Clinical Application of Homologous Recombination Deficiency (HRD) Biomarkers

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Conflicts of Interest

- Employee at Foundation Medicine
- Roche Shareholder

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Key Topics

1. Homologous Recombination Repair (HRR)
2. Clinical Validation of HRRm
3. HRD v HRRm
4. Clinical applications of HRD biomarkers

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Homologous Recombination Repair (HRR)

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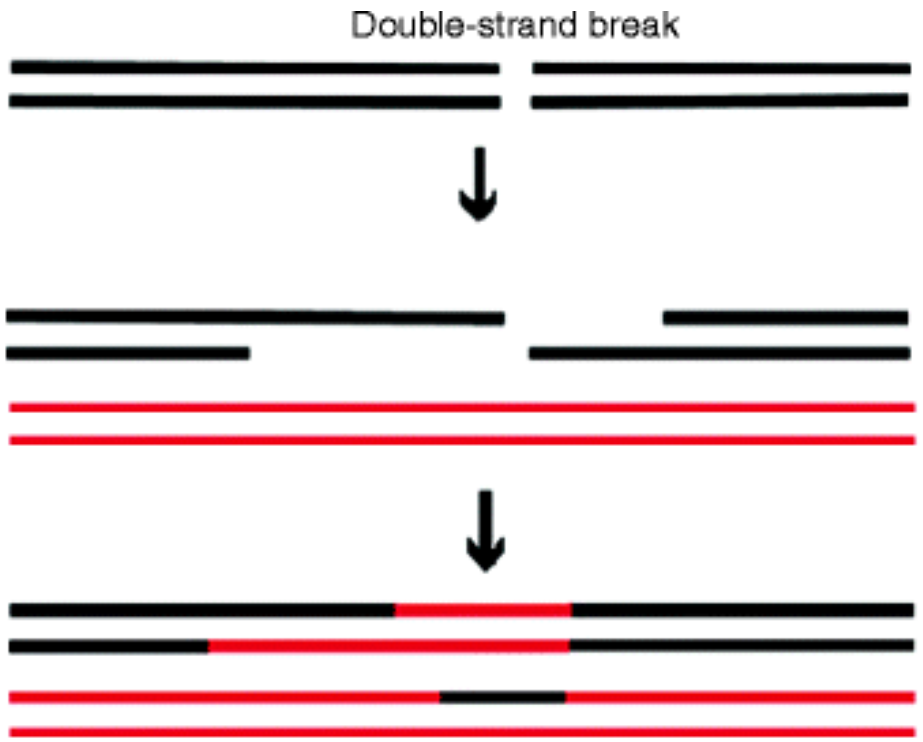
Homologous Recombination Repair (HRR)

Repair of Double Strand Breaks

- Homologous Recombination Repair (HRR) is a cellular pathway to repair double strand breaks using the sister chromatid as a guide
- HRR ensures **chromosomal integrity** and **cell viability** when double strand breaks occur since alternative mechanisms like non-homologous end joining are error-prone



1. Thacker 2017, Springer

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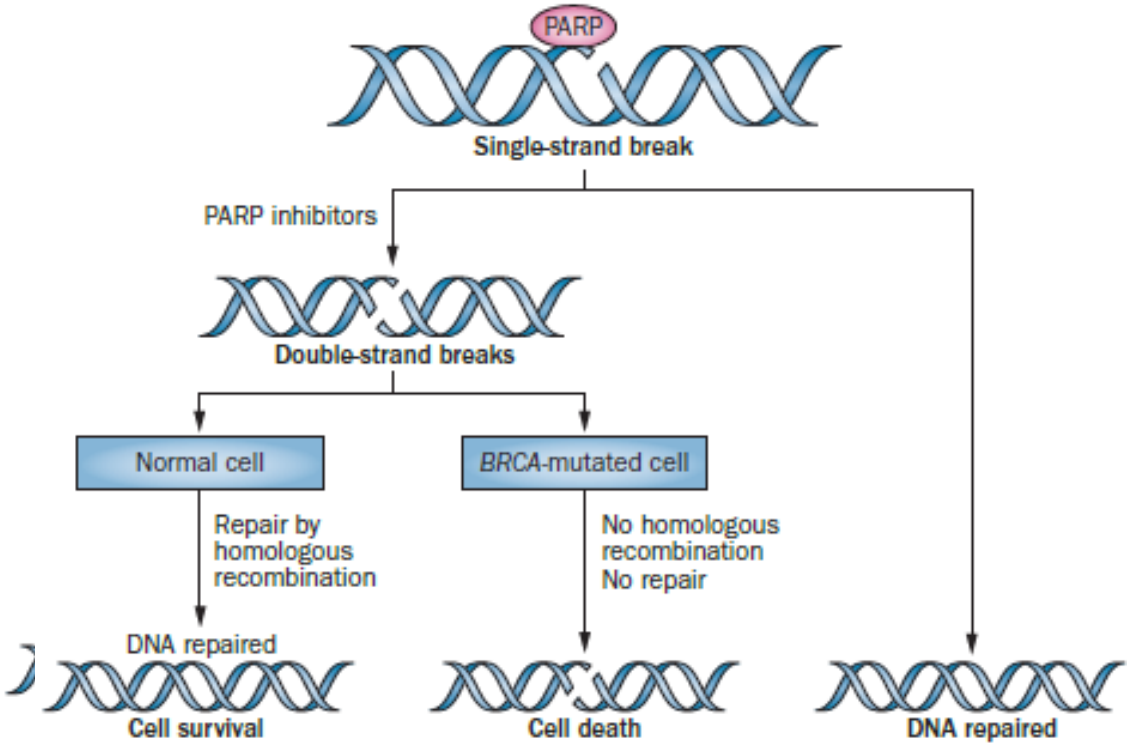
HRR Deficient Tumors are sensitive to Pt/PARPi

- HRR deficient tumors are sensitive to agents that induce double strand breaks: **'Synthetic lethality'**
- **Platinum chemotherapies** and Poly (ADP-ribose) polymerase **(PARP) inhibitors** induce double strand breaks and may benefit patients with deficiencies in HRR pathway



1. Sonnenblick 2015, Nature Rev. Clin

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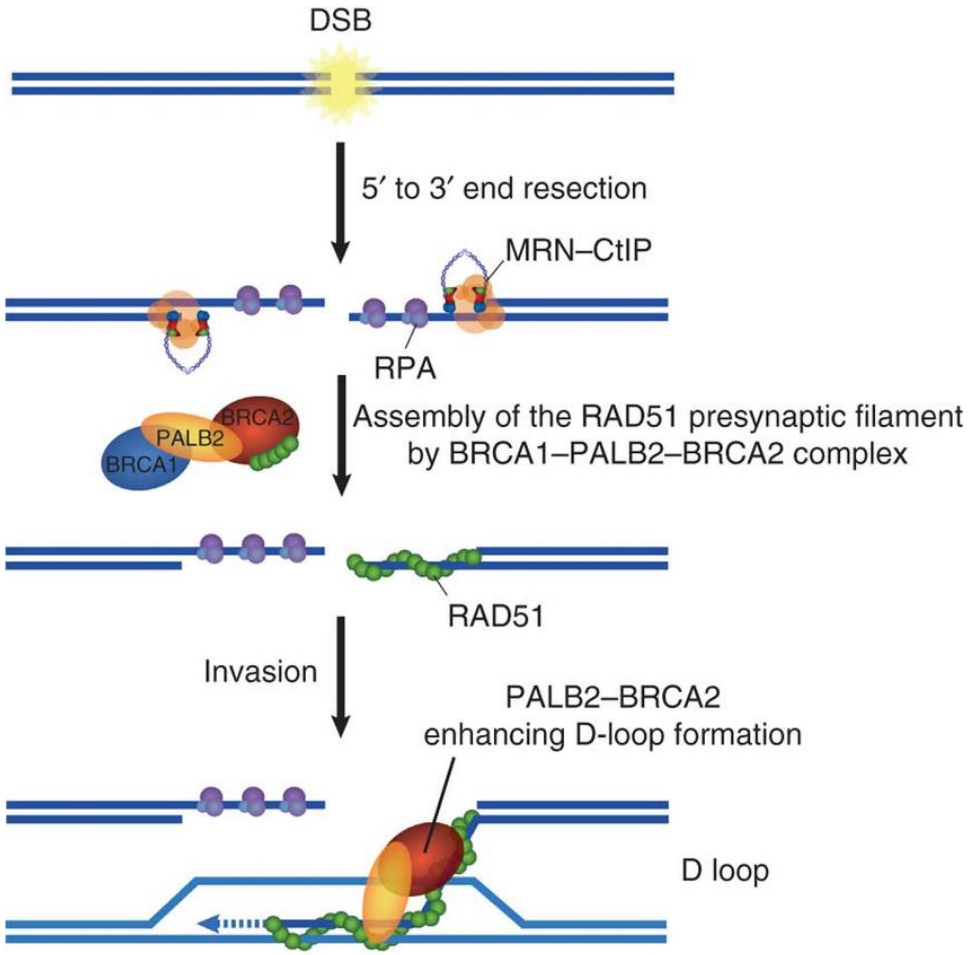
The HRR Pathway

- HRR requires the coordination of several proteins and protein complexes for efficient repair
- Mutations in HRR genes may lead to pathway inactivation, with strong evidence for ***BRCA1*** and ***BRCA2*** pre-clinically and clinically
- Growing body of evidence that mutation of additional pathway genes like ***RAD51*** and ***PALB2*** can impair HRR



