Settima edizione di

AIEOP...

...in Lab

G.d.L. Biologia Cellulare Molecolare
AIEOP
ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

The novel predisposition gene *SMARCA4* drives familial Neuroblastoma

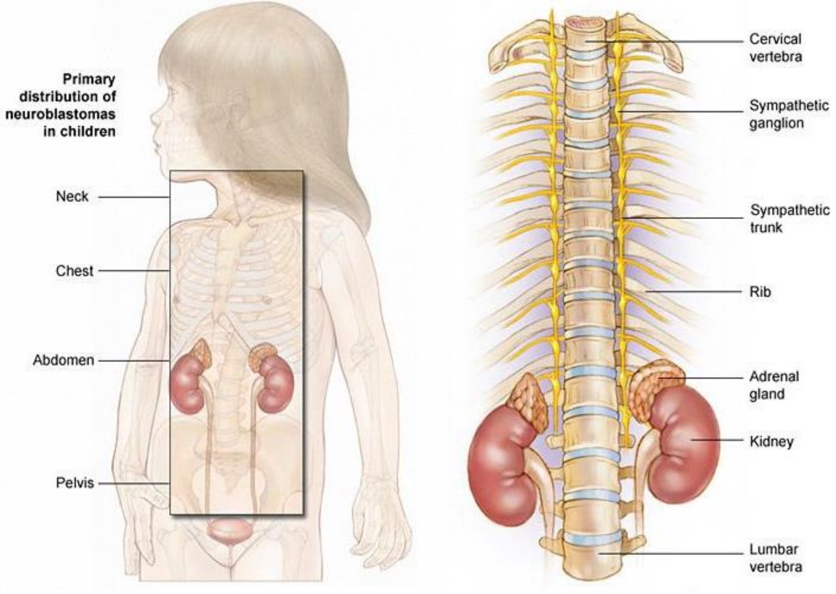
Mariagrazia Cantalupo

Milano, 22 e 23 maggio 2026

The author declares no potential conflicts of interest

FAMILIAL NEUROBLASTOMA

- **Familial Neuroblastoma** accounts for approximately **1-2%** of all cases
- It is inherited in an **Autosomal Dominant** manner with incomplete penetrance
- The genes most frequently associated with familial neuroblastoma are ***PHOX2B*** (implicated in ≈ 10% of familial cases) and ***ALK*** (implicated in ≈ 75% of familial cases)
- An estimated 15% of cases are currently genetically unexplained



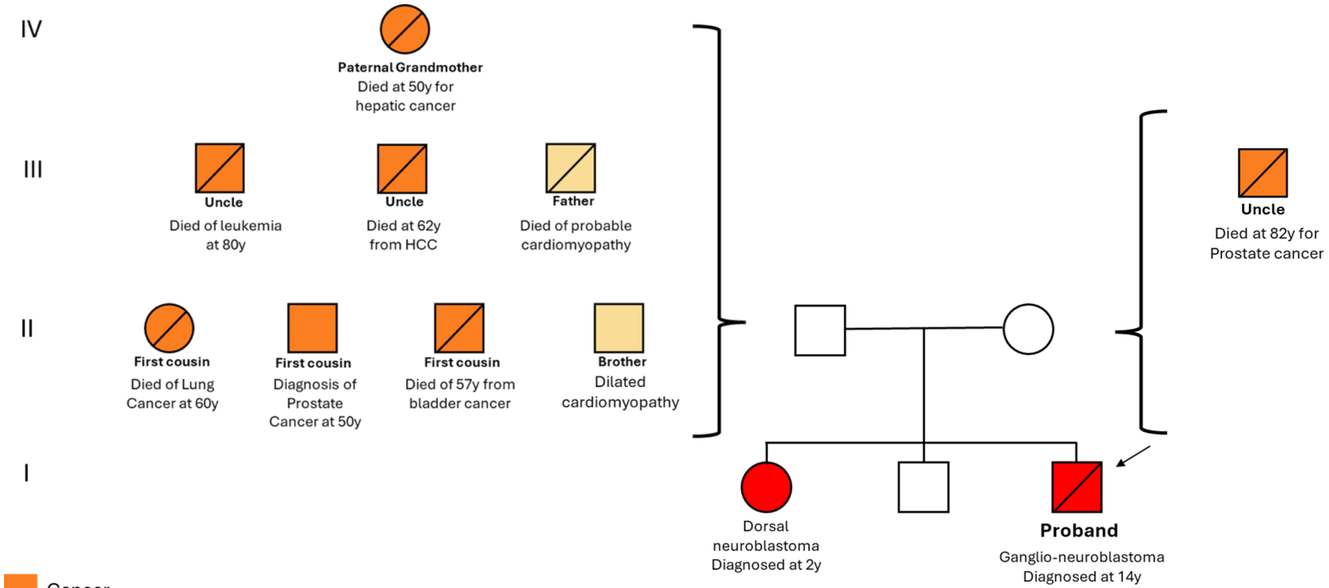
A CASE OF FAMILIAL NEUROBLASTOMA

🟧 Cancer

🟥 Neuroblastoma

🟨 Non oncological patient

A CASE OF FAMILIAL NEUROBLASTOMA



IV

Paternal Grandmother
Died at 50y for hepatic cancer

III

Uncle
Died of leukemia at 80y

Uncle
Died at 62y from HCC

Father
Died of probable cardiomyopathy

Uncle
Died at 82y for Prostate cancer

II

First cousin
Died of Lung Cancer at 60y

First cousin
Diagnosis of Prostate Cancer at 50y

First cousin
Died of 57y from bladder cancer

Brother
Dilated cardiomyopathy

I

Dorsal neuroblastoma Diagnosed at 2y

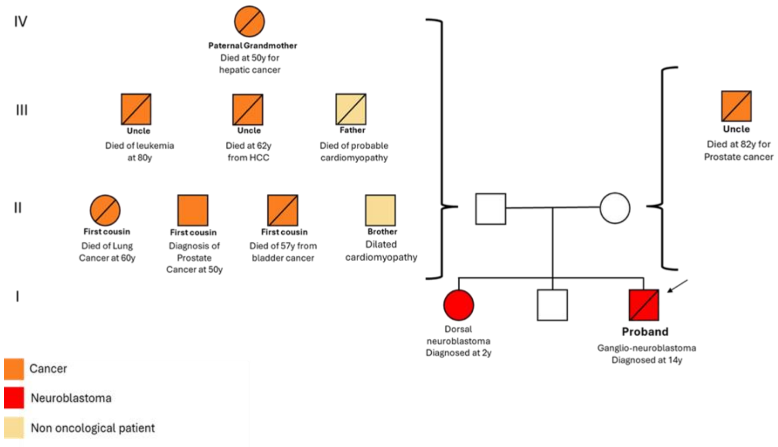
Proband
Ganglio-neuroblastoma Diagnosed at 14y

