NGS molecular profile of paediatric brain tumours research samples: results from 92 consecutive cases treated at Centro Hospitalar Universitário de São João

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Paediatric Cancer and Paediatric CNS tumours

- Paediatric cancer is the leading cause of death, beside accidents, in children aged 1-14y;
- Whilst global survival rates have improved significantly, they continue dismal for specific tumour types and for relapses/refractory disease;
- Moreover, survival rates are attained at the cost of high toxicity due to multimodal therapeutics;
- Paediatric CNS tumours represent ~25% of all paediatic tumours and are a heterogenous and challenging group of tumours;
- Have had tremendous advances in their molecular biology through several large-scale, pan-cancer studies. However, the effective utilization of molecular biomarkers in paediatric oncology clinical practice is still lagging behind what is already routinely performed in many adult cancer patients.

The 2021 WHO Classification of CNS tumours

The 2021 WHO Classification of Tumors of the Central Nervous System: a summary

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brain tumor | central nervous system | classification | diagnosis | World Health Organization

The 2021 WHO Classification of CNS tumours

Tumor Type	Genes/Molecular Profiles Characteristically Altered[a]
Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted	*IDH1*, *IDH2*, 1p/19q, *TERT* promoter, *CIC*, *FUBP1*, *NOTCH1*
Diffuse astrocytoma, *MYB*- or *MYBL1*-altered	*MYB*, *MYBL1*
Polymorphous low-grade neuroepithelial tumor of the young	*BRAF*, FGFR family
Diffuse midline glioma, H3 K27-altered	H3 K27, *TP53*, *ACVR1*, *PDGFRA*, *EGFR*, *EZHIP*
Diffuse pediatric-type high-grade glioma, H3-wildtype, and IDH-wildtype	IDH-wildtype, H3-wildtype, *PDGFRA*, *MYCN*, *EGFR* (methylome)
Pilocytic astrocytoma	*KIAA1549-BRAF*, *BRAF*, *NF1*
Pleomorphic xanthoastrocytoma	*BRAF*, *CDKN2A/B*
Chordoid glioma	*PRKCA*
Ganglion cell tumors	*BRAF*
Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters	Chromosome 14, (methylome)
Rosette-forming glioneuronal tumor	*FGFR1*, *PIK3CA*, *NF1*
Diffuse leptomeningeal glioneuronal tumor	*KIAA1549-BRAF* fusion, 1p (methylome)
Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)	*PTEN*
Supratentorial ependymomas	*ZFTA*, *RELA*, *YAP1*, *MAML2*
Spinal ependymomas	*NF2*, *MYCN*
Medulloblastoma, SHH-activated	*TP53*, *PTCH1*, *SUFU*, *SMO*, *MYCN*, *GLI2* (methylome)
Atypical teratoid/rhabdoid tumor	*SMARCB1*, *SMARCA4*
CNS neuroblastoma, *FOXR2*-activated	*FOXR2*
Desmoplastic myxoid tumor of the pineal region, *SMARCB1*-mutant	*SMARCB1*
Solitary fibrous tumor	*NAB2-STAT6*

WHO Classification of Pediatric tumours

REVIEW

A Summary of the Inaugural WHO Classification of Pediatric Tumors: Transitioning from the Optical into the Molecular Era

Stefan M. Pfister[1,2,3], Miguel Reyes-Múgica[4,5], John K.C. Chan[6], Henrik Hasle[7], Alexander J. Lazar[8], Sabrina Rossi[9], Andrea Ferrari[10], Jason A. Jarzembowski[11], Kathy Pritchard-Jones[12], D. Ashley Hill[13], Thomas S. Jacques[14,15], Pieter Wesseling[16,17], Dolores H. López Terrada[18], Andreas von Deimling[19,20], Christian P. Kratz[21], Ian A. Cree[22], and Rita Alaggio[9]

ABSTRACT Pediatric tumors are uncommon, yet are the leading cause of cancer-related death in childhood. Tumor types, molecular characteristics, and pathogenesis are unique, often originating from a single genetic driver event. The specific diagnostic challenges of childhood tumors led to the development of the first World Health Organization (WHO) Classification of Pediatric Tumors. The classification is rooted in a multilayered approach, incorporating morphology, IHC, and molecular characteristics. The volume is organized according to organ sites and provides a single, state-of-the-art compendium of pediatric tumor types. A special emphasis was placed on "blastomas," which variably recapitulate the morphologic maturation of organs from which they originate.

Significance: In this review, we briefly summarize the main features and updates of each chapter of the inaugural WHO Classification of Pediatric Tumors, including its rapid transition from a mostly microscopic into a molecularly driven classification systematically taking recent discoveries in pediatric tumor genomics into account.

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Classification of Pediatric CNS tumours

Gliomas, glioneuronal, and neuronal tumors
- *Pediatric-type diffuse low-grade gliomas*
  - Diffuse astrocytoma, *MYB* or *MYBL1*-altered new
  - Angiocentric glioma
  - Polymorphous low-grade neuroepithelial tumor of the young new
  - Diffuse low-grade glioma, MAPK pathway-altered new
- *Pediatric-type diffuse high-grade gliomas defined by H3 status*
  - Diffuse midline glioma, H3 K27-altered
  - Diffuse hemispheric glioma, H3 G34-mutant new
  - Diffuse pediatric-type high-grade glioma, H3-wild-type and IDH-wild-type new
  - Infant-type hemispheric glioma new
- *Circumscribed astrocytic gliomas*
  - Pilocytic astrocytoma
  - High-grade astrocytoma with piloid features new
  - Pleomorphic xanthoastrocytoma
  - Subependymal giant cell astrocytoma
  - Astroblastoma, *MN1*-altered
- *Glioneuronal and neuronal tumors*
  - Ganglioglioma
  - Desmoplastic infantile ganglioglioma/Desmoplastic infantile astrocytoma
  - Dysembryoplastic neuroepithelial tumor
  - Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC)[a] new
  - Diffuse leptomeningeal glioneuronal tumor
  - Multinodular and vacuolating neuronal tumor new
- *Ependymal tumors*
  - Supratentorial ependymoma
  - Supratentorial ependymoma, *ZFTA* fusion-positive
  - Supratentorial ependymoma, *YAP1* fusion-positive new
  - Posterior fossa ependymoma
  - Posterior fossa ependymoma, Group PFA new
  - Posterior fossa ependymoma, Group PFB new
  - Spinal ependymoma, *MYCN*-amplified new
  - Myxopapillary ependymoma

Choroid plexus tumors
- Choroid plexus papilloma
- Atypical choroid plexus papilloma
- Choroid plexus carcinoma

