
# Omics driven pathology in gynecological malignancies

*Gian Franco Zannoni*

# OMICS IN GYNAECOLOGICAL CANCERS

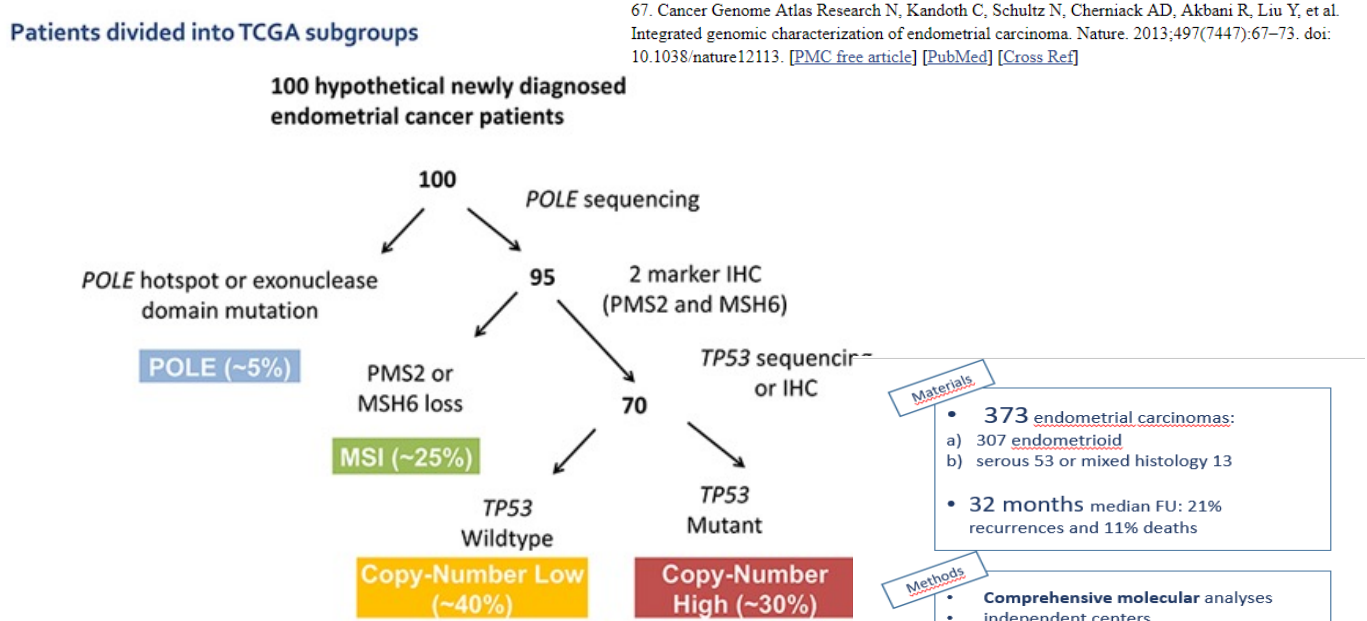
- ***Diagnostic confirmation***
- Validation of the classification
- Identification of new tumour entities
- Identification of new diagnostic biomarkers
- Elucidation of pathobiology
- Identification of new potential targets

**The correct histological diagnosis is the key to plan the appropriatest trial for the patient**

# …OMICS IN ENDOMETRIAL CANCERS

***The most comprehensive molecular study of ECs to date has been The Cancer Genome Atlas (TCGA) project, which included a combination of whole genome sequencing, exome sequencing, microsatellite instability (MSI) assays, and copy number analysis***

67. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67–73. doi: 10.1038/nature12113. [PMC free article] [PubMed] [Cross Ref]




Materials

- 373 endometrial carcinomas:
  a) 307 endometrioid
  b) serous 53 or mixed histology 13
- 32 months median FU: 21% recurrences and 11% deaths

Methods

- **Comprehensive molecular** analyses
- independent centers
- 6 genomic or proteomic platforms
- **MSI** testing on all samples using 7 repeat loci

# 4 SINGLE CLASSIFIERS

***POLE (ultramutated)***

- High mutation rates *(PTEN, PIK3R1, PIK3CA, FBXW7, and KRAS)*
- Hotspot mutations in the exonuclease domain of POLE
- Few copy-number aberrations
- *Favourable outcome*

***Microsatellite-instable (MSI hypermutated)***

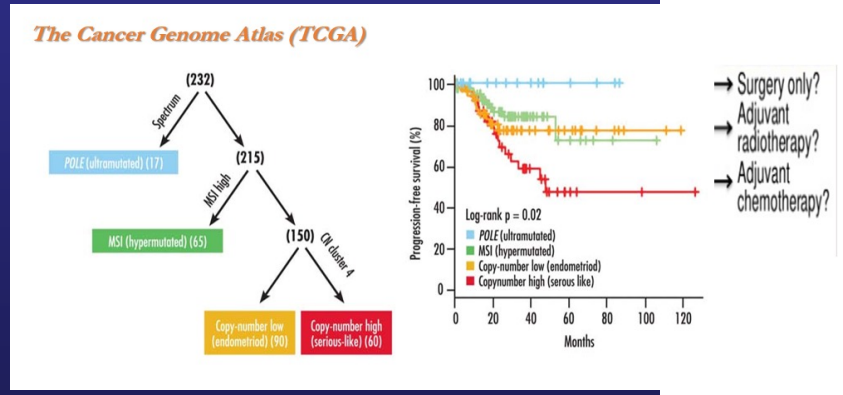
- MLH1 promoter methylation
- High mutation rates *(RPL22 frameshift deletions, and KRAS and PTEN)*
- Few copy-number aberrations

***Copy-number low (endometrioid)***

- MSS grade 1 and 2
- Low mutation rates *(frequent CTNNB1 mutations)*

***Copy-number high (serous-like)***

- Low mutation rates *(recurrent TP53, FBXW7, and PPP2R1A mutations, infrequent PTEN and KRAS mutations)*
- Extensive copy-number aberrations
- *Poor outcome*



# MOLECULAR SURROGATES

New classification of endometrial cancers: the development and potential applications of genomic-based classification in research and clinical care

A. Talhouk and J. N. McAlpine

*Gynecologic Oncology Research and Practice* 2016 3:14 | https://doi.org/10.1186/s40661-016-0035-4 

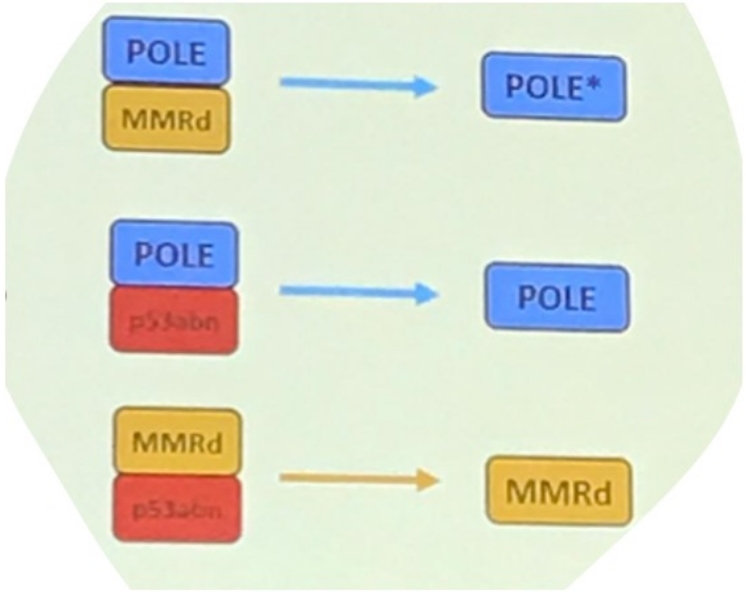
- **IHC on 4 MMR proteins** MLH1, MSH2, MSH6 and PMS2
- Genetic testing for Lynch syndrome

***Microsatellite- stable (MSS):*** 0 markers were altered
***Low level MSI (MSI-L) :*** 1-2 markers (less than 40%) were altered
***High level MSI (MSI-H) :*** 3 o + markers (greater than 40%) were altered.
Mismatch repair deficiencies can result from an inherited cancer syndrome (e.g., Lynch), acquired/somatic mutations, epigenetic events (methylation of one of the genes involved in mismatch DNA repair, most commonly MLH1)

- CN status was defined by **three genetic loci:** FGFR (4p16.3), SOX17 (8q11.23), and MYC (8q24.12)

- **Aberrant/abnormal (abn) p53** by genetic testing or IHC for complete loss or overexpressing (2+), able to separate CN-high (p53 abn) from CN-low (normal p53) subtypes

***Currently, there is no surrogate for POLE mutation but the targeted sequencing for the common mutations in this gene could be used rather than whole-genome or panel testing (mutation analysis of the exonuclease domain of POLE - exons 9, 11, 13, 14)***




*Multiple classifiers*

*Other molecular alterations*

## HORMONAL RECEPTORS

- Contradictory results regarding the prognostic value of ER/PR expression within TGCA molecular subgroups, due to application of multiple cutoff values for ER/PR expression

- 1% or 10% of positive tumor nuclei
- staining-intensity index of 3 (on a 0–9 scale)

- Recently, the ENITEC collaboration study has proposed an EC-specific classification for ER and PR expression categorized into three groups:

- HR group–low HR expressing (ER/PR expression: 0%–10%)
  *unfavorable outcome (5-year DSS 75.9%–83.3%)*
- IR group (ER/PR expression: 20%–80%)
  *intermediate outcome (5-year DSS 93.0%–93.9%)*
- LR group–high HR expressing (ER/PR expression: 90%–100%)
  *favorable outcome (5-year DSS 97.8%–100%)*

