Hereditary syndromes, genetic testing and gynaecological cancers

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IEO
European Institute
of Oncology

Disclosures

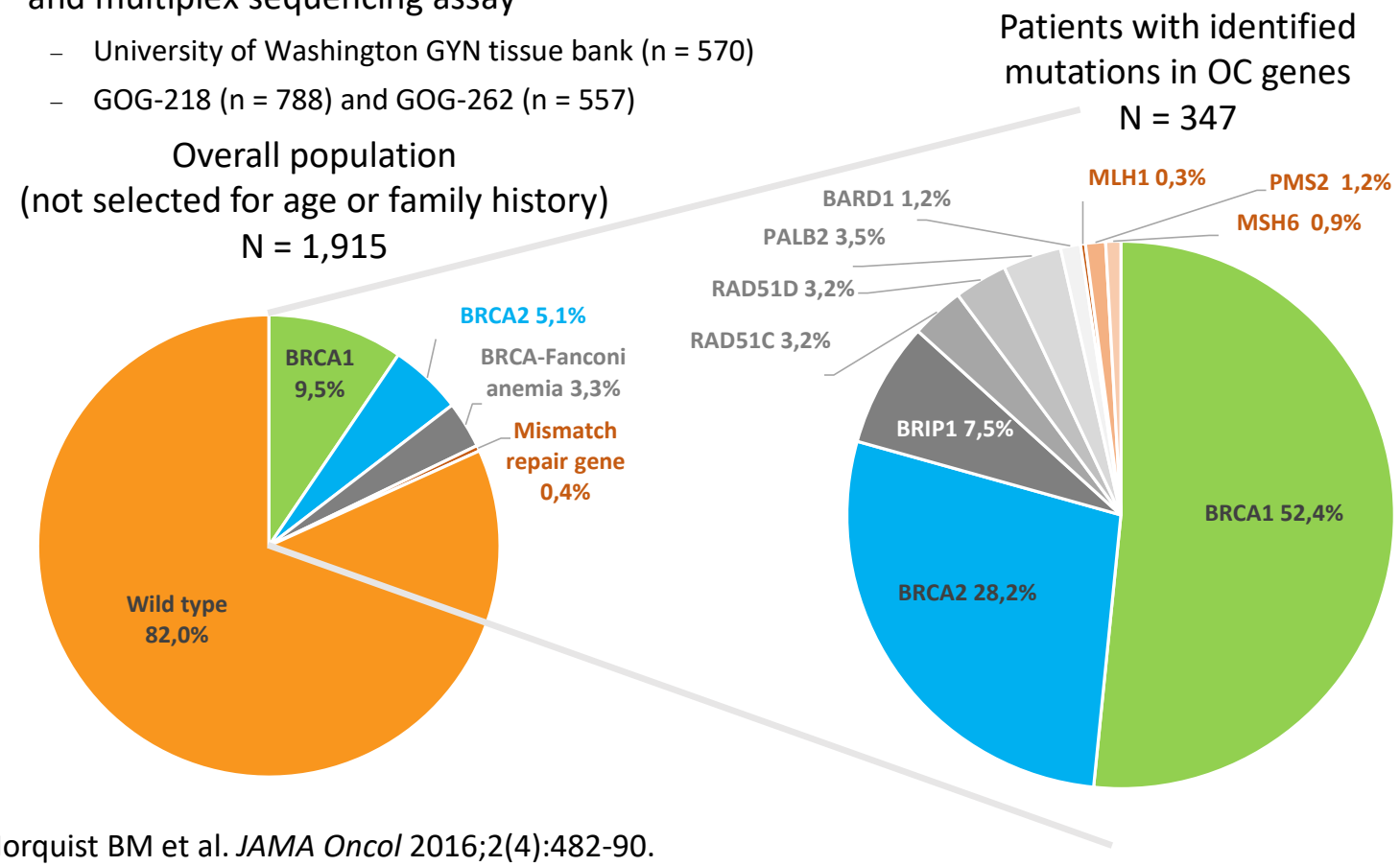
- I have acted as a consultant for AstraZeneca, Clovis, Tesaro, Roche, Pharmamar , Pfizer & Takeda
- I provided lectures for Astra Zeneca, Roche, Tesaro, Pharmamar, Takeda

Gynecological Cancer Susceptibility Syndromes

Syndrome	Genes associated
Hereditary breast ovarian cancer	*BRCA1*, *BRCA2, RAD51C, RAD 51D, BRIP1*
Hereditary nonpolyposis colon cancer (Lynch): Endometrial and ovarian cancer	*MLH1*, *MSH2*, *MSH6, PMS2 (EPCAM)*
Cowden syndrome: Endometrial Carcinoma	*PTEN*
Ovarian Small Cell Carcinoma	SMARCA4
Sertoli-Ledig ovarian tumors	*DICER1*
Peutz-Jeghers: Sex cord tumor with annular tubules (SCTAT), MDA cervix, endometrial carcinoma	*STK11*

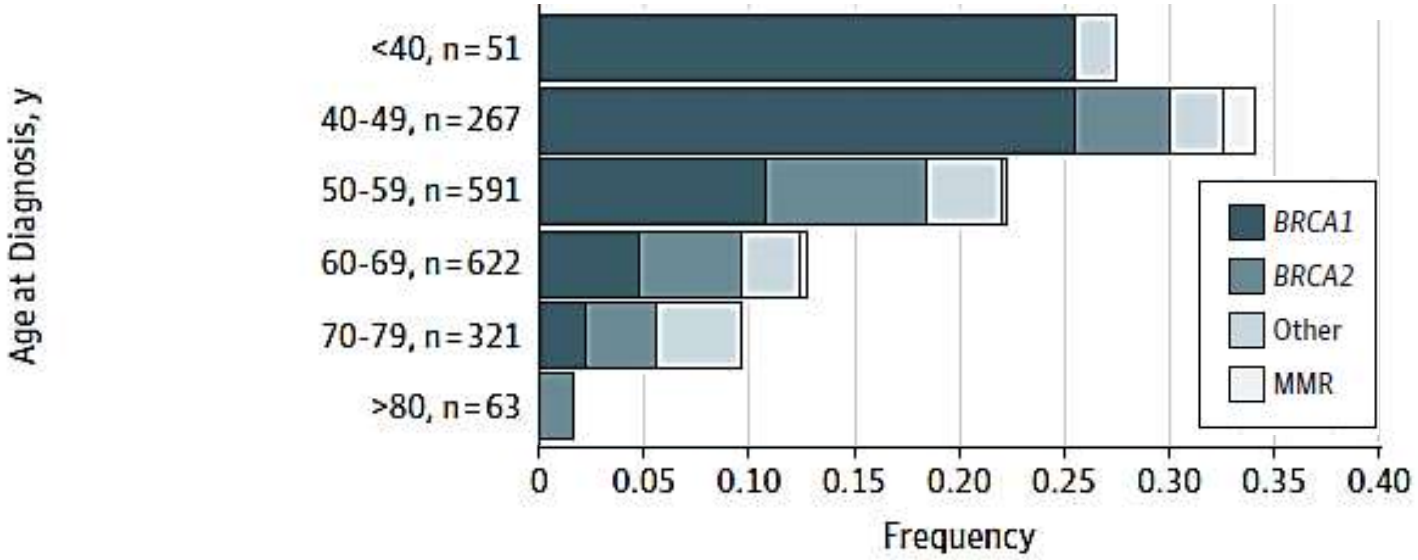
Summary of Germline DNA Mutations in OC

- Germline DNA sequenced from women with OC (N = 1,915) using a targeted capture and multiplex sequencing assay
  - University of Washington GYN tissue bank (n = 570)
  - GOG-218 (n = 788) and GOG-262 (n = 557)



