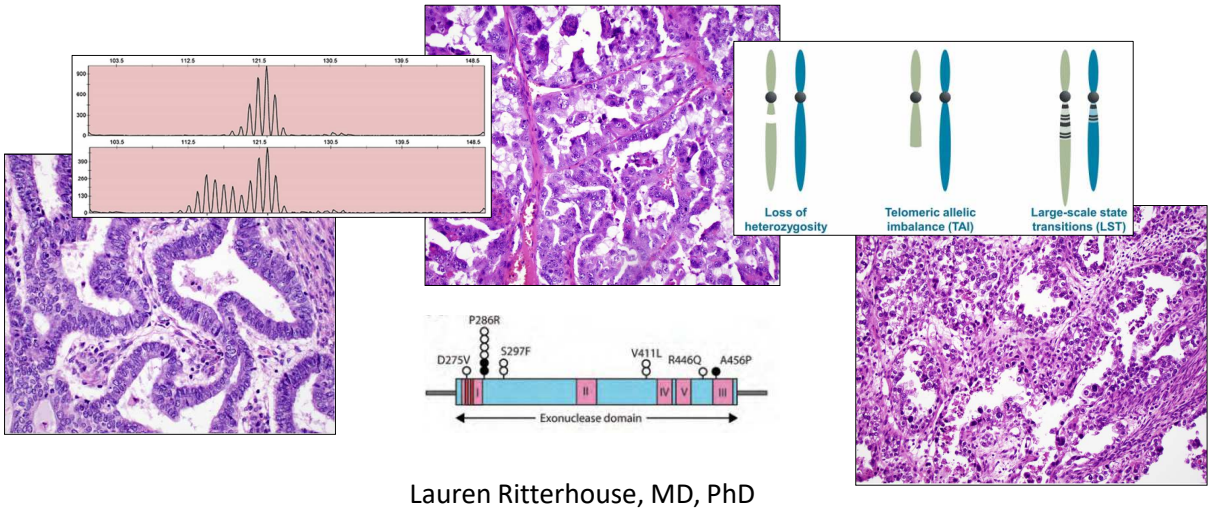
# Molecular diagnostics of gynecologic tumors

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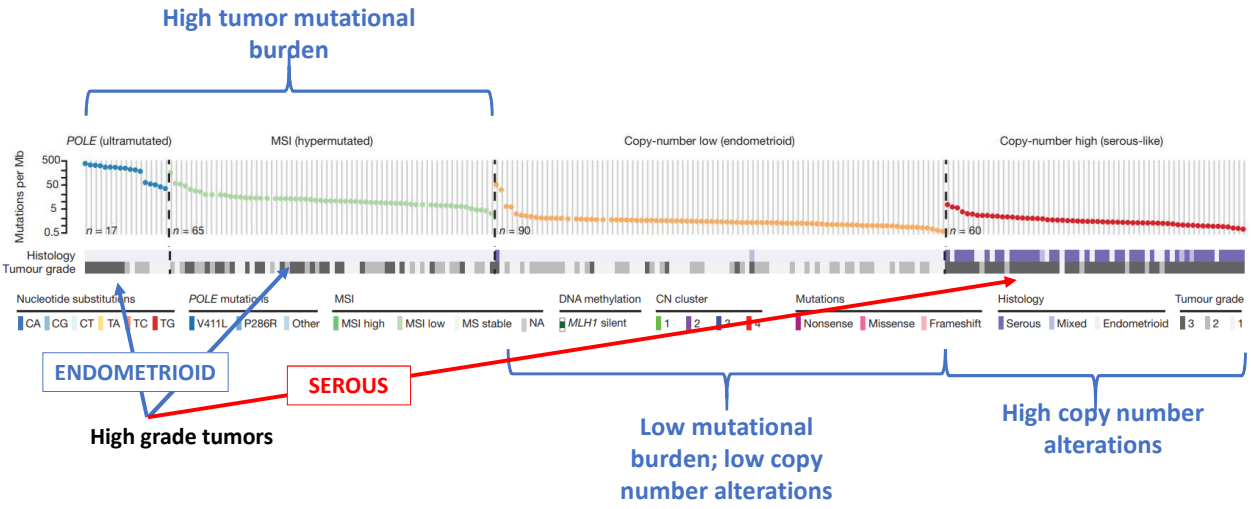
# Outline

- Uterine tumors
  - Endometrial
    - Molecular classification
      - *POLE* mutated
      - Microsatellite unstable (MSI/dMMR)
      - Copy number high
      - Copy number low
  - Malignant Mesenchymal Neoplasms
    - Leiomyosarcoma
    - Endometrial Stromal Sarcoma
- Ovarian tumors
  - Serous carcinomas
  - Homologous recombination deficiency (HRD)
  - Endometrioid/Clear cell carcinomas
- Inherited gynecologic tumors

# Endometrial carcinomas: Molecular classification




TCGA *Nature*. 2013 May 2;497(7447):67-73.

