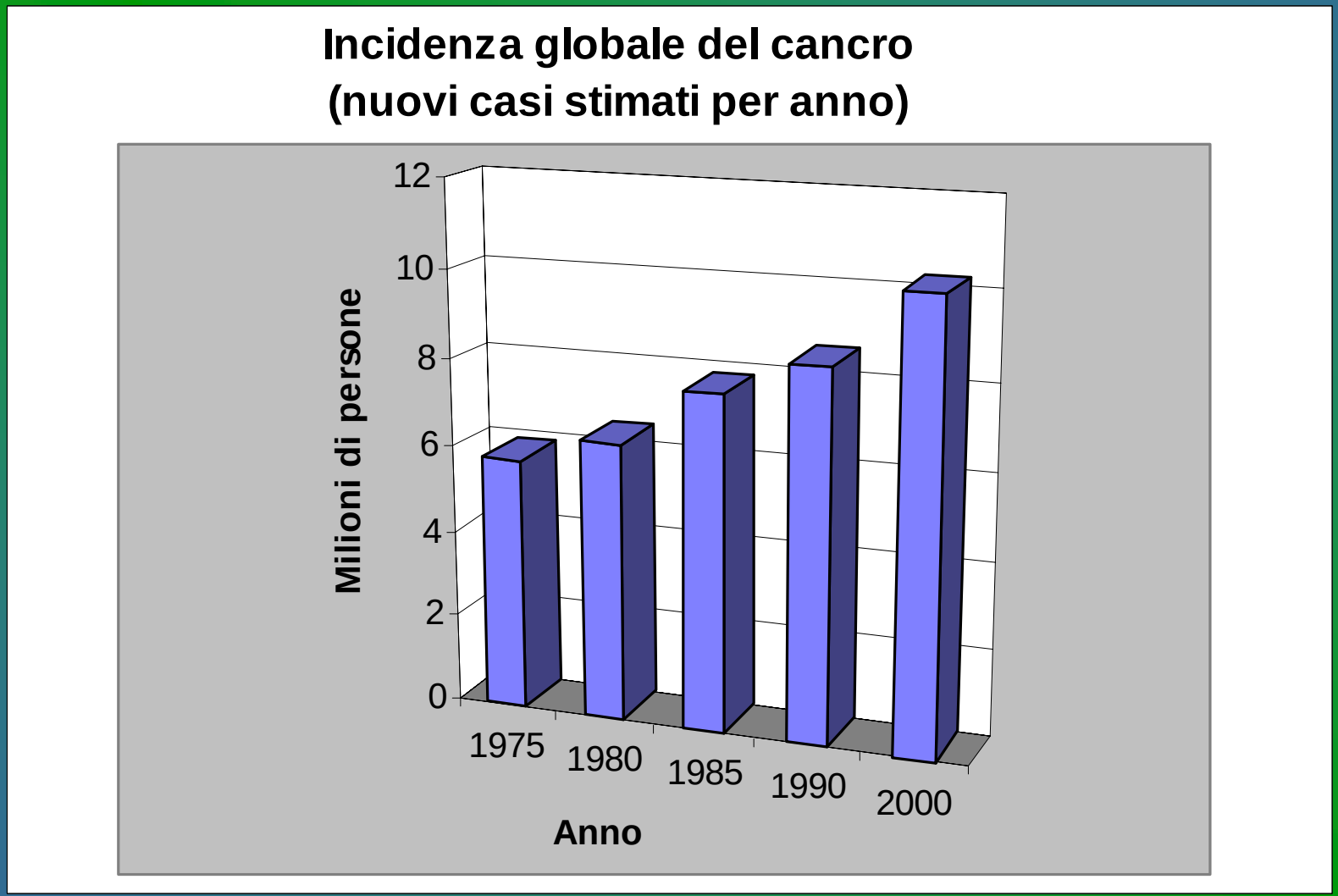
Contents

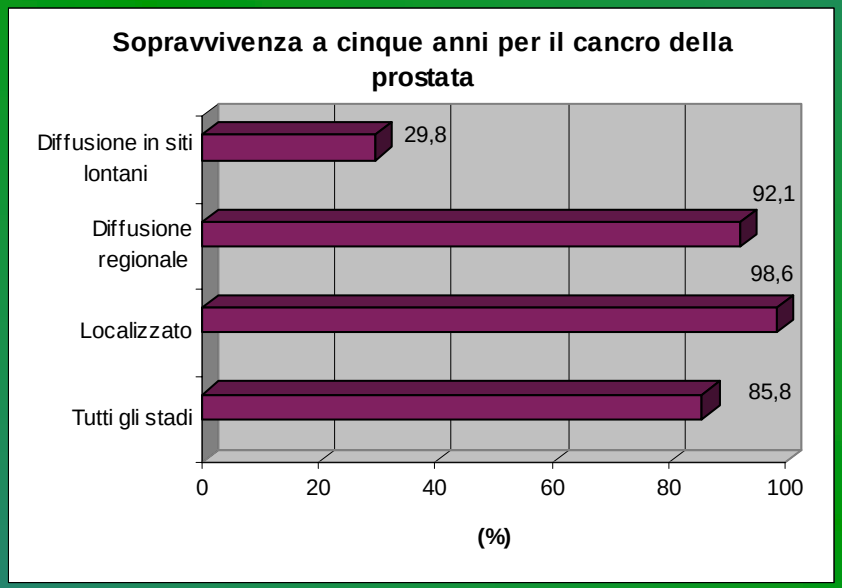
- **Introduction: biology and medicine, two separated compartments**
- **What we need to know:**
  - boring basics in DNA/RNA structure and overview of particular aspects of molecular biology techniques
  - How DNA is organized and differs in every individual
- **Molecular diagnostics of cardiovascular diseases**
  - Mutations in Factor V
  - Mutations in Factor II
  - Mutations in MTHFR gene
- **Breast cancer and BRCA1 and 2 genes**
  - Breast cancer in the industrialized countries
  - Breast cencer genes
  - sequence in selected areas
  - p53 and breast cancer
- **Pharmacogenomics: finding the right drug for a patient**
  - ADR: an emerging problem
  - structure of cytochromes
  - Example 1: TPMT-enzyme and the metabolism of azathioprines
  - Example 2: Clozapine in the treatment of psychiatric diseases
  - Example 3: CXP3A4 and the metabolism of anti-coagulant drugs

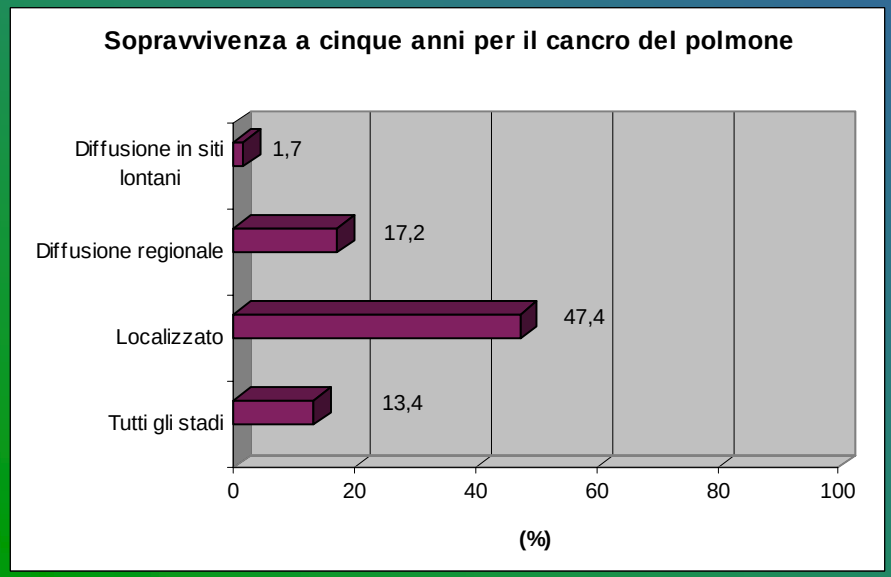
Breast cancer susceptibility genes BRCA1/2

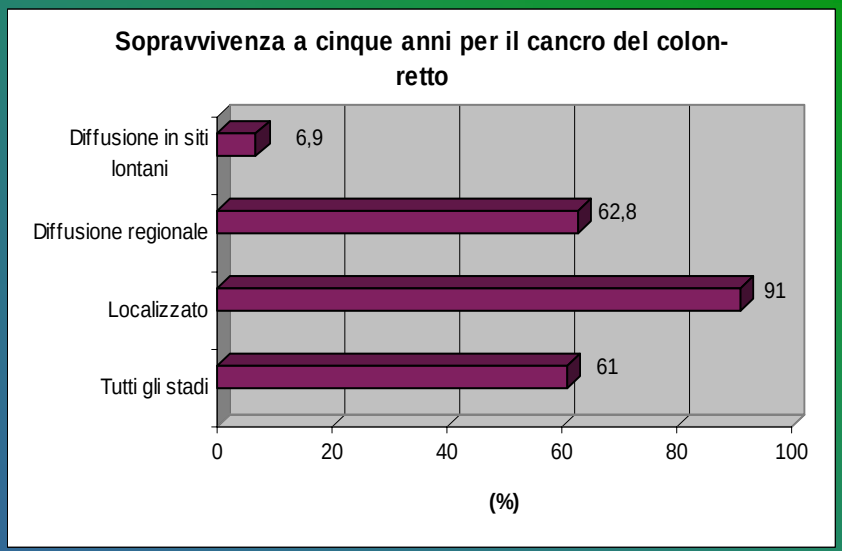
Global incidence of cancer

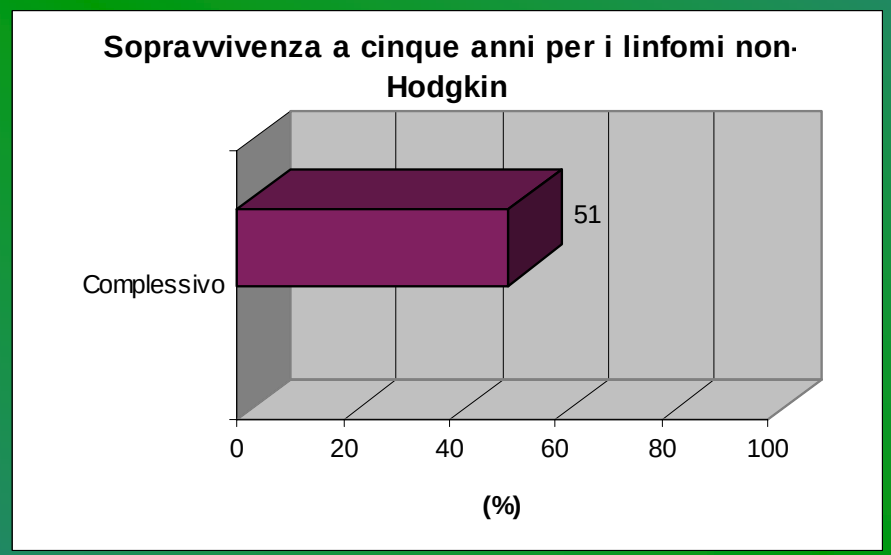


Survival at 5 years...

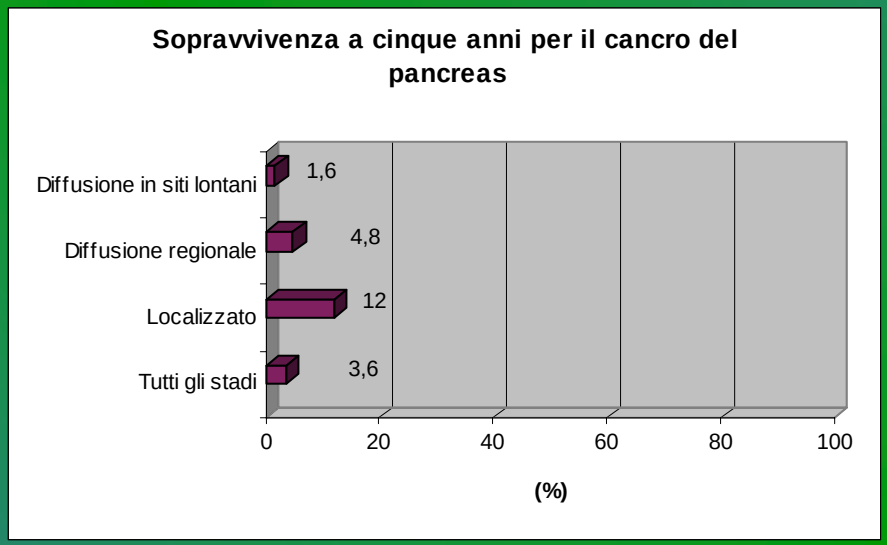


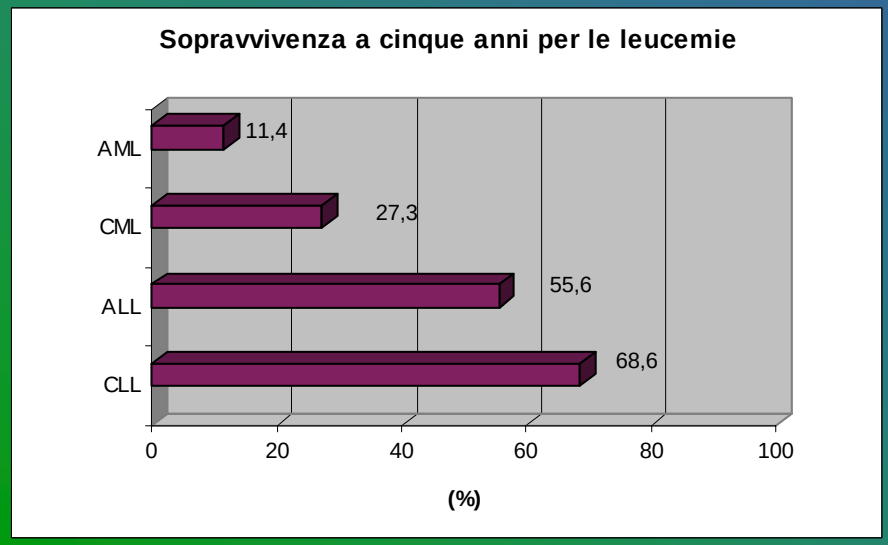


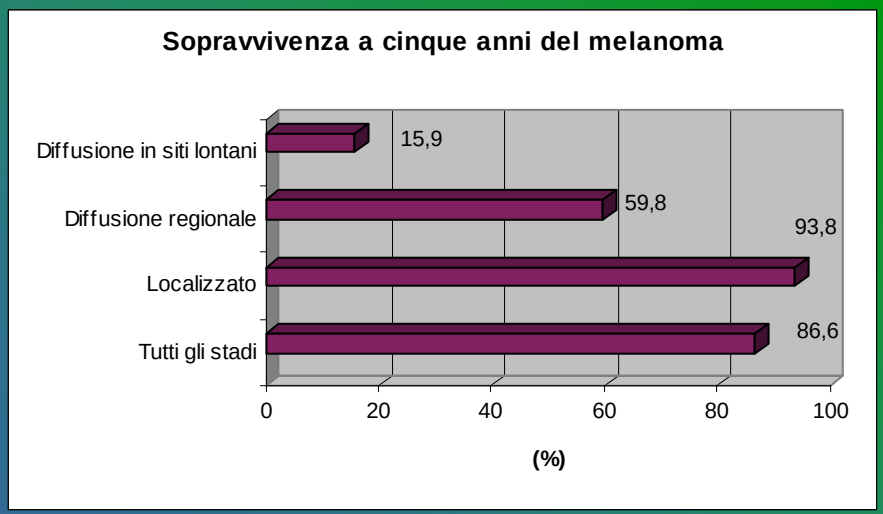


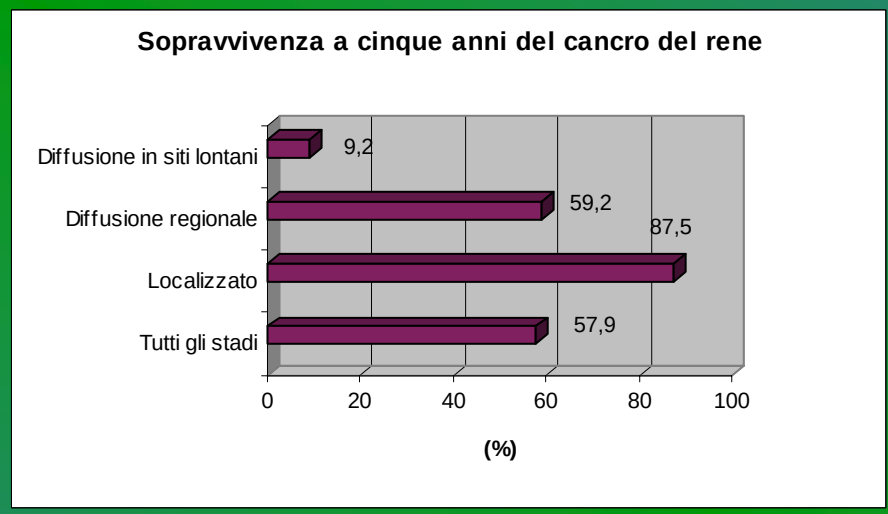


Survival at 5 years...