🟧 Cancer

🟥 Neuroblastoma

🟨 Non oncological patient

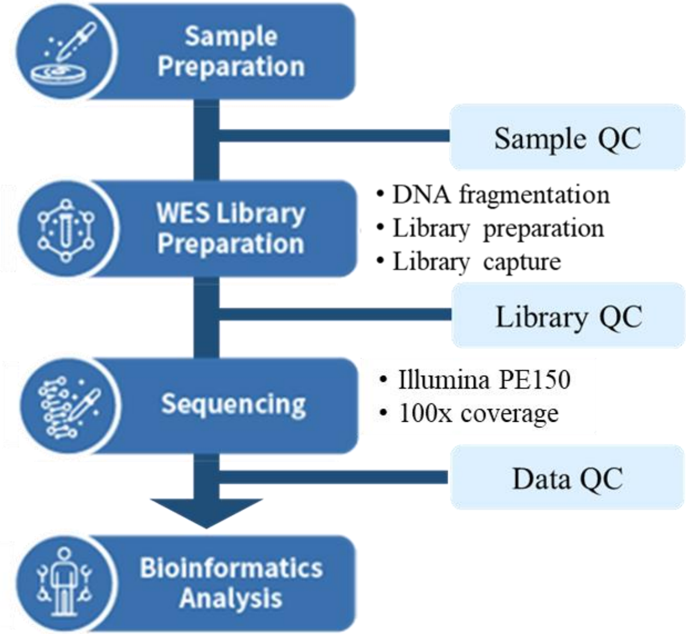
A CASE OF FAMILIAL NEUROBLASTOMA



- The proband was enrolled in the **PREME** program
- **Whole-Exome Sequencing** (WES) was performed on:
  - **Germline DNA** (father, mother, siblings)
  - **Primary tumor mass**
  - **Recurrent tumor mass**

GENETIC INVESTIGATION

Whole exome sequencing:

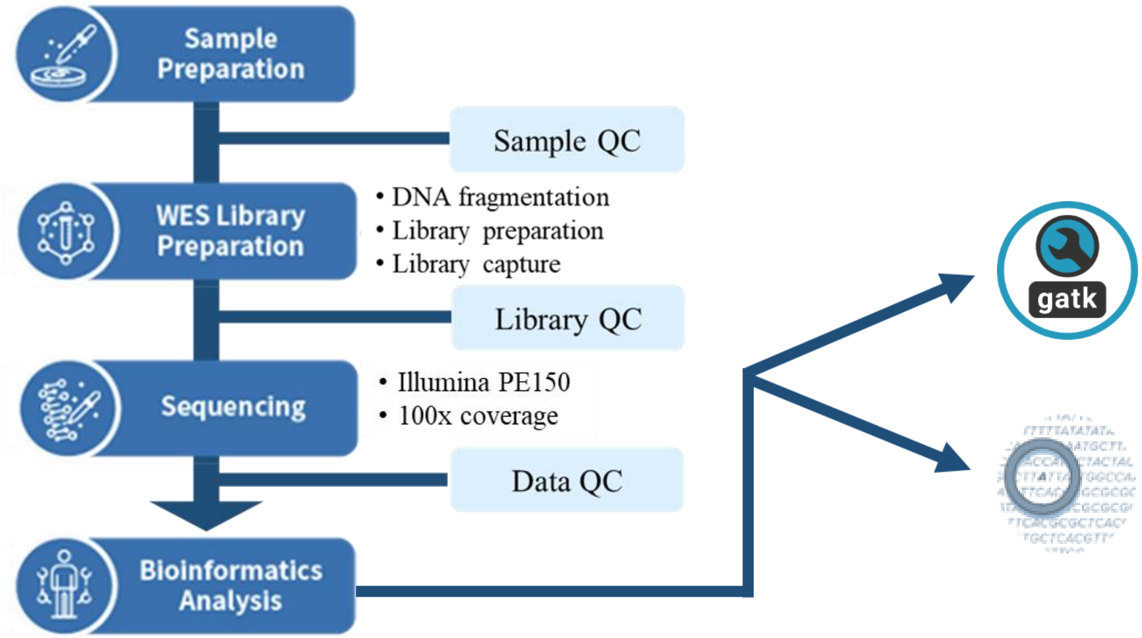


- DNA fragmentation
- Library preparation
- Library capture

- Illumina PE150
- 100x coverage

GENETIC INVESTIGATION

Whole exome sequencing:



Sample Preparation

Sample QC

WES Library Preparation

- DNA fragmentation
- Library preparation
- Library capture

Library QC

Sequencing

- Illumina PE150
- 100x coverage

Data QC

Bioinformatics Analysis

Variant Calling

SNV: gatk (v4.2.0.0)

CNV: cnvkit (v0.9.9)

Annotation

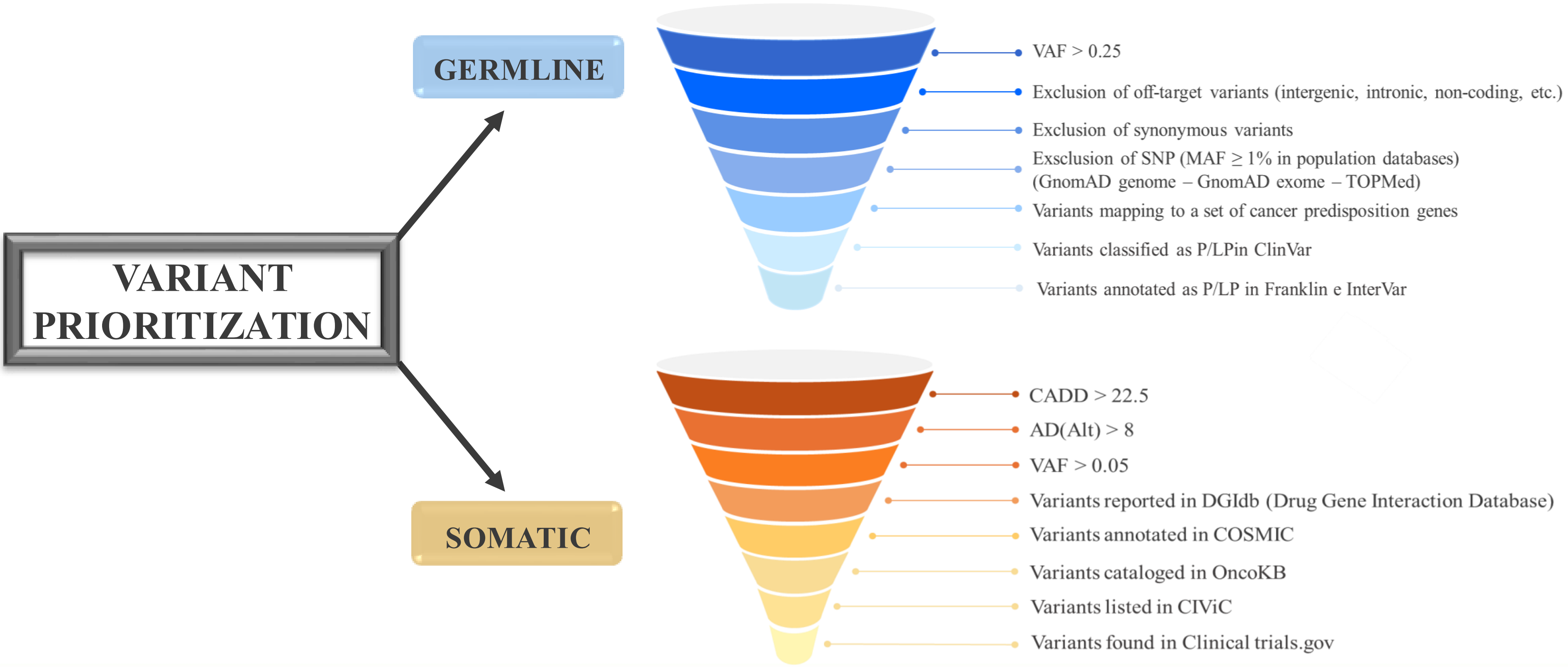
SNV: Annovar (v2019-10-24), vep (v99)