- ER ≤10% is more able to identify high-risk cases
- At the cutoff value of 80%, PR had a higher sensitivity, suggesting that PR is more able to identify a low-risk population

**L1-CAM+ >10%**

BJC British Journal of Cancer www.nature.com/bjc

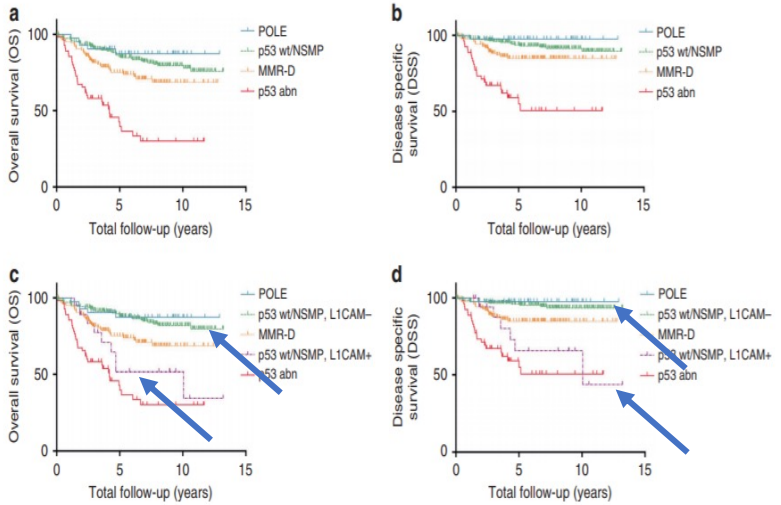
ARTICLE
Molecular Diagnostics

# L1CAM further stratifies endometrial carcinoma patients with no specific molecular risk profile

Felix KF Kommoss[1], Anthony N. Karnezis[2], Friedrich Kommoss[3], Aline Talhouk[2], Florin-Andrei Taran[4], Annette Staebler[5], C. Blake Gilks[6], David G. Huntsman[2], Bernhard Krämer[4], Sara Y. Brucker[4], Jessica N. McAlpine[7] and Stefan Kommoss[4]

| | | | | |
|---|---|---|---|---|
| ProMisE classification | | | | |
| POLE | 42 (9.3%) | 35 (9.8%) | 7 (7.2%) | <0.001 |
| MMR-D | 127 (28.1%) | 101 (28.5%) | 26 (26.8%) | |
| p53 wt/NSMP | 228 (50.4%) | 209 (58.9%) | 19 (19.6%) | |
| p53 abn | 55 (12.2%) | 10 (2.8%) | 45 (46.4%) | |
| LVSI | | | | |
| Negative | 388 (85.8%) | 315 (88.7%) | 73 (75.3%) | 0.005 |
| Positive | 60 (13.3%) | 38 (10.7%) | 22 (22.7%) | |
| Missing | 4 (0.9%) | 2 (0.6%) | 2 (2%) | |
| Adjuvant treatment | | | | |
| None | 171 (37.8%) | 151 (42.5%) | 20 (20.6%) | <0.001 |
| Any | 281 (62.2%) | 204 (57.5%) | 77 (79.4%) | |
| ESMO risk classification 2016 | | | | |
| Low | 230 (50.9%) | 213 (60%) | 17 (17.5%) | <0.001 |
| Intermediate | 64 (14.1%) | 58 (16.4%) | 6 (6.2%) | |
| High-intermediate | 27 (6%) | 22 (6.2%) | 5 (5.2%) | |
| High | 131 (29%) | 62 (17.4%) | 69 (71.1%) | |

| | | | | |
|---|---|---|---|---|
| Stage (FIGO 2009) | | | | |
| I | 365 (80.8%) | 303 (85.3%) | 62 (63.9%) | <0.001 |
| II–IV | 87 (19.2%) | 52 (14.7%) | 35 (36.1%) | |
| Tumour grade | | | | |
| Grade 1 | 282 (62.4%) | 267 (75.2%) | 15 (15.5%) | <0.001 |
| Grade 2 | 75 (16.6%) | 58 (16.3%) | 17 (17.5%) | |
| Grade 3 | 95 (21%) | 30 (8.5%) | 65 (67%) | |
| Histology | | | | |
| Endometrioid | 397 (87.8%) | 349 (98.3%) | 48 (49.5%) | <0.001 |
| Non-endometrioid | 55 (12.2%) | 6 (1.7%) | 49 (50.5%) | |




*British Journal of Cancer* (2018) 119:480–486; https://doi.org/10.1038/s41416-018-0187-6

**BETA-CATENIN**

## *CTNNB1 mutant EC as the fifth molecular EC subgroup: CTNNB1mut EC (after excluding MMRd and POLEmut)*

**HHS Public Access**
Author manuscript
*Mod Pathol*. Author manuscript; available in PMC 2017 September 10.

Published in final edited form as:
*Mod Pathol*. 2017 July ; 30(7): 1032–1041. doi:10.1038/modpathol.2017.15.

***CTNNB1* (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence**

*Tumors with CTNNB1 mutation were:*

- predominantly contained within the MSS, CNL, endometrioid cluster
- more likely have clinic-pathological characteristics commonly associated with lower clinical risk of recurrence

(younger age, lower FIGO grade, squamous differentiation, low TILs, less incidence of deep myometrial invasion, and less incidence of LVSI)

- had worse OS and recurrence-free survival
- had the lowest number of other concurrent mutations (KRAS, TP53, FGFR2 mutation)

# Immunohistochemical Assessment of β-Catenin in Endometrial Carcinoma

Angela Santoro, Damiano Arciuolo, Antonio Travaglino, Frediano Inzani, Gian Franco Zannoni, Antonio Raffone, Antonio Mollo

*American Journal of Clinical Pathology*, aqab207,
https://doi.org/10.1093/ajcp/aqab207
**Published:** 06 January 2022

In our previous meta-analysis, we assessed β-catenin as an immunohistochemical surrogate of CTNNB1 mutation

*Diagnostic accuracy 91%*
*Sensitivity 88%*
*Specificity 85%*

Herein, we report an update of our meta-analysis, performed by including only studies adopting NGS.

*Total accuracy (area under the curve on SROC curves) 96.4%*
*Sensitivity 85%*
*Specificity 98%*
*Positive likelihood ratio 28.41*
*Negative likelihood ratio 0.14*
*Diagnostic odds ratio 250,71*

According to these results, the use of NGS as reference standard confirms that nuclear β-catenin accumulation is an accurate surrogate of CTNNB1 mutation, with very high specificity.

frontiers | Frontiers in Oncology

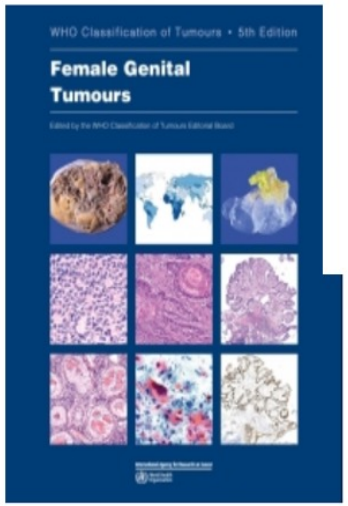
REVIEW
published: 08 April 2022
doi: 10.3389/fonc.2022.805613

# Current Prognostic and Predictive Biomarkers for Endometrial Cancer in Clinical Practice: Recommendations/Proposal from the Italian Study Group

OPEN ACCESS

**Edited by:**
*Priya Ranjit Bhosale,*

*Gian Franco Zannoni*[1,2*], *Emma Bragantini*[3], *Francesca Castiglione*[4], *Matteo Fassan*[5], *Giancarlo Troncone*[6], *Frediano Inzani*[1], *Anna Pesci*[7], *Angela Santoro*[1] and *Filippo Fraggetta*[8,9]




Female Genital Tumours
WHO Classification of Tumours, 5th Edition, Volume 4


Endometrioid adenocarcinoma NOS
- *POLE*-ultramutated endometrioid carcinoma
- Mismatch repair–deficient endometrioid carcinoma
- p53-mutant endometrioid carcinoma
- No specific molecular profile (NSMP) endometrioid carcinoma

Serous carcinoma NOS
Clear cell adenocarcinoma NOS
Carcinoma, undifferentiated, NOS
Mixed cell adenocarcinoma
Mesonephric adenocarcinoma
Squamous cell carcinoma NOS
Mucinous carcinoma, intestinal type
Mesonephric-like adenocarcinoma
Carcinosarcoma NOS

**…MORE THAN ONE HISTOTYPE:**
**the classic variants**

*cancers*

MDPI

*Review*

# New Pathological and Clinical Insights in Endometrial Cancer in View of the Updated ESGO/ESTRO/ESP Guidelines

Angela Santoro [1], Giuseppe Angelico [1], Antonio Travaglino [1], Frediano Inzani [1], Damiano Arciuolo [1], Michele Valente [1], Nicoletta D'Alessandris [1], Giulia Scaglione [1], Vincenzo Fiorentino [1], Antonio Raffone [2] and Gian Franco Zannoni [1,3,*]

