ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI

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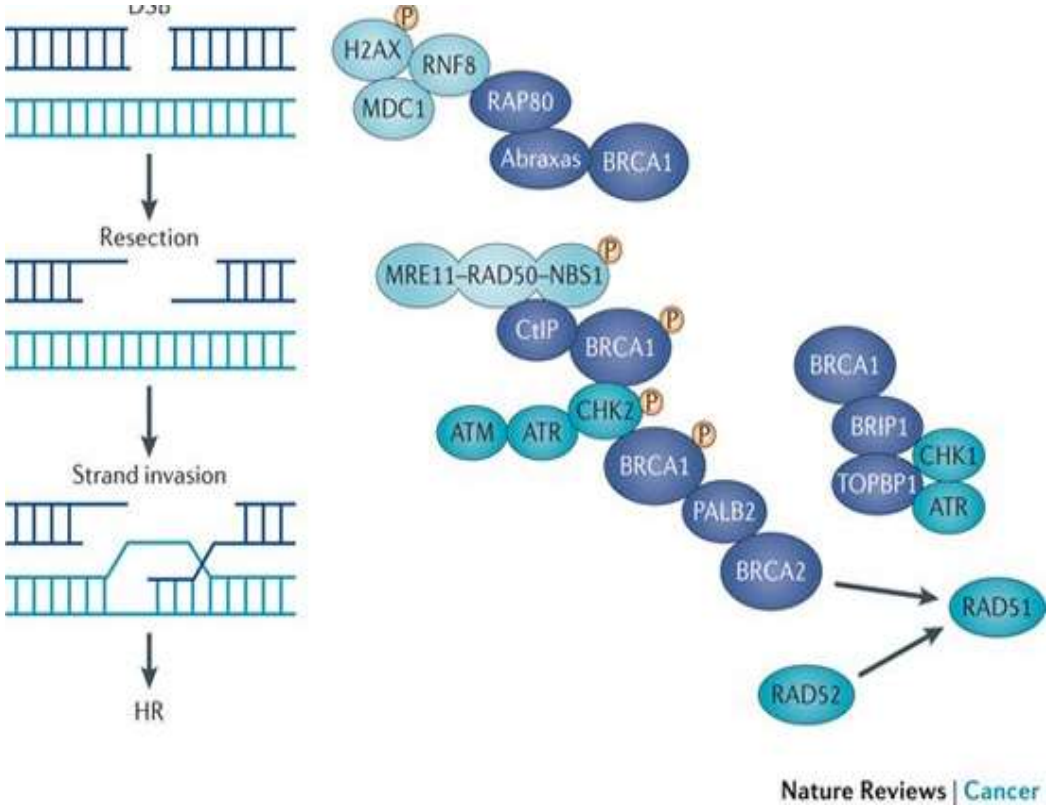
Il test per BRCA (somatico o germinale) e HRD

Cristin Roma

Why are BRCA mutations important in tumors?

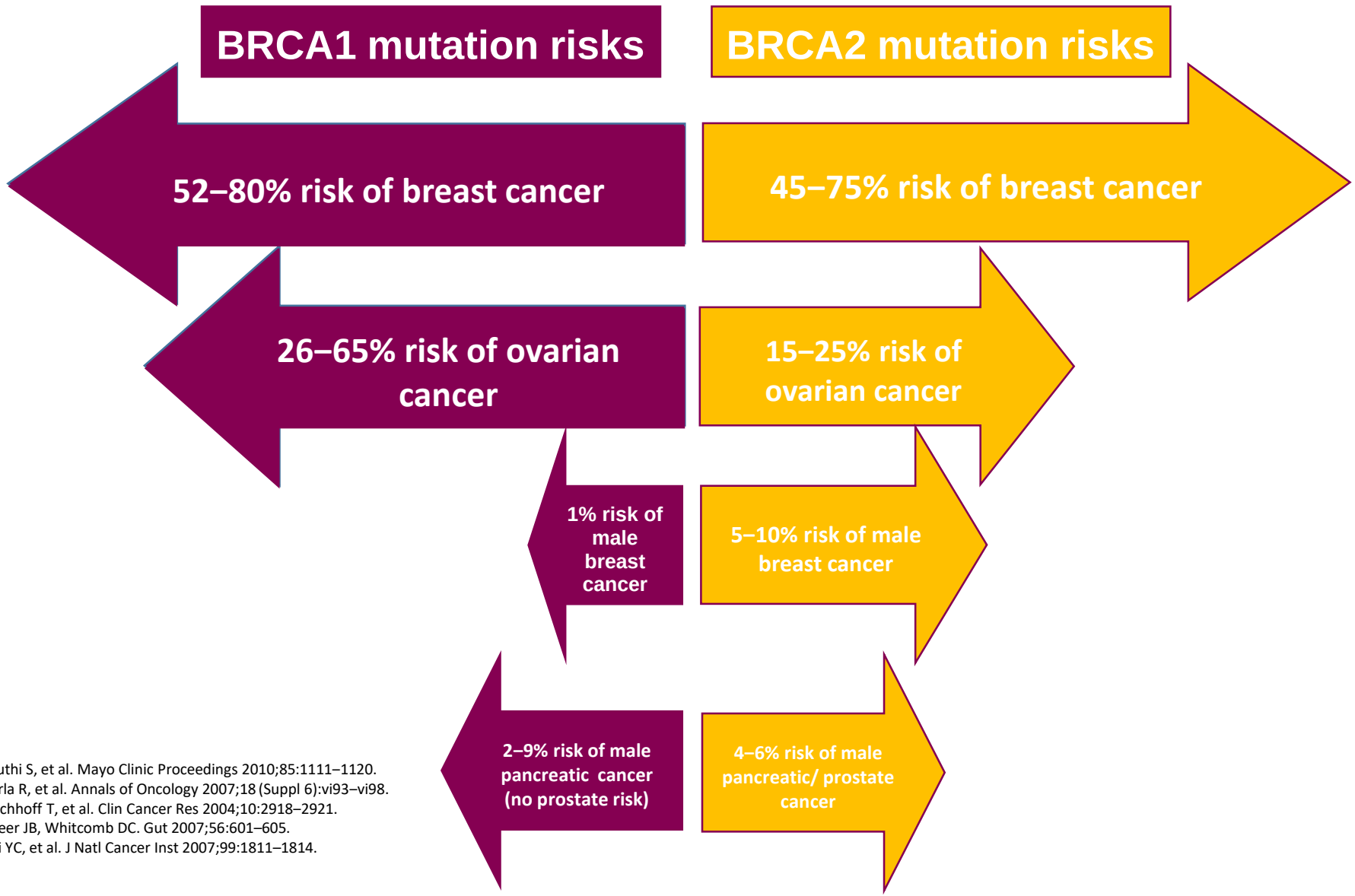
***BRCA*1 and *BRCA*2 genes are involved in DNA repair mechanisms**

