20.109 Spring 2016 Module 2 – Lecture 1
System Engineering (March 8th 2016)




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Leona Samson (Lectures)

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Key Experimental Methods for Module 2

- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

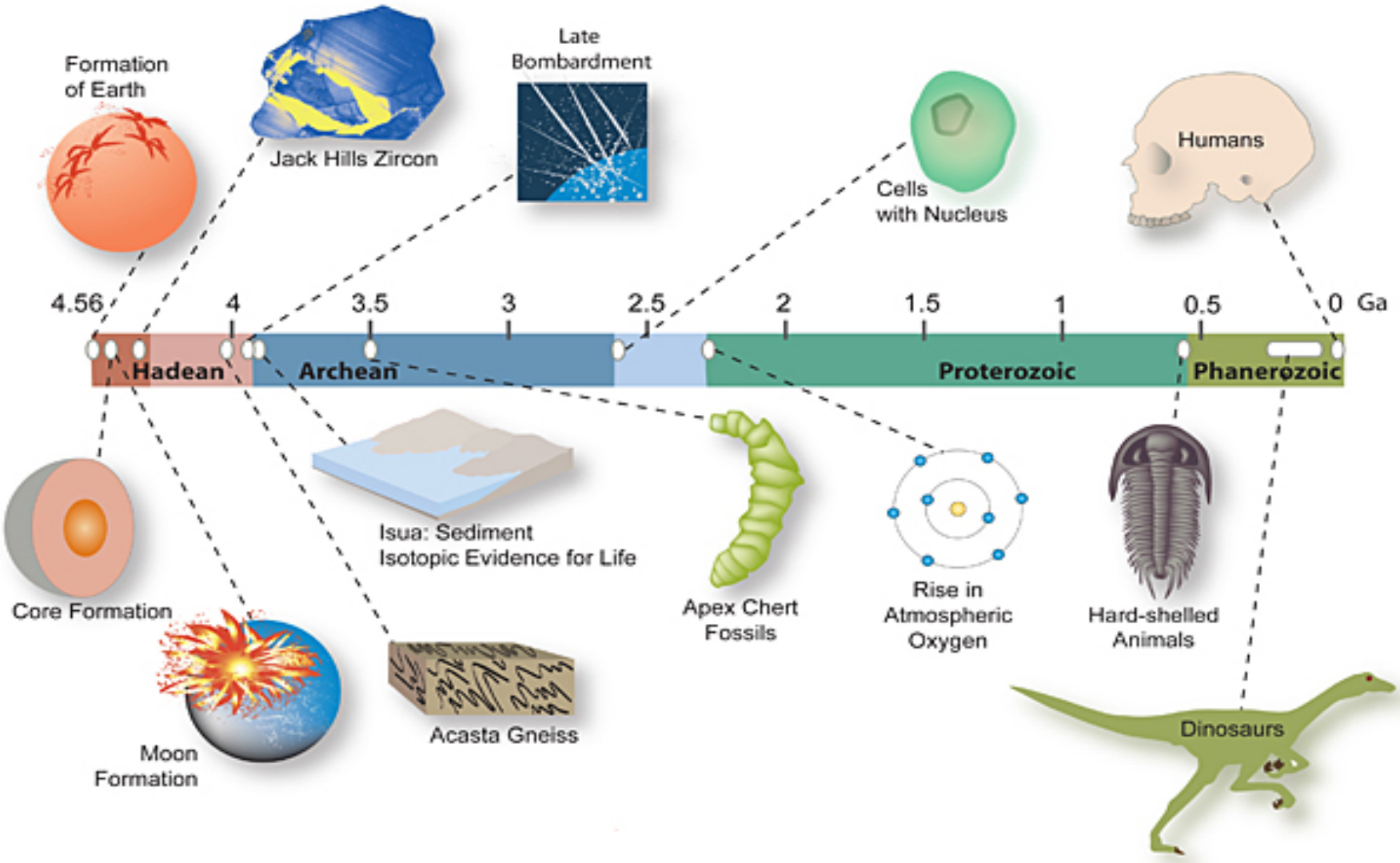
What experimental question will you ask in Module 2?

How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How will we actually measure DNA repair efficiency?

Evolution of life on Earth



http://astrobiology.com/2014/02/oldest-piece-of-earths-crust-suggests-a-cool-early-history.html

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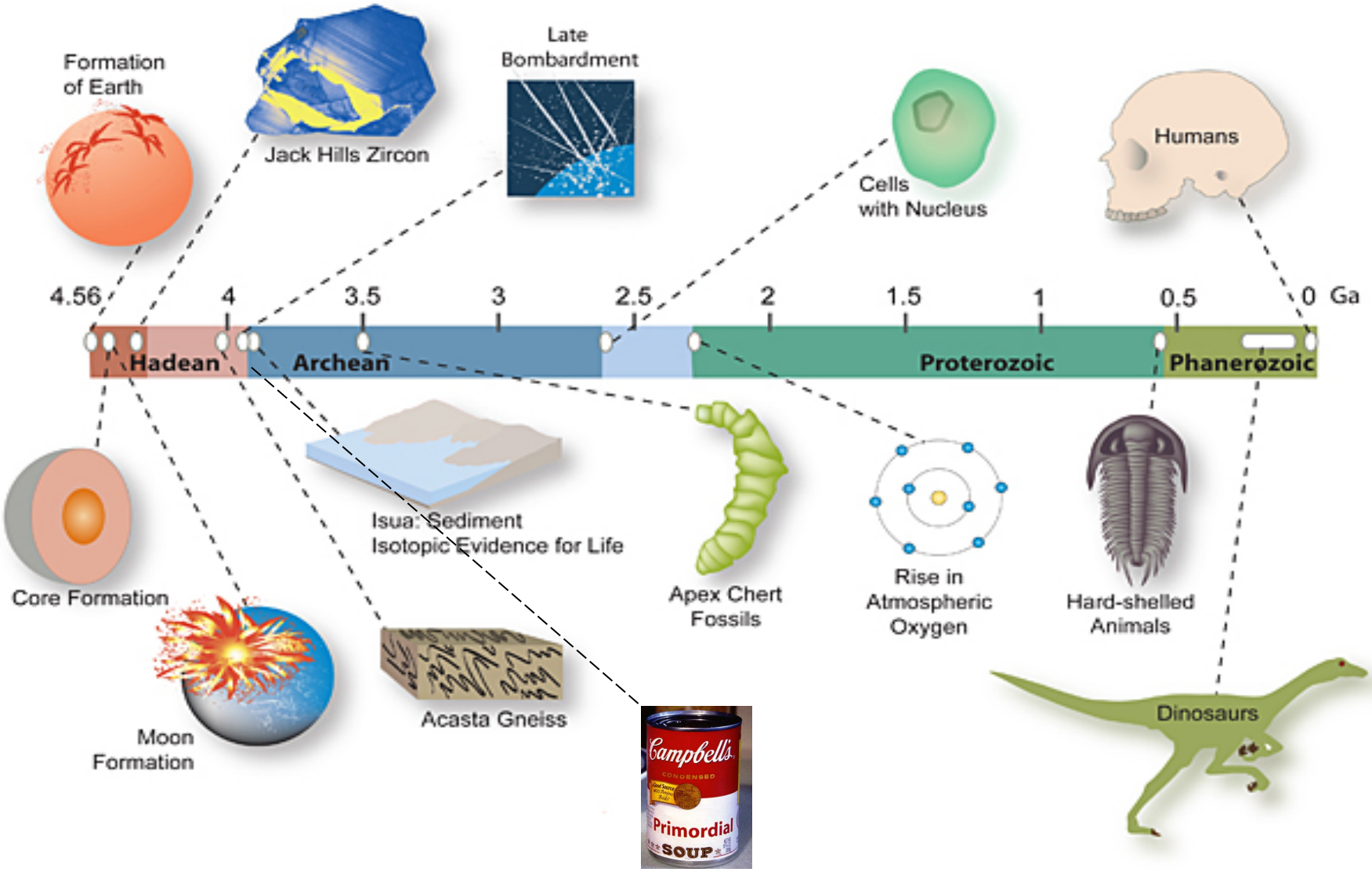

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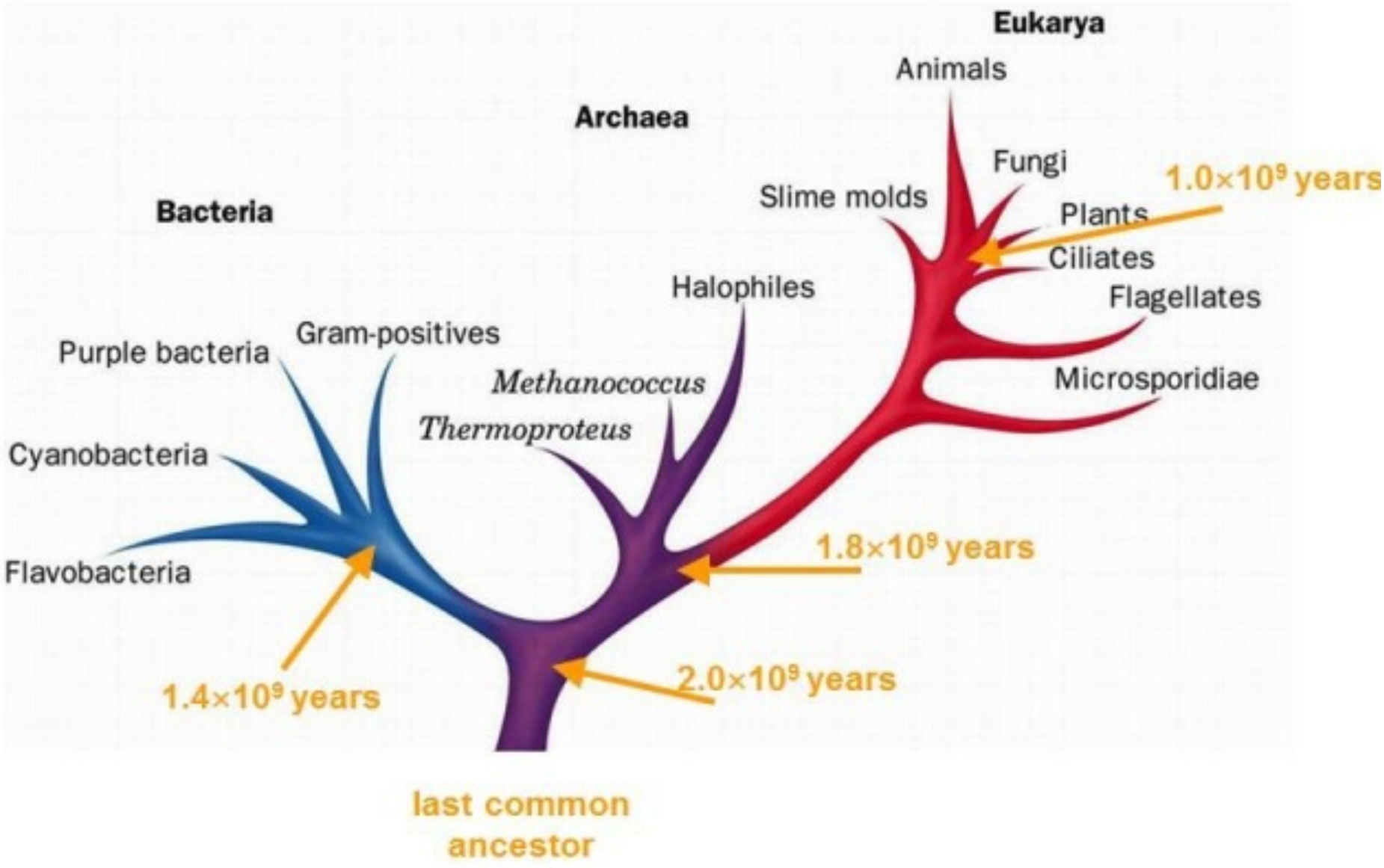
Andree Valley

All known life forms are based on DNA



http://biologicalphysics.iop.org/cws/article/lectures/47042

Central Dogma

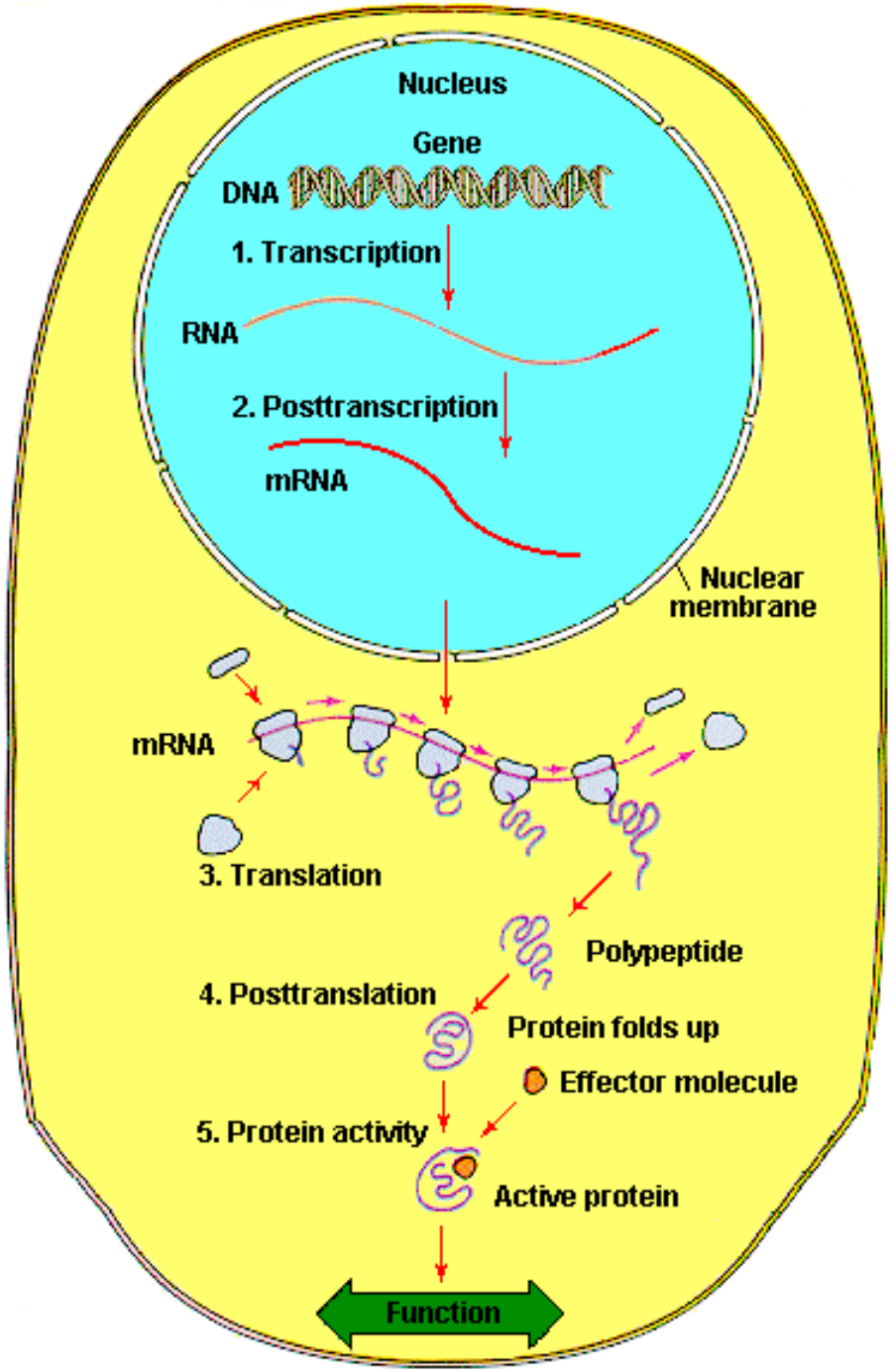
DNA

makes

RNA

makes

Protein



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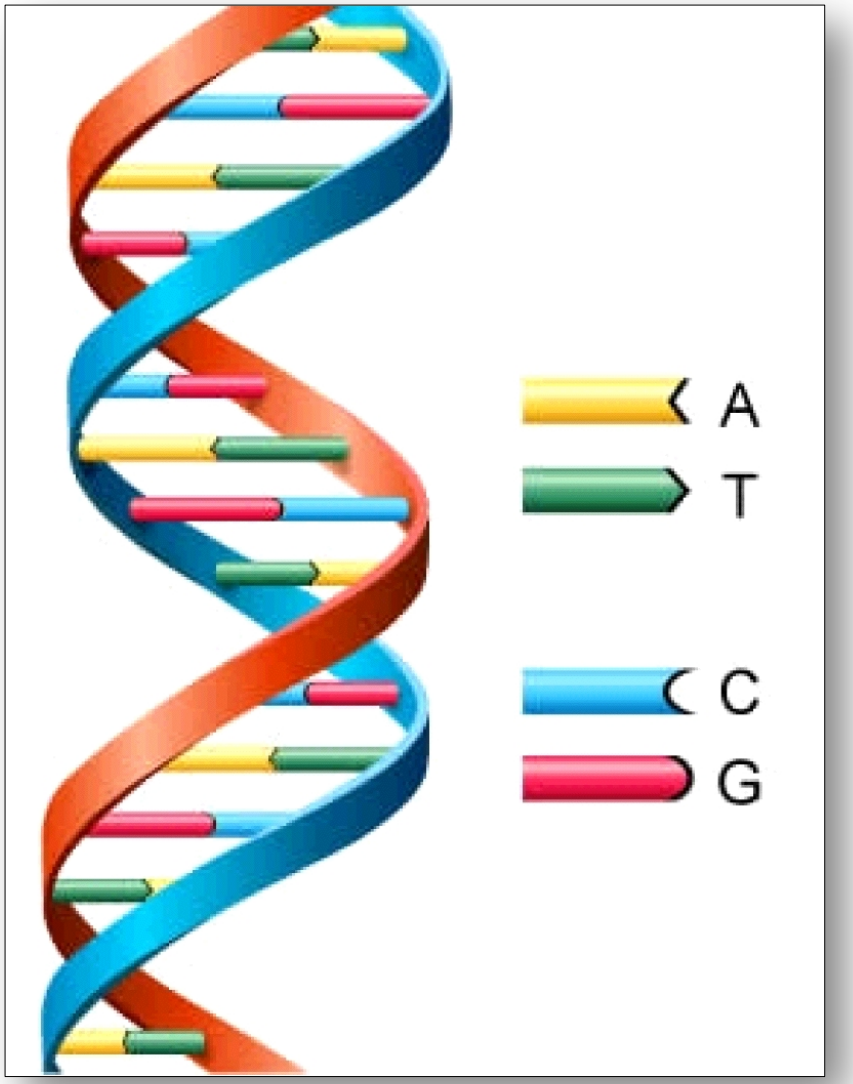
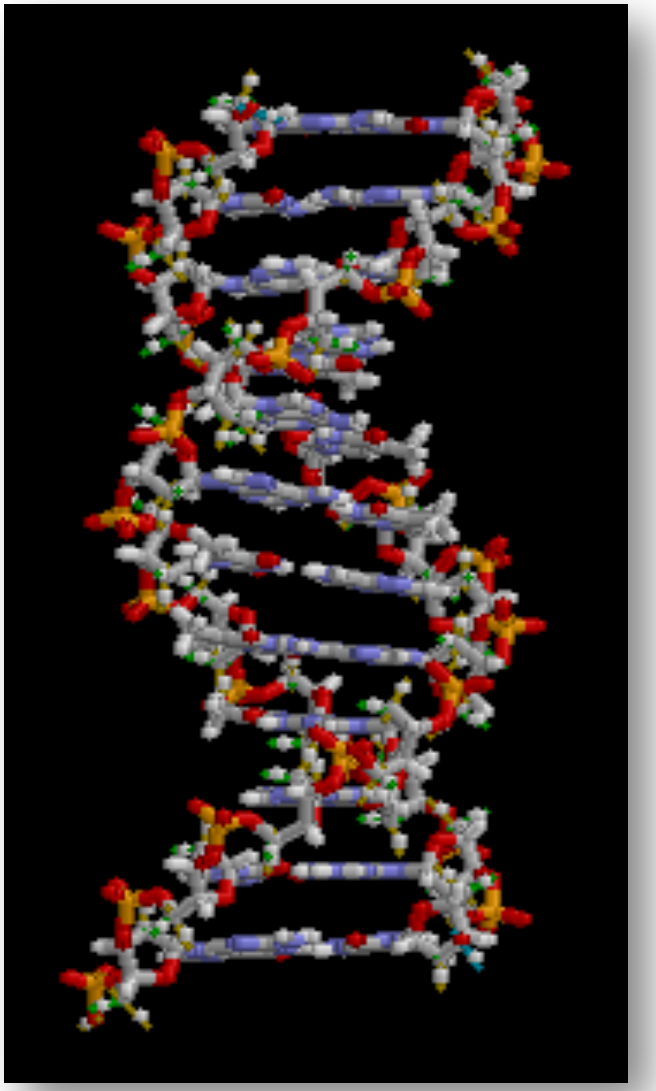
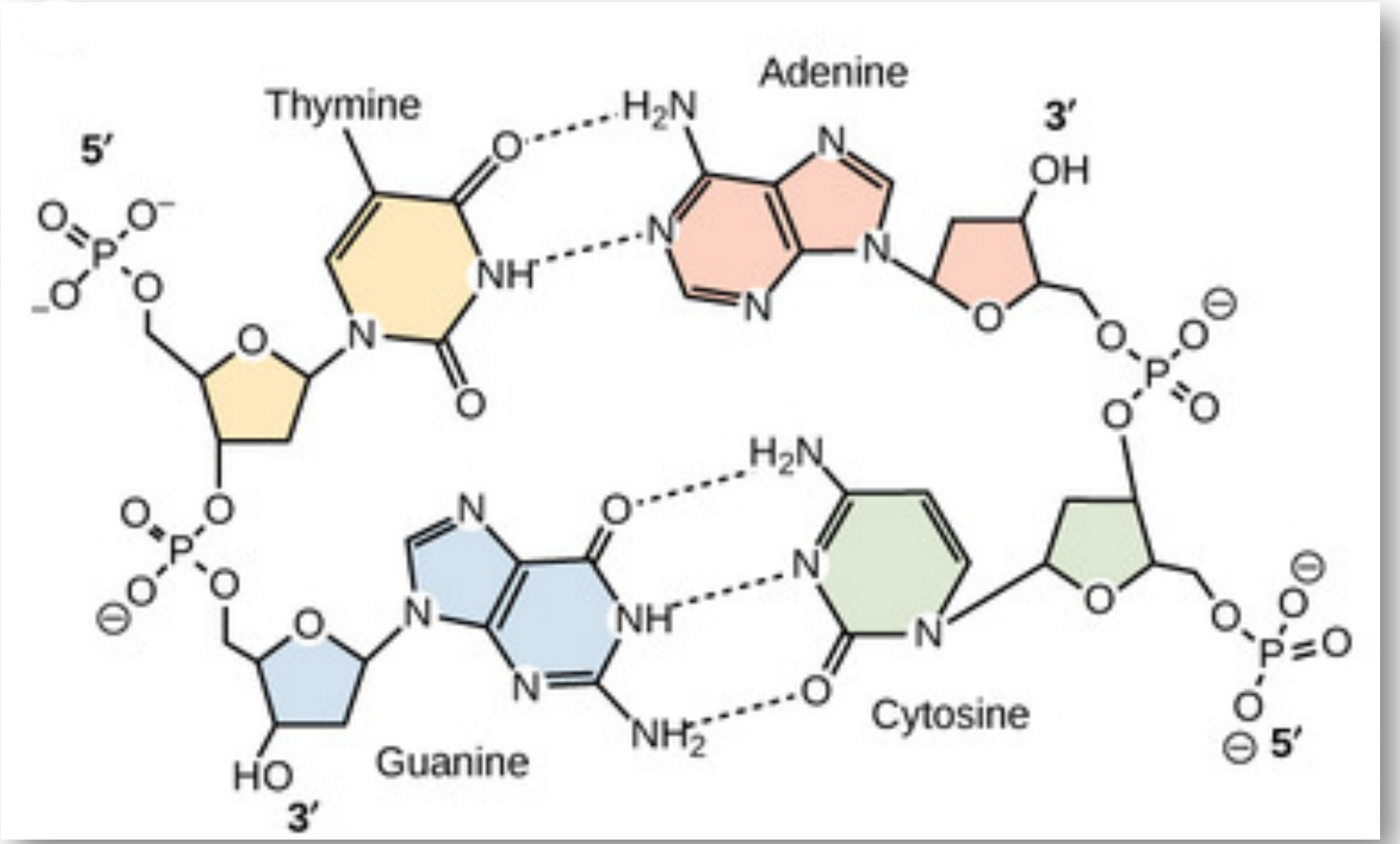




Each human cell has 6 billion base pairs of DNA

DNA Spontaneously Decays



In the time it takes to read this sentence your cells will have accumulated about 10 trillion DNA damage lesions throughout your body!

