Technical University of Denmark

Sequencing in cancer genomics

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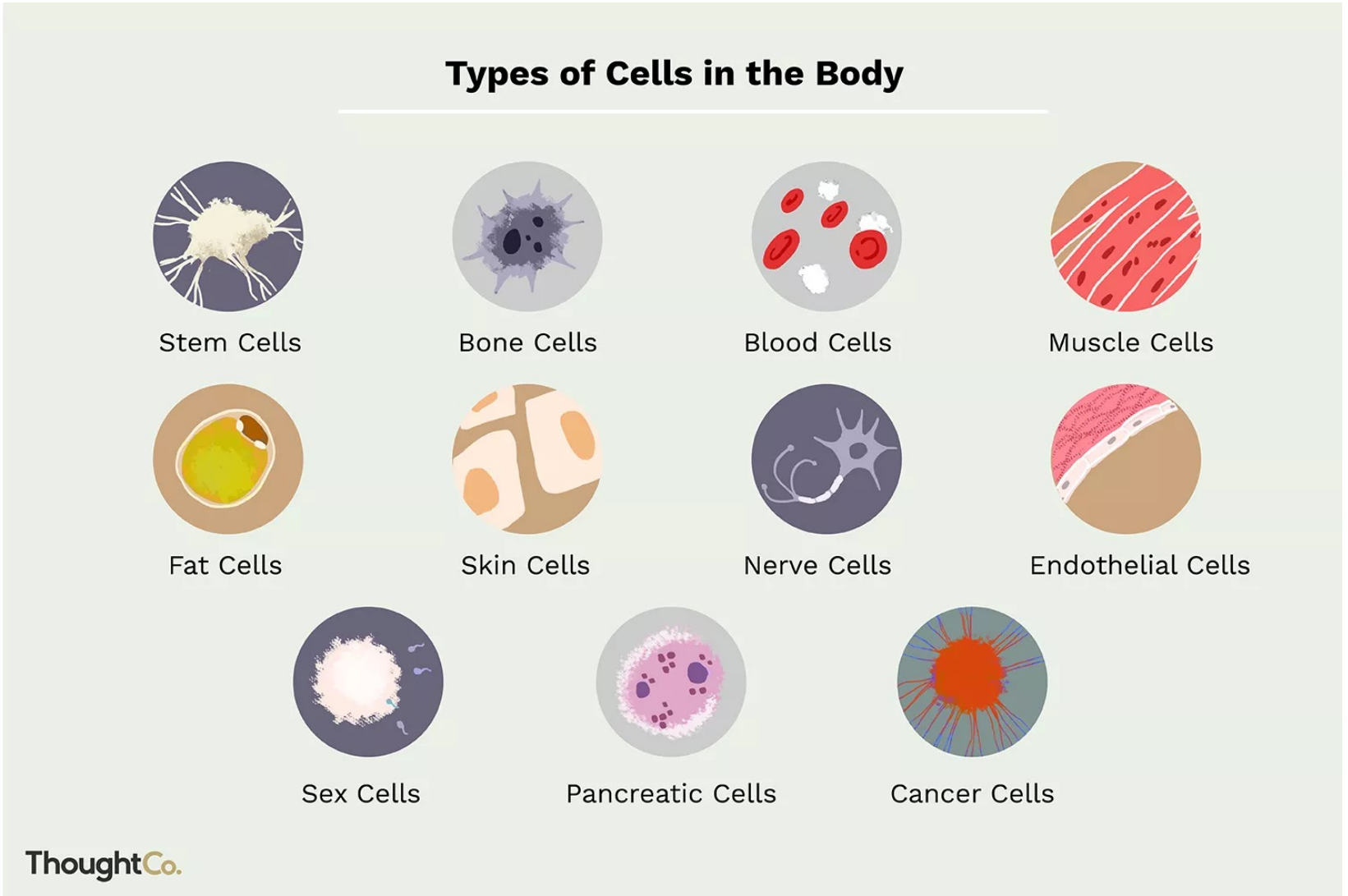
Outlines

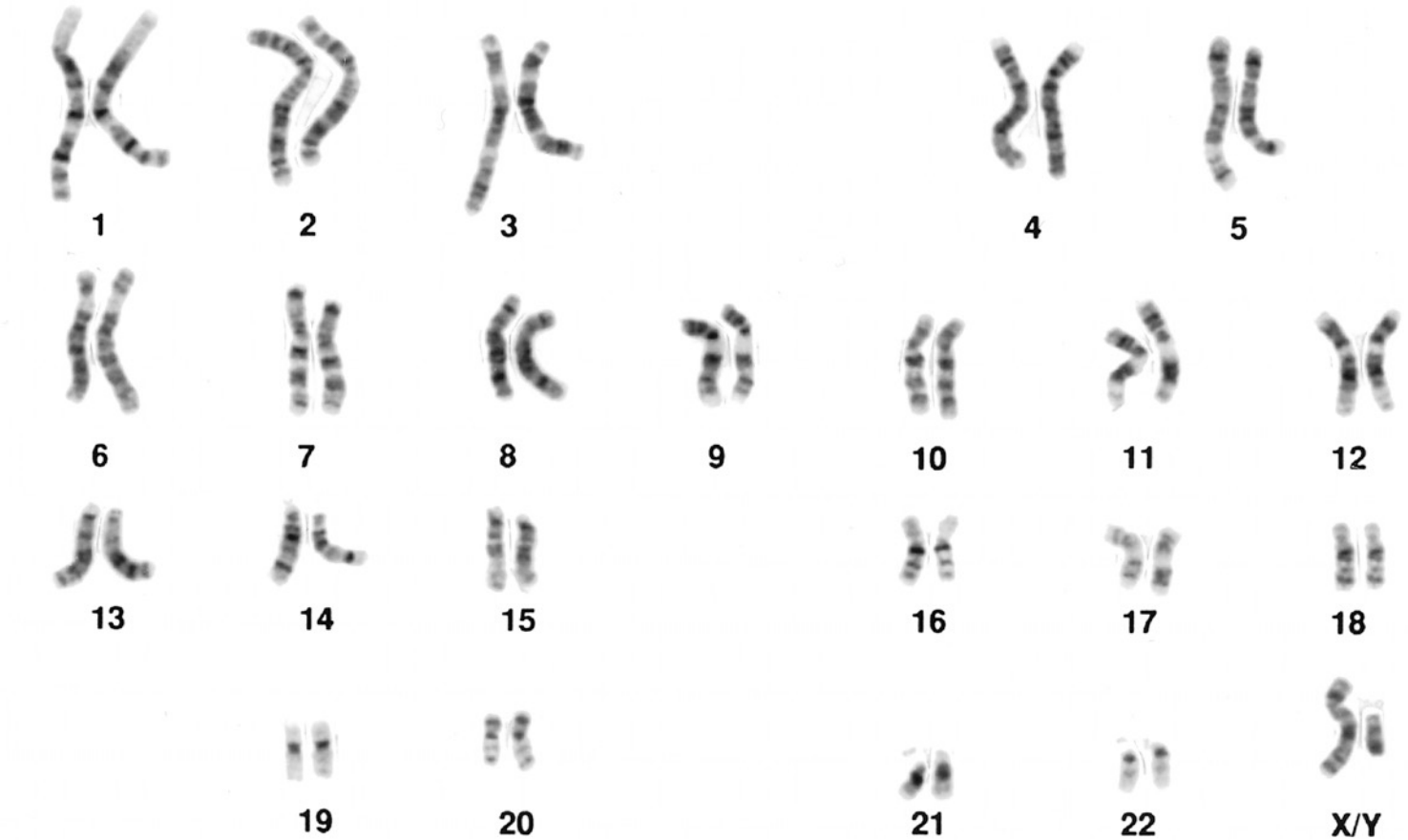
- Cancer
- Hallmarks of cancer
- Driver and passenger mutations
- Driver genes
- Exome and Whole Genome Sequencing: somatic variants
- RNA Sequencing and gene fusion detection and subtypes
- Databases for cancer research

Learning objectives

- To define how cancer develops and evolves
- To understand that cancer is not just one unique disease
- To identify alterations in the cancer genome
- To analyze cancer samples using sequencing approaches

