The Pediatric Preclinical Testing Consortium (PPTC)

Malcolm A. Smith, MD, PhD

Cancer Therapy Evaluation Program, NCI

NCI Pediatric Preclinical Testing Consortium

A program funded by the National Cancer Institute of the National Institutes of Health

PPTC Primary Objective

- The PPTC is designed to produce reliable preclinical in vivo data using genomically characterized patient-derived xenograft lines so that childhood cancer clinical researchers can better prioritize which agents to pursue in pediatric clinical trials.

PPTC Key Characteristics

- Academic testing program led by research leaders for the cancers studied
- Large panels of genomically characterized models for the diseases studied
- Focus on in vivo testing to document proof-of-concept for specific responder hypotheses
- Streamlined template-based MTA process
- Particular areas of interest
  - Combinations designed to potentiate cytotoxic therapy
  - Agents targeting pediatric cancer molecular targets
  - Antibody-drug conjugates targeting pediatric-relevant surface antigens

Agent Targets Under Study

- EZH2
- Tubulin
- CHK1
- HDAC (with cytotoxic agents)
- NAMPT
- Wee1 with cytotoxic agents
- XPO1
- Proteasome inhibitor
- LSD1 inhibitor
- DNMT1
- Multitargteted kinase
- DLL3
- DLK1
- Menin
- ROR1
- AKR1C3
- MET
- LRRC15

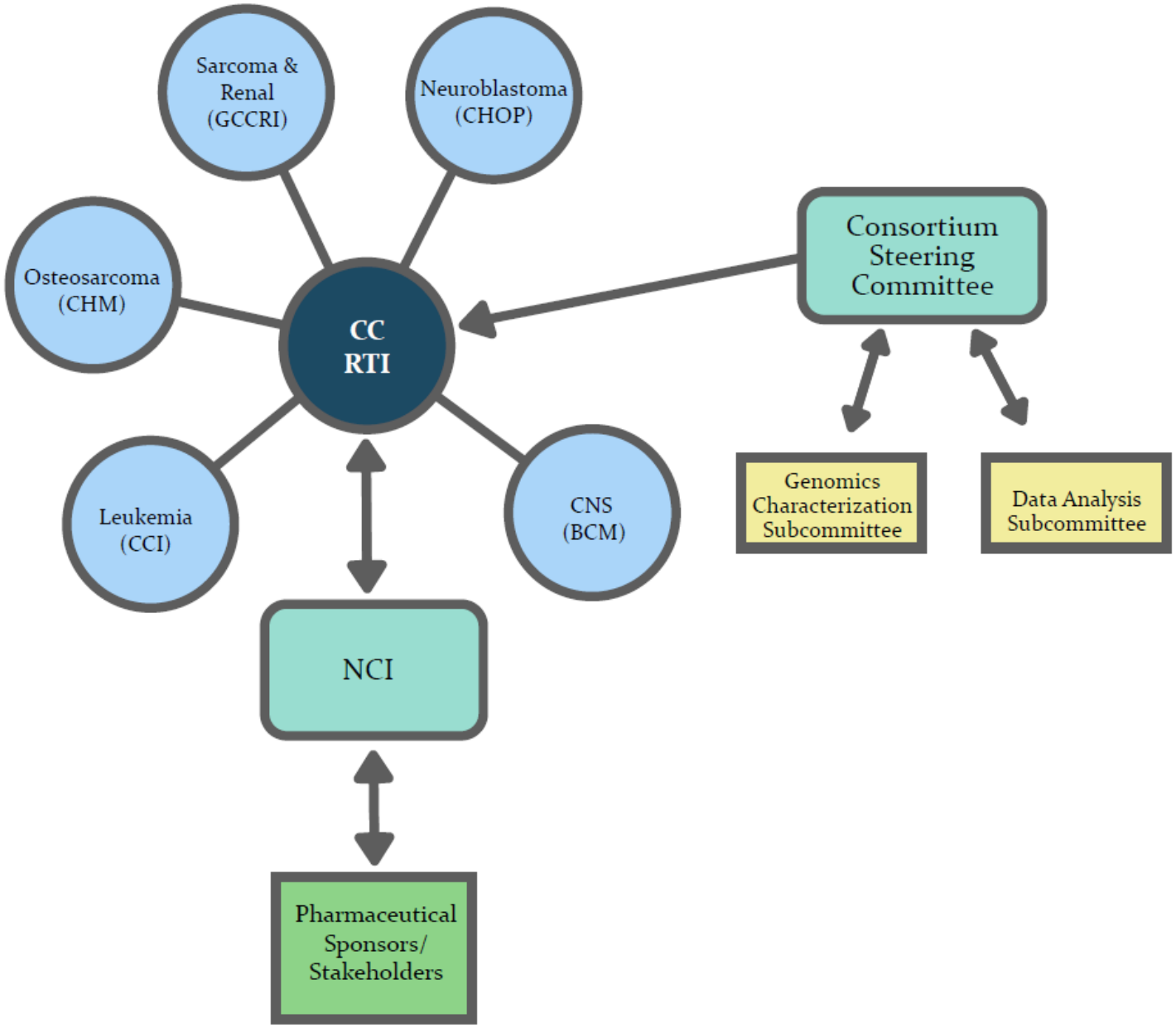
Evaluating Agents Through PPTC

- Confidentiality agreement put in place
- “Agent Proposal” prepared (see application at http://www.ncipptc.org/application)
- Agent reviewed by PPTC Steering Committee
- Research Plan developed by PPTC and finalized after review/edits by Collaborator
- MTA with NCI (see http://www.ncipptc.org/documents)
- Provision of agent to PPTC Coordinating Center at RTI
- In vivo testing of agent at relevant Research Program(s)
- Data sent by Research Program(s) to RTI for analysis by RTI statistical personnel
- Technical report prepared for collaborator approximately 12 months after drug receipt
- Abstracts describing results are submitted to international meeting (e.g., AACR) and manuscripts are submitted for publication (each after review by collaborating company)

PPTC Components

Role	Institution	PI
Coordinating Center	Research Triangle Institute (RTI)	Greg Gatto, PhD
Sarcoma & Renal	Greehey Children's Cancer Research Institute	Peter Houghton, PhD Raushan Kurmasheva, PhD
Neuroblastoma	Children's Hospital of Philadelphia	John Maris, MD Yael Mosse, MD
Osteosarcoma	M.D. Anderson Cancer Center	Richard Gorlick, MD Andy Kolb, MD
Leukemia	Children's Cancer Institute Australia	Richard Lock, PhD
CNS	Baylor College of Medicine	Xiao-Nan Li, MD, PhD
Sponsorship & Funding	National Cancer Institute	Malcolm Smith, MD, PhD Beverly Teicher, PhD

