INTERPRETATION OF NGS TUMOR DATA

David Tamborero, PhD

> Senior Researcher,
Translational Bioinformatics Team Leader

david.tamborero@ki.se

DavidTamborero

OBJECTIVES OF THE SLIDE SET

- Review general concepts for tumor **NGS data interpretation**
- Explain how to analyse NGS data of an **individual case**
- Hands-on with **resources** supporting the task

Introduction

- Functional relevance of variants
- Clinical actionability of variants
- Resources for variant interpretation

From cohorts to individual tumors

n=N scenario

(retrospective) Analysis of tumor cohorts to generate **new knowledge**

n=1 scenario

(prospective) Analysis of a tumor individual based on **current knowledge**

EXAMPLE OF **COHORT VS INDIVIDUAL ANALYSIS**

n =N

> Analyse **patterns** of mutations across (large) cohorts of sequenced tumors

Discovery of ~ 80 unique ***mutational signatures***

(capturing the different mutational processes that [tumor] cells undergo)

n =1

> Identify which of these **known** mutational processes have occurred in this tumor individual

From cohorts to individual tumors

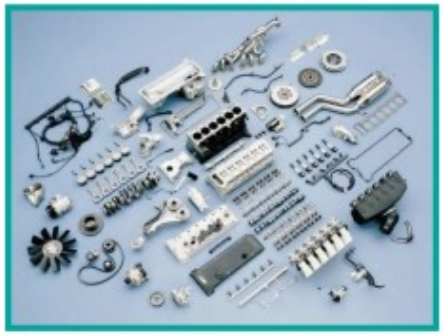
n=N scenario

(retrospective) Analysis of tumor cohorts to generate **new knowledge**

n=1 scenario

(prospective) Analysis of a tumor individual based on **current knowledge**

HIGH-THROUGHPUT DIAGNOSTIC ASSAYS

	DNA	RNA	Protein
			
Method	Genomics	Transcriptomics	Proteomics
Information	Genetic sequence	Genetic activity	Phenotype
Research Questions	Mutations	Altered activity	Altered function

HIGH-THROUGHPUT DIAGNOSTIC ASSAYS

DNA

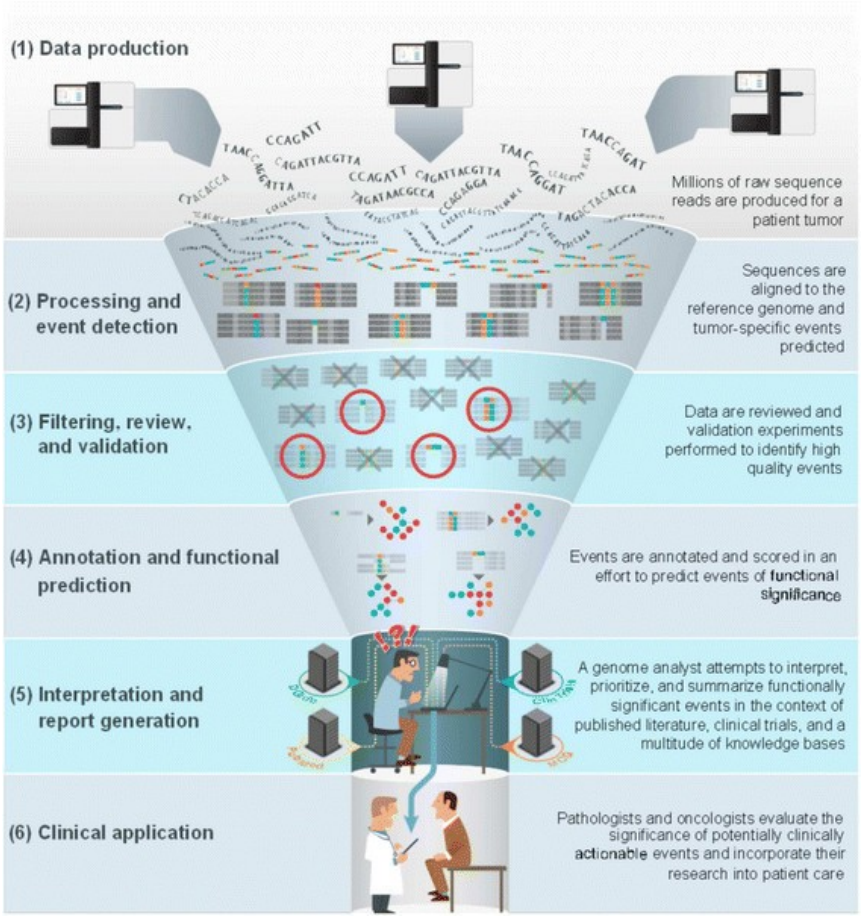
Method	**Genomics**
Information	**Genetic sequence**
Research Questions	**Mutations**

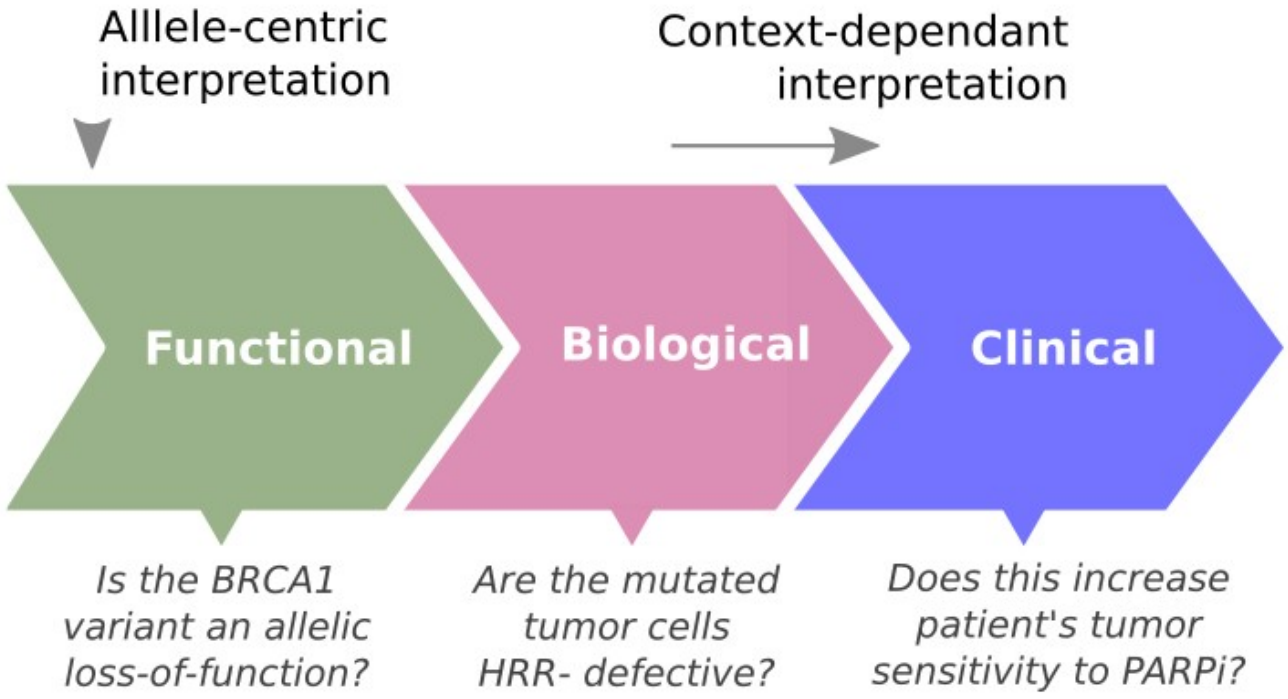
> Single nucleotide **mutations**, **indels**, copy number **alterations** and/or **fusions**