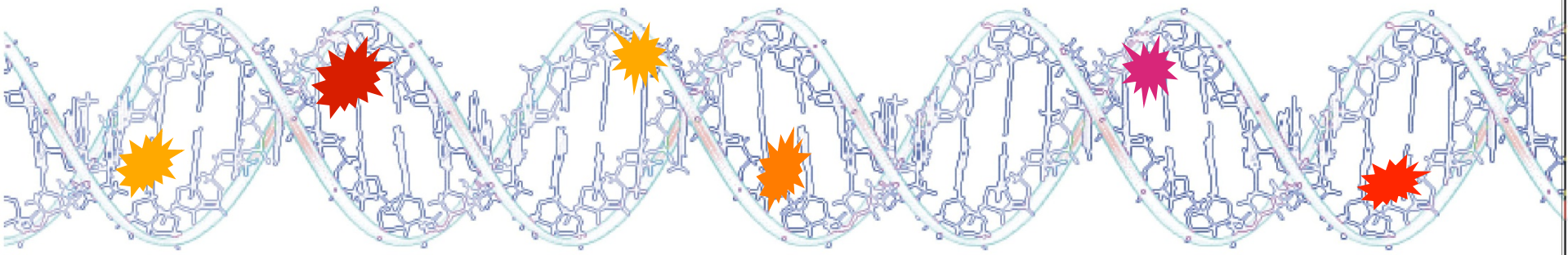
Assumptions:
20,000 lesions per cell per day
10^{13} cell in the human body
4 seconds to read the sentence

DNA is constantly being damaged by external and internal agents

Sunlight

Radiation

Inflammation



Chemicals in air, food, water

Normal metabolites

Reactive Oxygen species

Courtesy of Bevin Engelward

Environmental exposures to potentially harmful agents – DNA damaging agents

Harmful agents

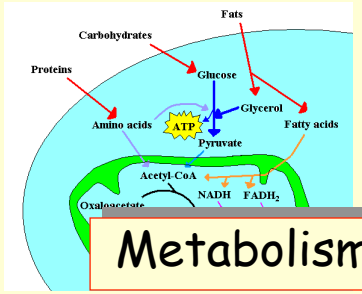

Eat


Medicine


Drink


Ambient

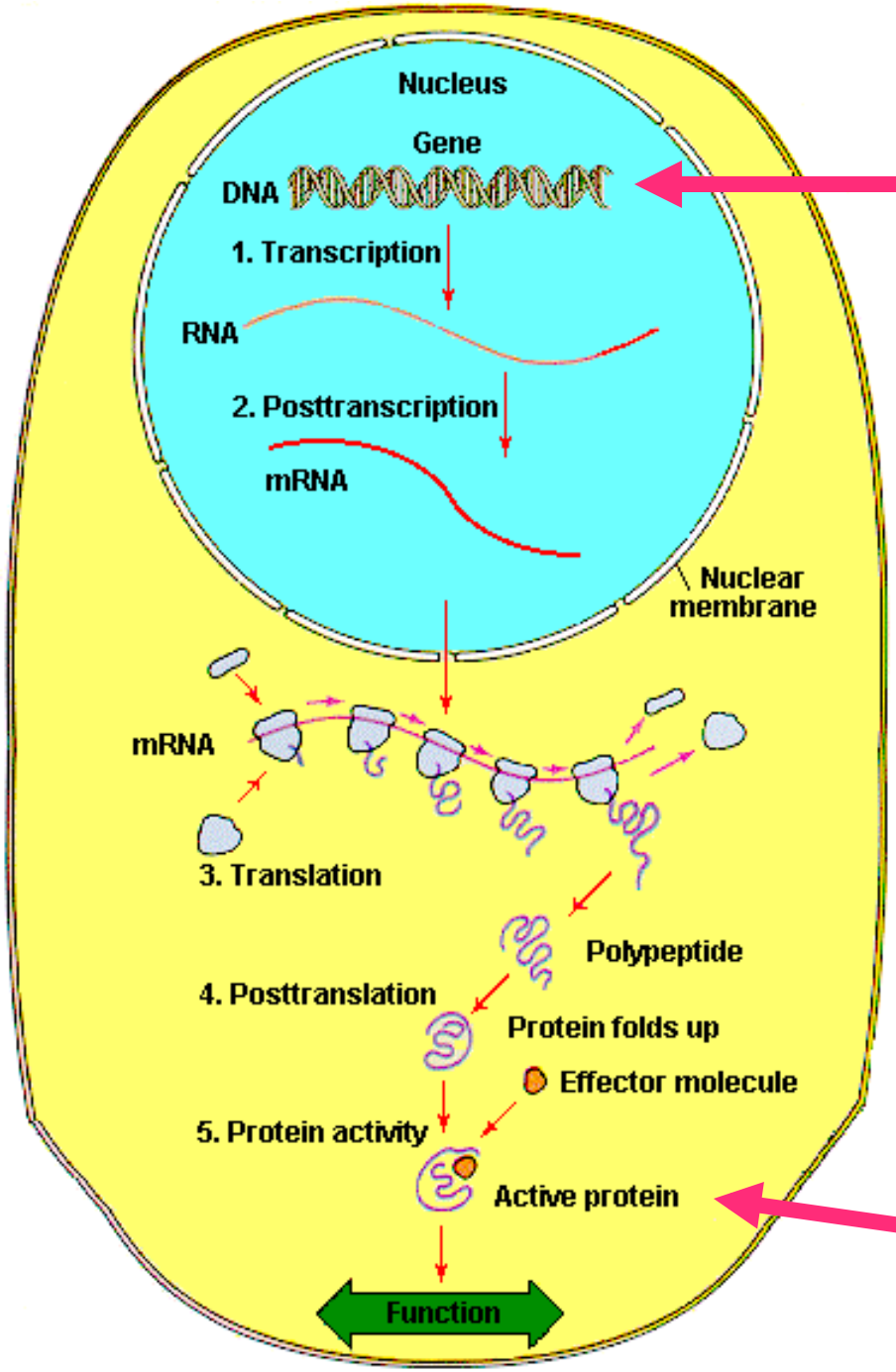

Breathe



Metabolism

People have different exposures

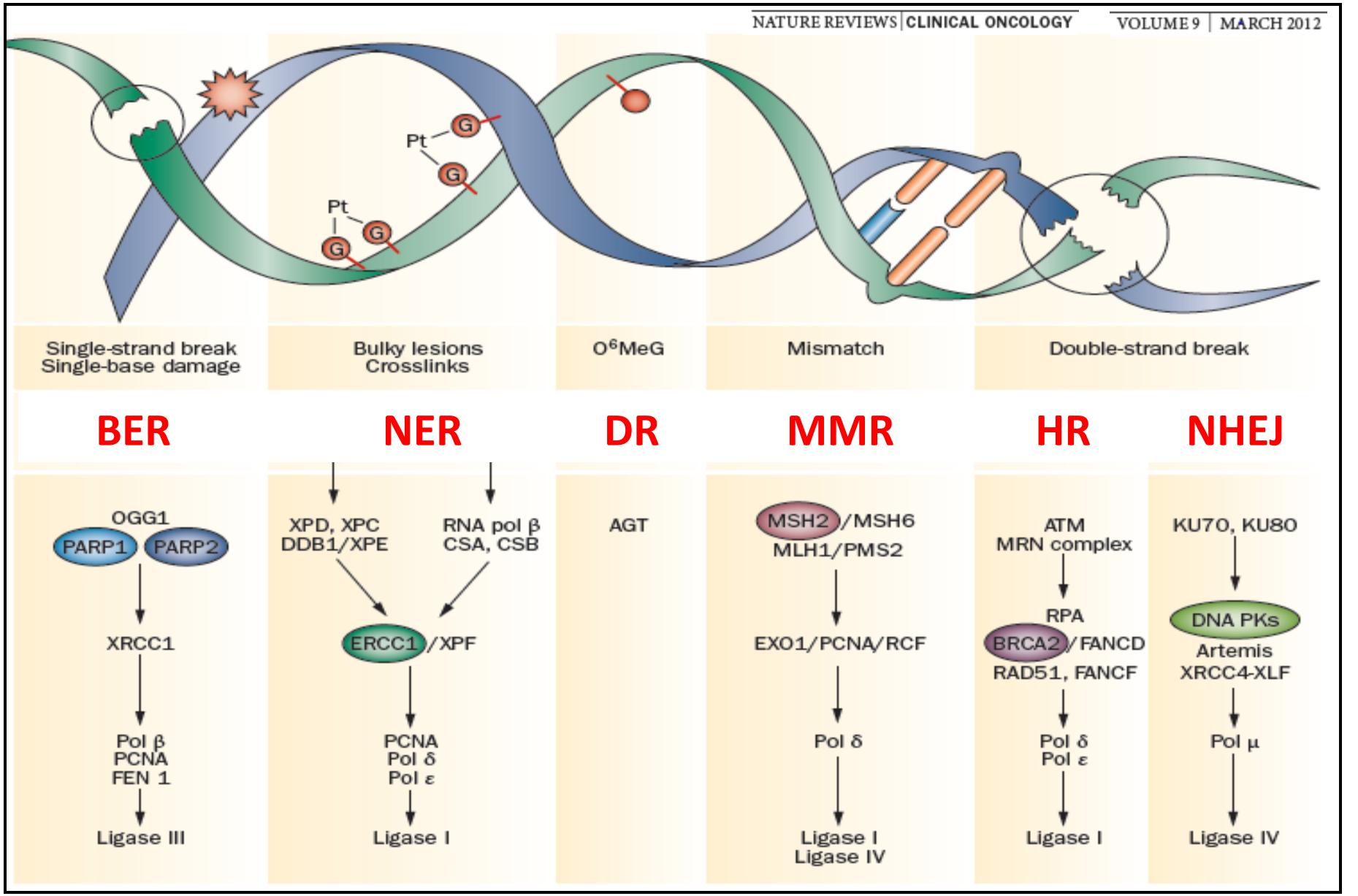
People have different responses



Damage to DNA can lead to permanent changes in the genetic information (mutations)

Inactive proteins or proteins with altered function are produced

Six Major DNA Repair Pathways



The Nobel Prize in Chemistry 2015

Photo: A. Mahmoud

Tomas Lindahl

Prize share: 1/3

Photo: A. Mahmoud

Paul Modrich

Prize share: 1/3

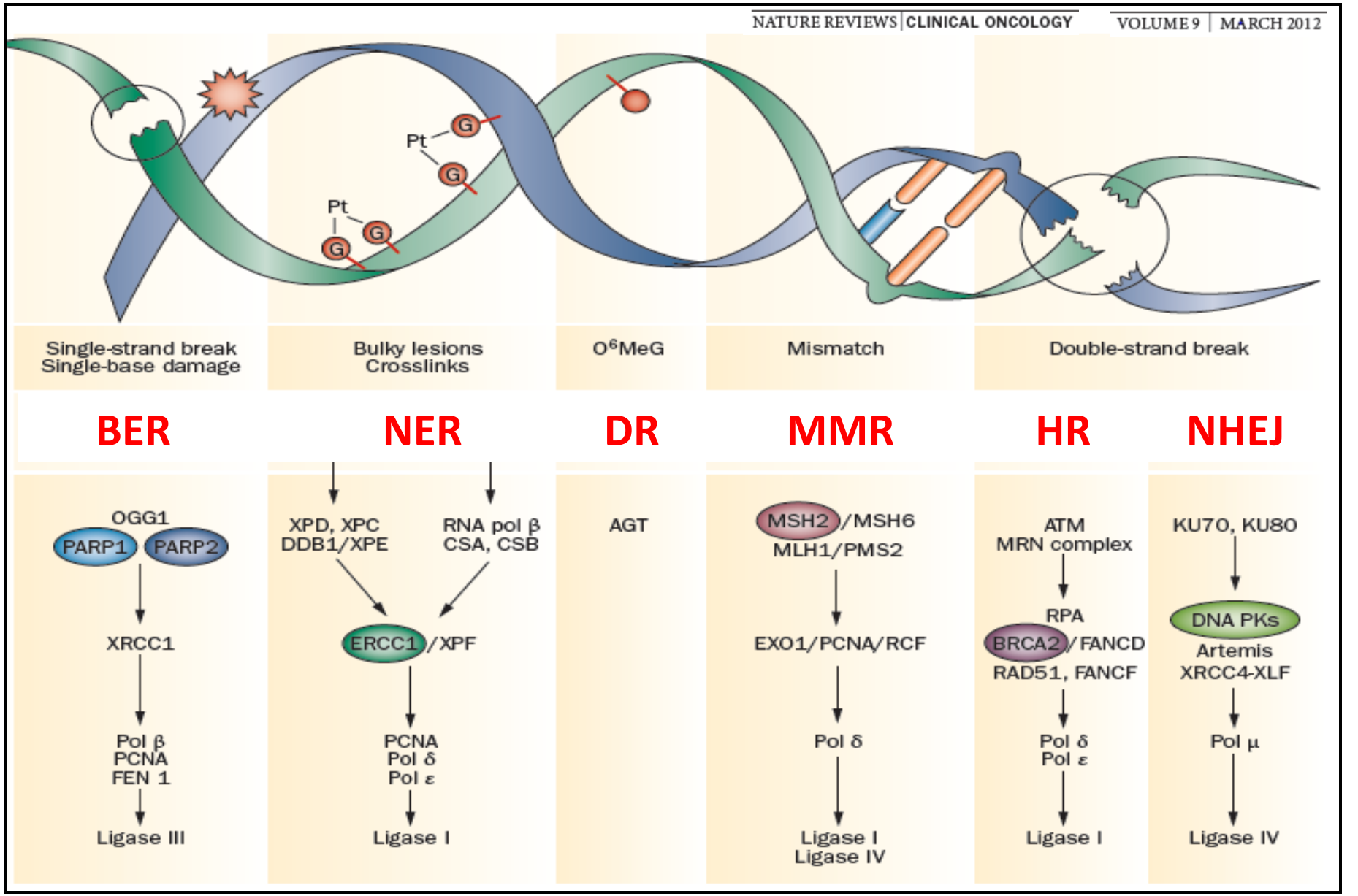
Photo: A. Mahmoud

Aziz Sancar

Prize share: 1/3

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *"for mechanistic studies of DNA repair"*.

Six Major DNA Repair Pathways



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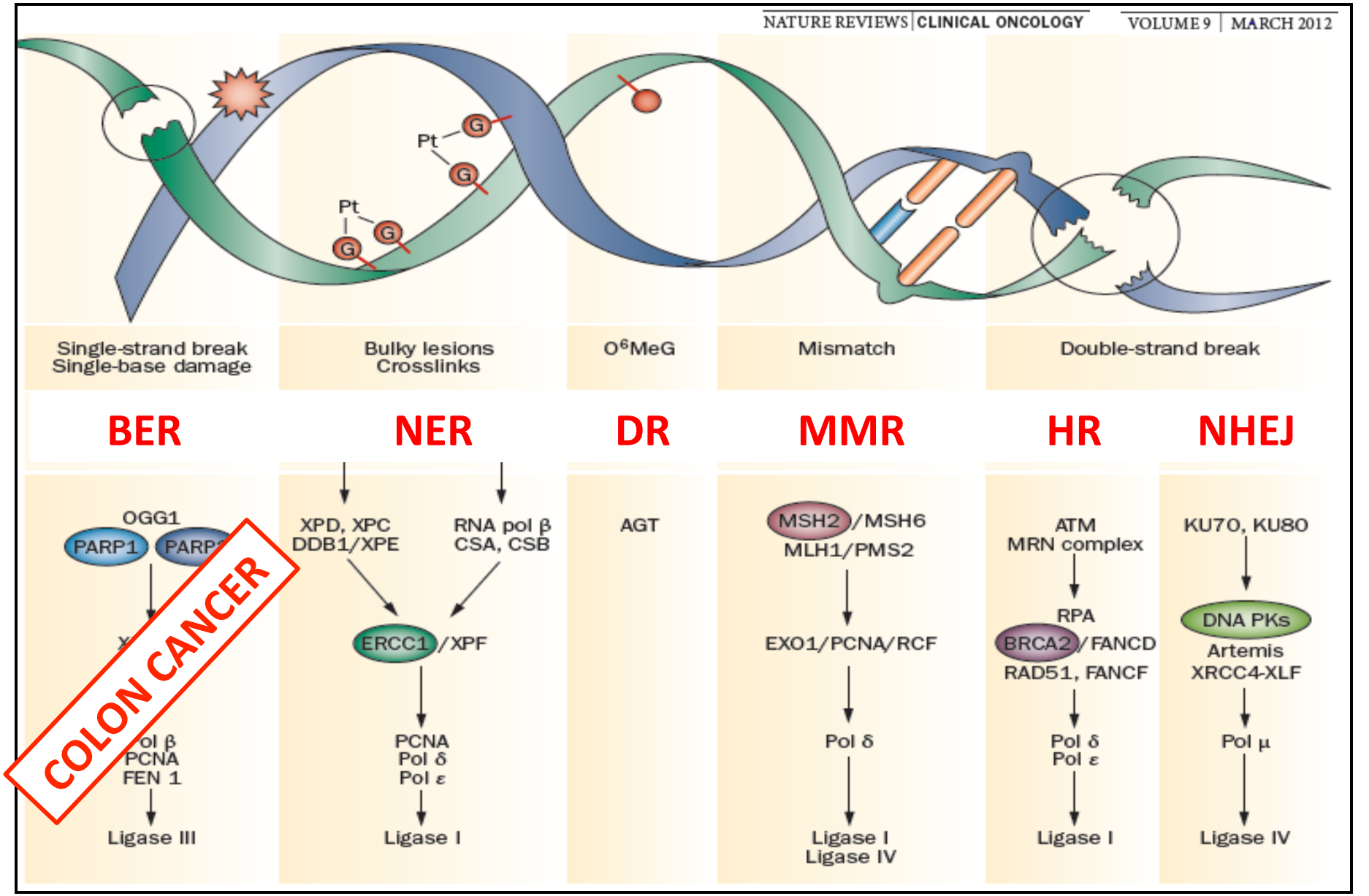

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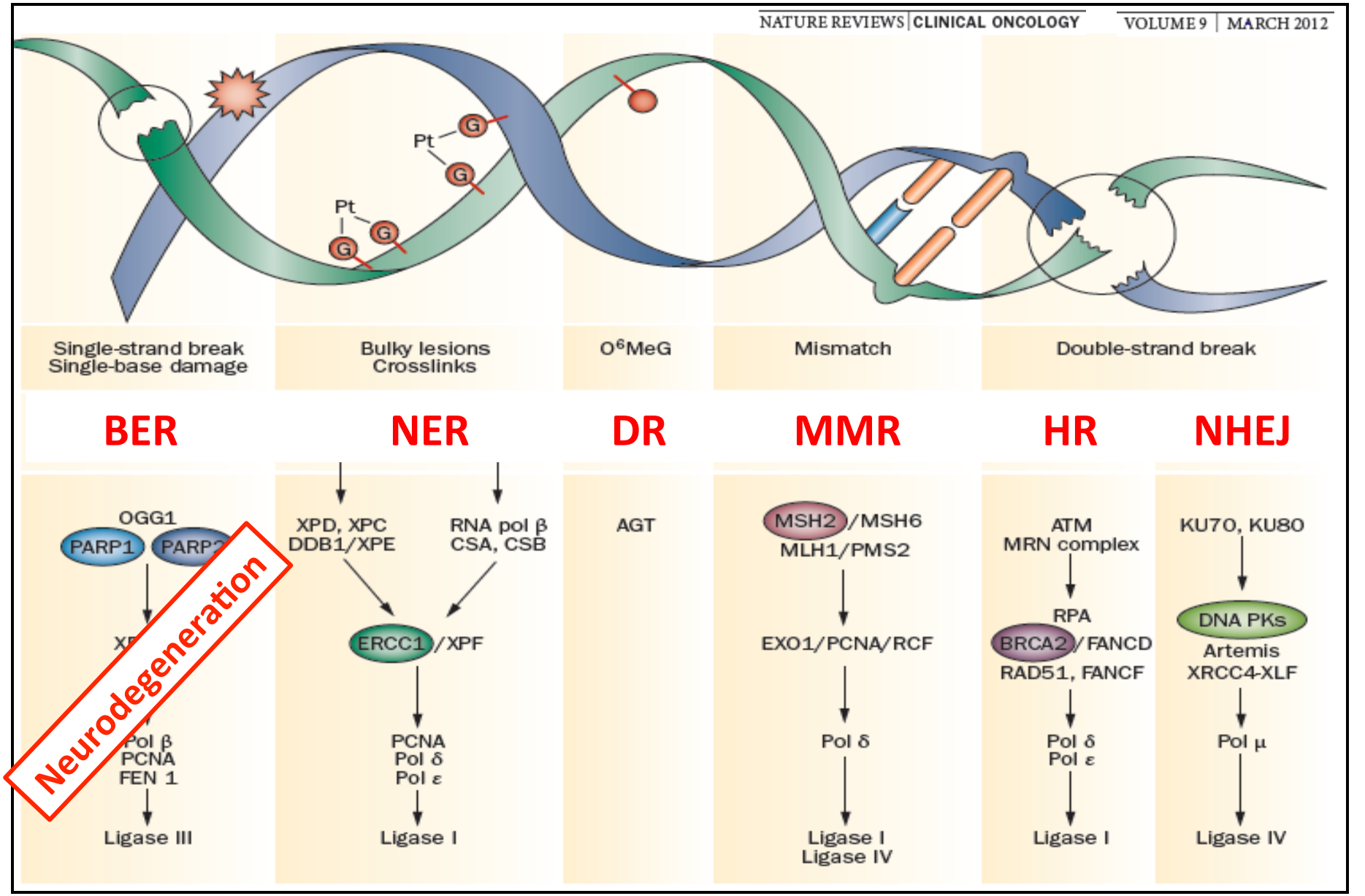