CNS embryonal tumors
- *Medulloblastomas, molecularly defined*
  - Medulloblastoma, WNT-activated
  - Medulloblastoma, SHH-activated & *TP53*-wild-type
  - Medulloblastoma, SHH-activated & *TP53*-mutant
  - Medulloblastoma, non-WNT/non-SHH
- *Medulloblastoma, histologically defined*
  - Medulloblastoma, histologically defined
- *Other CNS embryonal tumors*
  - Atypical teratoid/rhabdoid tumor
  - Cribriform neuroepithelial tumor[a] new
  - Embryonal tumor with multilayered rosettes
  - CNS neuroblastoma, *FOXR2*-activated new
  - CNS tumor with *BCOR* internal tandem duplication new
  - CNS embryonal tumor NEC/NOS

Pineal region tumors
- Pineoblastoma

Melanocytic tumors
- Meningeal melanocytosis and melanomatosis

Tumors of the sellar region
- *Pituitary endocrine tumors*
  - Pituitary adenoma/PitNET
  - Pituitary blastoma new
- *Craniopharyngiomas*
  - Adamantinomatous craniopharyngioma

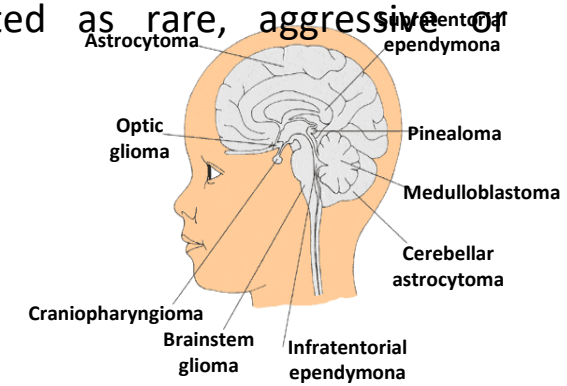
Aims of the Project

Perform a retrospective **comprehensive genetic screening** in all diagnosed **paediatric CNS tumours**, treated at Centro Hospitalar Universitário de São João (Jan 2018-present).

The fundamental goal of this protocol is to evaluate the future value of **a tool to assist in the diagnosis and prognosis**, while supporting the clinician in identifying the best possible therapy.

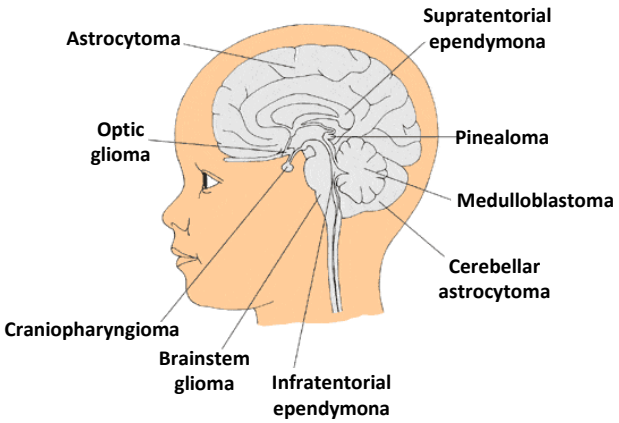
Inclusion criteria are:

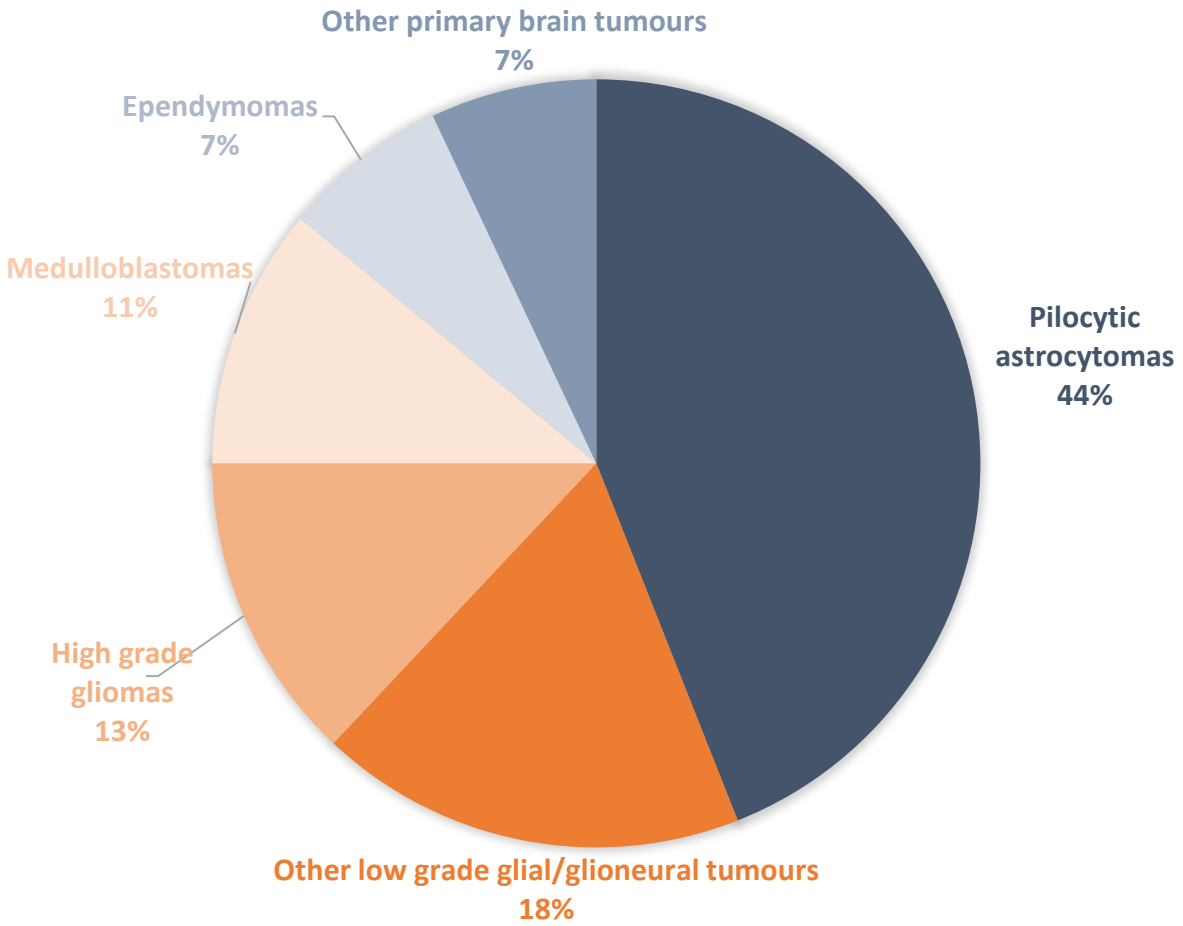
- Age 18 years or below;
- Newly diagnosed brain tumour, with histologic confirmation, irrespective of location;
- Previously diagnosed brain tumour, whenever it presented as rare, aggressive or irresectable disease;
- Availability of tumour material;
- Signed informed consent.