PPTC Organization



PPTC Funding

- Total funding of approximately $2.7 million per year
- Testing of 6 to 10 new agents (or combinations of agents) annually across relevant, well-characterized preclinical models.
- Limited funding for PK/PD studies

PPTC Material Transfer Agreements

- Company executes MTA with CTEP/NCI for provision of agent for Pediatric Preclinical Testing Consortium only. No other uses of agent under this MTA.
- NCI has executed MTAs with each of participating institutions and a collaboration agreement with the PPTC Coordinating Center
- No significant modifications to MTAs are accepted from companies; terms of institution MTAs and company MTAs are consistent
- Institution awards were based on acceptance of MTA terms

MTA Terms

- Use agent as provided without making any modifications to agent or attempting to analyze compound provided
- Institution indemnifies company for its use of material and data
- Company indemnifies institution for its use of data resulting from the project
- Company granted right to use all data and results for any purpose

Publications and IP Issues

- 45 day company review of all manuscripts and 10 day abstract review
- Institution may not submit without written approval from CTEP to do so after any and all NCI and company comments are addressed
- NCI IP Option to Collaborator included in Institution MTA and Coordinating Center Collaboration Agreement.
  - Company receives non-exclusive royalty free license to any invention for commercial purposes, and first option to negotiate an exclusive or co-exclusive royalty bearing license for commercial purposes.
  - Company will cover patent prosecution and maintenance costs.
  - Company has 3 months to notify institution of interest in obtaining an exclusive license. Extended time period to decide if invention will be useful.
- Unauthorized Use. Company receives a royalty-free exclusive or co-exclusive license.

NCI PPTC
Pediatric Preclinical Testing Consortium

ABOUT MEMBERS APPLICATION RESOURCES CONTACT

PEDIATRIC PRECLINICAL TESTING CONSORTIUM

The NCI PPTC addresses key challenges associated with the development of new therapies for children with cancer by developing reliable preclinical testing data for pediatric drug candidates that can be used to inform new agent

Genomics

Alex's Lemonade Stand Foundation Pediatric Preclinical Genomic Characterization Project

The Pediatric Preclinical Testing Consortium (PPTC) gratefully acknowledges the support of Alex's Lemonade Stand Foundation (ALSF) for the characterization of patient-derived xenograft (PDX) childhood cancer models with advanced genomic technology.

- The ALSF contribution allowed the PPTC, in collaboration with the Baylor College of Medicine and Nationwide Children's Hospital, to generate 152 new whole exome sequences, 105 new SNP array genotypes, and 237 RNA sequences.
- The data will be available to all academically qualified petitioners through the database of Genotypes and Phenotypes (dbGaP). ✱
- The data will be merged with existing Pediatric Preclinical Testing Program (PPTP) PDX sequencing data at the cBio portal, so that complete genomic data will be available on 237 PDX models across 23 unique childhood cancers. ✱
- A goal of the PPTC is to freely share data and models, and additional instructions will be provided when the data are fully available at the cBio Portal.

✱ The Alex's Lemonade Stand Foundation Preclinical Genomic Characterization project data is available to support your research. Acknowledgment of this resource is required in any resulting publications including journal articles, institutional media releases and newsletters or presentations with the following text:

> *This research used data from Alex's Lemonade Stand Foundation Pediatric Preclinical Genomic Characterization project.*

Notification of any printed acknowledgements must be sent to the Foundation in a timely manner (Grants@AlexsLemonade.org). The Foundation logo is available upon request from the same email.

Pediatric Preclinical Testing Consortium (Maris, 2018)

As part of an overall strategy for improving therapies for child-hood cancers, the PPTC seeks to develop models for the types of tumors that will be encountered in early phase clinical testing by establishing patient derived xenografts (PDXs) from high-risk childhood cancers refractory to current standard of care treatments. Genomic profiling of these models is required to enable PPTC investigators to develop robust "responder hypotheses" when drug activity is observed. With funding provided by Alex's Lemonade Stand Foundation, we genomically characterize a major subset of 265 PDX models. We use whole exome sequencing, transcriptome sequencing, and SNPArray to characterize the tumor models. The focus on DNA and RNA sequencing data mirrors the current standard practice in most clinical diagnostics lab that use these technologies to detect the spectrum of targetable mutations, gene amplifications, and gene fusion events relevant to preclinical drug development.

Study Summary | Clinical Data | Mutated Genes | Copy Number Alterations

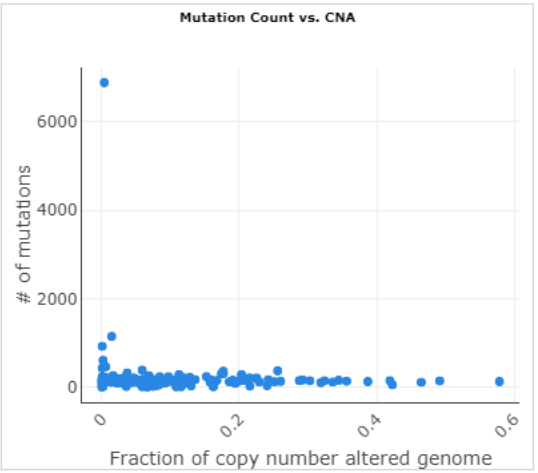
Selected: 265 samples / 265 patients query genes - click to expand Query Select cases by IDs

CANCER_TYPE	#	Freq
ALL	90	33.96%
Osteosarcoma	36	13.58%
Neuroblastoma	35	13.21%
Medulloblastoma	21	7.92%
Wilms	13	4.91%
Ewing Sarcoma	10	3.77%
ATRT	8	3.02%
Embryonal Rhabdomyosarcoma	7	2.64%
PNET	7	2.64%
Alveolar Rhabdomyosarcoma	7	2.64%
Glioblastoma	6	2.26%

Search...

CANCER_TYPE_DETA...	#	Freq
BCP-ALL	37	13.96%
Osteosarcoma	36	13.58%
Neuroblastoma	35	13.21%
Medulloblastoma	21	7.92%
T-ALL	19	7.17%
Ph-likeALL	15	5.66%
Wilms	13	4.91%
MLL-ALL	10	3.77%
Ewing Sarcoma	10	3.77%
PNET	8	3.02%
ATRT	8	3.02%

Search...



