TUMOR GENETICS AND IMPLICATIONS FOR TREATMENT

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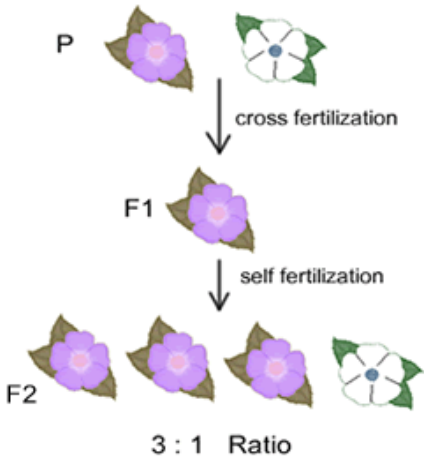
GERMLINE VERSUS SOMATIC

Gregor Mendel & Germline Genetics



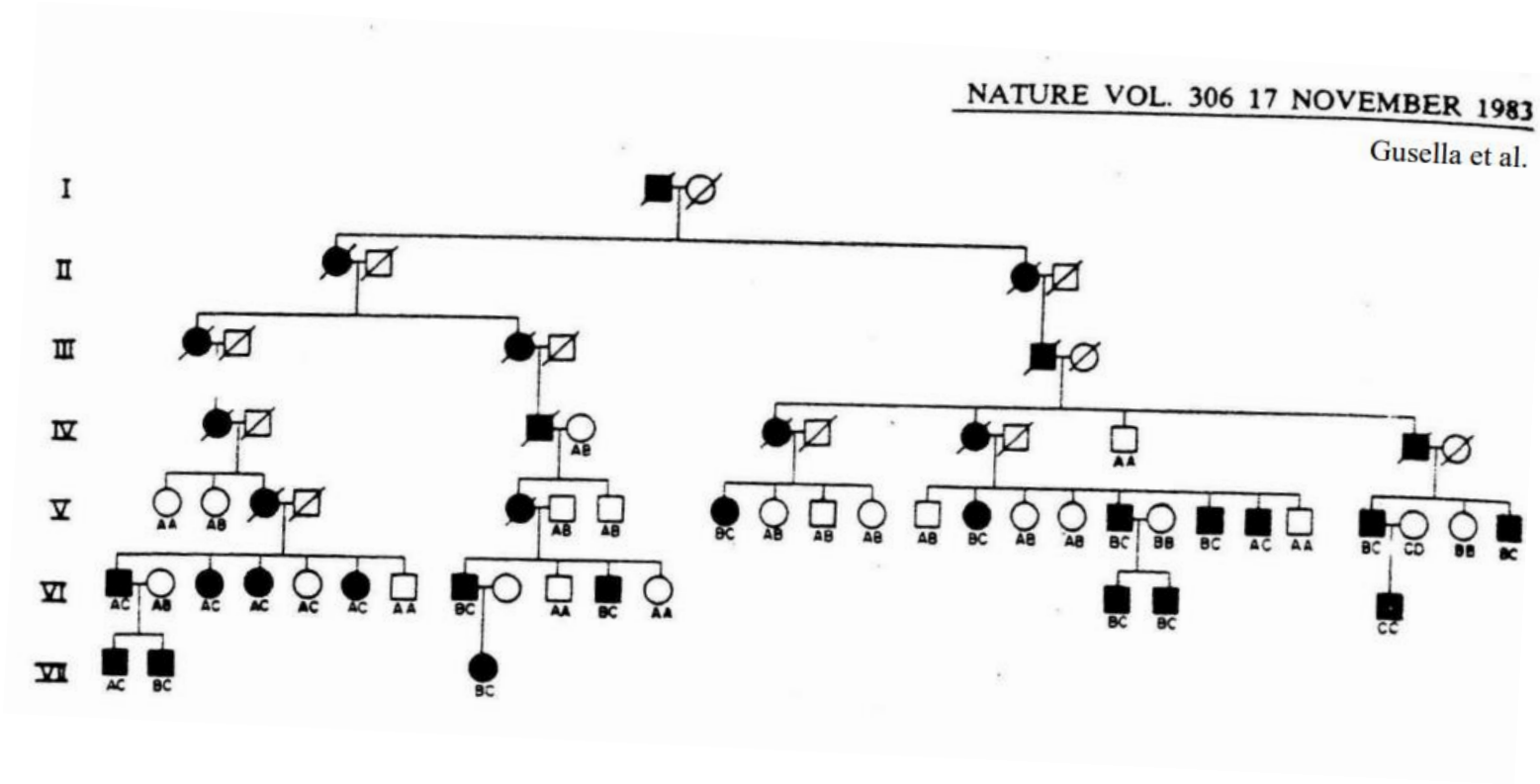
Law of segregation – Each gamete contains one or the other of two allelomorphic factors (alleles) later found to fit meiotic separation of pat and mat chromosomes

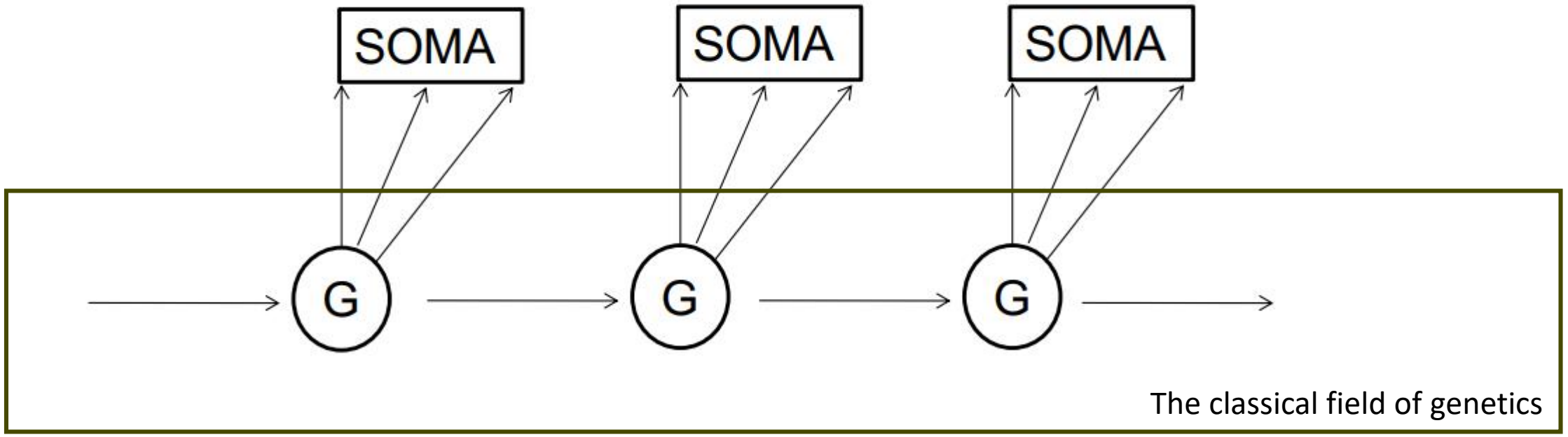
Law of independent assortment – Pairs of alleles from different genes enter gametes independetly of one another except if genes closely located on same chromosome (linkage)

The Principle of Dominance and Recessiveness

GENETICS: A GERMLINE POINT OF VIEW

Example of huntington (AD inheritance)




SOMA
SOMA
SOMA
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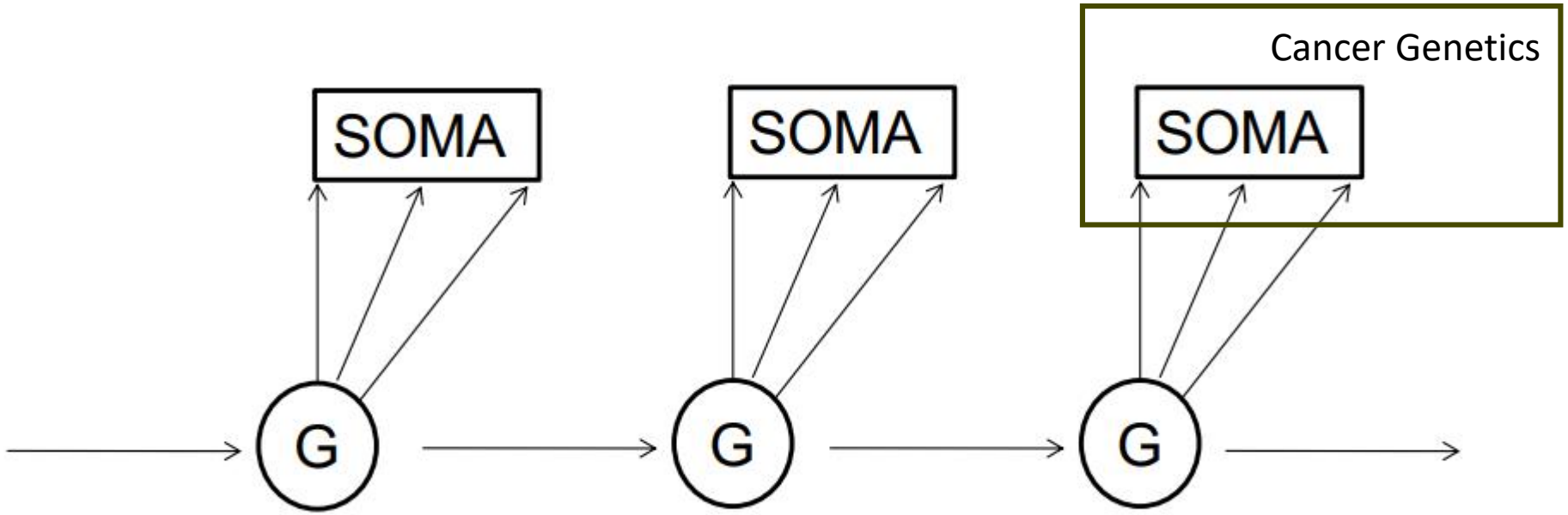
G
The classical field of genetics


Cancer Genetics
SOMA
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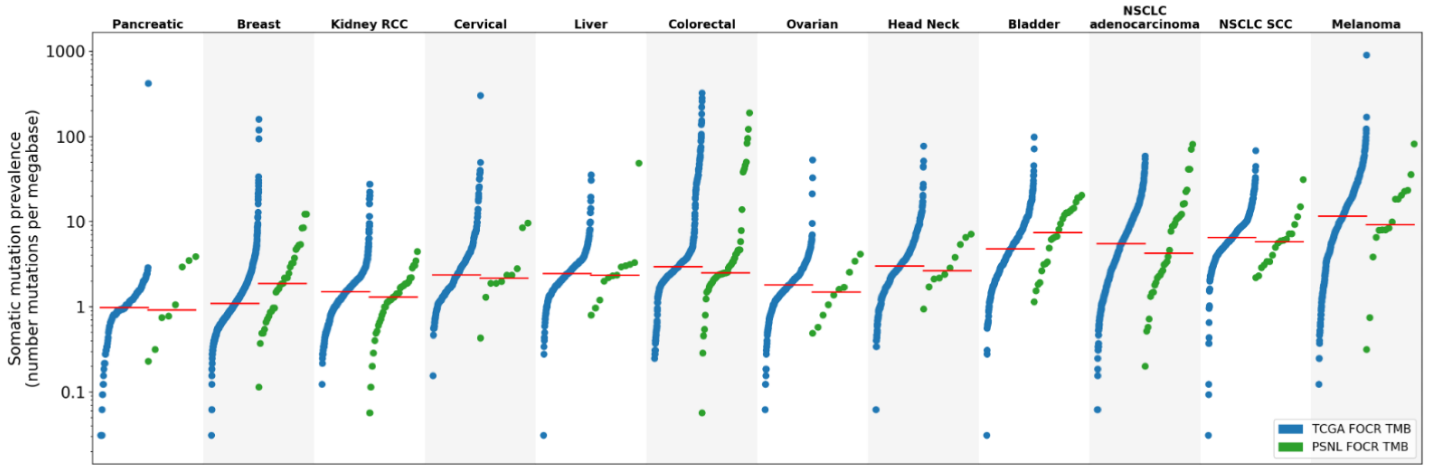
CANCER IS A GENETIC DISEASE

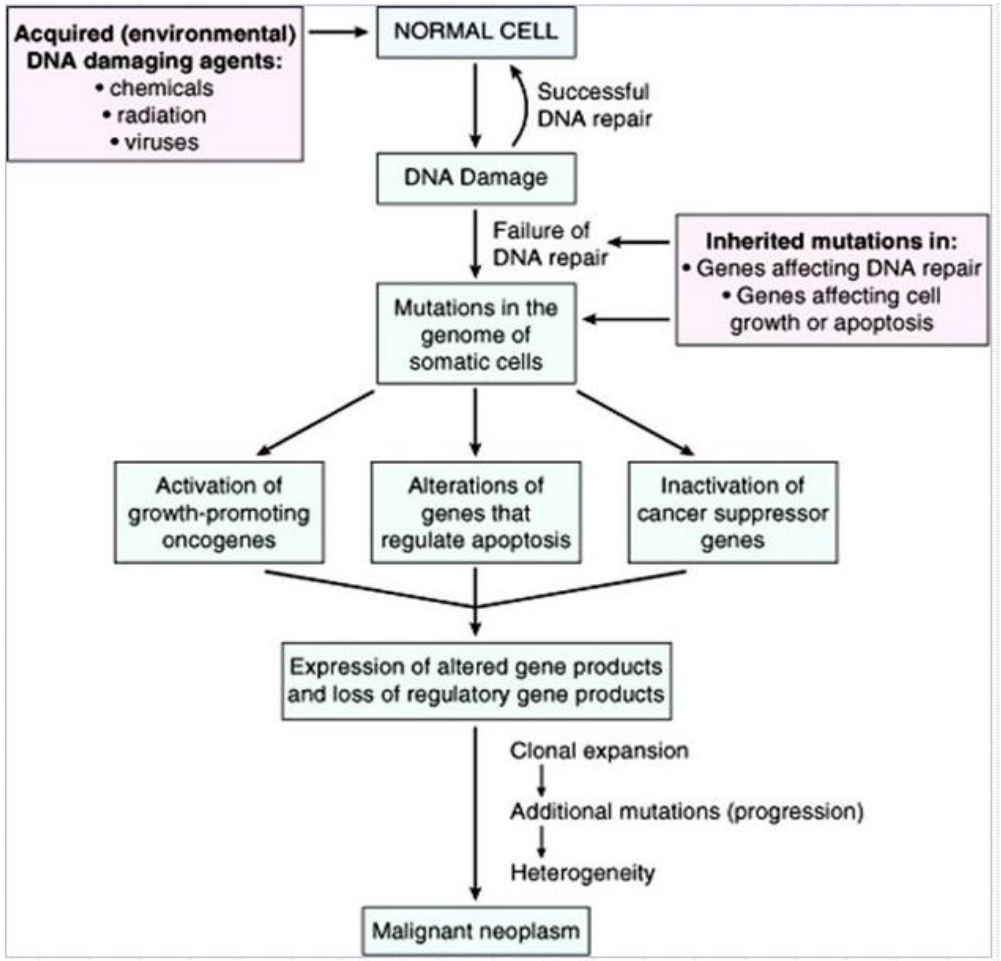
Per generation: 60-70 *de novo* single nucleotide variants in the genome

Per cancer: 32.000 – 3.200.000 *de novo* single nucleotide variants

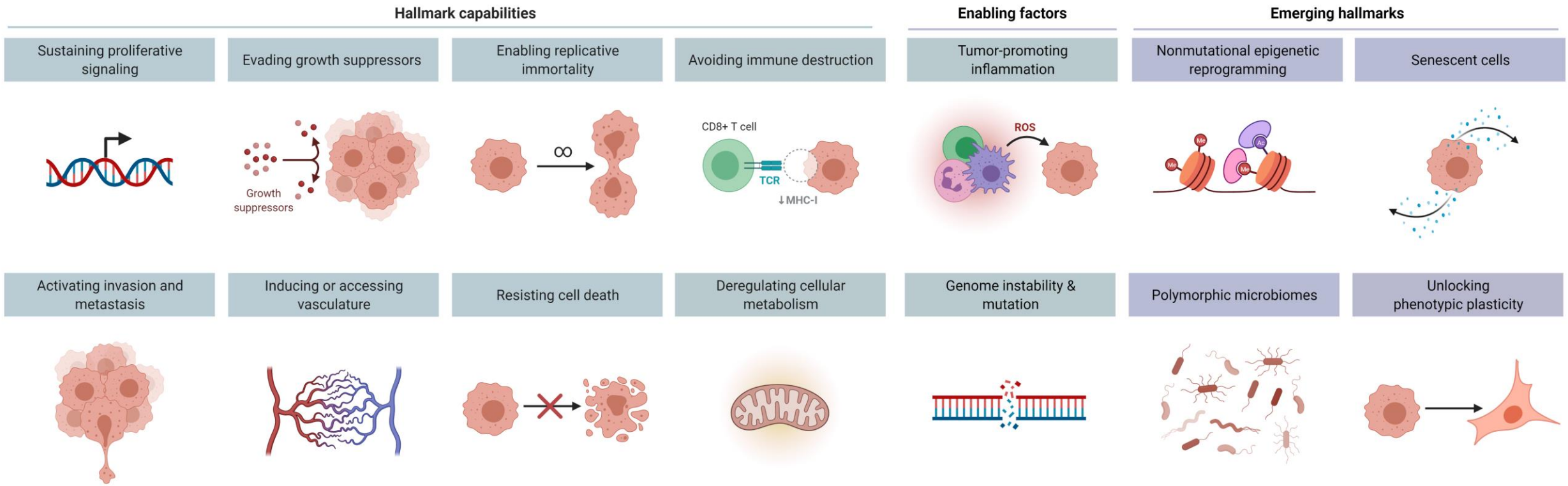


Each dot represents a single patient sample. The horizontal red lines indicate the median number of mutations in each respective cancer and cohort. The vertical axis (log-scaled) shows the number of mutations per megabase, segregated by the various cancer types investigated. The estimation of TMB was determined utilizing the FOCR 'Uniform TMB Calculation Method' (Merino et al. 2020).

- **Cohorts**:
  - FOCR Exome-derived TMB from TCGA samples (from Merino et al. 2020)
  - NeXT Exome-derived TMB using Personalis NeXT DB samples


Acquired (environmental) DNA damaging agents:
• chemicals
• radiation
• viruses
NORMAL CELL
Successful DNA repair
DNA Damage
Failure of DNA repair
Inherited mutations in:
• Genes affecting DNA repair
• Genes affecting cell growth or apoptosis
Mutations in the genome of somatic cells
Activation of growth-promoting oncogenes
Alterations of genes that regulate apoptosis
Inactivation of cancer suppressor genes
Expression of altered gene products and loss of regulatory gene products
Clonal expansion
Additional mutations (progression)
Heterogeneity
Malignant neoplasm

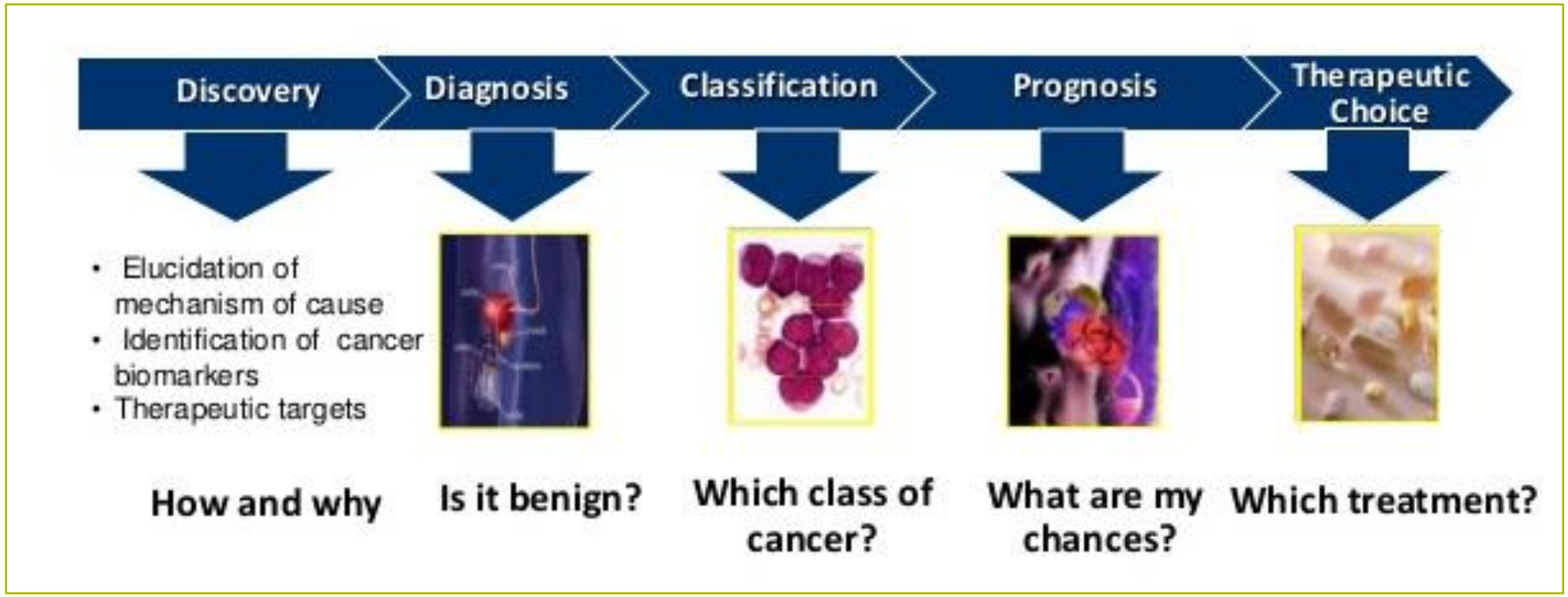
HALLMARKS OF CANCER



Douglas Hanahan and Robert Weinberg

TUMOR PROFILING

Why do we characterize tumors?



