

The importance of mutation analysis of the p53 tumour suppressor gene

Potential applications with cancer patients

James L. Gallarda, PhD

Roche Molecular Diagnostics

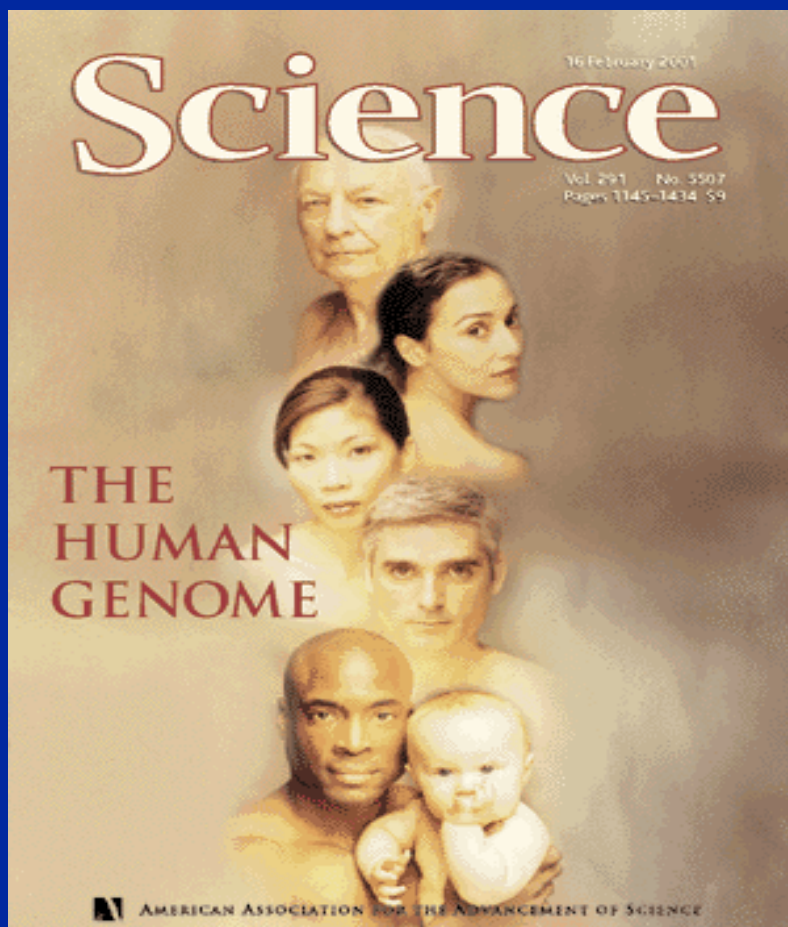
“If new refrigerators hurt 7% of customers and failed to work for another one-third of them, customers would expect refunds”

BJ Evans, DA Flockhart, EM Meslin Nature Med 10:1289, 2004

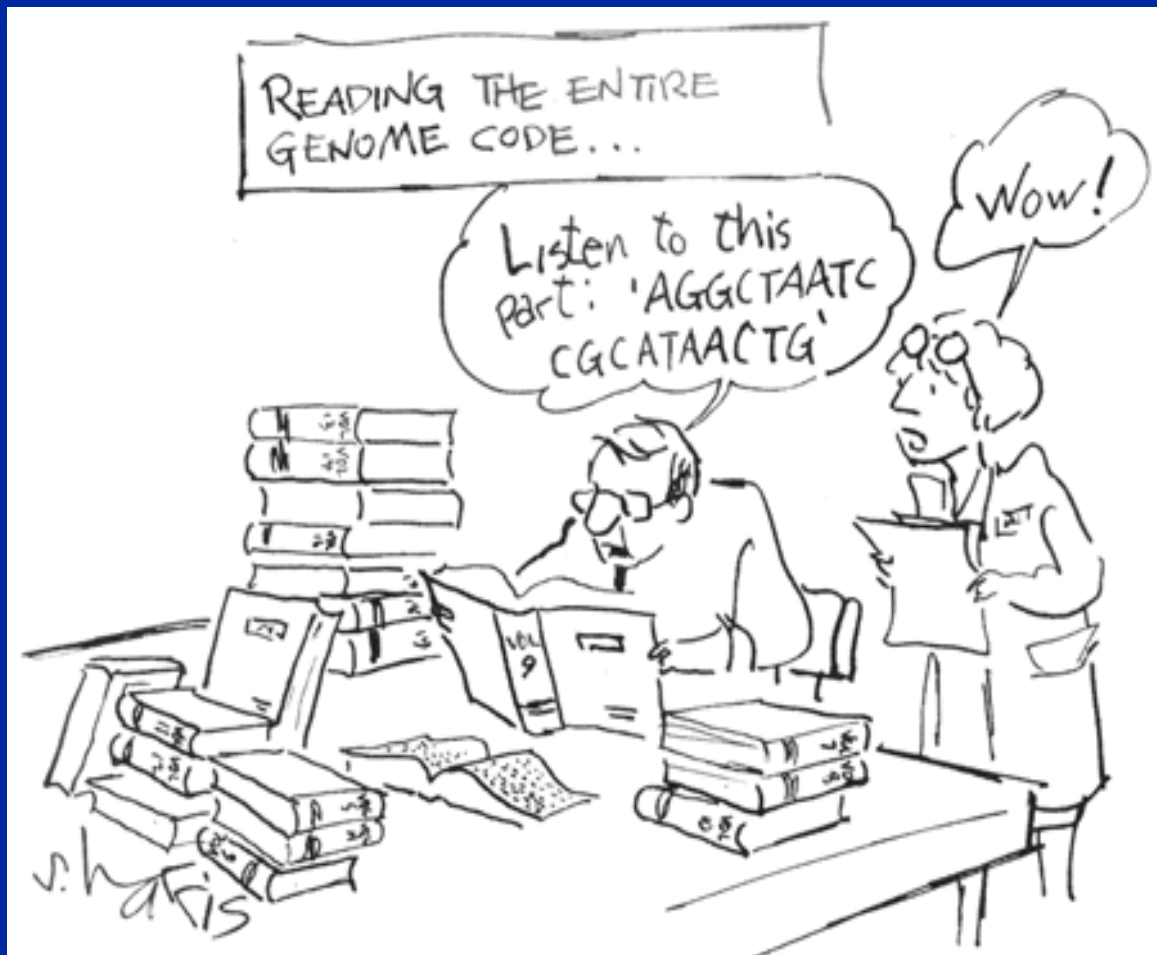


Future diagnostics and treatment will be personalized

based on the variations in the human genome and...



