

Cancer Biology I :

Topics covered

Week 1:

Hallmarks of cancer – an overview; **Mutations, oncogenes and tumor suppressor genes**

(Chapters 2, 4, 7 (Weinberg book))

Week 2:

Lecture 2 (Monday 14:15-16:00: room AAC132):

Genome instability and DNA repair of DNA double strand breaks; Synthetic lethality

Exercises: Wednesday 13:15-16:00: room CE1103

Week 3:

Lecture 3/Exercises: **DNA repair and the DNA damage response**

Week 4:

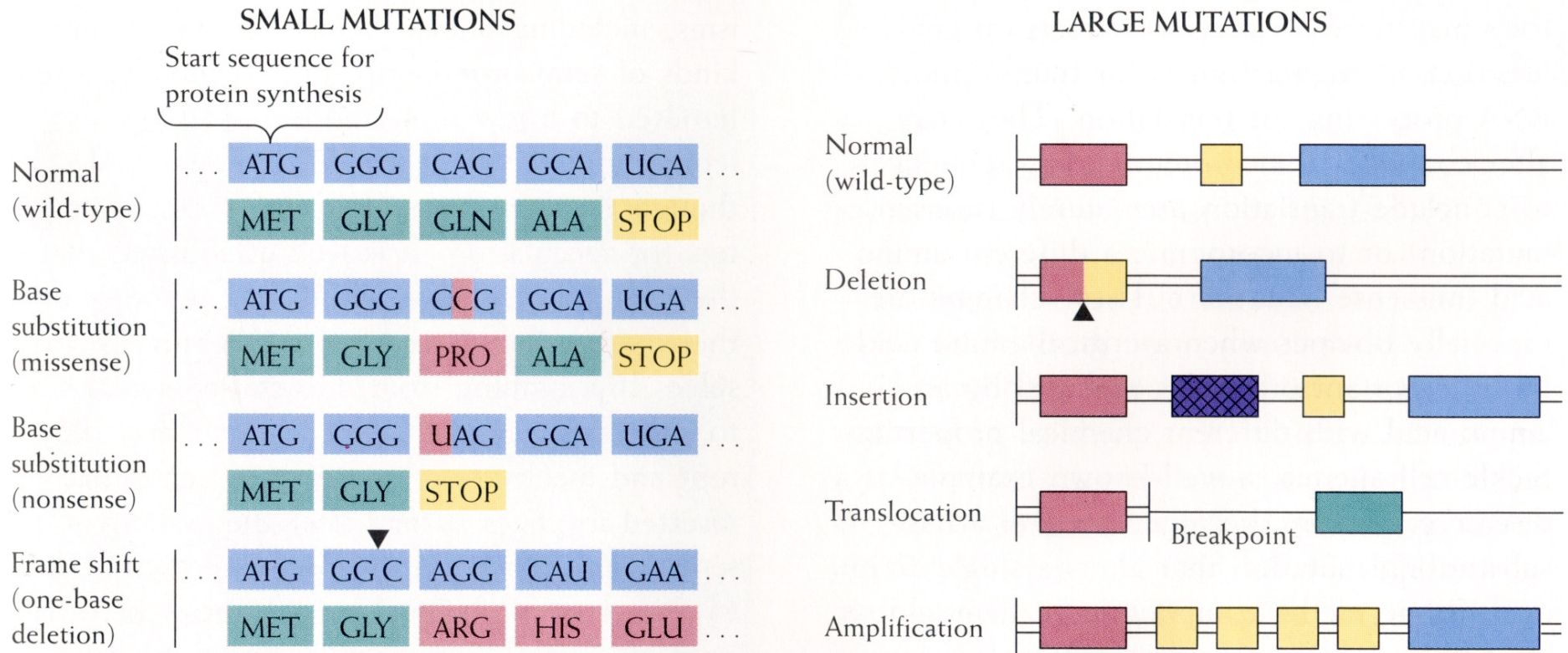
Lecture 4/Exercises: **p53 and apoptosis**

(Chapters 9 (Weinberg))

from Monday's Lecture

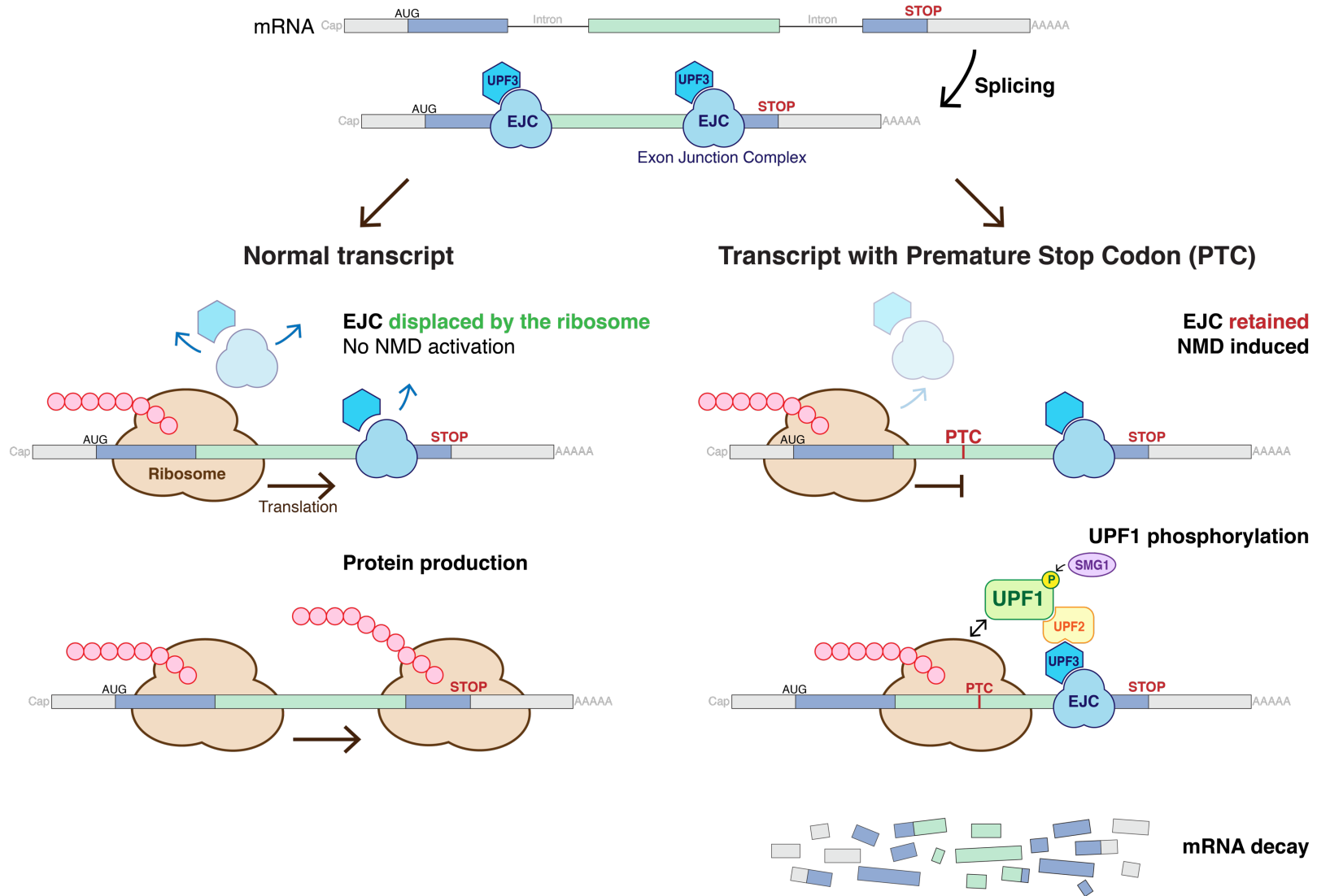
- The vast majority of human tumors is monoclonal. But: genetic heterogeneity due to genetic instability may mask the true monoclonal origin
- The multiplicity of defenses may explain why cancer is relatively rare during human lifetime (more than 6 specific changes must occur to make out of a normal cell a tumor cell)
- Human cancers express a mutator phenotype

Genetic Variations



Genetic variation can take the form of small or large mutations in an organism's DNA, as the scheme above illustrates. Large mutations may involve the insertion of foreign, perhaps viral, genetic information (purple box) or the exchange of information by translocation between two chromosomes at a breakpoint. Small mutations result from changes in a single base (nucleotide) or codon (nucleotide triplet, dictating a particular amino acid in the protein product).

Nonsense-mediated mRNA Decay (NMD)



mRNAs with premature stop codons are degraded by NMD

DNA Replication Errors are Extremely Rare

- Mutation rate of $1/10^9$ per nucleotide per cell division
 - Copying mistake by DNA polymerases (delta and epsilon): $1/10^5$
 - 3'-5' proofreading overlook: $1/10^2$
 - Mismatch repair enzymes overlook: $1/10^2$
- 10-50 double-strand DNA breaks occur per S phase
- Human genome: 6.4 billion bp

Human Cells can Tolerate Elevated Mutation Burdens

ARTICLES

<https://doi.org/10.1038/s41588-021-00930-y>

nature
genetics



OPEN

Increased somatic mutation burdens in normal human cells due to defective DNA polymerases

Philip S. Robinson ^{1,2,7}, Tim H. H. Coorens ^{1,7}, Claire Palles^{3,7}, Emily Mitchell¹, Federico Abascal ¹, Sigurgeir Olafsson¹, Bernard C. H. Lee^{1,4}, Andrew R. J. Lawson¹, Henry Lee-Six ¹, Luiza Moore ¹, Mathijs A. Sanders^{1,5}, James Hewinson¹, Lynn Martin³, Claudia M. A. Pinna ³, Sara Galavotti³, Raheleh Rahbari ¹, Peter J. Campbell ¹, Iñigo Martincorena ¹, Ian Tomlinson ^{6,8}  and Michael R. Stratton ^{1,8} 

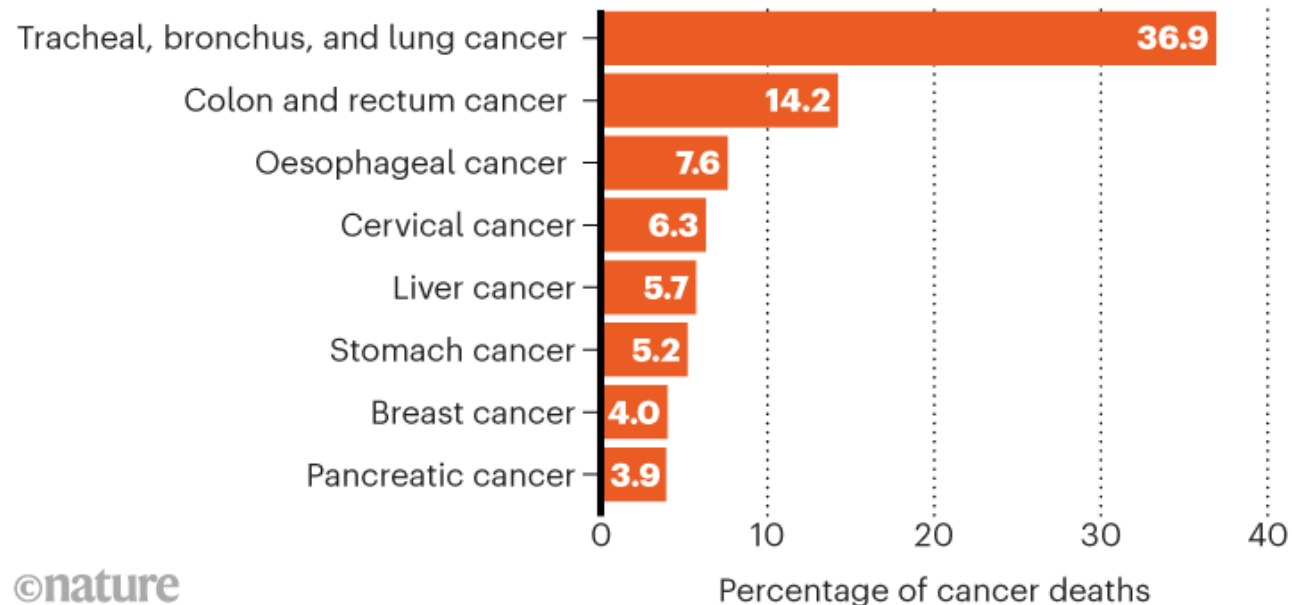
Nature Genetics, 53, 1424 (2021)

But: Germline *POLE/POLD1* mutations with defects in the exonuclease domain lead to loss of proofreading function. → familial cancer predisposition

44% of Cancer Deaths are Caused by Avoidable Risk Factors

CANCER DEATHS BY TUMOUR TYPE

In men and women, among cancers caused by preventable risk factors, tumours of the lung, trachea and bronchus were the leading cause of death. Smoking was the biggest risk factor associated with those cancer deaths.

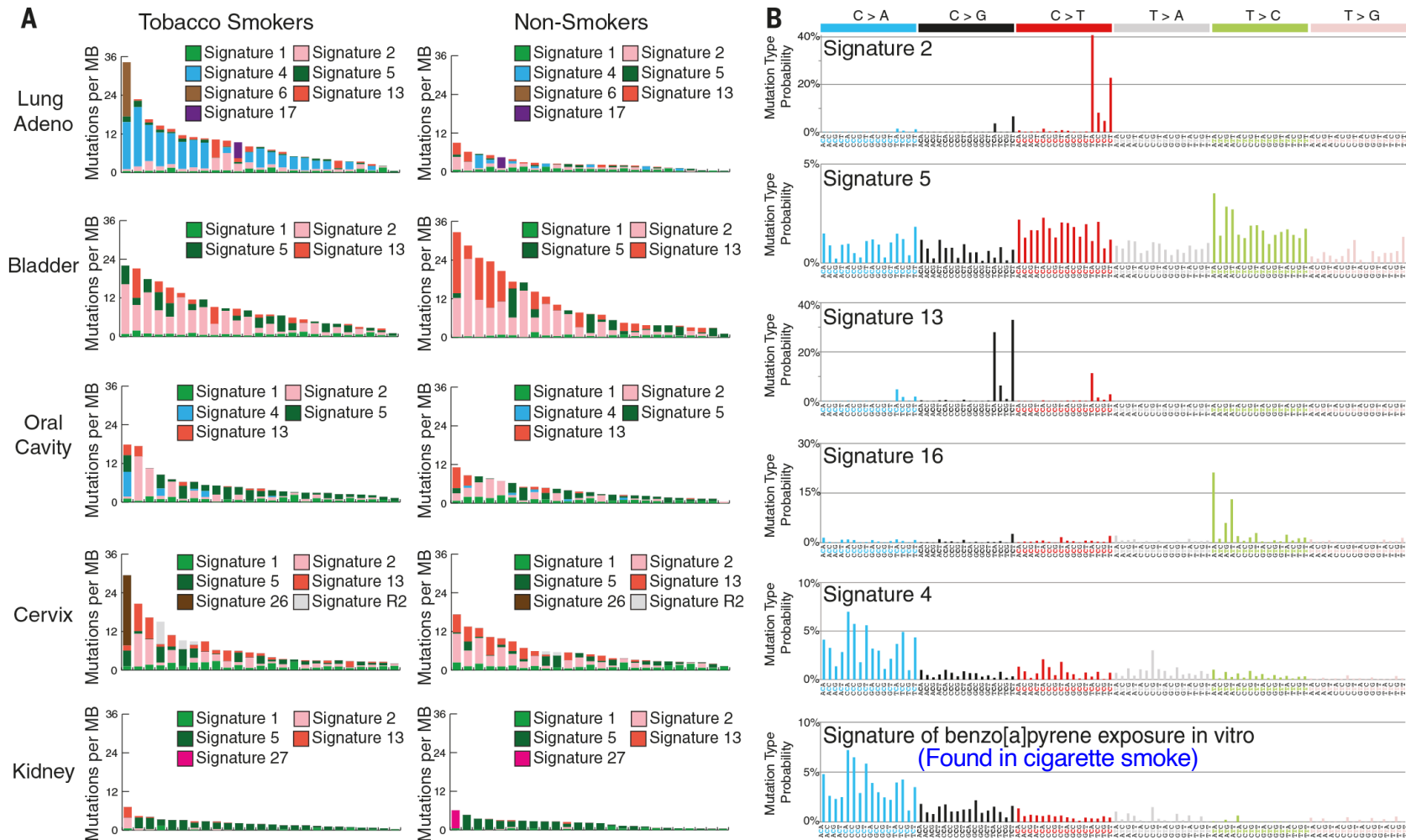


Source: Ref. 1

In 2019, half of all male deaths from cancer, and more than one-third in women, were due to preventable risk factors including tobacco and alcohol use, unhealthy diets, unsafe sex and workplace exposure to harmful products, such as asbestos.