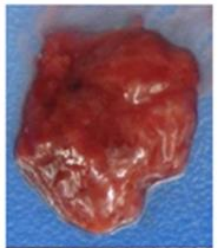
TUMOR GENETICS

How do we genetically characterize tumors?

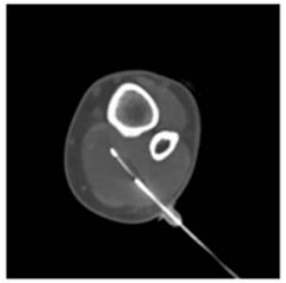
TUMOR PROFILING

SAMPLES – processing by anatomopathology

Sampling

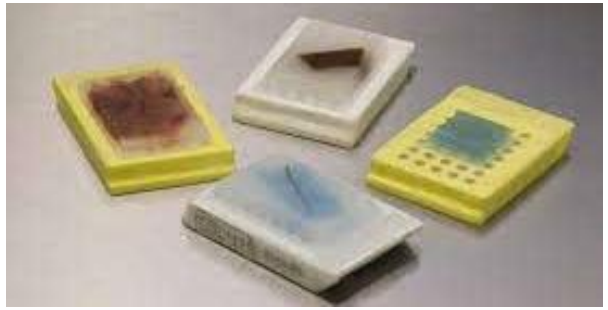
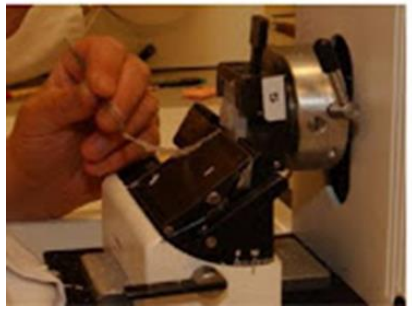


Resection



Needle biopsy

Bone marrow aspirate

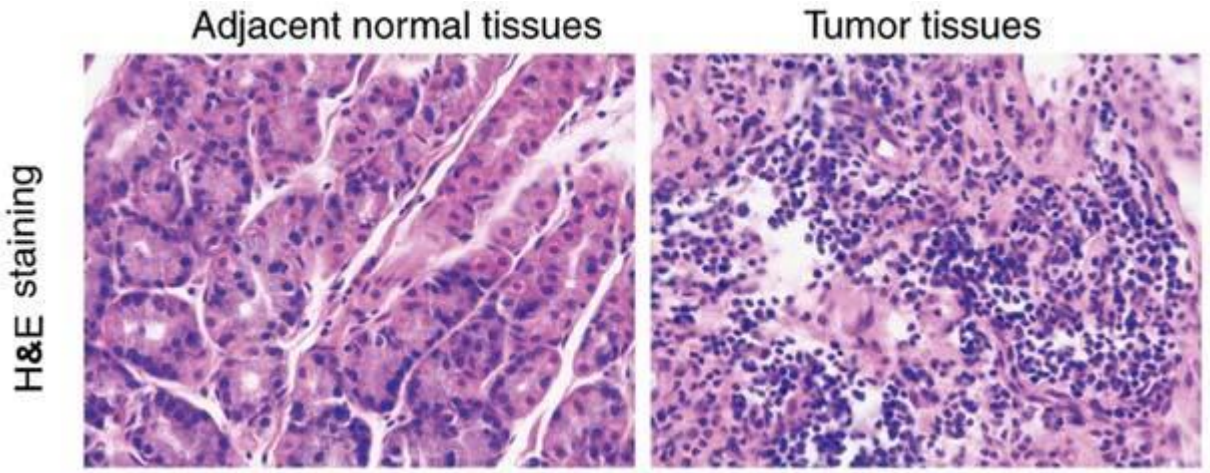

Formalin **F**ixed & **P**araffin **E**mbedded (FFPE)

- Preservation of tissue structure
- Storage and handling

TUMOR PROFILING

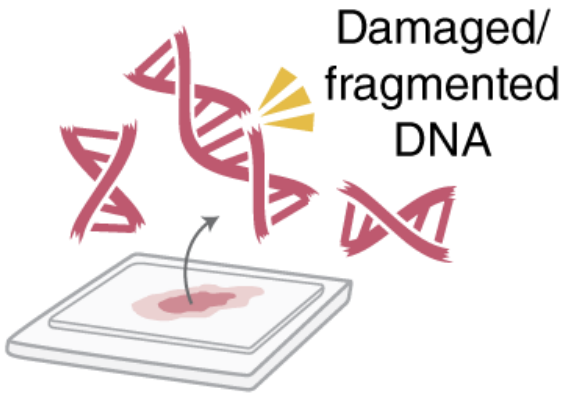
SAMPLES – processing by anatomopathology

Recommendations



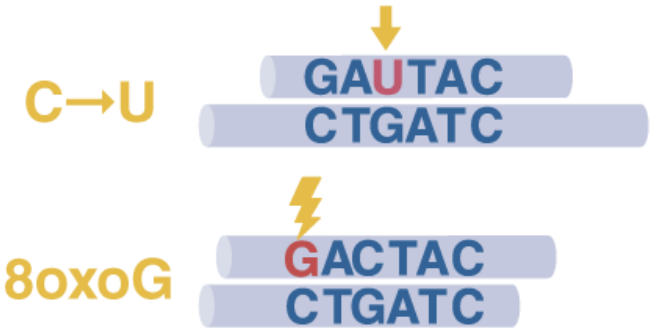
- min. 10% neoplastic cells ←→ germline = 100% cells
- tumor enrichment (macrodissection)
- no necrotic tissue
- fixation: min 6h, max 48-72h, in 10% neutral buffered formalin solution
- FFPE storage: max ± 3years

FFPE DNA LIBRARY PREP CHALLENGES



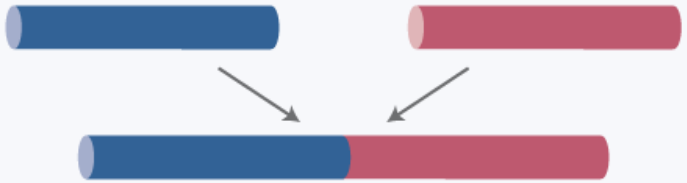
Low DNA input

Non-uniform DNA ends



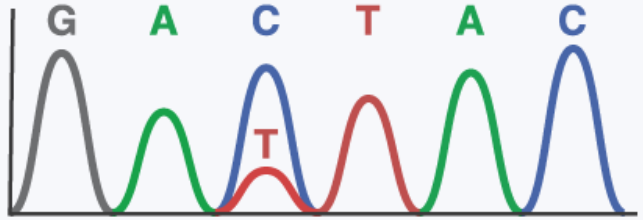
DNA damage

Negative effects on downstream sequencing



Chimeric reads

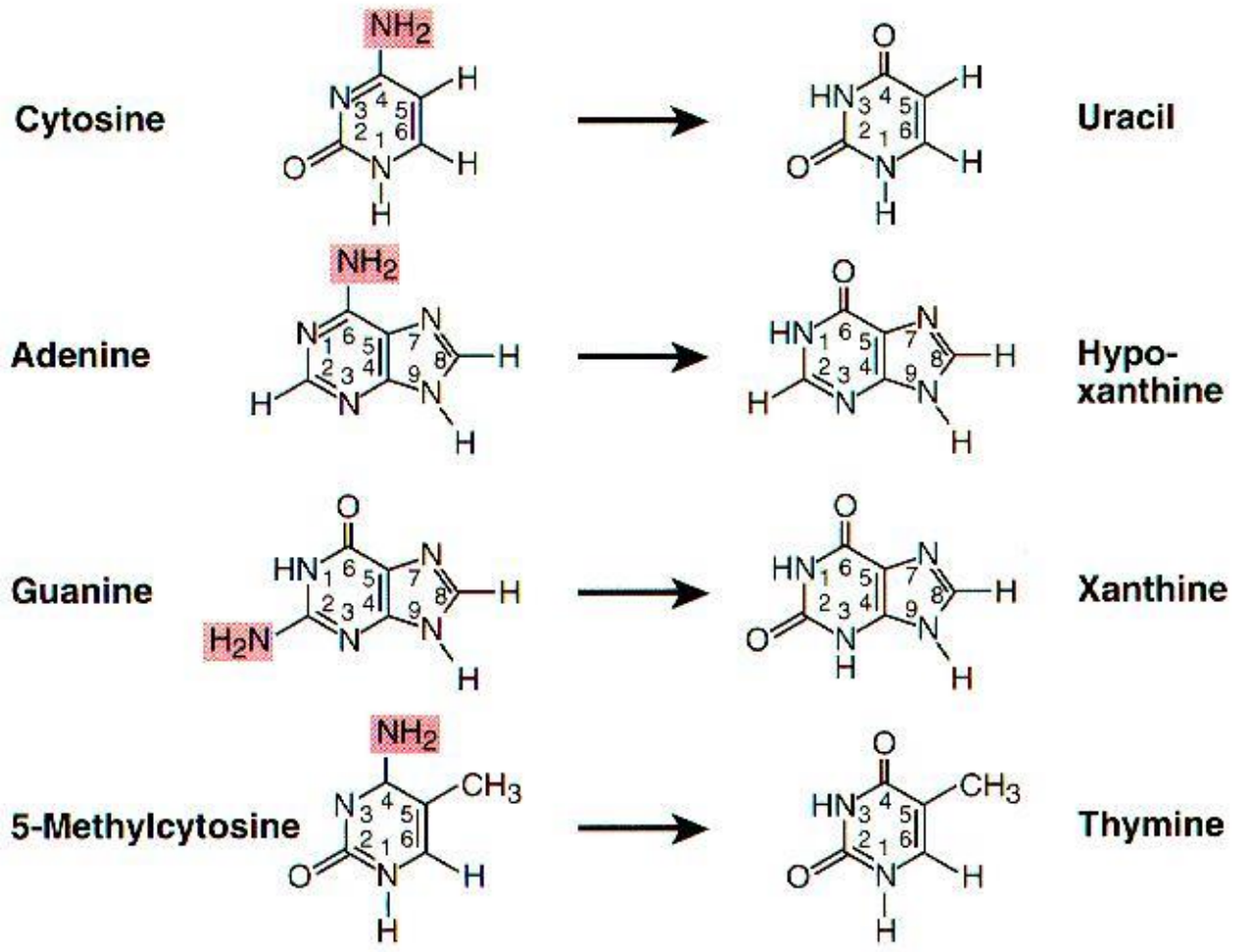
Result from single-stranded overhangs annealing with other DNA fragments



Sequencing artifacts & false positives

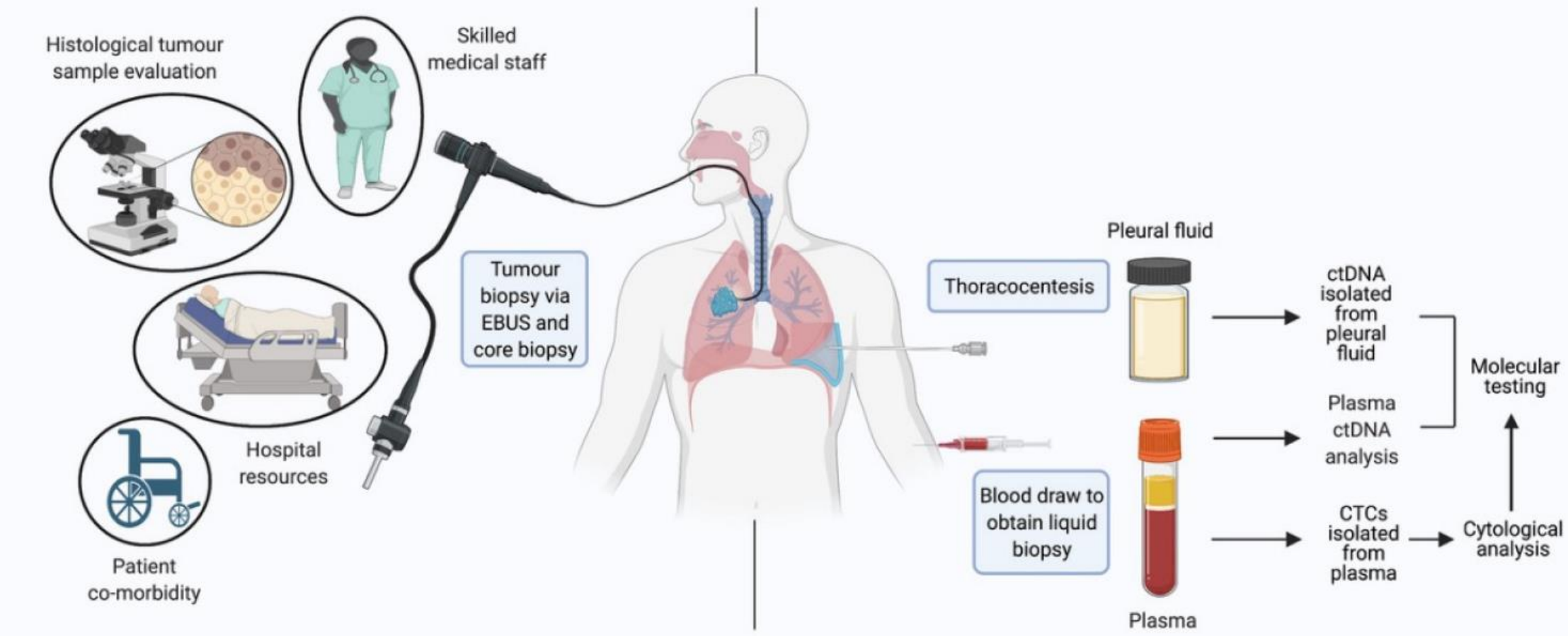
Cytosine deamination and oxidative damage can lead to the introduction of erroneous bases

DEAMINATION DUE TO FFPE STORAGE



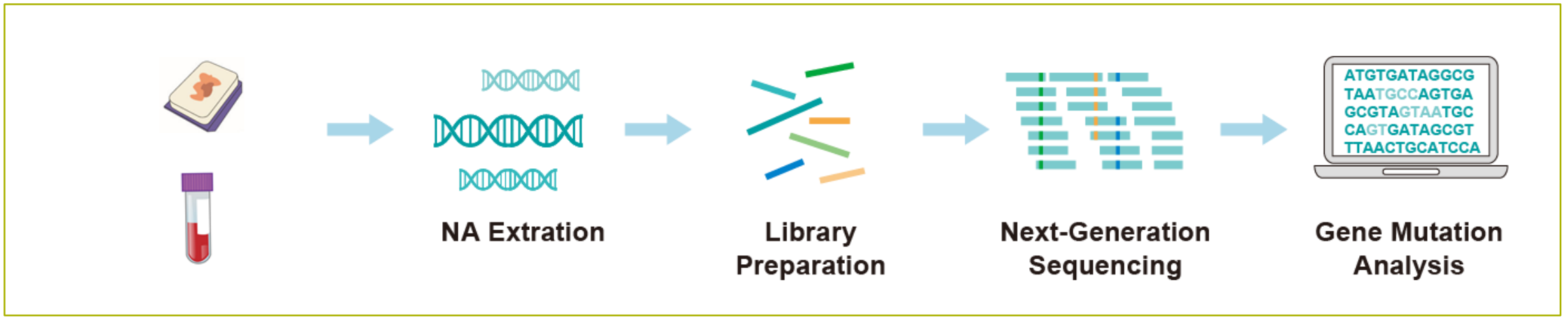
ALTERNATIVES TO TUMOR SAMPLES

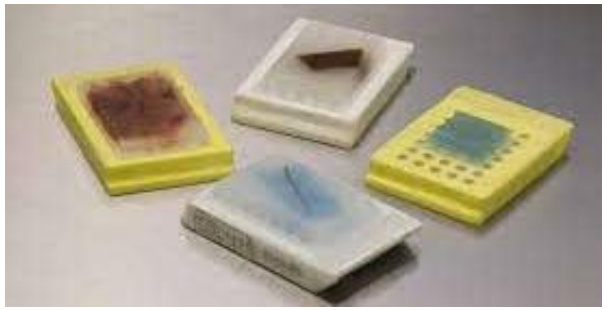
Liquid biopsies: circulating tumor DNA



Nagasaka, M., Uddin, M.H., Al-Hallak, M.N. *et al.* Liquid biopsy for therapy monitoring in early-stage non-small cell lung cancer. *Mol Cancer* **20,** 82 (2021). https://doi.org/10.1186/s12943-021-01371-1

MOLECULAR TUMOR PROFILING

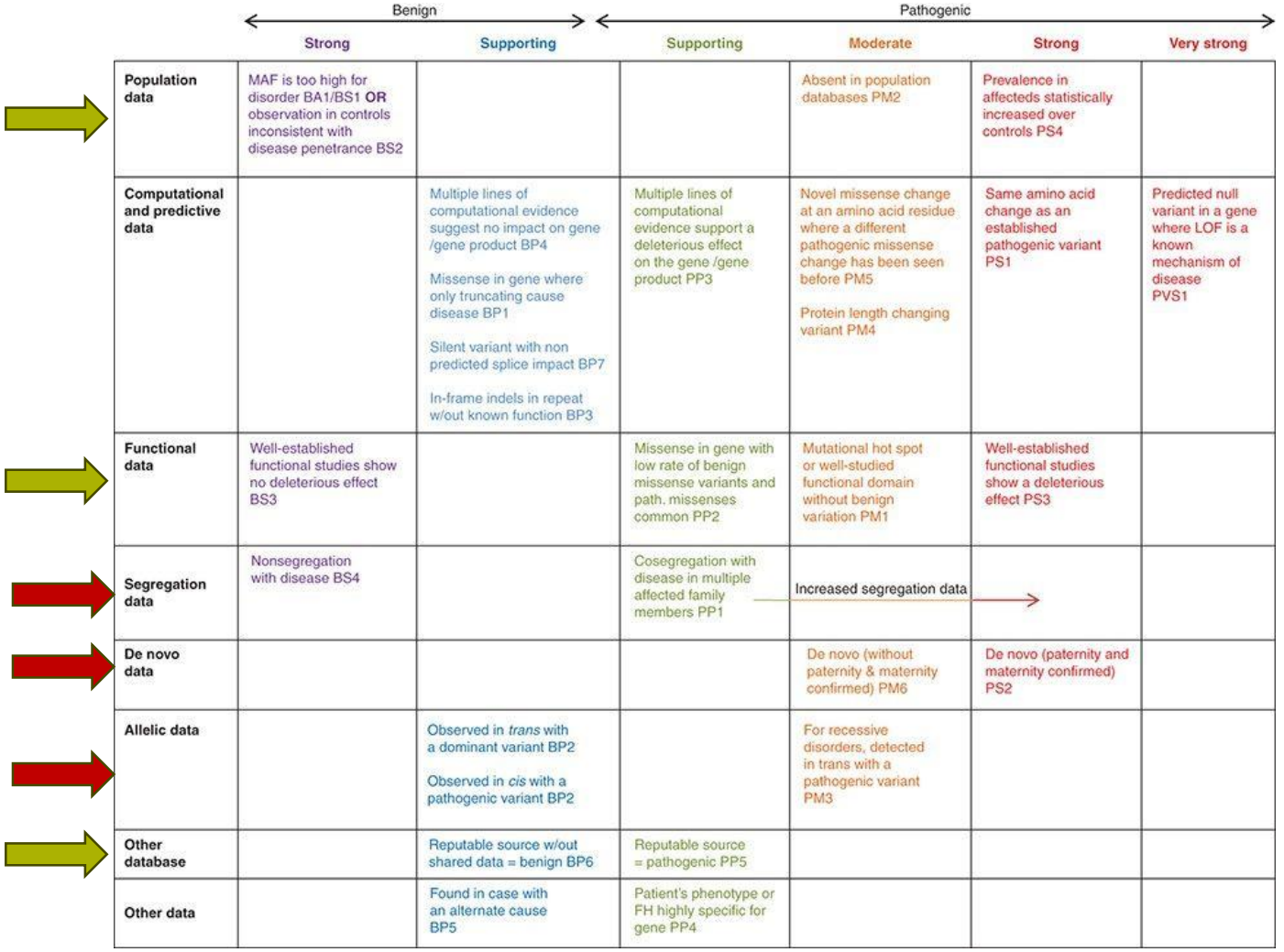




- Targeted Sequencing (hotspots)
- Panel Sequencing (3 - 500 genes)
- Exome Sequencing (all coding genes)
- RNAseq (eg. gene fusions, transcriptomics)