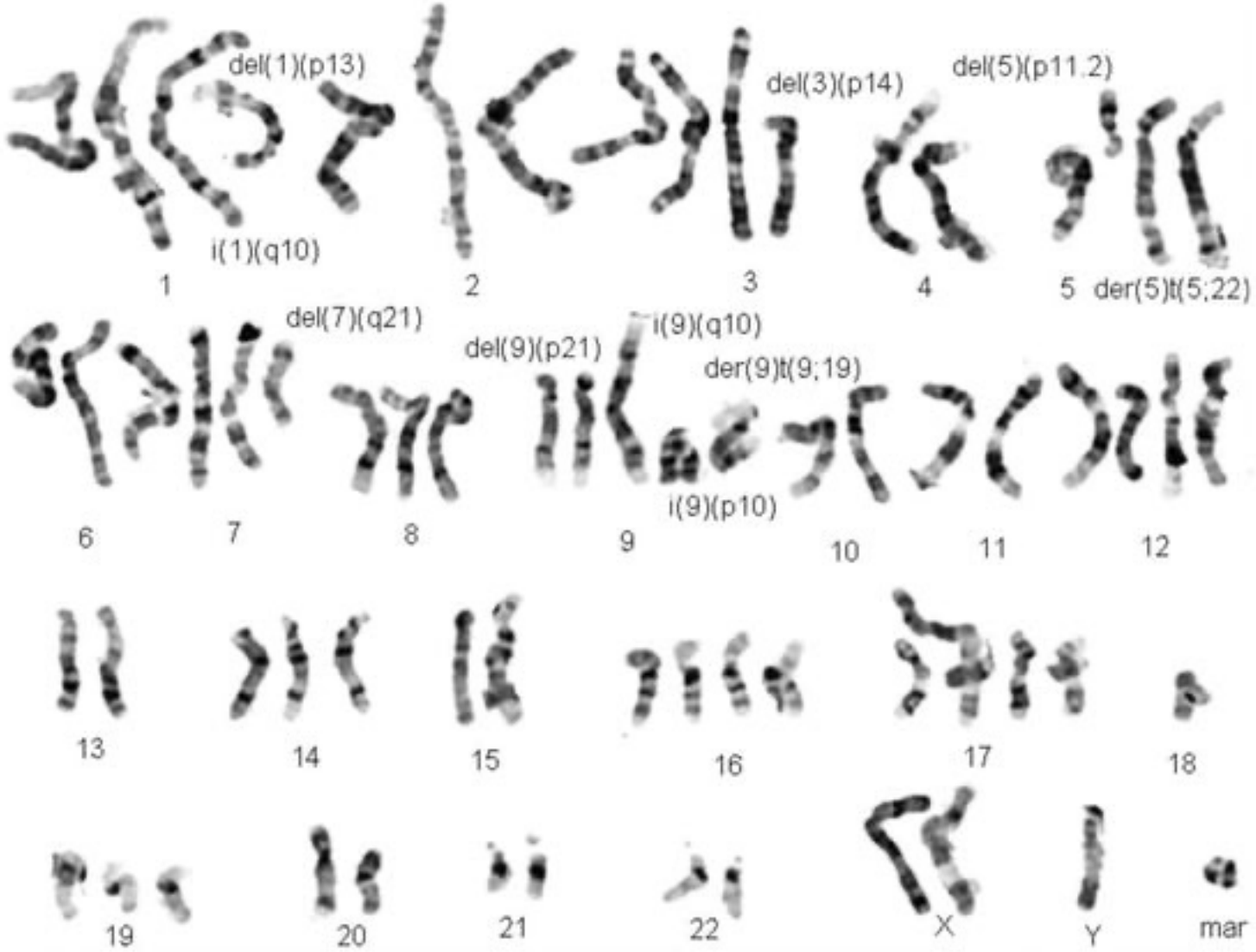
We are all made of cells





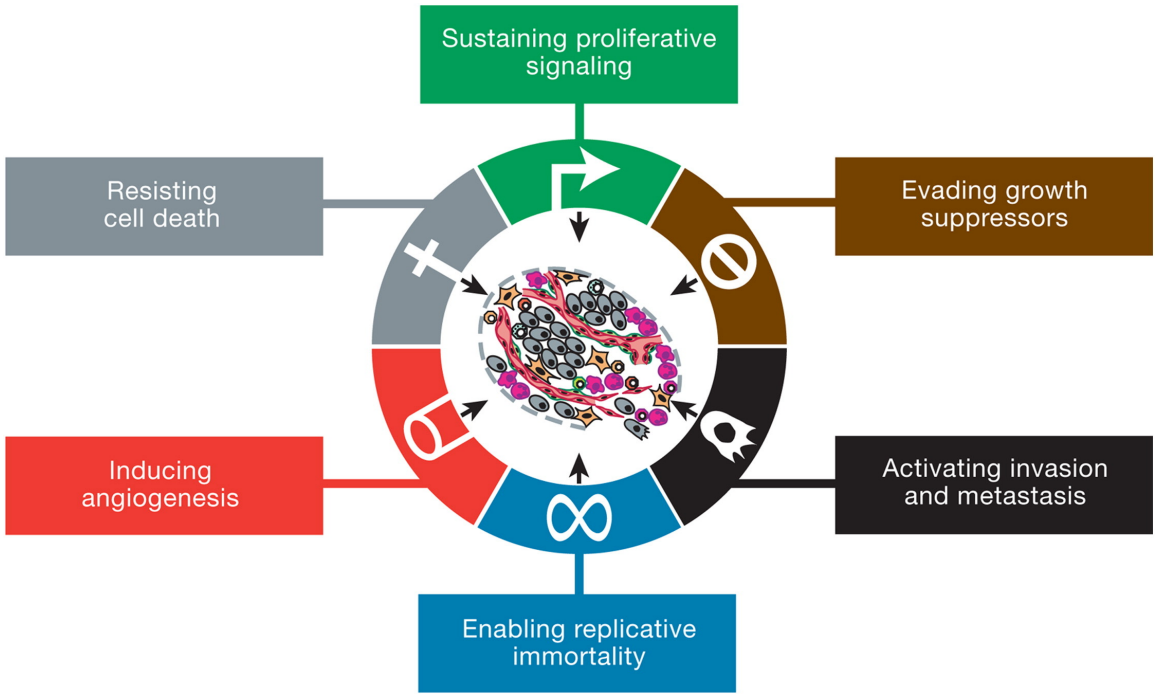
Normal Karyotype

National Cancer Institute, AV-9700-4394, 2001

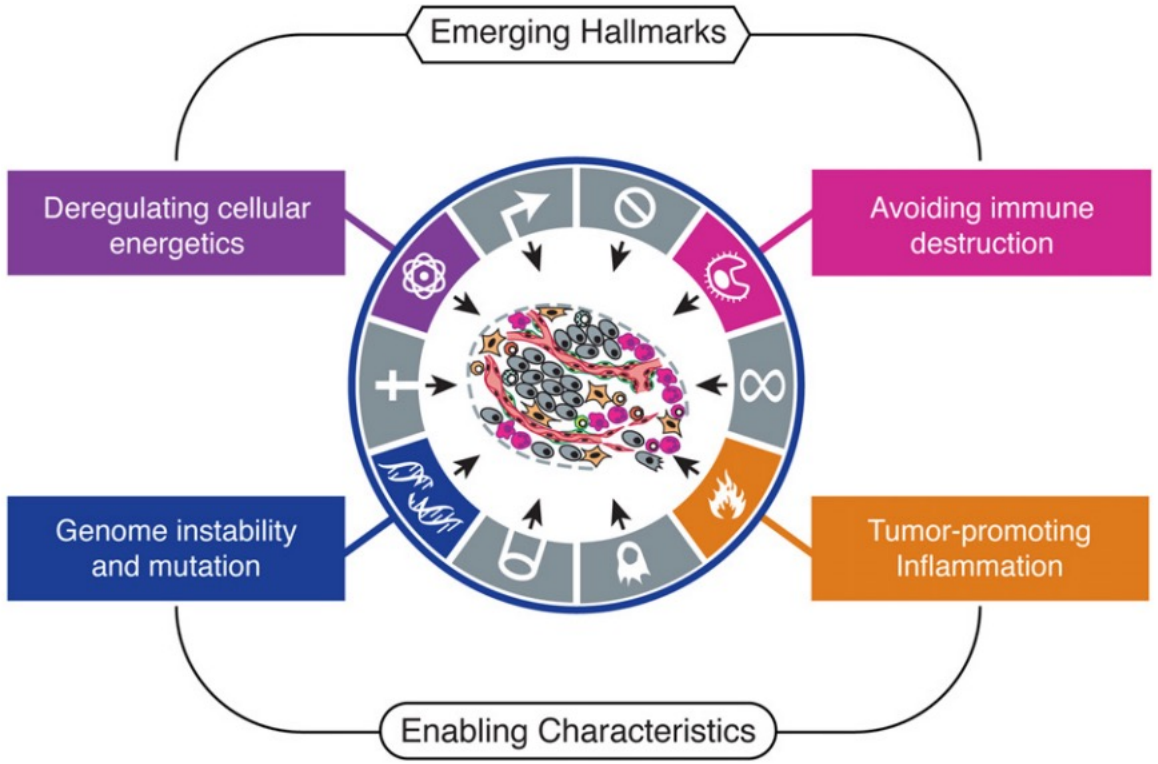


Karyogram of a representative G-banded Head and Neck Cancer metaphase (from Ribeiro et al. *Int. J. Mol. Sci.* **2019** ; https://doi.org/10.3390/ijms20194711)

Hallmarks of cancer



Weinberg and Hanahan Cell, 2000



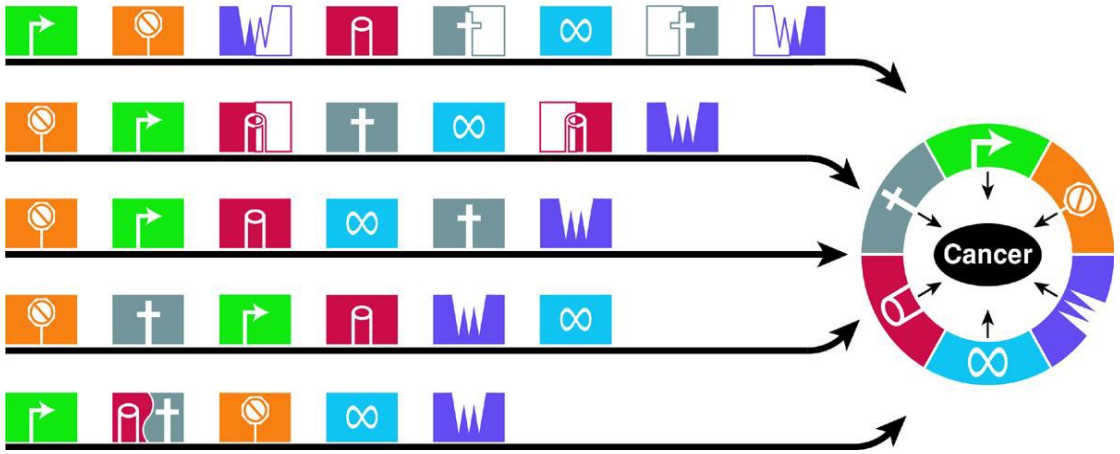
Weinberg and Hanahan Cell, 2011
Hanahan, Cancer Discovery, 2022

Multiple routes to cancer

A

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

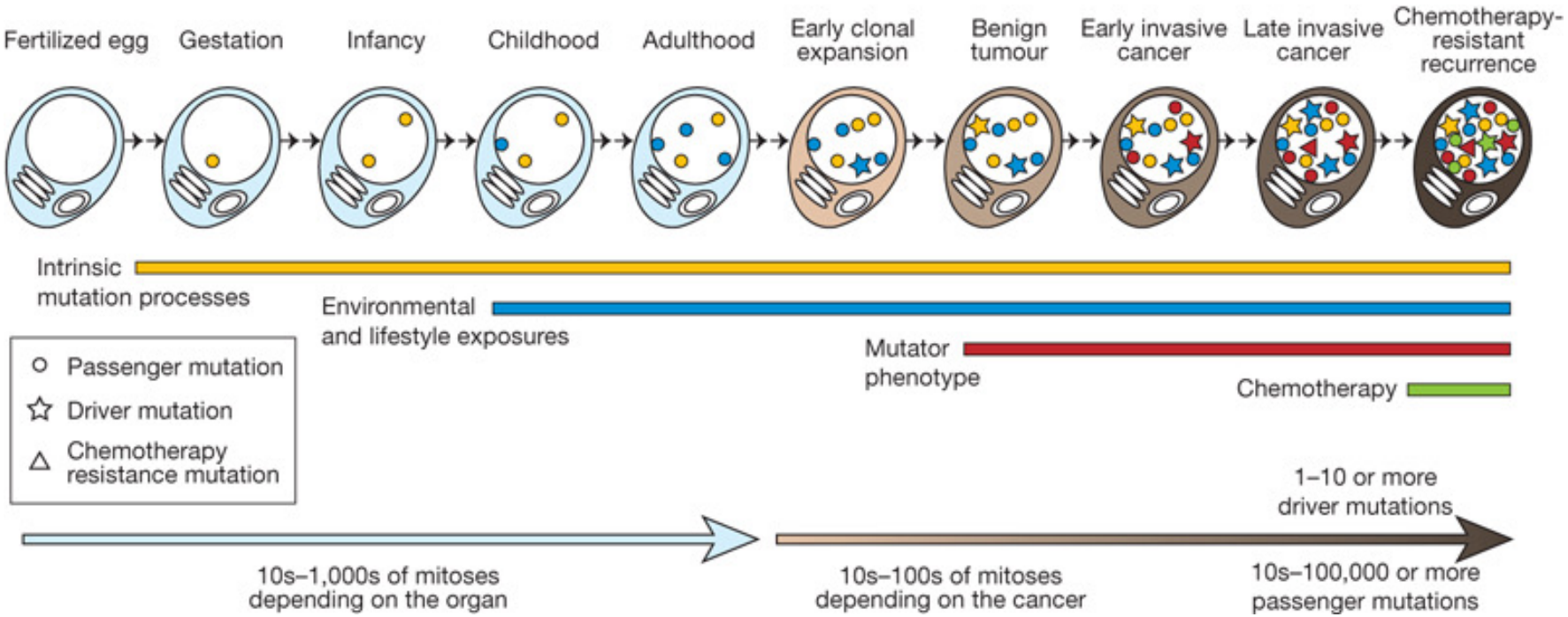
B



Circos plot and classes of alterations of interest

https://www.researchgate.net/figure/A-Circos-plot-showing-the-summary-of-somatic-genomic-alterations-in-the-three-HCC_fig1_314247140

