Settima edizione di

AIEOP...

...in Lab

G.d.L. Biologia Cellulare Molecolare

AIEOP

ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

Medicina di precisione nella caratterizzazione del sarcoma pediatrico

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Padova

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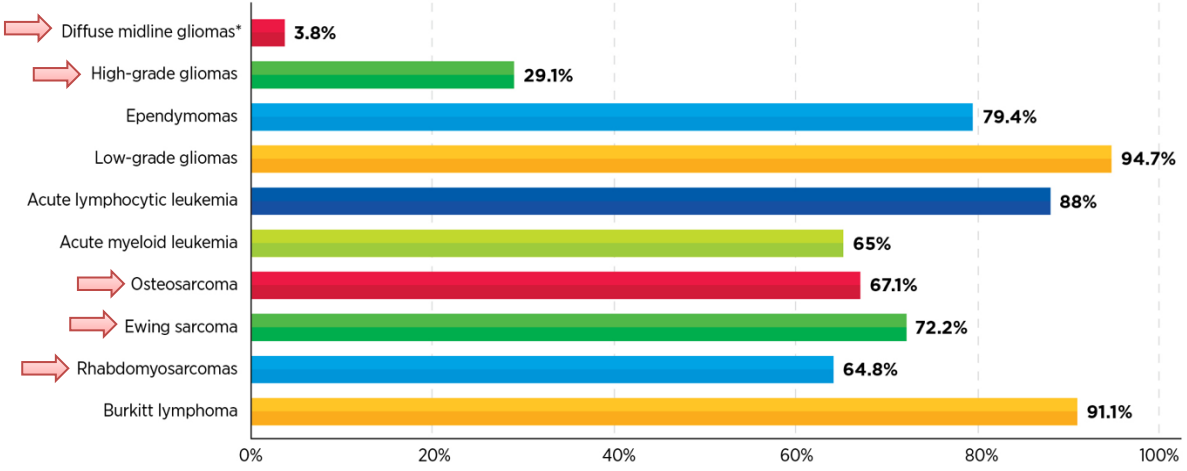
Disclosures of Paolo Bonvini

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
none	no	no	no	no	no	no	none

COURTESY BY IRP

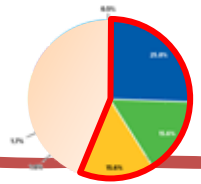


* 4-year overall survival.

AACR#PCPR

Survival reaches and exceeds **90% for some** cancers, such as Leukemias, Lymphomas, thyroid carcinoma, and retinoblastoma

Others, including high-grade gliomas and certain sarcomas, remain among **the deadliest**.



- Pediatric cancers are **rare** (15/100K), biologically distinct, and (**un)evenly studied** when compared to adult cancers.

- Survival gains are concentrated in the more common pediatric cancers, while **rare or more aggressive tumors** — characterized **by metastases at diagnosis** or poor response to therapy — continue to have **dismal outcomes (<30%).**

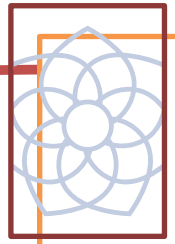
Clinical research led by **pediatric cancer–focused cooperative groups** has driven major breakthroughs in pediatric oncology.

Major advances included:

- improved methods **for staging** tumors,
- assessing tumor **size** and **spread**,
- optimizing **treatment** approaches

Despite such gains, survival rates have only increased, on average, by only about 0.5% annually since 2000.

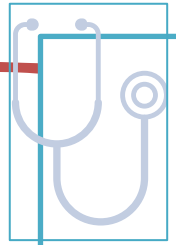
PROGRESSES AND CHALLENGES IN PEDIATRIC CANCER TREATMENT



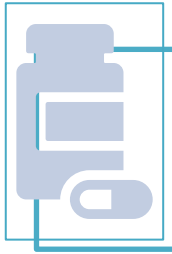
The use of surgery, radiotherapy, and chemotherapy continues to evolve, as more advanced forms of these treatments are developed.

A higher proportion of childhood and adolescent patients participate in clinical trials (20%-30%) than adults (7%)

With greater understanding of the biology of pediatric cancers, comes an increasing focus on personalized approaches



Cancer remains the leading cause of death in children of HICs; more than 60 % of survivors experience significant long-term effects of treatment.



Access to appropriate therapies can be limited by drug availability, clinical trial eligibility, and safety concerns.

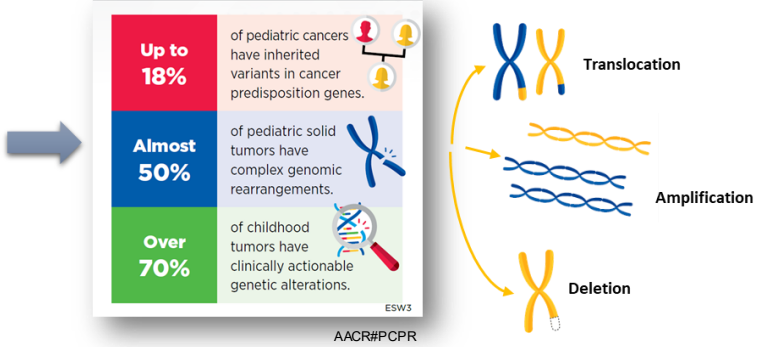
Most of the molecular drivers of pediatric cancers remain difficult to target (epigenetic and/or transcription factors).

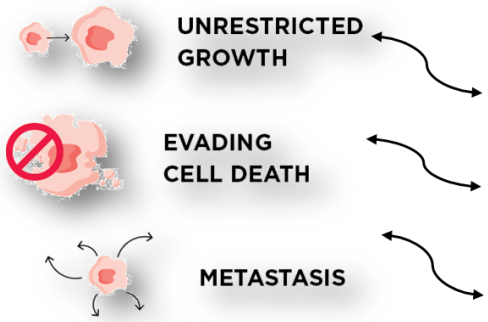
TRANSFORMING PEDIATRIC CANCER OUTCOME THROUGH PRECISION DIAGNOSTIC

(FROM ONE-SIZE-FIT APPROACH TOWARD TAILORED TREATMENT MODALITY)

Key Concepts in Pediatric Cancer Genetics:

- **Cancer is** a disease in which some of the body's cells acquire **DNA changes** that allow them to grow uncontrollably and spread afar.
- **Germline mutations** are typically present in every cell in the body and can be inherited from parents or occur *de novo*.
- **Somatic mutations** are acquired over an individual's lifetime, are restricted to selected cells, are pathogenic when changes in DNA sequence disrupt normal cellular functions.



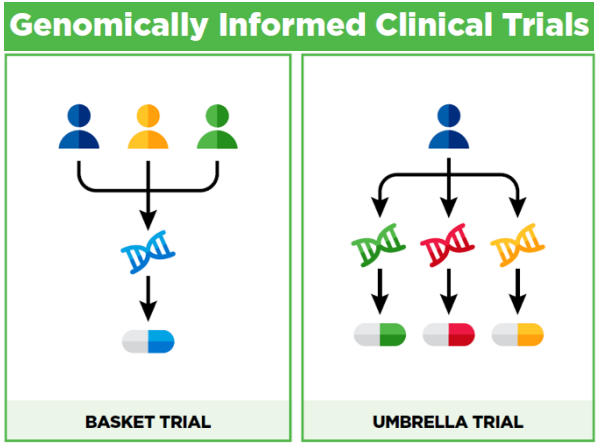


Key Features of Pediatric Cancer Genetics:

- Few cancer-specific **mutations**, frequently in genes involved **in transcription** and **epigenetic regulation**.
- Mutations **affect** survival signals that control **embryonic development**.
- Often occurs through pathways involved in cell **differentiation** and **DNA repair**.
- Metabolic changes resembles states of **rapid cell multiplication**.

Cancer-driving genomic alterations used to identify pediatric patients benefitting from an anticancer therapeutic

CANCER	MARKER	TARGET	DRUG	DRUG RESISTANCE
B-ALL	BCR::ABL1	**ABL1**	Dasatinib	ABL1 mut (T315I) /Amp
	KMT2A-rearranged	**MEN-1**	Revumenib	MEN-1 (M327I, T349M)
AML	KMT2A-rearranged	**CD33**	Gemtuzumab (ADC)	CD33 loss; MDR1/P-gp
Hodgkin Lymphoma (HL)	CD30	**CD30**	Brentuximab (ADC)	CD30 loss; MDR1/P-gp
ALCL; NB	NPM::ALK; ALK amp/mut	**ALK**	Lorlatinib	ALK mut (G1202R) / Amp; others (KRAS/Met/EGFR)
DLBCL; BL; BLL; mature B-ALL	various	**CD20**	Rituximab	CD20 loss; MDR1/P-gp
HR-NB	MYCN amp	**GD2**	Dinutuximab	GD loss; MDR1/P-gp; ALK mut; MYCN amp
HR-NB; HB; WT; JMML	MYCN amp; MYC act; ODC1 act	**ODC1**	Eflornithine	PTS (polyamine) upreg.; ODC1 loss/amp; CDK1/2 act;
pLGG (CNS)	BRAF mut / rearranged	**BRAF V600E**	Tovorafenib	BRAF Famp; MEK1/2 mut; RTK act.
pHGG (CNS)	H3K27M mut (DMG)	**ClpP ; DRD2/DRD3**	Dordaviprone	ClpP mut; PDGFRA act; PI3K/AKT/mTOR mut/act
NF1 tumors	NF1 loss	**MEK1/2**	Selumetinib	KRAS/NRAS mut; MEK1/2 mut; PI3K/AKT/mTOR mut
PTC/MTC/DTC; RCC	BRAF V600E; RET-fus; NRAS mut; NTRK1-fus; ALK-fus	**RET; VEGFR2; MET; ROS1; TRKA**	Cabozantinib	BRAF mut/amp; MEK1/2 mut; RTK mut/amp; AXL act; PI3K/AKT/mTOR act
ATRT; MTRK; SYS; MPNST	SMARCB1/INI1 loss; PRC2 loss	**EZH2**	Tazemetostat	EZH2 mut/amp; EZH1 act; KDM6A/KDM6B loss;
inf. HGG; Thyroid cancer; IFS	NTRK1/2/3-rearrangements	**TRKA/B/C**	Larotrectinib; Entrectinib	TRK mut (G→R/C; F→L)/ amp; RAS mut; BRAF V600E; RTK amp/act



