Cancer Bio Course

Session 1: Introduction to course and basic techniques applied in basic cancer research

August 7th, 2024

Memorial Sloan Kettering Cancer Center

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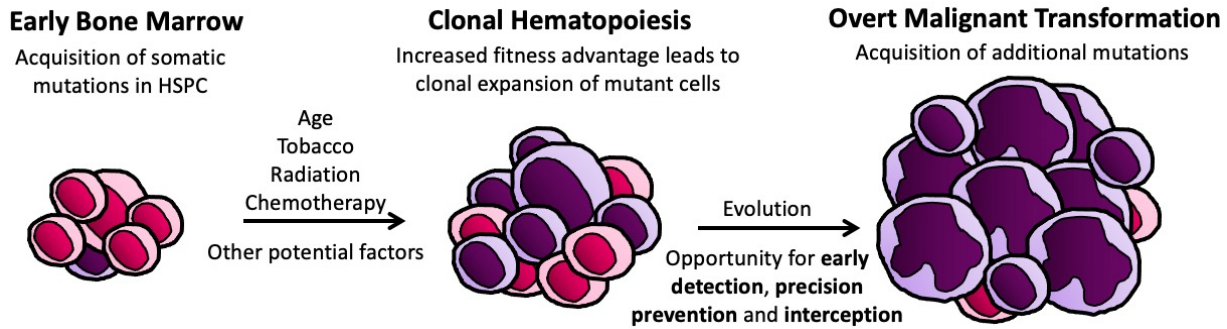
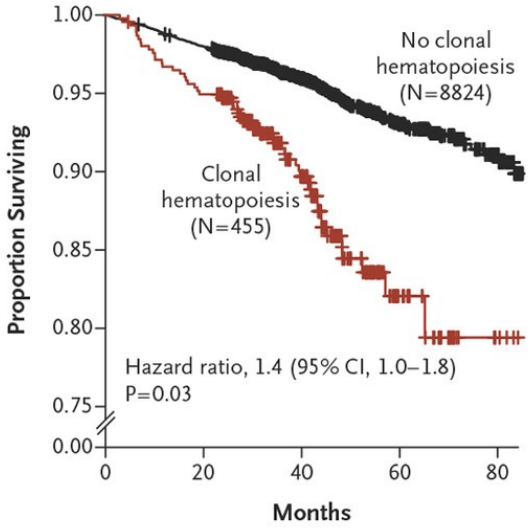
Human Oncology and Pathogenesis Program
sanchezp@mskcc.org

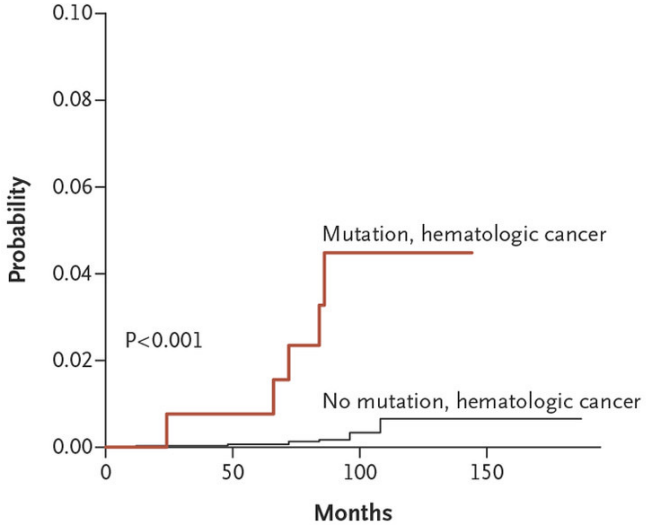
Questions I decided to tackle during my time at MSK



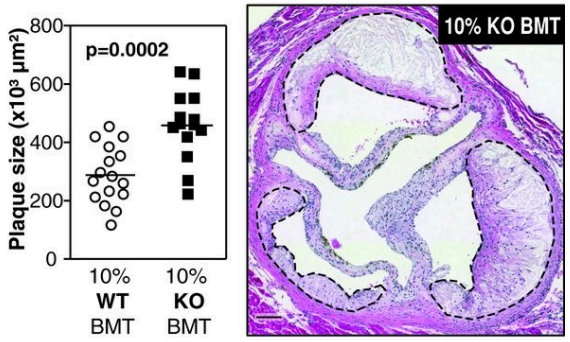
Genovese et al. NEJM 2014
Overall Mortality



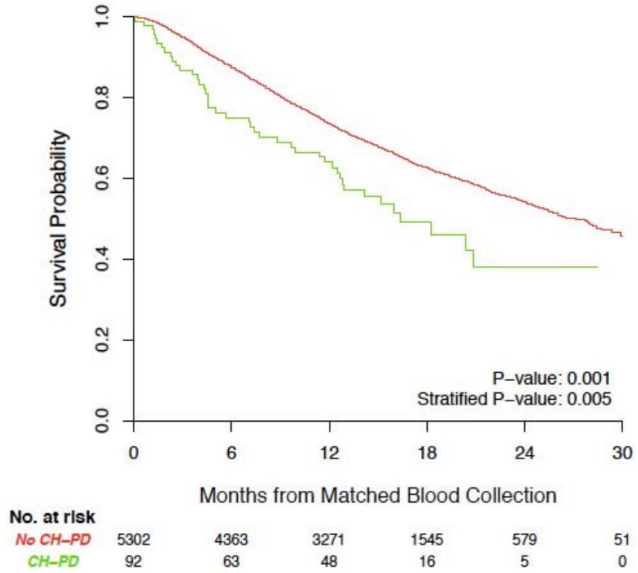
Jaiswal et al. NEJM 2014
Hematologic Malignancies



Fuster et al. Science 2017
Cardiovascular Disease



Coombs et al. CellStemCell 2017
Solid Tumors



PSV

Course structure

Scientific topics covered will include:

- Cancer as a disease
- Genetic and epigenetic mechanisms
- Computational biology and oncology
- Cancer signaling
- Cancer metabolism
- Metastasis
- Tumor modeling and heterogeneity
- Cancer types and microenvironments

This course will:

- Provide a review of advanced concepts in cancer biology
- Expose students to techniques and experimental design applied to basic-translational cancer research
- Potentiate the ability to perform critical analysis of basic-translational research
- Strengthen capacities to develop a research project

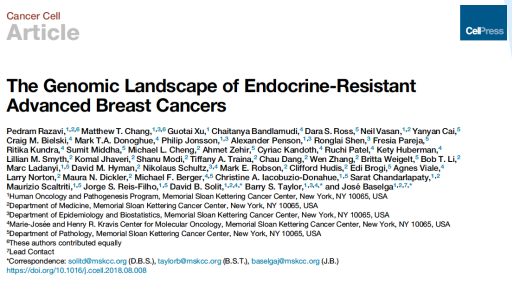
RECORDED LECTURES

IN-PERSON ACTIVITIES

Course structure

In-person activities:

- Session 1 – Introduction to course and basic techniques applied in basic cancer research
  - **Explanation of the question under research** - *why on earth did they decide to do this?*
  - **Discussion figure by figure –** *is this paper not as good as authors think?:*
    - What is the point of each figure/panel?
    - Are there any missing experimental conditions?
    - Are results interpretable?
    - Do the results support the conclusions by the authors?
    - Would you have done anything differently?
    - Are there any missing experiments?
    - What are the limitations of the work?
    - What experiments could be done as a follow-up to the paper?
- Session 2 – Paper discussion
- Session 3 – Paper discussion
- Session 4 – Paper discussion
- Session 5 – Guided live research activity


Cancer Cell
Article

The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers


Article

Lung adenocarcinoma promotion by air pollutants


RESEARCH BRIEF

Jak2^{V617F} Reversible Activation Shows Its Essential Requirement in Myeloproliferative Neoplasms

Course evaluation

Class participation and attendance (33%)

• All scholars are expected **to attend all sessions**. A scholar must notify the Bridge team and instructor prior to class if they will absent. This notice should be sent by email.

Take Home (67%)

• A research question (project) will be assigned to you, in groups of 2-3 people.

• You will have to propose a series of experiments to address that research question (i.e. a light version of the Research Strategy section of a grant) – **Max. 2 pages**

• **Due date is September 18th, 2024**

• Your work will be reviewed and feedback will be provided by October 2nd, 2024. You will have the chance to made modifications according to the comments provided (**due October 15th, 2024**).

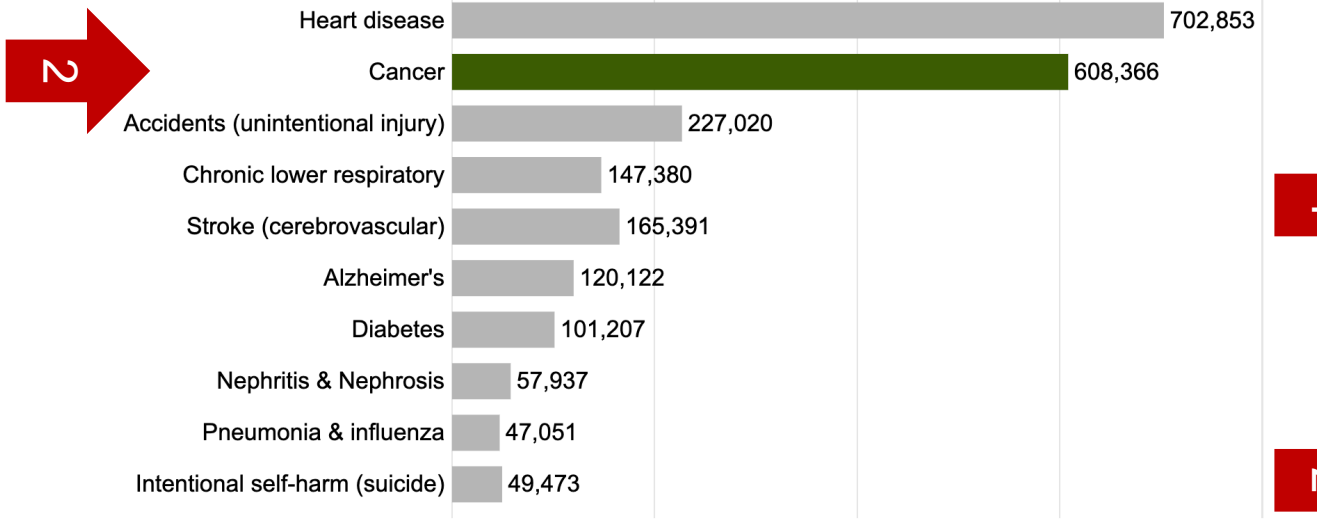
• Your revised work will be graded.

Course evaluation

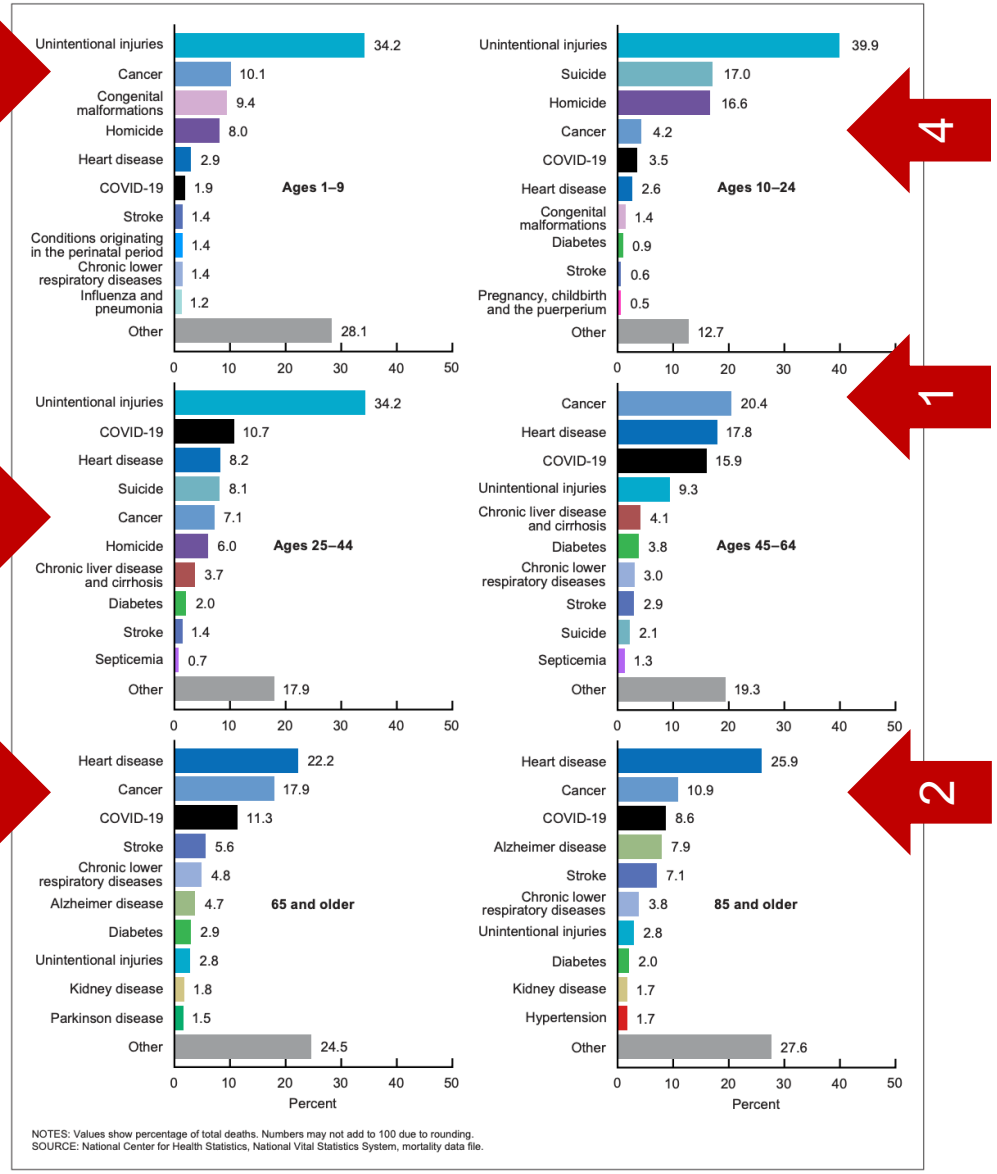
Letter Grade	Range
A	85-100
A-	82-85
B+	78-82
B	75-78
B-	72-75
C+	68-72
C	65-68
C-	62-65
F	<62

Cancer 101

Cancer 101: Why do we study cancer?



