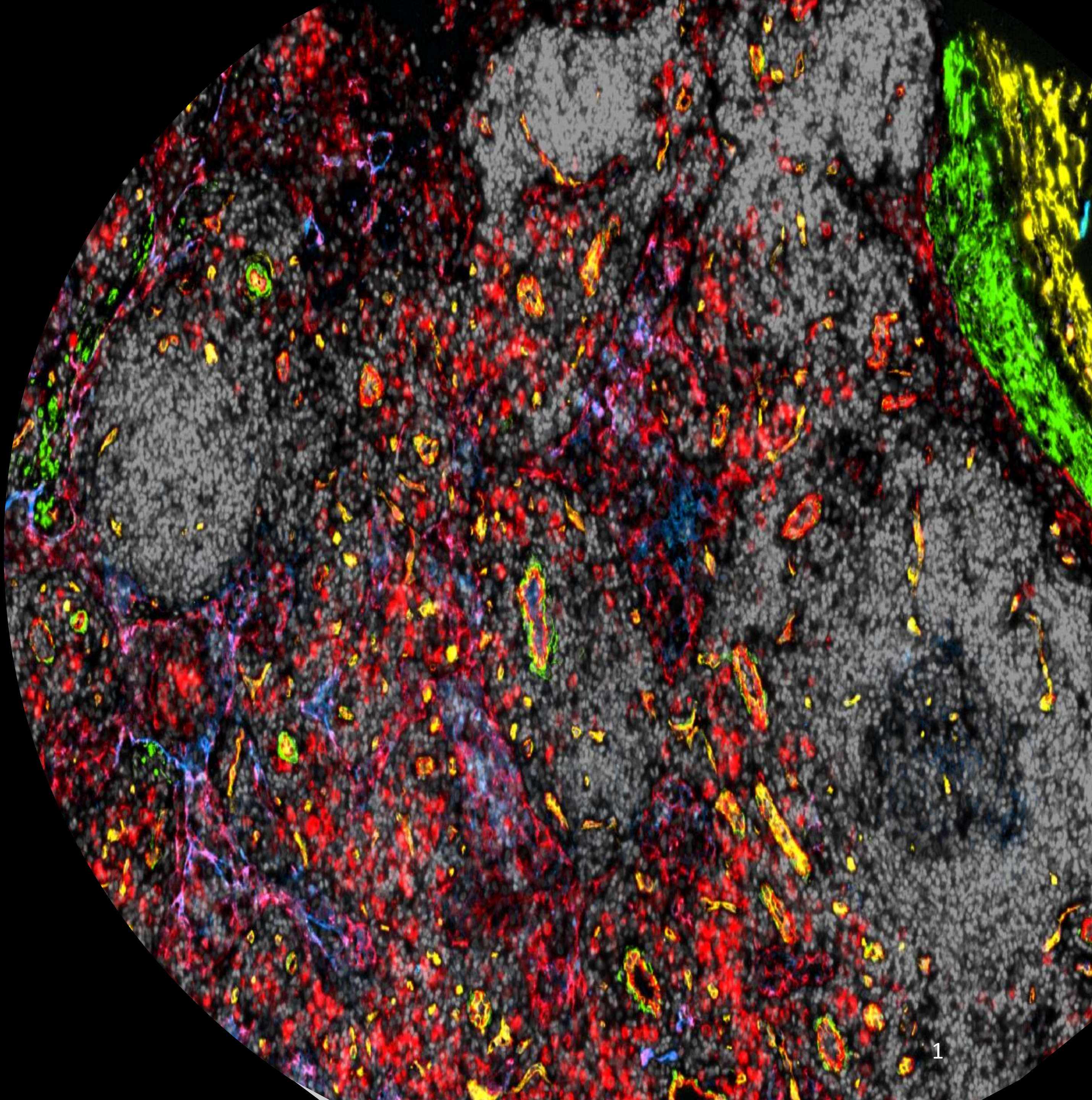
Cancer Biology I

Part-II

Fall semester 2024
Week 8



My career path …

Master in Biology

Specialization in physiology and medicine

PhD

Post-Doc

Assistant Professor

Associate Professor

Director of ISREC@EPFL

2004

2008

Nov. 2014

Oct. 2021

Oct. 2022

🇮🇹

University of Rome

SAPIENZA
UNIVERSITÀ DI ROMA

Master thesis topic:
Cell Cycle control in cancer

🇮🇹

University of Rome

ISTITUTO SUPERIORE DI SANITÀ

UNIVERSITA' degli STUDI di ROMA TOR VERGATA

6 months internship

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

PhD thesis topic:
Retroelements in cancer

🇺🇸

Memorial Sloan Kettering Cancer Center

New York

Memorial Sloan Kettering Cancer Center

Topic:
lymphoma genetics

🇨🇭

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

School of Life Science
Swiss Institute for Experimental Cancer Research

EPFL

Topics: *Functional Genomics, Chromatin 3D structure, and therapies in cancer*

Main research areas in Oricchio lab

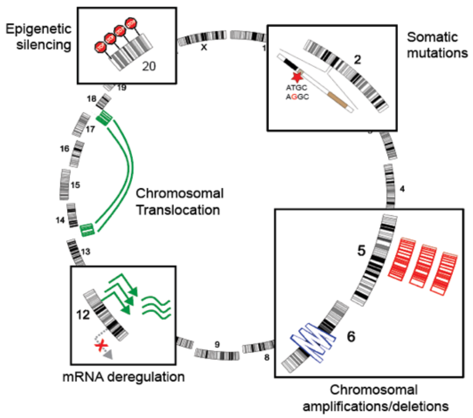
Areas of research

CANCER GENOMICS

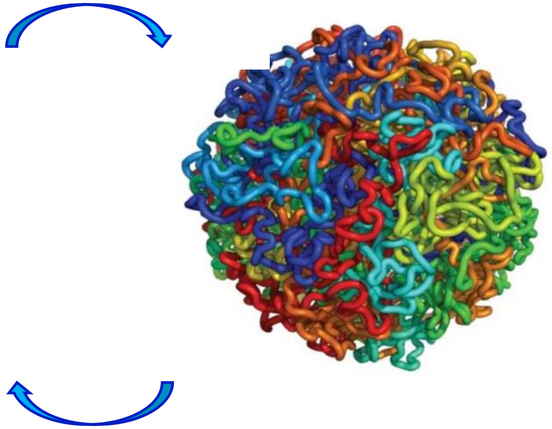
CHROMATIN
3D STRUCTURE

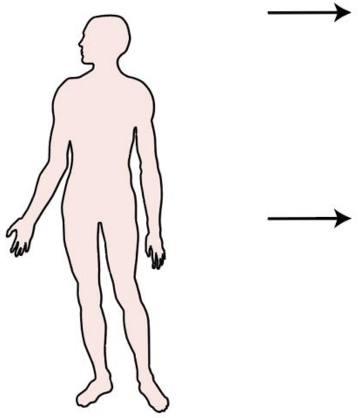
THERAPIES

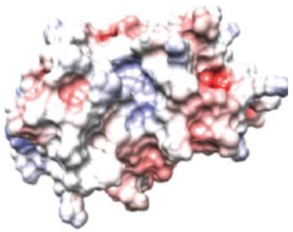
Genomic alterations in cancer cells



Chromatin organization

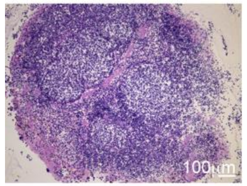






Target identification

therapy development



Different models to study response to therapies

Zufferey, Taveranri et al. *Genome Biology 2018,*
Donaldson, Sungalee, Zufferey, Tavernari et al. *Nature Genetics 2019,*
Sungalee et al. *Nature Genetics 2021,*
Liu et al. *Nature Communications 2021*,
Lambuta, Nanni et al., *Nature 2023*

Battistello et al. *Blood 2019,*
Dheilly, Battistello et al. *Cancer Cell 2020,*
Petruzzella et al. *Nature Chem. Bio. 2024,*
Santamaria-Martinez et al. *Nature Comm 2024*
Bruand et al. *in revision*
Epiney et al. *submitted*

AGENDA

Nov 3rd: Cancer genomics- mutations

Nov 10th: Cancer genomics-copy number alterations, heterogeneity, tumor evolution

Nov 17th: Cancer Epigenetics- chromatin 3D structure, cell plasticity

Nov 24th: – Major signaling pathways leading to cancer

Dec 1st: Cancer Therapies – chemo and targeted therapies

Dec 8th: Introduction to immunotherapies –

Dec 15th: Exam
(If it conflicts with another exam, it is possible to do the exam on Dec 17th)

Dec 17th: discussion of unclear points and career development discussion towards a
PhD or not…(if the exam will be on Dec 17th)

Exercise

- Two TAs will help in the discussion of the paper

Alice Pellegrino
Dea Muller

I provide a list of questions that can guide the discussion and help to understand key point of the paper

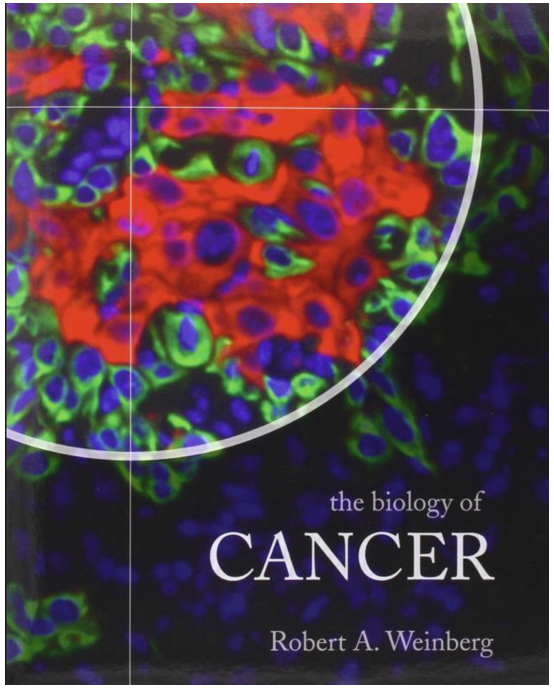
- Wednesday, Nov 19th and Wednesday, Dec 10th:

I will post two short mock exam sessions with questions from the previous years

Exam Information

- Exam questions will be in English, but you're allowed to answer in French (*if you do, please use capital letters*)
- *Day to be confirmed:* Dec 15 or 17th , 2 - 4 PM (*room will be communicated*)
- ~10 open questions
  - 10 points for each question
  - <u>2 questions will be on the papers that you will read during the exercises</u>
- The exam will count for 50% of your final grade

If you want to read on specific topics:



Some information if this book

Send an email:

I can send you recent Reviews written by prominent cancer scientists in the fields

We can discuss interest for your lab immersion and master thesis project

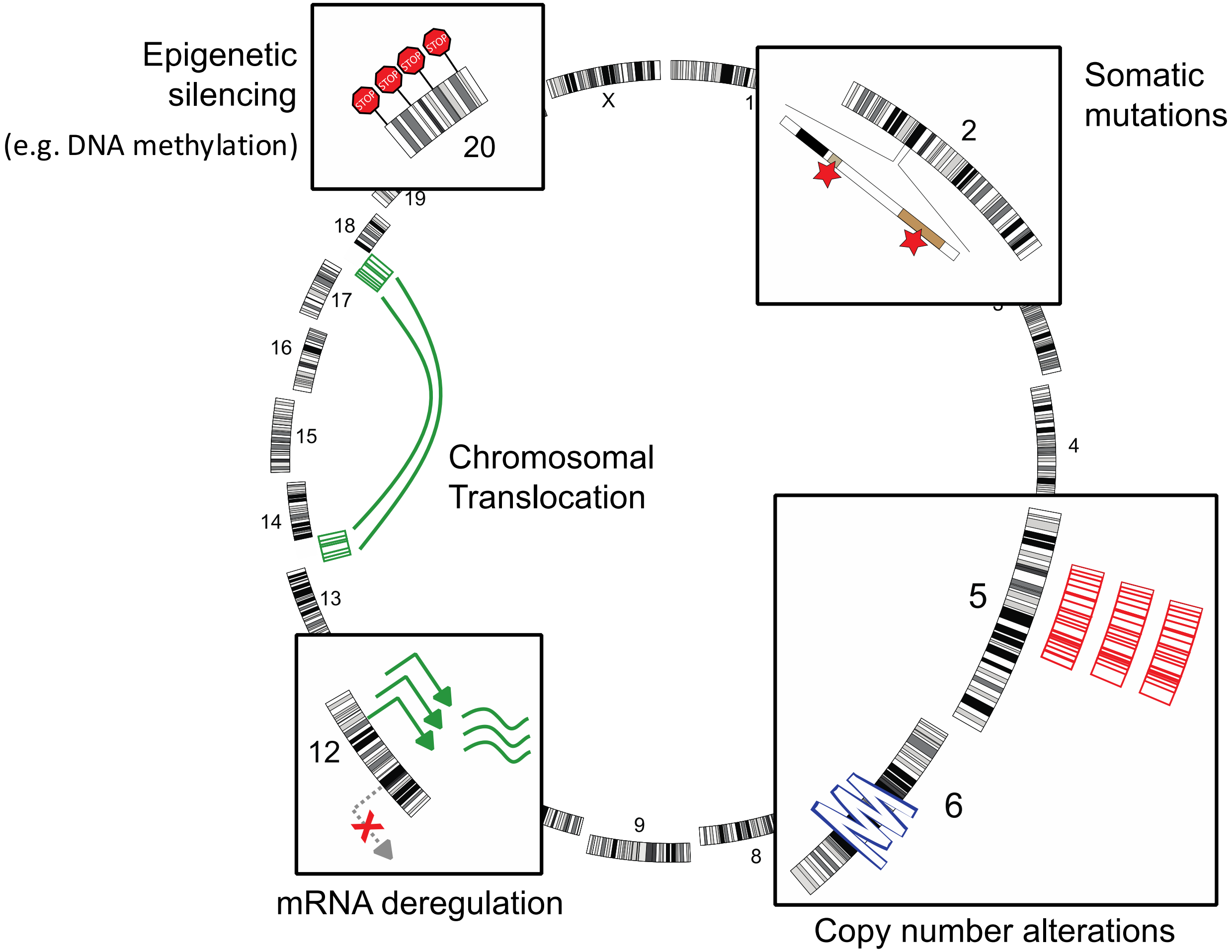
Why do we develop cancer?

1. Cancer is a disease of the genome

Cancer genomics: to study the acquisition of alterations in the genome (i.e. in our DNA) that can cause cancer development

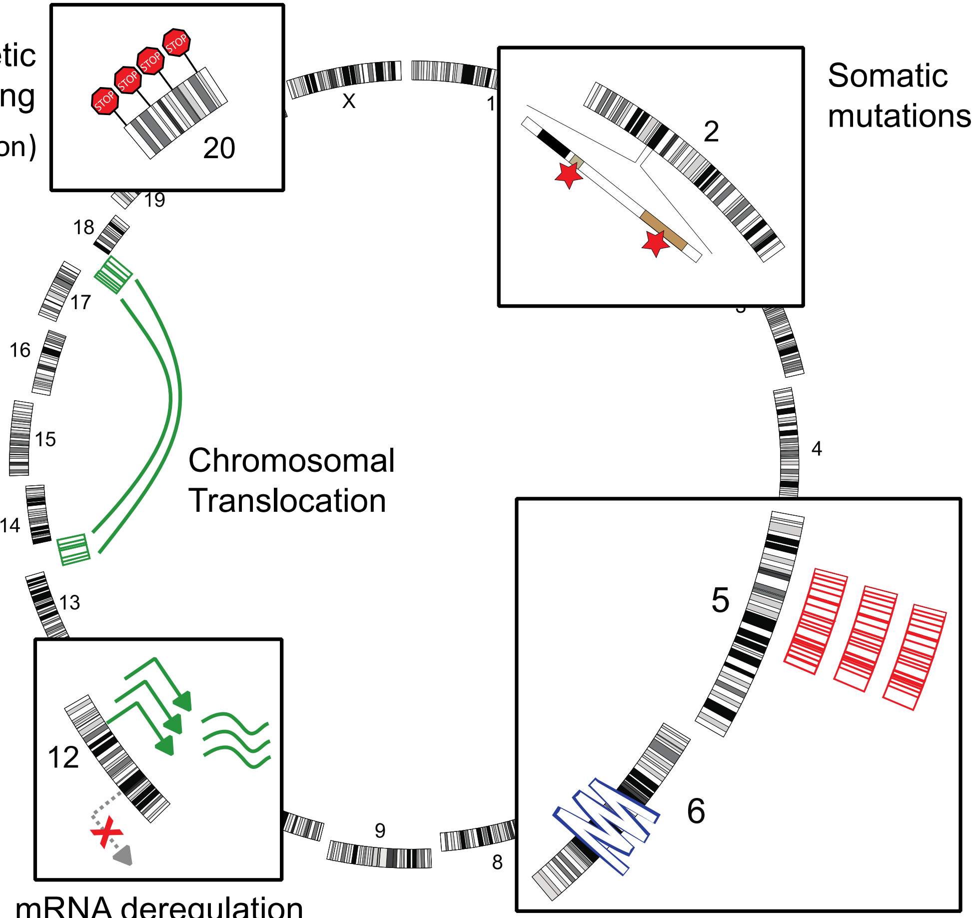
What are they?

Cancer Genomic Alterations



What are they?