Paediatric CNS tumours study cohort

Paediatric CNS tumours (n=92)





In this cohort, the majority of tumours are **pilocytic astrocytomas** (44%)

Methods: NGS With Oncomine Childhood Cancer Research Assay

Oncomine Childhood Cancer Research Assay Panel - 203 genes

Comprehensive mutation coverage (86)				
ABL1	CSF1R	GATA2	MAP2K2	RAF1
ABL2	CSF3R	GNAQ	MET	RET
ALK	CTNNB1	H3F3A	MPL	RHOA
ACVR1	DAXX	HDAC9	MSH6	SETBP1
AKT1	DNMT3A	HIST1H3B	MTOR	SETD2
ASXL1	EGFR	HRAS	NCOR2	SH2B3
ASXL2	EP300	IDH1	NOTCH1	SH2D1A
BRAF	ERBB2	IDH2	NPM1	SMO
CALR	ERBB3	IL7R	NRAS	STAT3
CBL	ERBB4	JAK1	NT5C2	STAT5B
CCND1	ESR1	JAK2	PAX5	TERT
CCND3	EZH2	JAK3	PDGFRA	TPMT
CCR5	FASLG	KDM4C	PDGFRB	USP7
CDK4	FBXW7	KDR	PIK3CA	ZMYM3
CIC	FGFR2	KIT	PIK3R1	
CREBBP	FGFR3	KRAS	PPM1D	
CRLF2	FLT3	MAP2K1	PTPN11	

CNV (28)	
ALK	IGF1R
BRAF	JAK1
CCND1	JAK2
CDK4	JAK3
CDK6	KIT
EGFR	KRAS
ERBB2	MDM2
ERBB3	MDM4
FGFR1	MET
FGFR2	MYC
FGFR3	MYCN
FGFR4	PDGFRA
GLI1	PIK3CA
GLI2	

Full exon coverage (44)		
APC	GATA3	RUNX1
ARID1A	GNA13	SMARCA4
ARID1B	ID3	SMARCB1
ATRX	IKZF1	SOCS2
CDKN2A	KDM6A	SUFU
CDKN2B	KMT2D	SUZ12
CEBPA	MYOD1	TCF3
CHD7	NF1	TET2
CRLF1	NF2	TP53
DDX3X	PHF6	TSC1
DICER1	PRPS1	TSC2
EBF1	PSMB5	WHSC1
EED	PTCH1	WT1
FAS	PTEN	XIAP
GATA1	RB1	

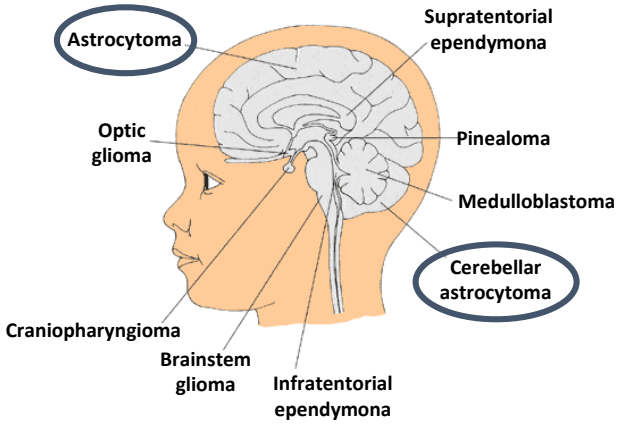
Fusion and expression (97)				
ABL1	FGFR2	MEF2B	NUP214	SSBP2
ABL2	FGFR2	MET	NUP98	STAG2
AFF3	FGFR3	MKL1	NUTM1	STAT6
ALK	FLT3	MLLT10	NUTM2B	TAL1
BCL11B	FOSB	MN1	PAX3	TCF3
BCOR	FUS	MYB	PAX5	TFE3
BCR	GLI1	MYBL1	PAX7	TP63
BRAF	GLIS2	MYH11	PDGFB	TSLP
CAMTA1	HMGA2	MYH9	PDGFRA	TSPAN4
CCND1	JAK2	NCOA2	PDGFRB	UBTF
CIC	KAT6A	NCOR1	PLAG1	USP6
CREBBP	KMT2A	NOTCH1	RAF1	WHSC1
CRLF2	KMT2B	NOTCH2	RANBP17	YAP1
CSF1R	KMT2C	NOTCH4	RECK	ZMYND11
DUSP22	KMT2D	NPM1	RELA	ZNF384
EGFR	LMO2	NR4A3	RET	
ETV6	MAML2	NTRK1	ROS1	
EWSR1	MAN2B1	NTRK2	RUNX1	
FGFR1	MECOM	NTRK3	SS18	

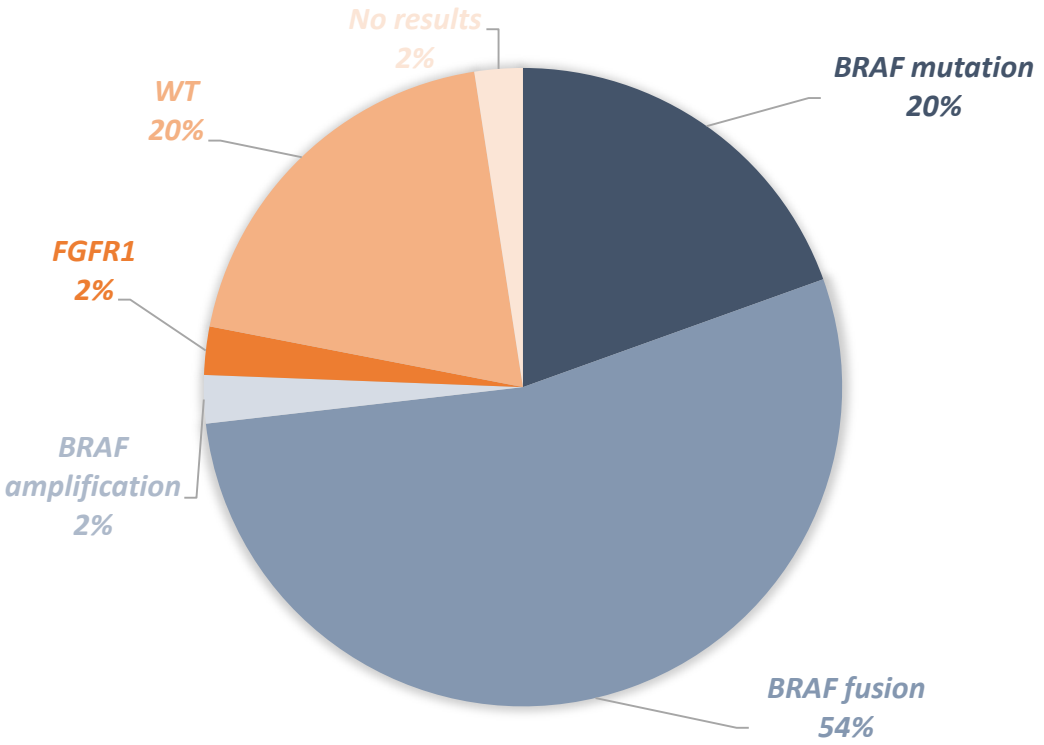
Gene expression
BCL2
BCL6
FGFR1
FGFR4
IGF1R
MET
MYCN
MYC
TOP2A