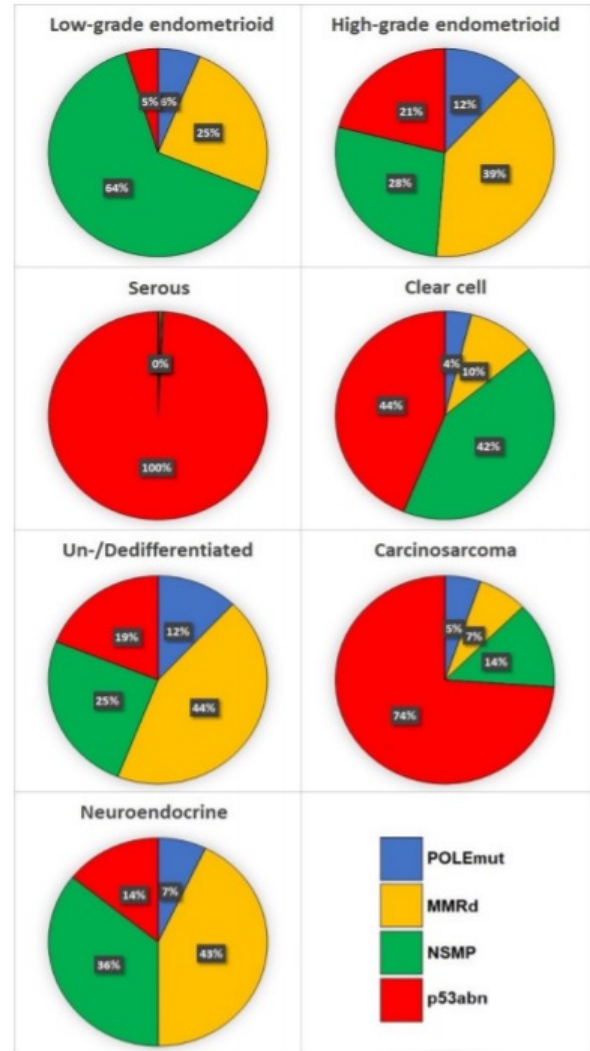



**Figure 1.** Distribution of TCGA molecular groups according to endometrial carcinoma histotype.

# *Lynch Syndrome (MMRd – MSI)*

- an autosomal dominant disorder resulting from constitutional mutations, affecting the DNA MMR genes MLH1, MSH2, MSH6, and PMS2
- cancers can arise in the endometrium, ovary, colorectum, stomach, small bowel, gallbladder, hepatobiliary tract, pancreas, renal pelvis and/or ureter, bladder, kidney, brain, or prostate

- Endometrial and ovarian cancers occurring in this setting often arise in YOUNGER WOMEN and endometrial cancer is the index cancer in slightly more than 50% of cases

Mismatch repair–deficient (dMMR) tumours show an increase in the point mutation rate, especially within repetitive stretches of DNA called ***microsatellites***; this manifests as microsatellite instability (MSI)

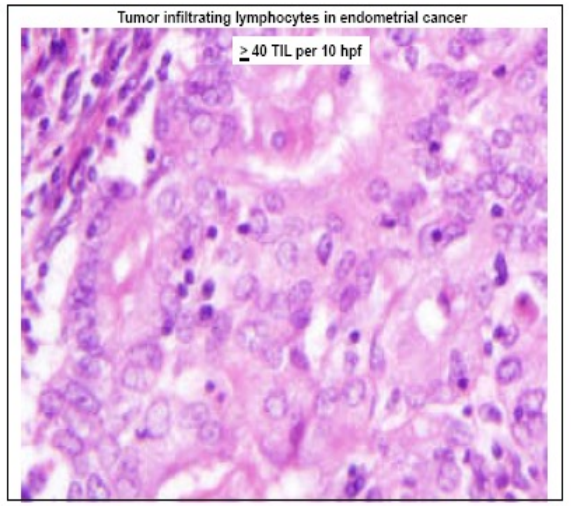
## MORPHOLOGICAL FEATURES

- 2% of EC
- 45-55 ys

***The gross appearance and the histological features have distinctive features:***

***LOWER UTERINE SEGMENT ORIGIN***
***PERITUMORAL AND TUMORAL INFILTRATING LYMPHOCYTES***
***SYNCHRONOUS OVARIAN CANCER (CCC)***
***HIGHER GRADE WITH UNDIFFERENTIATED COMPONENT***

- ***> LVI***
- ***> DEEP MYOINVASION***
- ***> STAGE***




***BUT....***

***SPORADIC ECs MMRd with:***

***- MLH1 HYPERMETHYLATION***
***- 2 SOMATIC MUTATIONS MMR gene***
***- 1 SOMATIC MUTATION MMR gene + LOH***
***- EPIGENETIC SILENCING OF MSH6, POST NAD CHT/RT***

**25-30%**

ASCO
752 Volume 40, Issue 7
Journal of Clinical Oncology



original reports

# Pembrolizumab in Patients With Microsatellite Instability–High Advanced Endometrial Cancer: Results From the KEYNOTE-158 Study

David M. O'Malley, MD[1]; Giovanni Mendonca Bariani, MD[2]; Philippe A. Cassier, MD[3]; Aurelien Marabelle, MD, PhD[4]; Aaron R. Hansen, MBBS[5]; Ana De Jesus Acosta, MD[6]; Wilson H. Miller Jr, MD, PhD[7,8]; Tamar Safra, MD[9,10]; Antoine Italiano, MD, PhD[11,12]; Linda Mileshkin, MBBS[13]; Lei Xu, PhD[14]; Fan Jin, MD[14]; Kevin Norwood, MD[14]; and Michele Maio, MD[15]

**CONCLUSION** Pembrolizumab demonstrated robust and durable antitumor activity and encouraging survival outcomes with manageable toxicity in patients with previously treated, advanced MSI-H/dMMR endometrial cancer.

MERCK

Media > News releases > News release

## FDA Approves Merck's KEYTRUDA® (pembrolizumab) for Patients With MSI-H/dMMR Advanced Endometrial Carcinoma, Who Have Disease Progression Following Prior Systemic Therapy in Any Setting and Are Not Candidates for Curative Surgery or Radiation

The objective response rate (ORR) was 46% (95% CI, 35-56) for patients who received KEYTRUDA, including a complete response rate of 12% and a partial response rate of 33%, at a median follow-up time of 16.0 months (range, 0.5 to 62.1 months).
Of the responding patients (n=41), 68% had responses lasting 12 months or longer, and 44% had responses lasting 24 months or longer. Median duration of response (DOR) was not reached (range, 2.9 to 55.7+ months).

# … ESGO/ESTRO/ESP 2020 GUIDELINES

Histopathology

*Histopathology* 2020, **76**, 52–63. DOI: 10.1111/his.14015

REVIEW

## Incorporation of molecular characteristics into endometrial cancer management

Lisa Vermij,[1] Vincent Smit,[1] Remi Nout[2] & Tjalling Bosse[1]

[1]*Department of Pathology, Leiden University Medical Center, and* [2]*Department of Radiation Oncology, Leiden University Medical Center, Leiden, the Netherlands*

INTEGRATED HISTOMOLECULAR EC CLASSIFICATION

POLE pathogenic variants:
*Pro286Arg*
*Val411Leu*
*p.Ser297Phe*
*p.Ala456Pro*
*p.Ser459Phe*

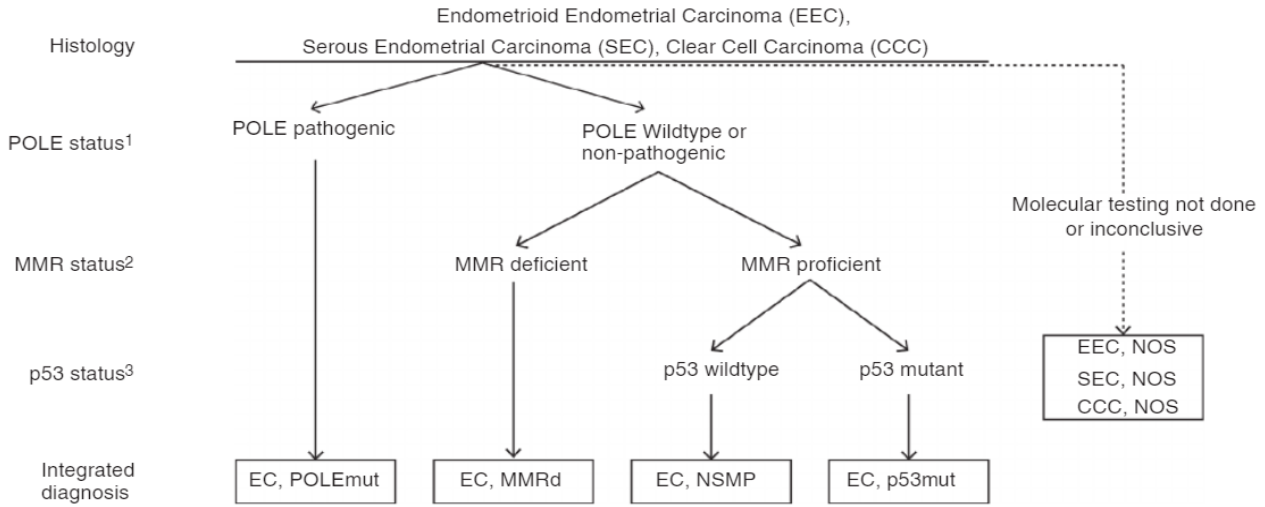



**Figure 1.** Diagnostic algorithm for a 'histomolecular' endometrial cancer classification. [1]Pathogenic polymerase-epsilon (*POLE*) variants include: P286R, V411L, S297F, A456P and S459F. [2]Mismatch repair protein (MMR) deficiency is defined by the loss of one or more MMR-proteins (MLH1, PMS2, MSH2 and MSH6). [3]p53 immunohistochemistry (IHC) is an acceptable surrogate marker for *TP53* mutational status in MMR-proficient, *POLE* wild-type endometrial cancer (EC).[50]

# … THE FIRST ITALIAN SURVEY

PATHOLOGICA 2022;114:189-198;
DOI: 10.32074/1591-951X-775

Guidelines

## Biomarker characterization in endometrial cancer in Italy: first survey data analysis

Gian Franco Zannoni[1,2], Angela Santoro[2], Nicoletta D'Alessandris[2], Giulia Scaglione[2], Frediano Inzani[2], Giuseppe Angelico[3], Emma Bragantini[4], Alessia Piermattei[2], Federica Cianfrini[2] Brigitte Bisaro[5], Matteo Fassan[6] and Members of PAGINE (SIAPEC) - Collaborators*

IHC based method for the biomarker analysis in EC emerges as the preferred and adopted diagnostic tool by the majority of investigated labs.

