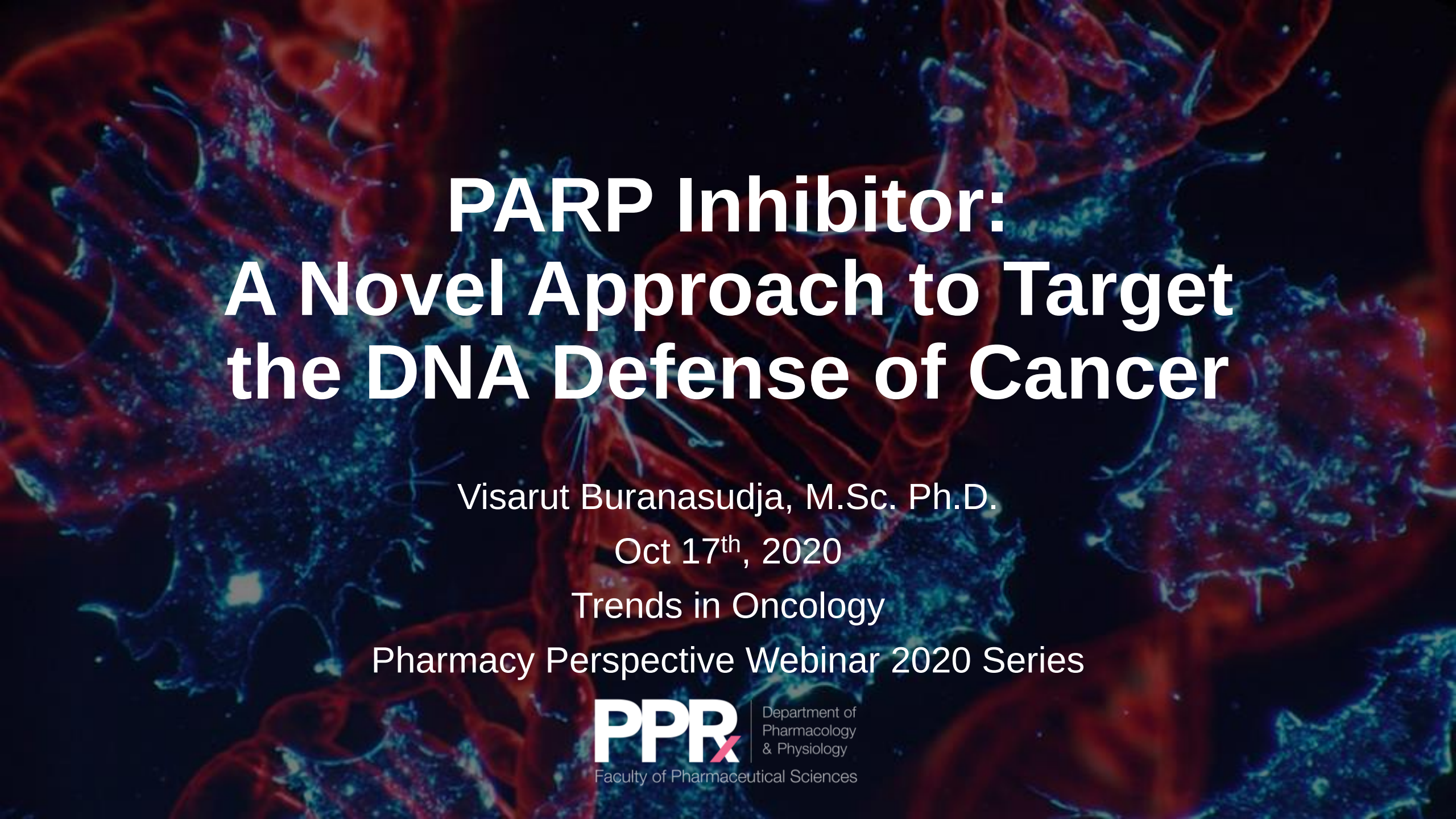


PARP Inhibitor: A Novel Approach to Target the DNA Defense of Cancer

Visarut Buranasudja, M.Sc. Ph.D.

Oct 17th, 2020

Trends in Oncology

Pharmacy Perspective Webinar 2020 Series

PPRx | Department of Pharmacology & Physiology
Faculty of Pharmaceutical Sciences

Outlines

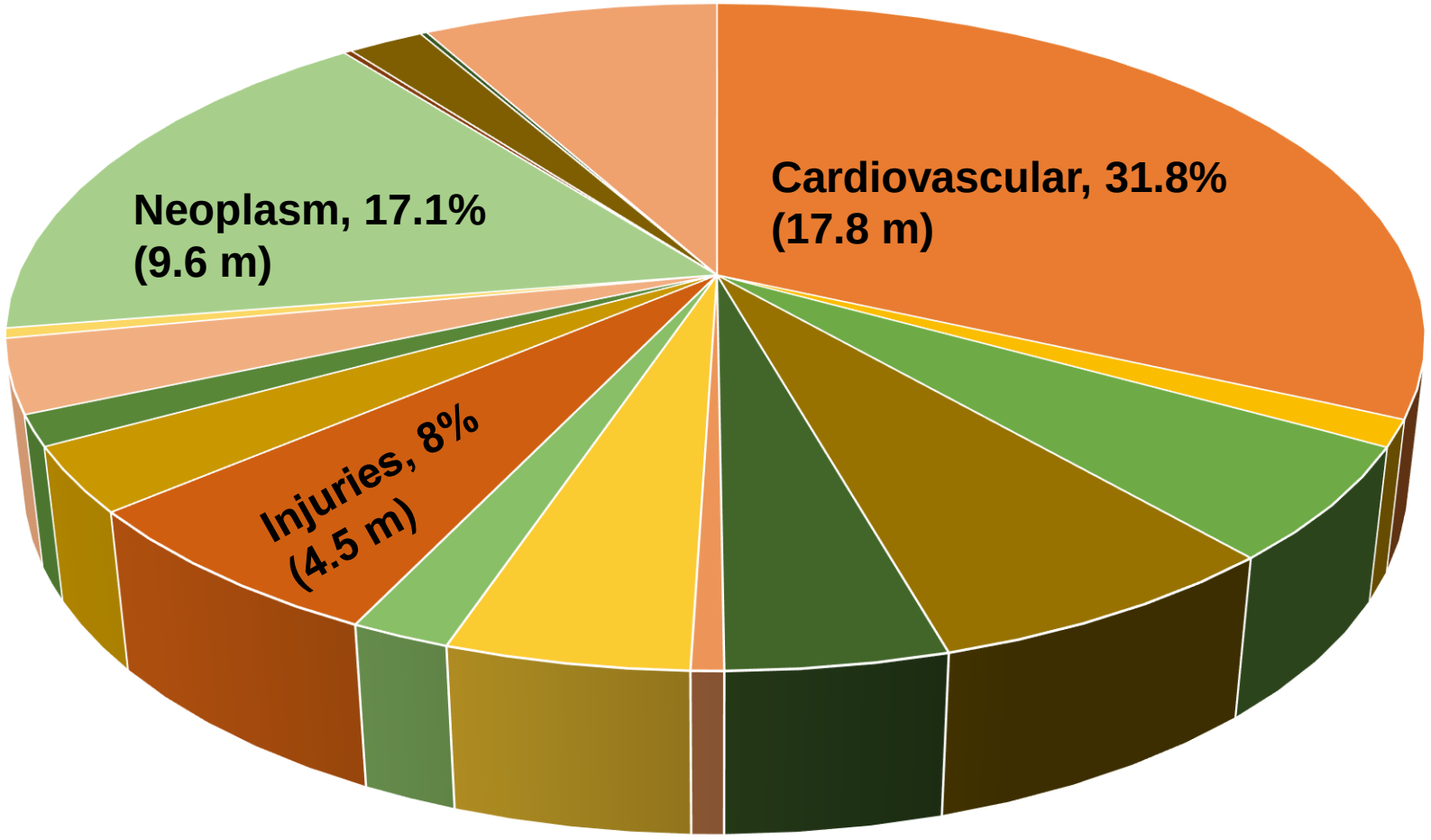
- Genome instability as a hallmark for cancer
- DNA damage responses (DDRs)
  - *BRCA* mutations in cancers
  - PARPs as a master regulator for DDRs
- PARP inhibitors (PARPi)
  - Synthetic lethality therapy
  - Approved indications of PARPi
  - Resistance to PARPi
  - Future directions

What is “Cancer” ?

Cancer is collection of related genetic-disease that results when abnormal cells divide without control and can invade into surrounding tissue.

Cancer: Global Health Crisis

Cancer: 2nd Leading Cause of Death (2017)

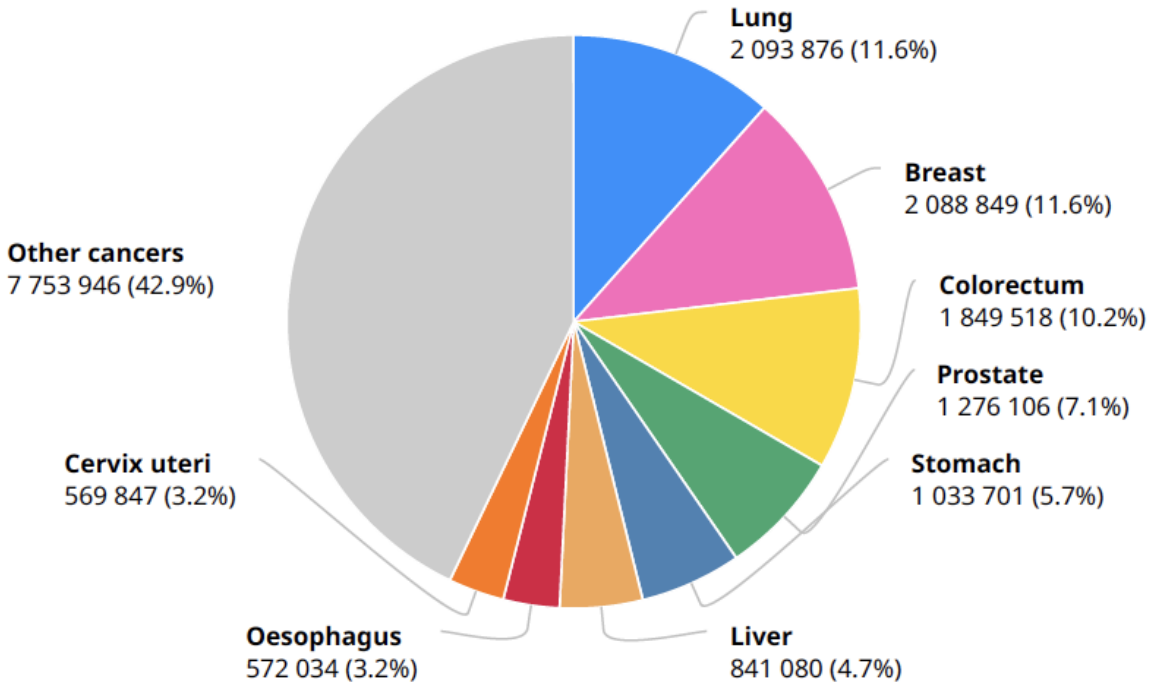


- **Cardiovascular diseases**
- Neglected tropical diseases and malaria
- Neurological disorders
- Mental disorders
- Chronic respiratory diseases
- Digestive diseases
- Substance use disorders
- Diabetes and kidney diseases
- HIV/AIDS and sexually transmitted infections
- Respiratory infections and tuberculosis
- Enteric infections
- Other infectious diseases
- Maternal and neonatal disorders
- Nutritional deficiencies
- **Neoplasms**
- Musculoskeletal disorders
- Other non-communicable diseases
- Skin and subcutaneous diseases
- **Injuries**

Data were obtained from http://ghdx.healthdata.org/gbd-results-tool (Accessed 10/07/20).

