20.109 Spring 2017 Module 2 – Lecture 1
Gene Expression Engineering (March 9th 2017)




Noreen Lyell

Leslie McLain

Maxine Jonas

Rob Wilson

Leona Samson (Lectures)

Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

What experimental question will you ask in Module 2?

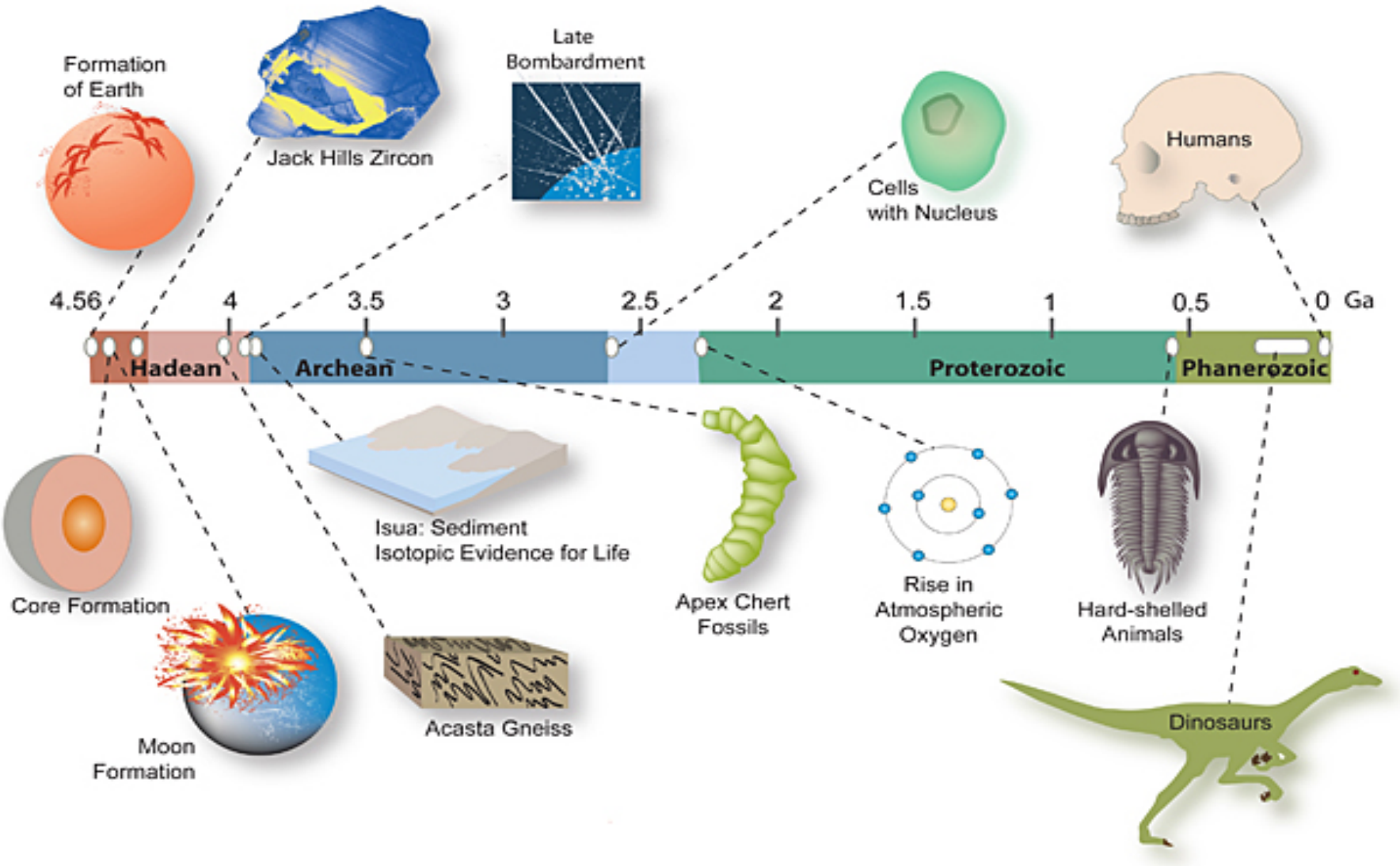
How does DNA repair affect the ability of cancer chemotherapy drugs to kill cancer cells?

How does cancer chemotherapy affect gene expression?

This raises the following questions

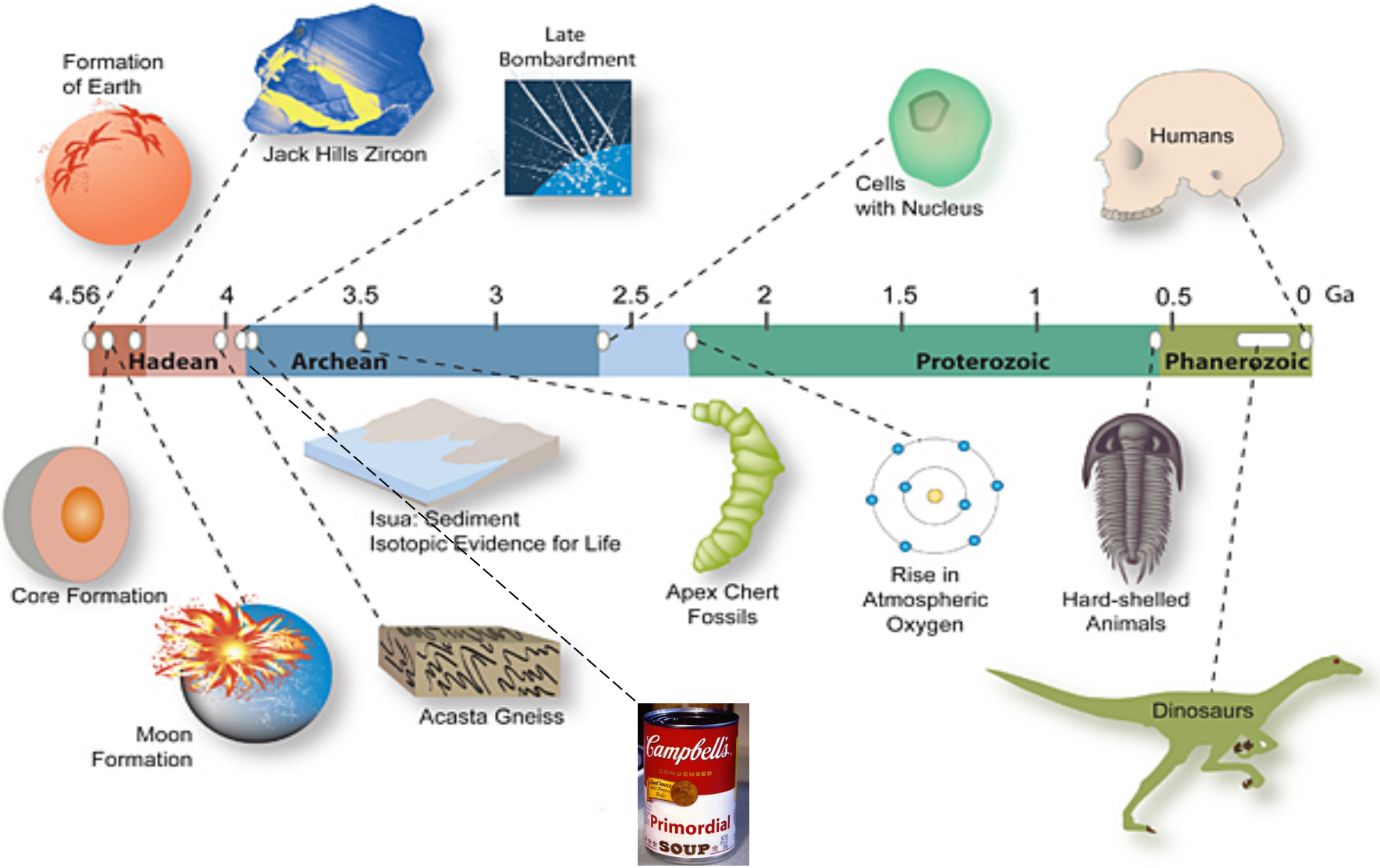
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?

