臺中榮民總醫院
Taichung Veterans General Hospital

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The use of molecular testing in ovarian cancer

focus on Synthetic Lethality & PARP I

台中榮民總醫院
婦女醫學部
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日期：20220813
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座長：王鵬惠，呂建興

Why do we need molecular testing in AEOC?

First-line Management of Ovarian Cancer

2003

Chemotherapy

No further improvement in survival with chemotherapy alone since the introduction of platinum–taxane chemotherapy[1,2]

Population	Study	Treatment	PFS
Optimal Stage 3	GOG 158	IV Pac & Carb	21 mos
	GOG 114	IV Pac & Cis	22 mos
	GOG 158	IV Pac & Cis	19 mos
	GOG 172	IV Pac & Cis	18 mos
Suboptimal 3 & 4	GOG 111	IV Pac & Cis	18 mos
	GOG 162	IV Pac Cis	12 mos
	GOG 152	IV Pac Cis	11 mos
All Stage 3 & 4	GOG 182	IV Pac/Carbo x 8	16 mos

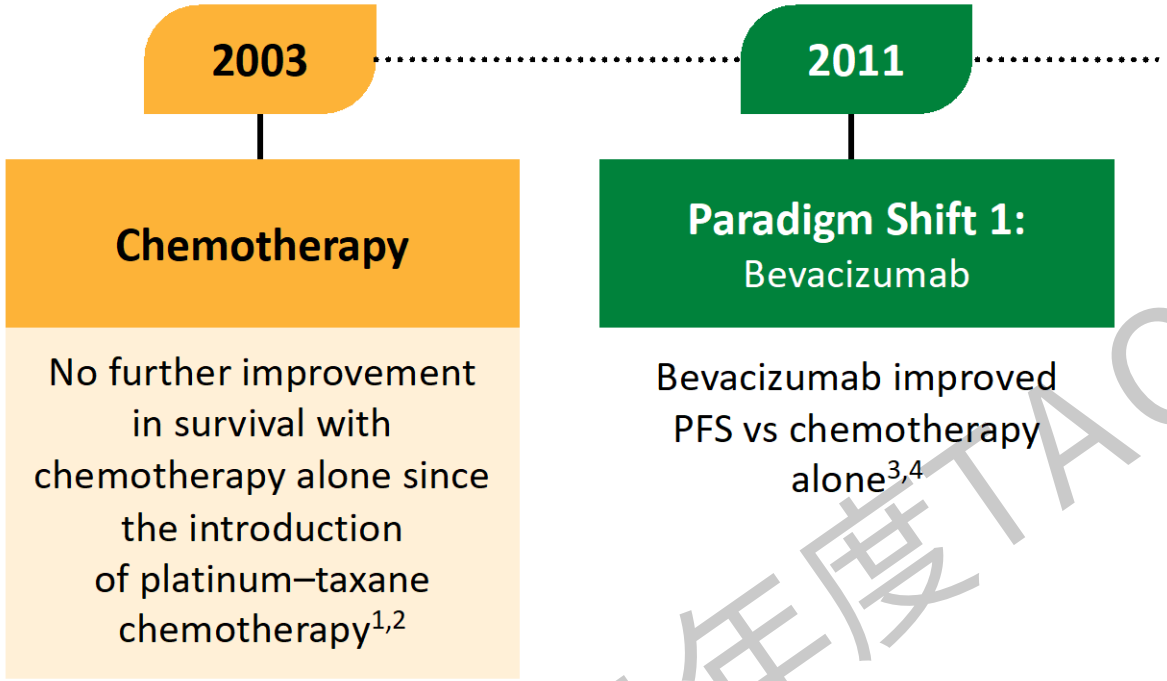
<2 yrs

<1.5 yrs

Several studies with PARP inhibitors as maintenance for newly diagnosed advanced ovarian cancer[5-8]

1. McGuire. NEJM. 1996;334:1. 2. du Bois. J Natl Cancer Inst. 2003;95:1320. 3. Burger. NEJM. 2011;365:2473.
4. Perren. NEJM. 2011;365:2484. 5. Friedlancer. Lancet Oncol. 2021;22:632. 6. Ray-Coquard. NEJM. 2019;381:2416.
7. Gonzalez-Martin. NEJM. 2019;381:2391. 8. Aghajanian. Gynecol Oncol. 2021;162:375.

First-line Management of Ovarian Cancer



Maintenance with bevacizumab can improve PFS, but approximately 4 months, in exchange for 5 cycles of concurrent therapy and single-agent maintenance extending beyond a year, without objective evidence of clinical benefit in terms of quality of life, time without symptoms or toxicity, or increased OS.

(Charlie et al, 2019)

Several studies with PARP inhibitors as maintenance for newly diagnosed advanced ovarian cancer[5-8]

1. McGuire. NEJM. 1996;334:1. 2. du Bois. J Natl Cancer Inst. 2003;95:1320. 3. Burger. NEJM. 2011;365:2473.
4. Perren. NEJM. 2011;365:2484. 5. Friedlancer. Lancet Oncol. 2021;22:632. 6. Ray-Coquard. NEJM. 2019;381:2416.
7. Gonzalez-Martin. NEJM. 2019;381:2391. 8. Aghajanian. Gynecol Oncol. 2021;162:375.

Why do we need molecular testing in AEOC?

- Taxol +carboplatin Q3W x6→ SOC of OC since 2003
  - Stage 3, Optimal debulking operation→ PFS< 2yrs
  - Stage 3, suboptimal or stage 4→PFS< 1.5 yrs

- Maintenance with bevacizumab can improve PFS, but approximately 4 months.

Molecular testing in ovarian cancer

Synthetic Lethality & PARP i

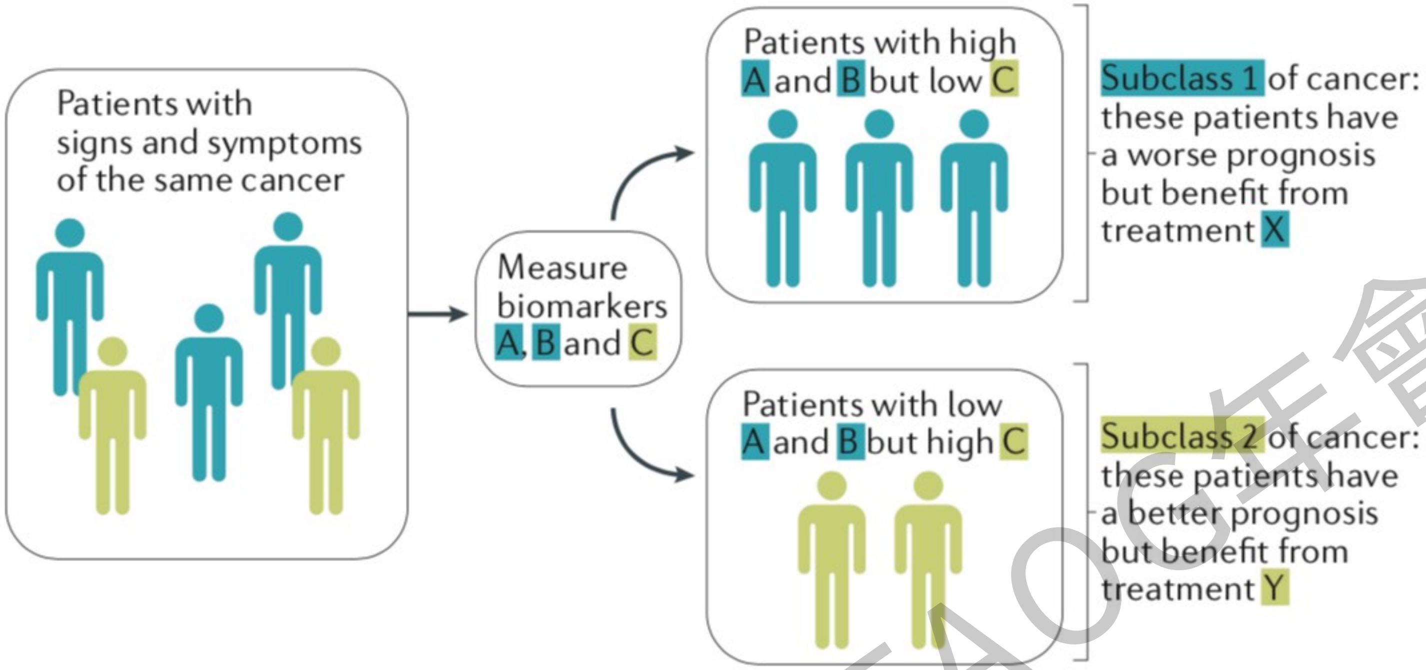


Figure 1 | **Classifying patients into new, specific taxa.** Patients with the same signs and symptoms of cancer often have different outcomes. The precision medicine approach provides a research strategy to develop biomarkers that can be used to classify patients with the same cancer into finer taxa (subclass 1 versus subclass 2) by biomarkers that predict prognoses derived from the synthesis of large amounts of data to identify discriminating biomarkers. For example, patients in subclass 1 who have a worse prognosis (that is, have biomarkers that are associated with poor survival) may be given a more aggressive treatment (treatment X) versus those in subclass 1 who have a better prognosis (that is, have biomarkers that are associated with good outcome) and require a less aggressive therapy (treatment Y). Additionally, the converse may be true where individuals with a worse prognosis are provided less aggressive therapy if no benefit

Histology
Grading
Staging
Platinum sensitive
Optimal or suboptimal
.....

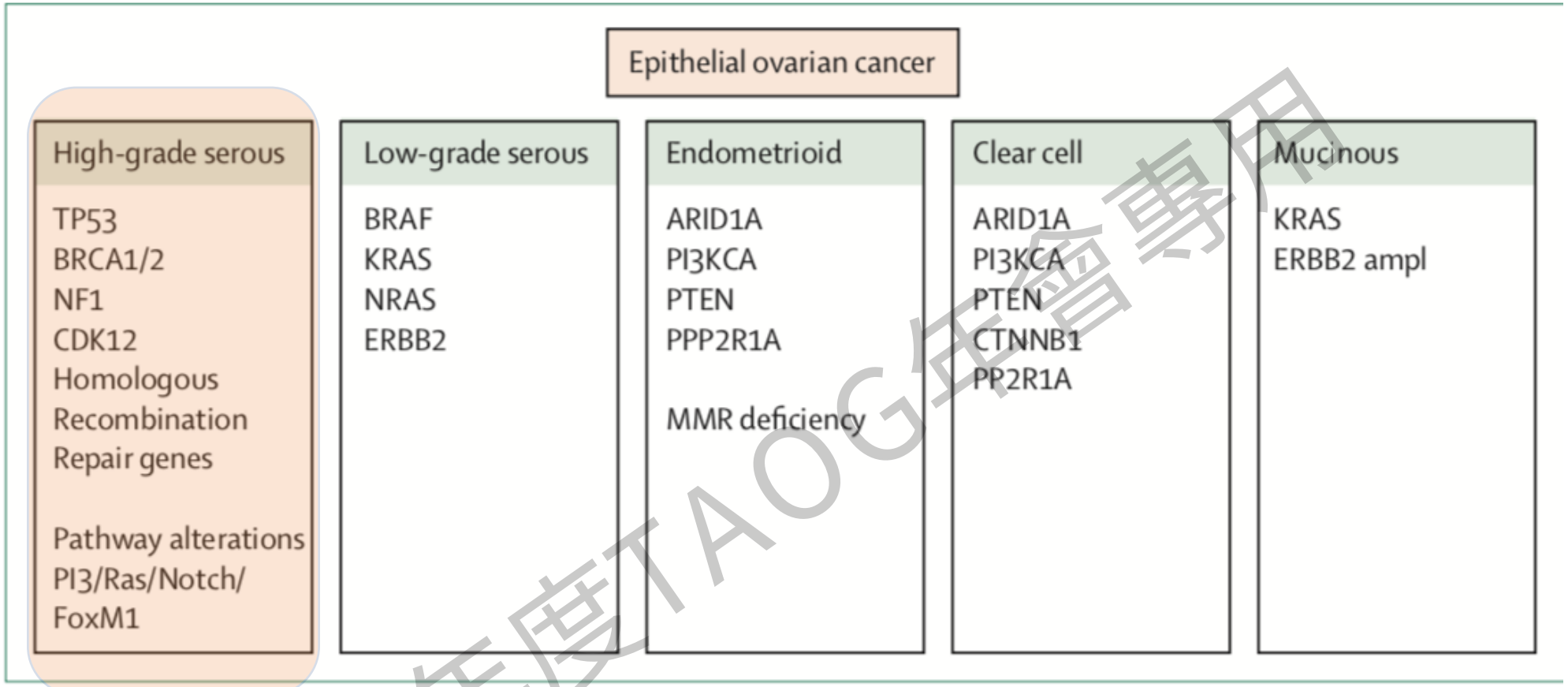


Figure 2: **Epithelial subtypes and associated mutations**
Adapted from Banerjee and colleagues[25] by permission of AACR.