CNV: AnnotSV (v3.0.7)

GENETIC INVESTIGATION

VARIANT PRIORITIZATION

GENETIC INVESTIGATION



VARIANT PRIORITIZATION

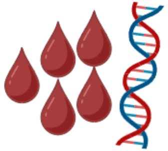
GERMLINE

- VAF > 0.25
- Exclusion of off-target variants (intergenic, intronic, non-coding, etc.)
- Exclusion of synonymous variants
- Exsclusion of SNP (MAF ≥ 1% in population databases) (GnomAD genome – GnomAD exome – TOPMed)
- Variants mapping to a set of cancer predisposition genes
- Variants classified as P/LPin ClinVar
- Variants annotated as P/LP in Franklin e InterVar

SOMATIC

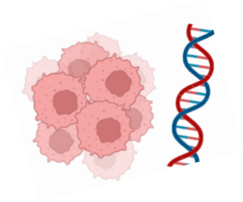
- CADD > 22.5
- AD(Alt) > 8
- VAF > 0.05
- Variants reported in DGIdb (Drug Gene Interaction Database)
- Variants annotated in COSMIC
- Variants cataloged in OncoKB
- Variants listed in CIViC
- Variants found in Clinical trials.gov

RESULTS OF GENETIC INVESTIGATION



GERMLINE

- missense variant in *SMARCA4* (NM_003072.5:c.2779T>G; p.Phe927Val)



SOMATIC

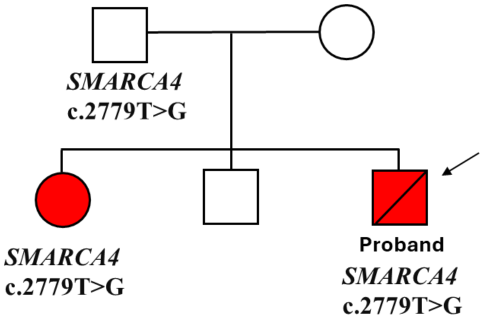
1. **PRIMARY TUMOR**

- Chr19 deletion (start: 9946018; end: 12076026)

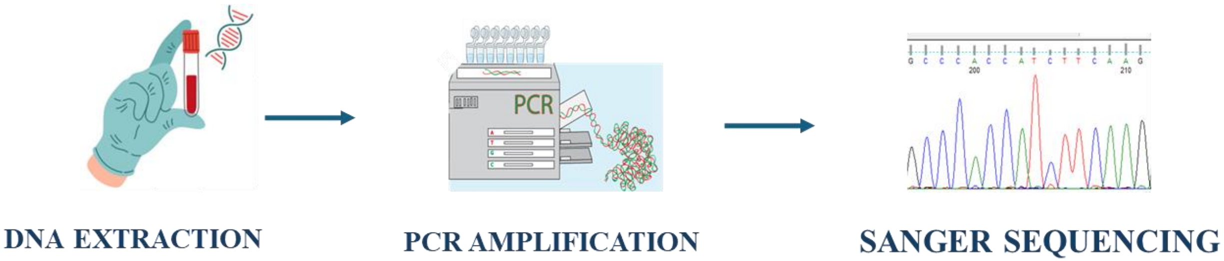
2. **RECURRENT MASS**

- nonsense mutation in *TP53* (NM_001126118:c.155G>A; p.Trp52X)

VALIDATION OF *SMARCA4* GERMLINE VARIANT



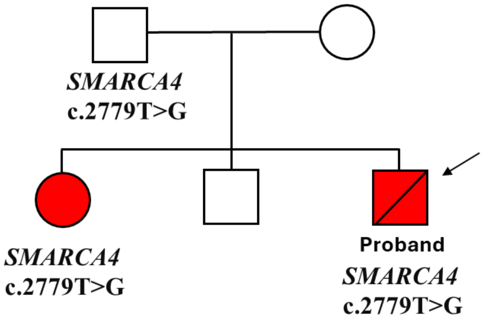
VALIDATION OF *SMARCA4* GERMLINE VARIANT



DNA EXTRACTION

PCR AMPLIFICATION

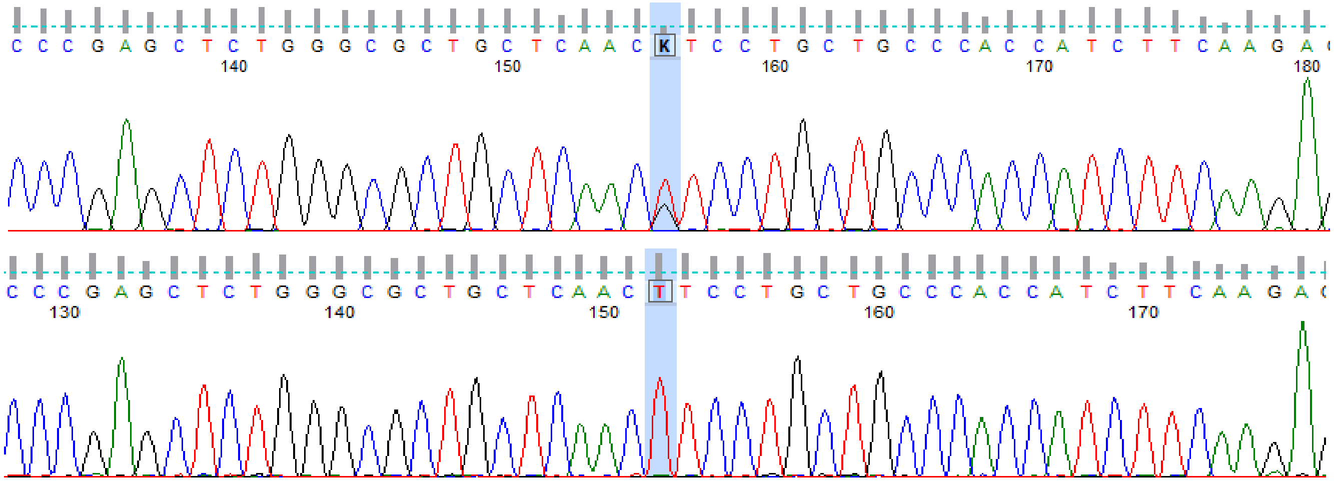
SANGER SEQUENCING



SMARCA4
c.2779T>G

SMARCA4
c.2779T>G

Proband
SMARCA4
c.2779T>G



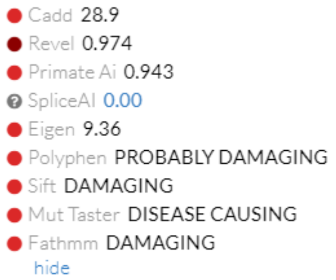
Proband, father, sister:
***SMARCA4* c.2779T>G**