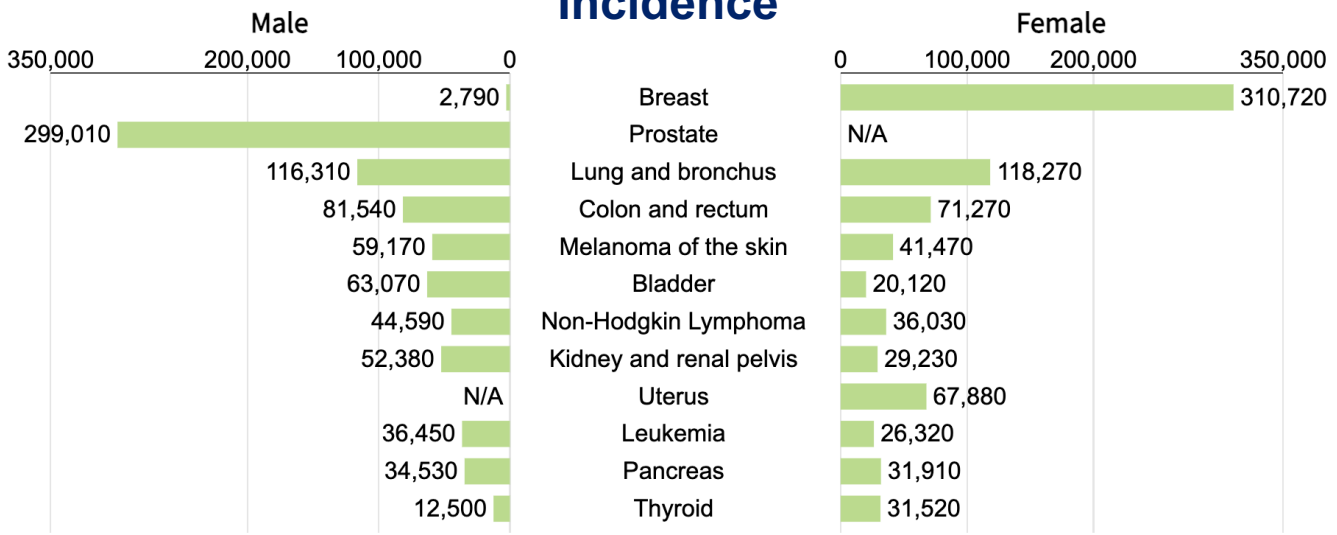
NCHS Data Brief, Number 492, December 2023



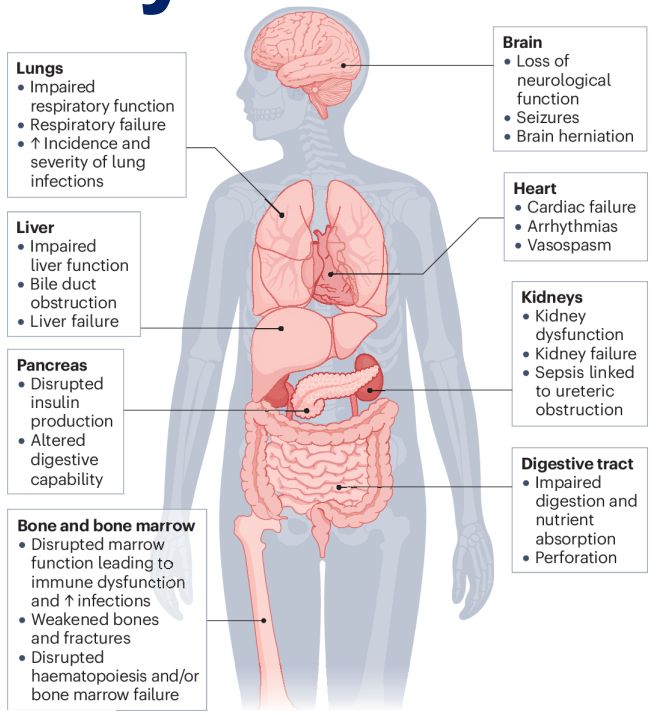
National Vital Statistics Reports Volume 73, Number 4 April 8, 2024 Death Leading Causes for 2021

Cancer 101: Cancer diagnosis and mortality

Incidence

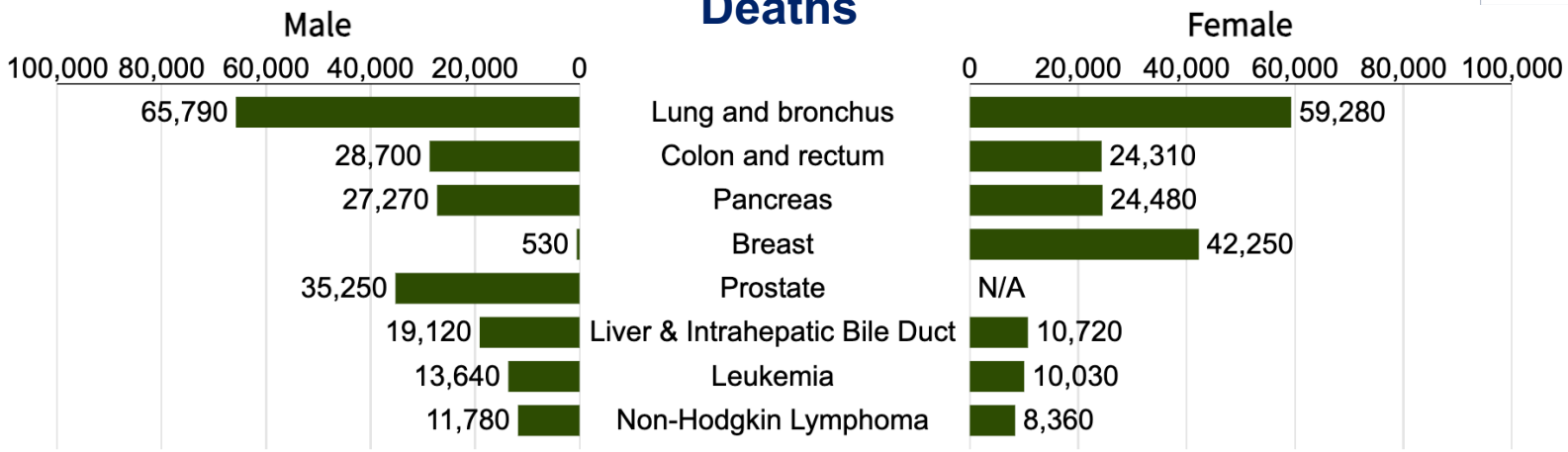


Source: Cancer Facts & Figures 2024, American Cancer Society (ACS), Atlanta, Georgia, 2024.



Boire, A., Burke, K., Cox, T.R. *et al.* Why do patients with cancer die?. *Nat Rev Cancer* **24**, 578–589 (2024).

Deaths

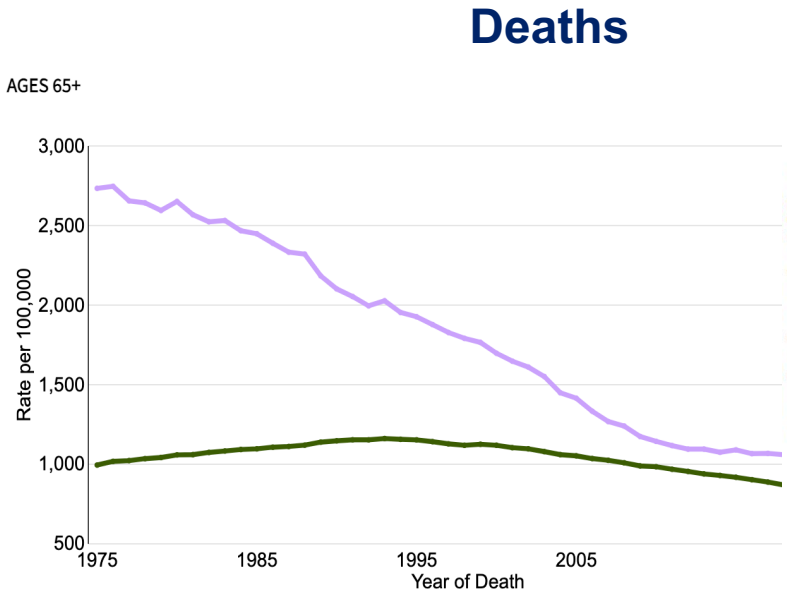


Source: Cancer Facts & Figures 2024, American Cancer Society (ACS), Atlanta, Georgia, 2024.

2 types of treatment:
- Local
- Systemic

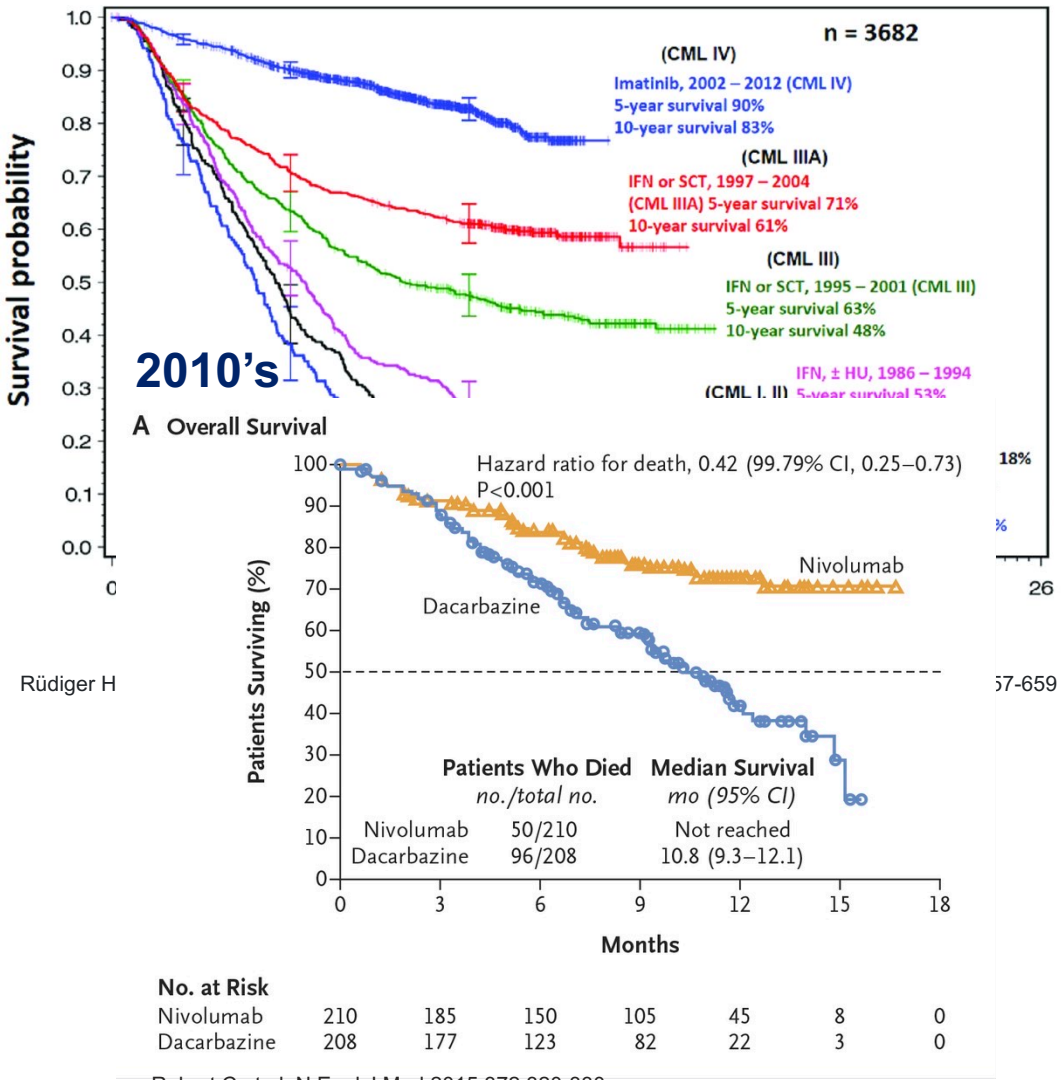
Cancer 101: How are we doing so far?

Mary Lasker. Washington Post 1969



Source: U.S. Mortality Files, National Center for Health Statistics, Centers for Disease Control
Rates are age-adjusted.

Richard Nixon. Rockefeller Research Laboratories, 1971

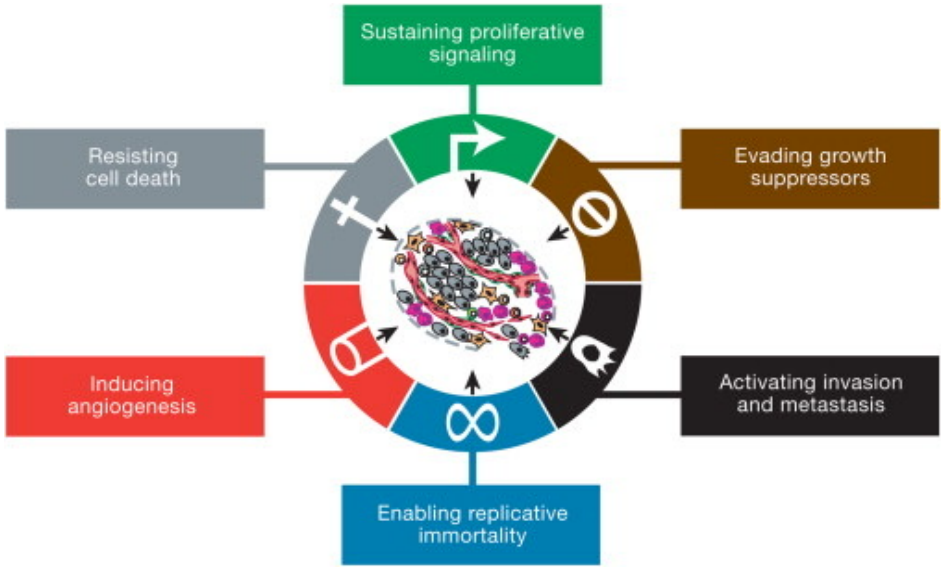


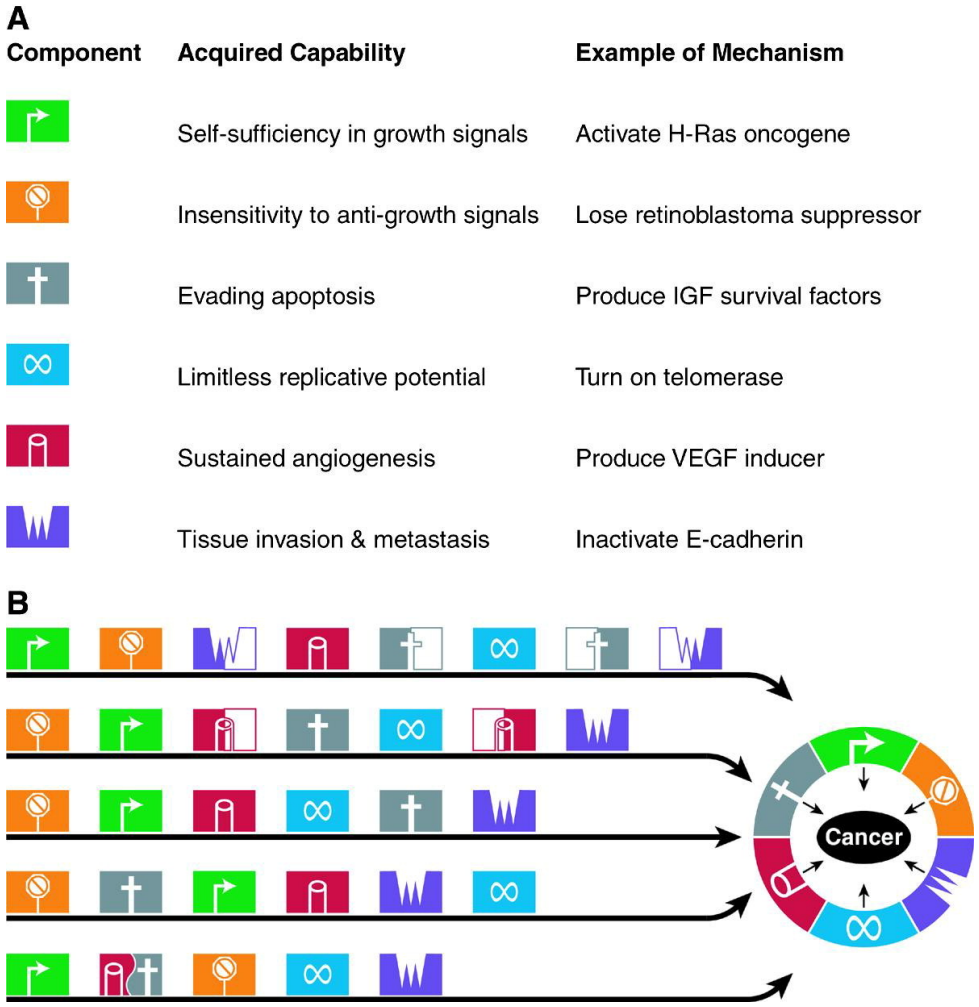
Robert C et al. N Engl J Med 2015;372:320-330

"Whether it was Cancer or Alzheimer's or another condition […] We clearly needed fundamental basic research to understand those diseases before we could hope to cure them." — Benno Schmidt 1995

Cancer 101: What is a cancer cell?