BRCA基因(BREAST CANCER GENE)的功能與影響

- BRCA1和BRCA2基因分別於1990年和1994年發現， 最初發現與遺傳性乳癌相關，所以被命名為Breast Cancer 1(BRCA1)基因和BRCA2基因
- BRCA1基因位於17q21.31，含有24個exon； BRCA2基因位於13q13.1，含有27個exon
- 與雙股ＤＮＡ損傷 Error-free 修復有關（homologous recombination repair)

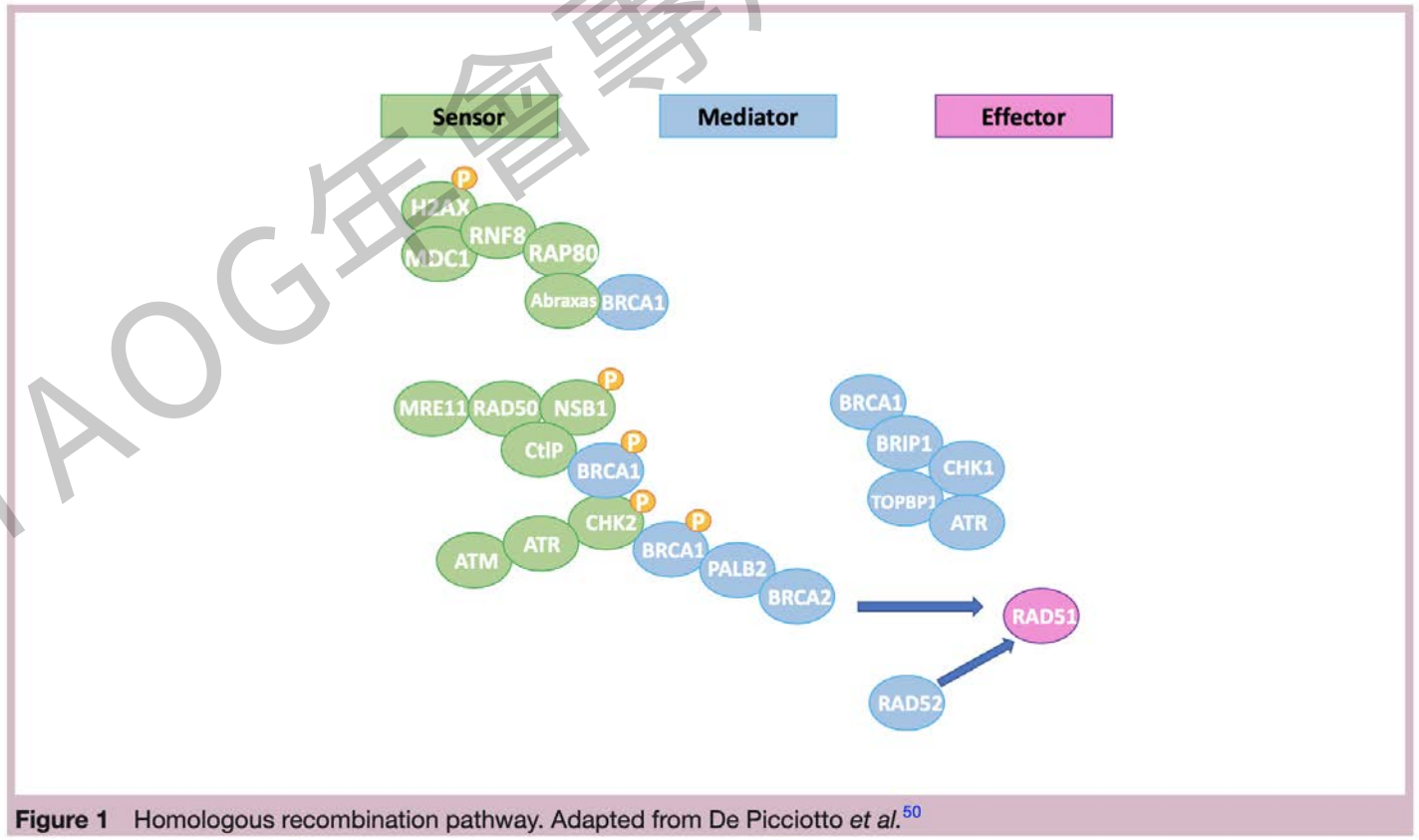


Figure 1 Homologous recombination pathway. Adapted from De Picciotto *et al.*[50]

Roy R, Chun J and Powell SN. Nat Rev Cancer 2012;12(1): 68-78.

Synthetic lethality

GENETICS OF NATURAL POPULATIONS. XIII. RECOMBINATION AND VARIABILITY IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

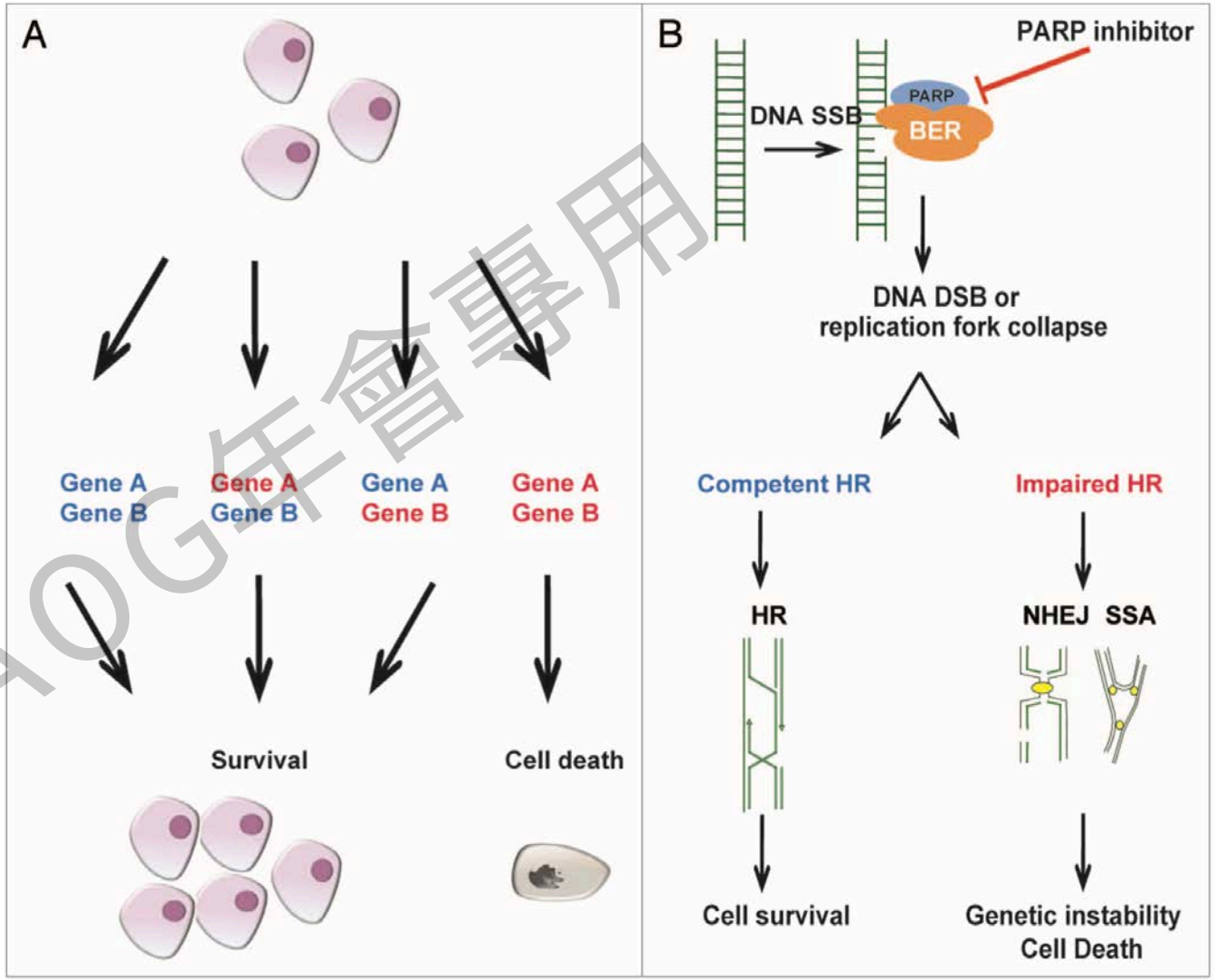
TH. DOBZHANSKY

Columbia University, New York

Received December 5, 1945

Particularly interesting is the appearance of "synthetic" lethal and semi-lethal chromosomes, which arise through crossing over between chromosomes lacking these properties. One chromosome has a dominant effect on the development rate of its carriers; no such effects were present in the ancestral chromosomes. At least two chromosomes have "synthetic" effects on the visible morphology of the flies.

GENETICS 31: 269 May 1946



Cell Cycle 10:8, 1192-1199; April 15, 2011

Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy

Hannah Farmer[1,2*], Nuala McCabe[1,2*], Christopher J. Lord[2*], Andrew N. J. Tutt[2,3], Damian A. Johnson[2], Tobias B. Richardson[2], Manuela Santarosa[2†], Krystyna J. Dillon[4], Ian Hickson[4], Charlotte Knights[4], Niall M. B. Martin[4], Stephen P. Jackson[4,5], Graeme C. M. Smith[4] & Alan Ashworth[1,2]

Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

Helen E. Bryant[1], Niklas Schultz[2], Huw D. Thomas[3], Kayan M. Parker[1], Dan Flower[1], Elena Lopez[1], Suzanne Kyle[3], Mark Meuth[1], Nicola J. Curtin[3] & Thomas Helleday[1,2]

Alan Ashworth FRS

Thomas Helleday PhD

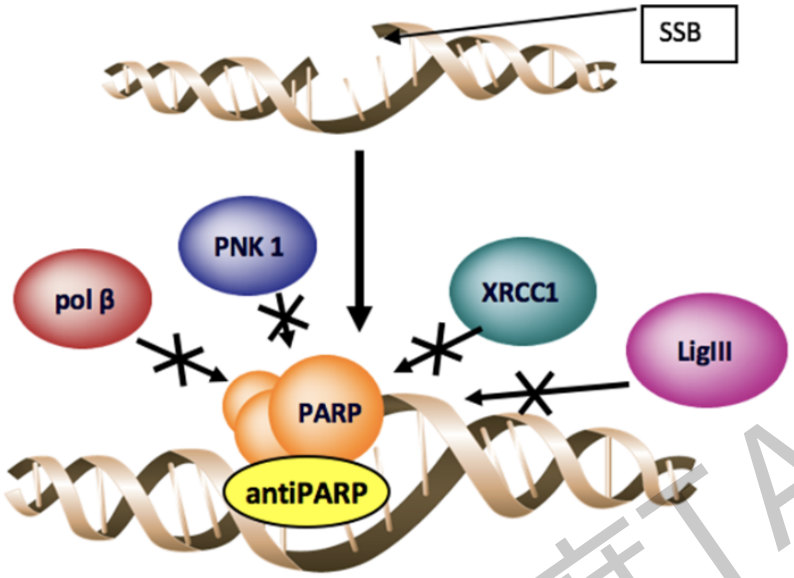
Table 1. Representative synthetic lethal interactions among genes in preclinical studies

Gene	Chromosome	Cellular process and mechanism	SL partners	Cancer type	Reference
PARP1 (mutant)	1q41.42	Regulate cell proliferation and differentiation; repair DNA single- and double-strand breaks.	BRCA1/2	Breast, ovarian, pancreatic and liver cancer; leukemia	6,50,132,138
			RAD51	Ovarian cancer; HCC	139,140
			ATG5	Ovarian cancer	141
			CDK5	Cervical and breast cancer	142,143
TP53 (mutant)	17p13.1	Major tumor suppressor; regulate the cell cycle, senescence, and apoptosis.	ATM	Glioma	54
			ATR	CLL; osteosarcoma, colon and breast cancer	55,56
			WEE1	HNSCC	57
			CHK1	NSCLC, B-ALL	58,144
			BCL-2	AML	59
			SLC711	NSCLC; renal, esophagus, cervical and gastric cancer	145
			mTOR	Pancreatic adenocarcinoma; lung and breast cancer	102
			AURKA	Liver cancer	146
			PIP4KB	Breast Cancer	147
KRAS (mutant)	12p12.1	Transcriptional activator that regulates endothelial cells endothelin-1 gene expression.	CDC6	Colon cancer	63
			GATA2	Colon cancer; NSCLC	63,64
			SLC25A22	Colorectal cancer	65
			PLK1 and ROCK	Lung and pancreatic cancer	66
			CD274	Colon and lung cancer	67
MYC (mutant)	8q24.21	Regulate cell cycle progression, transcription, and apoptosis.	4EBP1	Hematological cancer	68
			SAE1/2	Breast cancer	69
			AURKB	T-ALL	70
			PIM1	Breast cancer	71
			CDK9	HCC	72
ARID1A (mutant)	1p36.11	Target SWI/SNF complexes, which regulate chromatin remodeling. SWI/SNF complexes are involved in controlling the cell cycle, DNA replication, and repairing DNA damage.	ARID1B	Ovarian cancer	79
			EZH2	Ovarian cancer	148
			PARP1	Breast and colon cancer	149
MAD2 (overexpress)	4q27	A component of the mitotic spindle assembly checkpoint that prevents the onset of anaphase until all chromosomes are properly aligned at the metaphase plate.	PP2A	Lung and liver cancer; malignant lymphoma	73
CKS1B (overexpress)	1q21	Codes for a conserved regulatory subunit of cyclin-CDK complexes that function at multiple stages of cell cycle progression	PLK1	Breast cancer	41
TDP1 (overexpress)	14q32.11	Encode the protein that repairs stalled topoisomerase I-DNA complexes and repair of free-radical mediated DNA double-strand breaks.	HDAC1/2 RPD3	Fibrosarcoma; rhabdomyosarcoma	74

Signal Transduction and Targeted Therapy (2020)5:241

PARP INHIBITORS

Poly(ADP-ribose) polymerase and DNA Repair

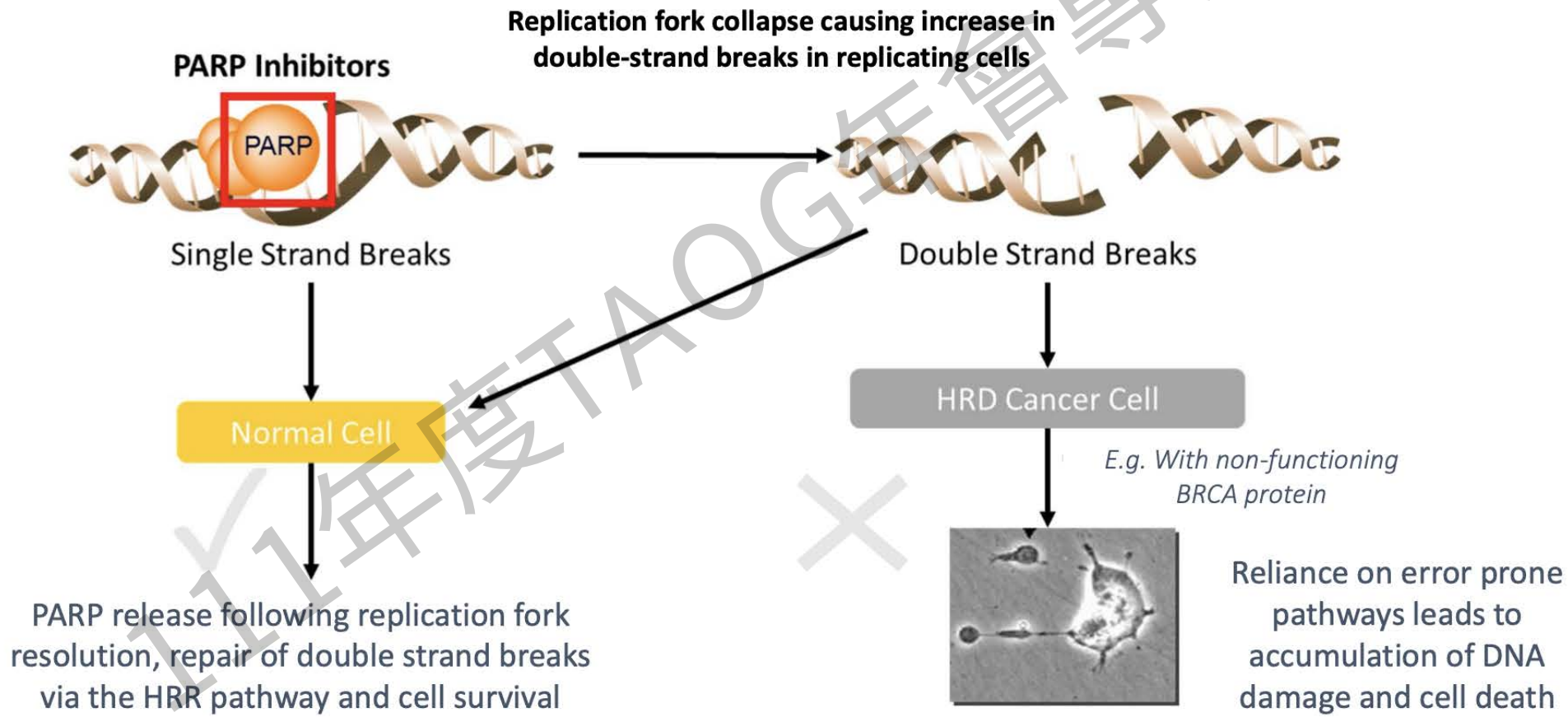


- PARP is a key regulator of DNA damage repair processes
- Involved in DNA base-excision repair (BER)
- Binds directly to DNA damage
- Produces large branched chains of poly(ADP-ribose)
- Attracts and assists BER repair effectors

