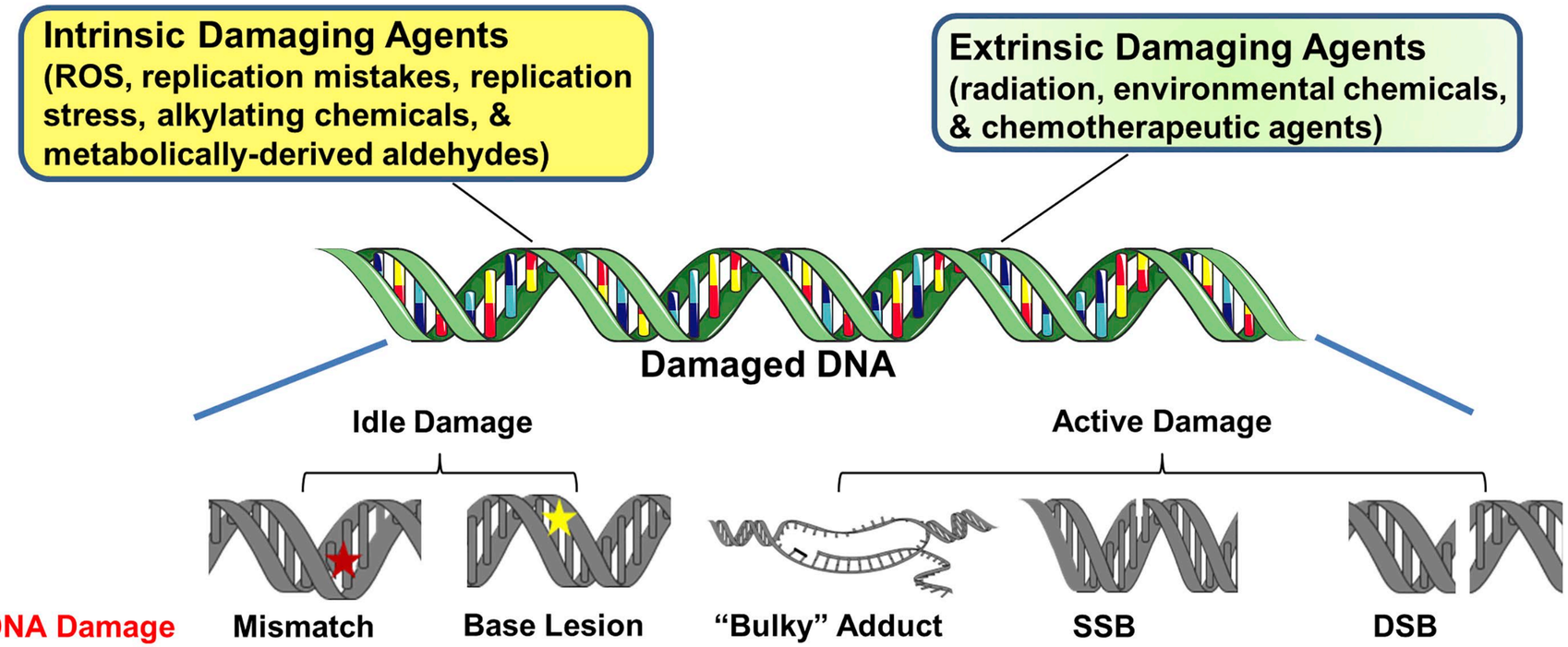
A novel DNA repair assay to investigate the mode-of-action of genotoxic compounds

Giel Hendriks

DNA damage and DNA repair pathways



DNA Damage	Mismatch	Base Lesion	"Bulky" Adduct	SSB	DSB
Molecular Effect	Mutagenesis	Mutagenesis	Blocked transcription Blocked replication Mutagenic bypass	Blocked transcription Faithful recombination	Blocked transcription Replication fork collapse Genomic instability
Cellular Consequence	Transformation	Transformation	Transformation Death	Death	Transformation Death
Disease Outcome	Cancer	Cancer Immunodysfunction	Cancer Neurological disease Premature aging	Neurological disease	Cancer Neurological disease Immunodysfunction
Responsible Repair Pathway	MMR	BER	NER	SSBR	DSBR

Adapted from: Tiwari and Wilson, 2019

DNA repair and disease

DNA Repair Pathway	Genetic Defects	Inheritable disease	Major Clinical Phenotypes
MMR	MSH2, MSH6, MLH1, PMS1, PMS2	Lynch syndrome	cancer predisposition
BER	MUTYH, NTHL1, UNG	Colorectal cancer, Hyper IgM syndroom	cancer predisposition, immunological defects
GG-NER	XPA, XPB, XPC, XPD, XPE, XPF, XPG, ERCC1	Xeroderma pigmentosum	cancer predisposition (particularly UV-induced skin melanoma), some instances of neurological disease
TC-NER	CSA, CSB, XPB, XPD, XPF, XPG, ERCC1, TTD-A	Xeroderma pigmentosum, Cockayne syndrome	developmental defects, premature aging, neurological abnormalities, no cancer predisposition
SSBR	APTX, TDP1, PNKP, XRCC1	Ataxia-oculomotor apraxia	neurological disease, no cancer predisposition
NHEJ	LIG4, XLF, Artemis, DNA-PKcs, XRCC4, ATM, MRE11, NBS1	SCID, Ataxia telangiectasia, Nijmegen breakage syndrome	cancer predisposition, immunodeficiency, neurological disease
HR	BRCA1/2, RAD50, RAD51, PALB2, FANCA, FANCC, and FANCG	Breast/ovarian cancer, Fanconi's anemia	cancer predisposition, aplastic anemia

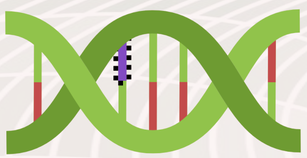
DNA Repair-Profiler

- Panel of mammalian DNA repair-deficient cell lines
- Investigate the impact of DNA repair on the toxicity of genotoxic compounds
- Gain insight into the type of DNA damage induced by genotoxic compounds
- Assess which DNA repair pathway repairs mutations caused by compounds

Base damages

Base Excision Repair (BER)

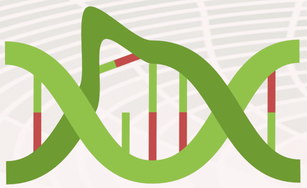
XRCC1



Damaged base

Nucleotide Excision Repair (NER)

XPD
CSB



Damage-induced conformational change in DNA

DNA double strand breaks

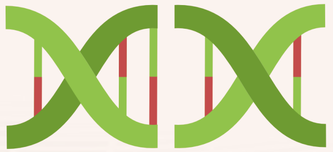
Homologous recombination (HR)

BRCA2

Genetic information of homologous DNA is needed

Non-homologous end-joining (NHEJ)

KU80

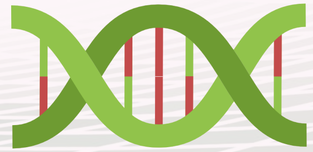


Genetic information of homologous DNA is not needed

DNA mismatches

DNA mismatch repair (MMR)

MSH2

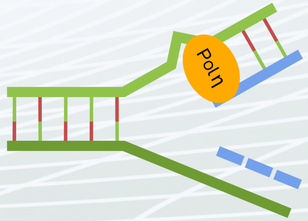


Incorrect base pair

DNA replication blocks

Translesion synthesis (TLS)

Polη



DNA lesion blocking replication bypassed by Polη

DNA Repair-Profiler cell lines

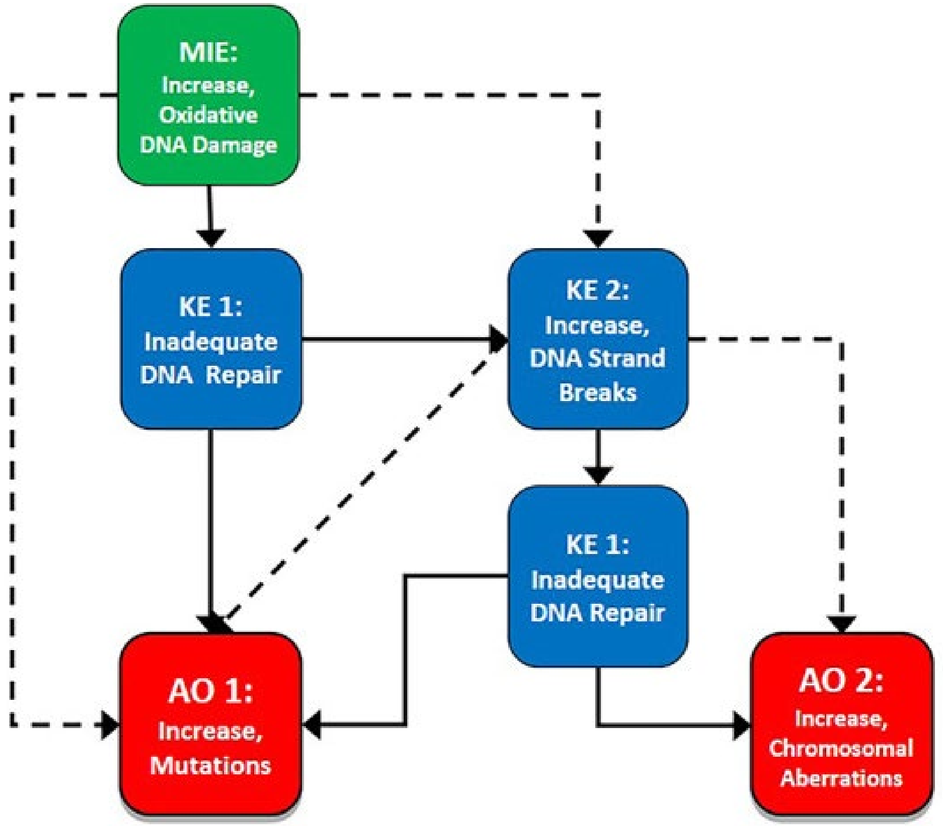
Cell line	Mutation	Species	DNA repair pathway	Reference
AA8	Wild type	Chinese hamster ovary		Thompson et al. (1982). Mutation Research 95: 427-440.
V79	Wild type	Hamster lung fibroblast		Zdzienicka et al. (1987). Mutation Research 178(2):235-244.
MEF	Wild type	Mouse embryonic fibroblast		Temviriyanukul et al. (2012). DNA repair 11(6):550-558.
IB10	Wild type	Mouse embryonic stem cells		Hendriks et al. (2014). Human Mutation 35(11):1382-1391.
EM9	Xrcc1	Chinese hamster ovary (AA8)	BER	Thompson et al. (1982). Mutation Research 95: 427-440.
UV20	Ercc1	Chinese hamster ovary (AA8)	NER	Yang et al. (1991). Mutagenesis 6(6):449-453.
UV5	Xpd	Chinese hamster ovary (AA8)	NER	Yang et al. (1991). Mutagenesis 6(6):449-453.
UV61	Csb	Chinese hamster ovary (AA8)	NER	Yang et al. (1991). Mutagenesis 6(6):449-453.
VC8	Brca2	Hamster lung fibroblast (V79)	HR	Zdzienicka et al. (1987). Mutation Research 178(2):235-244.
UV40	Fancg	Chinese hamster ovary (AA8)	HR	Busch et al. (1996). Mutation Research 363(3):209-22, Liu et al. (1997) PNAS 94: 9232-9237.
XR-V9B	Ku80	Hamster lung fibroblast (V79)	NHEJ	Pergola et al. (1993). Molecular and Cellular Biology 13(6):3464-3471.
XR-1	Xrcc4	Chinese hamster ovary (AA8)	NHEJ	Stamato et al. (1983). Somatic Cell Genetics, 9, 165-173.
Xpv	Pol eta (Xpv)	Mouse embryonic fibroblasts	TLS	Temviriyanukul et al. (2012). DNA repair 11(6):550-558.
Msh2	Msh2	Mouse embryonic stem cells	MMR	De Wind et al. (1995). Cell 82: 321-330.