Mutated Genes (243 profiled samples)

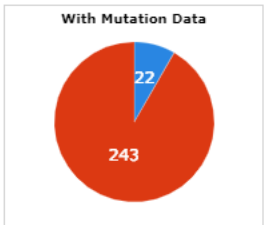
Gene	# Mut	#	Freq
TTN	129	88	36.21%
TP53	57	53	21.81%
MUC16	50	41	16.87%
FLG	37	35	14.40%
OBSCN	40	32	13.17%
KMT2D	36	32	13.17%
MUC17	33	29	11.93%
USH2A	29	28	11.52%
PLEC	33	28	11.52%
FAT3	28	25	10.29%
NOTCH1	34	25	10.29%

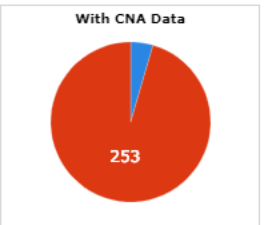
Search...

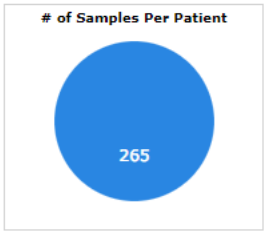
CNA Genes (253 profiled samples)

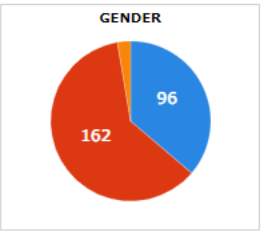
Gene	Cytoband	CNA	#	Freq
MYCN	2p24.3	AMP	25	9.88%
ACVR2A	2q22.3-...	AMP	4	1.58%
MYC	8q24.21	AMP	3	1.19%
IRS2	13q34	AMP	3	1.19%
EIF4A2	3q27.3	AMP	2	0.79%
EPHB3	3q27.1	AMP	2	0.79%
ETV5	3q27.2	AMP	2	0.79%
MECOM	3q26.2	AMP	2	0.79%
GLI1	12q13.3	AMP	2	0.79%
IGF1R	15q26.3	AMP	2	0.79%
MEF2A	15q26.3	AMP	2	0.79%

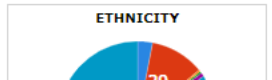
Search...











SITE_OF_INITIAL_T...	#	Freq
Unknown	144	54.24%

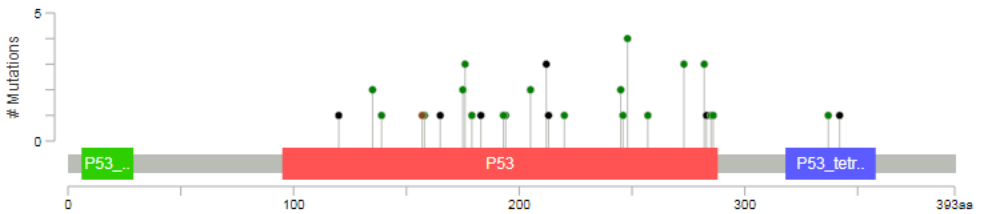
Modify Query

Pediatric Preclinical Testing Consortium (Maris, 2018)
All cases in study (**265** samples) / **1** Genes

Gene Set / Pathway is altered in 53 (20%) of queried samples

OncoPrint | Cancer Types Summary | Plots | Mutations | Co-Expression | Tumor vs Normals | Enrichments | Network | CN Segments | Download | Bookmark

TP53



TP53
UniProt: <html><head>
Transcript: ENST00000269305

Somatic Mutation Frequency: 20.0%

32 Missense 12 Truncating
1 Inframe 12 Other

View 3D Structure

57 Mutations (page 1 of 3)

Columns

Sample ID	Cancer Type	Protein Change	Annotation ▼	Mutation Type	Copy #	COSMIC	Allele Freq (T)	# Mut in Sample
ALL-121	T-ALL	***R273C***		**Missense**	Diploid	1312	0.35	926
IC-1621GBM	Glioblastoma	***R273C***		**Missense**	Diploid	1312	0.62	6883
Rh-30R	Alveolar Rhabdomyosarcoma	***R273C***		**Missense**	Diploid	1312	0.39	50
ALL-121	T-ALL	***R248Q***		**Missense**	Diploid	1298	0.40	926
ES-1	Ewing Sarcoma	***R248Q***		**Missense**	Diploid	1298	0.44	180
NCH-MB-1	Medulloblastoma	***R248Q***		**Missense**	Diploid	1298	0.43	130
PAKSWW	Ph-likeALL	***R248Q***		**Missense**	Diploid	1298	0.99	183
OS-55	Osteosarcoma	***E285K***		**Missense**	Diploid	141	1.00	156
KT-18	Wilms	***R282W***		**Missense**	Diploid	536	1.00	86
Rh-12	Embryonal Rhabdomyosarcoma	***R282W***		**Missense**	Diploid	536	0.91	124
KT-13	Wilms	***C176Y***		**Missense**	Diploid	261	0.97	118
IC-6634GBM	Glioblastoma	***R175H***		**Missense**	Diploid	992	0.97	25
OS-44	Osteosarcoma	***R175H***		**Missense**	Diploid	992	0.97	133
OS-54-OHS-x	Osteosarcoma	***E286K***		**Missense**	Diploid	113	1.00	311
ICb-5610MB	Medulloblastoma	***H193R***		**Missense**	Diploid	196	1.00	124
OS-46	Osteosarcoma	***H179Q***		**Missense**	Diploid	329	0.99	157
COG-N-519x	Neuroblastoma	***G245S***		**Missense**	Diploid	607	1.00	146

Modify Query

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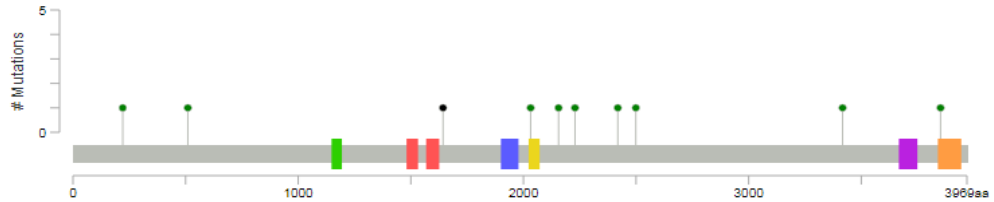
Gene Set / Pathway is altered in 20 (7.5%) of queried samples

OncoPrint | Cancer Types Summary | Plots | Mutations | Co-Expression | Tumor vs Normals | Enrichments | Network | CN Segments | Download | Bookmark