Mutations in these genes cause loss of function of BRCA proteins



Several tumors are associated with BRCA mutations but only breast and ovarian carcinoma currently are included in clinical practice

Cancer risk and BRCA mutations



Pruthi S, et al. Mayo Clinic Proceedings 2010;85:1111–1120.
Ferla R, et al. Annals of Oncology 2007;18 (Suppl 6):vi93–vi98.
Kirchhoff T, et al. Clin Cancer Res 2004;10:2918–2921.
Greer JB, Whitcomb DC. Gut 2007;56:601–605.
Tai YC, et al. J Natl Cancer Inst 2007;99:1811–1814.

BRCA mutations in breast and ovarian cancers

- Breast Cancer:

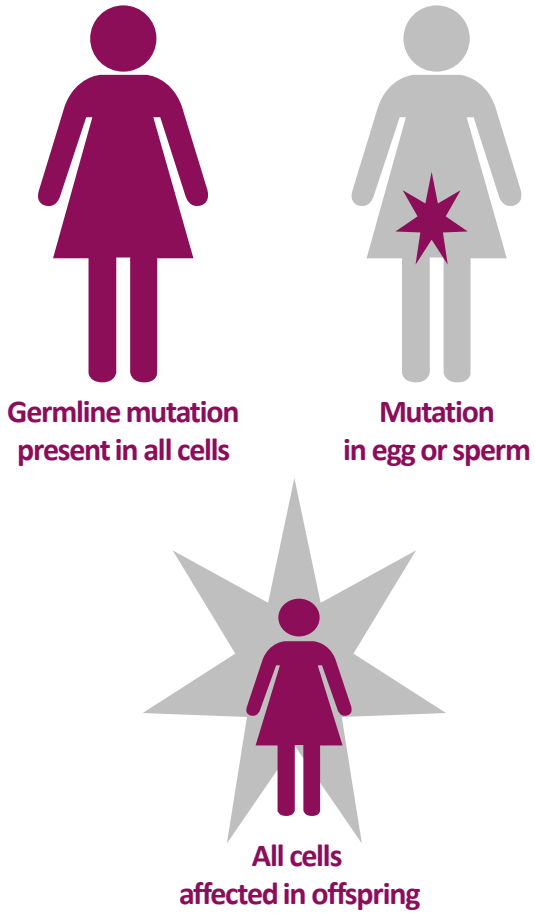
approximately 5–10% of cases are hereditary, with germline BRCA1/2 mutations being the most common (25-50%)

- Ovarian Cancer:

About 23-25% of cases of high-grade, epithelial ovarian carcinoma are related to mutated BRCA, 2/3 are germline and 1/3 are somatic

Germline mutations

- **A germline mutation can be passed on to the next generation[1]**
- **In oncology, germline mutations contribute to the likelihood of developing certain cancers and play a significant role in the response to both chemotherapy and targeted anticancer agents[2]**



- 1. National Cancer Institute. http://www.cancer.gov/cancertopics/understandingcancer/cancergenomics.
2. Filipski KK, *et al. Front Genet* 2014;5:73.

Autosomal Dominant Transmission (AD)

Figure 3. The normal cells of BRCA carriers carry one normal copy and one mutated copy of the BRCA gene.

Figure 4. Tumour cells in BRCA carriers have no normal copies of the BRCA gene, and are therefore BRCA deficient.

Figure 5. The multi-step model of carcinogenesis allows for loss of tumour suppressor genes playing an important role in the process.

Germline mutation (in all cells) of a single gene copy

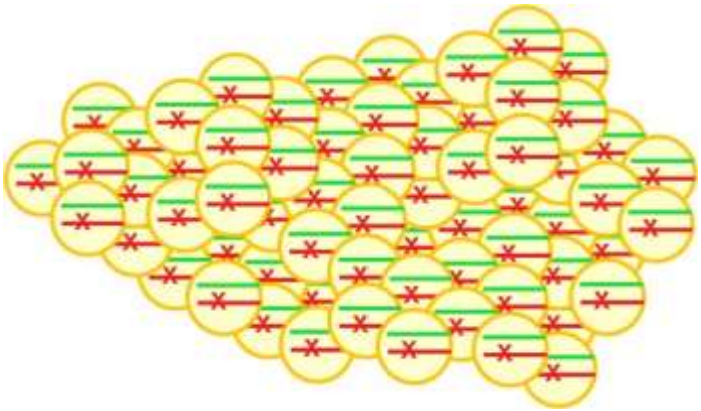
•A mutated copy of gene is inherited (Autosomal Dominant Transmission)

but

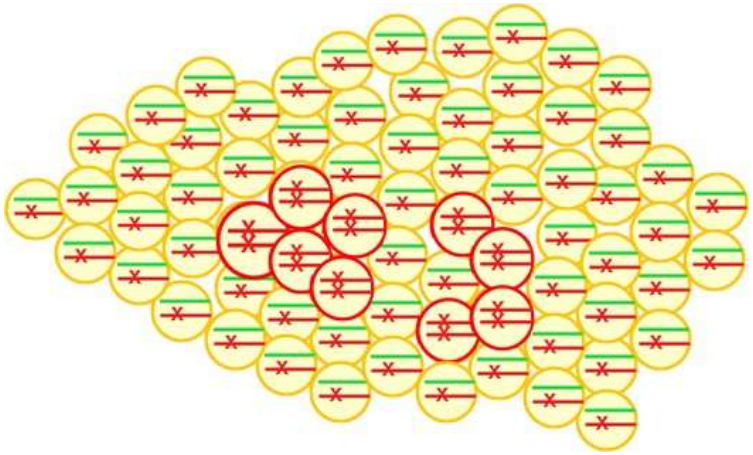
•a mutation in a second not mutated copy is necessary for tumor development *("second hit")*

