

Molecular Tumor Boards: translating molecular data in a therapy

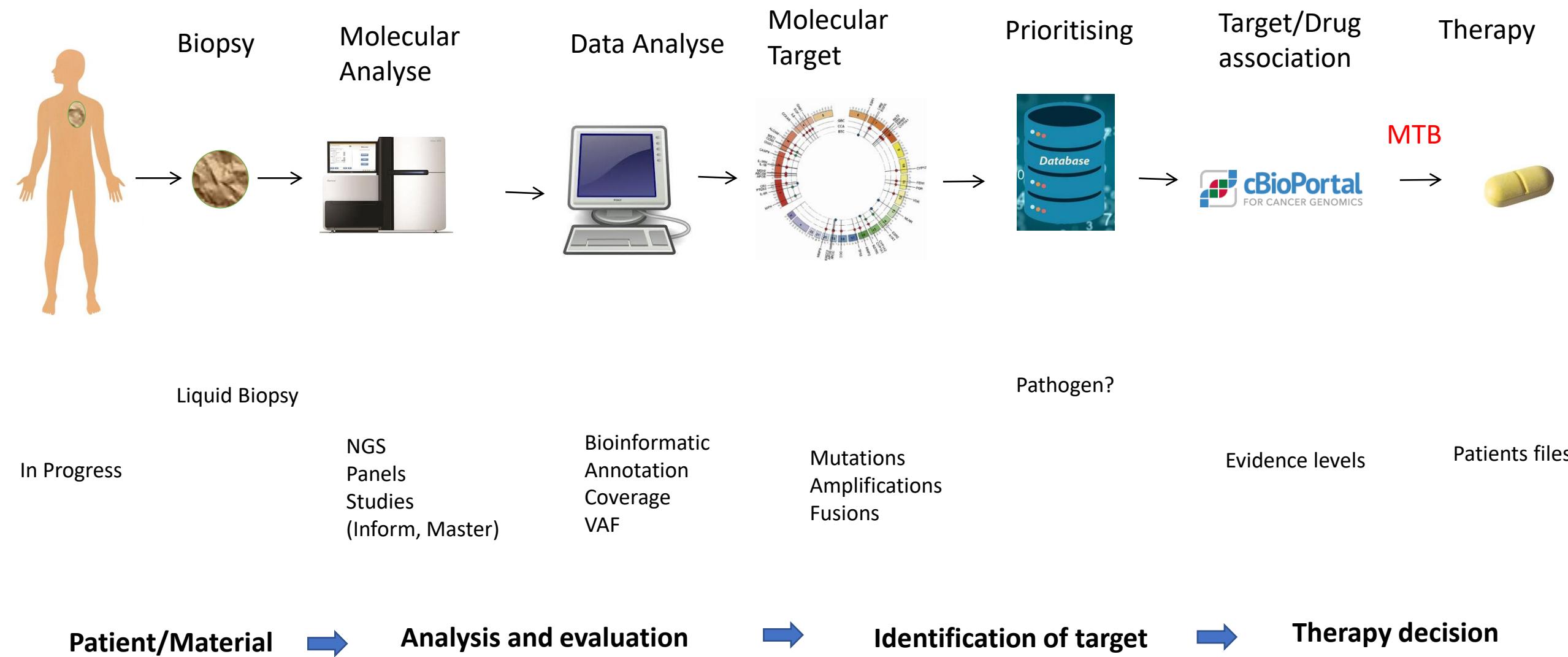
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University Medical Center of the Johannes Gutenberg-University Mainz

Member of the molecular tumor boards of the UMM Mainz

Coordinator of the immune-oncology boards of the UMM Mainz

Process in a MTB



Targets in Cancer

Melanoma



About 50 % of melanomas harbor a BRAF (V600E) mutation

The BRAF (V600E) mutation is a driver for the tumor growth in melanoma

Targeting BRAF (V600E) with specific drugs (Vemurafenib) impairs cell growth

2011 FDA
Vemurafenib
zulassung für
Melanoma mit
BRAF (V600E)

„ib“= small molecules

Content

1-How molecular analysis are changing the classification, prognosis and therapy

Exercise

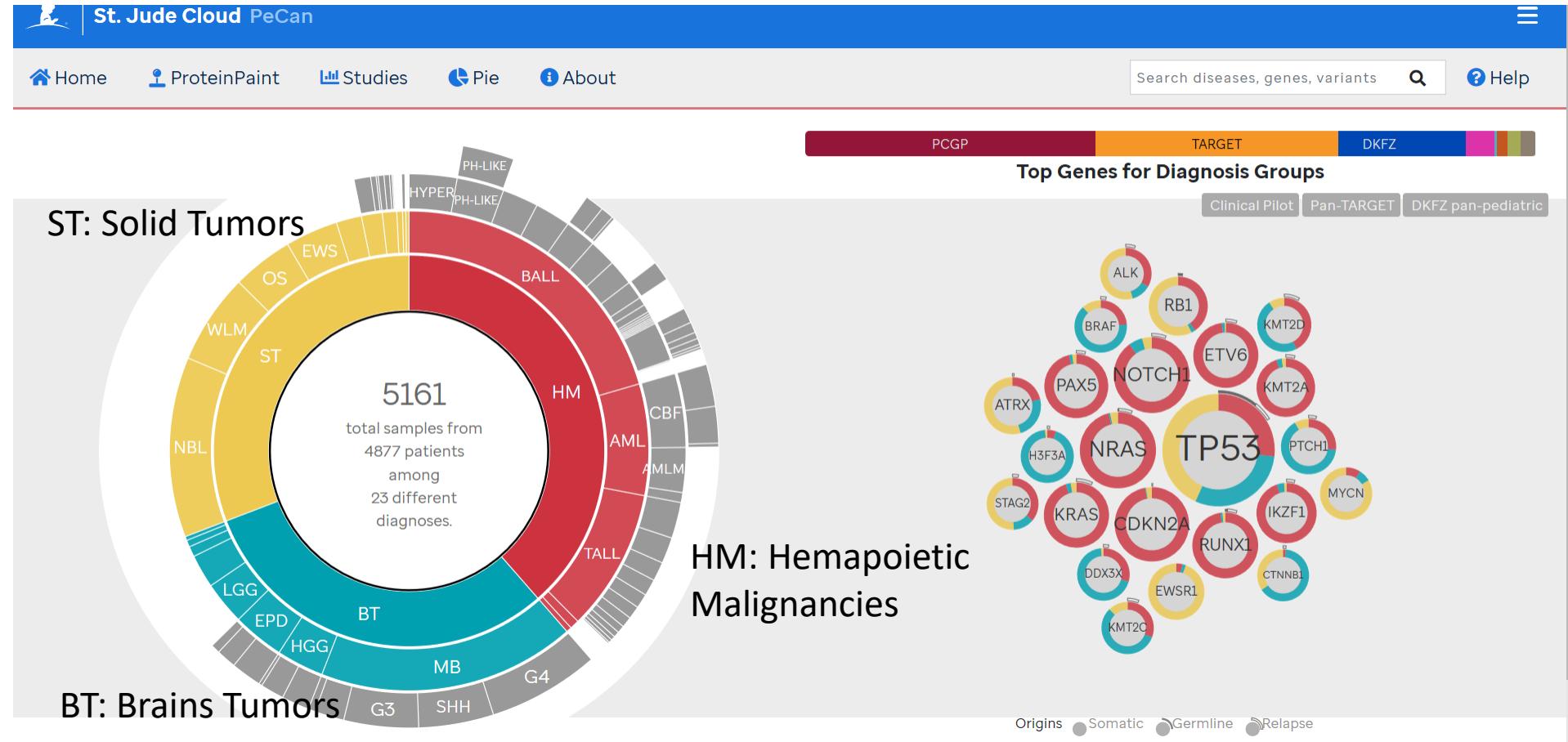
2-How molecular data are used for the selection of a personalised therapy

Exercise

<https://docs.google.com/spreadsheets/d/1ZHr02JC8YcSpzksFcYmVX1MQerBLb04IIJf1GVGc6Ak/edit?usp=sharing>

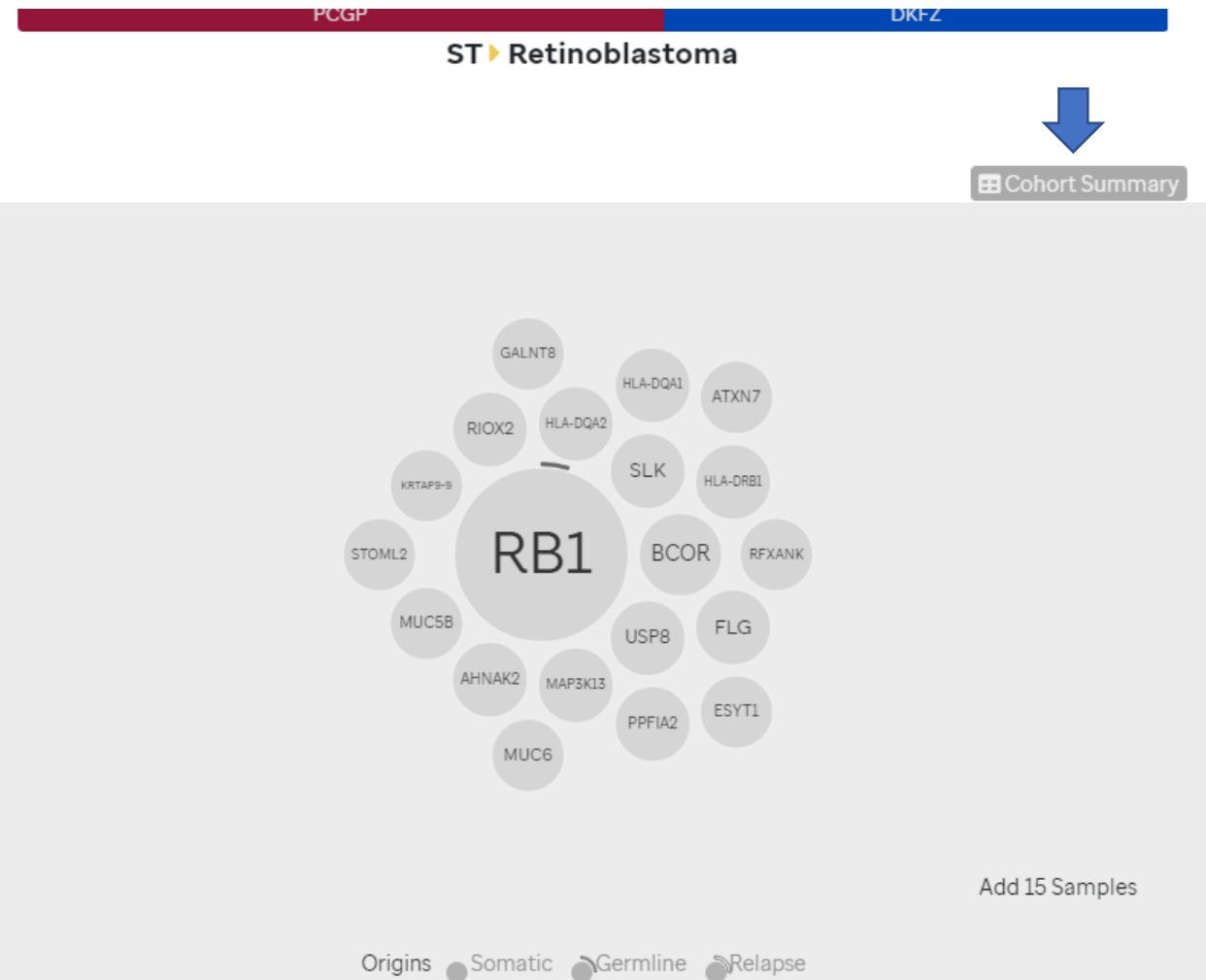
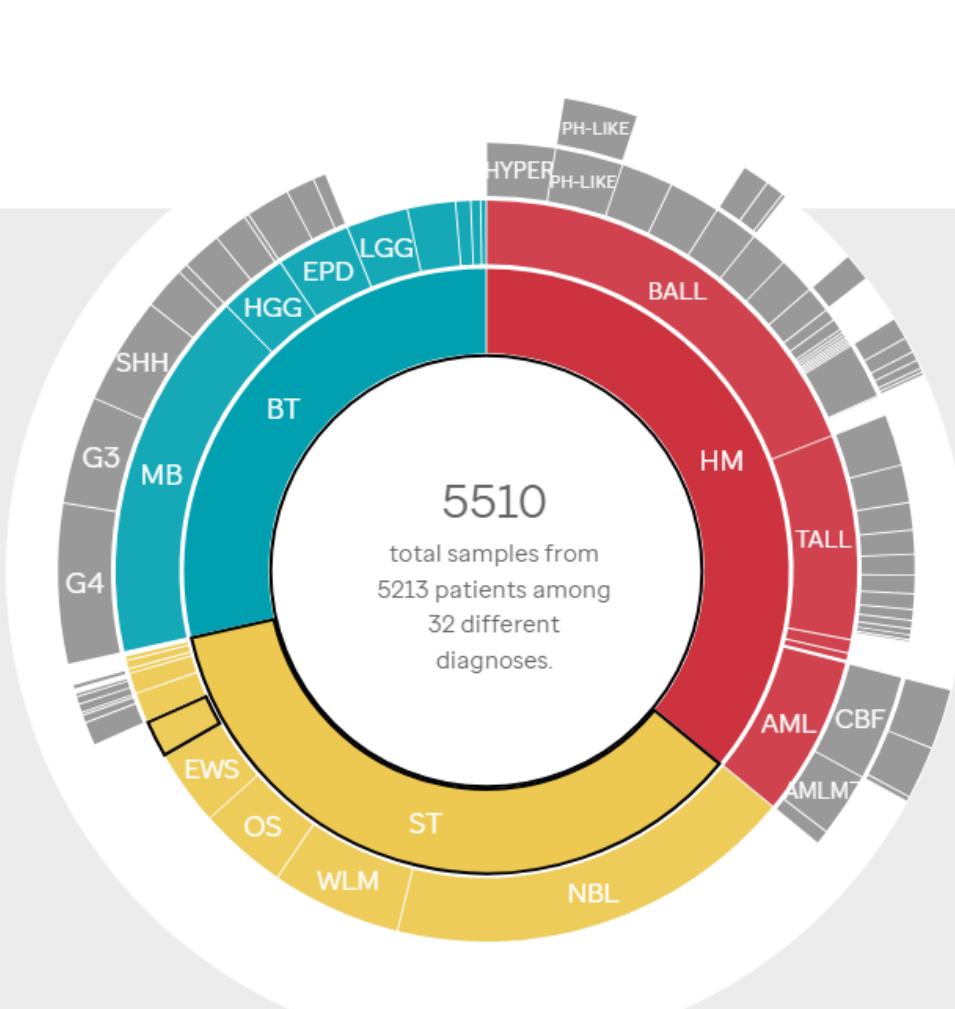
Which aberrations are expected in my patient?

St. Jude PeCan Data Portal <https://pecan.stjude.cloud/>

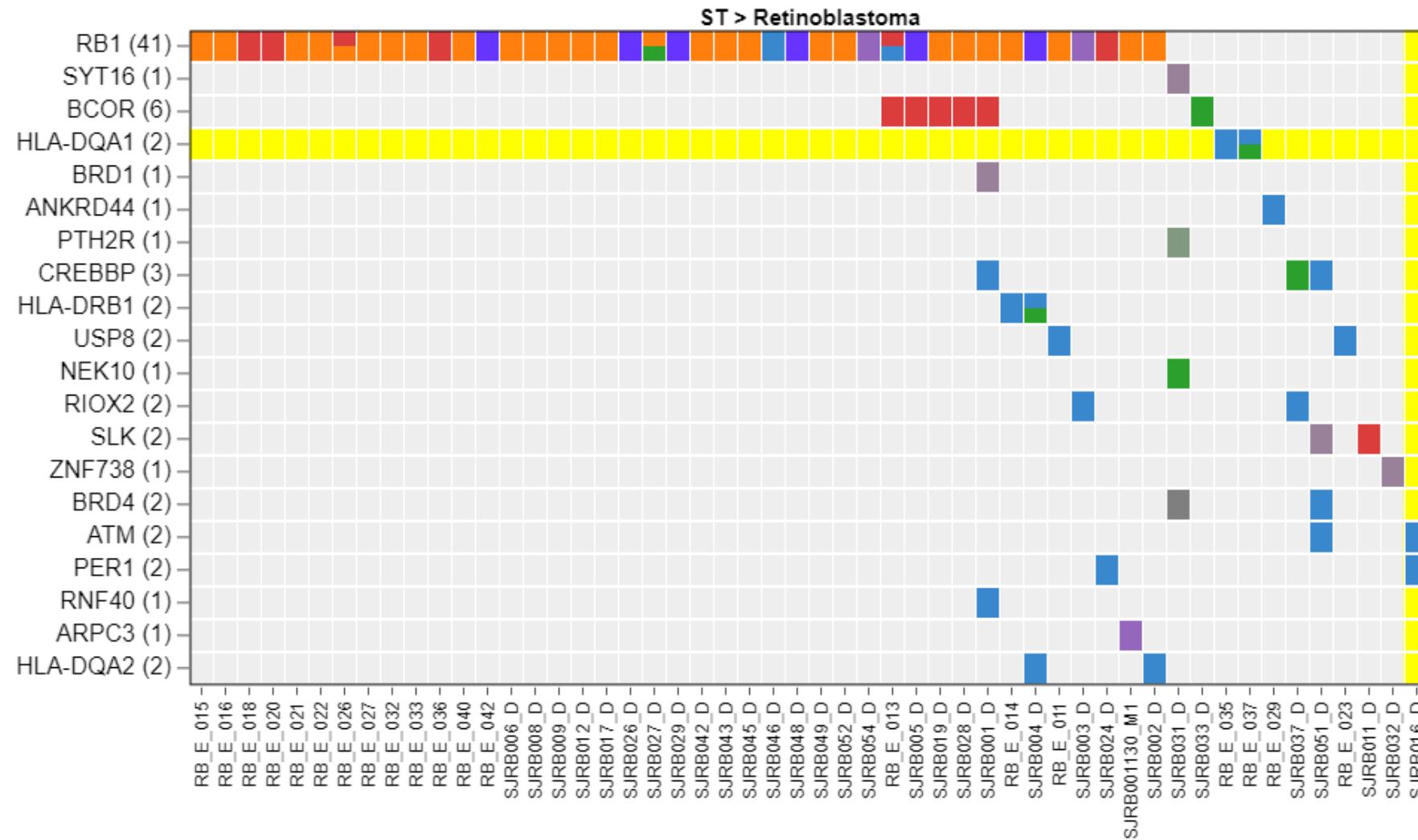


Retinoblastoma

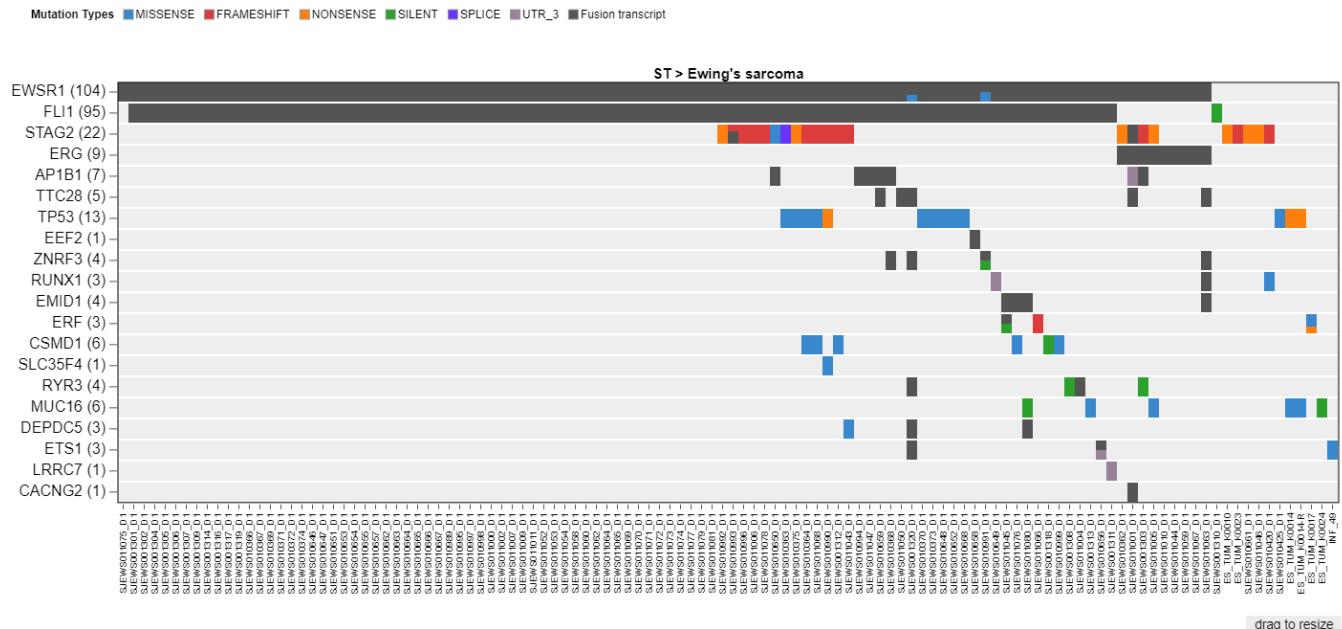
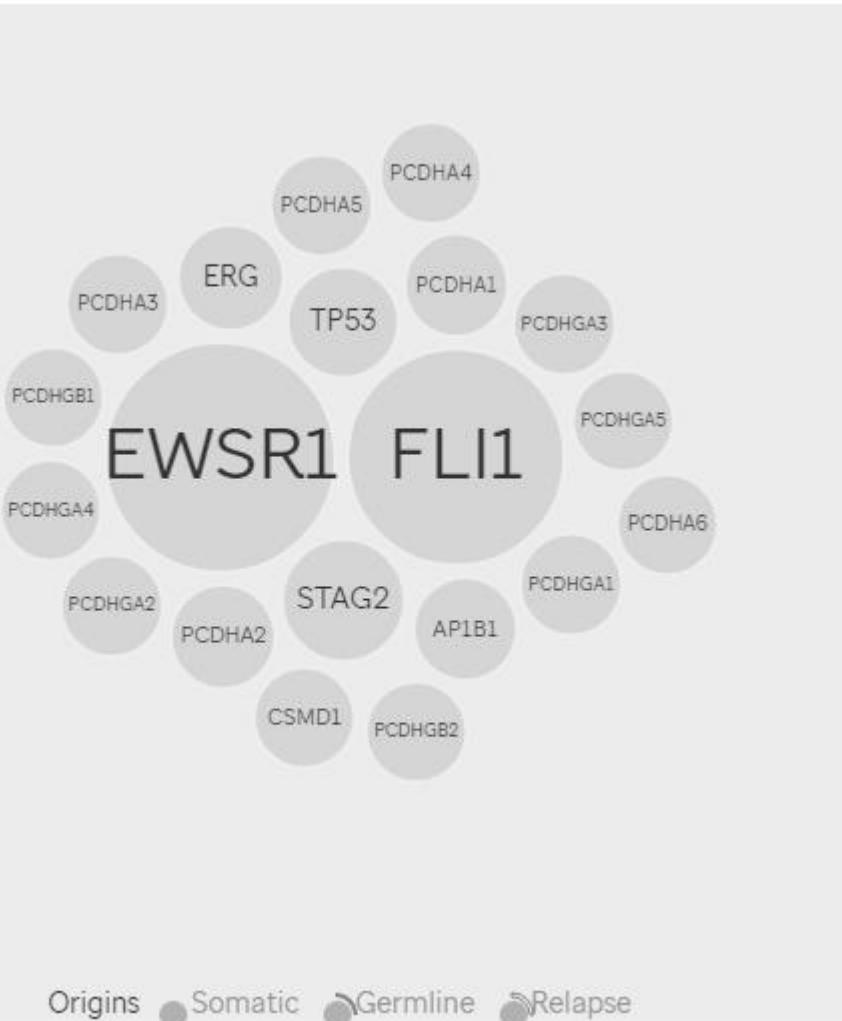
Retinoblastoma