KMT2A



KMT2A
UniProt: <html><head>
Transcript: ENST00000389506

Somatic Mutation Frequency: 7.5%

9 Missense 1 Truncating
0 Inframe 11 Other

View 3D Structure

21 Mutations (page 1 of 1)

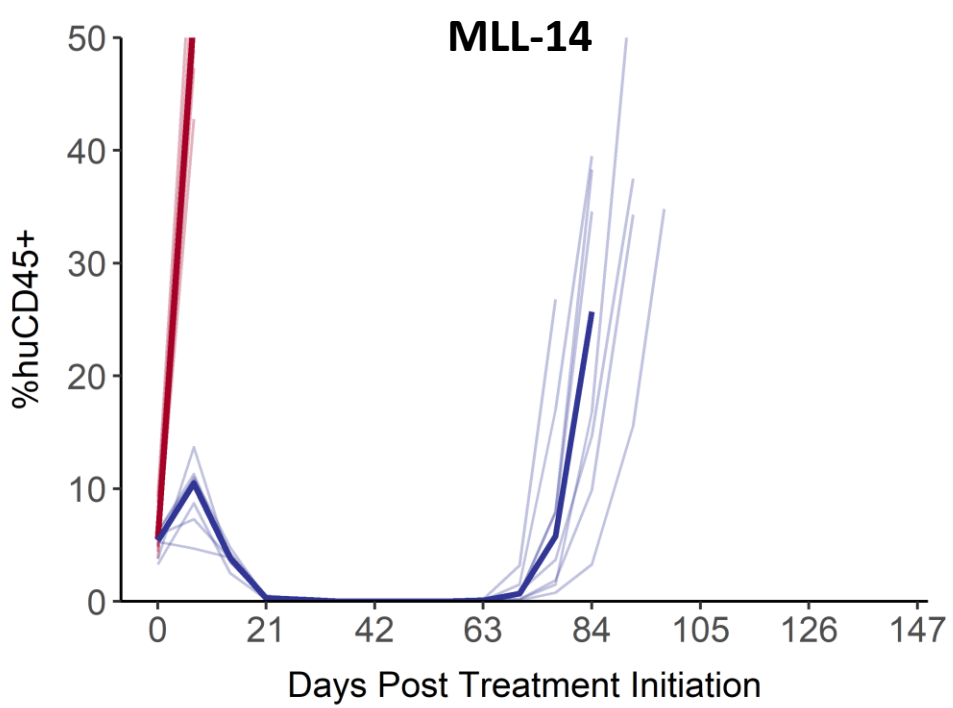
Columns

Sample ID	Cancer Type	Protein Change	Annotation ▼	Mutation Type	Copy #	COSMIC	Allele Freq (T)	# Mut in Sample
ALL-54	BCP-ALL	***L1643fs***		**FS ins**	Diploid		0.30	463
MLL-2	MLL-ALL	***KMT2A-AFF1***		Fusion	Diploid			107
MLL-7	MLL-ALL	***KMT2A-AFF1***		Fusion	Diploid			117
MLL-86	MLL-ALL	***KMT2A-AFF1***		Fusion				163
MLL-1	MLL-ALL	***KMT2A-EPS15***		Fusion	Diploid			145
MLL-3	MLL-ALL	***KMT2A-GAS7***		Fusion	Diploid			162
ALL-03	MLL-ALL	***KMT2A-MLLT1***		Fusion	Diploid			134
MLL-14	MLL-ALL	***KMT2A-MLLT1***		Fusion	Diploid			95
MLL-6	MLL-ALL	***KMT2A-MLLT1***		Fusion	Diploid			109
MLL-8	MLL-ALL	***KMT2A-MLLT1***		Fusion	Diploid			134
MLL-5	MLL-ALL	***KMT2A-MLLT10***		Fusion	Diploid			5
ETP-5	ETP-ALL	***KMT2A-MLLT4***		Fusion	Diploid			152
BT-27	PNET	***K221N***		**Missense**	Diploid		0.26	118
ALL-39	T-ALL	***T3417I***		**Missense**	Diploid		0.45	264
COG-N-480x	Neuroblastoma	***G2499E***		**Missense**	Diploid		0.54	106
IC-1621GBM	Glioblastoma	***I3852S***		**Missense**	Diploid		0.34	6883
IC-1621GBM	Glioblastoma	***D2032N***		**Missense**	Diploid		0.59	6883
ICb-2555MB	Medulloblastoma	***H510R***		**Missense**	Diploid		0.43	184
ICb-5610MB	Medulloblastoma	***S2229T***		**Missense**	Diploid		1.00	124
PAKVKK	Ph-likeALL	***T2156I***		**Missense**	Diploid		0.43	142
SK-NEP-1	Ewing Sarcoma	***E2419K***		**Missense**	Diploid		0.42	147

Showing 1-21 of 21 Mutations

Menin Inhibitor for MLL-Rearranged Infant ALL

- The menin-MLL interaction is critical for the oncogenic activity of MLL fusion proteins
- Blocking the menin-MLL interaction represents an attractive therapeutic strategy
- VTP-50469 is a potent small molecule inhibitor of the menin-MLL interaction



Lock, et al. Abstract 3187, AACR 2018

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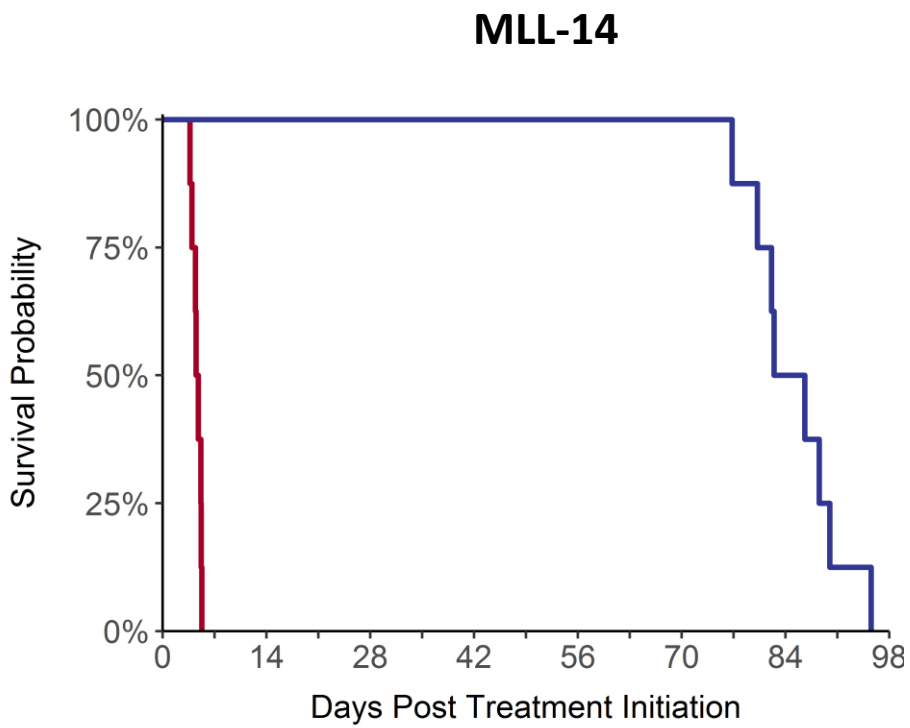

