An introduction to cancer cell genetics

Basic Course in Molecular Pathology

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CANCER

What is cancer?

‘Group of diseases characterized by changes in DNA that alter cell behavior, leading to uncontrollable growth and malignancy ’

Complex disease!

200 types

Molecular subtypes

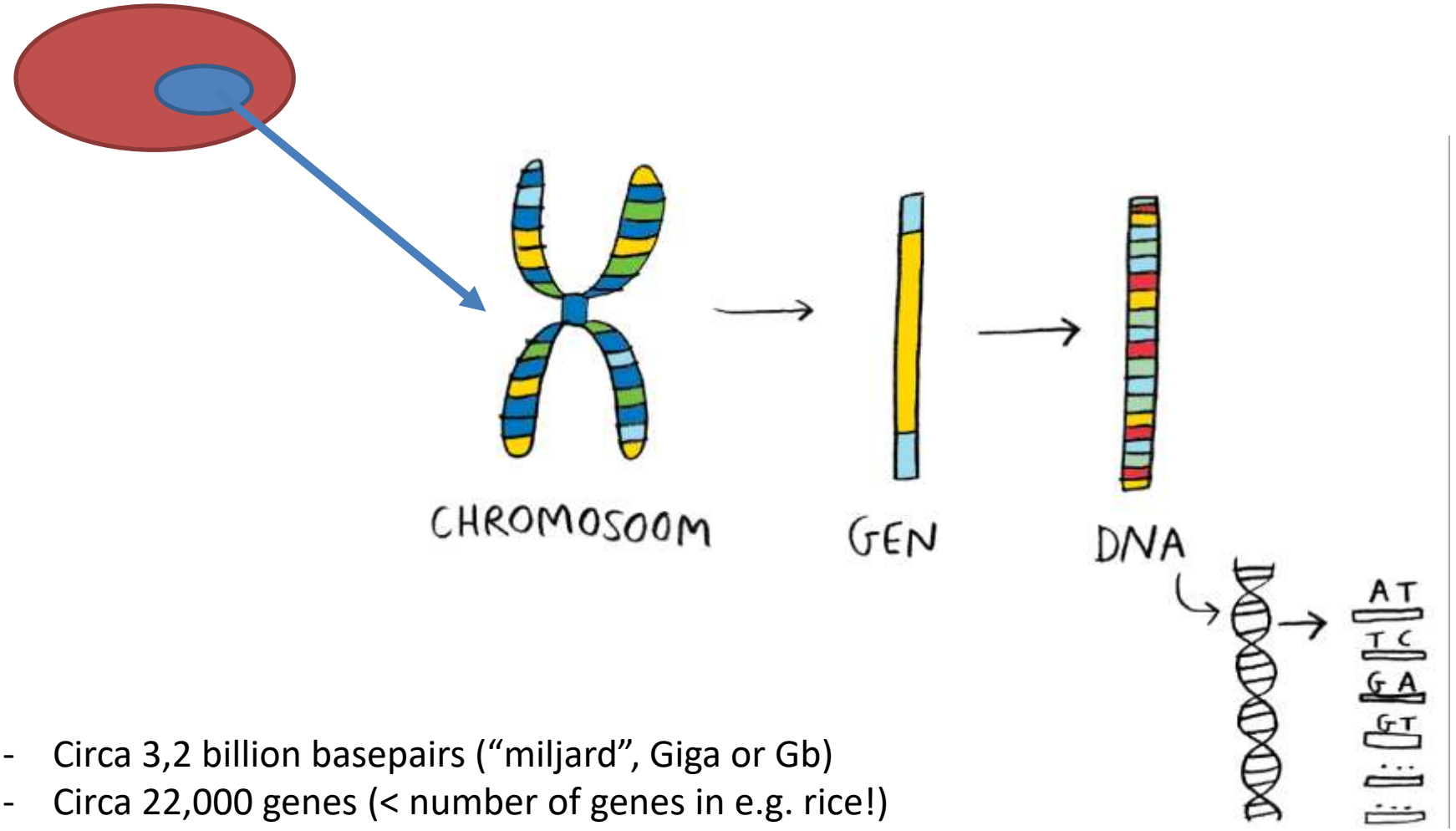
-> yet often same principles

TECHNOLOGICAL & GENOMIC REVOLUTION

- Gregor Mendel **1865**: “inheritance’
- Boveri, Morgan, Bateson **1900**: “Chromosomes, Genetics, Gene terminology” rediscovery
- Watson, Crick, Wilkins & Franklin: Structure of DNA **1953**
- Frederick Sanger **1977**: determination of base sequences (DNA sequencing); semi-automated sequencing machine (Leroy Hood, 1986)
- Human Genome Project NIH & Craig Venter: 1990 – **2001**

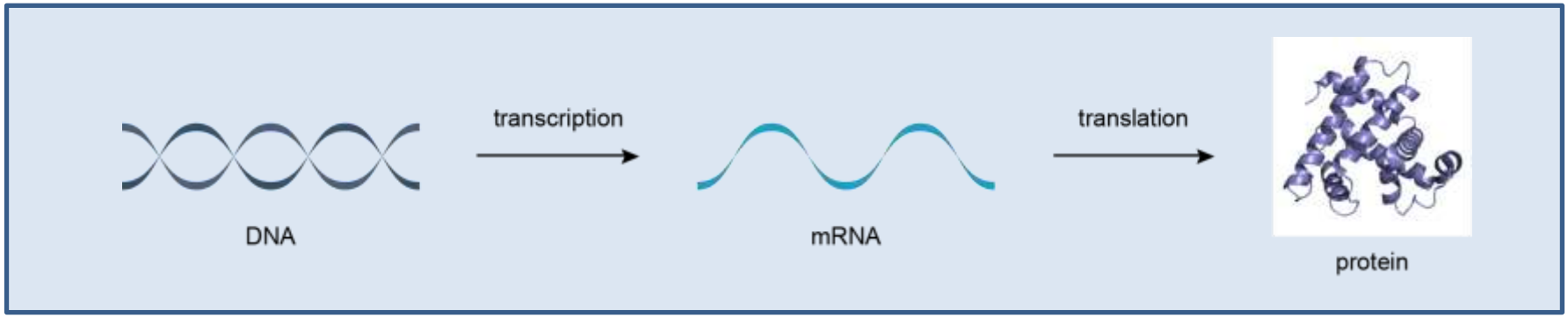
  - 15 & 16 february 2001: Published in Nature & Science
  - Determination of base sequences in human genome (cDNA)
  - More than 3 billion nucleotides
  - Identify and map genes: circa 21.000 genes

THE HUMAN GENOME



- Circa 3,2 billion basepairs (“miljard”, Giga or Gb)
- Circa 22,000 genes (< number of genes in e.g. rice!)
- A, C, G & T (DNA)
- Human genome = diploid (2 x 23 chromosomes)
- In total 6 picogram DNA per nucleus

“CENTRAL DOGMA”

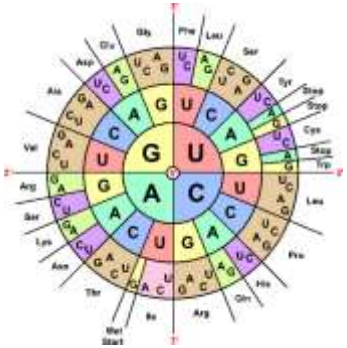


GTGCATCTGACTCCTGAGGAGAAG
CACGTAGACTGAGGACTCCTCTTC

DNA (transcription)

GUGCAUCUGACUCCUGAGGAGAAG

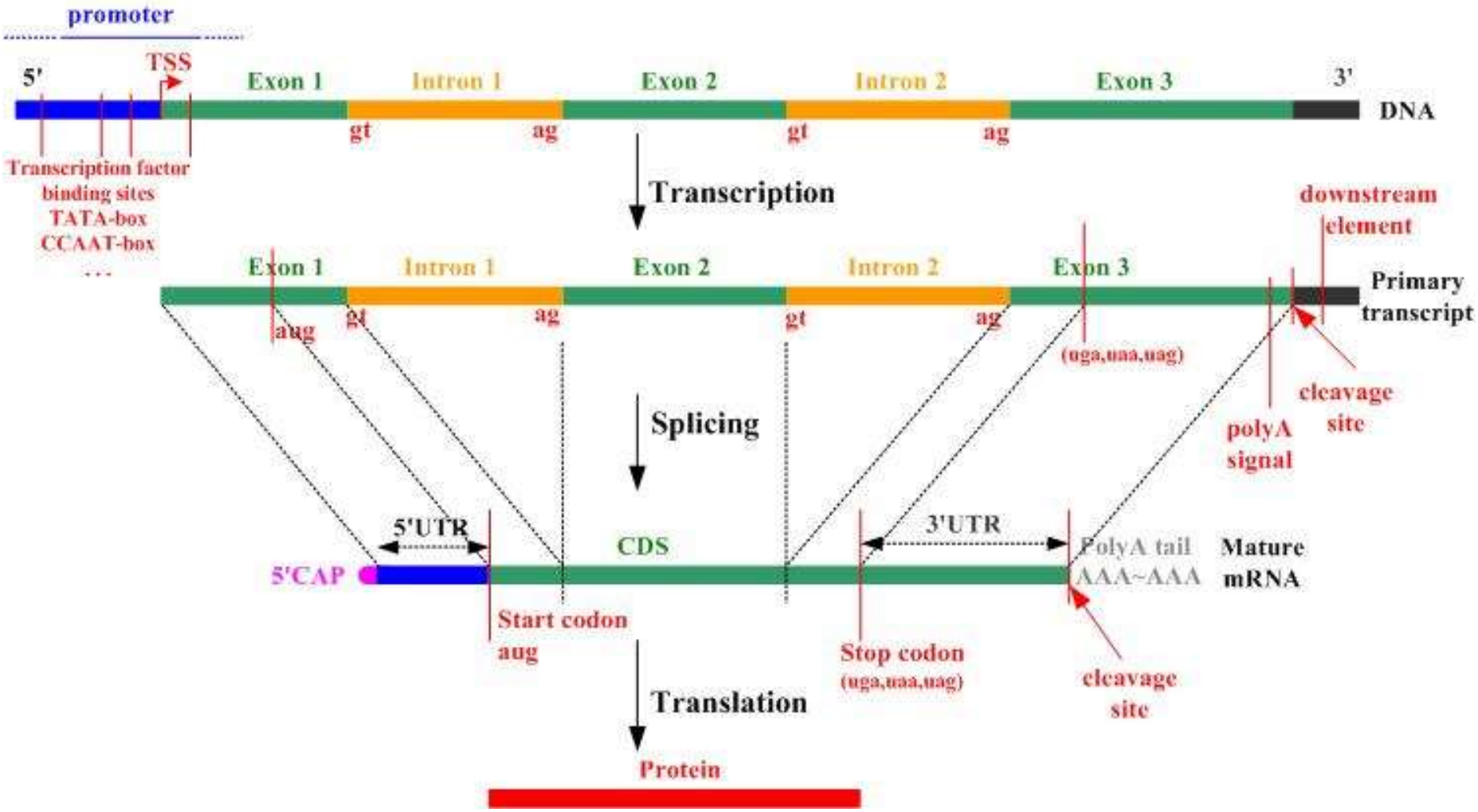
RNA (translation)



--V----H----L---T-----P----E-----E---K--

Protein

STRUCTURE OF A GENE



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Cancer Genome Landscapes

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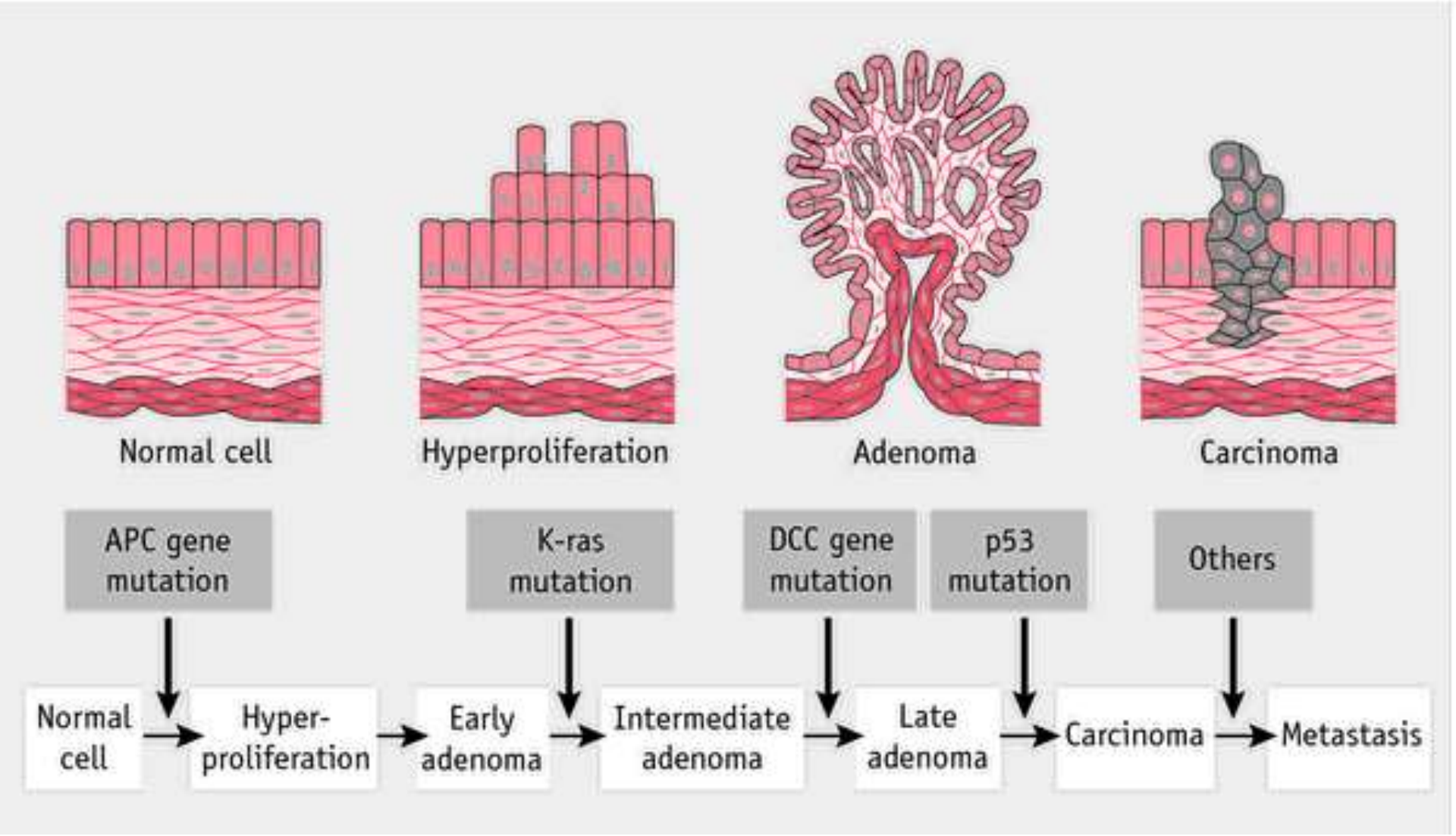
Abstract

Over the past decade, comprehensive sequencing efforts have revealed the genomic landscapes of common forms of human cancer. For most cancer types, this landscape consists of a small number of "mountains" (genes altered in a high percentage of tumors) and a much larger number of "hills" (genes altered infrequently). To date, these studies have revealed ~140 genes that, when altered by intragenic mutations, can promote or "drive" tumorigenesis. A typical tumor contains two to eight of these "driver gene" mutations; the remaining mutations are passengers that confer no selective growth advantage. Driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell fate, cell survival, and genome maintenance. A better understanding of these pathways is one of the most pressing needs in basic cancer research. Even now, however, our knowledge of cancer genomes is sufficient to guide the development of more effective approaches for reducing cancer morbidity and mortality.