Applications of DNA Repair-Profiler

- Predict genotoxicity of compounds
- Determine the mode-of-action of genotoxic substances
- Build evidence for adverse outcome pathways

- Characterization of novel medicines, e.g., oncology drugs

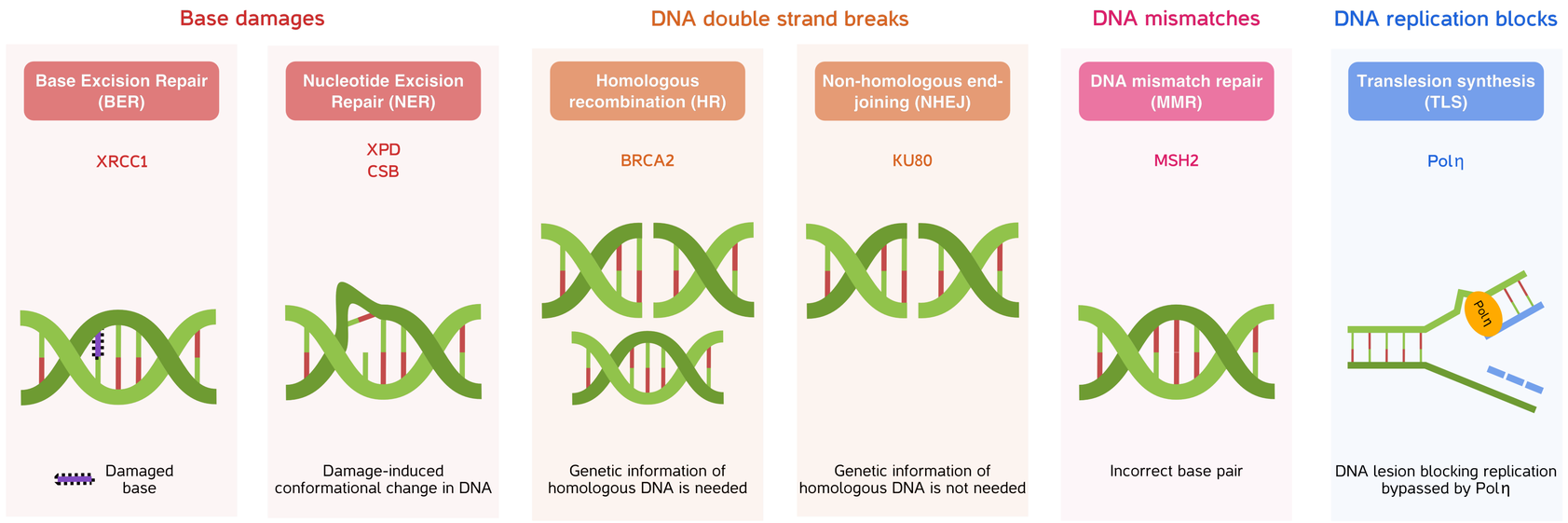
- Determine relevance of repair pathways
  - Sensitivity of specific tumors for chemotherapeutics



Adapted from: Cho, *et al.,* 2022

Overview of assays

DNA repair pathways



Assays

Cytotoxicity

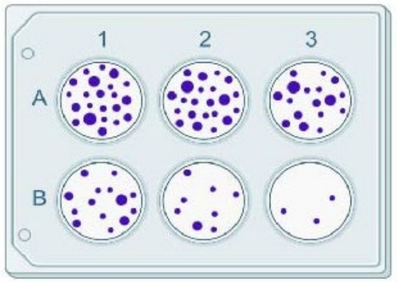
- Cell viability assays
- Clonogenic survival assay

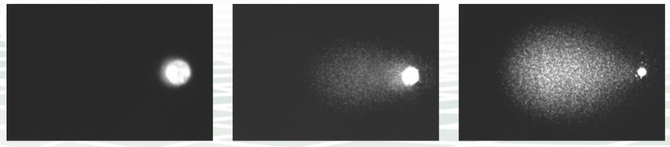
DNA damage

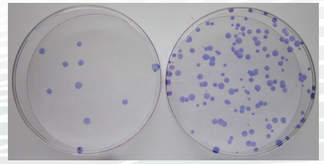
- (FPG-modified) Comet assay

Gene mutations

- HPRT assay

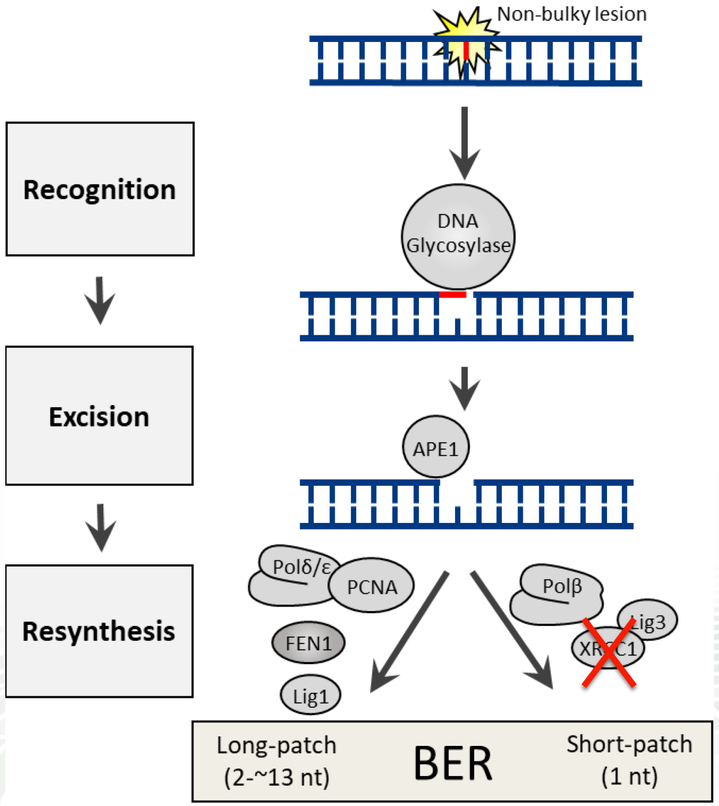




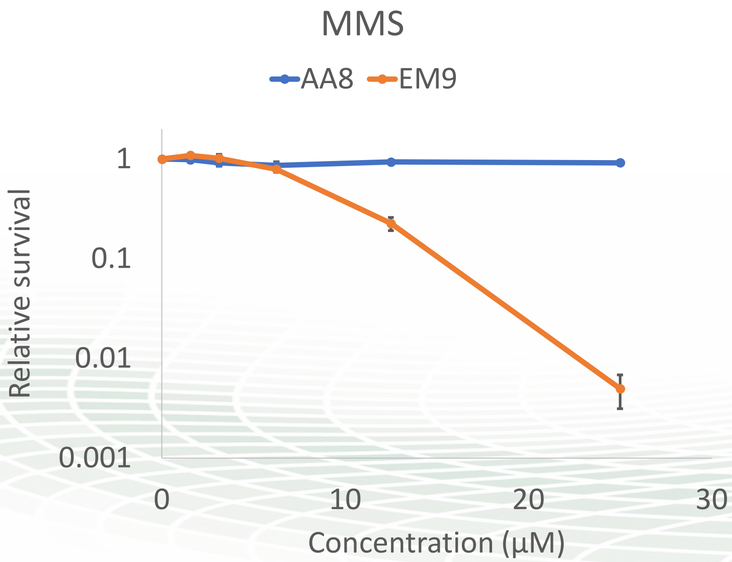


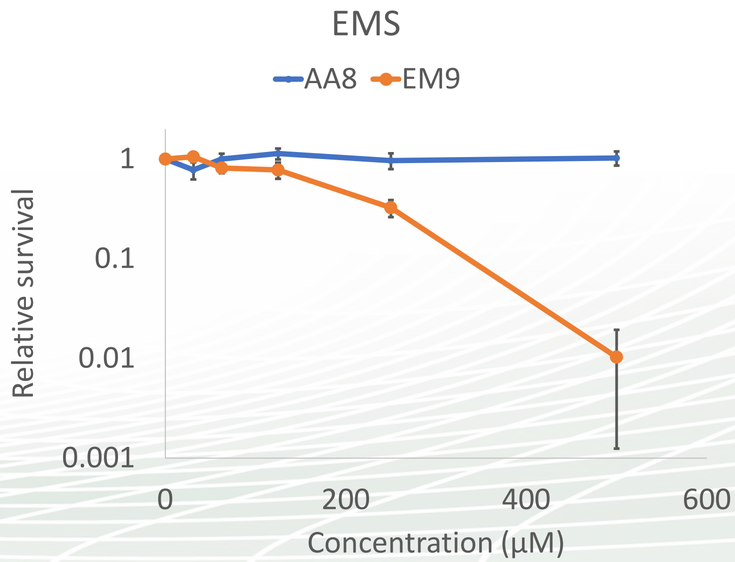
Base Excision Repair

- Removal of small base damages that cause no/minor DNA helix distortion



Cell line	Mutant	Origin
AA8	Wild type	Chinese hamster ovary
EM9	Xrcc1	Chinese hamster ovary