..will rely on a four-letter alphabet



The tumour suppressor “p53” gene

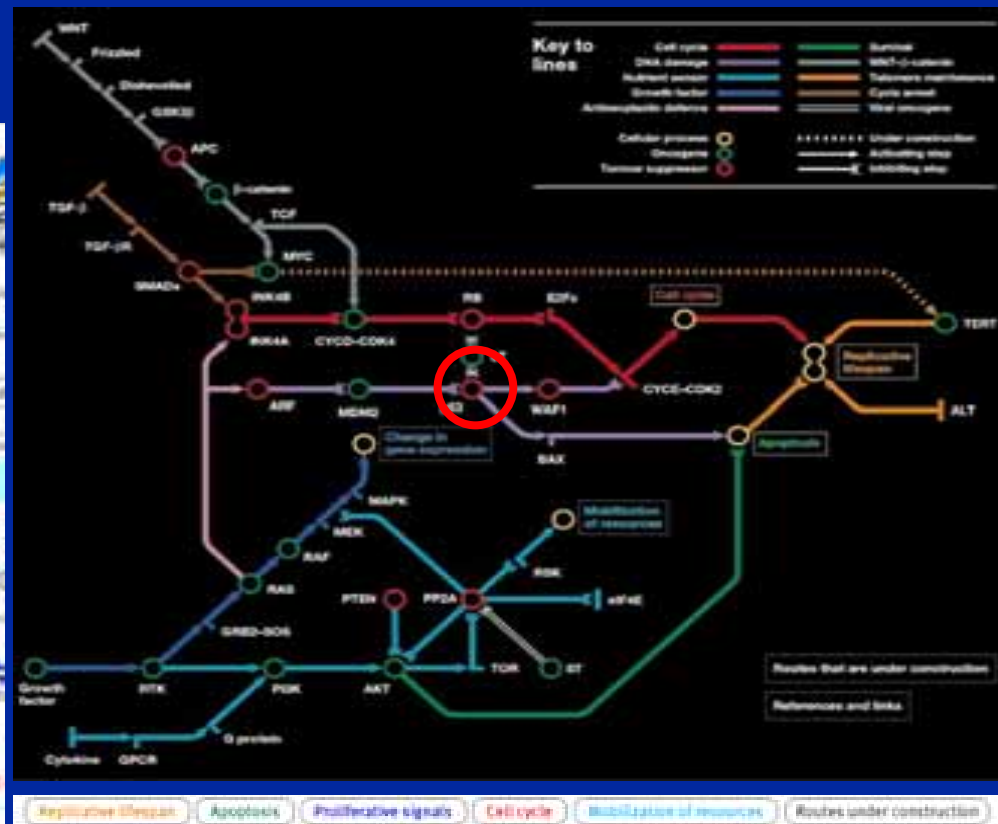
“Guardian of the genome”

Complex Networks

Subways and cells

nature
REVIEWS CANCER

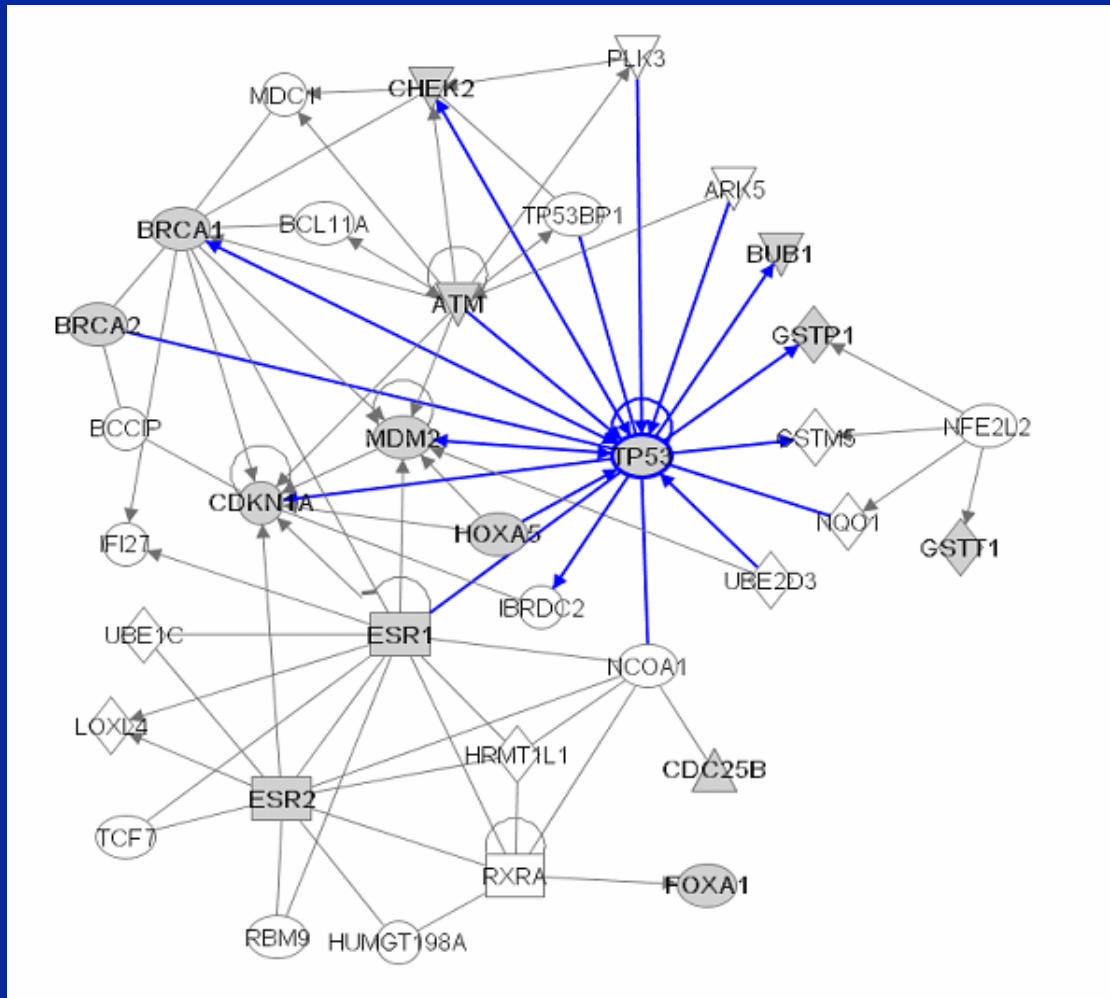
A subway map of cancer pathways
William C. Hahn and Robert A. Weinberg