MULTISTEP CARCINOGENESIS



Sequential accumulation of mutations affecting oncogenes and tumor suppressors (mainly)

DRIVER VS PASSENGER!

- Driver gene mutation: a mutation that directly or indirectly confers a selective growth advantage to the cell in which it occurs

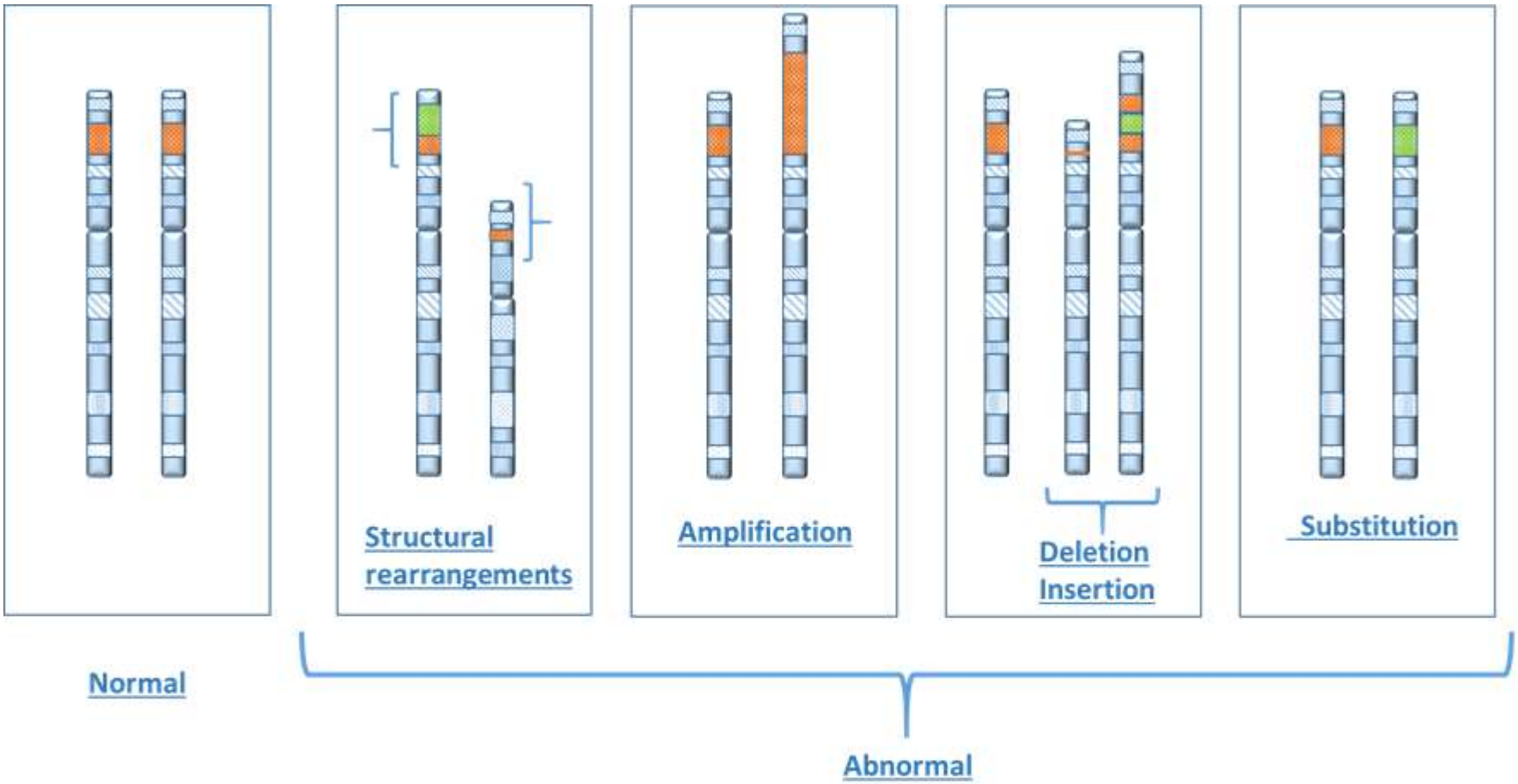
- Passenger mutation: a mutation that has NO direct or indirect effect on the selective growth advantage of the cell in which it occurs

VOGELSTEIN B ET AL. SCIENCE 2013

- Next generation sequencing => massive amount of data produced over diverse tumor types
- Adult cancers: range 10-1500 mutations!
  - Mutagens (UV, smoking)
  - Age (many passenger mutations!)
  - Epithelial cell of origin > non-replicating cells (glial cells)
- Pediatric cancers: 10 or less on average
- Most mutations are passenger mutations !
- Most human cancers are caused by 2 to 8 sequential (driver) alterations that develop over a course of 20 to 30 years

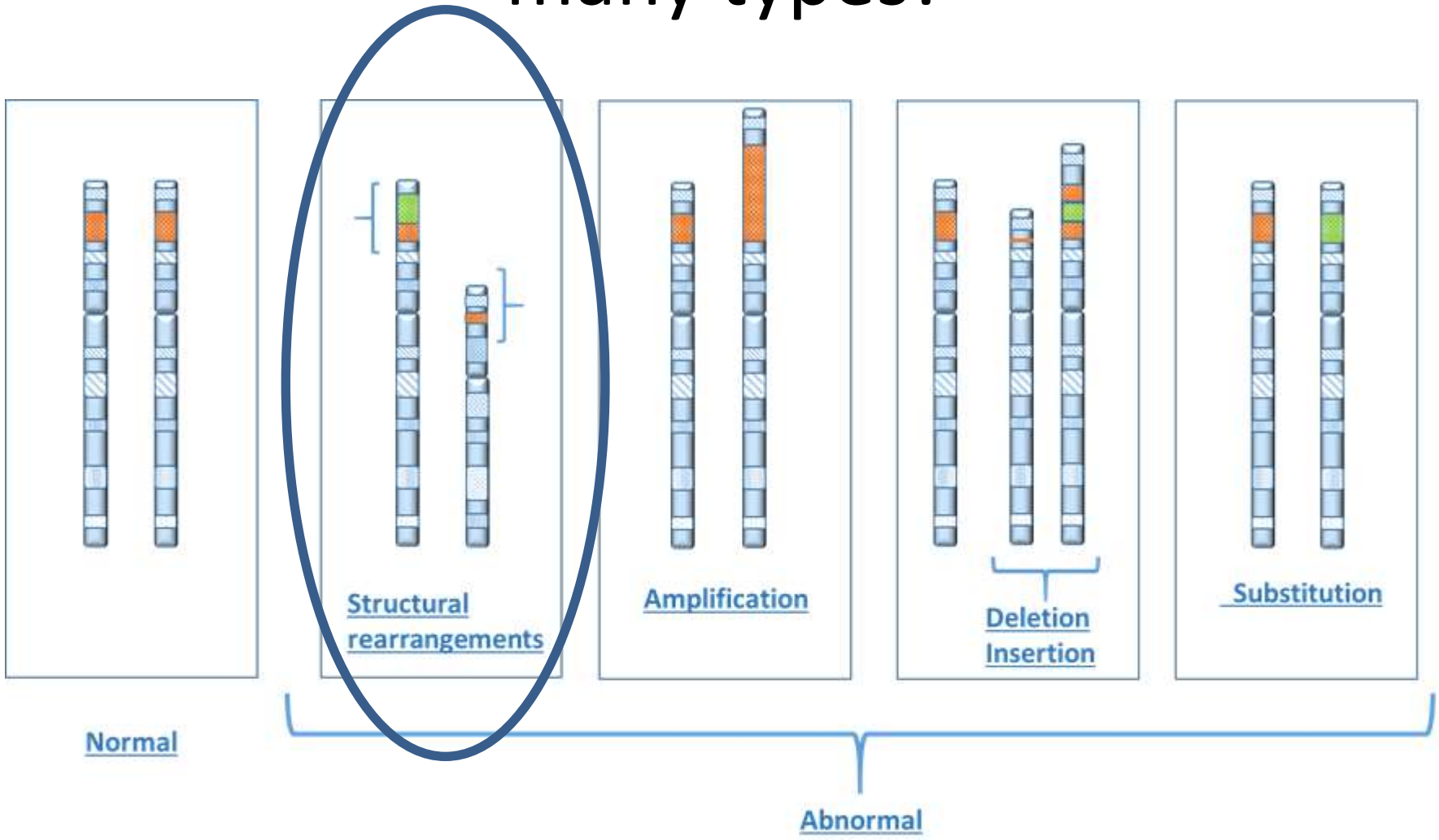
GENETIC ALTERATIONS IN CANCER GENES: MANY TYPES!

Various alterations in the cancer DNA including:



- Structural aberrations (translocations, inversions)
- Copy number alterations (duplication, amplifications)
- Deletions, insertions
- Base substitutions (point mutations)

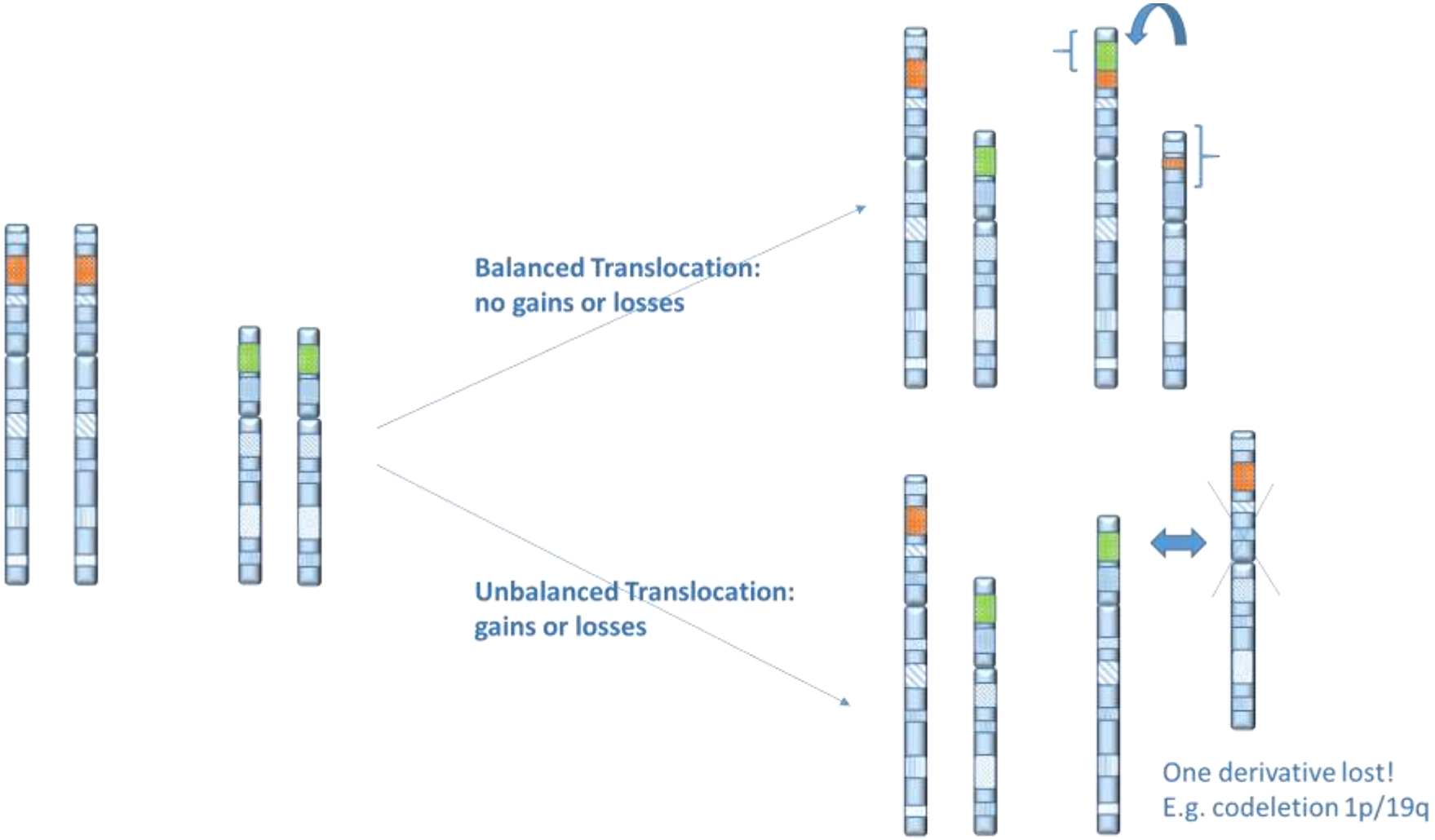
Genetic alterations in cancer genes: many types!



