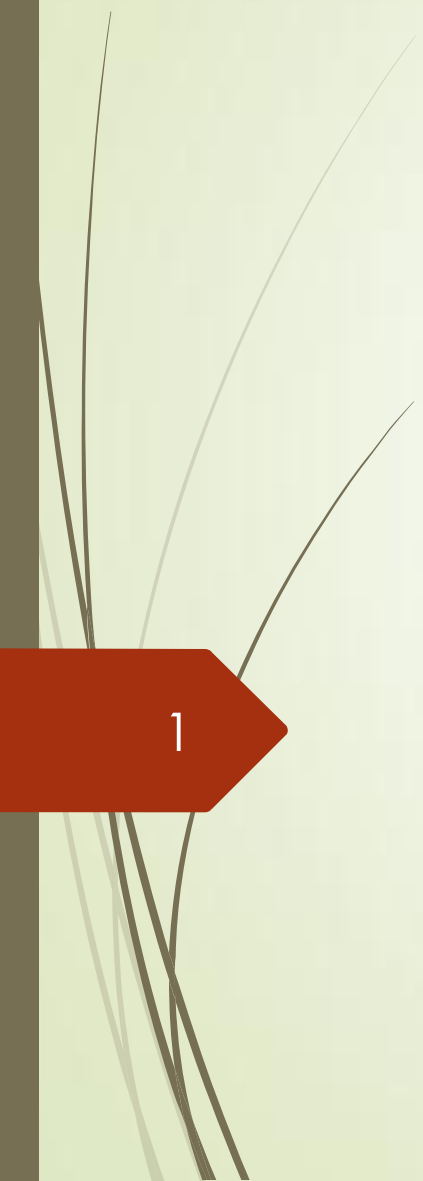
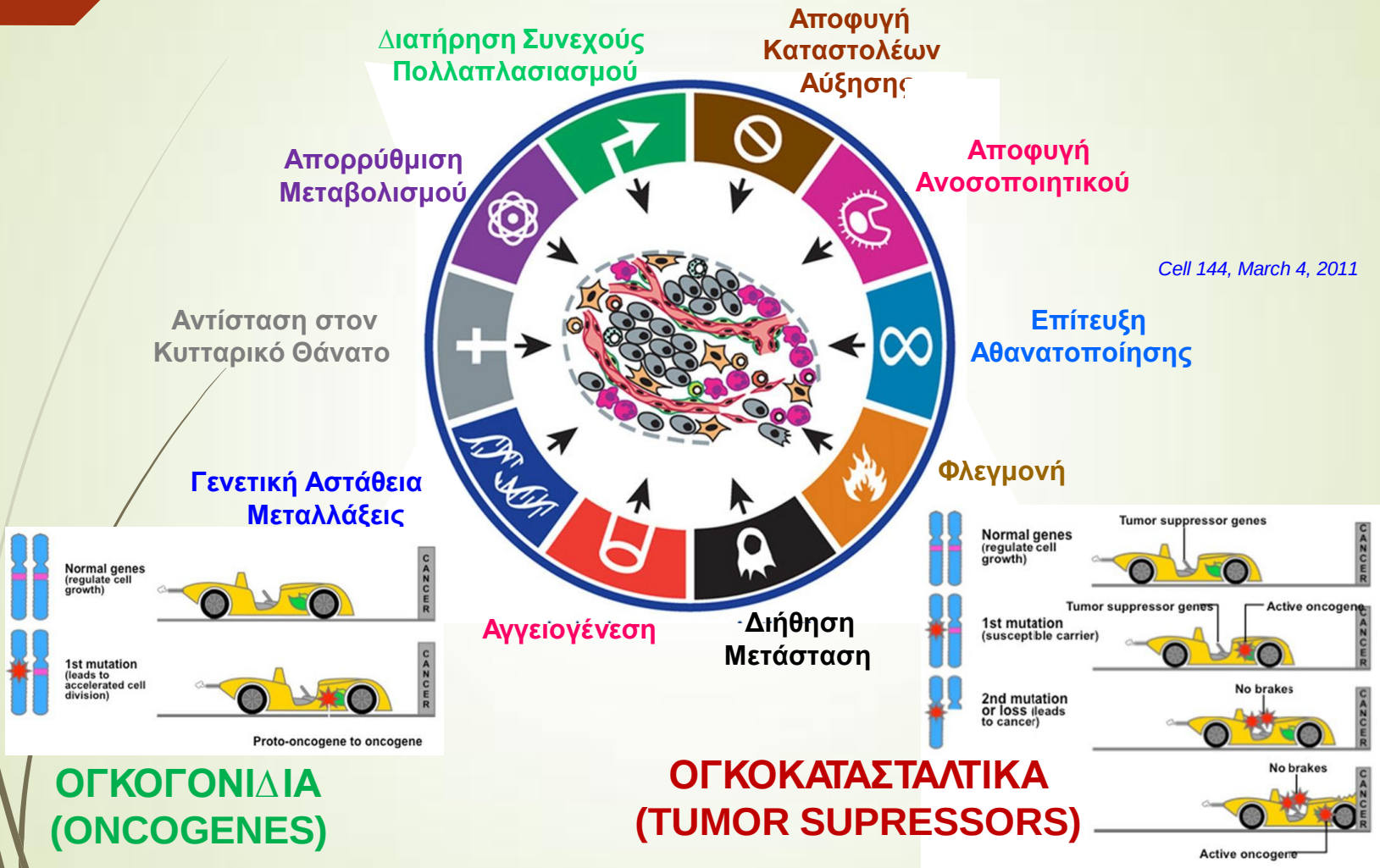