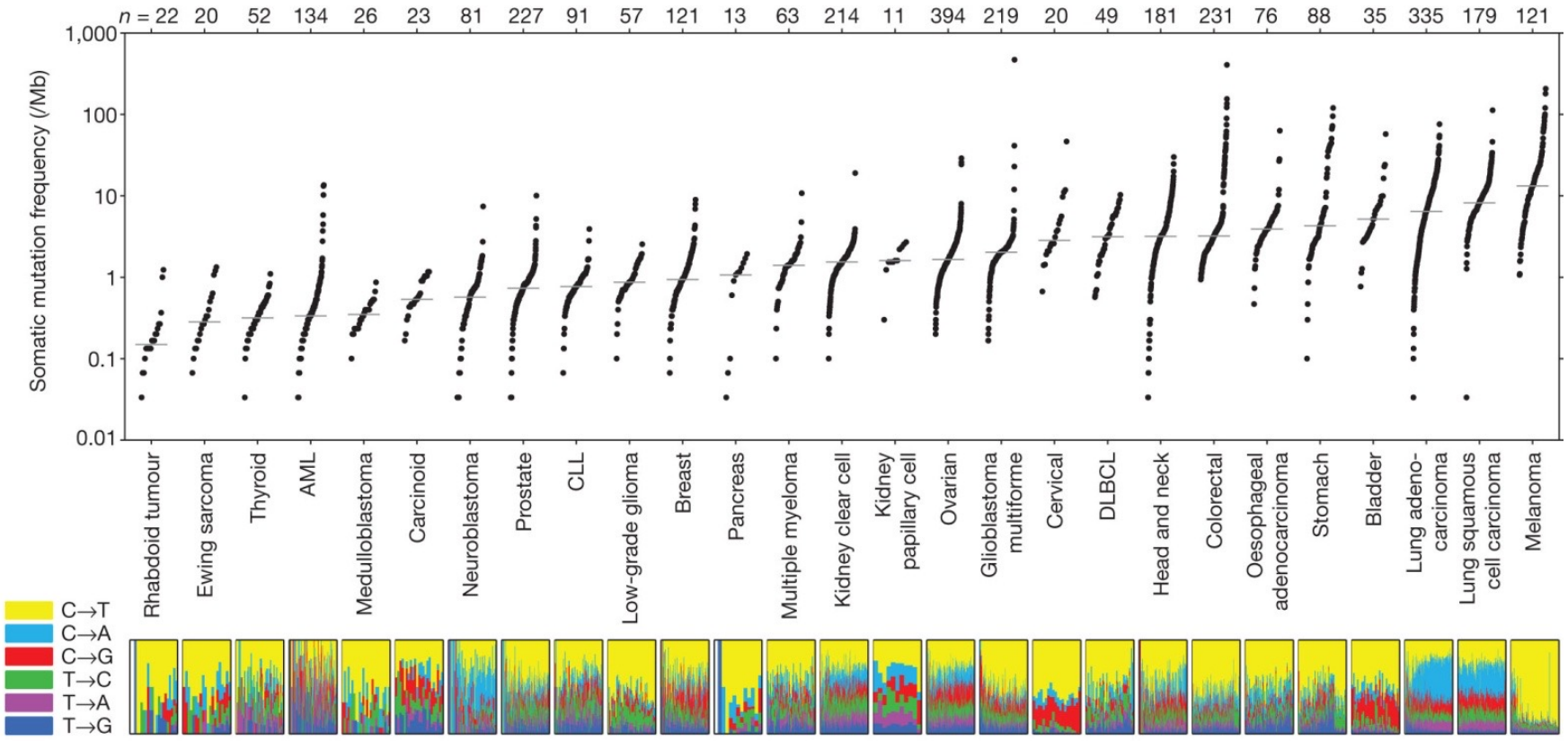
Driver and passenger mutations in cancer



MR Stratton *et al. Nature* **458**, 719-724 (2009) doi:10.1038/nature07943

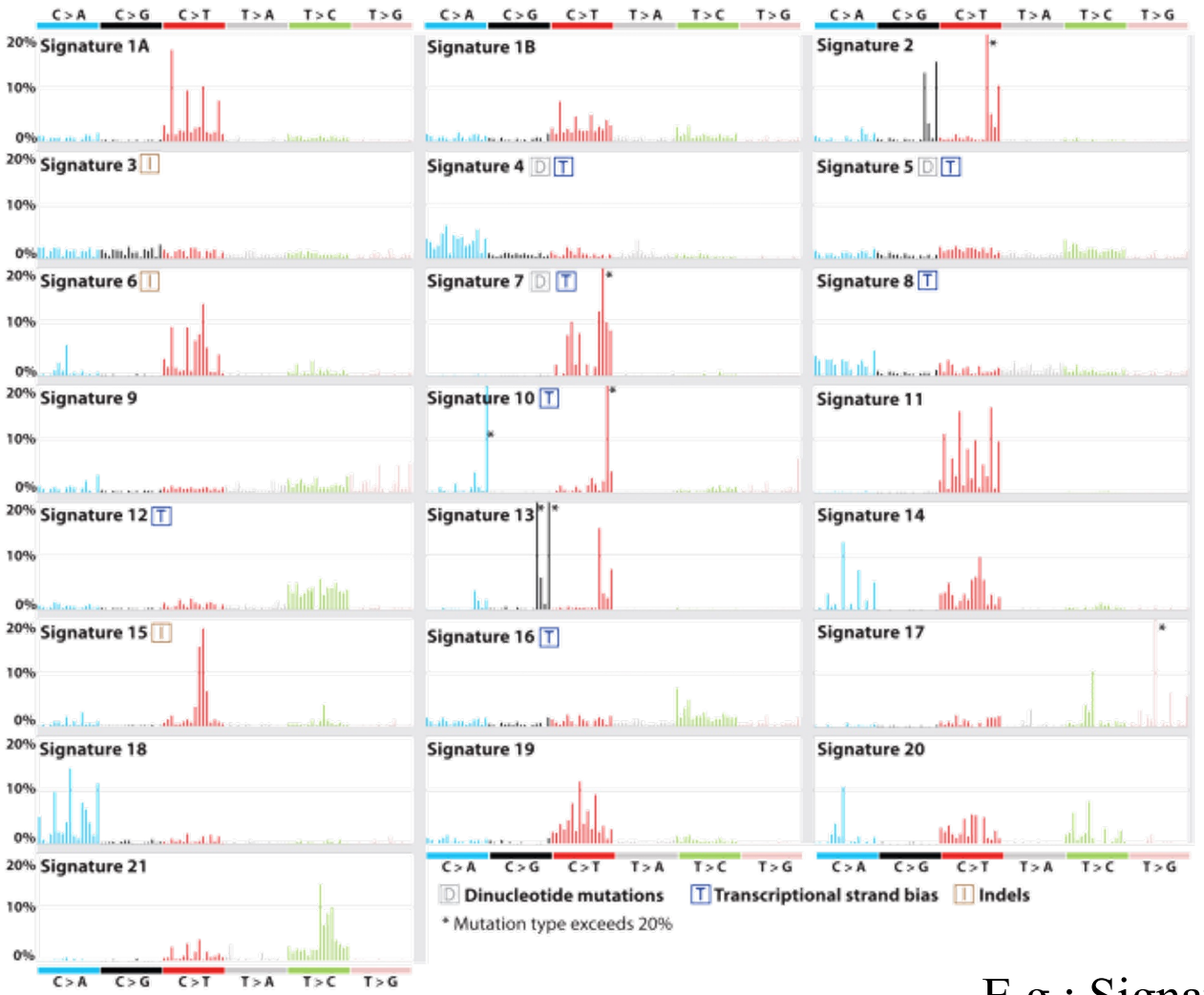
- Combination of germline and somatic mutations
- Driver and passenger mutations
- Reccurence of mutations

Mutation burden in different cancer types and mutational signature



MS Lawrence *et al. Nature* (2013) doi:10.1038/nature12213

Mutational signatures in different cancer types



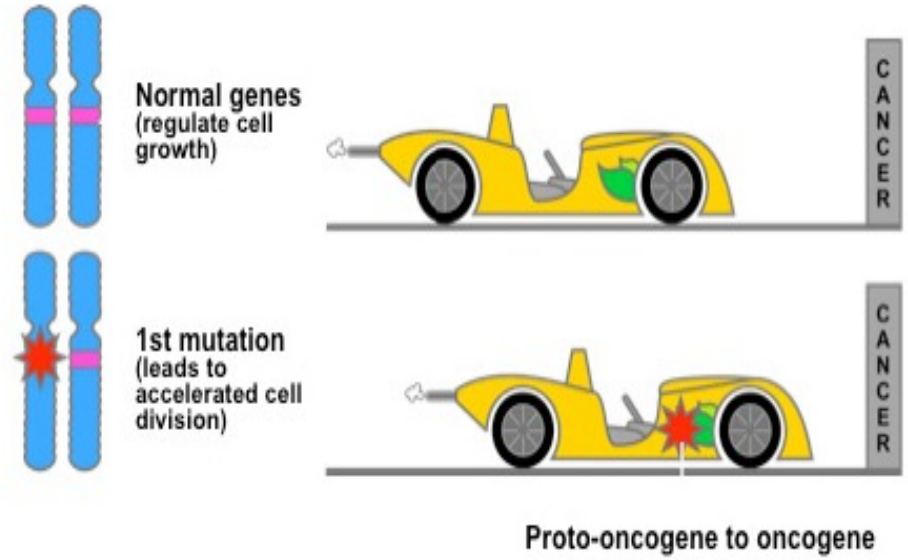
E.g.: Signature 4

- Smoking induced mutations
- Lung cancers

Alexandrov, Nature et al., 2013

Need for speed: **Oncogenes** vs Tumour suppressors

Oncogenes:
- Mutated proto-oncogenes
- Turn abnormal cell growth on
- 70 protooncogenes
- gain of function genes
- primarily somatic activated
- [throttle pedal in a car]



"Oncogenes are mutated genes whose PRESENCE can stimulate the development of cancer"

Examples: HER-2/neu. RAS, MYC, SRC, hTERT

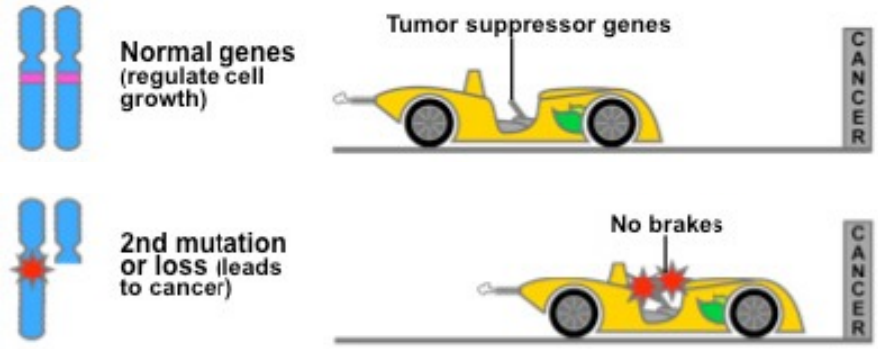
RAS, or SRC are protein kinases ➔ Cell cycle regulation

(Adapted from Nat. Cancer Inst.)