Evolution of life on Earth



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

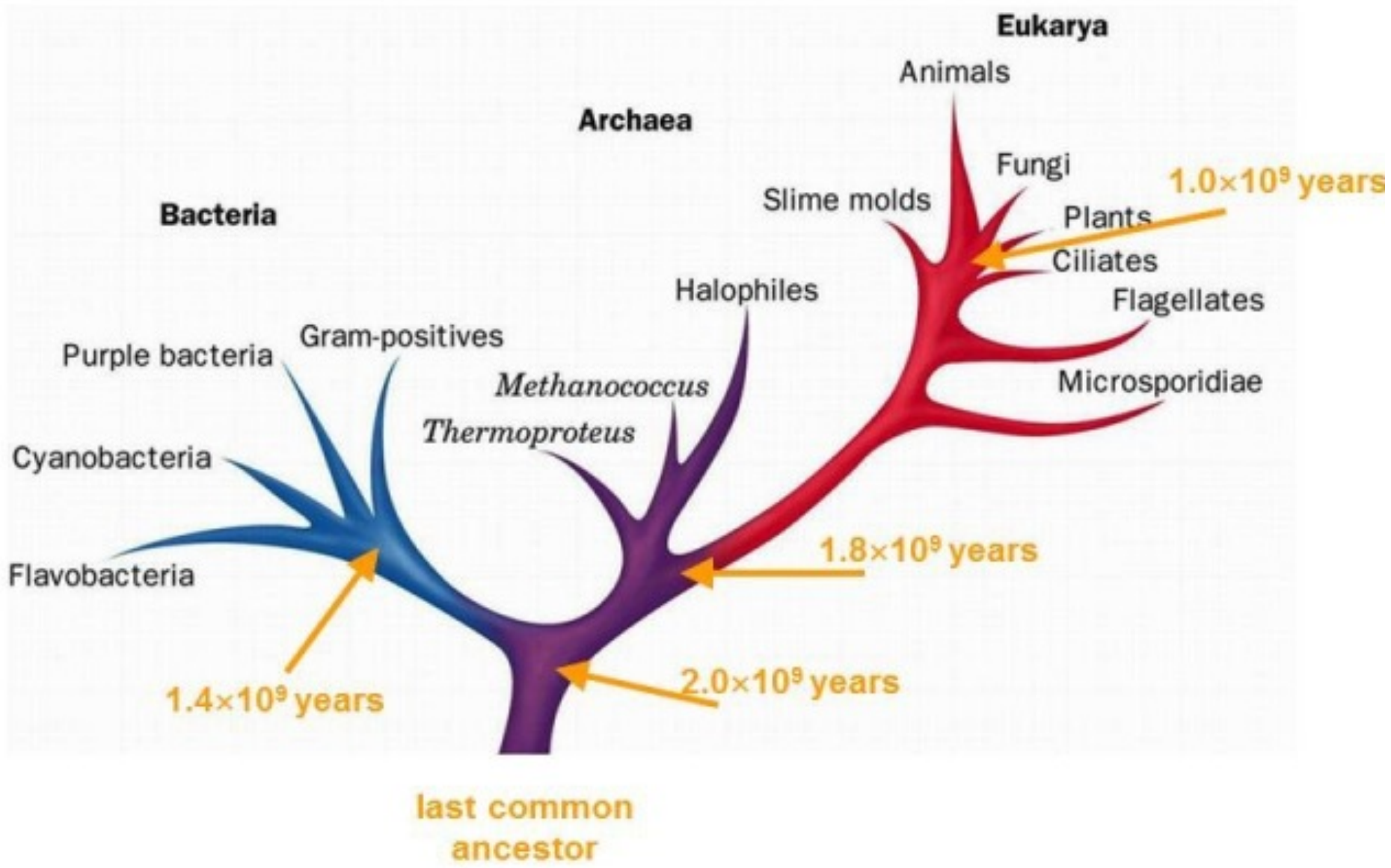
Evolution of life on Earth



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

Andree Valley

All known life forms are based on DNA



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

Central Dogma

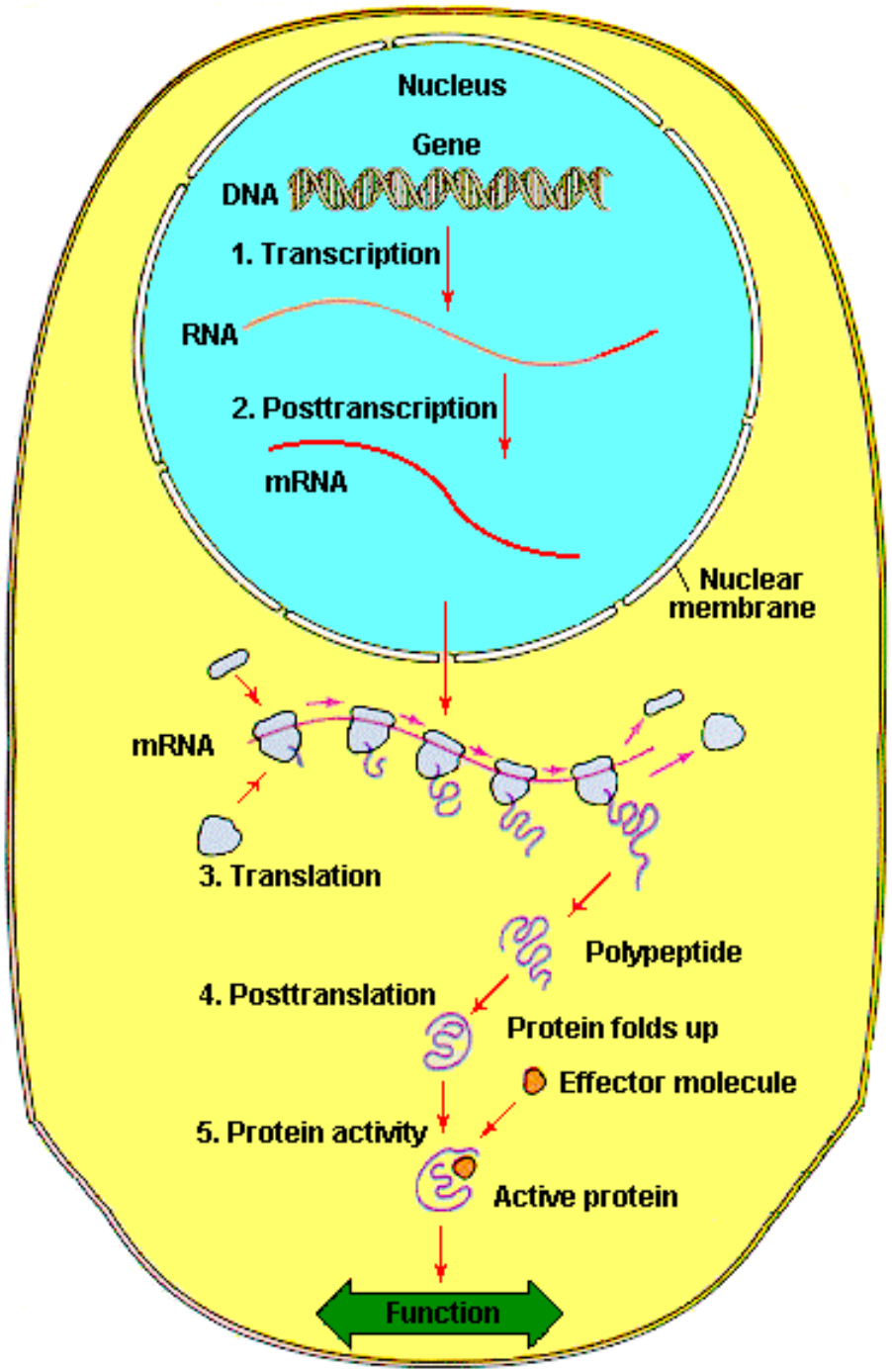
DNA

makes

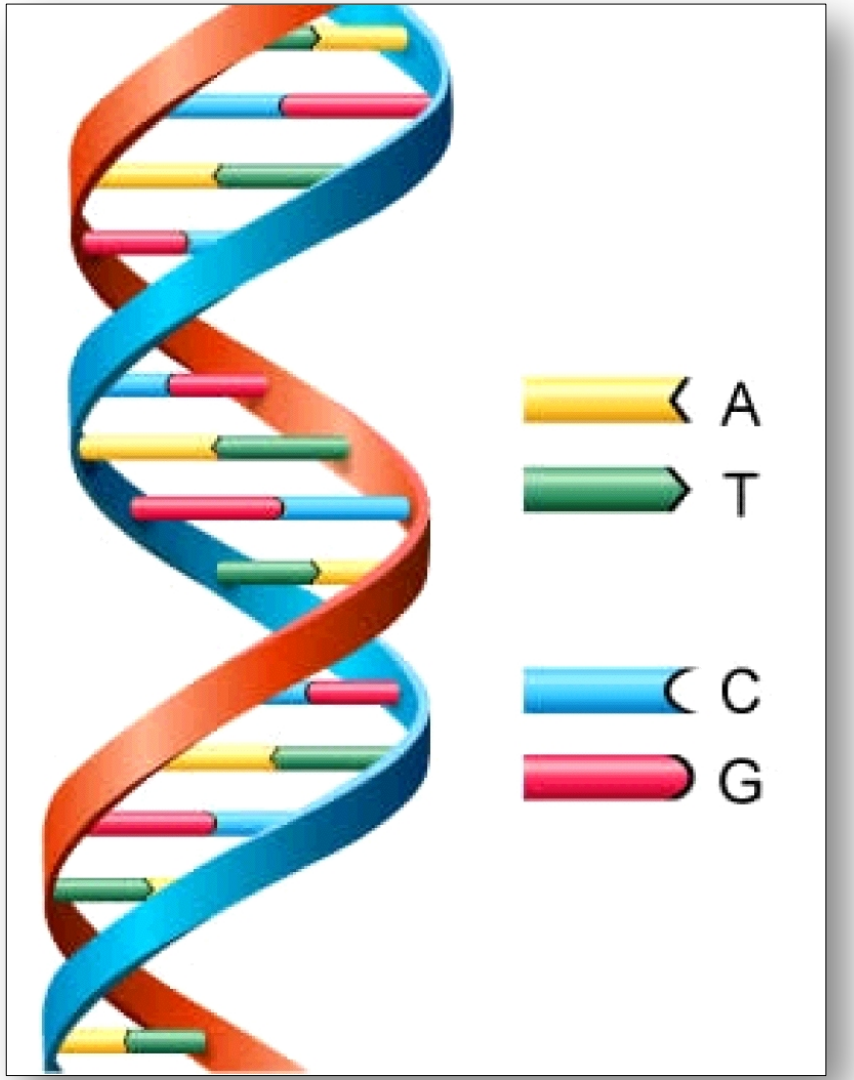
RNA

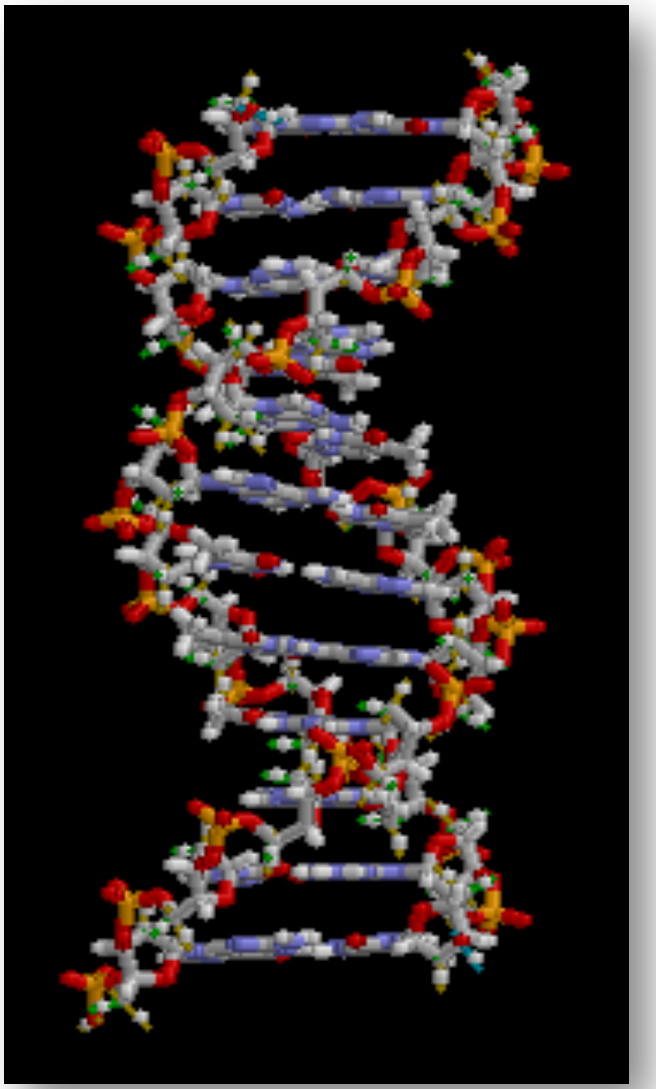
makes

Protein



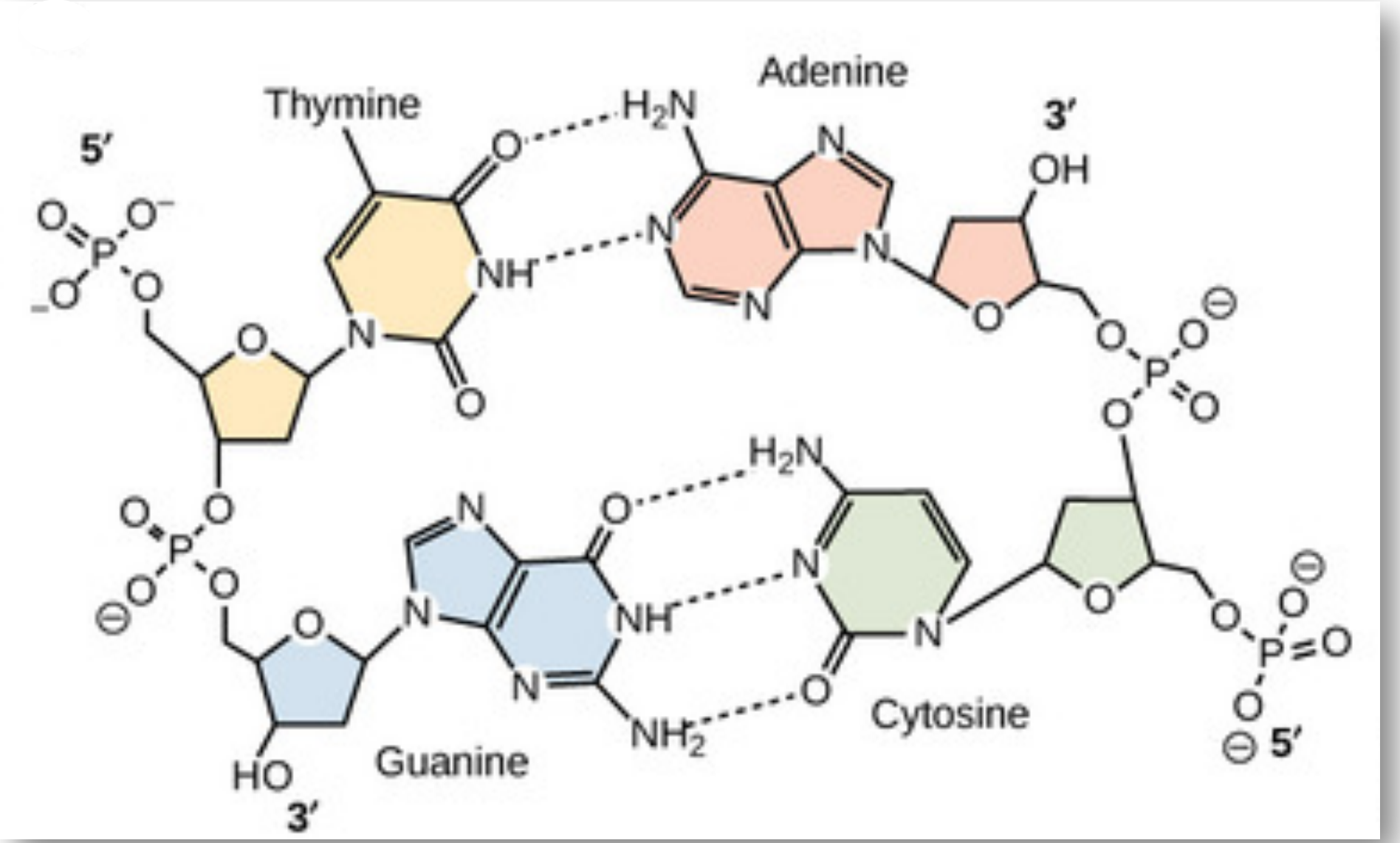
All known life forms are based on DNA





Each human cell has 6 billion base pairs of DNA

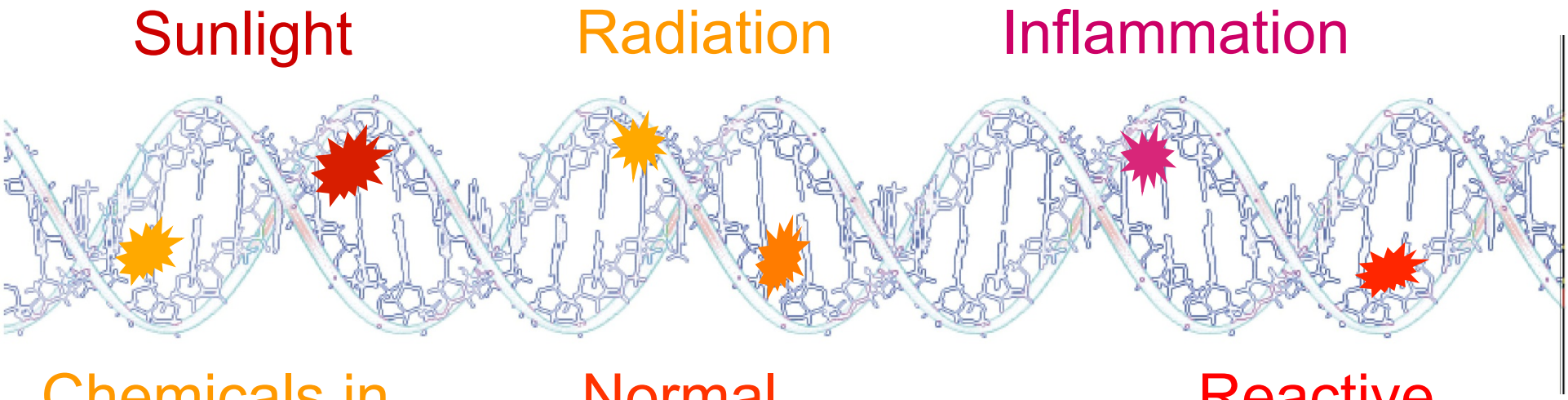
DNA Spontaneously Decays



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} cells in the human body
4 seconds to read the sentence

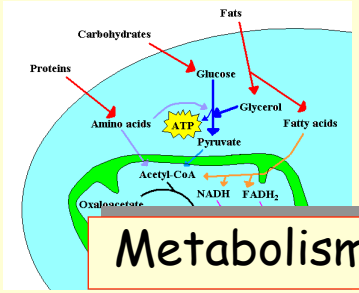
DNA is constantly being damaged by external and internal agents



