20.109

LABORATORY FUNDAMENTALS IN BIOLOGICAL ENGINEERING

MODULE 2

EXPRESSION ENGINEERING

Lecture # 6

Leona Samson

April 9th 2009

DNA Repair and Human Populations

Leona D. Samson

Biological Engineering Department, MIT

Biology Department, MIT

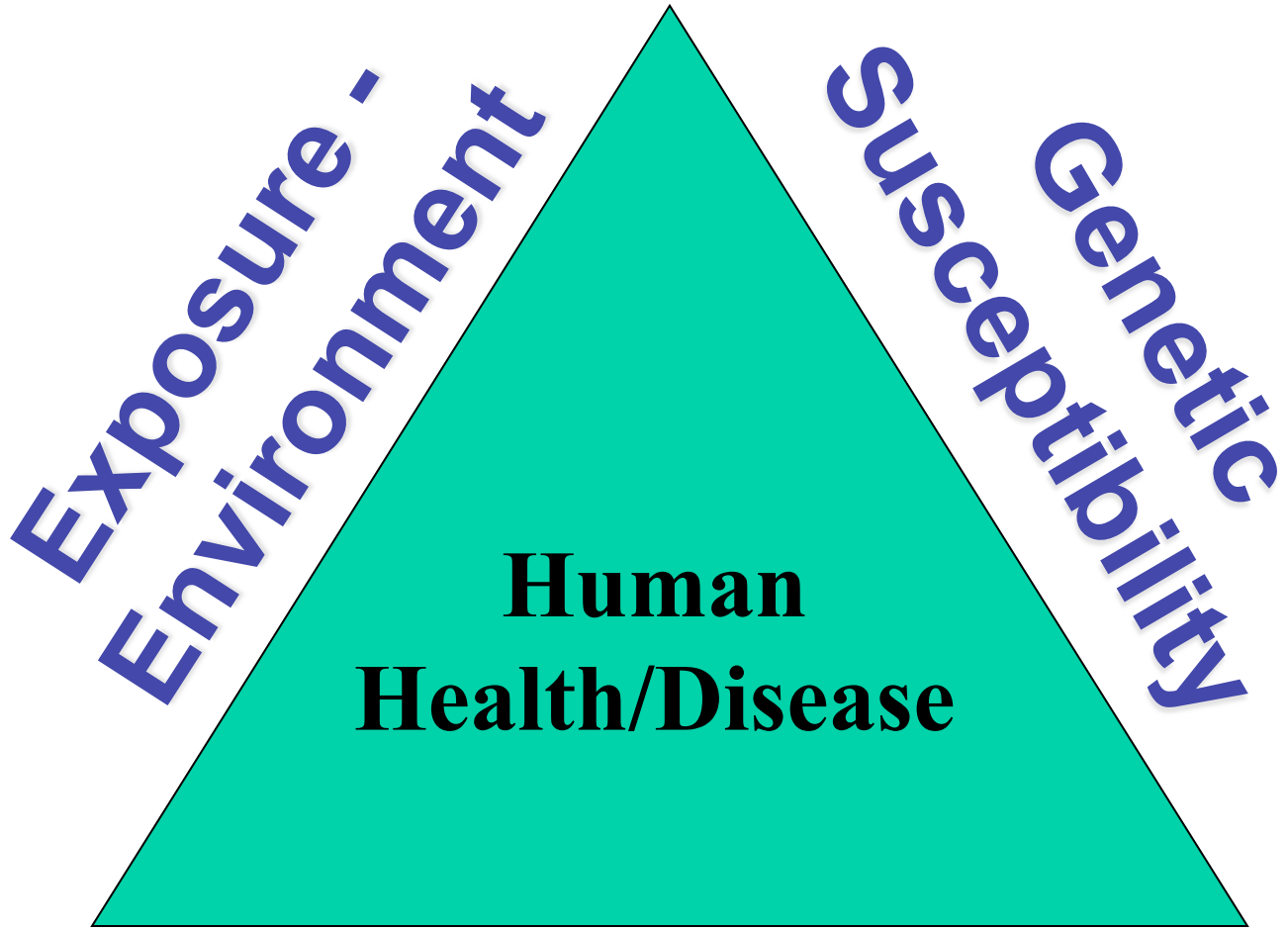
Center for Environmental Health Sciences, MIT

Koch Institute for Integrated Cancer Research, MIT

Computational and Systems Biology Initiative, MIT

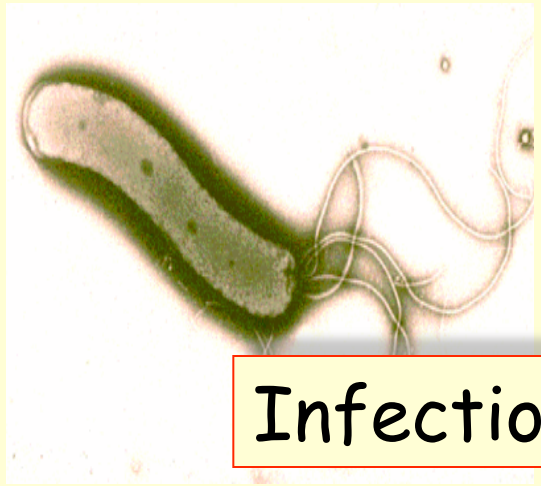
Broad Institute, Harvard & MIT

Toxic agents in our environment
Gene-Environment Interaction



Time/Age/Behavior

Environmental Exposures



Food: www.boarhouse.ru
Drink: www.terlyn.com
Air: www.npl.co.uk
Pharmaceutical: www.butterworth-labs.co.uk
Sun: www.epa.gov
Helicobacter: microbewiki.kenyon.edu

Environmental exposures to potentially harmful agents

Harmful agents


Eat


Medicine


Drink


Absorbed


Breathe


Infection

People have different exposures

People have different responses

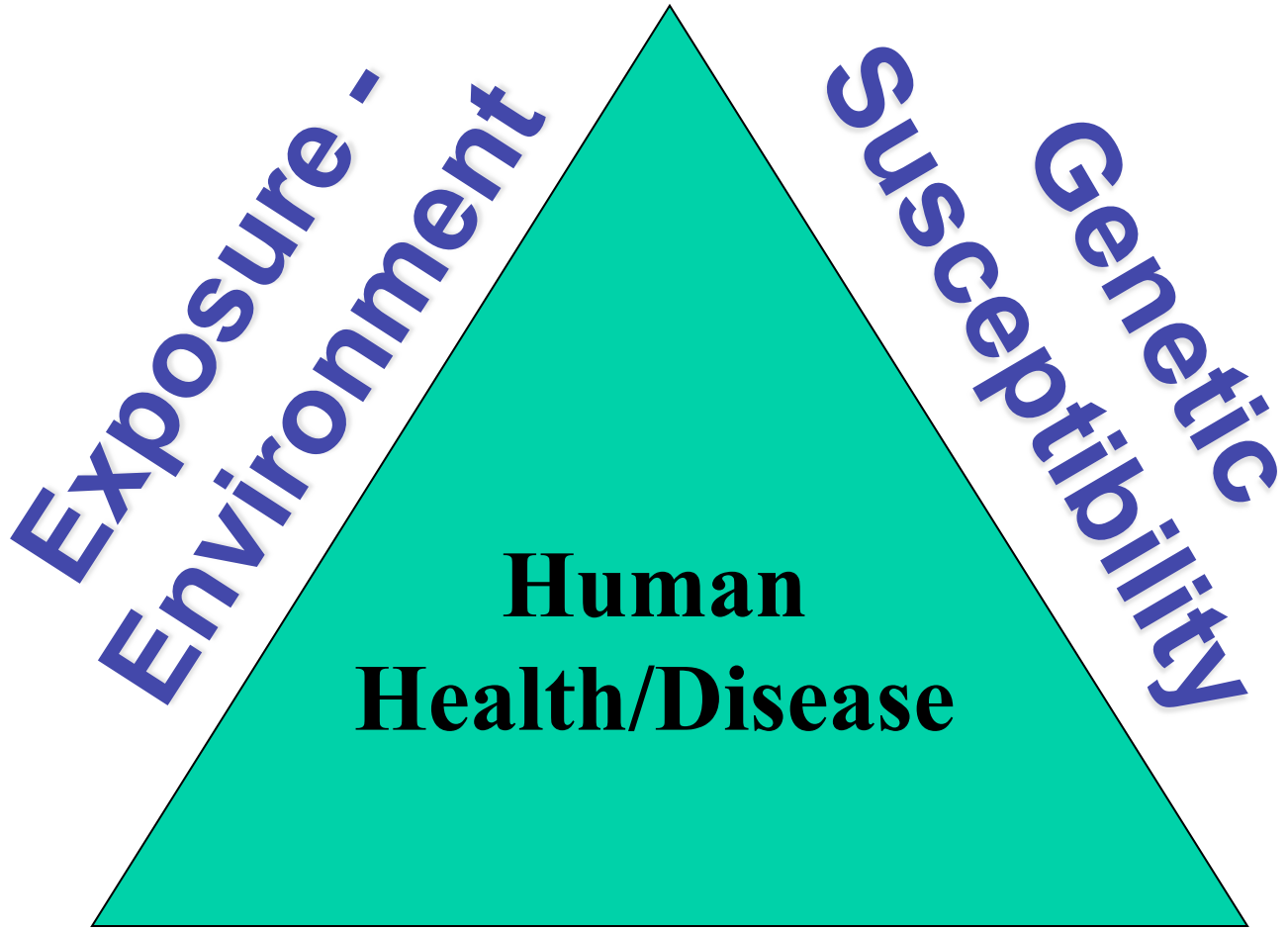
2007 – Breakthrough of the year

Natural sequence variation

single nucleotide polymorphisms (SNPs) every 1000 base pairs.

Compare two people – have about ~ 3 million SNP variants!

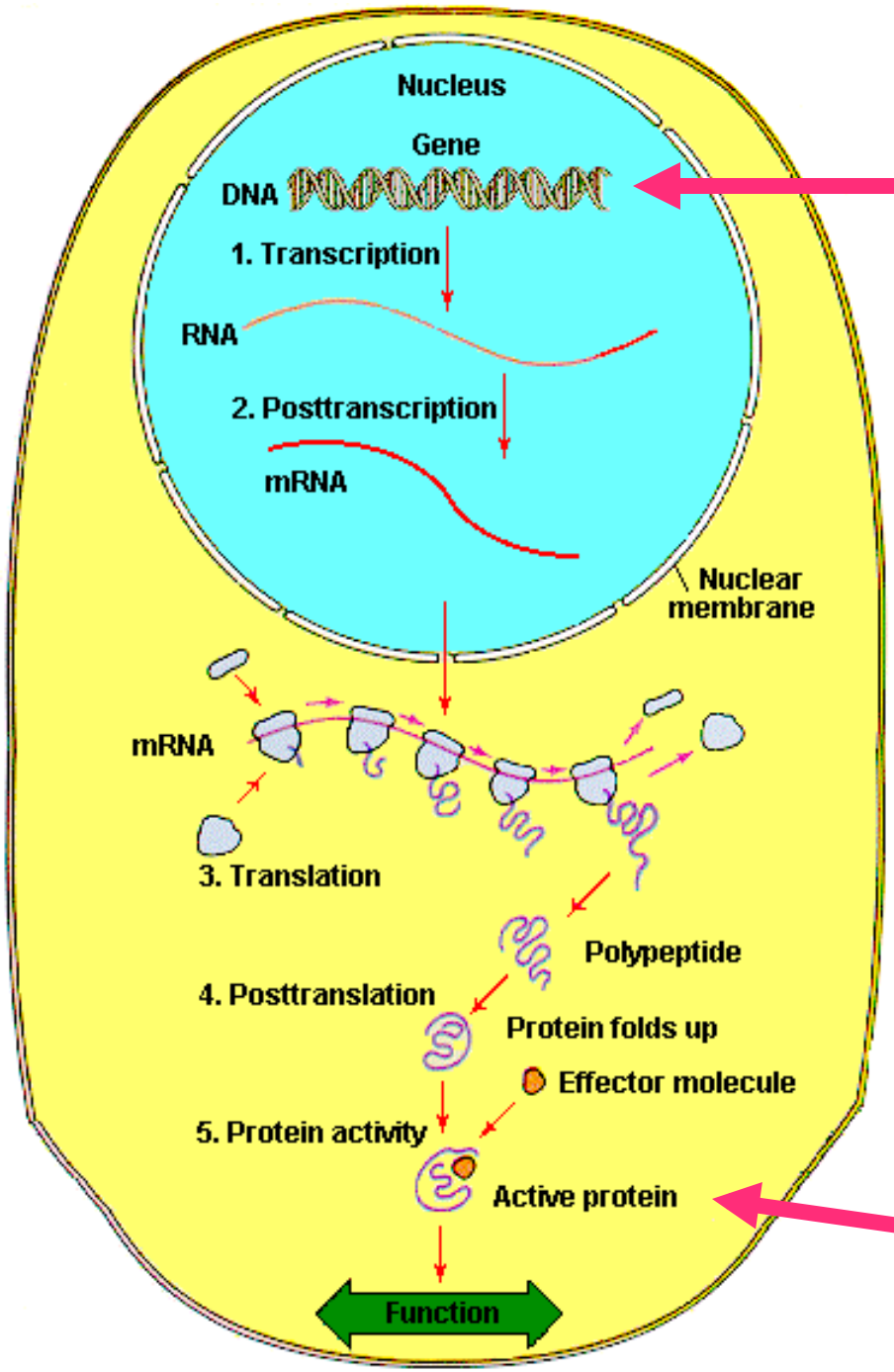
Toxic agents in our environment
Gene-Environment Interaction



Time/Age/Behavior

DNA is constantly being damaged by endogenous and exogenous agents

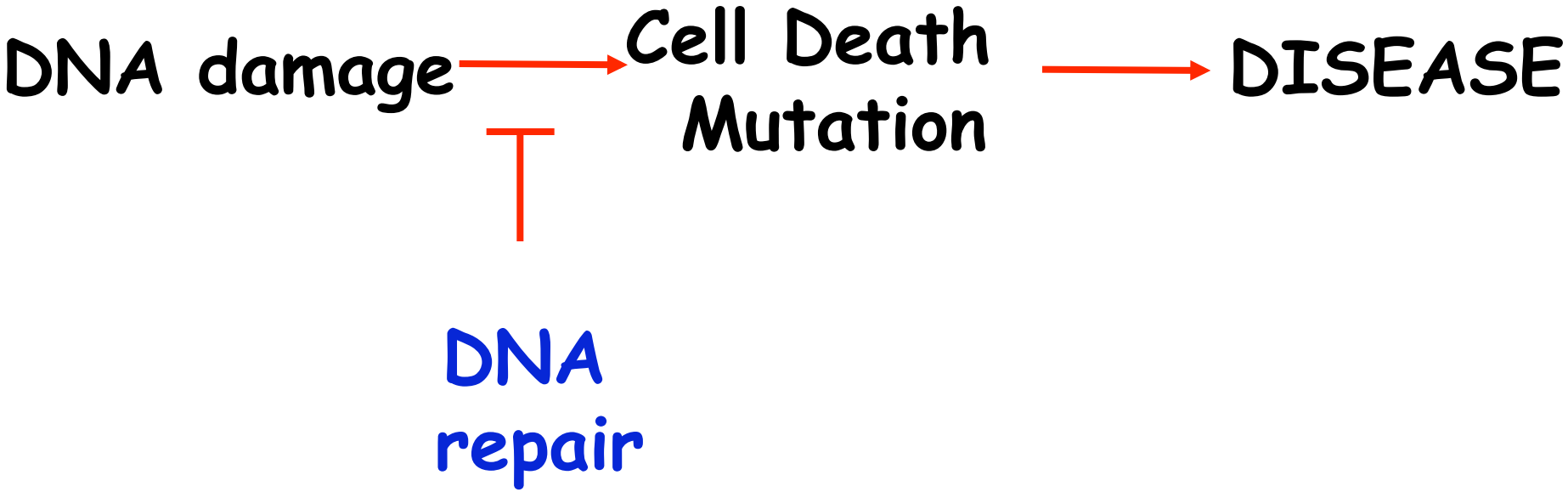
Courtesy of Bevin Engelward

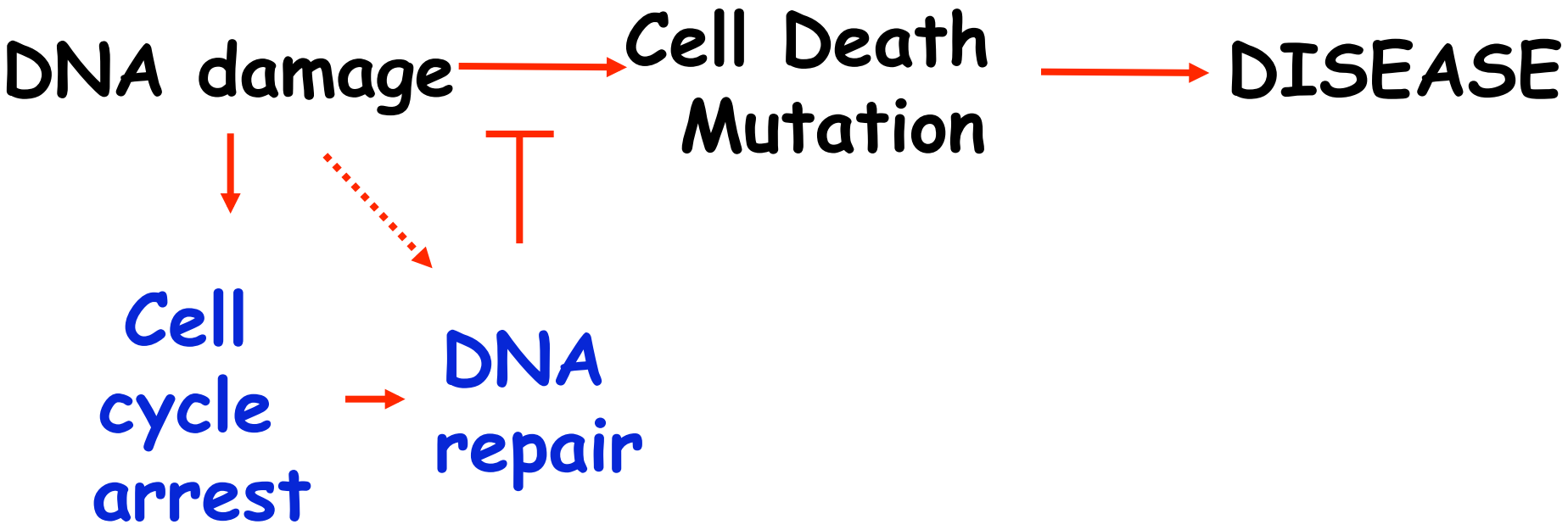


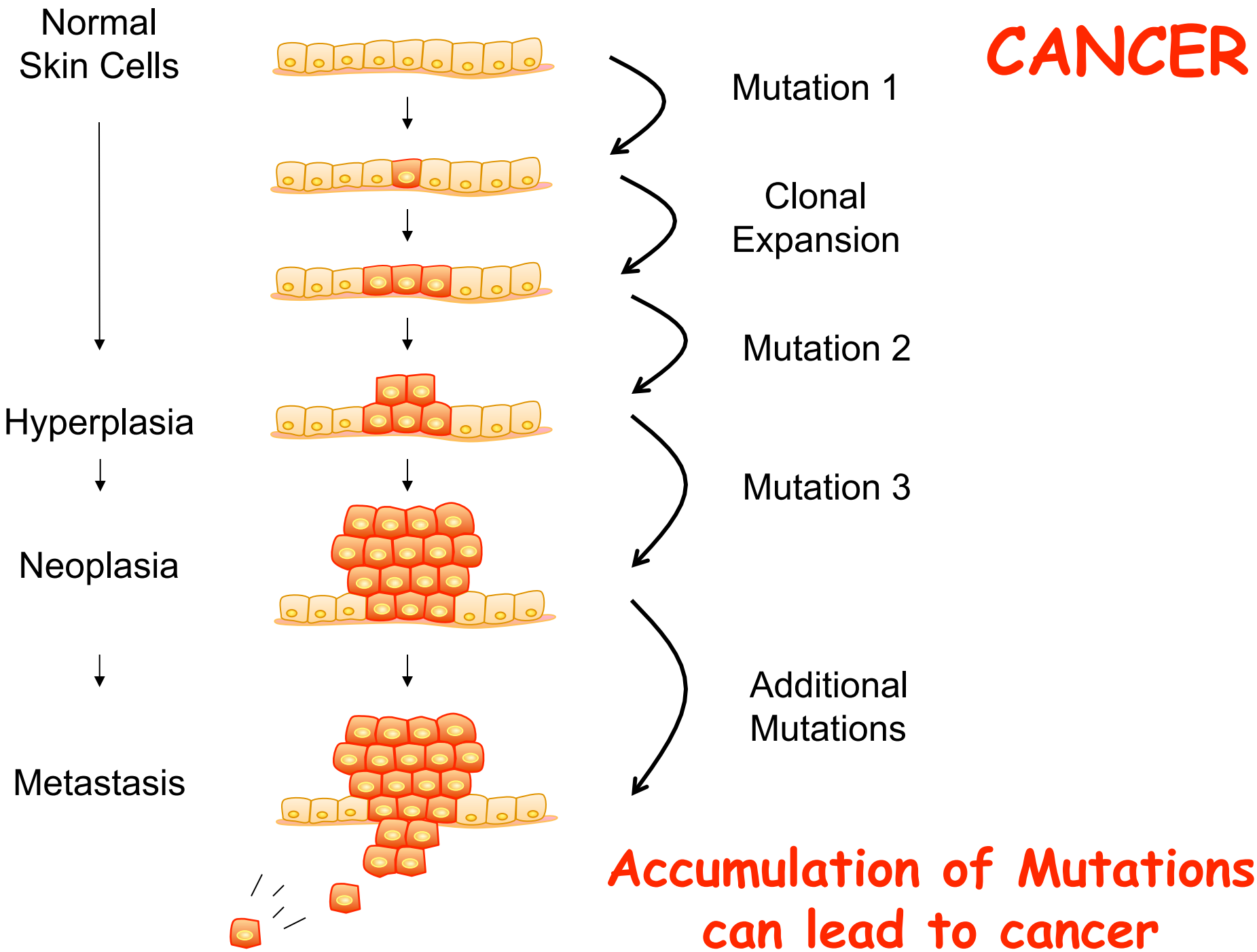
Damage to DNA can create permanent changes in the genetic information