- High sensitivity & specificity (>99%)
- Up to 1% allel frequency

ACMG GUIDELINES ARE NOT SUITABLE

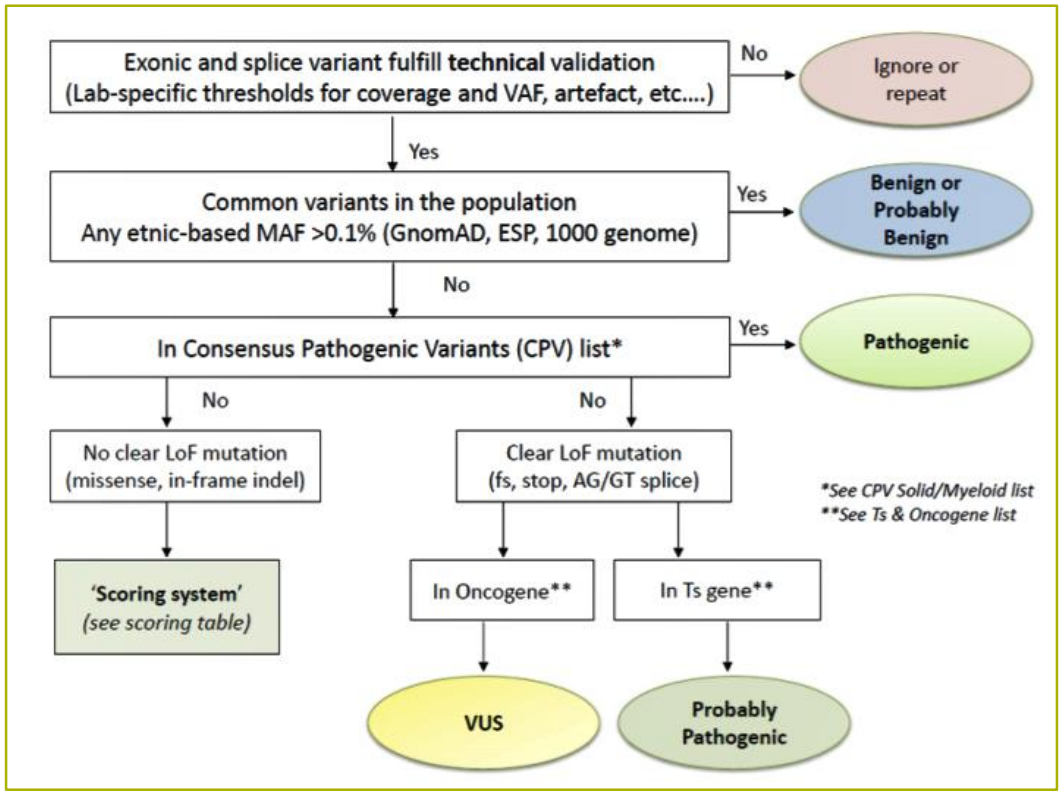


	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

TUMOR PROFILING

ComPerMed - Biological classification variants (snv & small indels)

Richards et al. Genet Med 2015



➡ assay-specific requirements (QC, thresholds, artefacts, …)

➡ 'common variants' - any ethnic based MAF > 0,1% => probably benign (class 2) / benign (class 1)

➡ 'consensus pathogenic variants' => pathogenic (class 5)

➡ <u>LoF (stop, fs, splice site)</u>
In TSG => probably pathogenic (class 4)
In oncogene => VUS (class 3)

<u>No clear LoF (missense, in frame indel):</u>
Scoring: Cosmic db, in silico prediction of structure and function, functional studies in literature, …
=> probably pathogenic (class 4) or VUS (class 3)

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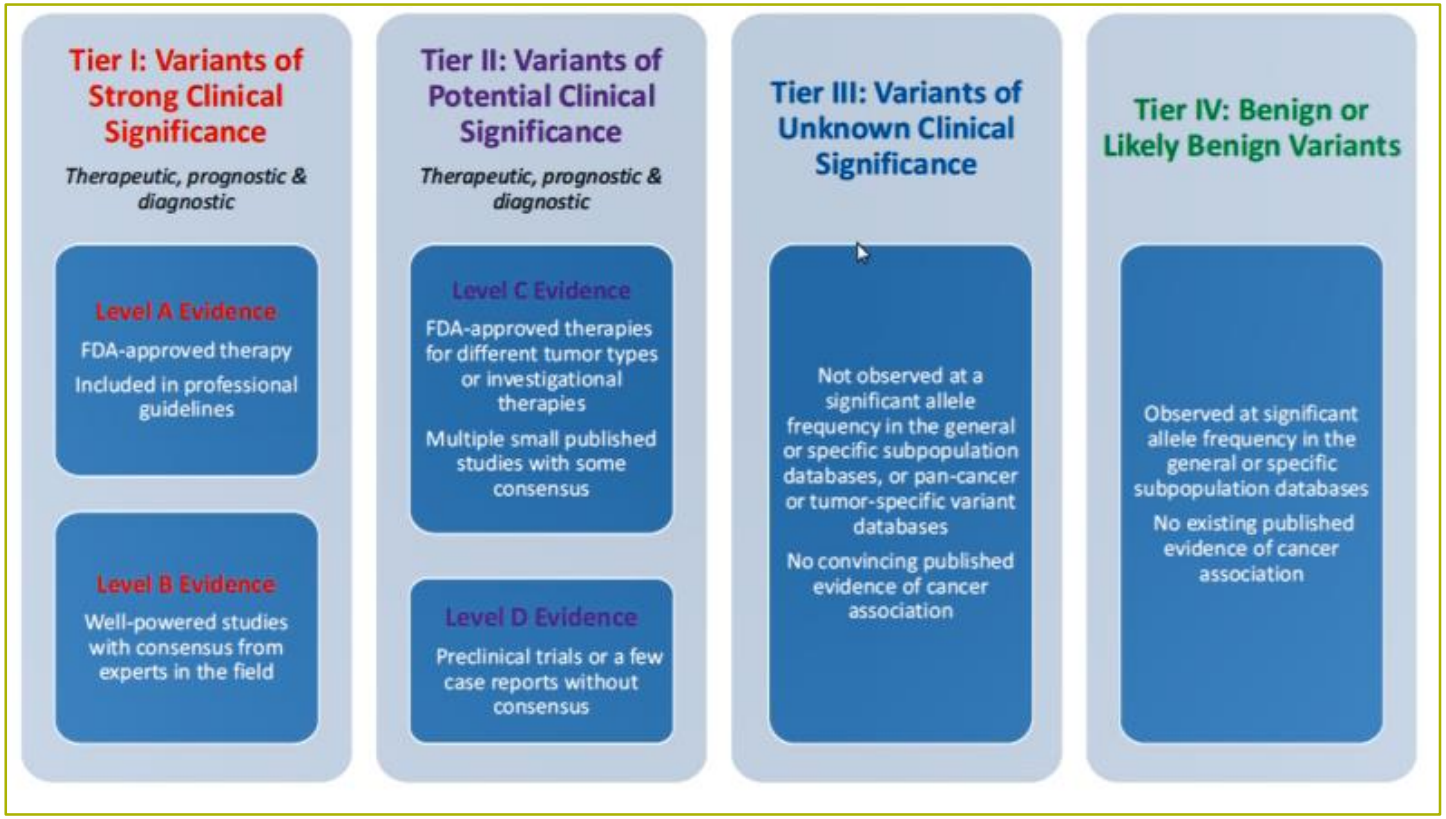
TUMOR PROFILING

ComPerMed - Clinical classification variants (snv & small indels)

Li et al. J Mol Diagn 2017

strong clinical significance (tier I)
FDA approved - specific tumor
Professional guidelines - specific tumor
Consensus in literature - specific tumor

potential clinical significance (tier II)
Preclinical studies
No consensus in literature
Inclusion criteria in Clinical Trial
FDA approved for another tumor type



class 5
class 4

class 5
class 4

class 5
class 4
class 3

class 2
class 1

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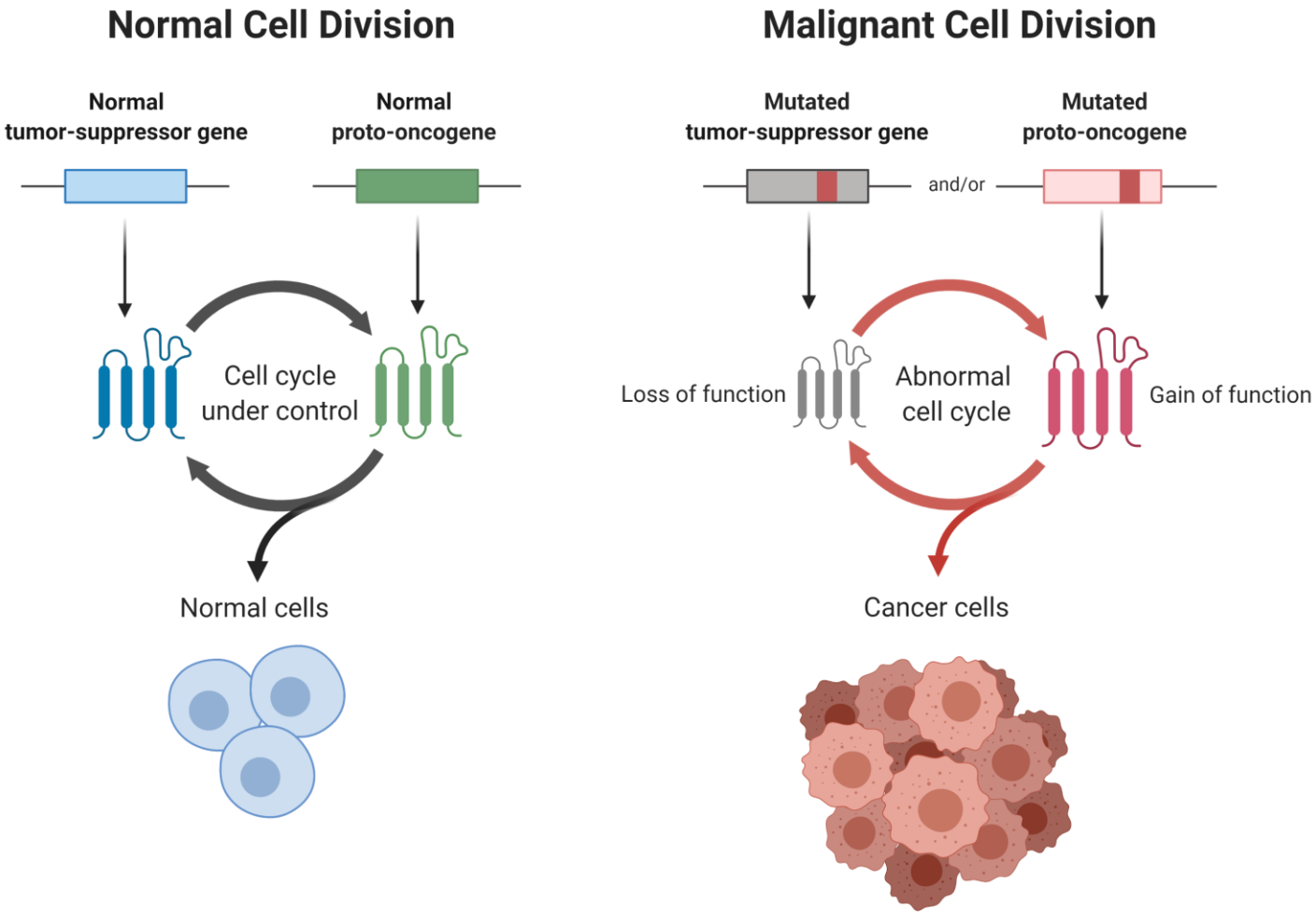
TUMOR GENETICS: WHAT?

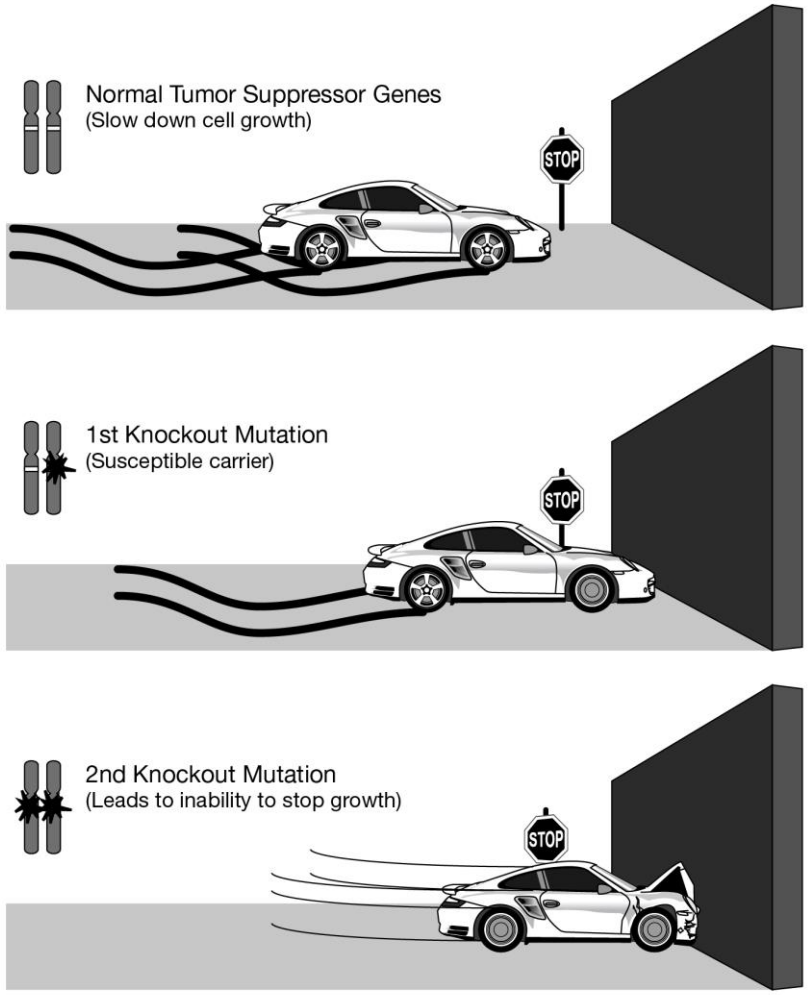
Relevant pathways in cancer

THE PRINCIPLE OF DOMINANCE AND RECESSIVENESS



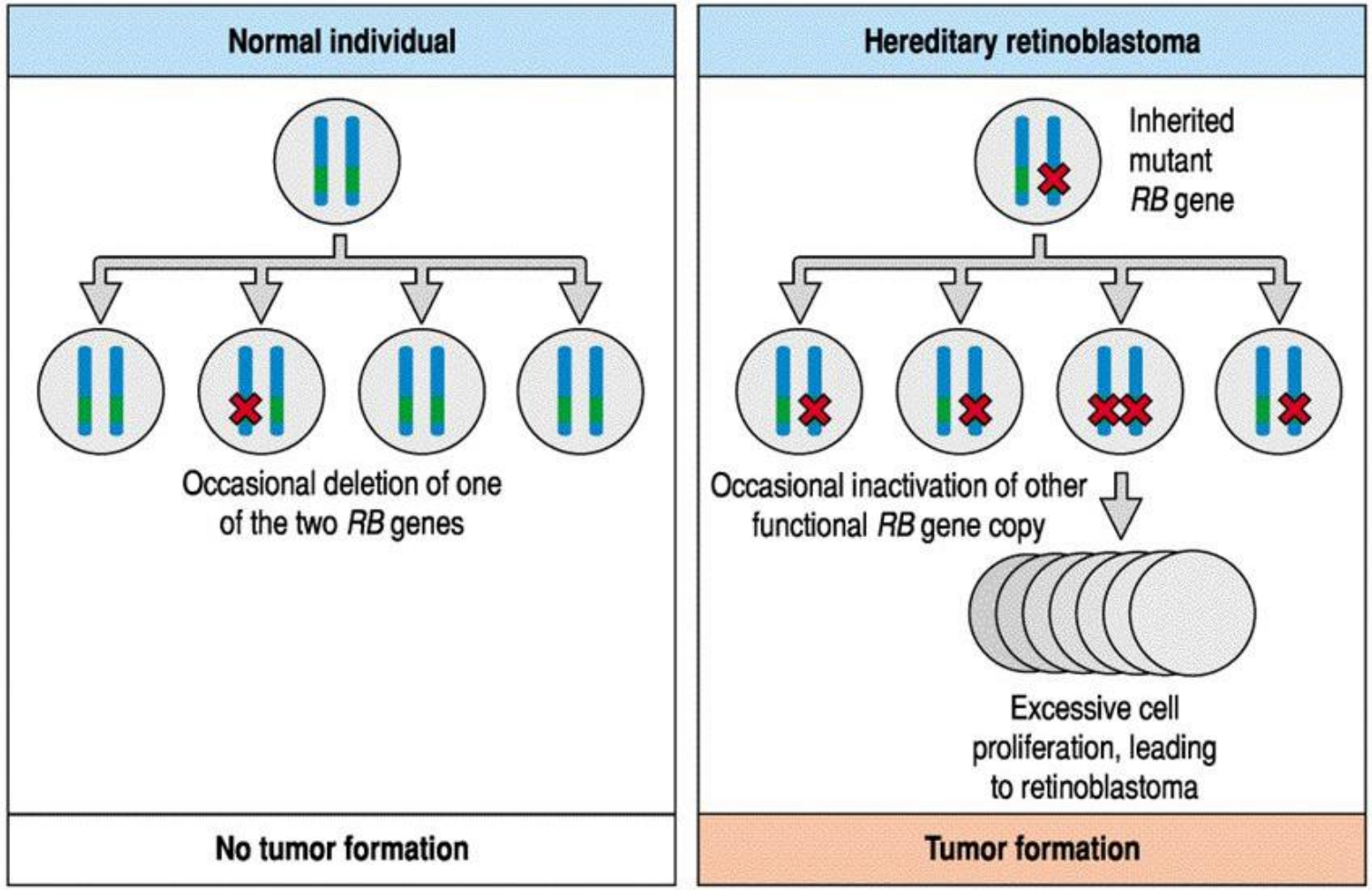
KUNDSON'S TWO HIT HYPOTHESIS

Tumor Suppressor Genes

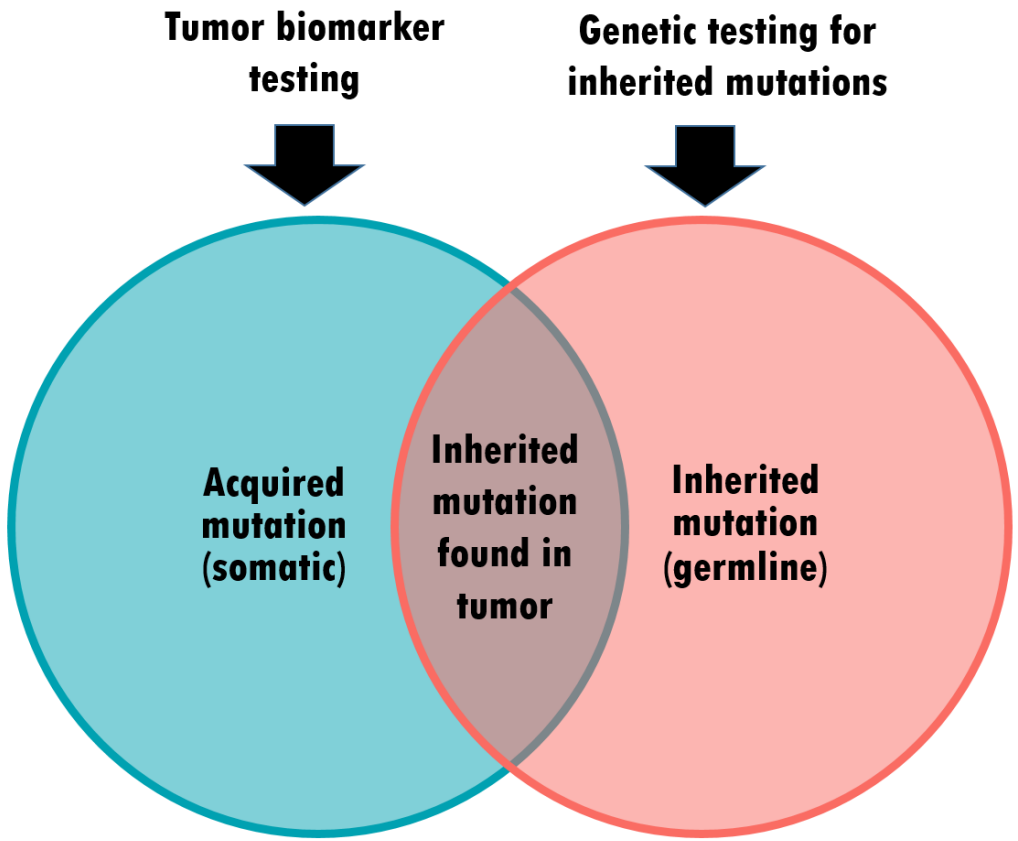


KUNDSON'S TWO HIT HYPOTHESIS

Tumor Suppressor Genes



SUSPICION HEREDITARY CANCER



Recognize !

Approach and considerations

Action !

