

Cancer Gene Panel DNA testing

Duc-Minh Nguyen-Le, MD

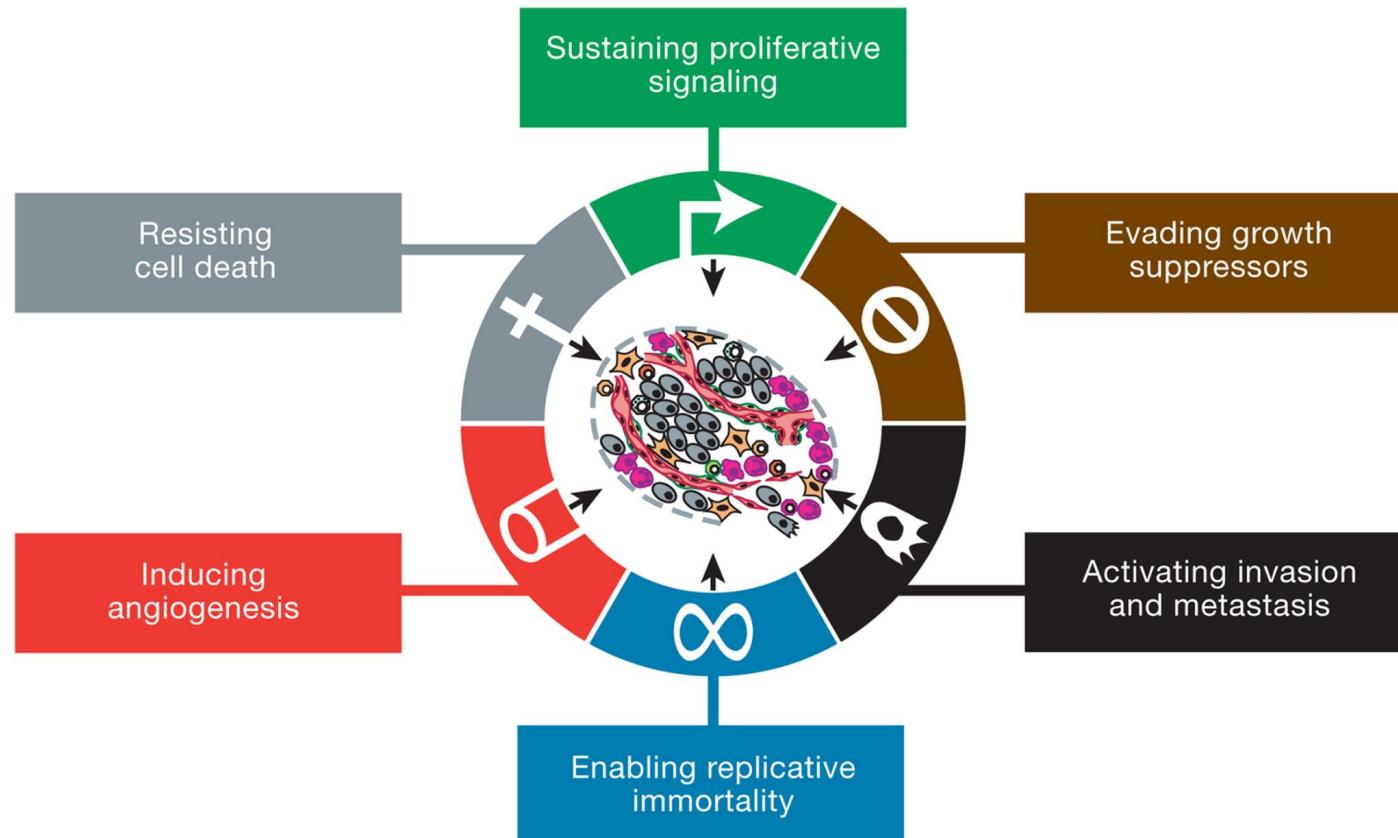
2025 Jan 03

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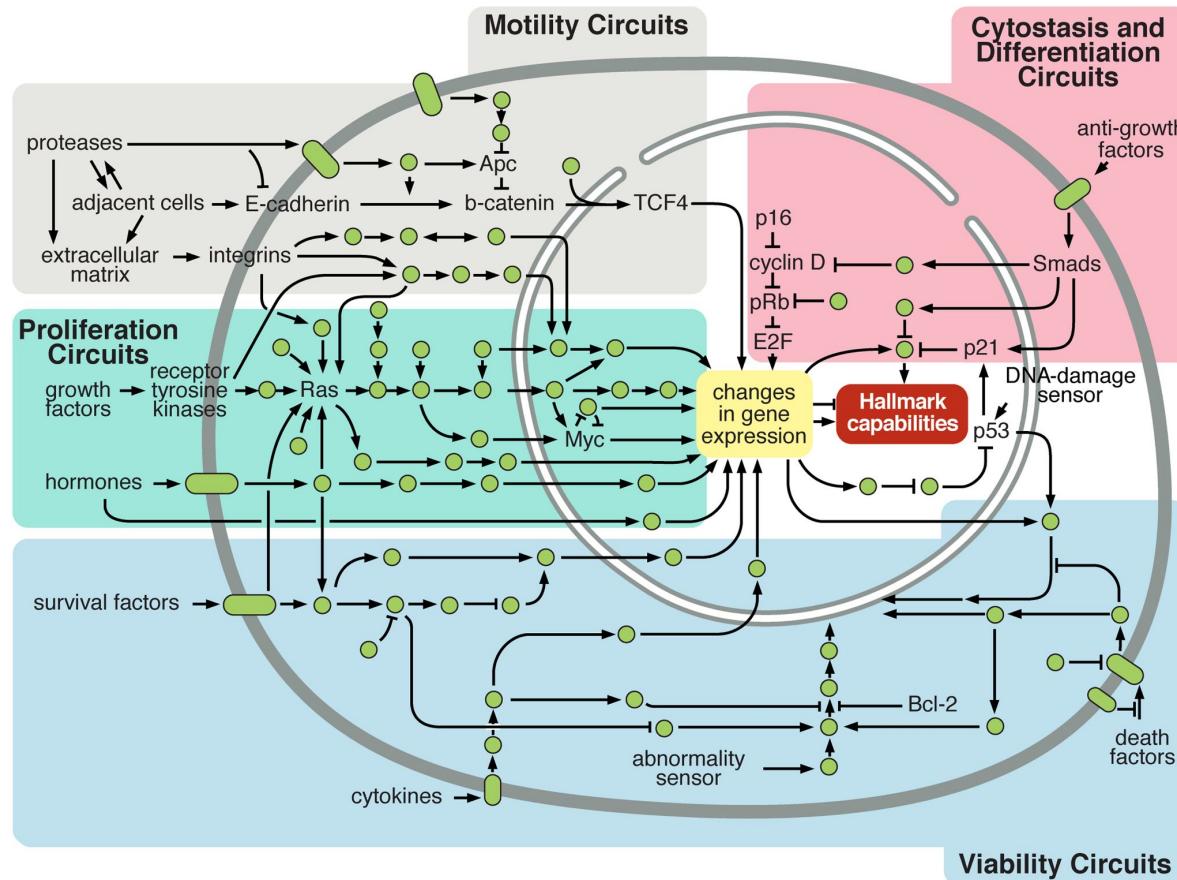
1. Cancer disease: What have we known?
2. Principles in cancer gene panel testing
3. Introduction to somatic variant discovery

Background

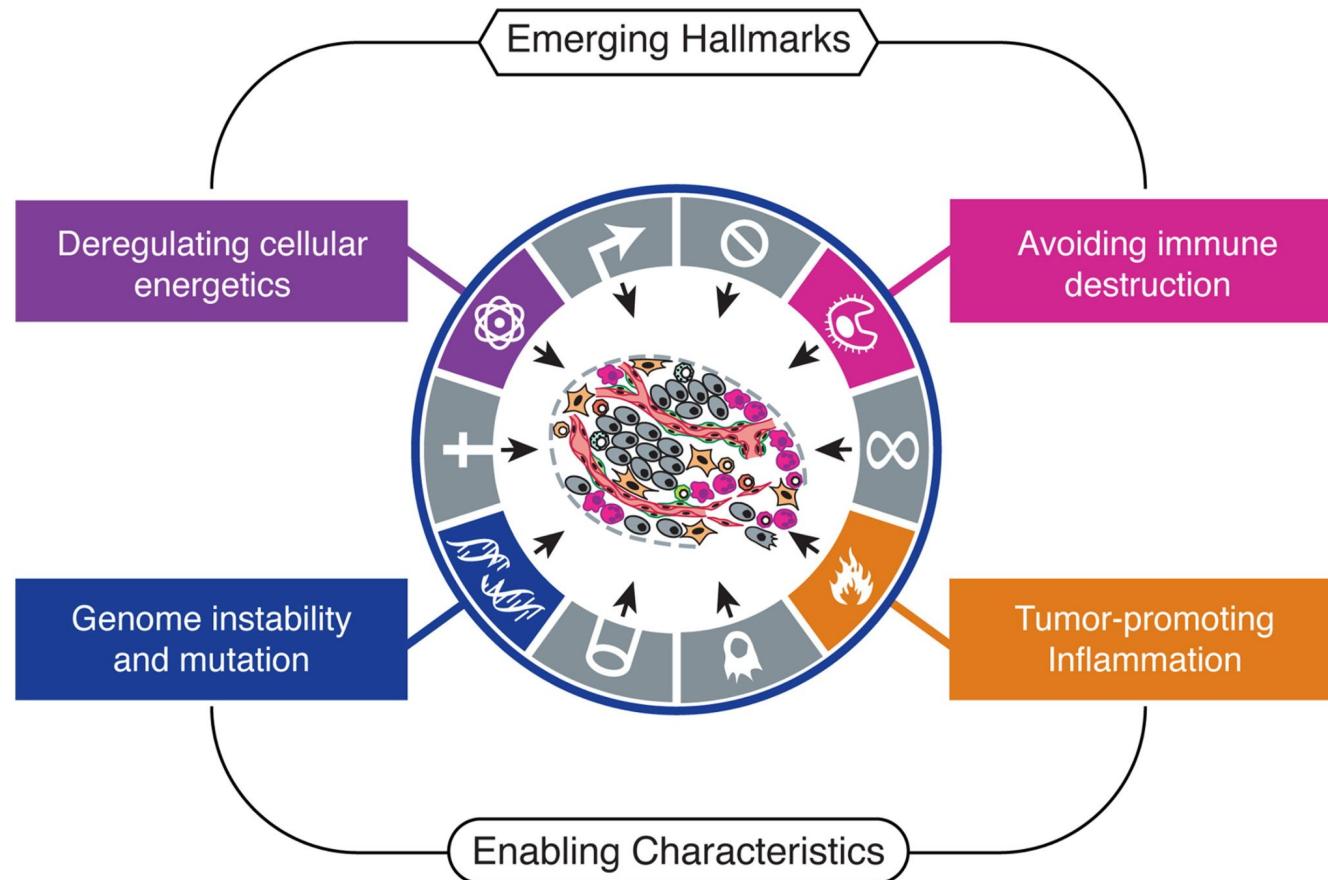
The development of cancer, is characterized by the sequential accumulation of molecular alterations within previously non-malignant cells



Intracellular Signaling Networks Regulate the Operations of the Cancer Cell

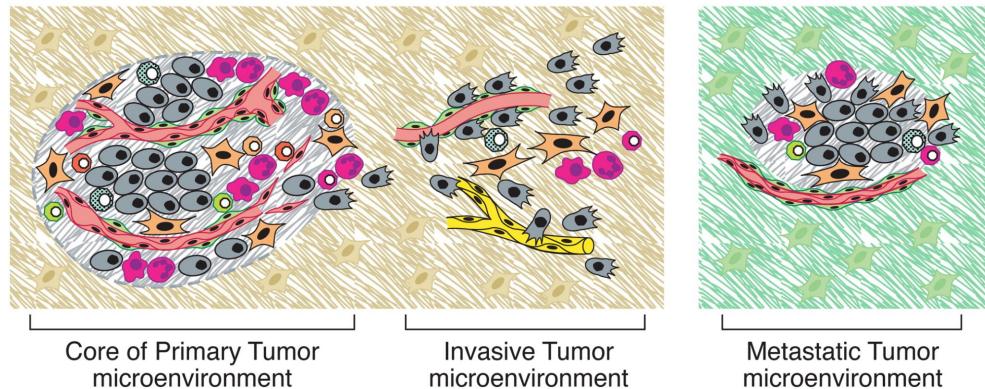
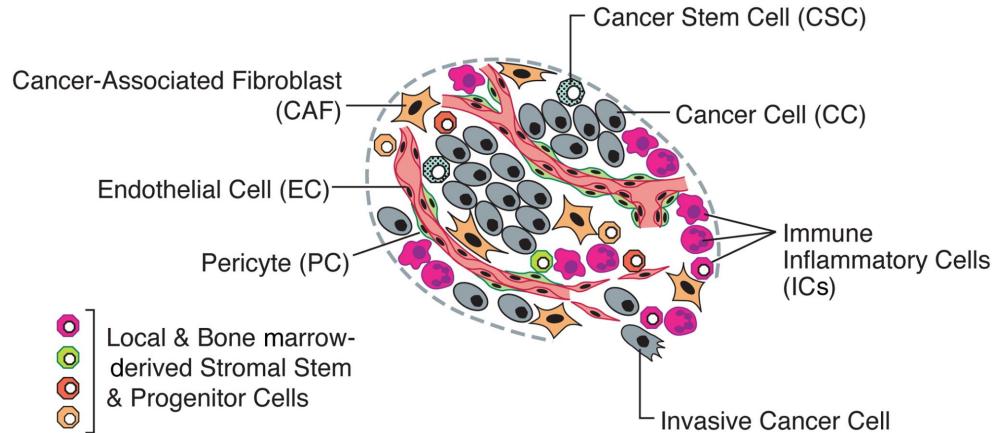


Emerging Hallmarks and Enabling Characteristics

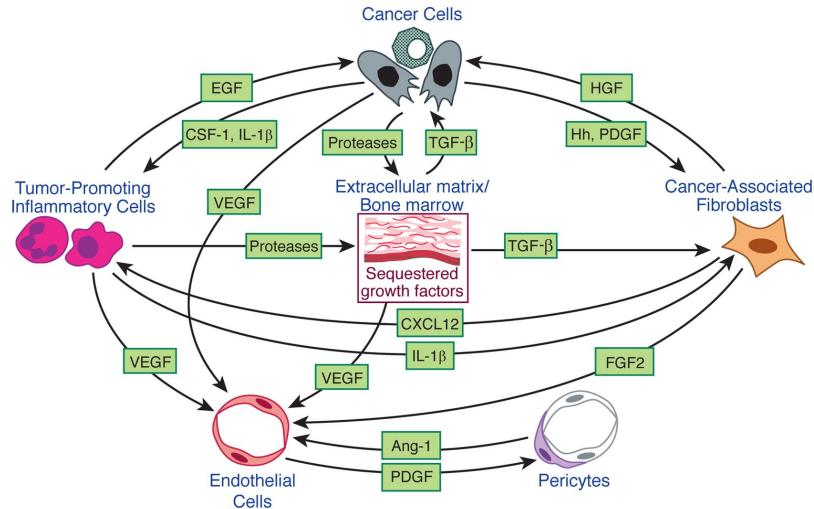


The Cells of the Tumor Microenvironment

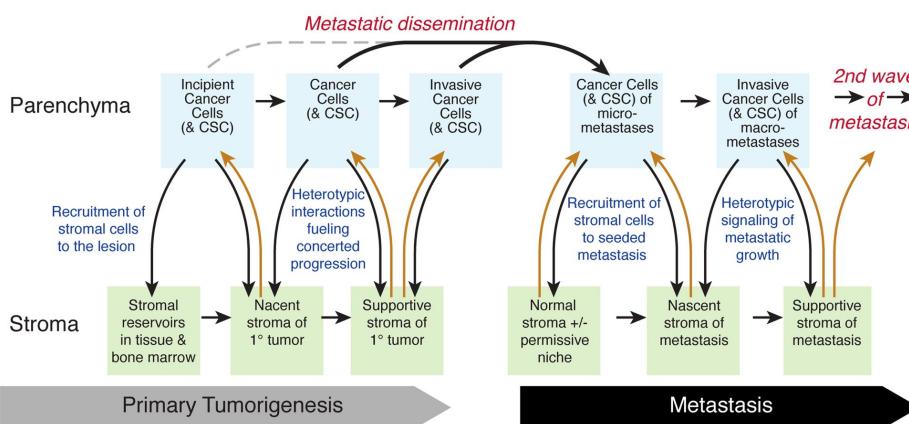
- Solid tumors are composed of a diverse collection of cell types, including both tumor cells (parenchyma) and supporting tissue (stroma), with the immune cell component exhibiting both tumor-promoting and tumor-inhibiting activities, all contributing to tumor growth and progression
- Tumor progression is driven by evolving microenvironments created by diverse stromal cell types and extracellular matrix, which change as the tumor invades local tissue and metastasizes, with surrounding normal cells also likely influencing these microenvironments