http://www.nature.com/nrc/journal/v2/n5/weinberg_poster/index.html

Networks in a cancer cell

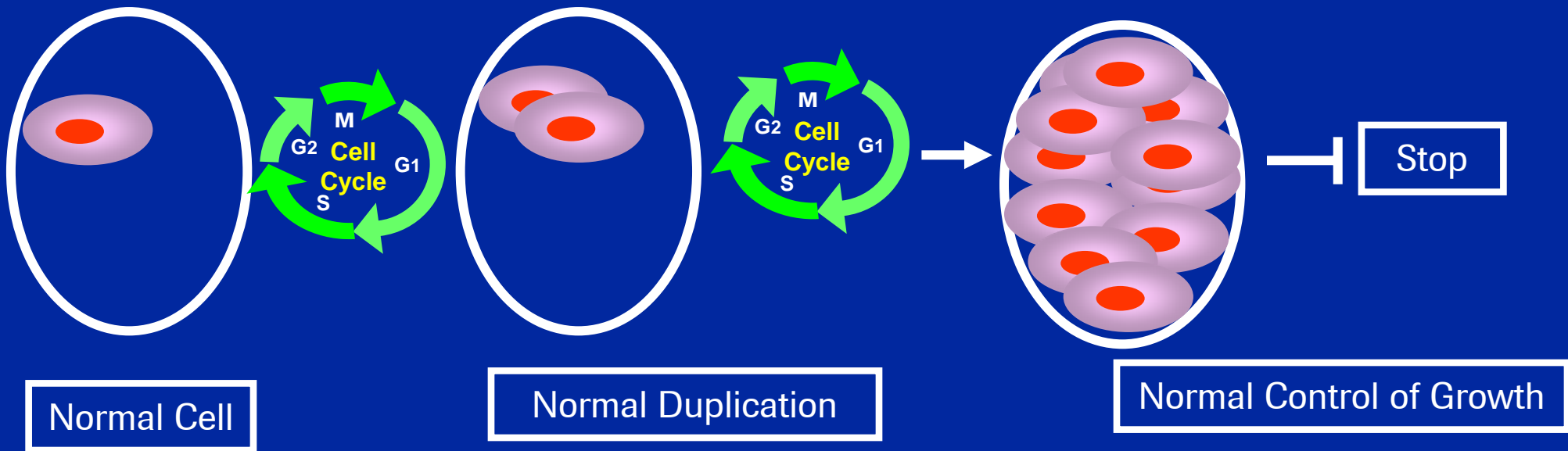
The position of p53



p53 is a central hub in the cell growth pathway

Normal cell growth

maintained by a regulated series of programmed events (cell cycle)