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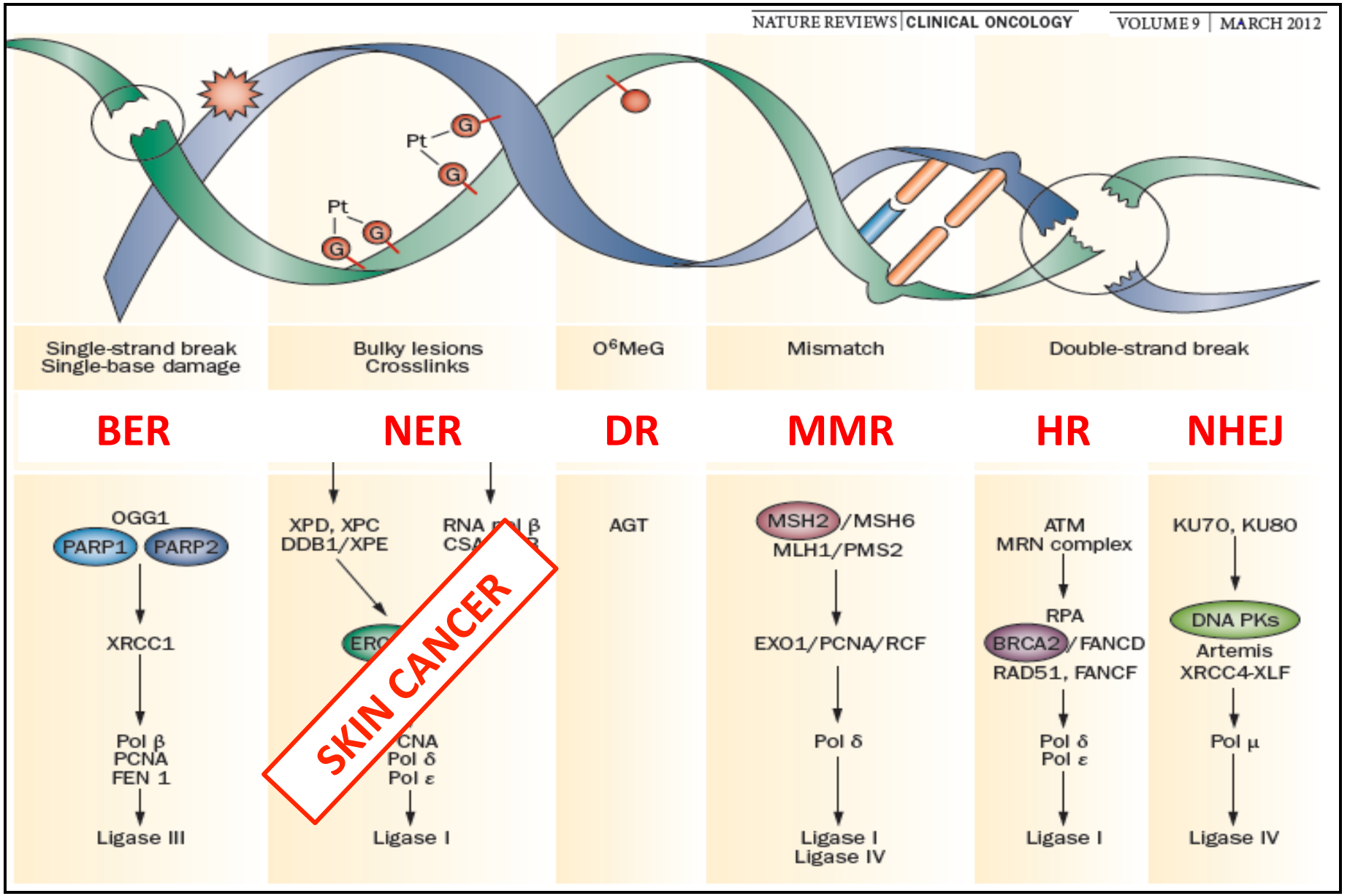

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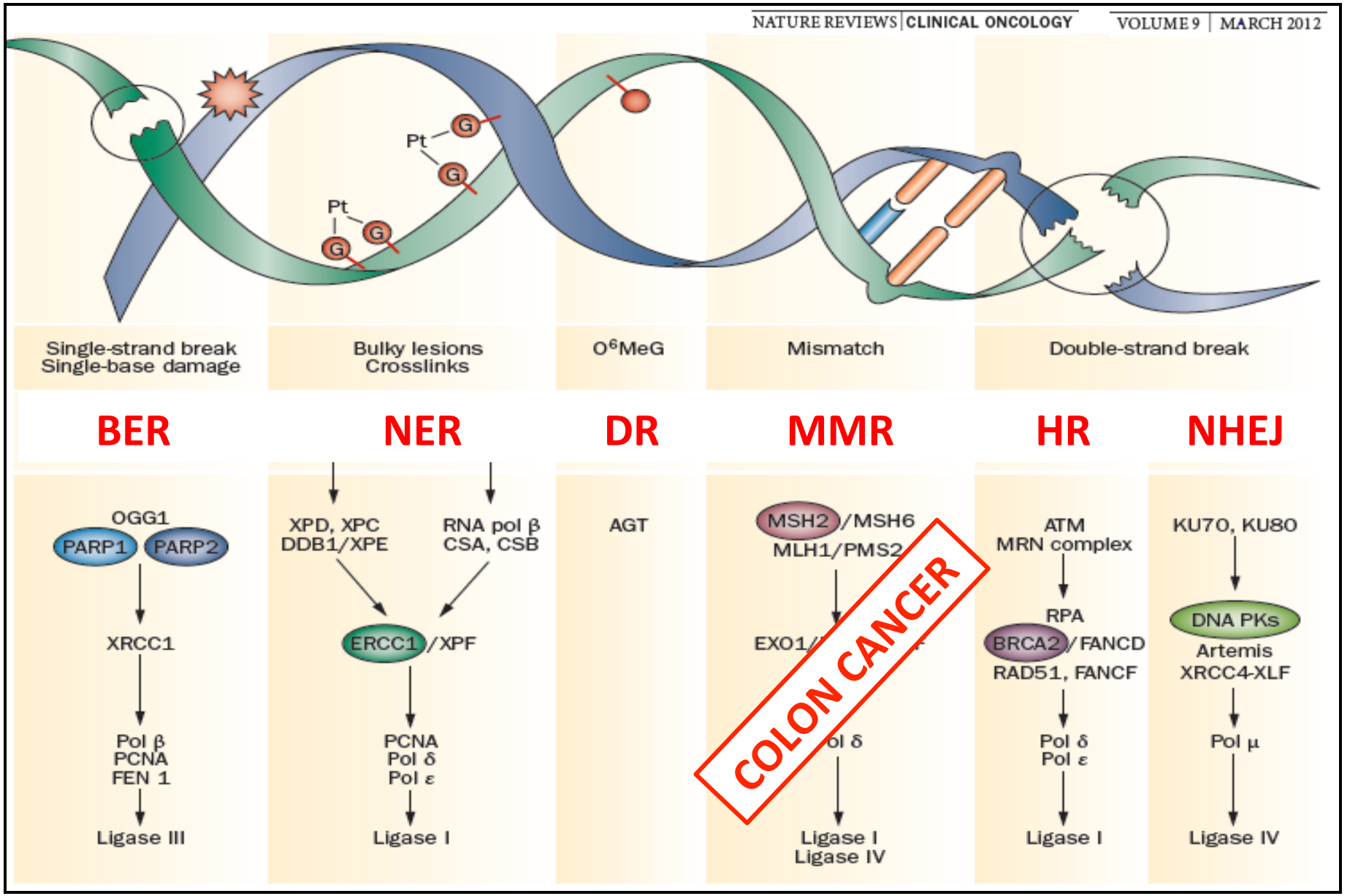

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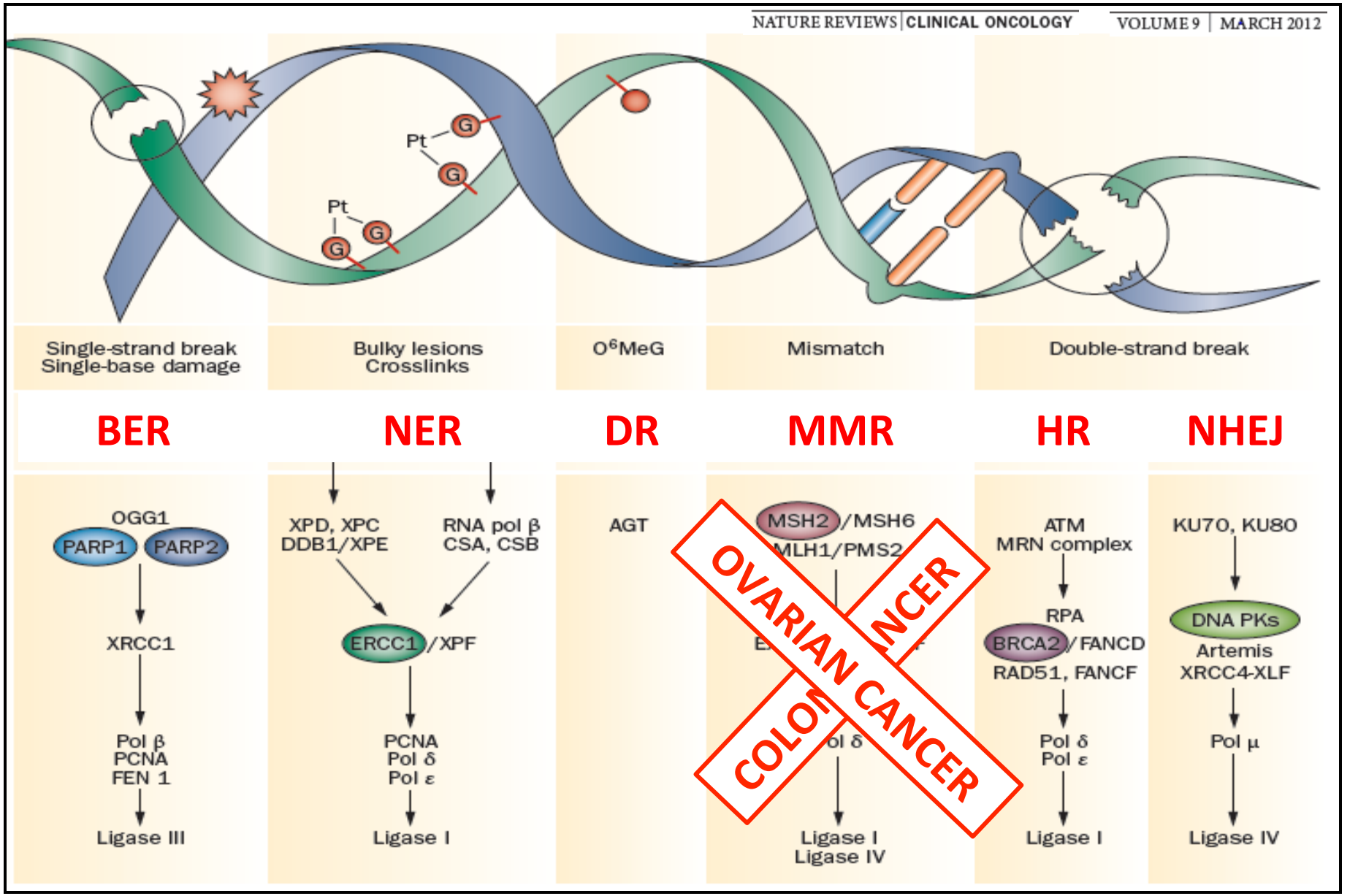

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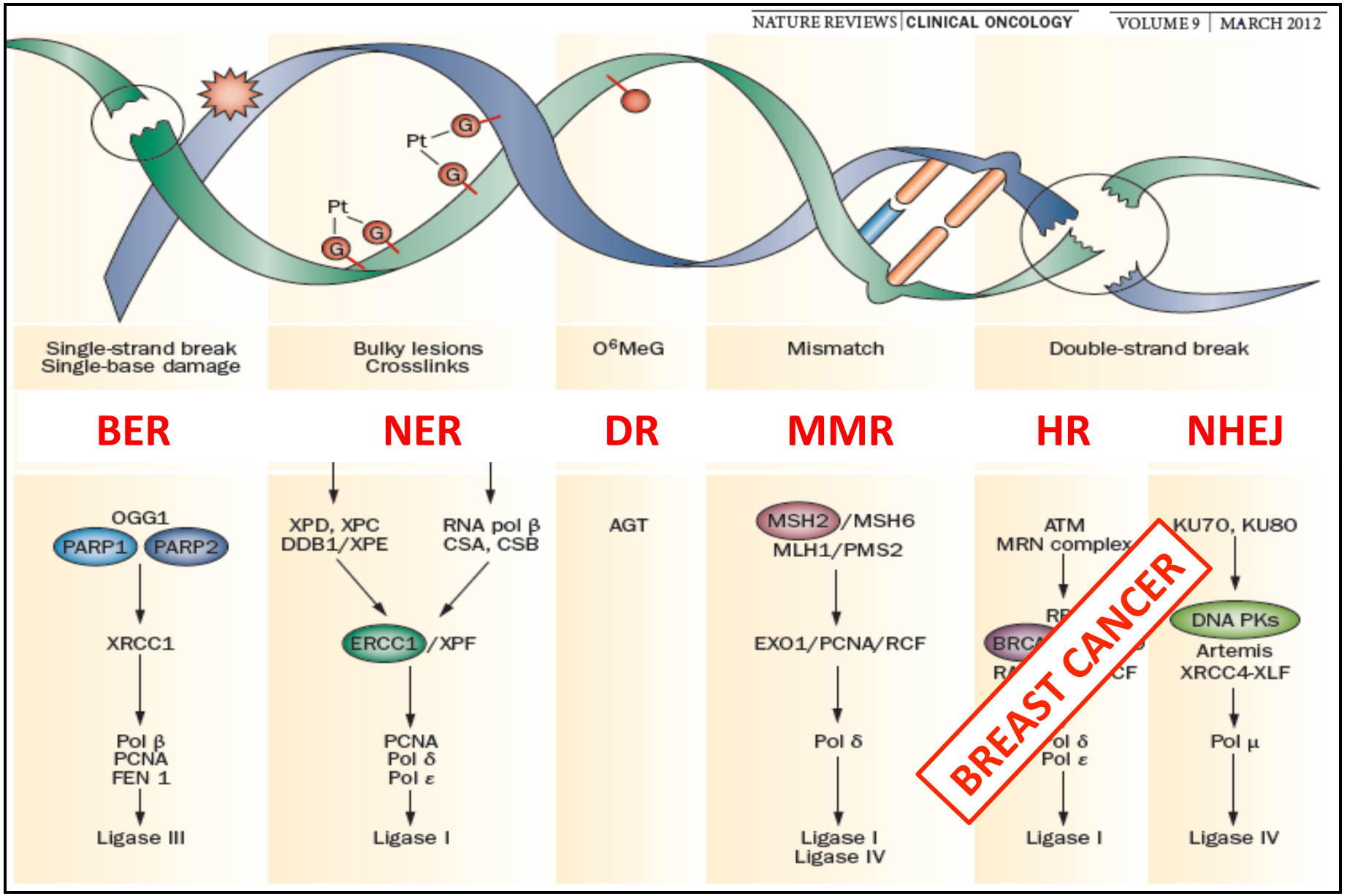

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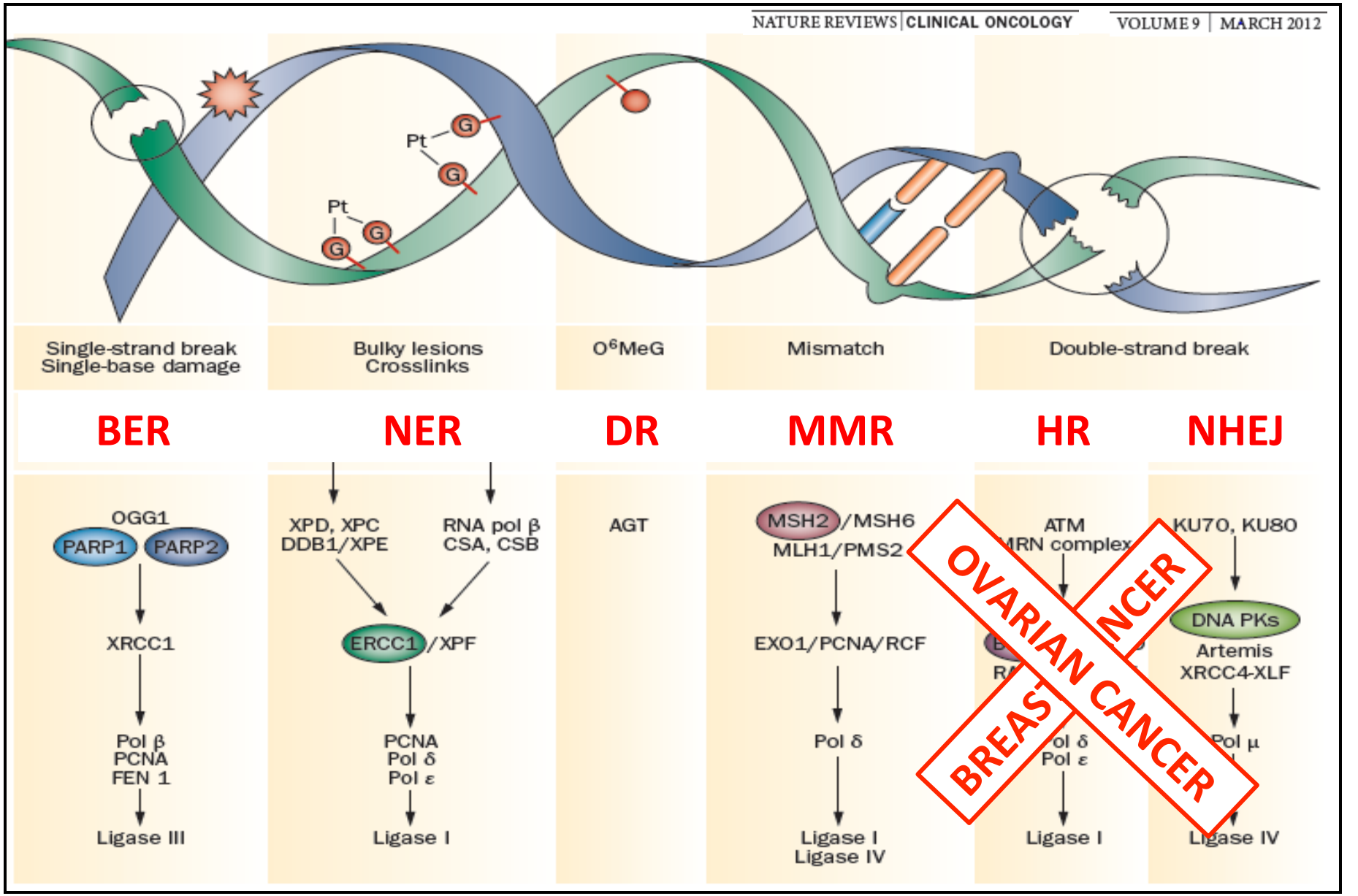

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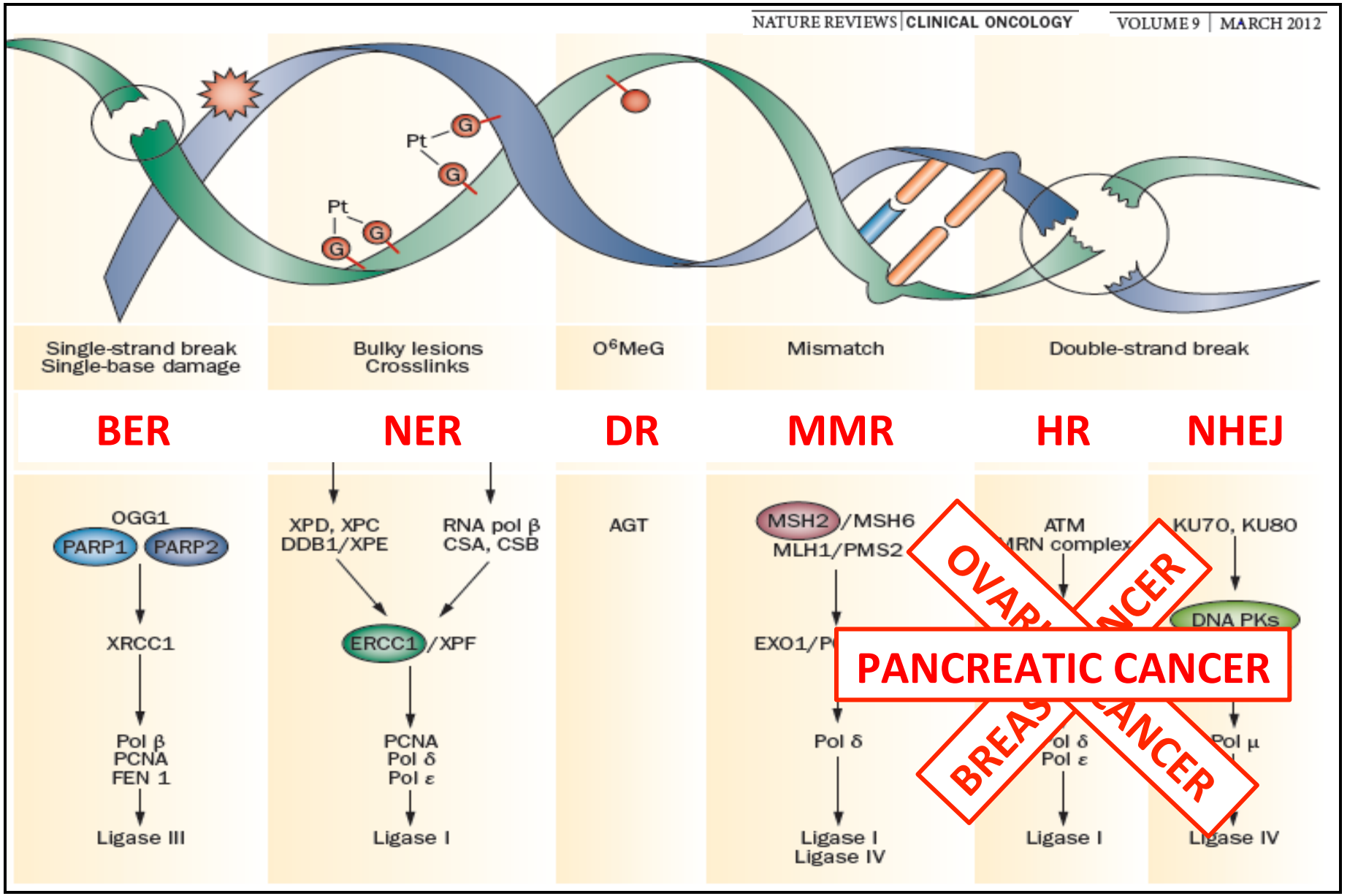