Ruth Plummer MD PhD

Steve Jackson FRS FMedSc

Patients whose tumours harbour germline or somatic BRCA mutations have equivalent PARPi sensitivity



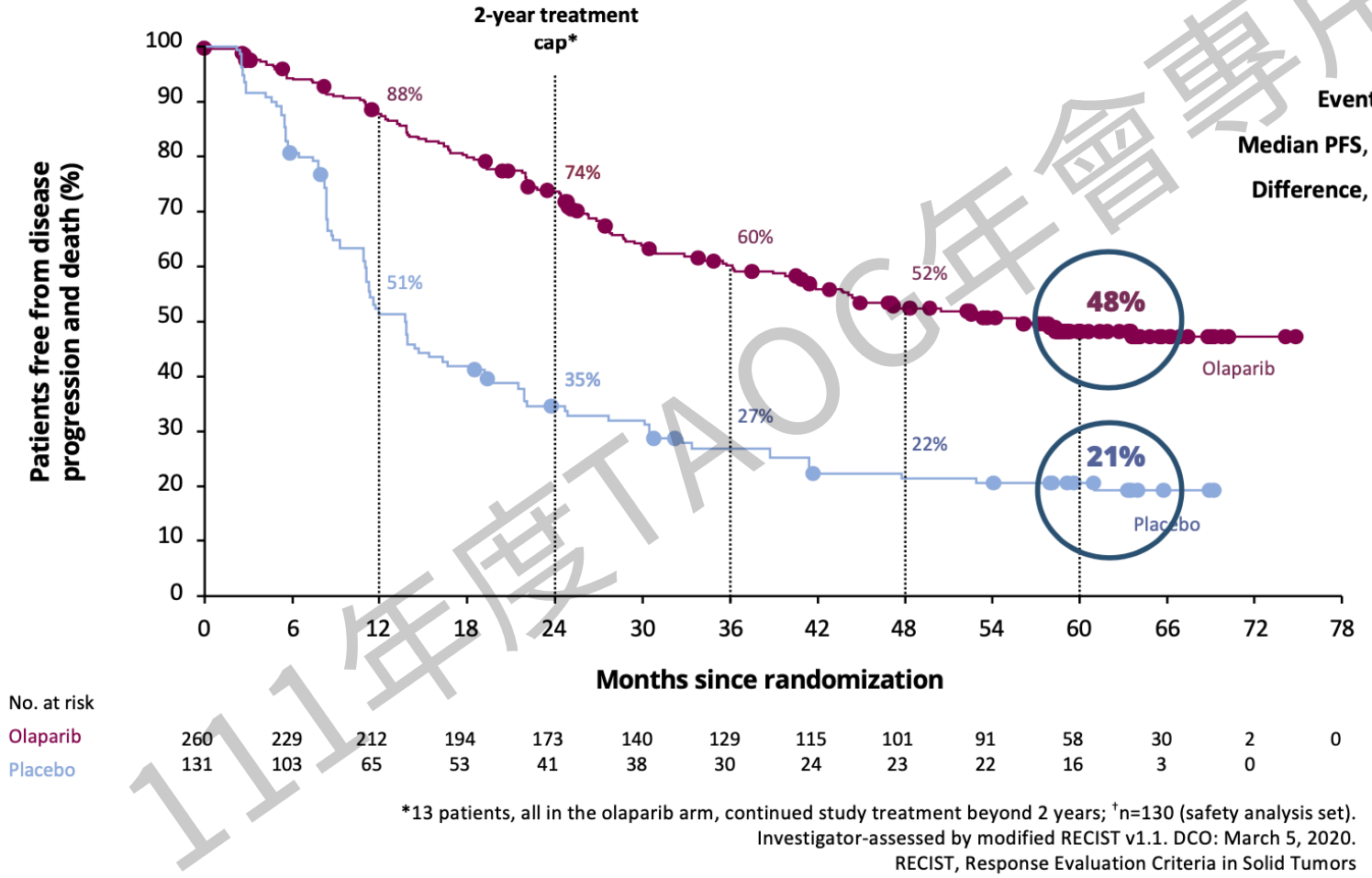
HRR=Homologous recombination repair; HRD=Homologous recombination deficient; PARP=Poly (ADP-ribose) polymerase

1. Adapted from: O'Connor MJ. *Mol Cell.* 2015;60:547–60

Table 2. Pharmacokinetic characteristics of PARP inhibitors.

	Olaparib	Niraparib	Rucaparib
Posology	300 bid	300 mg	600 bid
Bioavailability	NA	73%	30–45%
AUC 0-24	42,000 h ng/mL	NA	1690 h ng/mL
Cmax	58,000 ng/mL	3 h	1940 ng/mL
Tmax	1–3 h	NA	1.9 h
Plamatic Clearence	8.6 L/h	16.5 L/h	13.9–18.4 L/h
Volume of Distribution	167 L	1311 L	113–262 L
Half-life	11.9 h	48-51 h	25.9 h
Co-Administration with Food	Food assumption delays Tmax of about 2 h	No influence	After a highly lipidic meal, Cmax is increased by 20% and AUC of 38%, while Tmax is delayed by 2.5 h
Plasmatic Protein Binding	Dose-dependent: bound fraction decreases from 91% at 1 microg/mL concentration to 82% to qo microg/mL and to 70% at 40 microg/mL	83%	70.2%
Metabolism	CYP3A4/5 are enzymes primarily responsible for metabolism	Carboxilestherasis are the enzymes primarily responsible for metabolism	CYP2D6 and CYP1A2 e CYP3A4 are the enzymes primarily involved in metabolism
Substrate of	P-gp (clinically non-significant)	P-gp, BRCP, MATE1/2 (clinically non-significant)	P-gp and BCRP
Cytochromes and Transporters Inhibition	Induction of CYP1A2, 2B6 e 3A4	Inhibition of MATE1/2 e and mild inhibition of OCT1	Moderate inhibition of CYP1A2
Cytochromes and Transporters Inhibition	Moderate inhibition of CYP3A, P-gp, BCRP, OATP1B1, OCT1, OCT2, OAT3, MATE1, MATE2K	None	Mild inhibition of CYP2C9, CYP2C19, CYP3A E P-gp
Renal Impairment	Severe renal impairment (ClCr < 30 mL/min): not recommended Moderate renal impairment (CrCl 31–50 mL/min): dose reduction to 300 mg × 2 Mild renal impairment (ClCr 51–80 mL/min): no dose adjustment	Severe renal impairment (ClCr < 30 mL/min): not recommended Moderate renal impairment (CrCl 31–50 mL/min): no dose adjustment Mild renal impairment (ClCr 51–80 mL/min): no dose adjustment	Severe renal impairment (ClCr < 30 mL/min): not recommended Moderate renal impairment (CrCl 31–50 mL/min): no dose adjustment Mild renal impairment (ClCr 51–80 mL/min): no dose adjustment
Hepatic Impairment	Mild or moderate hepatic impairment (child pug A or B): no dose adjustment Severe hepatic impairment (child pug C): not recommended	Mild or moderate hepatic impairment (child pug A or B): no dose adjustment Severe hepatic impairment (child pug C): not recommended	Mild or moderate hepatic impairment (child pug A or B): no dose adjustment Severe hepatic impairment (child pug C): not recommended

SOLO1: Progression-Free survival of maintenance olaparib in women with high grade ovarian cancer and a *BRCA mutation*



	Olaparib (N=260)	Placebo (N=131)
Events, n (%)	118 (45)	100 (76)
Median PFS, months	**56.0**	**13.8**
Difference, months	42.2	
	HR 0.33 (95% CI 0.25–0.43)	

Olaparib (N=260)	Placebo (N=131)
Median-follow up for PFS	
4.8 years	**5.0** years

*13 patients, all in the olaparib arm, continued study treatment beyond 2 years; †n=130 (safety analysis set).
Investigator-assessed by modified RECIST v1.1. DCO: March 5, 2020.
RECIST, Response Evaluation Criteria in Solid Tumors

Bradley et al SGO 2021; Banerjee et al Lancet Oncol 2021

Lynparza™ olaparib tablets 150 mg 2020年11月1日起納入健保給付

健保給付條文

- 1. 卵巢、輸卵管或原發性腹膜癌：
- （1）單獨使用於具下列所有條件的病患做為維持治療，限用兩年：
- A.對第一線含鉑化療有治療反應後使用。
- B.具生殖細胞或體細胞BRCA1/2 致病性或疑似致病性突變。
- C.FIGO (International Federation of Gynecology and Obstetrics) Stage III or IV disease。
- （2）須經事前審查核准後使用，每次申請事前審查之療程以6 個月為限，再次申請必須提出客觀證據（ 如：影像學）證實無惡化，才可繼續使用。
- 2. 三陰性乳癌：
- （1）單獨使用於曾接受前導性、術後輔助性或轉移性化療，且具生殖細胞BRCA1/2致病性或疑似致病性突變之三陰性(荷爾蒙接受體及HER2 受體皆為陰性)轉移性乳癌病人。
- （2）須經事前審查核准後使用，每次申請事前審查之療程以3 個月為限，再次申請必須提出客觀證據（ 如：影像學）證實無惡化，才可繼續使用。
- 3.每日最多使用4 粒。

Reference: 衛生福利部中央健康保險署109.10.05公告-健保審字第1090062685號

First-line Management of Ovarian Cancer

2003

Chemotherapy

No further improvement in survival with chemotherapy alone since the introduction of platinum–taxane chemotherapy[1,2]

2011

Paradigm Shift 1:
Bevacizumab

Bevacizumab improved PFS vs chemotherapy alone[3,4]

2018

Paradigm Shift 2:
PARP inhibitors for *BRCA*-mutated ovarian cancer

Olaparib	**SOLO-1**[5] *NCT01844986*

但只有少數病人受惠

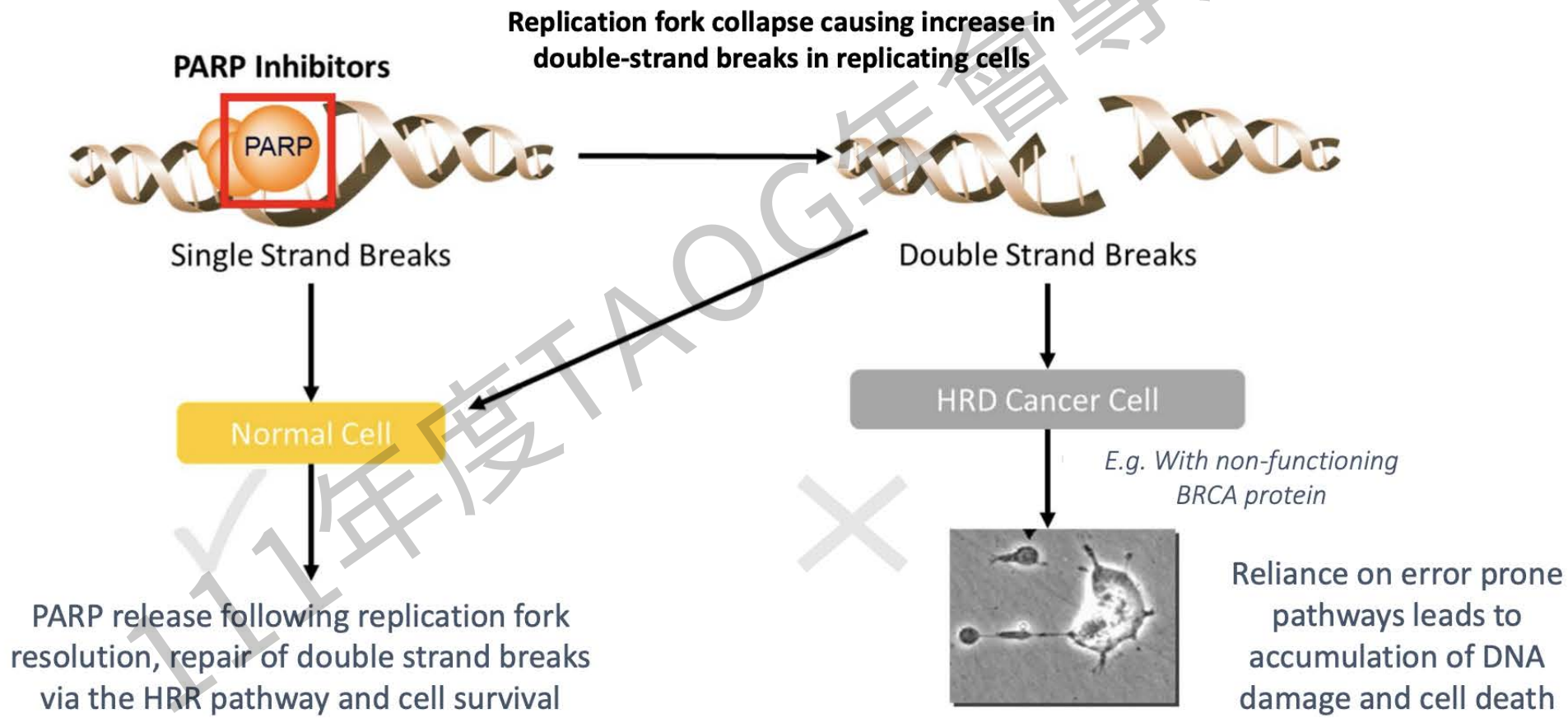
1300(OC in Taiwan)
650(stage 3,4)
400(HGS, HGE)
80(BRCA)
60(platinum sensitive)

Several studies with PARP inhibitors as maintenance for newly diagnosed advanced ovarian cancer

1. McGuire. NEJM. 1996;334:1. 2. du Bois. J Natl Cancer Inst. 2003;95:1320. 3. Burger. NEJM. 2011;365:2473.
4. Perren. NEJM. 2011;365:2484. 5. Friedlancer. Lancet Oncol. 2021;22:632. 6. Ray-Coquard. NEJM. 2019;381:2416.
7. Gonzalez-Martin. NEJM. 2019;381:2391. 8. Aghajanian. Gynecol Oncol. 2021;162:375.

