BRCA1/2 Mutations: What is Changing in Molecular Diagnostics

Negrar, 08/06/2017

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TOPICS

- DNA Damage Repair (DDR): an overview
- BRCA1/2 as actors of the same molecular pathway
- NGS technologies to enhance molecular diagnostics
- Issues in reporting BRCA1/2 variants

*The prime Objective for every life-form is to deliver its genetic material, **intact and unchanged**, to the next generetion.*

Human Body
~ 10^{13} cells

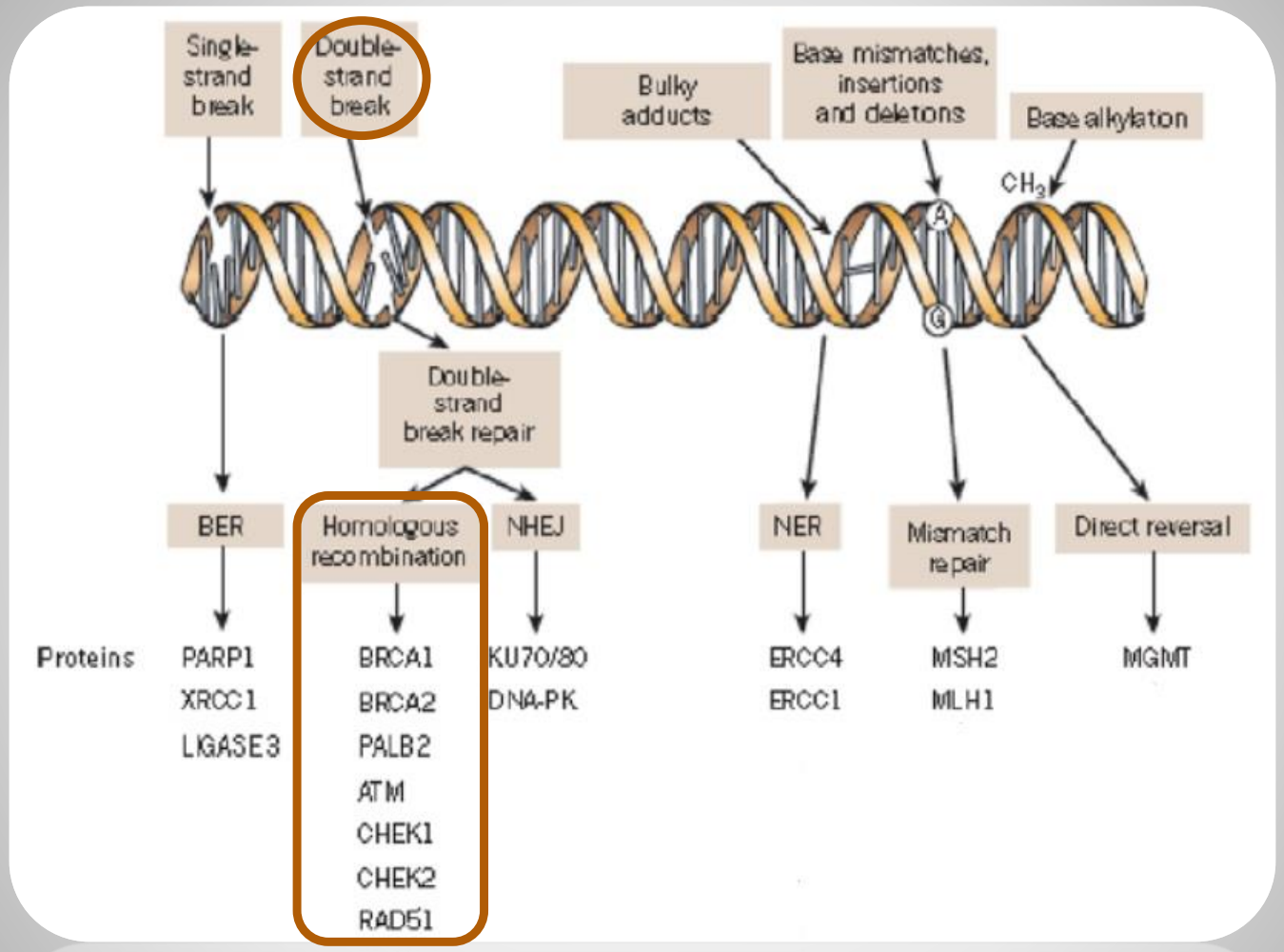
Thousand
DNA lesions
per day

Endogenous
Processes

Environmental
Agents

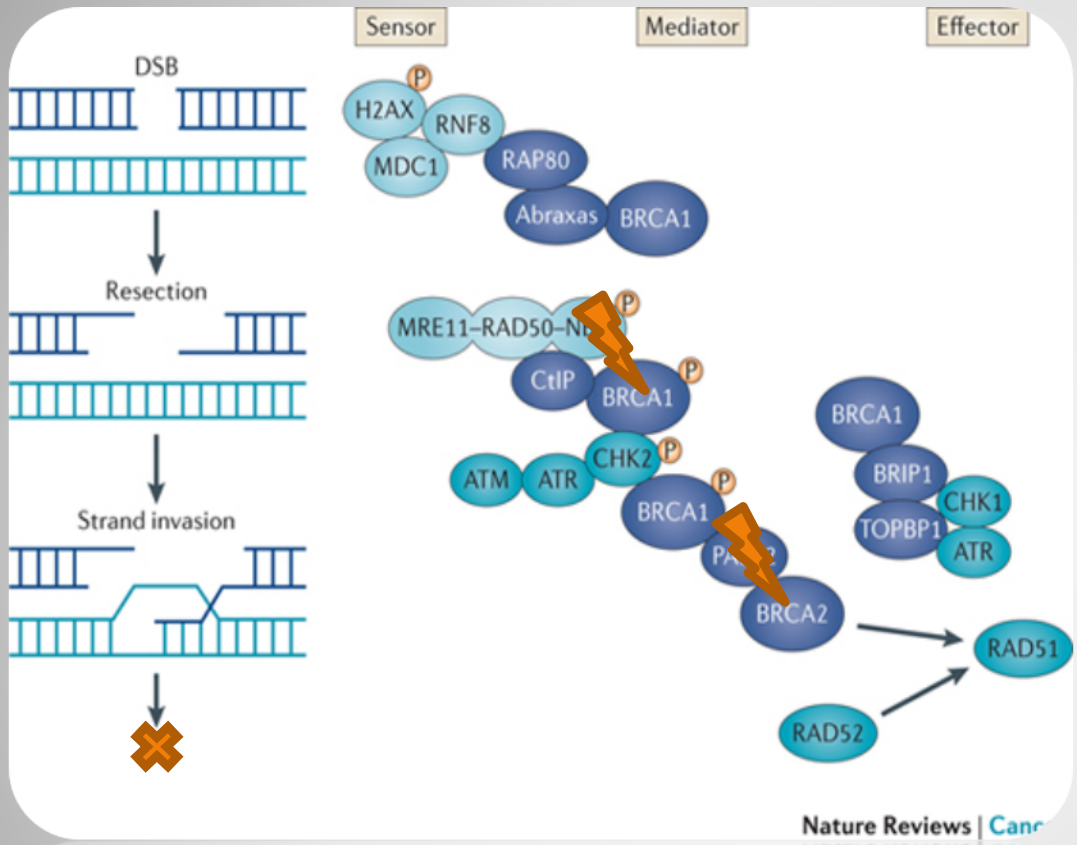