# Endometrial carcinomas: Molecular classification

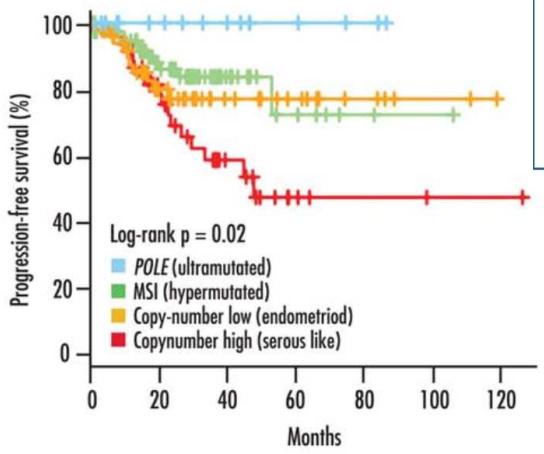
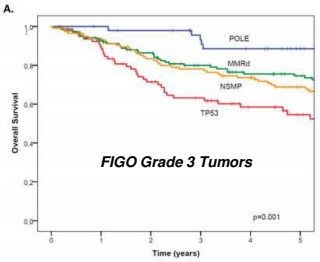



***Table 1***
**TCGA molecular subtypes of endometrial carcinoma**

| | Histotypes | Number of Mutations | Copy Number Alterations | Specific Genes Recurrently Altered |
|---|---|---|---|---|
| *POLE*-mutated (ultramutated) | Endometrioid (G3 > G2-1) | Very high | Low | *POLE, PTEN, PIK3R1, PIK3CA, FBXW7, KRAS, TP53* |
| MSI (hypermutated) | Endometrioid (G2-3 > G1) | High | Variable (low to intermediate) | *PTEN, KRAS, ARID1A* |
| Copy number: low | Endometrioid (G1-2 > G3) | Low | Variable (low to intermediate) | *CTNNB1, PTEN* |
| Copy number: high (serous-like) | Serous | Low | High | *TP53, FBXW7, PPP2R1A* |

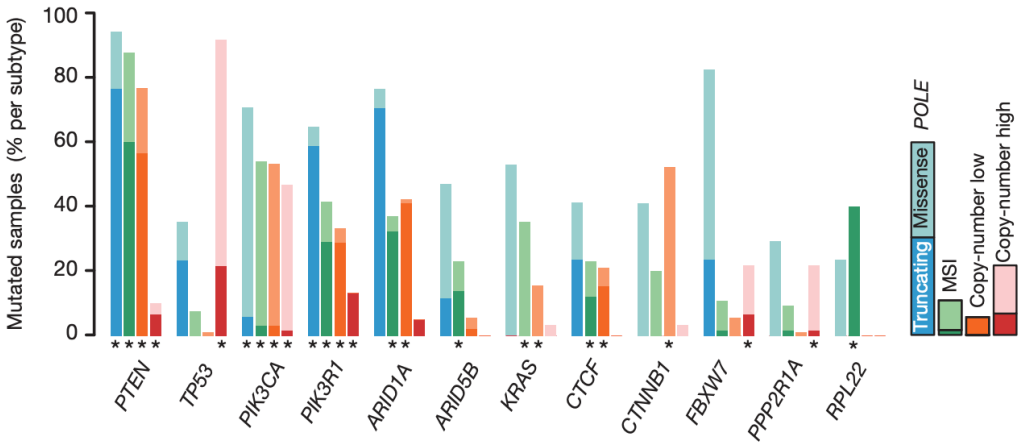



Am J Surg Pathol. 2018 May; 42(5): 561–568.

Surg Pathol Clin. 2016 Sep;9(3):405-26.

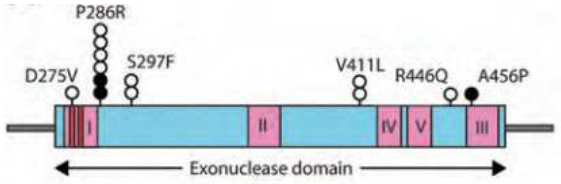
TCGA *Nature*. 2013 May 2;497(7447):67-73.

# Endometrial carcinomas: Molecular classification




TCGA *Nature*. 2013 May 2;497(7447):67-73.

