Oncogenetics

Clinical genetics

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Neoplasm (a reminder) basic facts :

- **overproliferating group of cells (neoplasm, cancer)**
  - **autostimulation of cell division**
  - **decreased sensivity to growth-inhibition signals**
  - **crippled apoptosis**
  - **induction of angiogenesis**
- **cell migration:**

migration:	**no**	↔	**local (invasion)**	↔	**distant (metastasis)**
tumor:	*benign*	↔		↔	*malignant*

Neoplasms – pathomorphologic classification:

Benign

Malignant

- **from epithelial tissue – carcinoma**
- **from connective tissue – sarcoma**
- **from lymphocyte precursors – lymphoma**
- **from leukocyte precursors – leukaemia**
- **from glial cells – glioma and alikes**
- **from fetal tissue - hamartoma**

Etiology of neoplasms: cells, chromosomes

- **abnormal cells**
  - **in a blood of leukemic patient** *(Virchow 1845, Bennett 1845)*
  - **in a bone marrow of leukemic patient** *(Neumann 1855)*
- **chromosomal abnormalities**
  - **abnormalities of mitotic spindle** *(Hanseman 1890)*
  - **neoplasm originates from a single cell with chromosomal abnormalities**

Etiology of neoplasms: viruses

- **Rous and epidemic chicken neoplasm**
  - **tissue homogenate transmits neoplasm**
  - **filtration does not prevent**
  - **cause is an 'oncogenic' virus** *(1911; Nobel 1966)*
- **isolation of RSV virus (retrovirus)**
  - **neoplasms are virally transmitted?**
  - **'oncogenic' viruses transfer oncogenes - first discovery: *src* from RSV** *(Duesberg 1970, Wang 1976);* **neoplasms are 'genetic' diseases?**
  - **the oncogene itself is sufficient, virus is dispensable**

Etiology of neoplasms: heritable susceptibility (1)

- **Lynch syndrome**
  - **1913 – Aldred Warthin**
    a case of his tailor, who succumbed to depression, convinced that she would inevitably perish to cancer of reproductive organs or colon, as everybody in her family – indeed, she soon died of uterine cancer
  - **1962 – Henry Lynch**
    his alcohol-addicted patient explained that he would not stop drinking, because his fate is dim – all his relatives are dying of cancer, mostly colorectal. He actually died soon of adrenal neoplasm.

Lynch described two such families in 1966.

Etiology of neoplasms: environmental factors

- **some environmental factors may cause cancer**
  - **scrotum cancer in chimney sweepers** *(Pott 1775)* **– contact with soot**
  - **lung cancer in smokers** *(Müller 1939, Wynder 1950)*
  - **skin cancer and ultraviolet – confirmed in mice studies**
- **these environmental factors are mutagenic**
  - **soot contains polycyclic aromatic hydrocarbons (PAHs) known to damage DNA**
  - **cigarette smoke also contais PAHs**
  - **ultraviolet directly and indirectly damages DNA**

Etiology of neoplasms - conclusion: underlying genetic changes !

- chromosomal abnormalities – lead to gene fusions and/or copy number alterations
- ‘oncogenic’ viruses appear as such, because they carry transforming genes (oncogenes)
- susceptibility to cancer formation can be heritable
- some environmental factors are mutagenic

THUS:

- cancer is due to genetic abnormalities, inherited or acquired

Etiology of neoplasms: genetic abnormalities – what is affected?

Genes related to cancer fall into one of two classes:

- **oncogene** *(1969)*: **a gene (viral gene?!) that can transform a normal cell into a cancerous one**
  - oncogene is a modified cellular gene (protooncogene) *(Stehelin 1976)*, stolen by a virus and subjected to an activating mutation (that increases its function)
  - differentiating between oncogene and protooncogene is no longer critical, since in some cases there is really no difference (explanation in a moment)
- **tumor suppresor gene: gene, whose inactivation supports carcinogenesis**

Genetics of neoplasms: milestones of molecular biology

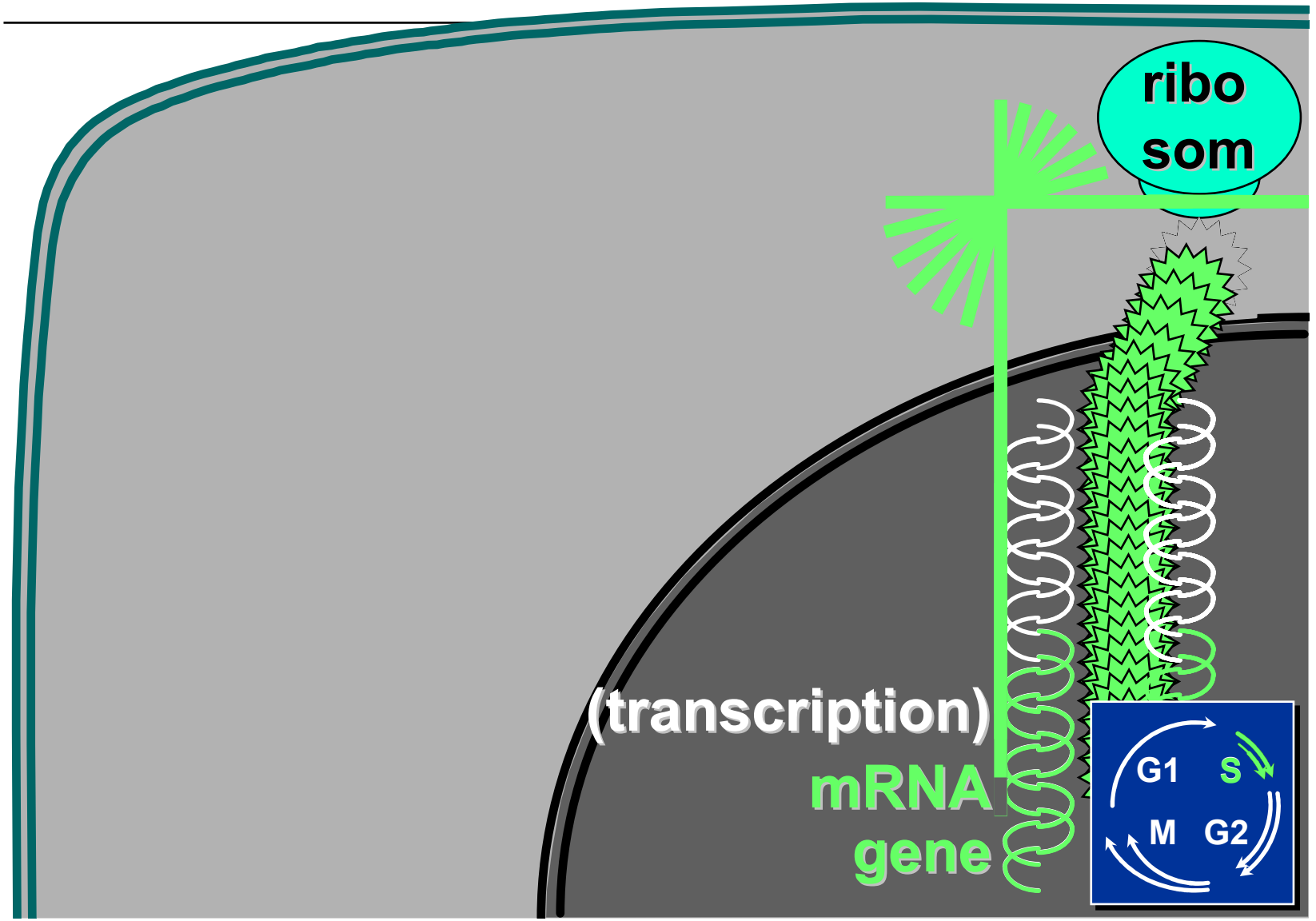
- **heritability of traits** *(~1866)*
- **DNA as a carrier of genetic information** *(1944)*
- **structure of DNA** *(Crick & Watson 1953)*
- **genetic code, mRNA**
  (Crick, Brenner, Watts-Tobin, Leder, Nirenberg 1961)
- **sequencing of RNA** *(Friers 1972)*
- **hybridization of NA** *(Southern 1975)*
- **sequencing of DNA** *(Sanger 1977)*
- **polymerase chain reaction (PCR)** *(Mullis 1984)*
- **human genome decoded** *(2001)*

Genetics of neoplasms: (proto)oncogenes – activation mechanisms

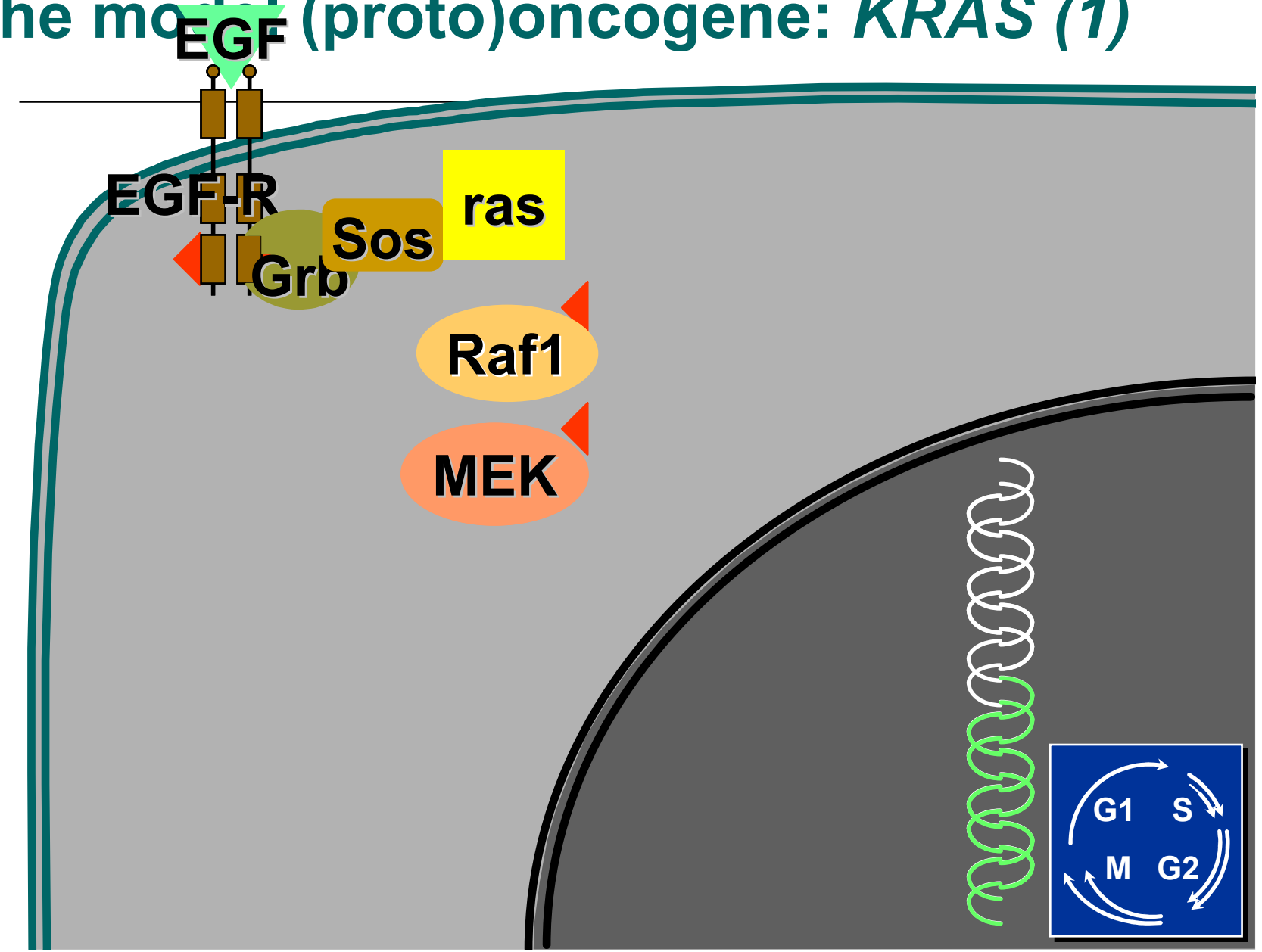
Change increases the activity of encoded protein:

- **increase the amount of normal protein**
  - **gene amplification (even >100 copies)**
  - **gene translocation into another chromosomal region** (i.e., under influence of a very powerful promoter)
  - **mutation increasing stability of a protein or mRNA**
- **transform the molecule into a more active one**
  - **single nucleotide substitutions (SBS) – only missense ones, those that change the sense of a code**
  - **deletions or nonsense mutations leading to a loss of an autoinhibitory domain** *(if such one exists)*
  - **fusions of the oncogene with another gene**

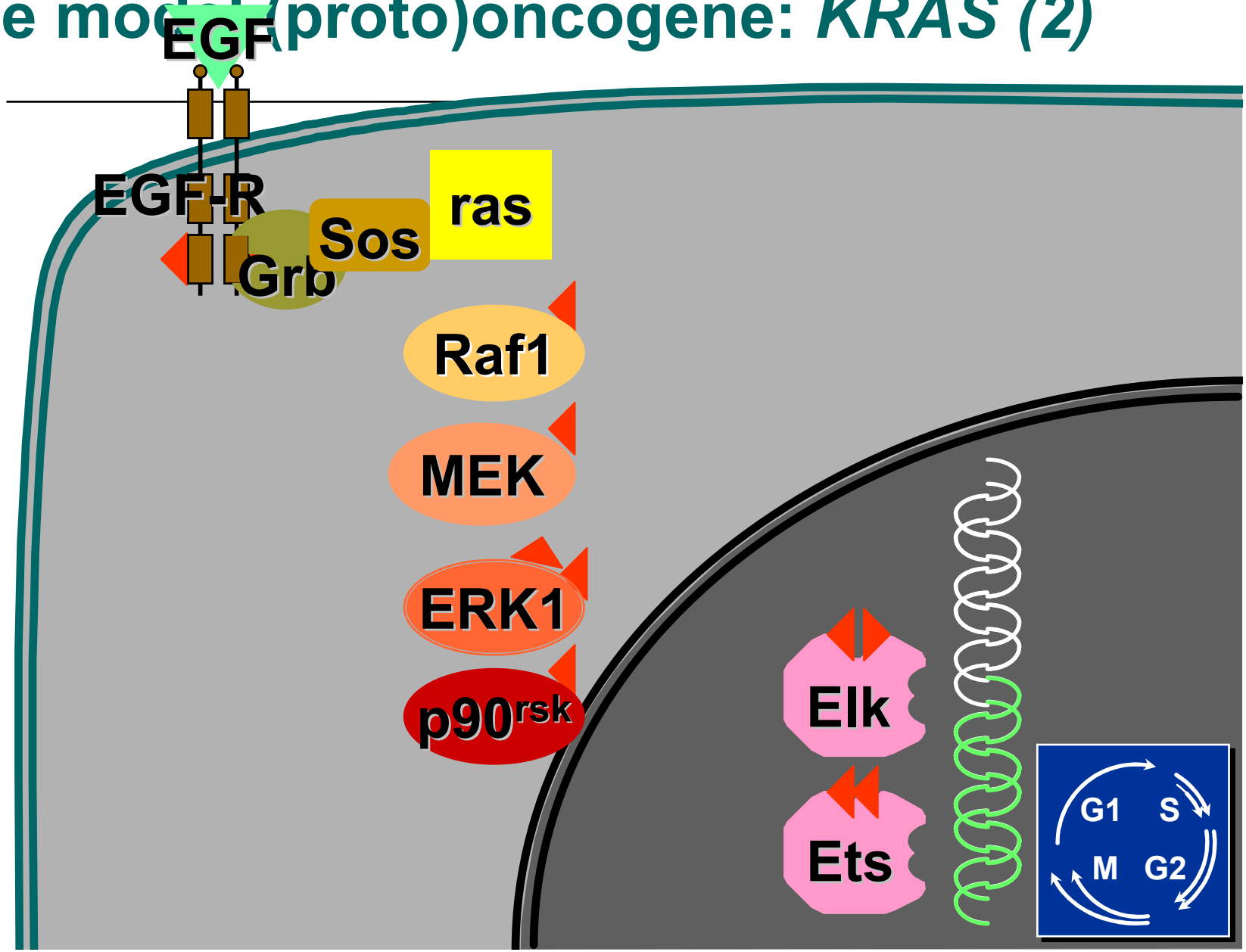
Genetics of neoplasms – the model (proto)oncogene: *KRAS (0)*



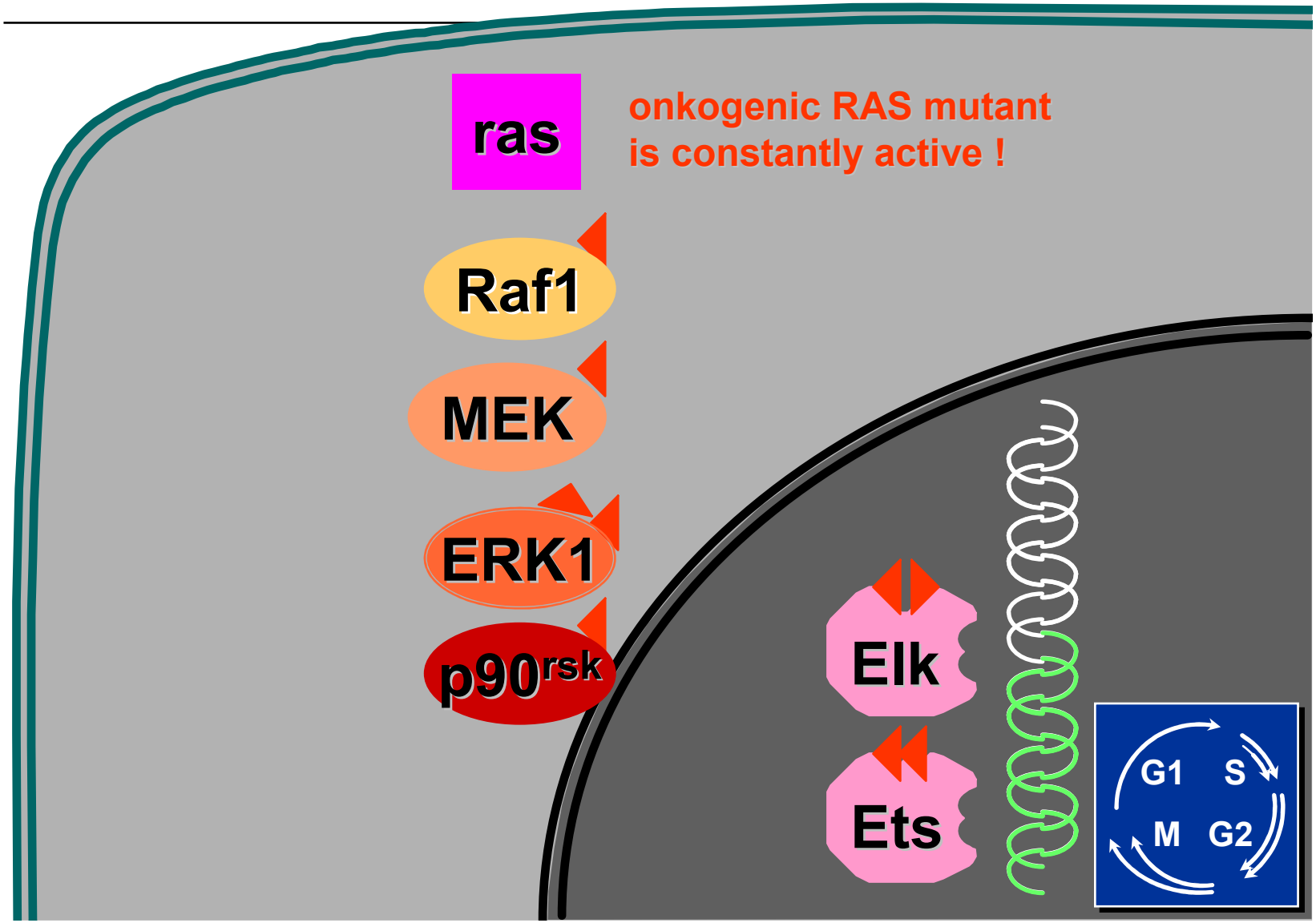
Genetics of neoplasms – the model (proto)oncogene: *KRAS (1)*



Genetics of neoplasms – the model (proto)oncogene: *KRAS (2)*



Genetics of neoplasms – the model (proto)oncogene: *KRAS (3)*



Genetics of neoplasms: functions of (proto)oncoges

Mostly encode elements of pathways conveing the signal from growth factors into the cell nucleus

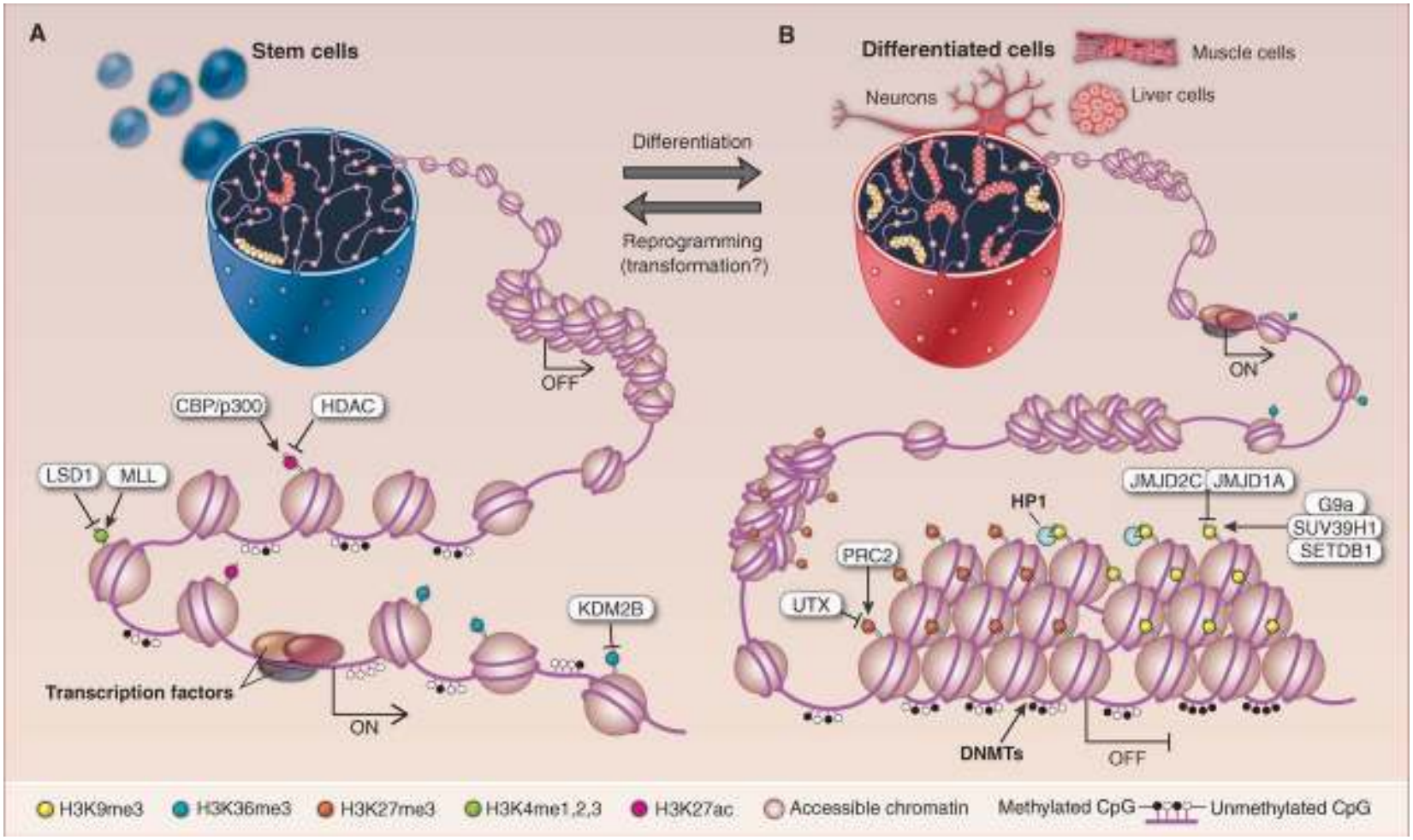
- **growth factors**
  - Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), Patelet-derived Growth Factor (PGDF), Insulin-like Growth Factor (IGF-1), Transforming Growth Factor β (TGFβ)
- **receptors for growth factors (protein kinases)** such as: EGFR, PDGFR, TGFBR
- **cooperating proteins,** such as GTPases, i.e.: RAS
- **cytoplasmic protein kinases**, such as: SRC
- **trascription factors,** such as: MYC

Genetics of neoplasms: tumour suppressor genes – INactivation

Change decreases the activity of encoded protein

- **gene silencing**
  - **epigenetic change, not a mutation!**
  - **reversible – that's exceptional!**

Genetics of neoplasms: tumour suppressor genes – INactivation



- **DNA methylation and histone modification -> tightly packed DNA -> inaccessible ('silenced') genes**

[Suva, Science 2013]

Genetics of neoplasms: tumour suppressor genes – INactivation

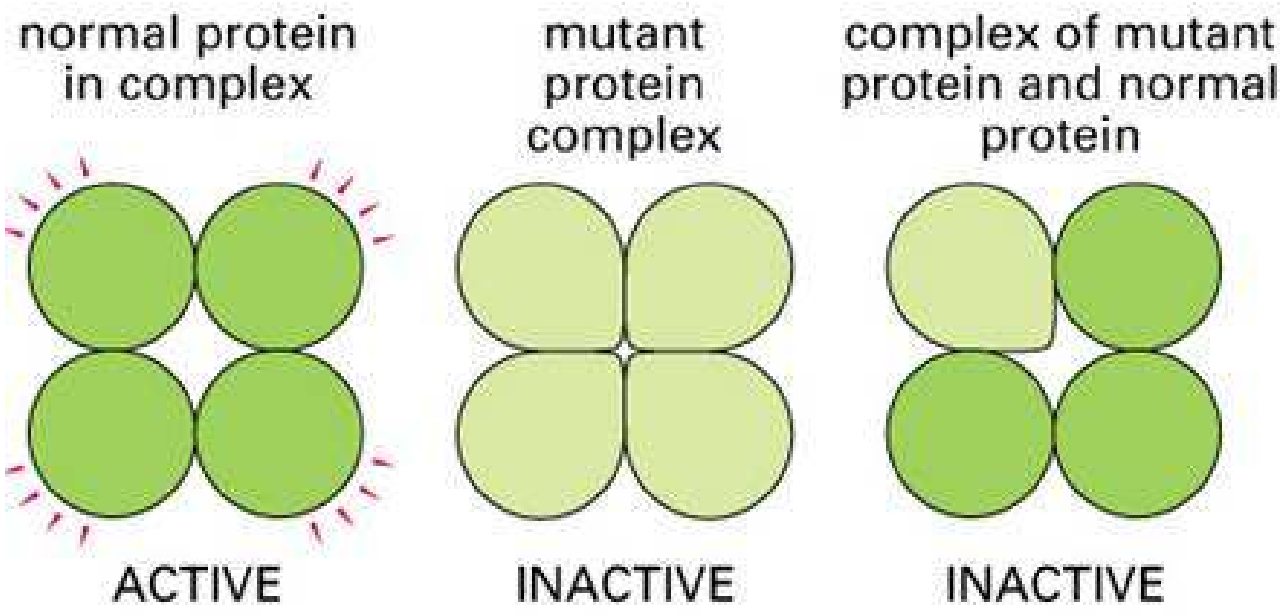
Change decreases the activity of encoded protein

- **gene silencing**
  - **epigenetic change, not a mutation!**
  - **reversible – that's exceptional!**
- **deletion of the gene or its fragment**
- **nonsense mutation**

Must affect both alleles – 'two hit' theory *(Knudson 1971)* **– exceptions:**

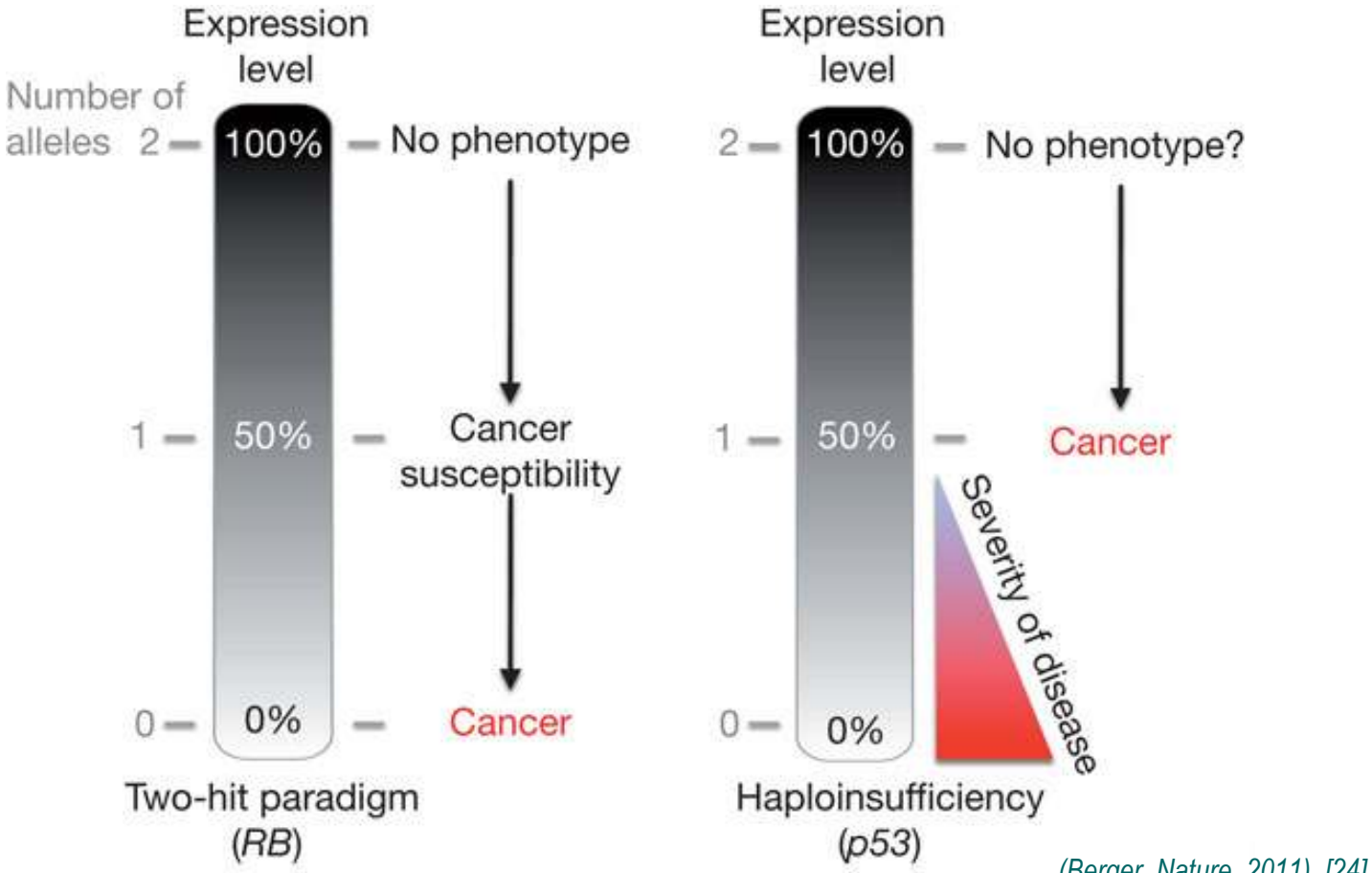
- **dominant negative mutation**
- **haploinsufficiency**