Cancer Genomic Alterations



Epigenetic silencing

(e.g. DNA methylation)

Somatic mutations

Chromosomal Translocation

mRNA deregulation

Copy number alterations

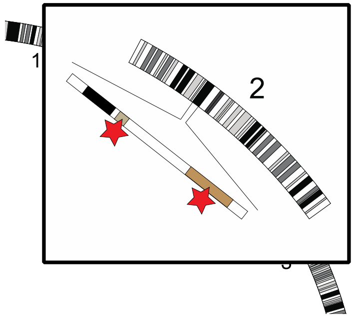
You like my dogs

↓

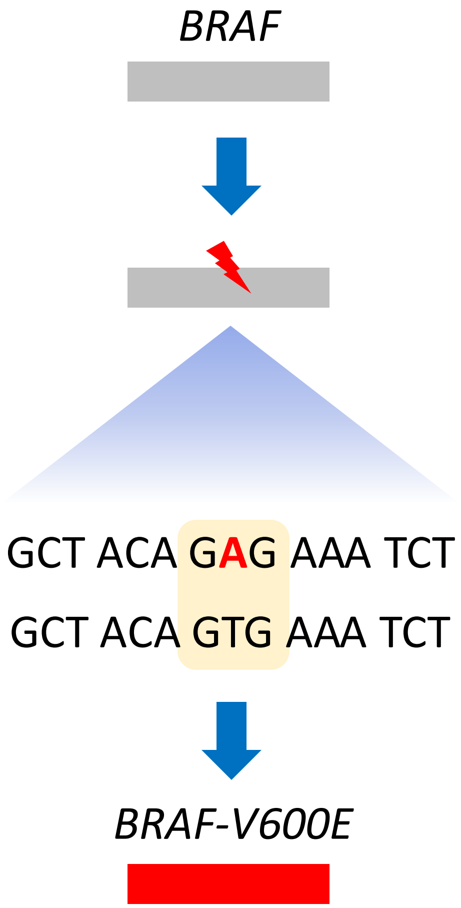
You like my dots

Mutations

- Single nucleotide changes



Somatic
mutations



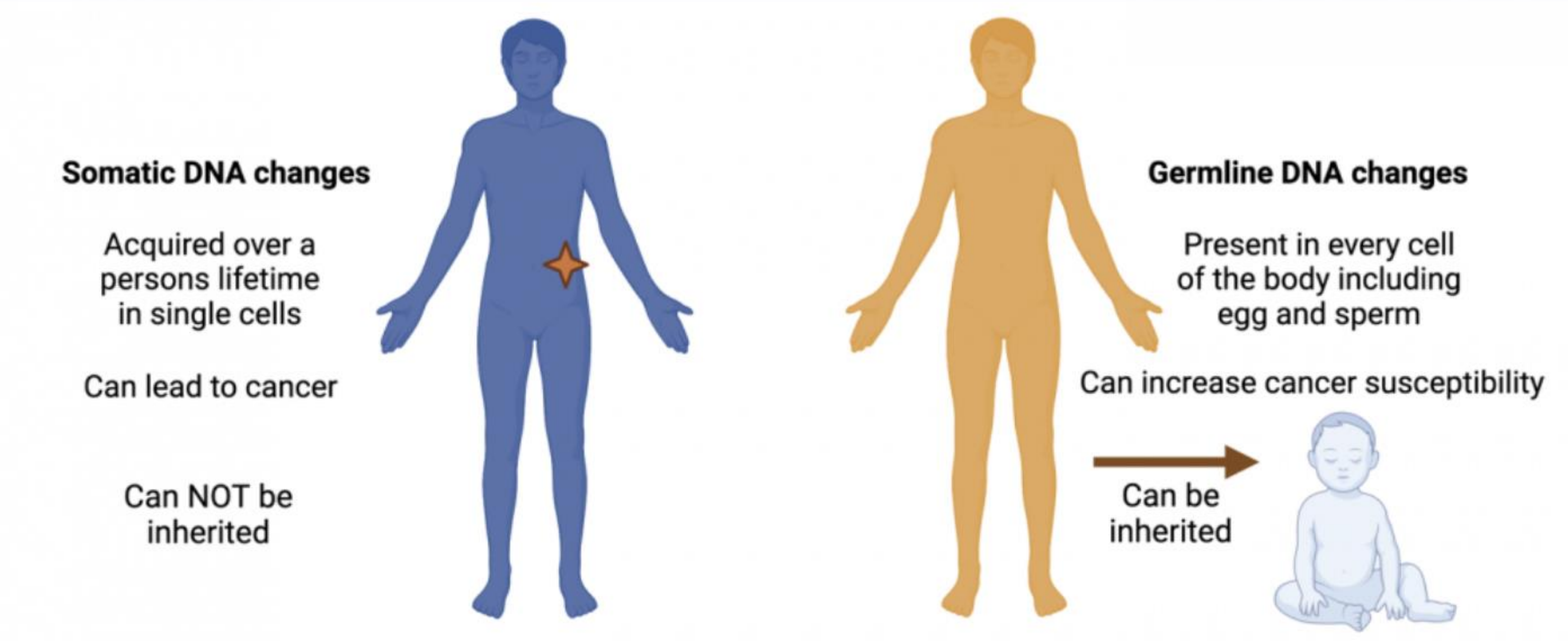
BRAF

GCT ACA GAG AAA TCT

GCT ACA GTG AAA TCT

BRAF-V600E

Mutations



Somatic DNA changes

Acquired over a persons lifetime in single cells

Can lead to cancer

Can NOT be inherited

Germline DNA changes

Present in every cell of the body including egg and sperm

Can increase cancer susceptibility

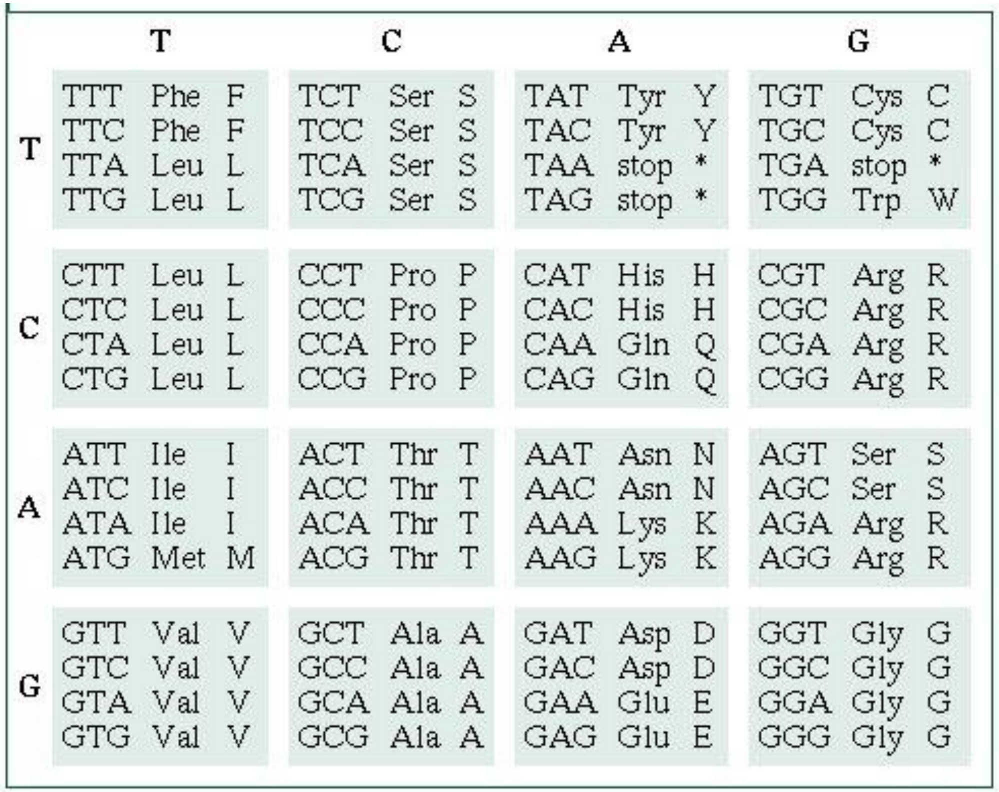
Can be inherited

How can we find mutations?

How can we find somatic mutations?

Somatic Mutations have the same properties of germline mutations

- Single nucleotide changes
  - Missense Mutations
    - GAG = Valine
    - GTG = Glutamate
  - Silent Mutations
    - TCT = Serine
    - TCC = Serine
  - Nonsense Mutations
    - TAC = Tyrosine
    - TAG = **Stop Codon!**



	T	C	A	G
T	TTT Phe F	TCT Ser S	TAT Tyr Y	TGT Cys C
	TTC Phe F	TCC Ser S	TAC Tyr Y	TGC Cys C
	TTA Leu L	TCA Ser S	TAA stop *	TGA stop *
	TTG Leu L	TCG Ser S	TAG stop *	TGG Trp W
C	CTT Leu L	CCT Pro P	CAT His H	CGT Arg R
	CTC Leu L	CCC Pro P	CAC His H	CGC Arg R
	CTA Leu L	CCA Pro P	CAA Gln Q	CGA Arg R
	CTG Leu L	CCG Pro P	CAG Gln Q	CGG Arg R
A	ATT Ile I	ACT Thr T	AAT Asn N	AGT Ser S
	ATC Ile I	ACC Thr T	AAC Asn N	AGC Ser S
	ATA Ile I	ACA Thr T	AAA Lys K	AGA Arg R
	ATG Met M	ACG Thr T	AAG Lys K	AGG Arg R
G	GTT Val V	GCT Ala A	GAT Asp D	GGT Gly G
	GTC Val V	GCC Ala A	GAC Asp D	GGC Gly G
	GTA Val V	GCA Ala A	GAA Glu E	GGA Gly G
	GTG Val V	GCG Ala A	GAG Glu E	GGG Gly G

Somatic Mutations

- **Frame-shift mutations:** insertion or deletion that change the reading frame
- **Deletion:** deletion of 1 or more nucleotides

ACC	AGC	TGC	ACT
Thr	Ser	Cys	Thr

ACC	AGC	TGA	CT
Thr	Ser	**stop**	

- **Insertion**: Addition 1 or more extra-nucleotides

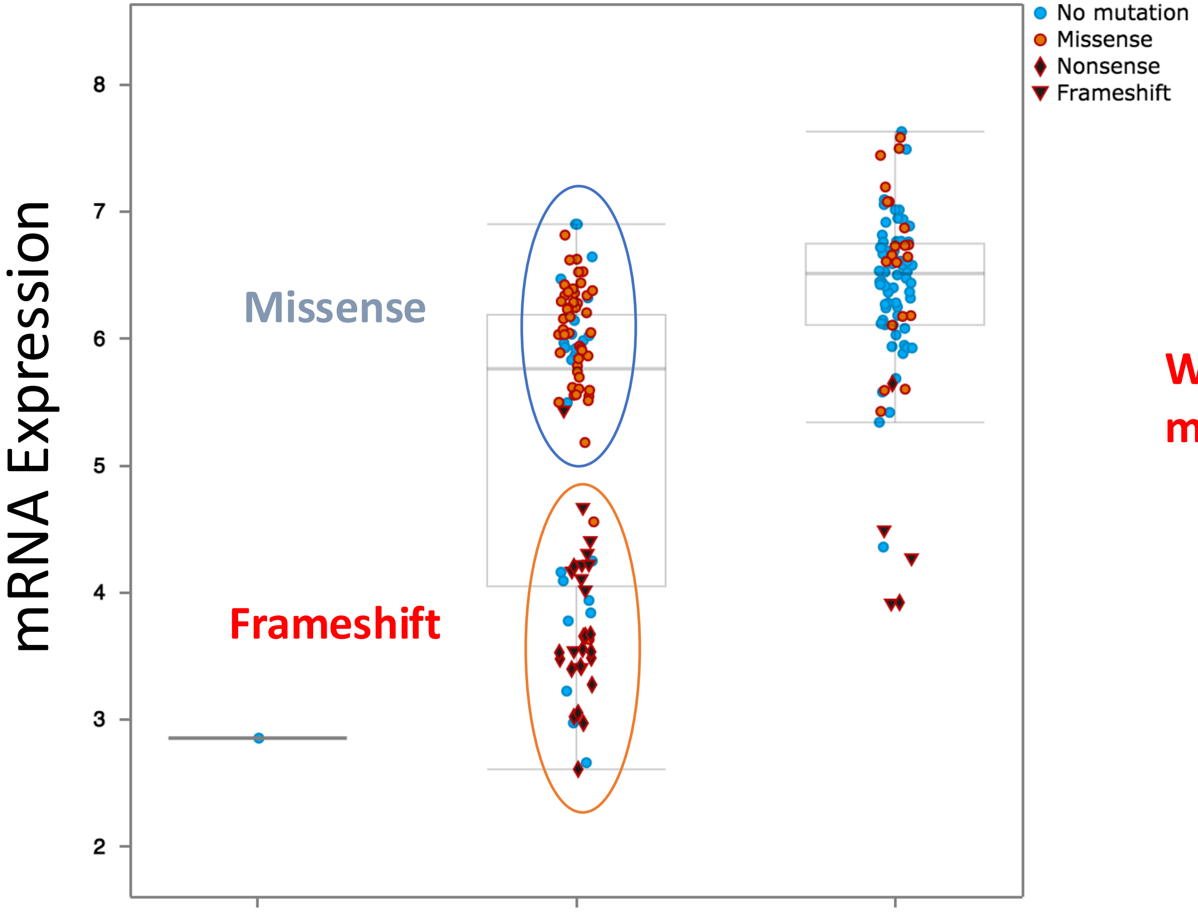
ACC	AGC	TGC	ACT
Thr	Ser	Cys	Thr

ACC	AGC	TGC	CAC	CT
Thr	Ser	Cys	His	

Truncating Mutations

(inactivating a **tumor suppressor**)

- **TP53 mutations in Colorectal cancer patient**



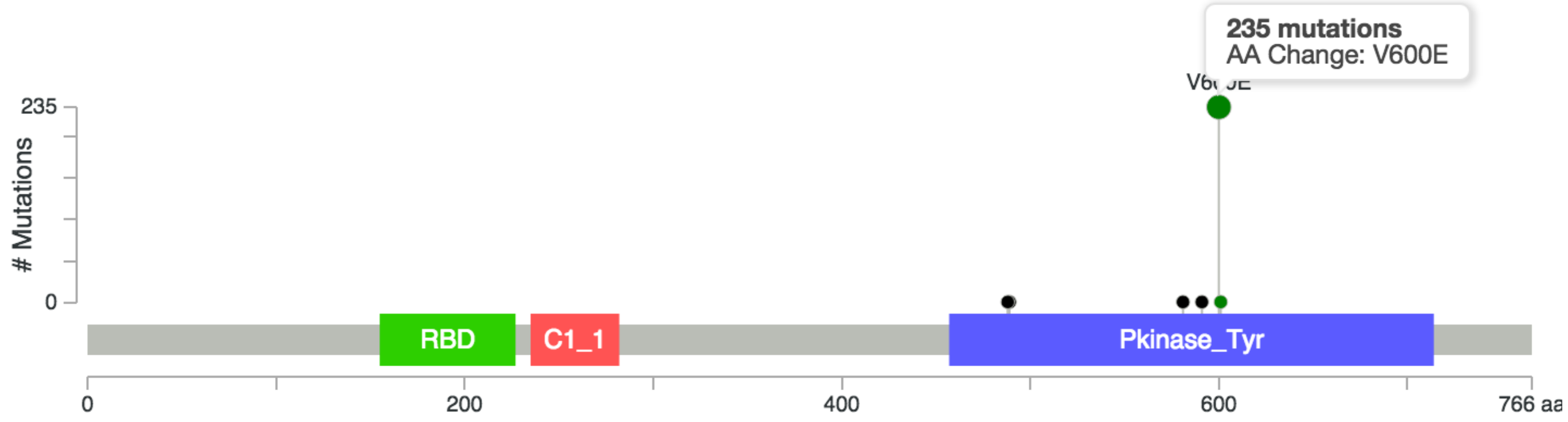
Why this difference between missense and frameshifts?