Inactive proteins or proteins with altered function are produced


DNA damage
Cell Death
Mutation
DISEASE


DNA damage
Cell Death
Mutation
DISEASE
DNA
repair


DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair


Normal
Skin Cells
Hyperplasia
Neoplasia
Metastasis
Mutation 1
Clonal
Expansion
Mutation 2
Mutation 3
Additional
Mutations
CANCER
Accumulation of Mutations
can lead to cancer

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915

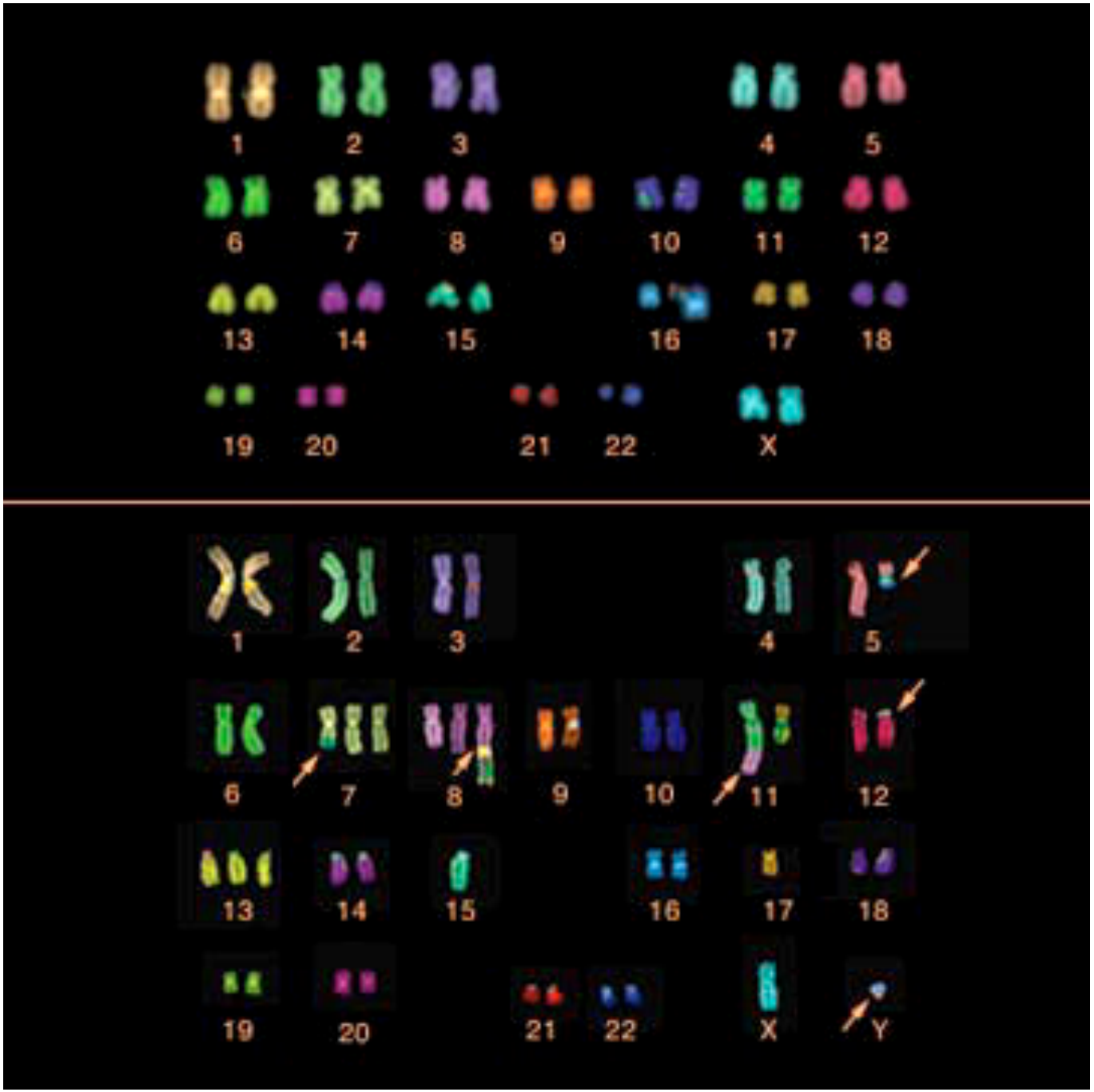
- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.
- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell
- Suggested that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

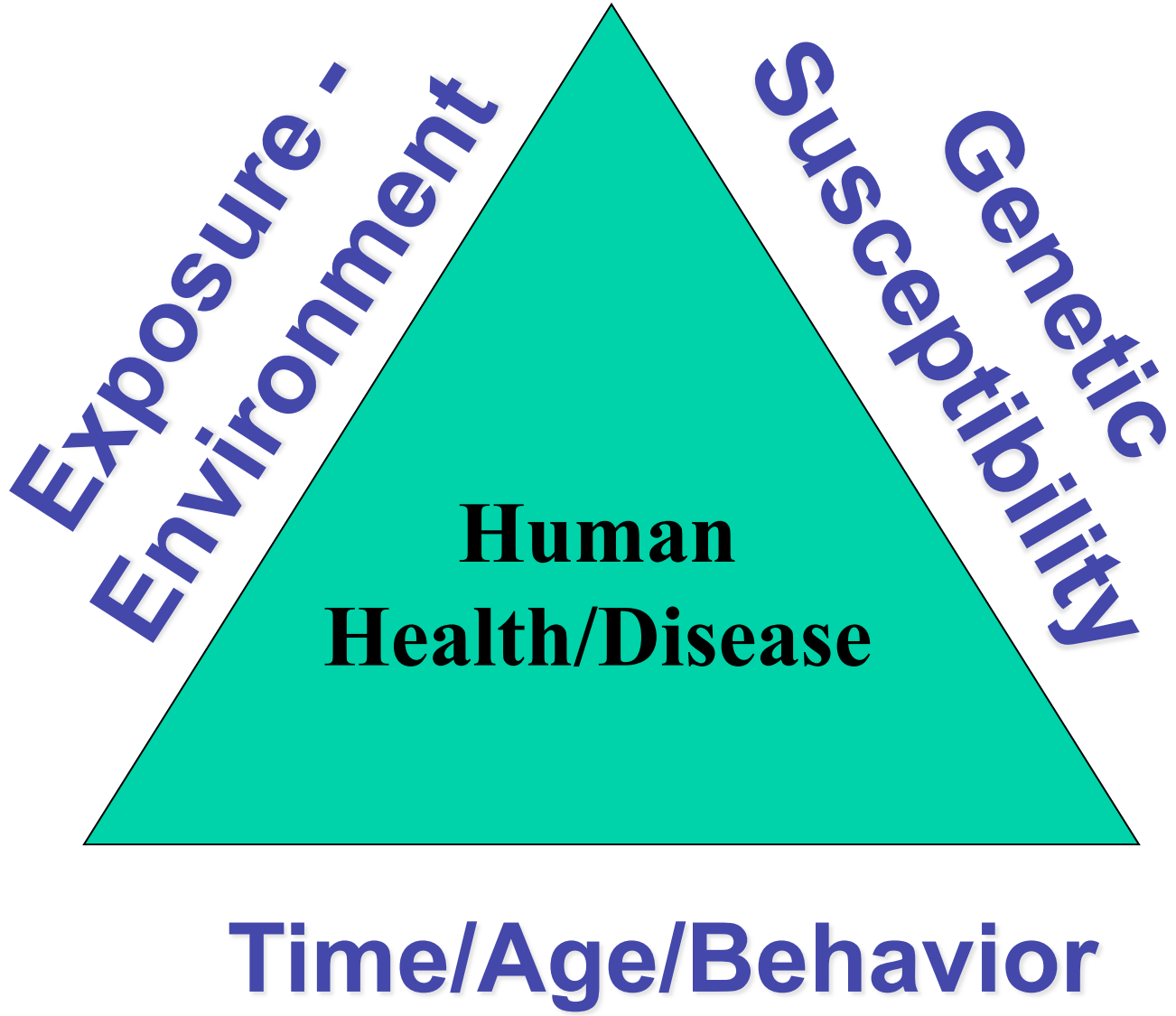


Chromosomes from a Normal cell

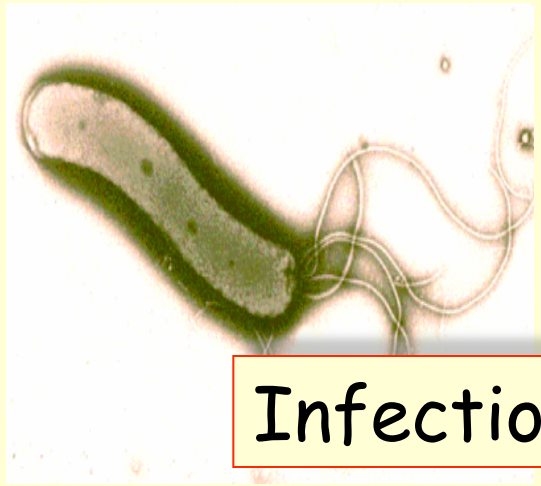
Chromosomes from a Tumor cell

Chromosomes from a Pancreatic Tumor Cell

Why do we care about DNA damaging agents in our environment??



Environmental Exposures



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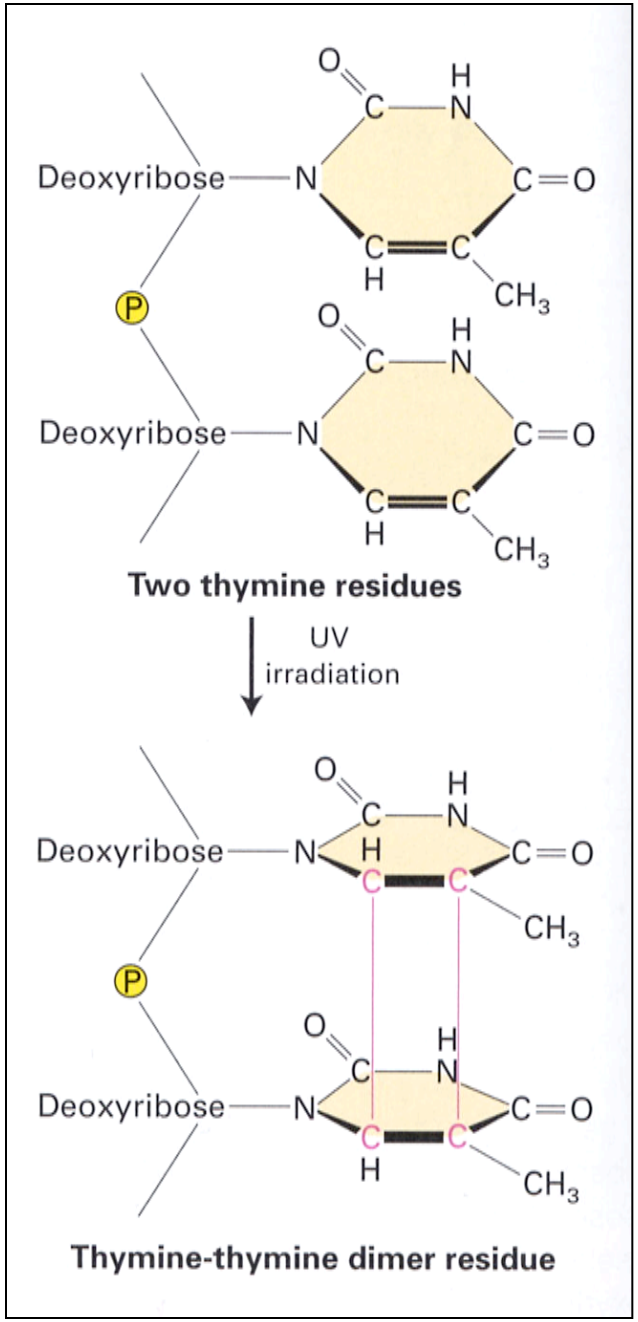
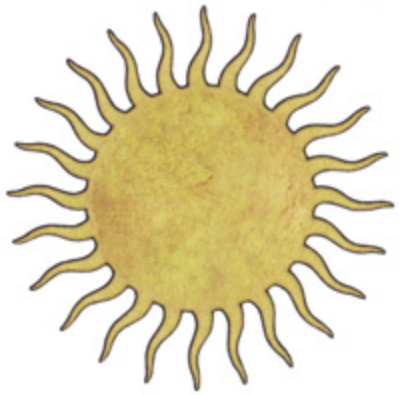
Sun: www.epa.gov
Helicobacter: microbewiki.kenyon.edu

One dramatic example - Xeroderma Pigmentosum

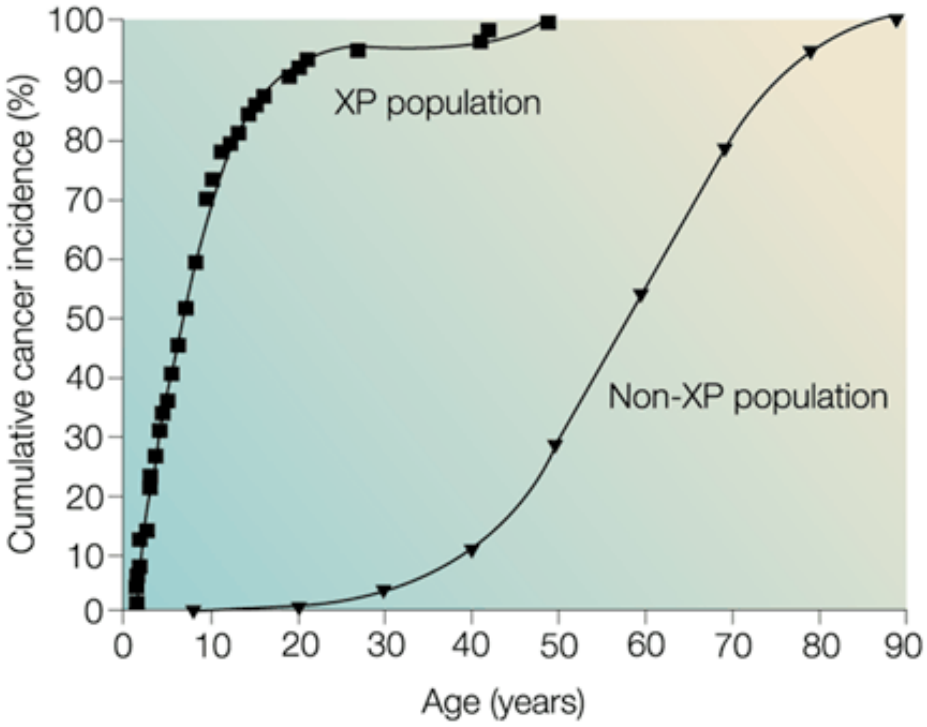




Before

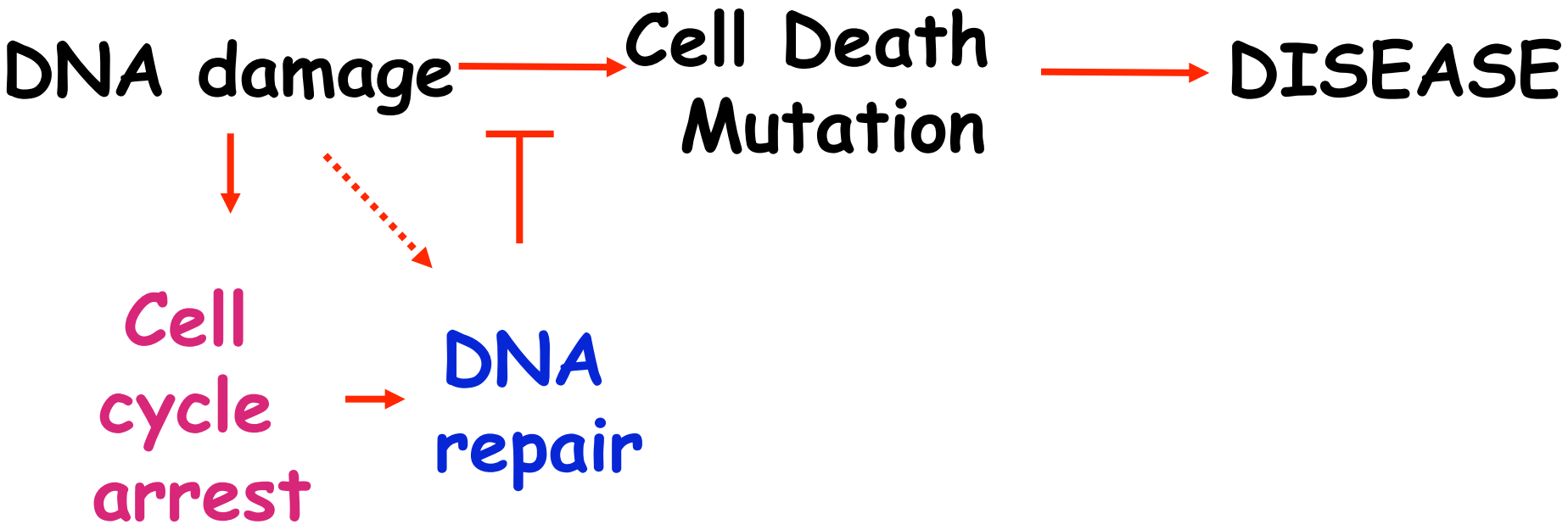
After

Lack of DNA Repair speeds up the carcinogenic process, presumably because mutations accumulate more rapidly