1. Buisson R, Dion-Cote et al. Nature structural & molecular biology. 17. 1247-54.

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Clinical Validation of HRRm

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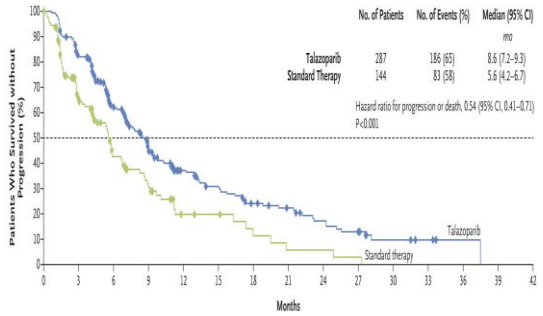
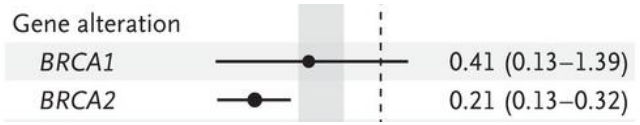
BRCA1/2 Mutation is a Biomarker of PARPi response

- ***BRCA1*** and ***BRCA2*** mutations predict sensitivity to PARPi in many tumor types, including breast, ovary, prostate, and pancreatic cancers
- Evidence that **germline** and **somatic** mutations are associated with efficacy in ovary, breast, prostate
- Approvals for many PARPi, including olaparib, niraparib, and rucaparib

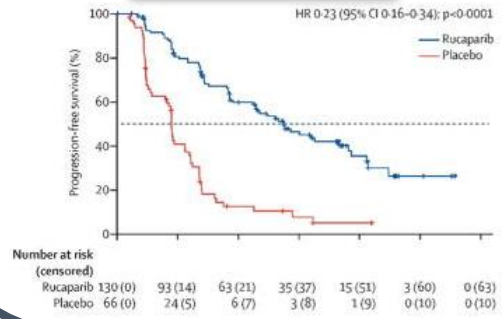
Breast



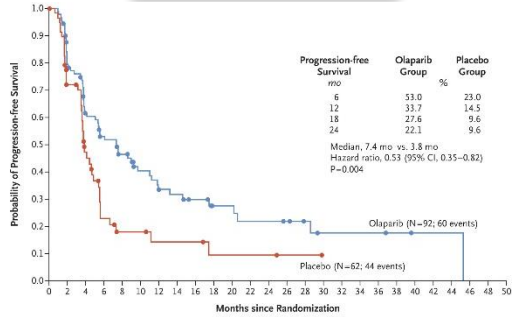
Prostate



Ovary



Pancreas



Ovary: ARIEL 3 Coleman et al., 2016; Pancreas: POLO, Golan et al, 2019; Prostate PROFOUND Bono et al, 2020; breast EMBRACA Litton et al 2018

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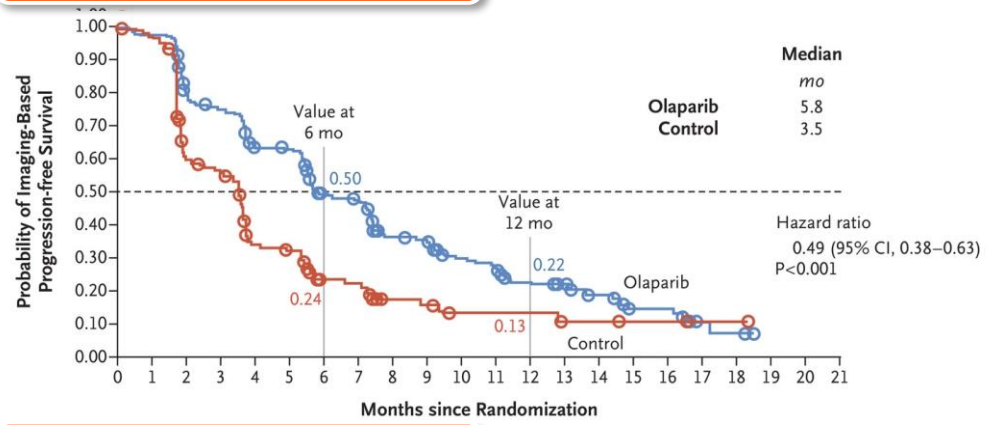
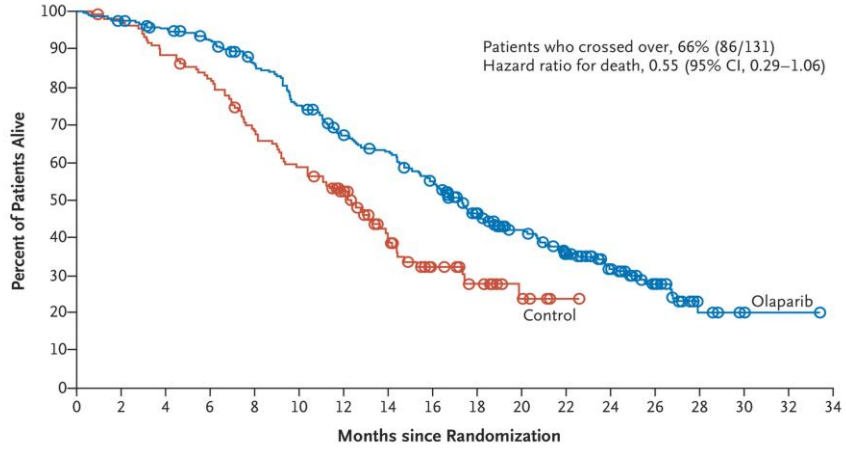
Prostate PARPi efficacy for a basket of HRR genes

- Enrollment for a broad basket of HRR genes: *BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R1, RAD51B, RAD51C, RAD51D, RAD54L BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R1, RAD51B, RAD51C, RAD51D, RAD54L*
- 2.3 month improvement in PFS
- F1CDx Companion Diagnostic claim in prostate cancer for olaparib with eligibility based on the basket of HRR genes

Progression Free Survival



Crossover-Adjusted OS



1. Bono et al. NEJM. 2020. Hussain et al, NEJM 2020.

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HRD v HRRm

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HRRm v HRD

- HRRm (HRR mutation) and HRD (Homologous Recombination Deficiency) are distinct but related terms
- Additional mechanisms for HRD exist beyond HRRm, including gene methylation
- Measuring/assessing HRD may help identify **"BRCAness"** patients who lack an HRR alteration but may respond to Pt/PARPi

HRRm: Mutation in one or more HRR pathway genes that may lead to functional deficiency in homologous recombination

HRD: Homologous recombination deficiency is a phenotype where tumor cells are not efficiently able to undergo HRR

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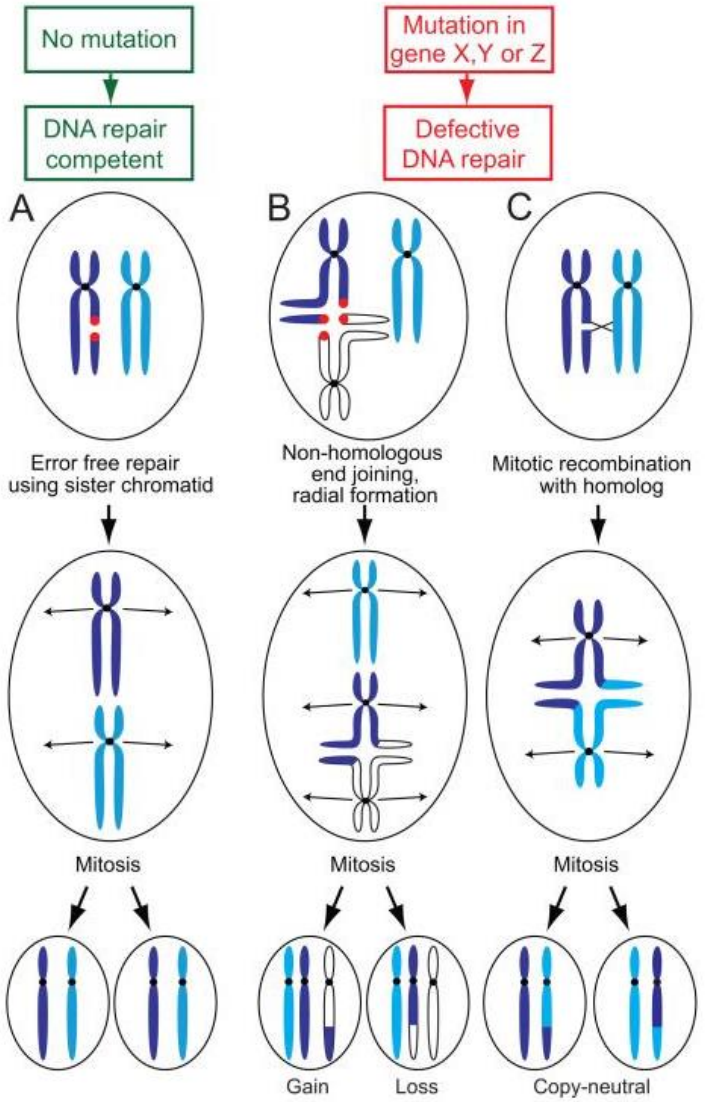
HRD may result in genomic scarring

- Alternative repair pathways (NHEJ and mitotic recombination) leave genomic scars on the genome
- Most common scars are **LOH segments** (LOH1 or LOHx)
- Focal segments (not whole arm)