The second mutation is somatic

The ‘double-hit’ hypothesis of tumorigenesis



The ‘first-hit’ is germline mutation
All body cells heterozygous for *BRCA*

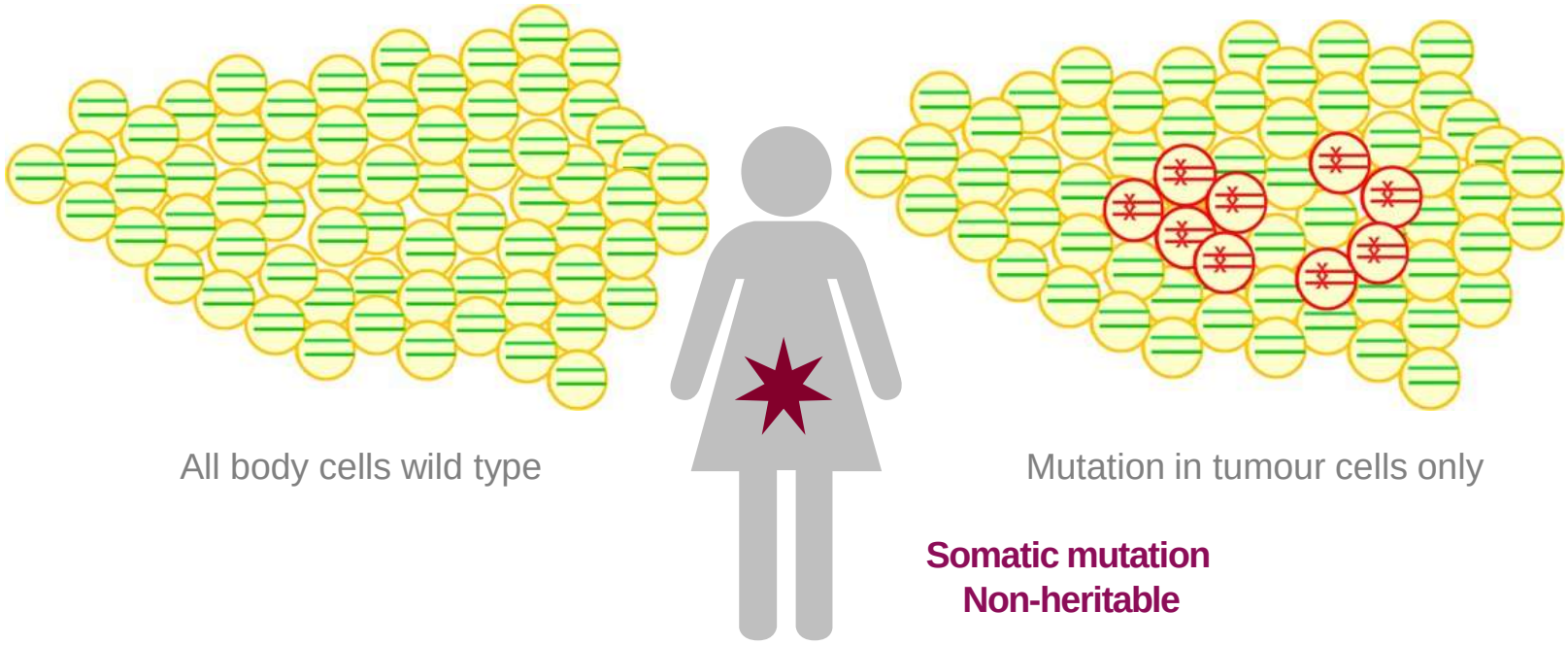


The ‘second-hit’ is a mutation in tumour cells that results in loss of heterozygosity

81% of *BRCA1* and 72% of *BRCA2* mutations are accompanied by heterozygous loss

Brozek I et al. *J Appl Genet* 2009; 50(4): 379–384

Tumour specific somatic mutation in women without germline *BRCA* mutation



A somatic mutation cannot be inherited, and does not occur in reproductive cells

- **Although an individual with a germline mutation may also develop a somatic mutation**

TEST BRCA: what's the scope?

- ✓ **BRCA test for the diagnosis of hereditary predisposition to cancer**

-Define germline alteration in affected patients

-Offer a predictive test for the family members

- Cancer prevention with adequate prevention programs in family members who also carry the mutation

- ✓ **BRCA test predictive of response to treatment with inhibitors of the enzyme Poli (ADP-ribose) Polymerase (PARP)**

December 2014: European Medicines Agency (EMA) approved “Olaparib”

- 4.1 Therapeutic indications

Lynparza (Olaparib) is indicated as monotherapy for the maintenance treatment of adult patients with platinum-sensitive relapsed BRCA-mutated (germline and/or somatic) high grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in response (complete response or partial response) to platinum-based chemotherapy.