Refer to genetic counseling & genetic testing

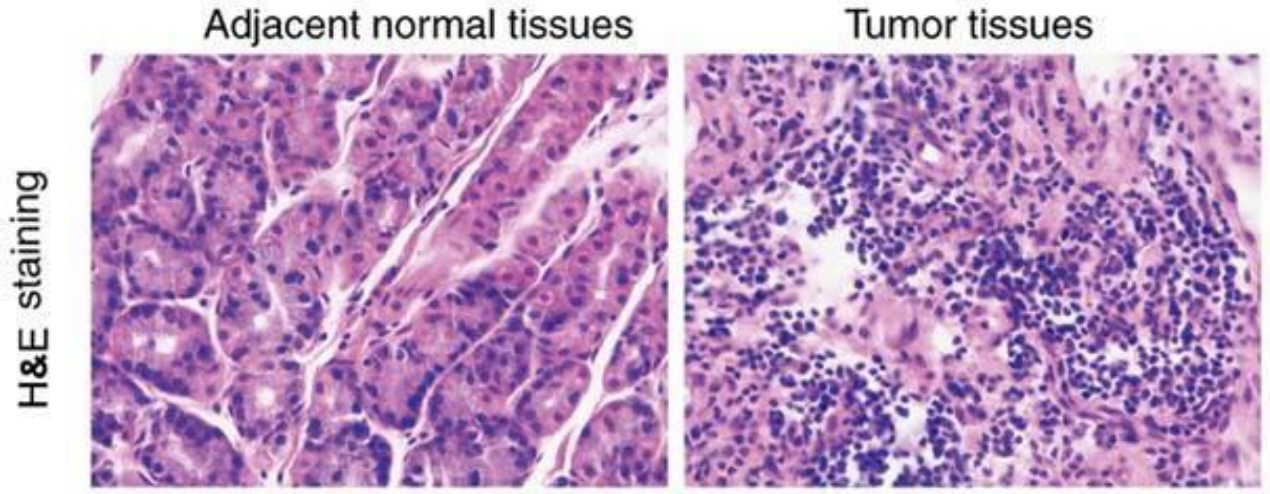
TUMOR PROFILING

Recognize potentially germline ?

Pathogenic variants in cancer susceptibility genes
Cancer is associated with cancer predisposition syndromes
Case dependent

- Young age at diagnosis (< 50 years)
- Cancer in family
- Multiple primary tumors in patient

Allelic frequency



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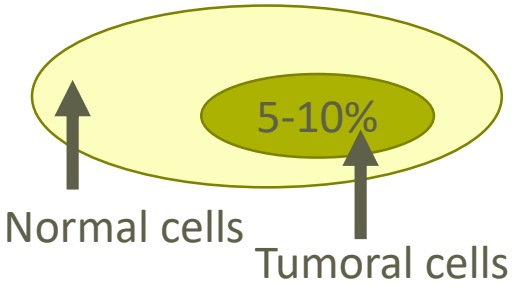
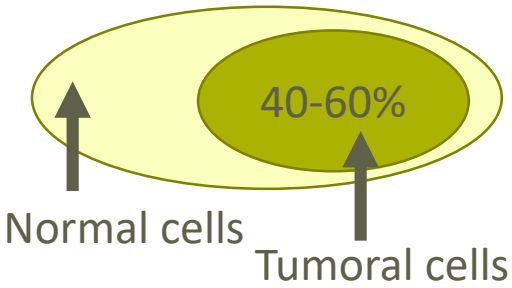
TUMOR PROFILING

Variant allele frequency vs tumor percentage in theory

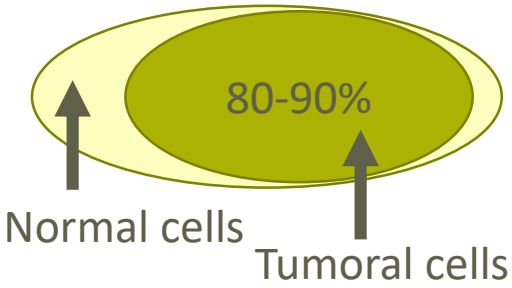
FFPE slide



Variant allele frequency	Somatic vs Germline
<25%	likely somatic
25-75%	likely germline -/+
>90%	likely germline +/+



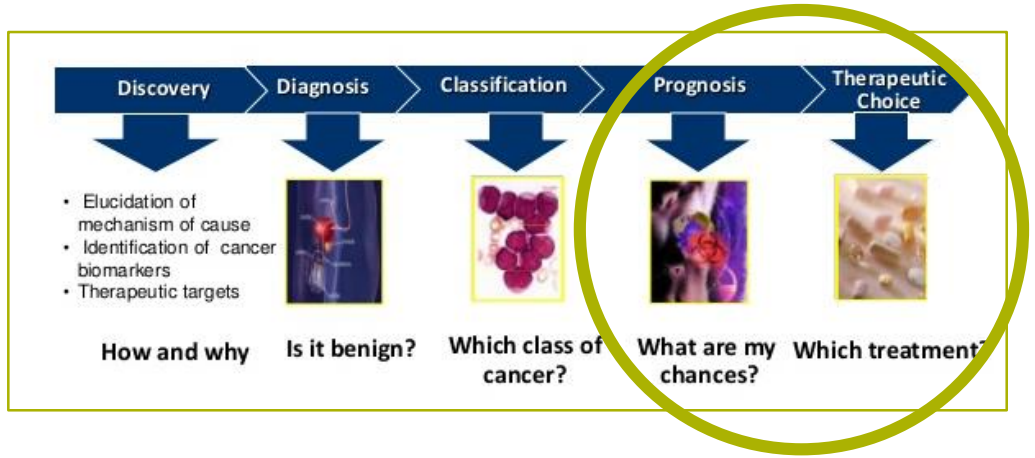
<25%	likely somatic (subclonal?)
25-75%	germline +/- or somatic
>90%	likely germline +/+

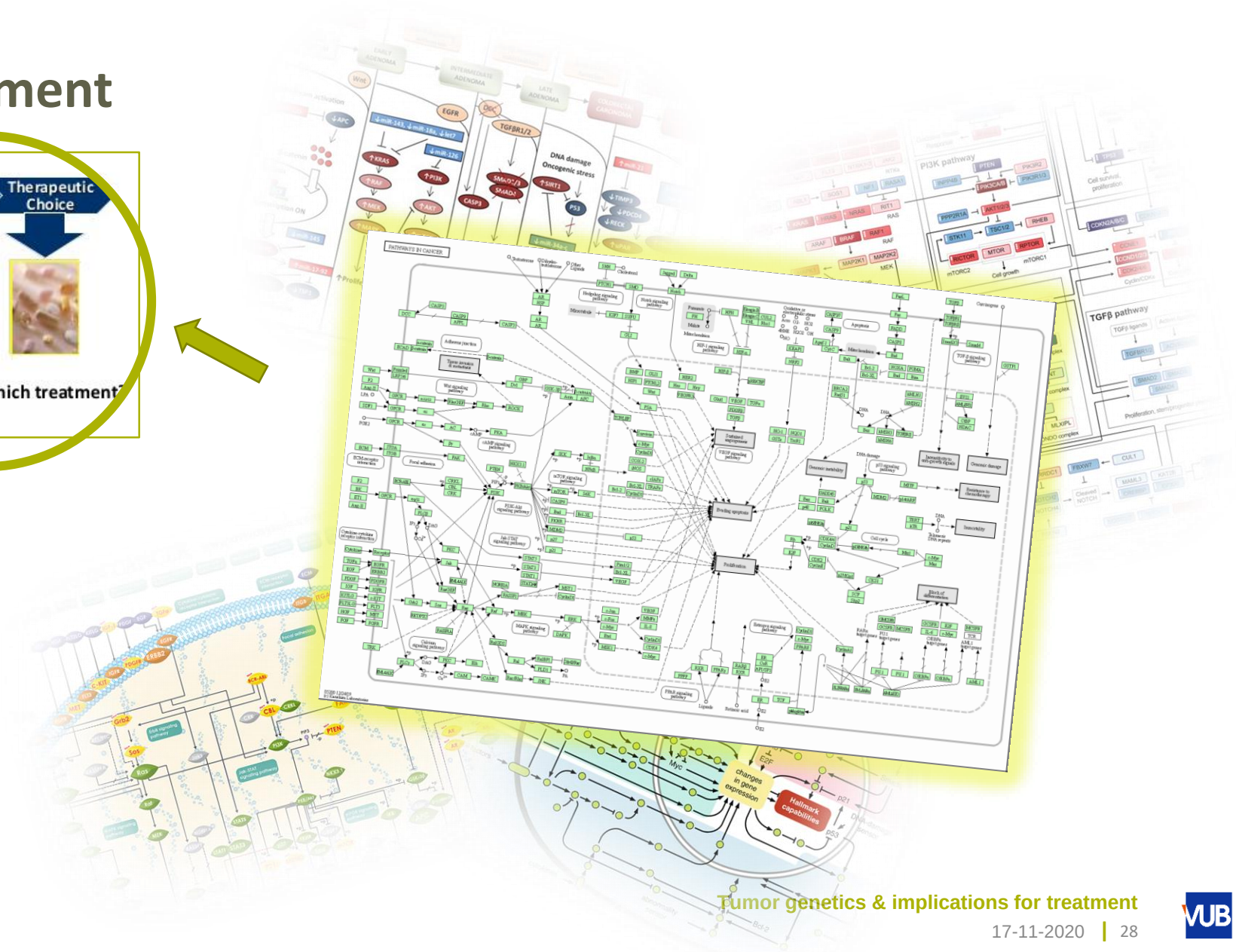


<25%	likely somatic (subclonal?)
25-75%	germline +/- or somatic
>90%	germline +/+ or somatic

TUMOR PROFILING

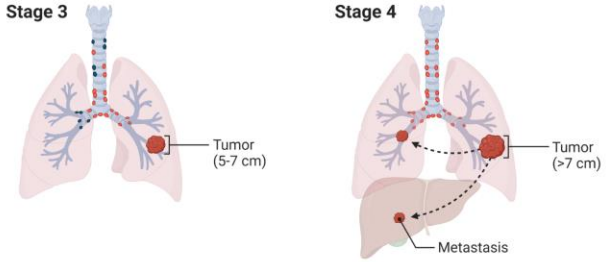
Implications for treatment

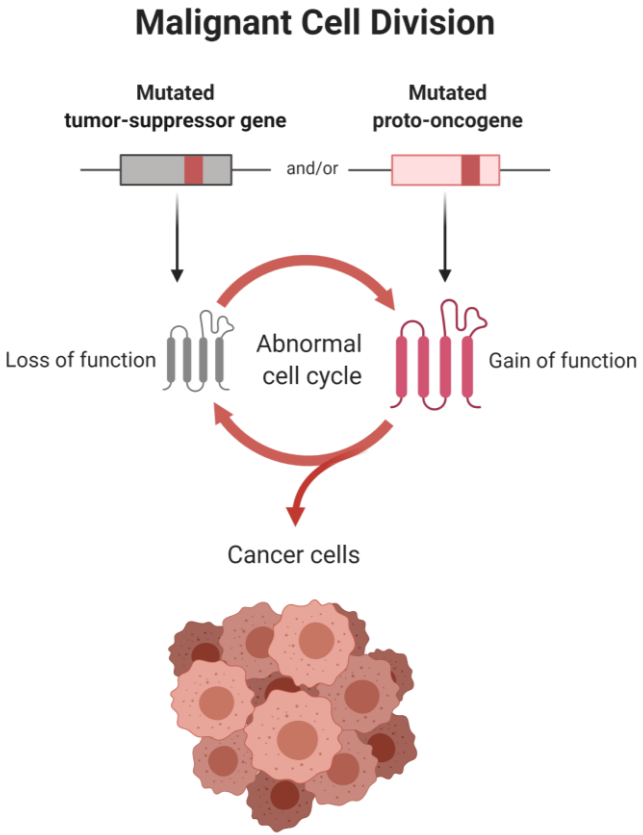




NON-SMALL CELL LUNG CANCER

TARGETED THERAPY

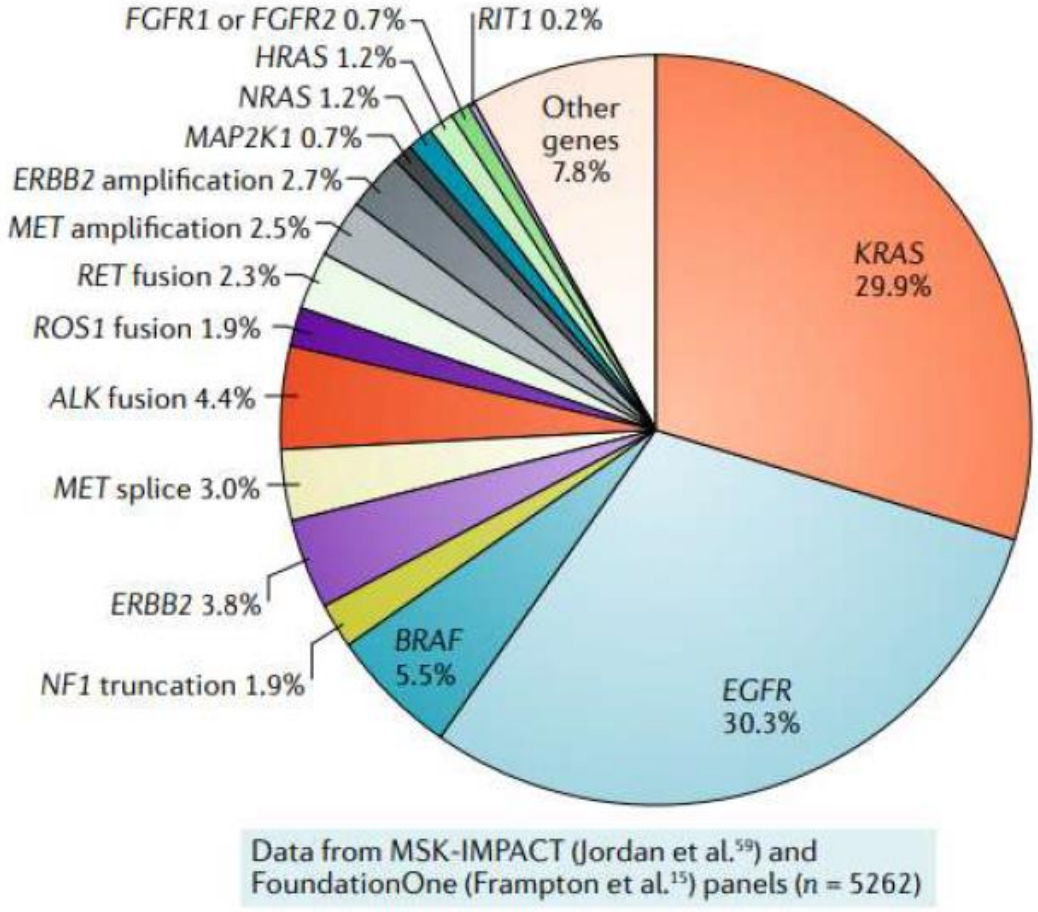


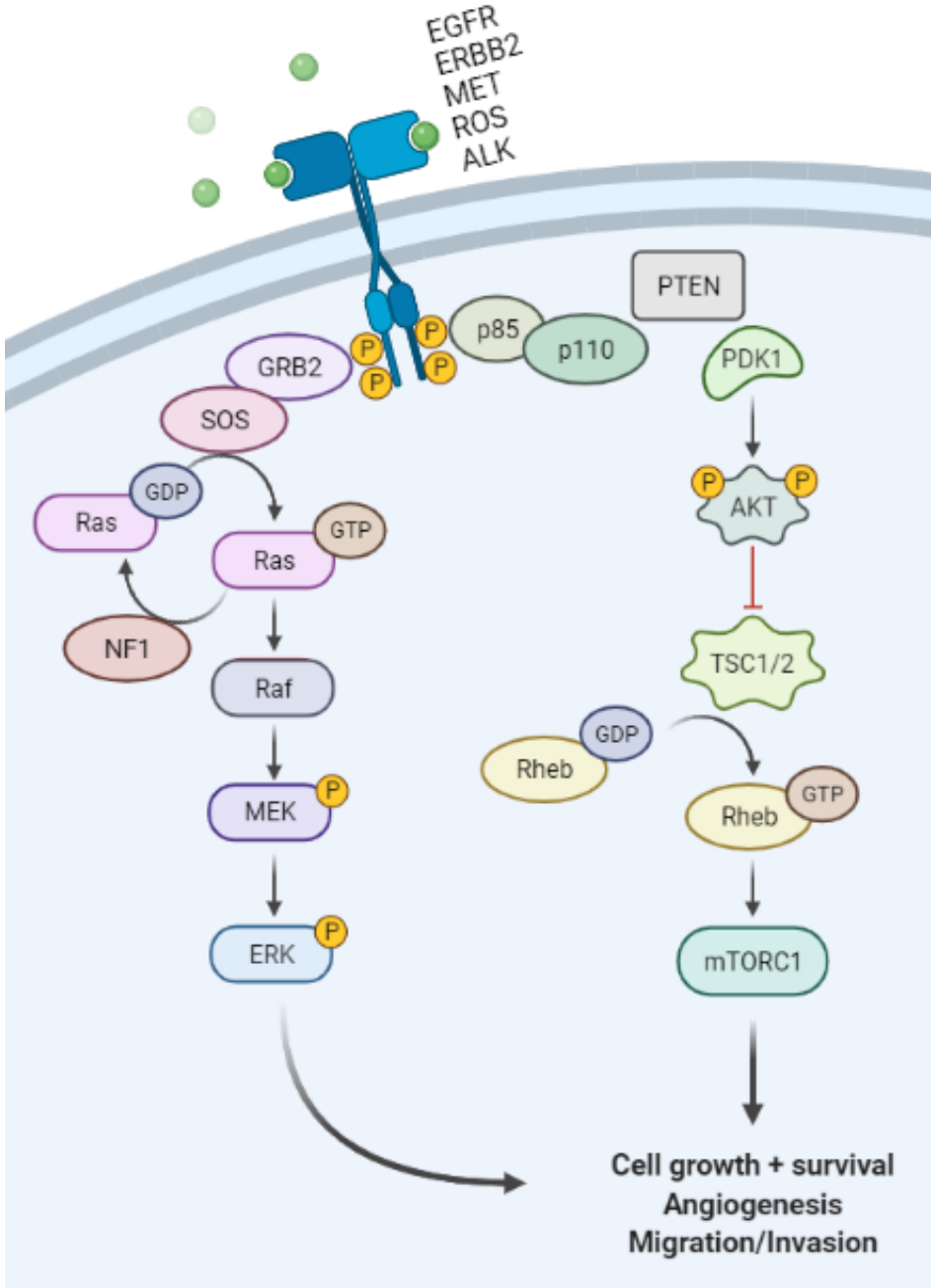


Targeted therapy focuses on the molecular inhibition of cancer-specific defects which generally underly the malignant transformation