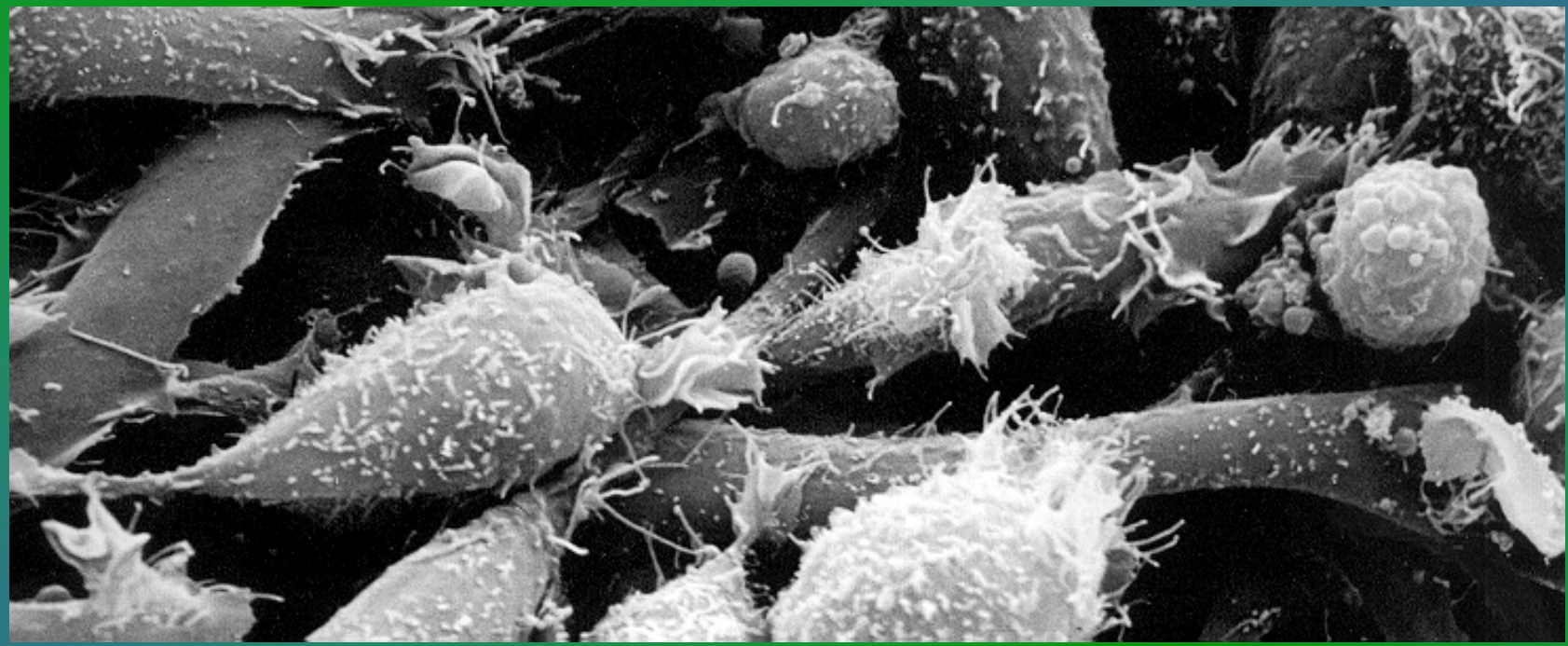




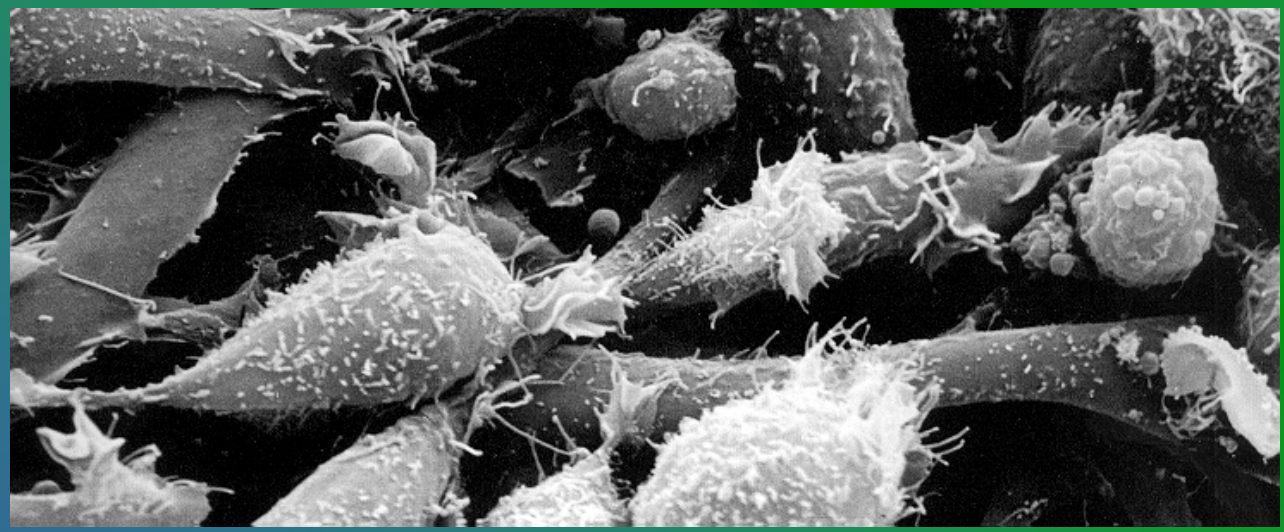




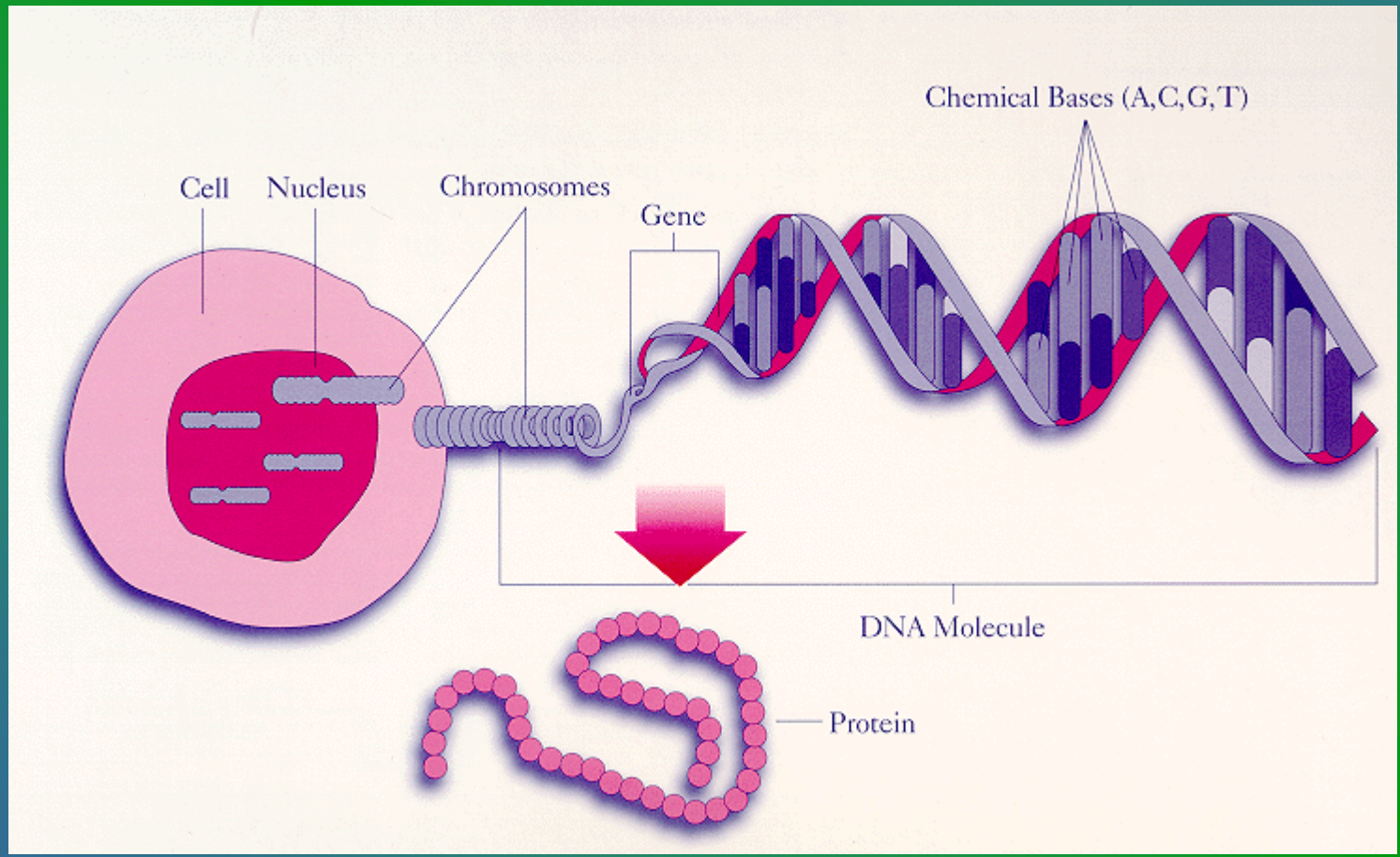
Tumor cells could look like this.



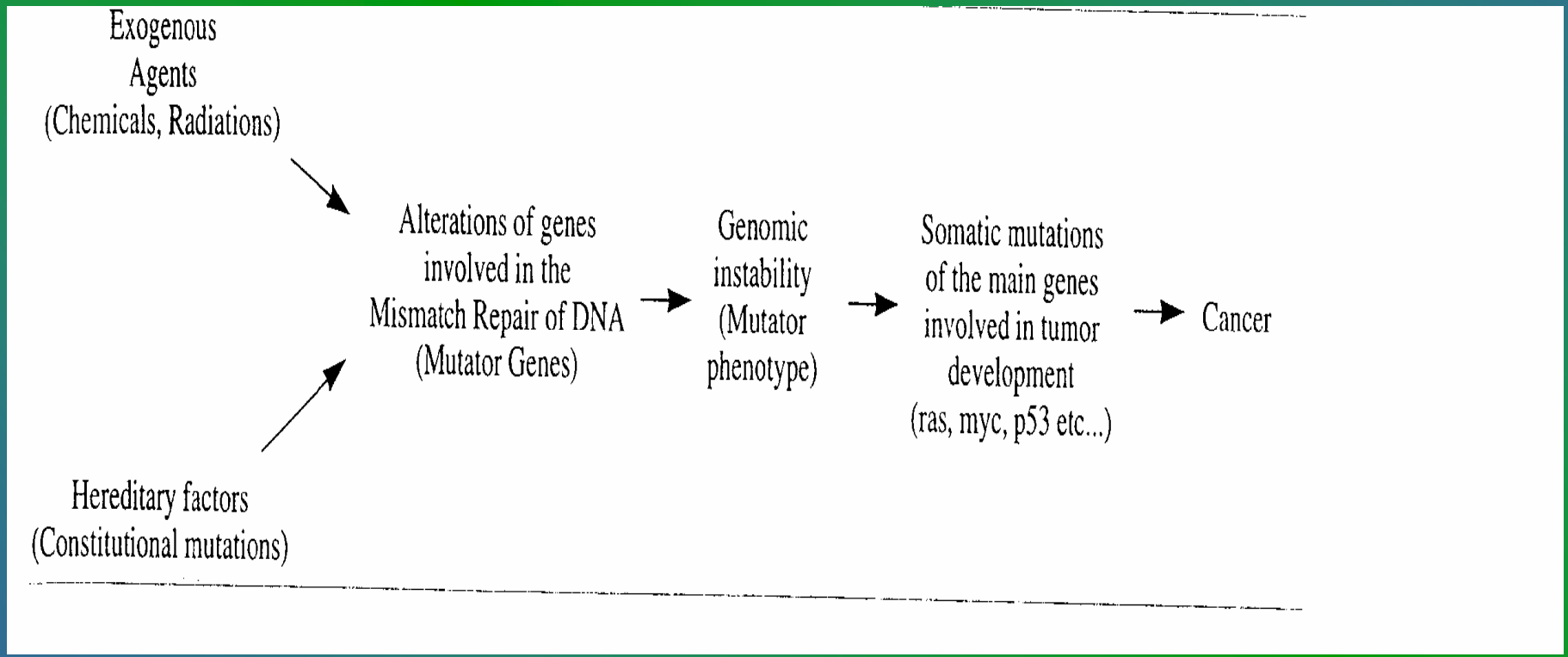
Just compare...