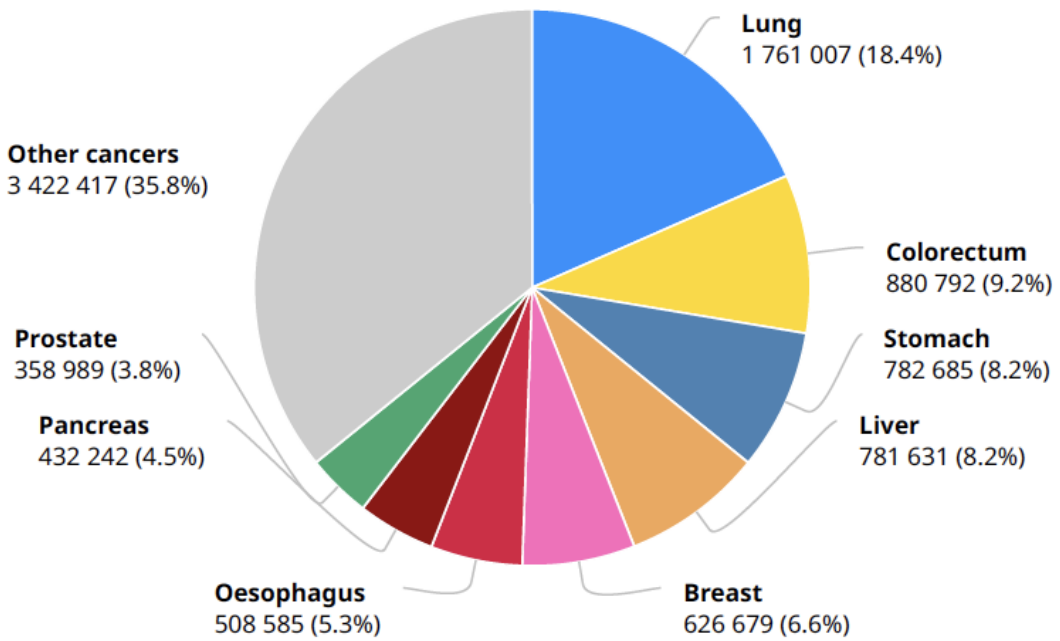
Cancer: Global Health Crisis

Number of new cases in 2018, both sexes, all ages



Total: 18 078 957 cases

Number of deaths in 2018, both sexes, all ages



Total: 9 555 027 deaths

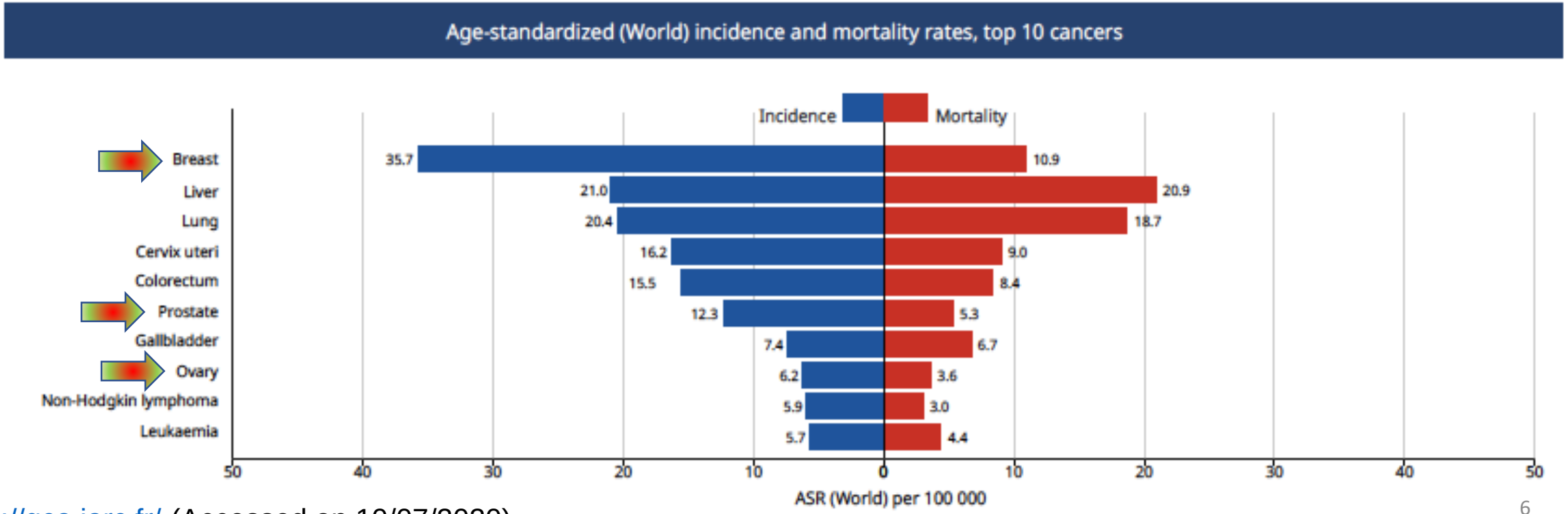
https://gco.iarc.fr/ (Accessed on 10/07/2020)

Cancer: Global Health Crisis

Thailand (2018):

Number of new cases: 170,995

Number of deaths: 114,199



https://gco.iarc.fr/ (Accessed on 10/07/2020)

Hallmarks of Cancer

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Review

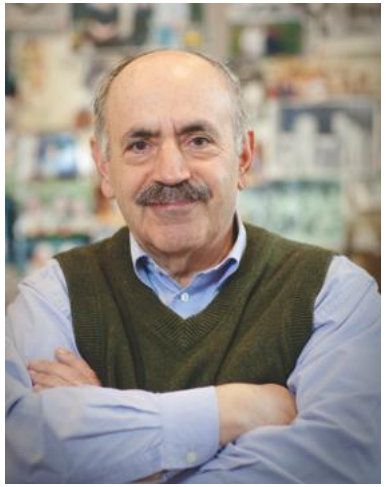
Douglas Hanahan
- 1st transgenic mouse models of cancer

Cell

Leading Edge
Review

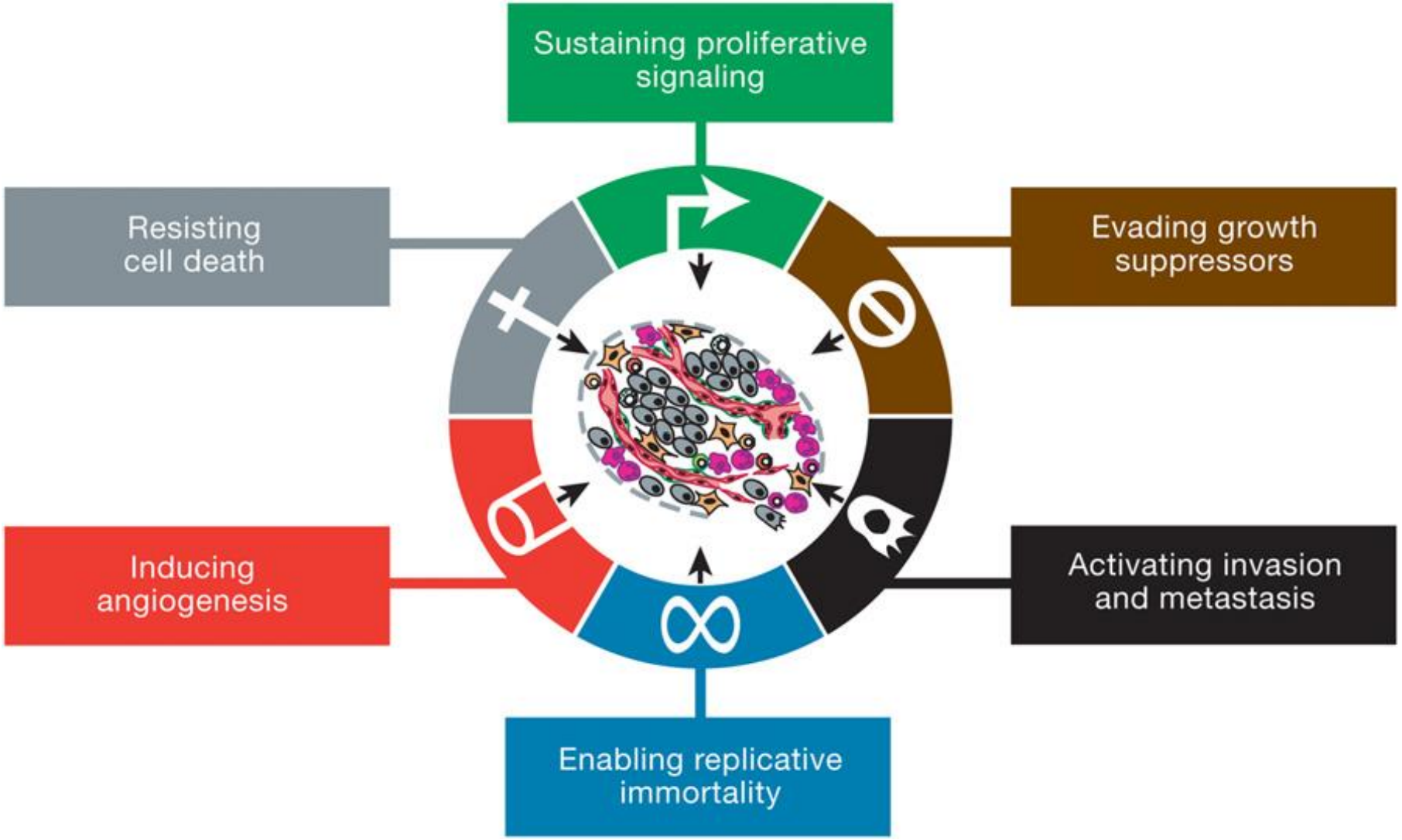
Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
[3]Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA
*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)
DOI 10.1016/j.cell.2011.02.013