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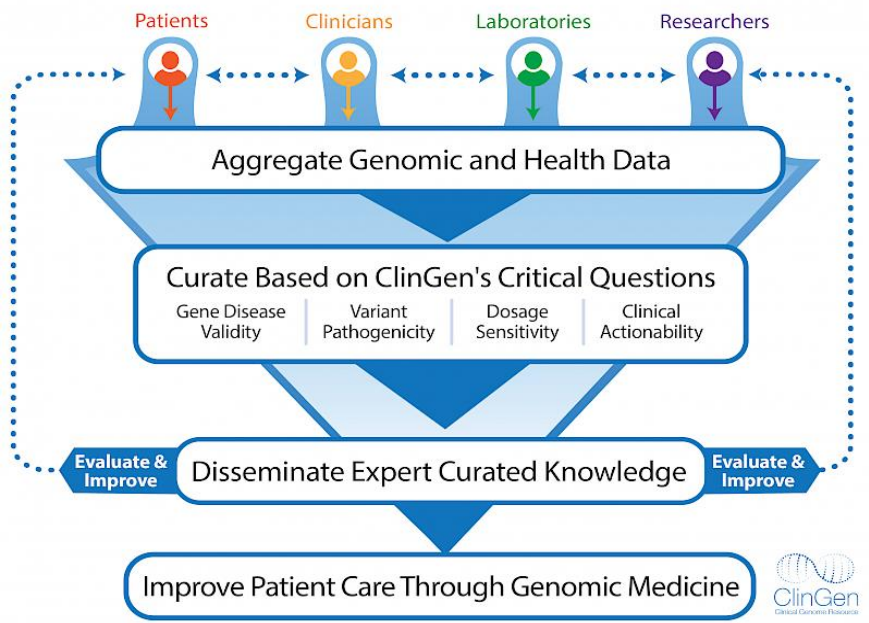
One drug is tested against a particular genetic mutation across **different cancer types**.

Different drugs are tested against multiple genetic mutations within the **same cancer type**.

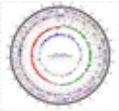
ADVANCES IN PEDIATRIC CANCER TREATMENT THROUGH CANCER **MOLECULAR PROFILING**

1. ***Precision medicine*** *programs aim to match cancer patients to the most effective therapy based on molecular features.*

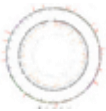


2. **Personalized treatment** plans can be achieved by combining information and data unique to each individual (e.g., clinical, functional, genomic and transcriptomic data) to target drugs.

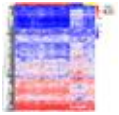
HIGH-THROUGHPUT TECHNOLOGIES DECODING PEDIATRIC CANCER COMPLEXITY



WHOLE-GENOME SEQUENCING (**WGS**): provides the entire sequence of the normal/cancer cell genome; reveals both common and rare variants in coding and non-coding regions.



WHOLE-EXOME SEQUENCING (**WES**): provides the sequence of the protein-coding regions of the genomes; identifies clinically relevant alterations (SNV; CNV; INDELs).

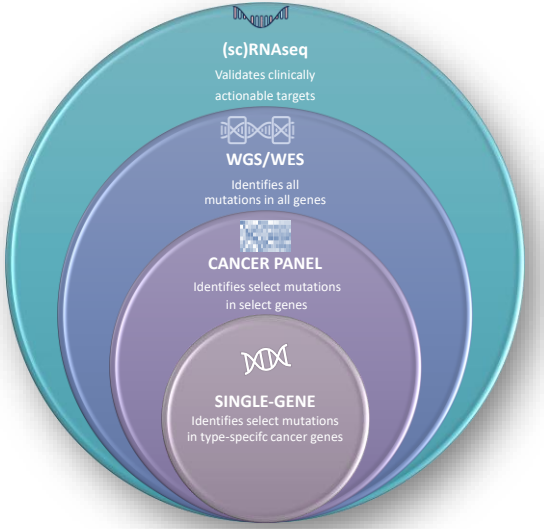


DNA METHYLATION PROFILING: detects chemical modifications to DNA; identifies patterns driving cancer; provides biomarkers for prognosis.

RNA SEQUENCING: measures gene expression, detects gene fusions, identifies transcript variants that can be used to refine diagnosis.

SINGLE-CELL ANALYSIS: examines all above; captures differences between tumor and healthy cells; reveals tumor heterogeneity.

MOLECULAR TECHNOLOGIES DECODING PEDIATRIC CANCER COMPLEXITY – CLINICAL VALUE



	SINGLE-GENE	CANCER PANEL	WES	WGS	RNA-seq
Input material	**DNA/RNA**		**DNA**	**DNA**	**RNA**
Target	Single variant	50-500 genes	All exons	Entire genome	Transcriptome
Scope	Ultra-targeted	Targeted	Broad	Widest	Expression-focused
Detects mutations	✓ Known only	✓ Known only	✓ Coding	✓ All regions	⚠ Indirectly
Novel variants	✗ No	✗ No	⚠ Partial	✓ Yes	⚠ Partial
Gene expression	⚠ Single gene	✗ No	✗ No	✗ No	✓ Excellent
Absolute quantification	✓ Yes	✗ No	✗ No	✗ No	✗ No
Rare variant detection	🔥 Exceptional (<0.1%)	⚠ ~1-5%	⚠ ~5%	⚠ ~5%	✗ Poor
CNV detection	⚠ Targeted	⚠ Partial	⚠ Partial	✓ Yes	✗ No
Fusion genes	⚠ Known only	⚠ Known only	✗ No	⚠ Limited	✓ Yes
Splicing variants	✗ No	✗ No	✗ No	✗ No	✓ Yes
TMB assessment	✗ No	⚠ Partial	✓ Good	✓ Best	✗ No
MSI detection	✗ No	⚠ Limited	✓ Good	✓ Best	✗ No
Sensitivity	🔥🔥 Highest	🔥 Very hight	Moderate	Moderate	High
Cost	$ Low-Moderate	$ Low-Moderate	$$ Moderate	$$$ High	$$ Moderate
Data complexity	Very low	Low	High	Very high	High
Turnaround time	⚡⚡ Very fast	⚡ Fast	Moderate	Slow	Moderate
Clinical use	✓ Routine	✓ Growing	✓ Growing	⚠ Limited	⚠ Research mostly
Liquid biopsy	✓ Excellent	✓ Good	⚠ Possible	⚠ Possible	✗ Limited

SINGLE-GENE → ultra-sensitive, best for liquid biopsy & MRD monitoring
CANCER PANEL → fast, cheap, clinically actionable for known variants
WES → best balance for comprehensive coding variant discovery
WGS → most complete, gold standard for research
RNA-seq → uniquely captures gene expression, fusions & splicing

Precision oncology in pediatric cancer is:

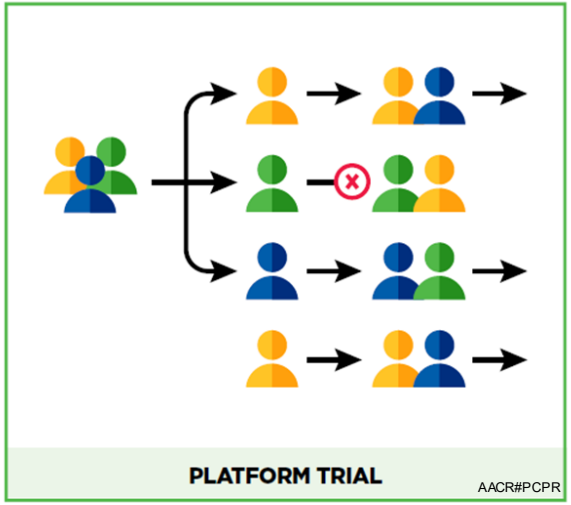
- Feasible
- Informative
- Clinically useful (but still limited)

Precision oncology in children with high-risk cancers can improve:

- Diagnosis
- Therapeutic targets
- Treatment options
- Clinical outcomes (but still limited)

Precision oncology is effective if :

- The genetic alteration is an actionable clear oncogenic driver
- A drug that directly inhibits the driver exists and is available
- The driver inhibition is likely to halt tumor growth
- The matched targeted therapy is given in early-phase clinical trials



Genomically informed clinical trial that matches children with targeted therapies based on their tumor's genetic profile, using multiple basket trial arms focused on the most common altered pathways.

HOW MUCH MOLECULAR TUMOR PROFILING HAS IMPACTED ON SURVIVAL OF CHILDREN AND ADOLESCENT WITH **SARCOMA** ?

LucrezIA: «Great question. This is a honest answer...»

- Diagnostic accurancy: High
- Molecular classification: High
- Clinical trial matching: Moderate-High
- Personalized Therapy: Moderate
- Overall survival improvement: Moderate rather than dramatic !