Mother and unaffected brother: **WT**

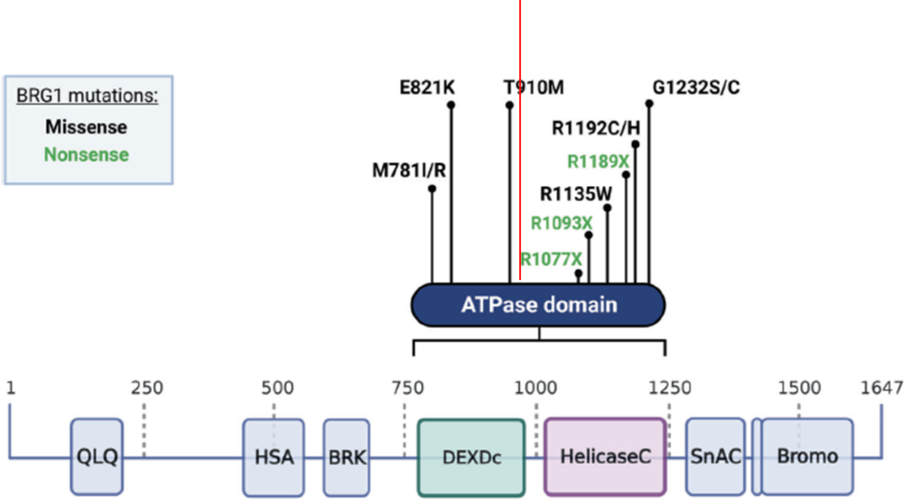
CLASSIFICATION OF *SMARCA4* VARIANT

SMARCA4, chr19:11132563-T-G
NM_003072.5;c.2779T>G; p.Phe927Val
missense variant

OMIM: Rhabdoid tumor predisposition syndrome 2; Coffin-Siris syndrome 4
Pattern: Dominant
ClinVar: not reported
Intervar: LP
Franklin: LP



- ● Cadd 28.9
- ● Revel 0.974
- ● Primate Ai 0.943
- ? SpliceAI 0.00
- ● Eigen 9.36
- ● Polyphen PROBABLY DAMAGING
- ● Sift DAMAGING
- ● Mut Taster DISEASE CAUSING
- ● Fathmm DAMAGING



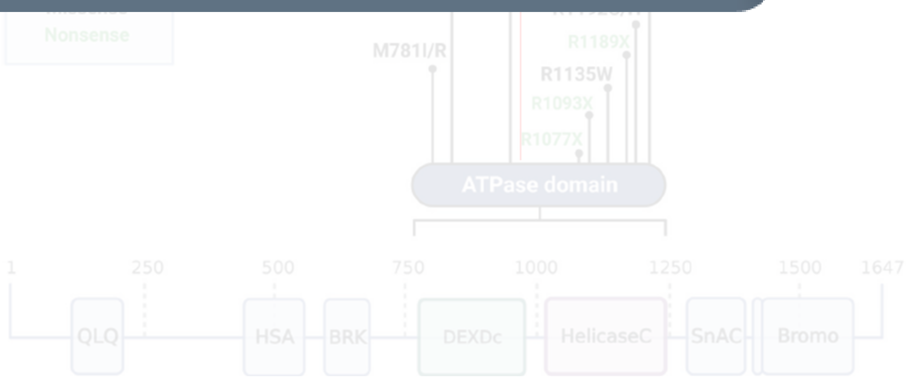
CLASSIFICATION OF *SMARCA4* VARIANT

SMARCA4, chr19:11132563-T-G
NM_003072.5;c.2779T>G; p.Phe927Val
mis

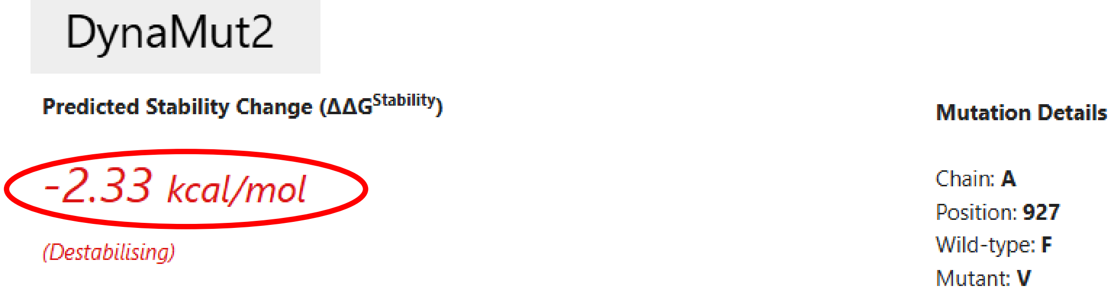
- Cadd 28.9
- Revel 0.974
- Primate Ai 0.943
- SpliceAI 0.00
- Eigen 9.36
- Polyphen PROBABLY DAMAGING

The variant was classified as <u>Likely Pathogenic</u> according to ACMG guidelines

OMIM: Rhabdoid tumor predisposition syndrome 2; Coffin-Siris syndrome 4
Pattern: Dominant
ClinVar: not reported
Intervar: LP
Franklin: LP



IN SILICO FUNCTIONAL IMPACT PREDICTION OF *SMARCA4* VARIANT



DynaMut2

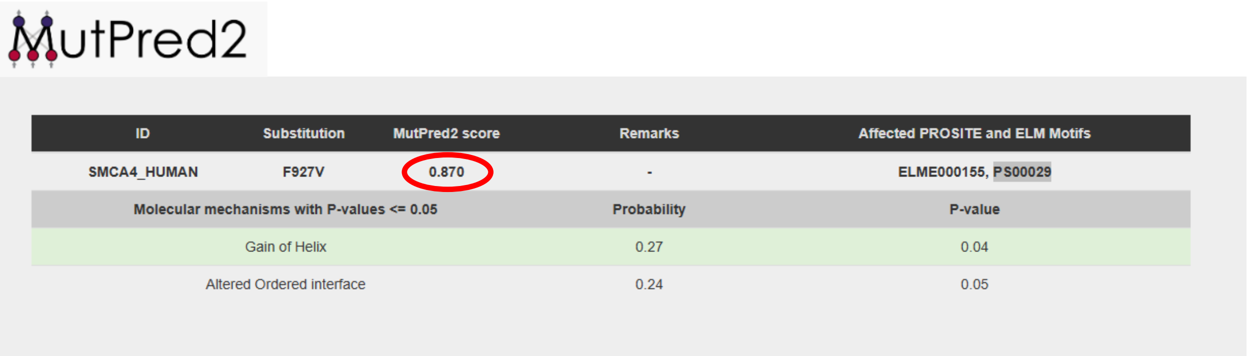
Predicted Stability Change ($\Delta\Delta G^{Stability}$)