Need for speed: Oncogenes vs **Tumour suppressors**

Tumour suppressor genes:
- Stop the cell cycle, G1 phase
- Slow the cell cycle before S phase
- Can induce apoptosis

- primarily somatic de-activated
- loss-of-function mutations
- [brake pedal in a car]



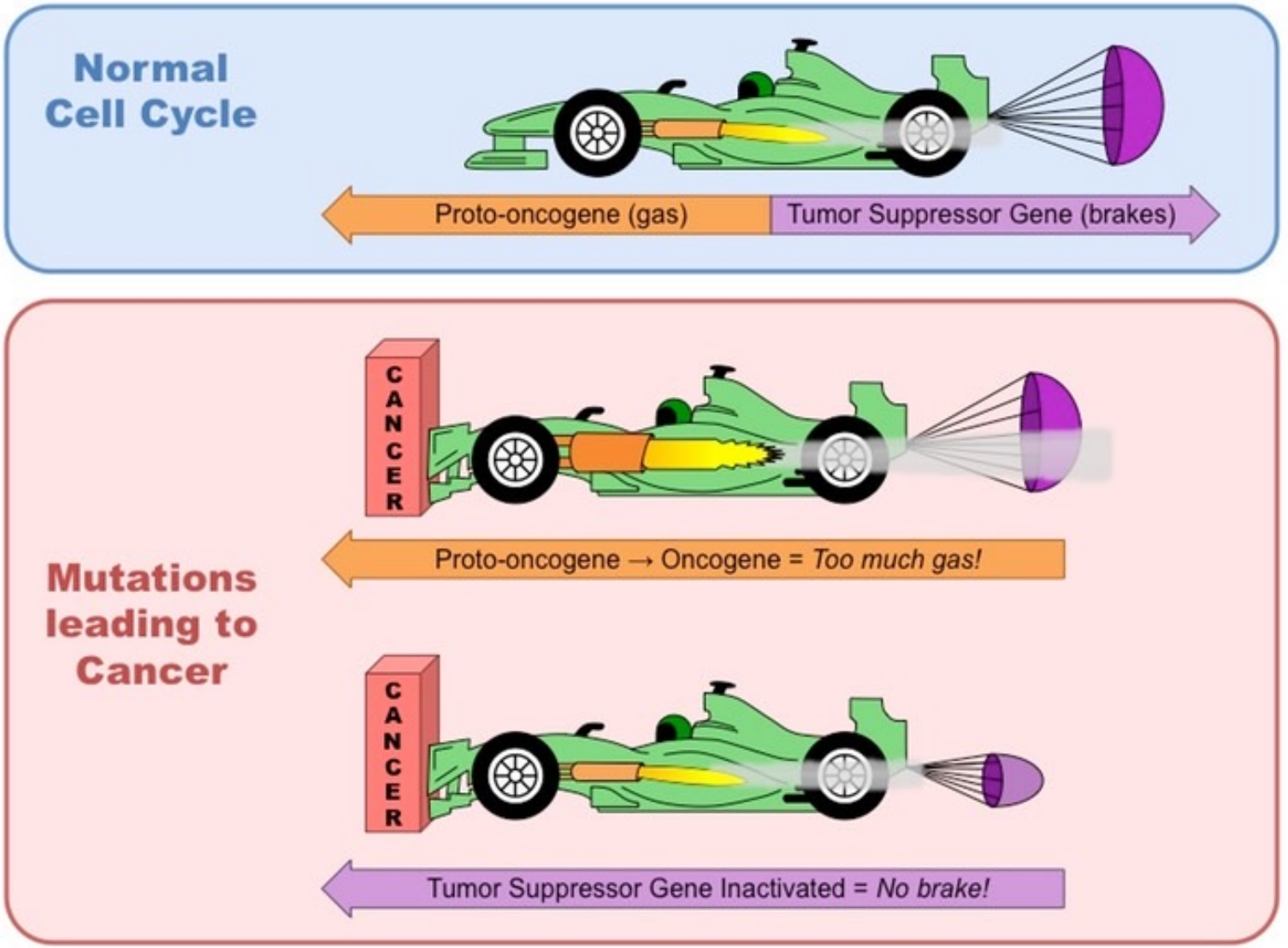
"Tumour suppressors are normal genes whose ABSENCE can stimulate the development of cancer"

Examples: p53, Rb, APC

Sometimes, a single functional copy (heterozygous) is enough to prevent cancer

(Adapted from Nat. Cancer Inst.)

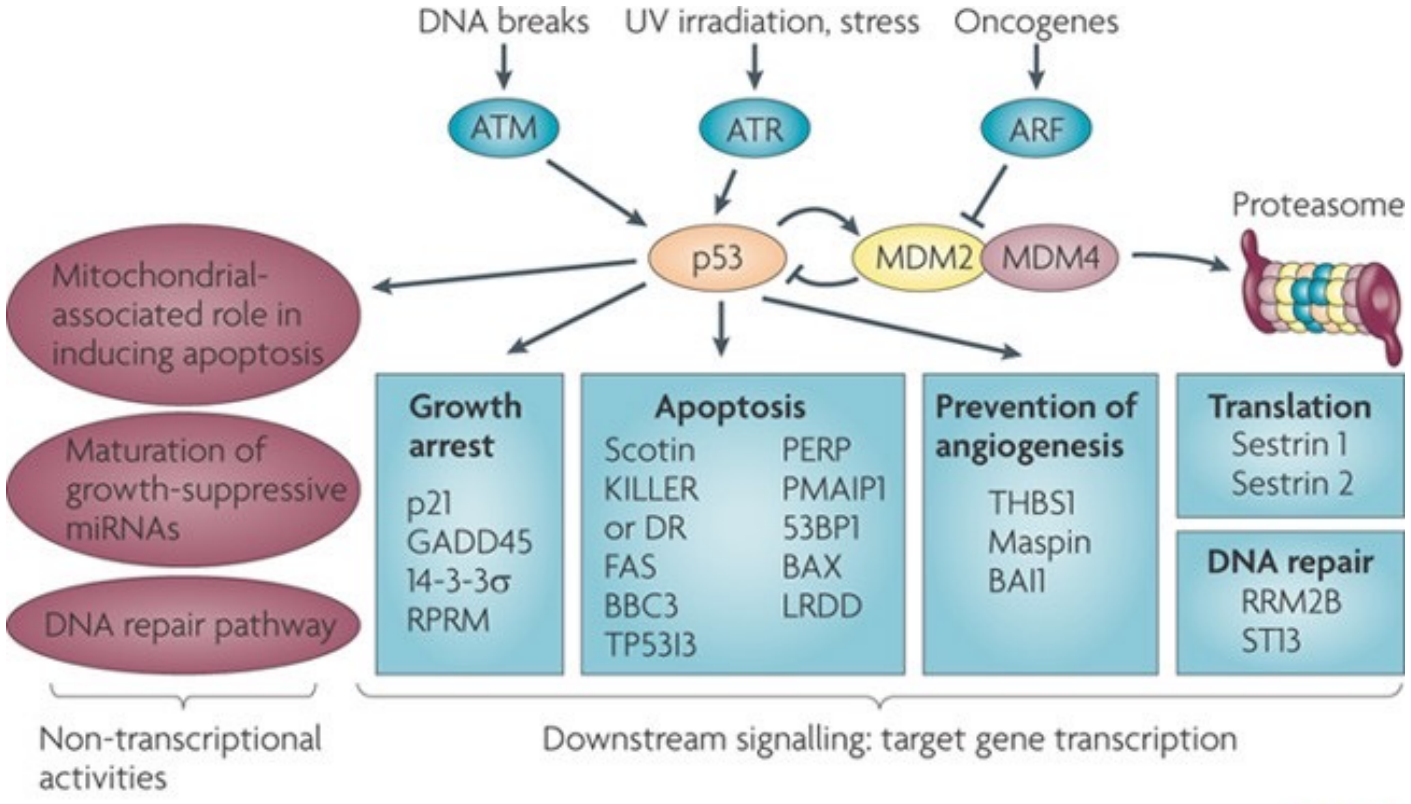
Oncogenes vs Tumour suppressors



(ib.bioninja.com.au)