Cancer arises from gene mutations



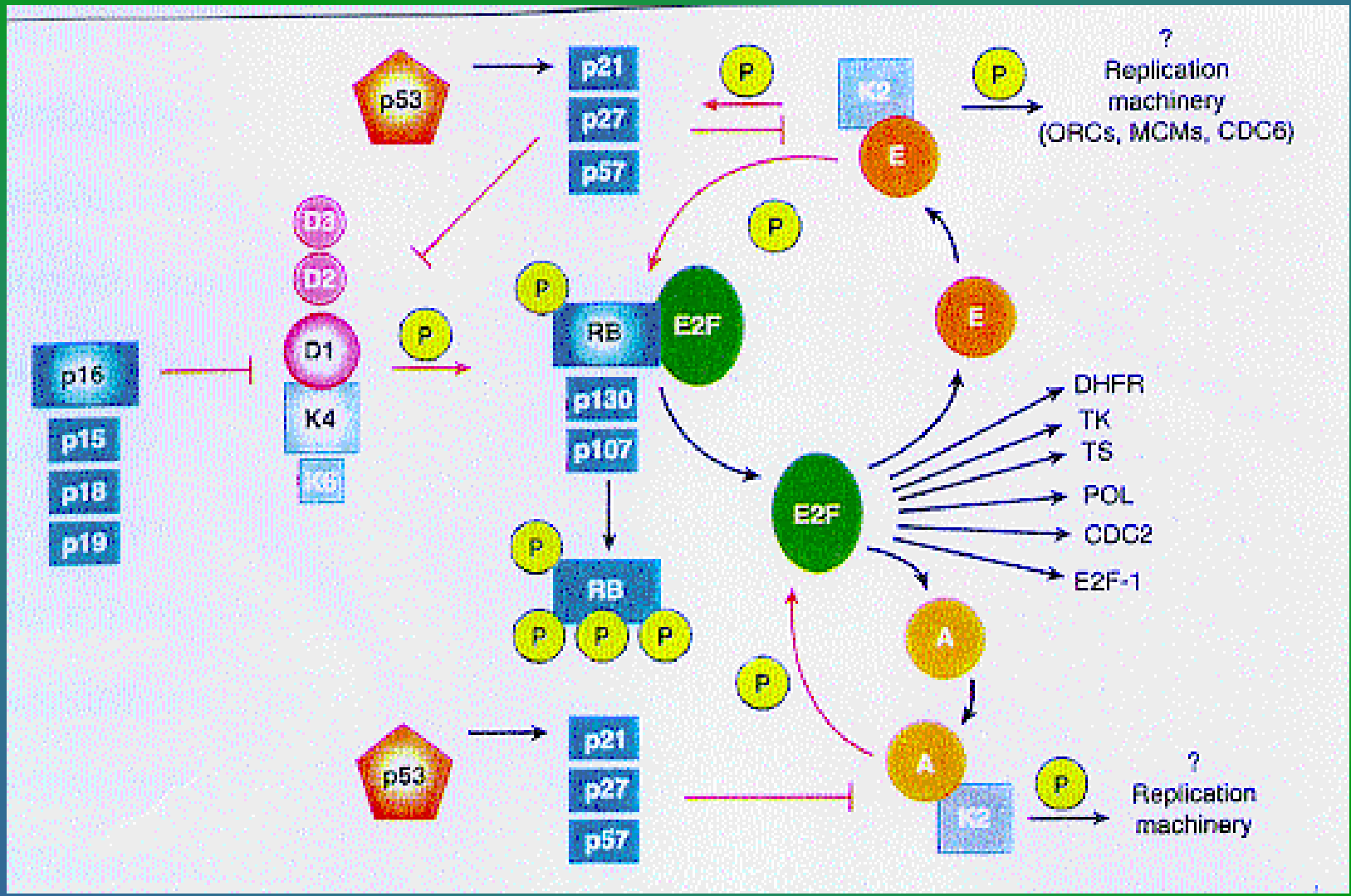
Cancer is a multistep process resulting from an accumulation of mutations



Tumor cells...

- Do not respond to control mechanisms
- Do not need growth factors (or less)
- They divide in an infinite way
- They invade other tissues and induce capillary growth

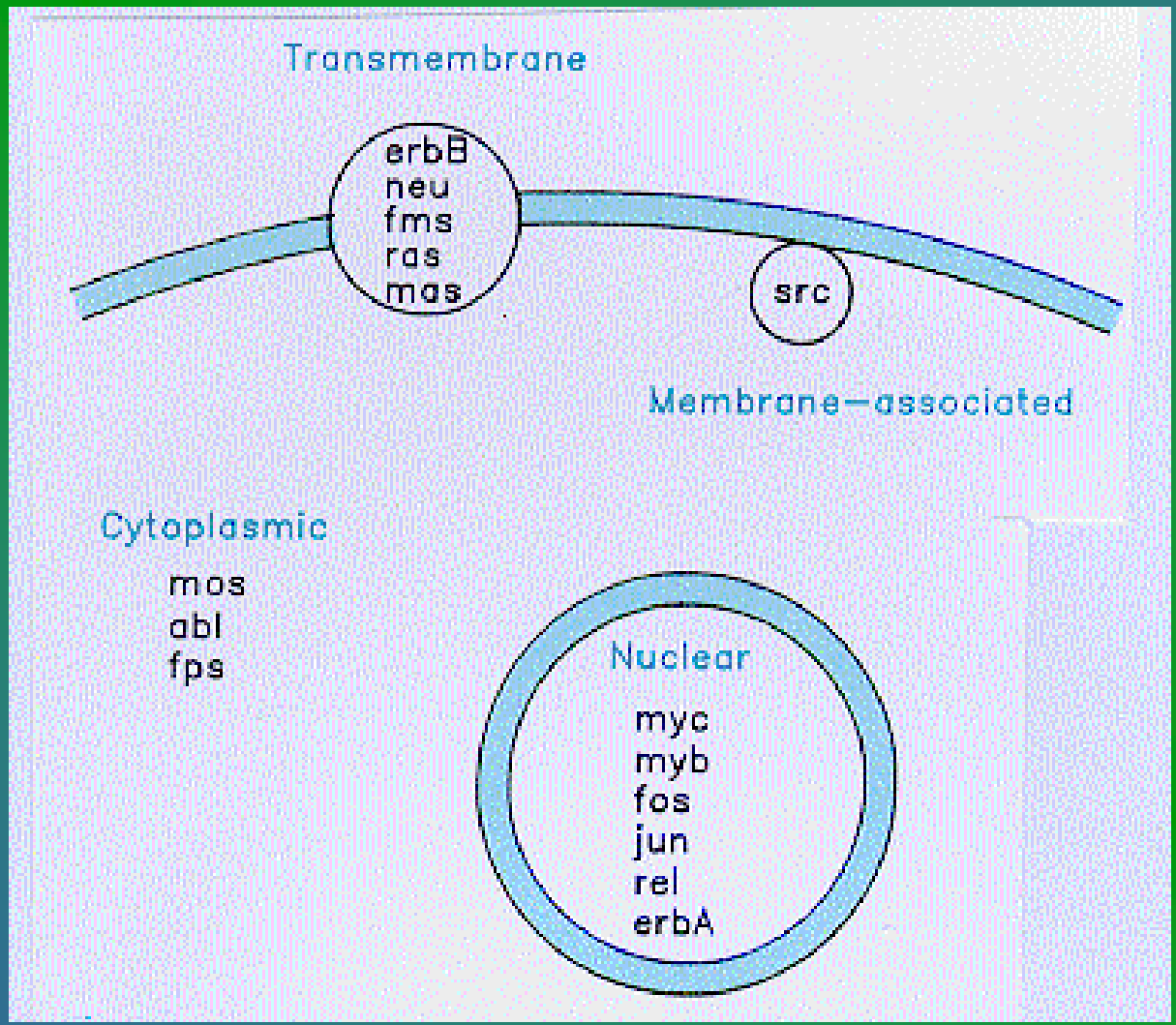
In the jungle of cell regulatory molecules



Which are critical in:

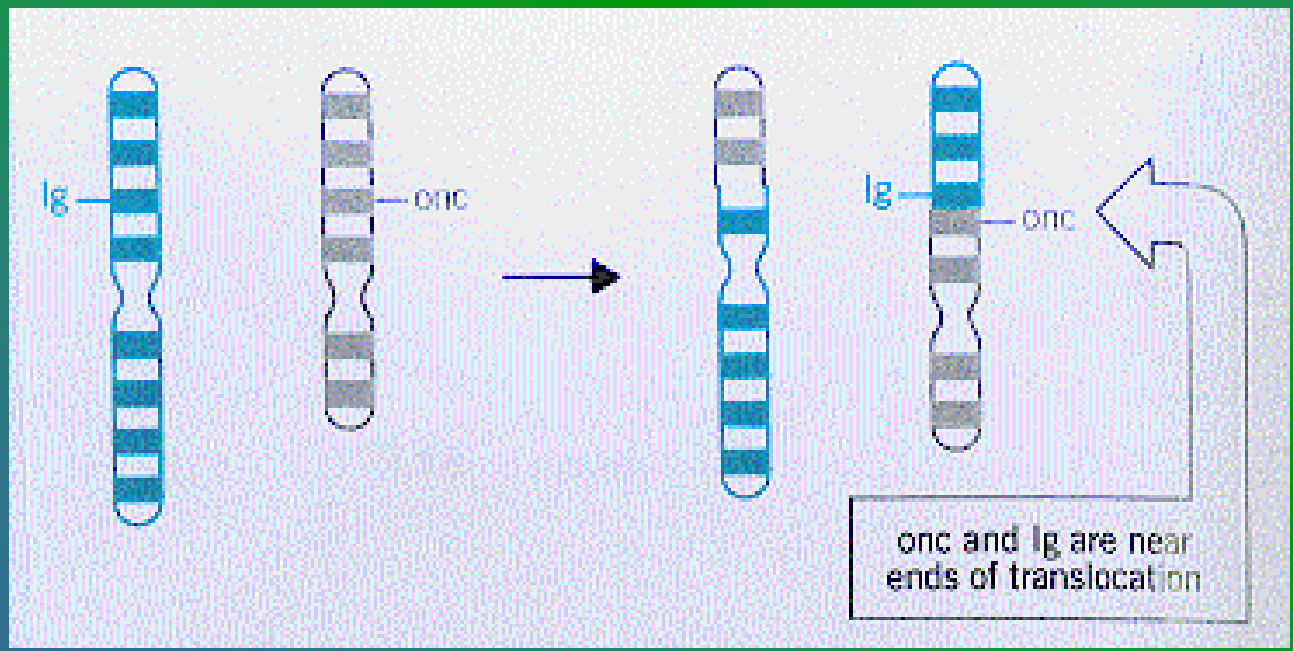
- Cell growth control genes
- genes which has the capability, if mutated, to transform the cell (oncogenes)
- genes coding for proteins that negatively control the cell cycle (tumor suppressor genes)

Cellular oncogenes



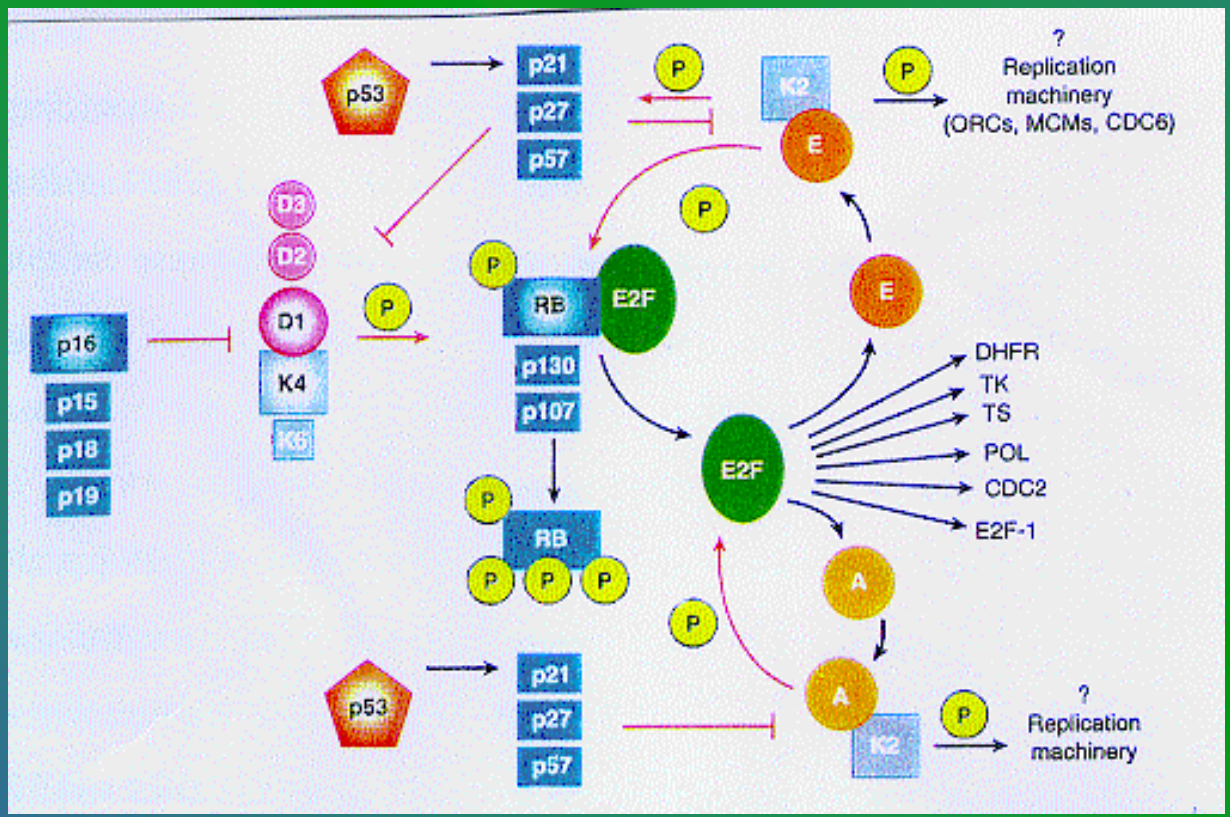
Factors that could activate a proto-oncogene

- Insertions (viral promoter before a proto-oncogene)
- Chromosomal translocations
- Amplifications



Tumor suppressor genes

Nuclear proteins and transcription activators like p53, p21, p16, Rb, Brca1 and Brca2



The Li-Fraumeni syndrome

- Germinal mutation on one of the two p53 genes and loss of the other allele
- p53 is a tumor suppressor gene
- produces a wide spectrum of primary tumors like sarcomas, breast carcinoma, brain tumor
- Diagnosed by sequencing the p53 gene

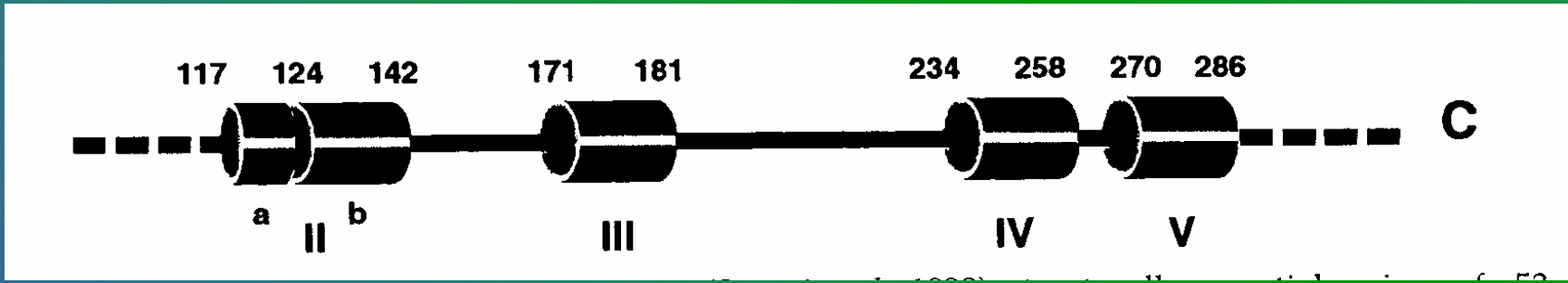
The p53 gene in sporadic tumors

Tumor Type	n	*p53* Mutations	Tumor Type	n	*p53* Mutations	Tumor Type	n	*p53* Mutations
Lung	897	56%	Prostate	87	30%	Carcinoid	61	11%
Colon	960	50%	Hepatocellular	716	29%	Melanoma	70	9%
Esophagus	279	45%	Brain	456	25%	Parathyroid	13	8%
Ovary	386	44%	Adrenal	31	23%	Cervix	350	7%
Pancreas	170	44%	Breast	1536	22%	Neuroblastoma	212	1%
Skin	220	44%	Endometrium	224	22%	Wilms'	41	none
Gastric	314	41%	Mesothelioma	23	22%	Testes	40	none
Head and neck	524	37%	Renal	102	19%	Pituitary	27	none
Bladder	308	34%	Thyroid	299	13%	Pheochromocytoma	47	none
Sarcoma	339	31%	Hematologic	1916	12%			