"Cells": Basic unit of life

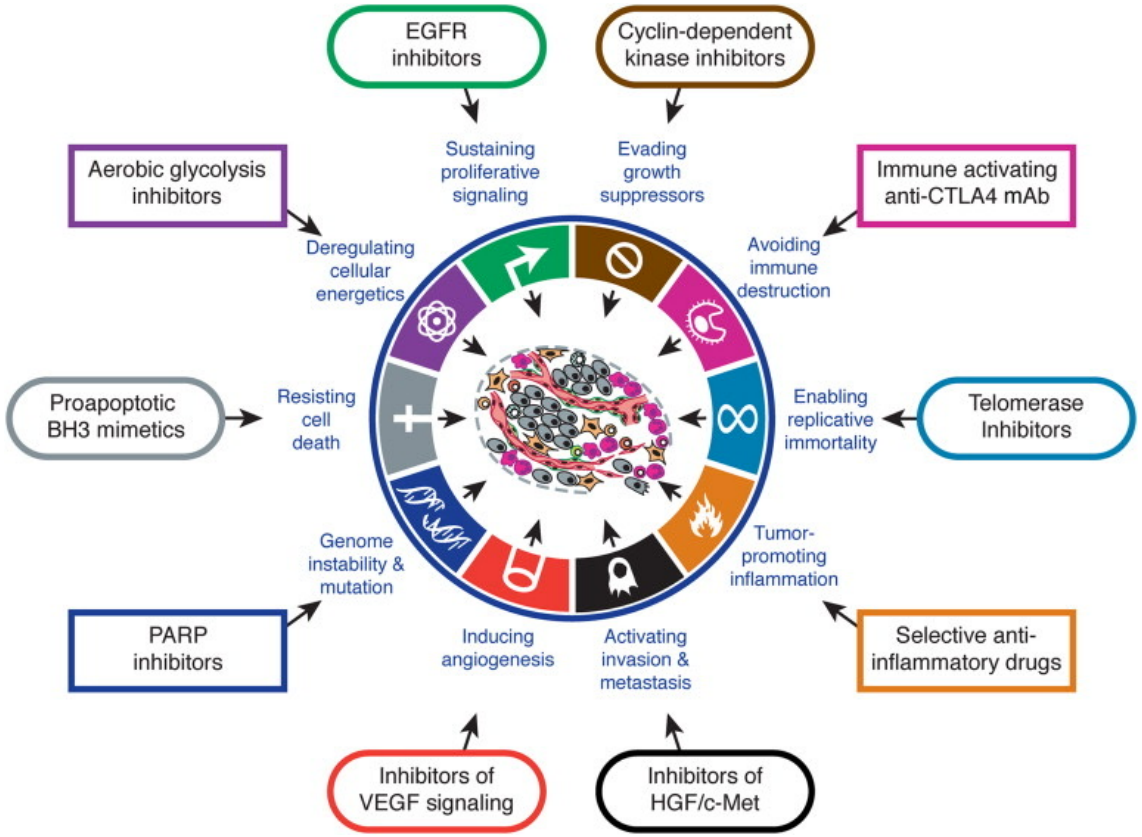




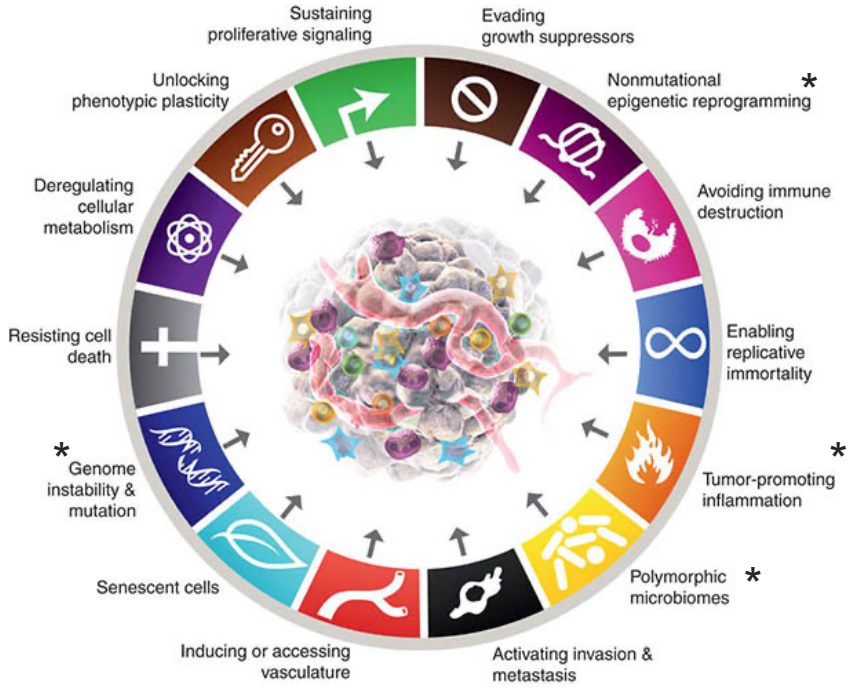
Hallmarks of Cancer. 2000 (Cell), 2011 (Cell), 2020 (Cancer Discovery)

Cancer 101: What is a cancer cell?

"Cells": Basic unit of life



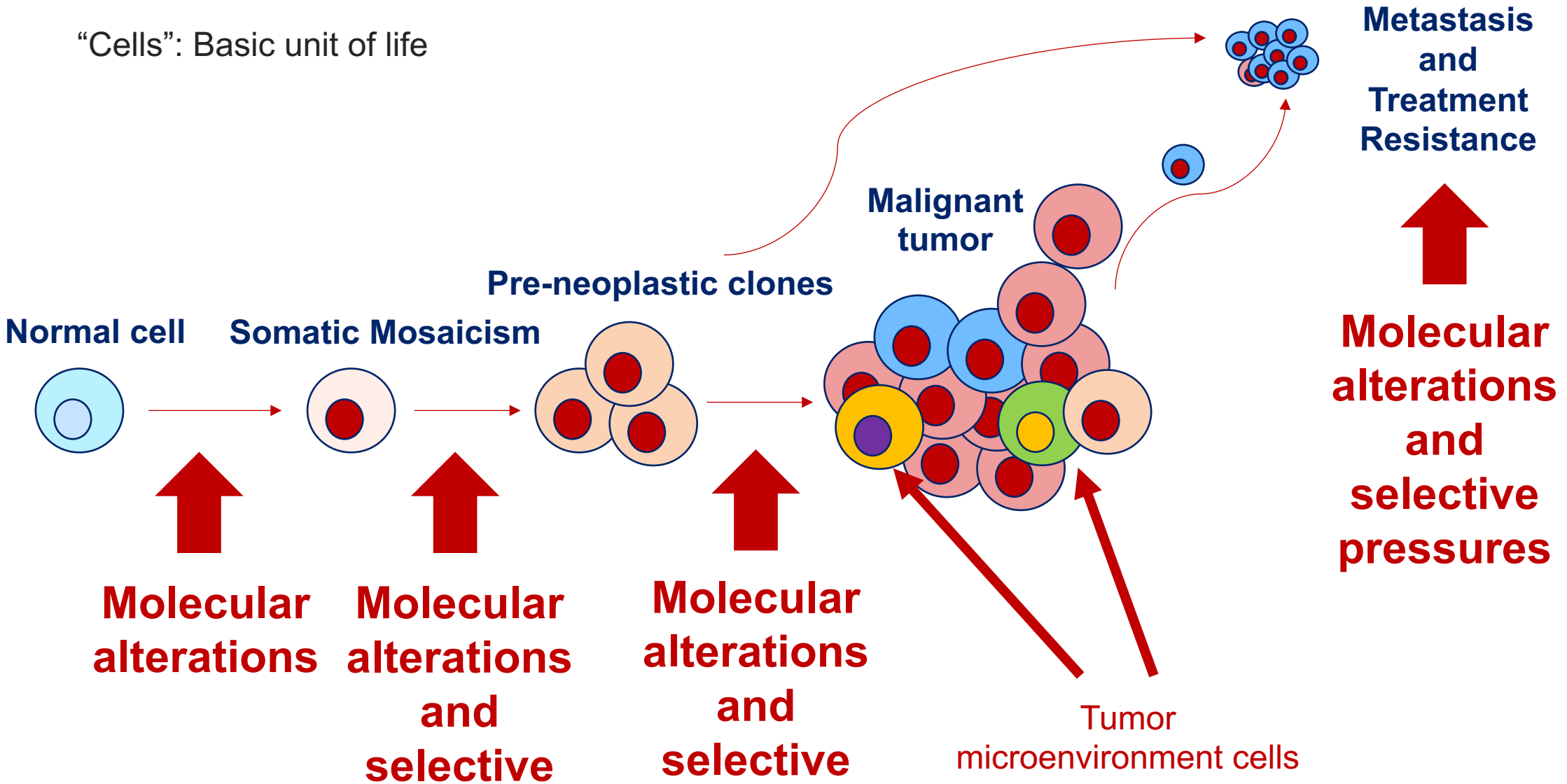
Cancer Hallmarks and Enabling Characteristics*



Hallmarks of Cancer. 2000 (Cell), 2011 (Cell), 2020 (Cancer Discovery)

