DNA-Breaks DAMAGE REPAIR

BASIC COURSE OF MOLECULAIRE PATHOLOGY

BSP

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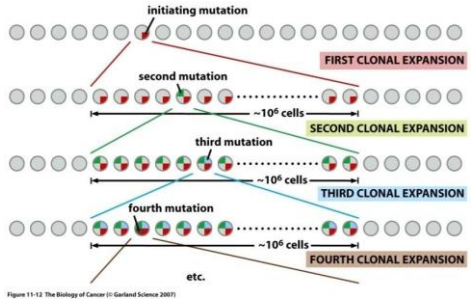
MAY 2022

OUTLINE

- INTRODUCTION
  - Basic concepts oncogenesis
  - Cancer genetics/heredity
- DNA DAMAGE REPAIR (DDR) CONCEPTS
  - DNA Repair (DNA –Breaks)
  - Synthetic Lethality
- HOMOLOGOUS RECOMBINATION REPAIR defects (HRRd)
  - Predictive biomarker / Cancer subtype
  - Predictive Hereditary risk factor of cancer/ Cancer subtype
  - Detection/ testing
- CONCLUSIONS

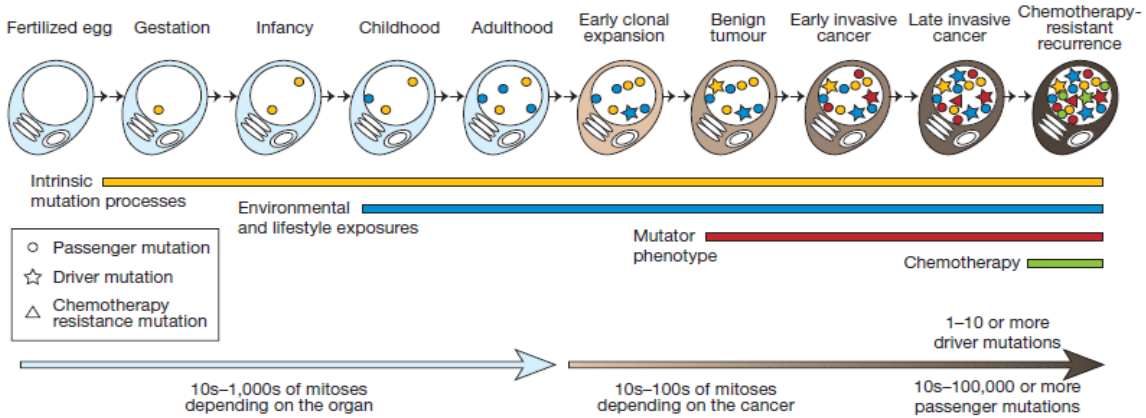
Cancer is a multi-step disease



the number of cells in humans (10^{13-14})

the number of mitosis during life (10^{16})

Cancer genetics : a continuous process



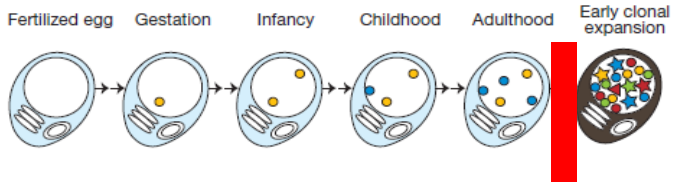
Genetic instabilities in human cancers

Christoph Lengauer, Kenneth W. Kinzler & Bert Vogelstein

Whether and how human tumours are genetically unstable has been debated for decades. There is now evidence that most cancers may indeed be genetically unstable, but that the instability exists at two distinct levels. In a small subset of tumours, the instability is observed at the nucleotide level and results in base substitutions or deletions or insertions of a few nucleotides. In most other cancers, the instability is observed at the chromosome level, resulting in losses and gains of whole chromosomes or large portions thereof. Recognition and comparison of these instabilities are leading to new insights into tumour pathogenesis.

NATURE | VOL 396 | 17 DECEMBER

Cancer genetics : a not so continuous process



Cancers are genetic diseases

- **The neoplastic phenotype is heritable**
  - Virchow – 1858 – « omnis cellulae cellula » (every cell arises from a cell)
  - David van Hansemann - 1890 – abnormal mitosis in tumor cells
  - Theodor Boveri - 1914 – First modern theory of cancer genetic :

    « …tumor growth is based on …a particular, incorrect chromosome combination which is the cause of abnormal growth characteristics passed on daughter cells » Zur Frage der Entstehung Maligner Tumoren.

Mecanisms of alterations in human tumors

Oncogenes

- Activating mutations
- Gene amplifications
- Translocations
- Insertions (virus, ALU, HERV…)

Tumor suppressor genes

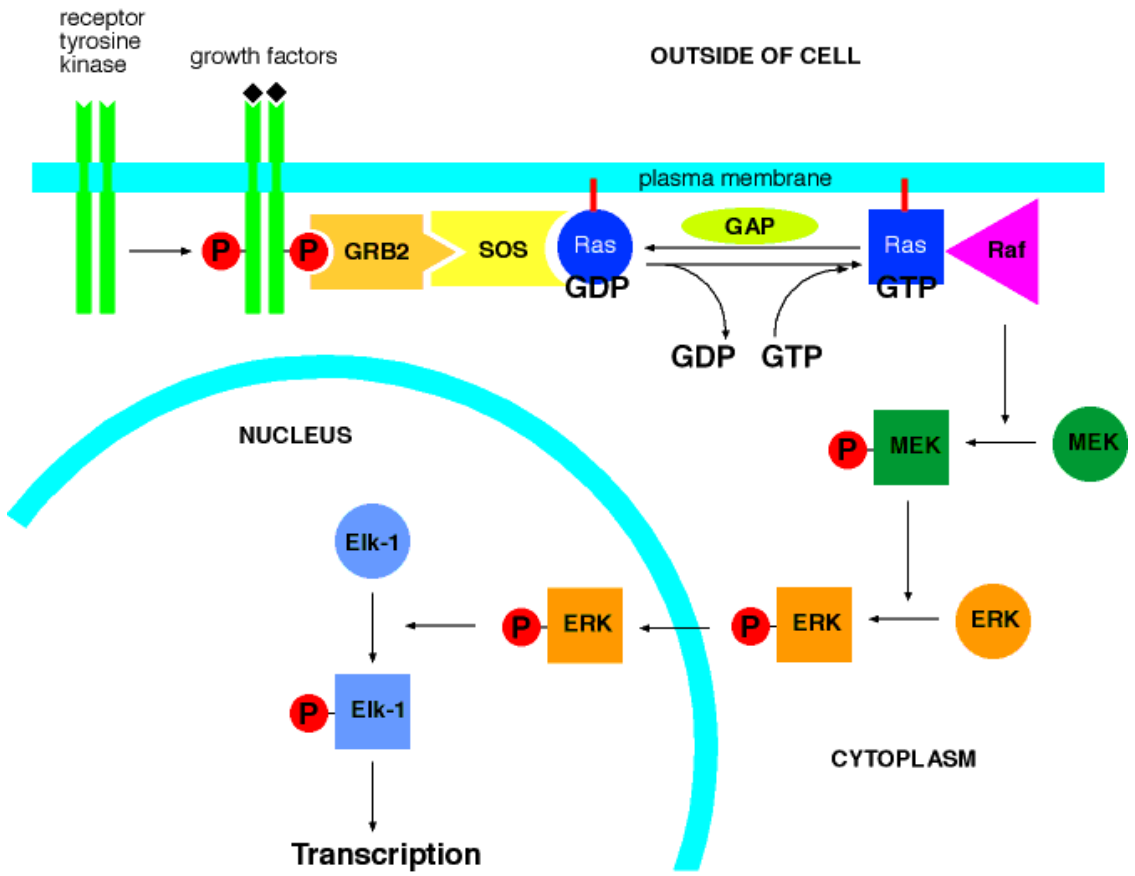
- Inacativating mutations
- Deletions (+/- larges)
- Epigenetic alterations
- Insertions (virus, ALU, HERV…)

Hereditary factors of cancer : the tumor suppressor genes

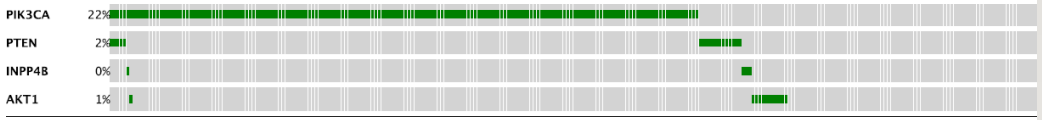
The anti-oncogenes or Tumor Suppressor Genes

- Broca - 1866 : Breast cancer families
- Harris - 1969 : Recessive properties of cancer
- Knudson - 1971 : "two-hit hypothesis"
- Comings - 1973 : Hypothesis of recessive mutations of the same locus
- Wilson et al - 1973 : 13q14 deletion and hereditary retinoblastoma
- Cavenee et al - 1983 : LOH in 13q
- Friend et al - 1986 : Identification of RB1

Oncogenes and Tumor Suppressor Genes converge in biologic pathways



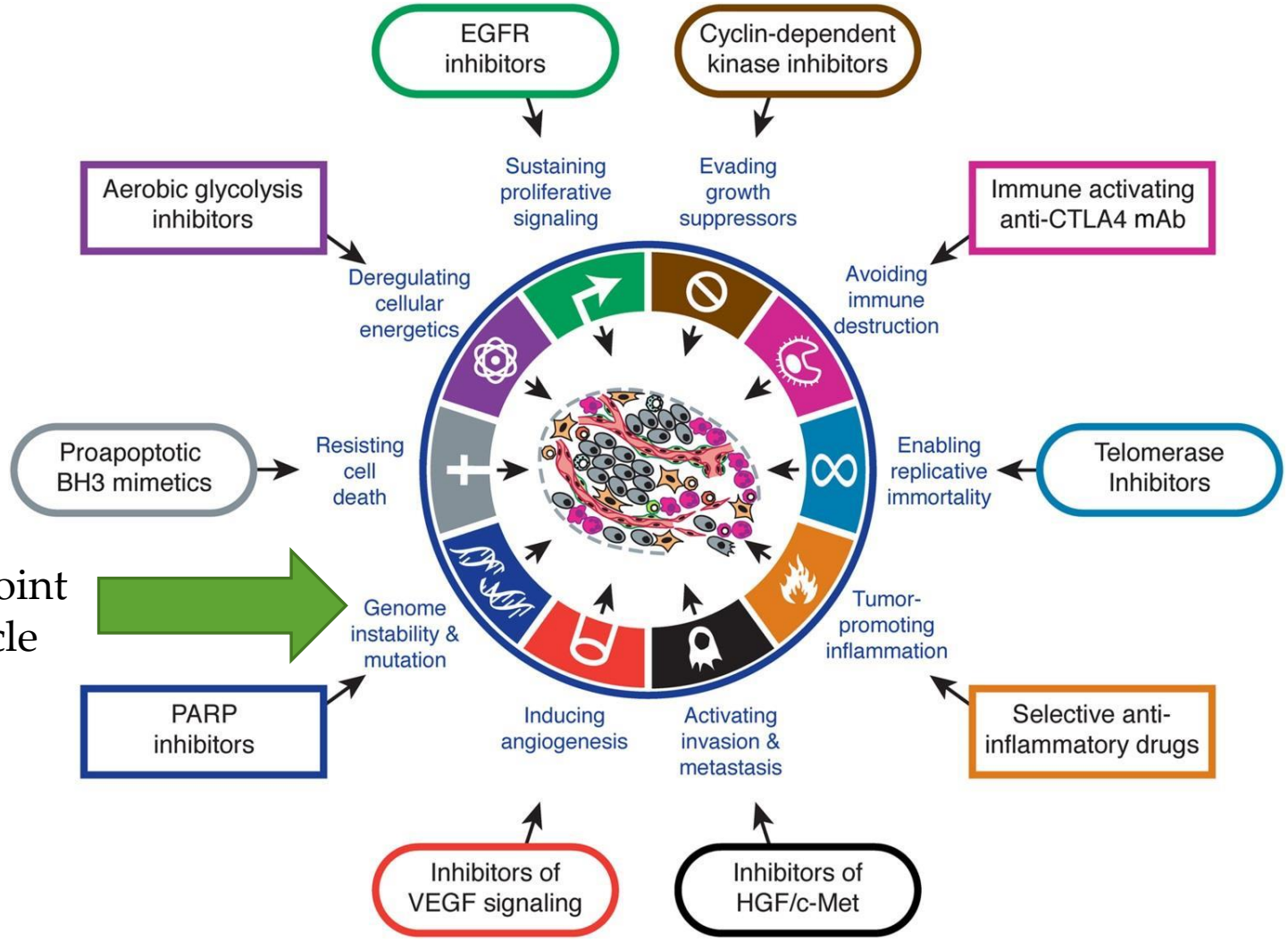
Pathways rather than genes altered in cancers



QUESTION ?

- GENETIC
- HEREDITARY
- THE SAME ?????

Hallmarks of Cancer: The Next Generation
Douglas Hanahan, Robert A. Weinberg



Check point
Cellc cycle

Two different flavors of tumor suppressor genes

Gatekeepers:
"brakes" inhibiting cell growth and division
RB1, CDKN2A, …
Recessive : 2 alterations are needed

Caretakers :
Control fidelity of DNA replication, maintenance, repair, correct cell division, etc.
If inactivated,

- increased rate of mistakes in DNA processing
- faster evolution to malignancy

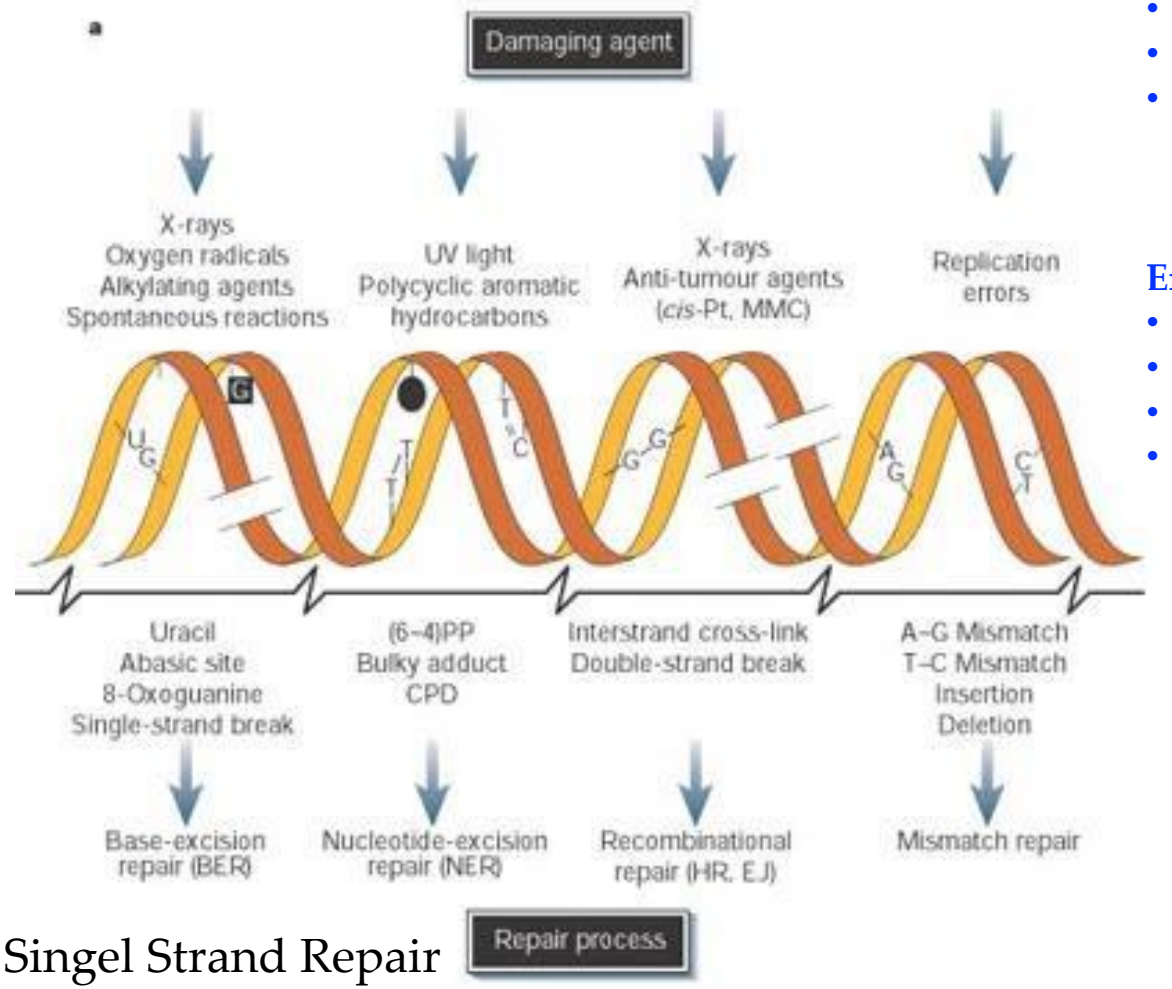
Recessive : 2 alterations are needed

Anomalies of DNA repair leads t
***Senescence**
***Apoptosis**
***Cancer**

DNA DAMAGE REPAIR pathways

- SINGEL STRAND BREAKS –REPAIR (SSB-R)
  - BER (Base Excision Repair)
  - NER (Nucleotide Excision Repair)
- DOUBLE STRAND REPAIR (DSB-R)
  - NHEJ (Non-Homologous End-Joint)
  - MMEJ (Micro-homologous End-Joint)
  - HR (Homologous Recombination)
- MISSMATCH RECOMBINATION REPAIR (MRR)