Robert Weinberg
- Discover Ras, Rb

Hallmarks of Cancer



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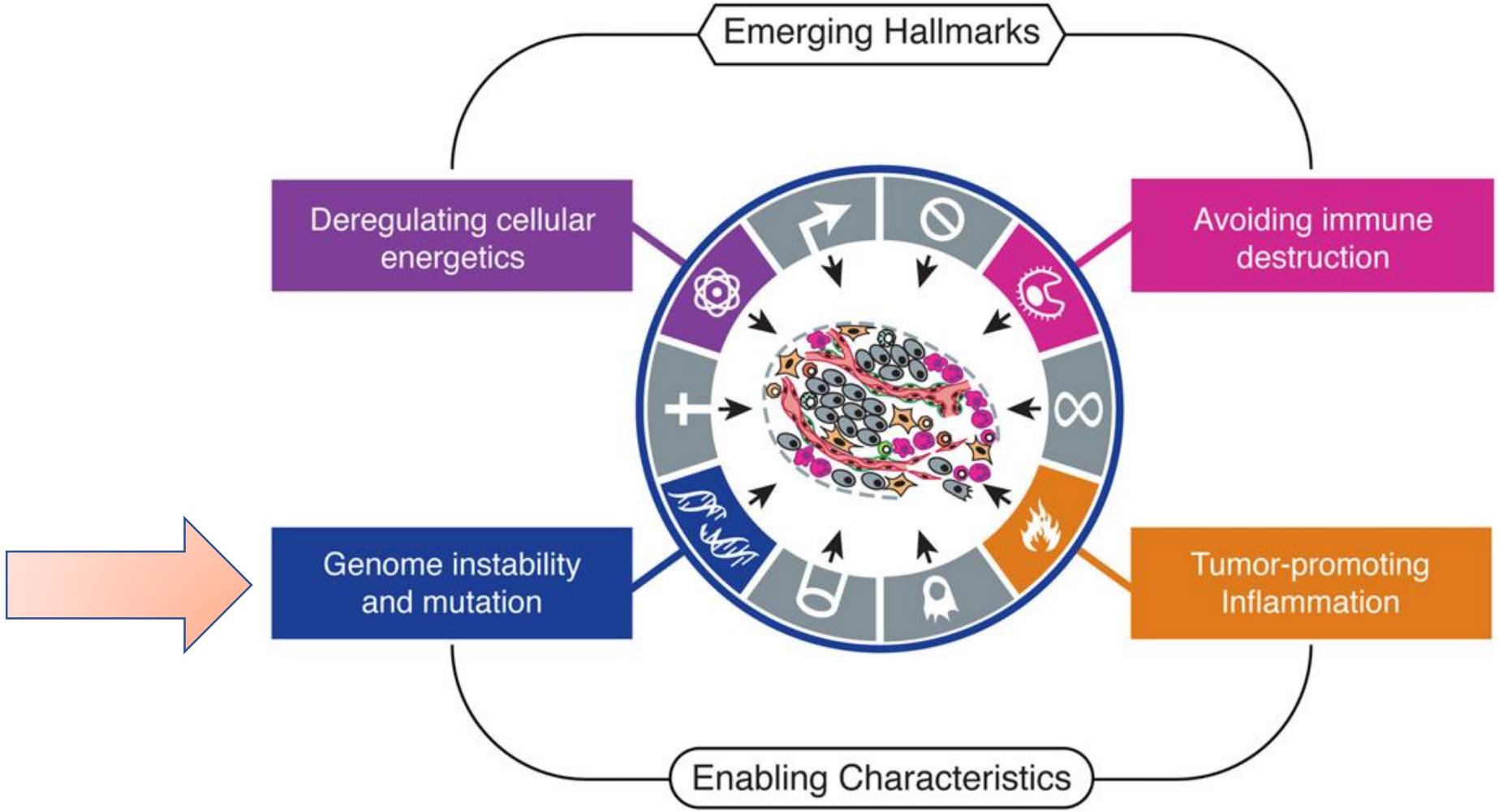
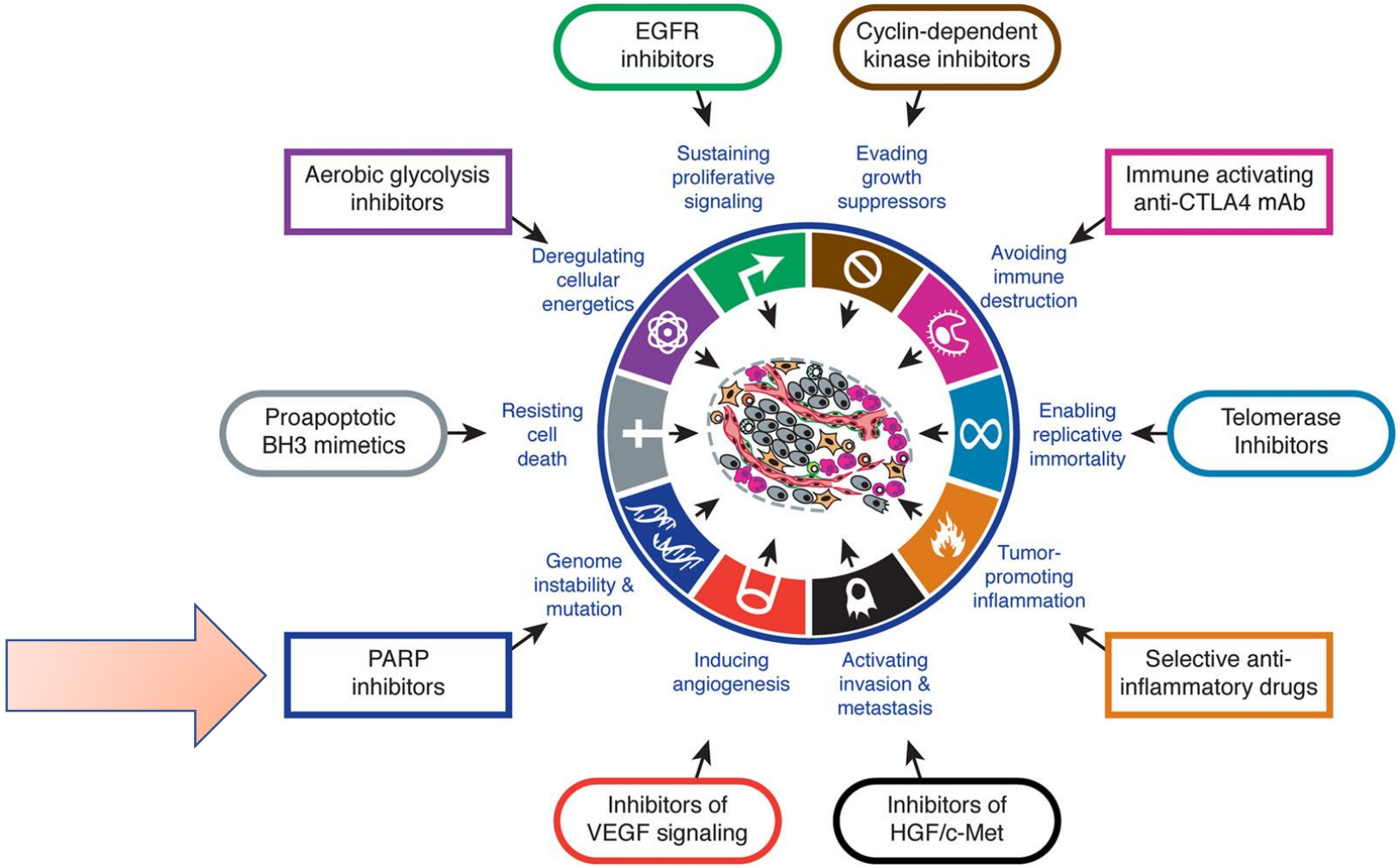
Hallmarks of Cancer



PMID: 21376230

Therapeutic Targeting of the Hallmarks of Cancer



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DNA Damage Responses

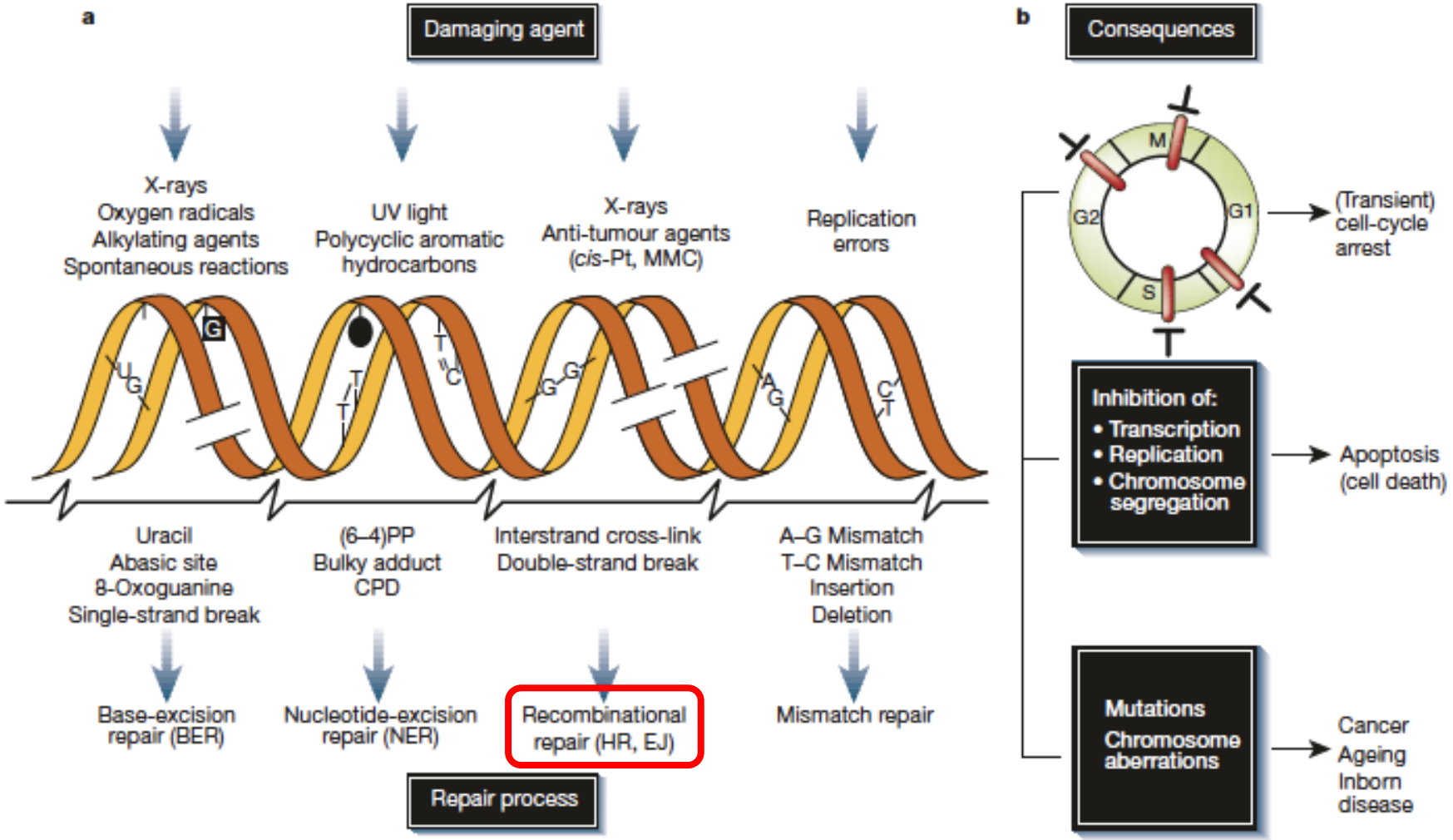
- Cells are exposed constantly to various genotoxic stresses that can lead to DNA damage.

- Healthy cells defend themselves against the deleterious effects of DNA damage by **DNA damage responses (DDR)**, that recognize DNA damage, stall the cell cycle, and mediate DNA repair, thus maintaining the integrity of the genome.

DNA Damage Responses

- If left unrepaired or are repaired incorrectly, DNA damage can give rise to mutations, deletions, amplifications, and chromosomal translocations, leading to development of cancers.

DNA Damage Responses



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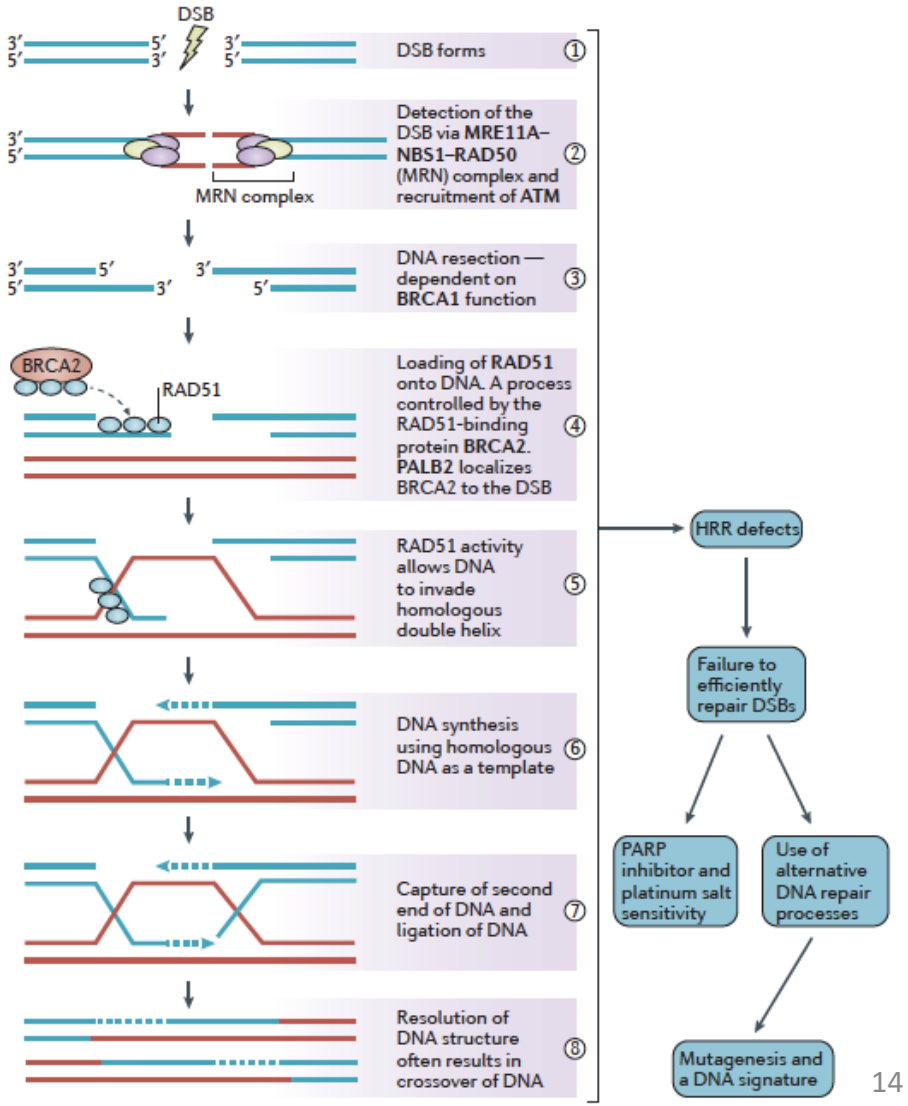
Homologous Recombination (HR)

- A conservative form of DNA repair, in that it restores the DNA sequence to **its original form**.
- **High-fidelity, error-free** damage reversal
- BRCA1 and BRCA2 are essential for HR.