-2.33 kcal/mol

(Destabilising)

Mutation Details

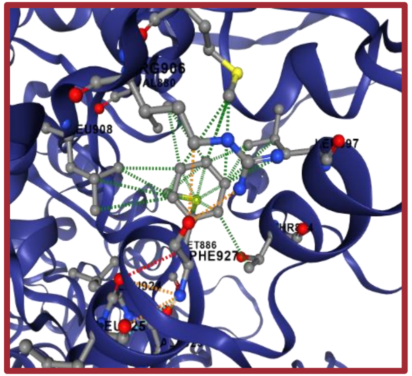
Chain: **A**
Position: **927**
Wild-type: **F**
Mutant: **V**



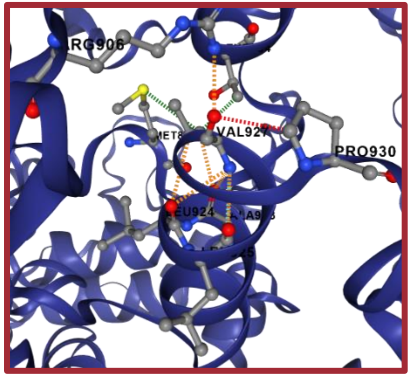
MutPred2

ID	Substitution	MutPred2 score	Remarks	Affected PROSITE and ELM Motifs
SMCA4_HUMAN	F927V	0.870	-	ELME000155, PS00029
Molecular mechanisms with P-values <= 0.05			**Probability**	**P-value**
Gain of Helix			0.27	0.04
Altered Ordered interface			0.24	0.05

WT



F927V



IN SILICO FUNCTIO… IMPACT PREDICTION OF S… RIANTS

DynaMu

Predicted Stability Change ($\Delta\Delta G^{Stability}$)

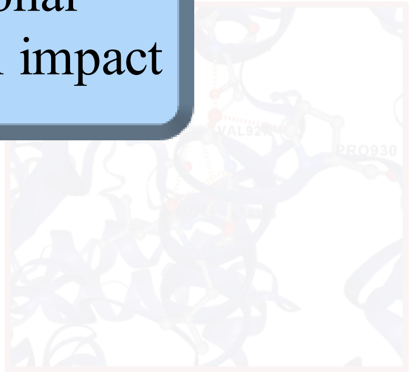
-2.33 kcal/mol

(Destabilising)

Wild-type: **F**

WT

27V



Ongoing studies are currently focused on the functional characterization of this variant to elucidate its biological impact

MutPred

ID	Substitution	MutPred2 score	Remarks	Affected PROSITE and ELM Motifs
SMCA4_HUMAN	F927V	0.870	-	ELME000155, PS00029

Molecular mechanisms with P-values <= 0.05	Probability	P-value
Gain of Helix	0.27	0.04
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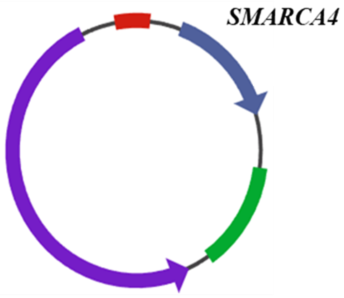
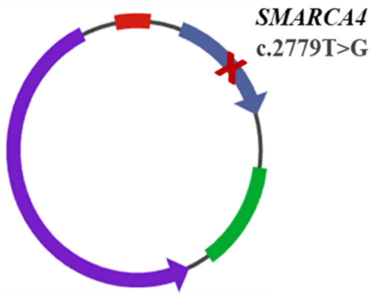
SMARCA4 VARIANT OVEREXPRESSION WORKFLOW



VS



Study the impact of the variant on the protein

- IP + ATPase Assay
- Immunofluorescence

Evaluation of complex integrity and remodeling activity

- Co-IP
- ChIP-Seq
- ATAC-Seq
- RNA-Seq

Tumorigenicity assays

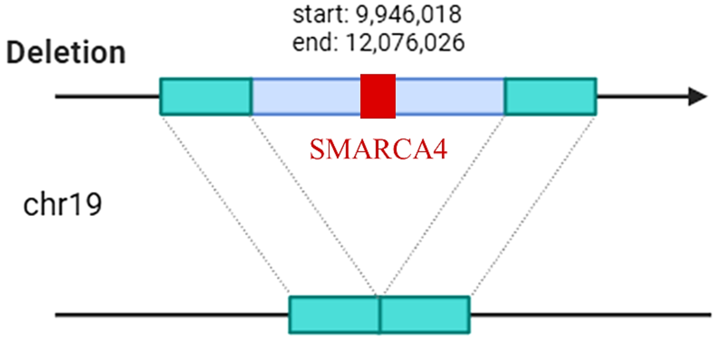
- Proliferation assay
- Invasion assay
- Neurobasal assay
- Scratch assay

RESULTS OF GENETIC INVESTIGATION: SOMATIC

PRIMARY TUMOR

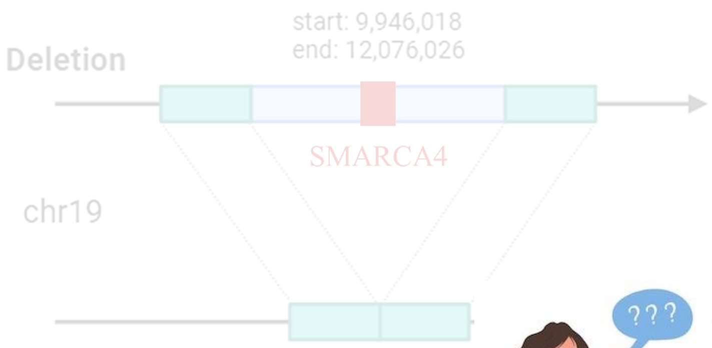
chr19 deletion (start: 9946018; end: 12076026)

The large deletion on chr19 is of critical interest as it encompasses the entire *SMARCA4* tumor suppressor gene (chr19: 11,071,598 - 11,176,071)



RESULTS OF GENETIC INVESTIGATION: SOMATIC

PRIMARY TUMOR
chr19 deletion (start: 9946018; end: 12076026)