DNA REPAIR PATHWAYS



Singel Strand Repair

Exogenous factors:

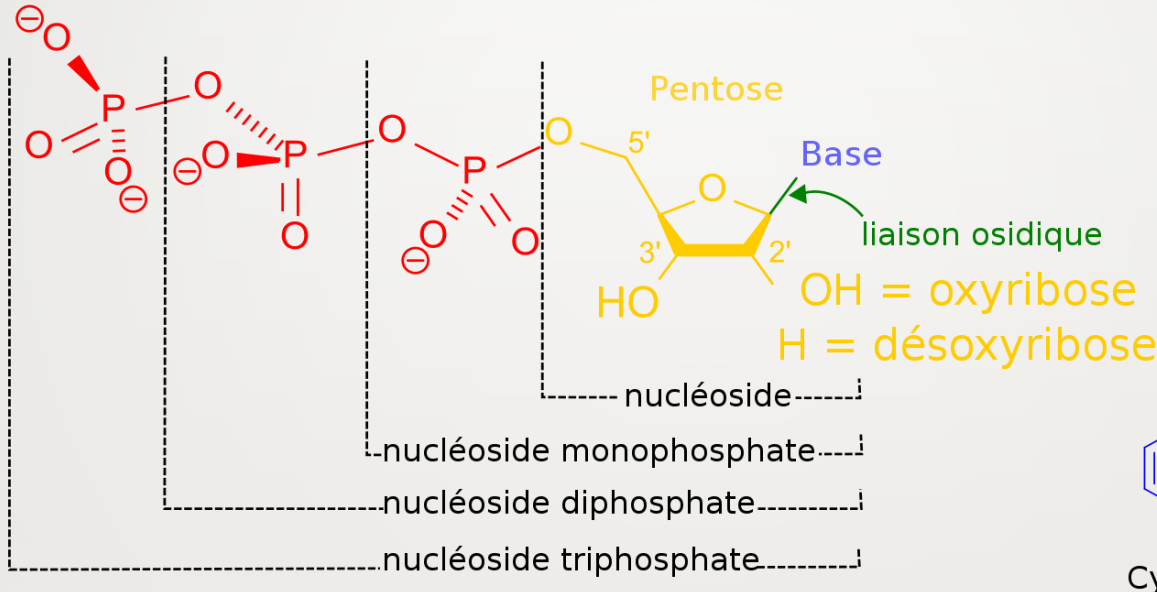
- UV radiation
- Ionizing radiation
- Genotoxic chemicals

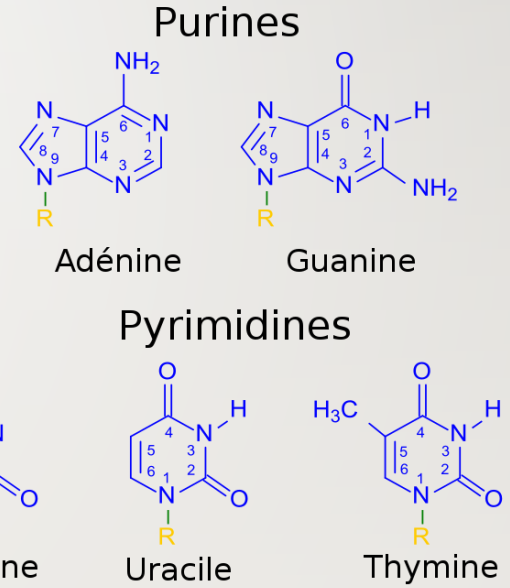
Endogenous factors:

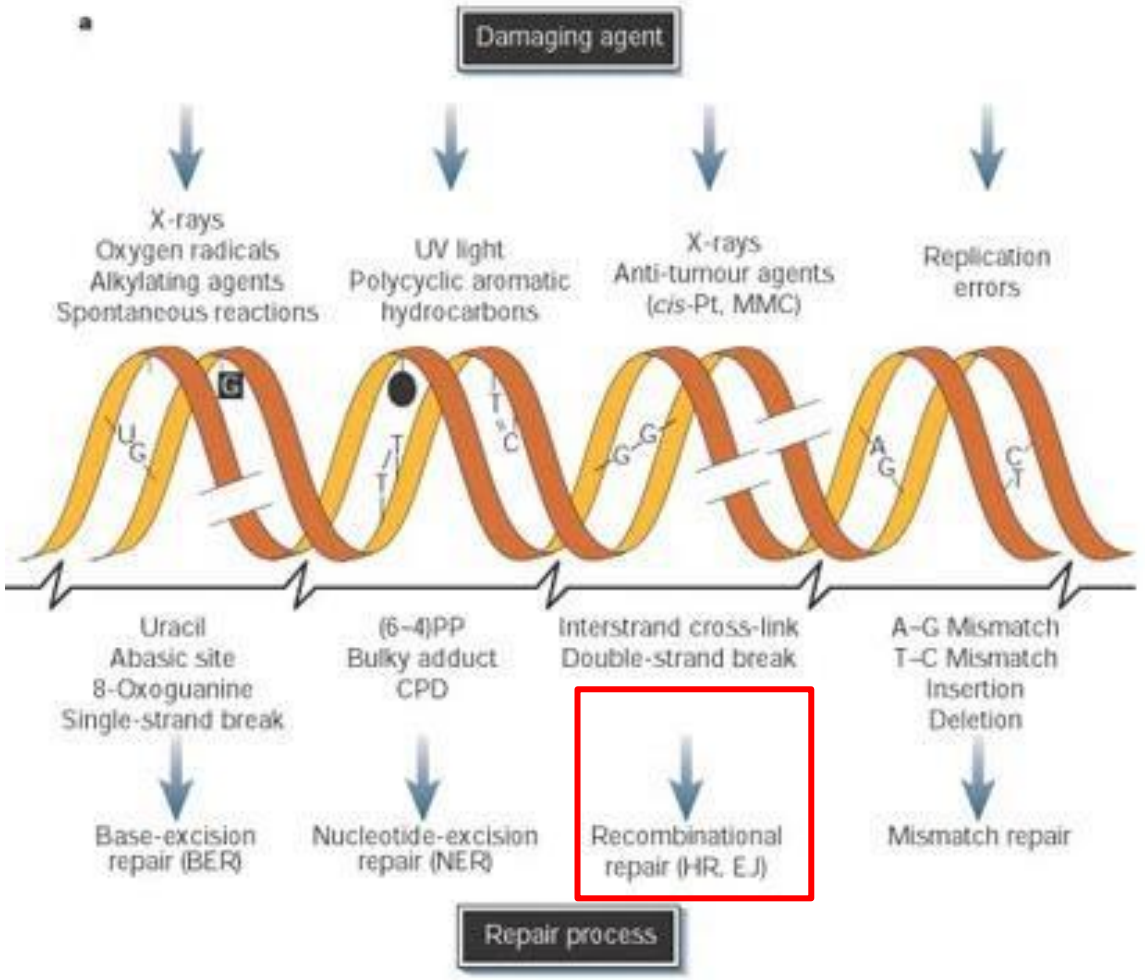
- Spontaneous or enzymatic reactions
- Chemical modifications
- Replication errors
- Replication stress

QUESTION? Base/ Nucleotide

- Nucleotide: Base+ sugar+ Phosphates

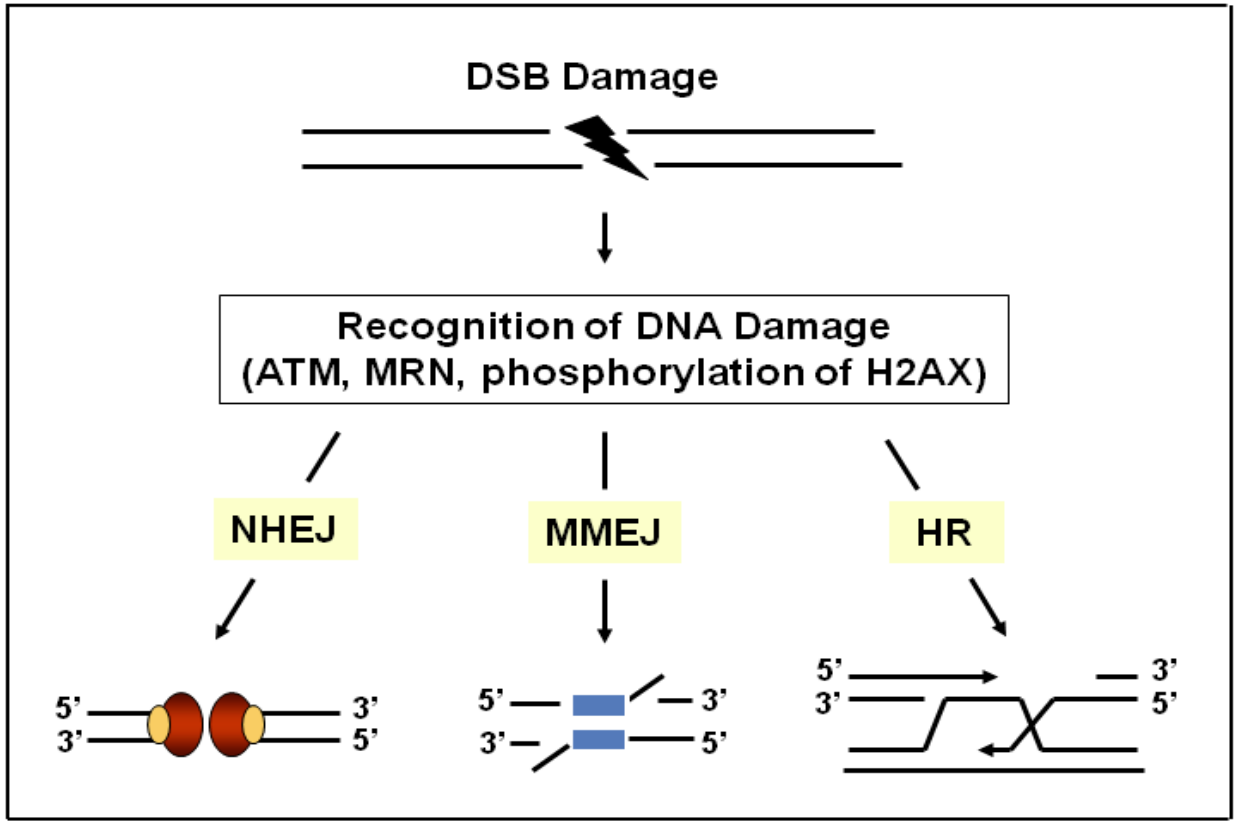


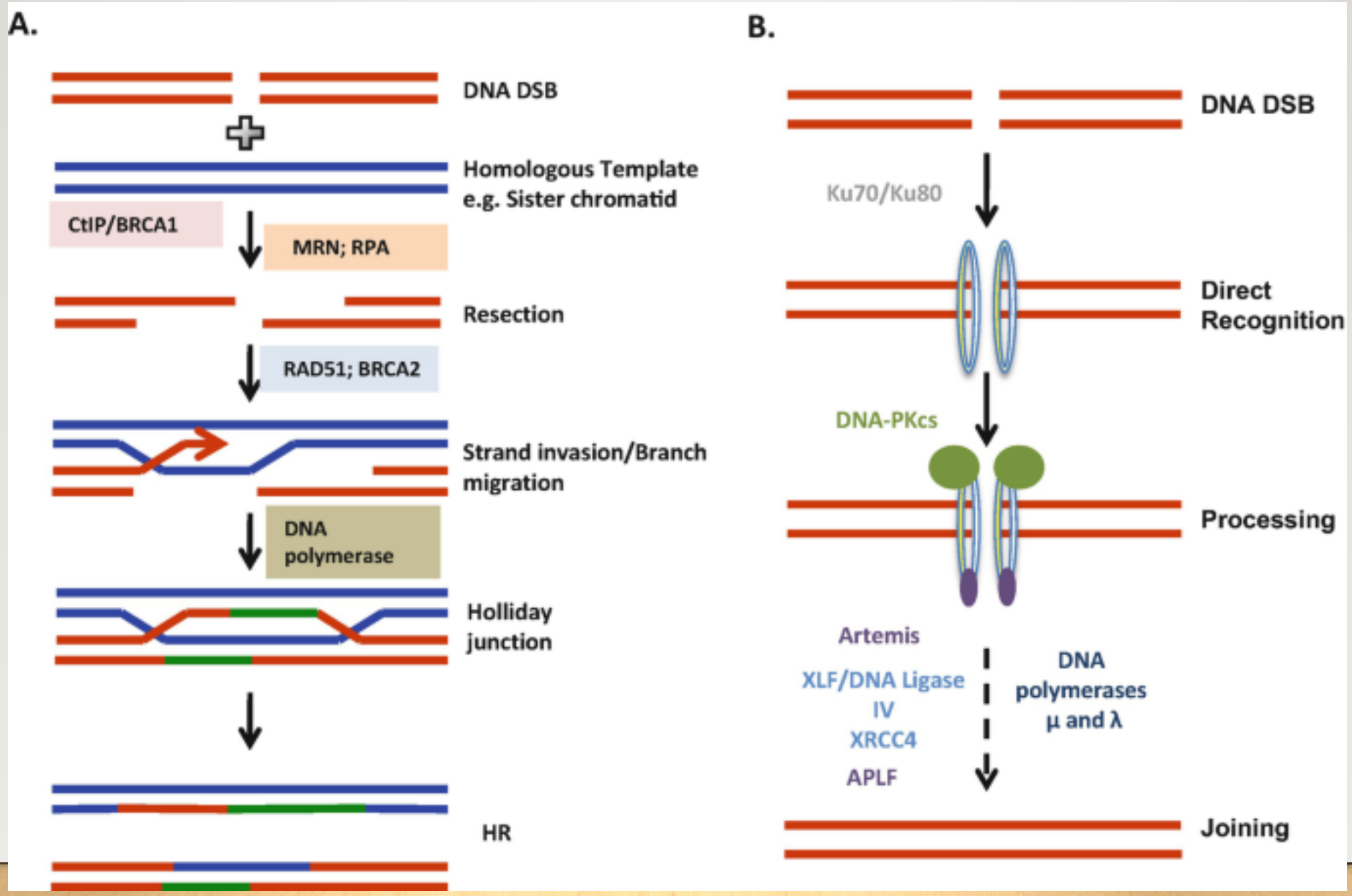



Damaging agent
X-rays
Oxygen radicals
Alkylating agents
Spontaneous reactions
UV light
Polycyclic aromatic hydrocarbons
X-rays
Anti-tumour agents
(cis-Pt, MMC)
Replication errors
Uracil
Abasic site
8-Oxoguanine
Single-strand break
(6–4)PP
Bulky adduct
CPD
Interstrand cross-link
Double-strand break
A–G Mismatch
T–C Mismatch
Insertion
Deletion
Base-excision repair (BER)
Nucleotide-excision repair (NER)
Recombinational repair (HR, EJ)
Mismatch repair
Repair process