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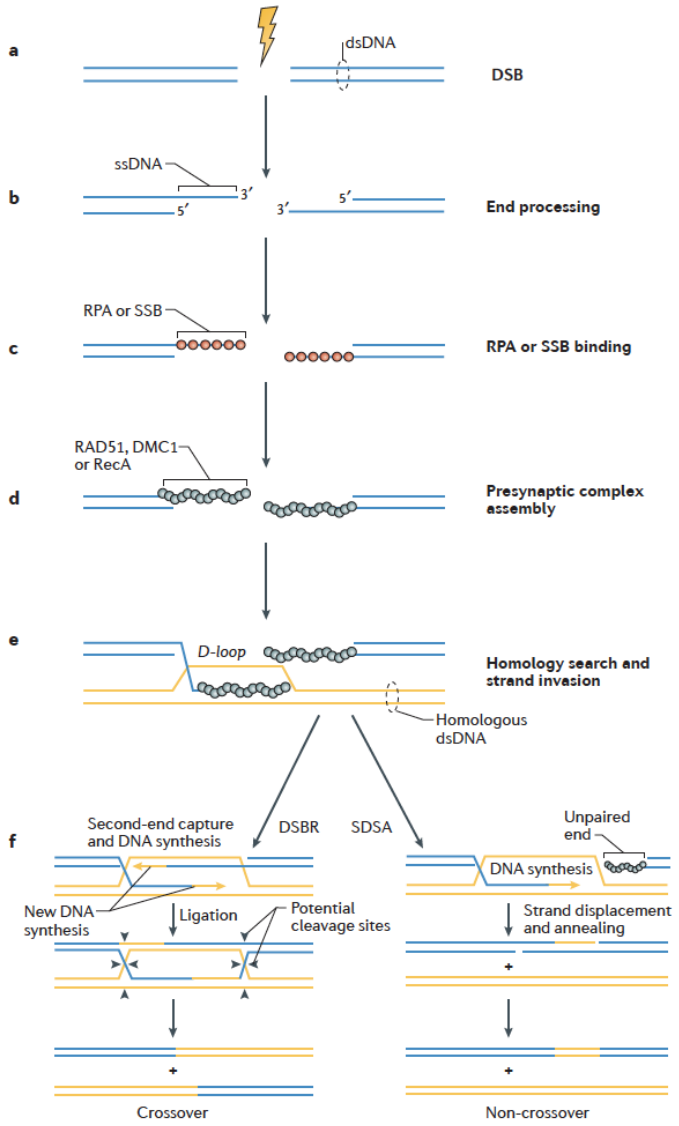
Homologous Recombination (HR)



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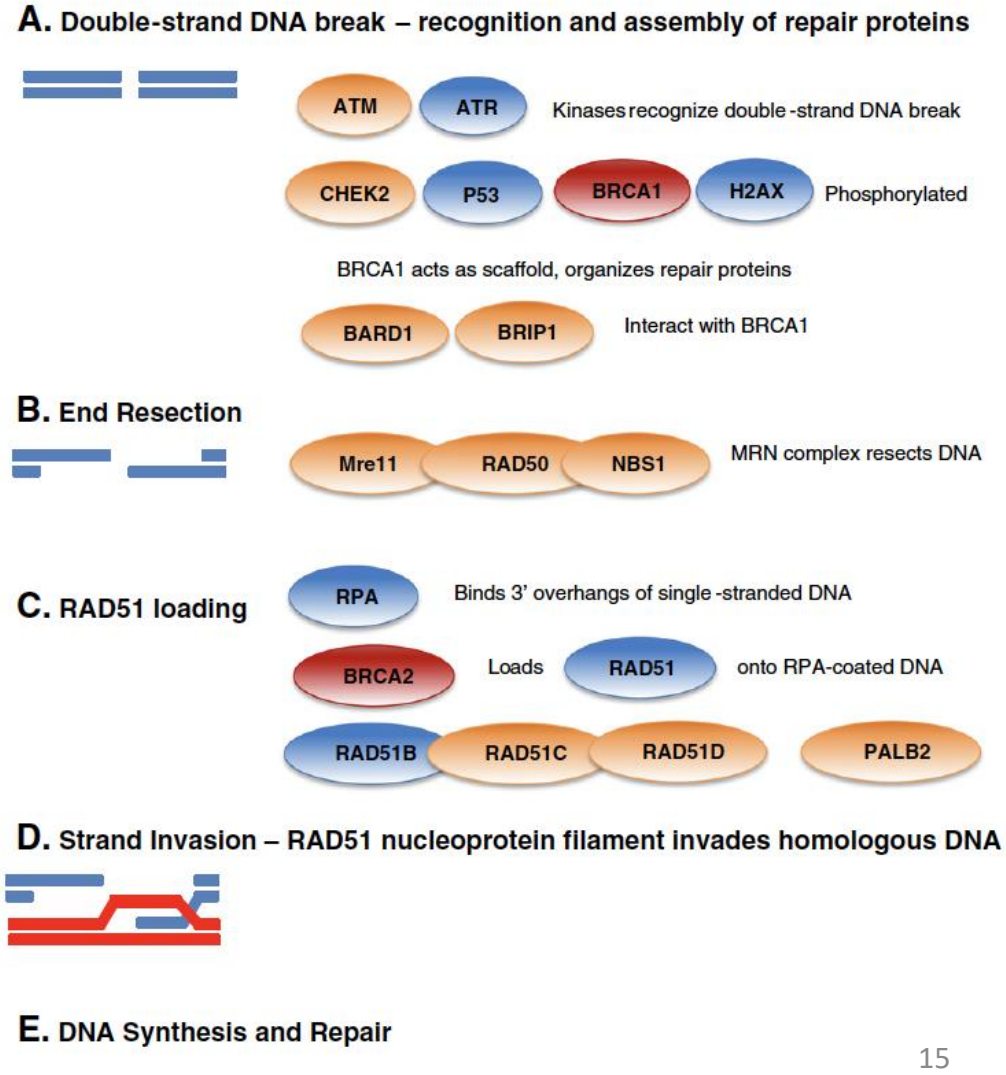

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BRCA Mutations in Cancers

- *BRCA1* and *BRCA2* are tumor suppressor genes that, when heterozygously mutated in the germ line, confer a considerably higher risk of several forms of cancer including breast, ovarian, pancreatic and prostate cancer.

- Functional BRCA1 and BRCA2 proteins are crucial to the repair of double-stranded DNA breaks by HR.

BRCA Mutations in Cancers

- Loss of *BRCA1* and *BRCA2* lead to defect in HR, resulting in mutations.
- Some of the mutations that arise in this way may foster cancer initiation or progression, potentially explaining at least in part why mutations in *BRCA1* and *BRCA2* increase cancer risk.

BRCA Mutations in Cancers

- DNA repair deficiencies associated with *BRCA1/2* loss represent the **Achilles' heel** of these cancer cells, which may be exploited in therapies with DNA-damaging drugs, such as platinum-based compounds, or agents that inhibit specific DNA-repair pathways *e.g.* **PARP inhibitors**.

PARPs: Master Regulator for DNA Damage Response

- The poly(ADP-ribose) polymerase (PARP) enzymes are important **sensors** of DNA damage that bind to single-stranded DNA breaks and other types of DNA damage and subsequently act as **signal transducers** in the DRR pathway.

- PARP enzyme family catalyzes protein post-translational ribosylation modification and utilizes nicotinamide adenine dinucleotide (**NAD^+**) as a substrate to perform **mono- or poly-ADP-ribosylation (PAR) modification** on target protein.

PARPs: Master Regulator for DNA Damage Response

- There are at least 18 members of the PARP family that are encoded by different genes and share homology in a conserved catalytic domain.

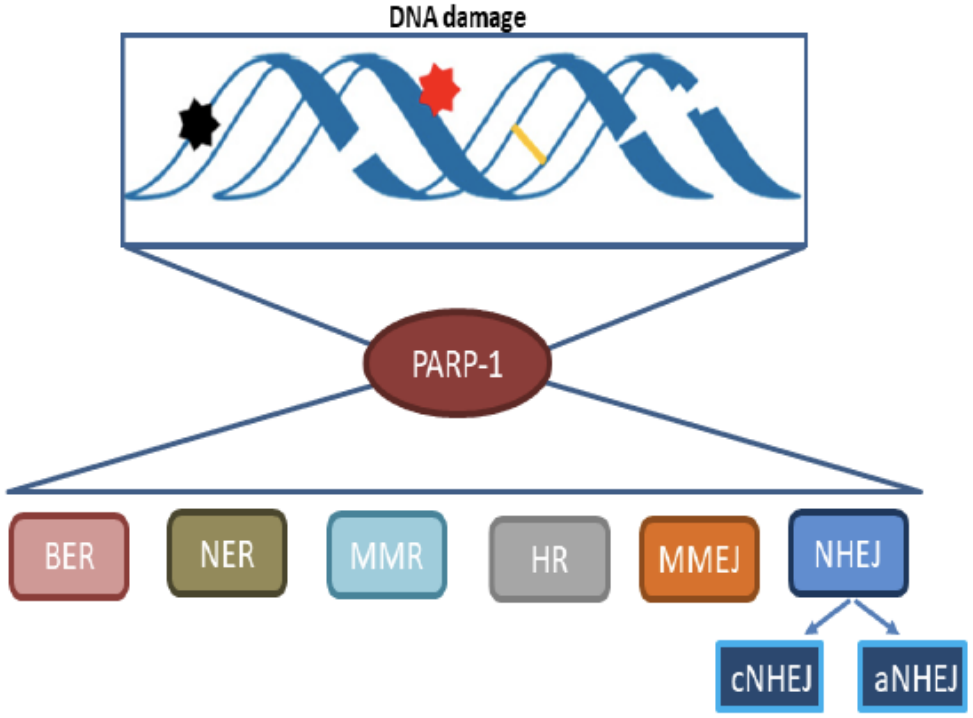
- **PARP1** is the most abundant and dominant member of PARP family.
- PARP1 is highly expressed and generates the majority of PAR polymers.
- PARP1 plays a critical roles in DNA damage responses and genomic stability.