JAMA

"Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth"
(JAMA. 2015;314(9):913-925. doi:10.1001/jama.2015.10080)

Diagnosis Impact

102 patients enrolled / 91 eligible

63 BSTS (69%), **28 Leu/Lymph** (31%)

- **Localized BSTS 65%**
- **Metastatic BSTS 36.5%**

15% (**14**/91) **diagnosis change**

25% (**23**/91) **therapy change**

Diagnosis	No. of Patients			Age, y			No. of Patients Sequenced	
	Total	Male	Female	Range	Mean	Median	At Relapse or Progression	At Diagnosis
All patients	102	52	50	0-22	10.6	11.5	81	21

Genomic Landscape (WES/RNAseq)

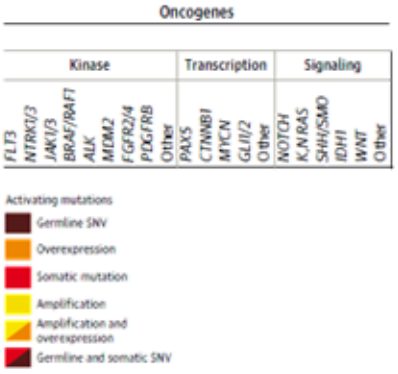
46% SNV/Indels (BRAF, FGFR4, FLT3, NRAS,IDH1, GNAS, CTNNB1, MYCN)

35% gene fusions (ATIC-ALK, ETV6-ABL1, LMNA-NTRK1, AFF1-MLL, EWSR1-FLI1, PAX3/7-FOXO1)

15% homozygous deletions (SMARCB1, KDM6A, TP53, AMER1, CDKN2A/B)

8% amplifications (PDGFRA, KRAS, MDM2, MYBL1)

12% germline mutations (PDGFRA, MITF, DICER1, BAP1, BARD1)



Therapeutic Impact

22% actionable **SNV/Indels**

10% actionable **fusions**

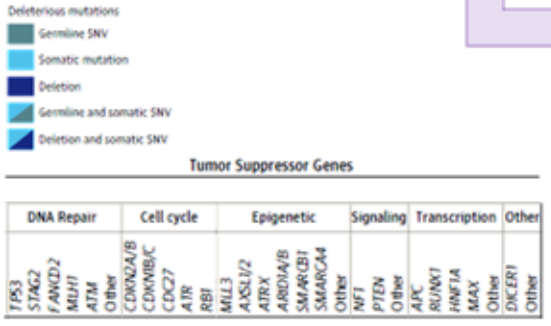
9% actionable **homozygous deletions**

3% actionable **amplifications**

15% (**14**/91) patients treatment changes

10% (**9**/91) patients for genetic counseling

10% (**9**/91) patients with durable clinical response



6. MOLECULAR PROFILING - PRECISION MEDICINE PROGRAMS

ZERO

2020

- **247** pediatric cancer patients
- **WGS/RNA-seq/Methylation**
- ~50% rel/ref. cancer
- **93,7% at least 1 relevant molecular alteration**
- 16,2% had germline mutations
- 5,2% newly diagnosed
- **71,4% had actionable targets**
- **17% NGS-based therapy**
- **13 patients had clinical benefit (5,2%)**

DOI: 10.1038/s41591-020-1072-4

iTHER

2022

- **253** pediatric cancer patients
- **WGS/WES/RNA-seq/Met**
- 79,1% rel/ref. cancer
- **90,3% at least 1 relevant molecular alteration**
- 16% had germline mutations
- 3,5% newly diagnosed
- **81,9% had actionable targets**
- **13,9% NGS-based therapy**
- **3 patients had clinical benefit (5,7%)**

DOI: 10.1016/j.ejca.2022.09.001

INFORM

2021

- **519** out of 926 patients with >2y follow-up
- **WGS/WES/RNA-seq/Met**
- 91,8% rel/ref. cancer
- **71,2%** with metastases
- 91,3% non-CNS+CNS cancer
- 9,6% newly diagnosed
- **43% had actionable targets**
- **28,3% NGS-based therapy**
- **20 patients had clinical benefit (3,8%)**

Priority level	
Very high	20 (13.6)
High	31 (21.1)
Moderate	24 (16.3)
Intermediate	33 (22.4)
Borderline	26 (17.7)
Low	6 (4.1)
Very low	2 (1.4)

DOI: 10.1158/2159-8290.CD-21-0094

MAPPYACT

2022

- **787** rel/ref pediatric cancer patients
- **WES/RNA-seq/cfDNA-seq**
- **69% patients at least 1 actionable target**
- 7% had germline mutations
- 2-3% newly diagnosed
- **82,4% had actionable targets**
- **30% NGS-based therapy**
- **18 patients had PR (17%)**
- **27 patients had SD (25%)**
- **10 patients developed drug resistance**

DOI: 10.1158/2159-8290.CD-21-1136

MOLECULAR PROFILING - PRECISION MEDICINE PROGRAMS

ZERO — 2020

Strengths
- **Multi-omic study**
- **Improved diagnosis**
- Identified molecularly matched treatment options

Limitations
- Many targets had no drug available
- Many patient could not access it
- **Small number of patients were treated with matched therapy**
- **No survival improvement observed**

iTHER — 2022

Strengths
- High success rate of sequencing
- Majority of detectable alterations
- **Significant numbers of actionable targets**

Limitations
- Smaller subset receiving targeted therapy
- Very few patients beneffitting from targeted therapies
- **Most of the successful treatments included chemotherapy**
- **Limited overall survival**

INFORM

Strenghts
- Comprehensive molecular profiling
- **Alterations ranked using** a 7-level target **prioritization algorithm**
- **Mean turnaround time 25,4 days**

Limitations
- Strong correlation between presence of alteration and likelihood of treatment response in a small subset of patients (8%)
- **Improved PFS only in patients with top-tier targets**

MAPPYACT

Strengths
- Many treatments were given in early-phase clinical trials
- ctDNA sequencing is informative

Limitations
- Many patients did not receive matched therapy due to:
  - Disease progression or death
  - Lack of access to targeted drugs
- **Only a small fraction (~10%) of alterations had strong clinical evidence**

SUCCESS



	G4K	ZERO	iTHER
CR	FLT3i+pan-Tki (**AML**)	mTORi; BRAFi* (**CNS**); MEKi; PARPi+anti-PDL1 mAb*(**CNS**)	MEKi+chemo*(**ALL**); Proteos.i+chemo+HSCT (**ALL**);
PR	MEKi*(**Mel**)	NTRKi (**STS**);FGFRi (**CNS**);mTORi+ chemo* (**STS**); ALKi* (**NB**); mTORi + anti-VEGFA mAb*(**CNS**); EGFRi	CDK4/6i+chemo*(**ES**)
SD	anti-PD1 mAb (**GBM**)*; mTORi (**ACP**)*	MEKi*(**CNS**); MEKi+BRAFi (**CNS**); BCL2i (**STS**); VEGFRi + chemo (**Solid**); PDGFRi (**CNS**); anti-PD1 mAb + anti-CTLA4 mAb* (**CNS**); ABLi* (**CNS**); ALKi+ chemo* (**NBL**); FGFRi+ chemo (**STS**); mTORi + CDK4/6i* (**CNS**);	CDK4/6i+chemo*(**ES**); PARPi+chemo*(**ES**); BCL2i+chemo*(**NB**); CDK4/6i+chemo*(**RMS**); ALKi*(**NB, RMS**);
PD	JAKi (**MPAL, B-ALL**); mTORi (**T-ALL, AA**); EGFRi (**Meningioma**); BRAFi (**HB**); pan-Tki (**AA**);	anti-PD1 mAb + anti-CTLA4 mAb (**Solid**); mTORi + chemo (**HM**); PARPi (**Solid**); EGFRi (**CNS**); MEKi (**STS**); HDACi (**Solid**); AURKAi (**Solid**)	ALKi (NB, RMS); ALKi+mTORi (**NB**); anti-PDL1 mAb+HDACi (**HGG**); CDK4/6i+chemo (**MB**); CDK4/6i (**NB, RMS**); PARPi+chemo (**HGG**); mTORi (**HGG**); TKi+chemo (**AML**)

* Late Relapse

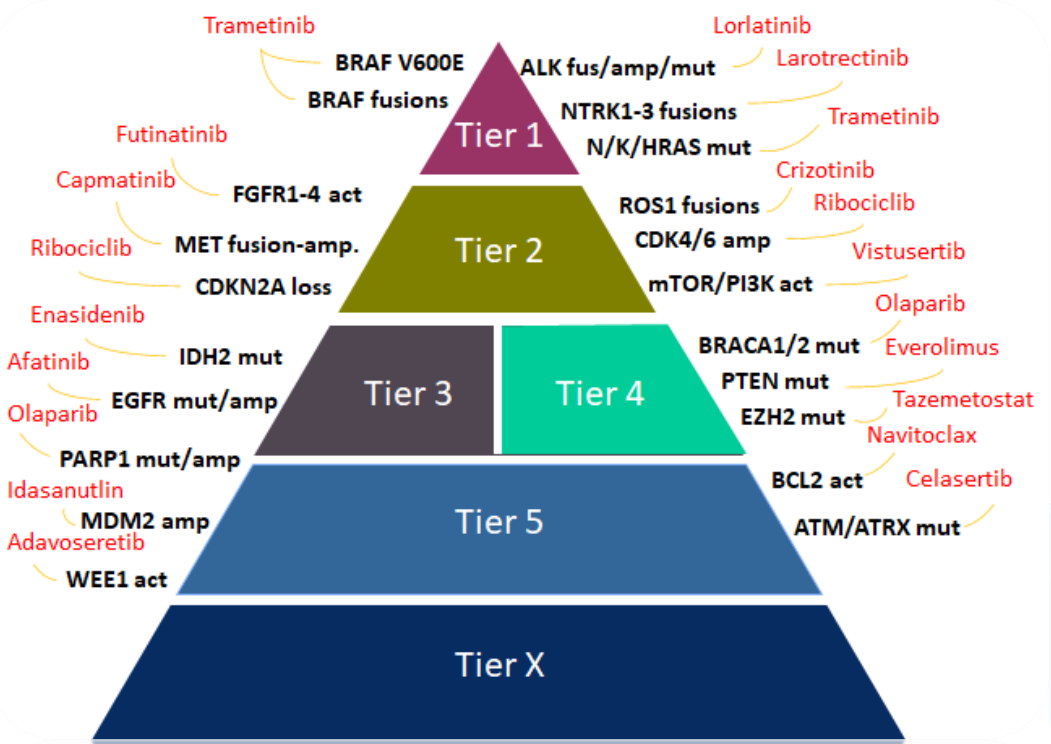
* **CDK6** amplification

* **RAD51** upregulation

* **RAS/MAPK** activation
ALK 2nd mutation

ESCAT: ESMO Scale of Clinical Actionability for molecular Targets

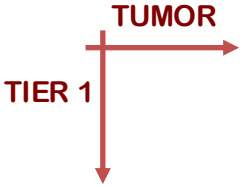
(European Society for Medical Oncology)