Beyond BRCAm

Patients whose tumours harbour germline or somatic BRCA mutations have equivalent PARPi sensitivity

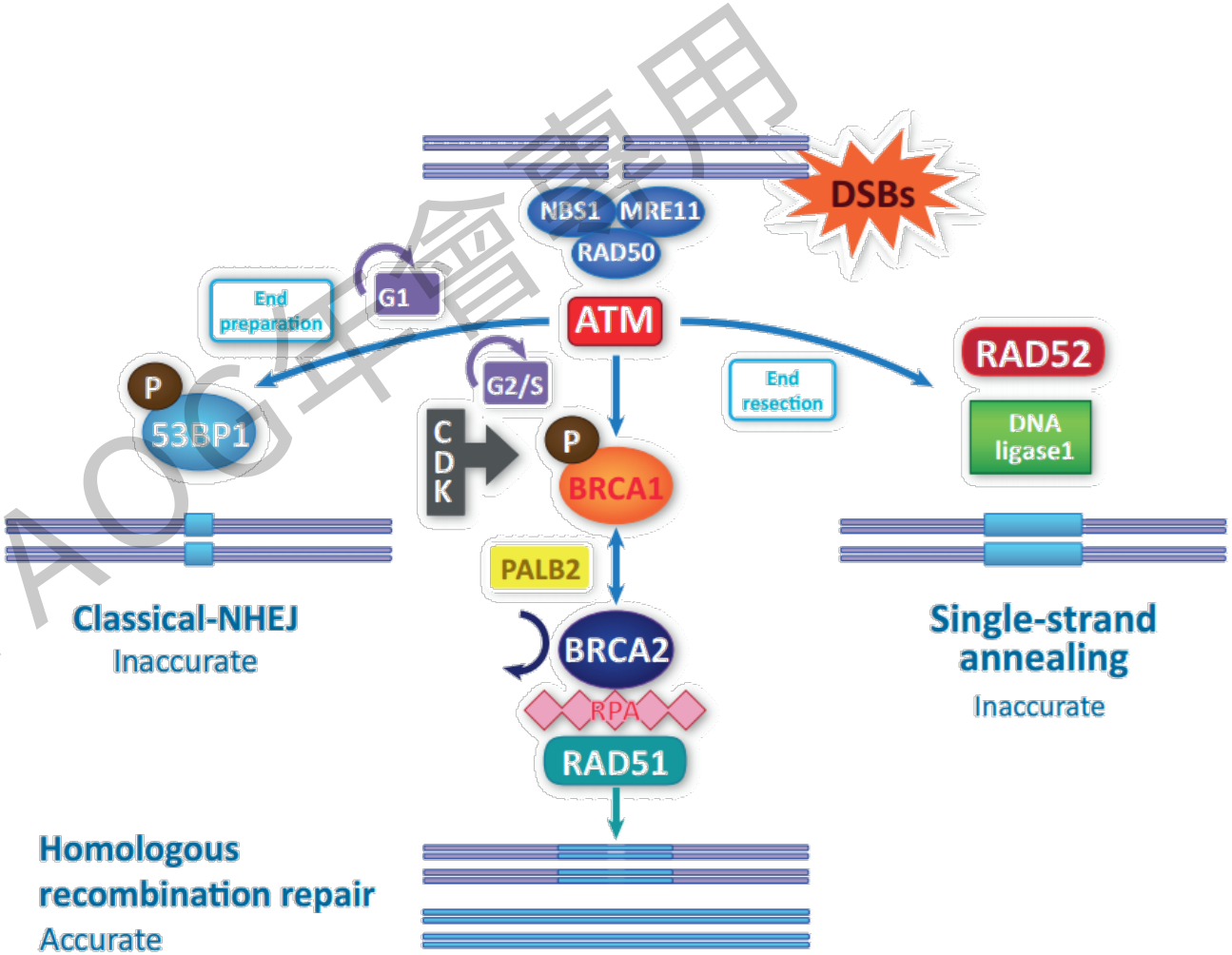


HRR=Homologous recombination repair; HRD=Homologous recombination deficient; PARP=Poly (ADP-ribose) polymerase

1. Adapted from: O'Connor MJ. *Mol Cell.* 2015;60:547–60

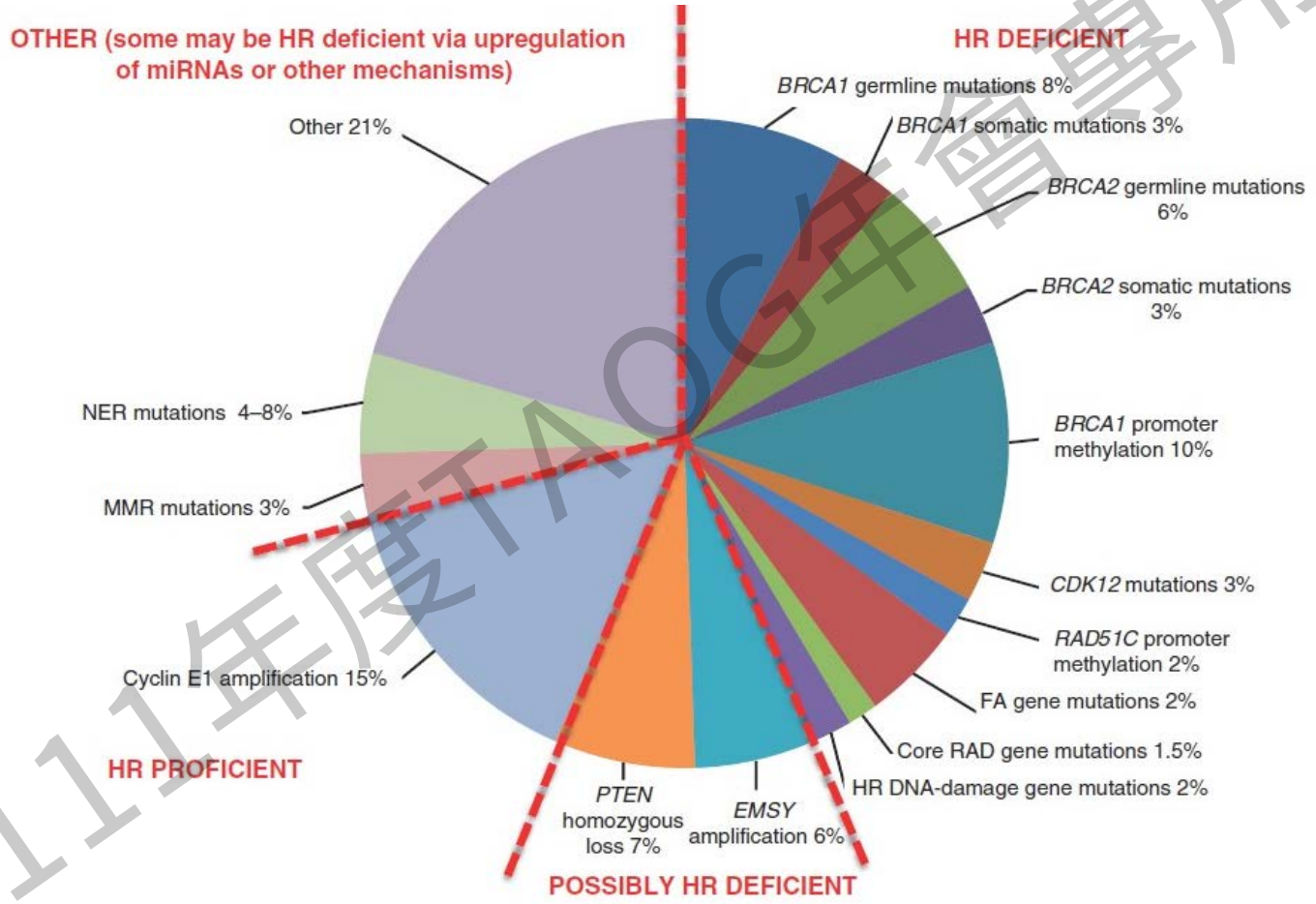
導致HRD的原因不僅限於BRCA1/2基因突變

- “BRCAness”的概念，描述無BRCA突變，但具有和BRCA突變腫瘤類似表現型的HRD
- Homologous Recombination (HR)過程中主要涉及到BRCA1/2，RAD51，以及BRCA2定位基因 (PALB2) 等編碼的蛋白
- 這些BRCA以外的HR相關基因的改變也可能導致HRD，包括基因突變、表現調控及其他未知原因



1. Lord CJ, et al. Nat Rev Cancer. 2016; 16(2): 110-20 Turner N, et al. Nat Rev Cancer. 2004; 4(10): 814-9. 2. Ashworth A. J Clin Oncol. 2008; 26(22): 3785-90. 3. O’ Kane GM, et al. Trends Mol Med. 2017; 23(12): 1121-1137.

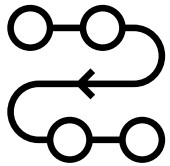
約50%高分化漿液性上皮型卵巢癌存在同源重組修復基因缺(HRD)



1. Konstantinopoulos PA, et al. Cancer Discov. 2015;5(11):1137–54.

Terminology consistent with community language, labels and guidelines

It is important to distinguish between phenotype and test



Homologous recombination repair (HRR): the cellular mechanism to repair DNA double strand breaks

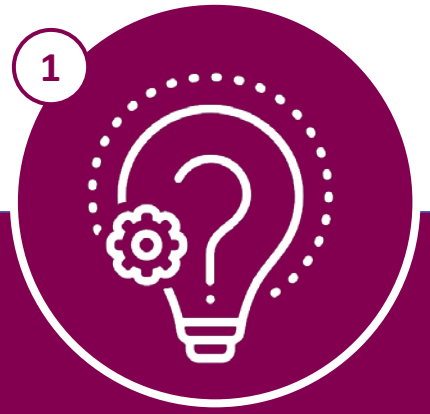
Homologous recombination deficiency (HRD): the phenotype of a cell/tumor that has impaired ability to conduct HRR (for example due to loss of function of genes involved in the HRR pathway)

Genomic instability test: a molecular diagnostic test to assess HRD phenotype (for example the Myriad myChoice® CDx test)

HRD-positive: a tumor which is identified as HRD based on a molecular diagnostic test (for example a genomic instability test)
HRD-negative: a tumor which is identified as HRD-negative based on a molecular diagnostic test

Genomic instability in the context of HRD testing in Ovarian Cancer refers to genomic alterations resulting from homologous repair deficiency (HRD) and not microsatellite instability resulting from mismatch repair (dMMR).

Three approaches to identify HRD



Cause of HRD

Function of HRR

Effect of HRD

Three approaches to identify HRD

Causes of HRD

*BRCA1/2*m

Germline / Tumor

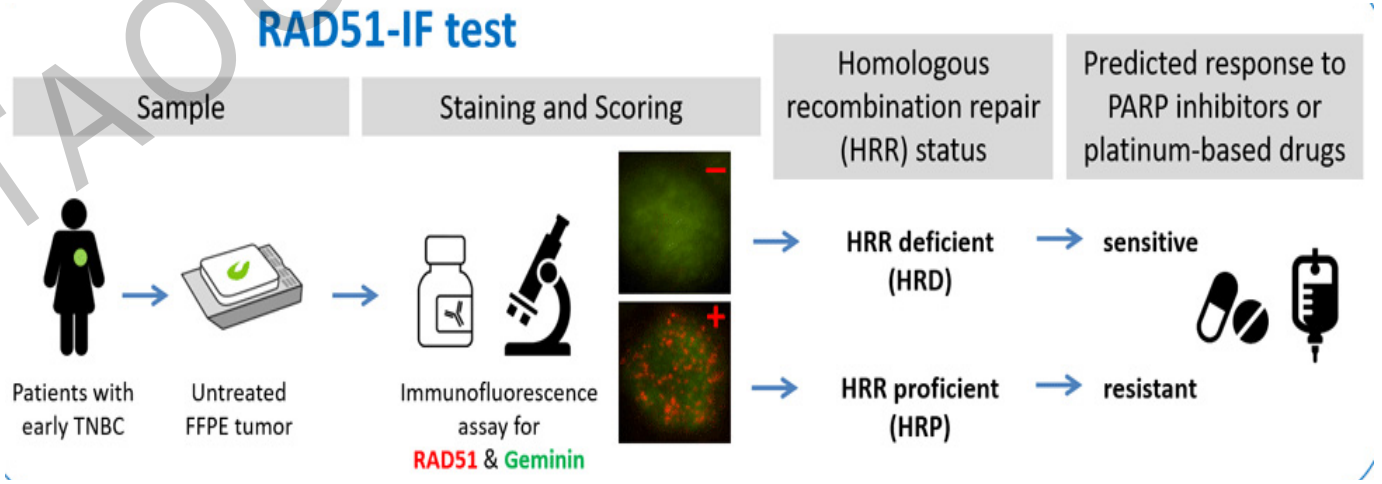
- Including point mutation / InDel detected by NGS
- Large DNA deletion detected by MLPA

HRRm
Gene panels

- Loss of function of key HRR genes (tumor test)
- 15 genes in AZ panel:

BRCA1, ***BRCA2***, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*

Function of HRR



1. O'Kane GM et al. Trends Mol Med. 2017;23(12):1121-1137. 2. Hoppe MM, et al. J Natl Cancer Inst. 2018;110(7):704-713

2. Serra Elizalde V, Llop-Guevara A, Pearson A, et al. Detection of homologous recombination repair deficiency (HRD) in treatment-naïve early triple negative breast cancer (TNBC) by RAD51 foci and comparison with DNA-based tests.

3. Llop-Guevara A, Vladimirova V, Schneeweiss A, et al. Association of RAD51 with Homologous Recombination Deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): analysis of the GeparSixto randomized clinical trial.