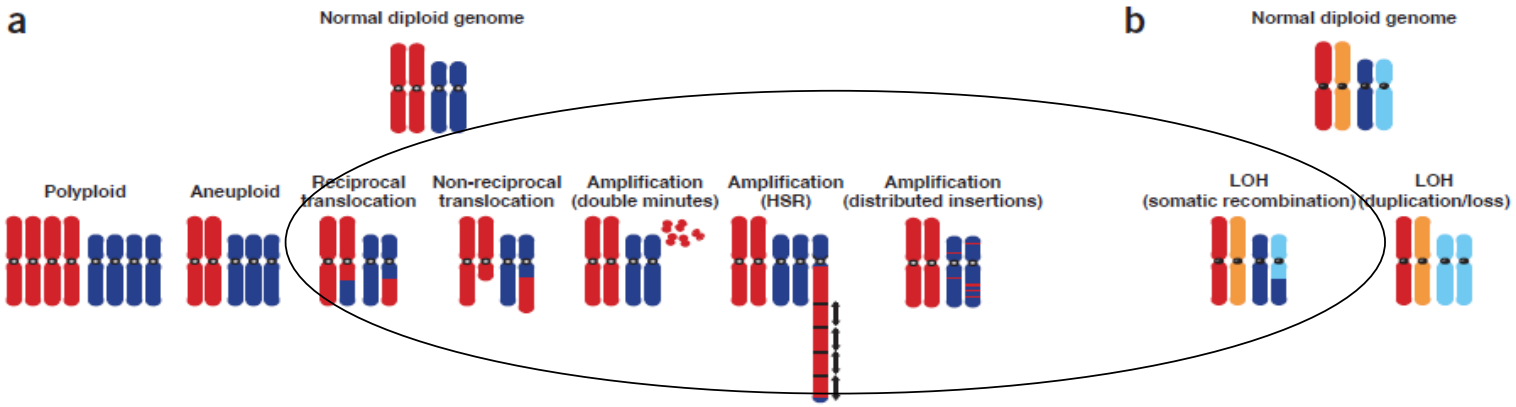
DSB repair

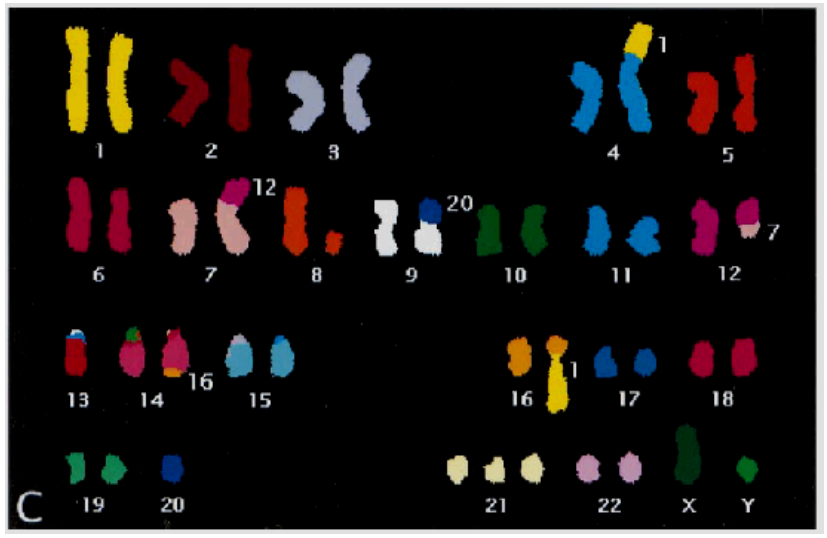
- Non Homologous End Joining (NHEJ)
- Alternative End Joining (AltEJ) / Microhomology-Mediated End Joining (MMEJ)
- Homologous Recombination (HR)

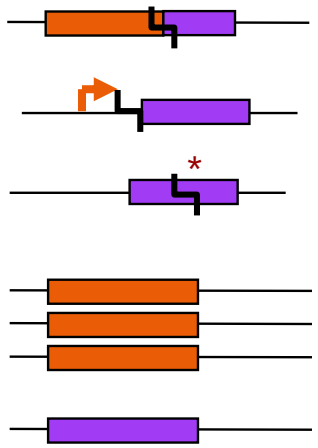



A.
DNA DSB
Homologous Template
e.g. Sister chromatid
CtIP/BRCA1
MRN; RPA
Resection
RAD51; BRCA2
Strand invasion/Branch
migration
DNA
polymerase
Holliday
junction
HR
B.
DNA DSB
Ku70/Ku80
Direct
Recognition
DNA-PKcs
Processing
Artemis
XLF/DNA Ligase
IV
XRCC4
APLF
DNA
polymerases
μ and λ
Joining

Consequences of DNA double-strand breaks (DSB)







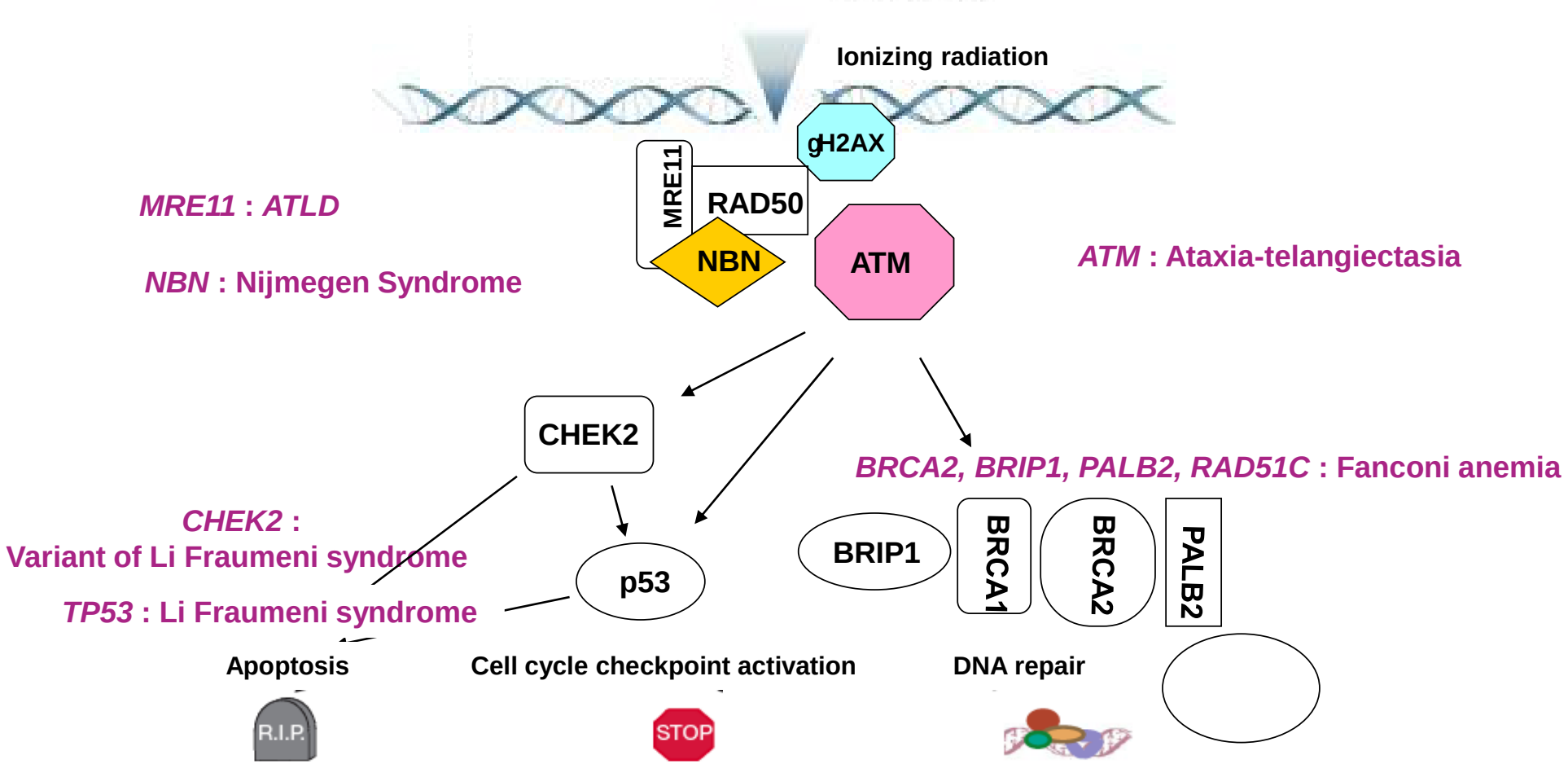
Gene fusion

Gene surexpression

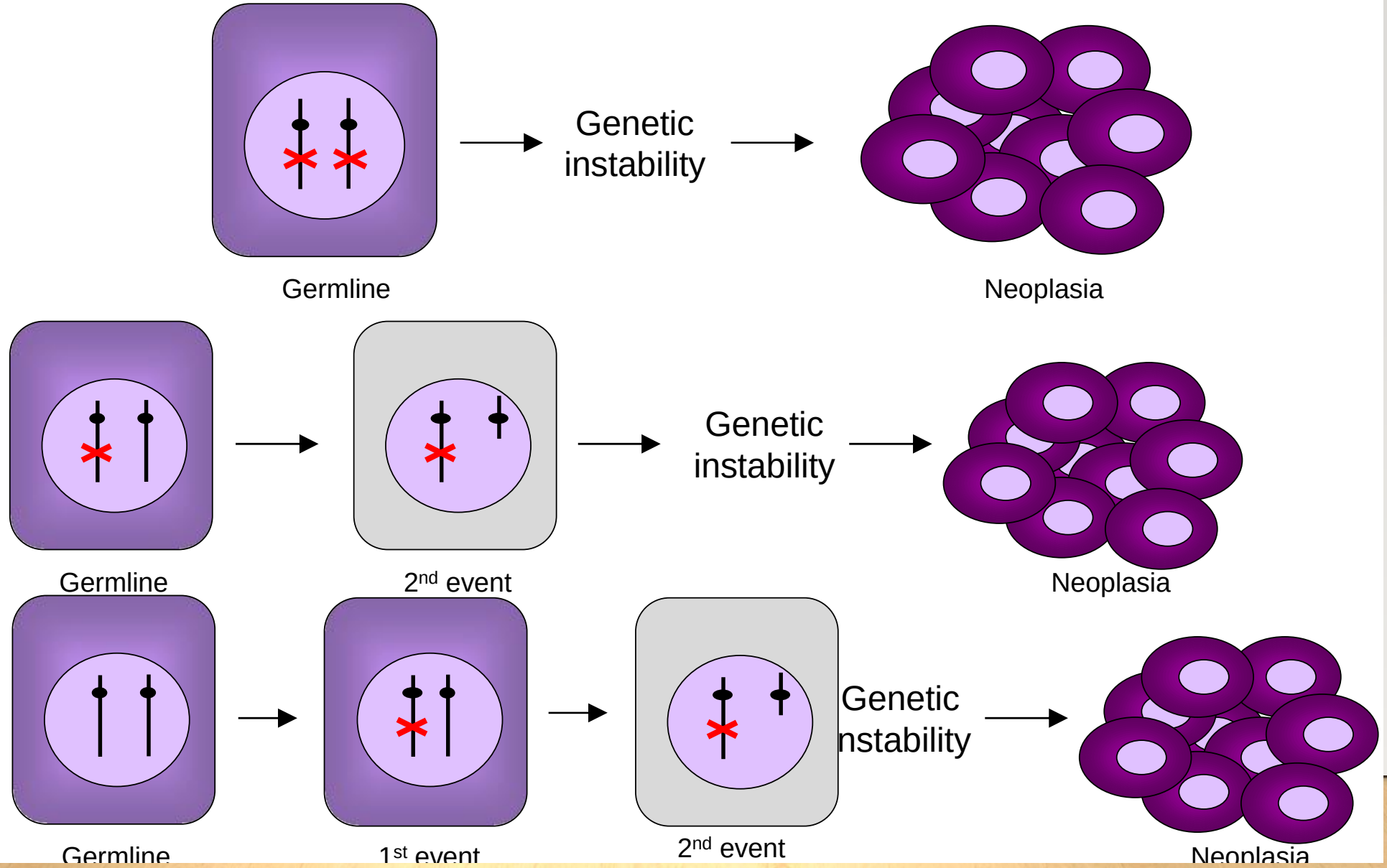
Amplification

Haploinsufficiency

DDR defects in human pediatric diseases

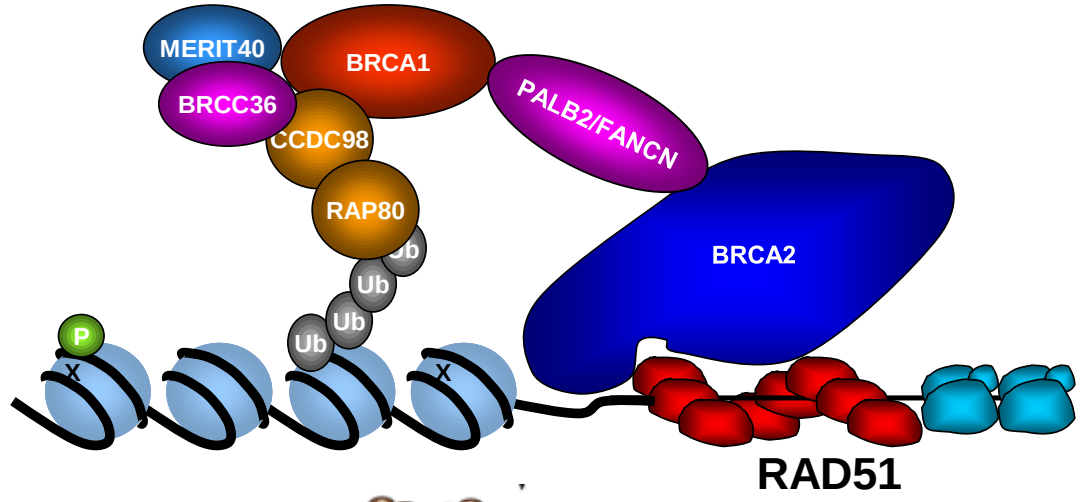


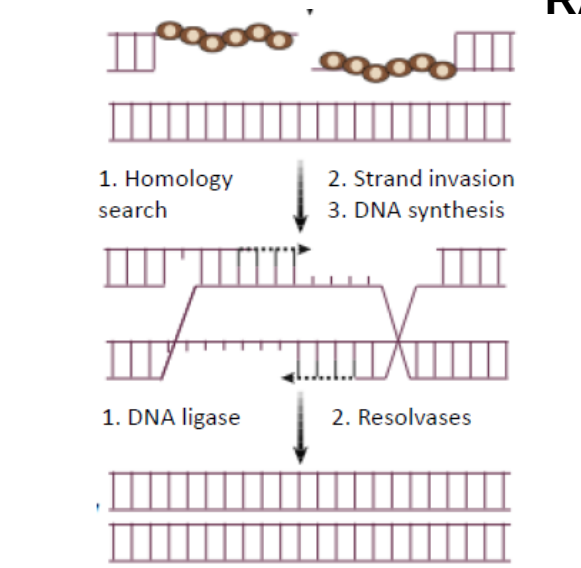
DDR defects in human pathologies



Homologous Recombination

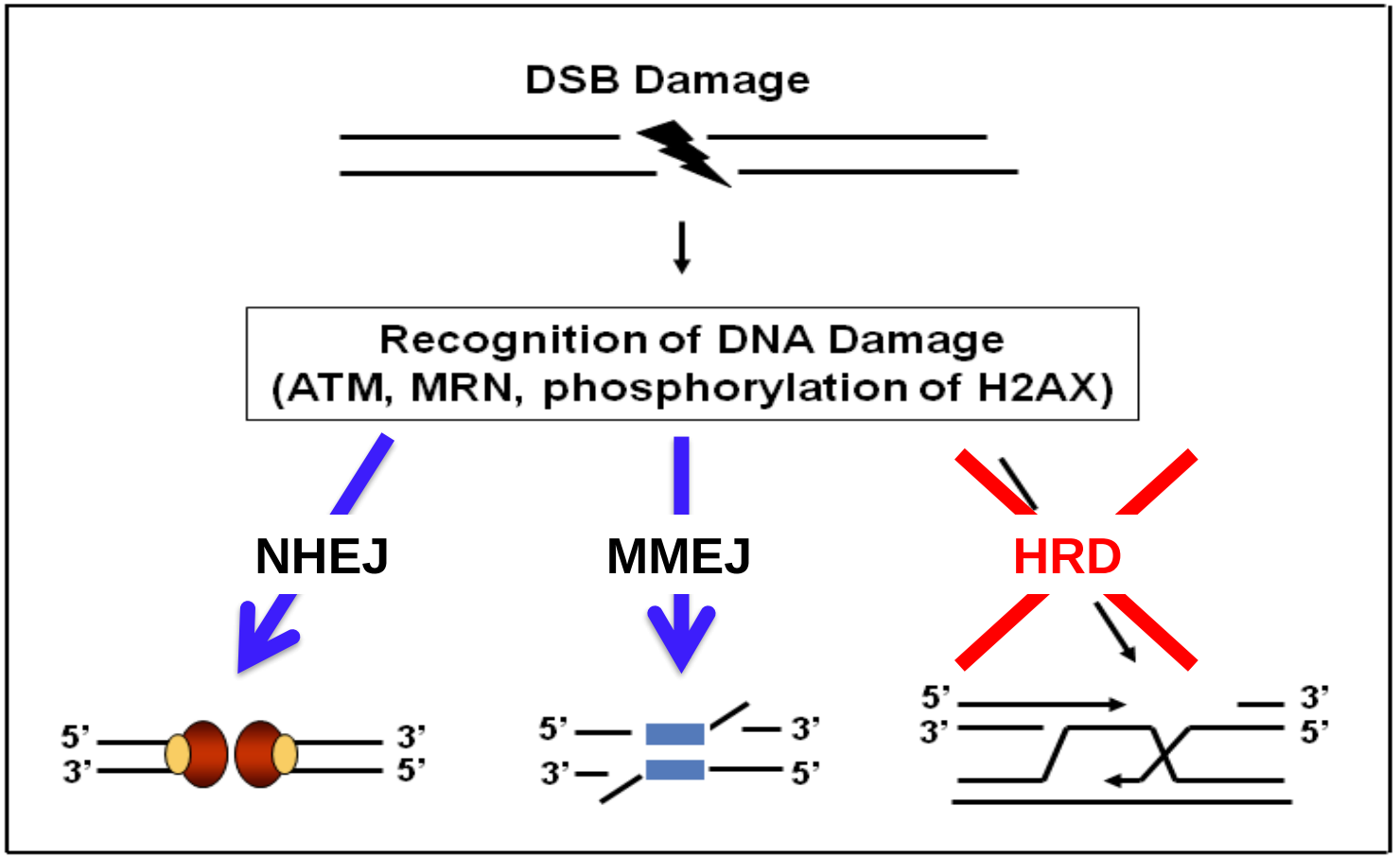
Key roles of the main breast/ovarian susceptibility genes