The most common prognostic assessment strategy in EC in Italy includes analysis of:
- MMR by IHC in all samples
- MLH1 promoter hypermethylation
- other biomarkers such as p53

The complete panel, including POLE analysis, is adopted only by a minority of labs, because this molecular test is not still reimbursed by the National Health Service.

It may be possible to restrict POLE sequencing to low-risk EC showing abnormal or subclonal p53 staining and omitted in advanced (stage III-IV) ECs since adjuvant therapy is always performed regardless of molecular classification.

# CONCLUSIONS

**Molecular classifiers** combined with **Clinical risk groups** and **Pathological parameters** showed an improved ability to discriminate outcomes

Both pathologic and molecular classifications may be integrated in pathology report

*'Independent of 'histomolecular' type, certain histopathological characteristics, such as the extent of LVSI and stage, do not have a molecular surrogate and will remain essential in the pathological assessment of a hysterectomy with EC.*
*The value of histological type and FIGO grade is less certain, but it is recommended to still report on these in the years to come'*

British Journal of Cancer (2015) 113, 299–310 | doi: 10.1038/bjc.2015.190

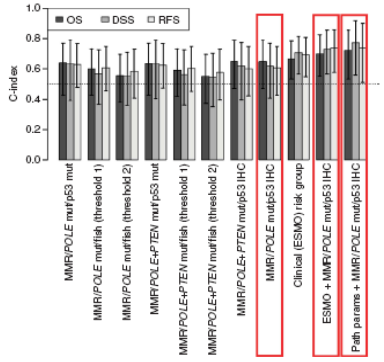
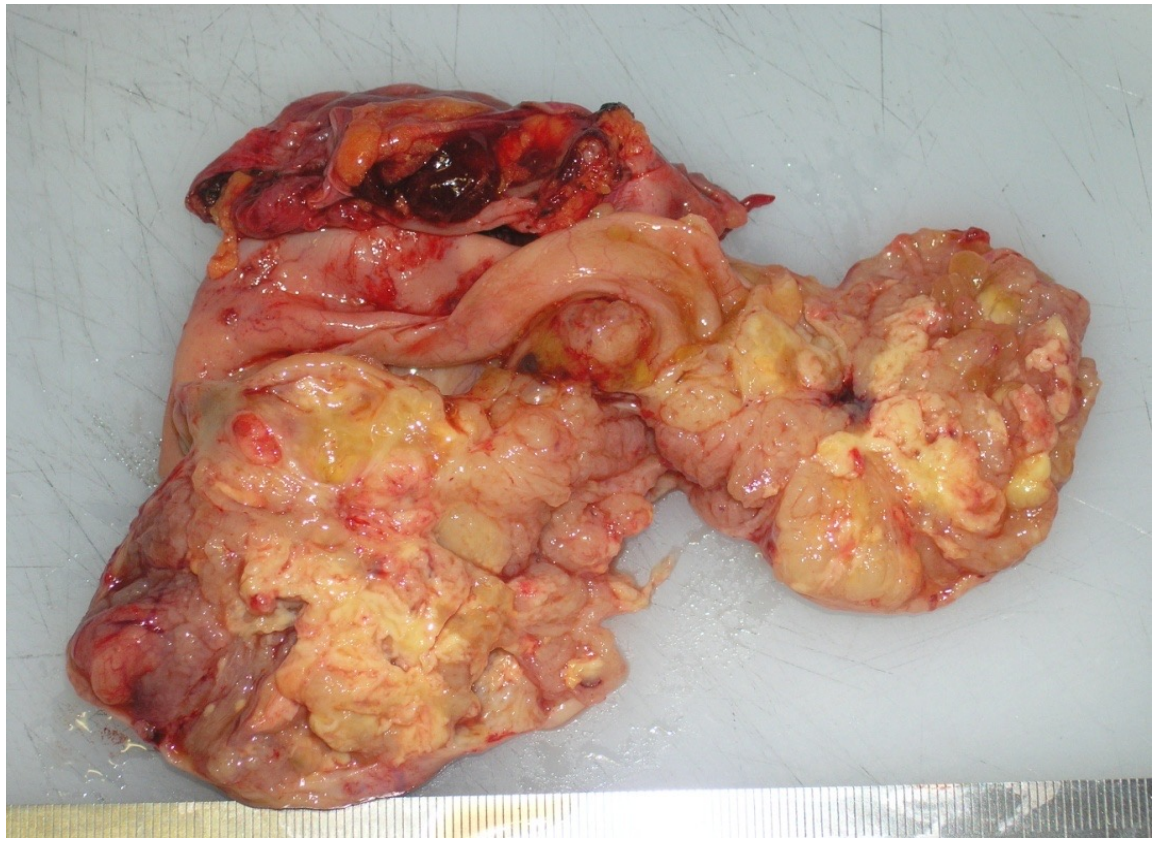



Figure 2. Harrell's C-Index for Models 1 to 8, ESMO clinical risk group, and combined molecular and risk groups or pathologic parameters as applied to the Vancouver cohort ($n=143$). A C-index of 0.5 (dotted line) indicates that the model has no discriminative ability and a C-index of 1 indicates that a model perfectly distinguishes between those who have an event and those who do not. The pragmatic model chosen to move forward with is outlined in red. Also outlined are the indices for the molecular classifier combined with clinical risk groups or pathological parameters, suggesting an improved ability to discriminate outcomes when taken together.

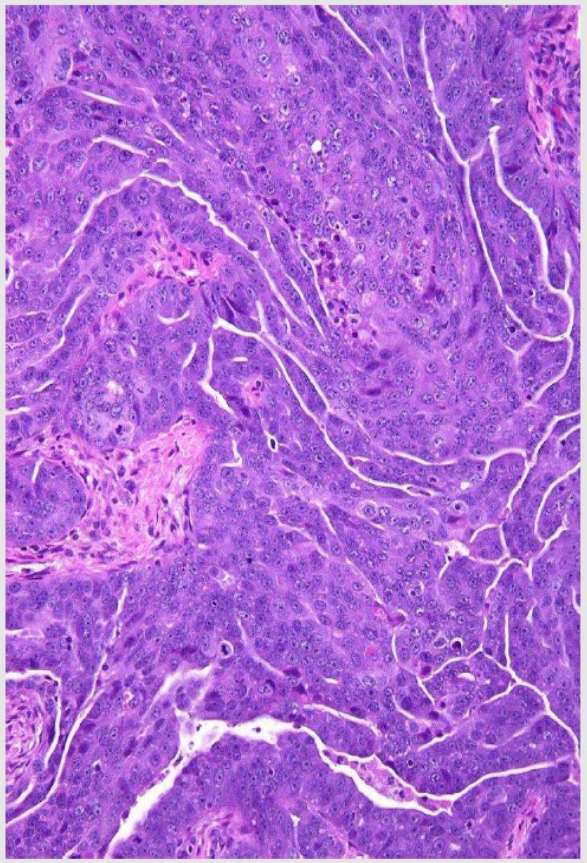
Vermij L, Smit V, Nout R & Bosse T
(2020) *Histopathology* **76**, 52–63. https://doi.org/10.1111/his.14015
**Incorporation of molecular characteristics into endometrial cancer management**

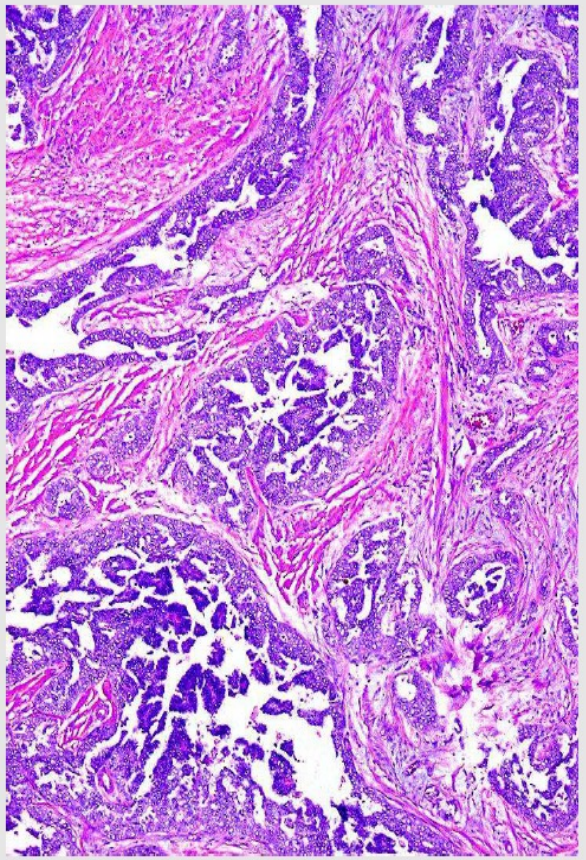
Multi-disciplinary EC patients management
Target therapies