Research Use Only

Genomic profiling results

Pilocytic Astrocytomas (n=41)

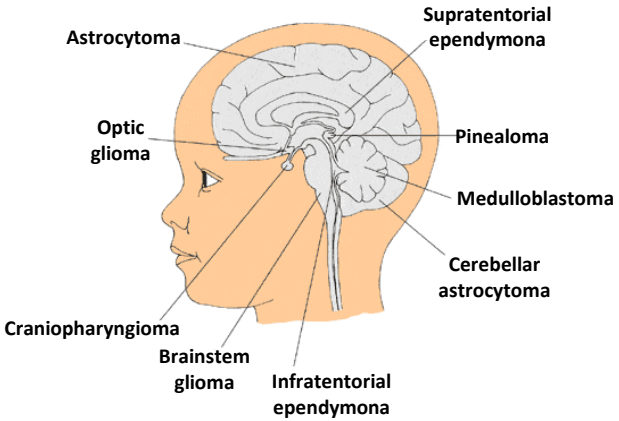


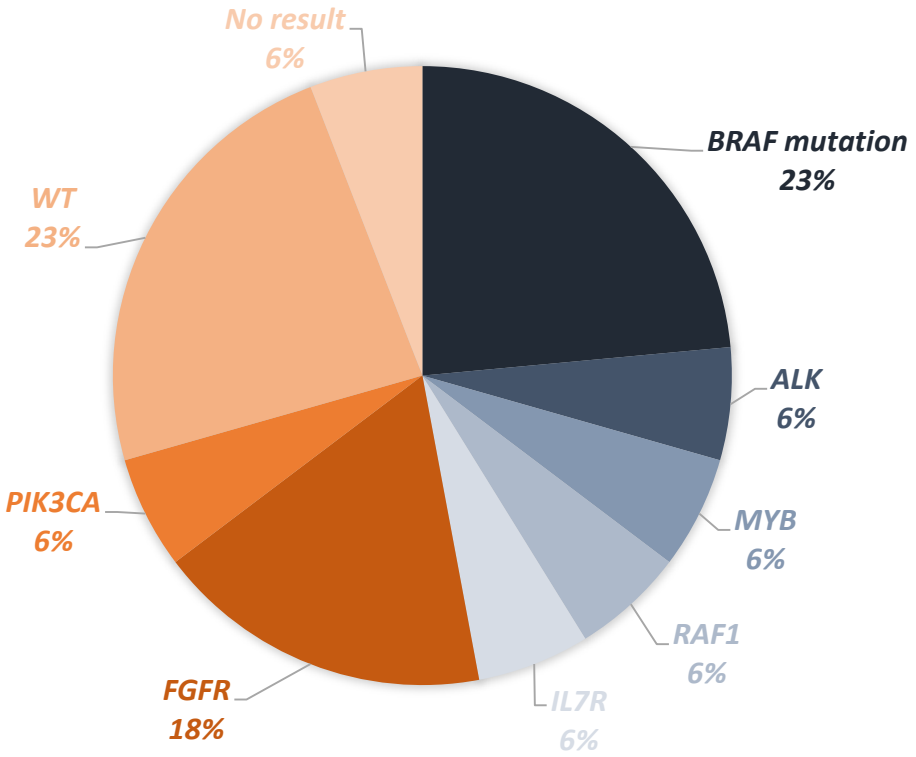


The majority of variants (54%) were ***BRAF* fusions**

Genomic profiling results

Other Low Grade Glial/ Glioneural Tumours (n=17)