S.P. Jackson, J. Bartek – Nature 2009

DNA MANTEINANCE



H Fleury et al., Oncotarget, 2016

DNA DAMAGE REPAIR



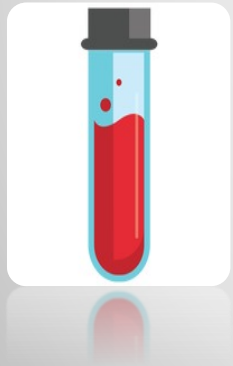
Oncosuppressor genes involved in HR

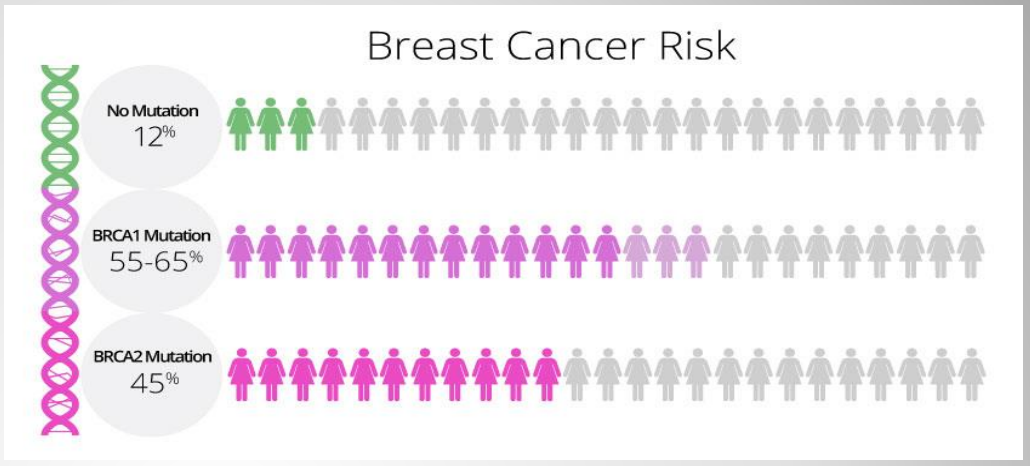
BRCA1/2 causative mutations may disrupt DSB repair leading to genetic instability