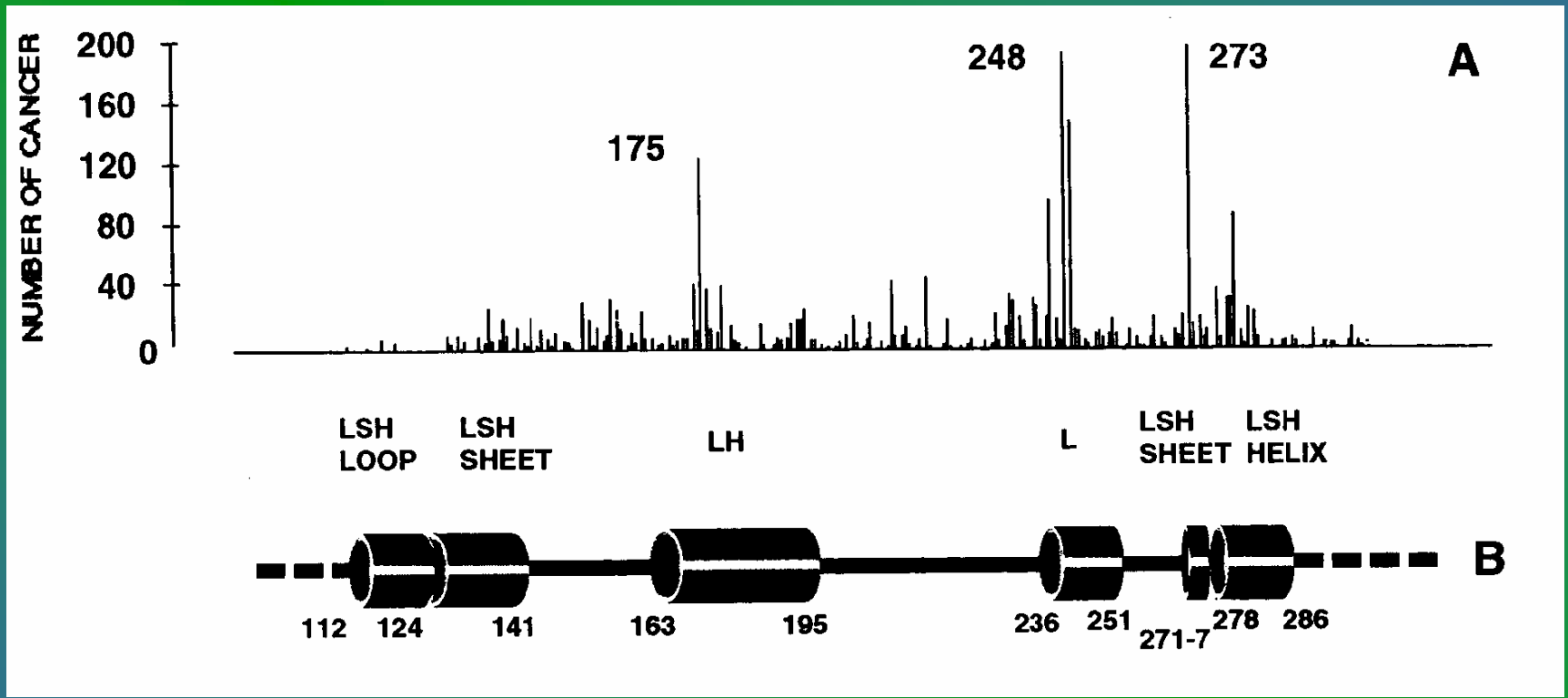
n = number of tumors of each cell type evaluated for *p53* mutation by PCR-based techniques. Courtesy of Curtis C. Harris, M.D.

p53

- On chromosome 17
- composed of 11 exons of which 5,6,7 and 8 are highly conserved
- P53 is a nuclear phosphoprotein
- has 4 functional domains

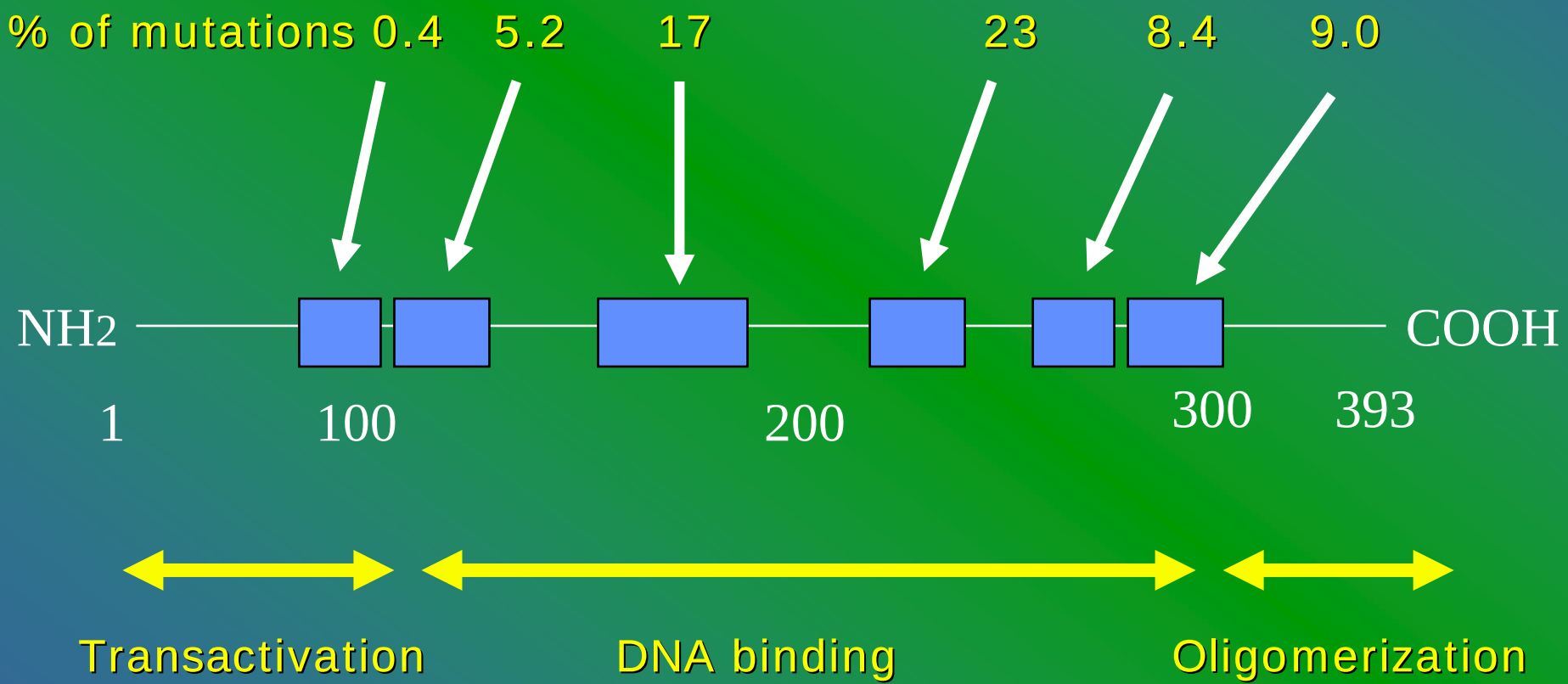


98% of p53 mutations...

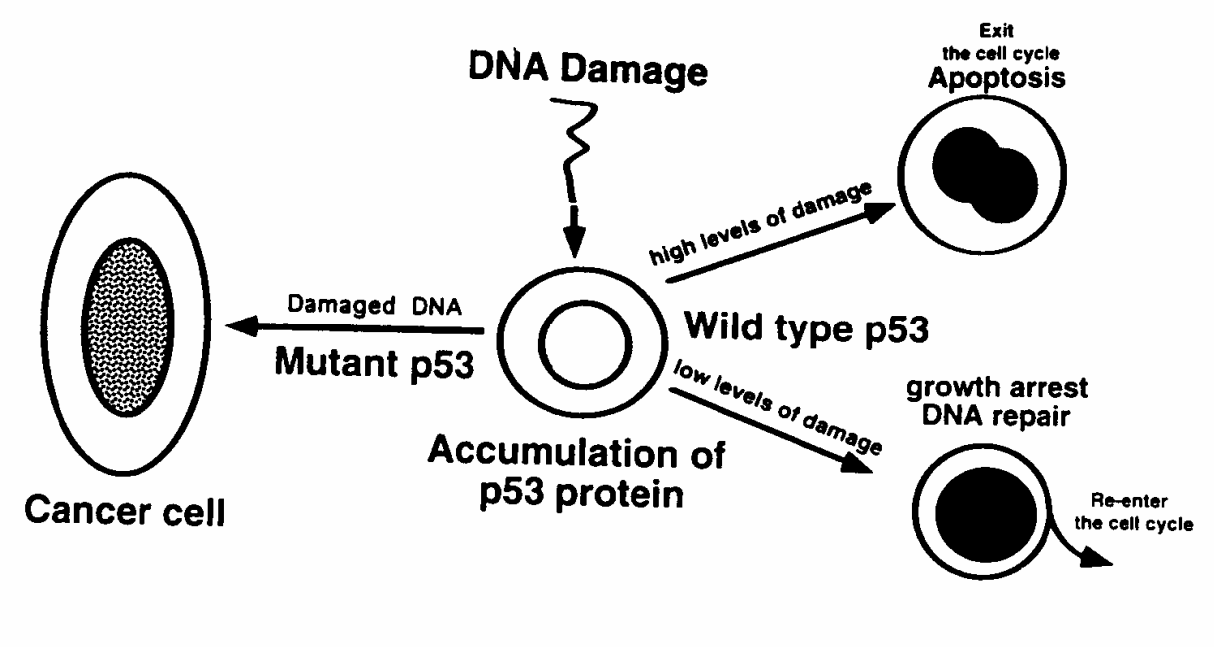


are in the conserved regions.

Structure of the p53 protein
(B. Vogelstein and W. Kinzler, Nature 370, 1994)



P53 in DNA repair



- P53 accumulates upon DNA damage
- wild-type p53 promotes growth arrest or apoptosis
- mutant p53 can lead to a cancer cell

Cell cycle regulation...critical steps

The cell cycle is regulated by complicated feed-back mechanisms

Foundamental is to keep equilibrium between dying and new cells

Cells of a multicellular organism divide with different frequencies

Tissue cells normally divide only inside the tissue

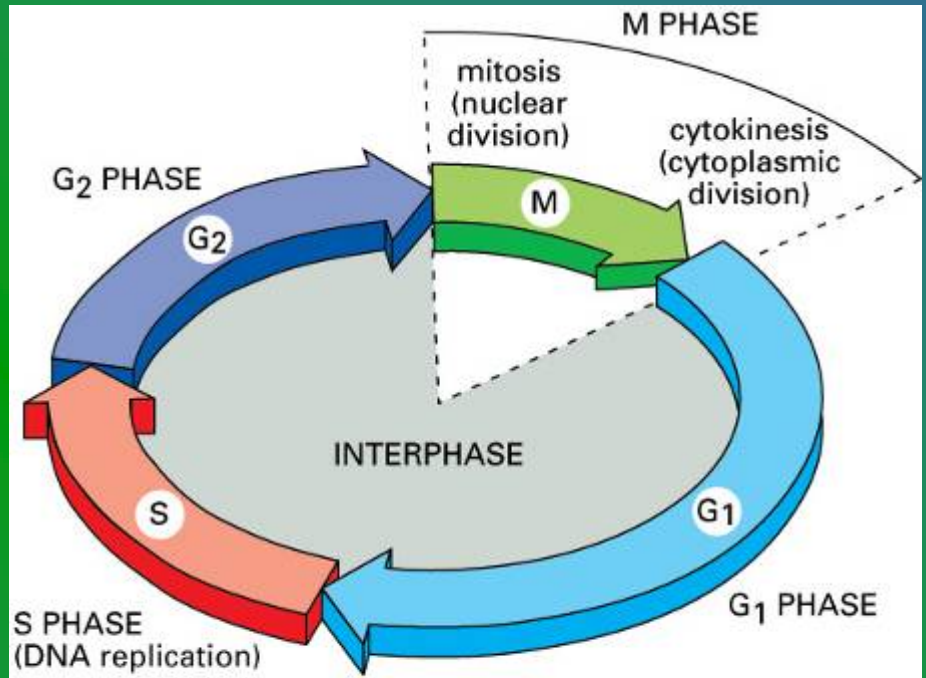
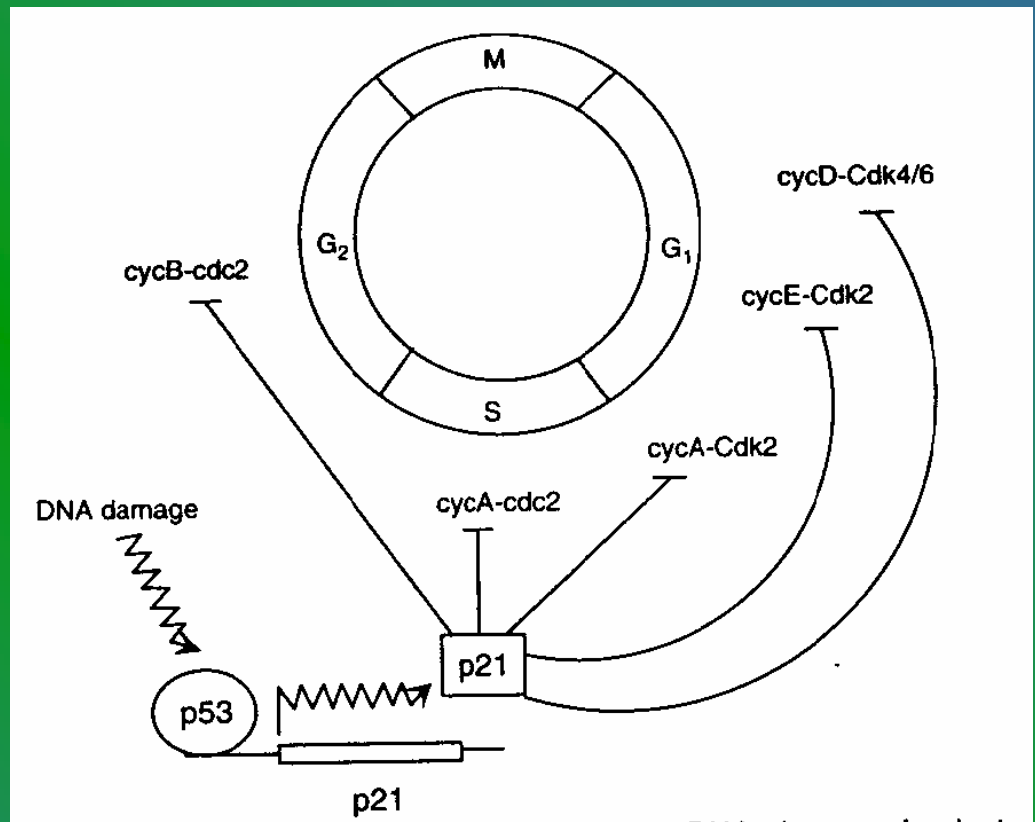


Figure 17–3. Molecular Biology of the Cell, 4th Edition.