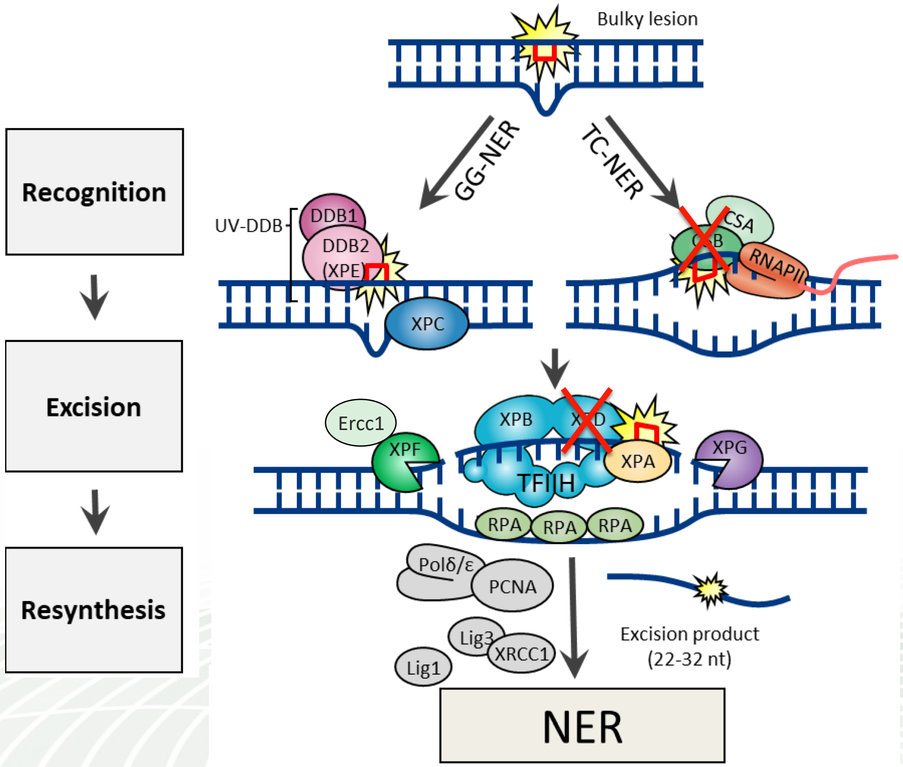




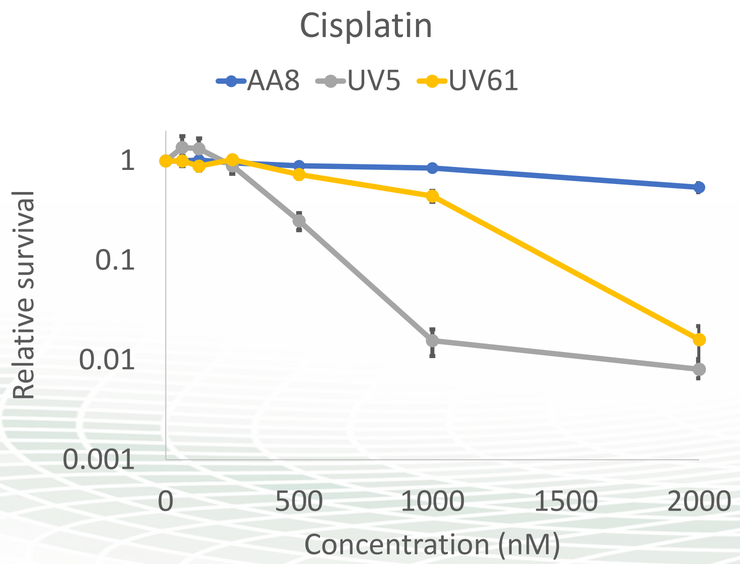
Adapted from: Lee and Kang, 2019

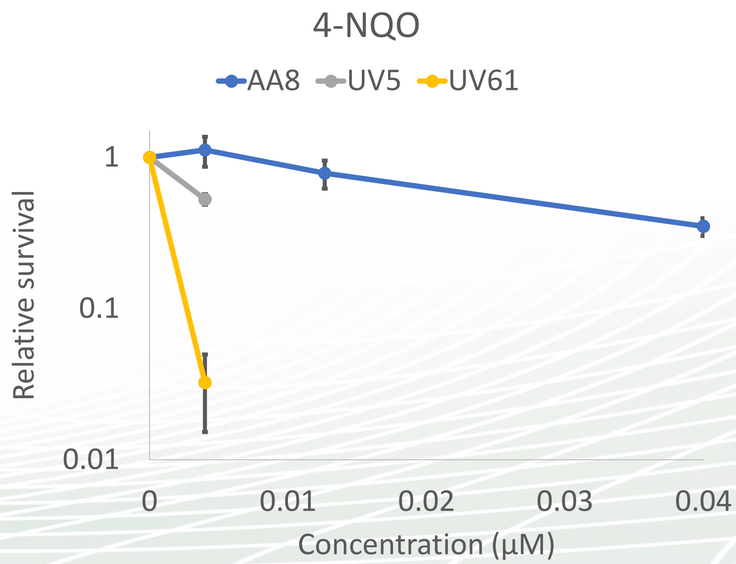
Nucleotide Excision Repair

- Removal of bulky, helix distorting DNA lesions



Cell line	Mutant	Origin
AA8	Wild type	Chinese hamster ovary
UV5	Xpd (NER)	Chinese hamster ovary
UV61	Csb (TC-NER)	Chinese hamster ovary



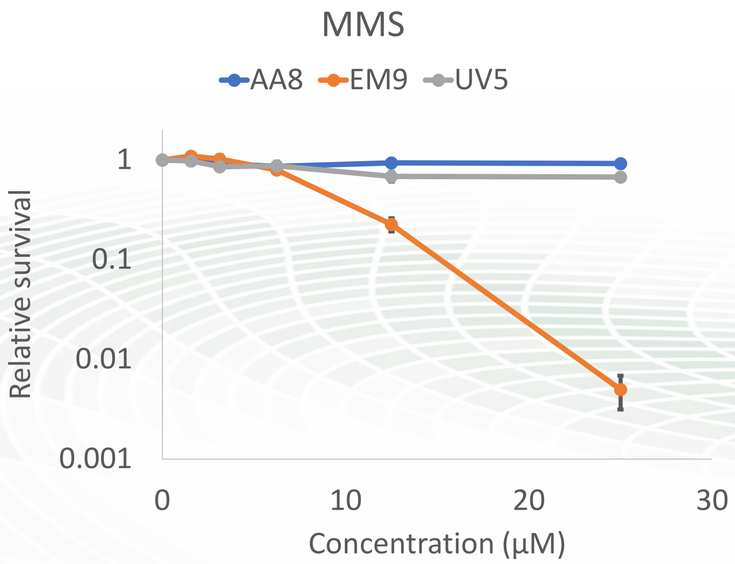


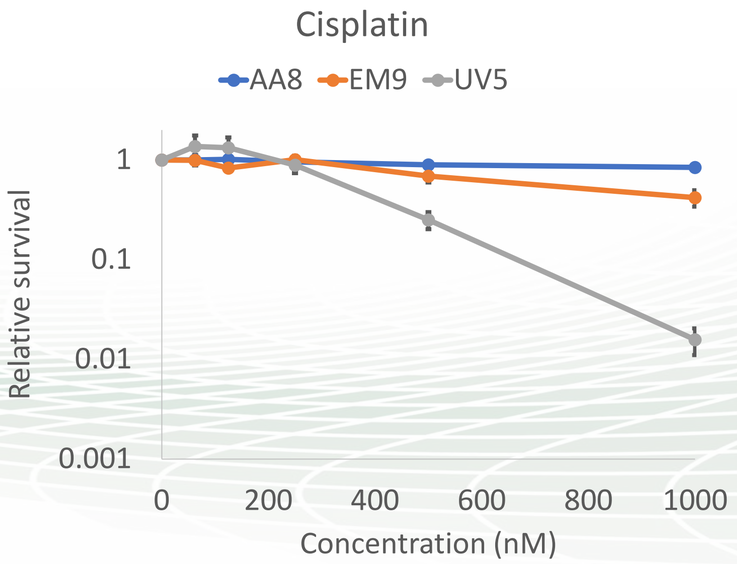
Adapted from: Lee and Kang, 2019

Cytotoxicity of repair mutants is highly specific

- BER specificially removes base damages and smaller DNA lesions
- NER specifically removes bulky adducts and helix-distorting lesions

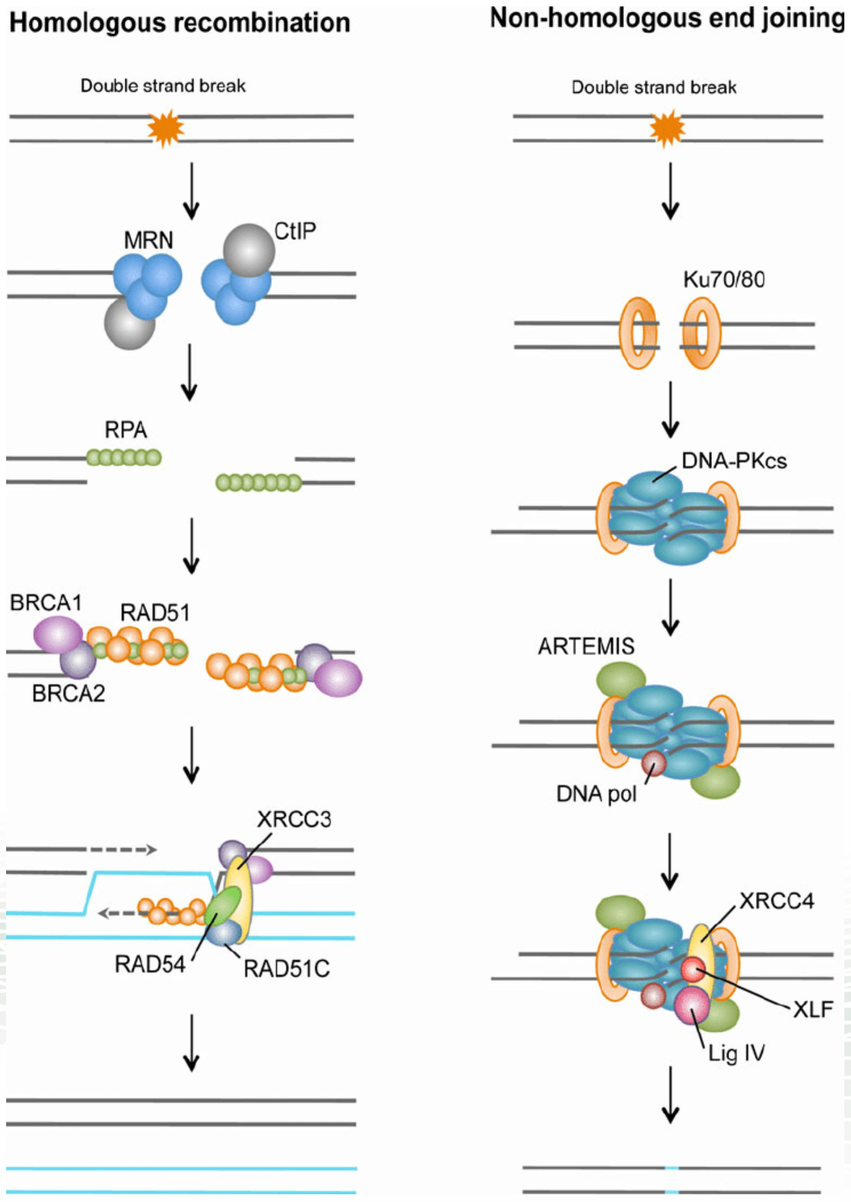
Cell line	Mutant	Origin
AA8	Wild type	Chinese hamster ovary
UV5 (NER)	Xpd	Chinese hamster ovary
EM9 (BER)	Xrcc1	Chinese hamster ovary