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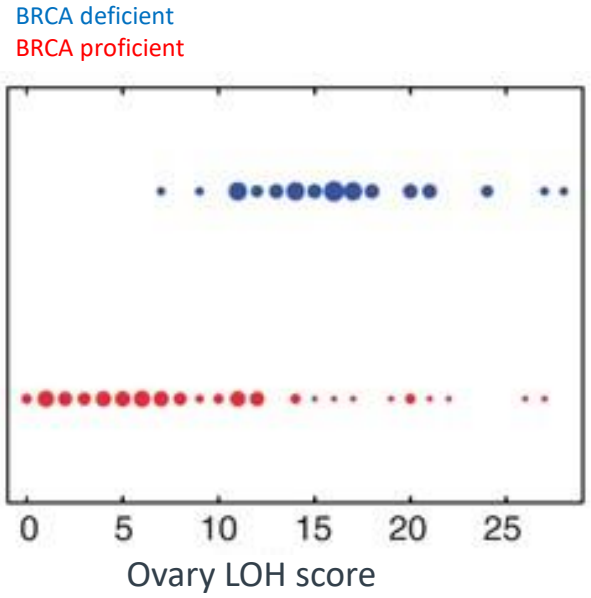
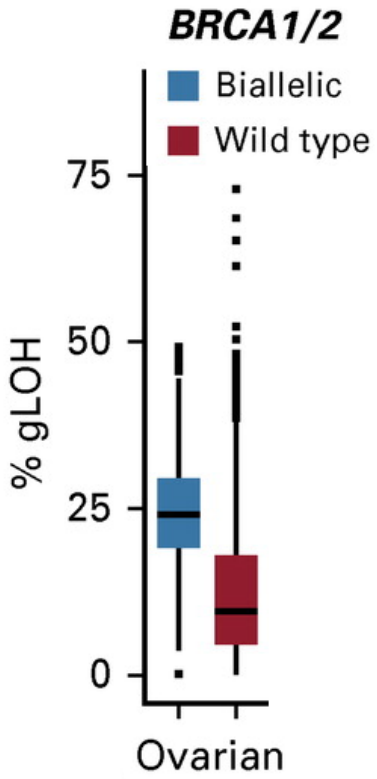
Genomic scarring (gLOH) correlates with *BRCA* status

- Several publications associating focal LOH fragments with *BRCA1/2* inactivation
- Suggested that a scarring approach could identify HRD tumors





1. Abkevich 2012 BJC

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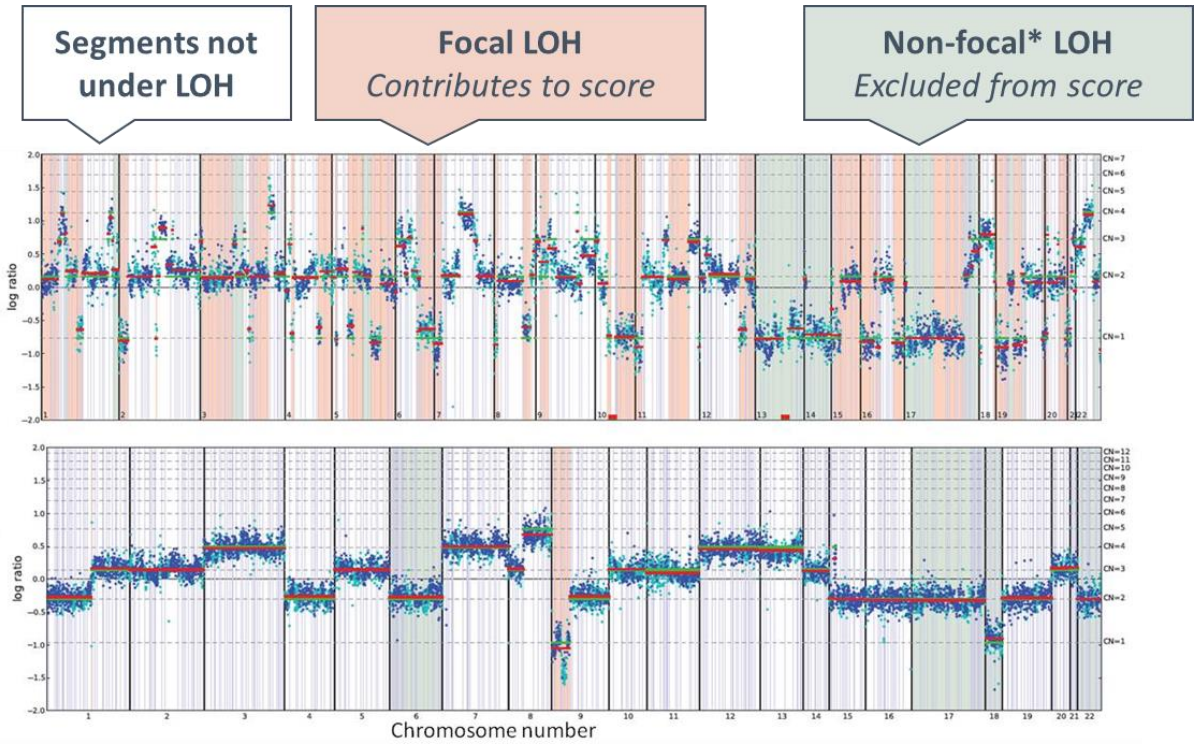
FMI development of a gLOH HRD algorithm

- HR defects can result in accumulation of focal LOH segments
- FMI measures HRD with a genome-LOH (gLOH) biomarker



LOH-High (≥ 16%)
Most segments under LOH are **focal** and **contribute** to the LOH score. Genomic LOH profile **exceeds** 16%.

LOH-Low (< 16%)
Most segments under LOH are **chromosome-level events** and are **excluded** from the LOH score. Genomic LOH profile is **below** 16%.

1. Swisher et al., 2016; 27908594, Coleman et al., 2016; ASCO Abstract 5540, Elvin et al., 2017; ASCO Abstract 5512

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Clinical applications of HRD biomarkers

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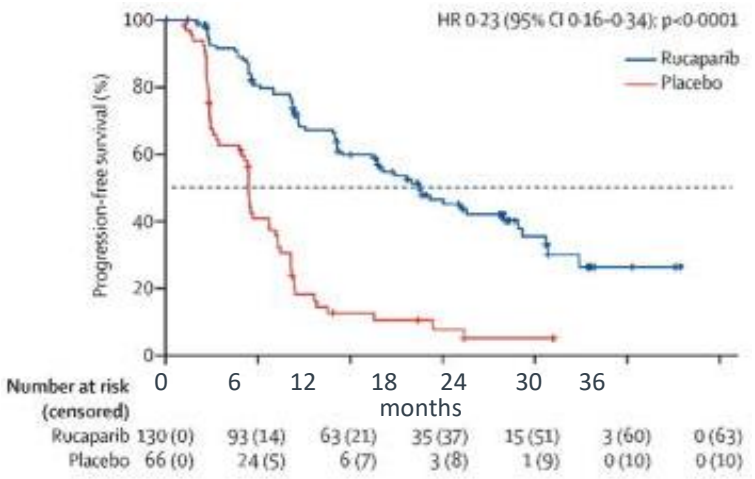
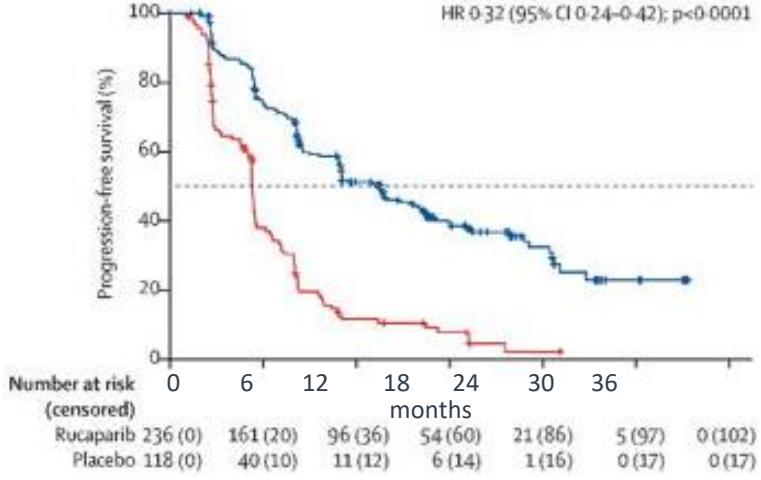
Validation of the gLOH HRD algorithm in ARIEL3

- ARIEL3: Ph3 trial in high grade, Pt-sensitive ovarian cancer; rucaparib v placebo
- Pre-specified gLOH cutoff of 16%; examined in overall and BRCA-WT populations

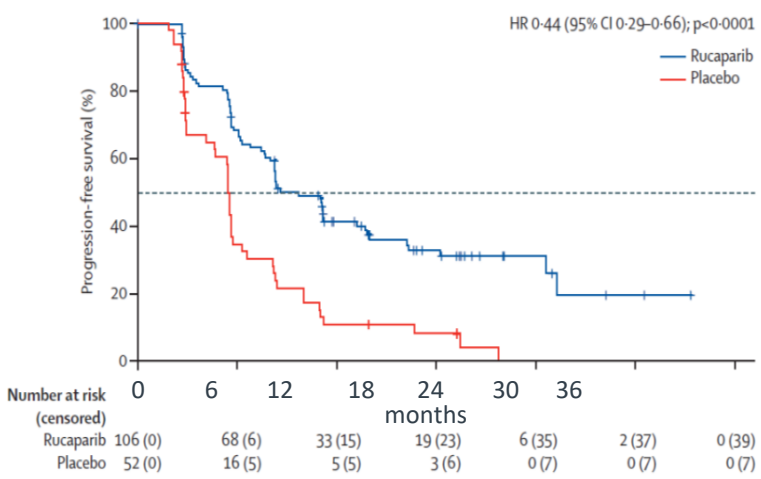
BRCA-mut



gLOH-H



BRCA-WT/gLOH-H



1. Coleman et al., 2016

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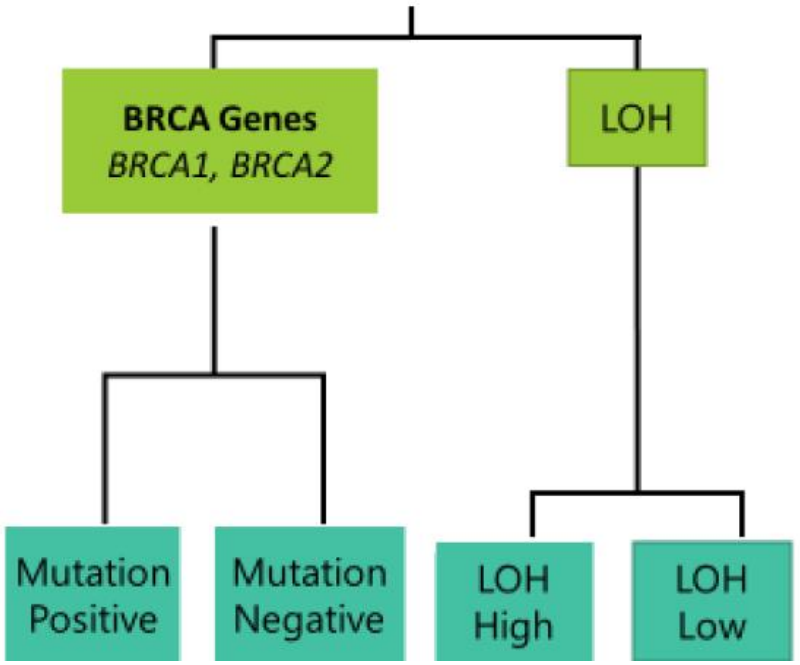
HRD Complementary Diagnostic in Ovarian Cancer