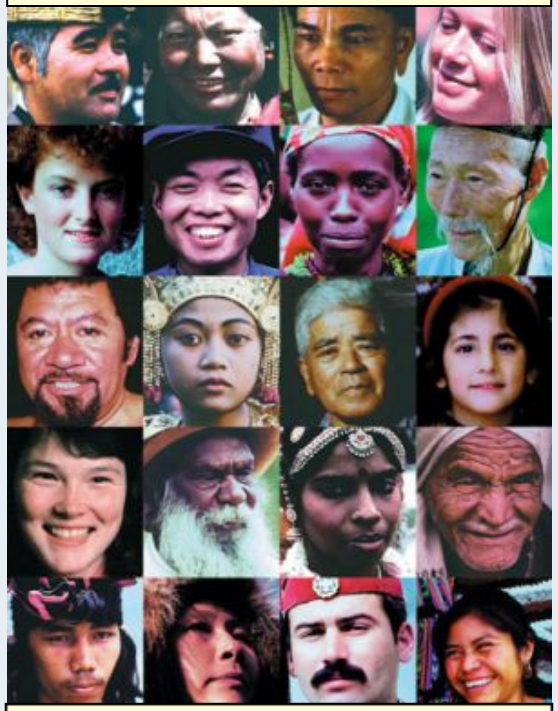
Courtesy of Bevin Engelward

Environmental exposures to potentially harmful agents – DNA damaging agents

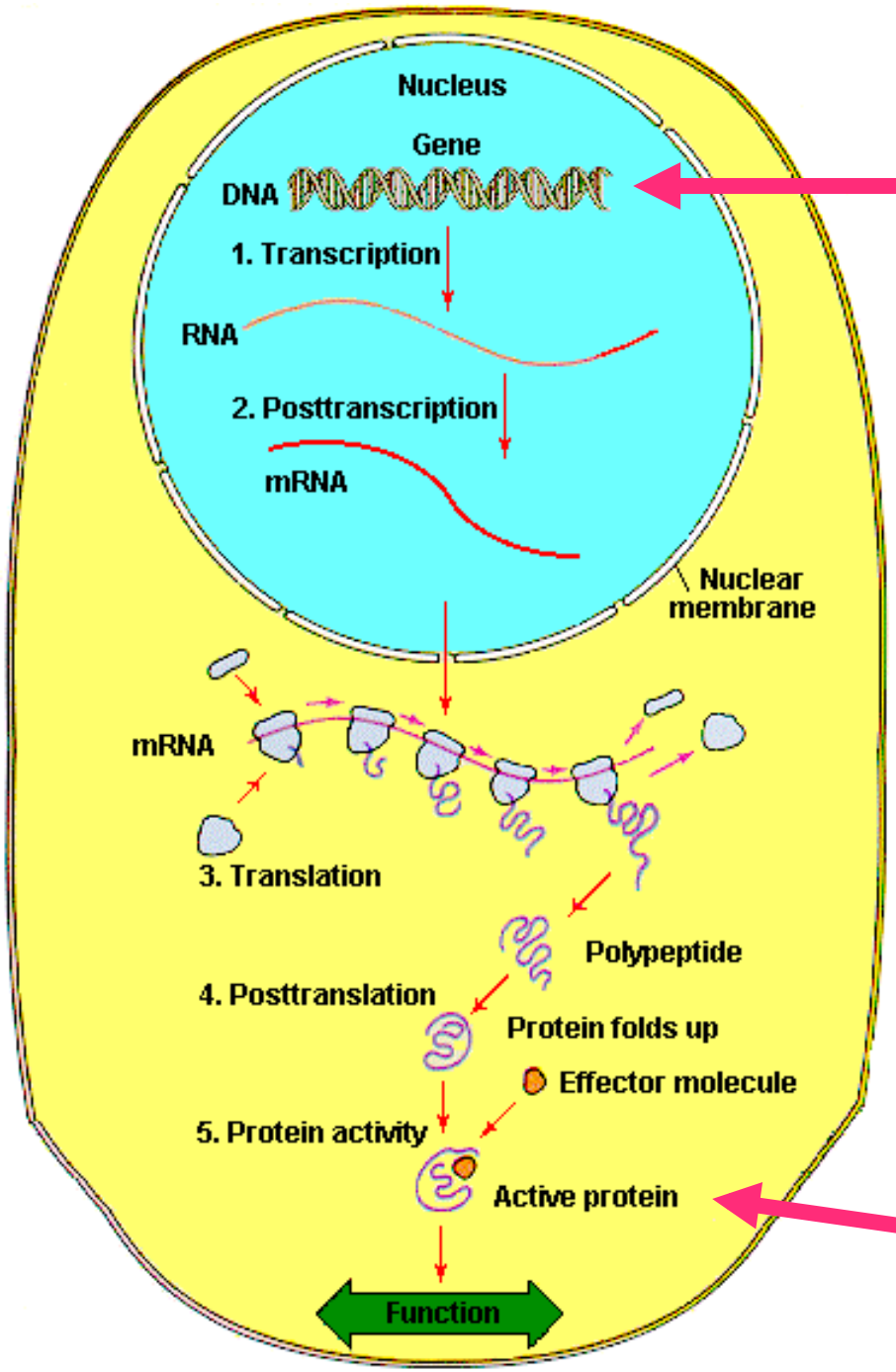
Harmful agents



People have different exposures



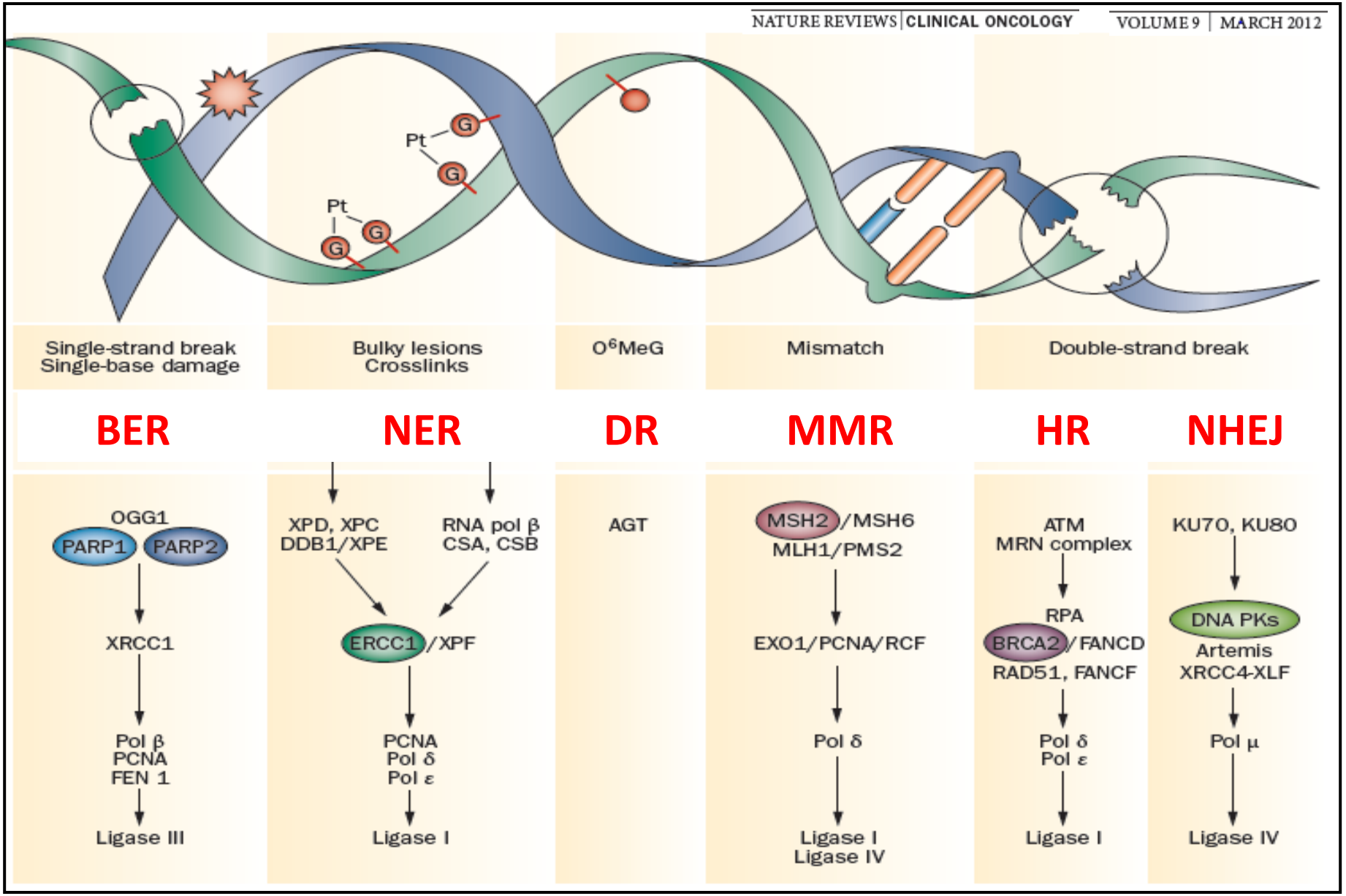
People have different responses



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

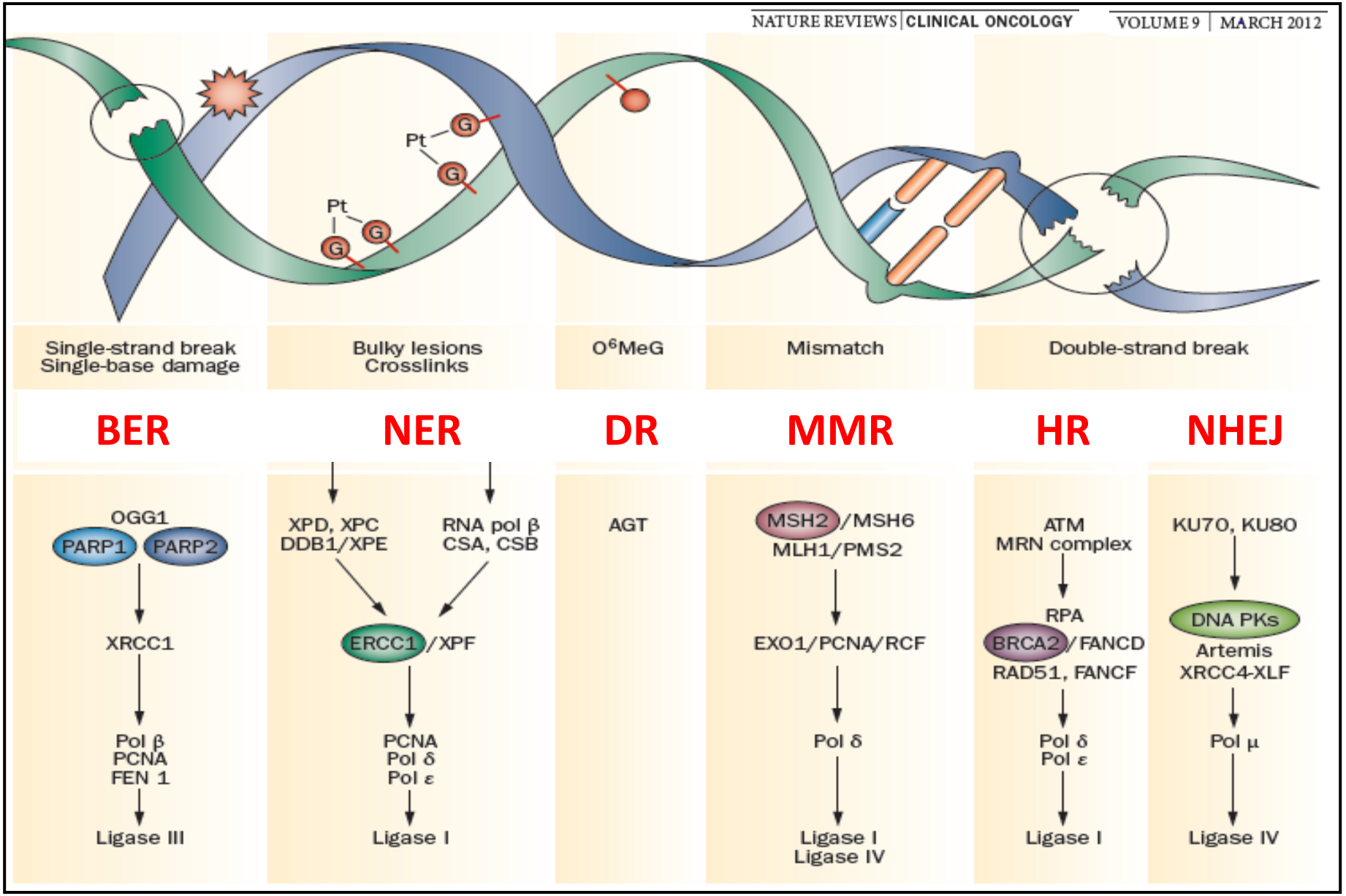
Photo: A. Mahmoud

Aziz Sancar

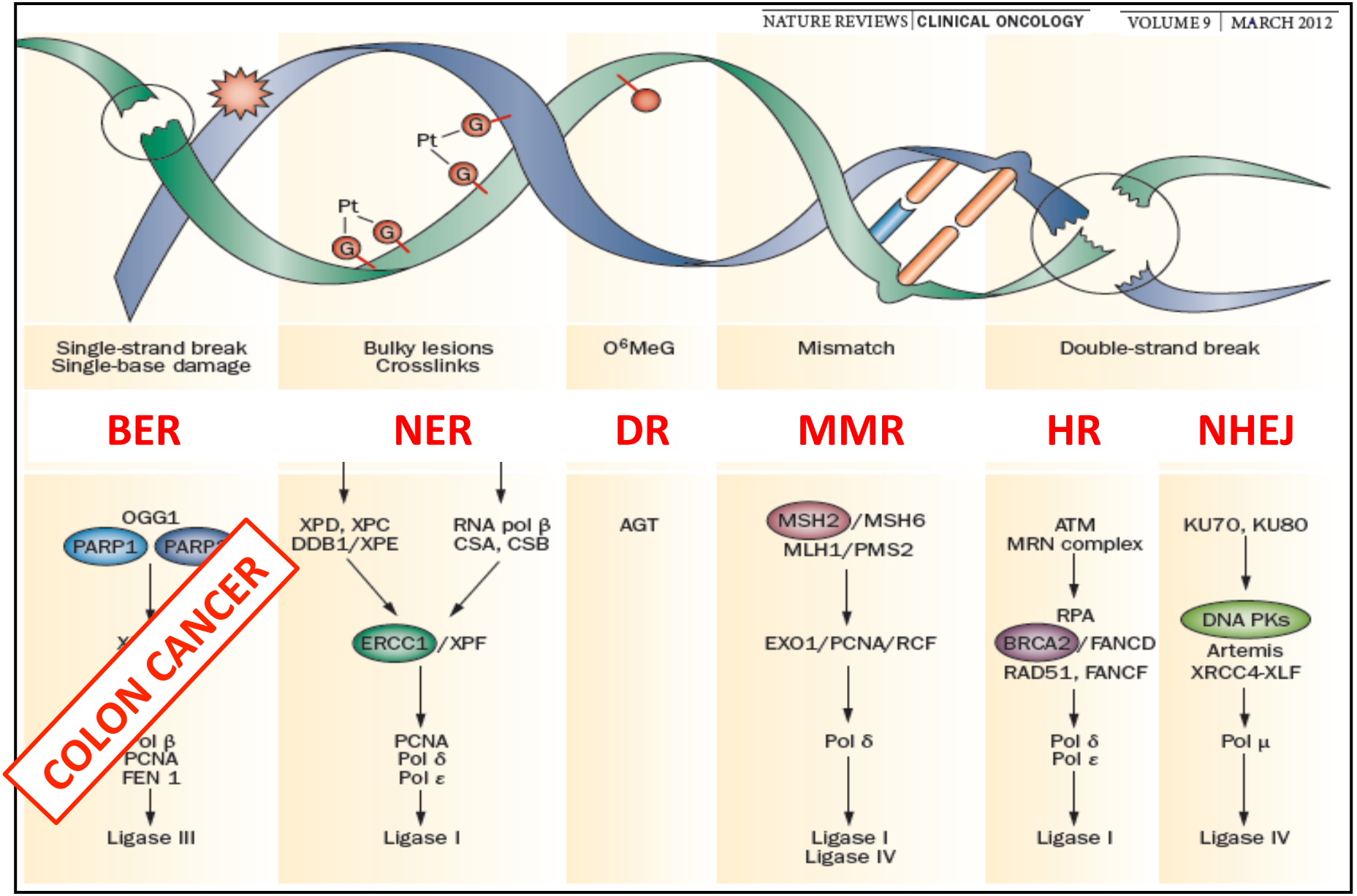
Prize share: 1/3

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

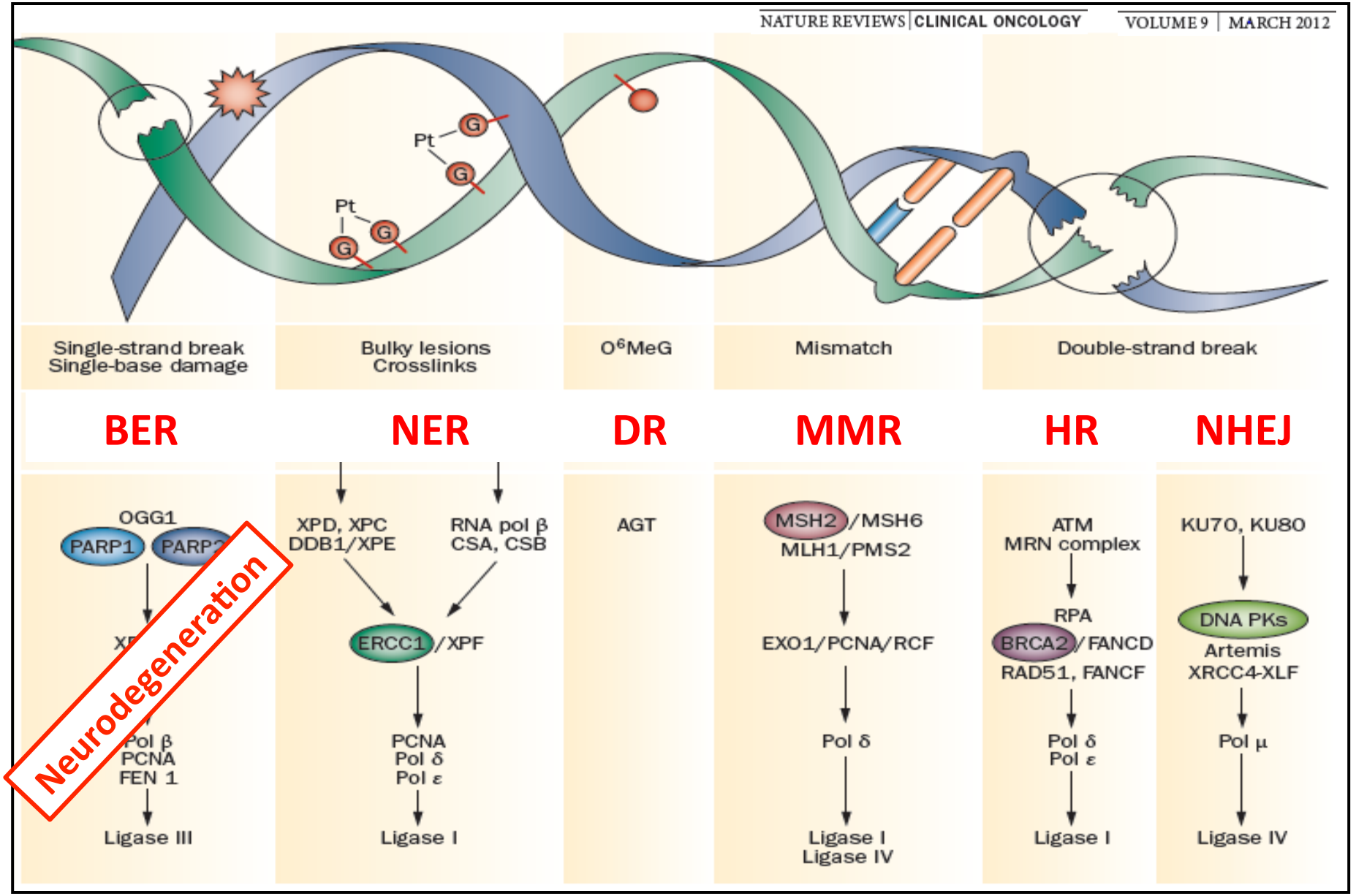
Six Major DNA Repair Pathways



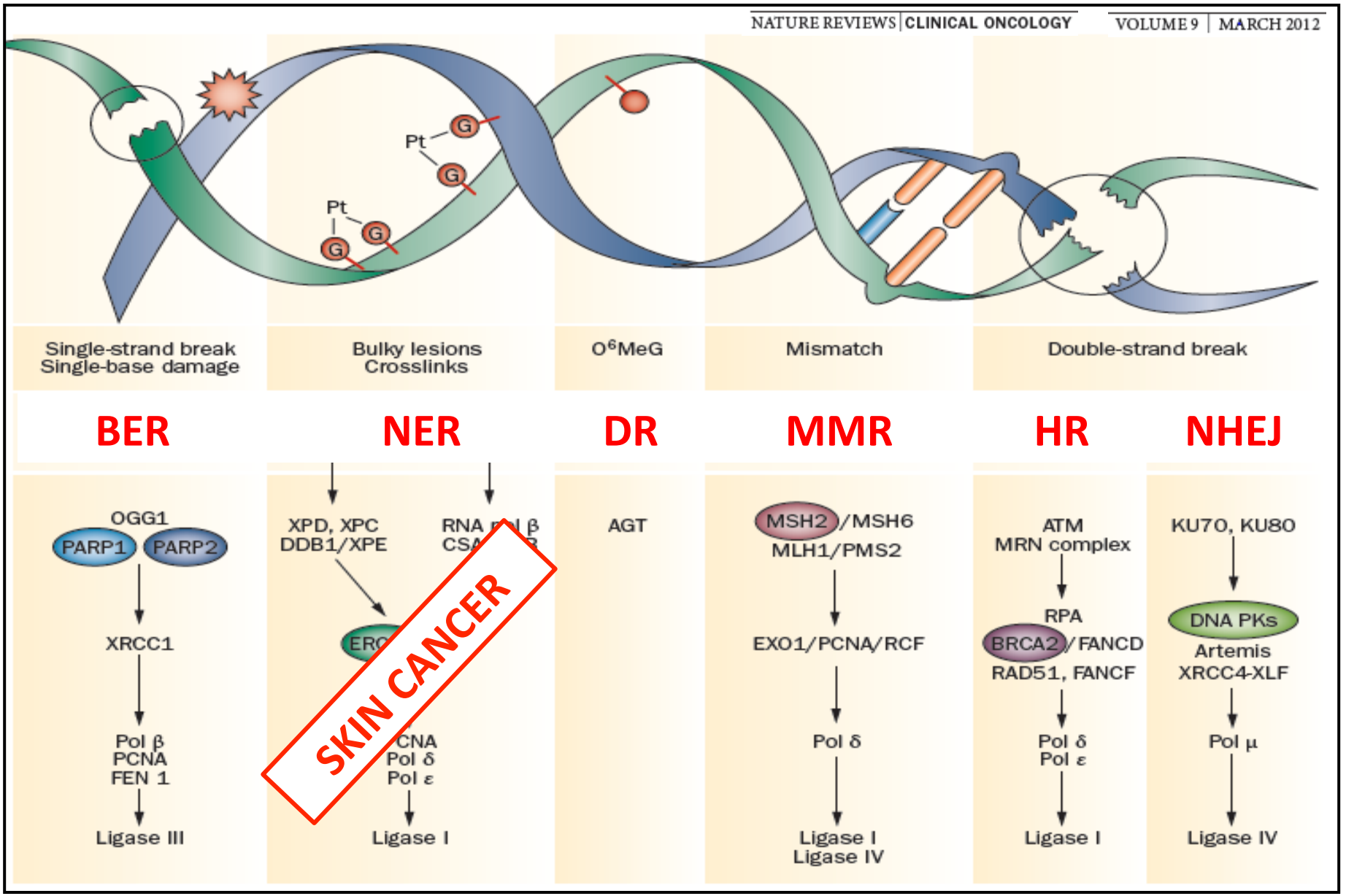
Six Major DNA Repair Pathways



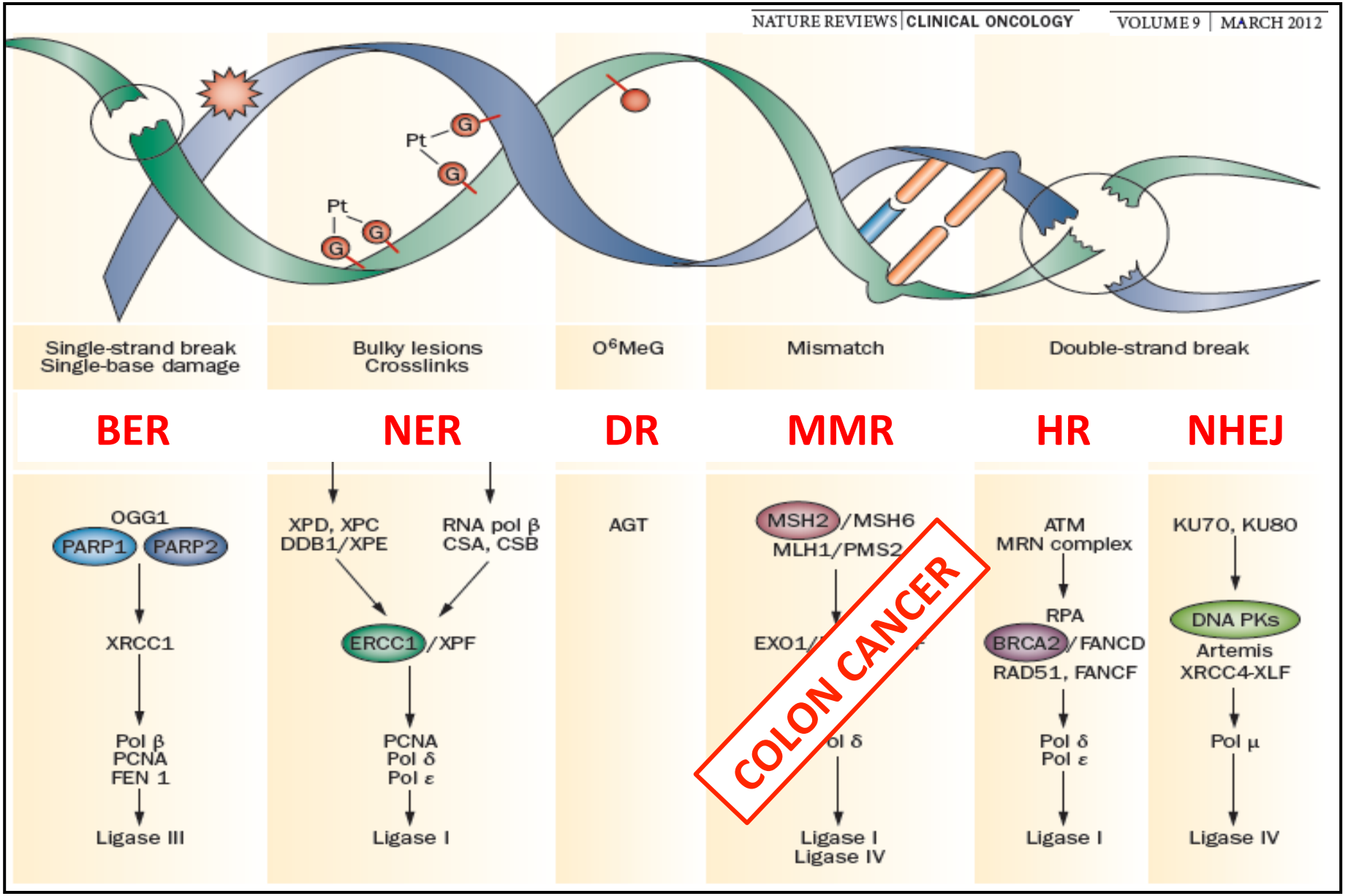
Six Major DNA Repair Pathways



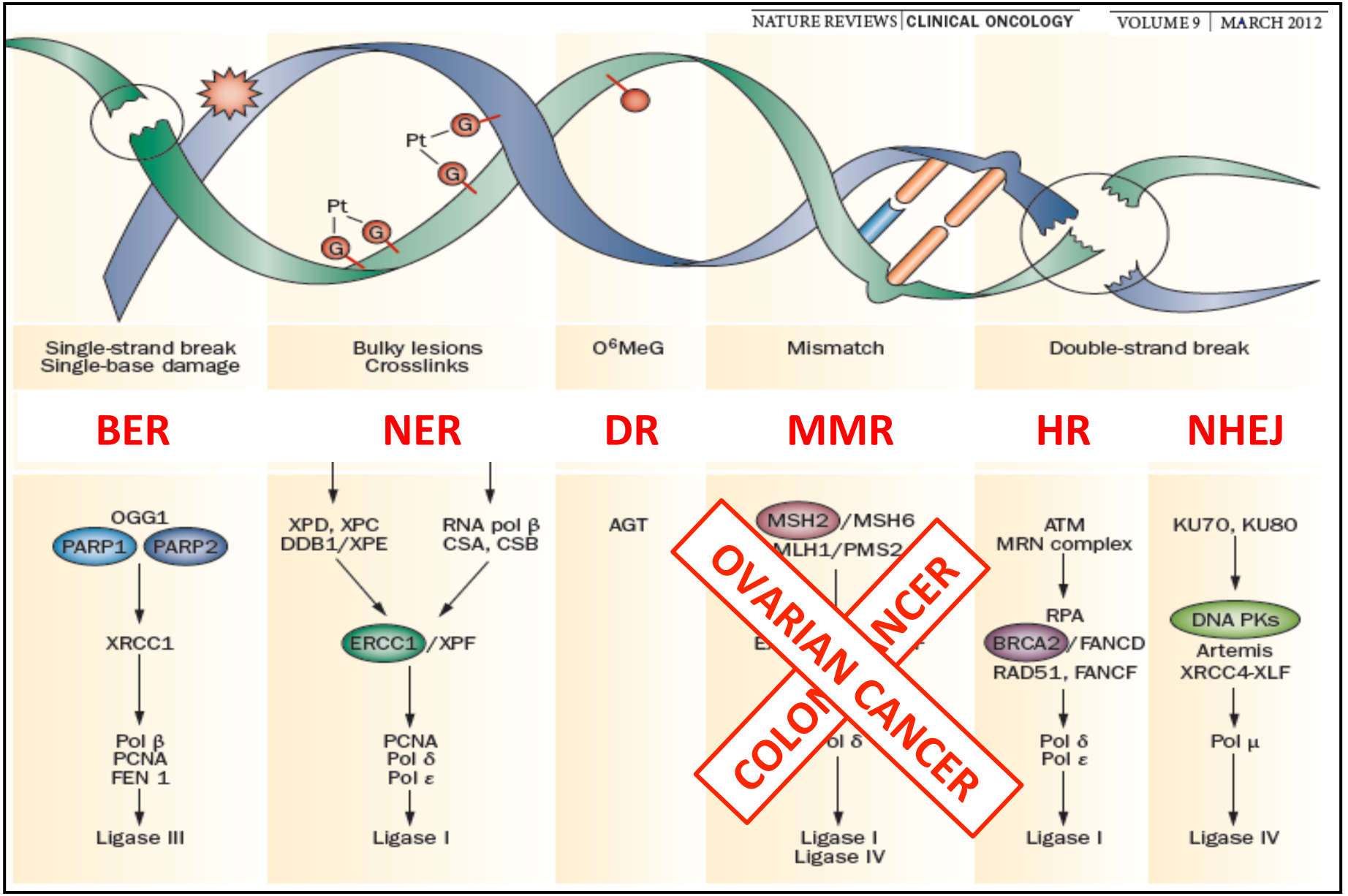
Six Major DNA Repair Pathways



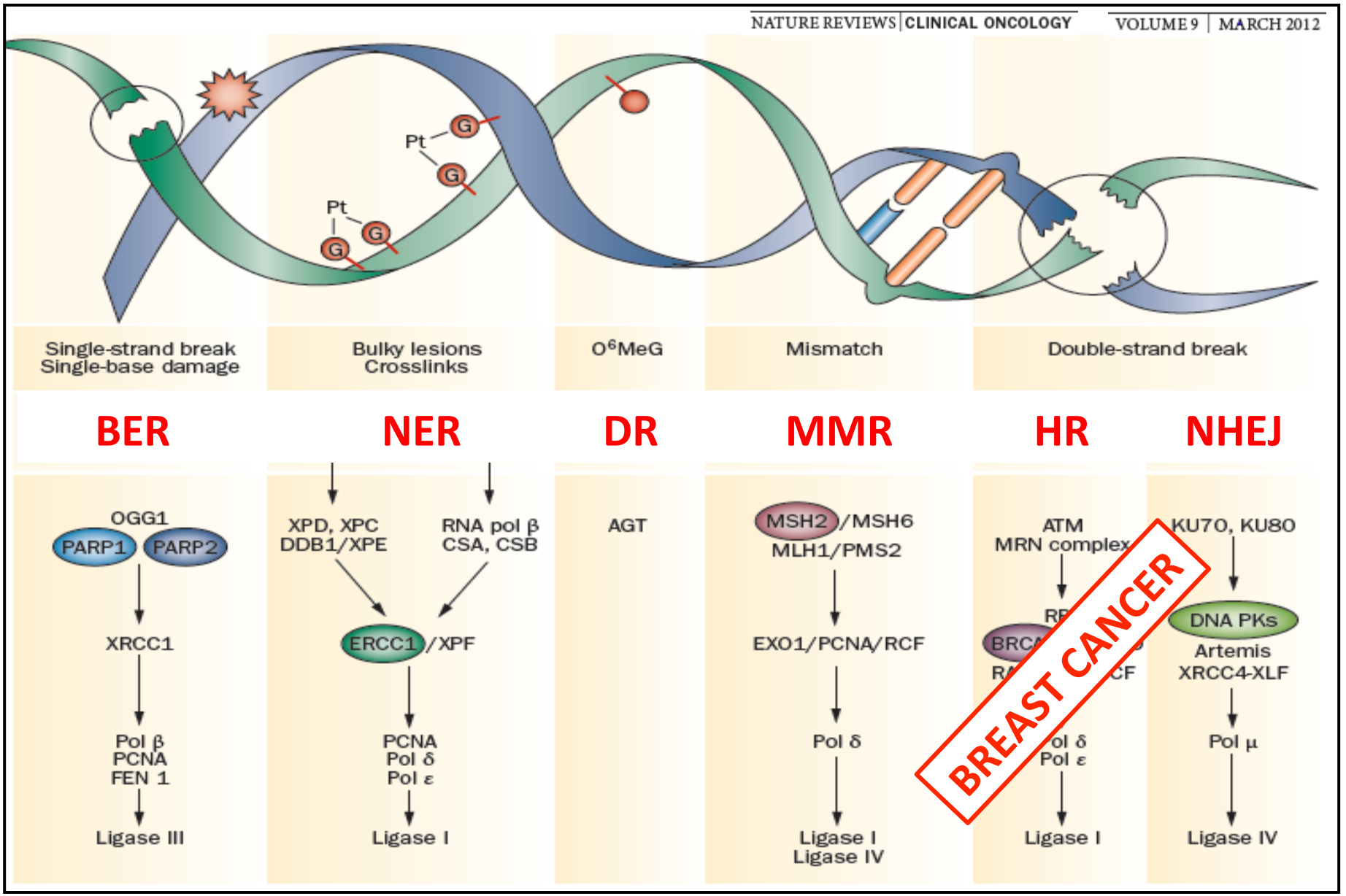
Six Major DNA Repair Pathways



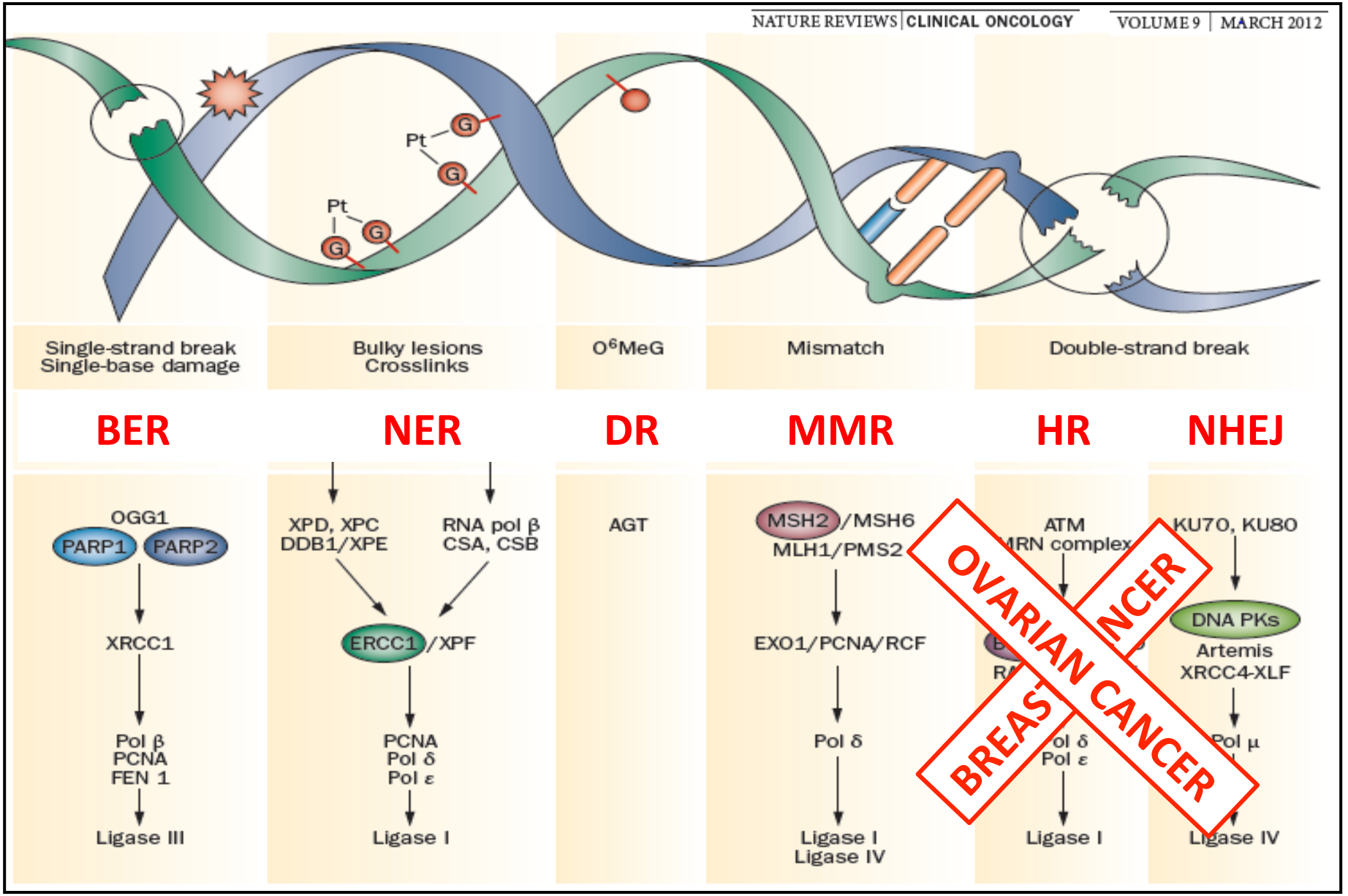