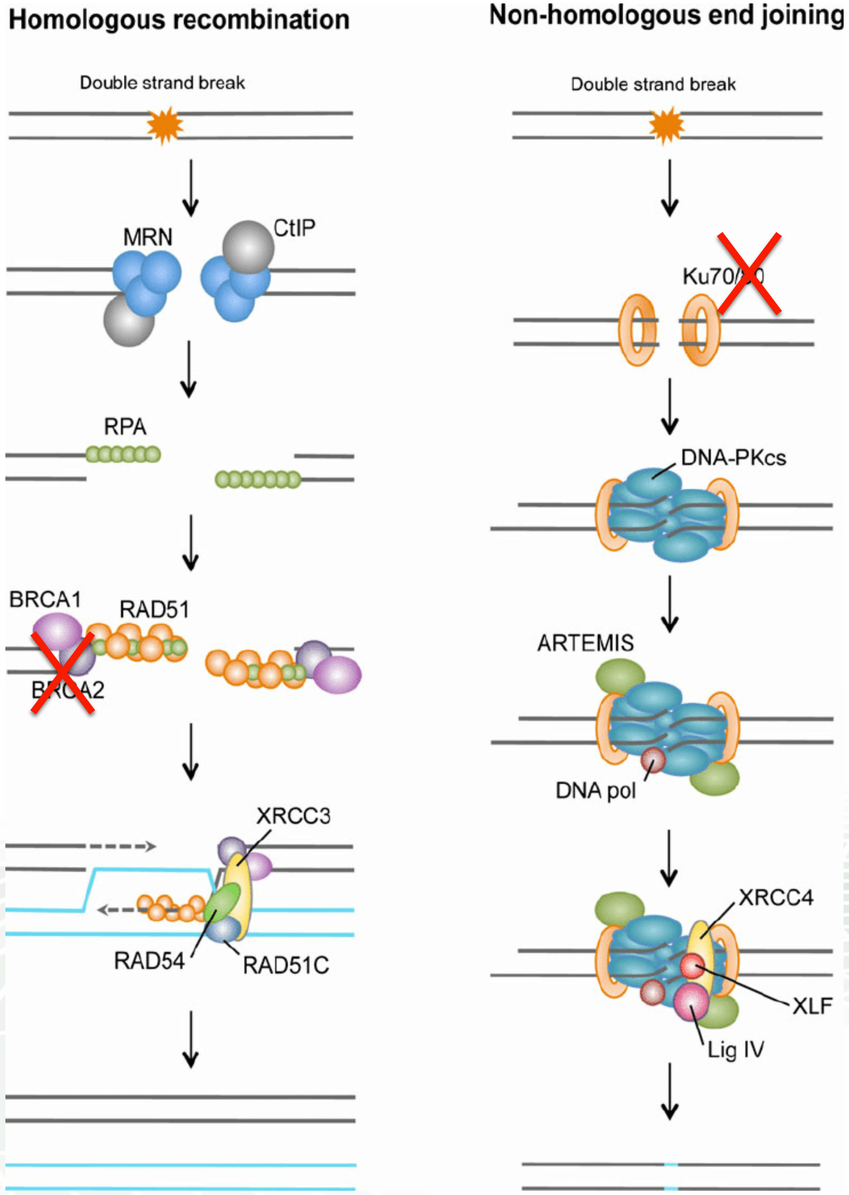
DNA double strand break repair

- NHEJ and HR both repair DSBs
- NHEJ is cell cycle independent
- HR requires a sister chromatid
- HRis restricted to S-phase

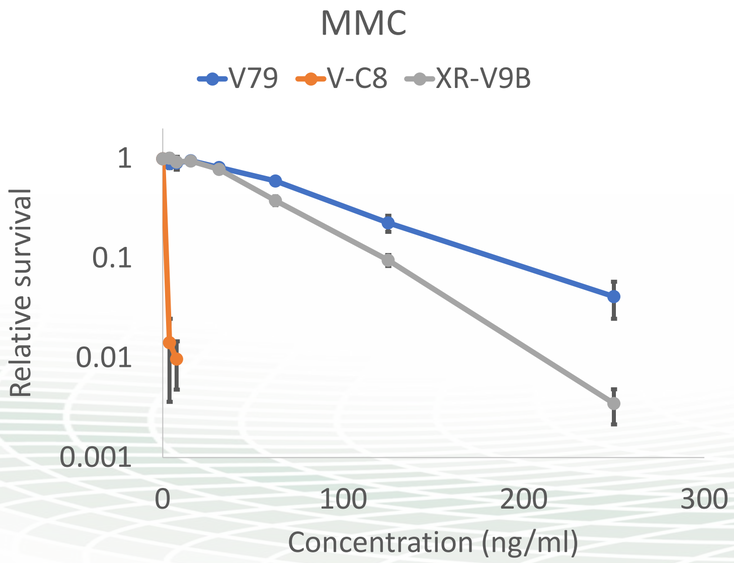


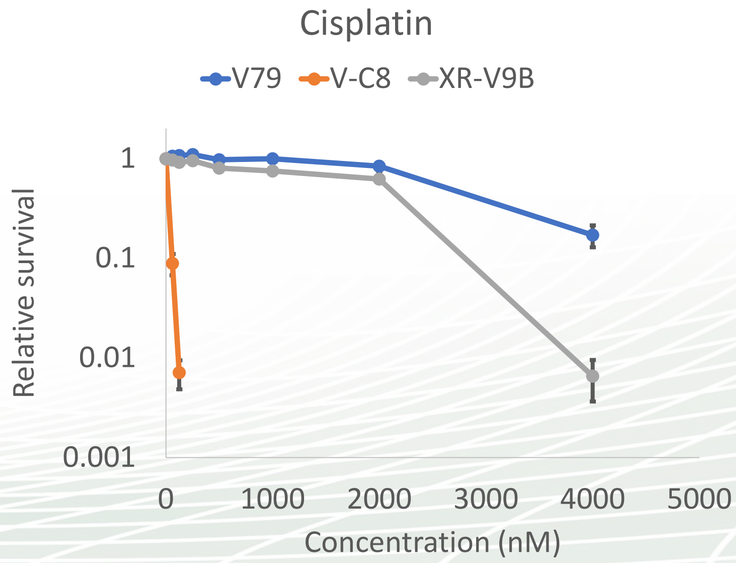
Adapted from: Brochier and Langley, 2013

DNA double strand break repair



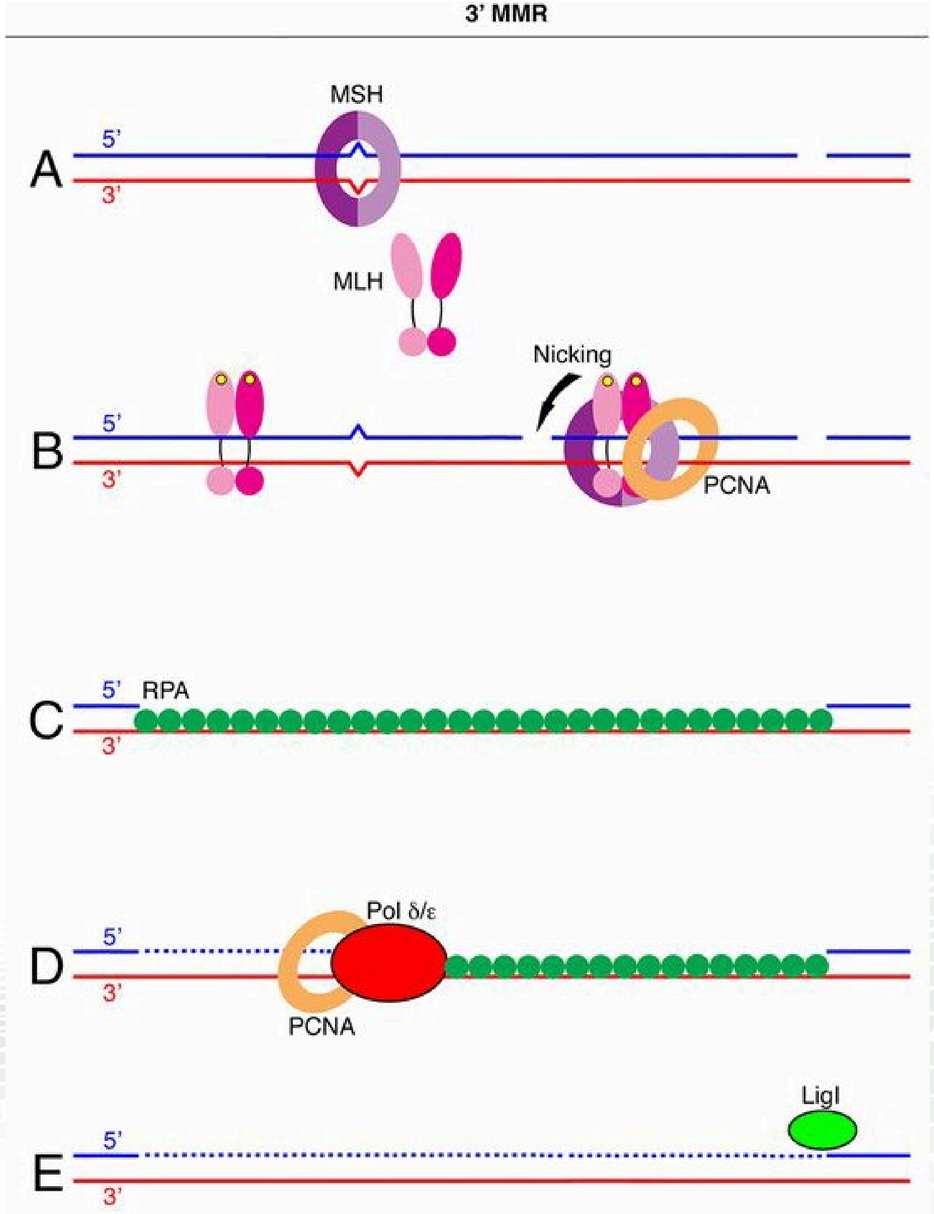
Cell line	Mutant	Origin
V79	Wild type	Hamster lung fibroblast
VC8	Brca2 (HR)	Hamster lung fibroblast
XR-V9B	Ku80 (NHEJ)	Hamster lung fibroblast





DNA Mismatch Repair

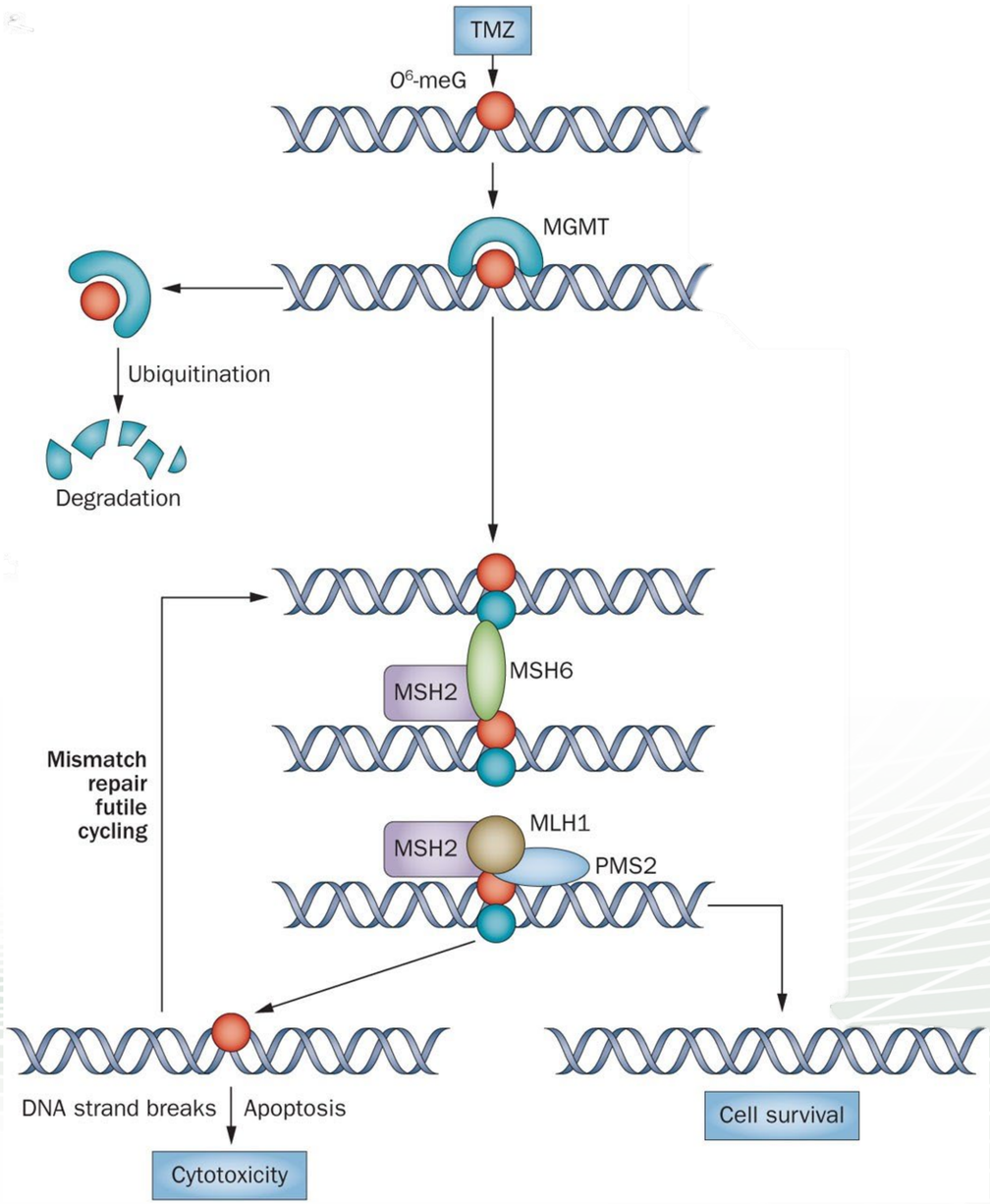
- MMR removes base-base mismatches and insertion/deletion mispairs generated during DNA replication
- Spontaneous or DNA damage-induced mismatches
- Repeated MMR cycles cause cytotoxicity