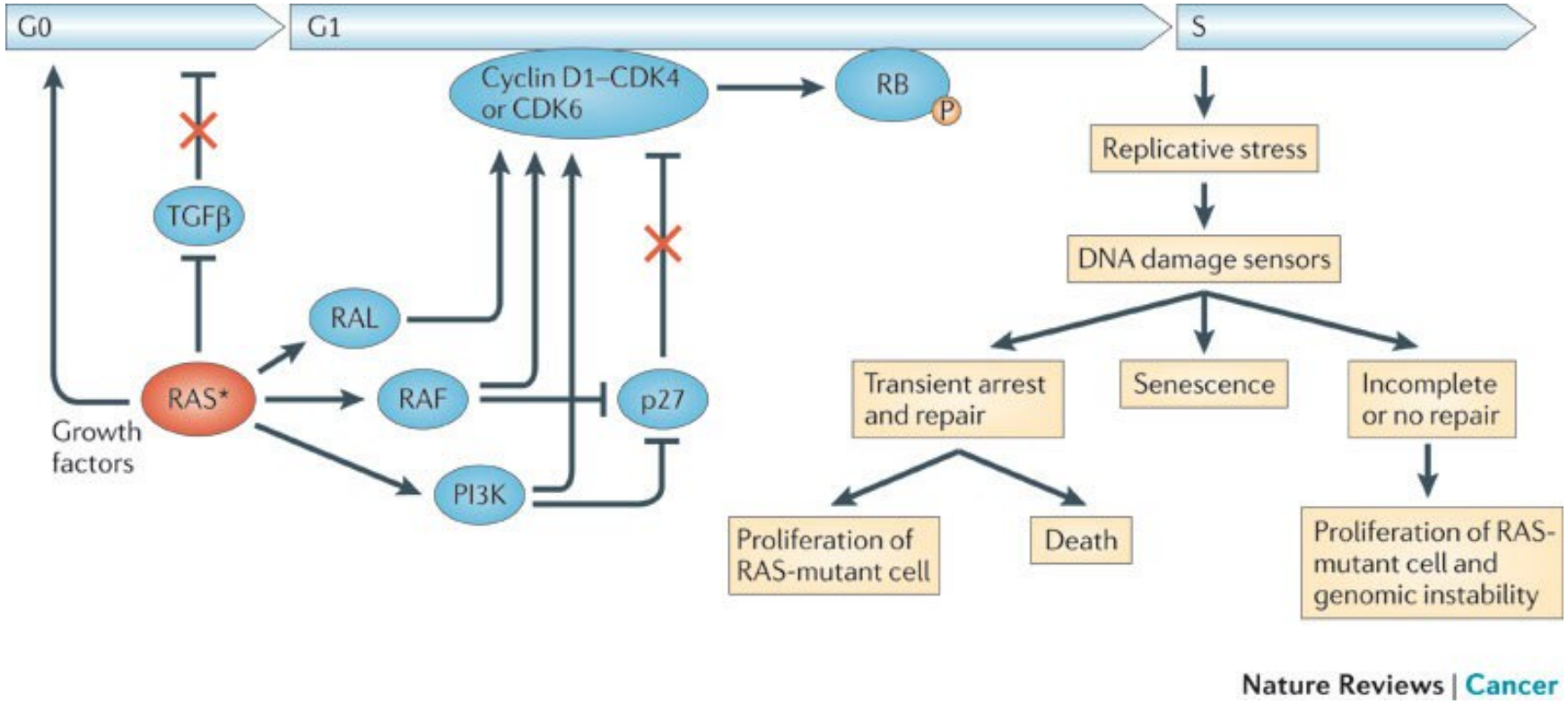
EXAMPLE OF TUMOUR SUPPRESSOR: P53

- Guardian of the human genome
- Key link between DNA damage and repair/apoptosis
- Mutations cause loss of function and promotes tumour emergence and growth



Brown CJ et al. 2009, Nature Reviews, https://doi.org/10.1038/nrc2763

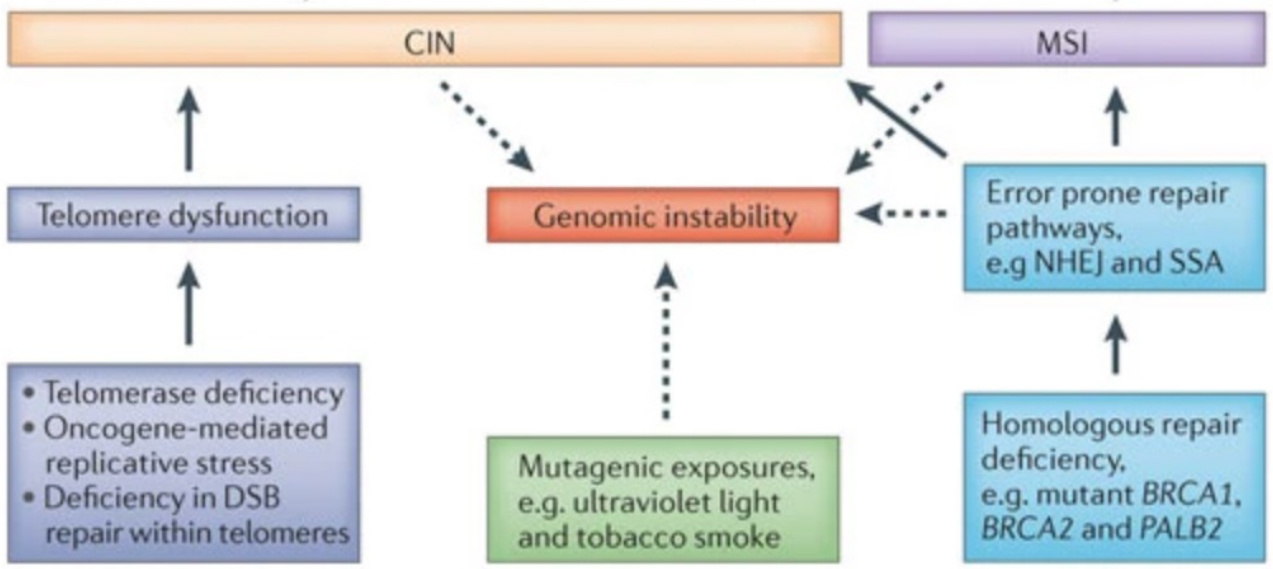
EXAMPLE OF ONCOGENES: RAS family



Small GTPases that are involved in cell cycle and cell-growth pathways
Mutations can make them more active and overcome their regulation, sustaining continuous growth and proliferation

Mutator genes: master mutational switches

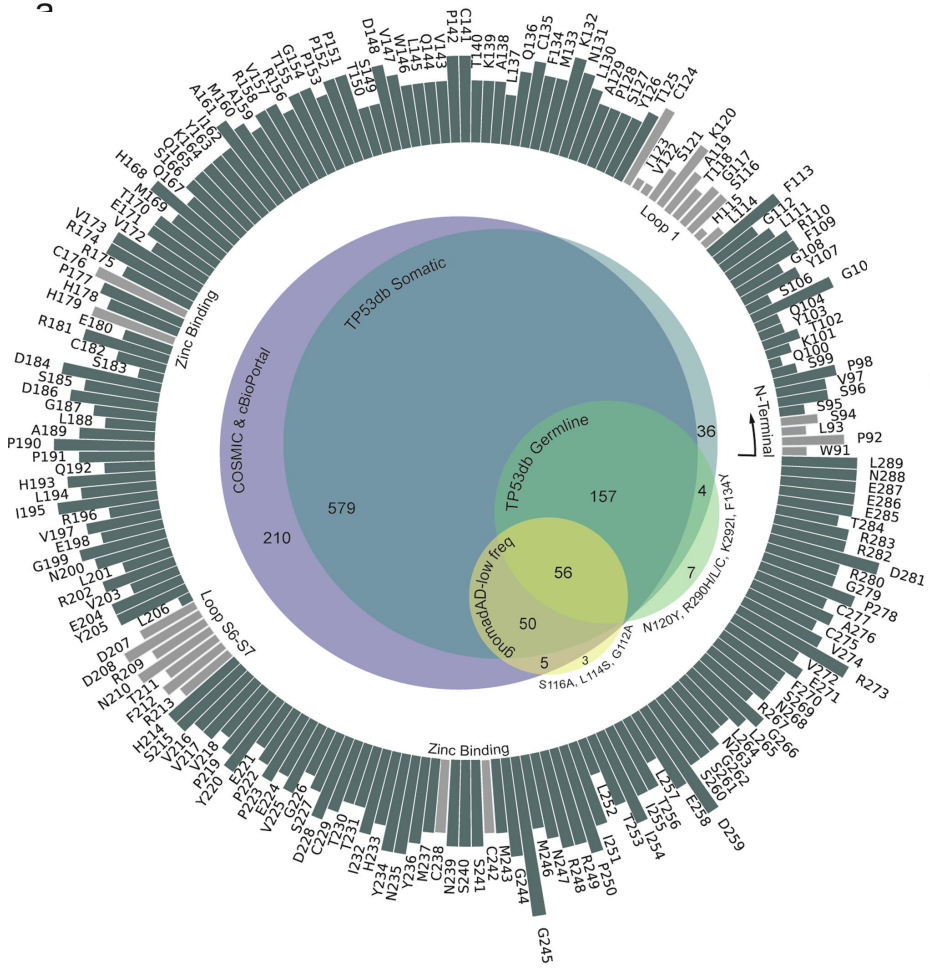
- Mutations in genes that decrease repair efficiency
- Increase of tumour mutation rate
- Involved in DNA repair, or chromatin stability

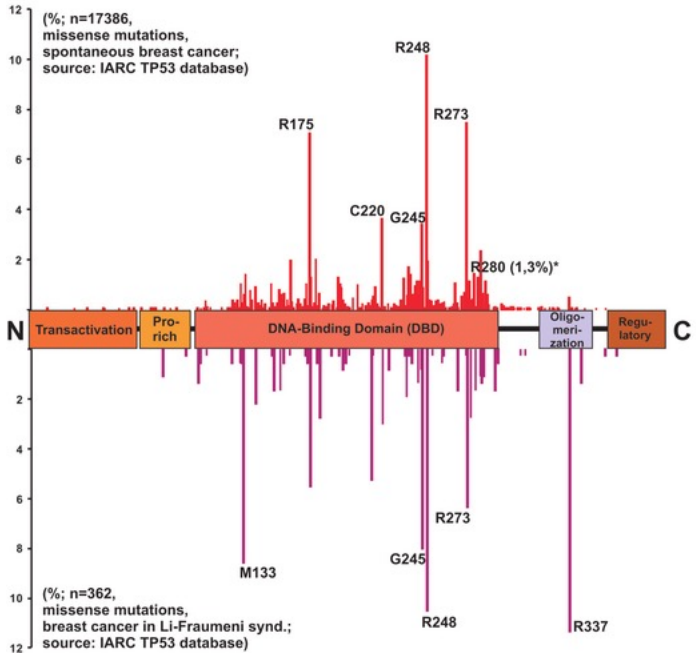


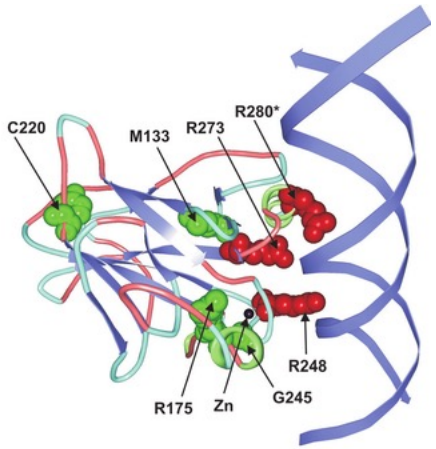
Yates and Campbell, Nat Rev Genetics, 2012, https://doi.org/10.1038/nrg3317