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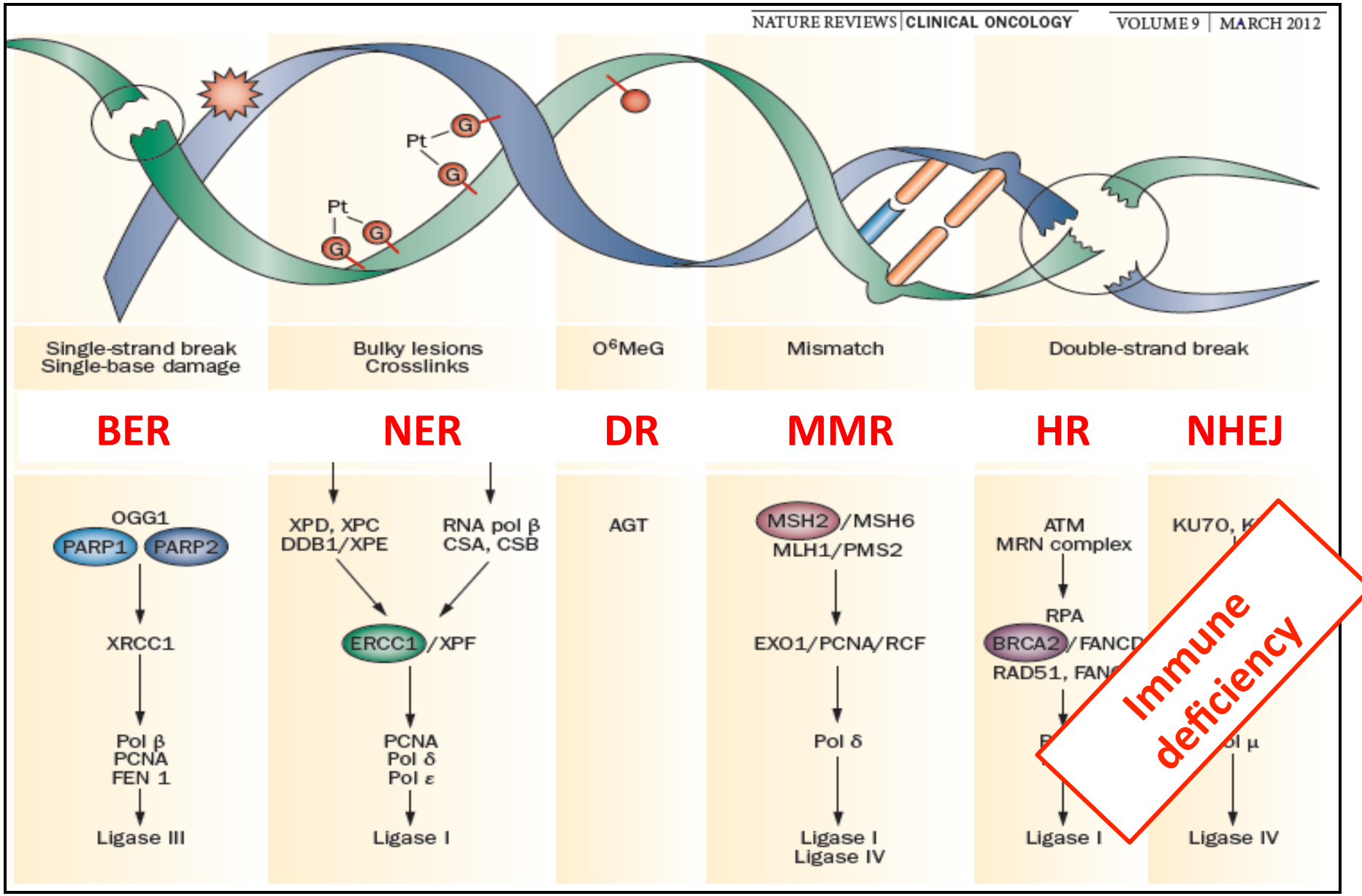

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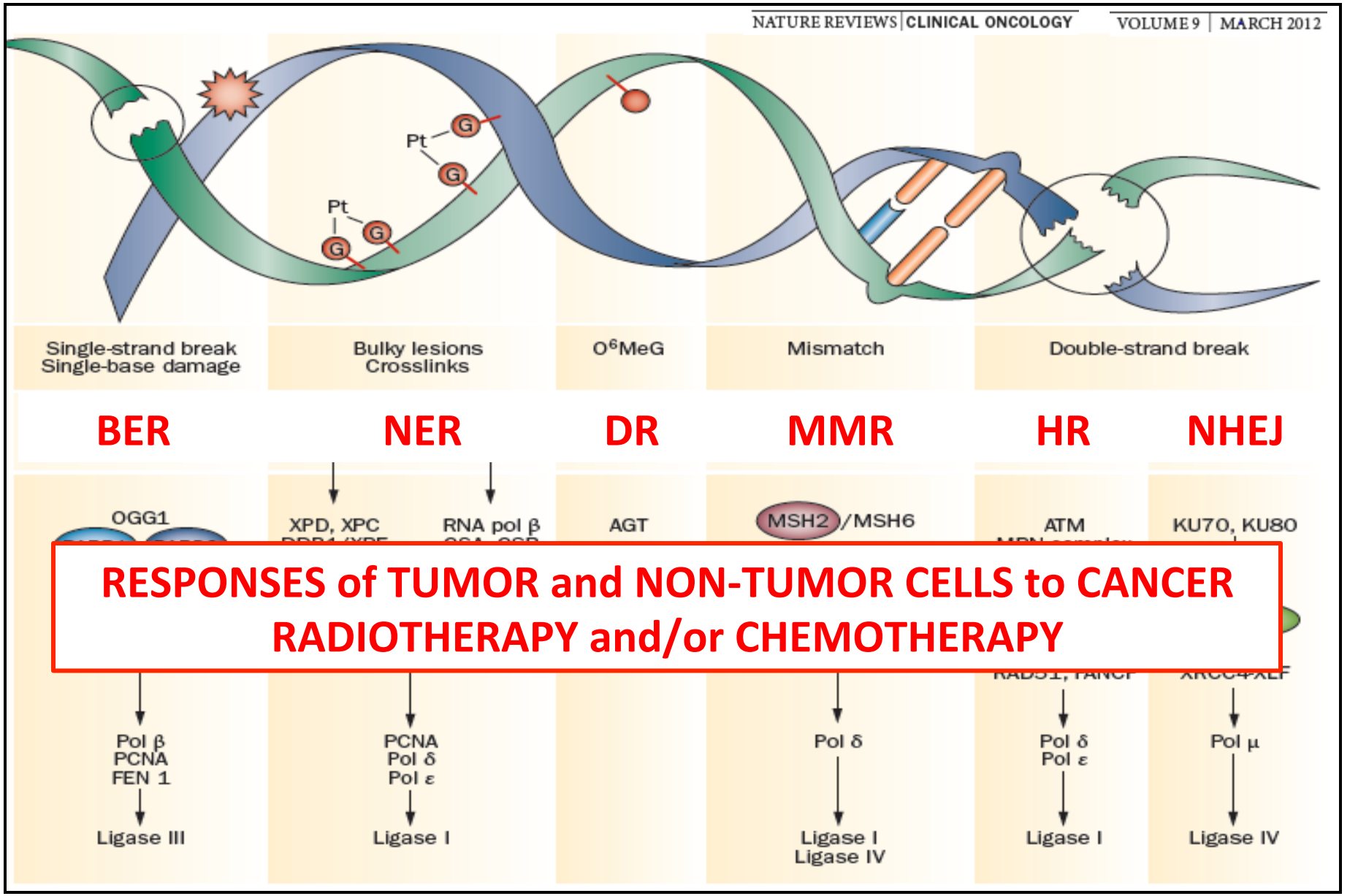


Some cancer Chemotherapy agents and all Radiotherapies CAUSE DNA DAMAGE

Environmental exposures to potentially harmful agents – DNA damaging agents

Harmful agents

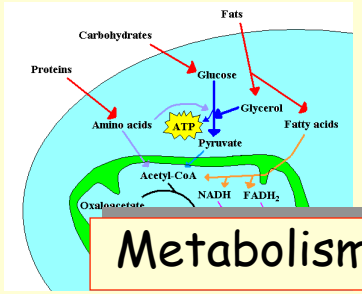

Eat


Medicine


Drink


Ambient


Breathe



Metabolism

People have different exposures

People have different responses

2007 – Breakthrough of the year

Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

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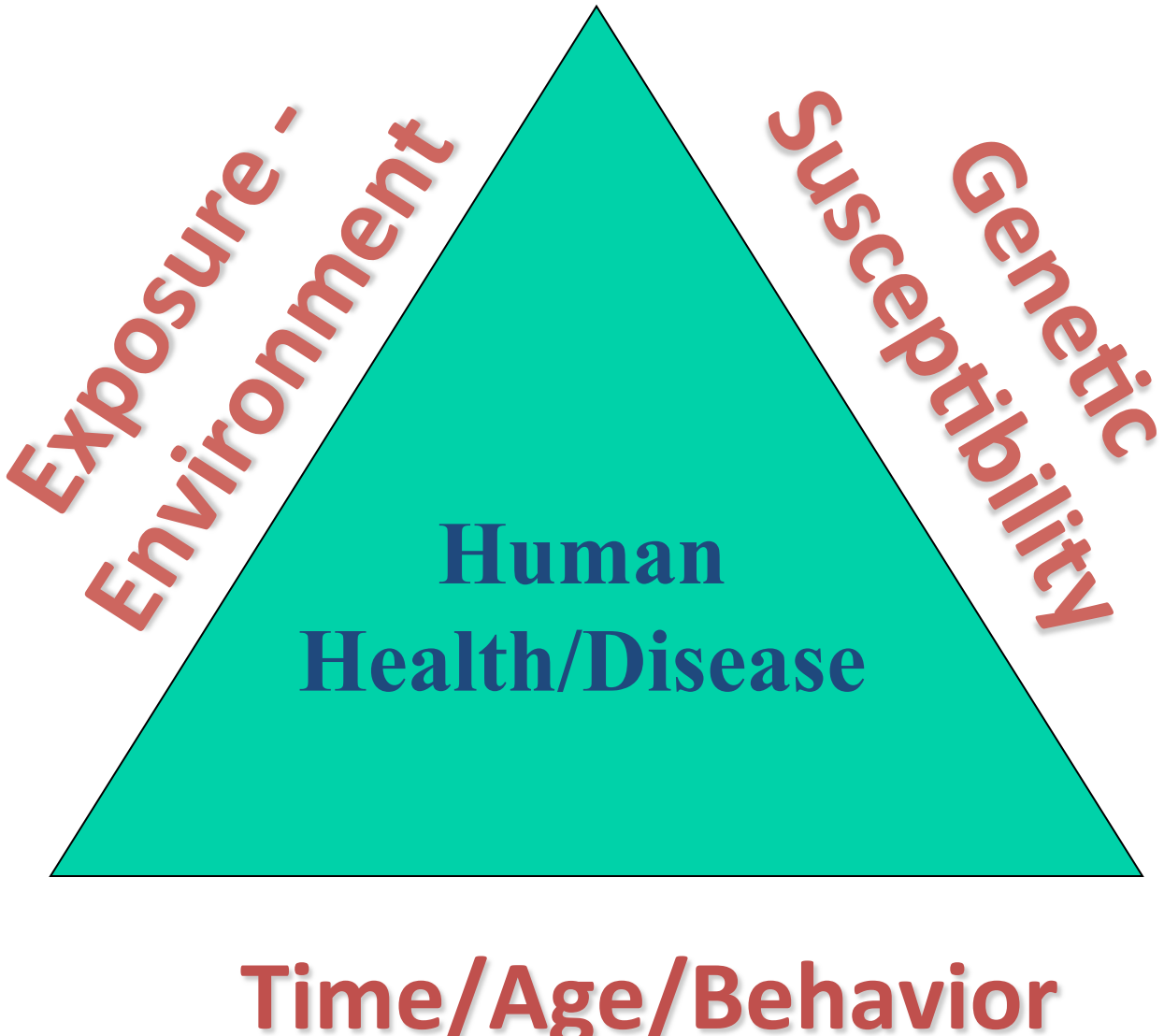
Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

Compare two people – have about ~ 6 million SNP variants!

Toxic agents in our environment
Gene-Environment Interaction



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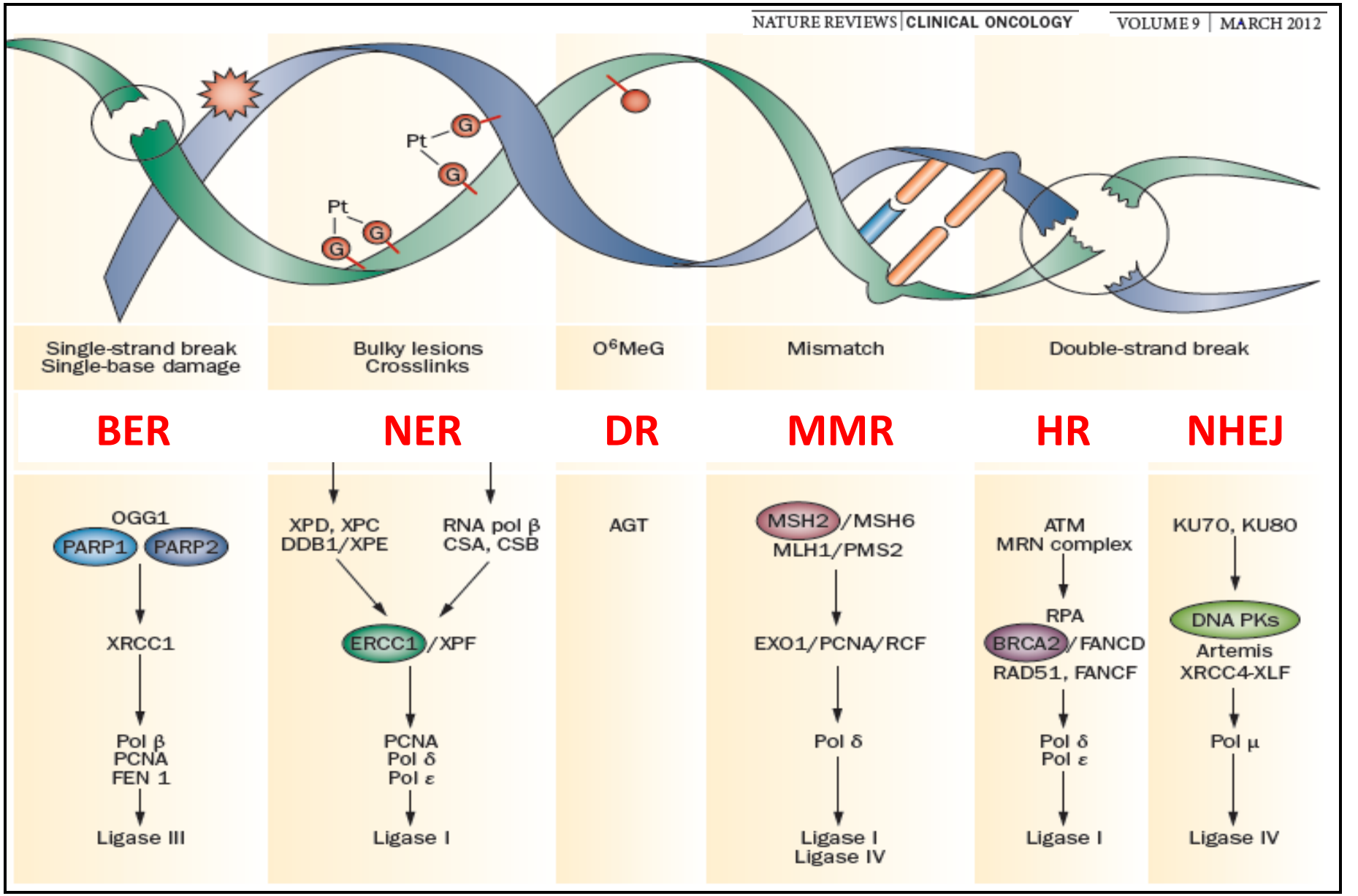

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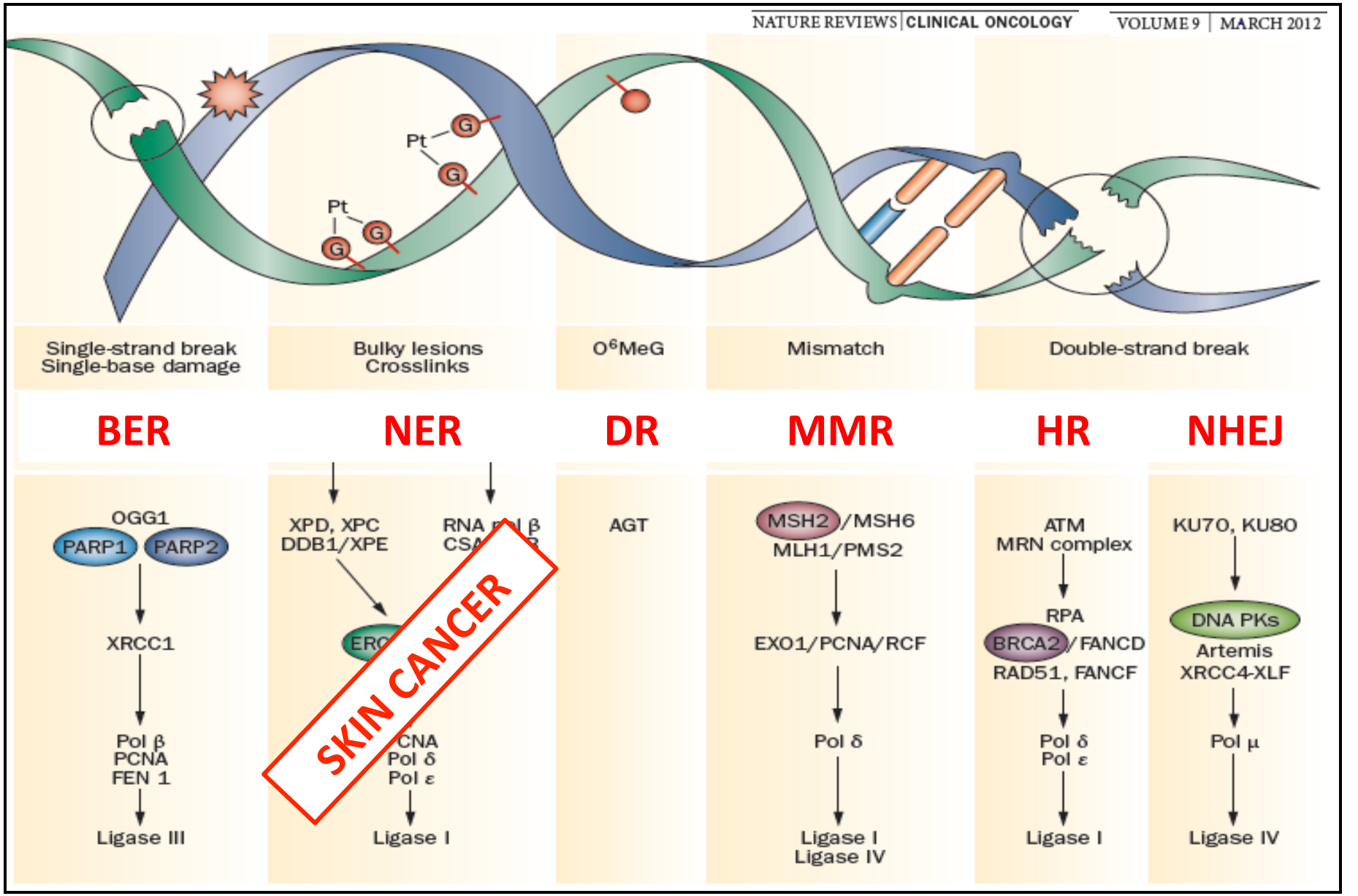

What are the known risk factors for Skin Cancer?

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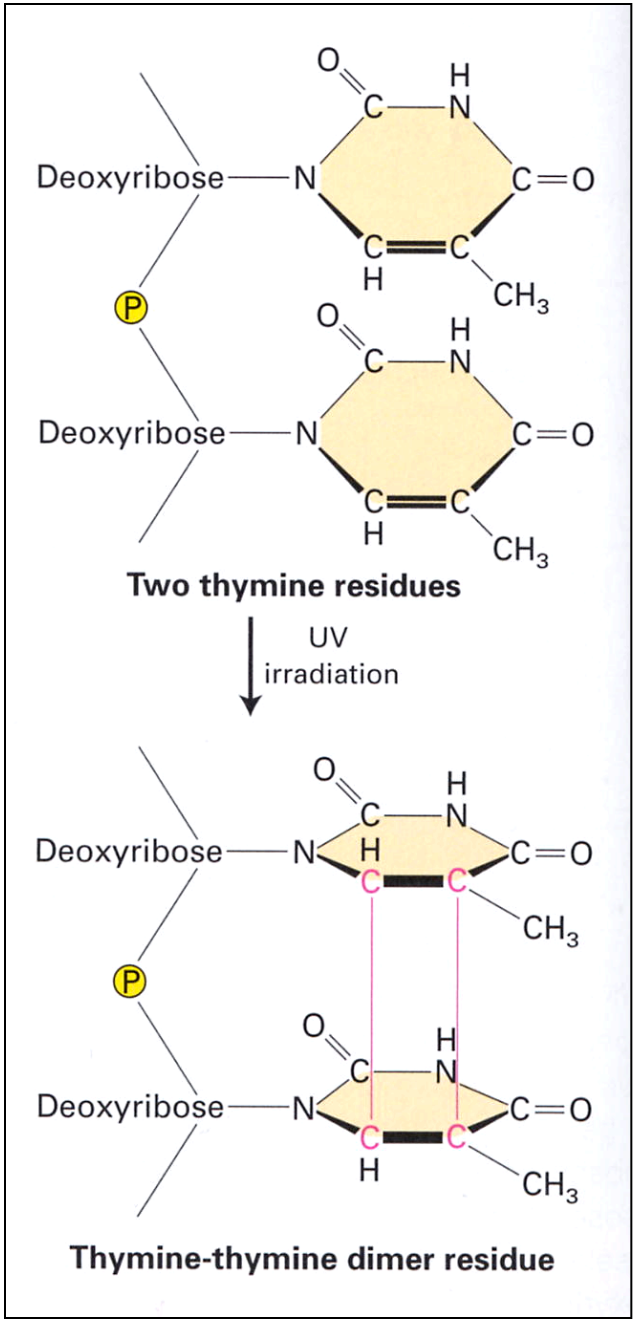
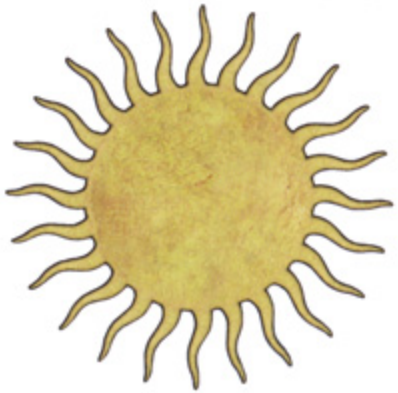
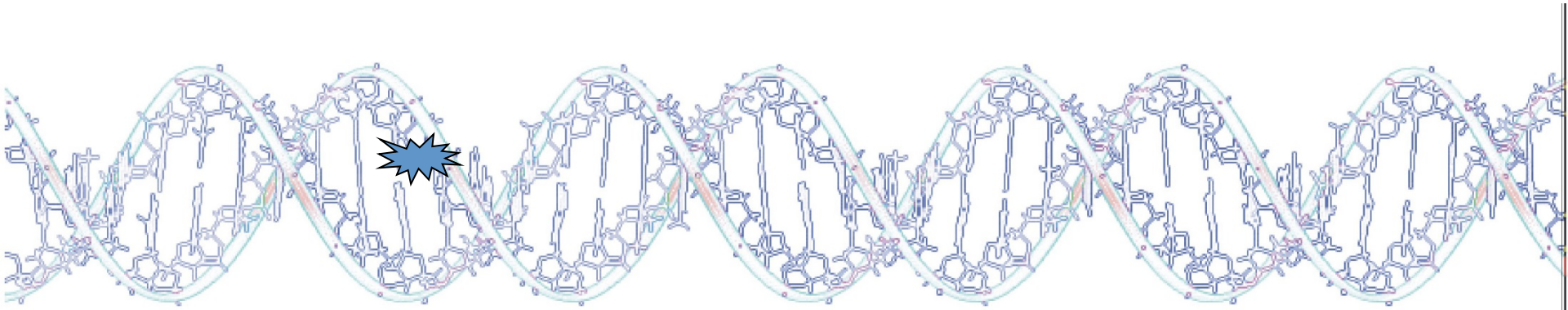
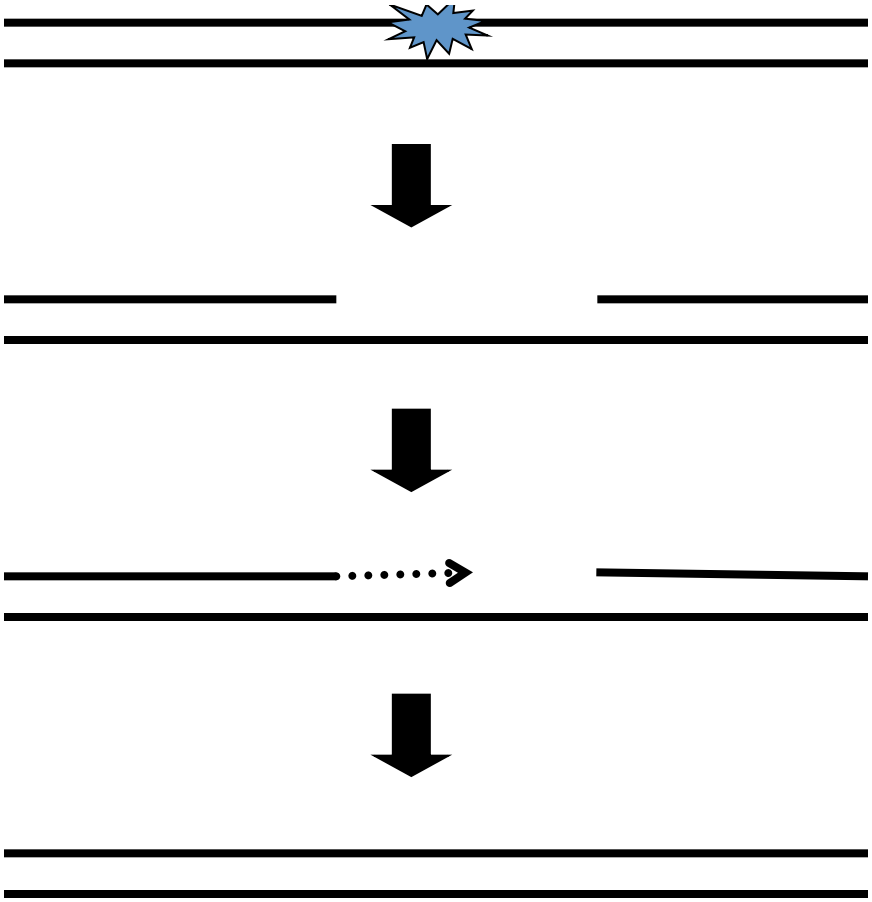