HOTSPOT mutations

(activating an **oncogene**)

BRAF V600E mutations in Thyroid Carcinoma (399 patients)

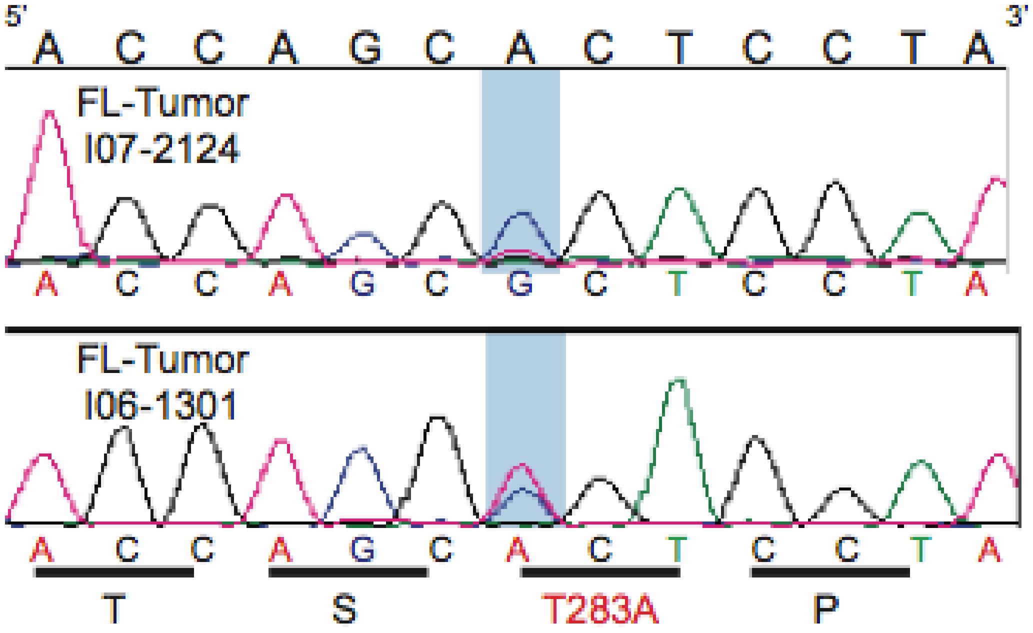
GTG = Valine (V)
GAG = Glutamate (E)

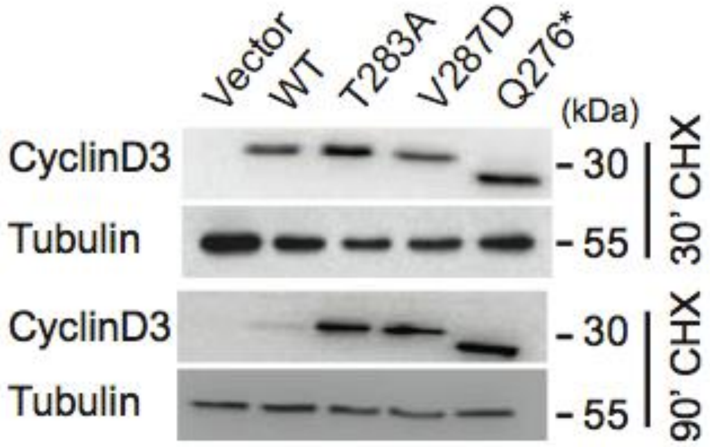


Truncating Mutations

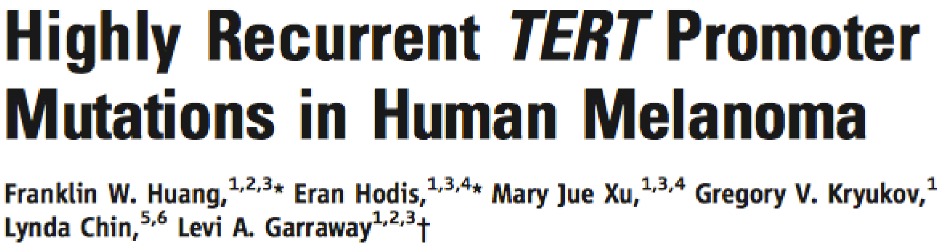
(activating an oncogene)

In Lymphoma mutation in CyclinD3 occurs in ~10% of the cases





Non-coding Mutations



Highly Recurrent *TERT* Promoter Mutations in Human Melanoma

Franklin W. Huang,[1,2,3]* Eran Hodis,[1,3,4]* Mary Jue Xu,[1,3,4] Gregory V. Kryukov,[1]
Lynda Chin,[5,6] Levi A. Garraway[1,2,3]†

(Science, 2013)

TERT promoter

C228T

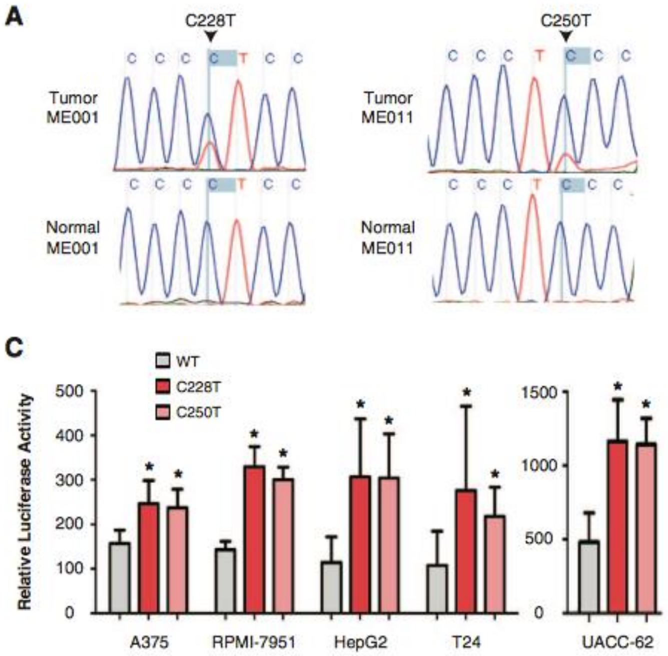
+1

CCCCTTCCGGG
GGGGAAGGCCC

C250T

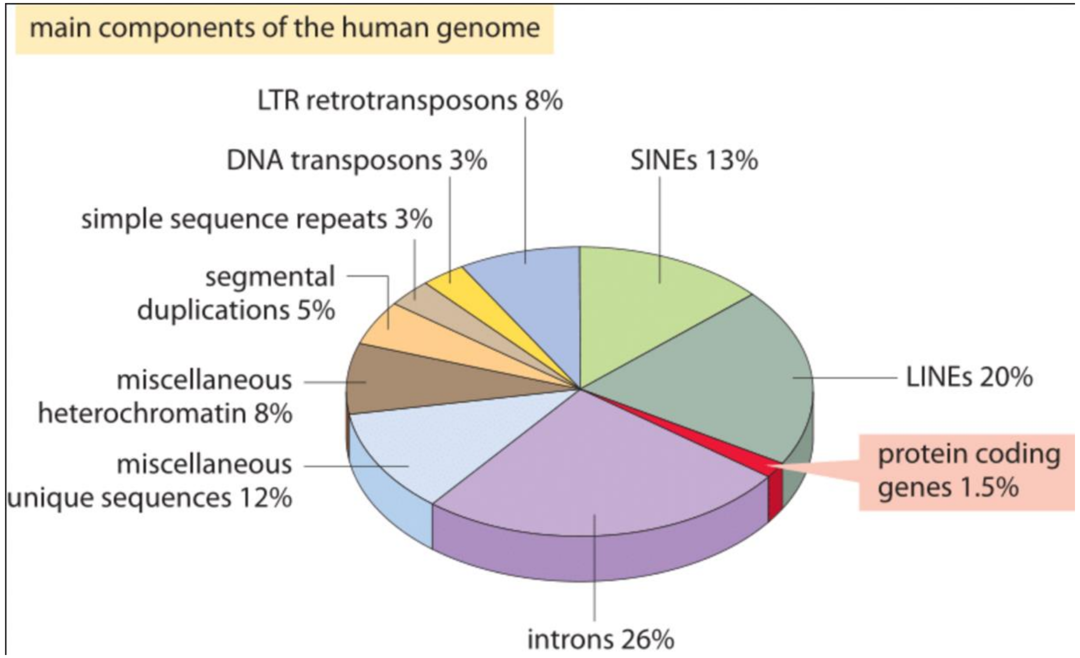
+1

CCCCTTCCGGG
GGGGAAGGCCC



What should we sequence?

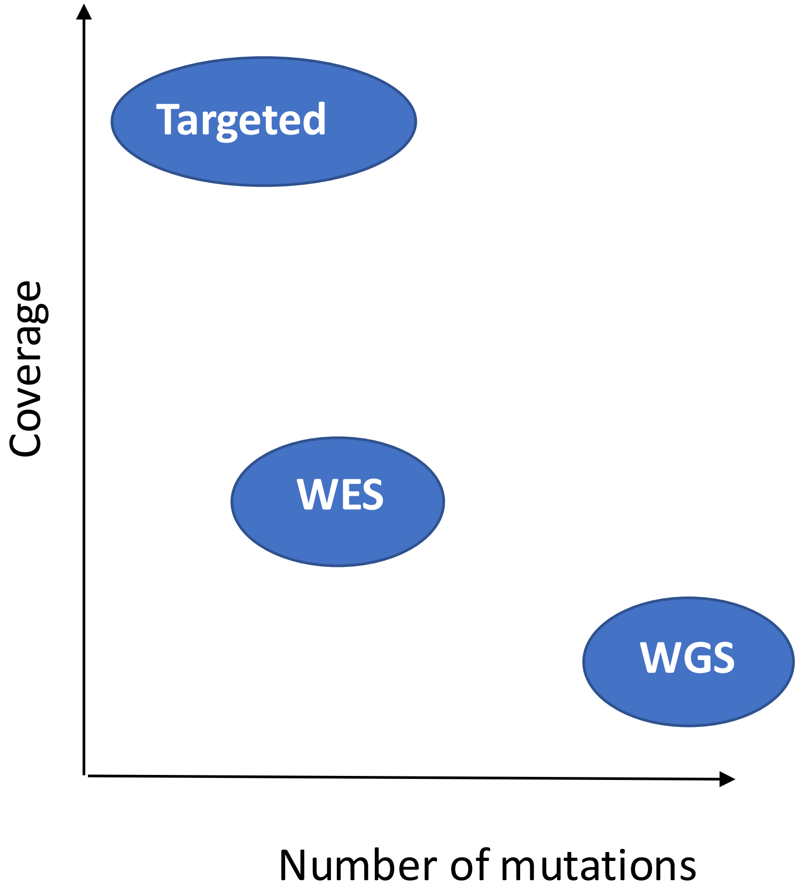
How much should we sequence?



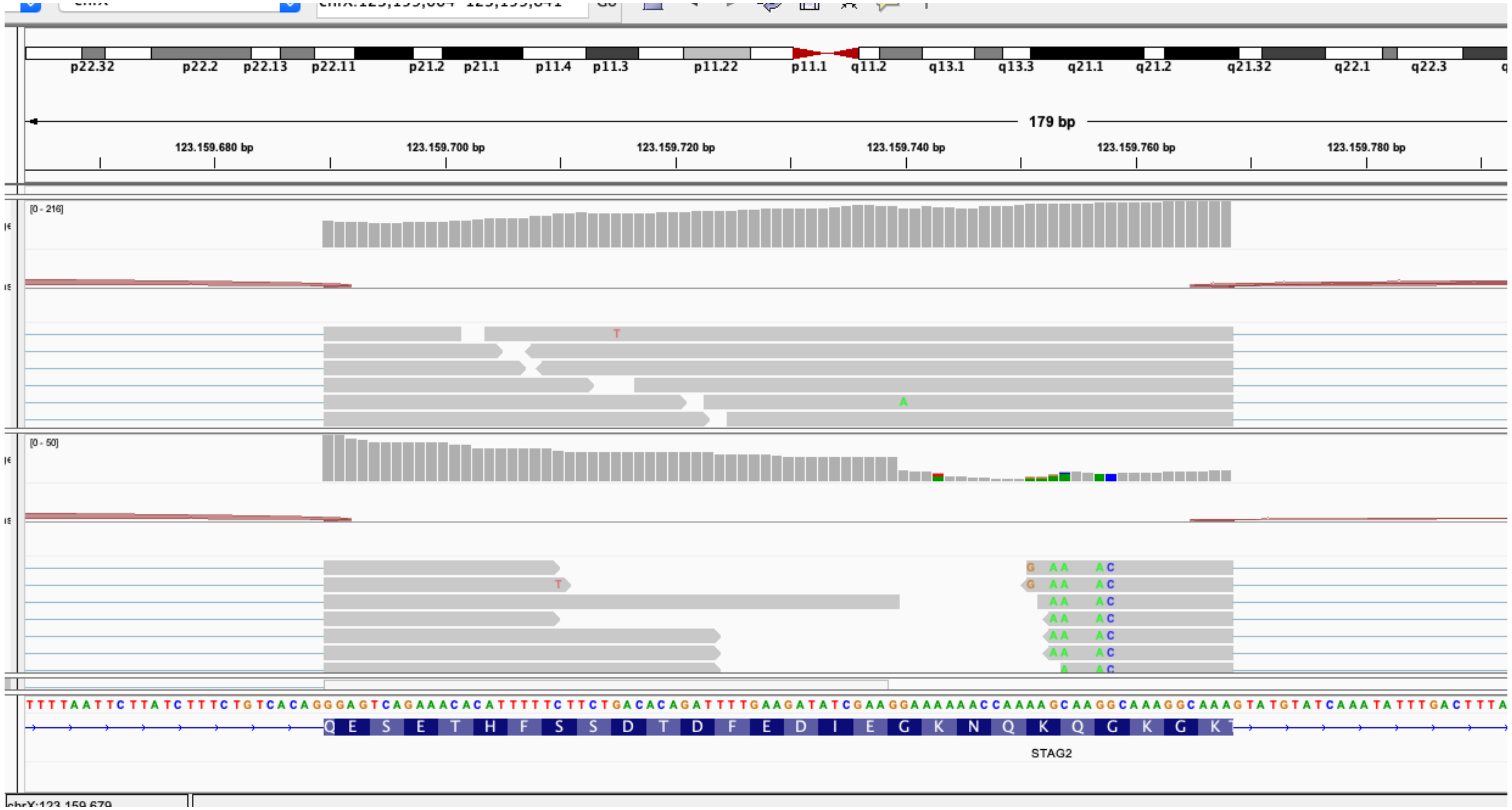
Cancer Genomics

- Sequencing the blood and tumor biopsy
- **Targeted sequencing –** select a limited number of genes that will be sequenced (e.g. the most frequent mutated genes)
- **Whole-exome sequencing – sequence the coding genome**
- **Whole-genome sequencing- sequence coding and non-coding regions**