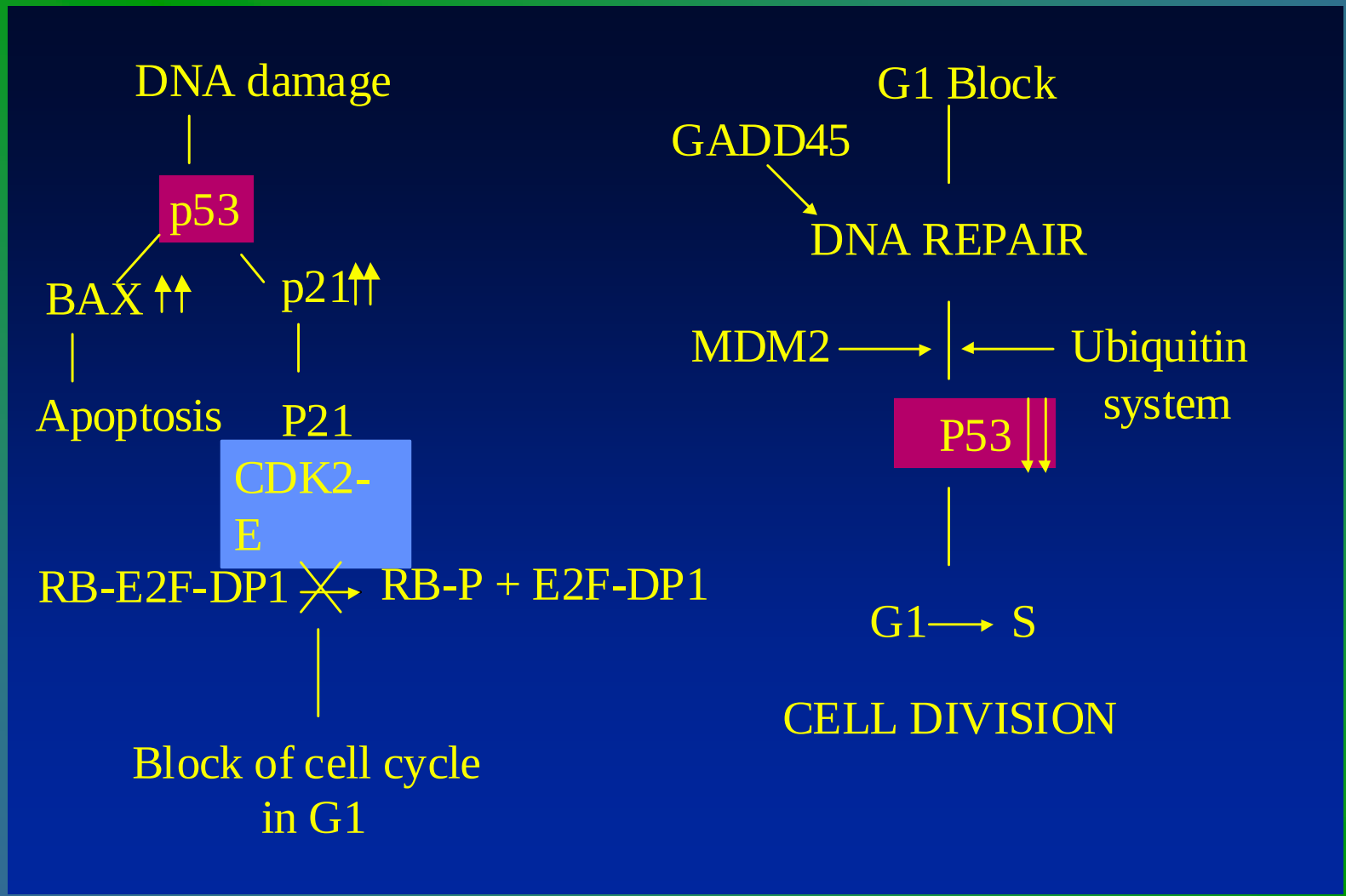
Growth arrest by p53

- DNA damage leads to an accumulation of transcriptionally active p53 that induces expression of p21 mRNA and protein.
- P21 acts as an universal inhibitor of cyclin-dep kinases affecting cell cycle progression at many points

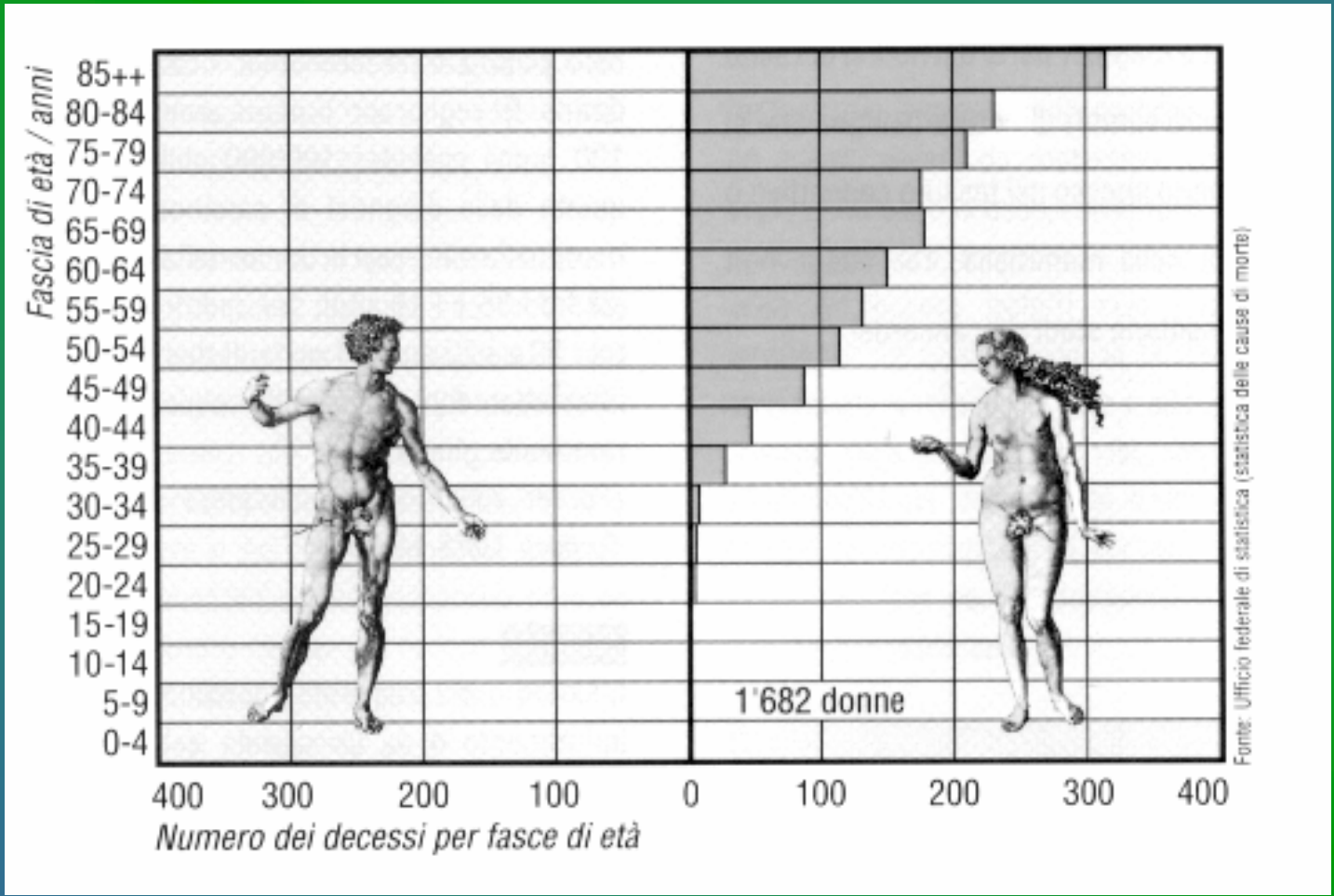


. p21 arrests the cell cycle at many points. DNA damage leads to

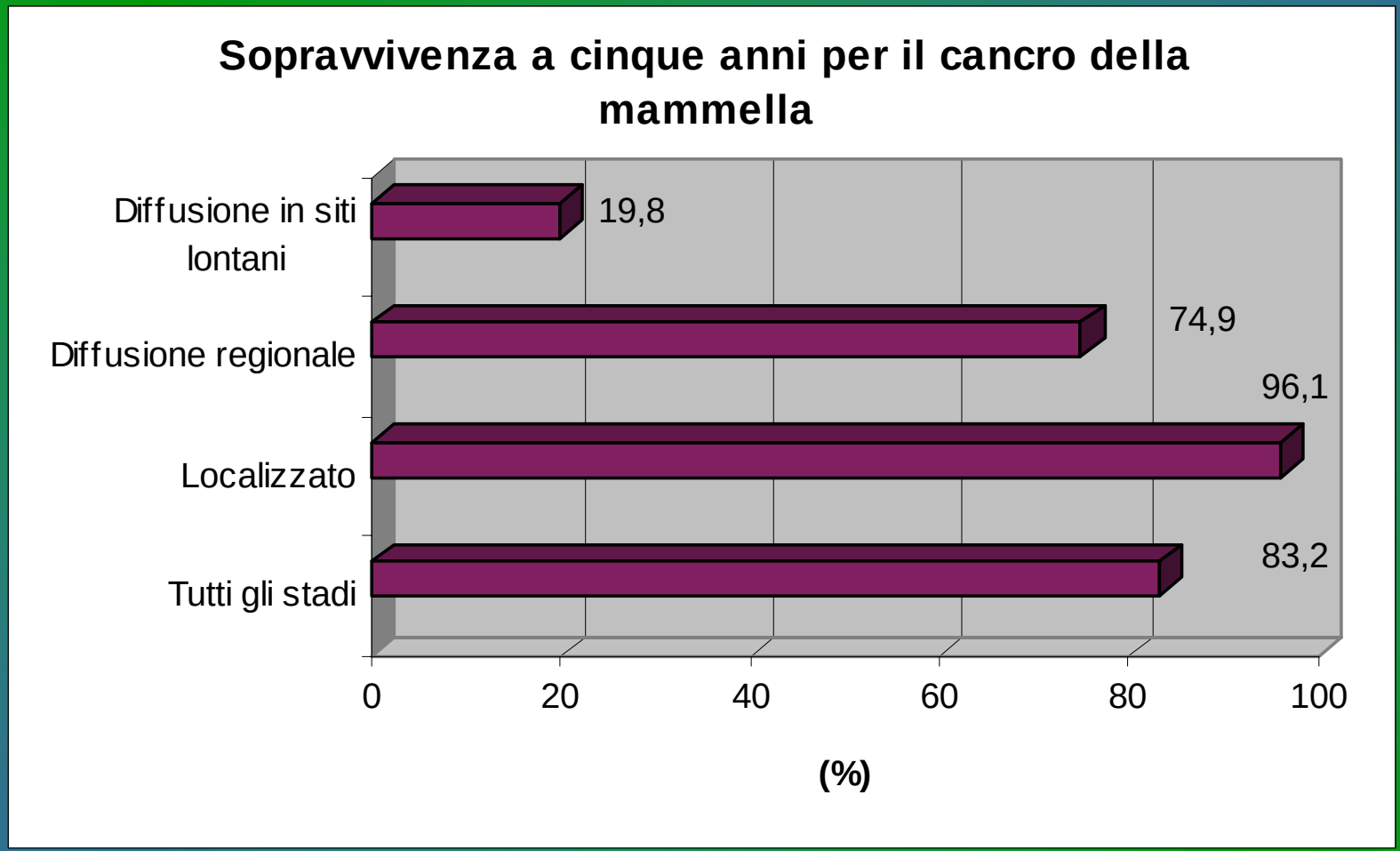
P53 up and down



Age distribution of mortality cases for breast cancer in Switzerland (1990-1993)