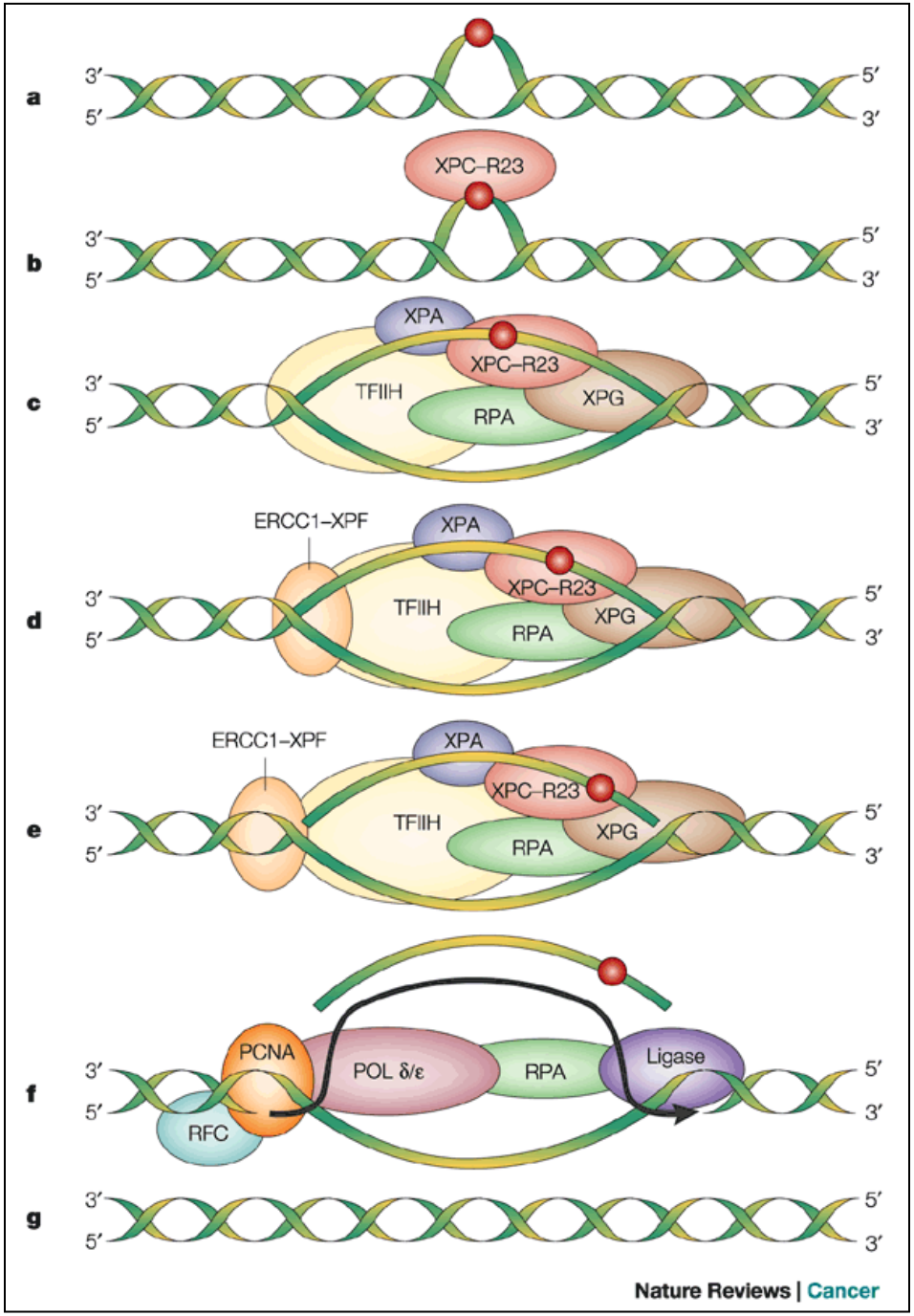
Before

After





Nucleotide Excision Repair



Nucleotide Excision Repair Proteins

XPA

XPB

XPC

XPD

XPE

XPF

XPG

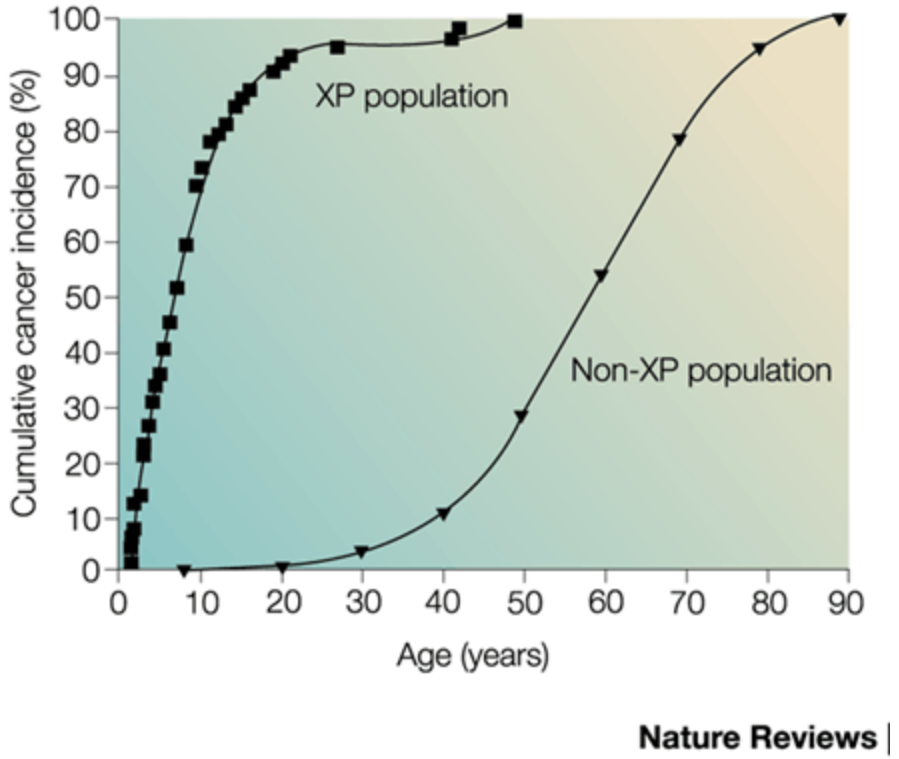
Errol C. Friedberg
Nature Reviews Cancer 1, 22-33 (2001)

Xeroderma Pigmentosum

Grossly Deficient in Nucleotide Excision Repair

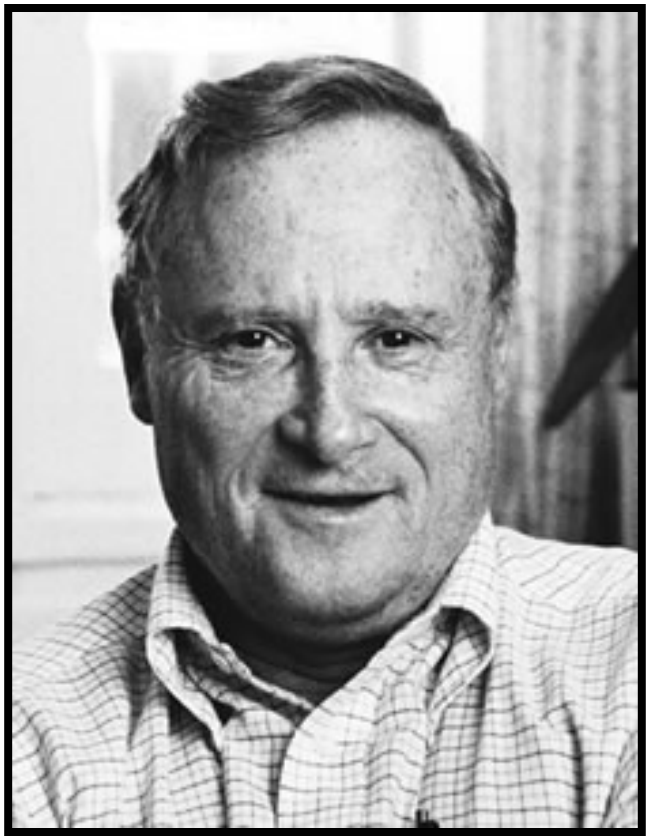
2000-fold increased risk of skin cancer

Lack of DNA repair accelerates the onset of cancer

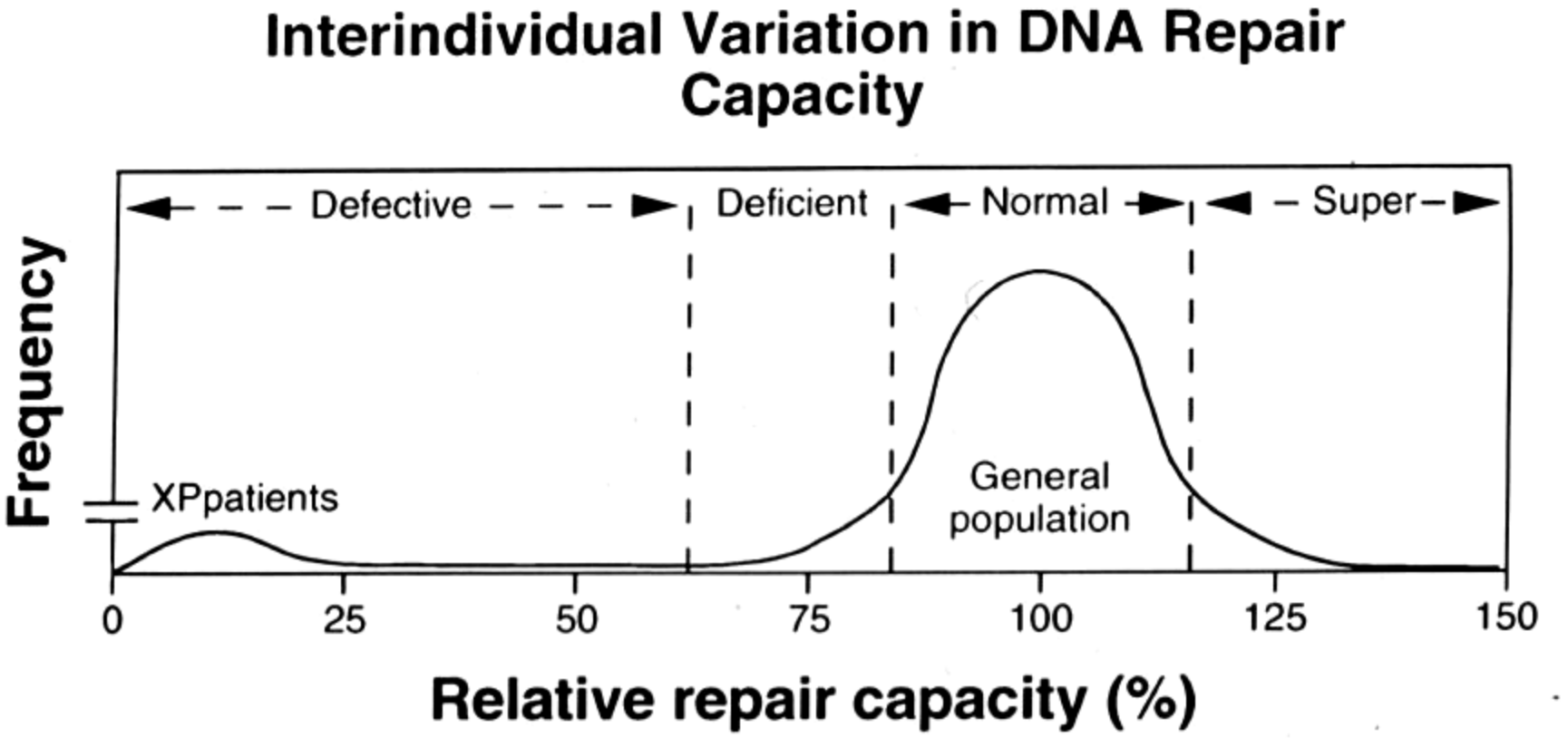


Nature Reviews

Larry Grossman wondered whether there is variation in DNA repair Capacity in the General Population



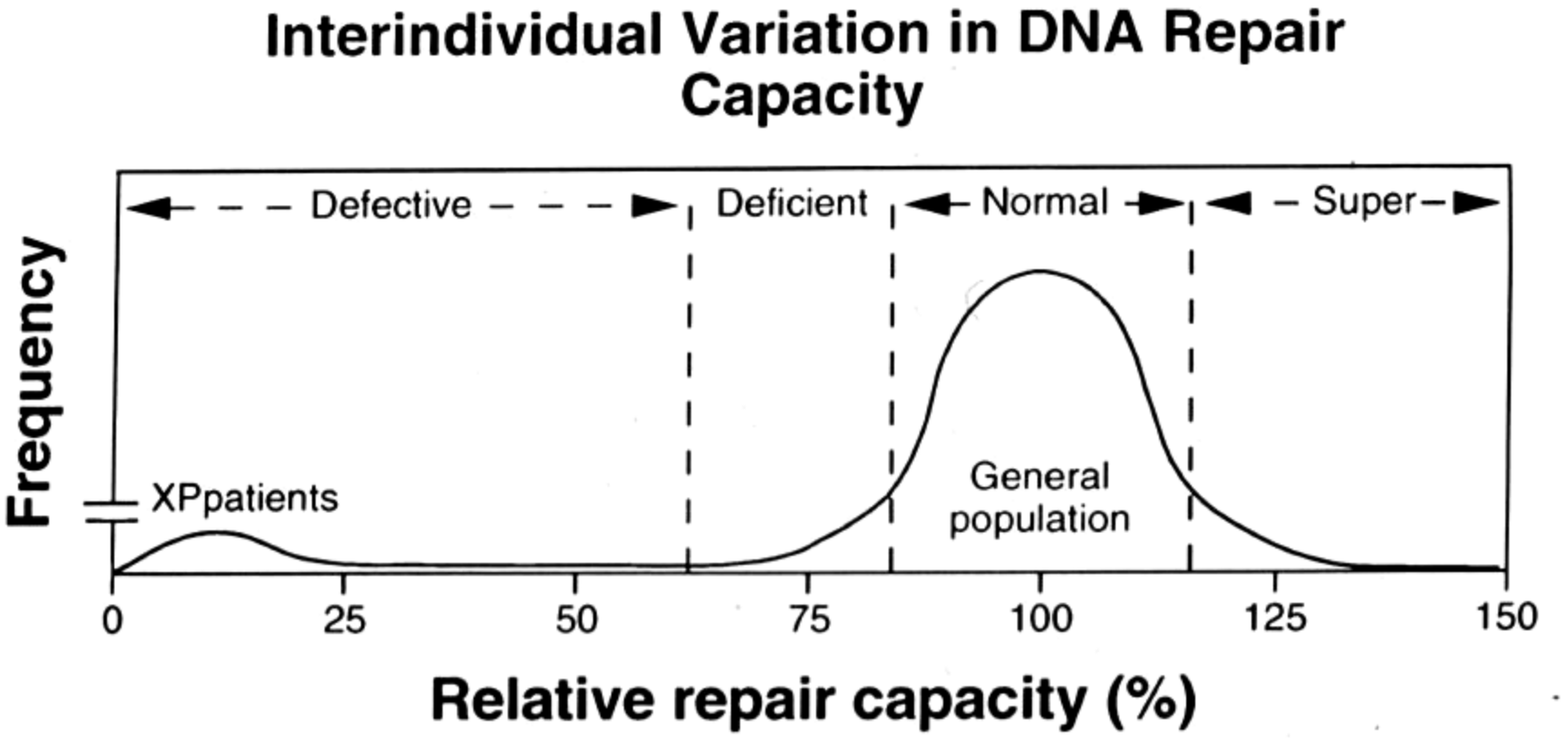
Dr. Lawrence Grossman
(1924–2006)



Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving a theoretical maximum of how many cases worldwide with 2,000-fold increased risk

Even if just 1% of the population is relatively repair deficient, could have how many with several-fold increased risk



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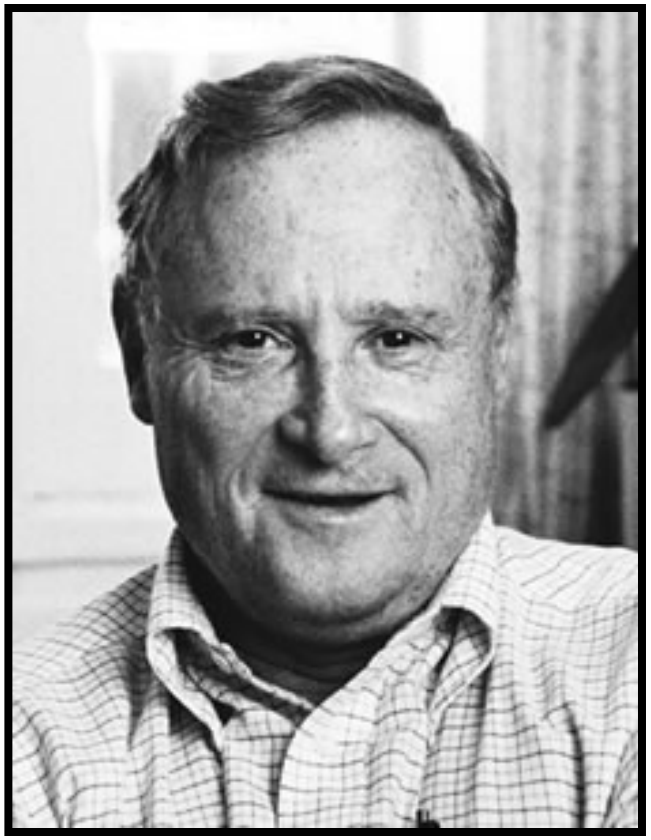
XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

A functional assay was developed by:



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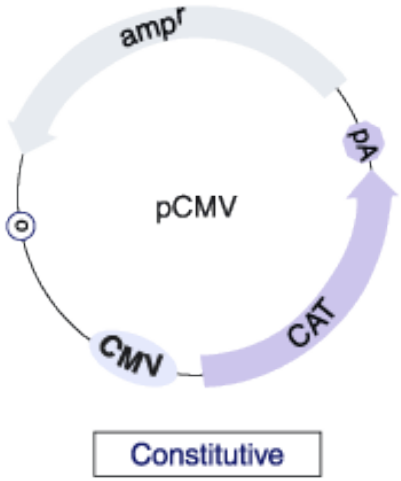
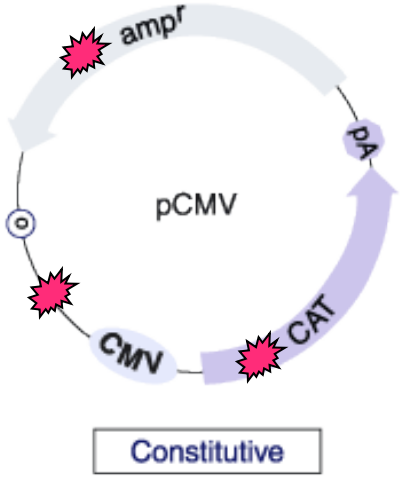
Dr. Qingyi Wei