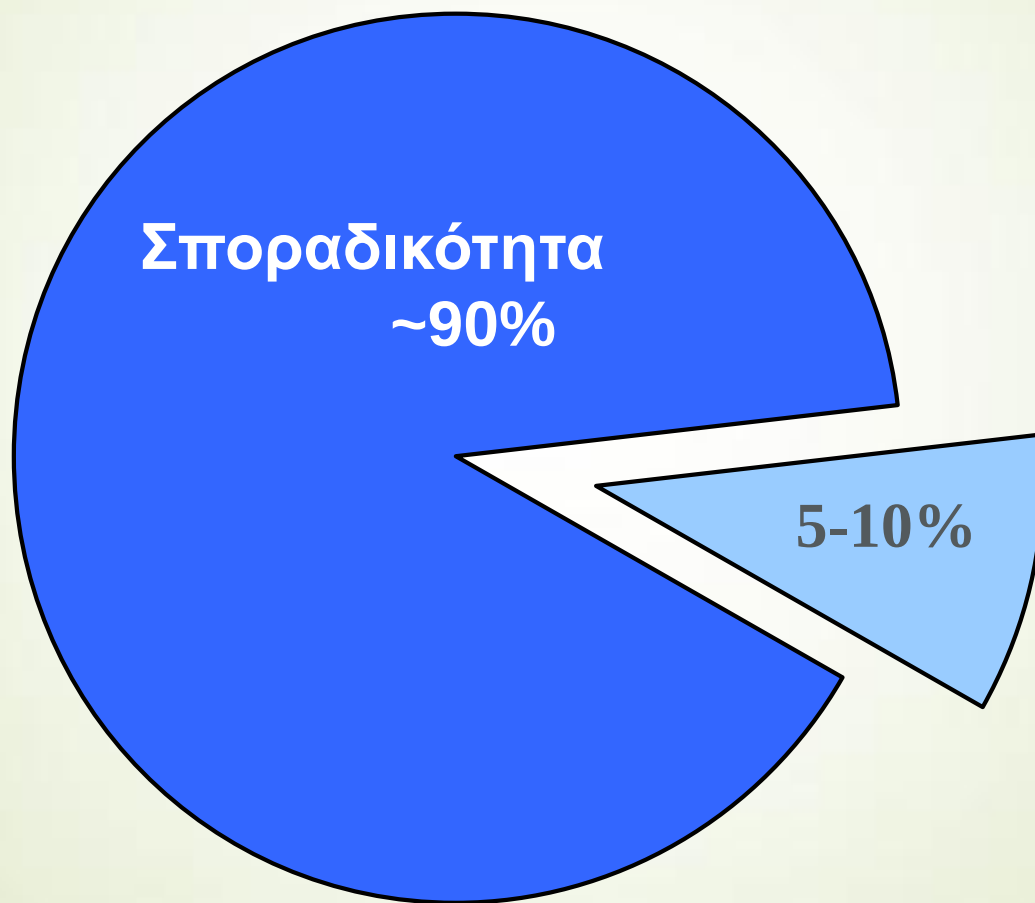


ΜΟΡΙΑΚΟ ΠΡΟΦΙΛ ΤΟΥ ΟΓΚΟΥ ΟΓΚΟΓΟΝΙΔΙΑ ΟΔΗΓΟΙ ΚΛΗΡΟΝΟΜΟΥΜΕΝΟΙ ΚΑΡΚΙΝΟΙ

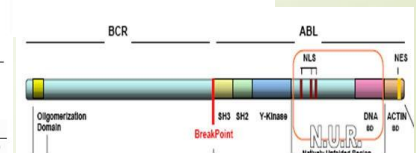
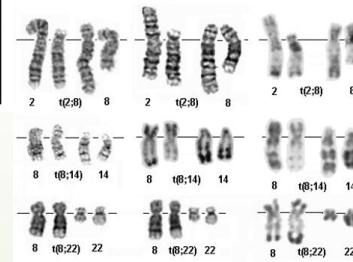
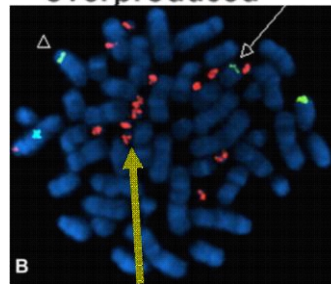
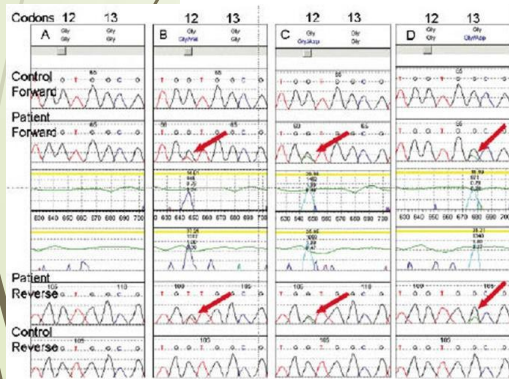
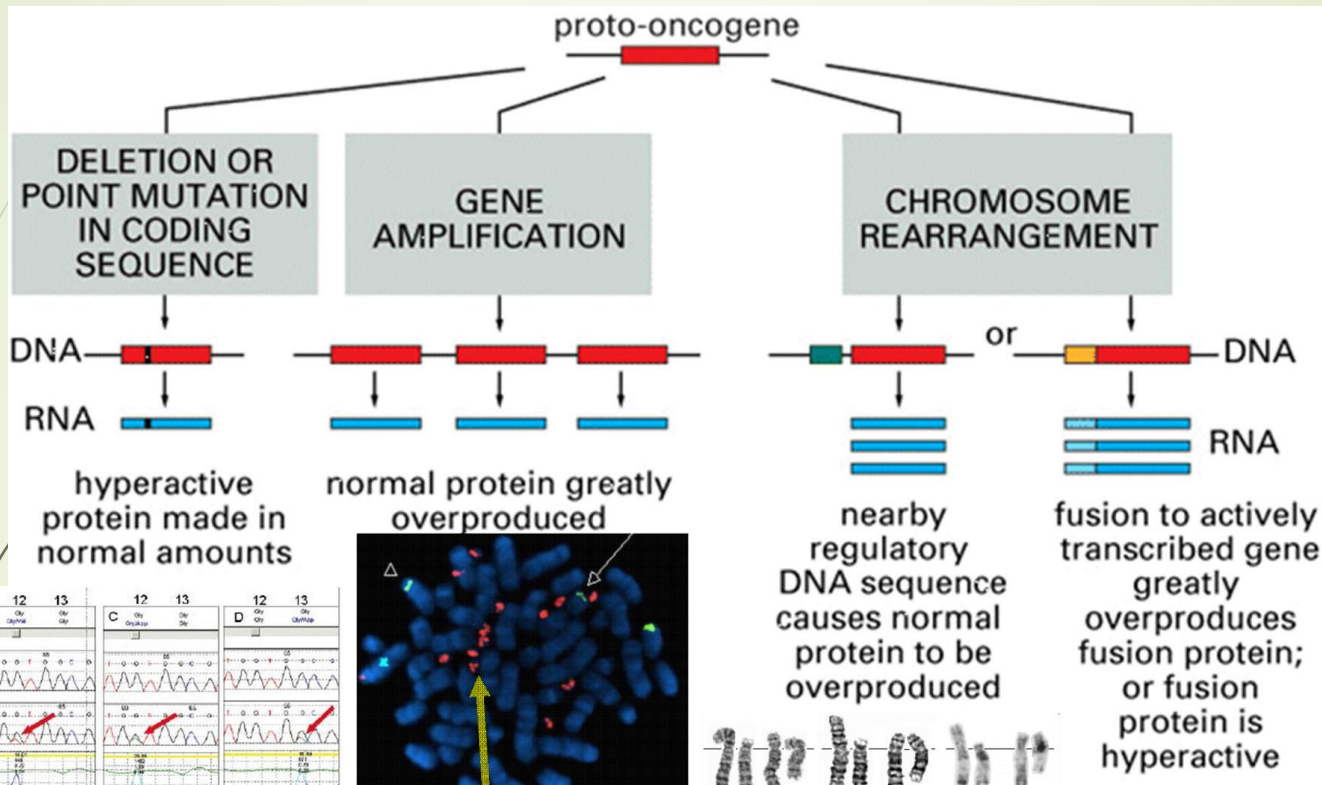
ΚΑΡΚΙΝΙΚΟΣ ΦΑΙΝΟΤΥΠΟΣ

2





Τρόποι Ενεργοποίησης Ογκογονιδίων



ΠΟΤΕ BRCA;

Αντζελίνα Τζολί φαινόμενο



National
Comprehensive
Cancer
Network®

NCCN Guidelines Version 1.2017 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:
 - ◊ Triple negative breast cancer
 - ▶ Diagnosed at any age with:
 - ◊ ≥2 close blood relatives with breast cancer, pancreatic cancer, or prostate cancer (Gleason score ≥7) at any age
 - ◊ ≥1 close blood relative^d with breast cancer diagnosed ≤50 y
 - ◊ ≥1 close blood relative^d with ovarian^e carcinoma
 - ◊ 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 ovarian carcinoma at any age or 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 ovarian carcinoma at any age or 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
- *BRCA1/2* mutation detected by tumor profiling in the absence of germline mutation analysis
- 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](#)

^eIncludes fallopian tube and primary peritoneal cancers. *BRCA*-related ovarian cancers are associated

Oncogenes and cell death - apoptosis

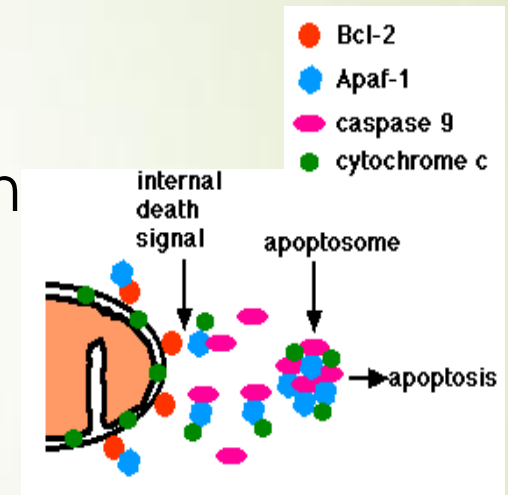
6

A variety of environmental stimuli can lead to the activation of apoptosis or programmed cell death. Cancers - characterized by ↓ rates of apoptosis.

DNA damage, growth factor withdrawal + oncogene activation
heat shock
+ activation of death receptors →
activation of caspase cascade
and cell death.

Cancers - not ↑ cell proliferation - ↓ rate of cell death.

PASG -Proliferation-associated SNF2-like gene= SNF2 family of proteins = DNA dependent ATPases involved in transcription, recombination, and DNA repair (↓ activity of other genes by methylation, or modifying histones.



SNF - sucrose non-fermenter

Pancreatic carcinomas point mutation

Human pancreatic carcinomas mostly show a loss of part of chrom. 18.

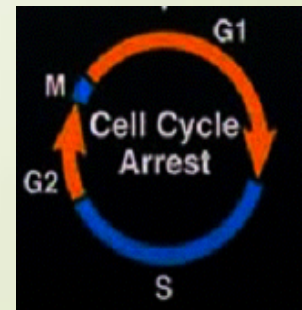
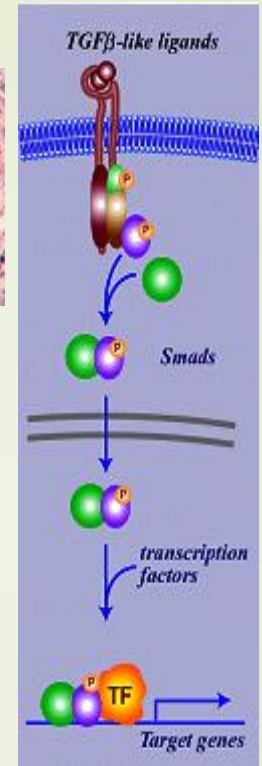
Point-mutations are supposed - in the K-RAS and INK4A (proto-oncogenes), p53 and DPC4 /SMAD4 (anti-oncogenes).

There is a whole family of SMAD proteins, cytoplasmic signaling proteins, all involved in signal transduction of TGF- β (transforming growth factor β) related pathways.

Main alteration - interplay with the cell cycle.

All proteins (K-RAS, INK4a, p53 and TGF- β) regulate directly or indirectly G1 progression, $\rightarrow$ to E2F dependent S phase entry.

