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Targeting poly(ADP-ribose) metabolism to potentialize radiotherapy

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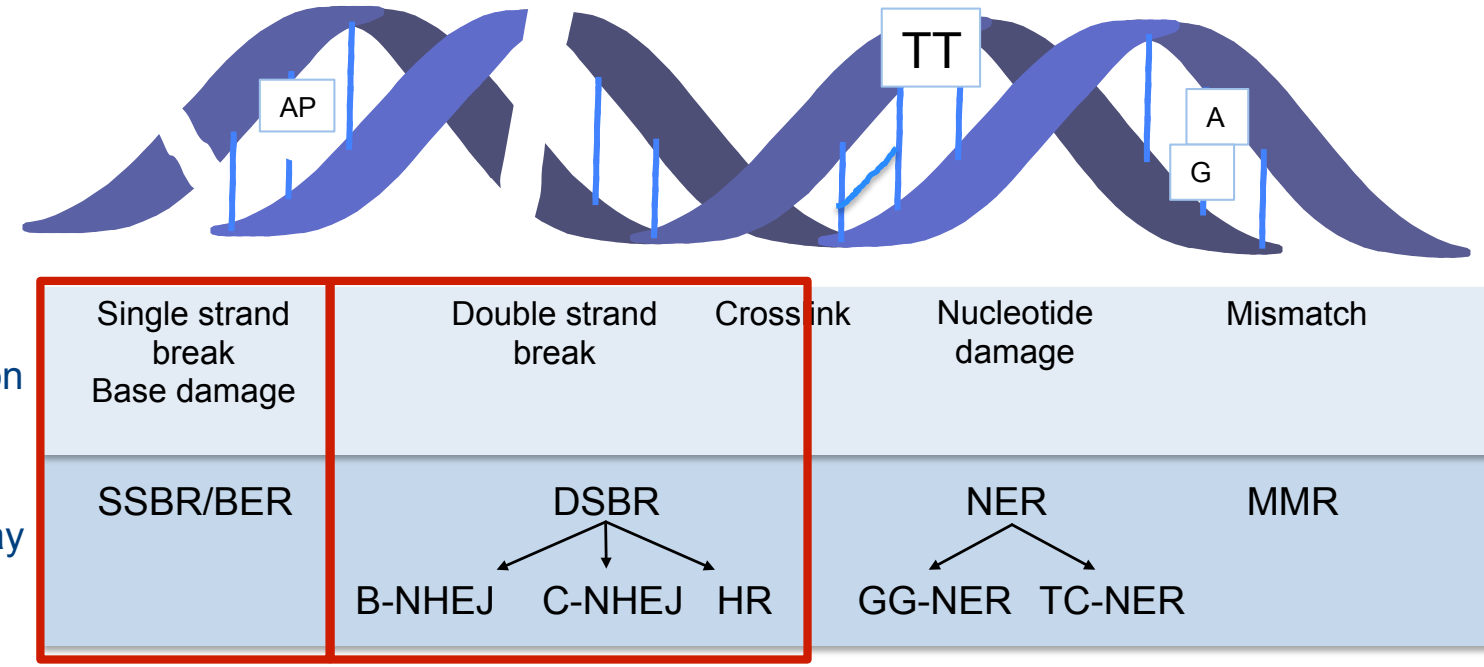
Illkirch

DNA damages induced by IR and corresponding repair pathways

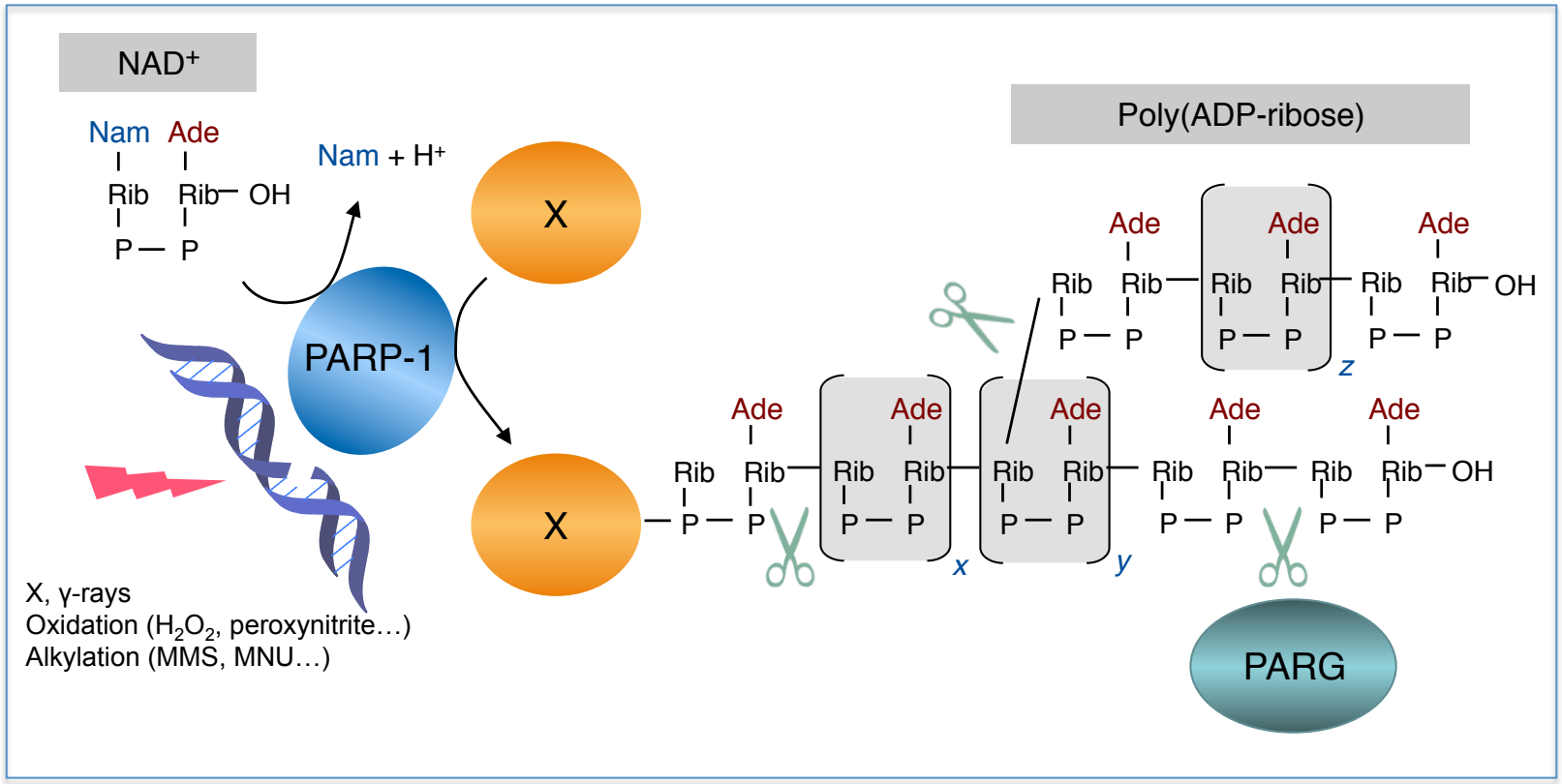


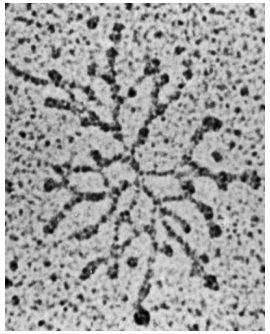
Ionizing radiations: damages /Gy/cell:

2.000 oxidized bases
1.000 SSB
200 crosslinks (proteins or DNA)
40 DSB

need for efficient detectors of DNA damages
to quickly restore DNA integrity !