Somatic and germline mutation testing: Sampling

If a patient is referred for tumour testing and a mutation (or mutations) is identified, a blood sample will need to be screened to determine if the patient has an inherited germline mutation

Tumour (germline and somatic)

FFPE

Snap-frozen

Germline

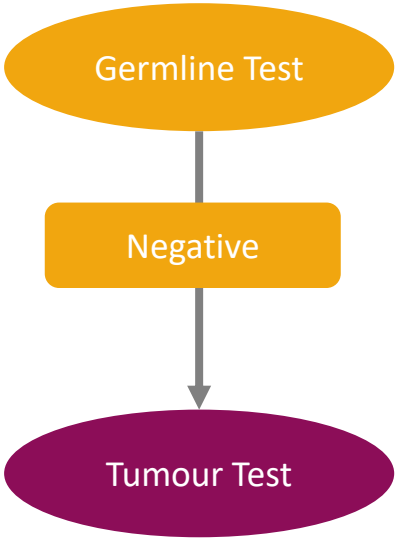
Blood sample

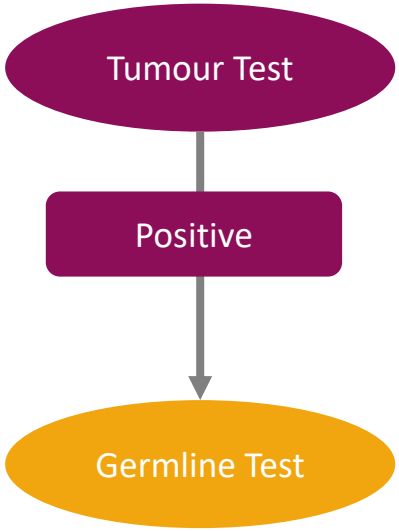
Buccal swab

FFPE, formalin-fixed paraffin-embedded.
Personal communication from Dr Emma Howard, Genomic Diagnostics Laboratory, St Mary's Hospital, Manchester, UK.

The relationship between germline and tumour testing

A woman with ovarian cancer who is found to be *BRCA* wild type on germline testing would need a subsequent tumour test to establish whether or not she has a somatic *BRCA* mutation





A subsequent germline test in a woman with ovarian cancer in whom tumour testing detects a somatic *BRCA* mutation can determine whether there are implications for her relatives

Beginning with a tumour test rather than a germline test has the following advantages:

- It will be conclusive as to whether or not an individual is *BRCA* mutation-positive (a negative germline test is inconclusive)
- Fewer patients will require two rounds of *BRCA* testing (a greater number of women with ovarian cancer will test negative on germline testing than will test positive on tumour testing)

Challenges to tumour testing

Routine tumour testing currently faces challenges in sample preparation and standardisation of testing methodologies:

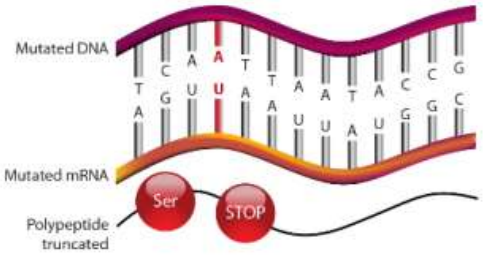
- **Sensitivity should strictly speaking be limit of mutation detection**
  - **Tumour samples have normal cells present**
- **FFPE samples prone to degradation**
  - **If using a PCR-based method, short amplicons (<160) should be used as little amplification will occur if the DNA is degraded**
- **Limited quantity of DNA extracted from the FFPE sample**

Which method?

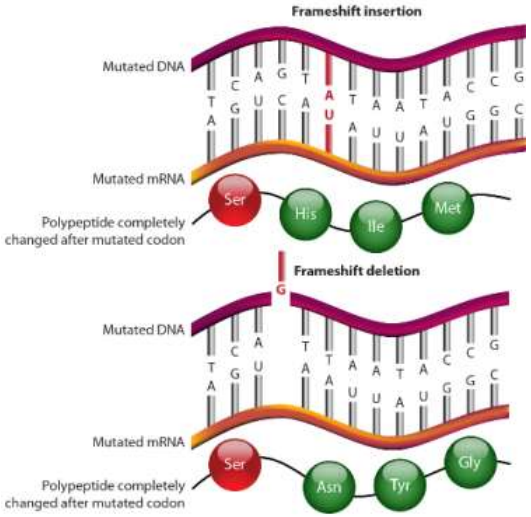
BRCA test		
	Detected variants	Method
Peripheral blood	Germline	Sanger Sequencing, NGS
Tumor tissue	Germline and Somatic	NGS

Mechanisms of mutation in *BRCA1* and *BRCA2*

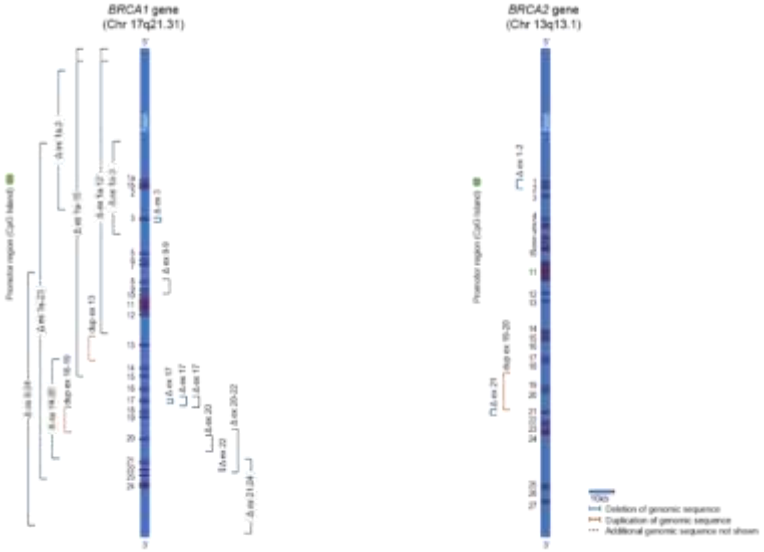
Point mutation (change in one base pair)



Frameshift (insertion or deletion of a base pair)



Large rearrangements (deletion or duplication of one or more exons)



NEXT GENERATION SEQUENCING

Change the paradigm in molecular diagnostic

-Sequencing of multiple DNA regions

- Analyze a large number of targets in a single run (multiplex analysis)