- Based on the results of the ARIEL3 trial, HRD was added as a **complementary diagnostic** for rucaparib in ovarian cancer
- HRD is a **composite biomarker**, taking into account *BRCA1/2* status and gLOH score
- Included on the Foundation Medicine CDx report

BRCA status	LOH Status	HRD Status
BRCA+ (either sBRCA or gBRCA)	LOH high	**HRD +**
	LOH low	
BRCA -	LOH high	
BRCA -	LOH low	**HRD -**

HRD biomarker



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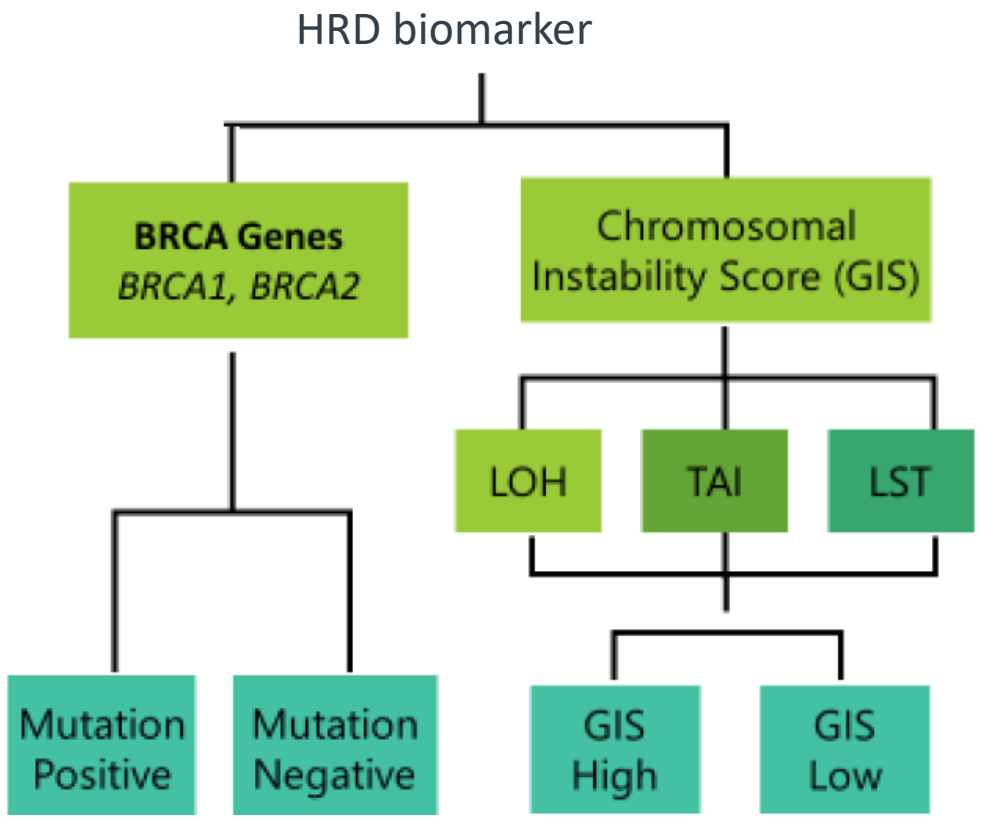
Myriad HRD Biomarker in Ovarian Cancer

- Myriad MyChoice HRD is also a **composite biomarker**, including genomic scarring methods (gLOH, TAI, LST) and *BRCA1/2* status
- **Companion diagnostic biomarker** in ovarian cancer for niraparib and maintenance olaparib

BRCA status	GIS Status	HRD Status
BRCA+ (either sBRCA or gBRCA)	GIS high	**HRD +**
	GIS low	
BRCA -	GIS high	
BRCA -	GIS low	**HRD -**



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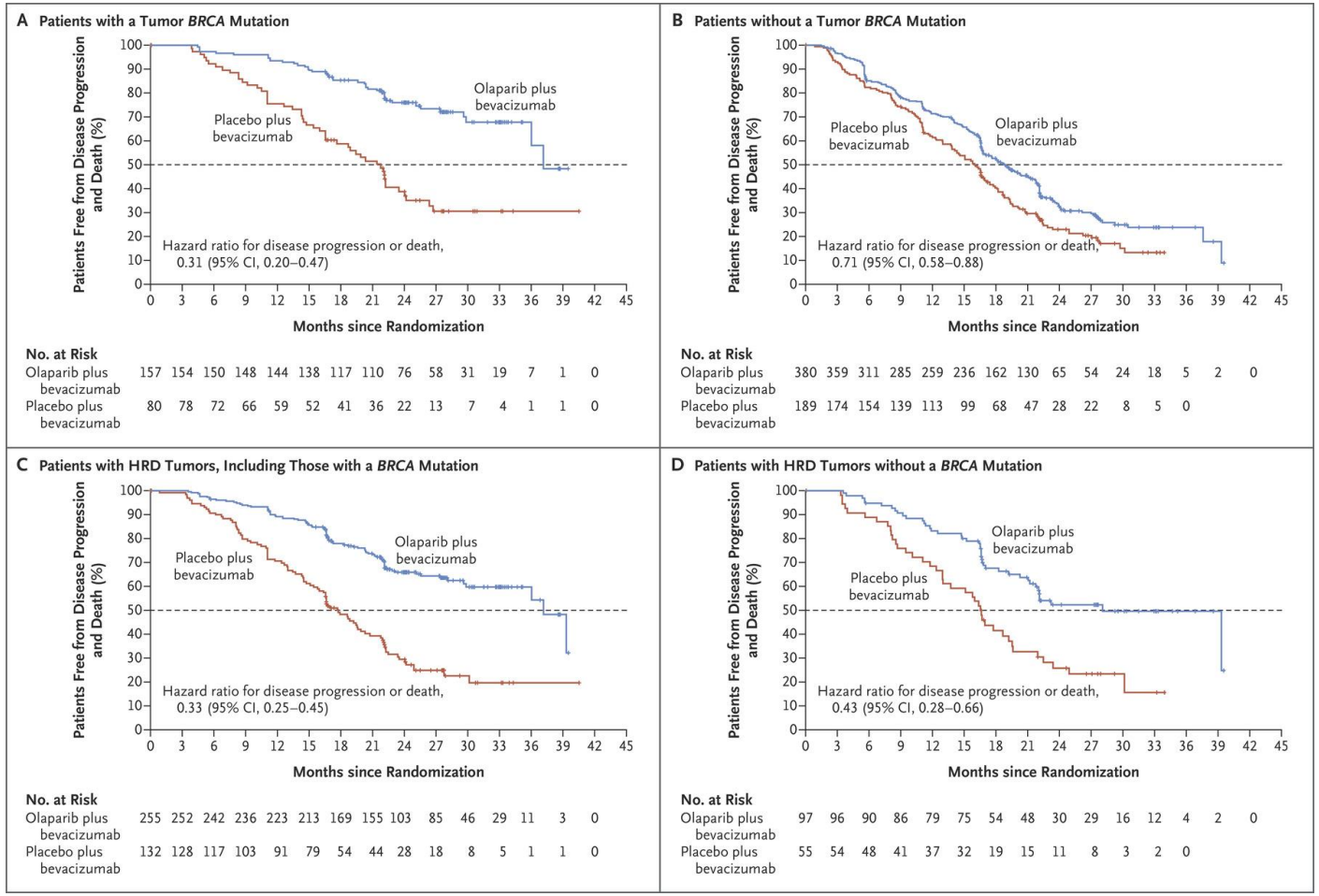
Myriad HRD Biomarker in Ovarian Cancer

- Myriad MyChoice HRD identifies BRCAwt patients who benefit from olaparib in PAOLA-1



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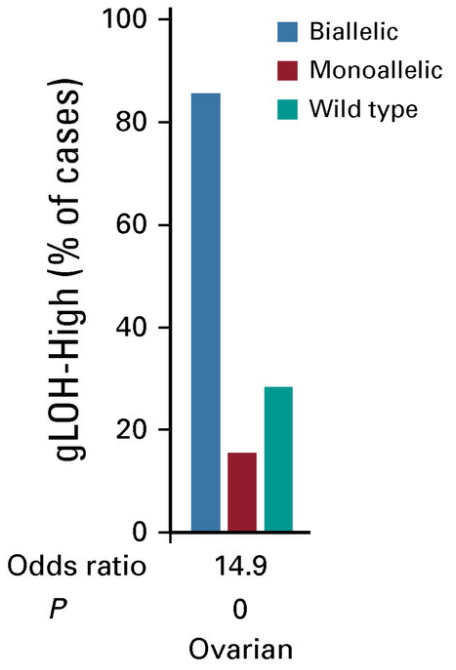
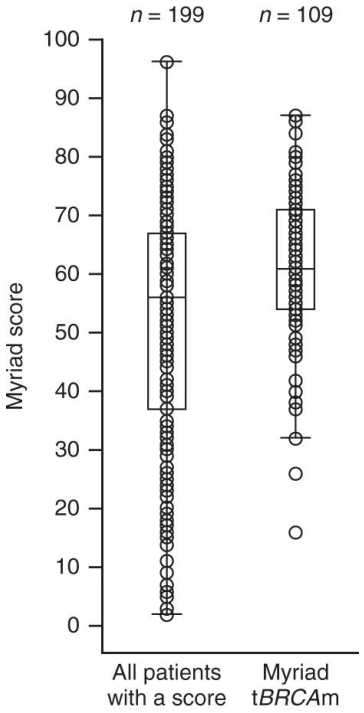
Myriad and Foundation Medicine Approaches