PAOLA-1 studied olaparib plus bevacizumab maintenance in newly diagnosed advanced ovarian cancer

- FIGO Stage III–IV high-grade ovarian cancer*
- Surgery (upfront or interval)
- Platinum taxane-based chemotherapy
- ≥3 cycles of bevacizumab†

NED/ CR/PR

Olaparib (300 mg BID) x 2 years
+ bevacizumab†

2:1 randomisation; **N=806**

Stratification by tBRCA status‡ and 1L treatment outcome

Placebo x 2 years
+ bevacizumab†

2 years' maintenance treatment

Primary endpoint

- Investigator-assessed PFS (RECIST 1.1)

Pre-specified exploratory endpoints

- PFS in pre-defined subgroups, including tBRCAm, HRRm (including BRCAm) by Myriad myChoice® CDx

*Serous or endometroid (also includes fallopian tube and primary peritoneal cancer) or non-mucinous BRCAm
†Bevacizumab: 15 mg/kg, every 3 weeks for a total of 15 months, including when administered with chemotherapy
‡By central labs
1L=first-line; BID=twice daily; BRCAm=BRCA mutation; CDx=companion diagnostic test; CR=complete response; FIGO=International Federation of Gynecology and Obstetrics; HRRm=homologous recombination repair gene mutation; NED=no evidence of disease; PFS=progression-free survival; PR=partial response; RECIST=Response Evaluation Criteria in Solid Tumours; tBRCA=tumour BRCA; tBRCAm=tumour BRCA mutation

1. Ray-Coquard I, et al. *N Engl J Med*. 2019;381:Clinical Study Protocol; 2. Study NCT02477644. Available at: https://clinicaltrials.gov/ct2/show/NCT02477644. Accessed March 2021

Study Objective and Design(PAOLA-1)

Exploratory analysis
- PFS (RECIST v1.1) by investigator in patients with a non-BRCA HRRm

- Tumors analyzed using the Myriad myChoice® HRD Plus assay*

Exploratory gene panels

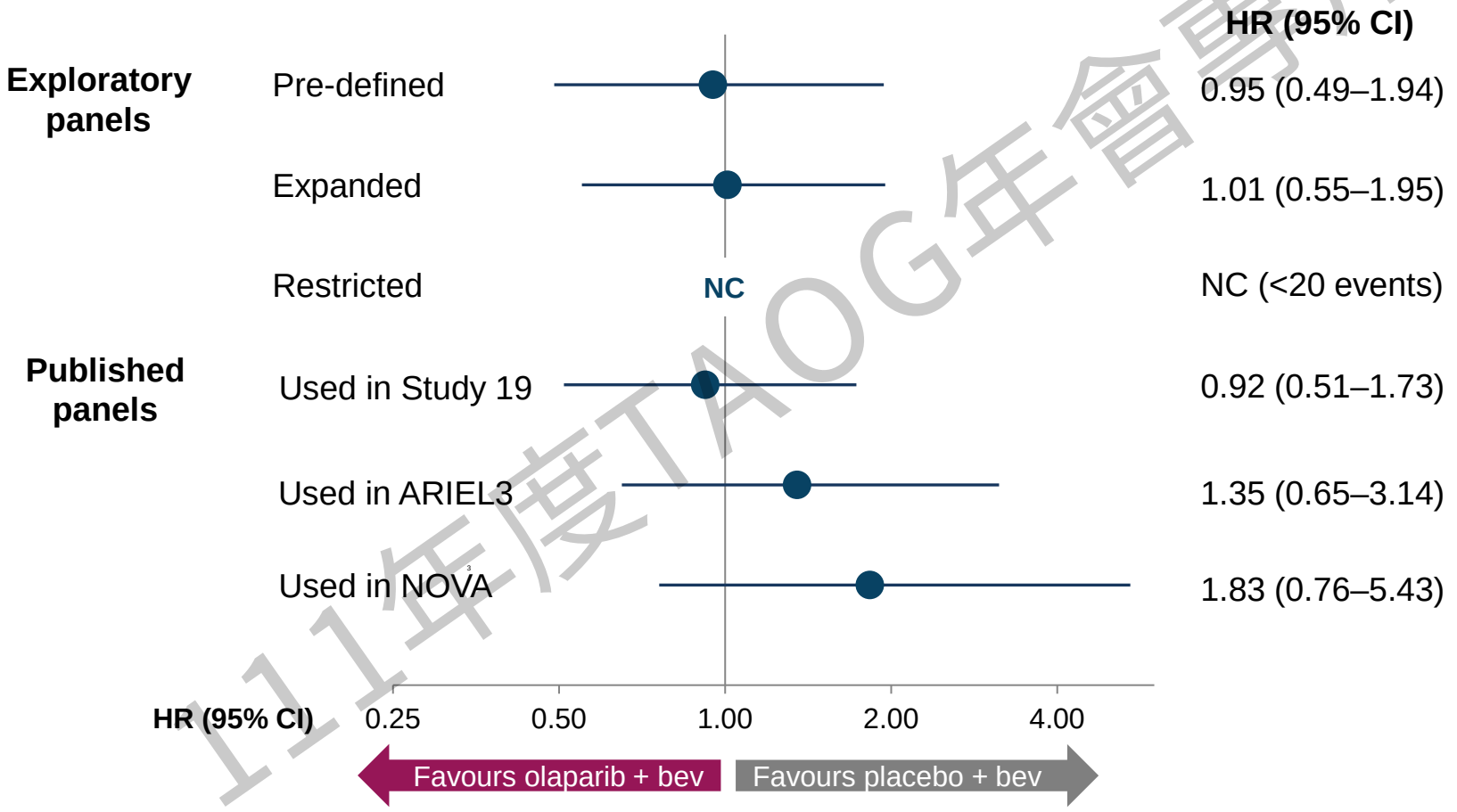
Pre-defined (13 genes)	**Expanded** (18 genes)	**Restricted** (5 genes)
ATM, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*	Pre-defined panel plus five additional genes involved in HRR: *BLM*, *FANCA*, *FANCI*, *FANCM*, *NBN*	Five genes with highest median Myriad genomic instability scores: *BLM*, *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*

Published gene panels

Used in Study 19 (26 genes)[2]	**Used in ARIEL3** (19 genes)[3]	**Used in NOVA** (11 genes)[4]

All genes considered were in both the gene panel noted and the Myriad myChoice®HRD Plus assay.
*HRD was defined by the presence of a genomic instability score ≥42 and/or BRCAm.
RECIST, Response Evaluation Criteria in Solid Tumors.
1.Ray-CoquardIetal.*N EnglJ Med*2019;381:2416 – 28; 2.Hodgson et al. *BJC*2018;119:1401 – 09; 3. Coleman et al. *Lancet*2017;390:1949 – 61; 4. Mirza et al. *ASCO*2019 (abstract 5568)

Non-BRCA HRRm was not predictive of improved PFS, regardless of gene panel in 1L OC(PAOLA-1)



bev=bevacizumab; CI=confidence interval; HR=hazard ratio; HRRm=homologous recombination repair gene mutation; NC=not calculable; PFS=progression-free survival

Pujade-Lauraine E, et al. Presented at the SGO Annual Meeting 2021; 19–25 March; Virtual

Effect of HRD

Effect of HRD

Two different HRD test methodologies: (1) LOH, TAI and LST (2) GI

Myriad myChoice® CDx

FoundationOne® CDx

ACT HRD

HRD → Genomic Instability

LOH

TAI

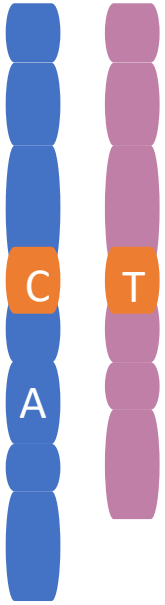
LST

SOPHiA GENETICS

Genomic Integrity (GI)

Genomic Instability and SNP Testing

- Genomic instability (e.g. LoH) tests are NOT based on gene panels, but are specialized tests that require pan-genome SNP coverage



Loss of Heterozygosity

Design principles for SNP selection:

1. SNPs are evenly distributed across the genome.
2. SNPs should be from the regions that are unique in the genome.
   - ➢ Low complexity region will complicate the analysis.
3. SNPs have good population allele frequency (GMAF); in other words a significant proportion of the selected SNPs should be heterozygous

The SNP density can determine the minimum size of LoH segment detected.
A sparse SNP assay can miss LOH segments

Abkevich V et al. Br J Cancer 2012 ;107: 1776–1782; Swisher EM et. al. Lancet Oncol. 2017 Jan;18(1):75-87.

LOH: Presence of a single allele

TAI: A discrepancy in the 1:1 allele ratio at the end of the chromosome (telomere)

LST: Transition points between regions of abnormal and normal DNA or between two different regions of abnormality

Myriad myChoice CDx Test

HRR Gene Panel Test

Mutations in Homologous Recombination Repair (HRR) pathway genes

ATM
BARD1
BRIP1, CDK12, CHEK1
CHEK2, FANCL, PALB2,
PPP2R2A, RAD51B,
RAD51C, RAD51D, RAD54L

Genomic Instability Test

Multiple genetic biomarkers/composite scores

BRCA1
BRCA2

Loss Of Heterozygosity (LOH)

Allelic Imbalance (AI)

Large-scale State Transitions (LSTs)

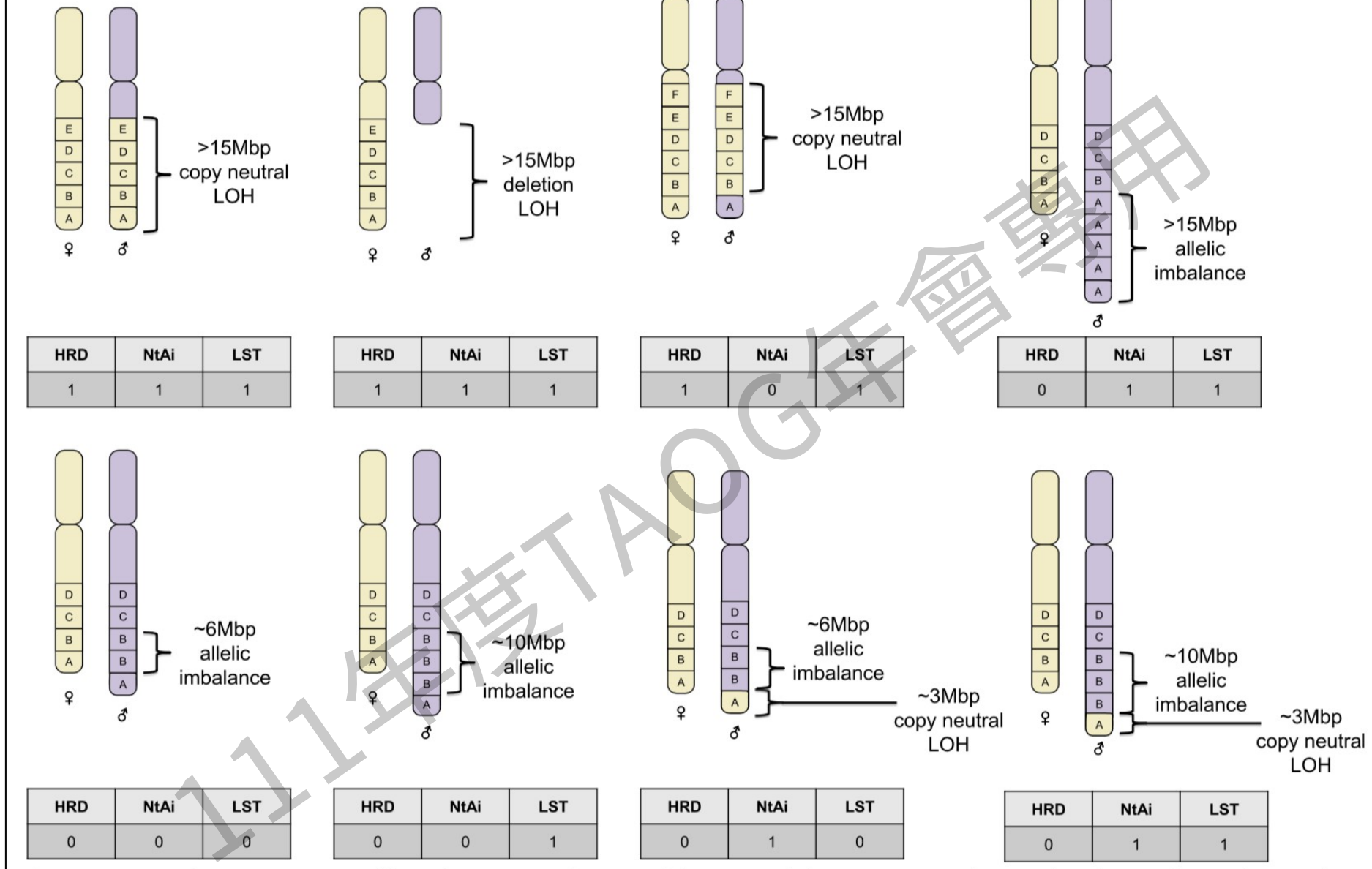
Myriad GIS
(Genomic Instability Score)

myChoice approved by FDA in Oct, 2019 as Companion Diagnostic for Niraparib for treatment of 4L+ ovarian (QUADRA)[1]

myChoice was also evaluated in the NOVA[2] and PRIMA studies[3,4]

Myriad myChoice® CDx[5]
Test positive (HRD+) is BRCAm and/or a Genomic Instability Score ≥42

1. Moore, K.N. et. al. *Lancet Oncol* 2019; 20: 636–48; 2. Mirza MR et al. N Engl J Med. 2016;375:2154-2164; 3. González Martín A et al. Presented at ESMO 2019. 27 September – 1 October., Barcelona, Spain. Abstract #LBA1; 4. González Martín A, et al. N Engl J Med. 2019;381:2391-2402; 5. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P190014



HRD	NtAi	LST
1	1	1

HRD	NtAi	LST
1	1	1

HRD	NtAi	LST
1	0	1

HRD	NtAi	LST
0	1	1

HRD	NtAi	LST
0	0	0

HRD	NtAi	LST
0	0	1

HRD	NtAi	LST
0	1	0

HRD	NtAi	LST
0	1	1

Figure 2 Scoring by genomic scars of homologous recombination deficiency and drug response. Eight examples of various forms of structural copy number aberrations and rearrangements are given, whereby each box, lettered A to F, represents a genomic segment of approximately 3 Mbp in length. Below the chromosomes, the three genomic scars - homologous recombination defect (HRD), telomeric allelic imbalance score (NtAi), and large-scale transition (LST) - are listed along with the respective integer count for the scar (0 = not seen, 1 = detected once). LOH, loss of heterozygosity.