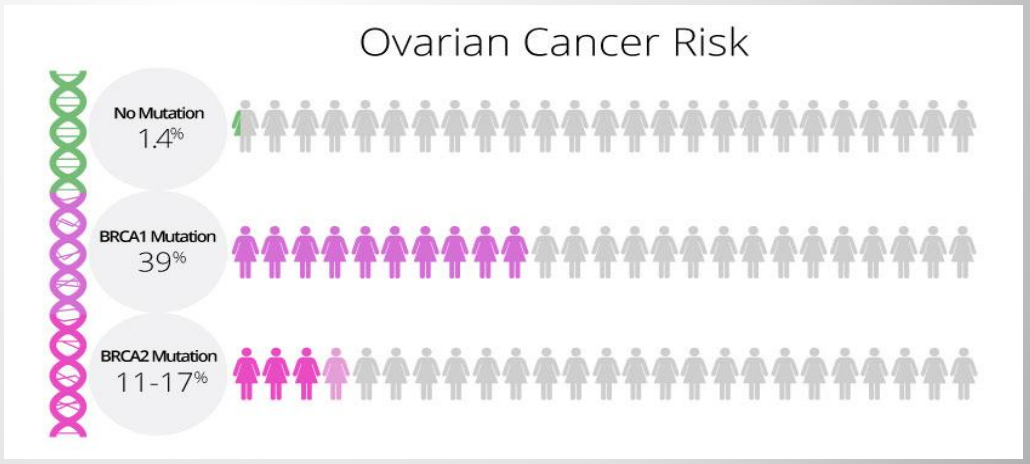
Possible tumor development

BRCA1/2 IN HR

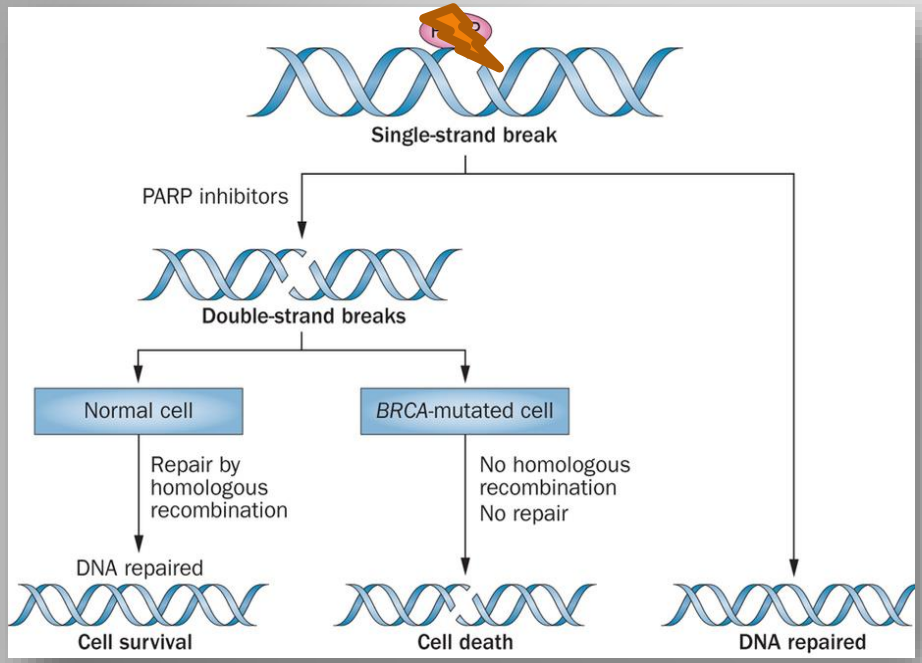
Inherited germline BRCA1 and BRCA2 mutations can be assessed from blood sample DNA







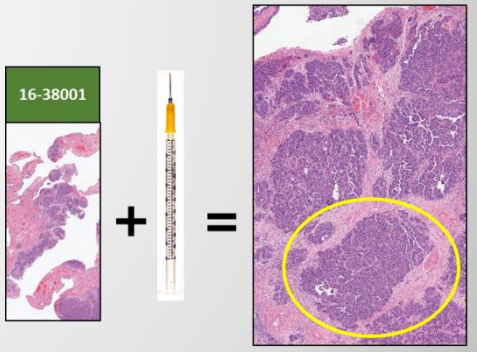
BRCA1/2 IN HEREDITARY BREAST & OVARIAN CANCER



Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by *BRCA* status in a randomised phase 2 trial