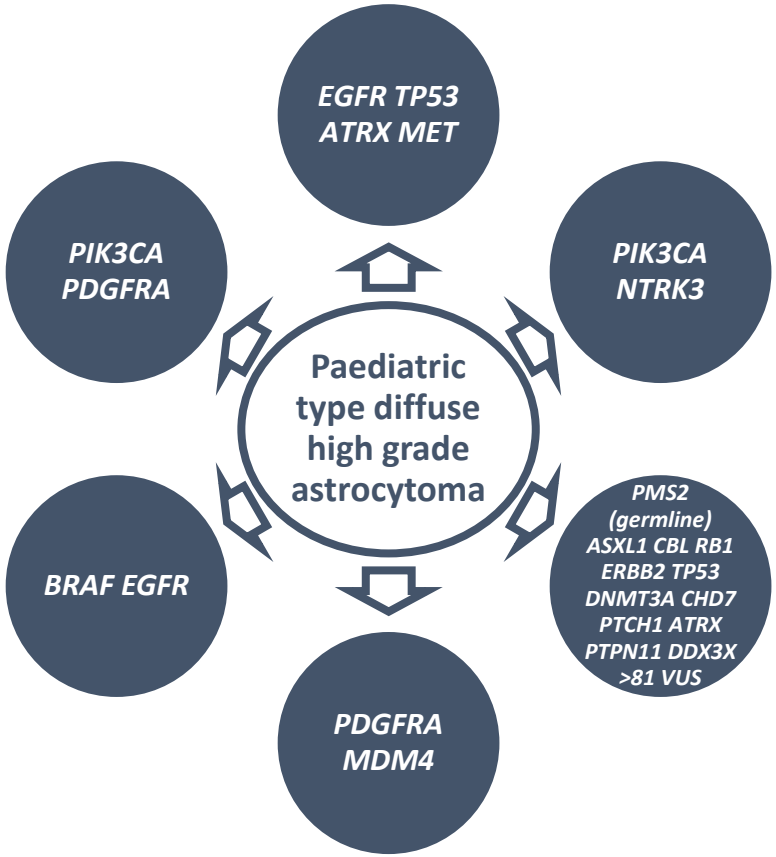


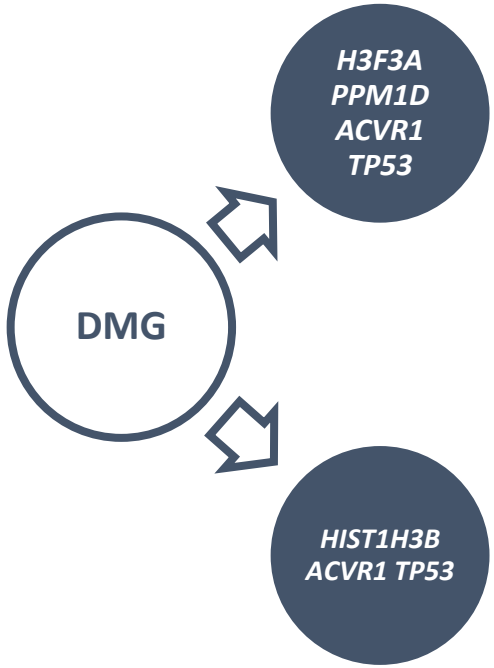


The majority of variants (23%) were ***BRAF* mutations**

Genomic profiling results

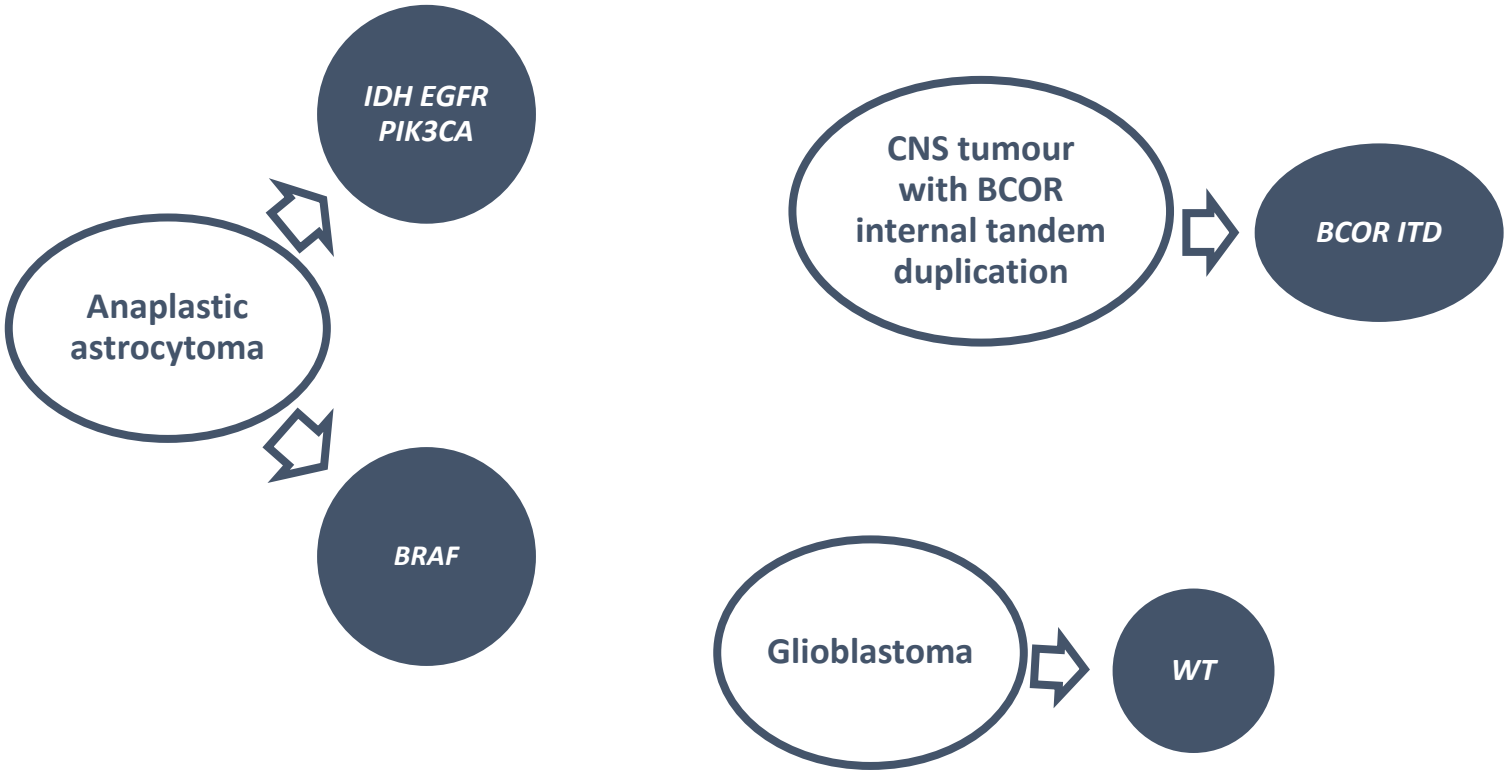
High Grade Gliomas (n=12)