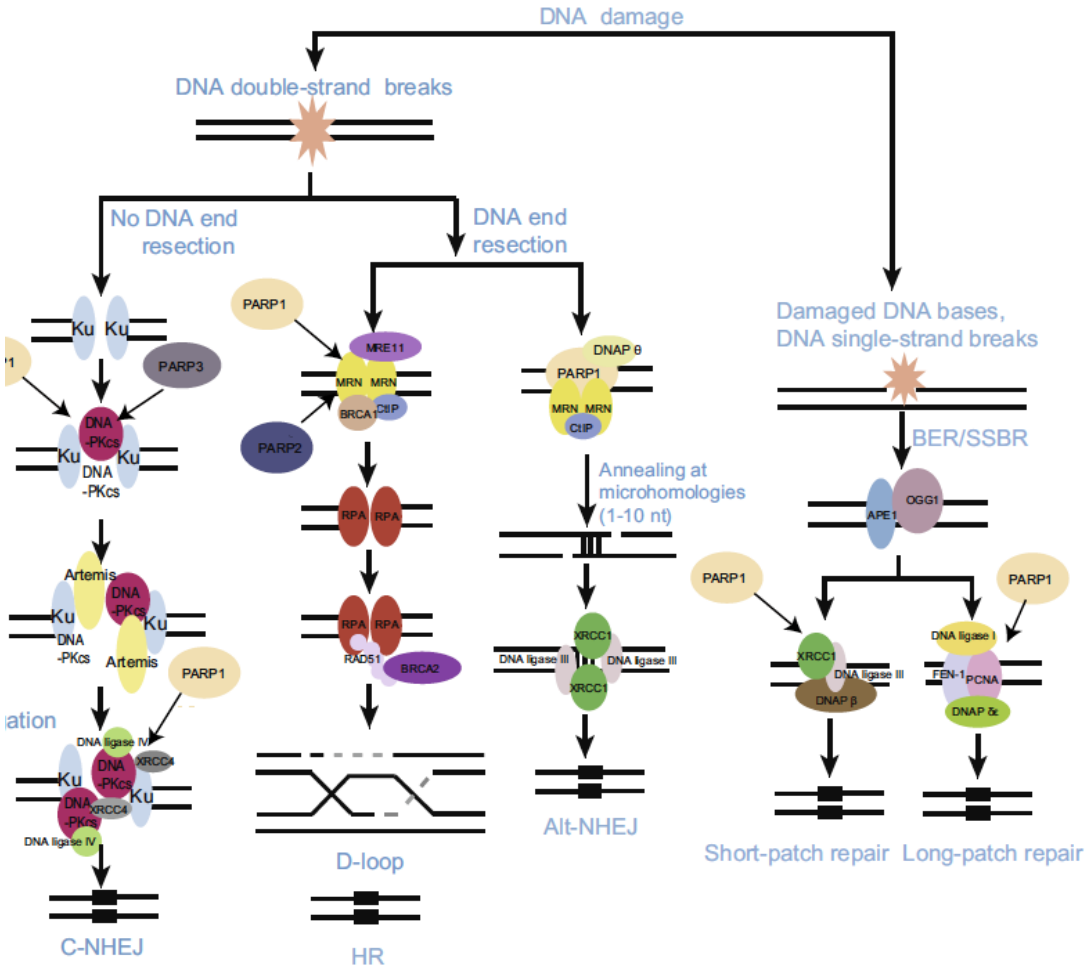
PARPs: Master Regulator for DNA Damage Response

- Upon DNA damage, PARP1 is recruited to site of damage and is activated.
- Activated PARP1 post-translationally catalyze covalent attachment of poly(ADP-ribose) (**PAR**) polymer (**PARylation**) to substrate proteins.
  - PARP1, histones, transcription factors, chromatin modulators
- The PARylation due to PARP1 is using **NAD^+** as an **ADP-ribose donor**.
- PAR polymer is essential for recruitment of DNA repair machinery.
- As a result, DNA damage is repaired, and cell viability is maintained.

Hence, PARP1 is an attractive target for cancer therapy.

PARPs: Master Regulator for DNA Damage Response





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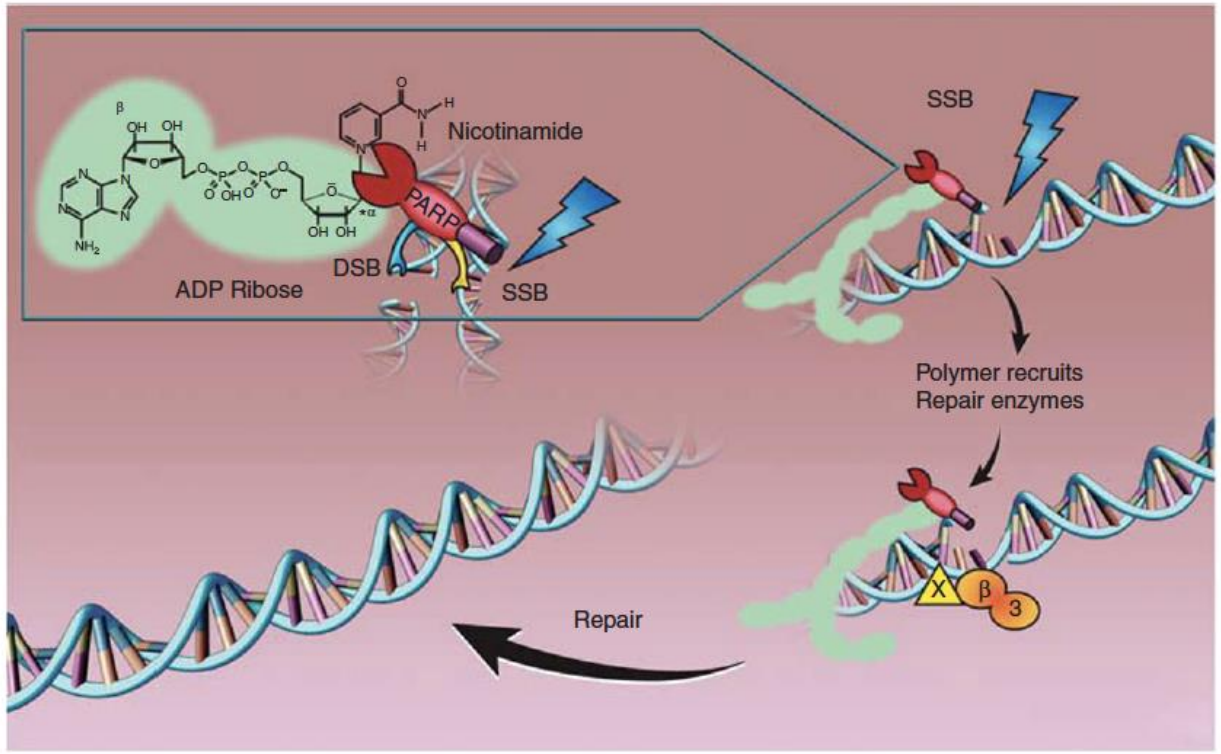
PARPs: Master Regulator for DNA Damage Response



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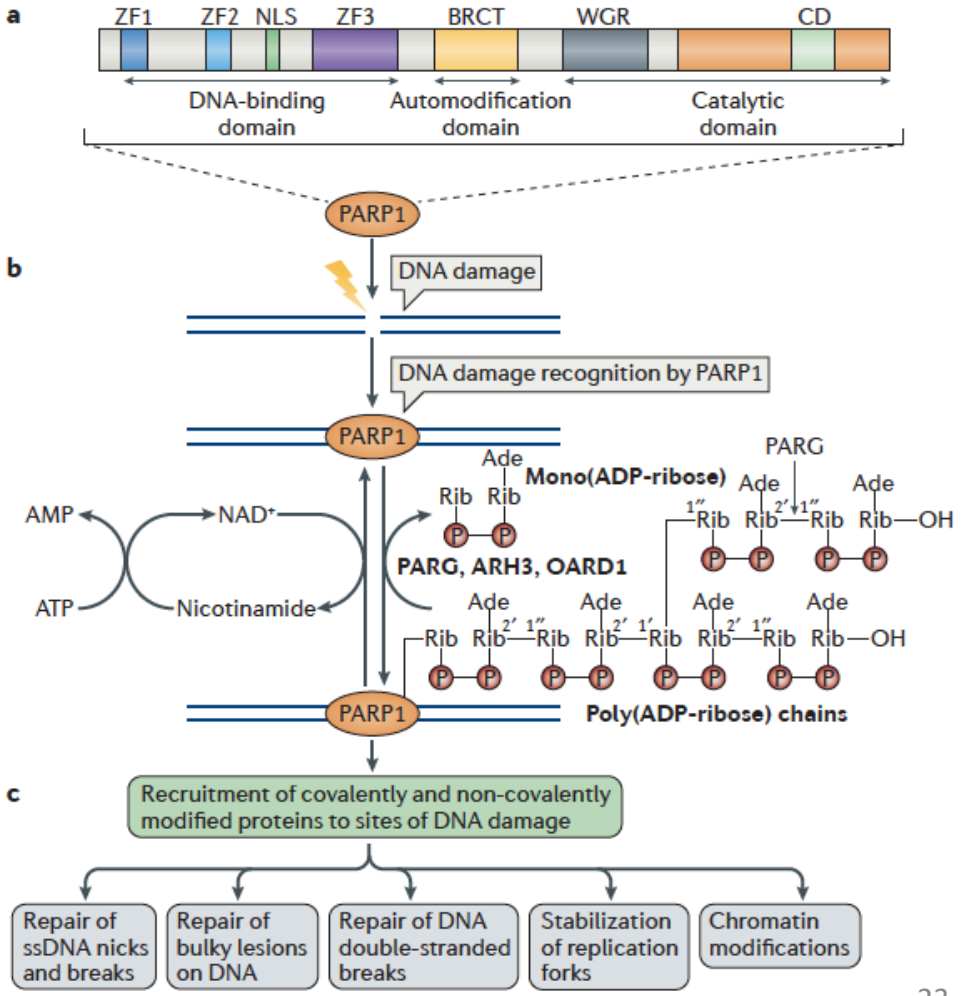

