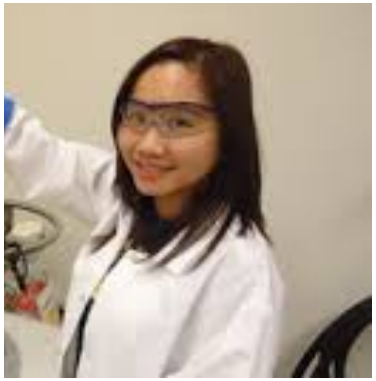
20.109 Spring 2015 Module 2 – Lecture 4
System Engineering and Protein Foundations

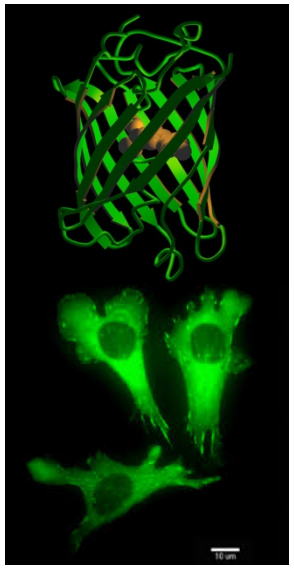


Shannon Hughes
Noreen Lyell
Leslie McLain
Nova Pishesha (TA)
Leona Samson (Lectures)

Zachary Nagel (help with development) Alex Chaim

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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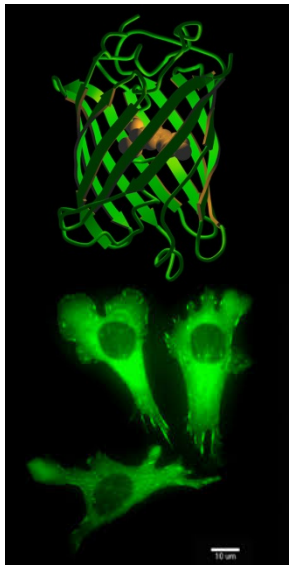

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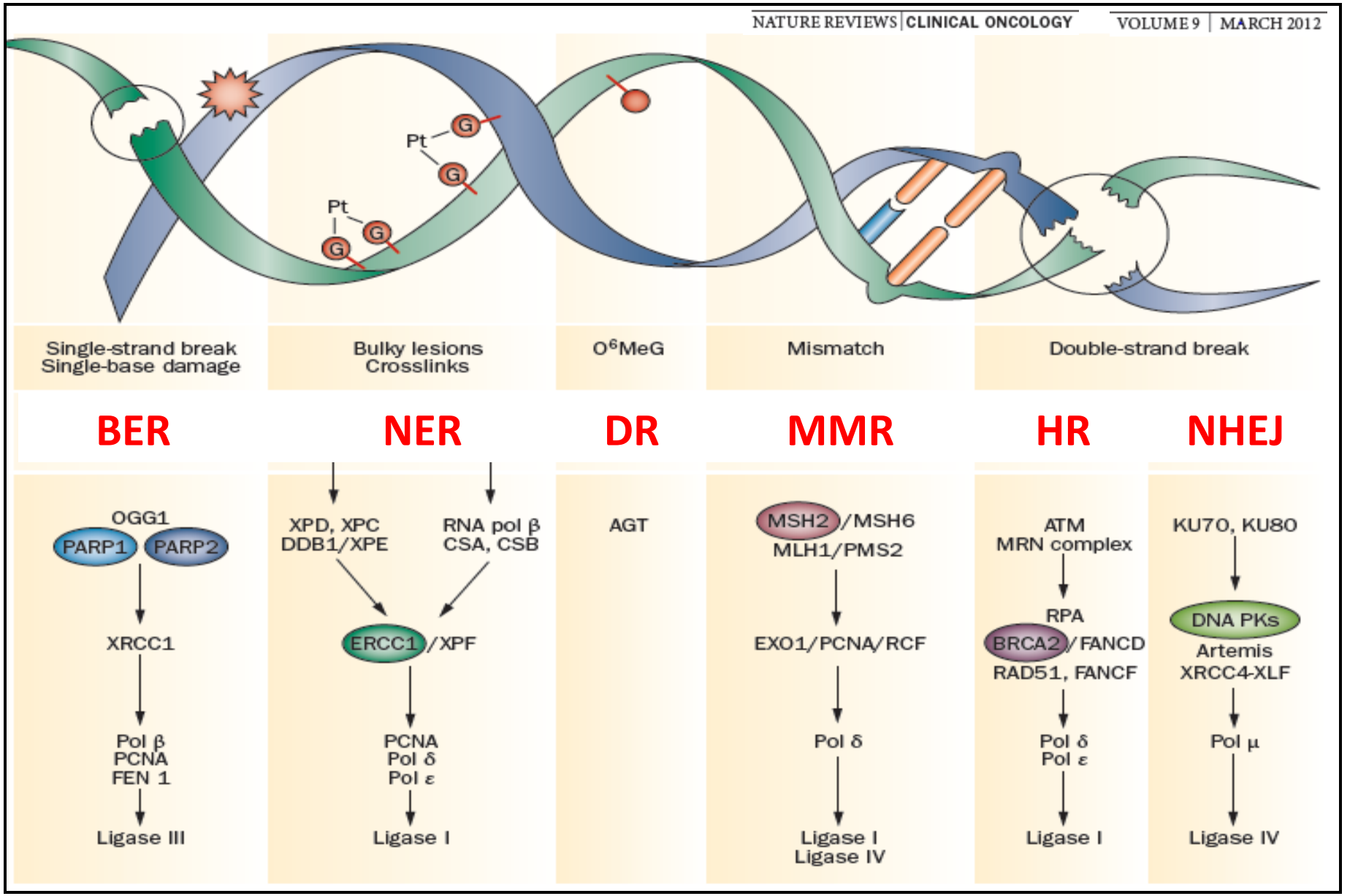
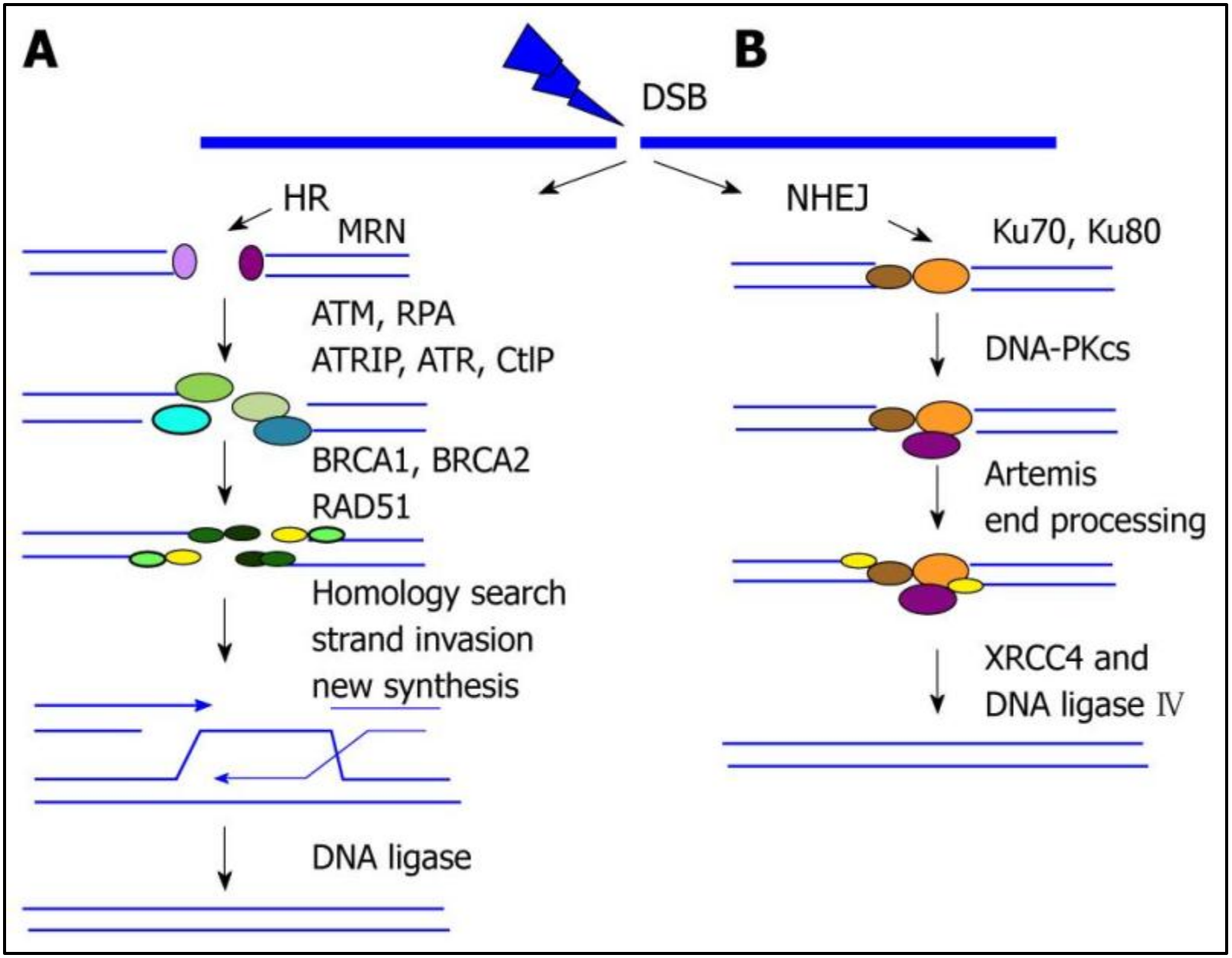
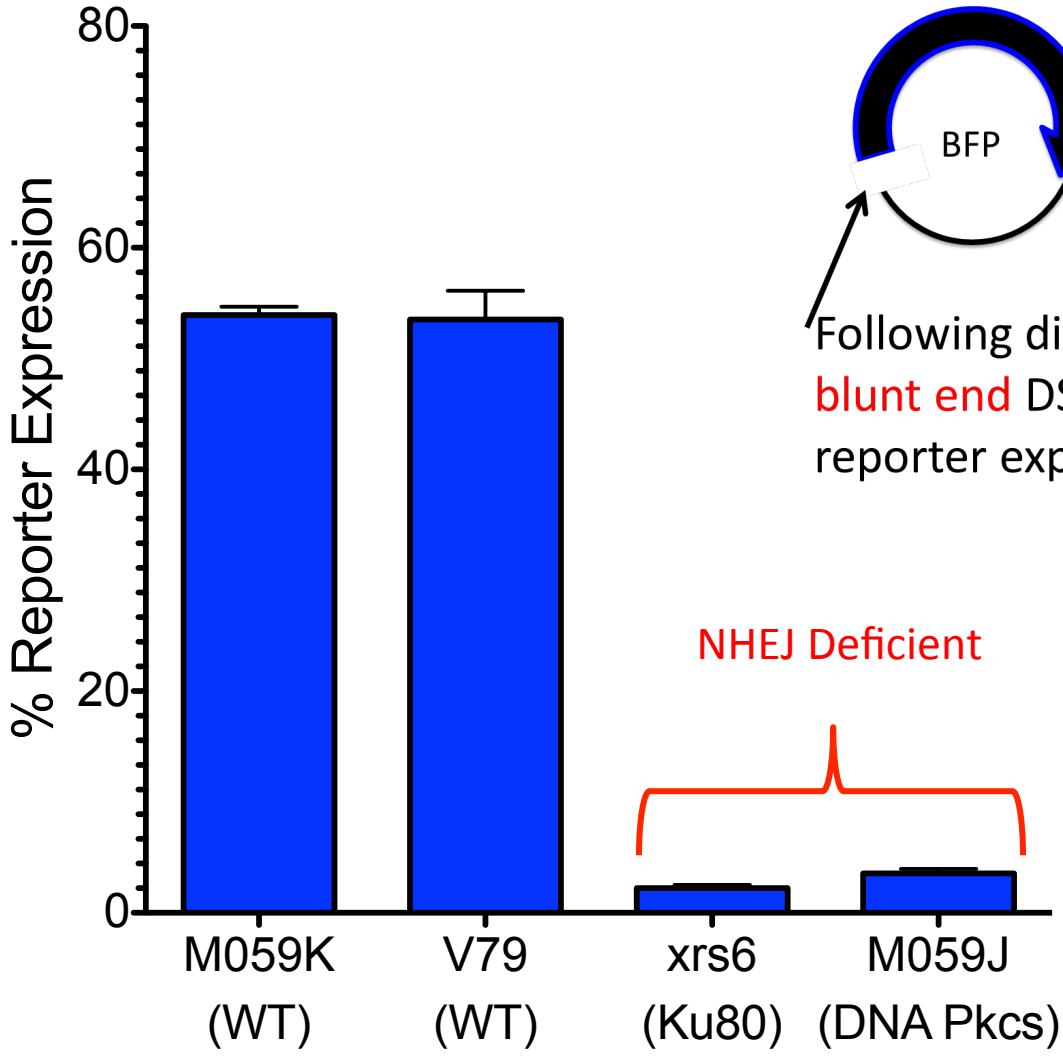
Six Major DNA Repair Pathways