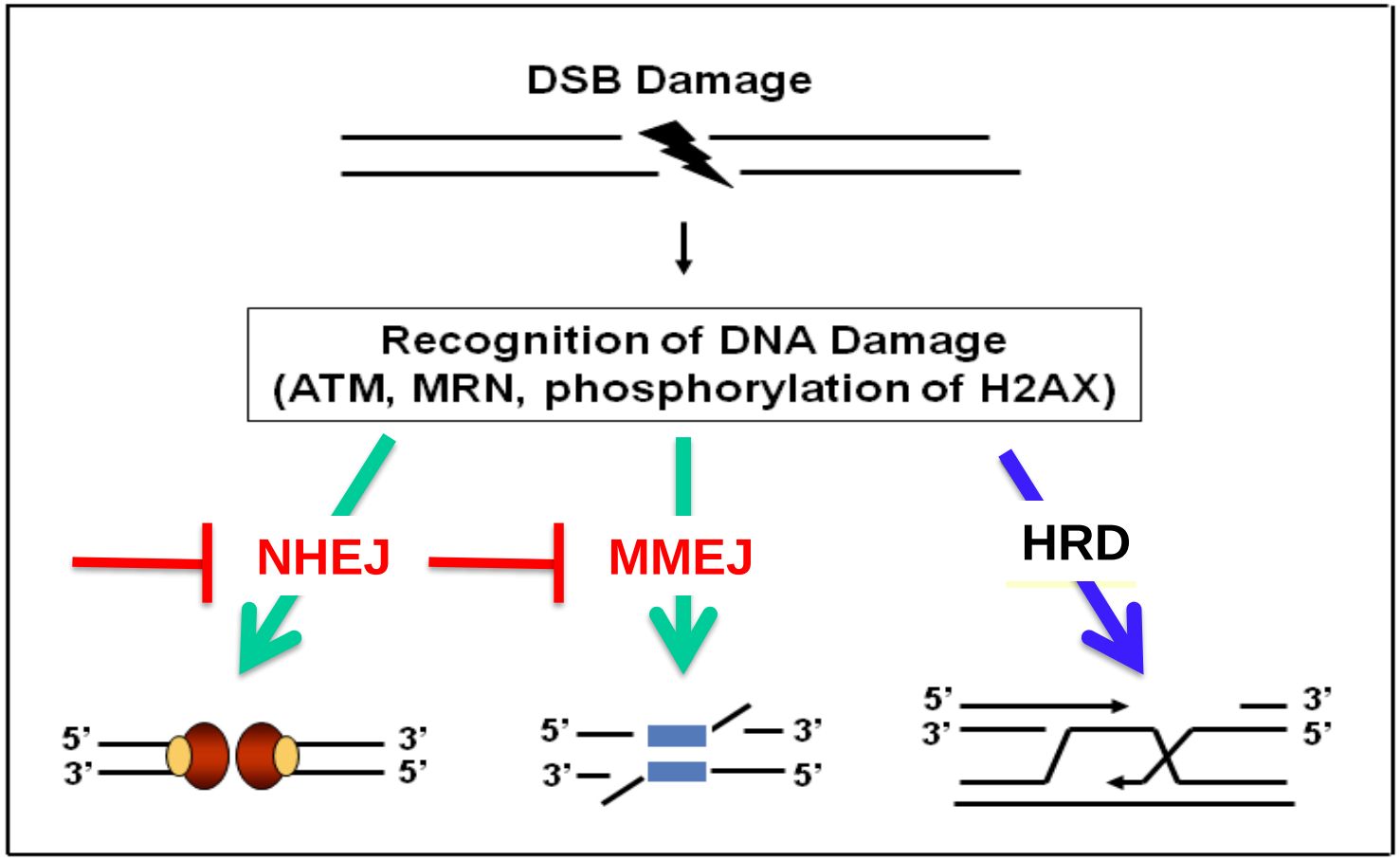


HOMOLOGOUS RECOMBINATION PATHWAY
(error free)

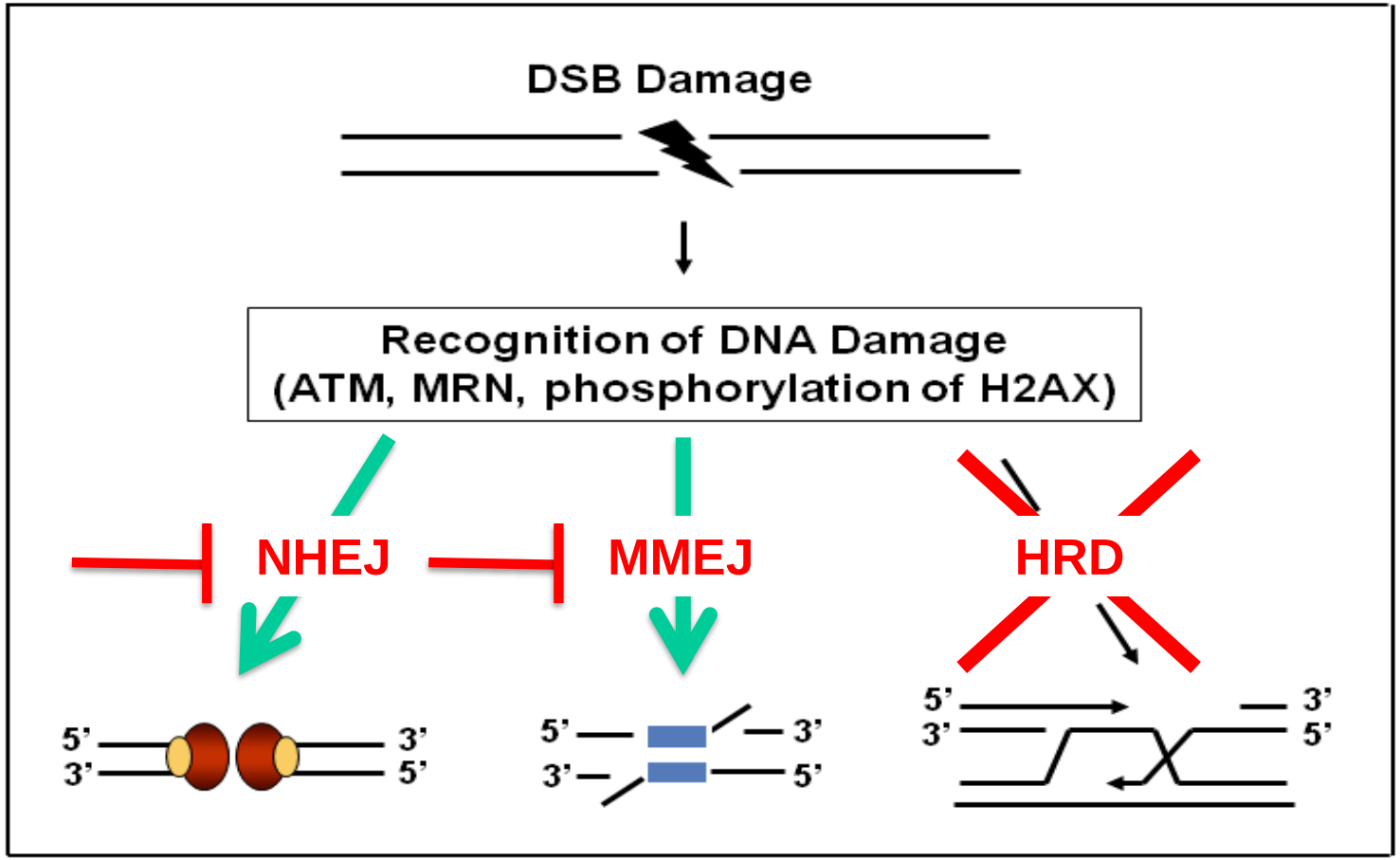
Synthetic letality



Synthetic letality



Synthetic letality



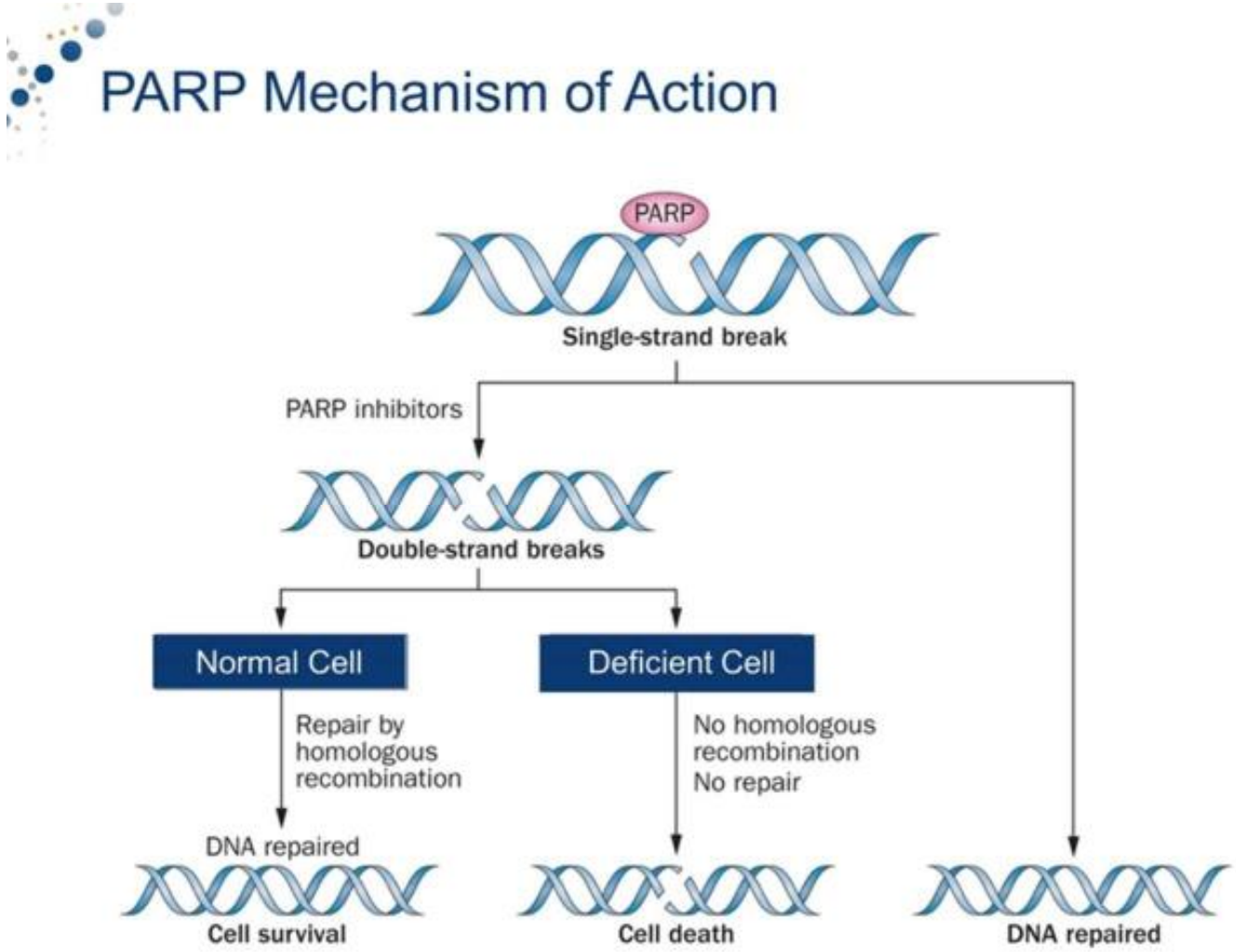
Why it is important to identify BRCAness/HRD ?

PREDICTIF FACTOR (Biomarker for targeted therapy / Synthetic Lethality)

Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase.
Bryant et al. Nature. 2005;434:913-7.
4286 citations

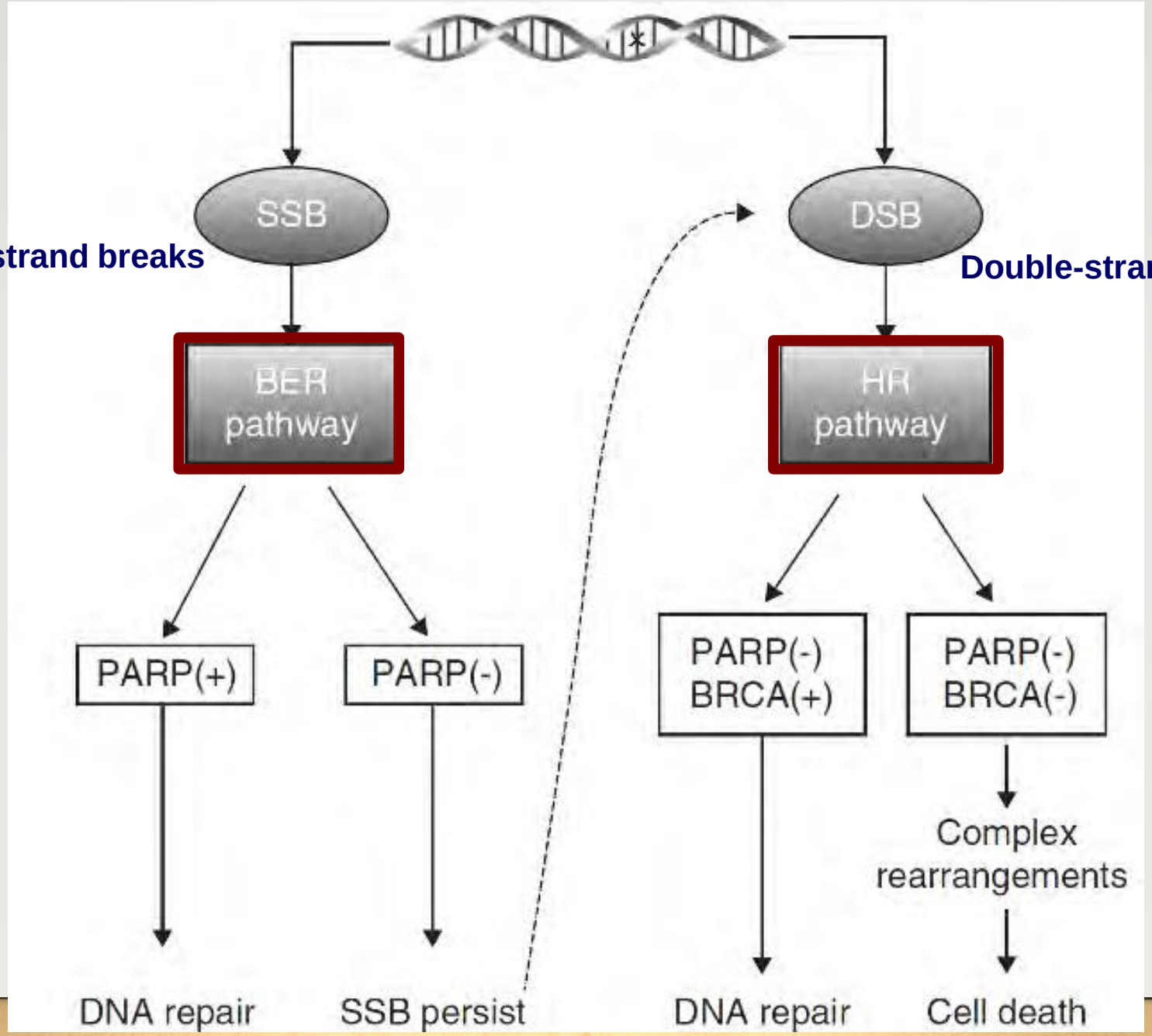
Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy.
Farmer et al. Nature. 2005;434:917-21
4323 citations

Homologous Recombination Deficiency: Therapeutic Relevance



Single-strand breaks

Double-strand breaks



Rodon et al, Expert opinion 2009

HRR Defect (HRRd) Biomarker?