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

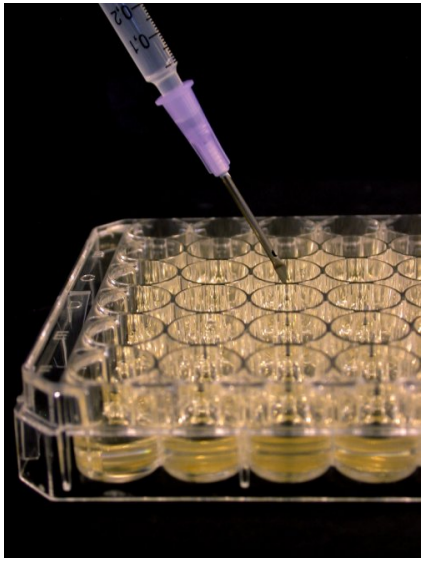
Athas & GROSSMAN
Cancer Res. 1991

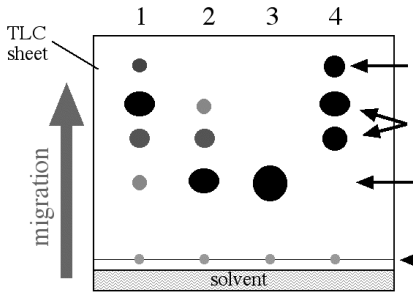


+ UV light



Transient transfection peripheral blood lymphocytes

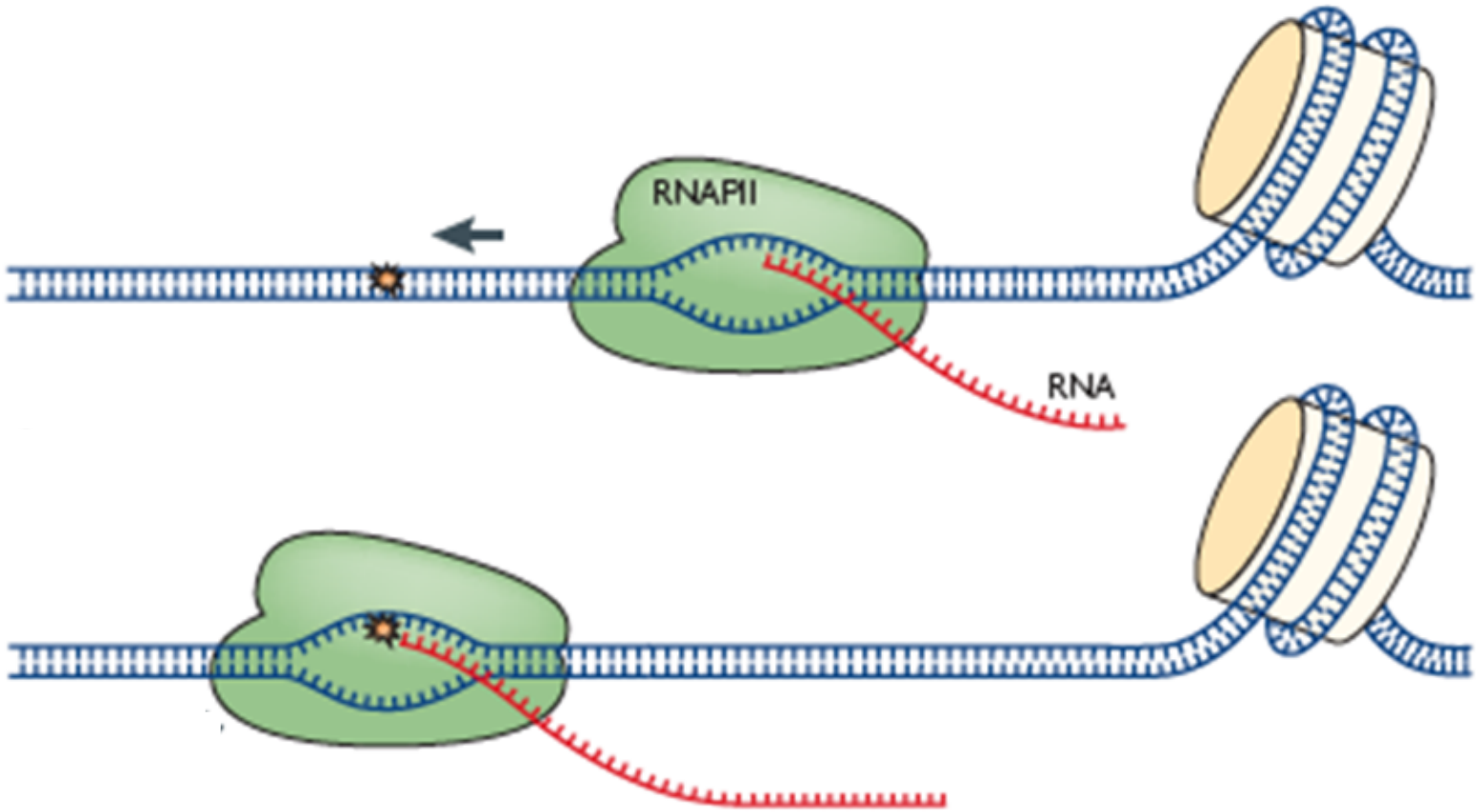




Time to repair

CAT Assay

RNA Polymerase II is exquisitely sensitive to DNA lesions



Nature Reviews Molecular Cell Biology **9**, **958-970**

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

Athas & GROSSMAN
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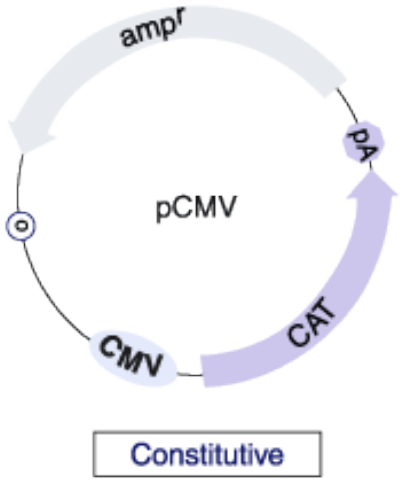
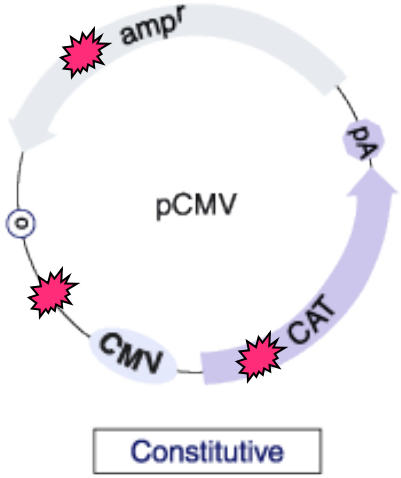


+ UV light



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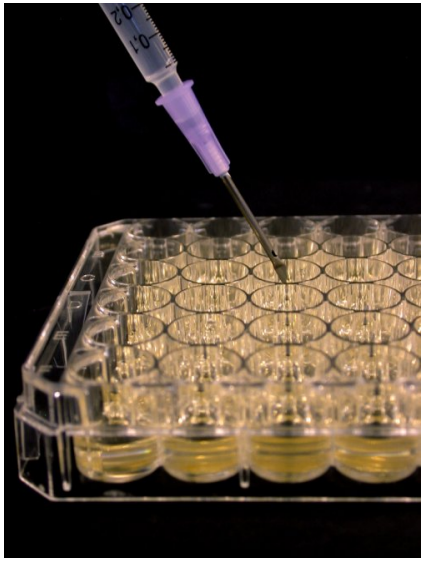
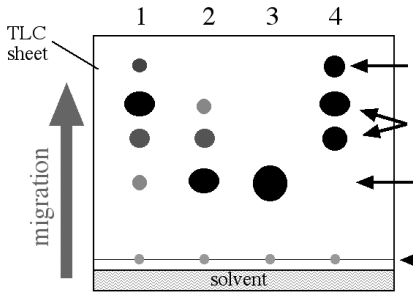
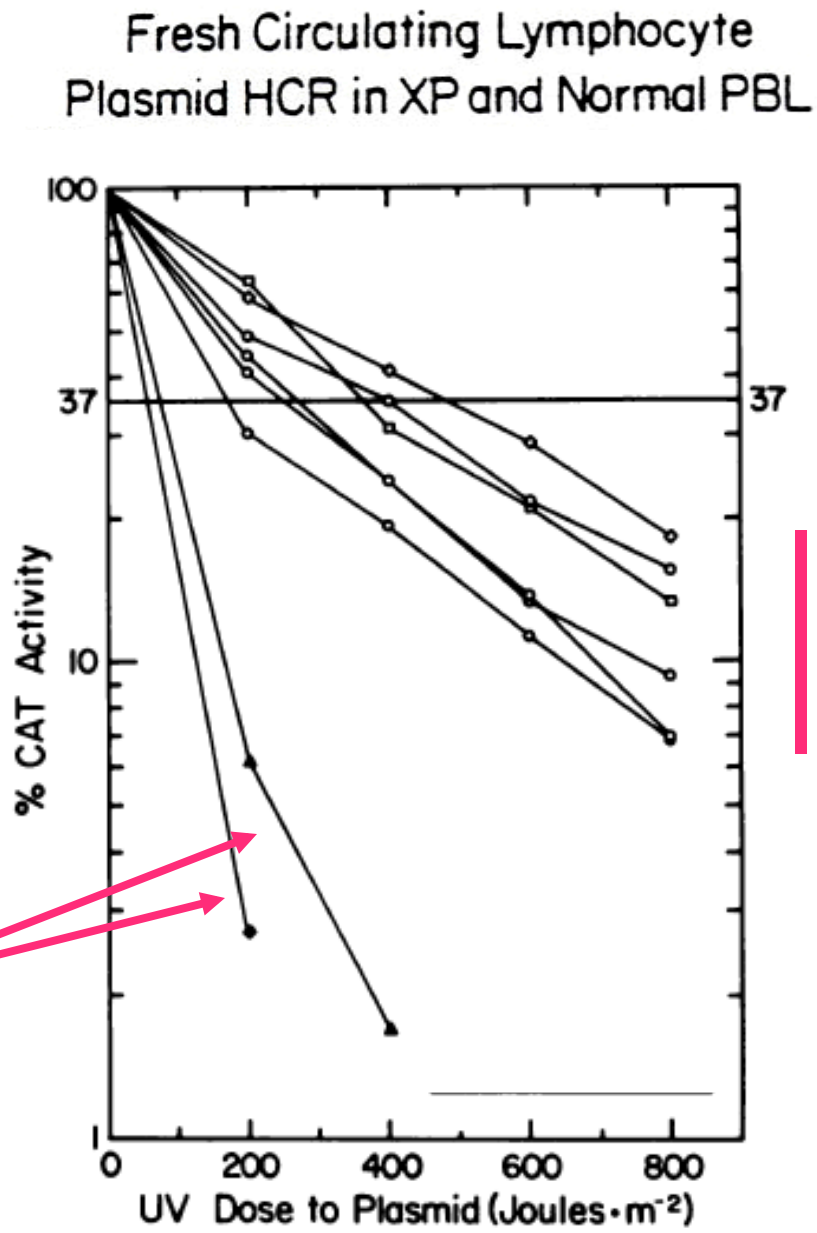




Time to repair

CAT Assay

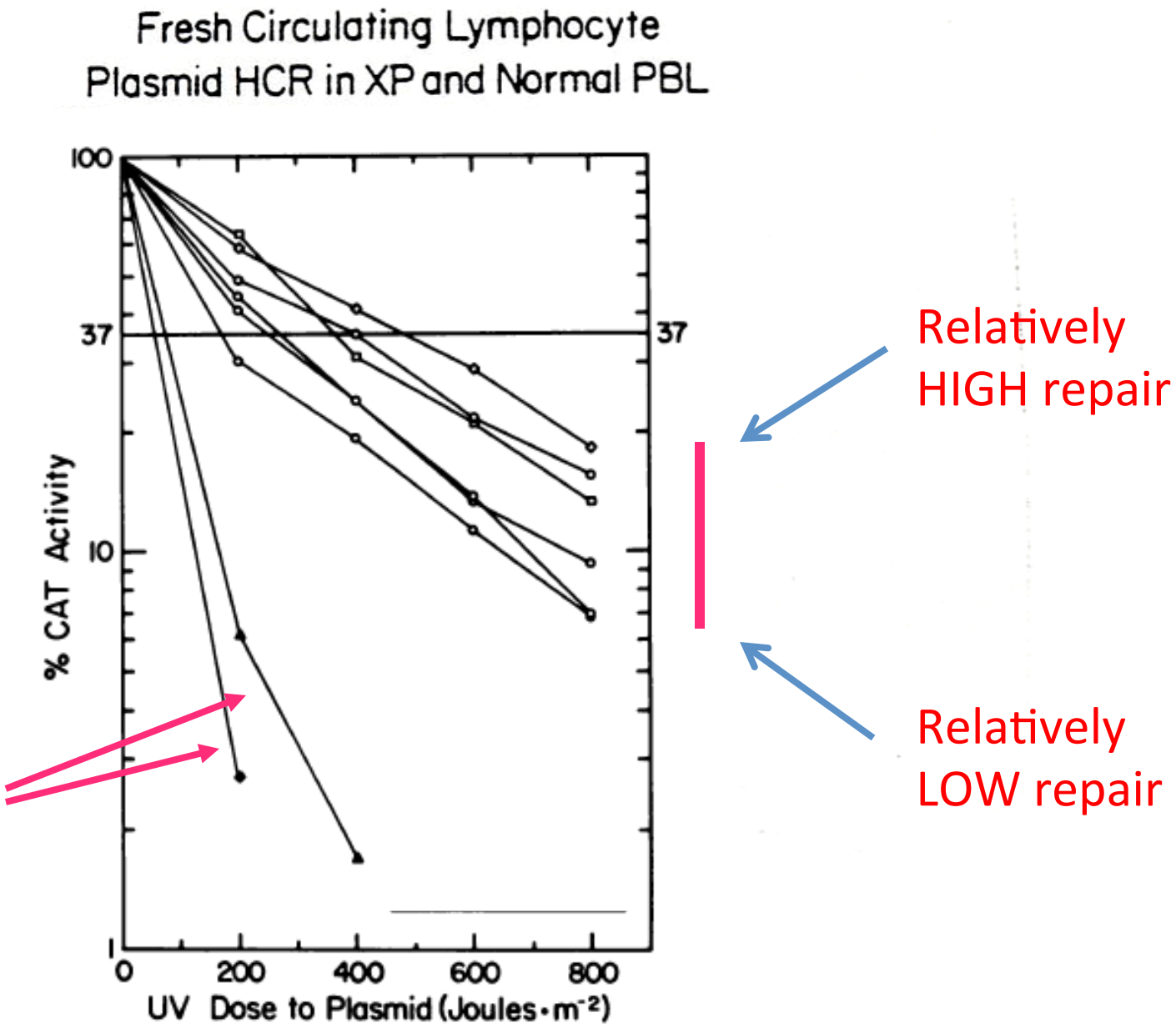


Cells from ‘healthy” people

Cells from XP patients

Athas & GROSSMAN

[CANCER RESEARCH 51, 5786–5793, November 1, 1991]



Athas & GROSSMAN

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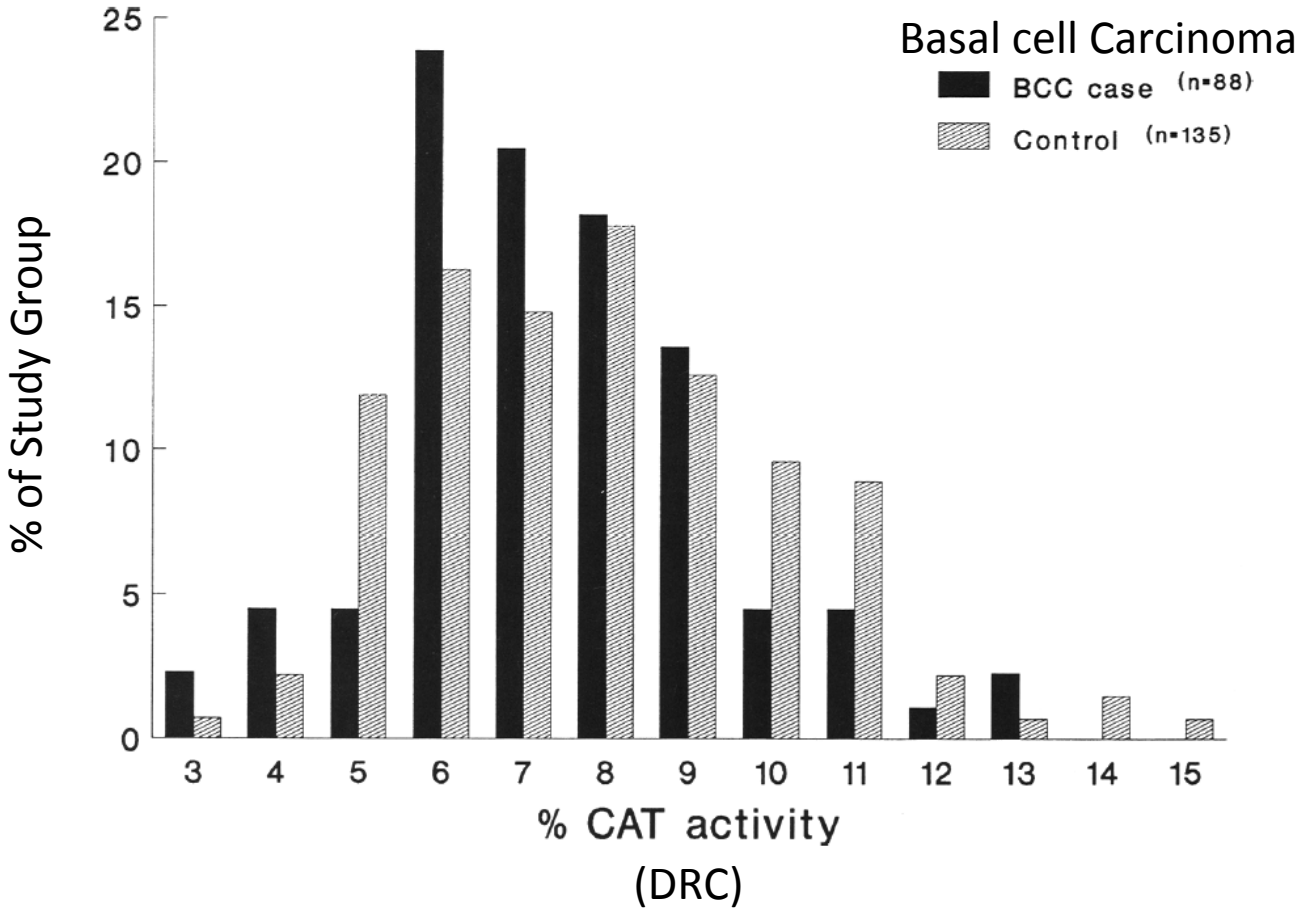
Case-Control Study monitoring DNA Repair Capacity (DRC) by Host Cell Reactivation (HCR) of plasmids containing DNA damage



[CANCER RESEARCH 54, 437-44(i, January 15, 1994]

Qingyi Wei, Genevieve M. Matanoski, Evan R. Farmer, Mohammad A. Hedayati, and Lawrence GROSSMAN

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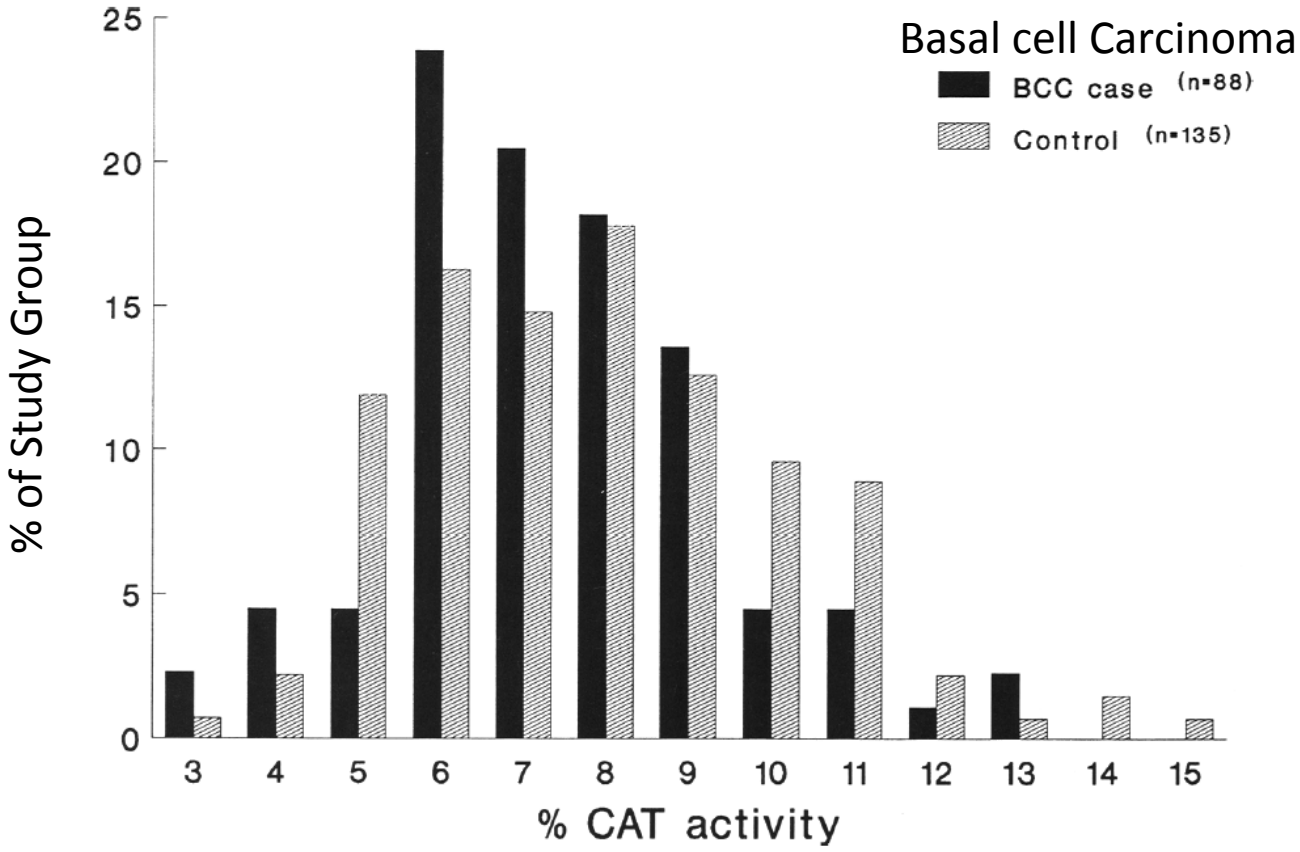
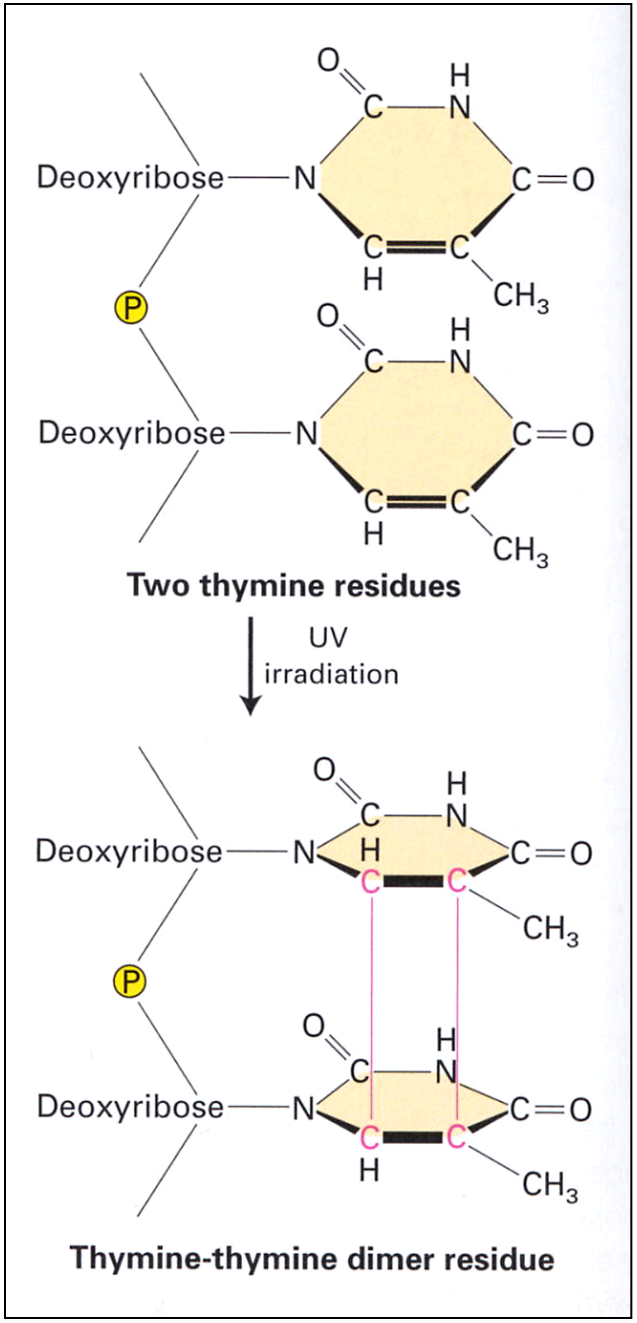
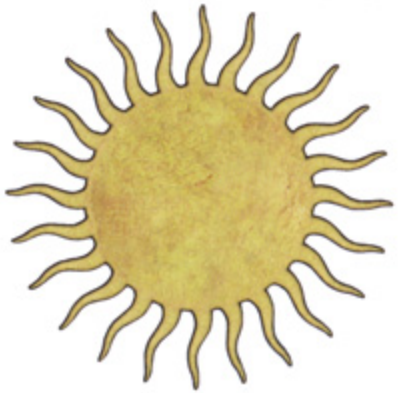


LOW Repair HIGH Repair

[CANCER RESEARCH 54, 437-44(i, January 15, 1994]

Qingyi Wei, Genevieve M. Matanoski, Evan R. Farmer, Mohammad A. Hedayati, and Lawrence GROSSMAN

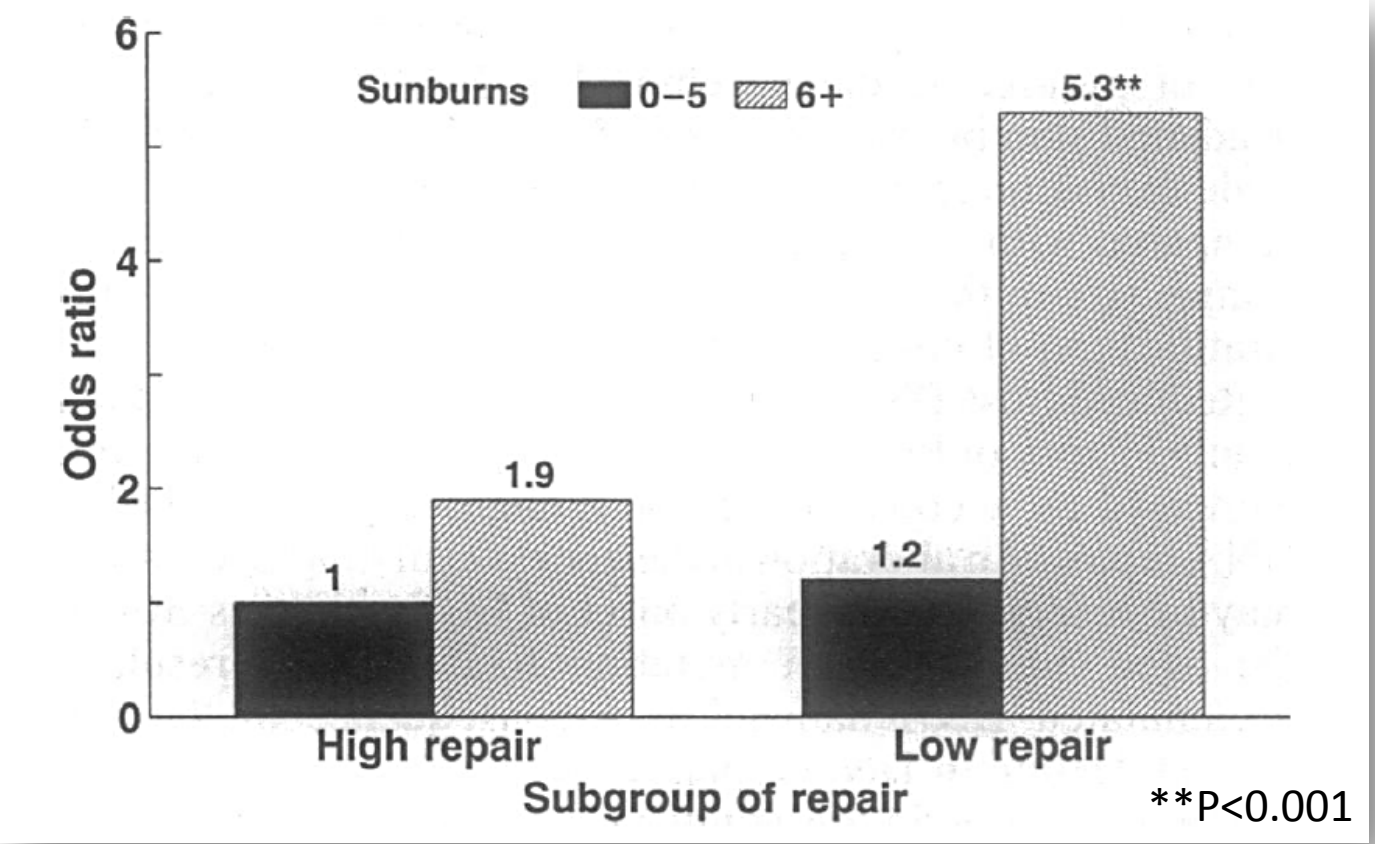




Before

After

Low NER status combined with excessive sun exposure is very dangerous



Wei Q, Matanoski GM, Farmer ER, Hedayati MA, **GROSSMAN L.** Proc Natl Acad Sci U S A. 1993 90:1614-8.