# Endometrial carcinomas: *POLE*-mutated

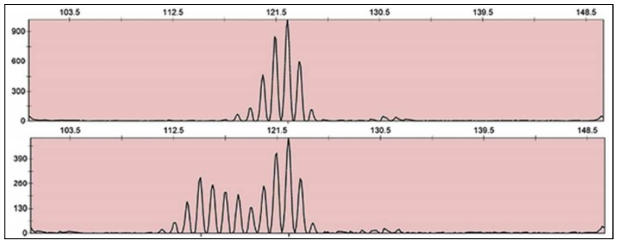



***POLE*-mutated Carcinomas (Key Points)**

| Molecular Features | Morphologic Features | Clinical Features |
|---|---|---|
| Ultramutated (mutation rate: median 232 mutations per megabase pair)<br>Minimal CNVs<br>Mutation in the exonuclease domain of *POLE* (hotspots at P286R and V411L) | Endometrioid, but may have high-grade features bordering on serous carcinoma (nuclear atypia)<br>Eosinophilic cytoplasm | 5%–10% of all endometrial carcinomas |
| Increased C > A transversion frequency<br>*PTEN*, *PIK3R1*, *PIK3CA*, *FBXW7*, *KRAS* mutations common<br>*TP53* mutation common, rare indels<br>Increased predicted neoantigen load<br>Majority are microsatellite stable | Lymphovascular invasion common<br>Increased tumor infiltrating lymphocytes levels<br>Higher mitotic index | Wide age range<br>Favorable prognosis in most studies |

Hum Mol Genet. 2013 Jul 15;22(14):2820-8.
Surg Pathol Clin. 2016 Sep;9(3):405-26.

# Endometrial carcinomas: Microsatellite Instability

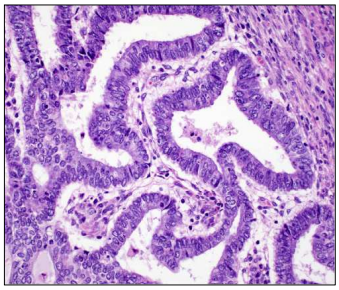



***MSI (Hypermutated) (Key Points)***

| Molecular Features | Morphologic Features | Clinical Features |
|---|---|---|
| Hypermutated (mutation rate: median 18 mutations per megabase pair; 10-fold higher than MSS tumors) | Most are endometrioid | Subset are caused by hereditary endometrial cancer (Lynch syndrome) |
| C > T at NpCpG sites and numerous small indels | FIGO grades 2–3 > grade 1 | 20%–40% of all endometrial carcinomas |
| Low numbers of CNVs | | |
| *KRAS*, *ARID1A*, *ARID5B*, *PTEN* mutations | | Prognosis similar to low copy number; intermediate between *POLE*-mutated and high-copy-number carcinomas |
| *RPL22* deletions | Increased TIL levels | |
| Few *TP53*, *FBXW7*, *CTNNB1*, *PPP2R1A* mutations | | |
| *MLH1* hypermethylation | | |
| Increased predicted neoantigen load | | |

Surg Pathol Clin. 2016 Sep;9(3):405-26.

# Endometrial carcinomas: Low Copy Number

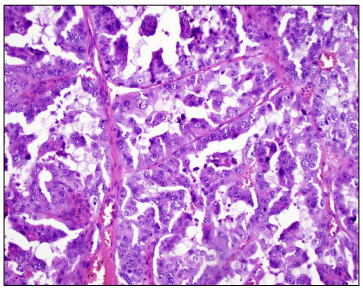


***Low Copy Number (Key Points)***

| Molecular Features | Morphologic Features | Clinical Features |
|---|---|---|
| Few CNVs<br>Few SNVs | Endometrioid, predominantly FIGO grade 1–2 | Most common subtype<br>Prognosis similar to MSI; intermediate between POLE-mutated and high-copy-number carcinomas |
| Frequent *CTNNB1* mutations (>50%), only gene mutated more frequently in low-copy-number group compared with MSI group | | |
| *PTEN*, *PIK3CA*, *PIK3R1*, and *ARID1A* are common mutations | | |

Surg Pathol Clin. 2016 Sep;9(3):405-26.

# Endometrial carcinomas: High Copy Number



***High Copy Number (Key Points)***

| Molecular Features | Morphologic Features | Clinical Features |
|---|---|---|
| High levels of CNVs<br>Low numbers of SNVs | Predominantly serous histotype | Older age at presentation |
| Frequent TP53 mutation (>90%)<br>*PIK3CA*, *FBXW7*, *PPP2R1A* mutations | Prominent nuclear atypia | Higher stage at presentation |
| Uncommon *PTEN* and *ARID1A* mutations<br>Gene amplifications: *CCNE1*, *MYC*, *PIK3CA*, *CDKN2A*, *ERBB2* | Subset of high-grade endometrioid carcinoma (FIGO grade 3) | Poor prognosis |

Surg Pathol Clin. 2016 Sep;9(3):405-26.

# Endometrial carcinomas: Prognostic/Predictive biomarkers

***Key Points***
**PROGNOSTIC BIOMARKERS IN UTERINE CANCER**

| Biomarker | Histotype | Prognosis |
|---|---|---|
| High copy number | Serous, high-grade endometrioid | Worse PFS |
| ER/PR loss | All | Worse DSS |
| *TP53* mutation | All | Worse PFS and DSS |
| *PIK3CA* mutation | High-grade endometrioid | Worse DSS |
| *CCNE1* amplification | High-grade endometrioid | Worse PFS and OS |
| *POLE* mutation | Endometrioid | Improved PFS |

*Abbreviations:* DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival.