Signaling Interactions in the Tumor Microenvironment during Malignant Progression

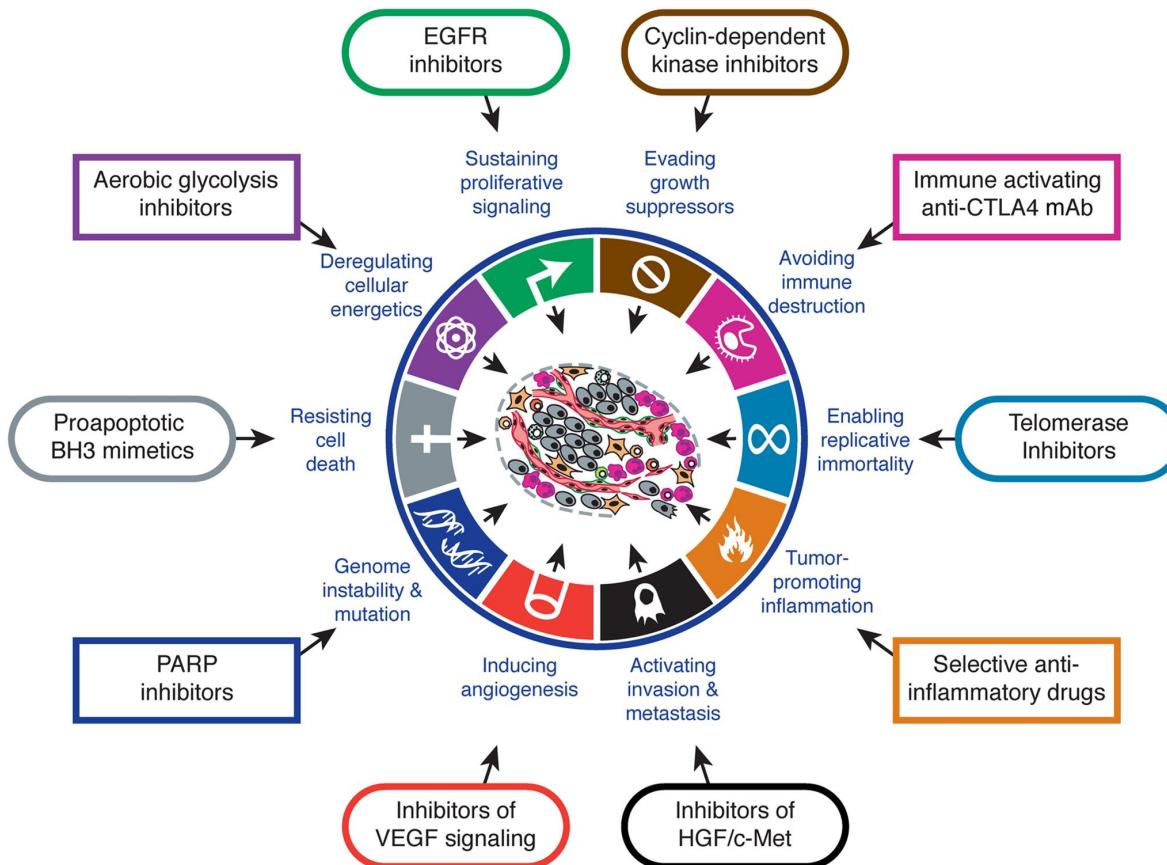


- Reciprocal signaling between cancer cells and stromal cells within the tumor microenvironment drives tumor progression, with these interactions evolving over time and contributing to increasingly aggressive phenotypes, including metastasis.
- The propensity for metastasis can be established early and influenced by both the cell's origin and oncogenic changes, while certain organ sites offer favorable conditions ("metastatic niches") for colonization, and cancer stem cells may play a role in various stages of tumor development and spread

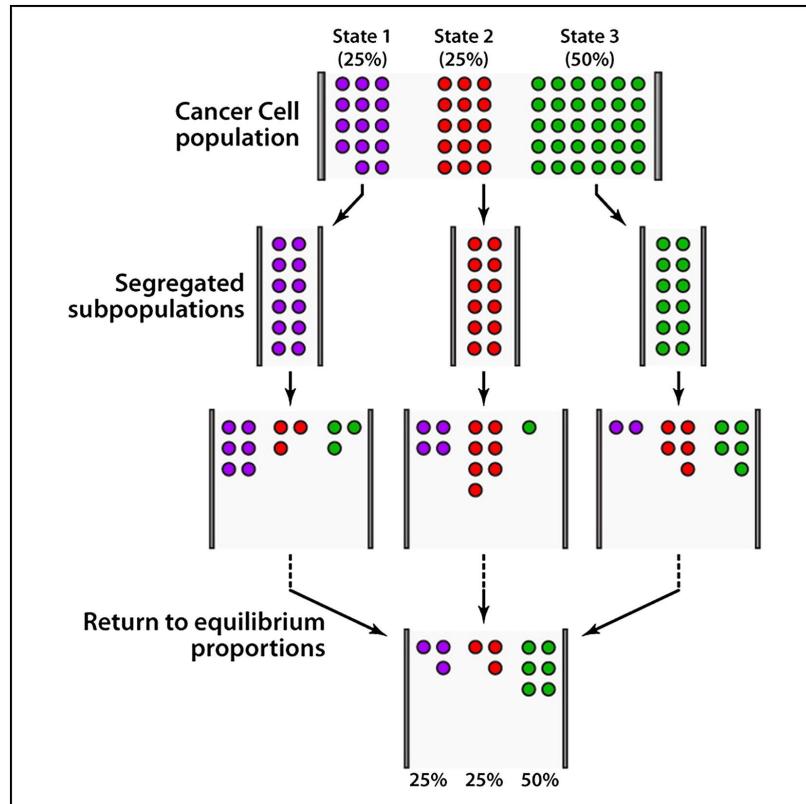


Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646-674

Therapeutic Targeting of the Hallmarks of Cancer



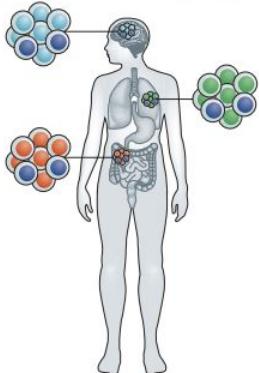
The stochasticity of molecular alterations drives continuous tumor evolution, generating cellular heterogeneity characterized by diverse genetic and epigenetic features



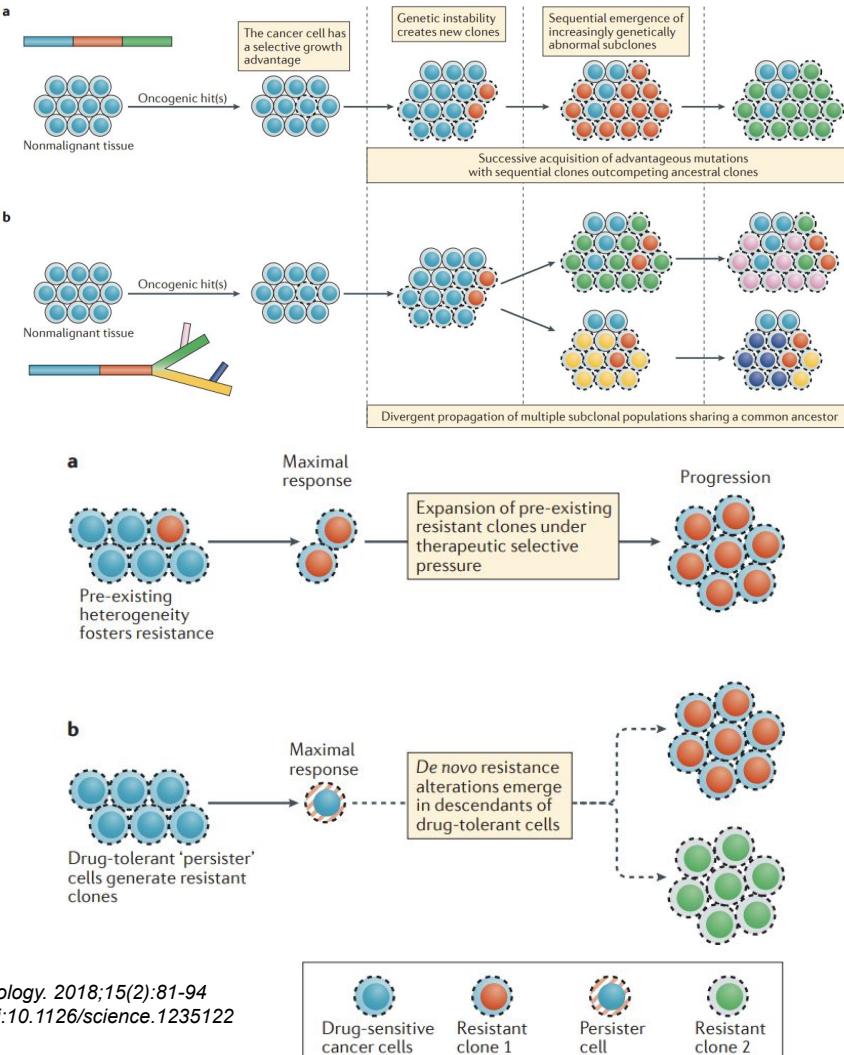
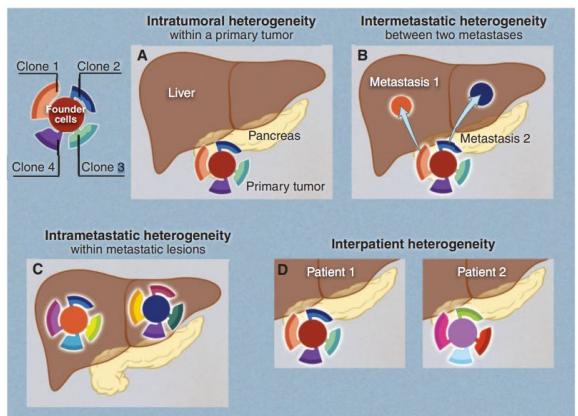
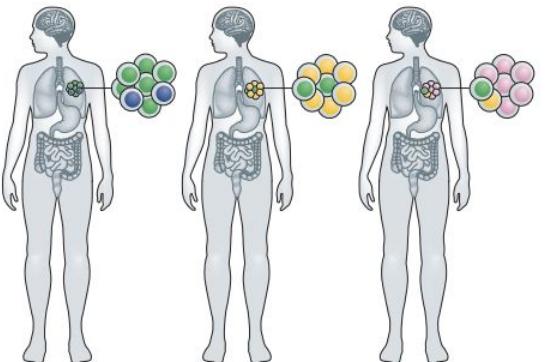
- Cancer cell populations interconvert between phenotypic states.
- Cell-state transitions can be described with a stochastic Markov model.
- Markov model predicts convergence to equilibrium phenotypic proportions
- Cancer stem cells can arise de novo from non-cancer stem cells

Tumor heterogeneity

a Spatial heterogeneity

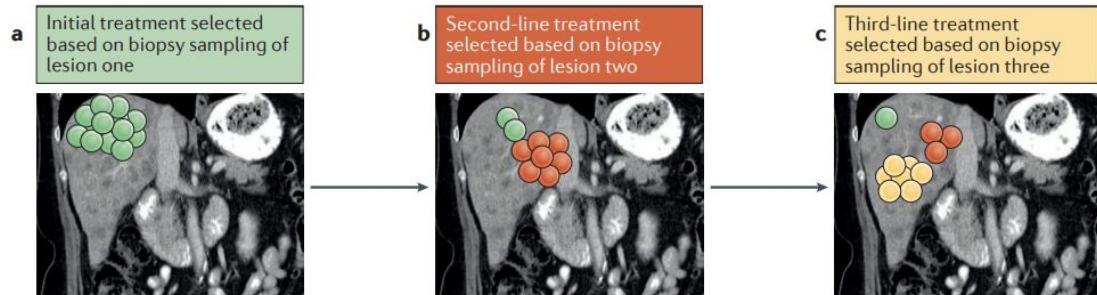


b Temporal heterogeneity



Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nature Reviews Clinical Oncology*. 2018;15(2):81-94
 Vogelstein, Bert et al. "Cancer genome landscapes." *Science (New York, N.Y.)* vol. 339,6127 (2013): 1546-58. doi:10.1126/science.1235122

Application of longitudinal plasma profiling

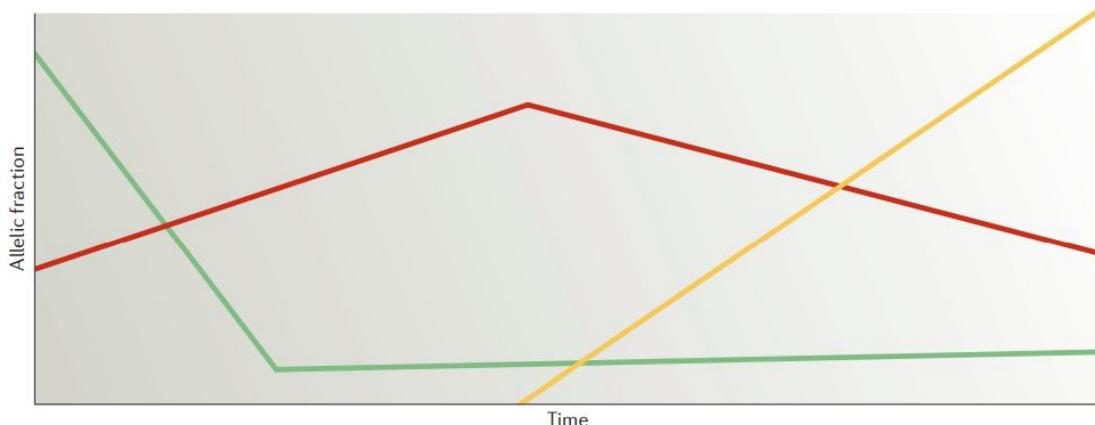


d

Plasma allelic fraction of variant at lesion one decreases, but allelic fraction of variants at other sites increases

Plasma allelic fraction of variant at lesion one is undetectable. The allelic fraction of variant at lesion two decreases in response to treatment

After therapy for lesion two is initiated, plasma allelic fraction of variant at lesion three continues to increase, corresponding to the appearance of a new lesion on imaging



Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nature Reviews Clinical Oncology*. 2018;15(2):81-94

Genes in oncogenesis

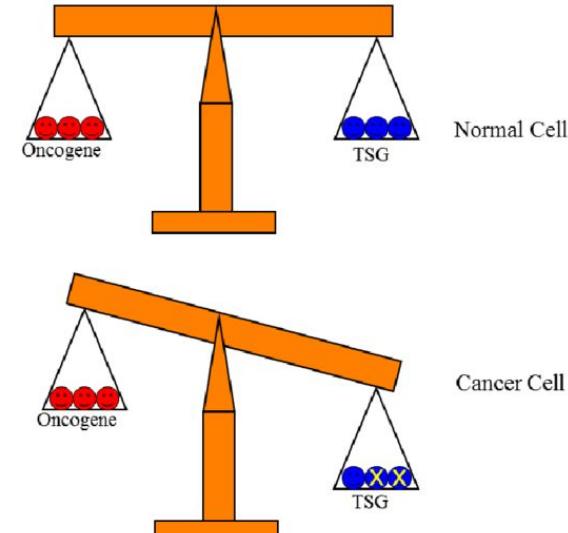
Oncogenes:

- Proto-oncogenes:
 - Normal genes regulating cell proliferation
 - Under control: Stable
 - Mutated: Gain of function => Oncogenes

Tumor suppressor genes:

- Gate keepers: control cell proliferation
- Care-takers: repair DNA damage
- Mutated: Loss of function

Functional balance/imbalance of Oncogene and Tumor suppressor gene (TSG) in normal and cancer Cells