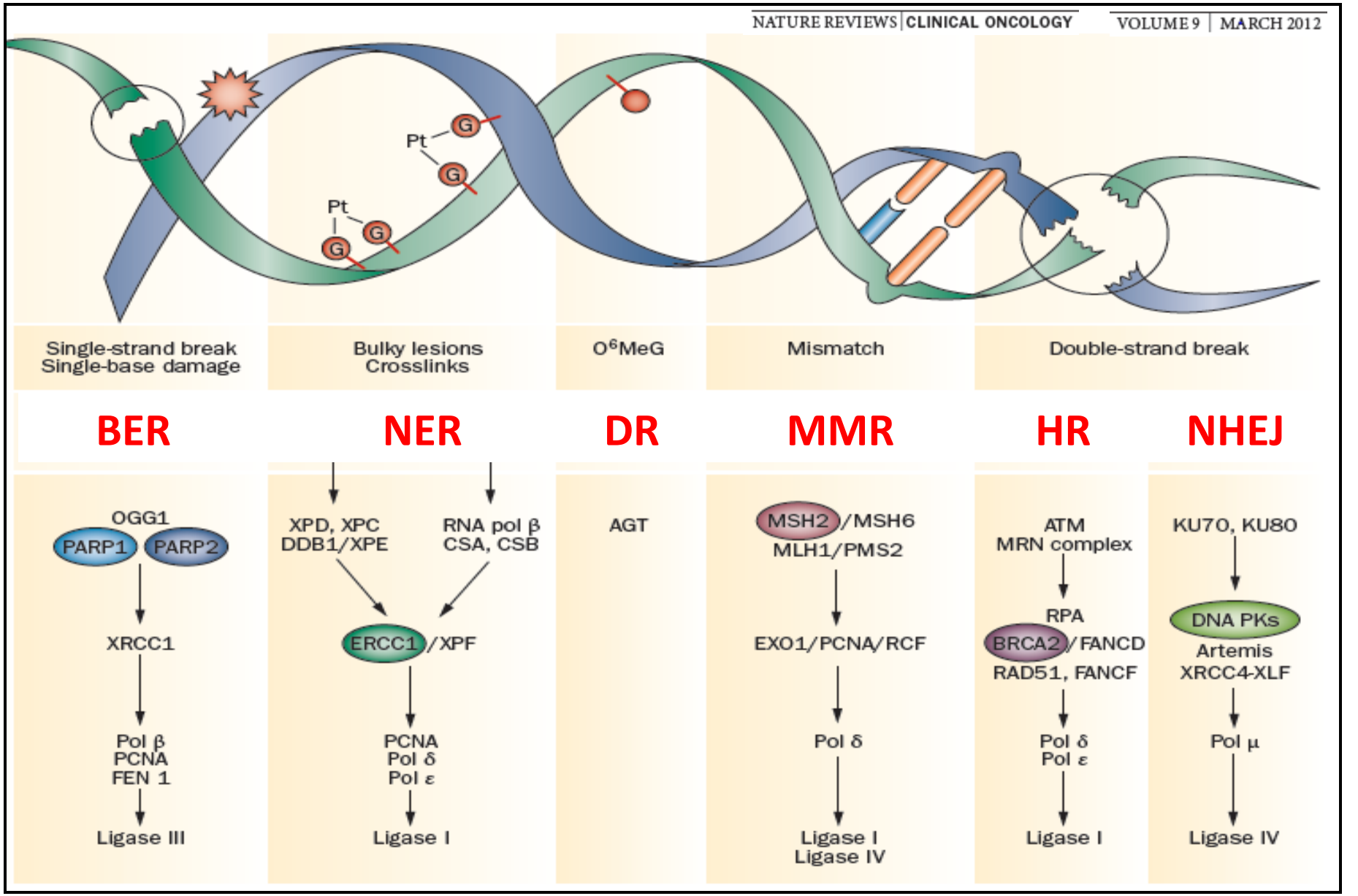
What experimental question will you ask in Module 2?

How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

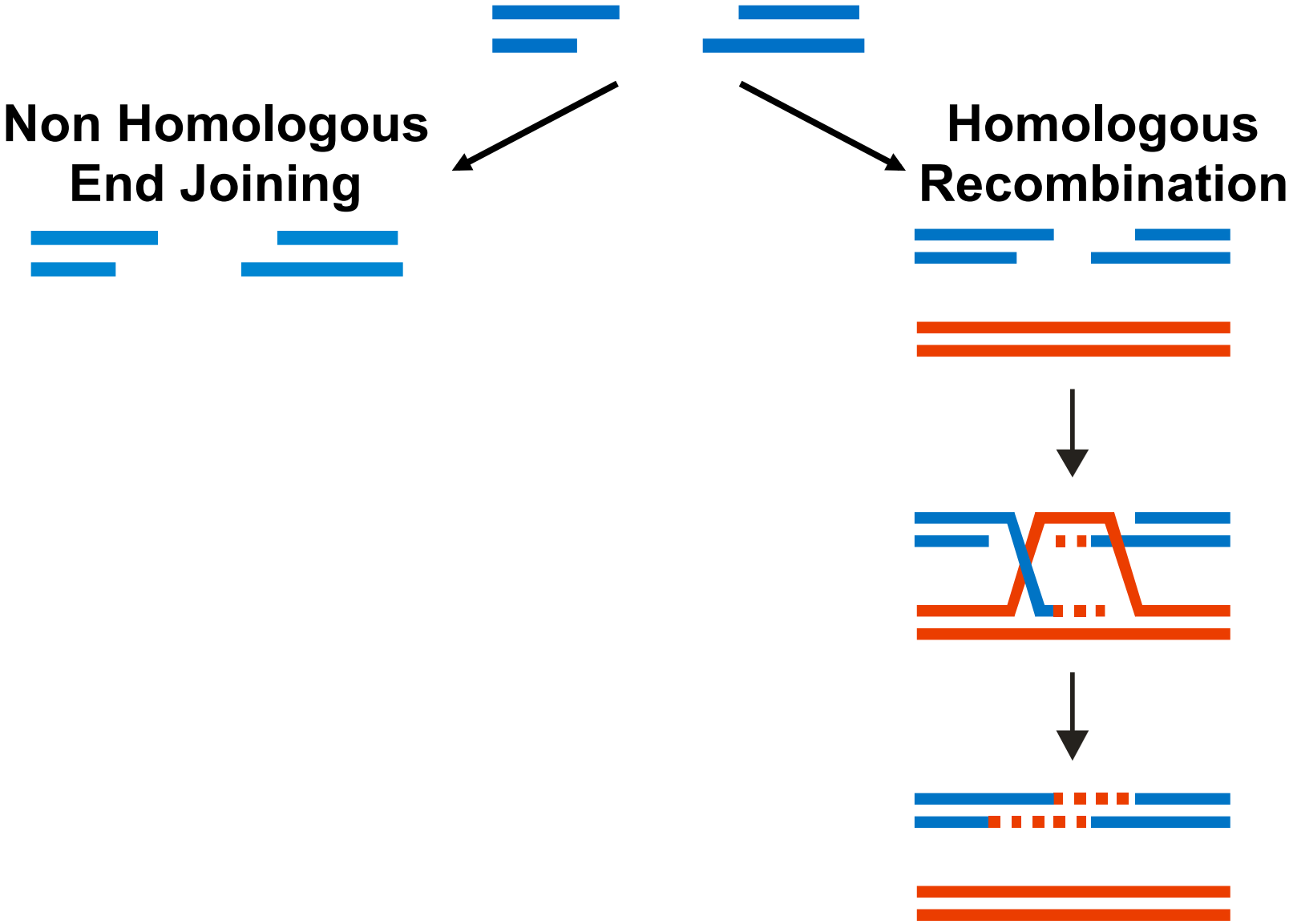
This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How will we actually measure DNA repair efficiency?

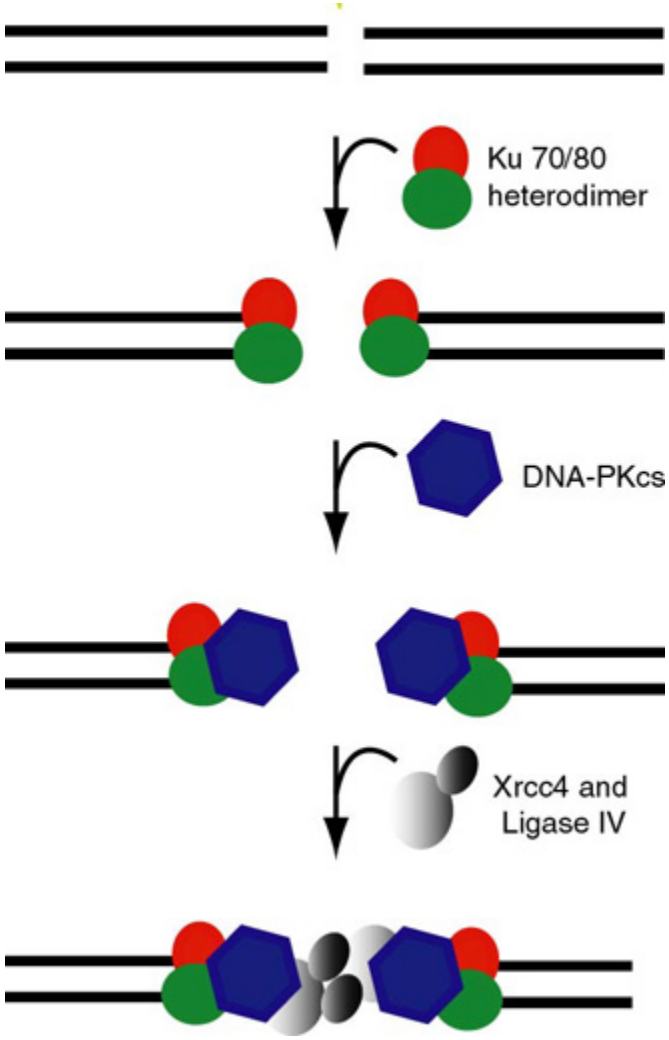
Six Major DNA Repair Pathways



DNA double-strand break repair



Non-Homologous End Joining (NHEJ)



Ku70
Ku80

DNA-PKcs

Xrcc4
Ligase IV

Key Experimental Methods for Module 1

- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

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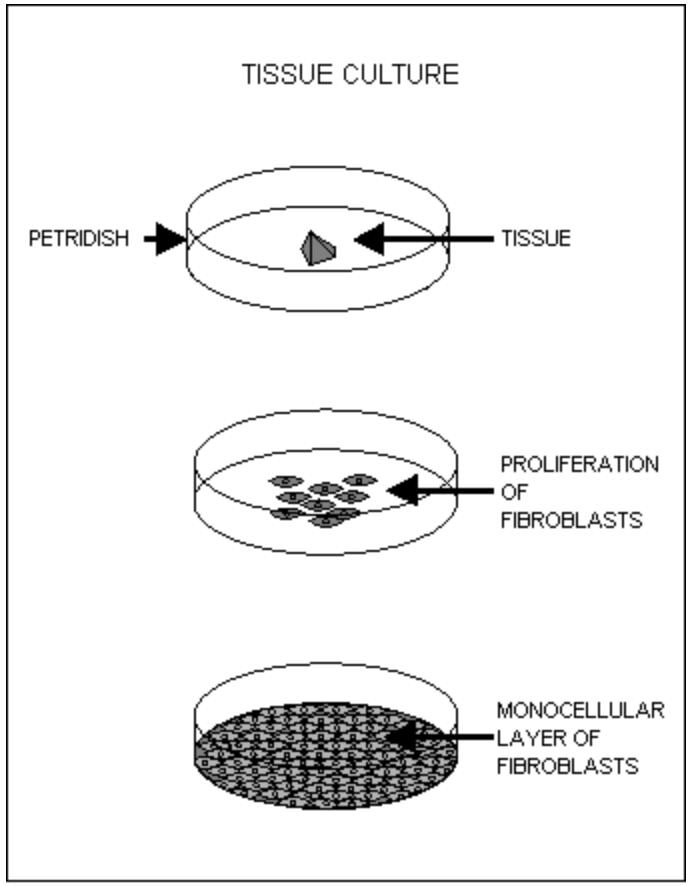
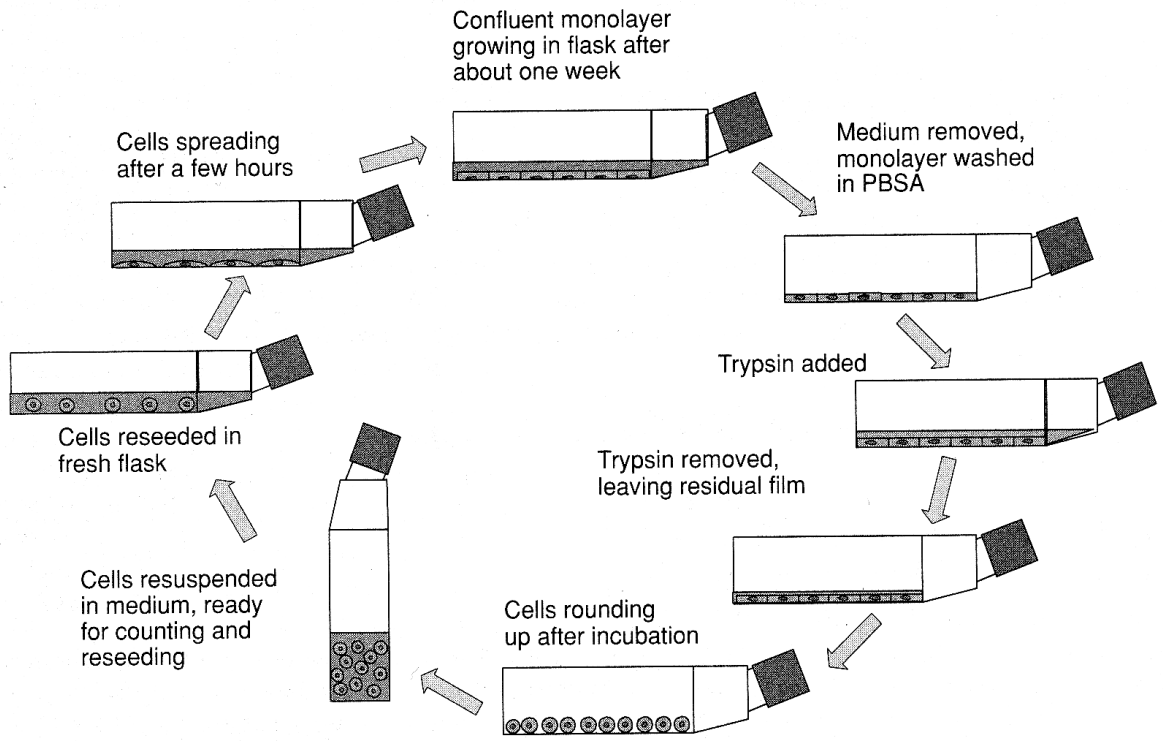
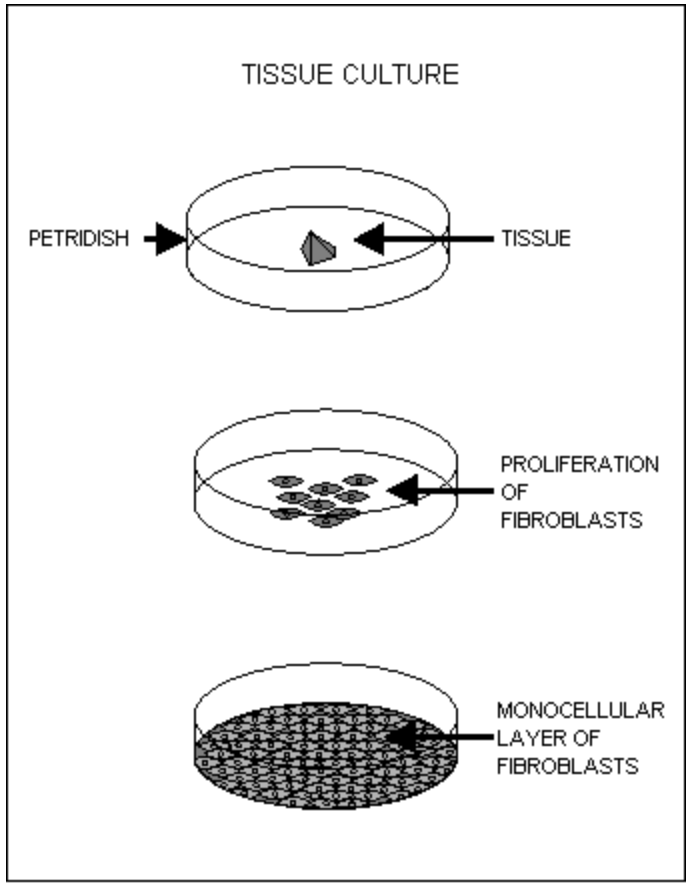
How do you grow mammalian cells?

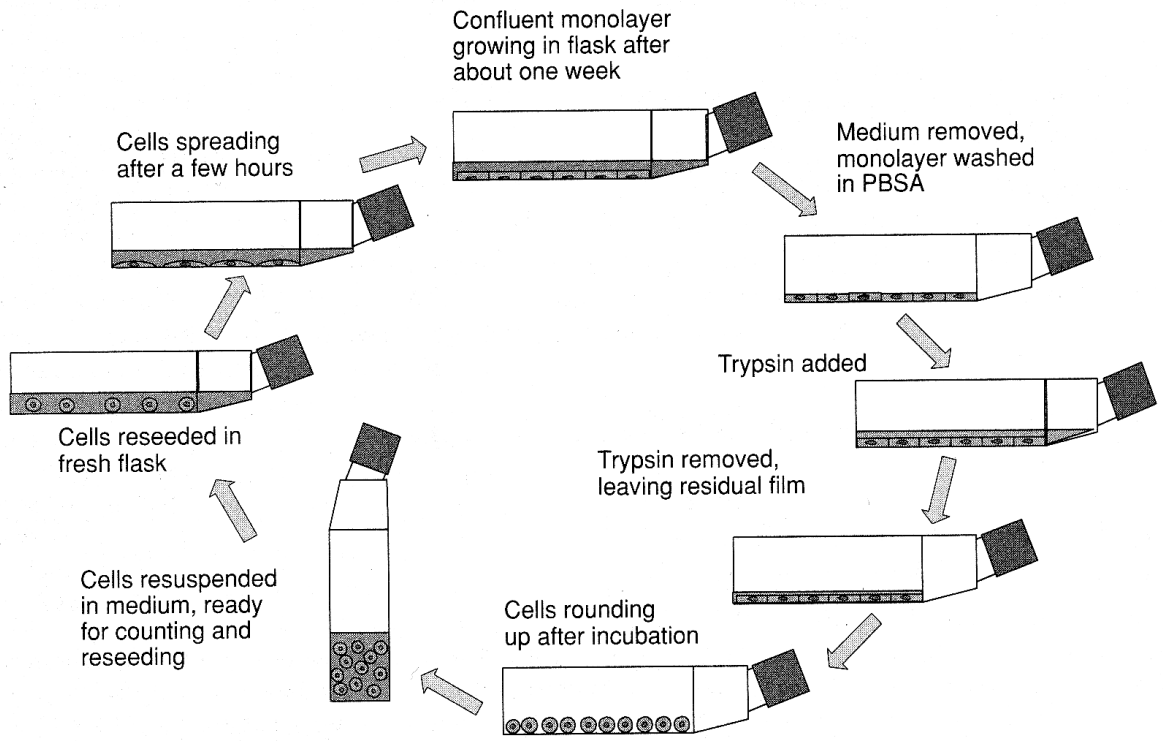




From Freshney's "Culture of Mammalian Cells"

How do you grow mammalian cells?