Six Major DNA Repair Pathways



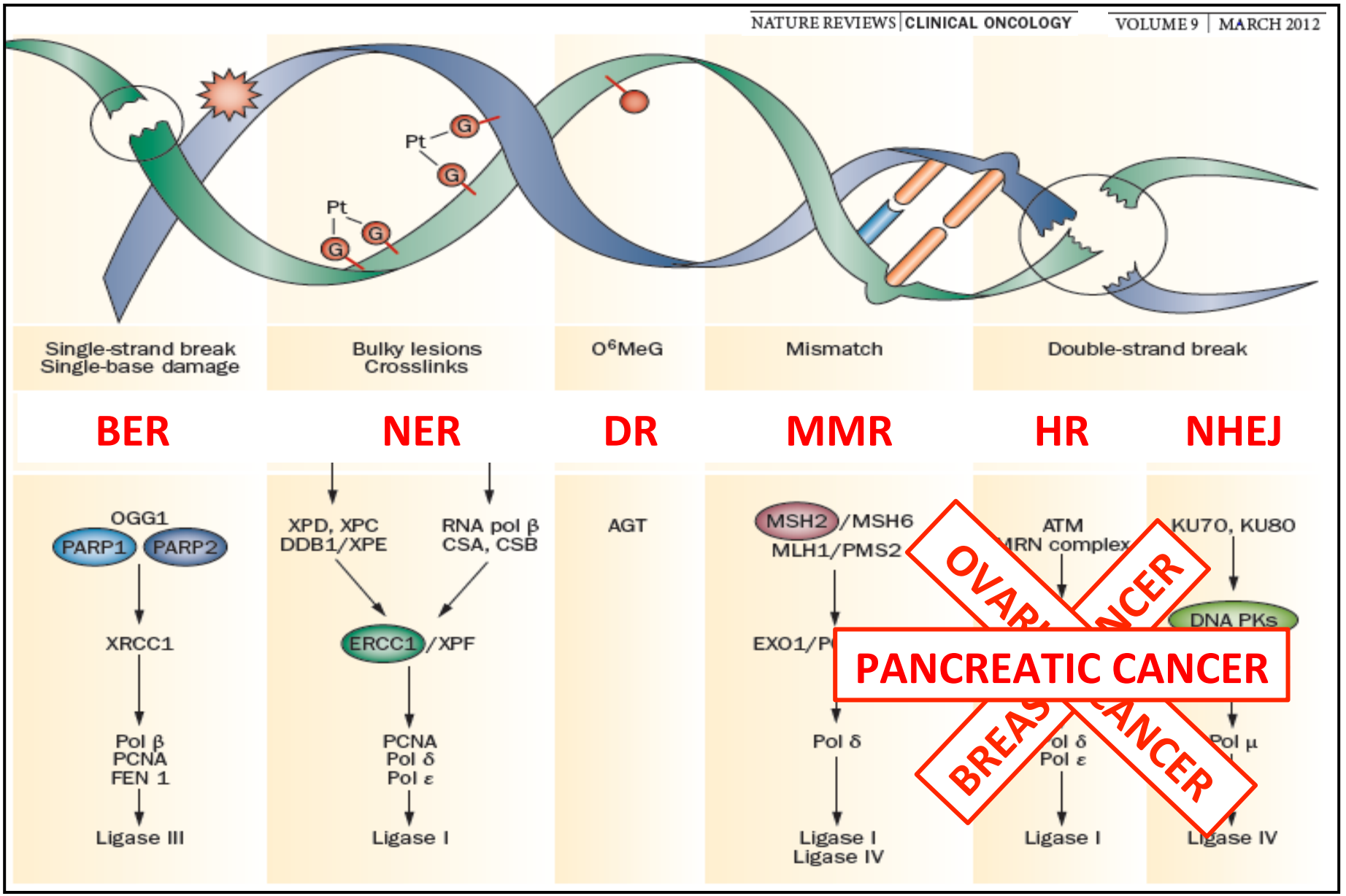
Six Major DNA Repair Pathways



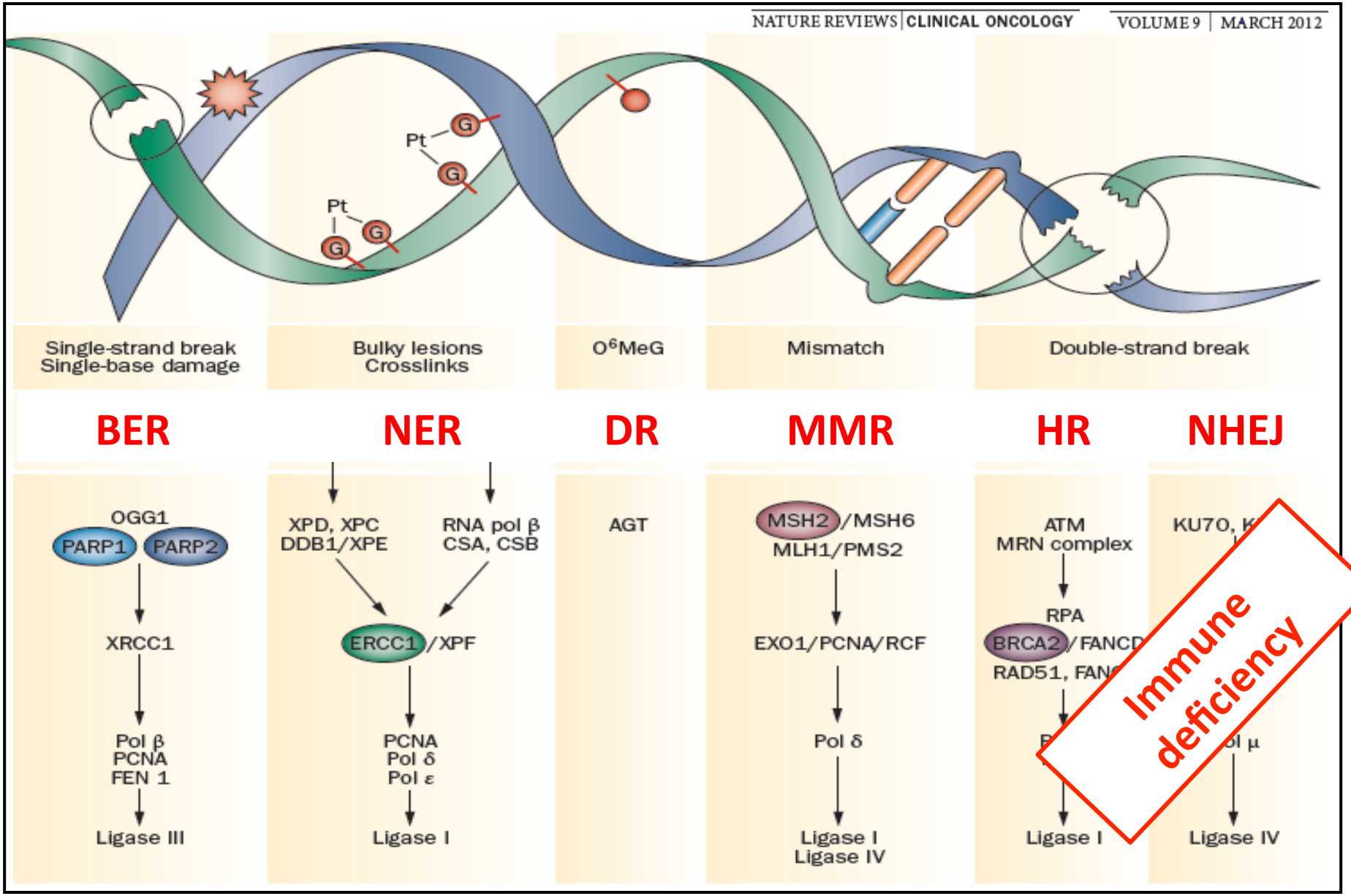
Six Major DNA Repair Pathways



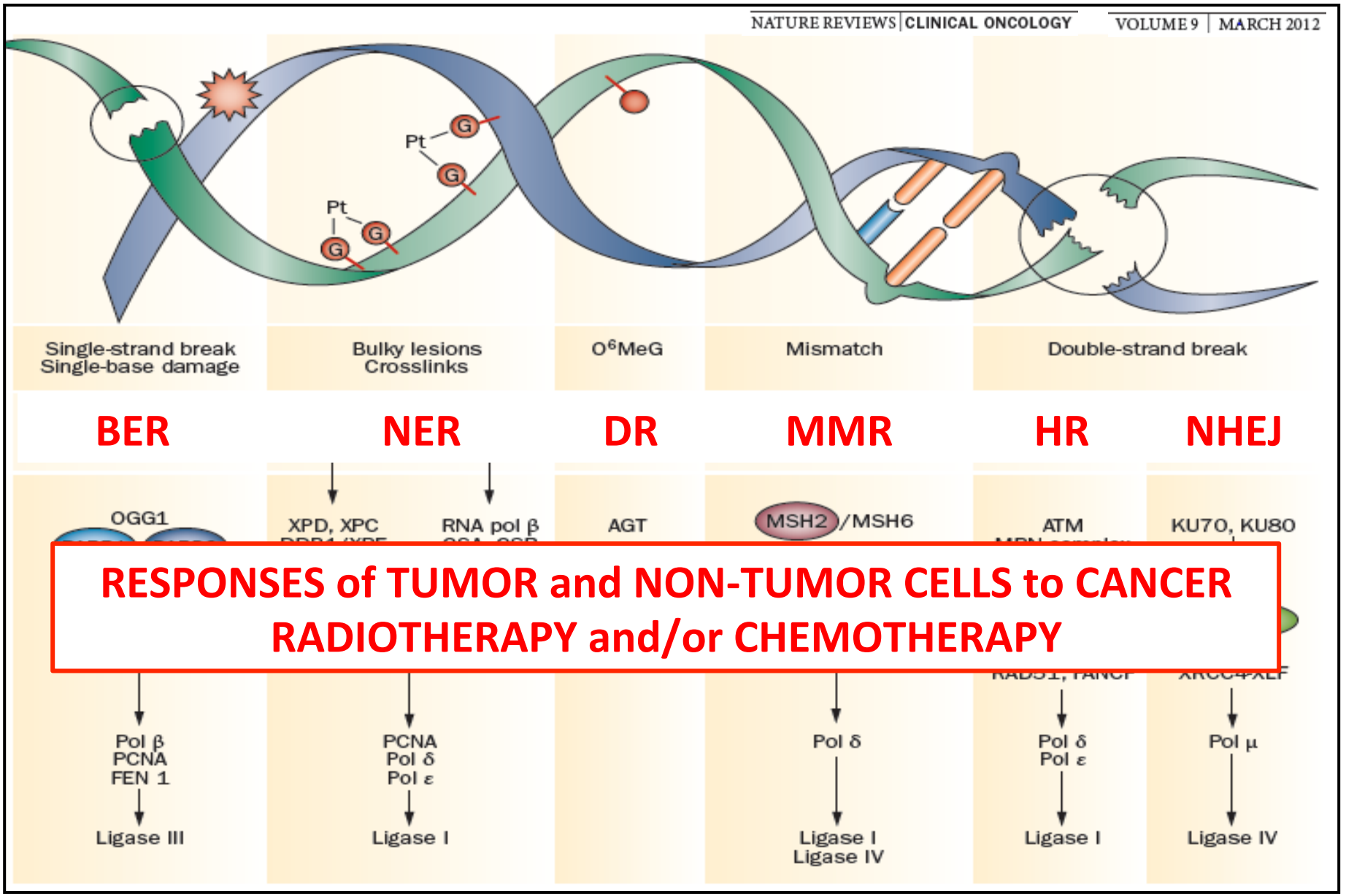
Six Major DNA Repair Pathways



Six Major DNA Repair Pathways



Six Major DNA Repair Pathways

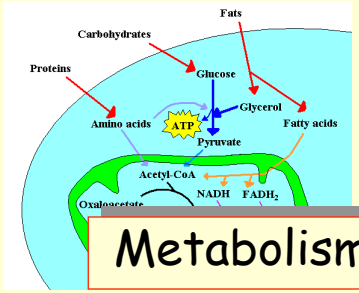


Some cancer Chemotherapy agents and all Radiotherapies

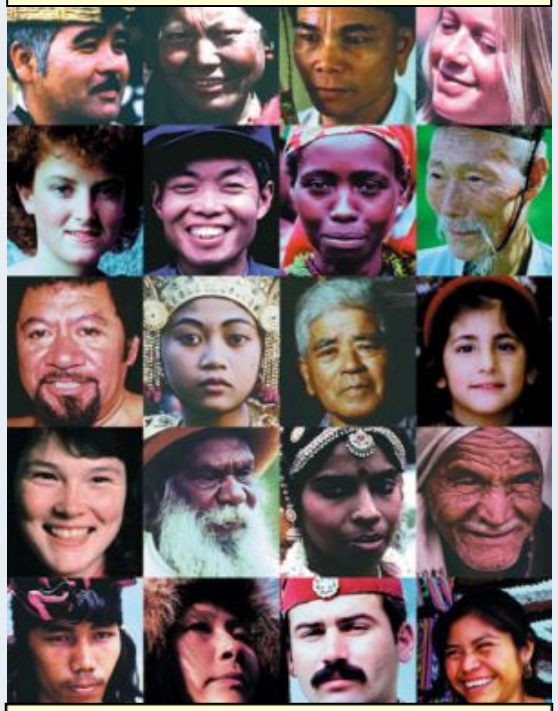
CAUSE DNA DAMAGE

Environmental exposures to potentially harmful agents – DNA damaging agents

Harmful agents



People have different exposures



People have different responses

2007 – Breakthrough of the year

Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

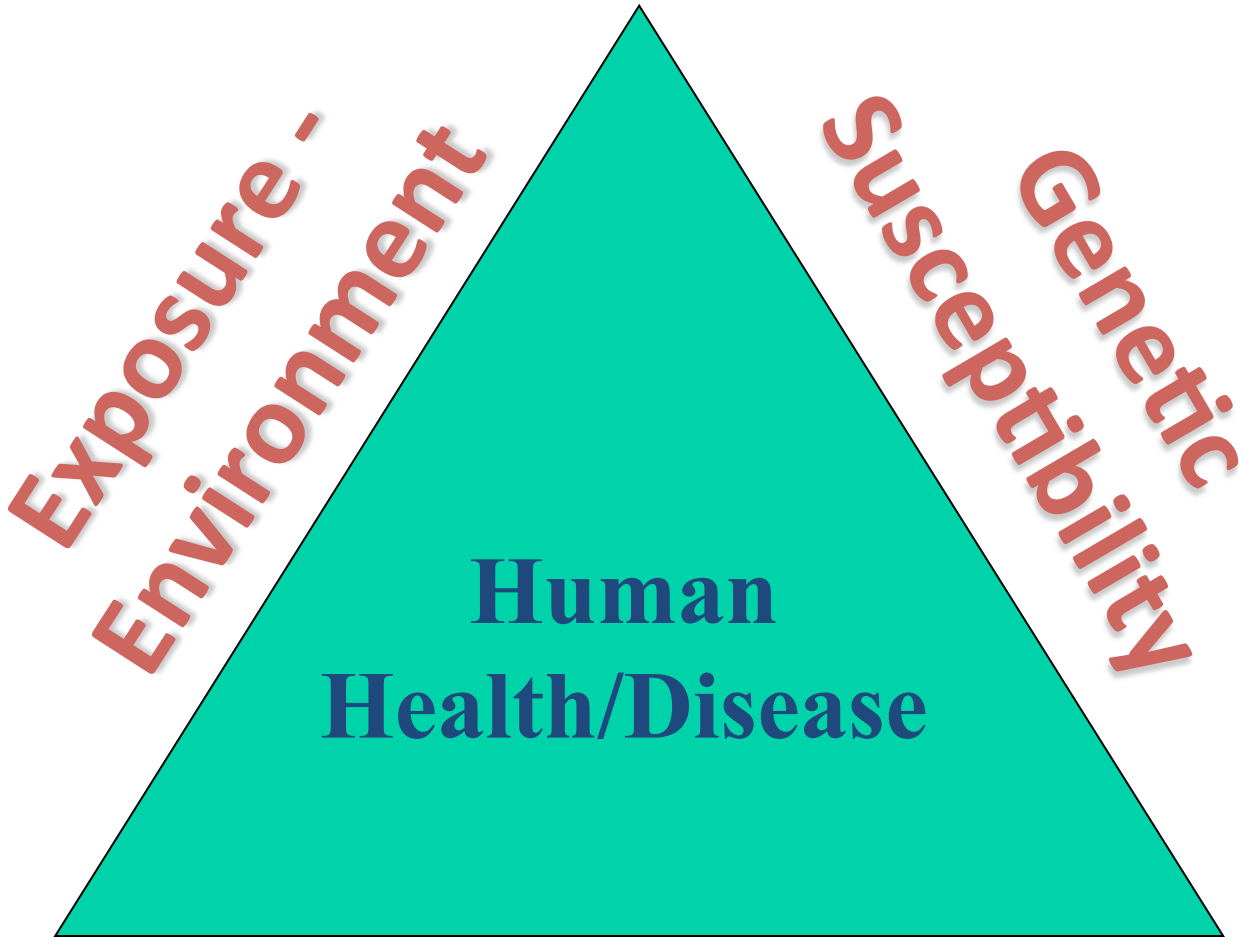
2007 – Breakthrough of the year

Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

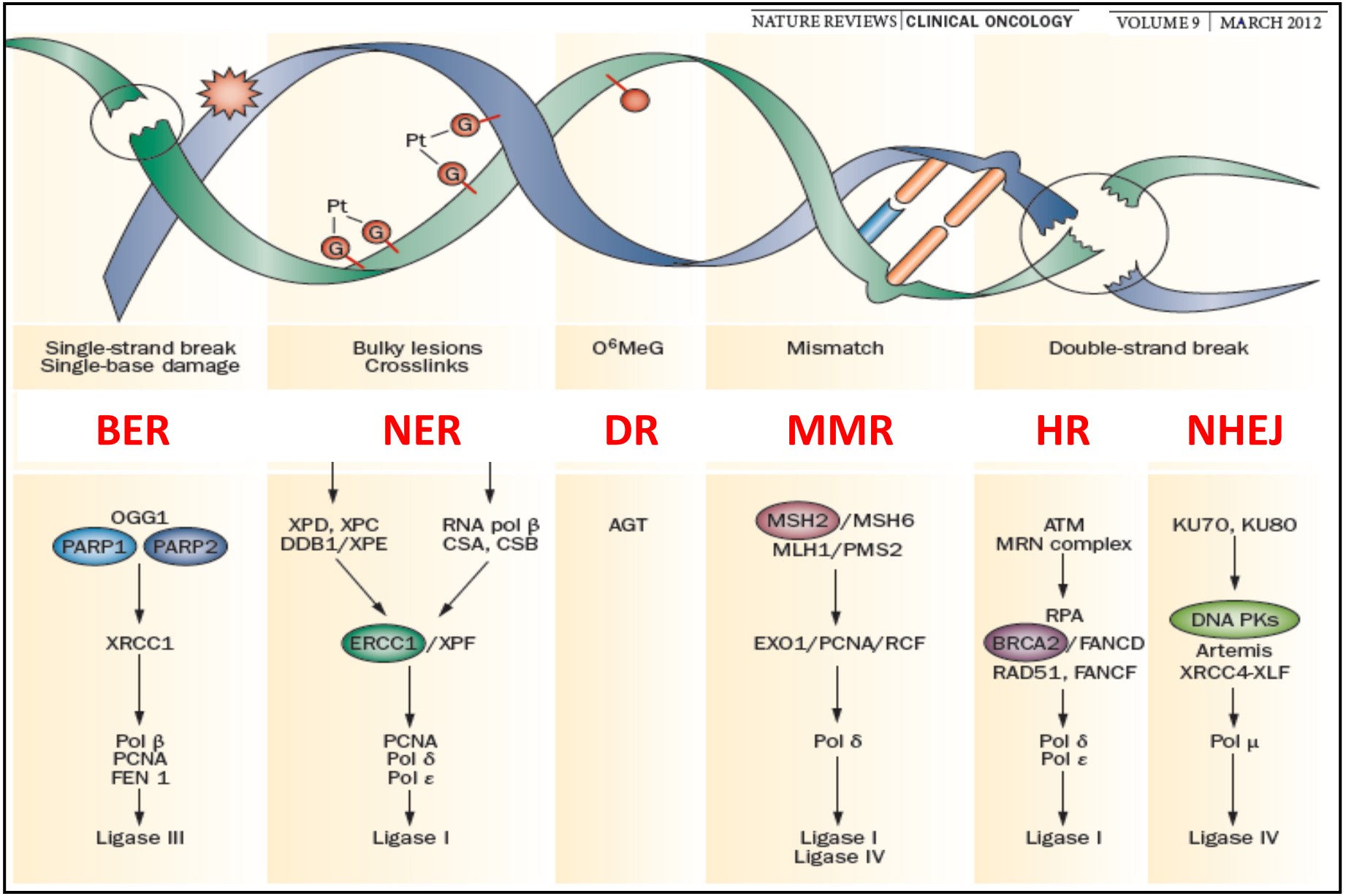
Compare two people – have about ~ 6 million SNP variants!