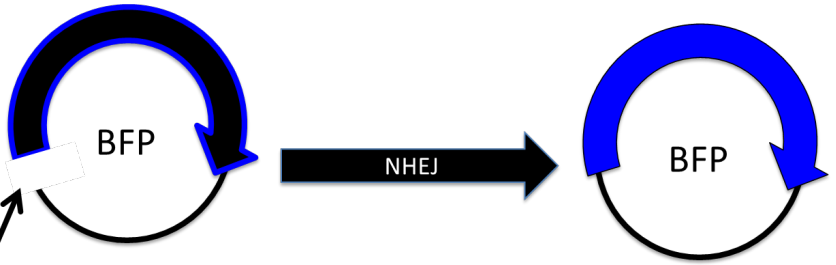


DNA Double Strand Break (DSB) Repair



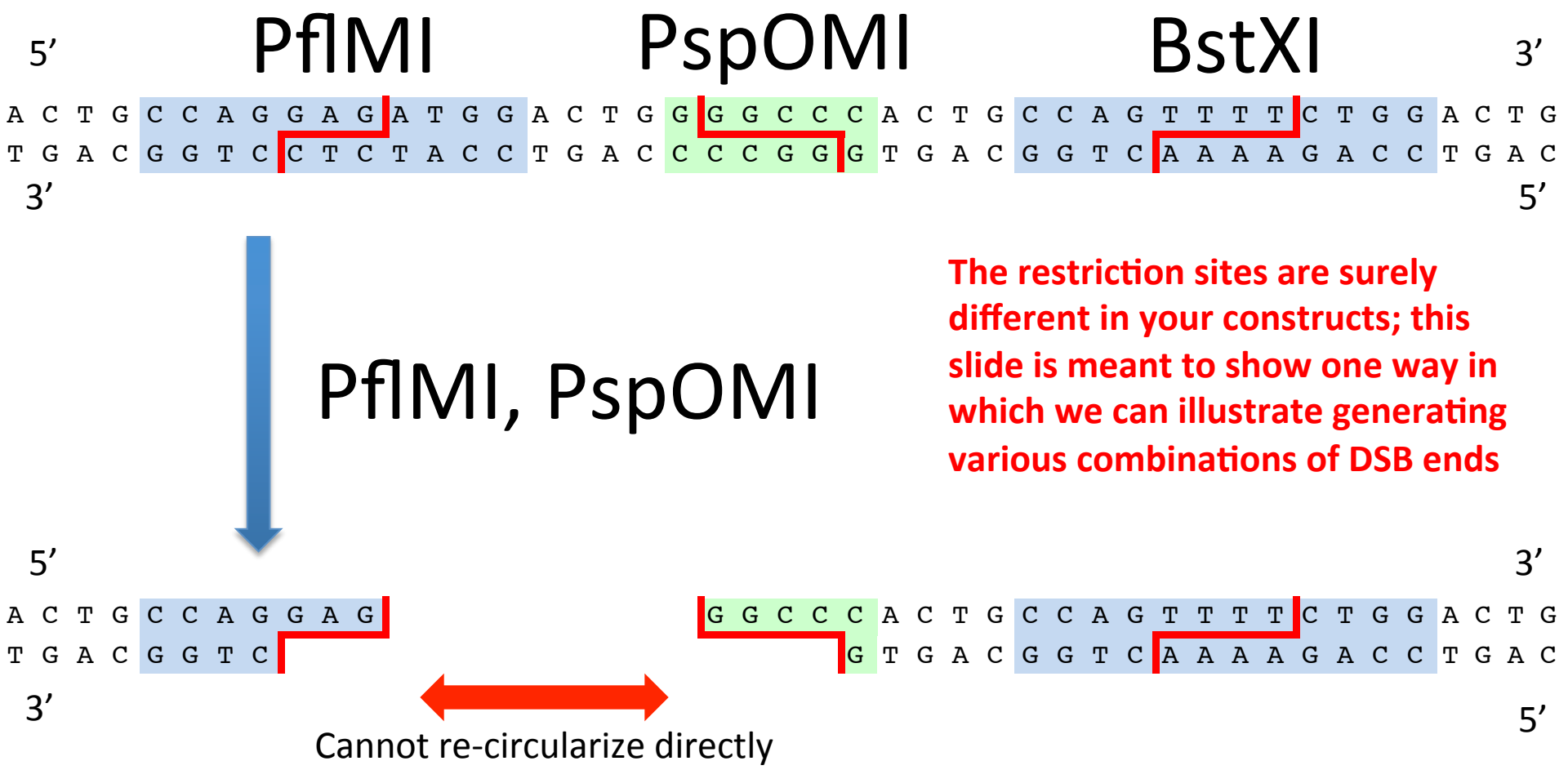
NHEJ HCR in WT and NHEJ defective cells at 18 hours post-transfection:





Following digest, the substrate contains a blunt end DSB that prevents fluorescent reporter expression

Double digest to produce DSBs with ends that are not compatible with ligation:



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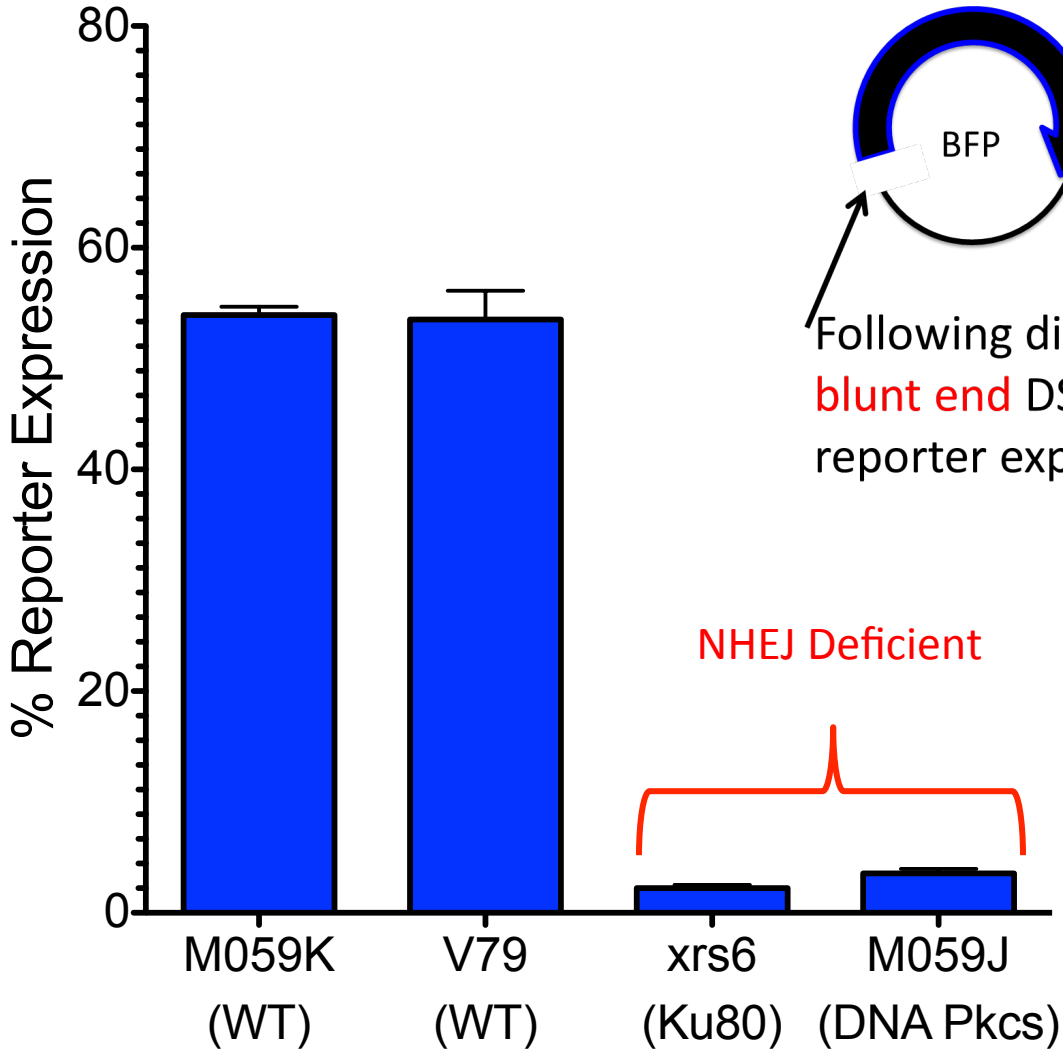

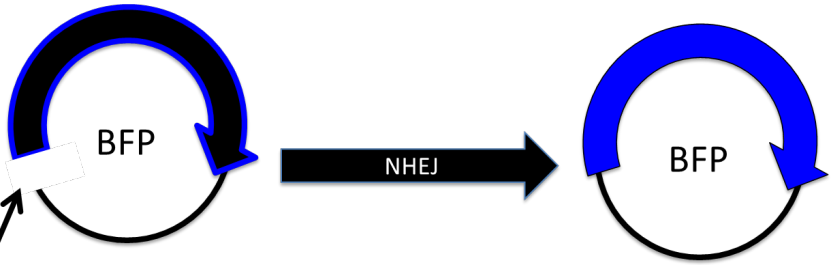

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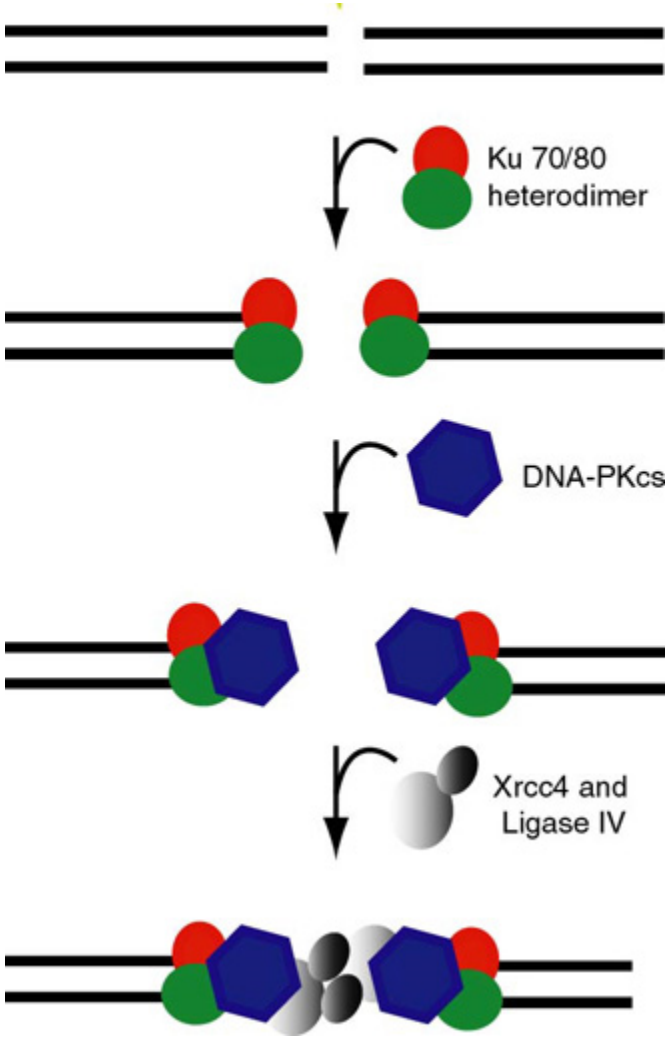
Non-Homologous End Joining (NHEJ)