Knudsona theory – exeptions **(1)**
Dominant negative mutation



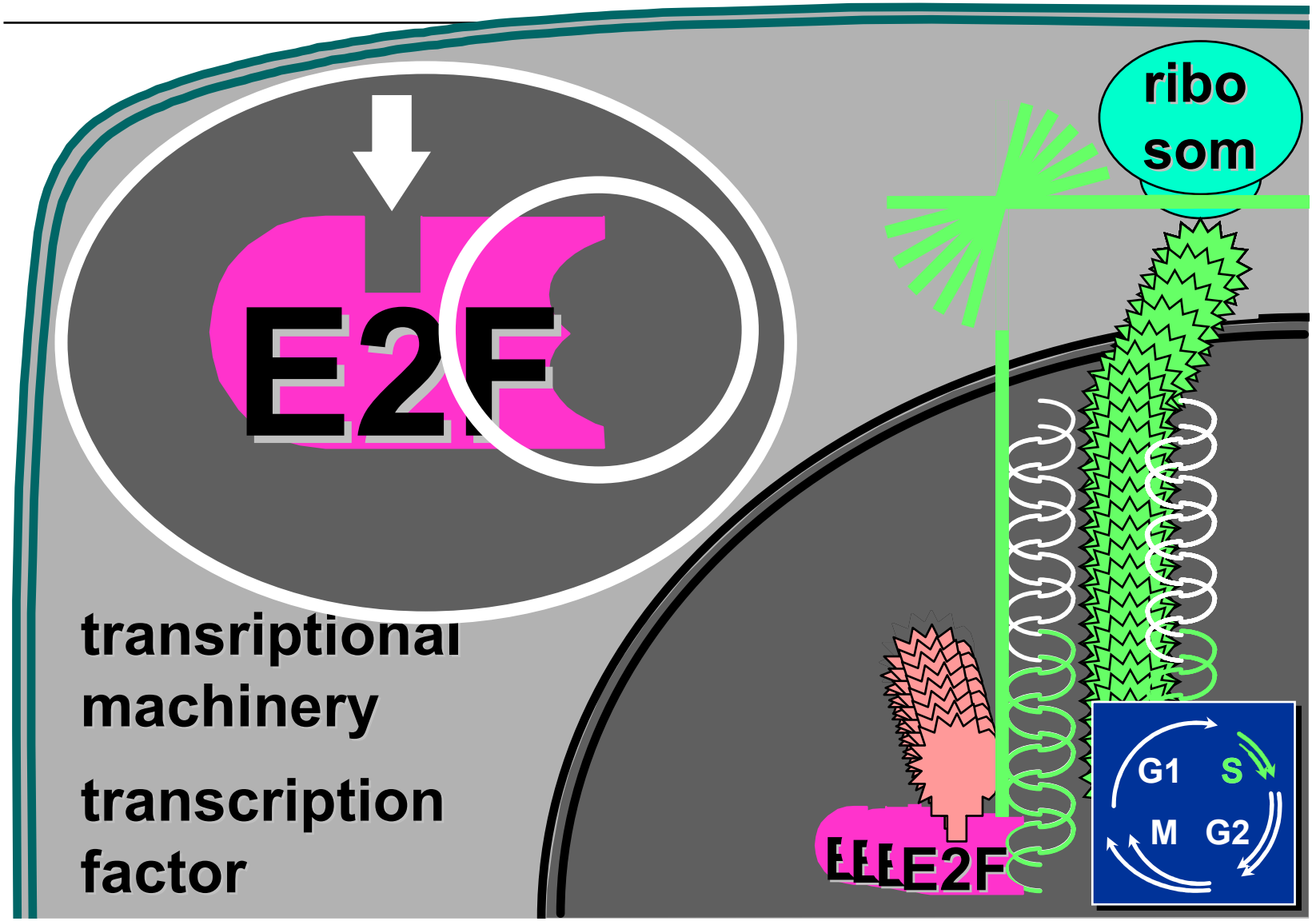
Molecular Biology of the Cell, 4th edition

Knudsona theory – exeptions **(1)** **Haploinsufficiency**

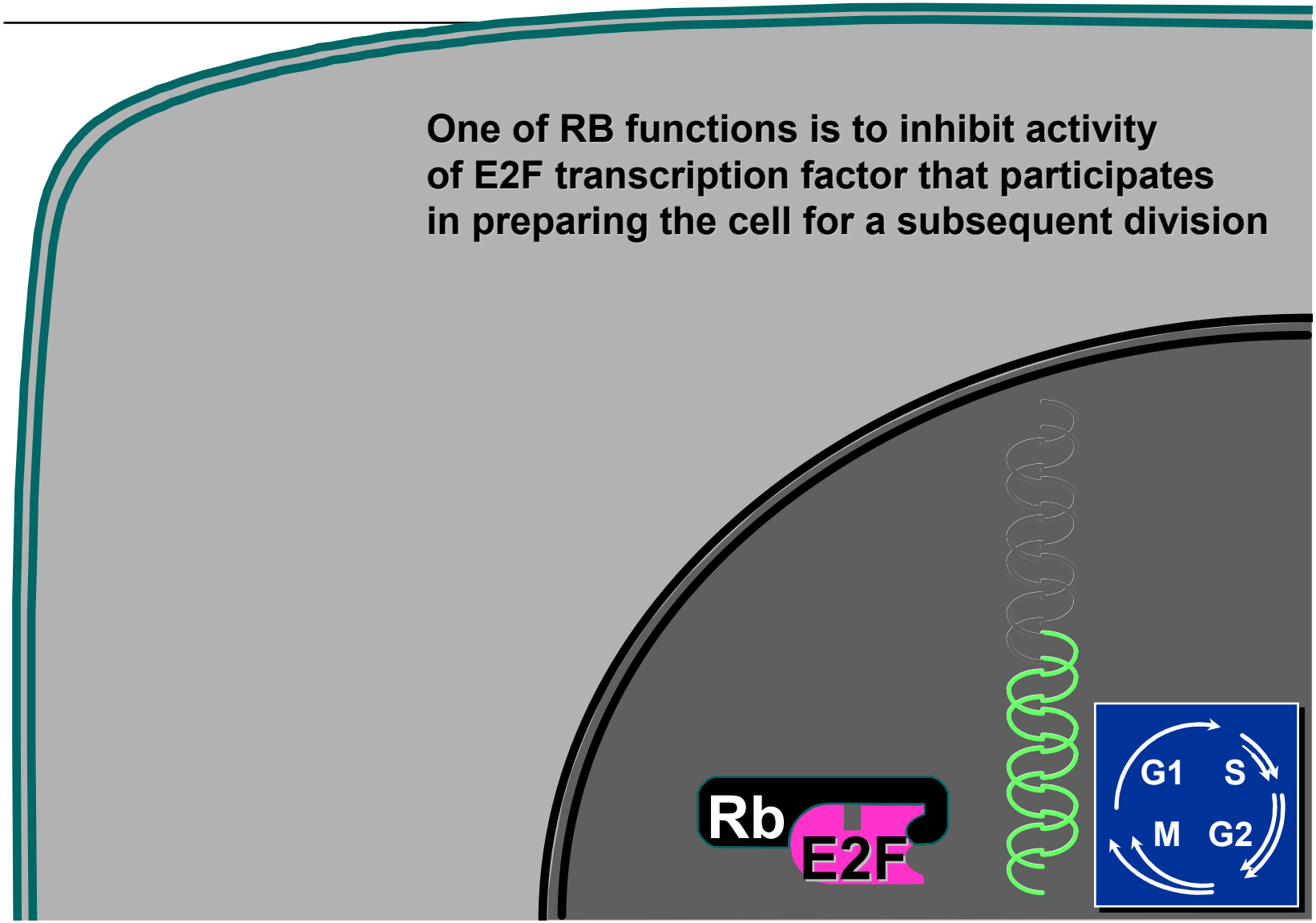


(Berger, Nature, 2011) [24]

Genetics of neoplasms: suppressor genes – *RB (1)*

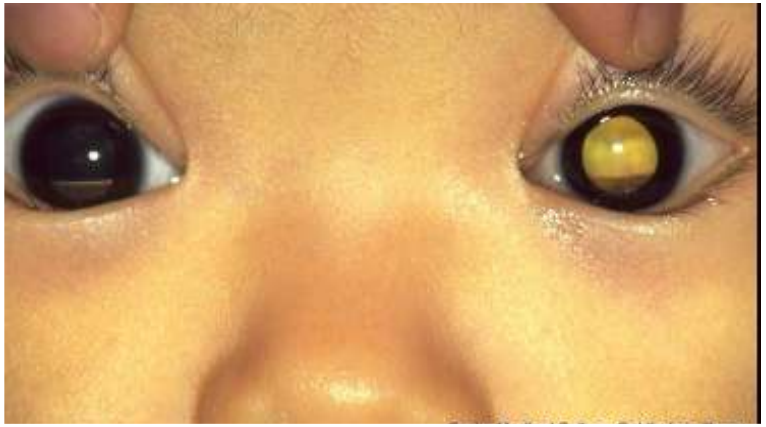


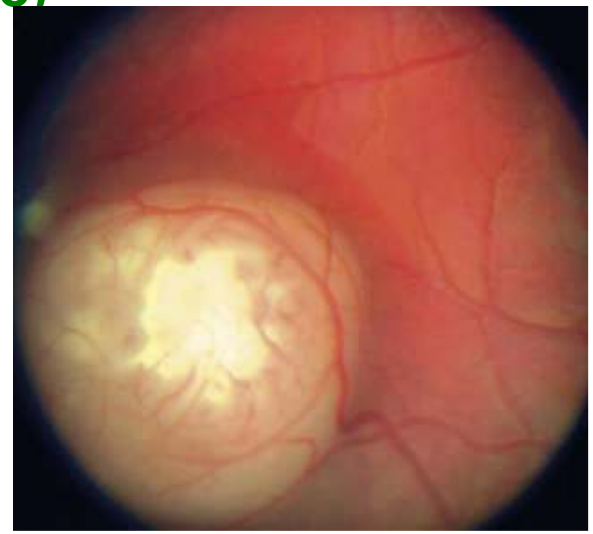
Genetics of neoplasms: suppressor genes – *RB (2)*



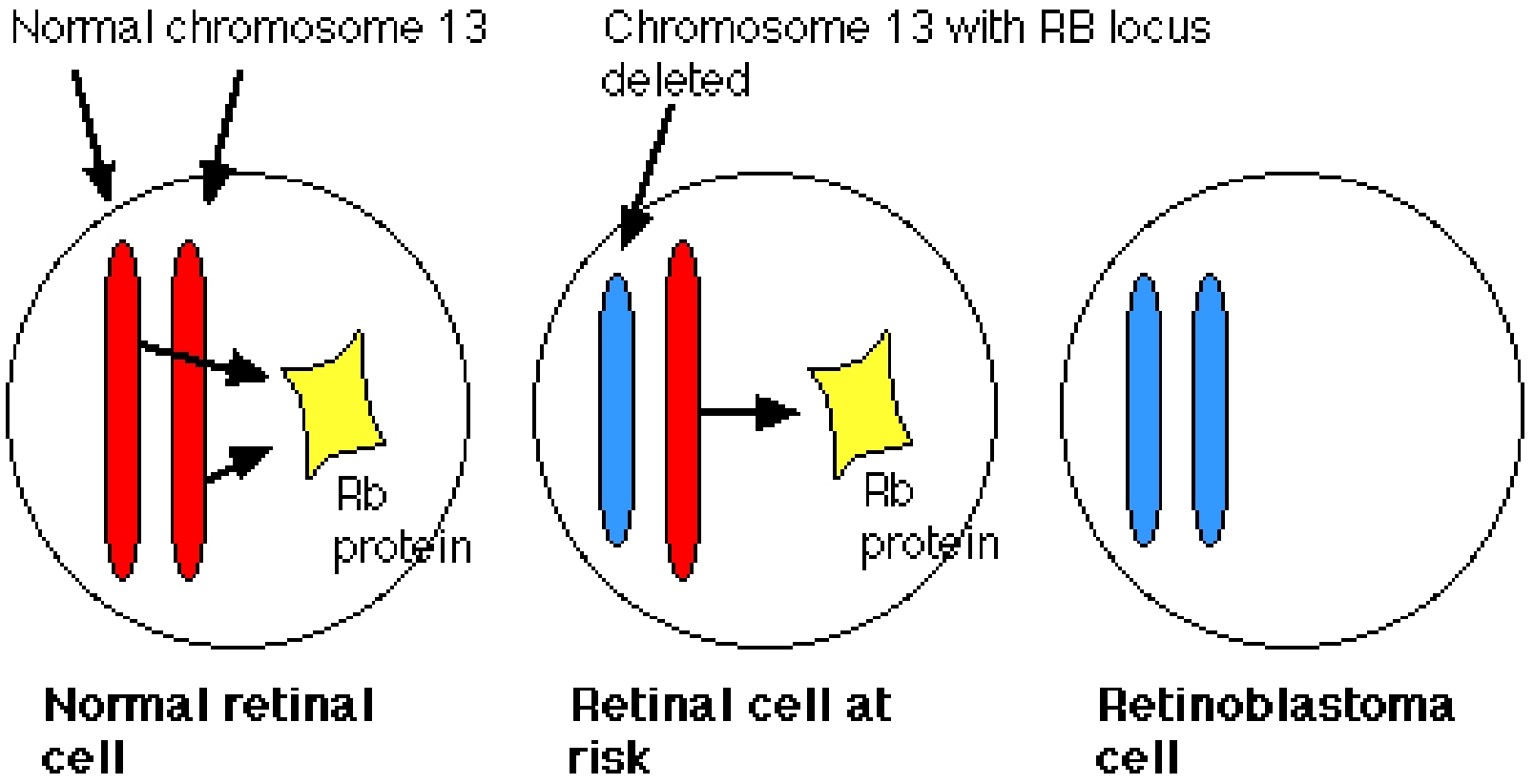
Genetics of neoplasms: suppressor genes – *RB (3)*

- defect of RB leads to retinoblastoma
- 1/25 000
- white reflection (leukocoria)
- 250 new cases yearly in US
  - 25-30% bilateral (average age 12 mo)
  - unilateral (average age 21 mo)





Genetics of neoplasms: suppressor genes – *RB (4)*



Genetics of neoplasms: suppressor genes – *RB (5)*

- 30% germinal mutation in unilateral cases
- control examinations until 7 y. old
- bilateral cases:
  - 50% risk of passing an affected allele
  - 45% risk of developing a disease (high risk of a second somatic mutation)
- unilateral cases:
  - 7-15% risk of passing an affected allele (germinal mutation is possible !)
  - 90% of children are first cases in the family

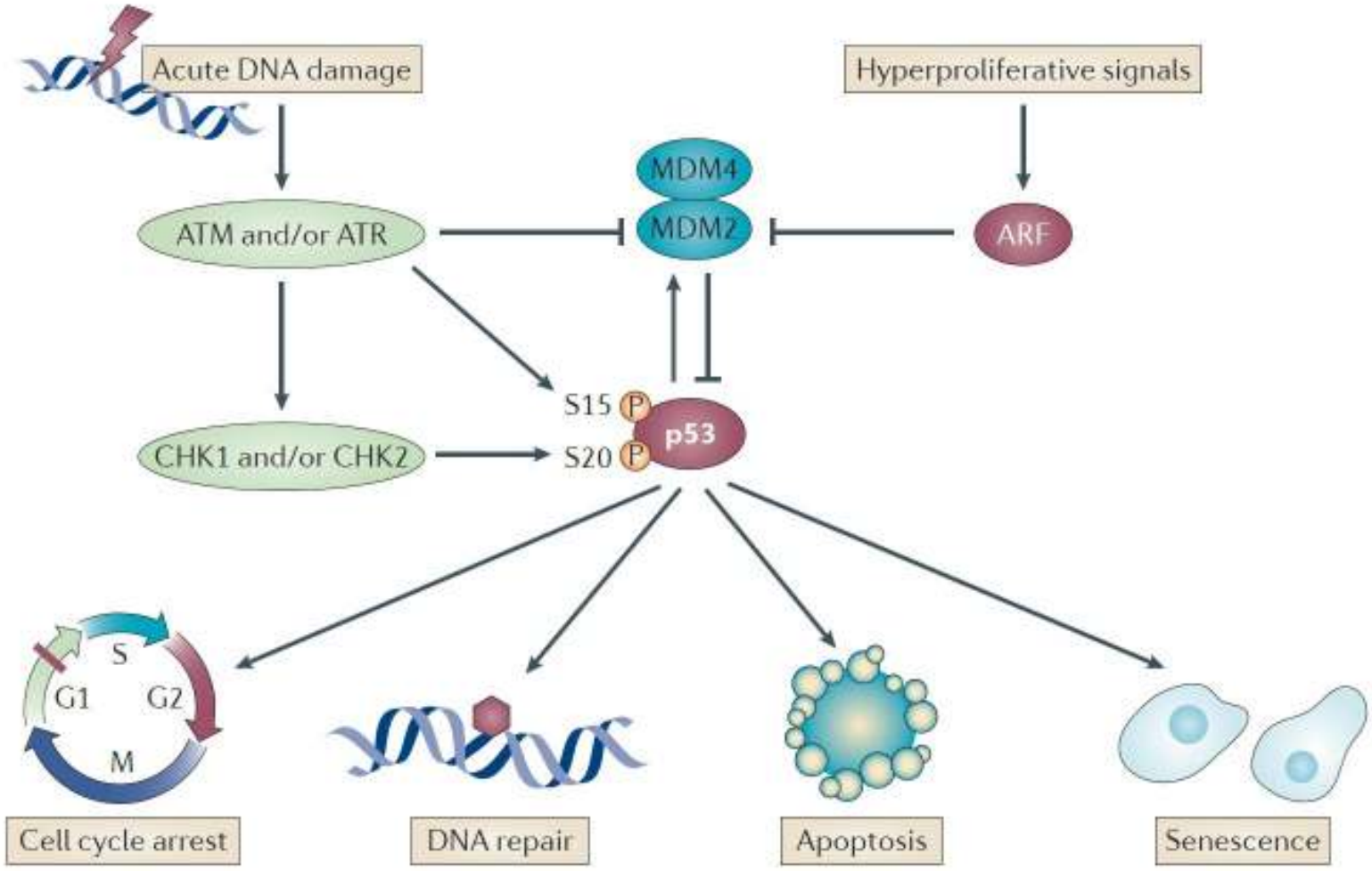
Genetics of neoplasms: tumor suppressor functions

- **braking the cell cycle progress:** RB
- **DNA integrity control:**
  ATM, CHK1
- **chromatin state:**
  SMARCA4, ARID1A, ARID1B, PBRM1, ARID2
- **stopping the cell cycle in case of DNA damage:**
  TP53
- **DNA repair:** BRCA1

Genetics of neoplasms: tumor suppressor – *TP53*

- transcription factor (many interactions)
- regulates cell cycle, apoptosis, supervises genome integrity
- basic role: gatekeeper of entry into S phase
- „guardian of the genome”
- active in tetrameric form
- inherited mutations => Li-Fraumeni syndrome (sarcoma, leukemia, brain tumors, breast cancer; hypersensitivity to ionizing radiation)

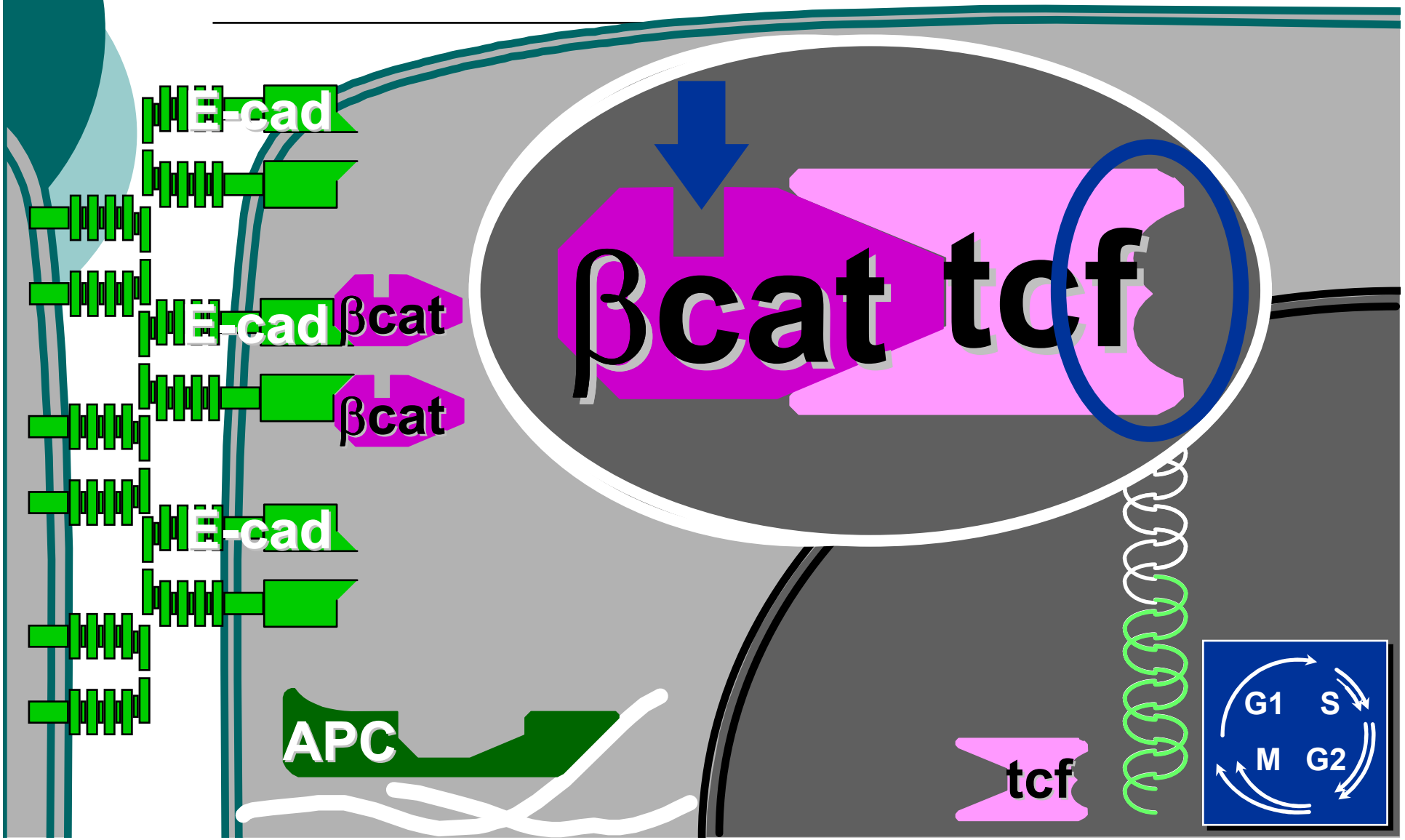
Genetics of neoplasms: tumor suppressor – *TP53*



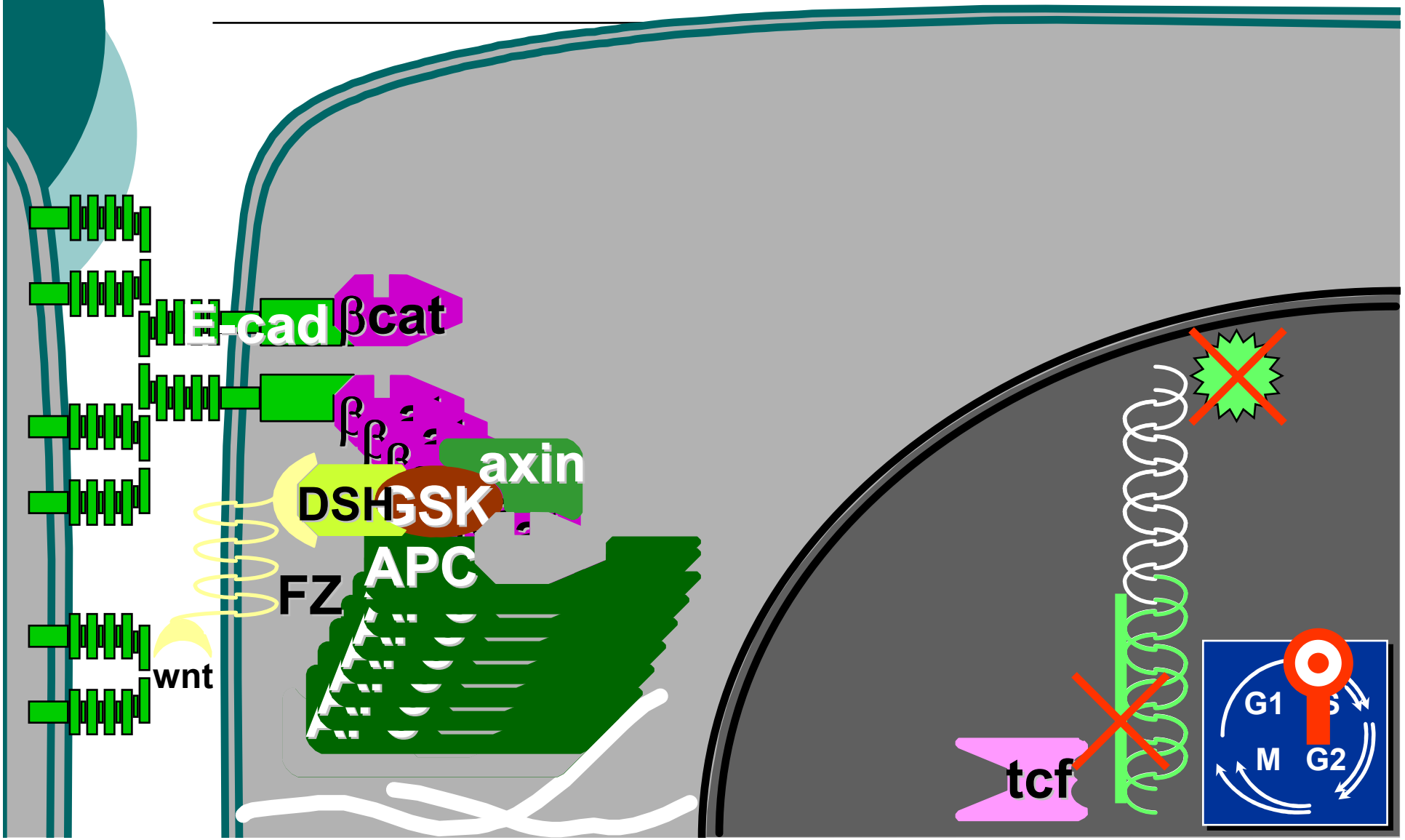
Etiology of ‘inherited’ neoplasms: defect of a one of a hundred genes

- **inherited mutations of certain genes determine a substantial increase of cancer susceptibility**
- **susceptibility vary depending on type of cancer and type of mutation**
- **predisposing genes:**
  - **COSMIC: 89 genes**
    [http://cancer.sanger.ac.uk/cosmic/census/tables?name=gmuts]
  - **Rahman: 115 genes**
    [Rahman, Nature 2014]