Toxic agents in our environment
Gene-Environment Interaction

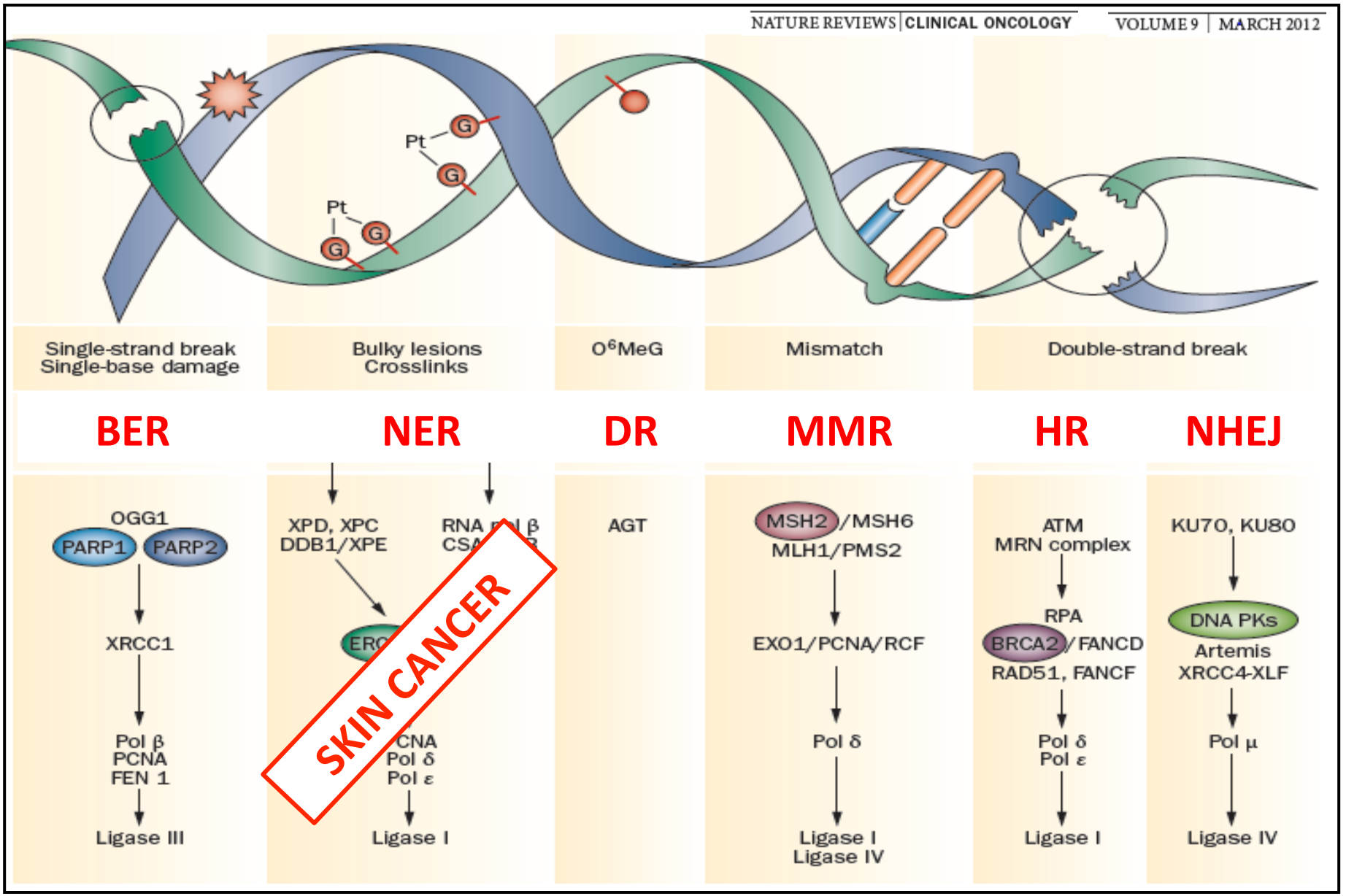


Time/Age/Behavior

Six Major DNA Repair Pathways

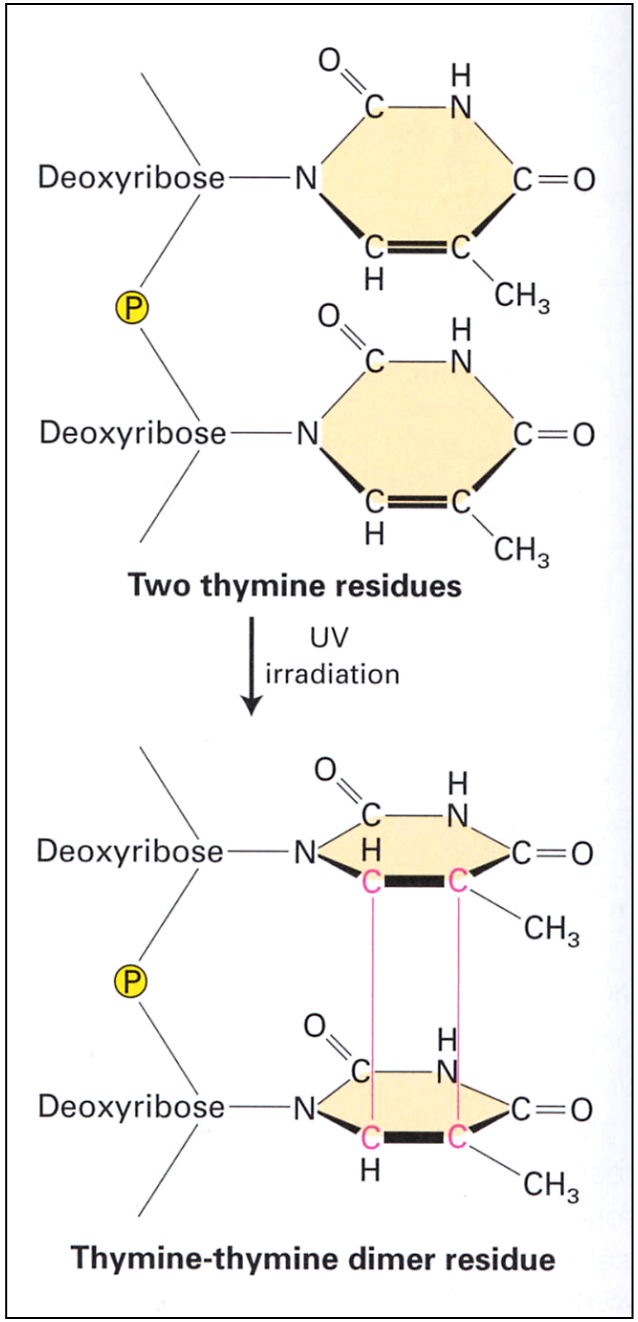


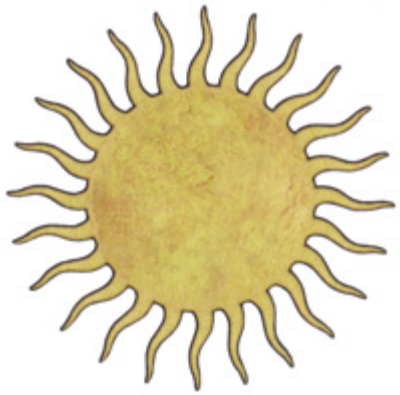
Six Major DNA Repair Pathways



What are the known risk factors for Skin Cancer?

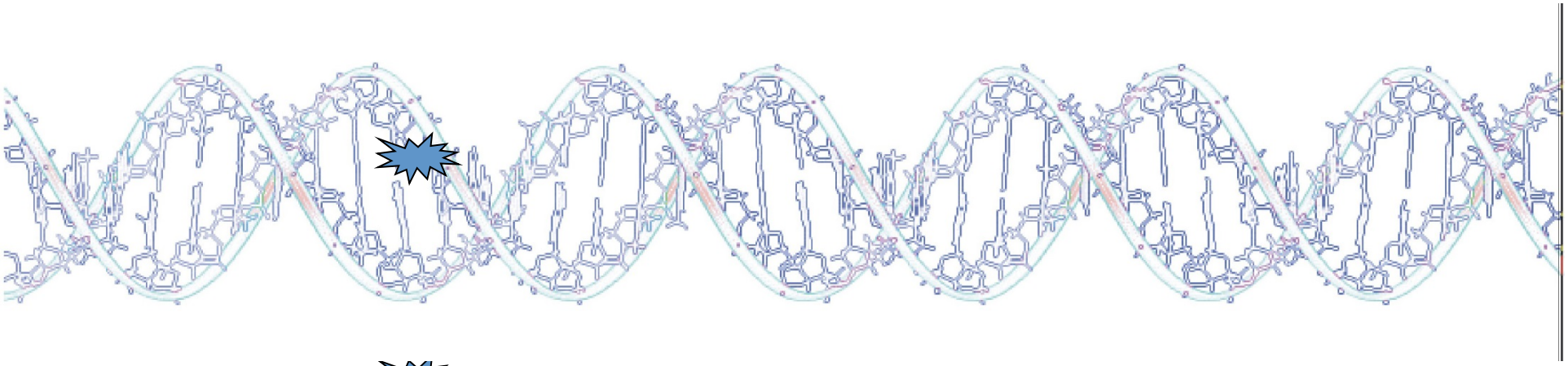
What are the known risk factors for Skin Cancer?

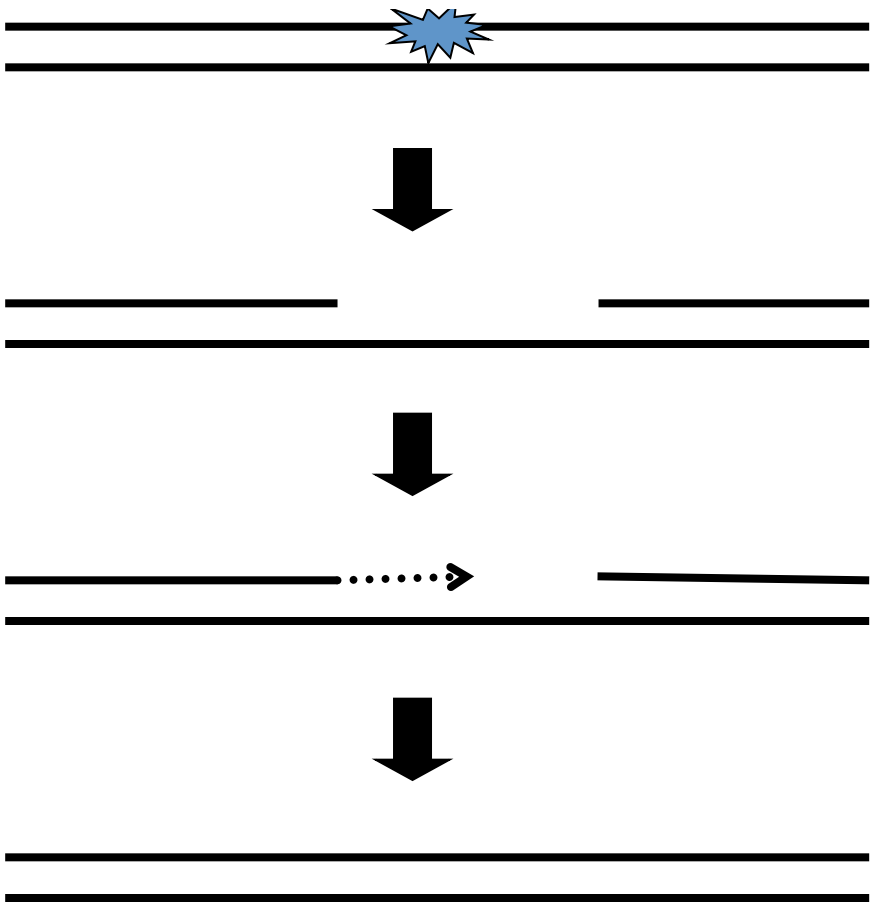




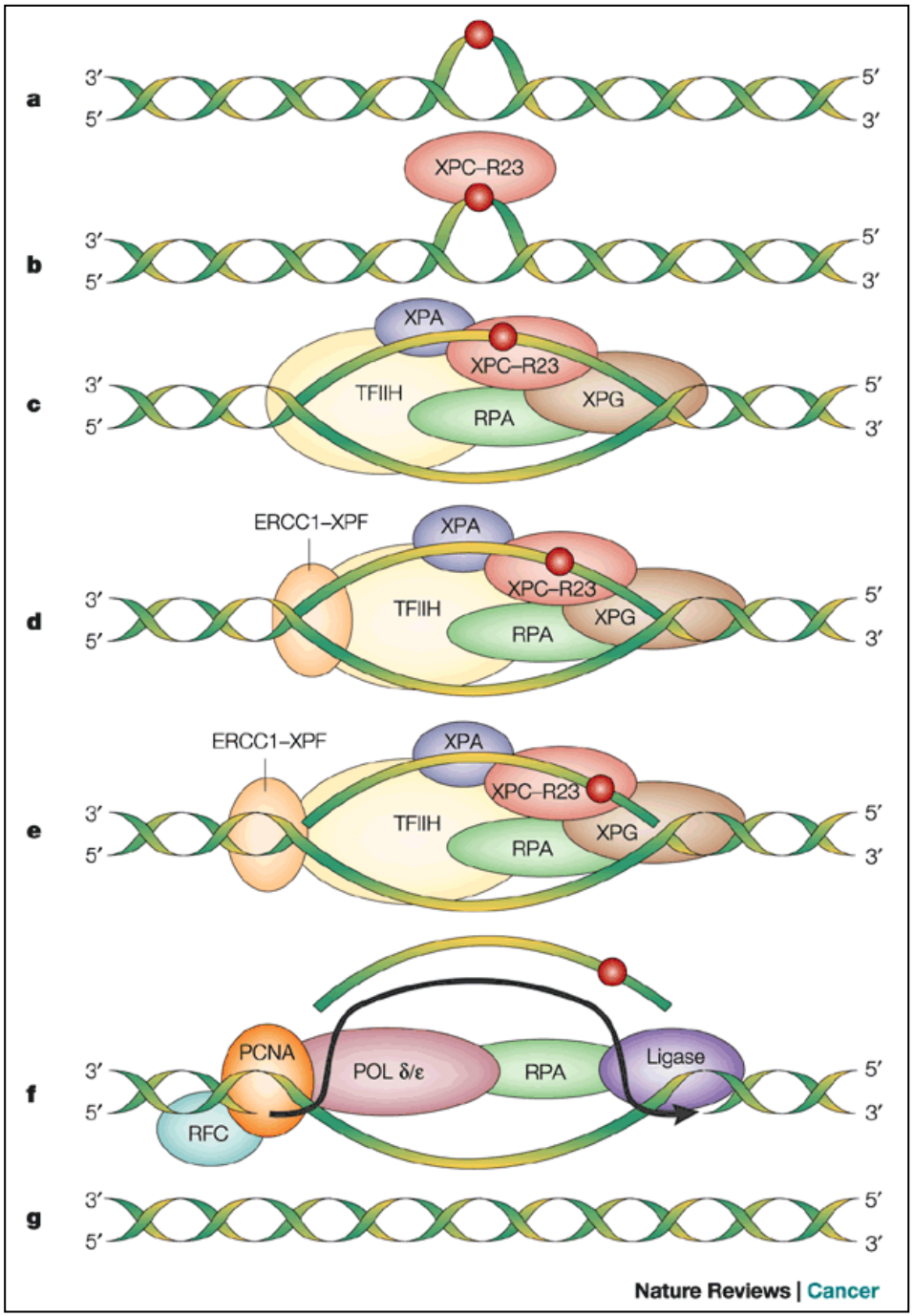
Before

After





Nucleotide Excision Repair



Nucleotide Excision Repair Proteins

XPA
XPB
XPC
XPD
XPE
XPF
XPG

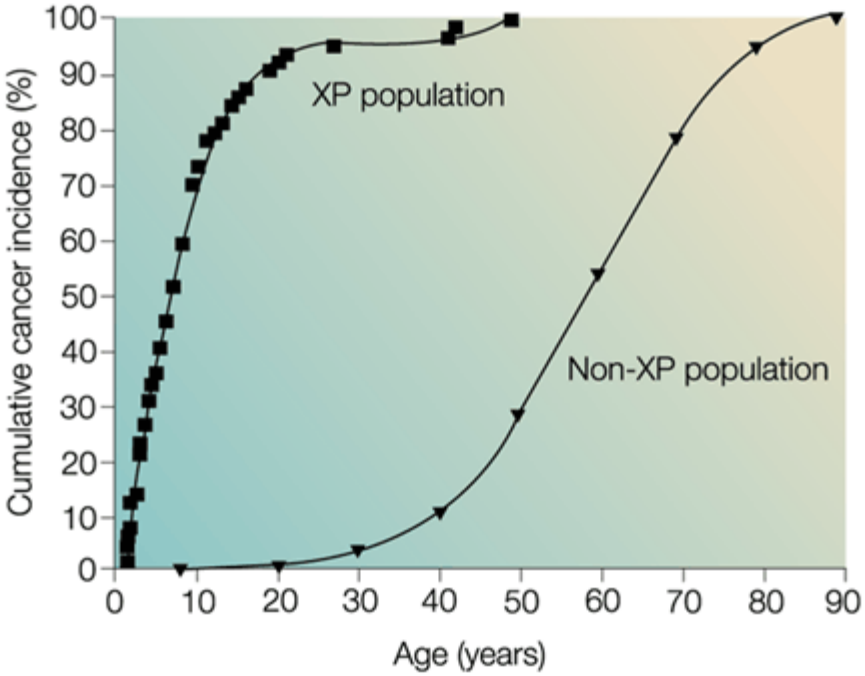
Errol C. Friedberg
Nature Reviews Cancer 1, 22-33 (2001)

Xeroderma Pigmentosum

Grossly Deficient in Nucleotide Excision Repair

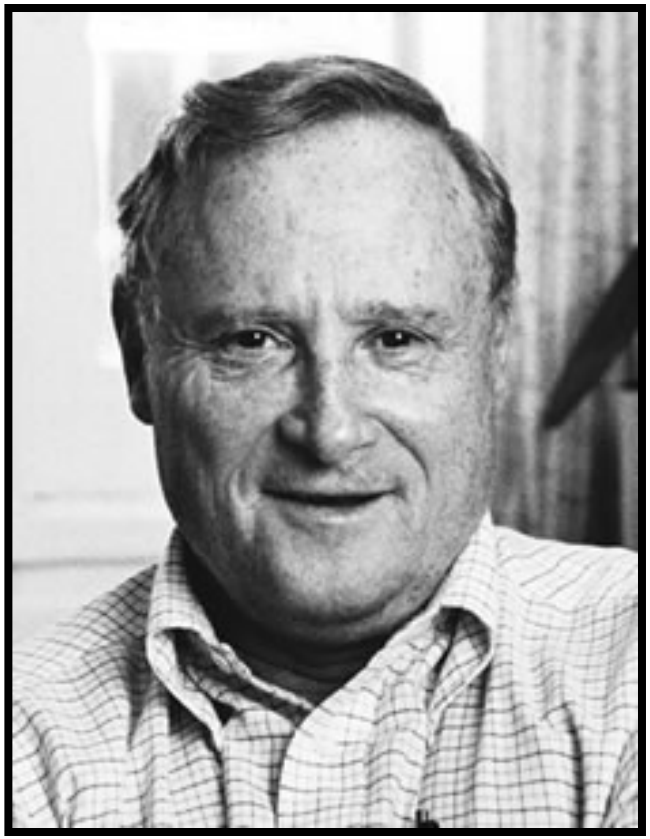
2000-fold increased risk of skin cancer

Lack of DNA repair accelerates the onset of cancer

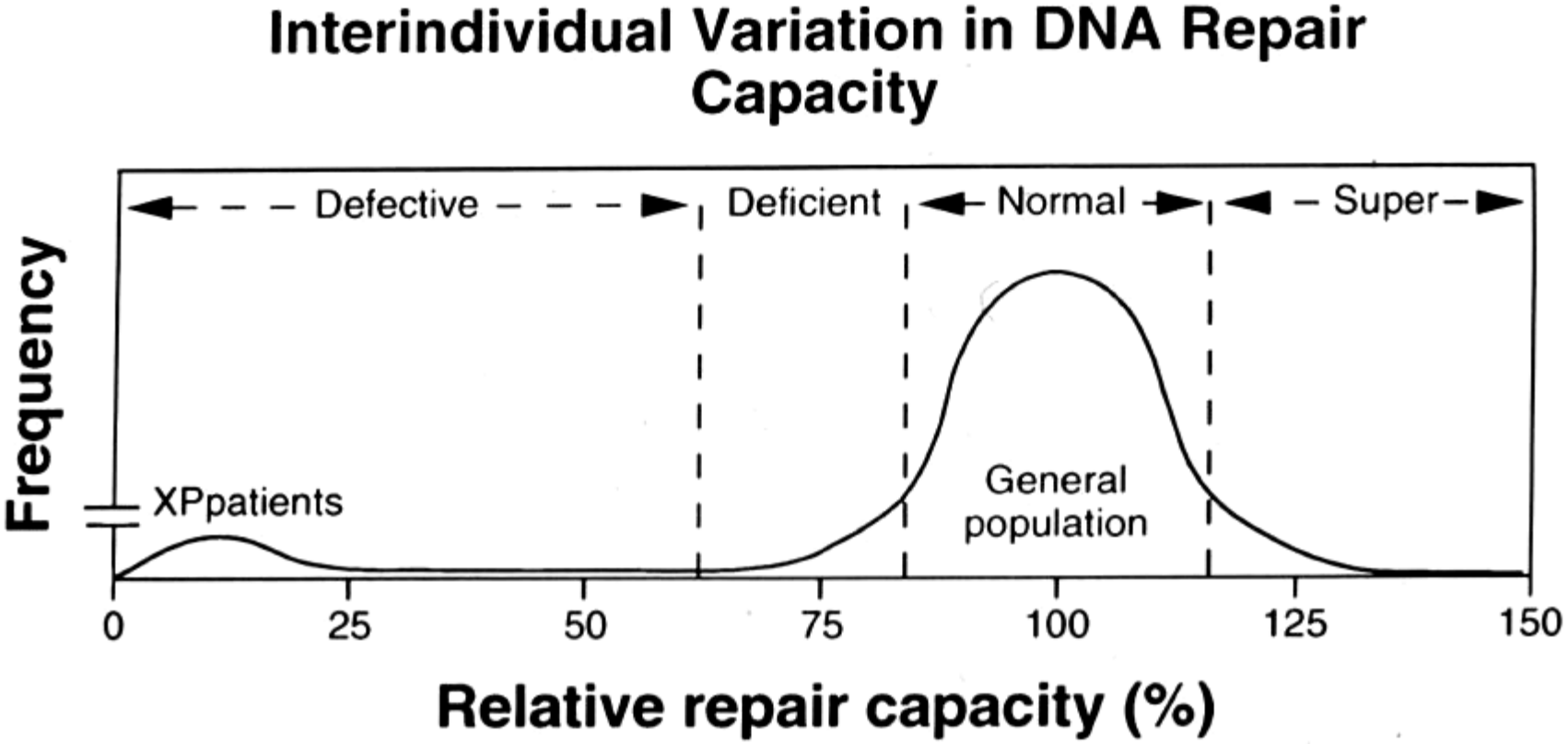


Nature Reviews |

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



Dr. Lawrence Grossman
(1924–2006)

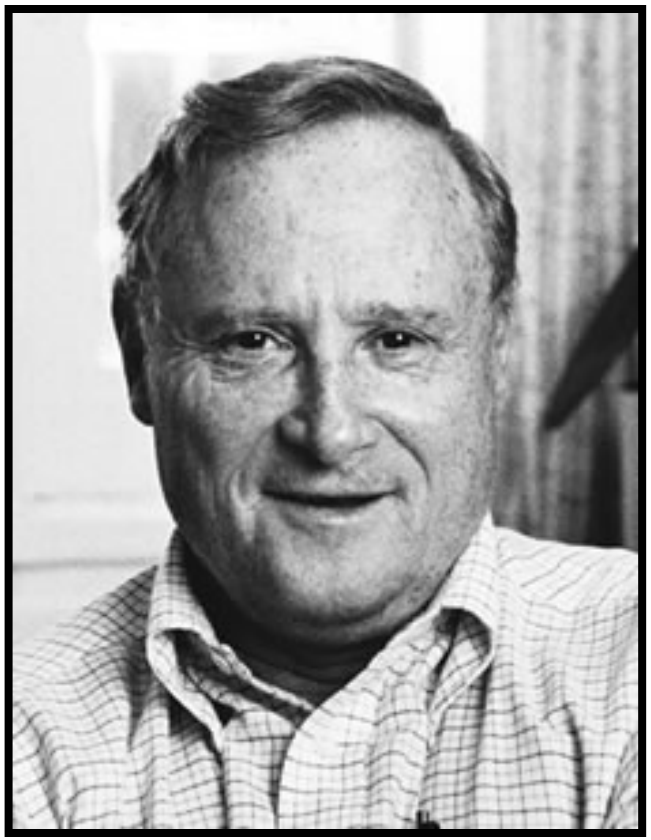


Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving a theoretical maximum of ~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:

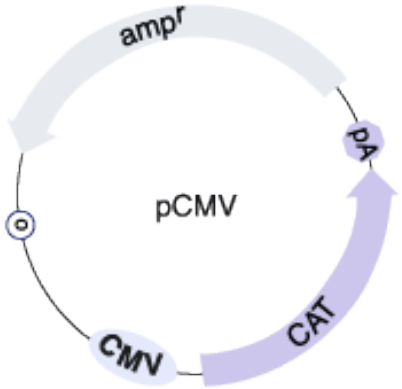


Dr. Lawrence Grossman
(1924–2006)