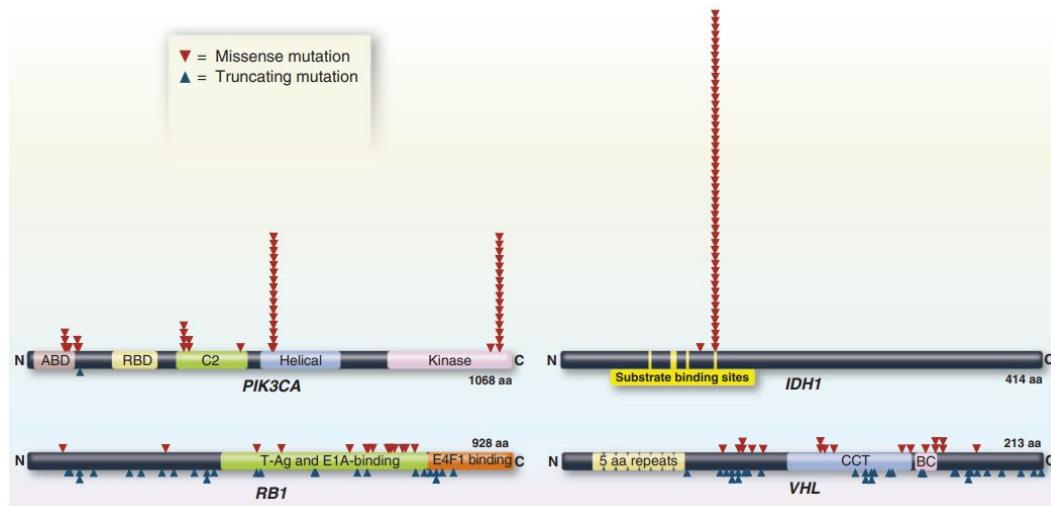
Mutational characteristics in Oncogenes and Tumor Suppressor Genes (TSG)

Mutation in Oncogenes

- Mutation hotspots
- Missense mutations => Gain of function of the protein

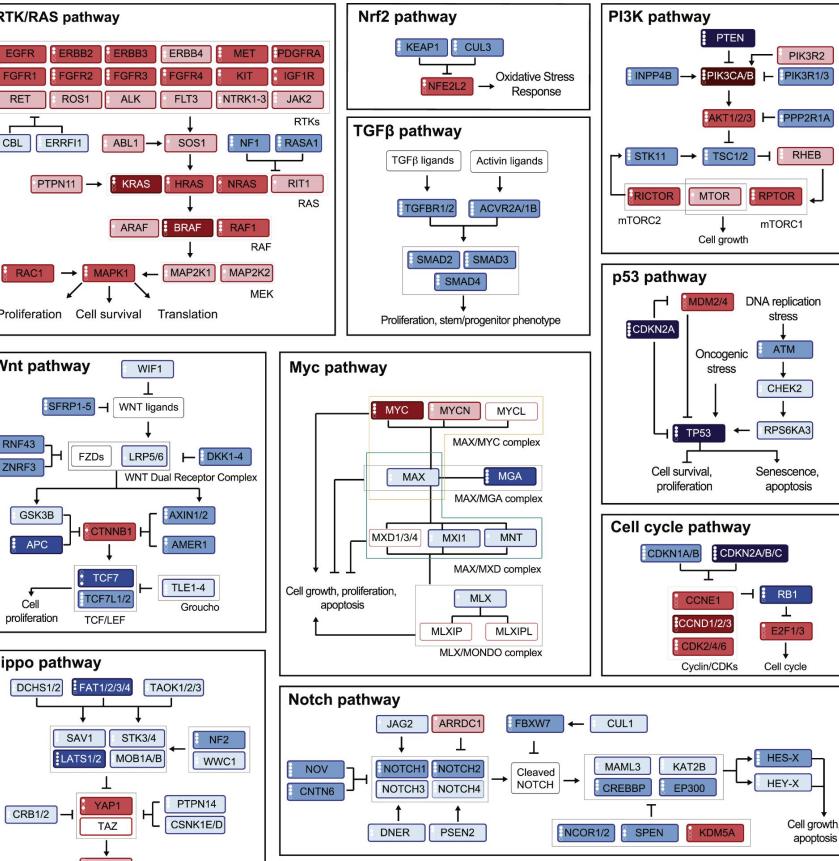
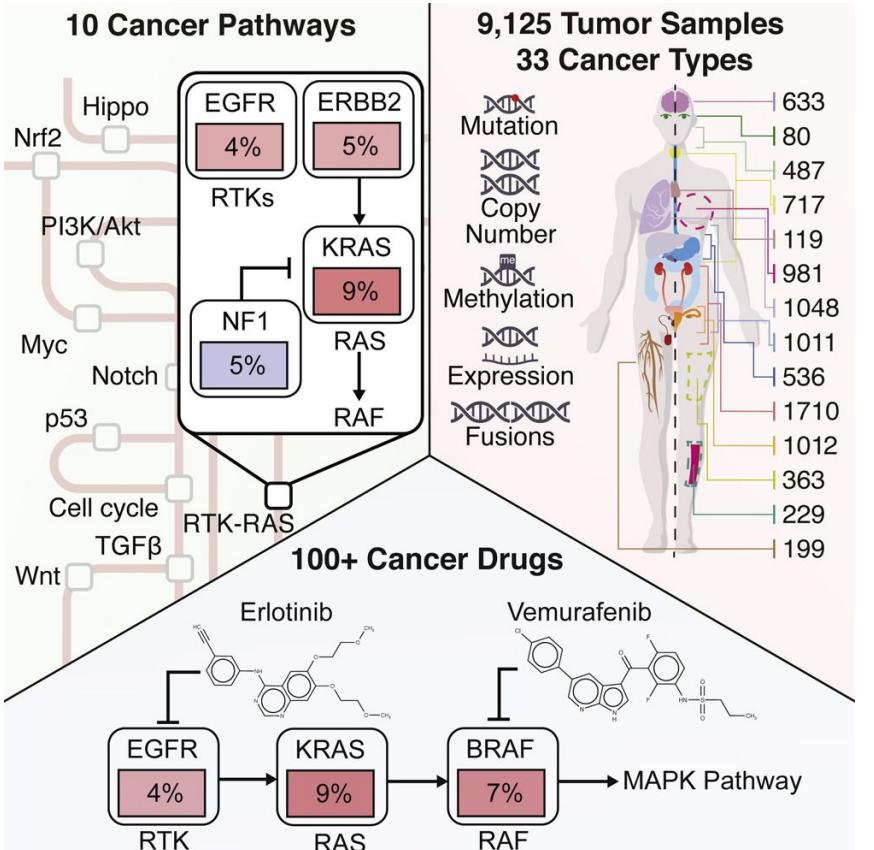
Mutations in Tumor suppressor genes

- Across the whole gene length
- Missense or Nonsense (truncating) mutations => Loss of function of the protein



Distribution of mutations in two oncogenes (PIK3CA and IDH1) and two tumor suppressor genes (RB1 and VHL)

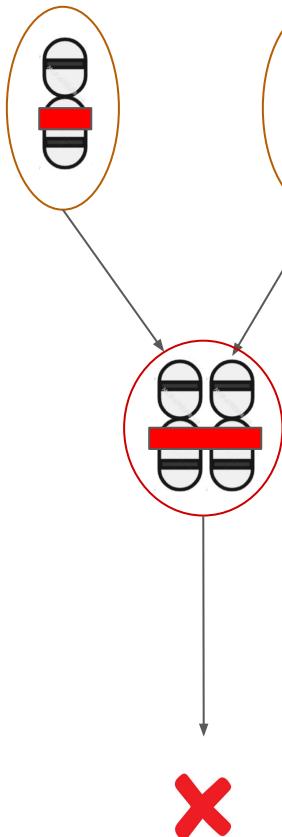
Biological pathways in cancer



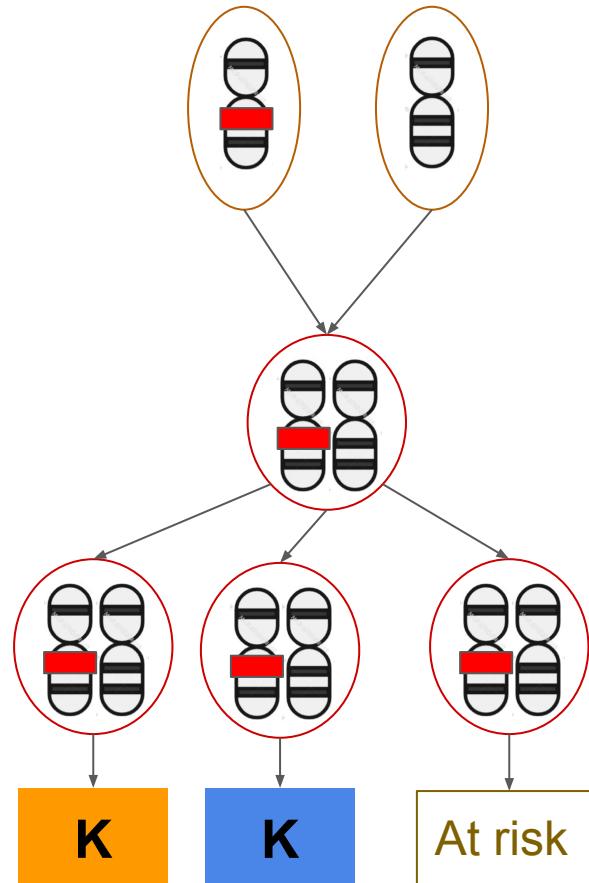
Sanchez-Vega, Francisco et al. "Oncogenic Signaling Pathways in The Cancer Genome Atlas." Cell vol. 173,2 (2018): 321-337.e10. doi:10.1016/j.cell.2018.03.035

Oncogenes - Pathogenesis

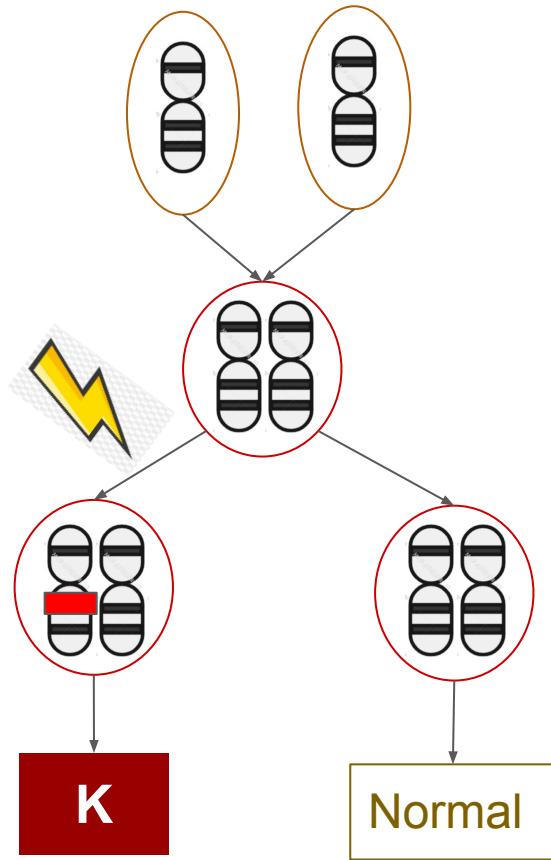
Germline mutation



Germline mutation

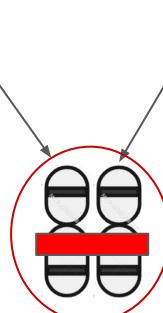
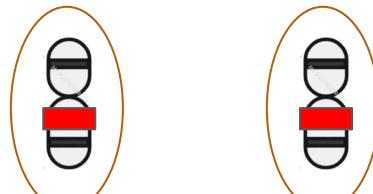


Somatic mutation

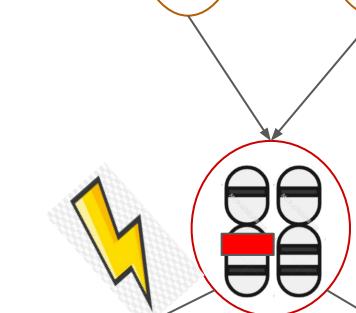
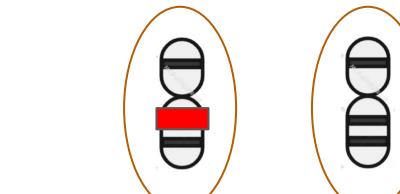


TSGs - Pathogenesis

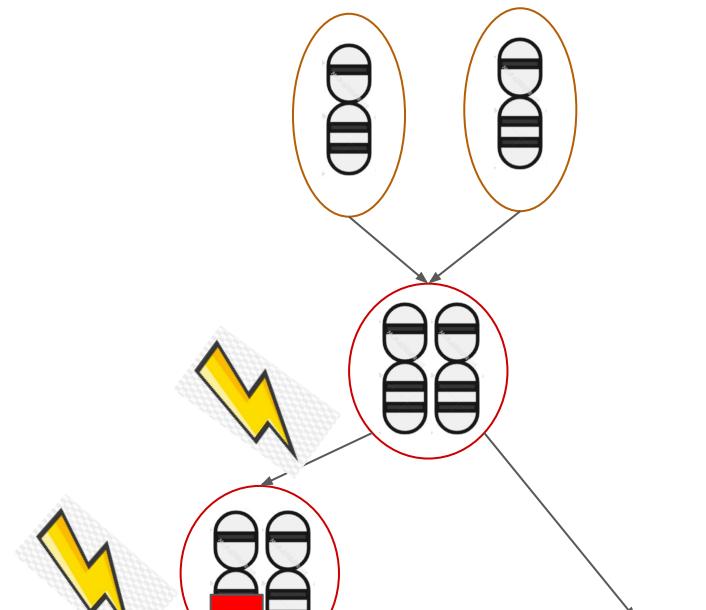
Germline mutation



Germline mutation



Somatic mutation



K

At risk

K

At risk

Normal

Dual oncogenic and tumour suppressive functions

- While cancer genes are generally classified as either oncogenes or tumor suppressors, some genes can exhibit both functions depending on the context.¹
- This duality often stems from the production of multiple isoforms that are further modified and interact with other proteins.²
- In diploid organisms, gain-of-function (GOF) mutations in oncogenes are typically dominant (single events are sufficient to promote tumorigenesis), while loss-of-function alterations are recessive in TSGs (requires two inactivation events).³

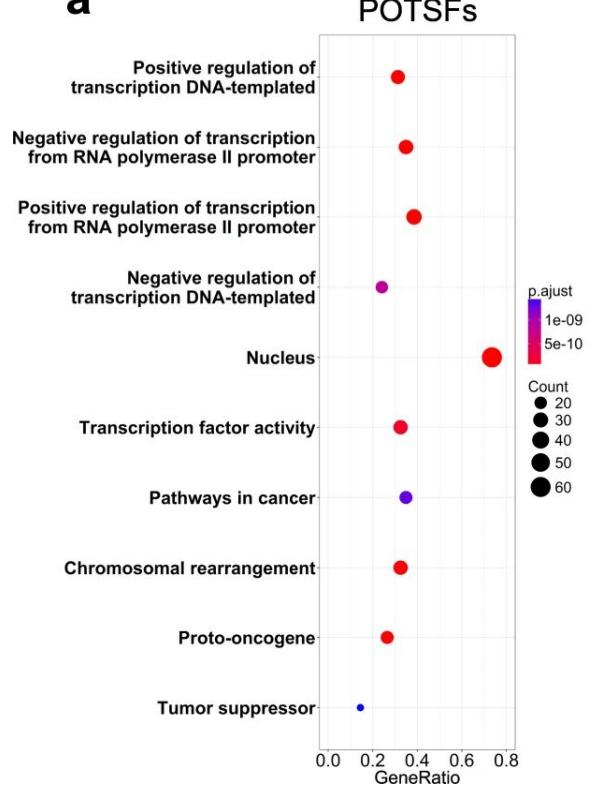
(1) Shen L, Shi Q, Wang W. Double agents: Genes with both oncogenic and tumor-suppressor functions. *Oncogene*. 2018;7(3):25

(2) Aranko AS, Oeemig JS, Kajander T, Iwai H. Intermolecular domain swapping induces intein-mediated protein alternative splicing. *Nature Chemical Biology*. 2013;9(10):616-622

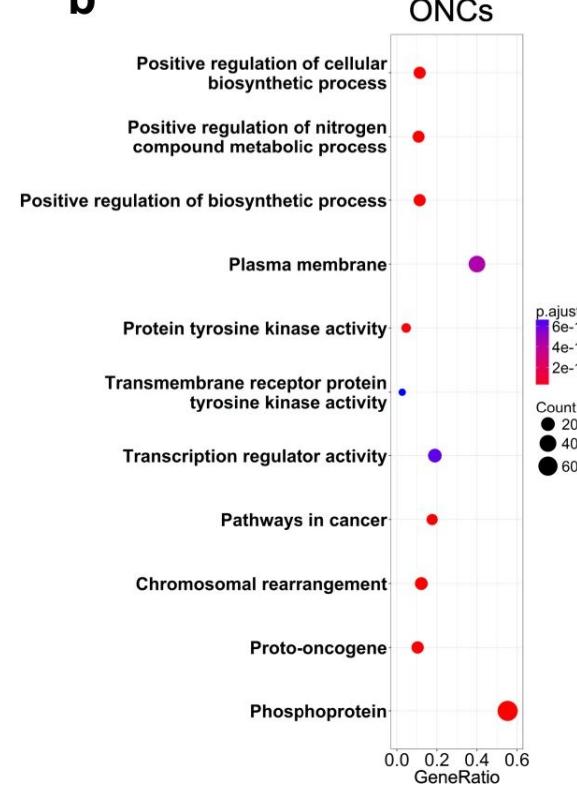
(3) Knudson AG. Two genetic hits (more or less) to cancer. *Nature Reviews Cancer*. 2001;1(2):157-162