www.esmo.org

TIER I → Target actionable con beneficio clinico dimostrato
TIER II → Target actionable con evidenza clinica preliminare
TIER III → Target con evidenza biologica/preclinica
TIER IV → Co-alteration / significato non autonomo
TIER V → Alterazioni di resistenza
TIER X → Non actionable / significato sconosciuto

European Proof-of-concept Therapeutic Stratification Trial of Molecular Anomalies in Relapsed or Refractory Tumors



1. Strong scientific evidence
2. Associated with clinical benefit
3. Specific for drugs recommended by clinical guidelines

ESMART TIER-1 TARGETS IN PEDIATRIC SOLID TUMORS

ESMART - TIER 1	RMS	EWS	DSRCT	SS	OS	MRT	HBK	WT	NB	MB	HGG
ACVR1 (mut)											T1_DIPG☆☆☆
ALK (amp/mut/fus)	T1☆	T1	T1	T1	T1	T1	T1	T1	T1☆☆☆	T1_G4☆☆	T1☆
ATRX (mut/loss)										T1_G3/4☆☆☆	T1_G34☆☆☆
ATRX/ALT (mut)											T1☆☆☆
AURKA (act)	T1	T1							T1☆☆☆	T1_G3/G4☆☆	
BET / BRD4 (act)									T1☆☆☆	T1_G3☆☆☆	
CCND1/CCNE1 (amp)							T1☆☆				
CDK4 (amp)	T1	T1	T1	T1☆☆	T1☆☆	T1	T1	T1	T1	T1_G4☆☆	T1
CDK6 (amp)										T1_G4☆☆☆	
CDK7 (act)										T1_G3/4☆☆	
CDK7/CDK12 (act)										T1_G3/G4☆☆	
CDK7/CDK9 (act)										T1_G3/4☆☆	
CDKN2A/B (del)	T1	T1	T1	T1☆☆☆	T1	T1	T1	T1	T1	T1_G4☆☆	T1
CTNNB1 (mut/act)							T1☆☆☆	T1☆☆		T1_WNT-MB☆	
EZH2 (act)				T1☆☆☆		T1☆☆☆				T1_G4☆☆	T1_G3/4☆☆
FGFR1-4 (mut/act)	T1☆☆☆						T1☆☆				
FGFR4/FGF19 (ACT)	T1☆☆☆						T1☆☆☆				
GD2 (act)									T1☆☆☆		
H3F3A G34R/V (mut)											T1_G34☆☆☆
H3K27M (mut)											T1_DMG☆☆☆
HEDGEHOG-HH (act)										T1_SHH☆☆☆	
HGF/cMET (act)											T1☆☆
IGF1R (act)	T1☆☆	T1☆☆☆	T1☆☆☆		T1		T1☆☆☆	T1☆☆			
KDM6A (mut)										T1_G4☆☆☆	
MDM2 (act/TP53wt)						T1☆☆	T1☆☆	T1☆☆	T1☆☆	T1☆☆	T1☆
MET (amp/act)											T1☆☆
MYC/BRD4(amp/act)									T1☆☆☆	T1_G3☆☆☆	
MYC/MYCN (amp/act)									T1☆☆☆	T1☆☆☆	
MYCN (amp)									T1☆☆☆	T1_G4☆☆	
PARP1 (act/amp)	T1	T1	T1	T1	T1	T1				T1	T1
PARP1 (TP53mut/ATRXmut)	T1	T1☆☆☆	T1	T1	T1	T1				T1	T1☆☆
PDGFRA (act)			T1☆☆☆					T1			T1_DMG☆☆☆
PI3K/mTOR (act/mut)	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1☆☆☆
PRC2 (act)				T1☆☆☆		T1☆☆☆				T1_G4☆☆	T1_G34☆☆
RAR/ATRA (act)									T1☆☆☆		
RAS/MAPK (mut)	T1☆☆☆										
RB1 (CDK4/6 act)	T1	T1	T1	T1☆☆☆	T1	T1	T1	T1	T1	T1_G4☆☆	T1
SHH/SMO (mut/act)										T1_SHH☆☆☆	
SMARCB1 (loss/mut)						T1☆☆☆					
VEGFR (act)		T1	T1	T1	T1☆☆	T1	T1☆☆	T1			
WEE1 (act/TP53wt)	T1	T1☆☆	T1/T2☆☆☆								
WEE1/ATR (act/DDR)	T1	T1☆☆									
BRAF (V600E)	T3	T3	T3	T3	T3	T3	T3	T3	T3	T3_SHH☆	T2☆☆☆