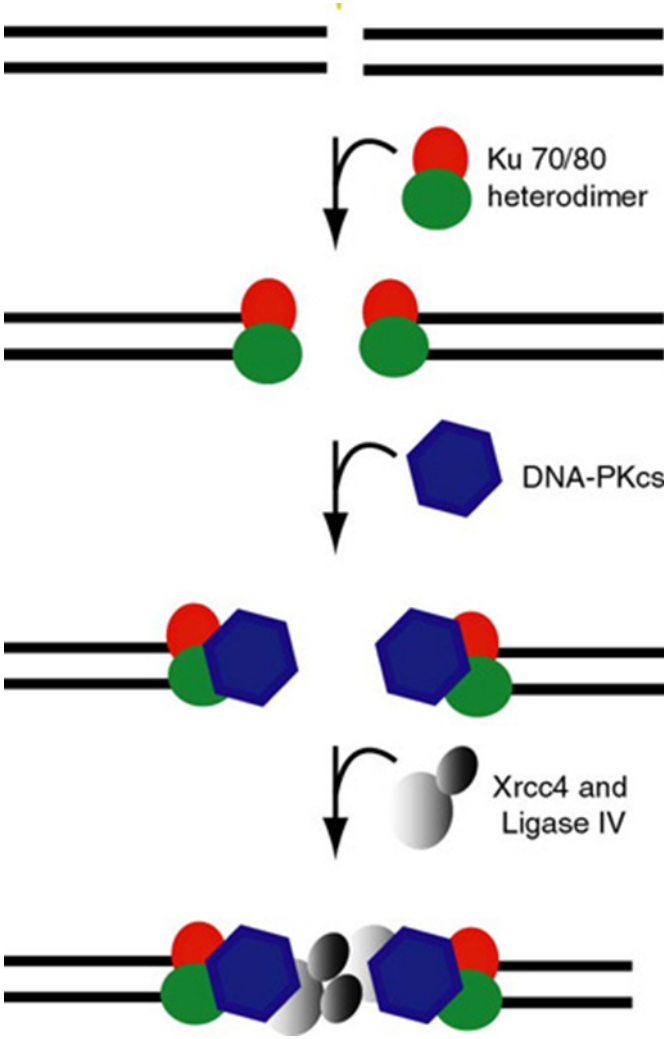


Ku70
Ku80

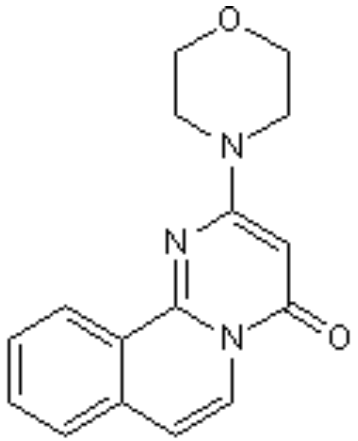
DNA-PKcs

Xrcc4
Ligase IV

NHEJ Inhibitor – Compound 401 Specifically Inhibits DNA-PKcs and thus NHEJ

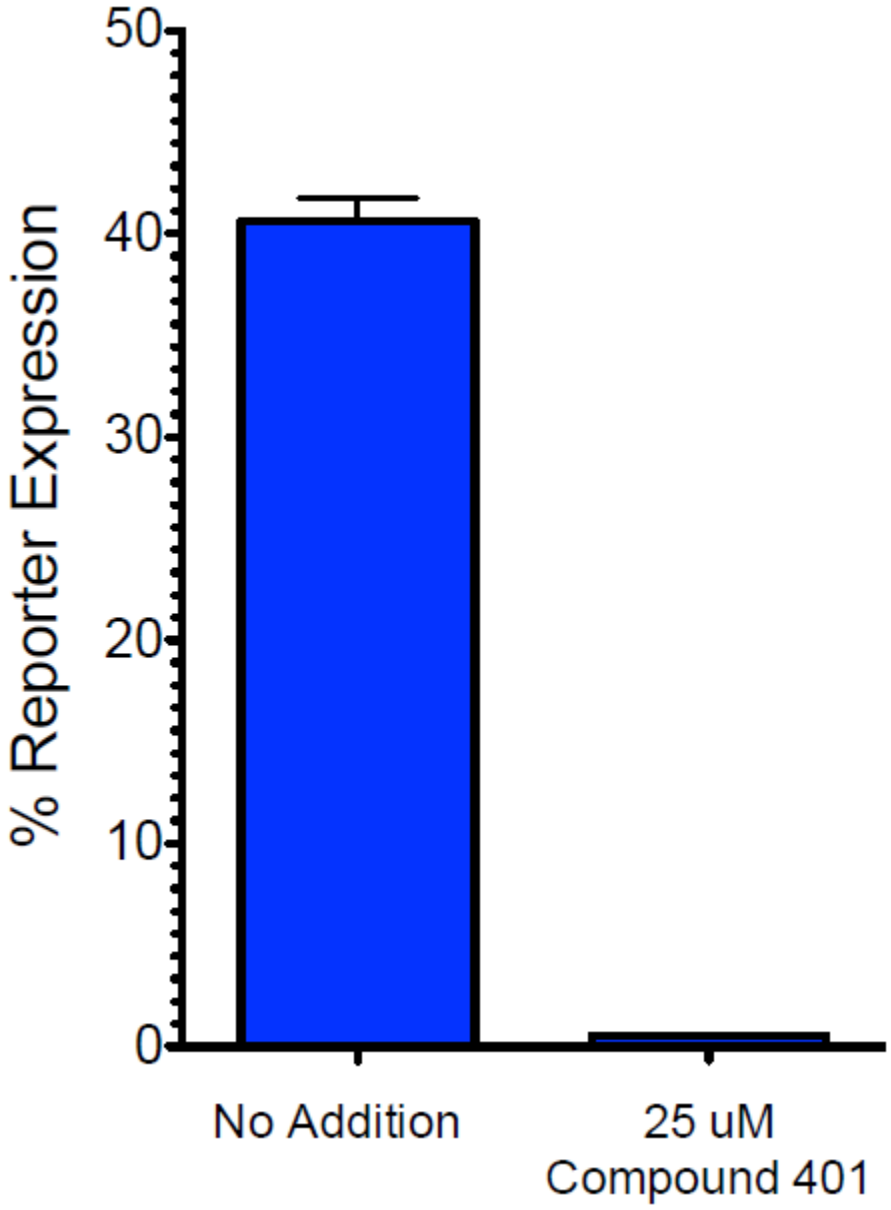


Compound 401

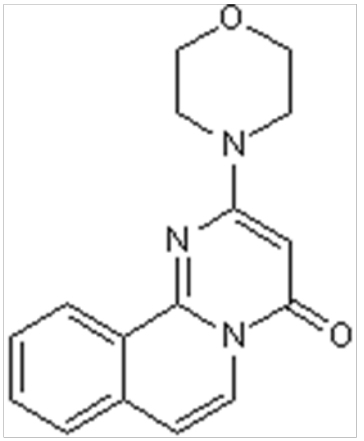


Chemical Name:
2-(4-Morpholinyl)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-one

NHEJ in Human Lymphoblastoid Cells



Compound 401



Chemical Name:
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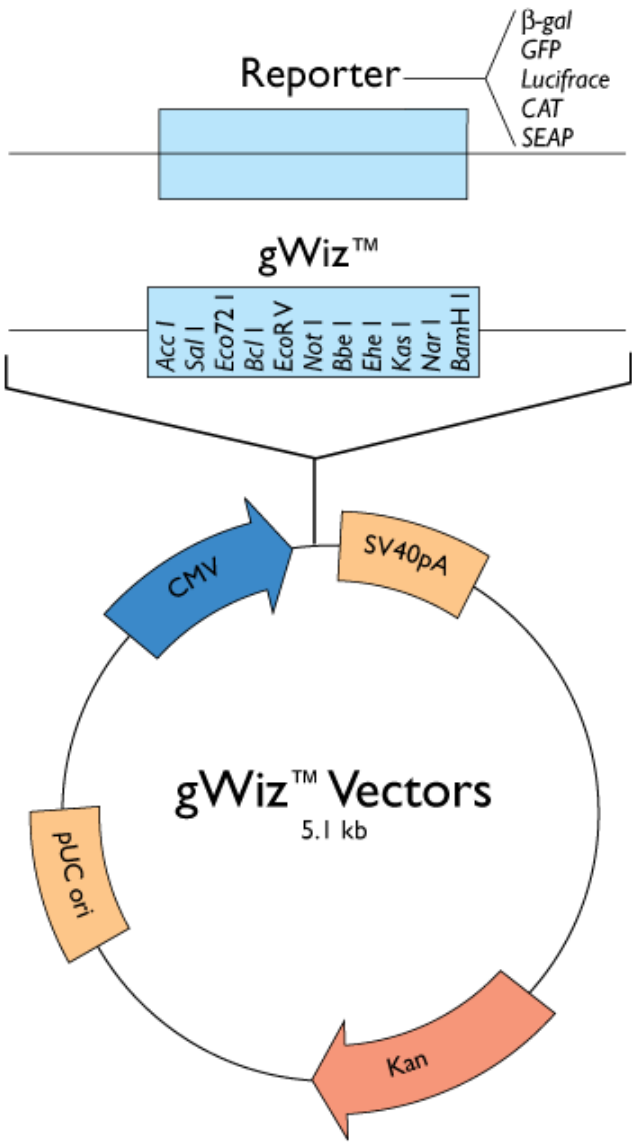
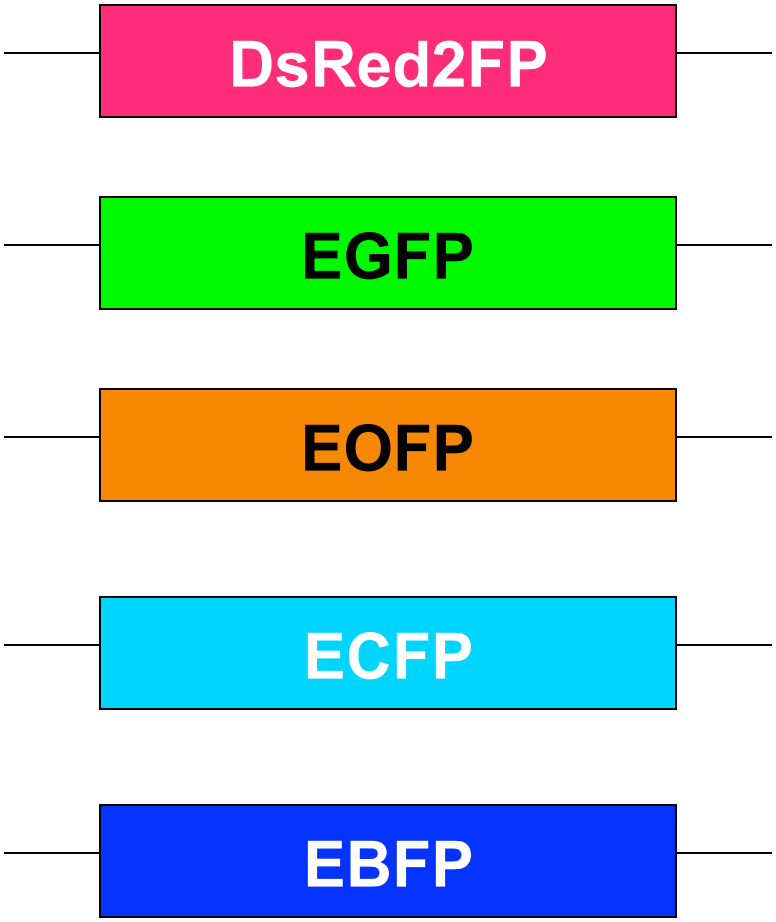
Reactivation of damaged DNA – multiplexed



Each Fluorescent Protein gene will harbor a different type of DNA damage



http://www.genlantis.com/corp/images/gWiz_vectmap.gif

NHEJ Inhibitor – Compound 401 Specifically Inhibits DNA-PK and thus NHEJ

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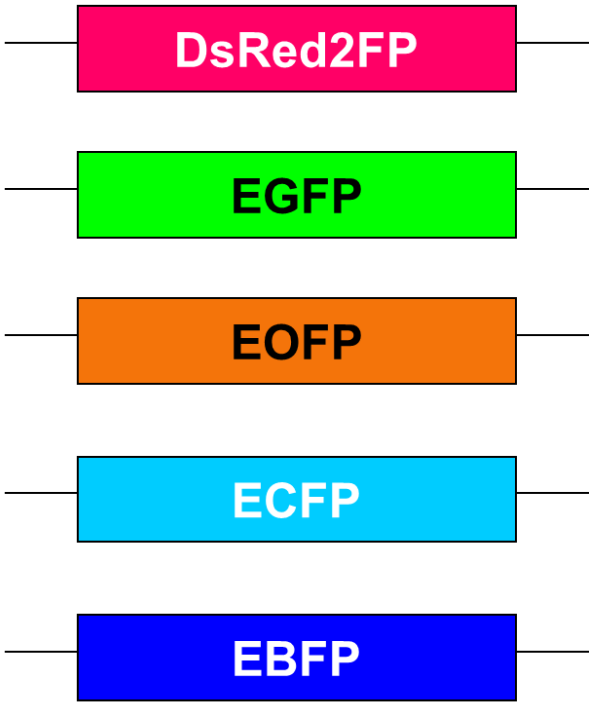
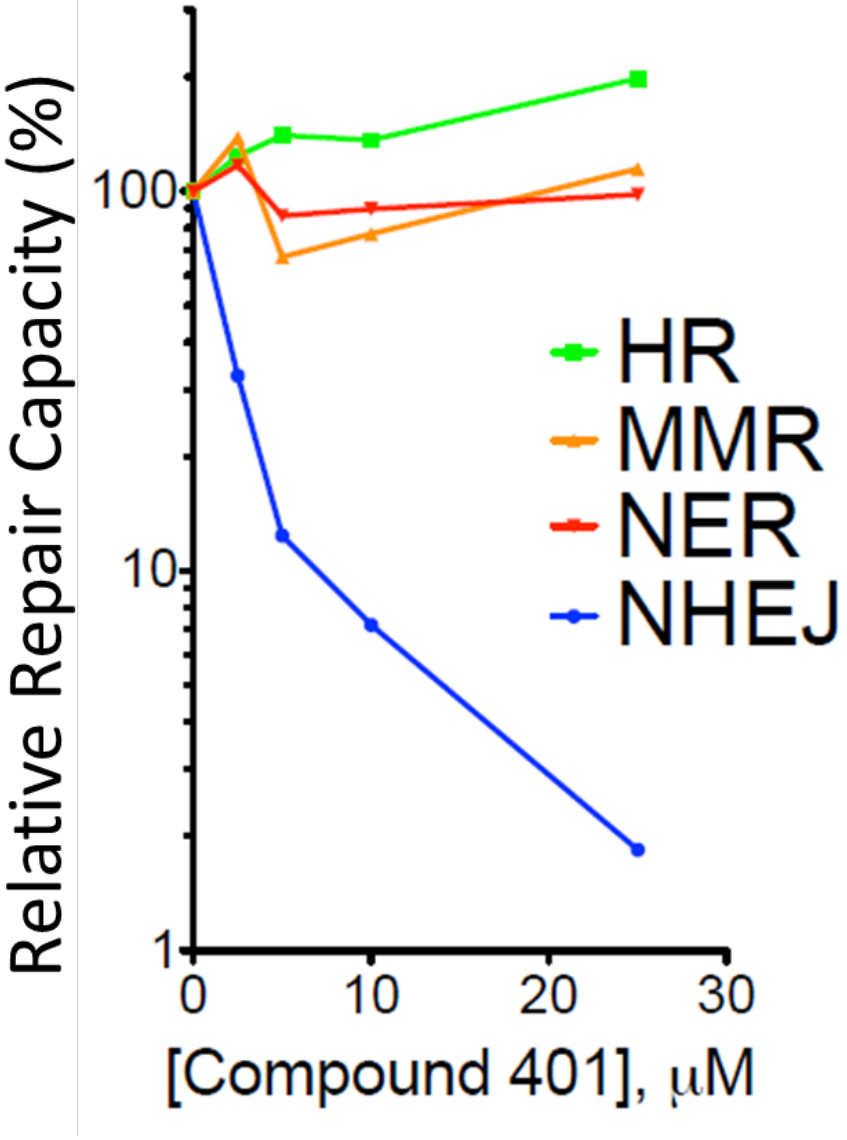
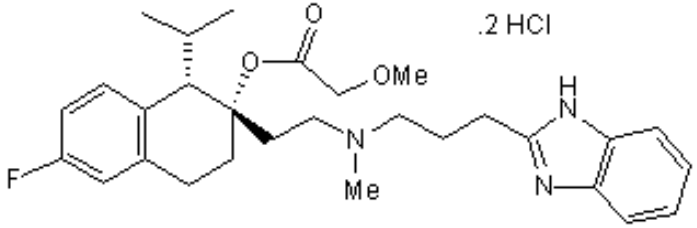


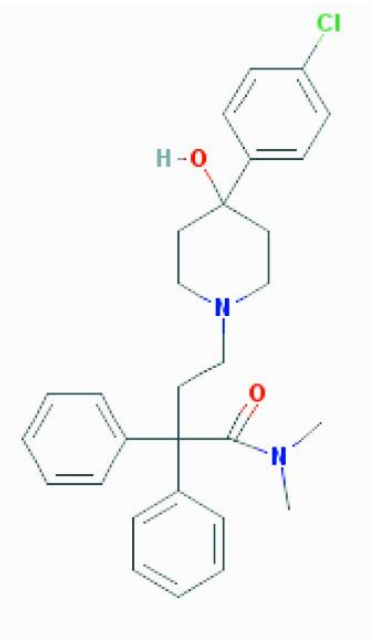


Four Different DNA-PKcs Inhibitors that work in Human Cells – will they work in CHO cells?