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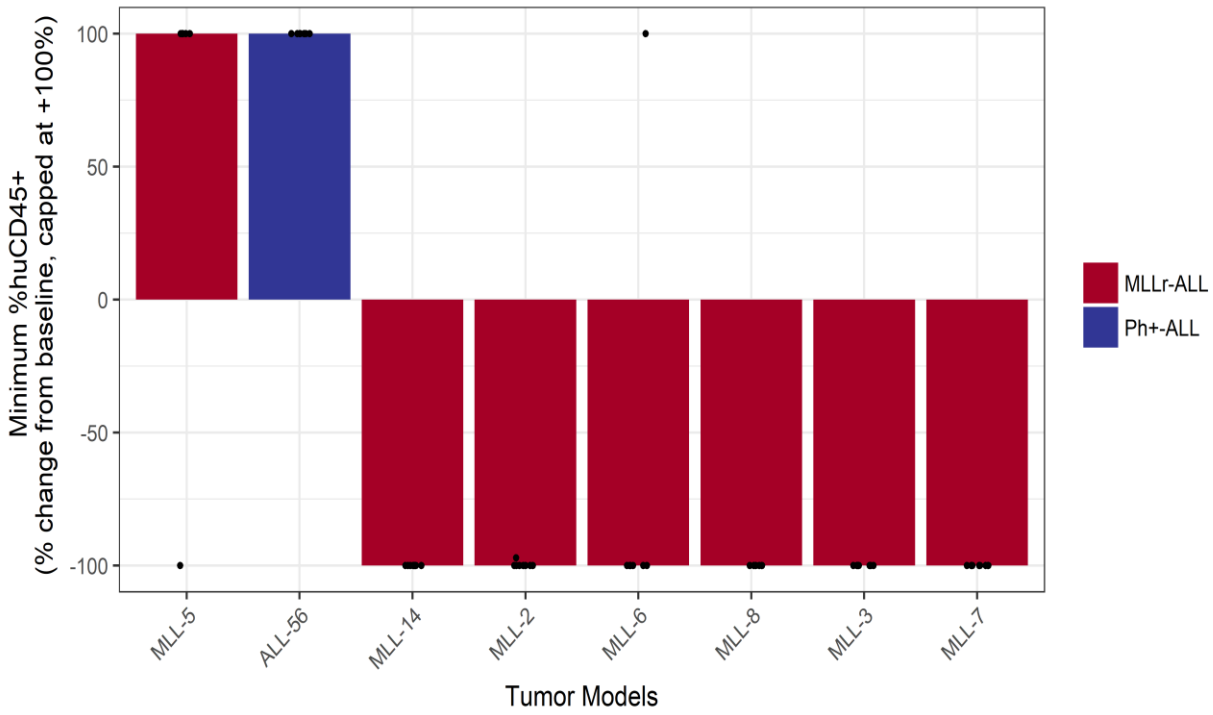
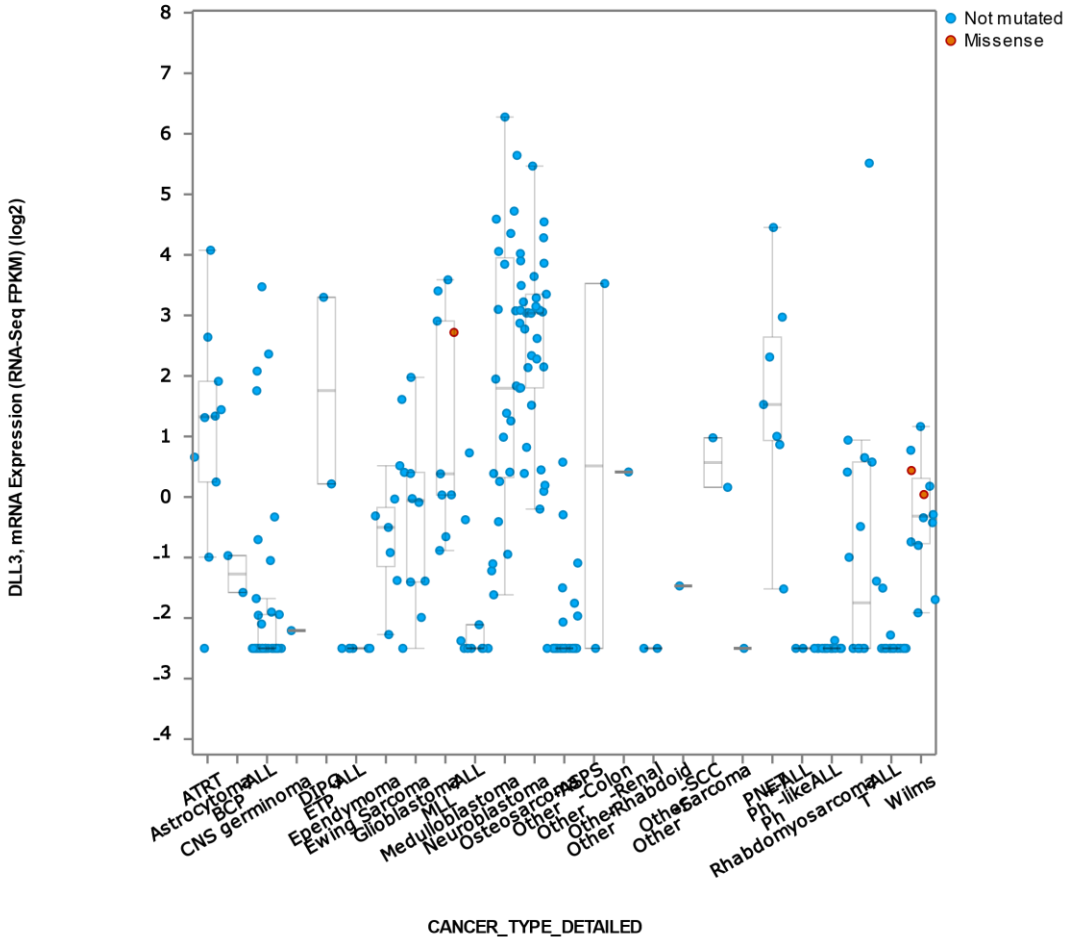
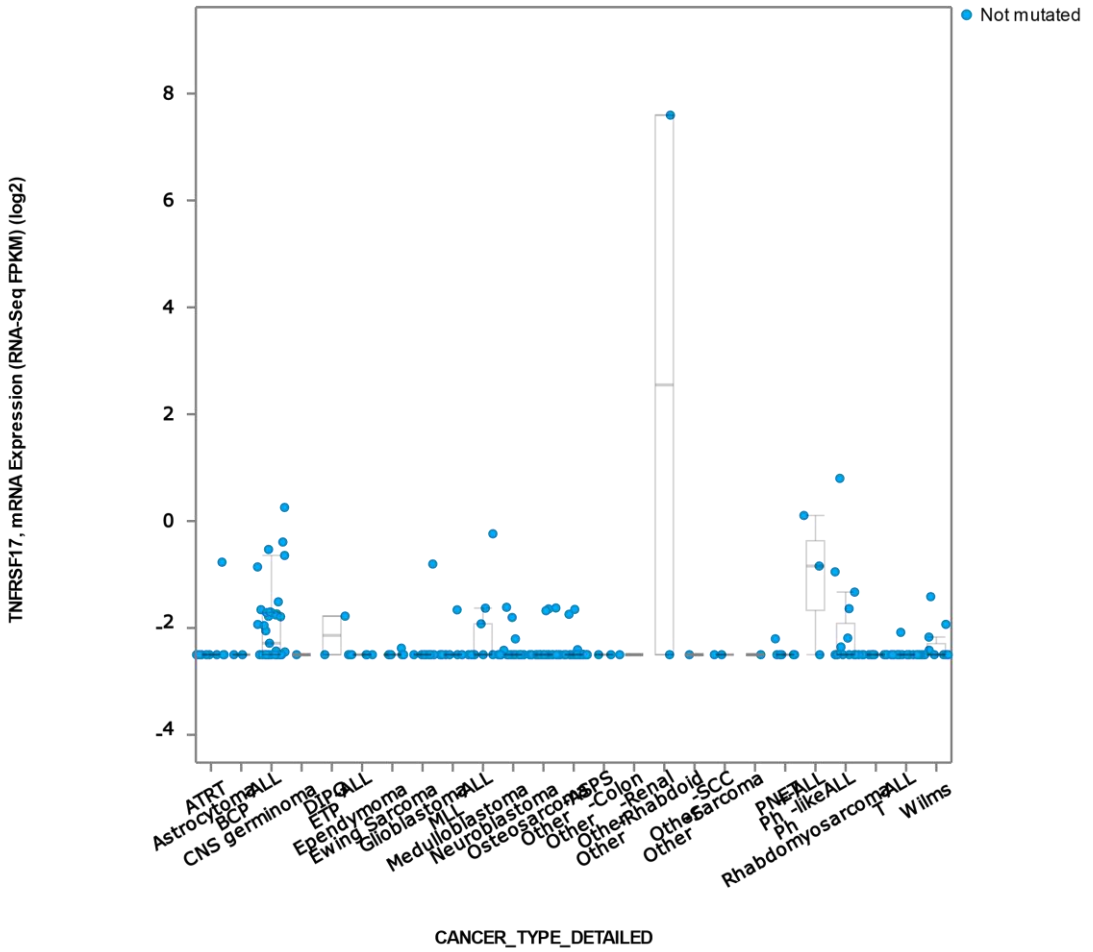