Adapted from : Hsieh and Zhang, 2017

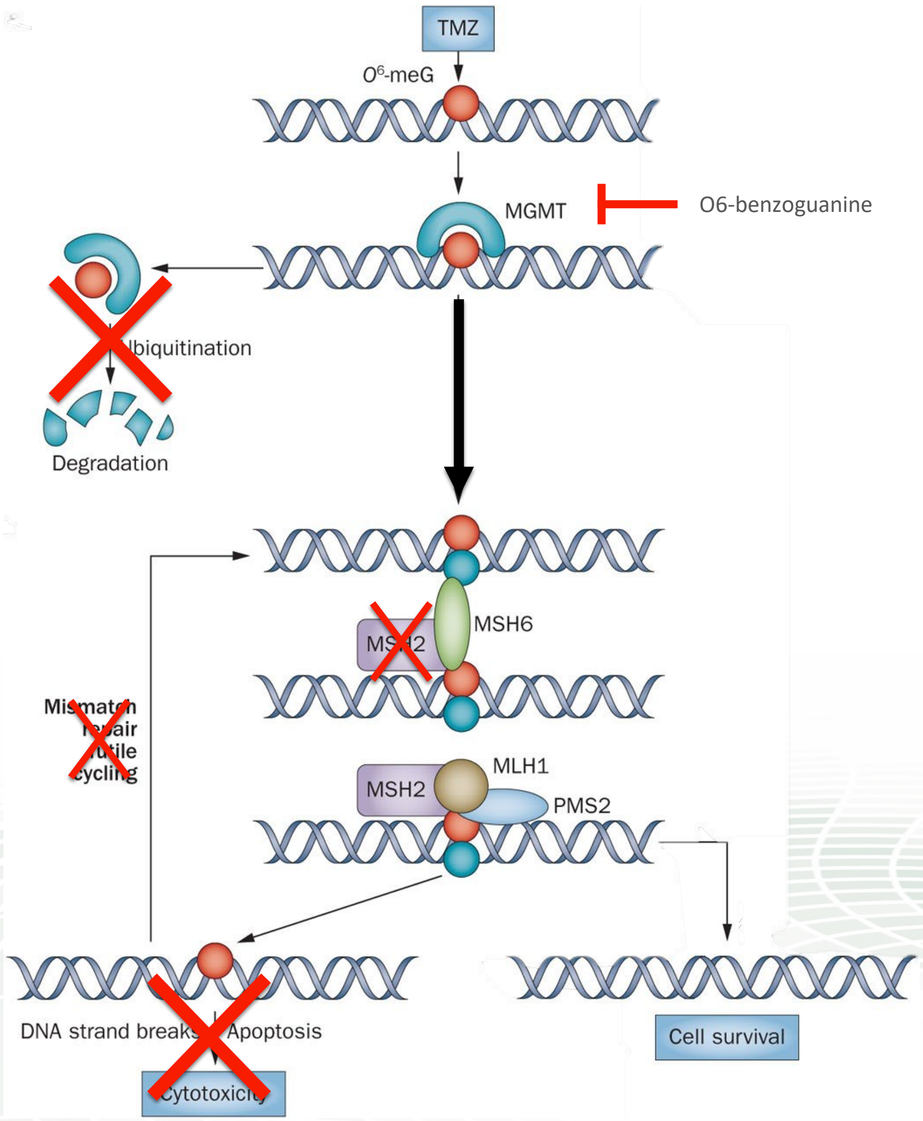
DNA Mismatch Repair

- Repair of mismatched at sites of DNA damage

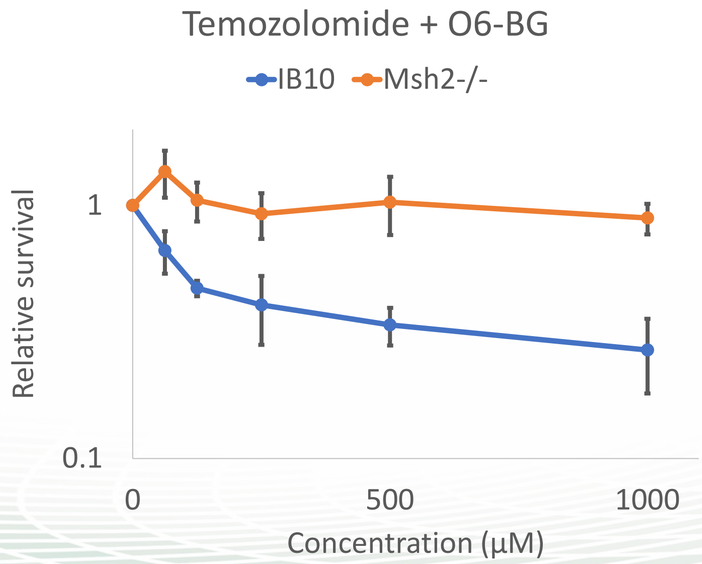


Adapted from: Wick, *et al.,* 2014

DNA Mismatch Repair



Cell line	Mutant	Origin
IB10	parent	Mouse embryonic stem cell
MSH2-/-	MSH2	Mouse embryonic stem cell



Treatment in presence O6-benzoguanine to inhibit MGMT

DNA damage by N-nitrosamines

- Important nitrosamines, formed DNA alkylation adducts, and their sources
- N-nitrosamines cause various DNA lesions that are substrate for different DNA repair pathways

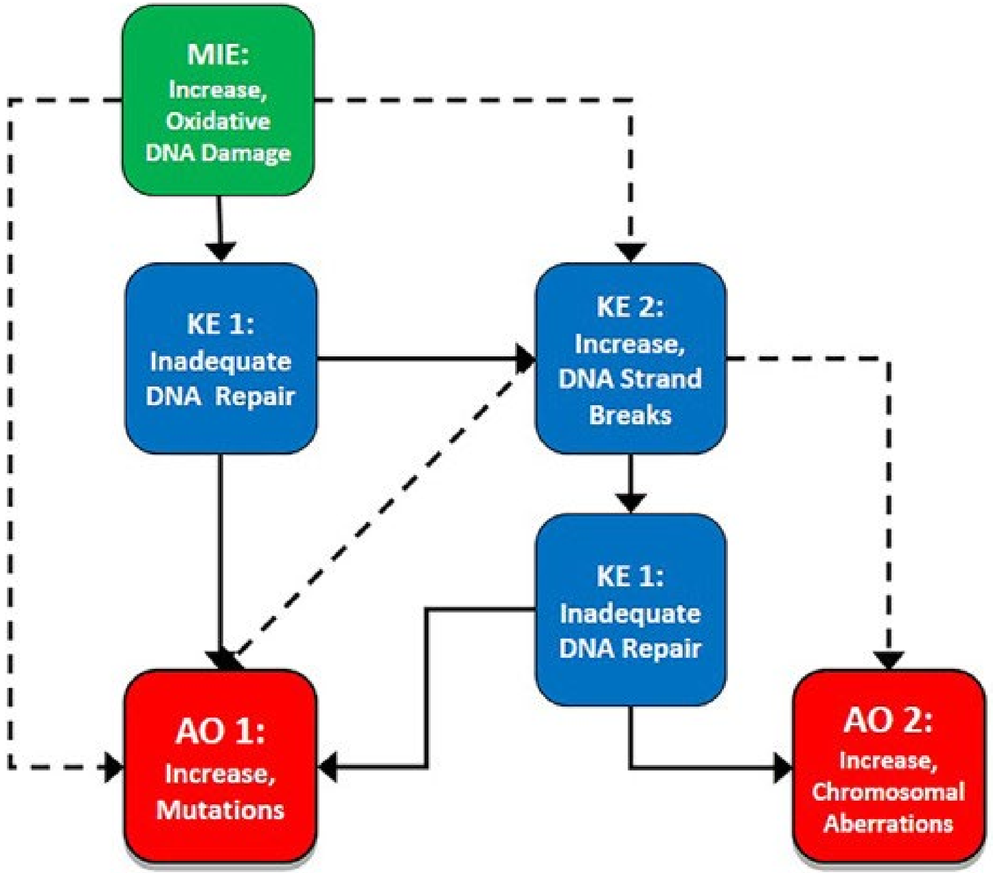
Nitrosamines	Abbreviation	Major DNA Alkylation Adducts	Sources
N-nitrosodimethylamine	NDMA	N7-MeG, N3-MeA O^6-MeG, O^2-MeT, O^4-MeT	Food, drugs, tobacco smoke
N-nitrosodiethylamine	NDEA	N7-EtG, N3-EtA, O^6-EtG, O^2-EtT, O^4-EtT	Food, drugs
N-nitrosopiperidine	NPIP	7-(2-oxopropyl)-N1,N^2-etheno-G, N^2-(3,4,5,6-tetrahydro-2H-pyran-2-yl)-2′-G	Food
N-nitrosopyrrolidine	NPYR	N7,8-ButanoG, N7-(4-Oxobutyl)-G, O^4-(4-OH-Butyl)-T, and others	Food
N-nitrosodiethanolamine	NDELA	O^6-OHEtG and others; glyoxal adducts	Cosmetics
N-nitroso-*N*-methyl-4-aminobutanoic acid	NMBA	unknown	Drugs
N-nitrosodiisopropylamine	NDIPA	unknown	Drugs
N-nitrosoethylisopropylamine	NEIPA	unknown	Drugs
N-nitrosomethylphenylamine	NMPA	unknown	Drugs
N-nitrosovarenicline	-	unknown	Drugs
N-nitrososalbutamol	-	unknown	Drugs
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; (nicotine-derived nitrosamine ketone)	NNK	N7-MeG, N3-MeA, N3-MeG, O^6-MeG, O^4-MeG, O^6-pobG	Tobacco smoke
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; (nicotine-derived nitrosamine alcohol)	NNAL	N7-MeG, N3-MeA, N3-MeG, O^6-MeG, O^4-MeG, O^6-pobG	Tobacco smoke
N′-nitrosonornicotine	NNN	O^6-pobG	Tobacco smoke
N′-nitrosoanabasine	NAB	unknown	Tobacco smoke
N′-nitrosoanatabine	NAT	unknown	Tobacco smoke