FDA NEWS RELEASE

**FDA approves first cancer treatment for any solid tumor with a specific genetic feature**

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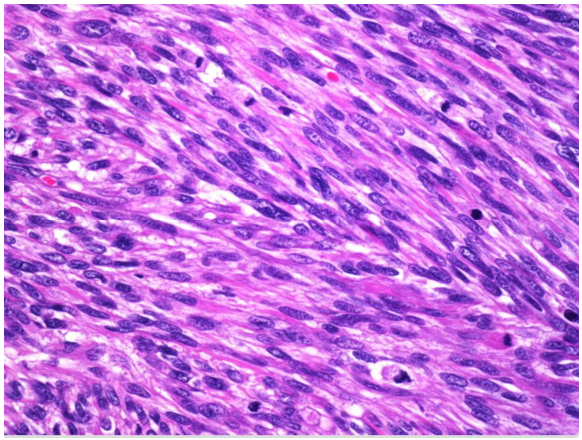
**For Immediate Release:** May 23, 2017

FDA approved ***pembrolizumab*** on May 23, 2017, for the treatment of adult and pediatric patients with:
unresectable or metastatic, microsatellite instability-high (**MSI-H**) or mismatch repair deficient (**dMMR**) solid tumors
progressed following prior treatment
who have no satisfactory alternative treatment options

Surg Pathol Clin. 2016 Sep;9(3):405-26.

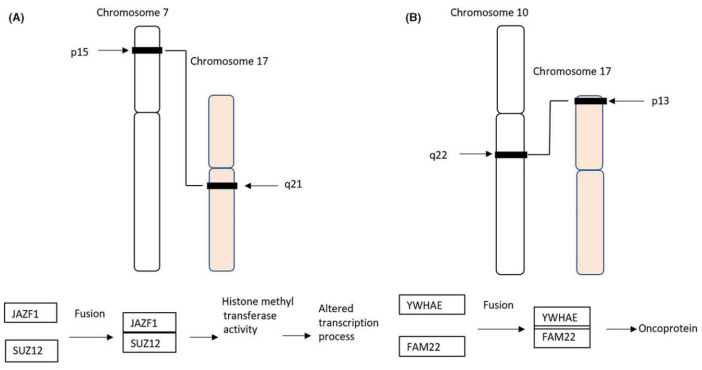
# Mesenchymal Tumors: Leiomyosarcomas

- Markedly complex karyotypes
- Recurrent karyotypic alterations include gain of **1q**, **17p**, and **Xp**
- Loss of heterozygosity for **10q** (containing *PTEN*) and/or **13q** (containing *RB1*) is present in greater than 50% of LMS
- *TP53* mutations may also be present in greater than 50% of cases



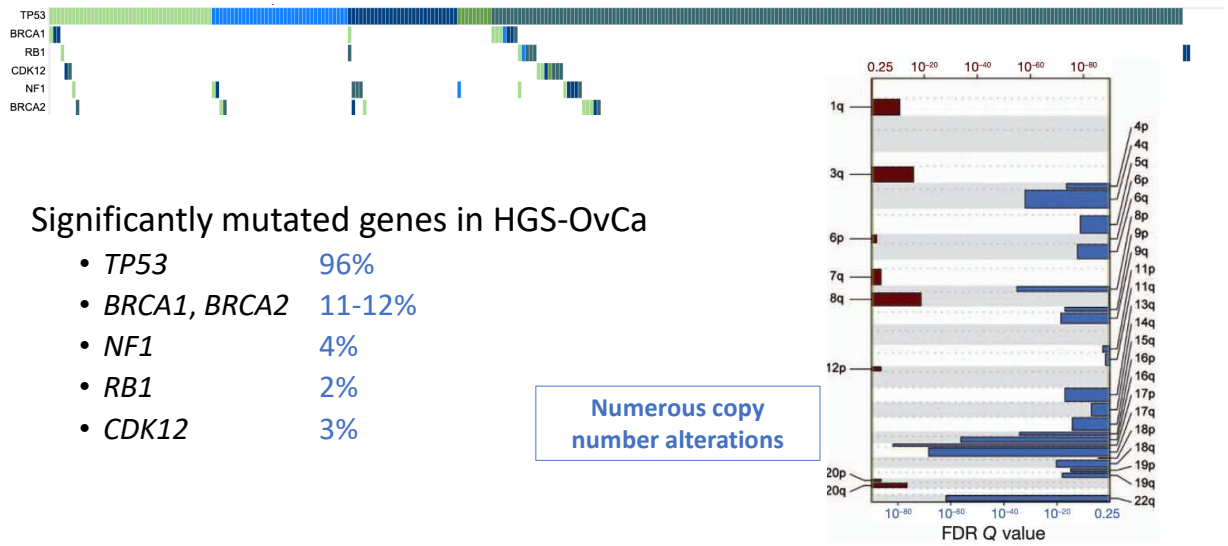
# Mesenchymal Tumors: Endometrial Stromal Sarcoma

- Highly recurrent translocations resulting in gene fusions
- *JAZF1-SUZ12* is the most common gene fusion in low-grade (LG) ESS (25% to >90% of cases)
- *JAZF1-PHF1* gene fusion, is present in up to 28% of ESS
- Small subset of ESS may have *PHF1* rearrangements resulting in fusion with genes other than *JAZF1* or *MEAF6*, most notably *EPC1* on 10p11
- *YWHAE-FAM22A/B* gene fusions high-grade ESS (HGESS)




Cancer Sci. 2018 Jun;109(6):1743-1752.