Lock, et al. Abstract 3187, AACR 2018

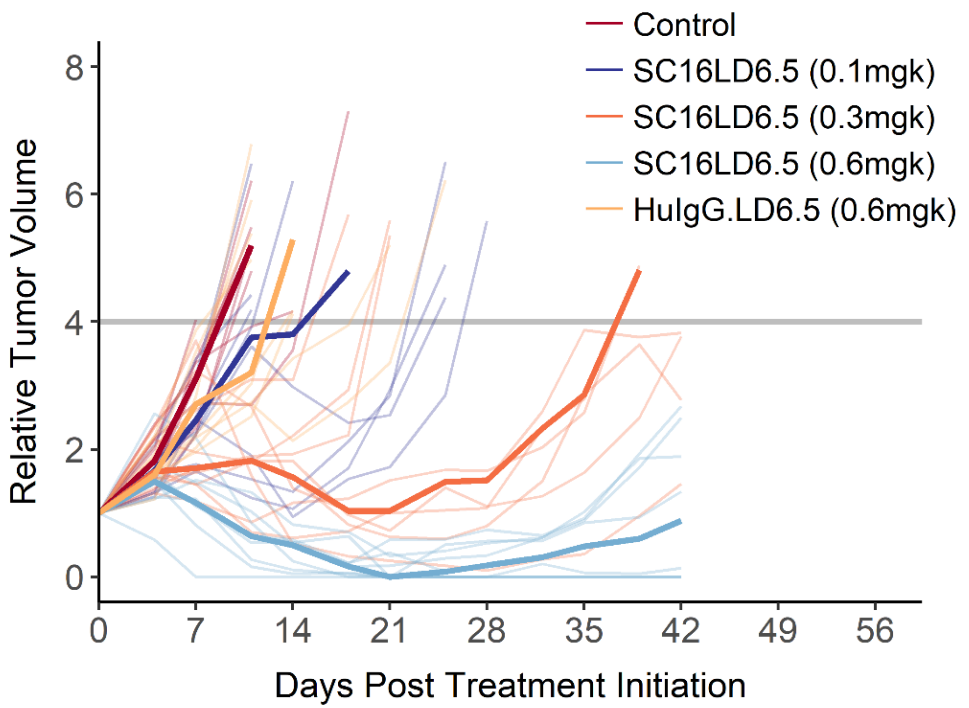
PPTC RNA-seq Results: DLL3 and BCMA (TNFRSF17)