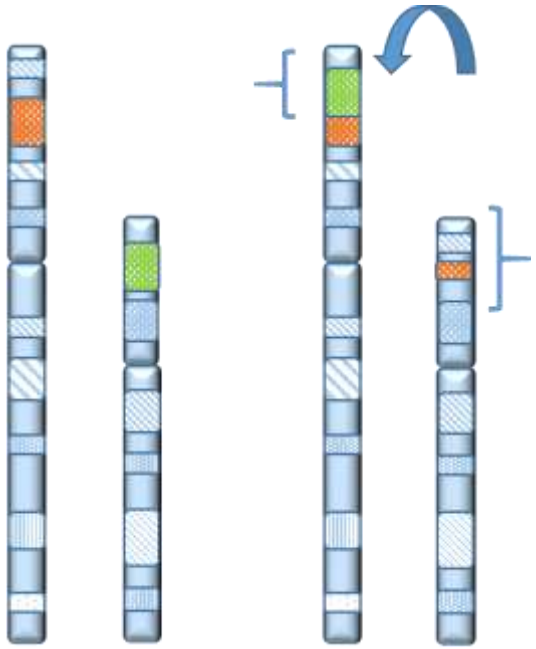
TRANSLOCATIONS

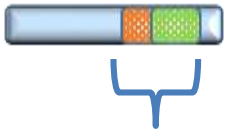
- Somatic and often very specific (pathognomonic)
- Lymphoma, leukemia, soft tissue tumors… benign tumors!
- Often recurrent and only aberration
- 2 functional types of transforming translocations
  - New fusion genes formed (and corresponding fusion proteins with transforming characteristics)
  - Intact gene placed under control of irrelevant promotors or enhancers => uncontrolled transcription (e.g. t14;18)
- 2 structural types of translocation: balanced versus unbalanced

STRUCTURAL TRANSLOCATION TYPES



BALANCED TRANSLOCATION, RECIPROCAL

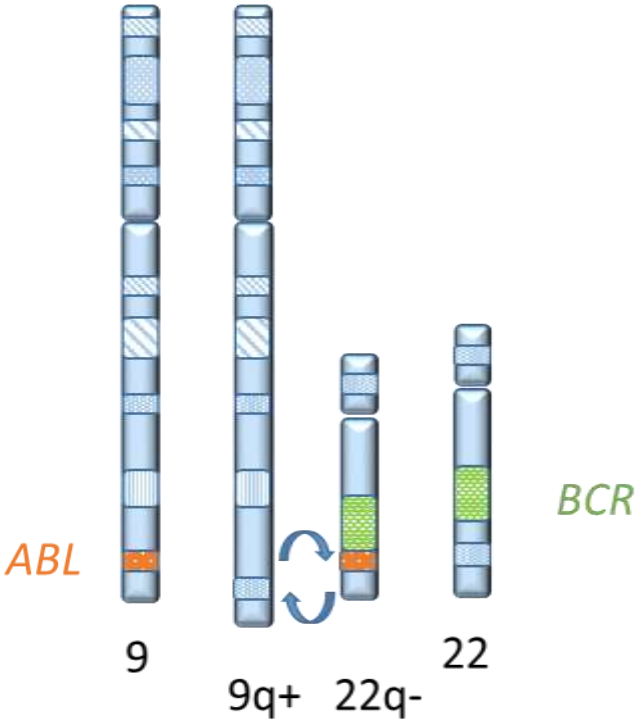




Fusion gene with oncogenic properties

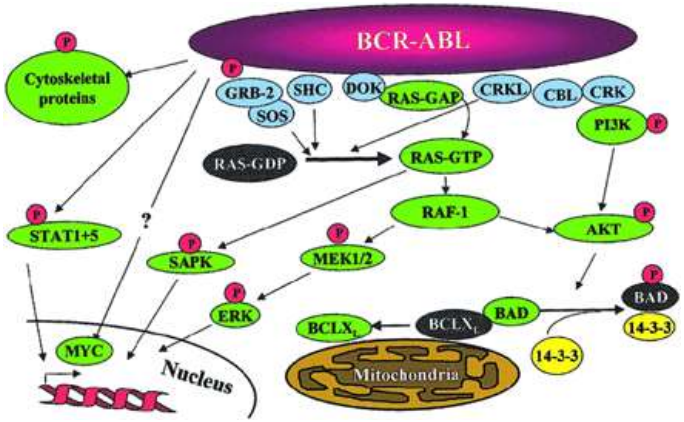
Gene under control of other promotor region ✹ => more active transcription

CHRONIC MYELOID LEUKEMIA



t(9;22)(q34;q11):
Philadelphia chromosome (*Ph*)

Constitutively active *BCR-ABL* tyrosine kinase



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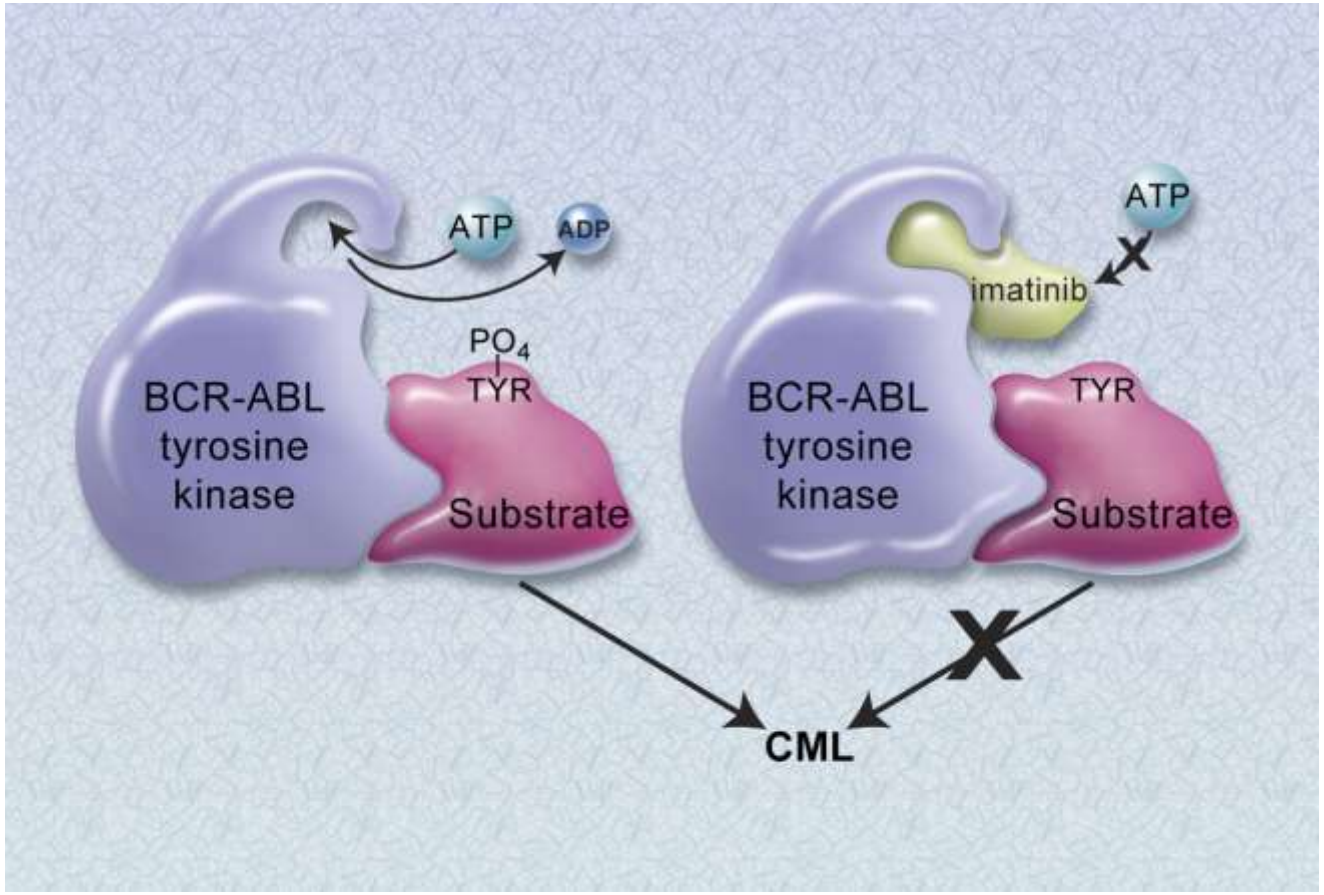
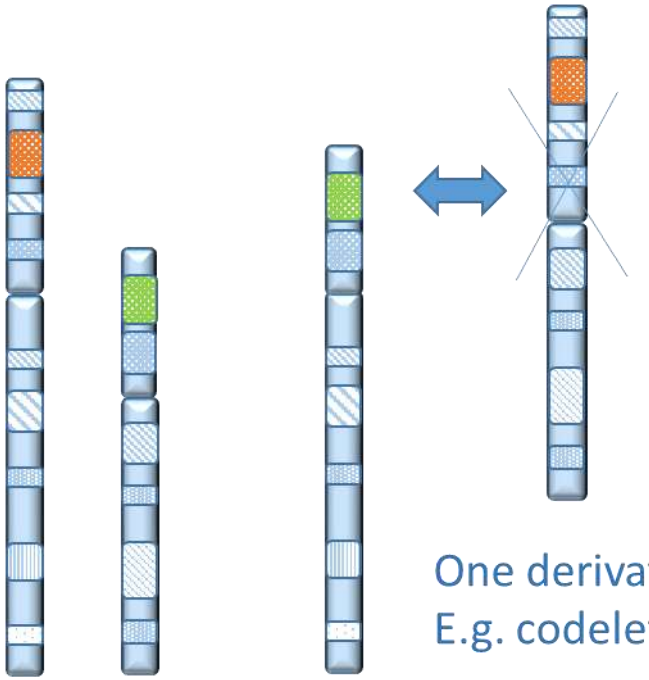


Mechanism of action of imatinib. The latter blocks the binding of ATP to the BCR-ABL tyrosine kinase. Druker B et al. Blood 2008.

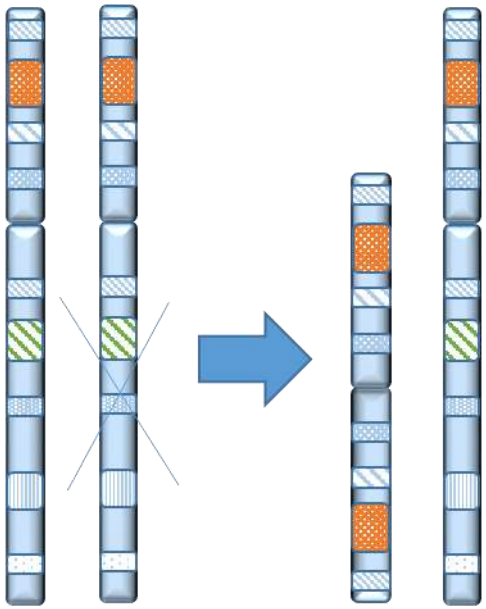
UNBALANCED TRANSLOCATION: CODELETION 1P/19Q



One derivative lost!
E.g. codeletion 1p/19q

- 1994: unbalanced translocation (1;19)(q10;p10)
- Gliomas with oligodendroglial morphology
- Favourable prognosis and good response to chemo- and radiotherapy (Louis DN et al. 2014, WHO Classification of Tumours of the CNS 2016).

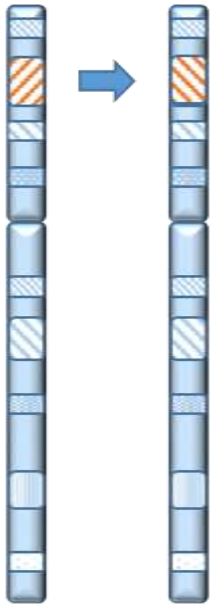
UNBALANCED TRANSLOCATION: ISOCHROMOSOME



Loss of one arm and duplication of the other

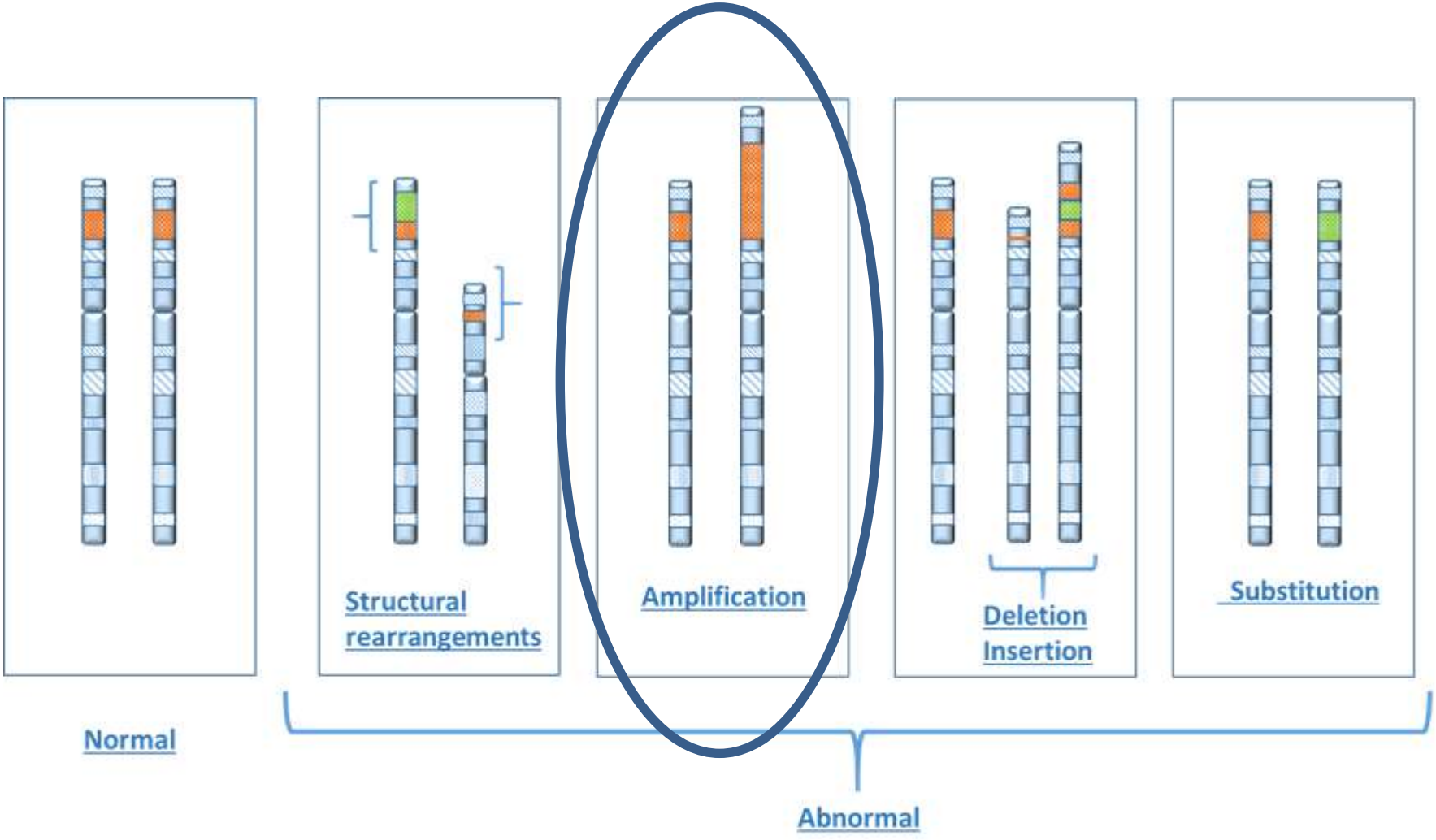
- Two copies of either the long (q) arm or the short (p) arm
- simultaneous duplicaton and deletion of genetic material
- Partial Trisomy and Monosomy (TSG)!
- Example: i(17q)

STRUCTURAL ABERRATIONS: INVERSION



- New fusions can be created
- E.g. ALK rearrangement in NSCLC

GENETIC ALTERATIONS IN CANCER GENES: MANY TYPES!