DPC- deleted in pancreatic cancer; SMAD -"Sma-2,, (Mothers Against Decapentaplegic) in *Caenorhabditis elegans* + *Drosophila* mad protein



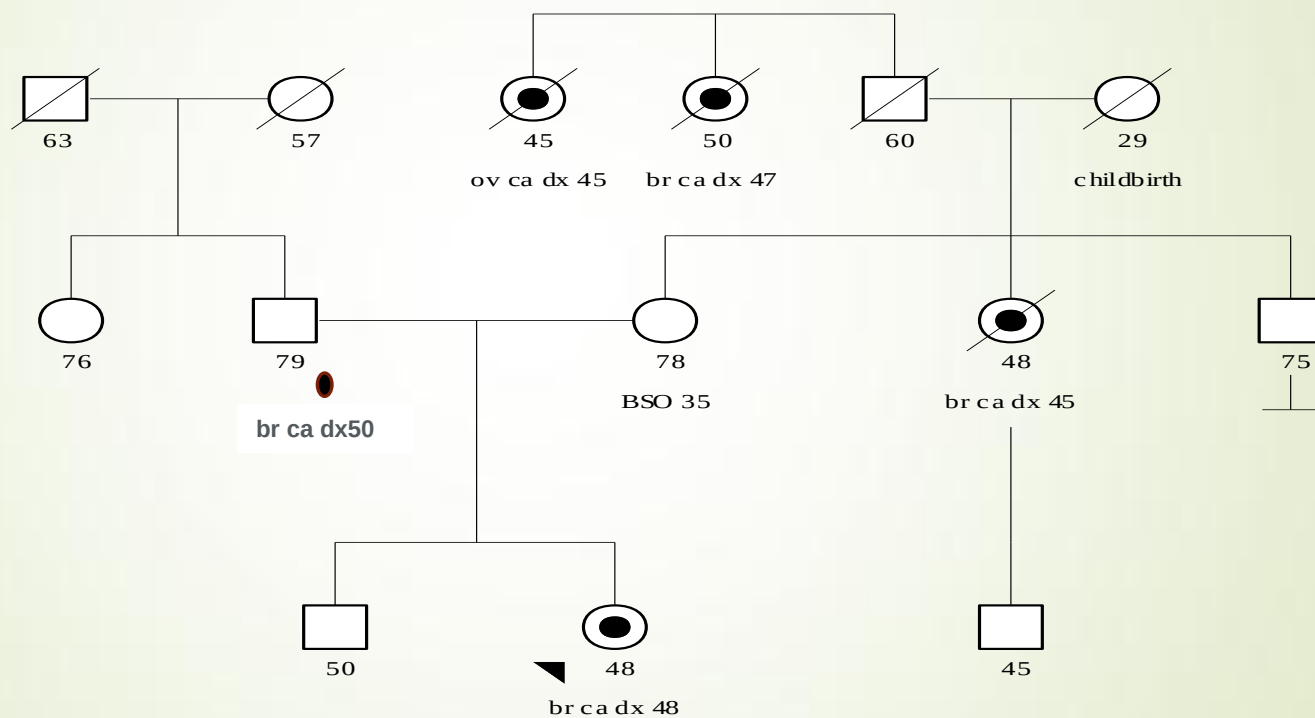
CONFIDENTIAL

Comprehensive BRCAAnalysis®
BRCA1 and BRCA2 Analysis Result

**MYRIAD.****Test Results and Interpretation****NO MUTATION DETECTED**

<u>Test Performed</u>	<u>Result</u>	<u>Interpretation</u>
BRCA1 sequencing	No Mutation Detected	No Mutation Detected
5-site rearrangement panel	No Mutation Detected	No Mutation Detected
BRCA2 sequencing	No Mutation Detected	No Mutation Detected

2008



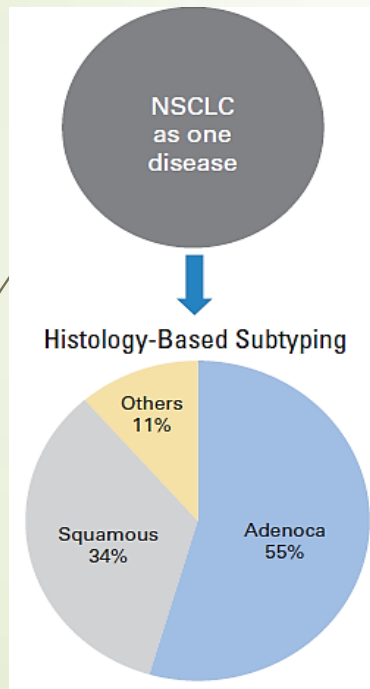
BRCA θετικός

Σύνδρομα κληρονομικής προδιάθεσης

- Γνωστά >50 σύνδρομα κληρονομικής προδιάθεσης για καρκίνο
- Τα περισσότερα κληρονομούνται κατά τον επικρατή χαρακτήρα
- Γαμετικές (germline) μεταλλάξεις
- De novo γαμετικές μεταλλάξεις (δεν ανιχνεύονται στους γονείς)
- Αυξάνουν την προδιάθεση για περισσότερους του ενός καρκίνου.

Traditional Classification and Treatment of NSCLC

Classification



Treatment: “One-Size-Fits All” Approach



BRCA Deletion/Duplication Results: ΝΕΕΣ ΤΕΧΝΙΚΕΣ

CONFIDENTIAL



AMENDED REPORT

BRCAAnalysis[®] Rearrangement Test
Full Gene BRCA1-BRCA2 Large Rearrangement Analysis Result



Test Results and Interpretation

POSITIVE FOR A DELETERIOUS MUTATION

<u>Test Performed</u>	<u>Result</u>	<u>Interpretation</u>
BRCA1 full gene rearrangement	No Mutation Detected	No Mutation Detected
BRCA2 full gene rearrangement	Exon 16 Rearrangement	Deleterious

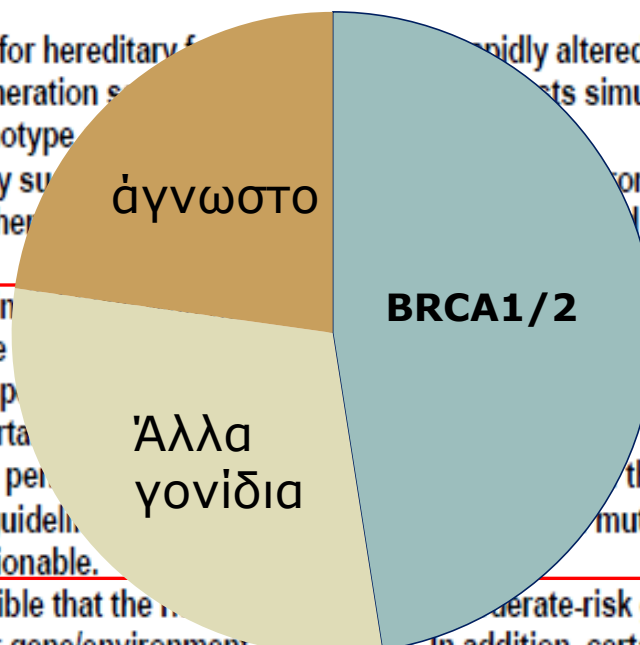
NCCN Guidelines Version 1.2017

Genetic/Familial High-Risk Assessment: Breast and Ovarian

MULTI-GENE TESTING

Overview of multi-gene testing

- The recent introduction of multi-gene testing for hereditary cancer susceptibility has rapidly altered the clinical approach to testing at-risk patients and their families. Based on next-generation sequencing, these tests simultaneously analyze a set of genes that are associated with a specific family cancer phenotype.
- Patients who have a personal or family history suggestive of a specific cancer syndrome are most appropriately managed by genetic testing for that specific syndrome. When a specific cancer syndrome is not identified, then multi-gene testing may be more efficient and/or cost-effective.
- There may be a role for multi-gene testing in individuals whose personal or family history remains suggestive of a specific cancer syndrome but for whom a specific gene mutation has not been identified.
- As commercially available tests differ in the specific genes included, the specific laboratory and test panel is important.
- Multi-gene testing can include “intermediate” penetrance genes, for which the degree of cancer risk and there are no clear guidelines for management. multi-gene tests are necessarily clinically actionable.
- As is the case with high-risk genes, it is possible that the risk associated with moderate-risk genes may not be entirely due to that gene alone, but may be influenced by gene/gene or gene/environment interactions. In addition, certain mutations in a gene may pose higher or lower risk than other mutations in that same gene. Therefore, it may be difficult to use a known mutation alone to assign risk for relatives.
- In many cases the information from testing for moderate penetrance genes does not change risk management compared to that based on family history alone.
- Mutations in many breast cancer susceptibility genes involved in DNA repair may be associated with rare autosomal recessive conditions.
- There is an increased likelihood of finding variants of unknown significance when testing for mutations in multiple genes.
- It is for these and other reasons that multigene testing is ideally offered in the context of professional genetic expertise for pre- and post-test counseling.