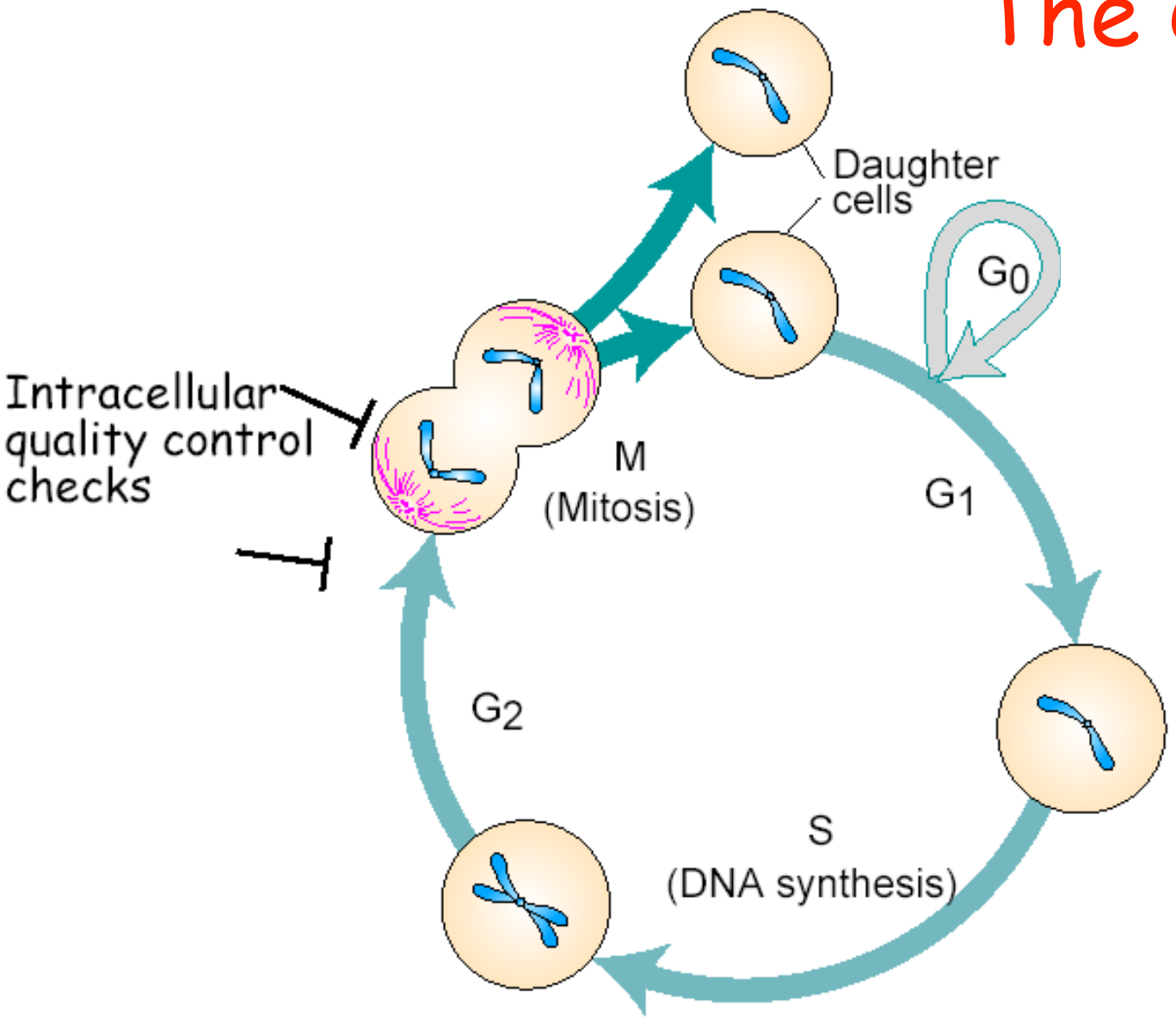


Nature Reviews

Another dramatic example – deficiencies in DNA damage induced cell cycle arrest

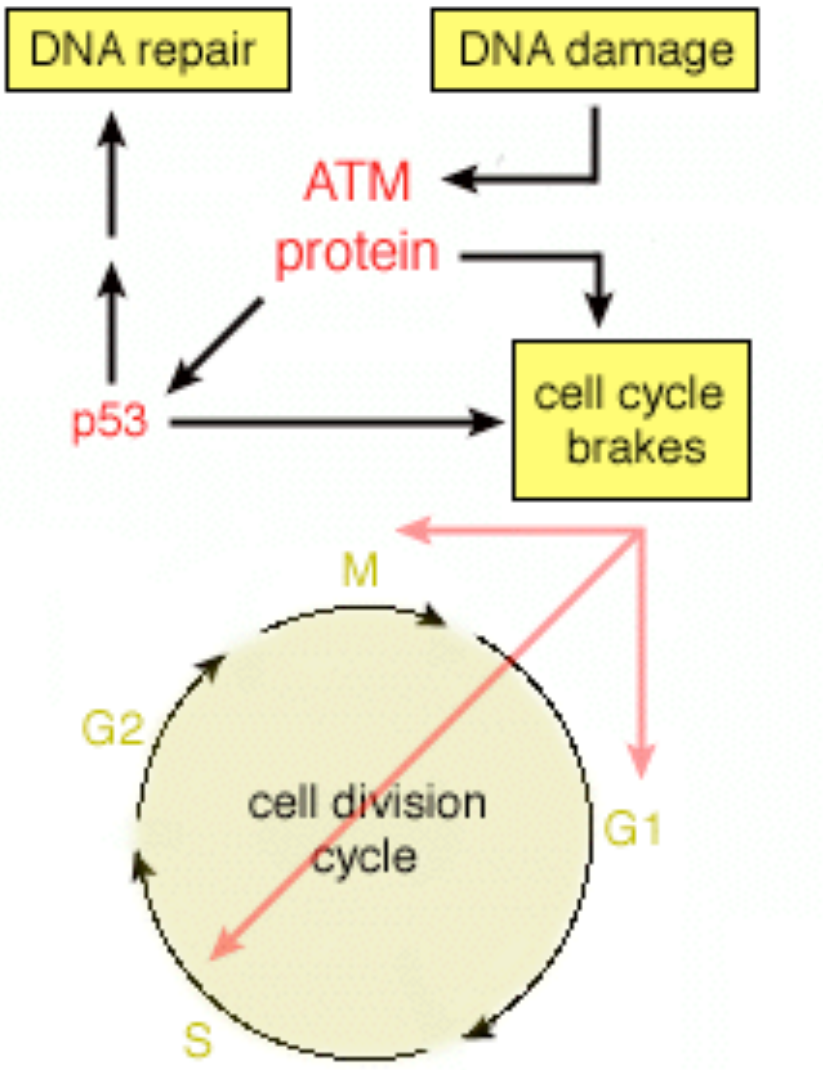


The Cell Cycle



Duplication of chromosomes
DNA Replication

Ataxia Telangiectasia



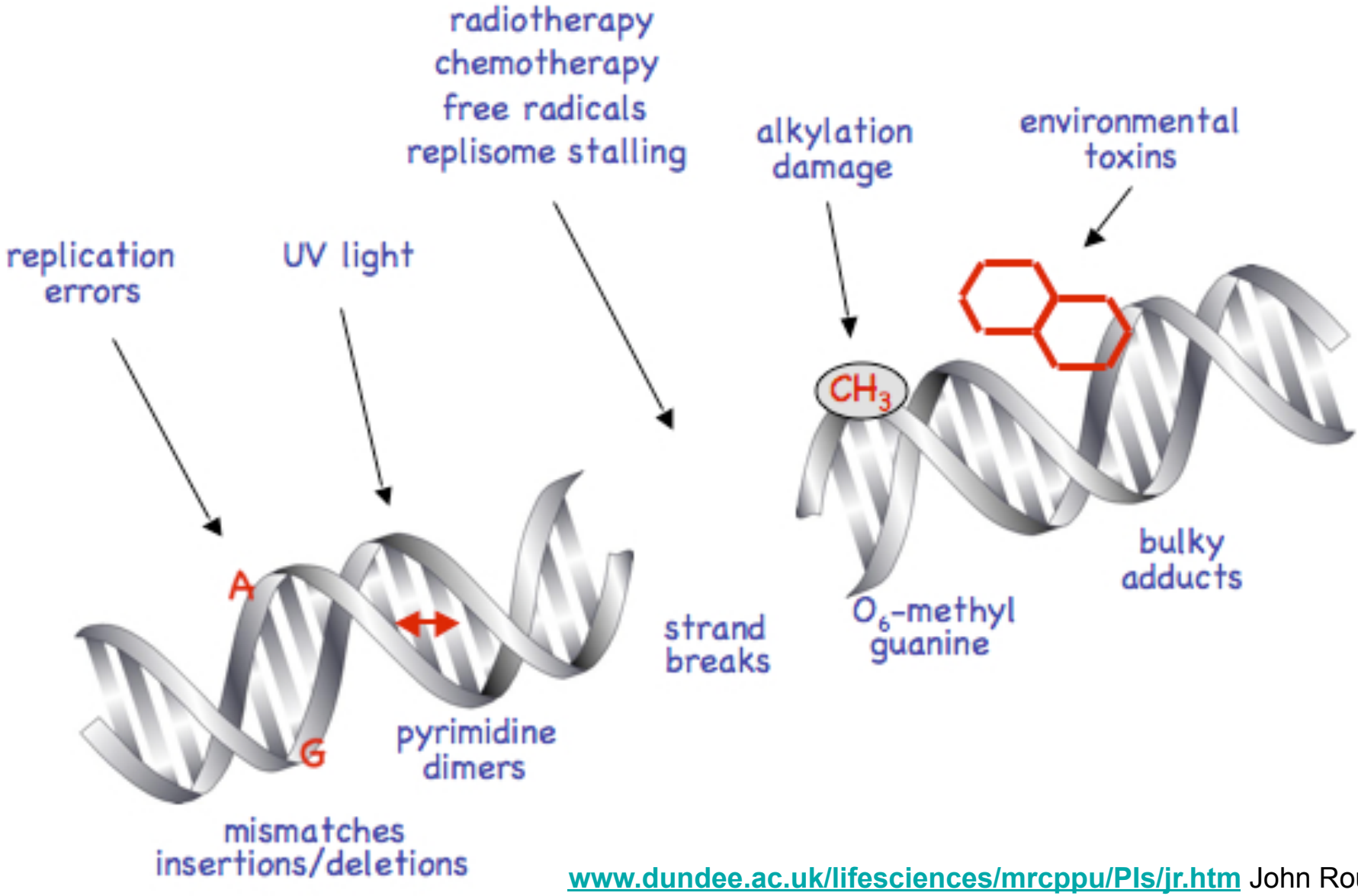
The ATM protein mediates responses to DNA damage, in particular those that control progression through the cell cycle.

THE FIRST SIGNS of ataxia telangiectasia (A-T) usually appear in the second year of life as a lack of balance and slurred speech. It is a progressive, degenerative disease characterized by cerebellar degeneration, immunodeficiency, radiosensitivity (sensitivity to radiant energy, such as x-ray) and a predisposition to cancer.

Ataxia Telangiectasia – Cancer Prone

Defective DNA Damage Responses can affect both neurodegeneration and cancer susceptibility

DNA damage from many different sources



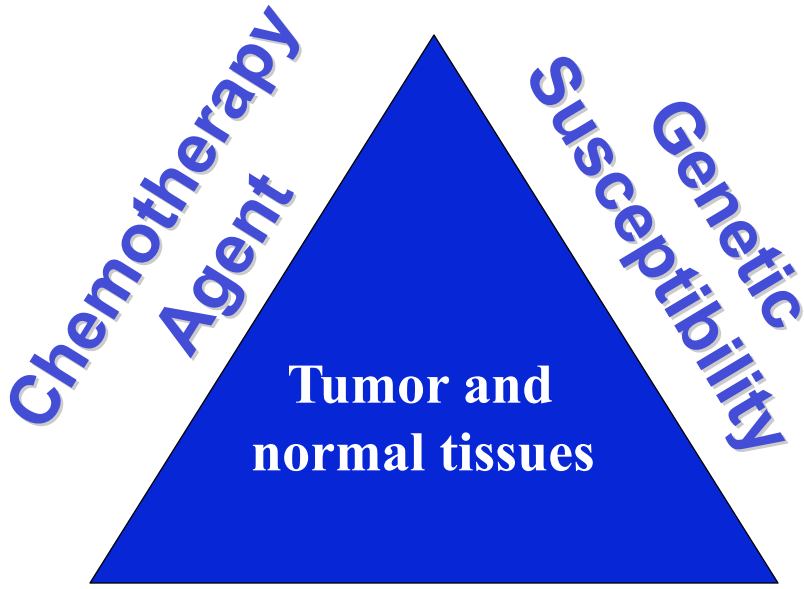
www.dundee.ac.uk/lifesciences/mrcppu/PIs/jr.htm John Rouse

DNA damaging agents for treating cancer Chemotherapy and Radiotherapy

Human Susceptibility

Time/Age/Behavior

Tumor Susceptibility



Treatment Regimen

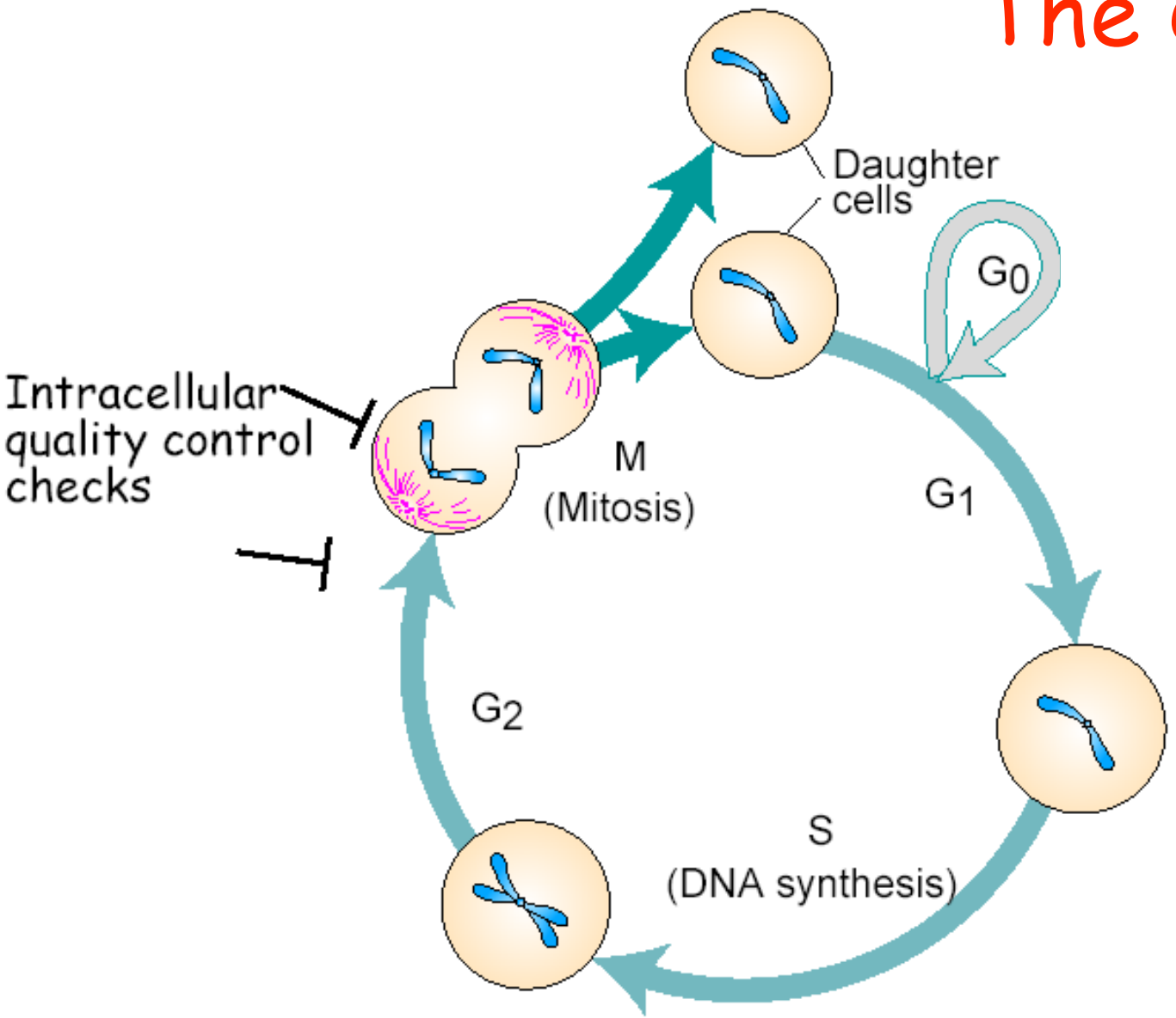
How do genes get mutated?

How can we stop them from being mutated?

How do genes get mutated?

How can we stop them from being mutated?

The Cell Cycle

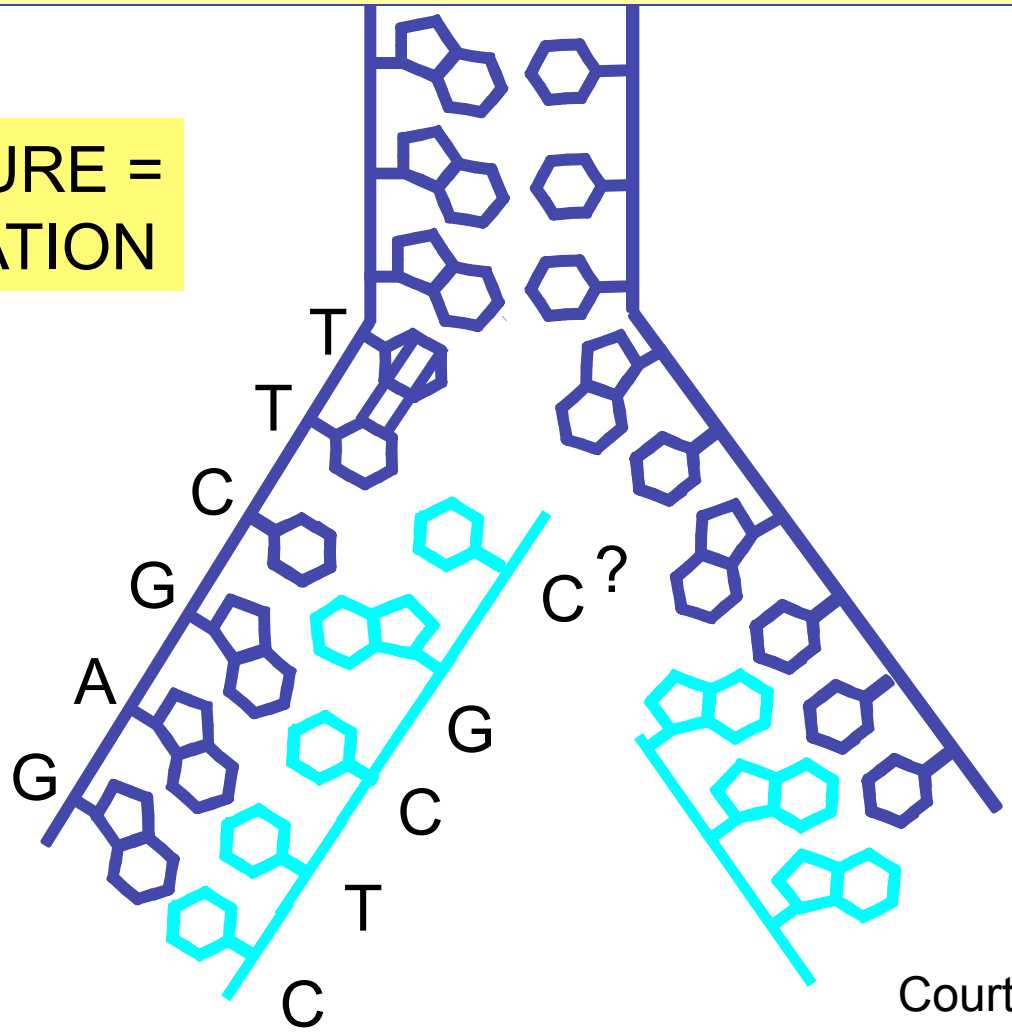


Duplication of chromosomes
DNA Replication

DNA Damage Causes Mutations

...So, Sunlight can lead to DNA damage that causes Mutations

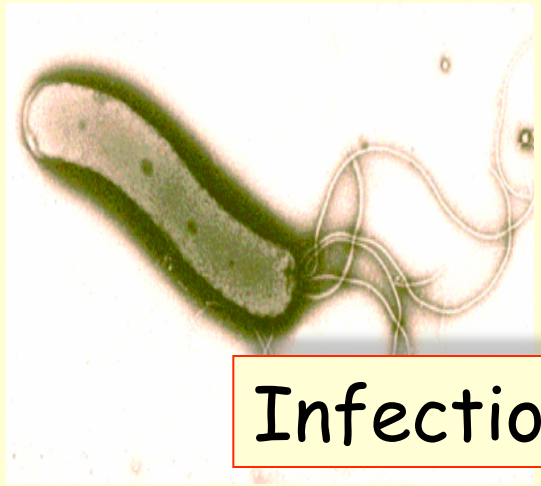
STRUCTURE = INFORMATION



A → C

Courtesy of Bevin Engelward

Environmental Exposures



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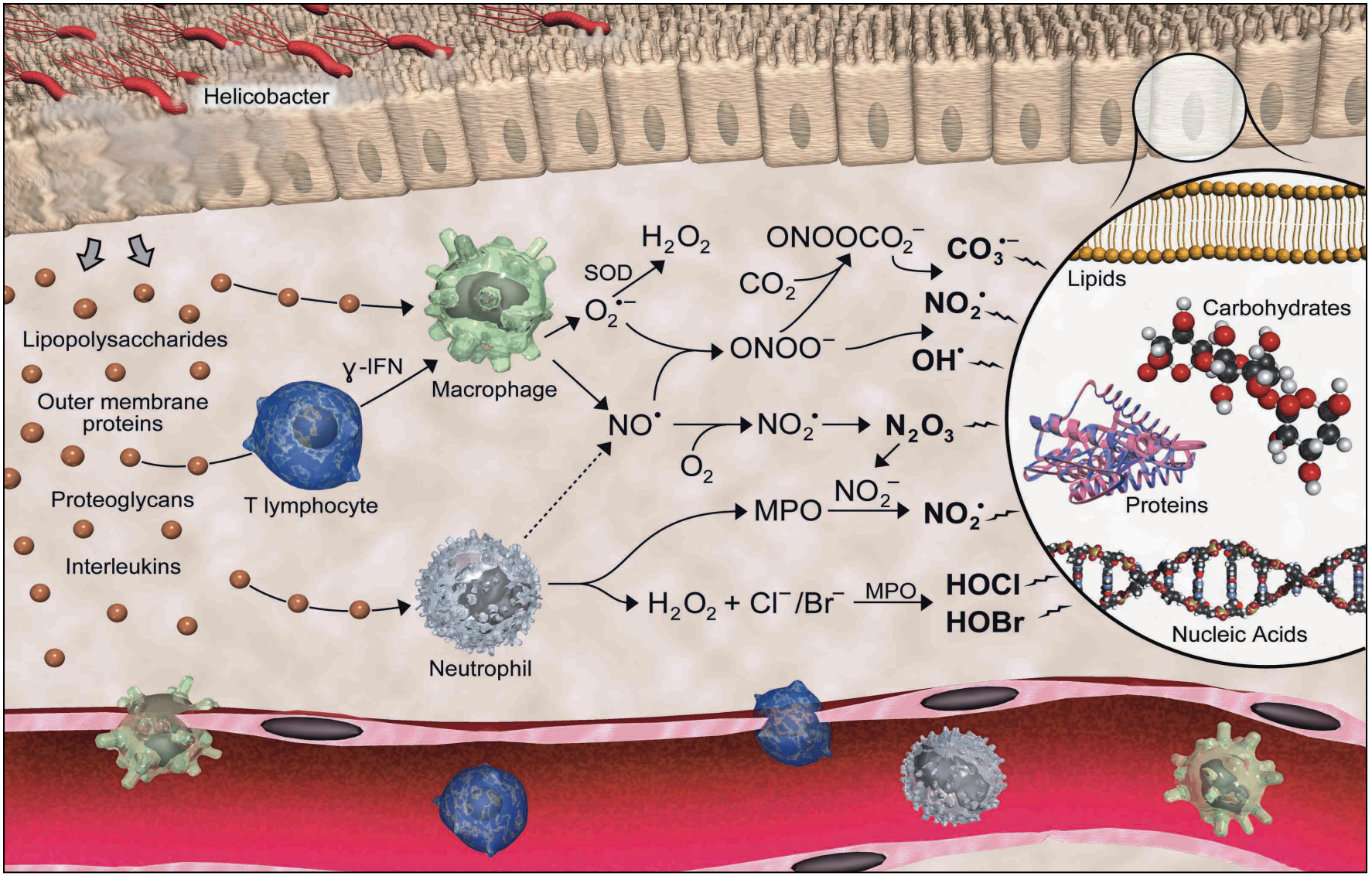
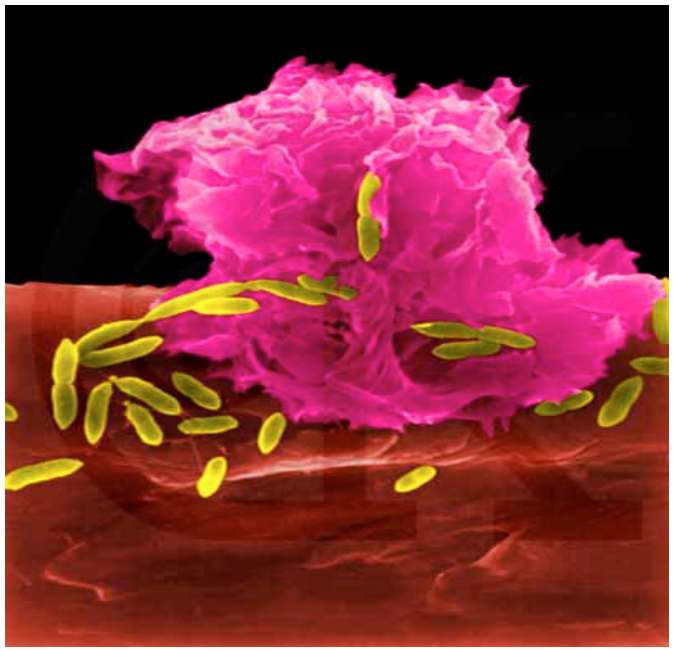
Helicobacter: microbewiki.kenyon.edu