> Use in the clinics: cancer gene **panels** and **WGS/WTS***

* Hodder et al, Nature Medicine 2024 (*pediatric*)
Watkins et al, BMJ 2024 *(sarcomas)*

n=1 INTERPRETATION OF GENE MUTATIONS



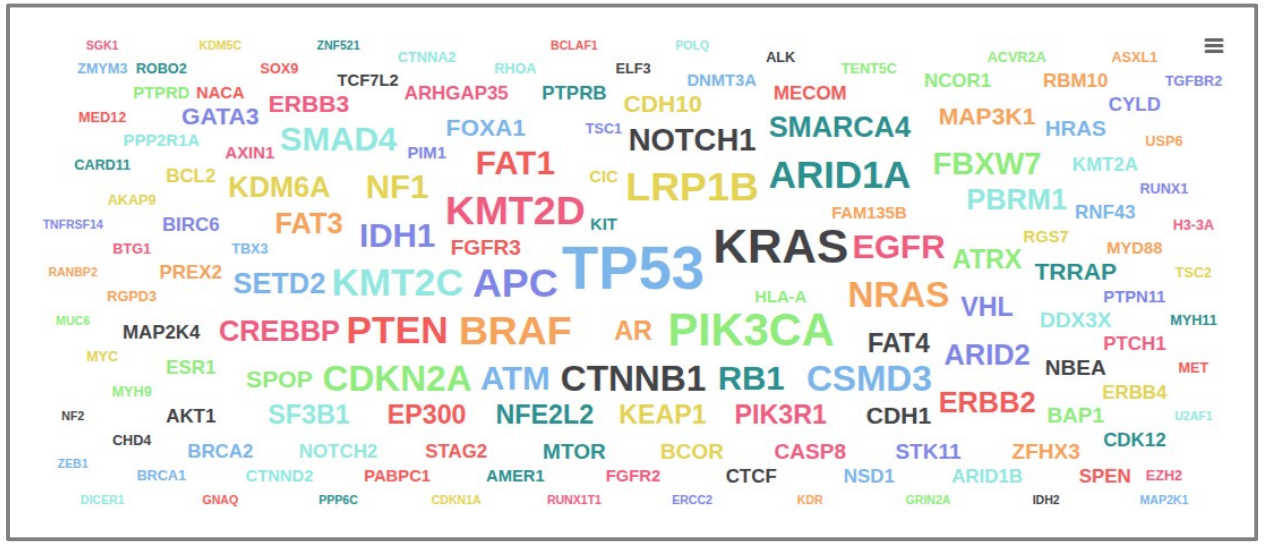


- Good et al. Genome Biology 2014

- Introduction
- **Functional relevance of variants**
- Clinical actionability of variants
- Resources for variant interpretation

~600-700 CANCER GENES DESCRIBED

> Most recurrently mutated **cancer genes**:



Intogen website, 2025

ONCOGENES

> WT function **promotes** cell growth, division and survival

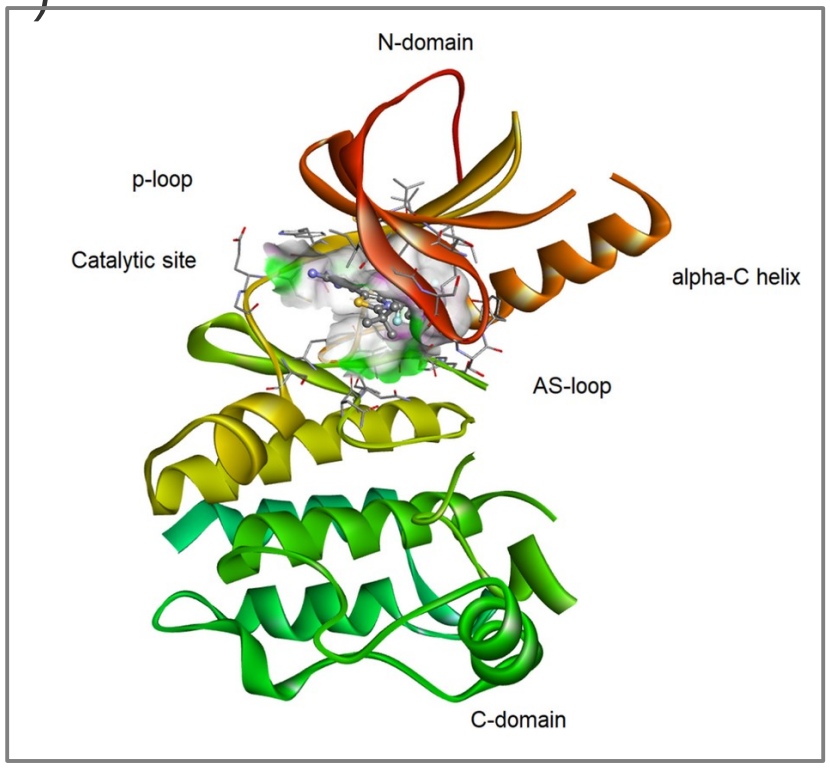
TUMOR SUPPRESSORS

> WT function **inhibits** cell cycle and **ensures** apoptosis and DNA damage

HOW A MUTATION CAN BE TUMORIGENIC?

> Example: **BRAF p.V600E (gain-of-function)**

(chr7:g.140453136A>T
)



BRAF V600E with a ligand bound
(PDB ID: 4XV2)

> BRAF is a kinase that in WT is phosphorylated via RAS-regulated (extracellular) growth signaling

> The mutation codes for a glutamic acid, which mimics the effect of adding phosphate groups

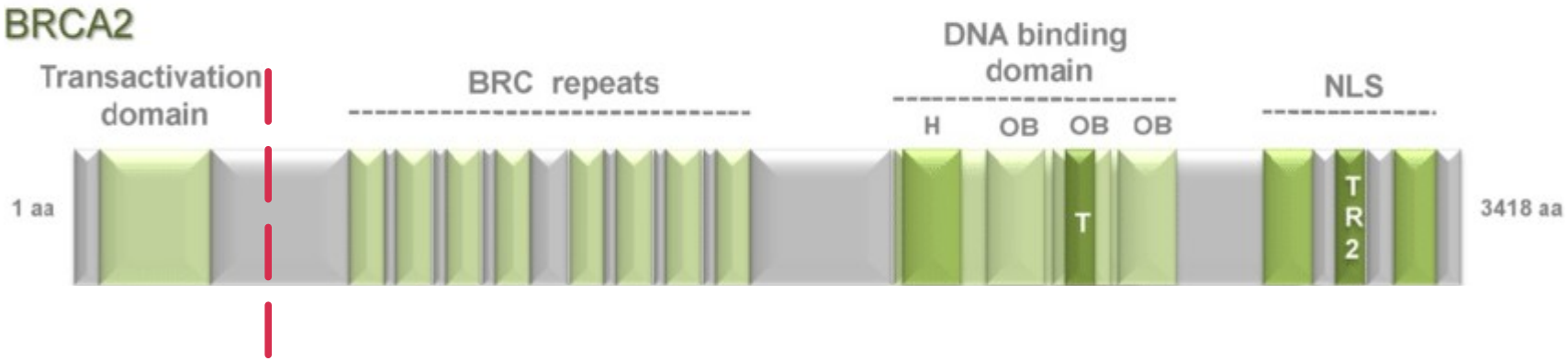
> The protein is ‘tricked’ to flip into a constitutively active form

> Cells with this mutant allele are in over-active proliferation/survival signaling state

HOW A MUTATION CAN BE TUMORIGENIC?

> Example: **BRCA2 p.Gln366Ter (loss-of-function)**

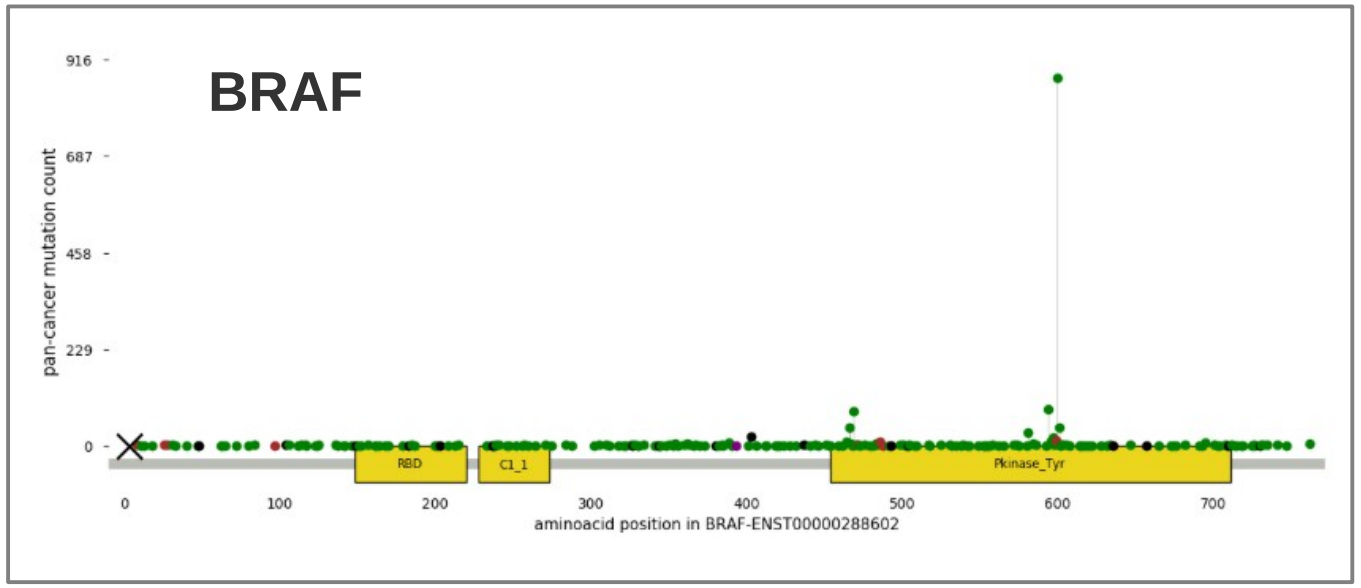
(chr13:g.32906712C>T
)