Normal Cell

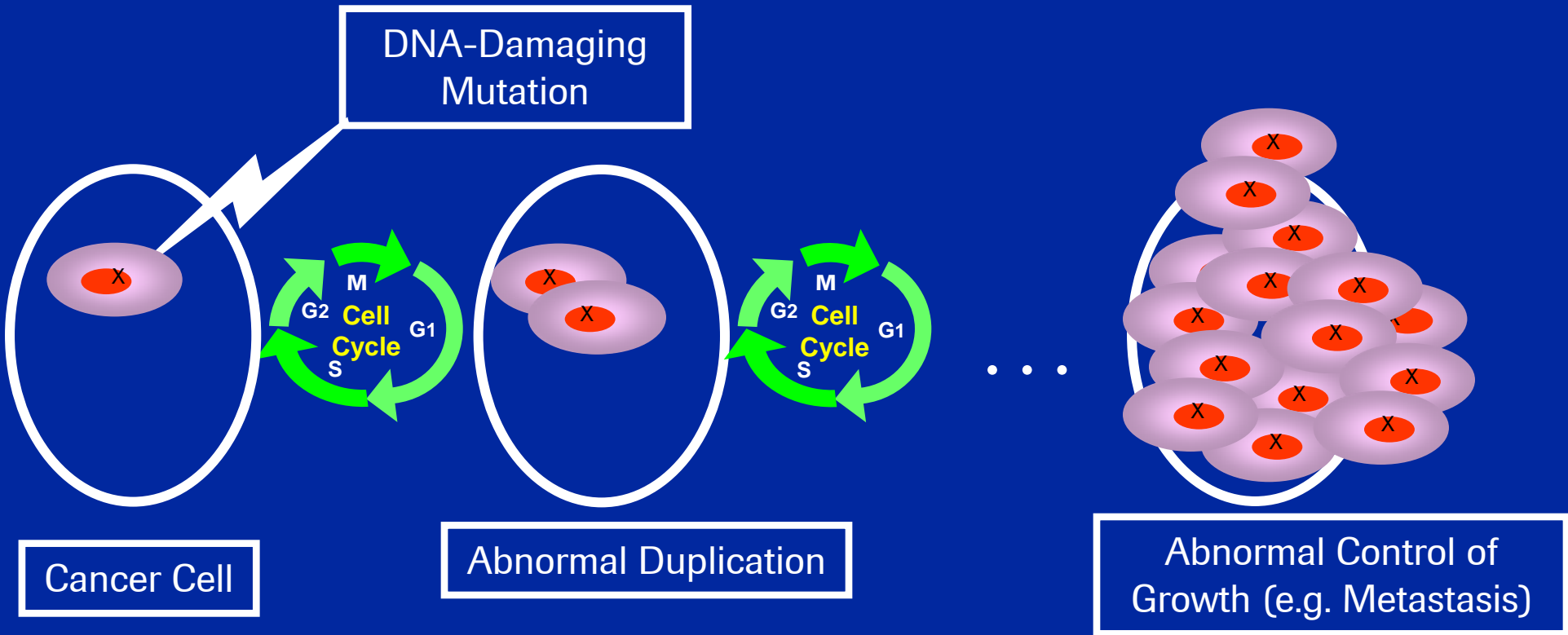
Normal Duplication

Normal Control of Growth

Normal Cell Growth

Abnormal cell growth

is no longer properly regulated in cancer



Cancer Cell

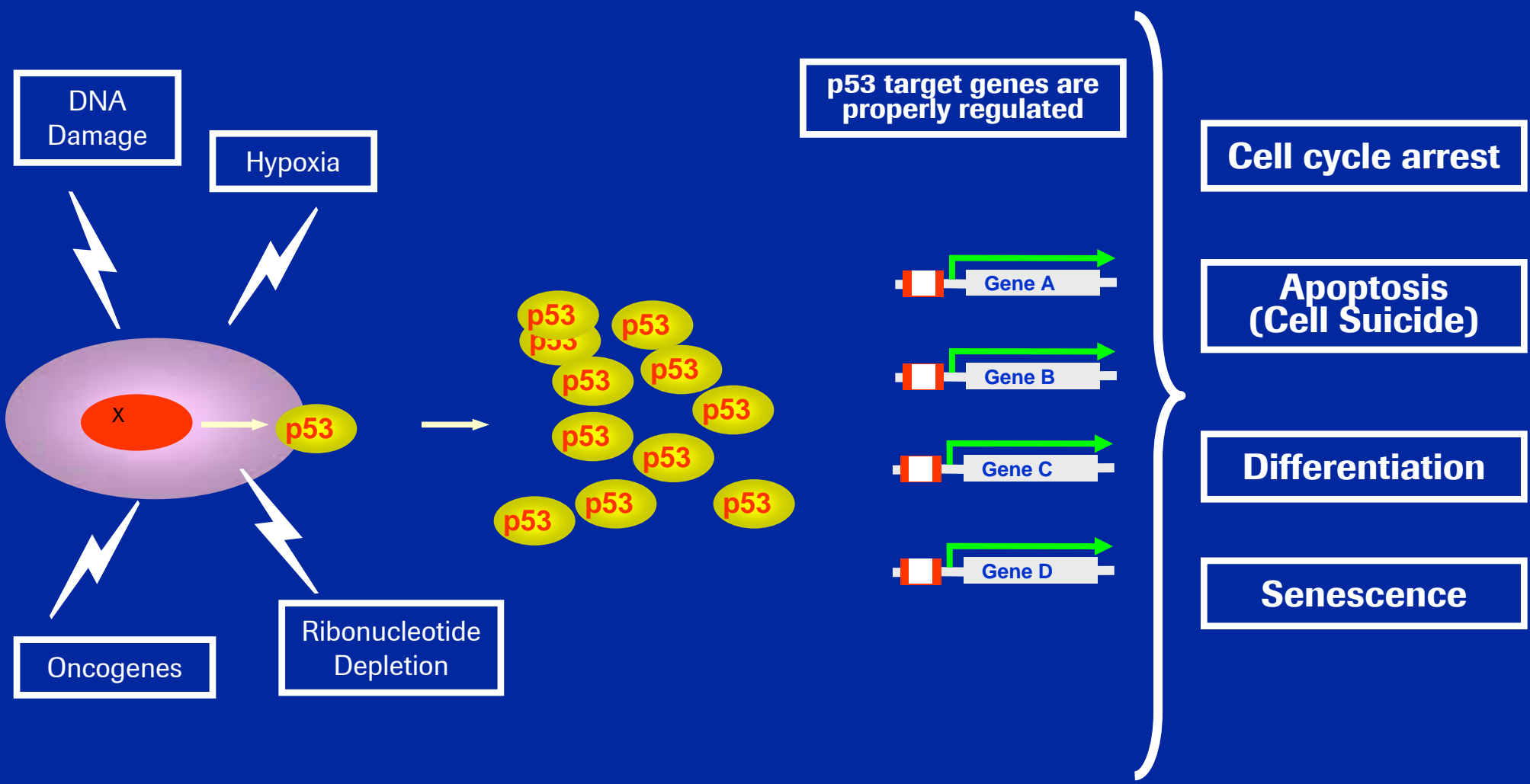
Abnormal Duplication

Abnormal Control of Growth (e.g. Metastasis)

Abnormal Cell Growth



p53 regulates cellular response to stress



DNA Damage

Hypoxia

p53 target genes are properly regulated

Cell cycle arrest

Apoptosis (Cell Suicide)

