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IMProving cAre and Knowledge through Translational research

Basics in cancer cell biology

- DNA repair

Ken A. Olaussen

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OUTLINE

DNA damage response and DNA repair pathways
DNA repair and cancer
DNA repair biomarkers in breast cancer
DNA repair as anticancer target
DNA repair and targeted therapies
Perspectives and conclusions

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Cells are continuously submitted to DNA damage

- High fidelity of DNA replication machinery : = 10^{-10} spontanous mutations/base
- Endogenous metabolic activities and environmental factors are continously affecting DNA's integrity (oxidative stress, alkylating agents, UV radiation ...)
- Lesions: chemical modification of DNA bases, abasic sites, base mismatches, chemical alkylation (adducts), intra-and inter-strand cross links, single-and double-strand breaks

Table 1. DNA Lesions Generated by Endogenous and Exogenous DNA Damage

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day
Depurination	AP site	10000[a]
Cytosine deamination	Base transition	100–500[a]
SAM-induced methylation	3meA	600[a]
	7meG	4000[a]
	O^6meG	10–30[b]
Oxidation	8oxoG	400–1500[c]

Ciccia & Elledge, Molecular Cell, 2010

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- Exogenous origins of DNA damage
- DSBs more dangerous and less frequent

Lesions/day

Exogenous DNA Damage	Dose Exposure (mSv)	DNA Lesions Generated	Estimated Numb
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

Ciccia & Elledge, Molecular Cell, 2010

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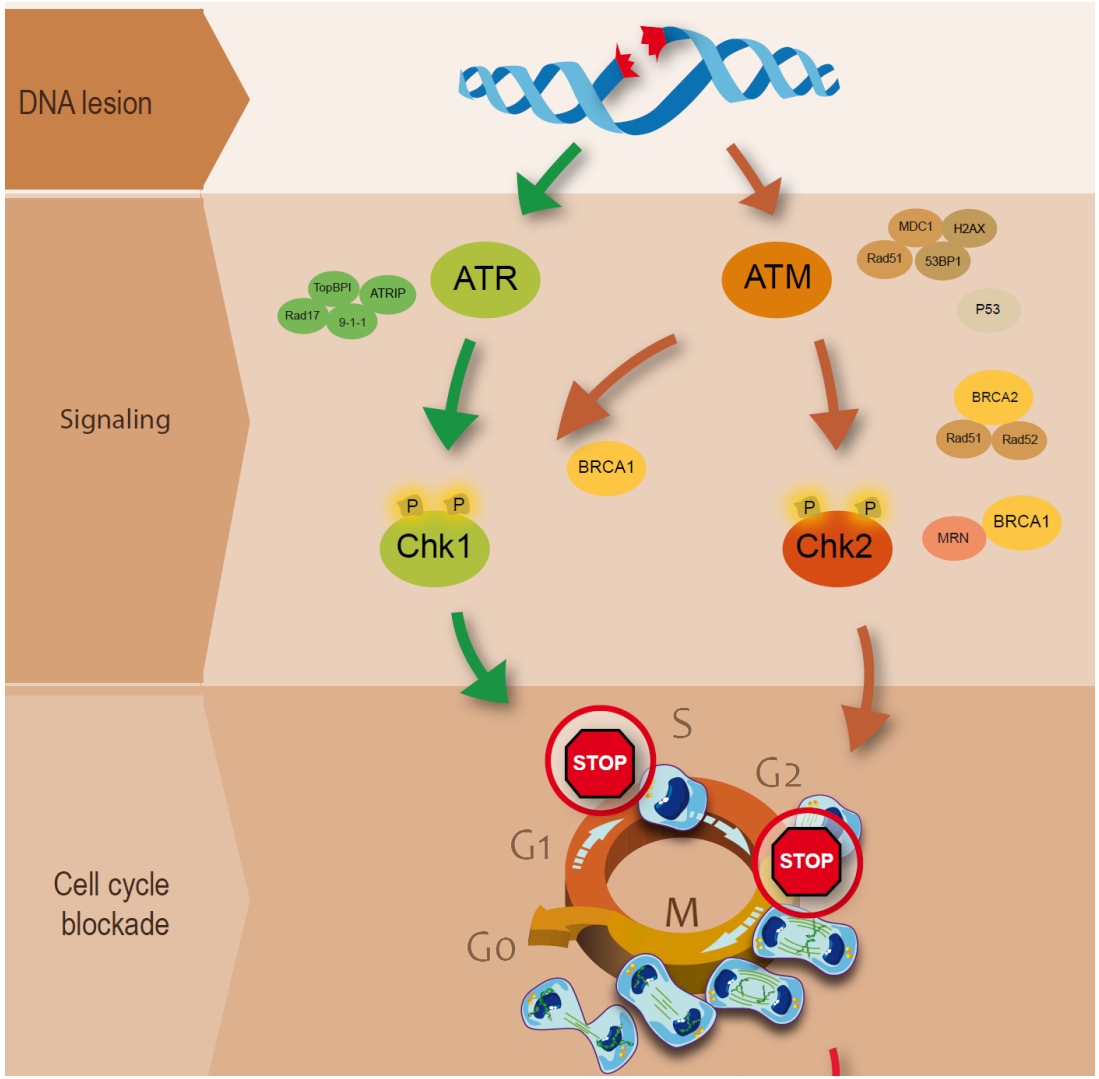
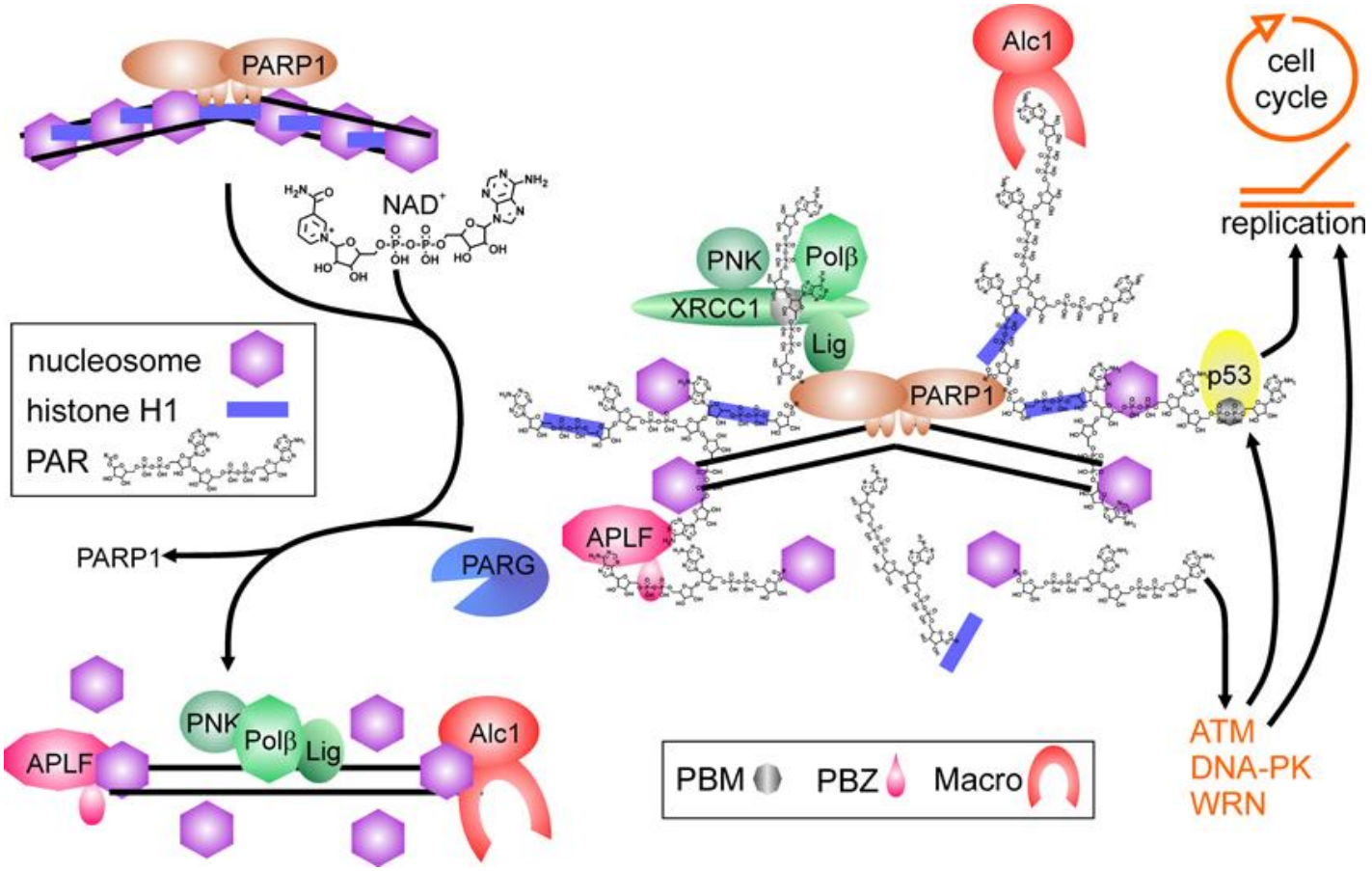
DNA Damage Response (DDR)