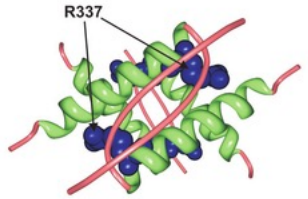
Coding variants– exome sequencing

- Example: p53 – majority of mutations are in the DNA binding domain





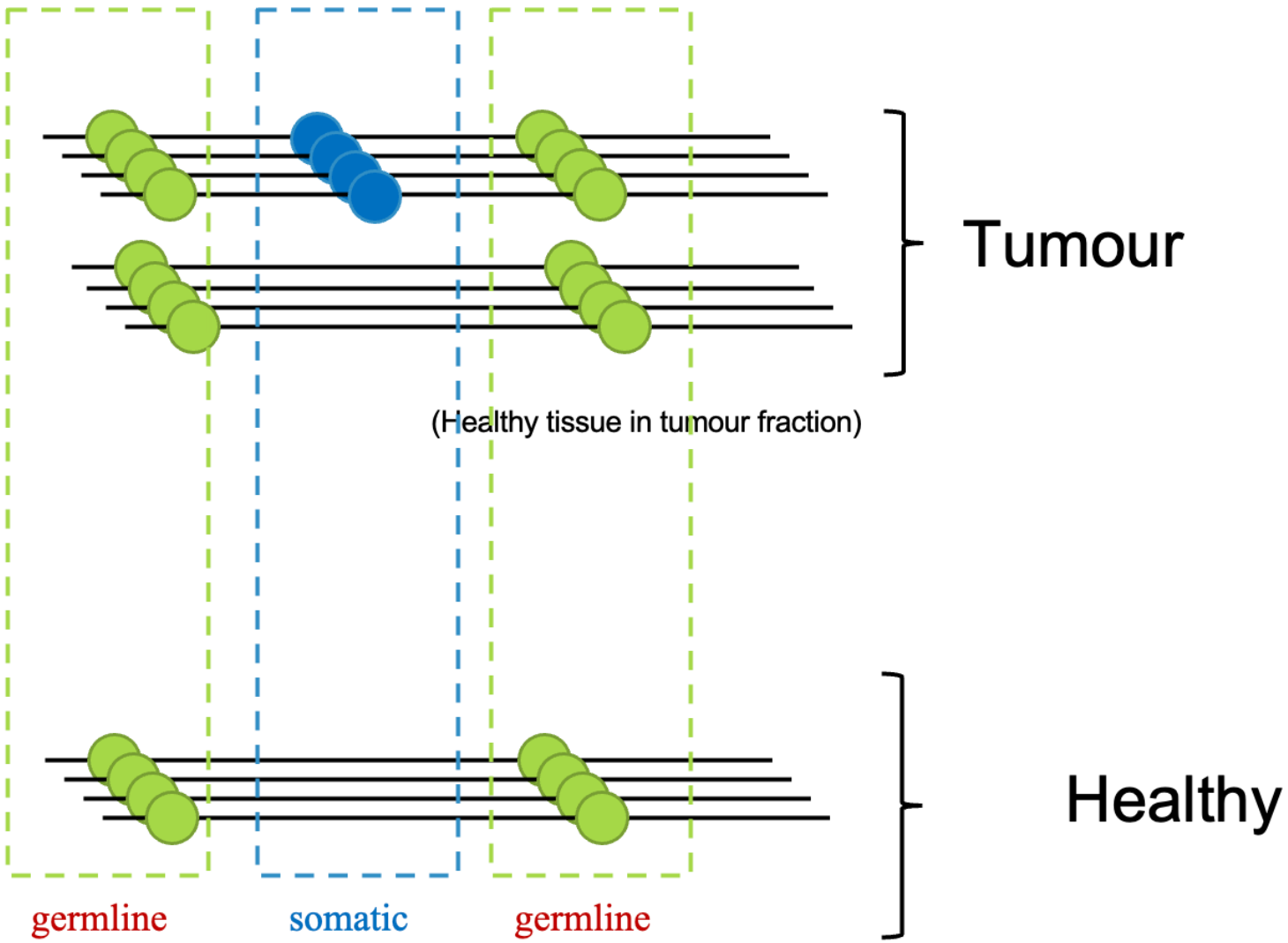




Somatic mutations and sequencing

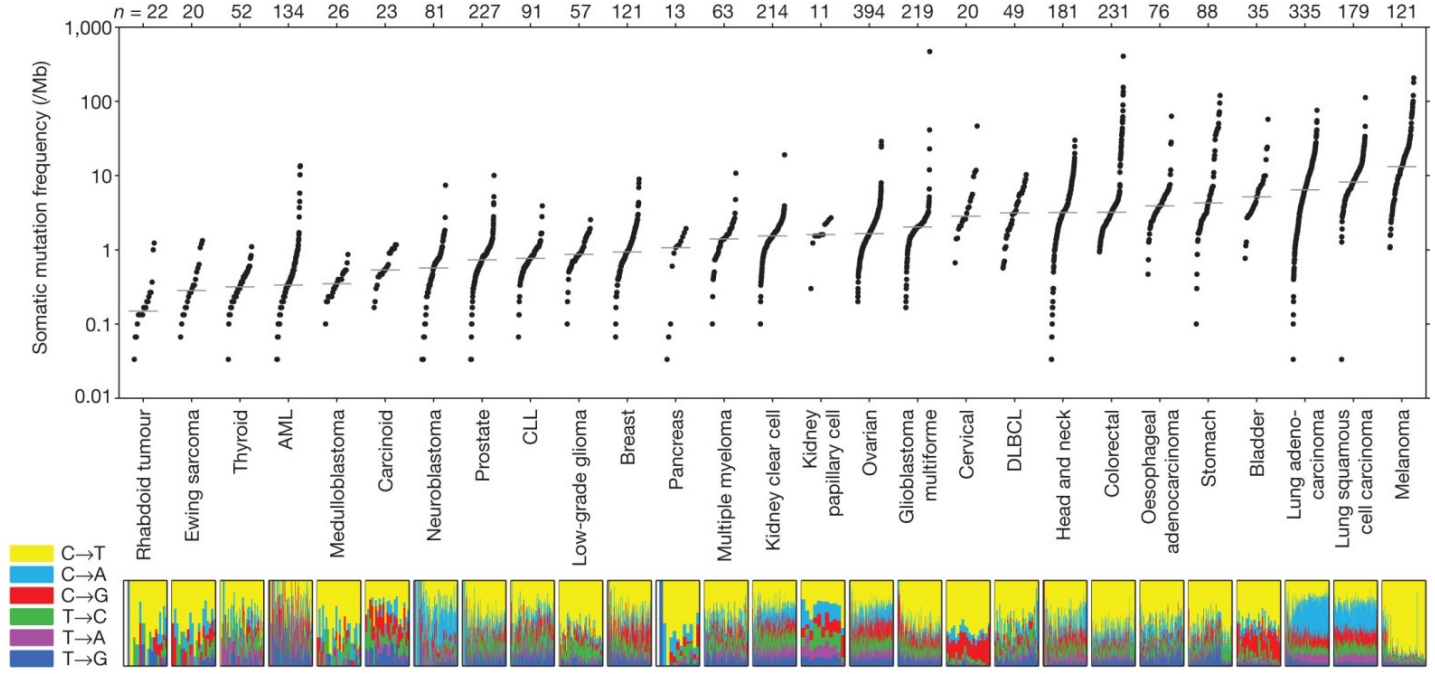
- Paired normal and tumour samples
- Challenges due to tumour purity
- Often difficult to have sources of normal samples
- Recurrent mutations, hotspots implicated in cancer
- Clonal heterogeneity (different mutations in the same tumour)
- Challenges: low allele frequency, small fraction of reads with mutations
- Need sensitive variant calling algorithms
- MuTecT2 (bayesian framework)
- Functional annotations

Matched samples for variant calling



Background mutation rate

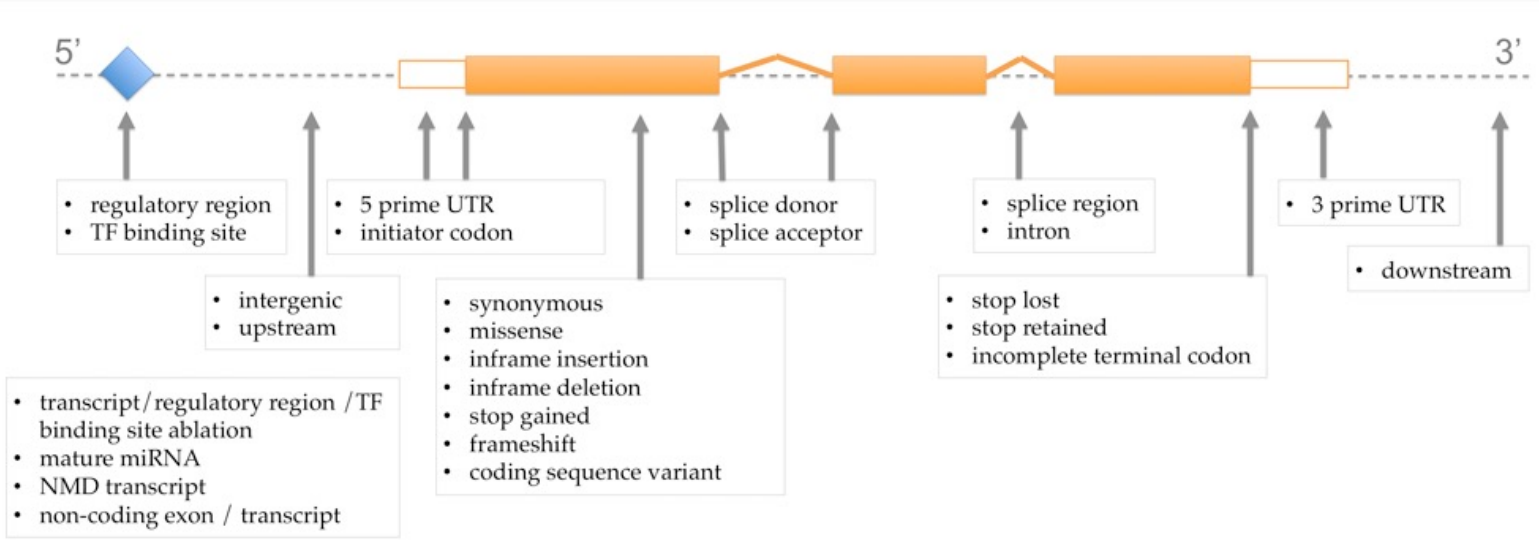
- adjust for background mutation rate
- different sources of differences in mutation rate
  - cancer-wise
  - Patient-wise
  - Genome region–wise



Non-coding variants and whole genome sequencing

LIFE 10e, In-Text Art, Ch. 4, p. 65

Coding mutations: Affect regions that **translate** to protein
Non-coding mutations: Affect regions **that do not translate** to protein



Non-coding variants and whole genome sequencing

- Even more challenging to find drivers
- Recurrence principle can be used similar to coding
- Variable background mutational rates within non-coding regions
- Scattered mutations
- Little knowledge of their functional impact

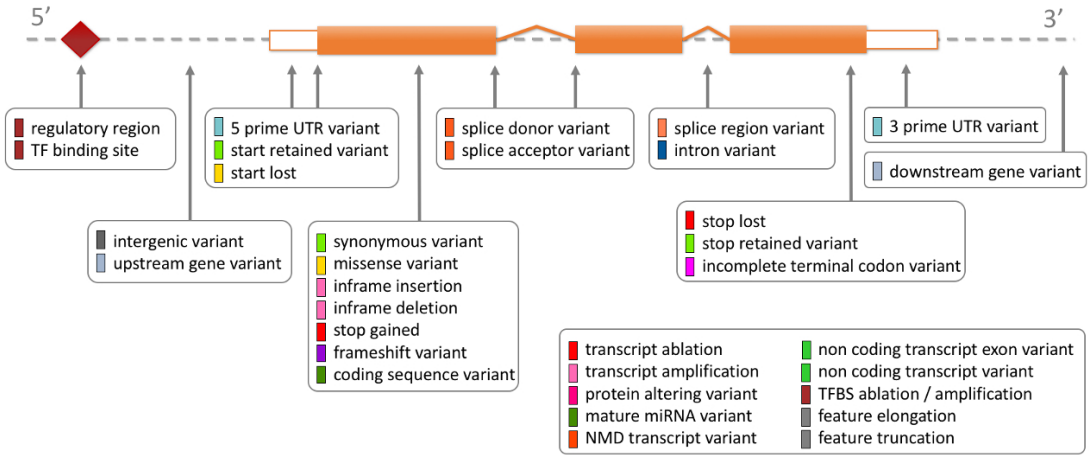
Pipelines for annotation of variants (Oncotator)