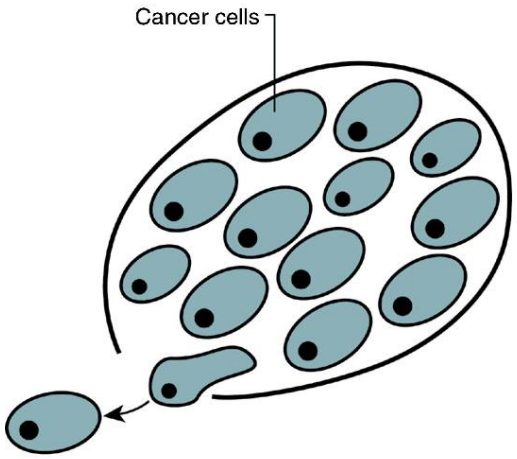
Cancer 101: Tumor evolution

"Cells": Basic unit of life

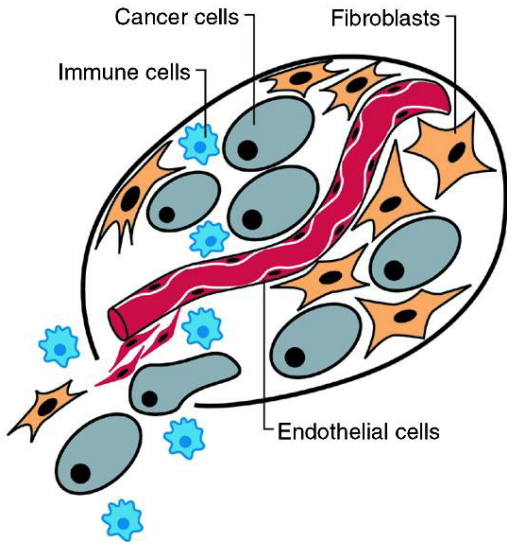


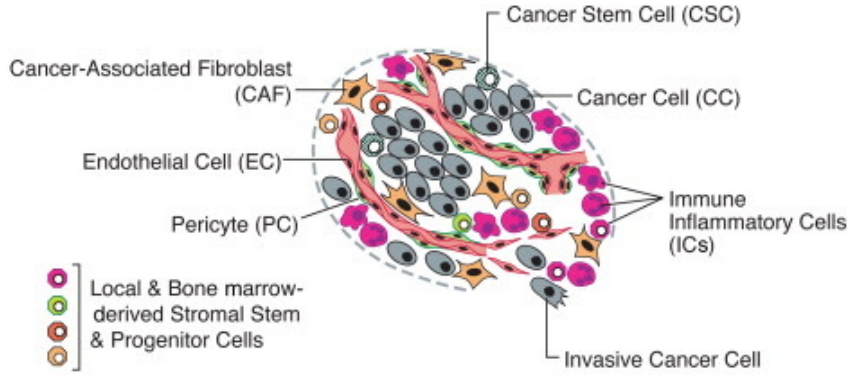
The importance of context

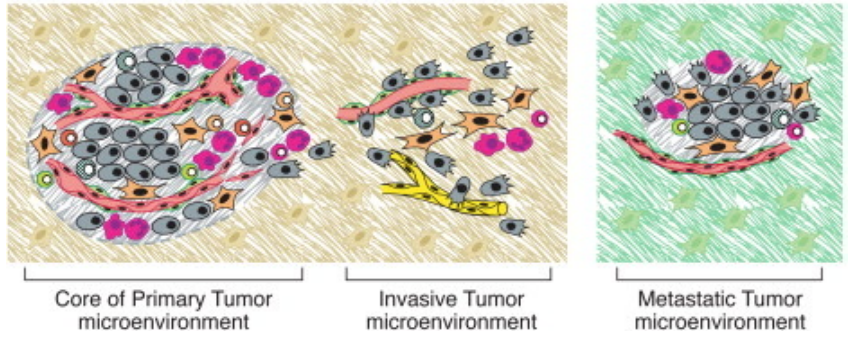
The Reductionist View

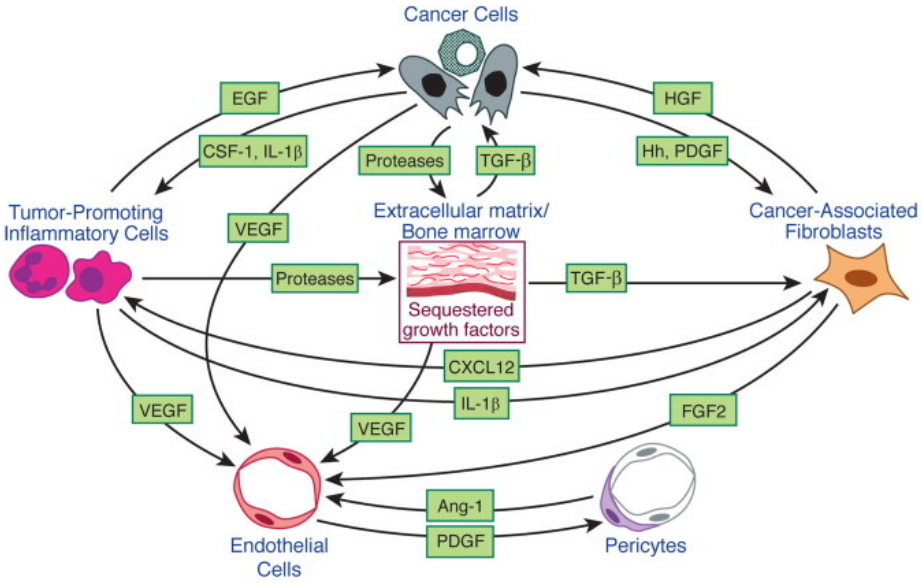


A Heterotypic Cell Biology

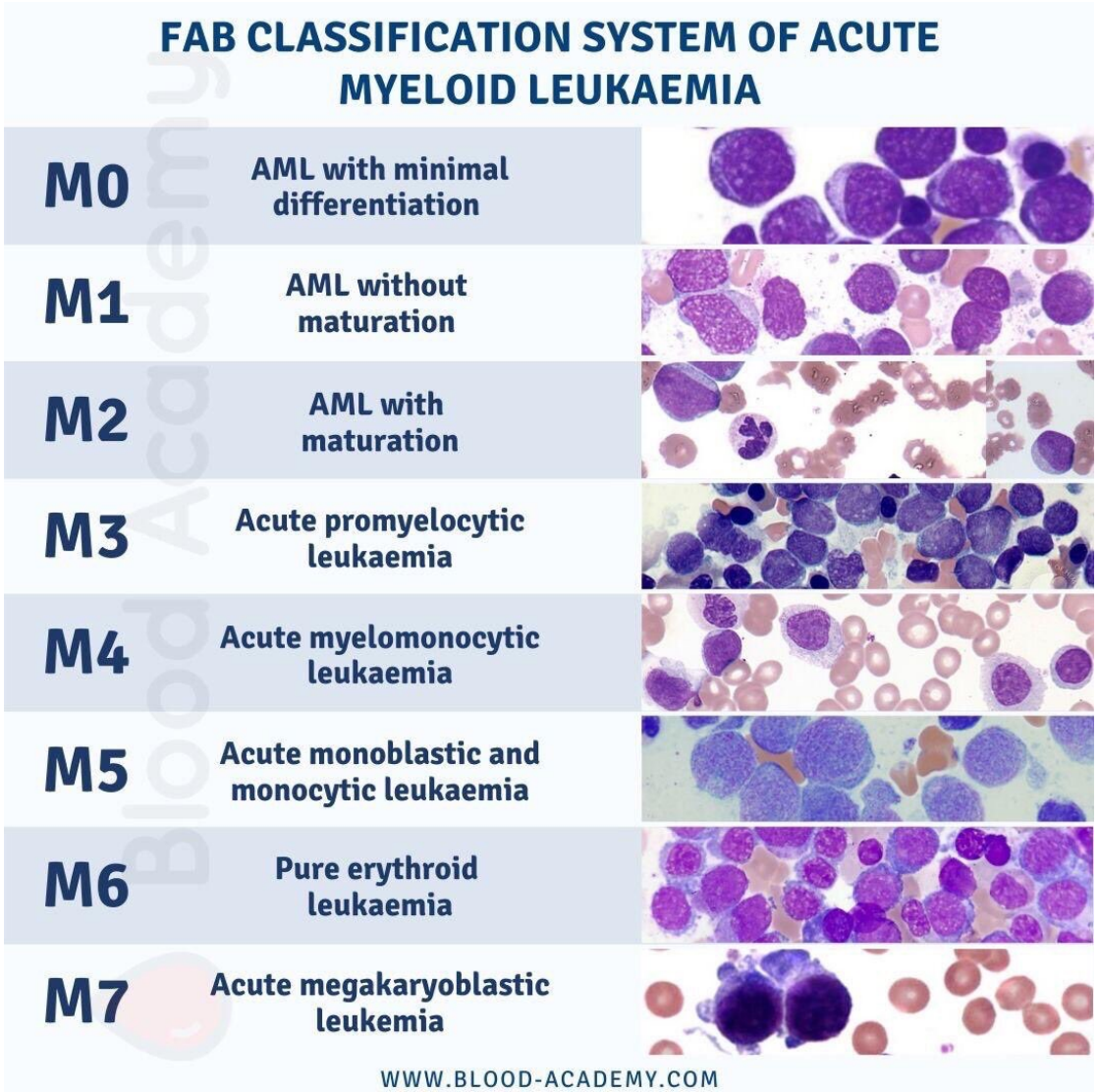




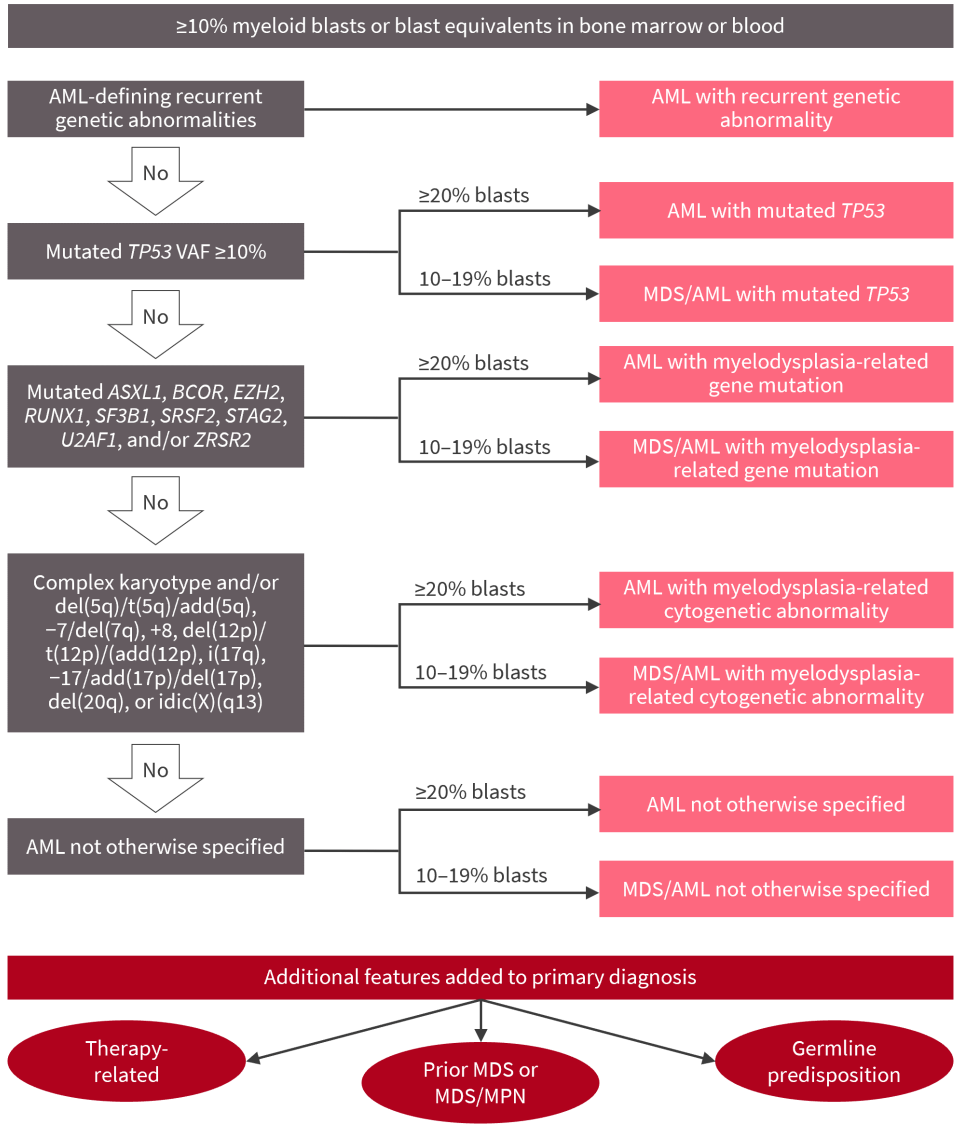




Historical classification of blood cancer
MORPHOLOGY-based: How things look?



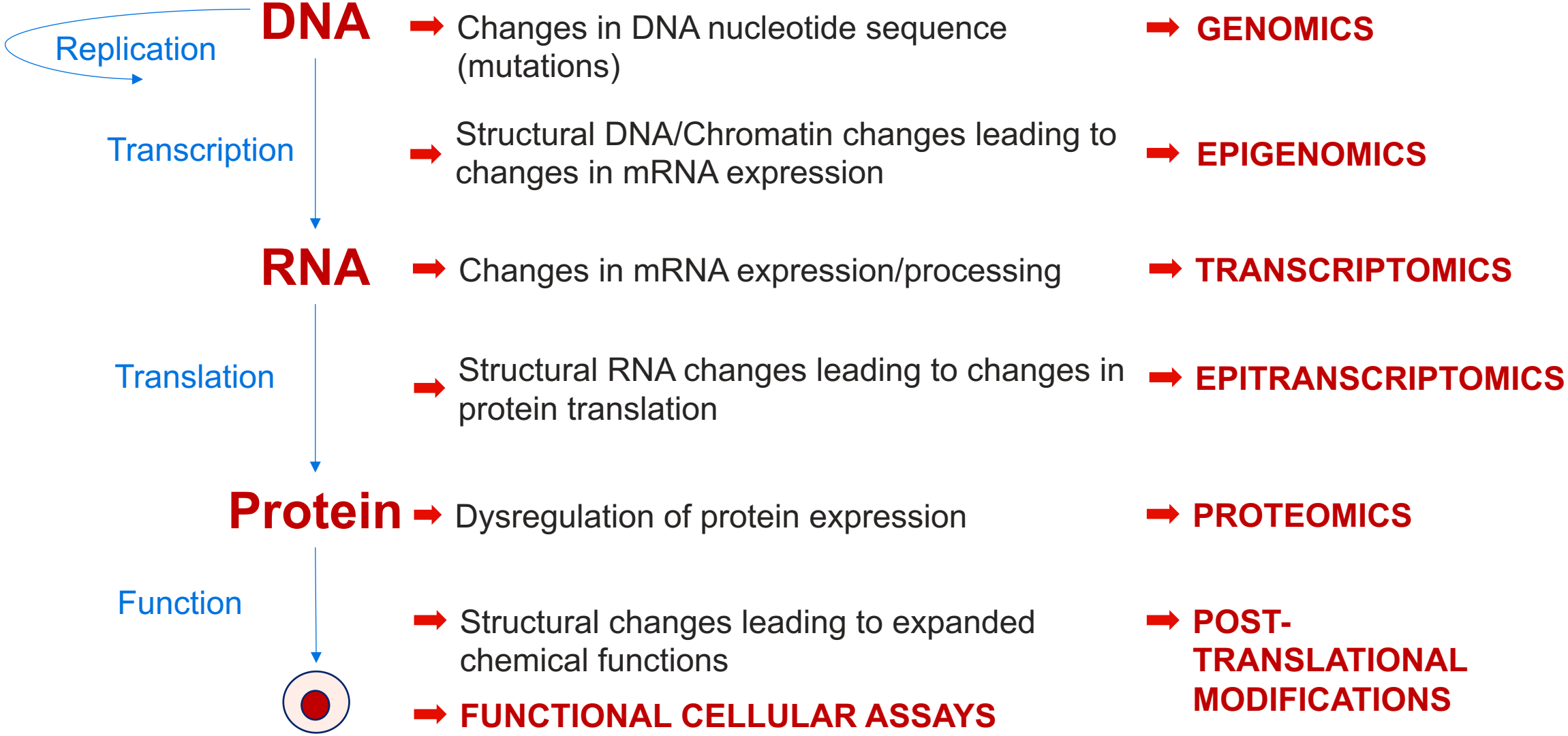
Current classification of blood cancer
MOLECULAR-based: What genetic abnormalities do you carry?



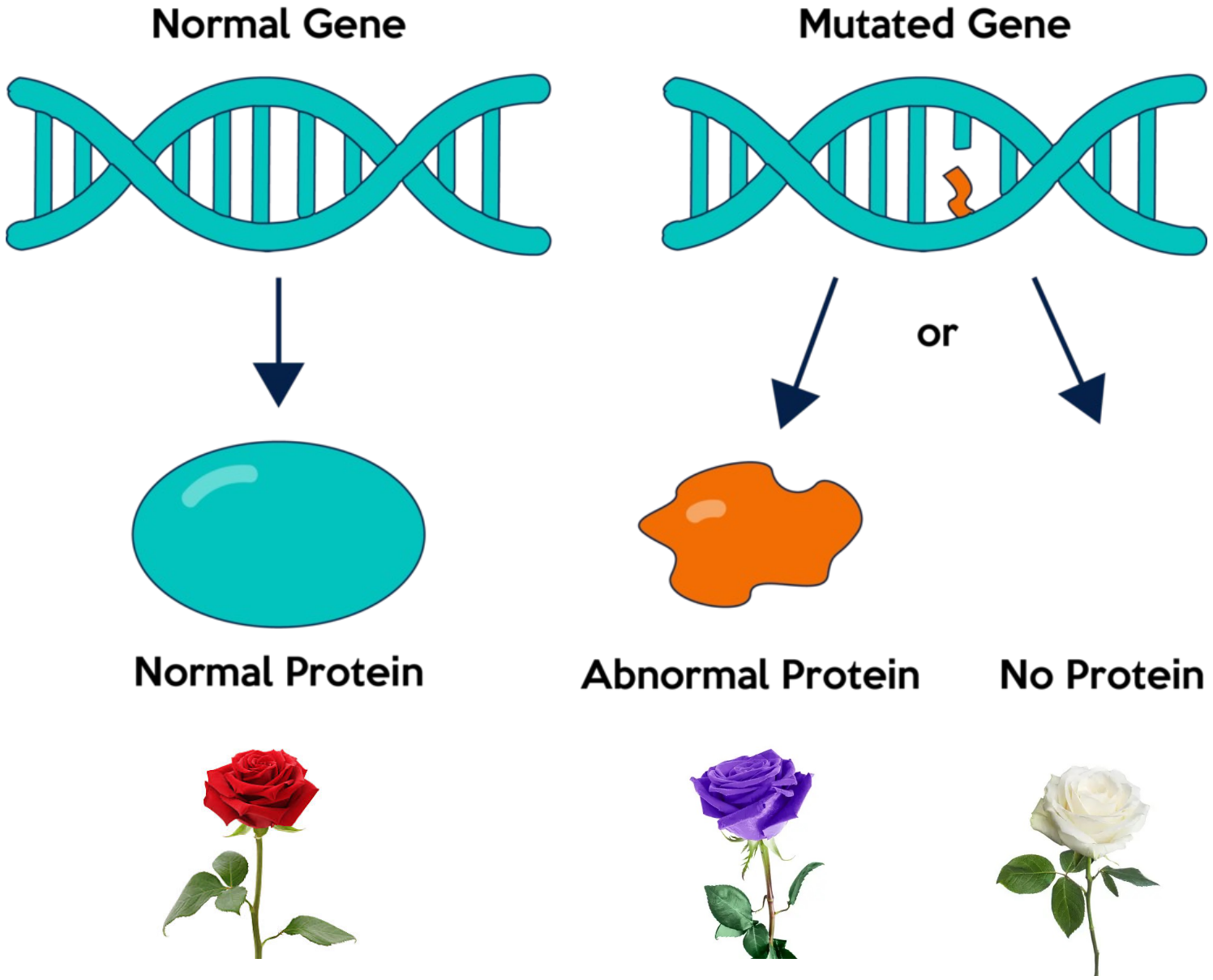
ELN 2022

Cancer 101: Main Molecular alterations

"Genes": Basic units of heredity

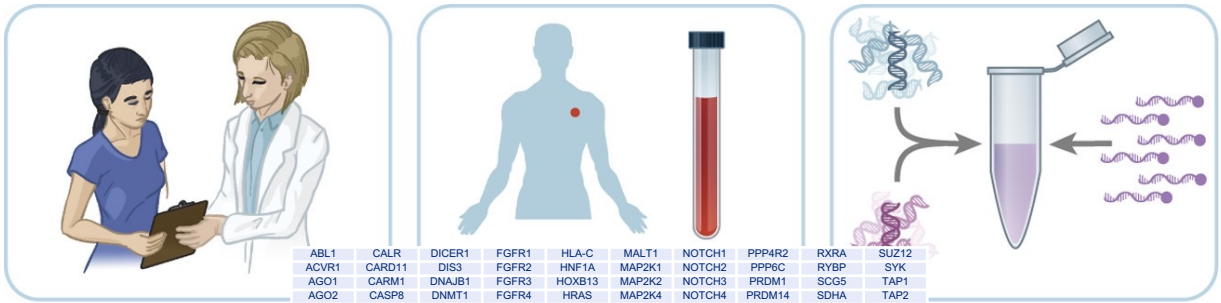


Mutations in the DNA have functional consequences



MSK-IMPACT

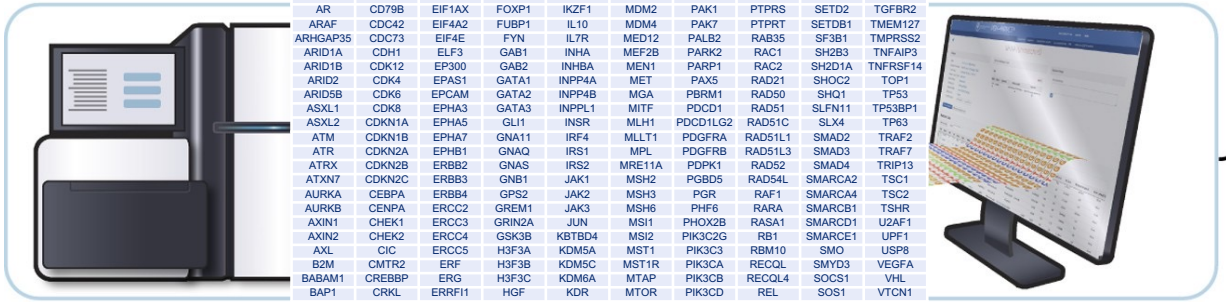
(Integrated Mutation Profiling of Actionable Cancer Targets