PARP-1: an efficient sensor of DNA breaks





PAR (EM)
G. de Murcia, 1983

Poly(ADP-ribose) polymerase: PARP-1

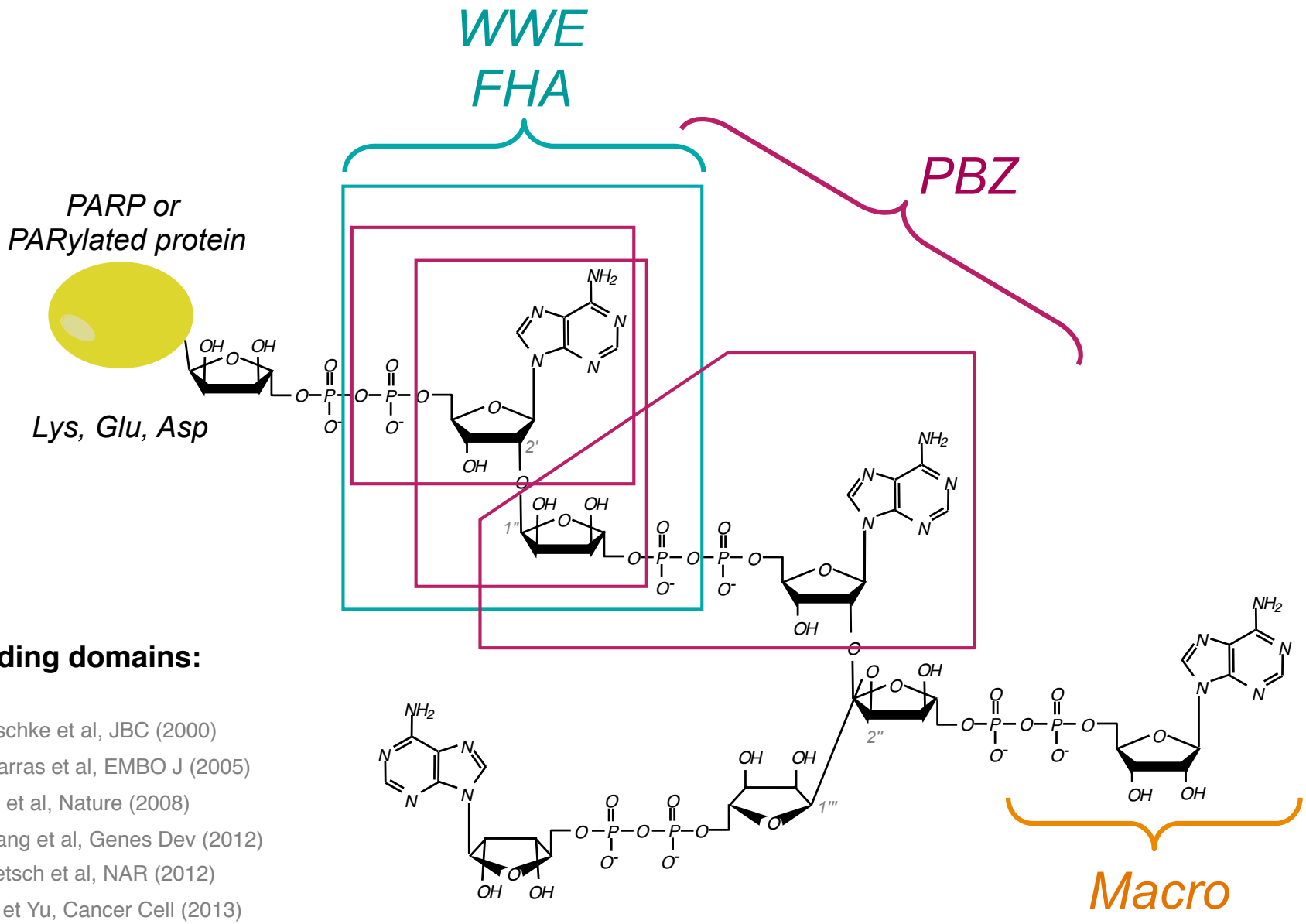
Poly(ADP-ribose) glycohydrolase

- immediate response
- highly energy consuming
- transient

Role of PAR:

- modification of the structural and functional properties of target proteins
- scaffold molecule regulating protein localisation and interactions

PAR bound to proteins: covalent modification or non-covalent binding



PAR binding domains:

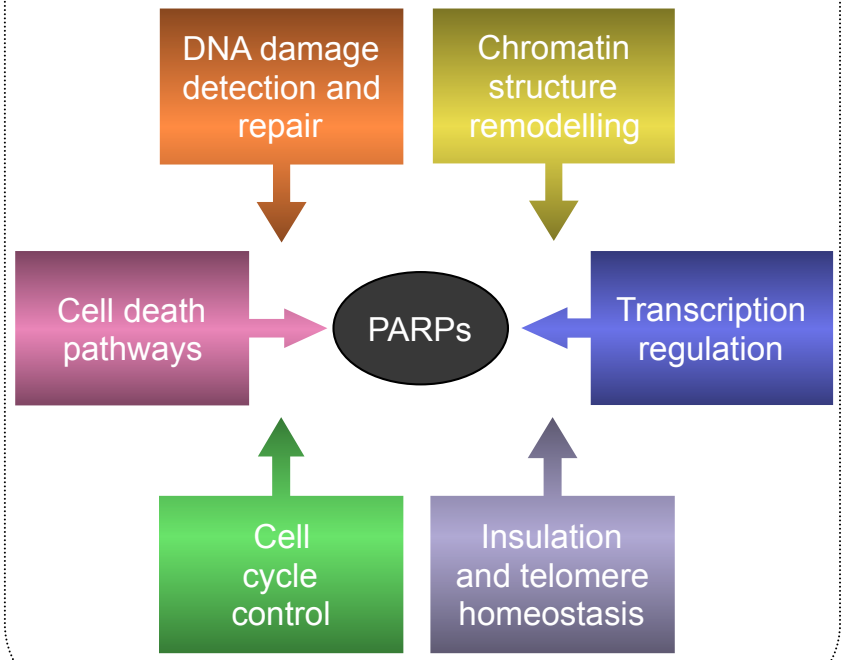
PBM Pleschke et al, JBC (2000)
macro Karras et al, EMBO J (2005)
PBZ Ahel et al, Nature (2008)
WWE Wang et al, Genes Dev (2012)
RRM Krietsch et al, NAR (2012)
BRCT Li et Yu, Cancer Cell (2013)
FHA Li et al, Genes Dev (2013)

Kalisch et al, TIBS 2012

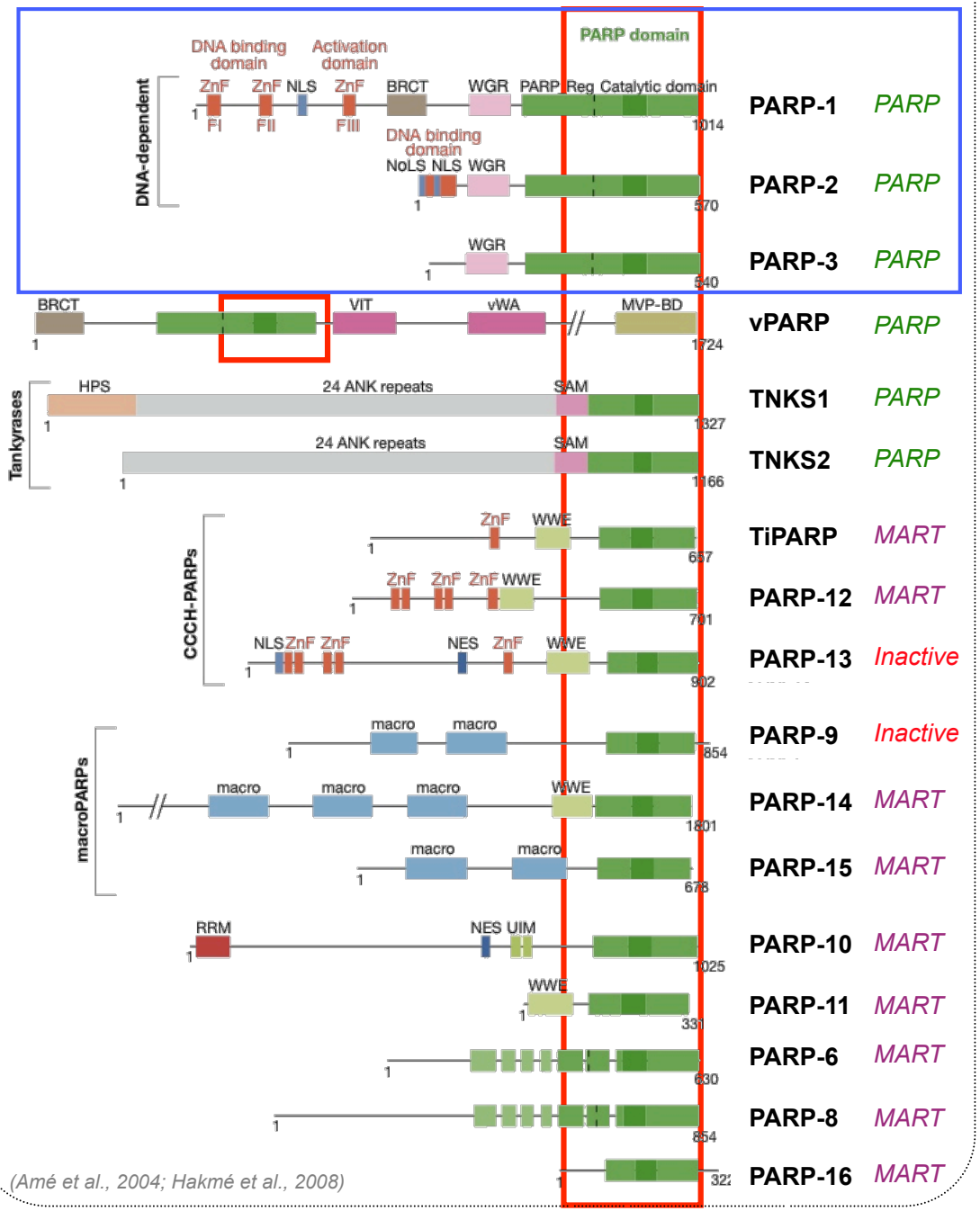
The PARP family

PARP-1, PARP-2, PARP-3
Activated by damaged DNA

… involved in diverse cellular processes



17 members, a conserved catalytic domain

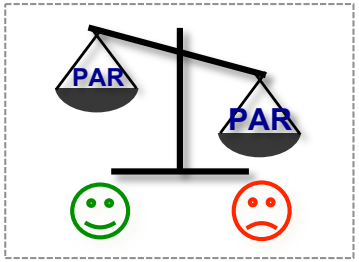


(Amé et al., 2004; Hakmé et al., 2008)