Sequencing a tumor



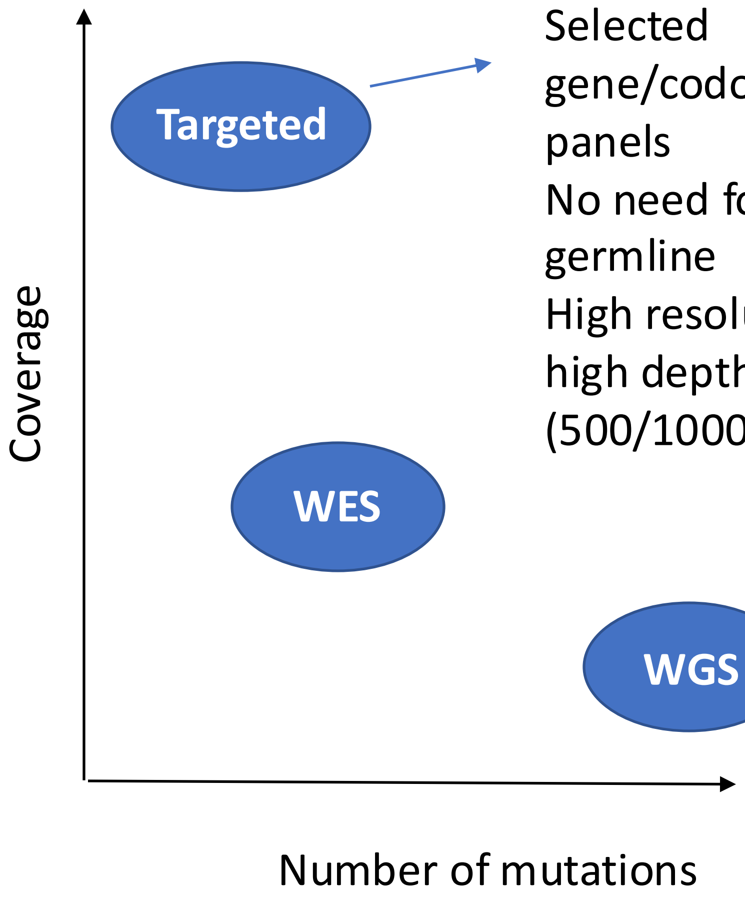
Coverage versus number of mutations



Wild-type

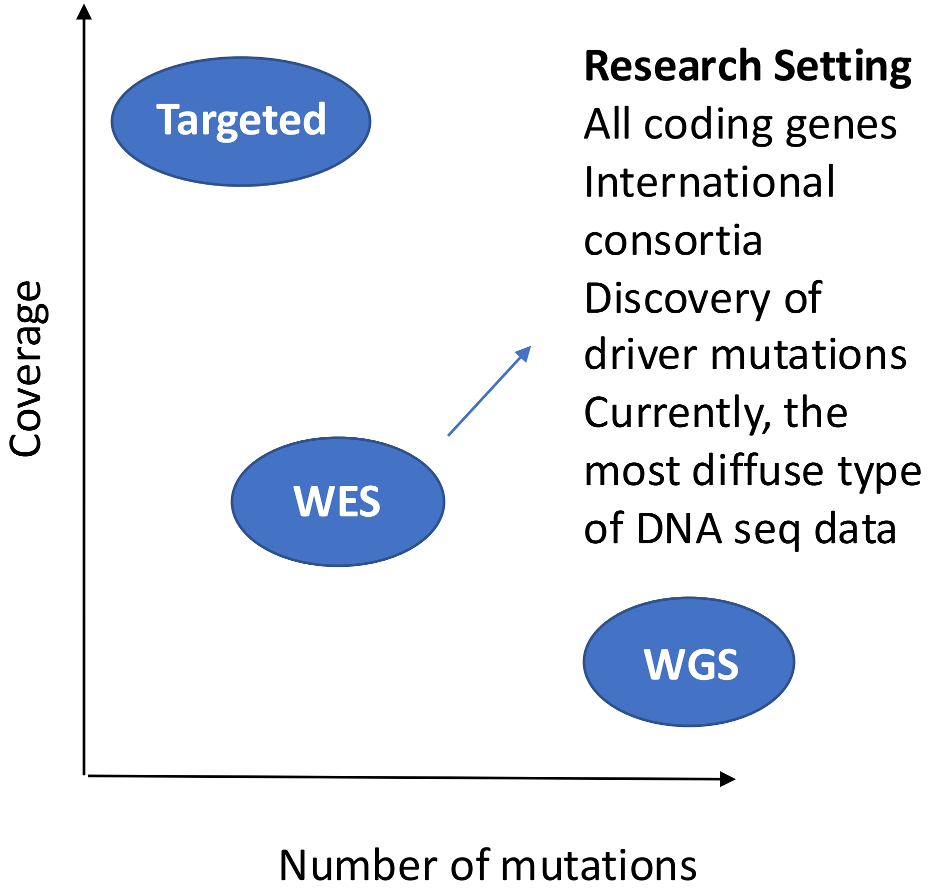
Mutated

Targeted Sequencing



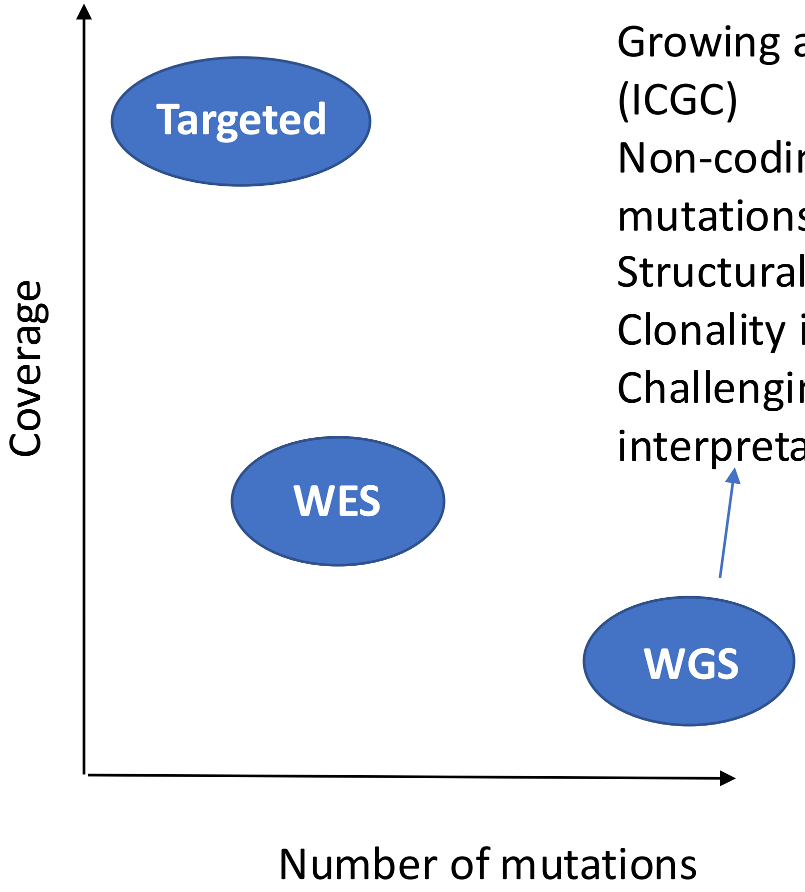
Clinical Setting
Selected gene/codon panels
No need for germline
High resolution / high depth (500/1000x)

Whole Exome Sequencing



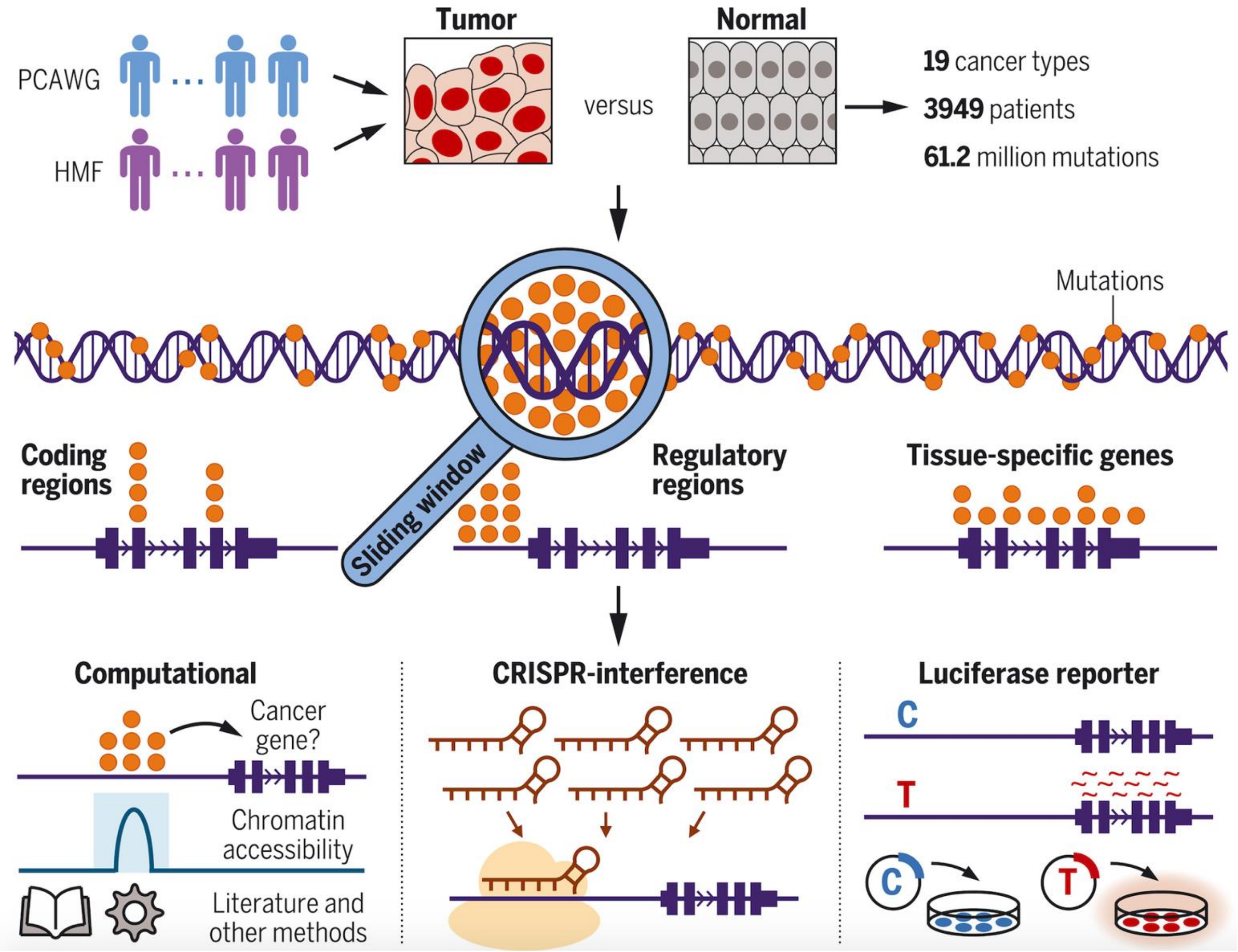
Research Setting
All coding genes
International consortia
Discovery of driver mutations
Currently, the most diffuse type of DNA seq data

Whole genome sequencing



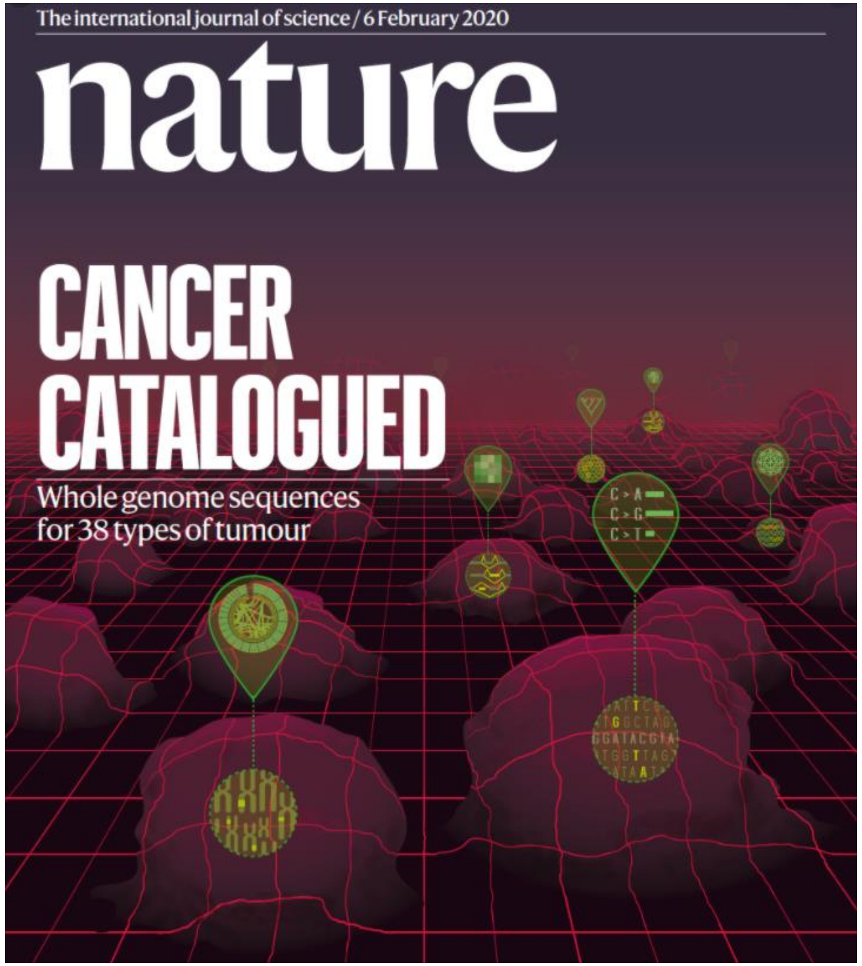
Research Setting
Growing availability (ICGC)
Non-coding mutations
Structural variants
Clonality inference
Challenging interpretability..

Non-coding Mutations



(Dietlein et al. Science 2022)

Pan-Cancer Analyses of Whole Genomes

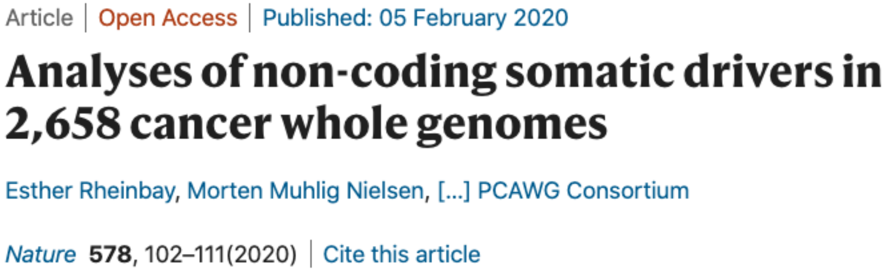


The international journal of science / 6 February 2020

nature

CANCER CATALOGUED

Whole genome sequences for 38 types of tumour



Article | Open Access | Published: 05 February 2020

Analyses of non-coding somatic drivers in 2,658 cancer whole genomes

Esther Rheinbay, Morten Muhlig Nielsen, [...] PCAWG Consortium

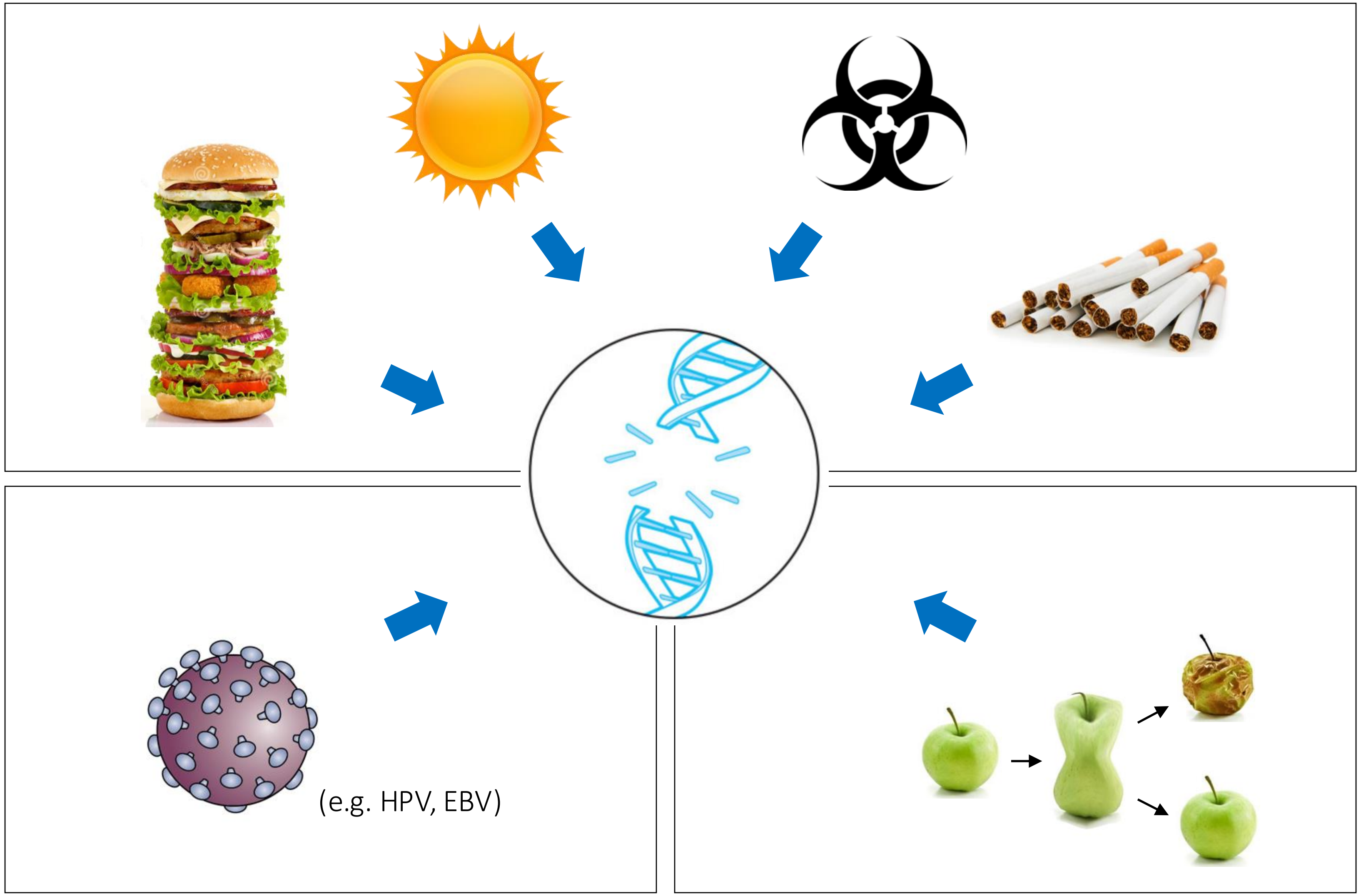
Nature **578**, 102–111(2020) | Cite this article

Paucity of non-coding drivers in cancer

Why?

How do mutations emerge?

How do *mutations* emerge?