- Low input DNA quantity

-High coverage per single base

- Identification of SNPs, InDels and CNV

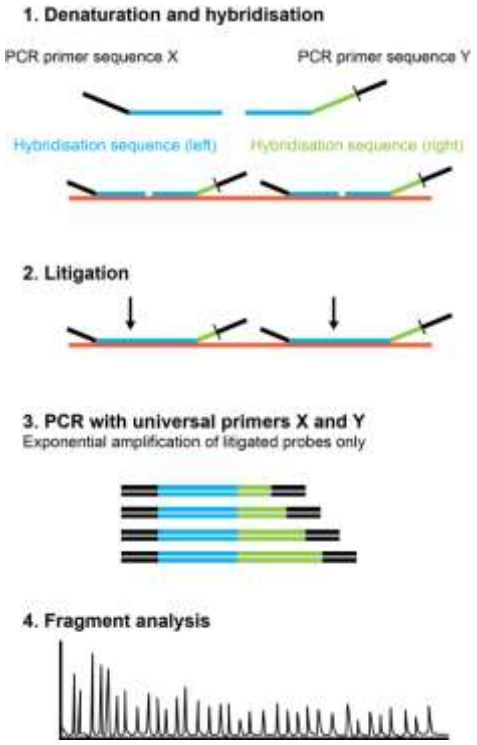
Methods of detecting large rearrangements: Multiplex ligation-dependent probe amplification (MLPA)

Most analysis methods cannot detect large rearrangements and a second technology is often required to detect these[1–3]

MLPA has rapidly become a popular method[2]

Four-step process, followed by data analysis, with a fast turnaround time[3]

Advantages[1–3]	Disadvantages[1–3]
• Low sample consumption • Fast turnaround time • Simple workflow • Widely available kit	• Can be affected by poor DNA sample quality • Care required to ensure robust scoring of single-exon deletions • Limited information on the location of the deletion/duplication breakpoints • Highly trained technical staff required



Used with permission from MRC-Holland b.v.

Figure taken from www.mlpa.com.

1. Hogervorst F, *et al. Cancer Res* 2003;63:1449–53; 2. Larsson N, *et al. EMQN Guidelines*. 2007; MRC-Holland b.v.;
3. https://mlpa.com/WebForms/WebFormMain.aspx?Tag=zjCZBtdOUyAt3KF3EwRZhNWLtcfv9pVl/tHJIM%5Cfa9FWO8KMqctOGIoqYwxaGF9Y.

NGS Technologies and Applications

Whole Genome and Whole Exome –*Investigative*

WG: (Human 3.2 GB) Chromosomal DNA is fragmented and all fragments sequenced
WE: (around 50MB out of the total of 3200MB human genome)Larger chromosomal regions are captured, fragmented, and all fragments sequenced

- Broad coverage of genes, massive information
- **High input DNA, higher cost, long turnaround time (TAT)**

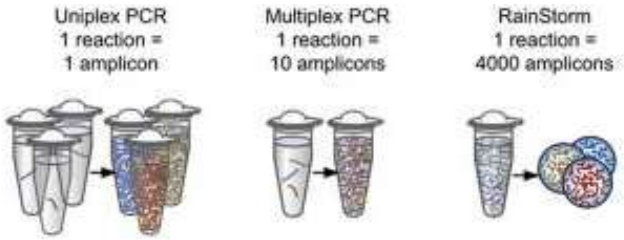
Targeted NGS Panels –*Diagnostic*

Resequencing of selected regions of interest (typically 1KB–1MB).
Small targeted gene regions are captured (hybridization) or amplified and sequenced.

- Mutations of genes with clinical significance
- Deeper sequencing
- **Low input DNA, lower cost, short TAT**
- Limited genomic coverage

Enrichment Methods

Multiplex PCR

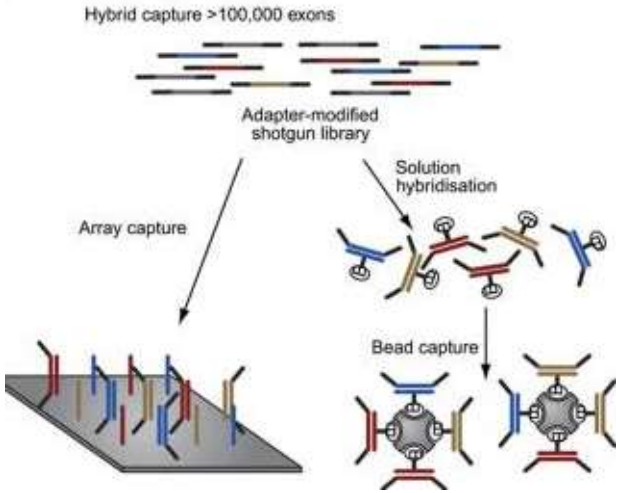


BRCA MASTR Plus Dx CE-IVD (Multiplicom)

Oncomine BRCA (Lifetechnologies)

GeneRead BRCA 1/2 Panel (Qiagen)