AMPLIFICATIONS

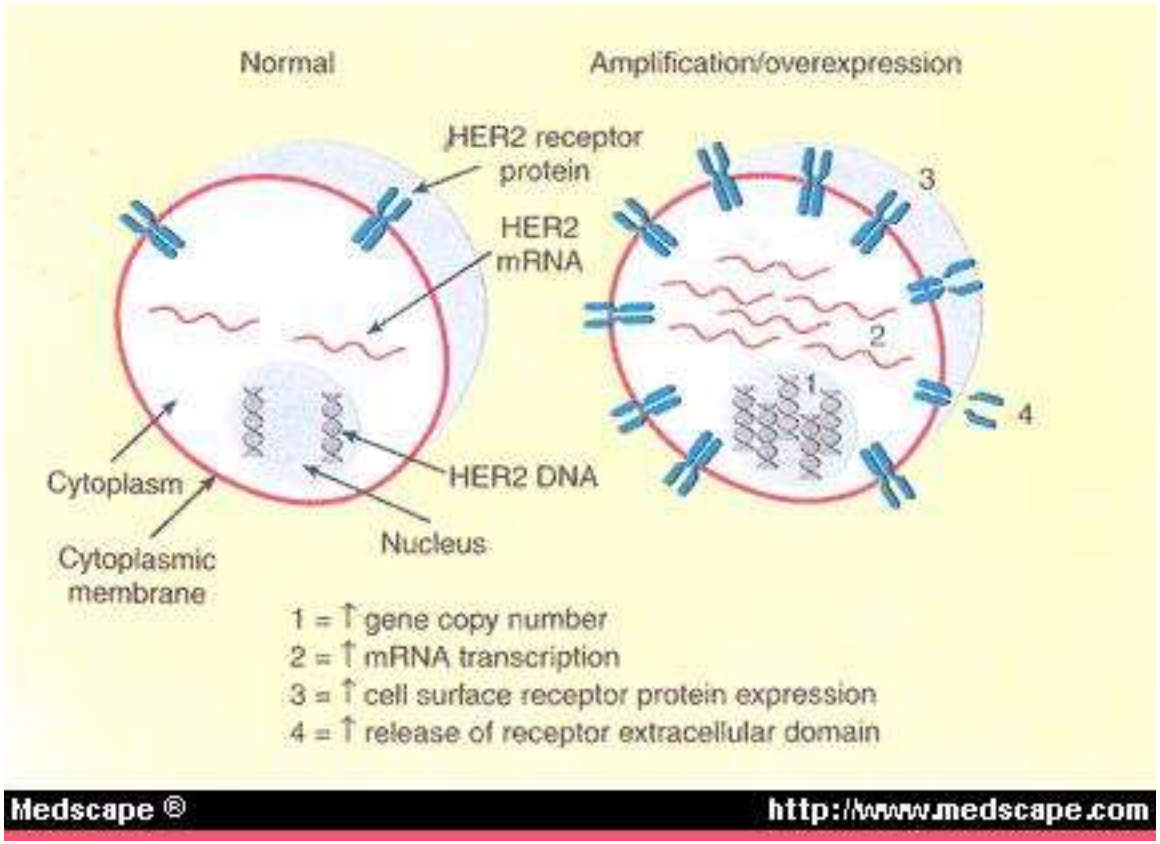
- “A genetic alteration producing a large number of copies of small segment (< few Mb) of the genome”

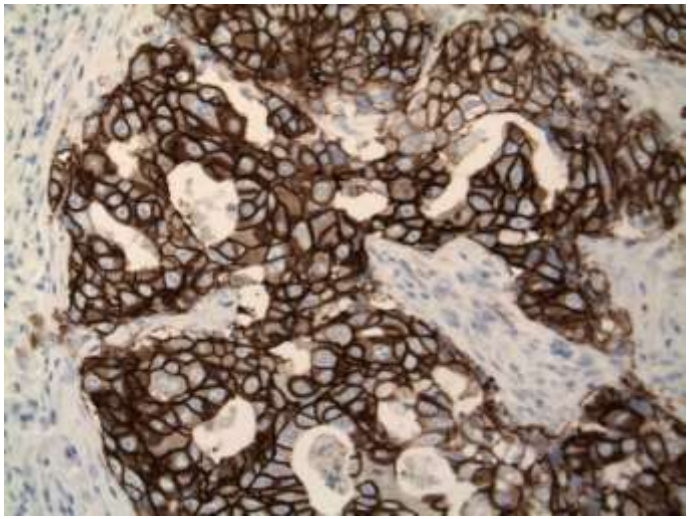
- Up to 100 copies of genes resulting in overexpression and activation

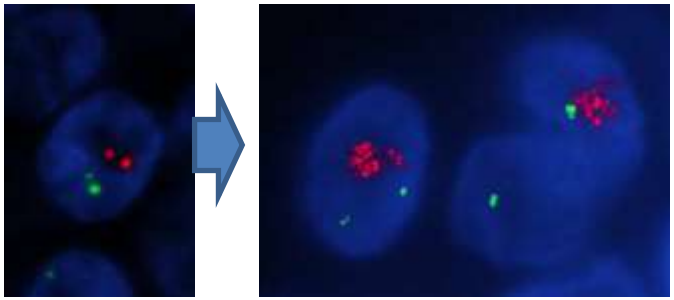
- Oncogenes! => encoded proteins mostly regulators of normal cellular growth and differentation