Nature, 2022: doi: <https://doi.org/10.1038/d41586-022-02355-x>

Mutational Signatures Tobacco Smoking

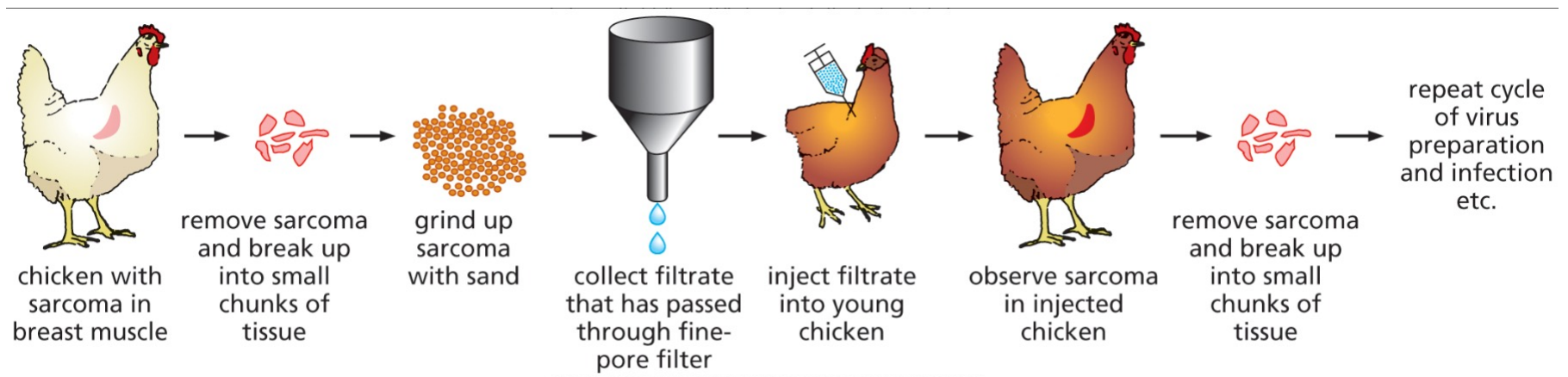


https://www.science.org at ERT Lausanne on September 03, 2022

Fig. 2. Mutational signatures associated with tobacco smoking. (A) Each panel contains 25 randomly selected cancer genomes (represented by individual bars) from either smokers or nonsmokers in a given cancer type. The y axes reflect numbers of somatic mutations per megabase. Each bar is colored proportionately to the number of mutations per megabase attributed to the mutational signatures found in that sample. The naming of mutational signatures is consistent with previous reports (16–18). (B) Each panel contains the pattern of a mutational signature associated with tobacco smoking. Signatures are depicted using a 96-substitution classification defined by the

substitution type and sequence context immediately 5' and 3' to the mutated base. Different colors are used to display the various types of substitutions. The percentages of mutations attributed to specific substitution types are on the y axes, whereas the x axes display different types of substitutions. Mutational signatures are depicted based on the trinucleotide frequency of the whole human genome. Signatures 2, 4, 5, 13, and 16 are extracted from cancers associated with tobacco smoking. The signature of benzo[a]pyrene is based on in vitro experimental data (19). Numerical values for these mutational signatures are provided in table S6.

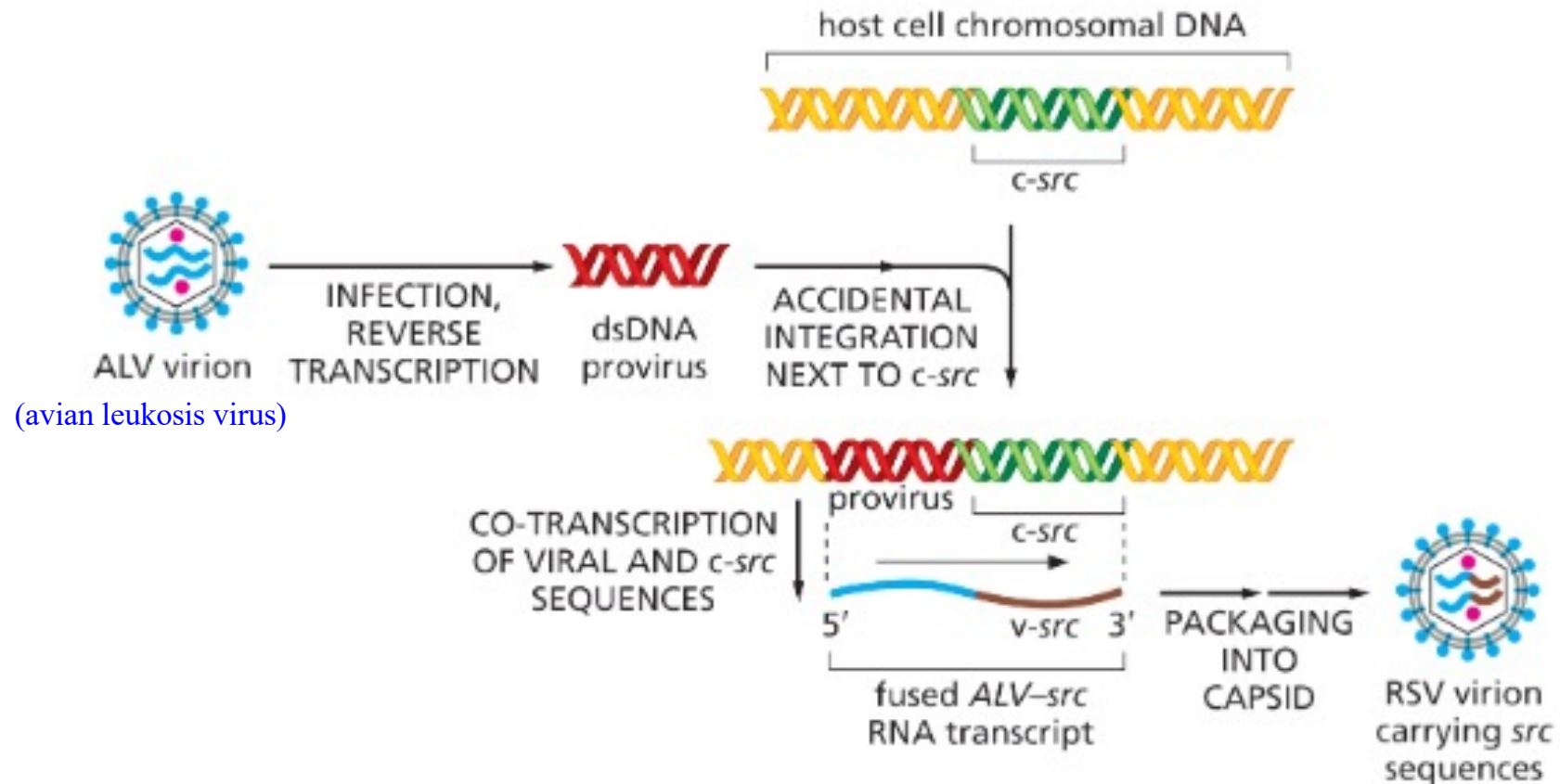
Viral Oncogenes



Only subcellular fragments passed through the filter. ... i.e. the cancer causing agent was a virus.

Figure 3.1 The Biology of Cancer 2023

Viral Oncogenes are Derived from Cellular Proto-oncogenes



Model of how ALV mistakenly captured *SRC* during the evolution of RSV

Figure 3.16 The Biology of Cancer 2023

Cellular Proto-Oncogenes

- 1970's: tumor viruses were suspected to be the cause of many human cancers. The study of tumor viruses led to the understanding of molecular principles in many human cancers.
- But: Of the > 100 tumor types, only cervical carcinoma (papilloma virus) and hepatomas (hepatitis virus) could be linked to viruses. Though, there are probably others...

Cellular Proto-Oncogenes

- Carcinogens function as mutagens.
- They mutate critical growth-controlling genes (proto-oncogenes).
- The mutated alleles function as active oncogenes.

Testing the Hypothesis

- 1) Development of gene transfer protocols
- 2) Extract DNA from cancer cells
- 3) Choose appropriate recipient cells