Differentiation

Senescence

Oncogenes

Ribonucleotide Depletion

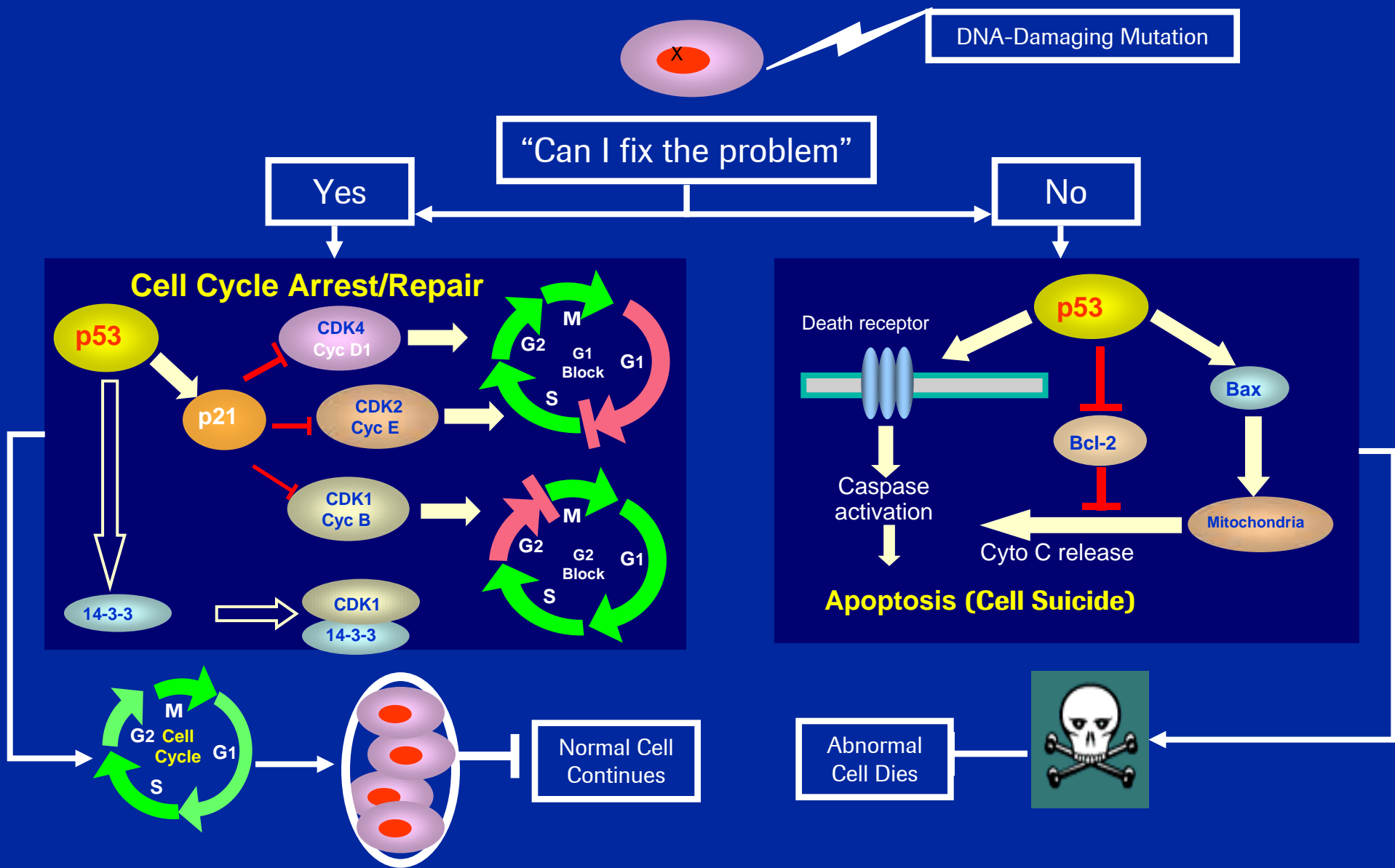
Gene A

Gene B

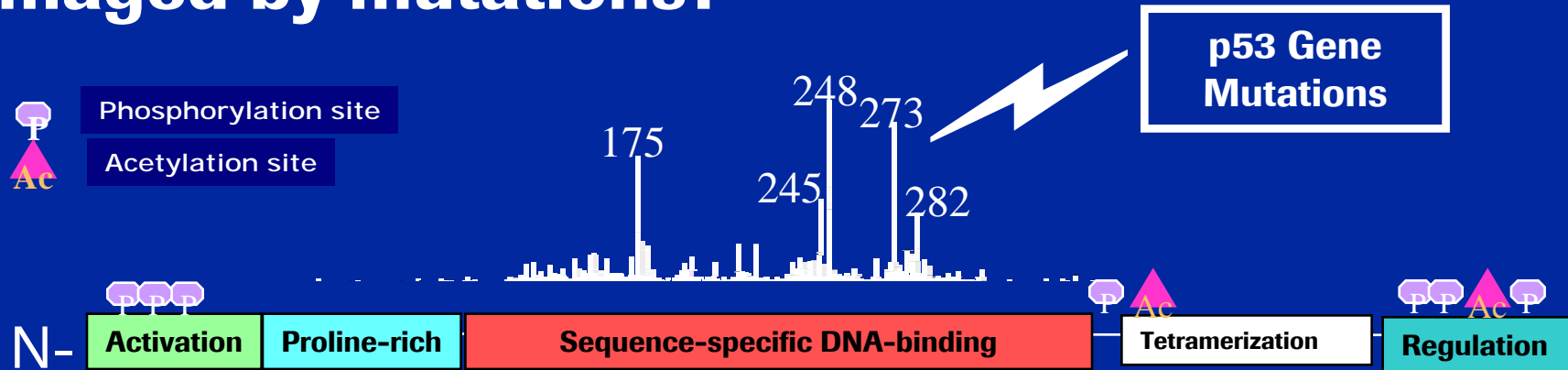
Gene C

Gene D

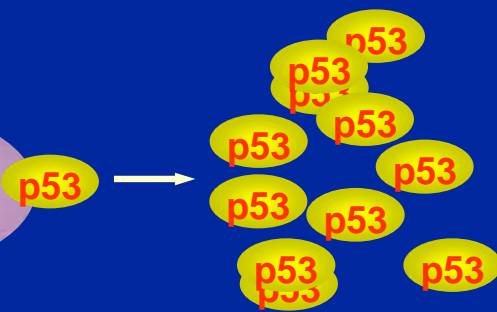
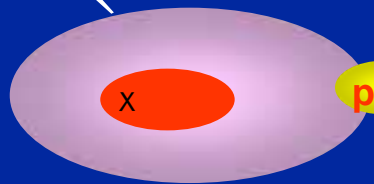
p53 regulates cellular response to stress



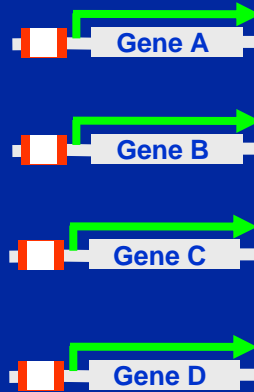
What happens if the p53 gene itself is damaged by mutations?



DNA-Damaging Mutation



p53 Target Genes Are Not Regulated



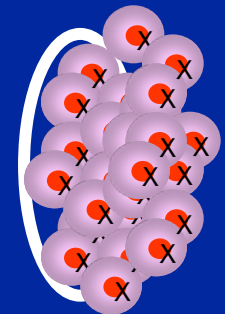
No Cell Cycle Arrest



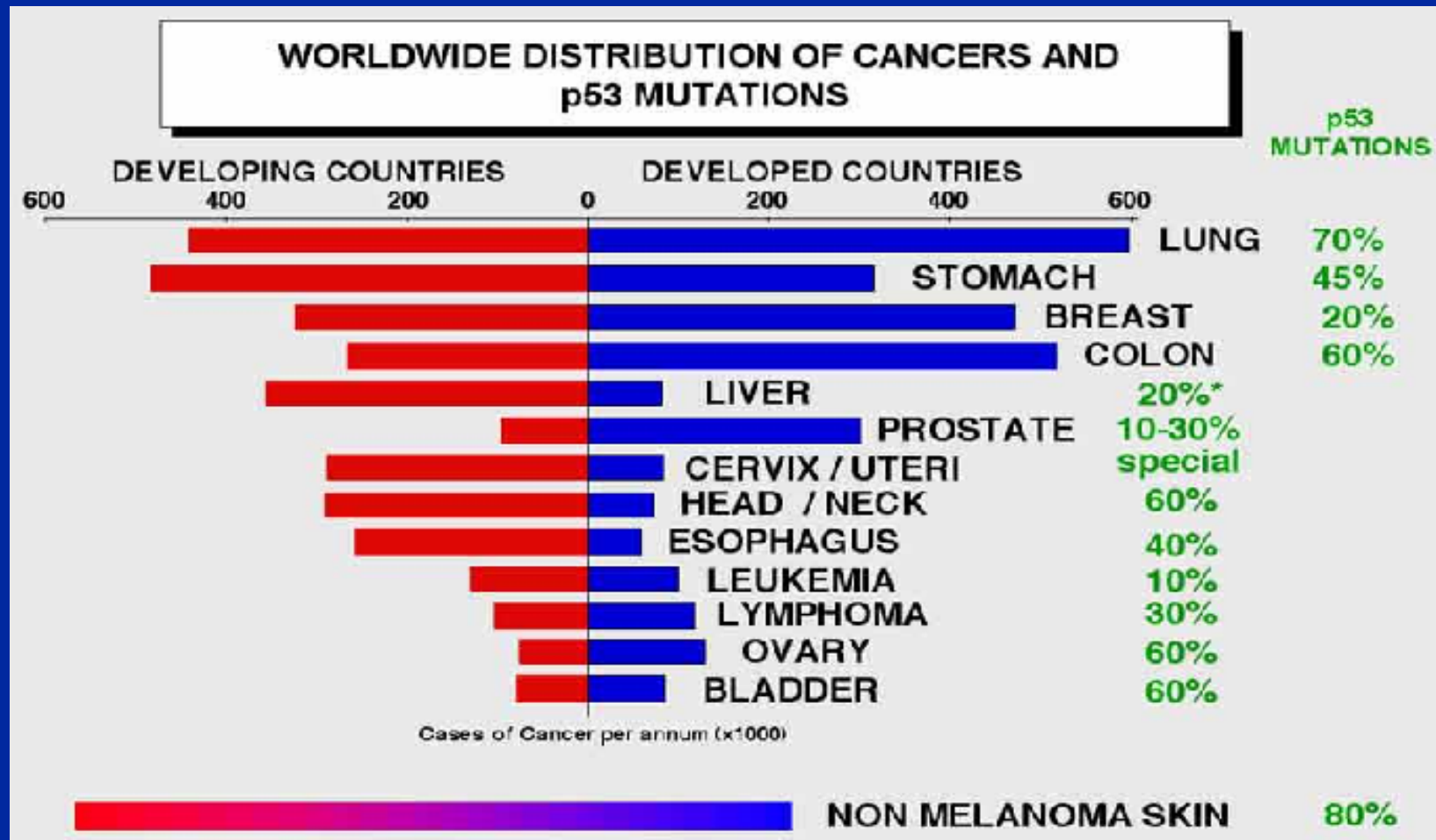
No Apoptosis (Cell Suicide)