Cancer Therapy: Clinical

Clinical Cancer Research

Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer

Melinda L. Telli[1], Kirsten M. Timms[2], Julia Reid[2], Bryan Hennessy[3], Gordon B. Mills[3], Kristin C. Jensen[1], Zoltan Szallasi[4,5,6], William T. Barry[6,7], Eric P. Winer[6,7], Nadine M. Tung[6,8], Steven J. Isakoff[6,9], Paula D. Ryan[9], April Greene-Colozzi[7], Alexander Gutin[2], Zaina Sangale[2], Diana Iliev[2], Chris Neff[2], Victor Abkevich[2], Joshua T. Jones[2], Jerry S. Lanchbury[2], Anne-Renee Hartman[2], Judy E. Garber[6,7], James M. Ford[1], Daniel P. Silver[6,7], and Andrea L. Richardson[6,7,10]

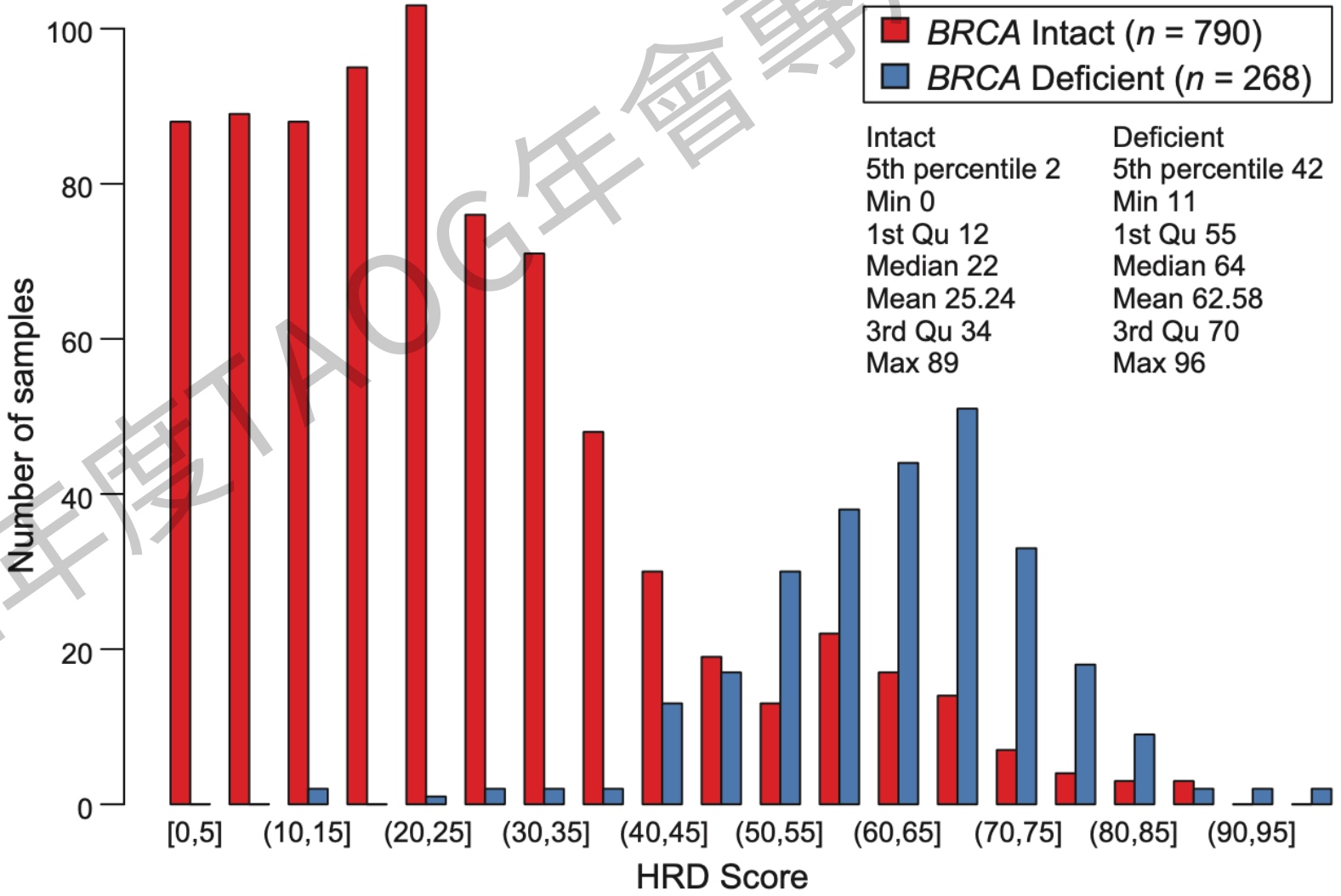


Figure 1.
HRD score distribution in the combined breast and ovarian training set. *BRCA*-deficient tumors include those with a *BRCA1/2* mutation and/or *BRCA1* methylation.

Three different subtypes by Myraid MyChoice

The effectiveness of PARPi has been evaluated in 3 different biological subtypes

1. BRCAm
2. BRCAw but HRD high
3. HRD-

Trial	SOLO-1	PRIMA	PAOLA-1	VELIA
Investigational arm	Olaparib	Niraparib	Olaparib+bevacizumab	Veliparib
BRCA mutated PFS HR (95% CI) Median PFS (PARPi vs control)	(n=391) 0.30 (0.23-0.41) NR vs 13.8 (56.0 vs 13.8 , ASCO 2020)	(n=233) 0.40 (0.27-0.62) 22.1 vs.10.9	(n=237) 0.31 (0.20-0.47) 37.2 vs 21.7	(n=200) 0.44 (0.28-0.68) 34.7 vs 22.0
HRD test positive non-BRCAm PFS HR (95% CI) Median PFS (PARPi vs control)	NA	(n=150) 0.50 (0.31-0.83) 19.6 vs 8.2	(n=152) 0.43 (0.28-0.66) 28.1 vs 16.6	(n=221) 0.74 (0.52-1.06) 22.9 vs 19.8
HRD test negative (proficient) PFS HR (95% CI) Median PFS (PARPi vs control)	NR	(n=249) 0.68 (0.49-.0.94) 8.1 vs 5.4	(n=277) 1.00 (0.75-1.35)	(n=249) 0.81 (0.60-1.09) 15.0 vs 11.5

GIS is the only HRD assay with front-line PARPi evidence beyond BRCA

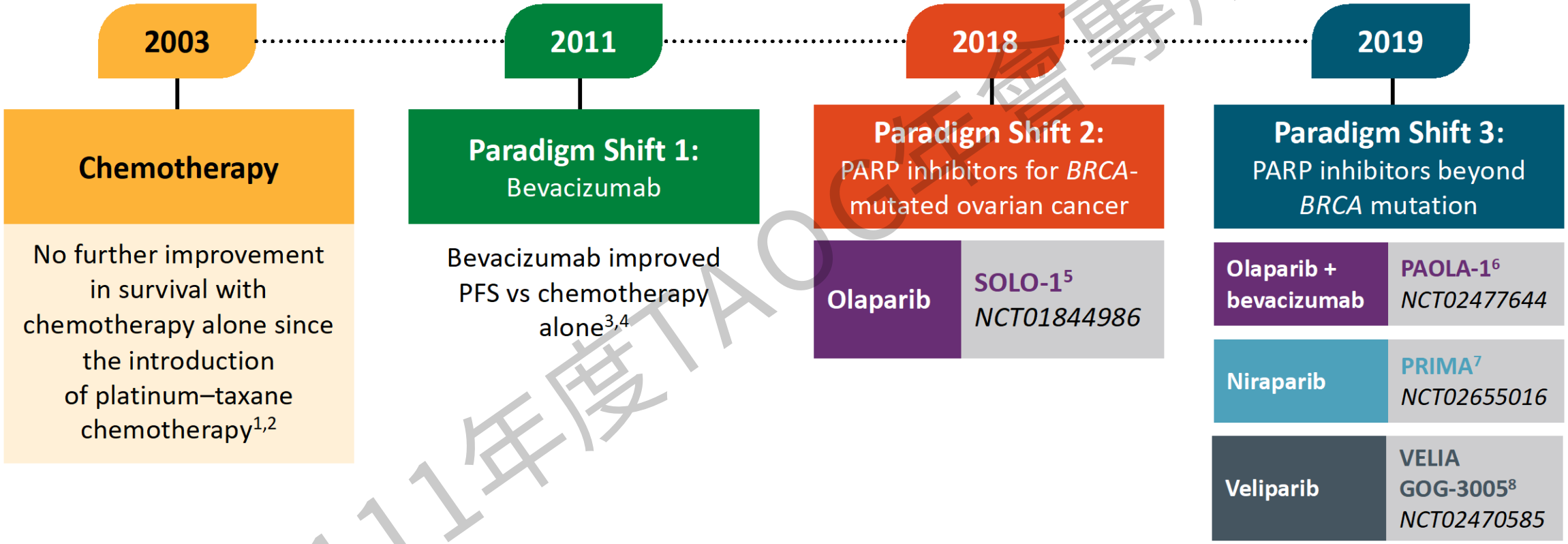
Validated in over 3,200 ovarian cancer patients in Phase III trials

myChoice® has been the test of choice
in 4 Phase III ovarian cancer trials for PARP inhibitors.
There are an additional 4 currently underway in 2,475 patients.

Phase III Trial	PARPi	Study Status	n	Treatment setting
PAOLA-1	Olaparib	Published	806	First-line
PRIMA	Niraparib	Published	733	First-line
VELIA	Veliparib	Published	1140	First-line
NOVA	Niraparib	Published	553	Recurrent
FIRST	Niraparib	Maturing	912	First-line
OPINION (IIIb)	Olaparib	Maturing	279	Recurrent
DUO-O	Olaparib	Recruiting	1056	First-line
OreO (IIIb)	Olaparib	Recruiting	228	Recurrent

1. Giornelli GH 2016 **2.** Ray-Coquard et al 2019 **3.** Gonzalez-Martin et al 2019.

First-line Management of Ovarian Cancer



Several studies with PARP inhibitors as maintenance for newly diagnosed advanced ovarian cancer[5-8]

1. McGuire. NEJM. 1996;334:1. 2. du Bois. J Natl Cancer Inst. 2003;95:1320. 3. Burger. NEJM. 2011;365:2473.
4. Perren. NEJM. 2011;365:2484. 5. Friedlancer. Lancet Oncol. 2021;22:632. 6. Ray-Coquard. NEJM. 2019;381:2416.
7. Gonzalez-Martin. NEJM. 2019;381:2391. 8. Aghajanian. Gynecol Oncol. 2021;162:375.

Assays for Homologous Recombination Deficiency (HRD)

FMI FoundationOne CDx

HRR Gene Panel Test

Mutations in Homologous Recombination Repair (HRR) pathway genes

ATM
BARD1
BRIP1, CDK12, CHEK1
CHEK2, FANCL, PALB2,
PPP2R2A, RAD51B,
RAD51C, RAD51D, RAD54L

BRCA1
BRCA2

Genomic Instability Test

Multiple genetic biomarkers/composite scores

Loss Of Heterozygosity (LOH)

Allelic Imbalance (AI)

Large-scale State Transitions (LSTs)

Initial version of FMI LoH test (FoundationFocus™ CDx BRCA LOH) was approved by FDA in April, 2018 as Companion Diagnostic for Rucaparib as maintenance therapy in ovarian PSR (ARIEL 3). LoH was subsequently transitioned across to the FoundationOne CDx gene panel test.[1,2]

FMI FoundationOne CDx™

F1CDx contains the following measures:

- variant detection in 324 genes (incl. HRR15)
- LoH (cut-off score ≥16)
- TMB, MSI

1. Coleman RL et al. Lancet. 2017;390:1949-1961. 2. https://www.fda.gov/medical-devices/recently-approved-devices/foundationone-cdx-p170019

Percent genomic LOH

To compute the percent genomic LOH for each tumour, LOH segments were inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 polymorphic SNPs sequenced in the Foundation Medicine's NGS-based T5a assay. Briefly, a comparative genomic hybridisation (ie, log-ratio profile of the sample) was obtained from the NGS sequencing data by normalising the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile was segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (C_i) and minor allele count (M_i) at each segment (i). A segment was determined to have LOH if $C_i \neq 0$ and $M_i = 0$. Low tumour content or low aneuploidy were the most common reasons for failure to pass the quality control to perform genomic LOH inference.

Two types of LOH segments were excluded from the calculation of percent genomic LOH: (1) LOH segments spanning ≥90% of a whole chromosome or chromosome arm, as these LOH events usually arise through non-HRD mechanisms (eg, mitotic nondisjunction[6]), and (2) regions in which LOH inference was ambiguous.

For each tumour, the percent genomic LOH was computed as 100 times the total length of nonexcluded LOH regions (x_i) divided by the total length of nonexcluded regions of the genome. In equation form:

$$Percent\ genomic\ LOH = 100 \times \frac{\sum_i x_i}{L_{genome} - L_{exclusions}}$$

Where

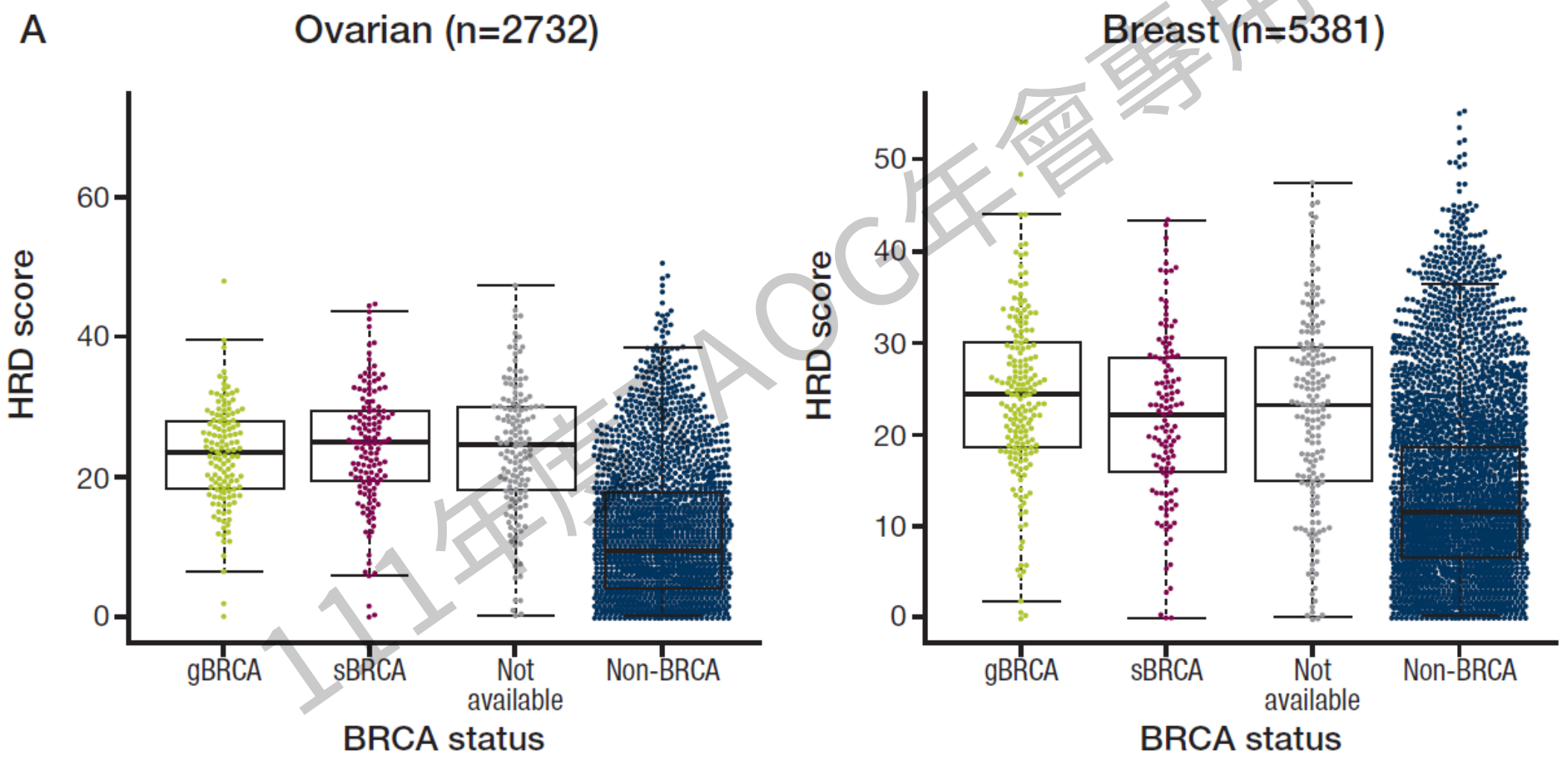
x_i: Length of eligible LOH at segment i