AMPLIFICATION: HER2/NEU

HER2 amplification => overexpression in breast ca

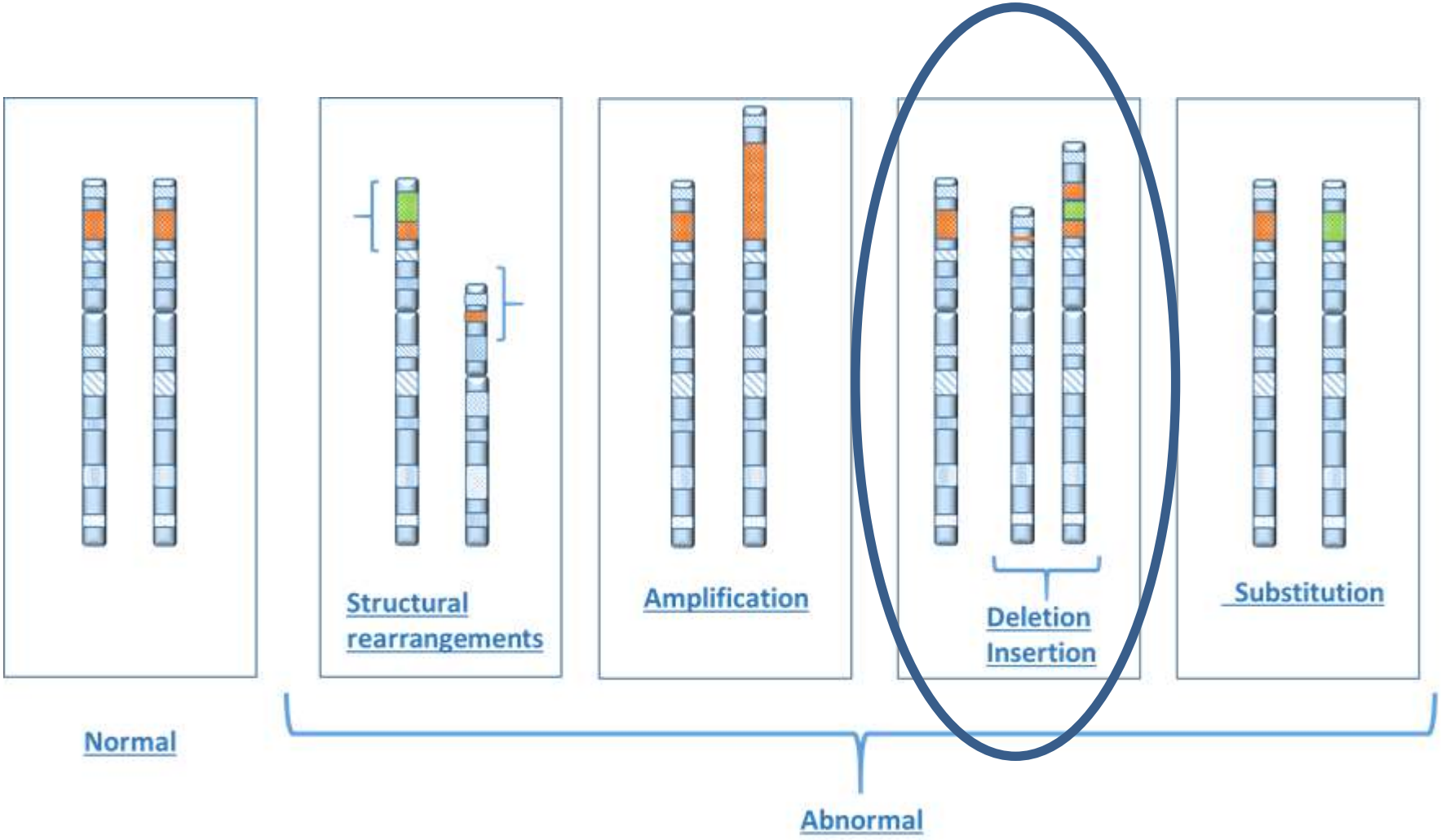




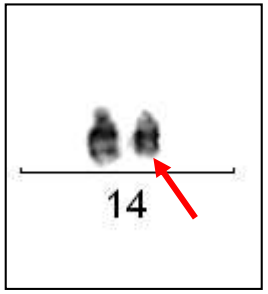


Normal FISH: amplified

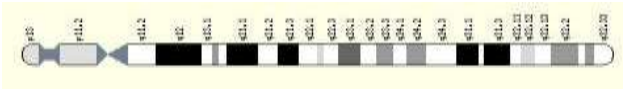
GENETIC ALTERATIONS IN CANCER GENES: MANY TYPES

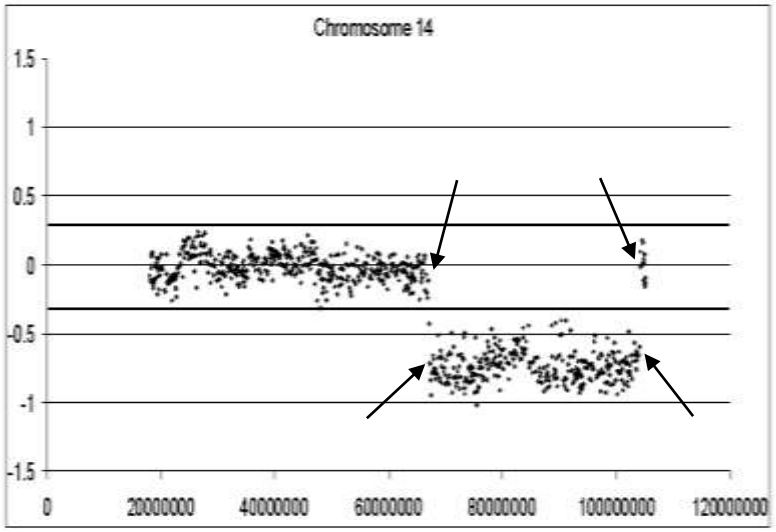


LARGE DELETIONS



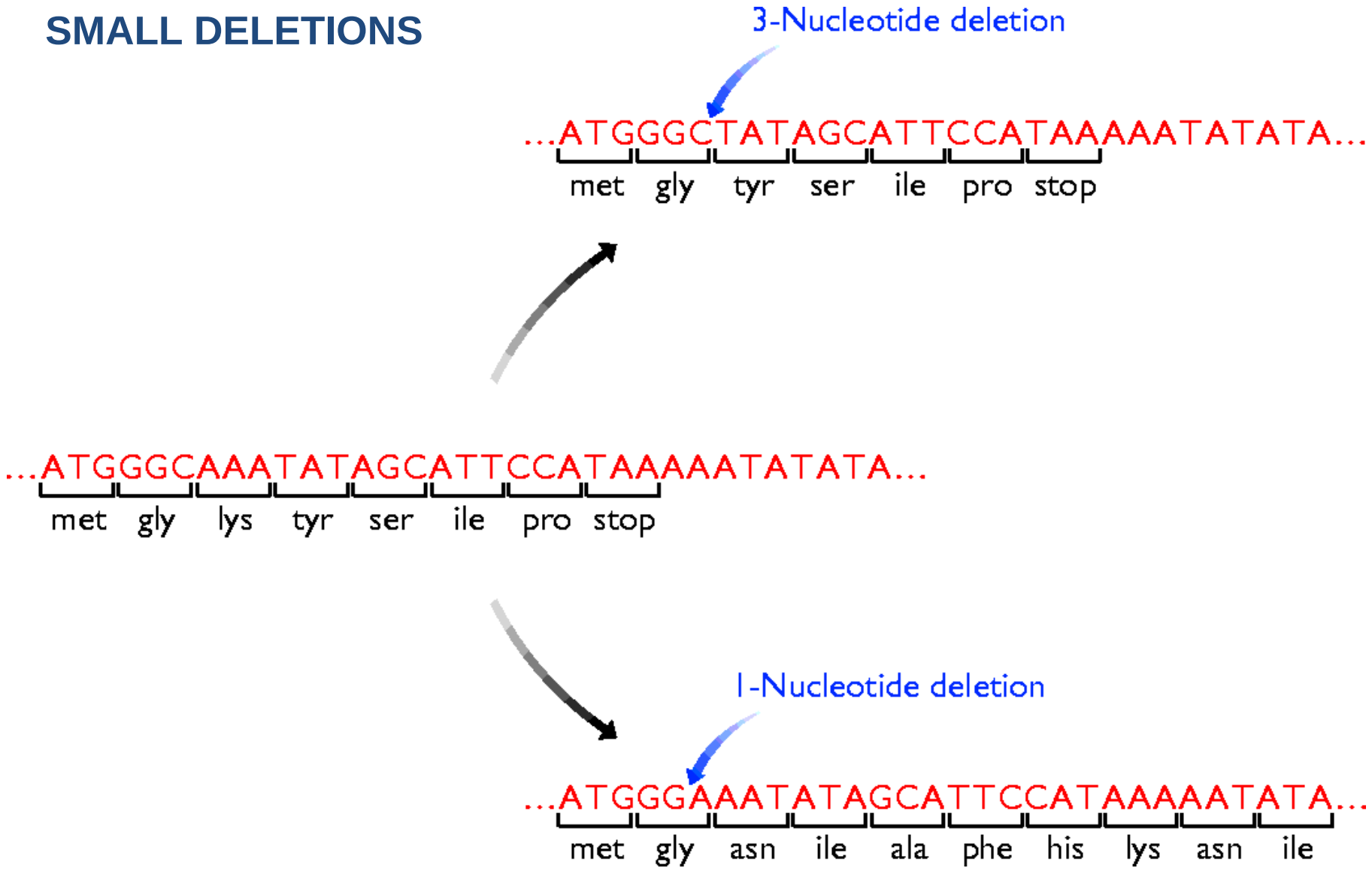
del(14)(q24.1q32.33)



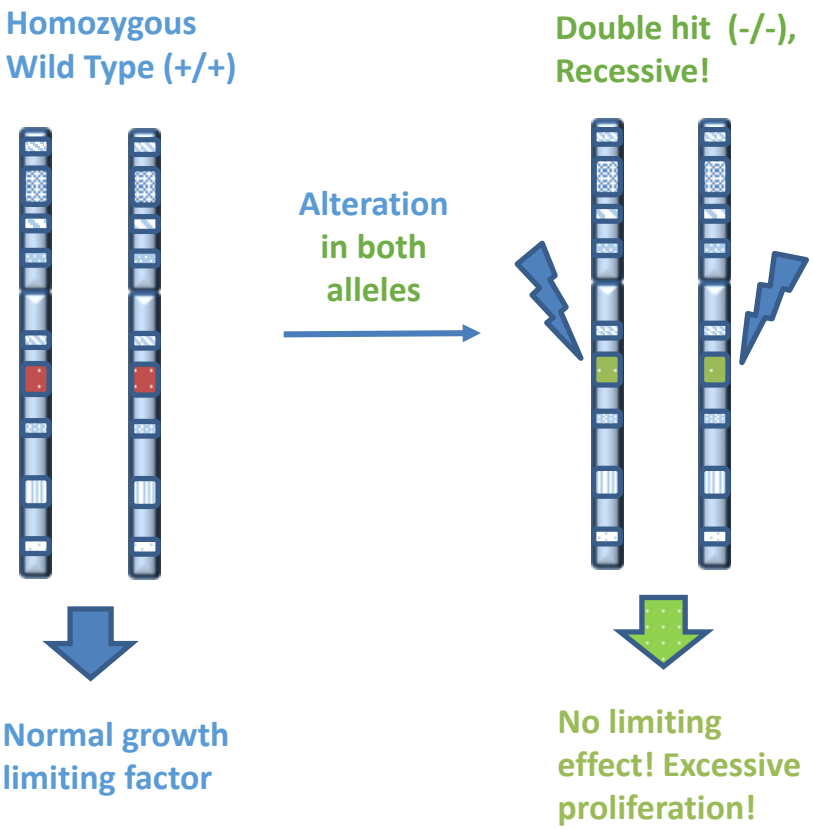


Pospisilova et al, Leukemia, 2007

SMALL DELETIONS

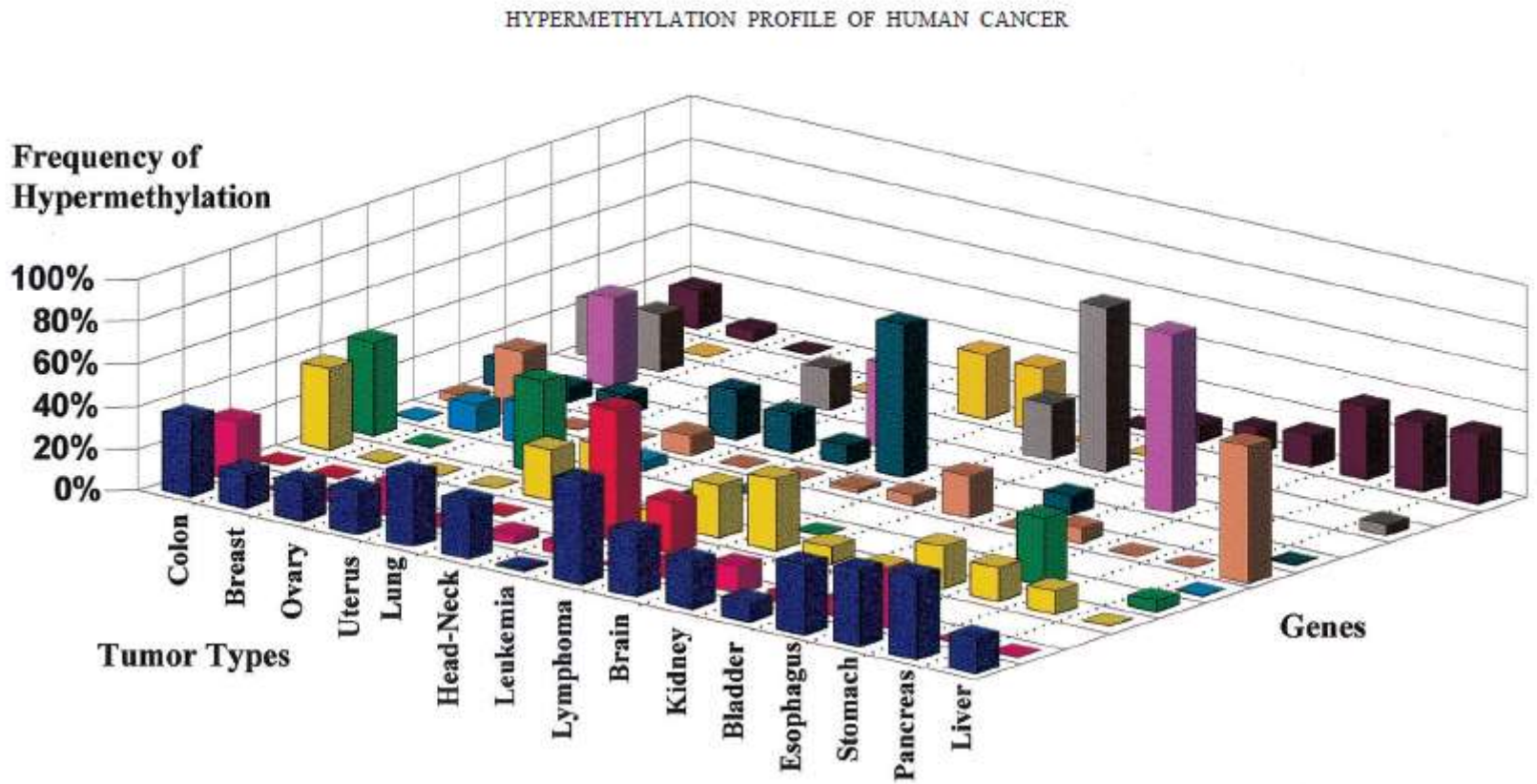


TUMOR SUPPRESSOR GENES



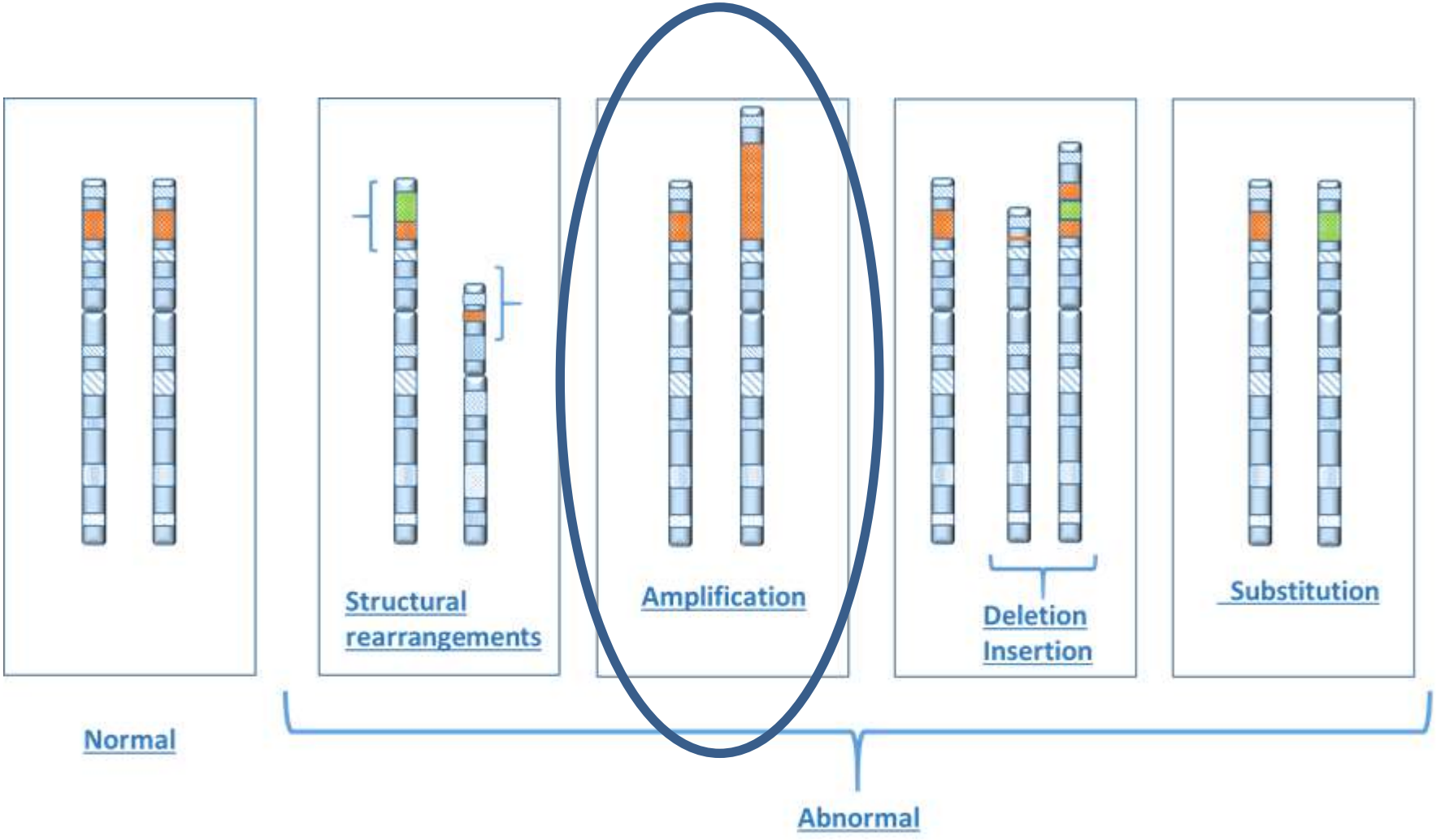
Alteration in one allele is not sufficient (recessive), Second hit

METHYLATION

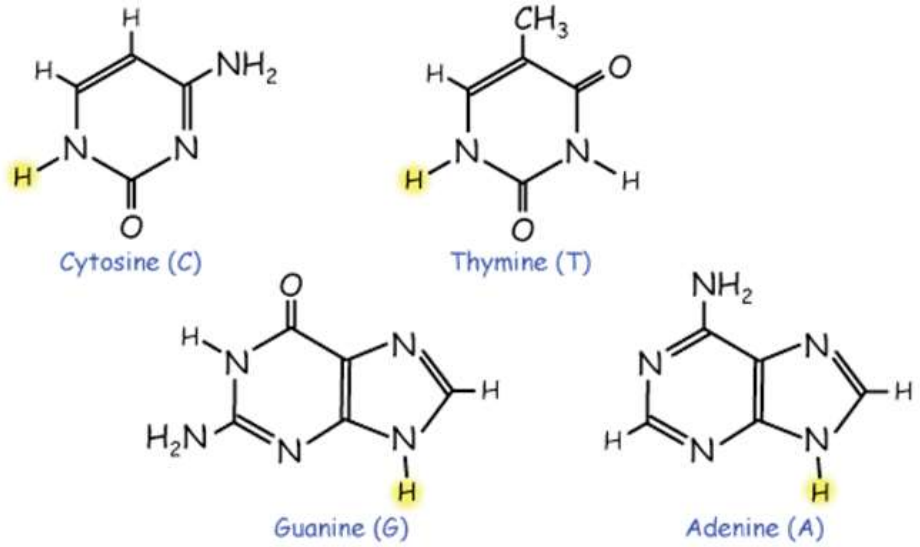


Esteller M et al. Cancer Research 2001

GENETIC ALTERATIONS IN CANCER GENES: MANY TYPES!