Mutation Types MISSENSE FRAMESHIFT NONSENSE SILENT PROTEINDEL SPLICE_REGION SPLICE UTR_3 UTR_5



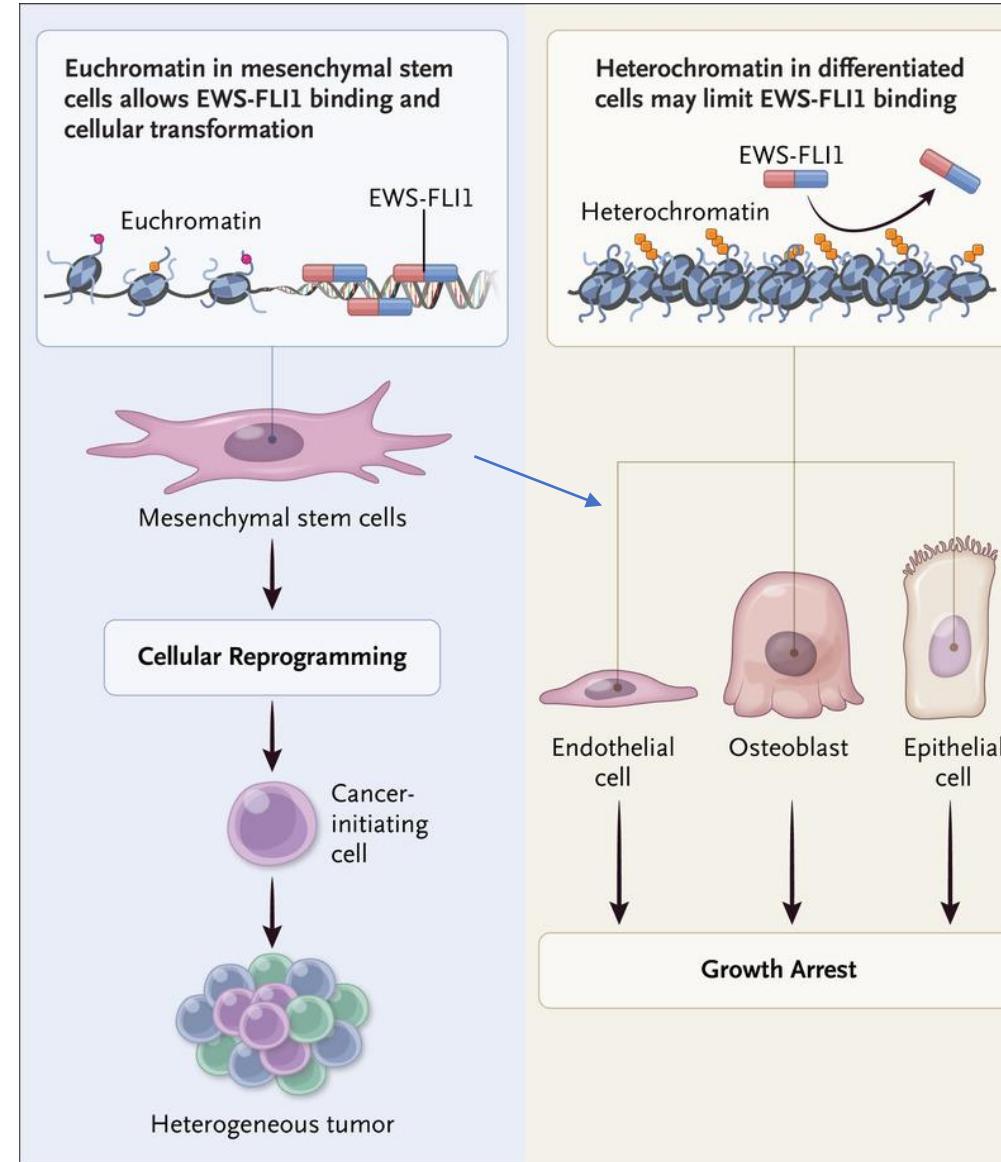
Ewing Sarcoma



Ewing Sarcoma



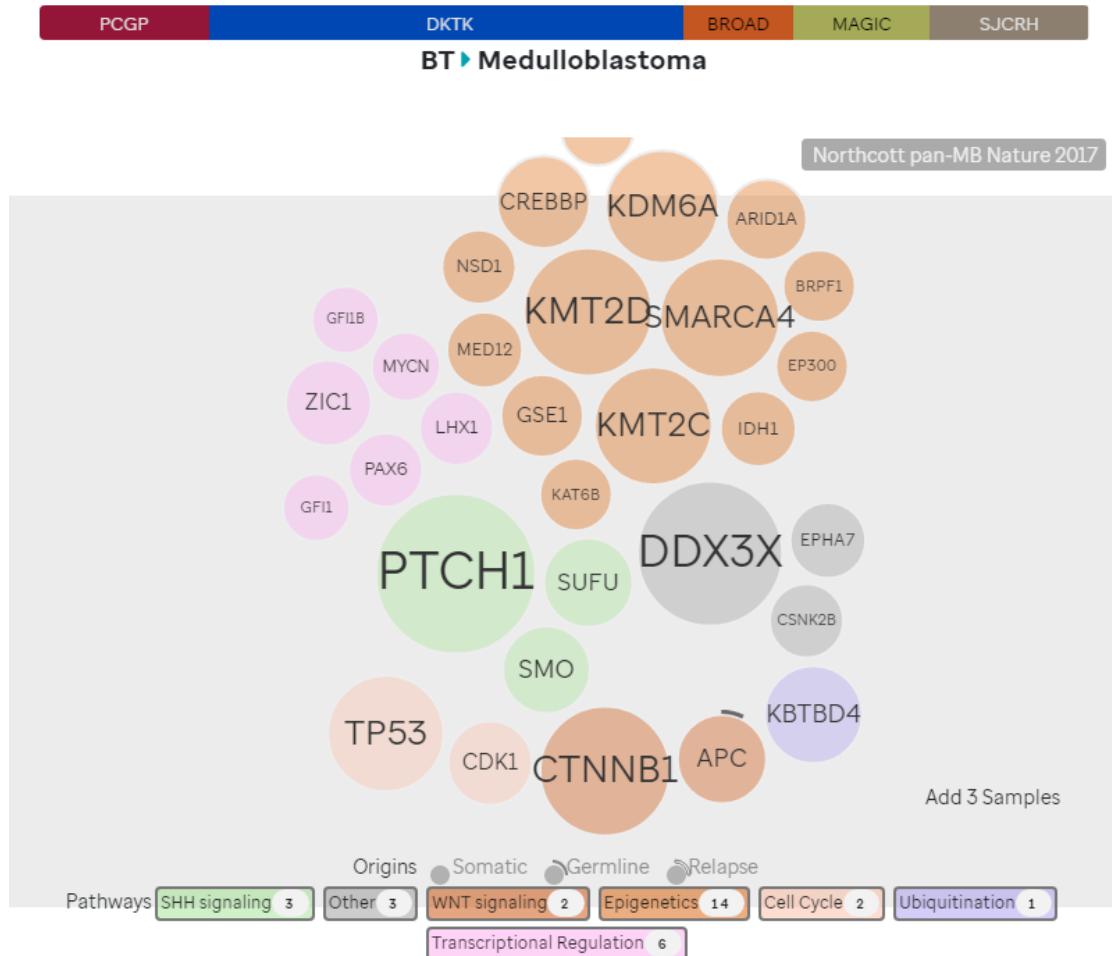
Ewing sarcoma is a type of cancer growing in bone or soft-tissues.



Genetic

Fusion of the EWS gene, generally with FLI1

Medulloblastoma



Mutation Types: MISSENSE, FRAMESHIFT, NONSENSE, PROTEINDEL, PROTEININS, SPLICE, Copy number gain, Copy number loss

Assay: 0, 1

Age: Adult, Child, Infant, NA

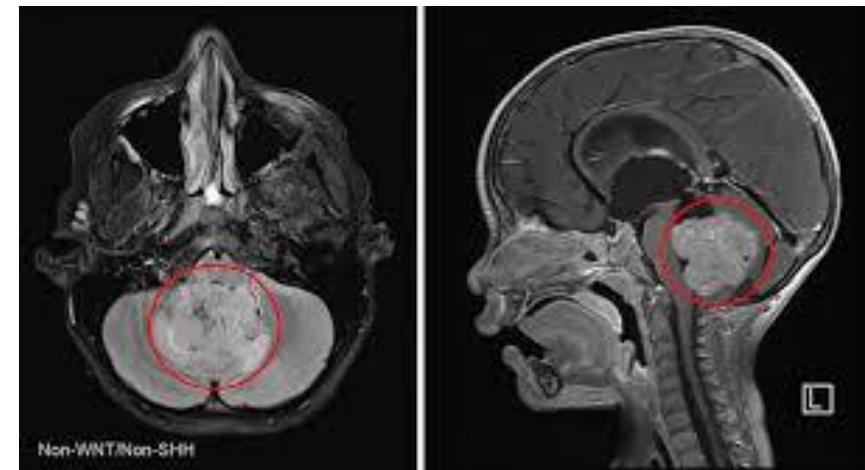
Ploidy: Diploid, Hyperploid, NA

Gender: F, K, M, NA, T



Medulloblastoma

	<u>Genetic</u>			
Demographics	WNT	SHH	Group 3	Group 4
Age				
Gender	$\sigma \sigma : \Omega \Omega$	$\sigma \sigma : \Omega \Omega$	$\sigma \sigma : \Omega$	$\sigma \sigma : \Omega$
Clinical Features				
Histology	Classic, Rarely LCA	Desmoplastic/nodular, Classic, LCA	Classic, LCA	Classic, LCA
Metastasis	Rarely M+	Uncommonly M+	Very frequently M+	Frequently M+
Prognosis	Very Good	Infants good, others intermediate	Poor	Intermediate
Genetics	<i>CTNNB1</i> Mutation	<i>PTCH1/SMO/SUFU</i> Mutation <i>GLI2</i> Amp, <i>MYCN</i> Amp	<i>MYC</i> Amp	<i>CDK6</i> Amp <i>MYCN</i> Amp
Gene Expression	WNT Signaling <i>MYC</i> +	SHH Signaling <i>MYCN</i> +	Photoreceptor/GABA <i>MYC</i> +++	Neuronal/Glutamatergic Minimal <i>MYC</i> / <i>MYCN</i>

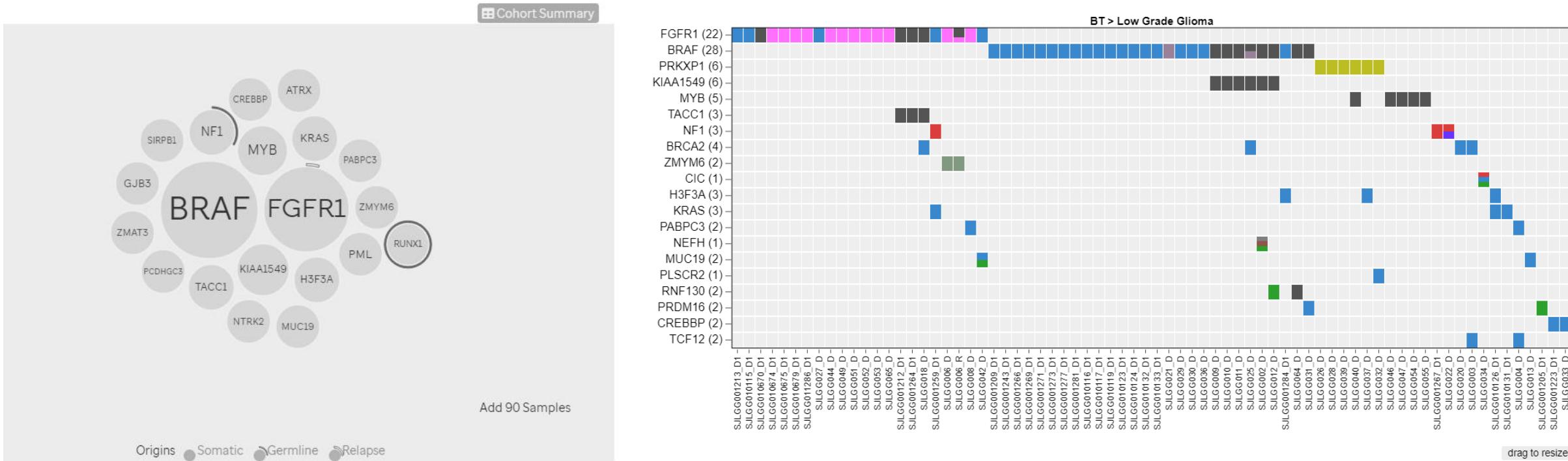


The good prognosis of WNT medulloblastoma in children allowed the initiation of a prospective study evaluating reduced intensity of irradiation in order to reduce late effects in this specific subgroup.

Inhibitors of the SHH pathway (Vismodegib) in clinical trials

Children with germline mutations in *PTCH1* are not irradiated because develop Basal-cell carcinoma

LGG



LGG-Low grade glioma

This is the most common type of brain tumour in children, representing 40% of all childhood brain and spinal cord tumours.

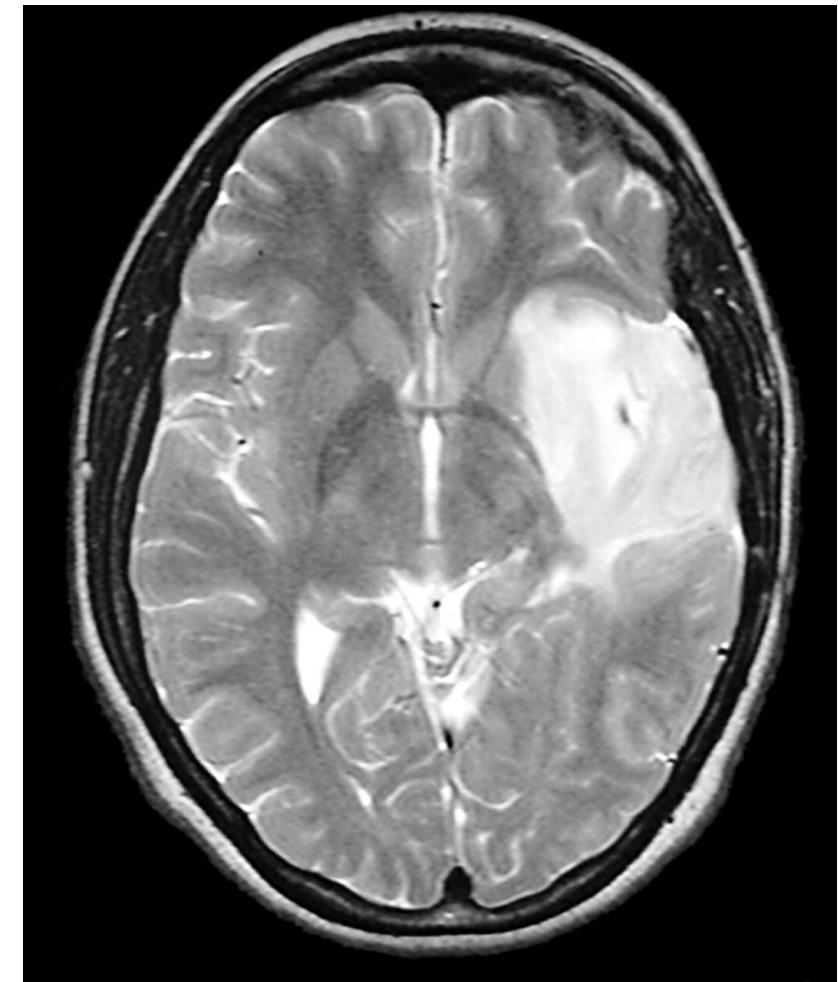
Slow growing tumors

Genetic

20% of patients with NF1 germlines mutations develop a LGG

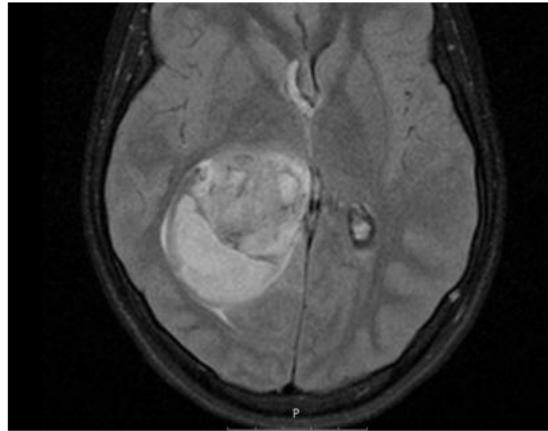
BRAFV600E mutations and BRAF fusions

Therapy?