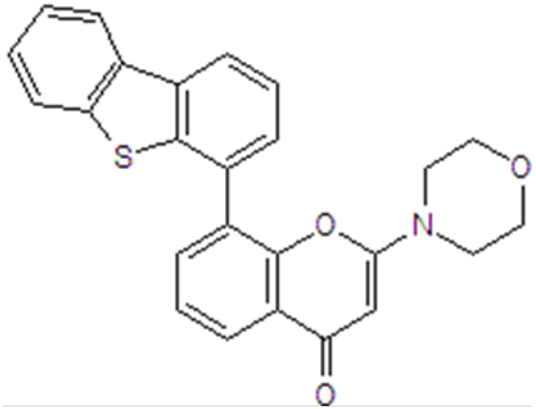
Drug	Mechanism of action	Vendor website	Literature reference	Fun fact
Mibefradil dyhydrochloride	Unknown (for NHEJ)	Tocris	Goglia et al.	Used clinically to treat angina
Loperamide hydrochloride	Unknown (for NHEJ)	Santa Cruz	Goglia et al.	You many know this as Imodium
NU-7441	DNA-PKcs inhibitor	Tocris	Zhao et al.	45 PubMed hits for NHEJ inhibition
DMNB	DNA-PKcs inhibitor	Santa Cruz	Durant et al.	Chemical derivative of vanilla



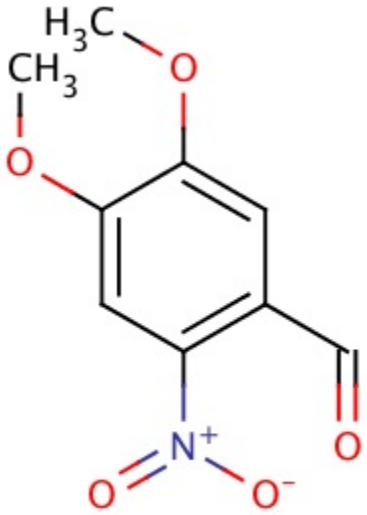
Mibefradil dihydrochloride



Loperamide Hydrochloride

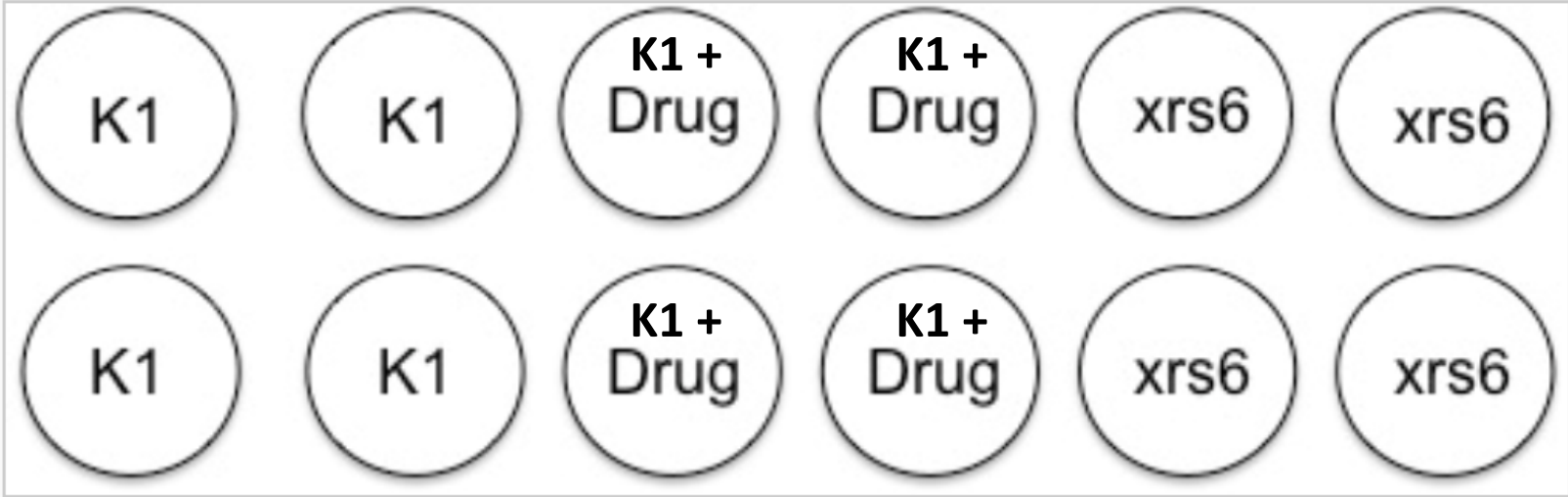


NU 7441



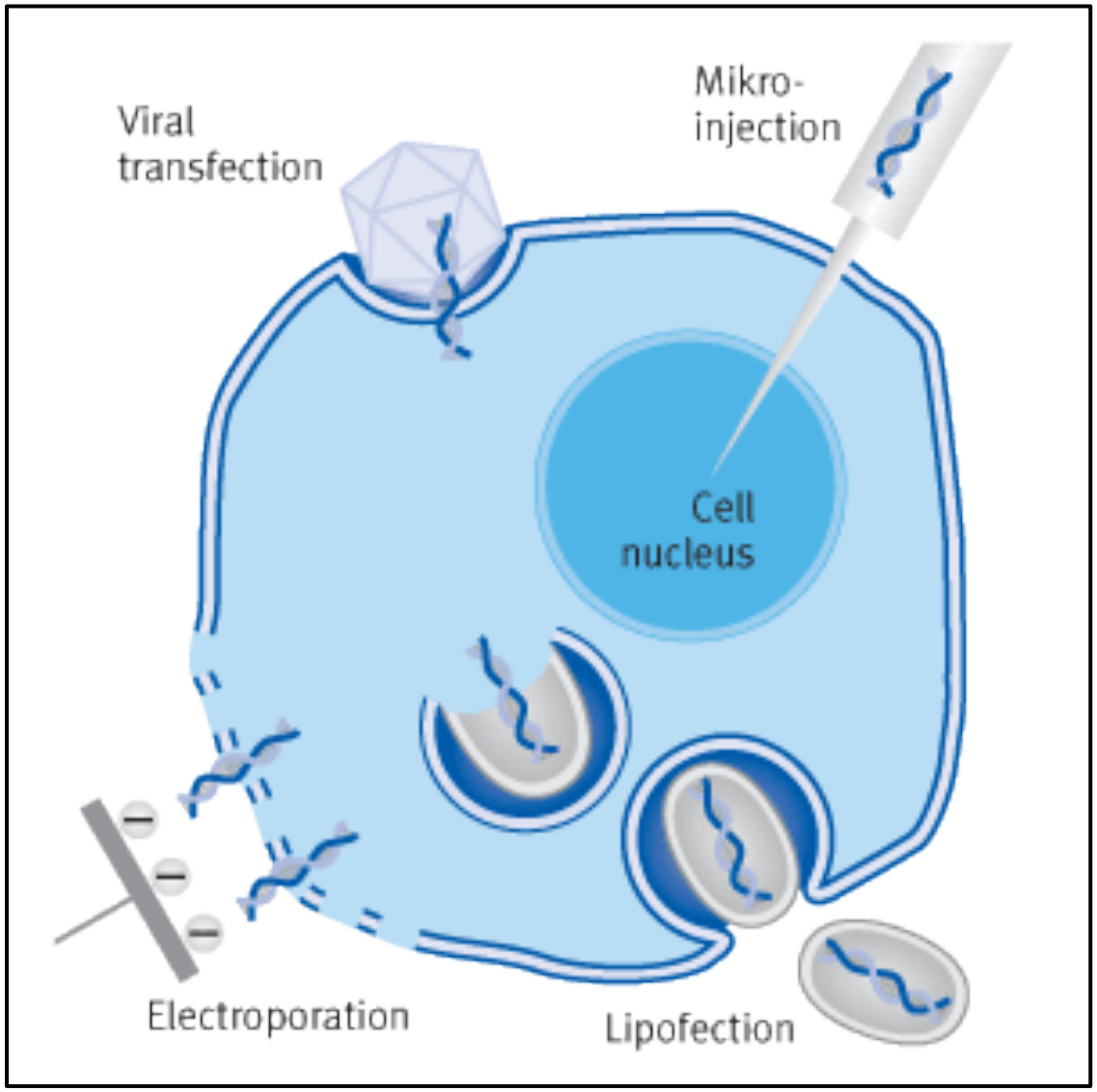
DMNB

Transfection – Experimental Design



Lipofection sample schematic (top half of a 24-well plate.) The top row will receive mixture A, and the bottom row mixture B. Each condition will be done in duplicate.

How can we get DNA into Mammalian Cells?



http://www.biontex.com/con_4_6_4/cms/front_content.php?changelang=3&idart=198

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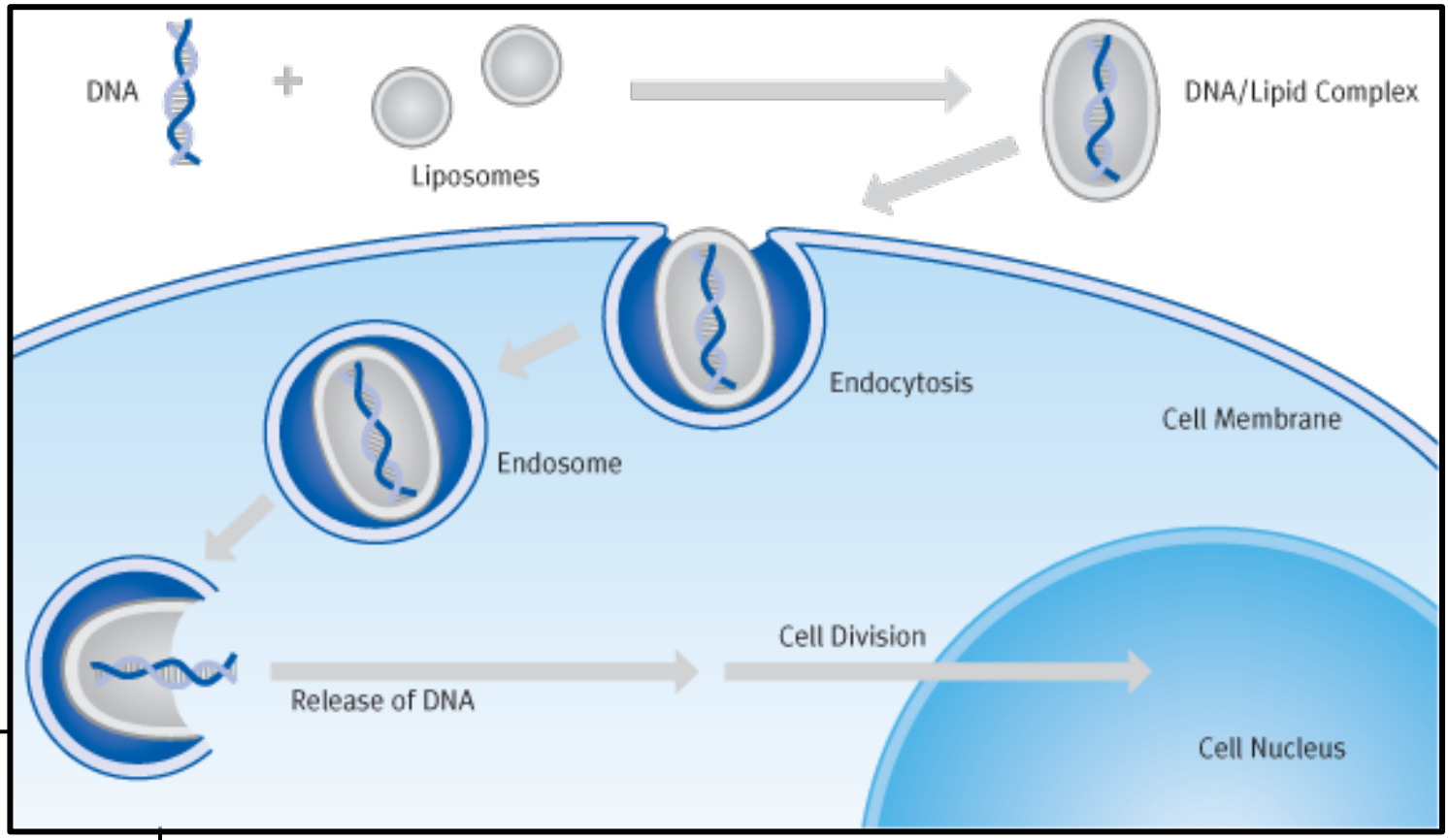

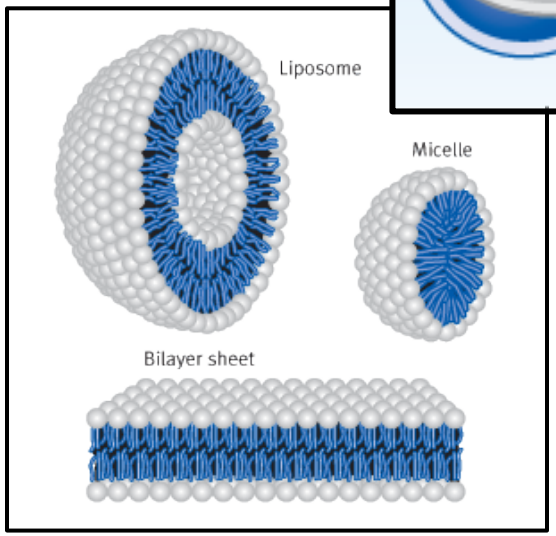

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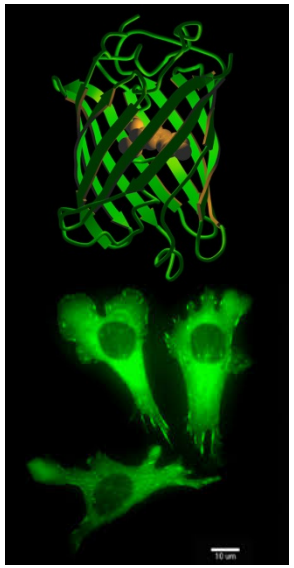
What experimental question will you ask in Module 2?