Exogenous mutagens

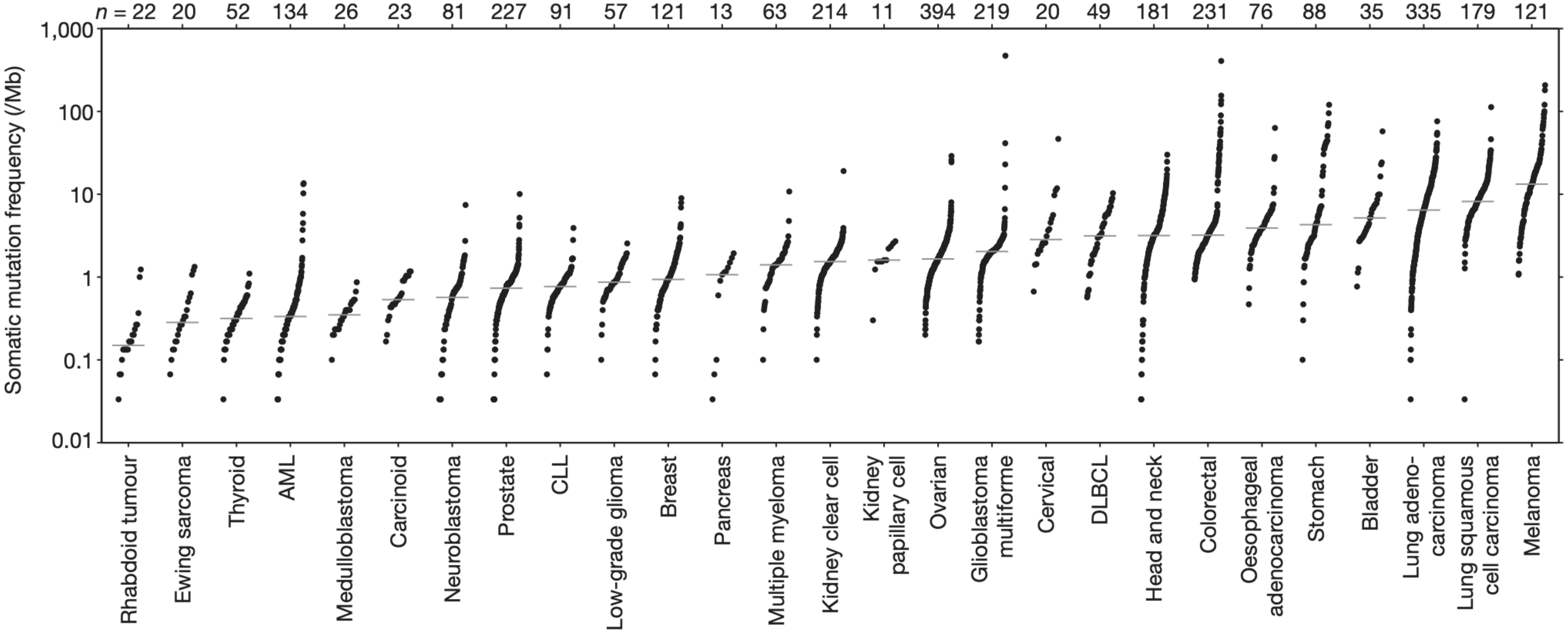
Viral infection

(e.g. HPV, EBV)

Unrepaired Replication Errors

What do you get after sequencing 1000 tumors?

of mutations per Mb

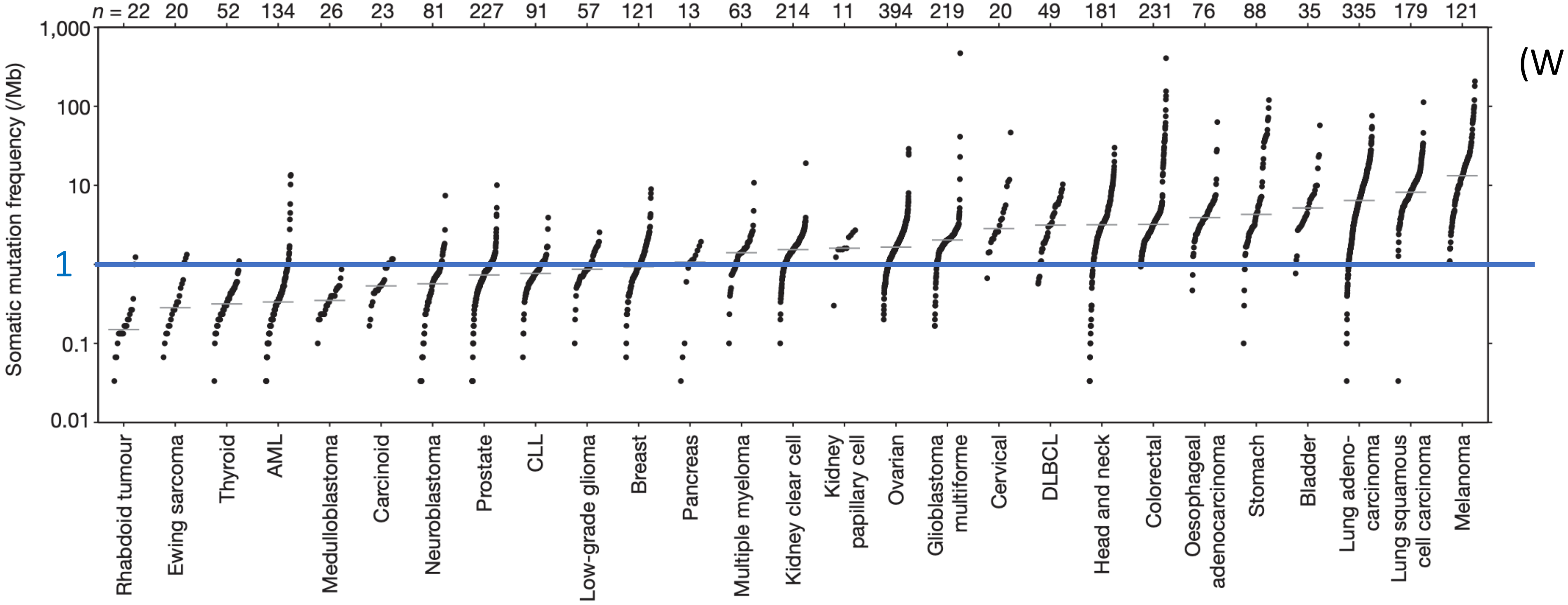


(Whole <u>exome</u> seq.)

(Lawrence et al, Nature 2013)

What do you get after sequencing 1000 tumors?

of
mutations
per Mb



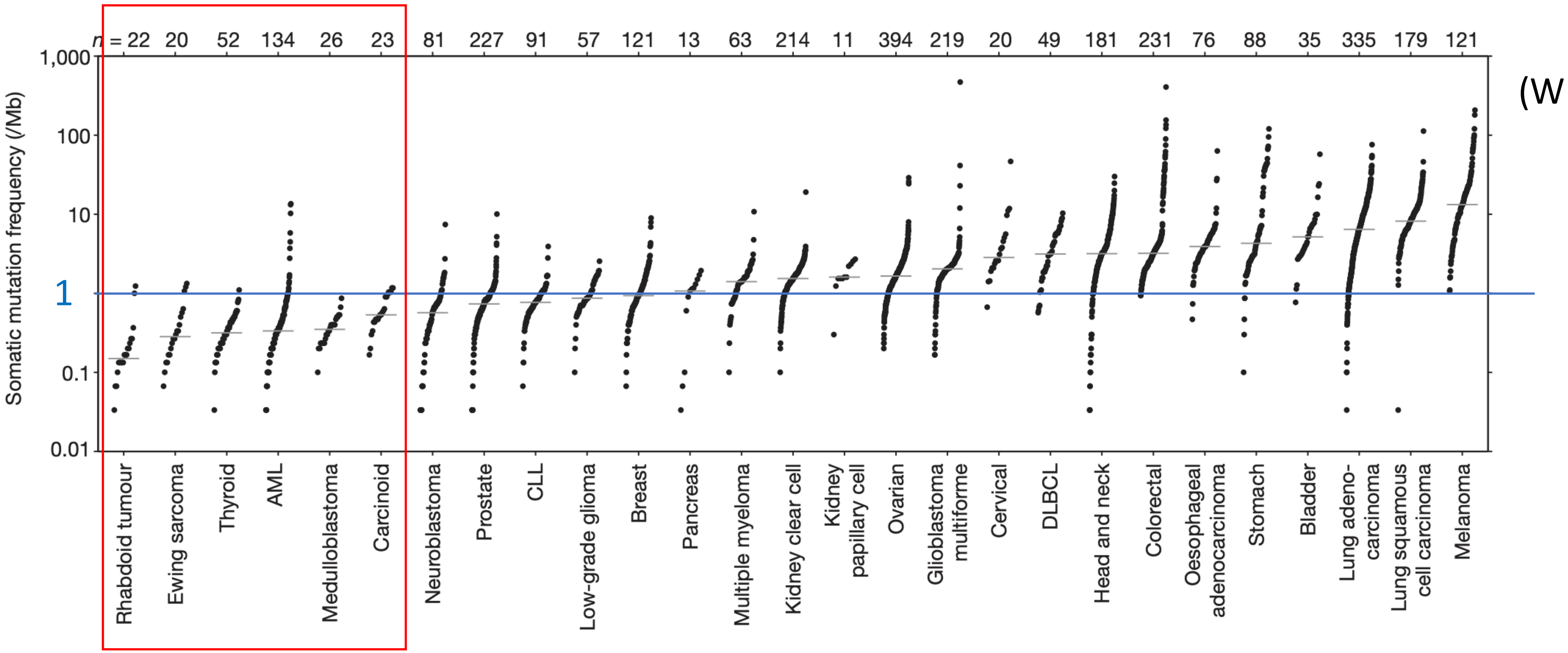
(WES)

1 → 1/Mb * 3000Mb ~ 3000 mutations
2% are coding sequences → ~60 mutations in gene sequences

(Lawrence et al, Nature 2013)

What do you get after all this sequencing?

Young patients / pediatric diseases



(WES)

# of mutations per Mb

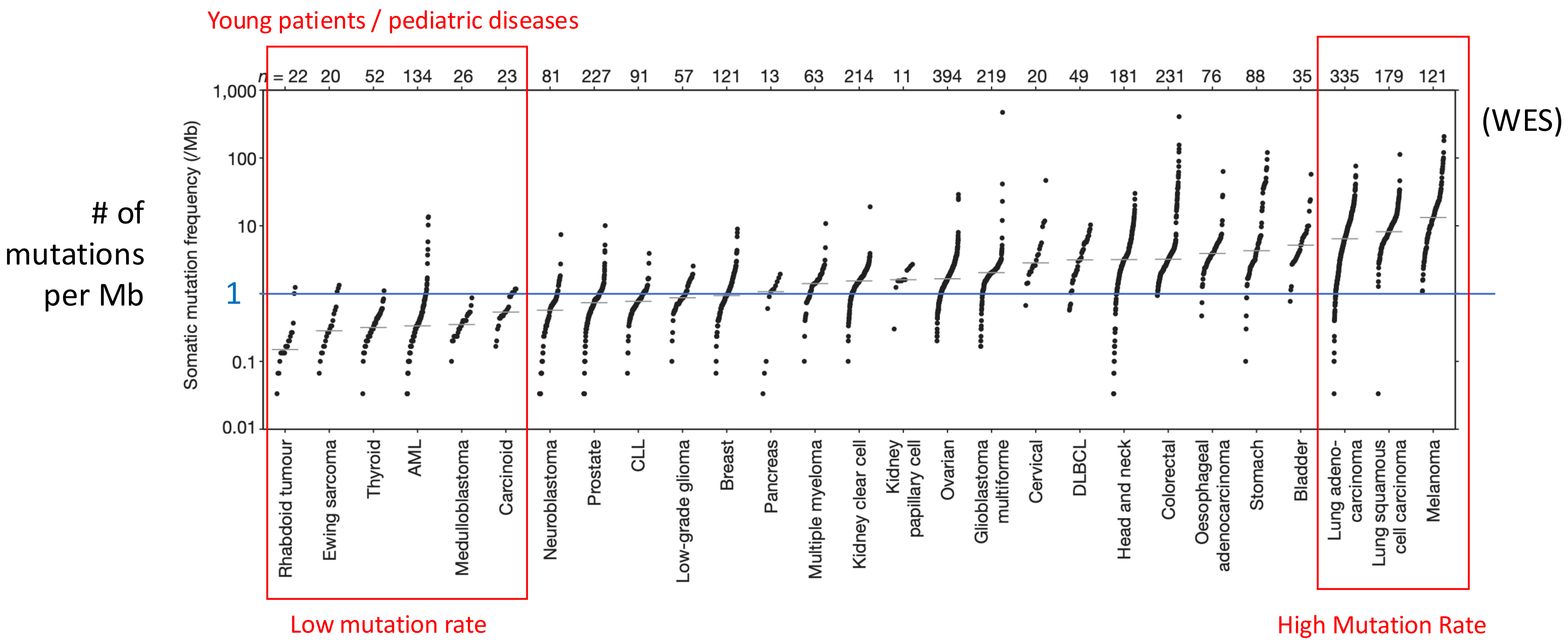
Low mutation rate

1 → 1/Mb * 3000Mb ~ 3000 mutations

2% are coding sequences → ~60 mutations in gene sequences

(Lawrence et al, Nature 2013)

What do you get after all this sequencing?



Why a difference between tumor that develops in children vs the one that develop in adults?

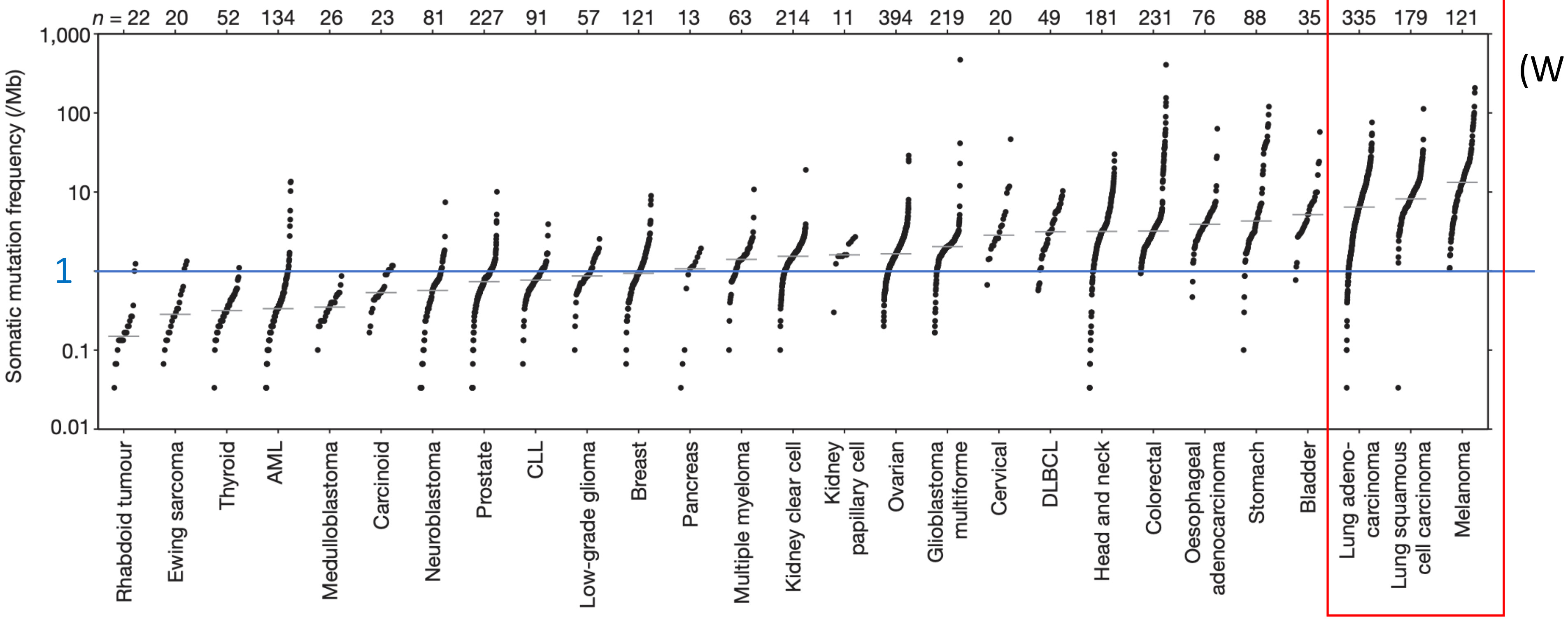
(Lawrence et al, Nature 2013)

What do you get after all this sequencing?


Exogenous Mutagens

# of mutations per Mb

1

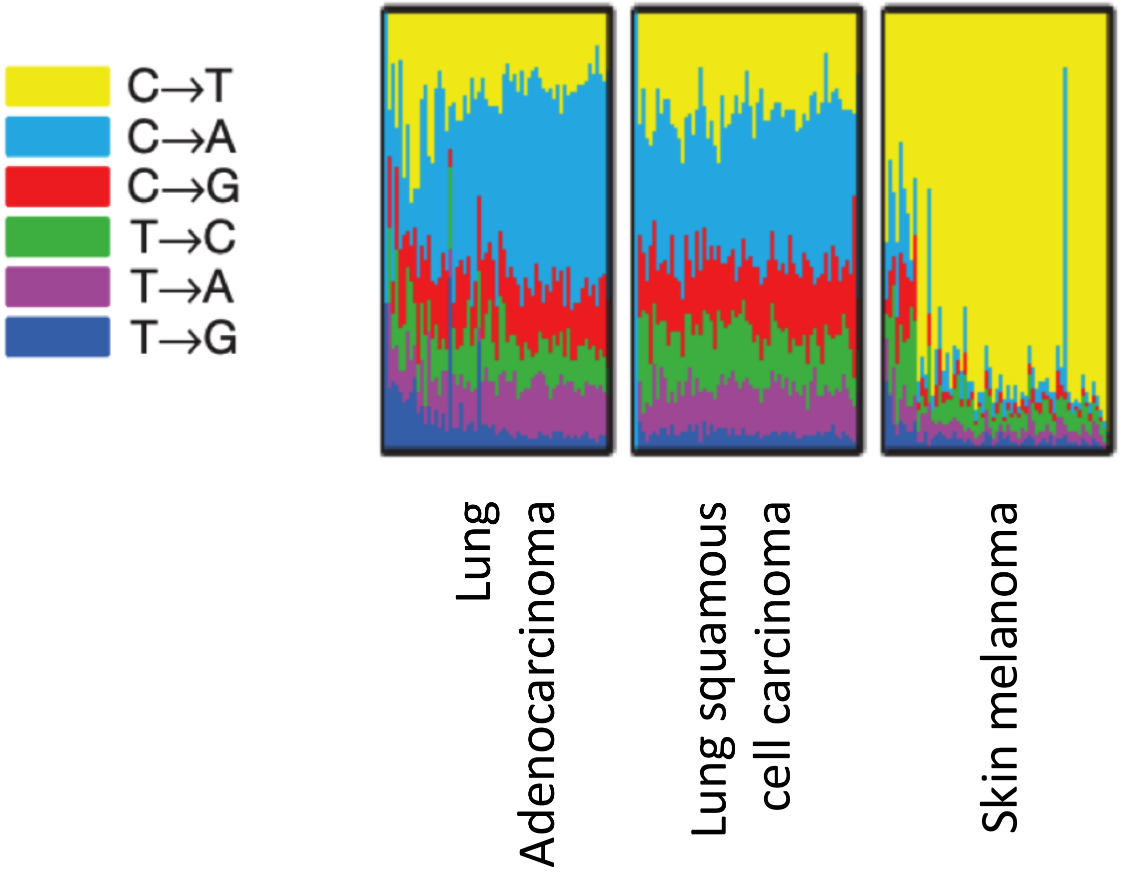


(WES)