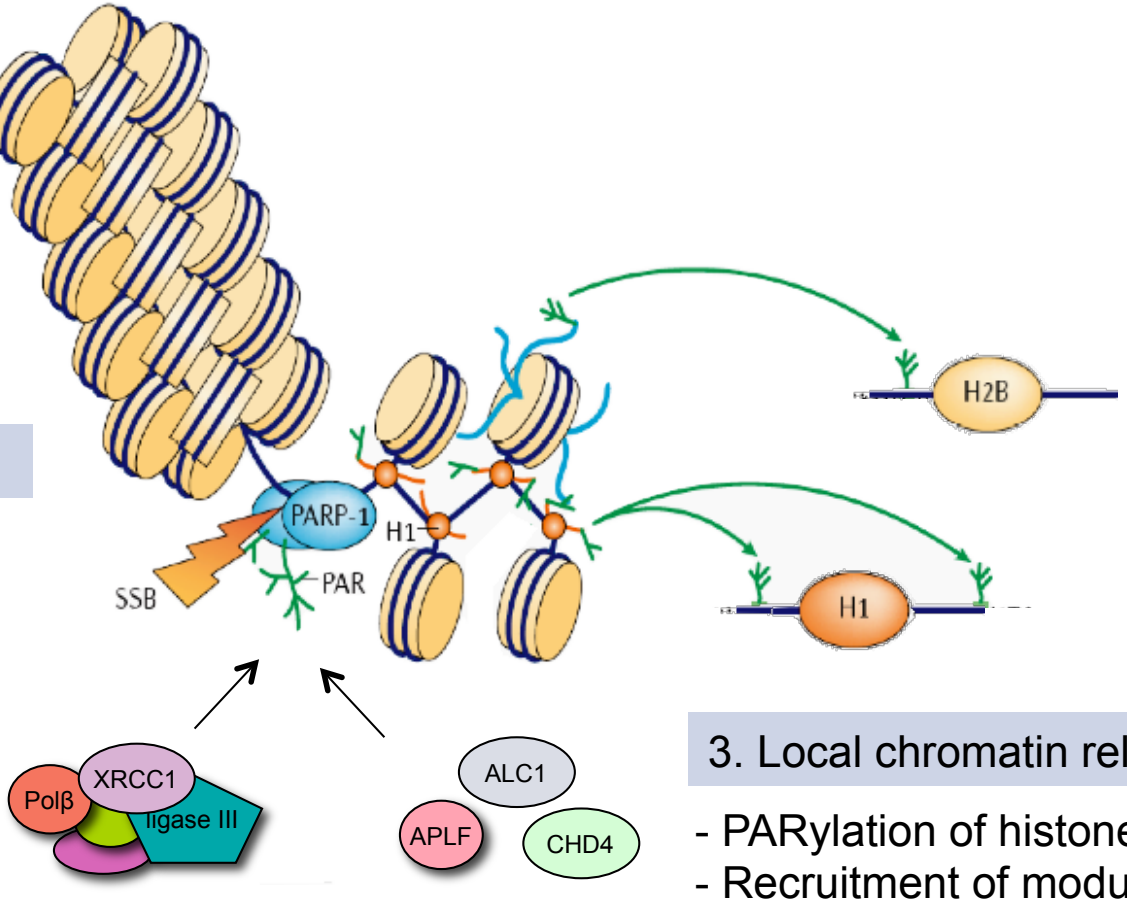
Functions of PARP-1 and PAR in the DNA damage response: Detection – Signaling - Recruitment – Repair

1. Detection of the break

2. PAR synthesis = signaling



Survival/death



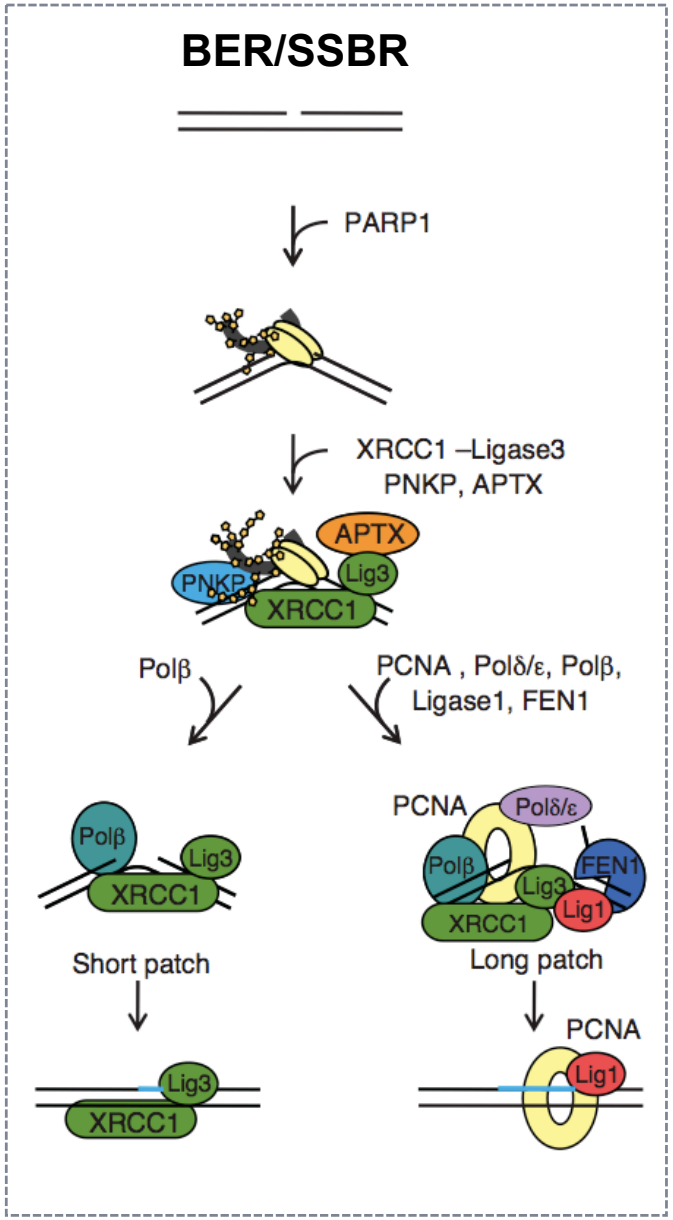
3. Local chromatin relaxation

- PARylation of histones
- Recruitment of modulators of chromatin structure

4. Recruitment of repair factors

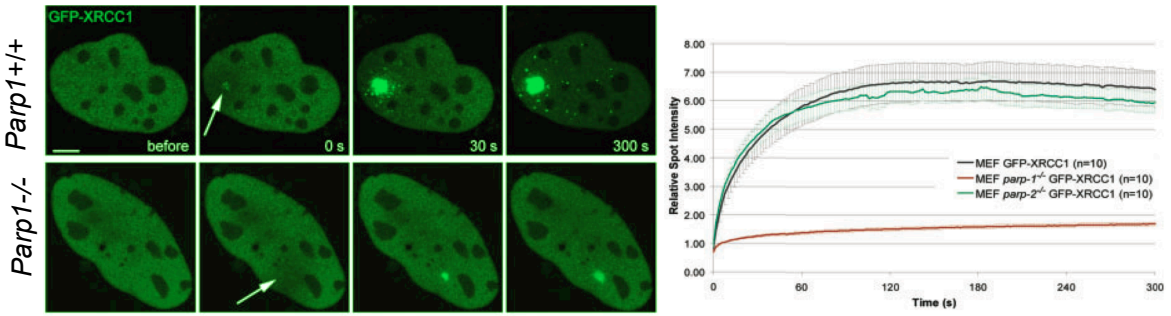
XRCC1: scaffold protein, interacts with and stimulates SSBR/BER proteins

Functions of PARP-1 and PAR in the DNA damage response: Detection – Signaling - Recruitment – Repair



Taken from Bryant et al, EMBO J (2005)

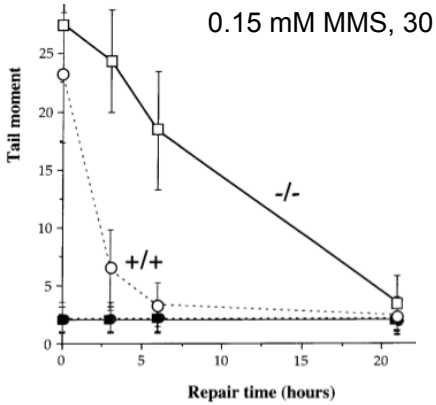
Recruitement of XRCC1 depends on PARP-1/PAR

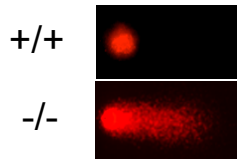


Mortusewicz et al. NAR, 2007

Okano et al., 2003; El-Khamisy et al., 2003, Godon et al, 2008, …

Parp1-/-: delay in BER/SSB repair





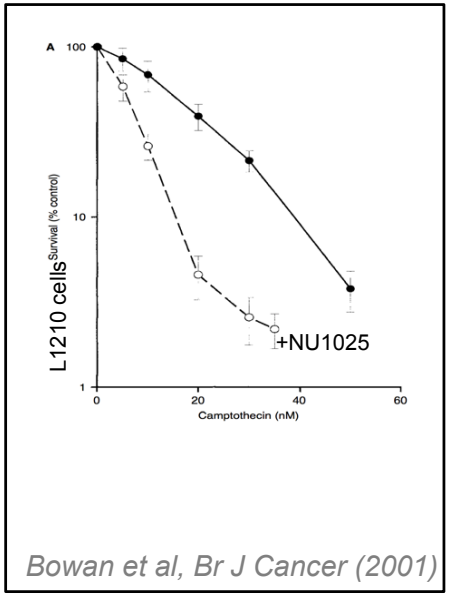
COMET assay
5h post MMS

Trucco et al, NAR 1998

PARP-1: required for efficient BER/SSBR

PARP-1 depletion or inhibition sensitizes cells and mice to genotoxic drugs generating SSB