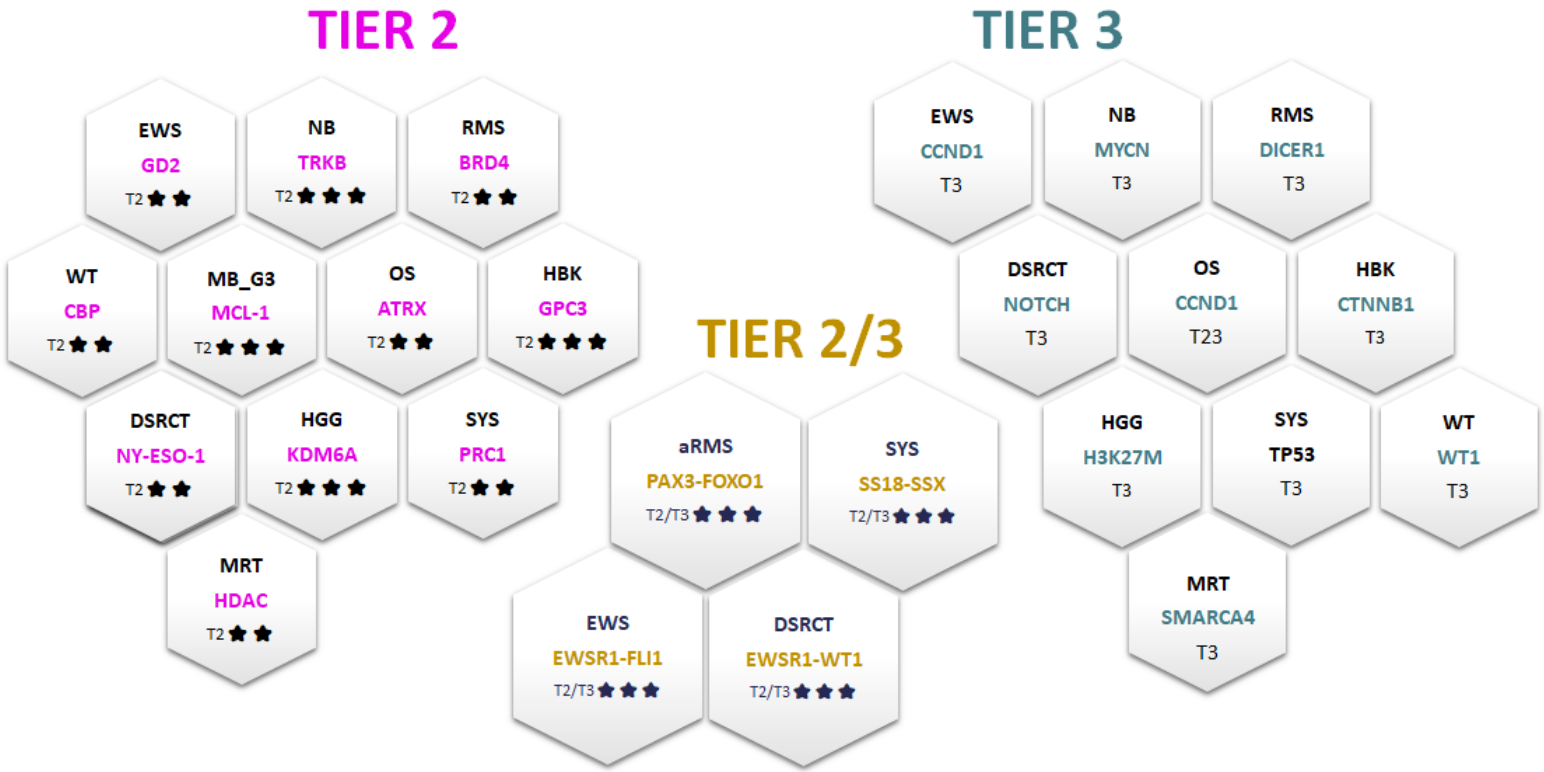
EVIDENCE OF CLINICAL BENEFIT: STRONG = ★★★ ; MODERATE = ★★ ; LIMITED = ★

ESMART TIER-1 TARGETS IN PEDIATRIC SOLID TUMORS

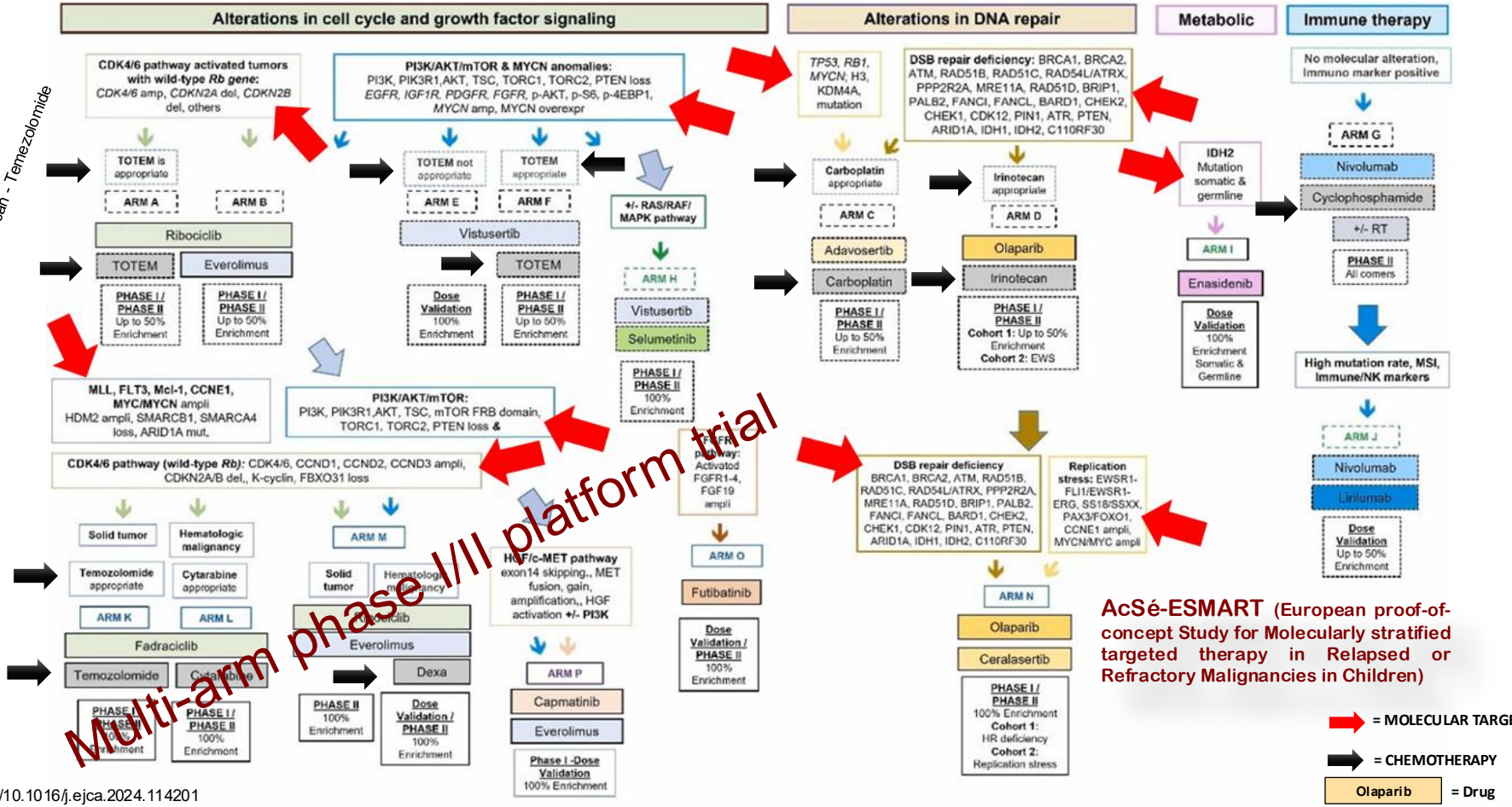
ESMART - TIER 1	RMS	EWS	DSRCT	SS	OS	MRT	HBK	WT	NB	MB	HGG
ACVR1 (mut)											T1_DIPG☆☆☆
ALK (amp/mut/fus)	T1☆	T1	T1	T1	T1	T1	T1	T1	T1☆☆☆	T1_G4☆☆	T1☆
ATRX (mut/loss)										T1_G3/4☆☆☆	T1_G34☆☆☆
ATRX/ALT (mut)											T1☆☆☆
AURKA (act)	T1	T1							T1☆☆☆	T1_G3/G4☆☆	
BET / BRD4 (act)									T1☆☆☆	T1_G3☆☆☆	
CCND1/CCNE1 (amp)							T1☆☆				
CDK4 (amp)	T1	T1	T1	T1☆☆	T1☆☆	T1	T1	T1	T1	T1_G4☆☆	T1
CDK6 (amp)										T1_G4☆☆☆	
CDK7 (act)										T1_G3/4☆☆	
CDK7/CDK12 (act)										T1_G3/G4☆☆	
CDK7/CDK9 (act)										T1_G3/4☆☆	
CDKN2A/B (del)	T1	T1	T1	T1☆☆☆	T1	T1	T1	T1	T1	T1_G4☆☆	T1
CTNNB1 (mut/act)							T1☆☆☆	T1☆☆		T1_WNT-MB☆	
EZH2 (act)				T1☆☆☆		T1☆☆☆				T1_G4☆☆	T1_G3/4☆☆
FGFR1-4 (mut/act)	T1☆☆☆						T1☆☆				
FGFR4/FGF19 (ACT)	T1☆☆☆						T1☆☆☆				
GD2 (act)									T1☆☆☆		
H3F3A G34R/V (mut)											T1_G34☆☆☆
H3K27M (mut)											T1_DMG☆☆☆
HEDGEHOG-HH (act)										T1_SHH☆☆☆	
HGF/cMET (act)											T1☆☆
IGF1R (act)	T1☆☆	T1☆☆☆	T1☆☆☆		T1		T1☆☆☆	T1☆☆			
KDM6A (mut)										T1_G4☆☆☆	
MDM2 (act/TP53wt)						T1☆☆	T1☆☆	T1☆☆	T1☆☆	T1☆☆	T1☆
MET (amp/act)											T1☆☆
MYC/BRD4(amp/act)									T1☆☆☆	T1_G3☆☆☆	
MYC/MYCN (amp/act)									T1☆☆☆	T1☆☆☆	
MYCN (amp)									T1☆☆☆	T1_G4☆☆	
PARP1 (act/amp)	T1	T1	T1	T1	T1	T1				T1	T1
PARP1 (TP53mut/ATRXmut)	T1	T1☆☆☆	T1	T1	T1	T1				T1	T1☆☆
PDGFRA (act)			T1☆☆☆					T1			T1_DMG☆☆☆
PI3K/mTOR (act/mut)	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1☆☆☆
PRC2 (act)				T1☆☆☆		T1☆☆☆				T1_G4☆☆	T1_G34☆☆
RAR/ATRA (act)									T1☆☆☆		
RAS/MAPK (mut)	T1☆☆☆										
RB1 (CDK4/6 act)	T1	T1	T1	T1☆☆☆	T1	T1	T1	T1	T1	T1_G4☆☆	T1
SHH/SMO (mut/act)										T1_SHH☆☆☆	
SMARCB1 (loss/mut)						T1☆☆☆					
VEGFR (act)		T1	T1	T1	T1☆☆	T1	T1☆☆	T1			
WEE1 (act/TP53wt)	T1	T1☆☆	T1/T2☆☆☆								
WEE1/ATR (act/DDR)	T1	T1☆☆									
BRAF (V600E)	T3	T3	T3	T3	T3	T3	T3	T3	T3	T3_SHH☆	T2☆☆☆

EVIDENCE OF CLINICAL BENEFIT: STRONG = ☆☆☆ ; MODERATE = ☆☆ ; LIMITED = ☆

EXAMPLES OF ESMART TIER 2 AND 3 TARGETS IN PEDIATRIC SOLID TUMORS



EVIDENCE OF CLINICAL BENEFIT: STRONG = ★★★ ; MODERATE = ★★ ; LIMITED = ★



doi.org/10.1016/j.ejca.2024.114201

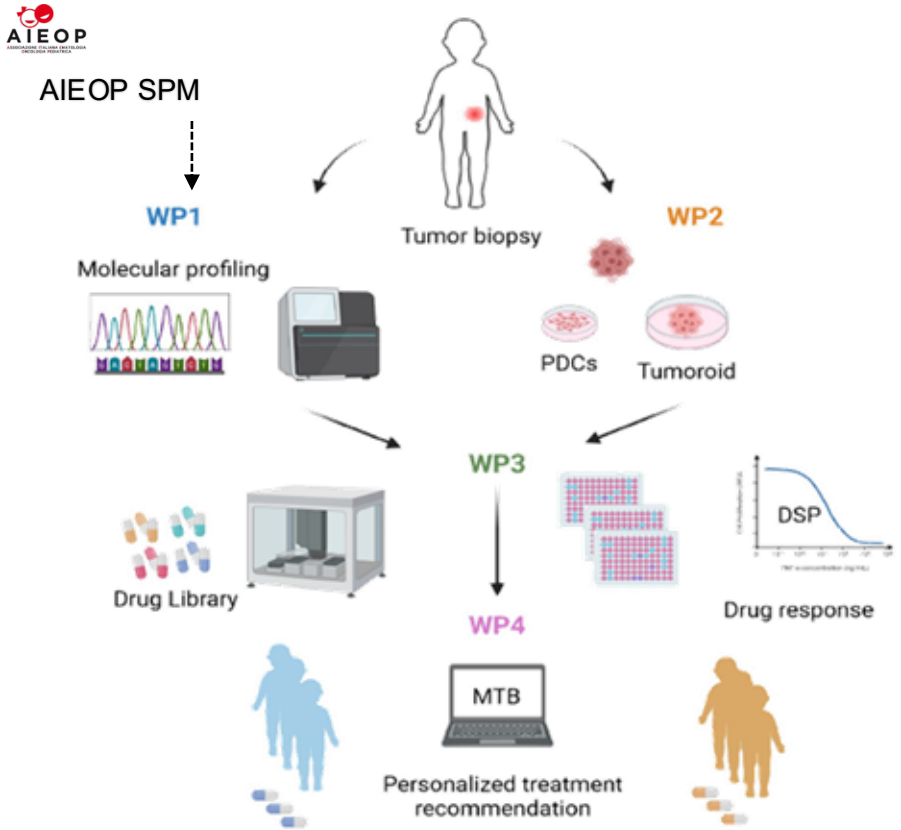
PERSONALIZED DRUG SENSITIVITY PROFILING PROGRAM FOR PROGRESSIVE AND RELAPSED PEDIATRIC SARCOMAS [**PRESTO**]

