Caroline Symmes Foundation Update

Riley Hospital for Children
Indiana University Health

INDIANA UNIVERSITY
SCHOOL OF MEDICINE

July 26, 2018

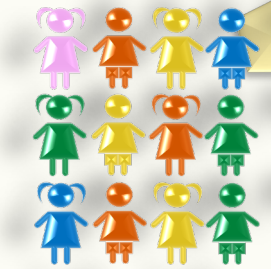
A Partnership!

The Caroline Symmes Cancer Endowment: Supporting pediatric cancer research

NICHD/Riley U54 Grant: Funding to identify innovative, targetable oncogenes in pediatric sarcomas

IU School of Medicine and Riley Children's Foundation: Helping to find new options to improve outcomes in children with aggressive disease.

THANK YOU SO MUCH FOR YOUR SUPPORT!



The Caroline Symmes Cancer Endowment
Riley Hospital for Children

NIH
Eunice Kennedy Shriver National Institute of Child Health and Human Development

Riley Children's Foundation

SCHOOL OF MEDICINE
INDIANA UNIVERSITY

Caroline Symmes Foundation: *A partner in helping develop a premier center for Pediatric Non-CNS Solid Tumors*

- Many pediatric, non-CNS solid tumor cancers continue to lag
- We are making great progress in trying to advance outcomes in aggressive pediatric solid tumors
- Broadening the net to include other non-CNS solid tumors
  - Wilms' tumor
  - Rhabdomyosarcoma
  - Osteosarcoma
  - Ewing's Sarcoma
  - Neuroblastoma
- With future hopes of designing and executing novel clinical trials to improve outcomes

- Research infrastructure and model development to begin unraveling the molecular underpinnings that drive aggressive solid tumors
  - Biobanking (Longitudinal Sample Collection)
  - Cell lines / Spheroids / PDX / Other models
  - Research-based genomics (germ line) and tumor genomics (Renbarger Lab) to identify relevant patient and tumor genetic characteristics to investigate at the bench (Pollok, et al)
  - Investigating targeted and combination therapies for better clinical outcomes (Pollok Lab)
  - Sequencing/pharmacogenetics (Renbarger Lab) Personnel needs (postdoc and research associate level scientists) to expand critical and needed infrastructure for scientists to be successful
  - Bioinformatics and systems pharmacology
- Education - Developing translational researchers/physician scientists
- Pilot research funding to follow-up on novel findings to build innovative niche
- Faculty / Physician and Research Scientists
  - Develop expertise/internal and external (more translational niche)
  - Structured recruitment to bridge clinical and research link
  - Structured recruitment for 'all phase' patient care

The Precision Genomics Program at Riley Hospital for Children at IU Health

The Precision Genomics Program at Riley at IU Health - Overview

The Precision Genomics Program at Riley at IU Health helps children with all types of relapsed or aggressive cancer receive new treatment options that may be effective when other treatment options no longer work.

The PG team uses genetic testing to identify DNA, RNA and proteins in cancer cells that can be precisely targeted with newer, targeted treatments.

The Precision Genomics Program is the only program of its kind in Indiana. Our multidisciplinary team includes pediatric oncologists, a nurse coordinator, pharmacist, genetic counselor, molecular geneticist and ethics worker.

The Precision Genomics Program at Riley at IU Health – Patients in Program Since 2016