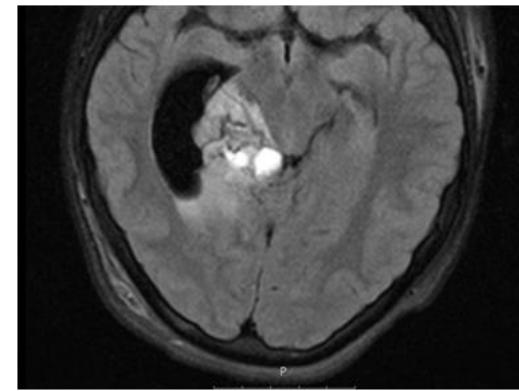


MRT Verlauf

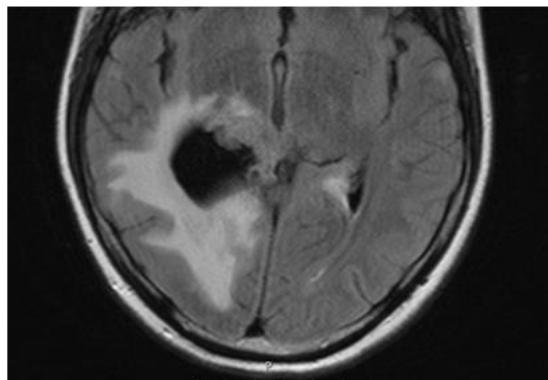
ED



Erste OP

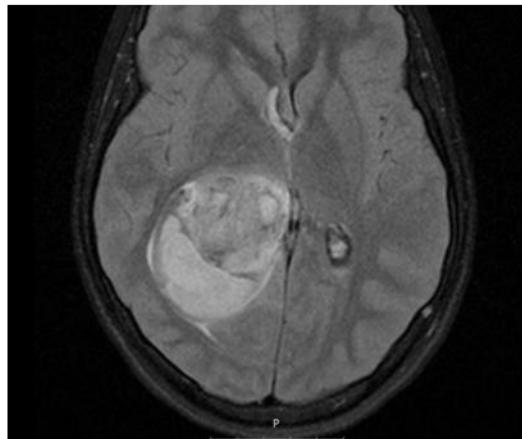


Progress

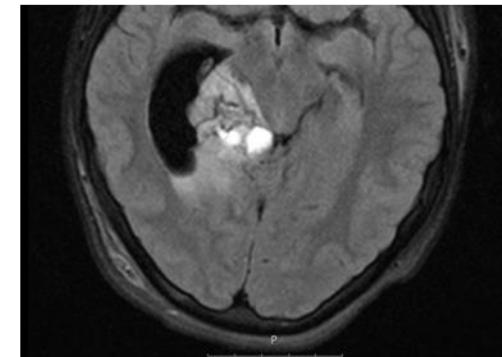


MRT Verlauf

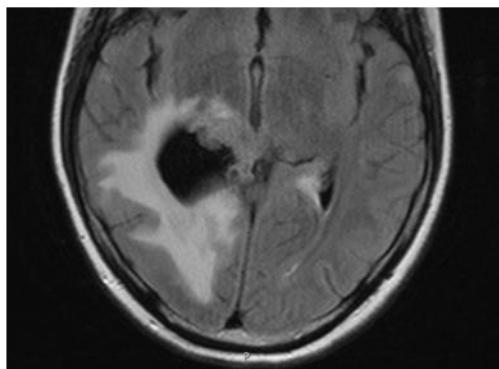
ED



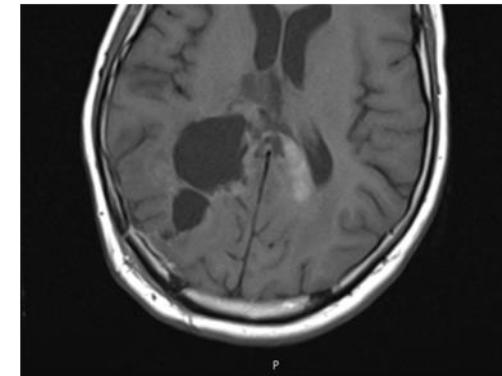
Erste OP



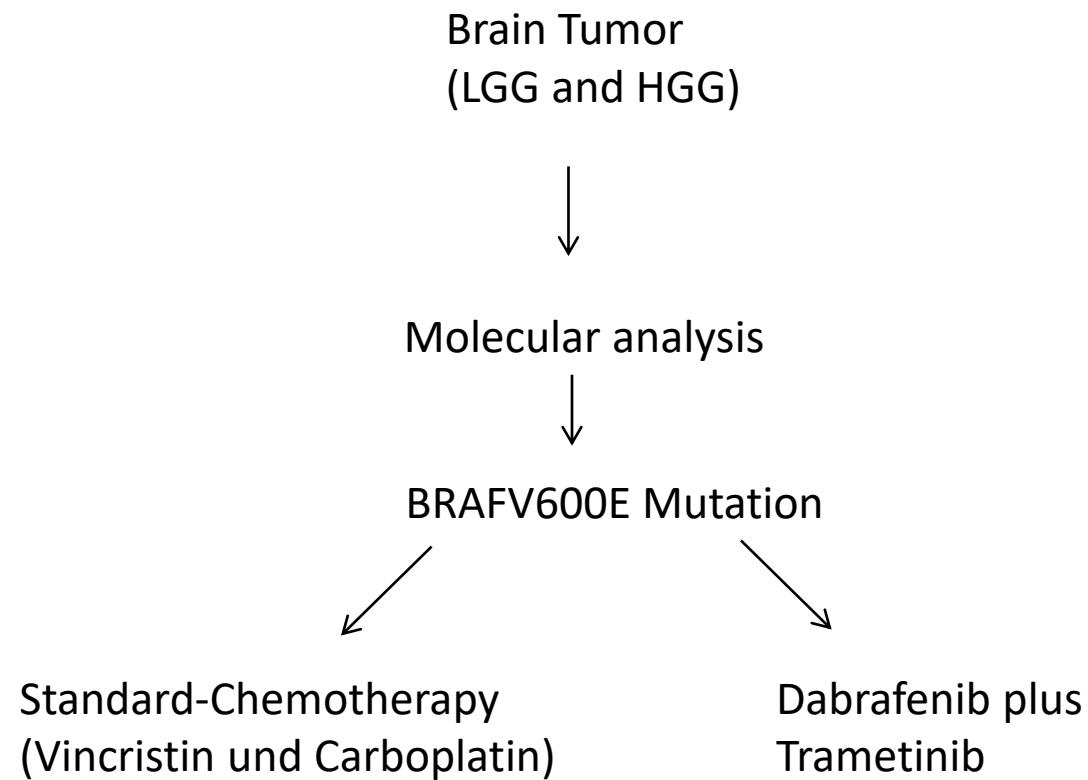
Progress



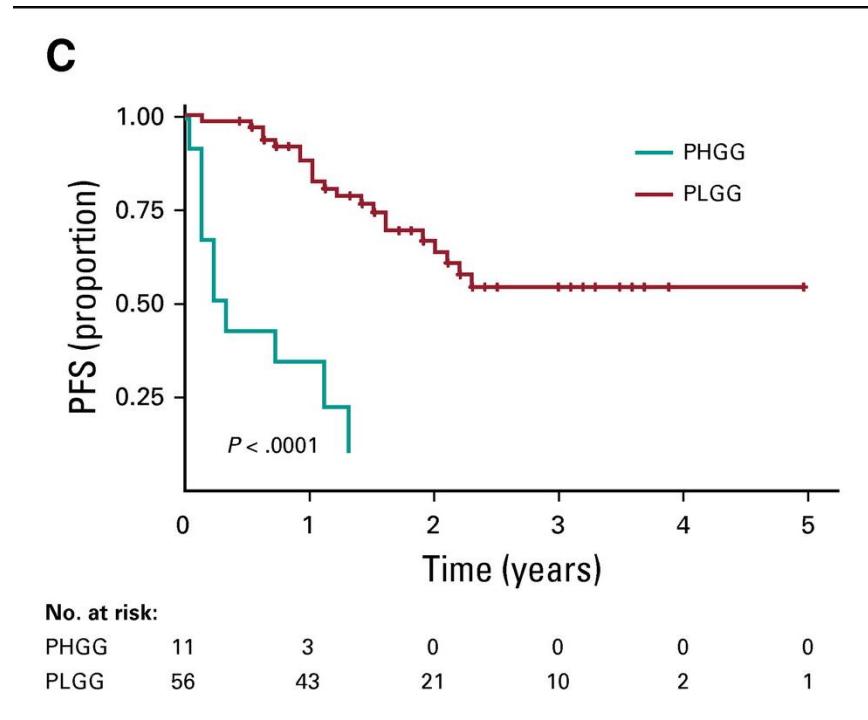
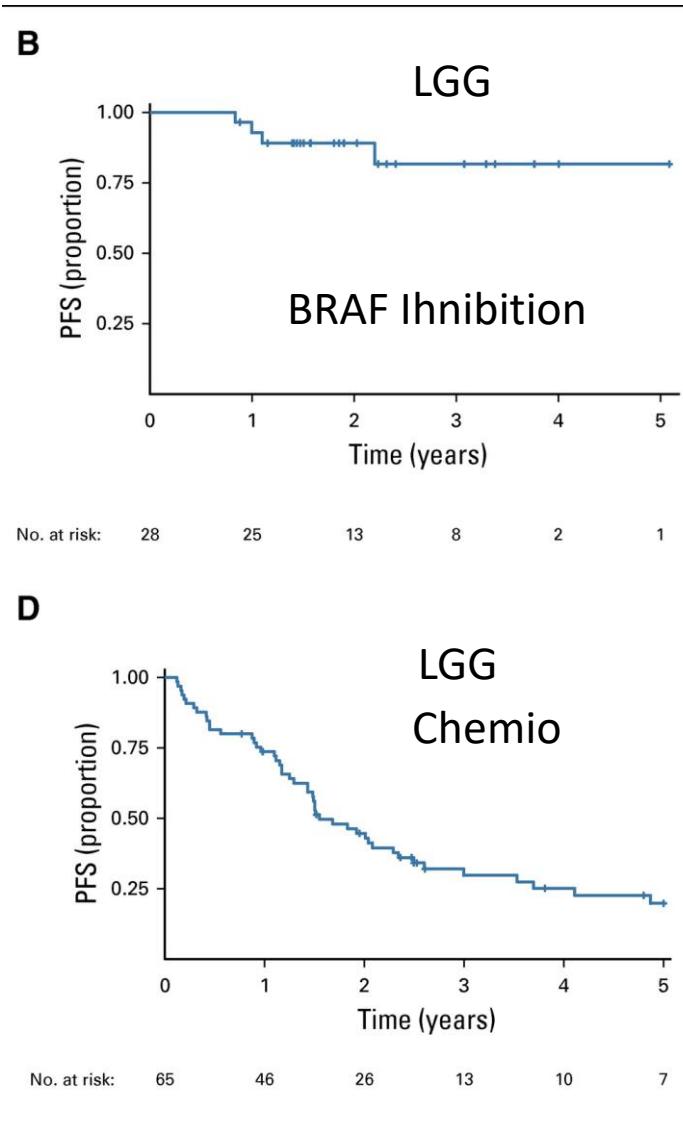
Vemurafenib



Brain tumors



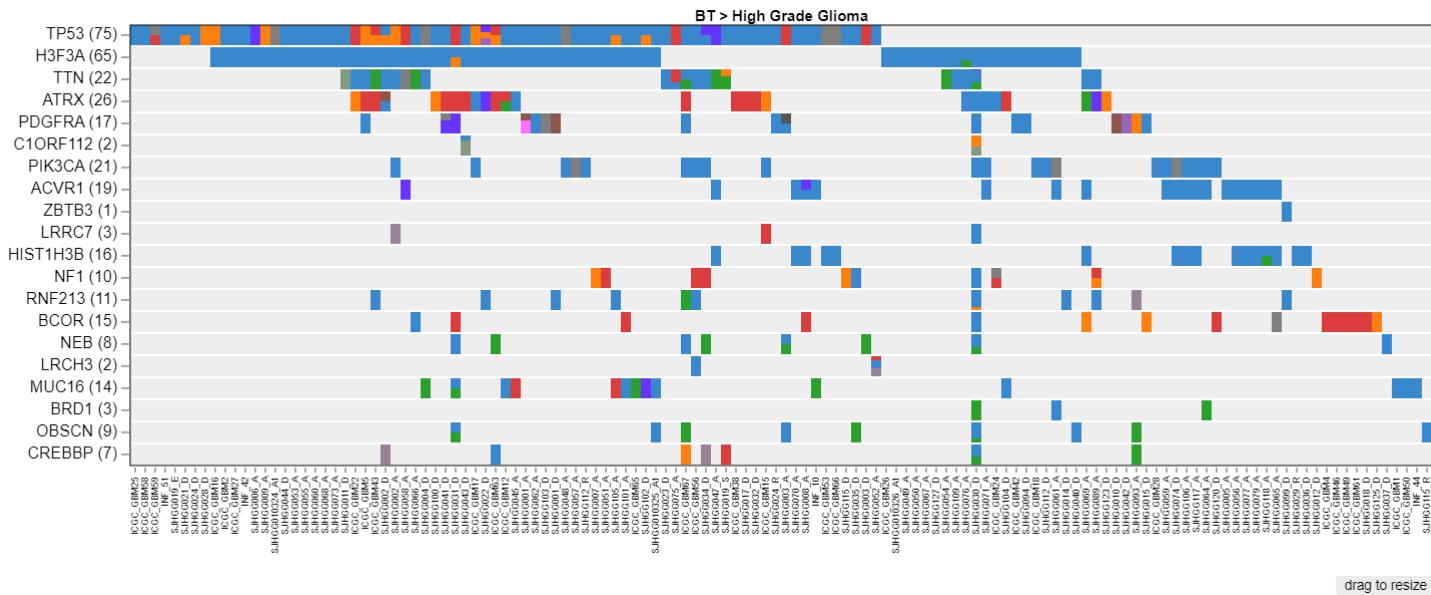
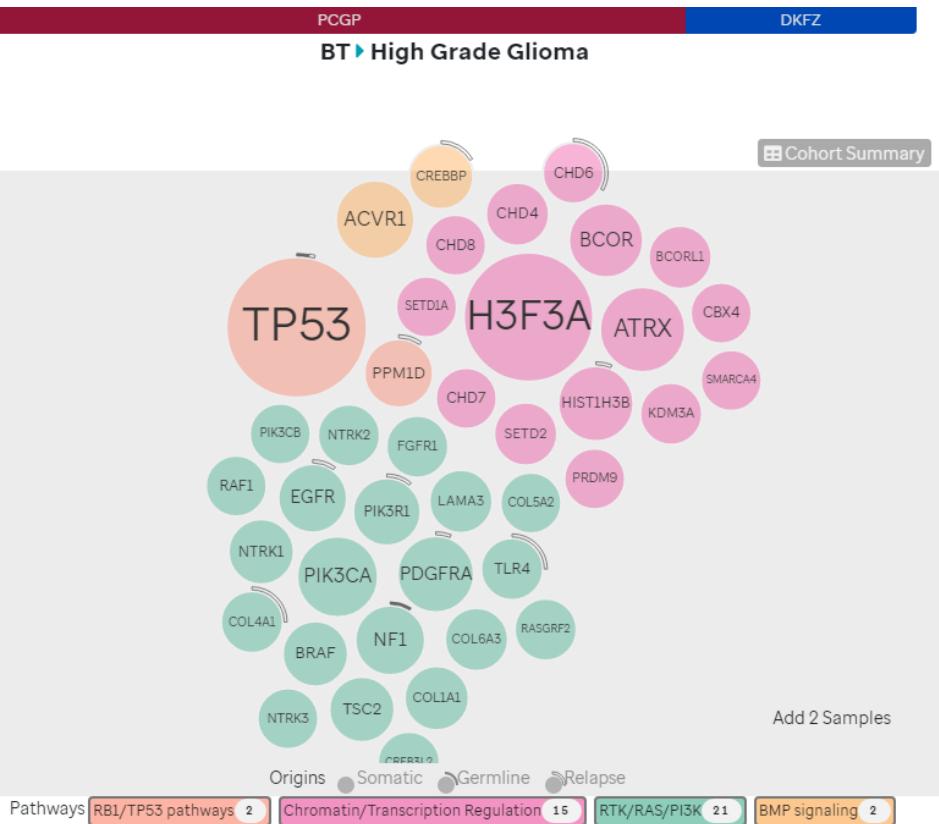
BRAF monotherapy in Pediatric Glioma



- Sixty-seven patients
- 29 centers from multiple countries.
- 5.6 years

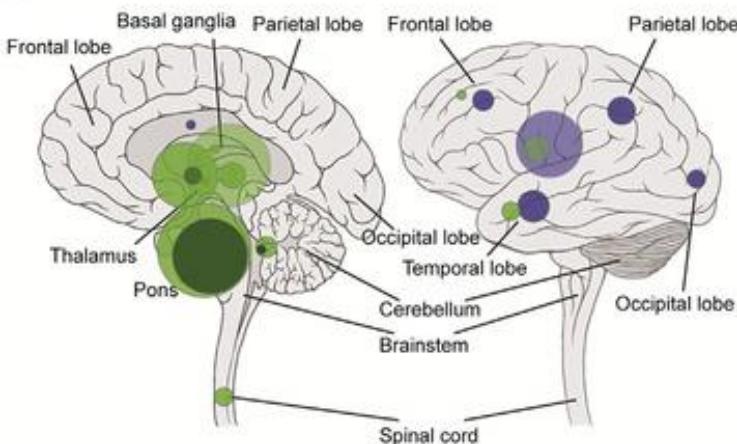
- However, it is important to note that BRAF inhibition leads to CR in a minority of tumors.
- After discontinuation of BRAF inhibition, 76.5% (13 of 17) of patients with PLGG experienced rapid progression. However, upon rechallenge with BRAF inhibition, 90% achieved an objective response.

HGG

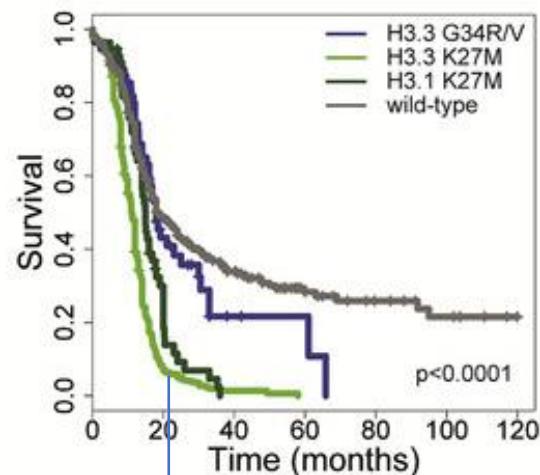


PONS Glioma with Histone mutations

D



F



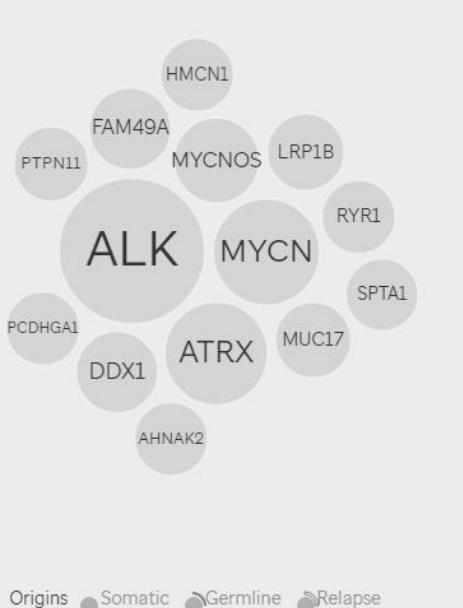
H3.3 K27M

Diffuse intrinsic pontine gliomas (DIPGs) (also known as pontine gliomas and brain stem gliomas) make up around 10% of all pediatric brain tumors

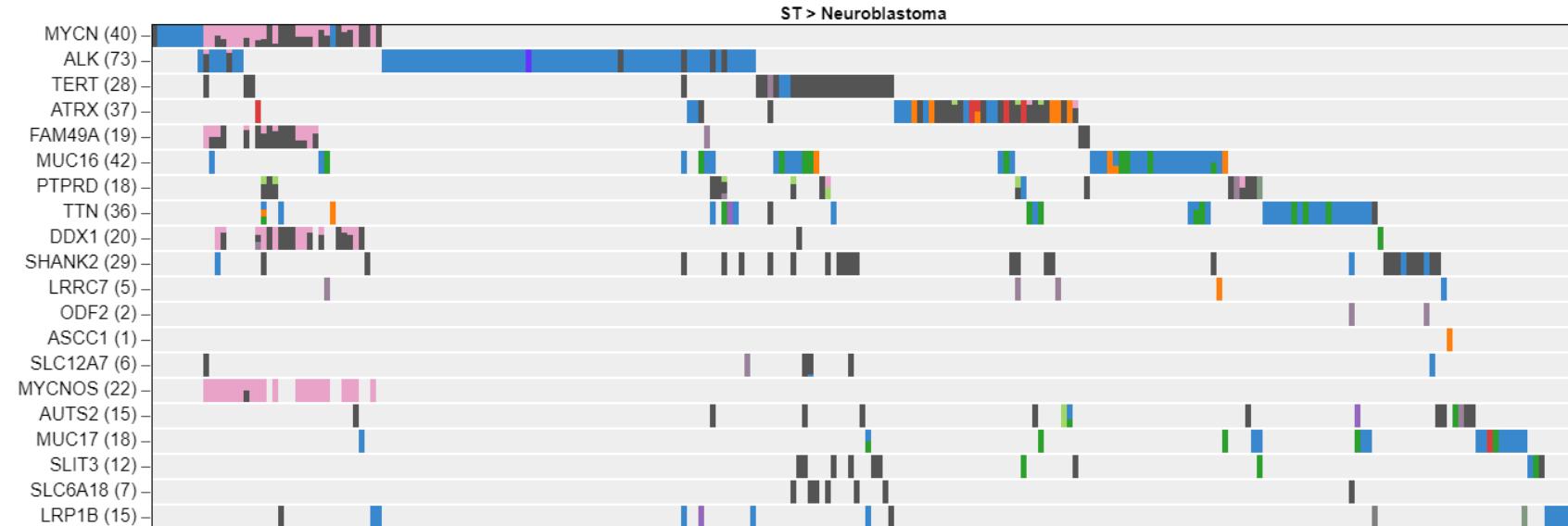
In spite of decades of investigation, these tumors remain refractory to therapy and result in a mean life expectancy of 9–12 months from diagnosis

Genetic:
a K27M mutation in
the H3F3A gene, which
encoded histone 3.3, is implicated
in a large proportion of DIPG cases

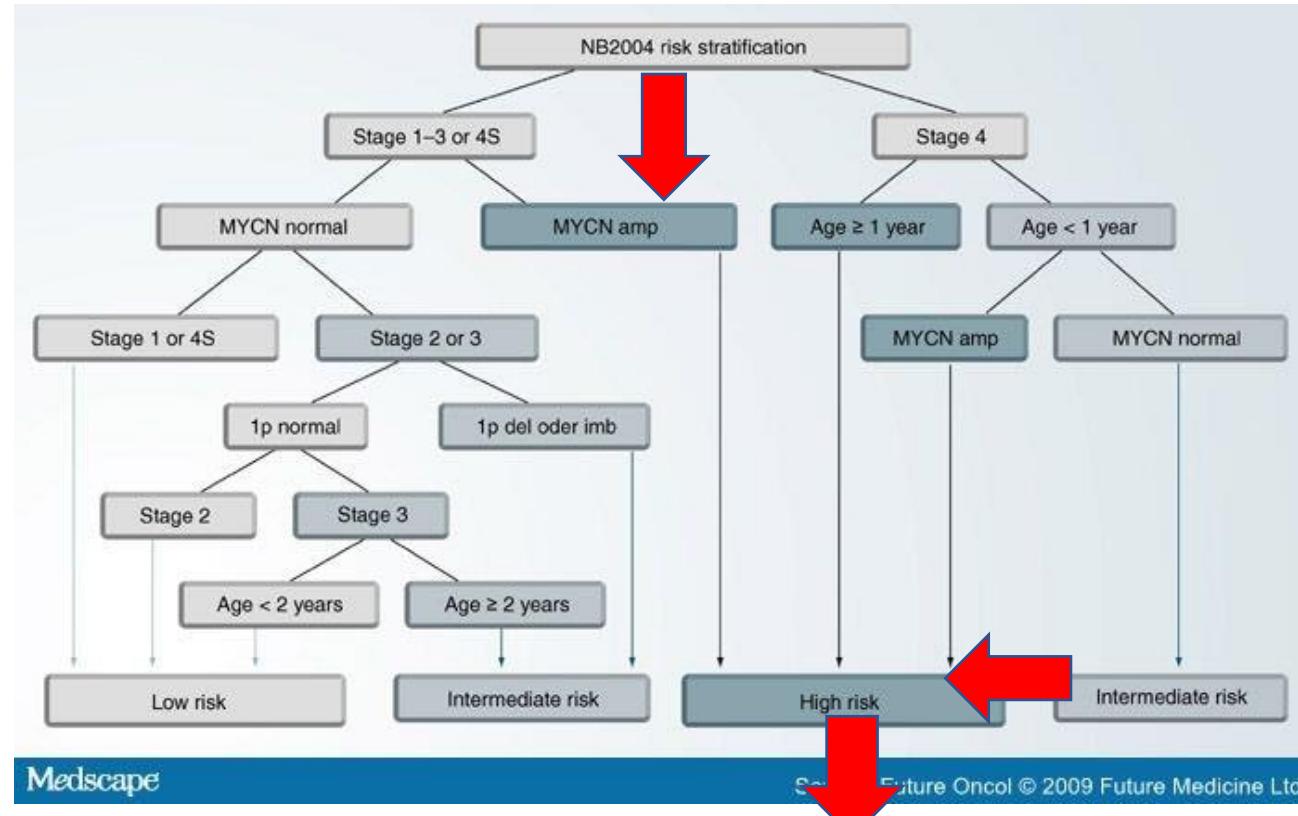
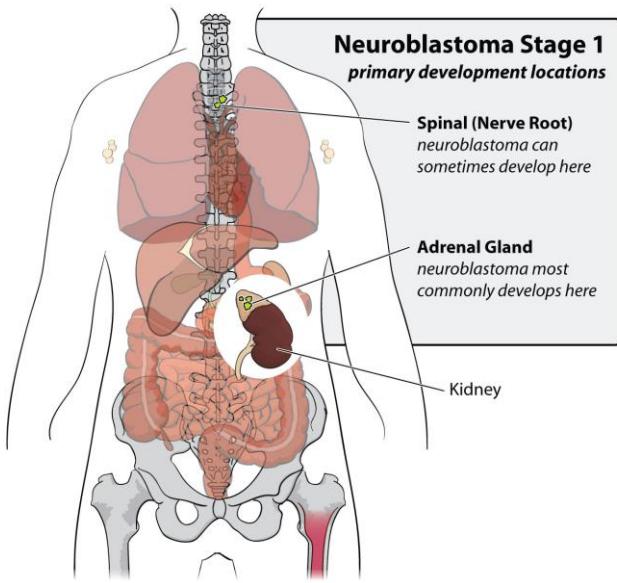
Neuroblastoma