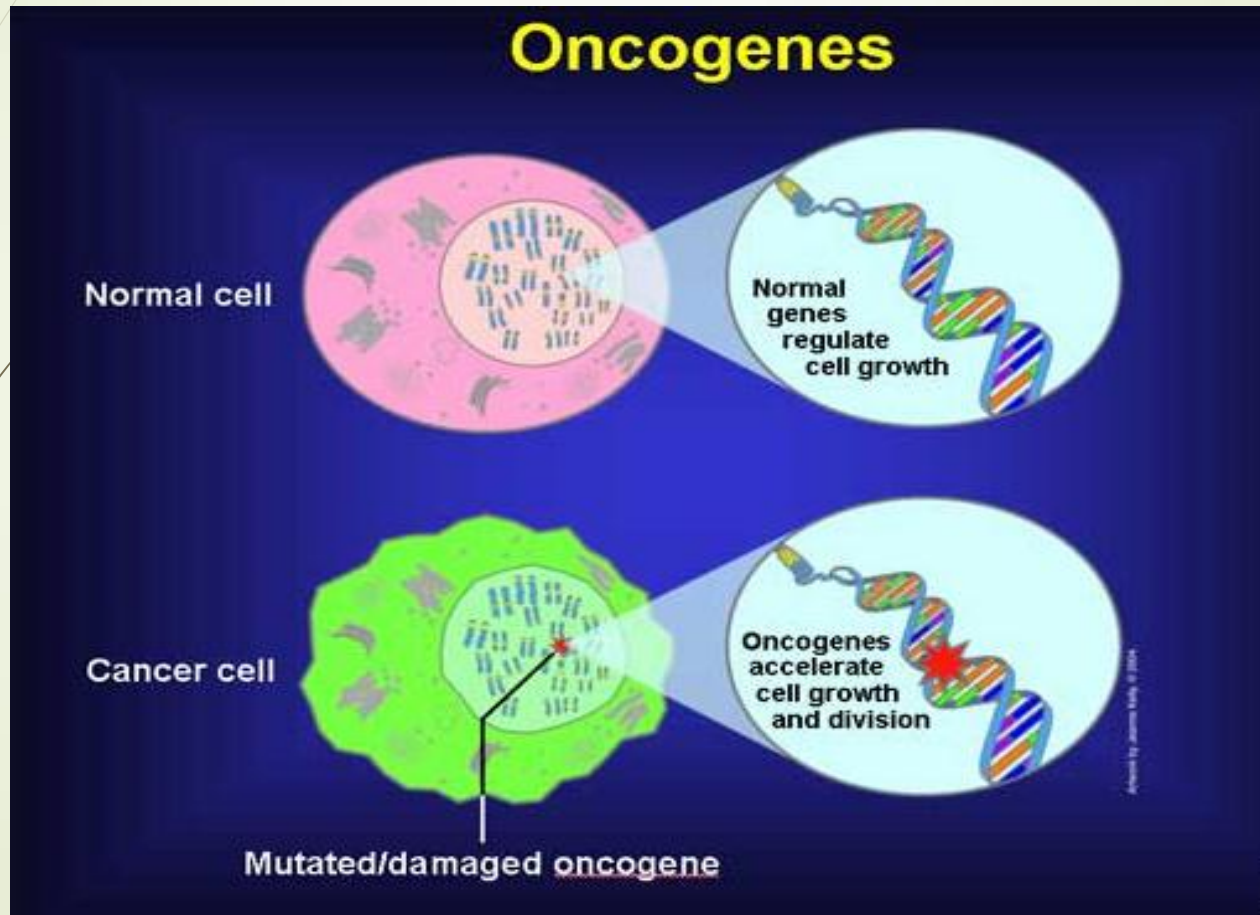


- The normal versions of genes in the first group (whose protein products stimulate or enhance the division and viability of cells)are called proto-oncogenes.
- The mutated or otherwise damaged versions of these genes are called oncogenes.
- The genes in the second group (whose protein products can directly or indirectly prevent cell division or lead to cell death) are called tumour suppressors.

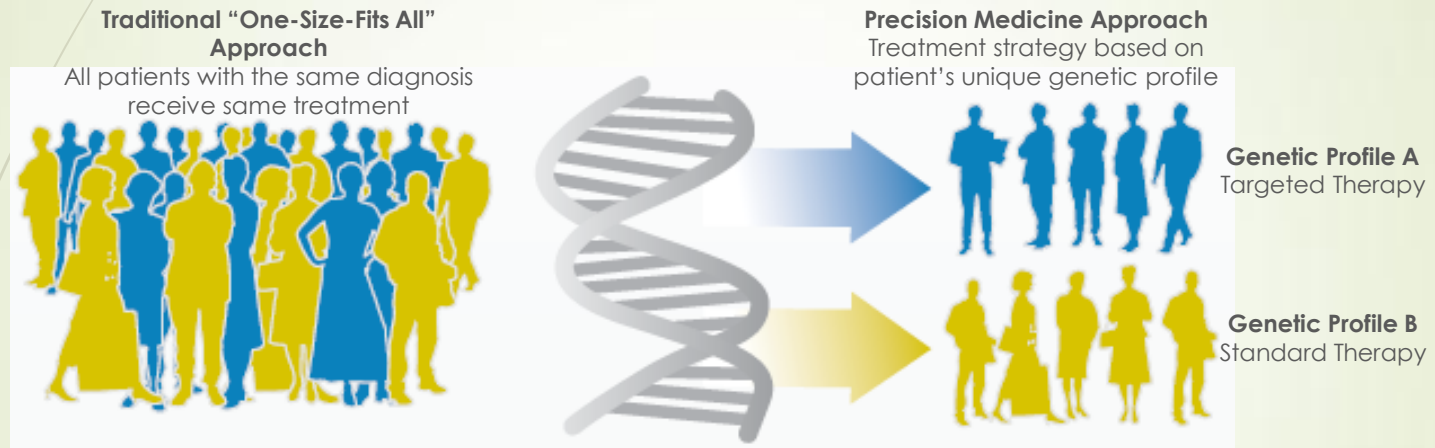
Oncogenes

- Genes associated with the stimulation of cell division.
- Cancers result from only one mutant allele of gene.

Oncogenes



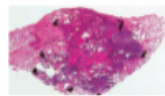
There is a Need for a More Precise Treatment Approach Based on Molecular Features in NSCLC



- Biologic diversity provides opportunities for exploitation of interpatient tumour heterogeneity by **ungrouping a population into molecularly defined subsets** in which mutations and/or abnormal gene expressions drive cancer cell growth and survival and can serve as drug targets¹
- Treatment decisions made based on tumour phenotype and genomic profile hold the promise to **maximize efficacy and minimize toxicity**¹

Revolution in Cancer Molecular Diagnosis Involves High-Throughput Technology

1. Histomorphologic Diagnosis:



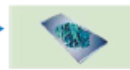
Clinical & Histology-Based Therapy (Compound-Based Therapy):
Use clinicopathologic factors to select available drugs for an individual patient

2. Molecular Diagnosis:

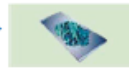
Archival FFPE tumor specimens



Archival cancer specimens



Macro- or microdissection of tumors



Extract tumor nucleic acids:



DNA and RNA

Representative technologies:

Single Biomarker Tests:

- Sanger DNA sequencing or pyrosequencing
- RT-PCR
- FISH
- IHC

Multiplex, Hotspot Mutation Tests:

- PCR-based SNaPshot
- PCR-based Mass Array SNP Sequenom

Initial High-Throughput Technologies:

- SNP/CNV DNA microarray
- RNA microarray
- Epigenetic modifications

Next-Generation Sequencing:

- Whole genome or exome capture sequencing (DNA)
- Whole or targeted transcriptome sequencing (RNA)
- Epigenetic profiling

Current Personalized Medicine (Target-Based Therapy V1.0):
Use single gene-based molecular tests to select specific drugs for an individual patient

Evolving Personalized Medicine (Target-Based Therapy V2.0):
Use multiplexed molecular tests with increased sensitivity and outputs for the therapeutically effective selection of available drugs for an individual patient