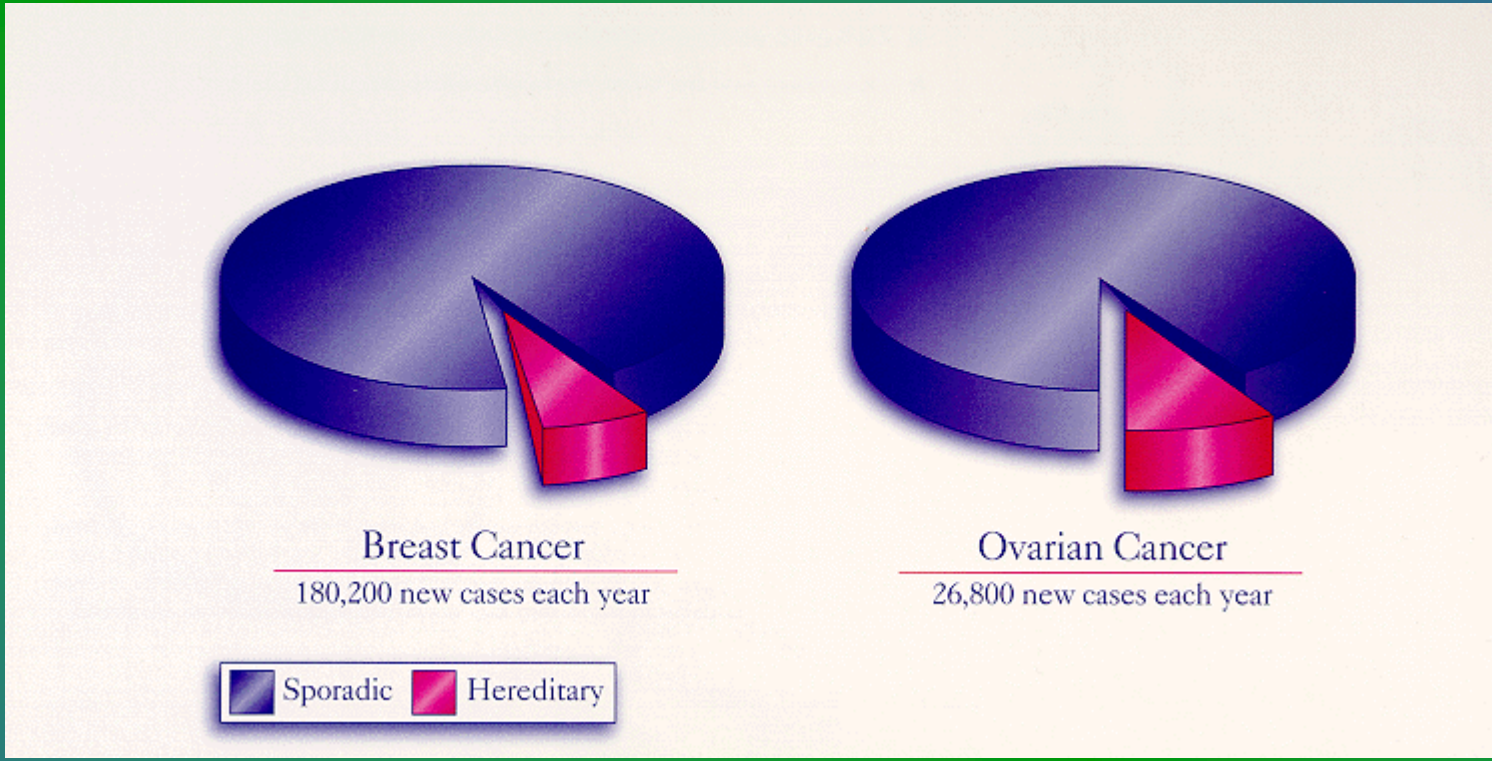
Breast cancer survival



Breast cancer

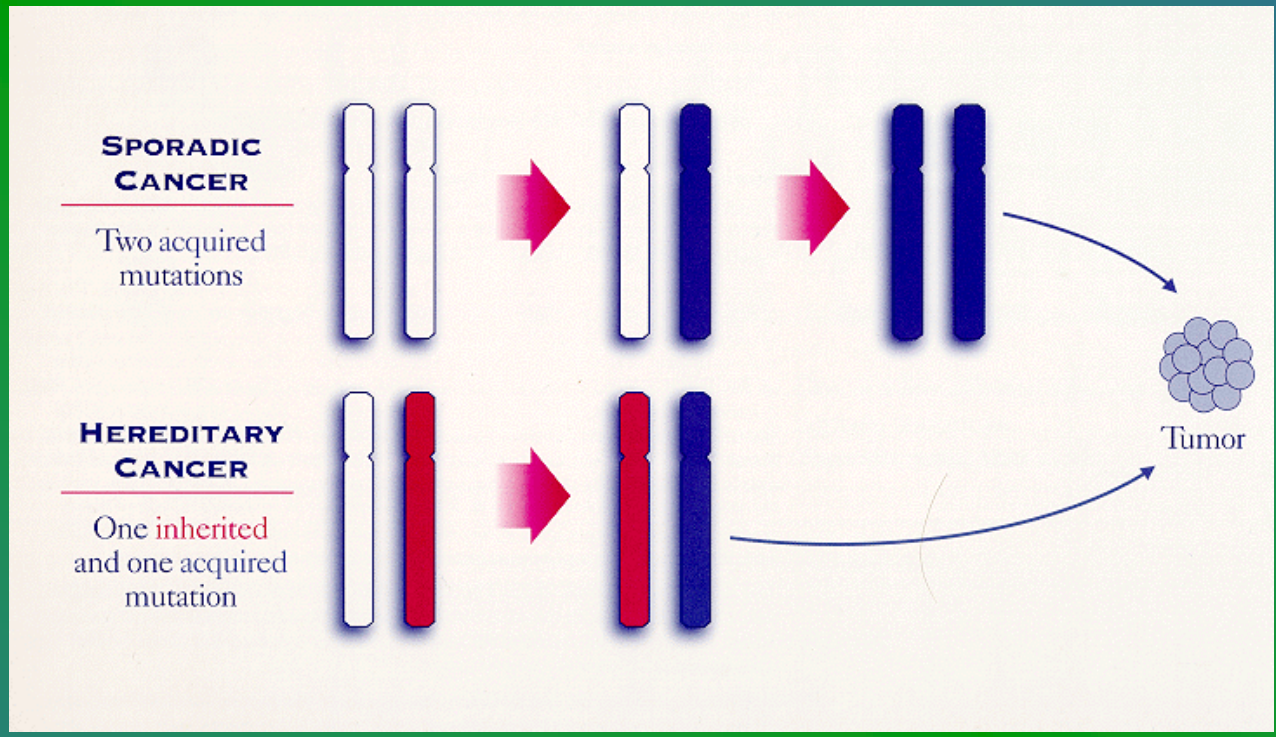
- In Switzerland every year 1700 women are dying of breast cancer
- This represent 23% of deaths of cancer in the women population
- Every year 100 new cases over 100’000 people are registered
- 20% of diagnoses concern women between 35 and 49 years of age
- 40% of diagnoses concern women between 40 and 70 years of age

Each year new cases...



Most breast and ovarian camcers are sporadic in nature. Only 10% of them are hereditary. Up to 7% of hereditary breast cancers and 9-10% of hereditary ovarian cancers are caused by inherited mutations in the BRCA1 and BRCA2 genes.

Gene mutations may be inherited or acquired during a person's life.



Most cancers develop from random mutations that develop in body cells during aperson's lifetime. Somatic mutations are not passed to offspring. Only a small percentagr of cancers are hereditary and result from germline mutations. Germline mutations are passed on from one generation to the next.

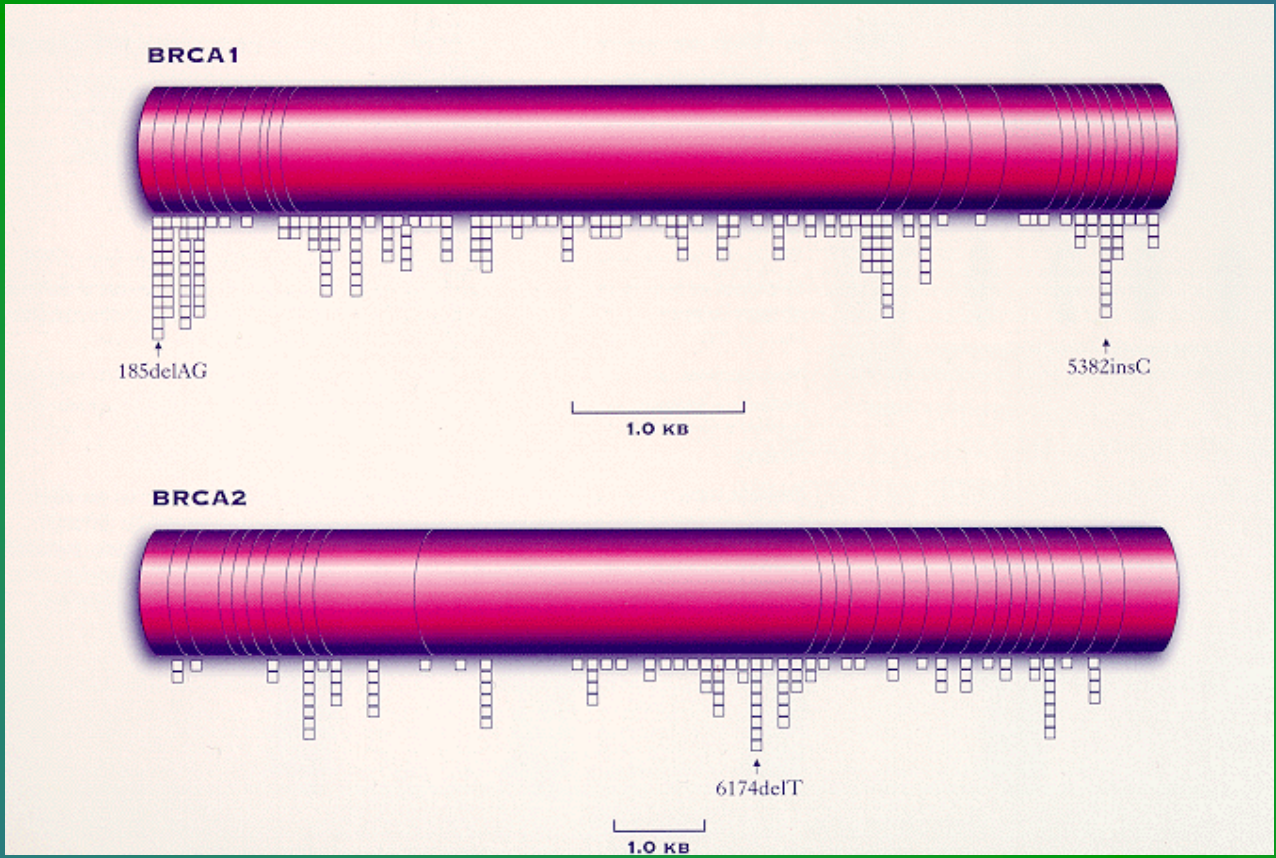
BRCA1 and BRCA2 genes

- BRCA1 and BRCA2 are tumor suppressor genes, encoding a protein capable of negatively regulating tumor growth
- 15849 basepairs of coding sequence
- population frequency of BRCA1 mutations is 1/800 women
- some 10% of all breast cancers are hereditary
- the mutations are present in 90% of hereditary breast and ovary tumors

BRCA1/2 (suite)

- the germinal mutation on one of the two alleles leads to LOH
- Diagnostics: protein truncation test (PTT) and sequence
- risk of breast cancer: 85% before age 70 and 50% before age 40.

The two genes.

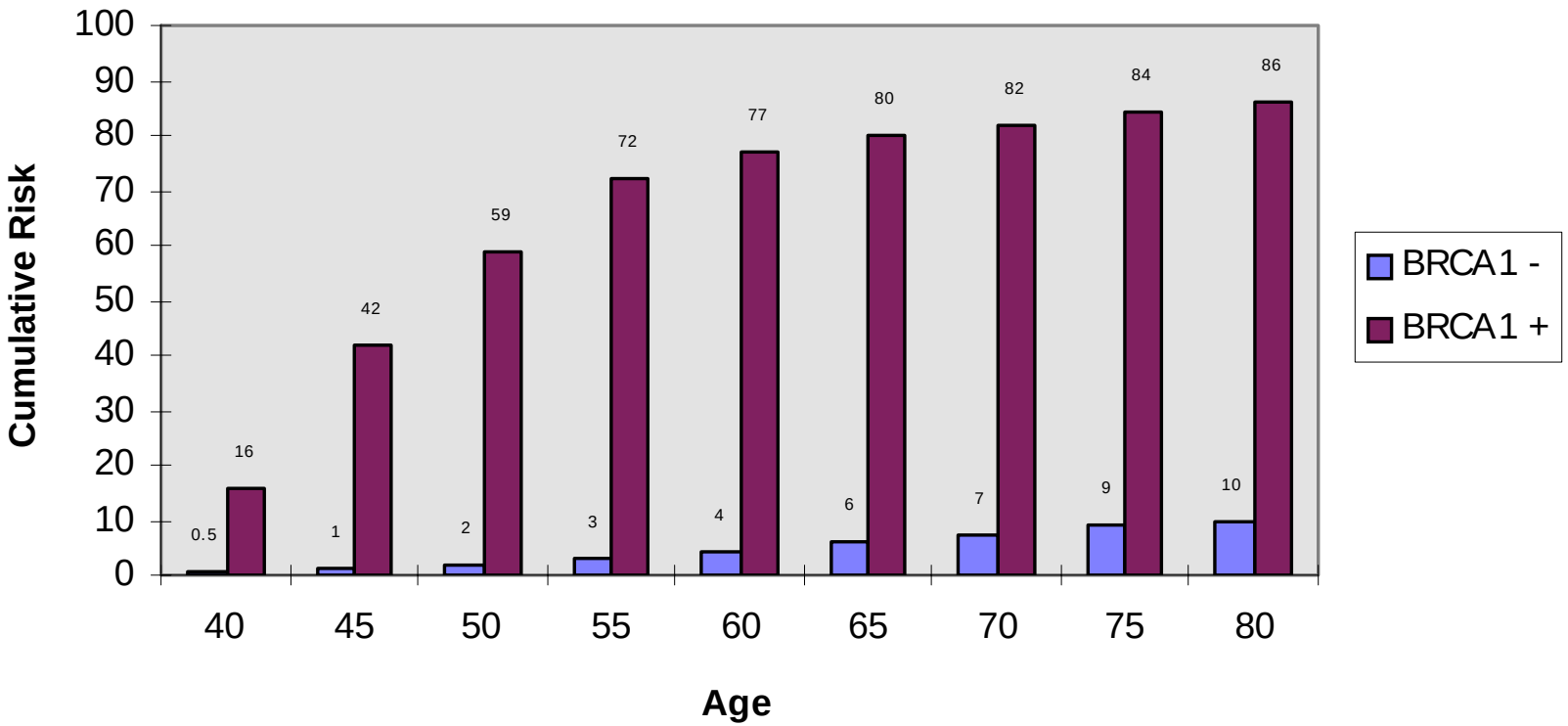


Chr 17

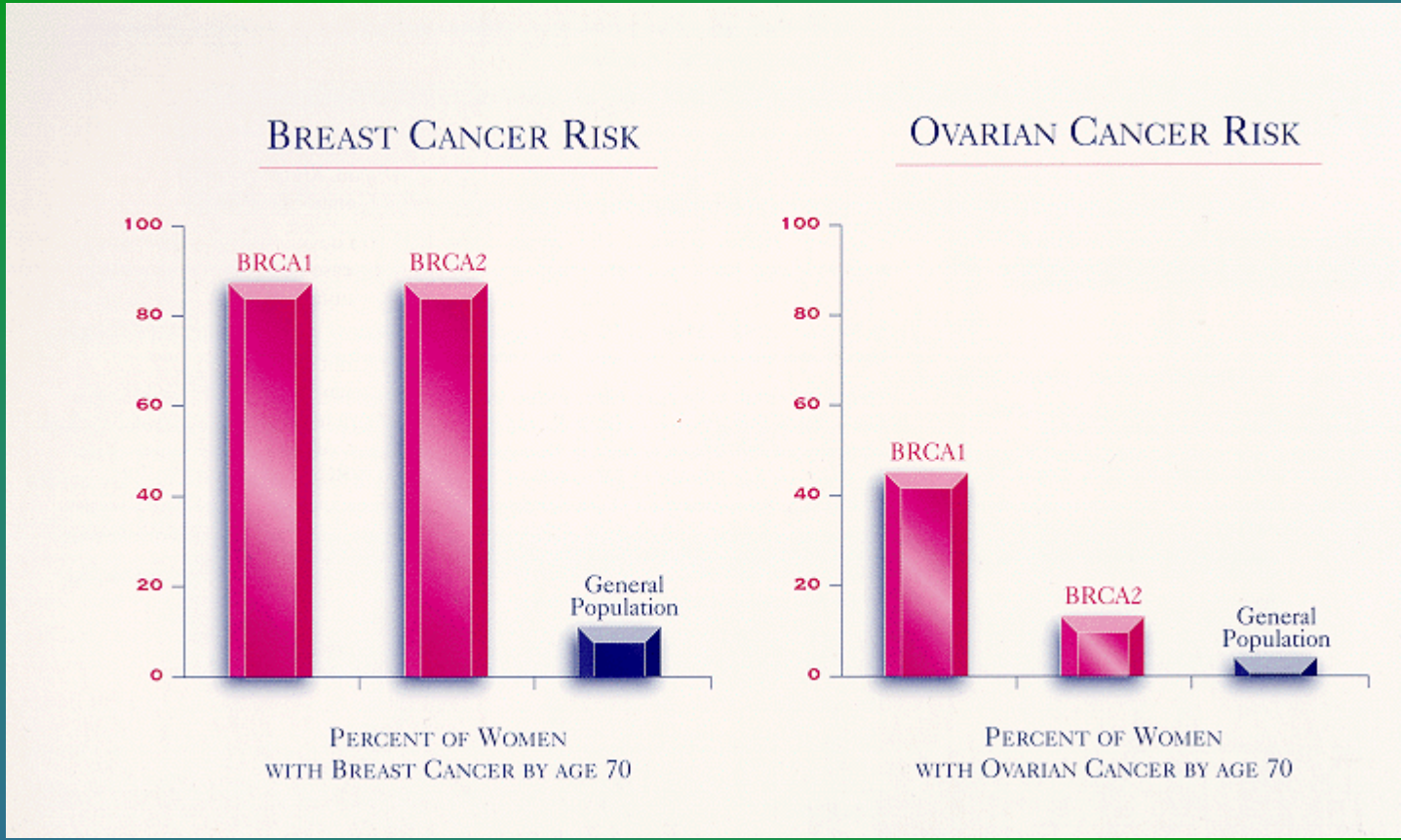
Chr 13

More than 200 different cancer predisposing mutations have been identified throughout the BRCA1 and 2 genes. Only a few mutations have been shown to occur in multiple families. Of special importance are the three mutations found in the Ashkenazi Jewish population (185delAG, 538insC and 6174delT).