# High Grade Serous Ovarian Carcinoma



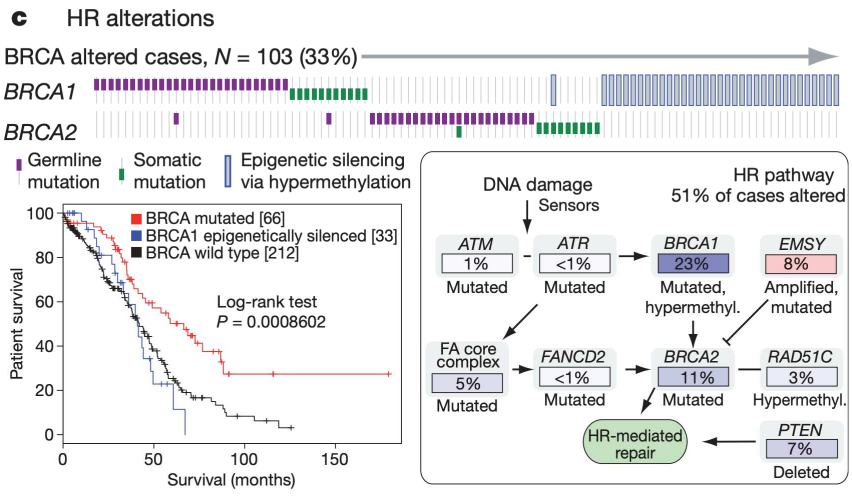

Significantly mutated genes in HGS-OvCa

- *TP53* 96%
- *BRCA1, BRCA2* 11-12%
- *NF1* 4%
- *RB1* 2%
- *CDK12* 3%

**Numerous copy number alterations**

Nature. 2011 Jun 29;474(7353):609-15.

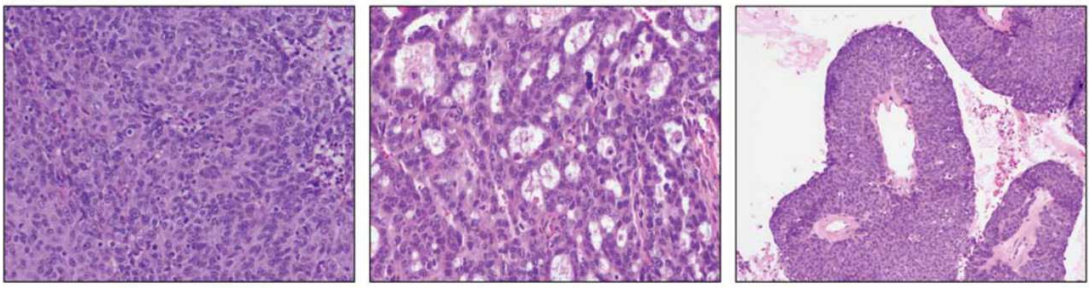
# High Grade Serous Ovarian Carcinoma: Homologous Recombination Deficiency




Nature. 2011 Jun 29;474(7353):609-15.

# High Grade Serous Ovarian Carcinoma: Molecular / Morphologic Correlation

SET morphology



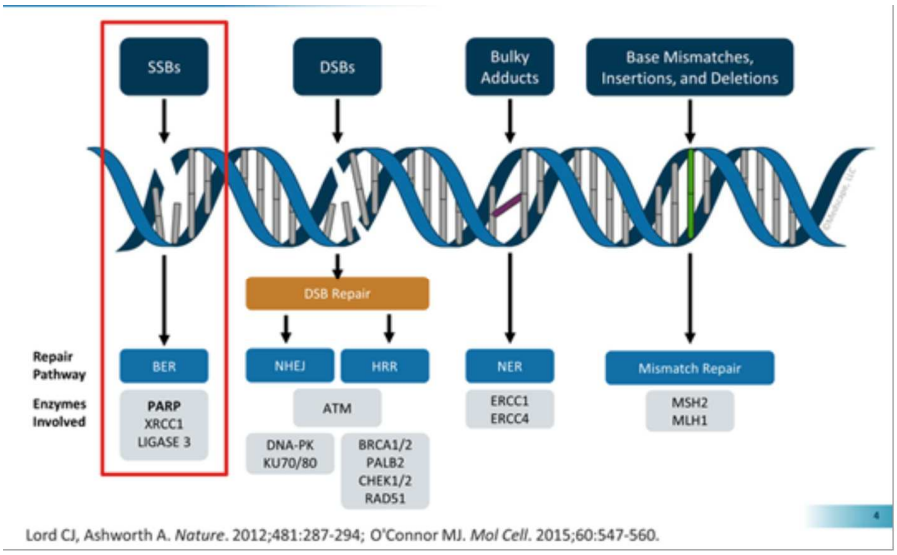
Solid | Pseudo-Endometrioid | Transitional