160

Total Patients

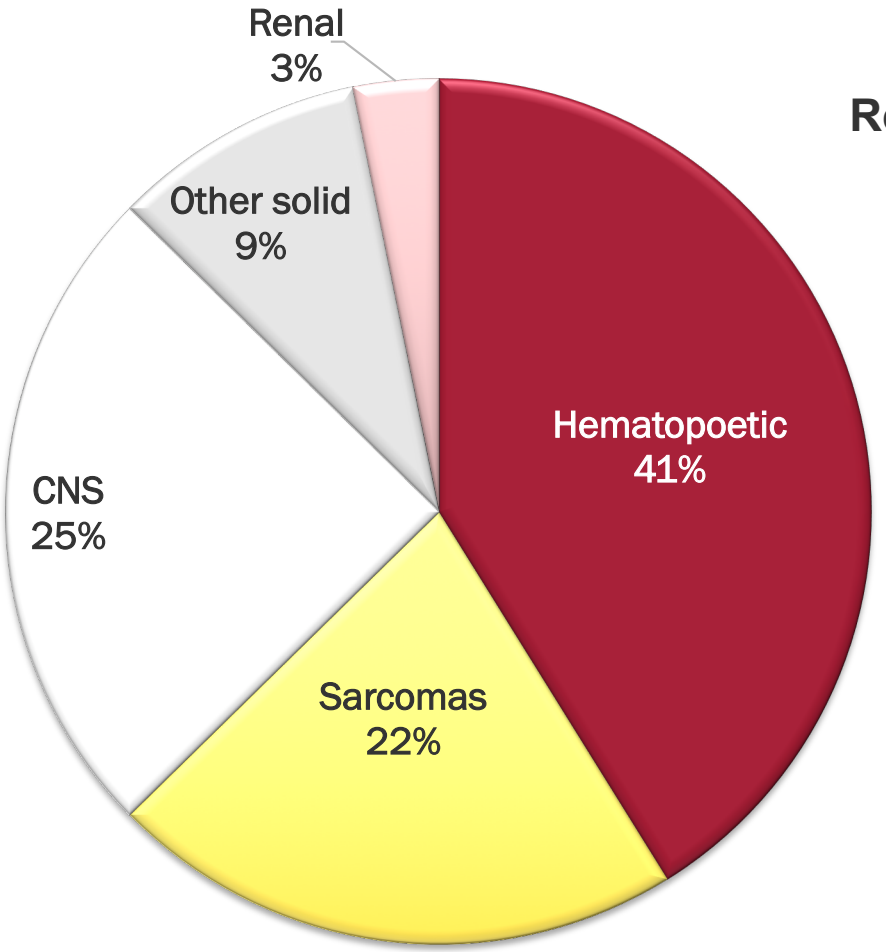
128

Precision Genomics Reports

96

Tailored Treatment recommendations

The Precision Genomics Program at Riley at IU Health – Disease Distribution of Patients



Renal Cancer Patients:

Wilms tumor (1)

- Biomarker- CA IX +
- Drug recommendations – Bevacizumab and Doxorubicin

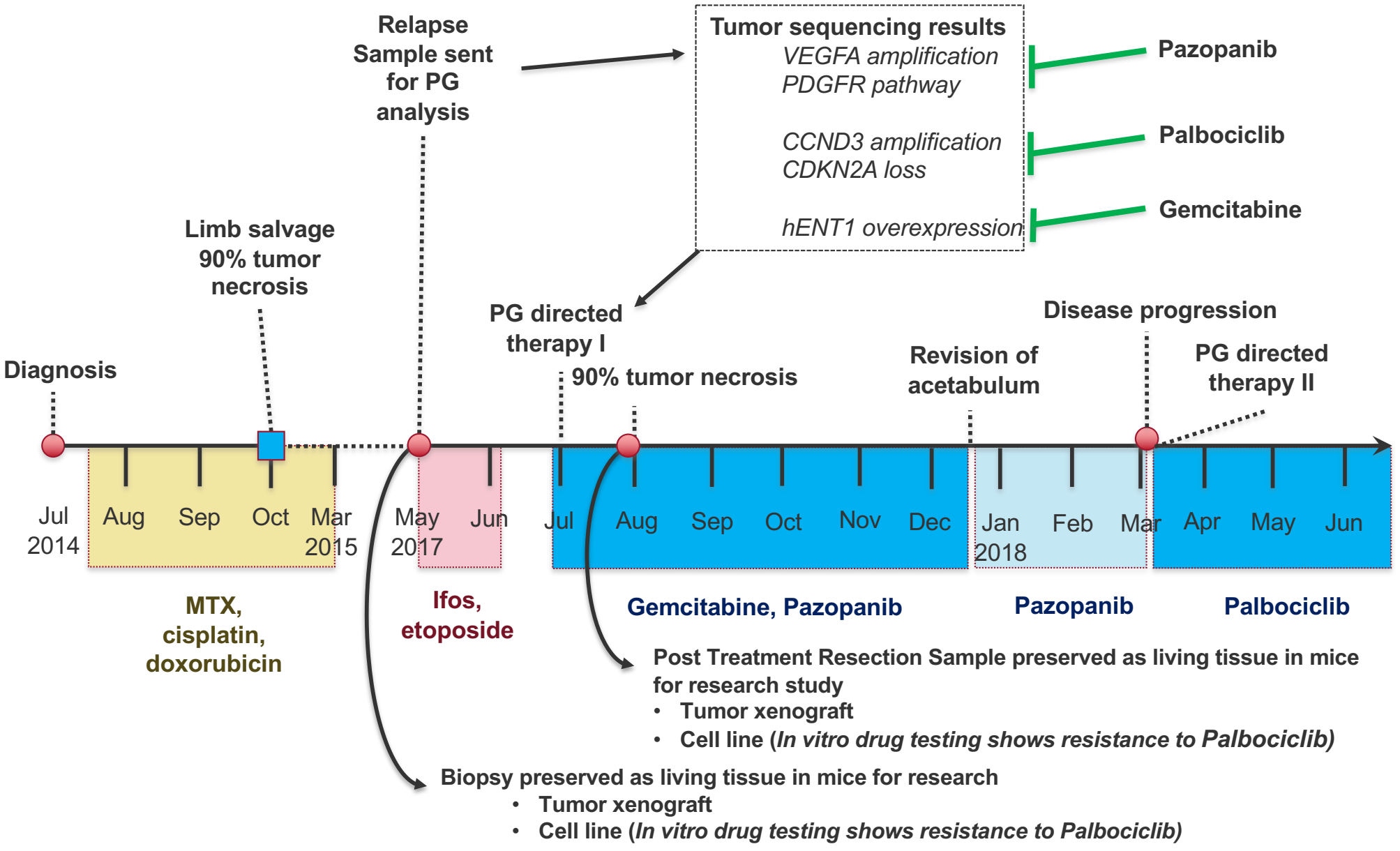
Renal cell carcinoma (1)