- **PROGNOSTIC BIOMARKER ?**
  - Prediction of survival (PFS/OS/Risk) but not of a treatment
- **PREDICTIVE BIOMARKER ?**
  - Prediction of sensibility/resistance to a treatment
- **BOTH**
  - Predictive Risk of Hereditary Cancer
  - Predictive for PARPi therapy

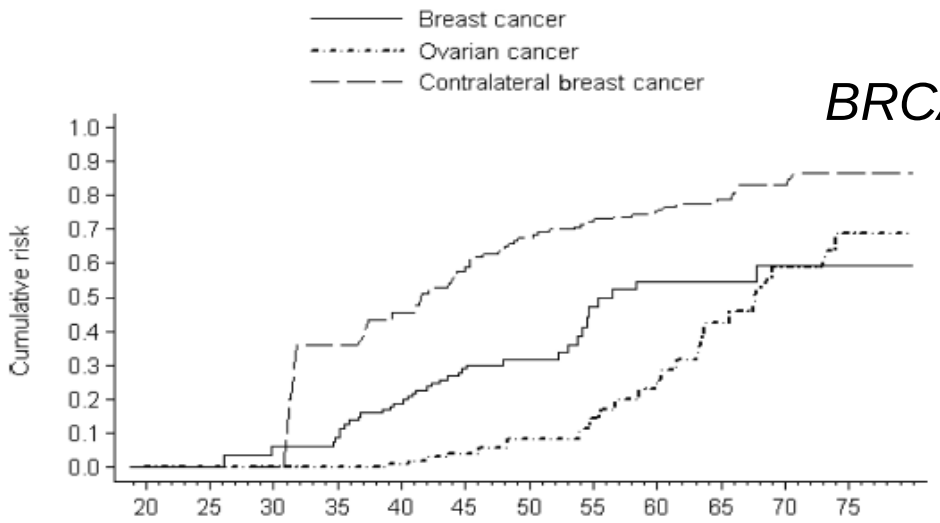
HRR- D Type cancers

- **BREAST**
- **OVARIAN**
- **PROSTATE**
- **PANCREAS**
- **Other cancers have HRR-D**

EMBRACE

PROGNOSTIC FACTOR /HEREDITARY OF CANCER

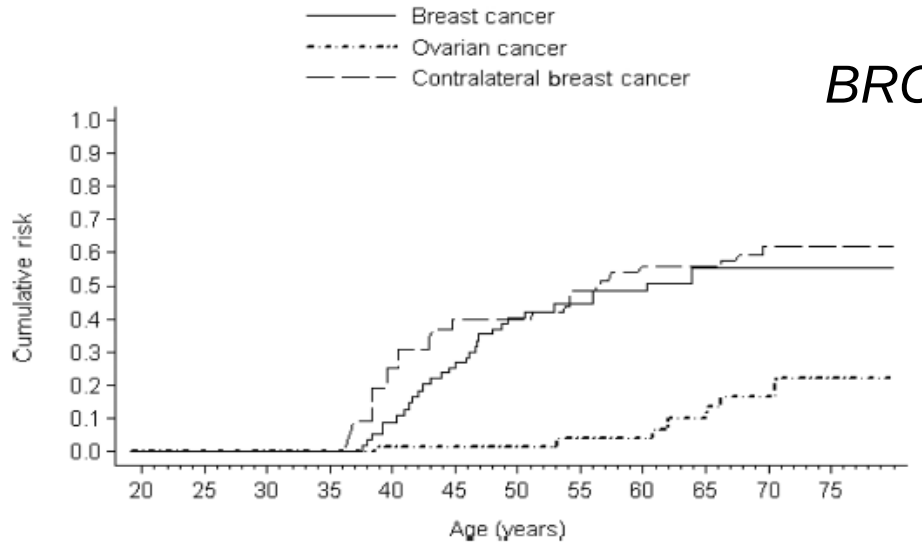
A



BRCA1

Cumulated risk at 70y
Breast K : 60%
Breast controlateral K : 83%
Ovarian K : 59%

B



BRCA2

Cumulated risk at 70y
Breast K : 55%
Breast controlateral K : 62%
Ovarian K : 16%

Mavaddat et al. JNCI, 2013

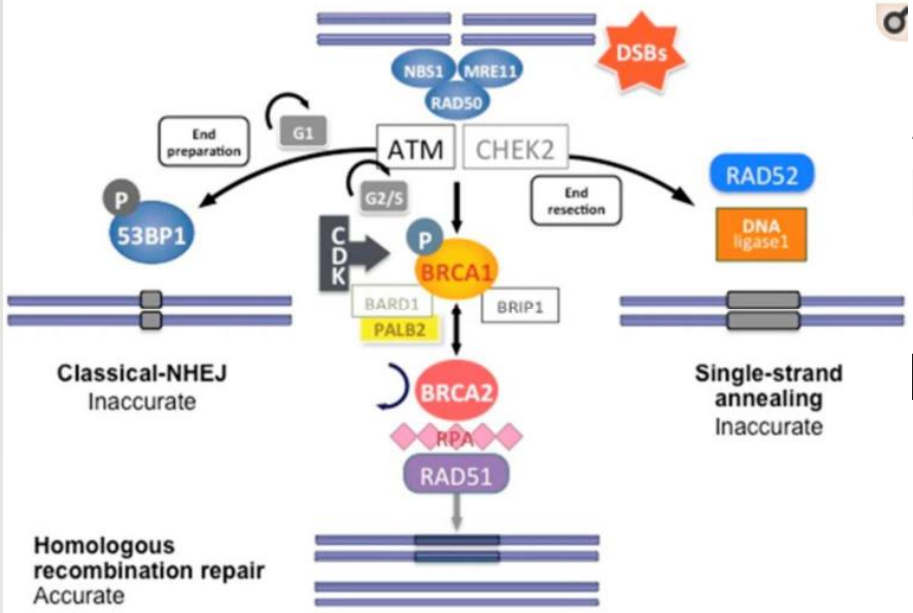
Inherited Gynecologic Tumors: Hereditary Breast and Ovarian Cancer Syndrome

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

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

HRR-D BREAST CANCER

BRCA1/2 are tumor suppressor genes



BRCA1/2 proteins involved in DNA ds break repair

Mutations in these genes cause

- 75% to 80% of hereditary breast cancer
- 5% to 10% of all breast cancer

[Dos Santos et al. Int J Mol Sci. 2020 Jun; 21(11): 3850.]

HRR-D BREAST CANCER

Predicting Response to PARP Inhibition

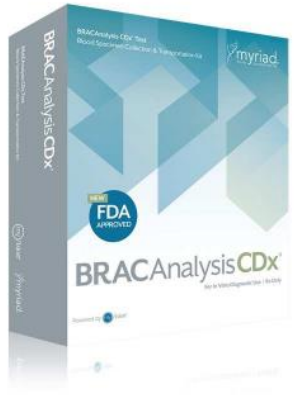
In January 2018, FDA approved olaparib (PARP inhibitor) for the treatment of patients with **germline** *BRCA*-mutated (g*BRCA*m), *HER2* negative metastatic BC

-failed prior chemotherapy

FDA approved Companion Dx (Myriad)

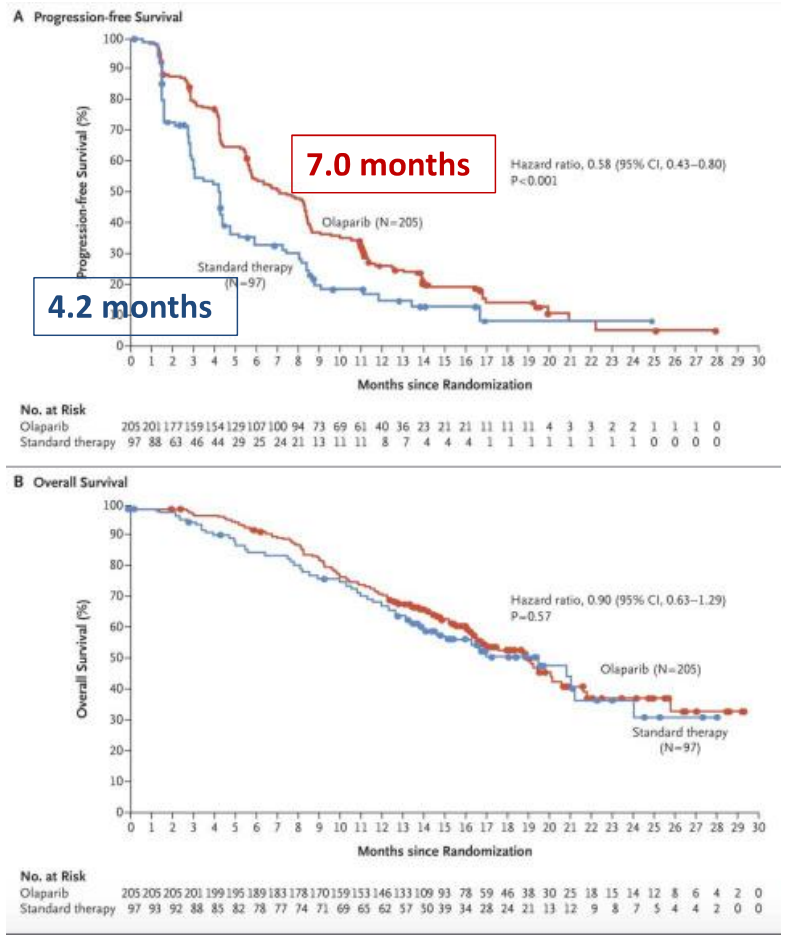
--Single-nucleotide variants and small insertions and deletions Identified by PCR and Sanger sequencing

--Large deletions and duplications detected using multiplex PCR



www.myriad.com

HRR-D BREAST CANCER



Robson et al, N Engl J Med 2017; 377:523-533

OlympiAD Trial

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation

Mark Robson, M.D., Seock-Ah Im, M.D., Ph.D., Elżbieta Senkus, M.D., Ph.D., Binghe Xu, M.D., Ph.D., Susan M. Domchek, M.D., Norikazu Masuda, M.D., Ph.D., Suzette Delaloge, M.D., Wei Li, M.D., Nadine Tung, M.D., Anne Armstrong, M.D., Ph.D., Wenting Wu, Ph.D., Carsten Goessl, M.D., et al.

Phase III trial that randomized 302 pts with **g*BRCA*m**, *HER2*- met BC to olaparib vs physician's choice of chemotx

median PFS was significantly longer with olaparib monotherapy than with standard chemotherapy (7.0 vs. 4.2 months)

HRR-D BREAST CANCER

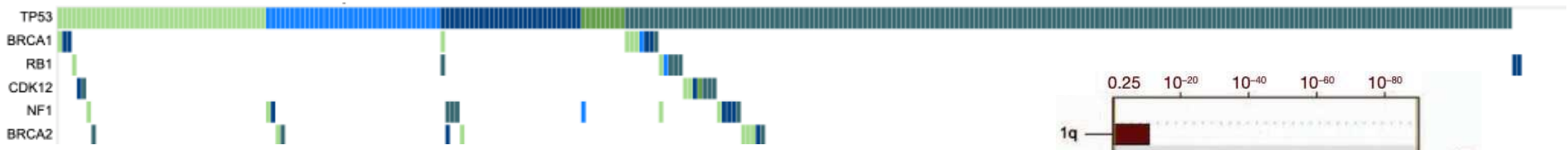
Interesting Question
What about patients with somatic mutations?

- Does a somatic mutation in *BRCA1/2* also predict response to PARP inhibition?
  - Prior studies in Ovarian Cancer showed response to PARPi in both germline and somatic *BRCA*-mutated cancers
- somatic *BRCA*1/2 mutations are present in ~3% of breast cancers
- Recent phase II study has shown that PARP inhibition is an effective treatment for patients with metastatic BC and **somatic** *BRCA1/2* mutations

Tung et al. TBCRC 048. J Clin Oncol 38: 4274-82, 2020.

OVAIRAN CARCINOMA

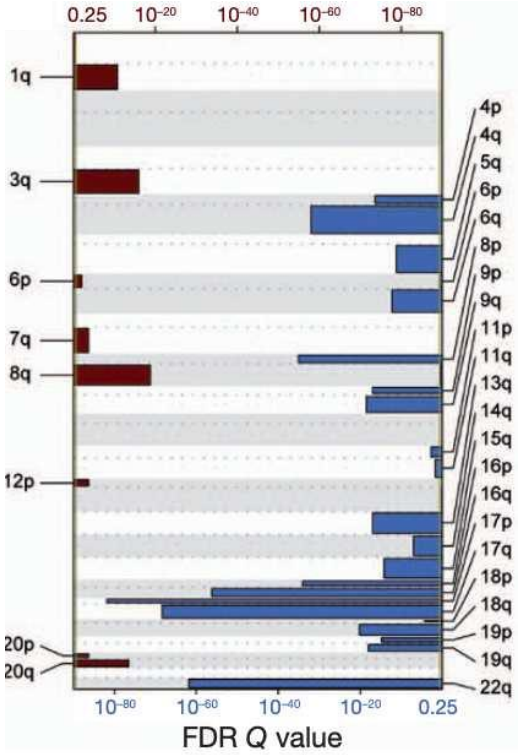
High Grade Serous Ovarian Carcinoma



Significantly mutated genes in HGS-OvCa

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

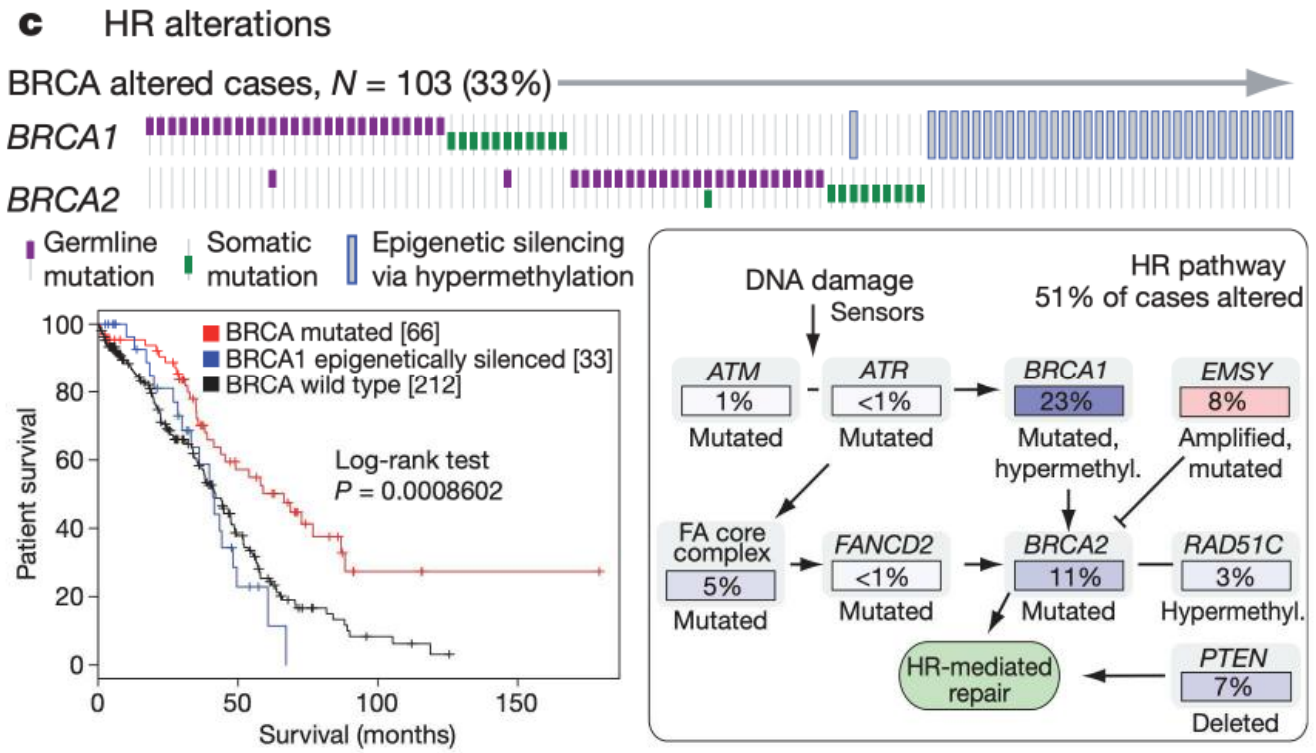
Numerous copy number alterations



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

OVAIRAN CARCINOMA

High Grade Serous Ovarian Carcinoma: Homologous Recombination Deficiency

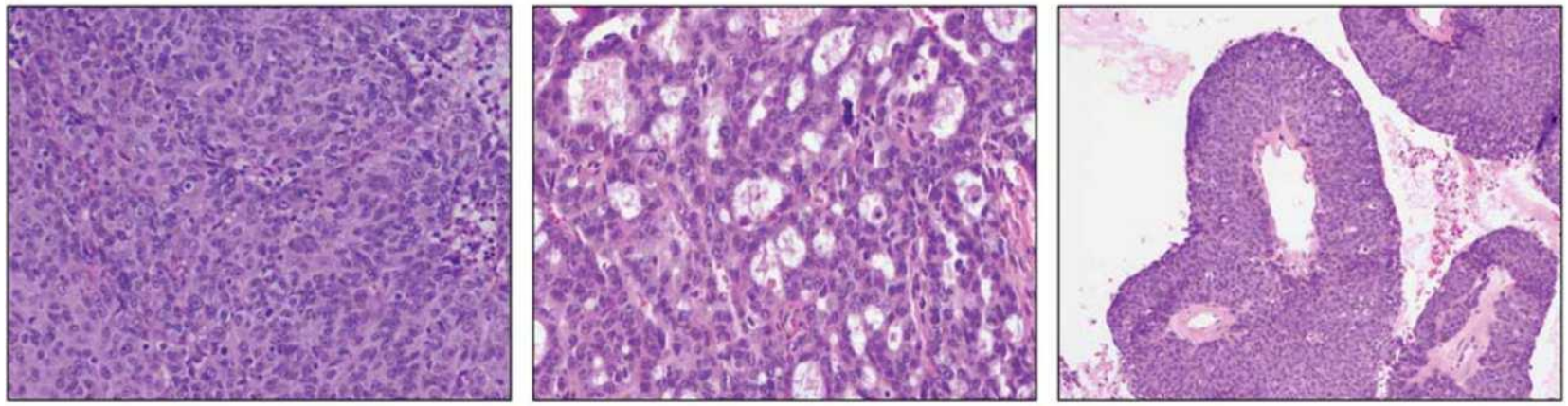


Nature. 2011 Jun 29;474(7353):609-15.