NON-SMALL CELL LUNG CANCER

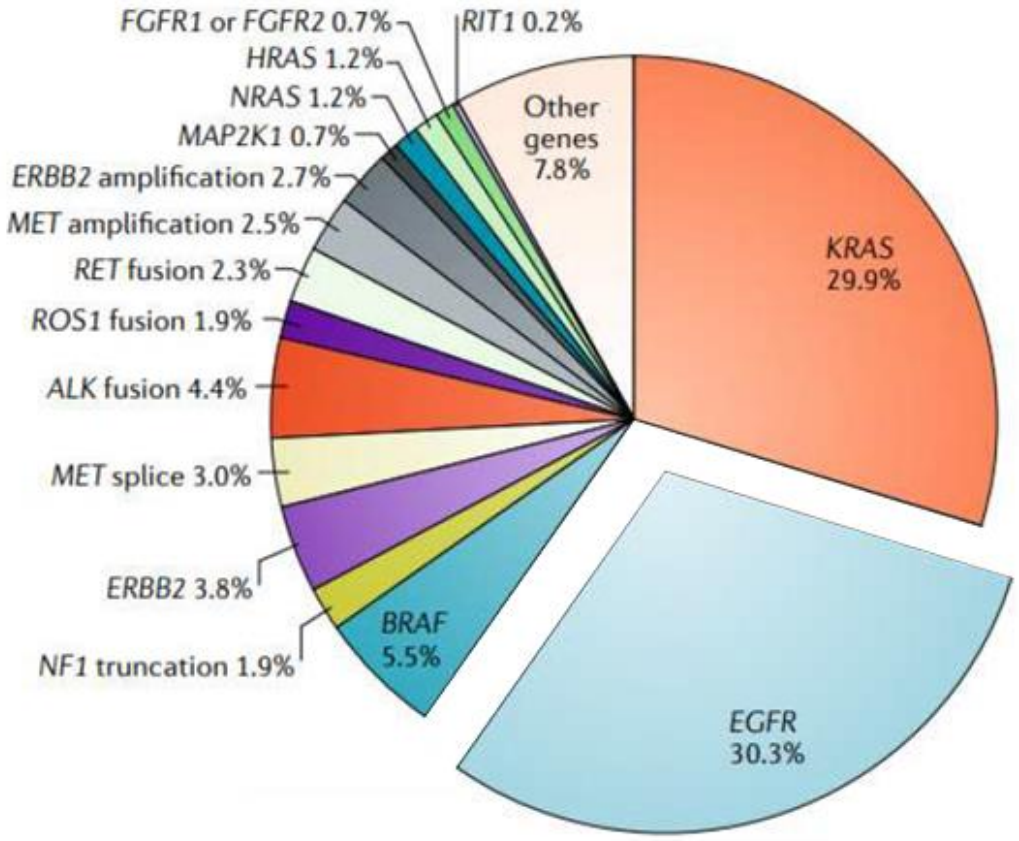
TARGETED THERAPY

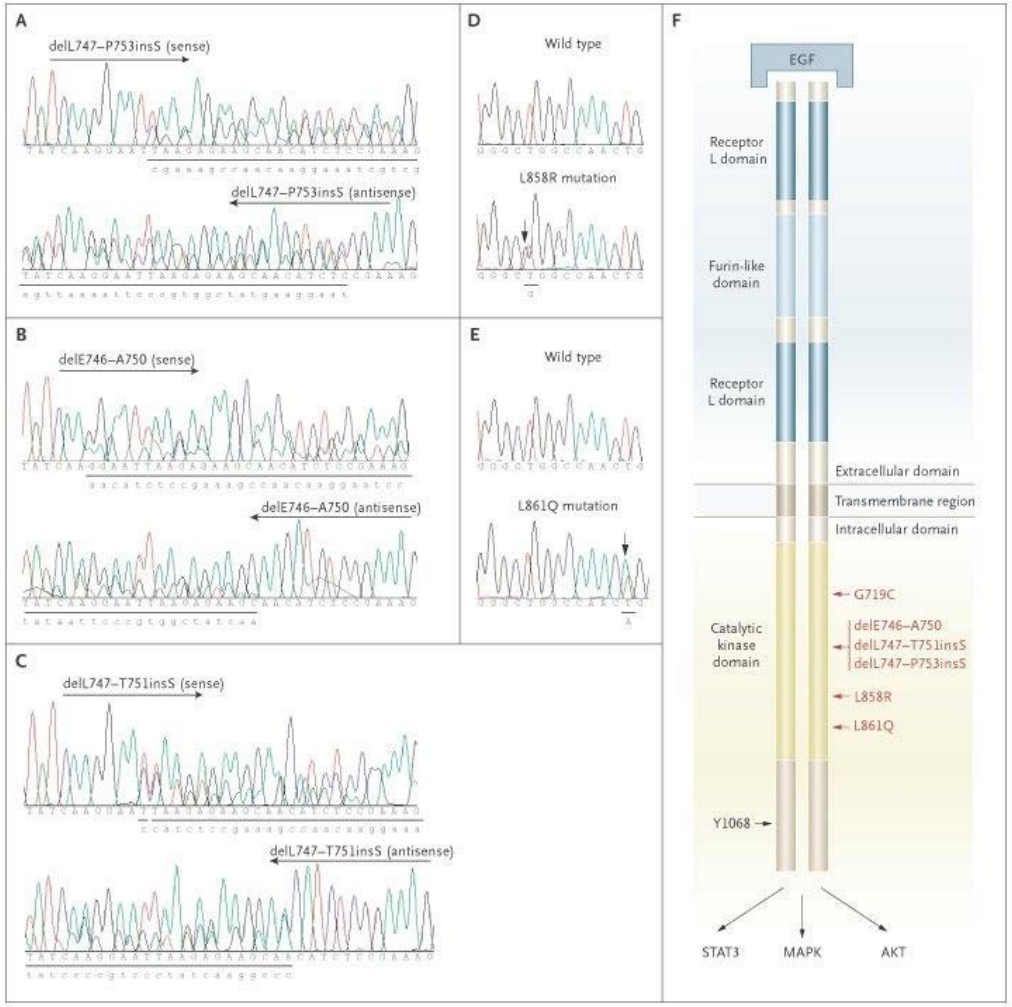


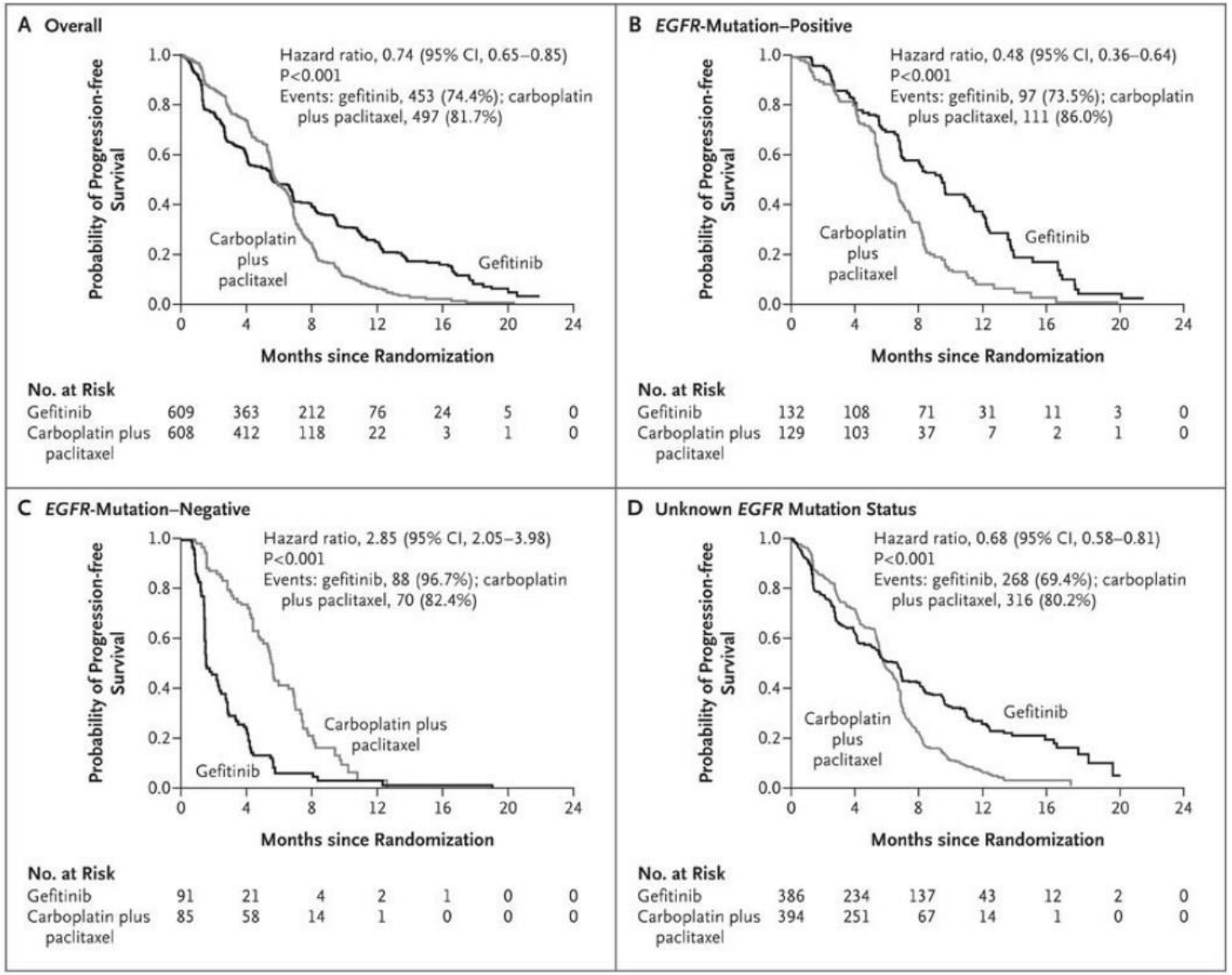


Skoulidis F et al. *Nature Reviews Cancer 2019 19:9,* 2019;19:495–509.

EGFR TARGETED THERAPY







IPASS trial

- Unselected NSCLC patients: response rates of 9-19% (PMID: 12748244, PMID: 14570950)
- Selected NSCLC patients (EGFR mut): response rates of >70% (PMID: 20573926)

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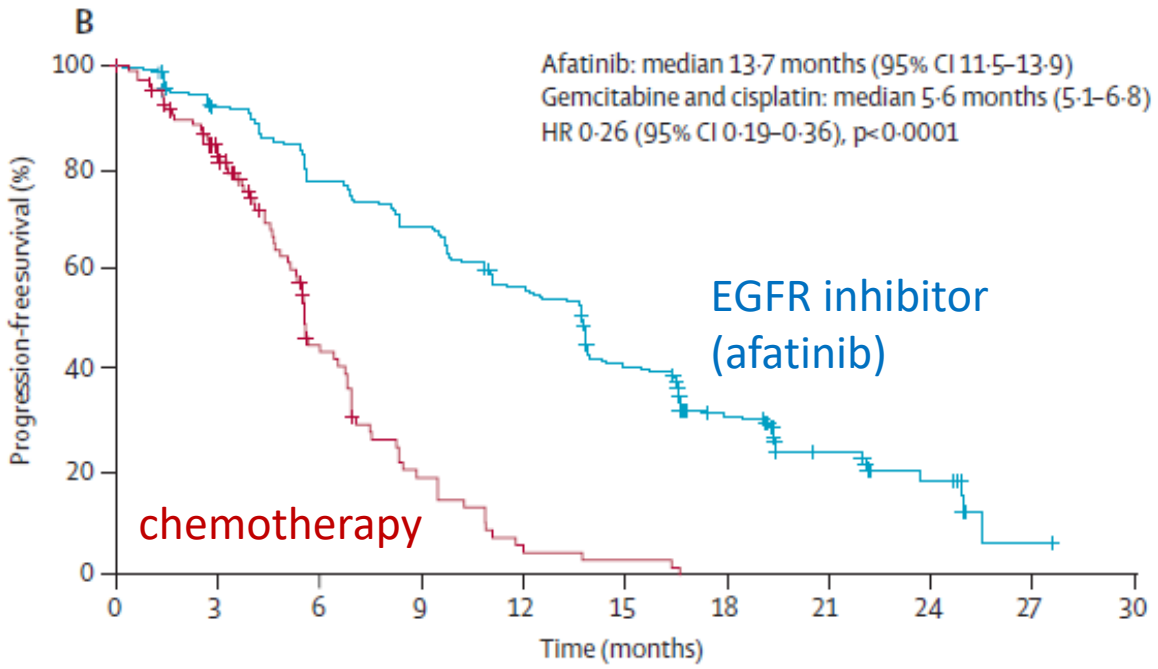
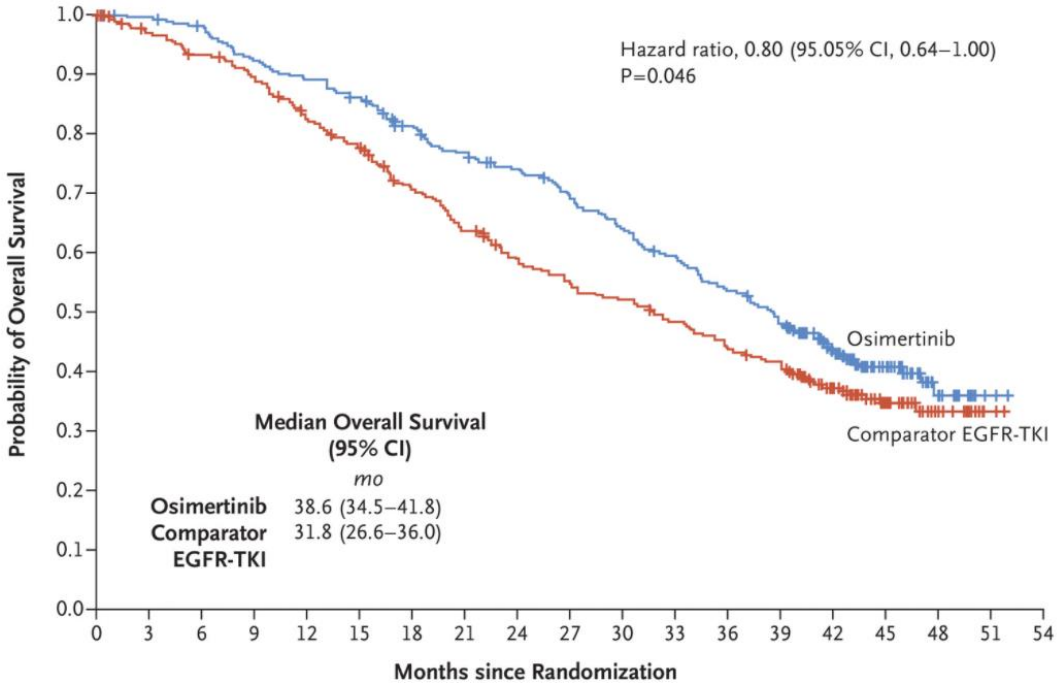
VUB

SMALL MOLECULE INHIBITORS

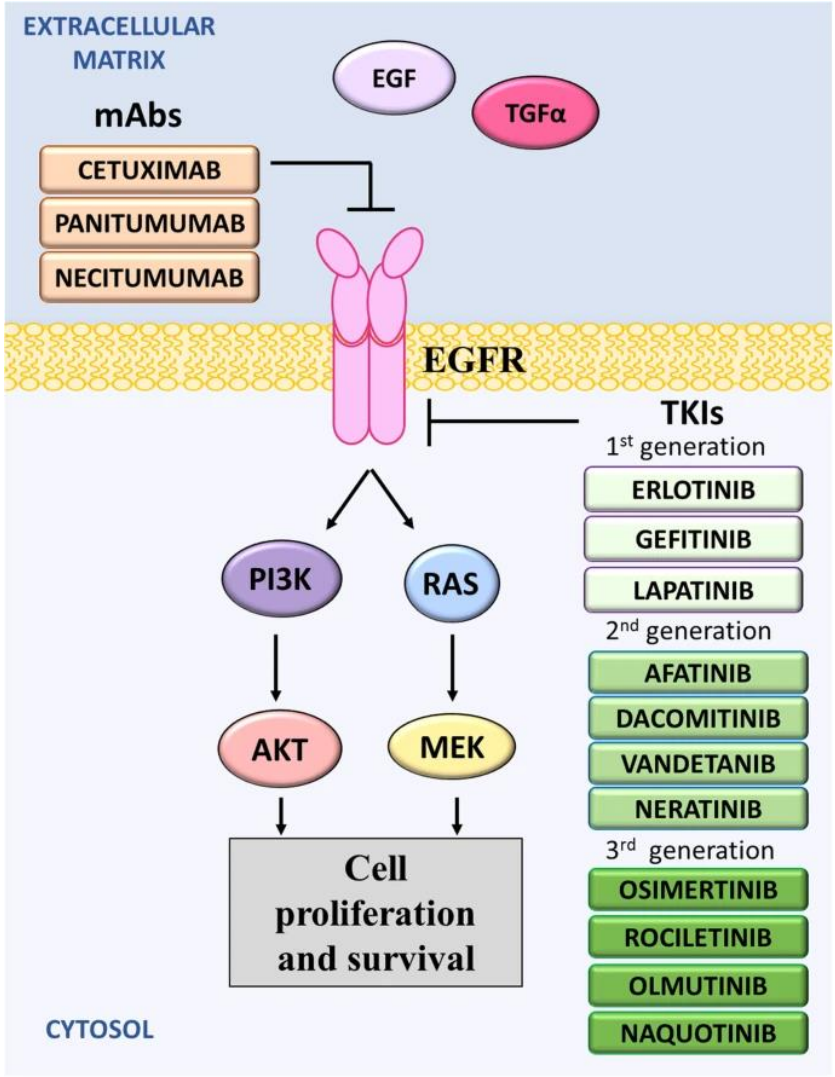


Clinical trial: LUX-Lung 6



Clinical trial: FLAURA

TARGETING EGFR/HER TYROSINE KINASE PATHWAY



Indications

EGFR/HER activating mutations

- Ex19 in-frame del/ins
- EGFR TKD L858R, S781I, S768I, L861Q, G719X
- EGFR extracellular domain A289V

EGFR/HER amplifications

Contra-indications

EGFR "resistance" mutations

- Ex20 in frame insertions (resistance to 1st and 2nd second generation TKI)
- T790M (acquired resistance to 1st and 2nd generation TKI)
- C797S (acquired resistance to 3rd generation TKI)
- D761Y, L747S, T854A (uncommon resistance mutations)
- G465R, S492R (resistance to mAbs)

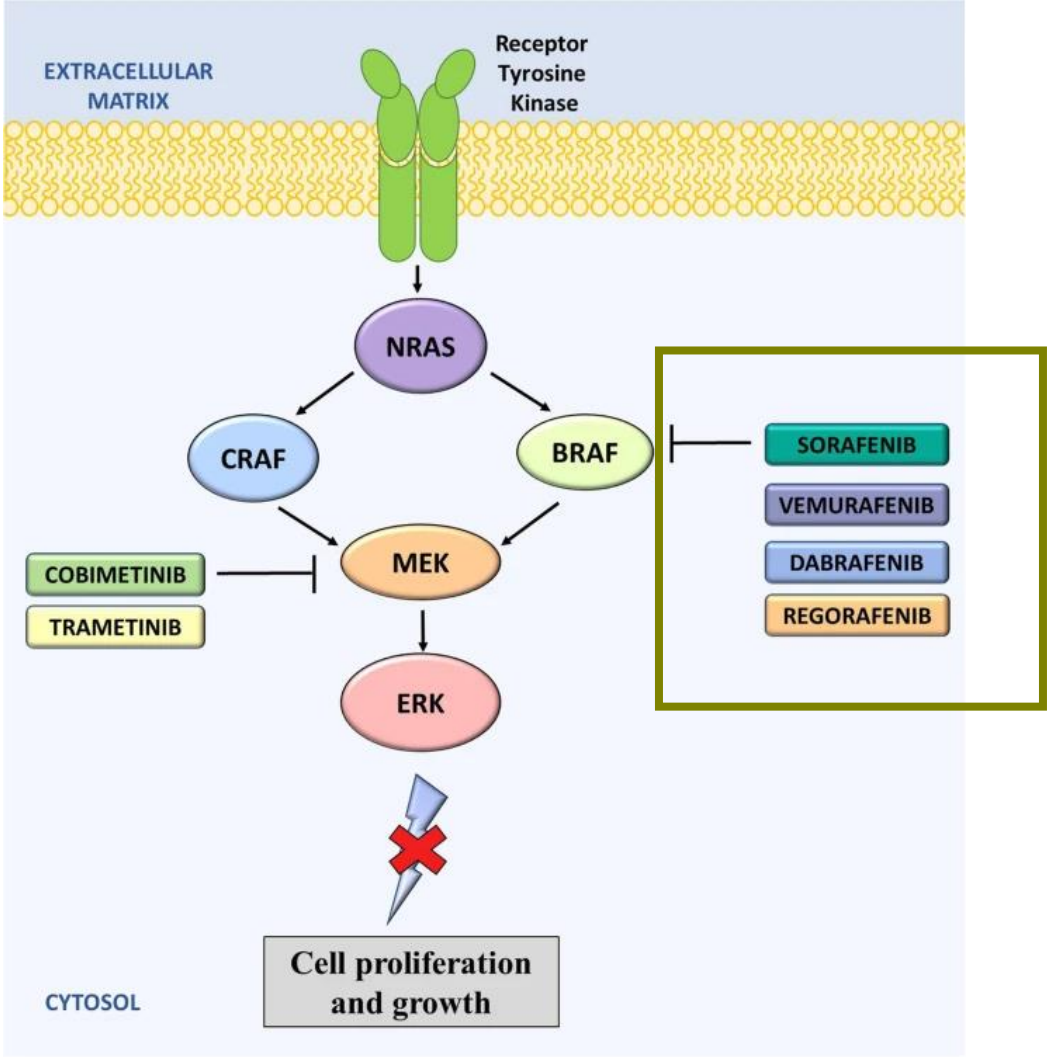
MET amplifications

Loss of NF1/PTEN

Activating KRAS/NRAS/BRAF mutations

Clinical development of targeted and immune based anti-cancer therapies. Seebacher et al., 2019

TARGETING BRAF AND MEK



Indications

BRAF activating mutations

- V600E
- non-V600E (D594G/V, G469A/V, in-frame del exon 12)