Inflammation-induced DNA damage

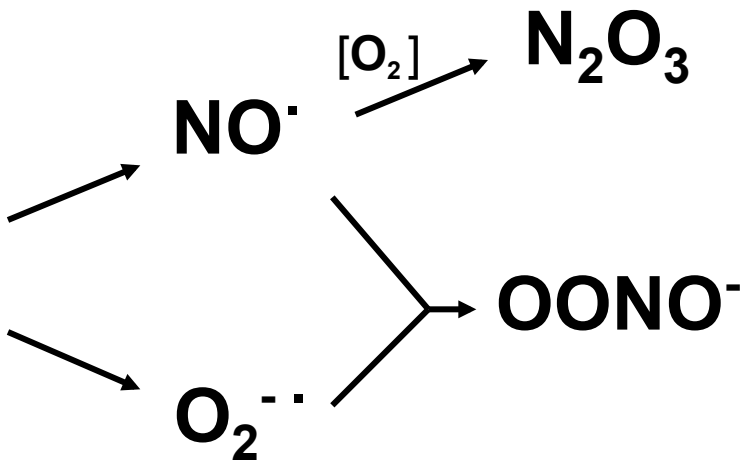


Oxidized or deaminated bases (including several AAG substrates)

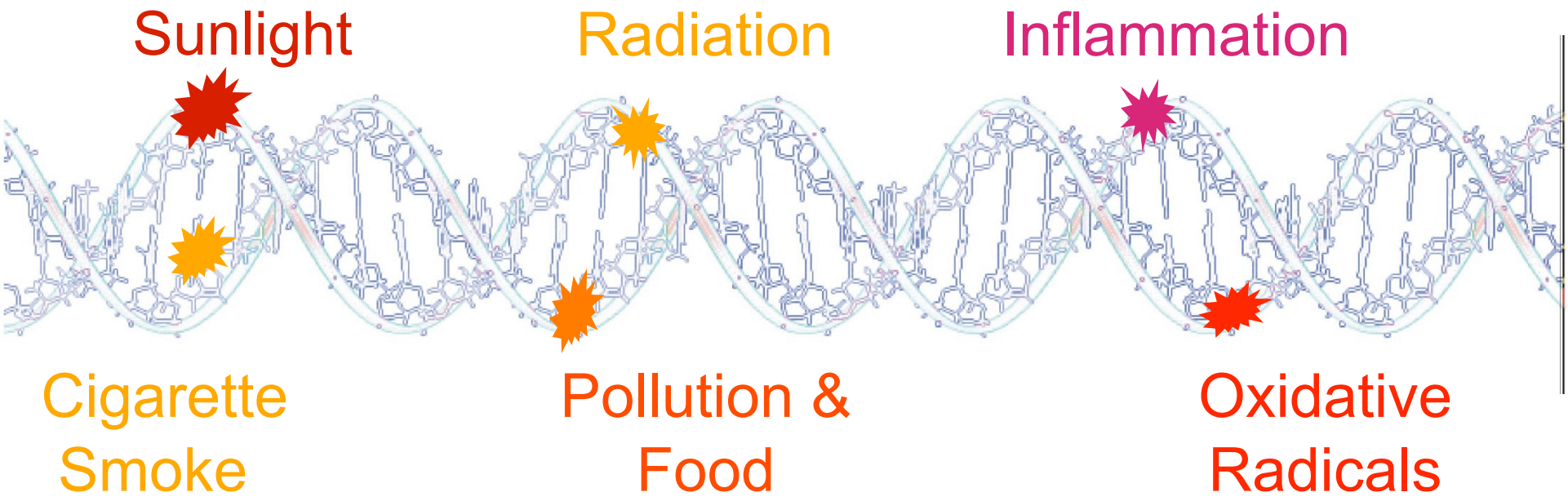
Activated
Macrophage



SEM (x10.000)
www.DennisKunkel.com



Macrophages bombard invading microbes and adjacent tissues with genotoxic chemicals

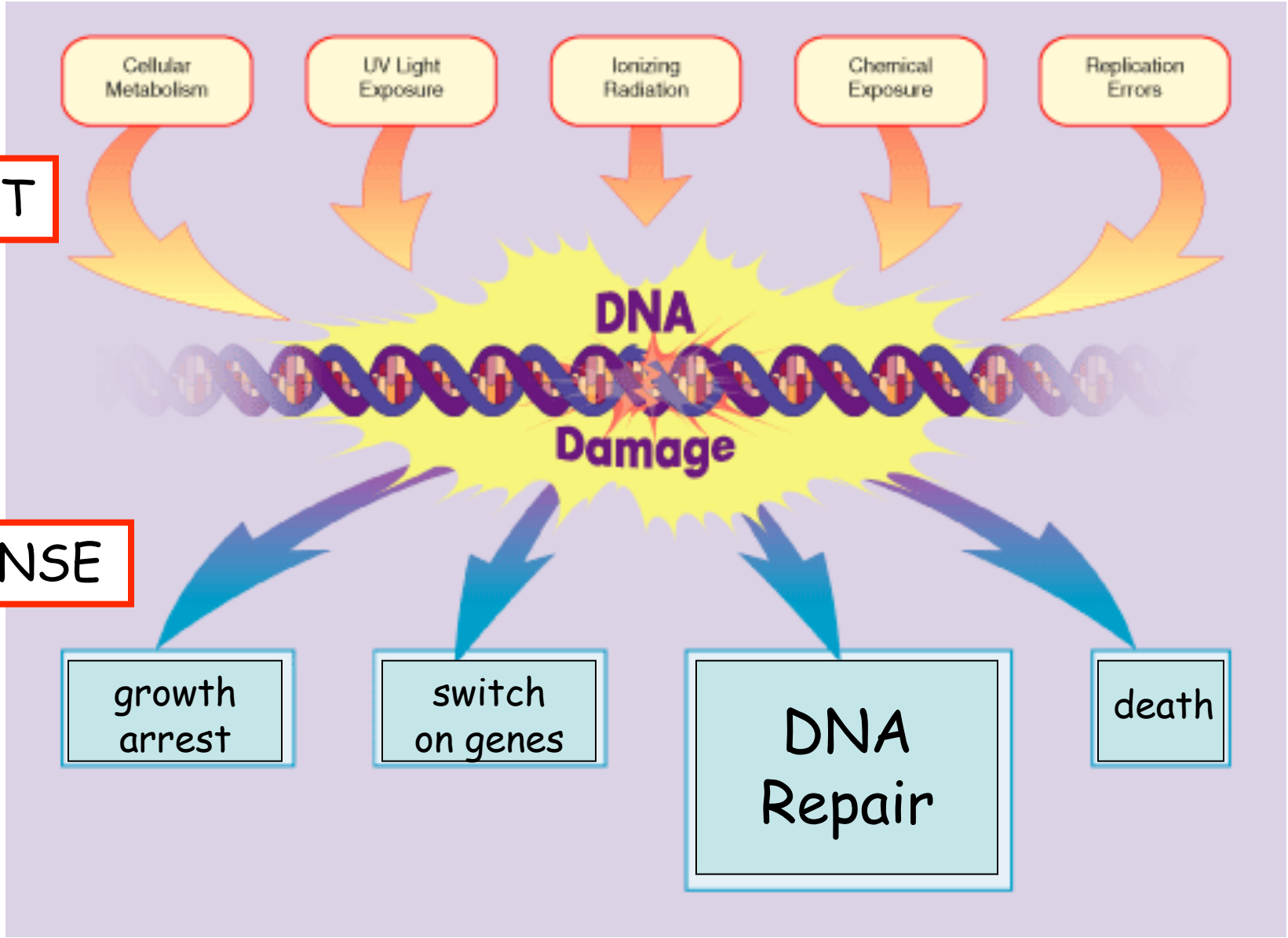

Sunlight
Radiation
Inflammation
Cigarette Smoke
Pollution & Food
Oxidative Radicals

How do genes get mutated?

How can we stop them from being mutated?

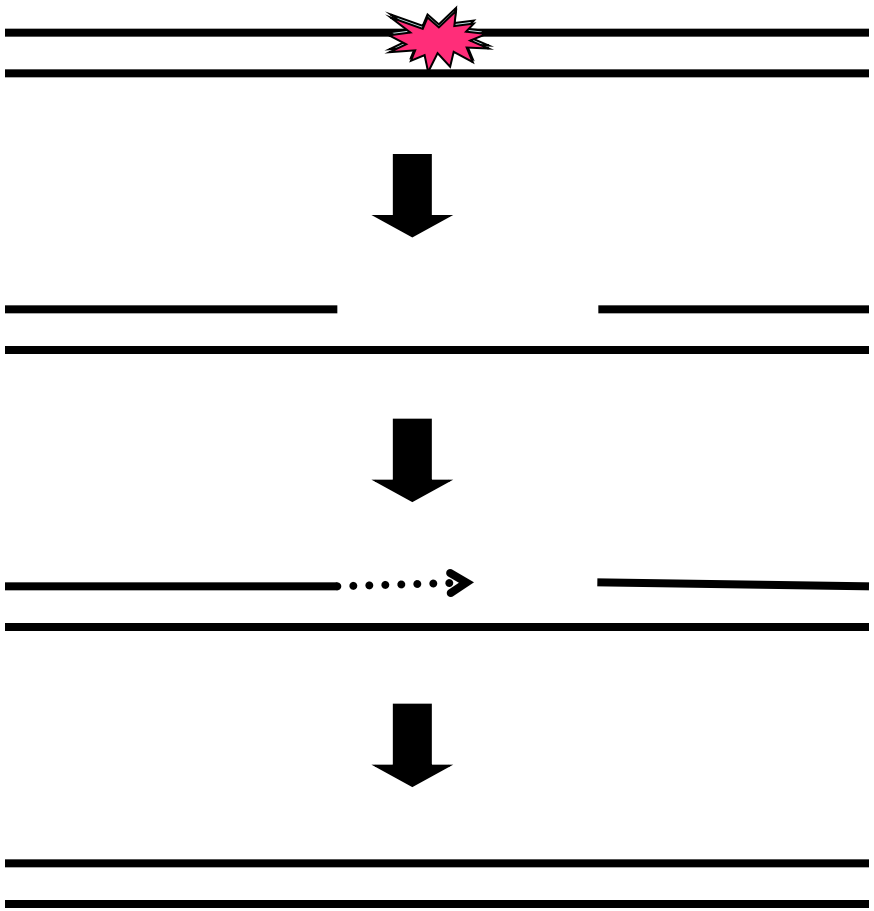
INSULT

RESPONSE



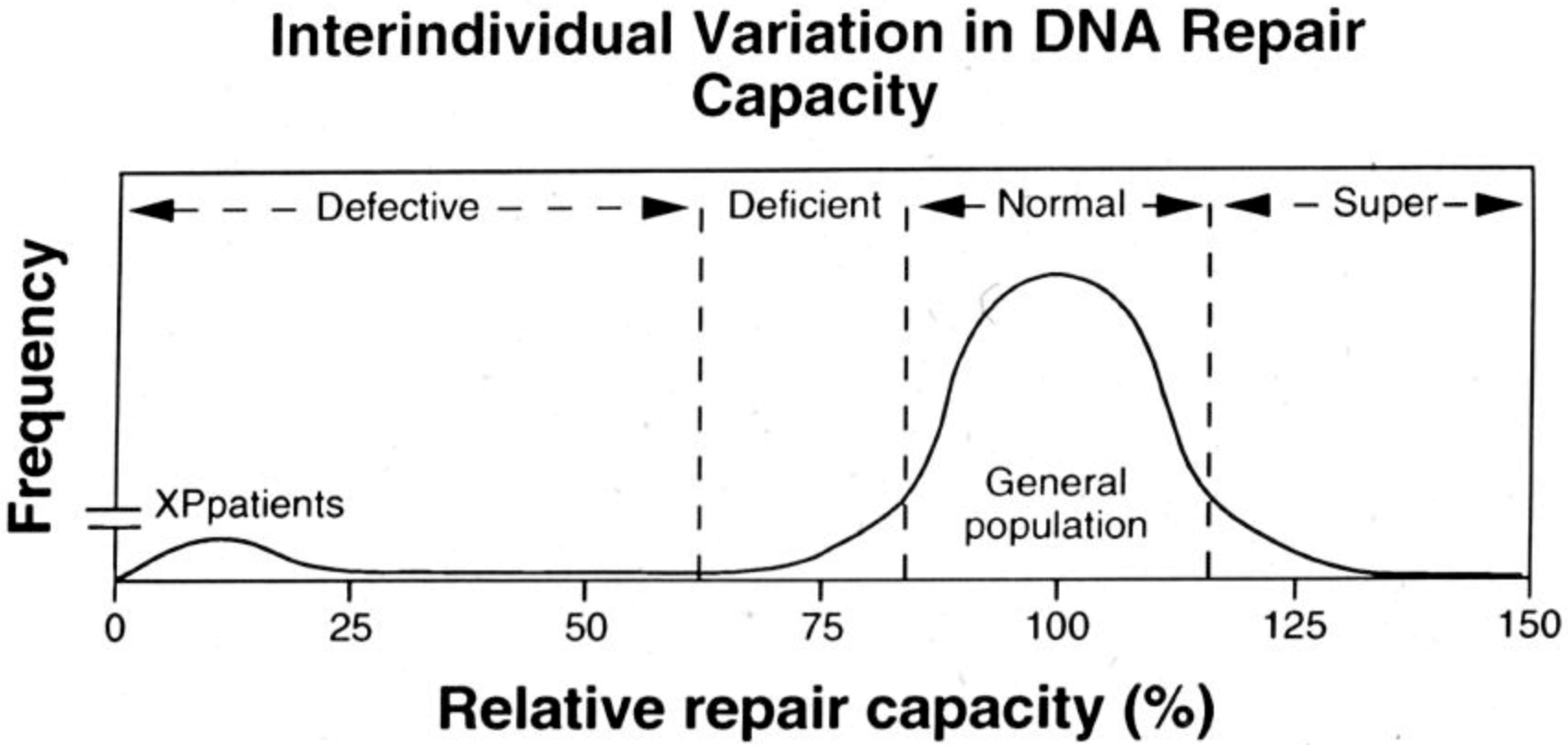
All these responses to DNA damage serve to prevent mutations accumulating, and thus prevent CANCER

DNA Repair



- Direct Repair
- Base Excision Repair
- Nucleotide Excision Repair
- Transcription Coupled Repair
- Mismatch Repair
- Recombination Repair

Xeroderma Pigmentosum ~ 1/250,000



Wei *et al.*, Clinical Chemistry, Vol. 41, No. 12, 1995

DNA Repair Strategies

- Direct Reversal

  Photolyase, Methyltransferase,Oxidative demethylase

- Excision Repair

  Base excision, nucleotide excision, mismatch repair

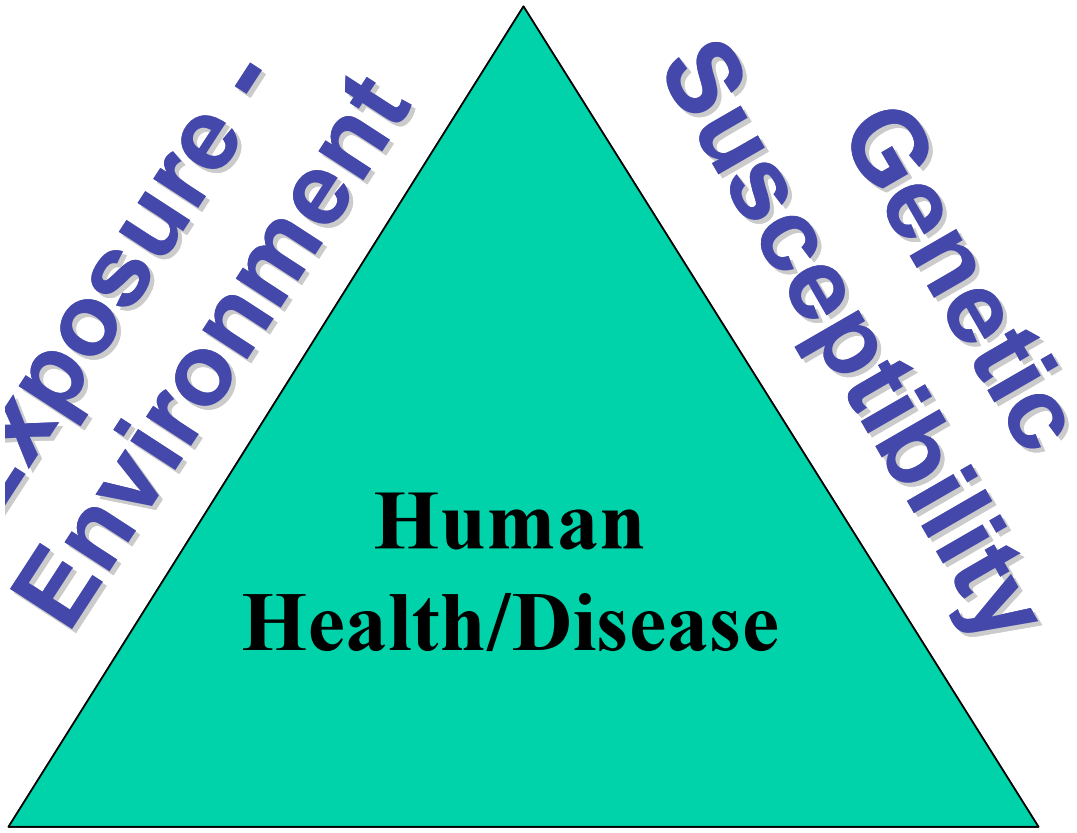
- Lesion Avoidance

  Translesion synthesis, DNA recombination

- Double strand break repair

  Homologous recombination, Non-homologous end joining

Many genes can influence whether or not an environmental exposure leads to disease