Proto-oncogenes with tumor-suppressor function (POTSFs), oncogenes (ONCs), and tumor-suppressor genes (TSGs)

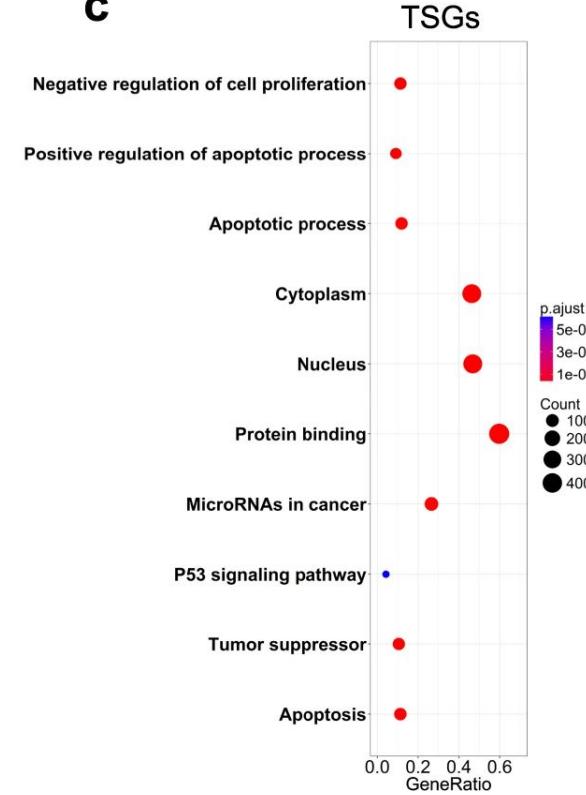
a



b



c



Recessive state

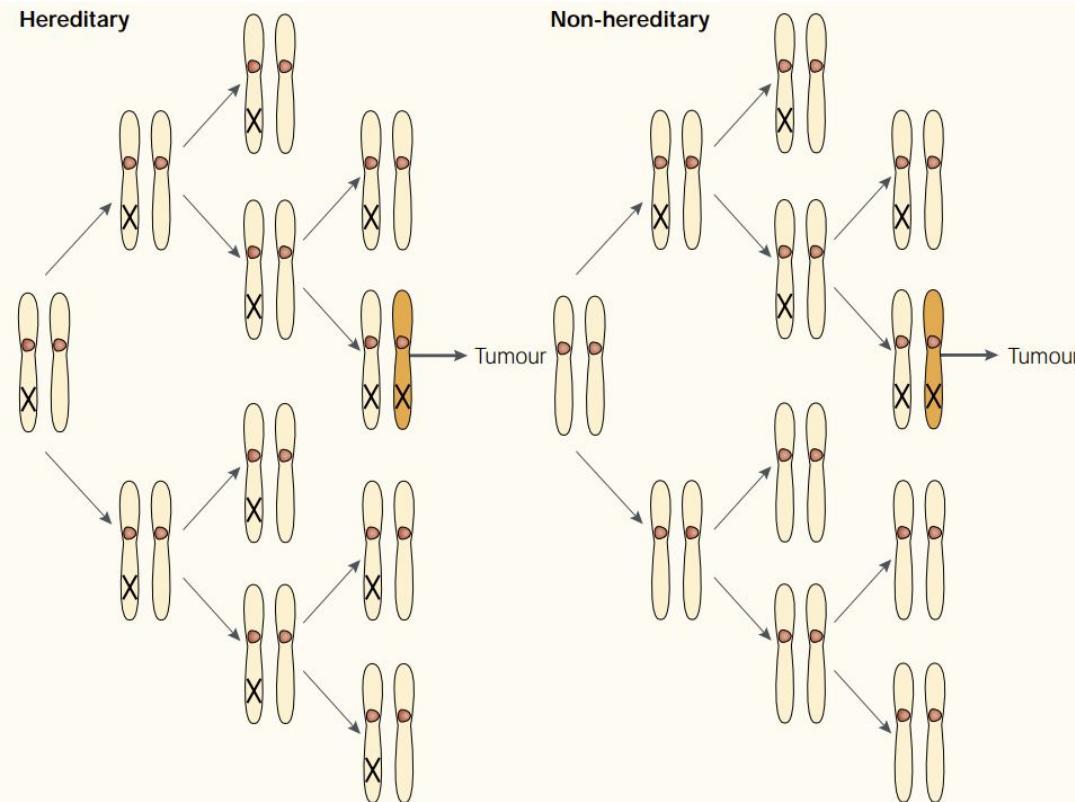
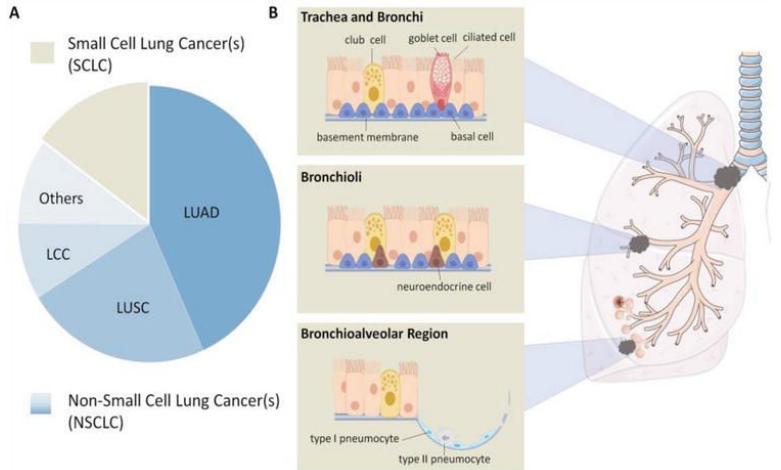


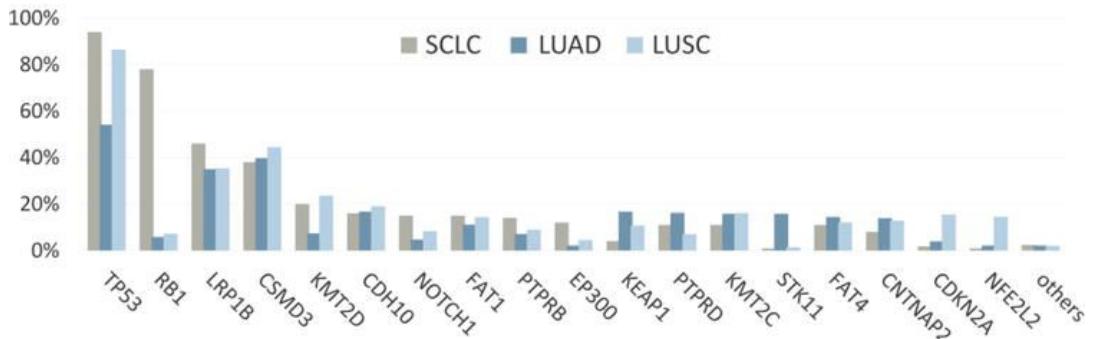
Figure 4 | Two-hit tumour formation in both hereditary and nonhereditary retinoblastoma. A 'one-hit' clone is a precursor to the tumour in nonhereditary retinoblastomas, whereas all retinoblasts (indeed, all cells) are one-hit clones in hereditary retinoblastoma.

Knudson AG. Two genetic hits (more or less) to cancer. *Nature Reviews Cancer*. 2001;1(2):157-16

TSG mutation spectrum in lung cancer

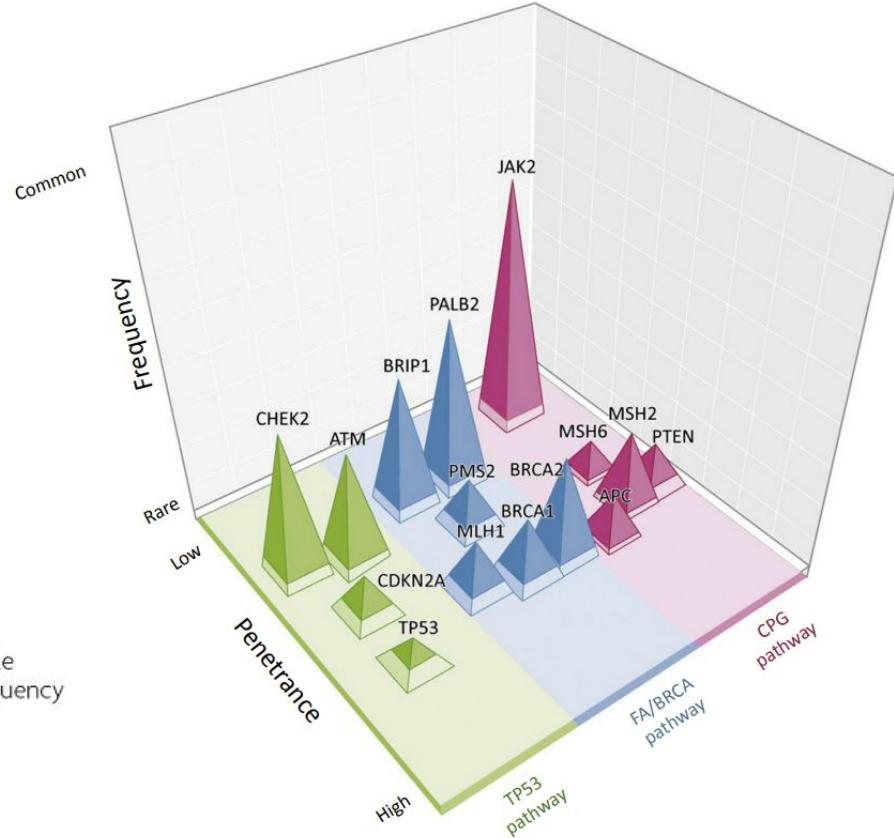
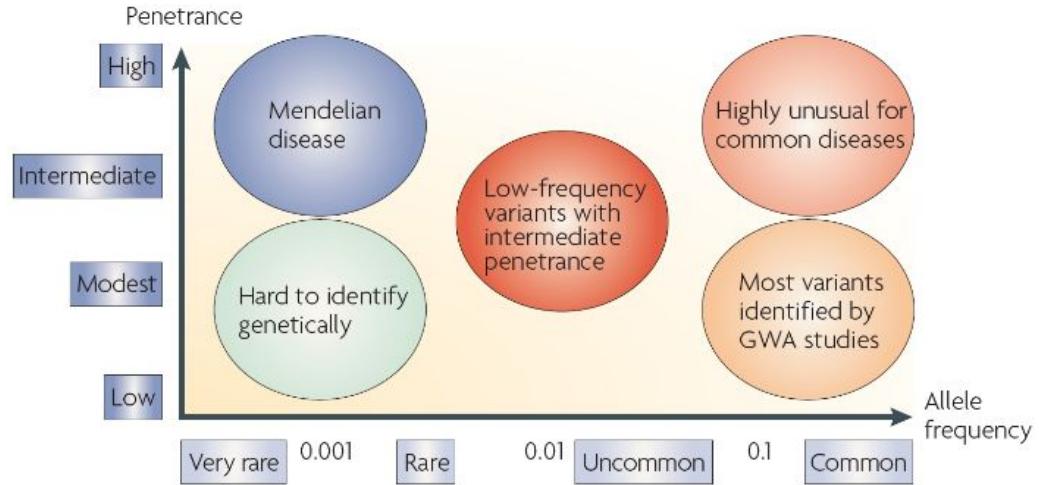


Mutational Frequency of TSG in Lung Cancer



Gene	Main function	Role as TSG	Role as oncogene
<i>TP53</i>	TF: regulates cell cycle, DNA repair, senescence and apoptosis	TSG in several tissues: frequently lost through mutations [34]	Misense mutations confer gain-of-function oncogenic properties [31]
<i>NF1B</i>	TF: crucial in lung development	Underexpressed in NSCLC and associated with poor survival in LUAD [32]	Amplified and OE in SCLC: inducing chromatin reprogramming during metastasis [33]
<i>NOTCH1/NOTCH2</i>	Transmembrane receptors: proliferation, differentiation and survival	Inactivated by inhibitor ligands and through mutations, especially in SCLC [34]	Maintains stem cell features; promotes proliferation in LUAD [35]
<i>NFE2L2</i>	TF: cellular defense mechanism against oxidative stress	Protects lung tissue against exposure to oxidative stress [36]	Mutational activation: aids cells to escape from endogenous tumour suppression [37]
<i>NKX2-1</i>	TF: essential for lung development	Acts as a TSG in KRAS-driven p53-mutant LUAD [38]	Enhanced oncogenic signals in EGFR-driven LUAD [39]
<i>STK11</i>	Serine-threonine kinase: regulation of energetic metabolism and cell polarity	Mutational inactivation promotes cancer development [40]	OE maintains metabolic homeostasis and attenuates oxidative stress [40]
<i>TGFβ</i>	Cytokine: regulates development, differentiation and homeostasis	Expression loss leads to growth arrest in early-stage lung and other cancers [41]	OE promotes tumour growth in advanced cancer stages [42]
<i>TUSC3</i>	Endoplasmic reticulum protein in magnesium uptake, glycosylation and embryonic development	Hypermethylation; expression loss in NSCLC; inhibits cell proliferation and promotes apoptosis [43]	OE in NSCLC accelerates cancer growth; induces EMT [44]
<i>WT1</i>	TF: role in urogenital system development	Loss of function enhances cell viability and proliferation in Wilms' tumour [45]	OE promotes survival in KRAS-mutated NSCLC [46]
<i>MALAT1</i>	Long non-coding RNA	OE reduces invasiveness in PTEN expressing tumours [47]	OE associated with chemotherapy resistance in NSCLC [48]
<i>miR-125b</i>	microRNA	OE induces apoptosis [49]	OE promotes metastasis [50]
<i>miR-378</i>	microRNA	OE reverses chemoresistance to cisplatin in LUAD [51]	OE is associated with invasion and brain metastasis [52]