Nonviral Oncogenes

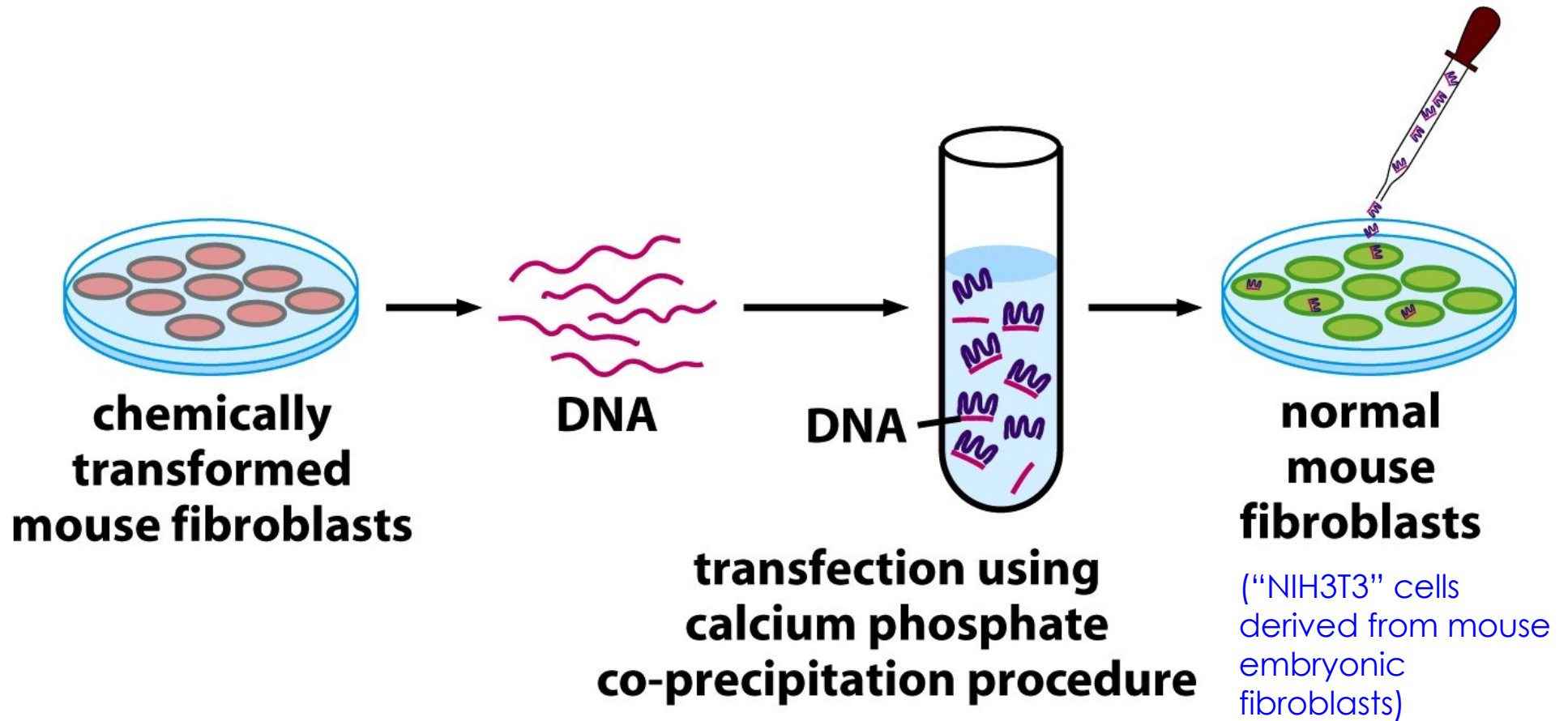


Figure 4.1 The Biology of Cancer 2023

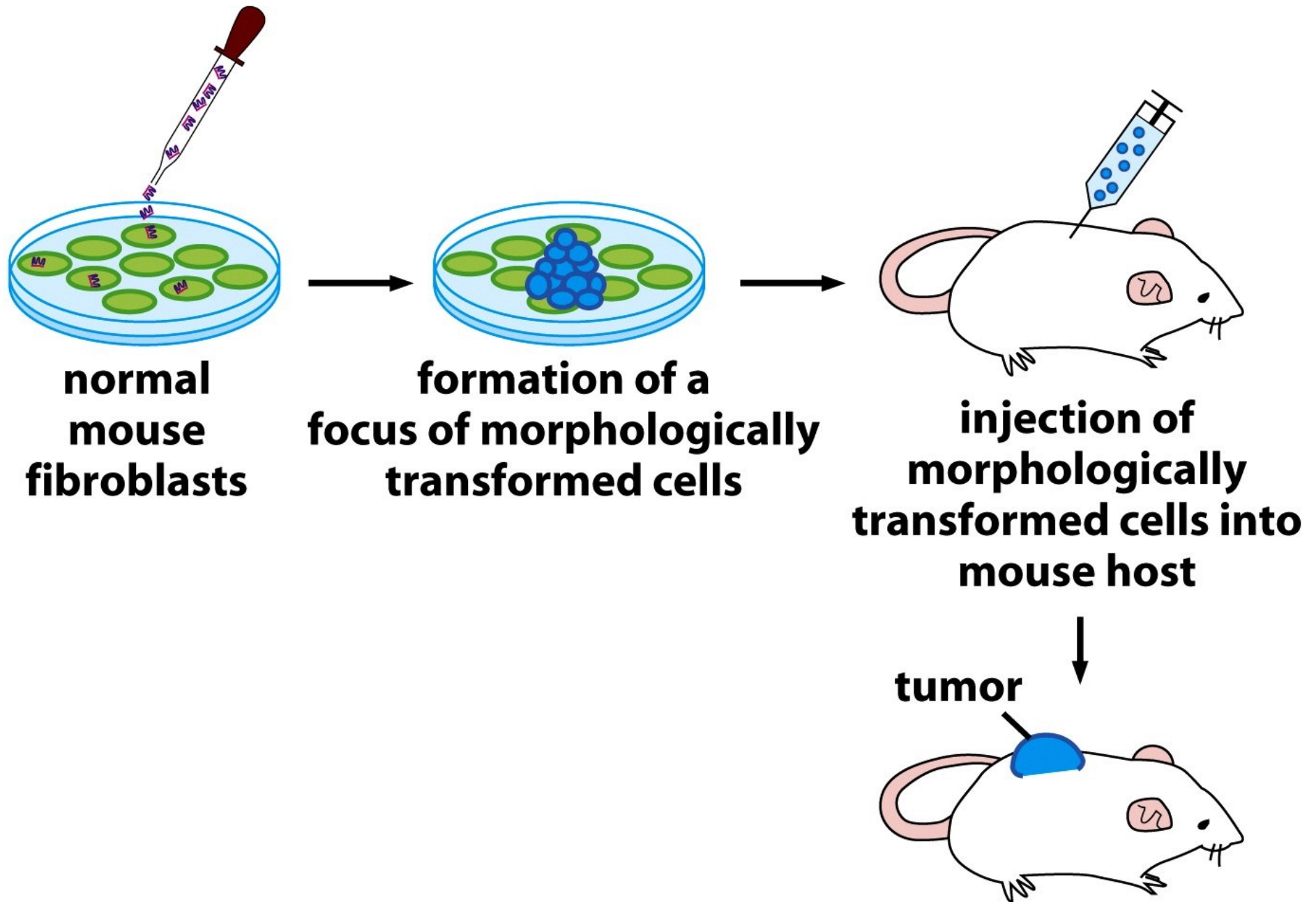


Figure 4.1 The Biology of Cancer 2023

Transformed NIH3T3

Untransformed NIH3T3

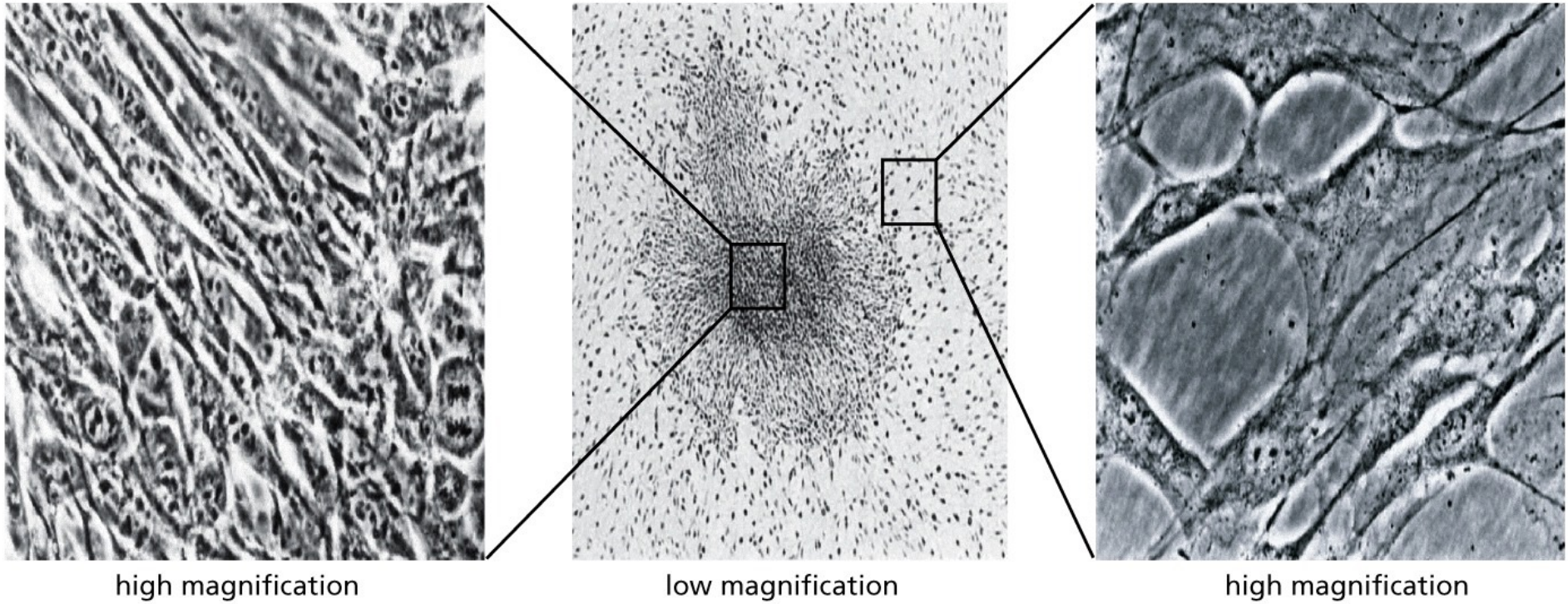
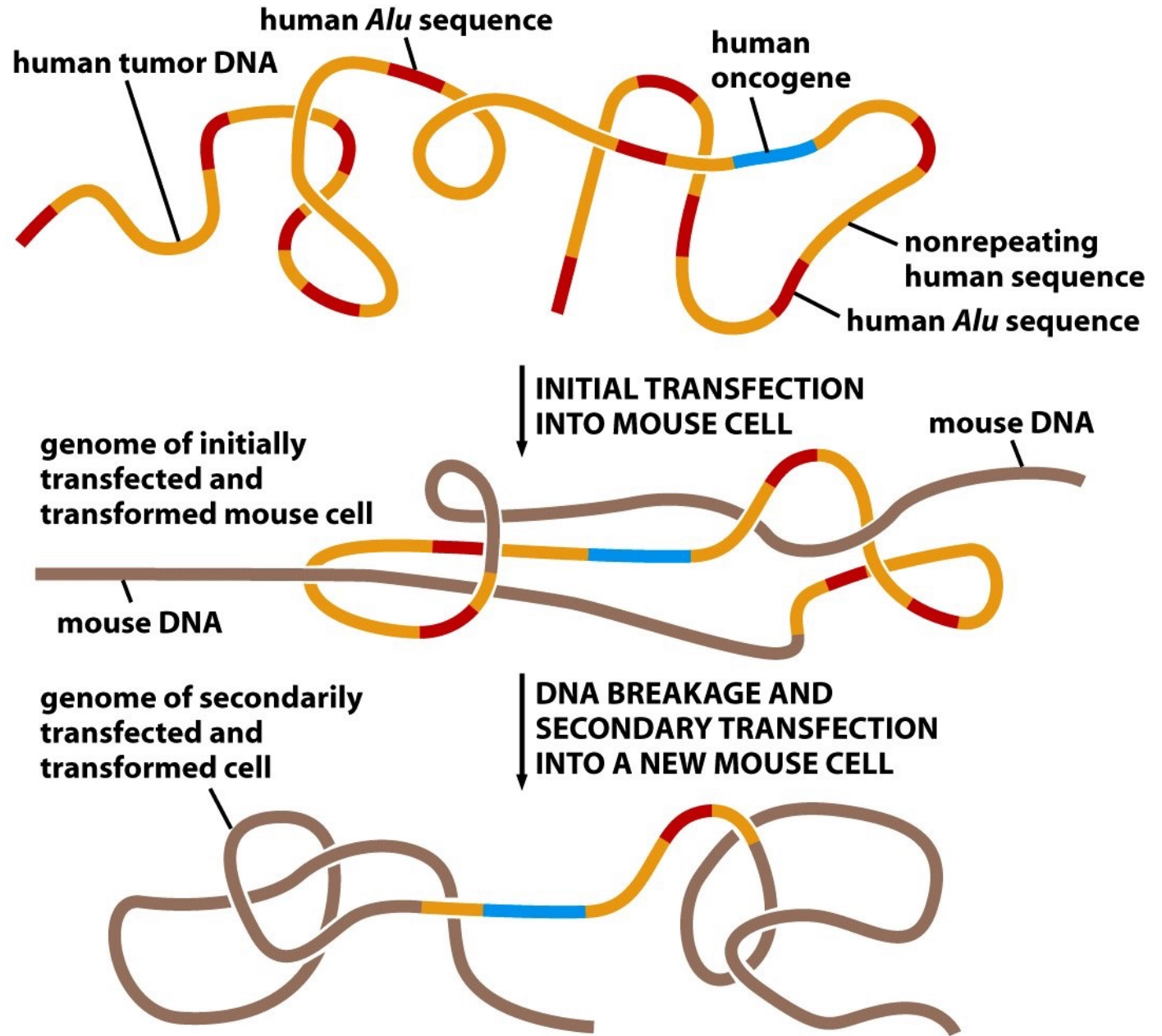


Figure 4.2 The Biology of Cancer (© Garland Science 2014)

Figure 4.1 The Biology of Cancer 2023

Cloning of Transfected Human Oncogenes

(DNA from bladder carcinoma cells)



Mutation Responsible for H-ras Oncogene Activation

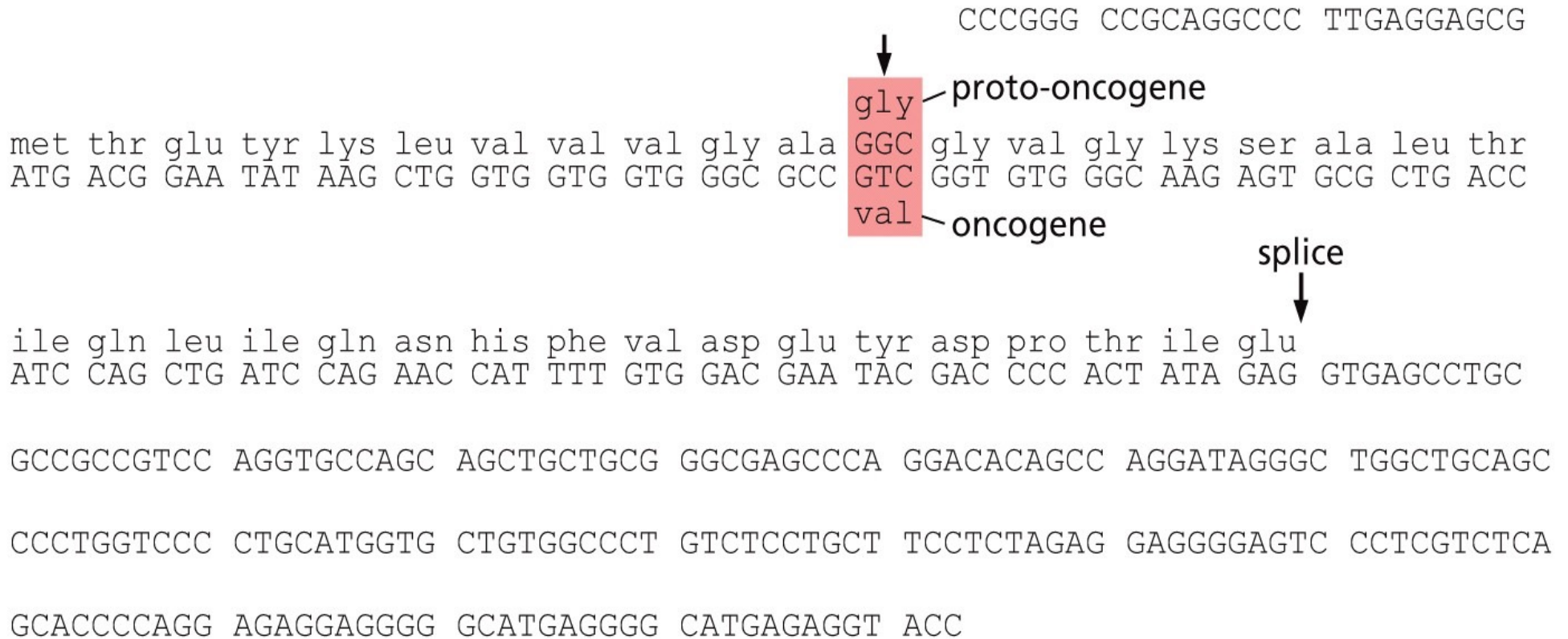


Figure 4.5 The Biology of Cancer 2023

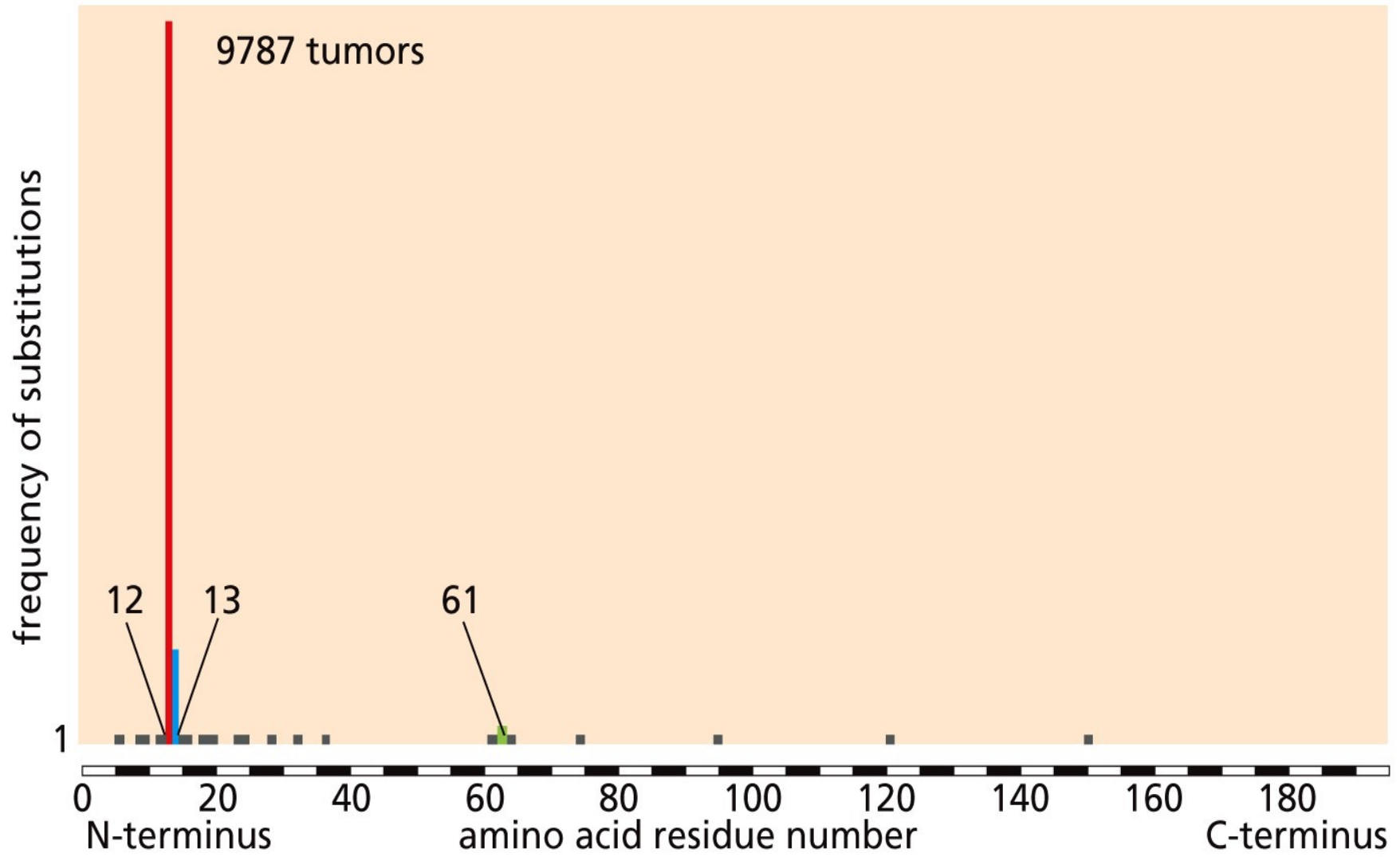


Figure 4.10 The Biology of Cancer (© Garland Science 2014)

Amplification of the *erb2/neu/HER2/ERBB2* Oncogene in 30% of Breast Cancer

Erb2/Neu: membrane surface-bound receptor tyrosine kinase; drives the cell cycle; protects from apoptosis