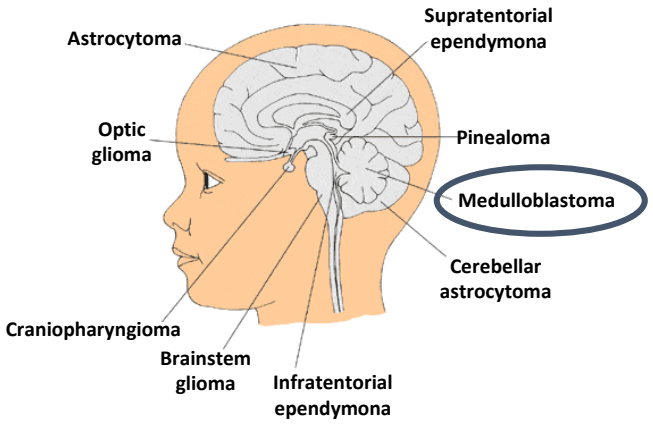
Genomic profiling results

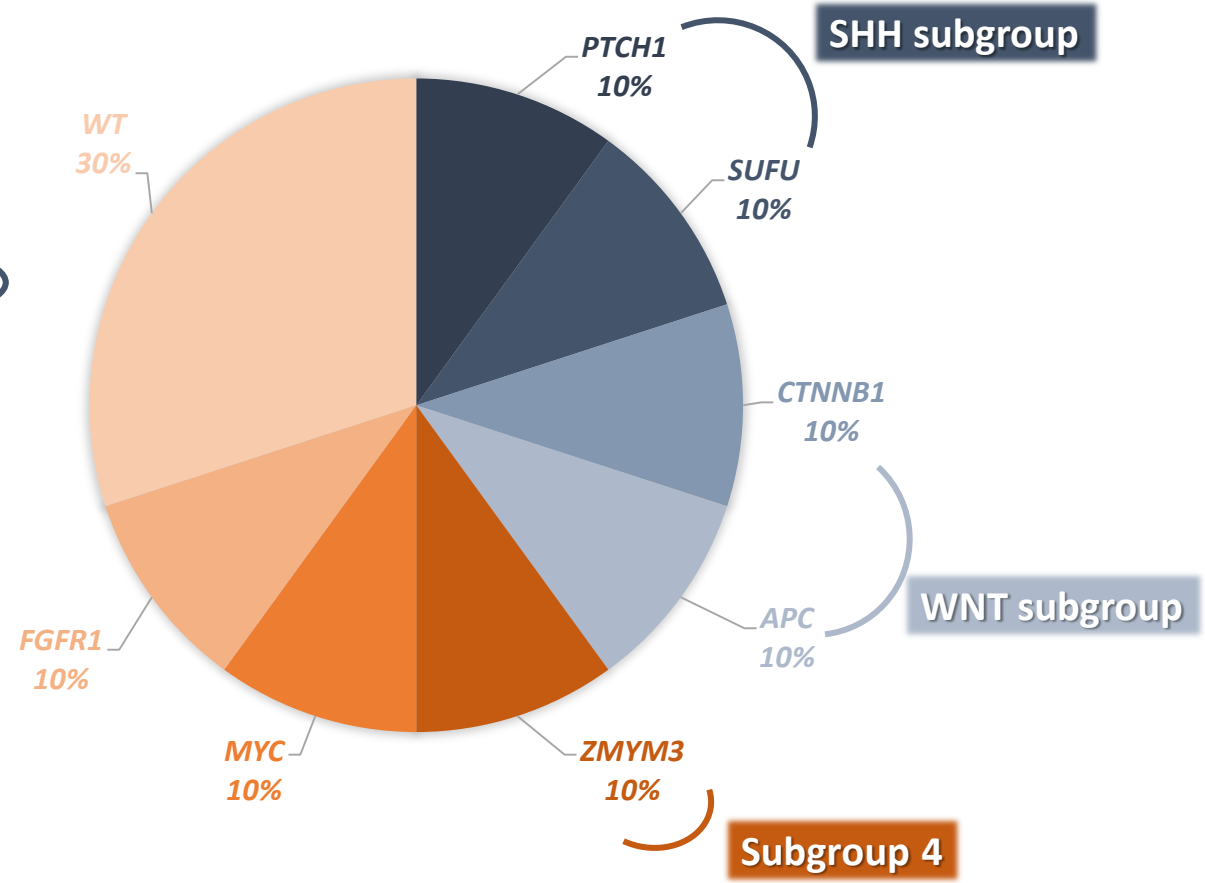
High Grade Gliomas (n=12)



Genomic profiling results

Medulloblastomas (n=10)

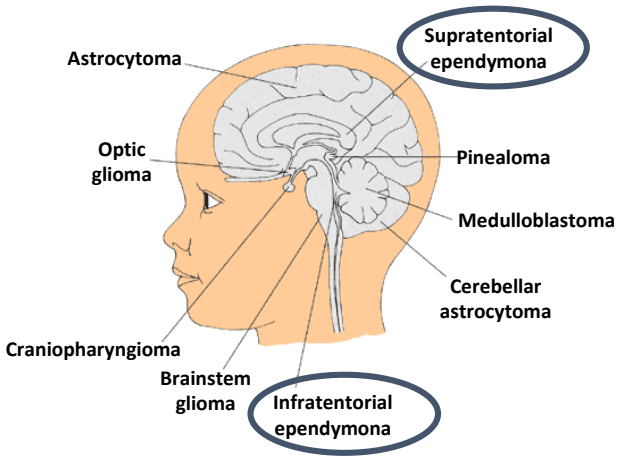


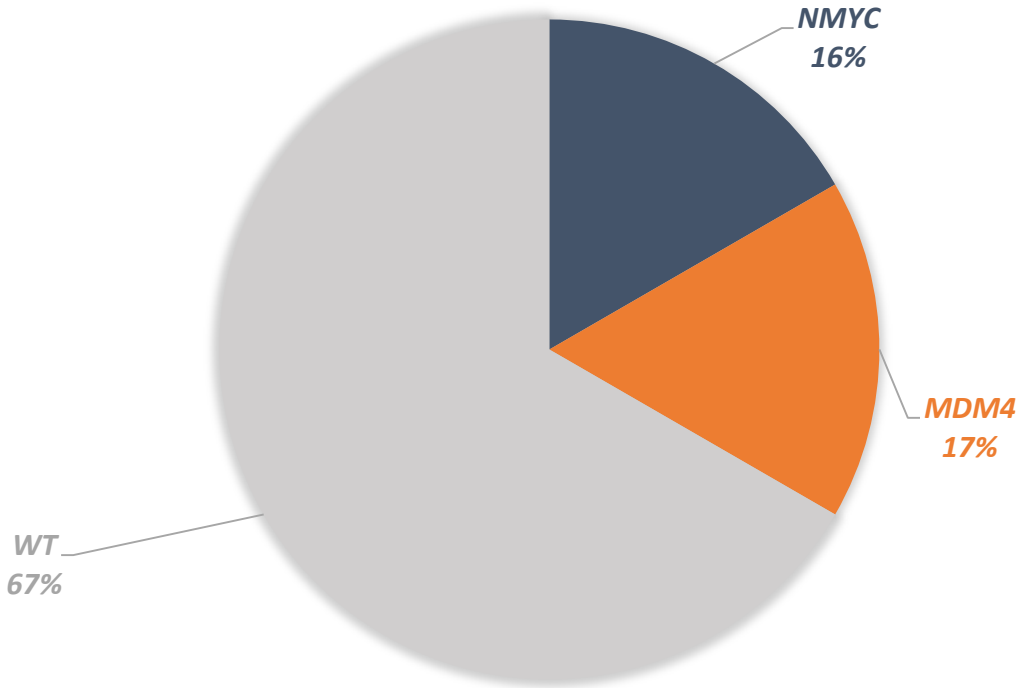


For medulloblastoma, **3 subgroups** (**SHH**, **WNT** and **ZMYM3**) were identified

Genomic profiling results

Ependymomas (n=6)