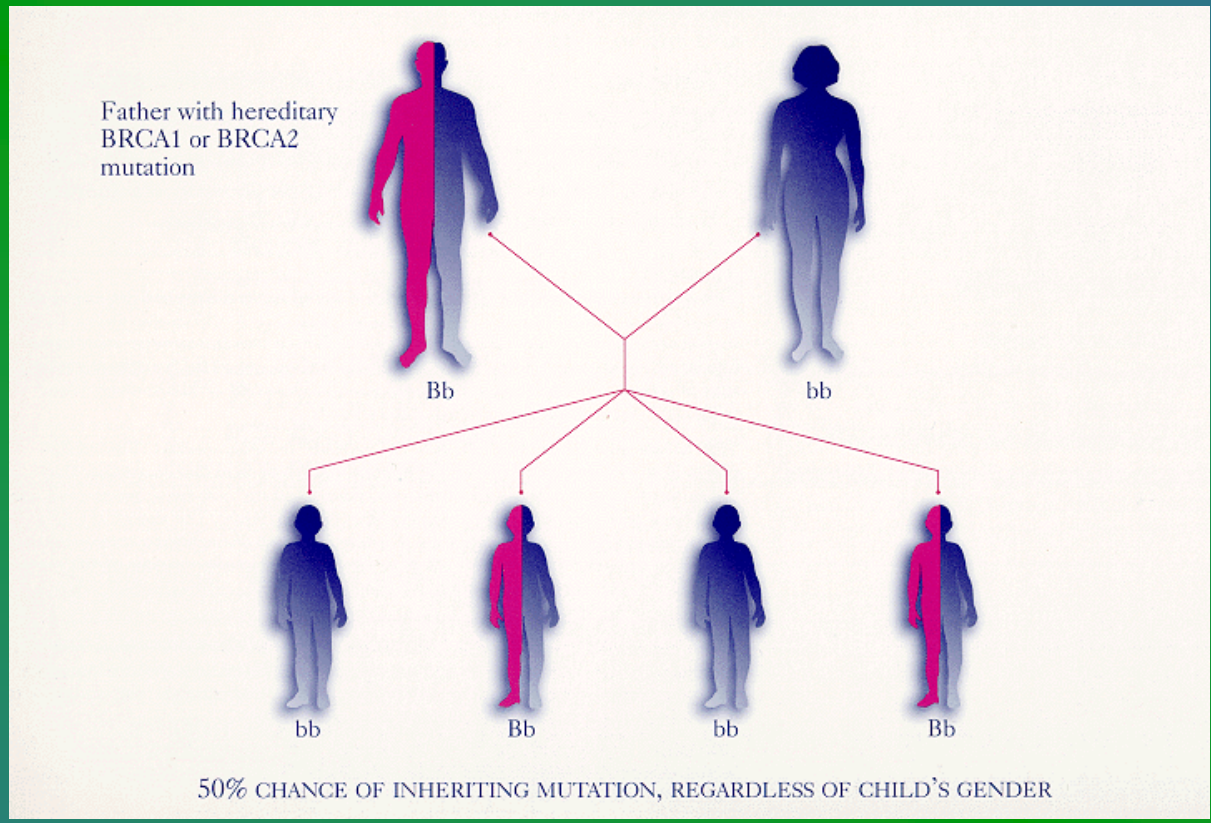
Cumulative risk of getting breast cancer in BRCA1+ women and BRCA1- women



Particularly nasty mutations.



Either parent can passon BRCA1/2 mutations



BRCA1/2 mutations are dominant and affetc multiple generations within a family. When a parent carries the mutation the children have 50% chance of inheriting the mutation.

A person may have a BRCA1 or 2 mutation but never develop cancer.

In many tumors disease arise following the production of a truncated non-functional protein

Table 1. Applications of PTT in Human Molecular Genetics.

Disease	% Truncating Mutations††	Gene
Familial Adenomatous Polyposis	95%	*APC*
Hereditary desmoid disease	100%	*APC*
Ataxia telangiectasia	90%	*ATM*
Hereditary breast and ovarian cancer	90% 90%	*BRCA1*, *BRCA2*
Cystic Fibrosis	15%	*CFTR*
Duchenne Muscular Dystrophy	95%	*DMD*
Emery-Dreifuss Muscular Dystrophy	80%	*EMD*
Fanconi anaemia	80%	*FAA*
Hunter Syndrome	~50%	*IDS*
Hereditary non-polyposis colorectal cancer	~80% ~70%	*hMSH2* *hMLH1*
Neurofibromatosis type 1	50%	*NF1*
Neurofibromatosis type 2	65%	*NF2*
Polycystic Kidney Disease	95%	*PKD1*
Rubinstein-Taybi Syndrome	10%	*RTS*

†† *The percentage of truncating mutations reported which should be detectable using PTT.*

The protein truncation test (PTT)

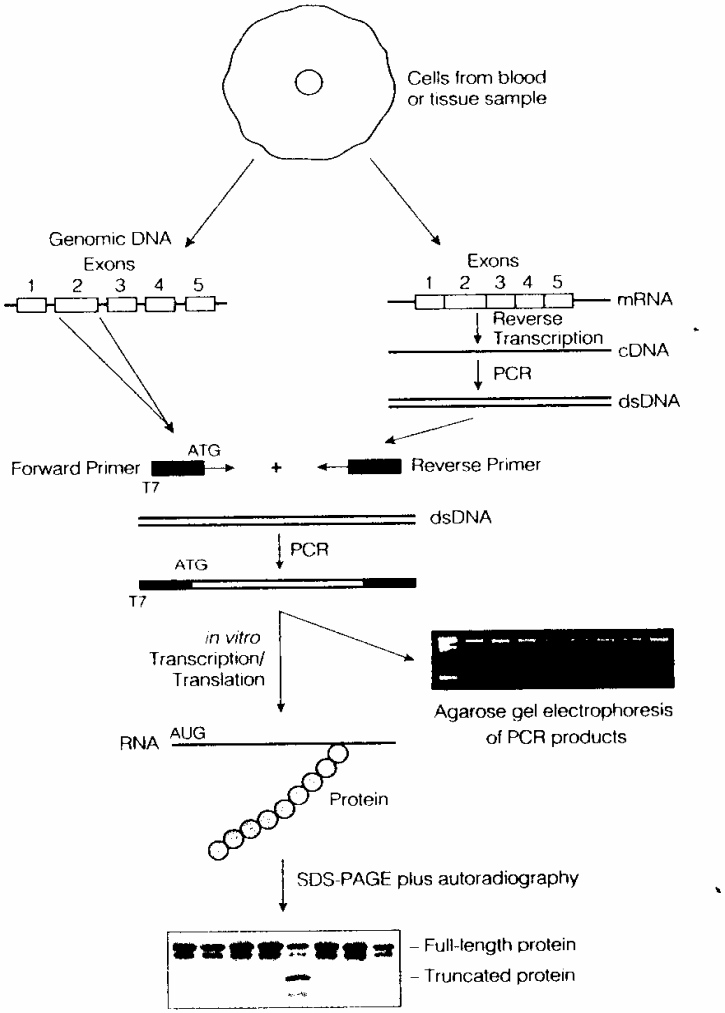
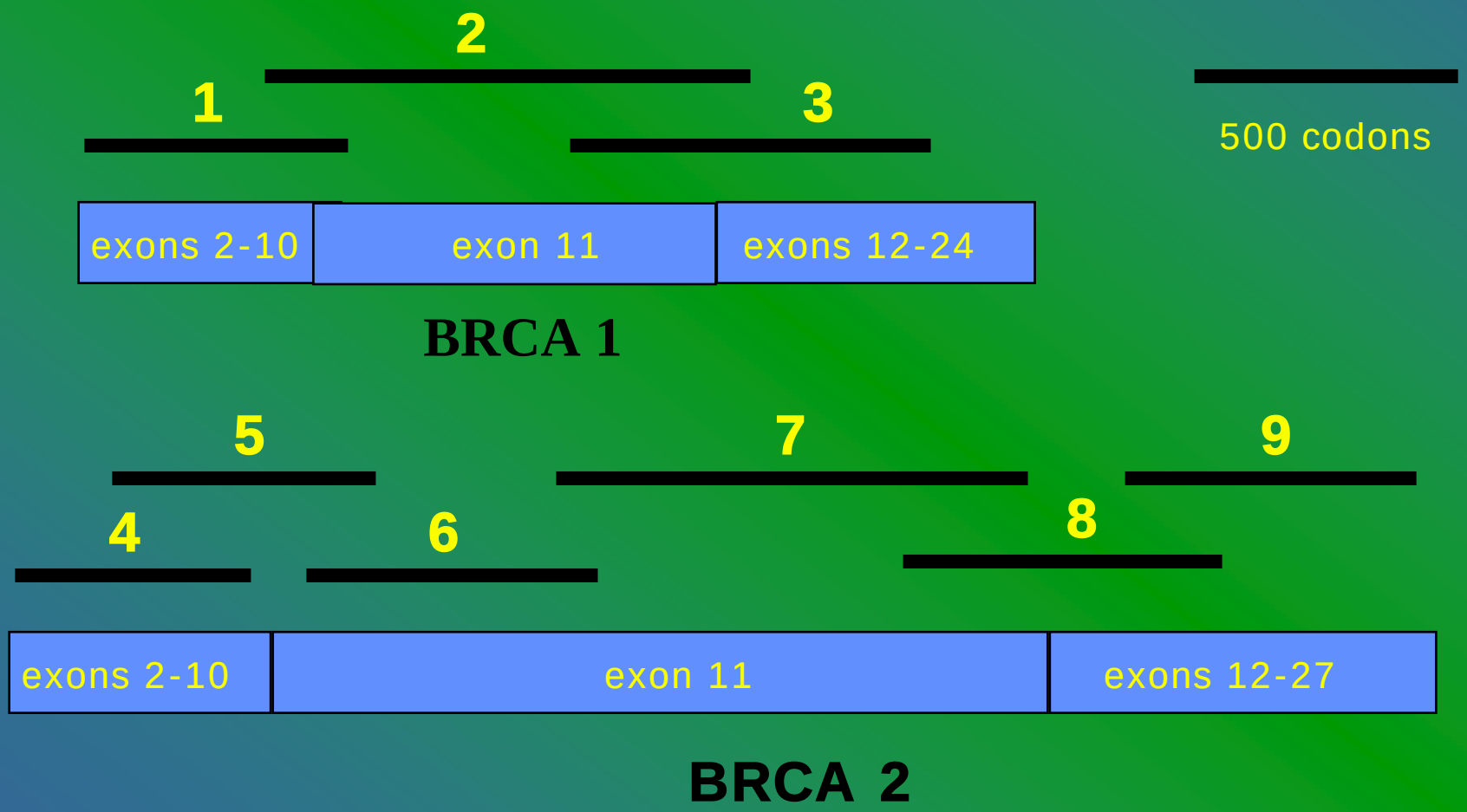


Figure 1. Schematic diagram of Protein Truncation Test.

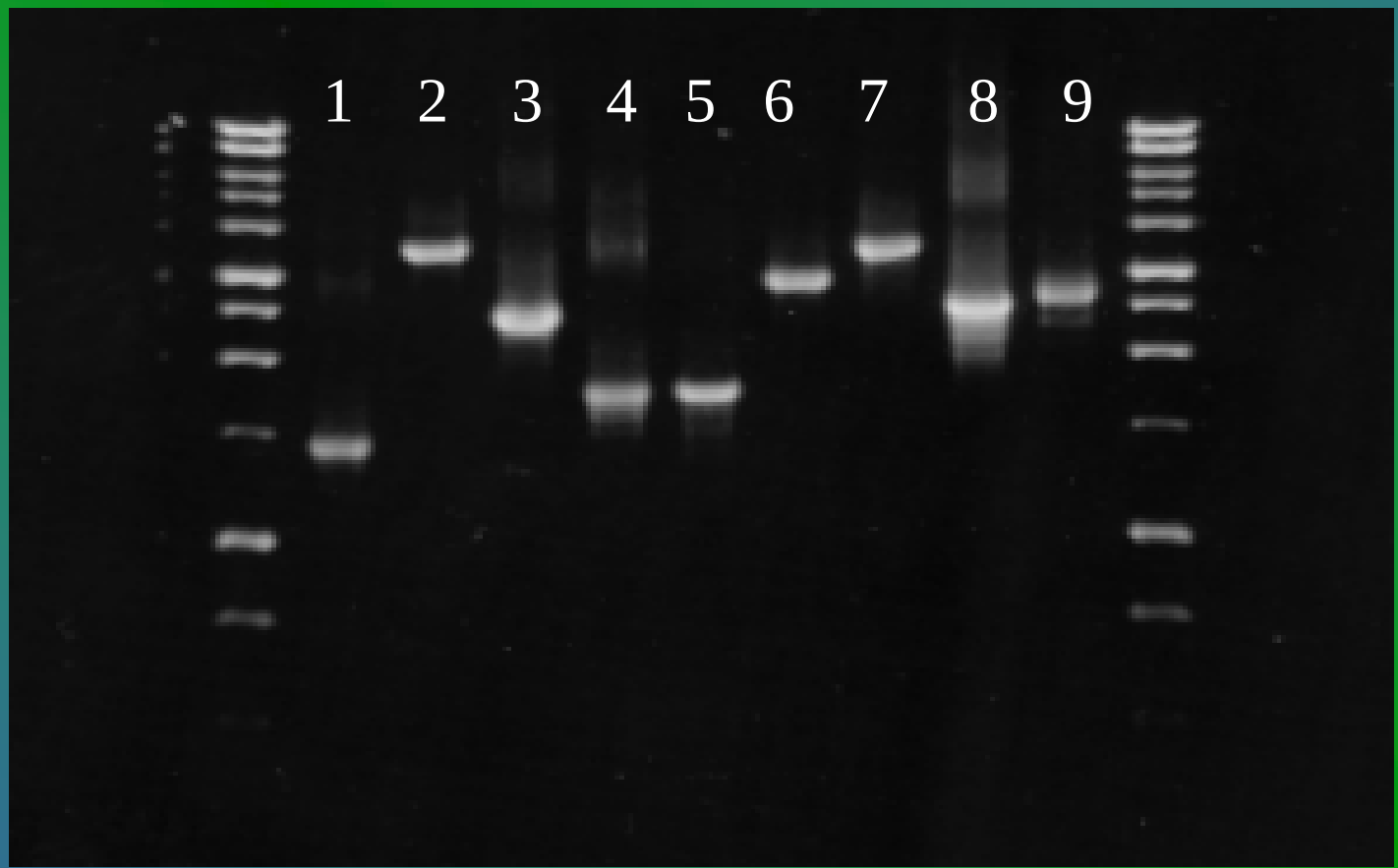
Amplification of the two BRCA1 and 2 genes



The test...first PCR

- The entire 15849 bp sequence for BRCA1/2 is divided in 9 overlapping fragments ranging in size from 473 to 1148 codons and having overlaps of 250 codons
- Nested PCR using cDNA as template is used to amplify segments 1, 3, 4, 5, 8 and 9 (48% of the coding sequence)
- Standard PCR is used to amplify segments 2, 6 and 7 from genomic DNA (52 % of the coding sequence)