Antibody-Drug Conjugate Targeting DLL3 for Neuroblastoma

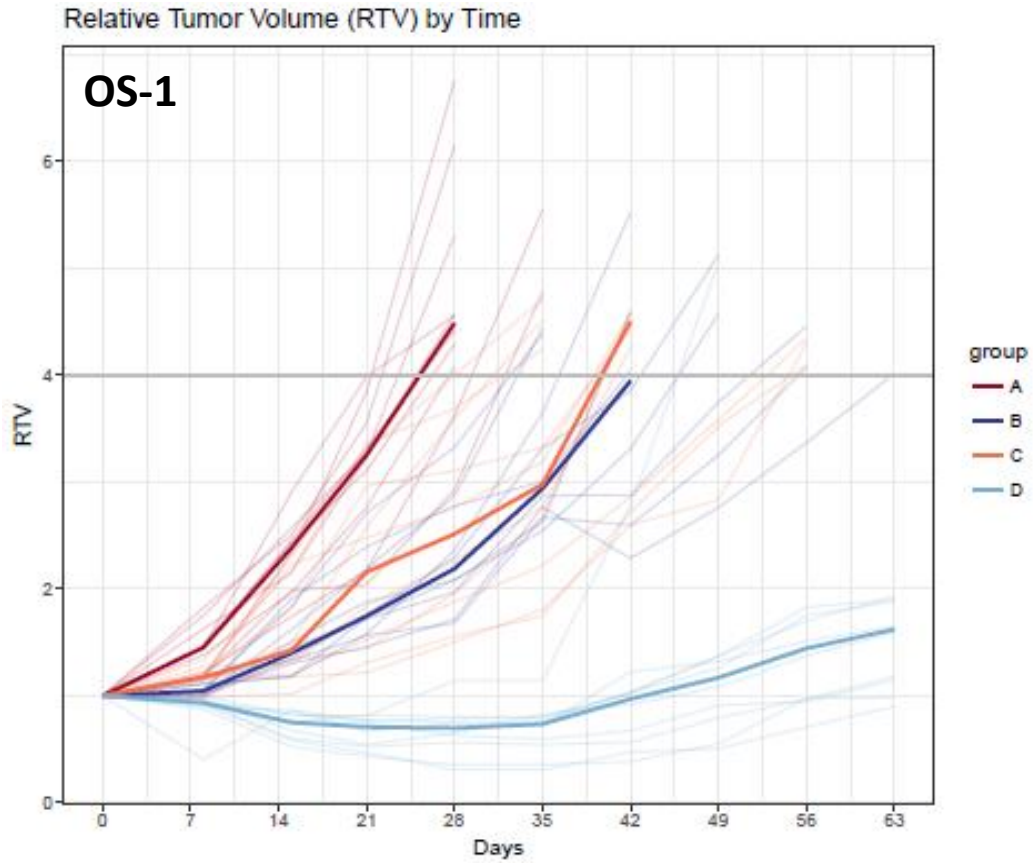
- Delta-like 3 (DLL3) is robustly and differentially expressed on the cell surface of neuroblastoma
- Rova-T (SC16LD6.5) is an ADC targeting DLL3 composed of the monoclonal antibody SC16 conjugated to the DNA-damaging D6.5 pyrrolobenzodiazepine (PBD) dimer toxin
- Rova-T shows anti-tumor activity in patients with small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) with high DLL3 protein expression
- PPTC showed substantial tumor-regressing activity for neuroblastoma PDXs



Sano, et al. Abstract LB-136, AACR 2018

Wee1 Inhibition Potentiates Irinotecan Activity for Selected Preclinical Models

- AZD1775 is a potent and selective ATP-competitive inhibitor of WEE1
- AZD1775 has primarily been studied for its ability to potentiate the genotoxic activity of anticancer agents
- PPTC showed potentiation of irinotecan activity, with most consistent effect observed for osteosarcoma PDXs