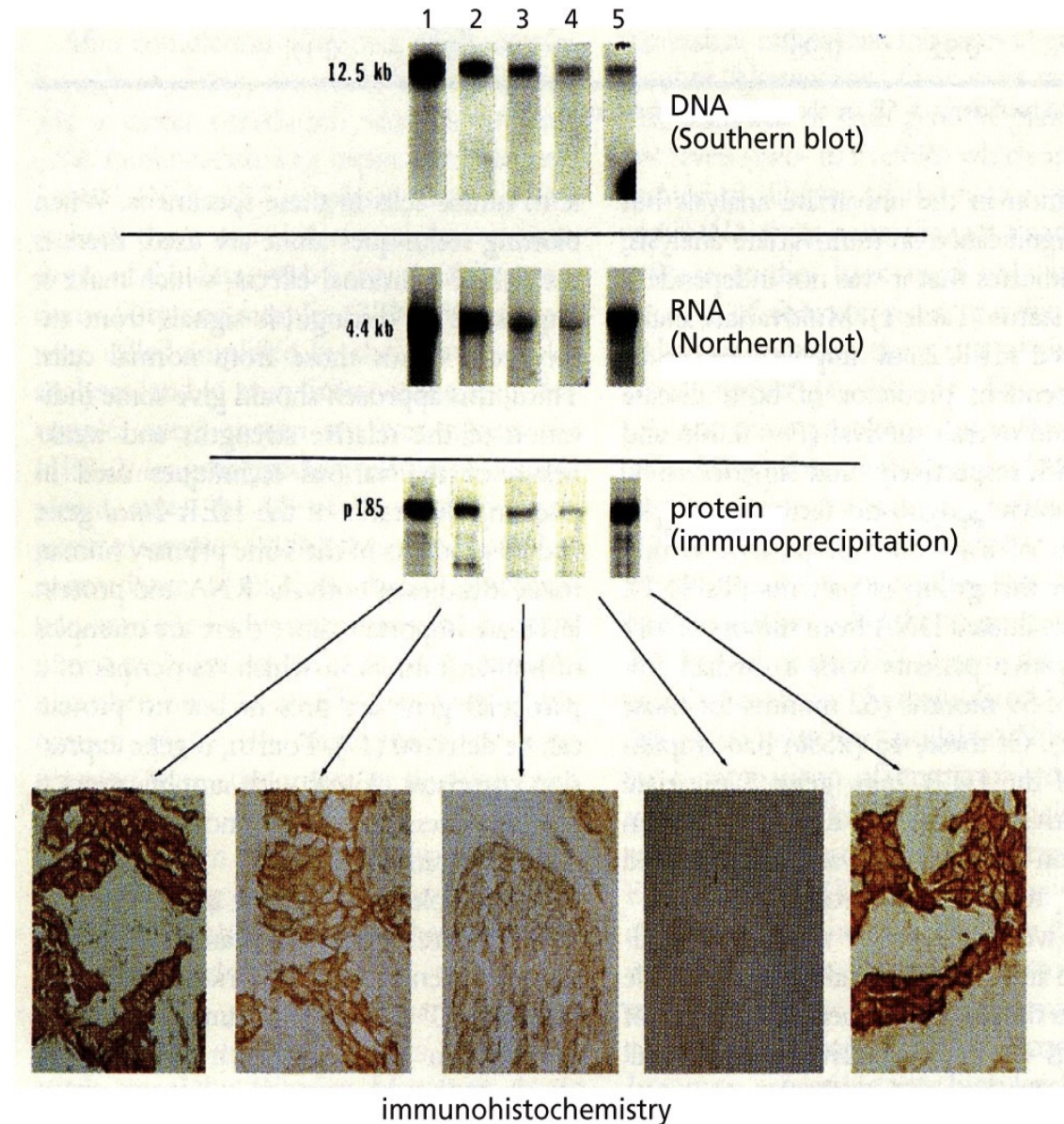


Figure 4.3 The Biology of Cancer 2023

Kaplan-Meier Plot: Relapse after Diagnosis and Treatment

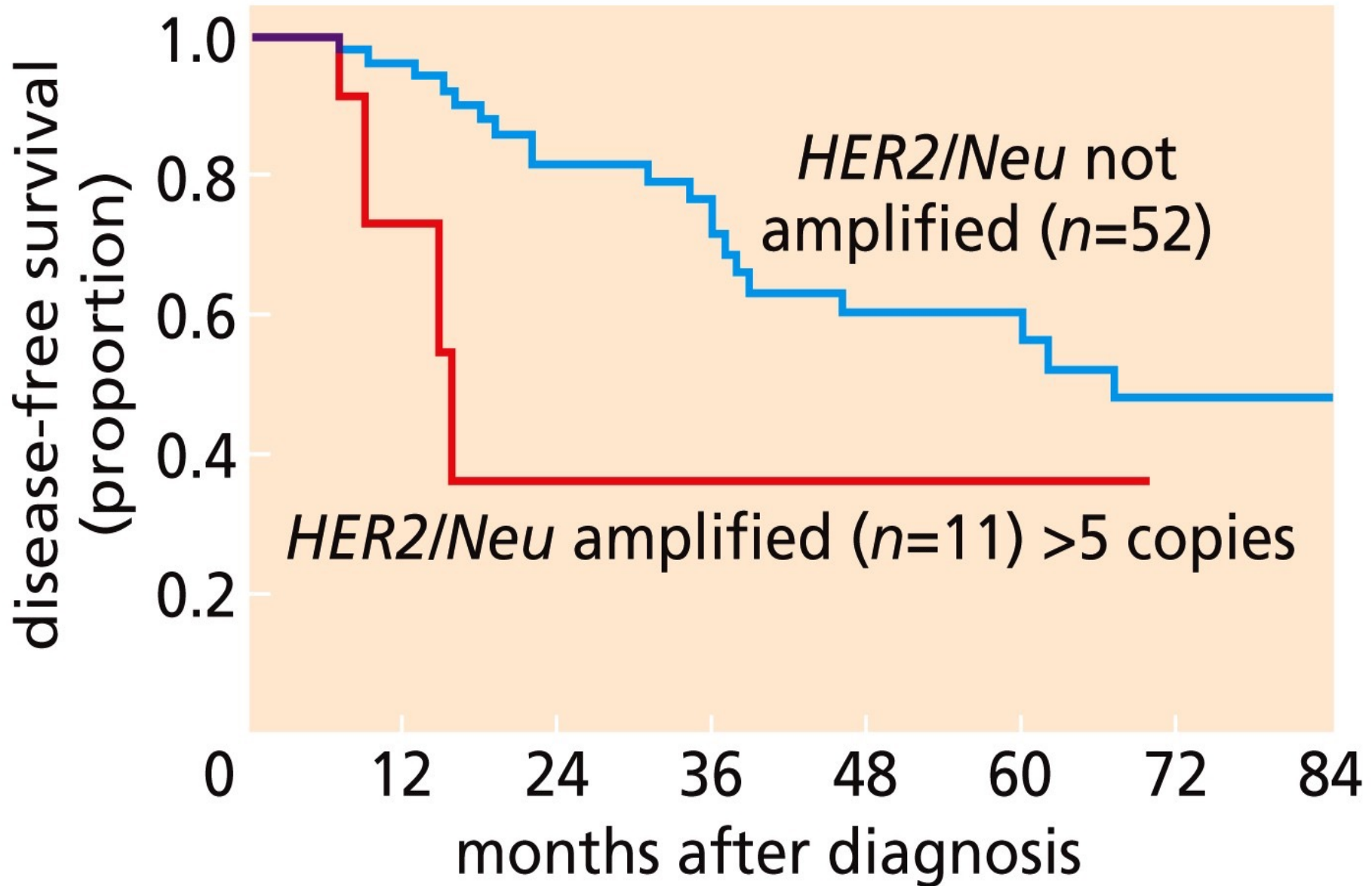
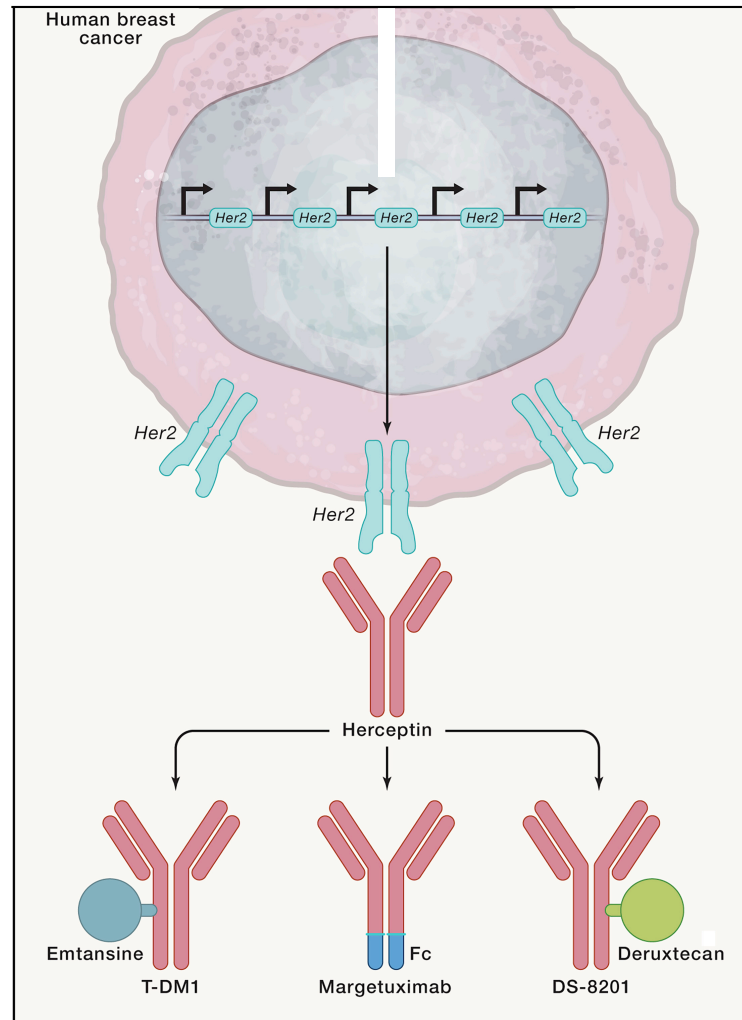


Figure 4.1 The Biology of Cancer 2023

Monoclonal Antibodies (“Herceptin”) Against HER2 for Treatment of Breast Cancer



From Sawyers Cell 179, 8 (2019)

N-myc Amplification in Pediatric Neuroblastoma

The gene is detected by fluorescence in situ hybridization (FISH) with fluorescent dye-labeled N-myc specific oligonucleotide probes.

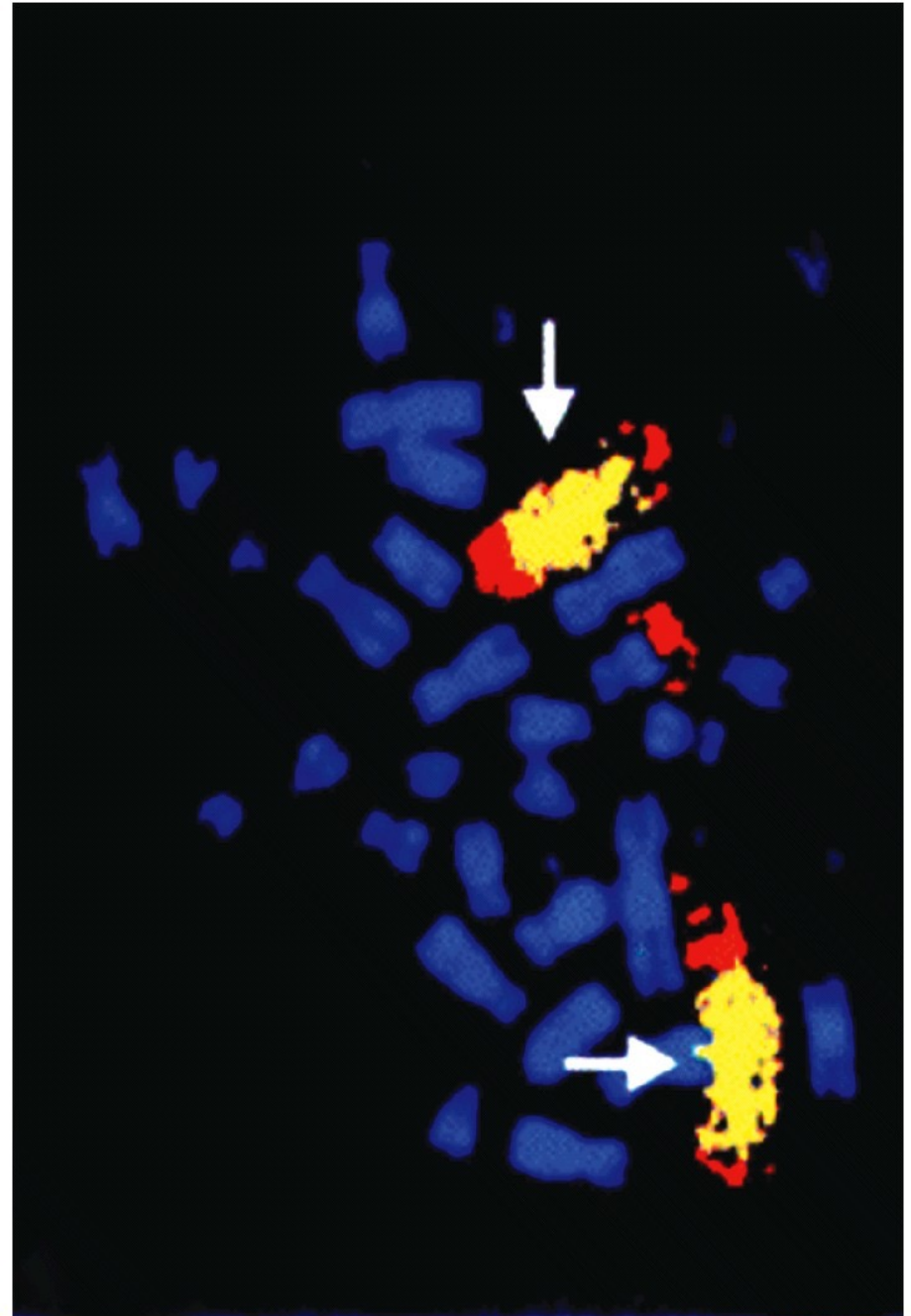


Figure 4.7 The Biology of Cancer 2023

Kaplan-Meier Plot: Event-Free Survival of Children Suffering from Neuroblastoma

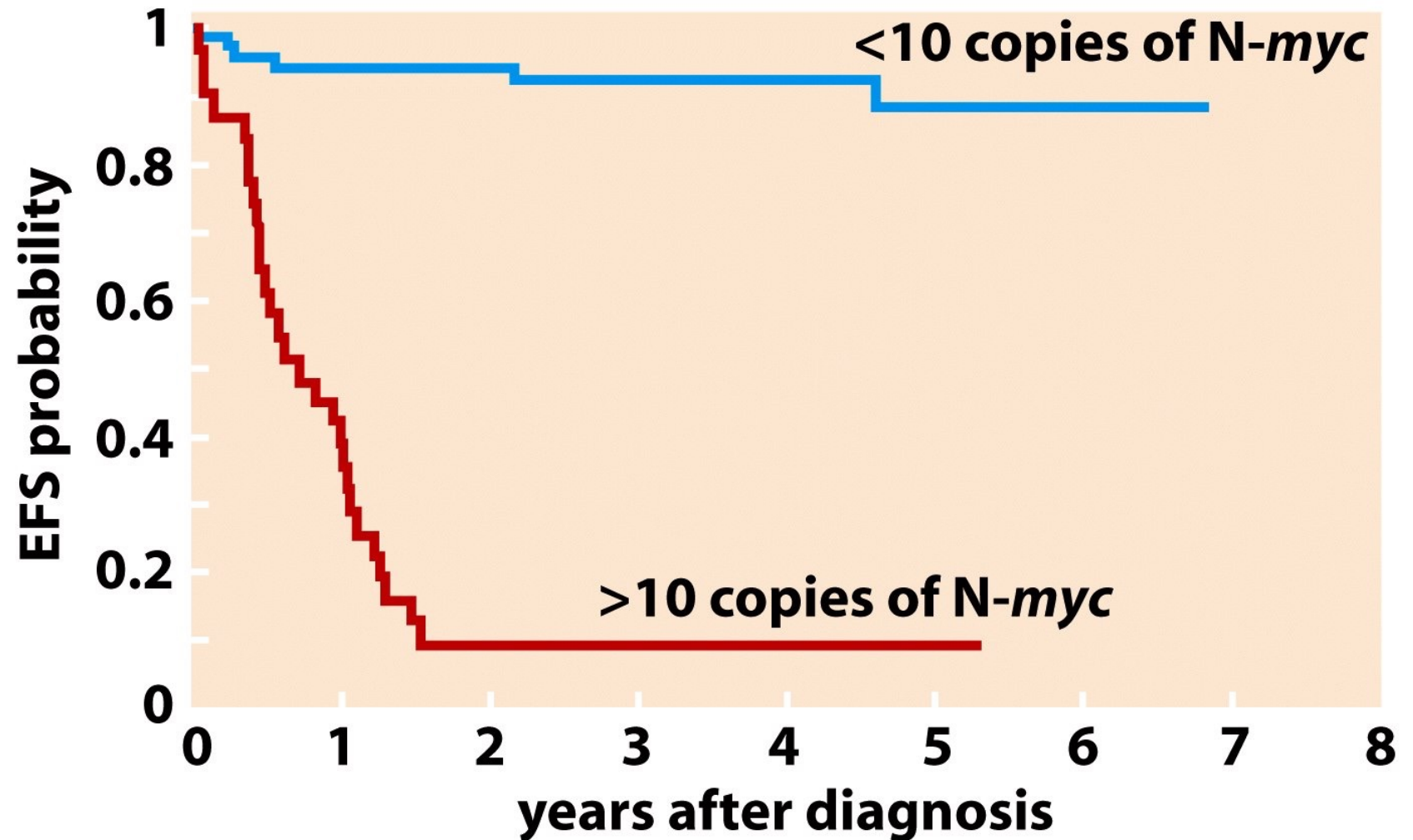


Figure 4.7 The Biology of Cancer 2023

Burkitt's Lymphoma: Translocation Brings *c-myc* Gene under Control of an Ig Gene