OVAIRAN CARCINOMA

High Grade Serous Ovarian Carcinoma: Molecular / Morphologic Correlation

SET morphology



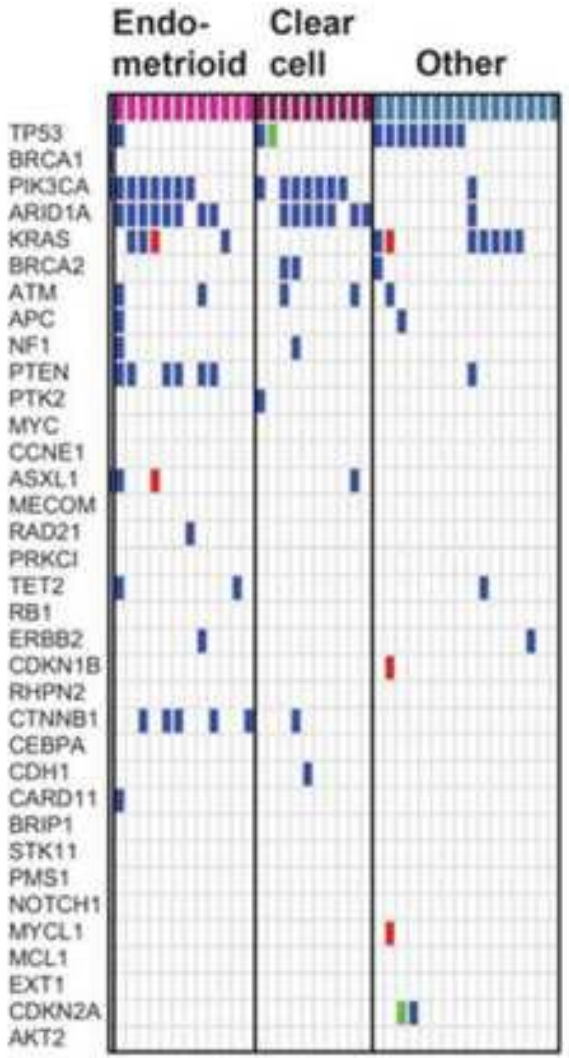
Solid | Pseudo-Endometrioid | Transitional

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

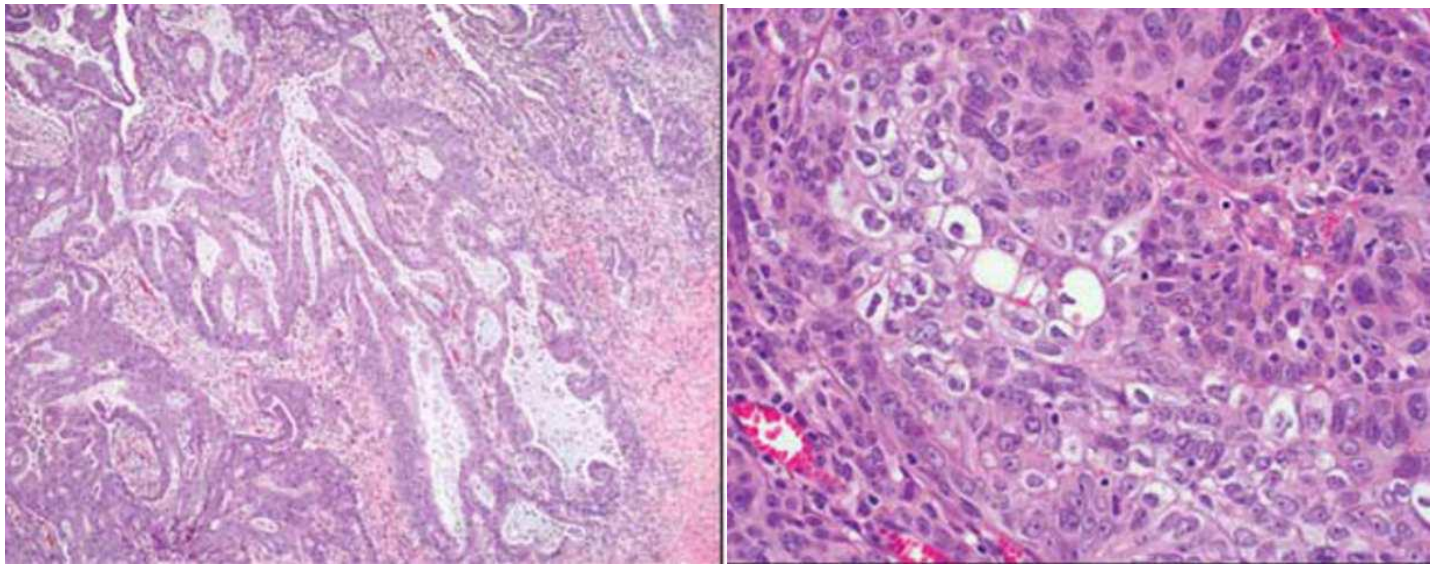
Mod Pathol. 2016 Aug;29(8):893-903.

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

OVAIRAN CARCINOMA



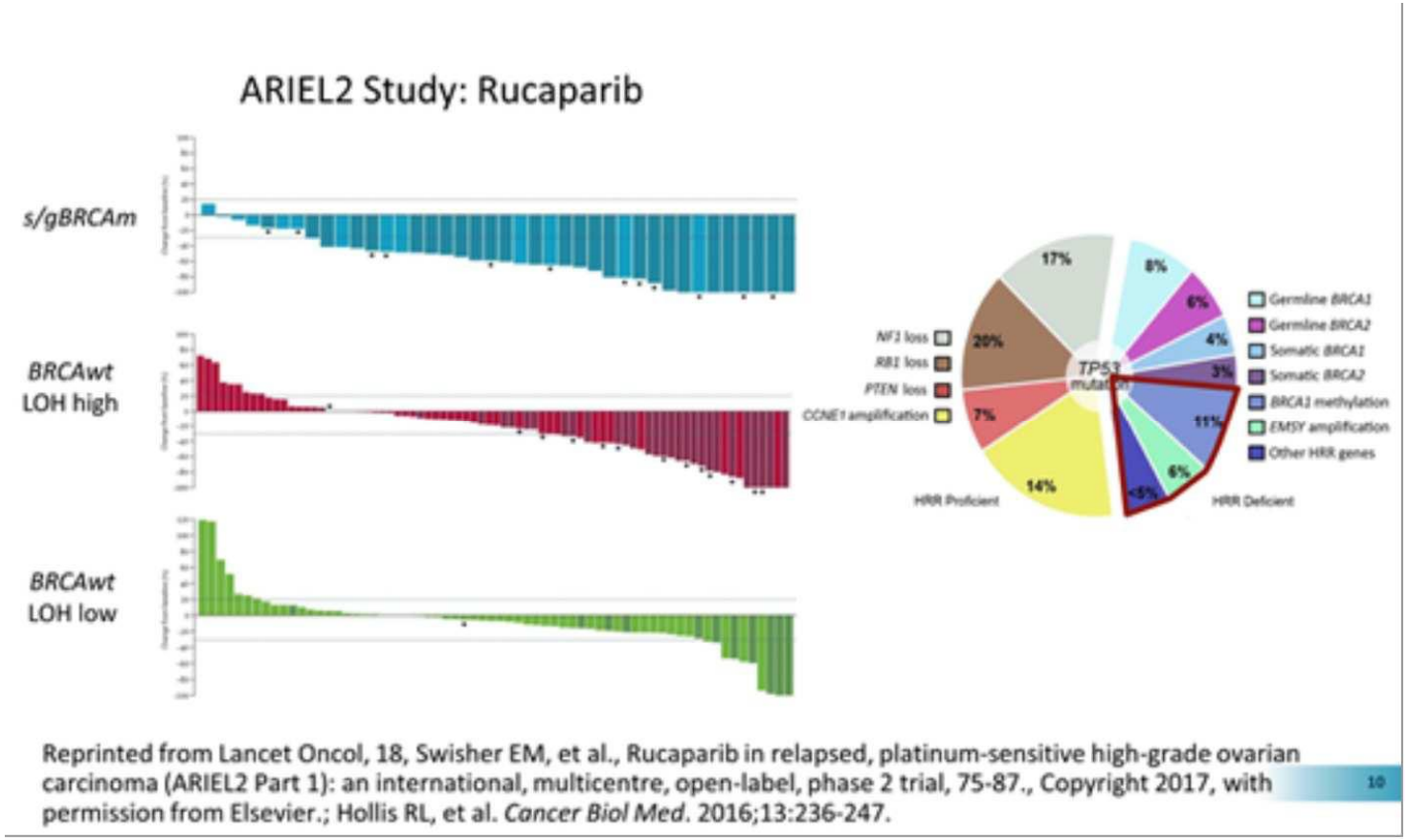
Non-serous Ovarian Carcinomas



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

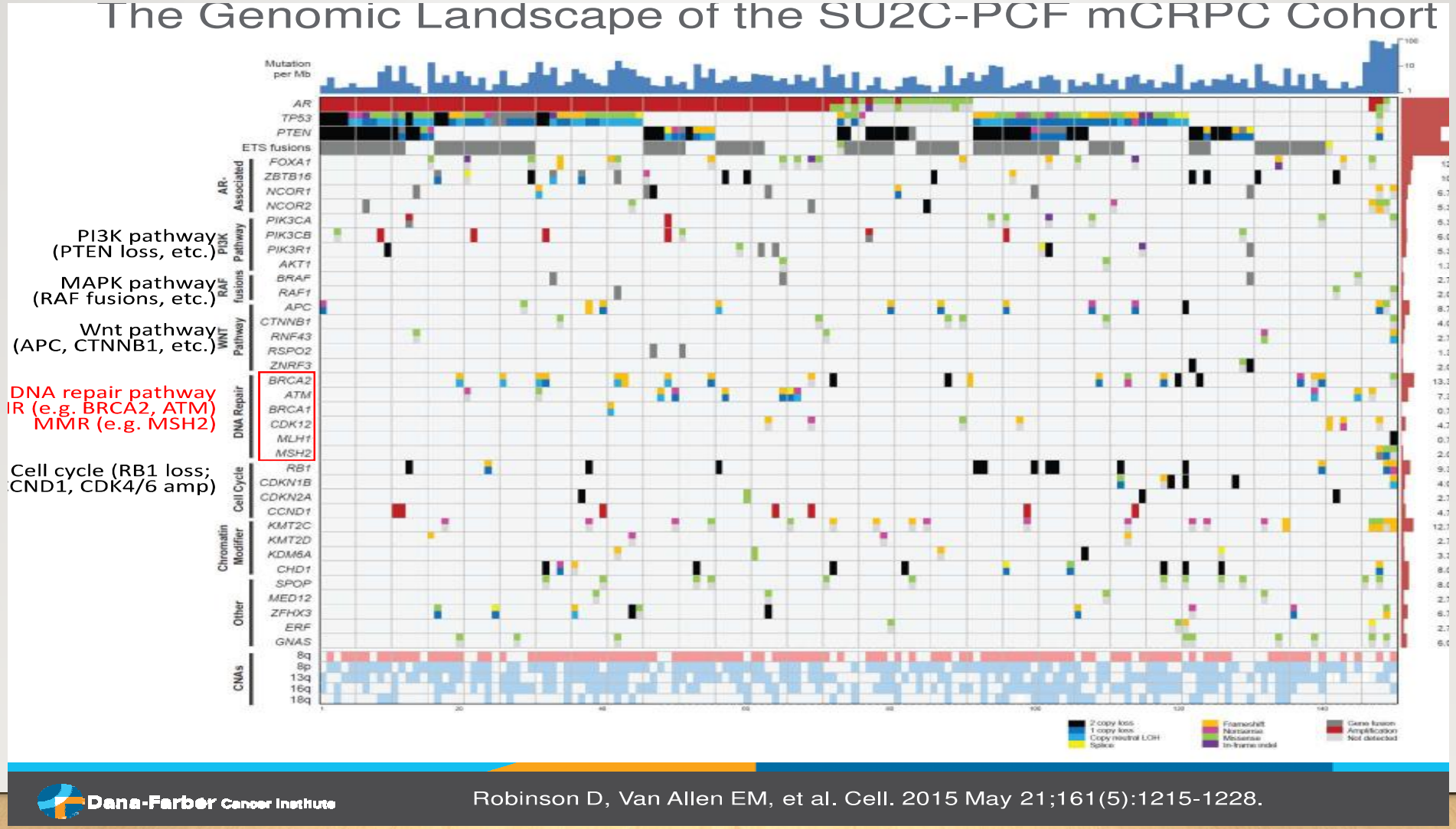
OVAIRAN CARCINOMA

Homologous Recombination Deficiency: Predict Response to PARP Inhibitors



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

PROSTATE CARCINOMA

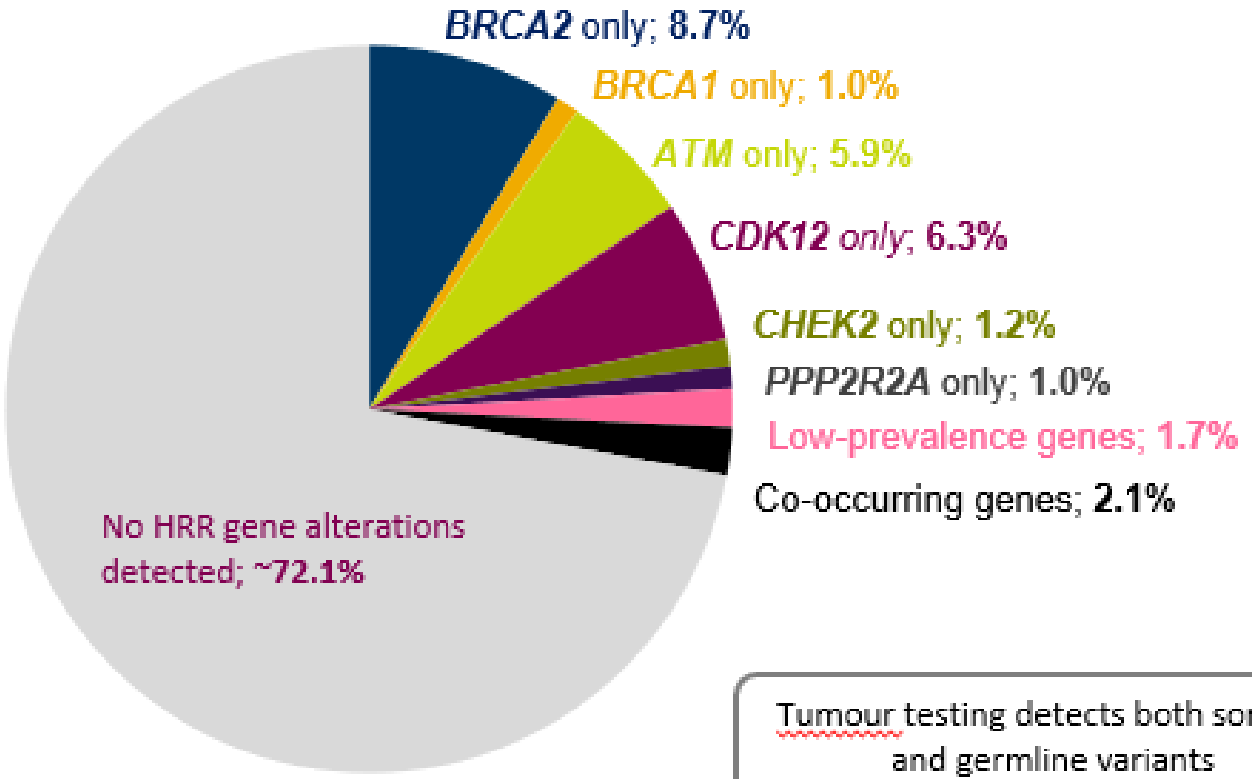


Dana-Farber Cancer Institute

Robinson D, Van Allen EM, et al. Cell. 2015 May 21;161(5):1215-1228.