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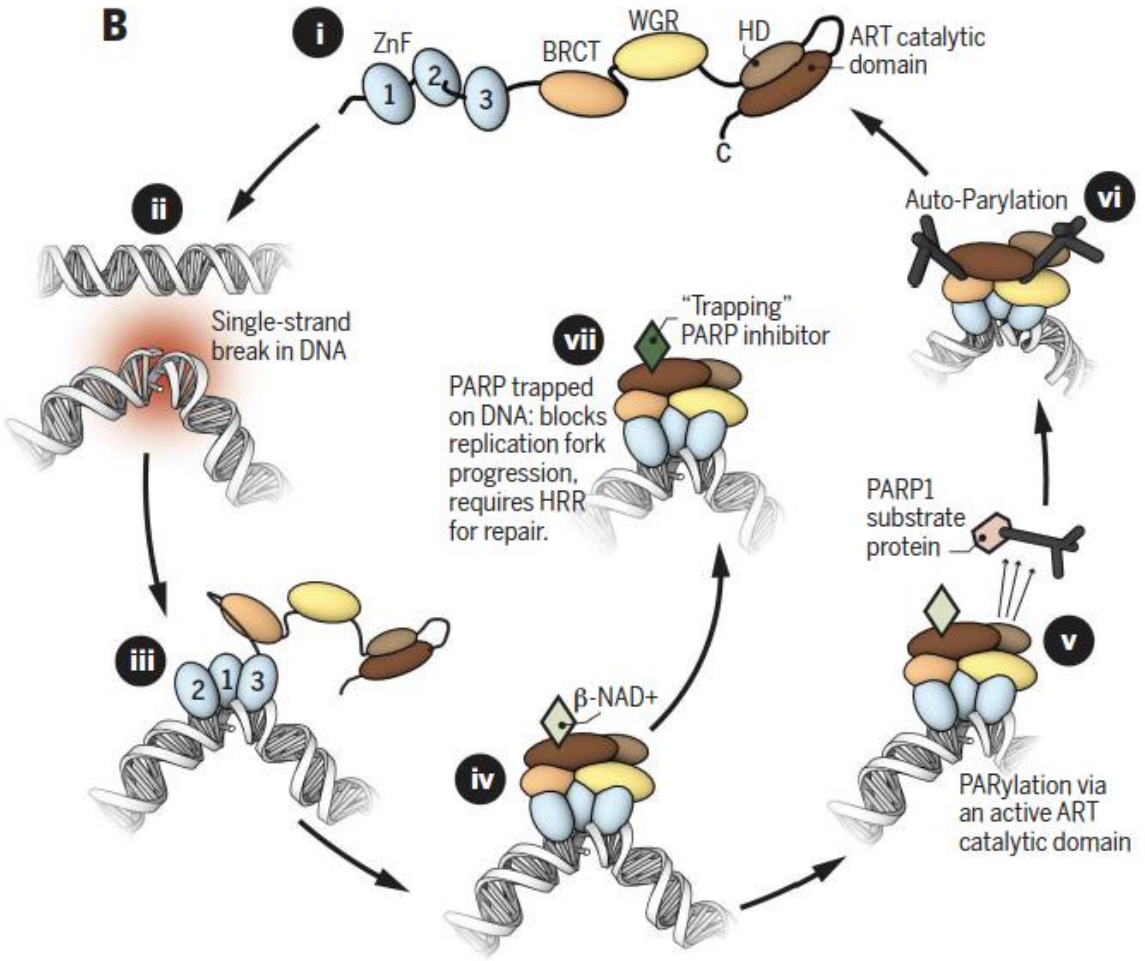
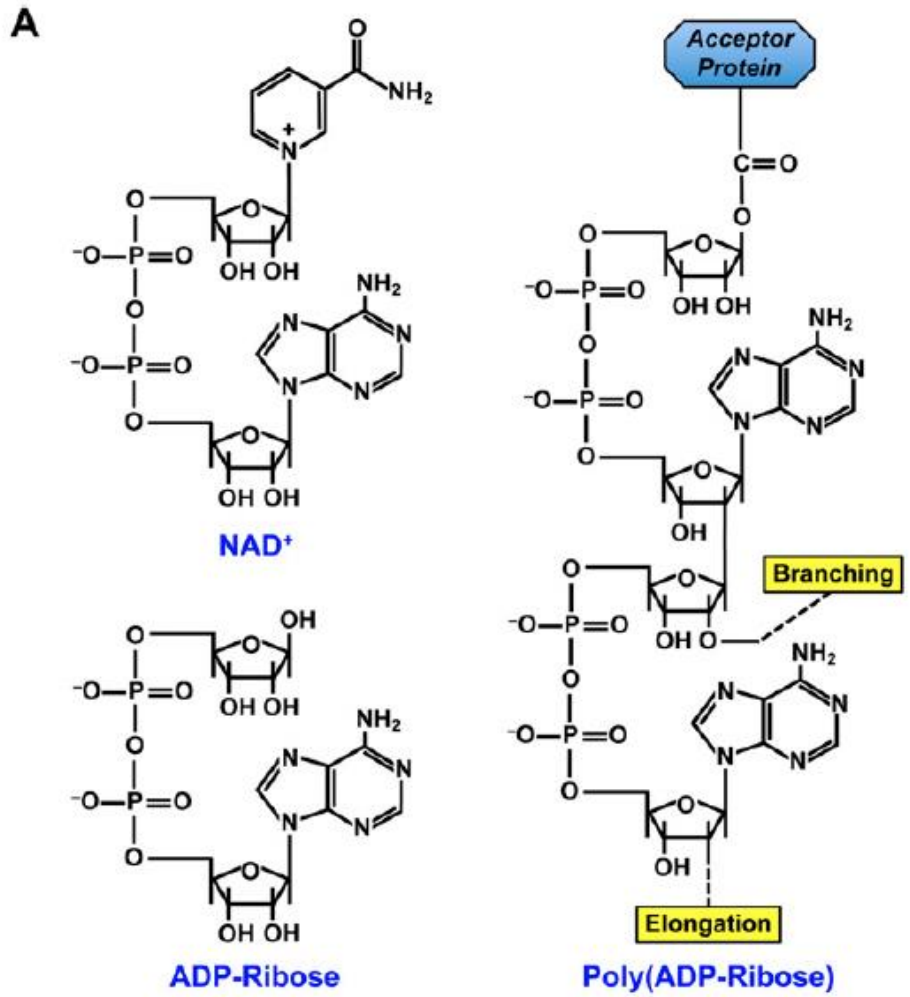
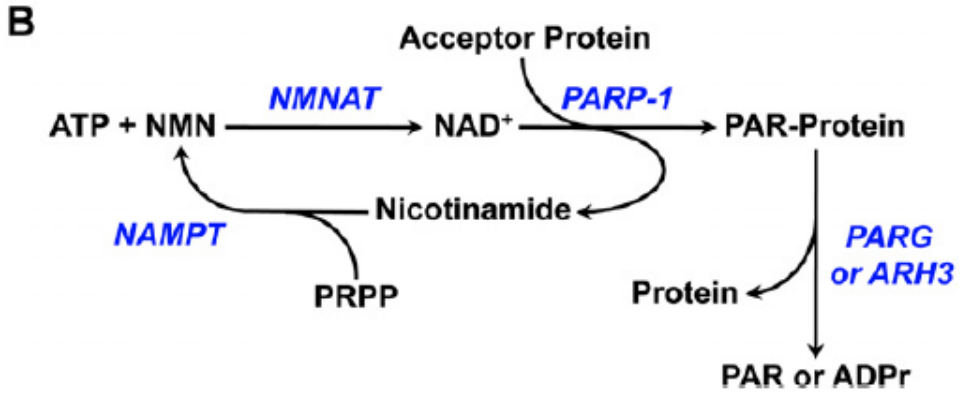
PARPs: Master Regulator for DNA Damage Response



PMID: 28302823

PARPs: Master Regulator for DNA Damage Response





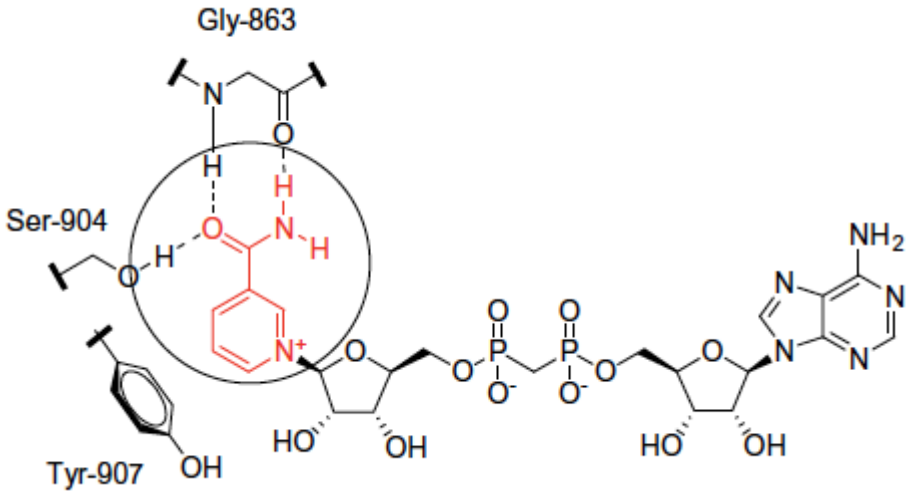
PARG = PAR glycohydrolase

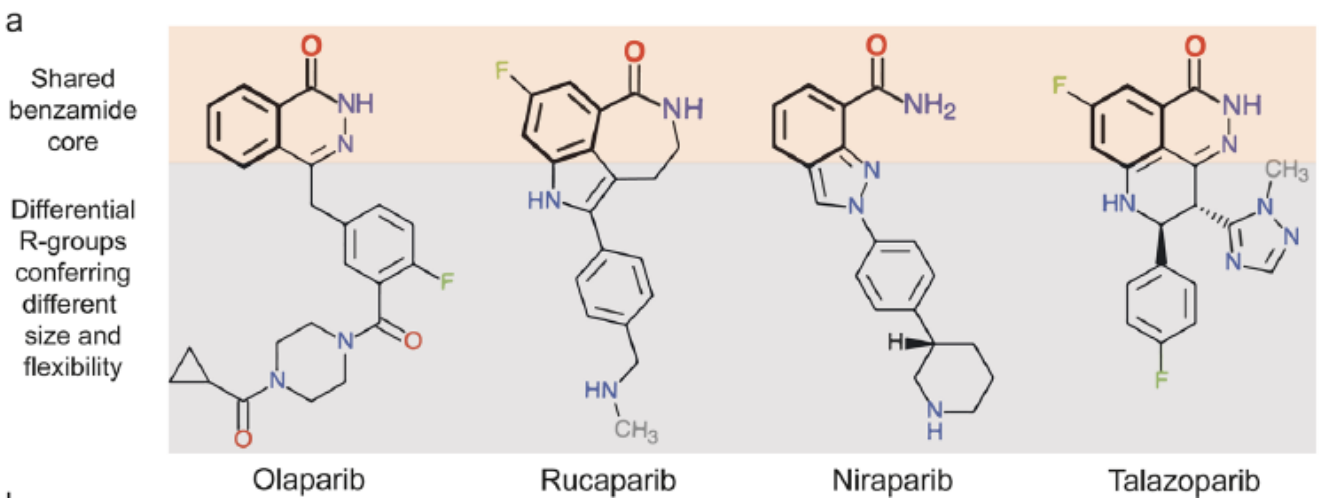
PMID: 22391446

PARP Inhibitors

- PARP inhibitors are designed to compete with **NAD^+** at the catalytic site of PARP.
- **All FDA-approved PARPi imitate the nicotinamide moiety of NAD^+,** and bind to the PARP catalytic domain, inhibiting auto modification and subsequent release of the enzyme from the site of DNA damage.

PARP Inhibitors





Nicotinamide of NAD^+ binds to active site of PARP1.

The **benzamide core pharmacophore** shared by all clinical PARPi.

PMID: 20045315

PMID: 32066817

PARP Inhibitors

b

	Olaparib	Rucaparib	Niraparib	Talazoparib
PARP1	1 - 19 nM	0.8 - 3.2 nM	2 - 35 nM	0.6 - 1.1 nM
PARP2	1 - 251 nM	28.2 nM	2 - 15.3 nM	4.1 nM
PARP3	46 - 230 nM	512 nM	296 - 1,300 nM	62.8 nM
PARP4	410 nM	839 nM	330 - 446 nM	254 nM
TNKS1	1,230 - 3,534 nM	25 - 144 nM	570 - 2,355 nM	322 nM
TNKS2	1,700 - 2,340 nM	14 - 890 nM	5,130 nM	108 nM
PARP10	1,250 - 9,300 nM	570 nM	1,900 nM	7,800 nM
PARP12	10,050 nM	6,650 nM	790 nM	
PARP14		8,830 nM	17,300 nM	25,500 nM
PARP15	17,600 nM	32,600 nM	29,200 nM	47,400 nM
PARP16	5,100 nM			1,900 nM

Literature IC_{50} values

1-10 nM
10-100 nM
100-1,000 nM
1,000-10,000 nM
Weak inhibition (> 10,000 nM)
No inhibition
Not determined

PMID: 32066817

PARP Inhibitors

- PARP inhibitors (PARPi) first entered the clinic in 2003 in combination with DNA-damaging cytotoxic agents on the basis of preclinical data showing both chemo- and radiopotentiation with this class of compounds.

- However, all of the early studies demonstrated the same clinical challenge—inhibiting the repair of DNA strand breaks also **enhances normal tissue toxicity**, especially myelosuppression, which is dose limiting with many cytotoxic agents.