POINT MUTATIONS (SINGLE NUCLEOTIDE VARIANT OR SNV)

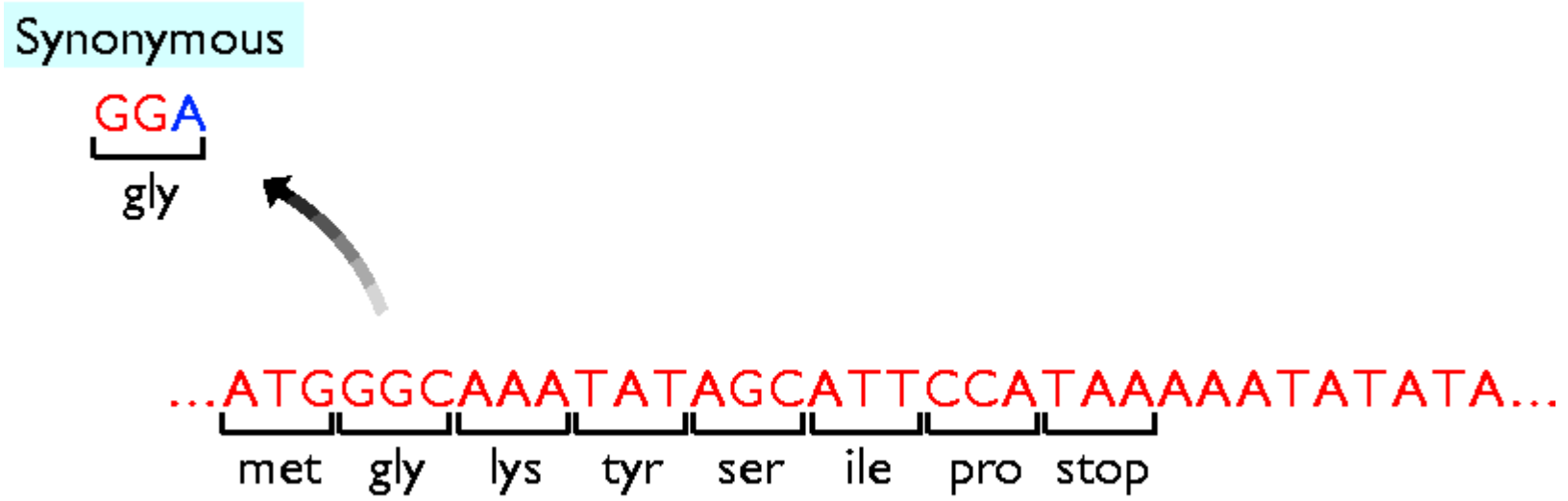


…ATGGGCAAATATAGCATTCCATAAAAATATATA…

met gly lys tyr ser ile pro stop

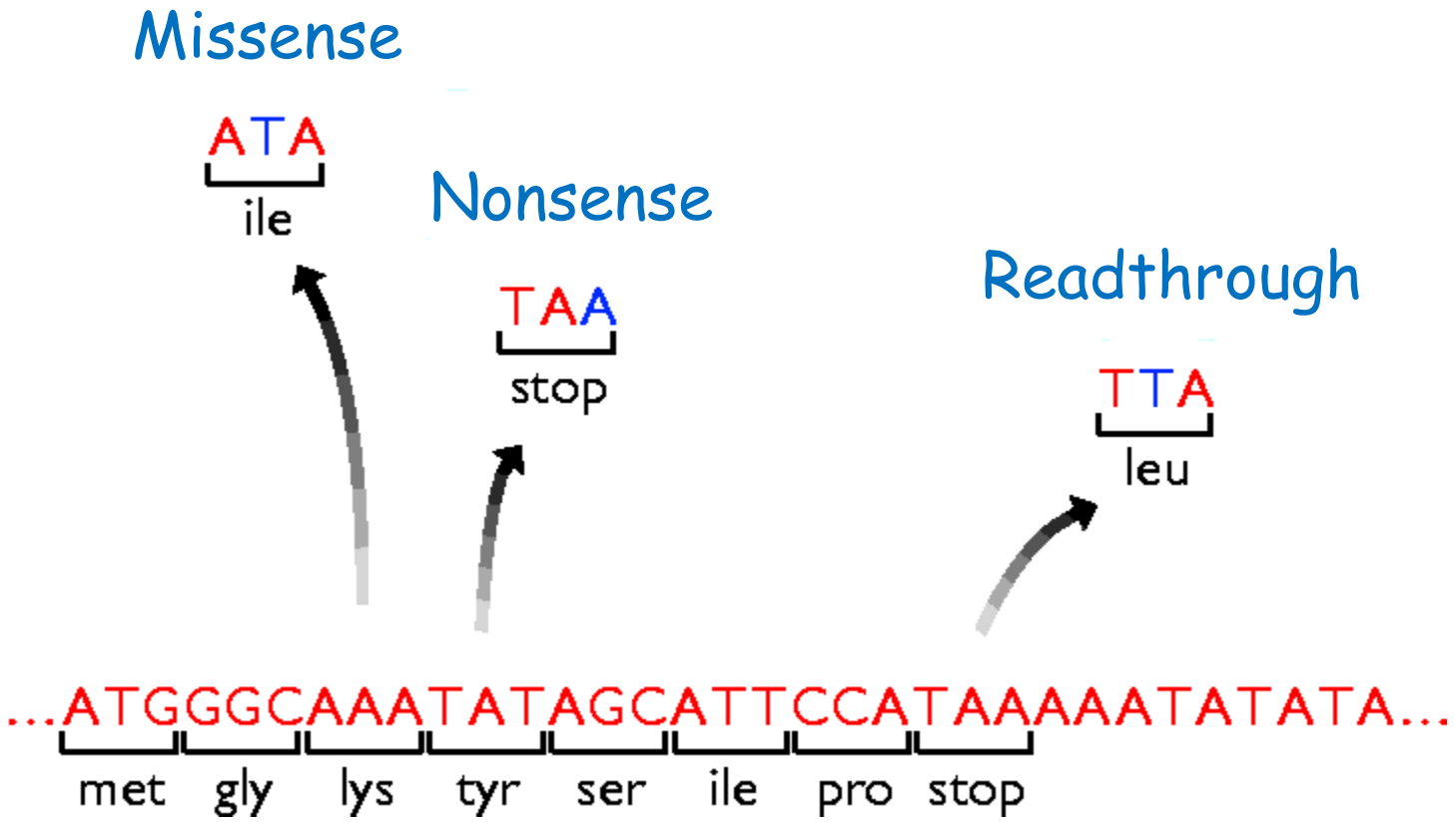
SYNONYMOUS MUTATION

- A mutation that does not alter the encoded amino acid sequence of a protein



NON SYNONYMOUS MUTATION

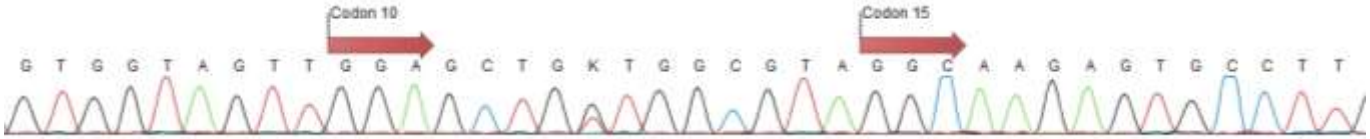
- A mutation that alters the encoded amino acid sequence of a protein



COLORECTAL CANCER

Reference (KRAS exon 2)

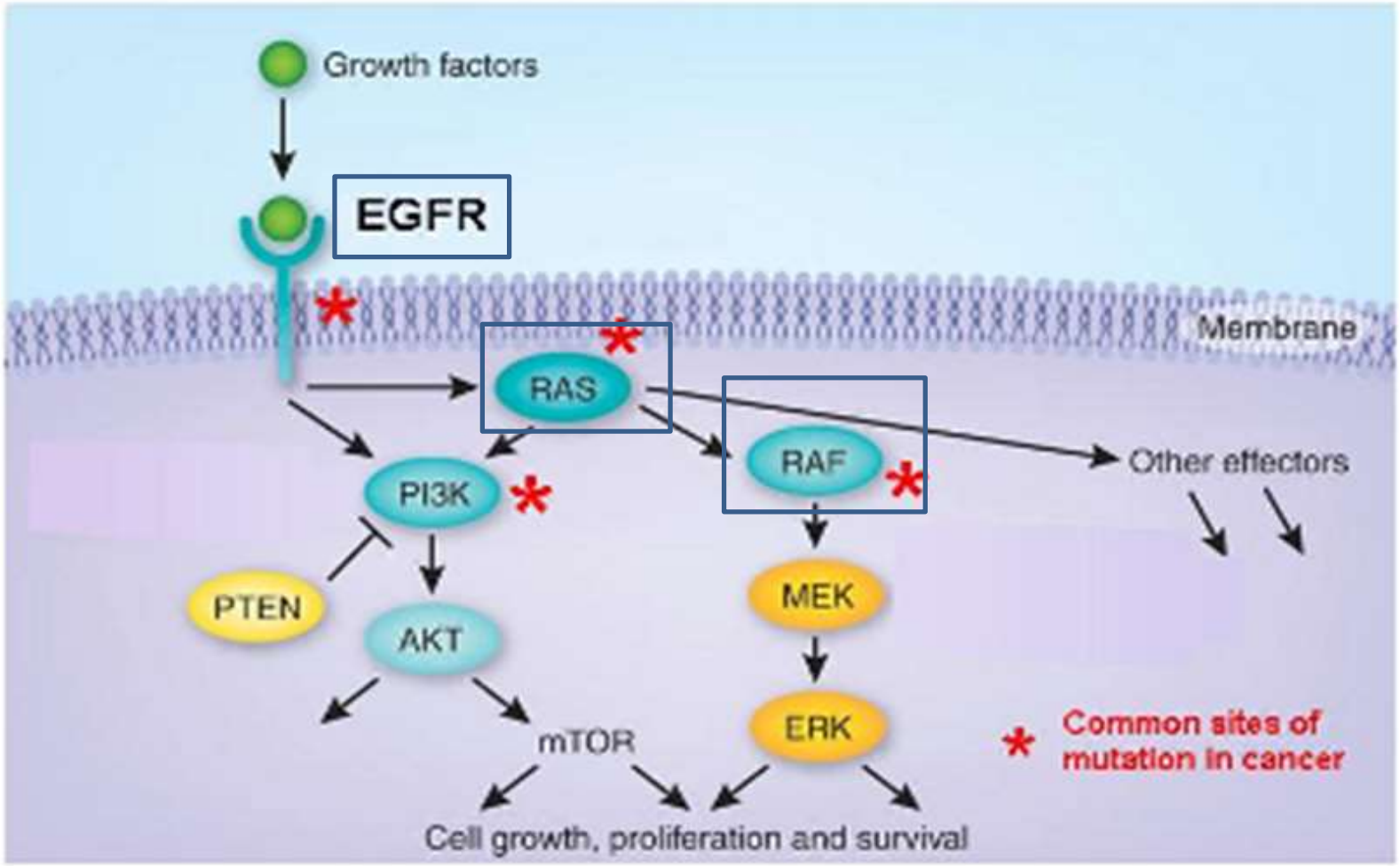
Patient



DNA sequence => presence of another basepair apart from G:

- GGT => GTT at DNA level (codon 12)
- Gly => Val at protein level
- KRAS p.Gly12Val (p.G12V)

RAS-RAF-MEK-ERK PATHWAY



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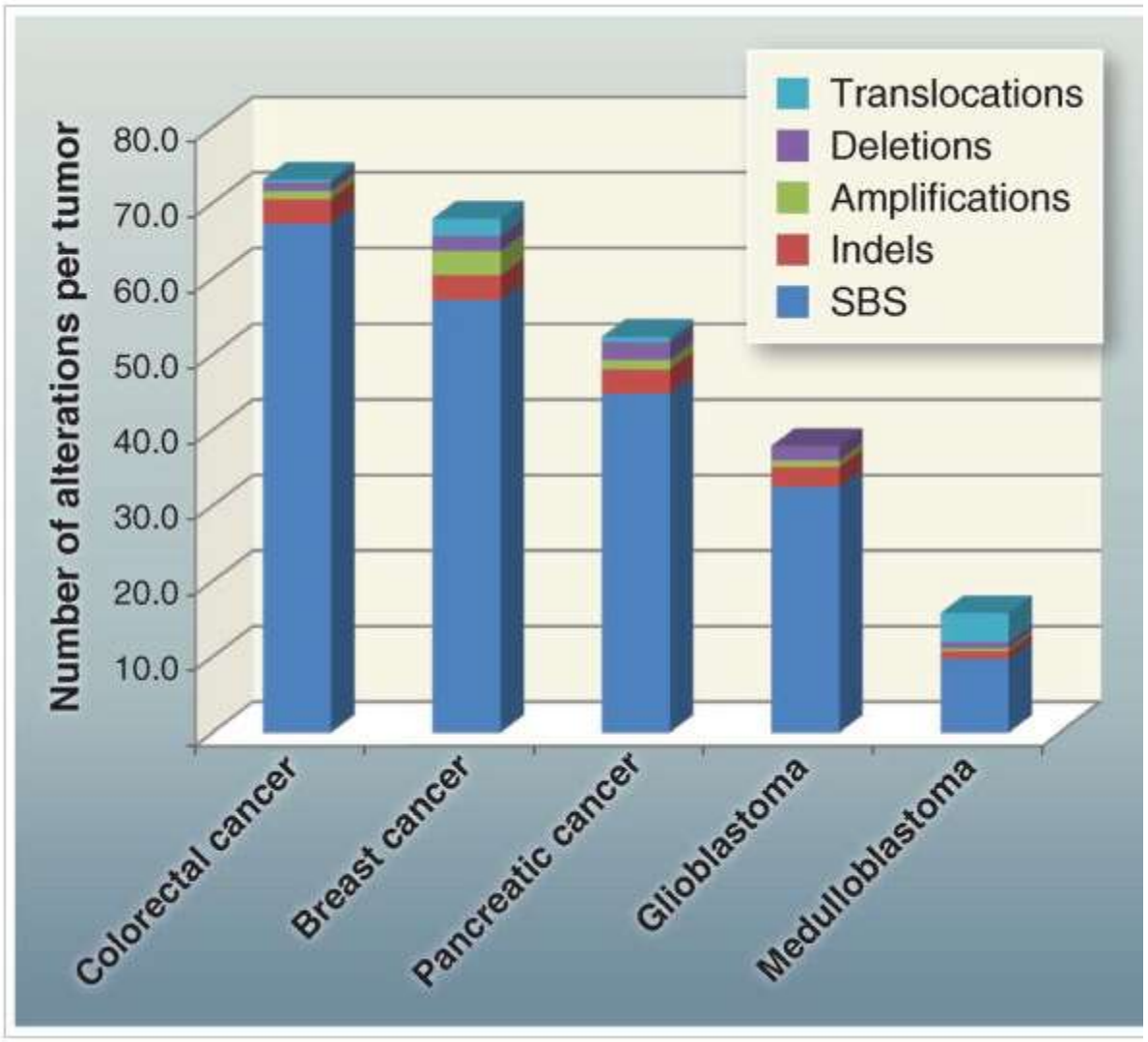