Mutation Types MISSENSE FRAMESHIFT NONSENSE SILENT PROTEINDEL SPLICE_REGION SPLICE UTR_3 UTR_5 Fusion transcript Copy number gain Copy number loss



Neuroblastoma



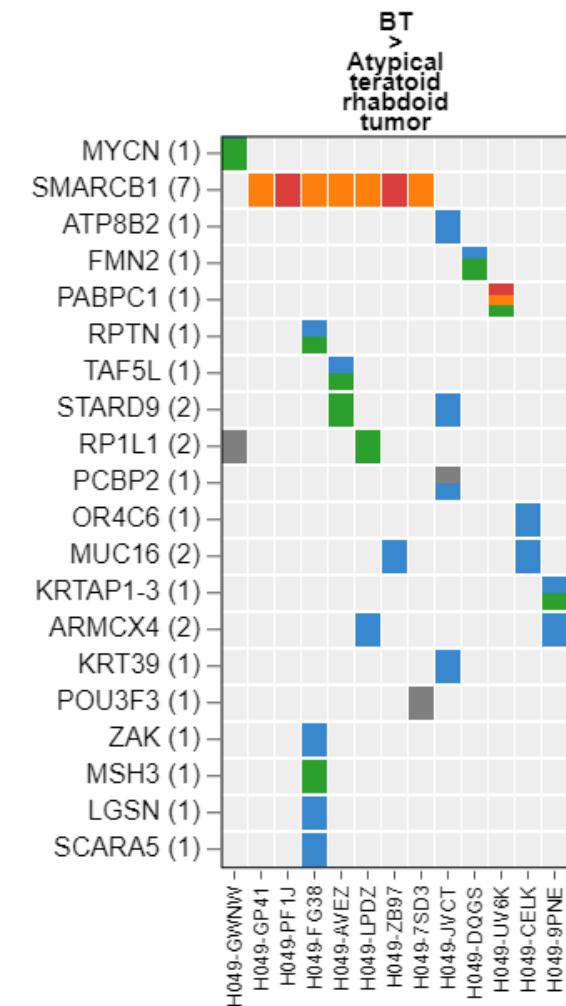
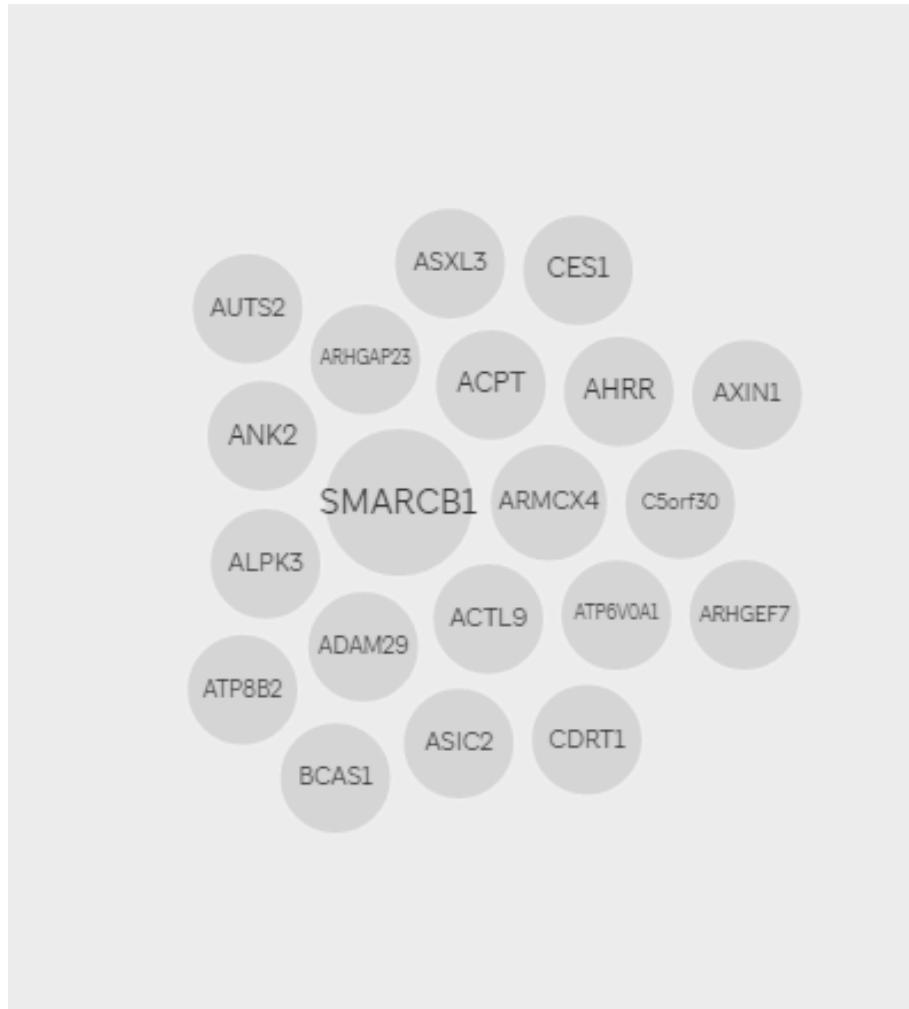
Genetic:

- MYCN copy gain (transcription factor)
- ALK mutations

High-dose chemotherapy (HDCT)
with autologous stem cell support

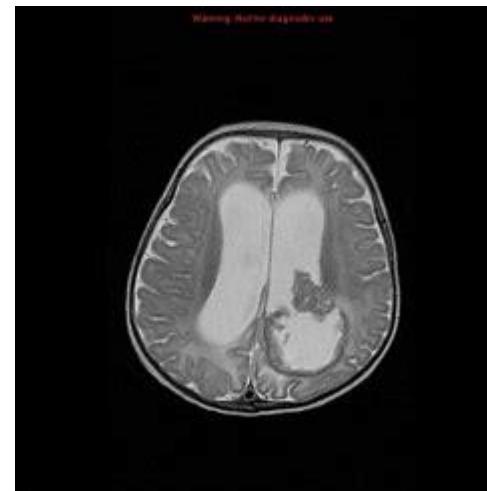
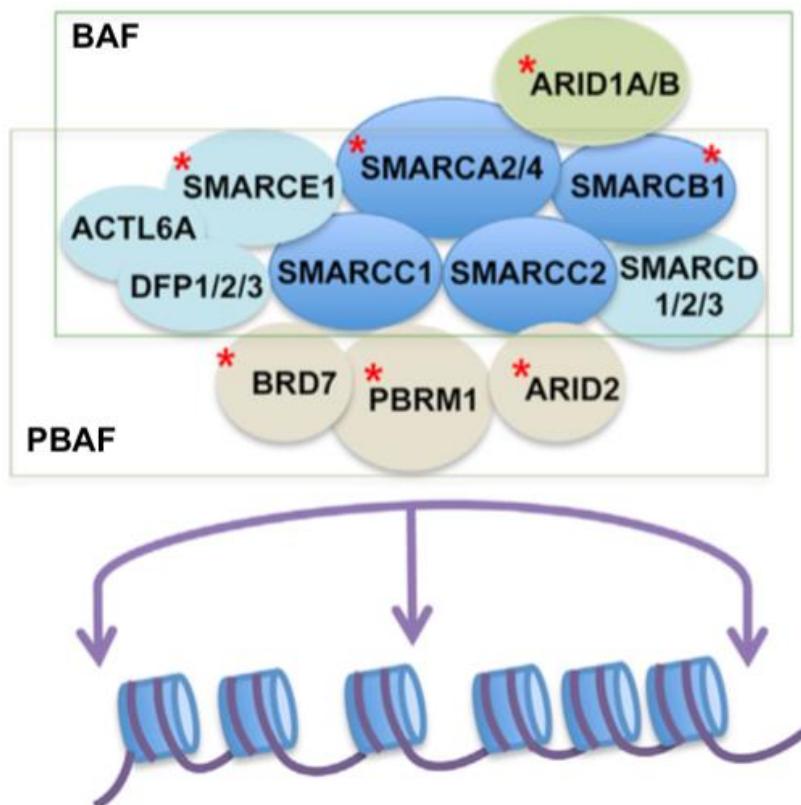
Specific inhibitors for ALK mutations
as SOC (lorlatinib)

ATRT



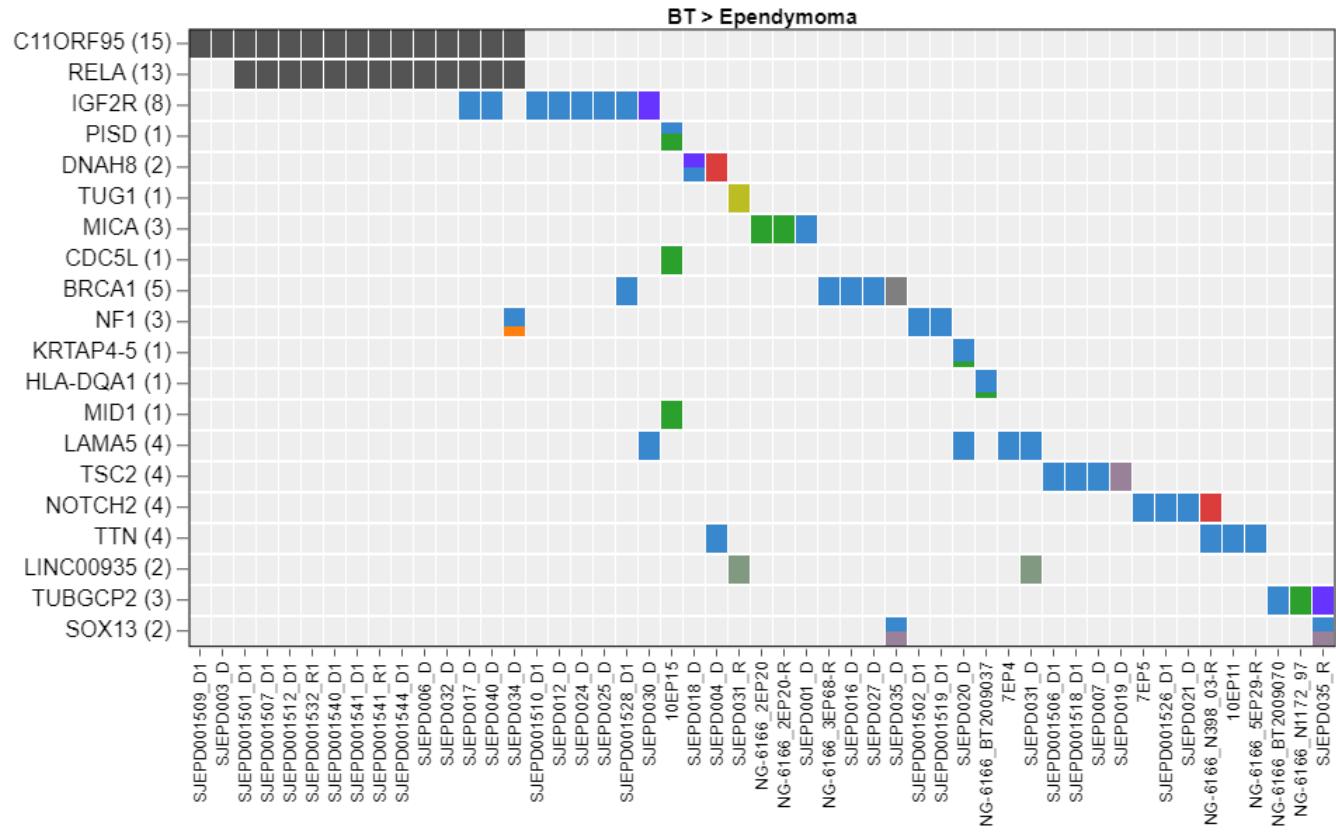
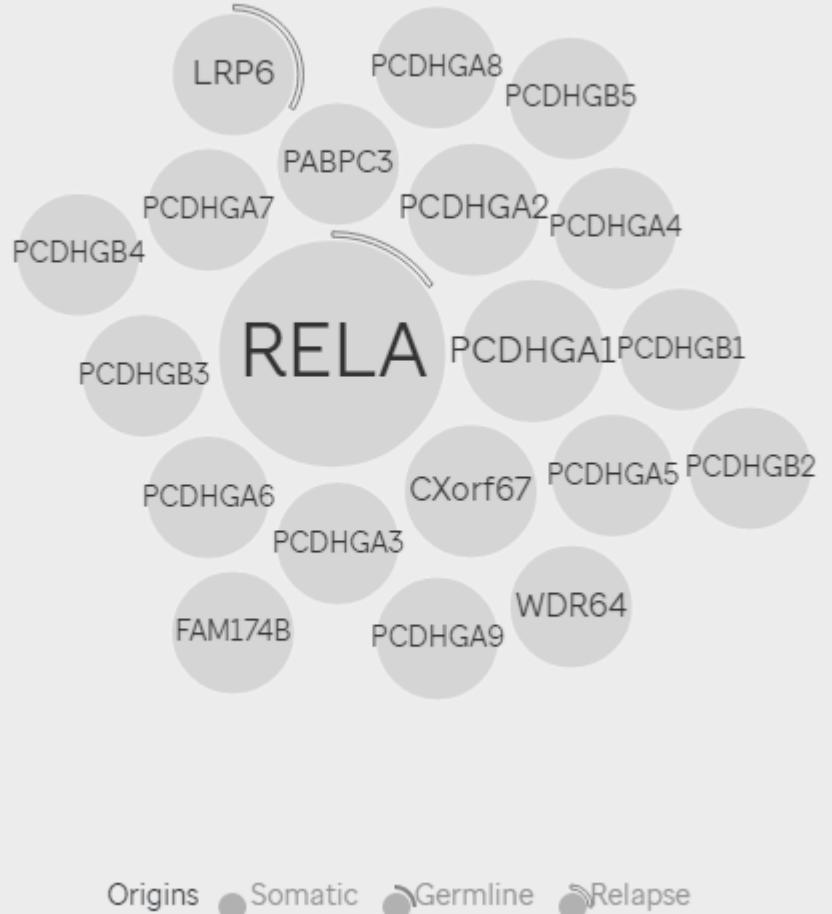
ATRT

Mammalian SWI/SNF complex (mBAF)



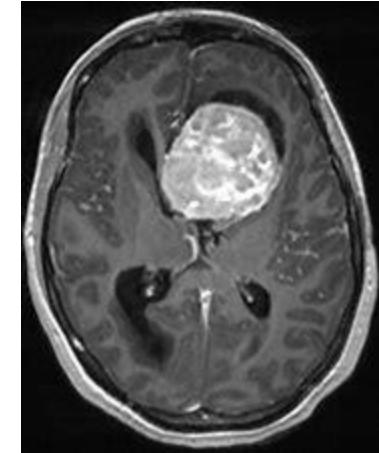
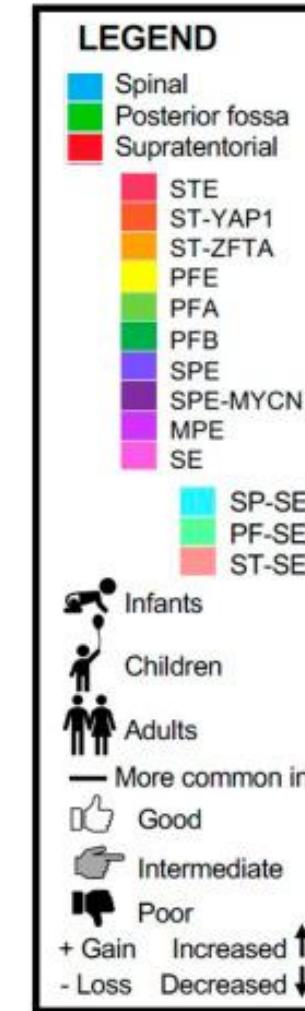
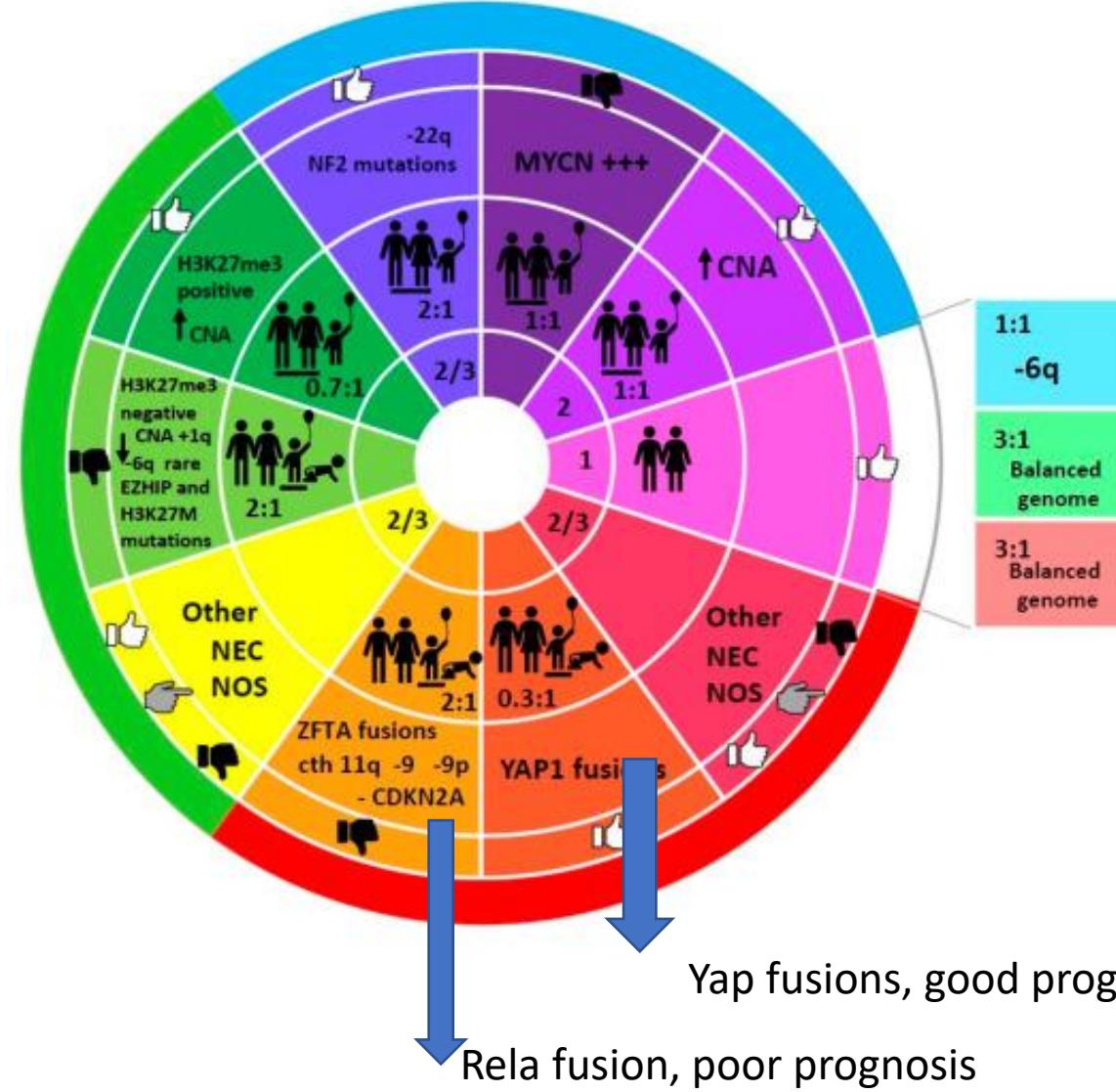
- ATRT is short for atypical teratoid rhabdoid tumor, is a very rare fast-growing and aggressive embryonal tumor of the brain and spinal cord
- ATRT is a rapidly progressing tumor, with most deaths occurring in the first 12 months after onset of symptoms
- The genetic landscape of AT/RTs is surprisingly simple: Inactivation of SMARCB1 or SMARCA4 by non sense or frame shift mutations
- Both proteins are involved in chromatin remodeling
- Tazemetostat in clinical study. Responses were observed in some patients (4/21)

Ependymoma



Ependymoma

Ependymoma molecular classification per WHO 2021



Take home message

High-throughput molecular analyses have allowed to

- Identify driver alterations (Fusions in Ewing Sarcoma and SMARCB1 mutation in ATRT)
- Define subtypes with different prognosis (Medulloblastoma with WNT alteration)
- Define subtypes with different answer to therapy (Neuroblastoma)
- Define molecular targets for therapies (BRAF in brain tumors)
- Identify germline mutations with impact on therapy and follow-up

Which analysis are used for therapy decision?