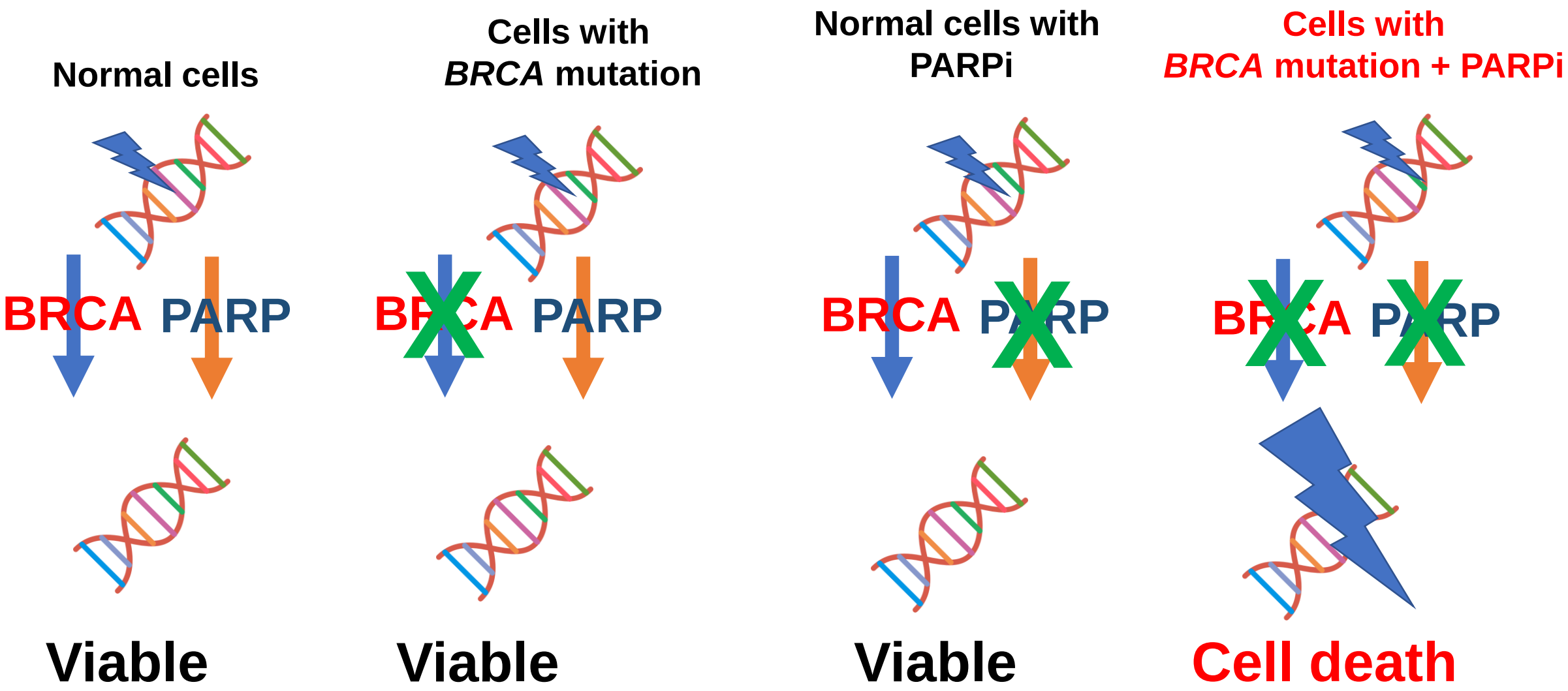
PARP Inhibitors

- In 2005, the first preclinical data were published that demonstrated the concept of **“synthetic lethality”** between *BRCA1/2* genetic defects and pharmacologic PARP inhibition, and these data suggested that there may be single-agent activity with this class of agents.

Synthetic Lethality Therapy

- Synthetic lethal interactions are a form of context- dependent essentiality, in which a genetic alteration, *e.g.* defect in a tumor suppressor gene, **causes a second gene to become essential for cell survival**.
- Pharmacological inhibition of the product of this second, synthetic lethal gene would be lethal to tumour cells but leave nonmalignant cells largely unaffected.
- Therefore, **synthetic lethality provides an approach that can be used to selectively target tumor cells and spare the patient's nonmalignant cells.**

Synthetic Lethality Therapy with PARPi



Synthetic Lethality Therapy with PARPi

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]

[1]*The Institute for Cancer Studies, University of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2RX, UK*
[2]*Department of Genetics, Microbiology and Toxicology, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[3]*Northern Institute for Cancer Research, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne, NE2 4HH, UK*

Poly(ADP-ribose) polymerase (PARP1) facilitates DNA repair by binding to DNA breaks and attracting DNA repair proteins to the site of damage[1–3]. Nevertheless, PARP1$^{-/-}$ mice are viable, fertile and do not develop early onset tumours[4]. Here, we show that PARP inhibitors trigger γ-H2AX and RAD51 foci formation. We propose that, in the absence of PARP1, spontaneous single-strand breaks collapse replication forks and trigger homologous recombination for repair. Furthermore, we show that BRCA2-deficient cells, as a result of their deficiency in homologous recombination, are acutely sensitive to PARP inhibitors, presumably because resultant collapsed replication forks are no longer repaired. Thus, PARP1 activity is essential in homologous recombination-deficient *BRCA2* mutant cells. We exploit this requirement in order to kill BRCA2-deficient tumours by PARP inhibition alone. Treatment with PARP inhibitors is likely to be highly tumour specific, because only the tumours (which are BRCA2$^{-/-}$) in BRCA2$^{+/-}$ patients are defective in homologous recombination. The use of an inhibitor of a DNA repair enzyme alone to selectively kill a tumour, in the absence of an exogenous DNA-damaging agent, represents a new concept in cancer treatment.

PMID: 15829966

Synthetic Lethality Therapy with PARPi

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]

[1]*Cancer Research UK Gene Function and Regulation Group and* [2]*The Breakthrough Breast Cancer Research Centre Institute of Cancer Research, Fulham Road, London SW3 6JB, UK*
[3]*Guy's Hospital, St Thomas' Street, London SE1 9RT, UK*
[4]*KuDOS Pharmaceuticals Ltd, Cambridge Science Park, Cambridge CB4 0WG, UK*
[5]*Wellcome Trust and Cancer Research UK, Gurdon Institute of Cancer and Developmental Biology, and Department of Zoology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK*

* These authors contributed equally to this work
† Present address: Division of Experimental Oncology1, CRO-IRCCS, Aviano 33081 PN, Italy

BRCA1 and BRCA2 are important for DNA double-strand break repair by homologous recombination[1], and mutations in these genes predispose to breast and other cancers[2]. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in base excision repair, a key pathway in the repair of DNA single-strand breaks[3]. We show here that BRCA1 or BRCA2 dysfunction unexpectedly and profoundly sensitizes cells to the inhibition of PARP enzymatic activity, resulting in chromosomal instability, cell cycle arrest and subsequent apoptosis. This seems to be because the inhibition of PARP leads to the persistence of DNA lesions normally repaired by homologous recombination. These results illustrate how different pathways cooperate to repair damage, and suggest that the targeted inhibition of particular DNA repair pathways may allow the design of specific and less toxic therapies for cancer.

