

Day 2- morning

Introduction to cancer genomics

30 October 2019

Dpt of Oncology
University of Lausanne



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SIB
Swiss Institute
of Bioinformatics



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of Bioinformatics



Analysis of mutations in somatic cells

- Point mutations
- Anywhere in the Genome, independent of effects
- Somatic not germline : in principle cancer versus control DNA seq (or no control DNA but reference database and eliminate all SNPs and focus on exom regions)
- Looking at the mutational position and one base before and one after
- Why are there 96 ?

Analysis of mutations in somatic cells

- Why are there 96 ?
- $4 \times 4 \times 4 = 64$
and central position to 3 others makes $64 \times 3 = 192$
why only half so many ?
- Which ones are missing ?
- "the mutated base is represented by the pyrimidine of the Watson-Crick base pair"
- SO : ...

Analysis of mutations in somatic cells

- Why are there 96 ?
- $4 \times 4 \times 4 = 64$
and central position to 3 others makes $64 \times 3 = 192$
why only half so many ?
- Which ones are missing ?
- "the mutated base is represented by the pyrimidine of the Watson-Crick base pair"
- so half is enough to be exhaustive,
because of the double stranded nature of DNA
And we do not know which strand was originally the first to have been mutated
(would there be a way to find out ?)

Analysis of mutations in somatic cells

- For each mutation that happened at a location with a Pyr in the center, there is one with a Pur in the center on the second DNA strand
- We can choose which one we count
 - those starting with T represent also those starting with A
 - those starting with C represent also those starting with G
- Is there a one to one bijective map ?
- The mutation A**C**G => A**T**G maps to which other one ?

Analysis of mutations in somatic cells

- For each mutation that happened at a location with a Pyr in the center, there is one with a Pur in the center on the second DNA strand
- We can choose which one we count
 - those starting with T represent also those starting with A
 - those starting with C represent also those starting with G
- Is there a one to one bijective map ?
- The mutation **ACG => ATG** maps to which other one ?
- equivalent to counting **CGT => CAT**
because of the antiparallel nature of DNA strands

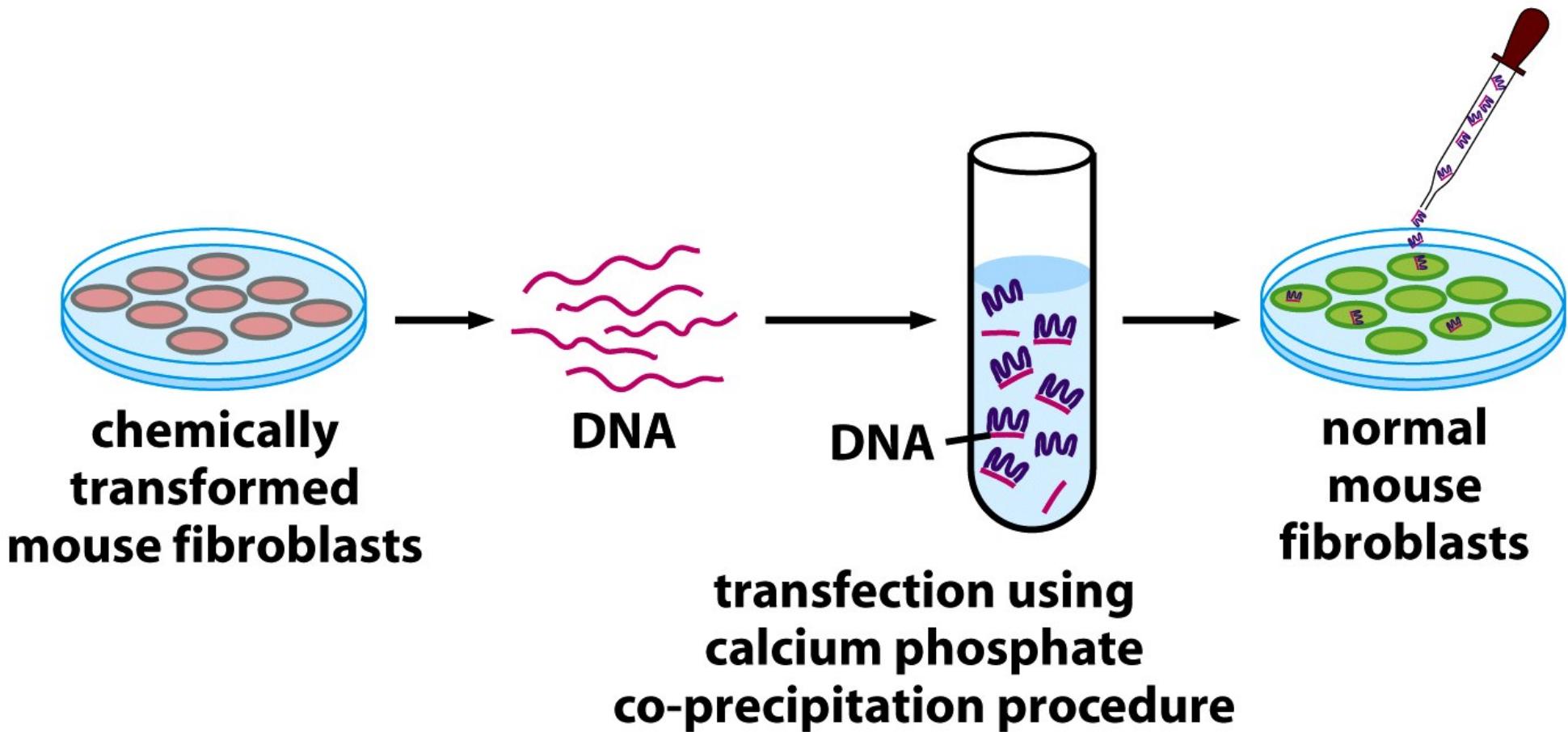
Introduction

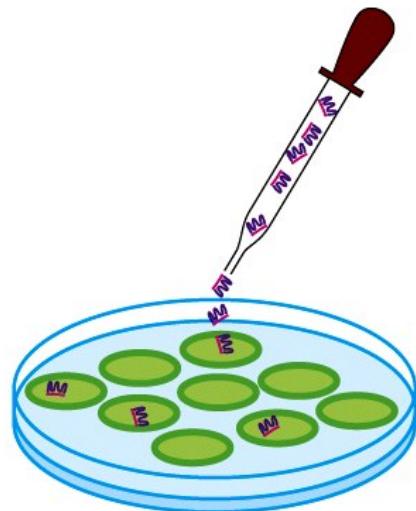
- **Cancer as a “somatic genetic disease”,
Changes in DNA as the central cause.**
- Which genes are responsible ?
- Which mutations ?
- How to find out ?
- Where do they come from ?
- Do different mutations cause different types of cancer ?
- If we know which mutations, does it help curing cancer ?

Introduction

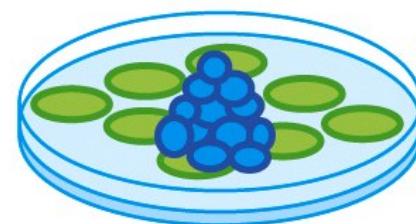
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The search for oncogenes

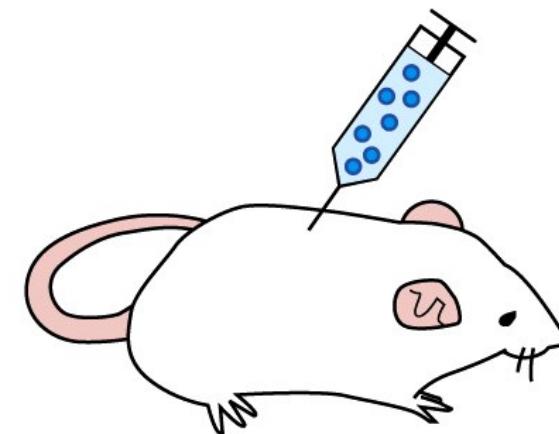




**normal
mouse
fibroblasts**



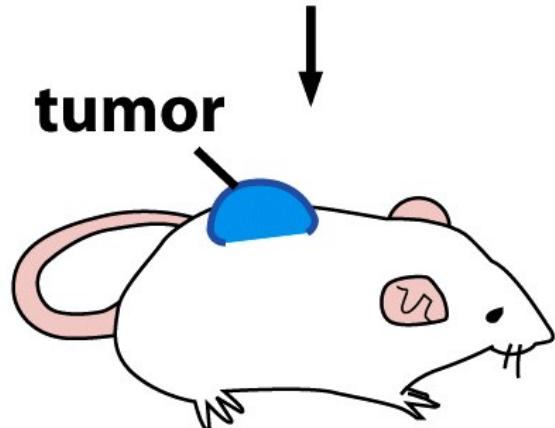
**formation of a
focus of morphologically
transformed cells**



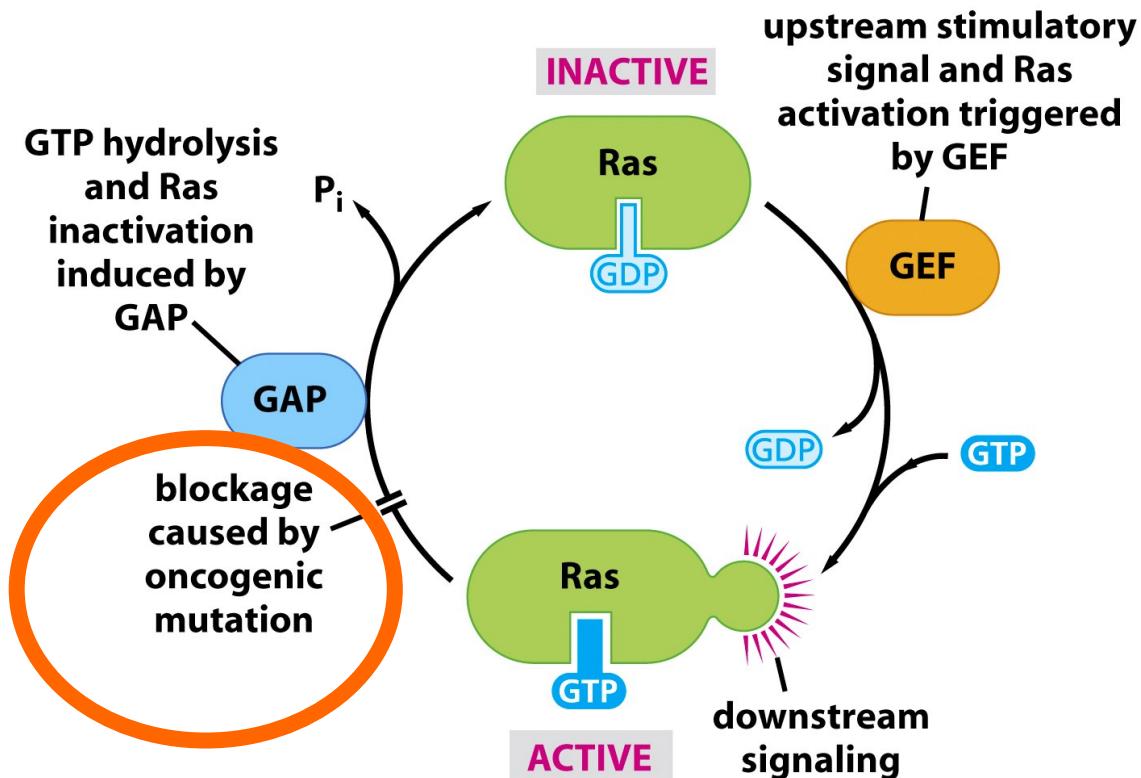
**injection of
morphologically
transformed cells into
mouse host**



tumor



RAS genes



RAS proteins are switches.

It gives (growth-promoting) signals to downstream proteins in the pathway when in the Ras-GTP state.