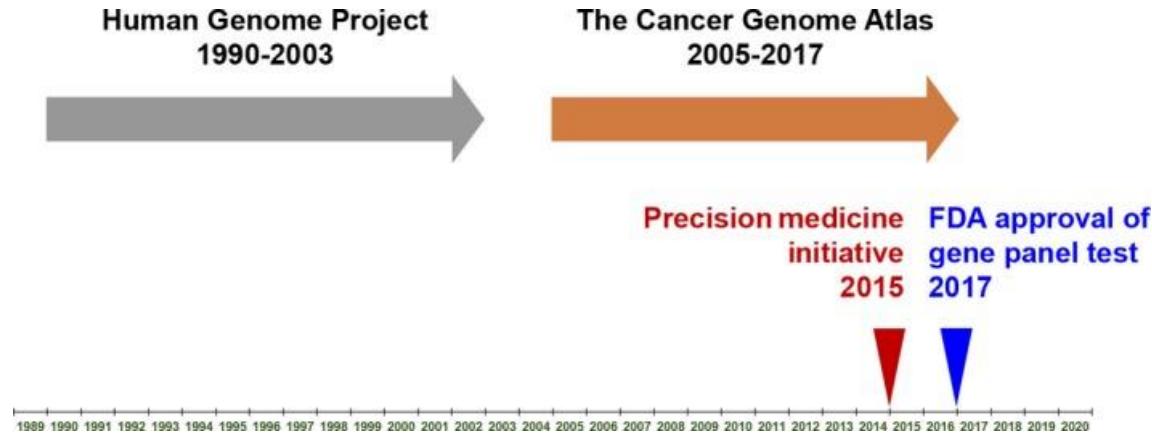
Frequency and Penetrance



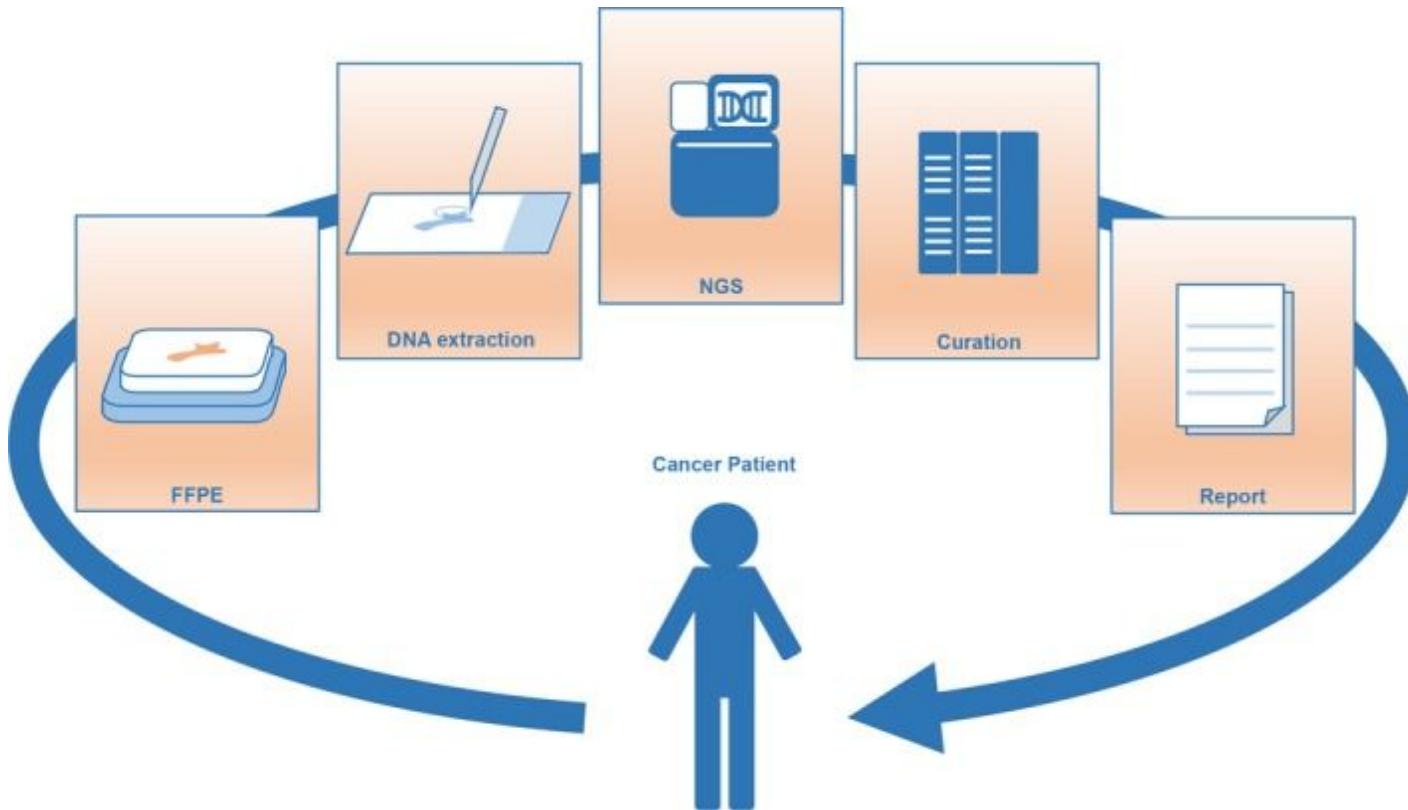
1. <https://www.nature.com/scitable/topicpage/multifactorial-inheritance-and-genetic-disease-919/>
2. Taeubner, Julia et al. "Penetrance and Expressivity in Inherited Cancer Predisposing Syndromes." *Trends in cancer* vol. 4,11 (2018): 718-728. doi:10.1016/j.trecan.2018.09.002

Principles in Cancer Gene Panel Testing

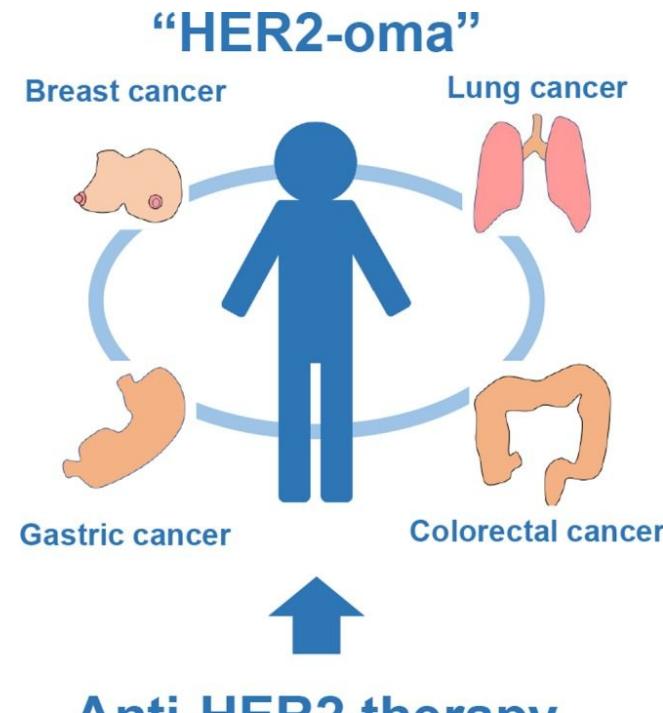
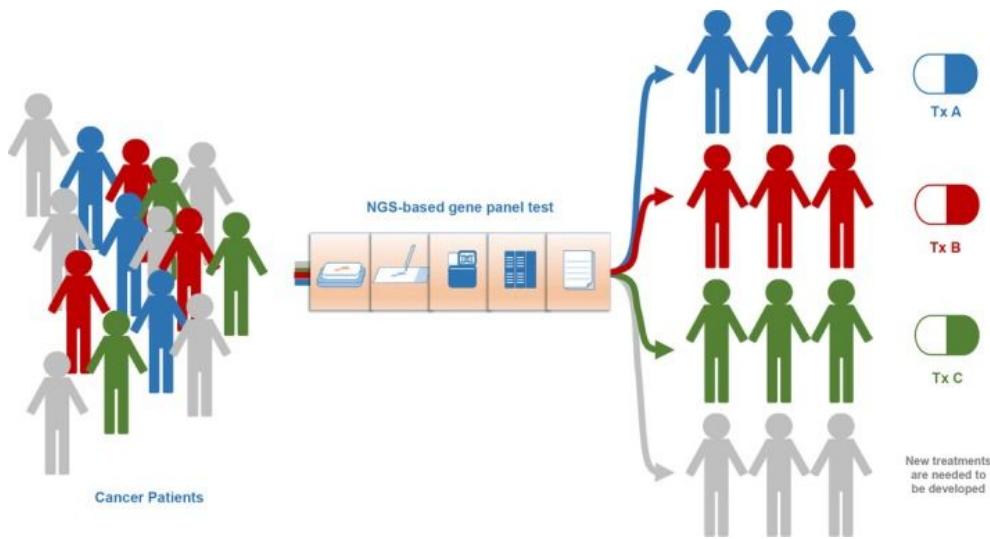
Determination of the human and cancer genomes



General procedure



Precision cancer medicine utilizing next generation sequencing (NGS)-based gene panel testing



Advantages of cancer gene panel testing

Increased clinical efficiency

- Quicker turnaround time than sequential testing
- Testing approach based less on a stringent phenotype
- Informed consent and sample collection at one clinic visit
- One time insurance review

Potential cost savings

- Lower per-gene cost
- Identification of co-occurrence of mutations in different genes

Improved detection of cancer susceptibility mutations in patients

- Atypical cancer phenotypes
- Absent family history information (adoption)
- Family history not meeting standard testing criteria
- Family history meeting >1 cancer family syndrome criteria

Technology

- Constant improvements in gene capture and analysis
- Flexible platform that can add additional genes

Disadvantages of cancer gene panel testing

Identification of mutations and variants in

- Moderate-penetrance genes
- High-penetrance genes unrelated to clinical features in patient and family members

Clinical utility not yet studied

- Some commercial health insurance plans designate panels as “investigational”
- Lack/unclear insurance coverage
- Lack of evidence-based management strategies for many of the rare syndromes or gene mutations

Increased prevalence of VUS

- Many genes on panels have not been widely tested in populations
- Commercial laboratories use separate processes to discern whether variant is clinically actionable
- Additional burden on clinical staff to store and track VUS results
- Patients and healthcare providers may mistakenly manage VUS as deleterious mutations

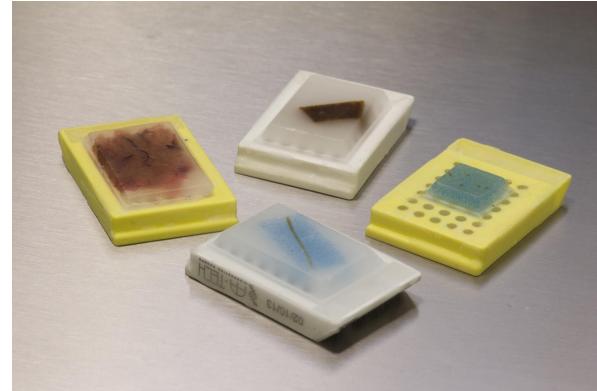
Technology

- Methodology rapidly changing, which pressures laboratory question/answer and test development processes
- Lab-specific processes may limit the interrogation of specific regions
- Difficult to compare analytical utility across several laboratories

Different methods and specimens for testing

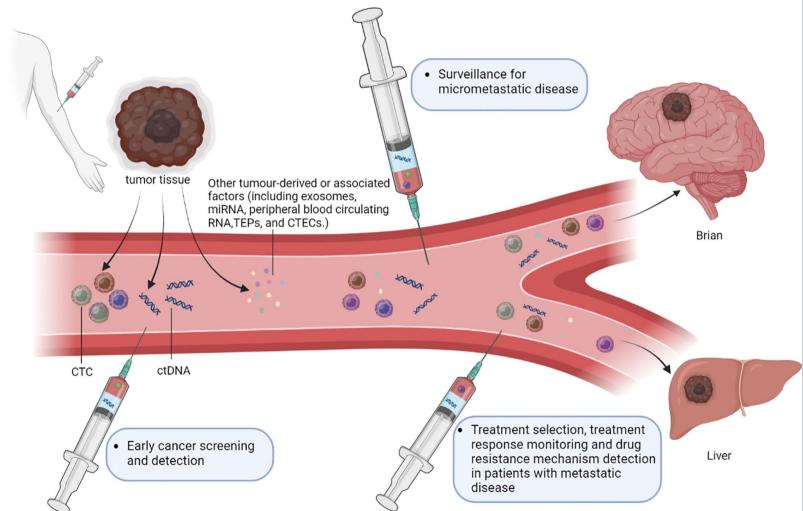
Mutations in tumors:

- Fresh tissues
- FFPE tissues
- Cytology samples
- ctDNA testing



Germline mutations:

- Blood (leukocyte DNA testing)
- Normal cell in mucous, saliva, etc.



Normal tissue



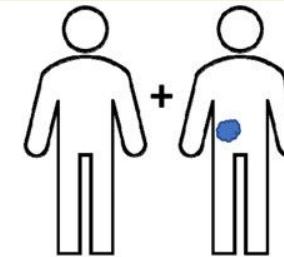
Germline result

Tumor tissue



Tumor result

Paired normal and tumor



Germline and somatic results

Pros	Assess inherited risk Cancer screening Counseling for families Reproductive planning	Inform diagnosis and prognosis Guide therapeutic decisions for targeted therapies Assess MSI for immunotherapies	Differentiation and integration of germline v somatic variants Guides therapy Allows genetic counseling, screening, and reproductive planning
	Limited to patients who meet guidelines Testing difficulties (eg, clonal hematopoiesis and mosaicism)	Inability to distinguish somatic v germline variants Inadequate surrogate for direct germline testing Need for further genetic testing and potential delays in care	Increased costs and resources, particularly related to genetic consents and counseling Specialized curation and interpretation by molecular pathologist

Testing in solid tumors

Target

- Cancer patient

Purposes

- Treatment selection
- Germline mutation screening

TABLE 2. Selected Genetic Alterations Linked to FDA Approvals as of June 2021^a

Genetic Alterations	Tumor Type	Targeted Therapeutics
FDA-approved treatments for specific genetic alterations in specific tumor types		
ALK fusions	NSCLC	Crizotinib, ceritinib, alectinib Brigatinib, lorlatinib
<i>BRAF</i> V600E	Melanoma	Dabrafenib, vemurafenib Dabrafenib + trametinib, encorafenib + binimetinib, vemurafenib + cobimetinib, trametinib
	Anaplastic thyroid cancer	Dabrafenib + trametinib
	NSCLC	Dabrafenib + trametinib
	CRC	Encorafenib + cetuximab
<i>BRAF</i> V600K	Melanoma	Dabrafenib + trametinib, encorafenib + binimetinib, vemurafenib + cobimetinib, trametinib
Deleterious or suspected ^a deleterious germline or somatic mutations in <i>BRCA1</i> and/or <i>BRCA2</i>	Ovarian cancer, fallopian tube cancer, peritoneal cancer Prostate cancer	Olaparib, ^a rucaparib, niraparib ^b Olaparib, ^a rucaparib ^b
Deleterious or suspected deleterious germline mutations in <i>BRCA1</i> and/or <i>BRCA2</i>	Ovarian cancer, pancreatic adenocarcinoma	Olaparib
	HER2-negative breast cancer	Olaparib, talazoparib
Deleterious or suspected deleterious germline or somatic mutations in <i>ATM</i> , <i>BARD1</i> , <i>BRIP1</i> , <i>CDK12</i> , <i>CHEK1</i> , <i>CHEK2</i> , <i>FANCL</i> , <i>PALB2</i> , <i>RAD51B</i> , <i>RAD51C</i> , <i>RAD51D</i> , and <i>RAD54L</i>	Prostate cancer	Olaparib
<i>EGFR</i> exon 19 deletions, L858R	NSCLC	Afatinib, dacomitinib, erlotinib, gefitinib, osimertinib
<i>EGFR</i> exon 20 insertions		Amivantamab
<i>EGFR</i> nonresistant mutations other than exon 19 deletions and L858R		Afatinib
<i>EGFR</i> T790M		Osimertinib

Testing in hereditary cancer syndromes

Target

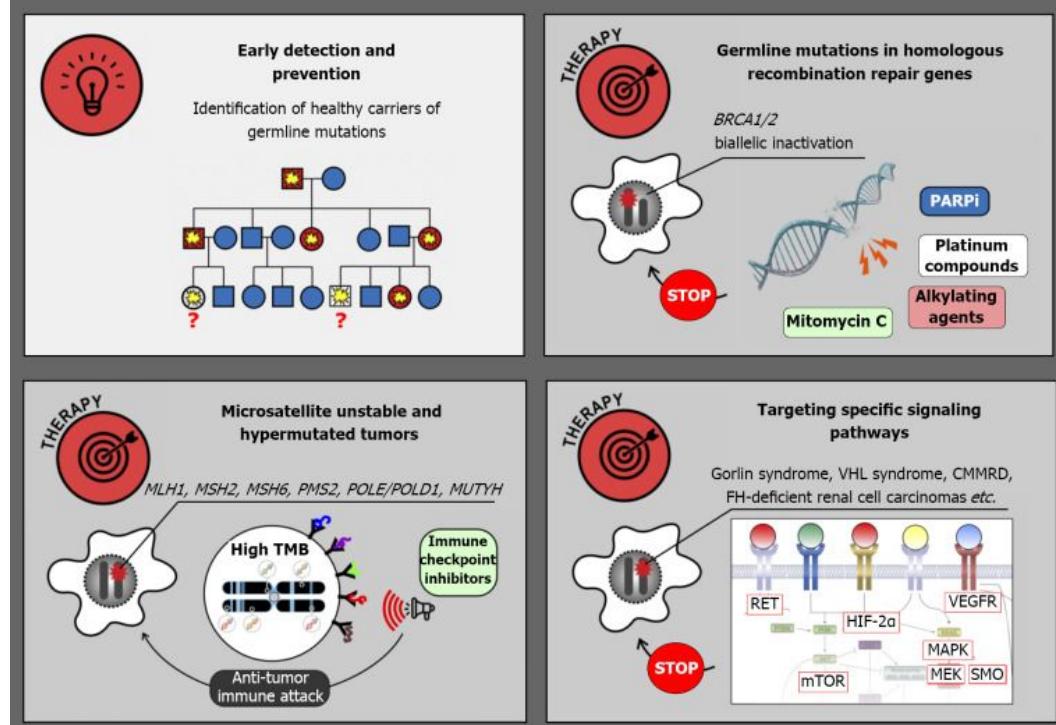
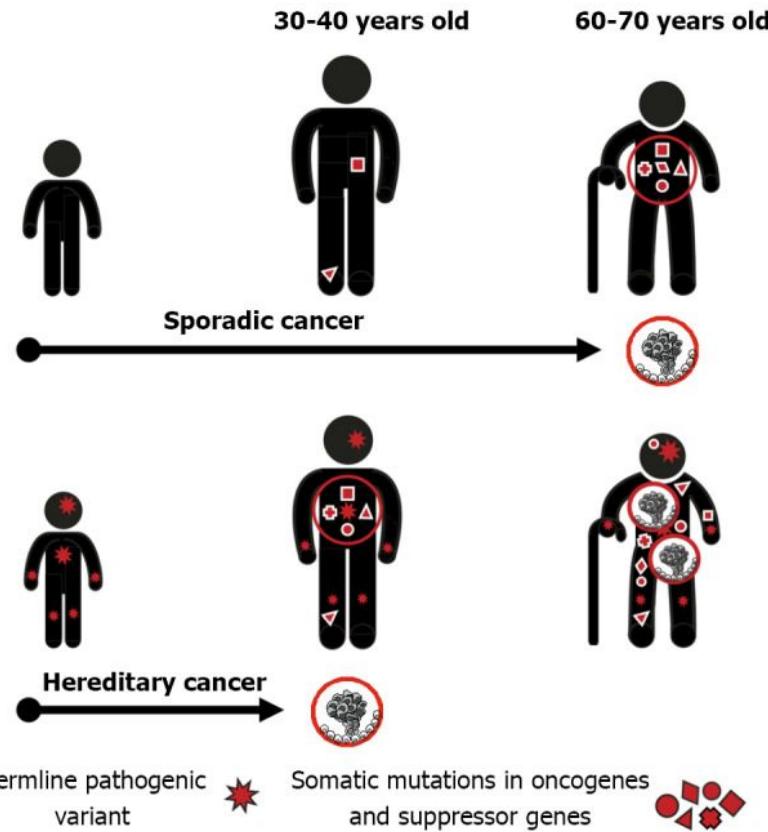
- Cancer patient
- Relatives of cancer patients having germline mutation
- General population (+/-)