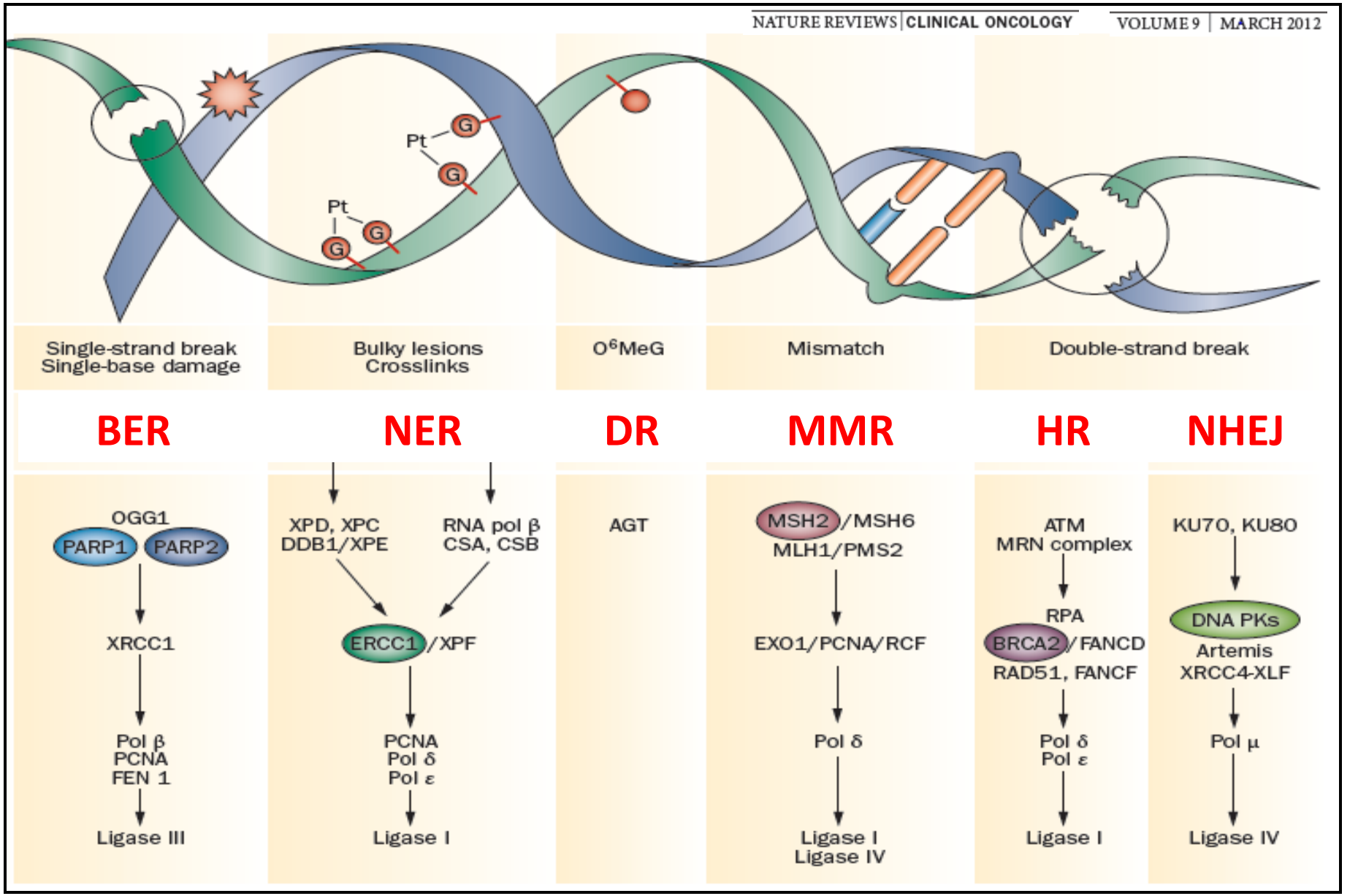


How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

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



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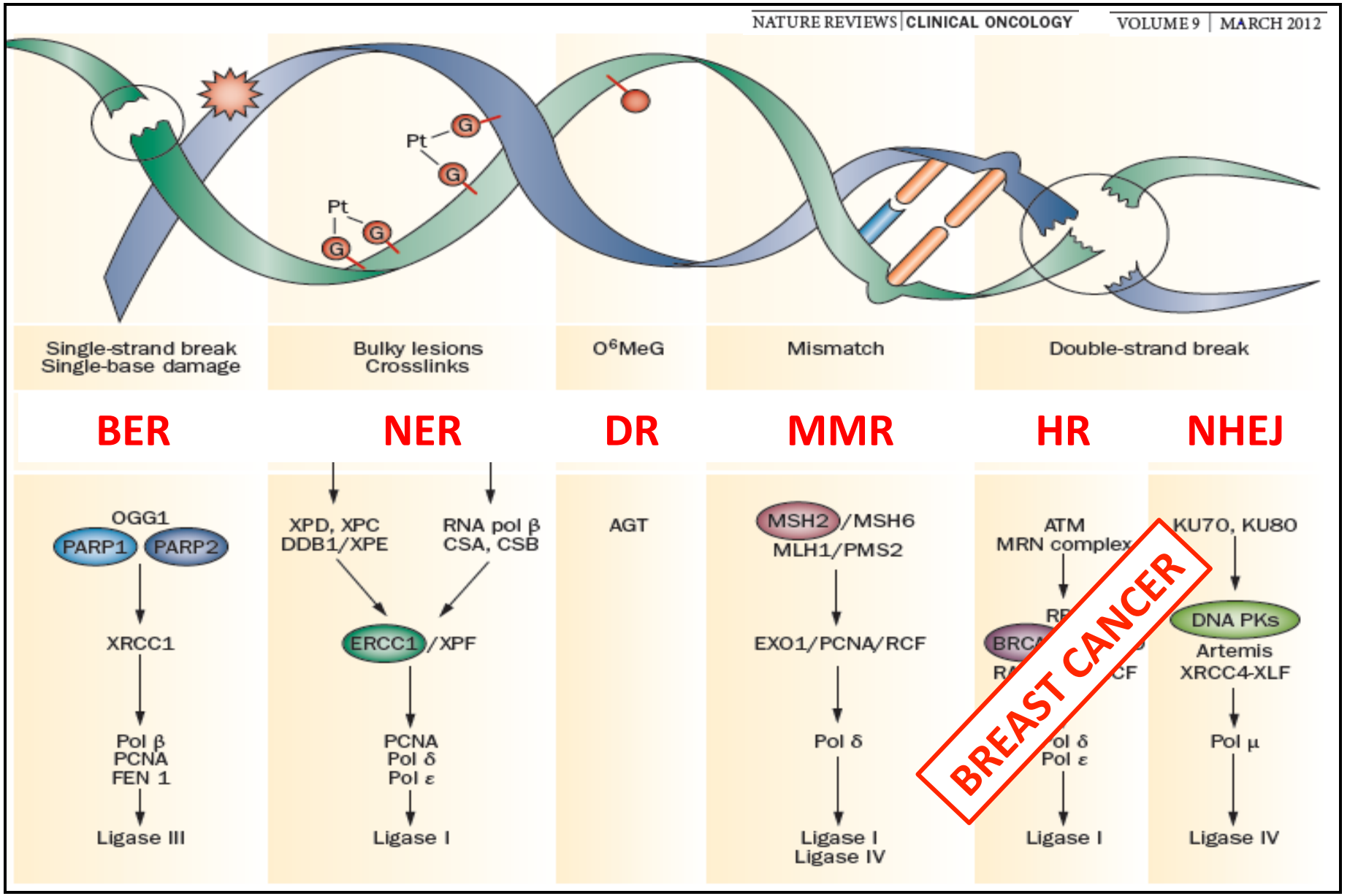

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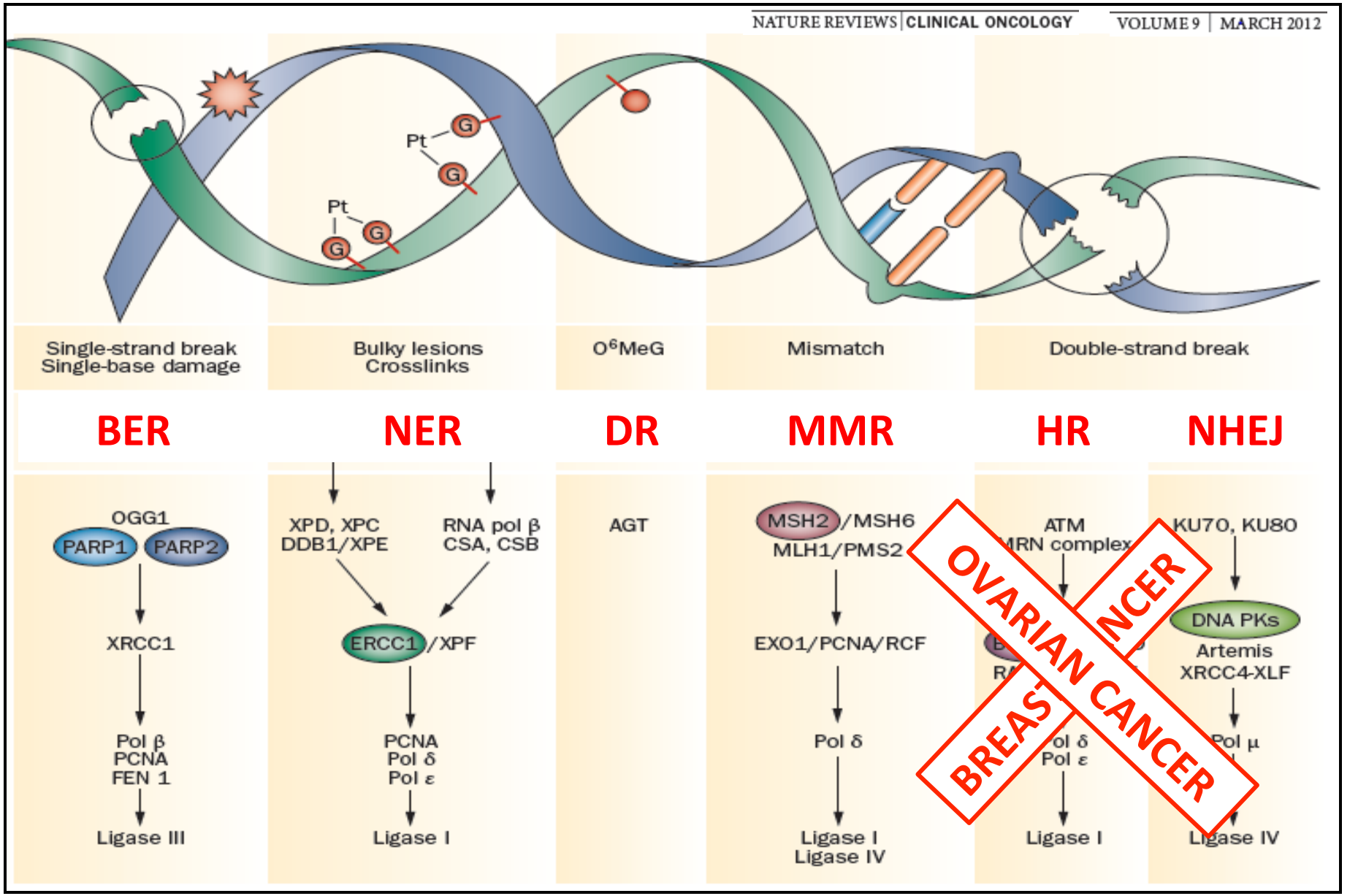