BRCA2 is the most common HRRm in prostate cancer and ~50% are derived from germline origin[1–6]

Prevalence of tumour HRRm identified from screened mCRPC population in PROfound (N=2,792 patients successfully sequenced)[2]



Tumour testing detects both somatic and germline variants

Prevalence of germline HRRm in patients with early or advanced prostate cancer

	Prevalence range
BRCA2[3,4]	3.5–5.3%
BRCA1[3,5]	0.9–1.3%
ATM[4,6]	0.3–2.0%
PALB2[3,5,6]	0.4–0.6%

Figure adapted from de Bono J et al. *Ann Oncol.* 2019[2]

HRR=homologous recombination repair; HRRm=homologous recombination repair mutation; mCRPC=metastatic castration-resistant prostate cancer.

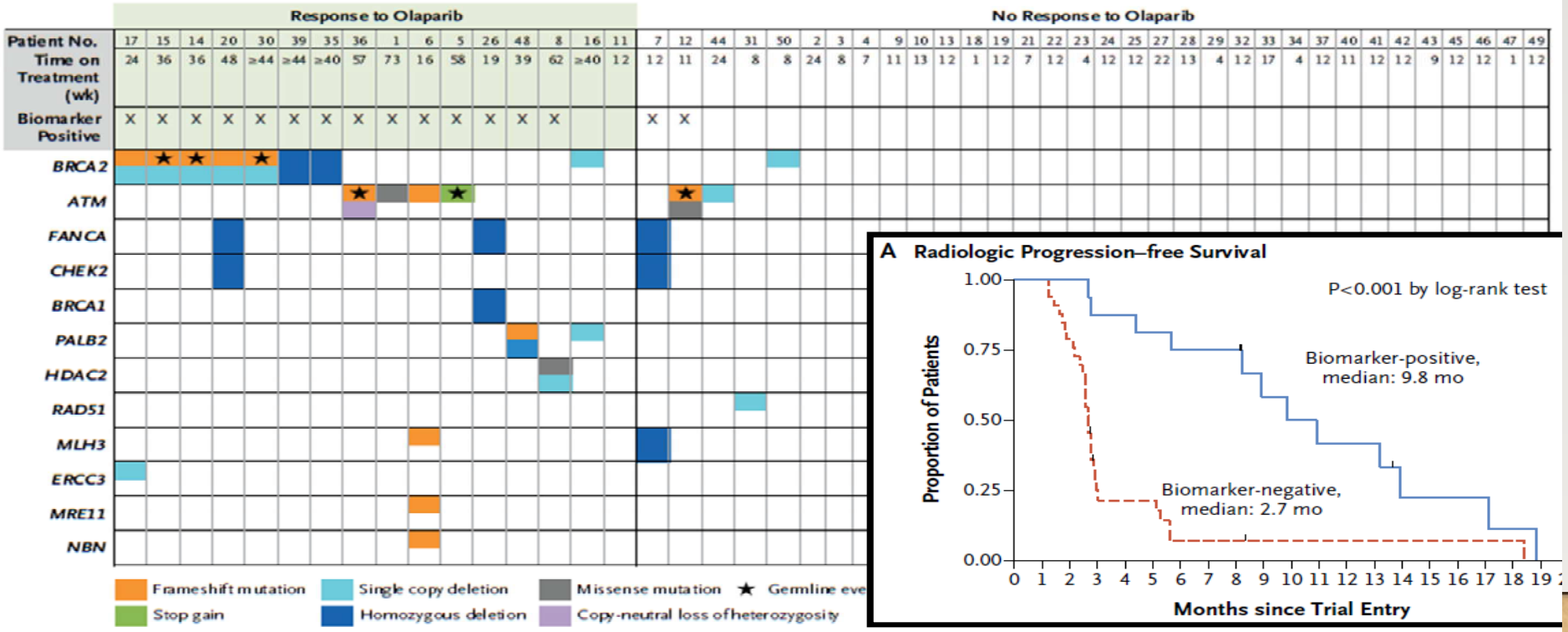
1. Lang SH, et al. Int J Oncol. 2019;55(3):597–616. 2. de Bono J, et al. Ann Oncol. 2019;30(suppl_5):v325-v355 (abst 847PD) associated poster; 3. Pritchard CC, et al. N Engl J Med. 2016;375:433–453; 4. Na R, et al. Eur Urol. 2017;71:740–747; 5. Nicolosi P, et al. JAMA Oncol. 2019;5:523-528; 6. Annala M, et al. Eur Urol. 2017;72:34–42

PROSTATE CARCINOMA

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 29, 2015 VOL. 373 NO. 18

DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer



Mateo J, et al. N Engl J Med. 2015 Oct 29;373(18):1697-708.

PROSTATE CARCINOMA

PROfound – Study Design

Key eligibility criteria
- mCRPC with disease progression on prior NHA, eg abiraterone or enzalutamide
- Alterations in ≥1 of any qualifying gene with a direct or indirect role in HRR*

Cohort A: *BRCA1, BRCA2 or ATM* N=245
- Olaparib 300 mg bid n=162
- Physician's choice‡ n=83

Cohort B: Other alterations N=142
- Olaparib 300 mg bid n=94
- Physician's choice‡ n=48

2:1 randomization
Open-label

Upon BICR progression, physician's choice patients were allowed to cross over to olaparib

Stratification factors
- Previous taxane
- Measurable disease

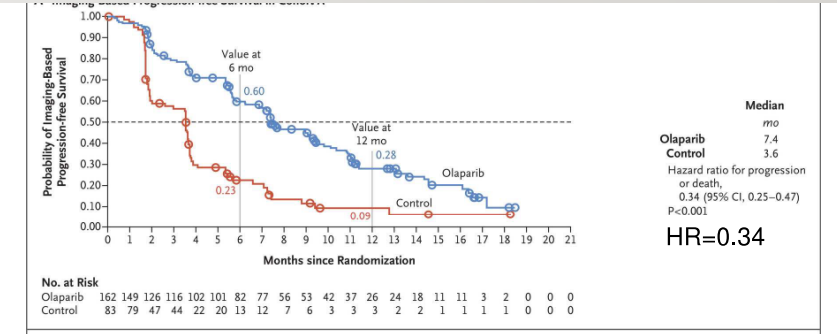
Primary Endpoint

Radiographic progression-free survival (rPFS) in Cohort A (RECIST 1.1 & PCWG3 by BICR)

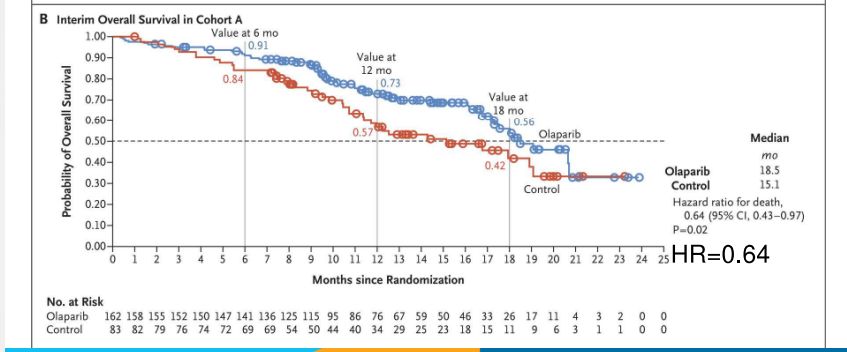
Key Secondary Endpoints
- rPFS in Cohorts A+B
- Confirmed radiographic objective response rate (ORR) in Cohort A
- Time to pain progression (TTPP) in Cohort A
- Overall survival (OS) in Cohort A

*An investigational Clinical Trial Assay, based on the FoundationOne® CDx next-generation sequencing test Developed in partnership with Foundation Medicine Inc, and used to prospectively select patients harboring alterations in *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D* and/ or *RAD54L* in their tumor tissue

‡Physician's choice of either enzalutamide (160 mg qd) or abiraterone (1000 mg qd + prednisone [5 mg bid])
BICR, blinded independent central review

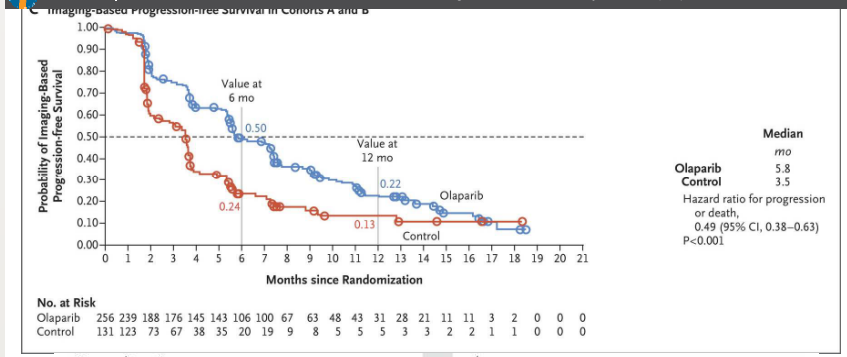


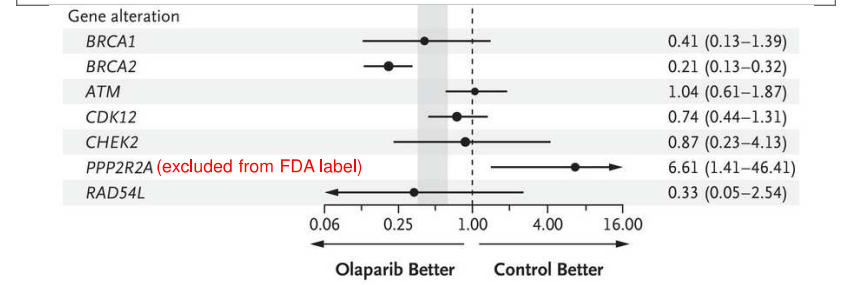
HR=0.34



HR=0.64

Dana-Farber Cancer Institute de Bono J, et al. N Engl J Med. 2020 May 28;382(22):2091-2102.





PROSTATE CARCINOMA

TRITON2: A Phase 2 Study of Rucaparib in Patients with Metastatic Castration-Resistant Prostate Cancer (mCRPC) Associated with Homologous Recombination Repair (HRR) Gene Alterations

Figure 5. Best Change from Baseline in PSA (n=84)

ESMO 2018

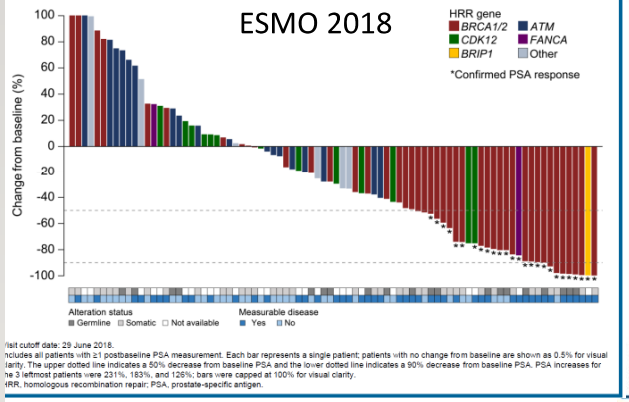


Figure 4. Best Change from Baseline in PSA in Rucaparib-Treated Patients with a *BRCA1/2* Alteration (n=96)

ESMO 2019

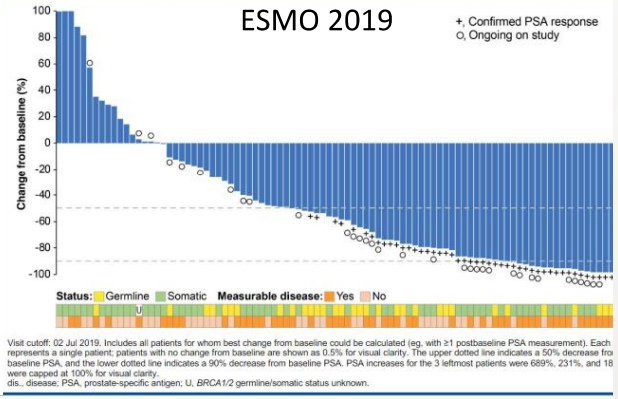
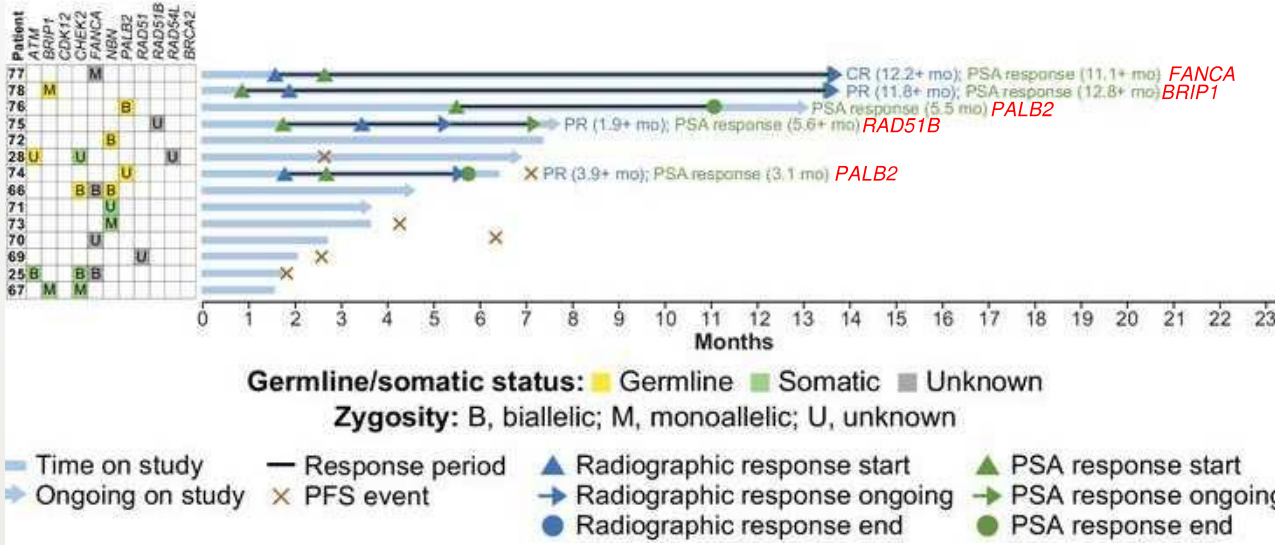


Table 4. Clinical Benefit Rates in Rucaparib-Treated Patients[a]

	DDR gene				
	BRCA1/2	*ATM*	*CDK12*	*CHEK2*	Other
6 mo, n/N (%) [95% CI]	47/84 (56.0) [44.7–66.8]	14/48 (29.2) [17.0–44.1]	3/14 (21.4) [4.7–50.8]	2/3 (66.7) [9.4–99.2]	6/12 (50.0) [21.1–78.9]
12 mo, n/N (%) [95% CI]	13/53 (24.5) [13.8–38.3]	2/25 (8.0) [1.0–26.0]	1/14 (7.1) [0.2–33.9]	0/1 (0) [0.0–97.5]	3/9 (33.3) [7.5–70.1]

Visit cutoff: 02 Jul 2019.
[a]Clinical benefit rate was the proportion of patients without radiographic progression per modified RECIST/PCWG3 criteria (per investigator assessment) who were ongoing with treatment through the indicated time interval.
CI, confidence interval; DDR, DNA damage repair; PCWG3, Prostate Cancer Clinical Trials Working Group 3; RECIST, Response Evaluation Criteria In Solid Tumors version 1.1.

Non-BRCA DNA Damage Repair Gene Alterations and Response to the Rucaparib: Analysis From the Phase II TRITON2 Study



Abida W, et al. Clin Cancer Res. 2020 Jun 1;26(11):2487-2496.