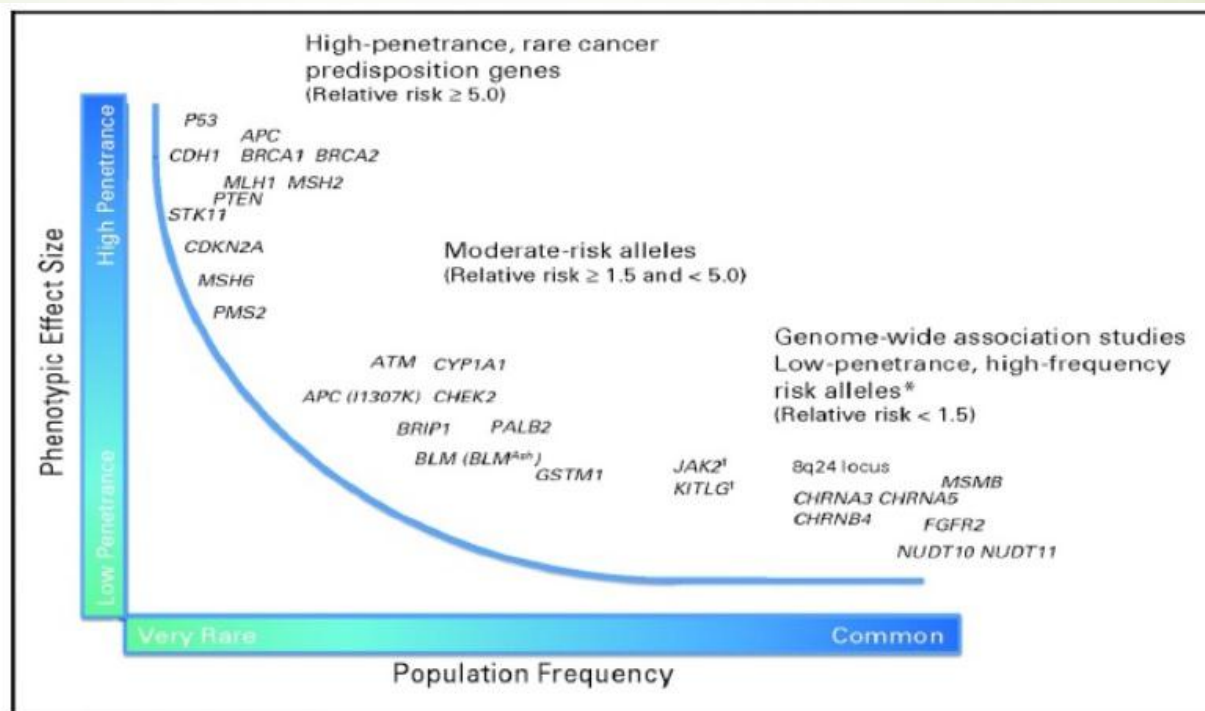
Future Personalized Medicine (Patient-Based Therapy):
Use an integrated genomic profile from high-throughput next-generation sequencing to tailor targeted treatment for an individual patient

The Importance of Validation in Biomarker Development

- Mutations are abundant in cancer cells
- “Driver” mutations are defined as mutations involved in development or progression of a tumour
 - Constitute the minority of mutations
 - A subset of drivers (such as EGFR or ALK) and their component cellular pathways may be “actionable” (i.e. have significant diagnostic, prognostic, or therapeutic implications in subsets of cancer patients and for specific therapies)
- Most mutations do not appear to play a role in cancer progression
 - “Passenger” mutations are indicative of a high mutation rate resulting from carcinogens and DNA instability

- Kras mutated and constitutively active in ~40% of colon cancers
- Large effort has gone into whole genome sequencing of tumors and comparison to non-tumor DNA from same patient
- What are main results?
 - several hundred oncogenes
 - several hundred tumor suppressor genes
 - organized in at least tens of pathways

Διεισδυτικότητα των γονιδίων & συχνότητα στον πληθυσμό

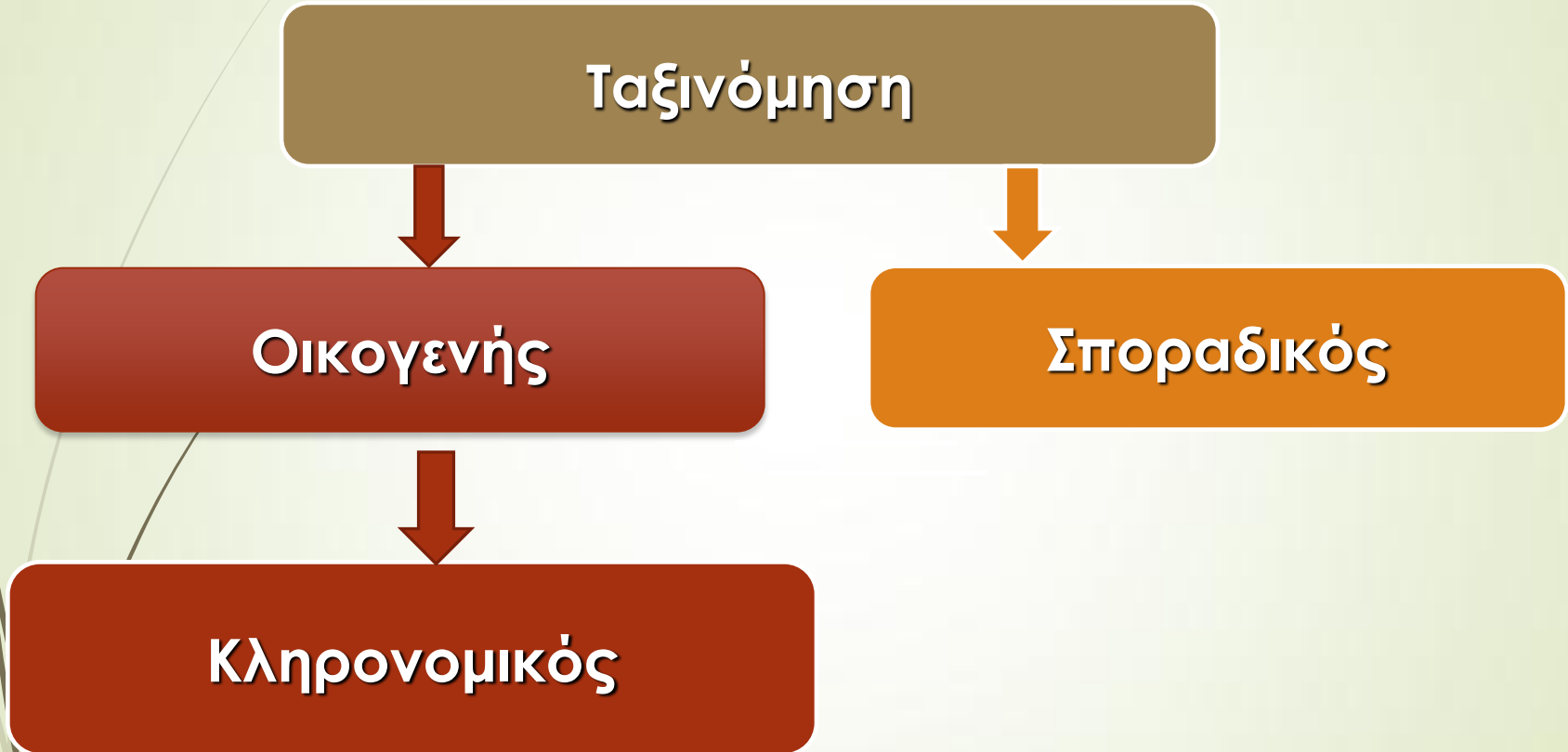


Stadler Z K et al. JCO 2010;28:4255-4267

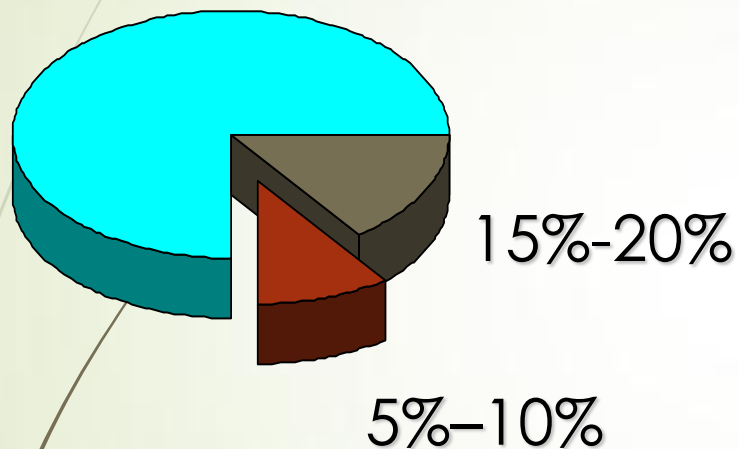
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JOURNAL OF CLINICAL ONCOLOGY

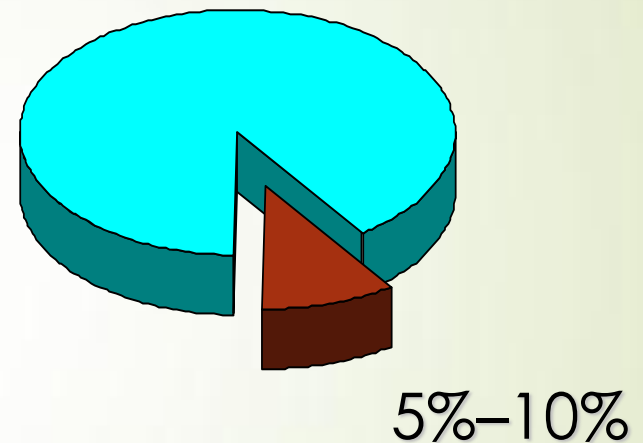
Καρκίνος του μαστού και ωοθηκών



Ποσοστό κληρονομικότητας;