- Myriad's and Foundation Medicine's approaches examine genomic scarring
- Both tests are clinically validated with meaningful benefit :
  - HR 0.32 and O.33 in ARIEL3 FMI and PAOLA-1 Myriad, respectively for biomarker + v -
  - HR 0.44 and 0.43 in ARIEL3 FMI and PAOLA-1 Myriad, respectively for biomarker + BRCA-WT
- Both tests identify a majority of *BRCA1/2* deficient tumors; FMI ~86% of biallelic *BRCA1/2*, Myriad tuned to ~95% sensitivity



Foundation Medicine
Sokol, 2020 JCO PO



Myriad Genetics
Hodgson, 2018 BJC

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Current Status of HRD Biomarkers

- HRRm detection is available from a number of comprehensive genomic profiling tests, including those from Myriad, Caris, Foundation Medicine, Tempus, in-house panel tests
- HRD tests are only available in ovarian cancers through Myriad and Foundation Medicine, incorporating HRRm and genomic scarring

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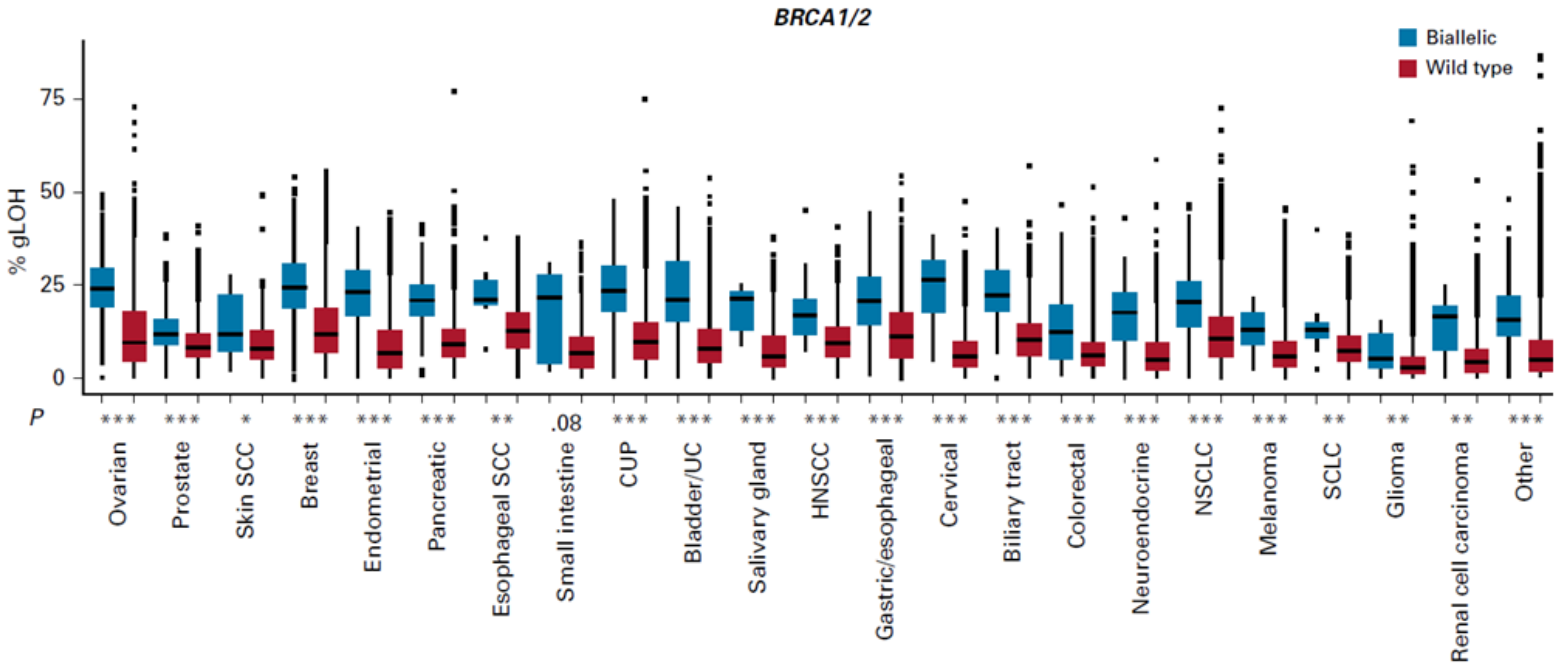
Future Status of HRRm/HRD Biomarkers

- Trials are planned investigating PARPi for additional HRR genes. We expect the landscape of HRRm approvals to expand in the coming years (>30 DDR genes covered)
- No clinical validation of HRD biomarkers outside of ovarian cancer, but research studies have examined associations of gLOH with HRRm beyond ovary



1. Sokol 2020 JCO PO

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Future Status of HRRm/HRD Biomarkers

- Expansion of HRD will **require clinical validation**
- **May require optimization** of the assay for different tissues

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Case Presentation

Umut Disel MD

Acibadem Health Group, Adana Hospital

Medical Oncology

Adana/Turkey

A case report; Ovarian cancer (ORD-0876496-01)

- 67 year old female patient. She appladmitted for abdominal swelling. With the diagnosis of ovarian cancer, the TAH + BSO operation was performed in September 2018.
- While the CA-125 test was 1200 at the time of diagnosis (pre-op), the postoperative level fell to 250. Final CA125 test level 12 after adjuvant chemotherapy was within normal range- (March 2019)
- Pathological examination was reported as high grade ovarian serous cancer. Because of stage III disease, adjuvant 6 cycles of paclitaxel and carboplatin chemotherapy was administered.

continued

- After 12 months adjuvant chemotherapy, relapse with increasing CA125 levels and some symptoms. Because of neuropathy in the second line setting, Gemcitabine plus Carboplatin plus Bevacizumab x 6 course was given then Bevacizumab maintenance. At 9th months of maintenance CA125 progressed - and F1CDX sent.
- Gem plus Carbo initiated again. (3th. line)
- Weak family history for same cancer.
- BRCA1/2 test for plasma (germline) negative (Inhouse test)
- She is never smoker.
- F1CDX and PDL1 staining was sent with first tissue from operation (time of the diagnosis) (September 2018)

Biomarker Findings

Loss of Heterozygosity score - 22.8%
Microsatellite status - MS-Stable
Tumor Mutational Burden - 8 Muts/Mb

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

CDK12 **Q244fs*93**
MET **amplification - equivocal†**
TP53 **D259Y**

2 Disease relevant genes with no reportable alterations: *BRCA1, BRCA2*

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† See About the Test in appendix for details.

continued

- After F1CDX report, Olaparib (lynparza) was started in the patient because of high LOH.
- She continues her treatment in the second month at a dose of 450mg/day without any problem.
- It continues to be treated with disease free and CA125 within normal limits.

Questions from presenter- Need to discuss

It is not surprising to see LOH score >16 (22) in this serous ovarian cancer case. Any Tumor supressor gene -HRD related genes alterations? Any explanations about that? Which gene/genes responsible for high LOH score?

MET amplification in this case (equivocal) functional? (Is this responsible for resistance to platinum agents?) or just a co-incidence?

CDK12 alteration- loss- what is the meaning of this alteration? PARP or platinum drugs sensitivity? Any immunotherapeutic efficacy prediction?

Finally Q about TMB 8 mut/Mb is higher than the median level of ovarian serous cancers. Any answer about this?- HRD genes and high TMB?

Case 2

ORD-0903204-01,
Ovary serous carcinoma

Dr. Jung-Yun Lee,

Gyn Oncologist

Severance Hospital, Yonsei University

South Korea

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ORD-0903204-01, Ovary serous carcinoma

Case History

- 58 year old female who was diagnosed with ovarian cancer, stage IIIC in May 2016.
- She underwent PDS+POAC #9 (June 2016~December 2016), Taxotere+Carboplatin CTx #9 (July 2017~March 2018), Caelyx+Avastin CTx #6 (August 2018~January 2019), Topotecan CTx #6 (January 2019~July 2019), then referred to our institution.
- Initial imaging taken in our hospital showed multiple variable sized hepatic metastasis.
- The patient started on Gemzar+Carboplatin CTx #6 (August 2019~December 2019). Follow-up imaging taken on January 2020 revealed overall diminished tumor burden, but increased size of hepatic mass. US guided liver mass biopsy was done, and the final pathology reported a few atypical cells, consistent with metastatic serous carcinoma from the ovary.
- The patient was enrolled in KGOG 3045 (AMBITION study) and treated with Durvalumab+Tremelimumab+Paclitaxel (January 2020~August 2020). Abd+Pel CT taken on August 2020 showed increased extent of hepatic metastatic nodules, therefore, she was eliminated from the trial and began Taxotere+Cisplatin CTx from September 2020.
- After 3 cycles of Taxotere+Cisplatin CTx, she had PR.