Table 1. Oncotator Datasources

Annotation Category	Resource	URL	Comments
Genomic	GENCODE	http://www.gencodegenes.org/	GENCODE/ENSEMBL transcripts and annotations for hg19
	ref_context		Can be used for artifact inference
	gc_content		Can be used for artifact inference
	Human DNA Repair Genes	http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html	Alteration in such genes can help explain higher overall mutation rates in specific samples
Protein	UniProt	http://www.uniprot.org/	Includes Drugbank & GO annotations
	dbNSFP	https://sites.google.com/site/jpopgen/dbNSFP	Contains pre-computed conservation scores, prediction classifications, and other information
Cancer Variant	COSMIC	http://www.sanger.ac.uk/genetics/CGP/cosmic/	
	Cancer Gene Census	http://www.sanger.ac.uk/genetics/CGP/Census/	
	CCLE	http://www.broadinstitute.org/ccle/home	Cancer cell line annotations. Can be used to identify cell line models containing variants of interest
	Familial Cancer Database	http://www.familialcancerdatabase.nl/	
	ClinVar	http://www.ncbi.nlm.nih.gov/clinvar/	
Non-Cancer Variant	dbSNP	http://www.ncbi.nlm.nih.gov/projects/SNP/	b142 release for human (9606)
	1000 Genomes	http://www.1000genomes.org/data	Phase 3 variant set
	NHLBI GO Exome Sequencing Project (ESP)	https://esp.gs.washington.edu/drupal/	

Ramos et al. 2015, Human Mutation, **https://doi.org/10.1002/humu.22771**

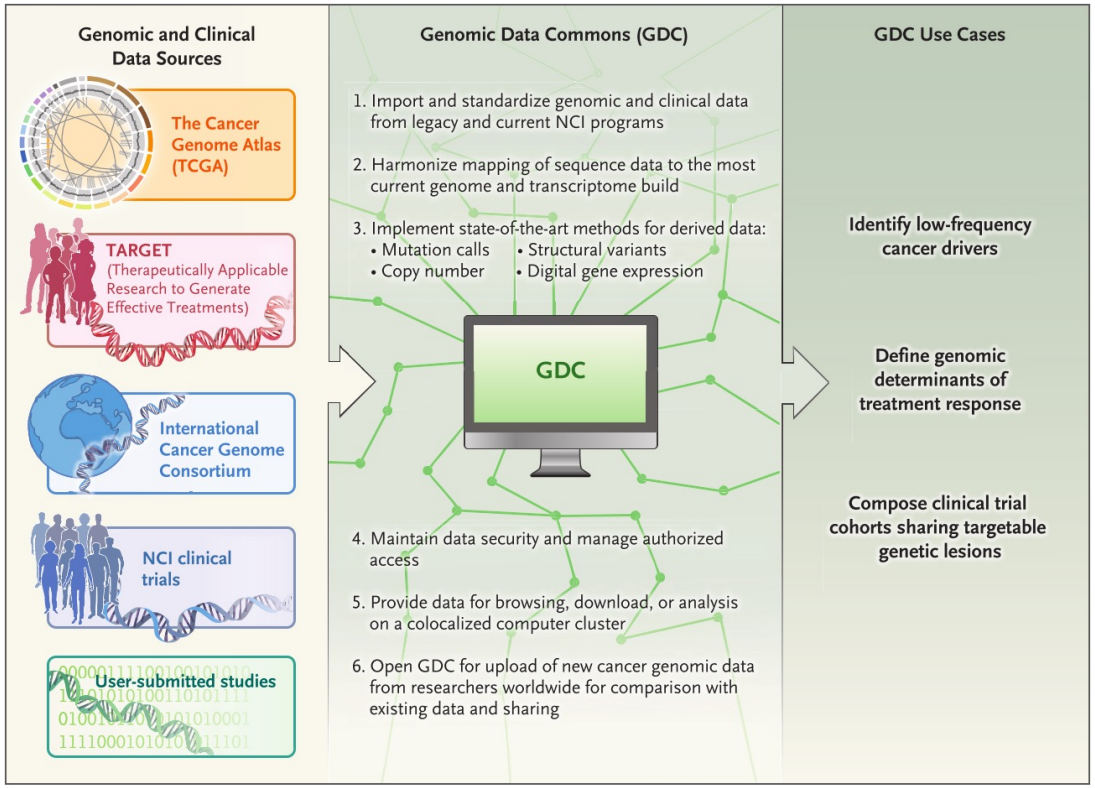
Ensembl Annotation for CONSEQUENCES



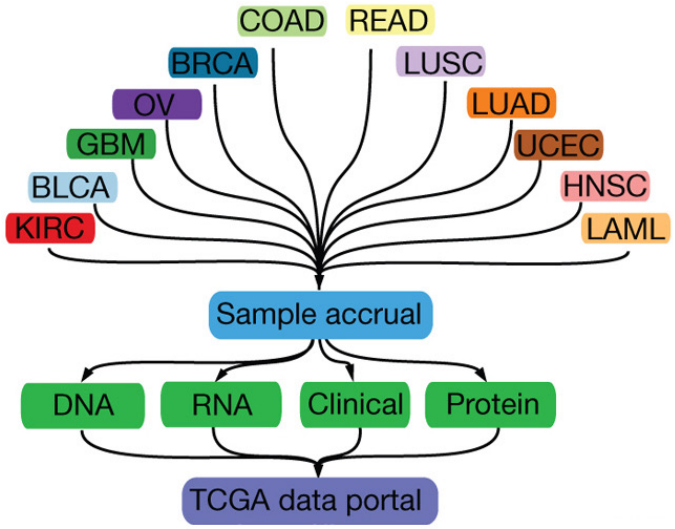
* SO term	SO description	SO accession	Display term	IMPACT
transcript_ablation	A feature ablation whereby the deleted region includes a transcript feature	SO:0001893	Transcript ablation	HIGH
splice_acceptor_variant	A splice variant that changes the 2 base region at the 3' end of an intron	SO:0001574	Splice acceptor variant	HIGH
splice_donor_variant	A splice variant that changes the 2 base region at the 5' end of an intron	SO:0001575	Splice donor variant	HIGH
stop_gained	A sequence variant whereby at least one base of a codon is changed, resulting in a premature stop codon, leading to a shortened transcript	SO:0001587	Stop gained	HIGH
frameshift_variant	A sequence variant which causes a disruption of the translational reading frame, because the number of nucleotides inserted or deleted is not a multiple of three	SO:0001589	Frameshift variant	HIGH
stop_lost	A sequence variant where at least one base of the terminator codon (stop) is changed, resulting in an elongated transcript	SO:0001578	Stop lost	HIGH
start_lost	A codon variant that changes at least one base of the canonical start codon	SO:0002012	Start lost	HIGH
transcript_amplification	A feature amplification of a region containing a transcript	SO:0001889	Transcript amplification	HIGH
inframe_insertion	An inframe non synonymous variant that inserts bases into in the coding sequence	SO:0001821	Inframe insertion	MODERATE
inframe_deletion	An inframe non synonymous variant that deletes bases from the coding sequence	SO:0001822	Inframe deletion	MODERATE
missense_variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the length is preserved	SO:0001583	Missense variant	MODERATE
protein_altering_variant	A sequence_variant which is predicted to change the protein encoded in the coding sequence	SO:0001818	Protein altering variant	MODERATE
splice_region_variant	A sequence variant in which a change has occurred within the region of the splice site, either within 1-3 bases of the exon or 3-8 bases of the intron	SO:0001630	Splice region variant	LOW
incomplete_terminal_codon_variant	A sequence variant where at least one base of the final codon of an incompletely annotated transcript is changed	SO:0001626	Incomplete terminal codon variant	LOW
start_retained_variant	A sequence variant where at least one base in the start codon is changed, but the start remains	SO:0002019	Start retained variant	LOW

(Pan)cancer genomic initiatives

THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute



NATIONAL CANCER INSTITUTE
GDC Data Portal



- A source of genomic profiling in cancer-patients across > 30 different cancer studies
- Similar approaches to other data from –omics initiatives
- Need of tools to understand the impact of the alterations at the molecular level
- Different layers of information

COSMIC
Catalogue Of Somatic Mutations In Cancer

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

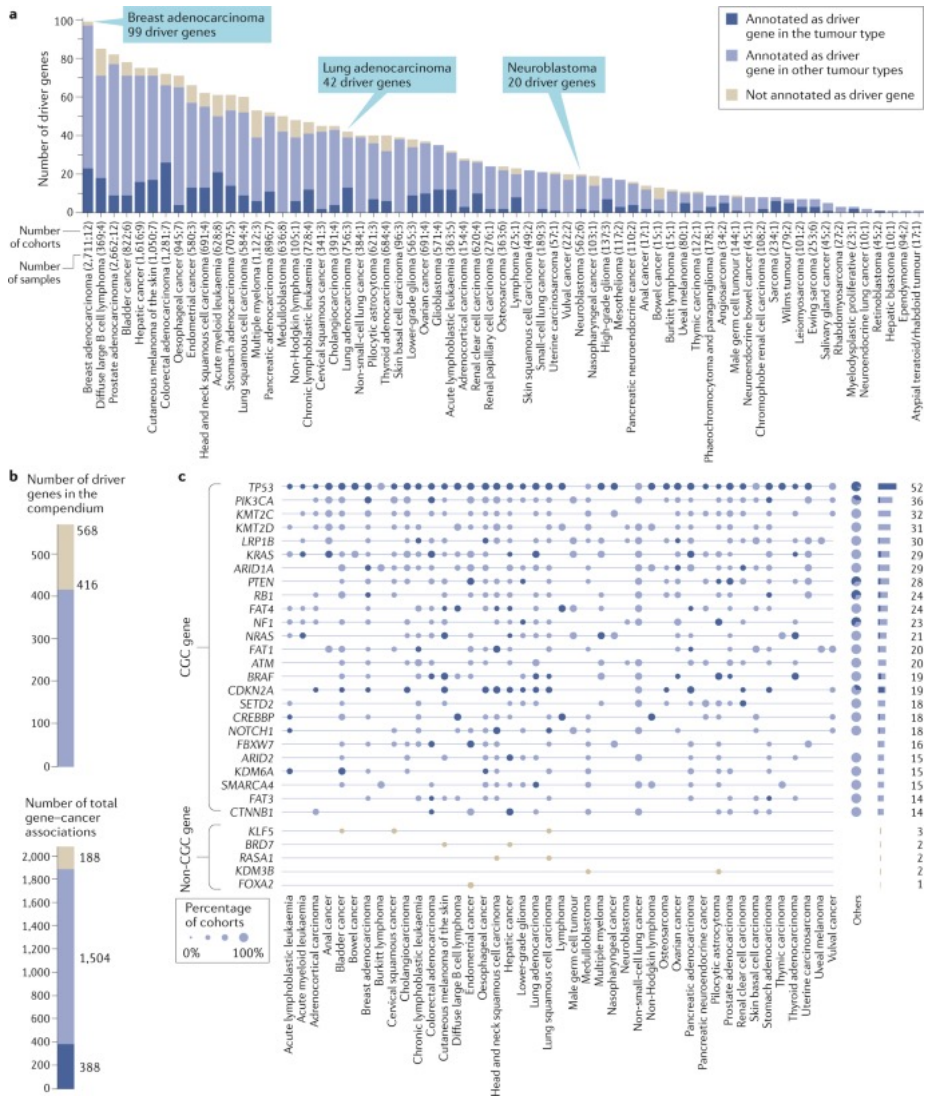
COSMIC-3D
An interactive view of cancer mutations in the context of 3D structures