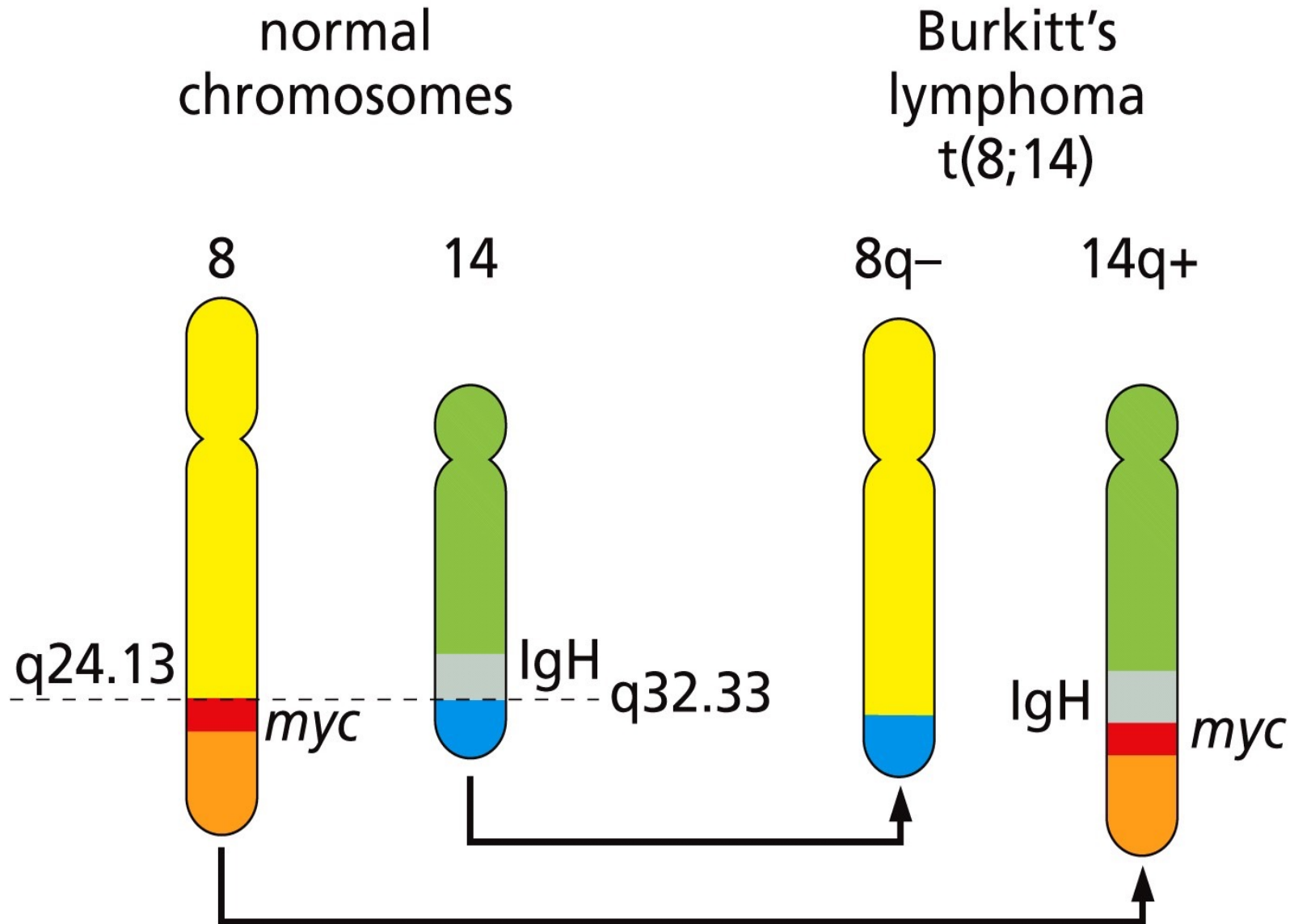


Figure 4.8 The Biology of Cancer 2023

Reciprocal Translocation

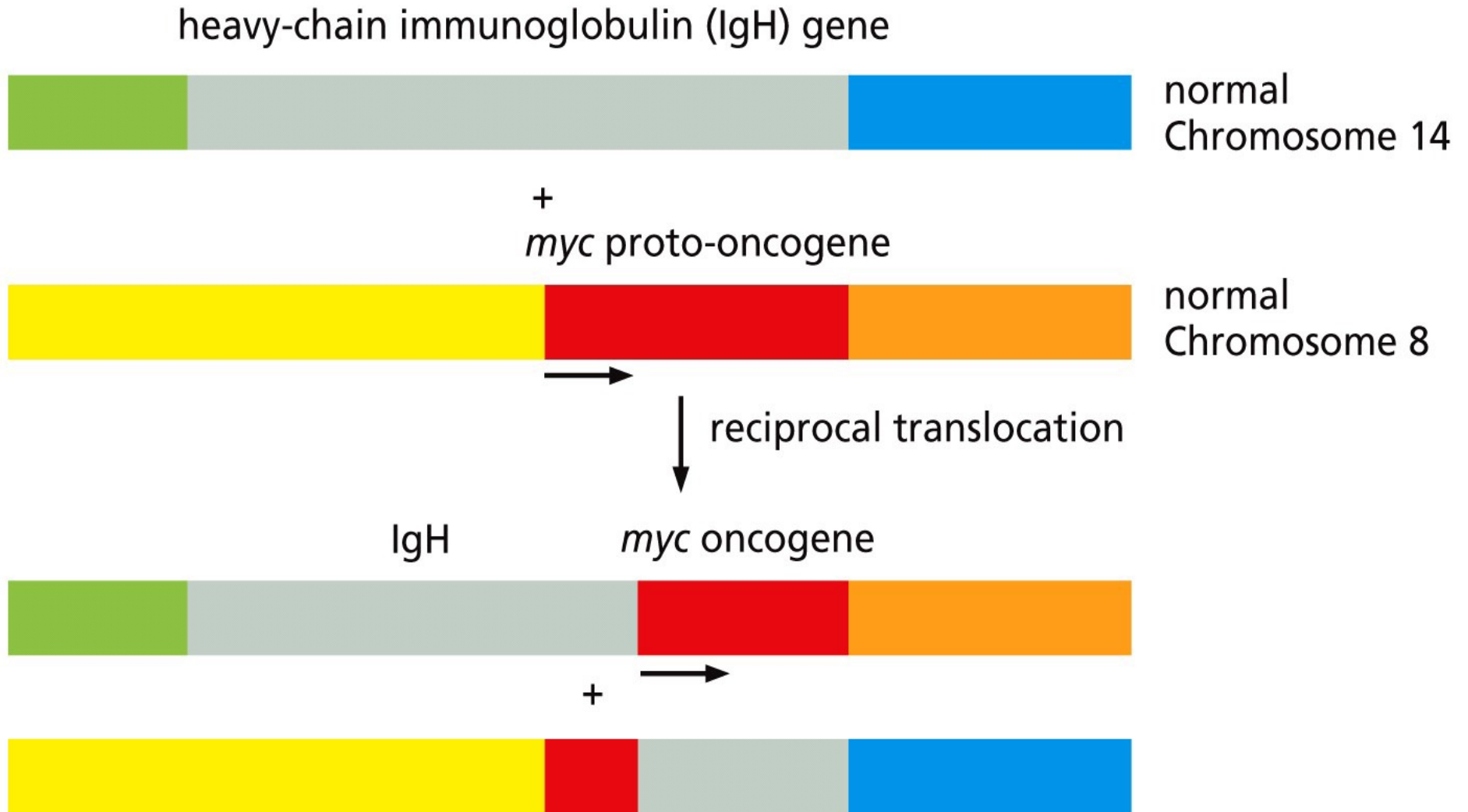
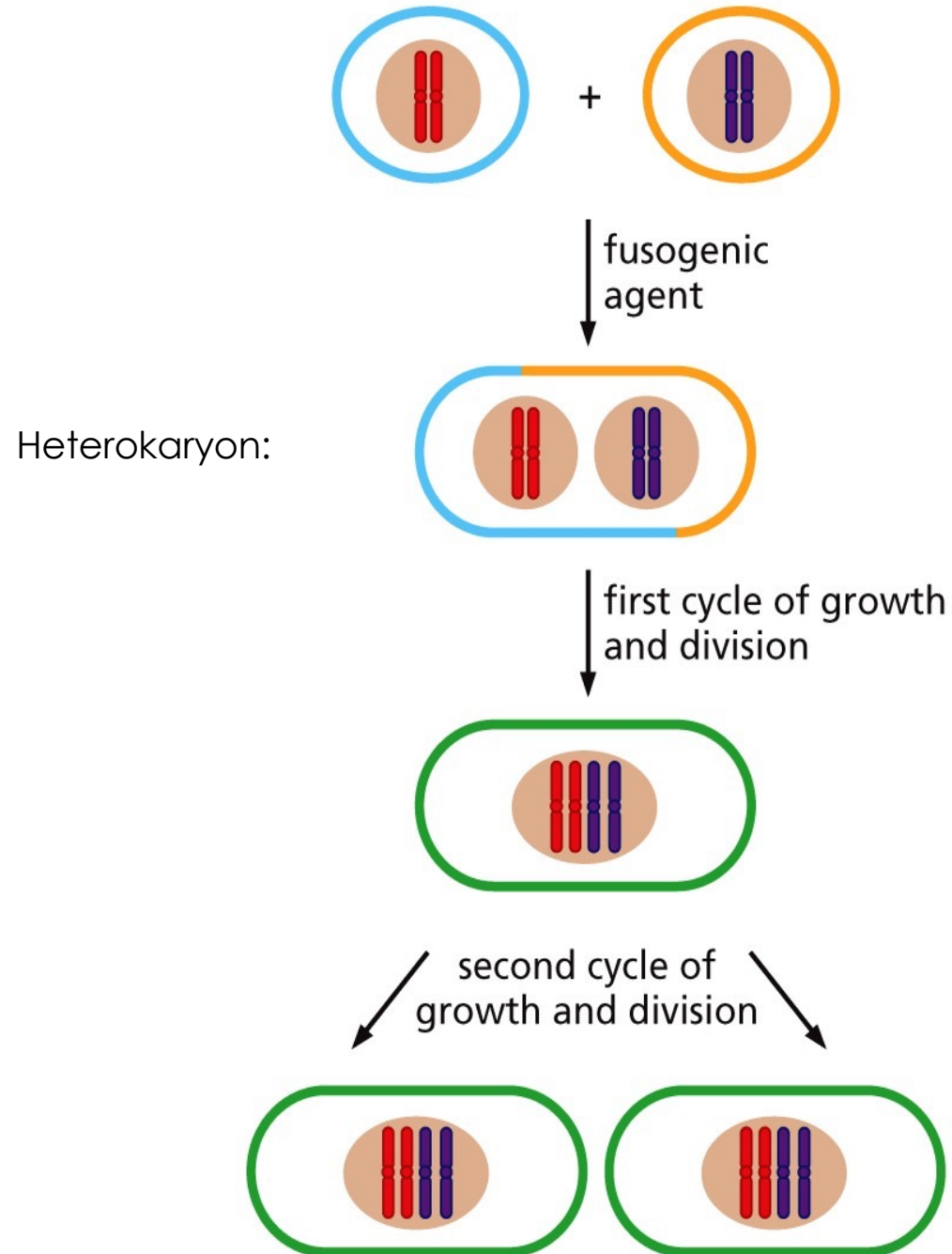


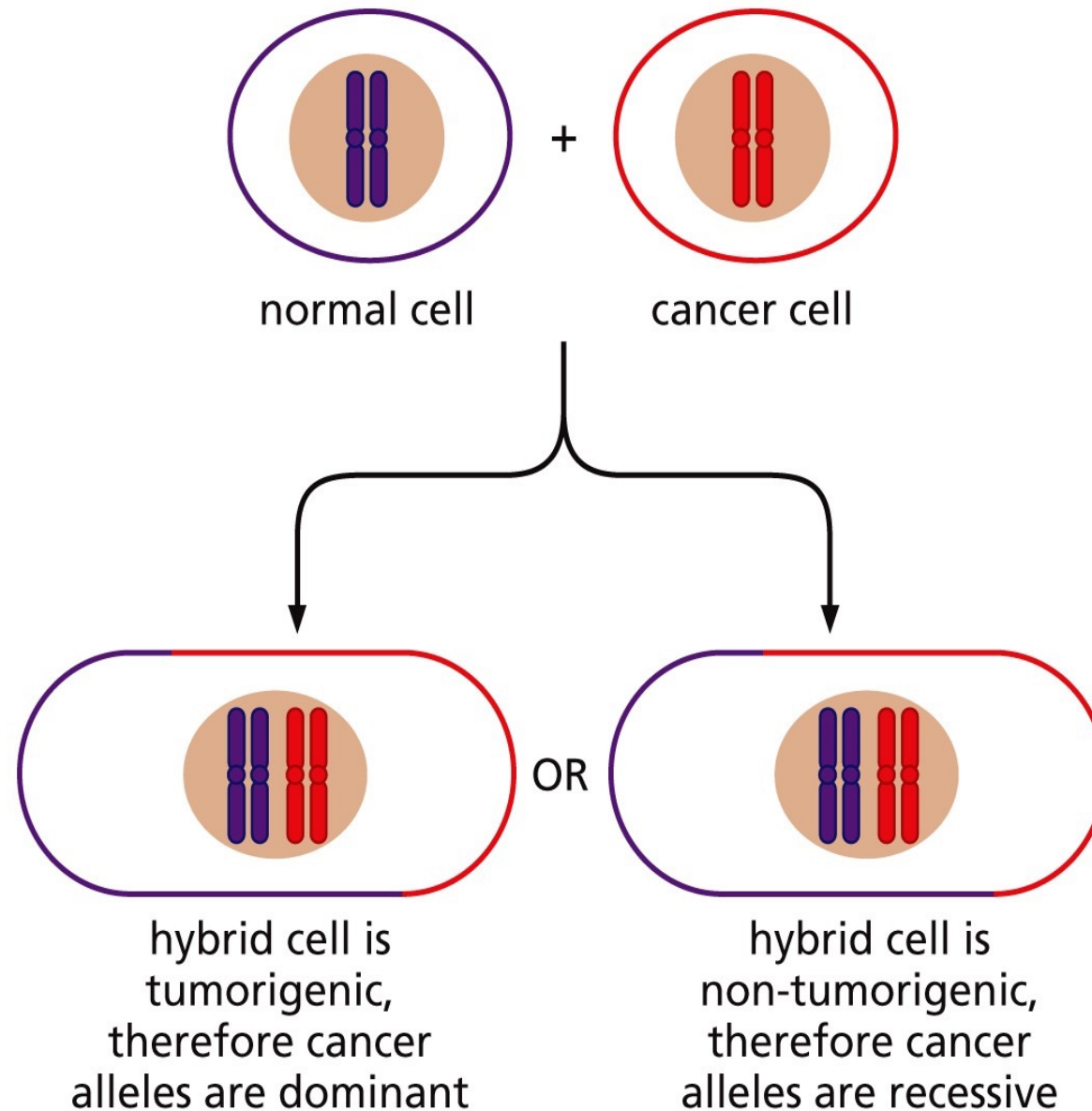
Figure 4.8 The Biology of Cancer 2023

Tumor Suppressor Genes

Experimental Fusion of Cells



Dominance and Recessiveness of the Tumorigenic Phenotype



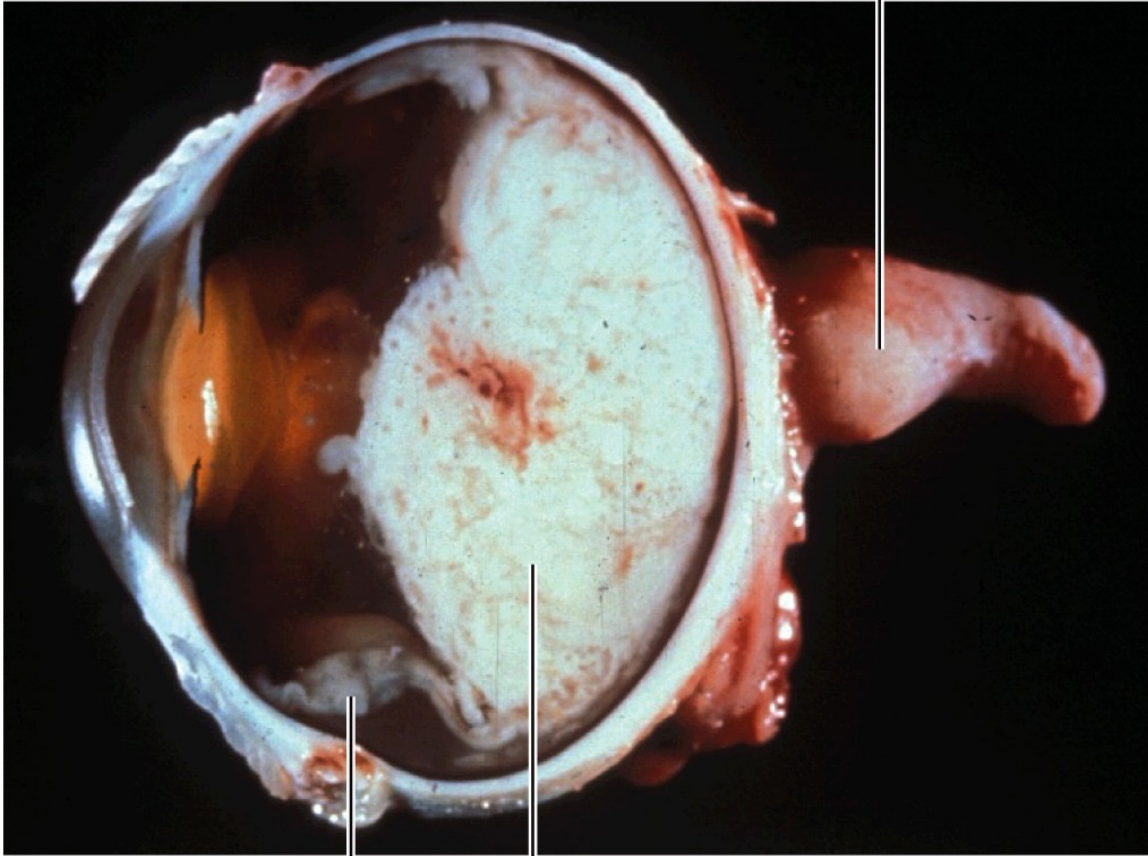
Tumor Suppressor Genes

Genes whose partial or complete inactivation, occurring in either the germ line or the genome of a somatic cell, leads to an increased likelihood of cancer development