RAS genes

Oncogenic activation of H-ras

Normal H-ras gene sequence:

5'..... ATGACGGAATATAAGCTGGTGGTGGTGGCGCCGGCGGTGTG 3'
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Val

H-ras gene sequence from bladder cancer sample:

5'..... ATGACGGAATATAAGCTGGTGGTGGTGGCGCCGTCGGTGTG 3'
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Val Gly Val



Onco-genes vs T-Sup-genes

Onco-genes

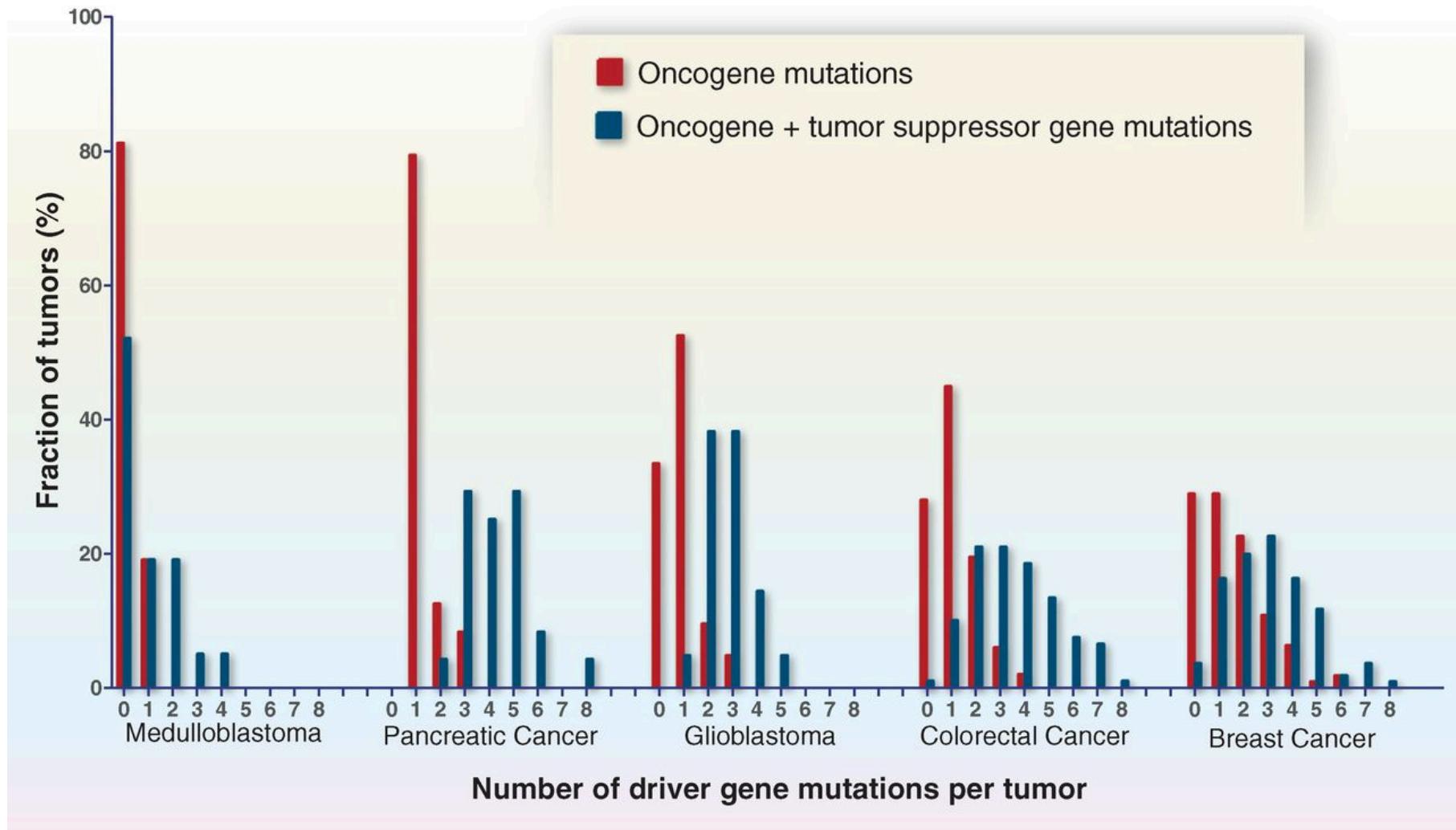
- Active: pro-tumor action
- **Hyper-activated** in tumors:
- Gain-of-function alleles (usually dominant)
- Usually One hit required
Specific hit
- Examples: **RAS**, SRC,
- More than 200 known

T-Sup-genes

- Active: anti-tumor action
- **In-activated** in tumors:
- Loss-of-function alleles
(recessive or dosage-dep.)
- Examples: **RB**, **p53**, BRCA
- “Gatekeepers” vs. “Caretakers”
- Two hits required, Unspecific inactivating hits
- More than 200 known
- Typical gene in familial cancer syndromes

What distribution of point mutations sites ?

Fig. Number and distribution of driver gene mutations in five tumor types.



Bert Vogelstein et al. Science 2013;339:1546-1558



Onco-genes vs T-Sup-genes

Onco-genes

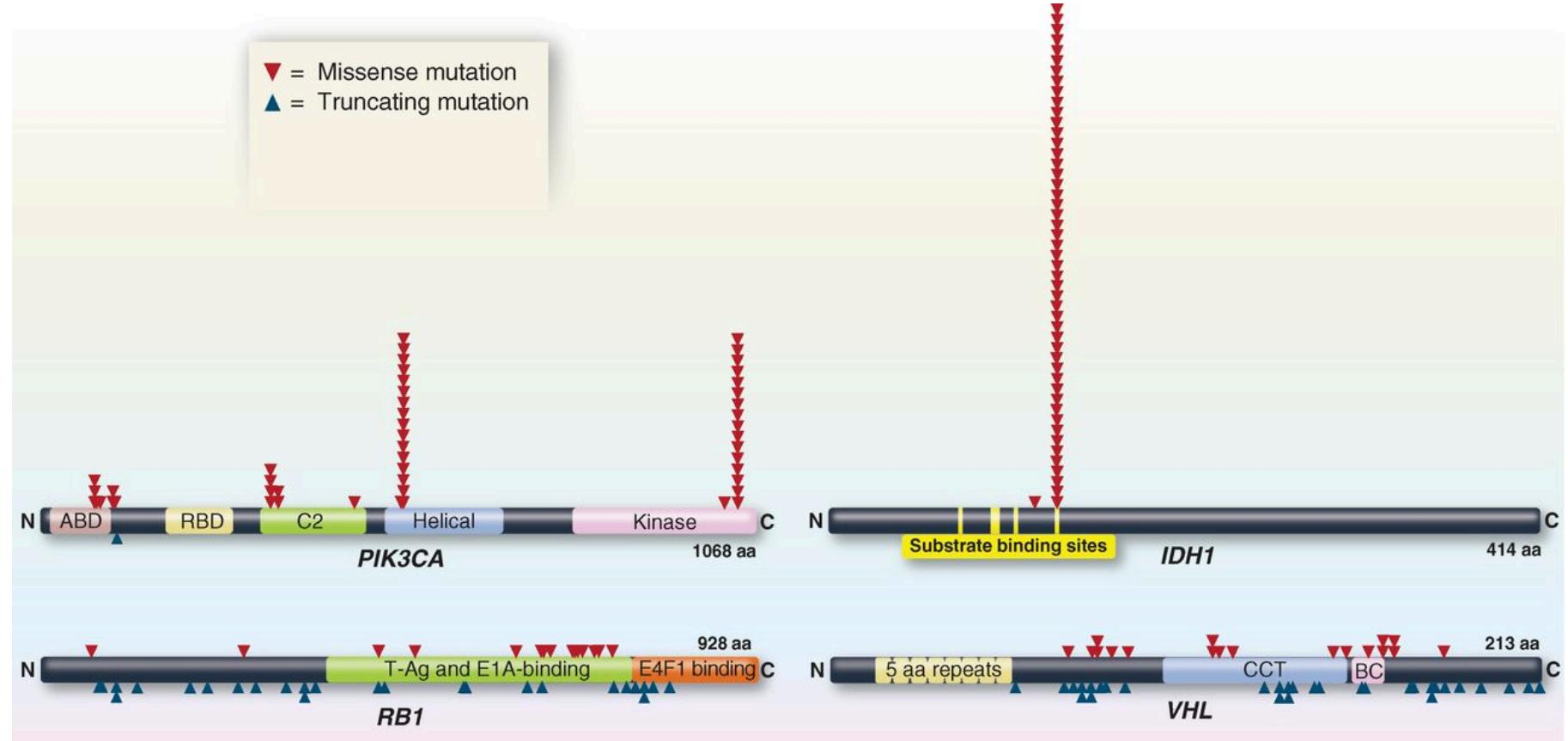
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What distribution of point mutations sites ?

Fig. 4 Distribution of mutations in oncogenes resp. tumor suppressor genes.