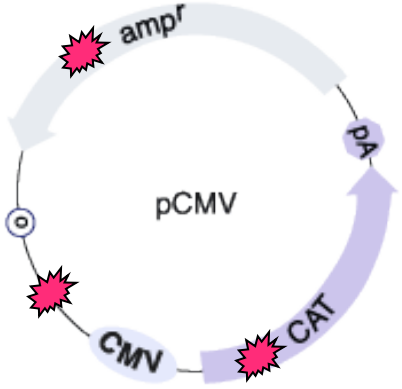
Dr. Qingyi Wei

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

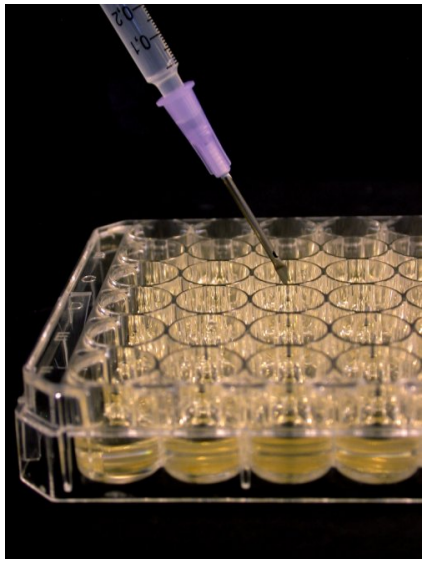
Athas & GROSSMAN
Cancer Res. 1991

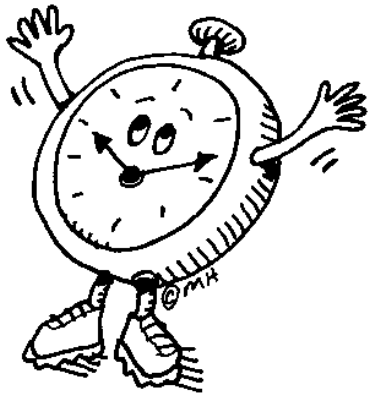


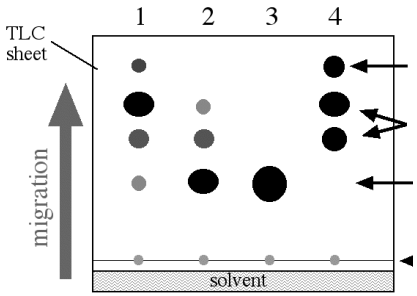
+ UV light



Transient transfection peripheral blood lymphocytes



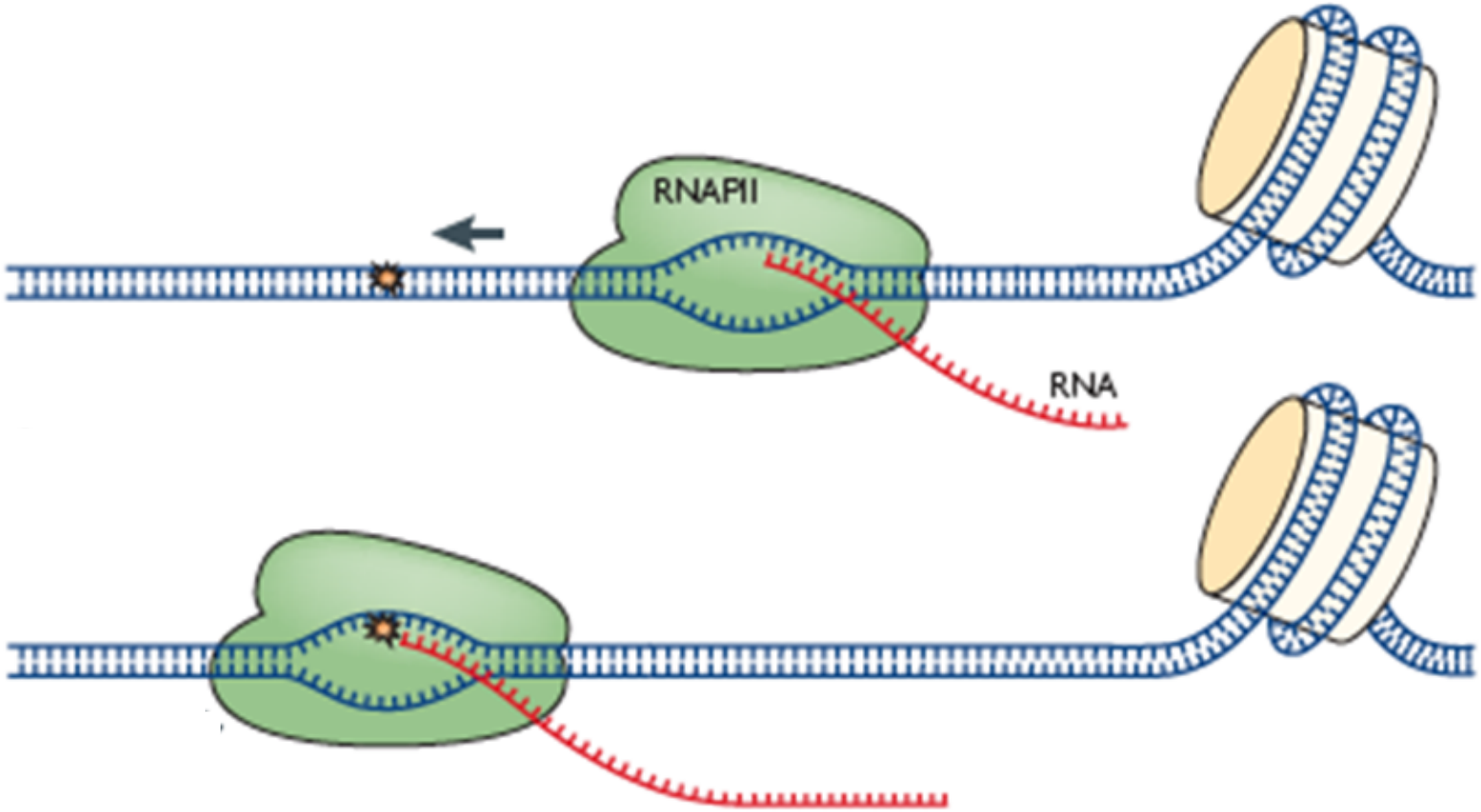




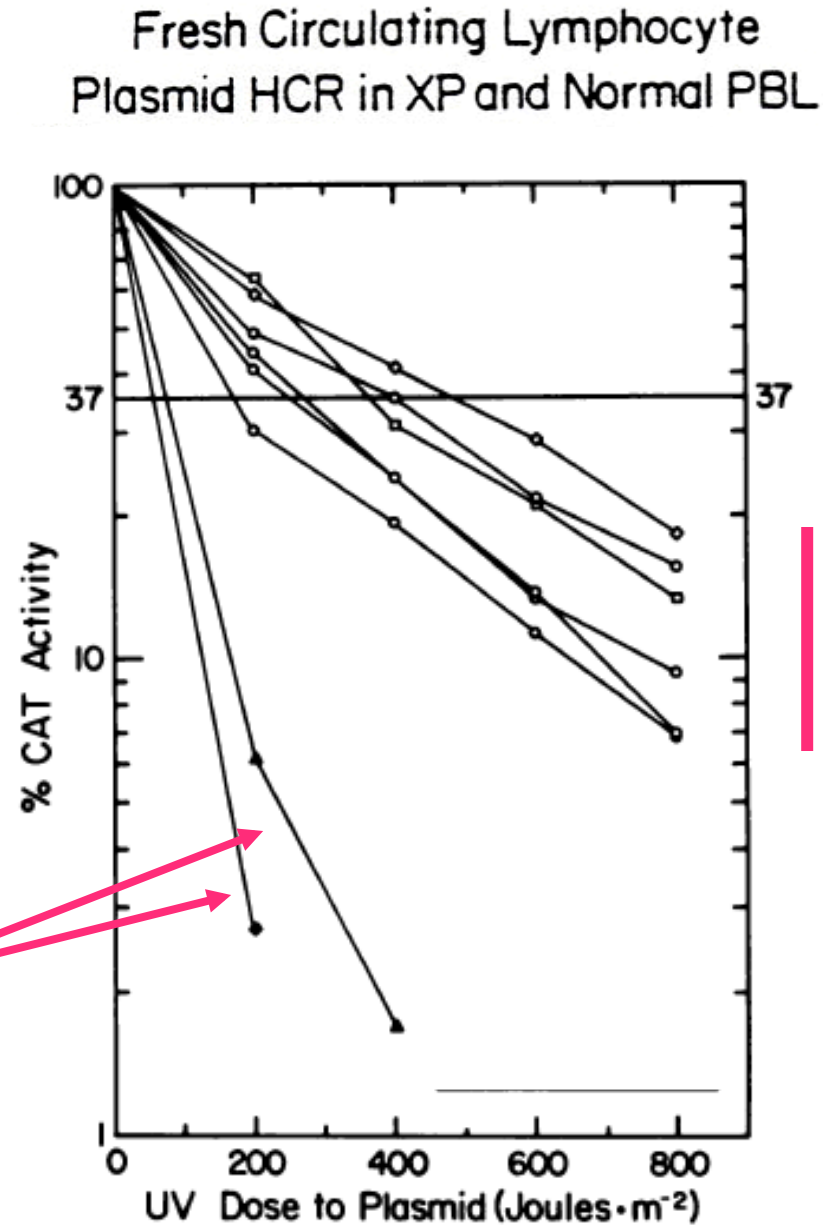
CAT Assay

Time to repair

RNA Polymerase II is exquisitely sensitive to DNA lesions



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

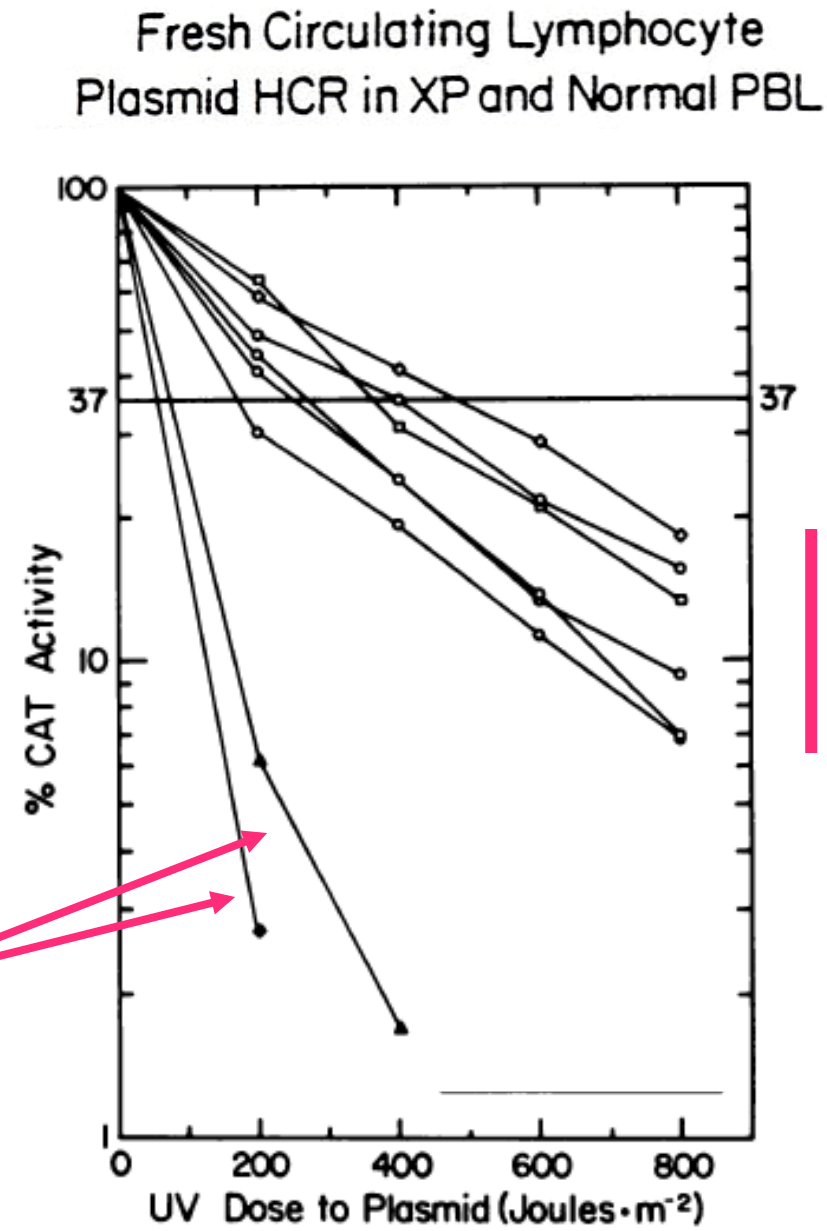


Cells from ‘healthy” people

Cells from XP patients

Athas & GROSSMAN

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



Relatively HIGH repair

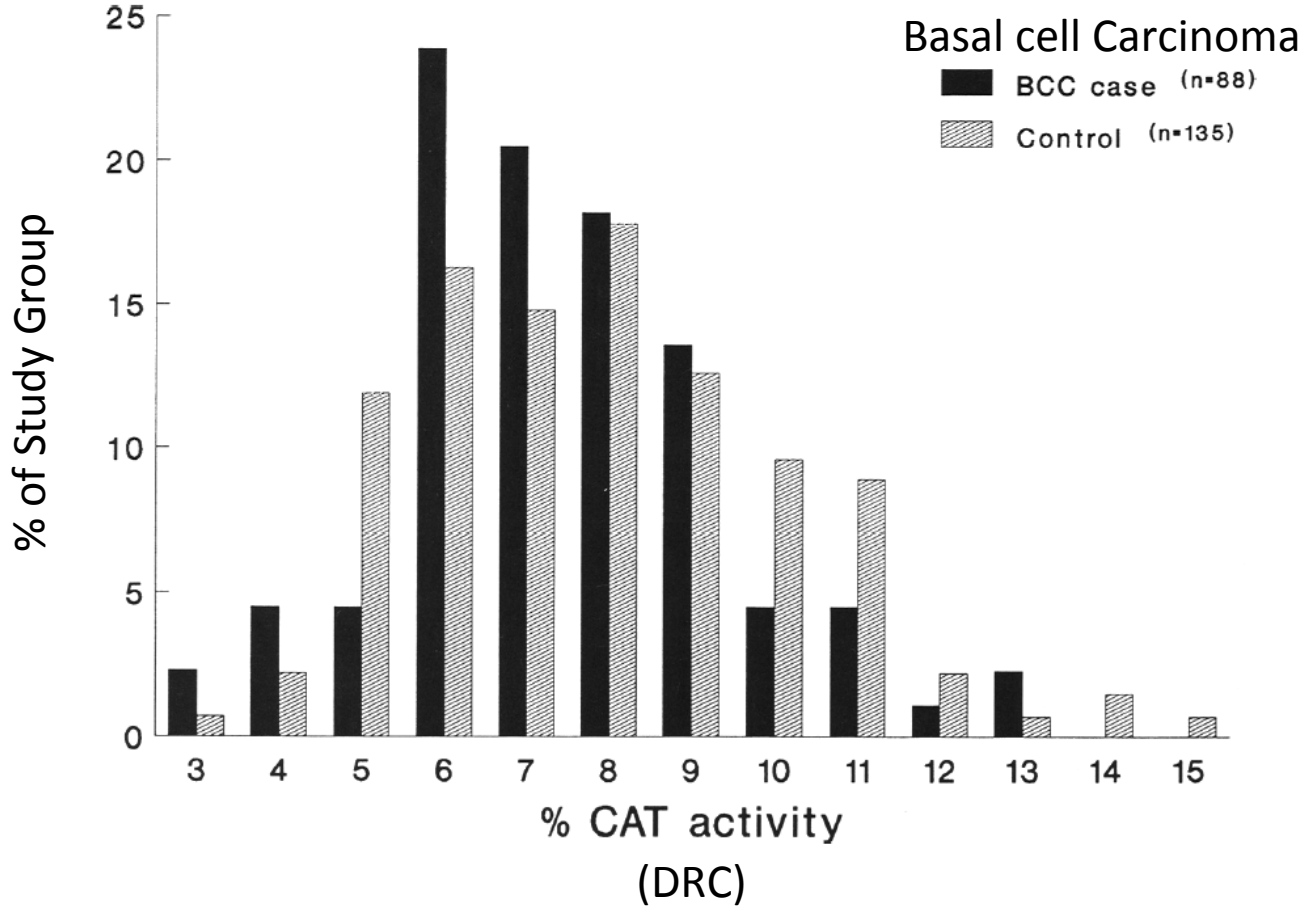
Relatively LOW repair

Cells from XP patients

Athas & GROSSMAN

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

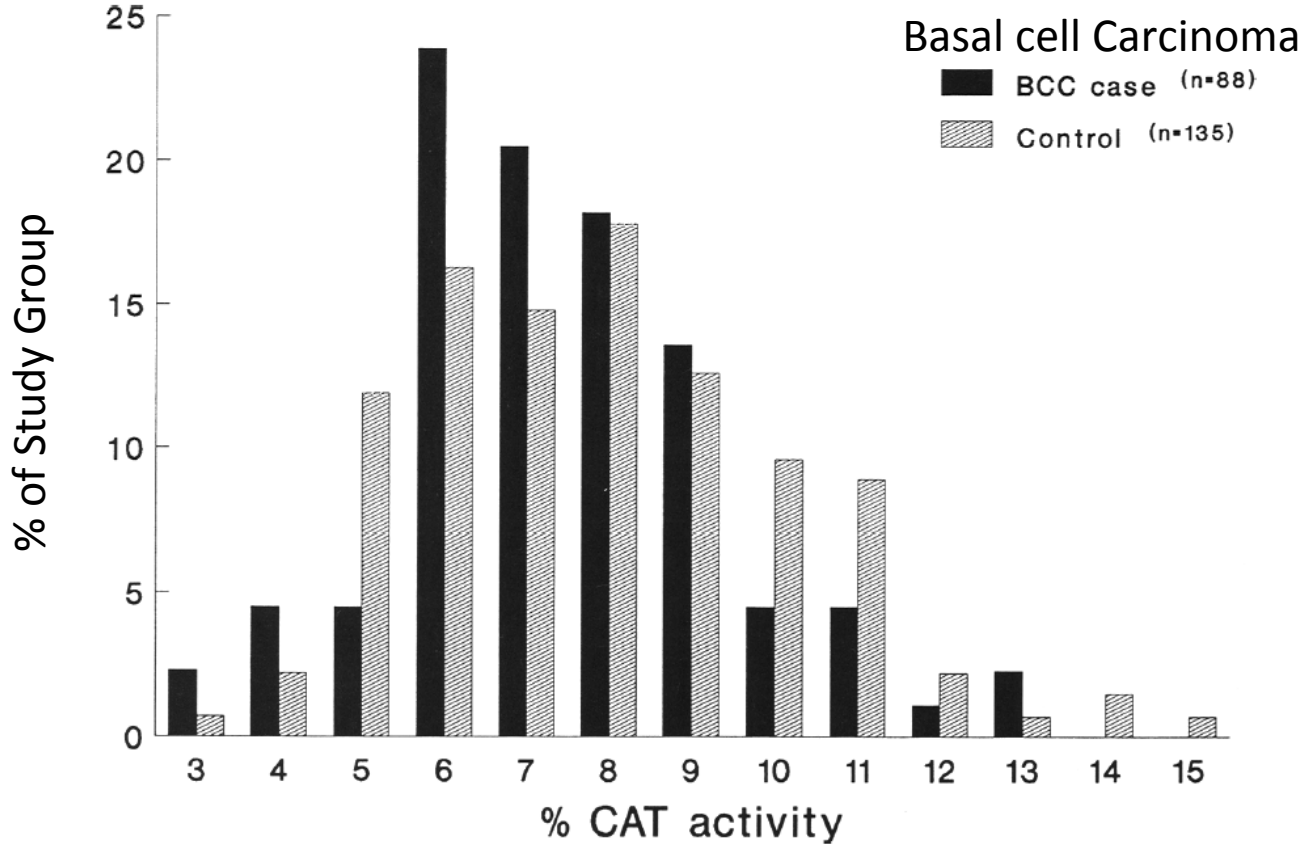
Case-Control Study monitoring DNA Repair Capacity (DRC) by Host Cell Reactivation (HCR) of plasmids containing DNA damage



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

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

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

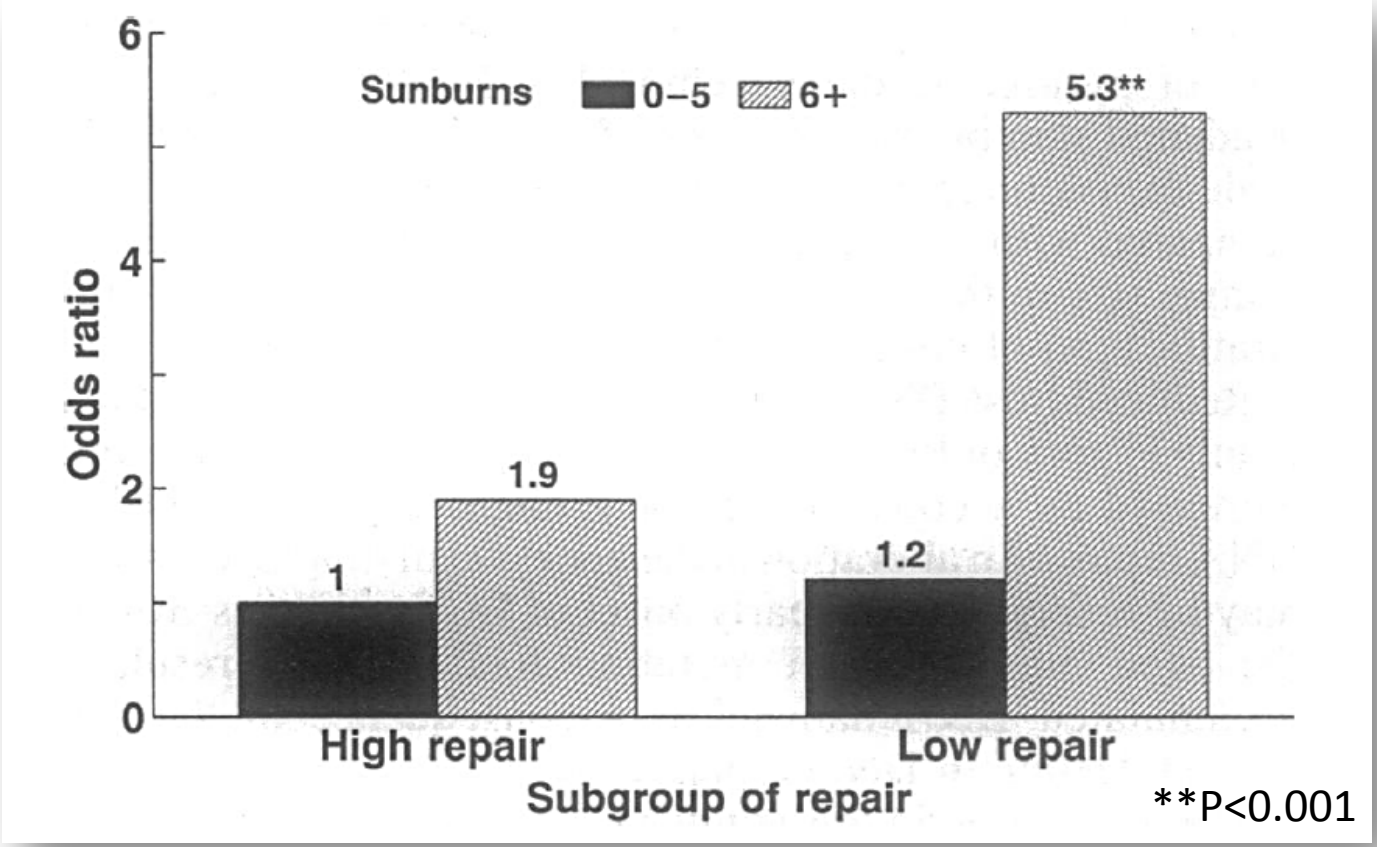


LOW Repair HIGH Repair

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

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

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?