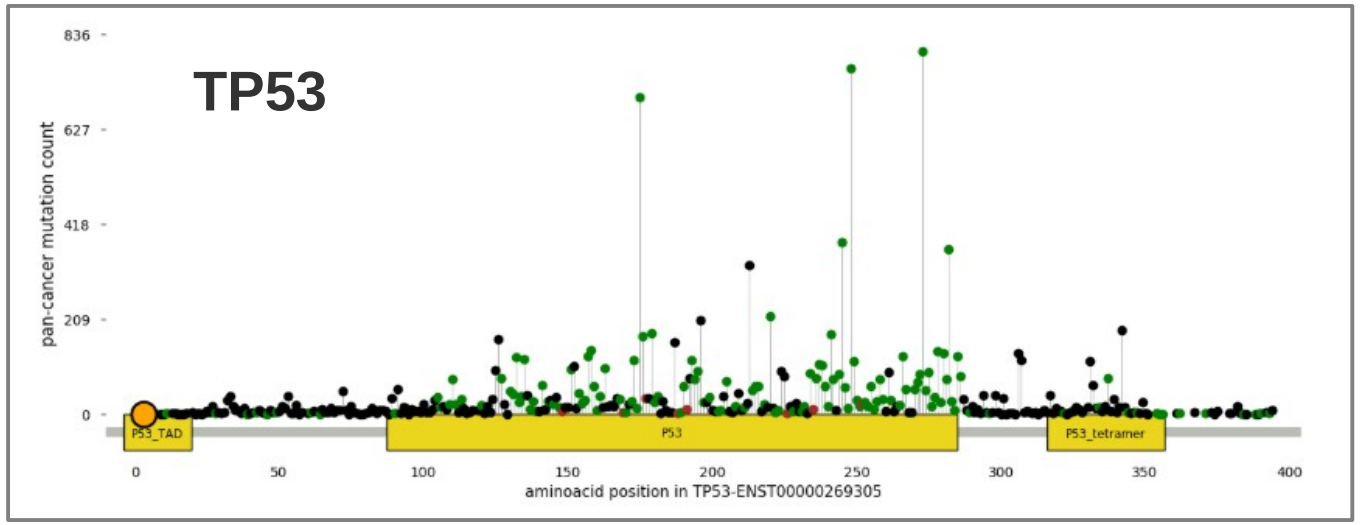


> The mutation creates an aberrantly premature stop codon

> Only a small mRNA segment is transcribed, preventing normal gene function.

MUTATIONS OBSERVED IN ~25,000 TUMORS





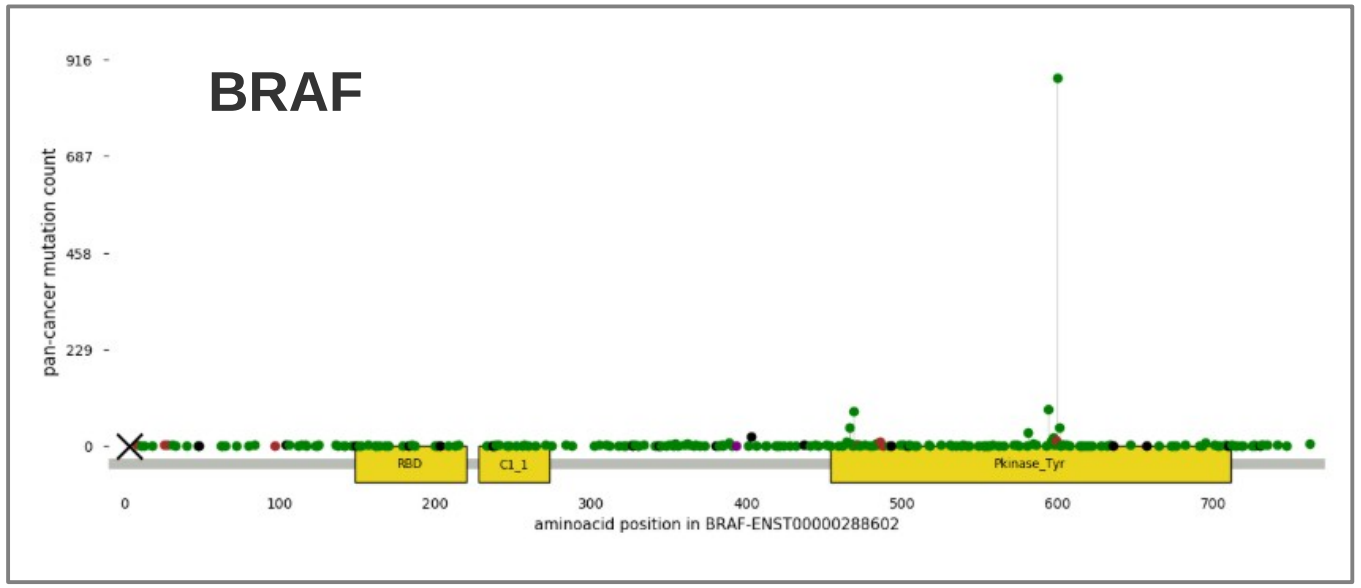
Public MTBP website, 2025

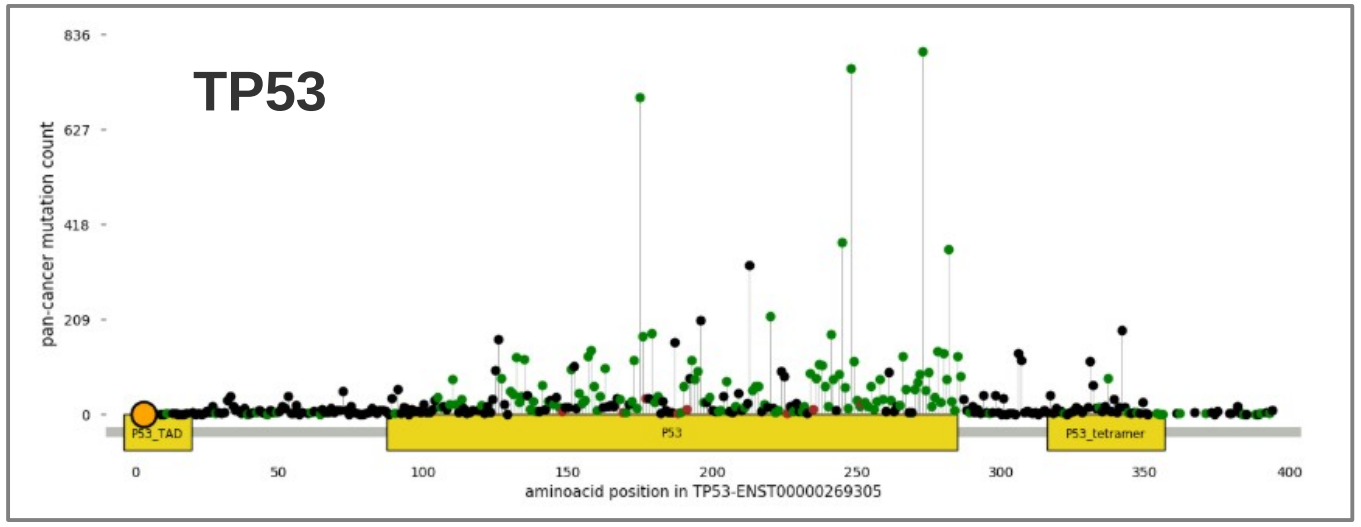
> Tumor mutations are **fixed** based on:

(a) how 'likely' are they to occur
*([somatic] **mutational processes**)*

(b) the **selective advantage** that they confer for clonal expansion

MUTATIONS OBSERVED IN ~25,000 TUMORS





> Tumor mutations are **fixed** based on:

(a) how 'likely' are they to occur
*([somatic] **mutational processes**)*

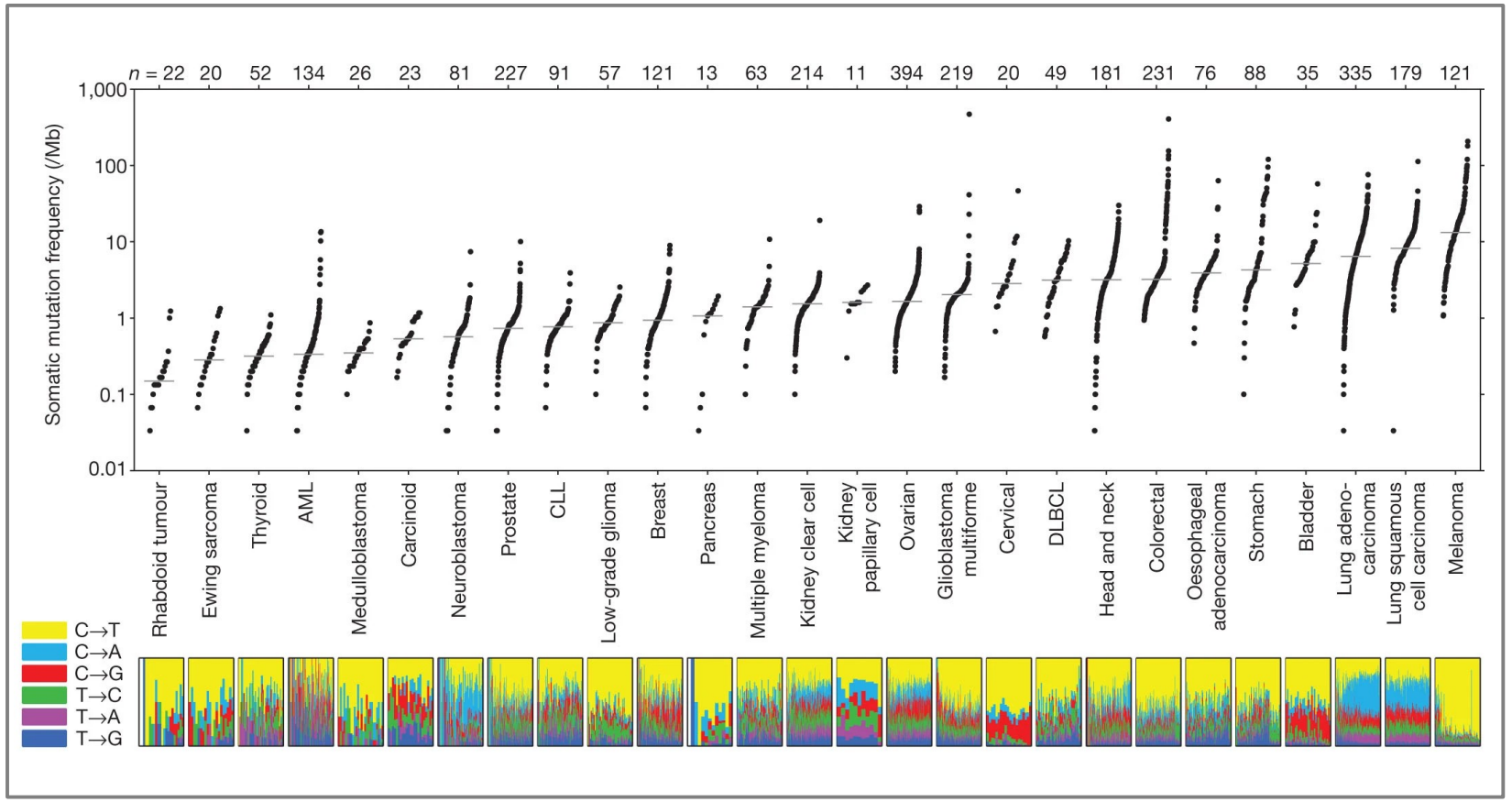
(b) the **selective advantage** that they confer for clonal expansion

Pan-cancer predominant mutation type:
- disrupting
- inframe
- missense
- other

Public MTBP website, 2025

HOW LIKELY IS A MUTATION TO OCCUR?

> Frequency and type of somatic mutations in first TCGA/ICGC release (~3,000 tumors):



Lawrence M, Nature 2013

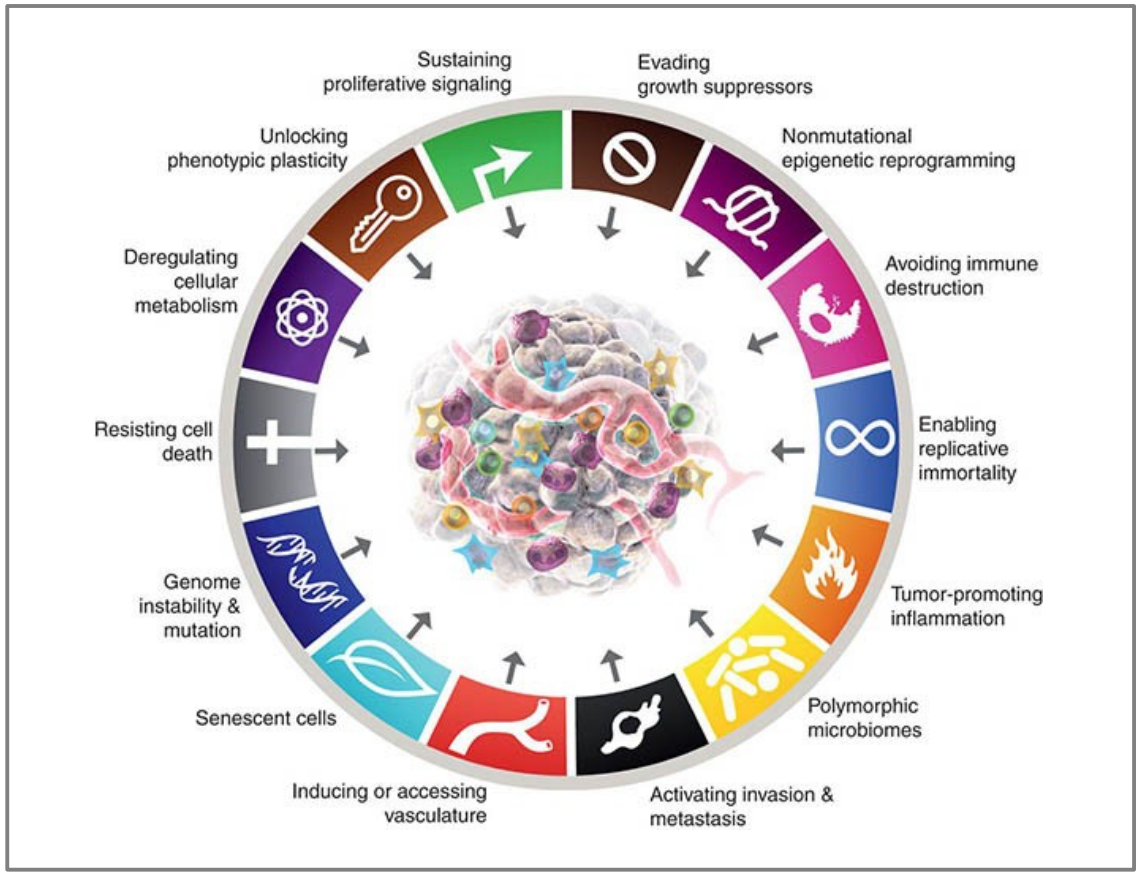
> **Mutational processes** in a cell are the result of:

(A) **Endogenous** and **exogenous** factors

(B) Performance of the **DNA damage repair**

WHAT IS A (CLONAL) SELECTIVE ADVANTAGE?

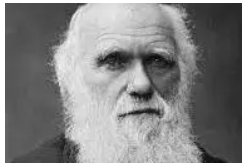
> Tumorigenic mutations make the cell to acquire **tumor hallmarks**:

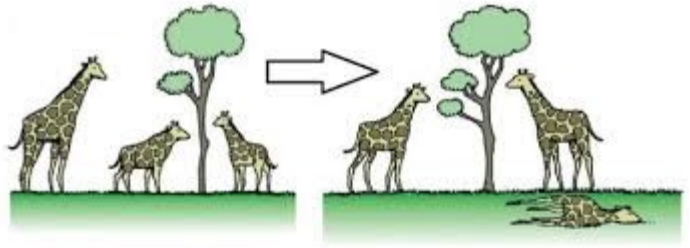


Hanahan D, Cancer Disc 2022

> These are otherwise **normal processes** that occur at the wrong place and/or time