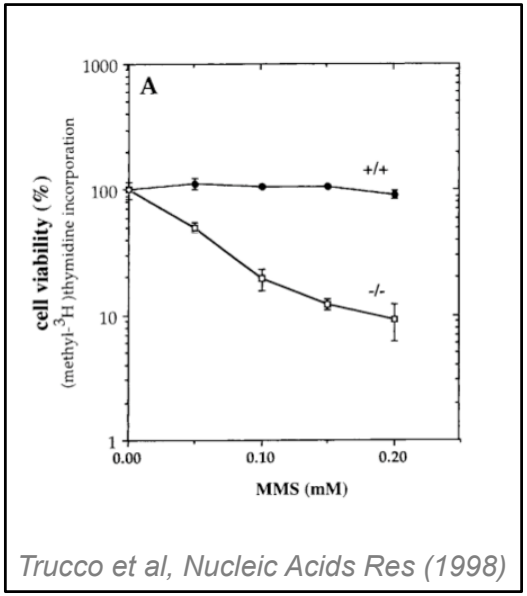
Camptothecine (CPT)



Bowan et al, Br J Cancer (2001)

PARP inhibitor (NU1025)

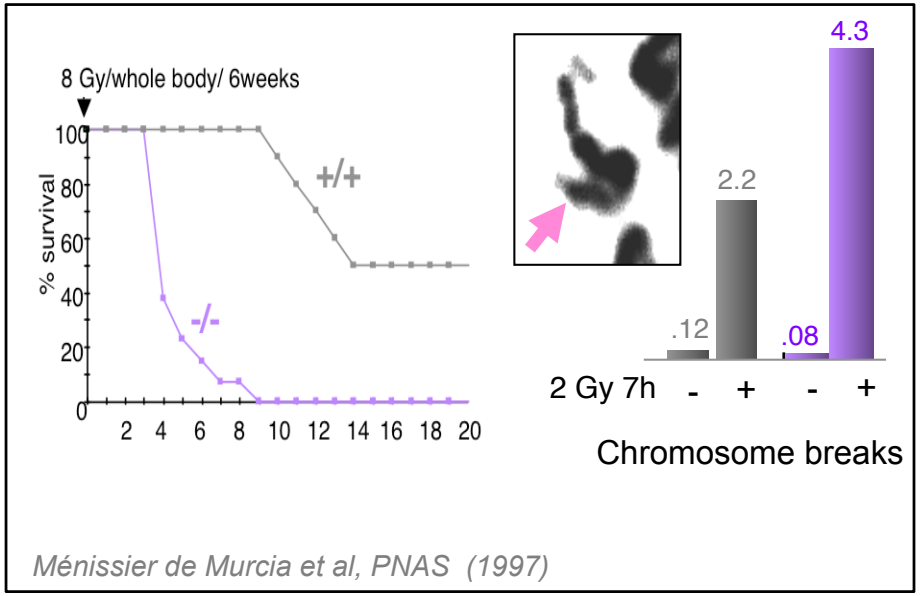
Alkylating agent (MNNG)



Trucco et al, Nucleic Acids Res (1998)

PARP-1$^{-/-}$ cells

Ionizing radiation (rayons X)



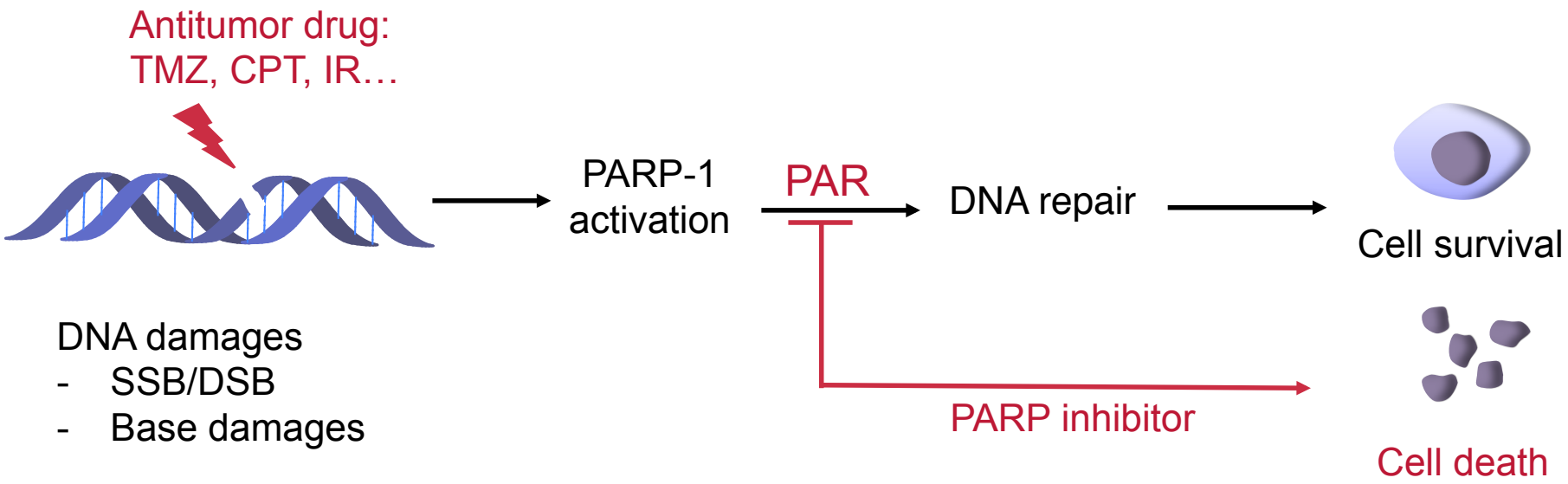
Ménissier de Murcia et al, PNAS (1997)

PARP-1$^{-/-}$ mice

Hypothesis:

PARP inhibition to potentialize radio- or chemotherapies

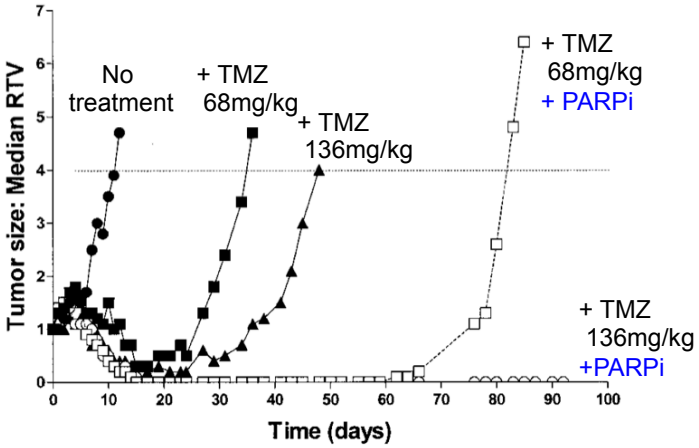
PARP inhibition in anticancer strategies: to potentialize radio or chemotherapies



PARP inhibitors as adjuvant in chemotherapy: clinical trials

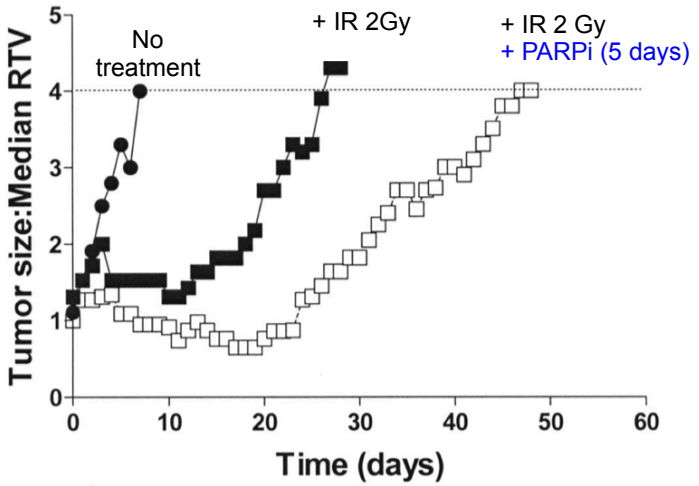
Xenografts of SW620 colorectal cancer cells:

TMZ+ PARPi (Rucaparib)



Taken from Calabrese et al, J Natl Cancer Inst (2004)

IR + PARPi (Rucaparib)



2003: First clinical assays Phase I (melanomas + TMZ+ PARPi)
Agouron Pfizer, La Jolla, CA; Curtin N., Newcastle, UK

