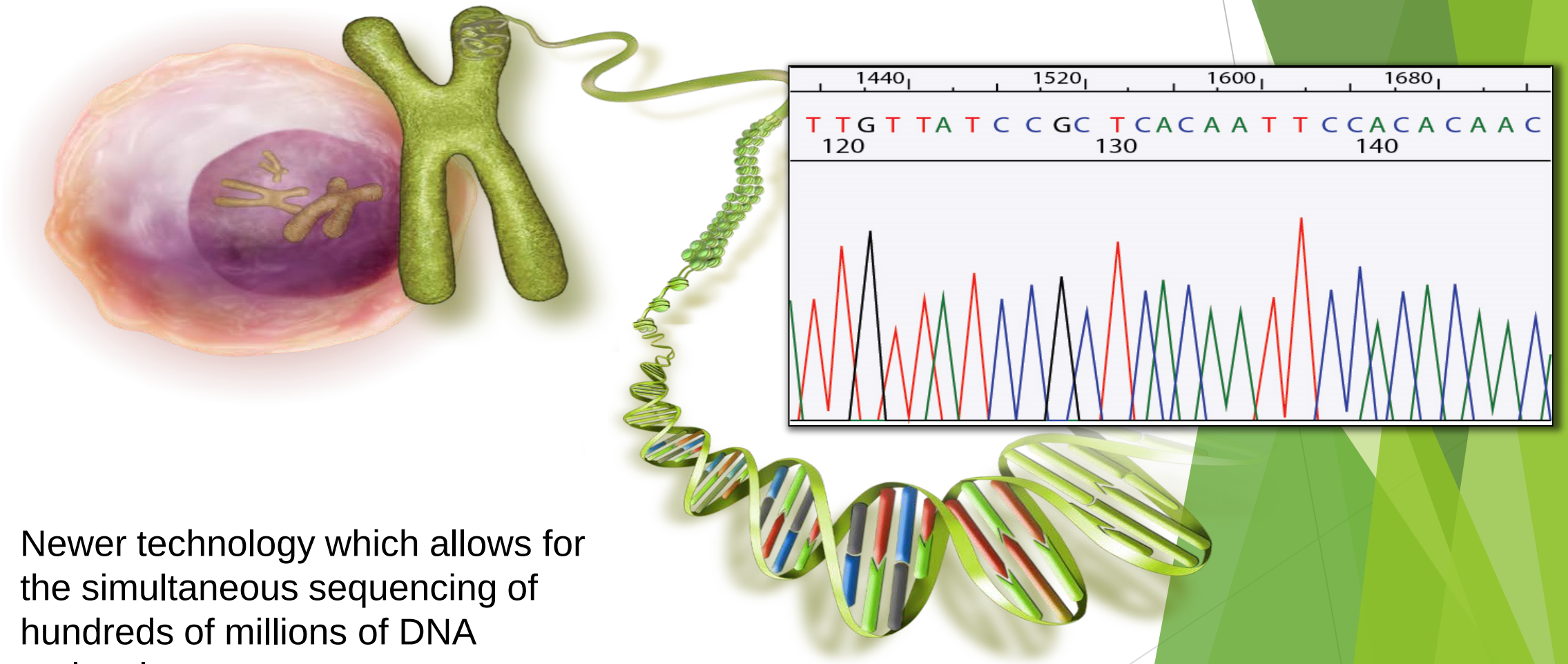
Next Generation DNA sequencing



Next Generation DNA sequencing



Next-Generation Sequencing (NGS)



- Newer technology which allows for the simultaneous sequencing of hundreds of millions of DNA molecules at once.

Next Generation DNA sequencing

1. Library preparation

Sequencing library is prepared by random fragmentation of DNA or cDNA sample, followed by 5' and 3' adapter ligation. This greatly increases the efficiency of the preparation and later amplification process. Adapter ligated fragments are then PCR amplified and gel purified

2. Cluster generation

Library is loaded into a flow cell where fragments are captured on a lawn of surface-bound or microsphere-bound oligos complementary to library adapters. Fragments are then amplified into distinct, clonal clusters through (bridge) amplification.

3. Sequencing

Sequencing by synthesis uses a terminator based method that detects single bases as they are incorporated into DNA template strands. As all 4 dNTP's are present in the reaction mix during the sequencing cycle, error rates are minimized resulting in highly accurate base-by-base data with minimal sequence context errors, even in areas of repetition and homopolymers.

4. Data analysis

Sequences can't be "read" and analyzed until fragment data is aligned to a reference genome . Depth of coverage determines quality of data and "meaningfulness" of the generated sequence.

AT EACH STEP ERRORS CAN OCCUR AFFECTING DNA SEQUENCE'S CLINICAL UTILITY!!!

Next Generation DNA sequencing

- Illumina sequencing by synthesis cartoon
- https://www.youtube.com/watch?v=womKfikWlxM

The Problem Today:

- **“MISREPRESENTATION OF A MISUNDERSTOOD TECHNOLOGY TO THE MISINFORMED”**

Monday December 07, 2015 *Dna Kit Predicts Health Issues*