Hybridisation



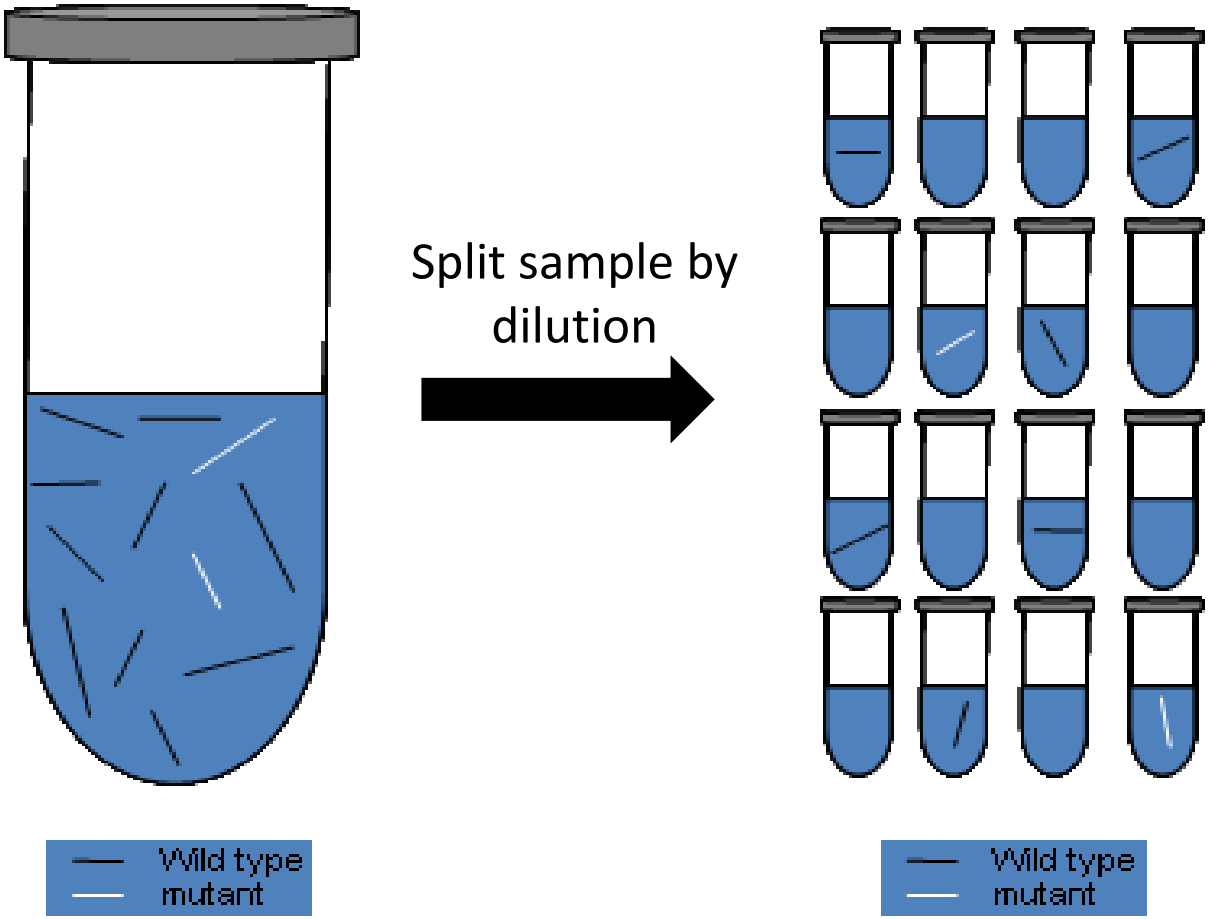
HaloPlexHS, 34 geni, custom panel, Agilent

TruSightCancer,94 geni e 286 SNPs, Illumina

Clonal amplification step

a) Conventional PCR

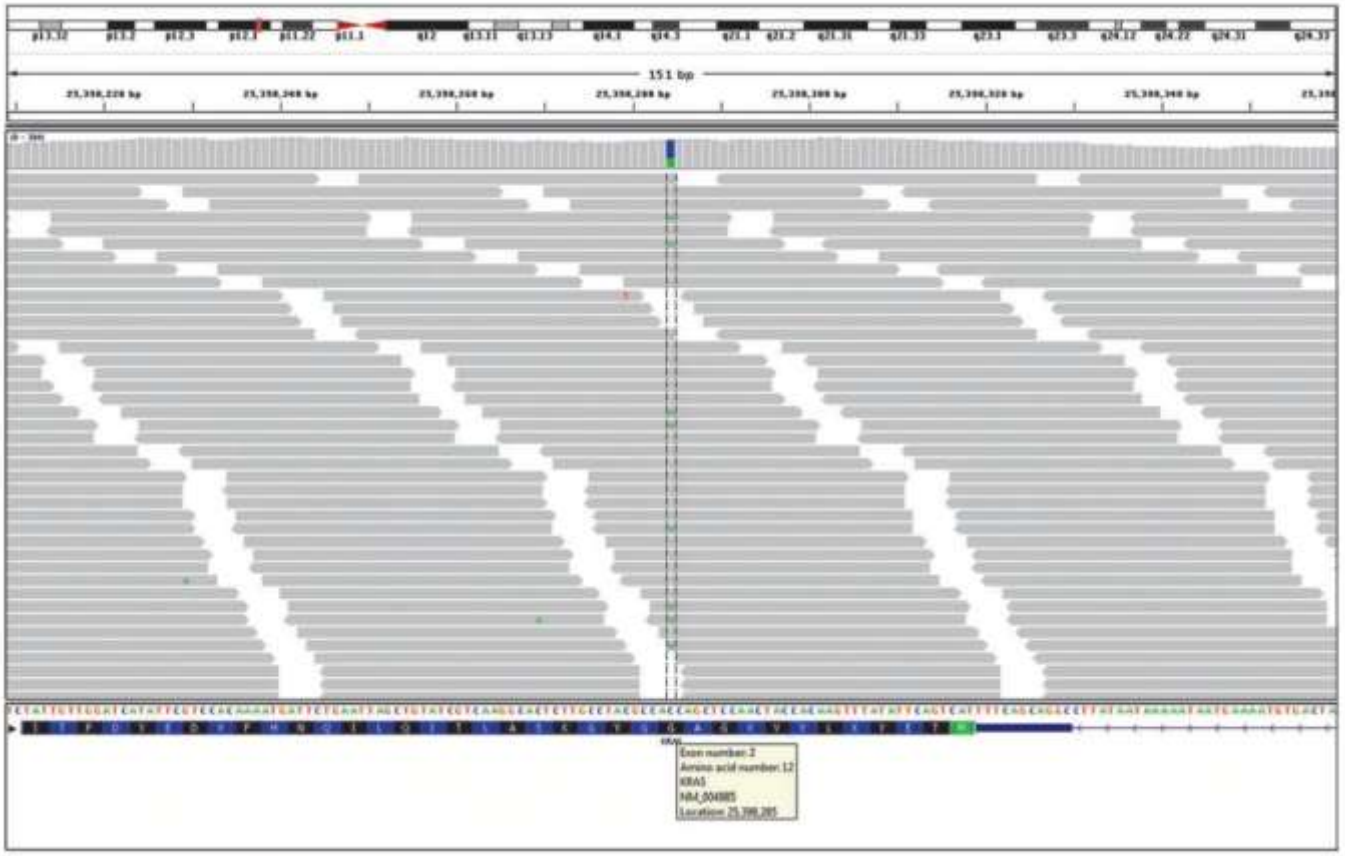
b) Clonal amplification



Slide courtesy of Nicola Normanno

NGS mutation analysis

In NGS methods, each molecule is sequenced independently, therefore there is not a mixed signal



Ross & Cronin. Am J Clin Pathol 2011;136:527–39.