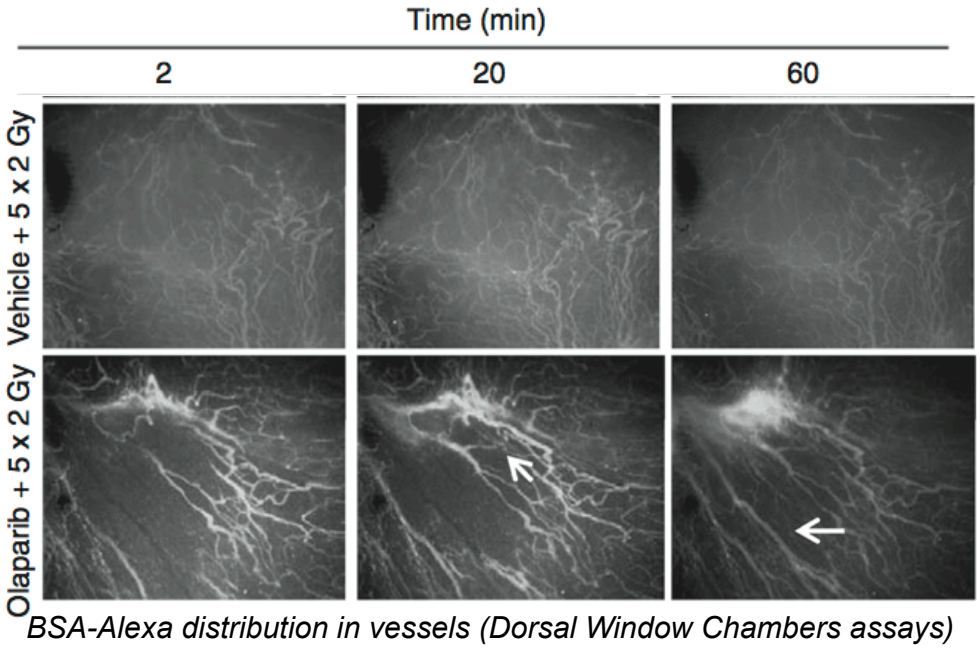
Phase I-II clinical trials in progress:
Melanoma, glioblastoma, breast, ovary, prostate, lung, leukemia…

PARPi + IR: glioma, NSCLC; HNSCC

PARP inhibitors improve vascular perfusion

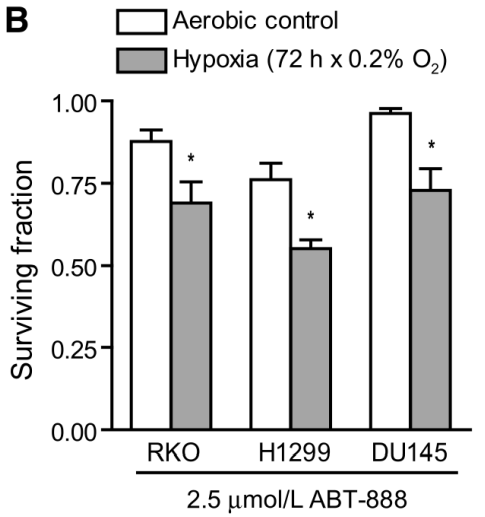
• PARPi or Parp1-/-:

- Vasodilatation, increased perfusion of tumors
- Increased oxygenation (decreased hypoxia)
- Sensitizes hypoxic malignant cells



BSA-Alexa distribution in vessels (Dorsal Window Chambers assays)

Taken from Senra et al. Mol Cancer Therapeutics (2011)

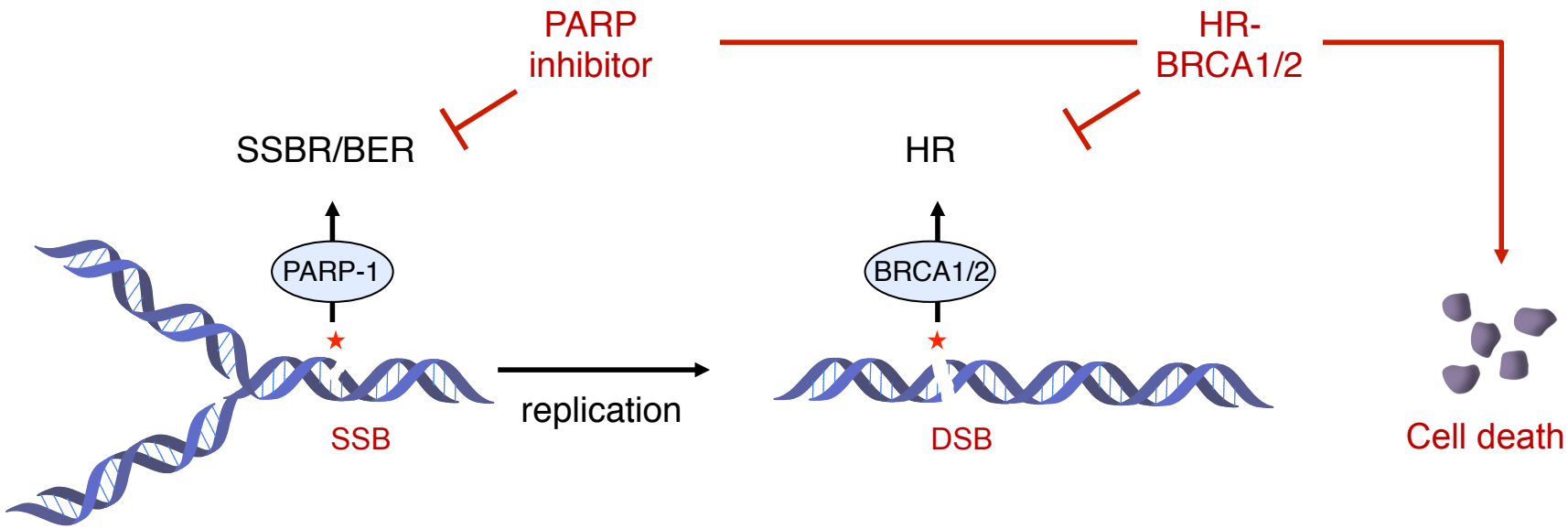


Taken from Chan et al. Cancer Cell (2010)

Contribute to the antitumoral effects of PARPi in combined therapies

PARP inhibitors to kill BRCA1/BRCA2 tumors by synthetic lethality

Breast/ovary cancers: genetic origin in 5-10%
Half have defect in BRCA1 or BRCA2 genes



Advantages of this therapeutic strategy :

- monotherapy
- targets only tumor cells (both BRCA alleles are mutated)
- Phase I-III clinical trials in progress: breast, ovary, prostate…

Phase II: Olaparib on 136 BRCA ovarian cancers: 82% reduction in the risk of disease progression or death and a median PFS of 11.2 vs 4.3 months

PARP inhibitors to kill BRCA1/BRCA2 tumors by synthetic lethality

Table 1 | **PARP inhibitors in late-stage development**

Company	Agent	Phase III indications
AstraZeneca (from the 2005 KuDOS Pharmaceuticals acquisition)	Olaparib (also known as AZD-2281)	• Ovarian cancer with *BRCA* mutations • One trial in relapsed disease, one in frontline setting • Both trials as maintenance therapy
Tesaro (licensed from Merck)	Niraparib (also known as MK4827)	• *BRCA* and non-*BRCA* platinum-sensitive serous ovarian cancer (NOVA trial), maintenance setting • Breast cancer trial in germline metastatic disease pending
BioMarin Pharmaceuticals	BMN 673	• Germline *BRCA*-mutant metastatic breast cancer
Clovis Oncology (licensed from Pfizer)	Rucaparib (also known as CO288, AGO14699 or PF01367338)	• BRCA and non-BRCA platinum-sensitive serous ovarian cancer, maintenance setting • Will separate non-*BRCA* patients based on HRD diagnostic
AbbVie	Veliparib (also known as ABT-888)	• Undisclosed to date

BRCA, breast cancer susceptibility; HRD, homologous recombination deficiency; PARP, poly(ADP-ribose) polymerase.

Chemo/radioresistance and side effects of PARP inhibition-based therapies

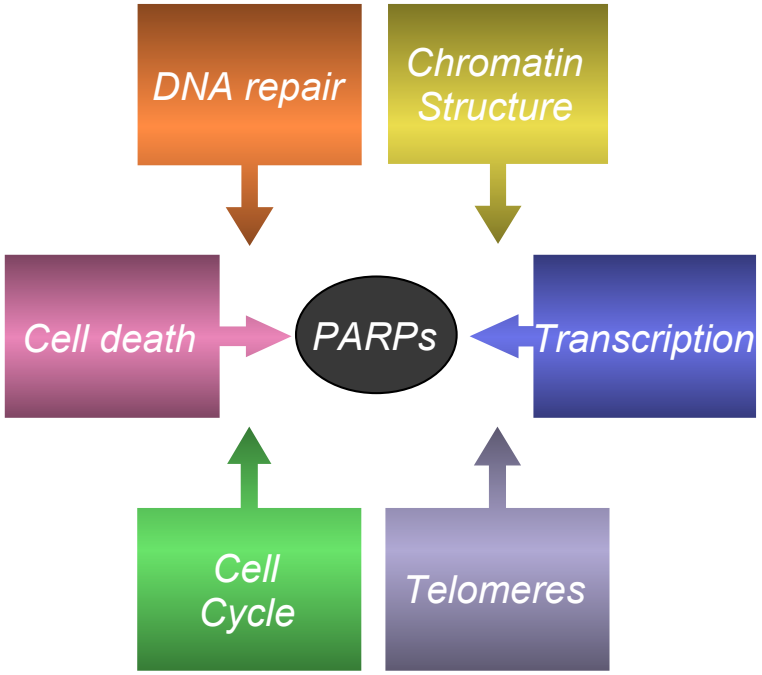
resistance

- Decreased intracellular availability of PARPi
  - expression of multidrug resistance P-glycoproteins involved in efflux of PARPi
- Reversion of the HR defect in synthetic lethality strategies:
  - Reverse mutation of BRCA
  - Overexpression of BRCA
  - Mutation of 53BP1 that shortcuts BRCA1 defect and restores HR