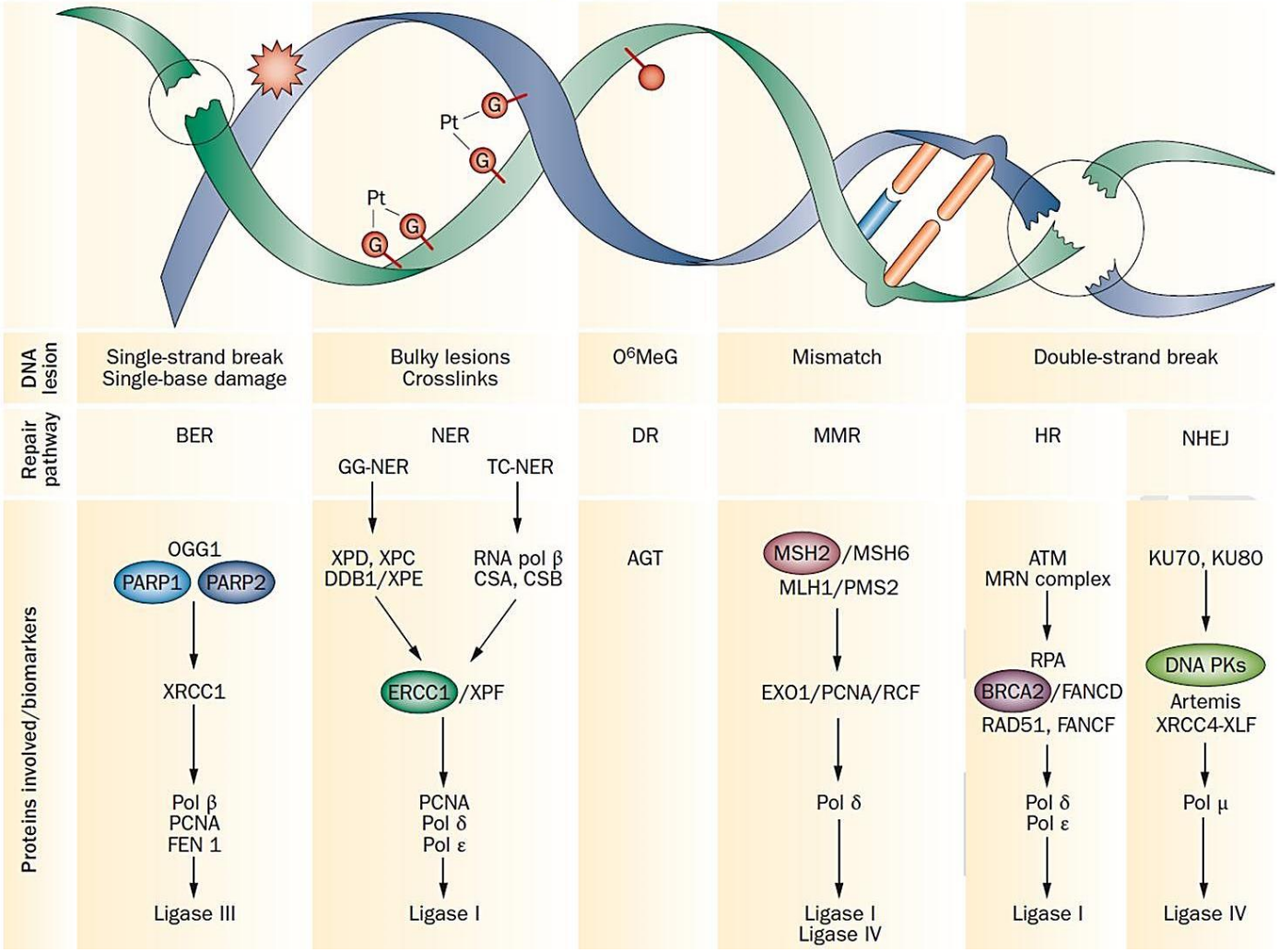


BER pathway depends on the automodification (poly(ADP-ribosyl)ation (PAR) of PARP1 to release DNA from nucleosomes and recruit repair proteins



Beneke, Front. Genet 2012

Major DNA repair pathways and biomarkers



Postel-Vinay, Nat Rev Clin Oncol 2012

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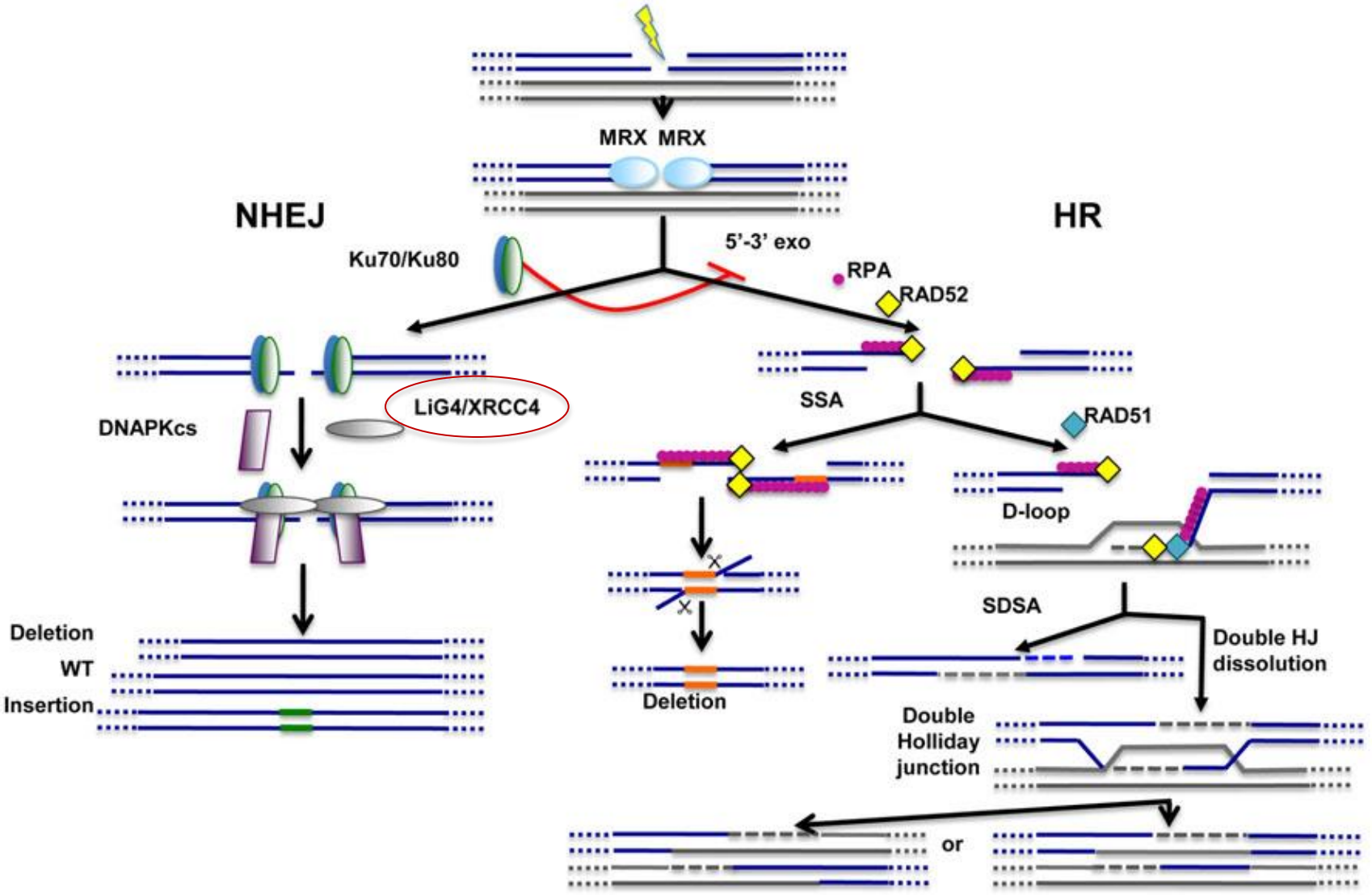
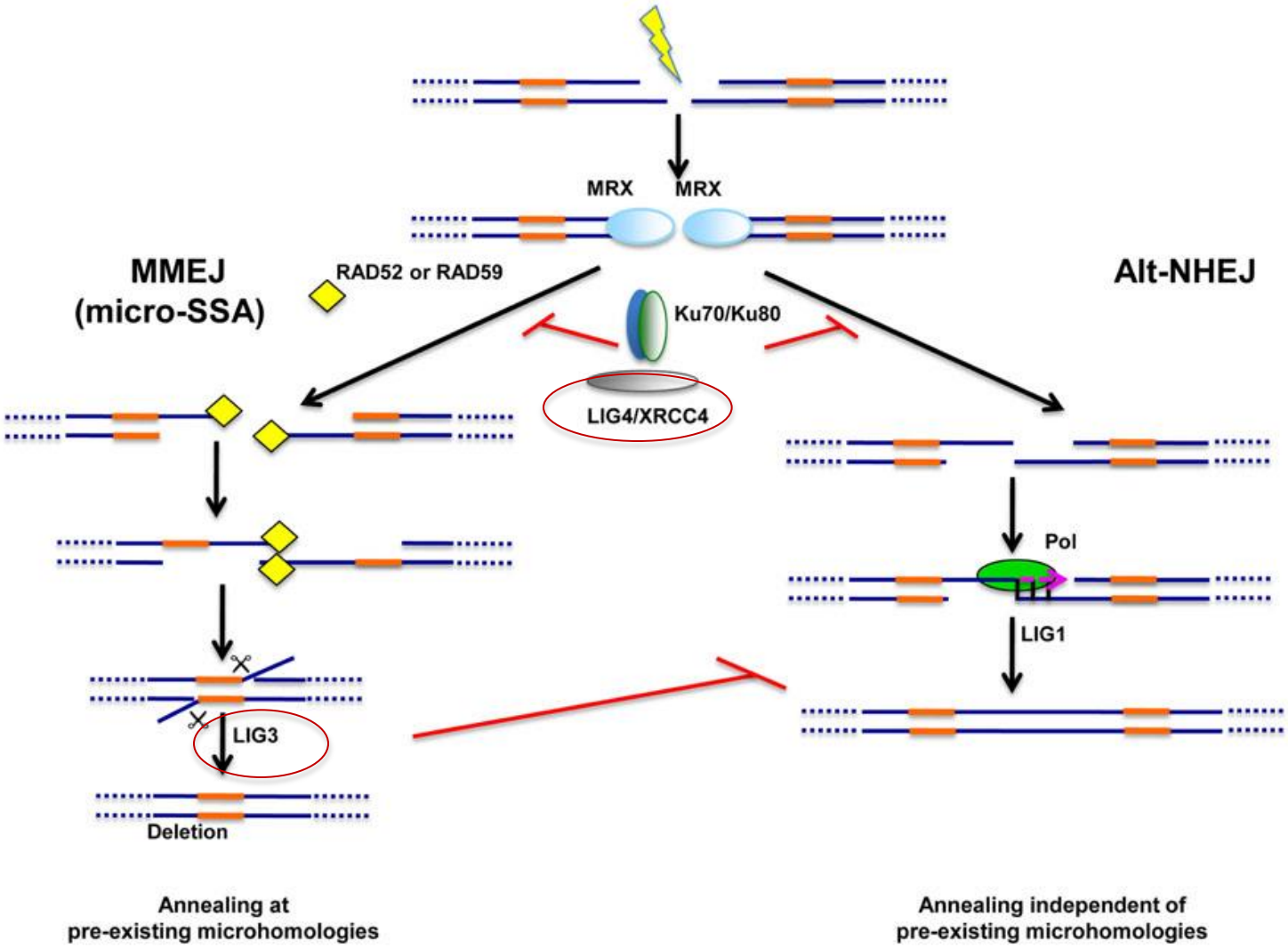
DSB repair pathways