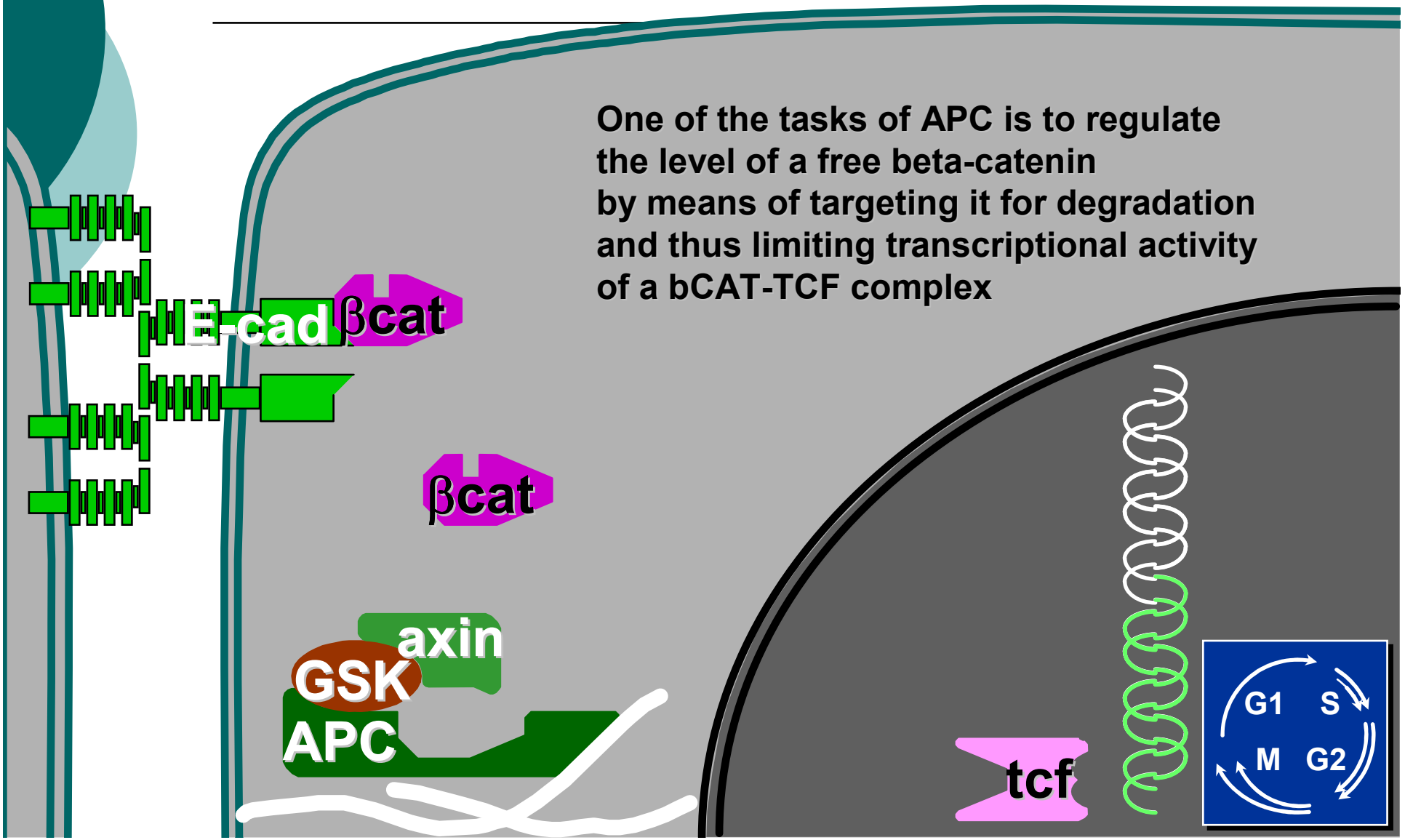
Inherited susceptibility example: APC => familial polyposis (1)



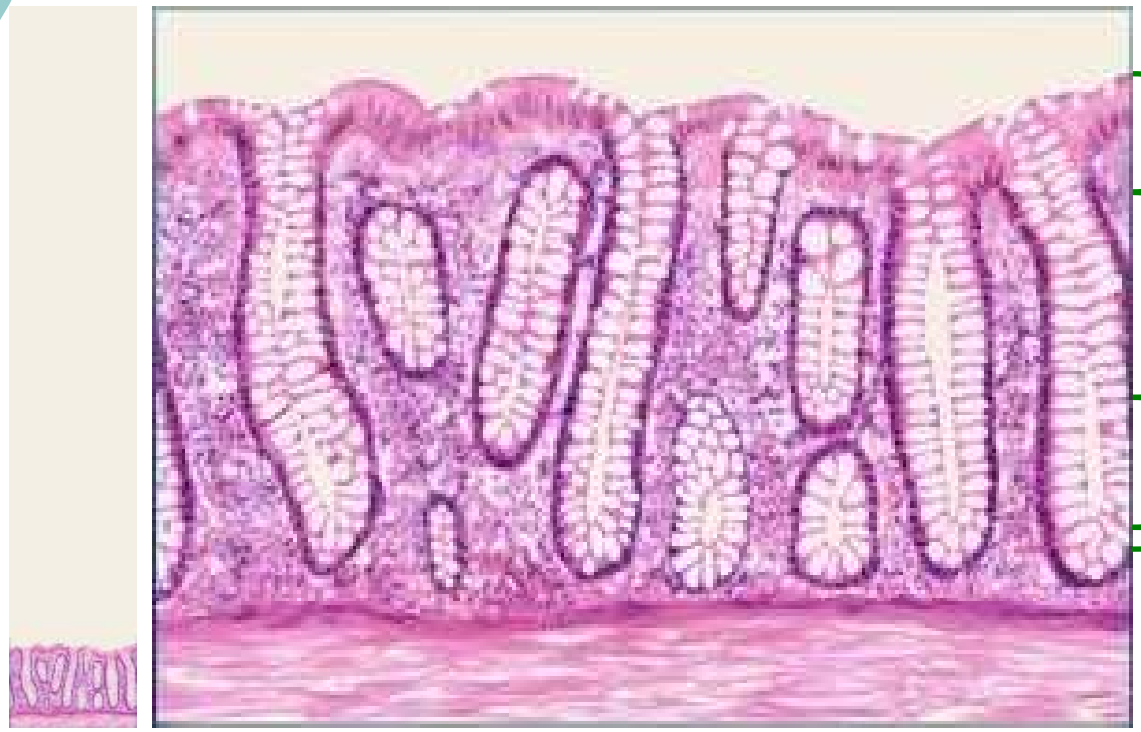
Inherited susceptibility example: APC => familial polyposis (2)



Inherited susceptibility example: APC => familial polyposis (3)



Inherited susceptibility example: APC => familial polyposis (4)



normal epithelium

(magnified)

detachment zone

maturation zone

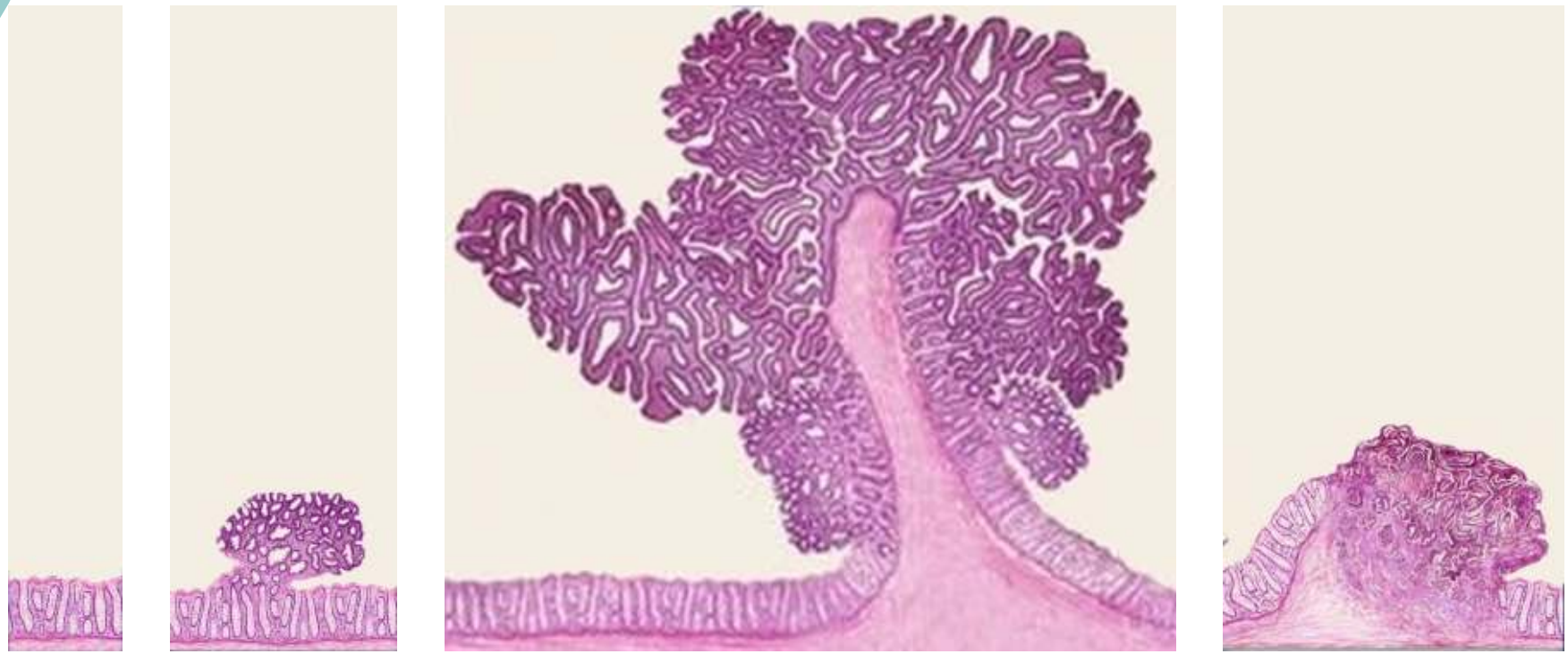
proliferation zone

cruypt stem cells

cell turnover: 3-5 days
(except for stem cells)

[Vogelstein, Science 2013]

Inherited susceptibility example: APC => familial polyposis (5)



normal epithelium

small adenoma

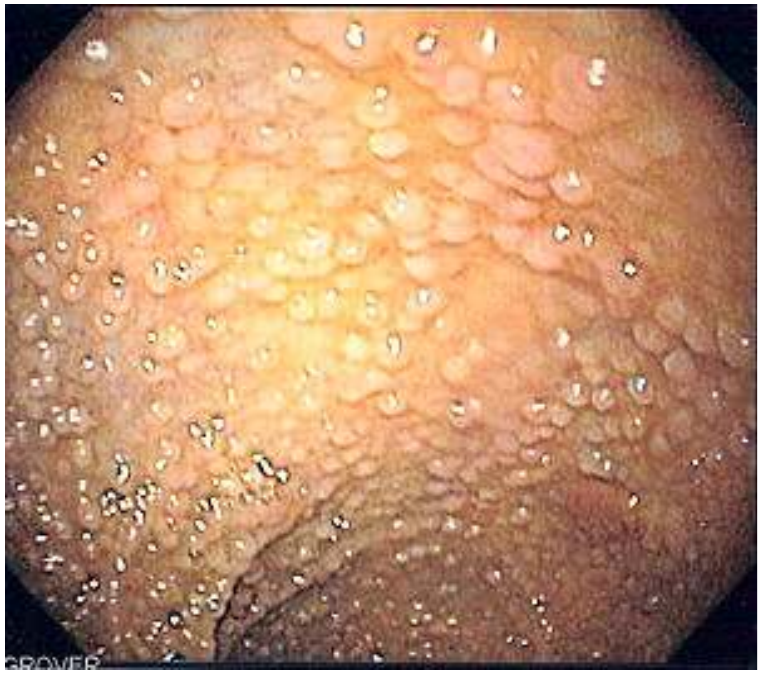
large adenoma

cancer

[Vogelstein, Science 2013]

Inherited susceptibility example: APC => familial polyposis (6)

- ***APC* 5q21**
- **inherited mutations: 2-3/10 000 newborns**
- **hundreds (or more) polyps in colorectum**
- **inherited mutations are responsible for ~1% cases of colorectal cancer**
- *somatic mutations in APC are present in ~80% cases of colorectal cancer*

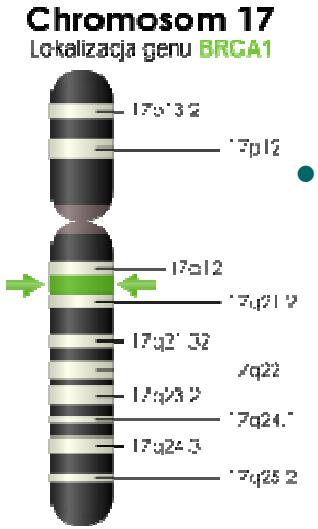


Inherited susceptibility example: susceptibility to breast cancer

- *BRCA1* and *BRCA2*
- Both encode proteins participating (among others) in:
  - homologous recombination and DNA repair
  - cell cycle control
  - developmental processes

Inherited susceptibility example: breast cancer susceptibility – *BRCA1:*

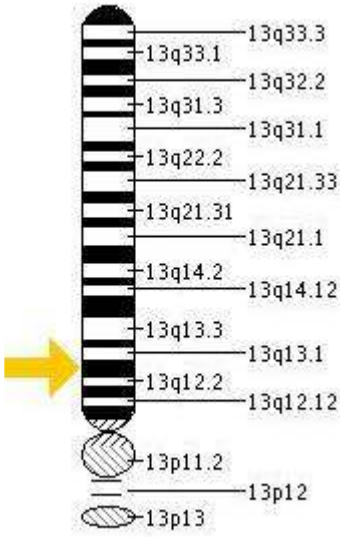
- chromosom 17, 24 exons,
- protein 1863 AA
- 5 functional domains
  - N-terminus – Zn finger – interaction protein–DNA
  - C-terminus – activation of transcription & DNA repair



- 55% of mutations in exon11

Inherited susceptibility example: breast cancer susceptibility – *BRCA1:*

- chromosome 13, 27 exons,
- protein 3418 AA
  - N-terminus – transcription activation domain
  - C-terminus – nuclear localisation signal



mutations mostly at both terminal parts

Inherited susceptibility example: breast cancer susceptibility – *BRCA1&2:*

- expression in all cells,
  the highest in thymus and testicles
- both genes active in mammary gland
  through pregnancy and
- inherited *BRCA1* mutations in women cause:
  - 50-80% lifetime risk of breast cancer
    (average age: ~40 y)
  - 40% lifetime risk of ovarian cancer
- inherited *BRCA2* mutations in men :
  - 200× increase of risk of breast cancer

Inherited susceptibility example: chromosomal instability syndromes

- **Breaks and rearrangements of various chromosomes – increased susceptibility to neoplasmsm**
  - **Bloom syndrome**
  - **Fanconi anaemia**
  - **ataxia-teleangiectasia (Louis–Bar syndrome)**

Chromosomal instability syndromes: Bloom syndrome

- **cause: mutation of *BLM* (15q26.1),**
- **heritability: autosomal recessive**
- **funtion of protein: DNA helicase**
  - **unwinds double helix of DNA** (during transcription, probably also participates in DNA repair)
  - **patients also overproduce superoxide radical**
- **molecular consequences of mutation:**
  - **10× increase in frequency of sister chromatid exchange, breaks and rearrangements of chromosomes**
- **effects:**
  - **neoplasms in up to 20% of patients; acute leukemias and lymphoproliferative diseases at an age below 25 y,**

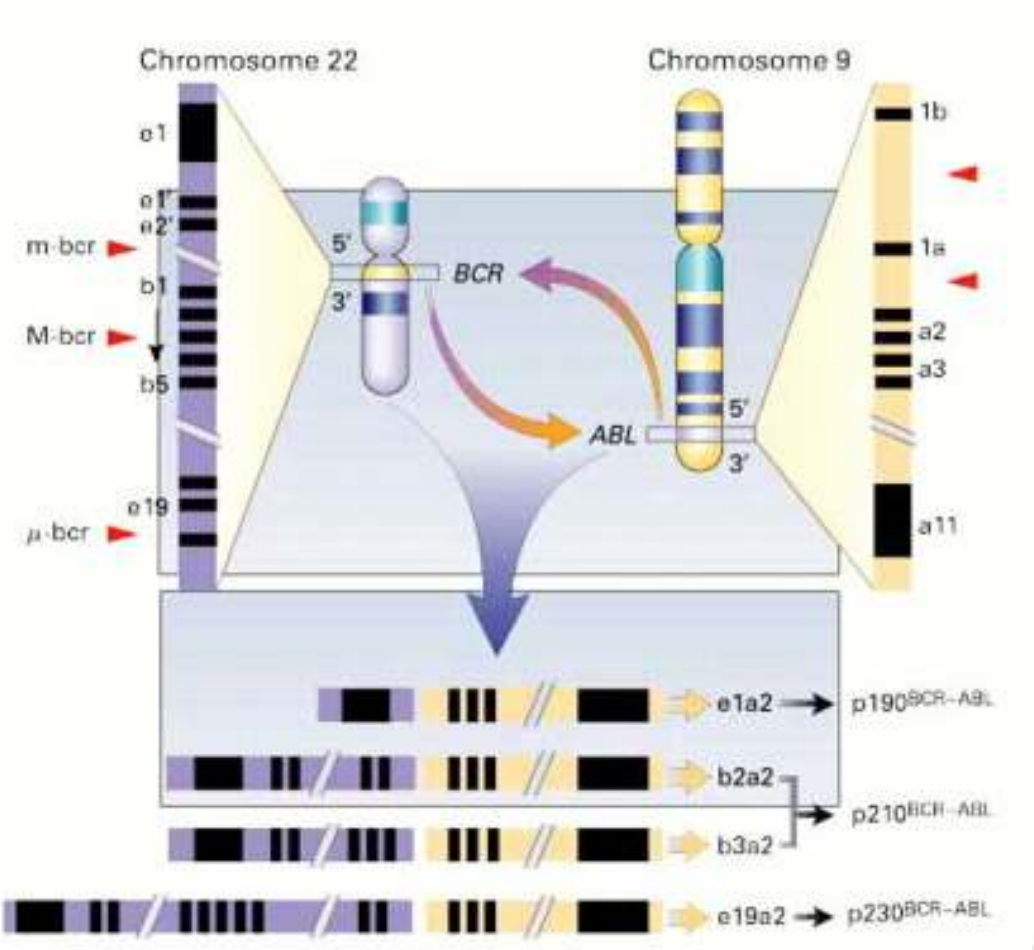
Chromosomal instability syndromes: Fanconi anaemia

- **A type of an inherited aplastic anaemia with accompanying skeletal malformations and predisposition to cancer (60% of cases: mutation of *FAA* gene)**
- **mutation impairs the repair of crosslink DNA damage**

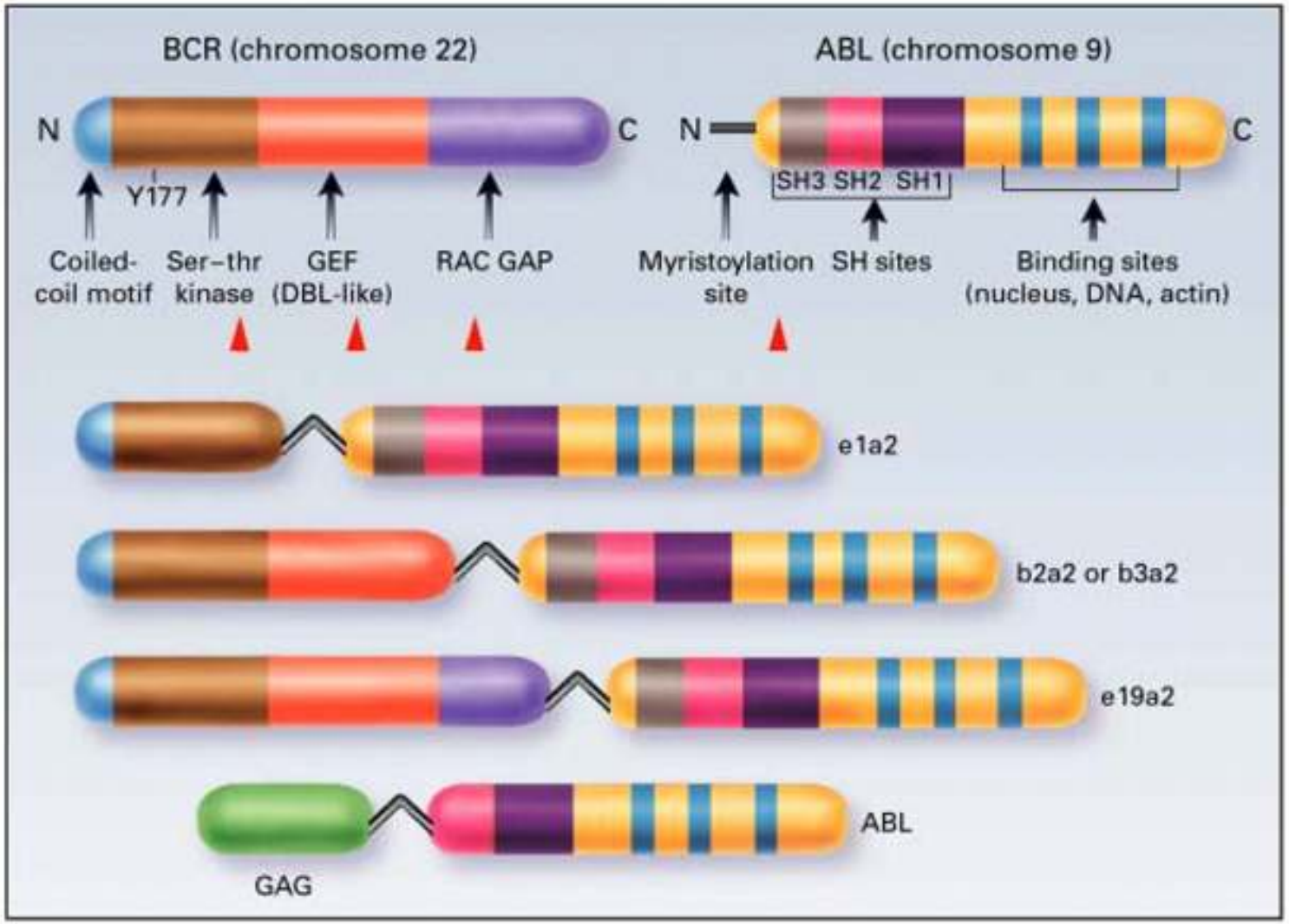
Chromosomal instability syndromes: ataxia-teleangiectasia

- **translocation 7-14 (breakage of 7p14), defect of *ATM***
- **the gene codes for a kinase participating in DNA repair, cooperating with TP53** (among others)
- **impaired cell cycle blockage normally resulting from DNA damage**
- **effects:**
  - **cerebellar abnormalities**
  - **immunodeficiency,**
  - **oversensitivity to radiation, chromosomal instabilities**

‘Single gene’ carcinogenesis – Bcr-Abl example (1)



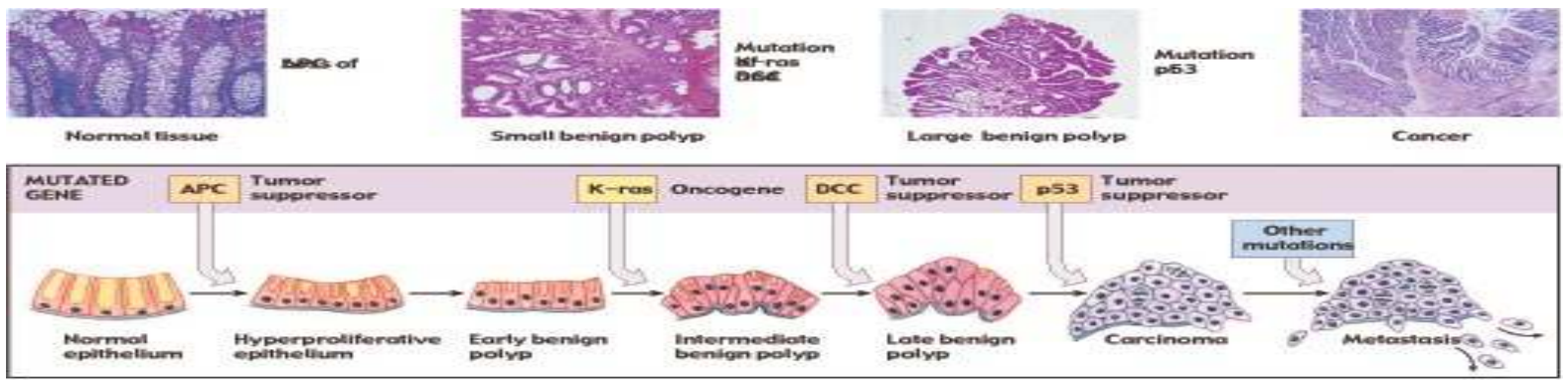
‘Single gene’ carcinogenesis – Bcr-Abl example (2)



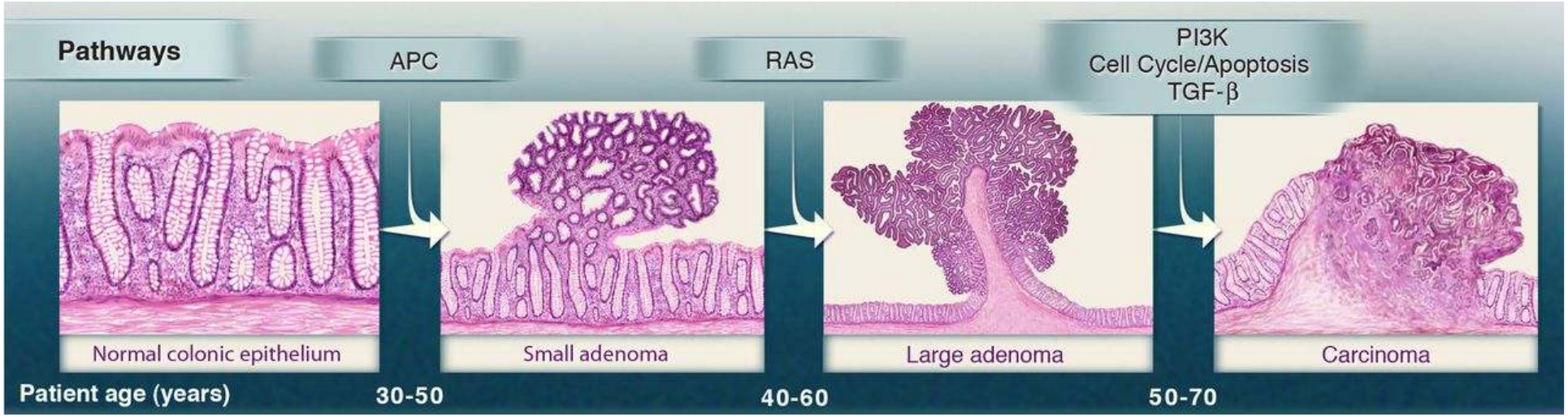
‘Single gene’ carcinogenesis – Bcr-Abl example (3)

- genetic modification of mice: introduction of the switchable *Bcr-Abl* gene fusion under tetracycline-regulated promoter
- switching the *Bcr-Abl* expression on resulted in rapid increase of blast cells in blood
  - turning the expression off in the early stage of leukemia resulted in full regression
    - turning on again caused leukemia to re-appear
  - turning the expression off in late stages of leukemia often had no effect

Linear path to neoplasm



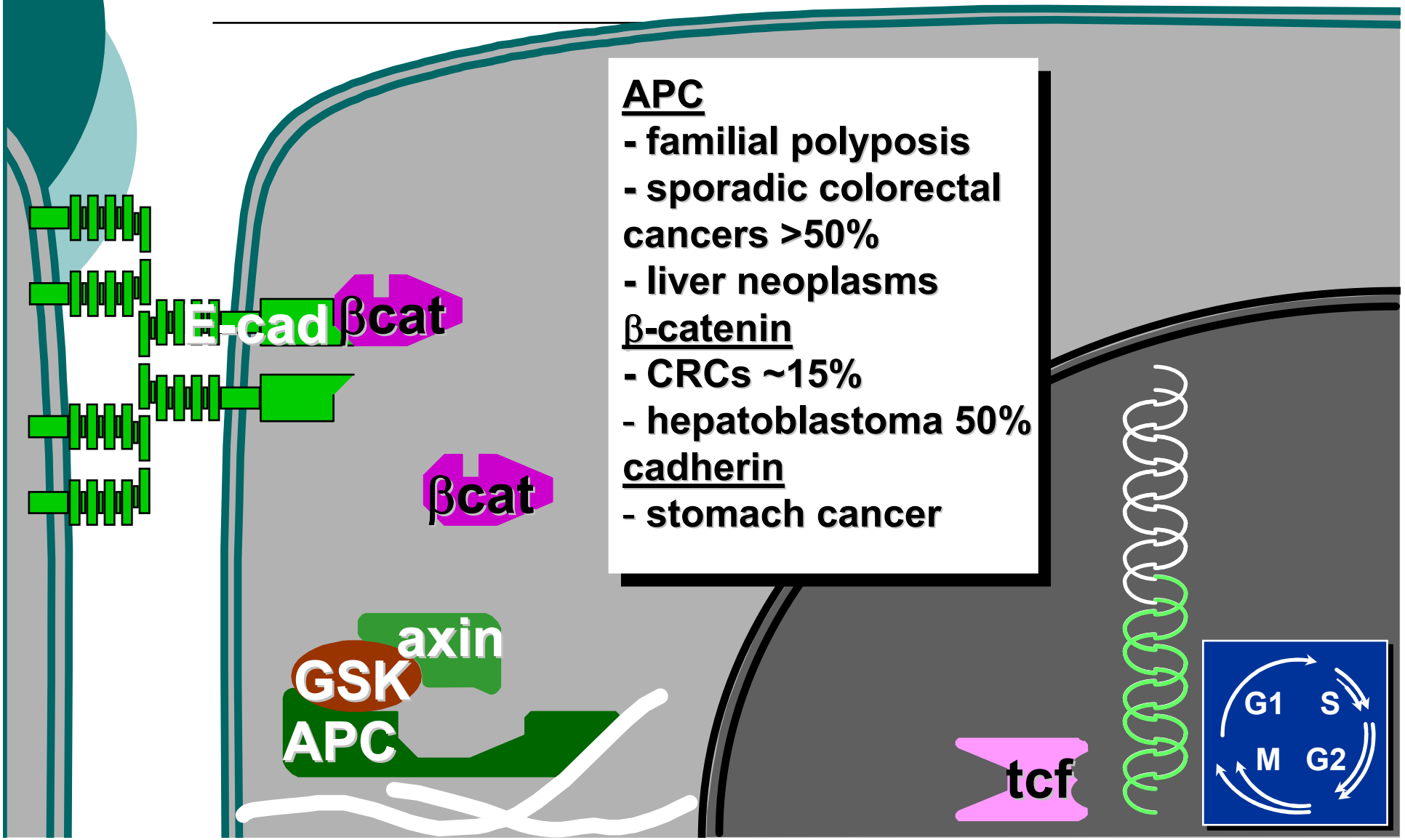
[Vogelstein, 199x]



[Vogelstein, Science 2013]

tumors with this set of mutations? **<10%!!**

Branched linear path to neoplasm – APC



Branched linear path to neoplasm – TGFb pathway

COLORECTAL CANCER

TGFβ-RII: >90% HNPCC
TGFb-RII: ~15% others

Smad 2: ~10%

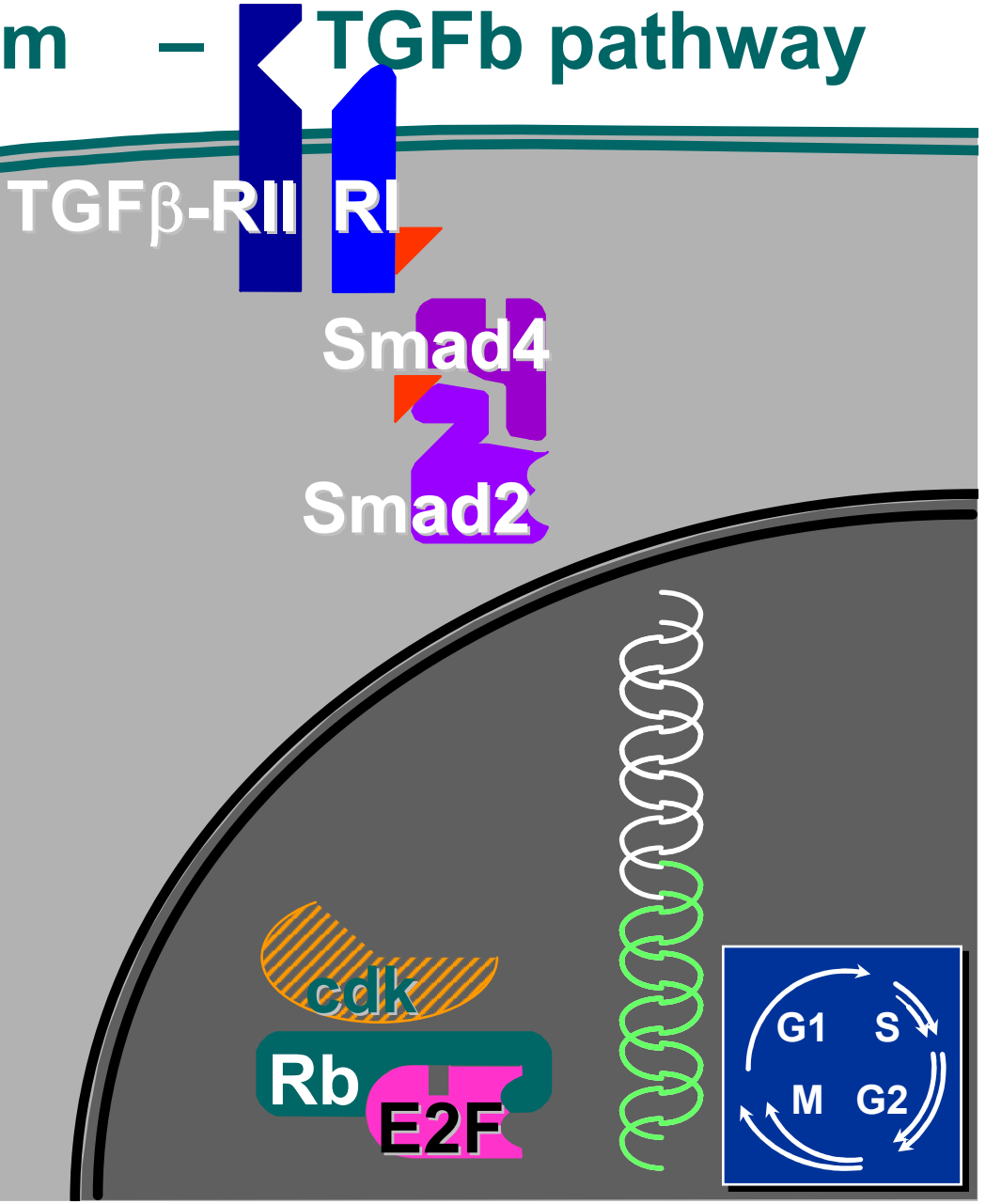
Smad 3: inherited mutation in mice – metastasing tumor

Smad 4: do 25% juvenile polyposis !