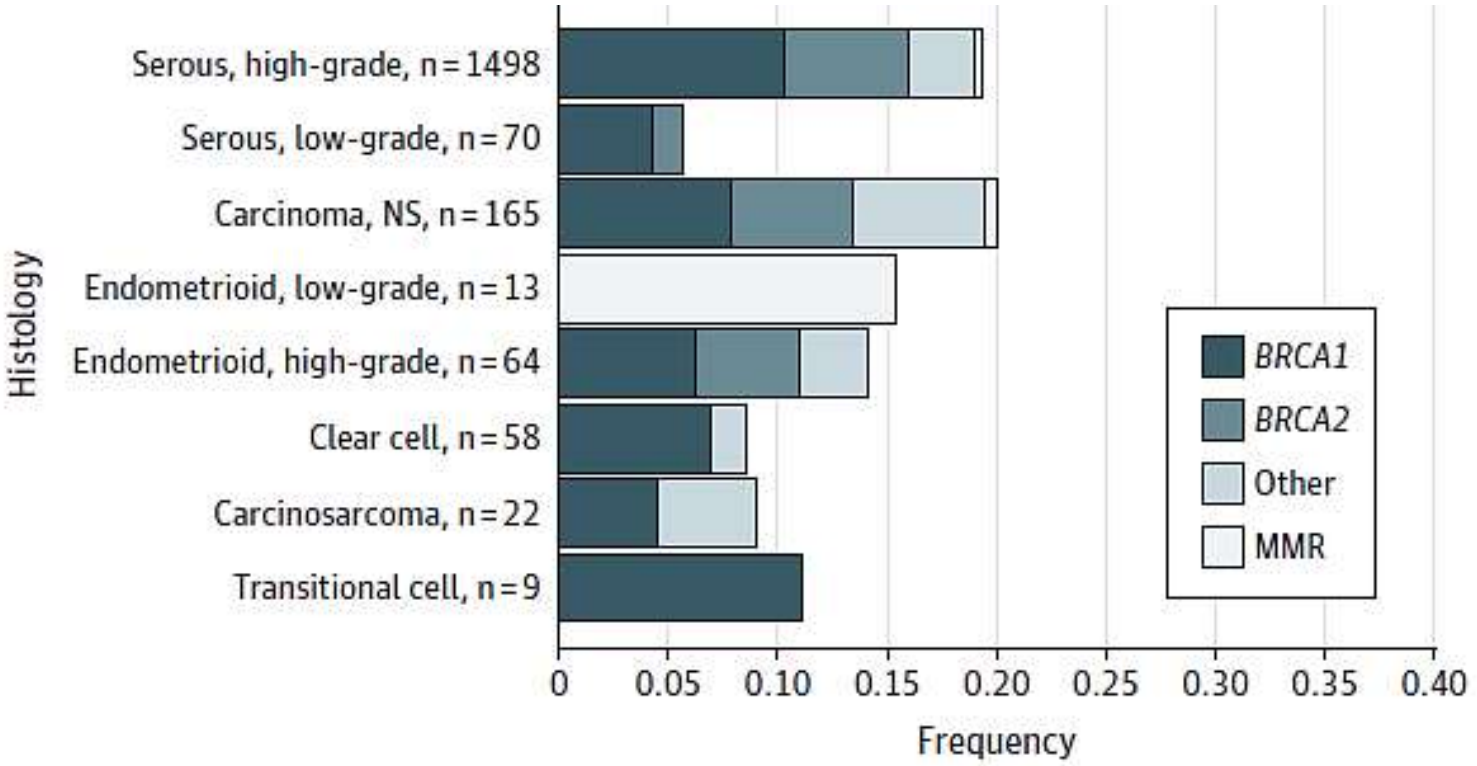
Norquist BM et al. *JAMA Oncol* 2016;2(4):482-90.

Germline Mutations in Ovarian Cancer



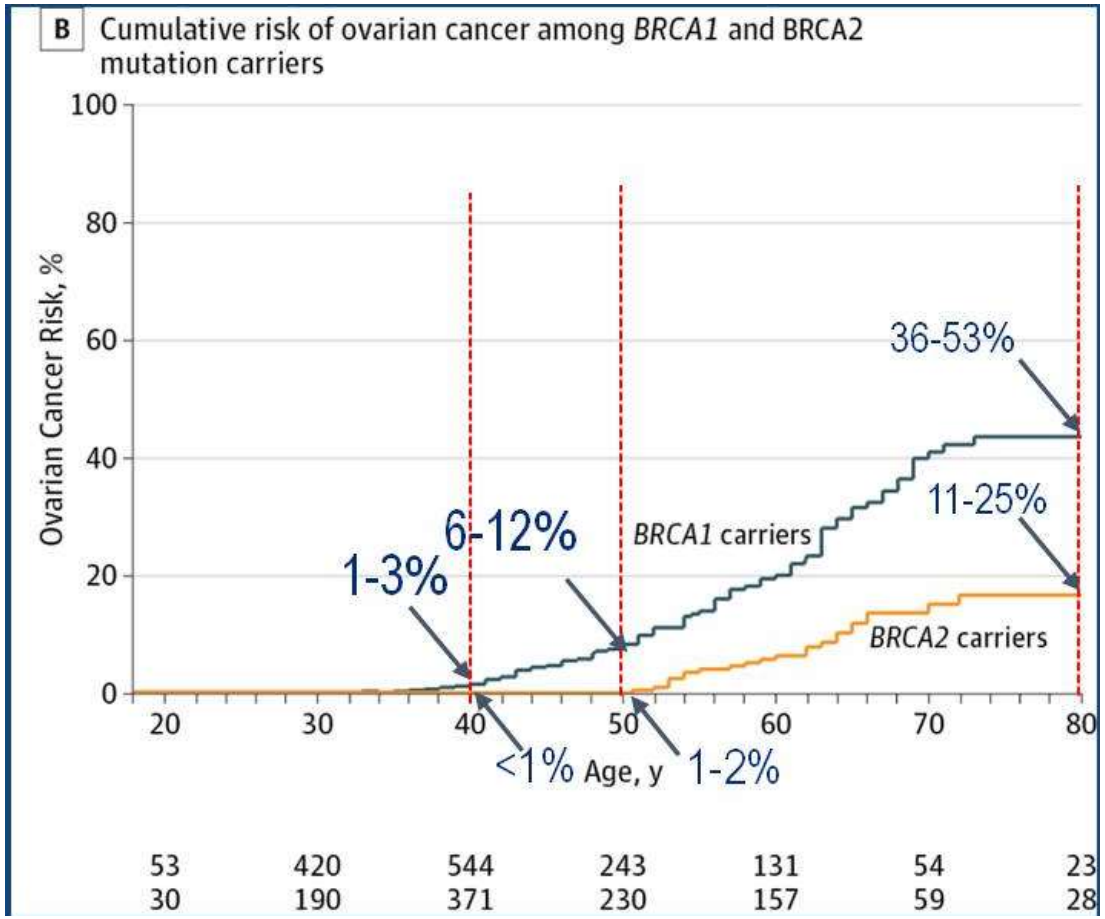
Norquist BM, et al. *JAMA Oncol*. 2016;2(4):482-490.

Germline Mutations in Ovarian Cancer



Norquist BM, et al. *JAMA Oncol*. 2016;2(4):482-490.

Cumulative Risks for Gynecologic Cancer in Carriers of BRCA Mutations (Prospective Cohort of 9856 Mutation Carriers)



Kuchenbaeker KB, et al. JAMA 2017;317:2401-16

Other Ovarian Cancer susceptibility genes Are these clinically actionable?

Gene	Frequency in Ov Ca	Relative Risk	Lifetime Risk	Reference
BRIP1	1.4%	9-10	10-15%	Rafnar et al, Nature Genet 2011, Ramus et al JNCI,2015
RAD51D	0.6%	6-12	8-15%	Loveday et al 2011, Nature Genet, Pelttari et al. J Med Gen 2012, Song et al, JCO 2015
RAD51C	0,5%	5-8	5-10%	Loveday et al 2012, Nature Genet, Pelttari et al. HMG 2011, Song et al. JCO2015
PALB2	0.6%	3-8	5-10%	Norquist et al, 2015
BARD1	0.2%	Wide CI	elevated	Norquist et al, 2015
Lynch	1%		8-10%	

Rationale for BRCA (and beyond) gene testing

BRCA1/2 Mutations in Ovarian Cancer

Who Should Be Tested?

Leading Oncology Societies Recommend Testing All Women With Ovarian Cancer

NCCN[a]
Genetic counseling and testing should be considered in women with a history of ovarian carcinoma, fallopian tube cancer, or primary peritoneal cancer

SGO[b]
Women diagnosed with epithelial ovarian, tubal, and peritoneal cancers should receive genetic counseling and be offered genetic testing, even in the absence of family history

ASCO®[c]
Genetic counseling and testing should be considered in women with epithelial ovarian, fallopian tube, or primary peritoneal cancer even in the absence of family history

a. NCCN Guidelines; b. Lancaster JM, et al. *Gynecol Oncol*. 2015;136:3-7; c. Lu JF, et al. *J Clin Oncol*. 2014;32:833-840.

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» Text update

Testing for a BRCA mutation

BRCA mutations are found in 5%-15% of ovarian cancer population studies [3]. Cohort studies have shown that the absence of a family history of breast/ovarian cancer is a poor negative predictor for a BRCA mutation [4, 5]. It is now recommended that patients with high-grade tumours are tested for germline BRCA mutation. Somatic mutations of BRCA are found in 5%-7% of ovarian cancer cases [6].

» Recommendation

- Patients with high-grade tumours should be tested for a germline BRCA mutation. Consideration should be given to testing tumours for a somatic BRCA mutation.

This update refers to the **Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up**, Ledermann JA, Raja FA, Fotopoulou C et al, Ann Oncol 2013; 24 (Suppl 6): vi24-vi32; and **Non-epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up**, Colombo N, Peiretti M, Garbi A et al, Ann Oncol 2012; 23 (Suppl 7): vii20-vii26.

Evolving role of mutation testing:
Why are patients with ovarian cancer being tested for BRCA?

Risk Assessment

- Women who harbour a BRCA mutation are more likely to suffer from breast cancer or ovarian cancer, in their lifetime, than those without a mutation
- Allows patients to take preventive action

Prognostic factor

- Important prognostic factor, other than stage and extent of surgical debulking
- Estimate PFS and OS according to BRCA status

Predictive factor
Option decisions

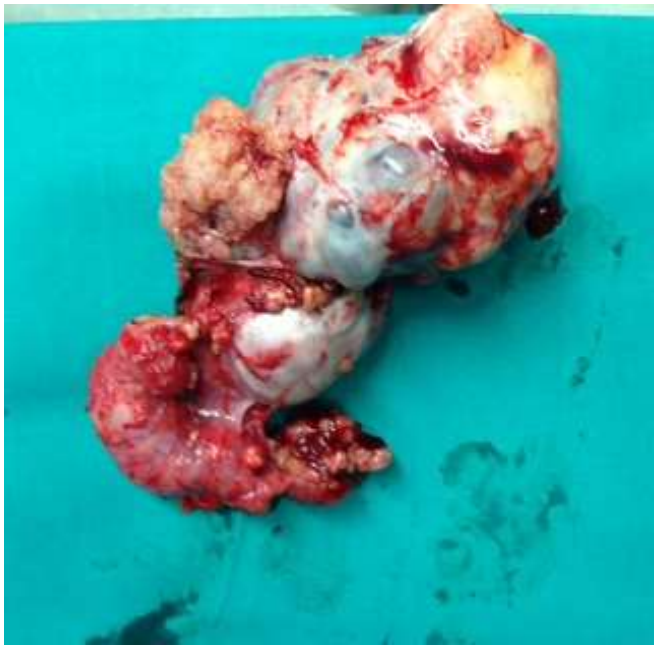
- Identification of patients who may be more sensitive to different treatment options

PFS: progression free survival
OS: overall survival


BRCA testing is important in risk management of family members
The cascade effect
Imperative to identify family members at risk

Strategies of prevention?