# …OMICS IN OVARIAN CANCERS



# High grade serous carcinoma





# High Grade Serous Carcinoma

## High Genomic instability (type II carcinoma)

**TP53 almost always mutated**

**BRCA1/BRCA2 inactivation in 30-40% of HGSC (somatic/germline mutations or promoter methylation)**

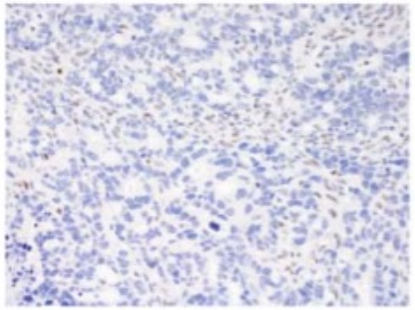
Widespread copy number changes
CCNE1, NOTCH3 activation
Rb, NF1 inactivation

# Optimized p53 immunohistochemistry is an accurate predictor of *TP53* mutation in ovarian carcinoma

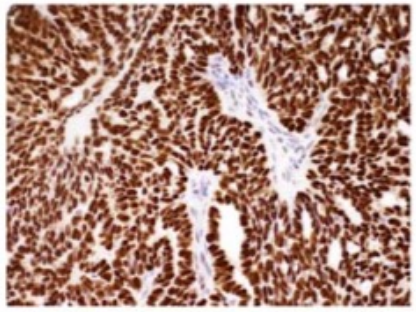
Martin Köbel,[1†] Anna M Piskorz,[2†] Sandra Lee,[1] Shuhong Lui,[1] Cecile LePage,[3,4] Francesco Marass,[2] Nitzan Rosenfeld,[2] Anne-Marie Mes Masson[3,4] and James D Brenton[2*]

## 3 aberrant immunophenotype of p53

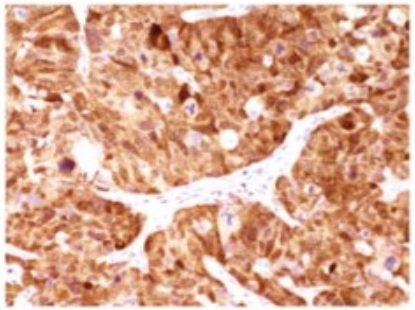
Abnormal



**P53 null-phenotype**

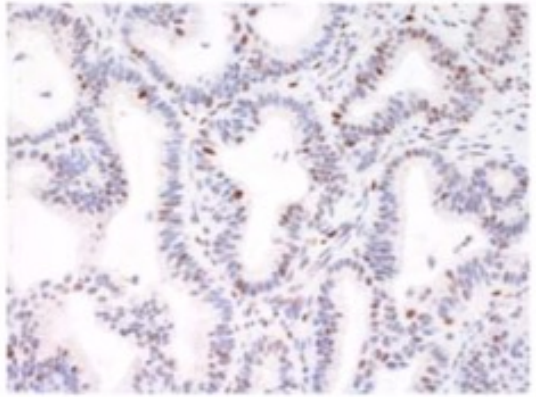


**P53 over-expression**



**Strong cytoplasmic staining in the absence of strong nuclear expression** (reflects the loss of function of p53, which is unable to enter the nucleus)

Normal



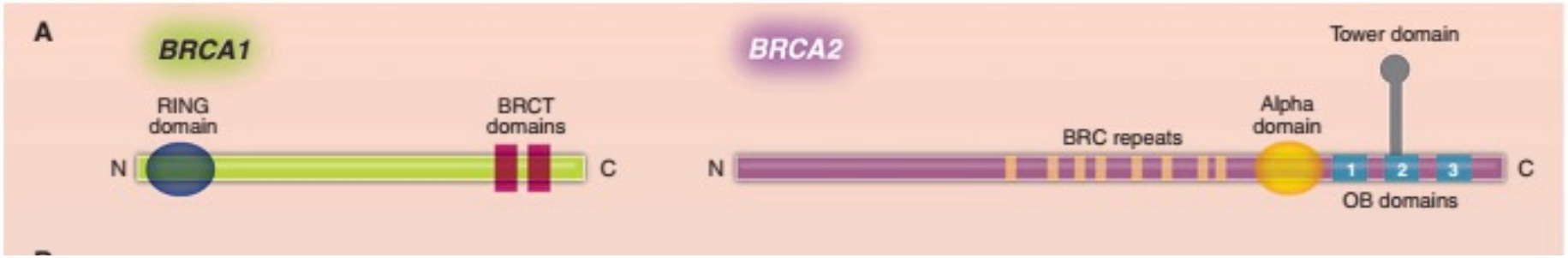
**Wild-type – 4% HGSCs**

*J Path: Clin Res* October 2016; **2:** 247–258

# Hereditary predisposition to Ovarian Cancer

## HEREDITARY BREAST-OVARIAN CANCER SYNDROME

Caused by BRCA1 or BRCA2 germline mutations; it represent 65-75% of all hereditary  ovarian cancer.




**17q21.31** **13q12.3**

Modern Pathology (2012) 25, 625–636



# Morphologic patterns associated with *BRCA1* and *BRCA2* genotype in ovarian carcinoma

Robert A Soslow[1], Guangming Han[2], Kay J Park[1], Karuna Garg[1], Narciso Olvera[1], David R Spriggs[3], Noah D Kauff[3,4] and Douglas A Levine[5]

[1]*Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA;* [2]*Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada;* [3]*Gynecologic Medical Oncology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA;* [4]*Clinical Genetics Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA and* [5]*Gynecology Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

**Table 1** Summary of morphologic characteristics of the training set organized by genotype

| | *Tumor-infiltrating lymphocytes per high-power field, median (range)* | *Mitotic index, median (range)* | *Solid, pseudoendometrioid, and transitional cell carcinoma-like (SET) features* | *Necrosis* | *Nuclear pleomorphism* | *Fallopian tube involvement* |
|---|---|---|---|---|---|---|
| *BRCA1* germline ($n=4$) | 31 (10–69) | 52 (40–71) | 2/4 | 3/4 | 2/4 | 1/1 |
| *BRCA1* somatic ($n=6$) | 29 (5–62) | 64 (19–105) | 4/6 | 3/6 | 3/6 | 5/5 |
| *BRCA1* methylated ($n=13$) | 52 (13–88) | 60 (17–102) | 10/13 | 7/13 | 7/13 | 4/5 |
| *BRCA2* germline ($n=4$) | 19 (5–43) | 62 (8–126) | 4/4 | 4/4 | 4/4 | 3/3 |
| *BRCA2* somatic ($n=4$) | 16 (12–60) | 35 (12–60) | 2/4 | 0/4 | 3/4 | 2/2 |
| *BRCA* unassociated ($n=12$) | 29 (2–140) | 37 (15–94) | 2/12 | 2/12 | 3/12 | 6/10 |
| *P*-value[a] | 0.034[b] | 0.012[b] | 0.0045 | 0.034 | 0.16 | 0.65 |
| Overall ($n=43$) | 36 (mean)<br>29 (median) | 53 (mean)<br>51 (median) | 24/43 | 19/43 | 21/43 | 21/26 |

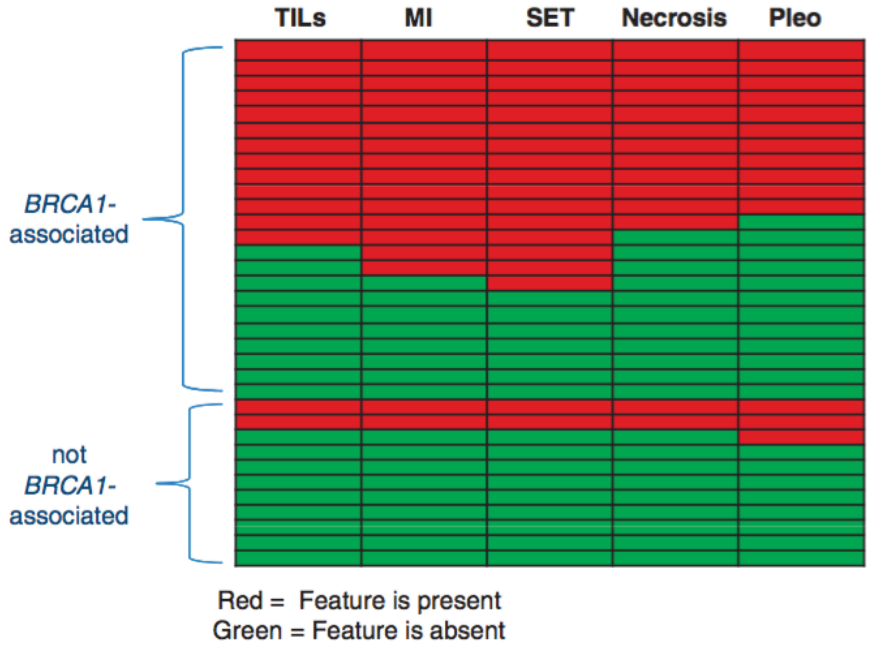
# Morphologic patterns associated with *BRCA1* and *BRCA2* genotype in ovarian carcinoma

Robert A Soslow[1], Guangming Han[2], Kay J Park[1], Karuna Garg[1], Narciso Olvera[1], David R Spriggs[3], Noah D Kauff[3,4] and Douglas A Levine[5]