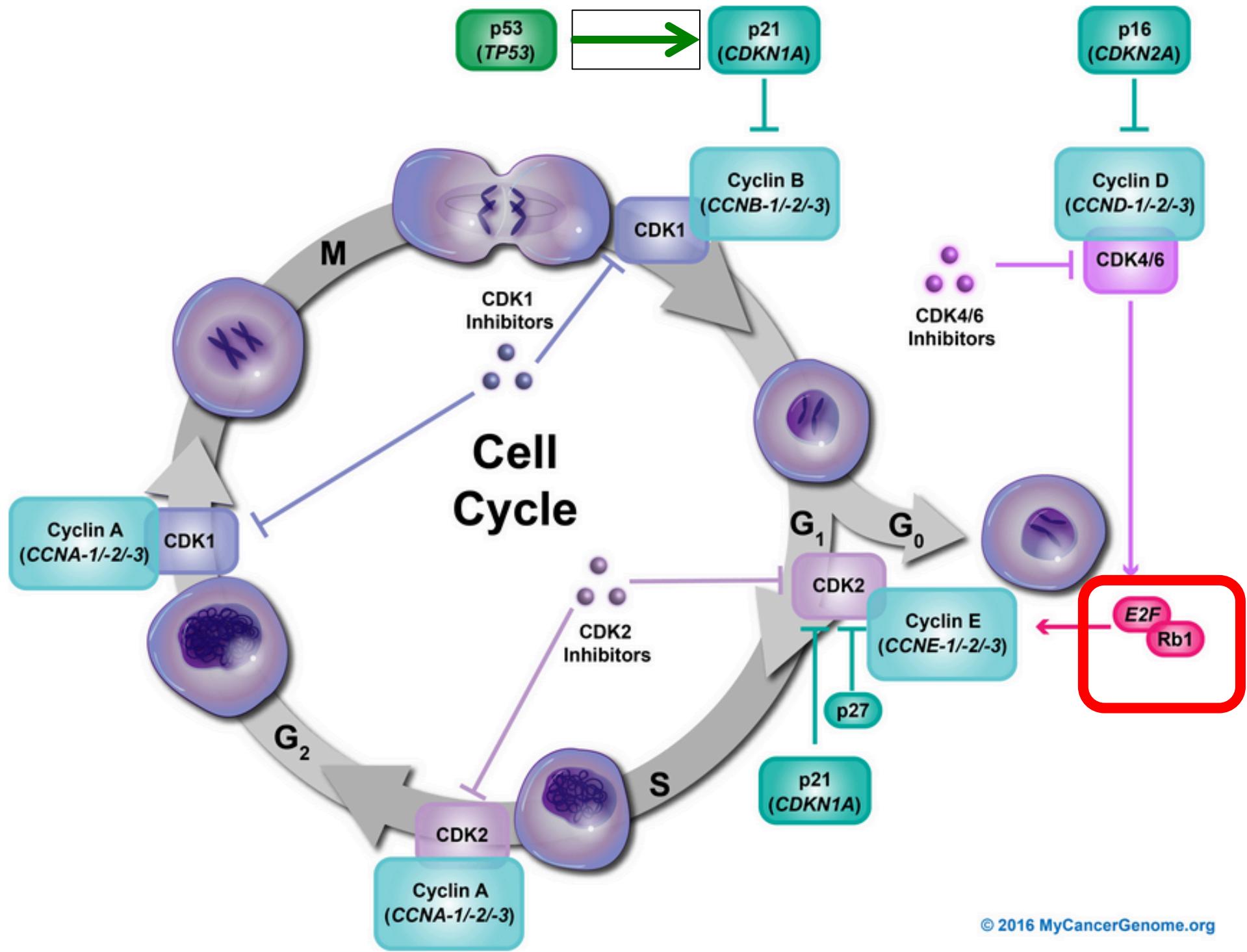


Bert Vogelstein et al. Science 2013;339:1546-1558

Science
AAAS

Introduction

- **Cancer as a “somatic genetic disease”,
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- Which genes are responsible ?
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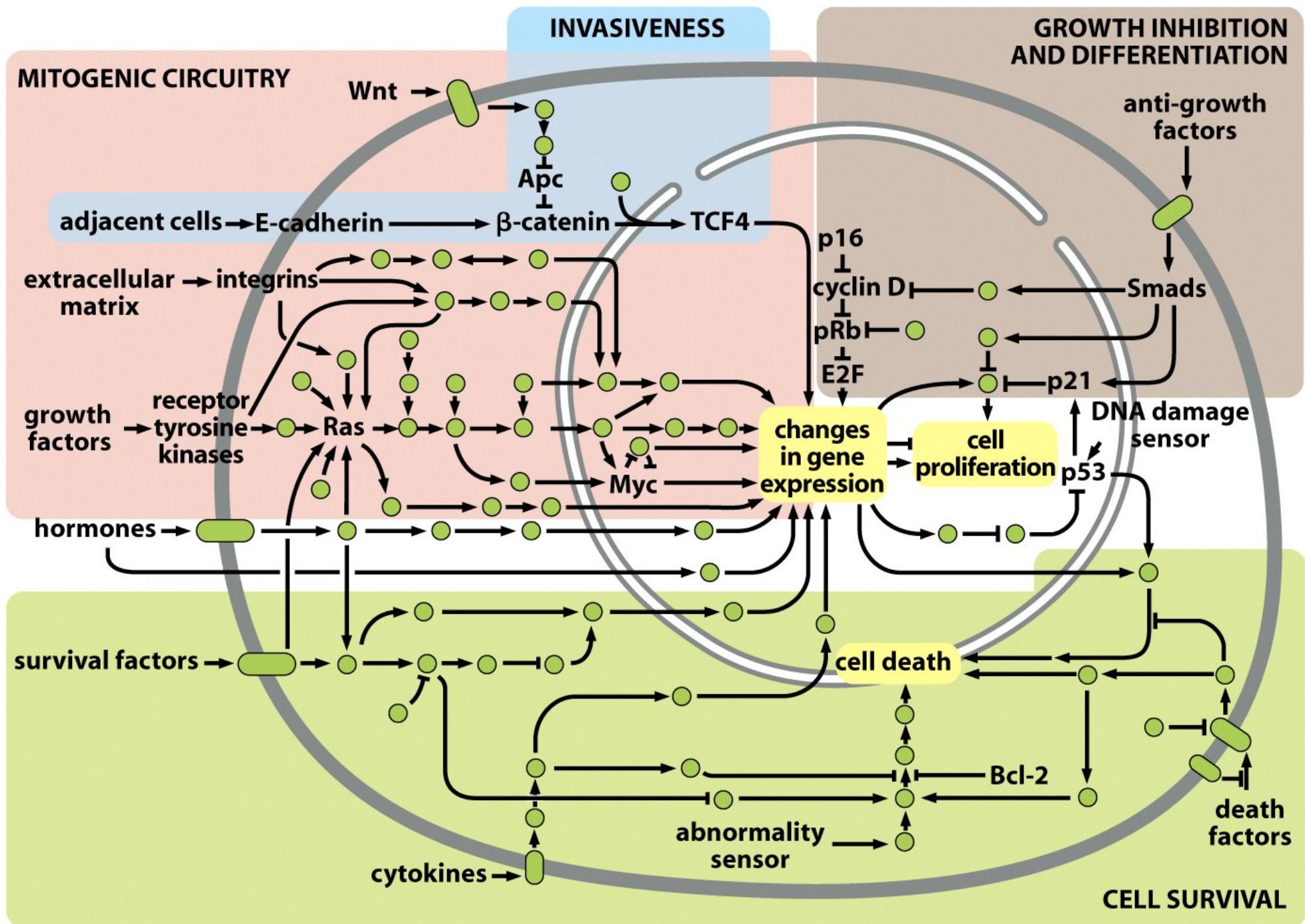
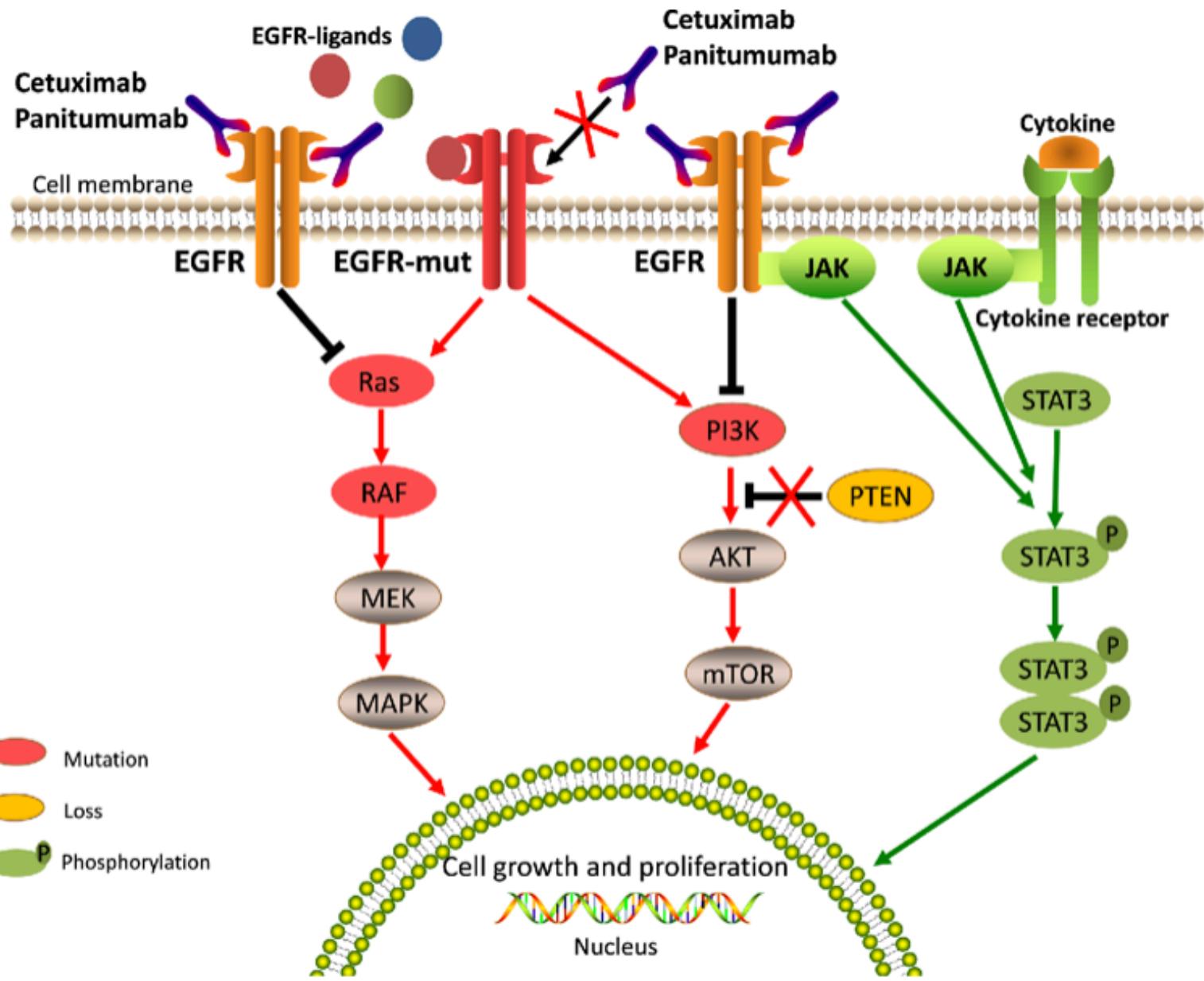


Figure 11.44 The Biology of Cancer (© Garland Science 2007)



Database of mutations in oncology

The screenshot shows the COSMIC website homepage. At the top is a navigation bar with links for Projects, Data, Tools, News, Help, About, Genome Version, a search bar, and a login link. Below the navigation is a banner for 'COSMIC v90, released 05-SEP-19'. The main content area includes a 'COSMIC News' section with a 'COSMIC Release v90' article, a 'Preview the COSMIC v90 release' section, and a 'COSMIC Annual Survey' section. There are also sections for 'Projects' (listing COSMIC, Cell Lines Project, COSMIC-3D, and Cancer Gene Census), 'Data curation' (listing Gene Curation, Gene Fusion Curation, Genome Annotation, Drug Resistance, and Mutational Signatures), and 'Tools' (listing Cancer Browser, Genome Browser, GA4GH Beacon, and COSMIC in BigQuery). A footer at the bottom contains links for COSMIC, Projects, Documentation, and Contact information, along with the Wellcome Sanger Institute logo.

COSMIC v90, released 05-SEP-19

COSMIC, the Catalogue Of Somatic Mutations In Cancer, is the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer.

Start using COSMIC by searching for a gene, cancer type, mutation, etc. below.

eg Braf, COLO-829, Carcinoma, V600E, BRCA-UK, Campbell SEARCH

Projects

COSMIC is divided into several distinct projects, each presenting a separate dataset or view of our data:

- COSMIC**
The core of COSMIC, an expert-curated database of somatic mutations
- Cell Lines Project**
Mutation profiles of over 1,000 cell lines used in cancer research
- COSMIC-3D**
An interactive view of cancer mutations in the context of 3D structures
- Cancer Gene Census**
A catalogue of genes with mutations that are causally implicated in cancer

Data curation

- Gene Curation** — details of our manual curation process
- Gene Fusion Curation** — details of our curation process for gene fusions
- Genome Annotation** — information on the annotation of genomes
- Drug Resistance** — curation of mutations conferring drug resistance
- Mutational Signatures** — a census of mutation signatures in cancer

Tools

- Cancer Browser** — browse COSMIC data by tissue type and histology
- Genome Browser** — browse the human genome with COSMIC annotations
- GA4GH Beacon** — access COSMIC data through the [GA4GH Beacon Project](#)
- COSMIC in BigQuery** — search COSMIC via the [ISB Cancer Genomics Cloud](#)

Help

- Downloads** — data that you can download from our SFTP site
- Documentation** — view our help documentation
- FAQ** — a compilation of our Frequently Asked Questions
- Release Notes** — information about the latest COSMIC release
- Licensing** — information about our licensing policy