Sources of DNA Alkylating Agents

Exogenous

Tobacco Smoke

Fuel Combustion Products

Food Constituents

Food Preservatives

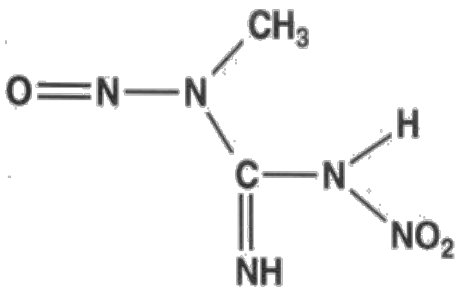
Chemotherapuetic Agents

Endogenous

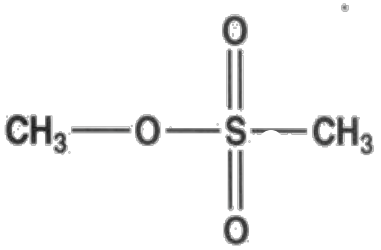
S-Adenosylmethionine

Nitrosation of Amines

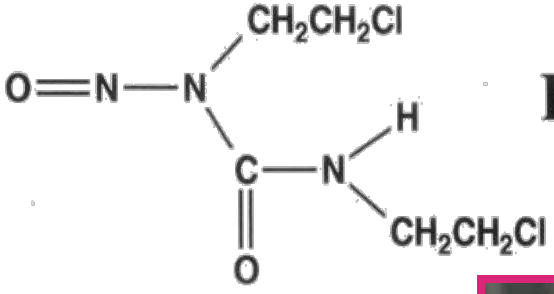
Lipid Peroxidation



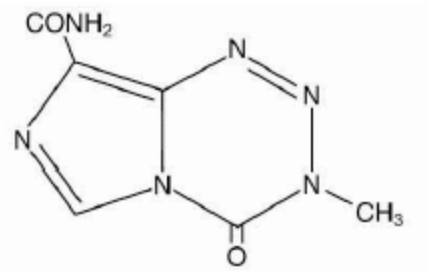
MNNG



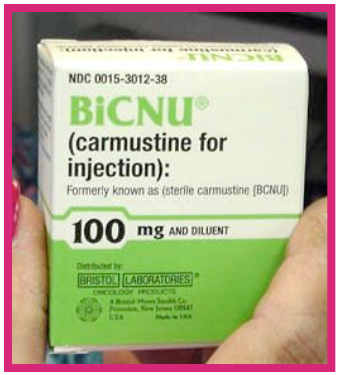
MMS



BCNU



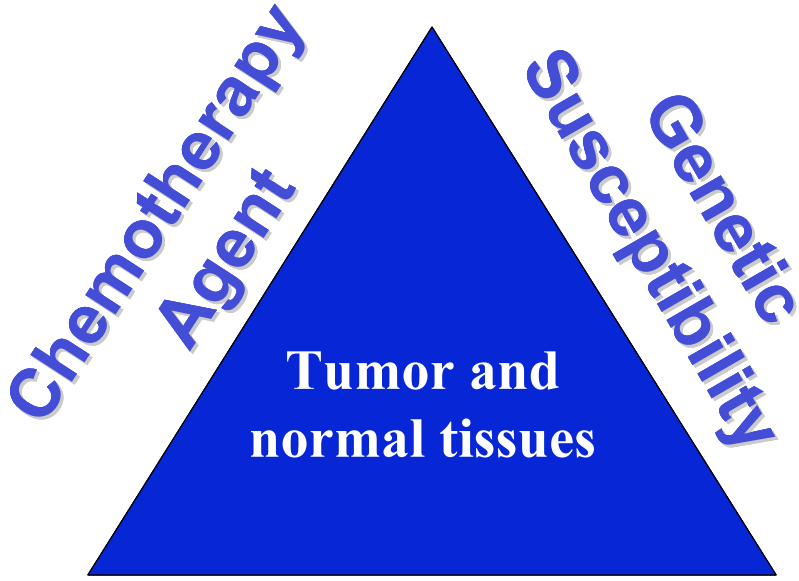
Temozolomide



Many genes can also influence whether or not cancer chemotherapy is effective

Gene-Environment Interaction

CANCER TREATABILITY



Environmental exposures to potentially harmful agents

Harmful agents


Eat


Medicine


Drink


Absorbed


Breathe


Infection

People have different exposures

People have different responses

Inter-individual responses to DNA damaging agents

Inter-individual responses to DNA damaging agents

- Goals
  - Establish range of inter-individual responses to DNA damaging agents
  - Identify genetic factors that may predict individual response to exposure

Inter-individual responses to DNA damaging agents

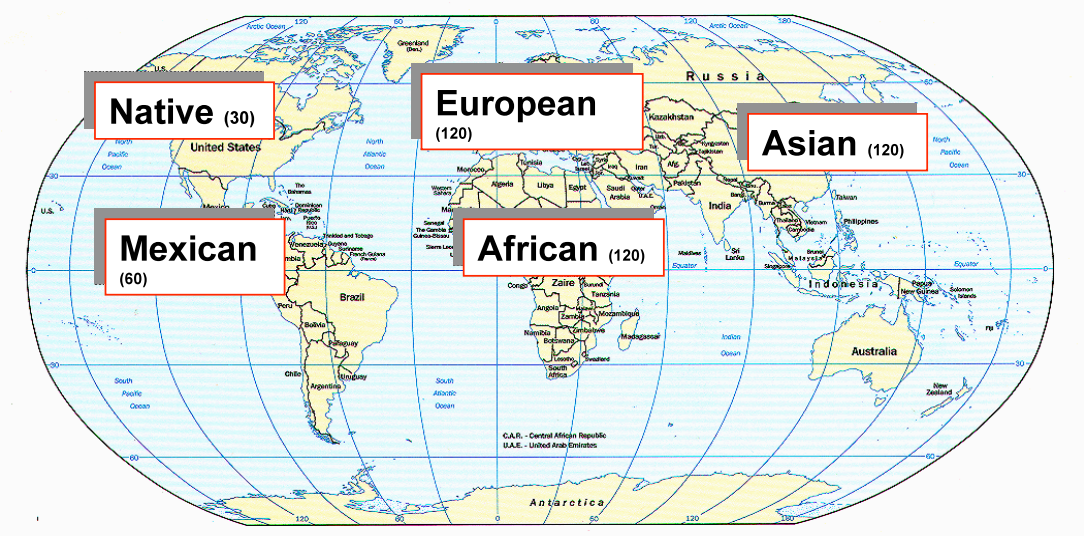



Rebecca C. Fry **Chandni Valiathan** **J. Peter Svensson** **Emma Wang**

PLUS

Brad J. Hogan, Sanchita Bhattacharya, James M. Bugni, Charles A Whittaker

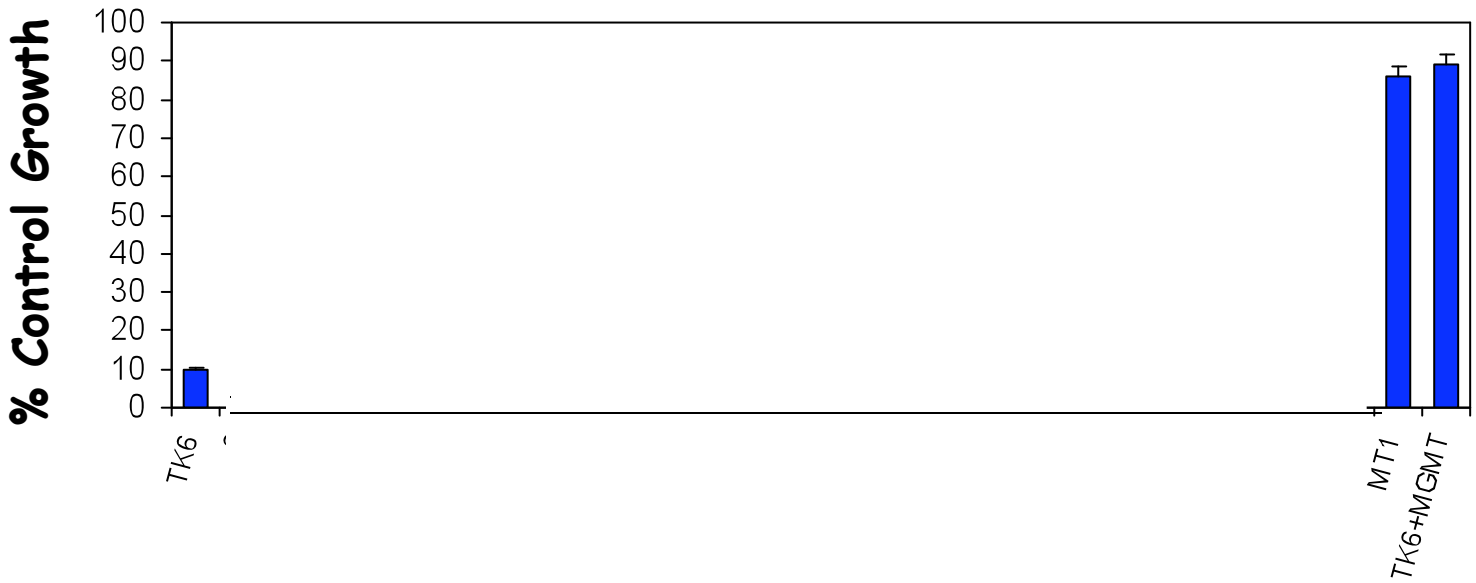
Coriell Cell Lines Representing a Healthy Genetically Diverse Population



450 healthy, unrelated individuals

24 cell lines – nested subset

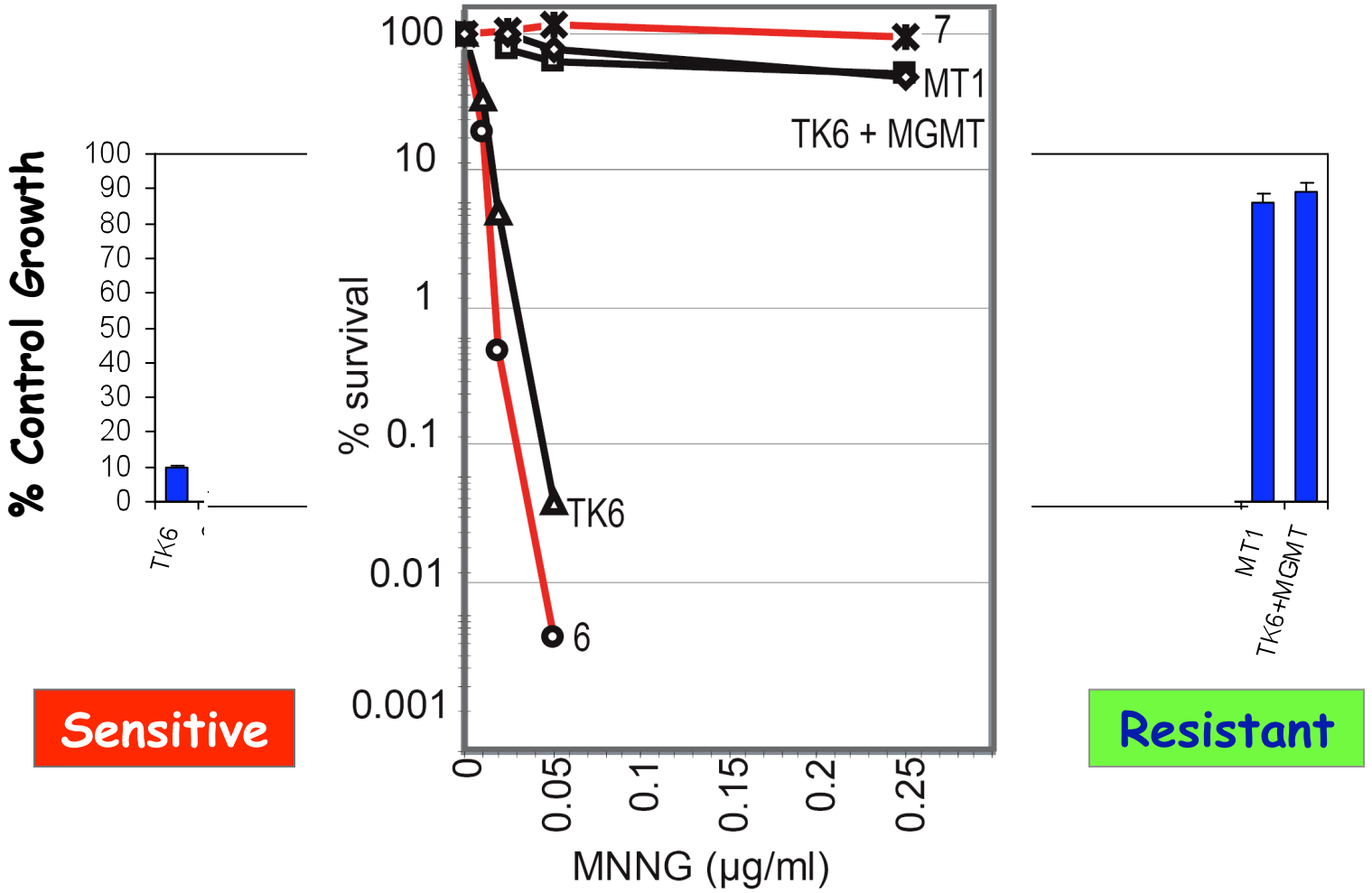
Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Control Cell Lines



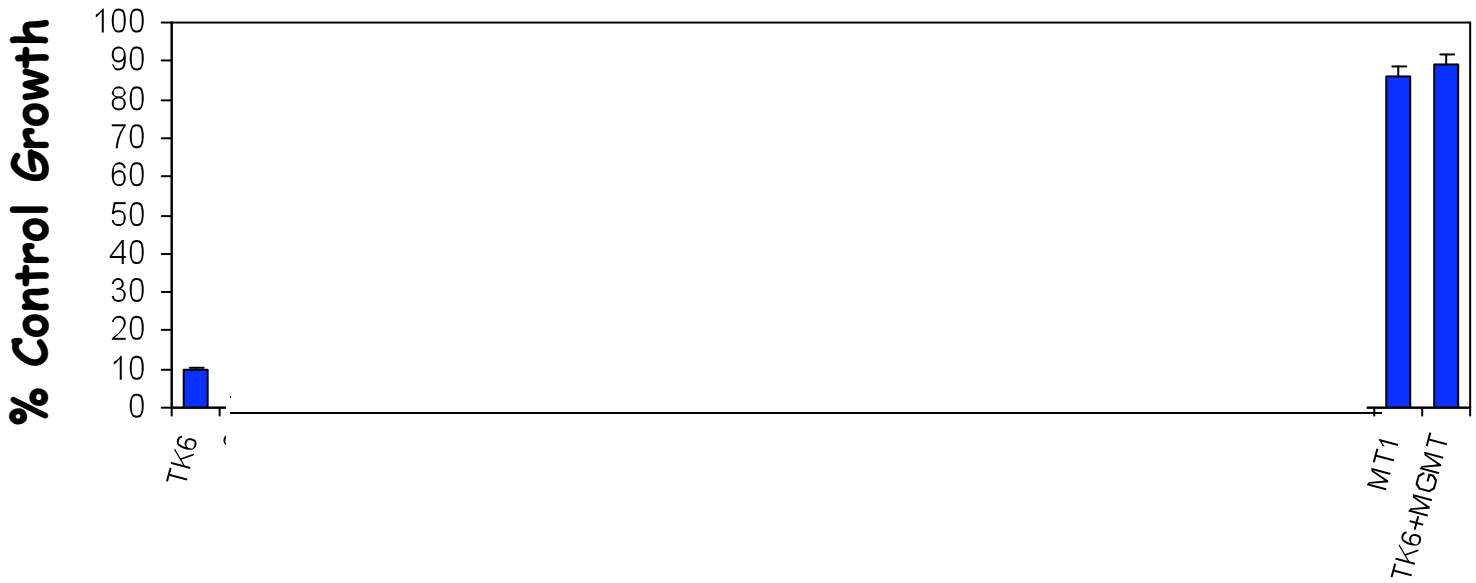
Sensitive

Resistant

Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Control Cell Lines



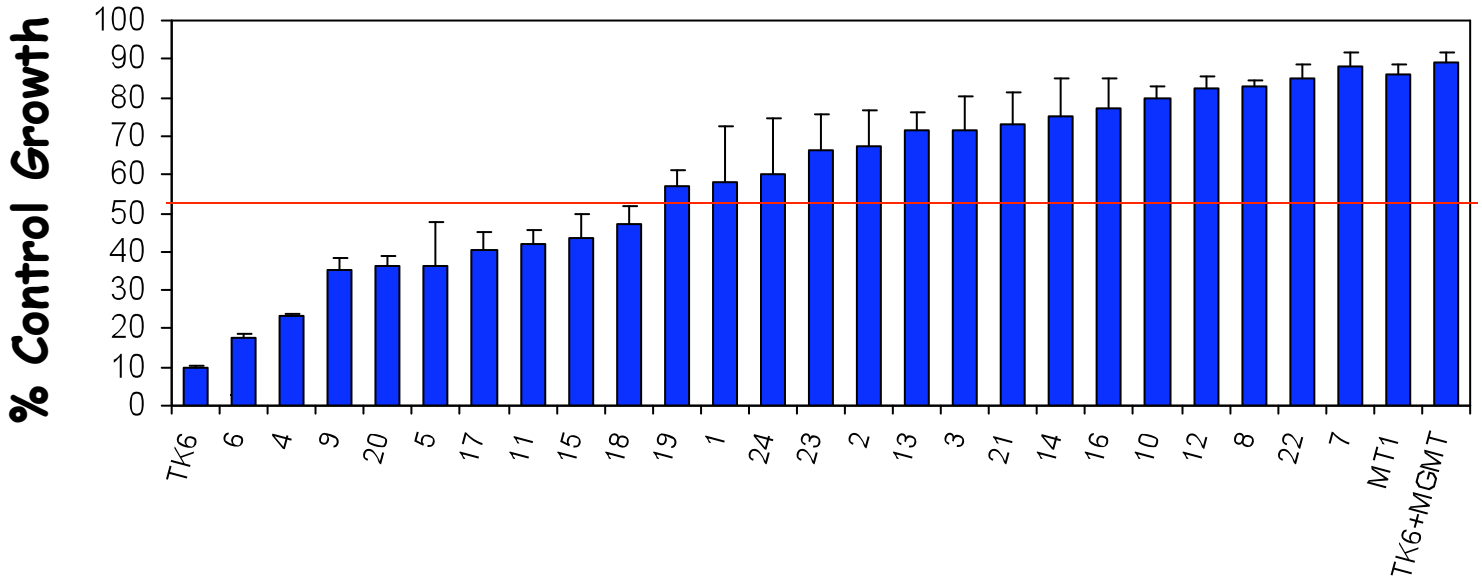
Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Control Cell Lines



Sensitive

Resistant

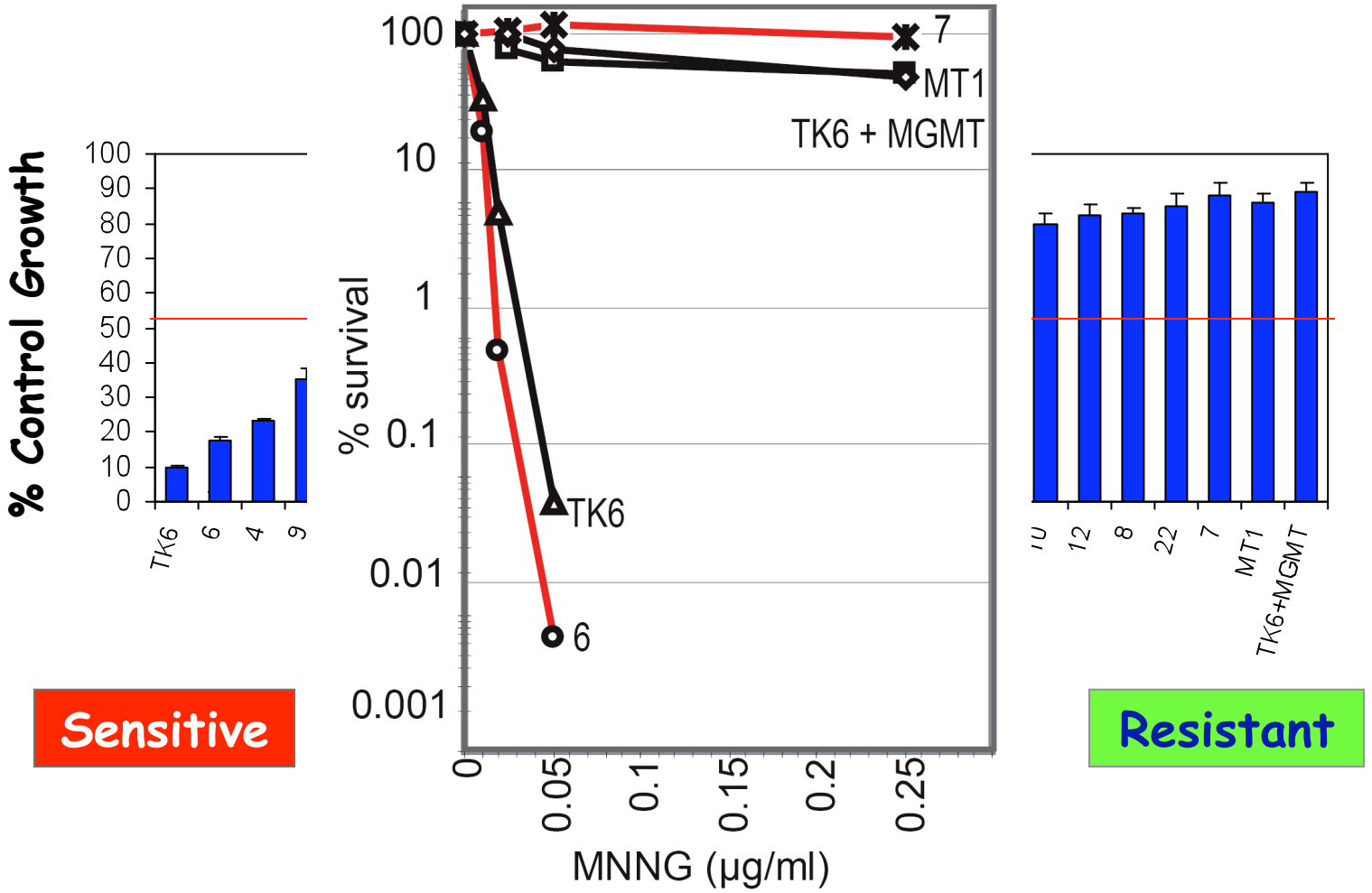
Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Coriell Cell Lines