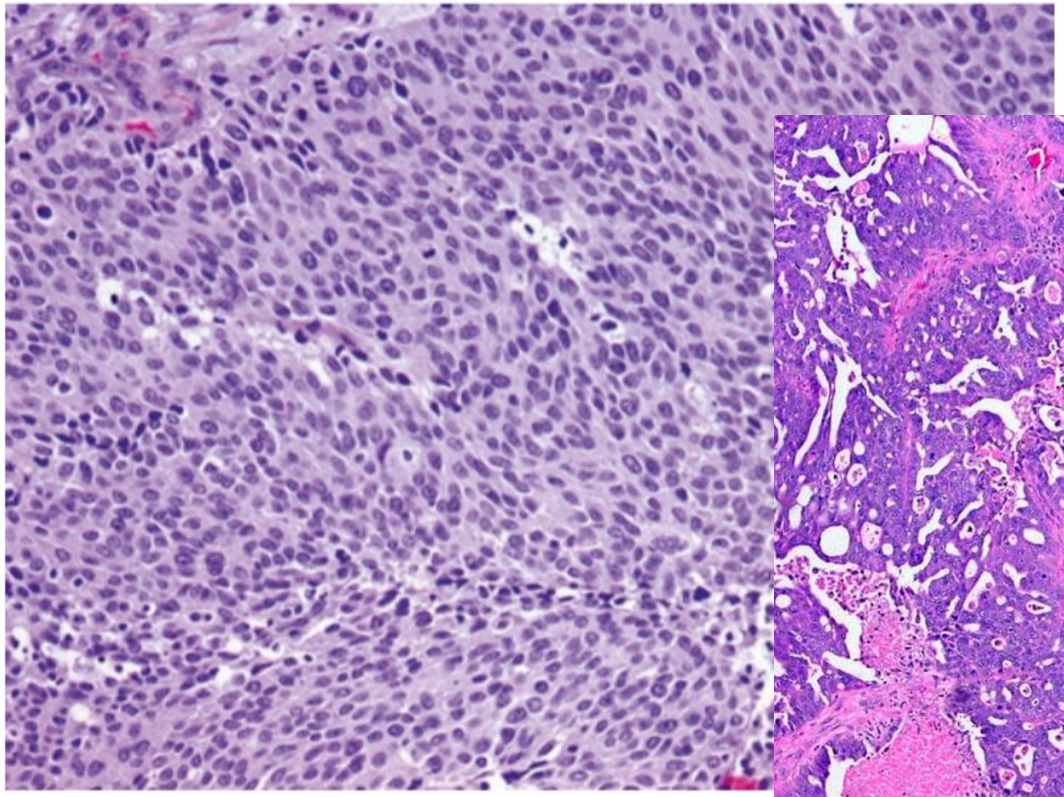
## Morphological Features of HGSC BRCA-related

(both germline and somatic mutations)

- **SET** (Solid/pseudoEndometrioid/Transitional) pattern
- **Tumor infiltranting lymphocytes** (TIL)
- **Tubal involvement**
- Severe Pleomorphism
- Higher Mitotic Count
- Frequent necrosis