Purposes:

- Germline mutation screening
- Cancer prevention

Syndrome	Acronym	Prevalence	Inheritance	Involved Genes
Hereditary paraganglioma-pheochromocytoma syndrome	HPPS	1–9:1,000,000	AD (SDHA, SDHB, SDHC, TMEM127) Paternal inheritance (SDHD, SDHAF2, MAX)	SDHA, SDHAF2, SDHB, SDHC, SDHD, MAX, TMEM127
Carney Complex	CNC	U	AD	PRKAR1A
Neurofibromatosis type 1	NF1	1:2600	AD	NF1
Neurofibromatosis type 2	NF2	1:60,000	AD	NF2
Schwannomatosis	SCHW	1:70,000	AD	SMARCB1, LZTR1
Multiple endocrine neoplasia type 1	MEN 1	1:10,000	AD	MEN1
Multiple endocrine neoplasia type 2A	MEN2A	1:44,000	AD	RET
Multiple endocrine neoplasia type 2B	MEN2B	1:700,000	AD	RET
Familial medullary thyroid carcinoma	FMTC	1:233,000	AD	RET
Multiple endocrine neoplasia type 4	MEN4	<1:1,000,000	AD	CDKN1B
Hyperparathyroidism-jaw tumor syndrome	HPT-JT	U	AD	CDC73
Parathyroid carcinoma syndrome	PC	U	AD	CDC73
HOXB13 hereditary cancer syndrome	HOXB13	U	AD	HOXB13 G84E

Mechanisms and management of hereditary cancer predisposition

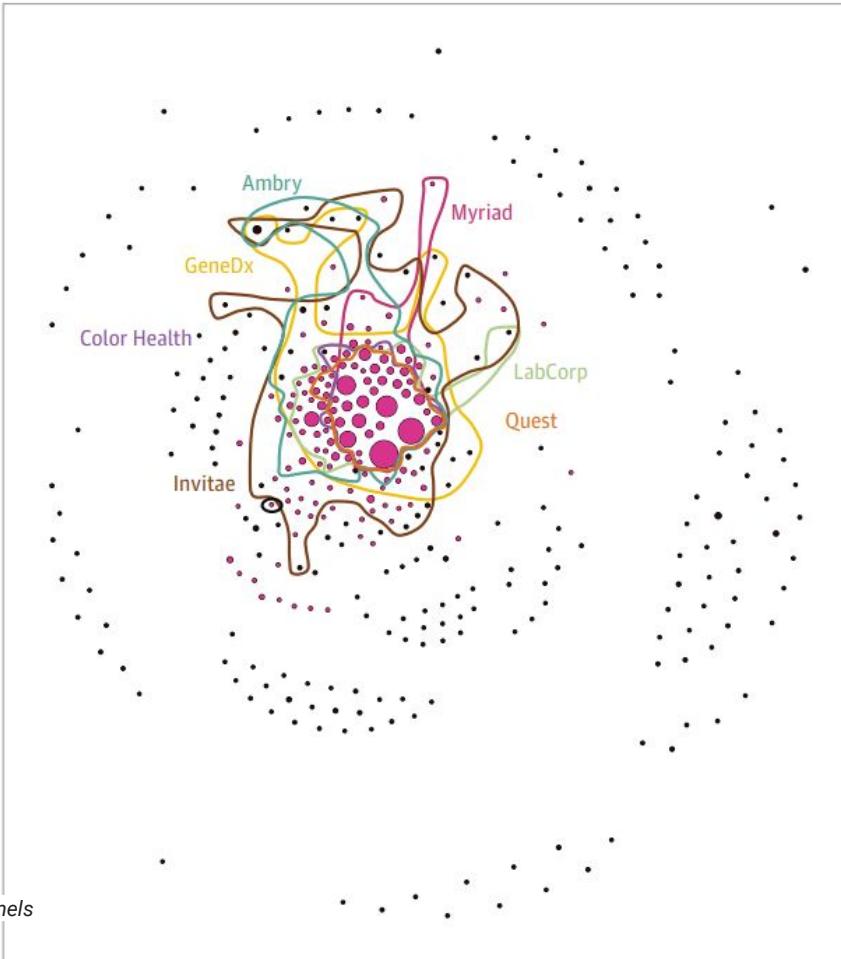


How many genes should be tested ?

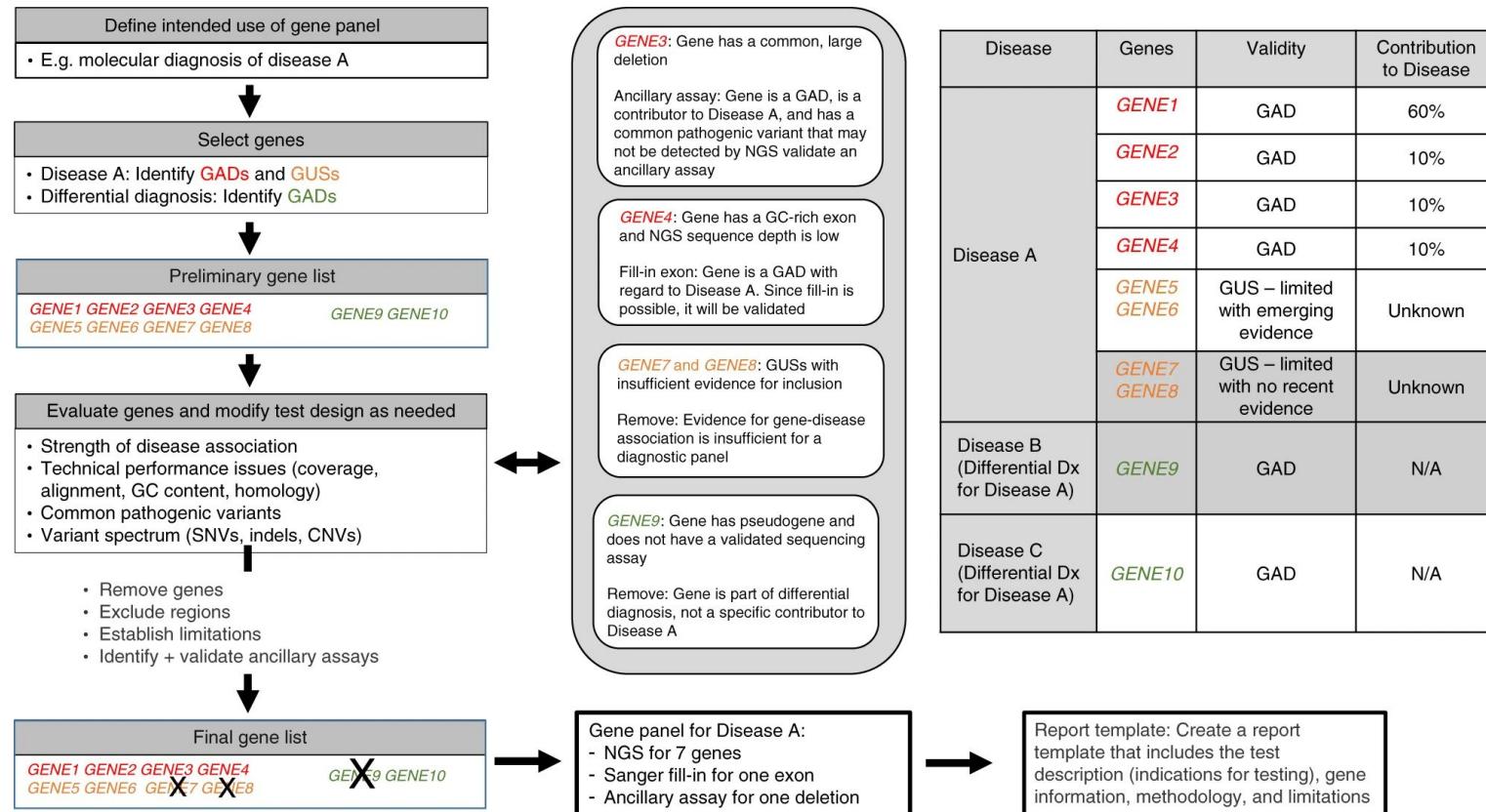
- **706** genes were included in at least 1 laboratory's panel
- **13** genes were included by all 17 companies
- **110** genes appeared in at least 1 clinical guidelines for hereditary cancer or had a ClinGen gene-disease relationship assessment

However, standards for which genes are offered in hereditary cancer panels and clinical guidelines are lacking

Figure. Network Depiction of Genes Appearing Together in a Test Panel, ClinGen Gene-Disease Evaluation, or Clinical Guideline



Workflow for design, evaluation, and implementation of a diagnostic gene panel

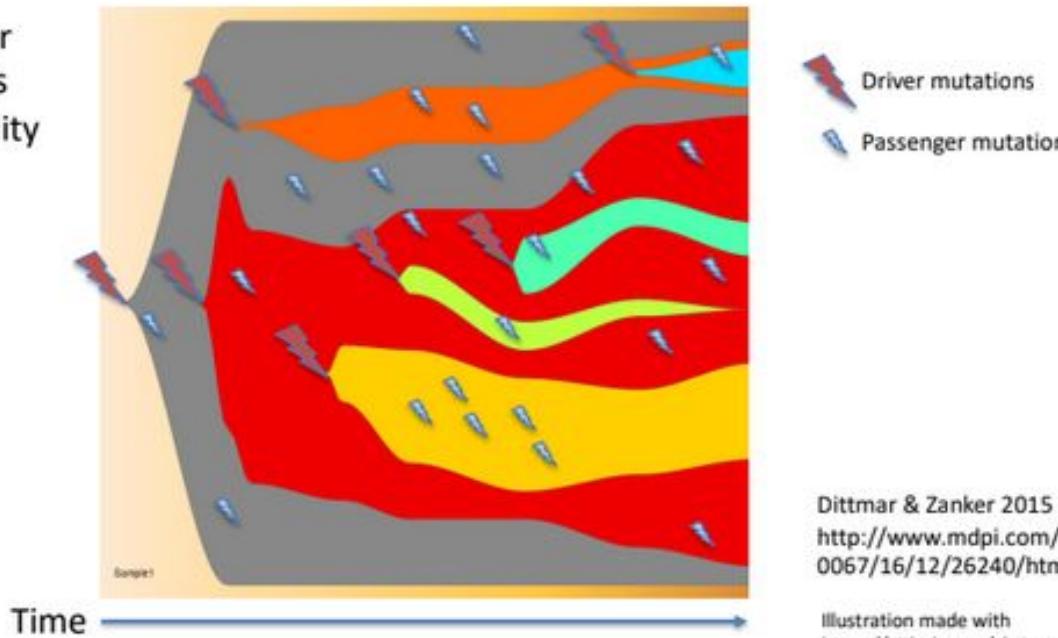


Introduction to Somatic Variant Discovery

Key considerations and workflow logic

Role of mutation events in tumor progression

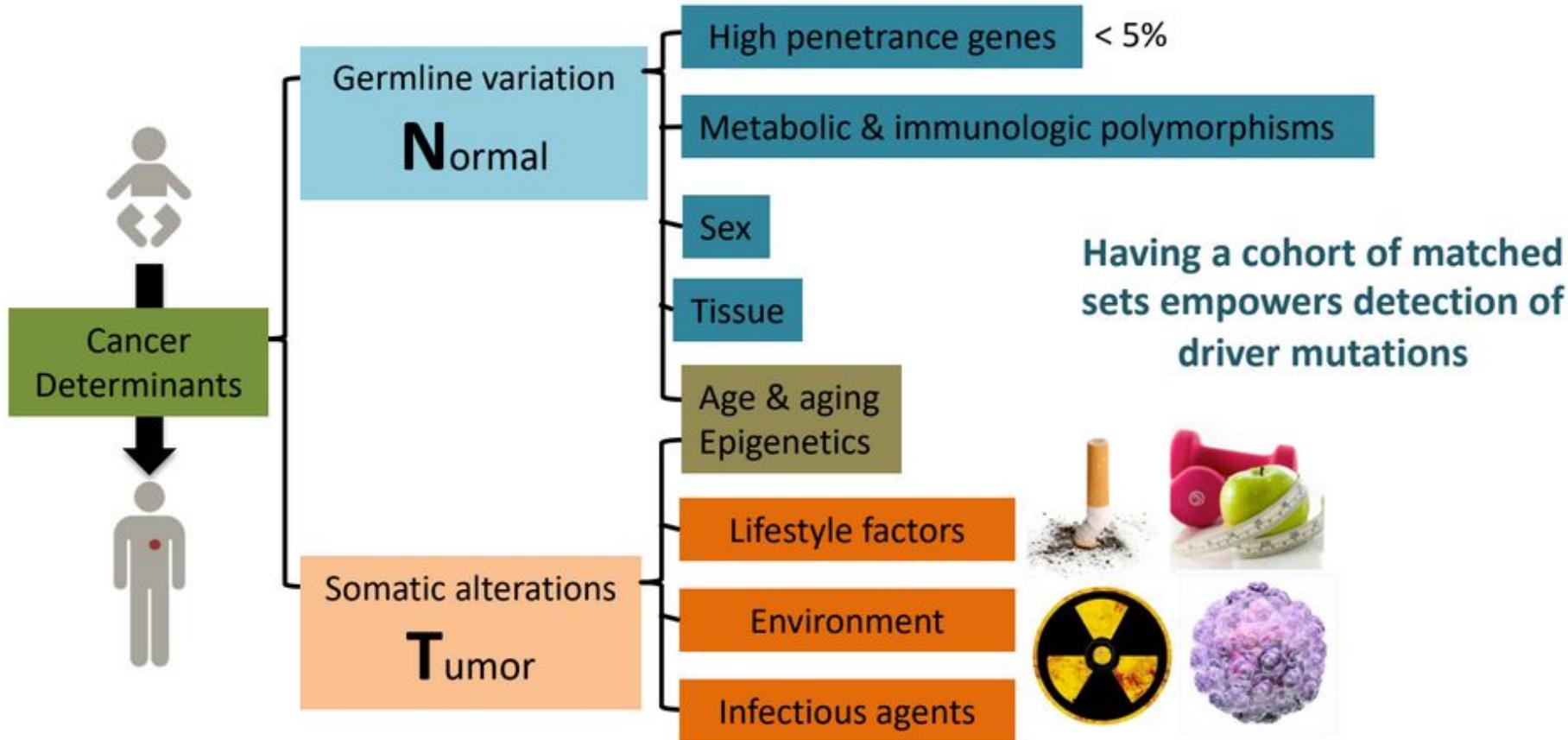
Increasing tumor heterogeneity as genomic instability increases