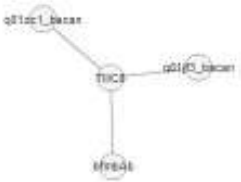
mut. Smad4 + mut. APC = adenomas turn malignant!

PANCREATIC CANCER

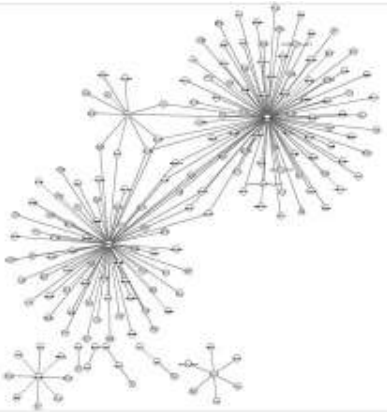
Smad 4 (=DPC4): >50%



A cell is a dense network of molecular interactions

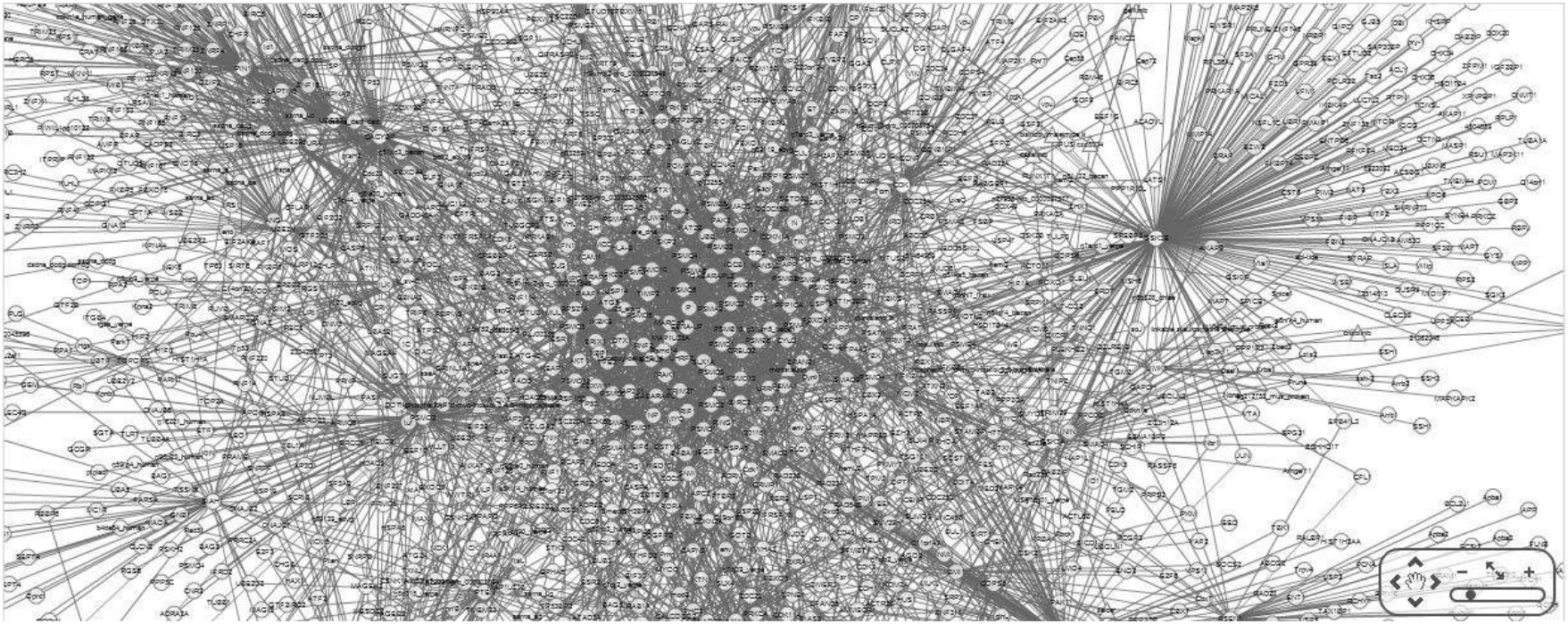


TMC8: 3

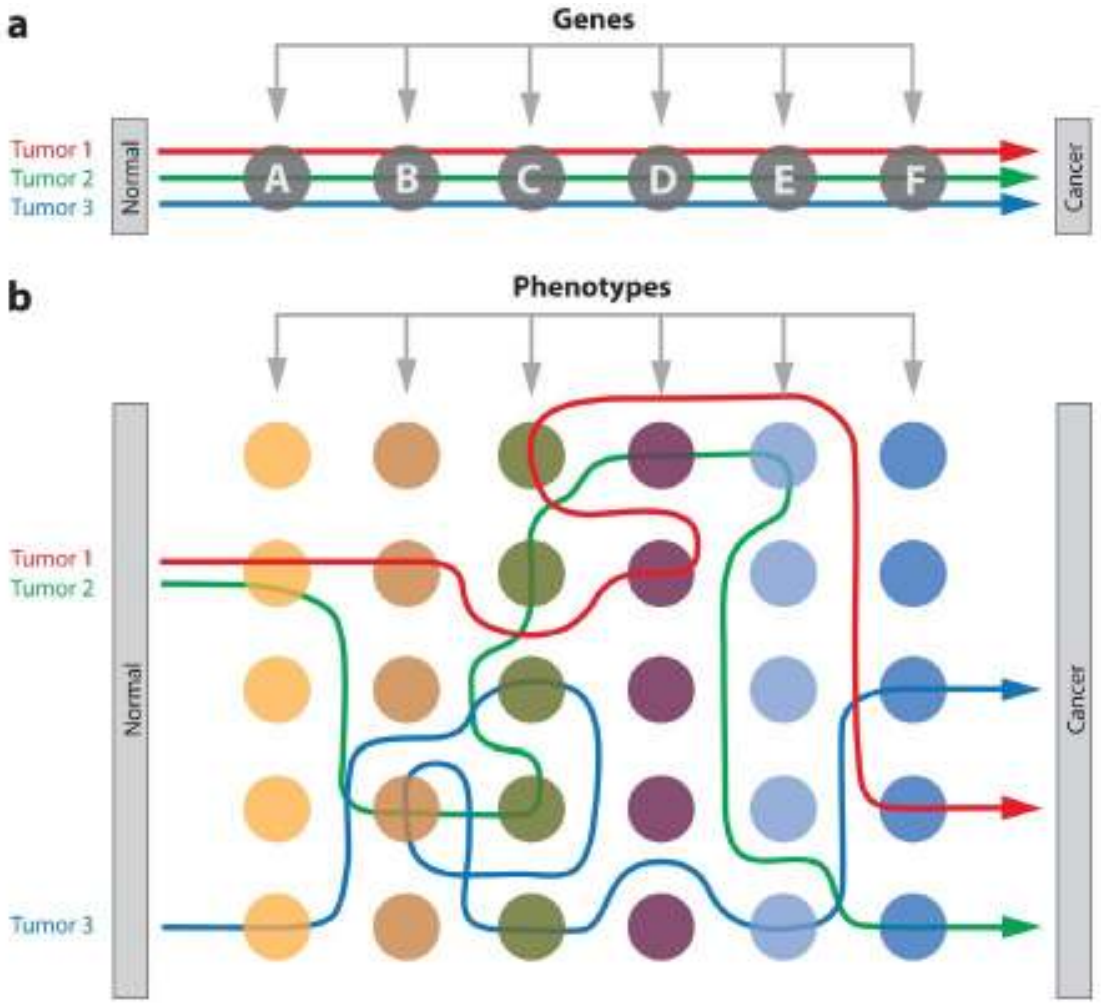


SMAD4: 307

APC: >8500 (a part is shown)

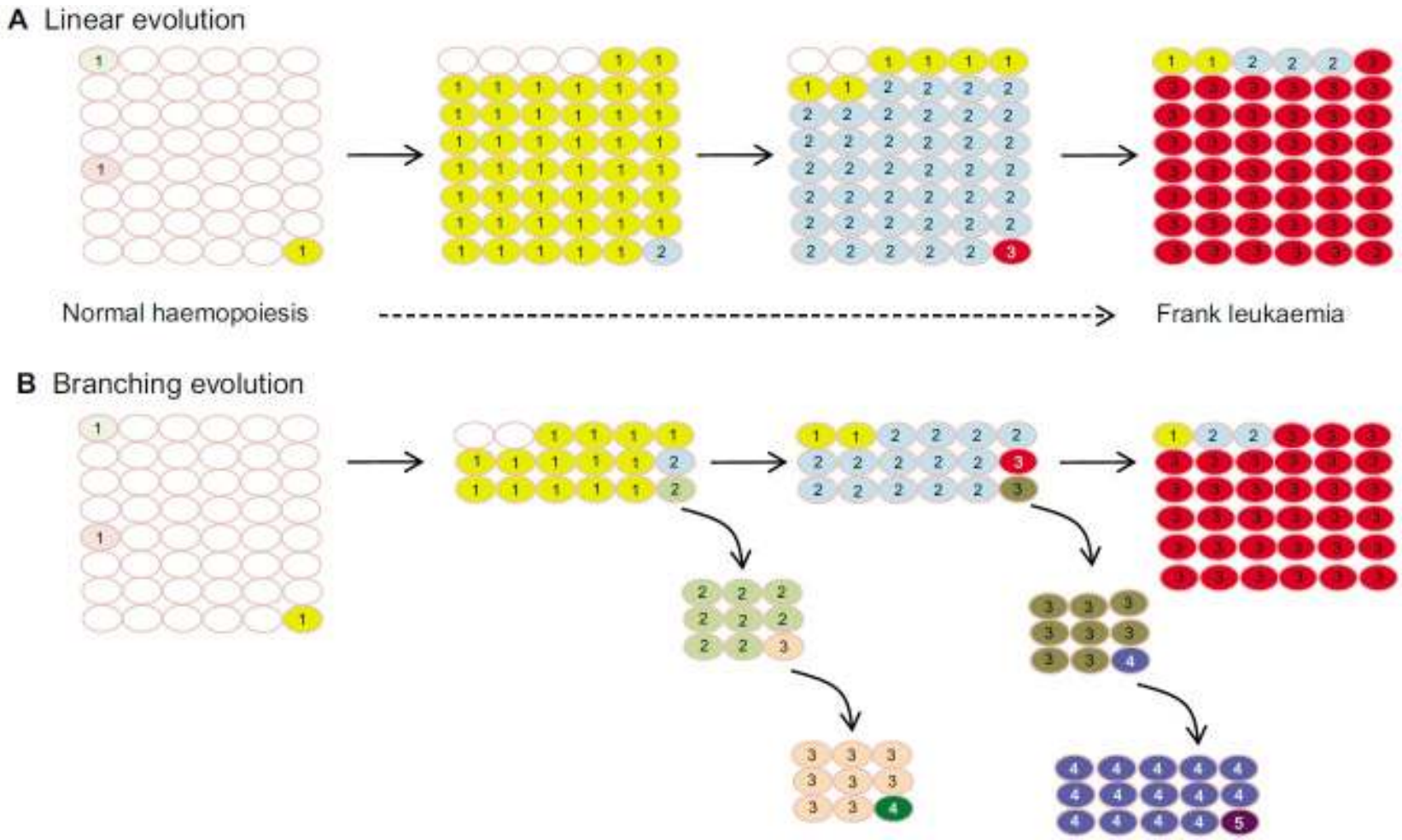


Multidirectional path to cancer



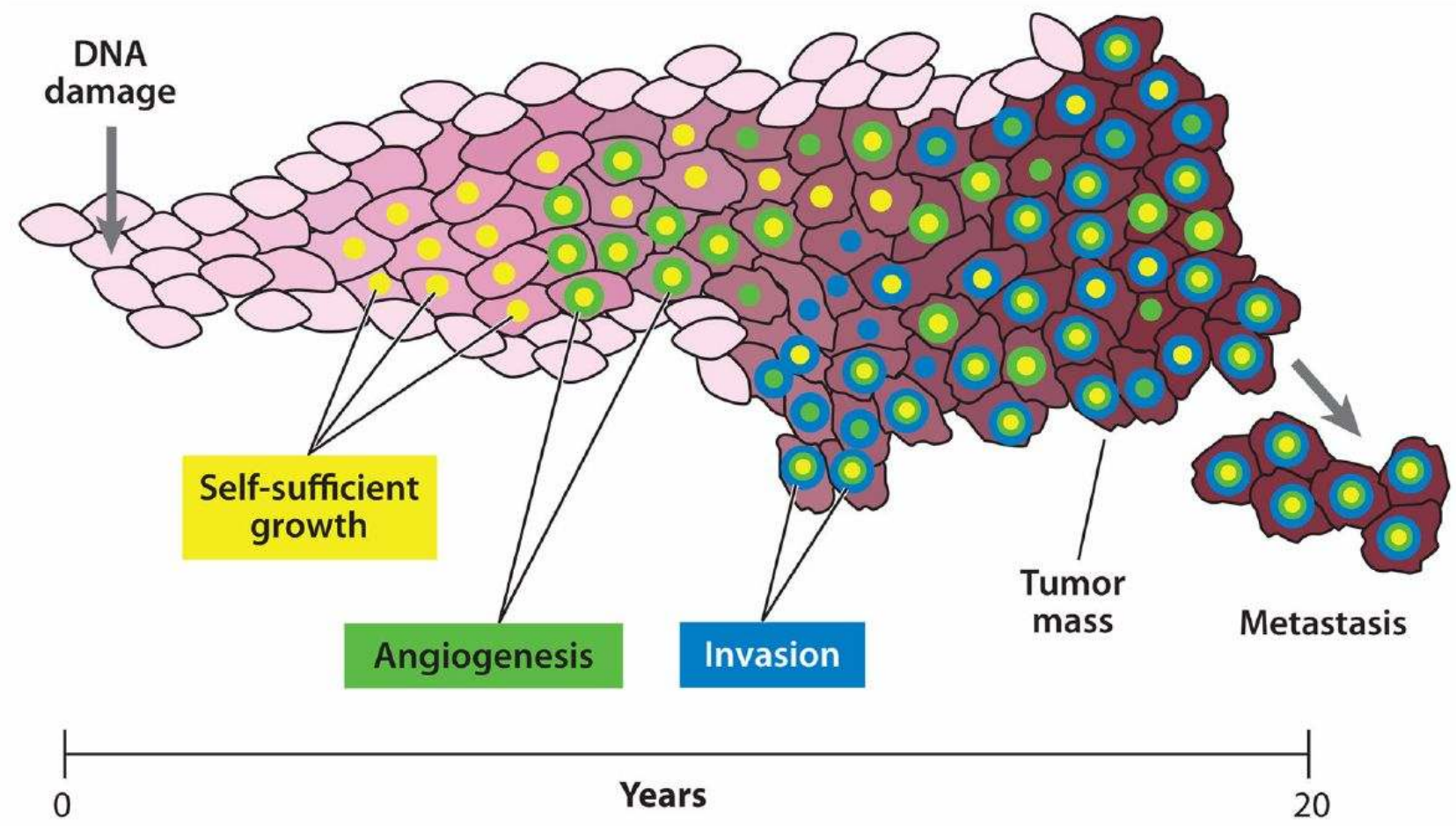
[Salk Annu Rev Pathol 2010]

Multidirectional path to cancer



[Grove, Dis Model Mech 2014]

Neoplasm heterogeneity



[Salk, Annu Rev Pathol 2010]

Genetics of cancer – stages of research (1)

- **random probing stage**
  - **identification of oncogenes** *(in viral DNA)* **and protooncogenes** *(in cellular DNA)*
  - **identification of tumor suppressors** *(linkage studies)*
  - **search for mutations in (proto)onkogenes and suppressor genes in various cancers** *(hit-or-miss)*
  - **studying interaction networks** *(searching for genes yet unknown to harbour cancerous mutations)*

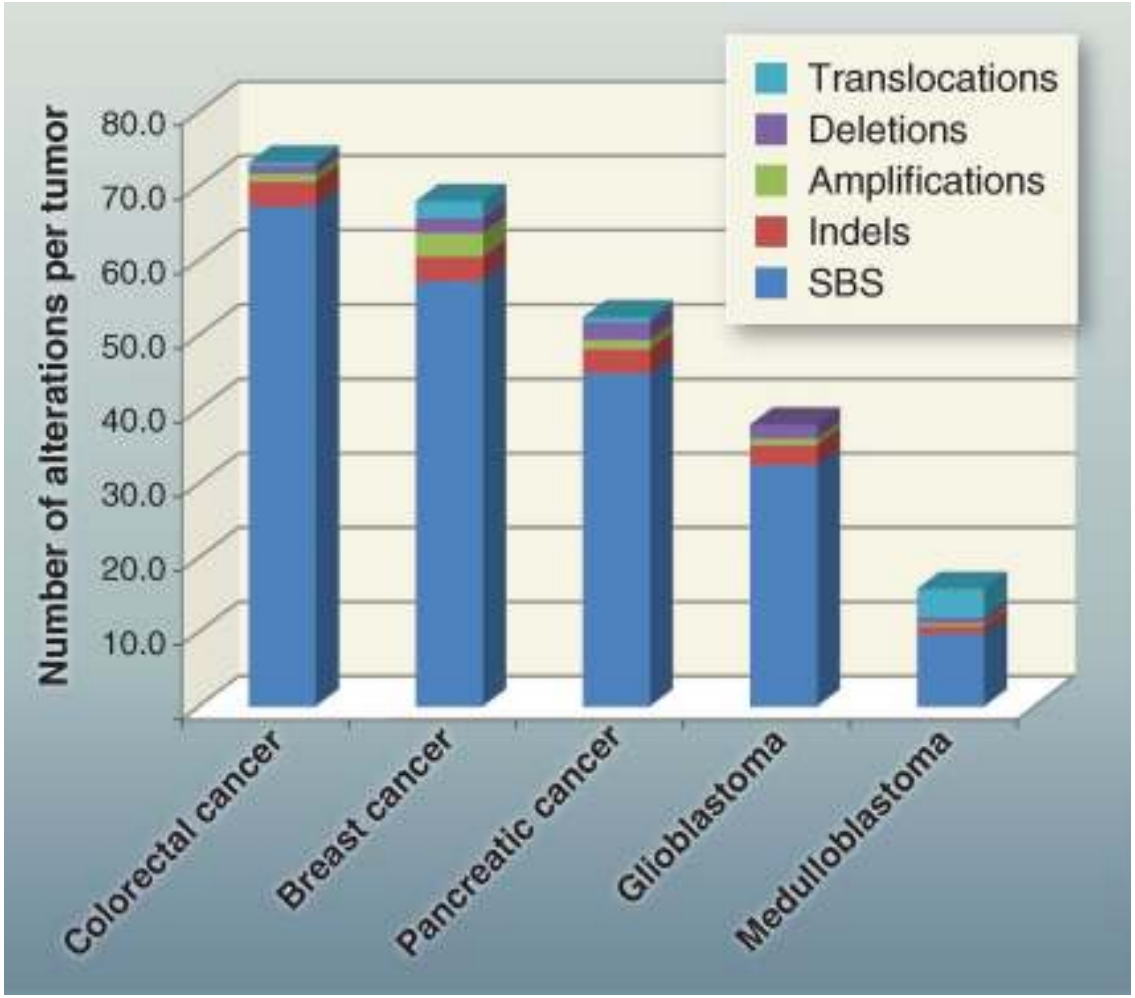
Genetics of cancer – stages of research (2)

- **complex studies (-omics studies)**
  - **microarrays**
    (transciptome, gene copy number variation (CNV), gene panels (sets), methylation arrays)
  - **new generation sequencing**
    (transcriptome, gene panels, exome, genome, methylome)

technology advances finally allowed us

- **to perform in-depth studies of cancer genetics**
- **to study ‘sporadic’ (non-familial) cases (>70%) and unexplained familial cases (~25?)**

Cancer genomics
- mutation types



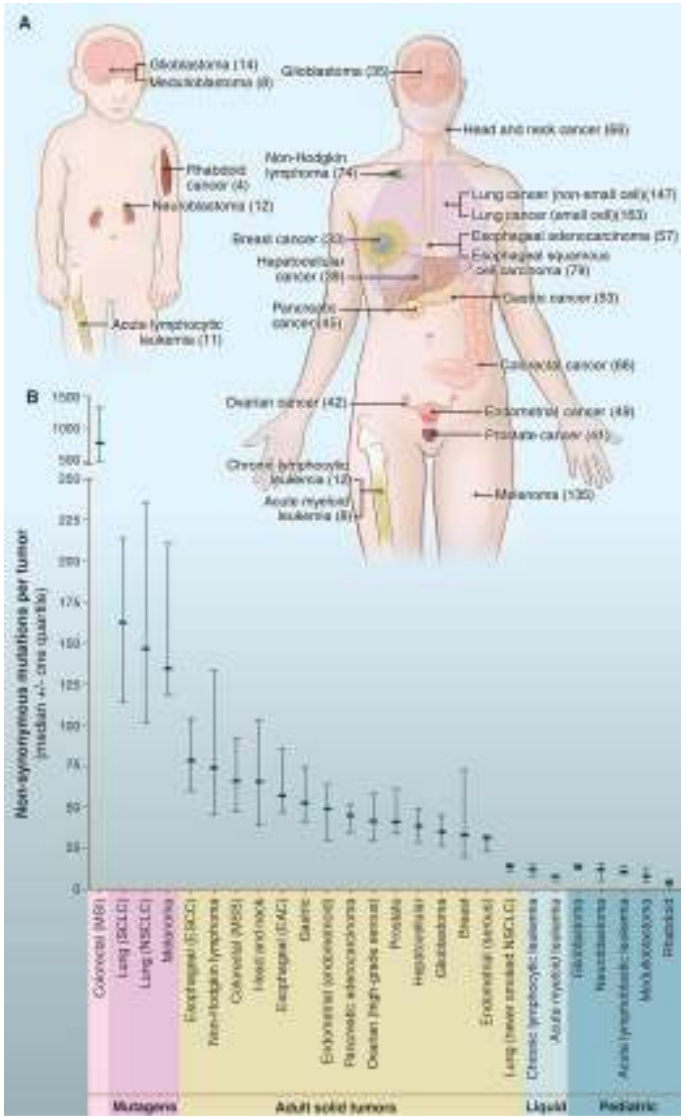
- neoplasms differ in their mutation profiles
- single base substitutions are dominant

[Vogelstein, Science 2013]

Cancer genomics
- frequency of all SBS

type	mut: /Mb	/genome
• medulloblastoma	0.15÷0.6	~1 000
• acute lymph. leukemia from T-cell precurs	0.3	<1 000
• chronic lymphocytic leukemia (CLL)	do 1.0	<3 250
• multiple myeloma	2.9	9 400
• prostate cancer	0.9	3 000
• breast cancer	1.2÷1.7	~4 700
• hepatocellular carcinoma (HCC)	4.2	<13 650
• colorectal cancer (CRC)	5.0	~16 250
• small cell lung cancer (SCLC)	7.4	~24 050
• non-small cell lung cancer (NSCLC)	17.7	~57 500
• melanoma (hairless skin)	3.0÷14.0	<45 500
• melanoma (hair)	5.0÷55.0	<160 000
• melanoma from UV exposure	111.0	360 000

Cancer genomics
- frequency of nonsynonymous SBS

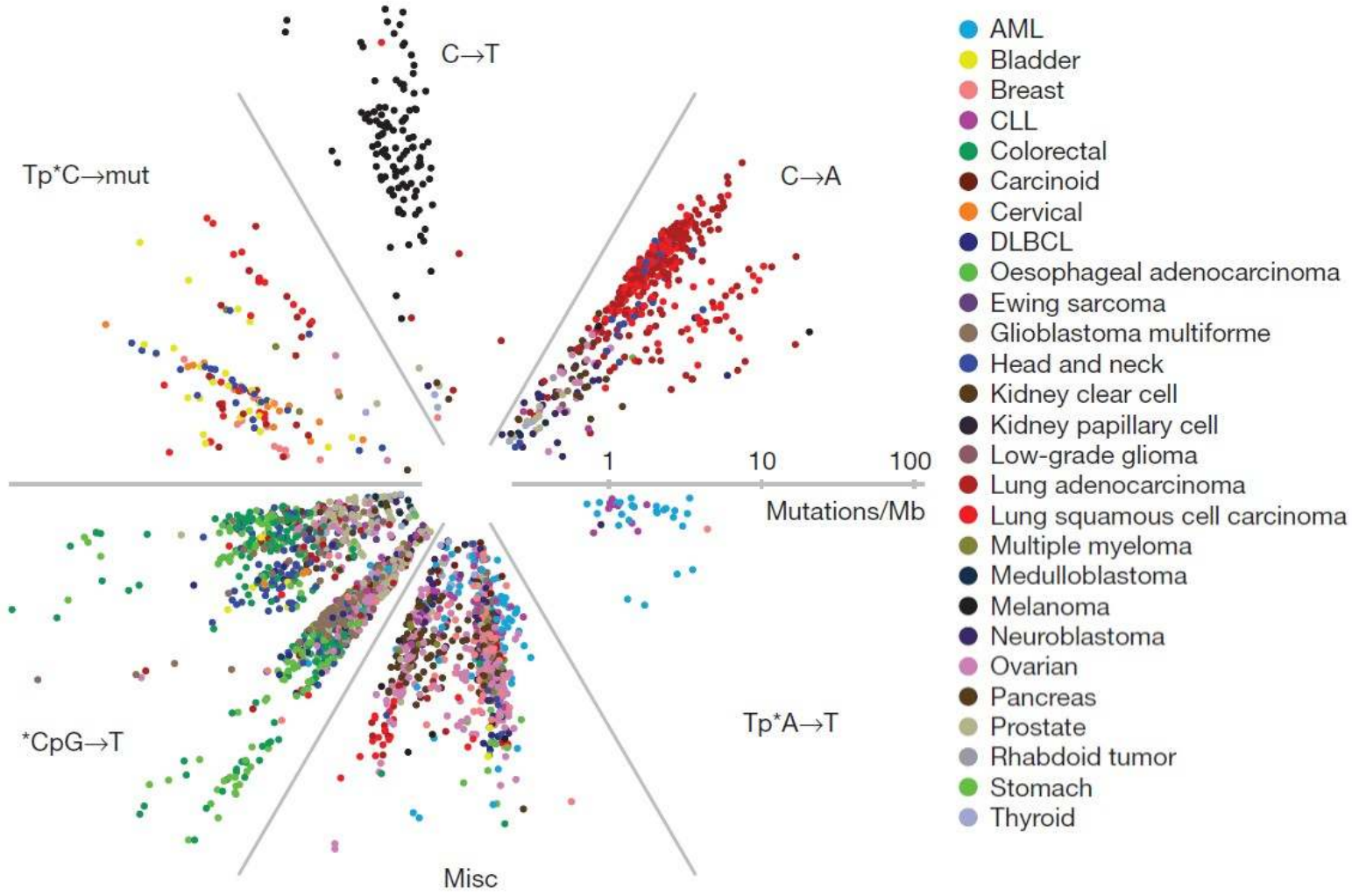


type	mut./genom
• medulloblastoma	do 1 000
• chronic lymphocytic leukemia	do 3 000
• breast cancer	~ 4 500
• hepatocellular carcinoma (HCC)	~13 500
• colorectal cancer(CRC)	~15 000
• small cell lung cancer (SCLC)	~24 000
• non-small cell lung cancer (NSCLC)	~57 000
• melanoma	~24 000

Less than 1% mut. = nonsynonymous!