1. Patient consen

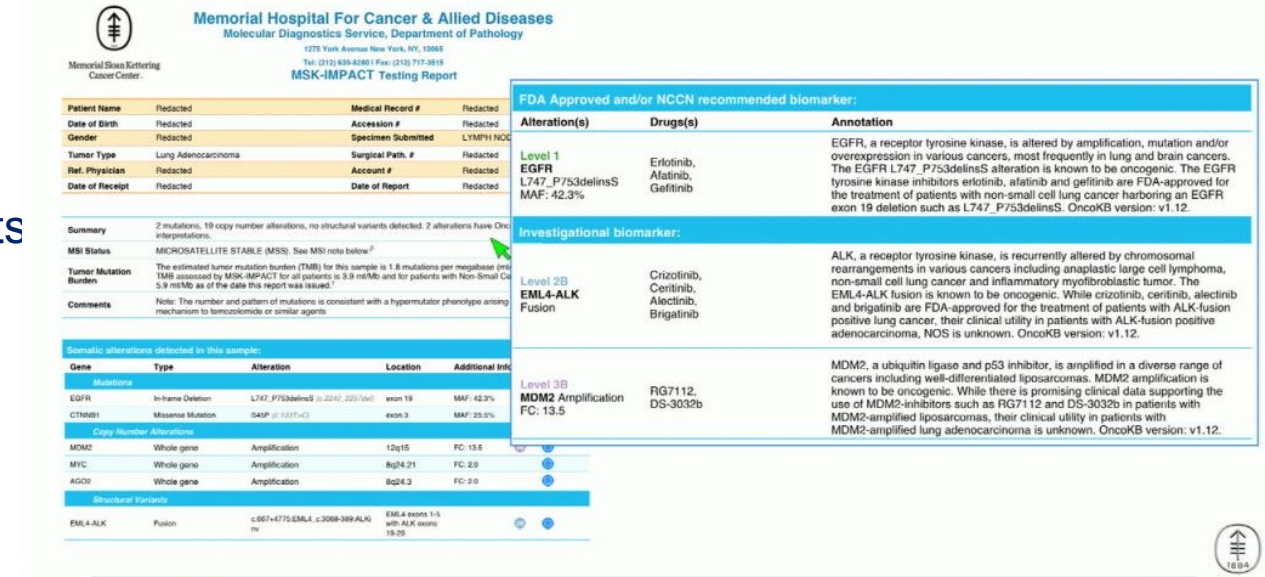
Sample preparation

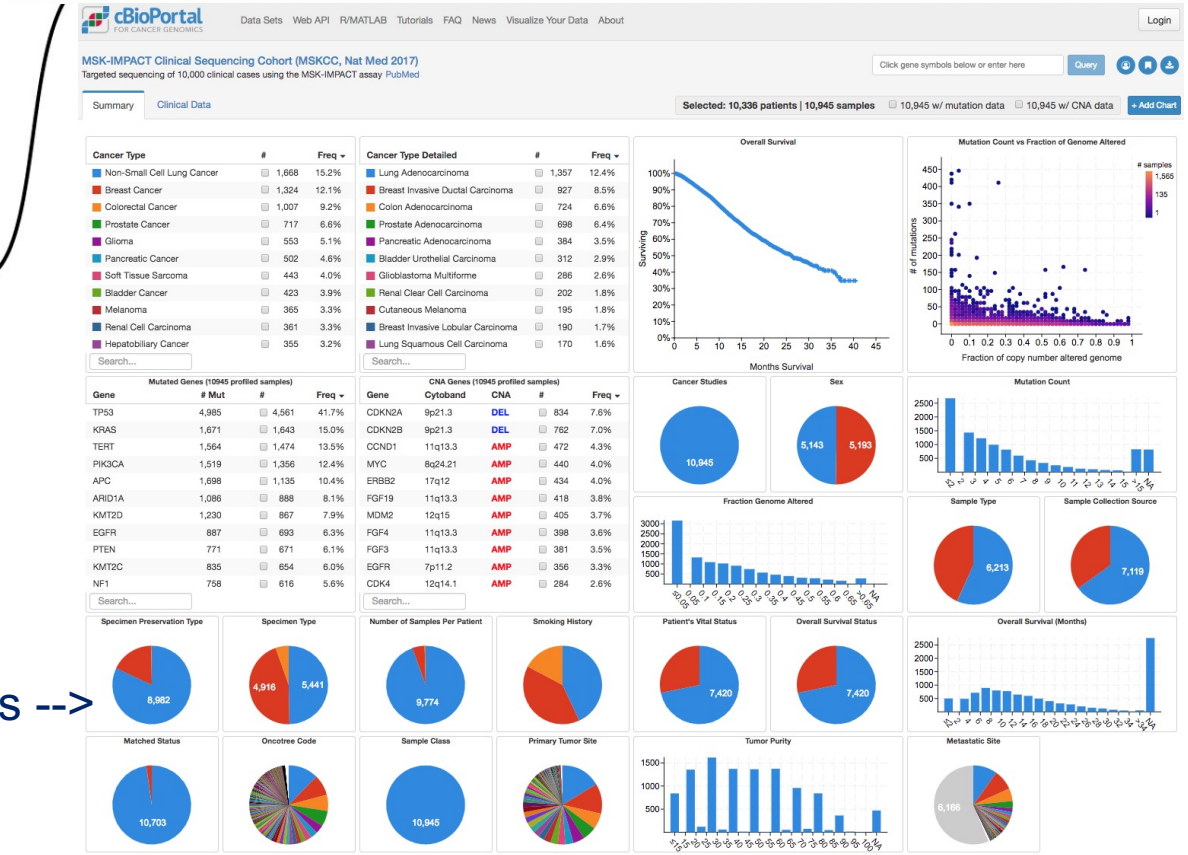
ABL1	CALR	DICER1	FGFR1	HLA-C	MALT1	NOTCH1	PPP4R2	RXRA	SUZ12
ACVR1	CARD11	DIS3	FGFR2	HNF1A	MAP2K1	NOTCH2	PPP6C	RYBP	SYK
AGO1	CARM1	DNAJB1	FGFR3	HOXB13	MAP2K2	NOTCH3	PRDM1	SCG5	TAP1
AGO2	CASP8	DNMT1	FGFR4	HRAS	MAP2K4	NOTCH4	PRDM14	SDHA	TAP2
AKT1	CBFB	DNMT3A	FH	ICOSLG	MAP3K1	NPM1	PREX2	SDHAF2	TBX3
AKT2	CBL	DNMT3B	FLCN	ID3	MAP3K13	NRAS	PRKAR1A	SDHB	TCEB1
AKT3	CCND1	DOT1L	FLT1	IDH1	MAP3K14	NSD1	PRKCI	SDHC	TCF3
ALB	CCND2	DROSHA	FLT3	IDH2	MAPK1	NTHL1	PRKD1	SDHD	TCF7L2
ALK	CCND3	DUSP4	FLT4	IFNGR1	MAPK3	NTRK1	PTCH1	SERPINB3	TEK
ALOX12B	CCNE1	E2F3	FOXA1	IGF1	MAPKAP1	NTRK2	PTEN	SERPINB4	TERT
ANKRD11	CD274	EED	FOXF1	IGF1R	MAX	NTRK3	PTP4A1	SESN1	TET1
APC	CD276	EGFL7	FOXL2	IGF2	MCL1	NUF2	PTPN11	SESN2	TET2
APLNR	CD79A	EGFR	FOXO1	IKBKE	MDC1	NUP93	PTPRD	SESN3	TGFBR1
AR	CD79B	EIF1AX	FOXP1	IKZF1	MDM2	PAK1	PTPRS	SETD2	TGFBR2
ARAF	CDC42	EIF4A2	FUBP1	IL10	MDM4	PAK7	PTPRT	SETDB1	TMEM127
ARHGAP35	CDC73	EIF4E	FYN	IL7R	MED12	PALB2	RAB35	SF3B1	TMPRSS2
ARID1A	CDH1	ELF3	GAB1	INHA	MEF2B	PARK2	RAC1	SH2B3	TNFAIP3
ARID1B	CDK12	EP300	GAB2	INHBA	MEN1	PARP1	RAC2	SH2D1A	TNFRSF14
ARID2	CDK4	EPAS1	GATA1	INPP4A	MET	PAX5	RAD21	SHOC2	TOP1
ARID5B	CDK6	EPCAM	GATA2	INPP4B	MGA	PBRM1	RAD50	SHQ1	TP53
ASXL1	CDK8	EPHA3	GATA3	INPPL1	MITF	PDCD1	RAD51	SLFN11	TP53BP1
ASXL2	CDKN1A	EPHA5	GLI1	INSR	MLH1	PDCD1LG2	RAD51C	SLX4	TP63
ATM	CDKN1B	EPHA7	GNA11	IRF4	MLLT1	PDGFRA	RAD51L1	SMAD2	TRAF2
ATR	CDKN2A	EPHB1	GNAQ	IRS1	MPL	PDGFRB	RAD51L3	SMAD3	TRAF7
ATRX	CDKN2B	ERBB2	GNAS	IRS2	MRE11A	PDPK1	RAD52	SMAD4	TRIP13
ATXN7	CDKN2C	ERBB3	GNB1	JAK1	MSH2	PGBD5	RAD54L	SMARCA2	TSC1
AURKA	CEBPA	ERBB4	GPS2	JAK2	MSH3	PGR	RAF1	SMARCA4	TSC2
AURKB	CENPA	ERCC2	GREM1	JAK3	MSH6	PHF6	RARA	SMARCB1	TSHR
AXIN1	CHEK1	ERCC3	GRIN2A	JUN	MSI1	PHOX2B	RASA1	SMARCD1	U2AF1
AXIN2	CHEK2	ERCC4	GSK3B	KBTBD4	MSI2	PIK3C2G	RB1	SMARCE1	UPF1
AXL	CIC	ERCC5	H3F3A	KDM5A	MST1	PIK3C3	RBM10	SMO	USP8
B2M	CMTR2	ERF	H3F3B	KDM5C	MST1R	PIK3CA	RECQL	SMYD3	VEGFA
BABAM1	CREBBP	ERG	H3F3C	KDM6A	MTAP	PIK3CB	RECQL4	SOCS1	VHL
BAP1	CRKL	ERRFI1	HGF	KDR	MTOR	PIK3CD	REL	SOS1	VTCN1
BARD1	CRLF2	ESR1	HIST1H1C	KEAP1	MUTYH	PIK3CG	REST	SOX17	WHSC1
BBC3	CSDE1	ETAA1	HIST1H2BD	KIT	MYC	PIK3R1	RET	SOX2	WHSC1L1
BCL10	CSF1R	ETV1	HIST1H3A	KLF4	MYCL1	PIK3R2	RFWD2	SOX9	WT1
BCL2	CSF3R	ETV6	HIST1H3B	KLF5	MYCN	PIK3R3	RHEB	SPEN	WWTR1
BCL2L1	CTCF	EZH1	HIST1H3C	KMT2A	MYD88	PIM1	RHOA	SPOP	XIAP
BCL2L11	CTLA4	EZH2	HIST1H3D	KMT2B	MYOD1	PLCG2	RICTOR	SPRED1	XPO1
BCL6	CTNNB1	FAM123B	HIST1H3E	KMT2C	NADK	PLK2	RIT1	SPRTN	XRCC2
BCOR	CTR9	FAM175A	HIST1H3F	KMT2D	NBN	PMAIP1	RNF43	SRC	YAP1
BIRC3	CUL3	FAM46C	HIST1H3G	KMT5A	NCOA3	PMS1	ROS1	SRSF2	YES1
BLM	CXCR4	FAM58A	HIST1H3H	KNSTRN	NCOR1	PMS2	RPS6KA4	STAG2	ZFHX3
BMPR1A	CXORF67	FANCA	HIST1H3I	KRAS	NEGR1	PNRC1	RPS6KB2	STAT3	ZNRF3
BRAF	CYLD	FANCC	HIST1H3J	LATS1	NF1	POLD1	RPTOR	STAT5A	ZRSR2
BRCA1	CYP19A1	FAT1	HIST2H3C	LATS2	NF2	POLE	RRAGC	STAT5B	
BRCA2	CYSLTR2	FBXW7	HIST2H3D	LMO1	NFE2L2	POT1	RRAS	STK11	
BRD4	DAXX	FGF19	HIST3H3	LYN	NFKBIA	PPARG	RRAS2	STK19	
BRIP1	DCUN1D1	FGF3	HLA-A	LZTR1	NKX2-1	PPM1D	RTEL1	STK40	
BTK	DDR2	FGF4	HLA-B	MAD2L2	NKX3-1	PPP2R1A	RUNX1	SUFU	



4. Sequencing

. Case review and sign out



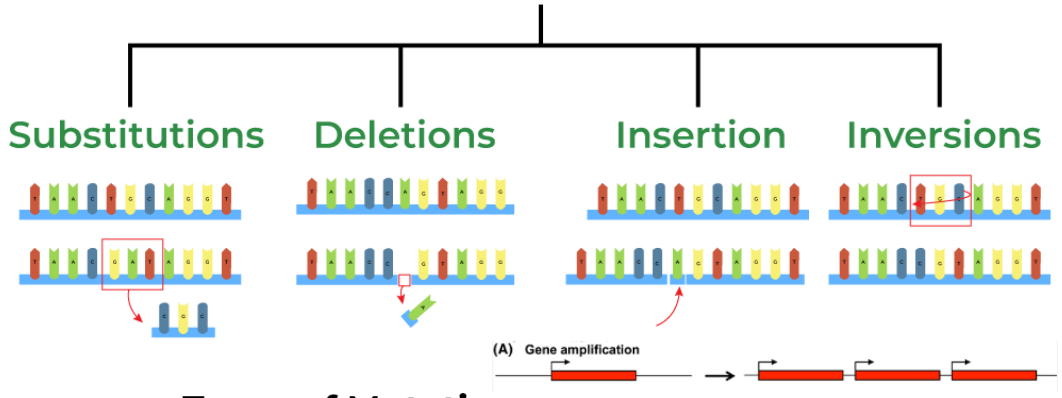


cBioPortal for Cancer Genomics -->

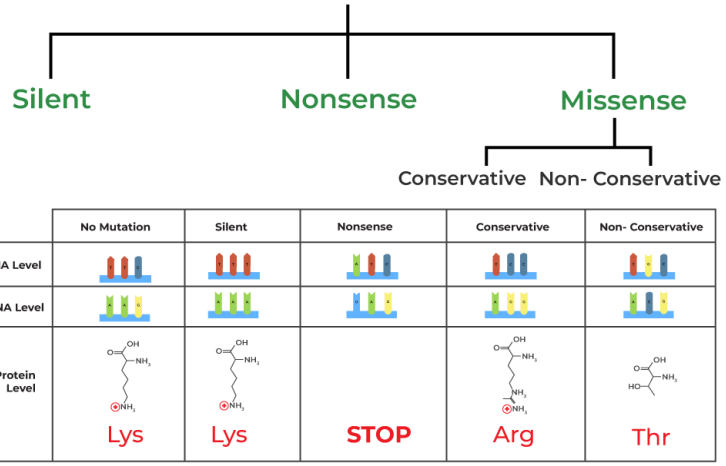
https://www.cbioportal.org

Cancer 101: Mutations glossary

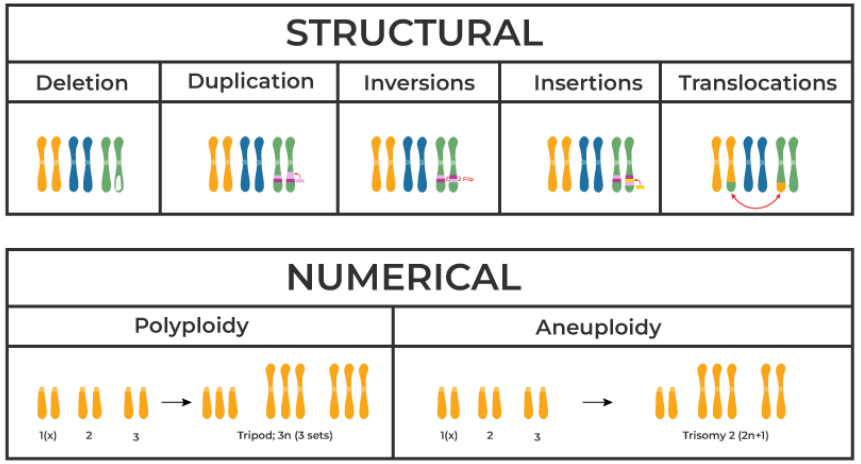
Types of Mutations
(At the DNA level)

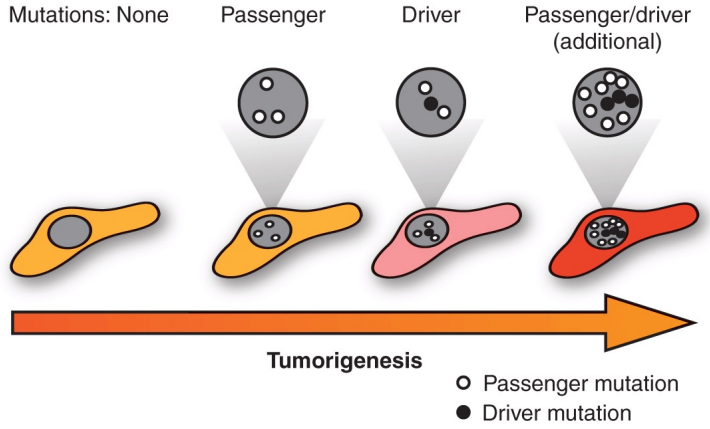


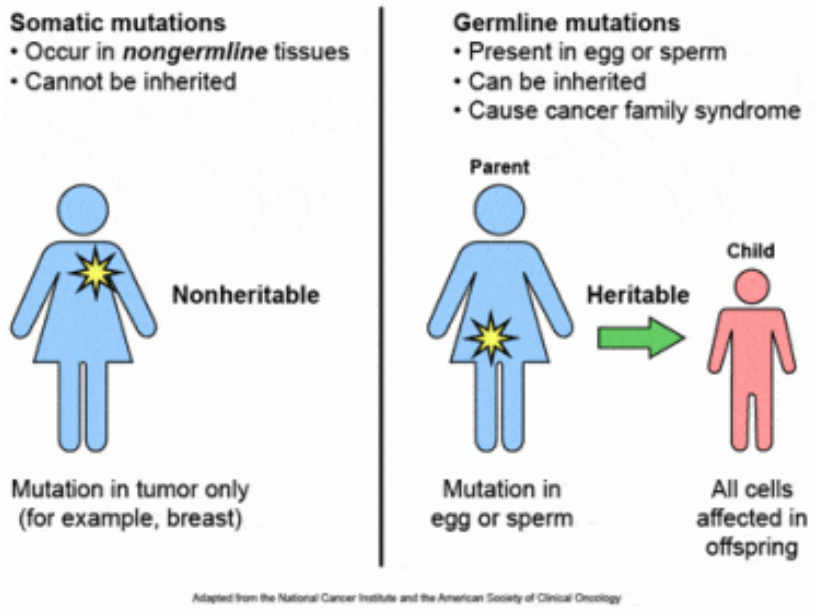
Types of Mutations
(At the Protein level)



Types of Mutations
(At the Chromosomal level)