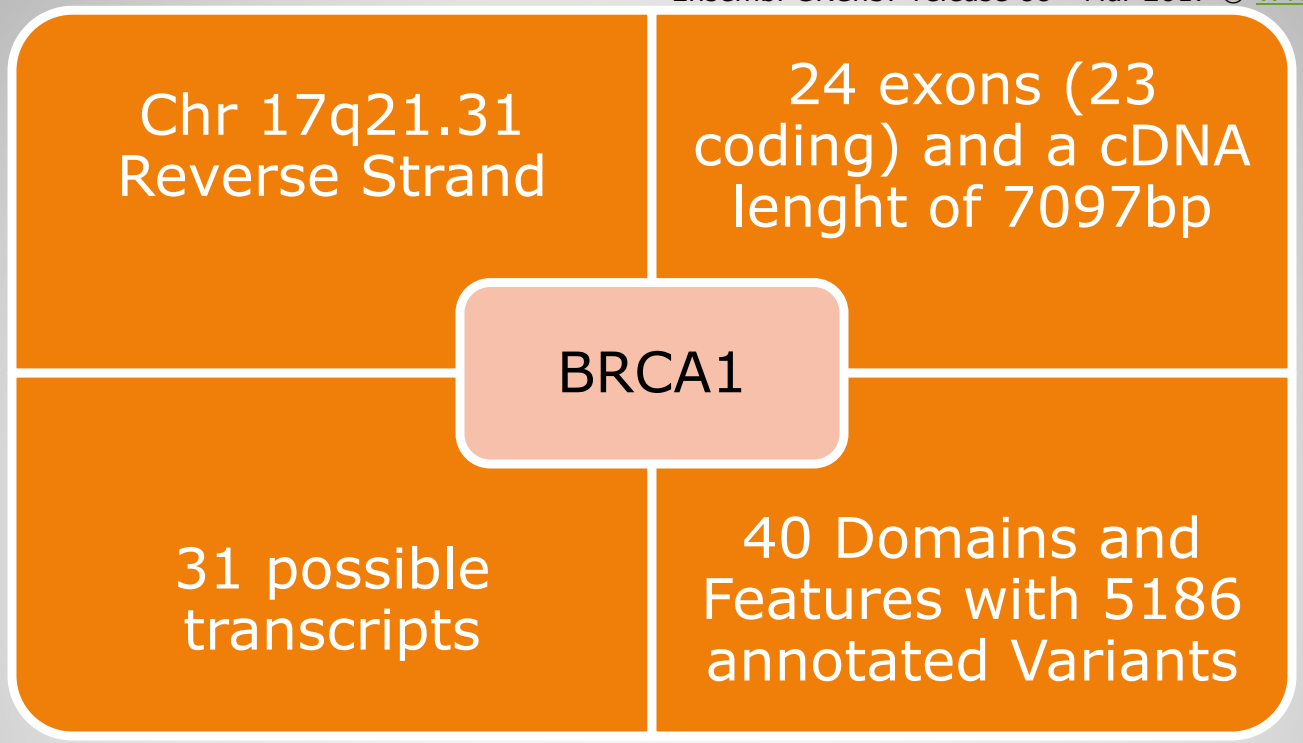
Jonathan Ledermann, Philipp Harter, Charlie Gourley, Michael Friedlander, Ignace Vergote, Gordon Rustin, Clare L Scott, Werner Meier, Ronnie Shapira-Frommer, Tamar Safra, Daniela Matei, Anitra Fielding, Stuart Spencer, Brian Dougherty, Maria Orr, Darren Hodgson, J Carl Barrett, Ursula Matulonis