DNA repair-Profiler case study: Oxidative DNA damage

- KBrO3, oxidizing agent
  - Positive in AMES
  - Positive *in vitro* MN, and CA
  - Positive *in vivo* MN

- MMS, alkylating agent
  - Positive in AMES test
  - Positive *in vitro* MLA, MN, and CA
  - Positive *in vivo* MN, CA, comet, and UDS

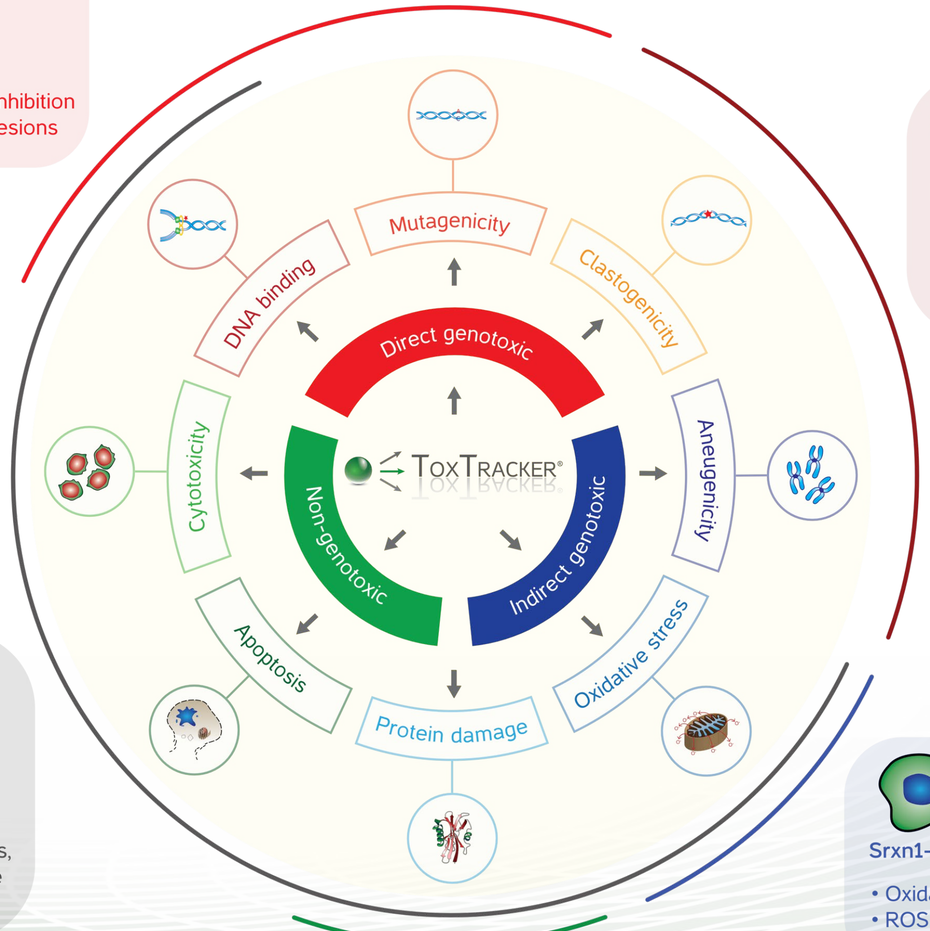
- What is the mode-of-action?
- Build evidence for AOP



Cho, *et al.*, 2022

The ToxTracker assay

- Stem cell-based reporter assay
- Accurate detection of genotoxicity
- Detect induction of DNA damage, oxidative stress and protein damage
- Insight into mode of action of genotoxic compounds



Bscl2-GFP

- DNA replication inhibition
- Mutagenic DNA lesions

Rtkn-GFP

- DNA double strand breaks
- Aneuploidy (ACE)
- Clastogens vs aneugens

Btg2-GFP

- p53 tumor suppressor activation
- DNA damage, oxidative stress, osmotic shock, ribonucleotide depletion, apoptosis

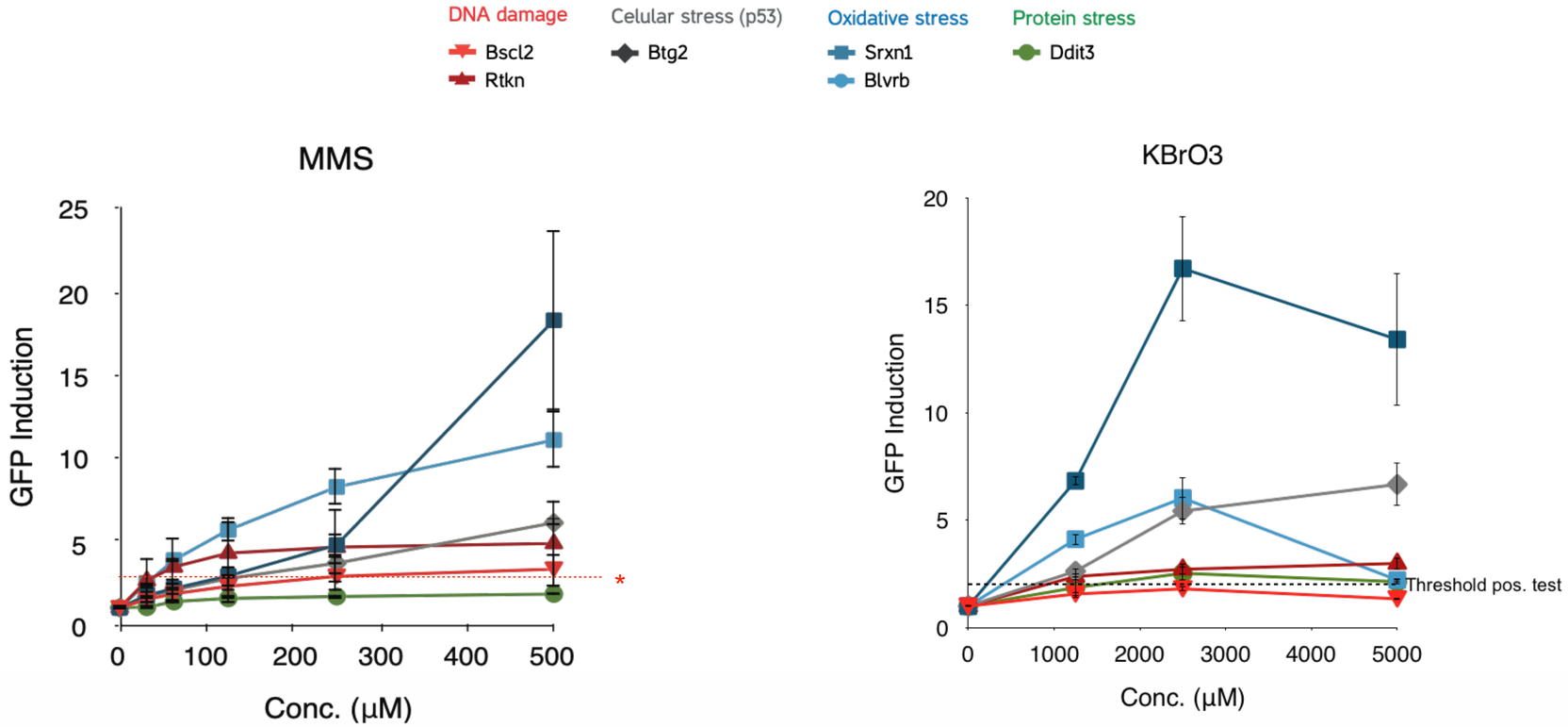
Srxn1-GFP **Blvrb-GFP**

- Oxidative stress
- ROS production
- Indirect genotoxicity (AO)

Ddit3-GFP

- Unfolded protein response
- ER stress

ToxTracker AO: DNA damage and oxidative stress

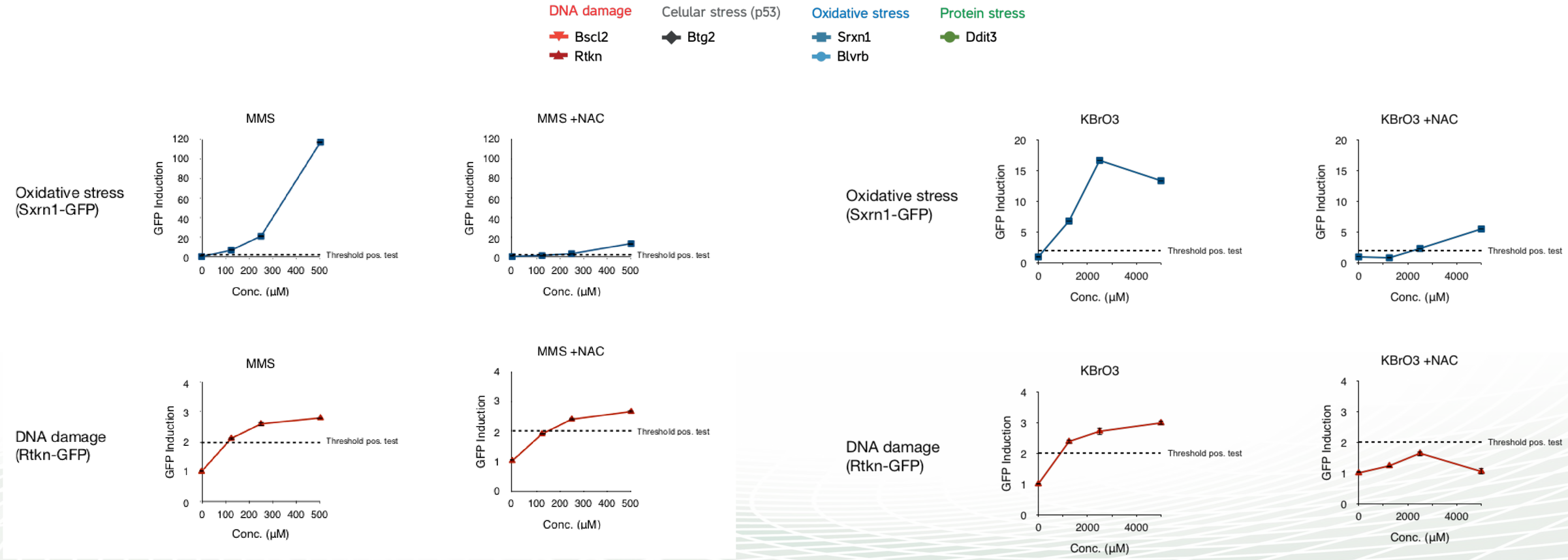


- MMS
  - Oxidative stress
  - Rtkn and Bscl2 activation
  - Directly genotoxic?

- KBrO3
  - Oxidative stress
  - Rtkn activation
  - Indirectly genotoxic?