[Vogelstein, Science 2013, tab.S1c]

Distribution of SBS in neoplasms – not quite random!



[Lawrence, Nature 2013]

- „nonrandomness" - each mutation reflects an underlying mechanism

Distribution of SBS in neoplasms – context matters

- **each substitution (such as C->T) can be further divided into subgroups according to its context** (neighbour bases)**:**
  - a**C**a **->** a**T**a a**C**c **->** a**T**c a**C**g **->** a**T**g a**C**t **->** a**T**t
  - c**C**a **->** c**T**a
  - g**C**a **->** g**T**a
  - t**C**a **->** t**T**a

  etc.
- **distribution of contexts can be studied in various types of cancer**

[Lawrence, Nature 2013]

Distribution of SBS in neoplasms – ~21 signatures

[Alexandrov, Nature 2013]

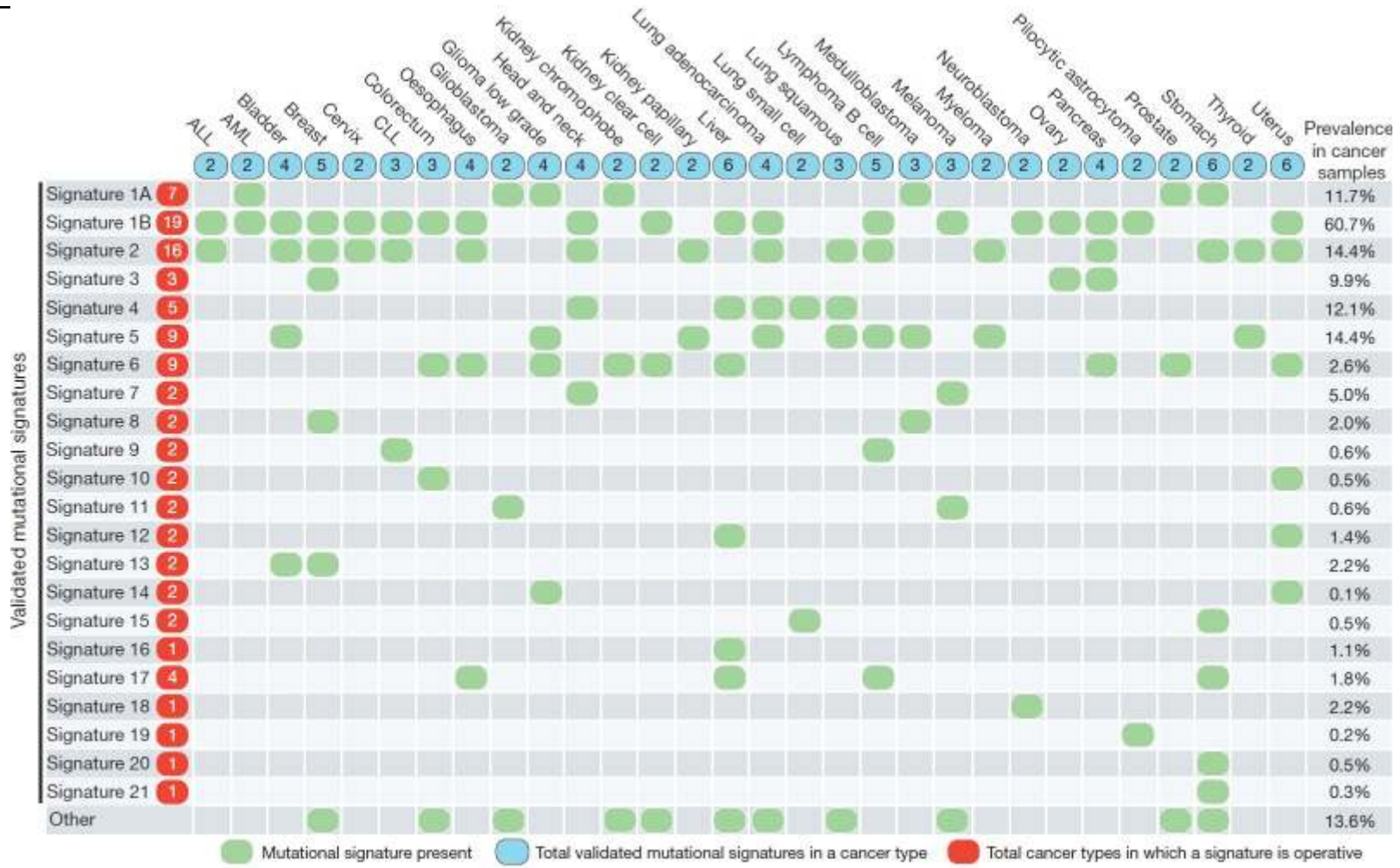
Distribution of SBS in neoplasms – ~21 signatures

age
age
APOBEC
BRCA1/2
smoking

MMR
UV

IgG hypermutations
DNA_Pol ε
Temozolomide

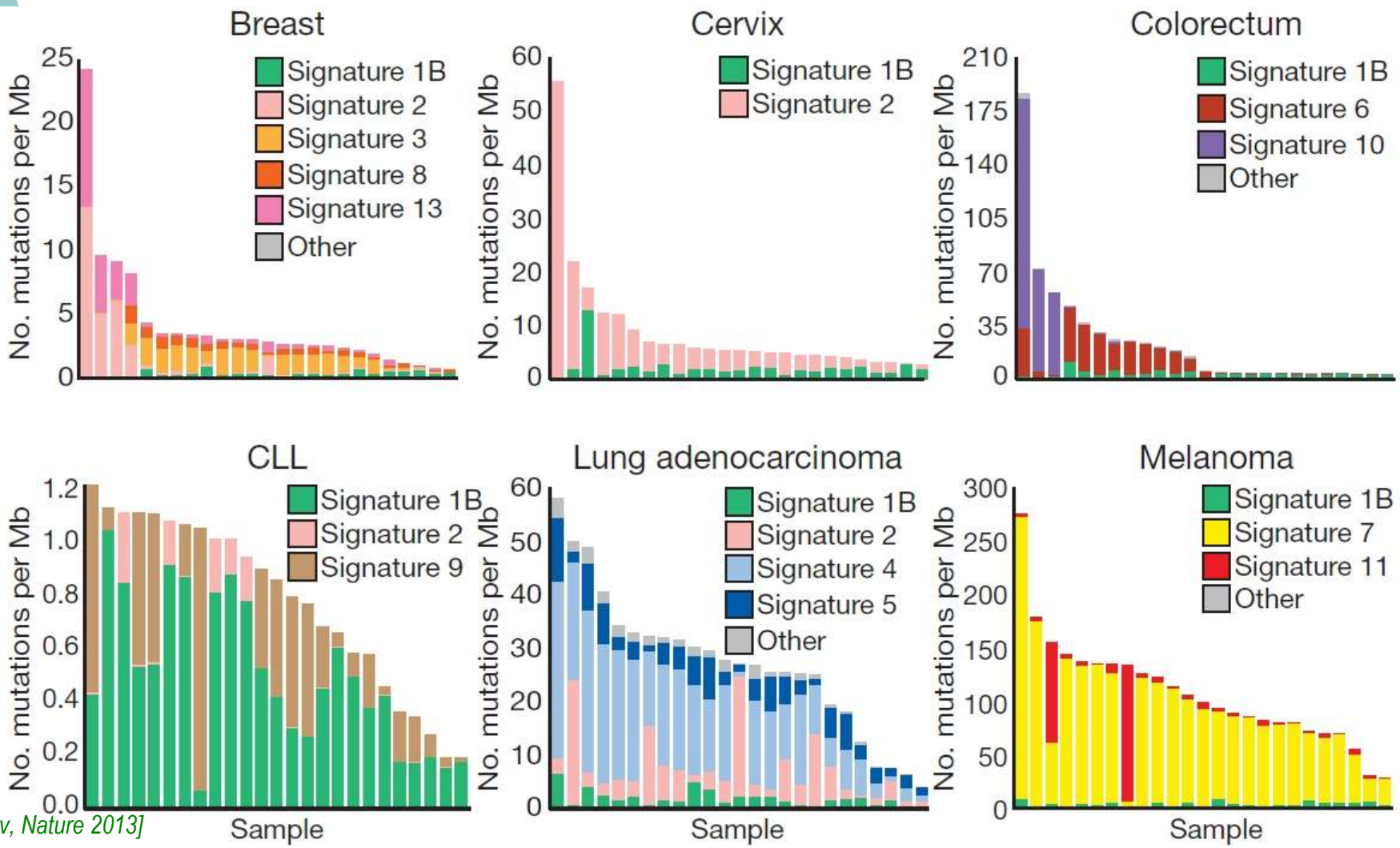
APOBEC



- Some of the signatures reflect known mechanisms of mutagenesis
- Others point towards yet-undiscovered ones ?

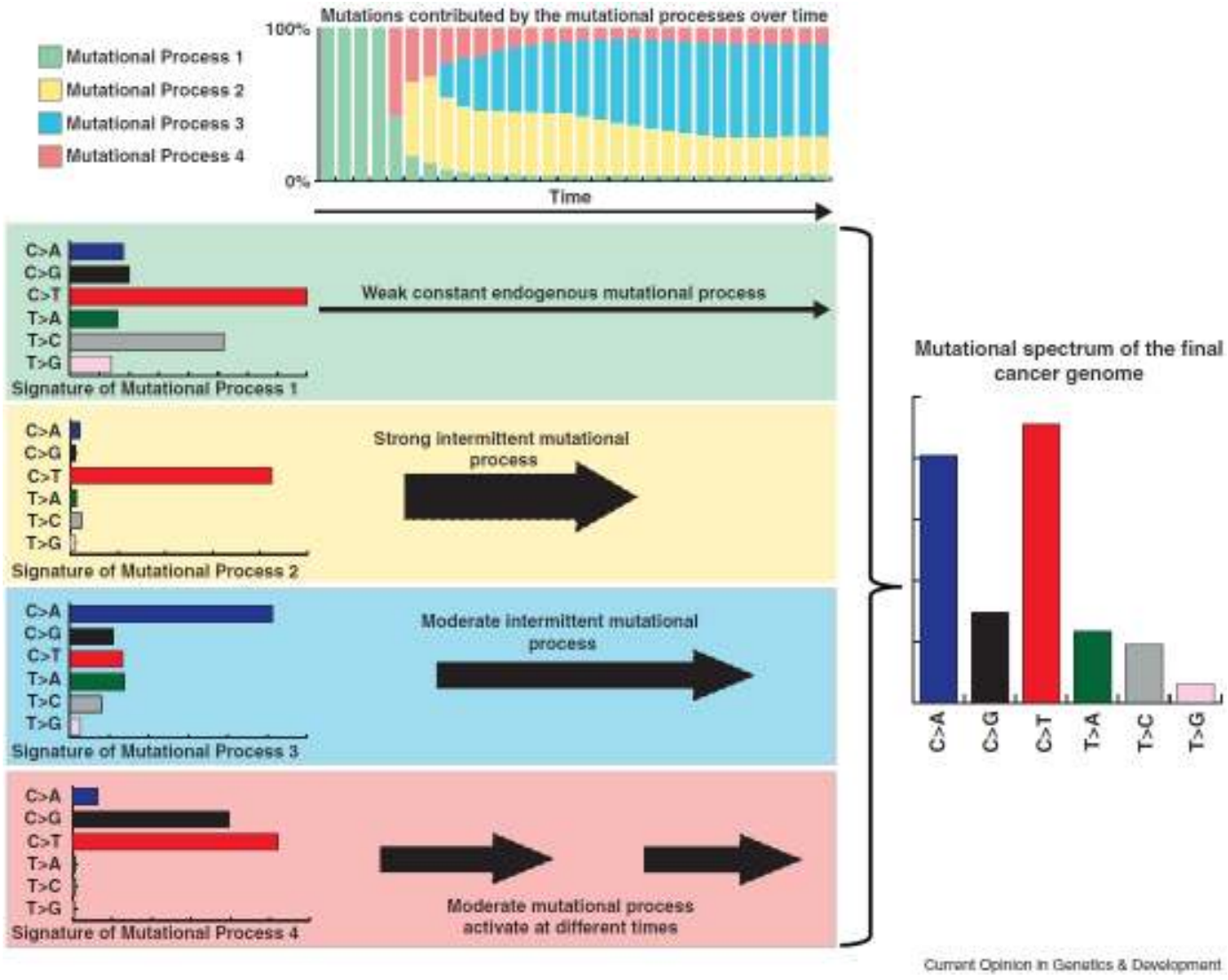
[Alexandrov, Nature 2013]

Distribution of various signatures in various neoplasms



[Alexandrov, Nature 2013]

Distribution of SBS in neoplasms – timing



[Alexandrov, Curr Opin Gen Dev 2014]

Multitude of mutations – drivers ↔ passengers

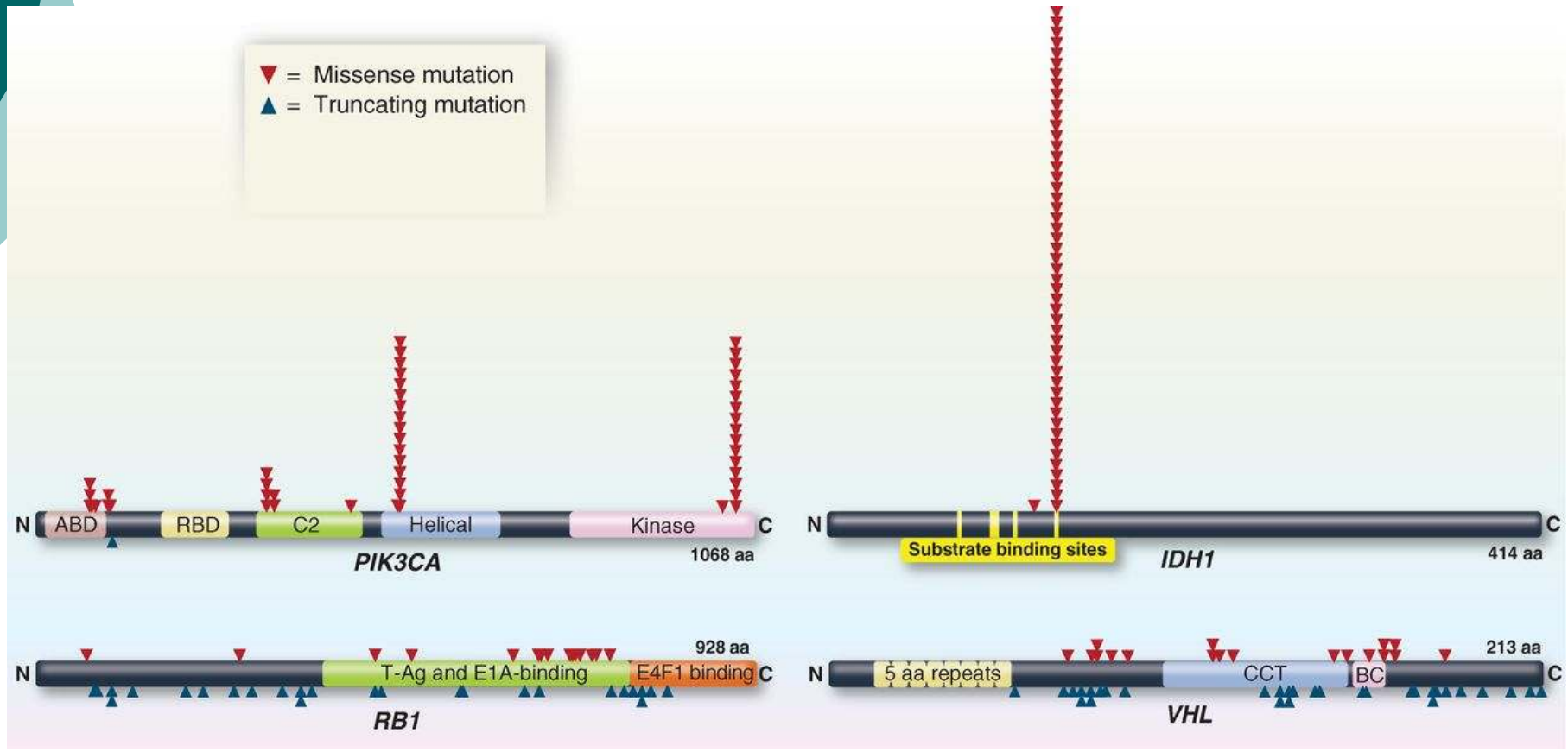
- **many genes mutated accidentally, with no importance for neoplasm growth => passengers**
- **how to tell the passenger from the driver?**
- **20/20 rule** *[Vogelstein, Science 2013]*:
  Mut-driver is a gene that can be classified as either oncogene or suppressor based on 20/20 rule

Multitude of mutations – drivers ↔ passengers

- **20/20 rule** *[Vogelstein, Science 2013]*:
  - **oncogene: >20% of small mutations cause missense changes and tend to focus in hot spots along the gene**
  - **suppressor: >20% of small mutations cause inactivation** (mostly nonsense mutations) **and tend to spread along the gene**

vast majority of known genes related to carcinogenesis easily fulfill these criteria

Multitude of mutations – drivers ↔ passengers



[Vogelstein, Science 2013]

Multitude of mutations – drivers

- **how many drivers (at present)**
  - **~500 (COSMIC database)** *(20/20 rule not aplied)*
  - **138** *(Vogelstein Science 2013) (20/20 rule aplied)*

- **What is important?**

that number is still increasing, but clearly approaches plateau (studies of new cancers result in ‘discovering’ already-known mutations)

Multitude of mutations – functional spectrum – DNA integrity

DNA control and repair:

- TP53, ATM;
- STAG2
- MLH1, MSH2, MSH6
- BRCA1, BRCA2;BAP;

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – DNA integrity

DNA control and repair:

(plus susceptibilities:

- **TP53, ATM;** CHEK2
- **STAG2**
- **MLH1, MSH2, MSH6** PMS1, PMS2
- **BRCA1, BRCA2;BAP;** BRIP1, PALB; FANCA, FANCC,FANCD2, FANCE, FANCF, FANCG
- ERCC2, ERCC3, ERCC4, ERCC5; XPA, XPC;
- BLM; NBS;
- WRN, RECQL4;

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – cell cycle, apoptosis

cell cycle and apoptosis:

- **CDKN2A**
- **RB1, TP53**
- **BCL2, CASP8, DAXX**
- **TRAF7, ABL1, CARD11, CDC73, CYLD, FUBP1, MYD88, NFE2L2, NPM1, PPP2R1A, SETBP1, TNFAIP3, MED12**

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – RAS pathway

RAS etc.:

- ALK, CSF1R, EGFR, ERBB2, FGFR2, FGFR3, FLT3, KIT, MET (HGFR), PDGFRA, RET(GDNF-R)
- HRAS, KRAS, NRAS
- GNA11,GNAQ,GNAS, NF1
- BRAF, MAP3K11, MAP2K1
- PTPN11
- CIC, CBL, B2M, CEBPA
- VHL

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – PI3K pathway

PI3K etc.:

- ALK, CSF1R, EGFR, ERBB2, FGFR2, FGFR3, FLT3, KIT, MET (HGFR), PDGFRA, RET(GDNF-R), TSHR
- HRAS, KRAS, NRAS
- GNA11,GNAQ,GNAS, NF1, TSC1, PTEN
- BRAF, MAP3K11, MAP2K1, PIK3, AKT,
- PTPN11
- CIC, CBL, B2M, CEBPA
- VHL,

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – MAPK pathway

MAPK etc.:

- ALK, CSF1R, EGFR, ERBB2, FGFR2, FGFR3, FLT3, KIT, MET (HGFR), PDGFRA, RET (GDNF-R), **TSHR**
- HRAS, KRAS, NRAS
- **GNA11**, **GNAQ**, GNAS, NF1
- BRAF, MAP3K11, MAP2K1, **MAP3K1**
- PTPN11
- CIC, CBL, **B2M**, **CEBPA**
- VHL, **TNFAIP3**

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – STAT pathway

STAT etc.:

- ALK, CSF1R, EGFR, ERBB2, FGFR2, FGFR3, FLT3, KIT, MET (HGFR), PDGFRA, RET(GDNF-R), CRLF2
- HRAS, KRAS, NRAS
- GNA11, GNAQ, GNAS, NF1
- BRAF, MAP3K11, MAP2K1, JAK1-JAK3
- PTPN11
- CIC, CBL, B2M, CEBPA
- VHL, MPL, SOCS

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – TGFb

TGFB path:

- ACVR1B
- GNAS
- SMAD2, SMAD4
- MED12
- EP300, FOXL2, GATA1, GATA2

(in inherited also:

- BMPR1A)

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – Notch i HH

NOTCH etc. :

- **NOTCH1, NOTCH2**
- **FBXW7**
- **GATA1, GATA2**
- **EP300**

HH itp. :

- **PTCH1**
- **SMO**
- **SPOP**

(in inherited susceptib.:

- EXT1, EXT2, SUFU)

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – APC pathway

APC path:

- **CDH1, CTNNB1, APC, AXIN1;**
- **FAM123B, GNAS, NF2;**
- **RNF43, EP300, HNF1A, SOX9,**

(inherited susceptibility:

- PRKAR1A)

[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – transcription factors

Transcription factors:

- AR
- BCOR
- GATA3
- PHF6
- RUNX1
- SF3B1, SRSF2, U2AF1
- CREBBP
- KLF4

(inherited susceptibility:
- DICER1, PHOX2B, SBDS)

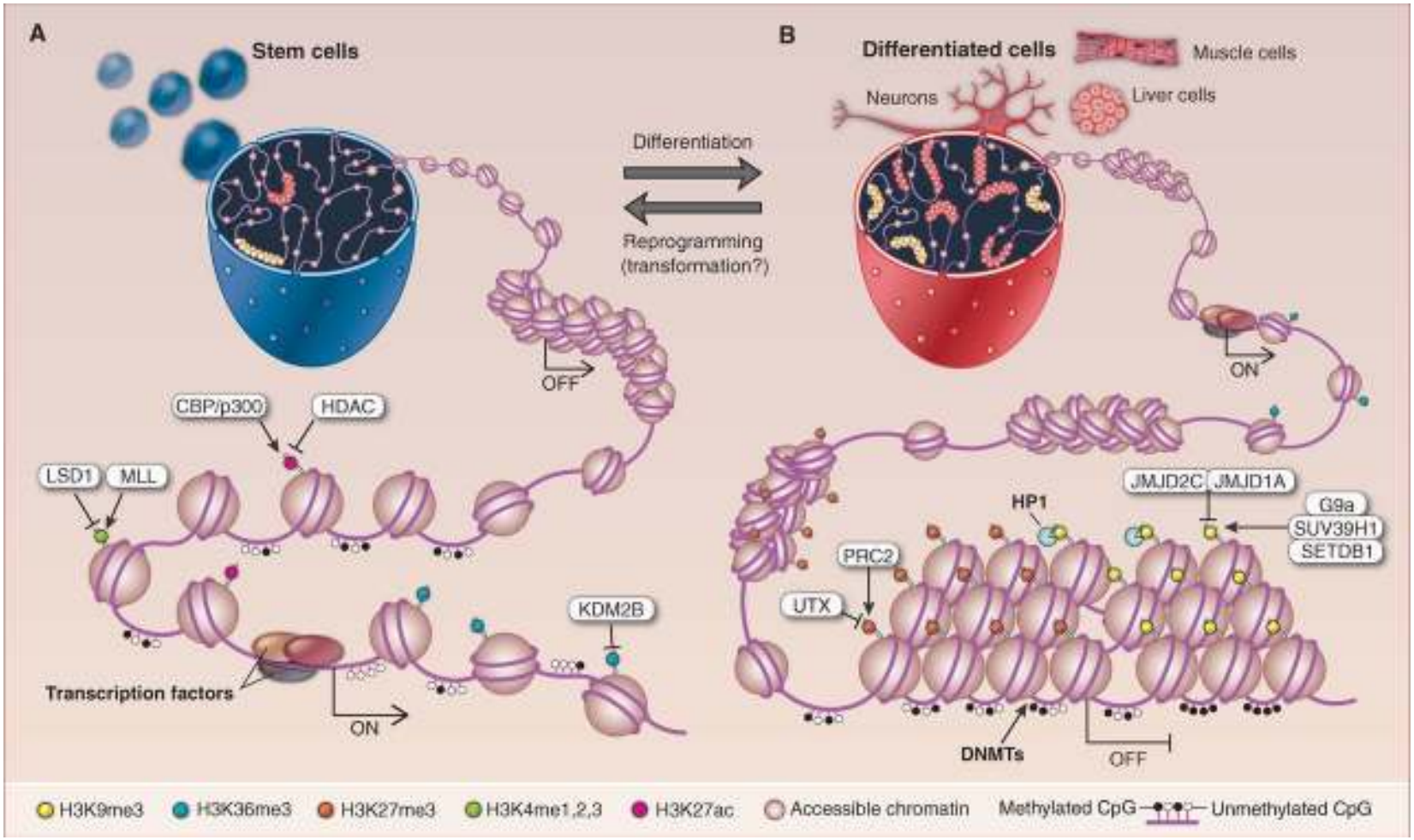
[Vogelstein, Science 2013, TabS2a]

Multitude of mutations – functional spectrum – chromatin state

- **More than half of newly discovered drivers is related to regulation of chromatin structure (histone modification, DNA methylation)**

[Vogelstein, Science 2013, TabS2a]

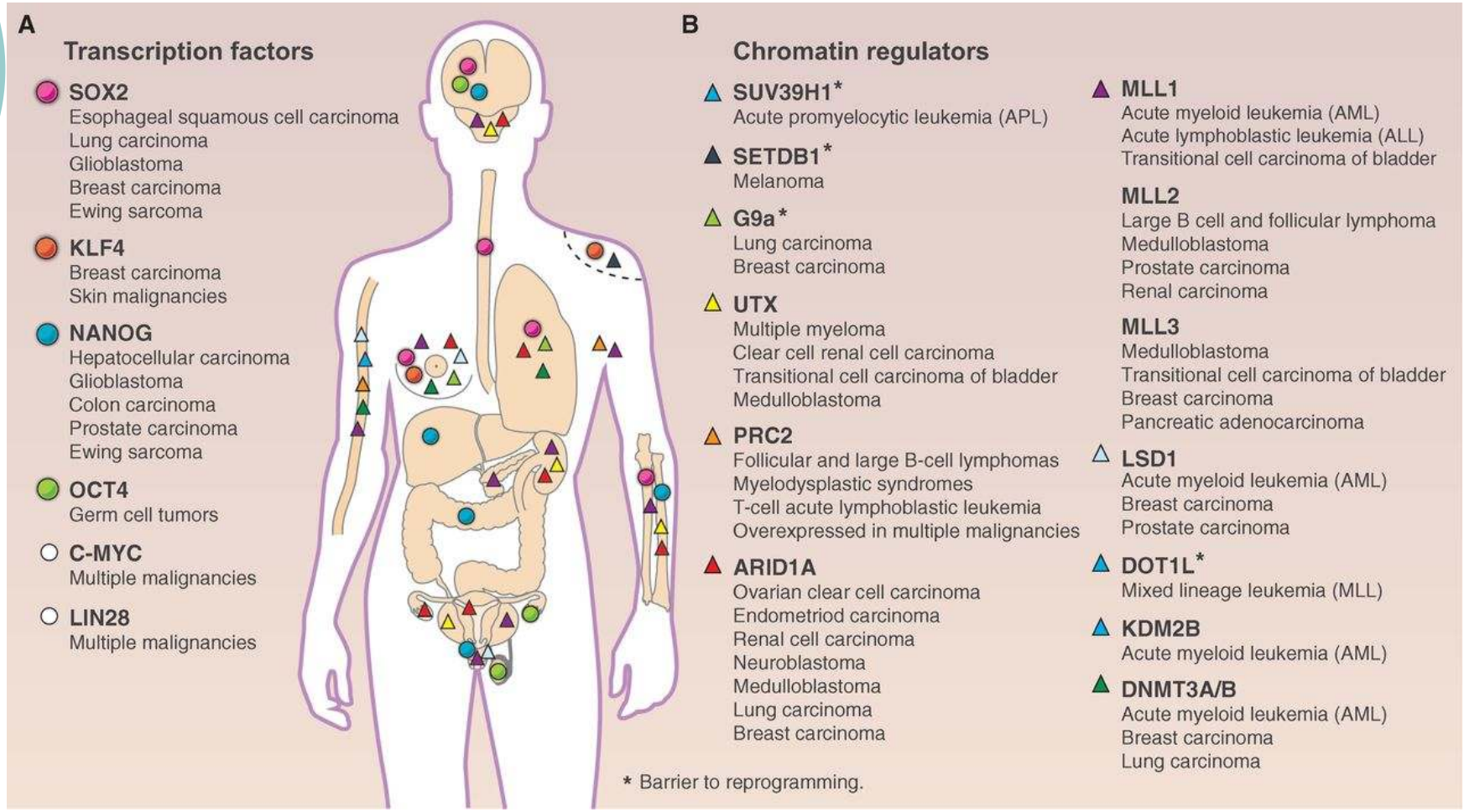
Multitude of mutations – functional spectrum – chromatin state