- ✓Intensified screening
- ✓Chemoprevention
- ✓Prophylactic surgery

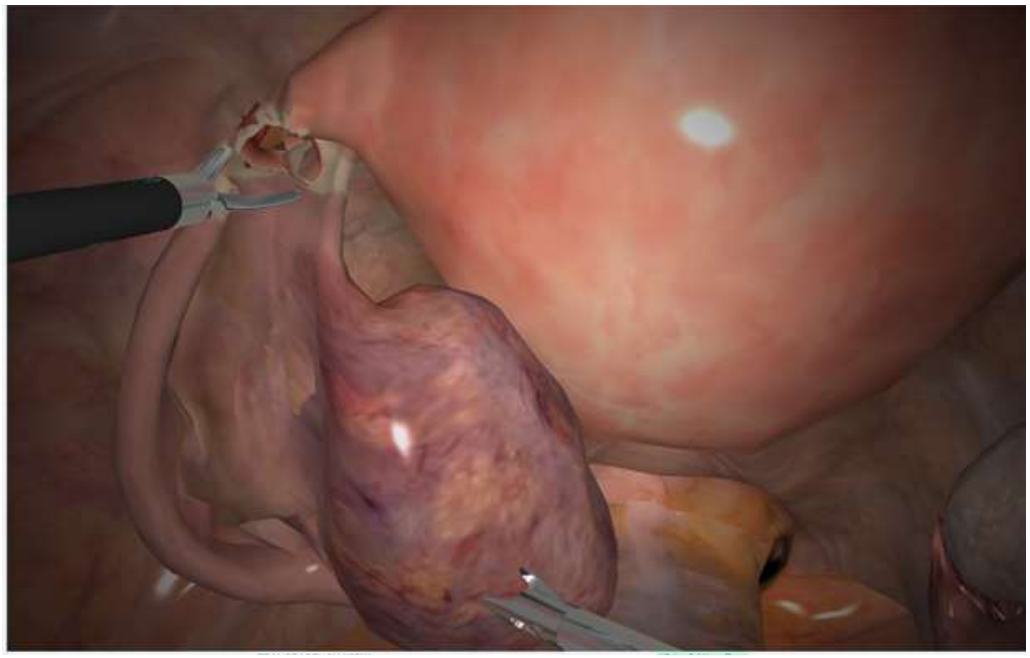


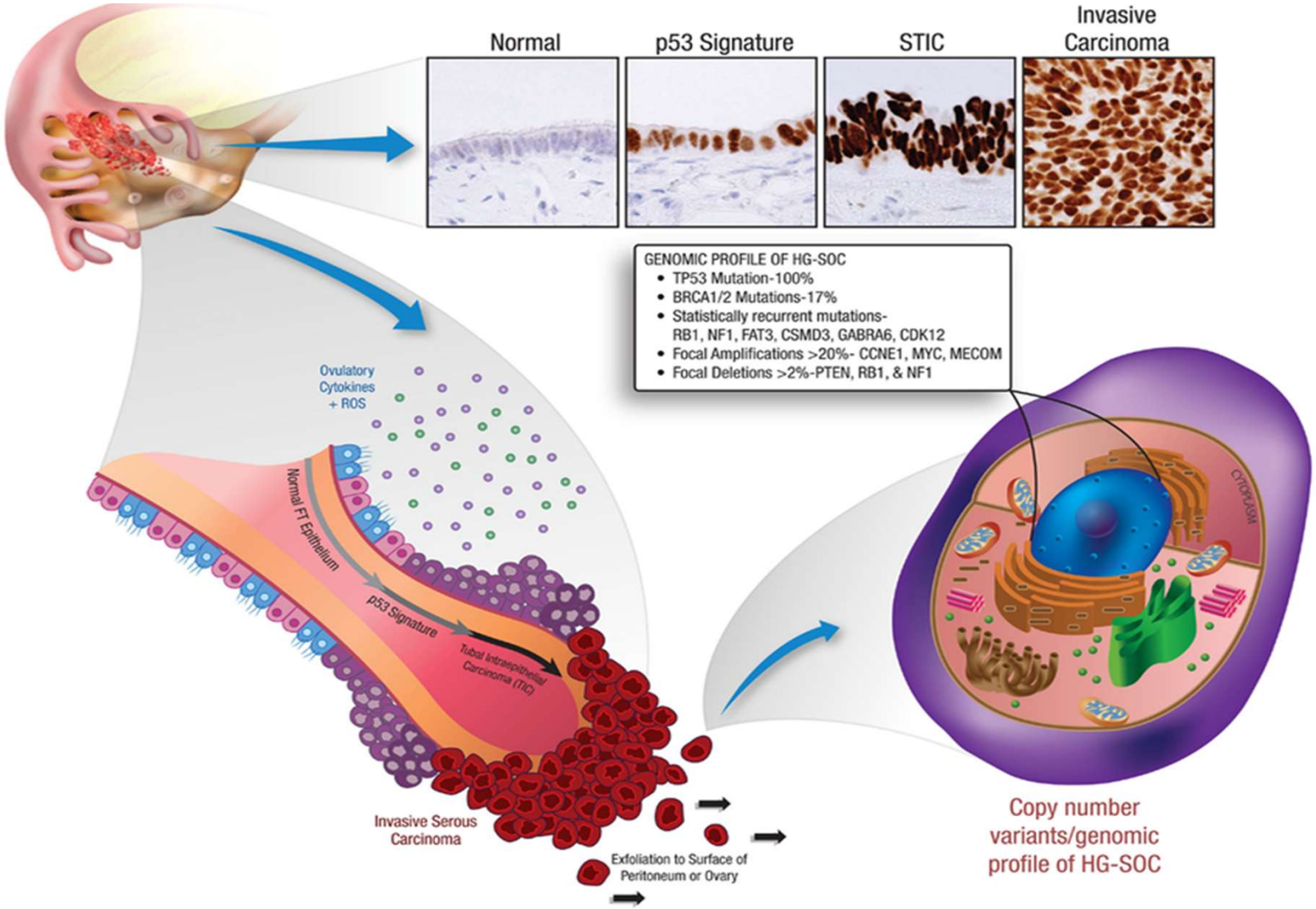
Chemoprevention

- Oral contraceptives (Ocs): 46% risk reduction of ovarian cancer in the general population
- Risk reduction related to the duration of use
- Protection persists for 15-30 years
- Moderately increased breast cancer risk, which tends to level off in the few years after stopping
- This could be of great concern in women at high risk for breast cancer.

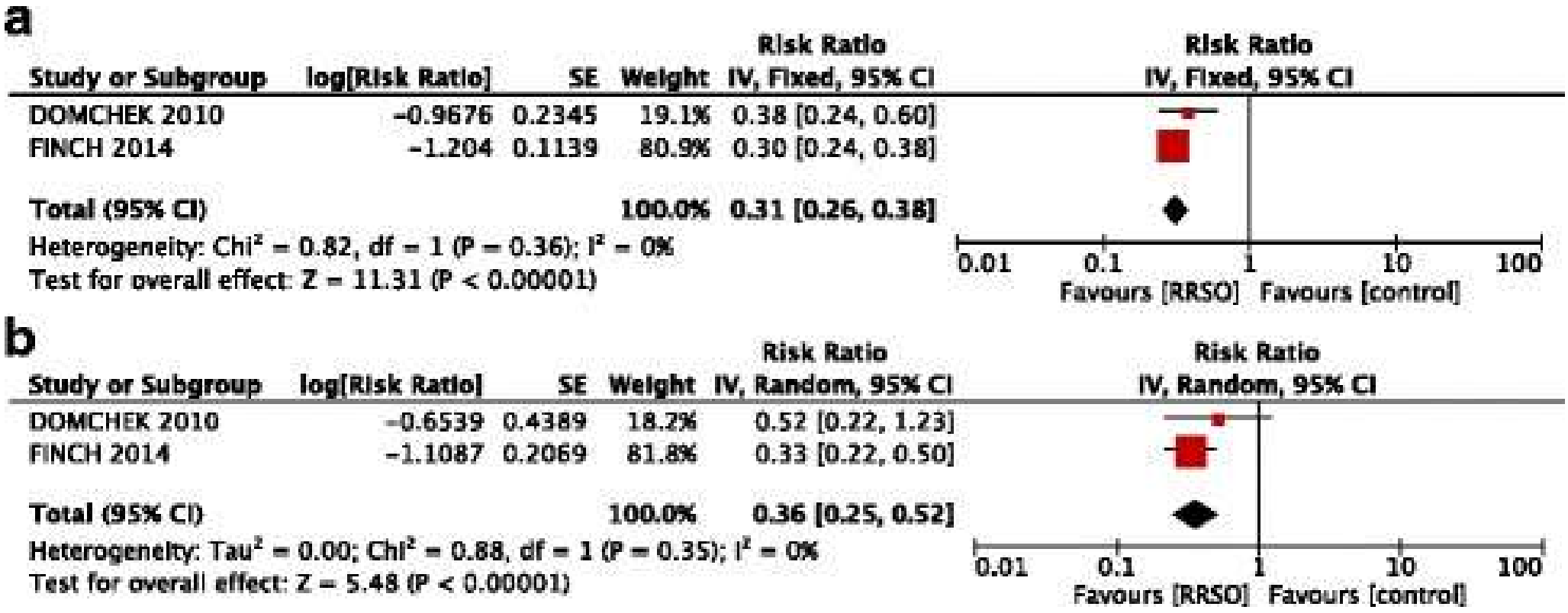

MAY 27, 2013
TIME
THE
ANGELINA
EFFECT
Angelina Jolie's double mastectomy puts genetic testing in the spotlight. What her choice reveals about calculating risk, cost and peace of mind
BY JEFFREY KLUGER & ALICE PARK