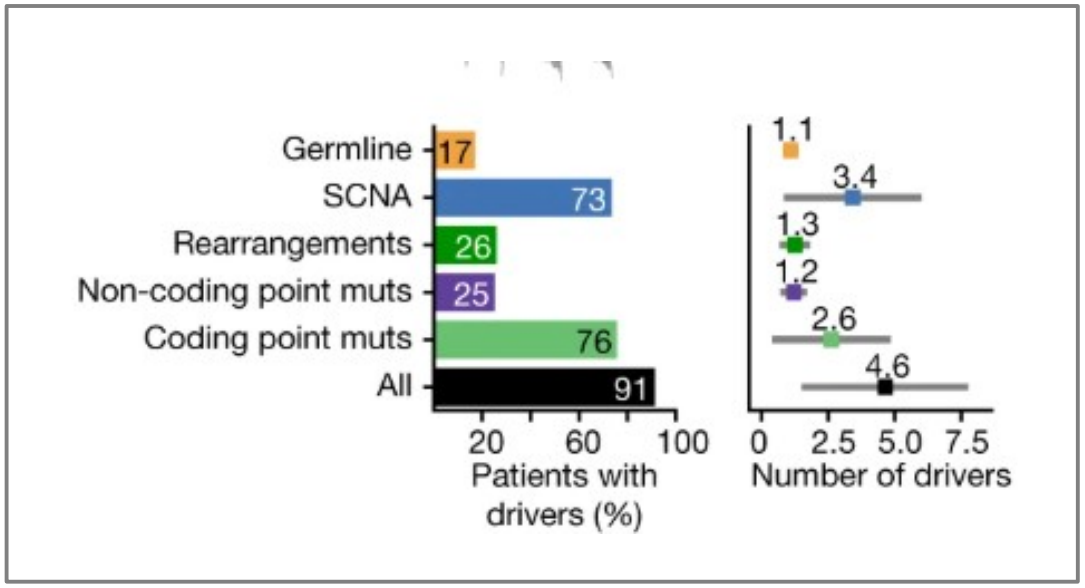
> They provide an edge to the malignant cells to outcompete others during **clonal expansion**





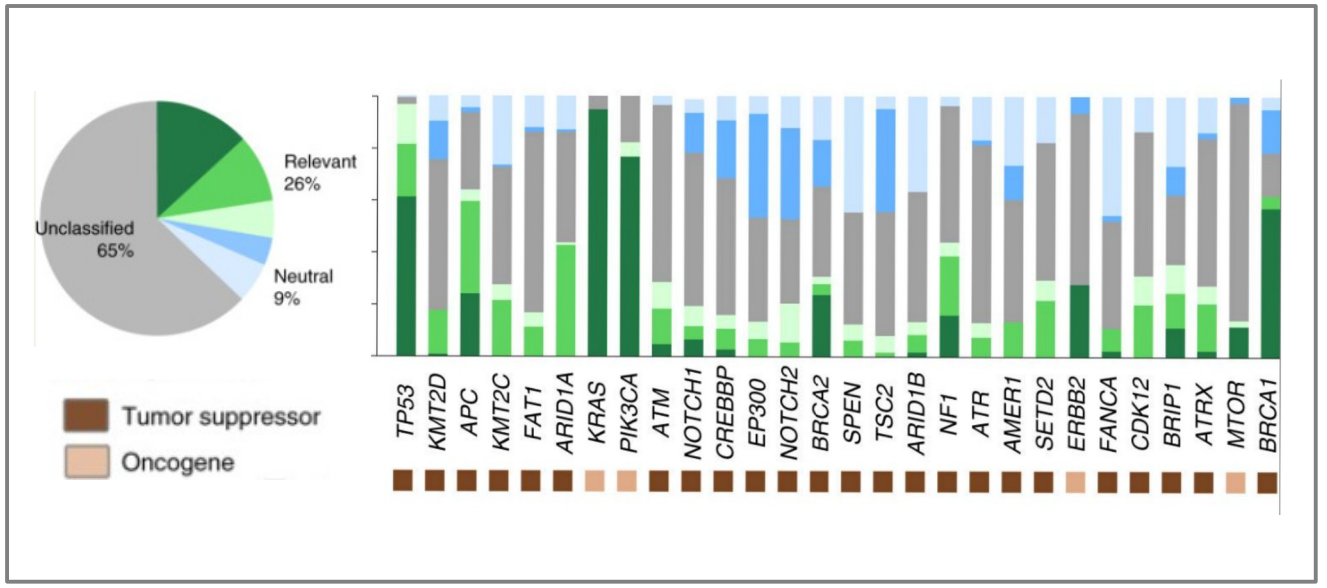
ARE ALL THE MUTATIONS THAT OCCUR IN CANCER GENES TUMORIGENIC?

> Tumors bear a mean of ~4 genomic alterations likely tumorigenic:



PCAWG, Nature 2020

> ~25% of the tumor mutations observed in cancer genes have evidence of being tumorigenic:

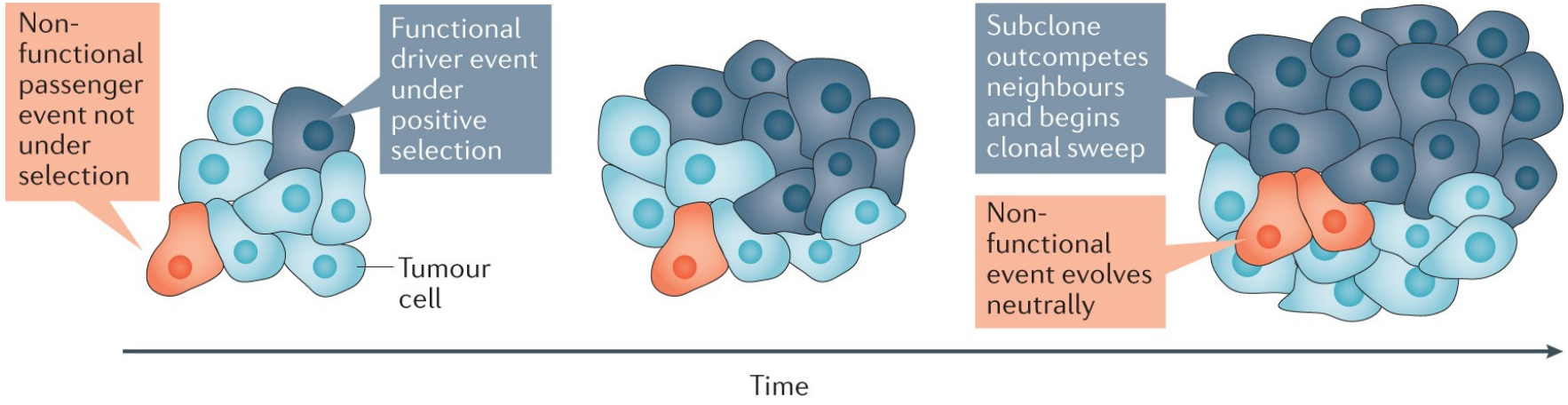


Tamborero D, Nature Cancer 2022

INTERPRETATION OF GENE MUTATIONS

> Importance of classifying mutations observed in a tumor by their **functional relevance**, as not all of them (<u>even in cancer genes</u>) are **tumorigenic**

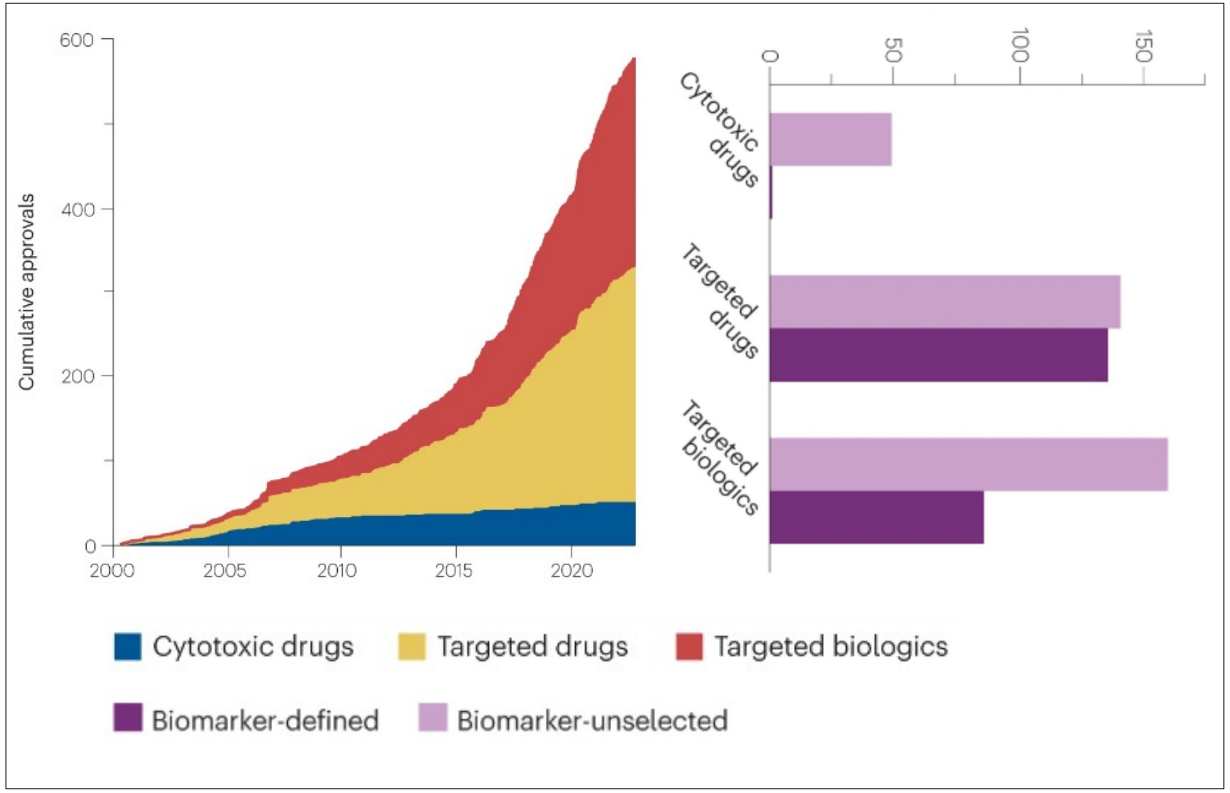
Driver vs **Passenger** mutations



- Introduction
- Functional relevance of variants
- **Clinical actionability of variants**
- Resources for variant interpretation