L_{genome}: Total length of genome with SNP coverage, which is $2{\cdot}78\times10^9$ base pairs

$L_{exclusions}$: Total length of genome excluded for LOH analysis

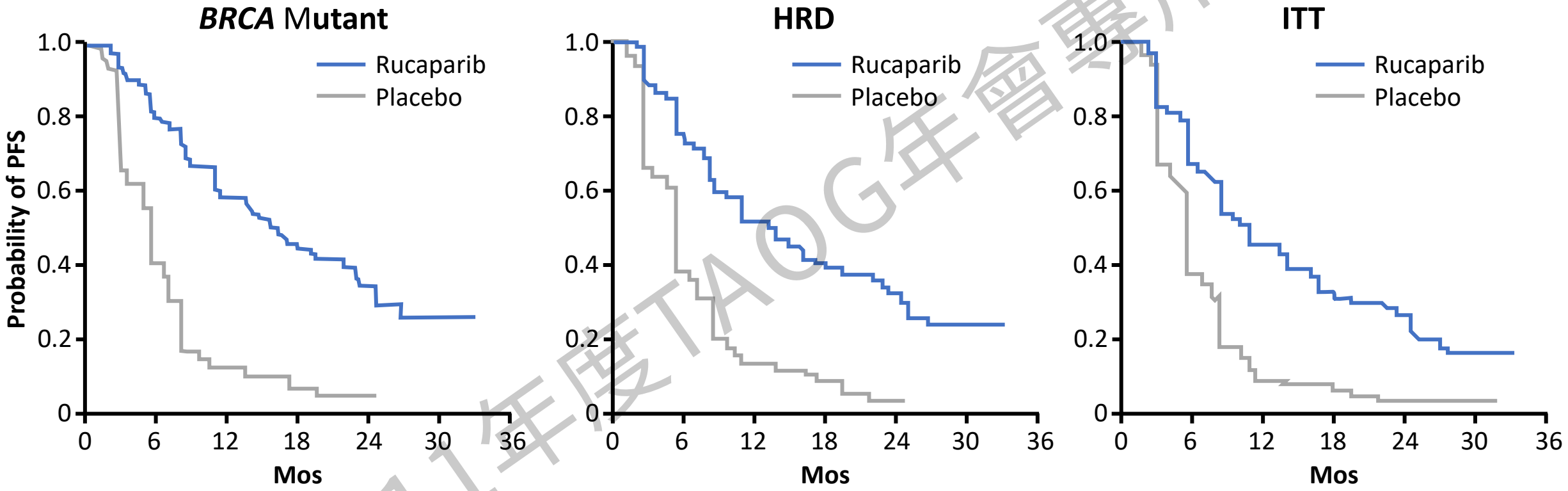
http://dx.doi.org/10.1016/S1470-2045(16)30559-9.

Figure 4. HRD scores by BRCA status in Foundation Medicine (similar fig.1 TCGA; germline is also similar to somatic)



40

ARIEL3: PFS



	Median, Mos	95% CI
Rucaparib (n = 130)	16.6	13.4-22.9
Placebo (n = 66)	5.4	3.4-6.7
HR: 0.23 (95% CI: 0.16-0.34; *P* < .0001)		

	Median, Mos	95% CI
Rucaparib (n = 236)	13.6	10.9-16.2
Placebo (n = 118)	5.4	5.1-5.6
HR: 0.32 (95% CI: 0.24-0.42; *P* < .0001)		

	Median, Mos	95% CI
Rucaparib (n = 375)	10.8	8.3-11.4
Placebo (n = 189)	5.4	5.3-5.5
HR: 0.36 (95% CI: 0.30-0.45; *P* < .0001)		

Coleman. Lancet. 2017;390:1949.

Slide credit: clinicaloptions.com

Biomarker of PARP inhibitors in Ovarian Cancer

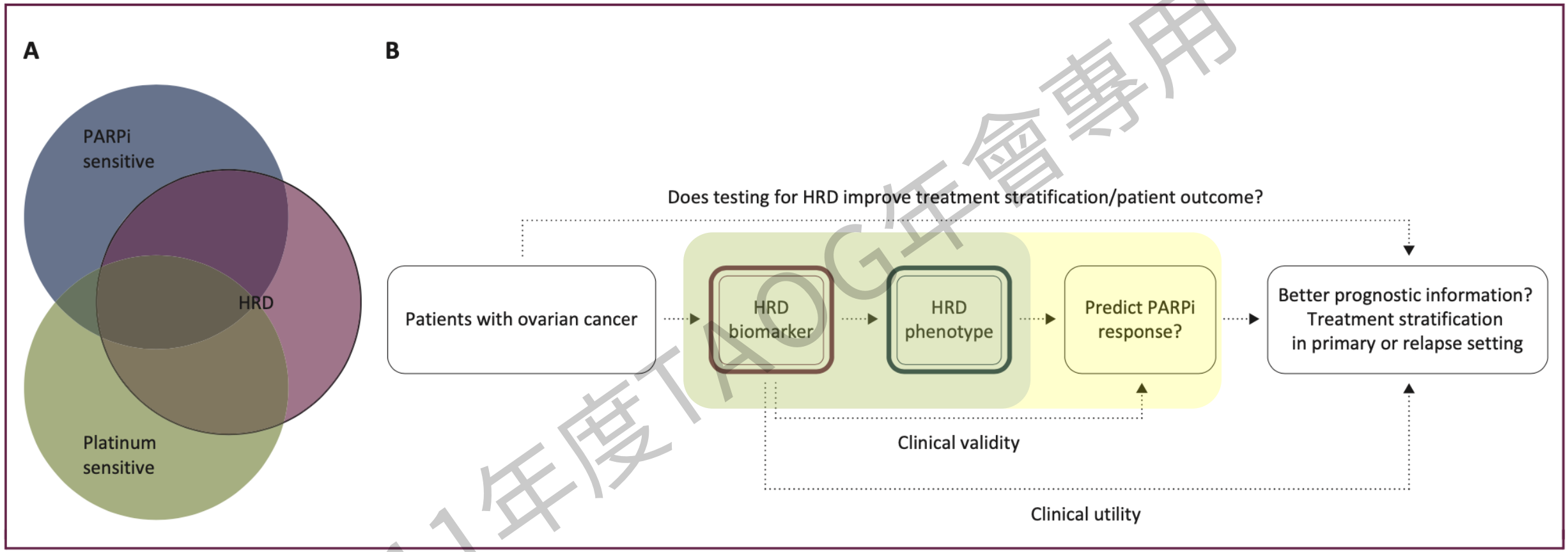


Figure 2. Rationale for using homologous recombination deficiency (HRD) tests to establish PARP inhibitor (PARPi) benefit in ovarian cancer.
(A) Tumours with evidence of HRD, determined using currently available tests, are more likely to respond to platinum salt chemotherapy and PARPis but factors such as resistance mechanisms mean overlap is incomplete. (B) Schema for assessing clinical validity and clinical utility of HRD biomarkers.

https://doi.org/10.1016/j.annonc.2020.08.2102

Effect of HRD

Effect of HRD

Two different HRD test methodologies: (1) LOH, TAI and LST (2) GI

Myriad myChoice® CDx

FoundationOne® CDx

ACT HRD

HRD → Genomic Instability

LOH

TAI

LST

SOPHiA GENETICS

Genomic Integrity (GI)

ACTHRD™ Performance

HRD Status		Comparator Assay			
		True Positive	True Negative	Invalid	Total
ACTHRD™	Positive	23	1	0	24
	Negative	0	10	0	10
	Invalid	1	0	1	2
	Total	24	11	1	36
Agreement Including Valid Results Only	PPA [95% CI]	100.00% [85.69%, 100.00%]			
	NPA [95% CI]	90.91% [62.26%, 99.53%]			
	OPA [95% CI]	97.06% [85.08%, 99.85%]			
Agreement Including Invalid Results	PPA [95% CI]	95.83% [79.76%, 99.79%]			
	NPA [95% CI]	90.91% [62.26%, 99.53%]			
	OPA [95% CI]	94.29% [81.39%, 98.42%]			

Definition of Positive with FDA-approved test:

BRCA1/2 mutation

or

GIS score ≥ 42

Definition of Positive with ACTHRD™:

BRCA1/2 mutation

or

LOH score ≥ 0.4

Effect of HRD

Effect of HRD

Two different HRD test methodologies: (1) LOH, TAI and LST (2) GI

Myriad myChoice® CDx

FoundationOne® CDx

ACT HRD

HRD → Genomic Instability

LOH

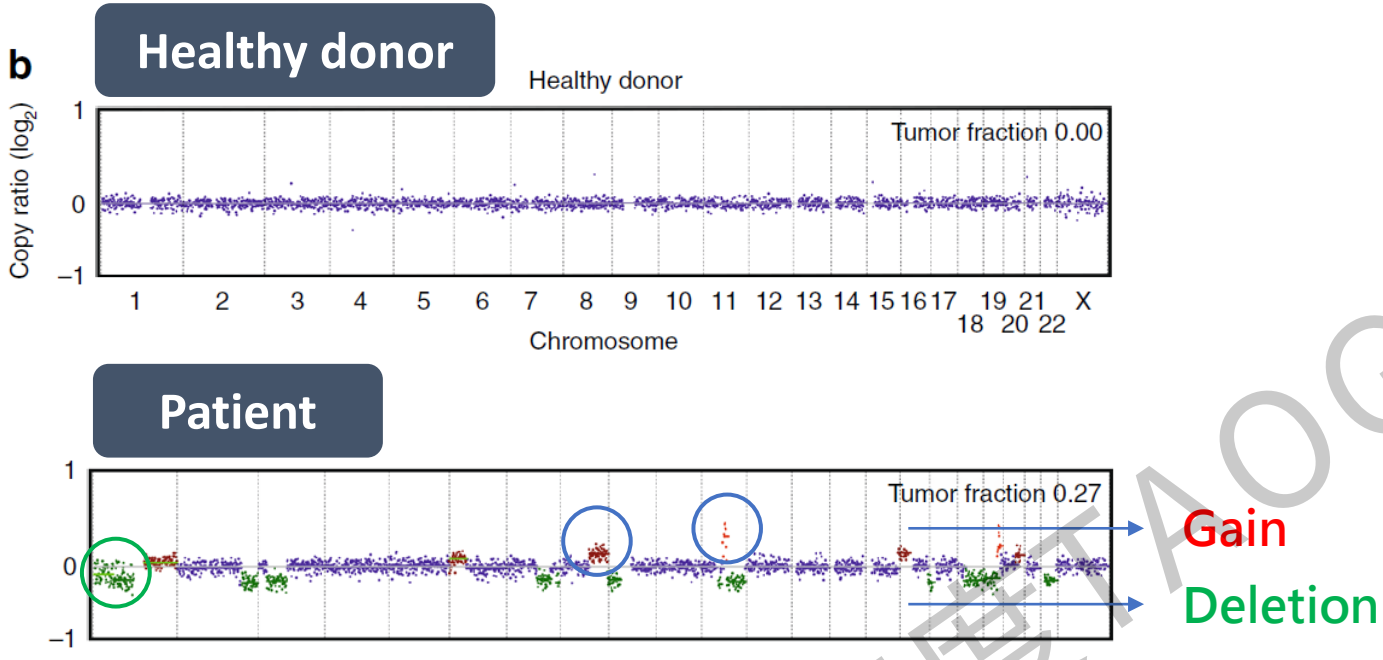
TAI

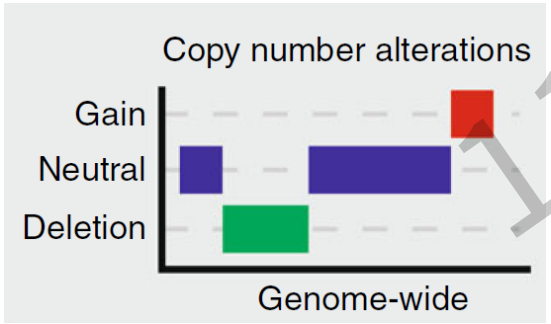
LST

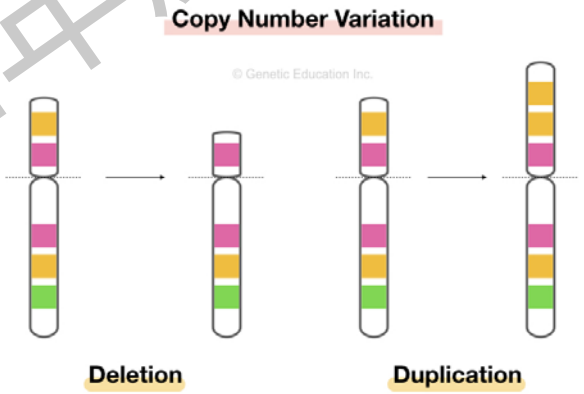
SOPHiA GENETICS

Genomic Integrity (GI)

Low-pass 全基因定序 (Low-pass WGS)







- 若 copy number 有 gain or deletion，表示發生 CNV (Copy Number Variation)，DNA修復功能可能有問題
- 已普遍應用於產前基因檢測 (NIPS)
- 高通量、檢測速度快、範圍廣