Risk-reducing salpingo-oophorectomy (RRSO)




Normal
p53 Signature
STIC
Invasive Carcinoma
GENOMIC PROFILE OF HG-SOC
• TP53 Mutation-100%
• BRCA1/2 Mutations-17%
• Statistically recurrent mutations-
RB1, NF1, FAT3, CSMD3, GABRA6, CDK12
• Focal Amplifications >20%- CCNE1, MYC, MECOM
• Focal Deletions >2%-PTEN, RB1, & NF1
Ovulatory Cytokines + ROS
Normal FT Epithelium
p53 Signature
Tubal Intraepithelial Carcinoma (TIC)
Invasive Serous Carcinoma
Exfoliation to Surface of Peritoneum or Ovary
CYTOPLASM
Copy number variants/genomic profile of HG-SOC

Forest plots of relative risk (RR) estimates for all-causes mortality associated with risk-reducing salpingo-oophorectomy in BRCA 1 (a) and BRCA 2 (b) mutation carriers



Marchetti et al: BMC Women Health 2014; 14: 150.

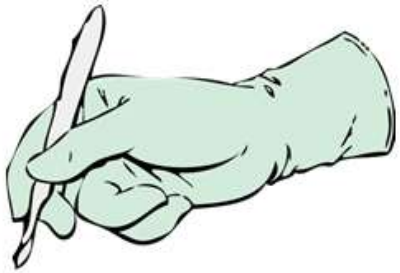
European Journal of Cancer 84 (2017) 159–167

Available online at www.sciencedirect.com

ScienceDirect

Conclusion: Our results suggest that HRT use in the first year after RRSO has beneficial effects in terms of minimising endocrine symptoms and sexual symptoms in premenopausal women who have undergone RRSO

Ravi F.M. Vermeulen [a], Marc van Beurden [a], Jacobien M. Kieffer [b], Eveline M.A. Bleiker [b], Heiddis B. Valdimarsdottir [c], Leon F.A.G. Massuger [d], Marian J.E. Mourits [e], Katja N. Gaarenstroom [f], Eleonora B.L. van Dorst [g], Hans W.H.M. van der Putten [h], Neil K. Aaronson [b,*]



$23,422 per patient for 25.71 quality-adjusted life-years (QALYs)

$68,392 per patient for 25.17 QALYs

$100,484 for 24.60 QALYs

Yang et.al. Famil Canc, 2011

Uptake of risk reducing among *BRCA carriers:* 60 - 90%

Occult cancers at the time of surgery:
2-18%

Finch et al. Womens Health 2012

Summary: Current NCCN Guidelines

Prophylactic Procedure	Gene or Syndrome
RRSO	BRCA1 BRCA2 BRIP1 RAD51C RAD51D +/- STK11/Peutz-Jeghers
Hysterectomy	Cowden +/- STK11/Peutz-Jeghers
Hysterectomy and RSSO	BRCA1 Lynch

Mary-Claire King, PhD,

- Every breast or ovarian cancer patient with a BRCA1 or BRCA2 mutation detected after diagnosis is a missed opportunity to prevent a cancer
- No woman with a mutation in BRCA1 or BRCA2 should die of breast or ovarian cancer
- **It is completely unnecessary !!!**

ABOG Lecturer, Sgo 2016

BRCA status provides information about prognosis and clinical outcomes

Germline BRCA mutation carriers – distinct clinical behaviour:

- Age at diagnosis: BRCA1 lower
  - Mean BRCA1 53.4 yrs, BRCA2 59.8 yrs, noncarrier 60.5yrs[1]
- Improved overall survival[2,3]
- Found in all non-mucinous epithelial ovarian cancers with greatest prevalence in high grade serous / endometrioid[1,5]
- Disease distribution
  - visceral metastases (liver, lung, splenic)[4]
- Support in option management

Mutation status	5-yr OS[2] %; HR	5-yr OS[3] %; HR
BRCA1	44; 0.73 *P*<.001	44; 0.76 *P* = .35
BRCA2	52; 0.49 *P*<.001)	61; 0.33 *P* = .003
BRCA non-carriers	36	25

1. Alsop K, et al. *J Clin Oncol.* 2012;30(21):2654-2663; 2. Bolton KL, et al. *JAMA.* 2012;307(4):382-390; 3. Yang D, et al. *JAMA.* 2011;306(14):1557-1565;
4. Gourley C, et al. *J Clin Oncol.* 2010;28(15):2505-2511. 5.Vergote et al 2016;69:127-134

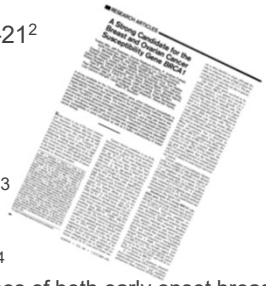
The search for BRCA

1990s

A region of chromosome 17 identifying as being associated with familial breast cancer[1]

1994

Localisation of the breast cancer susceptibility gene (BRCA1) on 17q12–21[2]



1994

Identification of BRCA1 by US scientists[3]

BUT BRCA1 mutations accounted for:[4]

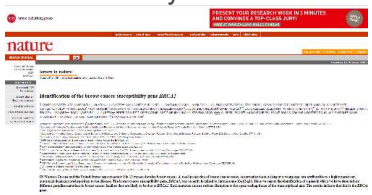
- Most, but not all, families with many cases of both early onset breast and ovarian cancer that set
- Just under half of all families affected by multiple breast cancer cases
- But no families affected by both male and female breast cancer

Search for BRCA2

BRCA2 localised to chromosome 13q12–13[5]

1995

Identification of BRCA2 by UK scientists[6]



1. Kat Arney High Impact Science Series: available at http://scienceblog.cancerresearchuk.org/2012/02/28/high-impact-science-tracking-down-the-brca-genes-part-1/
2. Smith SA, et al. Genes Chromosomes Cancer. 1994;10:71–6.
3. Miki Y, et al. Science 1994;266:666–671.
4. Futreal PA. Science. 1994;266:120–122.
5. Wooster R, et al. Science 1994;265:2088–90.
6. Wooster R, et al. Nature 1995;378:789–792.

After 10 years this defect was identified as a possible therapeutic target !!!

PARP-Inhibitors: Mechanism of Action

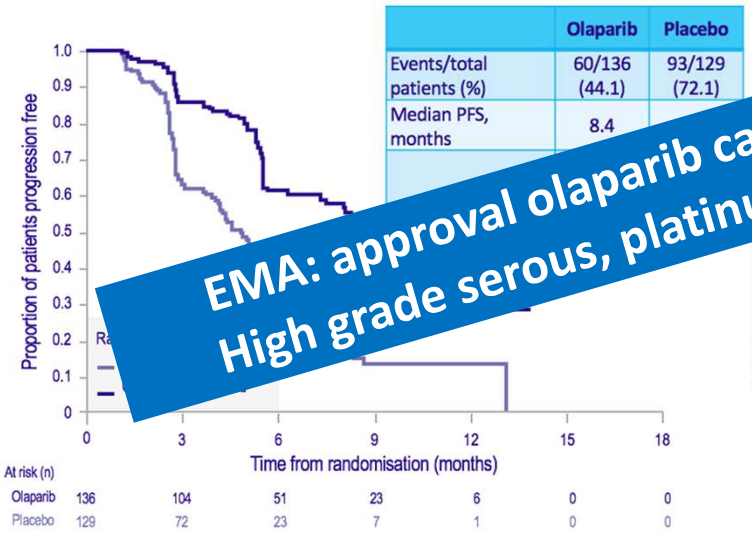
PARP inhibitors and predictive biomarkers

European Alliance for Personalised Medicine

Maintenance: olaparib

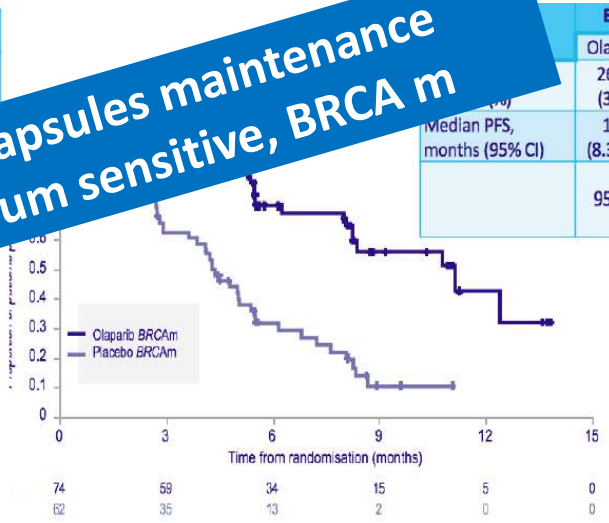
Study 19

Whole population with HGSOC



	Olaparib	Placebo
Events/total patients (%)	60/136 (44.1)	93/129 (72.1)
Median PFS, months	8.4	