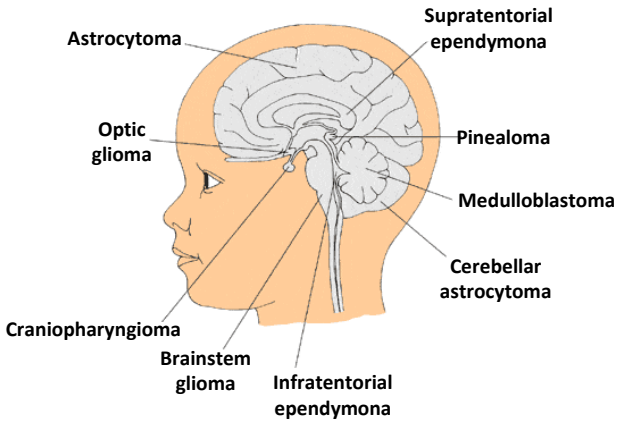


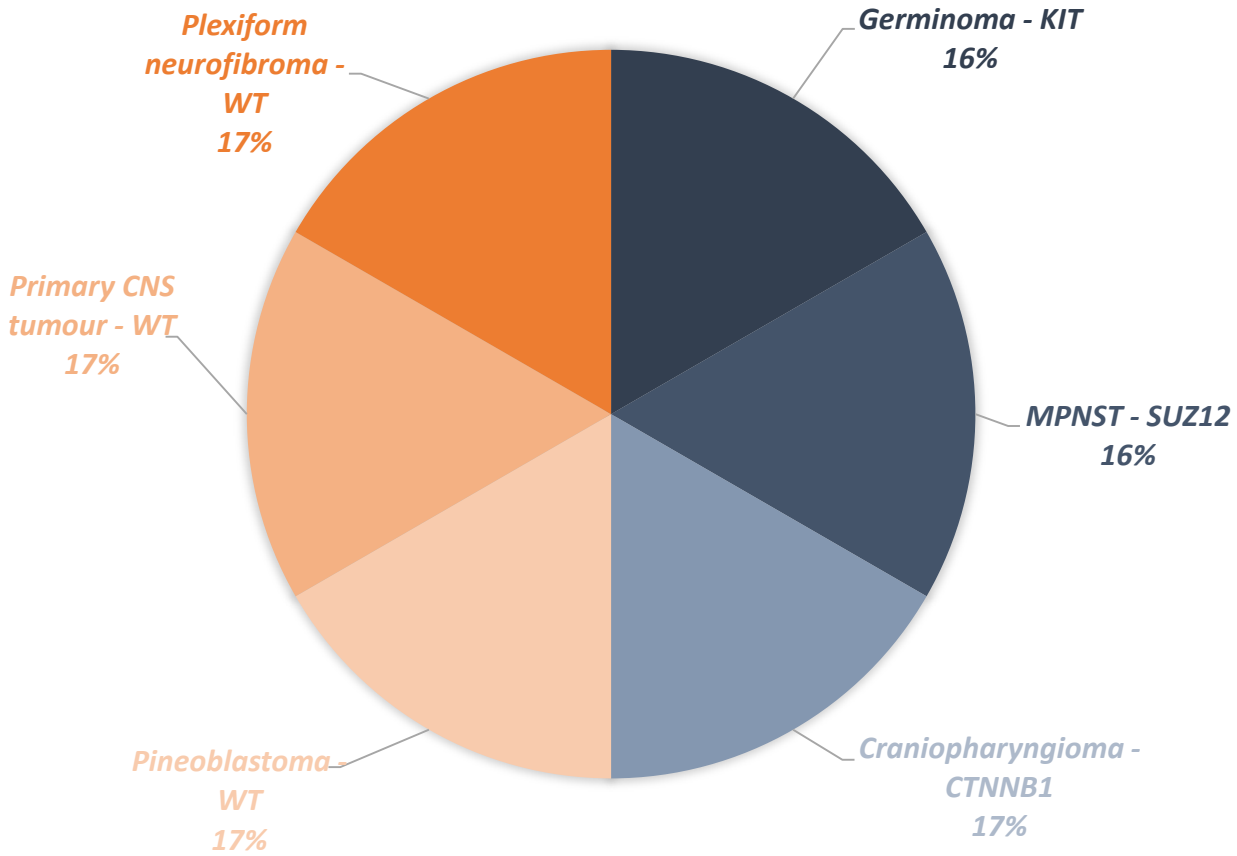


In **33%** of ependymoma cases a relevant genetic variant was identified

Genomic profiling results

Other Brain Tumours (n=6)