PMID: 15829967

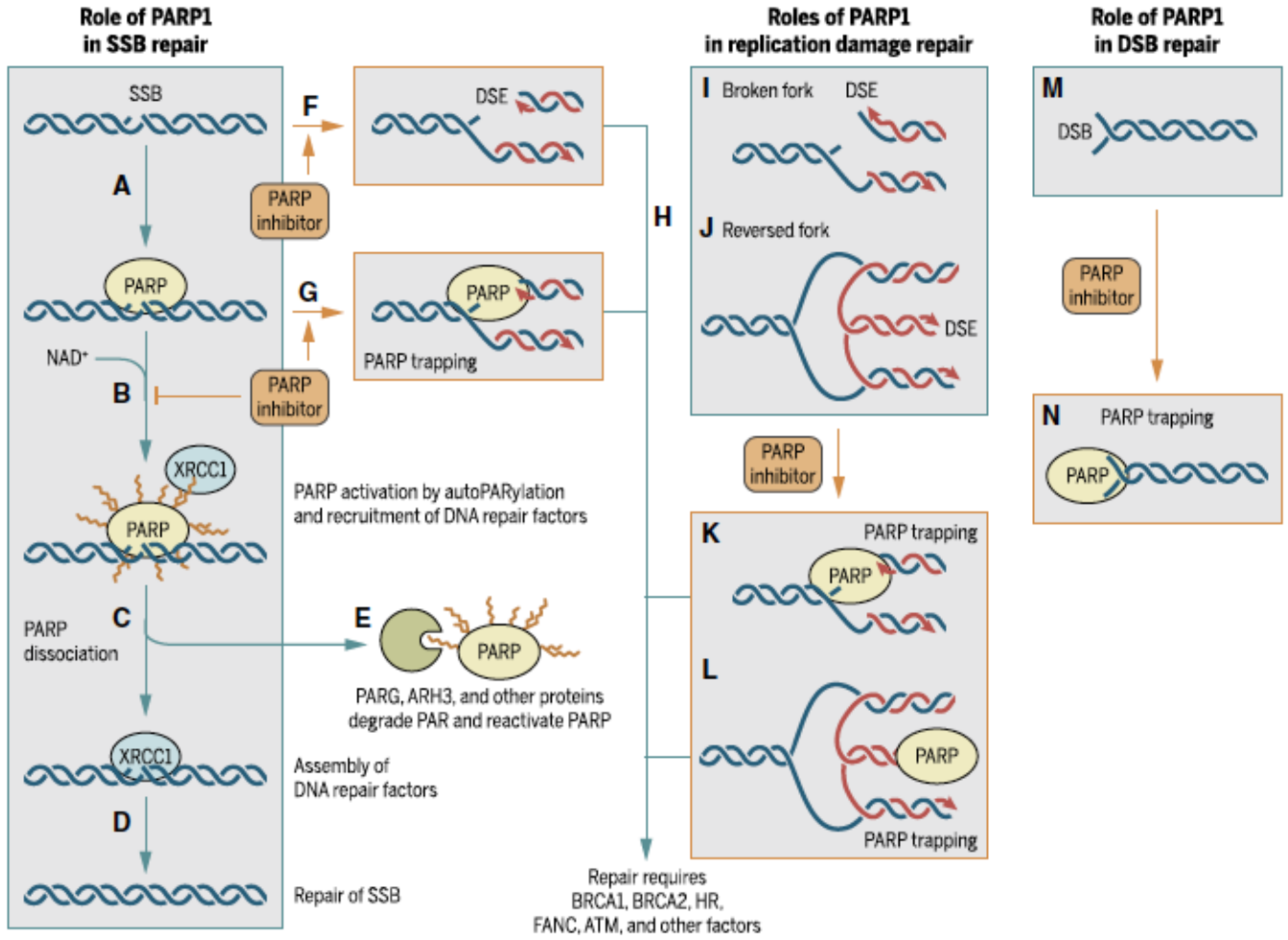
Synthetic Lethality Therapy with PARPi

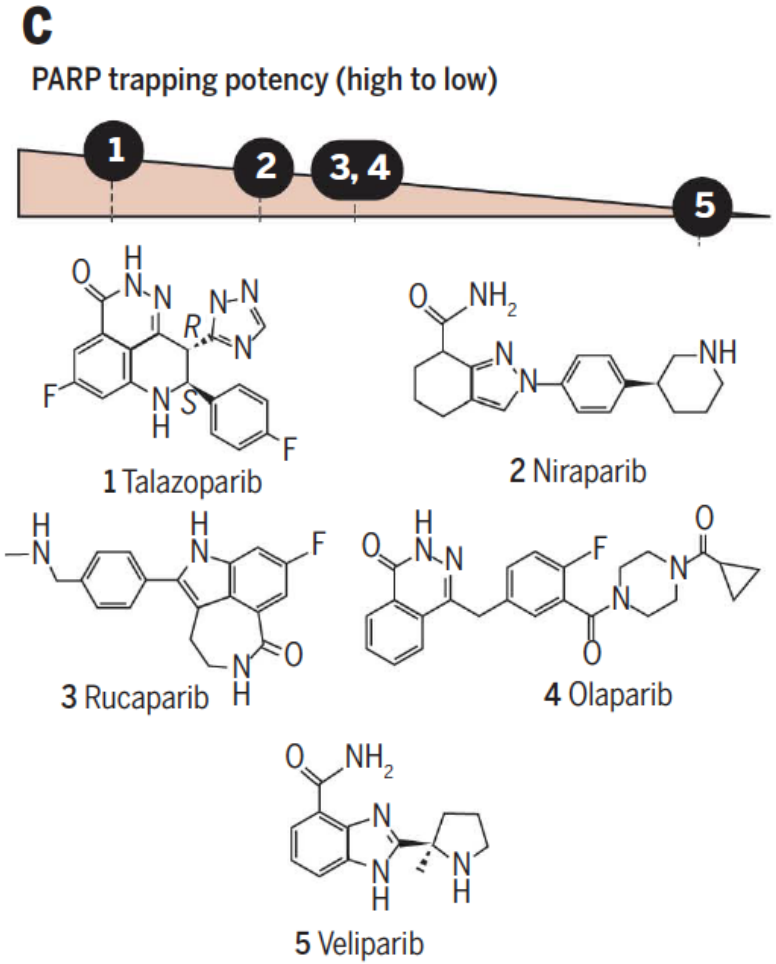
- Catalytic inhibition is not the only mechanism by which PARP inhibitors exert cytotoxic effects.
- **PARP Trapping** has been proposed as an additional pathway for cytotoxic effects of PARPi.

Synthetic Lethality Therapy with PARPi

- When DNA damage occurs in the presence of a PARPi, PARP1 binds to damage sites and remains tightly bound or trapped onto the chromatin.
- PARylation is inhibited, and PARP1 remains bound to the lesion, which progresses DNA double-strand breaks.
  - In HR–competent cells, DNA damage is repaired by the HR pathway, and cells remain viable.
  - In HR-defective cells such as *BRCA* 1/2 mutants, DSB damage is not repaired with high fidelity, resulting in cell death.

PARP Trapping: Additional Mechanism for PARPi





PMID: 27797957

PMID: 28302823

Timeline for PARP Inhibitors

1994
Discovery of *BRCA1*

1995
Discovery of *BRCA2*

2005
Synthetic lethality of PARPi

2014
FDA approved
olaparib
Lynparza™ olaparib

2016
FDA approved
rucaparib
Rubraca® (rucaparib) tablets

2017
FDA approved
niraparib
Zejula® niraparib

2018
FDA approved
talazoparib
TALZENNA®

PARP Inhibitors

- PARP inhibitors provided the **first clinical exemplification of synthetic lethality** in oncology.
- PARP inhibitors is the **first personalized medicine** in ovarian cancer and prostate cancer.

Approval Indications of PARPi (May 2018)

PARP inhibitor	Year of approval	Indication and expanded indication
Olaparib	2014	Treatment of patients with deleterious or suspected deleterious **germline BRCA mutated** (gBRCAm) **advanced ovarian cancer** who have been treated with three or more prior lines of chemotherapy.
	2017	Maintenance treatment of adult patients with recurrent **epithelial ovarian, fallopian tube, or primary peritoneal cancer**, who are in a complete or partial response to platinum-based chemotherapy.
	2018	Treatment of patients with deleterious or suspected deleterious **germline BRCA-mutated** (gBRCAm), HER2-negative metastatic **breast cancer** who have been treated with chemotherapy either in the neoadjuvant, adjuvant, or metastatic setting.
Rucaparib	2016	Treatment of patients with deleterious **BRCA mutation** (germline and/or somatic) associated **epithelial ovarian, fallopian tube or primary peritoneal cancer** who have been treated with two or more chemotherapies.
	2018	Maintenance treatment of recurrent **ovarian, fallopian tube, or primary peritoneal cancer** who are in a complete or partial response to platinum-based chemotherapy.
Niraparib	2017	Maintenance treatment of adult patients with recurrent **epithelial ovarian, fallopian tube, or primary peritoneal cancer** who are in complete or partial response to platinum-based chemotherapy.
Talazoparib	2018	Treatment of patients with deleterious or suspected deleterious **germline BRCA-mutated** (gBRCAm), HER2-negative locally advanced or metastatic **breast cancer**.

PMID: 32066817

Latest Approved Indications of PARPi

- ***Olaparib***
  - On December 19, 2018, the USFDA approved olaparib for the maintenance treatment of adult patients with deleterious or suspected deleterious **germline or somatic *BRCA*-mutated (*gBRCAm or sBRCAm*) advanced epithelial ovarian, fallopian tube or primary peritoneal cancer** who are in complete or partial response to first-line platinum-based chemotherapy.

Latest Approved Indications of PARPi

- ***Olaparib***
  - On December 27, 2019 the USFDA approved approved olaparib for the maintenance treatment of adult patients with deleterious or suspected deleterious **germline BRCA-mutated (gBRCAm) metastatic pancreatic adenocarcinoma**, as detected by an FDA-approved test, whose disease has not progressed on at least 16 weeks of a first-line platinum-based chemotherapy regimen.

Latest Approved Indications of PARPi

- ***Olaparib***
  - On May 8, 2020, the USFDA approved expanded the indication of olaparib to include its **combination with bevacizumab for first-line maintenance treatment** of adult patients with **advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer** who are in complete or partial response to first-line platinum-based chemotherapy and whose cancer is associated with **homologous recombination deficiency positive status defined by either a deleterious or suspected deleterious *BRCA* mutation, and/or genomic instability**.

Latest Approved Indications of PARPi

- ***Olaparib***
  - On May 19, 2020, the USFDA approved olaparib for adult patients with deleterious or suspected deleterious **germline or somatic homologous recombination repair (HRR) gene-mutated metastatic castration-resistant prostate cancer (mCRPC**), who have progressed following prior treatment with enzalutamide or abiraterone.

Latest Approved Indications of PARPi

- ***Niraparib***
  - On October 23, 2019,the USFDA approved for patients with **advanced ovarian, fallopian tube, or primary peritoneal cance**r treated with three or more prior chemotherapy regimens and whose **cancer is associated with homologous recombination deficiency (HRD)-positive status.** HRD is defined by either a deleterious or suspected deleterious *BRCA* mutation, or genomic instability in patients with disease progression greater than six months after response to the last platinum-based chemotherapy.

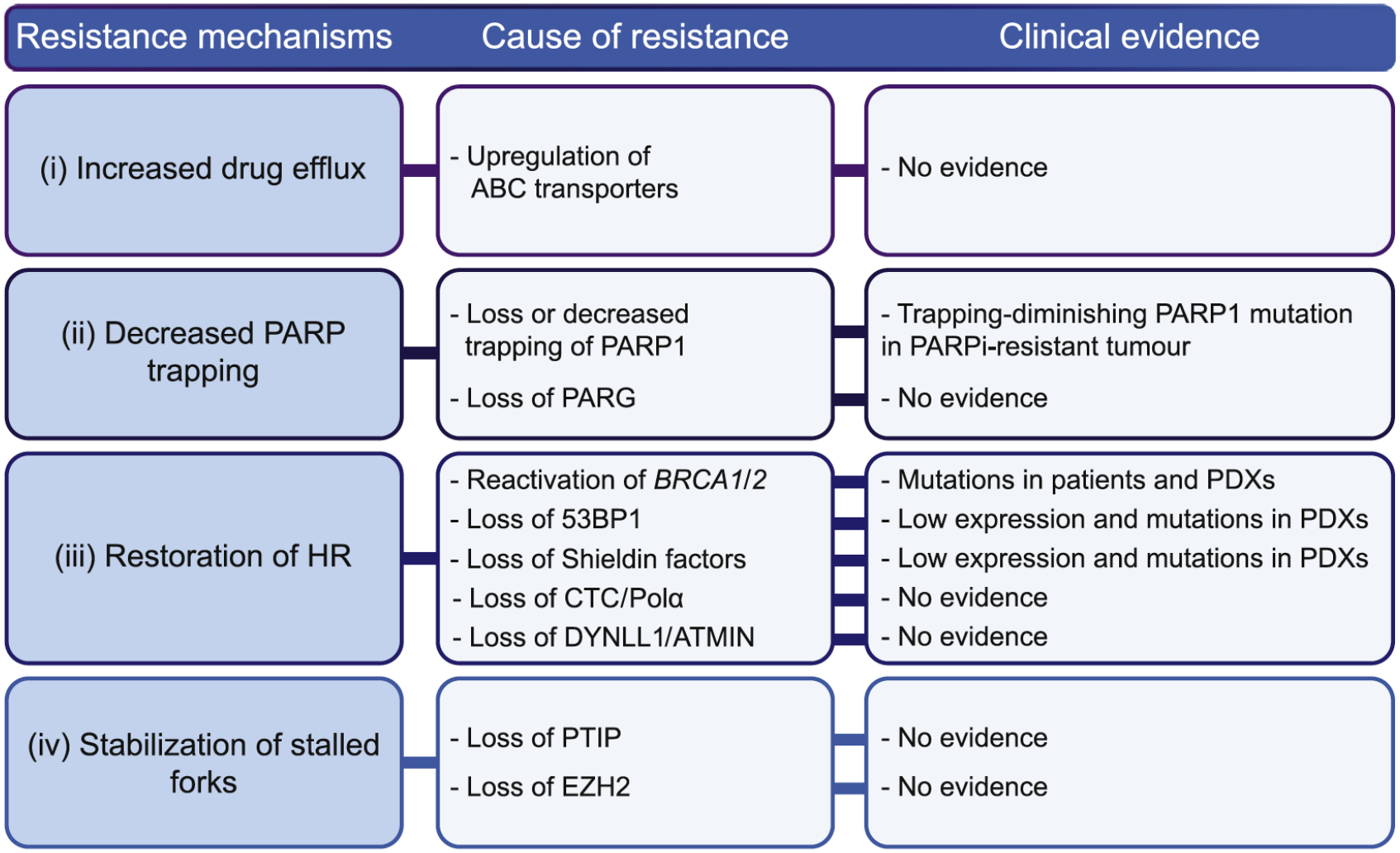
Latest Approved Indications of PARPi

- ***Rucaparib***
  - On May 15, 2020, the USFDA granted accelerated approval to rucaparib for patients with deleterious ***BRCA*** **mutation (germline and/or somatic)-associated metastatic castration-resistant prostate cancer** (**mCRPC**) who have been treated with androgen receptor-directed therapy and a taxane-based chemotherapy.

Resistances to PARPi

- Resistance to PARPi poses a significant barrier to the long-term survival and treatment options of patients with *BRCA1*- and *BRCA2*-mutated cancers.
- A large fraction of patients with tumors that are potentially amenable to therapy either do not respond to PARPi treatment or rapidly develop clinical resistance.
- Acquired resistance is becoming a significant problem.

Resistances to PARPi



Trends in Cell Biology

Future Directions

- Novel biomarker for prediction of PARP susceptibility and resistance
- Combination therapies with other agents
- New technology (*e.g.* CRISPR–Cas9 mutagenesis) and conceptual advances will speed the discovery of new clinically applicable synthetic lethal interactions.
- Synthetic lethality beyond *BRCA*
- Targeting other defects in DDR

Conclusions

- PARP inhibitors are promising strategy for treatment against HR-deficient tumors through **synthetic lethality**.
- Applying the synthetic lethal principle to identifying additional approaches to treating the disease has clear potential, and it is expected that a number of the synthetic lethal effects identified in preclinical studies will in time be able to be assessed in the clinic.

Q&A