Subpopulation with BRCA mutation



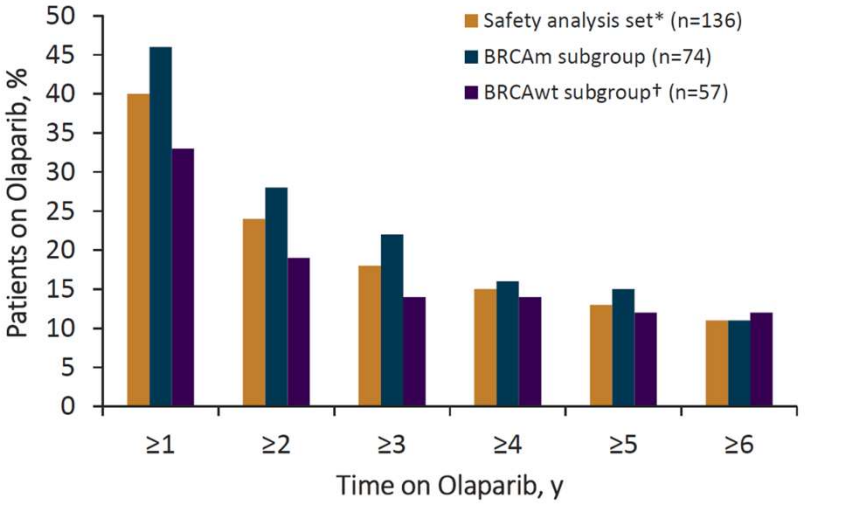
	BRCAm (n=136)	
	Olaparib	Placebo
	26/74 (35%)	46/62 (74%)
Median PFS, months (95% CI)	11.2 (8.3, NC)	4.3 (3.0, 5.4)
	HR=0.18 95% CI: 0.10, 0.31; P<0.0001	

EMA: approval olaparib capsules maintenance
High grade serous, platinum sensitive, BRCA m

Ledermann J et al. N Engl J Med 2012

Ledermann J et al. Lancet Oncology 2014

Significant long-term benefit in Study 19



Clear evidence of "super-responders"

Of the 15 patients who received olaparib for ≥6 years:

- Nine patients had a BRCAm, three of whom had a sBRCAm, and a slight preponderance of BRCA2 mutations was observed

Five patients were BRCAwt:

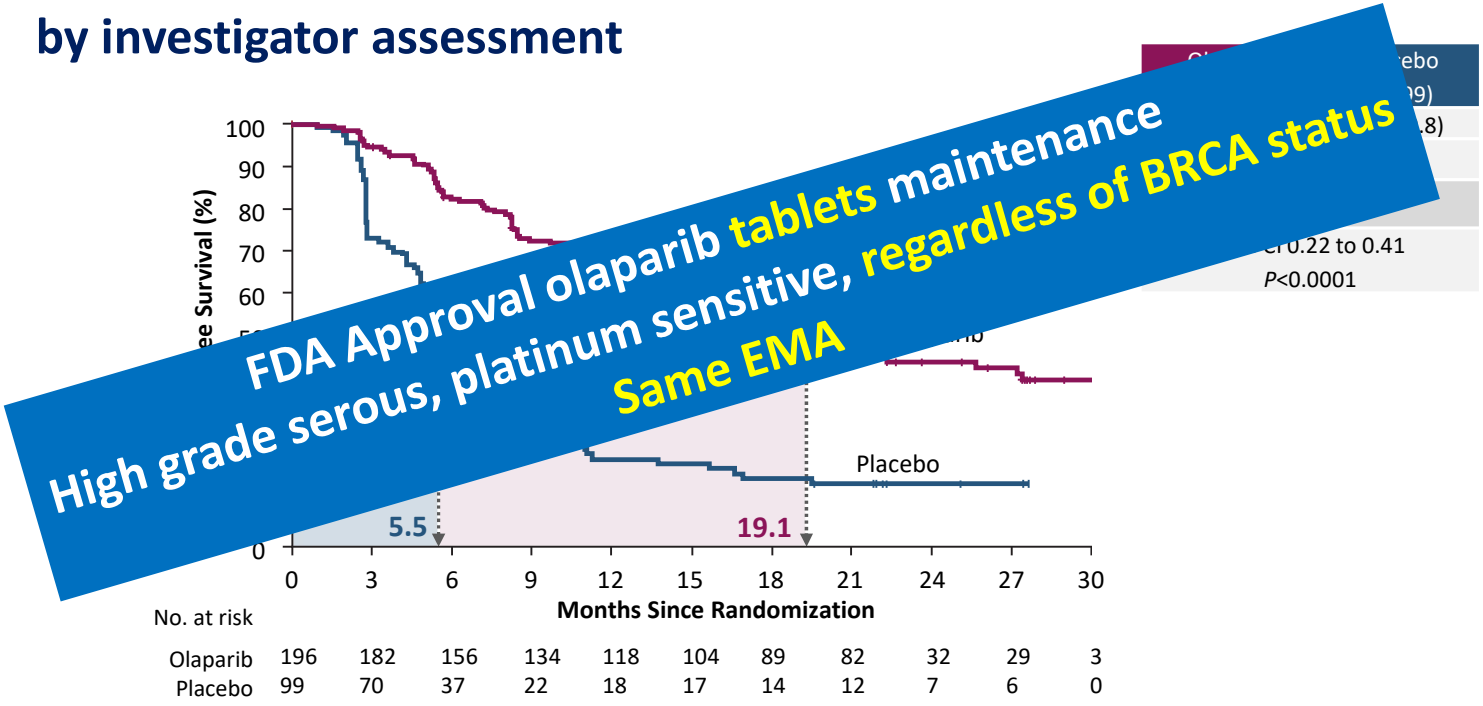
- One patient was found to have a RAD51B mutation
- Some patients had no HRR mutations and one patient also tested negative for HRD

One patient, who was germline BRCAwt, had no available tumor test results.

HRD, homologous recombination deficiency; HRR, homologous recombination repair.
Gourley C, *et al*. ASCO 2017. Abstract 5533 and poster presentation.

SOLO2/ENGOT-Ov21: study design

A statistically significant improvement was demonstrated in favour of olaparib for PFS by investigator assessment

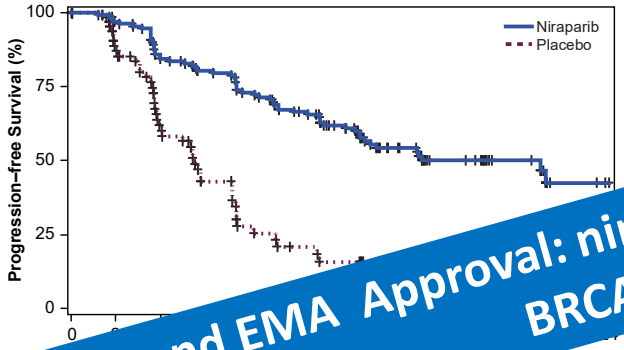


FDA Approval olaparib tablets maintenance
High grade serous, platinum sensitive, regardless of BRCA status
Same EMA

Platinum combination followed by iPARP

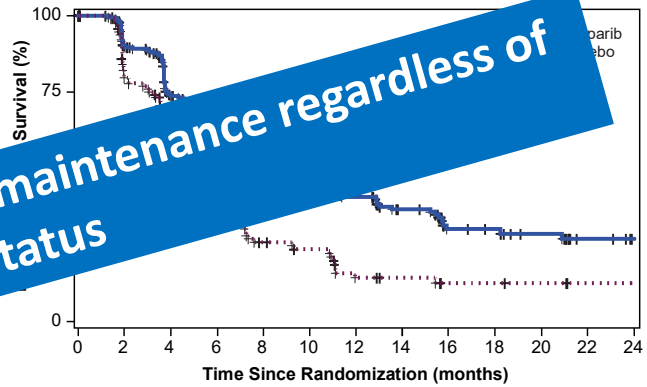
Niraparib: ENGOT ov16-NOVA primary end-point

PFS: gBRCAmut



Treatment	Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value
Niraparib (N=138)	**21.0** (12.9, NR)	**0.27** (0.173, 0.410) p<0.0001
Placebo (N=65)	**5.5** (3.8, 7.2)	