Side effects

- Nausea, fatigue
- Myelosuppression

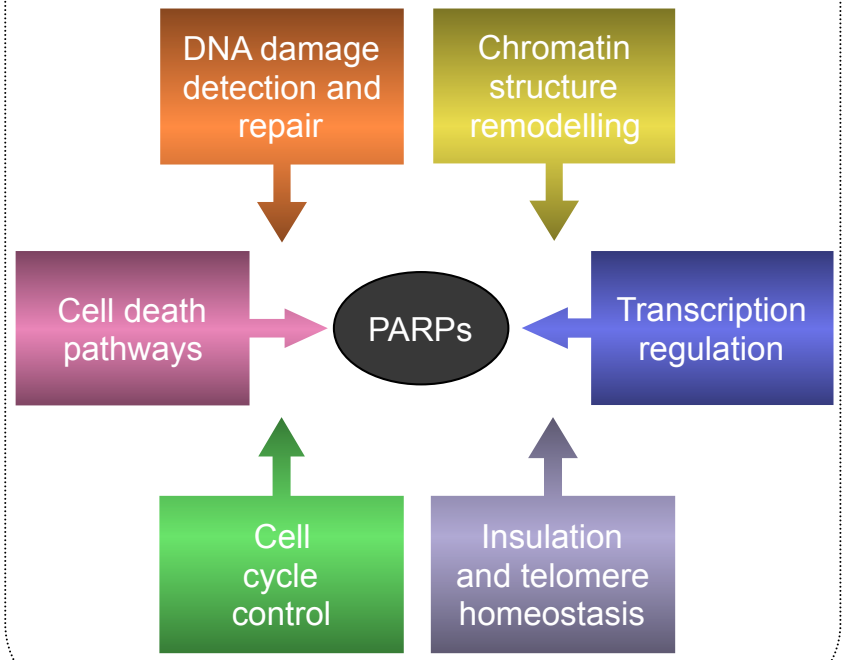
PARP inhibitors: non specific to PARP-1 !
Inhibits several PARPs, several pathways !



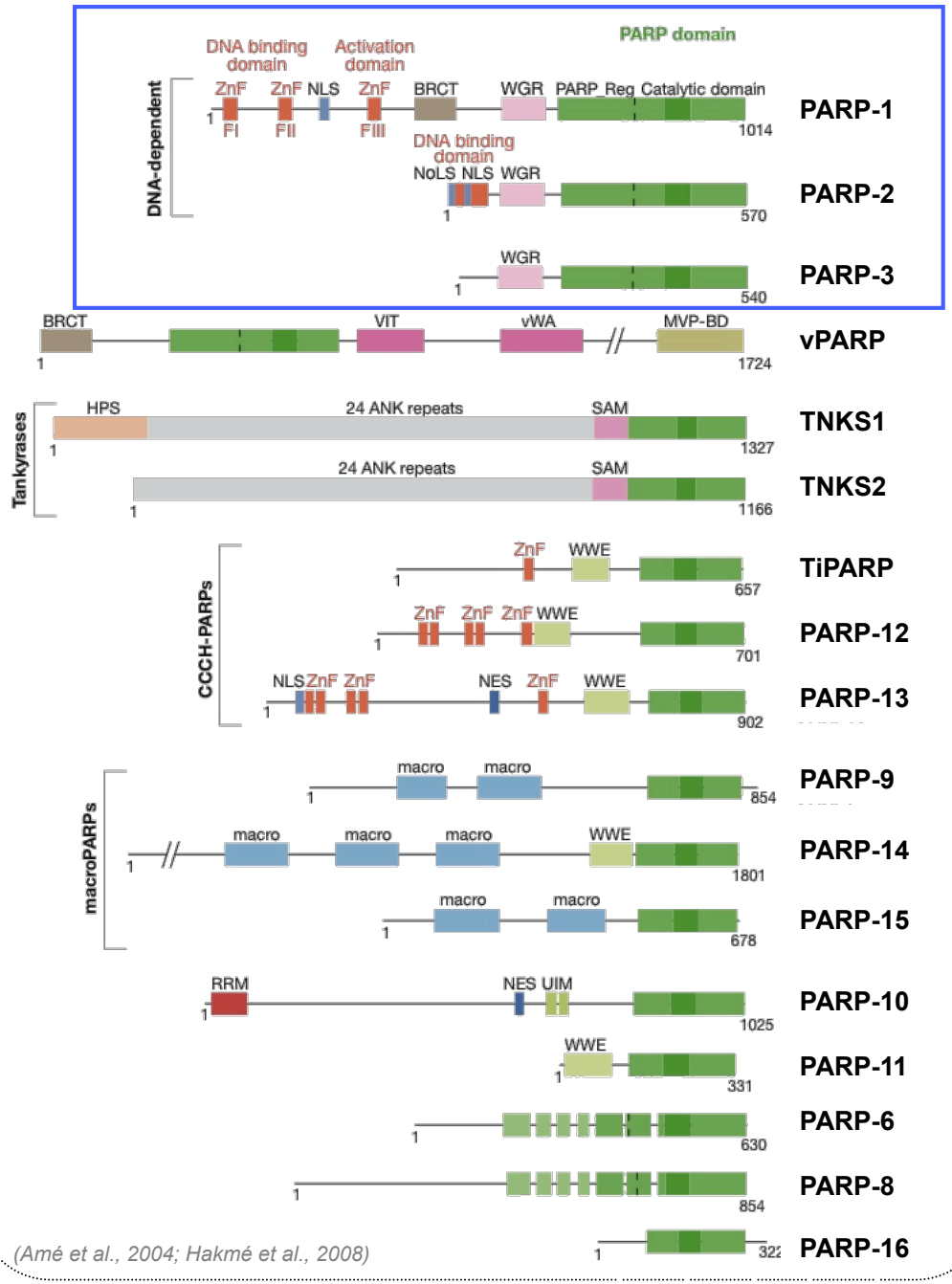
The PARP family

PARP-1, PARP-2, PARP-3
Activated by damaged DNA

… involved in diverse cellular processes

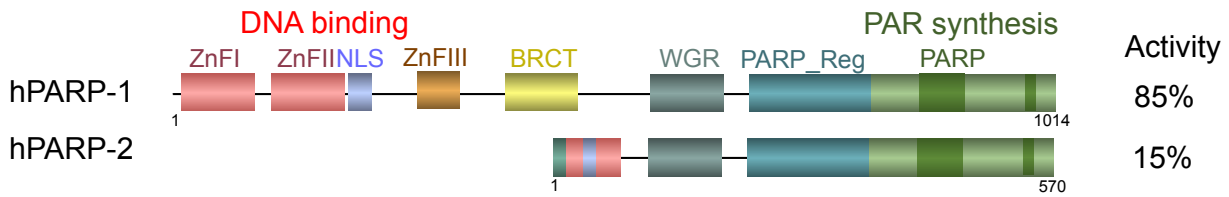


17 members, a conserved catalytic domain

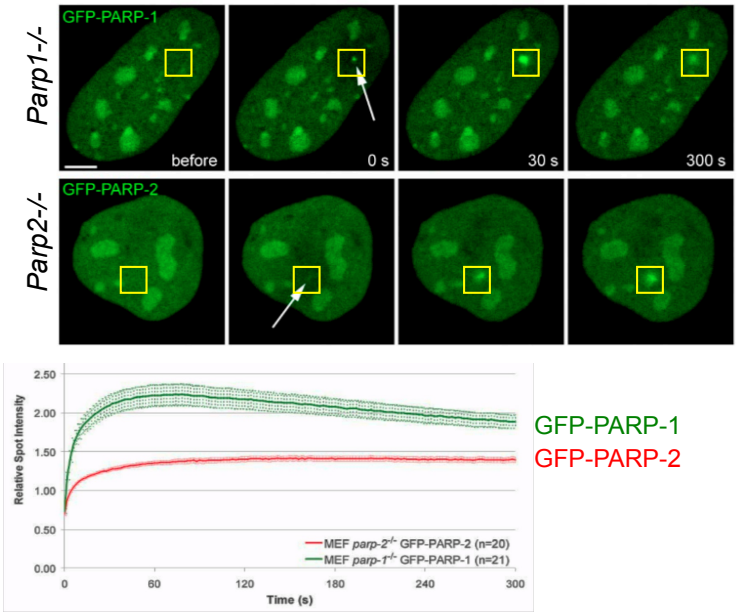


(Amé et al., 2004; Hakmé et al., 2008)

PARP-2 is involved in SSBR

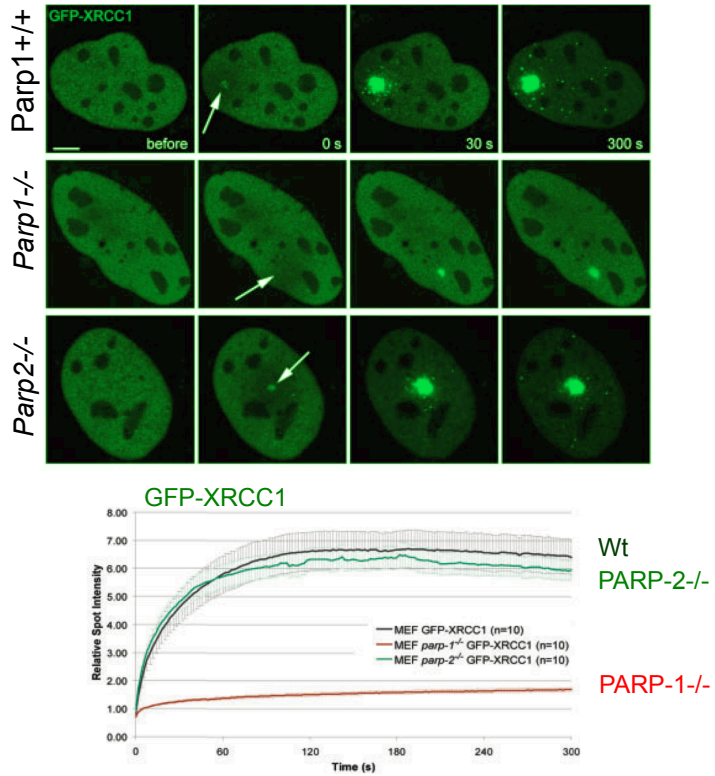


Slower but persistent recruitment of PARP-2



Hyp: PARP-2 acts at later steps of the repair process ?