Καρκίνος μαστού



Καρκίνος ωοθηκών

- Σποραδικός
- Οικογενής
- Κληρονομικός

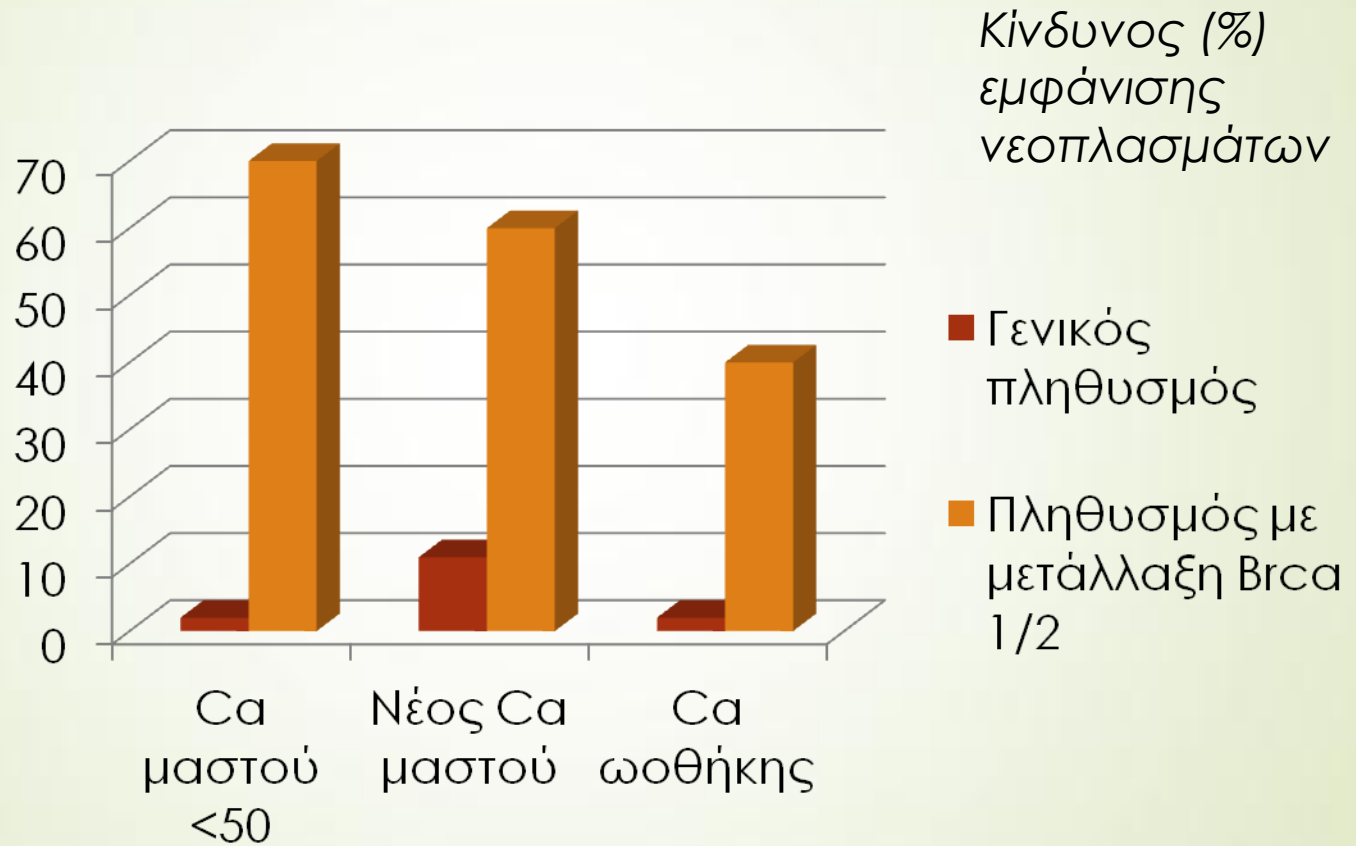
Κληρονομικό σύνδρομο μαστού και ωοθηκών

- ~90% του κληρονομικού ωοθηκικού καρκίνου
- 30-70% του κληρονομικού καρκίνου του μαστού
- Αυξημένο κίνδυνο εμφάνισης καρκίνου προστάτη
- Υπεύθυνα γονίδια : BRCA1, BRCA2
- Συχνά τριπλά αρνητικοί καρκίνοι του μαστού

Γονίδια σχετιζόμενα με τον καρκίνο μαστού & ωοθηκών

Gene	Associated syndrome	Major associated abnormalities
<i>BRCA1</i>	Hereditary breast cancer and ovarian cancer	Breast, ovarian, fallopian tube, male breast, pancreas, prostate cancers
<i>BRCA2</i>	Hereditary breast cancer and ovarian cancer	Breast, ovarian, fallopian tube, male breast, pancreas, prostate cancers
<i>TP53</i>	Li-Fraumeni	Breast cancer, soft tissue and bone sarcomas, leukemia, brain cancer, adrenocortical cancer, choroid plexus cancer, bronchoalveolar lung cancer
<i>PTEN</i>	Cowden/PTEN hamartoma	Hamartomas, papillomas of the lips and mucous membranes, acral skin keratoses, trichillemomas of skin, macrocephaly, breast cancer, endometrial cancer, non-medullary thyroid cancer, colon cancer, renal cell cancer
<i>CDH1</i>	Hereditary diffuse gastric cancer	Lobular breast cancer, diffuse gastric cancer
<i>STK11</i>	Peutz-Jeghers	Hamartomatous gastrointestinal tract polyps; characteristic mucocutaneous pigmentation; cancers of the breast, small bowel, stomach, colorectal, pancreas, lung, endometrium and ovary; sex cord tumor
Mismatch repair genes (<i>MSH2</i> , <i>MLH1</i> , <i>MSH6</i> , <i>PMS2</i> , <i>EPCAM</i>)	Lynch (hereditary nonpolyposis colon cancer)	Colon, endometrial, ovarian, stomach cancers

Σύνδρομο κληρονομικού Καρκίνου μαστού / ωοθηκών

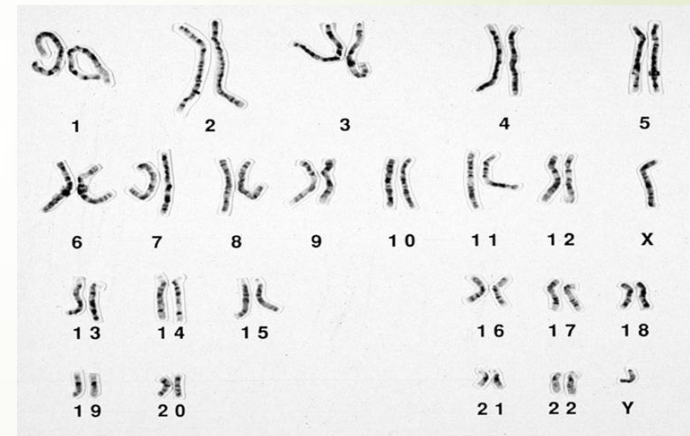


BRCA γονίδια

BRCA1 (17q21) 19941

BRCA2 (13q12) 19952

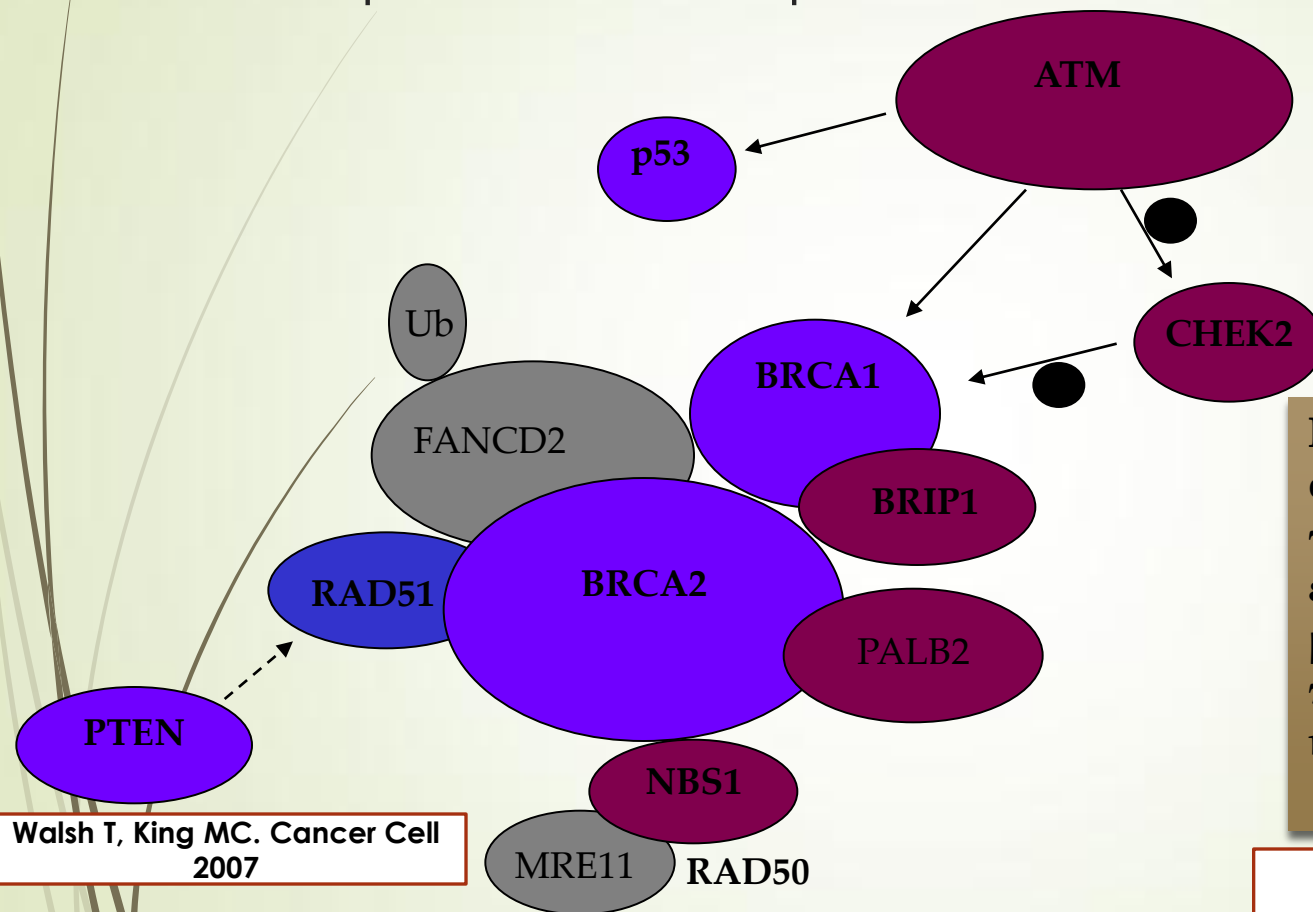
- Συχνότητα:
 - 1/500-1/800 (0.2%) στο γενικό πληθυσμό
 - 1/50 (2%) στη φυλή ασκενάζυ
- Διαφορετικές μεταλλάξεις εντοπίζονται σε διαφορετικούς πληθυσμούς