PFS: non-gBRCAmut

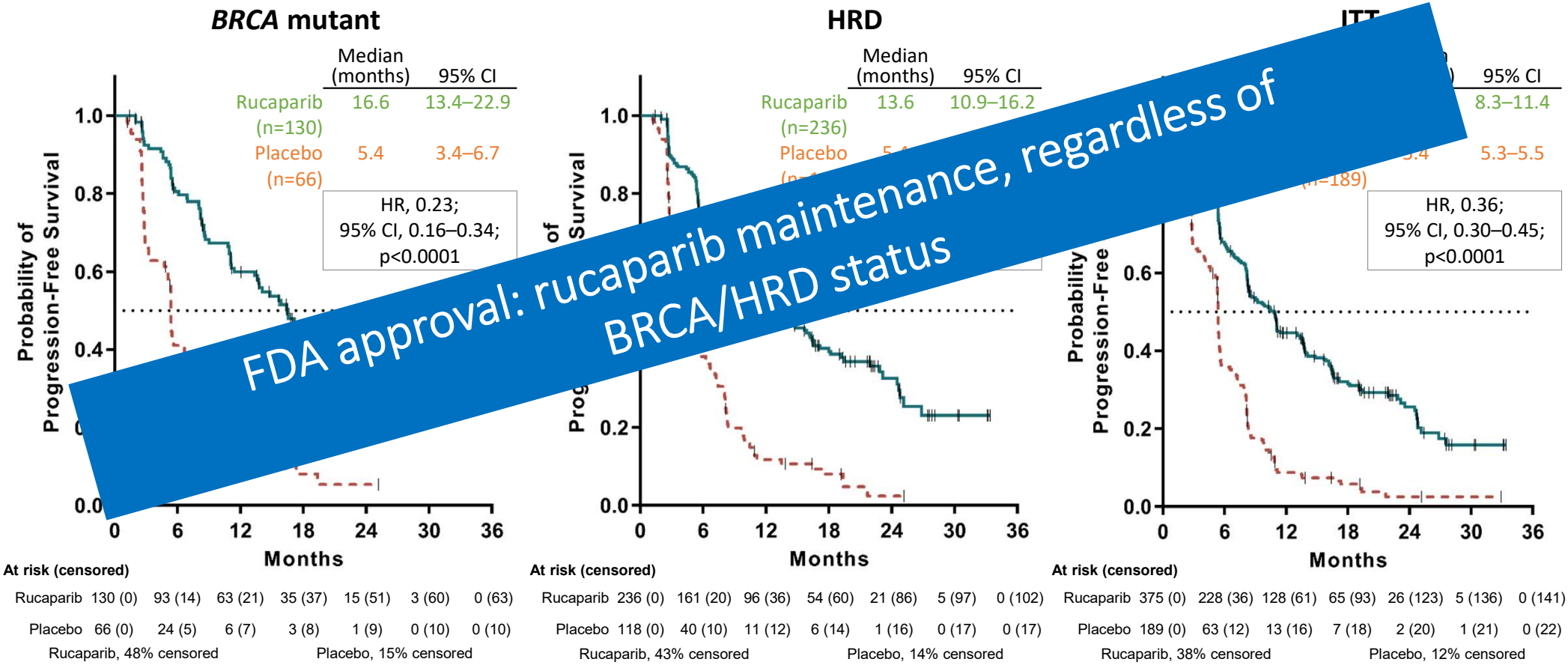


Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value
Niraparib (N=234)	**9.3** (7.2, 11.2)	**0.45** (0.338, 0.607) p<0.0001
Placebo (N=116)	**3.9** (3.7, 5.5)	

FDA and EMA Approval: niraparib maintenance regardless of BRCA/HRD status

Mirza MR et al. N Engl J Med 2016

ARIEL3: Investigator-assessed progression-free survival



FDA approval: rucaparib maintenance, regardless of BRCA/HRD status

Visit cut-off date: 15 April 2017.

1. Coleman RL, *et al. Lancet Oncol* 2017 [Epub ahead of print].

Despite the approval of PARP inhibitors as maintenance therapy in PSR OC patients regardless of *BRCA* status…

There is no doubt that the benefit magnitude is greater in g/s*BRCA*m

BRCA mutation remains a significative predictive biomarker of efficacy for PARP inhibitors

Germline versus Somatic Testing for BRCA mutations?

Somatic BRCA mutation are clinically relevant in ovarian cancer

BRCA mutations can be either germline or somatic

Germline BRCA mutations

- Blood sample
- Inherited mutations found in all body cells[1]

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

Somatic BRCA mutations

- Tumour sample
- Acquired mutations found only in tumour cells[2]
- **5-8%** of ovarian cancer patients harbour BRCA somatic mutation

Somatic BRCA mutations can be detected in a **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.

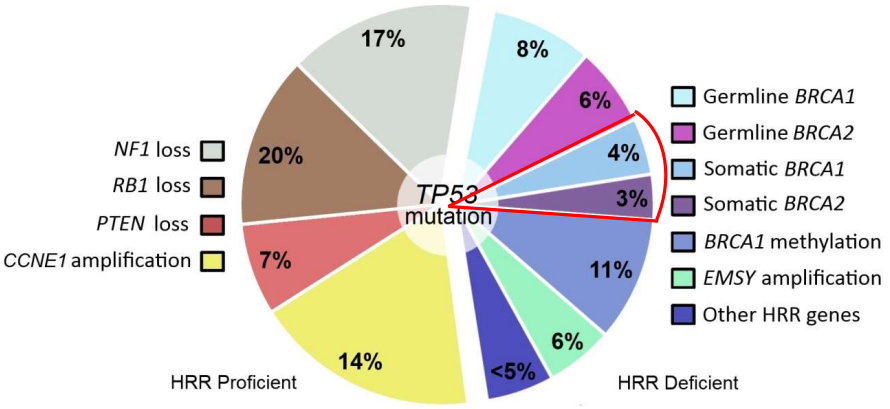
Selecting patients for PARP inhibitor treatment: consideration of somatic *BRCA*

Maintenance studies

Study	Agent	s*BRCA* PFS HR (95% CI)	g*BRCA* PFS HR (95% CI)
Study 19[1]	Olaparib vs placebo	0.23 (0.04-1.12)	0.17 (0.09-0.34)
NOVA[2]	Niraparib vs placebo	0.27 (0.08-0.90)	0.27 (0.17 to 0.41)

Treatment study

Study	Agent	s*BRCA* Response rate	g*BRCA* Response rate
ARIEL2[3]	Rucaparib vs placebo	63%	74%



Hollis RL, et al. Cancer Biol Med. 2016; 13:236-247.

If you do not test for somatic *BRCA* via tumour test you may miss information that can help support treatment decisions and guide benefit:risk profile of treatment

1. Dougherty BA, et al. Oncotarget. 2017;8(27):43653-43661. 2. Mirza MR, et al. NEJM. 2016; 375:2154-2164. 3. McNeish IA, et al. ASCO 2015. Abs 5508.

Beyond BRCA Mutations:
Homologous Recombination Deficiency

How to identify HRD ?

Look for the *Cause* of HRD	**Look for the *Effect* of HRD**
What genomic changes can cause defects in the homologous recombination repair pathway ?	What is the results in the genome of the defects in the homologous repair pathway ?
Assess the cause of HRD by looking for loss of function of key HRR genes	Identify the consequences of HRD by looking for patterns of genomic damage
***BRCA*, HRR Gene Panel**	**HRD Genomic Scar**

- **Loss of heterozygosity (LOH)**
- **Telomeric allelic imbalance (TAI)**
- **Large-scale state transitions (LST)**

Represent the burden of genomic instability
Acts as biomarkers of HR deficiency

Examples of Assays for Genetic Testing

Test	Companion diagnostics	Turnaround time
BRACAnalysis CDx®	Olaparib companion diagnostic test	2 weeks
FoundationFocus™ CDxBRCA test	Rucaparib companion diagnostic test — somatic and germline BRCA1/2	2 weeks
	Breast/ovarian panels	
Ambry Genetics BRCAplus™	6-gene panel	1-2 weeks
Ambry Genetics OvaNext™	25-gene panel	2-4 weeks
Invitae Breast/Gyn Guidelines-based panel	19-gene panel	1-3 weeks
Color Genomics™	19-gene panel	4-8 weeks
GeneDx Breast/Ovarian	21-gene panel	3 weeks
	Comprehensive panels	
Ambry Genetics CancerNext™	32-gene panel	2-3 weeks
GeneDx Comprehensive	32-gene panel	3 weeks
Myriad myRisk®	25-gene panel	2-4 weeks
Invitae Multi-Cancer	79-gene panel	1-3 weeks

GeneTests (www.genetests.org); Lynce F, Isaacs C. ASCO 2016 Education Book

Hereditary endometrial cancer: 3-5%
Endometrial Cancer susceptibility genes

Syndrome	Genes	Endometrial Cancer Risk	Ovarian Cancer Risk	Other Cancer Risk
Lynch Syndrome	*MLH1*	14-54%	11-20%	Colorectal, Stomach, Hepatobiliary,Urinary Tract, Small Bowel, Brain, Sebaceous Neoplasms, Pancreas
	MSH2	20-54%	15-24%	
	MSH6	16-71%	Elevated	
	PMS2	15%	Elevated	
	EPCAM	12-55%	Elevated	
Cowden Syndrome	*PTEN*	28%	No known risk	Female Breast, Thyroid, Kidney, Colorectal, Melanoma, Brain
Peutz-Jeghers	*STK11*	9%	18-20% SCTAT	Colorectal, Small intestine, Stomach, Pancreas, Breast, Lung, Adenoma Malignum of Cervix

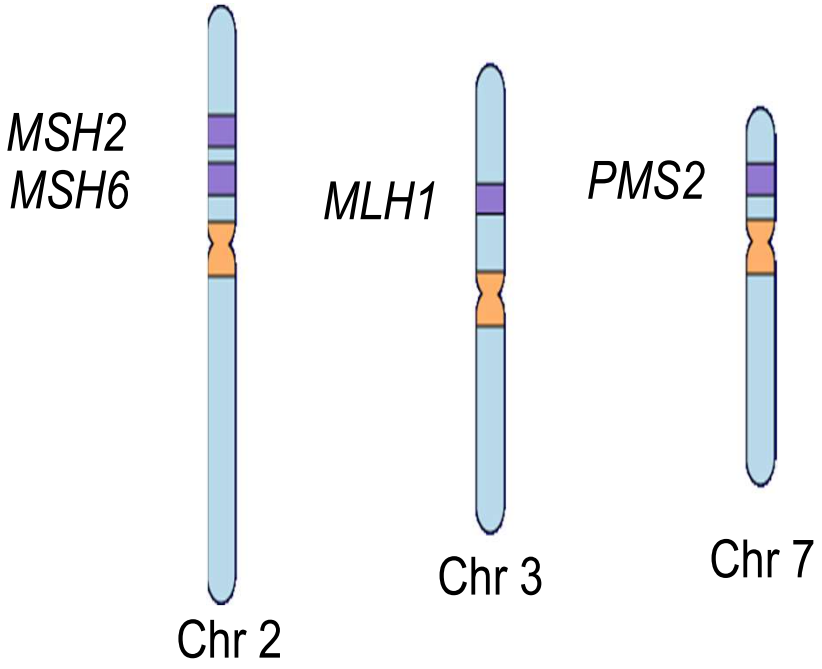
How common is Lynch Syndrome?