Adalsteinsson, V.A., et al., Nat Commun, 2017. 8(1): p. 1324

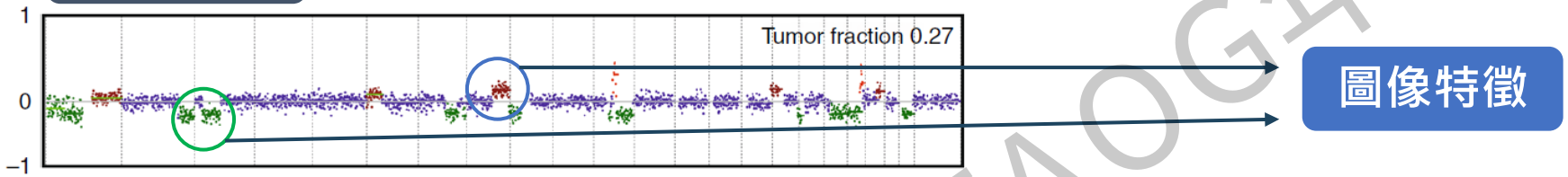
WGS: whole genome sequence, 全基因定序

如何尋找Low-pass 全基因定序圖像化特徵？跟誰做

AI 深度學習 Model

示意圖：

HRD +



Adalsteinsson, V.A., et al., Nat Commun, 2017. 8(1): p. 1324

利用經 Myriad 檢測為 HRD+ (超過3000人)之 low-pass WGS 圖形，來讓 AI 做深度學習：

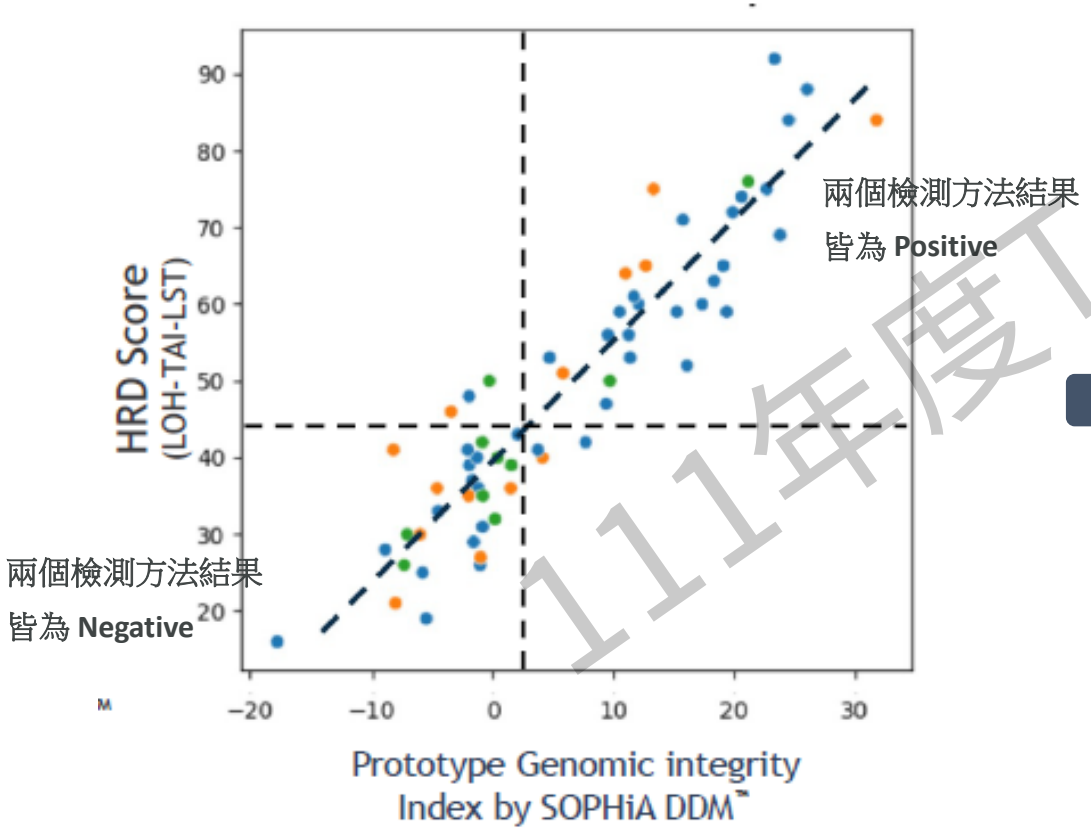
從Myriad-confirmed HRD+ 病人的 low-pass WGS 圖像，找出 HRD+ 在 low-pass WGS 中的圖像特徵，進而學習分析判斷 HRD status

Concordance Data (預計 2022 Q2 發表 paper)

SOPHiA GENETICS

Preliminary Data (Internal Study)

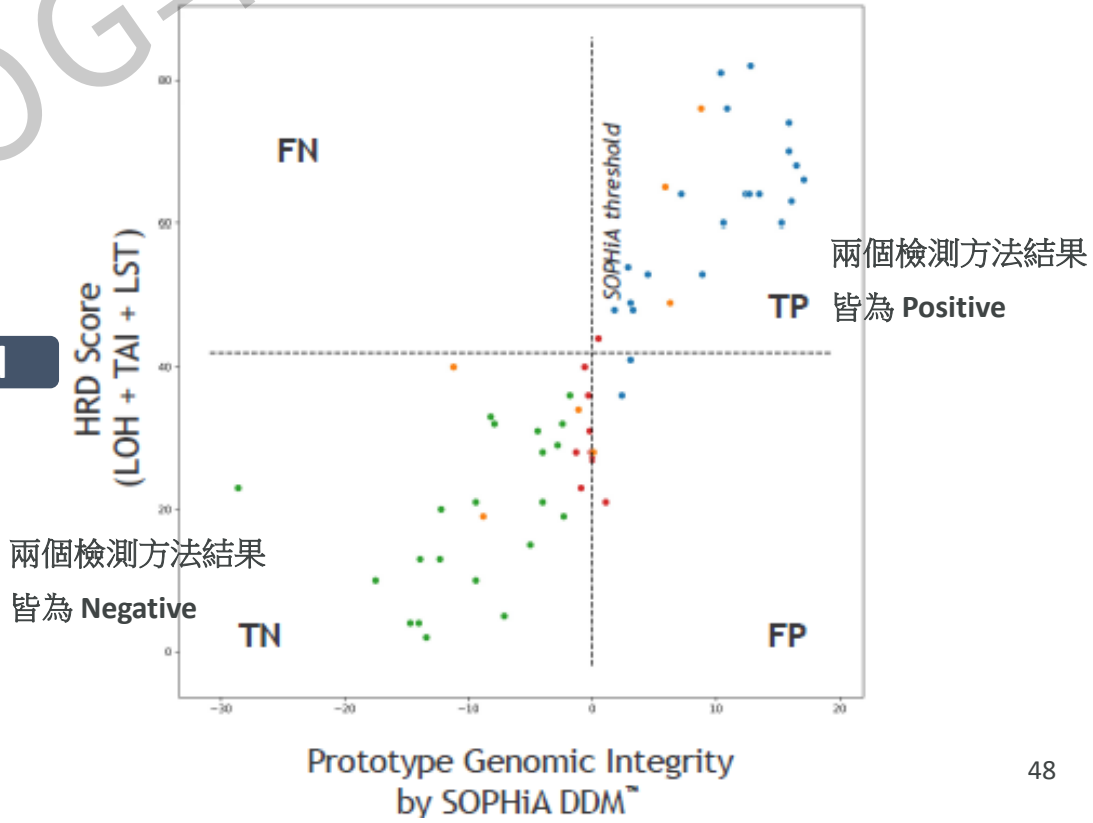
The SOPHiA solution was assessed using **62** high-grade serous Ovarian Cancer samples



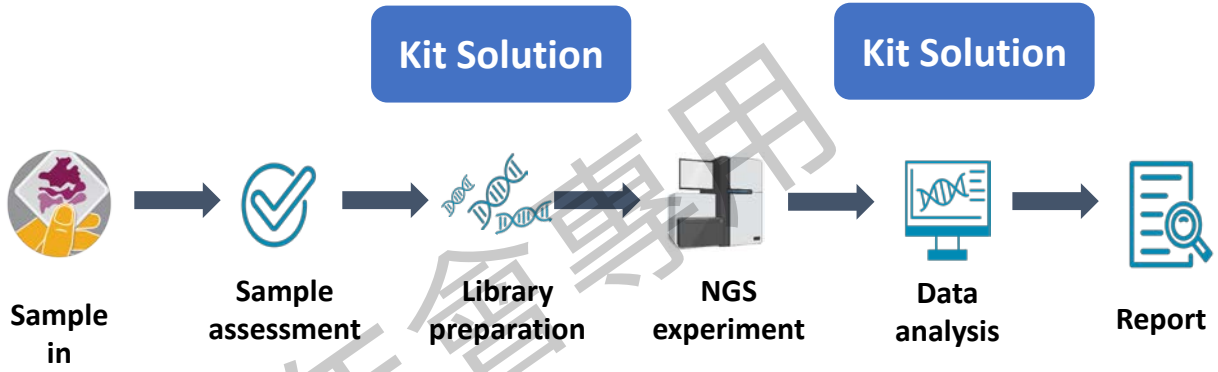
External Lab (will have peer to peer review publish)

- **53 samples** passed SOPHiA DDM™ sample QA
- Observed **concordance with HRD score** (LOH + TAI + LST) : **94%**
- 計畫收 **> 100 個 sample 於 2022 Q2 發表 paper**

Myriad

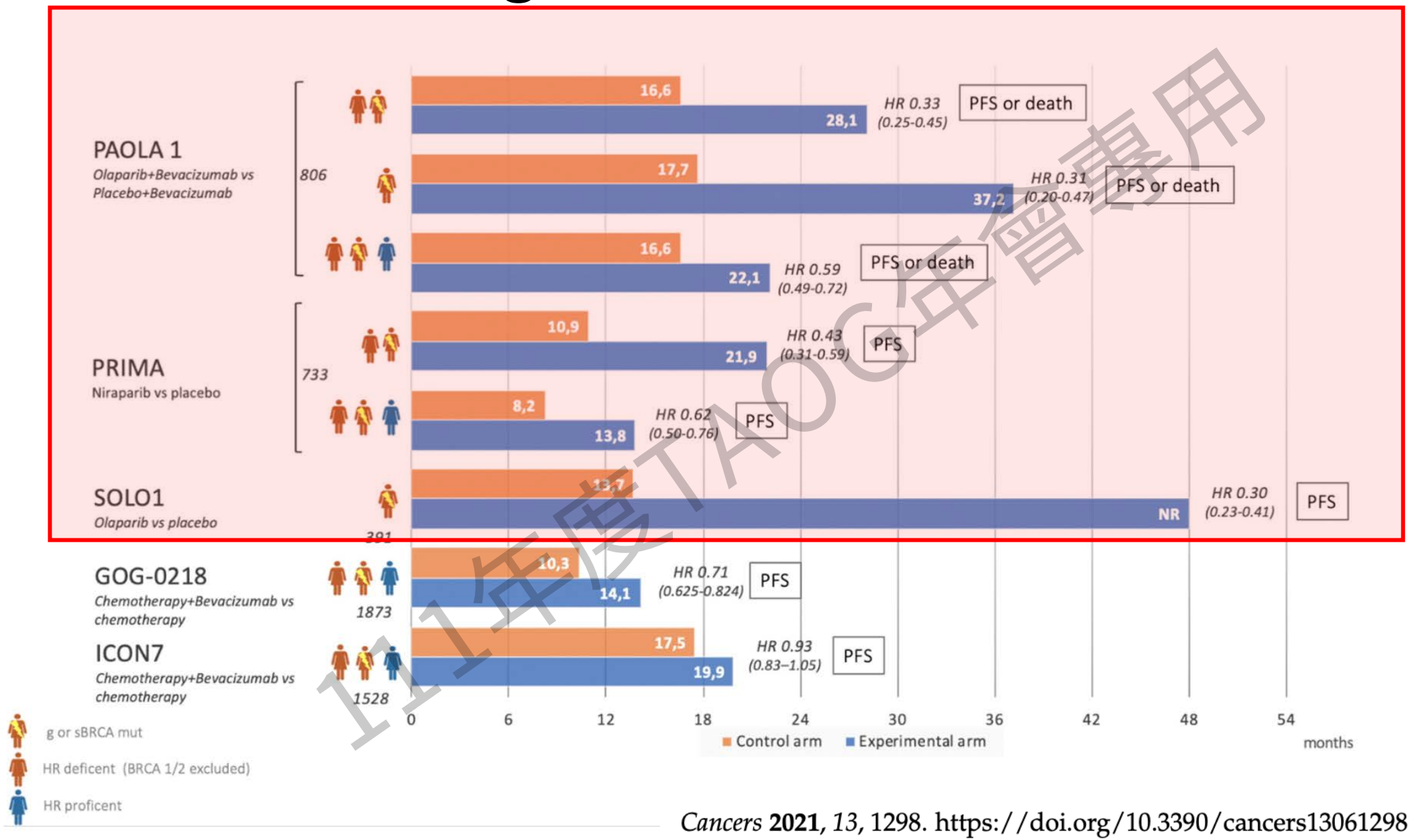


Current solution for HRD testing Overview



	Trial Central Lab	Trial Central Lab	Local LDT Solution	Kit Solution				
Company	Myriad	Foundation	ACT Genomics	Illumina	Amoy	SOPHiA	Roche (FMI)	Thermo Fisher
Availability In Taiwan	in market	in market	in market	2022.Q3	in market	in market	In market	2023 Q1
Validation/ Concordance	100% Global trial use: PAOLA-1 PRIMA	100% Global trial use: ARIEL3	95% (N=36) (concordance vs Myriad)	94.3 % (N=194) (concordance vs Myriad)	81.6% (N=98) (concordance vs Myriad)	> 90% (N=337) (concordance vs Myriad)	N/A	N/A

Take home message



Cancers **2021**, *13*, 1298. https://doi.org/10.3390/cancers13061298

Take home message

Trial	SOLO-1	PRIMA	PAOLA-1	GOG-218
Patients enrolled	HGSOC/HGEOC Stage III: with one attempt at optimal debulking surgery (PDS or IDS) Stage IV: with either a biopsy and/or PDS or IDS documented mutation in BRCA1 or BRCA2 predicted/ suspected to be deleterious	HGSOC/HGEOC Stage III: PDS with visible residual disease, NACT, or inoperable Stage IV: PDS regardless of residual disease, NACT or inoperable	HGSOC/HGEOC Stage III/IV: after completion of first-line surgery and platinum-based CT + bevacizumab; with/without residual disease	All histology type, but 84% HGSOC Stage III: with any gross (macroscopic or palpable) residual disease Stage IV Start with platinum chemotherapy
Number of included patients	391	733	806	1873
1L treatment	Response to Platinum chemotherapy	Response to Platinum chemotherapy	Response to Platinum chemotherapy + bevacizumab	Platinum chemotherapy+bevacizumab
FDA approval date	12/2018	4/2020	5/2020	6/2018
Median PFS (months)	56.0 vs 13.8 (ESMO 2020) Δ PFS: 42.2	13.8 vs 8.2 Δ PFS:5.6	22.1 vs 16.6 Δ PFS:5.5	14.1(CT + bev throughout) vs 10.3(CT) Δ PFS 4.7

- Trial 之前的選擇
- Histology
- Response to platinum

Thank You