Study (Inform, DKT Master)

- Whole-exome sequencing
 - RNA-sequencing
 - DNA Methylation array
- ↓
- All mutations
 - Mutational Burden
 - All fusions
 - All overexpressed genes
 - Signature
 - CNV
 - Tumor entity based on methylation analysis
 - Infiltration of immune cells

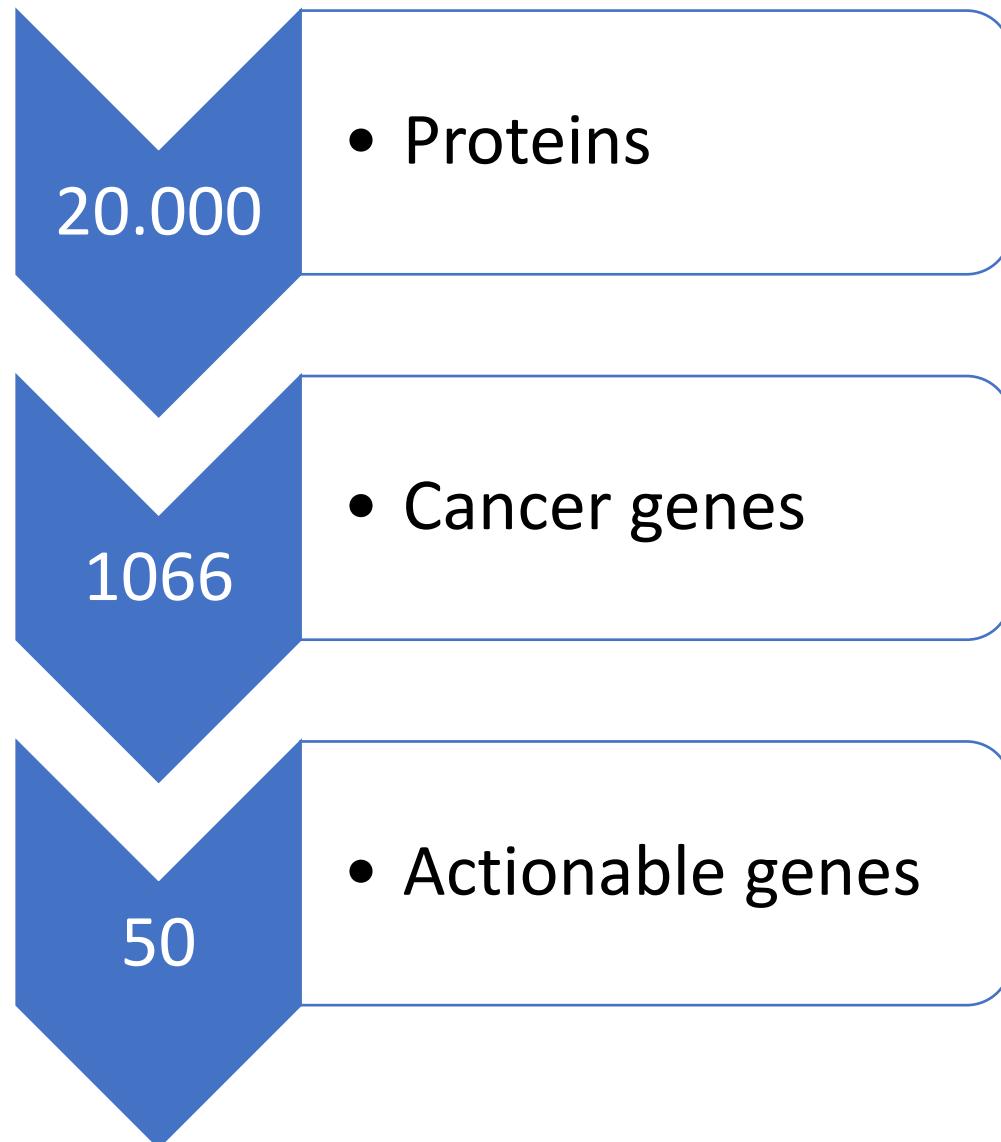
In House (Pathology)

- Panel analysis

↓
Only some Mutations
and fusions

→ **Comprehensive molecular analysis is done
only in some centres**

The druggable genome



Welcome to OncoKB™

MSK's Precision Oncology Knowledge Base
An FDA-Recognized Human Genetic Variant Database*

688
Genes

5729
Alterations

133
Cancer Types

111
Drugs

Search Gene / Alteration / Drug

Therapeutic Levels

Diagnostic Levels

Prognostic Levels

FDA Levels

① Level 1
FDA-approved drugs
43 Genes

② Level 2
Standard care
24 Genes

③ Level 3
Clinical evidence
27 Genes

④ Level 4
Biological evidence
25 Genes

⑤ Level R1/R2
Resistance
11 Genes



We can target only few genes

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MSK's Precision Oncology Knowledge Base

An FDA-Recognized Human Genetic Variant Database*

688

Genes

5729

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Clinical evidence

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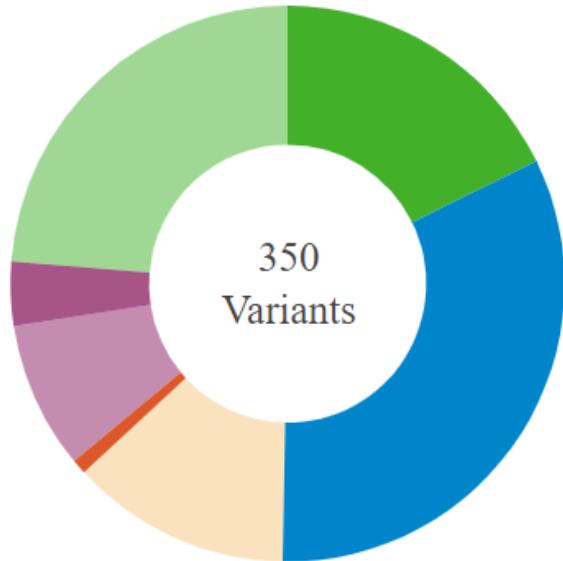
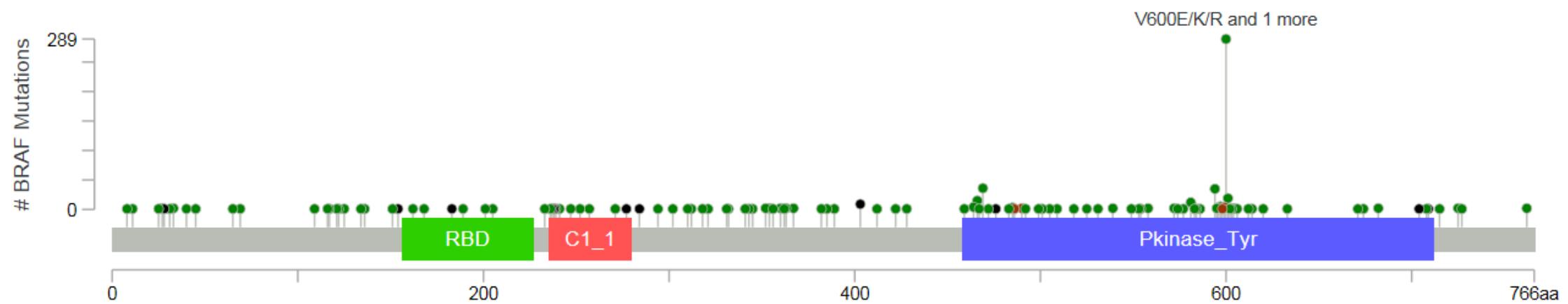
⑤ Level R1/R2

Resistance

11 Genes

-
- Most are mutations
 - Some fusions
 - 1 CNV (Her2)
- Most are mutations
 - Some fusions
 - 2 CNV (Her2 and Met)
- Most are mutations
 - Some fusions
 - 3 CNV (Her2 and Met and FGFR1)
- Most are mutations
 - Some fusions
 - 5 CNV

Molecular Target: Which mutation of a gene is pathogenic?

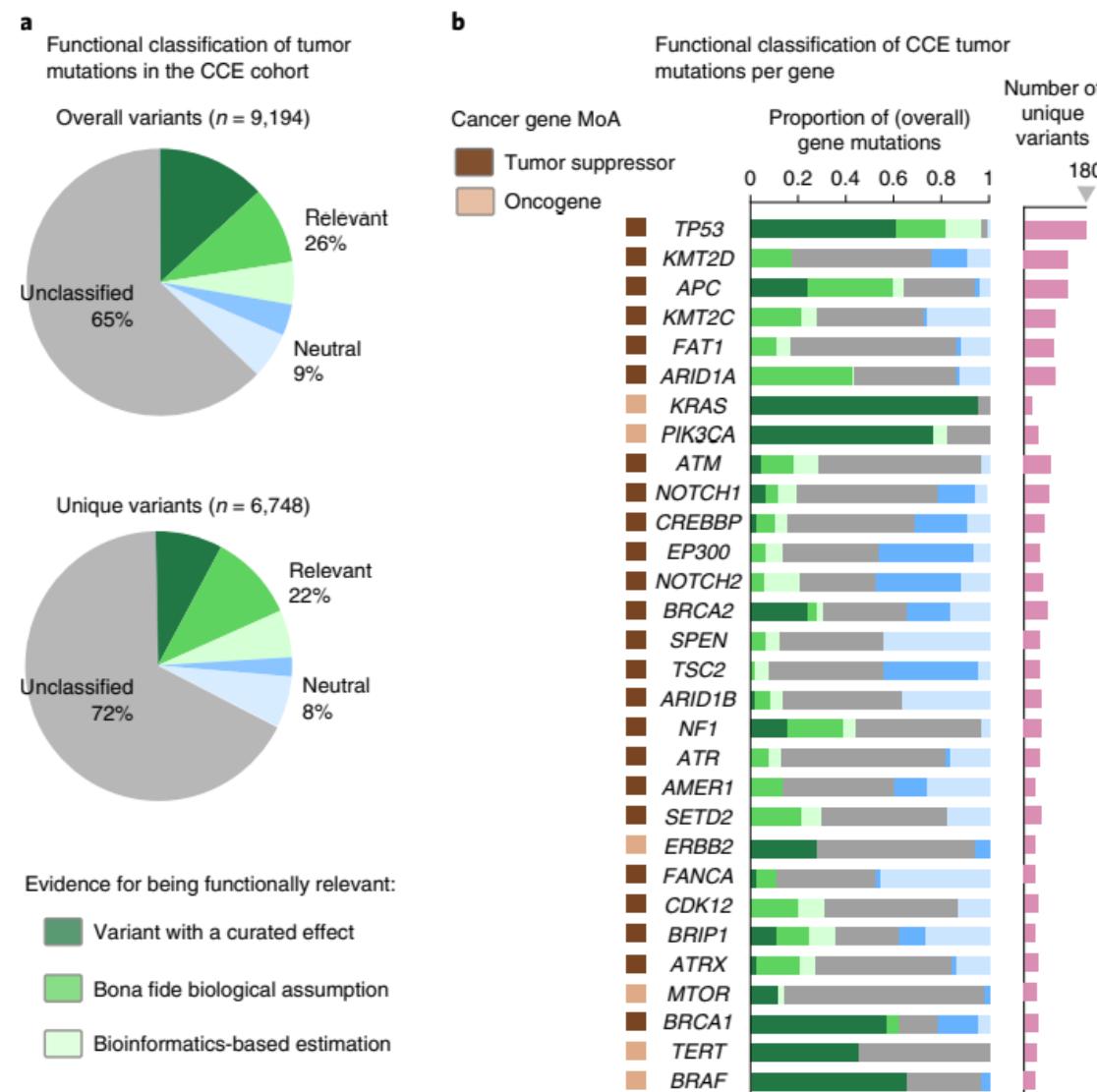


- gain of function
- gain of function - predicted
- loss of function
- loss of function - predicted
- no effect
- no effect - predicted
- unknown



We don't know all pathogenic mutations in a gene

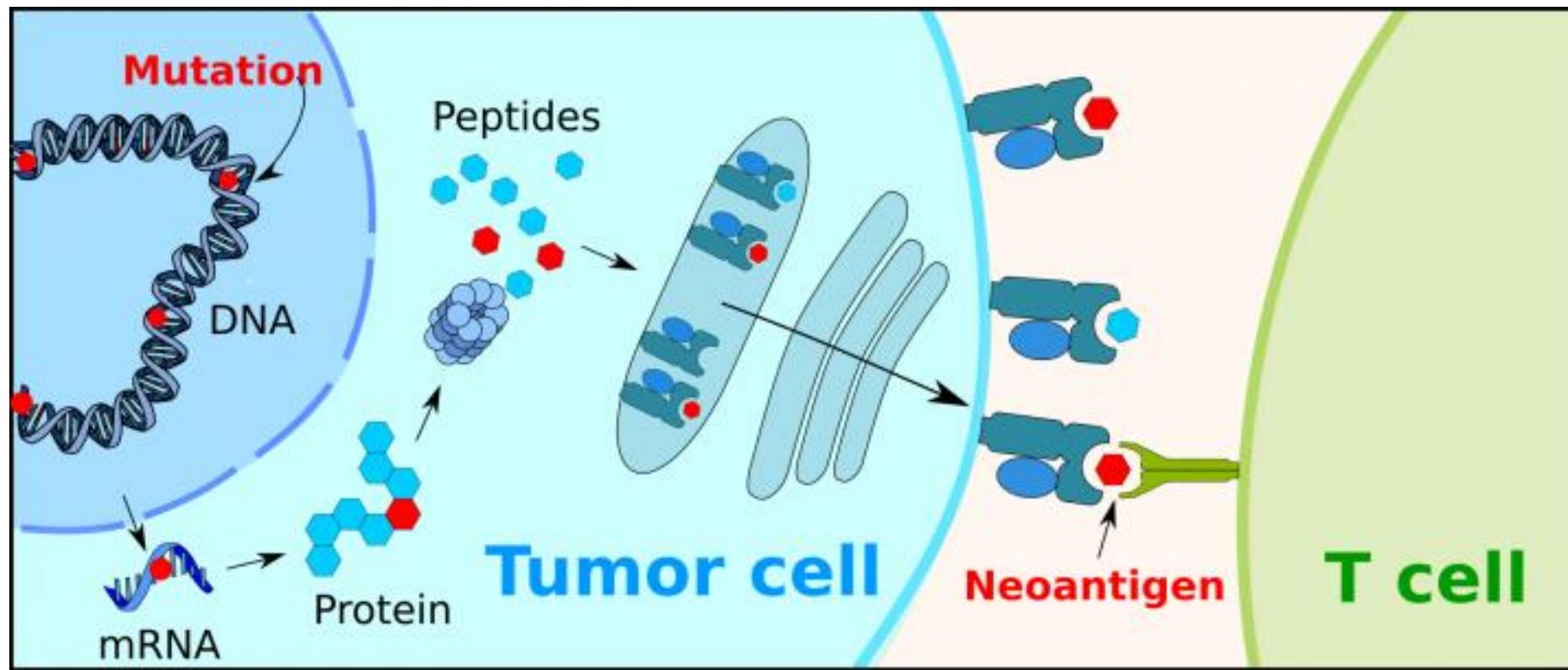
The druggable mutanome



Data bases to assess pathogenicity of a mutation

- COSMIC, <https://cancer.sanger.ac.uk/cosmic>
- ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar/>
- The Clinical Knowledgebase (CKB), <https://ckb.jax.org/>
- cBioportal, <https://www.cbioportal.org/>
- Varsome, <https://varsome.com/search-results/>

Mutational burden



→ **High mutational burden predict response to immunotherapy**

Transcriptomics

How to define upregulation?

Does the upregulation of one protein lead to the activation of a pathway?

Transcriptomics

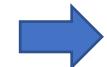


Unknown entity in 2016!

Biopsie

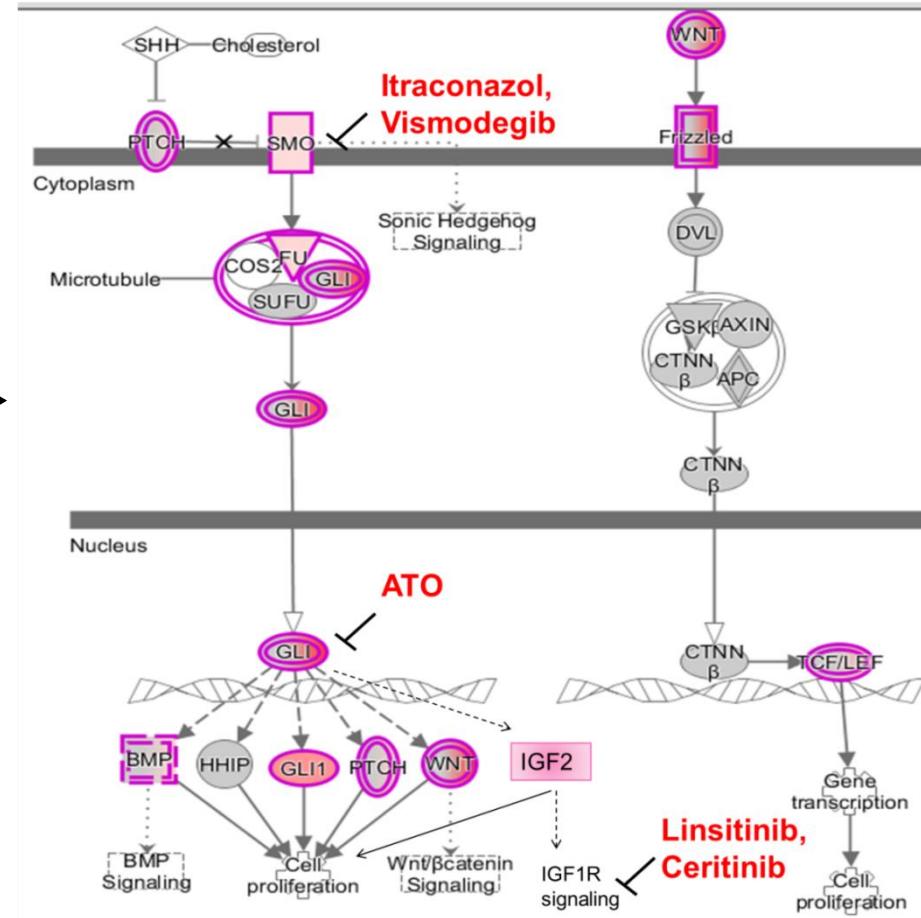


RNA
Sequencing



Pathway
analysis

Therapy



Mutational Signatures

Mutational signatures are characteristic combinations of mutation types arising from specific mutagenesis processes such as DNA replication infidelity, exogenous and endogenous genotoxin exposures, defective DNA repair pathways, and DNA enzymatic editing.

 Catalogue Of Somatic Mutations In Cancer 

Projects ▾ Data ▾ Tools ▾ News ▾ Help ▾ About ▾ Search COSMIC... **SEARCH**

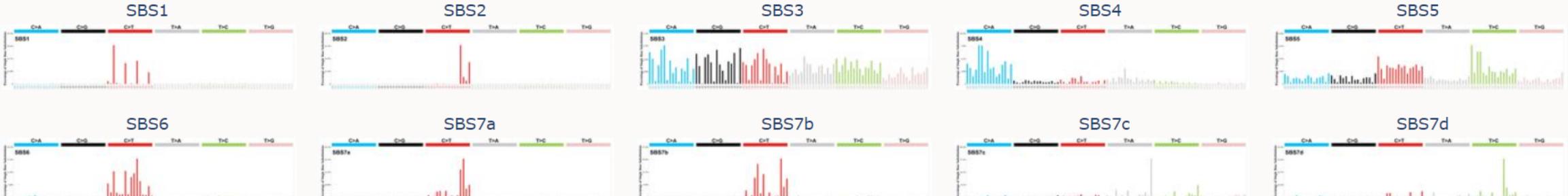
Mutational Signatures (v3.2 - March 2021)

Single Base Substitution (SBS) Signatures

Signature extraction methods

With a few exceptions, the current set of reference signatures were extracted using [SigProfiler](#) (as described in [Alexandrov, L.B. et al., 2020](#)) from the 2,780 whole-genome variant calls produced by the ICGC/TCGA [Pan Cancer Analysis of Whole Genomes \(PCAWG\)](#) Network. The stability and reproducibility of the signatures were assessed on somatic mutations from an additional 1,865 whole genomes and 19,184 exomes. All input data and references for original sources are available from synapse.org ID [syn11801889](#).

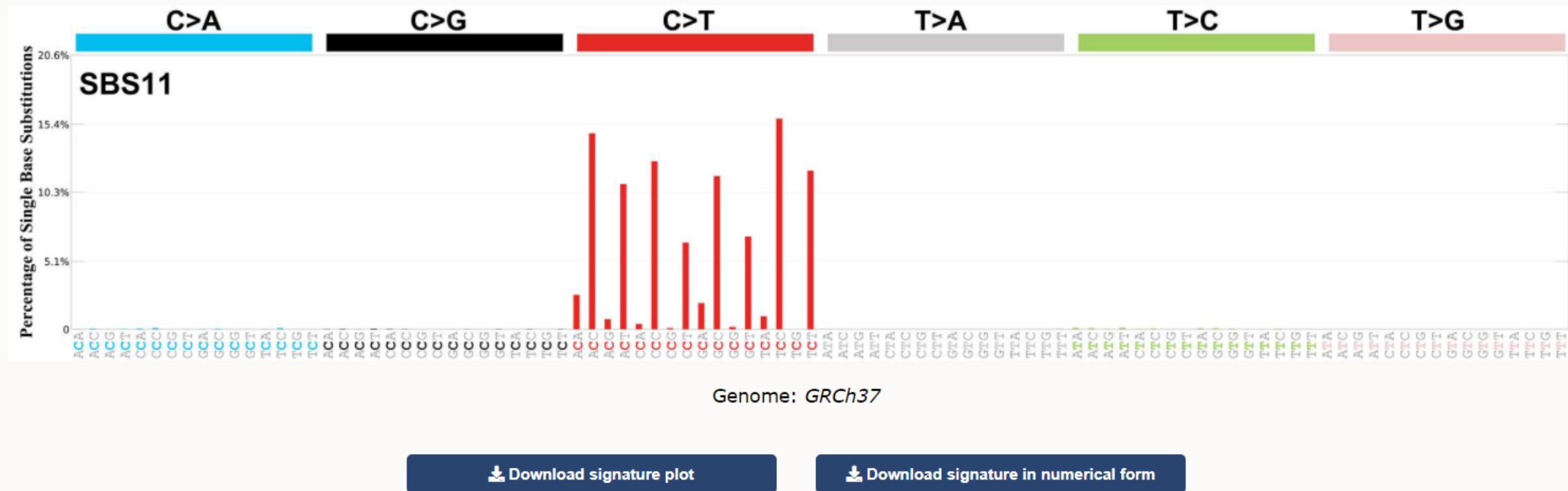
COSMIC mutational signatures are available in numerical form in our [data downloads page](#).