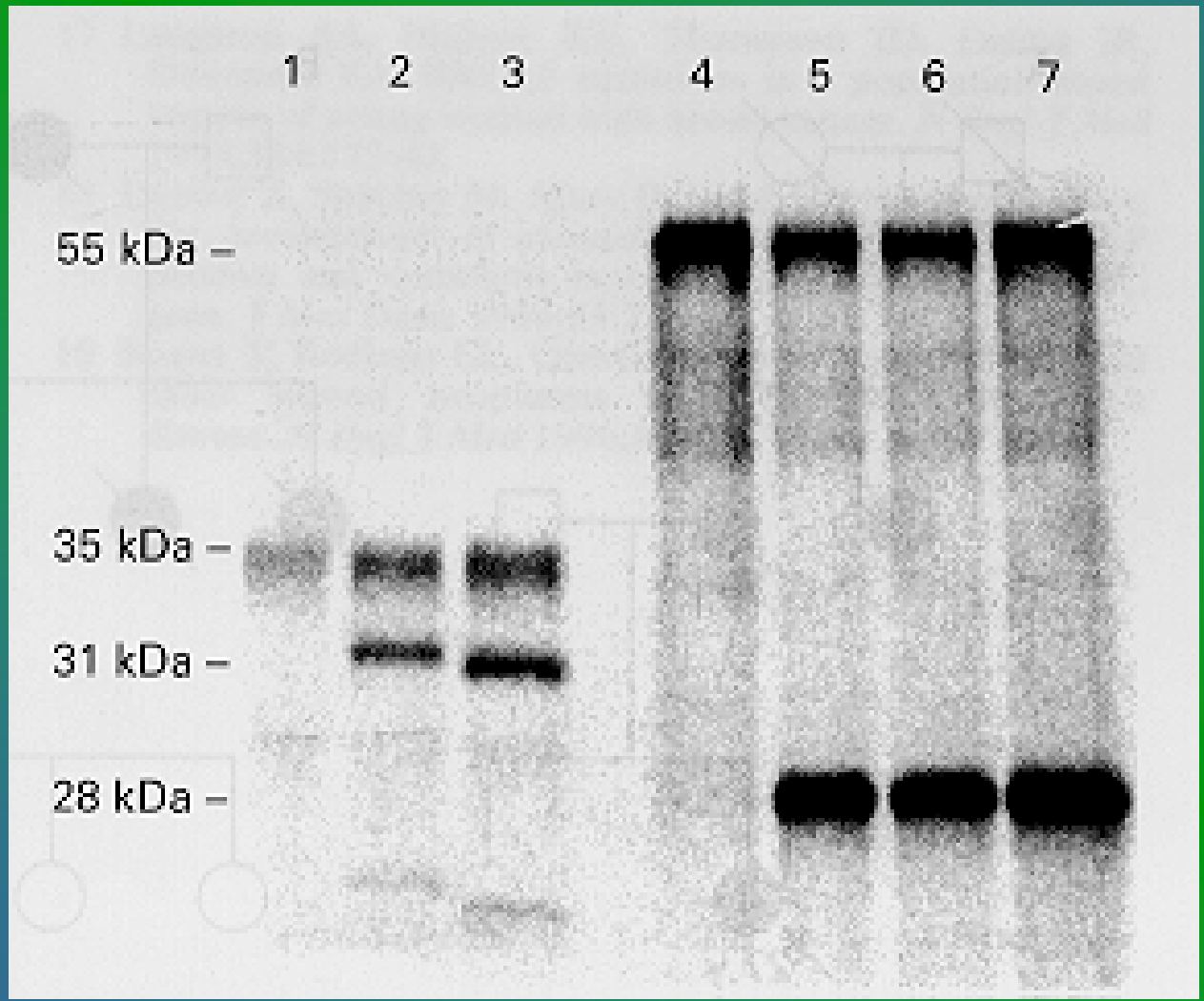
Amplification of the 9 fragments of DNA (3) and cDNA (6) for BRCA1/2 analysis



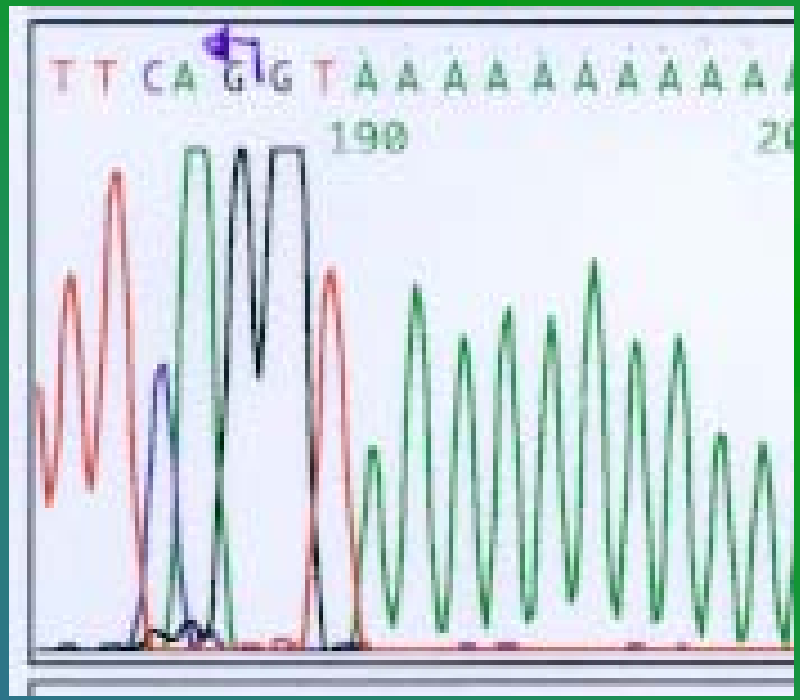
2nd, in vitro translation

- PCR products are transcribed and translated into radio-labeled proteins
- Translated products are analyzed on a 5-18% SDS-acrylamide gradient gel
- Limits of mutation detection on the SDS-PAGE are 10% of difference in protein size

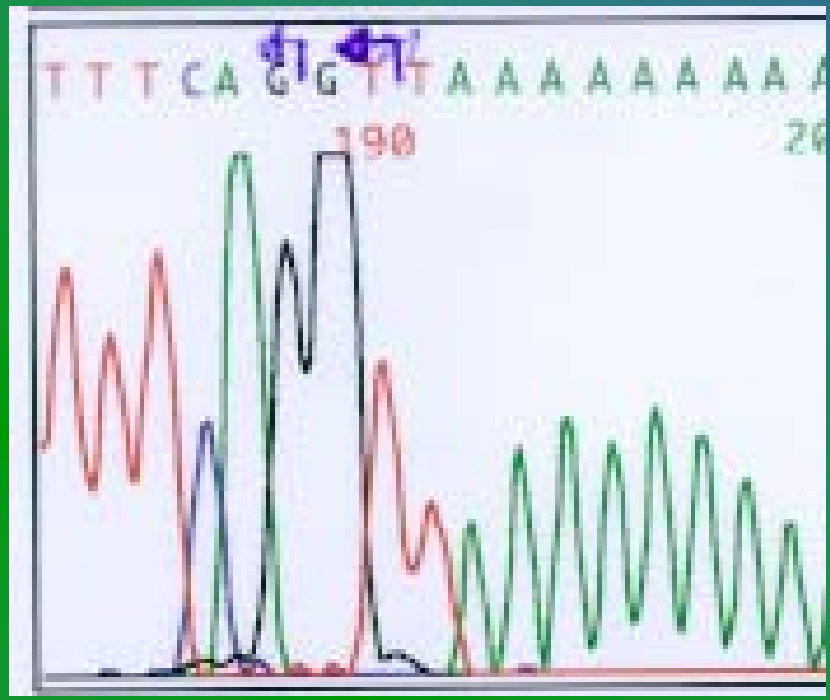
PTT results on an acrylamide gel



And at the end, the sequence

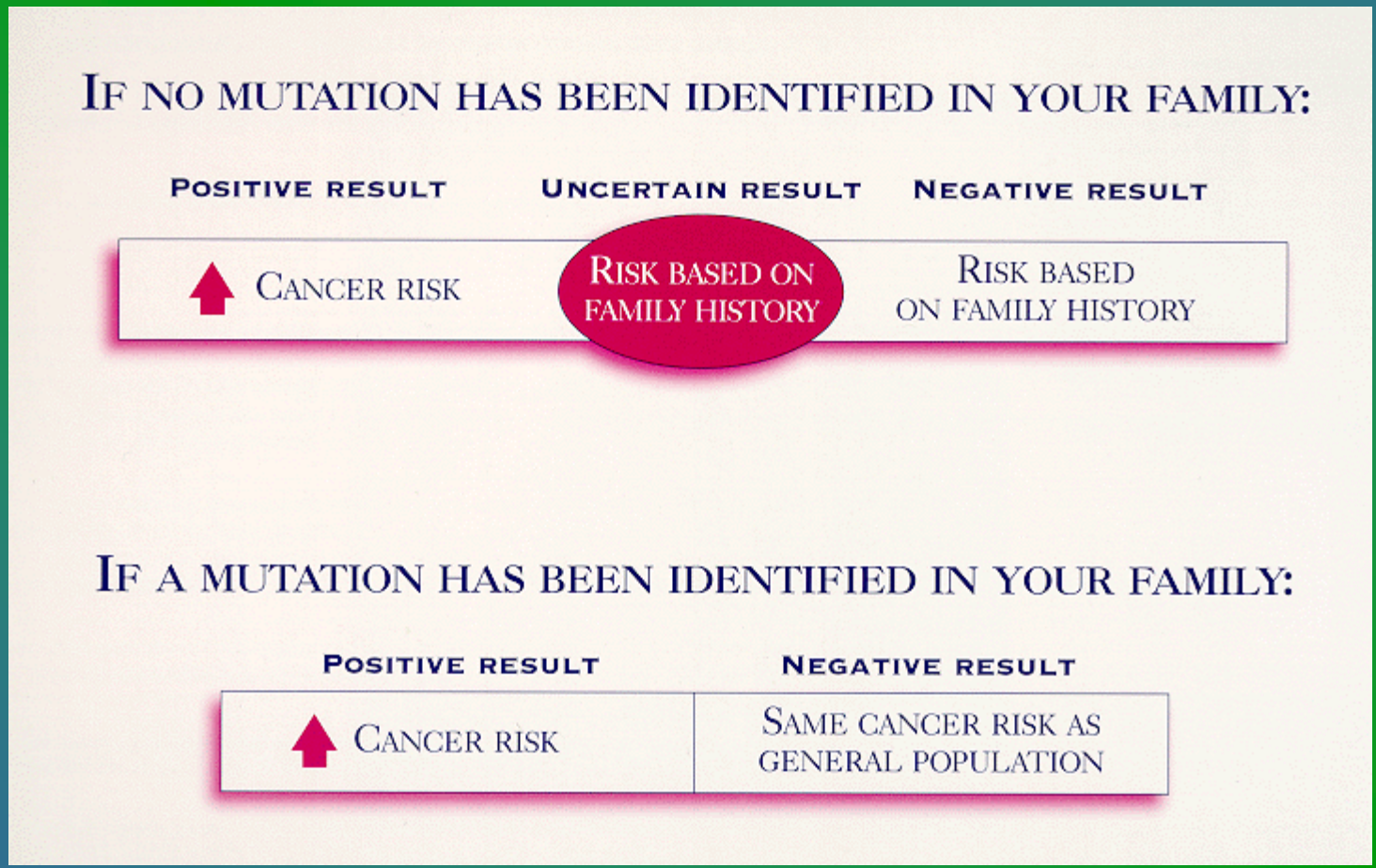


Wild-type



Mutated

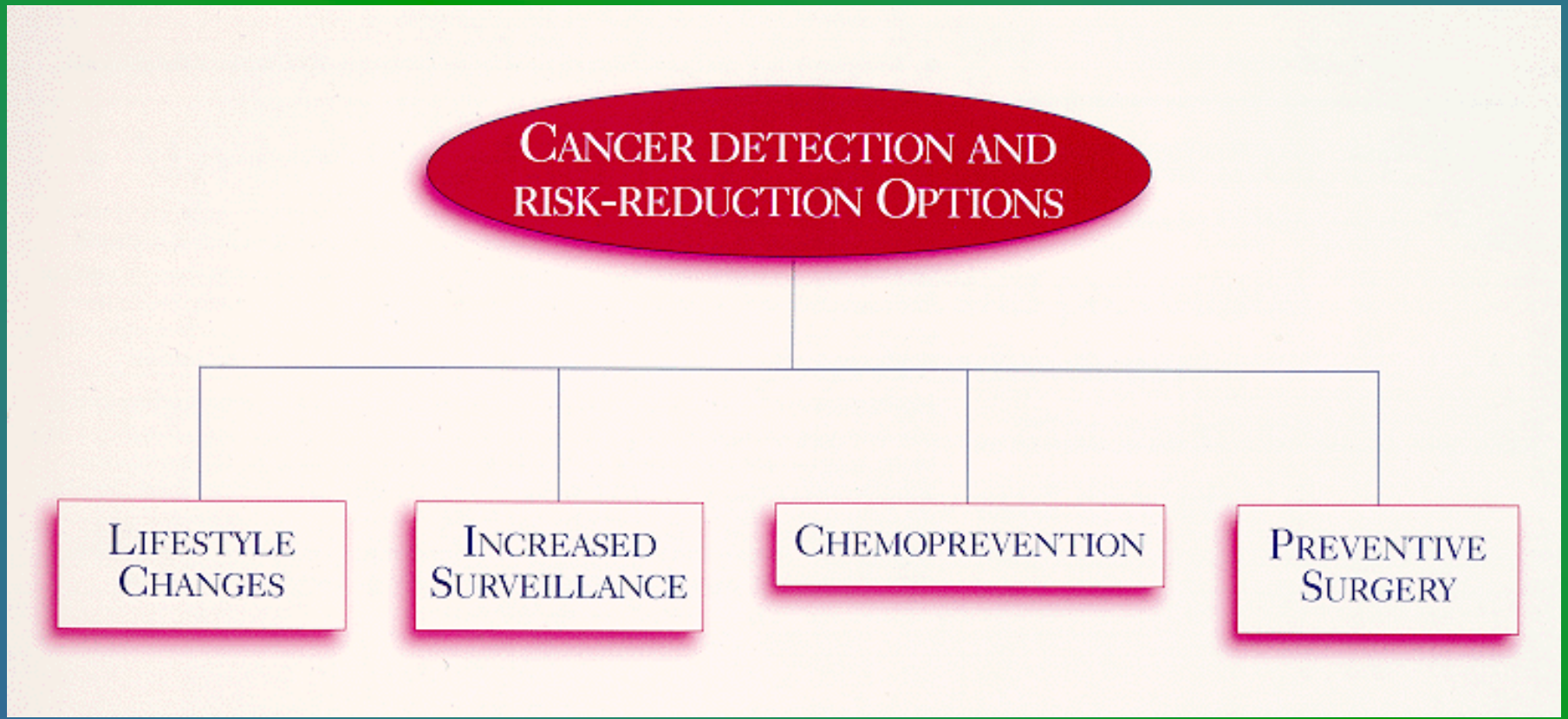
No mutations, good news.



Understanding possible test results

- A test result may be positive, negative or uncertain
- A positive result means a cancer susceptibility mutation was identified
- A negative result means no mutation was identified, but the individual has at least the same cancer risk as the general population
- An uncertain result means a gene alteration was identified but with unknown associated cancer risk. In this case the risk is based on family history

Consequences in case of positivity



A positive test indicates a probability not a certainity to develop cancer

- When a mutation is detected an individualized plan to reduce the risk of cancer or detect it as early as possible is developed
- To detect cancer early more frequent examinations and early mammography may be recommended
- to reduce cancer risk chemoprevention or preventive surgery may be considered

Therapeutic consequences for women carrying the BRCA1/2 mutations

- More frequent mammographies
- Oral contraceptives (NEJM 1998; 339:424-428)
- Tamoxifen (Nature Med. 1998; 4: 647)
- Bilateral mastectomy(Nature Med. 1998; 4: 647)

Benefits of Testing

- For carriers, allows early detection and prevention strategies
- Rules out noncarriers in high-risk families

Limitations of Testing

- Testing raises many psychological, social, and ethical concerns.
- Detection and prevention techniques are not 100% effective.

Futur prognostic factors

- Mutations in the p53 gene (tumor suppressor gene)
- Amplification of HER-2/neu gene
- Overexpression of Cyclin D1