High Mutation Rate

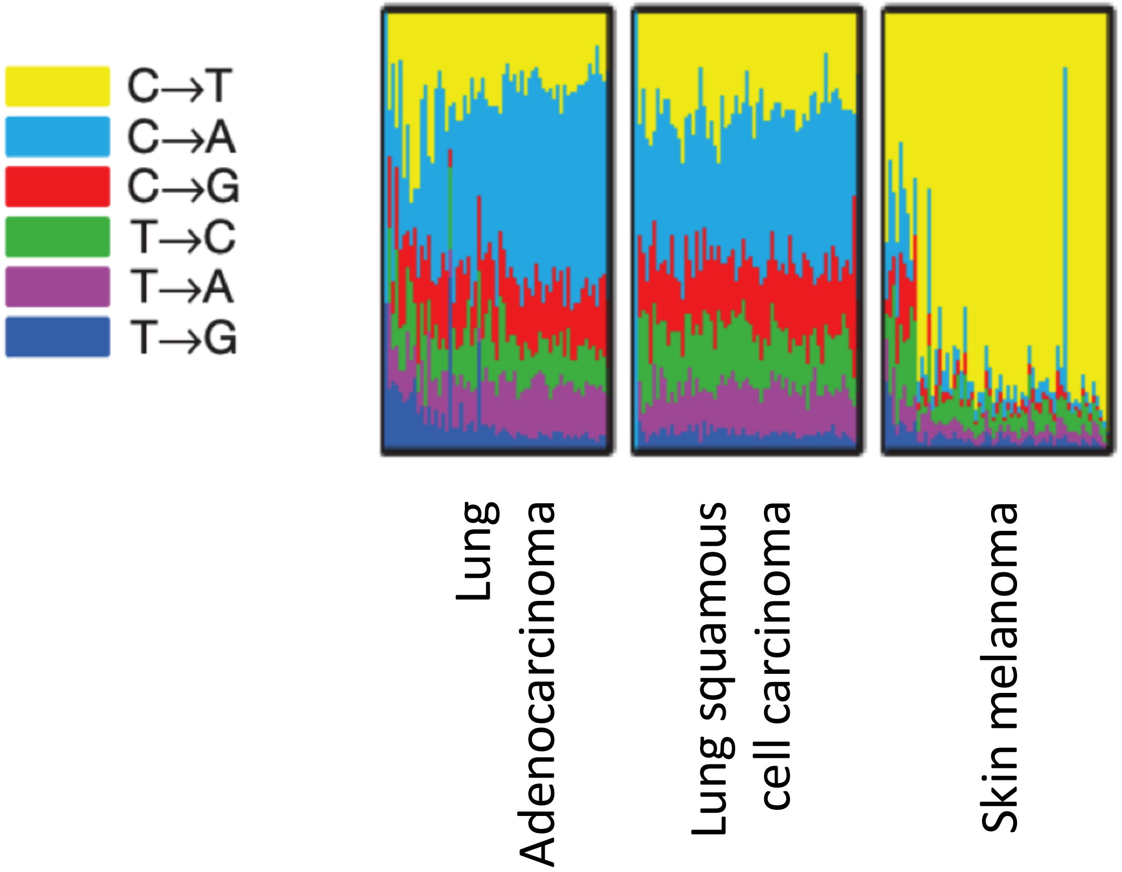
(Lawrence et al, Nature 2013)

Mutational Signatures



(Lawrence et al, Nature 2013)

Mutational Signatures



➡ C → A

➡ C → T

(Lawrence et al, Nature 2013)

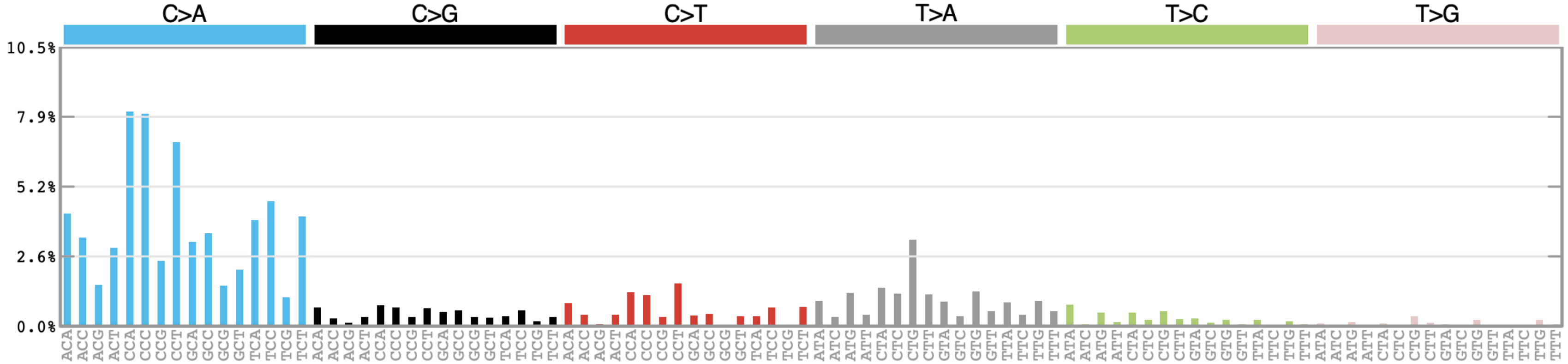
Mutational Signatures

C > A

Which nucleotide is before the mutated one

? C ?
>
? A ?

Which nucleotide is after the mutated one



Mutational Signatures

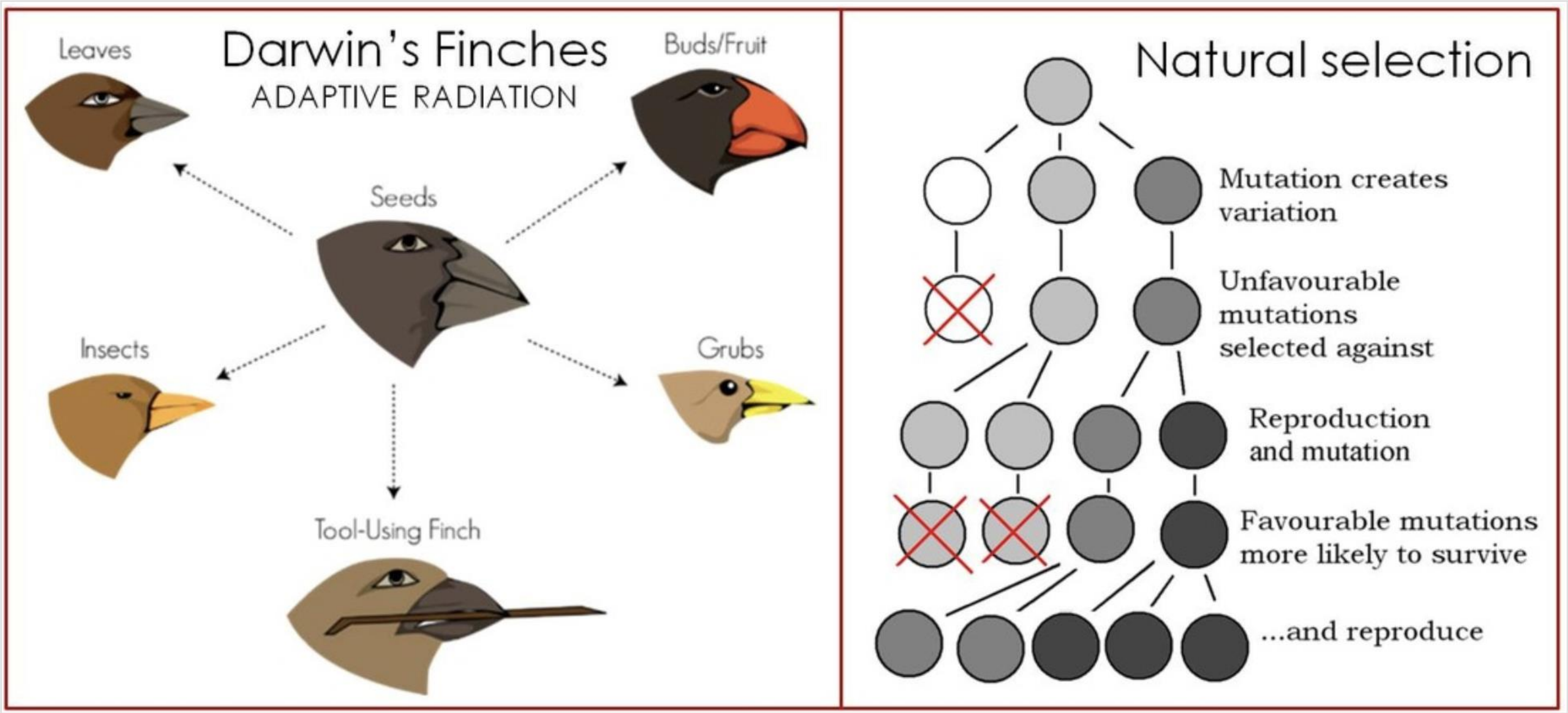
Spontaneous De-amination
(defective base excision repair)

Tobacco smoking

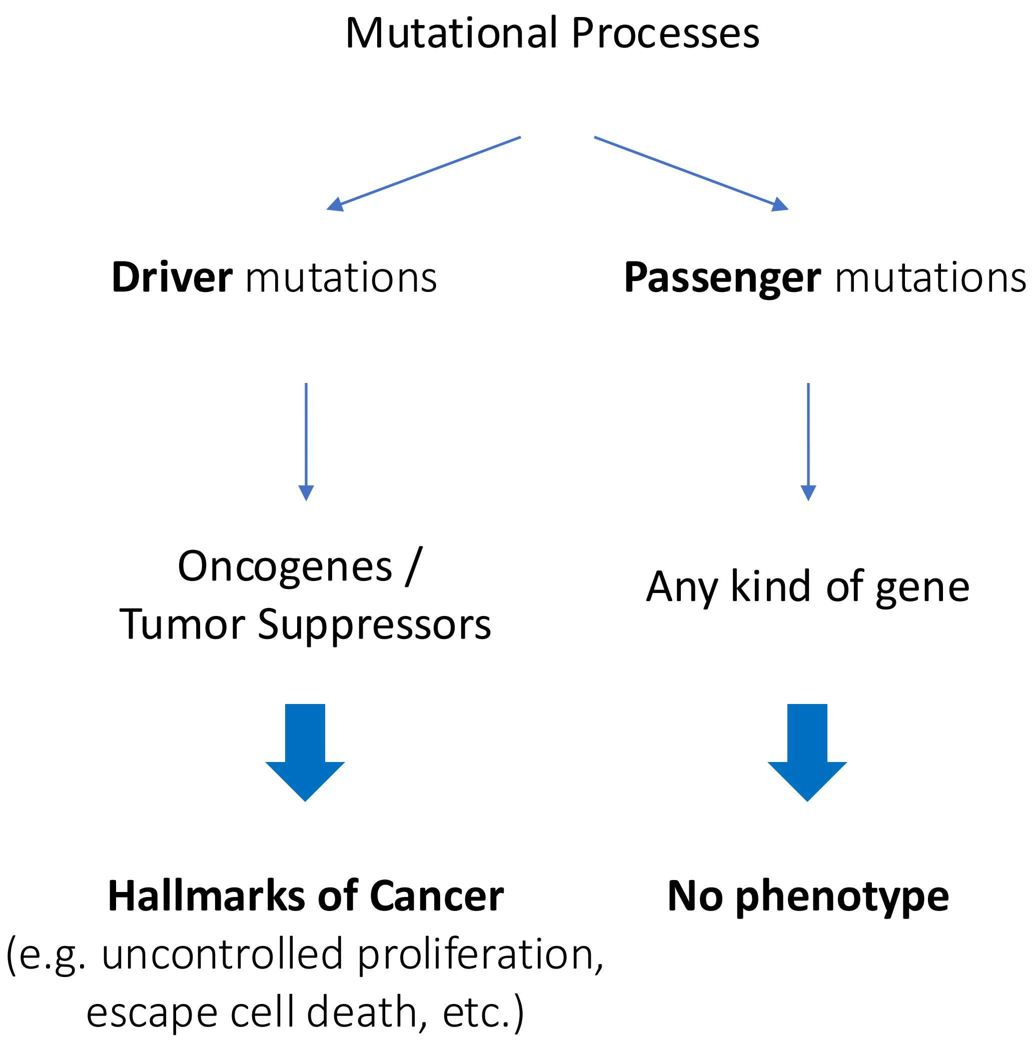
(Alexandrov et al., Nature 2013)

Are all mutations bad, and do they all cause cancer?

Mutation in species evolution



Driver vs Passenger mutations



Mutational Processes

Driver mutations

Oncogenes /
Tumor Suppressors

Hallmarks of Cancer
(e.g. uncontrolled proliferation,
escape cell death, etc.)

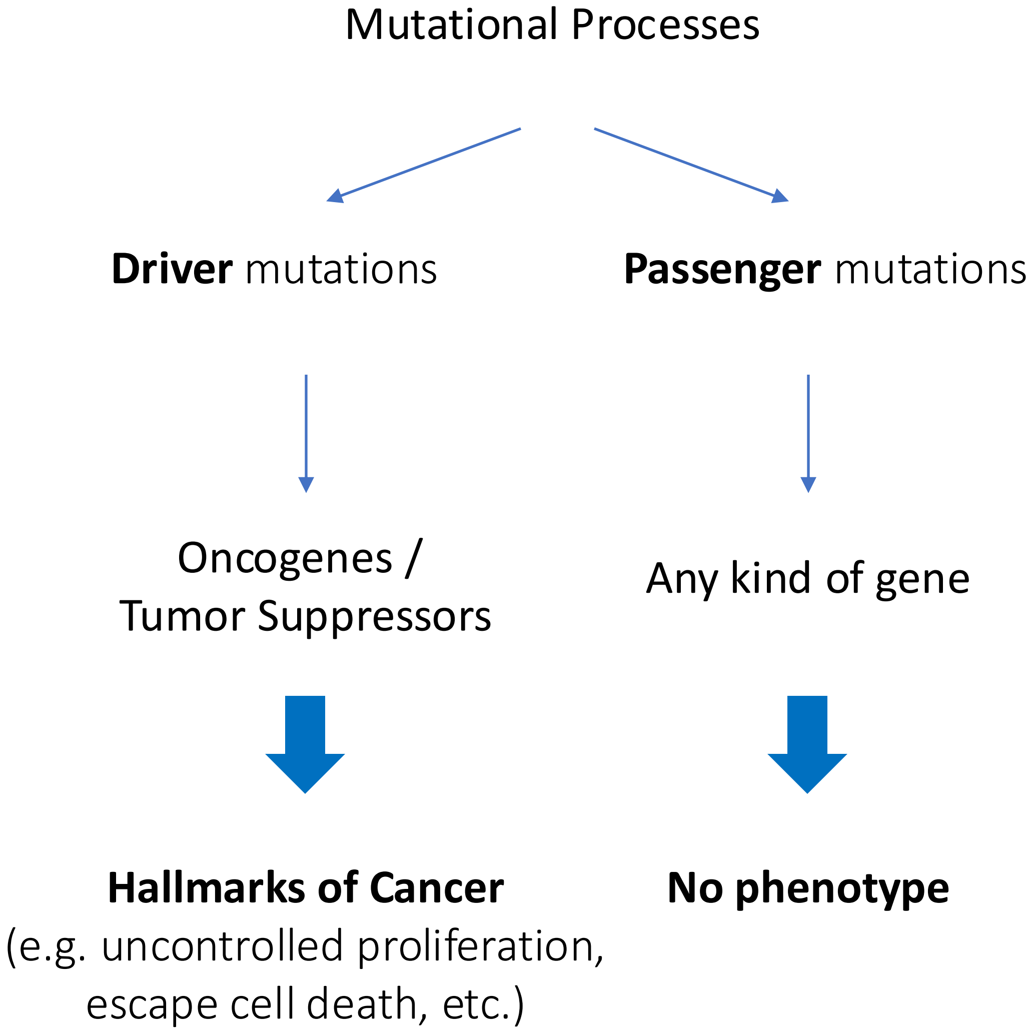
Passenger mutations

Any kind of gene

No phenotype

If you have a driver, usually
you also have a passenger

Driver vs Passenger mutations



Mutational Processes

Driver mutations

Passenger mutations

Oncogenes /
Tumor Suppressors

Any kind of gene

Hallmarks of Cancer
(e.g. uncontrolled proliferation,
escape cell death, etc.)