Possible Test Results

- ✓ Positive test: identifies a mutation with pathogenic significance;
- ✓ Not informative test: failure to identify mutations or identification of variants for which it is not possible to attribute a certain clinical significance (VUS);

The BRCA test will be extended to the relatives in case of positive germiline result

Variant interpretation

The interpretation of the clinical significance of the identified variants will be carried out following the guidelines of the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) (http://enigmaconsortium.org).

The ENIGMA consortium intends to carry out a systematic and centralized collection of the BRCA variants observed, in order to contribute to the best classification of the same in the various laboratories carrying out the BRCA test.

IARC/ACMG-AMP SCHEME

Classe	Descrizione	Strategie di Sorveglianza
1	Non patogenica (nessun significato clinico) $p < 0.001$	Sorveglianza come da risultato negativo
2	Bassa patogenicicità (Basso significato clinico) $0.001 < p > 0.049$	Sorveglianza come da risultato negativo
3	Significato clinico incerto $0.05 < p > 0.949$	Strategia scelta in base alla storia familiare ed altri fattori di rischio
4	Potenzialmente patogenica $0.95 < p > 0.99$	Sorveglianza per pazienti ad alto rischio
5	Alta Patogenicità $p > 0.99$	Sorveglianza per pazienti ad alto rischio

Plon SE , Hum Mut, 2008

Homologous recombination (HR)

- ✓ HR is a DNA repair mechanism responsible for repair of double-strand breaks (DSBs)
- ✓ BRCA1/2 genes are key components of HR-mediated DNA repair
- ✓ However, DNA repair capacity in the tumor may be altered through other mechanisms, such as somatic or germline mutation in other HR pathway genes, DNA methylation or attenuated mRNA expression
- ✓ Inherited and acquired defects in HR might serve as response biomarkers or as therapeutic targets in breast and ovarian cancers

Genotype: BROCA Gene Panel

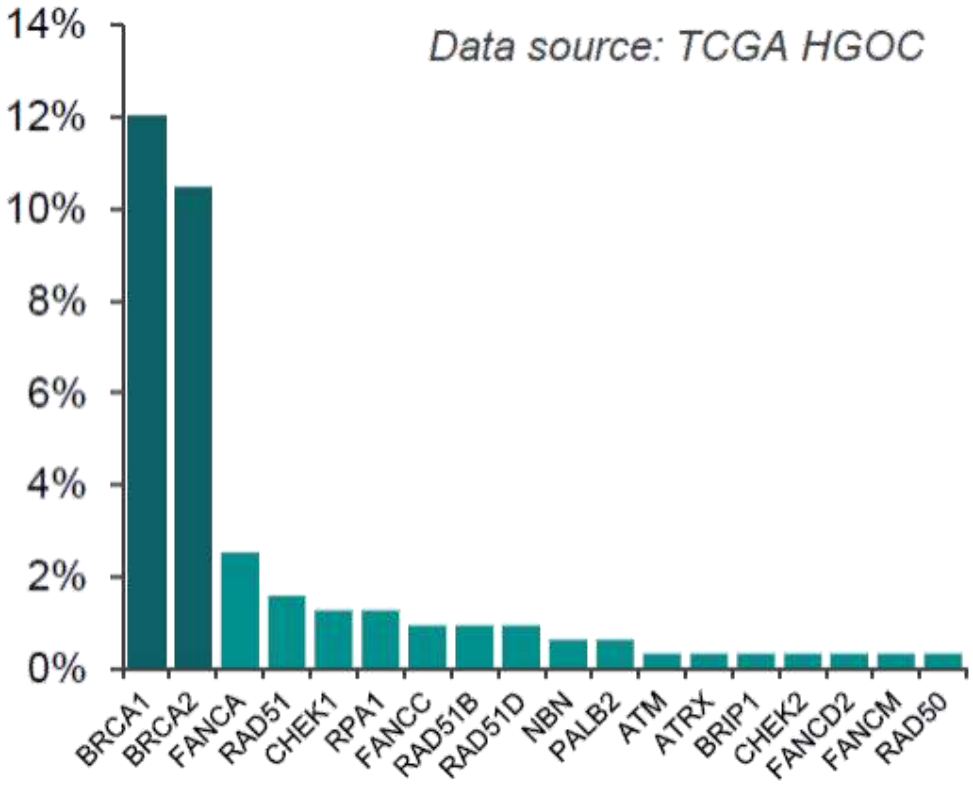
AKT1	CDK4	MEN1	**PIK3CA**	RB1
APC	CDKN2A	**MLH1**	PMS2	RET
ATM	CHEK1	**MRE11A**	POLD1	SDHB
ATR	**CHEK2**	**MSH2 (+EPCAM)**	POLE	SDHC
AXIN2	CTNNA1	MSH6	POT1	SDHD
BAP1	**FAM175A/Abraxas**	MUTYH	PRKAR1A	SLX4
BARD1	FH	**NBN**	PRSS1	SMAD4
BMPR1A	FLCN	NF1	PTCH1	**SMARCA4**
BRCA1	GALNT12	**RINT1**	**PTEN**	**STK11**
BRCA2	**GEN1**	RPS20	RAD51B	**TP53**
BRIP1	GREM1	**PALB2**	**RAD51C**	VHL
CDH1	HOXB13	PALLD	**RAD51D**	**XRCC2**

Genes in **blue** are associated with breast cancer
Genes in **red** are associated with both ovarian and breast cancers
Genes in **green** are associated with ovarian cancer

*Genes may have a role in one or more types of cancer.
http://tests.labmed.washington.edu/BROCA. Accessed April 25, 2016.