Starting material: Blood for germline, FFPE for somatic mutations



Manual microdissection

BRCA1/2 MUTATIONS AS THERAPY RESPONSE PREDISTORS



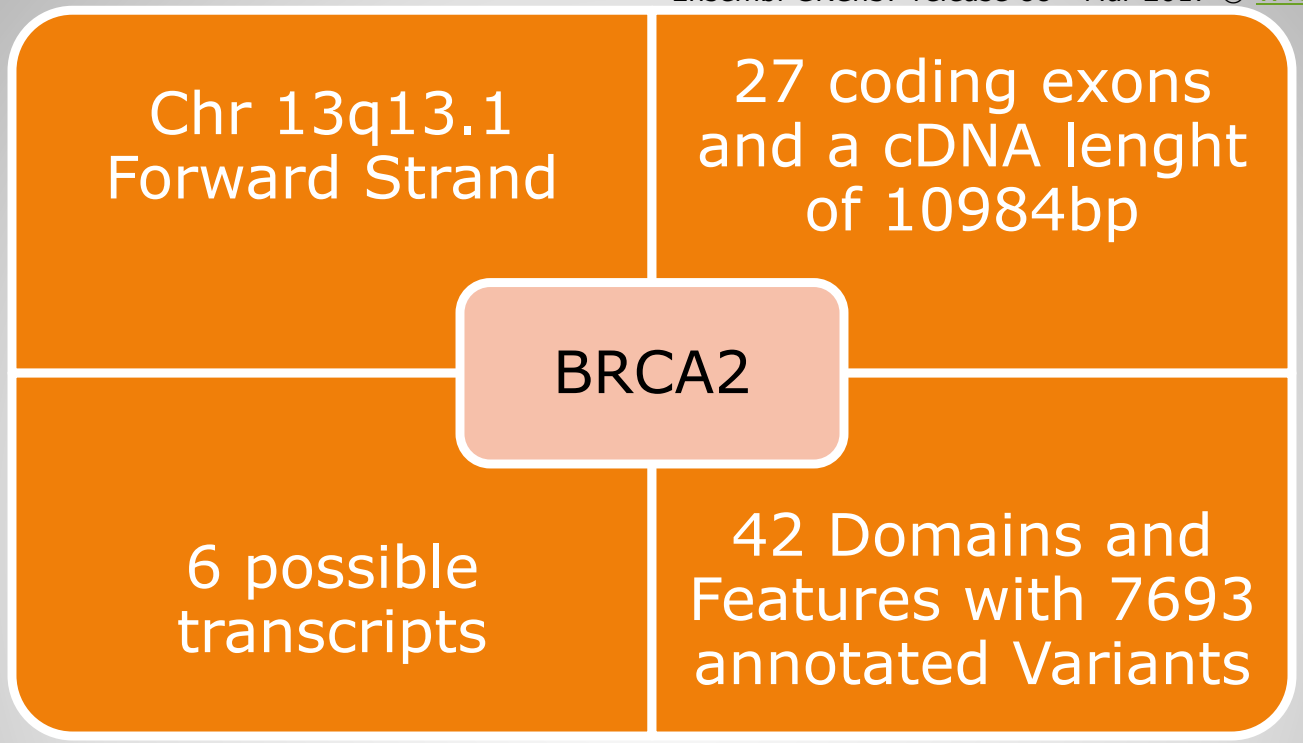
MAIN ISSUES

- -1 strand mapping
- Exon 4 splicing / exon numbering
- Lenght! (~40 Sanger Seq)
- Many transcripts
- No mutational hotspots

MAIN SOLUTIONS

- Complementarity (NGS)
- Exon 1 to 24 numbering!
- NGS Seq
- NCBI RefSeq: NM_007294.3
- NGS Seq

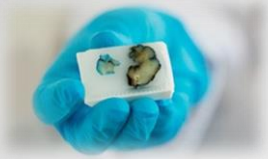
LET'S TALK ABOUT DNA...



MAIN ISSUES
- Lenght! (~40 Sanger Seq)
- Many transcripts
- No mutational hotspots

MAIN SOLUTIONS
- NGS Seq
- NCBI RefSeq: NM_000059.3
- NGS Seq

LET'S TALK ABOUT DNA...



DNA EXTRACTION

Breast and Ovary

CE-IVD

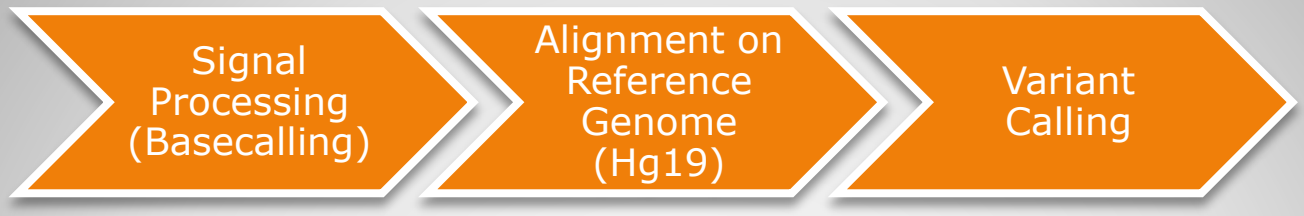
A validation program of 20 cases previously analyzed at IOV has been made in our lab, resulting in a 100% test-specificity