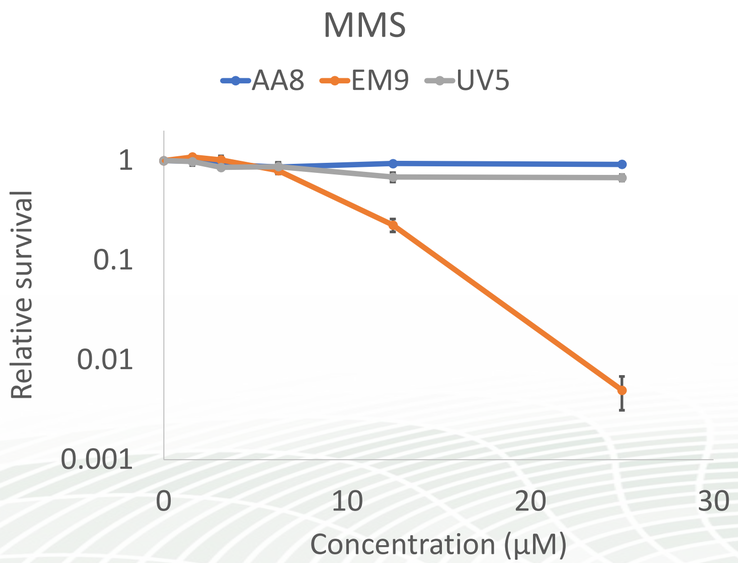
ToxTracker AO: DNA damage and oxidative stress

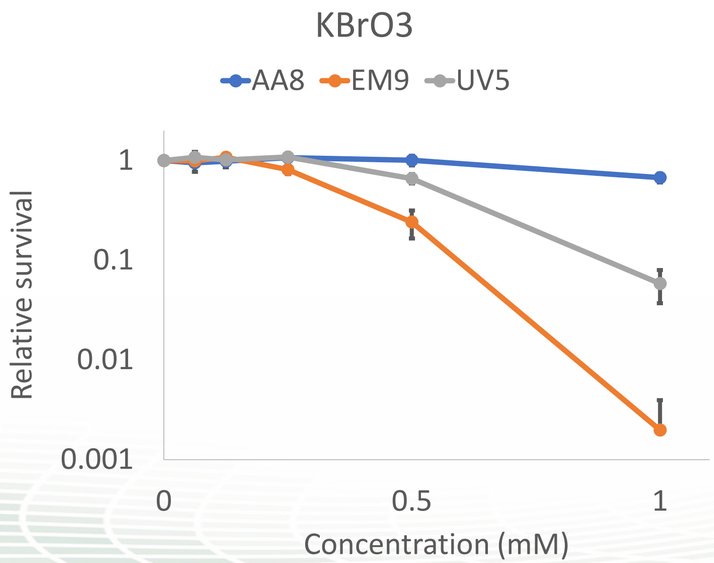
- ToxTracker assay in presence of ROS scavengers N-acetyl cysteine and reduced glutathione



DNA damage induction by MMS and KBrO3

Cell line	Mutant	Origin
AA8	Wild type	Chinese hamster ovary
UV5 (NER)	Xpd	Chinese hamster ovary
EM9 (BER)	Xrcc1	Chinese hamster ovary



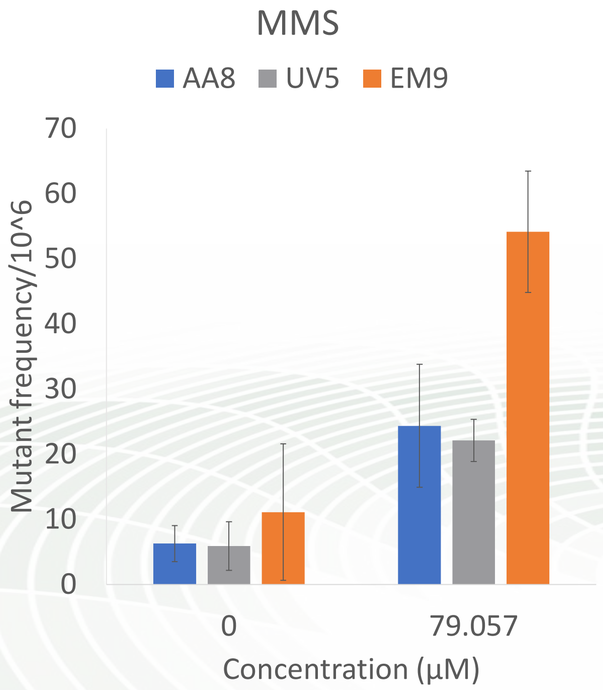


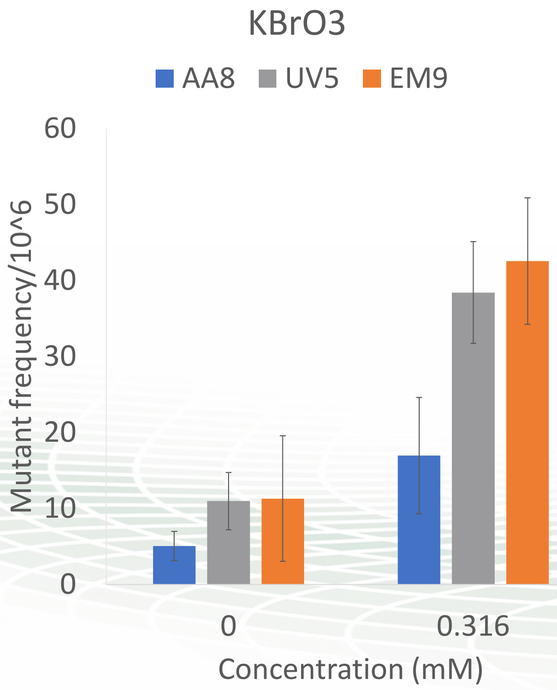
- MMS
  - Small, alkylating damage
  - BER
- KBrO3
  - BER
  - Different types of lesions
  - Also removed by NER

Gene mutation induction by MMS and KBrO3

Cell line	Mutant	Origin
AA8	Wild type	Chinese hamster ovary
UV5 (NER)	Xpd	Chinese hamster ovary
EM9 (BER)	Xrcc1	Chinese hamster ovary