Dittmar & Zanker 2015
<http://www.mdpi.com/1422-0067/16/12/26240/htm>

Illustration made with
<https://github.com/chrisamiller/fishplot>

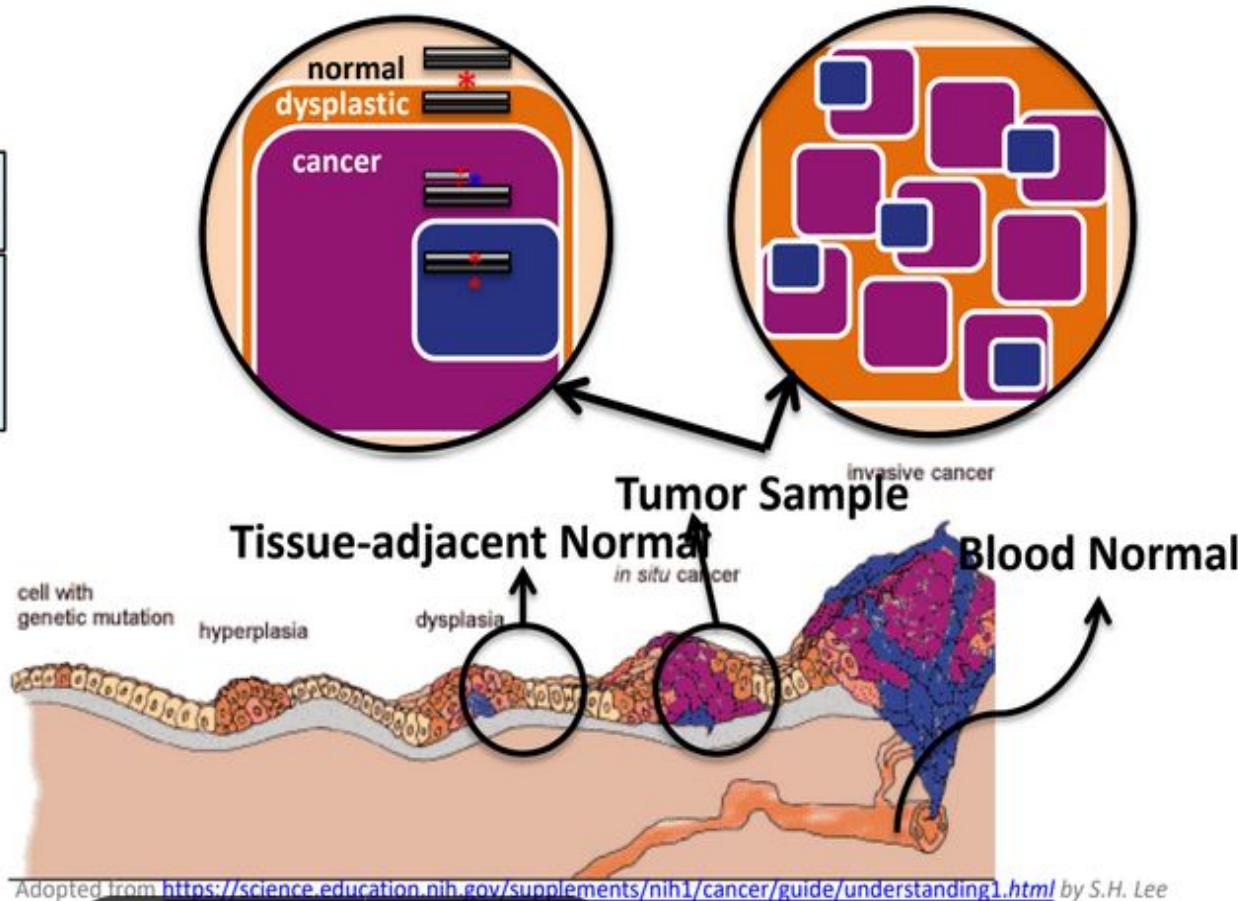
Cohorts of paired T-N data to detect driver mutations



Tumor and normal contamination and heterogeneity

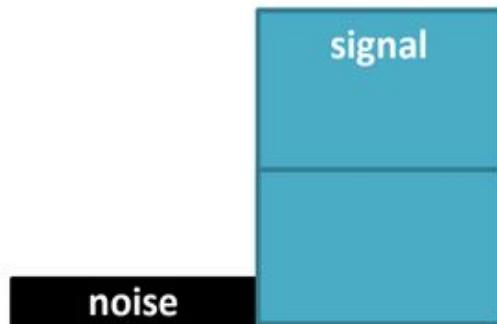
$$\text{Tumor purity} = \frac{(\text{tumor cells})}{(\text{normal} + \text{tumor cells})}$$

Tumor heterogeneity is based on polygenomic populations, segregated or intermixed, due to ongoing subclonal evolution.

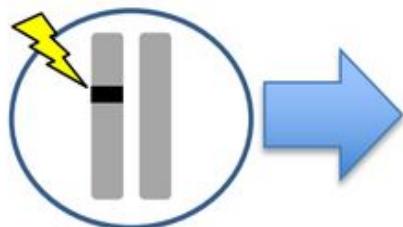


Amount of signal may be comparable to noise

Expectation for germline variants

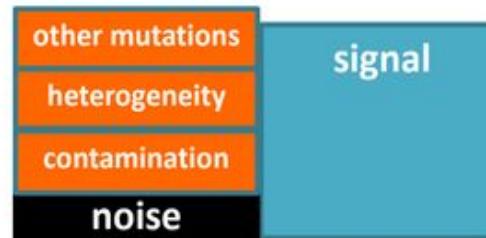


+ AF expected to follow ploidy

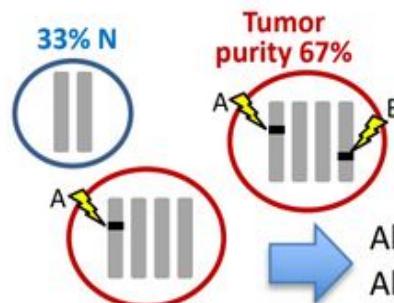


About 50% of reads will support the alternate allele

Expectation for somatic variants



+ no reliance on ploidy for AF

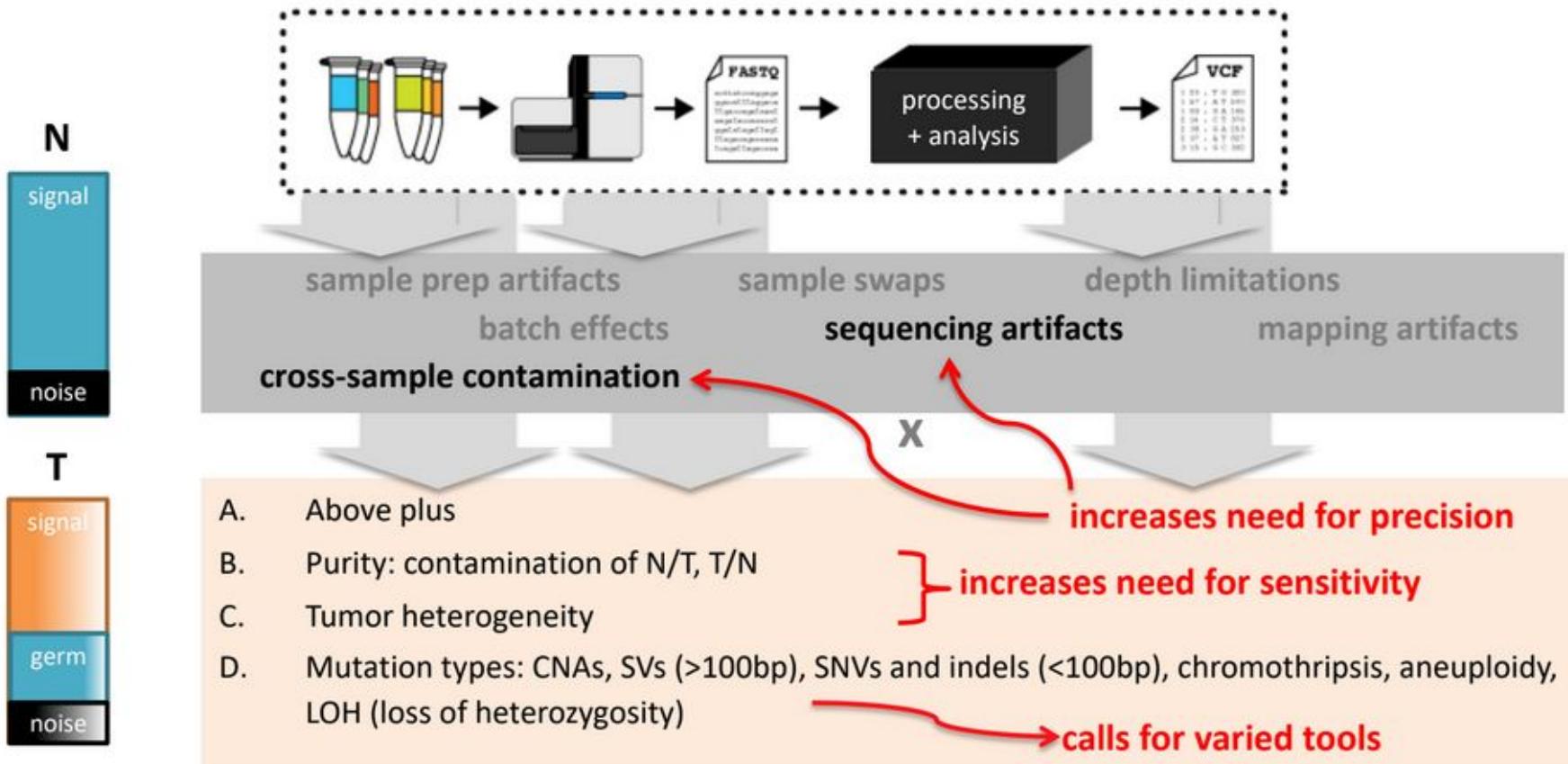


Example tumor sample:

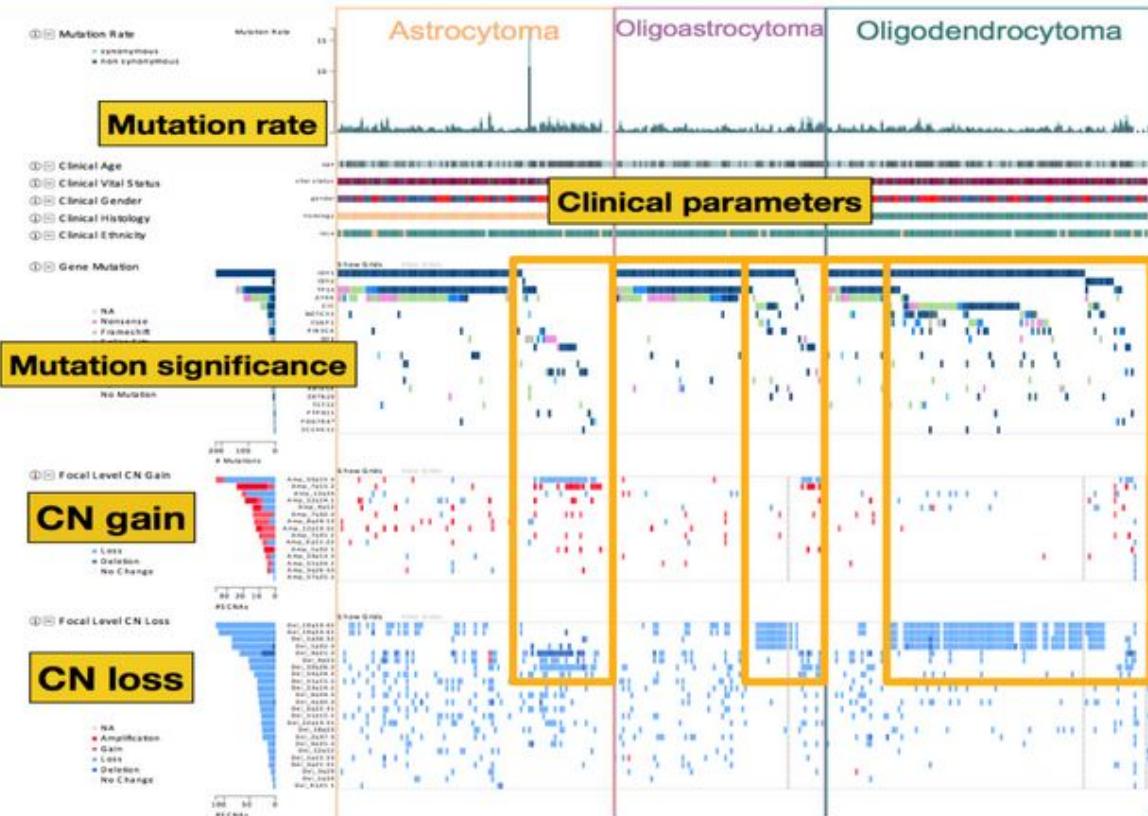
- Clonal SNV (A)
- Clonal copy number duplication
- Subclone of 50% of cancer cells (heterogeneity)
- Subclonal SNV (B)

About 20% of reads will support A
About 10% of reads will support B

Cancer-specific challenges confound analyses



A tumor's genomic alterations are multilayered

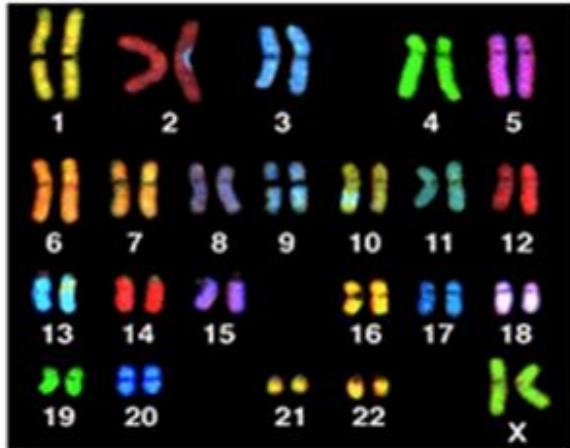


- Brain lower grade glioma (LGG) iCoMut plot from FireBrowse
 - Patients lacking characteristic mutations in IDH1/2, TP53 and ATRX have increased focal copy number alterations
- Cancer analysis must take into account a wide spectrum of alterations

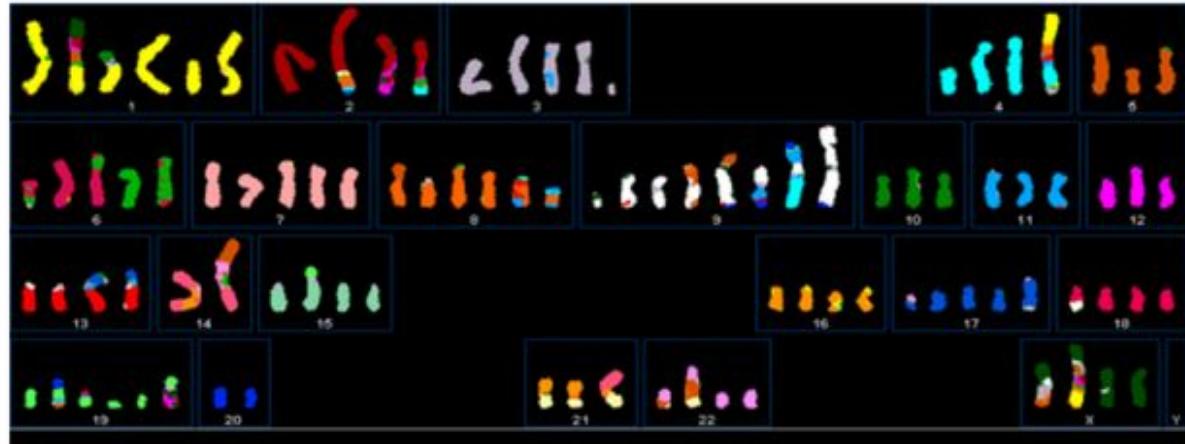
Courtesy of M. Noble

Somatic alterations can be dramatic

Normal Cell



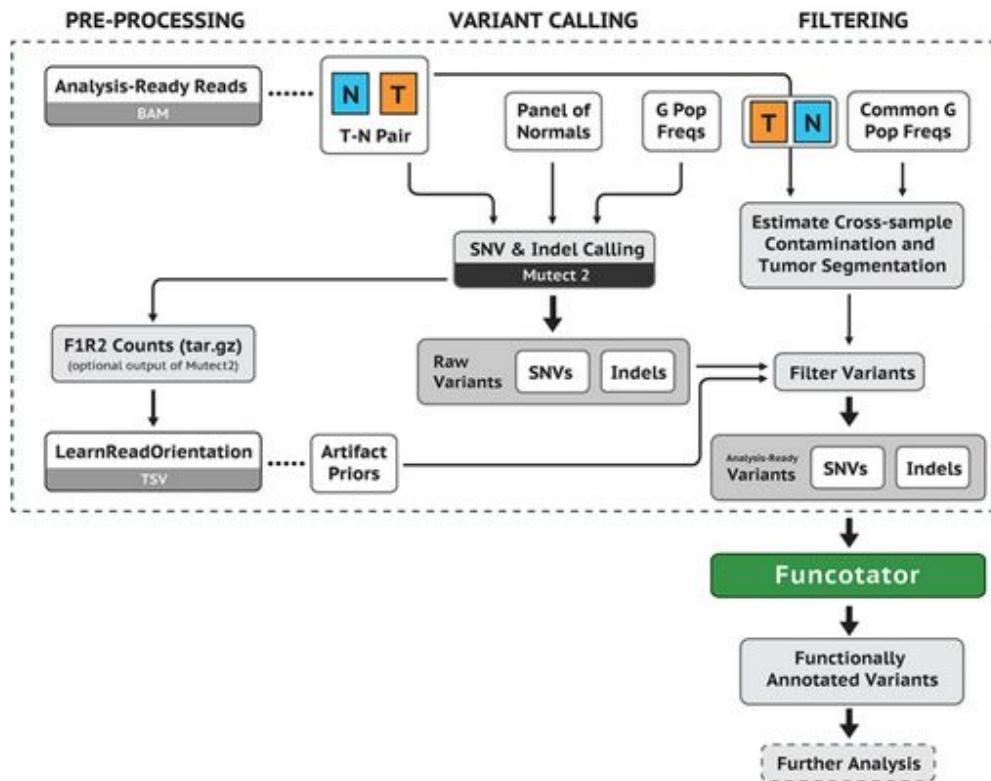
Cancer Cell Line HCC1954



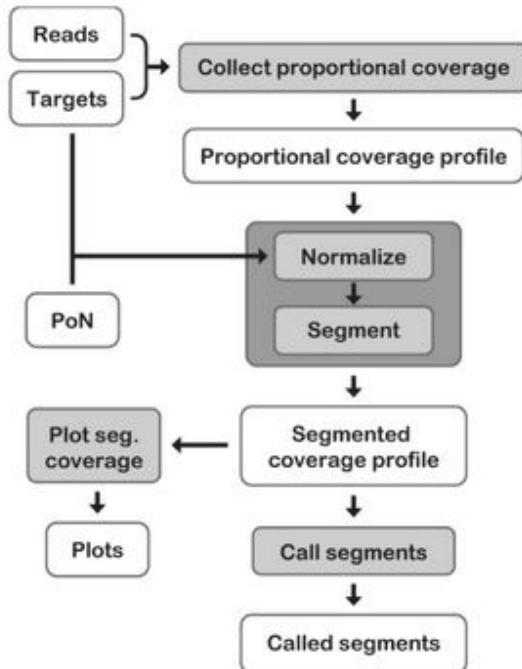
- Spectral karyotyping paints each chromosome pair with a color
- Alterations can vary dramatically between cancers and within cancers