Cancer Gene Census
A catalogue of genes with mutations that are causally implicated in cancer

Cancer Mutation Census
Classification of genetic variants driving cancer

Actionability
Mutations actionable in precision oncology

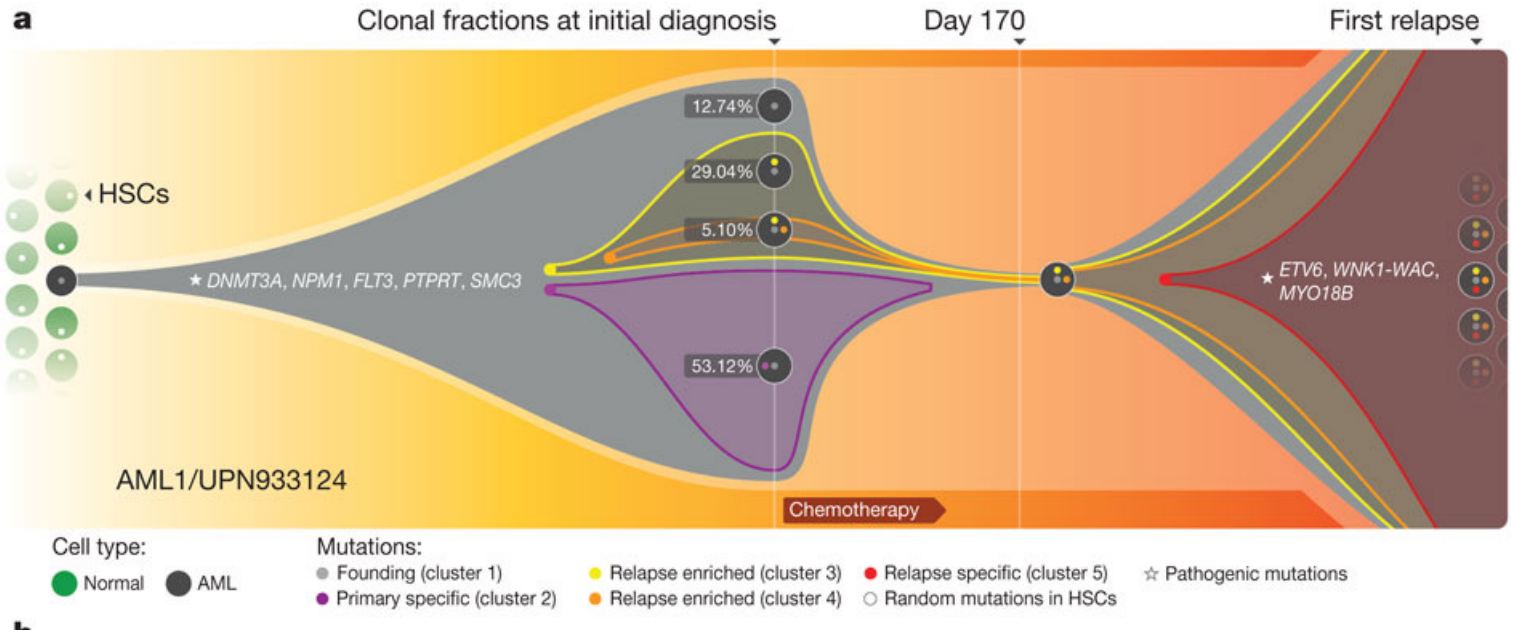
Large-scale cancer sequencing analyses and recurrent mutations

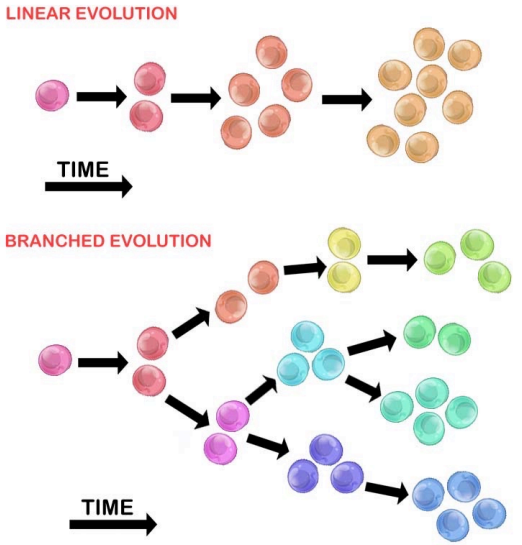


Martínez-Jiménez, F., Muiños, F., Sentís, I. *et al. Nat Rev Cancer* **20**, 555–572 (2020). https://doi.org/10.1038/s41568-020-0290-x

Clonal genomic heterogeneity

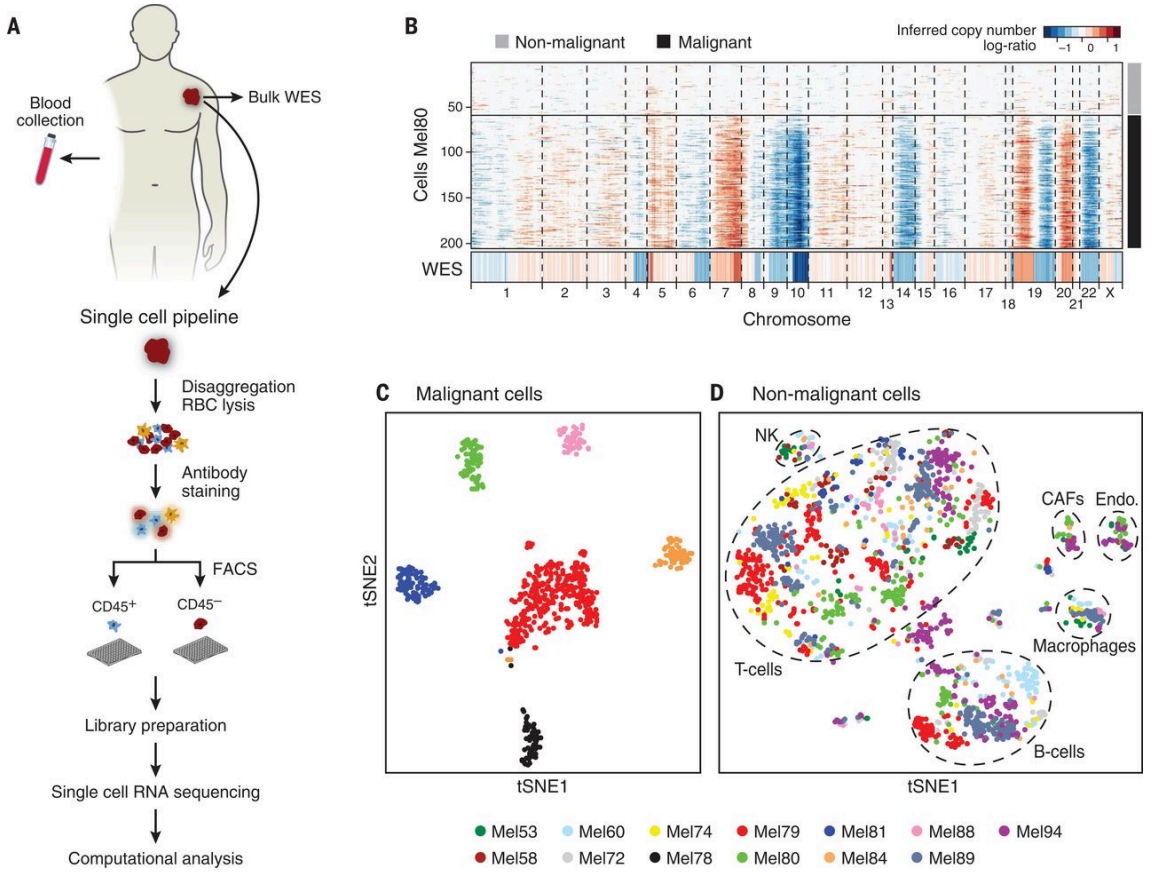
- Cancer is an evolutionary process driven by positive selection
- Large number of pre-cancerous cells constantly subjected to selection
- Many way a oncogenic pathway can be hit
- Genomic instability key source of heterogeneity
- Heterogeneity both in emergence of tumour and resistance to drugs





Ding et al. Nature, 2012, https://doi.org/10.1038/nature10738

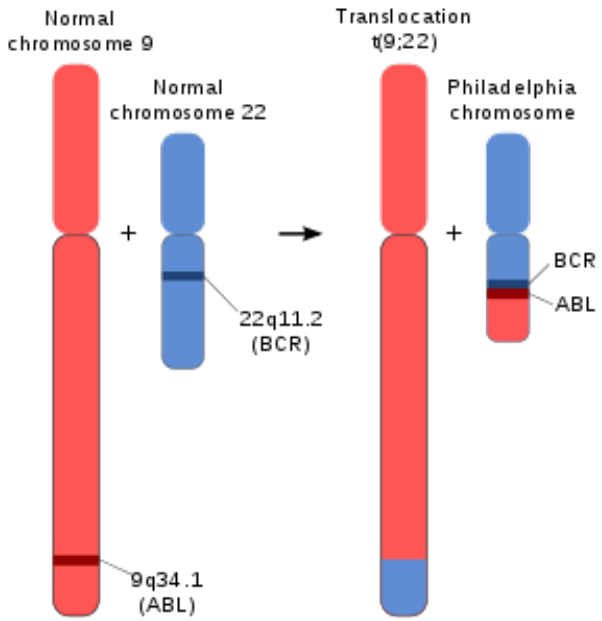
Single cell techniques to study diversity of clonal groups



Tirosh et al. 2016 Science, [DOI: 10.1126/science.aad0501](https://doi.org/10.1126/science.aad0501)

RNASEQ to identify gene fusions

- BCR-ABL fusion and Philadephia chromosome in leukemia
- Tumours driven by recurrent fusion events that create chimeric proteins that serve as oncogenes



Learning objectives

- To define how cancer develops and evolves
- To understand that cancer is not just one unique disease
- To identify alterations in the cancer genome
- To analyze cancer samples using sequencing approaches