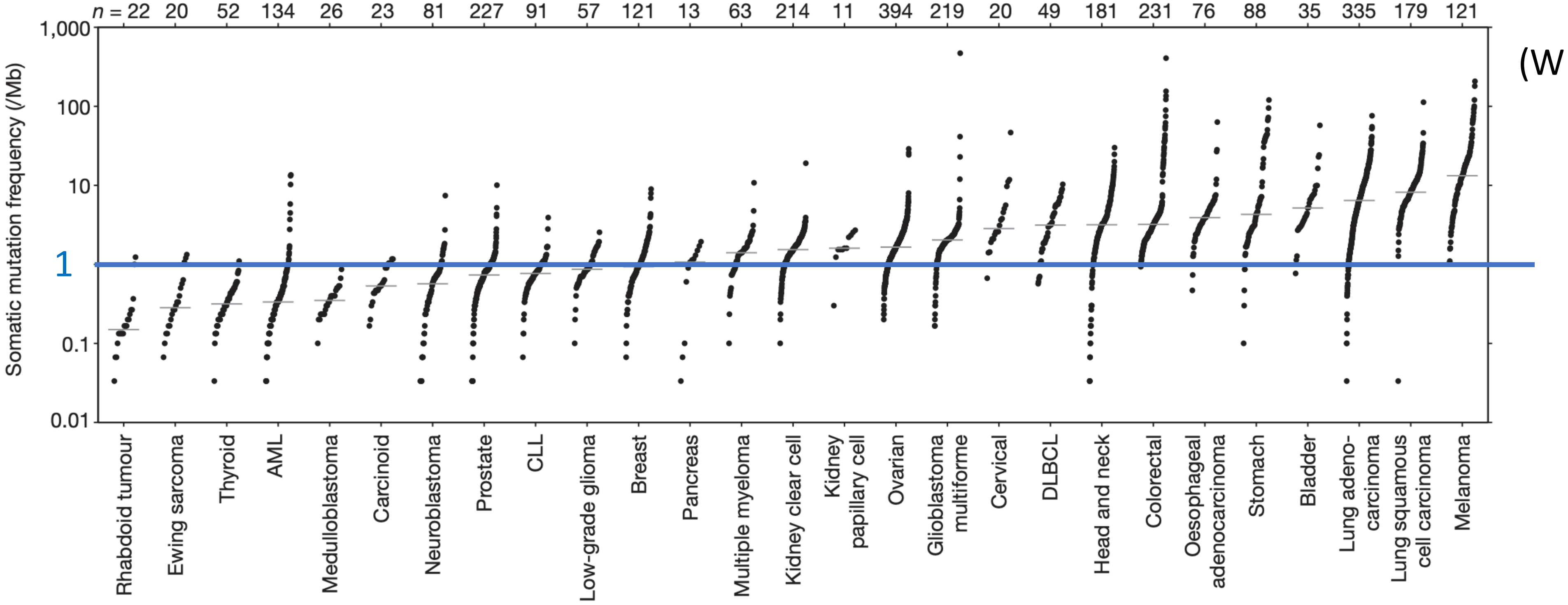
No phenotype

Often you have many more
passengers than drivers!

What do you get after sequencing 1000 tumors?

of
mutations
per Mb

1

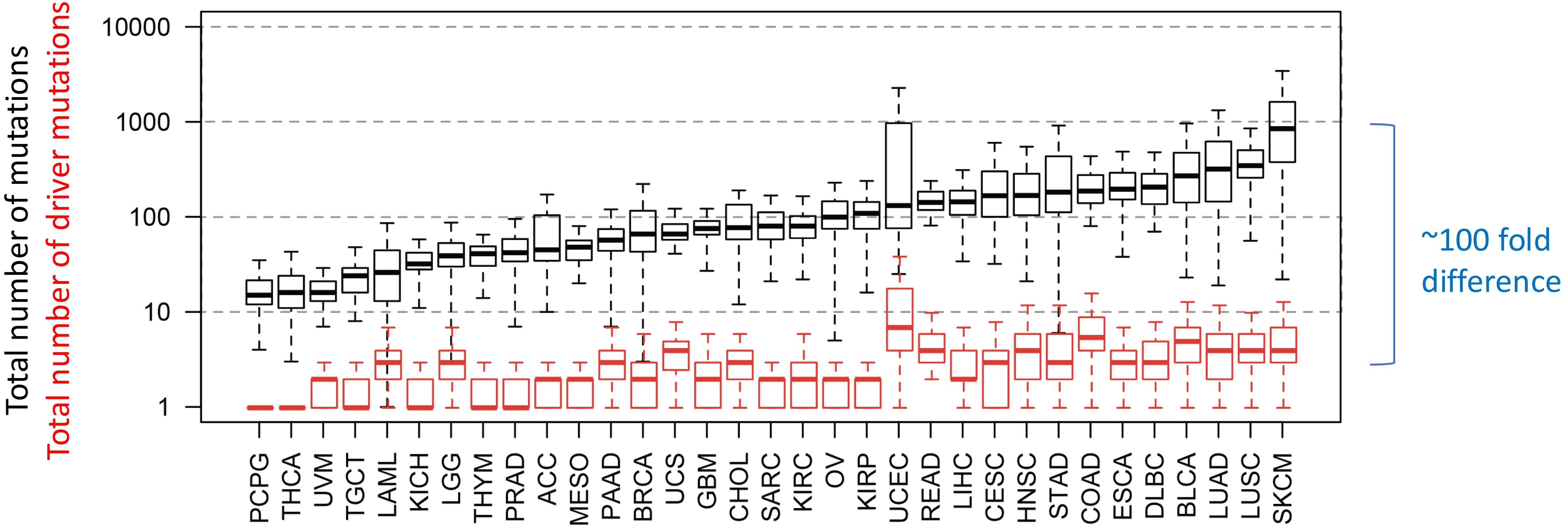


(WES)

1 → 1/Mb * 3000Mb ~ 3000 mutations
2% are coding sequences → ~60 mutations in gene sequences

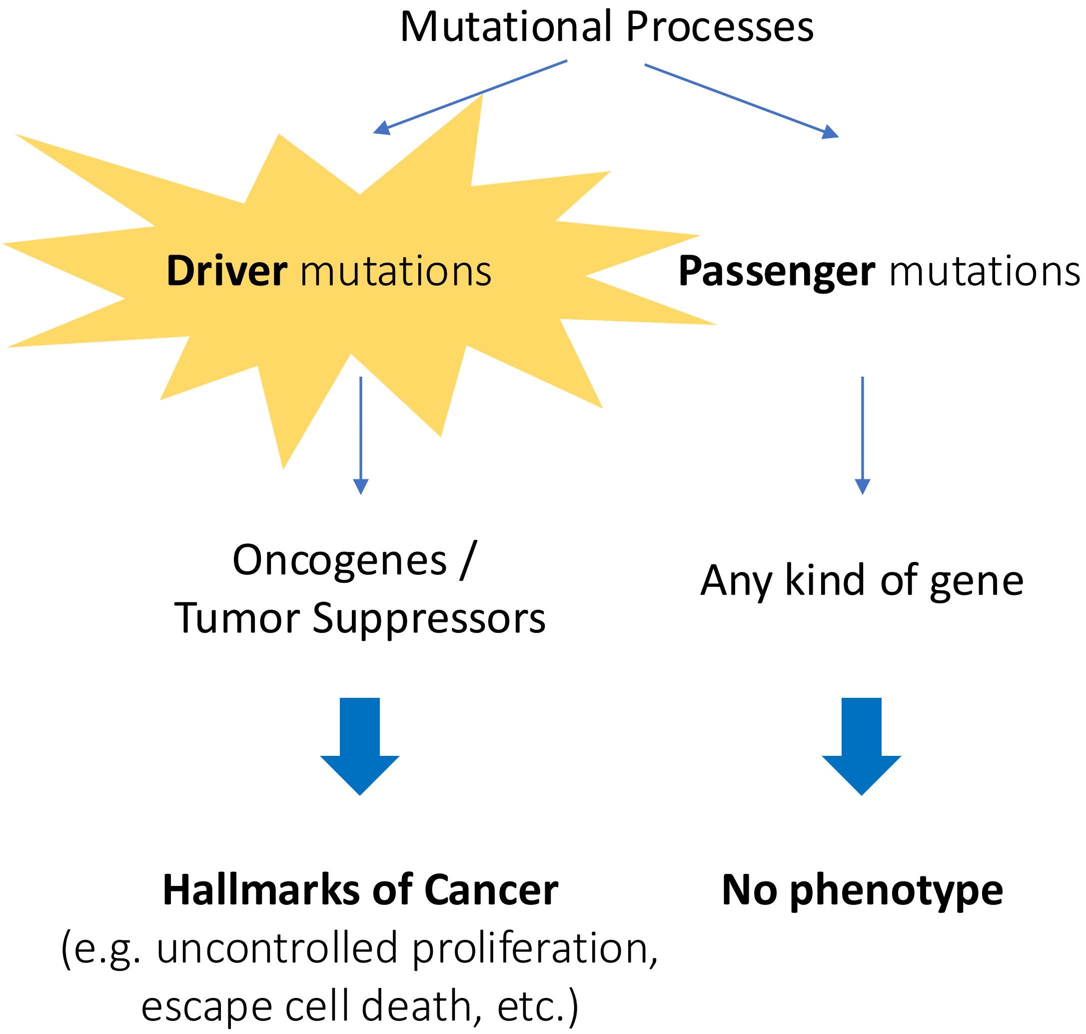
(Lawrence et al, Nature 2013)

Driver vs. passenger alterations



33 Tumor types, ~8,000 human tumors

Driver vs Passenger mutations



Mutational Processes

Driver mutations

Passenger mutations

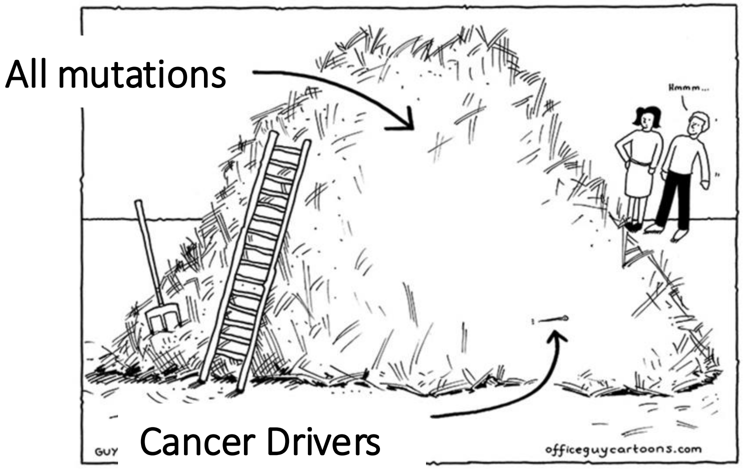
Oncogenes /
Tumor Suppressors

Any kind of gene

Hallmarks of Cancer
(e.g. uncontrolled proliferation,
escape cell death, etc.)

No phenotype

How do we identify cancer drivers?



How do we identify driver mutations?

- “driver alterations” vs. “passenger alterations”

- Design an experiment to prove a given alteration can induce cancer
  - Cell lines / Mouse models / tumor organoids / etc.

How do we identify driver mutations?

- “driver alterations” vs. “passenger alterations”

- Design an experiment to prove a given alteration can induce cancer
  - Cell lines / Mouse models / tumor organoids / etc.

- *However, it is unfeasible to test >10,000 mutations!*

In the genomics era, we need strategies to prioritize

How do we identify driver mutations?

- “driver alterations” vs. “passenger alterations”
- **Main idea**: *A cancer driver gene is a gene that is mutated more frequently than expected in a large tumor cohort*

How do we identify driver mutations?

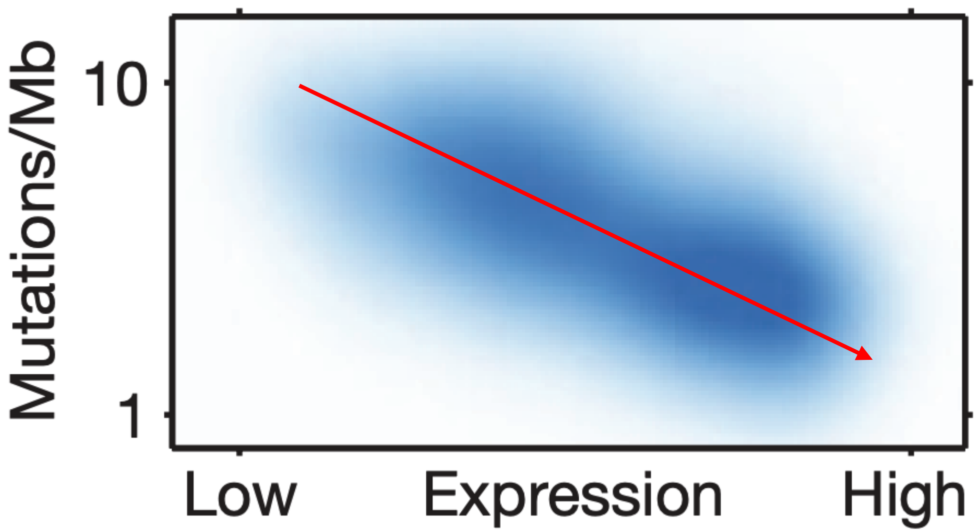
- “driver alterations” vs. “passenger alterations”

- **Main idea**: *A cancer driver gene is a gene that is mutated more frequently than expected in a large tumor cohort*

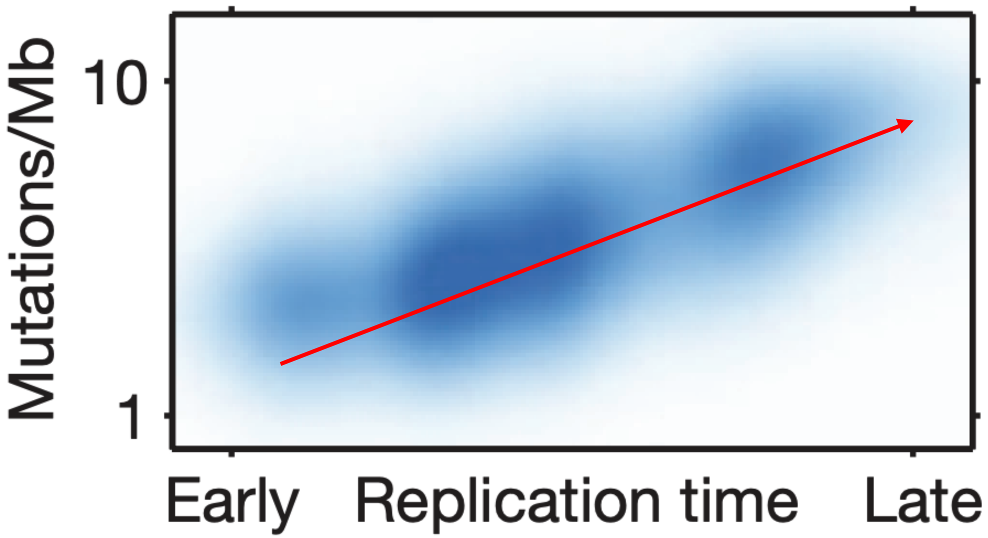
- Count the number of mutations observed in each gene in the cohort
- Determine the expected background mutation rate (BMR)
- Estimate significance of observed vs. expected number of mutations

What does influence the BMR?

Gene expression



Replication Time



What does influence the BMR?

- **Sample specific features**
  - Tissue-type
  - Impact of specific alterations

- **Underlying mutational processes**
  - (e.g. UV-light or tobacco consumption)

- **Regional genome properties:**
  - Gene expression
  - Replication time
  - Heterochromatin vs. euchromatin

Estimating Coding Drivers in 2020

Review Article | Published: 10 August 2020

A compendium of mutational cancer driver genes

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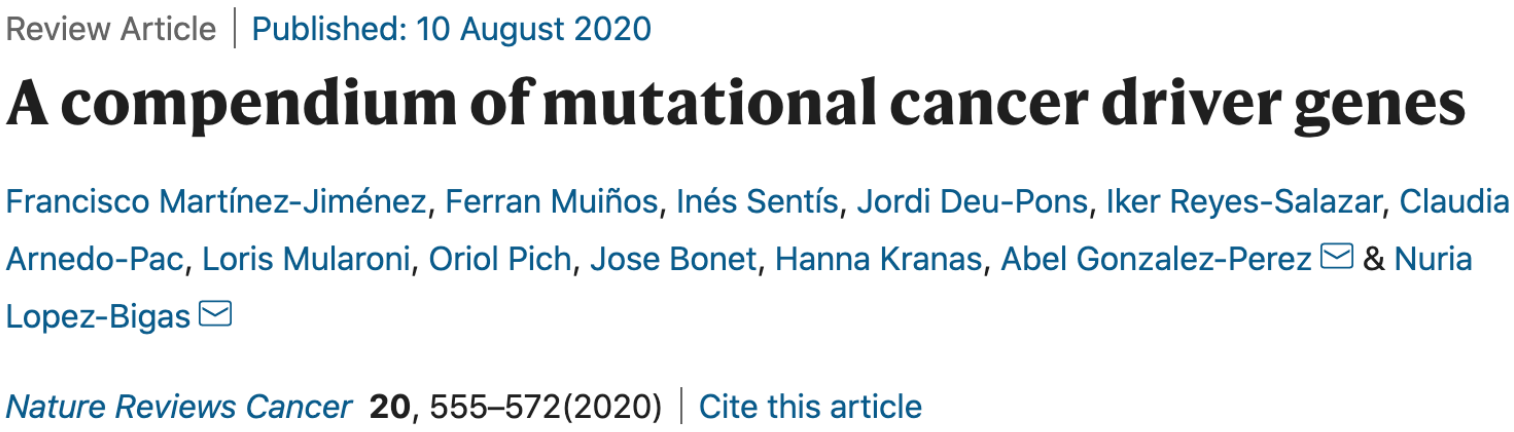

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What is the evidence of selection?