- Hprt gene mutation assay in DNA-deficient cell lines

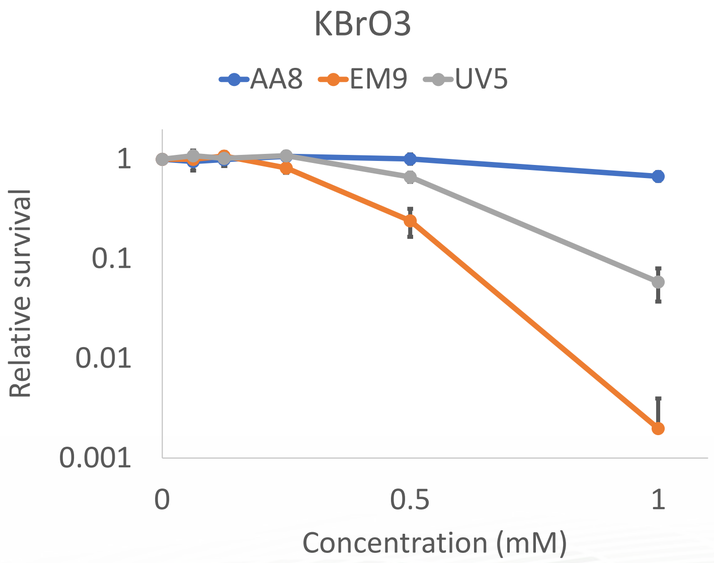


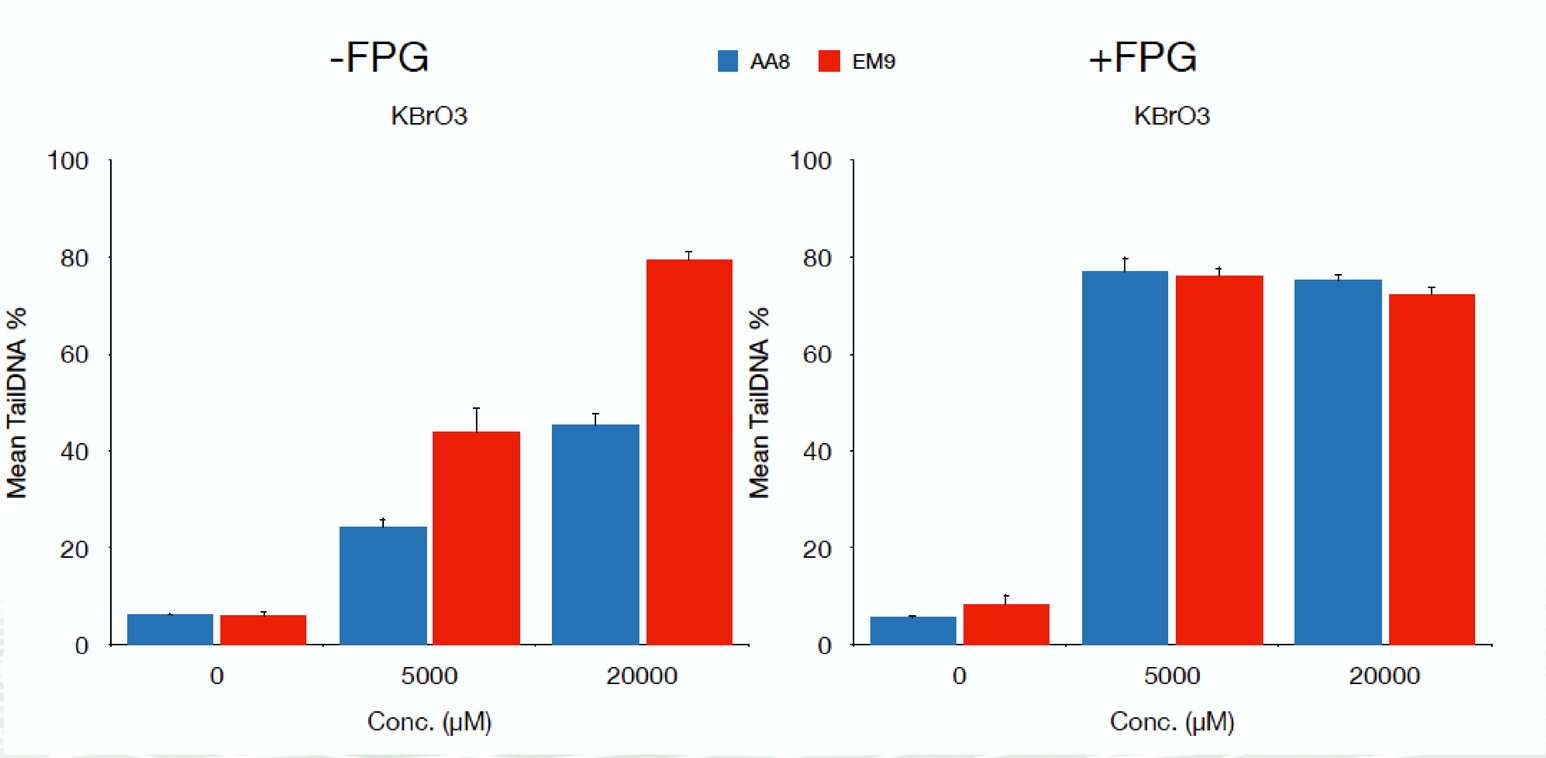


- KBrO3 and MMS
  - Absence of DNA leads to an increasesin gene mutations

Oxidate DNA lesions by KBrO3

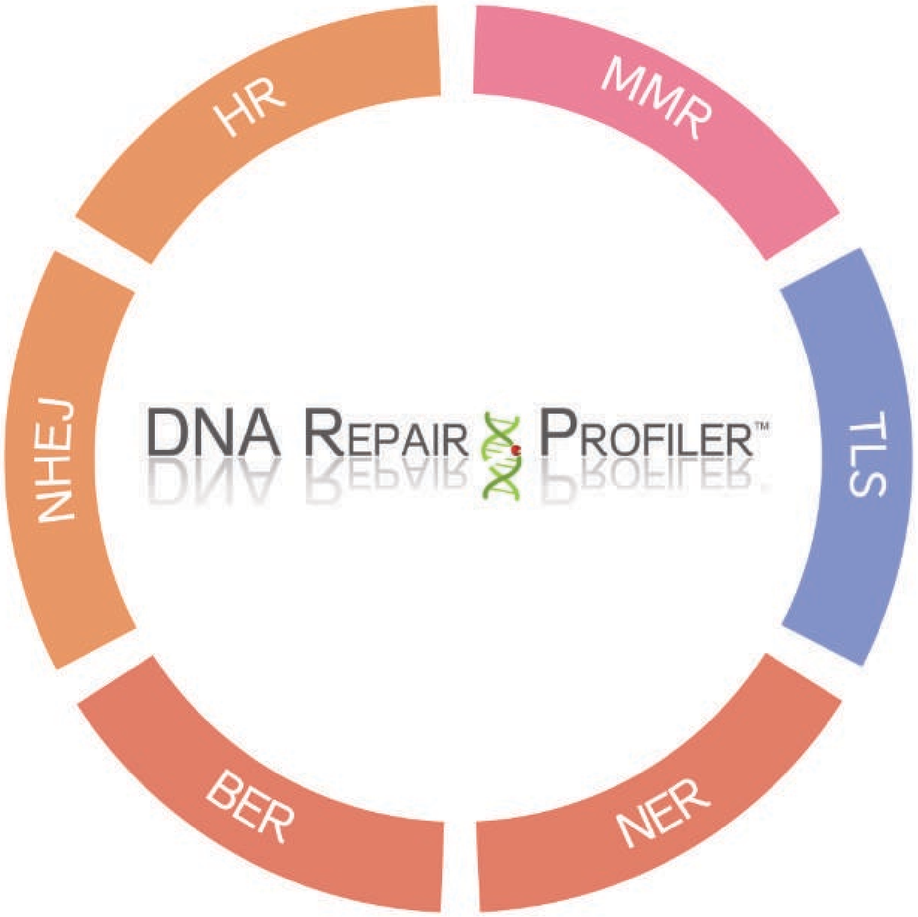
- FPG-modificed comet assay to detect oxidative DNA lesion





DNA Repair-Profiler

- DNA Repair-Profiler consists of a panel of DNA repair-deficient mammalian cell lines
- These can successfully determine the genotoxicity of substances
- The DNA repair-deficient mutants can be applied to investigate the types of DNA damage that is induced by genotoxic substances
- The DNA repair-deficient cell lines can be used to investigate DNA damage and gene mutation induction



The value of understanding

Thank you

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DNA Repair-Profiler case study II: hydrazine

- Rodent carcinogen
- IARC group 2a: probable human carcinogen

- Positive Ames test
- Equivocal results for CA and MLA
- Positive UDS
- Positive *in vitro* comet
- Negative mutation data

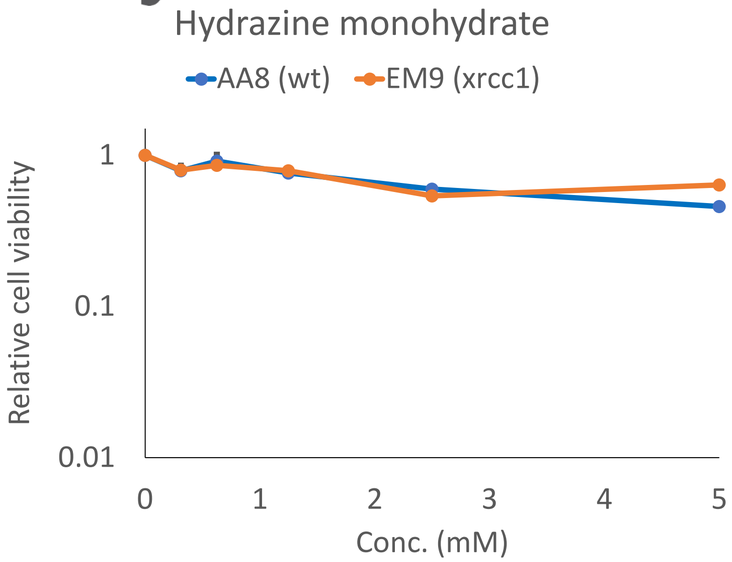
- Mechanistic insight into genotoxicity
- Understand involvement of different DNA repair mechanisms
- Does DNA repair deficiency lead to hydrazine-induced mutagenesis?

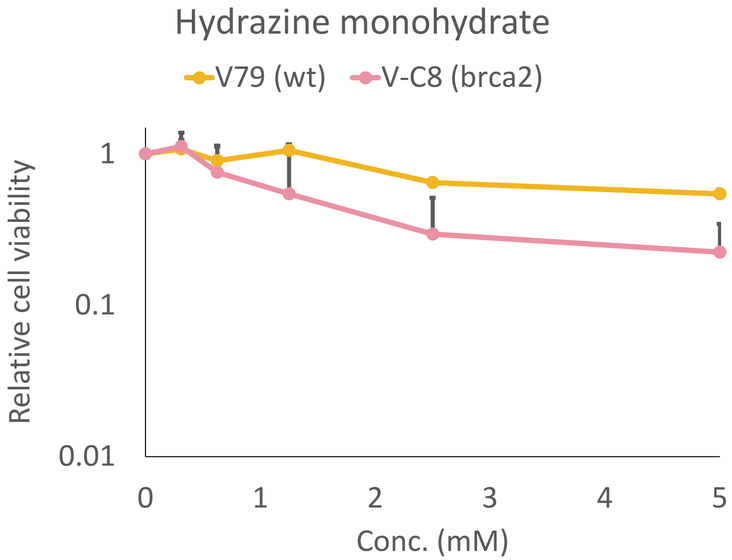
Table 2
Damage of nuclear DNA in primary cultures of rat lung cells exposed to test compounds

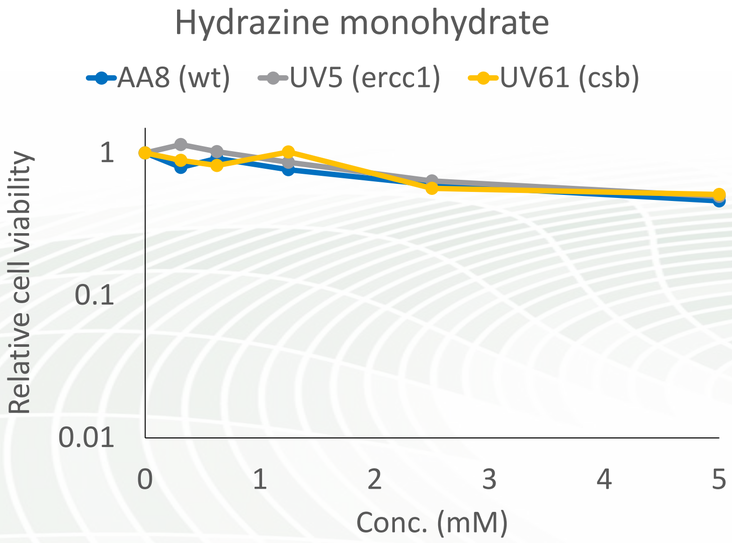
Treatment condition	Comet assay
	Tail moment
Solvent control	155 ± 53
HZ 0.25 mM	190 ± 107
HZ 0.5 mM	257 ± 100[b]
HZ 1.0 mM	312 ± 127[b]
HZ 2.0 mM	476 ± 179[b]
HZ 4.0 mM	643 ± 247[b]

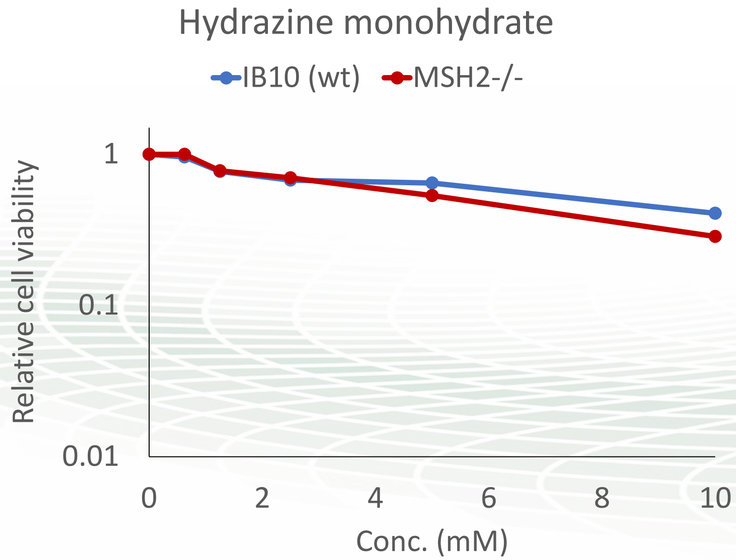
Adapted from Robbiano *et al.*, 2006

Case study: hydrazine





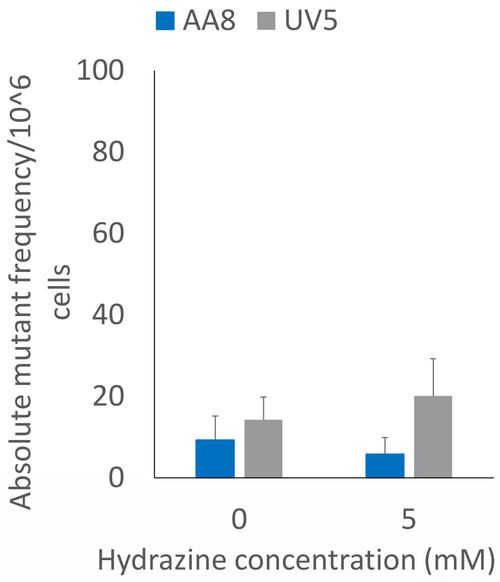


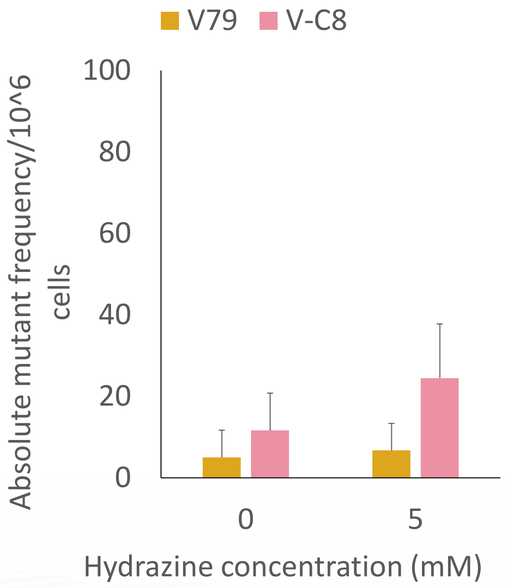


- No involvement of NER, BER, NHEJ, MMR
- Increased cytotoxicity in HR-deficient cells

Crosby *et al.*, 2022

Case study: hydrazine





- NER- and HR-deficient cells have a higher spontanous mutant frequency
- No significant mutation induction after hydrazine exposure in NER-deficient cells
- HR deficiency does not significantly increase mutant frequency
- Hydrazine is genotoxic but not mutagenic in mammalian cells

Crosby *et al.*, 2022