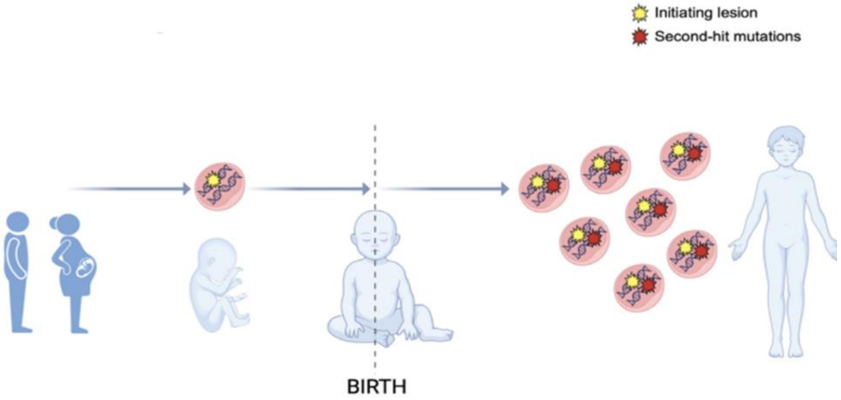


The large deletion on chr19 is of critical interest as it encompasses the entire *SMARCA4* tumor suppressor gene (chr19: 11,071,598 - 11,176,071)

Does this represent the somatic **second-hit event** responsible of tumorigenesis?

THE TWO-HIT HYPOTHESIS IN TUMORIGENESIS

Knudson Two-Hit Hypothesis suggests that the germline loss-of-function allele *SMARCA4* was complemented by a subsequent somatic event, the «second hit», to fully inactivate the tumor suppressor pathway and initiate malignancy



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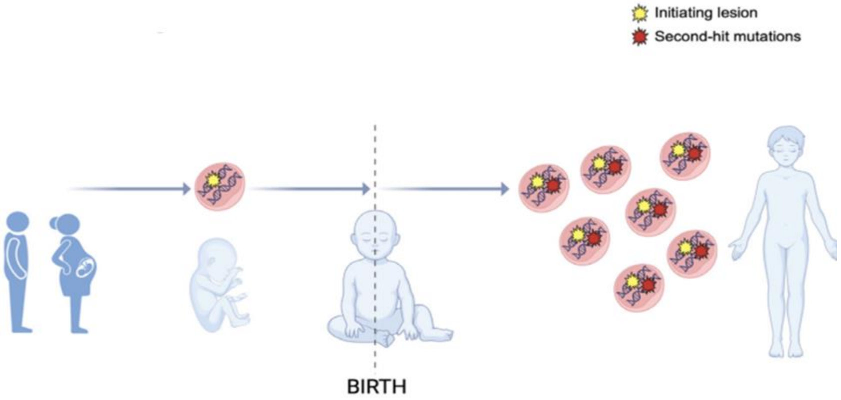
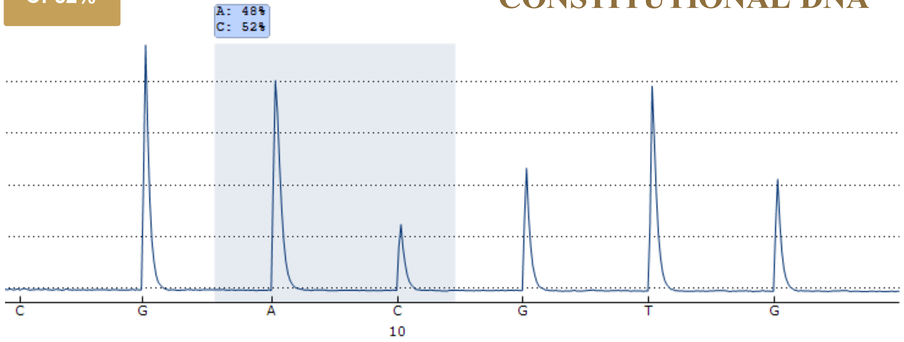


PYROSEQUENCING ANALYSIS
(P. Perri Laboratory)

PYROSEQUENCING ANALYSIS

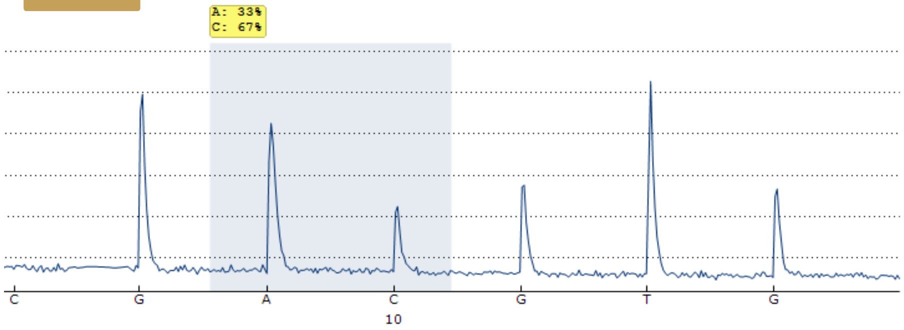
A: 48%
C: 52%

CONSTITUTIONAL DNA



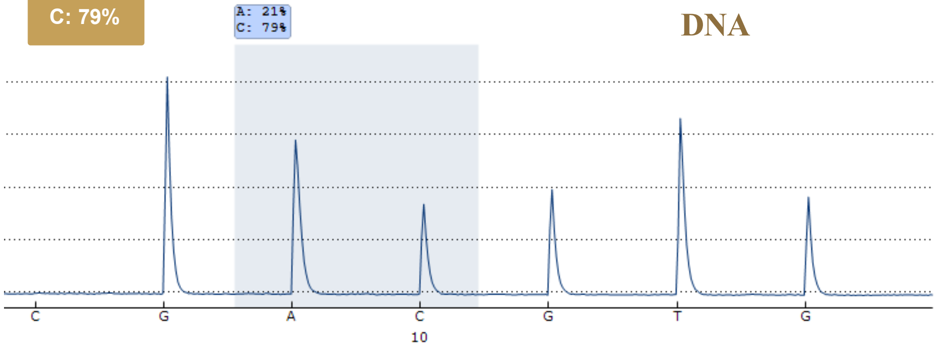
A: 33%
C: 67%

PRIMARY TUMOR DNA



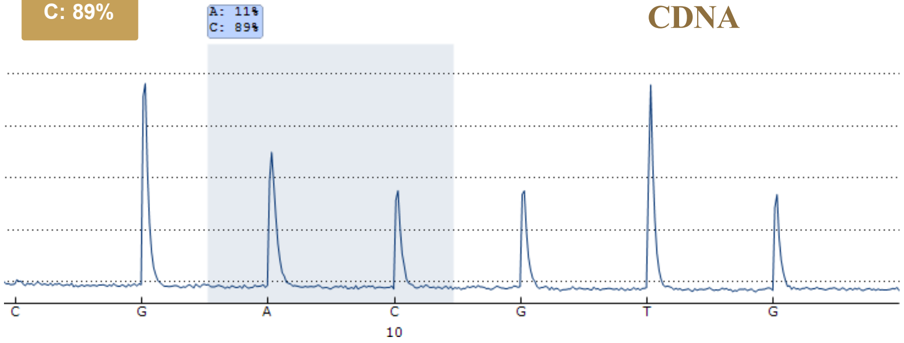
A: 21%
C: 79%

POST-TREATMENT TUMOR DNA



A: 11%
C: 89%

POST-TREATMENT TUMOR CDNA



RECURRENT MASS

- nonsense mutation in *TP53* (NM_001126118:c.155G>A; p.Trp52X)