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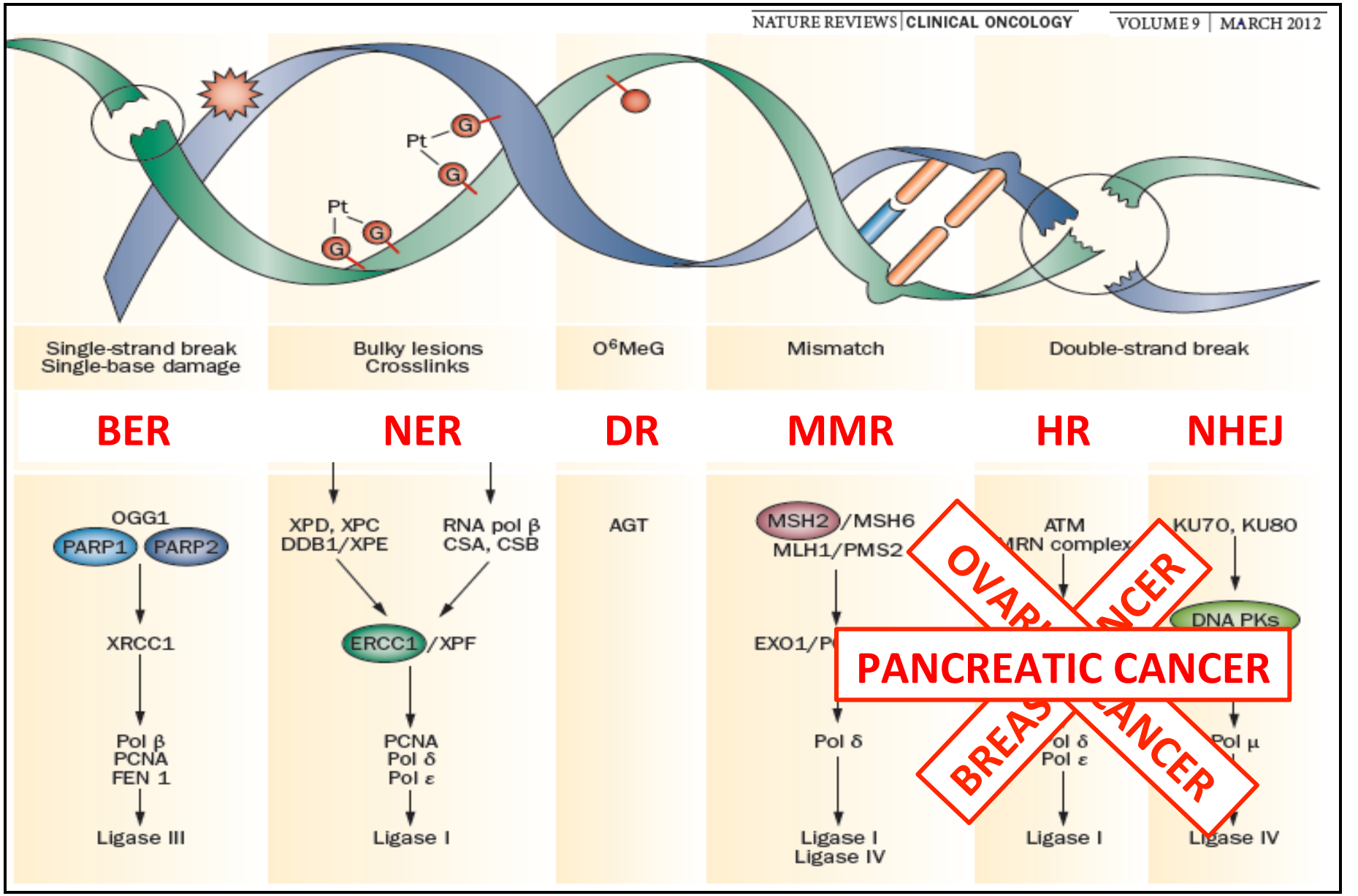
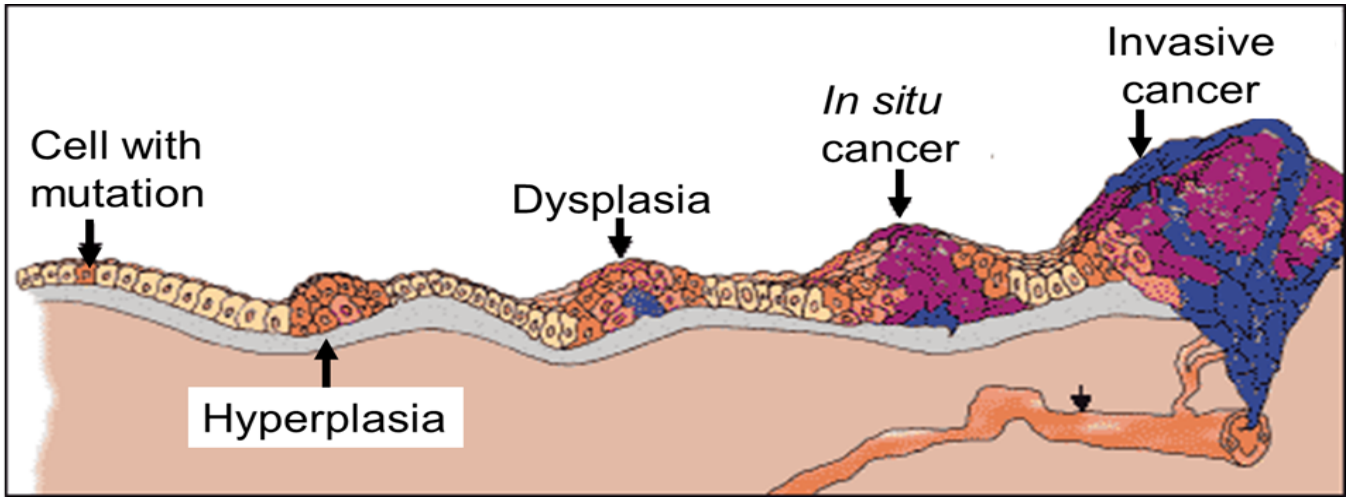
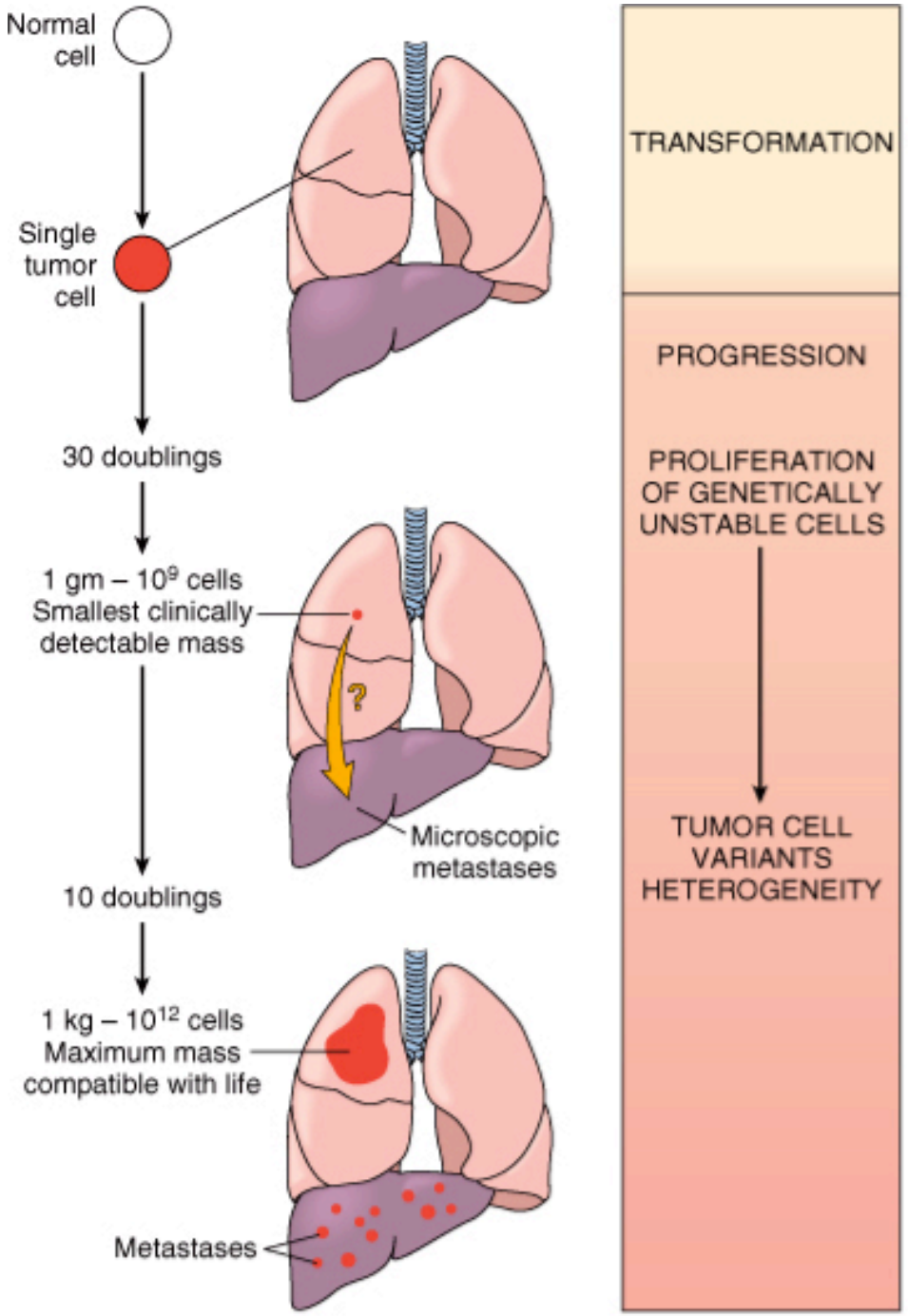


Cancers arise from the accumulation of heritable changes in gene function

Normal Epithelium → Neoplasia → Invasive Carcinoma → Metastasis

1st Mutation → 2nd Mutation → Additional Mutations

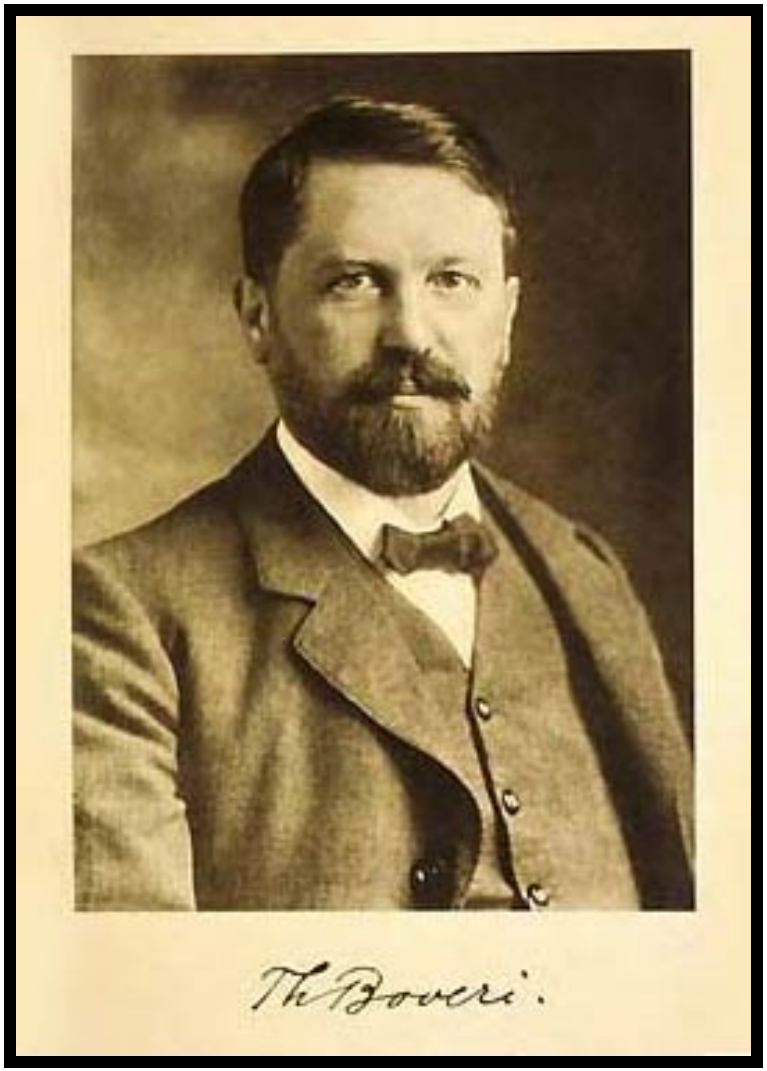




Multiple Mutations

More and more Mutations

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



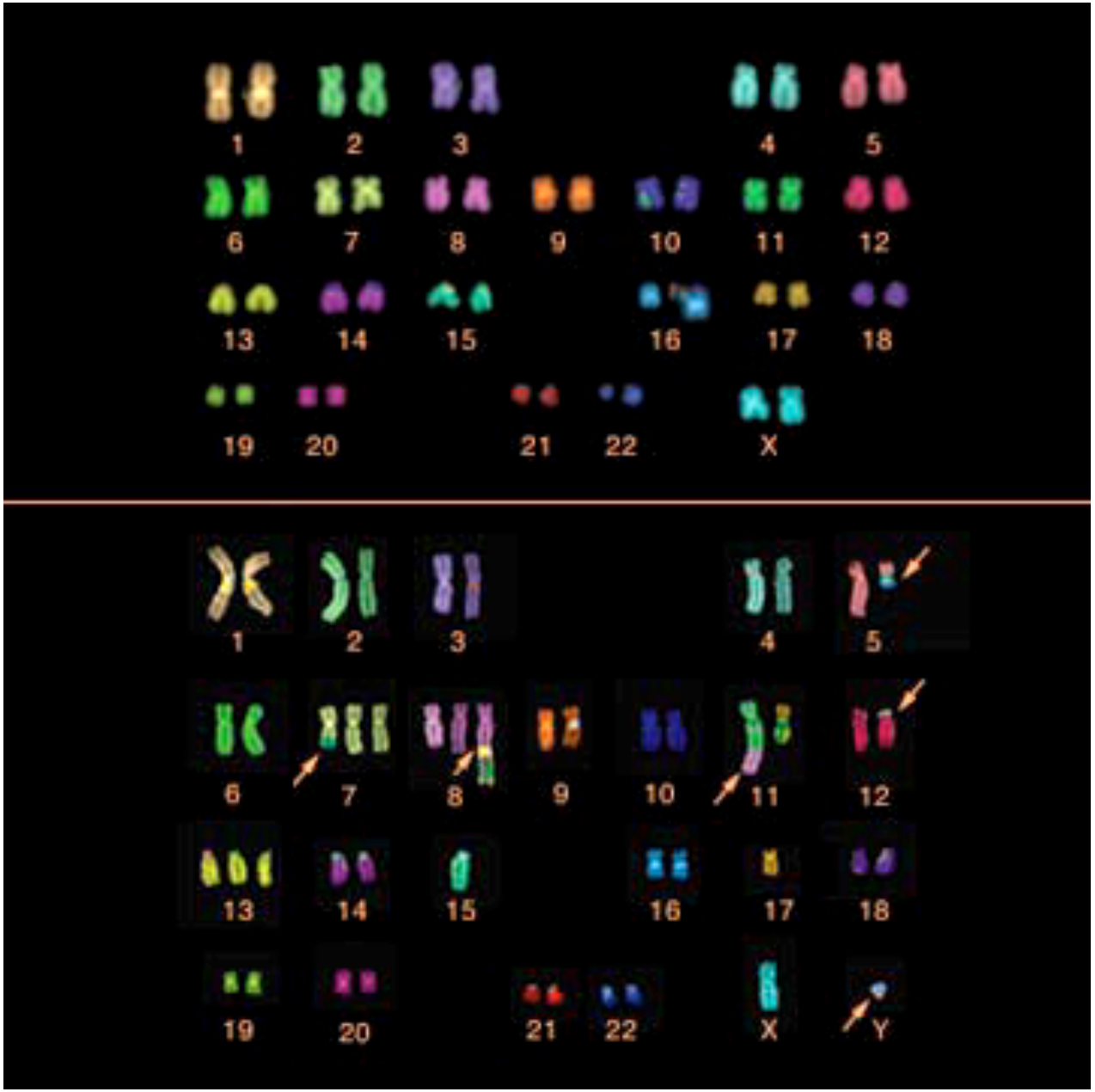
- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.
- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell
- Suggested that some chromosomes promote cell growth and others inhibit cell growth

Marcella O'Grady Boveri (1865-1950) also contributed

Marcella O'Grady Boveri (1863-1950) also contributed to Boveri's theory

She was the first woman student to graduate from MIT with a Biology Major in 1885!