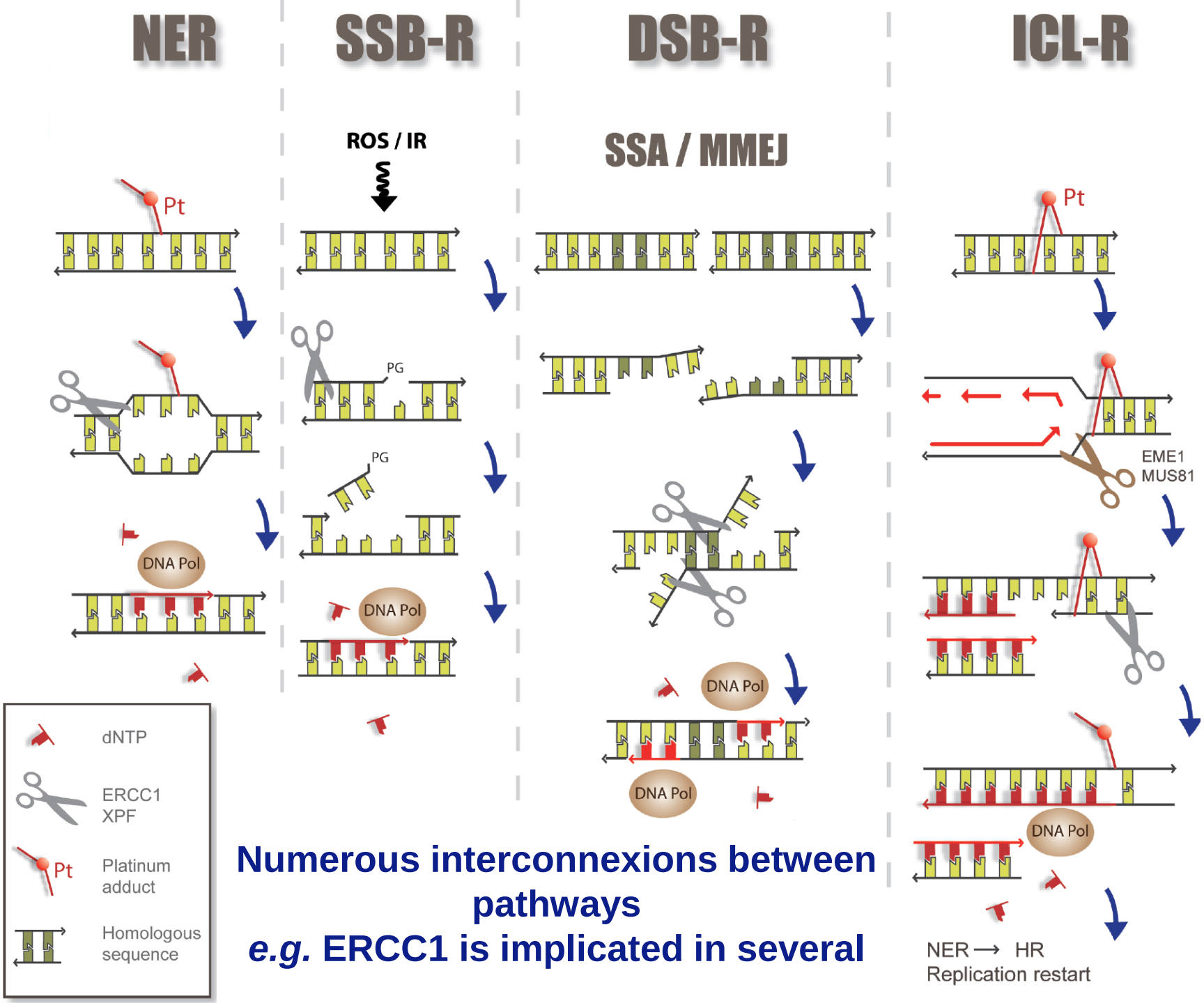


Decottignies, Front. Genet 2013

Alternative end-joining pathways



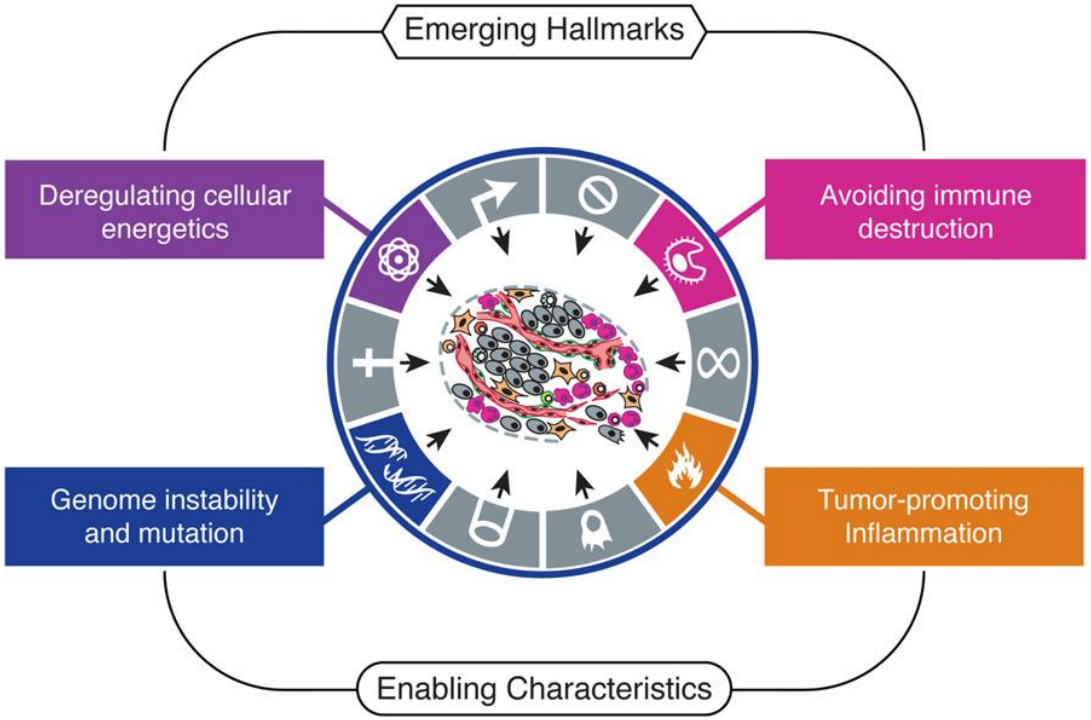
Decottignies, Front. Genet 2013



Numerous interconnexions between pathways

***e.g.* ERCC1 is implicated in several**

« Mutability endow cancer cells with genetic alterations that drive tumor progression »