Uncontrolled Cell Growth



p53 gene mutation prevalence in cancer



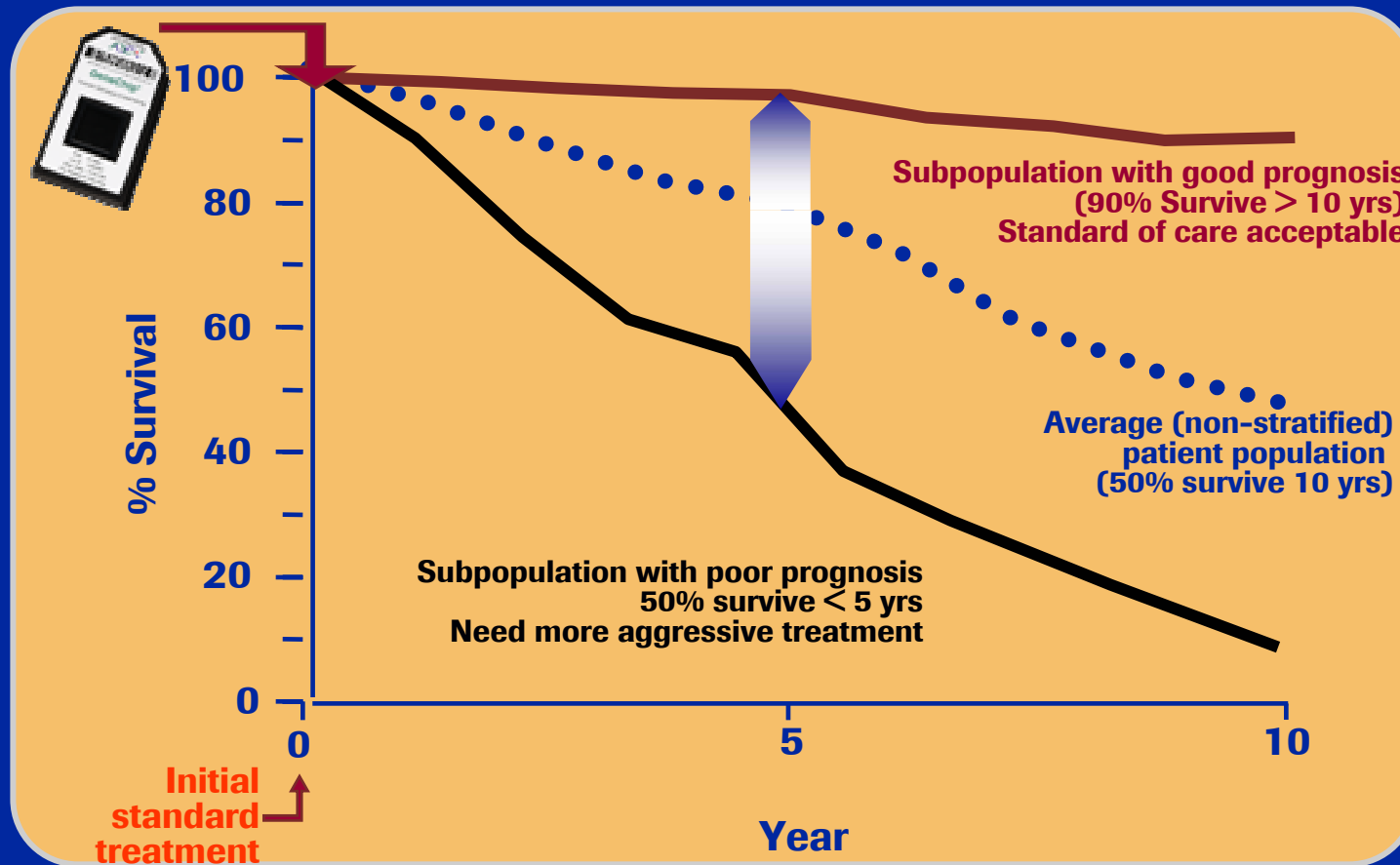
(Total of 14273 p53 mutations)

C. GALLOU, C. BÉROUD AND T. SOUSSI: p53 DATABASE 12/2001 ISSUE

http://p53.curie.fr/p53%20site%20version%202.0/p53%20in%20cancer/p53_databaseANAL.html

The goal

using p53 mutation testing to identify high risk patients who need more aggressive therapy





AmpliChip p53 Test

Test in Development

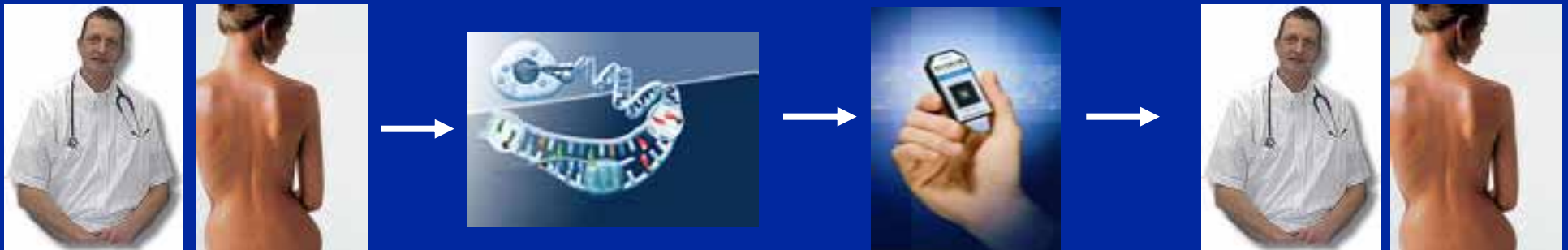
For research only

Not intended for use in *in vitro* diagnostics

The AmpliChip p53 Test

Detecting the mutation status of the p53 gene

- One test to detect > 6500 different possible mutations
- Can provide information relevant to normal or abnormal p53 “guardian of the genome” function
- The goal is to provide actionable information for the physician to better treat patients



How the test works...