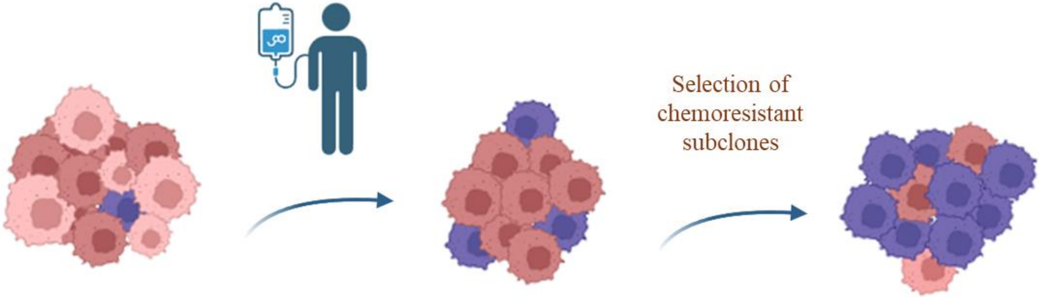
RECURRENT MASS

- nonsense mutation in *TP53* (NM_001126118:c.155G>A; p.Trp52X)

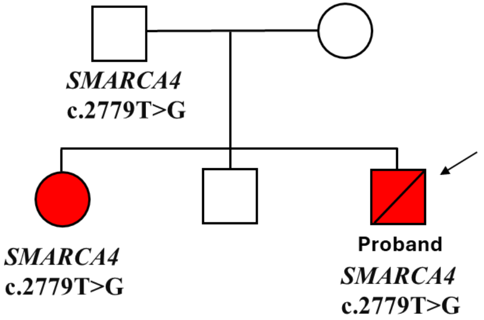
→ **CANCER DRIVER MUTATION IN TP53**

↓

Clonal selection of *TP53* mutated tumor cells

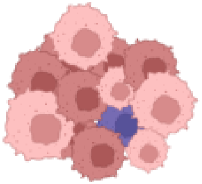


Germline variant
***SMARCA4* c.2779T>G**

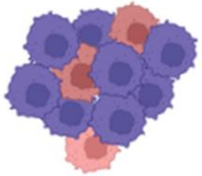


Second hit

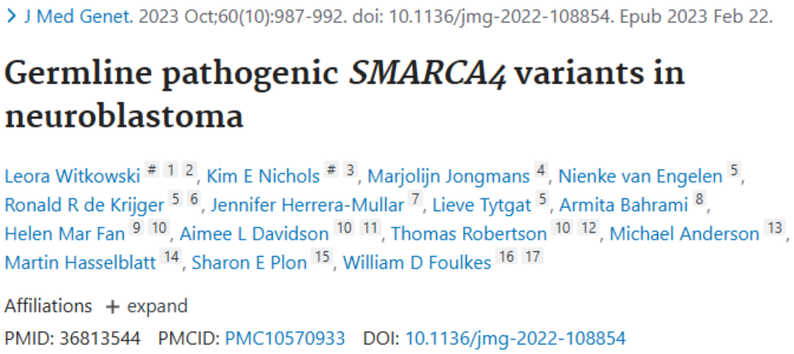
Loss of WT allele



Enrichment of *TP53* mutant clones under selective pressure



SMARCA4 AND NEUROBLASTOMA: REVIEW OF THE EXISTING LITERATURE



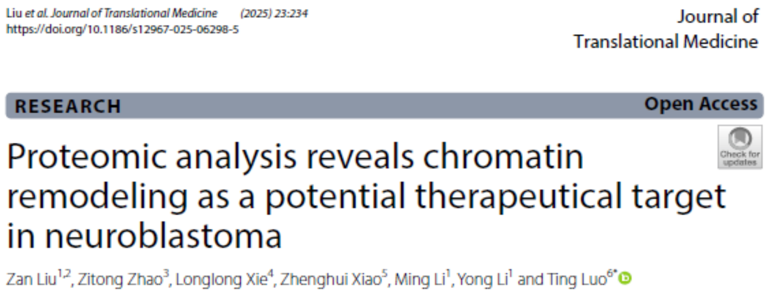
› J Med Genet. 2023 Oct;60(10):987-992. doi: 10.1136/jmg-2022-108854. Epub 2023 Feb 22.

Germline pathogenic *SMARCA4* variants in neuroblastoma

Leora Witkowski [#] [1] [2], Kim E Nichols [#] [3], Marjolijn Jongmans [4], Nienke van Engelen [5], Ronald R de Krijger [5] [6], Jennifer Herrera-Mullar [7], Lieve Tytgat [5], Armita Bahrami [8], Helen Mar Fan [9] [10], Aimee L Davidson [10] [11], Thomas Robertson [10] [12], Michael Anderson [13], Martin Hasselblatt [14], Sharon E Plon [15], William D Foulkes [16] [17]

Affiliations + expand

PMID: 36813544 PMCID: PMC10570933 DOI: 10.1136/jmg-2022-108854



Liu *et al. Journal of Translational Medicine* (2025) 23:234
https://doi.org/10.1186/s12967-025-06298-5

Journal of
Translational Medicine

RESEARCH **Open Access**

Proteomic analysis reveals chromatin remodeling as a potential therapeutical target in neuroblastoma

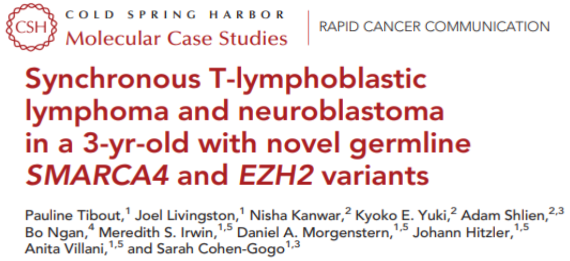
Zan Liu[1,2], Zitong Zhao[3], Longlong Xie[4], Zhenghui Xiao[5], Ming Li[1], Yong Li[1] and Ting Luo[6*]

eBioMedicine
2023;87: 104395

Inherited rare variants in homologous recombination and neurodevelopmental genes are associated with increased risk of neuroblastoma