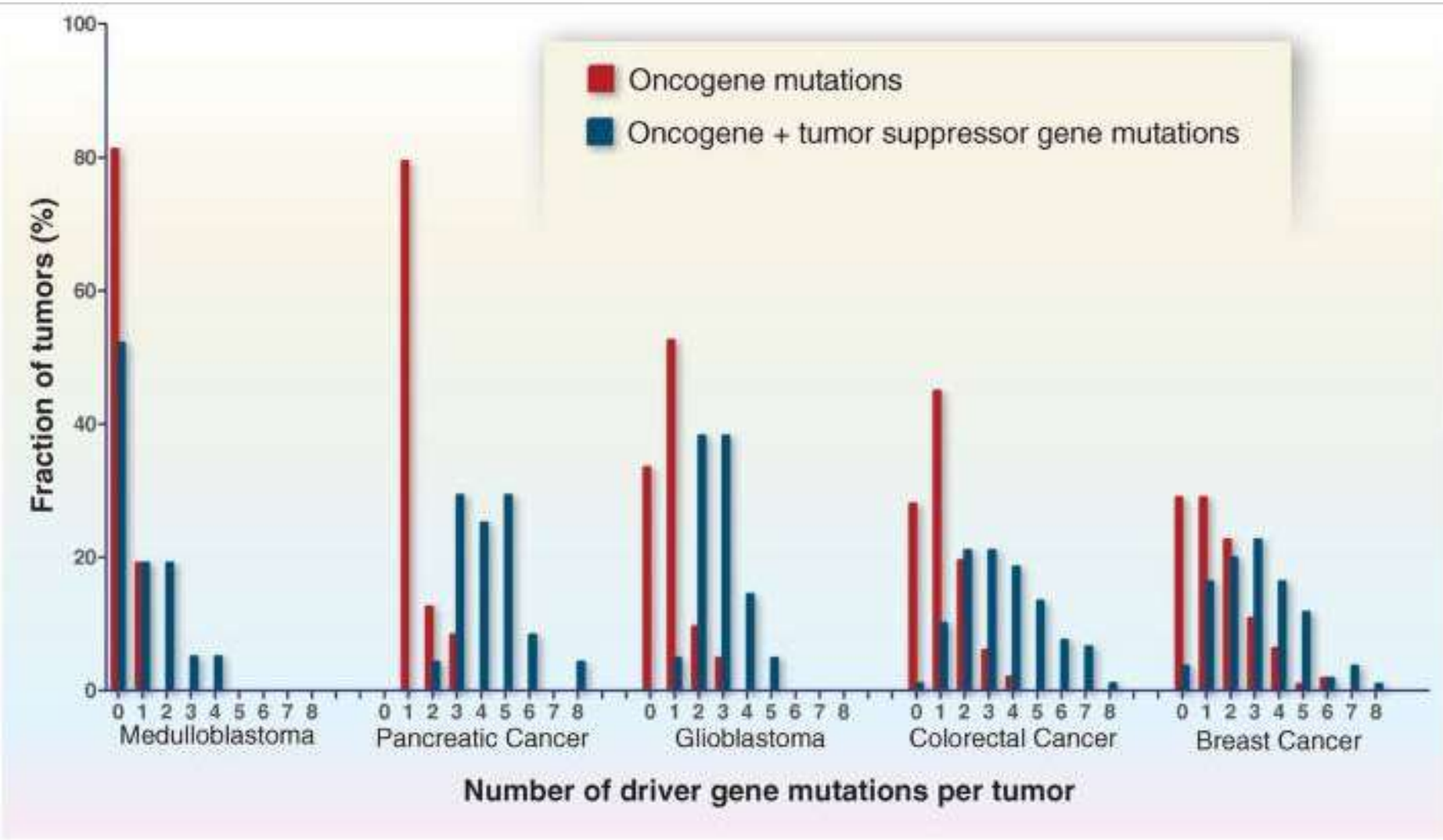
Vogelstein et al. Science 2013

ONCOGENE VS TUMOR SUPRESSOR GENE

- Oncogene:
  - > 20% of the mutations at recurrent positions and are missense
  - promote cell division
  - Gain of function mutation
  - One mutant allele is sufficient, dominant
  - > 75 oncogenes known to date

- Tumor supressor gene:
  - “A gene that, once inactivated, increases the selective growth advantage of the cell in which it resides”
  - Normal function: control or inhibit cellular proliferation
  - Loss of function mutation + second hit
  - > 20% of the recorded mutations in the gene are inactivating.

VOGELSTEIN B ET AL. SCIENCE 2013



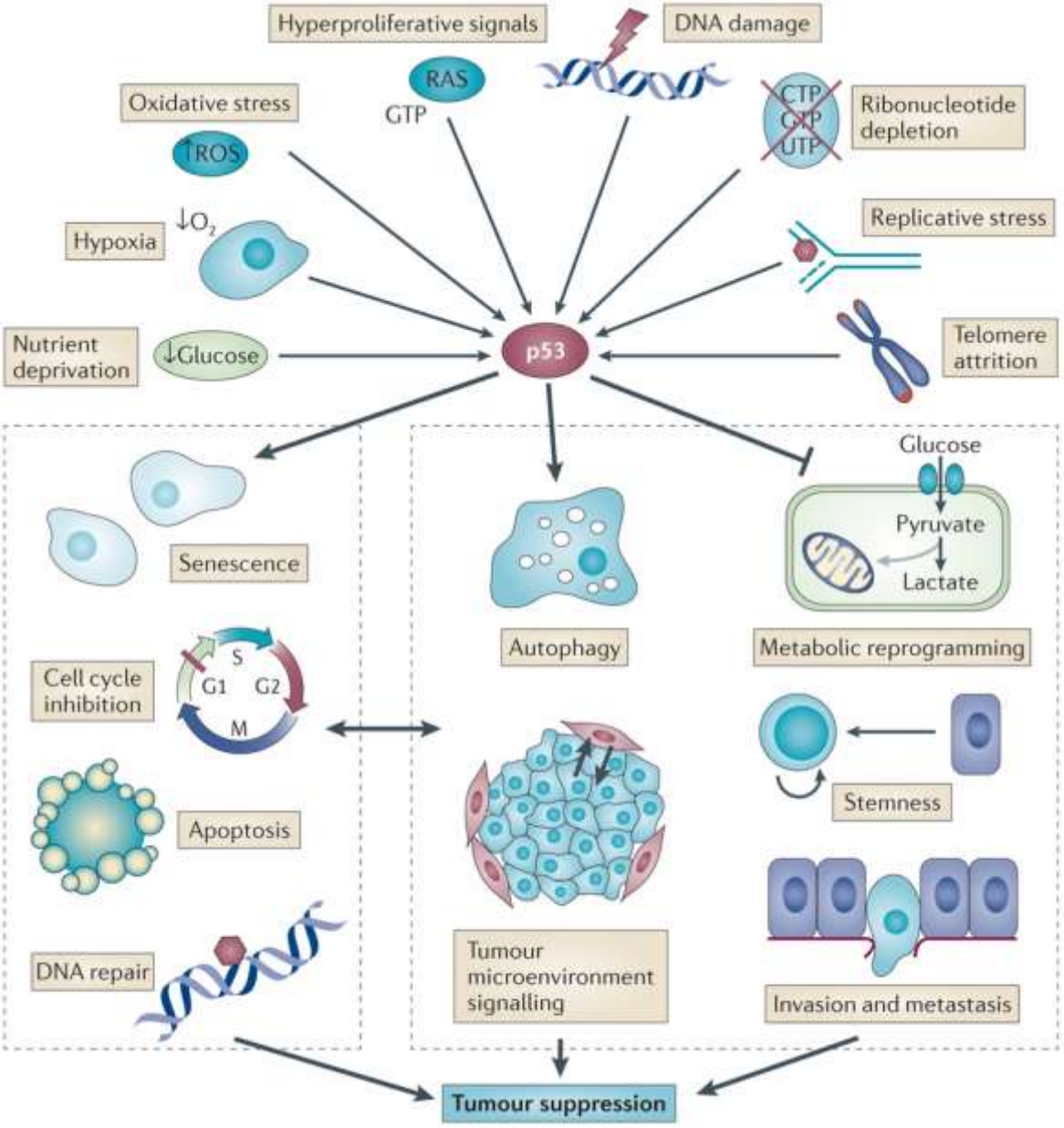
Vogelstein et al. Science 2013

TUMOR SUPRESSOR GENES

Gene (Chr)	Protein Function	Germline mutation (Cancer Syndrome)	Somatic mutations
RB (13q14)	Transcriptional repression (control E2Fs)	Retinoblastoma (retina stem cell)/Osteosarcoma	Observed in many tumors
TP53 (17p13.1)	Transcription factor	Li Fraumeni Syndrome	Observed in many tumors
APC (5p21)	Beta catenin degradation (IHC nuclear pos)	Familial Adenomatosis Polyposis Coli (FAP)	Colorectal, pancreas, stomach, prostate
BRCA1/2 (17q21/13q12)	DNA repair	Breast-Ovary Syndrome	Ovarian Cancer

TP53

- 1979 => protein of “53 kDA”
- Virus induced tumorigenesis: HPV => E6 protein inactivates p53
- 1990: > 50% human cancers contain mutations in *TP53* gene
- Transcription factor => “missense” mutations in DNA binding domain


Hyperproliferative signals
DNA damage
RAS
GTP
Oxidative stress
↑ROS
CTP
GTP
UTP
Ribonucleotide depletion
Hypoxia
↓O2
Replicative stress
Nutrient deprivation
↓Glucose
p53
Telomere attrition
Glucose
Pyruvate
Lactate
Senescence
Autophagy
Metabolic reprogramming
Cell cycle inhibition
S
G1
G2
M
Stemness
Apoptosis
Tumour microenvironment signalling
DNA repair
Invasion and metastasis
Tumour suppression

International Agency for Research on Cancer

World Health Organization

IARC TP53 Database

ABOUT | DATA | USER'S HELP | DATABASE RESOURCES | REFS CORNER | LINKS

You are here: Home / Data / **Gene variations**

SEARCH DATA
- TP53 gene variations
- TP53 somatic mutations
- TP53 germline mutations
- Cell lines
- Mouse models
- Exp. induced mutations

GET DATA
- Data downloads
- Selected statistics

Work best with:

Mutation conversion with: Mutalizer

Search List of TP53 Gene Variations

Reset | Go

cDNA description list
(i.e.: c.524G>A or 524G>A)

Protein description list
(i.e.: p.R175H or R175H)

Genomic description list
(i.e.: g.7578406C>T or 7578406C>T)

Use a delimited/enter key at end of line for querying multiple mutations.
Reference sequences used are: GenBank NC_000017.10 (genomic, hg19), NM_000546.4 (cDNA), UniProt P04637 (protein) For format conversions, we recommend to use Mutalyzer.