| *Histotype* | *HR* | *BRCA1/2* | *Non-BRCA HR* | |
|---|---|---|---|---|
| High-grade serous carcinoma ($n=138$) | 45% | 25% | 10% | *ATM* (6), *BRIP1* (5), *FANCC*, *FANCE*, *FANCG* |
| Classic ($n=40$) | 28% | 8% | 8% | *ATM*, *FANCC*, *FANCE* |
| Non-classic ($n=40$) | 70% | 45% | 10% | *ATM* (2), *BRIP1* (2) |
| Endometrioid carcinoma ($n=12$) | 25% | 0% | 25% | *ATM* (2), *RAD21* |
| Clear cell carcinoma ($n=10$) | 30% | 20% | 10% | *ATM* |
| Low-grade serous carcinoma ($n=7$) | 0% | 0% | 0% | |
| Mucinous carcinoma ($n=4$) | 25% | 25% | 0% | |

Mod Pathol. 2016 Aug;29(8):893-903.

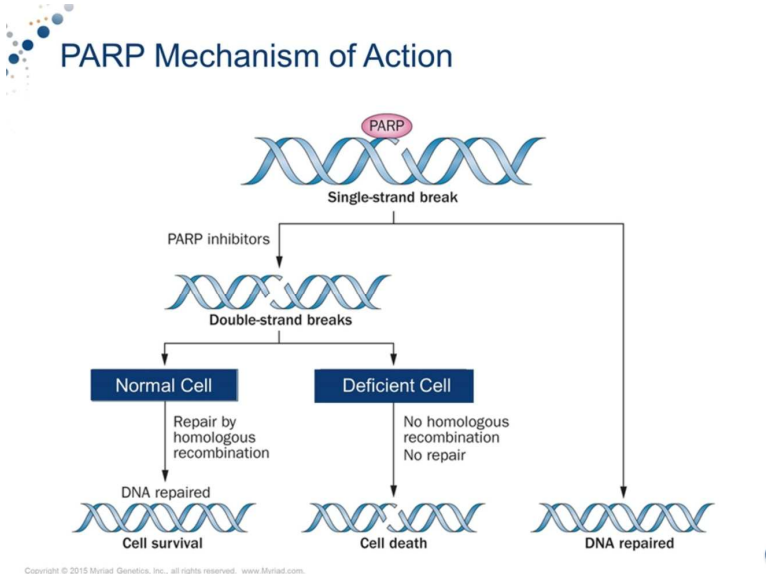
Mod Pathol. 2012 Apr;25(4):625-36.

# DNA Repair: Homologous Recombination

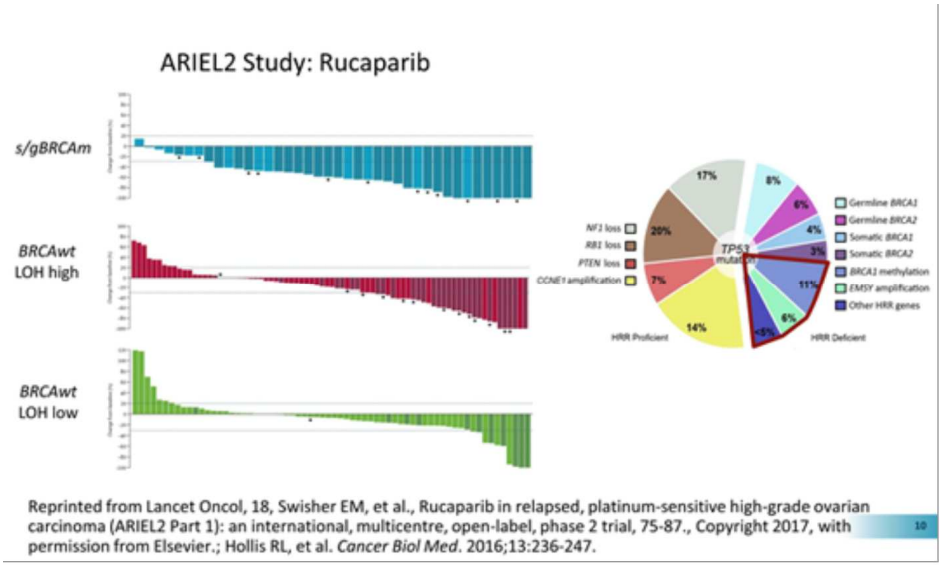



Lord CJ, Ashworth A. *Nature*. 2012;481:287-294; O'Connor MJ. *Mol Cell*. 2015;60:547-560.