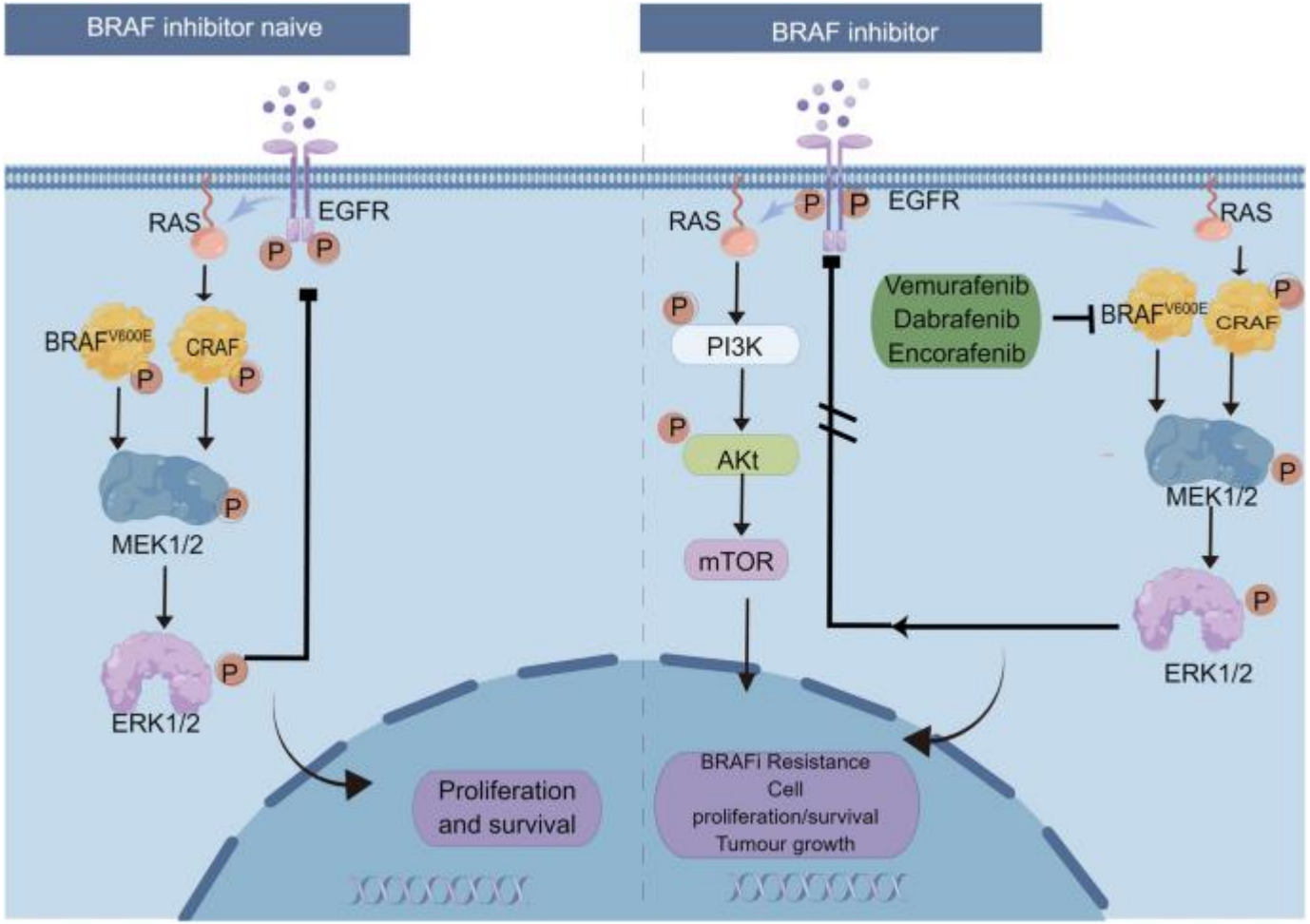
NRAS activating mutations

- G12X, G13X, A59T, Q61X

Contra-indications

- Loss of NF1/PTEN

Clinical development of targeted and immune based anti-cancer therapies. Seebacher et al., 2019



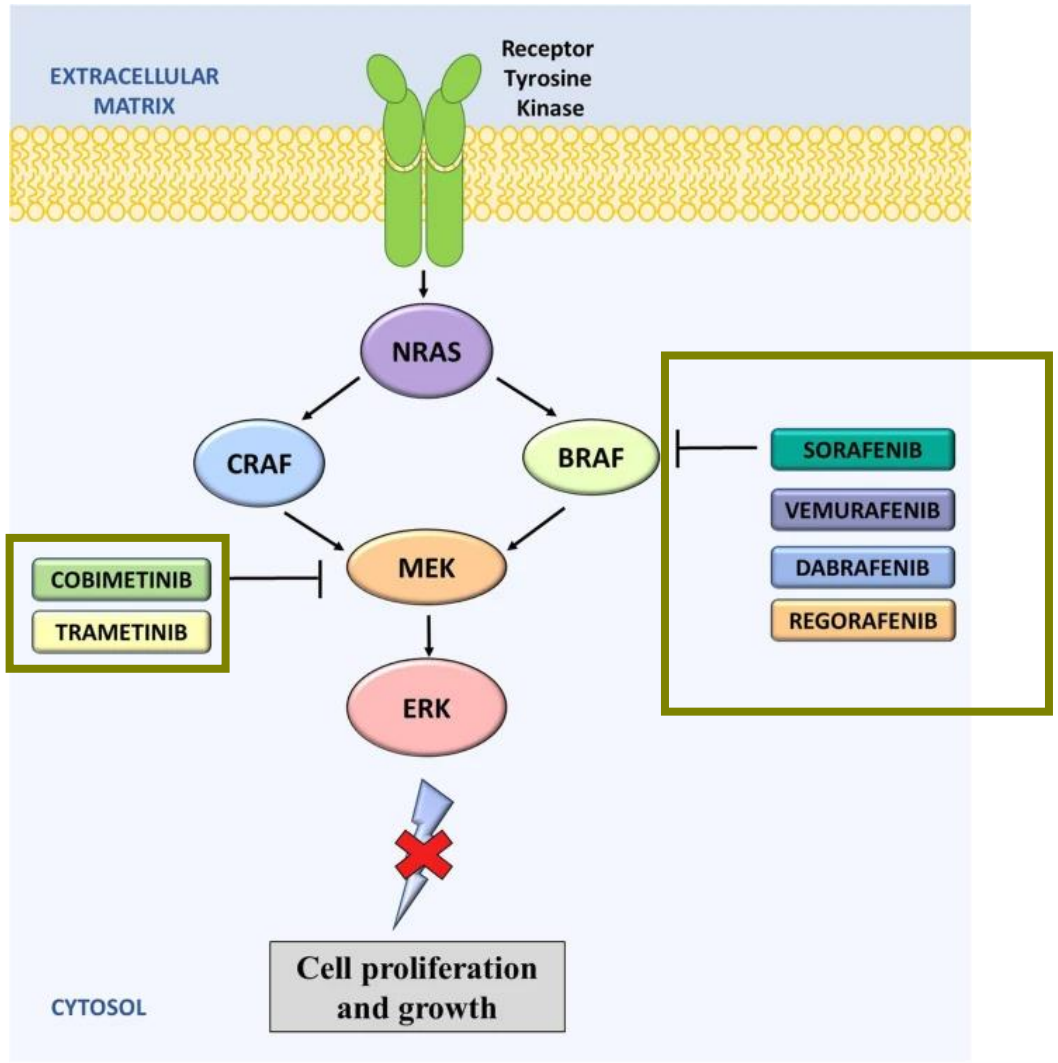
Published: 26 January 2012

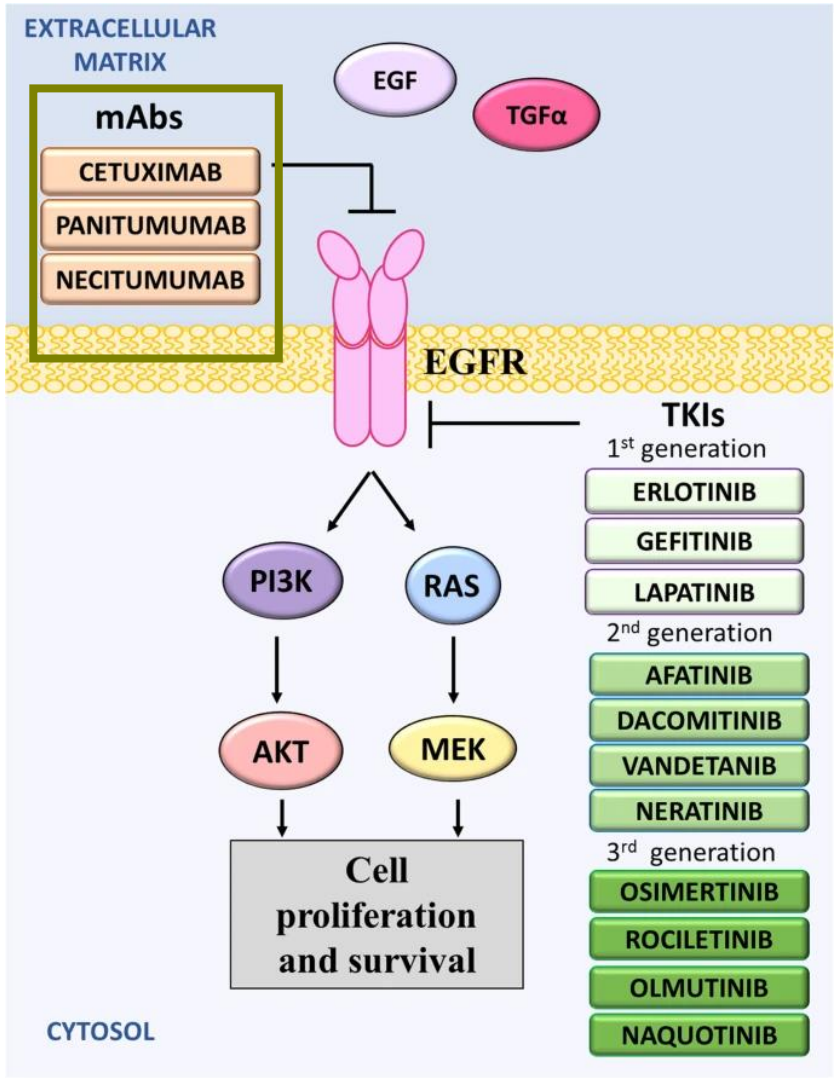
Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR

Anirudh Prahallad, Chong Sun, Sidong Huang, Federica Di Nicolantonio, Ramon Salazar, Davide Zecchin, Roderick L. Beijersbergen, Alberto Bardelli & René Bernards

Nature **483**, 100–103 (2012) | Cite this article

TARGETING BRAF AND MEK (+EGFR M-AB)

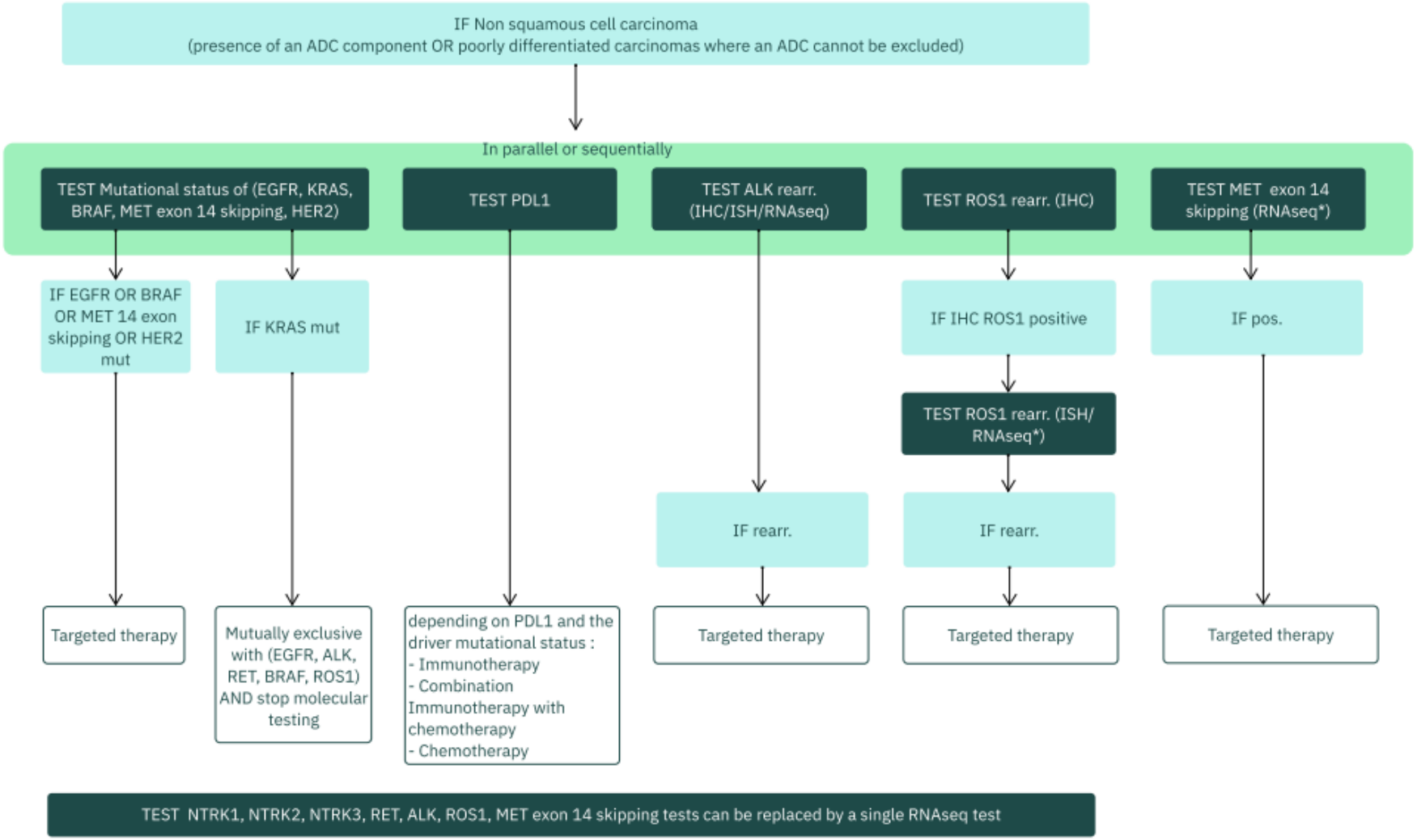




Clinical development of targeted and immune based anti-cancer therapies. Seebacher et al., 2019

Lung cancer

March 2022



These workflows are considered as a tool for good clinical practice. Some of the recommended molecular tests present in the workflows are not yet reimbursed by the INAMI/RIZIV.

Test level 1 & 2A : Molecular tests are recommended

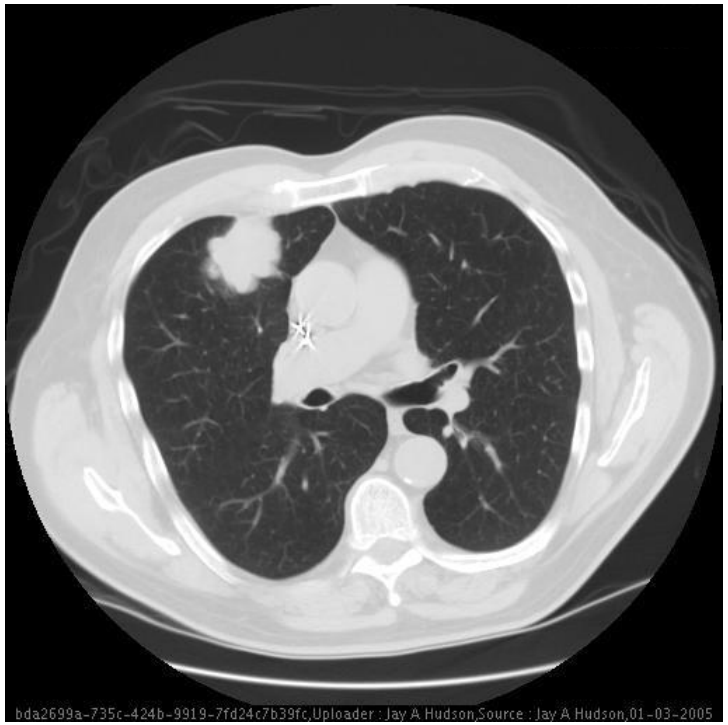
Test level 2B : Molecular tests are not yet recommened

Universitair Ziekenhuis Brussel

VUB

TUMOR PROFILING

58-year-old male - NSCLC - 20% neoplastic cells

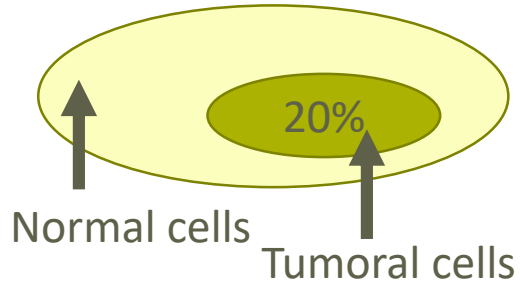


58 year-old male
Never smoker
Cough
Shortness of breath
CT shows mass in the lung

TUMOR PROFILING

58-year-old male - NSCLC - 20% neoplastic cells

Variant annotation		VAF	Biological classification
NM_005228.4(EGFR):c.2237_2255delinsT	p.(Glu746_Ser752delinsVal)	21% VAF	pathogenic
NM_006218.3(PIK3CA):c.3140A>G	p.(His1047Arg)	4.17% VAF	pathogenic
NM_000059.3(BRCA2):c.9117G>A	p.(Pro3039=)	56% VAF	likely pathogenic



TUMOR PROFILING

58-year-old male - NSCLC - 20% neoplastic cells

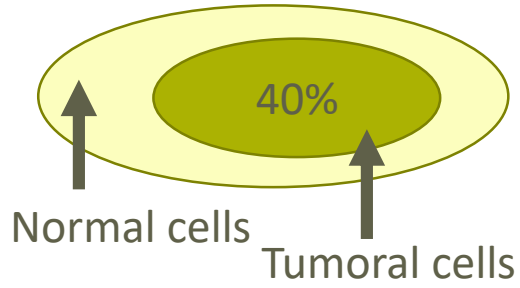
Variant annotation		VAF	Biological classification	
NM_005228.4(EGFR):c.2237_2255delinsT	p.(Glu746_Ser752delinsVal)	21% VAF	pathogenic	→ EGFR TKI
NM_006218.3(PIK3CA):c.3140A>G	p.(His1047Arg)	4.17% VAF	pathogenic	
NM_000059.3(BRCA2):c.9117G>A	p.(Pro3039=)	56% VAF	likely pathogenic	→ Olaparib Germline?

Patient harbours a NSCLC with a classical exon 19 deletion. First line treatment is EGFR TKI osimertinib.
Upon disease progression, explorative studies such as olaparib (not in study) or alpelisib (not in study) can be used. Alpelisib and Olaparib may not be effective in this cancer.
Genetic councelling for BRCA2 is recommended.

TUMOR PROFILING

64-year-old male - NSCLC meta - 40% neoplastic cells

Variant annotation		VAF	Biological classification
NM_000546.5(TP53):c.824G>T	p.(Cys275Phe)	34% VAF	likely pathogenic
NM_001127500.2(MET):c.3082+3A>G	p.?	43% VAF	likely pathogenic
NM_006218.3(PIK3CA):c.1342G>T	p.(Val448Leu)	12% VAF	VUS
NM_001982.3(ERBB3):c.3812del	p.(Gly1271Valfs*22)	26% VAF	VUS
NM_001127500.2(MET):c.3433G>T	p.(Glu1145*)	12% VAF	VUS

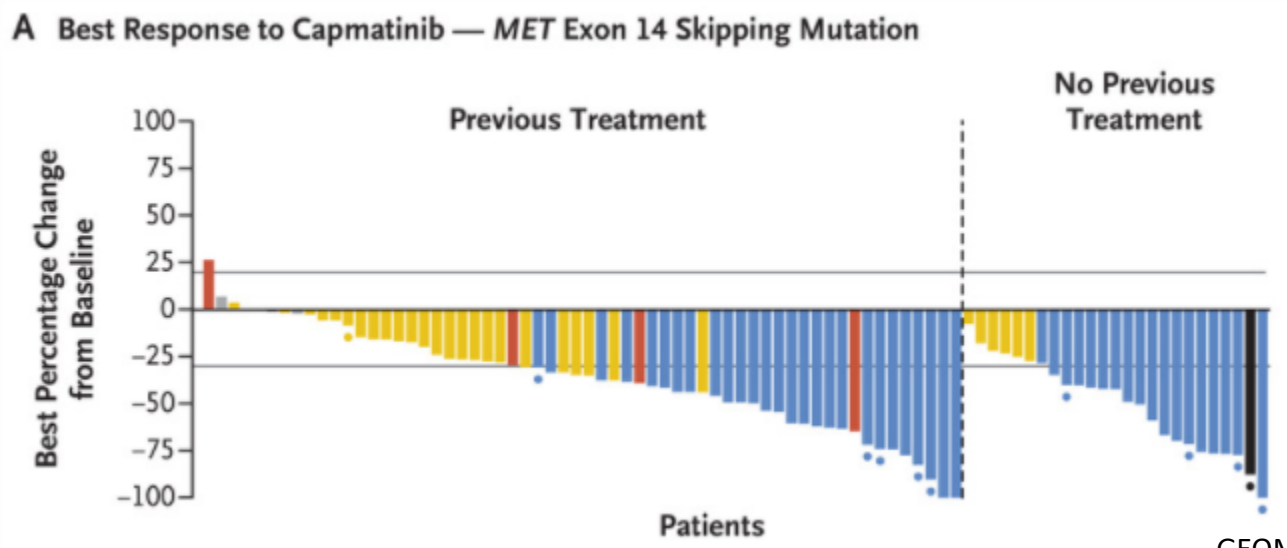


TUMOR PROFILING

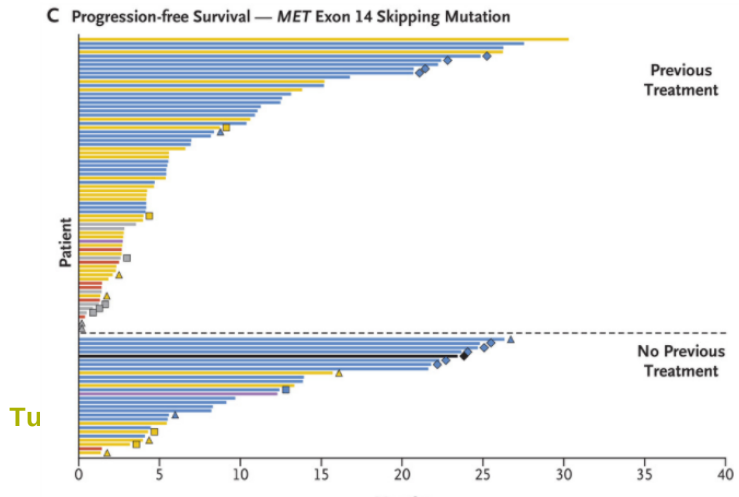
64-year-old male - NSCLC meta - 40% neoplastic cells

Variant annotation		VAF	Biological classification	
NM_000546.5(TP53):c.824G>T	p.(Cys275Phe)	34% VAF	likely pathogenic	
NM_001127500.2(MET):c.3082+3A>G	p.?	43% VAF	likely pathogenic	→ MET inhibitor
NM_006218.3(PIK3CA):c.1342G>T	p.(Val448Leu)	12% VAF	VUS	→ Alpelisib?
NM_001982.3(ERBB3):c.3812del	p.(Gly1271Valfs*22)	26% VAF	VUS	
NM_001127500.2(MET):c.3433G>T	p.(Glu1145*)	12% VAF	VUS	→ MET inhibitor?