PROSTATE CARCINOMA

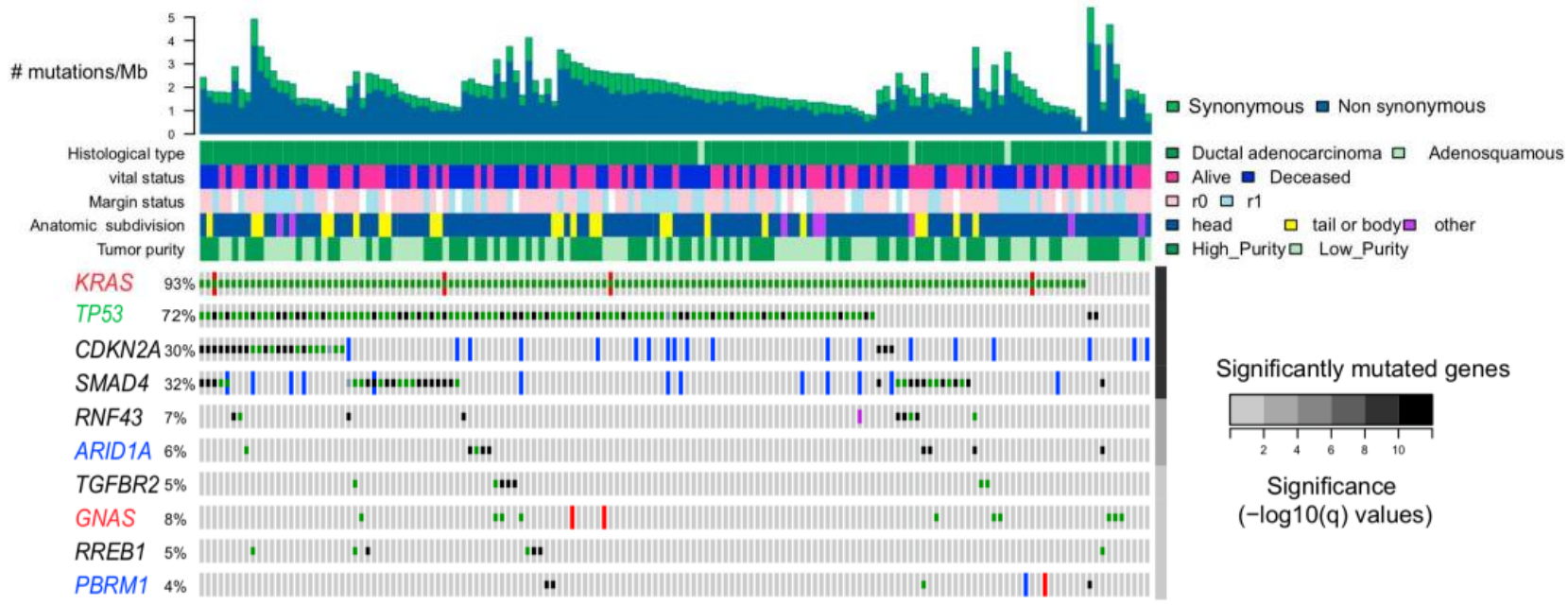
PARP inhibitors - Conclusions

- Approved by the US Food and Drug Administration in May 2020
  - Olaparib - mCRPC patients (**post AR-directed therapy**) with deleterious or suspected deleterious germline or somatic **homologous recombination repair gene mutation**
  - Rucaparib - mCRPC patients (**post AR-directed therapy AND taxane**) with deleterious germline or somatic **BRCA gene mutation**

- Clear benefit to patients with mutations in *BRCA2* (and likely *BRCA1*)
- Unclear benefit in patients with mutations in *ATM*, *CHEK2*, *CDK12*
- ? Benefit in patients with mutations in genes canonically involved in HR (*PALB2*, *FANCA*, *RAD51C/D, RAD52, RAD54L*)

PANCREAS CARCINOMA

Pancreatic adenocarcinoma landscape

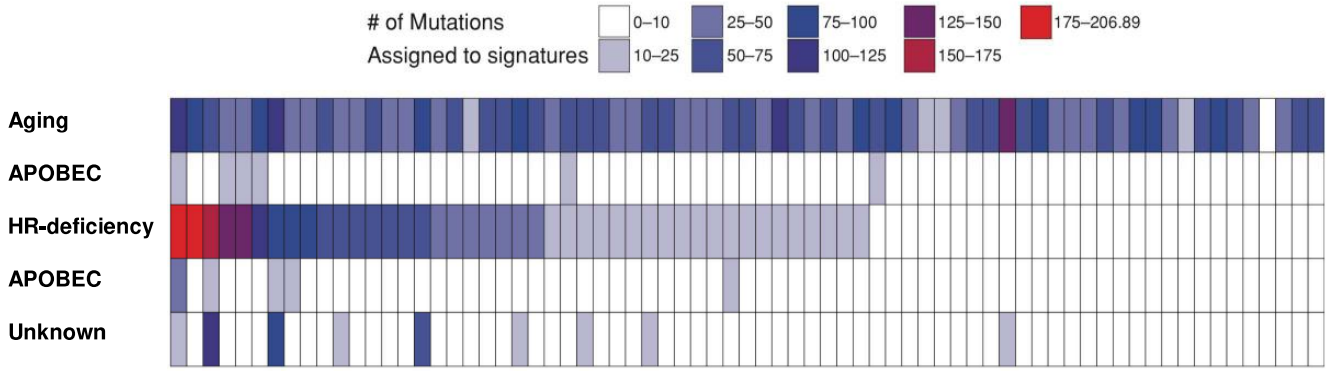


TCGA. 2017, Cancer Cell 32, 185–203.

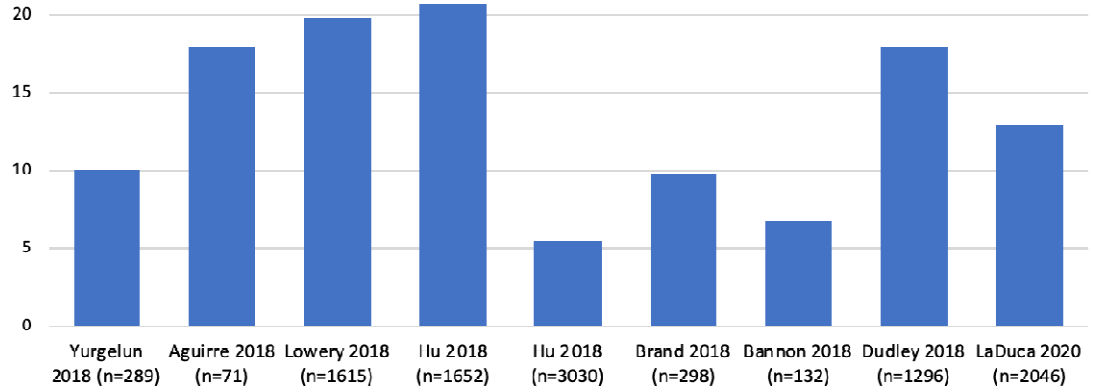
PANCREAS CARCINOMA

Homologous recombination deficient and germline mutations in PDAC

HR deficiency signature in metastatic PDAC



Rate of germline path/likely path alterations in PDAC patients



Germline BRCA1/2 mutant PDAC

2019

Olaparib approved for germline BRCA1/2 mutant metastatic PDAC that has not progressed on first-line platinum-based chemotherapy (POLO trial)

NCCN recommends germline testing for all pancreatic cancer patients.

Aguirre AJ, et al. Cancer Discov. 2018;8(9):1096-1111. Golan T, et al. N Engl J Med. 2019;381:317-27.

CONCLUSION HRR-D Biomarkers TODAY

Prognostic Biomarker / Risk Hereditary Cancer

- Germline BRCA1/2
- High Risk patients: Breast /Ovarian / Prostate/ Pancreas

Predictive Biomarker (Reimbursement for PARPi)

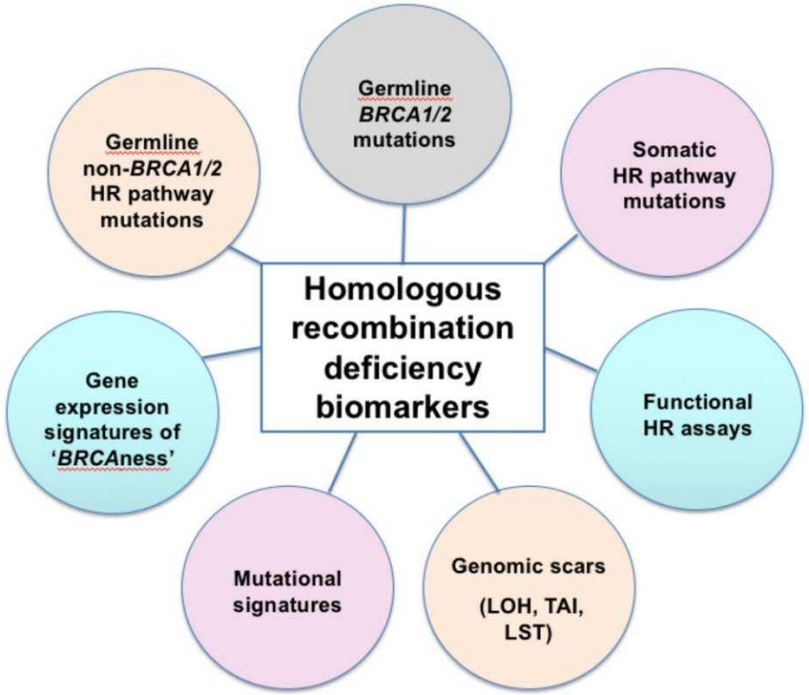
- Somatic/Germline BRCA1/2
  - Metastatic Castration Resistant Prostatic Cancer
- Germline BRCA1/2
  - Metastatic Breast Cancer (HER2 negative)
  - Metastatic Ovarian Cancer (High grade)
  - Metastatic Pancreas (Castration Resistant)

ANOMALIES DETECTIONS (DDR Biomarker)

DNA	RNA	PROTEINE
Amplifications	RNA quantity	Protein Quantity
Translocations	Alternative Transcripts	Protein Activity
Mutations		
CGH	RT-PCR	Wester-Blot
FISH	Transcriptional chip	Immunohistochemistry
DNA seq	RNA seq	Enzymatic Activity

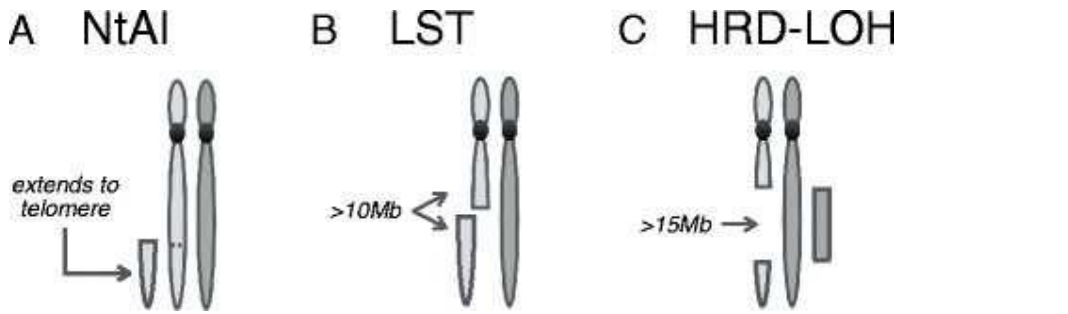
HRR –D Biomarker TEST

Homologous Recombination Deficiency : Biomarkers



HRR –D Biomarker TEST

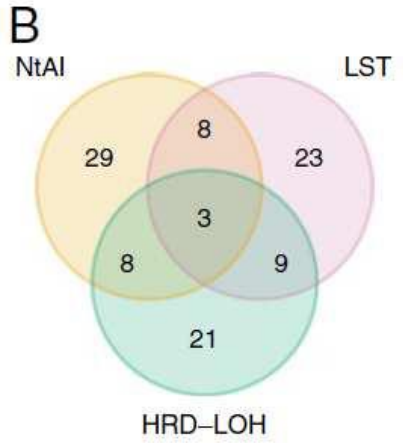
Homologous Recombination Deficiency Scores



Number of Telomeric Allelic Imbalances

Large Scale Transition

HRD-Loss of Heterozygosity



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

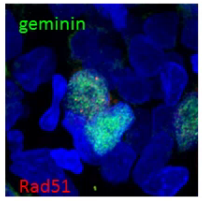
Marquard 2015 et al.

HRR –D Biomarker TEST

RAD51predict: Multiplexed IF functional test for HRD

Elisa Yaniz
Felix Blanc-Durand
Etienne Rouleau

« RAD51predict »

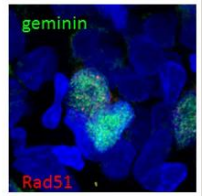


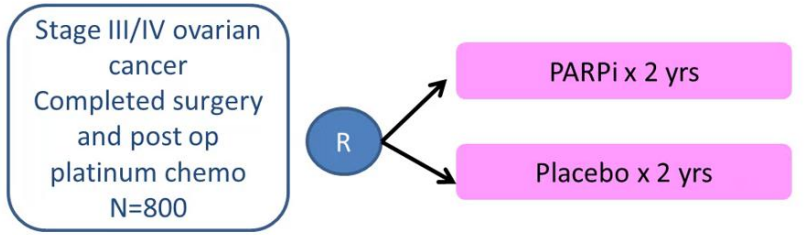
- Triple multiplexed IF (gH2ax/Gem/RAD51)
- Identify tumors with DSB, cells in S phase incapable of recruiting RAD51 (HRD)
- Evaluated in clinical trial of neoadjuvant platinum (N=150pts)
- Correlated to
  - platinum response (surrogate for HRD)
  - mutations in *BRCA* and other HR genes
- Results will be presented ASCO 2021

GUSTAVE ROUSSY CANCER CAMPUS GRAND PARIS

Next step (ongoing), Evaluation of « *RAD51predict* » in phase III randomized PAOLA trial

« RAD51predict »





- Validate in 600 tumors in RP3T PAOLA ola vs placebo
- Correlate to Myriad HRD test
- Evaluate predictive value for PARPi benefit

Elisa Yaniz

HRR –D Biomarker TEST
Gold standard NGS BRAC anomalies

Challenges in *BRCA* mutation testing

Strong family history
Known high-risk

Genetic counseling
Consent
Blood sample
Germline testing

Variant classification

Pathogenic
Likely pathogenic

VUS

Benign
Likely benign

NGS Testing of tumor tissue to detect targetable somatic variants

Possible germline variant is flagged

Pathogenic
Likely pathogenic

VUS

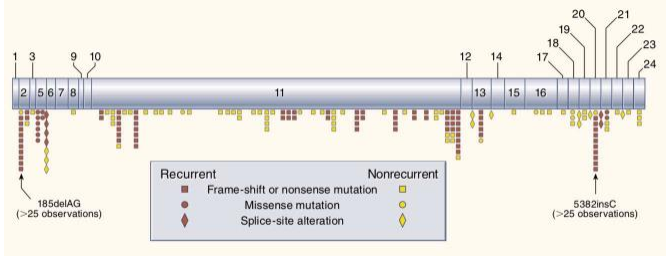
Benign
Likely benign

Many patients are tested initially because of family hx/high-risk

Others are not tested until a possible germline variant is identified by somatic mutation testing

Large number of Variants of Unknown Significance
Classifications change over time

Challenges in *BRCA* mutation testing



BRCA mutation types and technologies

3 broad classes of sequence changes:
- SNVS
- insertions/deletions
- large rearrangements

Many labs are transitioning to NGS
- advantages: increased throughput, multiplexing and lower costs
- disadvantage: lower sensitivity for large deletions/rearrangements (10%)

Collins FS. BRCA1 – Lots of Mutations, Lots of Dilemmas. NEJM 1996.

CONCLUSION DDR

- **Genetic/ Hereditary concept**
- **HRRd : Prognostic factor biomarker (ovarian , breast cancer)**
- **HRRd: Predictive factor biomarker**
  - **Synthetic lethality pathway**
  - **Sensibility PARPi (Breast, Ovarian, Prostatic, Pancreas cancer)**
  - **Resistance to chemotherapy (ovarian cancer)?**
- **Hereditary cancer biomarker: family implications/ follow up**
- **NGS: Gold standard**
  - **Challenges to solve**
  - **More techniques and evolution of NGS to detect significant HRRd-status**
- **BRCA 1/2 genes validated as predictive biomarker (PARPi) (reimbursement)**
  - **metastatic : breast, ovarian, prostate, pancreas**
- **But upcoming more DRR d –genes implicated as predictive biomarker:** ATM, CKEK 2, ……. depending on cancer subtype
- **HRR-D Test? Signatures?**