Recrutement of XRCC1 does not depend on PARP-2



Mortusewicz et al., NAR (2007)

- Interacts with and PARsylates XRCC1, polß, ligase III Schreiber et al., JBC (2002)
- Binds to DNA structures mimicking repair intermediates Kutuzov et al., Biochimie (2013)

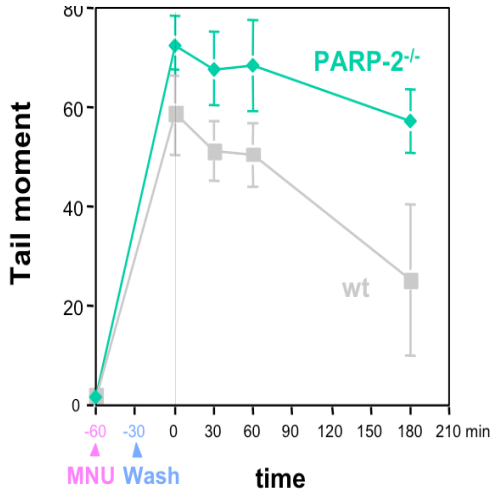
Parp2-/- mice and cells: increased sensitivity to IR

Parp2$^{-/-}$ mice and cells:

- Viable, not tumor prone

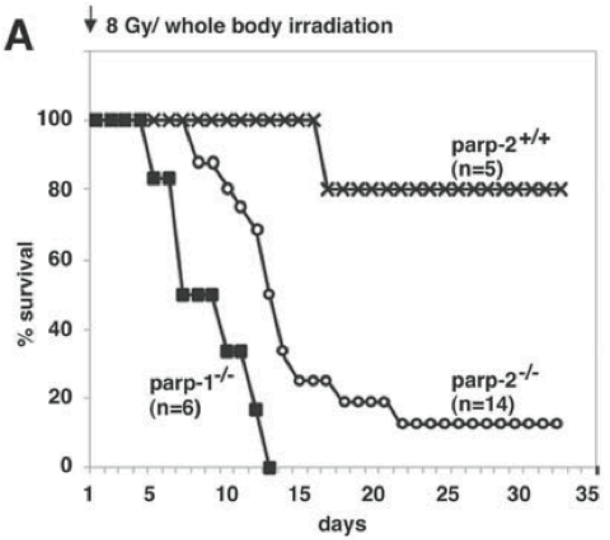
Upon genotoxic insult:

- Delay in SSB rejoining
- Increased apoptosis
- increased genome instability



Schreiber et al, JBC (2002)
Ménissier de Murcia et al, EMBO J (2003)

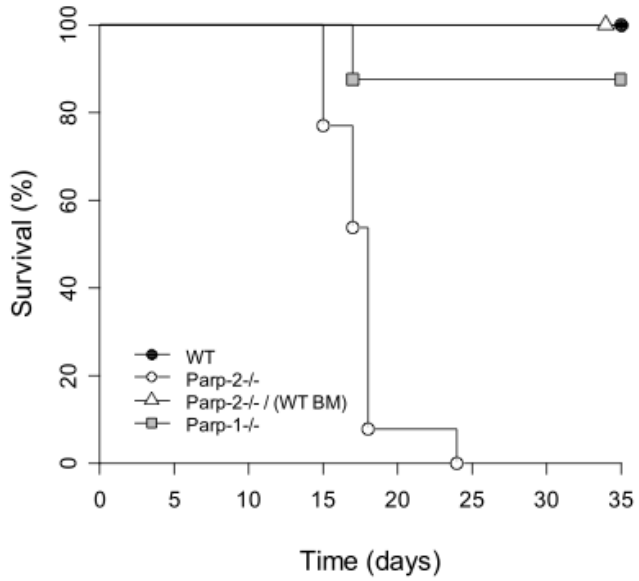
Radiosensitization of Parp2-/- mice



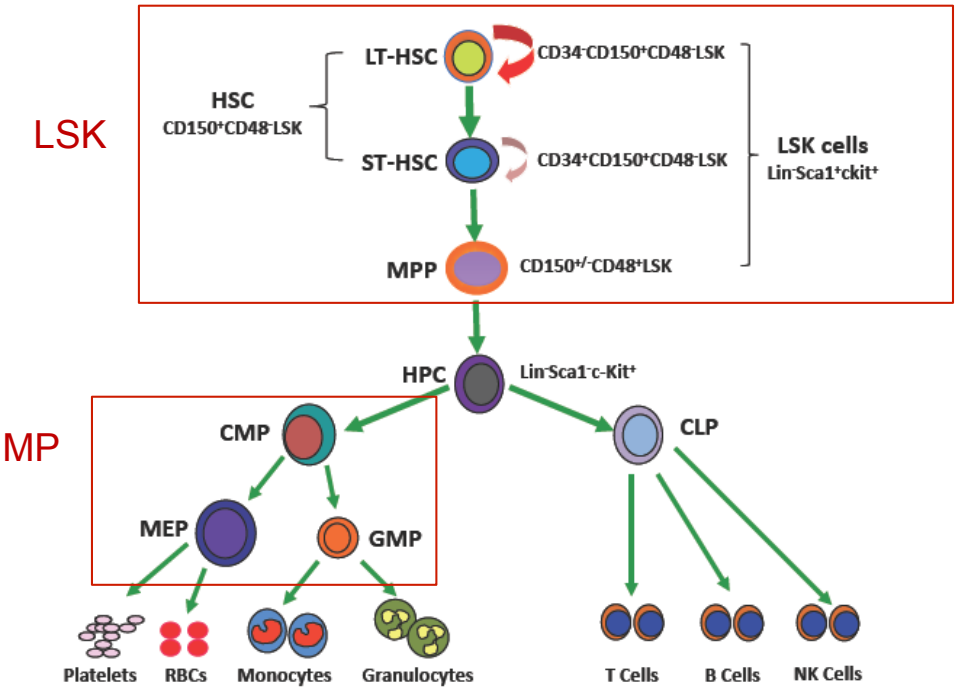
Ménissier de Murcia et al, EMBO J (2003)

Parp2-/- mice: radiosensitivity of hematopoietic/progenitor stem cells

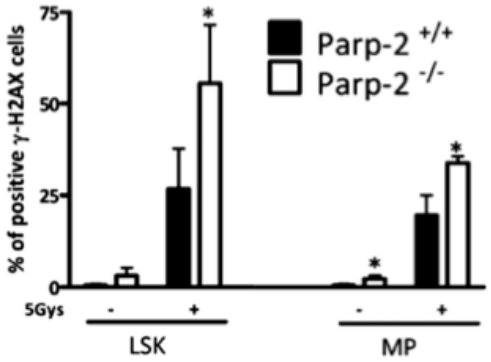
Parp2-/-, not Parp1-/- mice, are sensitive to sublethal irradiation (5 Gy)



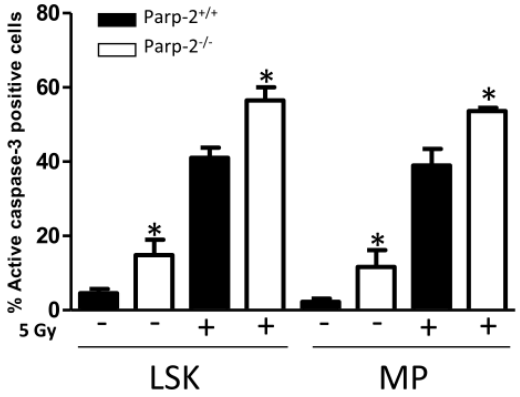
Bone marrow failure in irradiated Parp2-/- mice



Increased DNA breaks in HSPC cells



Increased apoptosis in HSPC cells



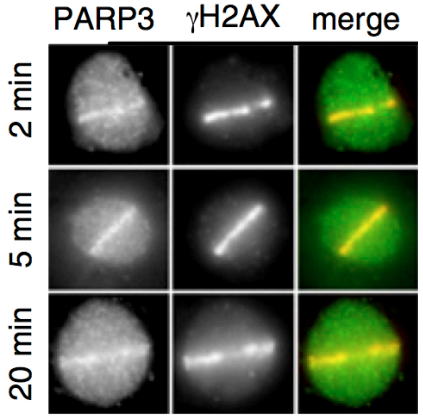
A possible explanation for the myelosuppression observed with PARPi in clinical trials ?