- **methylation of DNA & modification of histones -> ‘packed’ DNA -> inaccessible („silenced”) genes**

[Suva, Science 2013]

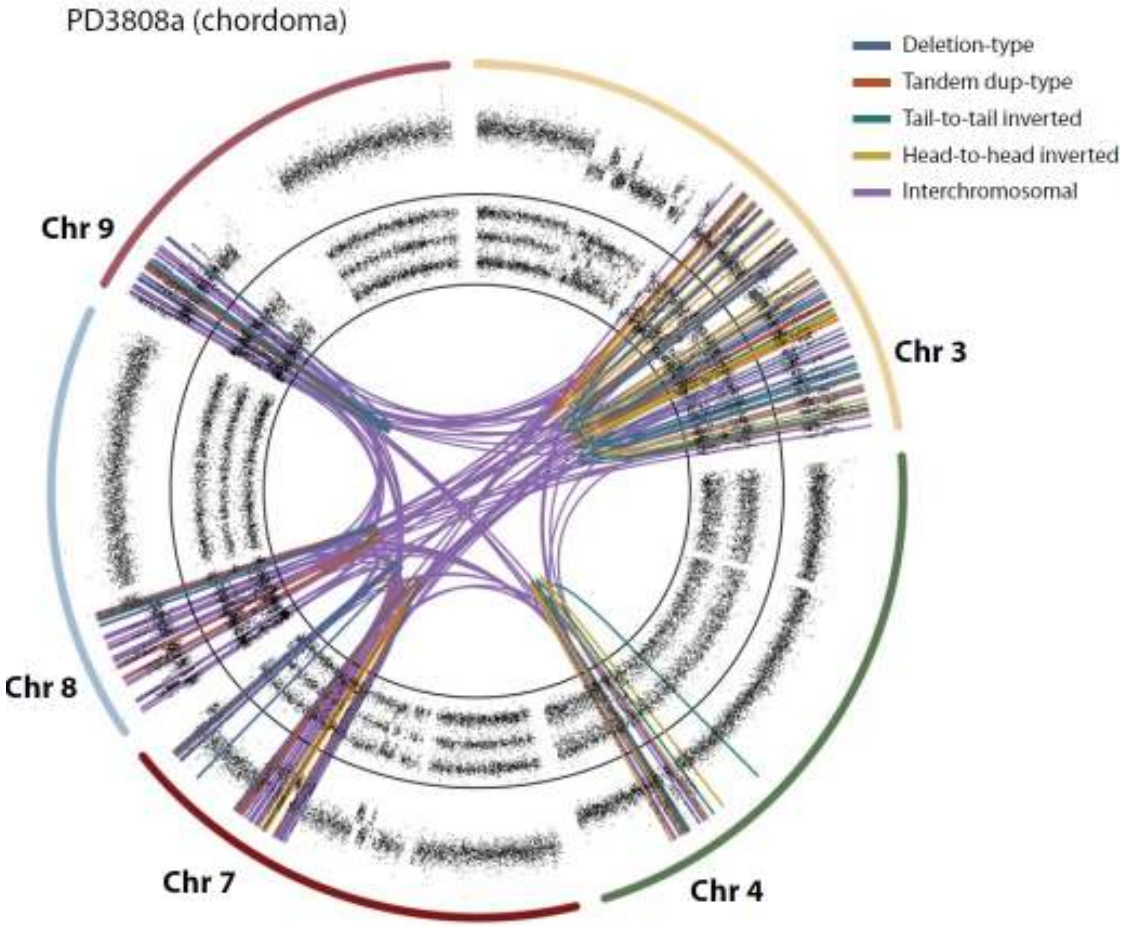
Multitude of mutations – functional spectrum – chromatin state



[Suva, Science 2013]

Mechanisms of carcinogenesis – large mutations – chromothripsis (1)

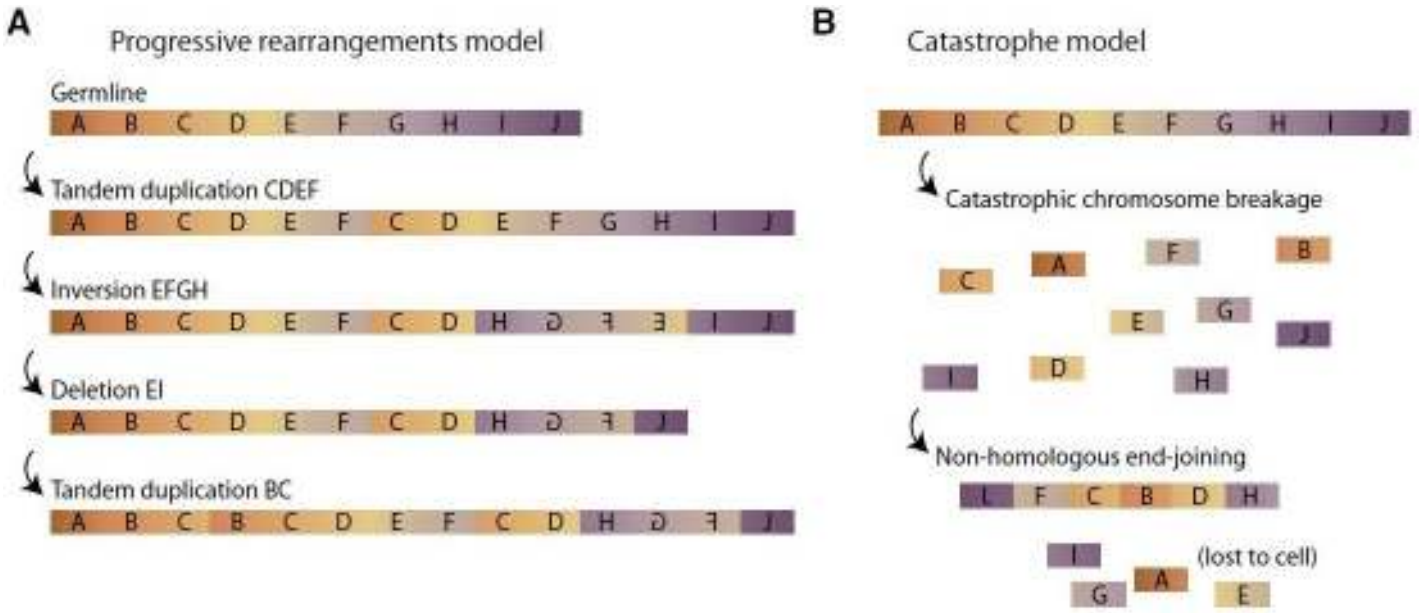
chromothripsis : shattering of chromosome(s) (or its part) followed by glueing of pieces at random
(Stephens, Cell 2011)



[Stephens, Cell 2011]

Mechanisms of carcinogenesis – large mutations – chromothripsis (2)

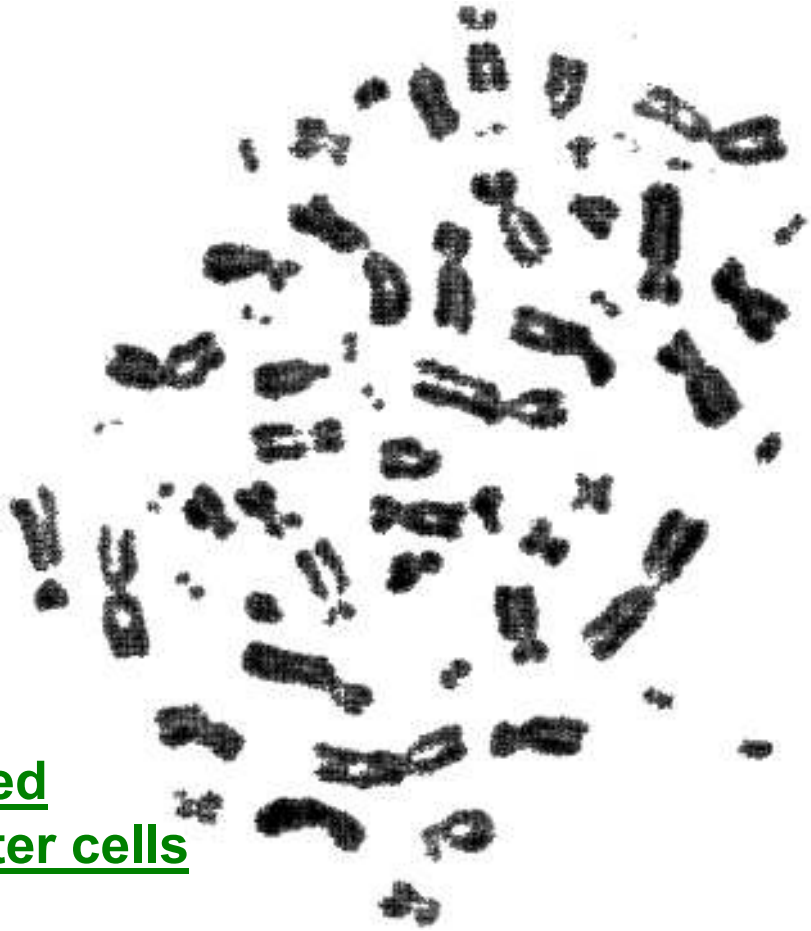
- **chromothripsis:**
  sudden mupltiple changes (B),
  not a gradual accumulation of changes (A)



[Stephens, Cell 2011]

Mechanisms of carcinogenesis – large mutations – chromothripsis (4)

- **double minute chromosomes**
  - small chromosome-like entities
  - discovered in neoplasms of children *(Cox, Lancet 1965)*
  - no centromeres
  - no telomeres
  - during cell division **separated randomly between daughter cells**



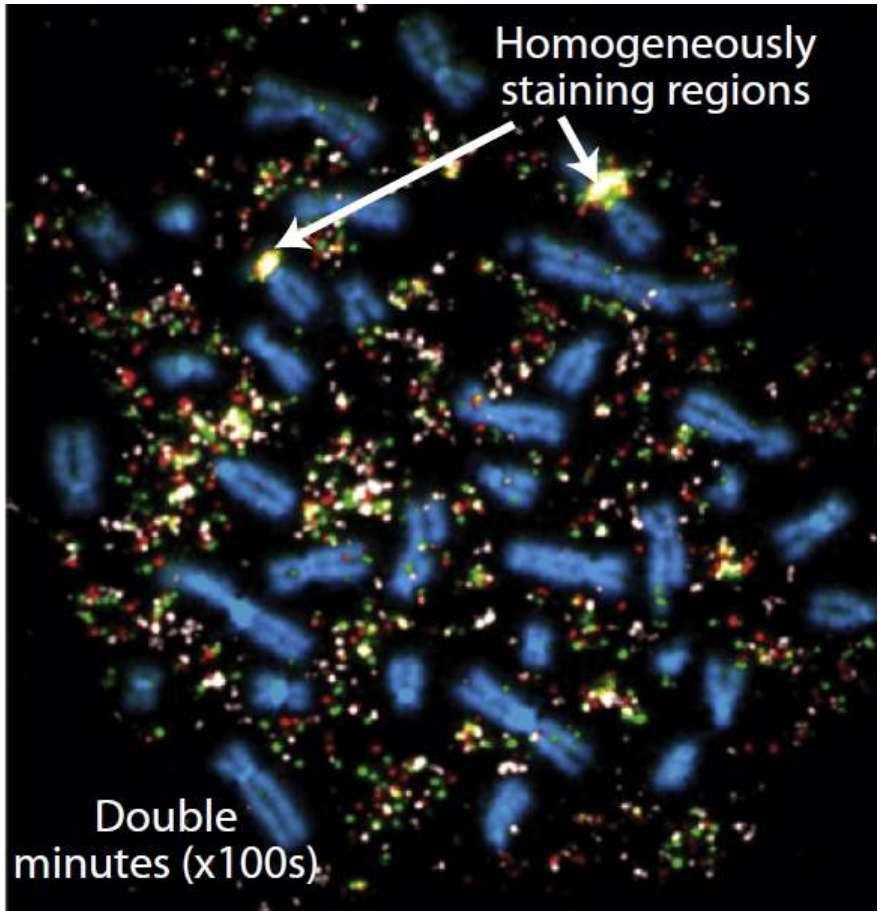
[Cox, Lancet 1965]

Mechanisms of carcinogenesis – large mutations – chromothripsis (5)

- **double minute chromosomes after chromothripsis** (example)

Read more:

- Korbel, Cell 2013; 152(6): 226
- Li, Nature 2014; 508(7): 494
  rob(15;21)(q10;q10)c
  2500× increased risk of ALL



[Stephens, Cell 2011]

Oncogenetics – usage

- **diagnosis, prophylaxis and follow-up**
- **treatment**
  - **selecting from existing drugs** (convent. / targeted)
  - **new indications for known drugs** *(repurposing)*
  - **discovering new targets => new drugs**
  - *(synthetic lethality)*
  - **methabolic vulnerabilities**

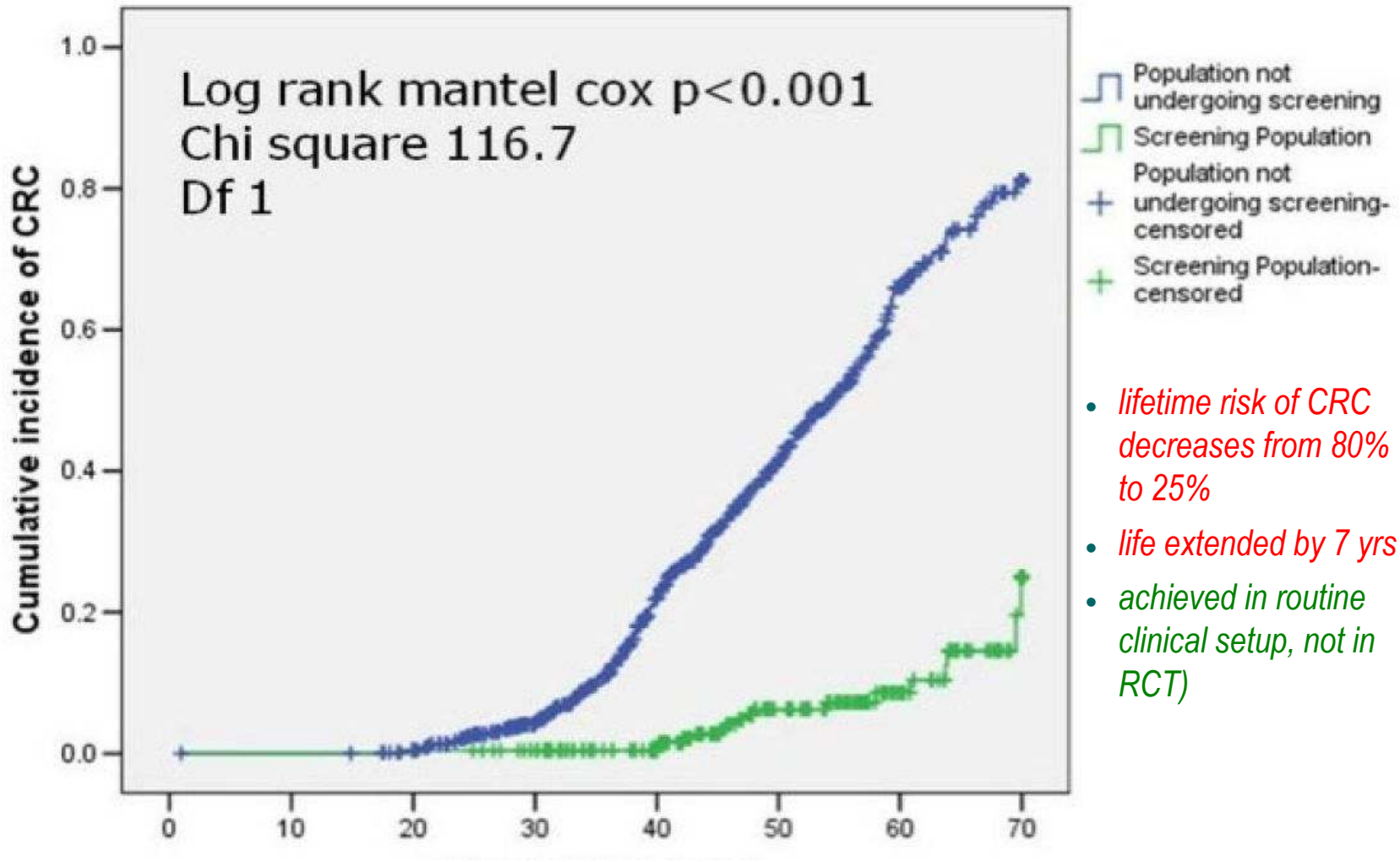
Oncogenetics in diagnostics – inherited predispositions

- **if the background of susceptibility is known, then screening is at present:**
  - **possible**
  - **relatively cheap**
- **BUT it may be indicated only if:**
  - **disease runs in the family**
    (or the cost of test further drops down)
  - **early detection will change the fate of the patient**
    (efficient prophylactic/therapeutic interventions exist)
  - **cost is adequate** (HTA)
    (some syndromes are simply too infrequent to)

Oncogenetics in prophylaxis – Lynch syndrome (1)

- **risk of cancer (lifelong cumulated) :**
  - **colon & rectum (30÷70%), small intestine, stomach, pancreas (4%), bile ducts**
  - **uterus (30÷60%), ovary (4-12%)**
  - **bladder (8%)**
- **known genetic basis** (possible to study)
- **prophylaxis and follow up** *(ESMO 2013)*
  - **aspirin 600mg/d => 60% decrease of CRC** *(Burn, Lancet 2011)*
  - **colonoscopy every 2y since 25 y => decrease of incidence of CRC from ~70% to ~10%** *(Jarvinen, Gastroenterology 2000)*
  - **ginecologic examinations, biopsy, USG, Ca125 level in blood yearly since 30–35 y old**

Oncogenetics in prophylaxis – Lynch syndrome (2)

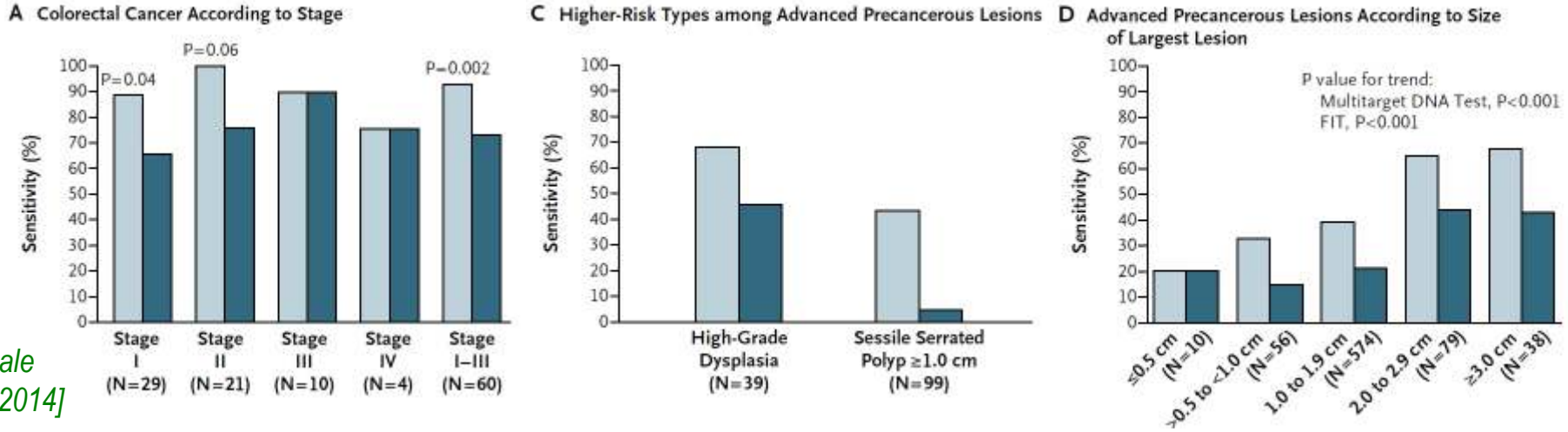


- *lifetime risk of CRC decreases from 80% to 25%*
- *life extended by 7 yrs*
- *achieved in routine clinical setup, not in RCT)*

[Newton, Colorect Dis 2014]

Oncogenetics in diagnostic – CRC (sporadic) (not inherited)

- **traditional screening: for 50y old and above**
  - **FIT (fecal immunochemical test), colonoscopy**
- **new test - DNA in stool** *(FDA 2014):*
  - **methylation of *NDRG4* & *BMP3*, mutations *KRAS & CATB***
  - **among 9989 pers: 65 (0.7%) cancers**
    757 (7.6%) precancerous conditions
  - **test DNA detected CRC in 92.3%, FIT in 73.8%, p=0.002**



[Imperiale NEJM 2014]

Oncogenetics in therapy – targeted therapy

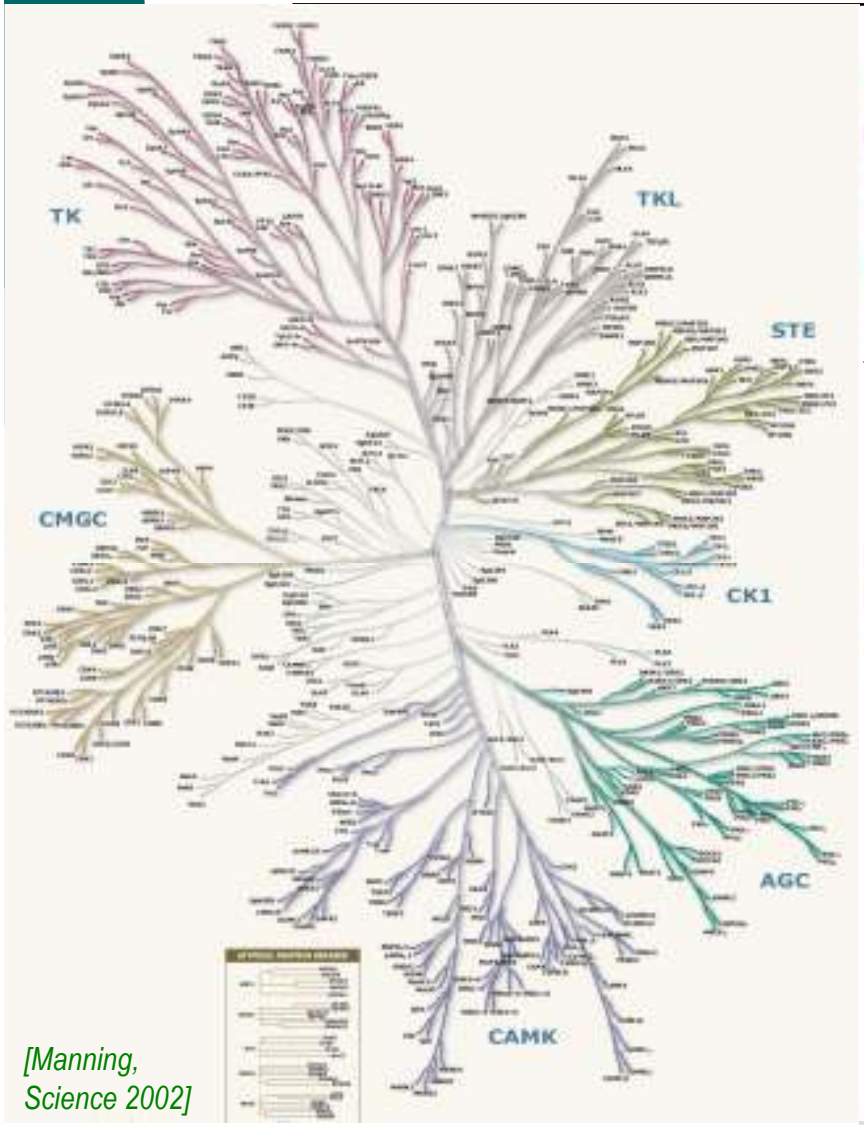
Targeted:

- **directed at the molecular level**
  - aiming at the molecule (not the disease!)
- **target shoud be a molecule playing major role in pathogenesis**
  - **protein** (most of the time)
  - **gene or RNA**
    (potentially; rarely used)

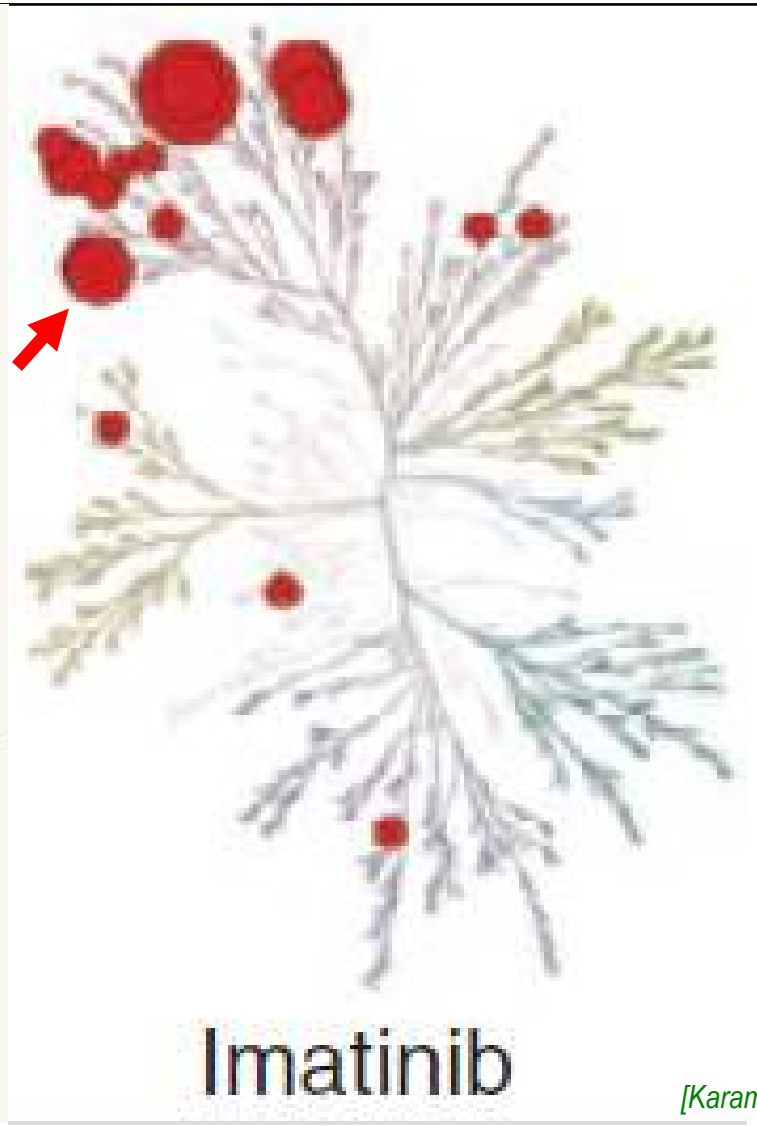
Oncogenetics in therapy – targeted therapy: oncogenes

- target: mRNA or protein encoded by an oncogene
  - mostly protein kinases
- aim: decreasing activity
- formula:
  - inhibitor of enzyme
    - small molecule inhibitors (-inib)
    - antibody inhibitors (-umab)
    - others

Oncogenetics in therapy – targeted therapy: kinase inhibitors



[Manning, Science 2002]



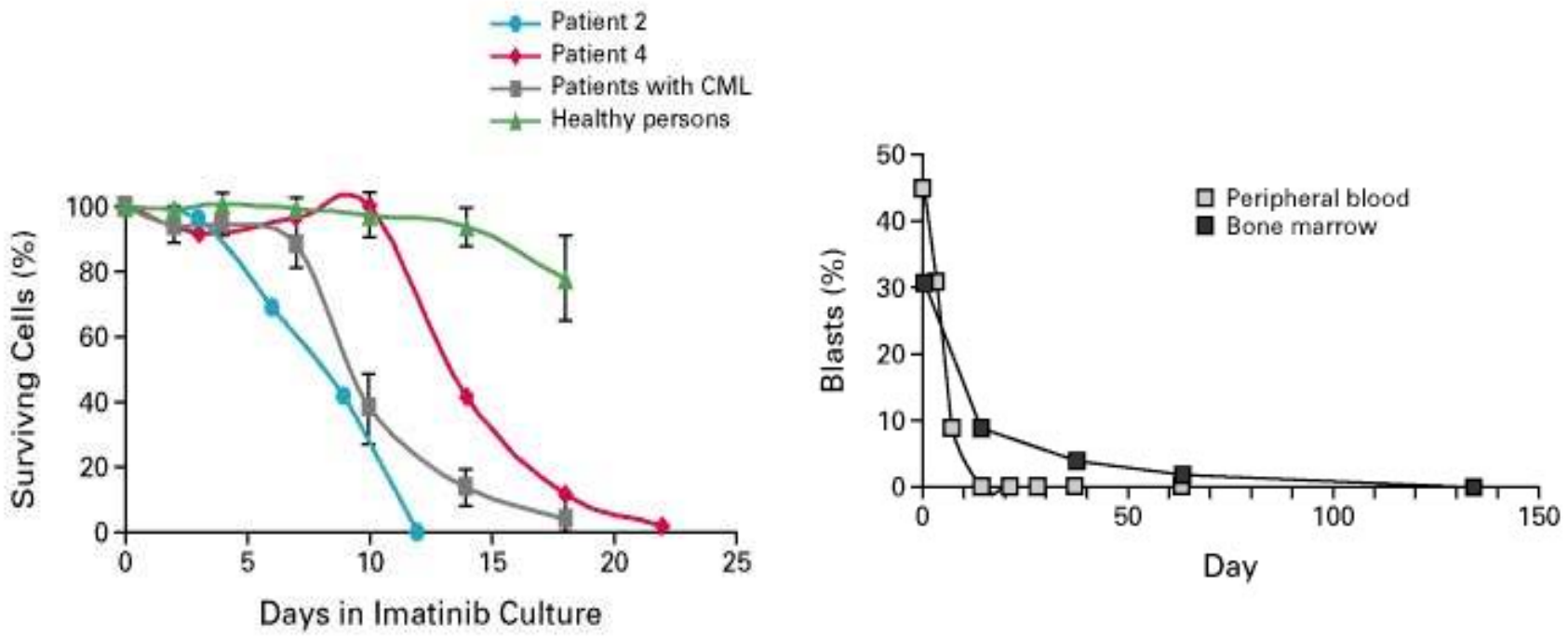
[Karaman, Nat Biotechnol 2008]