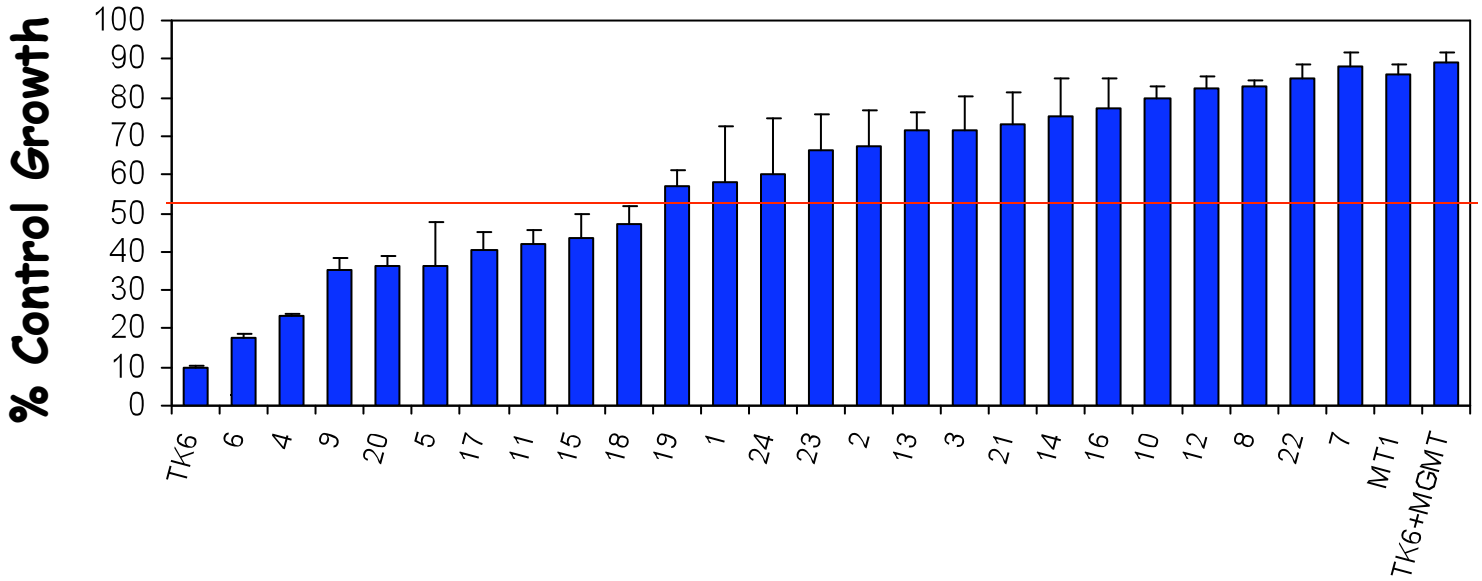
Sensitive

Resistant

Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Coriell Cell Lines

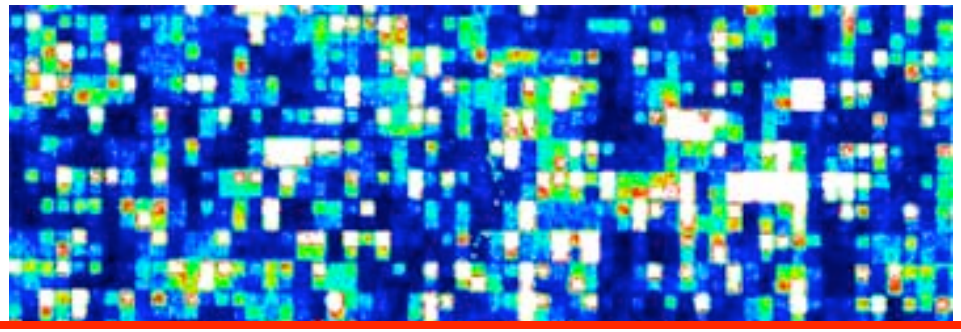


Extensive Range of Sensitivity in Cells Exposed to Alkylation Damage – Coriell Cell Lines

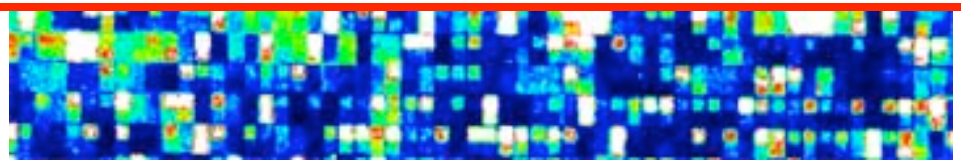


Sensitive

Resistant

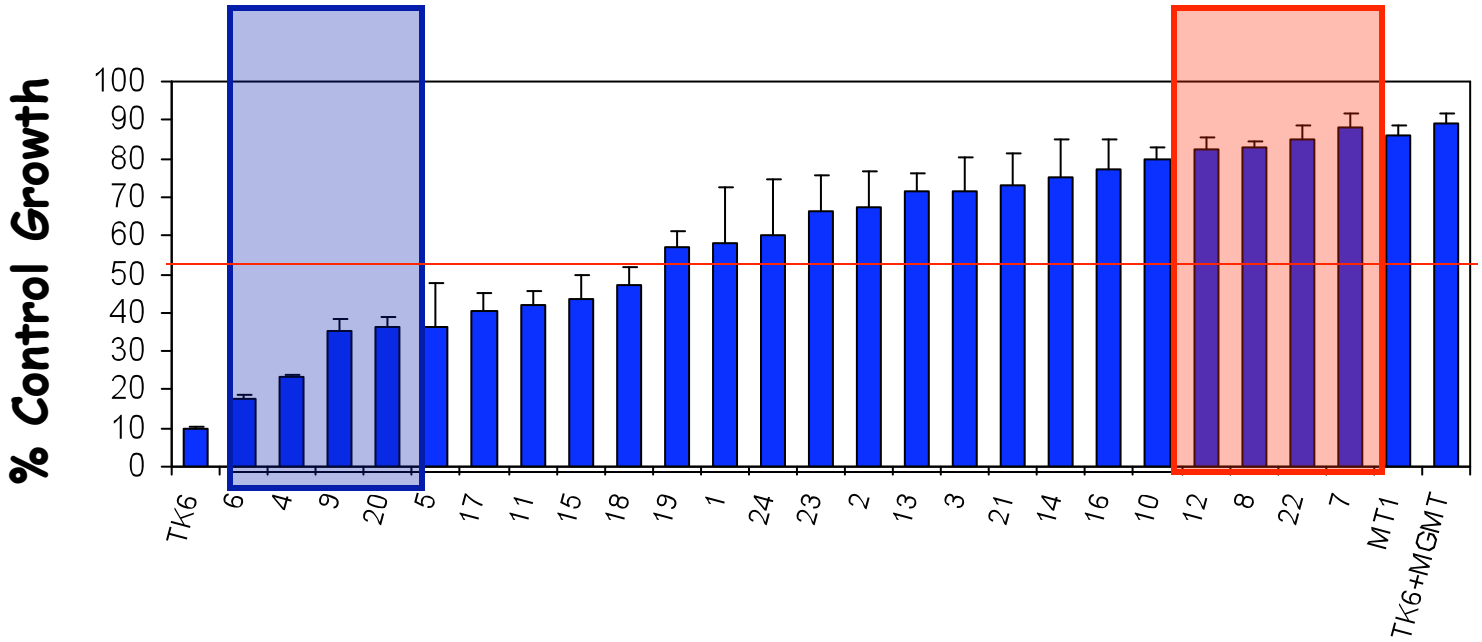


Using genomics to predict responses to alkylating agents



Genomics ⟷ Biological responses

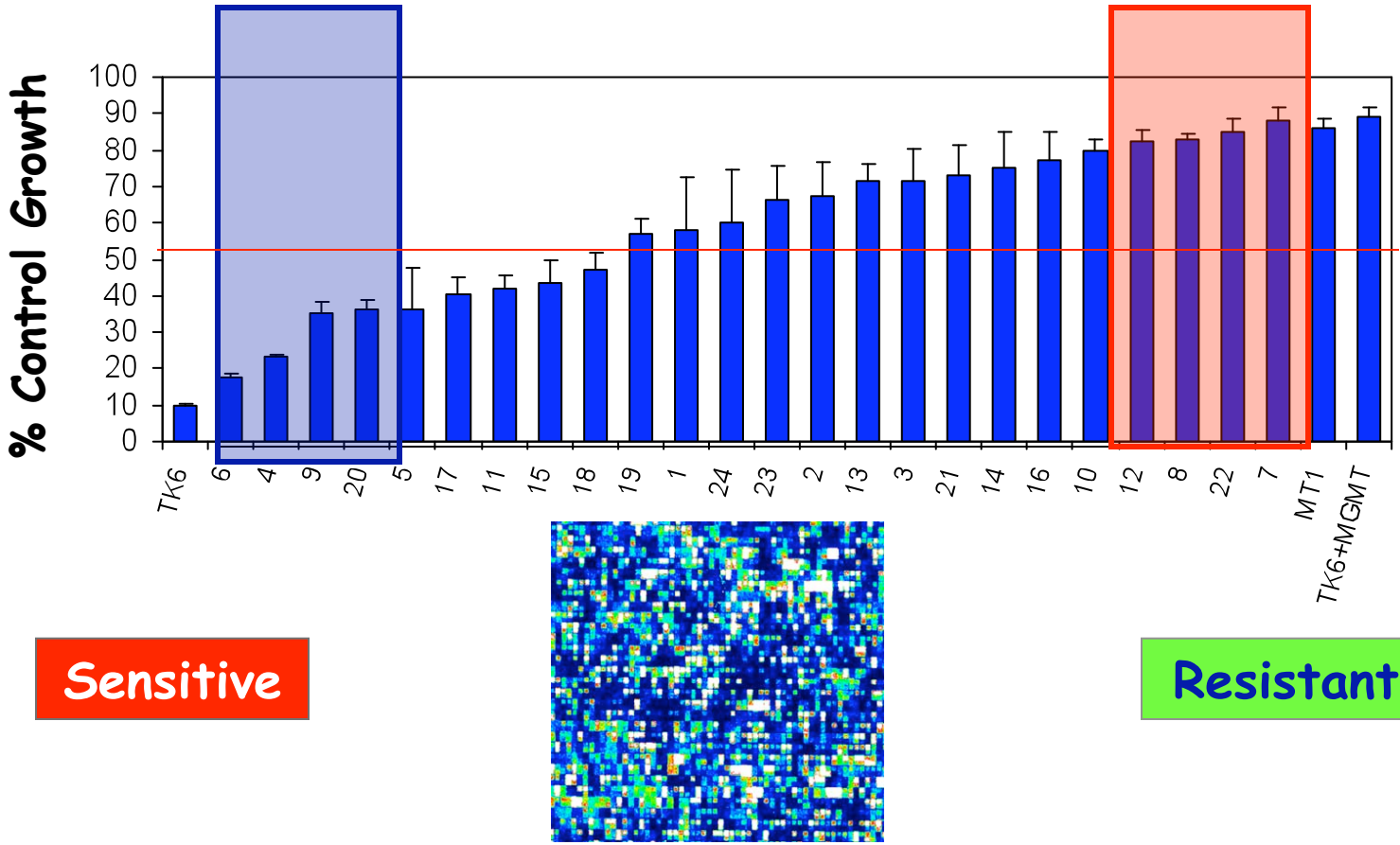
Training population comprised the most sensitive and most resistant human cell lines



Sensitive

Resistant

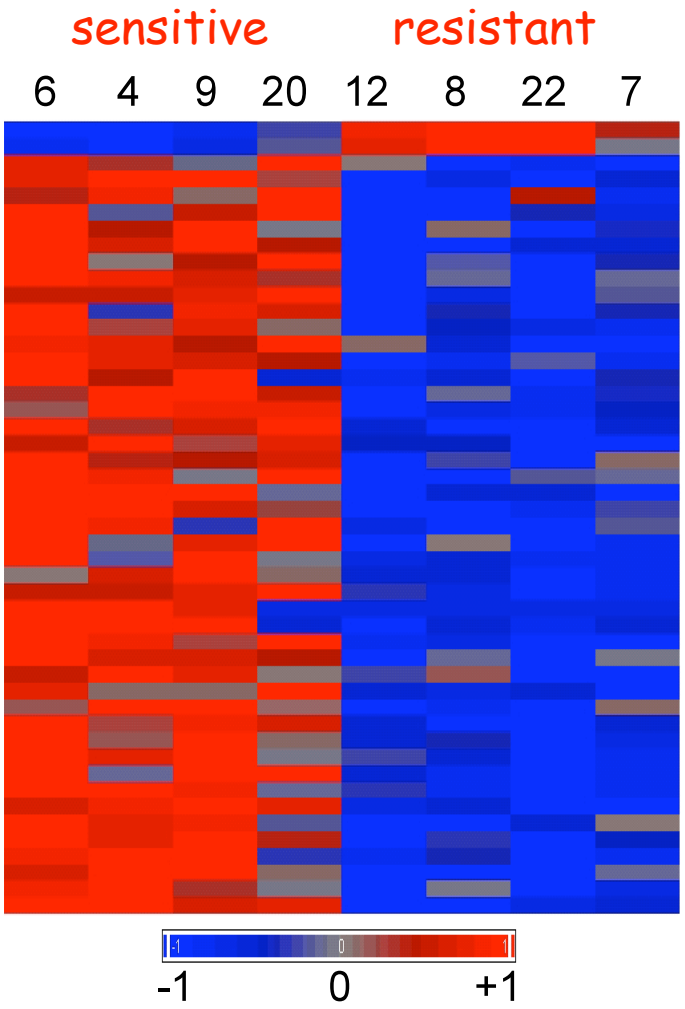
Training population comprised the most sensitive and most resistant human cell lines