Physician orders test



DNA extracted From patient's tumour



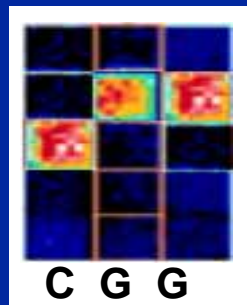
The p53 gene is sequenced from the tumour DNA

Mutation in Codon # 248



Normal Sequence

Mutation in Codon # 248



5' T G G G C A T G A A C C A G A G G C C C A 3'



G T A C T T G G A C T C C G G G T
 G T A C T T G G C C T C C G G G T
 G T A C T T G G G C T C C G G G T
 G T A C T T G G -- C T C C G G G T

The mutation found in the p53 Gene

Probes on the chip

Can p53 mutations predict outcome in advance

i.e. show clinical utility?

- “...**65/93** studies found that...p53 is a statistically significant factor of poor prognosis in various cancers...”

- “... **[14 of] 19** studies have found that the presence of a mutation was associated with a poor response to various chemotherapy or radiotherapy regimens in breast, head and neck, hematological, colorectal, ovarian, esophageal cancers and soft tissue sarcomas.”

Olivier et al. PROGNOSTIC AND PREDICTIVE VALUE OF *TP53* MUTATIONS IN HUMAN CANCER IN: “25 YEARS OF P53 RESEARCH”, KLUWER ACADEMIC PUBLISHERS IN PRESS, EDITORS: P. HAINAUT & K. WIMAN (2005)

What will it take to prove clinical utility of p53 mutation analysis?

- “... its use in clinical practice still requires validation studies to precisely define in which conditions it presents a real advantage over currently available markers....
- “...These studies should be conducted on a large scale and designed with the same standards as drug trials, with well-characterized cancer cases and well-documented treatment regimens and clinical information”.

Olivier et al. PROGNOSTIC AND PREDICTIVE VALUE OF *TP53* MUTATIONS IN HUMAN CANCER IN: “25 YEARS OF P53 RESEARCH”, KLUWER ACADEMIC PUBLISHERS IN PRESS, EDITORS: P. HAINAUT & K. WIMAN (2005)

Potential applications

Non-Small Cell Lung Cancer

Bladder Cancer



Non-Small Cell Lung Cancer (NSCLC)

p53 Gene Mutations Predict Poor Survival in Stage I Non-Small Cell Lung Cancer - Results of a Prospective Trial

**Steven A. Ahrendt, Ying Chuan Hu, Martin Buta, Michael P. McDermott,
Nicole Bonet, Stephen C. Yang, Li Wu, and David Sidransky**

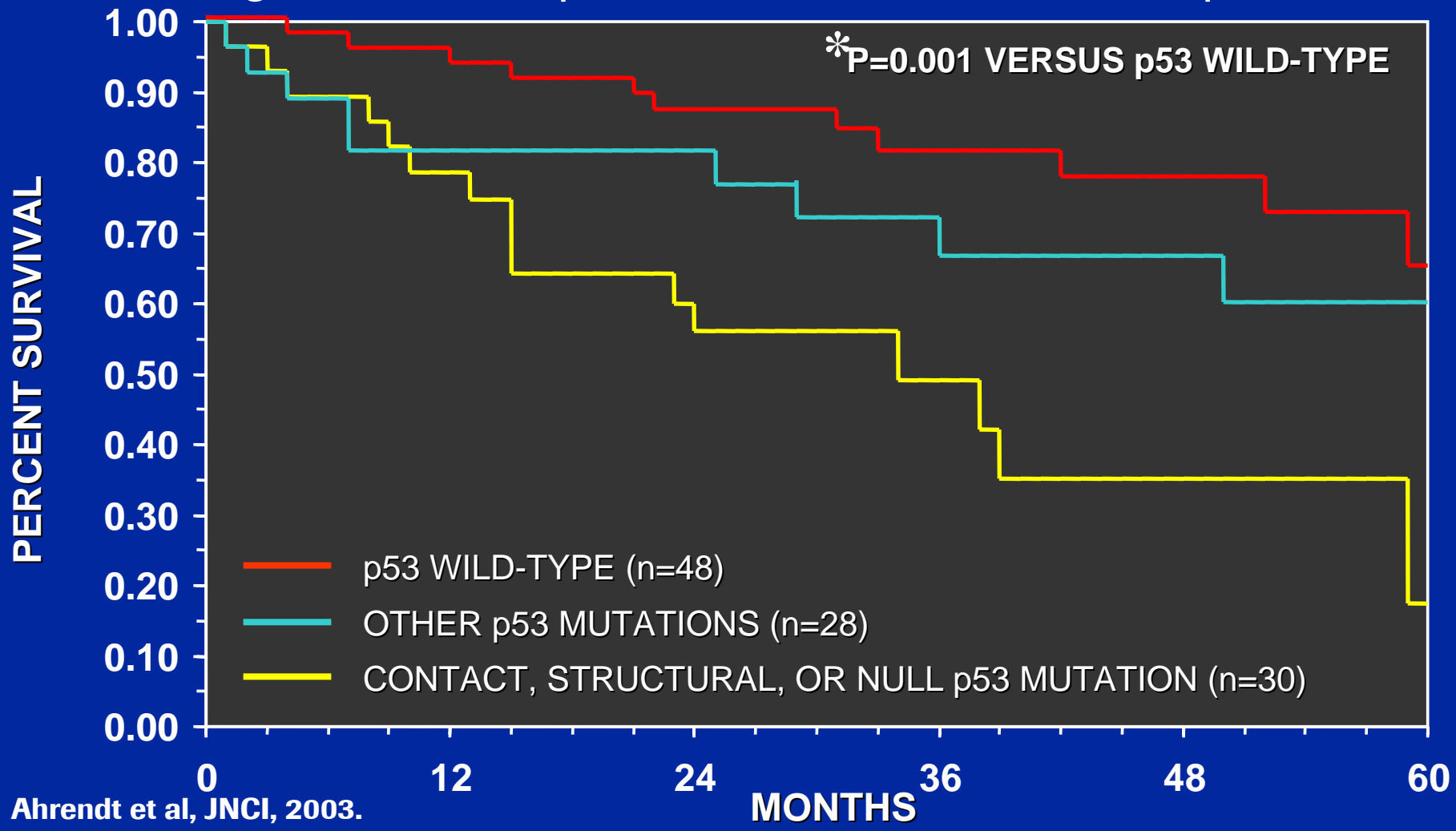
**University of Rochester
The Johns Hopkins University**

Ahrendt et al, JNCI, 2003.