J Med Genet. 1985;22(6):431-40.
Marcella O'Grady Boveri (1865-1950) and the chromosome theory of cancer



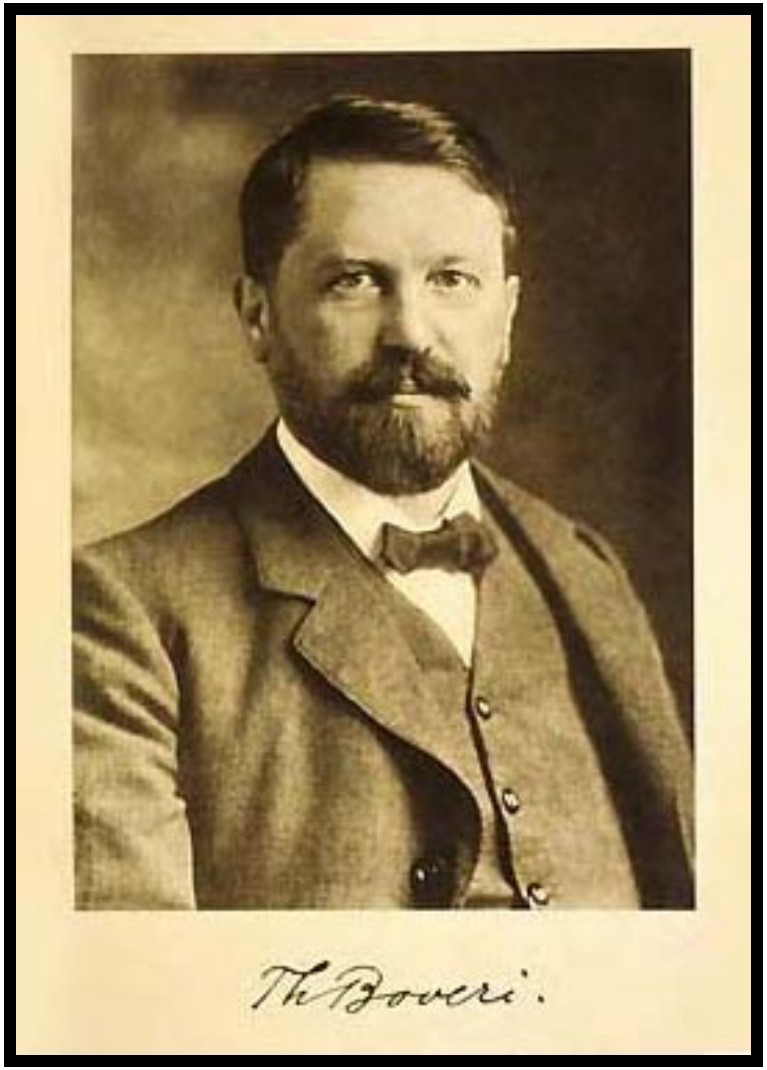
Chromosomes from a Normal cell

Chromosomes from a Tumor cell

Spectral Karyotyping (SKY)
"SKY Painted Chromosomes"

Chromosomes from a Pancreatic Tumor Cell

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



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Alterations (mutations) in different kinds of Genes cause Cancer

Oncogenes

genes that ordinarily promote cell proliferation but when mutated or overexpressed promote uncontrolled growth

Tumor suppressor genes

genes that ordinarily prevent inappropriate proliferation but when mutated allow uncontrolled growth

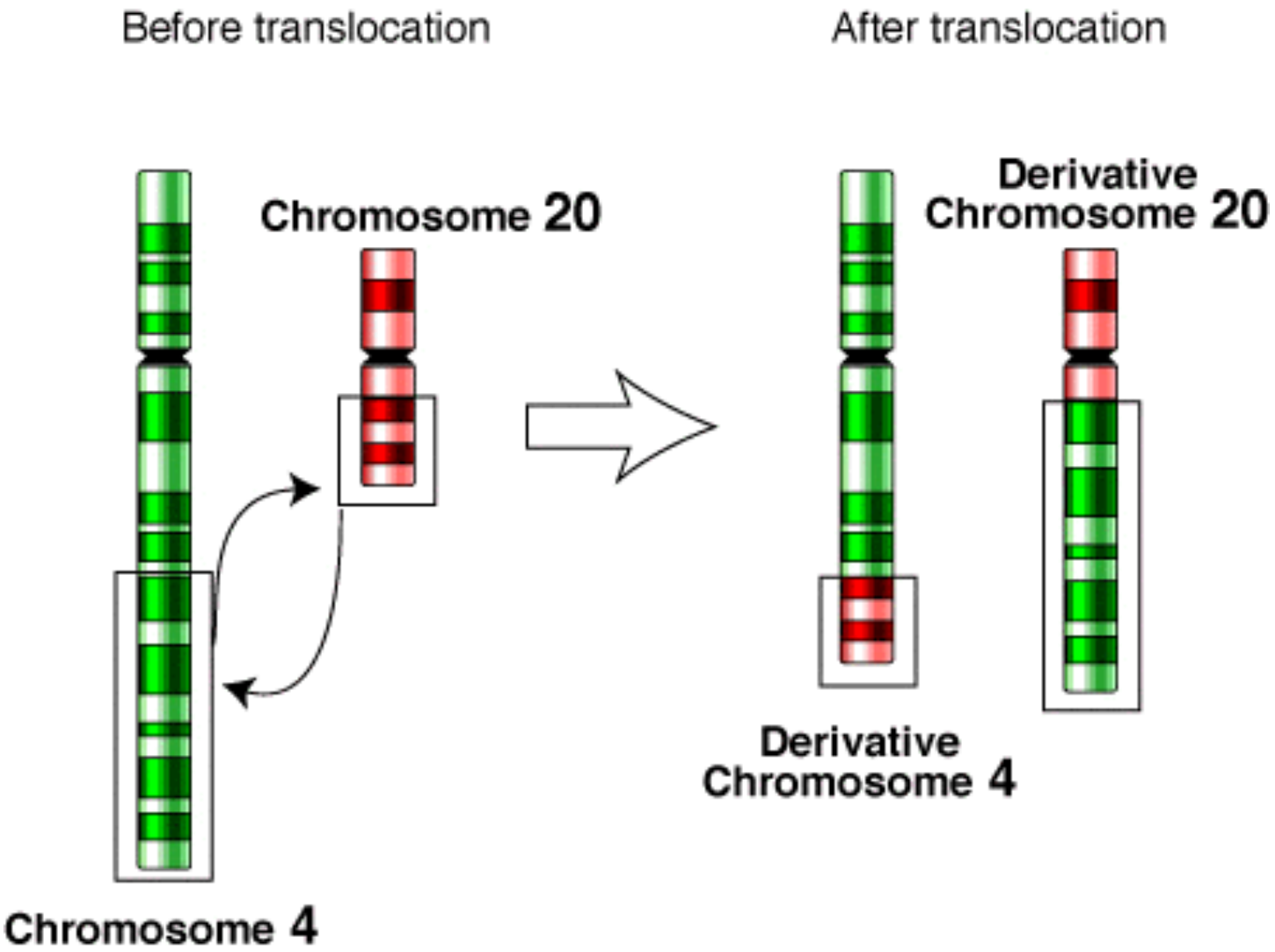
Mutator genes

genes that ordinarily prevent mutations; alterations in these genes allow increased mutation rates

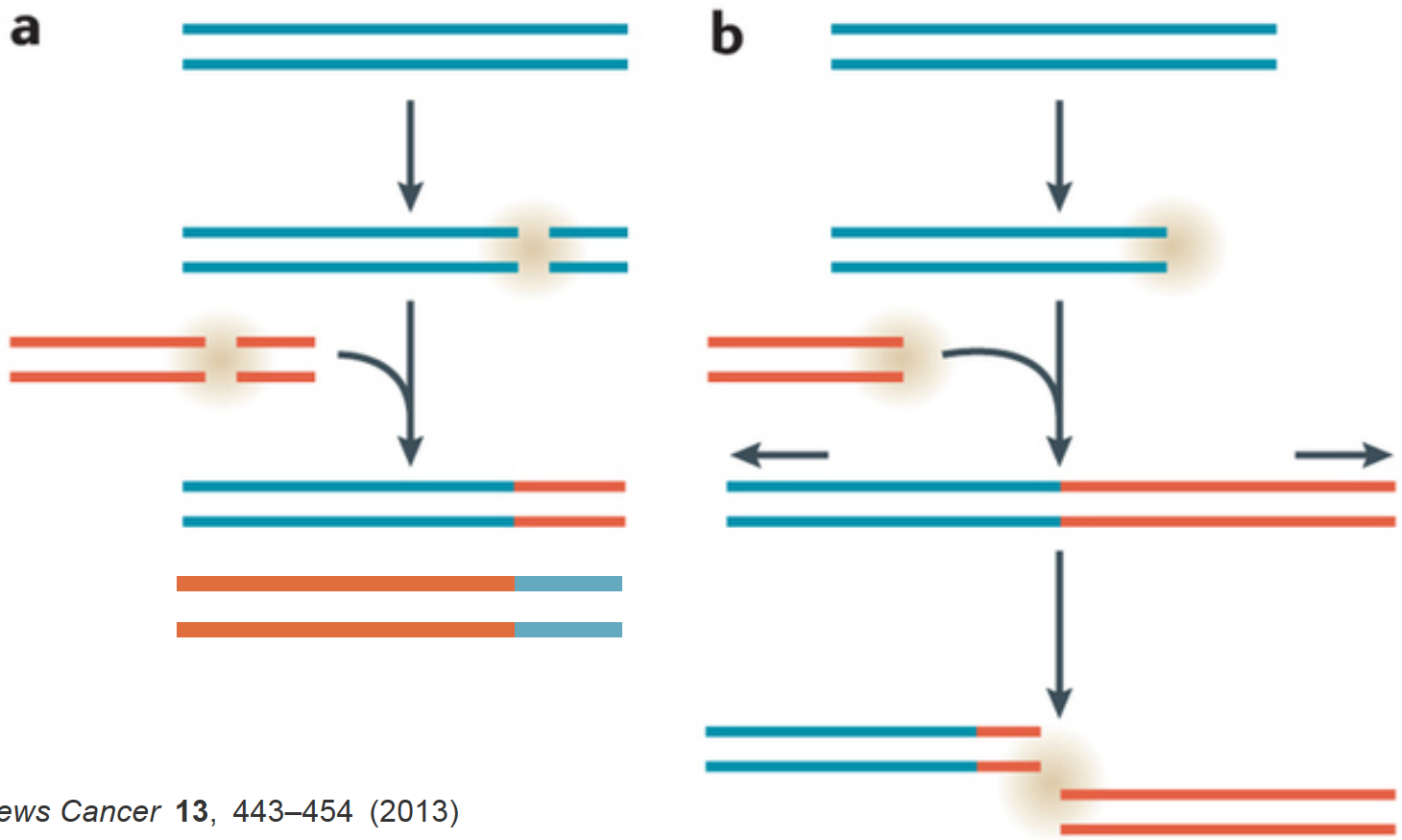

KEN BURNS PRESENTS
CANCER
THE EMPEROR OF ALL MALADIES
A FILM BY BARAK GOODMAN
BASED ON THE BOOK THE EMPEROR OF ALL MALADIES: A BIOGRAPHY OF CANCER
BY SIDDHARTHA MUKHERJEE
WATCH THE TRAILER