Patient with a MET exon 14 skipping. Crizotinib or capmatinib (pref.)
The PIK3CA and MET VUS are no indication/ contra indication for therapy.



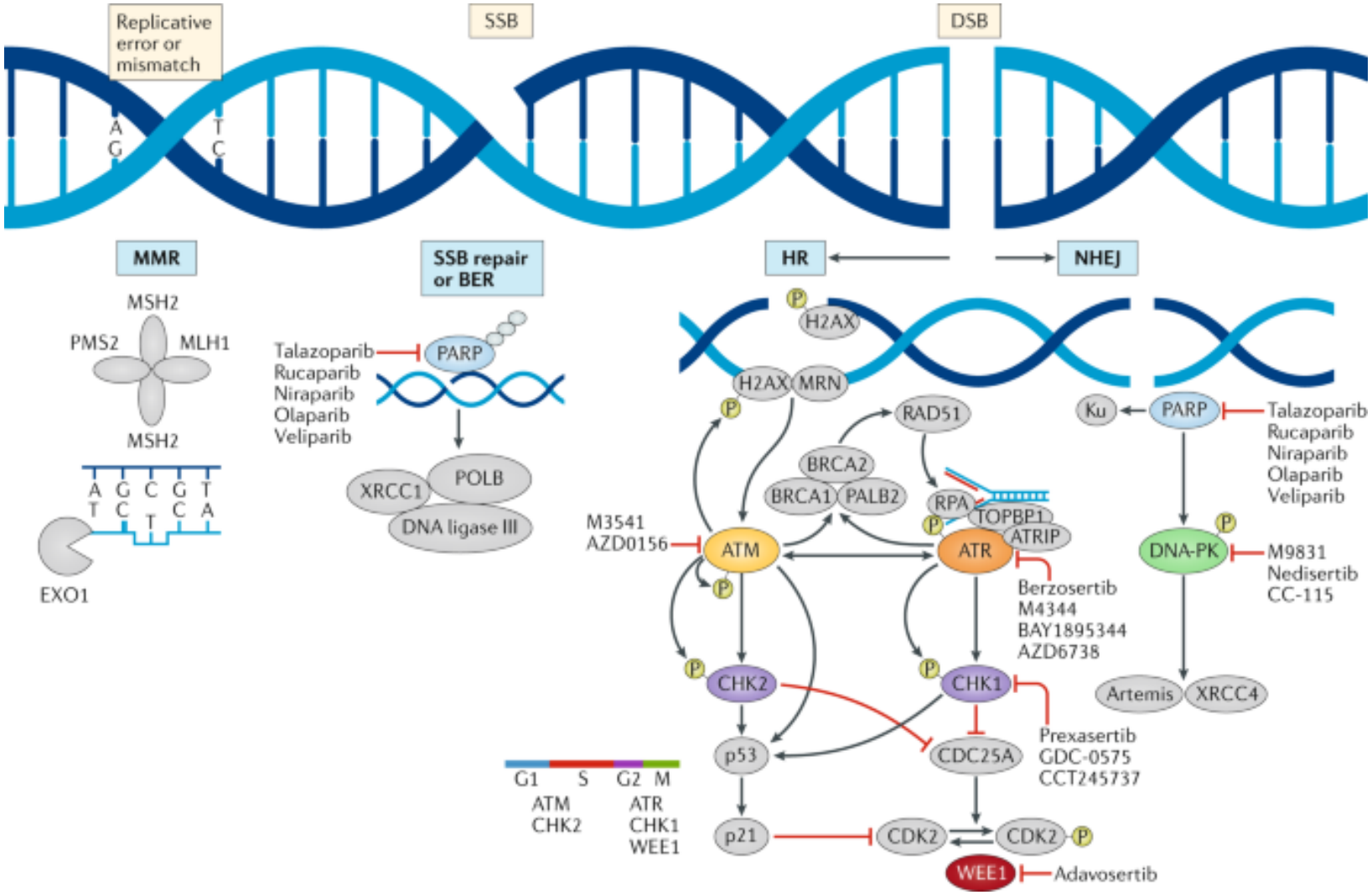
GEOMETRY mono-1 trial



Tu

DNA DAMAGE PATHWAY

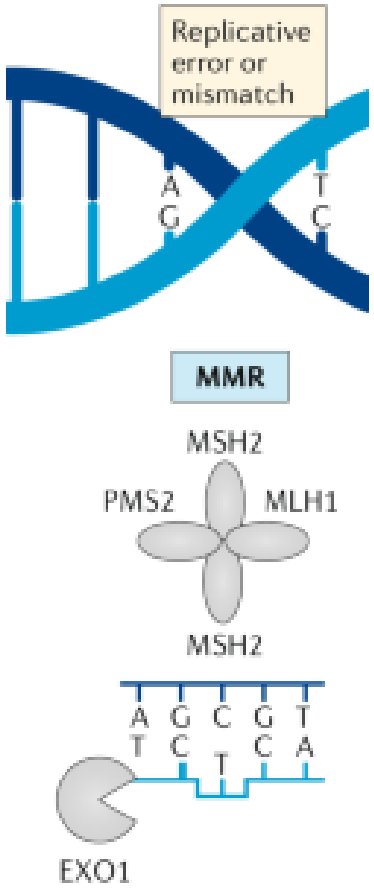
Implications for germline mutations and therapy

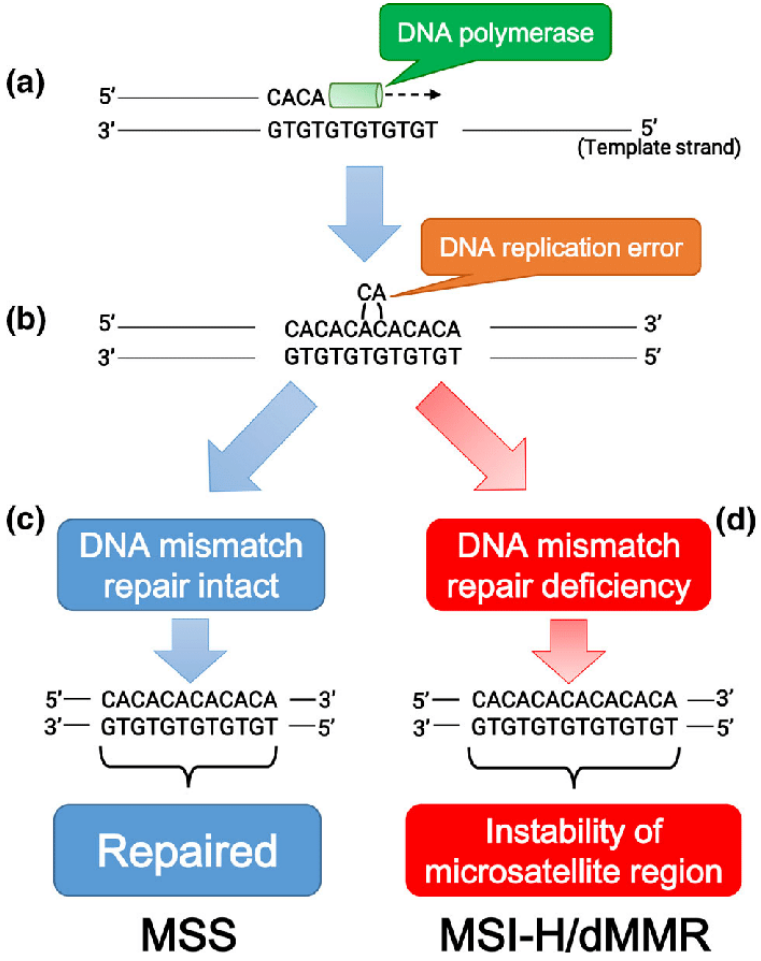


State-of-the-art strategies for targeting the DNA damage response in cancer. Pilié et al., 2019

DNA DAMAGE PATHWAY

Implications for germline mutations and therapy

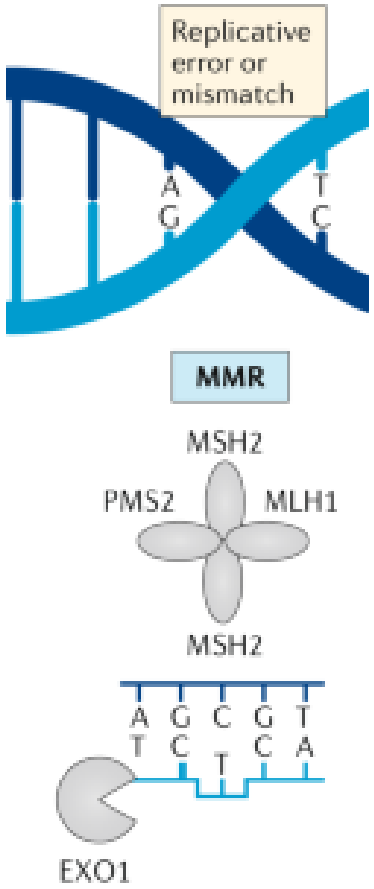




State-of-the-art strategies for targeting the DNA damage response in cancer. Pilié et al., 2019

DNA DAMAGE PATHWAY

Implications for germline mutations and therapy



Mutations in genes involved in mismatch repair or BER

- MSH2, MSH6, PMS2, MLH1
- MLH1 promoter methylation (non-heriditary)

Mutations in genes involved in DNA replication

- POLE, POLD1

Tumor types

Colorectal cancer
Gynaecological origin

Clinical implications

Associated with microsatellite instability

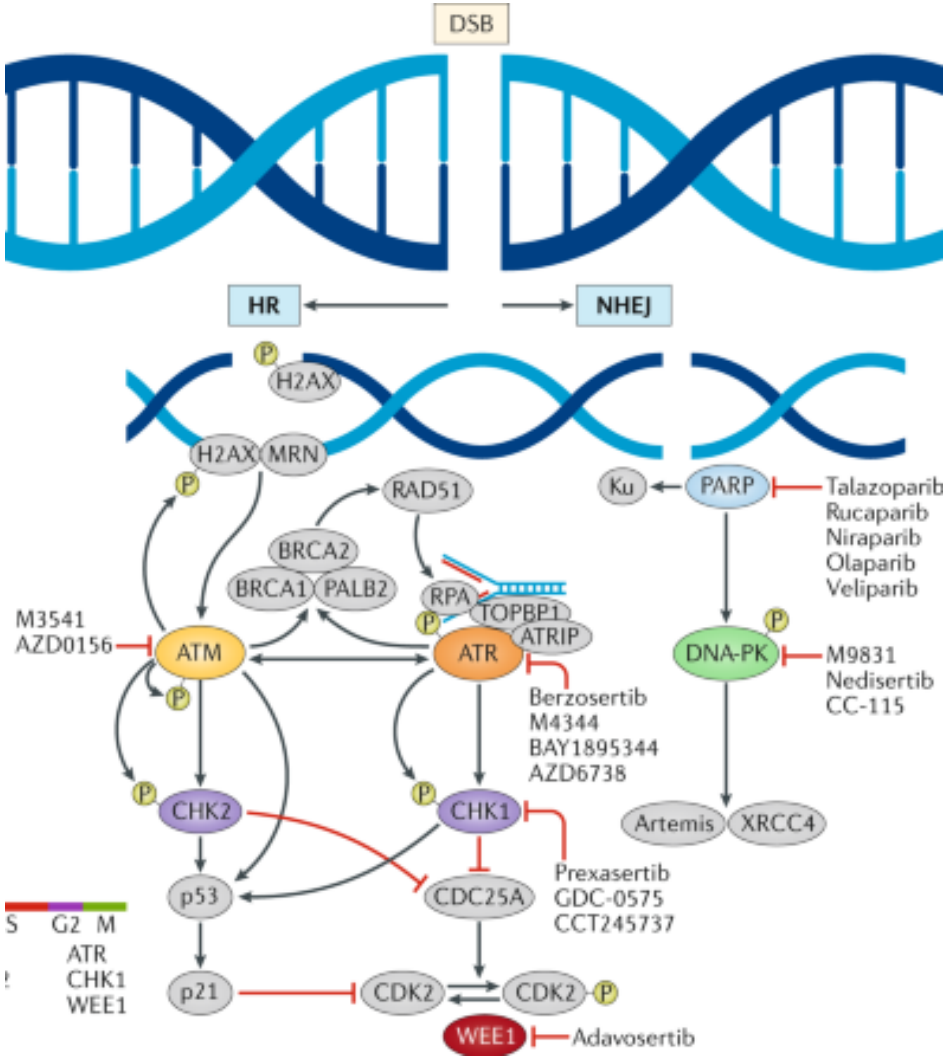
<u>Germline:</u> Lynch syndrome (HNPCC)
At risk for Colorectal cancer, gynaeco cancer, upper GI, urological

<u>Therapy:</u>
Indication for adjuvant chemotherapy
Indication for immunotherapy (checkpoint blockers)

State-of-the-art strategies for targeting the DNA damage response in cancer. Pilié et al., 2019

DNA DAMAGE PATHWAY

Implications for germline mutations and therapy



Mutations in genes involved homologous recombination

- BRCA1/2
- PALB2
- ATM, ATR
- CHEK1, CHEK2
- TP53
- BRIP1, RAD51, RAD54L, ...

Tumor types

Ovarian, prostate, breast and pancreatic cancers

Therapy implications:

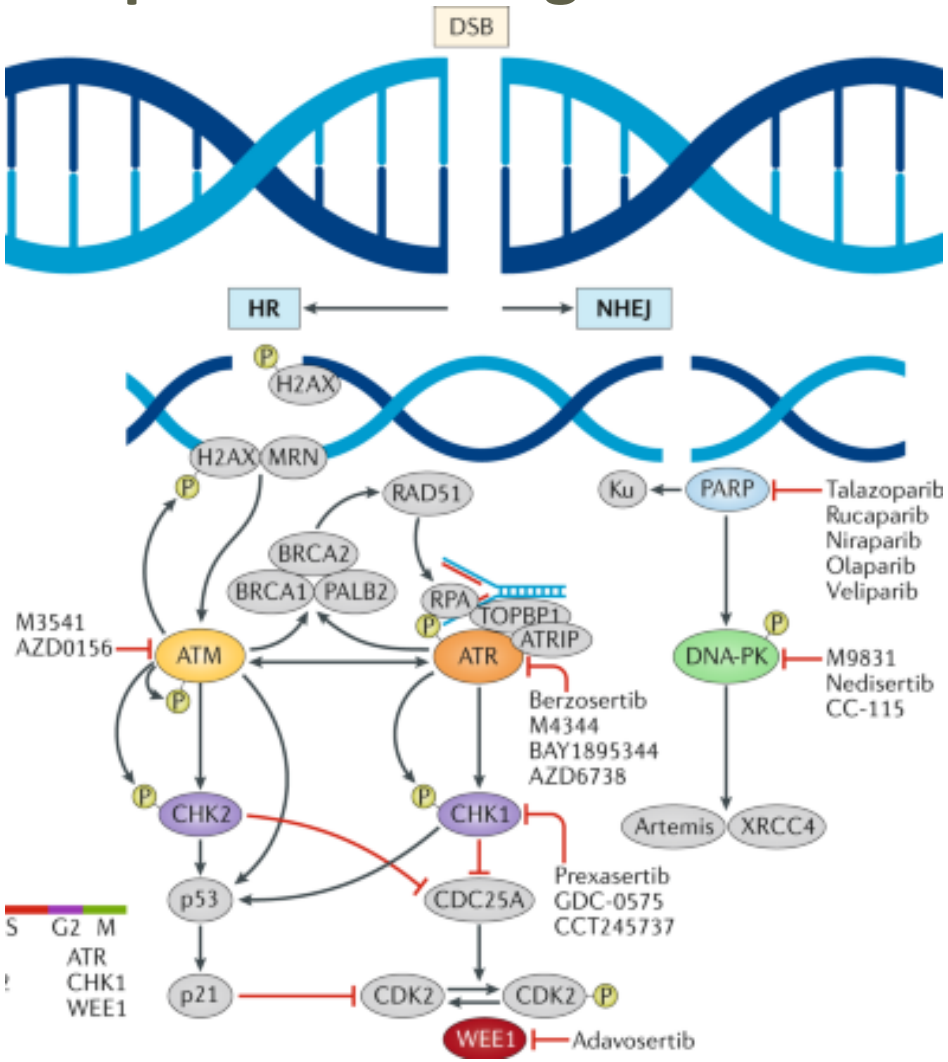
PARP inhibitors (eg. Olaparib) show good activity in tumors with defects in homologous recombination (synthetic lethality)

→ Olaparib (HR def. Status and/or BRCA1/2)

State-of-the-art strategies for targeting the DNA damage response in cancer. Pilié et al., 2019

DNA DAMAGE PATHWAY

Implications for germline mutations and therapy



Indications for genetic counseling

Germline BRCA1/2, PALB2: Heriditary breast and ovarian cancer:
At risk for breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, colorectal cancer

Germline ATM: ataxia-telangiectasia:
At risk for breast cancer, prostate cancer, pancreatic cancer, colorectal cancer, lymphoma, leukemia

Germline CHEK1, CHEK2:
At risk for breast cancer, prostate cancer, colorectal cancer

Germline TP53: Li - Fraumeni syndrome
Most commonly at risk for breast cancer, adrenocortical carcinoma, central nervous system, sarcoma

State-of-the-art strategies for targeting the DNA damage response in cancer. Pilié et al., 2019

TUMOR PROFILING

88-year-old male – CRC early stage - MSI unstable -

Variant annotation		VAF	Biological classification
NM_000546.5(TP53):c.524G>A	p.(Arg175His)	25% VAF	pathogenic
NM_007294.3(BRCA1):c.1961del	p.(Lys654Serfs*47)	11% VAF	likely pathogenic
NM_000179.2(MSH6):c.3261del	p.(Phe1088Serfs*2)	15% VAF	likely pathogenic
NM_000077.4(CDKN2A):c.220G>A	p.(Asp74Asn)	24% VAF	likely pathogenic
NM_003482.3(KMT2D):c.7061del	p.(Pro2354Leufs*30)	13% VAF	likely pathogenic
NM_003482.3(KMT2D):c.7780del	p.(Leu2594Trpfs*97)	13% VAF	likely pathogenic
NM_003482.3(KMT2D):c.9635dup	p.(Phe3213Valfs*2)	18% VAF	likely pathogenic
NM_000038.6(APC):c.1690C>T	p.(Arg564*)	18% VAF	likely pathogenic
NM_000038.6(APC):c.3925G>T	p.(Glu1309*)	22% VAF	likely pathogenic
NM_000038.6(APC):c.4057G>T	p.(Glu1353*)	18% VAF	likely pathogenic
NM_000051.3(ATM):c.7456C>T	p.(Arg2486*)	13% VAF	likely pathogenic
NM_003502.3(AXIN1):c.1597C>T	p.(Arg533*)	21% VAF	likely pathogenic
NM_006015.6(ARID1A):c.3524del	p.(Pro1175Hisfs*5)	24% VAF	likely pathogenic
NM_006015.6(ARID1A):c.5086_5093del	p.(Tyr1696Glnfs*28)	20% VAF	likely pathogenic
NM_002354.2(EPCAM):c.259del	p.(Ala87Profs*33)	18% VAF	likely pathogenic
NM_006231.3(POLE):c.2091dup	p.(Phe699Valfs*11)	6% VAF	likely pathogenic
NM_000321.2(RB1):c.1049+3A>T	p.?	23% VAF	likely pathogenic

TUMOR PROFILING

88-year-old male - CRC- 80% neoplastic cells - MSI unstable

Variant annotation		VAF	Biological classification
NM_000546.5(TP53):c.524G>A	p.(Arg175His)	25% VAF	pathogenic
NM_007294.3(BRCA1):c.1961del	p.(Lys654Serfs*47)	11% VAF	likely pathogenic
NM_000179.2(MSH6):c.3261del	p.(Phe1088Serfs*2)	15% VAF	likely pathogenic
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NM_006015.6(ARID1A):c.5086_5093del	p.(Tyr1696Glnfs*28)	20% VAF	likely pathogenic
NM_002354.2(EPCAM):c.259del	p.(Ala87Profs*33)	18% VAF	likely pathogenic
NM_006231.3(POLE):c.2091dup	p.(Phe699Valfs*11)	6% VAF	likely pathogenic
NM_000321.2(RB1):c.1049+3A>T	p.?	23% VAF	likely pathogenic

Do we expect Lynch syndrome?

Do we have an explanation for the MSI-high status?