# Homologous Recombination Deficiency: Therapeutic Relevance

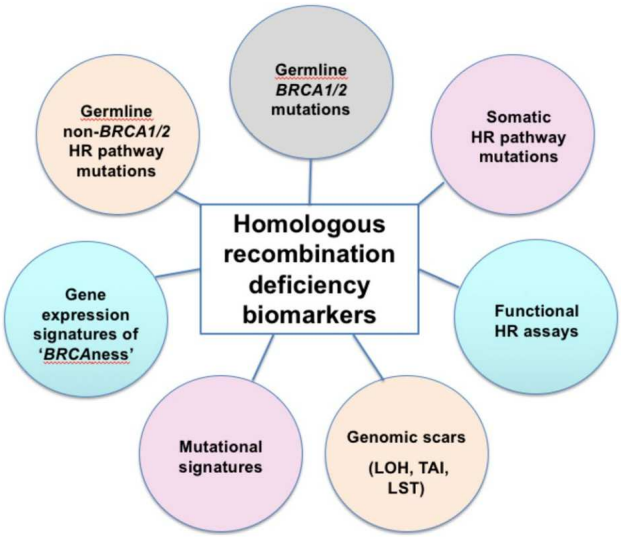



# Homologous Recombination Deficiency: Predict Response to PARP Inhibitors

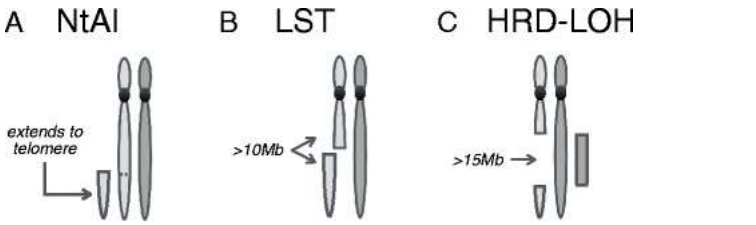



Reprinted from Lancet Oncol, 18, Swisher EM, et al., Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial, 75-87., Copyright 2017, with permission from Elsevier.; Hollis RL, et al. *Cancer Biol Med*. 2016;13:236-247.

## Homologous Recombination Deficiency : Biomarkers



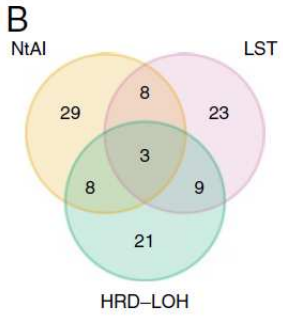

## Homologous Recombination Deficiency Scores




Number of Telomeric Allelic Imbalances

Large Scale Transition

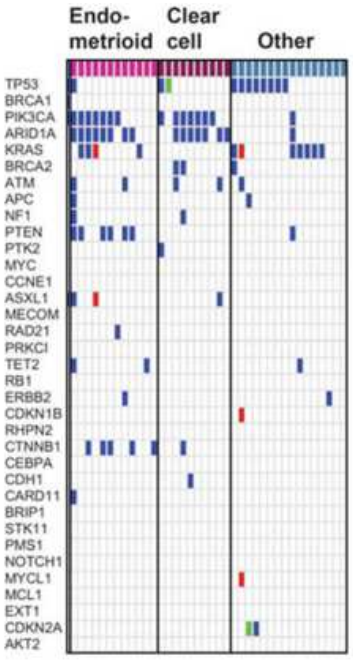
HRD-Loss of Heterozygosity

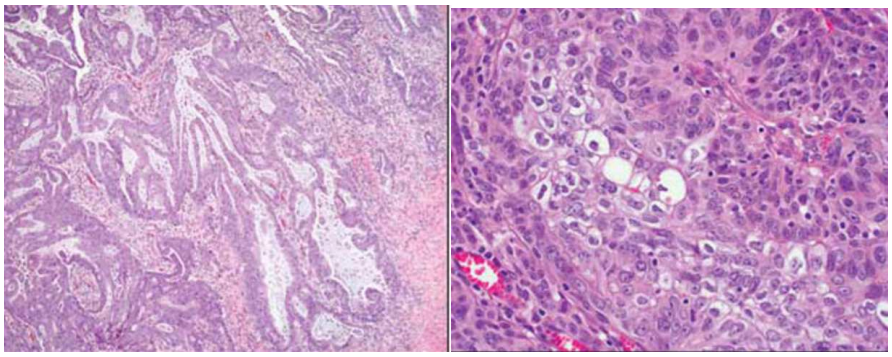



- All 3 signatures are defined differently, although there is some overlap
- All based on assumption that the measures are proportional to the number of times a tumor experienced error-prone DNA repair

Marquard 2015 et al.