Hanahan and Weinberg, Cell 2011

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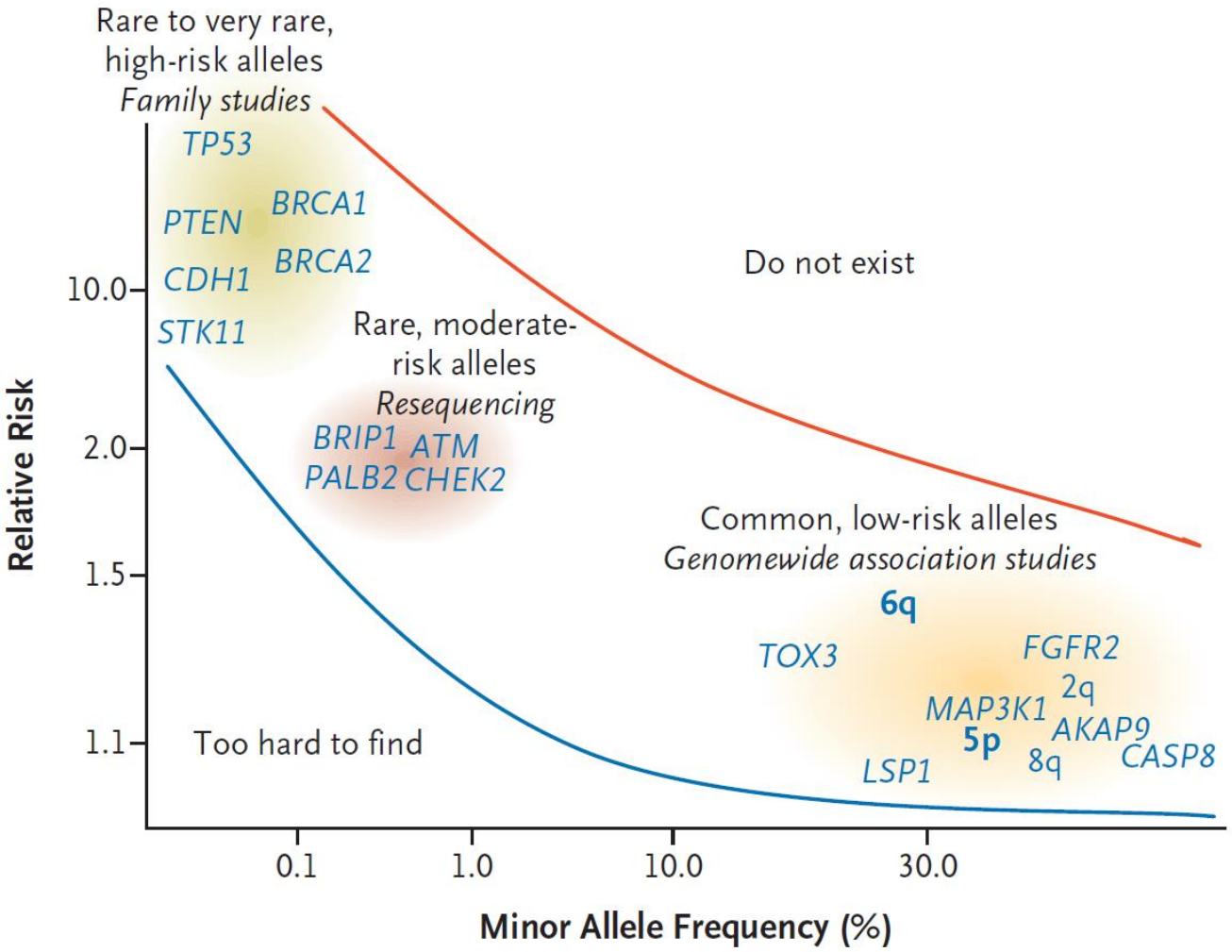
Risk alleles in breast cancer: several DNA-damage response or repair-related genes: BRCA1/2, TP53, ATM, CHEK2…



Foulkes, NEJM 2008

"The balance of life" theory supports that DNA repair mechanisms can be an anticancer target

DNA damage

Efficient DNA repair

Low DNA repair capacity

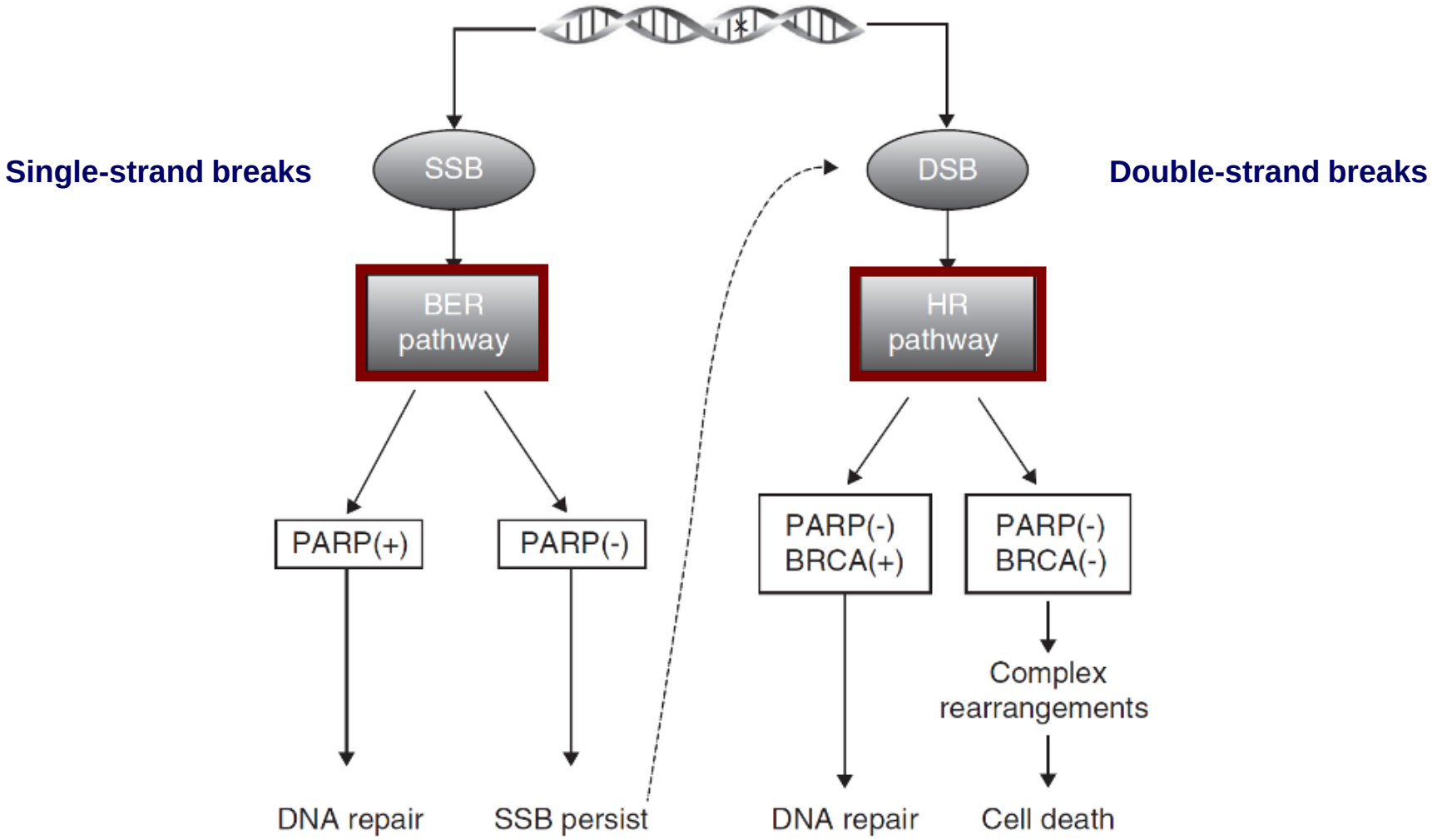
Genetic stability → CELL SURVIVAL

Genetic instability → Inhibit DNA repair !!!

Genetic instability → Genetic divergence (cancer)

Genetic instability → CELL DEATH

PARP inhibition is synthetic letal in BRCA1-deficient tumors because HR pathway is a back-up pathway for unrepaired SSBs



Rodon et al, Expert opinion 2009

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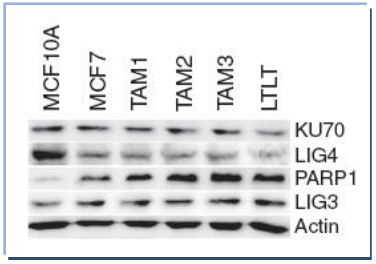
DNA Damage and Cellular Stress Responses

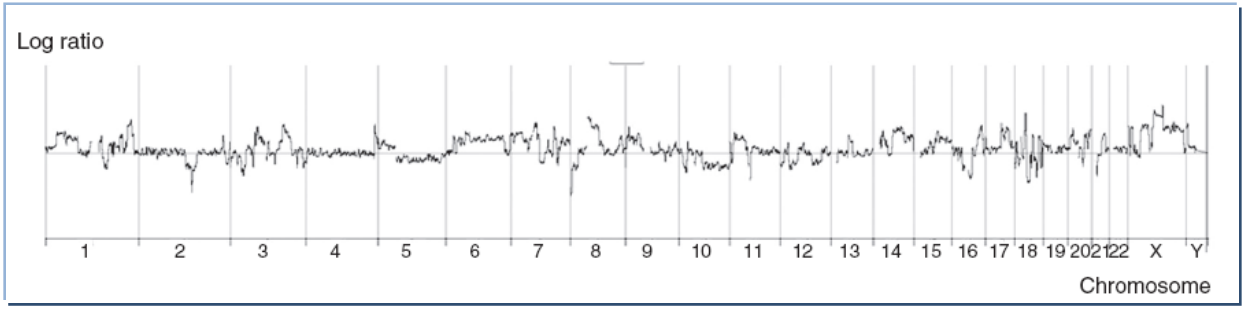
Molecular Cancer Research

Targeting Abnormal DNA Repair in Therapy-Resistant Breast Cancers

Lisa A. Tobin[1], Carine Robert[1], Pratik Nagaria[1], Saranya Chumsri[2], William Twaddell[3], Olga B. Ioffe[3], George E. Greco[5], Angela H. Brodie[4], Alan E. Tomkinson[6], and Feyruz V. Rassool[1]



Altered expression of DNA repair proteins in breast cancer cell lines with acquired resistance to the antiestrogens tamoxifen and letrosole



The increase of Ligase 3 favors error-prone ALT NHEJ resulting in large deletions and chromosomal translocations in therapy- resistant cells