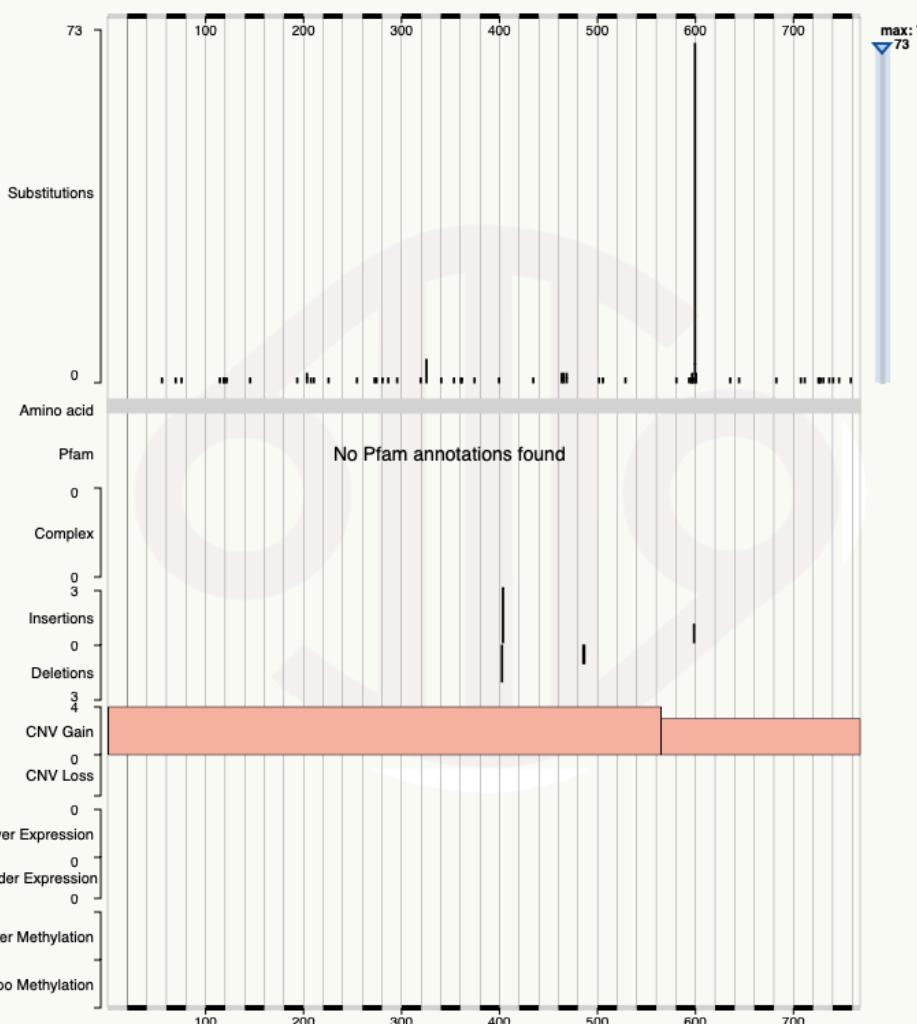
[https://
cancer.sanger.ac.uk/
cosmic](https://cancer.sanger.ac.uk/cosmic)

BRAF

Oncogene or TSG ?

Gene view

The gene view histogram is a graphical view of mutations across BRAF_ENST00000496384. These mutations are displayed at the amino acid level across the full length of the gene by default. Restrict the view to a region of the gene by dragging across the histogram to highlight the region of interest, or by using the sliders in the filters panel to the left. [Show more](#)

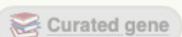
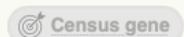


BRAF

Oncogene
or TSG ?

Overview

This section gives an overview of BRAF_ENST00000496384, along with links to any related data and resources.



COSMIC gene BRAF_ENST00000496384 (COSG496384)

Genomic coordinates [7:140719327..140924810](#) (negative strand)

Synonyms BRAF, BRAF1, ENSG00000157764.13, NM_001354609.1, NP_001341538.1

Characterization of cancer genomic heterogeneity by next-generation sequencing ... (Zhang 2018)



Precision Clinical Medicine, 1(1), 2018, 29–48

doi: 10.1093/pcmedi/pby007

Advance Access Publication Date: 14 June 2018

Review

REVIEW

Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment

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Characterization of cancer genomic heterogeneity by next-generation sequencing ...

Paragraphs:

1) Abstract / Introduction

1) Cancer genomic heterogeneity associated with clinical features

2) Identification of genomic heterogeneity in pan-cancer studies

3) Identification of genomic heterogeneity in hematological malignancy

4) Identification of genomic heterogeneity in solid tumors

5) Association of cancer genetic heterogeneity and therapeutic failures

6) Advances of **SCA technology** to uncover dynamic genetic heterogeneity

7)

8) Immune checkpoint inhibitors

9) Concluding remarks

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

Abstract

Main Points :

- 1) Tumour cells are very heterogeneous, (typically) even within one and the same tumour (same patient, same location)
- 2) Treatment response varies more than was expected, likely due (at least in part) to inter-tumoral and intra-tumoral heterogeneity
- 3) Trend: a tumour is more diverse, it tends to be more resistant to treatments
- 4) By precisely identifying the genetic-molecular characteristics of a tumour, we can better select the best treatment (precise, personalized medicine)

Terms :

- 1)

Questions ? :

- 1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“Introduction-1”

Main Points :

- 1) Fig. 1 , a tumour is a complex tissue
- 2) Treatment response varies more than was expected, likely due (at least in part) to inter-tumoral and intra-tumoral heterogeneity
- 3) Trend: a tumour is more divers, it tends to be more resistant to treatments
- 4) By precisely identifying the genetic-molecular characteristics of a tumour, we can better select the best treatment (precise, personalized medicine)

Terms :

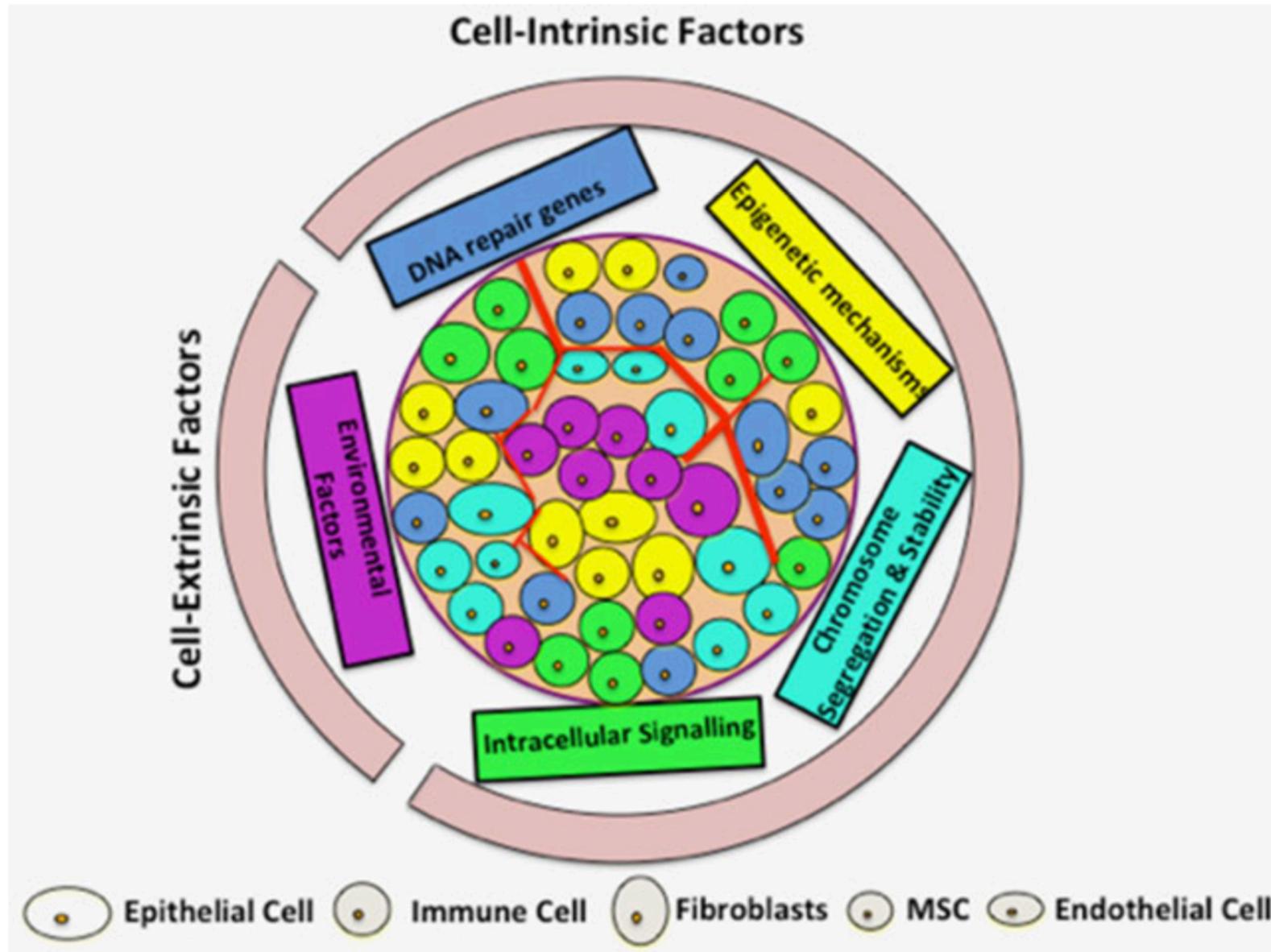
- 1)

Questions ? :

- 1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

1) Fig. 1 , a tumour is a complex tissue



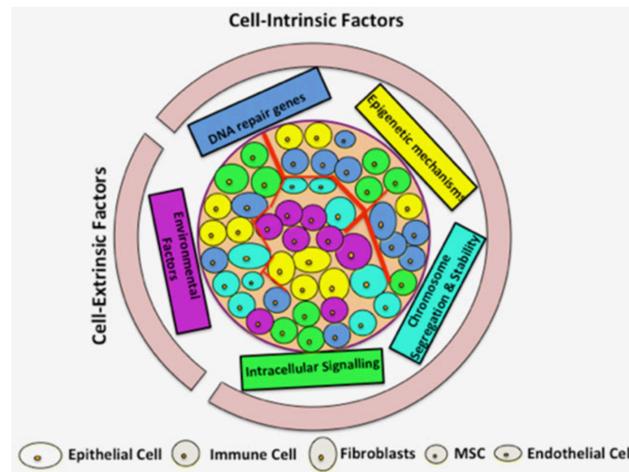


Figure 1. Interplay of key contributing factors to tumor heterogeneity. Both cell-intrinsic and cell-extrinsic factors contribute to tumor heterogeneity. Key cell-intrinsic factors include mutation, DNA-repair genes, epigenetic mechanisms, chromosome segregation and stability, as well as intracellular signaling. Non-genetic or phenotypic variations as a result of contributing cell-intrinsic factors are depicted by different cytoplasmic colors. Cell-extrinsic mechanisms affect and contribute to the unequal microenvironment, indirectly contributing to tumor heterogeneity. Multiple cell types and different inter- and intra-cell interactions within a tumor may exist (only representatives are shown here), hence selectively contributing to tumor heterogeneity.