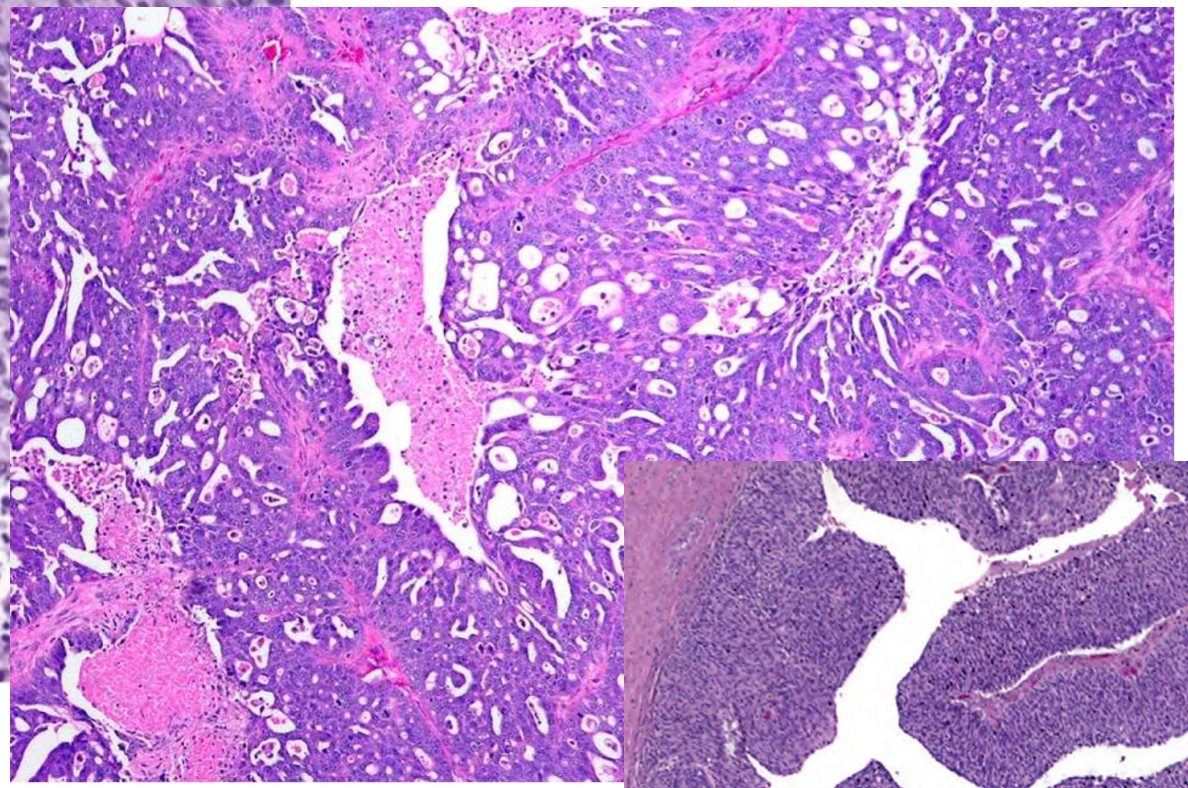


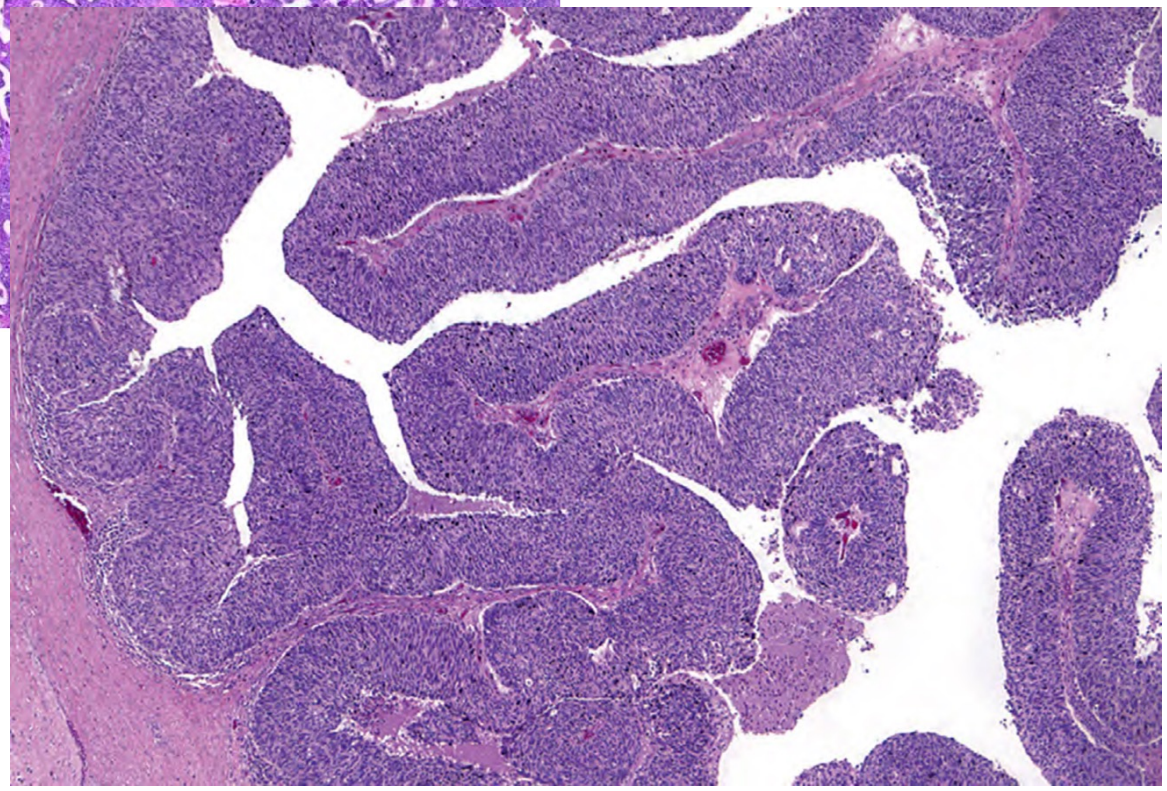

*Modern Pathology 2012; 25:625*



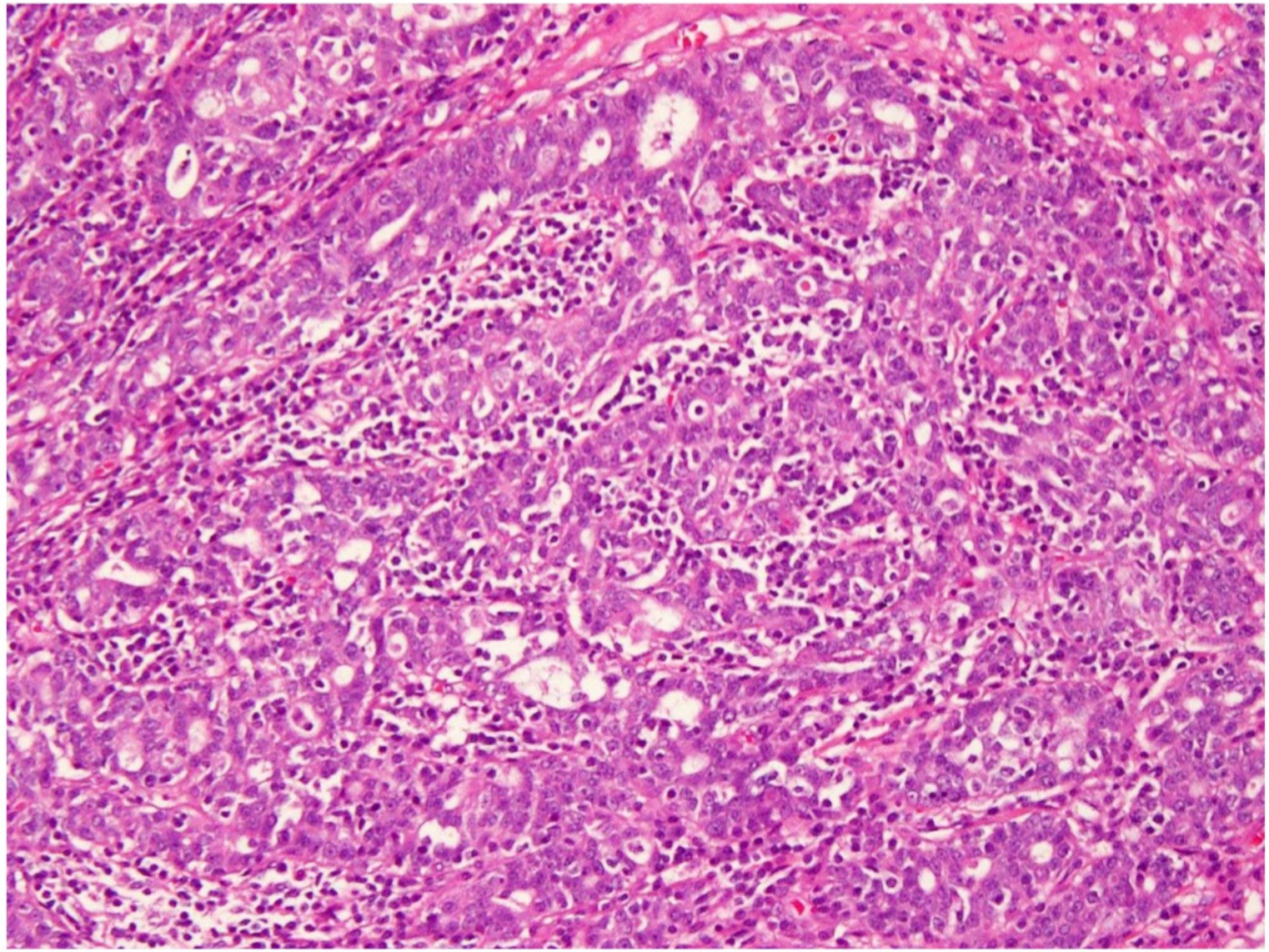
SET Solid

SET pseudo-Endometriod





SET-Transitional



TIL

# BRCA 1 - BRCA 2 and Ovarian cancer Patients

- ~ 25-30% of sporadic OvCa pts carry a BRCA mutation
- ~ 8% carries a SOMATIC mutation in absence of a germline
- More than 50% of germline showed LOH at somatic level

***Germline BRCA mutations***
- Blood sample
- Inherited mutations found in all body cells1

***Tumour BRCA mutations***
- Tumour sample
- Acquired mutations (somatic) found only in tumour cells2

**Germline** *BRCA* mutations can be detected in a **blood** sample[3]

**Somatic** *BRCA* mutations can be detected only in the **tumour** sample[3]


1. National Cancer Institute. http://www.cancer.gov/dictionary?cdrid=46384 [accessed January 2018].
2. National Cancer Institute. http://www.cancer.gov/dictionary?CdrID=46586. [accessed January 2018].
3. Vergote I et al. *Euro J Cancer* 2016; 69:127-1.

# What is the ideal sample to perform tBRCA1/2 assay?

- BRCA1/2 FFPE tumour testing should be performed on *PRIMARY TUMOURS (FFPE or FRESH)*

*It should be noted that the analysis of metastatic tissue at the time of progression may provide a more accurate indication of tumors likely to respond to PARPi treatment, due to the evidence supporting the association of revertant mutations and treatment resistance*

- Fresh-frozen specimens (FFS) provide better quality DNA
- Unfortunately, FFS are not routinely available from most referring centres
- FFPE is likely to be the most widely available sample type

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

**Performance of multiplicom's BRCA MASTR Dx kit on the detection of *BRCA1* and *BRCA2* mutations in fresh frozen ovarian and breast tumor samples**

**Cindy Badoer[1], Céline Garrec[2], Dirk Goossens[3], Gillian Ellison[4], John Mills[4], M. Dzial[1], H. El Housni[1], Sarah Berwouts[3], Paola Concolino[5], Virginie Guibert-Le Guevellou[2], Capucine Delnatte[2], Jurgen Del Favero[3], Ettore Capoluongo[5,6,*], Stephane Bézieau[2,*]**

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Ettore Capoluongo, **email:** ettoredomenico.capoluongo@policlinicogemelli.it

**Keywords:** BRCA1-BRCA2, next generation sequencing, fresh frozen tumors, ovarian carcinoma, olaparib

**Received:** July 16, 2016 **Accepted:** September 08, 2016 **Published:** October 25, 2016

Virchows Archiv
June 2016, Volume 468, Issue 6, pp 697–705

NGS-based BRCA1/2 mutation testing of high-grade serous ovarian cancer tissue: results and conclusions of the first international round robin trial

***HIGH QUALITY TUMOR TESTING IS FUNDAMENTAL***

# *How many tumoral cells are needed?*

- High percentage of neoplastic cells to detect somatic mutations
- A sample is recommended to contain a percentage of neoplastic cells that is at least 3 times the method's limit of detection

*(eg, methodology with 5% limit of detection requires the area of tumor sample selected for DNA extraction to contain ≥15% neoplastic cells).*

- This should  allow for overestimation of neoplastic cell content, particularly in samples with large areas of inflammation

*Each laboratory should establish the minimum proportion and number of neoplastic cells needed*

Gornjec et al. BMC Cancer (2019) 19:296
https://doi.org/10.1186/s12885-019-5535-2

BMC Cancer

RESEARCH ARTICLE Open Access

# Cytology material is equivalent to tumor tissue in determining mutations of *BRCA 1/2* genes in patients with tubo-ovarian high grade serous carcinoma

Andreja Gornjec[1], Srdjan Novakovic[2], Vida Stegel[2], Marko Hocevar[3], Ziva Pohar Marinsek[4], Barbara Gazic[5], Mateja Krajc[6] and Erik Skof[7*]

## What about cytology?

**Abstract**

**Background:** High-grade serous ovarian cancer is a detrimental disease. Treatment options in patients with a recurrent disease are dependent on *BRCA1/2* mutation status since only patients with known BRCA mutation are eligible for treatment with poly(ADP-ribose) polymerase inhibitors (PARPi). The aim of this study was to compare concordance of *BRCA* mutation analyses from cytological samples (CS) with *BRCA* mutation analyses from histological formalin fixed paraffin embedded (FFPE) samples.

**Methods:** Mutation analysis of *BRCA1* and *BRCA2* genes was performed in 44 women diagnosed with primary or recurrent high-grade ovarian cancer from three different samples: blood, cytological sample (ascites, pleural effusion and enlarged lymph nodes) and tumor tissue. Results from all three samples were compared.