Combination of mutations found in tumors after treatment with temozolomide

Mutational profile using the conventional 96 mutation type classification. This classification is based on the six substitution subtypes: C>A, C>G, C>T, T>A, T>C, and T>G, as well as the nucleotides immediately 5' and 3' to the mutation.

[Help](#)



Proposed aetiology

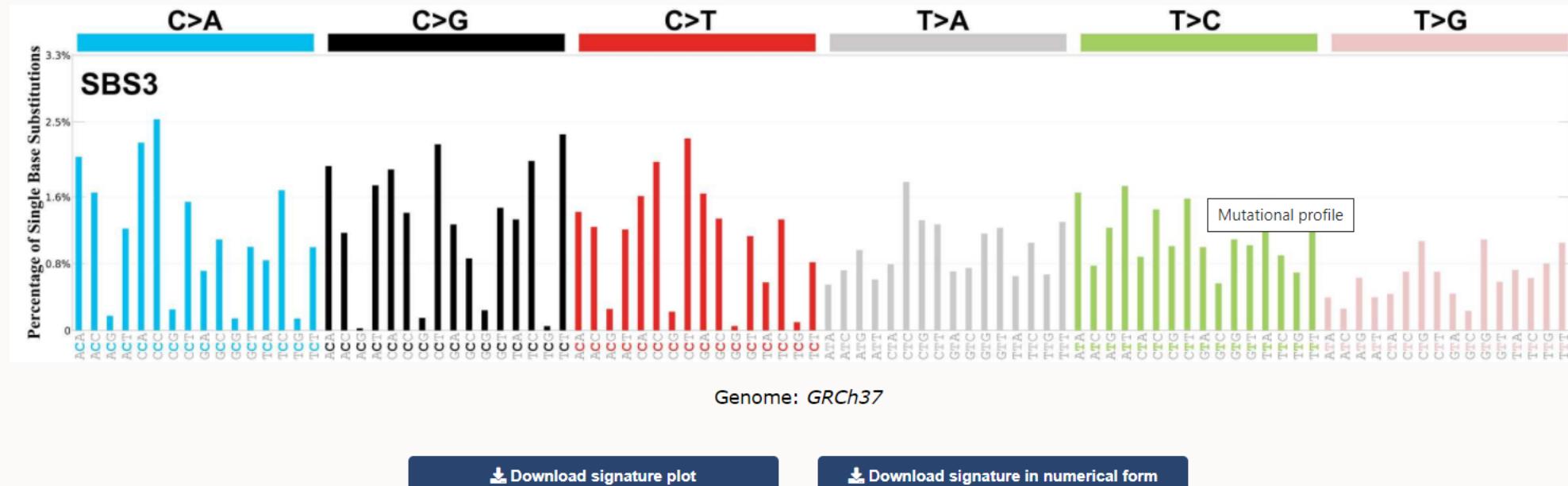
SBS11 exhibits a mutational pattern resembling that of alkylating agents. Patient histories indicate an association between previous treatment with the alkylating agent temozolomide and SBS11 mutations.

Combinations of mutation found in BRCAness signature

Mutational profile

Mutational profile using the conventional 96 mutation type classification. This classification is based on the six substitution subtypes: C>A, C>G, C>T, T>A, T>C, and T>G, as well as the nucleotides immediately 5' and 3' to the mutation.

[Help](#)



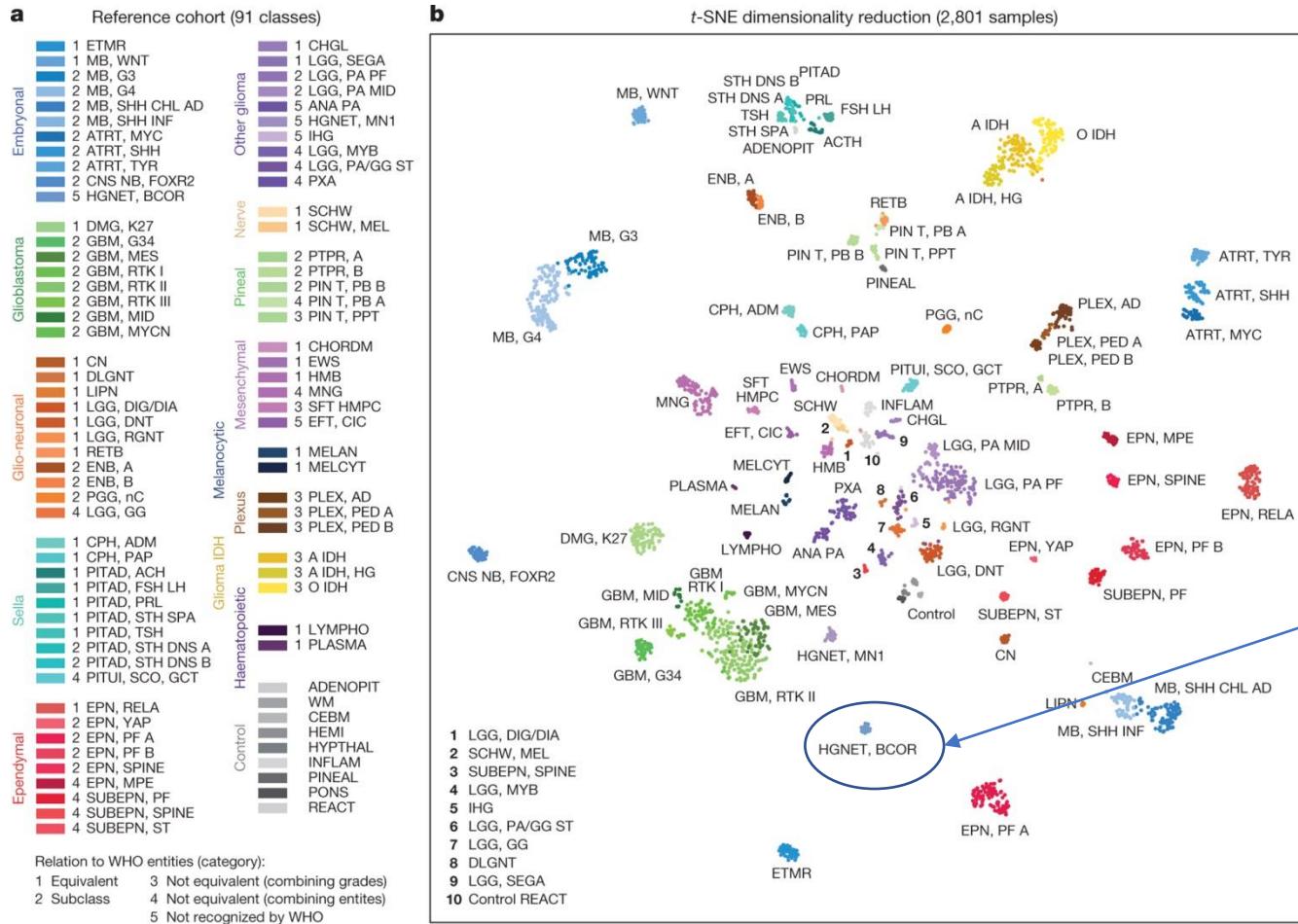
Proposed aetiology

Defective homologous recombination-based DNA damage repair which manifests predominantly as small indels and genome rearrangements due to abnormal double strand break repair but also in the form of this base substitution signature.

→ BRCAness signature (DNA repair mechanism) predict response to drugs otherwise used for patients with alterations in the BRCA 1 / 2 gene olaparib

Methylation analysis for tumor classification

DNA methylation-based CNS tumor classification



- Definition of new tumor entities
- Support pathologists by unclear diagnostic
- Also for sarcoma

Transcriptomics



Unknown entity → HGNET-BCOR

Biopsie

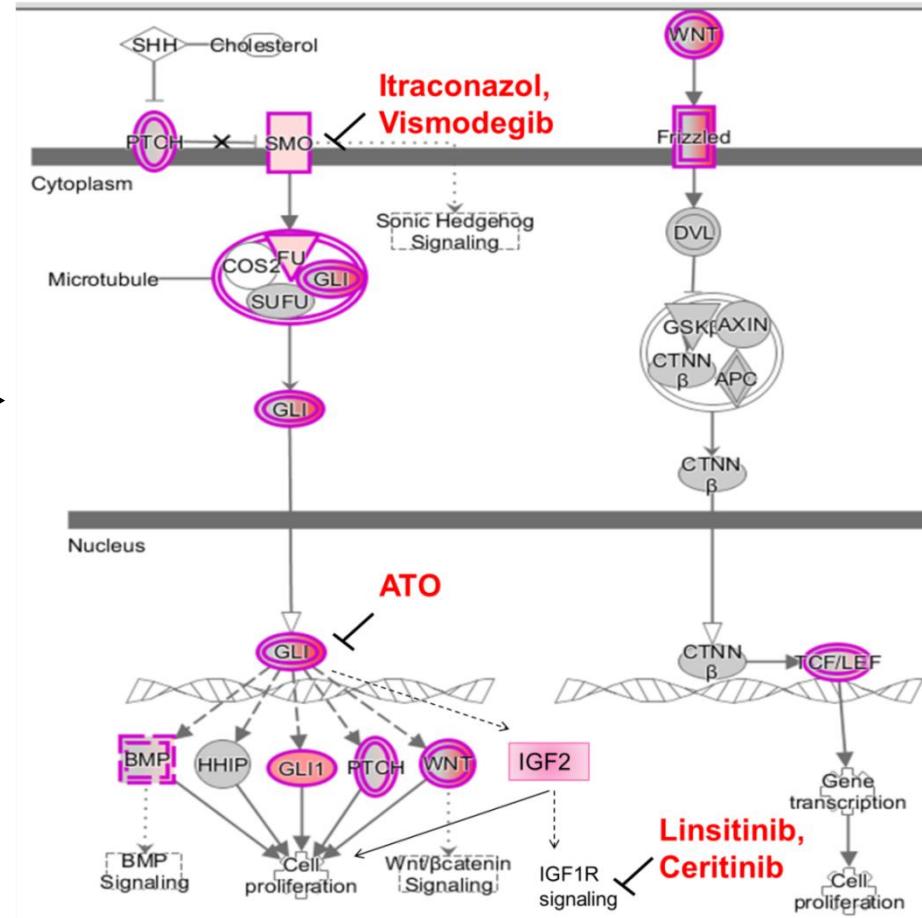


RNA
Sequencing



Pathway
analysis

Therapy



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Welcome to MolecularNeuropathology.org - The platform for next generation neuropathology.

This website represents the access point for DNA methylation-based classification of central nervous system tumors. For the scientific background and interpretation of the data, please see [Capper D, Jones DTW, Sill M, Hovestadt V et al., Nature. 2018 Mar 22; 555\(7697\):469-474.](#)

To implement the methylation profiling classifier you are required to generate and upload unprocessed IDAT-files of Illumina Human Methylation 450 BeadChip arrays or EPIC BeadChip arrays of your samples of interest. This data is then automatically compared to methylation data of a reference cohort comprising over 2800 neuropathological tumors of almost all known entities (currently over 80 tumor classes or subclasses included). Within a short time you will receive an E-

Upload statistic

Total cases: 89908

For classifier development: 68618

Involved parties

University Hospital Heidelberg

Neuropathology

Pediatric Oncology

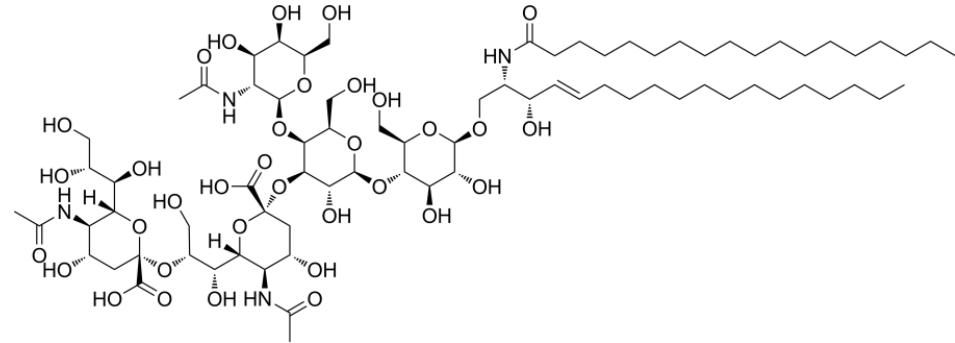
Neurooncology

Neurosurgery

Radiation Oncology

and Therapy

Not all aberrations are detectable bei NGS analysis!

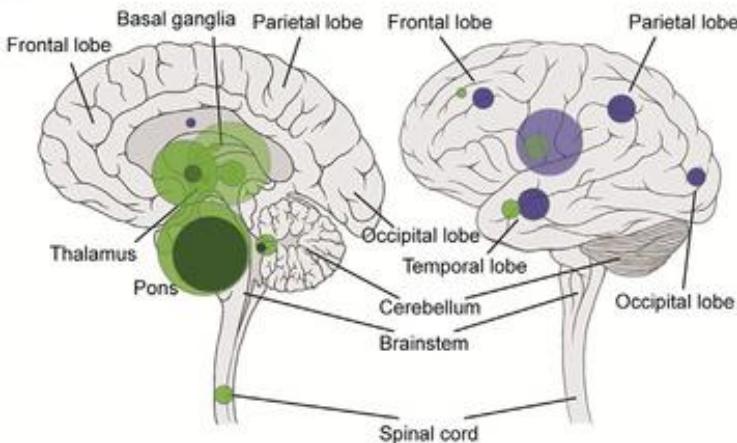


Lipids

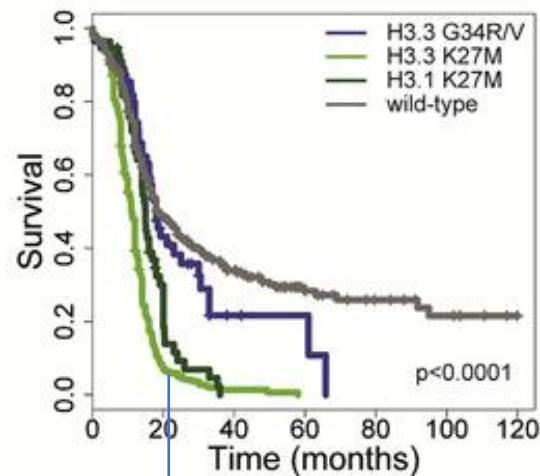
GD2

PONS Glioma with Histone mutations

D



F



H3.3 K27M



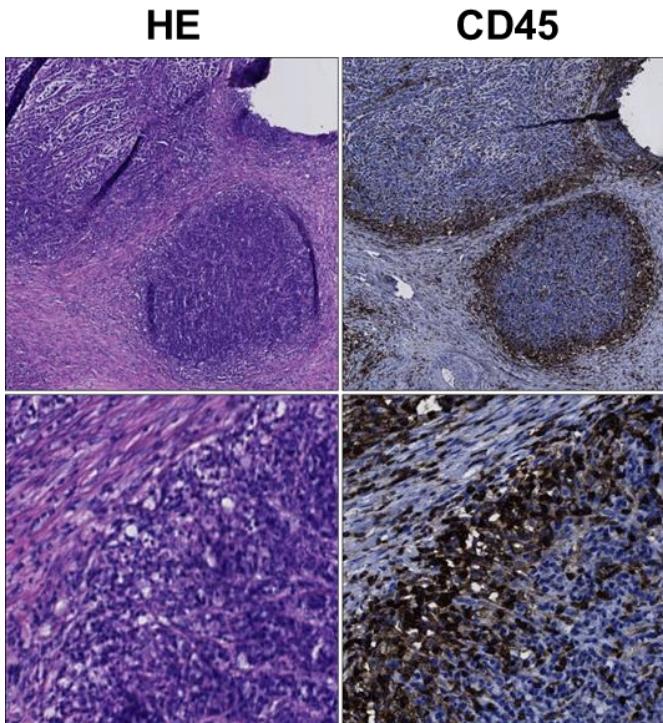
GD2

Diffuse intrinsic pontine gliomas (DIPGs) (also known as pontine gliomas and brain stem gliomas) make up around 10% of all pediatric brain tumors

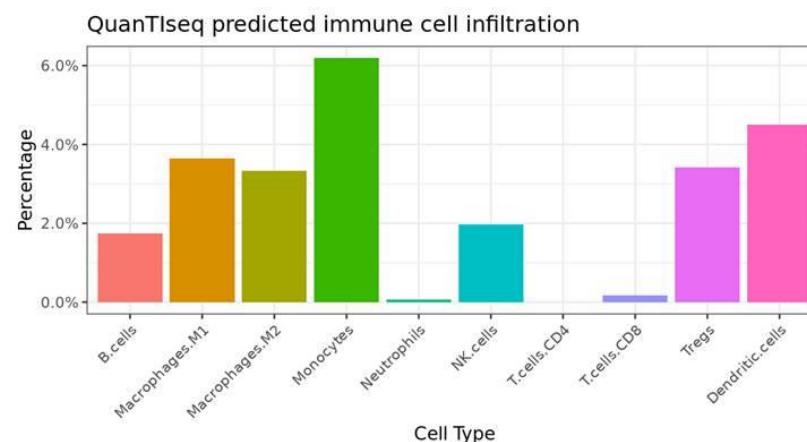
In spite of decades of investigation, these tumors remain refractory to therapy and result in a mean life expectancy of 9–12 months from diagnosis

Genetic:
a K27M mutation in
the H3F3A gene, which
encoded histone 3.3, is implicated
in a large proportion of DIPG cases

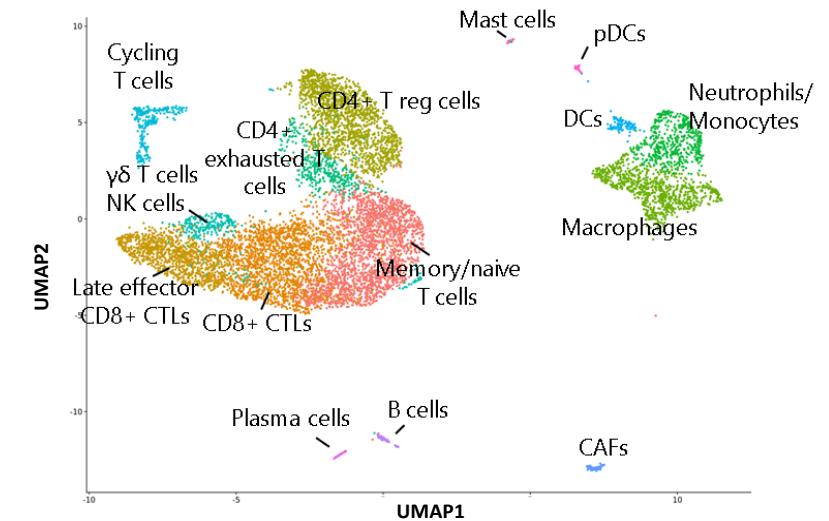
Infiltration of immune cells



Immunohistochemistry

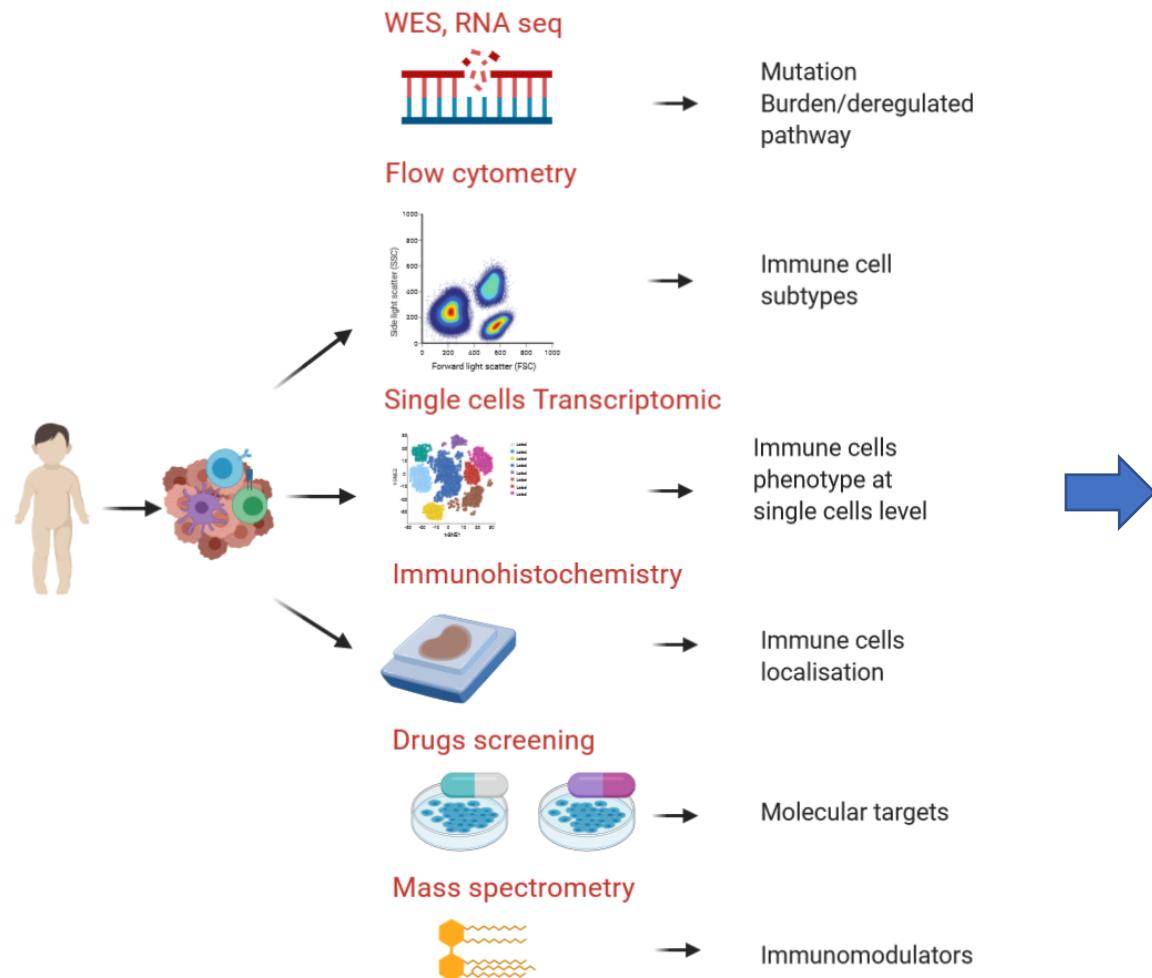


Prediction based on transcription data



Prediction based on transcription data at the single cells level

Predictive immune-oncology biomarkers identified by comprehensive molecular analysis