Μετάλλαξη-BRCA γονίδια

- Ογκοκατασταλτικά γονίδια
- Εμπλέκονται στη ρύθμιση του κυτταρικού κύκλου
- Εμπλέκονται στην επιδιόρθωση του DNA
- Οι μεταλλάξεις τους επιτρέπουν την συσσώρευση άλλων μεταλλάξεων και τη προαγωγή της καρκινογένεσης
- Αυτοσωματικό επικρατητικό πρότυπο μετάλλαξης

Μεταλλάξεις στα γονίδια BRCA1 & BRCA2 εξηγούν μόνο ένα ποσοστό των περιπτώσεων μαστού/ωοθηκών

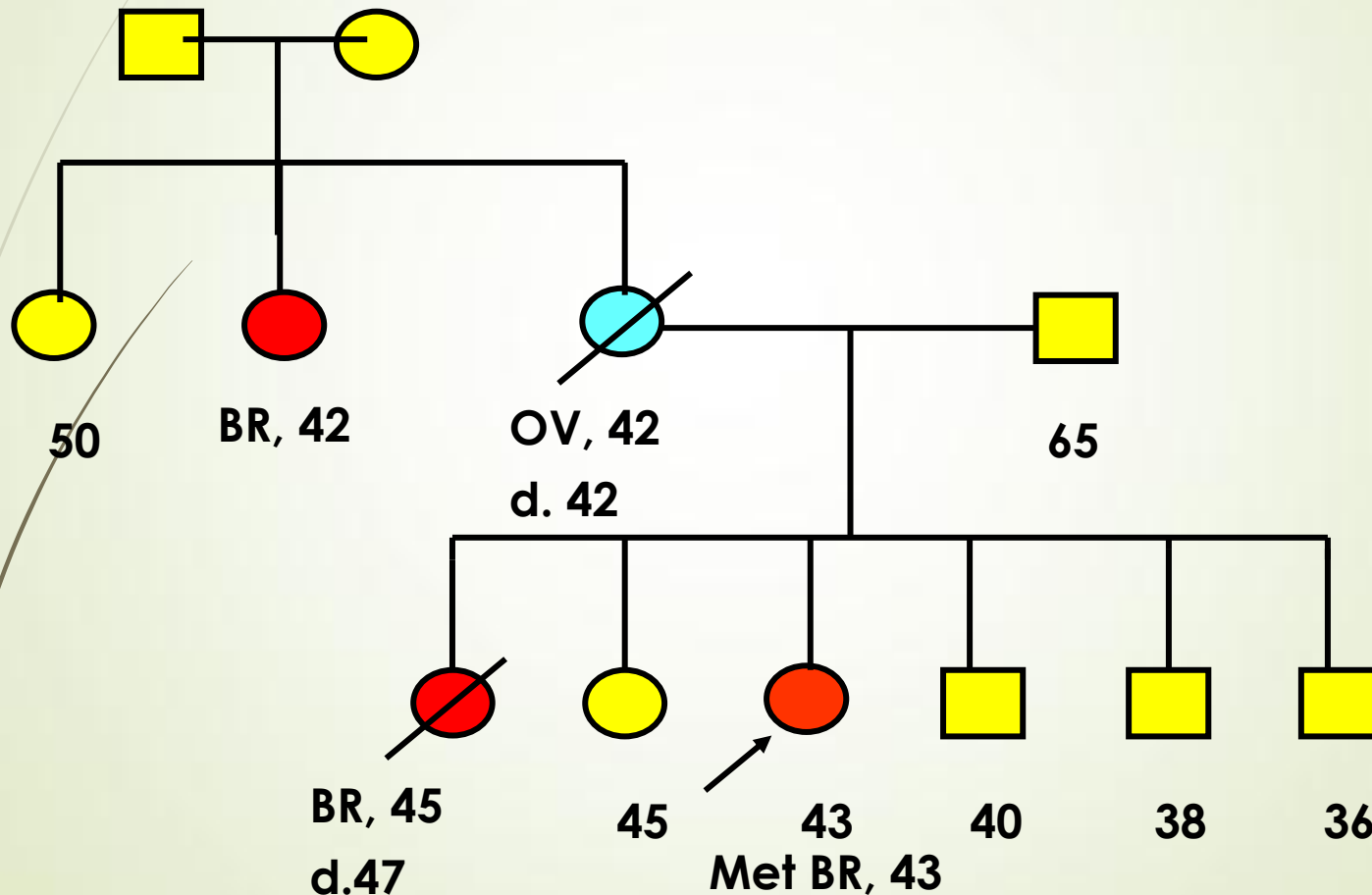


Κάθε μετάλλαξη είναι σπάνια και τουλάχιστον 20 γονίδια έχουν συσχετισθεί, μέχρι σήμερα, με την προδιάθεση για τον καρκίνο του μαστού

Walsh T, King MC. Cancer Cell 2007

McClellan J, King MC. Cell 2010

Κληρονομικό ιστορικό: Καρκίνος μαστού και ωοθηκών στο ίδιο γενεαλογικό δέντρο



Διαδικασία γενετικής συμβουλευτικής

- Αξιολόγηση στόχων & προσδοκιών συμβουλευόμενου
- Αξιολόγηση κινδύνου: λήψη οικογενειακού ιστορικού & φυσική εξέταση
- Διαφοροδιάγνωση
- Συζήτηση κληρονομικού συνδρόμου (κίνδυνος καρκίνου, στρατηγικές μείωσης κινδύνου)
- Επιλογές γονιδιακού ελέγχου (οφέλη, κίνδυνοι, περιορισμοί)
- Γονιδιακός έλεγχος
- Συνέχιση παρακολούθησης

Πληροφορίες ατομικού αναμνηστικού

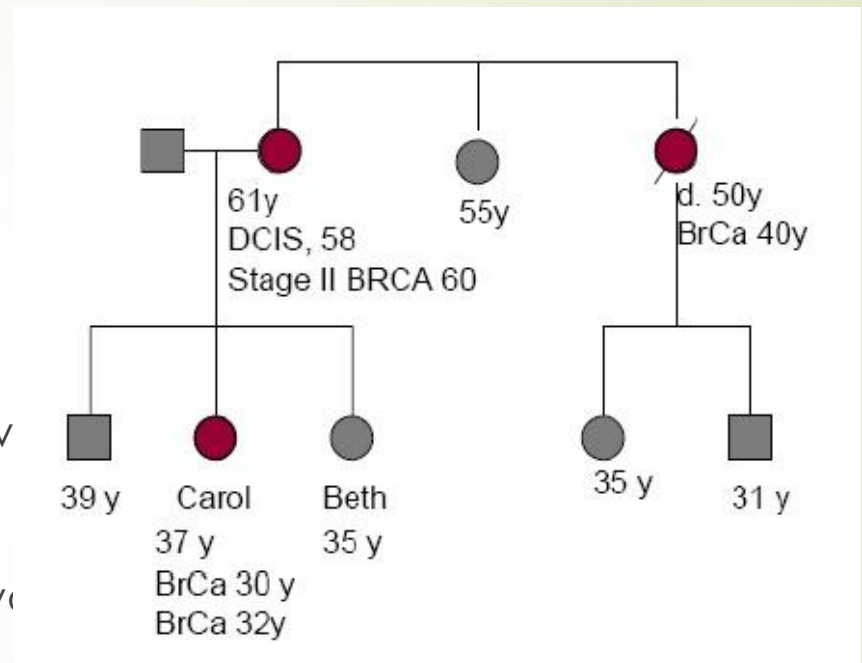
- Ηλικία
- Ατομικό αναμνηστικό καλοήθων/κακοήθων όγκων
- Μείζονα νοσήματα
- Νοσηλείες
- Χειρουργεία
- Βιοψίες
- Αναπαραγωγικό ιστορικό
- Προσυμπτωματικοί έλεγχοι για καρκίνο
- Περιβαλλοντικές εκθέσεις
- Εθνικότητα