- NGS allows massive parallele sequencing by multiplexing targets on a solid matrix
- 6 to 64 samples per-run may be combined on a single sequencing chip
- The entire wet-lab process takes virtually three working days
- IVD platforms are available

ion torrent by life technologies™

PGM - DX

NGS TECHNOLOGY IN BRCA1/2 SEQUENCING



- Torrent Suite (Ion Torrent provided Pipeline)
- SmartSEQ (4BASES provided pipeline)

VARIANTS ANNOTATION

- Variant Effect Predictor (Wellcome-Trust Sanger institute)
- SmartSEQ (4BASES)
- IonReporter (Thermo Fisher)

BIOINFORMATIC ANALYSIS

~15% of BRCA1/2 mutations are large deletions/duplications (longIN/DEL) involving one or more coding exones

MRC-Holland MLPA®

Bioinformatic plugins for copy number variations (CNVs) yet available but still not fully reliable

LONG INDEL ANALYSIS

DATABASES FOR BRCA1/2 VARIANTS INTERPRETATION

- **ClinVar (NCBI):** PROs - constantly updated with the most recent functional findings, rich of informations (e.g. ENIGMA classification). CONs - misses some variants.
- **UMD (QuestDiagnostics, Inserm):** PROs - well updated, good level of informations. CONs - misses lot of variants.
- **BIC (NIH): PROs:** - rich of annotated variants. CONs: not well updated (very high rate of VUS), poor of informations.

IARC and ACMG-AMP Reccomendations

CLASS	Probability of pathogenicity	Type of Variant	Testing for at-risk Relatives
5 - Pathogenic	>0,99	Frameshift, Large InDel, Nonsenses	YES
4 – Likely Pathogenic	0,95 – 0,99	Inframe InDel, Missense, Intronic	YES
3 – Unknown Significance	0,05 – 0,949	Variants with insufficient evidence to classify	NO
2 – Likely Benign	0,001 – 0,049	Missense, Intronic, Inframe InDel, Synonimus at splice site	NO
1 - Benign	<0,001	MAF>0.01, Synonimus, Missense, Intronic	NO

Evidence-based Network for the Interpretation of Germline Mutant Alleles (**ENIGMA**).

BRCA1/2 VARIANT REPORTING

- **PATHOGENIC**: presence of Pathogenic and Likely Pathogenic variants
- **BENIGN**: absence of any alteration, presence of polymorphisms (MAF>0,1), presence of Benign and Likely benign Variants.
- **VUS**: Presence of Variants Of Uncertain Significance 3 - 15%

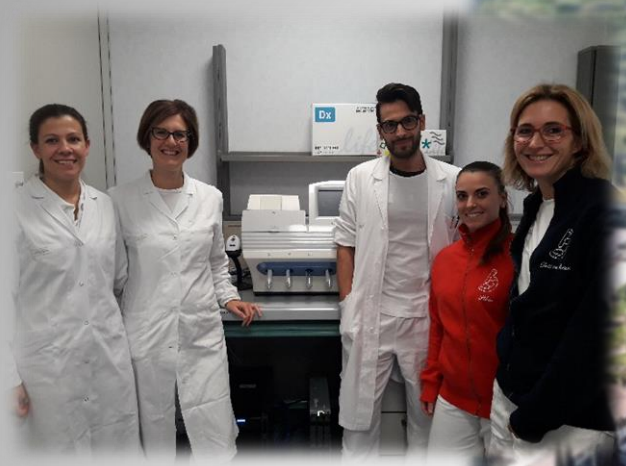
IARC / ACMG-AMP Reccomendations and ENIGMA have been essential in VUS rate reducing

VUS must be reviewed every 1 – 2 years

BRCA1/2 VARIANT REPORTING

SUMMARY

- BRCA1 and BRCA2 act as HR mediators
- Germline or somatic mutations in these genes may lead to genetic instability and cancer
- NGS approach is revolutioning complex analysis
- Lots of databases must be considered for functional analysis
- ENIGMA rules provides an objective classification of variants



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THANKS FOR YOUR ATTENTION