Fig. 2a

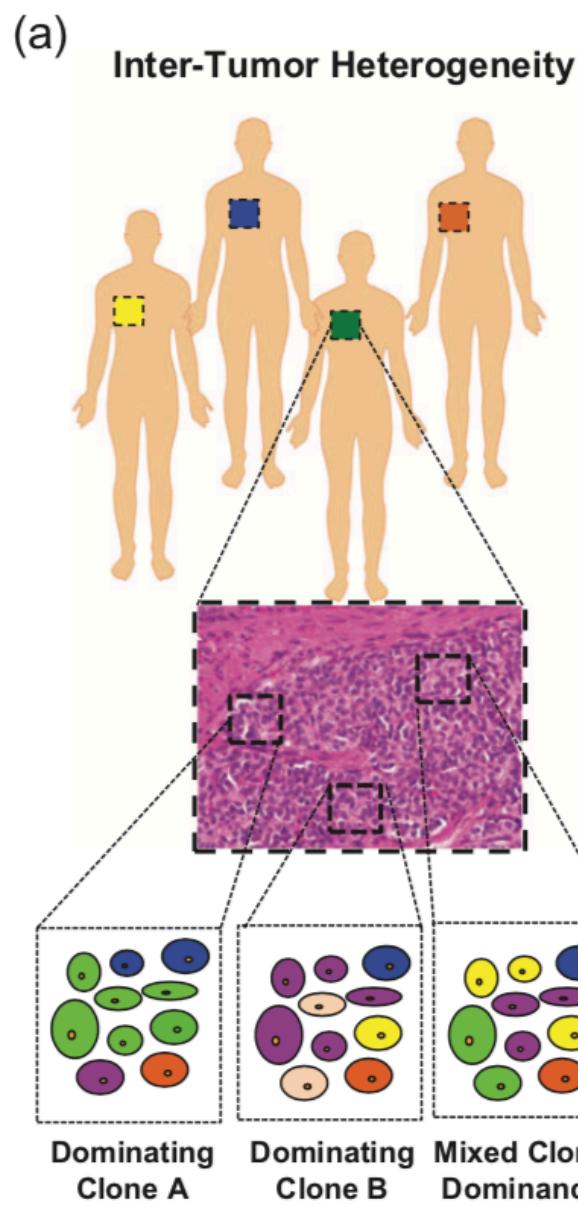


Figure 2. Contribution of tumor heterogeneity in cancer progression and metastasis. (a) Graphical representation of inter- and intra-tumor heterogeneity origins at macroscopic and microscopic levels. (b) Graphical summary of the two recognized heterogeneity models: clonal (stochastic) evolution and cancer stem cell (CSC), involving either monoclonal evolution or single progenitor, and polyclonal evolution or multiple progenitors, linking tumor cellular paths to different tumor heterogeneity. (c) Contributing role of tumor heterogeneity with respect to cancer progression and metastasis.

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“Introduction-2”

Main Points :

- 1) Fig. 2, a tumour is a complex tissue
- 2) Fig. 3, tumour evolution and treatments

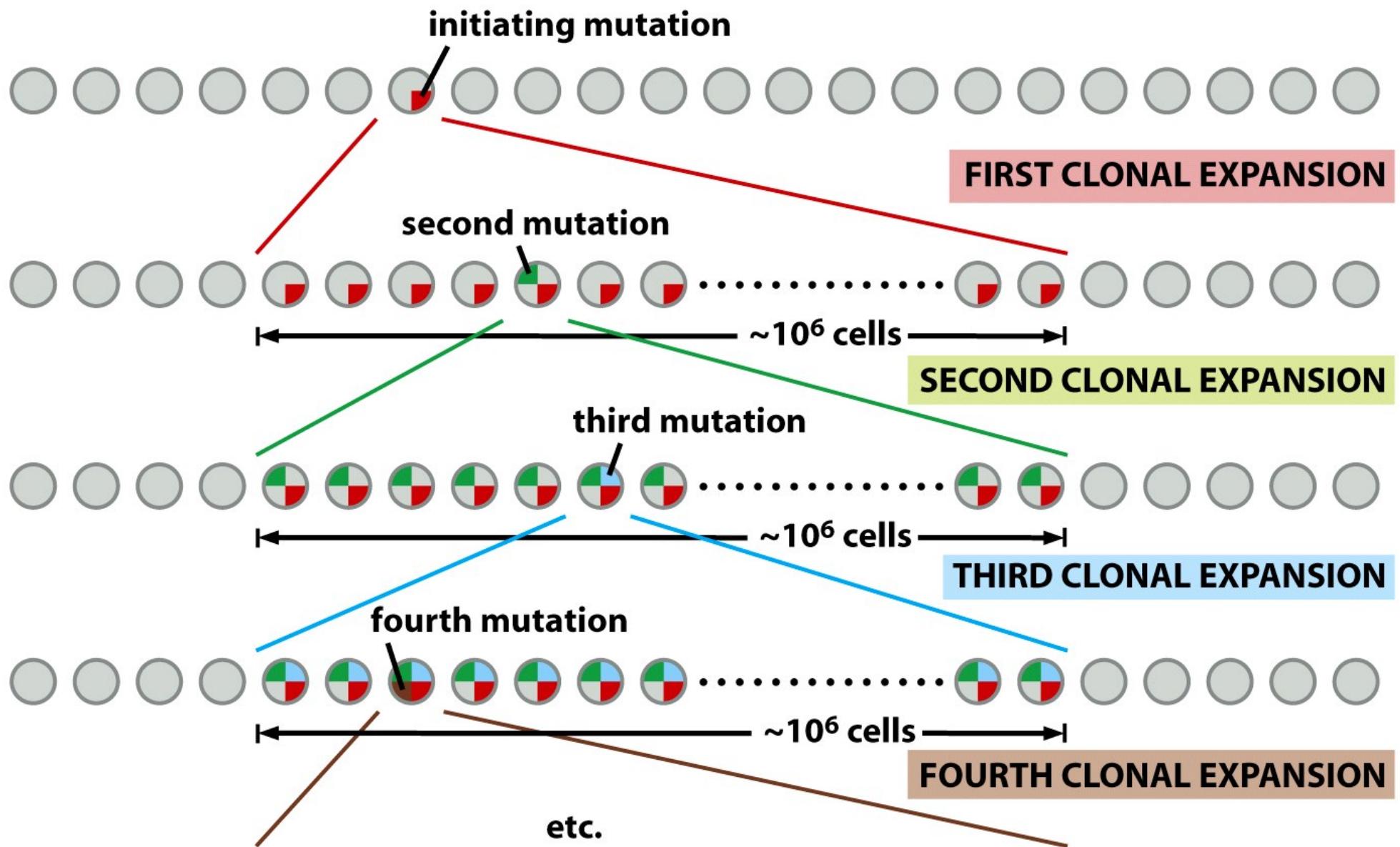
Terms :

- 1) Clonal Evolution Model vs. Cancer Stem Cell Model (hierarchical)

Questions ? :

- 1)...