Ferdinando Bonfiglio,[a,b] Vito Alessandro Lasorsa,[a,c] Sueva Cantalupo,[a,c] Giuseppe D'Alterio,[a,d] Vincenzo Aievola,[a,c] Angelo Boccia,[a] Martina Ardito,[e,j] Simone Furini,[f] Alessandra Renieri,[f] Martina Morini,[e,j] Sabine Stainczyk,[g,h,i] Frank Westermann,[g,h,i] Giovanni Paolella,[a,c] Alessandra Eva,[e] Achille Iolascon,[a,c] and Mario Capasso[a,c,]*

oa



COLD SPRING HARBOR
Molecular Case Studies | RAPID CANCER COMMUNICATION

Synchronous T-lymphoblastic lymphoma and neuroblastoma in a 3-yr-old with novel germline *SMARCA4* and *EZH2* variants

Pauline Tibout,[1] Joel Livingston,[1] Nisha Kanwar,[2] Kyoko E. Yuki,[2] Adam Shlien,[2,3] Bo Ngan,[4] Meredith S. Irwin,[1,5] Daniel A. Morgenstern,[1,5] Johann Hitzler,[1,5] Anita Villani,[1,5] and Sarah Cohen-Gogo[1,3]

npj | Genomic Medicine www.nature.com/npjgenmed

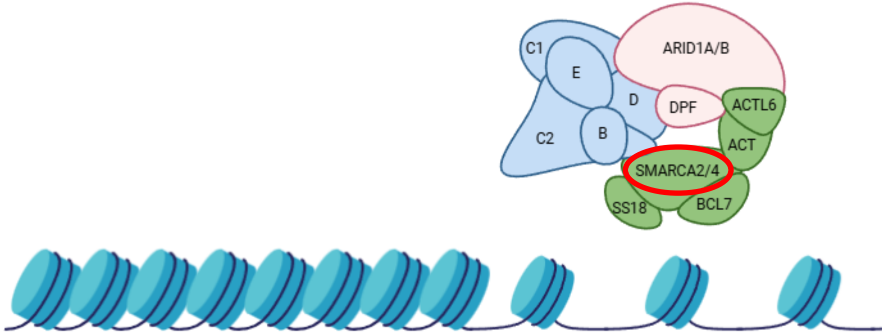
ARTICLE OPEN

Check for updates

19p loss is significantly enriched in older age neuroblastoma patients and correlates with poor prognosis

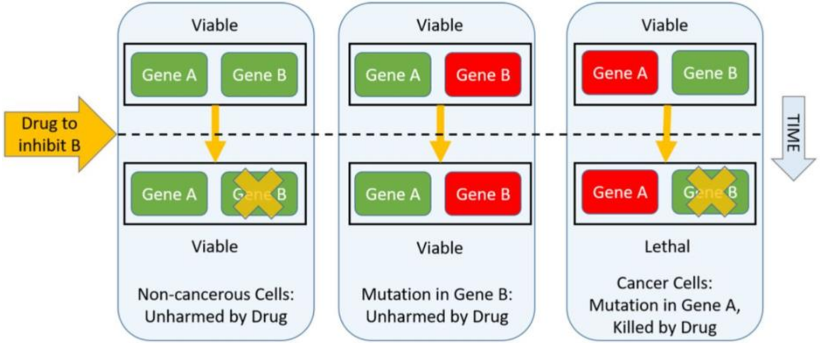
Vito Alessandro Lasorsa[1,2], Flora Cimmino[1,2], Marzia Ognibene[3], Katia Mazzocco[4], Giovanni Erminio[5], Martina Morini[6], Massimo Conte[7], Achille Iolascon[1,2], Annalisa Pezzolo[3,9] ✉ and Mario Capasso[1,2,8,9] ✉

SMARCA4 TUMOR SUPPRESSOR ROLE



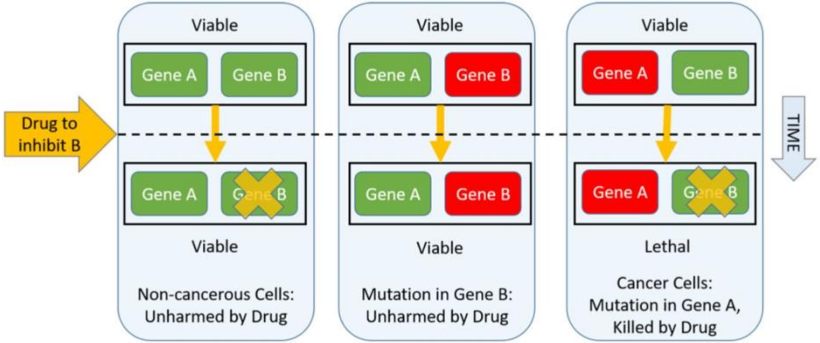
- *SMARCA4* (BRG1) is the ATPase subunit of the **SWI/SNF** chromatin remodeling complex
- *SMARCA4* acts as a critical **tumor suppressor** gene controlling global gene expression.
- *SMARCA4* loss generates a **synthetic lethality** dependency on its paralog, *SMARCA2* (BRM), opening a potential therapeutic window

SYNTHETIC LETHALITY



- Synthetic lethality is based on the principle that the inhibition of two paralogous genes is lethal, whereas the inhibition of either gene individually allows healthy cells to survive.
- In this strategy, tumor suppressor gene mutations are exploited to induce the death of cancer cells.

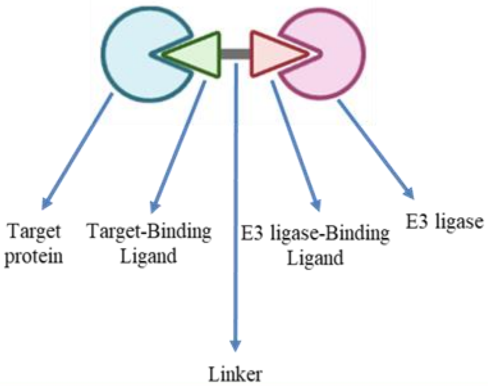
SYNTHETIC LETHALITY



- Synthetic lethality is based on the principle that the inhibition of two paralogous genes is lethal, whereas the inhibition of either gene individually allows healthy cells to survive.
- In this strategy, tumor suppressor gene mutations are exploited to induce the death of cancer cells.

- The dependency of *SMARCA4*-mutant cells on *SMARCA2* is being therapeutically exploited using ***SMARCA2*-targeting PROTACs**. These agents, that are already in clinical trials, are designed to not only inhibit SMARCA2 function but actively induce its targeted degradation.

PROteolysis-TArgeting Chimera



CONCLUSIONS

1. Our familial lineage analysis successfully traced the tumor evolution and pinpointed a germline predisposing variant in the *SMARCA4* gene
2. Functional studies are underway to elucidate the oncogenic effect of this variant and to assess the therapeutic susceptibility

Take Home Message

Characterizing the underlying causative genetic drivers is primary for revealing novel therapeutic vulnerabilities and paving the way for patient-centric Precision Medicine

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