Tobin et al, Mol Cancer Res, 2012

DNA Damage and Cellular Stress Responses

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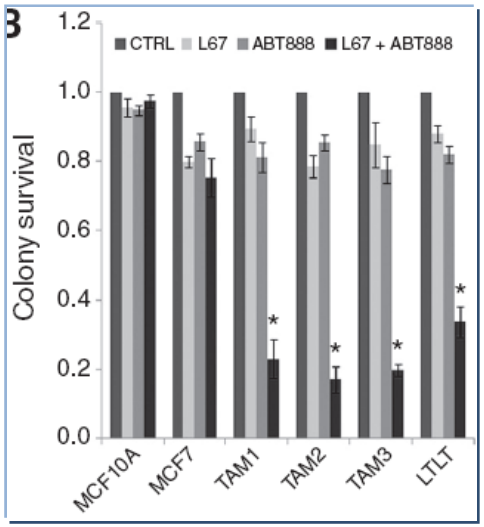
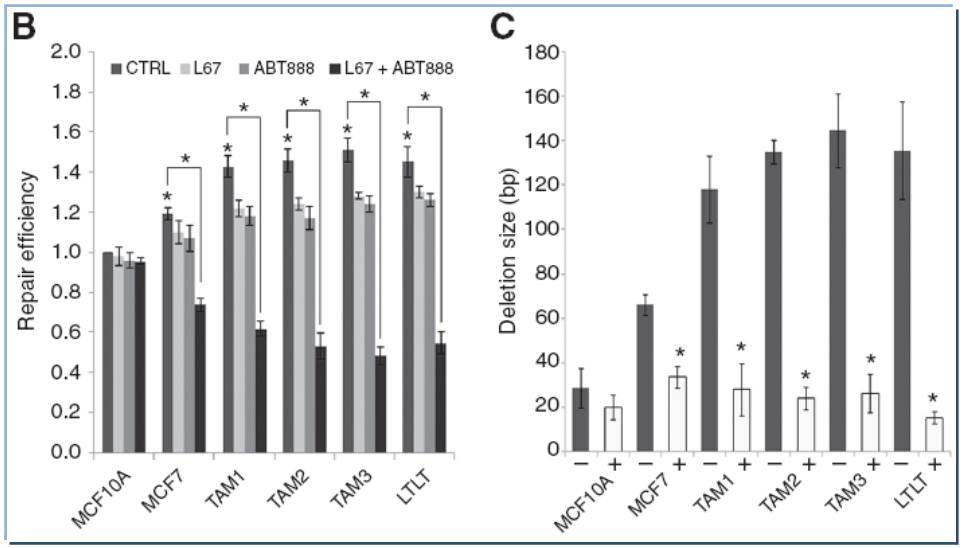


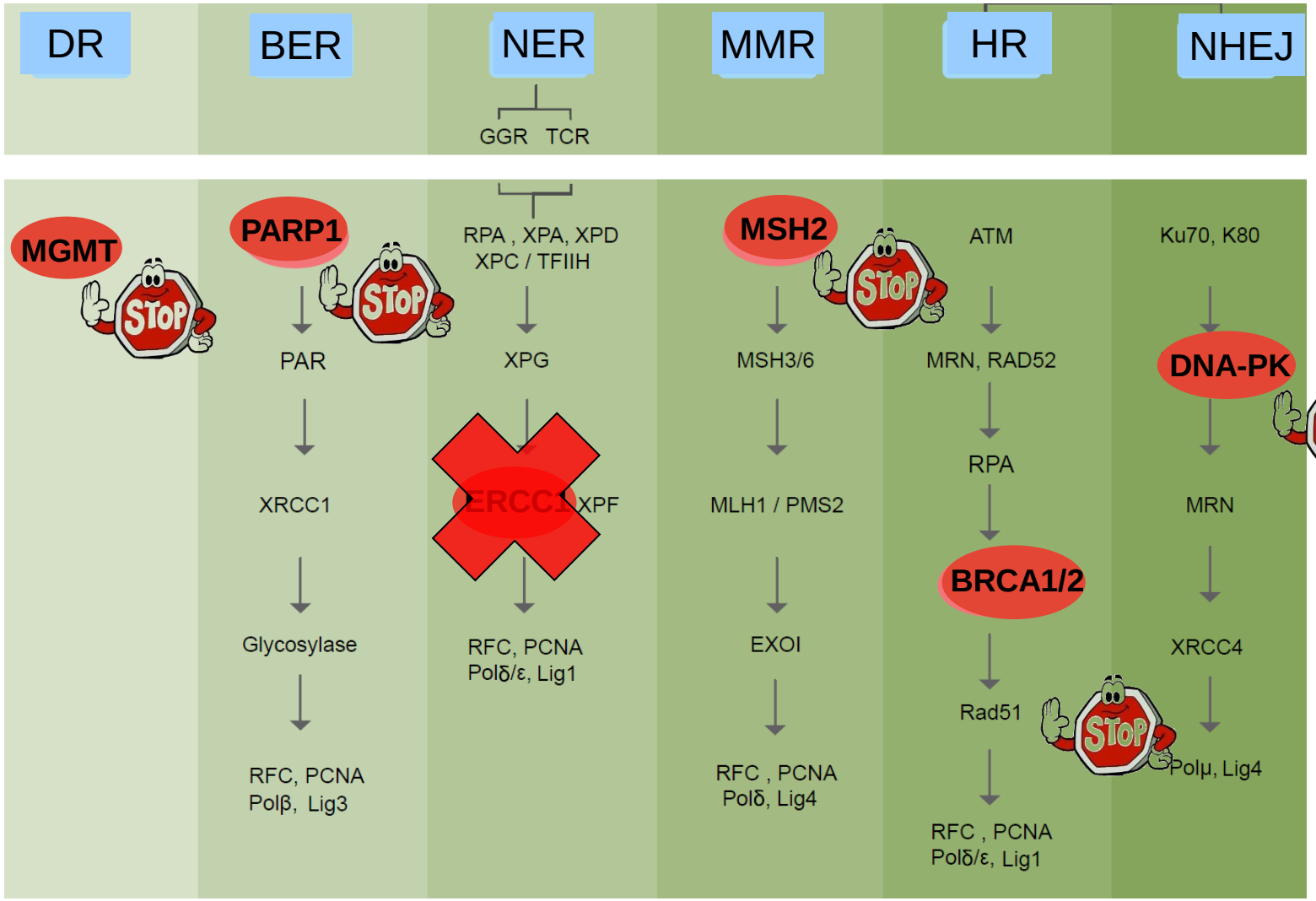
Cell lines with acquired resistance to antiestrogen therapeutics are hypersensitive to a combination of DNA ligase 3 (L67) and PARP inhibitors



The hypersensitivity of the combination is followed by loss of efficient DSB-repair (although less error-prone)

Tobin et al, Mol Cancer Res, 2012

ERCC1 deficient cell lines to explore synthetic letality with candidate drugs/pathways



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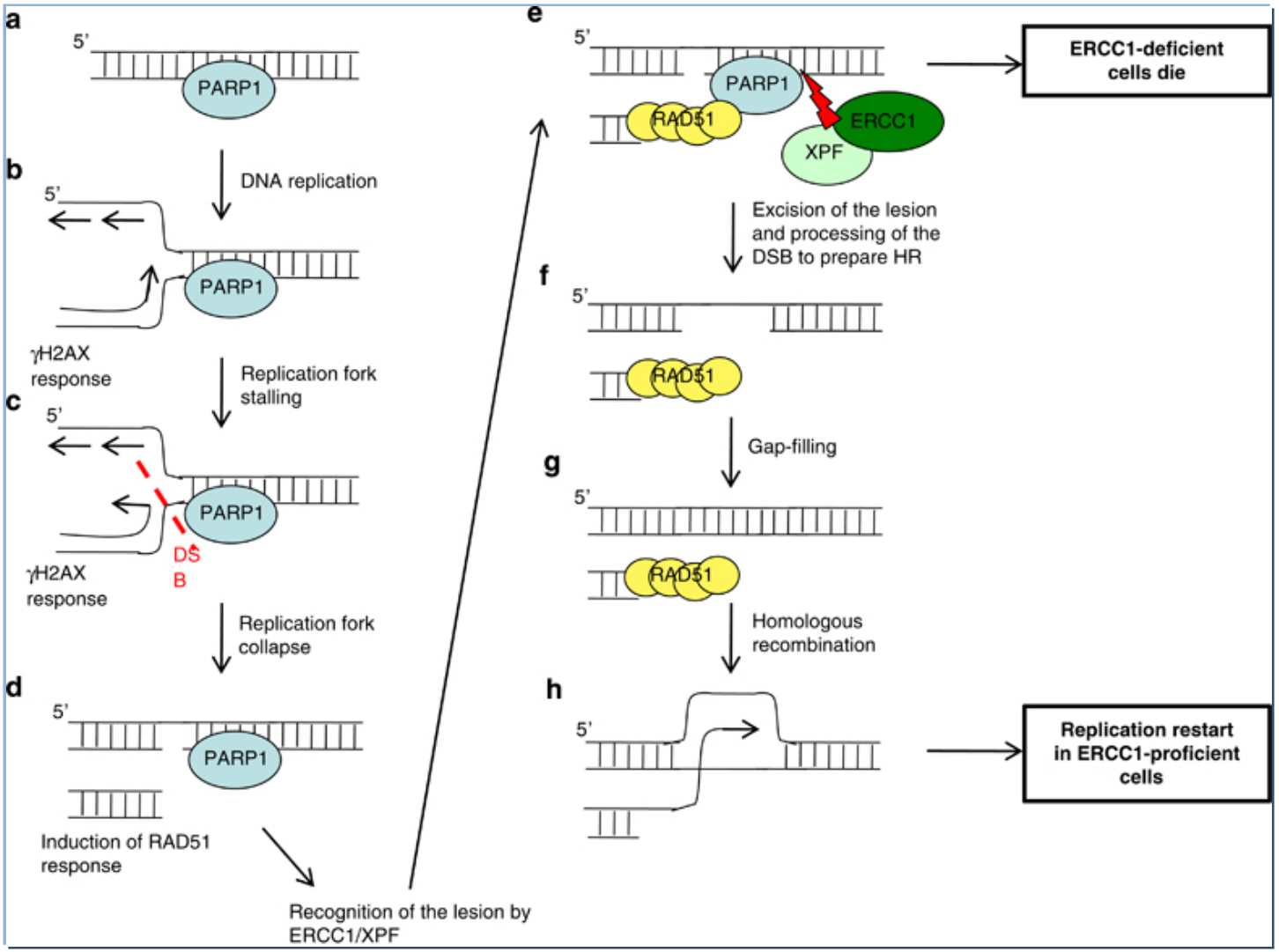
PARP1/2 inhibitors selectively kills ERCC1-deficient cells