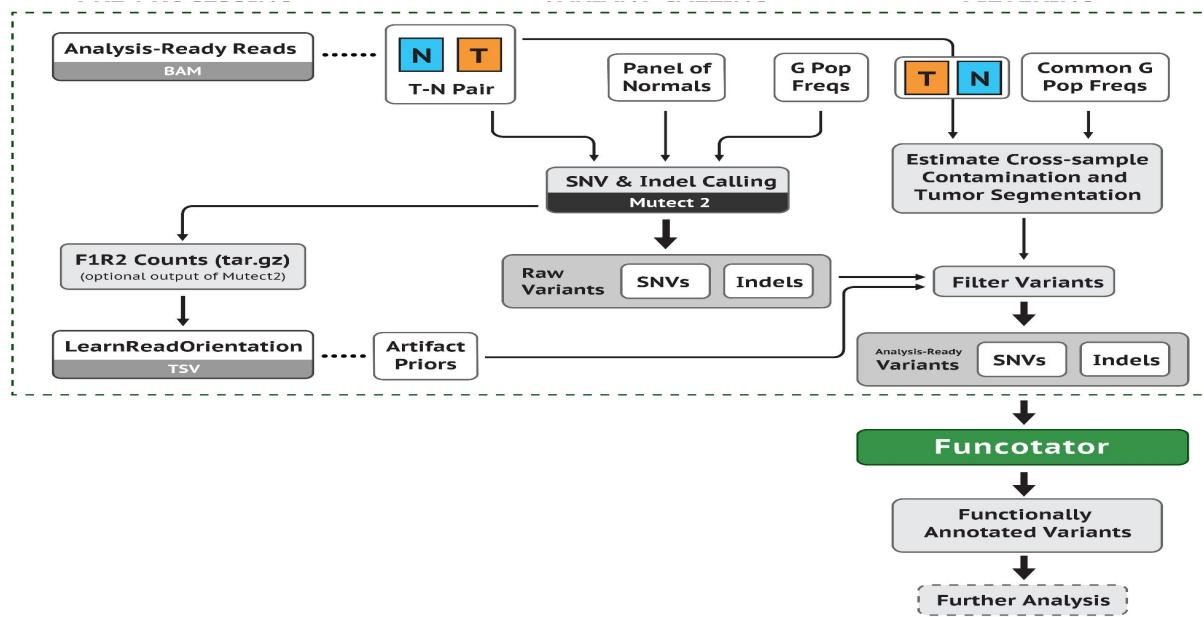
Somatic variant discovery workflow in GATK4

Somatic SNV and Indel Discovery



Somatic CNV Discovery





SOMATIC SNVs & INDELS

Logic of the Tumor-Normal workflow

Comparison to matched normal → subtraction of germline background



Tumor-only analysis

Panel of Normal (PON)

- It is possible to run the workflow without a matched normal in “tumor only mode” (normally used for PON creation).
- MUST have a good PON to eliminate common germline variation.
- Will still require extra filtering.

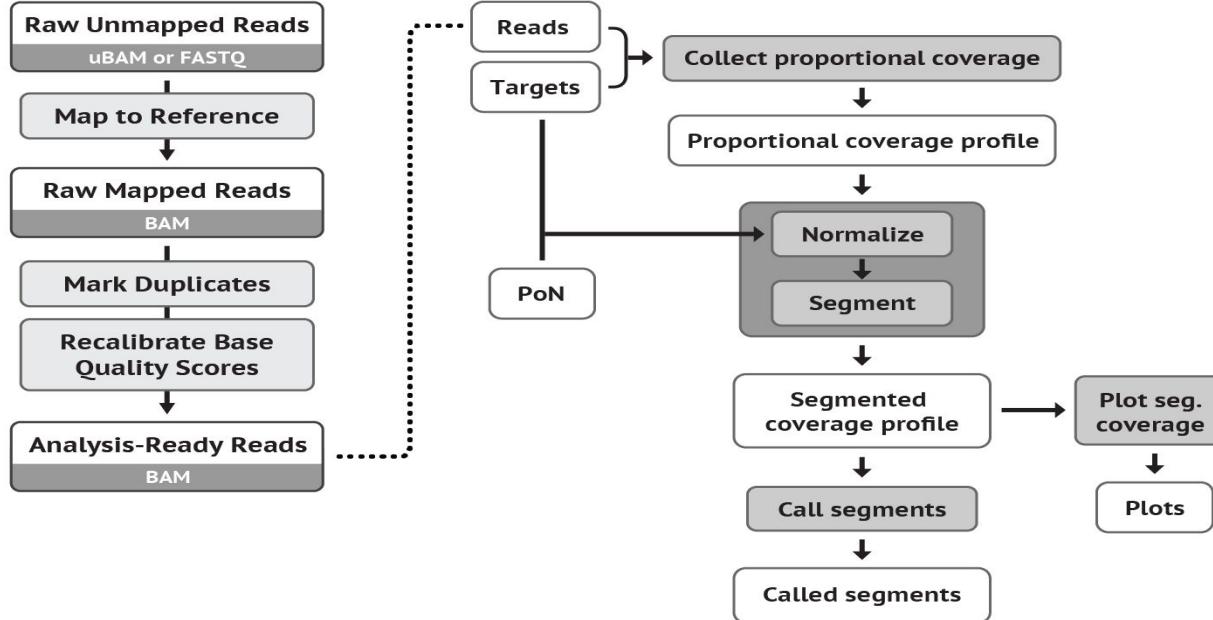
Panel of Normals for SNVs and Indels

- VCF of calls made from a set of *unrelated* “normal” samples.
- Main purpose:

Eliminate common/recurring *technical artifacts*.

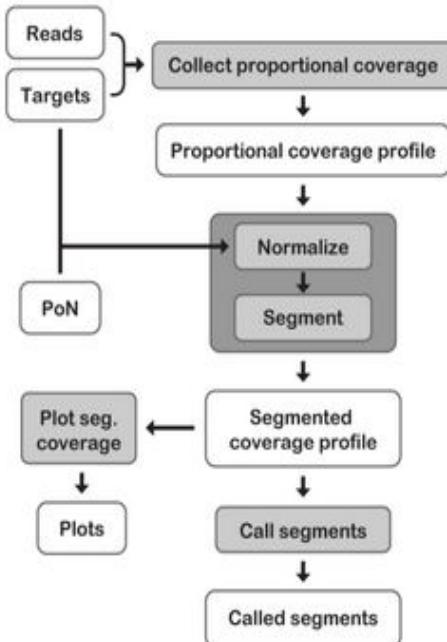
-> should use normals made using the same data generation techniques (e.g same capture kit for exomes, same sequencing platform, etc.)

- Secondary purpose: also eliminate *germline variants* not called in the matched normal (or approximate the normal if none is available)

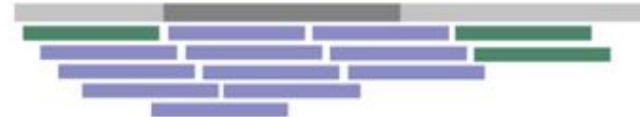


SOMATIC CNVs

Copy number: Coverage and Normalization

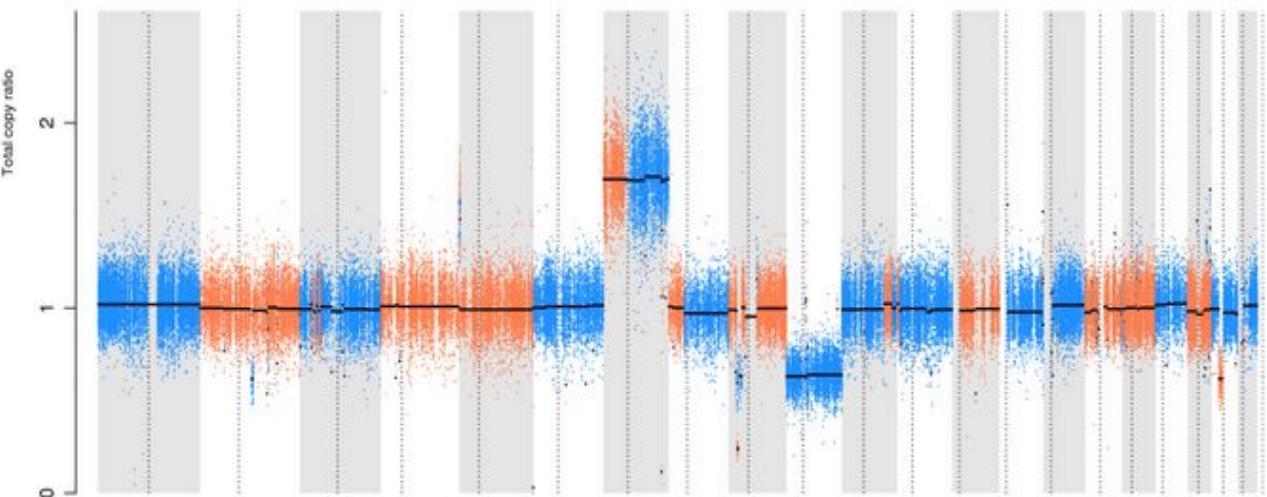


Collect proportional coverage

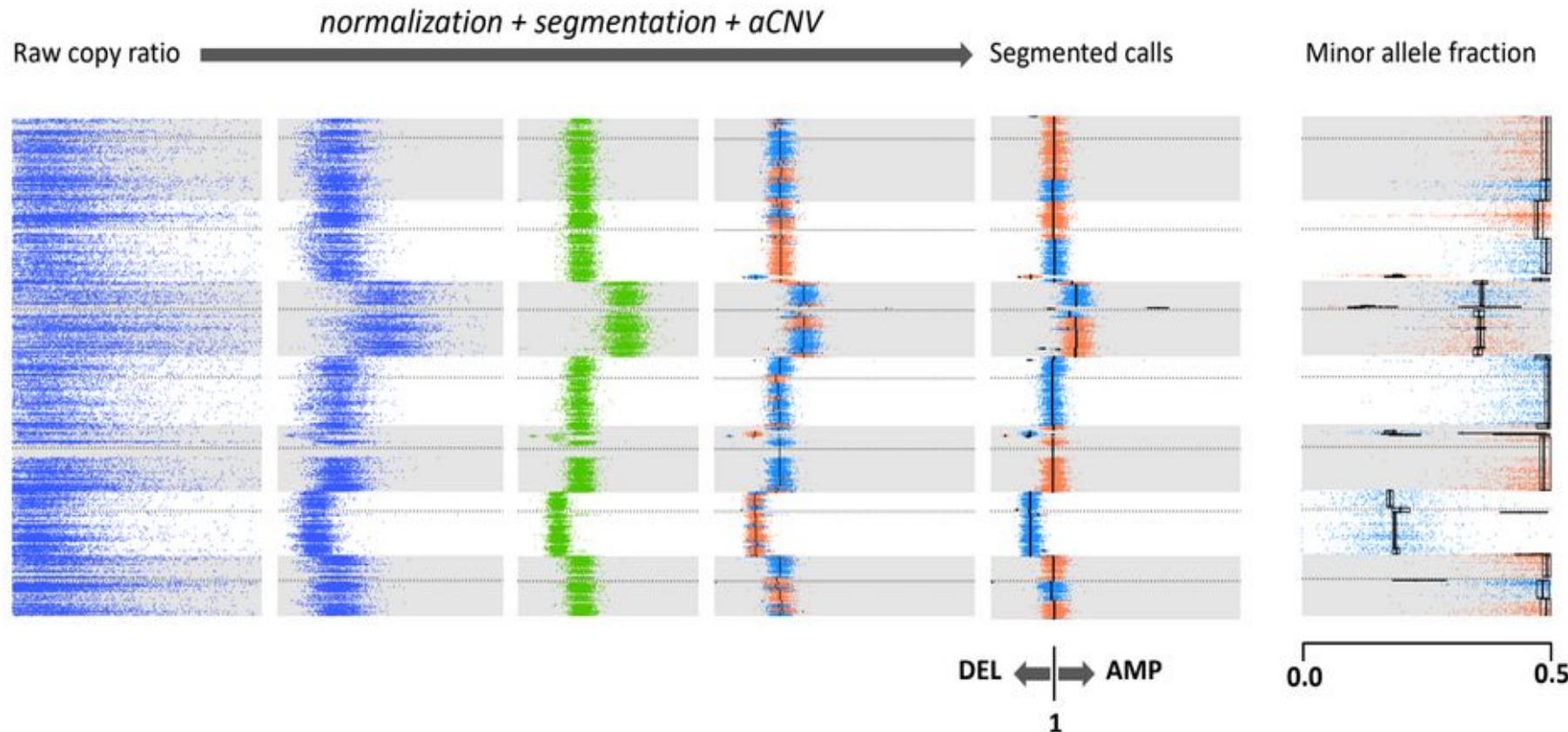


Normalize to remove noise

Identify segment boundaries

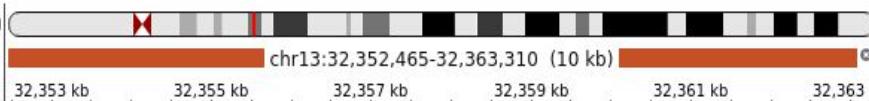
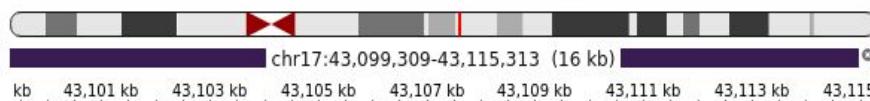


“Denoising” normalization process is essential



Panel of Normals for CNVs

- Made from a set of unrelated “normal” samples BUT **not** a vcf (unlike SNVs and Indels).
- Main purpose:
“Denoising” to compensate for variability in coverage.
-> should use normals made using the same data generation techniques (e.g same capture kit for exomes, same sequencing platform, etc.)



94265 [S11.unmrk.recal.bam]

0

chr17:43,104,204

Total Count 32991

A 225 (1%, 115+, 110-)

C 16 (0%, 10+, 6-)

G 9 (0%, 5+, 4-)

T 32741 (99%, 16240+, 16501-)

N 0

DEL 7

INS 0

MANE Transcripts

ENST00000357654.9

ENST00000380152.8

Refseq Genes

BRCA1

BRCA1

BRCA2

BRCA2

Thank you for your attention