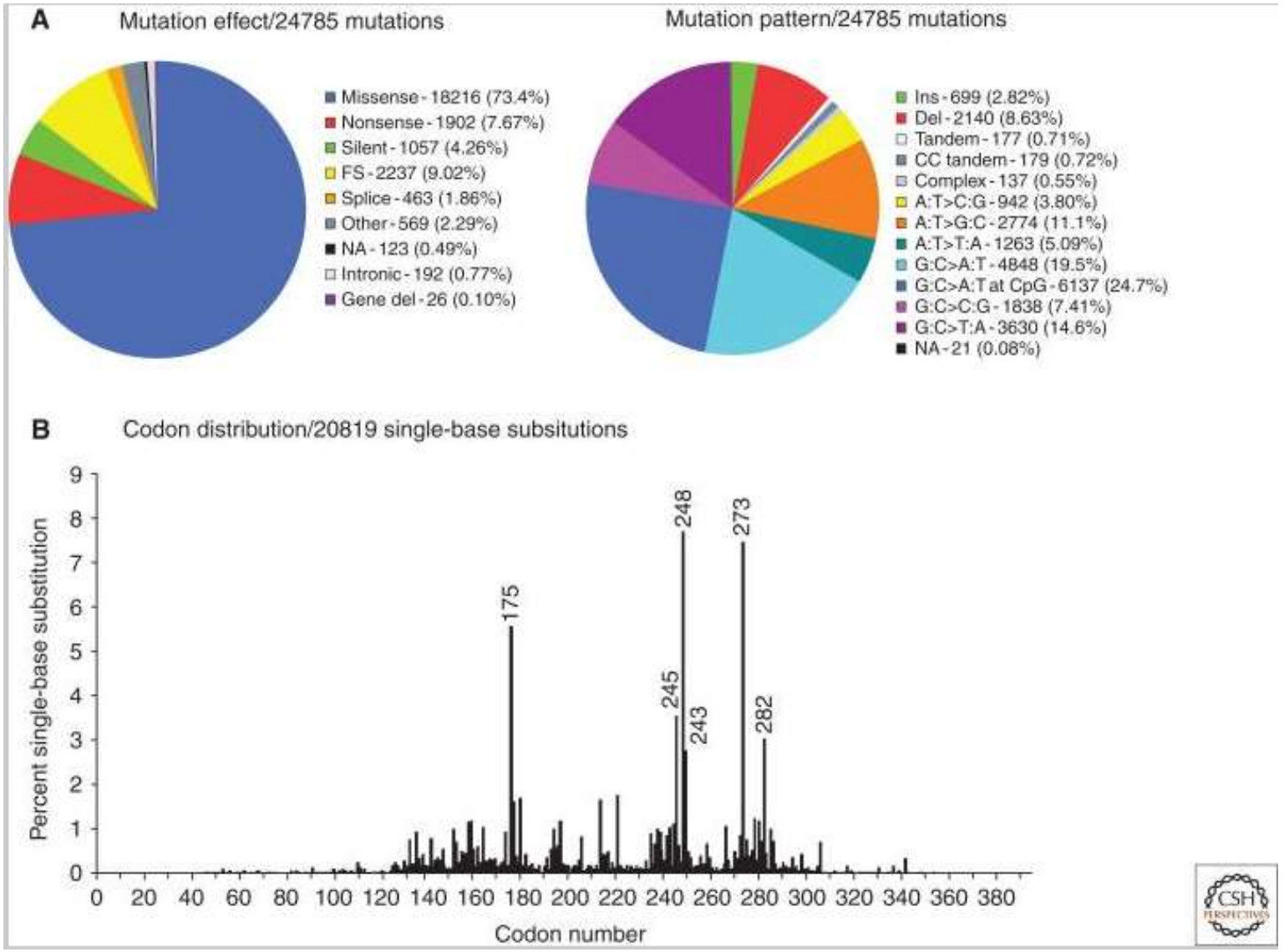
Search Single Gene Variation

Search by Mutation Features

Validated Polymorphisms

Download Datasets Of Mutation Phenotypes

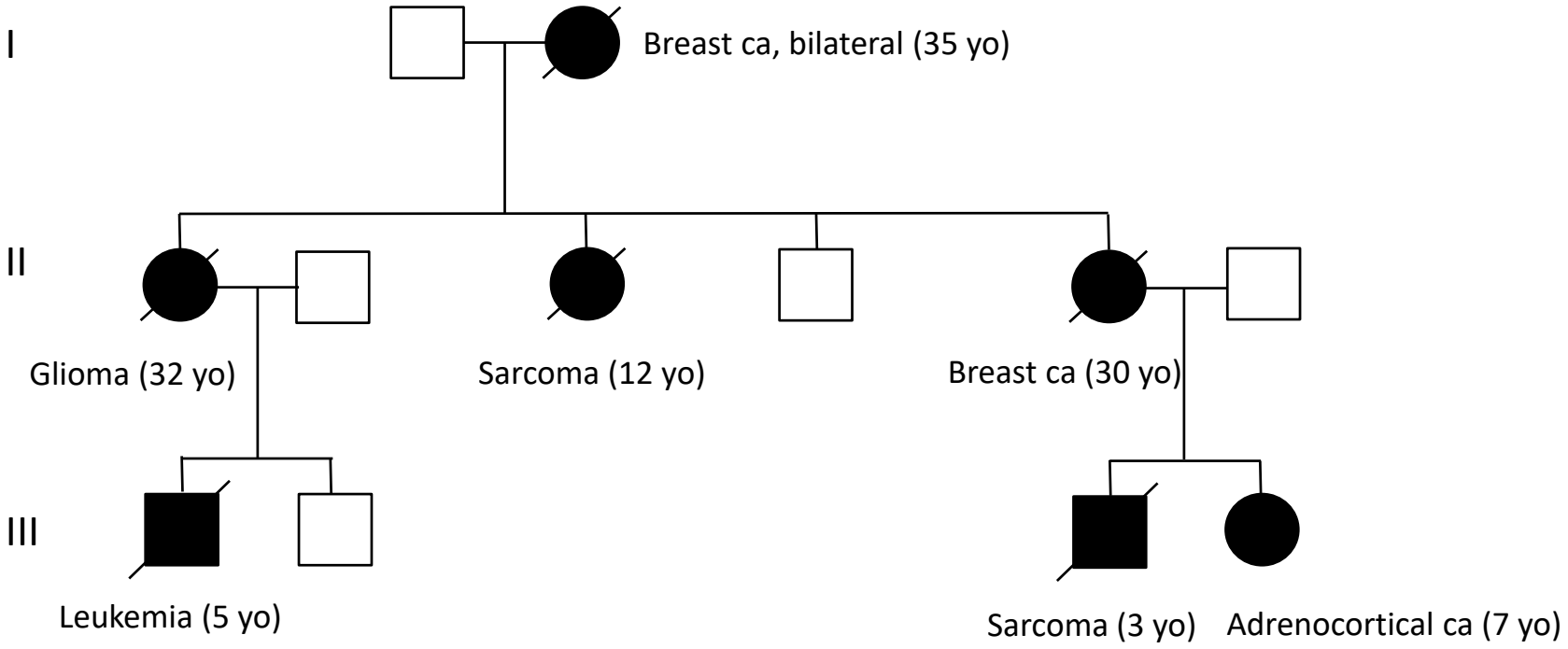
TYPES OF TP53 MUTATIONS IN HUMAN CANCERS (IARC)



LI FRAUMENI SYNDROME

- 50-70% of Li Fraumeni Syndrome families carry germline *TP53* mutations
- 50% chance of developing cancer at age 30
- Sarcoma (soft tissue, bone), breast cancer, brain tumors, adrenocorticoid carcinoma, leucemia…

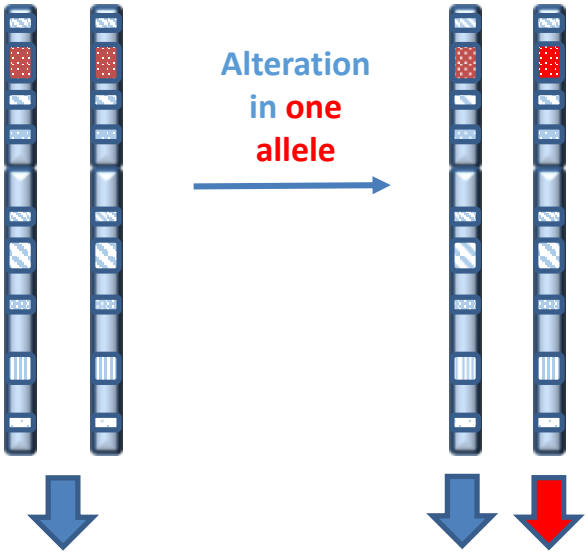
LI FRAUMENI SYNDROME



Oncogene

Homozygous
Wild Type (+/+):
Proto-oncogene

Heterozygous (+/-):
Oncogene (dominant)



Alteration
in one
allele

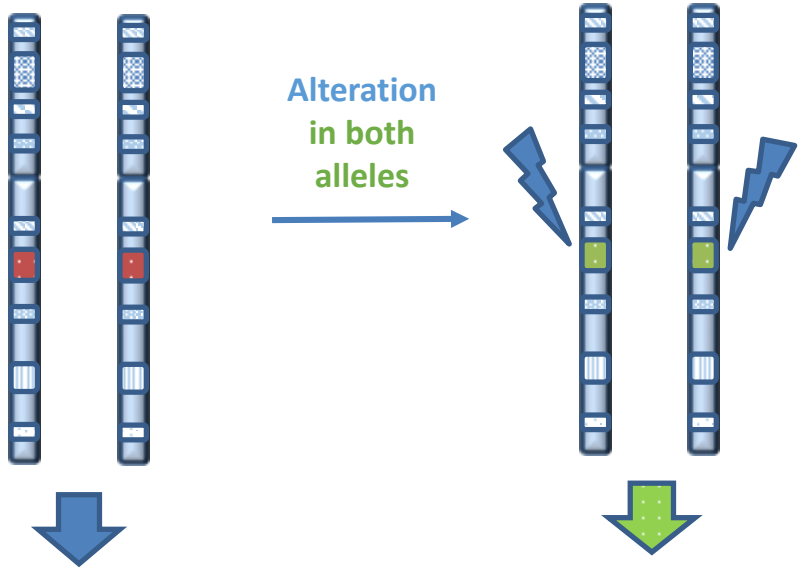
Normal growth
stimulating
factor

Abnormal growth
stimulating factor

Tumor Suppressor Gene

Homozygous
Wild Type (+/+)

Double hit (-/-),
Recessive!



Alteration
in both
alleles

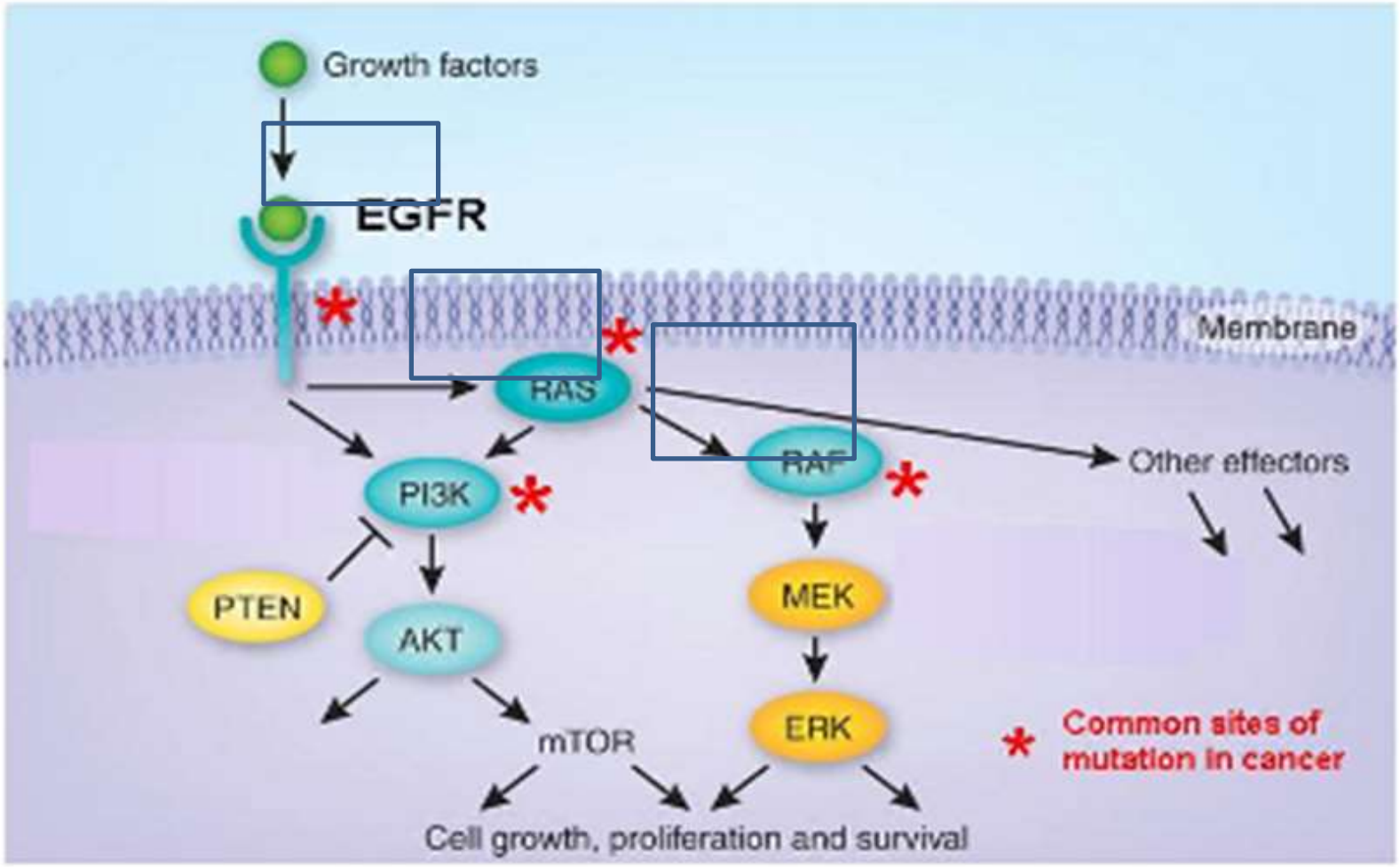
Normal growth
limiting factor

No limiting
effect! Excessive
proliferation!

ONCOGENES: SOME EXAMPLES

Gene	Function of Proto-Oncogene	Somatic mutations
EGFR	Growth factor receptor	Many tumors (lung, colorectal, breast, GBM,…)
HER2/neu	Growth factor receptor	Amplification in up to 20% of breast carcinomas, colon,..
Ras (KRAS, NRAS)	GTP binding and GTPase	Many tumors (lung, colon, melanoma,…)
Myc	Transcription factor	Neuroblastoma, lymphoma,…

RAS-RAF-MEK-ERK PATHWAY



THERAPEUTIC CHALLENGES

- Oncogenes => “activation of protein”, if enzymatic activity: targetable by drugs!
- Tumour suppressor genes: “inactivation of protein” => drugs to restore function?
  - Downstream in pathway!
    - APC inactivating mutations => constitutive activation of oncogenes such as CTNNB1 and CMYC
    - BRCA mutations => activation of DNA repair pathways downstream => PARP inhibitoren!

TAKE HOME MESSAGES

- Genetic alterations in cancer genes: many types:
  - Structural aberrations (translocations, inversions)
  - Copy number alterations (duplication, amplifications)
  - Deletions, insertions
  - Base substitutions (point mutations)
- Driver genes
  - Oncogenes
    - Gene variant (mutation) leading to constitutive activation => “gain of function mutation”
    - One mutant allele is sufficient, dominant
  - Tumor suppressor genes
    - A gene that, once inactivated, increases the selective growth advantage of the cell in which it resides
    - Inactivating mutation (“loss of function” mutation) + second hit