Houghton, et al. Abstract LB-B15, AACR-NCI-EORTC Molecular Targets Mtg

A. Control; **B. AZD1775**; **C. Irinotecan**; **D. AZD1775 + Irinotecan**

Wee1 Inhibition Potentiates Irinotecan Activity for Selected Preclinical Models

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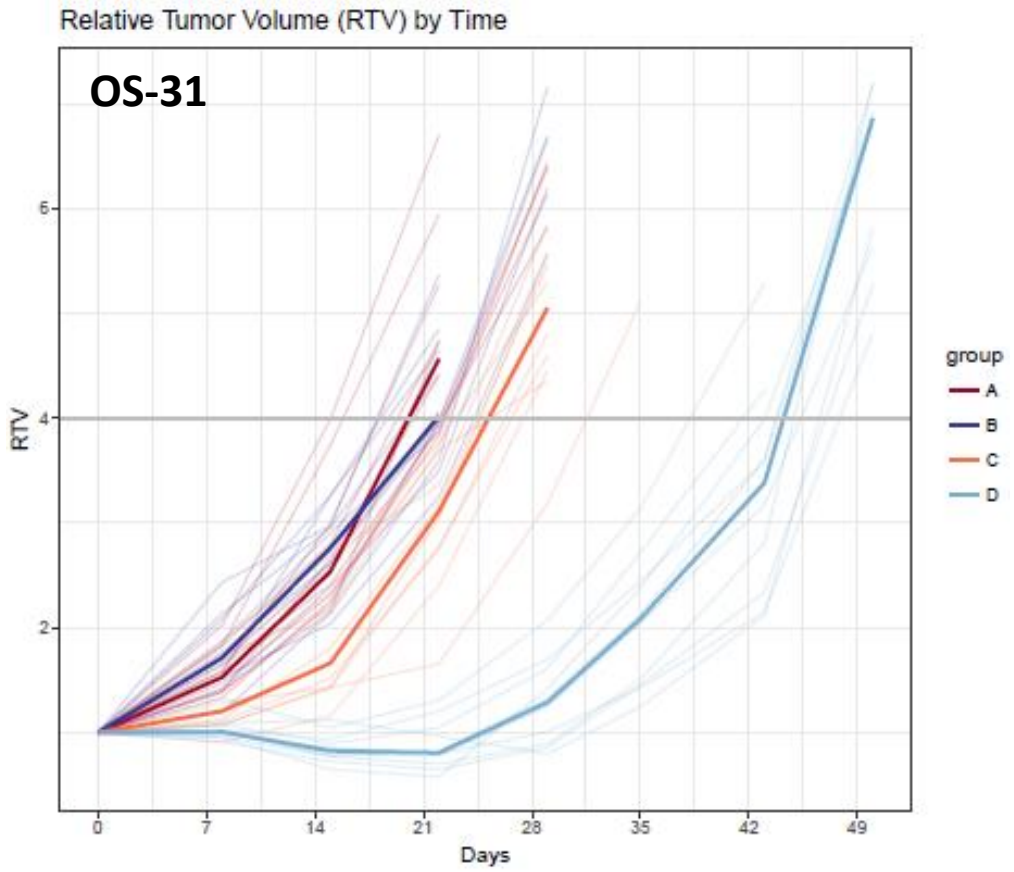
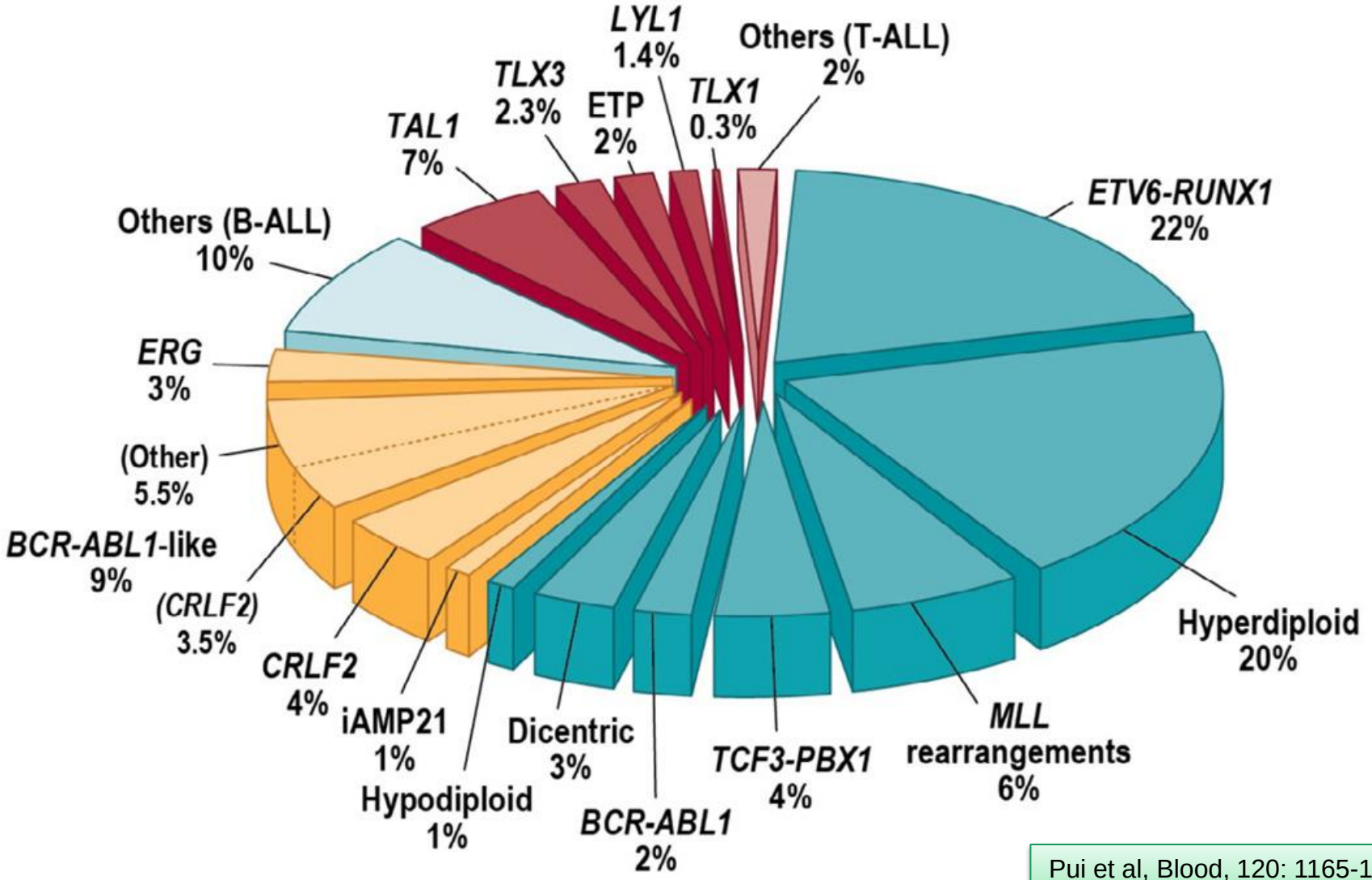


Houghton, et al. Abstract LB-B15, AACR-NCI-EORTC Molecular Targets Mtg

A. Control; **B. AZD1775**; **C. Irinotecan**; **D. AZD1775 + Irinotecan**

Heterogeneity of Pediatric ALL Subtypes



Pui et al, Blood, 120: 1165-1174, 2012

Single Mouse Trial – Pilot Study

- 80 xenografts inoculated in 2 mice each (batches of 40, 40 and 80 mice)
- All mice bled starting at 2 weeks after inoculation
- Treatment began for each mouse when it reached 1% huCD45^{+} cells in the peripheral blood
- Event defined as 25% huCD45^{+} cells in the peripheral blood or animal exhibiting leukaemia-related morbidity (defined as >50% infiltration of 2 or more organs from spleen, bone marrow, liver, kidney, brain or spinal cord)
- Other mice excluded from analysis
- Treatments included:
  - Topotecan (0.6 mg/kg IP daily x 5 x 2 weeks, repeated at 21 days) – topoisomerase I inhibitor
  - Birinapant (15 mg/kg IP every 3 days x 5) – SMAC mimetic, IAP degradation, TNF-mediated apoptosis
- When each mouse reaches event leukaemia cells harvested for SNP validation of identity

SMT Summary

- Single mouse data accurate to ± 1 ORM in >85% of mice tested
- Elimination of contaminated PDX stocks (thymoma) and propensity for NOD/SCID mice to develop thymoma (NSG) ➔ >90% testing success rate
- Possibility of PDX studies on an almost clinical trial scale due to the reproducibility of the ALL PDX model
- Can almost encompass the heterogeneity of ALL in a single experiment
- Can be used for large scale correlations with *in vitro* data
- Power to identify biomarkers of response to established and novel drugs in paediatric ALL when combined with exome-seq, RNA-seq and DNA copy number analysis
- Will present significant logistical challenges to implement routinely

NCI PPTC
Pediatric Preclinical Testing Consortium

ABOUT MEMBERS APPLICATION RESOURCES CONTACT

PEDIATRIC PRECLINICAL TESTING CONSORTIUM

The NCI PPTC addresses key challenges associated with the development of new therapies for children with cancer by developing reliable preclinical testing data for pediatric drug candidates that can be used to inform new agent prioritization decisions.

www.ncipptc.org