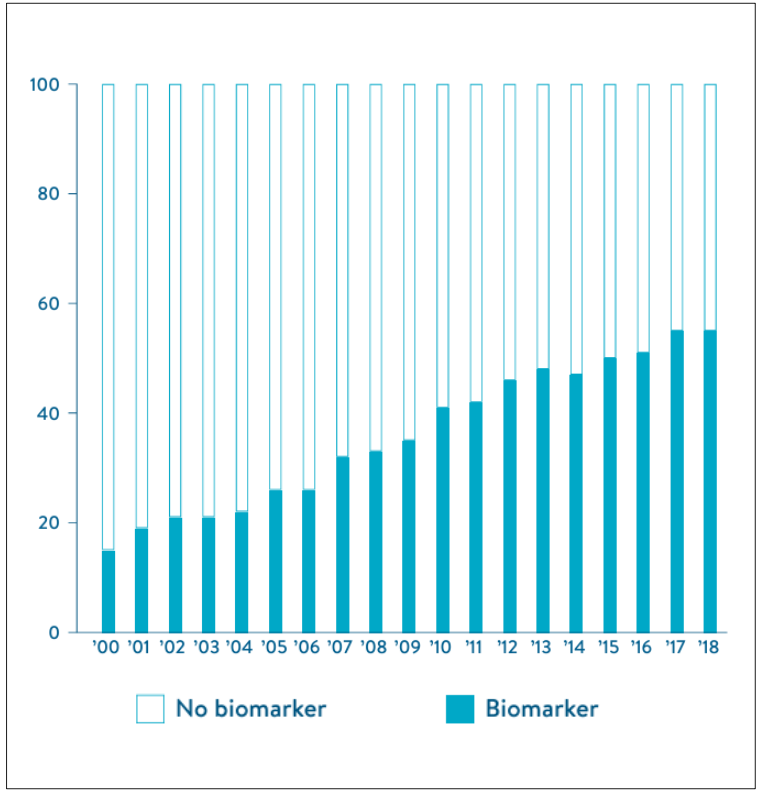
PRECISION CANCER ONCOLOGY

> **FDA drug approvals** (2000-2022)



Scott et al Nat Rev Drug Disc 2023

> **Clinical Trials** (2000-2018)



Vadas et al L.E.K. Consulting 2018

APPROVED DRUG GENOMIC BIOMARKERS

Drug Name	Biomarker(s)	Cancer Type(s)	Regulatory Approval
Imatinib	BCR-ABL fusion	Chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GIST)	FDA (2001), EMA (2002)
Gefitinib	EGFR exon 19 deletion, L858R mutation	Non-small cell lung cancer (NSCLC)	FDA (2003), EMA (2009)
Erlotinib	EGFR mutations	NSCLC, pancreatic cancer	FDA (2004), EMA (2005)
Lapatinib	HER2 overexpression	HER2-positive breast cancer	FDA (2007), EMA (2008)
Alpelisib	PIK3CA mutation	HR-positive, HER2-negative breast cancer (with fulvestrant)	FDA (2019), EMA (2020)
Selpercatinib	RET fusion, RET mutation	NSCLC, medullary thyroid cancer, other RET-altered cancers	FDA (2020), EMA (2021)
Entrectinib	NTRK fusion, ROS1 fusion, ALK fusion	Solid tumors (tissue-agnostic)	FDA (2019), EMA (2020)
Sotorasib	KRAS G12C mutation	NSCLC, colorectal cancer	FDA (2021), EMA (2022)
Krazati (adagrasib)	KRAS G12C mutation	Colorectal cancer (with cetuximab)	FDA (2024), EMA (2025)
Vorasidenib	IDH1 or IDH2 mutation	Grade 2 gliomas (astrocytoma or oligodendroglioma)	FDA (2024), EMA (2024)

Examples of genomic markers for FDA/EMA approved therapies

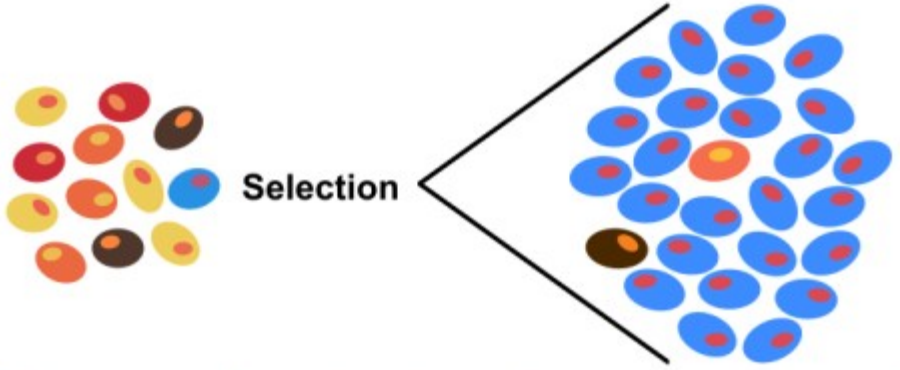
~190 prescriptions (genomic alteration + cancer type* + drug) approved for **standard-of-care**

**note also pancancer approvals*

PRIMARY AND SECONDARY RESISTANCE

> Drugs that target a specific **molecular mechanism** that the malignant cells are “addicted” to pursue the tumor collapse *(sparing healthy cells)*

> If this is not true for a significant number of tumor cells → **no clinical response**



> If this is not true for a (small) subset of cells → **clinical relapse**

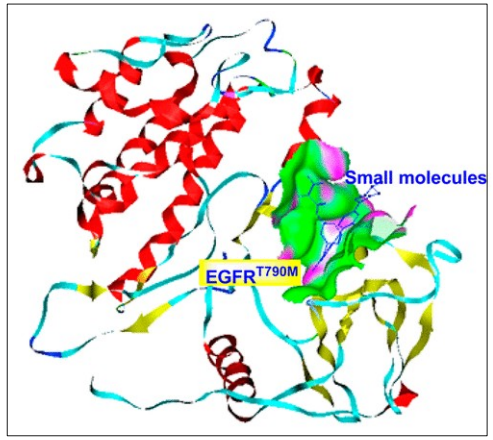
THERAPY-RESISTANT TUMOR CLONES



> Relapse to Vemurafenib in BRAF V600E melanoma

Wagle et al JCO 2011

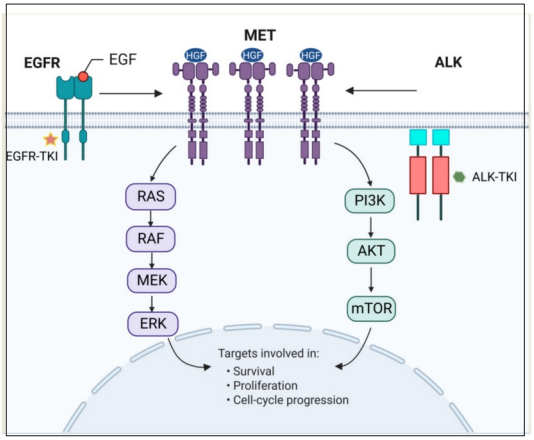
On-target resistance



> EGFR T790M prevents binding of 1st generation EGFR inhibitors

Song et al JMC 2016

Off-target resistance



> MET amplification as parallel driver to EGFR / ALK signaling

Qin et al Cancers 2023

ACTIONABILITY OF (DRIVER) MUTATIONS

> They can be **biomarkers of drug response**

> Confer **sensitivity** or **resistance** to a certain therapy

> Some are already approved for **SoC use**, others are tested in ongoing **clinical trials**

> In some institutions, genomics are also used for n=1 **off-label prescriptions**