PBS
WGBH

Mechanisms of Chromosome Translocation



Mechanisms of Chromosome Translocation

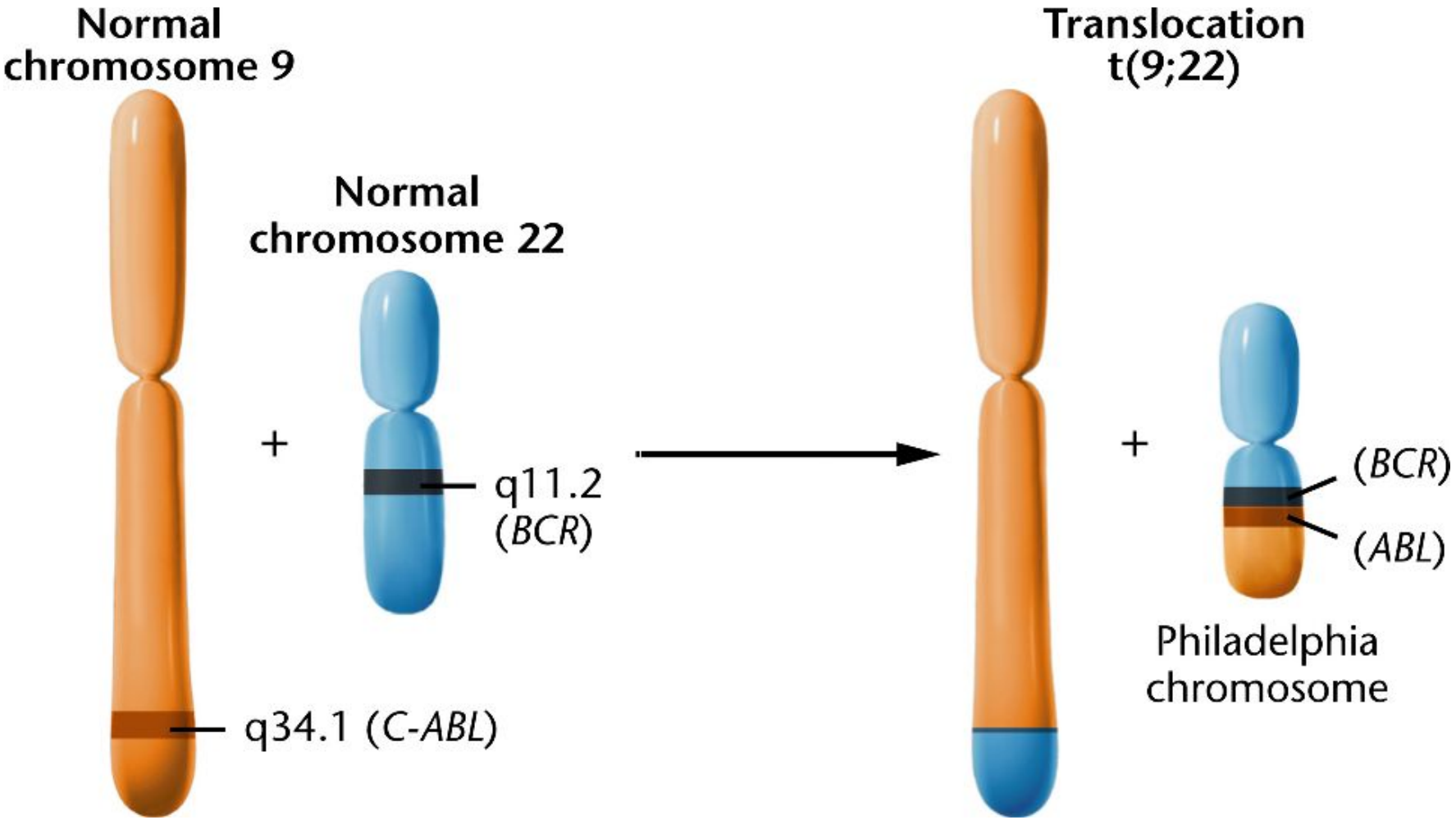


Nature Reviews Cancer **13**, 443–454 (2013)

a | Balanced reciprocal translocations from the fusion of two double-strand breaks that arise in the same cell; ligation of the free DNA ends is mediated by the non-homologous end-joining pathway. Red and blue strands represent different chromosomes.

b Telomere uncapping or attrition generates a DNA double-strand break response, which potentially leads to the fusion of telomeres, generating end-to-end fusions. During anaphase, dicentric fusion chromosomes are pulled apart, leading to the formation of translocations and double-strand breaks. Broken chromosomes act as substrates for additional rounds of fusion and breakage, generating increasingly complex translocations.

Chronic Myelogenous Leukemia (CML)

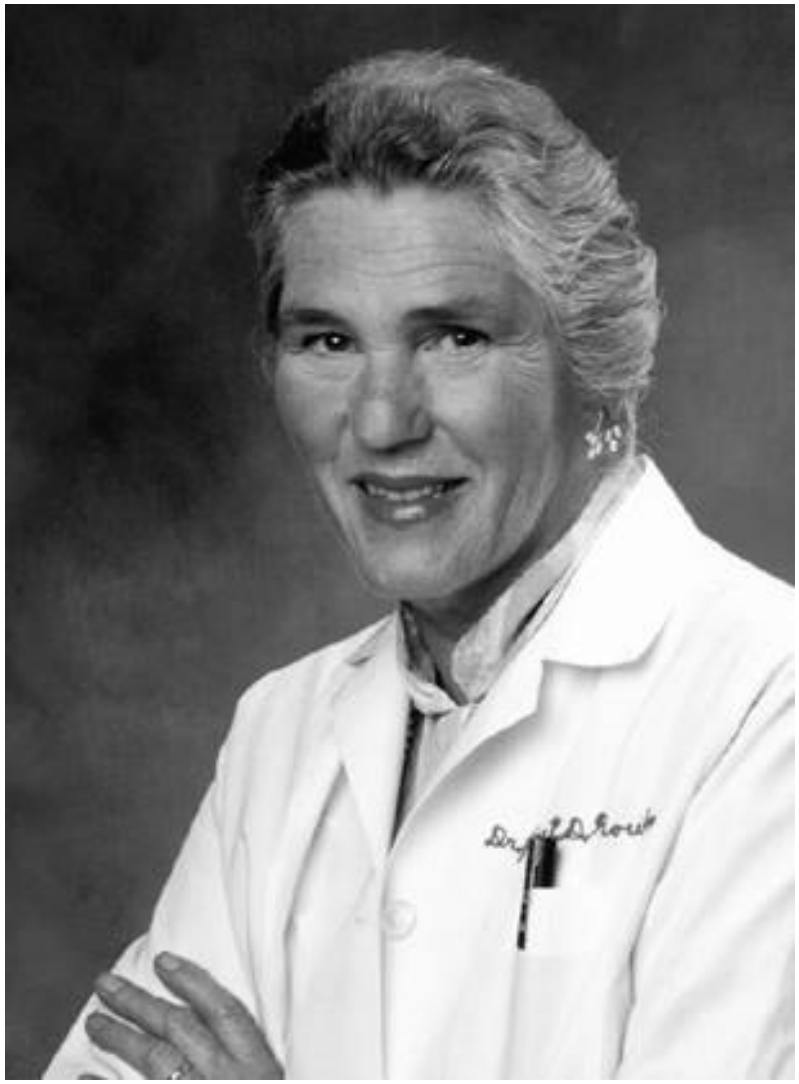


breakpoint cluster region protein (**BCR**)/ C-Abl non-receptor tyrosine kinase

Janet Rowley

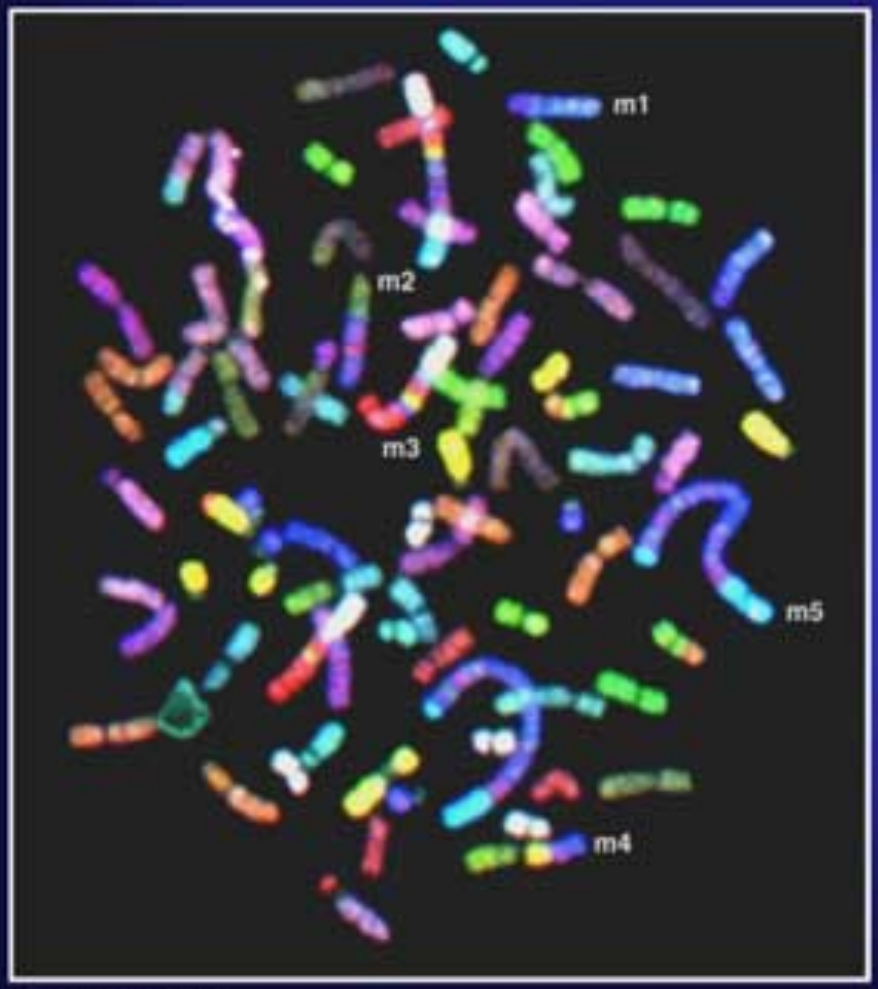
(April 5, 1925 – December 17, 2013)

American human geneticist and the first scientist to identify a chromosomal translocation as the cause of leukemia and other cancers.



Large Deletions or Insertions

SKY chromosome painting: breast cancer

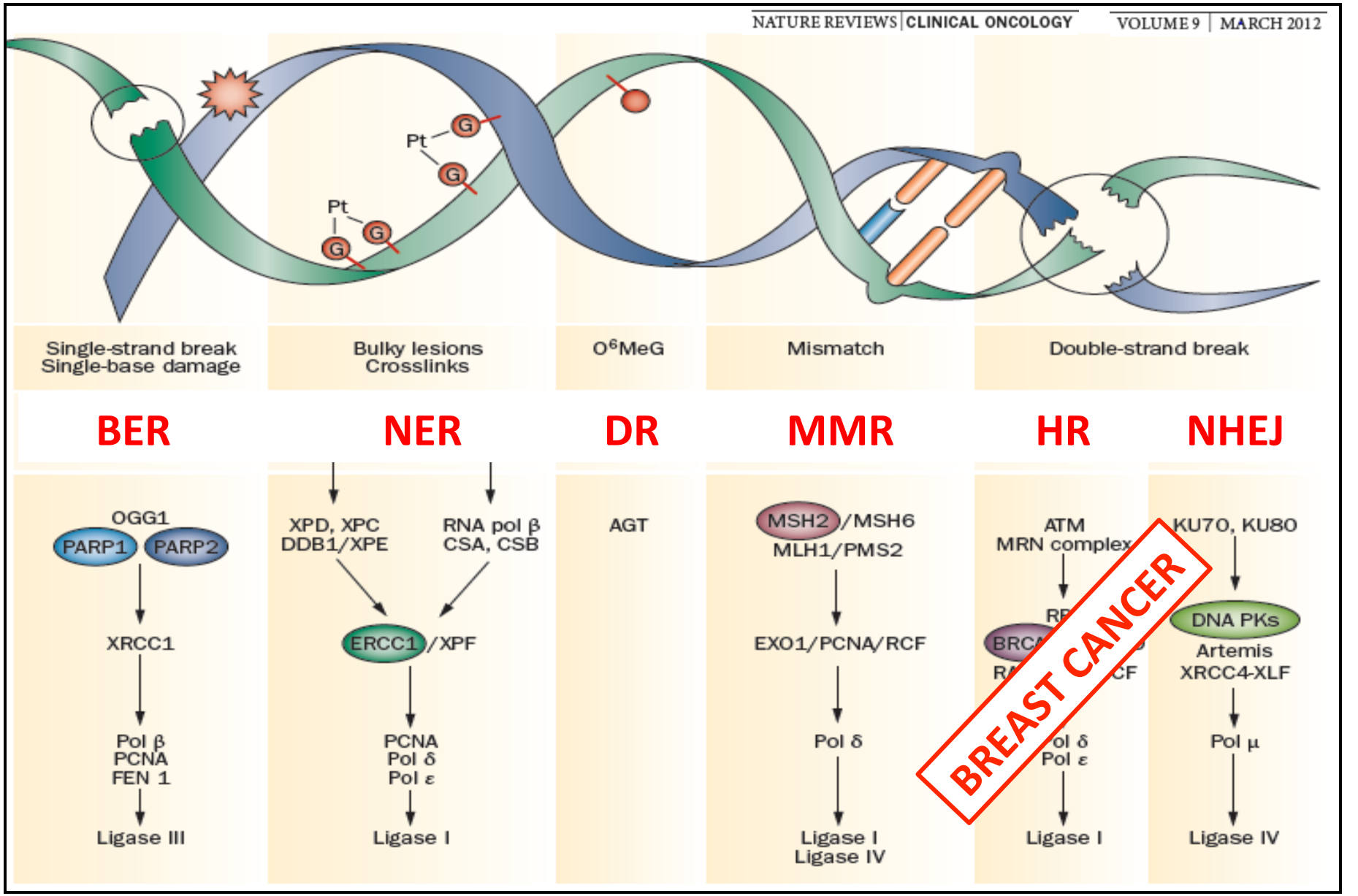


Artwork by Jeanne Kelly © 2004

Normal SKY chromosomes are not multicolored.

Chromosomes in breast cancer appear multicolored because they have exchanged genetic material.

Six Major DNA Repair Pathways



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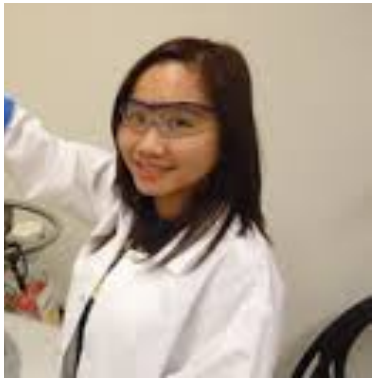

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