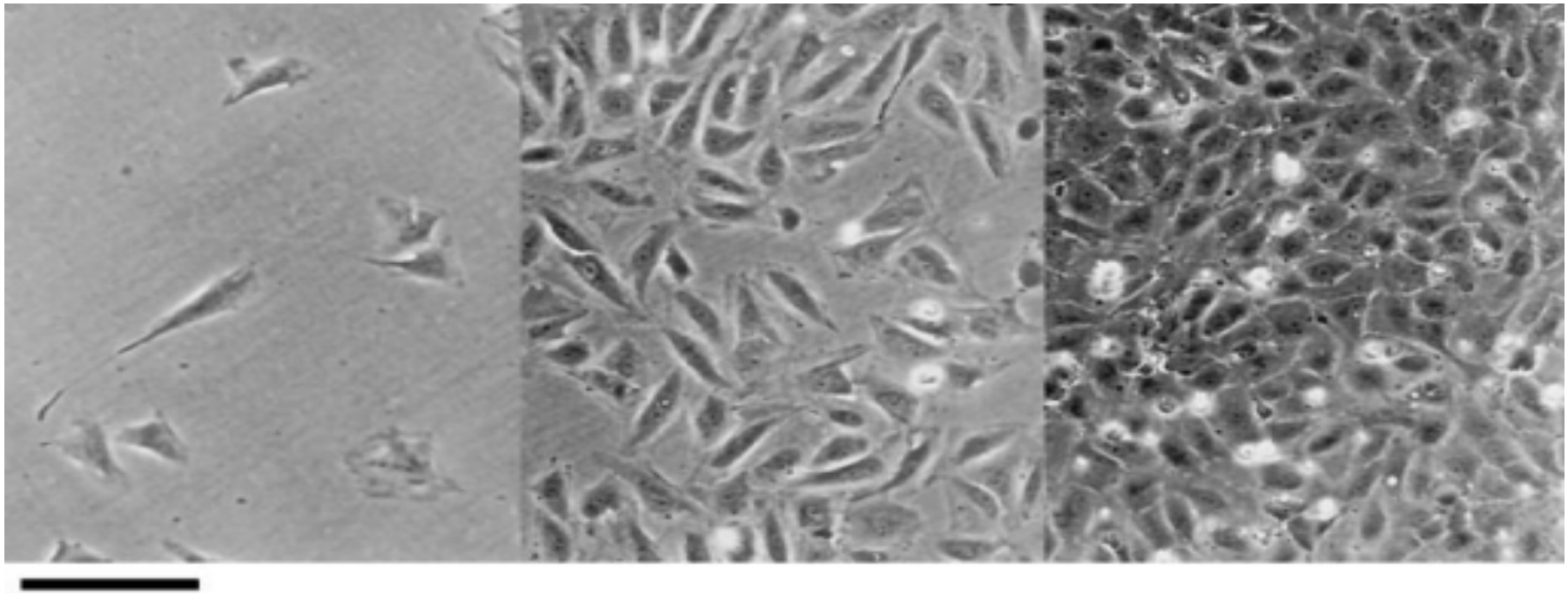




"Sub-Culturing"

From Freshney's "Culture of Mammalian Cells"

How do you grow mammalian cells?

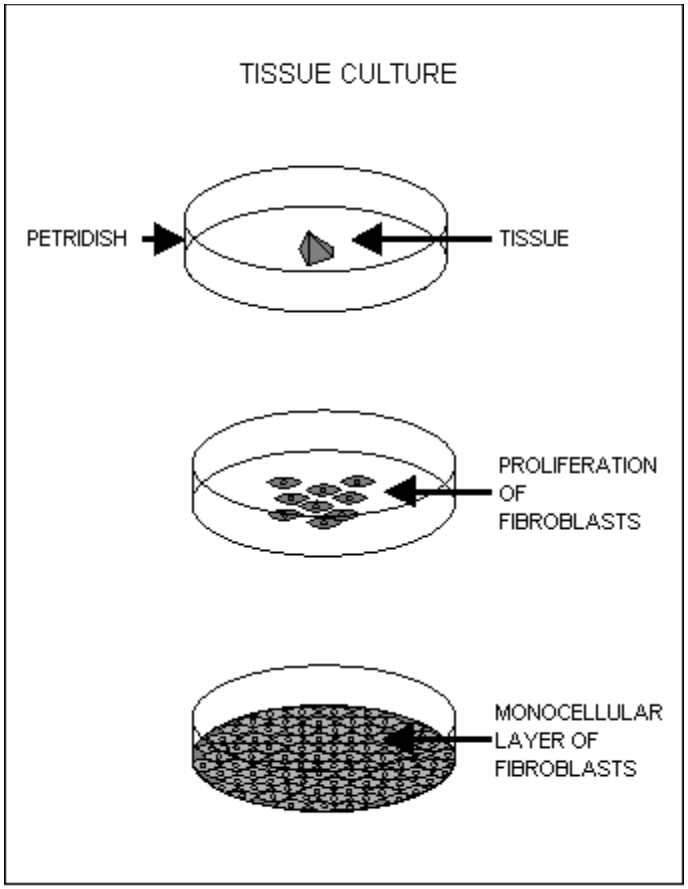


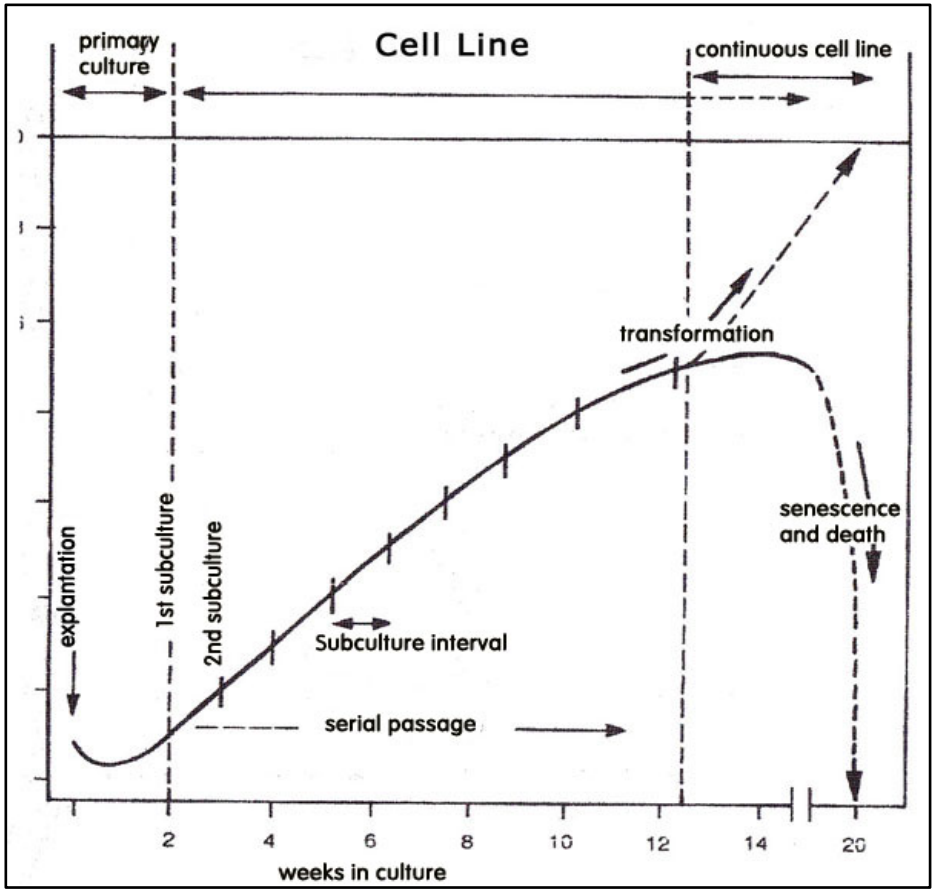
Just seeded

Growing

Confluent

How do you grow mammalian cells?





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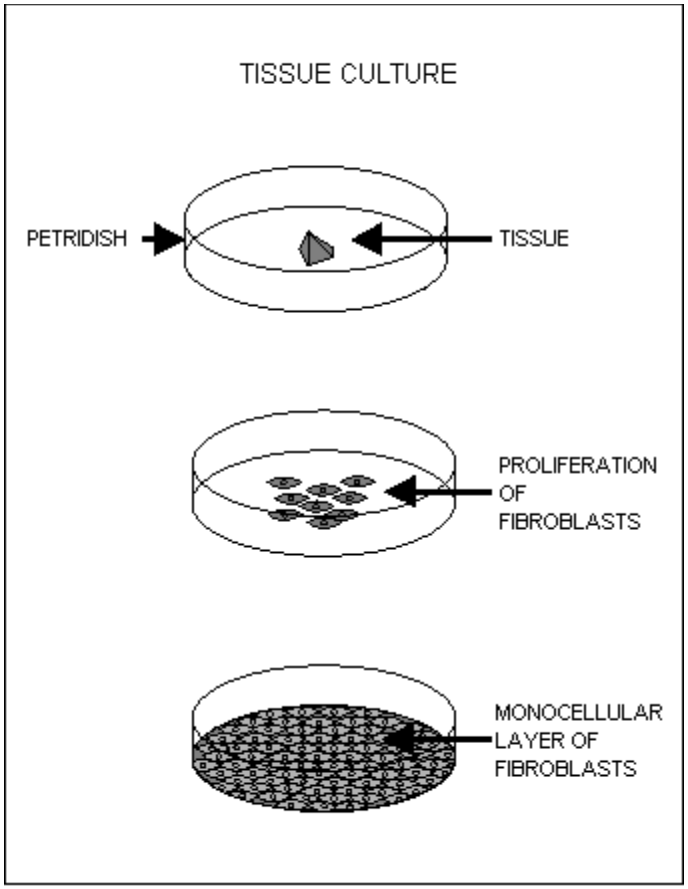
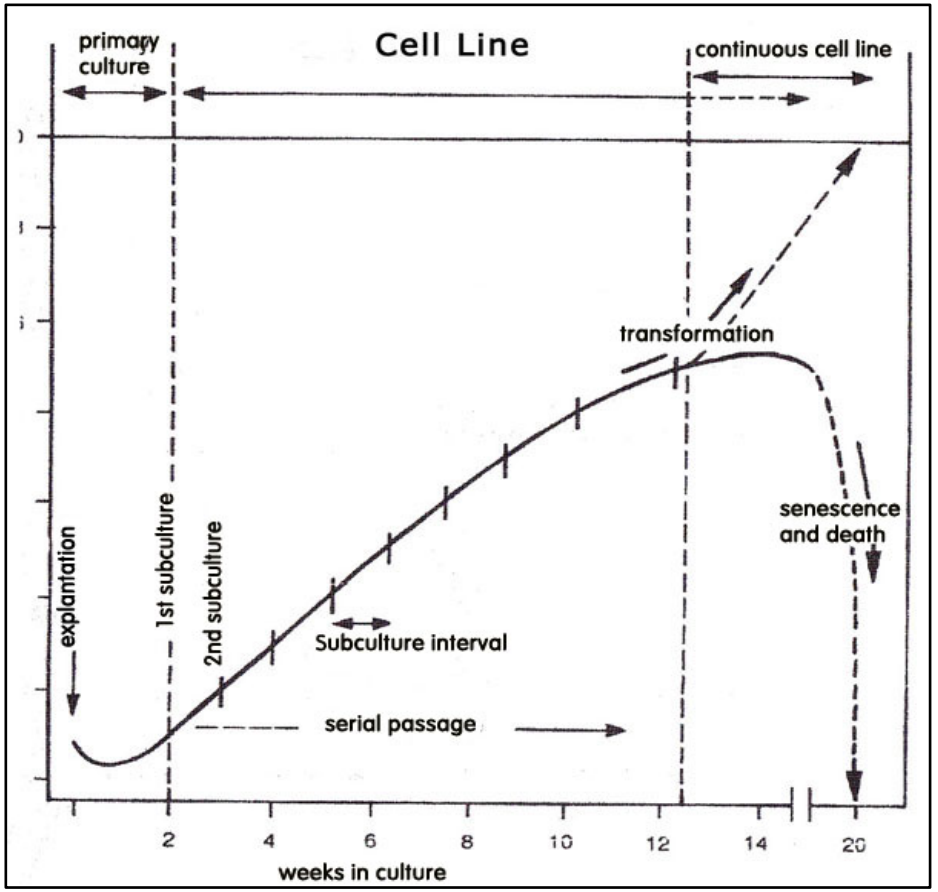
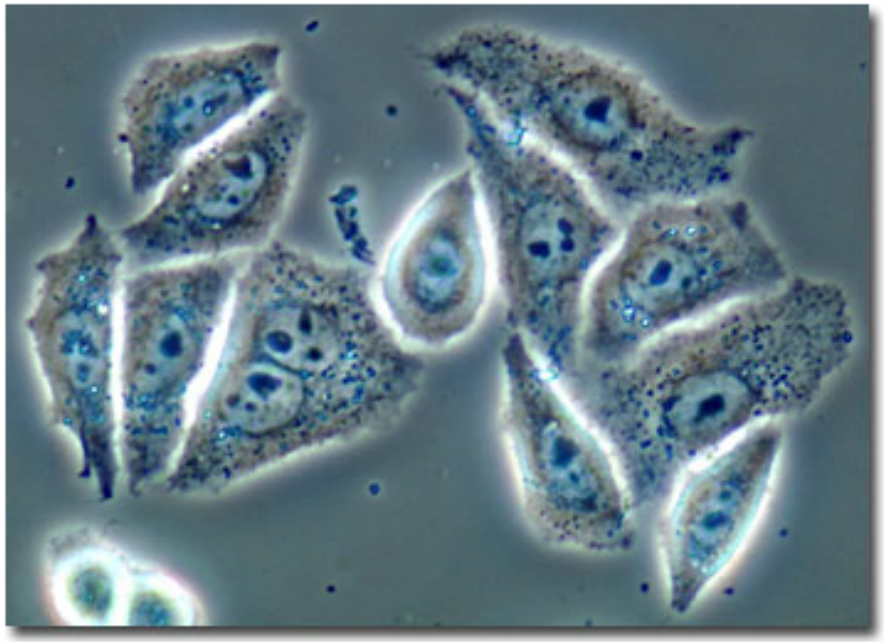




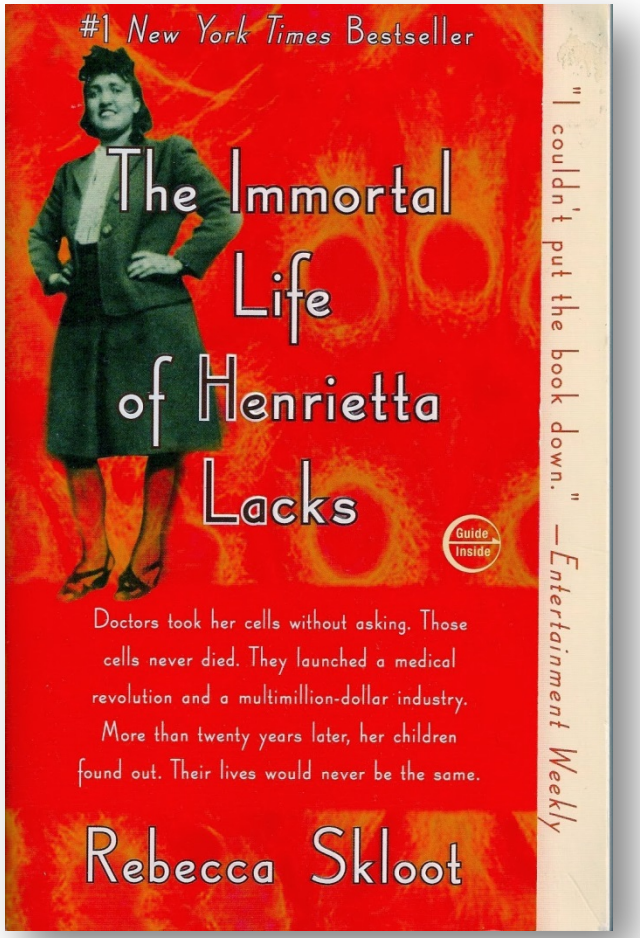
This is for normal tissue, what about tumor tissue?

From Freshney's "Culture of Mammalian Cells"

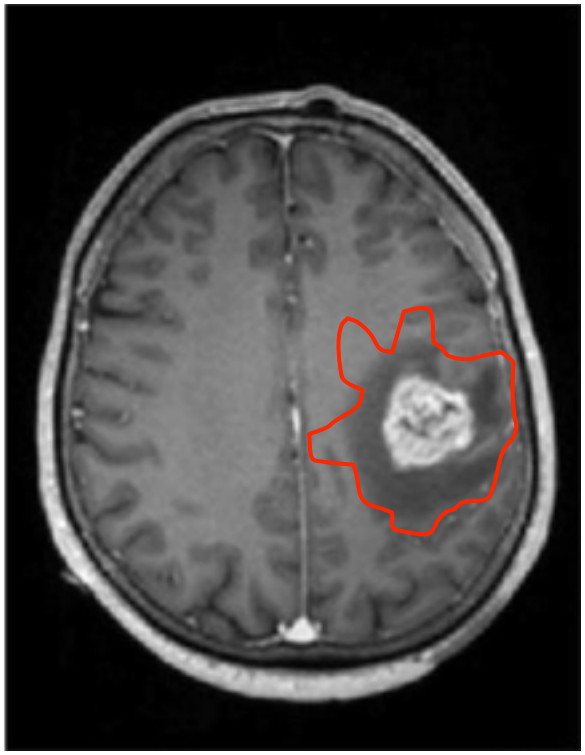


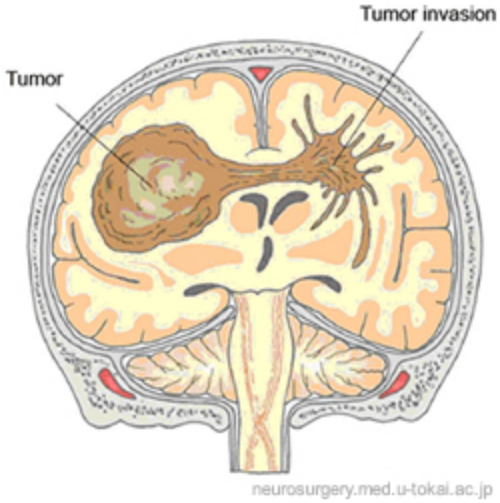
Phase Contrast

HeLa cells from the Nikon microscope web site



HeLa cells have been cultured continuously for scientific use since they were first taken from the ovarian tumor of **Henrietta Lacks** suffering from cervical cancer in the 1950s. They have been utilized for many purposes, including the development of a polio vaccine, the pursuit of a cure for diseases such as leukemia and cancer, and the study of the cellular effects of drugs and radiation.





Grade IV glioma
Glioblastoma Mulitiforme
GBM

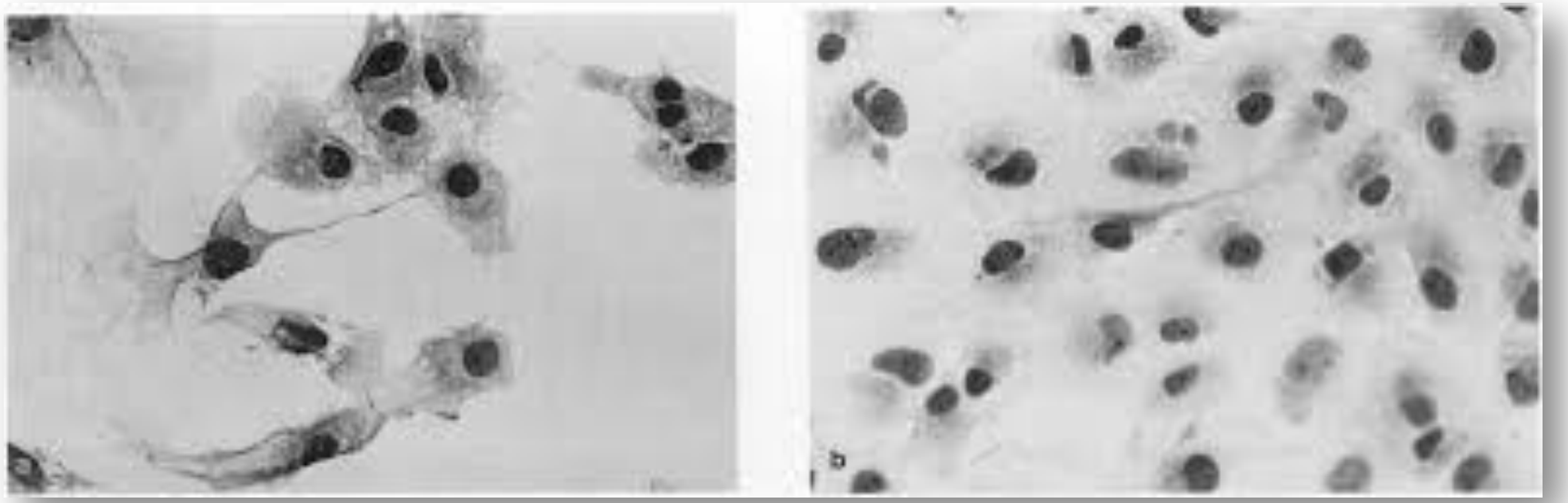
- Most common and malignant form of brain cancer
- Affects 10,000 people a year in North America alone
- Median survival is around 10 months after diagnosis
- Highly diffusive tumor type
- Treatment consists of surgical resection, radio- and chemotherapy

http://www.ajnr.org/content/32/1/67/F5.expansion.html

Isolation of Two Cell Lines from a Human Malignant Glioma Specimen Differing in Sensitivity to Radiation and Chemotherapeutic Drugs

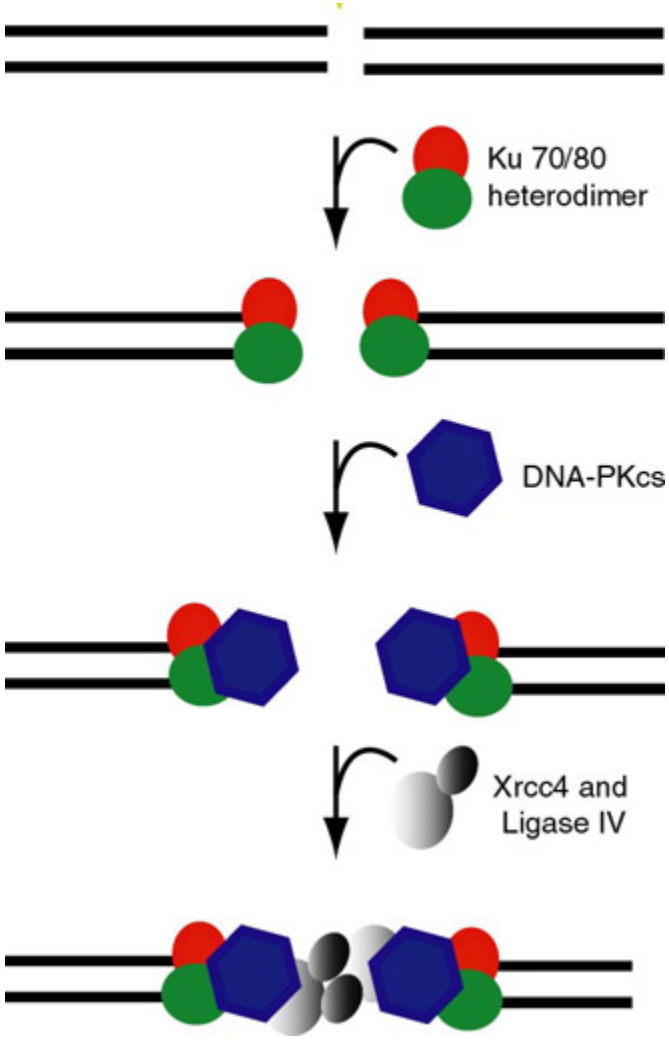
M. J. ALLALUNIS-TURNER,* G. M. BARRON,* R. S. DAY III,† K. D. DOBLER,† AND R. MIRZAYANS†

*Radiobiology Program, Department of Radiation Oncology, and †Molecular Oncology Program, Department of Medicine, Cross Cancer Institute, Edmonton, Alberta, Canada



M059J M059K

Non-Homologous End Joining (NHEJ)



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20.109 Spring 2016 Module 2 – Lecture 1
System Engineering (March 8th 2016)




Noreen Lyell

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Leona Samson (Lectures)

Zachary Nagel (help with development) Alex Chaim