> They can also be used as **diagnostic and/or prognostic** markers

> **Others**: secondary/incidental findings *(including clonal hematopoiesis)*

- Introduction
- Functional relevance of variants
- Clinical actionability of variants
- **Resources for variant interpretation**

n=1 VARIANT INTERPRETATION

Michael Ramirez

From cohorts to individual tumors

KNOWN VARIANT EFFECTS

> Based on existing **pre-clinical** and **clinical** data

> *"In vitro Ba/F3 cell studies show FGFR2 p.Cys62Tyr mutation to be activating as measured by increased transformation activity compared to wildtype."*

> *"RNA studies show that BRCA2 p.Val211Leu disrupt the normal splicing of the encoded transcript."*

> *"Germline TP53 p.Arg337Cys mutations have been demonstrated to cause Li-Fraumeni syndrome."*

> *"ST7 (e2)-MET (e2) fusion have been observed in several patients relapsed to ALK-inhibition therapy."*

KNOWLEDGEBASES

> Several international efforts to gather existing **knowledge on variant effects**

> **Scope** *(e.g. germline vs tumor somatic)*

> **Data model** *(e.g. format, classification framework)*

> **Curation and distribution** *(e.g. community vs in-house, license model)*

ClinVar
Clinically relevant variation

BRCA EXCHANGE

1000 genomes

gnomAD
Genome Aggregation Database

COSMIC
Catalogue Of Somatic Mutations In Cancer

CKB CANCER KNOWLEDGEBASE
POWERED BY GENOMENON

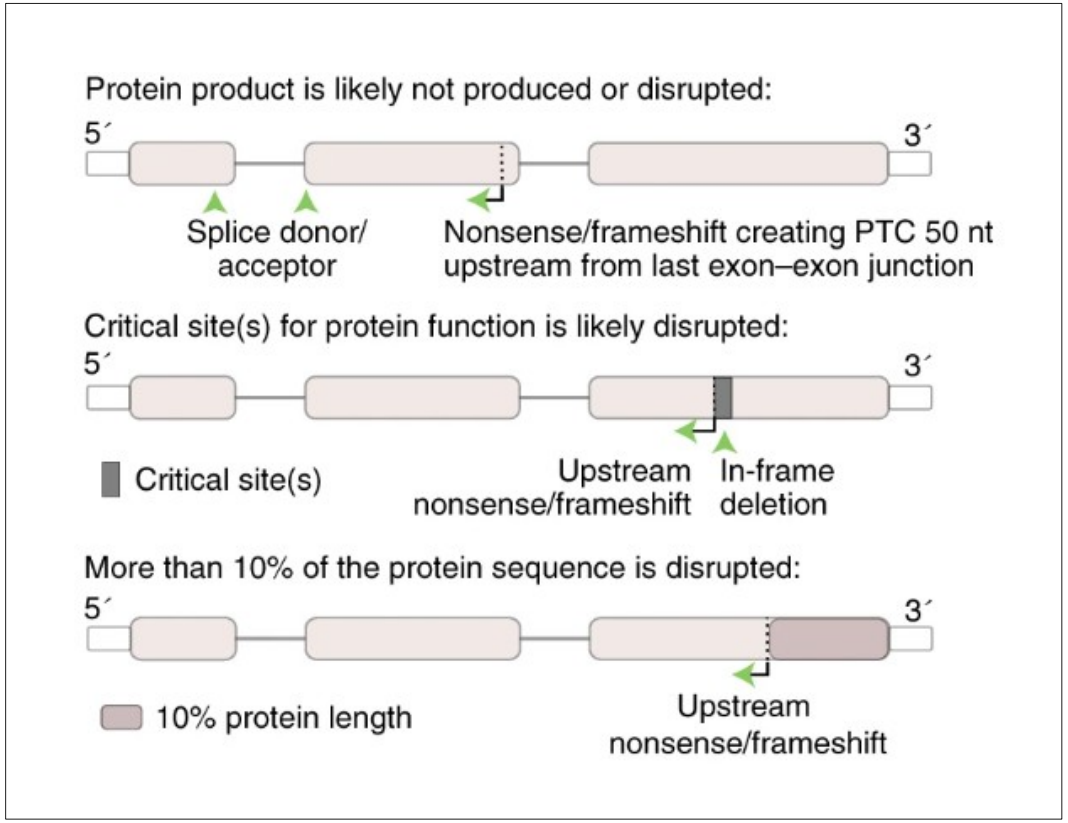
CIViC
CLINICAL INTERPRETATION OF VARIANTS IN CANCER

OncoKB
Precision Oncology Knowledge Base

VICC Variant Interpretation for Cancer Consortium

VARIANT EFFECT ASSUMPTIONS

> Some variants have been not characterized but their effect can be **(safely) assumed**



D Tamborero, Nature Cancer 2022

> Examples of mutations that likely disrupt the protein WT function

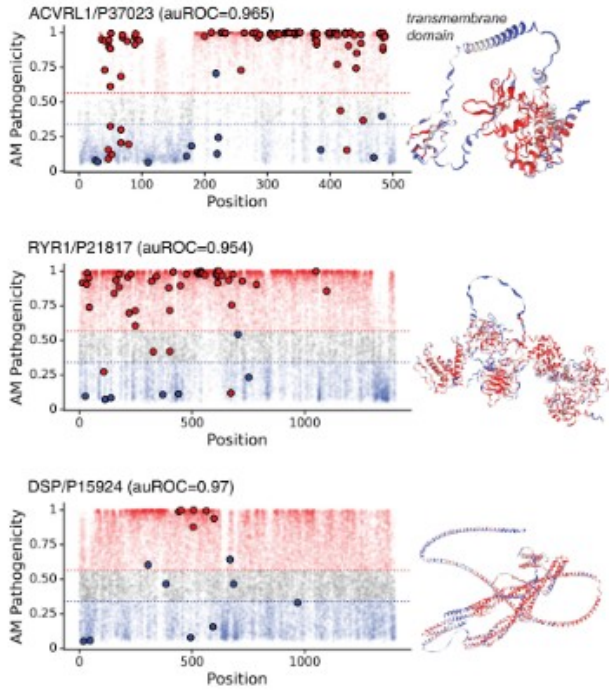
VARIANT PREDICTIONS

> When the variant effect is not known and cannot be assumed, **computational predictions** provide the **lowest** level of evidence

Accurate proteome-wide missense variant effect prediction with AlphaMissense

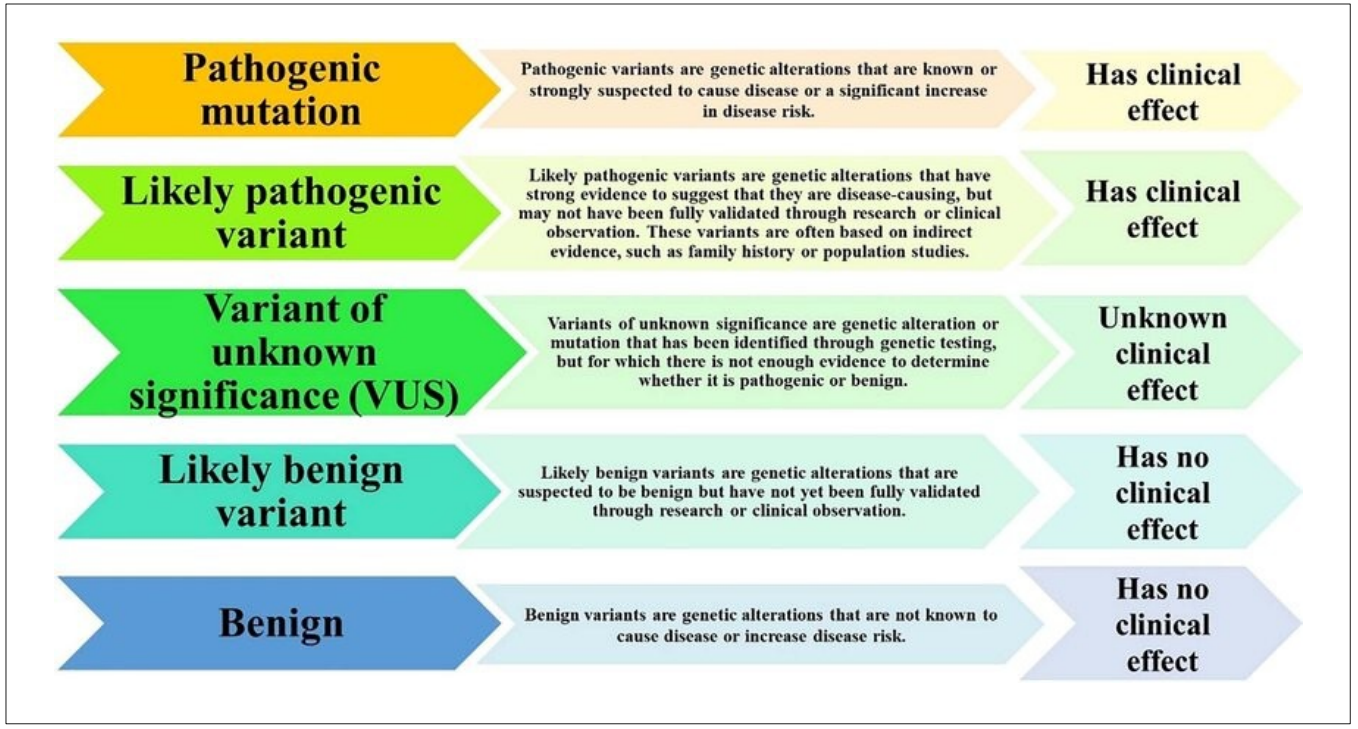
Science

ML-algorithm predicting the impact of a given mutation based on e.g. conformational changes in the affected protein