PARP is trapped onto the DNA by the PARP inhibitor leading to replication fork stalling and collapse



Postel-Vinay et al. Oncogene 2013

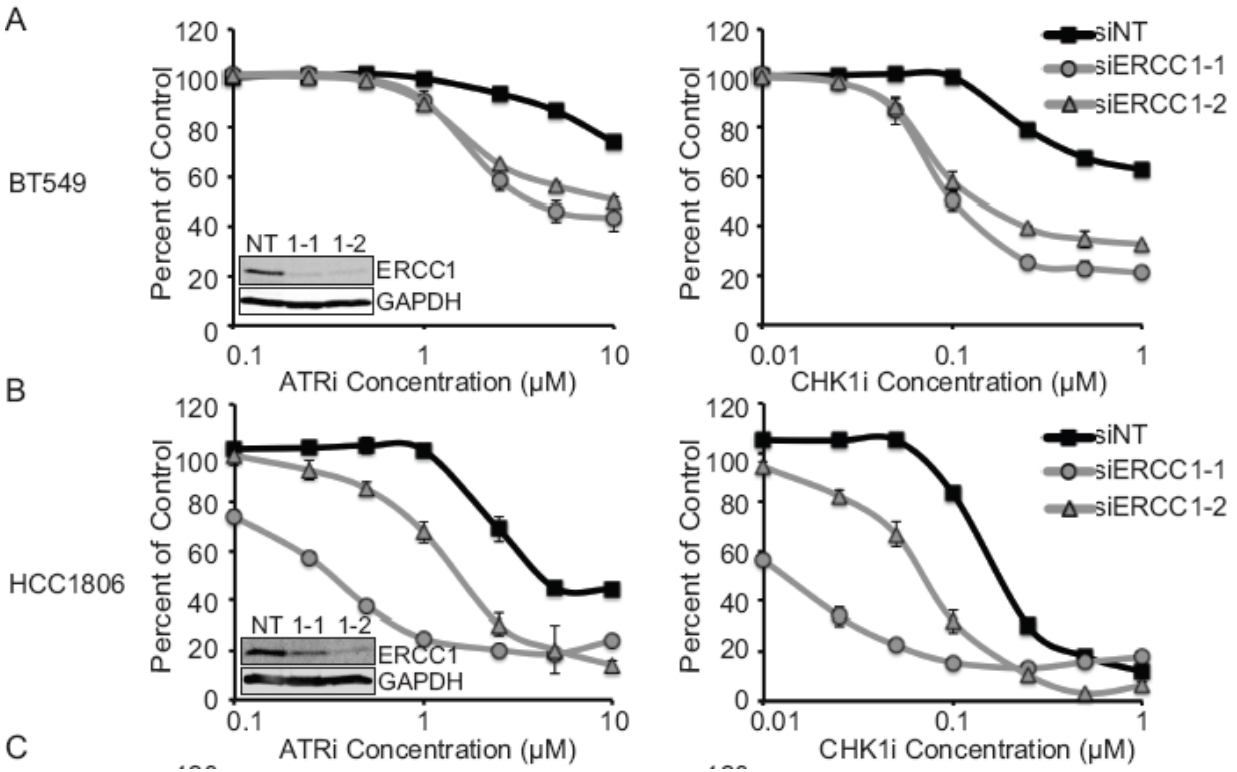
Cancer Research

ATR pathway inhibition is synthetically lethal in cancer cells with ERCC1 deficiency

Kareem N Mohni, Gina M Kavanaugh and David Cortez

Cancer Res Published OnlineFirst March 24, 2014.

Breast cancer cell lines

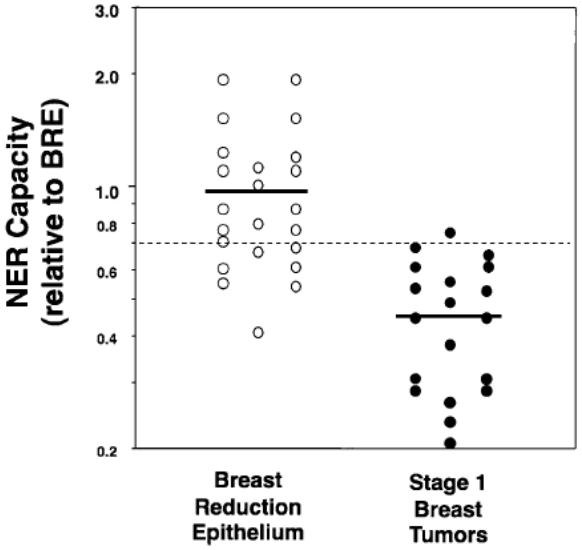


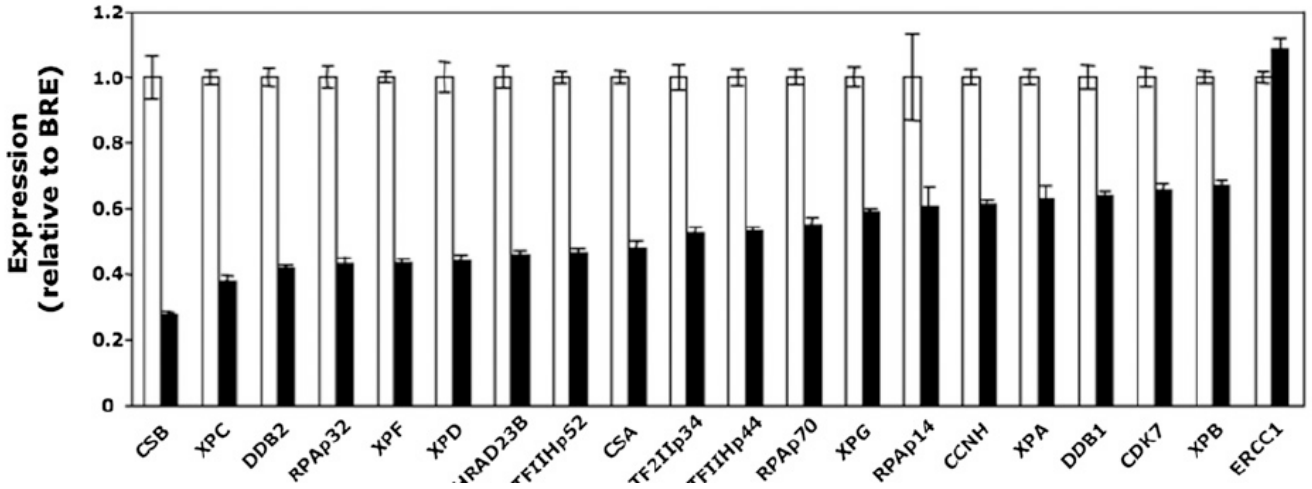
PNAS

Nucleotide excision repair deficiency is intrinsic in sporadic stage I breast cancer

Jean J. Latimer[a,b,1], Jennifer M. Johnson[b,c], Crystal M. Kelly[a], Tiffany D. Miles[b], Kelly A. Beaudry-Rodgers[d], Nancy A. Lalanne[b], Victor G. Vogel[b,e], Amal Kanbour-Shakir[f], Joseph L. Kelley[a], Ronald R. Johnson[g], and Stephen G. Grant[b,h]

[a]Department of Obstetrics, Gynecology and Reproductive Sciences, Magee–Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA 15213-3180; [b]Hillman Cancer Center, University of Pittsburgh Cancer Institute, Pittsburgh, PA 15232-1301; [c]Program in Biochemistry and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261; [d]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261; Departments of [e]Medicine, [f]Pathology, and [g]Surgery, Magee–Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA 15213-3180; and [h]Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15219-3130



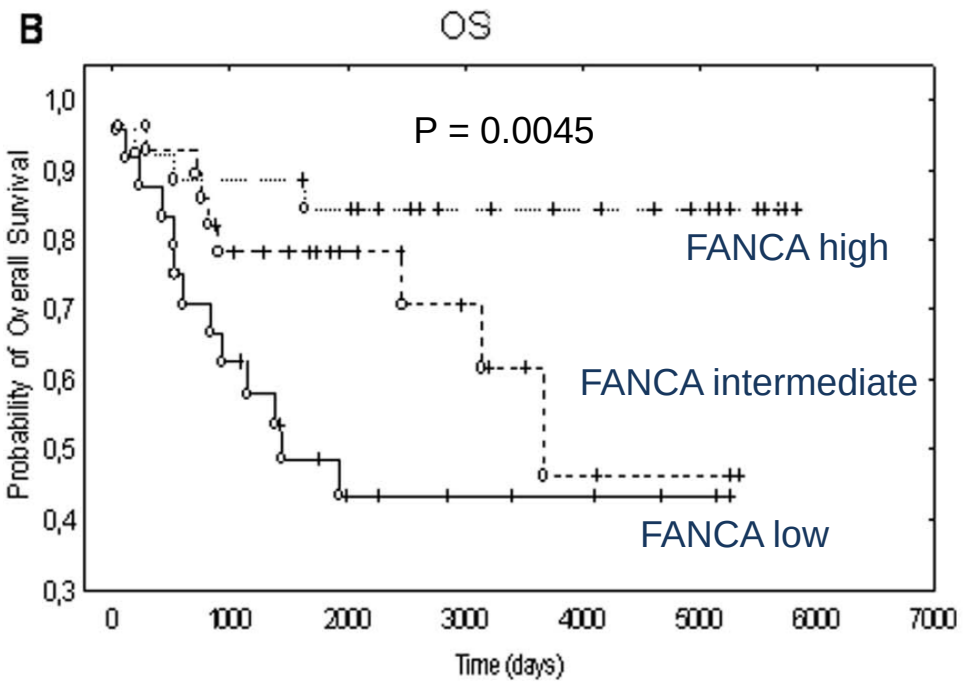


Remarkable loss of DNA repair proteins in TNBC compared to LABC

Table 3. Normalized and calibrated values (mean ± SD and median) of the different DNA repair genes in tumor samples.