TRANSCRIPTIONAL PROFILES – EXPRESSION OF ~ 20,000 GENES

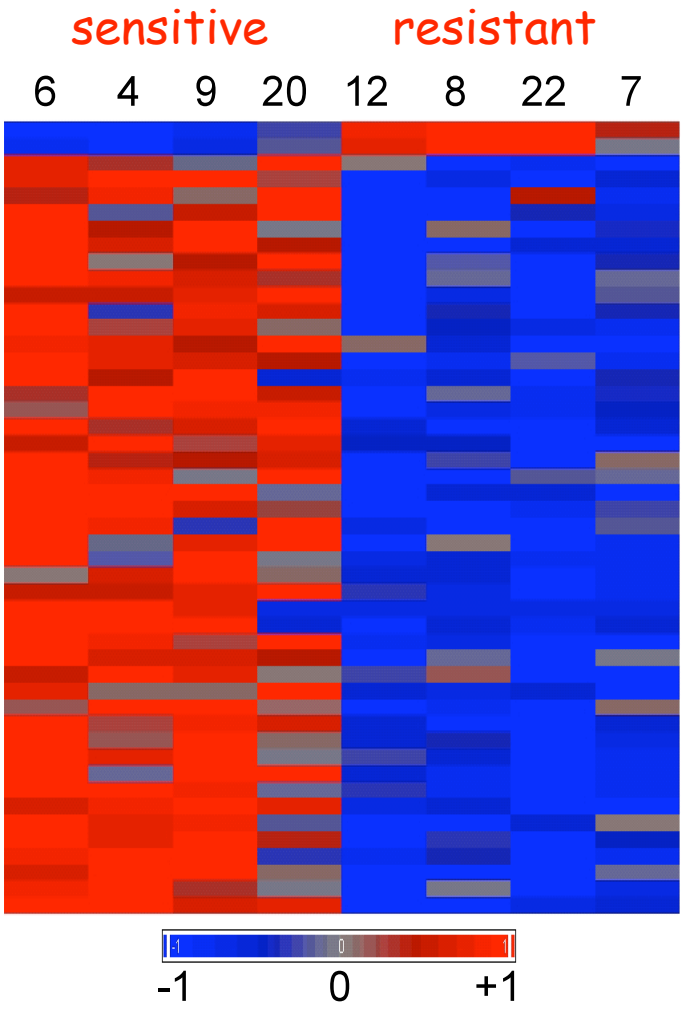
48 Basally Expressed Genes

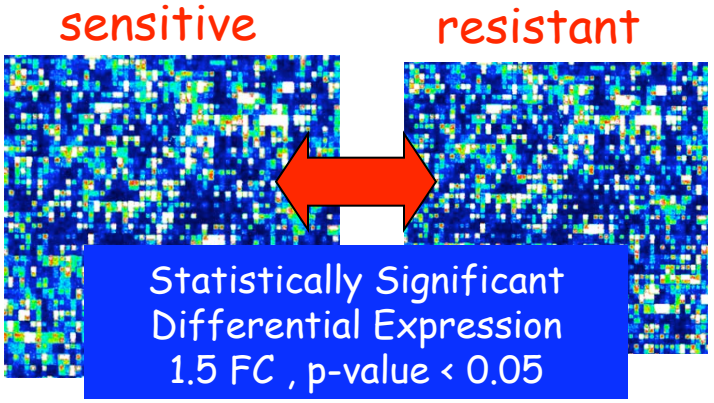
Training population

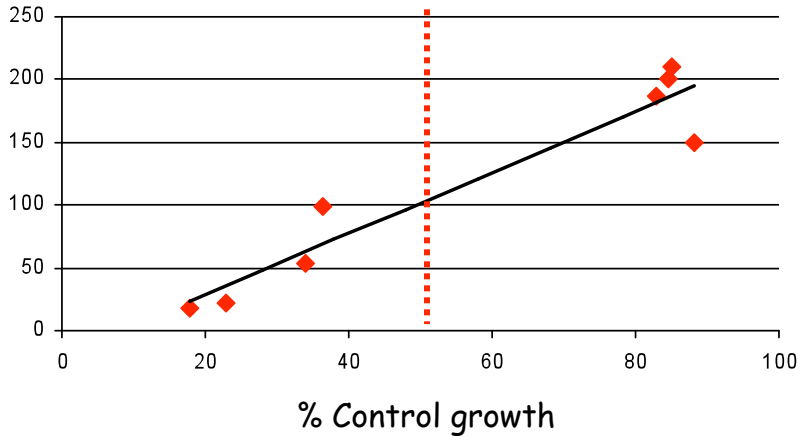


48 Basally Expressed Genes

Training population



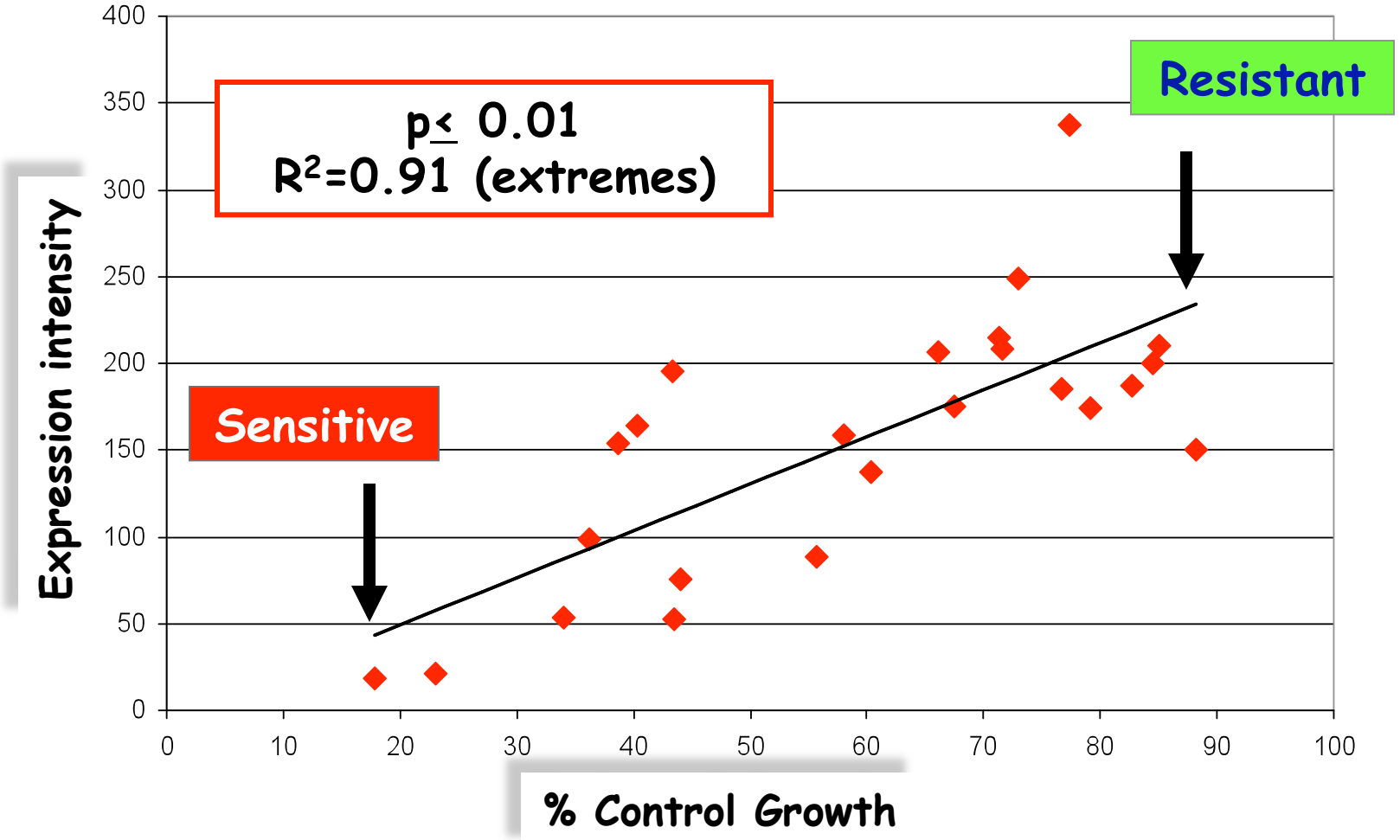




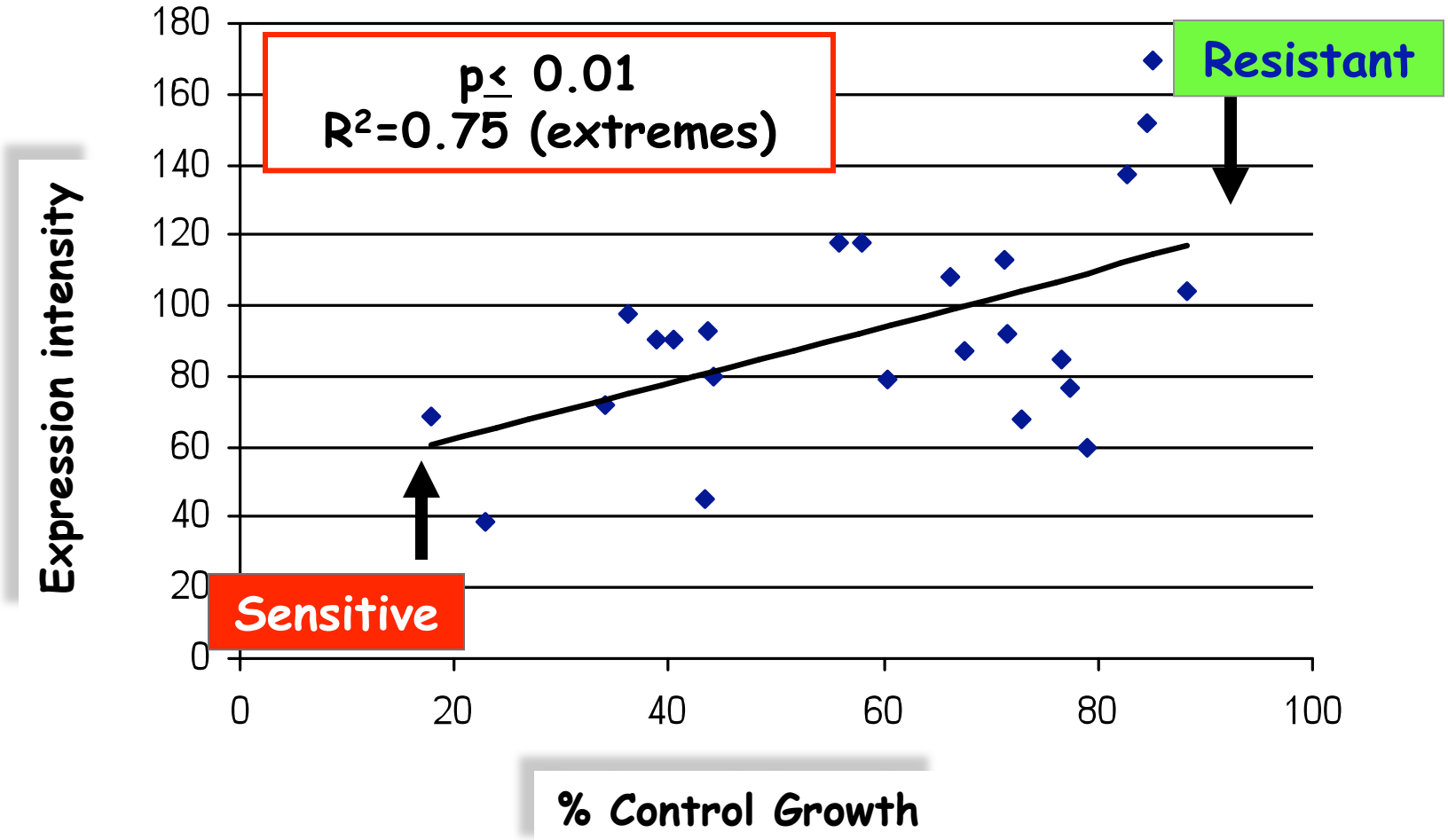
Statistically significant association ($p<0.01$) of % control growth and expression

remaining 16 human cell lines

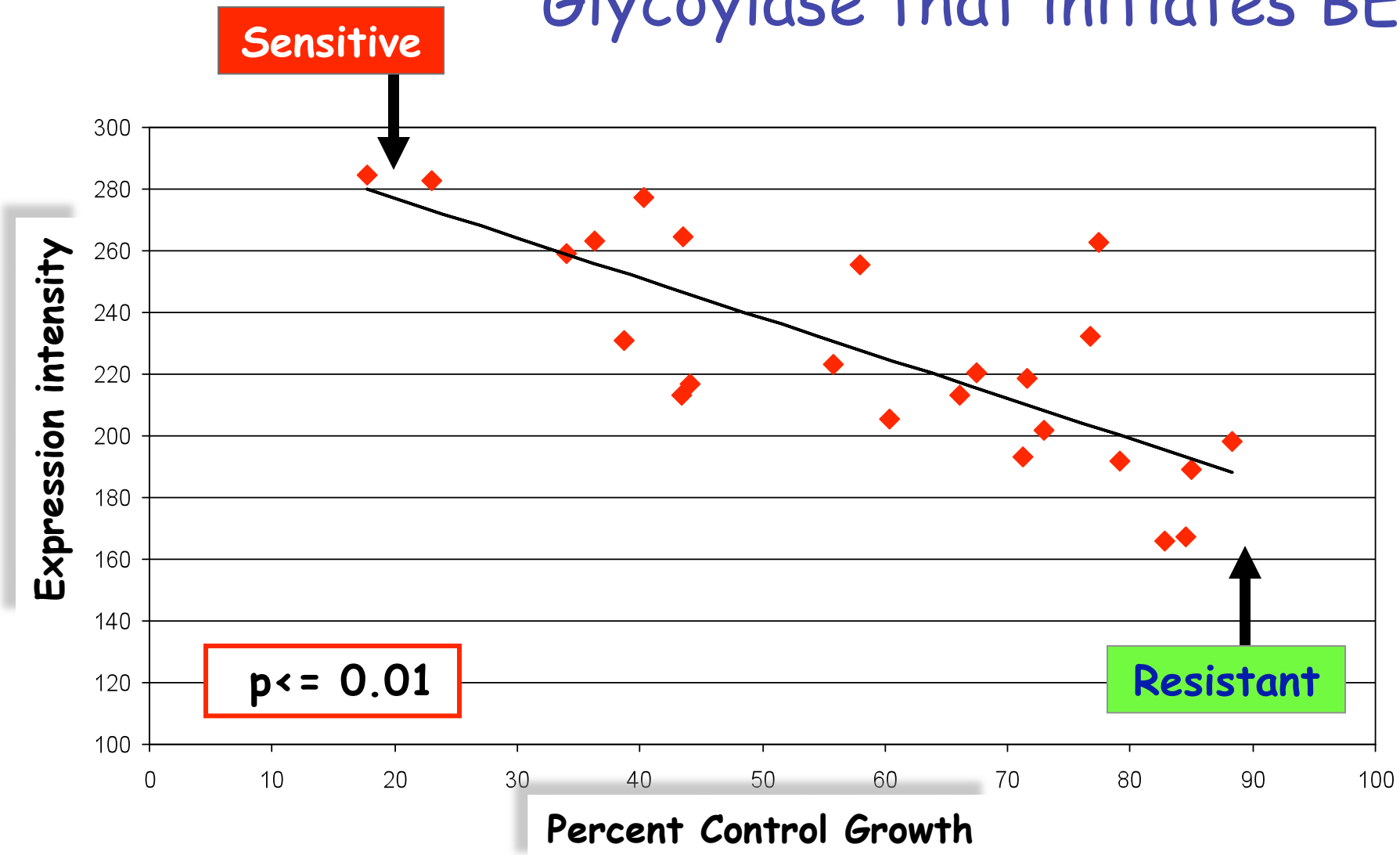
Most significant positive association was expression of MGMT



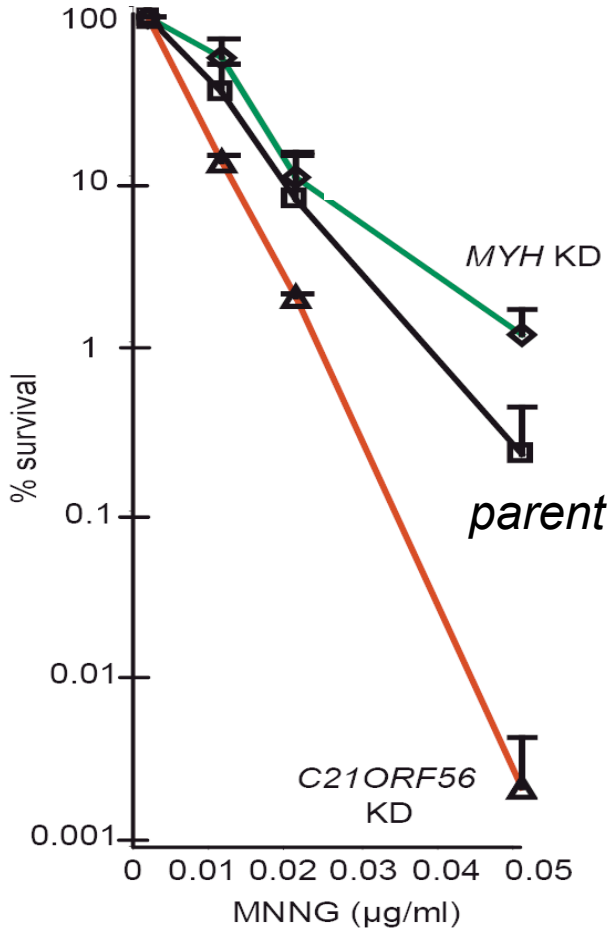
Second most significant positive association: orf of unknown function

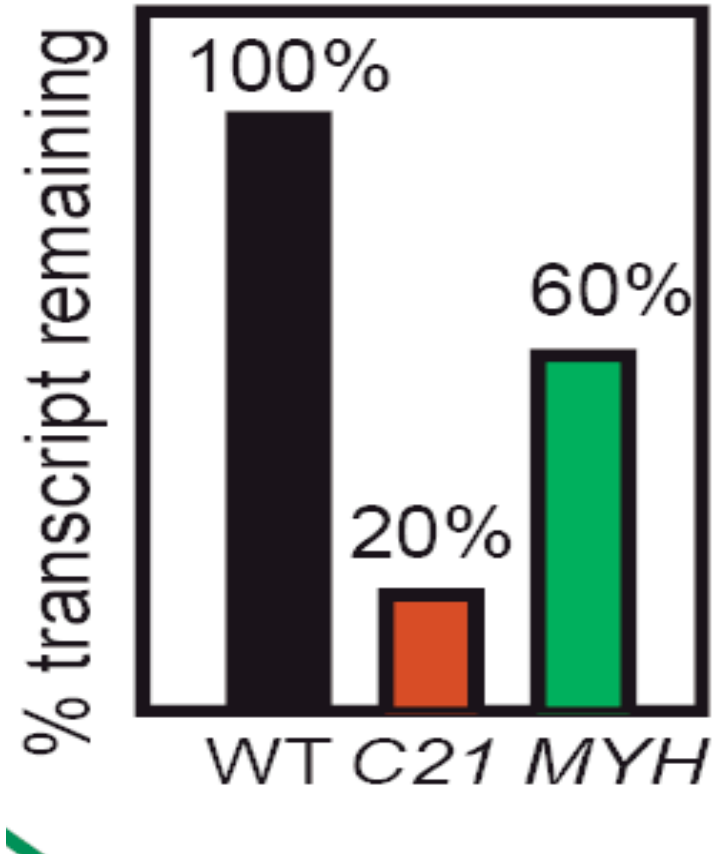


Most significant inverse association of expression with phenotype is MYH a DNA Glycoylase that initiates BER



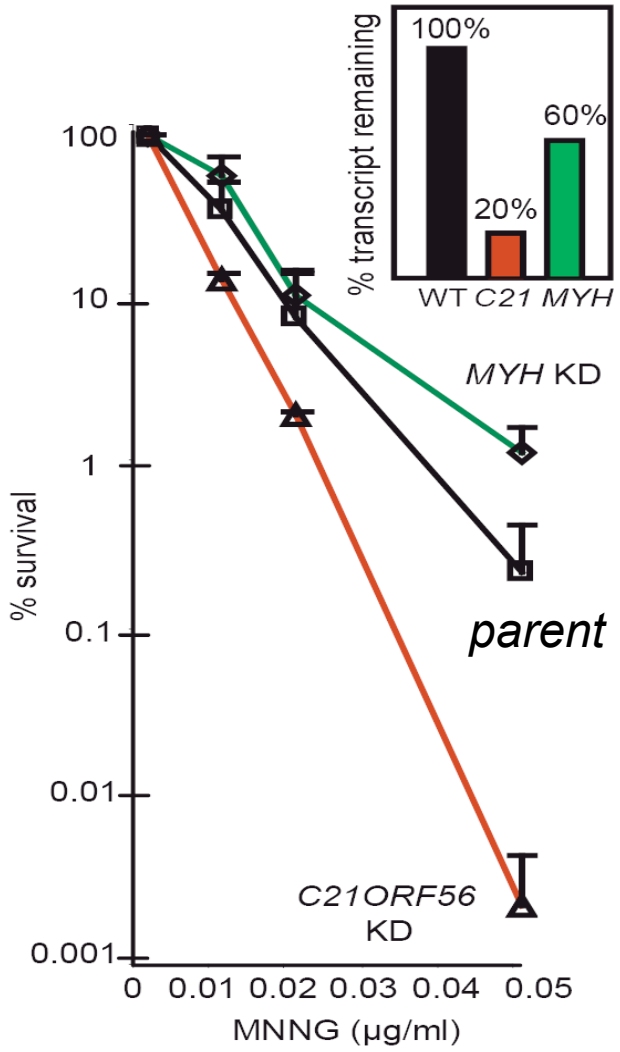
shRNA knock down of the transcript levels confirms that they do indeed modulate alkylation sensitivity



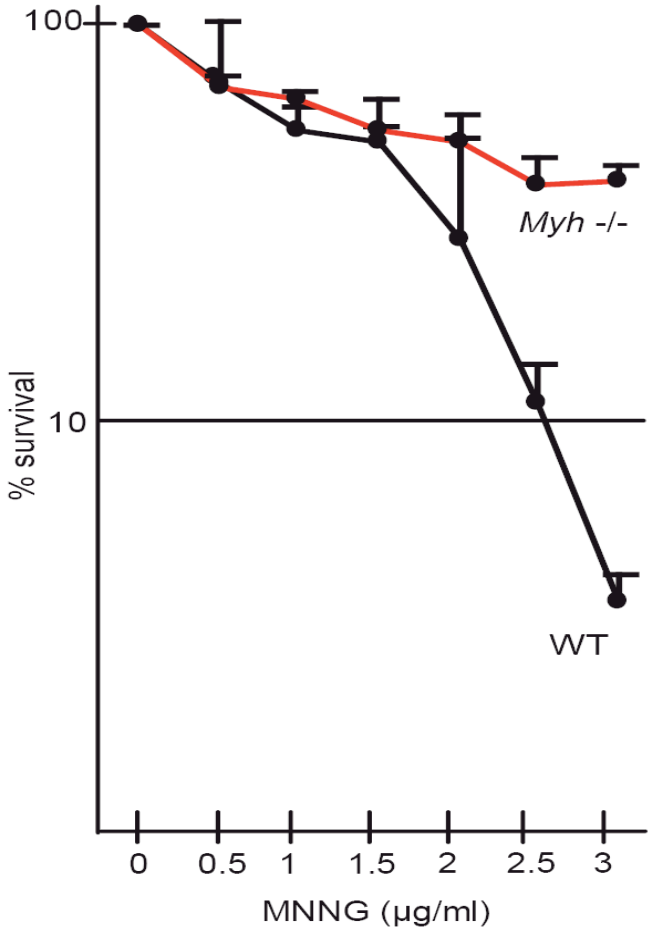


Human cells

shRNA knock down of the transcript levels confirms that they do indeed modulate alkylation sensitivity

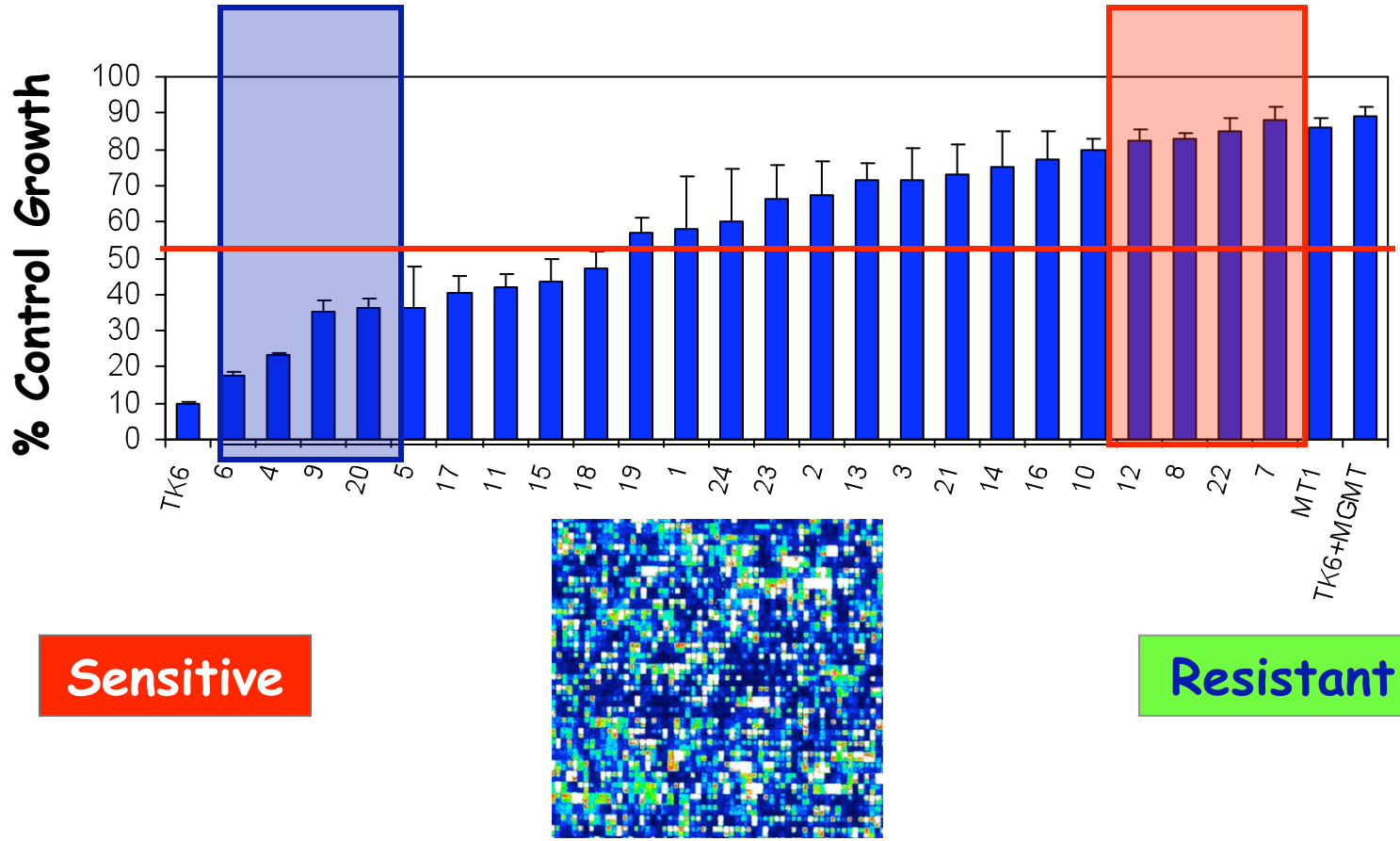


Human cells



Mouse cells

Training population comprised the most sensitive and most resistant human cell lines