Λήψη οικογενειακού ιστορικού

- Απαραίτητη η λήψη οικογενειακού ιστορικού των τριών τελευταίων γενεών
- Συμπλήρωση ερωτηματολογίου σχετικά με την καταγωγή, φυσικές δραστηριότητες
- Διατροφικές συνήθειες, το ιατρικό ιστορικό του ασθενούς
- Πλήρη κλινικο-παθολογικά δεδομένα
 - π.χ. Φορείς BRCA1 εμφανίζουν αρνητικούς υποδοχείς οιστρογόνων
 - ενώ Φορείς BRCA2 εμφανίζουν θετικούς υποδοχείς οιστρογόνων
- Η παθογόνος μετάλλαξη μπορεί να κληρονομηθεί εξ' ίσου από τον πατέρα και τη μητέρα
- Οι άρρενες φορείς στο BRCA1 συνήθως δεν αναπτύσσουν τη νόσο
- Οι γυναίκες φορείς μεταλλάξεων στο BRCA1 αναπτύσσουν τη νόσο πολύ νωρίτερα από τις φορείς στο BRCA2

Στρατηγική γονιδιακού ελέγχου

- Ο γονιδιακός έλεγχος πρέπει να γίνει στο άτομο που είναι πιο πιθανό να φέρει μετάλλαξη !!!
 - ήδη εμφάνισε καρκίνου μαστού ή/και ωοθηκών
 - εμφάνισε τον καρκίνο μαστού ή/και ωοθηκών στη μικρότερη ηλικία
 - αμφοτερόπλευρη εκδήλωση



Το πιο κατάλληλο άτομο δεν είναι πάντα διαθέσιμο !

Μαθηματικά μοντέλα Πιθανότητα φορείας μεταλλάξεων

- Αν πιθανότητα φορείας $> 10\%$ τότε πραγματοποιείται γονιδιακός έλεγχος
- BRCAPRO πιο αξιόπιστο
- Υπάρχουν διάφορα μοντέλα

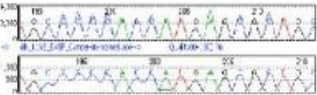
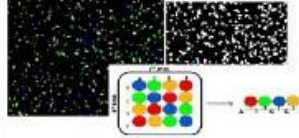
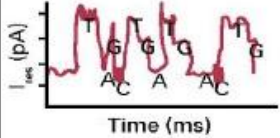
Πώς διενεργείται ο Γονιδιακός έλεγχος;

- Απλή λήψη αίματος
- Σε εξειδικευμένα εργαστήρια Γενετικής
- Με ειδικό εξωτερικό ποιοτικό έλεγχο
- Είναι διαπιστευμένα

Μέθοδος

- Η γενετική ανάλυση της αλληλουχίας των βάσεων των γονιδίων BRCA1 και BRCA2 (DNA sequencing): η εγκυρότερη μέθοδος για να ανιχνευθούν οι μεταλλάξεις που σχετίζονται με την κληρονομική προδιάθεση εμφάνισης καρκίνου του μαστού και ωοθηκών
- 99 % ευαισθησία
- Μέθοδος για τον εντοπισμό μεγάλων γενετικών αναδιατάξεων (ελλείψεων, αναδιπλασιασμών) που απενεργοποιούν τα γονίδια BRCA1 και BRCA2

Existing and emerging sequencing technologies.

	Sanger sequencing	Massively parallel sequencing	Emerging sequencing technologies
Amount of input DNA	High: micro- to nanogram 	Moderate: nanogram 	Low: picogram 
Library amplification method	Bacterial cloning 	PCR amplification, added sequences 	No amplification of template 
Sequencing reaction	Dideoxy chain termination	Sequencing-by-synthesis Sequencing-by-ligation	Sequencing-by-synthesis Sequencing-by-degradation
Capture of signal	Fluorescence-based imaging 	Sequential steps of imaging (fluorescence or light) and washing 	Direct inspection of the DNA molecule 
Read length	Moderate (800-1,000 bp)	Short (50-500 bp)	Long (1,000-10,000 bp)
Resolution of signal	Averaged across many copies of DNA	Averaged across many copies of DNA	Single-molecule resolution
Raw read accuracy	High	Moderate	Low
Cost per bp sequenced	High	Low	Very low

Γονιδιακός έλεγχος Που;

- Ηλικία -κάτω των 50 ετών.
- Τριπλά αρνητικός καρκίνος του μαστού
- Οικογενειακό ιστορικό
 - καρκίνου ωοθηκών
 - αντρικού καρκίνου μαστού
 - φυλή των Ασκενάζυ
- Δύο συγγενείς 1ου βαθμού με καρκίνο μαστού

Γονιδιακός έλεγχος Που;

- Πολλαπλά πρωτοπαθή νεοπλάσματα στο ίδιο άτομο
- Πολλαπλές διαγνώσεις κακοήθειας, συμβατές με συγκεκριμένα σύνδρομα (π.χ. μαστού & ωοθηκών ή παχέος εντέρου & ενδομητρίου)
- Διαγνώσεις σε πολλαπλές γενιές

Γονιδιακός έλεγχος Γιατί;

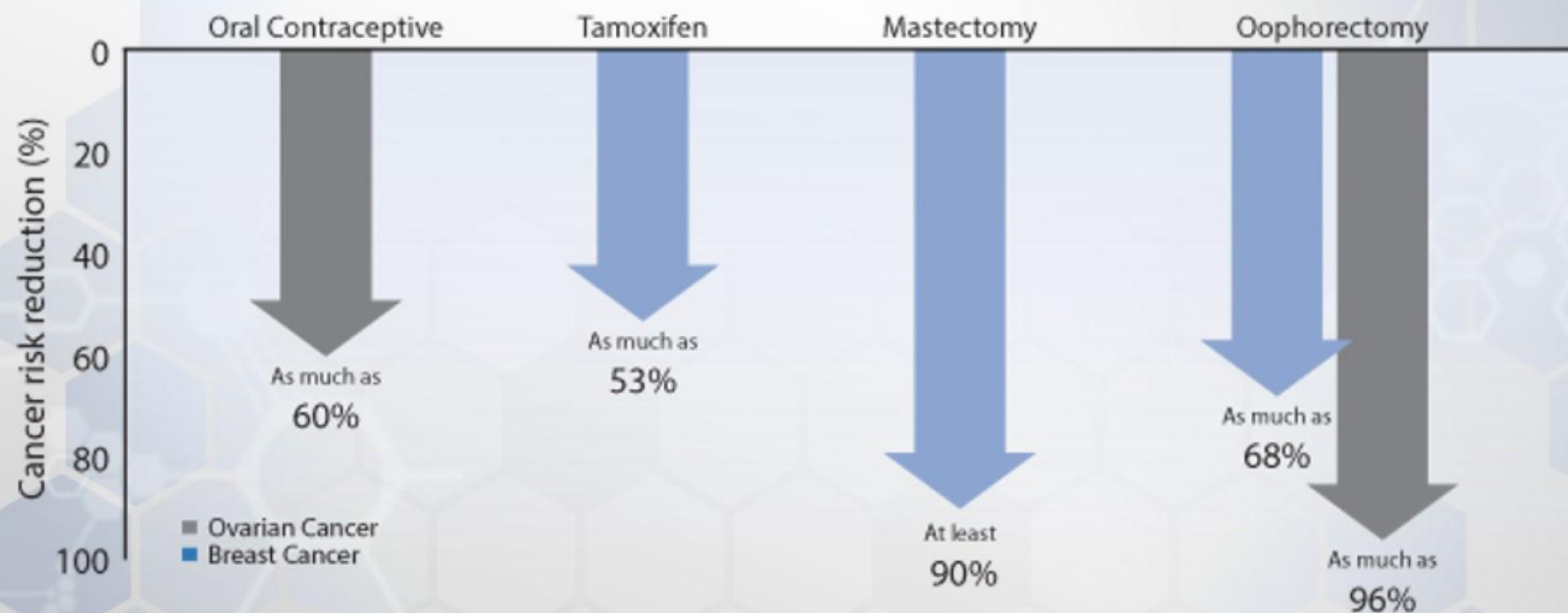
- Αναγνώριση του κληρονομικού καρκίνου
- Πρώιμη διάγνωση του καρκίνου
- Εντατική παρακολούθηση
- Προφύλαξη (χημειοπροφύλαξη - χειρουργική)



MYRIAD®

Optimized Treatment Reduces Risk in BRCA Positive Patients

NCCN recommended Medical Management Reduces Cancer Risks



Αποτελέσματα γονιδιακού έλεγχου

- Εύρεση μετάλλαξης → ΘΕΤΙΚΟ
- Δεν ανιχνεύεται μετάλλαξη → ΑΡΝΗΤΙΚΟ
- Εύρεση γενετικής αλλαγής άγνωστης σημασίας