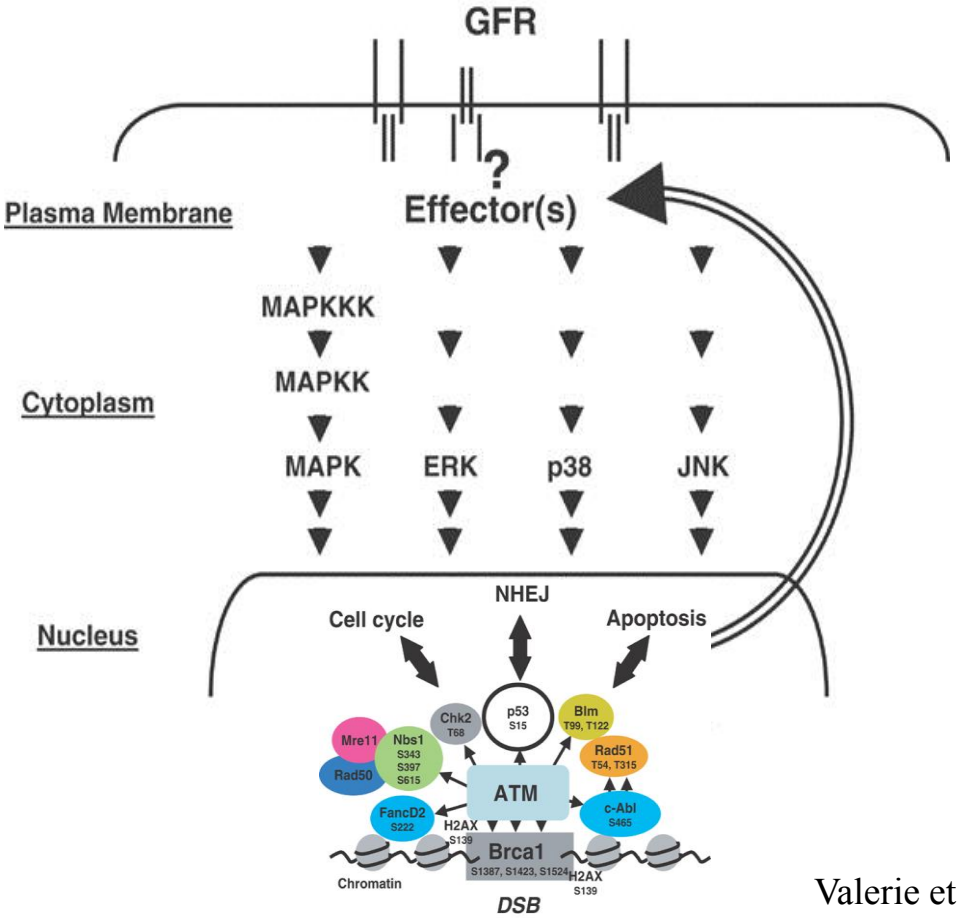
PATH WAY	GENE	TNBC (N = 80)		LABC (N = 70)		p value*
		Mean ± SD	Median	Mean ± SD	Median	
BER	*PARP1*	10.87±10.92	8.250	7.05±5.46	5.221	**0.0002**
NER	*ERCC1*	0.71±0.66	0.633	6.87±20.77	1.040	**<0.0001**
	XPA	0.10±0.08	0.080	0.16±0.21	0.104	**0.0309**
	XPF	23.35±55.61	5.108	68.93±107.05	21.996	**<0.0001**
	XPG	0.66±0.71	0.468	1.51±3.52	0.287	0.1534
	XPD	0.276±0.360	0.186	6.68±10.26	3.662	**<0.0001**
FA	*BRCA1*	0.028±0.025	0.020	0.83±3.36	0.062	**<0.0001**
	FANCA	0.138±0.279	0.057	0.27±0.71	0.094	0.8293
	FANCC	0.005±0.007	0.003	0.002±0.050	0.003	0.3627
	FANCD2	0.08±0.124	0.054	4.45±23.32	0.150	**<0.0001**
	FANCF	0.026±0.027	0.018	0.451±1.170	0.031	**<0.0001**
	PALB2	0.453±0.563	0.306	5.91±23.03	2.010	**0.0006**
SEN SOR	*CHK1*	0.179±0.300	0.108	11.51±46.44	1.779	**<0.0001**

FANCA mRNA expression in TNBC



Ribeiro E et al., PLoS ONE, 2013

DNA repair and targeted therapies



Valerie et al., Oncogene 2003

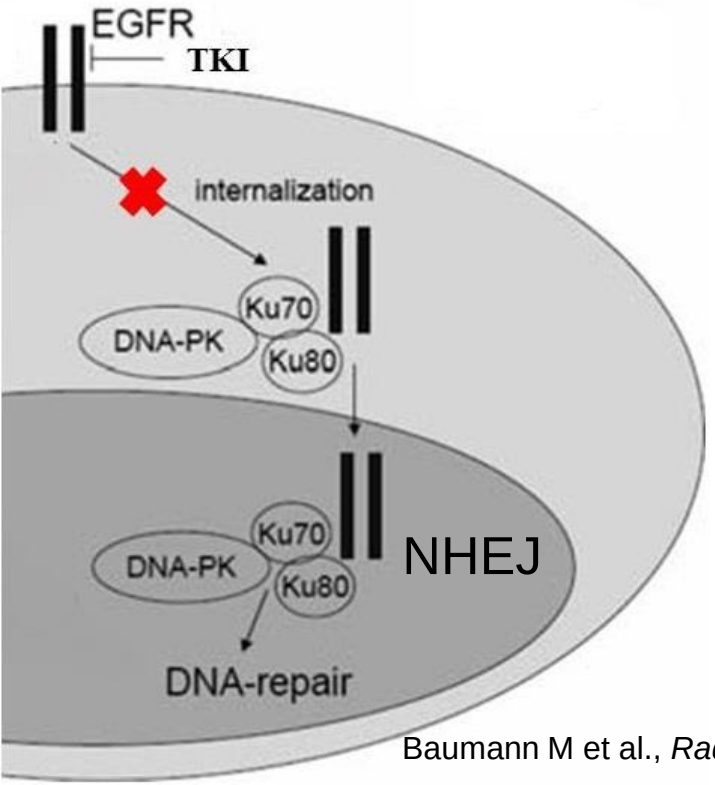
Recent example: EGFR cooperates with glucose transporter SGLT1 to enable chromatin remodeling in response to ionizing radiation. Dittmann K, et al. Radiother Oncol. 2013

Inhibiting PI3K/AKT and Ras/ERK pathways

Intracellular distribution of DNA repair proteins

Transcription of DNA repair genes

Phosphorylation of DNA repair proteins



Baumann M et al., *Radiother Oncol*, 2007

↘ NHEJ

↗ DSBs

↗ Radiosensitivity

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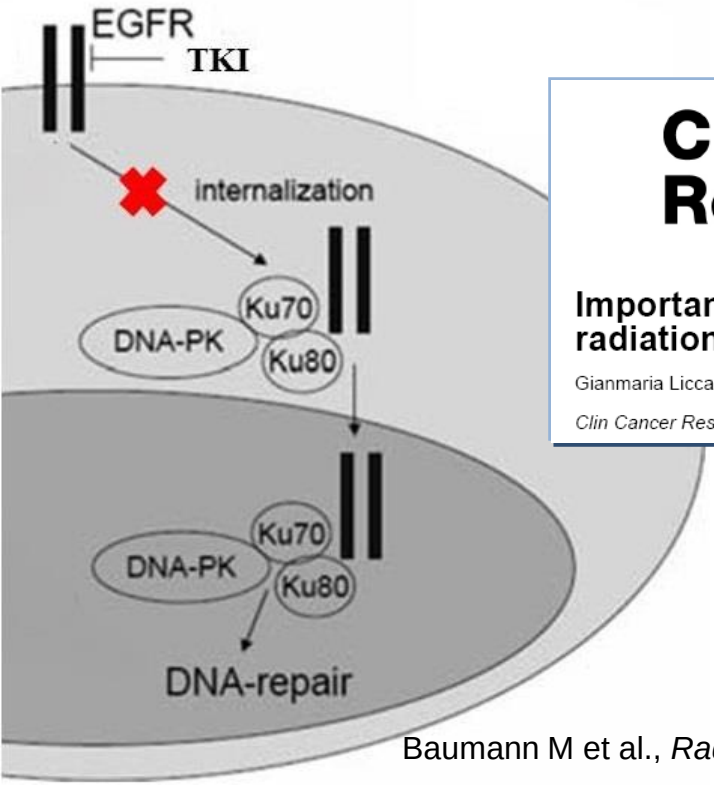

Clinical Cancer Research

Importance of EGFR/ERCC1 interaction following radiation-induced DNA damage

Gianmaria Liccardi, John A. Hartley and Daniel Hochhauser

Clin Cancer Res Published OnlineFirst April 29, 2014.