AIM

1. IDENTIFY ACTIONABLE GENETIC ALTERATIONS AND NOVEL BIOMARKERS USING NGS APPROACHES
2. ESTABLISH EX-VIVO PATIENT-DERIVED PRECLINICAL MODELS
3. PERFORM HIGH THROUGHPUT PRECLINICAL DRUG SCREENING
4. INTEGRATE CLINICAL, GENOMIC AND DRUG SENSITIVITY DATA

TARGET

1. PATIENTS WITH BSTS
2. DISEASE STATE: DISEASE PROGRESSION, RECURRENCE, RELAPSE AT ONSET, DRUG RESISTANCE



DNA EXTRACTION → DNA QUANTIFICATION AND QUALITY ASSESSMENT → **WHOLE EXOME SEQUENCING** → BIOINFORMATIC ANALYSIS

Qiagen AllPrep DNA/RNA/Protein Kit
- Tumor biopsy
- PBMC (healthy control)

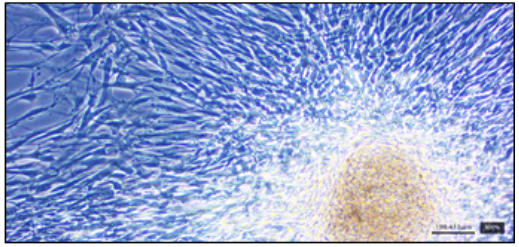
Qubit 1X dsDNA HS assay kit
- DNA concentration
- DNA integrity

- DNA amount > 300 ng

- Somatic mutation analysis

2024-2025

		DSP (Y/N)		
ARMS (5)	WES (Y/N)	Y	N	TOT
OS (4)	Y	7	14	21
ES; HBL (3)	N	1	5	6
PTC; PDC; SPT (2)	TOT	8	19	27
NOS; SYS; MRTK (1)				



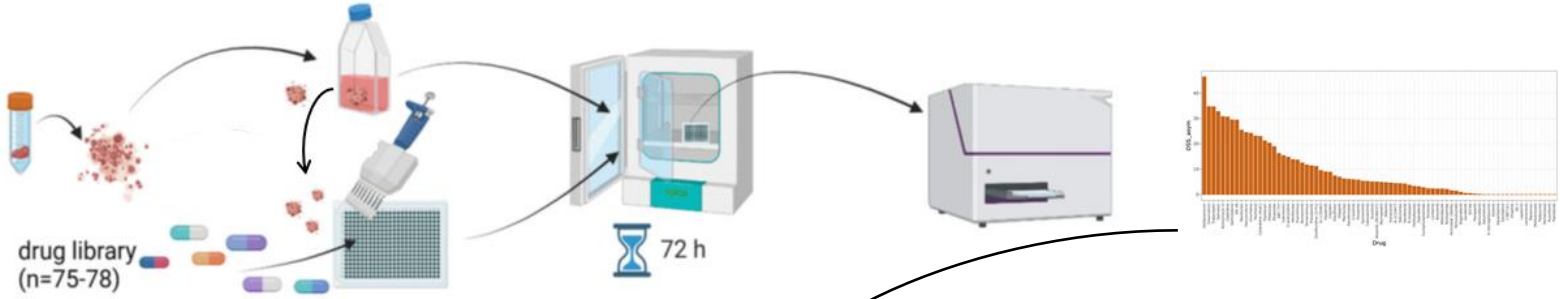
Tumor sample evaluation → Sample mincing → Enzymatic digestion → **PDC culture** → **DSP**

MOLECULAR PROFILING: **WHOLE EXOME SEQUENCING** – SNV/CNA/LOH

TUMOR	mut/Mb	HOTSPOTS SOMATIC VARIANTS	MOST RELEVANT VARIANTS	AMPLIFIED CANCER GENES	DELETED CANCER GENES	cnLOH CANCER GENES	PATHOGENIC / LIKELY PATHOGENIC	VARIANTS WITH UNCERTAIN SIGN. (VUS)	AMPLIFIED CANCER GENES	DELETED CANCER GENES	cnLOH CANCER GENES	DDR GENES
ARMS	0,51	0	1	146	32	0		PAPPA2	**ALK**; DNMT3A; **MYCN**; **CDK4**; RPTOR; PARP3; **CHEK1**; MSH2; MSH6;	LATS1; MST1		ARID1A Q1142*; RECQL4 R804Q
MRTK	0,51	1	1	206	31	9	**TP53**		KMT5A; **SIRT1**; **ABCB1**; NAE1;		**SMARCB1 (INI1)**	
SYS	0,27	1	2	0	675	0		MOB1B; NONO	**HRAS**; IGF2; FGFR3; **AKT1**	HLA-A/B/C; MSH3; MSH2; MSH6; MLH1; TAP1/TAP2; PTEN; CD274; BRCA2; RAD51B; RAD51D;		
HBK	**5,24**	2	8	754	12	0	**FGFR4**	FGFR4; TYRO3	BCL9; **MCL1**; TPM3; **CCNE1**; **NTRK1**; **KRAS**; ABCC3			
ES	0,61	0	7	84	13	1	ATR; KMT2D	PMS1; STAG2	**PARP1**; TRAF5; **NTRK1**; **YAP1**; ARNT; **MCL1**;	PPP4R2		
OS	0,29	3	2	10	11	2	**TP53**	HIST1H3B; SSBP1; TIMP2	**CCNE1**; CALR; CARM1	PPP4R2		

Drug Sensitivity Profiling (DSP)

Drug library preparation and seeding of PDC → Treatment 72h → Viability readout → Analysis



Drug Sensitivity Score (DSS)

- Drug potency
- (IC50) maximum
- Efficacy curve slope
- Area under the curve (AUC)
- Tested concentration range

→

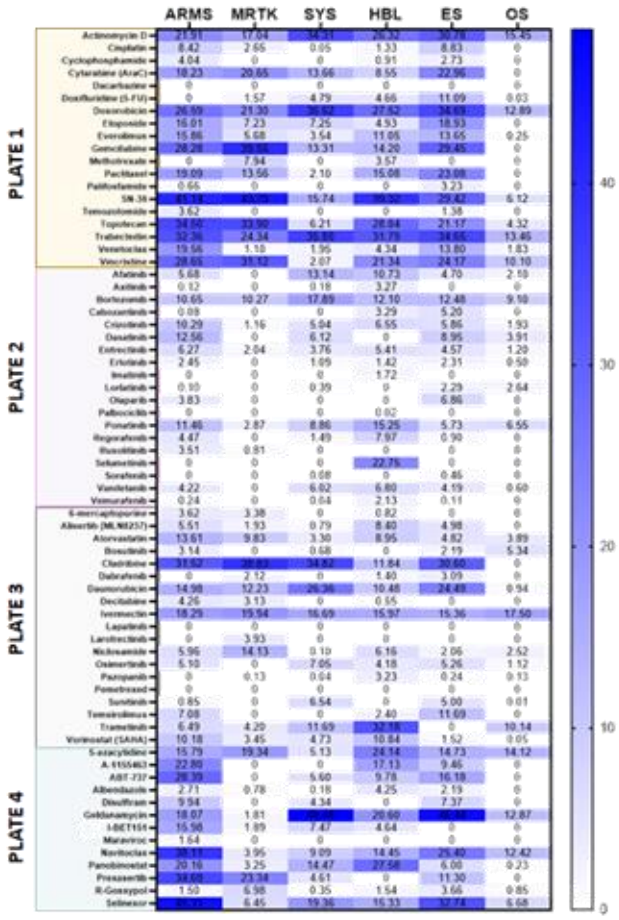
- High DSS → the sample is sensitive to the drug
- Low DSS → the drug has little effect
- DSS close to 0 → no significant activity

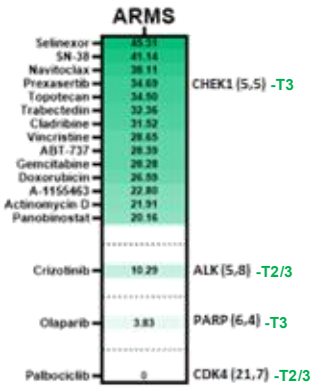
→

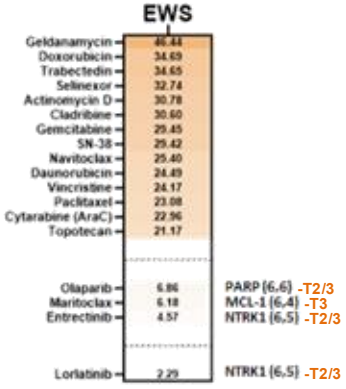
DSS	Interpretation*
0–5	minimal activity
5–10	moderate activity
>10	good sensitivity
>20	strong sensitivity