Cancer: clonal evolution theory



Linear vs Branched Evolution

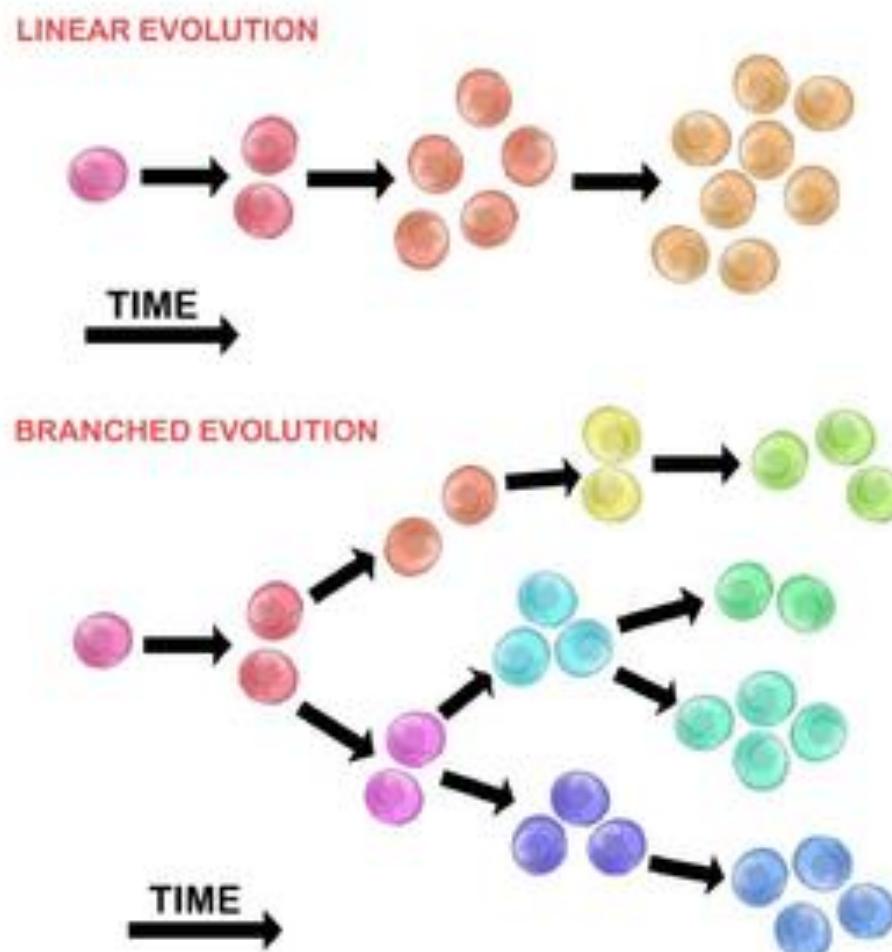


Fig. 2b

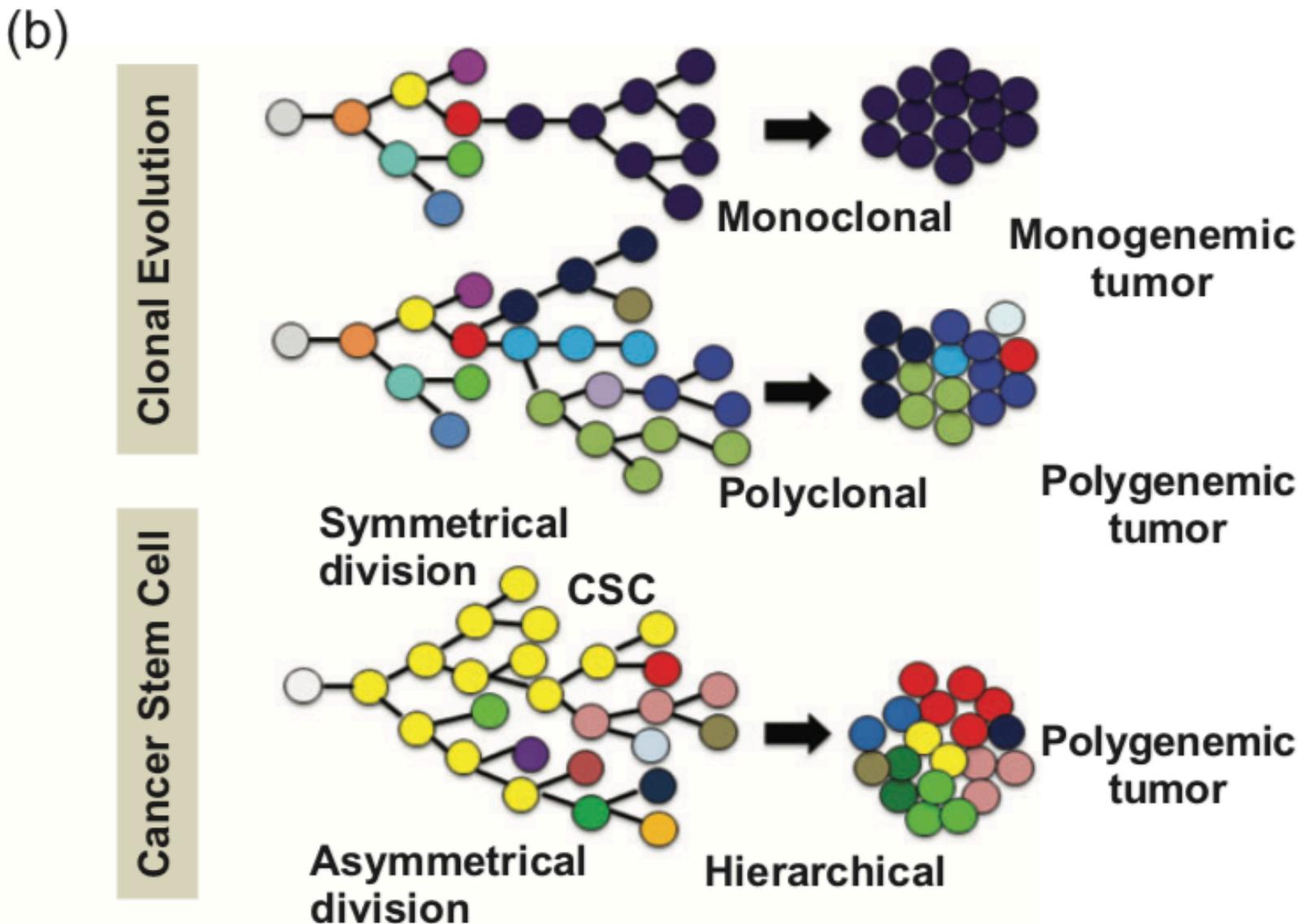


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Fig. 2c

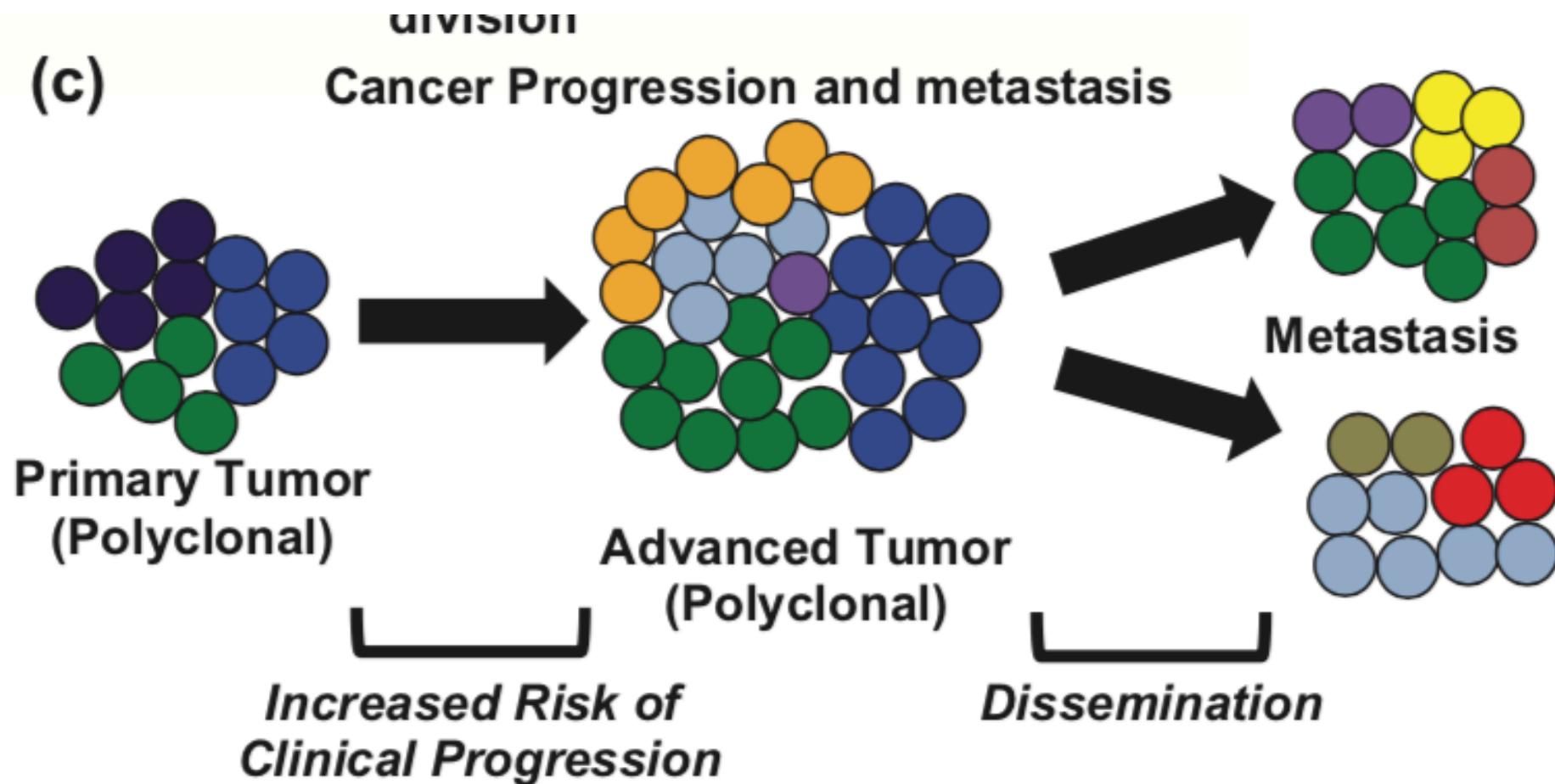


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Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“Introduction-3”

Main Points :

- 1) Fig. 3, tumour evolution and treatments ...
- 2) DNA of tumour cells is usually unstable (compared to normal cells): “**genetic instability**”: high degree of point mutations, chromosomal rearrangements etc. etc.
- 3) Factors inducing “genetic instability” include:
 - loss or mutation of genes necessary for DNA repair
 - or necessary for ordered mitosis, DNA replication and chromosome segregation to daughter cells
 - exposure to mutagenic factors like light and UV radiation, alpha particles, smoke, etc.

Terms ? :

- 1)...

Questions ? :

- 1)...

Fig. 3

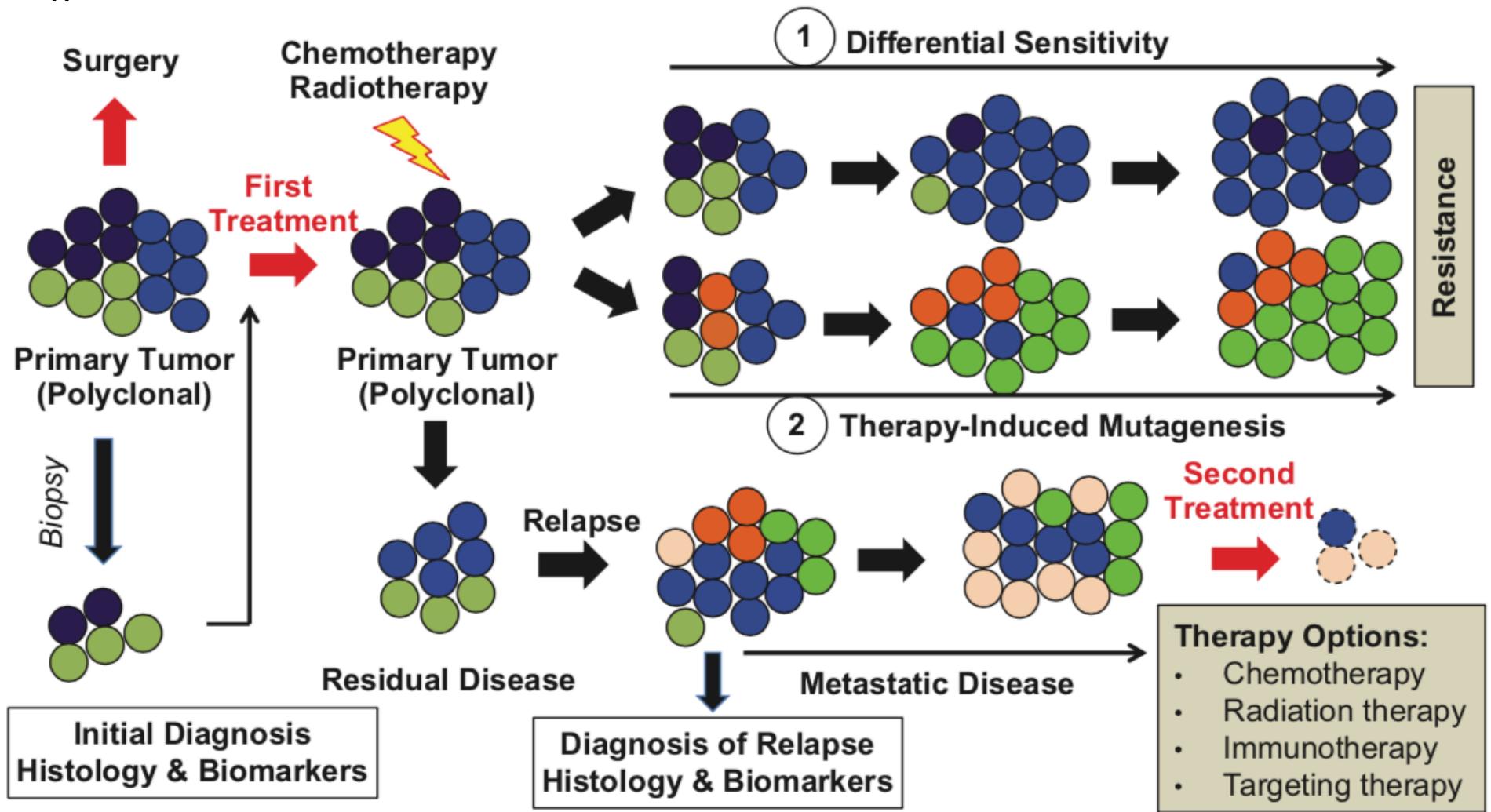


Figure 3. Role of tumor heterogeneity in biomarker prediction and tumor resistance to clinical therapy. Initial cancer diagnosis and first treatment depends on initial cell and molecular characterization, derived from a small tumor fraction (biopsy, here figure shows a complete representation, but in some cases, it may be biased). In most cases, the current first-line treatments can successfully eliminate dominating cancer clones, with the cost of selecting resistant tumor clones through either differential sensitivity (1) or therapy-induced mutagenesis (2). These resistant clones are capable of driving disease progression and eventually metastasis. Hence, the clonal composition of metastatic lesions may significantly differ from clones in the primary tumor. As a result, initial treatment choice may not be effective in progressive metastatic disease. This necessitates a new diagnosis and additional comparative steps after relapse, prior to second and usually combined treatment options (i.e., immunotherapy, selective pathway component targeting and/or gene therapy) (Adapted from Tellez-Gabriel et al., 2016; doi:10.3390/ijms17122142).

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“... clinical features and Genomic heterogeneity in pan-cancer studies”

Main Points :

- 1) non-silent coding mutations : many !!!
- 2) some important, most not (“**passengers**”)
- 3) Tumour heterogeneity is hugely heterogeneous: some have much more than others (mutation rates differ by factor > 1000-fold)
- 4) Factors inducing “genetic instability” include:
 - loss or mutation of genes necessary for **DNA repair**
 - or necessary for ordered mitosis, DNA replication and chromosome segregation to daughter cells (mitotic machinery, “proliferation genes”)
 - exposure to **mutagenic factors** like light and UV radiation, alpha particles, smoke, certain viruses, etc.

Terms ? :

- 1)...

Questions ? :

- 1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“... clinical features and Genomic heterogeneity in pan-cancer studies”

Main Points :

- 1) “a diverse genomic heterogeneity landscape across nine cancer types with a notable tendency for **highly heterogeneous tumors to have lower levels of immune cell infiltration or T cell infiltration**
- 2) How many genes are needed to make a normal cell become oncogenic ? : sometimes just one ?
- 3) Table 1: ?

Terms ? :

1) immune cell infiltration

Questions ? :

- 1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“... clinical features and Genomic heterogeneity in pan-cancer studies”

Main Points :

- 1) Genes found frequently mutated in cancer and believed to be **driver genes**: several different ones, partly common across tissues, partly rather specific ...
- 2) Common f.ex. : TP53 , PTEN , (K,H,N)RAS / BRAF ,
ERBB1-2 (EGFR) , PIK3CA
- 3) Specific f.ex. : APC , RB1 , BRCA1, BRCA2

Terms ? :

1)

Questions ? :

1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“Association of cancer genetic heterogeneity and therapeutic failures”

Main Points :

- 1) Example: KRAS mutations confer resistance to targeted anti-EGFR drugs:
we have seen this in detail in the earlier slides.