- Gatekeeper genes - operate to regulate cell proliferation or to regulate cell number by controlling cell differentiation or cell death. Loss of a gatekeeper gene removes an impediment to cell proliferation and thus to the appearance of populations of neoplastic cells
- Caretaker genes - encode a proteins that maintain the integrity of the genome and thereby prevent the accumulation of mutations and, in turn, the formation of neoplastic cells

Retinoblastoma: Develops from Retina Cell Precursor

thickening of optic nerve
due to extension of tumor



displaced
normal
retina retinoblastoma

Affects 1/20' 000 children

Figure 7.2 The Biology of Cancer 2023

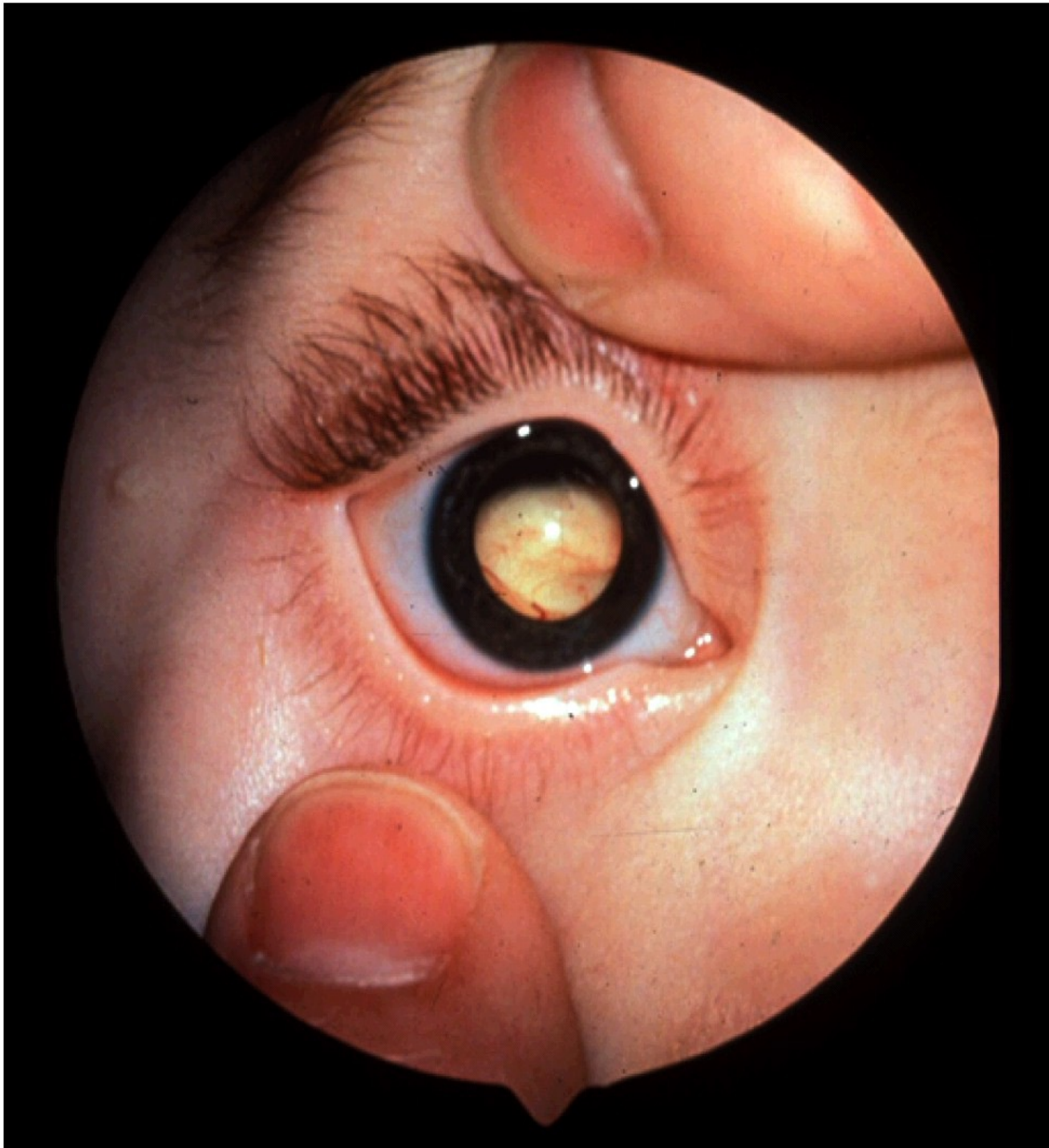


Figure 7.2 The Biology of Cancer 2023

Dynamics of Retinoblastoma Formation

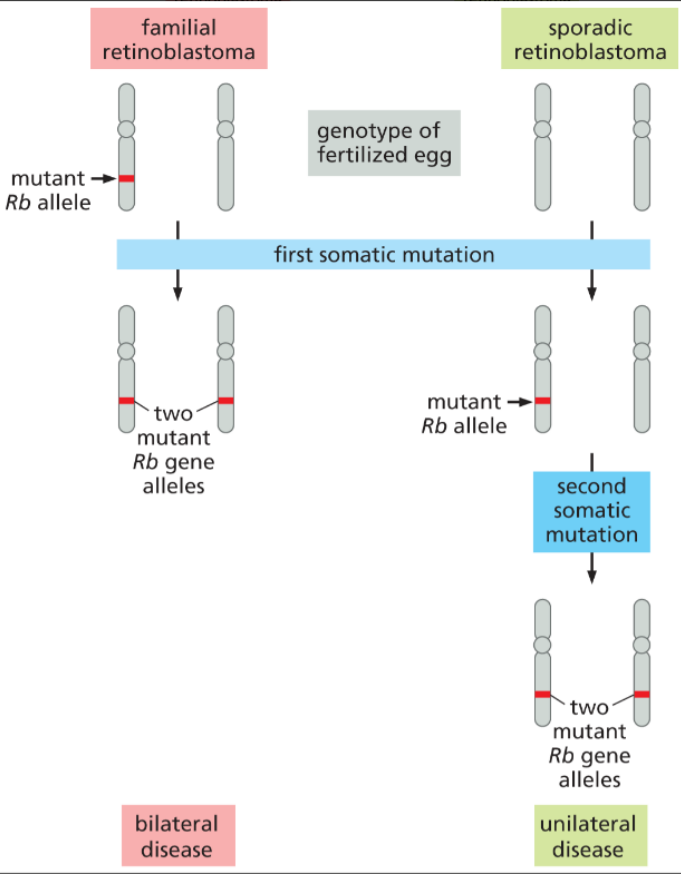


Figure 7.5 The Biology of Cancer 2023

Loss of Heterozygosity (LOH)

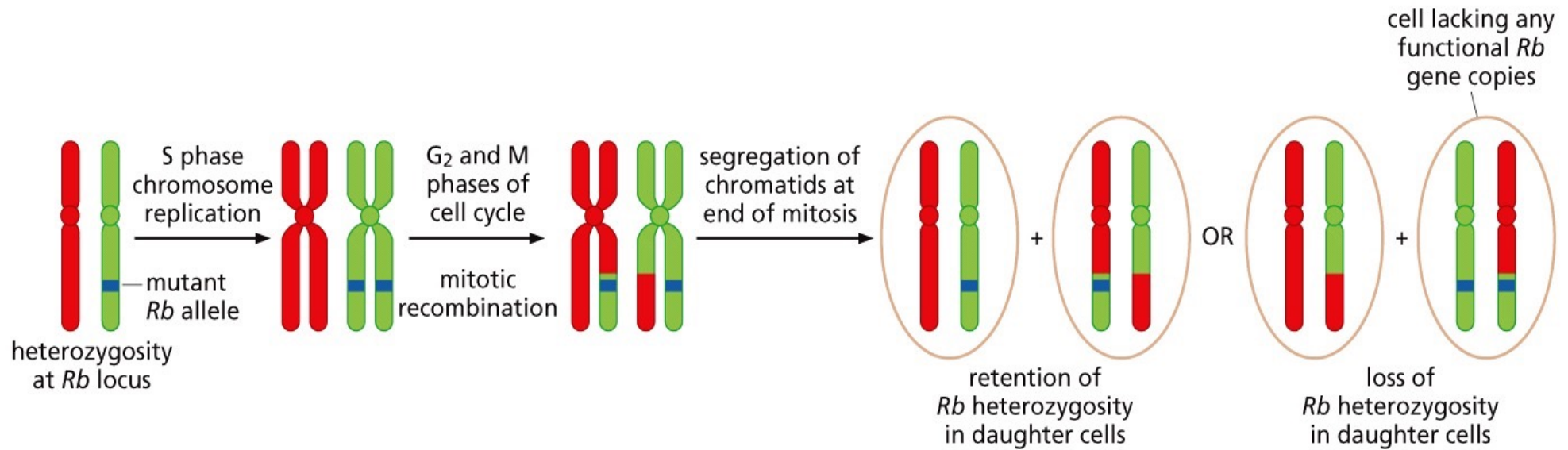
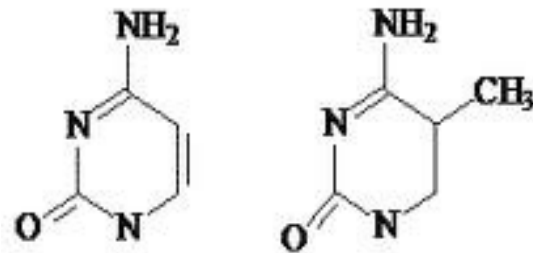
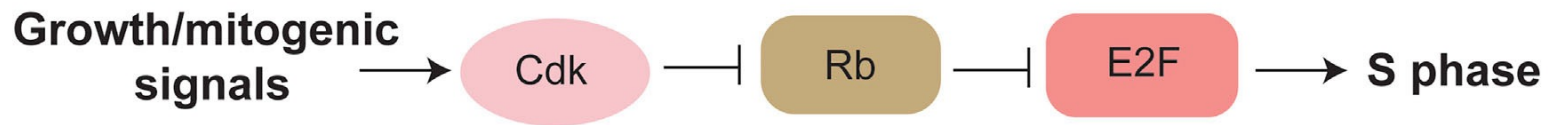


Figure 7.6 The Biology of Cancer 2023

- Nondisjunction: LOH through loss of an entire chromosome
- Promoter methylation can also lead to inactivation of tumor suppressor genes (MeCpG)





Cdk: cyclin-dependent kinases; phosphorylate Rb leading to Rb-dissociation from E2F transcription factor.

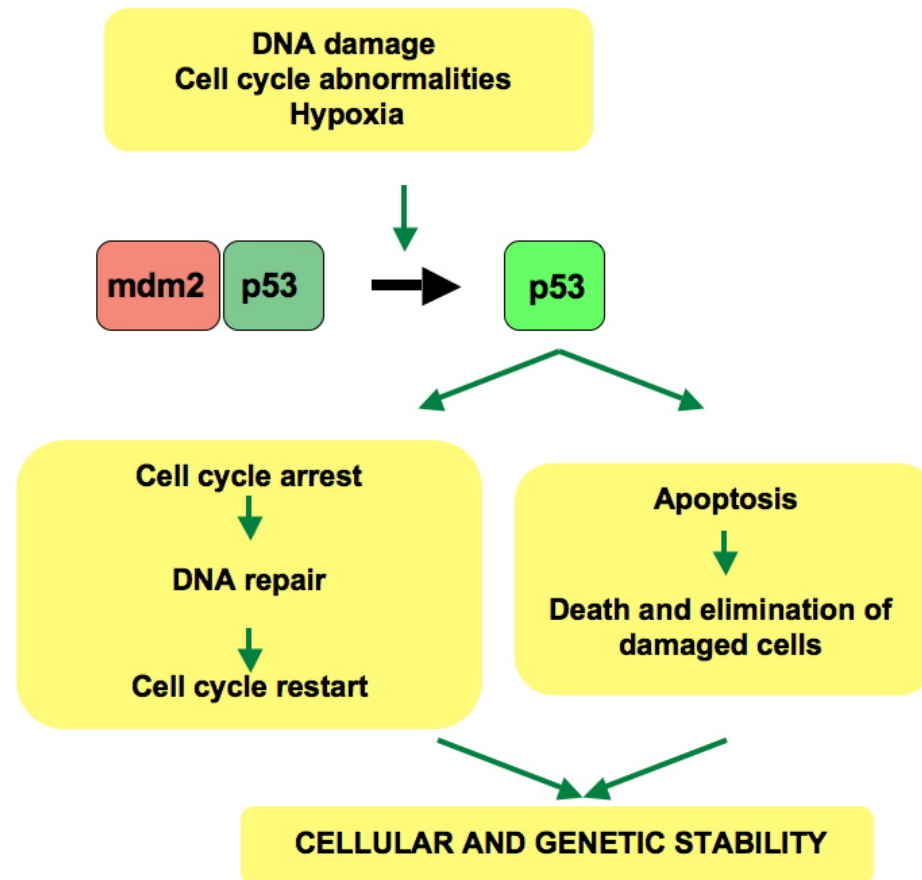
Rb: Retinoblastoma protein; binds and inhibits E2F when hypophosphorylated.

E2F: transcription factor inducing genes that are required for the G1 → S transition.

- Tumor suppressor gene: suppress increases in cell number, either by suppressing proliferation or by triggering apoptosis. Often called gatekeepers as opposed to the so-called caretakers, which work to maintain the integrity of the genome.