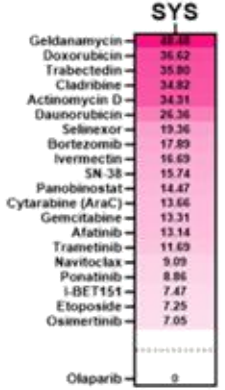
* May vary by lab

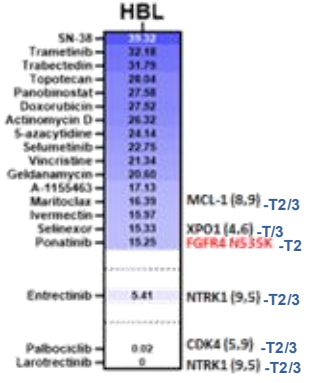
PLATE 1 ALKYLATING AGENTS, ANTIMETABOLITES	PLATE 2 KINASE INHIBITORS	PLATE 3 KINASE INHIBITORS, ANTIMETABOLITE, ANTIHELMINTIC	PLATE 4 REPURPOSING DRUGS, PRO-APOPTOTIC DRUGS, EPIGENETIC MODULTATORS,
Cisplatin	Olaparib	Vorinostat (SAHA)	Navitoclax
Dacarbazine	Palbociclib	Alisertib (MLN8237)	Panobinostat
Gemcitabine	Ponatinib	Osimertinib	Geldanamycin
Paclitaxel	Regorafenib	Pazopanib	Prexasertib
Temozolomide	Ruxolitinib	Pemetrexed	5-azacytidine
Topotecan	Sorafenib	Sunitinib	Albendazole
Actinomycin D	Vemurafenib	Temsirolimus	Disulfiram
Cyclophosphamide	Bortezomib	Bosutinib	JQ1
Cytarabine (AraC)	Crizotinib	6-mercaptopurine	Maraviroc
Doxifluridine (5-FU)	Dasatinib	Atorvastatin	ABT-737
Doxorubicin	Entrectinib	Daunorubicin	A-1155463
Etoposide	Imatinib	Ivermectin	A-1210477
Palifosfamide	Selumetinib	Larotrectinib	S55746
Methotrexate	Afatinib	Trametinib	(R)-(-)-Gossypol (AT-101) acetic acid
Vincristine	Axitinib	Niclosamide	I-BET151
SN-38 (Irinotecan)	Cabozantinib	Dabrafenib	Obatoclax Mesylate (GX15-070)
Trabectedin	Lorlatinib	Decitabine	Sabutoclax
Venetoclax	Vandetanib	Lapatinib	Marinopyrrole A (Maritoclax)
Everolimus	Erlotinib	Cladribine	Selinexor

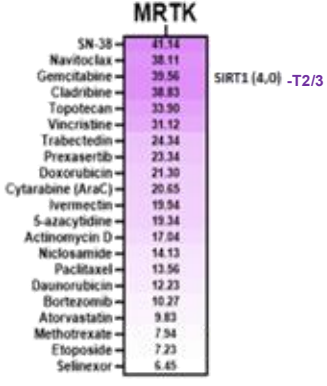

ARMS
MRTK
SYS
HBL
ES
OS
PLATE 1
PLATE 2
PLATE 3
PLATE 4
40
30
20
10
0

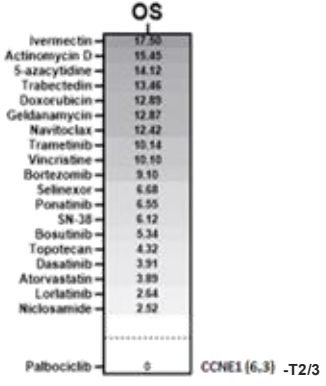

ARMS
Selinexor
SN-38
Navitoclax
Prexasertib
Topotecan
Trabectedin
Cladribine
Vincristine
ABT-737
Gemcitabine
Doxorubicin
A-1155463
Actinomycin D
Panobinostat
CHEK1 (5,5) -T3
Crizotinib
10.29
ALK (5,8) -T2/3
Olaparib
3.83
PARP (6,4) -T3
Palbociclib
0
CDK4 (21,7) -T2/3

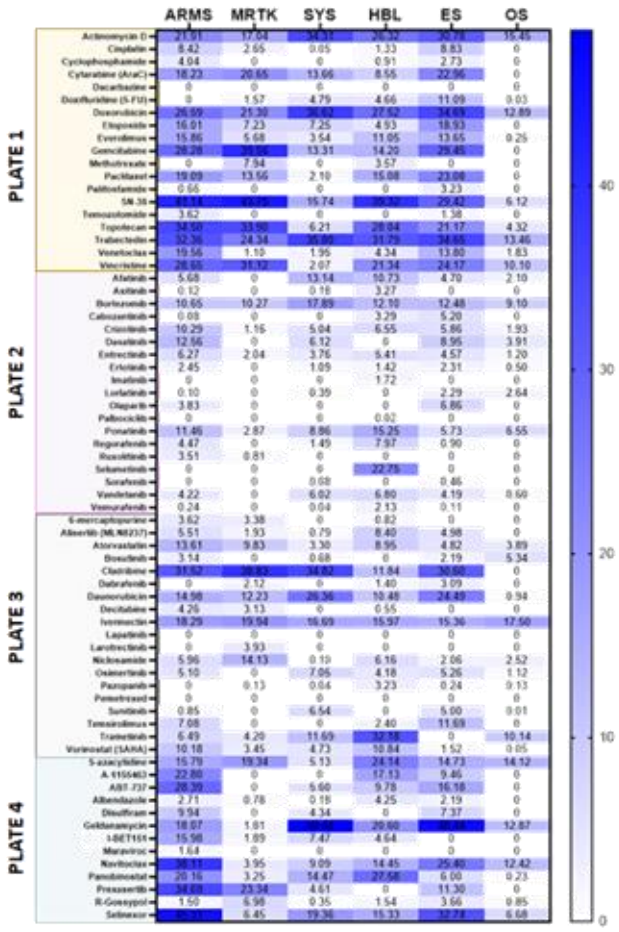

EWS
Geldanamycin
Doxorubicin
Trabectedin
Selinexor
Actinomycin D
Cladribine
Gemcitabine
SN-38
Navitoclax
Daunorubicin
Vincristine
Paclitaxel
Cytarabine (AraC)
Topotecan
Olaparib
6.86
PARP (6,6) -T2/3
Mariteclax
6.18
MCL-1 (6,4) -T3
Entrectinib
4.57
NTRK1 (6,5) -T2/3
Lorlatinib
2.29
NTRK1 (6,5) -T2/3

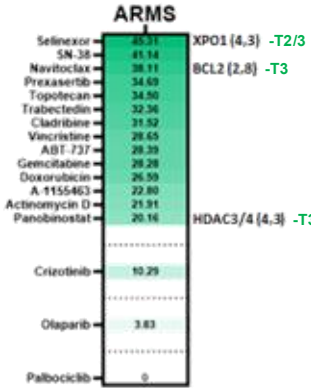

SYS
Geldanamycin
Doxorubicin
Trabectedin
Cladribine
Actinomycin D
Daunorubicin
Selinexor
Bortezomib
Ivermectin
SN-38
Panobinostat
Cytarabine (AraC)
Gemcitabine
Afatinib
Trametinib
Navitoclax
Ponatinib
I-BET151
Etoposide
Osimertinib
Olaparib
0

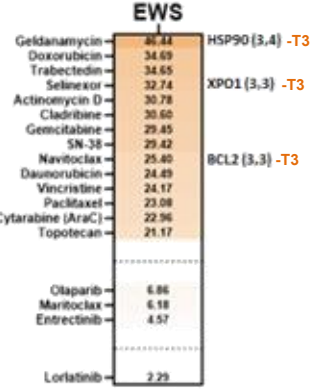

HBL
SN-38
Trametinib
Trabectedin
Topotecan
Panobinostat
Doxorubicin
Actinomycin D
5-azacytidine
Selumetinib
Vincristine
Geldanamycin
A-1155463
Mariteclax
Ivermectin
Selinexor
Ponatinib
MCL-1 (8,9) -T2/3
XPO1 (4,6) -T/3
FGFR4 N535K -T2
Entrectinib
5.41
NTRK1 (9,5) -T2/3
Palbociclib
0.02
CDK4 (5,9) -T2/3
Larotrectinib
0
NTRK1 (9,5) -T2/3

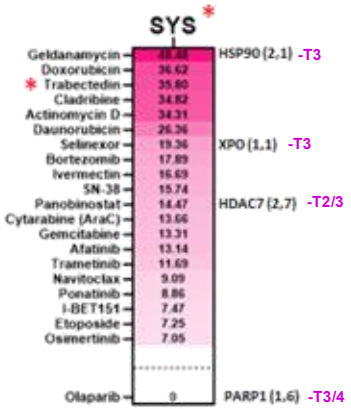

MRTK
SN-38
Navitoclax
Gemcitabine
Cladribine
Topotecan
Vincristine
Trabectedin
Prexasertib
Doxorubicin
Cytarabine (AraC)
Ivermectin
5-azacytidine
Actinomycin D
Niclosamide
Paclitaxel
Daunorubicin
Bortezomib
Atorvastatin
Methotrexate
Etoposide
Selinexor
SIRT1 (4,0) -T2/3