ORD-0903204-01, Ovary serous carcinoma

Additional Clinical Data (I) – NGS (August 2019), Uterus & BSO slides

NGS, 고형암 panel (Level II) Analysis Report

▣ 검체 정보

검체 번호	성별	나이	Unit NO.	환자명	장기명/진단	검체 유형
SR19-09647	[redacted]				Ovary/High-grade serous carcinoma	FFPE
의뢰의	의뢰의 소속			검체의 적절성여부	검체 접수일	결과보고일
이정윤	산부인과			적합 (Tumor%: 70 %)	20190829	20191018

▣ 검사결과

1. Variants of clinical significance

- SNVs & Indels

GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
TP53	Missense mutation	p.R248Q	91.1%	NM_000546.5:c.743G>A	NP_000537.3:p.Arg248Gln

- Fusion gene : None

- Copy number variation :

GENE	LOCATION	FOLD CHANGE	ESTIMATED COPY NUMBER
CCNE1	dup(19)(q12)	4.66	12.457
MDM4	dup(1)(q32.1)	2.815	7.186
MYC	dup(8)(q24.21)	2.504	6.297

2. Variants of unknown significance

- SNVs & Indels

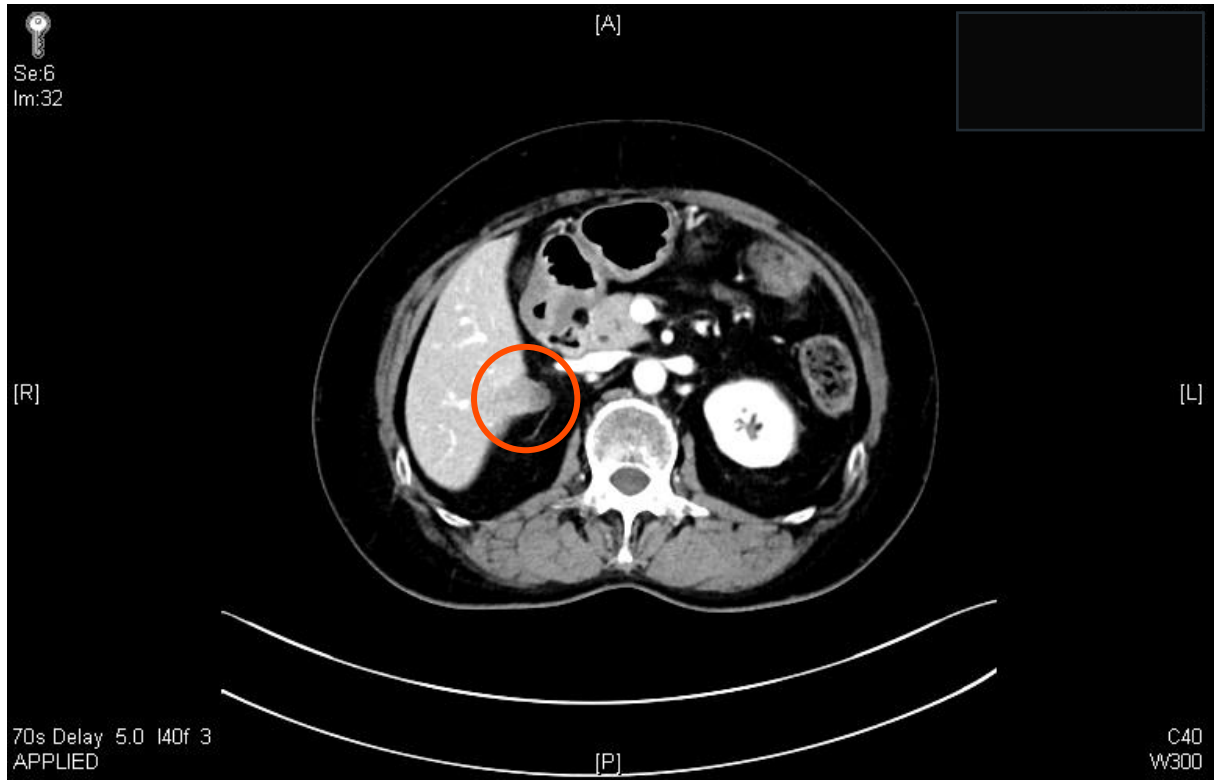
GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
CDK12	Missense mutation	p.R1008Q	92.5%	NM_016507.2:c.3023G>A	NP_057591.2:p.Arg1008Gln
CDKN2A	Missense mutation	p.D105E	61.5%	NM_001195132.1:c.315C>A	NP_001182061.1:p.Asp105Glu
FGFR3	Missense mutation	p.A502T	56.9%	NM_001163213.1:c.1504G>A	NP_001156685.1:p.Ala502Thr
ATM	Missense mutation	p.S854Y	53.3%	NM_000051.3:c.2561C>A	NP_000042.3:p.Ser854Tyr
KMT2A	Missense mutation	p.P54A	9.9%	NM_001197104.1:c.160C>G	NP_001184033.1:p.Pro54Ala

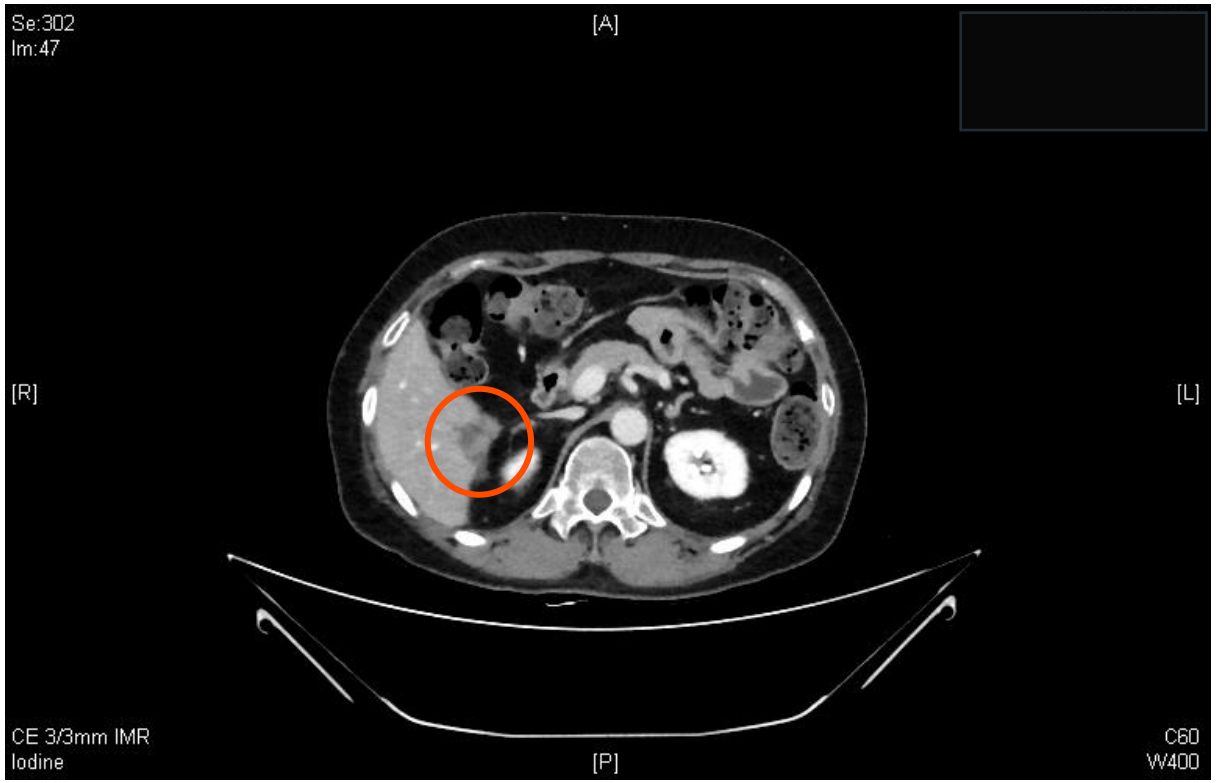
- Copy number variation :

GENE	LOCATION	FOLD CHANGE	ESTIMATED COPY NUMBER
CDK4	dup(12)(q14.1)	1.793	4.266
ERCC1	dup(19)(q13.32)	1.721	4.060
CHEK2	dup(22)(q12.1)	1.7	4.000
AKT2	dup(19)(q13.2)	1.659	3.883
ESR1	dup(6)(q25.1-25.2)	1.5	3.429
PIK3CB	dup(3)(q22.3)	1.491	3.403
FGF23	del(12)(p13.32)	0.583	0.809
FGFR4	del(5)(q35.2)	0.564	0.754
PDGFRB	del(5)(q32)	0.53	0.657
FGF4	del(11)(q13.3)	0.509	0.597
FGF6	del(12)(p13.32)	0.465	0.471
NRG1	del(8)(p12)	0.447	0.420

ORD-0903204-01, Ovary serous carcinoma

Additional Clinical Data (II) – Initial Abd CT (August 2019) & Follow-up Abd CT (January 2020)





The patient started on Gemzar+Carboplatin CTx #6 (August 2019~December 2019). Follow-up imaging taken on January 2020 revealed overall diminished tumor burden, but increased size of hepatic mass. US guided liver mass biopsy was done, and the final pathology reported a few atypical cells, consistent with metastatic serous carcinoma from the ovary.