General Population: 1/500-1/1000

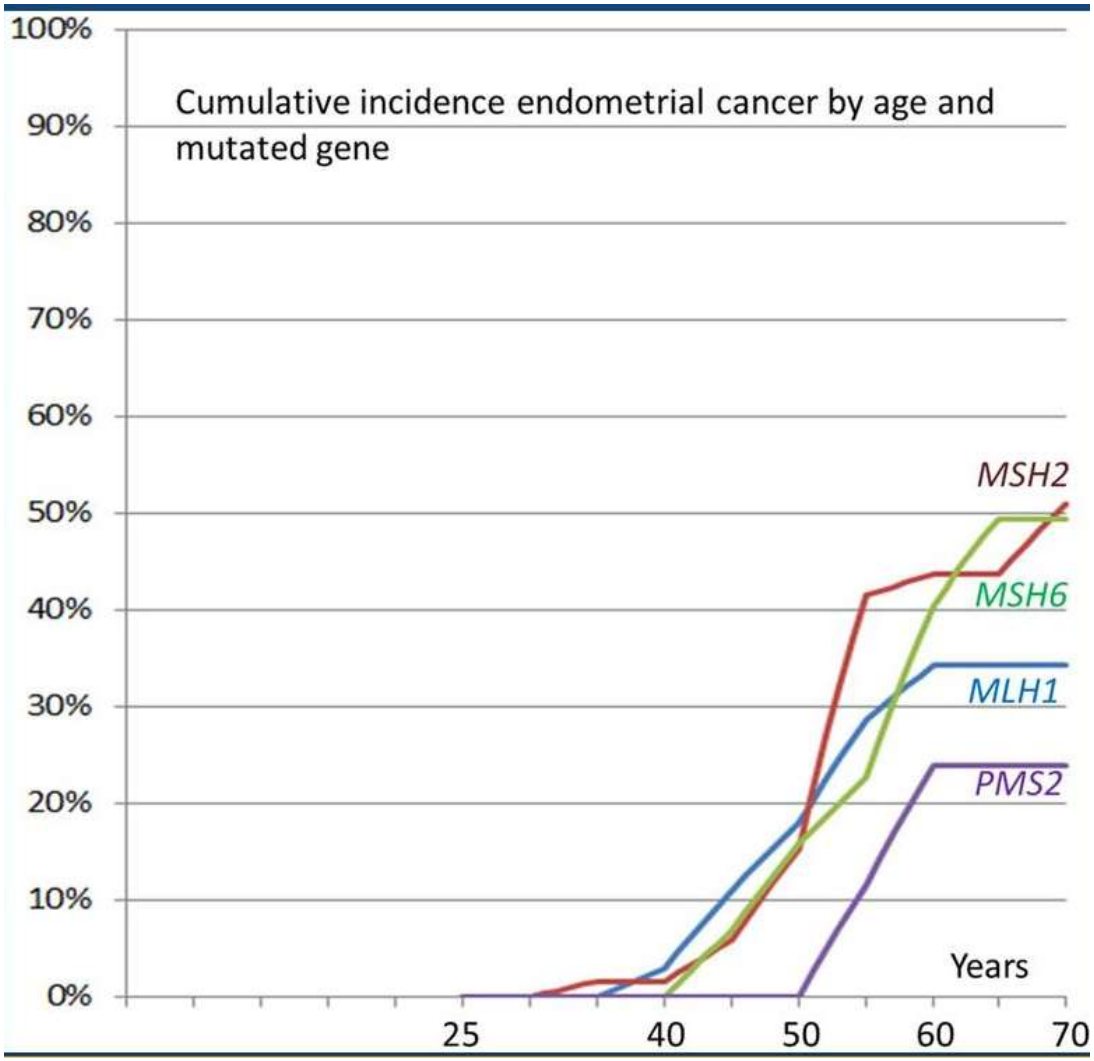
Patients with endometrial cancer: 2-3%

Patients with colon cancer: 2-3%

Lynch Syndrome/HNPCC



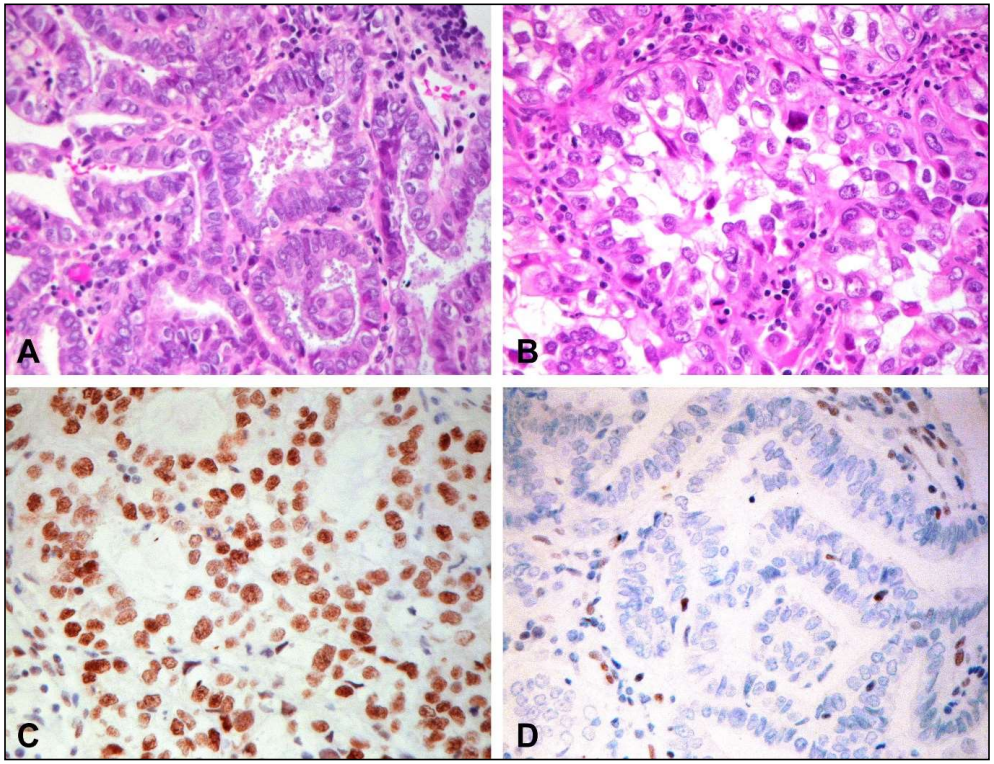
- Inherited cancer susceptibility syndrome
- Germline mutations in one of DNA mismatch repair genes, *MLH1, MSH2, MSH6* and *PMS2*
  - *EPCAM*
    - Large scale deletion of EPCAM at the 3' leads to epigenetic silencing of *MSH2*
    - Cell-specific effect – mosaic effect



Moller et al, Gut 2017, 66:464-472

IHC Testing in Endometrial Cancer

- Now recommended for all patients with EC
  - Loss of staining may be due to underlying germline MMR mutation
  - 25% of sporadic cases have MMR defect
  - Majority have epigenetic MLH1 loss by promoter methylation



IHC: MLH1 + IHC: MSH2 -

Risk of 2° cancer after Lynch associated endometrial cancer (win et al, 2013)

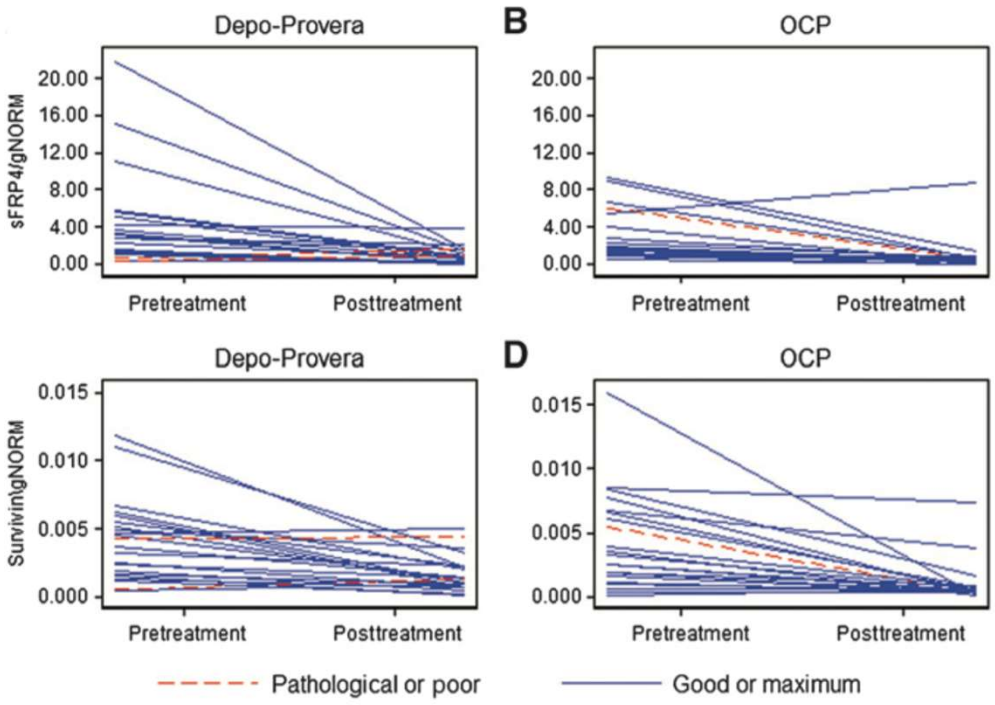
- **Colon Cancer :** **48%**
- **Ureteral or Kidney:** **11%**
- **Bladder:** **11%**
- **Breast:** **11%**