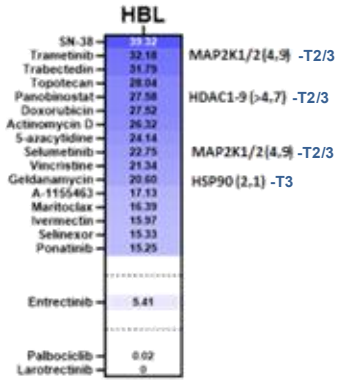

OS
Ivermectin
Actinomycin D
5-azacytidine
Trabectedin
Doxorubicin
Geldanamycin
Navitoclax
Trametinib
Vincristine
Bortezomib
Selinexor
Ponatinib
SN-38
Bosutinib
Topotecan
Dasatinib
Atorvastatin
Lorlatinib
Niclosamide
Palbociclib
0
CCNE1 (6,3) -T2/3

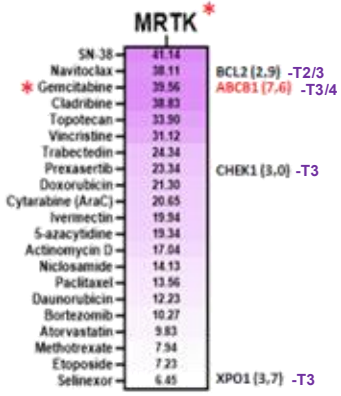

ARMS
MRTK
SYS
HBL
ES
OS
PLATE 1
PLATE 2
PLATE 3
PLATE 4
40
30
20
10
0

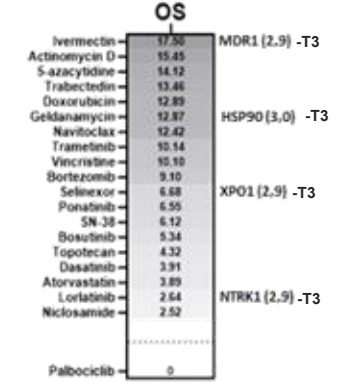

ARMS
Selinexor 45.31
SN-38 41.14
Navitoclax 38.11
Prexasertib 34.69
Topotecan 34.50
Trabectedin 32.36
Cladribine 31.52
Vincristine 28.65
ABT-737 28.39
Gemcitabine 28.28
Doxorubicin 26.59
A-1155463 22.80
Actinomycin D 21.91
Panobinostat 20.16
Crizotinib 10.29
Olaparib 3.83
Palbociclib 0
XPO1 (4,3) -T2/3
BCL2 (2,8) -T3
HDAC3/4 (4,3) -T3

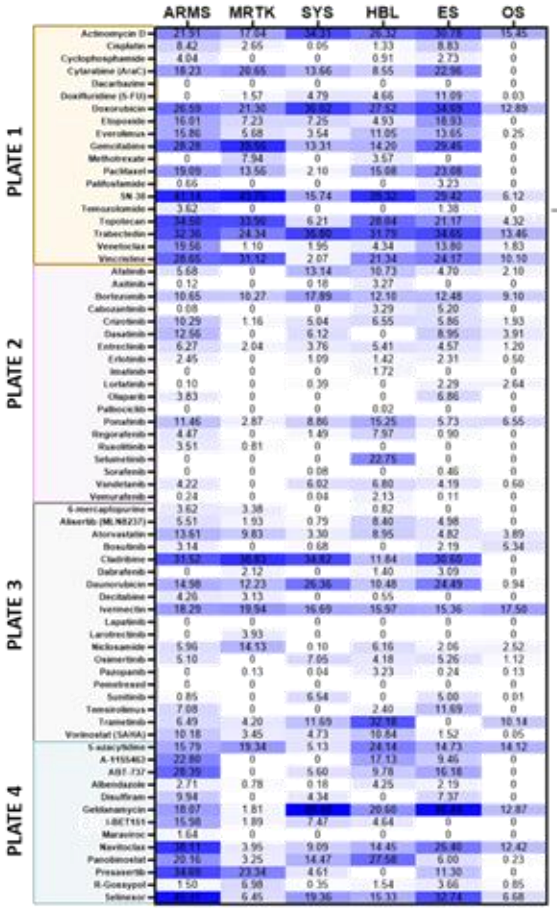

EWS
Geldanamycin 36.44
Doxorubicin 34.69
Trabectedin 34.65
Selinexor 32.74
Actinomycin D 30.78
Cladribine 30.60
Gemcitabine 29.45
SN-38 29.42
Navitoclax 25.40
Daunorubicin 24.49
Vincristine 24.17
Paclitaxel 23.08
Cytarabine (AraC) 22.96
Topotecan 21.17
Olaparib 6.86
Maritoclax 6.18
Entrectinib 4.57
Lorlatinib 2.29
HSP90 (3,4) -T3
XPO1 (3,3) -T3
BCL2 (3,3) -T3


SYS
Geldanamycin 38.44
Doxorubicin 36.62
Trabectedin 35.80
Cladribine 34.82
Actinomycin D 34.31
Daunorubicin 26.36
Selinexor 19.36
Bortezomib 17.89
Ivermectin 16.69
SN-38 15.74
Panobinostat 14.47
Cytarabine (AraC) 13.66
Gemcitabine 13.31
Afatinib 13.14
Trametinib 11.69
Navitoclax 9.09
Ponatinib 8.86
I-BET151 7.47
Etoposide 7.25
Osmertinib 7.05
Olaparib 0
HSP90 (2,1) -T3
XPO (1,1) -T3
HDAC7 (2,7) -T2/3
PARP1 (1,6) -T3/4


HBL
SN-38 33.32
Trametinib 32.18
Trabectedin 31.79
Topotecan 28.04
Panobinostat 27.58
Doxorubicin 27.52
Actinomycin D 26.32
5-azacytidine 24.14
Selumetinib 22.75
Vincristine 21.34
Geldanamycin 20.60
A-1155463 17.13
Maritoclax 16.39
Ivermectin 15.97
Selinexor 15.33
Ponatinib 15.25
Entrectinib 5.41
Palbociclib 0.02
Larotrectinib 0
MAP2K1/2 (4,9) -T2/3
HDAC1-9 (>4,7) -T2/3
MAP2K1/2 (4,9) -T2/3
HSP90 (2,1) -T3


MRTK
SN-38 41.14
Navitoclax 38.11
Gemcitabine 35.56
Cladribine 33.83
Topotecan 33.50
Vincristine 31.12
Trabectedin 24.34
Prexasertib 23.34
Doxorubicin 21.30
Cytarabine (AraC) 20.65
Ivermectin 19.54
5-azacytidine 19.34
Actinomycin D 17.04
Niclosamide 14.13
Paclitaxel 13.56
Daunorubicin 12.23
Bortezomib 10.27
Atorvastatin 9.83
Methotrexate 7.94
Etoposide 7.23
Selinexor 6.45
BCL2 (2,9) -T2/3
ABCB1 (7,6) -T3/4
CHEK1 (3,0) -T3
XPO1 (3,7) -T3


OS
Ivermectin 17.50
Actinomycin D 15.45
5-azacytidine 14.12
Trabectedin 13.46
Doxorubicin 12.89
Geldanamycin 12.87
Navitoclax 12.42
Trametinib 10.14
Vincristine 10.10
Bortezomib 9.10
Selinexor 6.68
Ponatinib 6.55
SN-38 6.12
Bosutinib 5.34
Topotecan 4.32
Dasatinib 3.91
Atorvastatin 3.89
Lorlatinib 2.64
Niclosamide 2.52
Palbociclib 0
MDR1 (2,9) -T3
HSP90 (3,0) -T3
XPO1 (2,9) -T3
NTRK1 (2,9) -T3



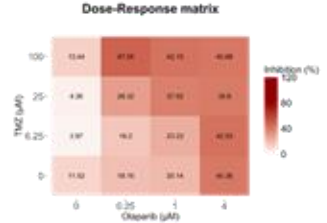
TMZ can upregulate certain molecular targets, potentially enhancing the effect of targeted drugs.

TMZ has shown activity across multiple pediatric tumor types.

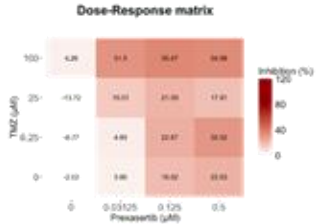
ARMS	MRTK	SYS	HBL	ES	OS
3.62	0	0	0	1.38	0

Temozolomide

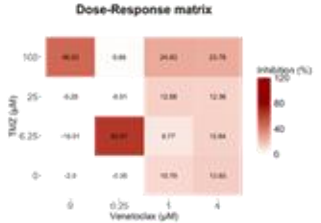
Olaparib (PARP1/2)



Prexasertib (CHK1/2)



Venetoclax (BCL2)



Phase I/II

Preclinical evidence

Very early evidence

ATR p.Glu125Ter (**T1**)
KMT2D p.Cys1471Tyr (**T3**)

Combination	Rationale	Clinical data	Toxicity
TMZ+Olaparib	★★★★	★★★	★★★
TMZ+Prexasertib	★★★★	★★	★★★★
TMZ+Venetoclax	★★★	★	★★★

In conclusion

- Molecular profiling of pediatric solid tumors (sarcoma et al.) is becoming increasingly important for identifying patients who may benefit from targeted therapies.
- Personalized treatment strategies based on individual genomic alterations are highly context- and function-dependent.
- Approaches that assess gene expression and functional activity need to be implemented.
- Methods that use patient-derived tumor cells to evaluate drug sensitivity and/or resistance may complement molecular profiling data but they require further improvements in drug selection and administration.

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