Terms ? :

- 1)...

Questions ? :

- 1)...

Characterization of cancer genomic heterogeneity by next-generation sequencing ...

“Immune checkpoint inhibitors ”

Main Points :

- 1)... tumor cells can impair the immune system's capacity to eradicate them by
 - immune suppressive effects
 - loss of targetable antigen expression.
- 2) The main concept of **immune checkpoint** targeting is to prevent receptors on the T cells and cancer cell ligands from binding to each other, hence disrupting signaling cascades that help cancer cells to evade T cell-mediated cell death.
- 3) Another strategy: „Cell engineering“, f.ex. **chimeric antigen receptor T cells** (CAR-T cells)

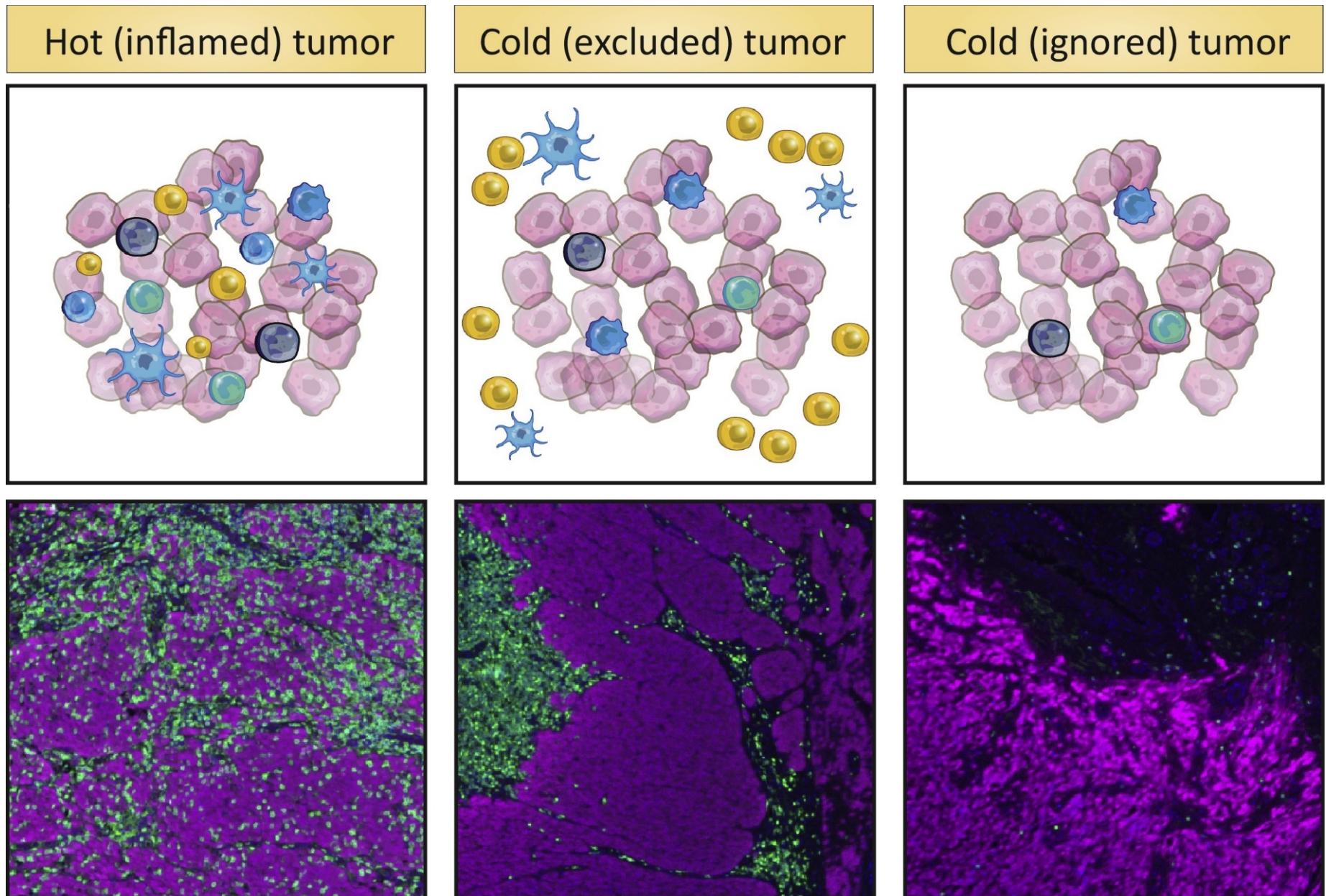
Terms ? :

- 1)...

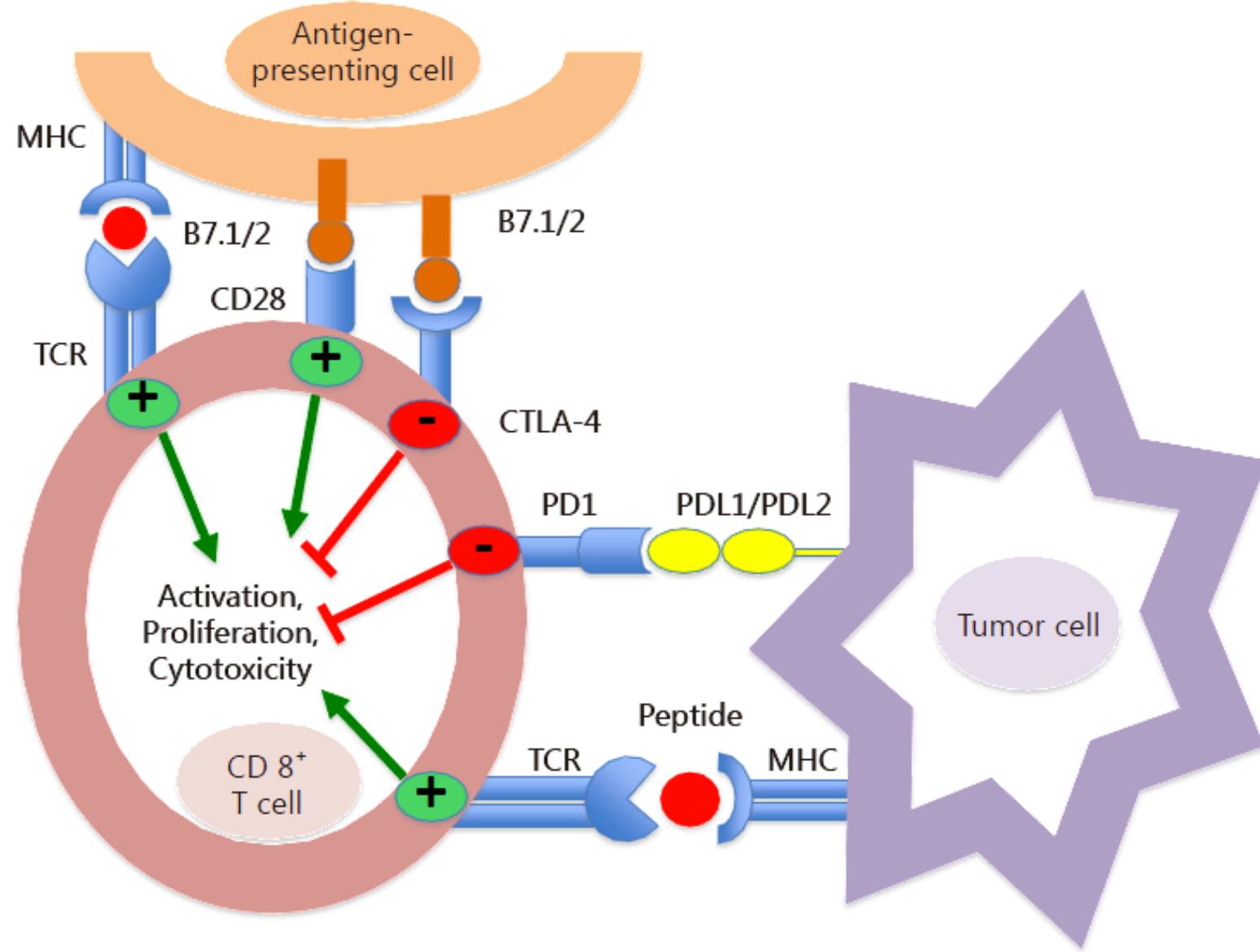
Questions ? :

- 1)...

Immune antitumor response



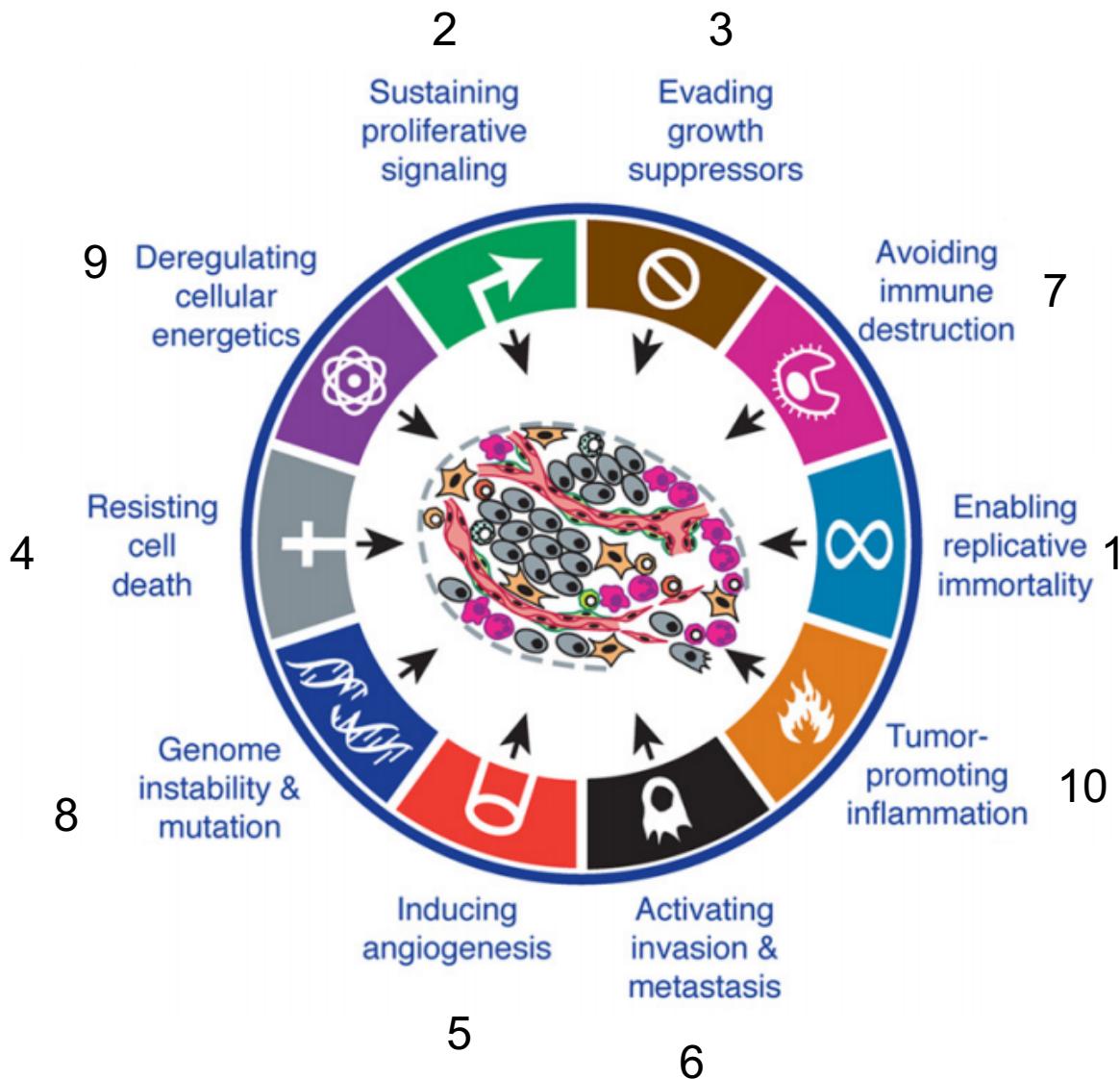
Immunotherapy by i-checkpoint inhibitors