BROCA Gene Panel Disadvantages

- Gene sequencing will not identify promoter methylation or other causes cancer
- Mutations in other HR genes are rare
- It is unclear which mutations in the HR pathway cause HRD
- The effect of these mutations on PARP inhibitor sensitivity may not be the same



HR=homologous recombination; HRD=homologous recombination deficiency.

“Genomic Scar”

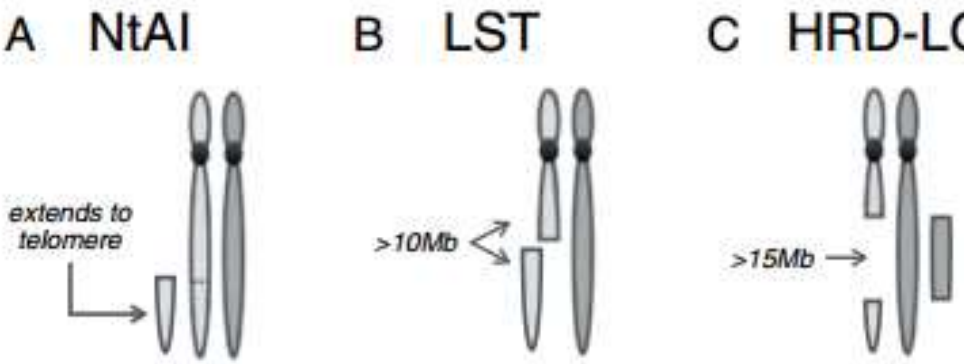


Figure 1 Overview of the type of genomic scars measured by each HR signature. Dark and light grey are used to indicate paternal and maternal chromosomes. **A**: Number of telomeric allelic imbalances (NtAI) counts the number of subtelomeric regions with allelic imbalance, that start beyond the centromere and extend to the telomere. **B**: Large-scale state transitions (LST) counts the number of chromosomal breaks between adjacent regions of at least 10 Mb. **C**: Homologous recombination deficiency score (HRD-LOH) measures the number of regions with LOH which are larger than 15 Mb, but shorter than the whole chromosome.

- ✓NtAI (Number of telomeric-allelic imbalance)
- ✓LST (large-scale state transitions)
- ✓LOH (loss of heterozygosity)

Marquard *et al. Biomarker Research* (2015) 3:9

Myriad Genetic test: 3-Biomarker HRD Score

(Nova trial – Niraparib)

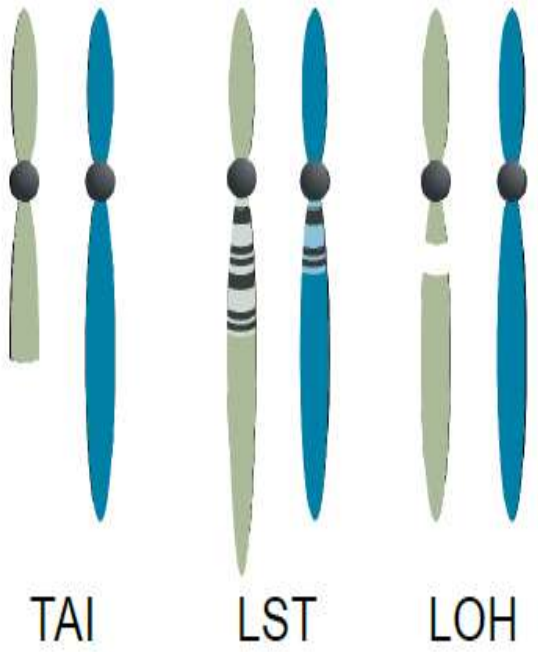
• An HR deficiency (HRD) score, which is a measure of genome instability, has been developed as the sum of three indipendent biomarkers:

✓TAI (telomeric-allelic imbalance)
✓LST (large-scale state transitions)
✓LOH (loss of heterozygosity)

• A score ≥ 42 (on a scale of 0-100) represents a positive score (loss of DNA repair function)

• Also tests for tBRCAm

• HRD score is calculated from SNP-derived whole genome profiling



G Mills et al San Diego SGO 2016

HRD causes genome-wide loss of heterozygosity (LOH) that can be measured by comprehensive genomic profiling based on NGS

FoundationFocus CDx *BRCA LOH (Ariel2- Rucaparib)*

HRD status was assessed using an algorithm two elements:

- tBRCAm status
- Genomic LOH (High or Low)

A tumor is defined as HRD negative if it is BRCAwt with low genomic LOH

LOH Status	Qualification Criteria	Methodology
LOH high	LOH score ≥ 16	Sequence analysis identifies LOH across the genome and summarizes it into a genomic LOH score
LOH low	LOH score < 16	Sequence analysis identifies LOH across the genome and summarizes it into a genomic LOH score

Recently EMA approved :

Lynparza (Olaparib) e Zejula (Niraparib)

as mantainance therapy for the platinum-sensitive ovarian cancer patients regardless of the BRCA mutational status

Advantages of BRCA test for all ovarian cancer patiens

✓The BRCA positive patients show an increased benefit from the PARP inhibitors treatment

✓Patients with germline BRCA mutations undergo tighter survelliance for the development of other tumors (associated with BRCA-linked inherited-familial syndromes)

✓This information is relevant for cancer prevention in relatives

Conclusions

- ✓ The BRCA test is important for both its preventive and predictive role
- ✓ Analysis of tumor tissue is essential for the identification of all the patients with ovarian cancer carrying BRCA mutations who may benefit from PARPi
- ✓ The somatic BRCA test must be performed with NGS
- ✓ The analysis of BRCA1 / 2 mutations does not include all tumors with HRD
- ✓ BRCA mutations remain predictive markers of response to PARP inhibitors, but better biomarkers are required for the identification of non-BRCA mutated patients able to benefit from PARPi therapy