Biomarker	Value in Responders	Value in IOB Patient
B cells/CD45+	> 7	1.4
Myeloids/CD45+	< ~3	16.7
CD8+ memory/CD3+	> ~2	6
CD4+ memory/CD3+	> ~2.5	23
TCF7+/TCF7- CD8+	> 1	1.5
cDC1s/HLA-DR+	> 2.5	0.5
NK cells/CD45+	> 2	3
Tertiary lymphoid structures	High density	Low density
Mutations/mb	≥ 20 mutations/mb	37,4/Mb
JAK, PTEN	wt	Mutated

Taking home message

- The druggable genome is small (Mutations and fusions)
- Methylation analysis is used only for tumour classification so far
- The number of mutations and the pattern of mutations predict answer to particular therapies
- The interpretation of transcriptome data remains difficult
- Not all relevant targets are detectable by genomic analysis

Part-2

Welcome to OncoKB™

MSK's Precision Oncology Knowledge Base

An FDA-Recognized Human Genetic Variant Database*

688

Genes

5729

Alterations

133

Cancer Types

111

Drugs

Alk fusions

Search Gene / Alteration / Drug

Therapeutic Levels

Diagnostic Levels

Prognostic Levels

FDA Levels

① Level 1

FDA-approved drugs

43 Genes

② Level 2

Standard care

24 Genes

③ Level 3

Clinical evidence

27 Genes

④ Level 4

Biological evidence

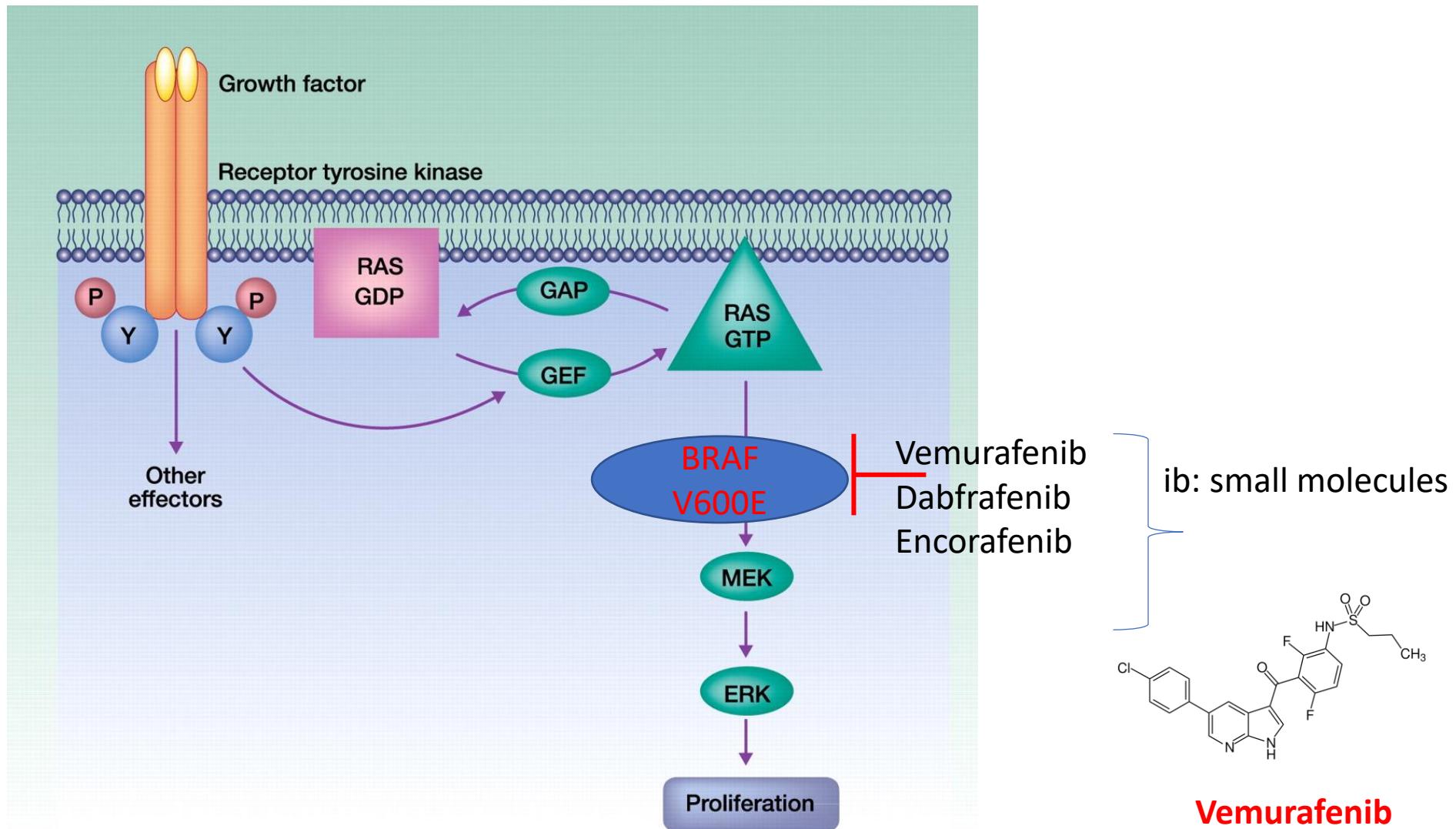
25 Genes

⑤ Level R1/R2

Resistance

11 Genes

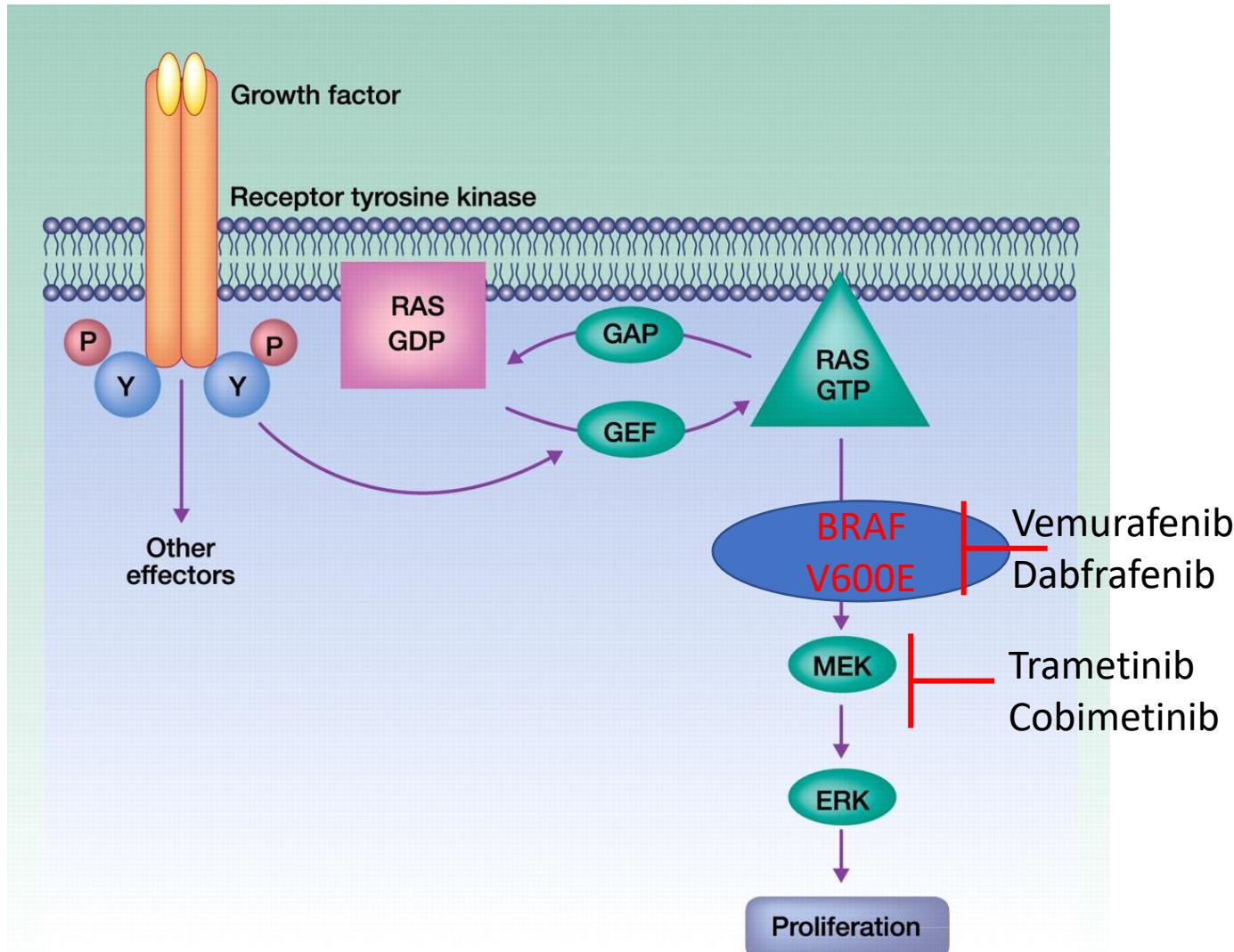
Dabrafenib ist auch ein BRAFV600E/K/M-spezifische Ihnibitor



Modified from: BRAF Inhibitors for the Treatment of Metastatic Melanoma: Clinical Trials and Mechanisms of Resistance.

Alexander Marzuka Alcalá and Keith T. Flaherty, DOI: 10.1158/1078-0432.CCR-11-0997 Published January 2012

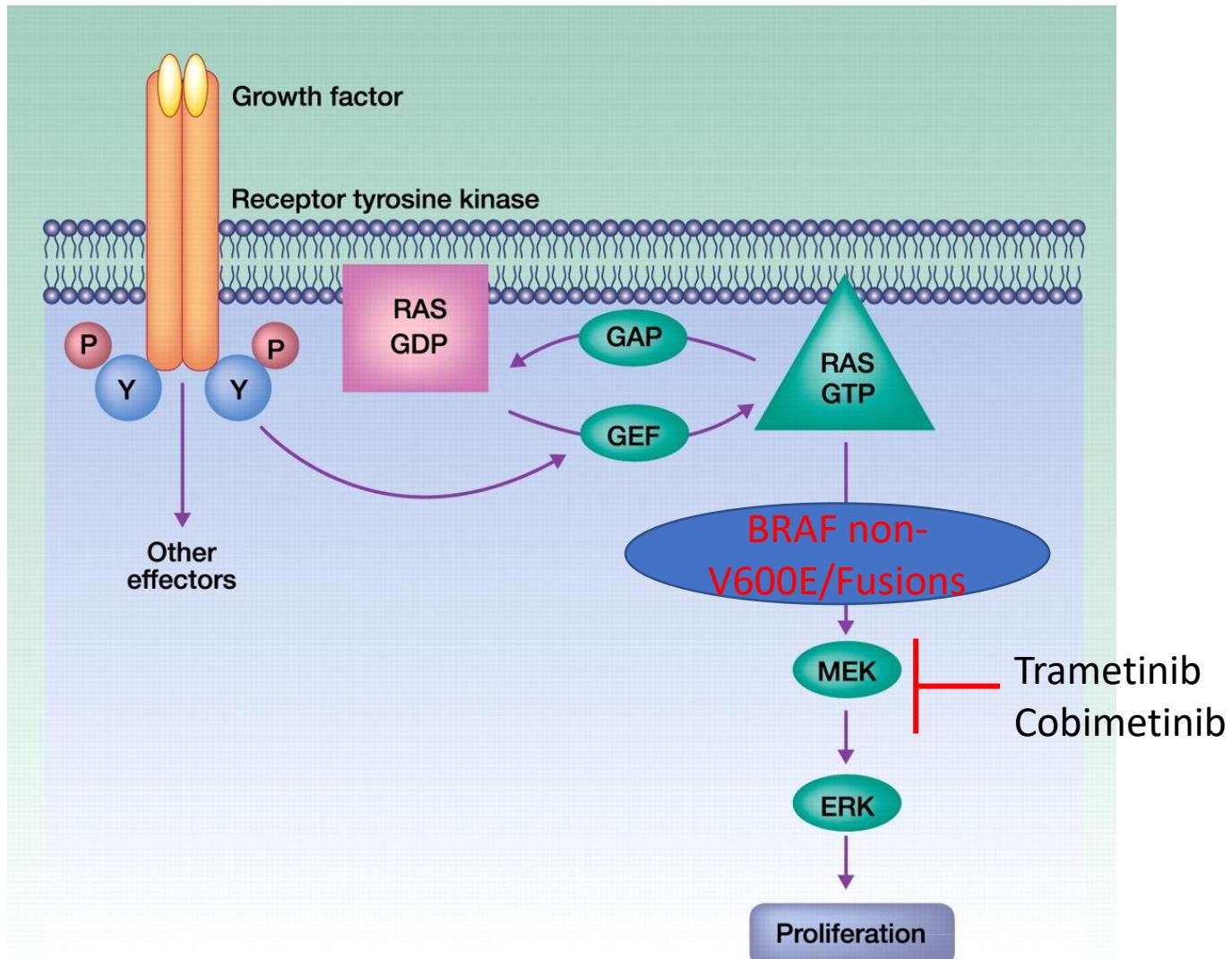
Die Inhibition funktioniert besser wenn auch MEK inhibiert wird



Although treatment with Vemurafenib or Dabrafenib produces some clinical benefit in nearly all patients with BRAF mutated melanomas, **more than 90% of them develop resistance to these drugs within one year**.

Therefore, two new drugs, **Trametinib and Cobimetinib** that target MEK downstream from the **BRAF in the MAP** kinase pathway were developed. These drugs, mainly when combined with Vemurafenib or Dabrafenib, do improve both the overall and the progression-free survival in melanoma patients

Die Inhibition funktioniert besser wenn auch MEK inhibiert wird

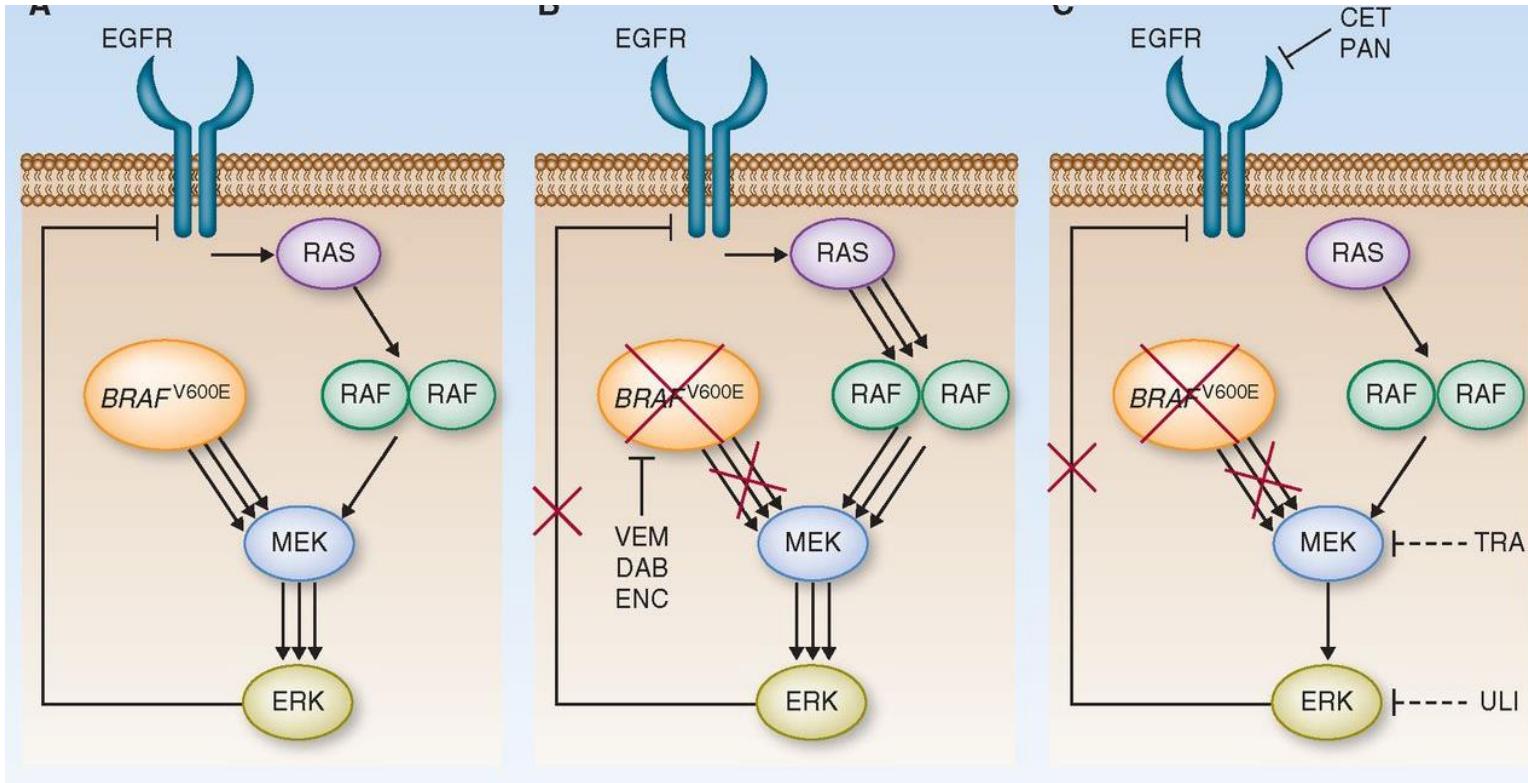


Modified from: BRAF Inhibitors for the Treatment of Metastatic Melanoma: Clinical Trials and Mechanisms of Resistance.

Alexander Marzuka Alcalá and Keith T. Flaherty, DOI: 10.1158/1078-0432.CCR-11-0997 Published January 2012

Feedback reactivation following BRAF inhibition in colon cancer

EGFR is driver in colon cancer and can be targeted with Cetuximab

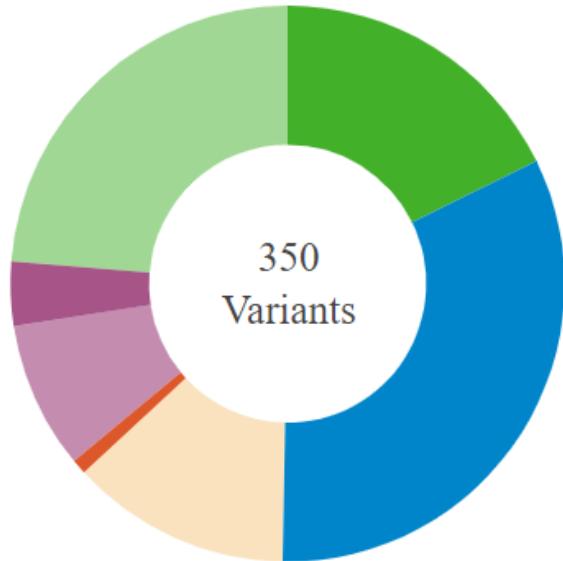
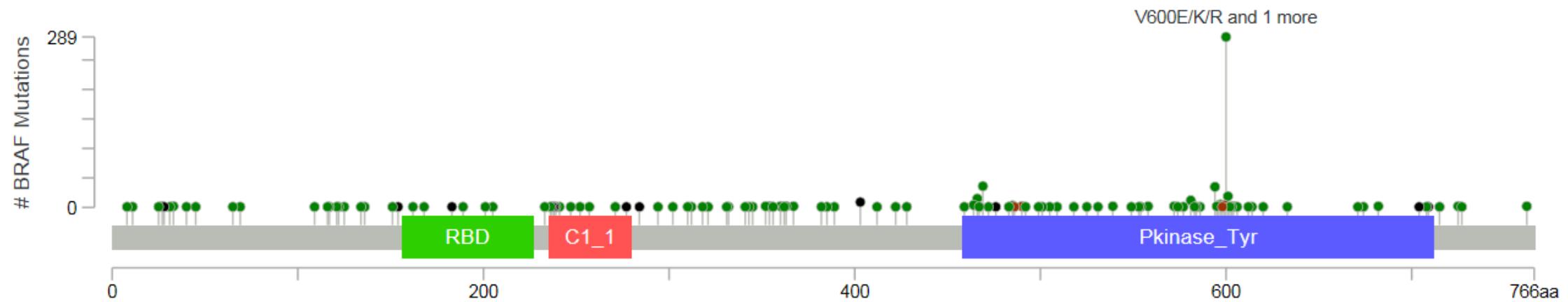


Constitutive activation of BRAF drives ERK activation. The activation of ERK leads to ERK-dependent negative feedback on EGFR activation

When mutated BRAF is inhibited, the ERK-dependent negative feedback is reduced, allowing for enhanced activation of EGFR and downstream RAS, leading to reactivation of the pathway

Targeting resistance through combination BRAF, MEK, and EGFR inhibition.
VEM, vemurafenib; DAB, dabrafenib;
ENC, encorafenib; TRA, trametinib;
ULI, ulixertinib; CET, cetuximab; PAN,
panitumumab

Which mutation is pathogenic?