NSCLC patients with normal (wild-type) p53

Longer survival of patients whose tumours have p53 mutations



Bladder Cancer

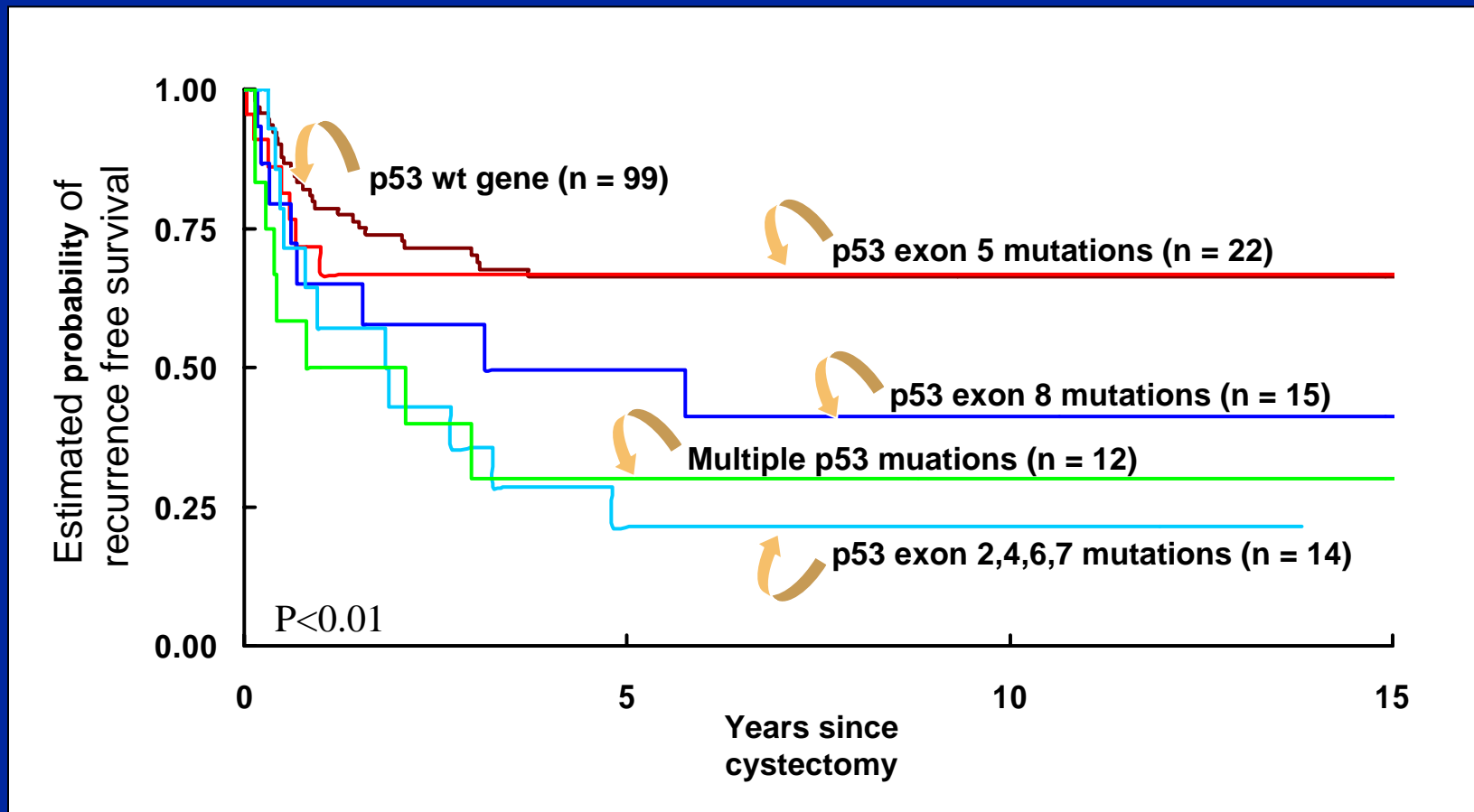


Status of p53 Mutations in Bladder Cancer

Richard J. Cote M.D., FRCPath
University of Southern California

Specific locations of mutations in p53 gene

Correlation with return of disease after surgery



Data kindly provided by R Cote, M.D., FRCPath USC



Ongoing prospective p53 targeted therapy clinical trial in bladder cancer

Roche participating

HYPOTHESIS:

- 1) p53 alterations in organ-confined transitional cell carcinoma of the bladder significantly increase the risk of recurrence and death;
- 2) Adjuvant chemotherapy will improve survival in these high risk patients.

Samples obtained in this prospective trial will be tested for p53 DNA mutations



Current research collaborations

- **Broad spectrum prevalence and prognostic studies in cancer**
 - Pierre Hainaut, International Agency for Research in Cancer, Lyon, France.
- **Bladder Cancer**
 - Richard Cote, USC, Los Angeles, CA: p53 prospective therapy prediction trial
 - Torben Orntoft, Aarhus University Hospital, Aarhus, Denmark: p53 prevalence & methodology studies
- **Lung Cancer**
 - Alfred Böcking, Hans Juergen Gote, Heinrich Heine University, Dusseldorf, Germany: p53 mutation prevalence study in stage I non-small cell lung cancer

Current research collaborations

- **Breast Cancer**

- Alastair M Thompson, Department of Surgery and Molecular Oncology, University of Dundee. p53 mutation prevalence in fresh-frozen tissue from breast cancer
- Howard Robin, Sharp Medical Labs, San Diego, CA: p53 mutation prevalence study in Stage I patients – Paraffin embedded tissues

- **Head and Neck Cancer**

- Maxine Partridge, Kings College, London, UK: p53 mutation prevalence study in head and neck cancer – Paraffin embedded tissues



Current research collaborations

- **Companion Diagnostics – Joint Programme with Roche Pharmaceuticals**
 - An Open Label Study of Capecitabine and Docetaxel as Neoadjuvant Treatment for Patients with Recently Diagnosed HER2-neu Negative Breast Cancer plus Trastuzumab for HER2-neu Positive Breast Cancer: AmpliChip p53 Test sequence analysis of p53 gene mutations to predict pathological responses
 - AmpliChip p53 sequence analysis as a companion therapy prediction diagnostic to mdm2/p53 antagonist drug