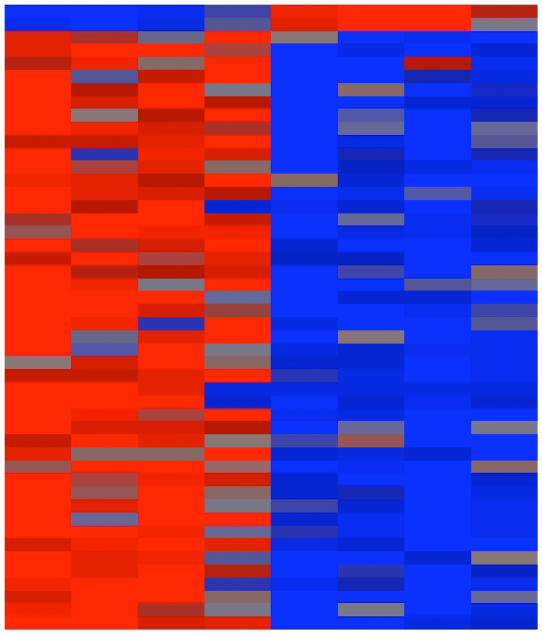
TRANSCRIPTIONAL PROFILES - EXPRESSION OF ~ 20,000 GENES

48 Basally Expressed Genes

Training population

sensitive resistant

6 4 9 20 12 8 22 7

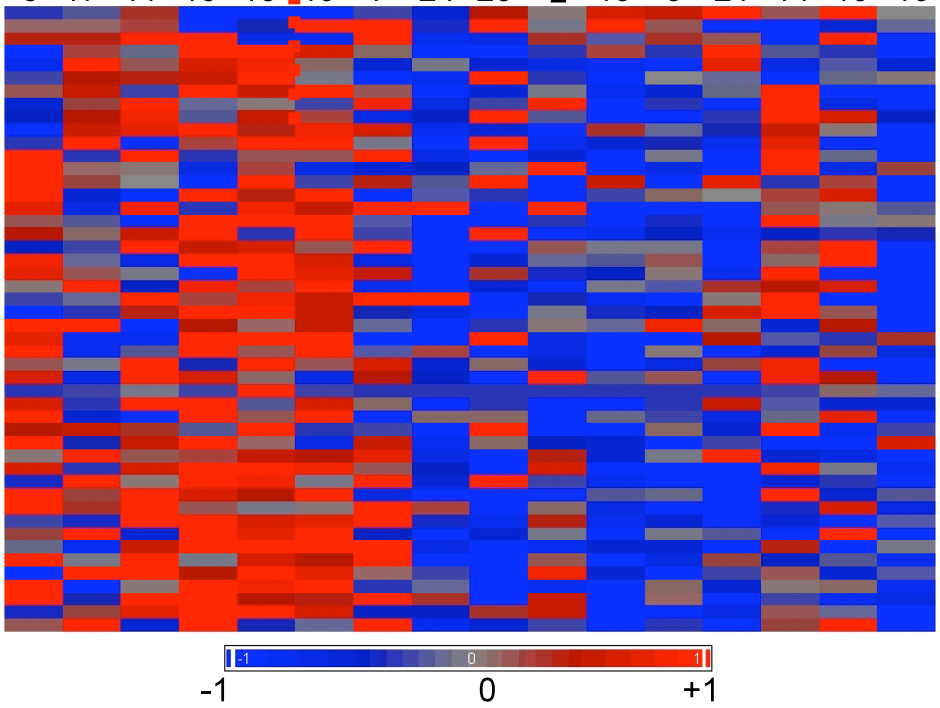


8 human cell lines

Test Population

sensitive resistant

5 17 11 15 18 19 1 24 23 2 13 3 21 14 16 10



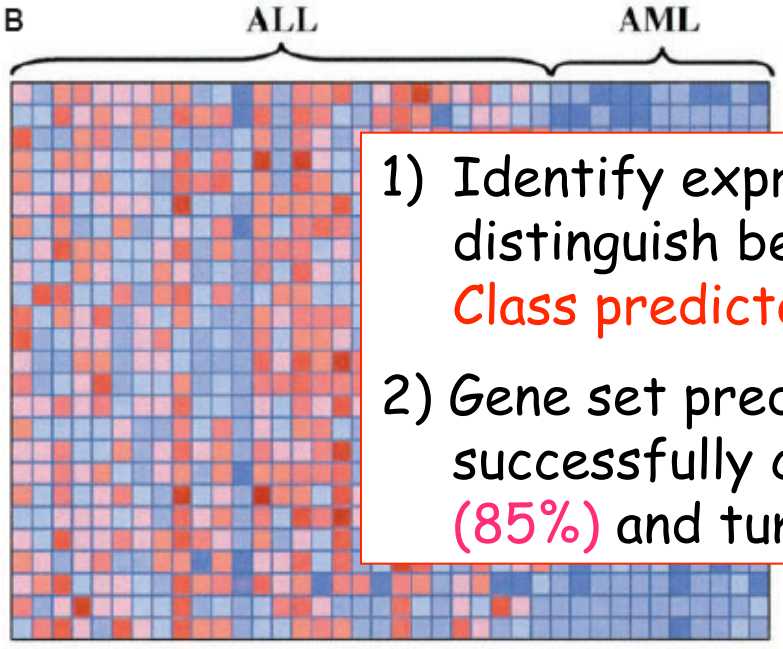
remaining 16 human cell lines

Classic Examples of Applying Class Prediction – to Distinguish tumor types

REPORTS

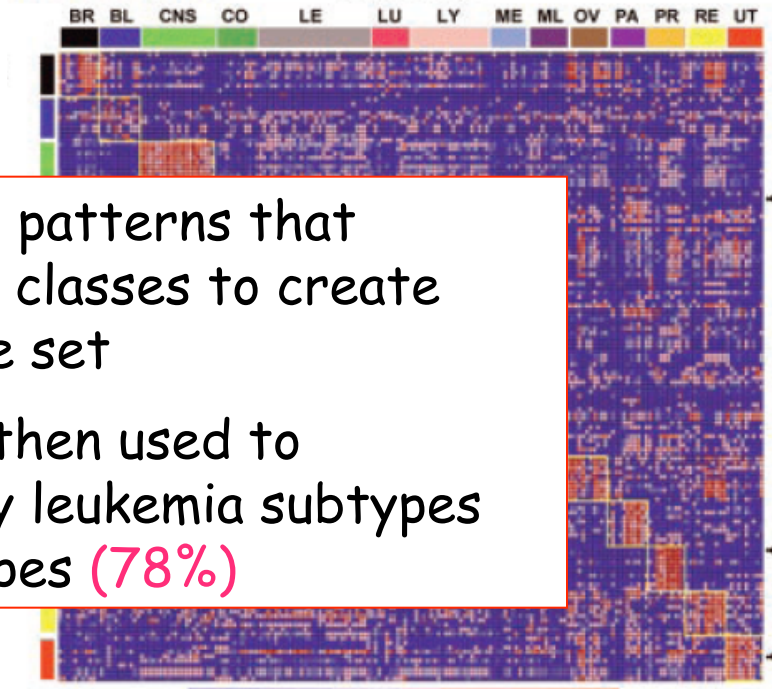
Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring

T. R. Golub,[1,2*†] D. K. Slonim,[1†] P. Tamayo,[1] C. Huard,[1] M. Gaasenbeek,[1] J. P. Mesirov,[1] H. Coller,[1] M. L. Loh,[2] J. R. Downing,[3] M. A. Caligiuri,[4] C. D. Bloomfield,[4] E. S. Lander[1,5*]



Multiclass cancer diagnosis using tumor gene expression signatures

Sridhar Ramaswamy*†, Pablo Tamayo*, Ryan Rifkin*‡, Sayan Mukherjee*‡, Chen-Hsiang Yeang*§, Michael Angelo*, Christine Ladd*, Michael Reich*, Eva Latulippe¶, Jill P. Mesirov*, Tomaso Poggio‡, William Gerald¶, Massimo Loda†‖, Eric S. Lander*,**, and Todd R. Golub*†††‡‡

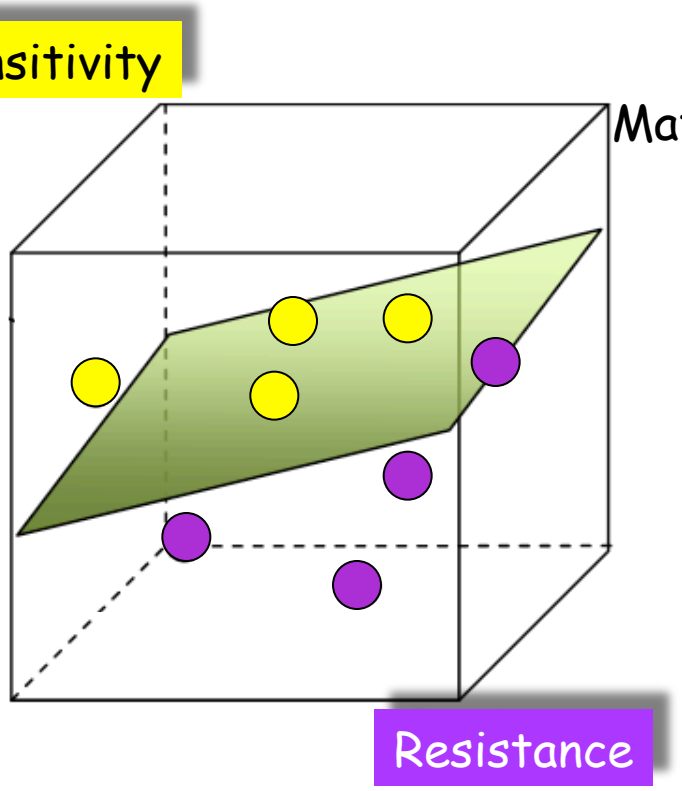


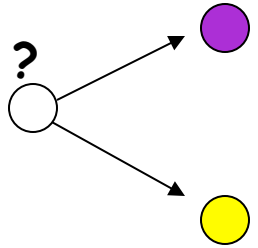
1) Identify expression patterns that distinguish between classes to create Class predictor gene set

2) Gene set predictor then used to successfully classify leukemia subtypes (85%) and tumor types (78%)

Two-class prediction algorithm: Support Vector Machine

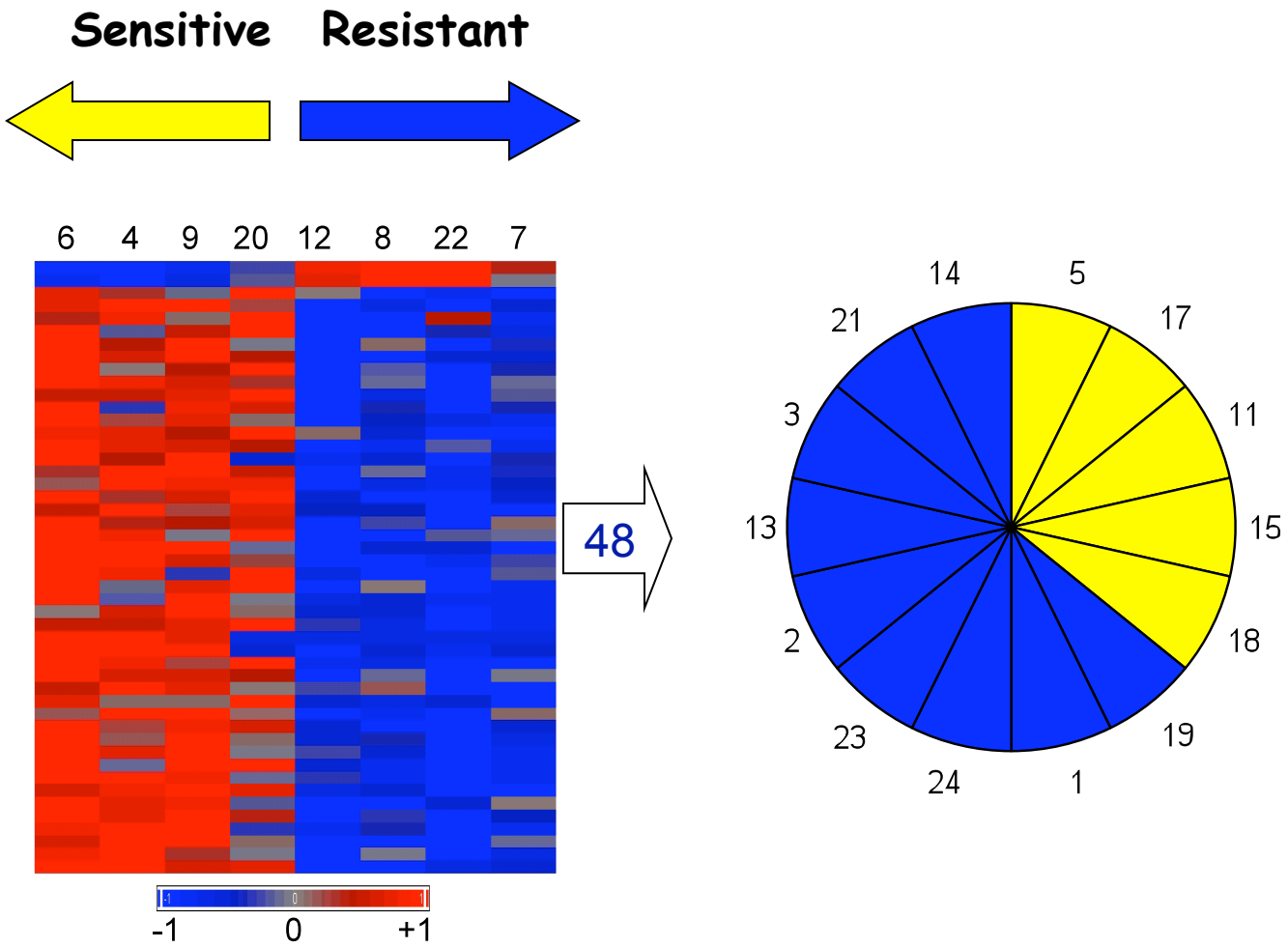
Training population of two groups with known responses to MNNG used to build gene set predictor



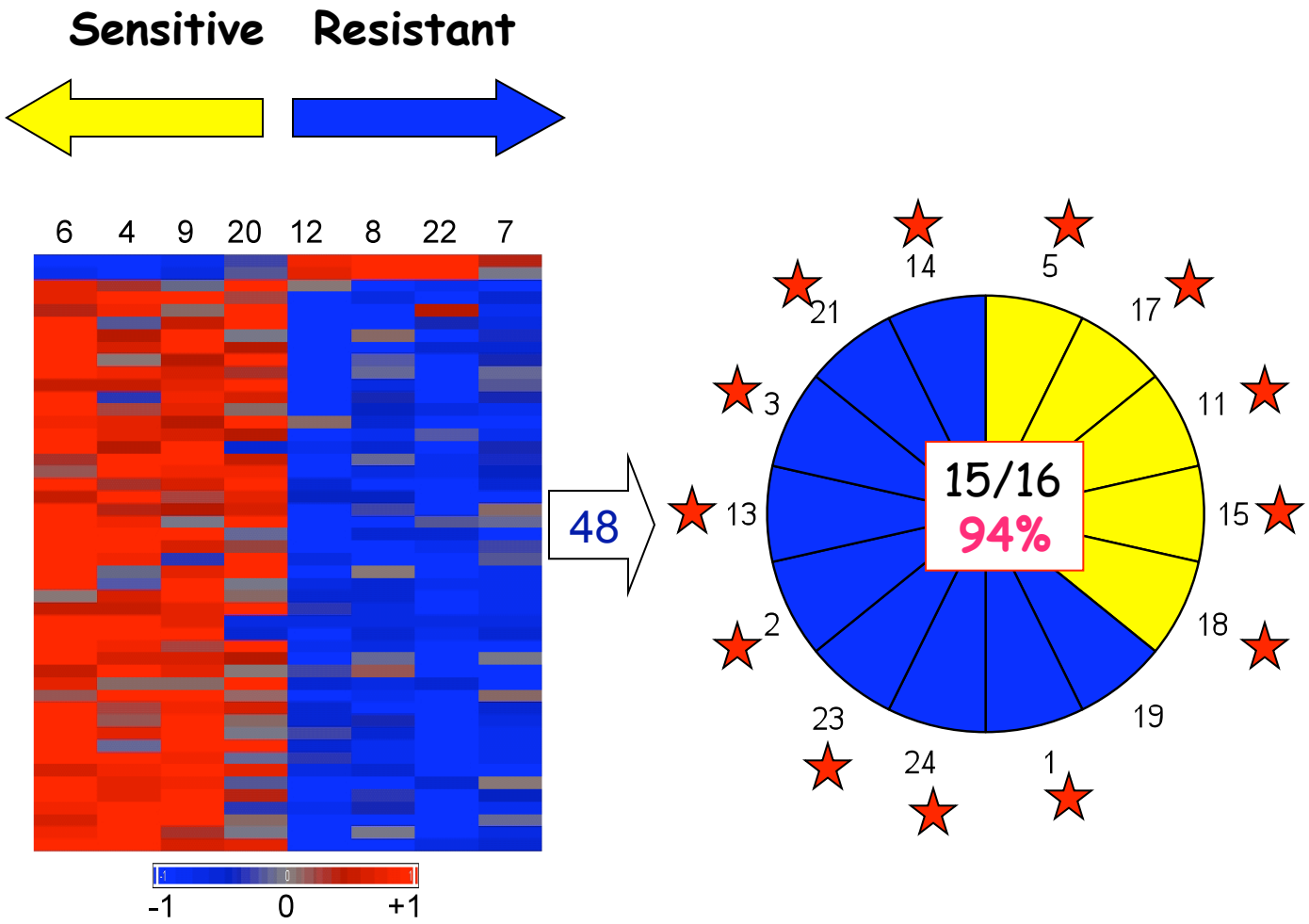


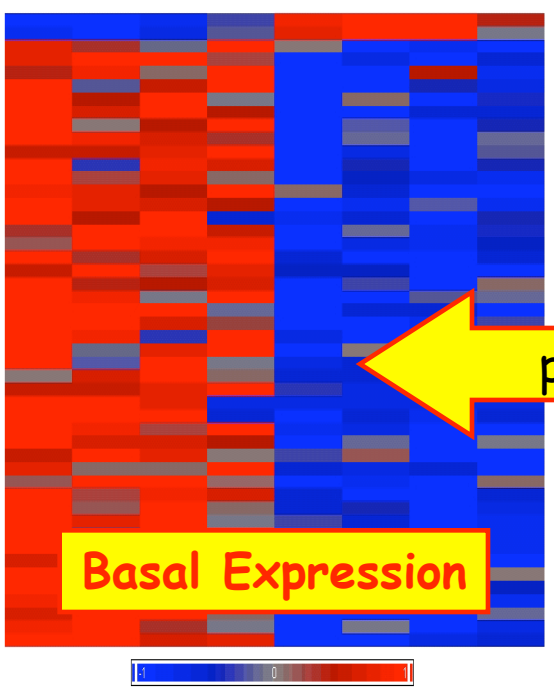
Predictor used to classify **test samples**

48 genes tested for prediction of alkylation sensitivity for the remaining test population

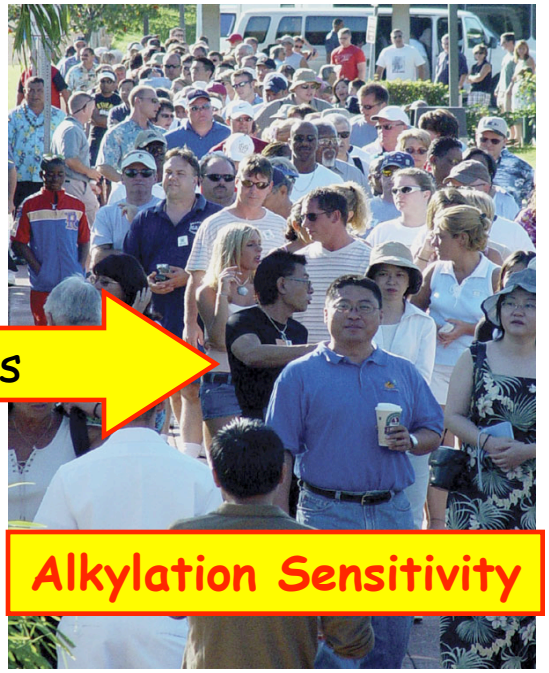


48 genes predict response to alkylation with 94% accuracy





predicts



Basal Expression

Alkylation Sensitivity

Basal gene expression can predict with high accuracy response to alkylation damage

Environmental exposures to potentially harmful agents

Harmful agents


Eat


Medicine


Drink


Absorbed


Breathe


Infection

People have different exposures

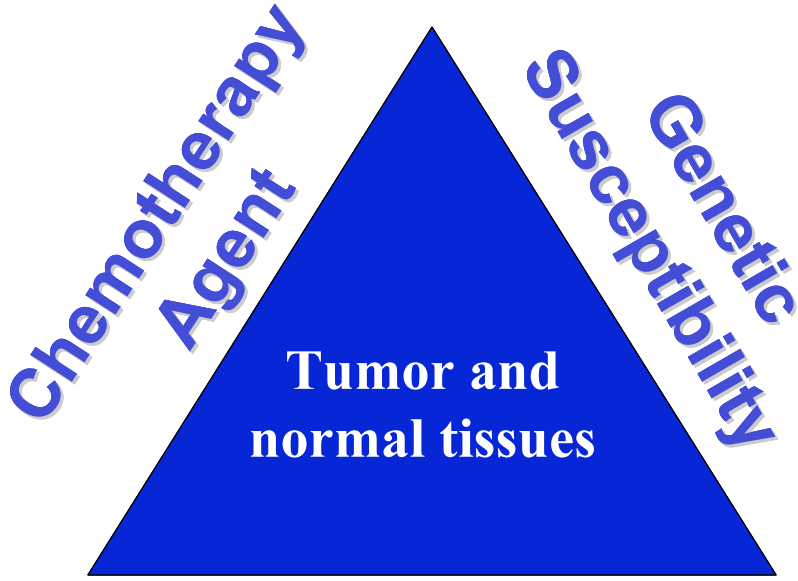
People have different responses

Determine what genes influence cancer susceptibility and whether cancer chemotherapy will be effective

Gene-Environment Interaction

Time/Age/Behavior

CANCER TREATABILITY



Treatment Regimen

Acknowledgements

Rebecca C. Fry
Chandni Valiathan
J. Peter Svensson
Brad J. Hogan
Emma Wang
Sanchita Bhattacharya
James M. Bugni
Charles A Whittaker

Funding from NCI, NIEHS and the ACS