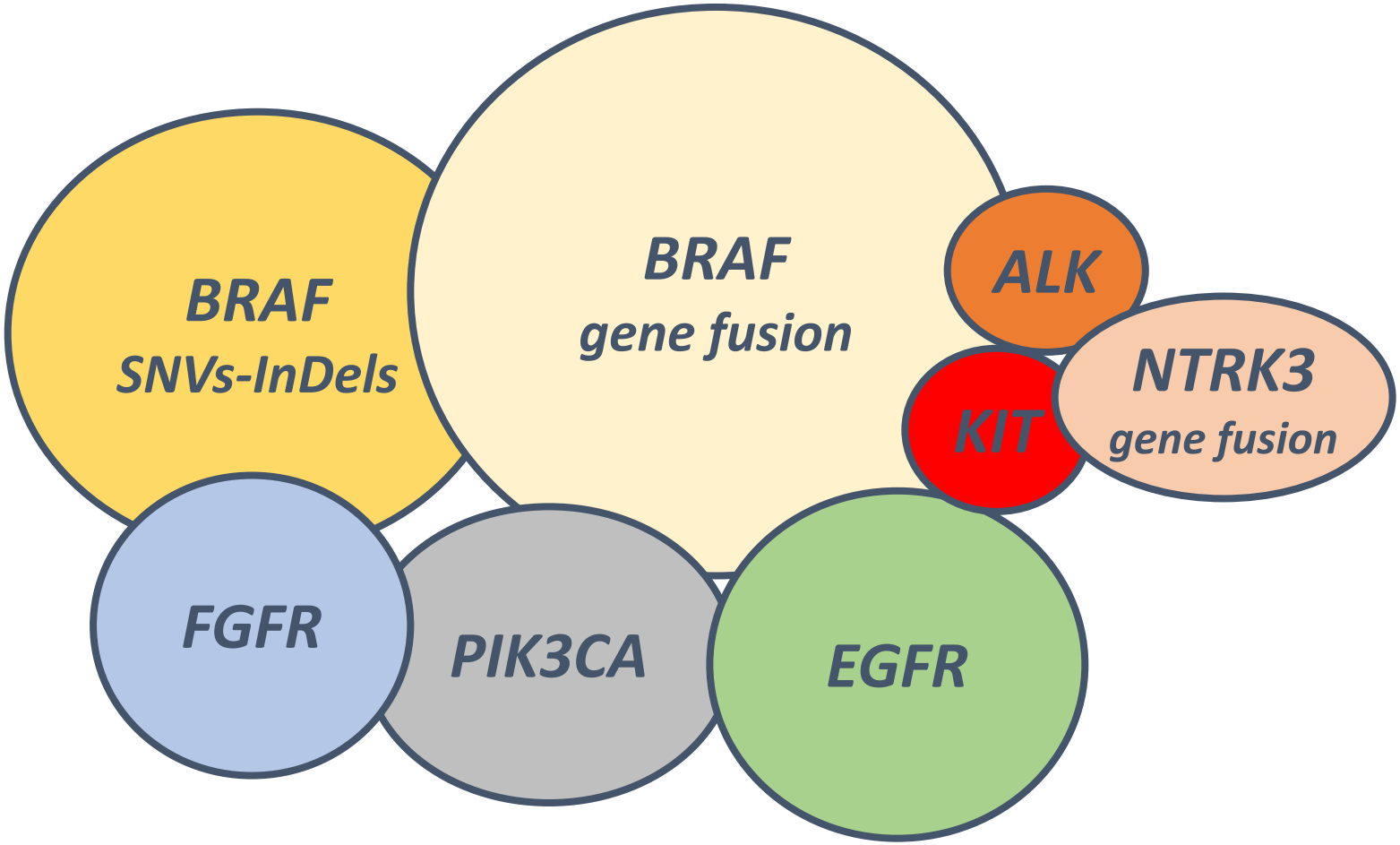


In other brain tumors the are **heterogeneity** in terms of **genomic findings**

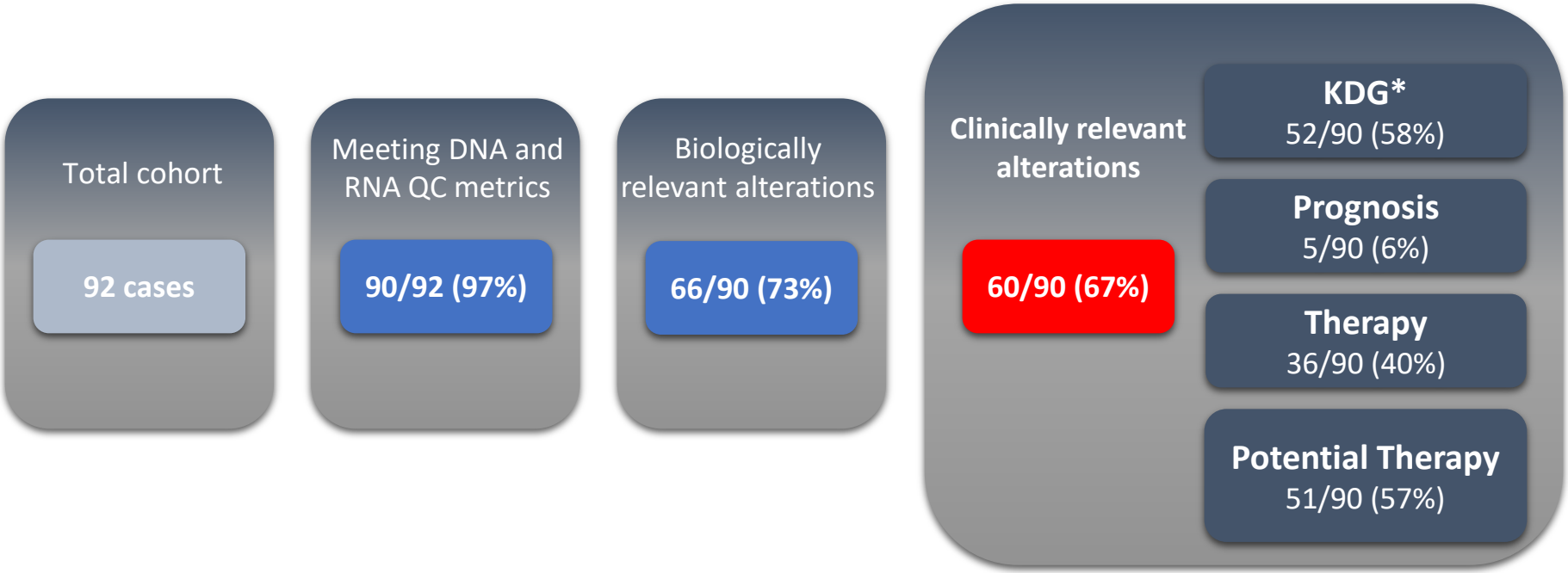
Notable Cases

Diagnosis	Alteration #1	Alteration #2	Alteration #3
PA	KIAA1549-BRAF	PTPRZ1-MET	EGFRVIII
PA	KIAA1549-BRAF	PAX8-PPARg	-
PA	KIAA1549-BRAF	CBL c.1096-1G>T	EGFRVIII
PA	KIAA1549-BRAF	ZCCHC8-WHSC1	C2orf44-FER
Ependymoma	*MYCN*	-	-
PTDHGA	*PMS2* (homoz)	hypermutant	

Alterations with potential actionable value



Study Overall Performance



***KDG:** key diagnostic gene (WHO 2021)*

Research cases with *BRAF*-V600 Mutant tumours

Pt	Age Dx	Age Tr	Sex	Topog.	Histology	option	Tr time	Response	Tox
1	12y	12y	F	IV vent / invasion BST	PA	first line	47 m	**improved**	-
2	8m	4y	F	Sup. selar	PA	rescue	42 m	**mixed**	Rash g1
3	4y	5y	F	Hemisph.	Met. GBM	rescue	2 m	**progr. died**	-
4	2y	12y	F	Sup. selar	PA	rescue	41 m	**improved**	Rash g1
5	4y	4y	M	Sup. selar	GG	first line	43 m	**improved**	Rash g1

Pt: *Patient;* ***Dx:*** *Diagnosis;* ***Tr****: Treatment;* ***Tox****: Toxicity;* ***PA****: Pilocytic Astrocytoma;* ***GBM****: Glioblastoma Multiforme;* ***GG****: Glioma*

Conclusions

- **NGS proved to be a valuable tool in the setting of PBT**, resulting from its significant capacity in revealing clinically meaningful molecular alterations, that can be potentially used for the diagnosis, prognosis and therapy selection
- **Low and high grade gliomas were the tumour types that benefitted more from NGS**, while ependymomas were the tumours less likely to harbour molecular alterations
- In terms of potential therapeutic targets, ***BRAF*** stands out, but ***FGFR*** genes might also be a potentially relevant target in LGG. In high grade gliomas, there were several potential targets (***EGFR***, ***PIK3CA***, ***PDGFRA***, ***MET***) although there is still little data about the efficacy of therapeutic agents directed at these targets

Thank you

PATIENTS AND FAMILIES

ThermoFisher SCIENTIFIC

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Lions Clube Boavista

LIGA PORTUGUESA CONTRA O CANCRO

ENSEMBLE VOCAL PRO MUSICA

Ensemble Vocal Pro Musica

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