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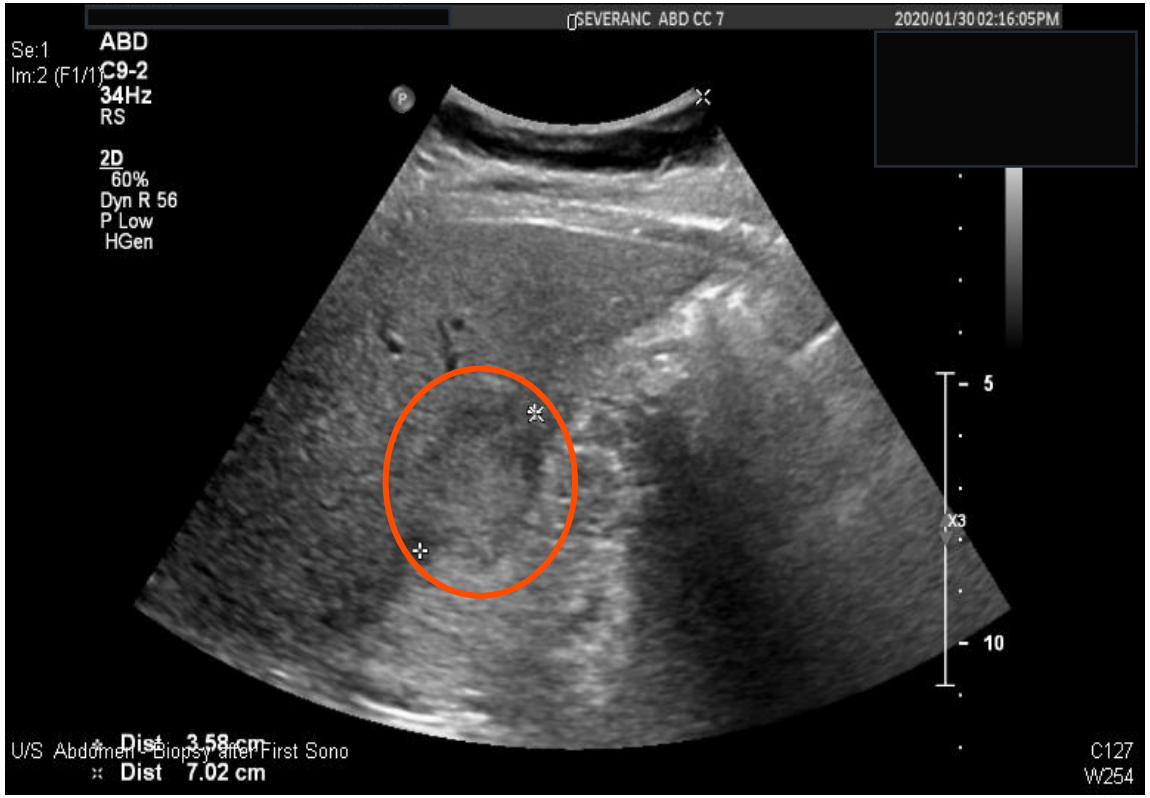
ORD-0903204-01, Ovary serous carcinoma

Additional Clinical Data (III) – US guided Liver mass Bx (January 2020)



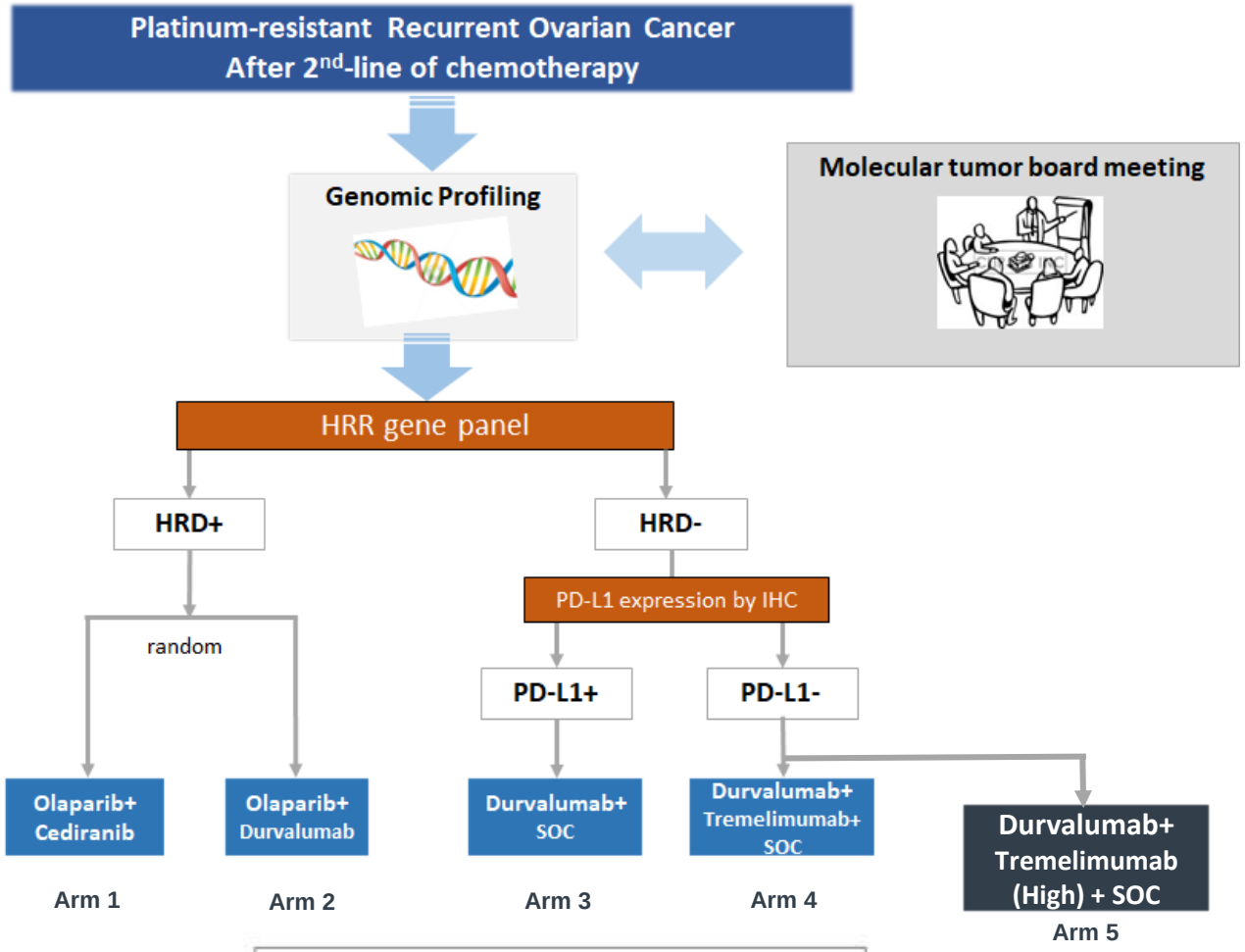
[Name Of Operation]
Liver, biopsy

[Pathological Diagnosis]
[Final report]
A few atypical cells expressing PAX8 and WT-1, consistent with metastatic serous carcinoma from the ovary, see note.

Note) The immunohistochemical stain results:
PAX8 and WT-1: positive in tumor cells

The patient was enrolled in KGOG 3045 (AMBITION study) and treated with Durvalumab+Tremelimumab+Paclitaxel (January 2020~August 2020).

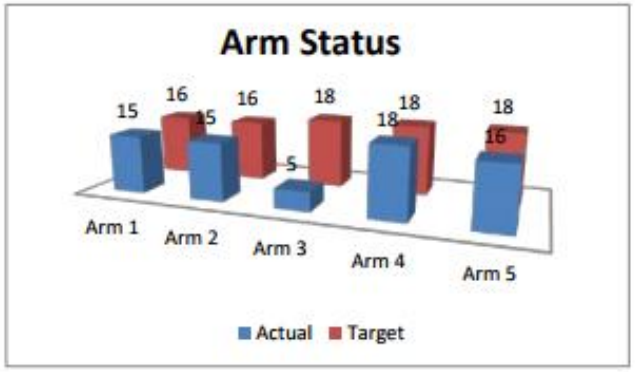
KGOG 3045 / AMBITION



Number	Gene Symbol
1	BRCA1
2	BRCA2
3	ATM
4	BRIP1
5	PALB2
6	RAD51C
7	BARD1
8	CDK12
9	CHEK1
10	CHEK2
11	FANCL
12	PPP2R2A
13	RAD51B
14	RAD51D
15	RAD54L

Primary endpoint: ORR
O+C or O+D is assumed with 30%
D+SOC or D+T+SOC is assumed with 25%
SOC, standard of care