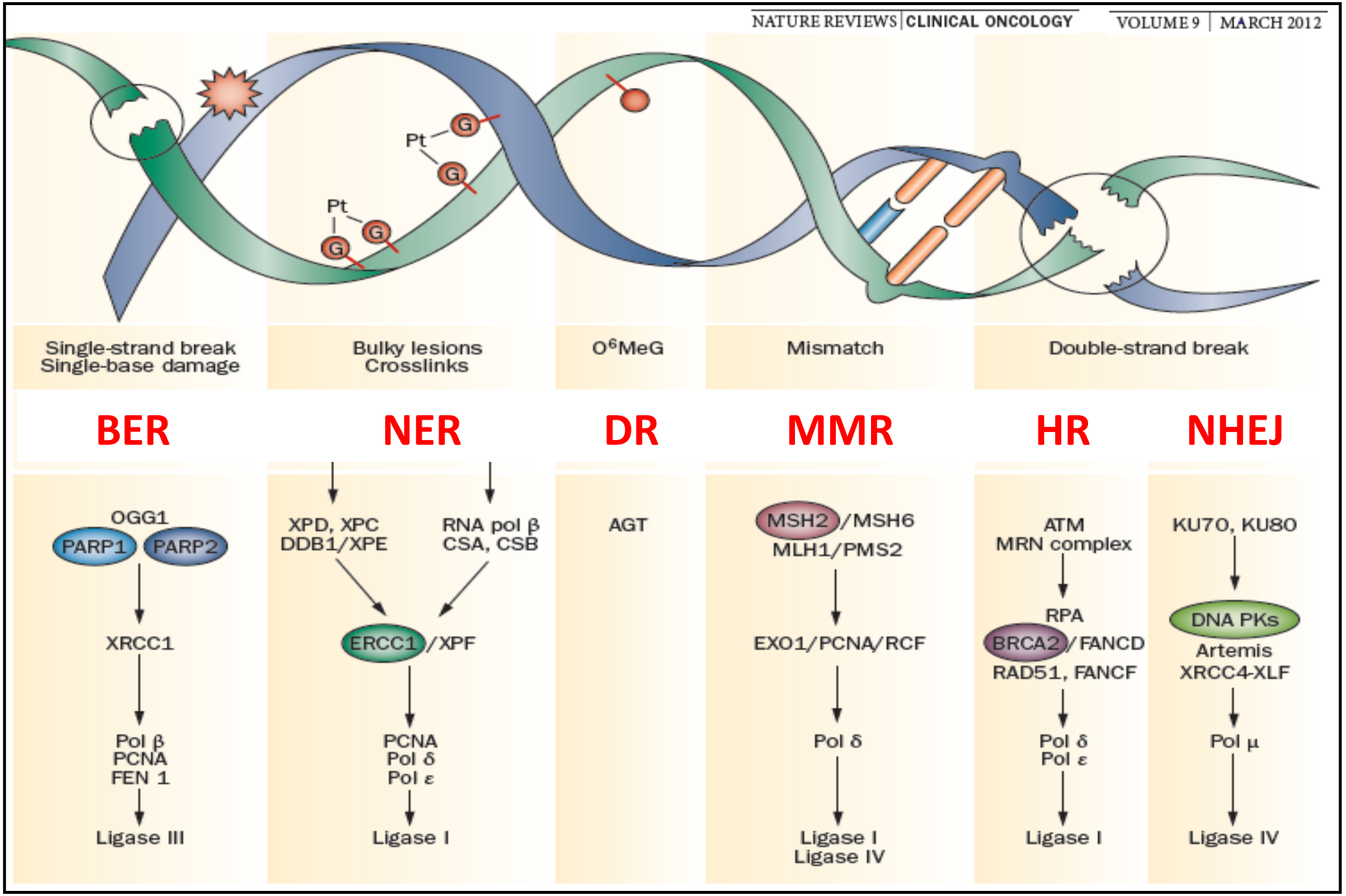
How does DNA repair affect the ability of cancer chemotherapy drugs to kill cancer cells?

How does cancer chemotherapy affect gene expression?

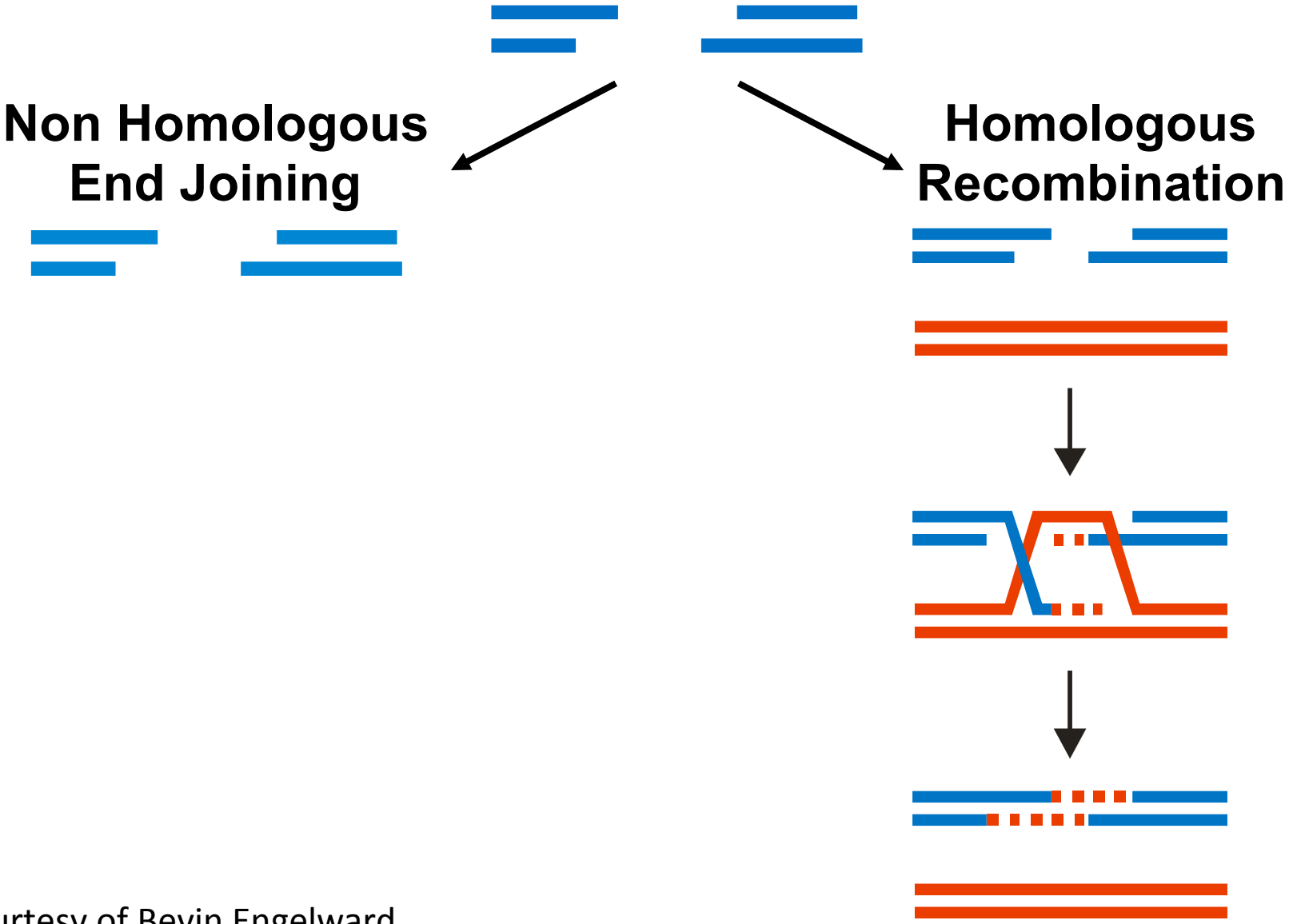
This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?

Six Major DNA Repair Pathways



DNA double-strand break repair

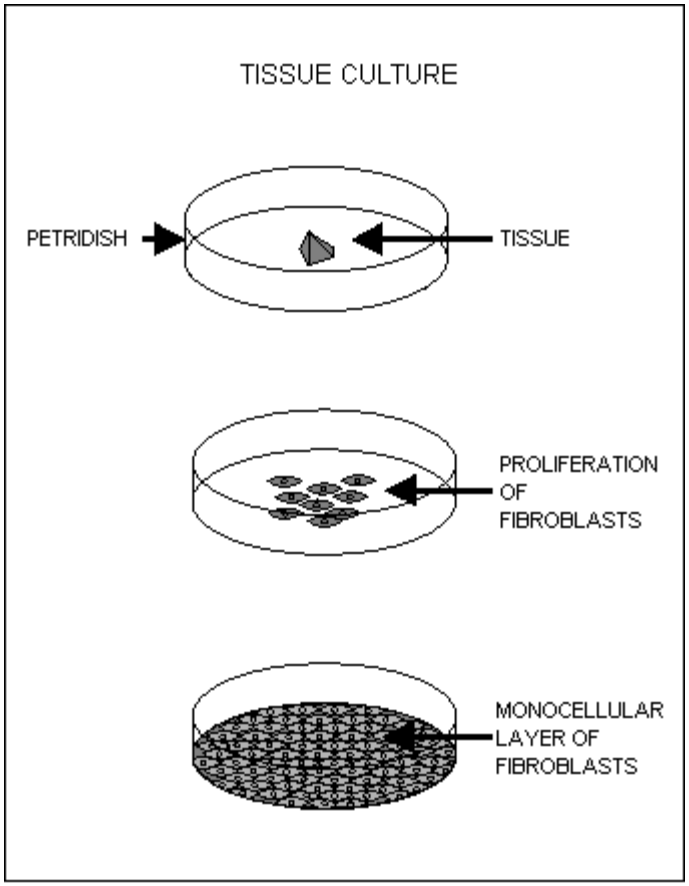


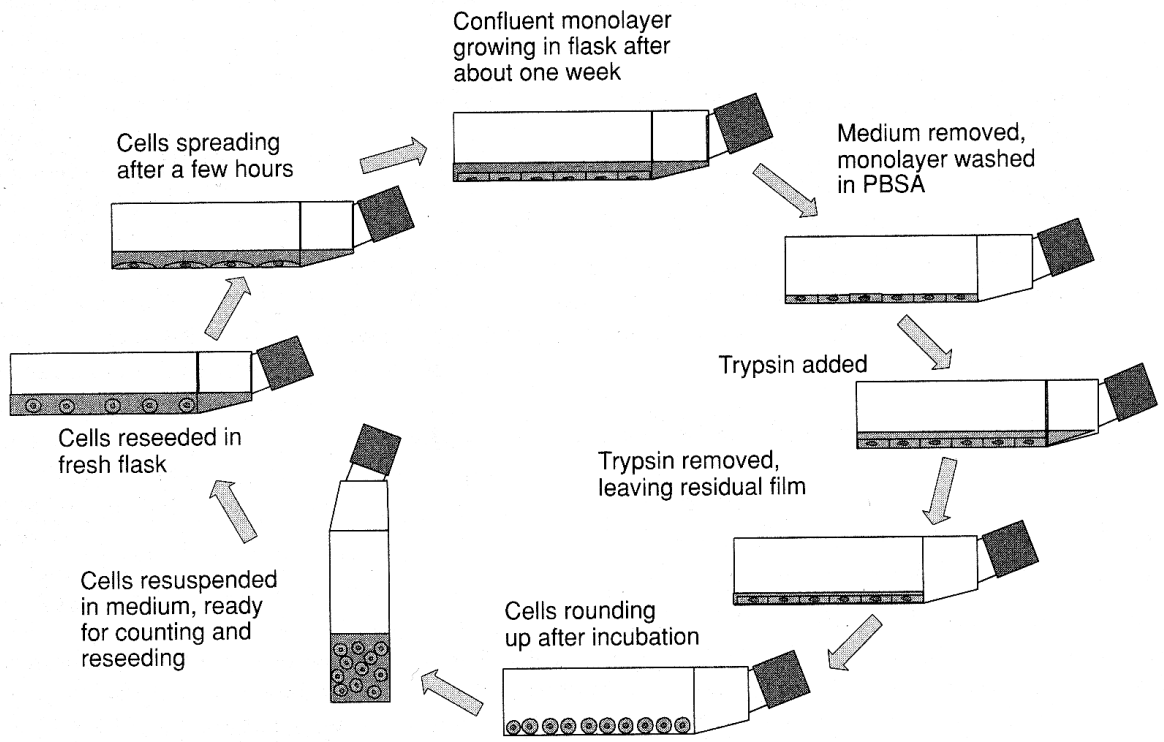
Courtesy of Bevin Engelward

Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

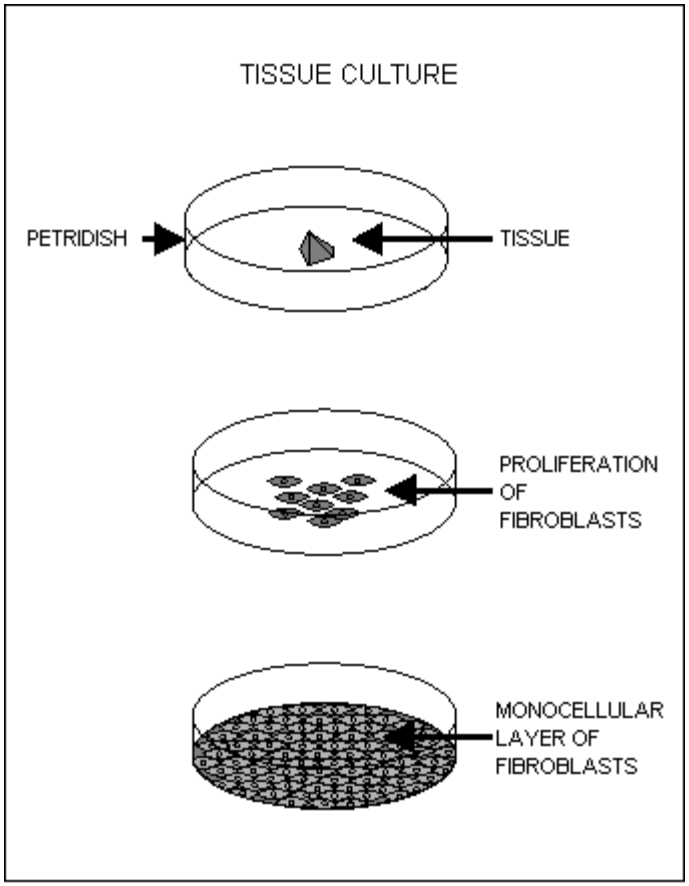
How do you grow mammalian cells?

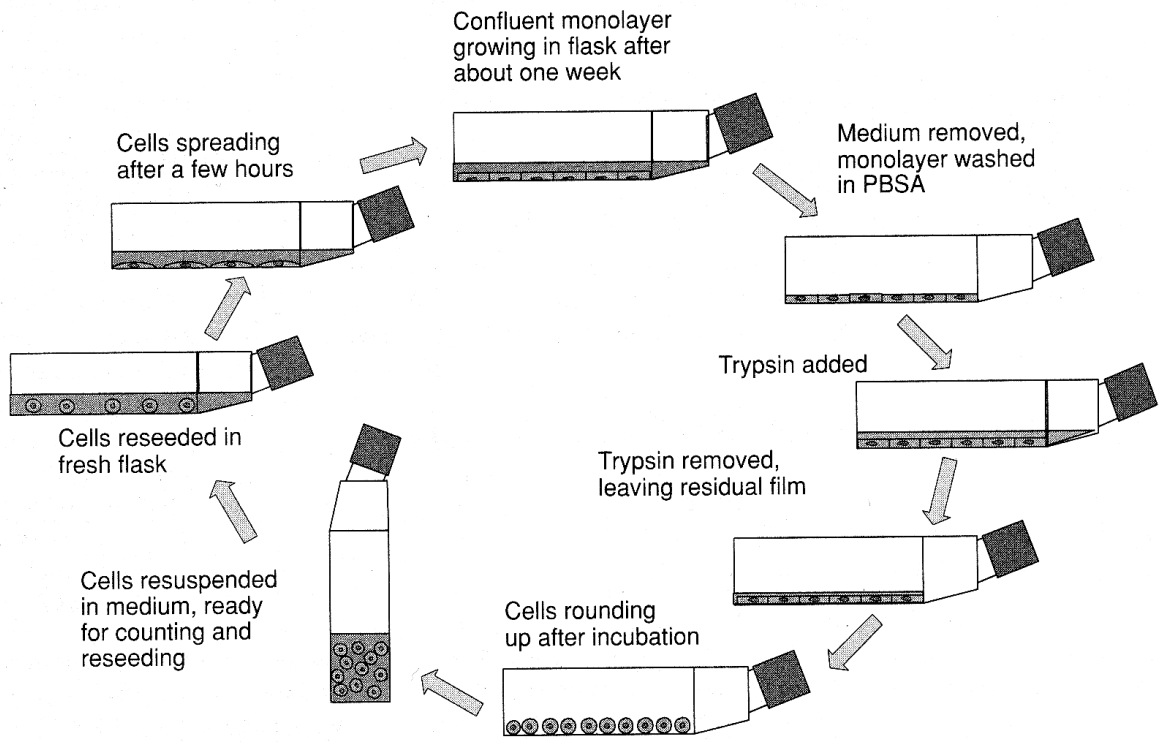




From Freshney's "Culture of Mammalian Cells"

How do you grow mammalian cells?

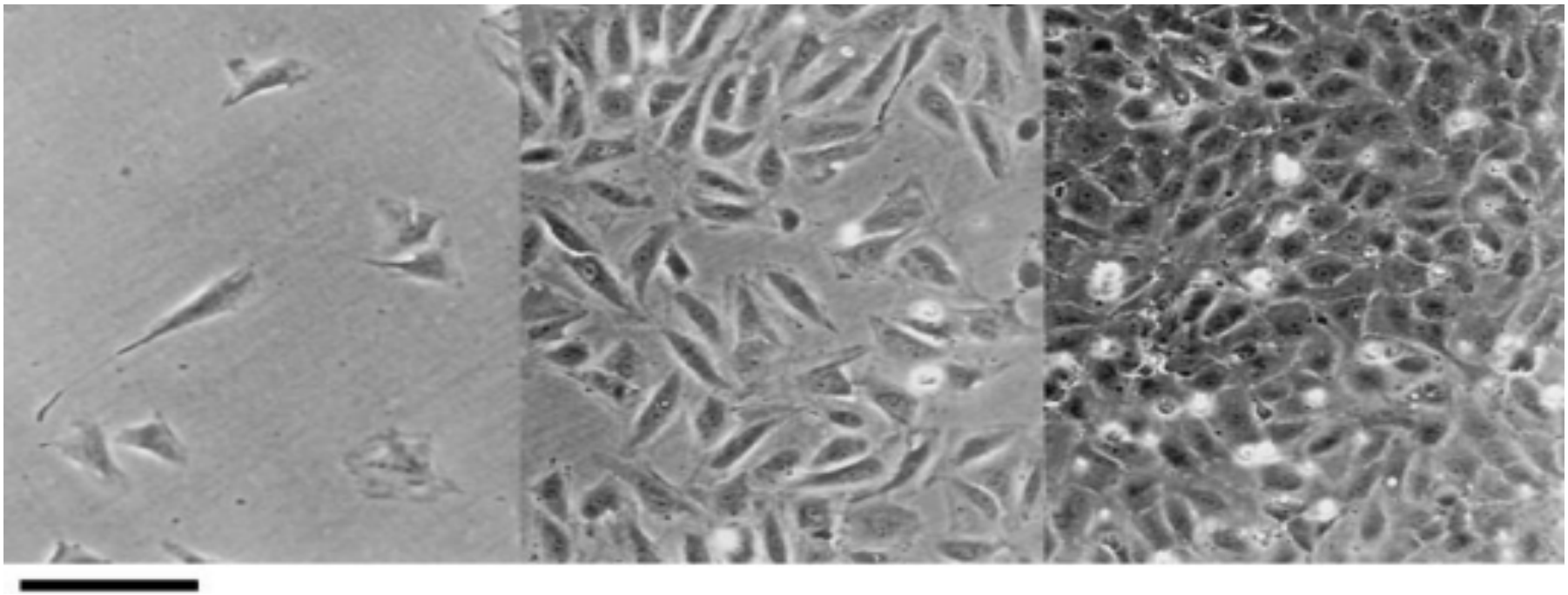




"Sub-Culturing"

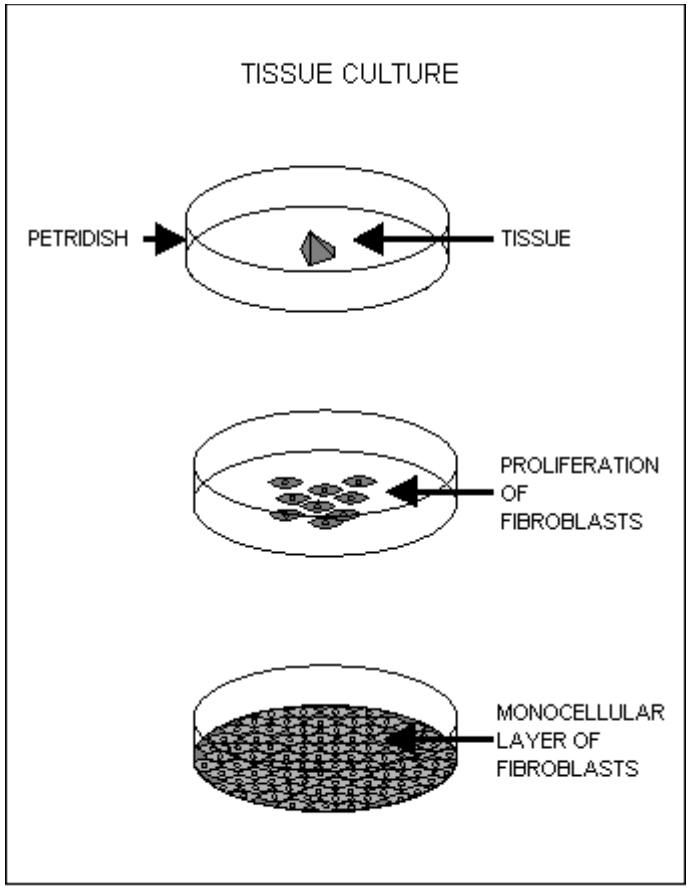
From Freshney's "Culture of Mammalian Cells"

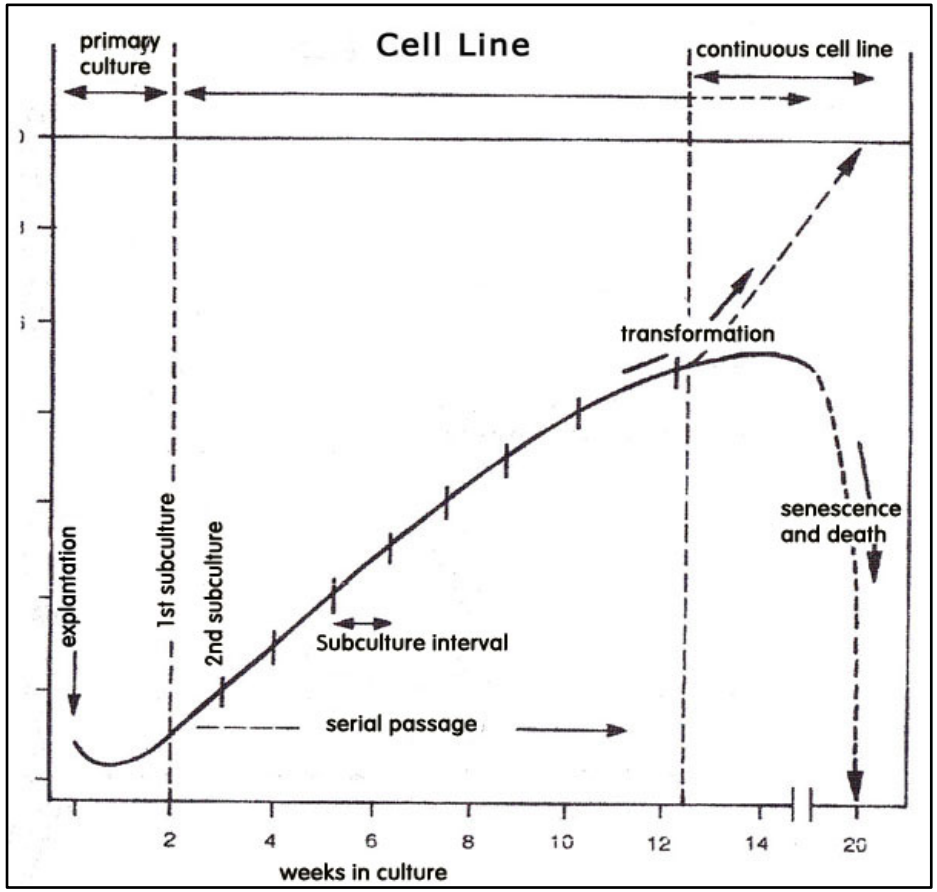
How do you grow mammalian cells?