Mutations in oncogenes are generally dominant, whereas mutations in tumor suppressor genes are recessive (p53 is a famous exception!).

p53: Master Guardian



p53 is a Transcription Factor

More than 300 target genes

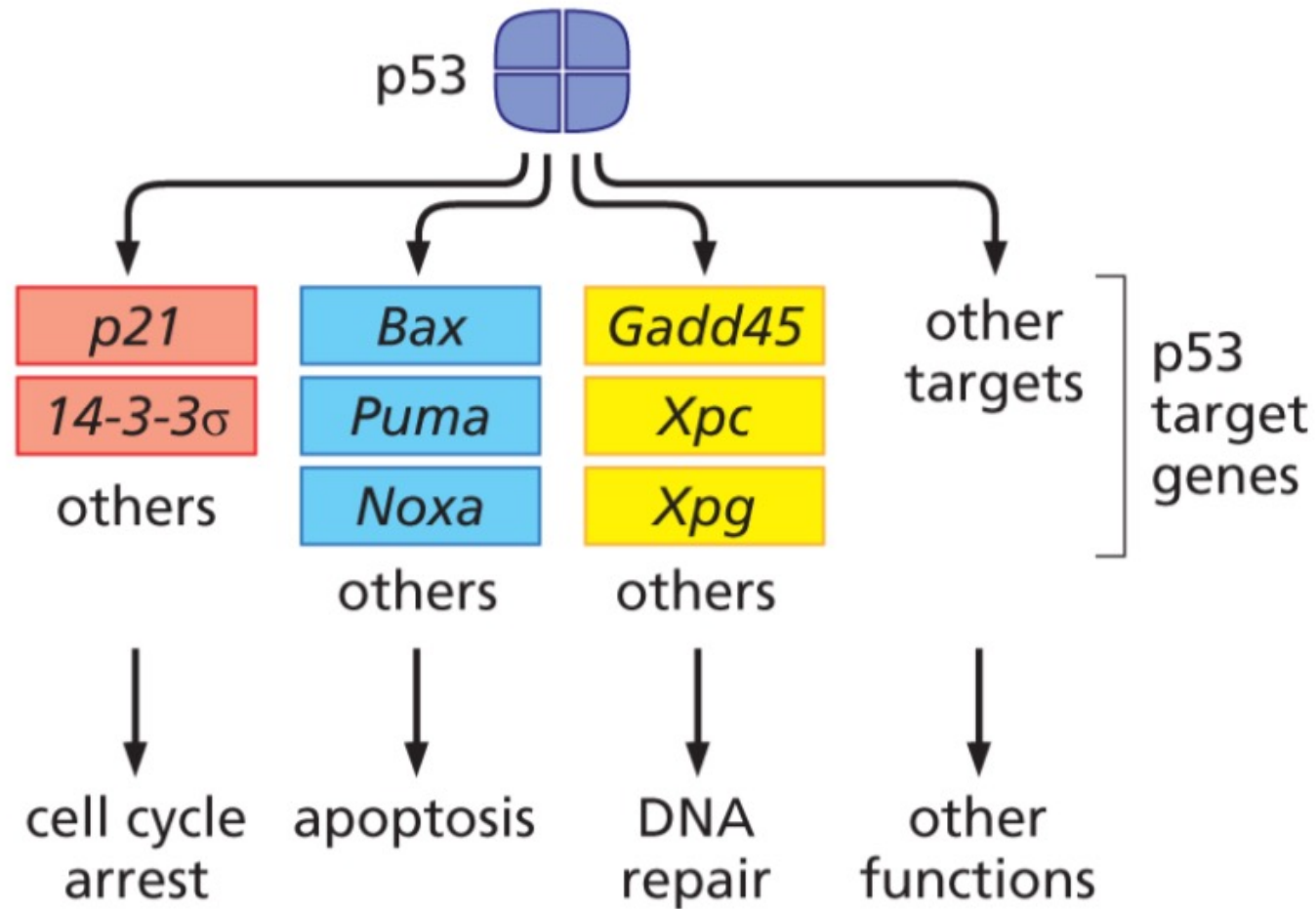


Figure 9.15. Weinberg, The Biology of Cancer

Consensus DNA Sequence Bound by p53

- Analyzed 1546 sites; consensus sequence: relative size of letter indicates frequency of DNA base at the position