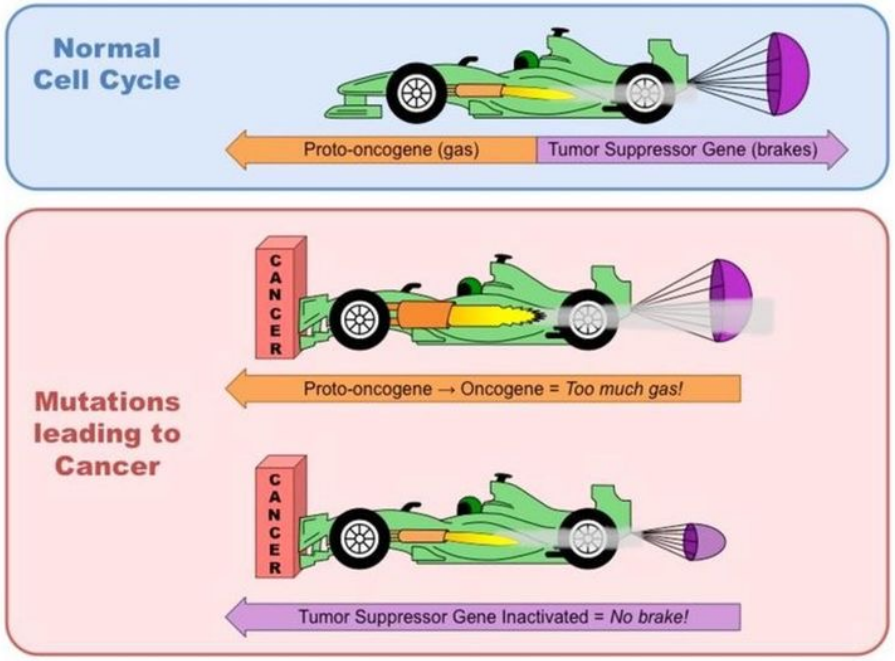


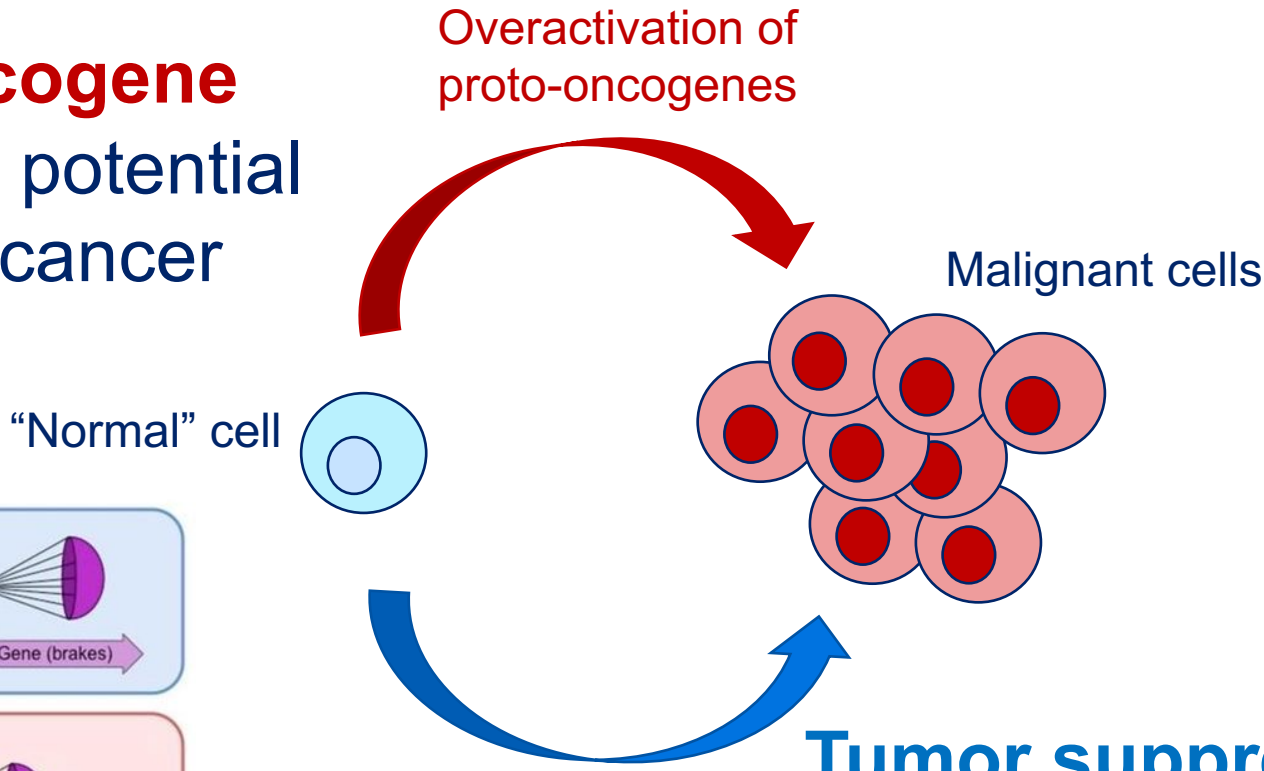




Cancer 101: Cancer is a genetic disease

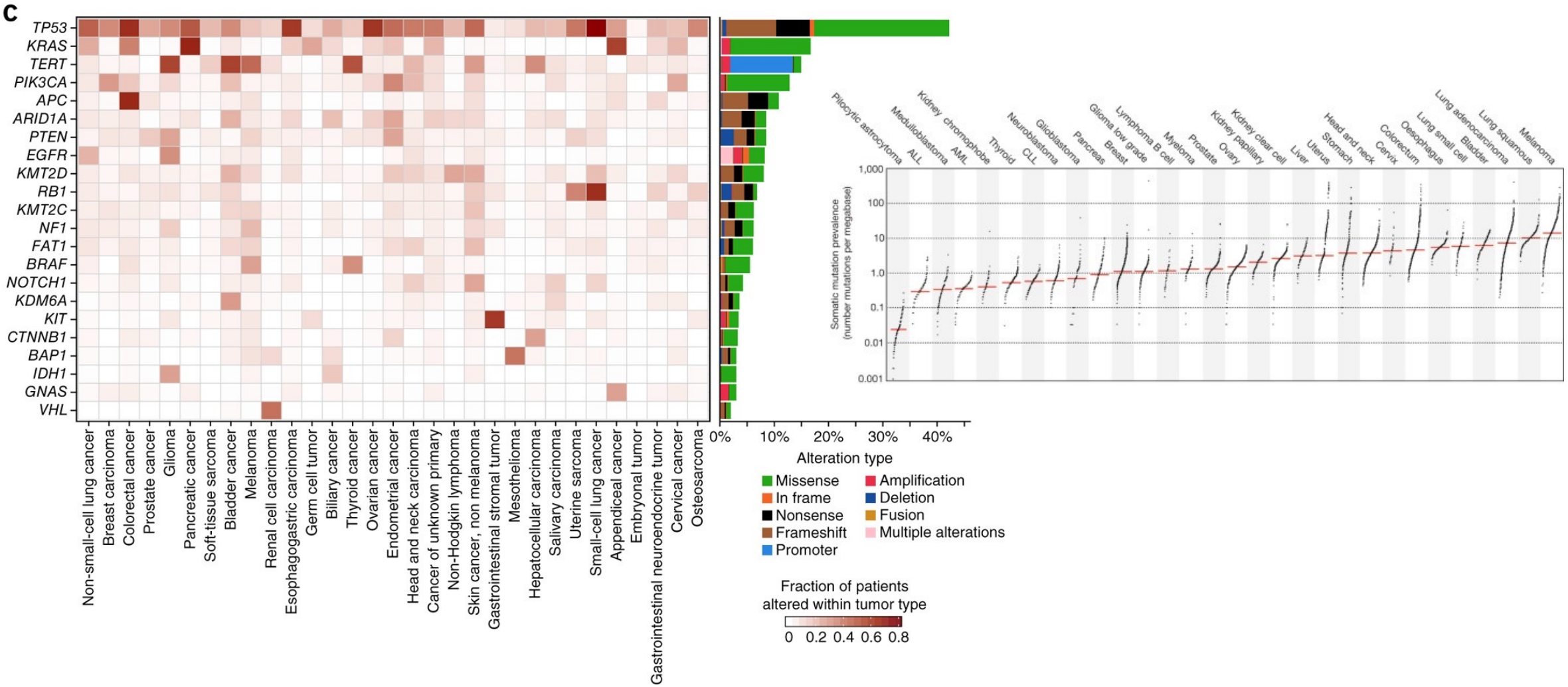
(Proto-)Oncogene
Gene with the potential to promote cancer





Tumor suppressor gene
Gene whose loss/dysfunction contributes to cancer

Commonly mutated cancer genes

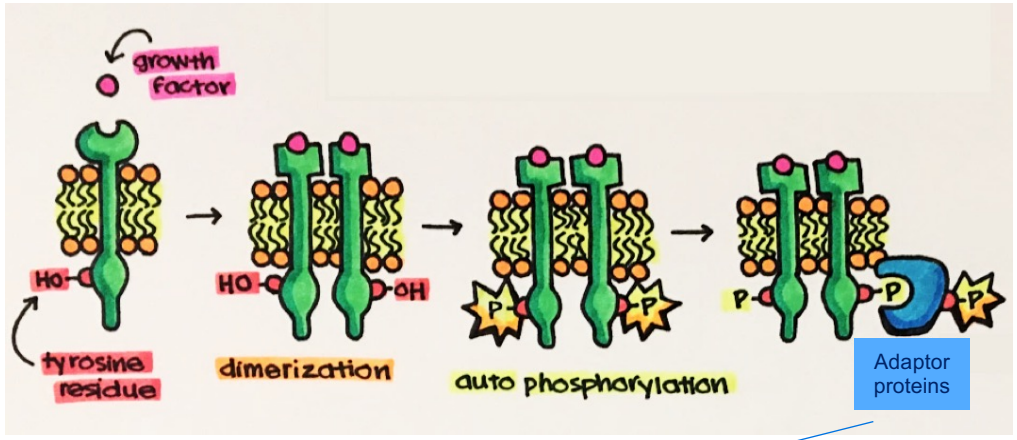


Nat Med 2017.
Nature 2023

Cancer Genes

Receptor tyrosine kinases (RTKs)

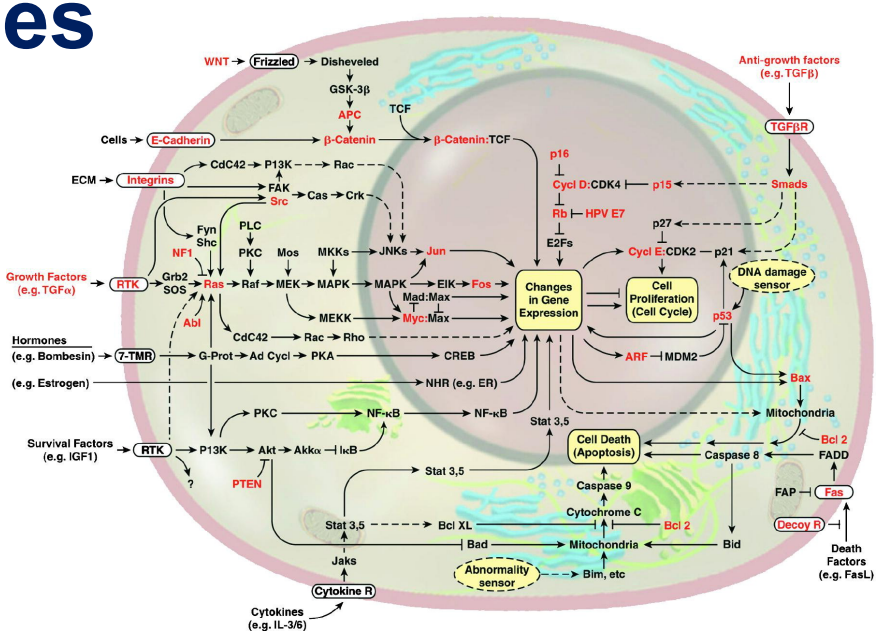
RTK activation

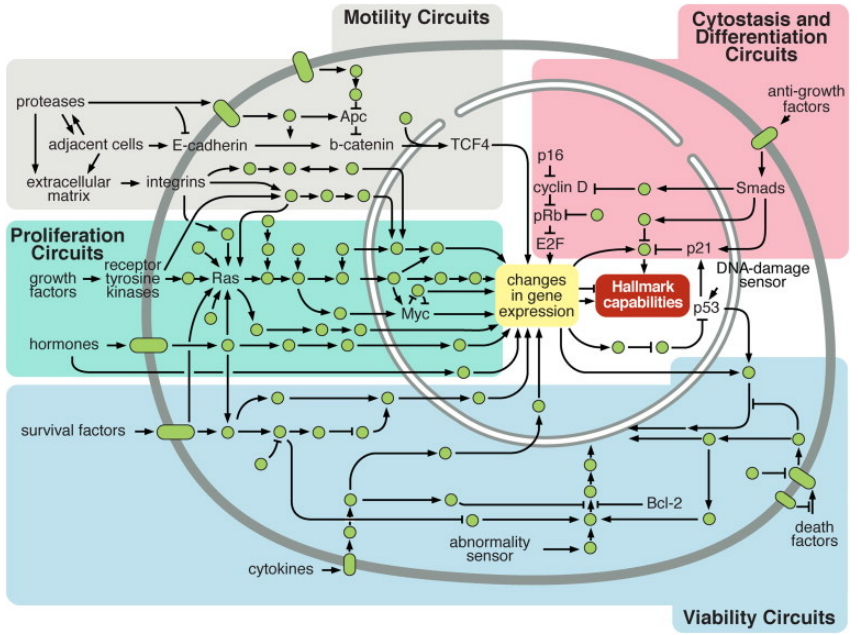


Signaling cascade

Activation/deactivation of TFs

Transcriptomic changes



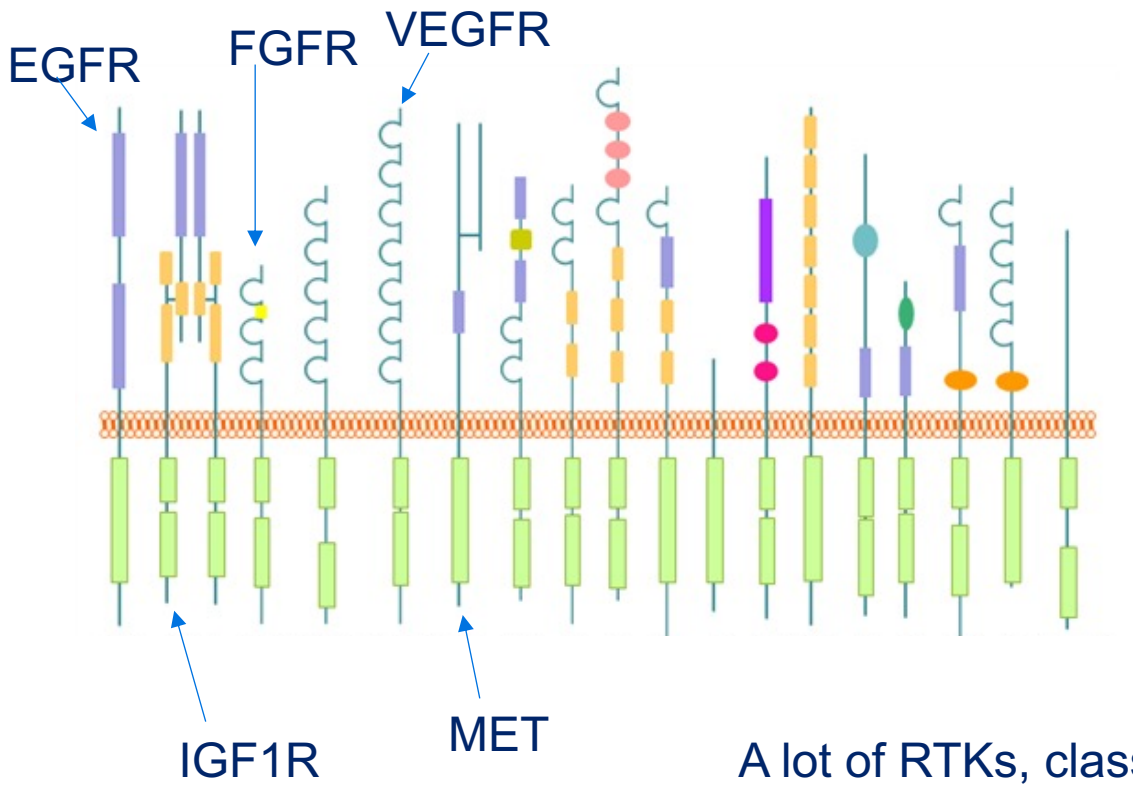


https://www.ncbi.nlm.nih.gov/books/NBK26822/

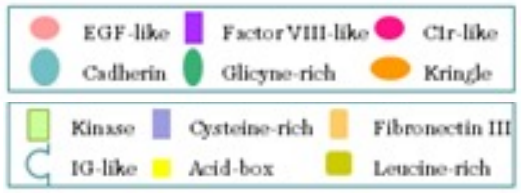
Adapted from Palmerini et al., Exp Rev of Anti-ineffective Ther 2014)