Open: recruiting
Status: FPI in Dec 2018
Target: 86 pts

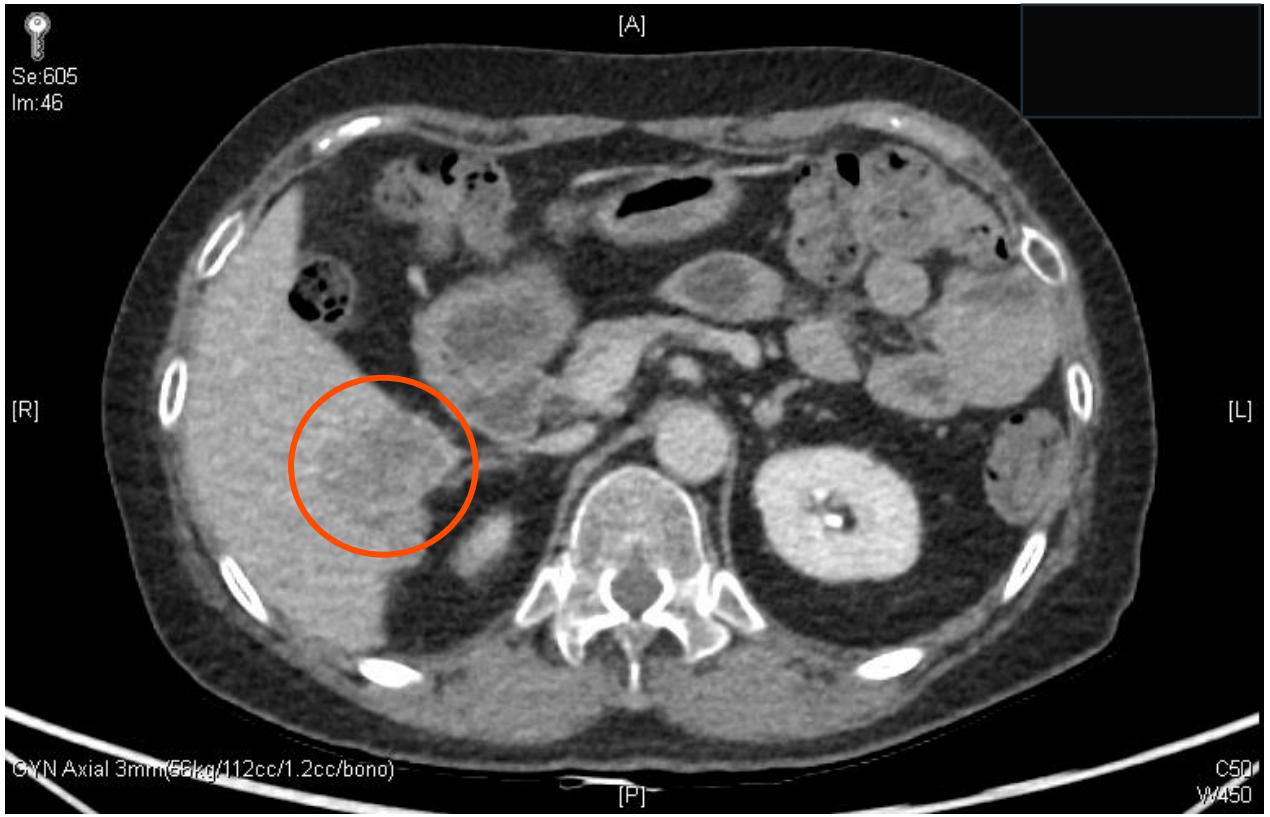


FOUNDATION MEDICINE

2020 Oct

ORD-0903204-01, Ovary serous carcinoma

Additional Clinical Data (IV) – Follow-up Abd-Pel CT & US guided Liver mass Bx (September 2020) **FMI**



Abd+Pel CT taken on August 2020 showed increased extent of hepatic metastatic nodules, therefore, she was eliminated from the trial

[Name Of Operation]
Liver, US-guided gun biopsy

[Pathological Diagnosis]
[Final report]

Presence of carcinoma, favoring metastatic serous carcinoma from the ovary, see note.

Note) 1. The immunohistochemical stain results:
PAX8: diffuse positive in tumor cells
2. 본 환자의 이전 liver 슬라이드 (SS20-06632) 를 리뷰하였습니다.

[Additional report]
+ Immunostaining result:
MLH1: No loss
MSH2: No loss
MSH6: No loss
PMS2: No loss
PD-L1 (SP263): Positive (10%)
HER2: Negative (1+)

Biomarker Findings

Loss of Heterozygosity score - 21.7%
Microsatellite status - MS-Stable
Tumor Mutational Burden - 4 Muts/Mb

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

AKT1 **amplification - equivocal†**
FGFR3 **A500T**
TP53 **R248Q**
C11orf30 (EMSY) **amplification**
CCNE1 **amplification**
EPHB1 **amplification - equivocal†**
MCL1 **amplification**
MDM4 **amplification - equivocal†**
MSH2 **G751R - subclonal†**
PRKCI **amplification**
TERC **amplification**

2 Disease relevant genes with no reportable alterations: *BRCA1, BRCA2*

† See About the Test in appendix for details.

4 Therapies with Clinical Benefit

0 Therapies with Lack of Response

28 Clinical Trials

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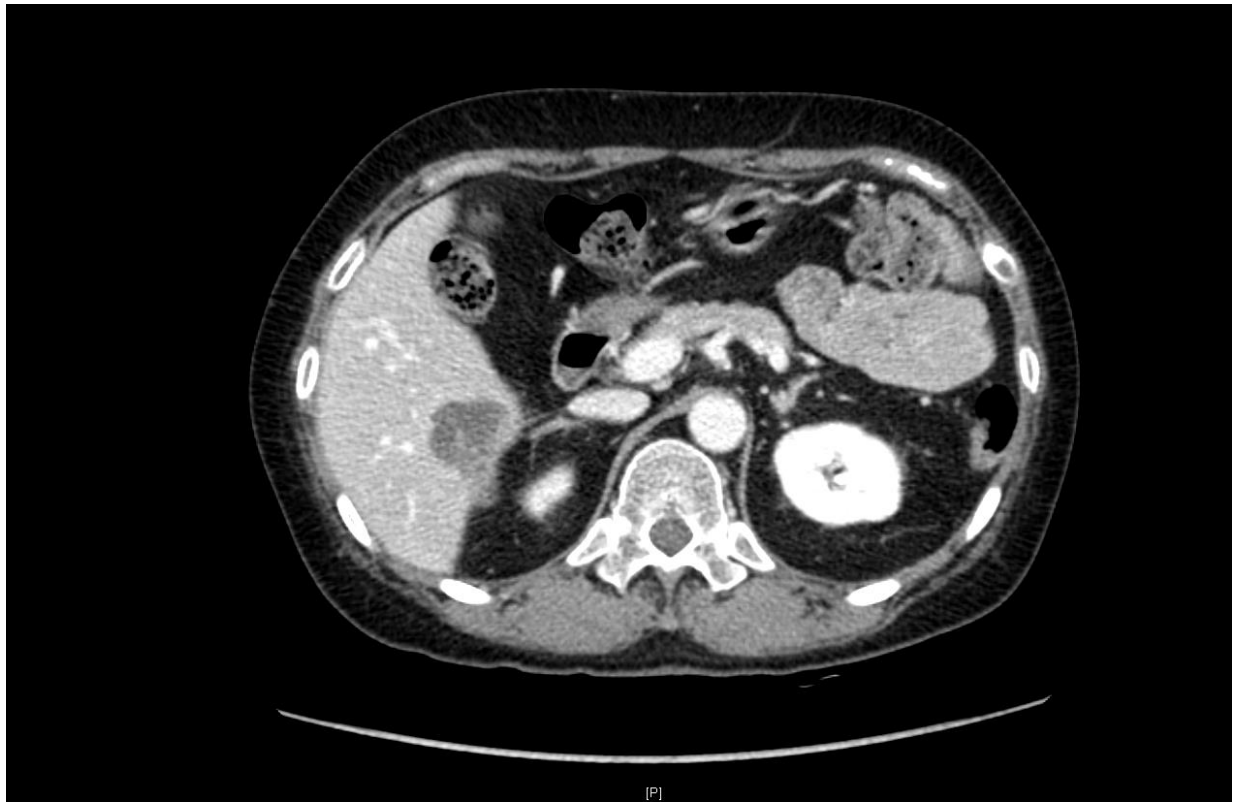
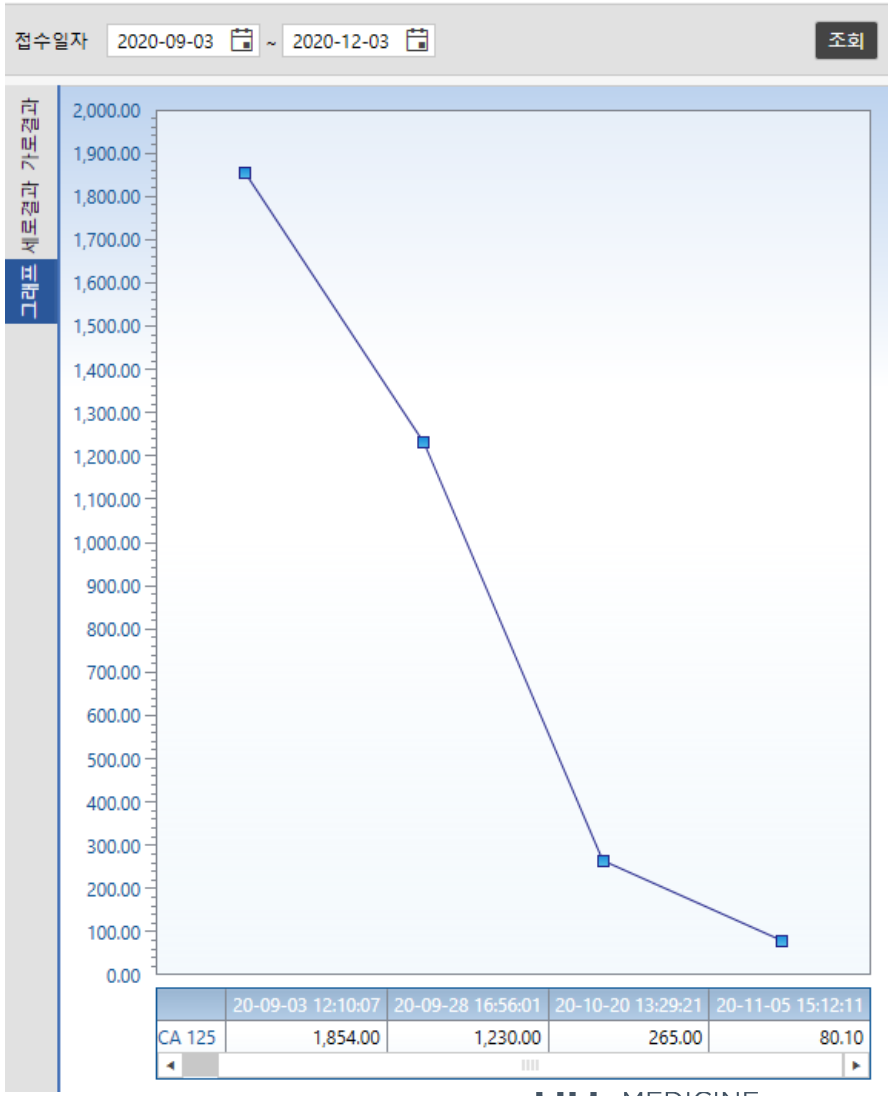
ORD-0903204-01, Ovary serous carcinoma

Additional Clinical Data (V) – Follow-up Abd-Pel CT (November 2020)





After 3 cycles of Taxotere+Cisplatin CTx, she had PR.
What is next?

Thank You

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