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Evidence of selection:

- Recurrence (it requires to estimate BMR)

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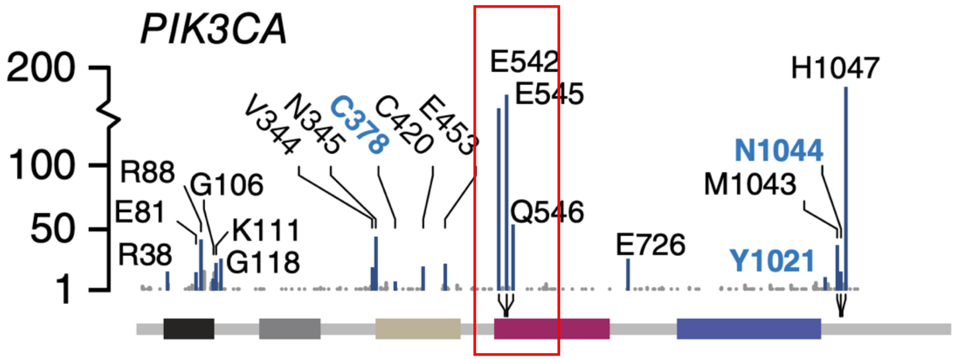
Evidence of selection:

- Recurrence (it requires to estimate BMR)
- Distribution of mutations (1D and 3D clusters)

1D cluster or mutation hotspot



Protein sequence

(Chang et al. Nat. Biotech 2016)

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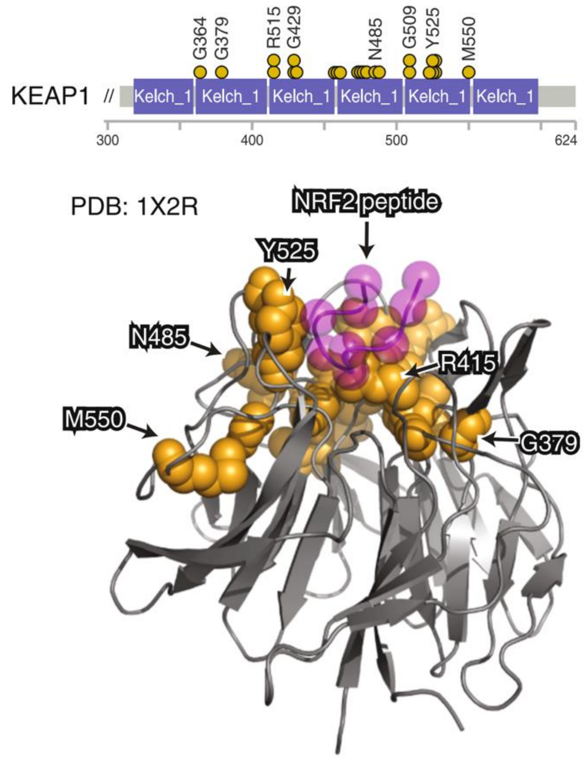
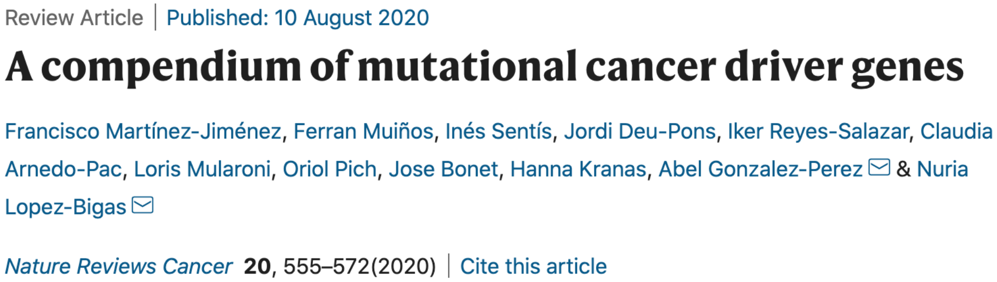


(Gao et al. Gen. Biology 2017)

Estimating Coding Drivers in 2020



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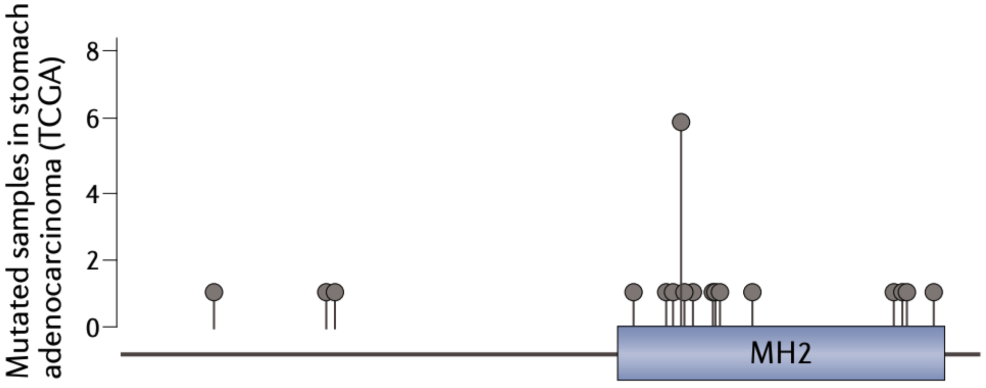
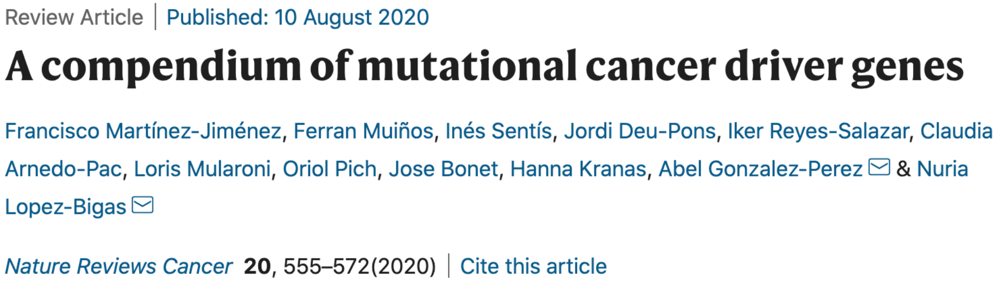
Evidence of selection:

- Recurrence (it requires to estimate BMR)
- Distribution of mutations (1D and 3D clusters)
- Mutations in functional domains



Gene	**Tumour type**	**PFAM domain**
SMAD4	Stomach adeno.	MH2 domain

Estimating Coding Drivers in 2020



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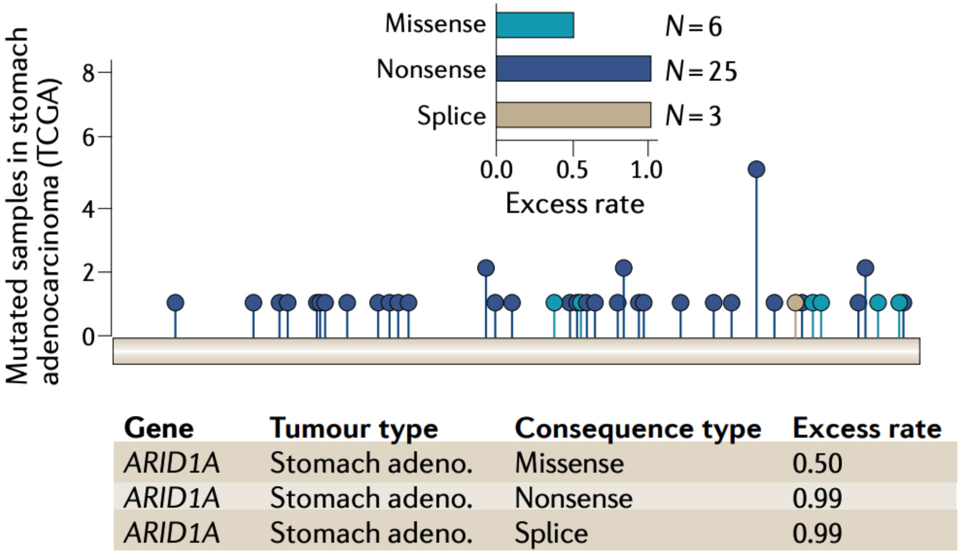
Evidence of selection:

- Recurrence (it requires to estimate BMR)
- Distribution of mutations (1D and 3D clusters)
- Mutations in functional domains
- *Functional impact bias*
    - *(also evolutionary conservation)*



Gene	Tumour type	Consequence type	Excess rate
ARID1A	Stomach adeno.	Missense	0.50
ARID1A	Stomach adeno.	Nonsense	0.99
ARID1A	Stomach adeno.	Splice	0.99

What did we learn about cancer from all these mutation analyses?

Exercise

Exercise: 1.15h-2h30 Prepare the paper presentation
2.30h-3h30 presentation of the paper

https://www.nature.com/articles/s41586-020-1965-x

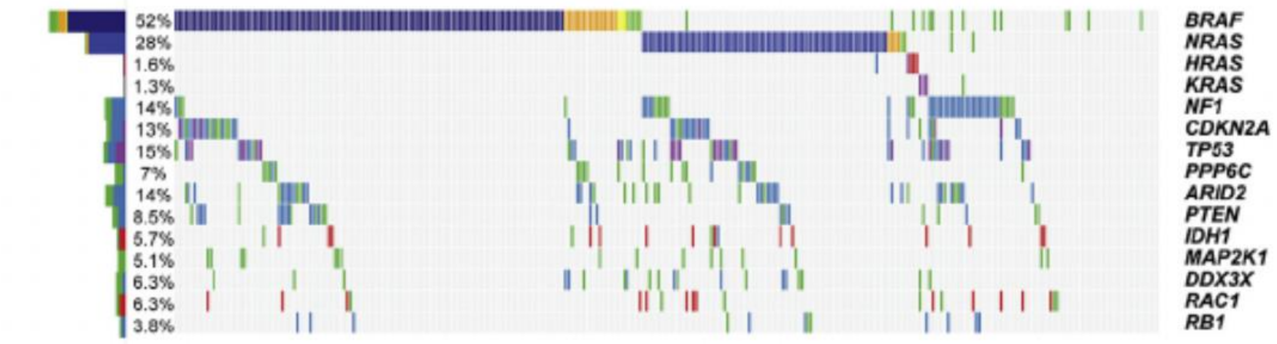
The pdf is in Moodle with a list of questions

Each group presents 1 figure, as it was organized in the previous exercise session.

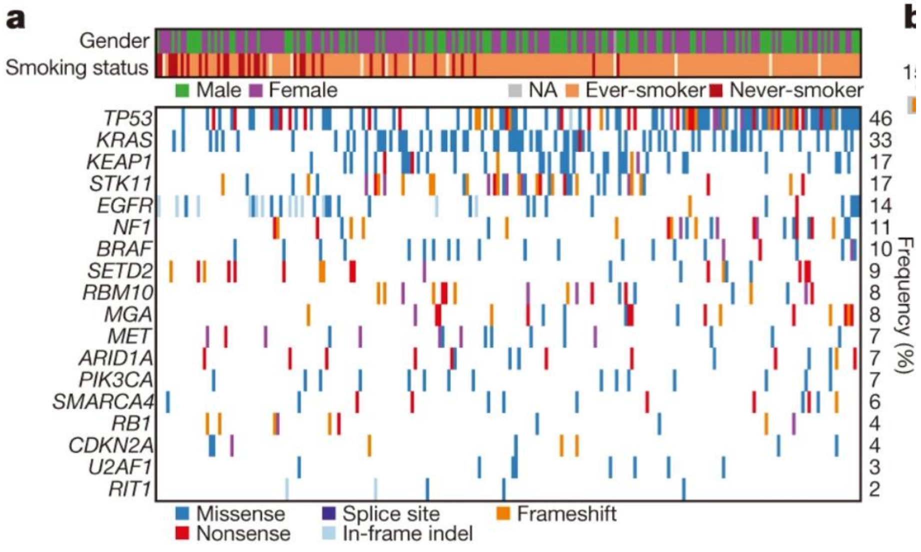
Address some of the questions

Cancer heterogeneity between different tumor types

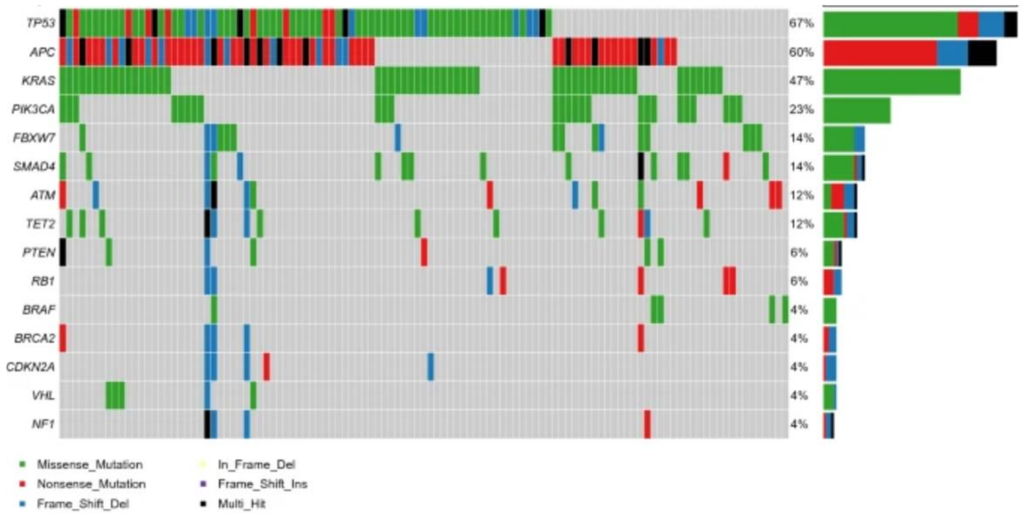
What are the most frequent mutated genes in each tissue?



Melanoma



Lung cancer

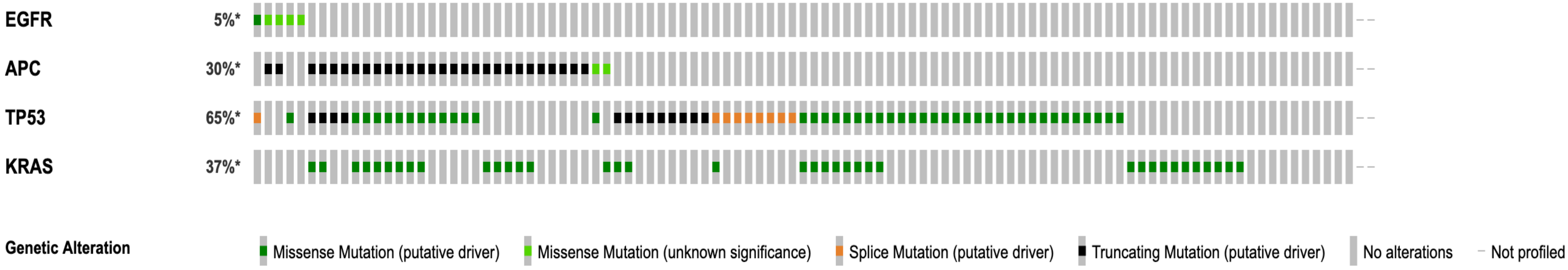


Colon cancer

Cancer inter-patient heterogeneity

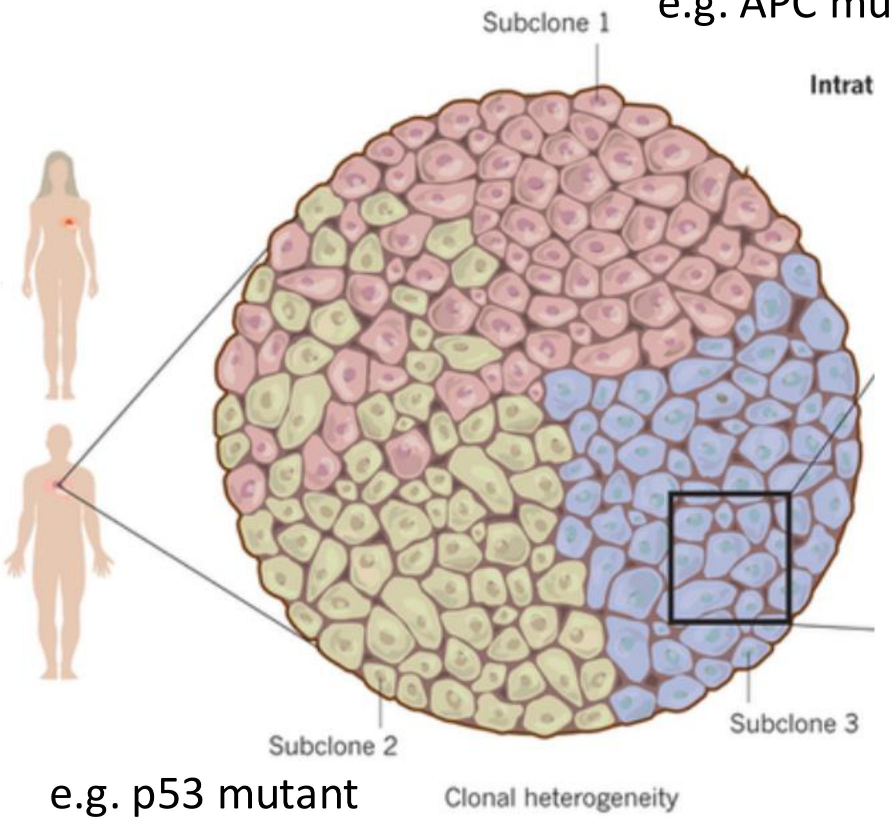
Same tumor: different mutation profile in each patient

103 patients colon cancer patient: each bar in this plot represents a patient



Cancer intra tumor heterogeneity

The same patient in different areas of the tumor have different mutations



e.g. APC mutant

e.g. KRAS mutant

e.g. p53 mutant

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