Cancer genes

Receptor tyrosine kinases (RTKs): RTK families



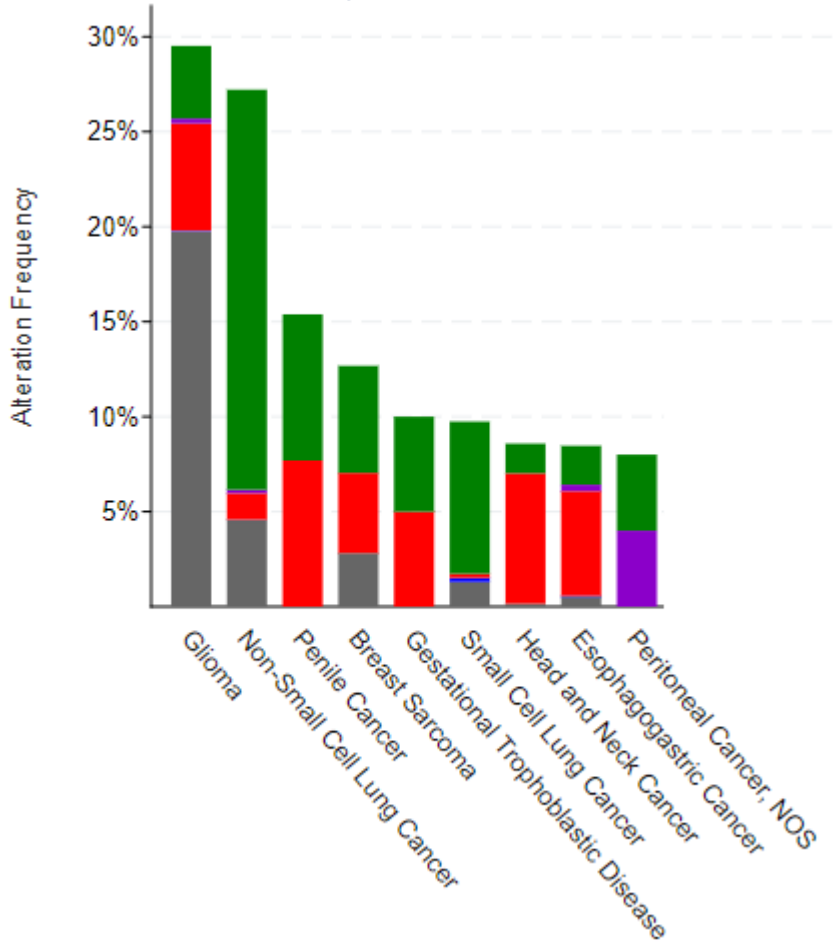
Domains



(Adapted from Critchley et al., Cells 2018)

A lot of RTKs, classified in families, with different functions and involvement in cancer

EGFR genomic alterations



Cancer genes

Receptor tyrosine kinases (RTKs): Pathways activated by RTKs

RAS/MAPK pathway

- Cell growth, cell cycle regulation
- Cell migration
- Angiogenesis

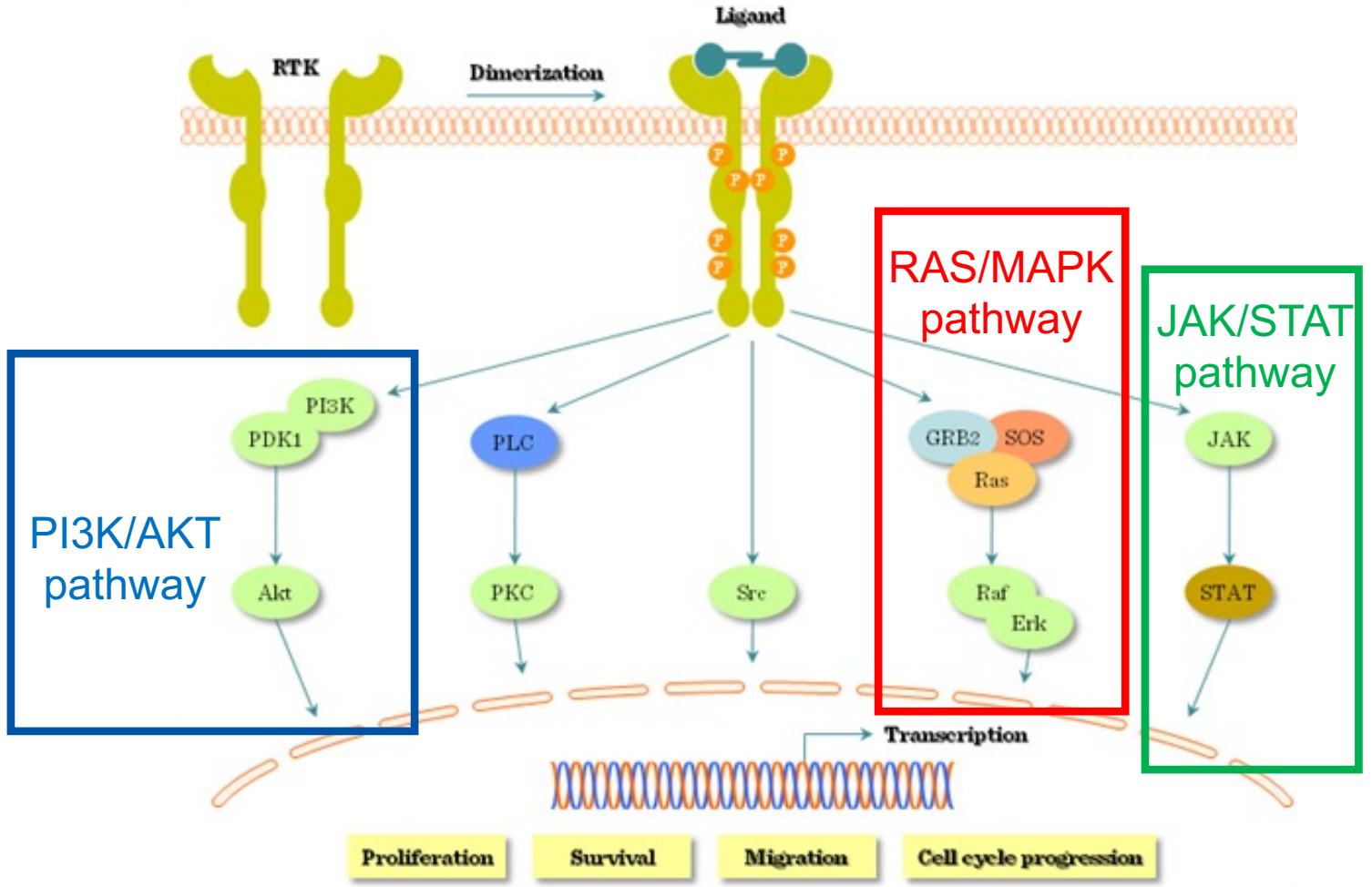
PI3K/AKT pathway

- Cell proliferation
- Apoptosis
- Metabolism
- Angiogenesis

JAK/STAT pathway

- Cell proliferation
- Apoptosis
- Immune response / inflammation
- Angiogenesis
- Metastasis

Some signaling pathways activated by



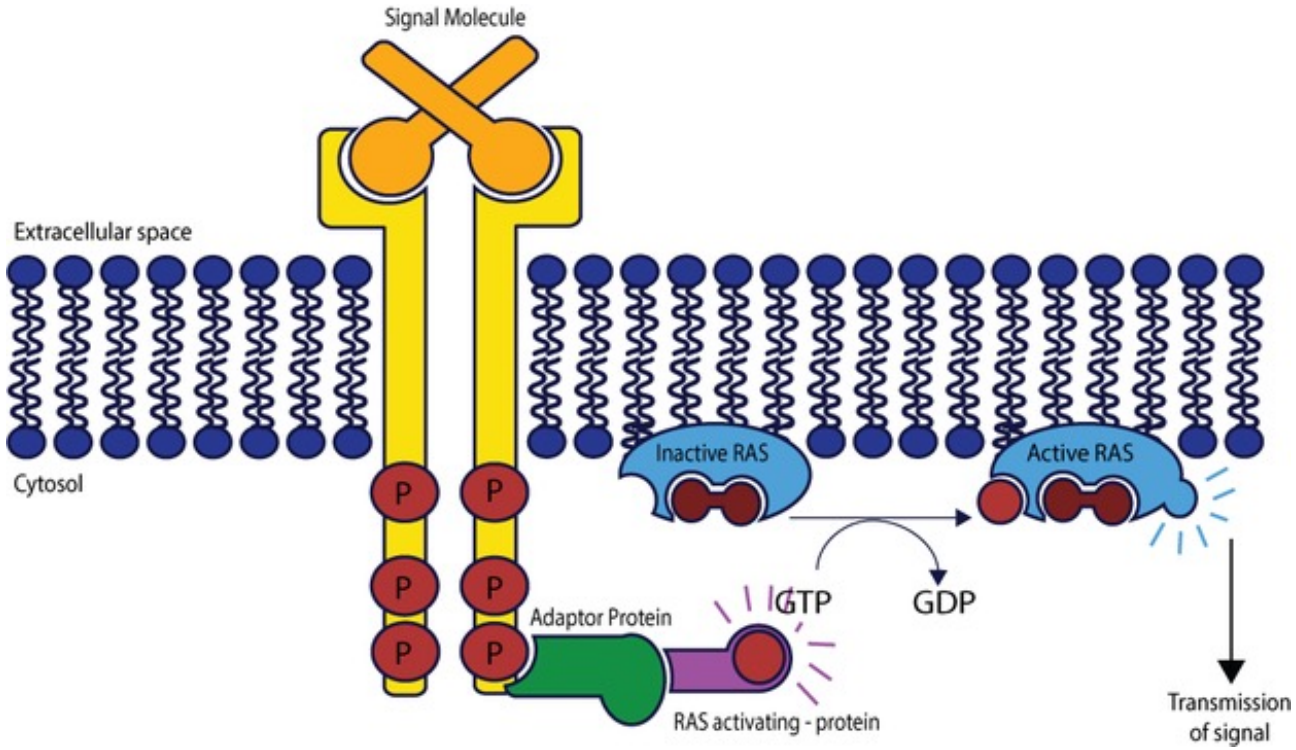
Adapted from Palmerini et al., Exp Rev of Anti-ineffective Ther 2014)

Cancer genes

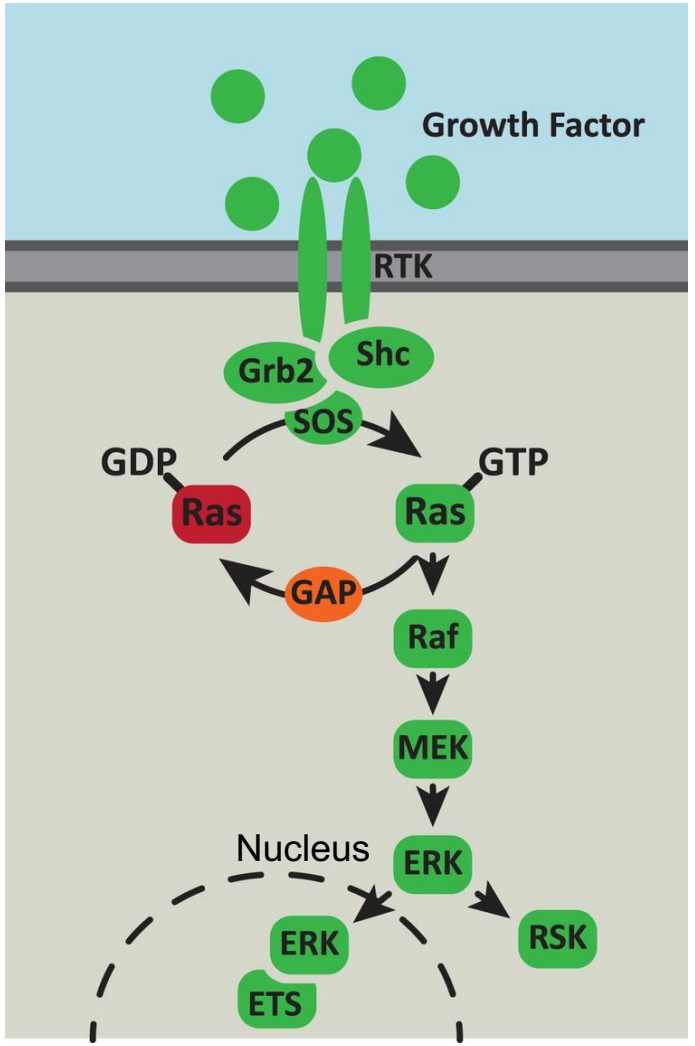
RAS (KRAS, NRAS, HRAS…)

Involved in…cellular proliferation, differentiation, and survival

RAS activation



MAPK pathway

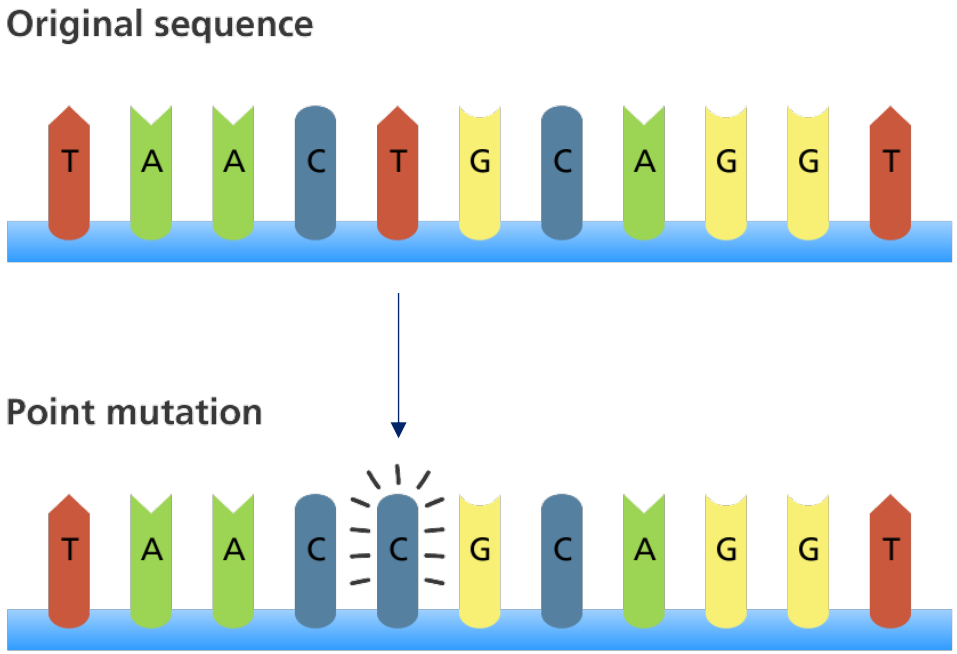


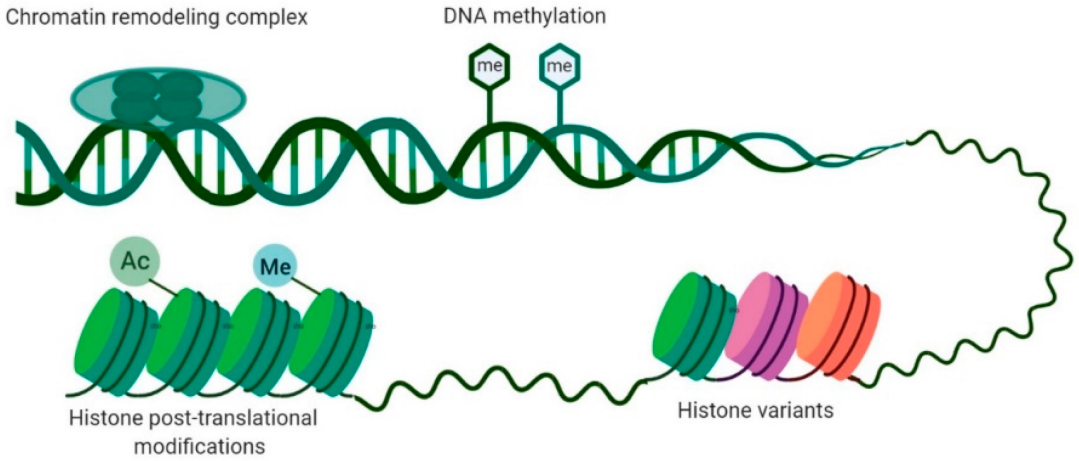
(Adapted from Addgene and Slack, Nutrition and healthy aging 2017)

Take home messages

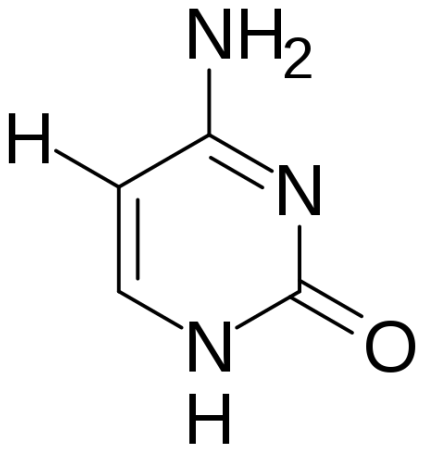
1. Cancer is a disease with a strong genetic component. Molecular alterations leading to loss of function of tumor suppressors (e.g. *TP53, RB1*) or to overactivation of proto-oncogenes (e.g. *RAS*, RTKs) may facilitate the occurrence of cancer or induce it.