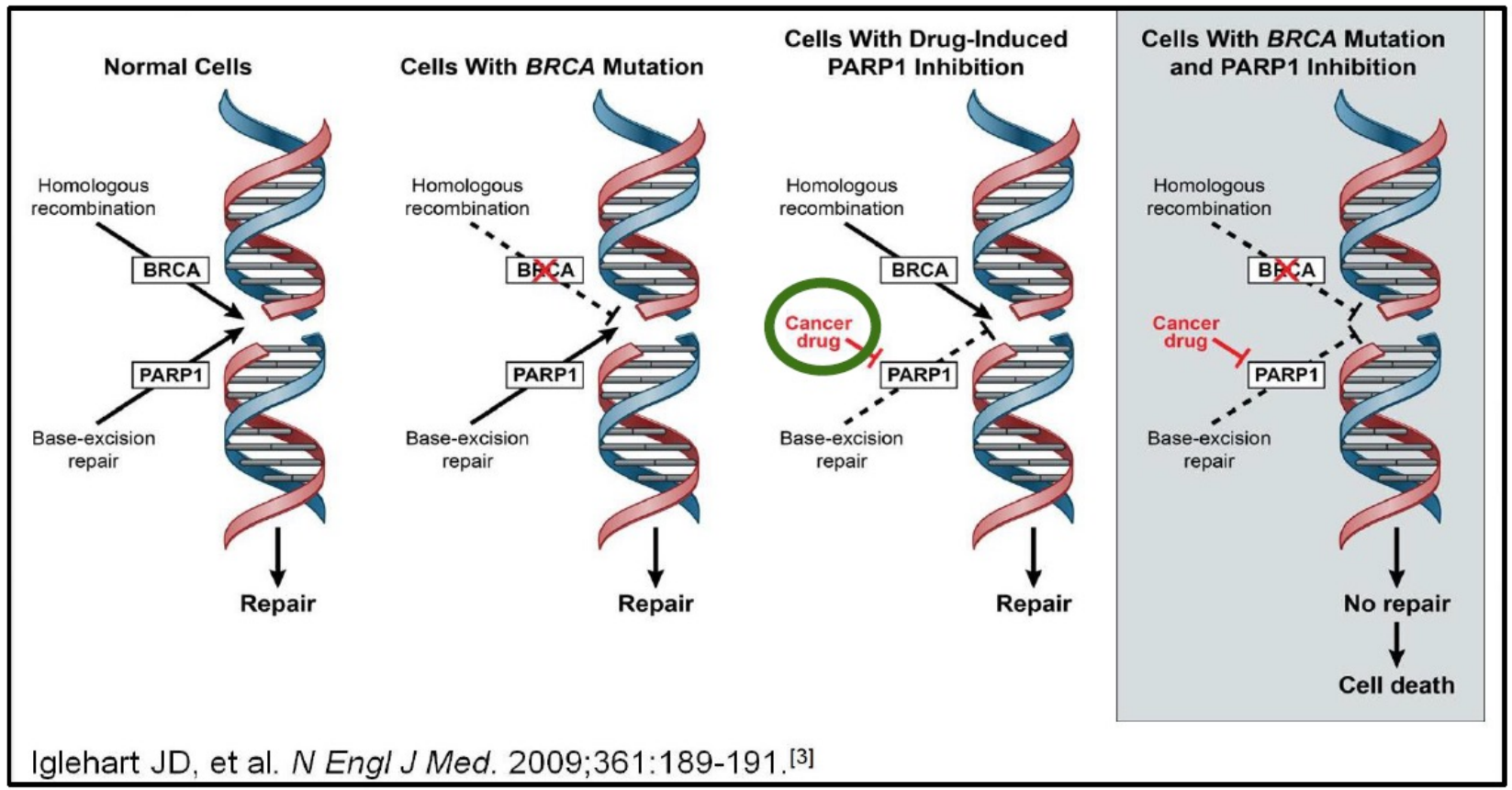
**Results:** Among 44 patients, there were 15 germline mutations and two somatic mutations. A 100% concordance was found between cytological and histologic samples.

**Conclusion:** There is a 100% concordance in *BRCA* mutation testing between cytological and histologic samples. *BRCA* mutation testing from CS could replace testing from FFPE tissue in clinical decision making in ovarian cancer patients.

**Trial registration:** The study was retrospectively registered at ISRCTN registry on 24/11/2015 - ISRCTN42408038.

**Keywords:** High-grade serous cancer, *BRCA1/2* mutation, *BRCA1/2* mutation testing, Cytological samples, Formalin fixed paraffin embedded samples

# BRCA-mutated: PREDICTIVE MARKER for a TARGET THERAPY with PARP INHIBITORS?




Iglehart JD, et al. *N Engl J Med*. 2009;361:189-191.[3]

MYRIAD myChoice® CDx

a FDA approved NGS-based *in vitro* diagnostic test assessing on DNA isolated from FFPE tumor tissue specimens:

- detection of single nucleotide variants, insertions and deletions, large rearrangement variants in protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes
- Genomic Instability Score (GIS) an algorithmic measurement of

***Loss of Heterozygosity (LOH)***
***Telomeric Allelic Imbalance (TAI)***
***Large-scale State Transitions (LST)***

The results of the test are used as an aid in identifying ovarian cancer patients with positive homologous recombination deficiency (HRD) status, who are eligible, or may become eligible because of a positive test result for ***deleterious or suspected deleterious mutations in BRCA1 or BRCA2 genes, or a positive Genomic Instability Score***, for treatment with the targeted therapy listed in Table 1 in accordance with the approved therapeutic product labeling.

**Table 1: Companion diagnostic indications**

| Tumor Type | Biomarker | Therapy |
|---|---|---|
| Ovarian Cancer | Myriad HRD (defined as deleterious or suspected deleterious mutations in BRCA1 and BRCA2 genes and/or positive Genomic Instability Score) | Lynparza® (olaparib)<br>Zejula® (niraparib) |

Detection of deleterious or suspected deleterious *BRCA1* and *BRCA2* mutations and/or positive Genomic Instability Score in ovarian cancer patients is also associated with enhanced progression-free survival (PFS) from Zejula® (niraparib) maintenance therapy.

FDA-approved for both first-line maintenance therapy and late line monotherapy for Zejula® (niraparib) for patients with ovarian cancer.

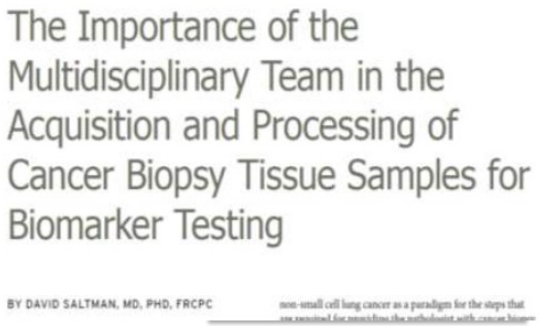
FDA-approved for first-line maintenance therapy for Lynparza ® (olaparib) for patients with ovarian cancer.

- Ovarian HGSC is a complex morphological diagnosis that required experienced pathologist

- Pathologists can be considered "diagnostic oncologists" and have a critical role as clinical consultants on the biology of disease.

- Tissue availability, ownership of archival tissue, type of diagnostic/biomarker test required, method of sample processing, concordance between different tests and testing centers, and tumor heterogeneity are necessary key-aspects to consider for adequate selection of patients and their samples

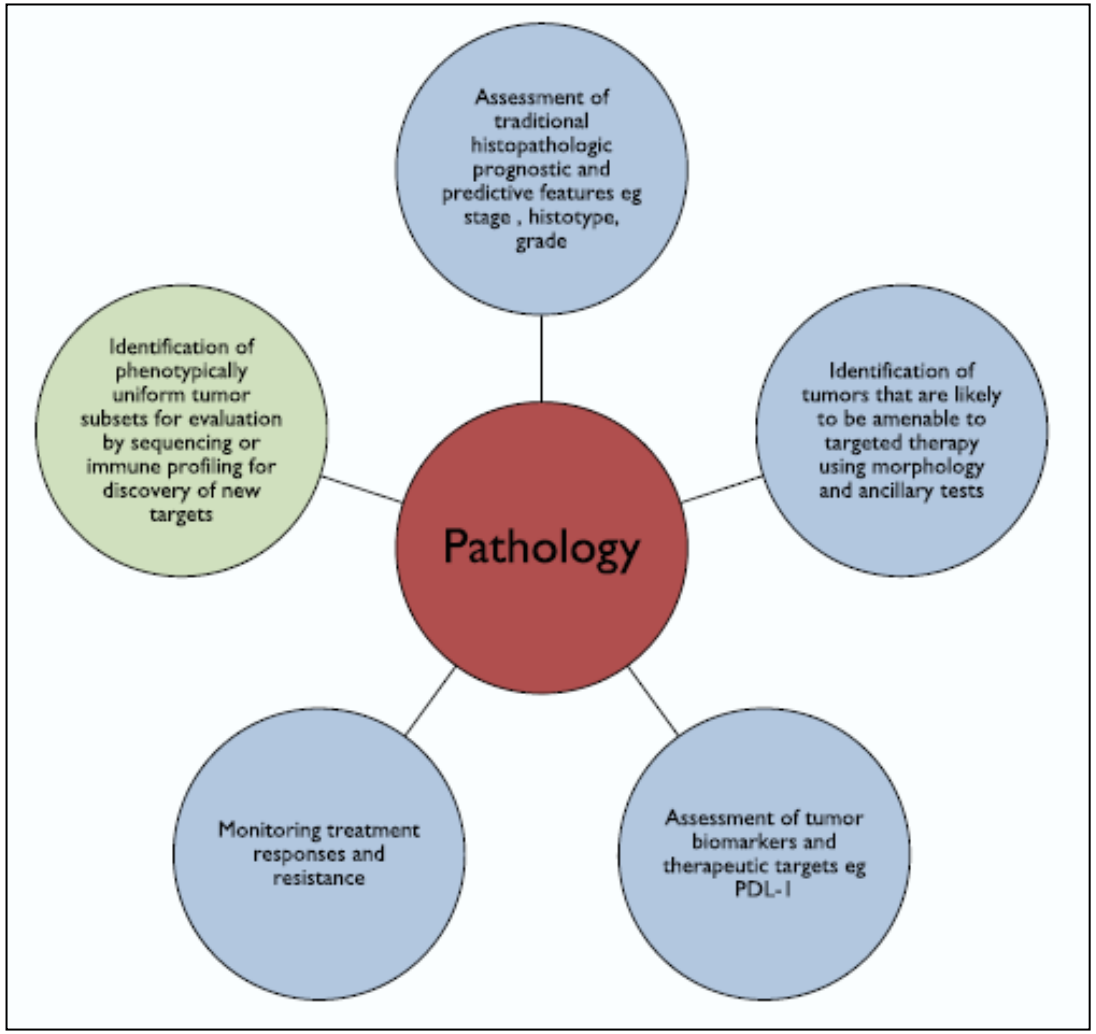
- It is important that cancer centres, pathology departments and molecular diagnostic laboratories develop effective communication strategies and standard operating procedures (SOPS) for the biomarker testing and reporting of results


The Importance of the Multidisciplinary Team in the Acquisition and Processing of Cancer Biopsy Tissue Samples for Biomarker Testing

BY DAVID SALTMAN, MD, PHD, FRCPC

# CONCLUSIONS

R. Murali et al. / Gynecologic Oncology xxx (2017) xxx–xxx




The roles of pathology in targeted therapy of women with gynecologic cancers

Thank You!