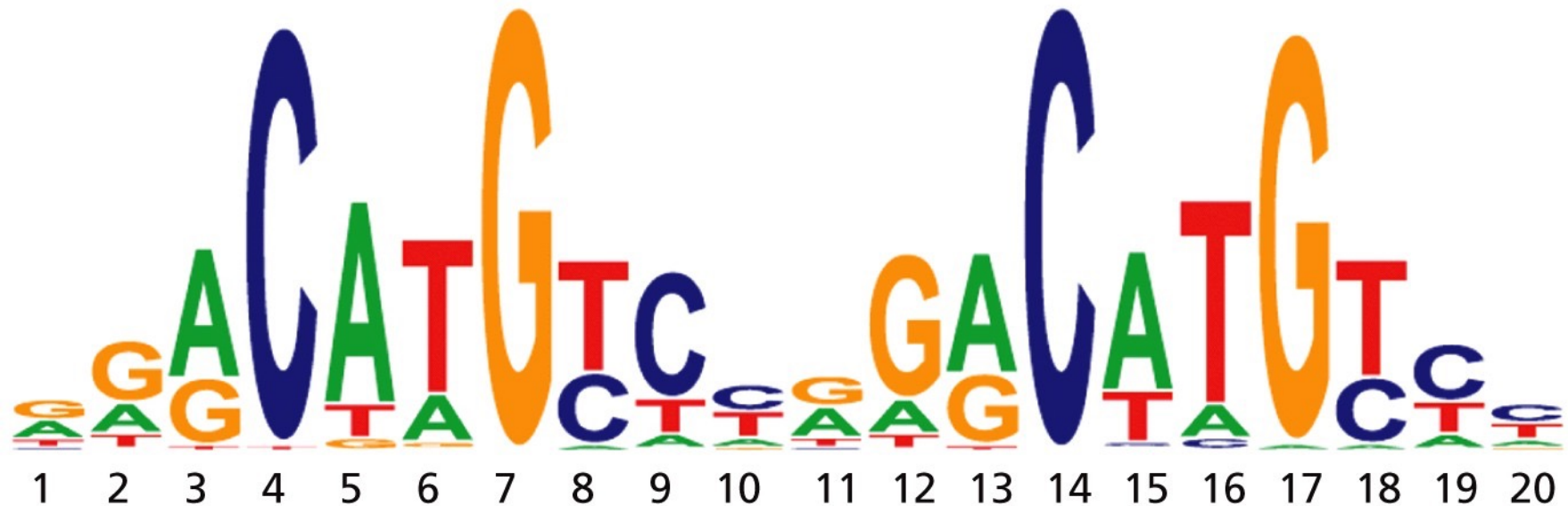


Figure 9.15 *The Biology of Cancer* 2023

TP53 is the most frequently Mutated Gene in Cancer

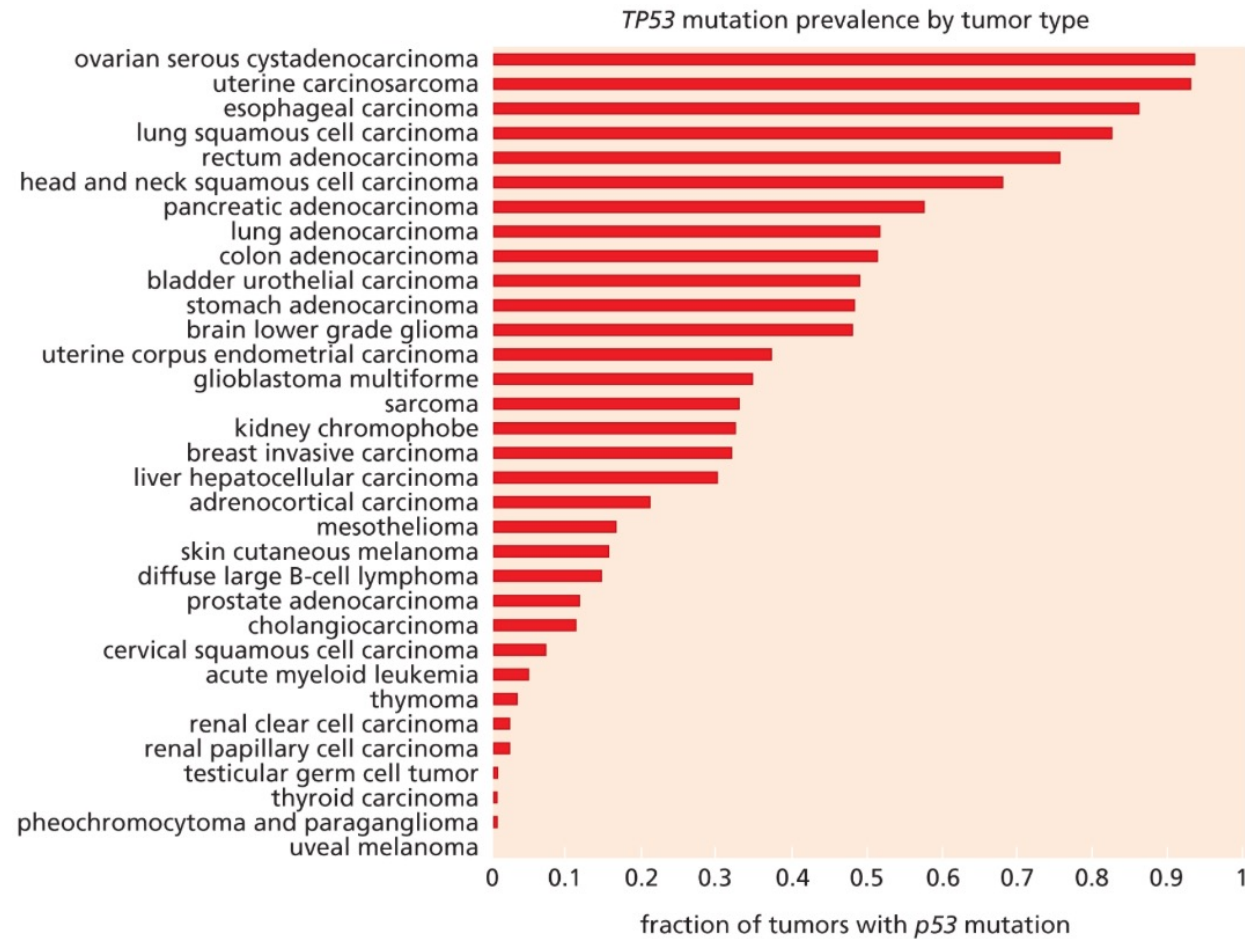


Figure 9.3 The Biology of Cancer 2023

Effects of Mutant p53 Alleles in the Mouse Germ Line

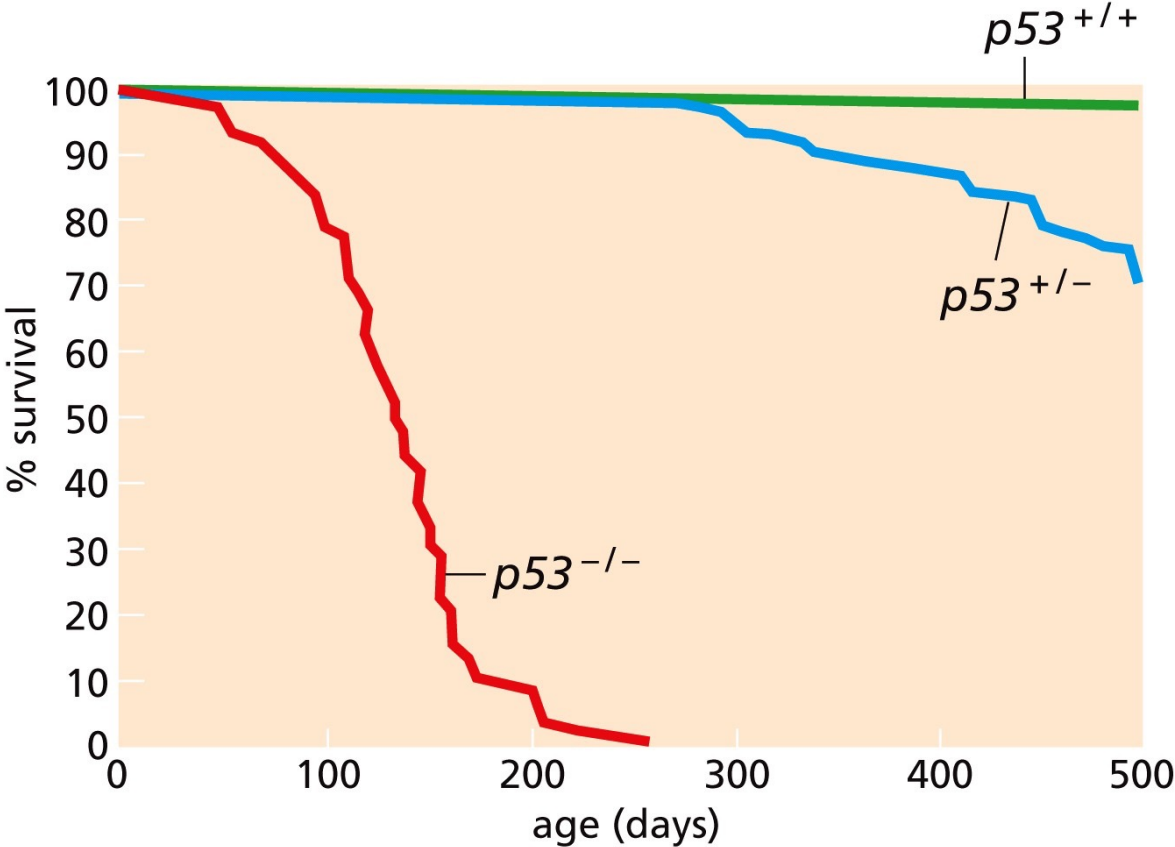


Figure 9.4 *The Biology of Cancer* 2023

Most of the Mutations in p53 are Missense

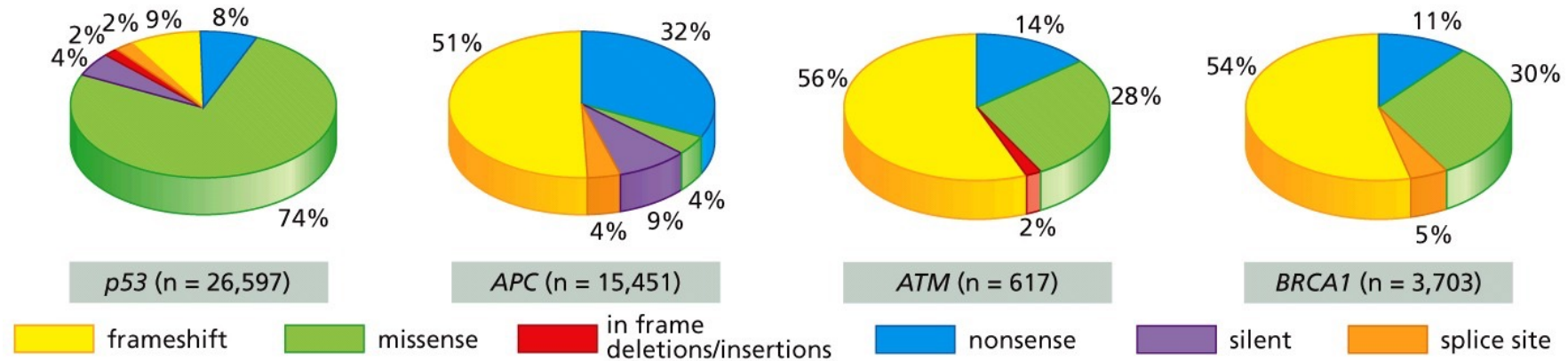


Figure 9.5 The Biology of Cancer 2023

p53: Binds DNA as a Tetramer

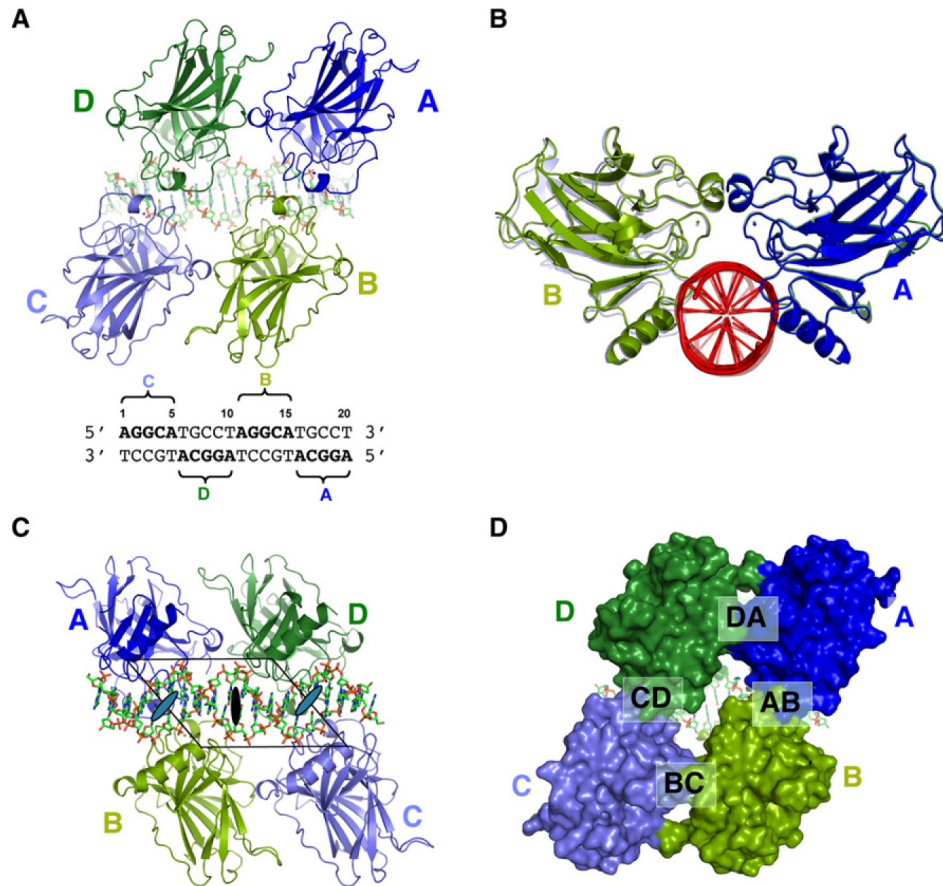


Figure 1. Overall Structure of the p53 Core Domain Bound to DNA as a Tetramer

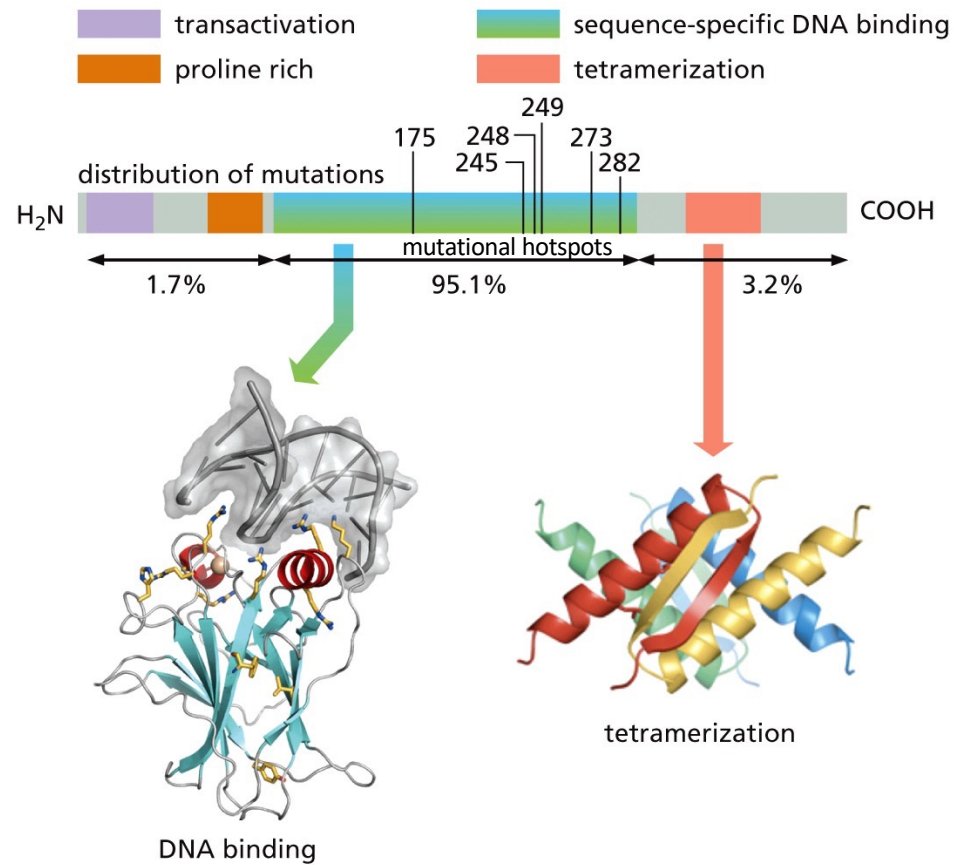
(A) The tetramer viewed from the protein side. The four monomers are colored in blue (A), light green (B), light blue (C), and green (D). The same color scheme is used throughout the illustration unless indicated otherwise. The DNA is in stick model with its sequence shown below. The four pentameric motifs (quarter site) and their corresponding monomers are indicated in the sequence.

(B) A view of the tetramer along the DNA axis. This view shows that the tetramer has a planer structure wherein the A-B dimer (front) and C-D dimer align almost perfectly along the DNA axis.

(C) The tetramer viewed from the DNA side. The parallelogram is shown together with the global two-fold axis (dark oval) and the two local dyad axes (gray ovals).

(D) A surface model of the tetramer view in the same orientation as (A). The four protein-protein interfaces are indicated.

Most of the Missense Mutations in p53 Affect DNA-binding Domain



Dominant-Negative Mutations in p53

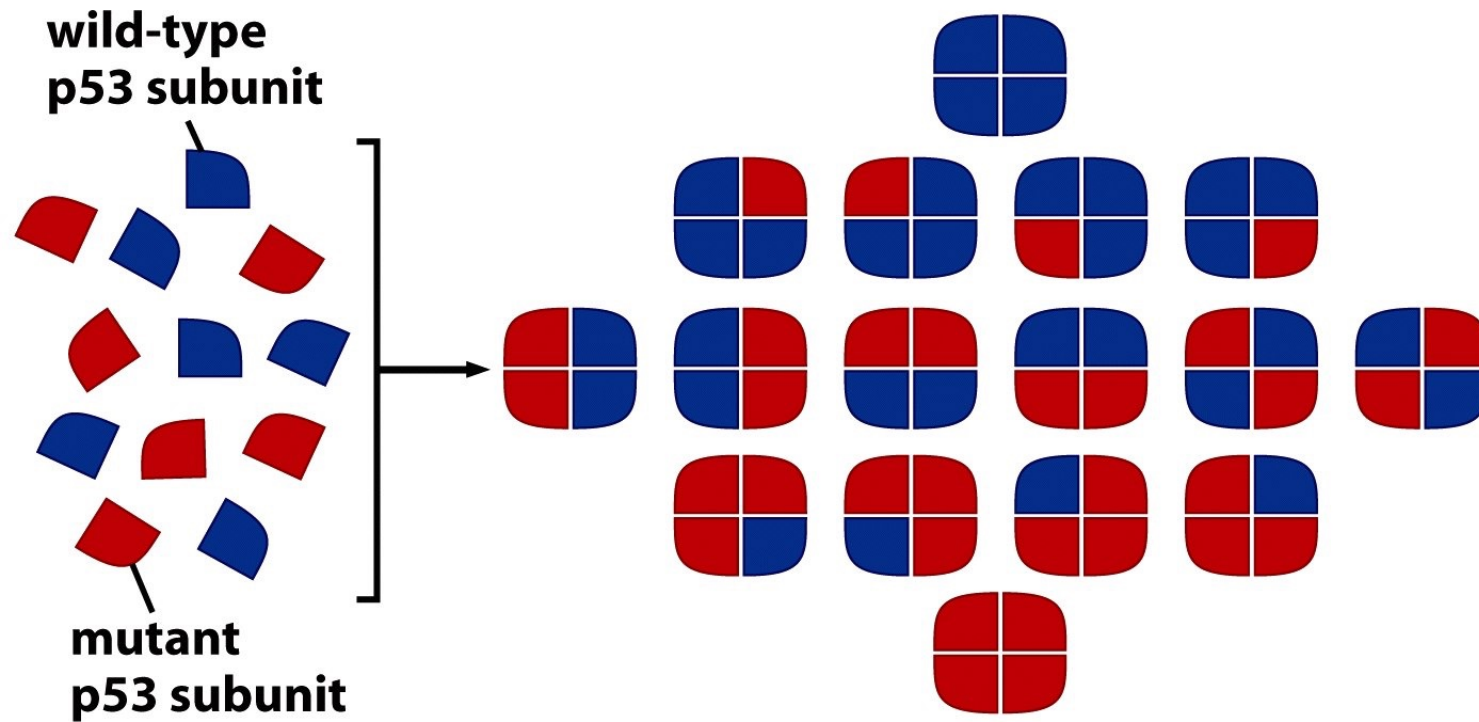
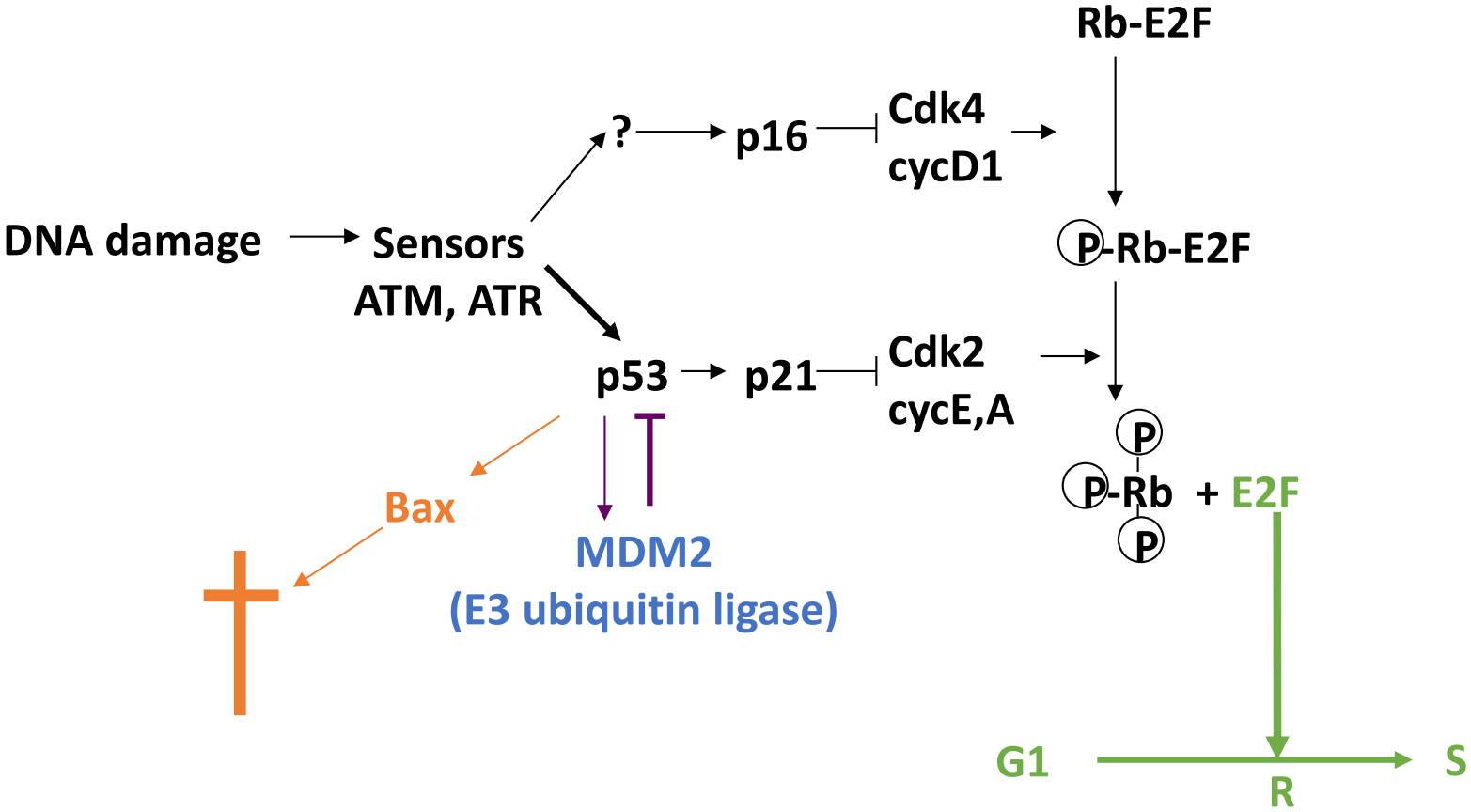


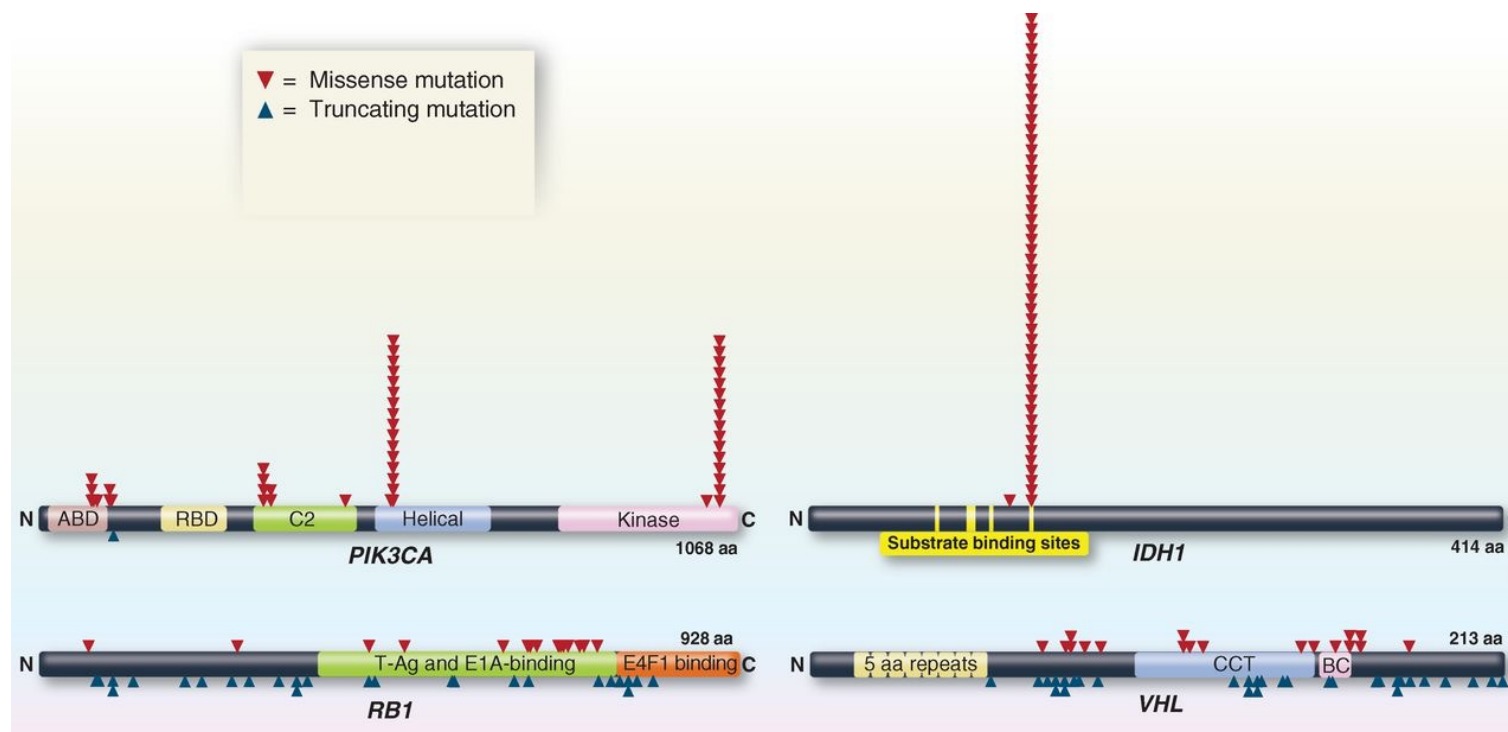
Figure 9.5 *The Biology of Cancer*

Illustration of dominant negative effect of p53 missense mutations in myeloid malignancies: Boettcher et al., *Science* **2019** 365: 599-604

Activation of p53 Upon DNA Damage



Distribution of Mutations in two Oncogenes and two Tumor Suppressor Genes



20/20 Rule

- Oncogene classification: >20% of recorded mutations are at recurrent positions and are missense
- Tumor suppressor gene classification: >20% of the recorded mutations in the gene are inactivating
- *TP53*: Oncogene score: 73%; TSG score: 20%
but classified as TSG because well-studied oncogenes rarely harbor premature stop codons

B Vogelstein et al. Science (2013);339:1546-1558

Key Concepts

- An oncogene is a mutated gene that contributes to the development of a cancer. In their normal, un-mutated state, oncogenes are called proto-oncogenes.
- Tumor suppressor genes, originally called antioncogenes, function to suppress the development of cancerous growth.
- Retinoblastoma protein (pRb) is a tumor suppressor protein that becomes dysfunctional in many types of cancer. In its active state, pRb is hypo-phosphorylated and able to act as a tumor suppressor by inhibiting cell cycle progression.

Key Concepts

- p53 is a transcription factor. As such, p53 can induce cell cycle arrest or apoptosis
- p53 turnover is blocked during cell-physiologic stress or DNA damage
- p53 mutations that occur in cancer are often dominant-negative
- p53 protein levels are controlled: to be discussed in detail later in the context of apoptosis! (week 4)

Exercise

Please form groups at: <https://framadate.org/hlzLhp9ckxDmLpCC>

BRCA2, TERT, RB, TP53, KRAS, ATM, ERBB2, RET

Explore www.cbioportal.org ; **quicksearch** your gene, check out **Cancer types summary, Mutations**

Also go to <https://www.oncokb.org/> and find out about possible drug treatments if available.

- Check out the mutation spectrum occurring in tumors in a given gene.
- Is there any pattern? What do you conclude? Is it a tumor suppressor or an oncogene?
- Do you expect that the mutations are dominant or are they recessive?
- Are there drug treatment options?