TUMOR PROFILING

69-year-old female - melanoma - 60% neoplastic cells

Variant annotation		VAF	Biological classification
NM_002524.5(NRAS):c.181C>A	p.(Gln61Lys)	50% VAF	pathogenic
NM_000314.7(PTEN):c.408T>A	p.(Cys136*)	35% VAF	likely pathogenic

→ **Therapy?**

TUMOR PROFILING

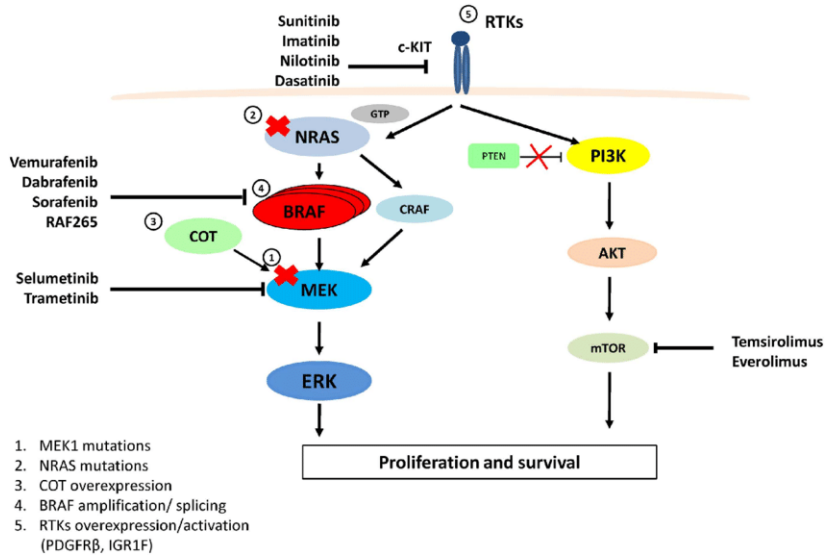
69-year-old female - melanoma - 60% neoplastic cells

Variant annotation		VAF	Biological classification
NM_002524.5(NRAS):c.181C>A	p.(Gln61Lys)	50% VAF	pathogenic
NM_000314.7(PTEN):c.408T>A	p.(Cys136*)	35% VAF	likely pathogenic

→ **MEK/BRAF inhibitor**

→ **Resistance BRAF inhibitor**

NRAS variants may induce sensitivity to BRAF/MEK inhibitors, albeit in a subpopulation of the cases.
PTEN LoF activates a parallel pathway, which may induce resistance to MAPK pathway inhibition.



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TUMOR PROFILING

65-year-old female – Endometrial Cancer - 40% neoplastic cells

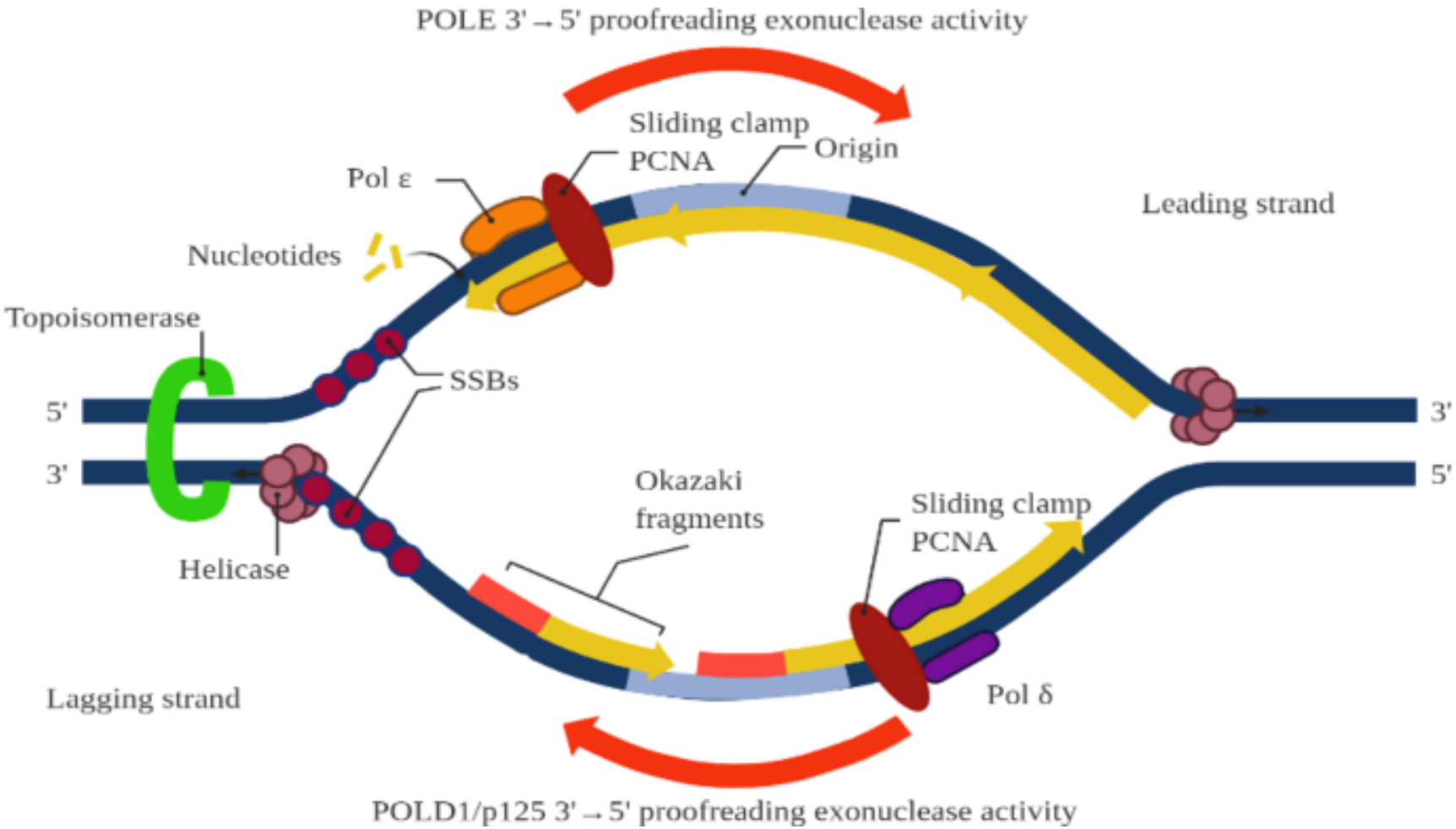
Variant annotation		VAF	Biological Classification
NM_006218.3(PIK3CA):c.1070G>A	p.(Arg357Gln)	11% VAF	Pathogenic
NM_006218.3(PIK3CA):c.1090G>A	p.(Gly364Arg)	12% VAF	Probably pathogenic
NM_181523.2(PIK3R1):c.1042C>T	p.(Arg348*)	32% VAF	Probably pathogenic
NM_006231.3(POLE):c.857C>G	p.(Pro286Arg)	18% VAF	Probably pathogenic
NM_015338.5(ASXL1):c.2941G>T	p.(Glu981*)	14% VAF	Probably pathogenic
NM_000051.3(ATM):c.1948G>T	p.(Glu650*)	14% VAF	Probably pathogenic
NM_000546.5(TP53):c.322G>A	p.(Gly108Ser)	18% VAF	Probably pathogenic
NM_000038.5(APC):c.4630G>T	p.(Glu1544*)	16% VAF	Probably pathogenic
NM_000038.5(APC):c.646C>T	p.(Arg216*)	14% VAF	Probably pathogenic
NM_000038.5(APC):c.6610C>T	p.(Arg2204*)	17% VAF	Probably pathogenic
NM_030621.4(DICER1):c.562G>T	p.(Glu188*)	14% VAF	Probably pathogenic

+ 150 extra variants of unknown significance

TUMOR PROFILING

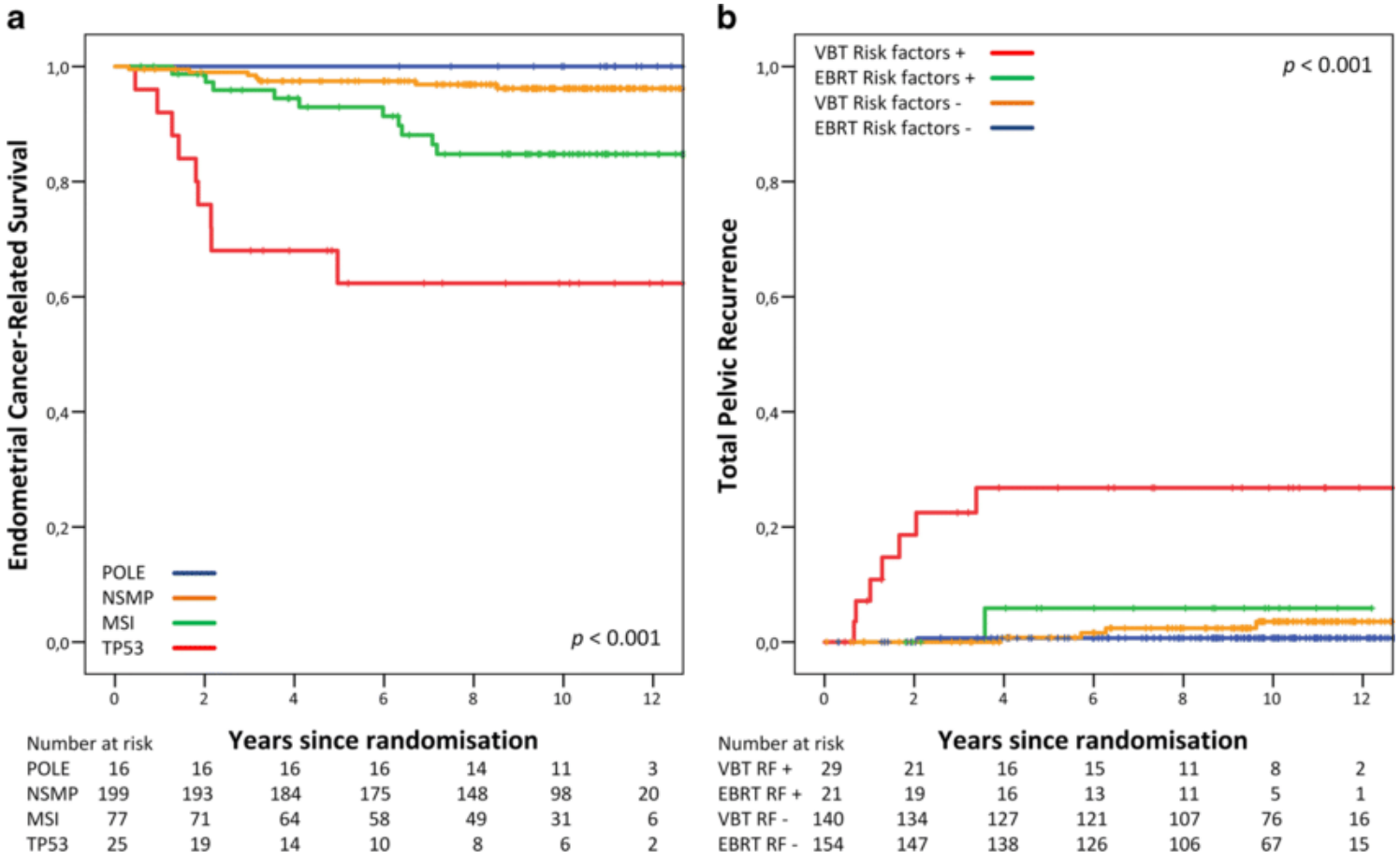
65-year-old female – Endometrial Cancer - 40% neoplastic cells

POLE driven hypermutator phenotype



TUMOR PROFILING

65-year-old female – Endometrial Cancer - 40% neoplastic cells



Wortman, Bastiaan & Nout, Remi & Bosse, Tjalling & Creutzberg, Carien. (2019). Selecting Adjuvant Treatment for Endometrial Carcinoma Using Molecular Risk Factors. Current Oncology Reports. 21. 10.1007/s11912-019-0825-z.

TUMOR PROFILING

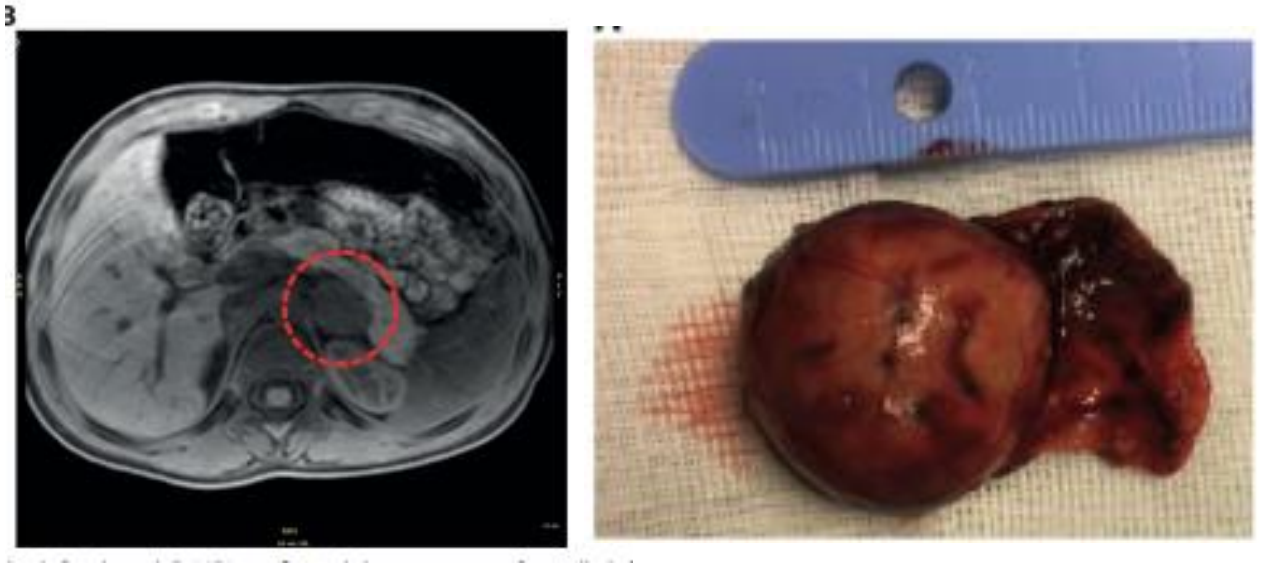
3-year-old boy - adrenal gland carcinoma - 80% neoplastic cells

Table 1 Hormone levels before and after left adrenalectomy

	Before surgery	1 month after surgery	References
LH (IU/L)	< 1.0	0.5	0.10–1.29
FSH (IU/L)	< 1.0	1.7	0.21–2.8
ACTH (ng/L)	24.1	24.4	8–10 hours: 7.2–63
IGF1-1 (µg/L)	270	156	23–212
Cortisol (µg/L)	70.4	78.1	7–10 hours: 62–180
DHEA-S (mg/L)	1.49	0.08	< 0.02–0.15
Estradiol (ng/L)	9.9	< 5	< 20
Androstenedione (ng/L)	2365	53	100–900
Testosterone (µg/L)	9.90	< 0.12	< 0.12
SHBG (nmol/L)	62.2	92.5	42.4–155.6

LH luteinizing hormone; *FSH* follicule stimulating hormone; *ACTH* adrenocorticotropic hormone, *IGF-1* insulin-like growth factor 1; *DHEA-S* dehydroepiandrosterone-sulfate, *SHBG* sex hormone binding globulin

2,5 year-old boy
6-month history of penile enlargement, pubic hear, frequent erections and rapid linear growth
No exposure to exogenous testosterone
Abdominal echo: hypoechogenic ovoid nodule in the left adrenal



TUMOR PROFILING

3-year-old boy - adrenal gland carcinoma - 80% neoplastic cells

Variant annotation		VAF	Biological Classification
NM_000546.5(TP53):c.473G>A	p.(Arg158His)	71% VAF	Likely pathogenic

→ **Germline?**
Therapy?

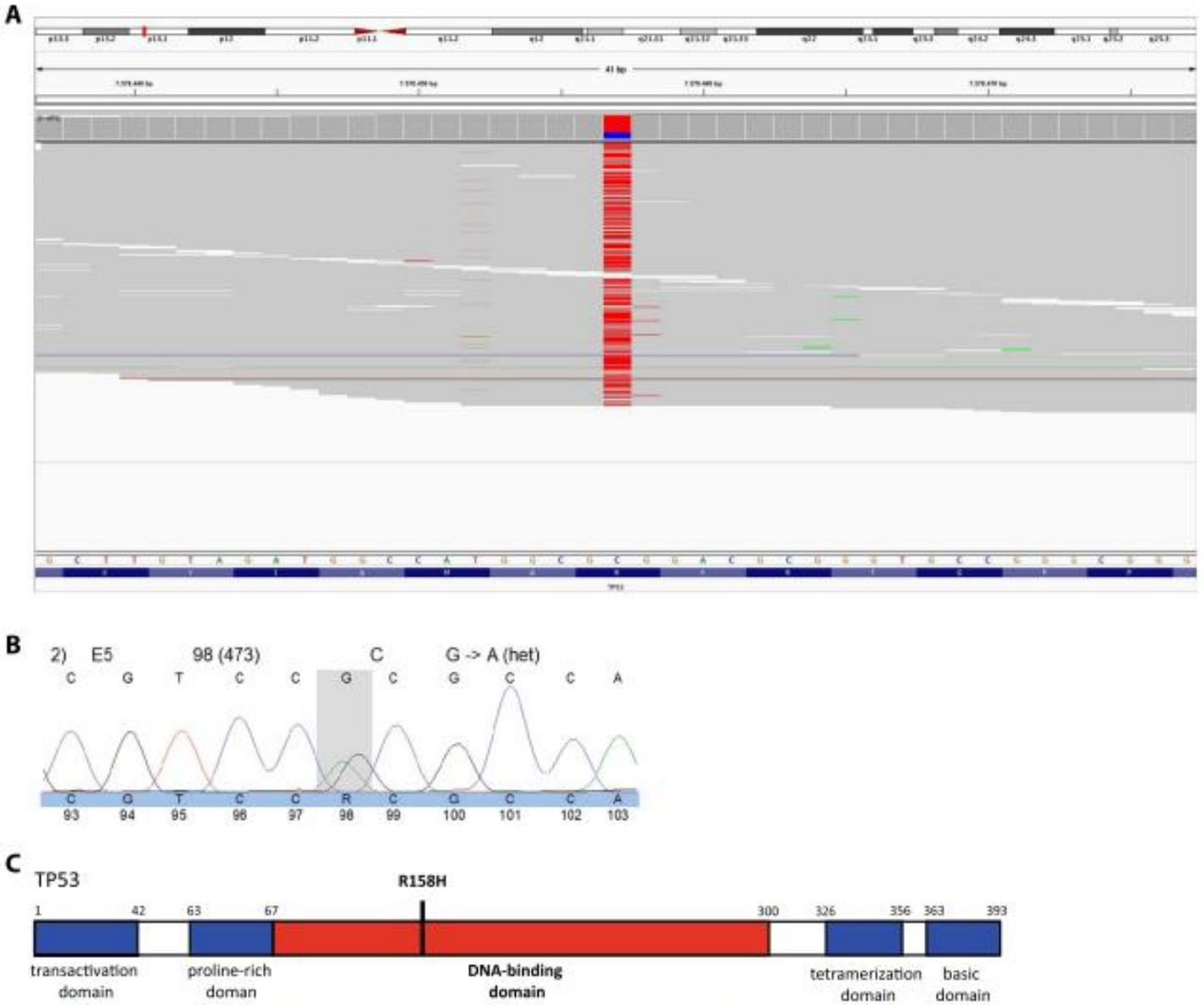


Fig. 3 Genetic testing. **A** Integrative Genomics Viewer (IGV) graphic showing a single nucleotide variant (SNV) in the *TP53* gene (G>A), producing p.Arg158His alteration. The reference sequence used is NM_000546.5. **B** *TP53* gene diagram shows that the R158H variant hits the functional part of the DNA-binding domain of P53 and imparts a transcriptional activity comparable to null variants. **C** Domain structure of p53. Adapted from Tanaka *et al.* [40]

TUMOR PROFILING

3-year-old boy - adrenal gland carcinoma - 80% neoplastic cells

Variant annotation		VAF	Biological Classification
NM_000546.5(TP53):c.473G>A	p.(Arg158His)	71% VAF	Likely pathogenic

→ **Germline Diagnosis of LFS**

Germline TP53 expected due to the young age of the patient and the high VAF (despite the tumor %).
Variant was confirmed as germline variant and patient diagnosed with li-fraumeni

Peripheral precocious puberty in Li–Fraumeni syndrome: a case report and literature review of pure androgen-secreting adrenocortical tumors

Sofie Ryckx[1,2*], Jean De Schepper[2], Philippe Giron[3], Ken Maes[3], Freya Vaeyens[3], Kaat Wilgenhof[4], Pierre Lefesvre[4], Caroline Ernst[5], Kim Vanderlinden[6], Daniel Klink[1], Frederik Hes[3], Jesse Vanbesien[2], Inge Gies[2] and Willem Staels[2,7]

TUMOR PROFILING - TAKE HOME MESSAGES

- **Diagnosis**
- **Prognosis**
- **Therapy decision**
- **Incidental Detection of germline mutations**

TUMOR PROFILING – QUESTIONS?

Freya Vaeyens

Ken Maes

Jelle Vlaeminck

TUMOR GENETICS AND IMPLICATIONS FOR TREATMENT

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