Kinases pedigree in human (left)

Imatinib inhibition profile in compare to all human kinases (right)

Oncogenetics in therapy – targeting oncogenes – imatinib (1)

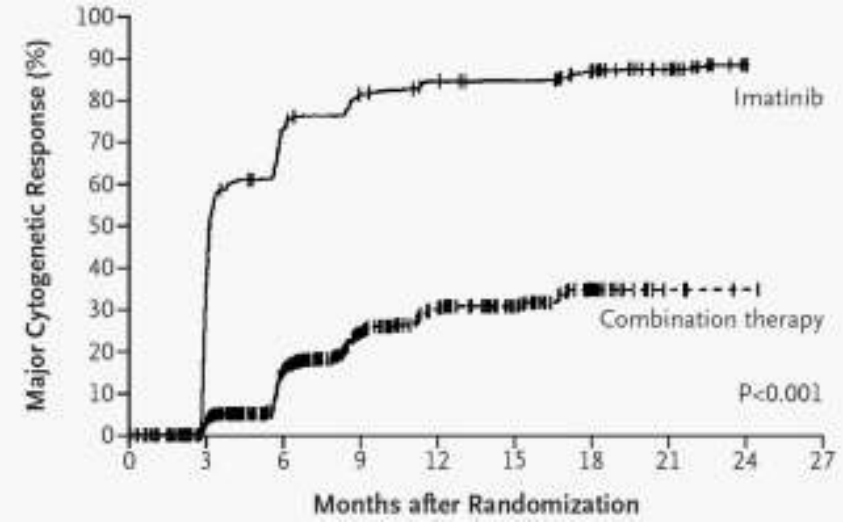
- **imatinib – first potent targeted drug inhibits activity of BCR-ABL fusion**

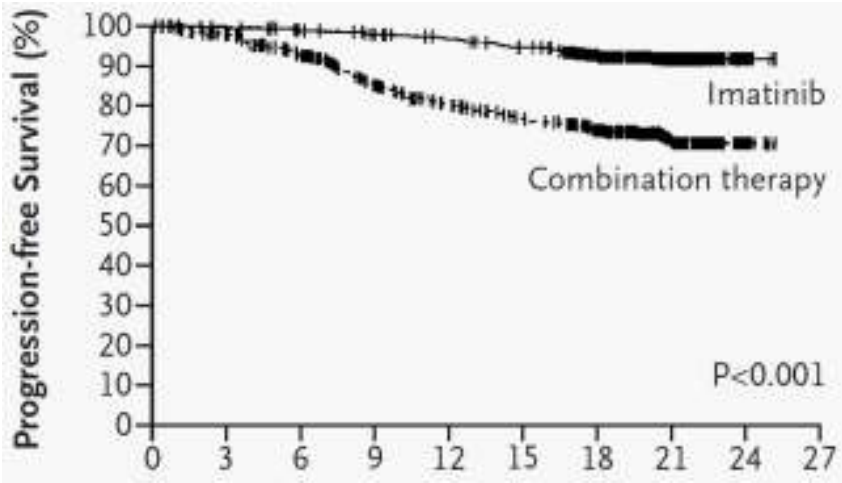


Blast cell survival rate after imatinib treatment, in vitro (left), blood and bone marrow (right)

Oncogenetics in therapy – targeting oncogenes – imatinib (2)

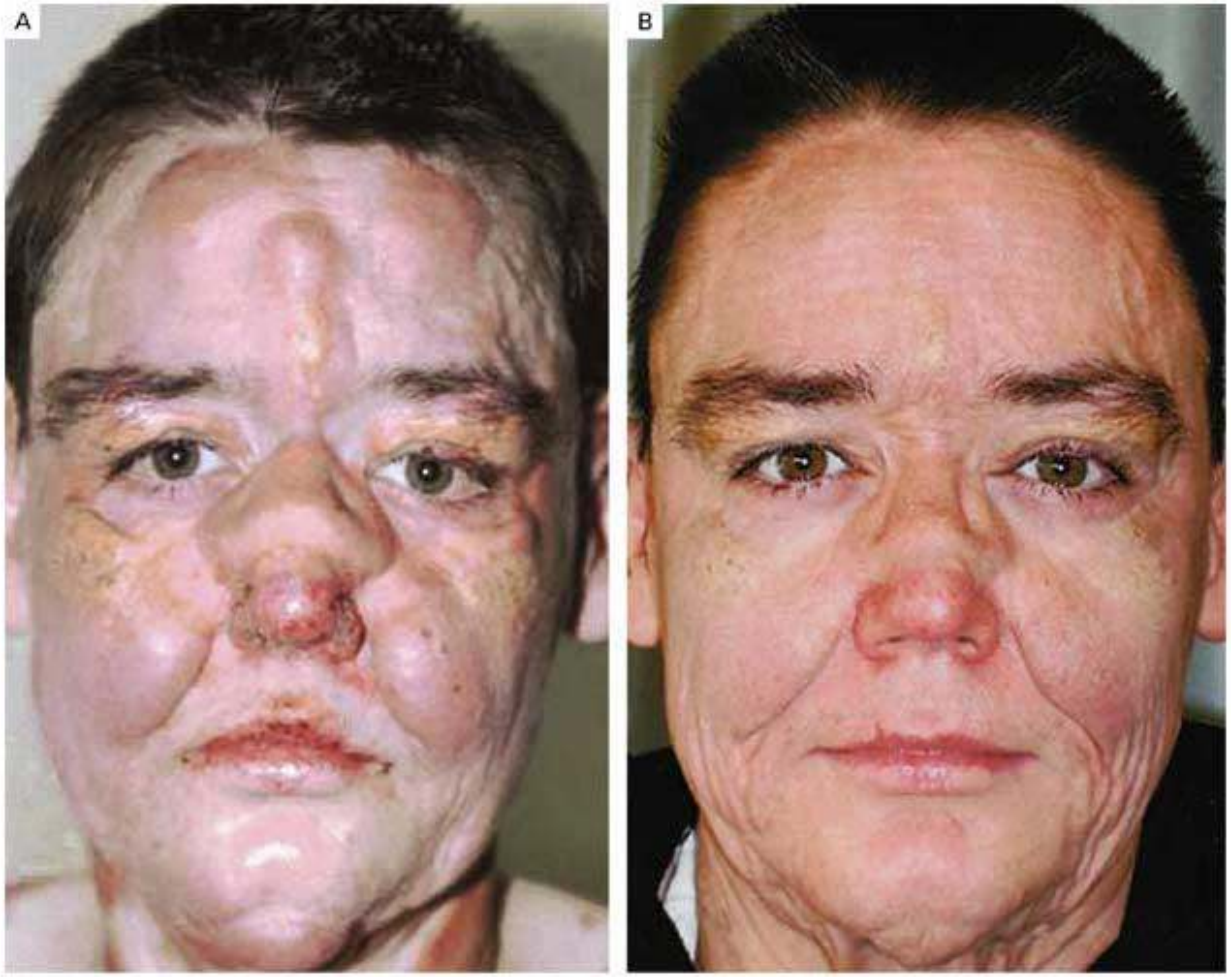
- **imatinib – first potent targeted drug inhibits activity of BCR-ABL fusion**





[O'Brien, NEJM 2003]

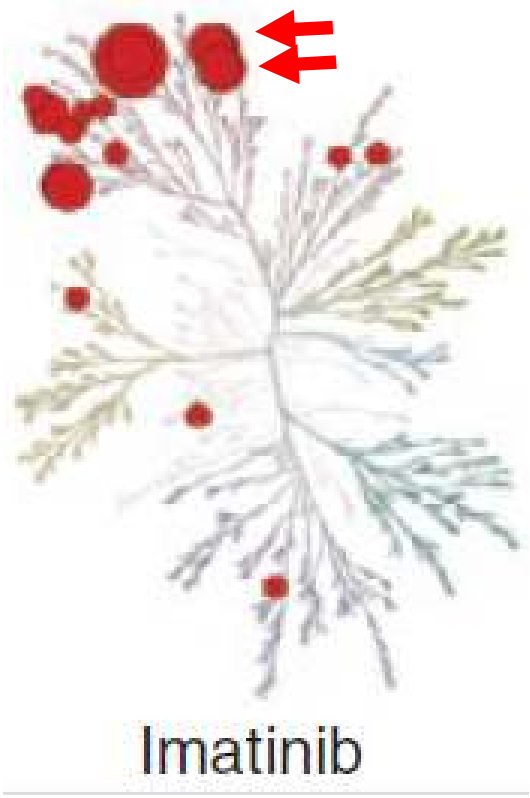
Oncogenetics in therapy – targeting oncogenes – imatinib (4)



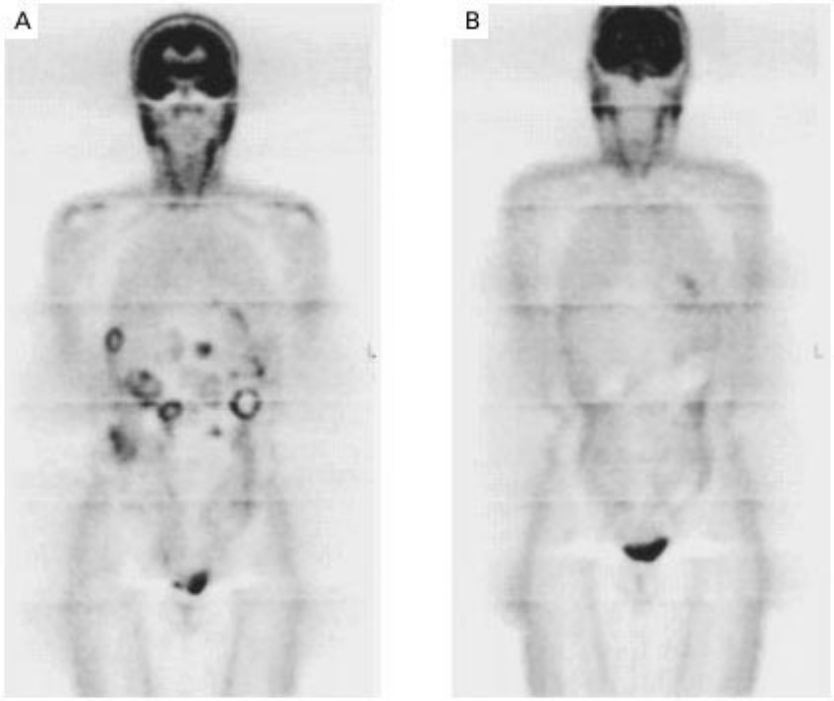
(before and after treatment)

Oncogenetics in therapy – expanding the drug indications

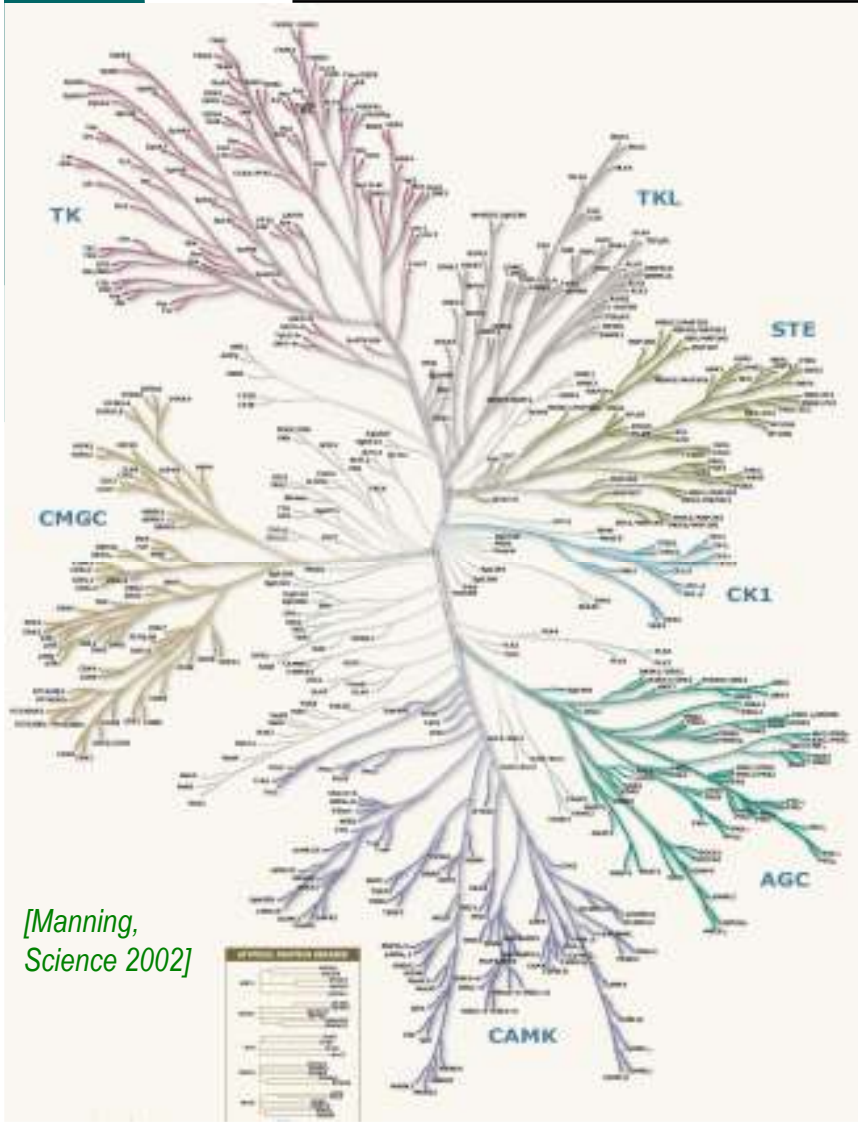
- imatinib inhibits also PDGF-R and KIT:



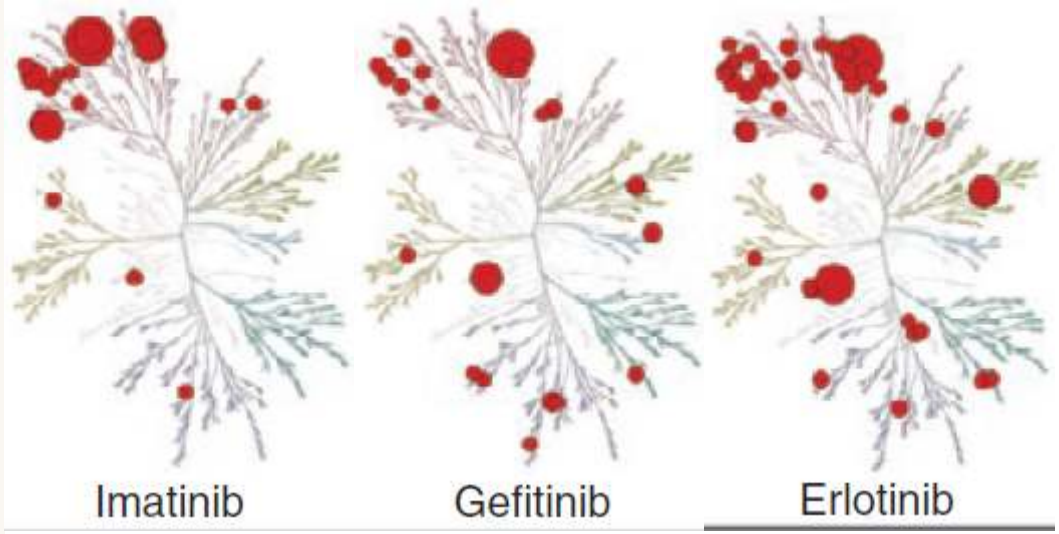
- *KIT* mutations are frequent in GIST tumors - imatinib administered:



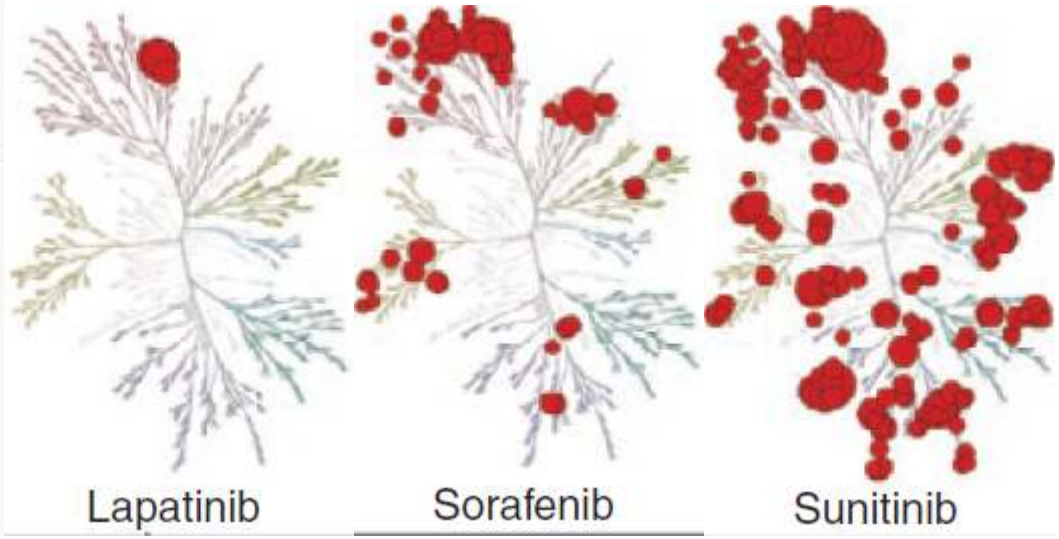
Oncogenetics in therapy – pointing at new possible targets



[Manning, Science 2002]

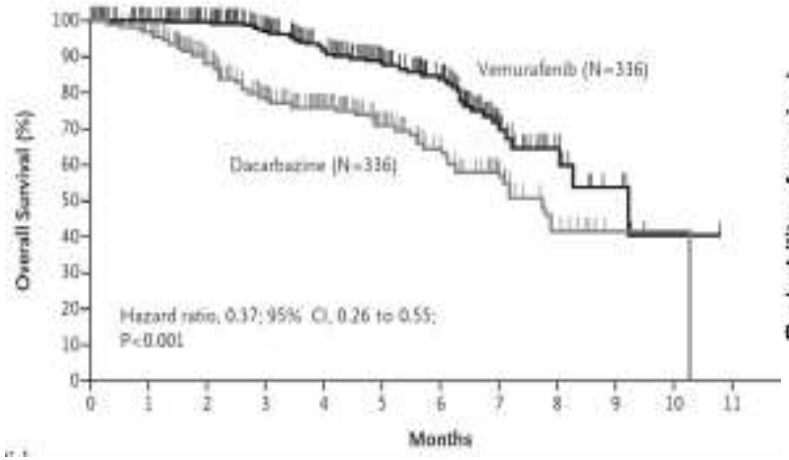


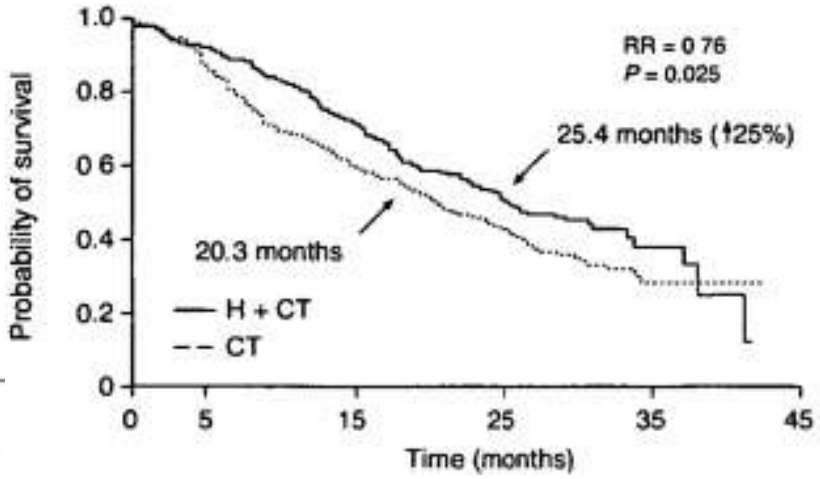
[Karaman, Nat Biotechnol 2008]



Oncogenetics in therapy – effectiveness of other kinase inhibitors

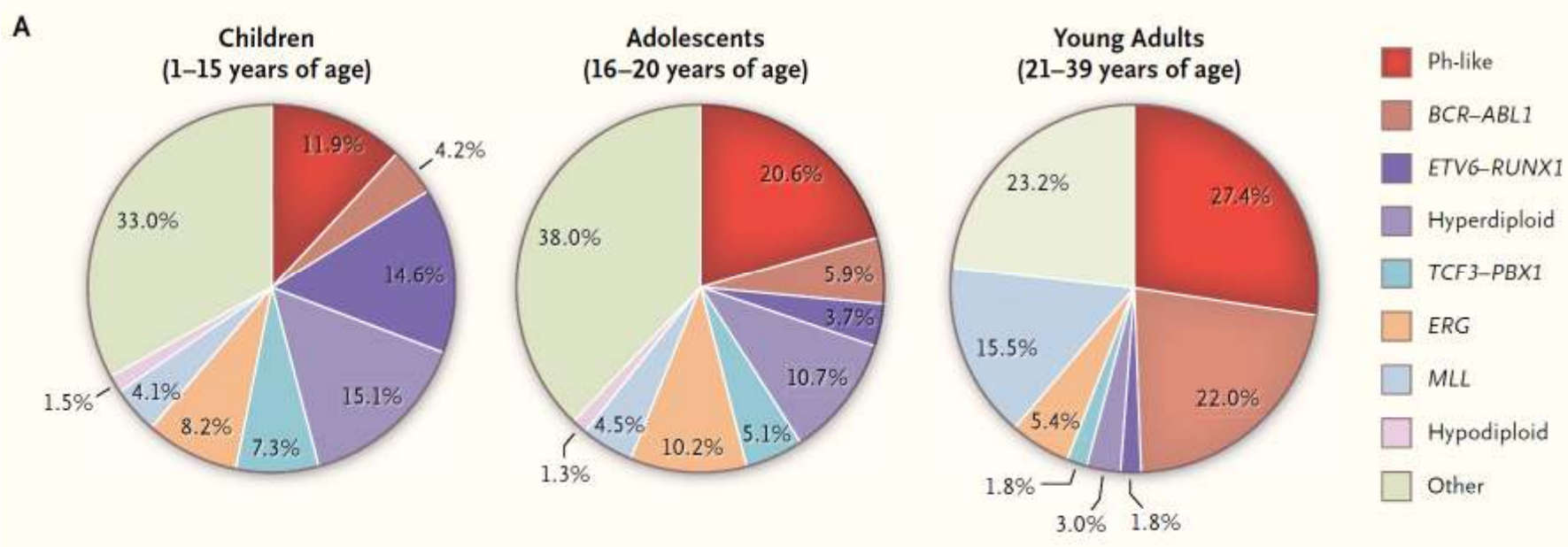
- **BRAF (Raf1) mutations in melanoma**
  - **V600E => vemurafenib** (Chapman, NEJM 2011)
- **EGFR mutations in breast cancer**
  - **trastuzumab (Herceptin)** (Eiermann, Ann Oncol 2001)





Oncogenetics in therapy – indicating new drugs

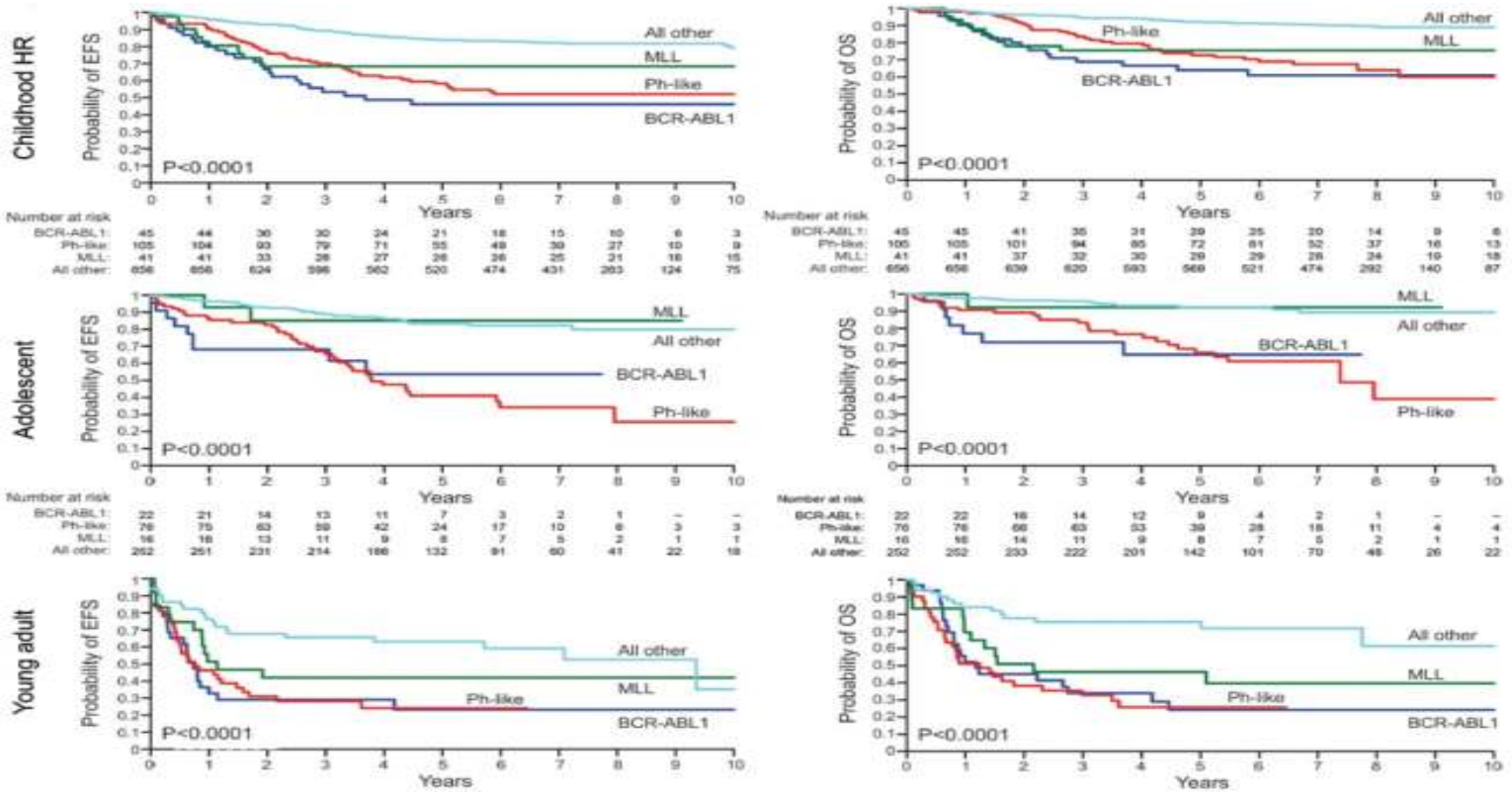
- **mutation spectrum in acute lymphoblastic leukemia (ALL) from B-cell precursors**



[Graubert, NEJM 2014]

Oncogenetics in therapy – indicating new drugs

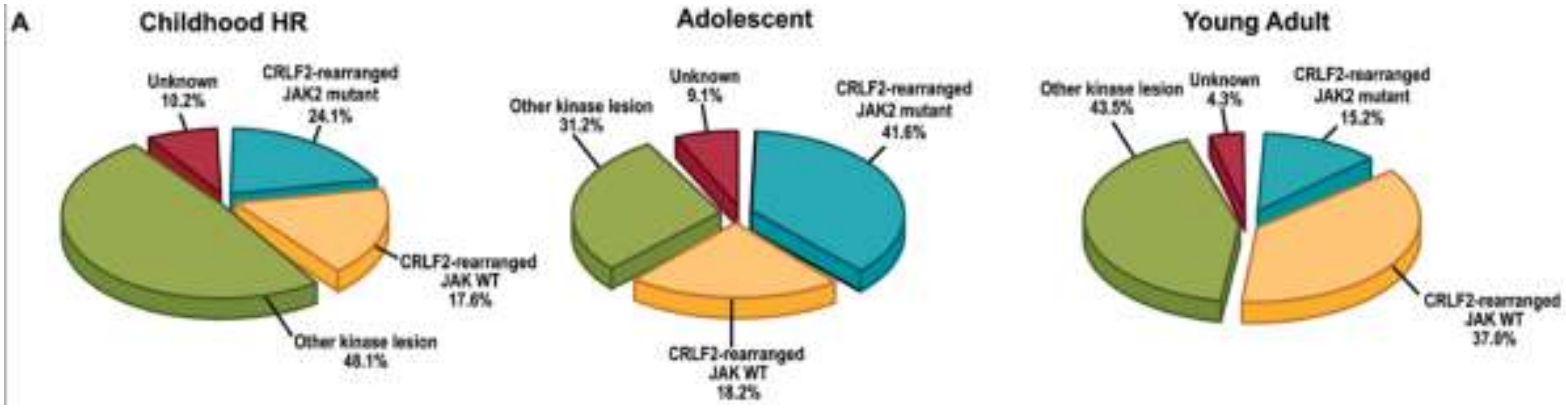
- **acute lymphoblastic leukemia (ALL) from B-cell precursors – prognosis**