Introduction

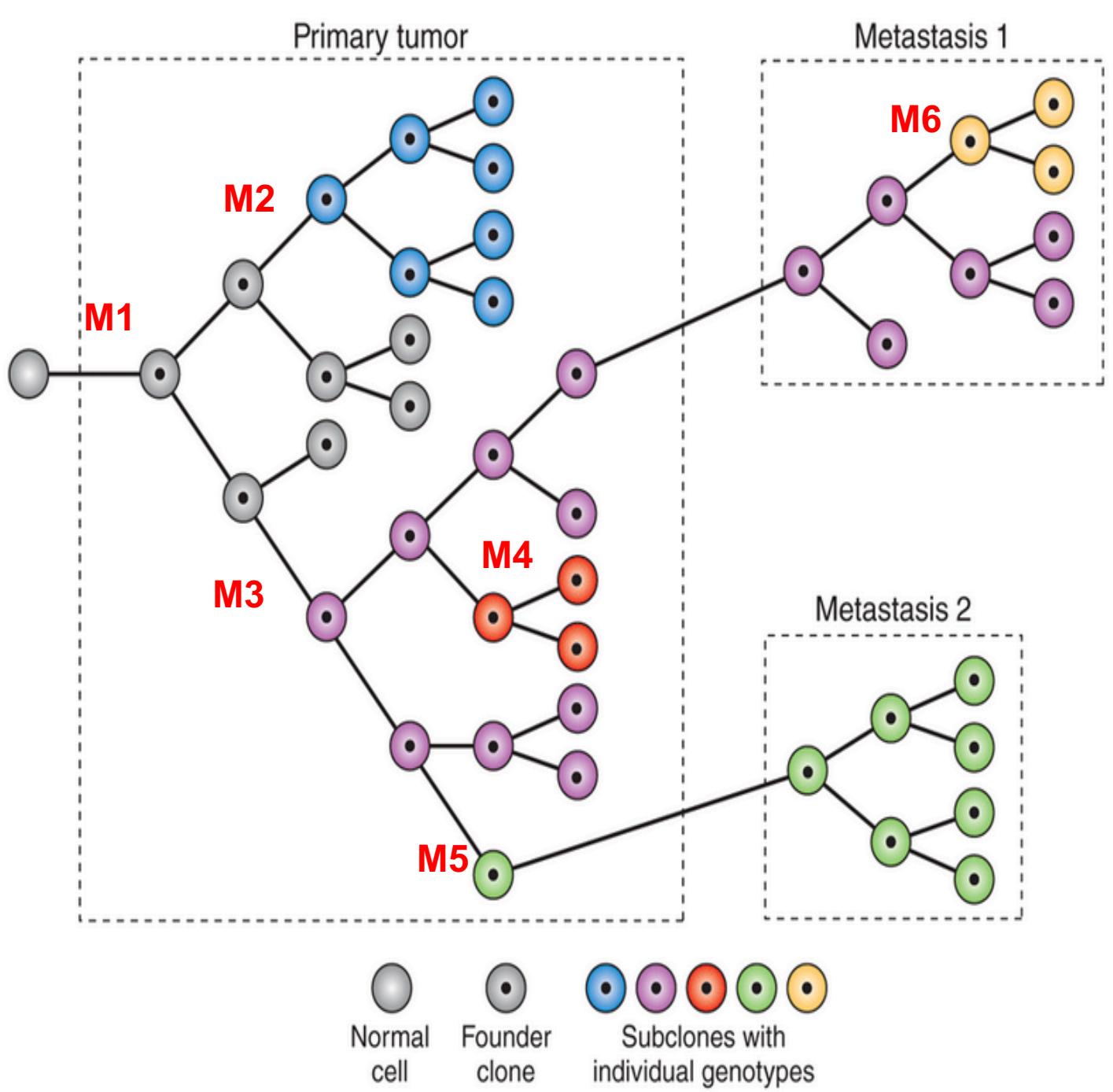
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Hallmarks of Cancer (Weinberg & Hanahan 2011)



1. Can divide forever
2. Can get growth signals
3. Can ignore growth-stop signals
4. Can evade Apoptosis
5. Can induce blood supply
6. Can invade and form metastases
7. Can avoid destruction by the immune system
8. Can mutate
9. Can adapt its (energetic) metabolism
10. Can use inflammation to its benefit

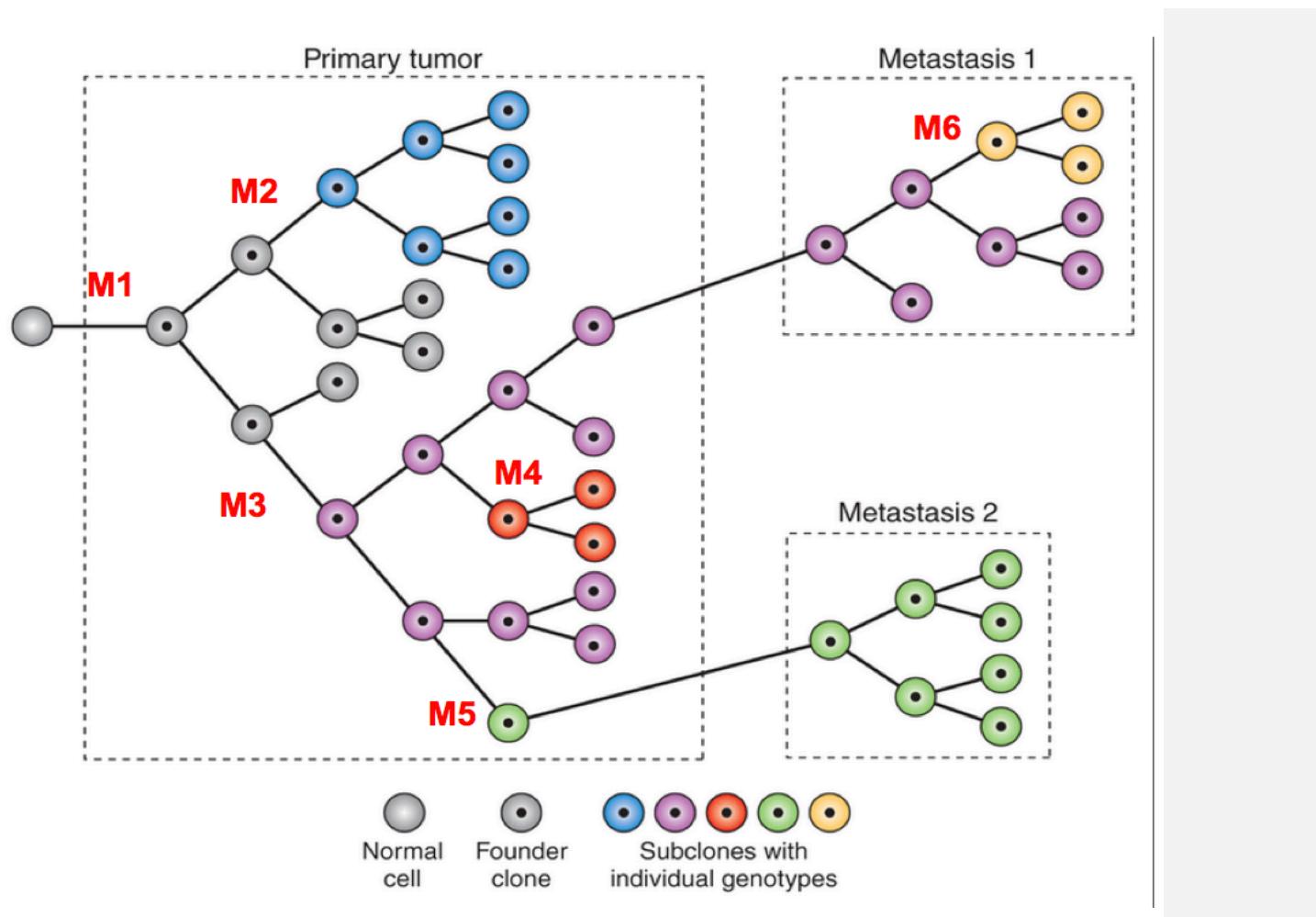
Subclones, mutations and their frequency in sequenced DNA



1) MUTATIONS IN CANCER:

- If we sequence the DNA of the primary tumor in the figure, which mutations we find with which allele frequency ?
- How do these frequencies change if the tumor purity is 25% ?
- And if we sequence the metastasis 1 resp. the metastasis 2 ?
- Are any found only in the primary or only in the mets ?

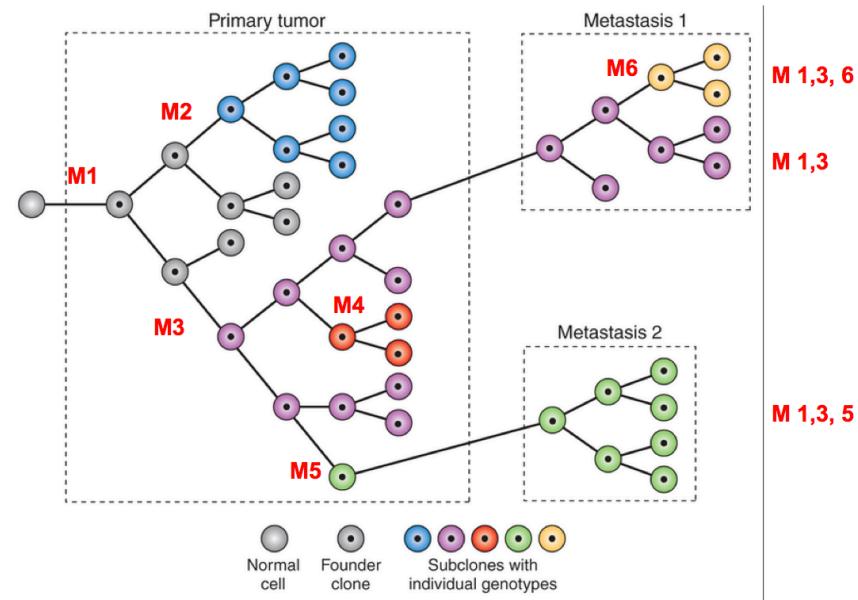
Exercise



Assumption: always mutation in one allele of two, no other events
(no amplifications, deletions, aneuploidies)

Exercise

	Primary / Met 1 / Met 2
M1	50% / 50% / 50%
M2	14% / 00% / 00%
M3	25% / 50% / 50%
M4	07% / 00% / 00%
M5	04% / 00% / 50%
M6	00% / 20% / 00%



25% purity: the mutations is diluted by “normal DNA”, the cancer allele is observed with only ¼ of the frequency estimated above.

For some of these mutations it can be problematically low, for example here M5 in the primary tumor. And the primary might be the only tumor that was sequenced, the only sufficiently large to be detected or accessible to biopsy (metastases in the brain usually are not).

Now for ex. M5 could be a KRAS mutation that conveys resistance to anti-EGFR treatment, and depending if it is detected or not, we might make a good choice of treatment or not. In the primary tumor its frequency here is about 1% (at 25% purity), and therefore reliable detection requires deep sequencing and low sequence error rate by the technology, and is therefore borderline to what can be done today .