DNA mutation vs DNA methylation:

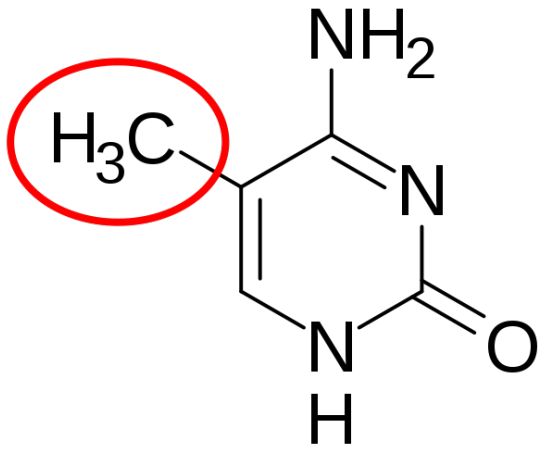




Epigenetics:
Methylation as an example of an epigenetic modification



cytosine



methylated cytosine

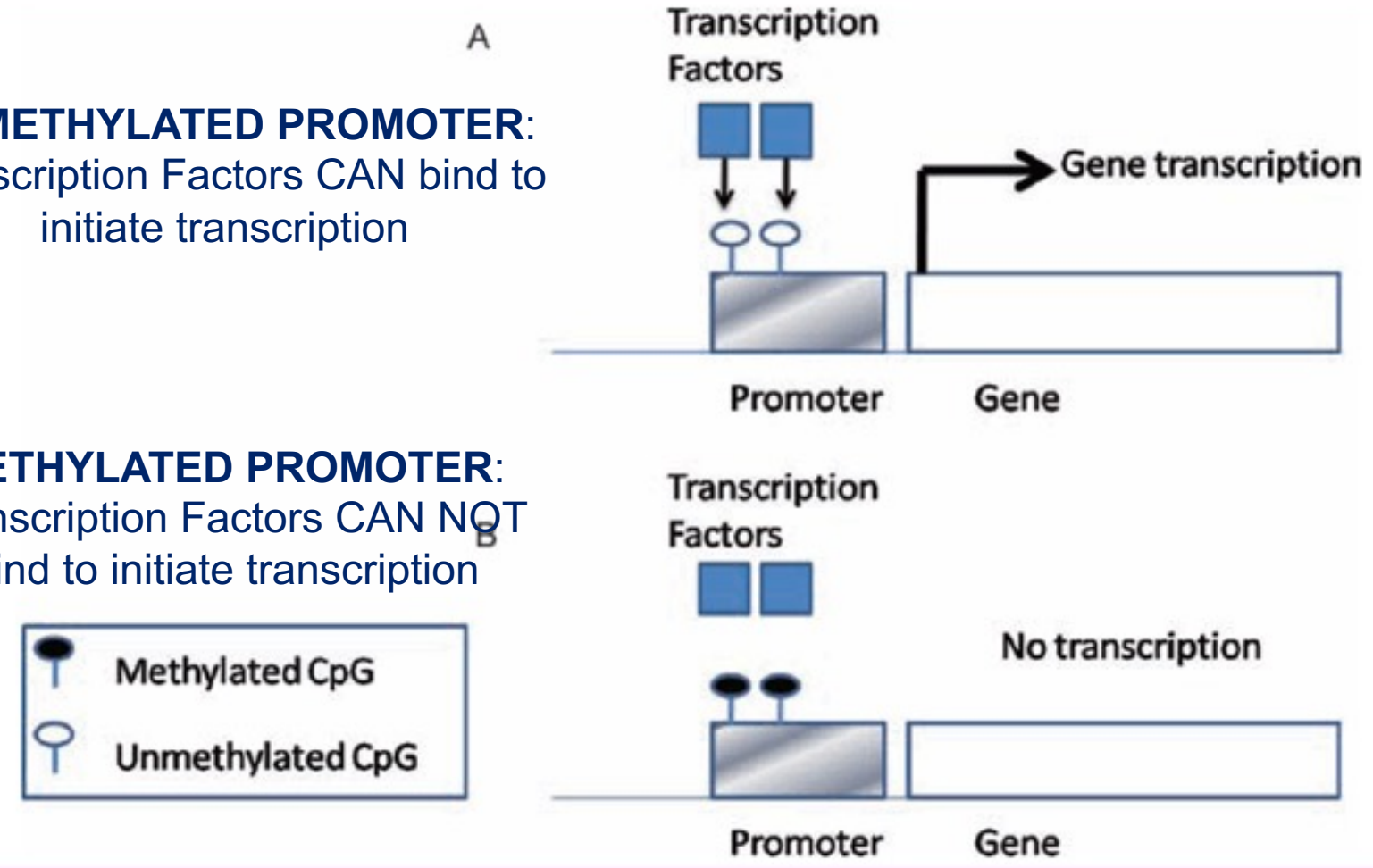
a vs á

Wikicommons
MDPI
Ambrosi JMB 2017
Kim EMM 2017
Lim TOG 2011
Whatisepigenetics.com

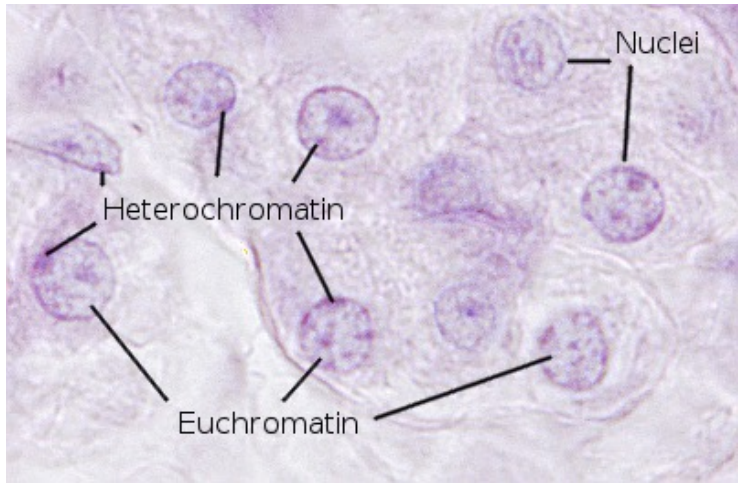
Effects of Promoter Methylation

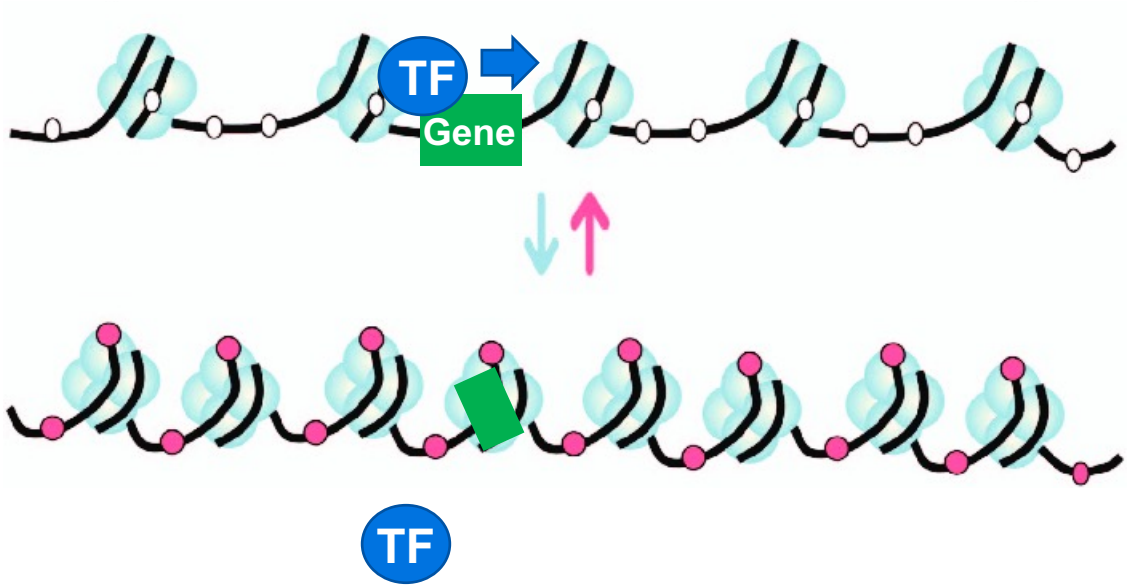
UNMETHYLATED PROMOTER:
Transcription Factors CAN bind to initiate transcription

METHYLATED PROMOTER:
Transcription Factors CAN NOT bind to initiate transcription



Alterations at the epigenetic/transcriptomic level





Open chromatin
(Transcription factor can access DNA and induce gene expression)

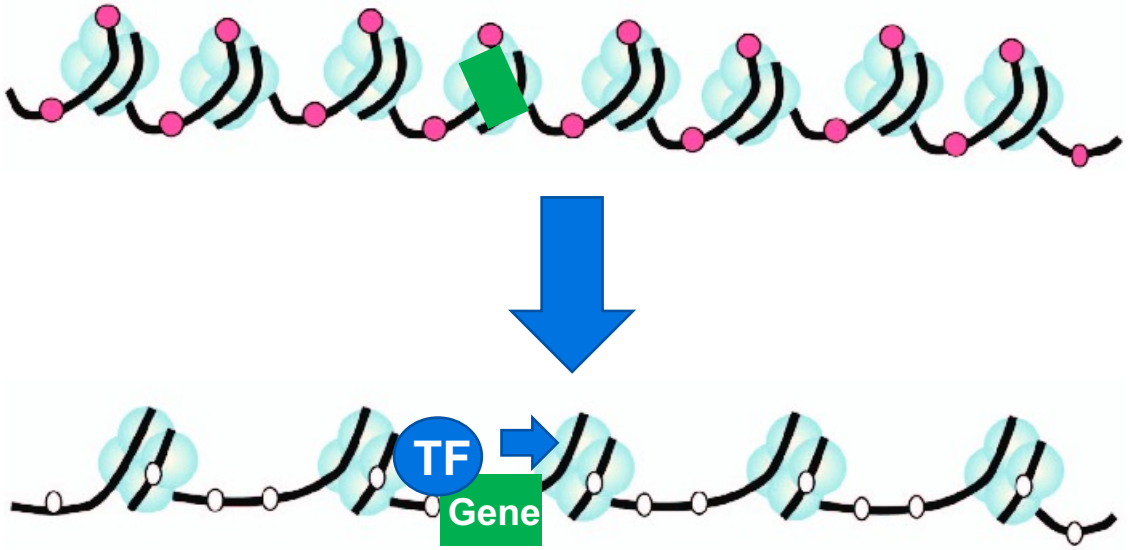
Condensed chromatin
(Transcription factor cannot access DNA)

Adapted from Wu et al., Cell & Mol Immunology 2018)

Interaction among different molecular alterations

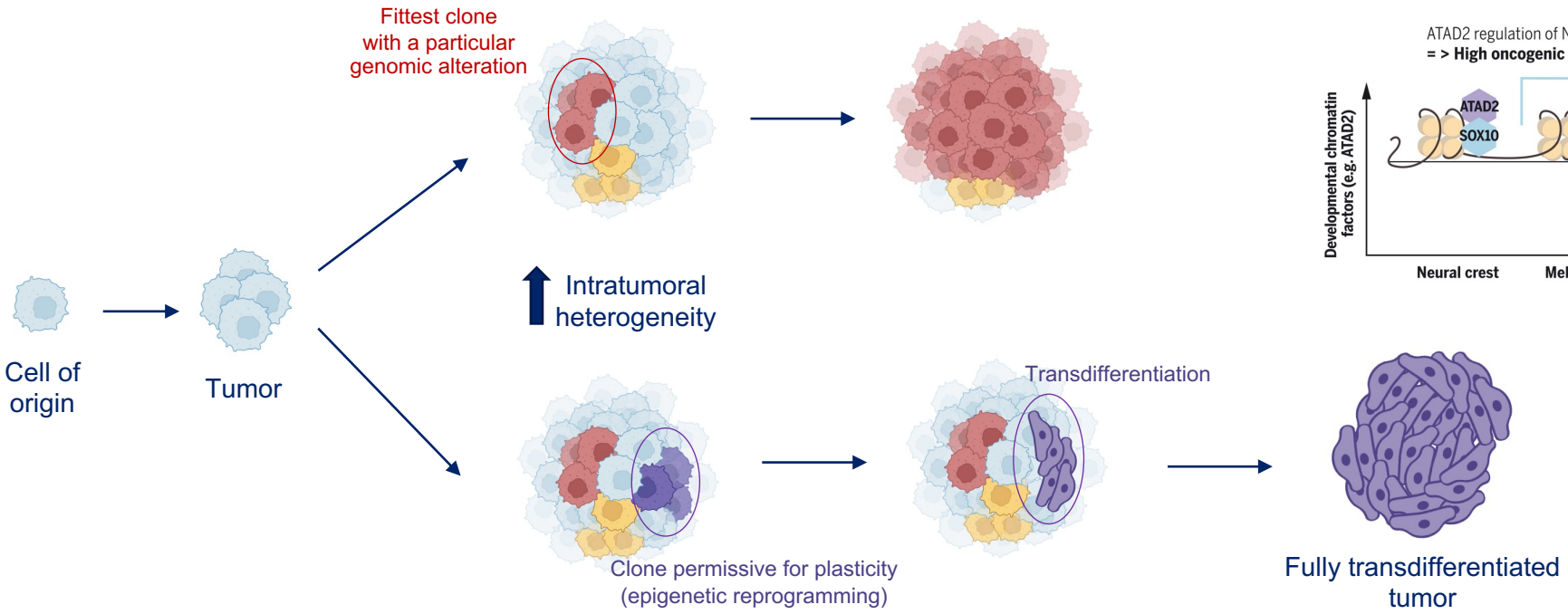
Example 1:
mutation on a transcription factor (TF) leading to inefficient DNA binding capacity (and then gene expression induction)

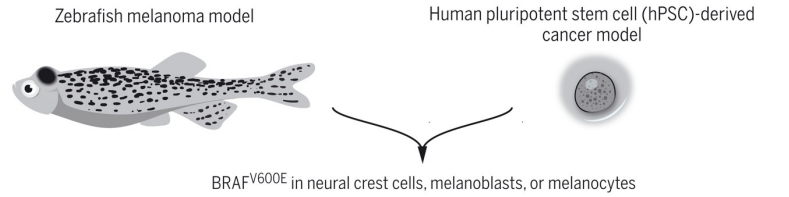
Example 2:
mutation on enzymes responsible to make the chromatin compact leading to open chromatin and increased expression of genes in those genomicloci

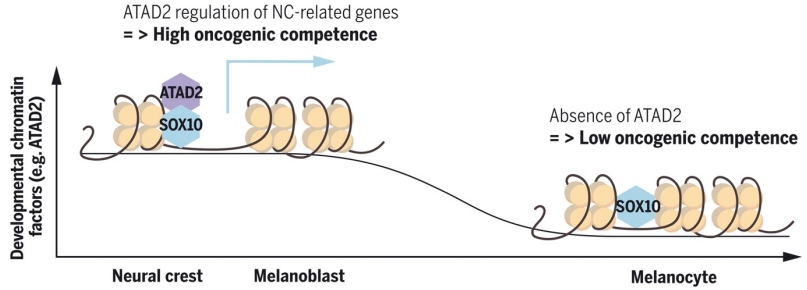


Adapted from Wu et al., Cell & Mol Immunology 2018

Tumor heterogeneity, plasticity, competence and evolution







Take home messages

1. Cancer is a disease with a strong genetic component. Molecular alterations leading to loss of function of tumor suppressors (e.g. *TP53, RB1*) or to overactivation of proto-oncogenes (e.g. *RAS*, RTKs) may facilitate the occurrence of cancer or induce it.

2. Tumors usually exhibit a number of different molecular alterations (genomic, epigenomic, transcriptomic,…) in a variety of genes, but not all of them contribute significantly to the oncogenic phenotype. It is important to identify which are driving the oncogenic phenotype (*drivers*) and study the molecular biology behind it (*mechanism*).

As part of your project, you are interested in modelling mutations that frequently occur in cancer patients. For this purpose, you are reviewing MSK-IMPACT clinical sequencing results using the cBioPortal platform. One of the genes that most commonly is found to be mutated in this dataset is TP53, a known tumor suppressor gene. What are the characteristics of these types of genes?

A) In cancer patients, inactivating mutations are the most frequent type of mutations occurring in tumor suppressors genes.

B) Loss of function in tumor suppressors genes cannot occur due to non-mutational processes like promoter methylation.

C) Loss of tumor suppressors decreases the likelihood of malignant transformation.

D) Mutation in tumor suppressors genes can only be somatic, and they don’t occur in the germline.

As part of your project, you are interested in **modelling** mutations that frequently occur in **cancer patients**. For this purpose, you are reviewing **MSK-IMPACT** clinical sequencing results using the **cBioPortal** platform. One of the genes that most commonly is found to be mutated in this dataset is **TP53**, a known **tumor suppressor** gene. What are the characteristics of these types of genes?

A) In cancer patients, **inactivating mutations** are the most frequent type of mutations occurring in tumor suppressors genes.

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C) Loss of tumor suppressors decreases the likelihood of **malignant transformation.**

D) Mutation in tumor suppressors genes can only be **somatic**, and they don't occur in the **germline**.

After acknowledging the importance of TP53 as a tumor suppressor, you are interested in understanding the effect of other tumor suppressors. Which of the following genes is not a tumor suppressor?

a) RB, a gene involved in controlling the pass through a cell cycle checkpoint.

b) APC, a gene involved in downregulating the expression of beta-catenin, a known proto-oncogene.

c) BRCA, a gene responsible for the reparation of DNA.

d) KRAS, a gene involved in promoting cell growth.

Thanks for your attention!

Any questions?

Memorial Sloan Kettering
Cancer Center