Need for more specific PARP-1 inhibitors ?

Farrés et al., Blood (2013)

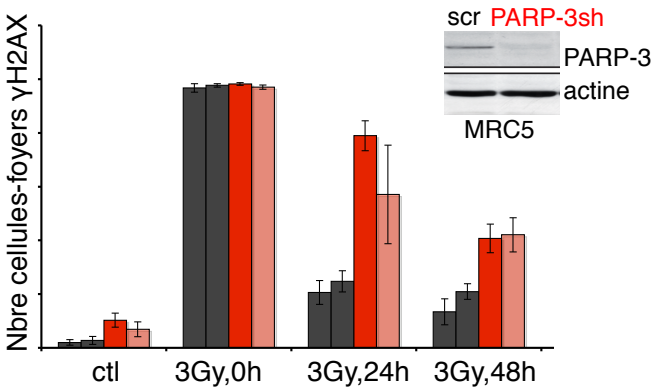
PARP-3 is involved in DSBR

Recruited to DNA damages

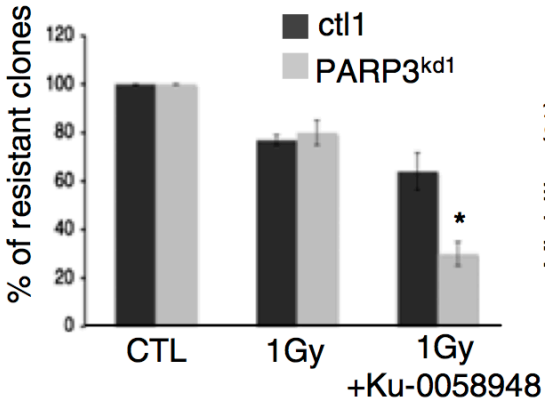


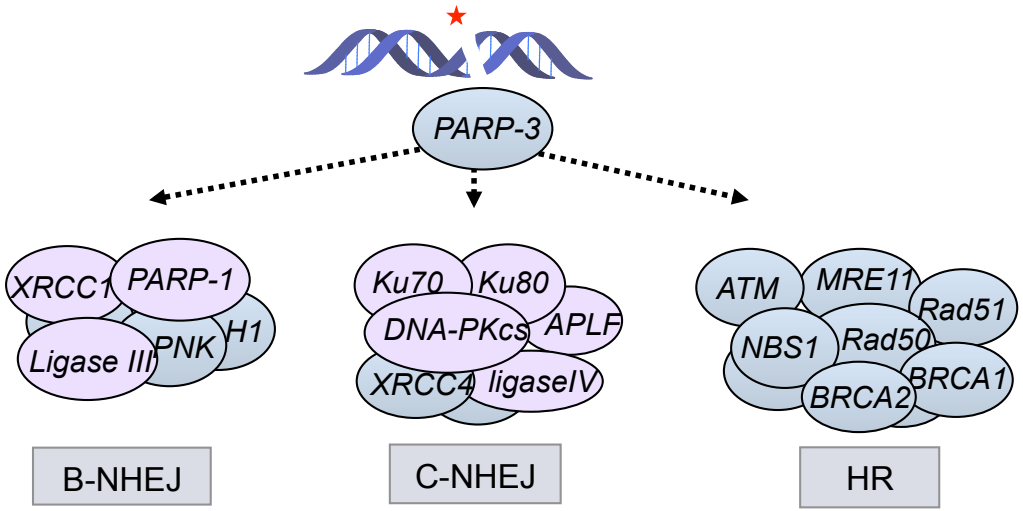
PARP3kd cells:

Defective DSB (not SSB) repair



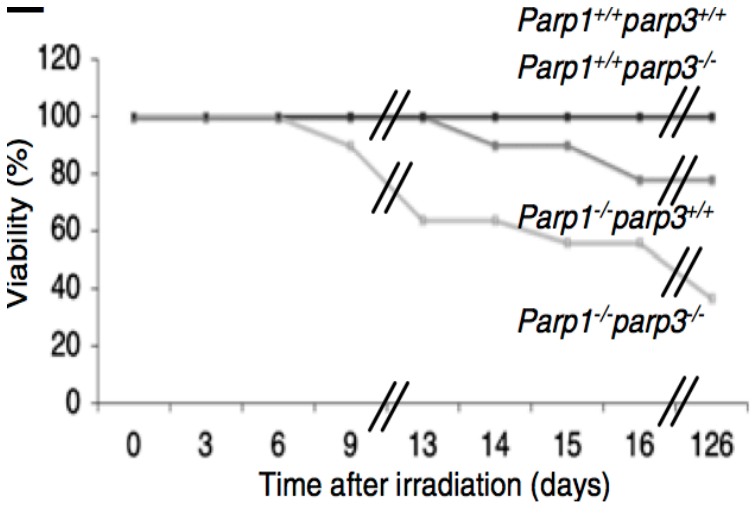
No increased radiosensitivity





Boehler et al. PNAS (2011)
Boehler et al. Cell Cycle (2011)
Beck et al, en révision

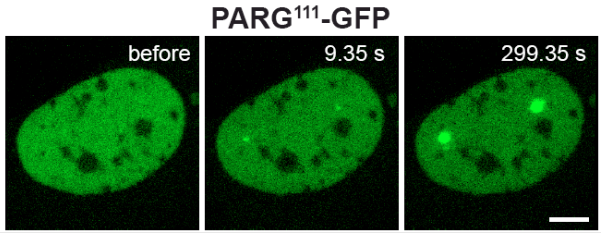
PARP-3$^{-/-}$/PARP-1$^{-/-}$: increased sensitivity to IR

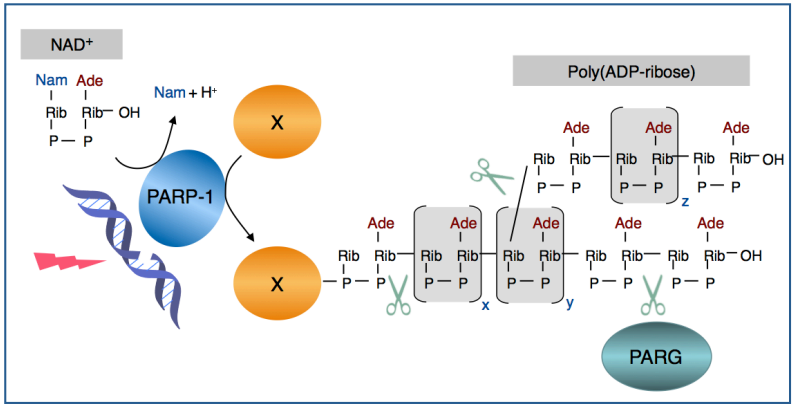


Boehler et al. PNAS (2011)
Boehler et al. Cell Cycle (2011)
Beck et al, en révision

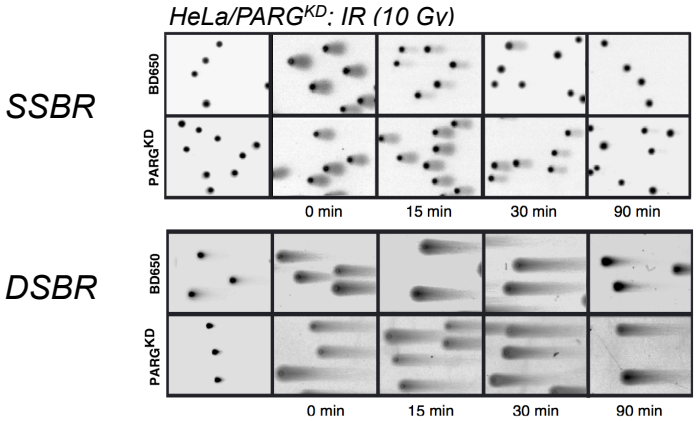
PARG, another therapeutic target for cancer therapy ?

Several isoforms (Parg-/-: lethal)
Recruited to DNA damages

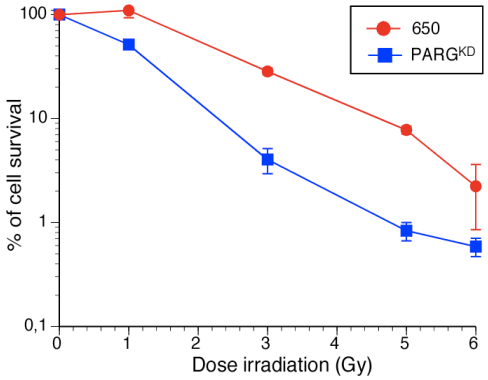




Delay in repair of radioinduced
SSB and DSB



Increased radiosensitivity



Sensitivity to
MMS, HU…

PARG inhibitors: to potentialize radio or chemotherapies ?

Amé, Fouquerel et al. JCS (2009)
Mortusewicz, Fouquerel et al (2011)

Targeting poly(ADP-ribose) metabolism in anticancer strategies

PARP inhibitors:

- To **potentialise** chemo and radiotherapies
- To target HR-deficient tumors (**synthetic lethal** with BRCA1, BRCA2,…)

But:

- Affects **several biological processes**
- May affect **several PARPs** (PARP-1, PARP-2, PARP-3…)

Need for:

- More **specific** inhibitors
- Define better the patients/tumors that will **benefit** from these therapies
- better **characterization** of all PARP family members
- Define better the positive and negative **effects** of PARPs inhibition

Poly(ADP-ribosyl)ation and Genome Integrity

Former PhD: Elise Fouquerel