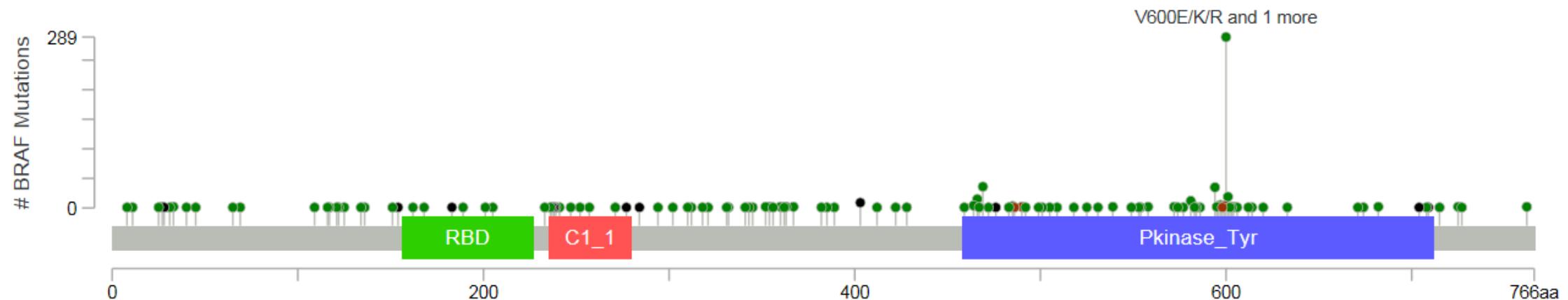


- gain of function
- gain of function - predicted
- loss of function
- loss of function - predicted
- no effect
- no effect - predicted
- unknown

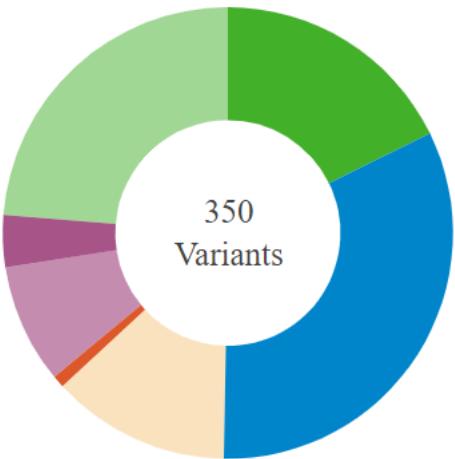


We don't know all pathogenic mutations in a gene

Which mutation is actionable?



350 Varianten



- gain of function
- gain of function - predicted
- loss of function
- loss of function - predicted
- no effect
- no effect - predicted
- unknown

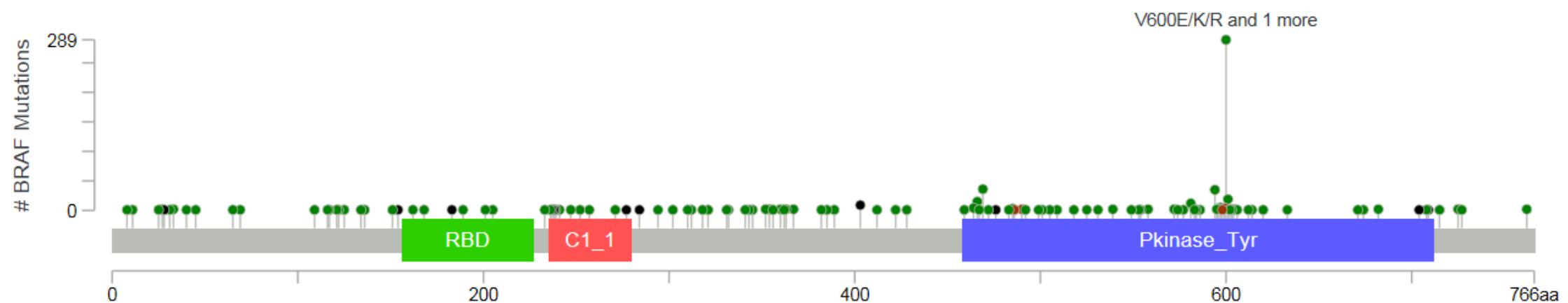


Actionable

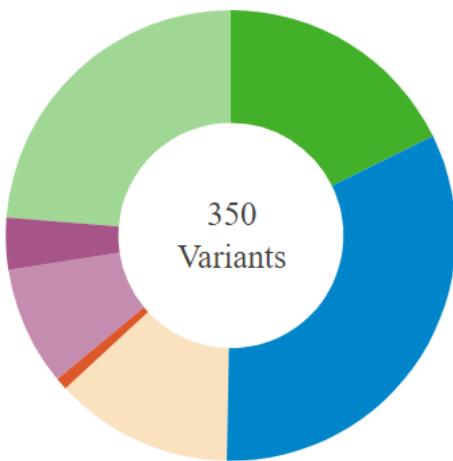
V600E	G464
K601	L597
G469	

We don't have have drugs for all pathogenic mutations in a gene

Which drug for which mutation?



350 Varianten



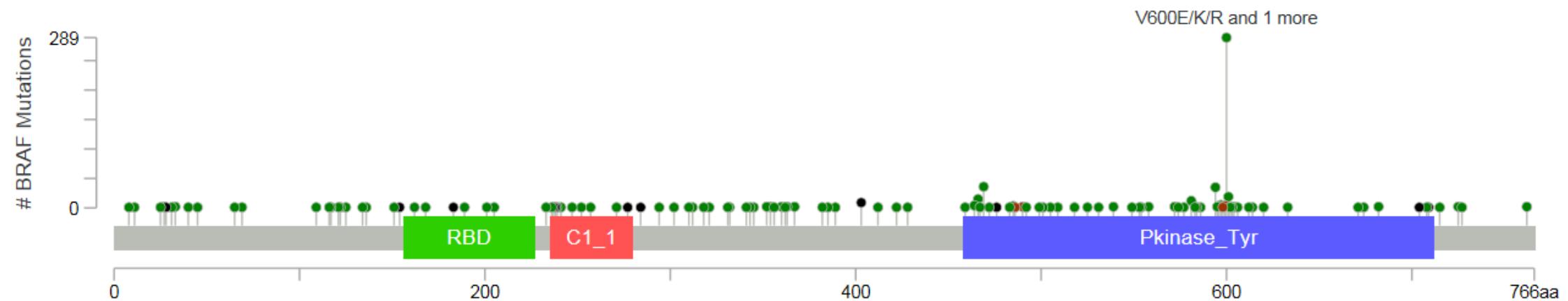
- gain of function
- gain of function - predicted
- loss of function
- loss of function - predicted
- no effect
- no effect - predicted
- unknown



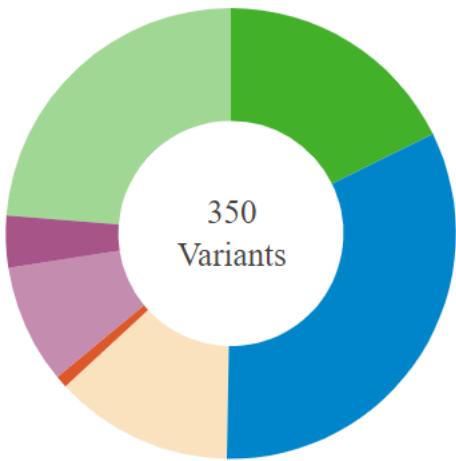
Actionable	Drug
V600E	Dabrafenib + Trametinib
Fusions	Trametinib
G469	PLX8394

The drug is dependent on the position

Which drug in which tumor entity?



350 Varianten



- gain of function
- gain of function - predicted
- loss of function
- loss of function - predicted
- no effect
- no effect - predicted
- unknown

Actionable



Actionable	Entität	Drug
V600E	Colorectal cancer	Encorafenib + Cetuximab
V600E	Melanoma	Dabrafenib + Trametinib

Different drugs/combination are required for the same gene at the same position in different entities

KRAS

Oncogene

Highest level of evidence: **Level 1 ①** · **Level R1 ④** · **Level Dx2 ②** · **FDA Level 2 ②**

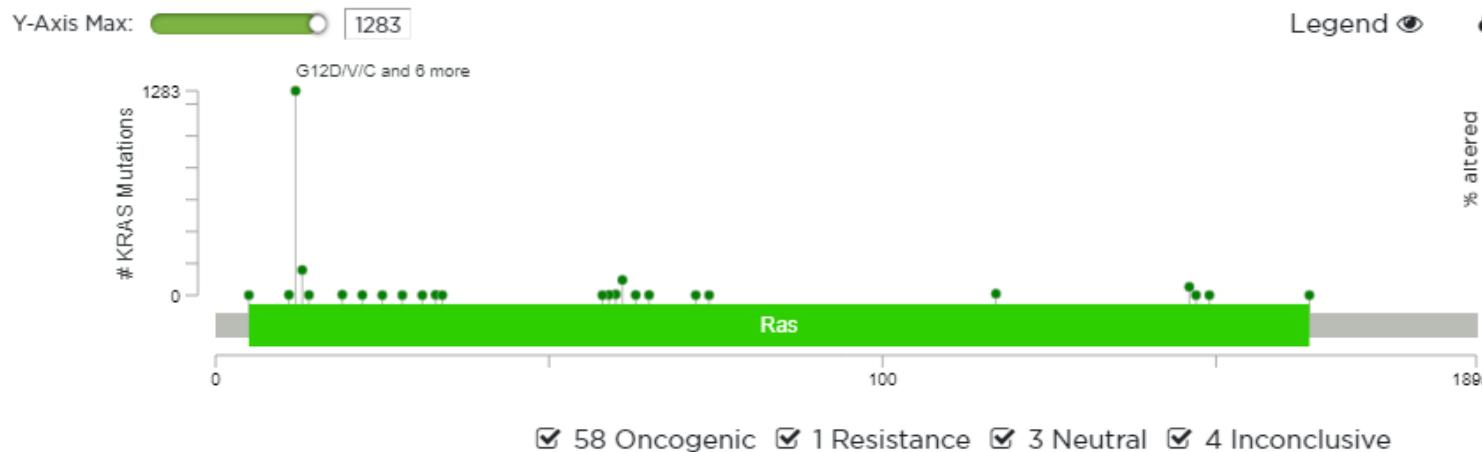
Also known as KRAS2, K-Ras4B, KRAS1

KRAS, a GTPase which functions as an upstream regulator of the MAPK pathway, is frequently mutated in various cancer types including lung, colorectal and pancreatic cancers.

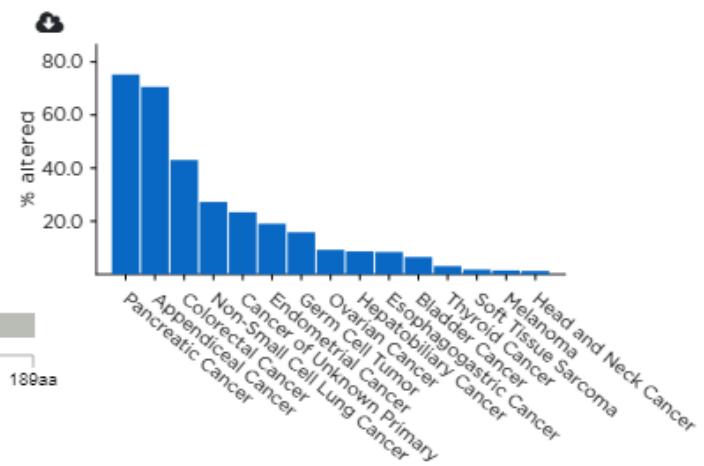
Show KRAS background 

NCBI Gene	3845
Ensembl Gene	ENSG00000133703 (GRCh37/GRCh38)
Location	Chr12:25357723-25403870 (GRCh37) Chr12:25205246-25250936 (GRCh38)
Ensembl Transcript	ENST00000311936 (GRCh37/GRCh38)
RefSeq	NM_004985.3 (GRCh37/GRCh38)

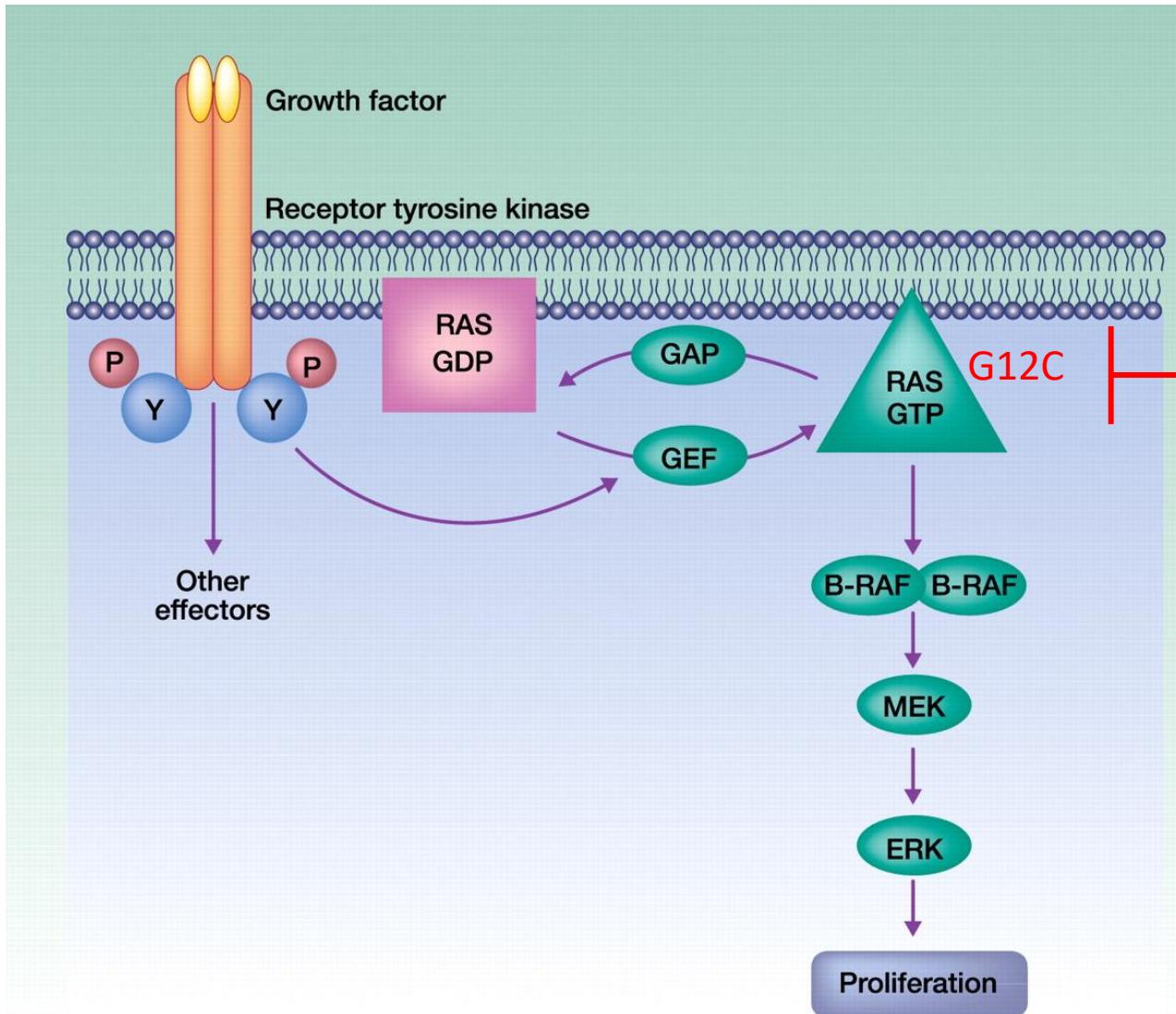
Annotated Mutations in MSK-IMPACT Clinical Sequencing Cohort (Zehir et al., Nat Med 2017)



Cancer Types with KRAS Mutations



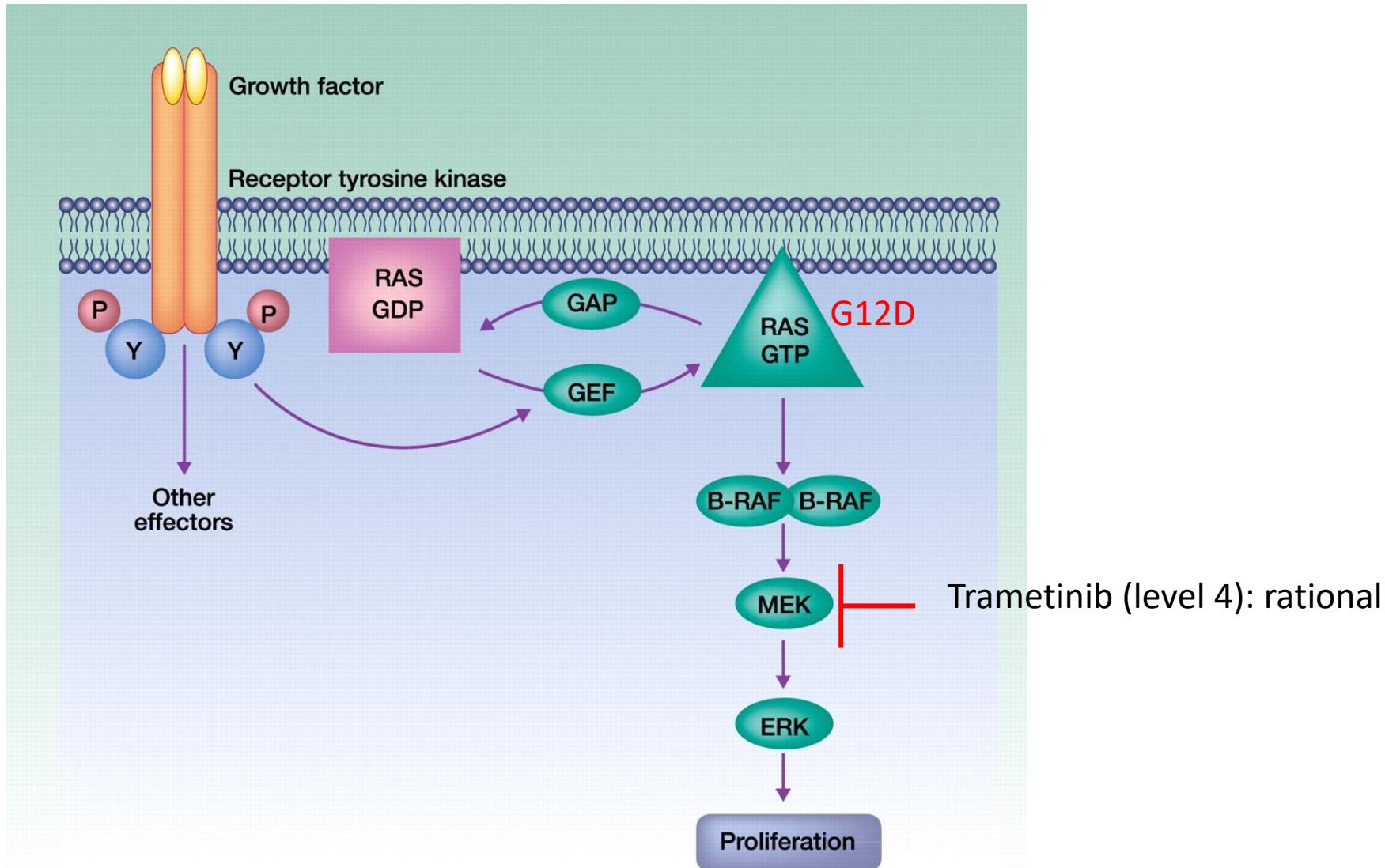
KRAS



Sotarasib: FDA released for non-small cell lung carcinoma (Level 1)

Adagrasib: in clinical testing (Level 3)

KRAS

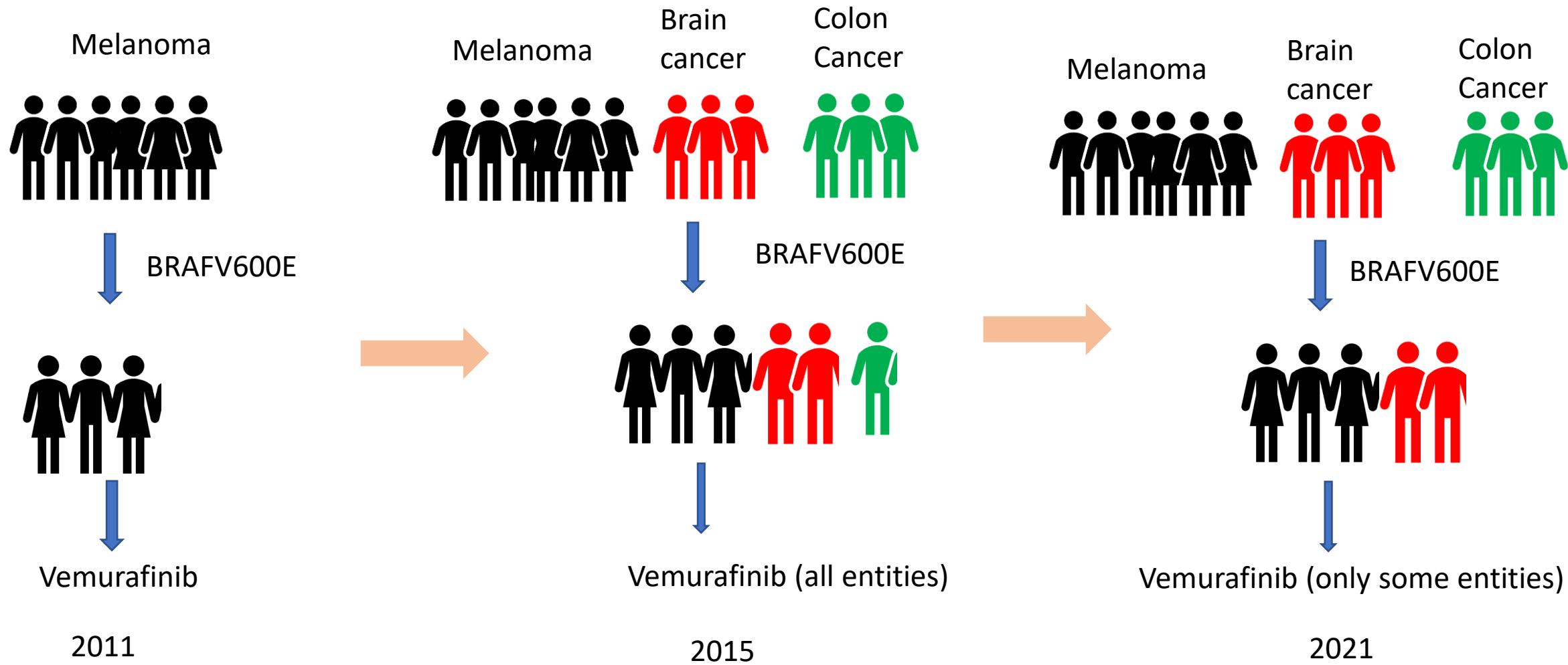


From tumor-agnostic cancer therapy to evidence-based personalised therapy

Entity specific biomarker-driven Medicine

Tumor-agnostic cancer therapy

Evidence-based personalised therapy



How many hystology-agnostic Therapies have been released so far?