→ Error-prone Alt-NHEJ

Baumann M et al., *Radiother Oncol*, 2007

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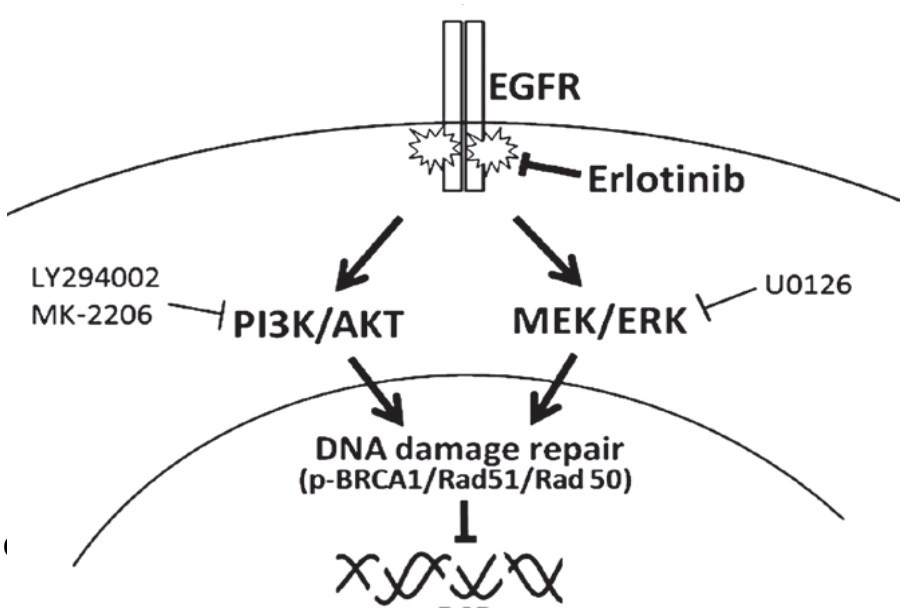


Rad51/Rad50 expression

HRR

DSBs

Kryeziu K et al., *Mol cancer ther*, 2013

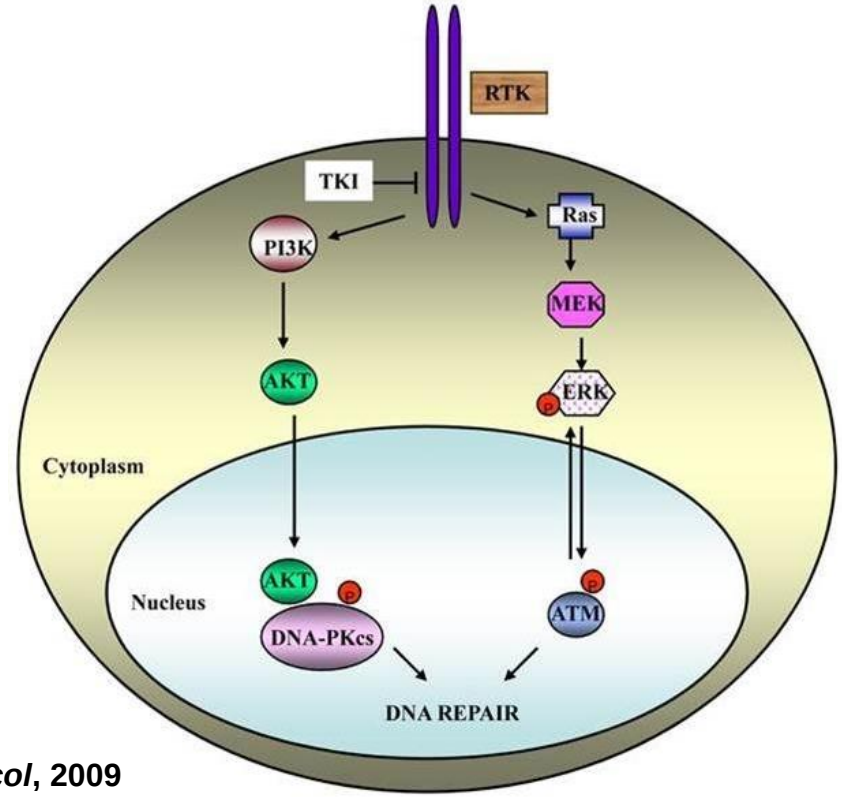
Inhibiting PI3K/AKT and Ras/ERK pathways

Intracellular distribution of DNA repair proteins

Transcription of DNA repair genes

Phosphorylation of DNA repair proteins

- **AKT and PI3K**
- **ERK**
- **DNA-PKcs phosphorylation (NHEJ)**
- **ATM phosphorylation (NHEJ and HRR)**



Meyn R et al., *Radiother Oncol*, 2009

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PI3K inhibition increases indicators of DNA damage such as γ-H2AX, but decreases Rad51 foci and BRCA1 expression allowing synthetic lethality approaches (with PARPi) in HRR proficient cells

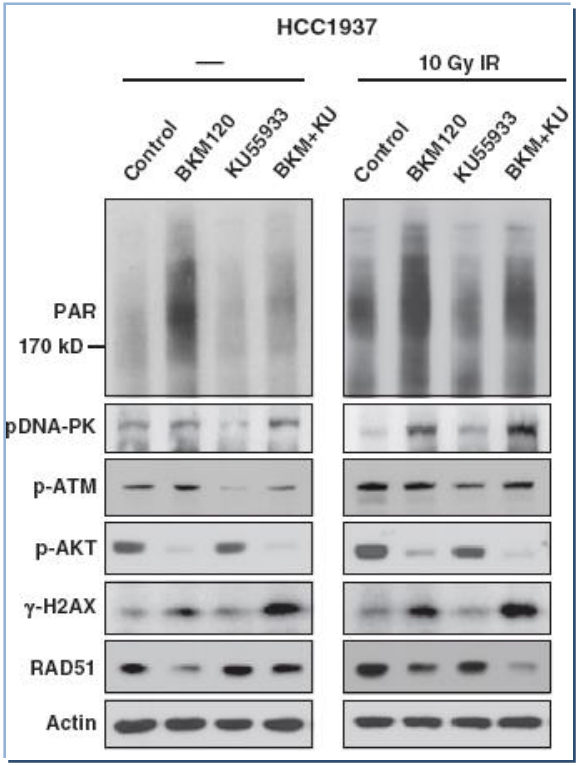
CANCER DISCOVERY

AACR

Combining a PI3K Inhibitor with a PARP Inhibitor Provides an Effective Therapy for BRCA1-Related Breast Cancer

Ashish Juvekar, Laura N. Burga, Hai Hu, et al.

Cancer Discovery 2012;2:1048-1063. Published OnlineFirst August 22, 2012.



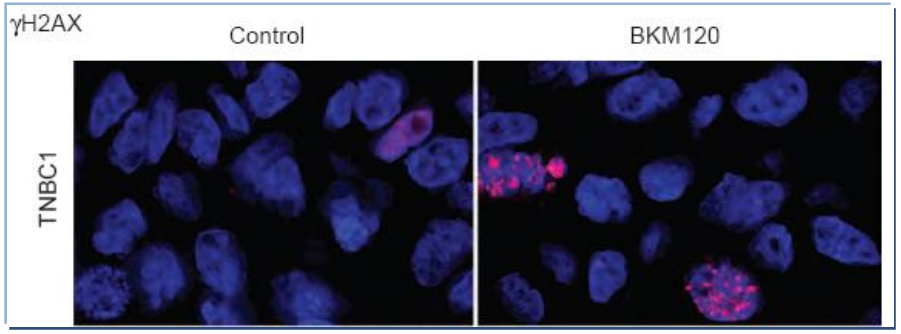
CANCER DISCOVERY

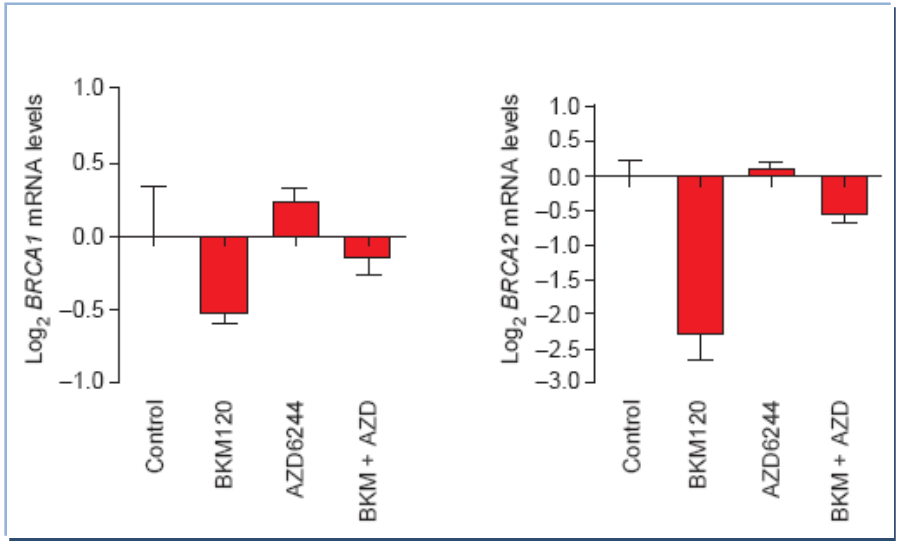
AACR

PI3K Inhibition Impairs BRCA1/2 Expression and Sensitizes BRCA-Proficient Triple-Negative Breast Cancer to PARP Inhibition

Yasir H. Ibrahim, Celina García-García, Violeta Serra, et al.

Cancer Discovery 2012;2:1036-1047. Published OnlineFirst August 22, 2012.





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Conclusive remarks

- DNA repair pathways important in cancer development and risk
- Complex interactions between DNA repair pathways give new therapeutic opportunities (synthetic letality)
- A more robust technology to select patients with DNA repair dysfonctionality is to be developed
- TKIs can change expression, intra-cellular distribution and activation of DNA repair proteins
- This indirect modulation inhibition of DNA repair might be combined with direct modulators (PARPi…)
- Better understanding of fundamental DNA repair mechanisms to be encouraged

Thank you !

Jean-Charles Soria
Fabrice André
Luc Friboulet
Sophie Postel-Vinay
Tony Sourisseau
Julien Adam
Florence Ponsonnailles
Angélique Robin
Nicolas Dorvault
Mei-Shiue Kuo-Cassin