[Roberts, NEJM 2014]

Oncogenetics in therapy – indicating new drugs

- **mutation spectrum in acute lymphoblastic leukemia (ALL) from B-cell precursors**

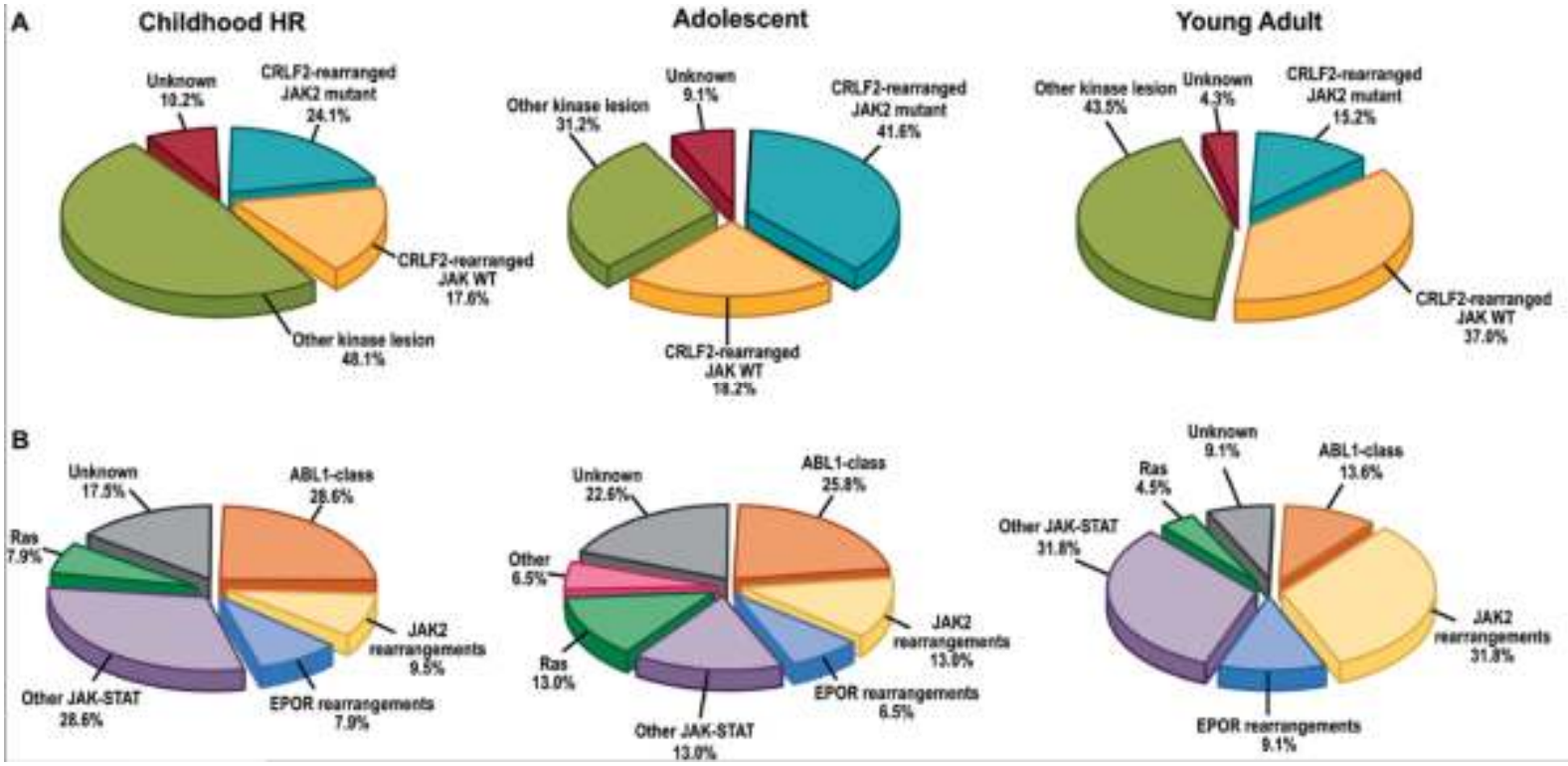


  - **dalszy podział grupy „inne”:**

[Roberts, NEJM 2014]

Oncogenetics in therapy – indicating new drugs

- **mutation spectrum in acute lymphoblastic leukemia (ALL) from B-cell precursors**



[Roberts, NEJM 2014]

Oncogenetics in therapy – indicating new drugs

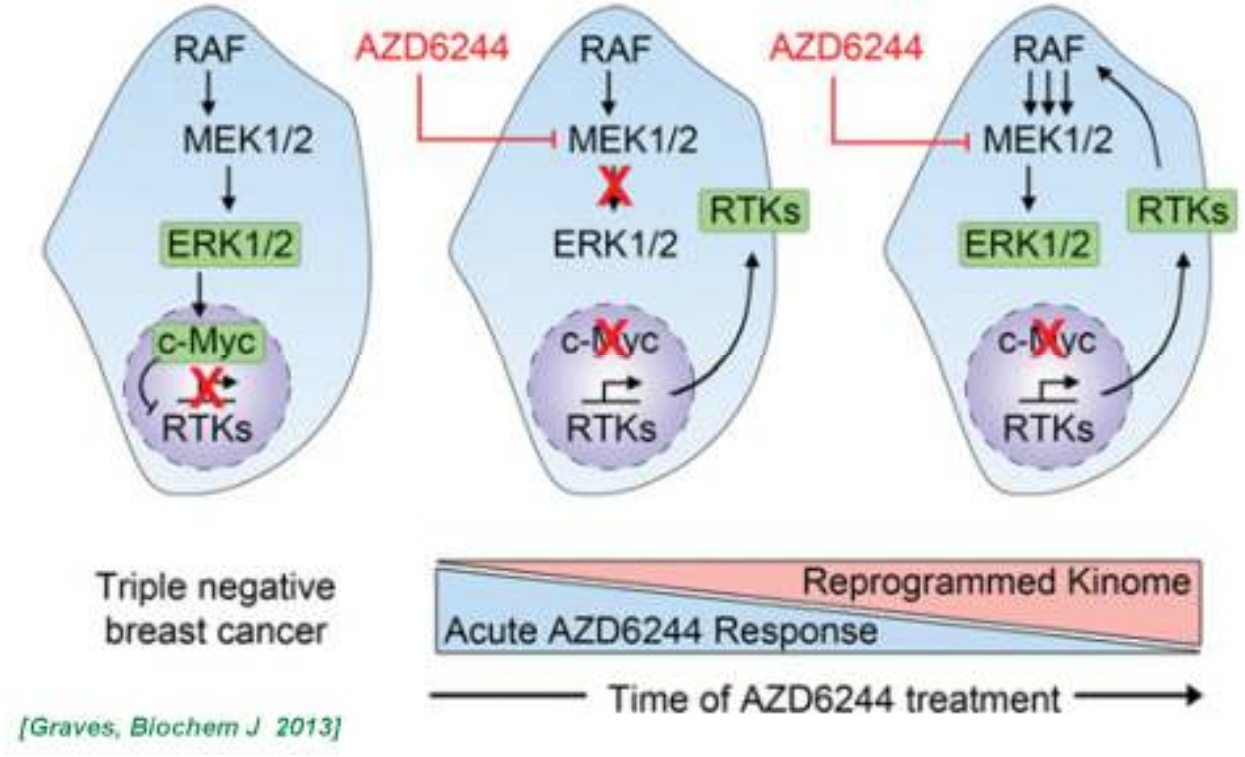
mutation spectrum in ALL from B-cell precursors (154 patients) – konkluzje:

- **91% patients: mutations of kinase genes**
- **drug effect – prognozed and verified on animals and cells:**
  - ***ABL1*, *ABL2*, *CSF1R*, *PDGFRB* fusions: dasatinib**
  - ***EPOR* or *JAK2* rearrangements: ruxolitinib**
  - ***ETV6–NTRK3* fusions: crizotinib**
- **clinically:**
  - **11 of 12 most serious cases – stable remission**

[Roberts, NEJM 2014]

Oncogenetics in therapy : alternative pathways

- **effect of inhibitors is decreasing quickly**



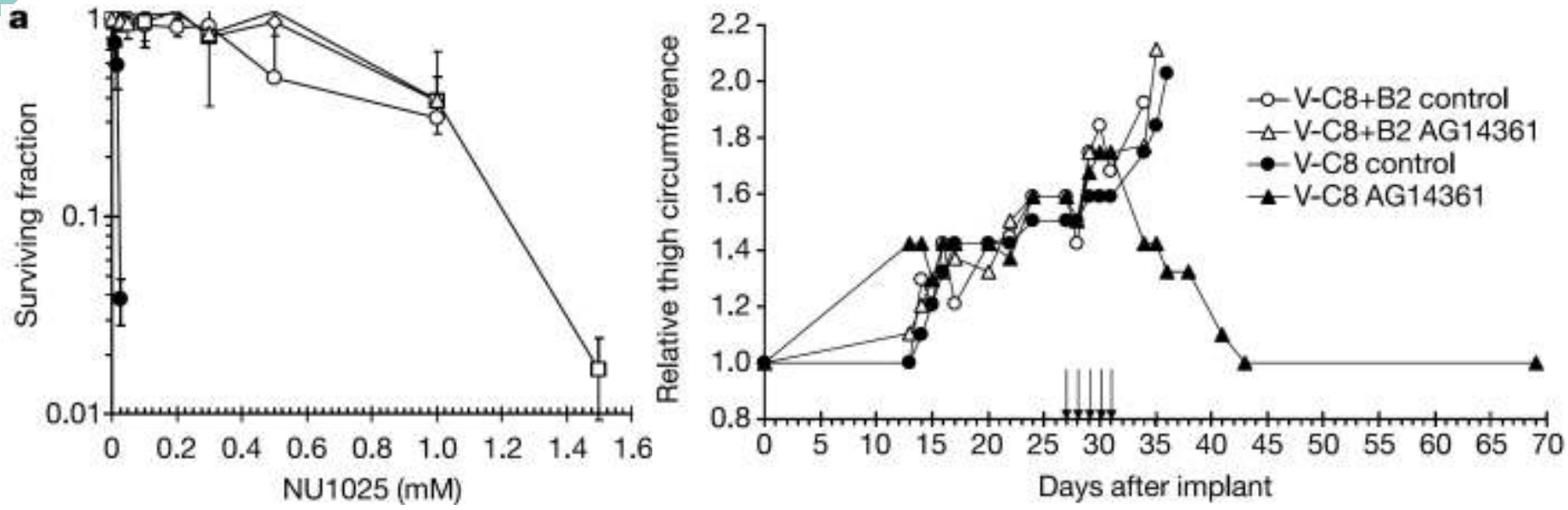
Oncogenetics in therapy: synthetic lethality

- **synthetic lethality –**
  <u>two distinct defects</u> are:
  - **separately: harmless**
  - **together: lethal** *[Bridges, 1922; Dobzhansky 1946]*
- **redundancy of metabolic pathways**
- **usage in oncology:** *[Hartwell, Science 1997]*
- **first success:**
  PARP inhibition in tumors with *BRCA2* mutation
  [Bryant, Nature 2005; Farmer, Nature 2005]
  ()

Oncogenetics in therapy: synthetic lethality

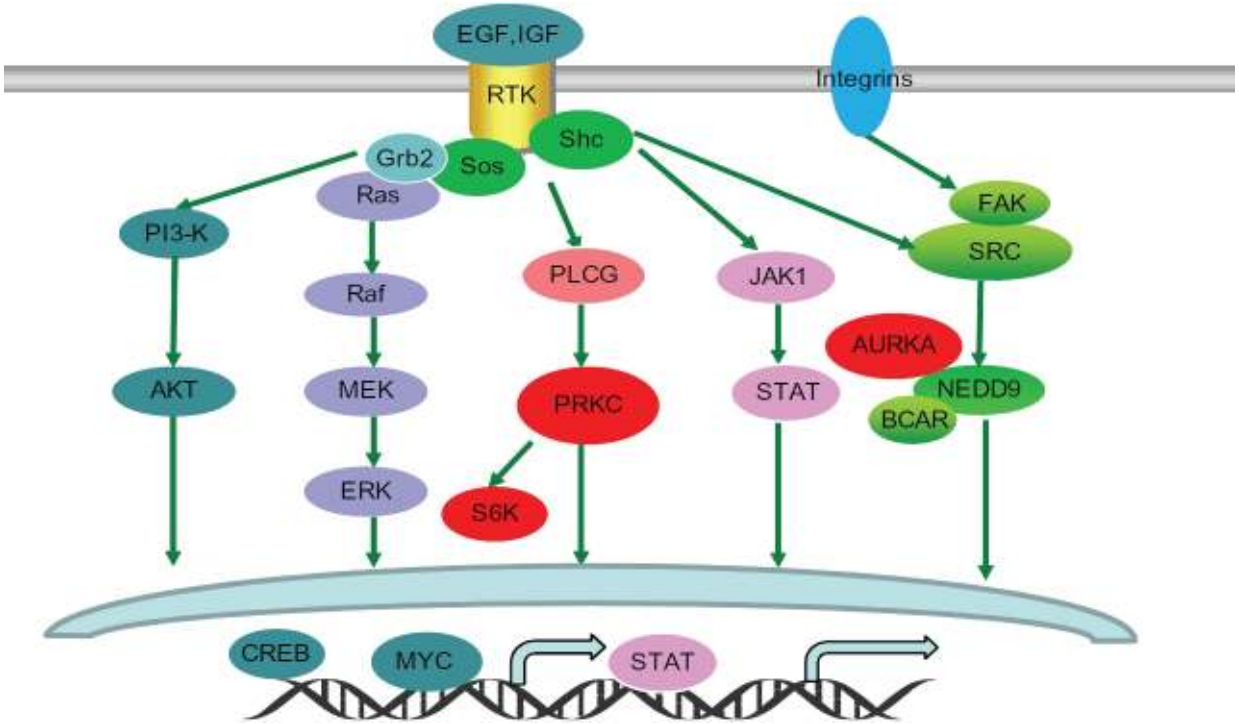
[Bryant, Nature 2005]

- **synthetic lethality**
- **PARP inhibition in cells with *BRCA2 mutation:***



Oncogenetics in therapy: synthetic lethality

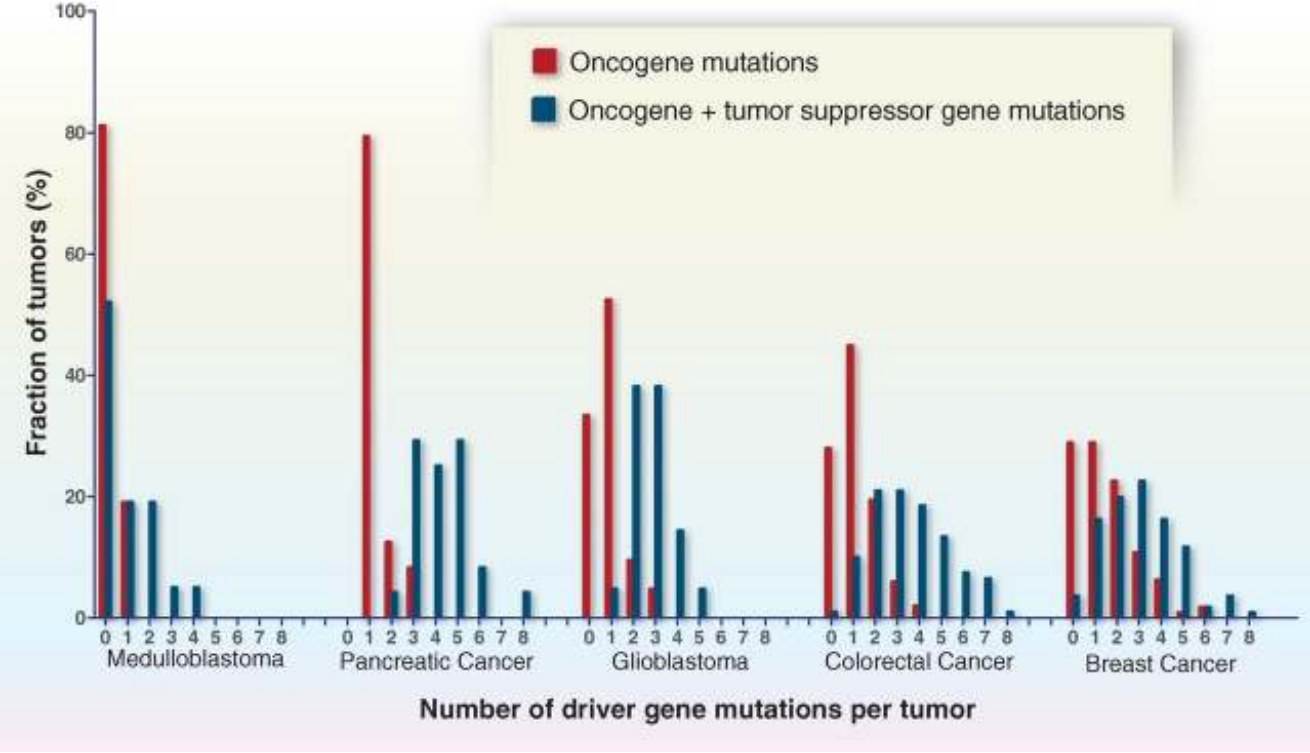
- **prognosed synthetic lethality in RAS (red = lethal (?) together with EGFR blockade)**



[Wu, Cancer Inform 2014]

Oncogenetics in therapy: mutations in oncogenes are rare!

- **leczenie celowane – głównie w onkogeny**
  - **problem: z reguły mało mutacji w onkogenach!**
    (wąski repertuar dostępnych terapii, trudno o leczenie skojarzone)



[Vogelstein, Science 2013]

Oncogenetics in therapy – targeted therapy: passengers (1)

passenger mutations:

- **no contribution to neoplastic transformation** (apparently useless in therapy)
- **remain in the genome of the clonal expansion** (probability of reversing mutation ~0)
- **may destroy genes coding elements of crucial metabolic pathways**
- **BUT**
  most of crucial metabolic pathways in mammals is at least duplicated (backup system)

Oncogenetics in therapy – targeted therapy: passengers (2)

metabolic vulnerability *[Muller, Nature 2012]*

- **If a major metabolic pathway is damaged by a passenger, cancer cell survival depends on metabolic pathway backup system**
- **Pharmacological inhibition of backup system may cause :**
  - **no difference in nomal cells**

    (main pathway is working well)
  - **death of cancer cells**

Oncogenetics in therapy – targeted therapy: passengers (3)

Glycolysis last step:

[normal nervous system cells]

2-phosphoglycerate

(ENO1) ↓↓ ↓ (ENO2)

phosphoenolpyruvate

↓

energy and survival

[1-5% glioma]

2-phosphoglycerate

(deleted) ↓↓ ↓ (ENO2)

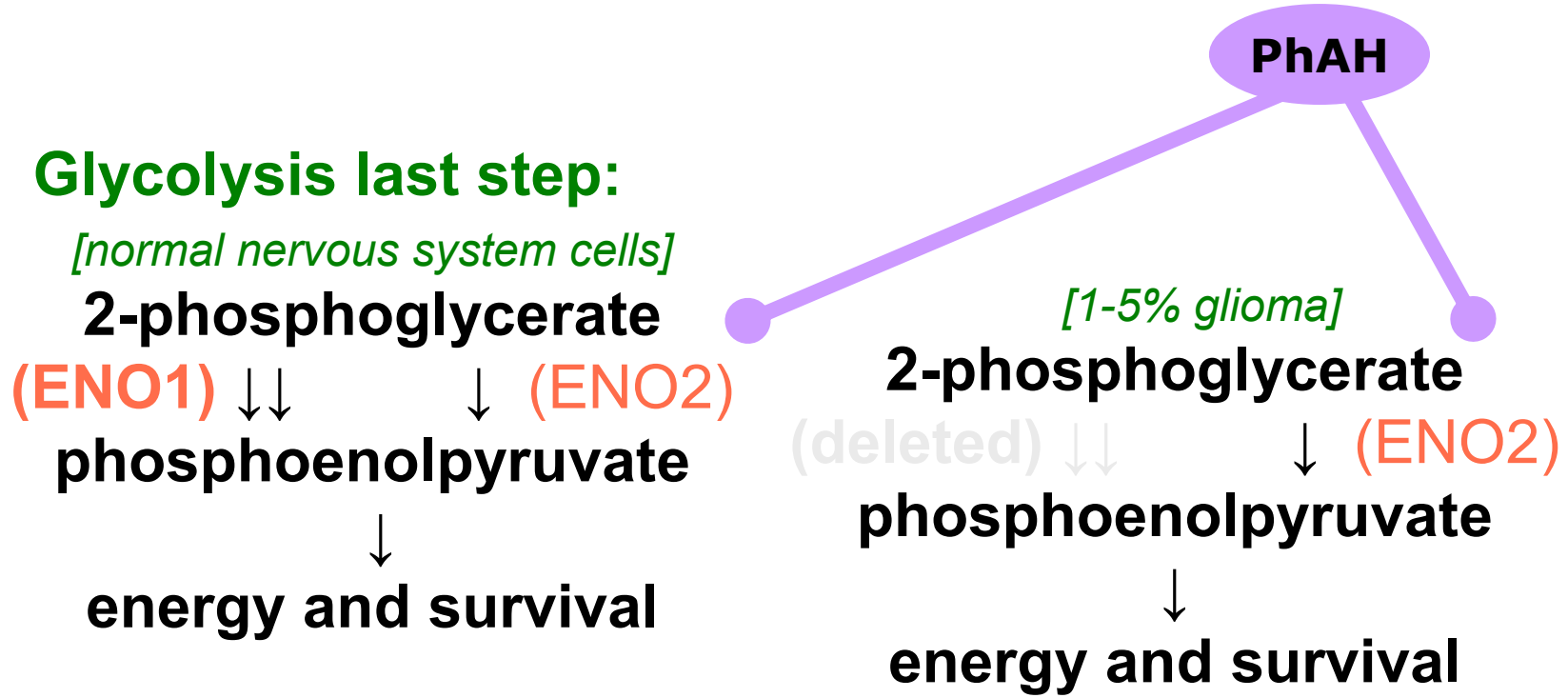
phosphoenolpyruvate

↓

energy and survival

[Muller, Nature 2012]

Oncogenetics in therapy – targeted therapy: passengers (4)



[Muller, Nature 2012]

Oncogenetics in therapy – targeted therapy: passengers (5)

Glycolysis last step:

[normal nervous system cells]

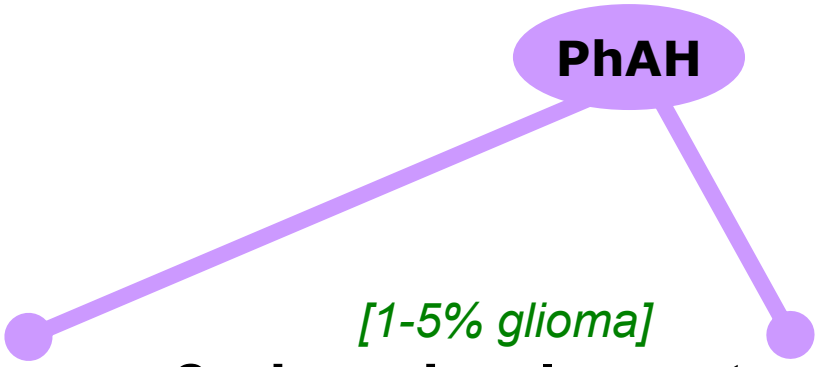
2-phosphoglycerate

(ENO1) ↓↓ ↓ (ENO2)

phosphoenolpyruvate

↓

energy and survival



[1-5% glioma]

2-phosphoglycerate

(deleted) ↓↓ ↓ (ENO2)

phosphoenolpyruvate

↓

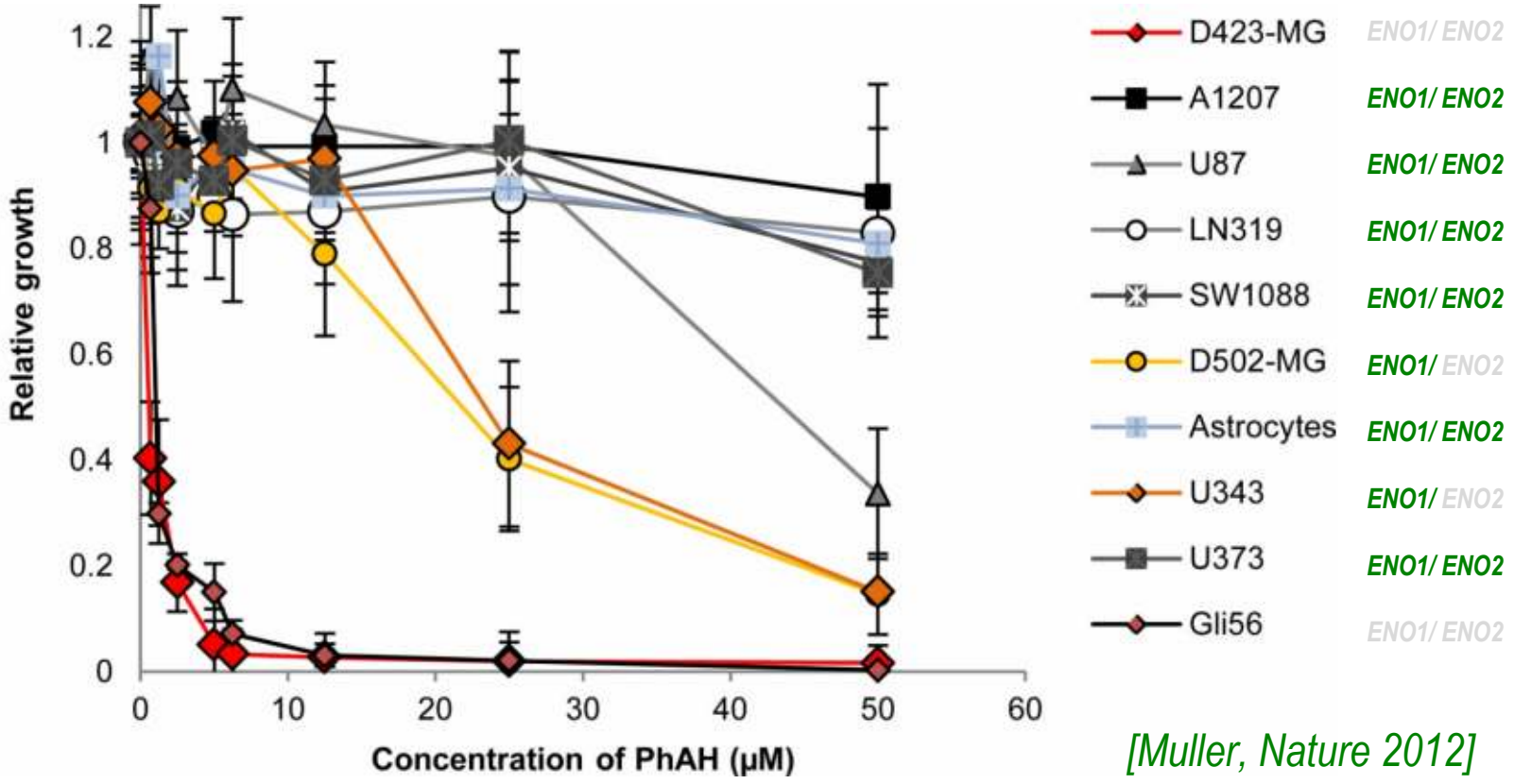
energy and survival

DEATH

[Muller, Nature 2012]

Oncogenetics in therapy – targeted therapy: passengers (6)

ENO inhibition in vitro



[Muller, Nature 2012]

Oncogenetics in therapy – targeted therapy: passengers (7)

Prospects for usage [Aksoy 2014]**:**

- **homozygous deletions of genes coding for crucial metabolic enzymes:**
  - **482 z 972 of cancerous cell lines**
  - **1019 z 5971 cancers**
  - **overall 4104 metabolic vulnerabilitese**
- **~45% of alternative enzymes could be inhibited by at least one drug already accepted by FDA**

Oncogenetics in therapy – targeted therapy: passengers (8)

Most frequent vulnerabilities [Aksoy 2014]:

non-oncologic drugs:

- **EXTL2, EXTL3 – 173/5971 (3%) – UDP-N-acetylglicosamine**
- **CPT1C, CPT1B, CPT2, CPT1A – 90/5971 (1.5%) – L-carnithine**
- **GOT1, GOT2, GOT1L1 – 65/5971 (1%) – maleic**
- **ATP2C1, ATP2C2 – 57/5971 (1%) – halotane**
- **ACAT1, ACAT2 – 39/5971 (0.7%) – sulphasalazin**

anticancer drugs:

- **TOP2B, TOP2A – 70/5971 (1%) – np. doxorubicin, etoposide**
- **DHFR, DHFRL1 – 68/5971 (1%) – np. methotrexate**
- **IKBKE, TBK1, IKBKB, CHUK – 46/5971 (0.8%) – AsO_3**
- **LIG1, LIG3, LIG4 – 43/5971 (0.8%) – bleomycin**

CONCLUSION: no universal method (applicability <<1%)