Just seeded Growing Confluent

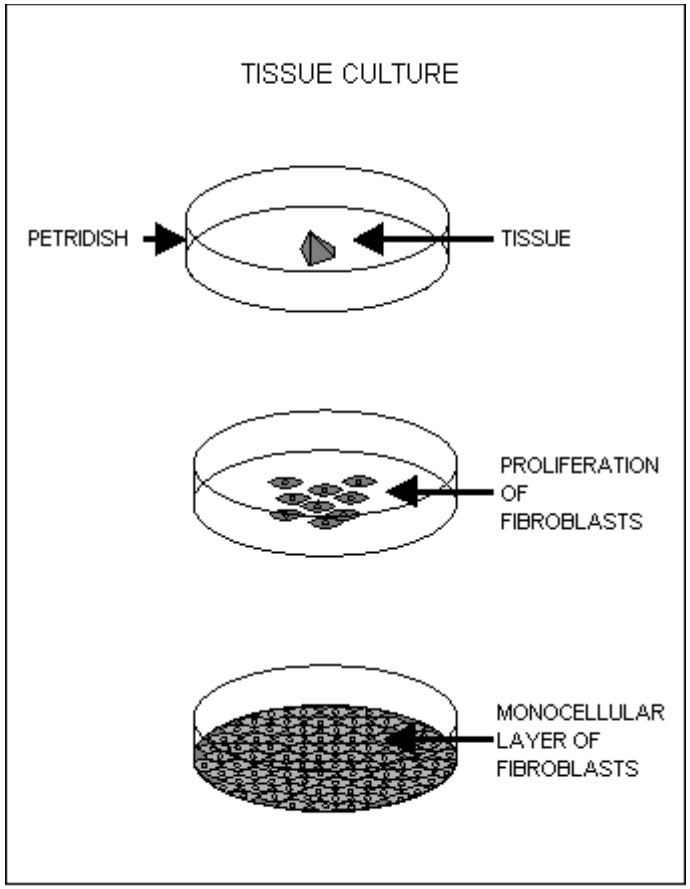
How do you grow mammalian cells?

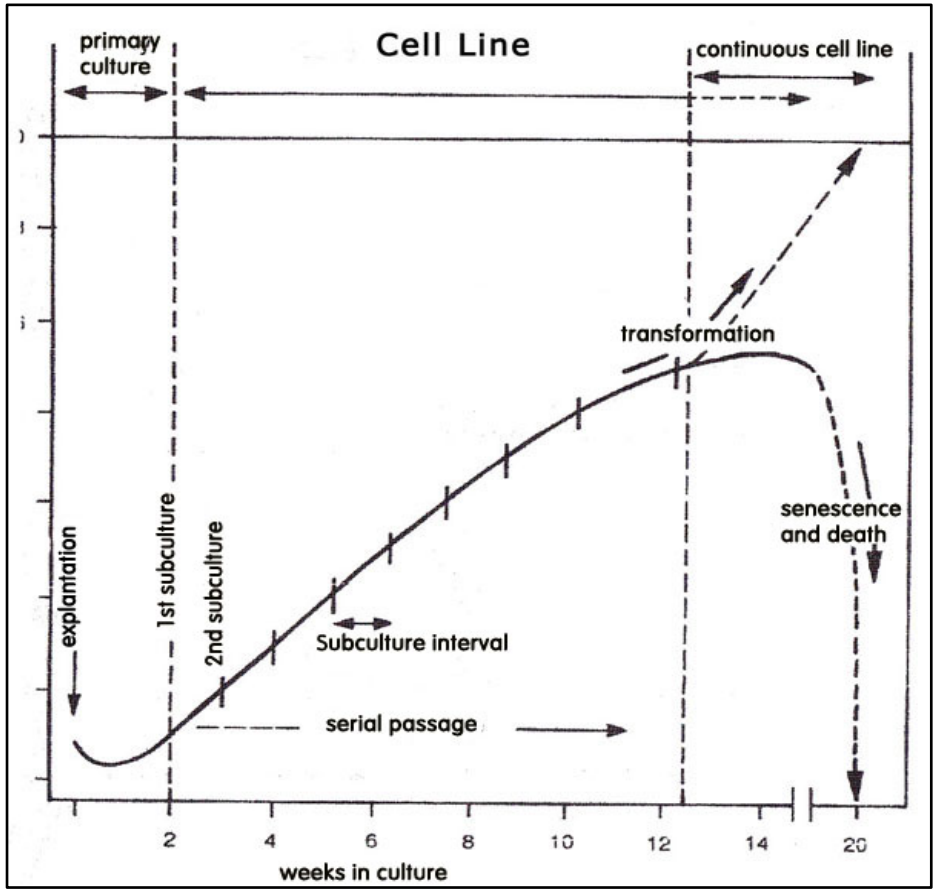




From Freshney's "Culture of Mammalian Cells"

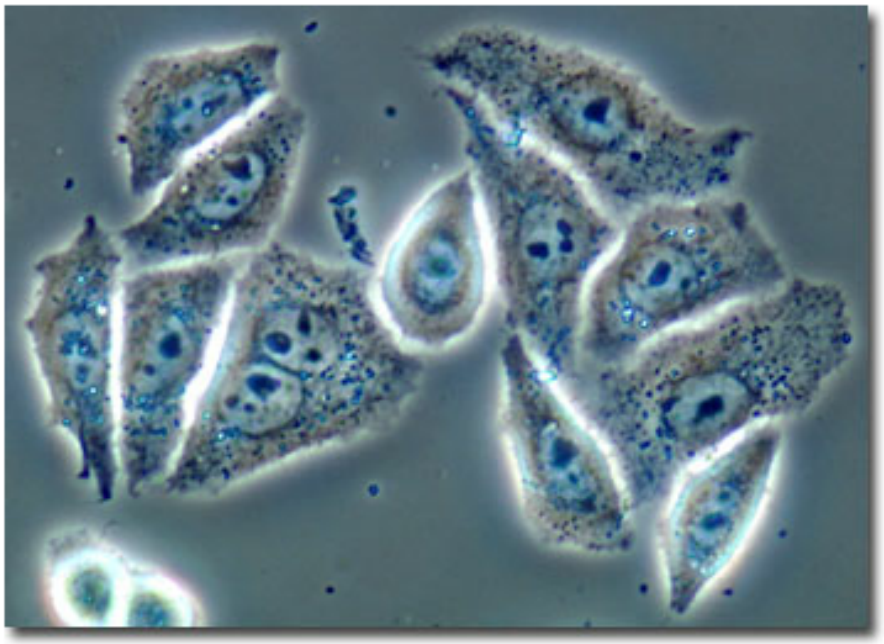
How do you grow mammalian cells?





This is for normal tissue, what about tumor tissue?

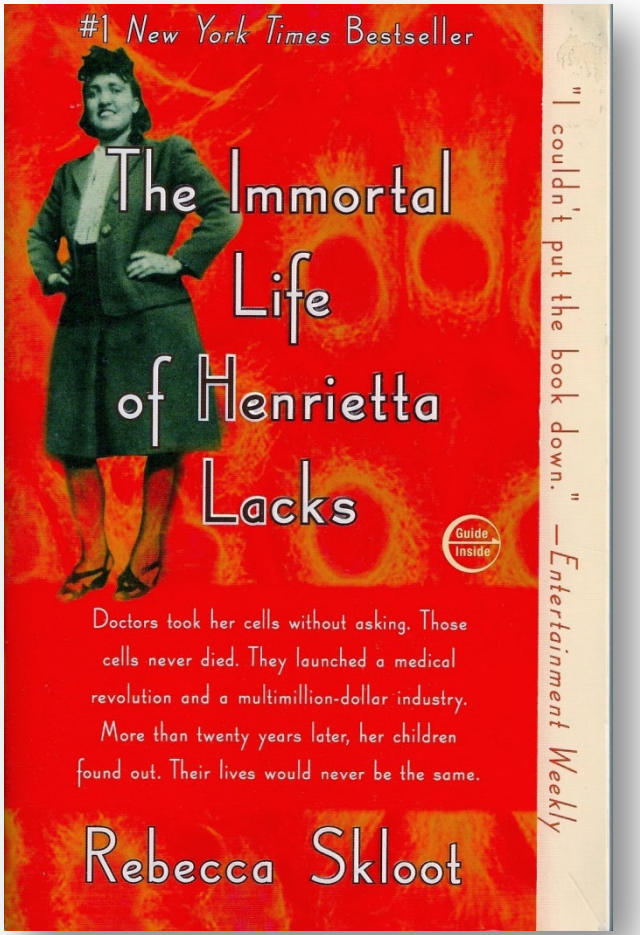
From Freshney's "Culture of Mammalian Cells"

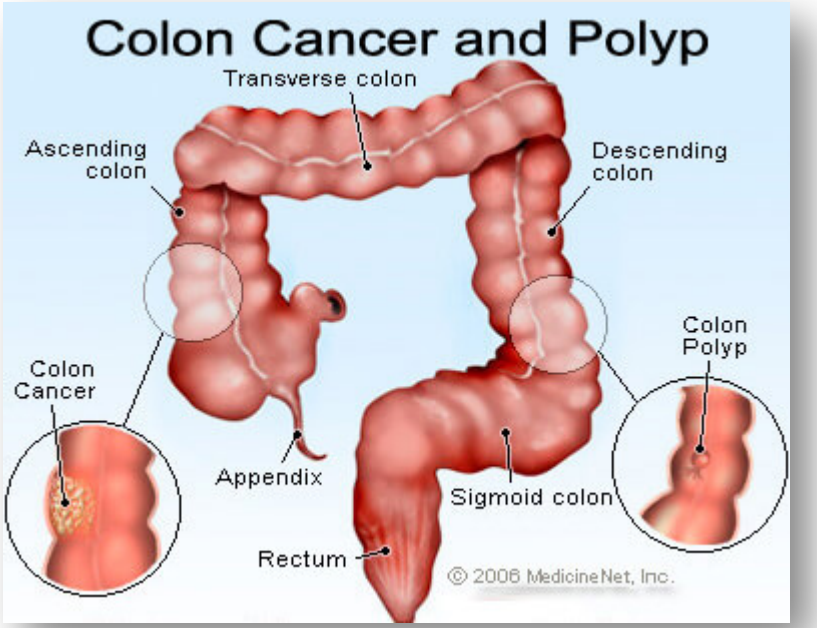


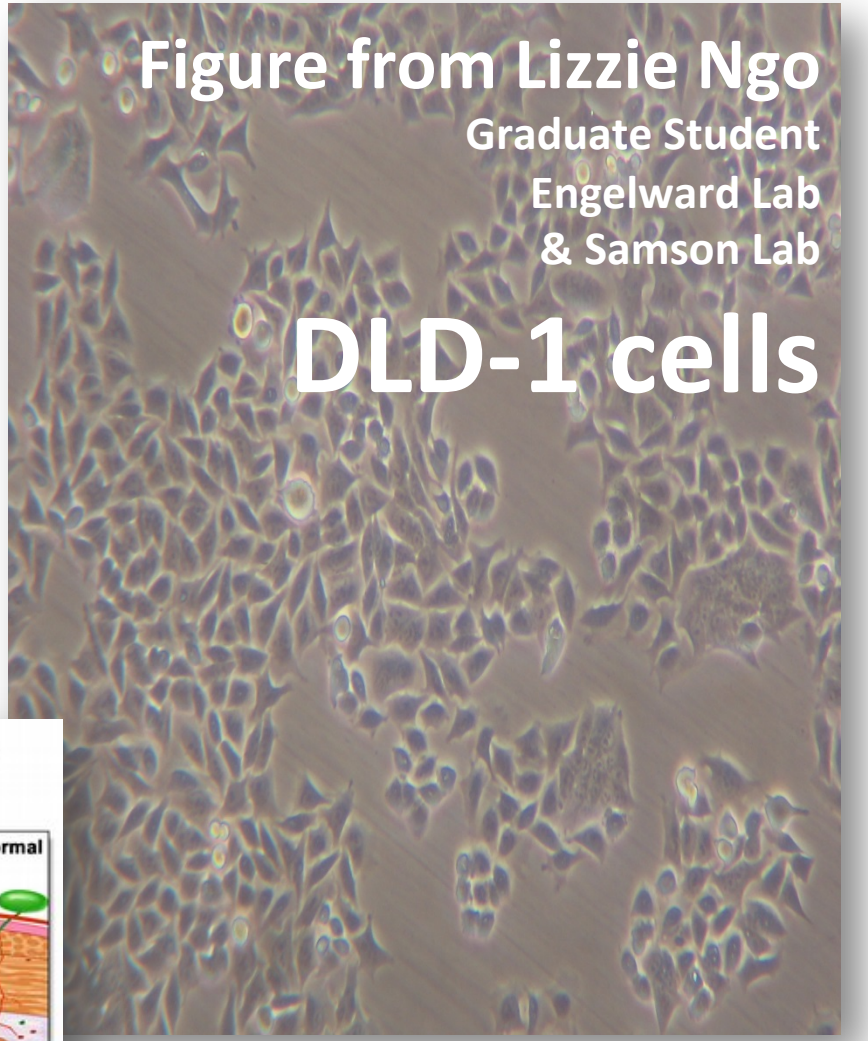
Phase Contrast

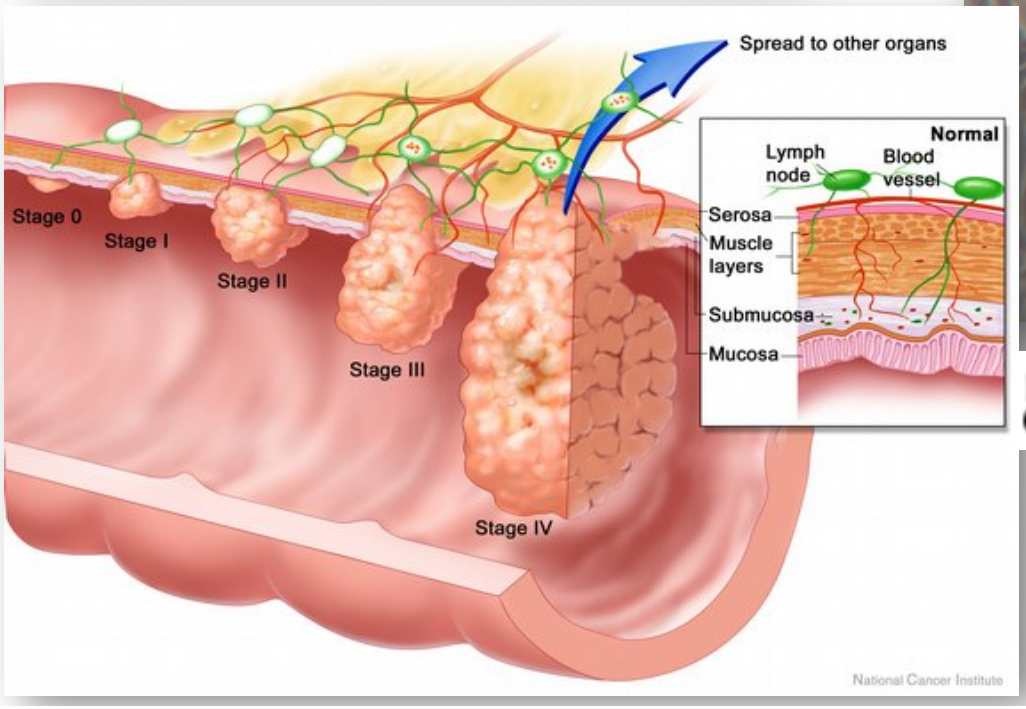
HeLa cells from the Nikon microscope web site

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.







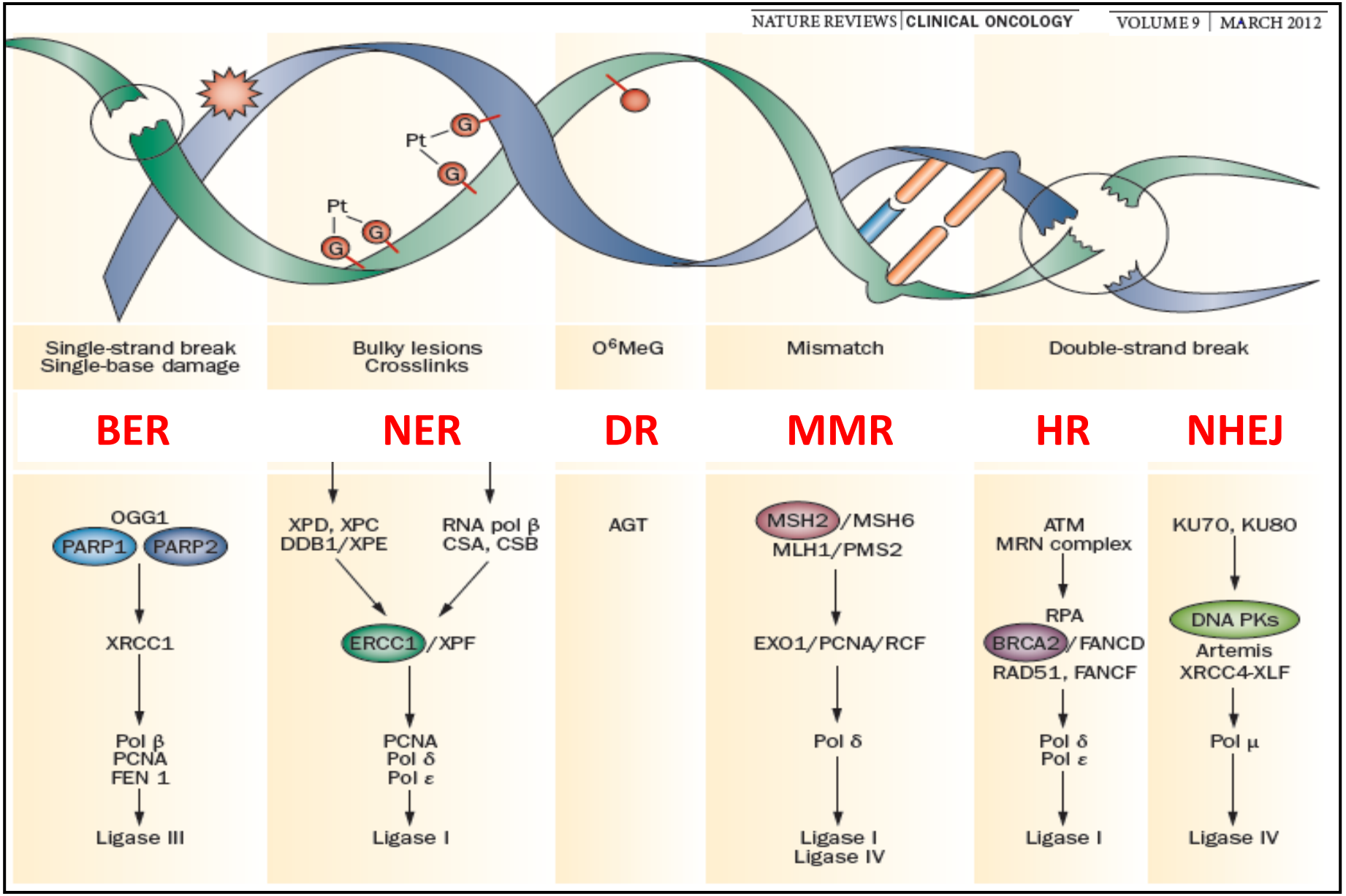


[CANCER RESEARCH 39, 1020–1025, March 1979]
0008-5472/79/0039-0000$02.00

https://www.google.com/search?q=colon+cancer&rlz=1T4GGHP_enUS635US636&source=lnms&tbm=isch&sa=X&ved=0ahUKEwj1hMLwrsfSAhWX0YMKHSFIB-EQ_AUICCgB&biw=1451&bih=669&dpr=1.75#imgrc=3X3SToZHyQtBfM:

https://www.google.com/search?q=colon+cancer&rlz=1T4GGHP_enUS635US636&source=lnms&tbm=isch&sa=X&ved=0ahUKEwj1hMLwrsfSAhWX0YMKHSFIB-EQ_AUICCgB&biw=1451&bih=669&dpr=1.75#imgrc=SVmB_b-jLkVYQM:

Six Major DNA Repair Pathways



Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

20.109 Spring 2017 Module 2 – Lecture 1
Gene Expression Engineering (March 9th 2017)




Noreen Lyell

Leslie McLain

Maxine Jonas

Rob Wilson

Leona Samson (Lectures)