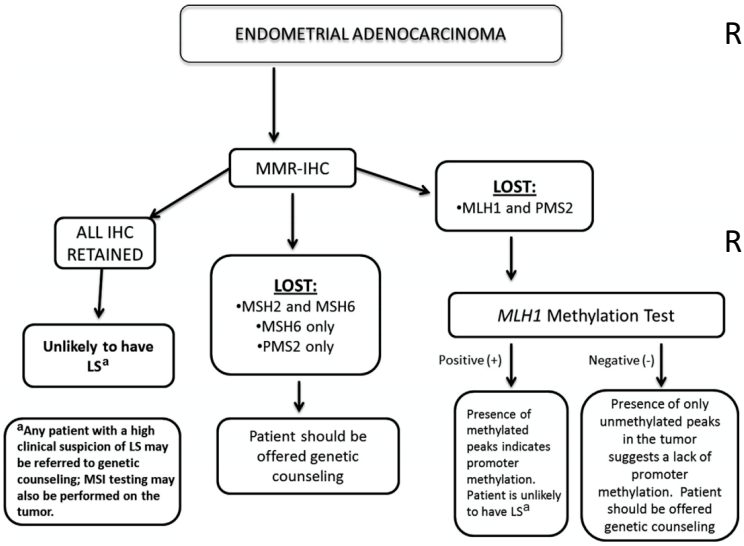
# Non-serous Ovarian Carcinomas



Mod Pathol. 2016 Aug;29(8):893-903.

# Inherited Gynecologic Tumors: Lynch syndrome




Risk for endometrial cancer:
- 15% *PMS2*
- 44% *MSH6*
- 25-60% *MLH1/MSH2*

Risk for ovarian cancer
- 4-13% *MLH1/MSH2*

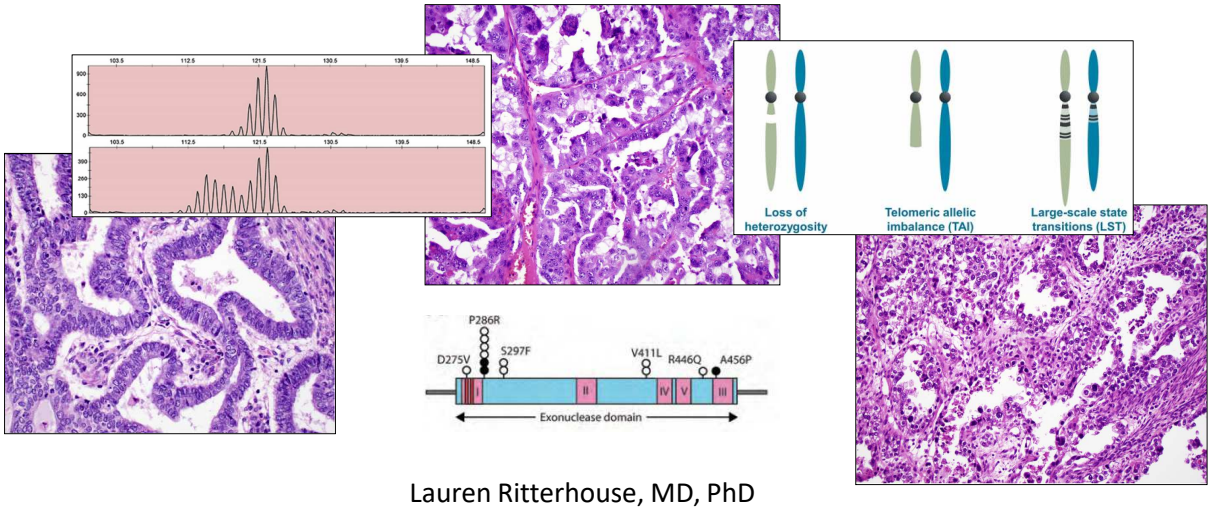
Surg Pathol Clin. 2016 Sep;9(3):405-26.

# Inherited Gynecologic Tumors: Hereditary Breast and Ovarian Cancer Syndrome

| Syndrome | Gene | Incidence | Cancers |
|---|---|---|---|
| **Hereditary breast and ovarian cancer syndrome** | BRCA1<br>BRCA2 | 1/300-800<br>Ashkenazi: 1/40 | Breast, ovary, melanoma, prostate, pancreatic |
| **Hereditary ovarian cancer syndrome** | RAD51C<br>RAD51D<br>BRIP1 | Unknown | Ovary |
| **Lynch syndrome** | MLH1<br>MSH2<br>MSH6<br>PMS2<br>EPCAM | 1/660-2000 | Uterine, colon, ovary, pancreatic, gastric, small intestine, central nervous system, renal, sebaceous |
| **Cowden syndrome** | PTEN | 1/200,000 | Breast, uterine, thyroid, colon, renal, sebaceous |
| **Li-Fraumeni syndrome (LFS)** | P53 | Unknown | Sarcomas, breast, adrenal, brain, lung, endometrial |
| **Peutz-Jeghers** | STK11 | 1/25,000-300,00 | Gastrointestinal, breast, ovarian, sex cord stromal, uterine, cervical (adenoma malignum) |

Obstet Gynecol Clin North Am. 2018 Mar;45(1):155-173.

# Molecular diagnostics of gynecologic tumors

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