FROM VARIANT ANNOTATION TO CLASSIFICATION

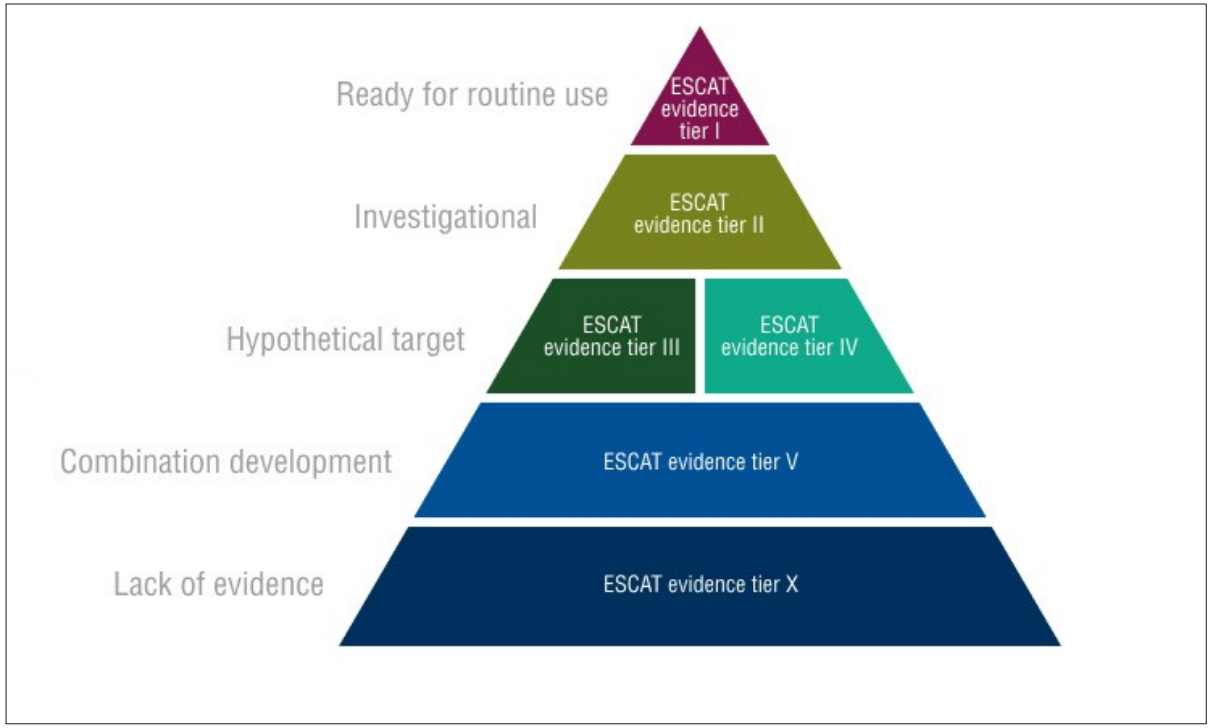
> Underlying evidence required to **associate** the variant with a **(clinical) action**



> ACMG/AMP classification framework for **variant pathogenicity** in **germline** genetic diseases

FROM VARIANT ANNOTATION TO CLASSIFICATION

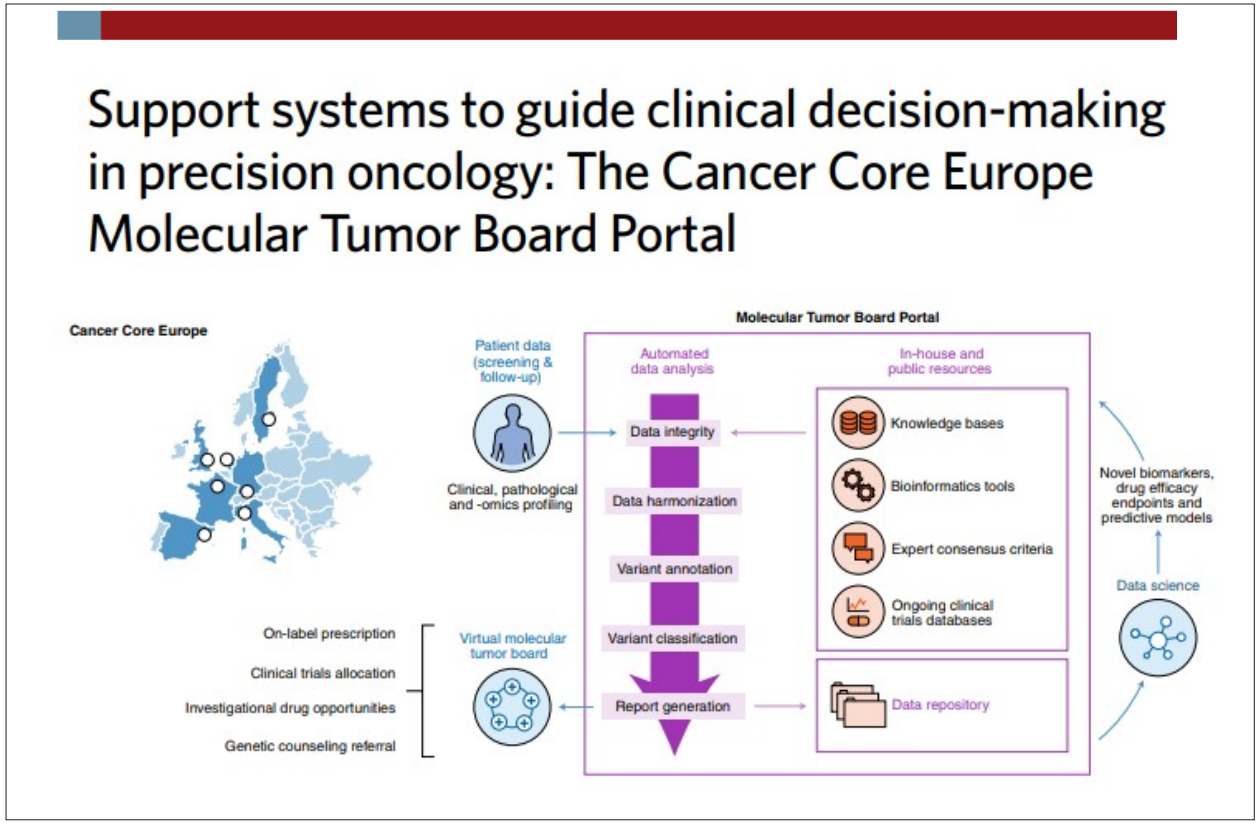
> Underlying evidence required to **associate** the variant with a (clinical) action



> ESMO classification framework for ranking **variant actionability**

CLINICAL DECISION SUPPORT SYSTEMS

> **Automates** the **annotation** of variants based on multiple resources and their **classification** according to predefined criteria



Molecular Tumor Board Portal

D Tamborero, Nature Medicine 2020

CONCLUSIONS

> **n=1 interpretation** is about using **existing** knowledge and methods to support the **most informed** downstream application

> It is key to understand all the **pros/cons** of each **resource** that is employed

> Importance of using computational tools to **scale** the process following a **consistent** workflow

LehtiöLab

PCM The Personalised Cancer Medicine Program at Karolinska Institutet

Karolinska Institutet | KAROLINSKA UNIVERSITETSSJUKHUSET

Cancer Proteogenomics and Clinical Translation

Cancer Core Europe

BARNCANCER FONDEN

Cancerfonden

Vetenskapsrådet

RADIUMHEMMETS FORSKNINGSFONDER

THE ERLING-PERSSON FAMILY FOUNDATION

Knut and Alice Wallenberg Foundation

#ERAPerMed | DART | PRIME-ROSE | RESCUER | SWEDISH FOUNDATION for STRATEGIC RESEARCH

EU JOINT ACTION JANE NETWORKS OF EXPERTISE ON CANCER | PCM4EU | eosc cancer | ONCOBIOME | Stockholms läns landsting

KAROLINSKA UNIVERSITY HOSPITAL