NCCN: endometrial screening recommendations in patients with Lynch syndrome

- Limited Data
- Educate patients about abnormal vaginal bleeding and unusual vaginal discharge, and use EMB for diagnosis if symptoms develop
- Consider EMB every 1-2 years
- Transvaginal ultrasound may be considered; limitations in premenopausal women due to changes in endometrial stripe in normal menstrual cycle
- Consider risk reduction agents

Risk Reduction: Chemoprevention

- OCPs have 50% reduction
- Progestins decrease proliferative gene effects
- Retention of organs until childbearing is reasonable
- ASA (600 mg/day)
  - No benefit for LS-colon cancer at 4 years but maybe an effect with long term administration (CAPP-3 trial underway)



Lu KH, et al. *Cancer Prev Res (Phila).* 2013;6(8):774-781.

Lynch Syndrome: Risk reducing surgery

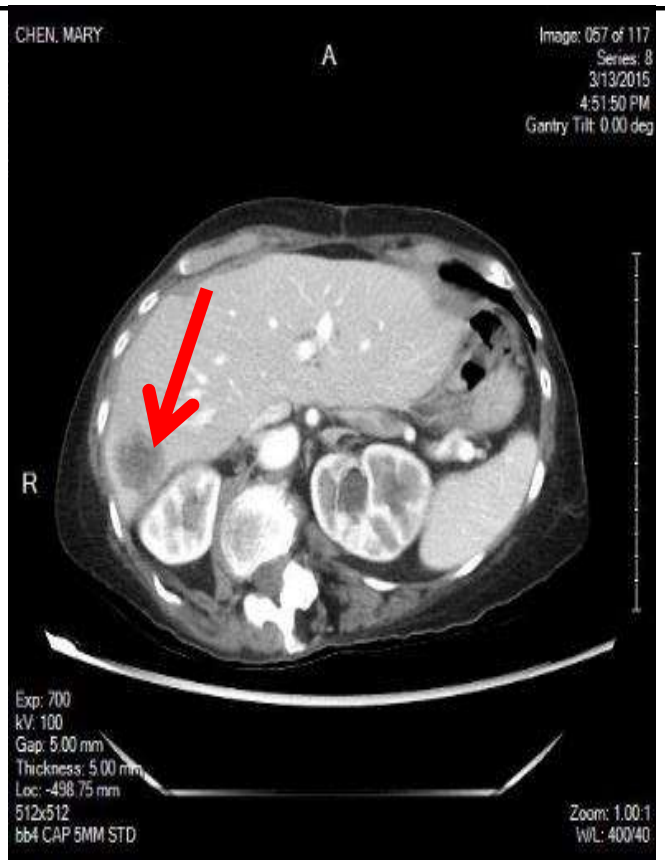
- Decreases incidence, but no evidence that it reduces mortality («Hysterectomy can be considered»)
- Timing can be personalized
  - Gene and family history
  - Consider simultaneous surgery in women undergoing colon cancer surgery
- Issues to consider
  - Both uterus and ovaries ? (*MSH6,*)*PMS2*
  - Hormone replacement?

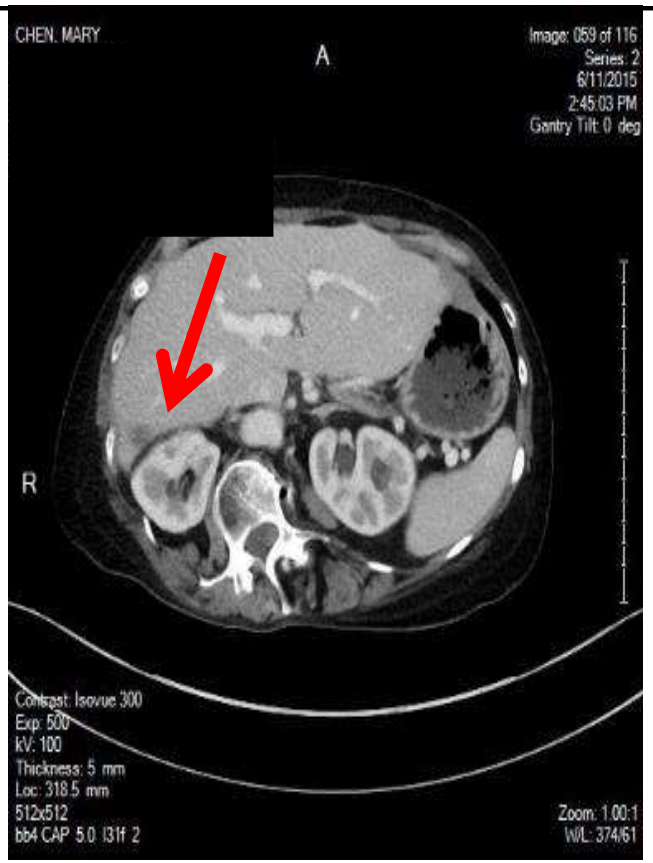
Therapeutic Implications of LS Cancers

- After the FDA approval of Pembrolizumab for MSI-H tumors, the assessment of MSI status is becoming standard of care in advanced colon and endometrial cancer

- **Progressive identification of individuals with Lynch syndrome !!!**

Endometrial Cancer: Partial Responder after 20 wks on Pembrolizumab





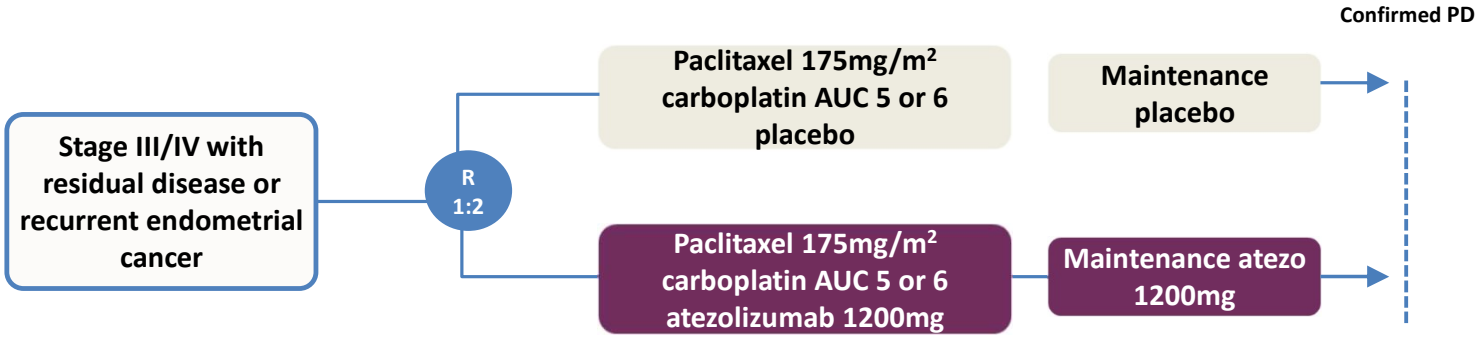
Amanda Nickles Fader: San Diego SGO 2016

Pembrolizumab

- Phase Ib trial KEYNOTE-028 evaluating RR in patients with refractory PD-L1+ solid tumors
  - Cohort endometrial cancer patients (N=24)
  - PR+SD=26%
- Phase Ib trials KEYNOTE-028/016/158 evaluating RR in patients with MSI or MMR deficient solid tumors
  - Cohort endometrial cancer patients (N=14)
  - Objective response rate 46.%
  - Duration of response 1.9 to 22.1 months

FDA 2017; Ott PA et al. JCO 2017

ENGOT-en7/MaNGO/AtTEnd
Study Design



Stratified by:

- Country of the experimental center
- Histological type (endometrioid vs. other types)
- Disease (recurrent disease vs advanced disease at primary diagnosis)
- MS status (MSS vs MSI vs non-evaluable)

Study Duration: accrual 2 years; Follow-up: 2 years

Total Sample Size: 550 evaluable patients

Takeaway messages

- **The most important Gynecological Cancer Susceptibility Syndromes include hereditary breast-ovary, Lynch, Cowden and Peutz-Jeghers syndromes**
- **BRCA testing in the patient population informs patient management decisions and should be performed in all patients with ovarian cancer**
- **Tumour BRCA testing is becoming more widely utilised to increase patient selection**
- **In the next 5 years, new diagnostic technologies (HRRm gene panel, HRD genomic scar, ctDNA) will be more utilised**
- **The cascade effect will allow the identification of family members at risk for whom effective prevention measures are available**
- **Lynch remains the main cause of hereditary endometrial cancer**
- **IHC for MMR and MSI is now recommended in all patients with endometrial cancer as may have therapeutic implications**
- **As the price of sequencing decreases, direct germline testing of endometrial cancer patients may become reality**

HRD: Homologous recombination deficiency
HRR: homologous recombinational repair