- Biomarkers- hENT1 + / ERCC1 -
- Drug recommendations – Cisplatin and Gemcitabine

Renal medullary carcinoma (3)

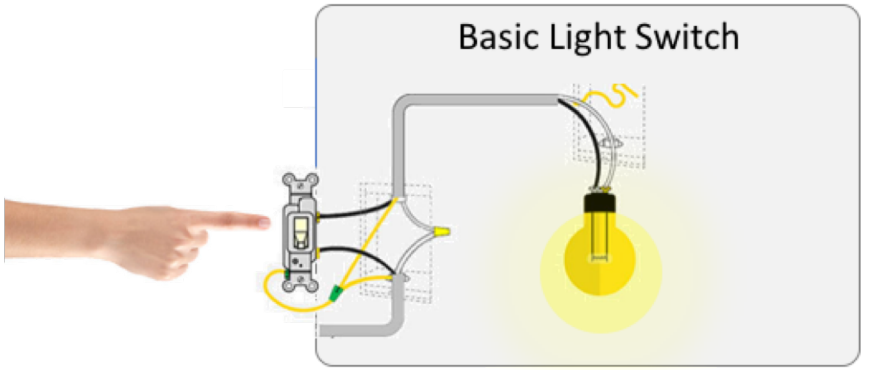
- Biomarkers- PD-L1 +/ CTLA4 + / VEGFA / PDGFRB / MET
- Drug recommendations - Pembrolizumab + Epacadostat, Pazopanib and Cabozantinib

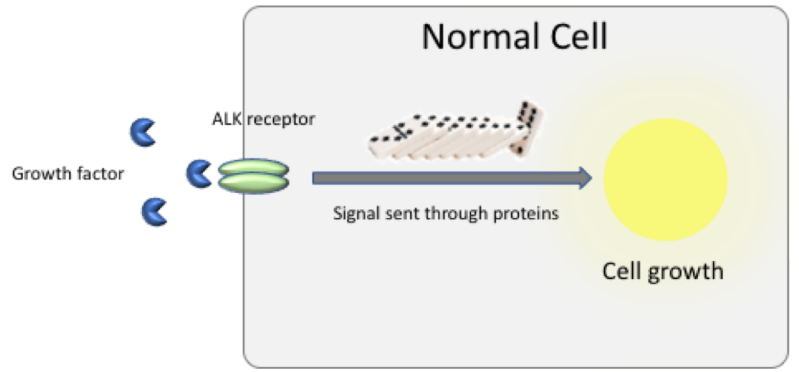
Precision Medicine in Pediatric Cancer – Osteosarcoma

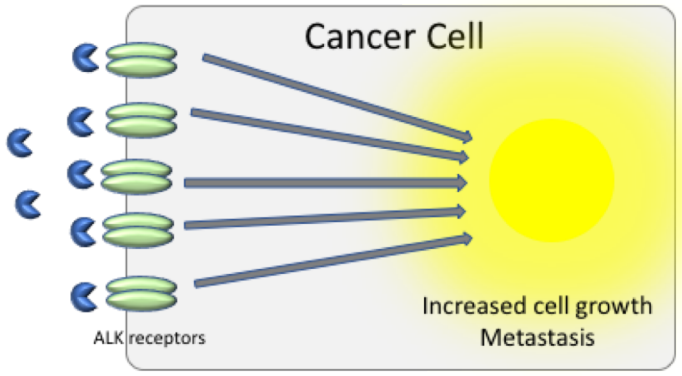


Precision Medicine in Pediatric Cancer – Primitive Neuroectodermal Tumor

- Six yr old girl, diagnosed in September 2015 with metastatic Primitive neuroectodermal tumor (PNET)
  - Surgical resection of ovarian mass
  - Started chemotherapy AEWS0031 regimen B in October 2015.
  - Radiation from February to April 2016
- Relapsed disease detected in March 2016
  - Irinotecan plus temozolomide was offered and declined due to quality of life concerns at end of life
- **Entered Precision Genomics program May 2016**
  - **ALK protein was detected in the tumor, leading to recommendation of oral Crizotinib; parental consent granted**
  - **After one month of Crizotinib (June-July 2016), lesions appeared to be decreasing in size**
  - **Disease continues to be stable after 24 months with continued compliance with Crizotinib**

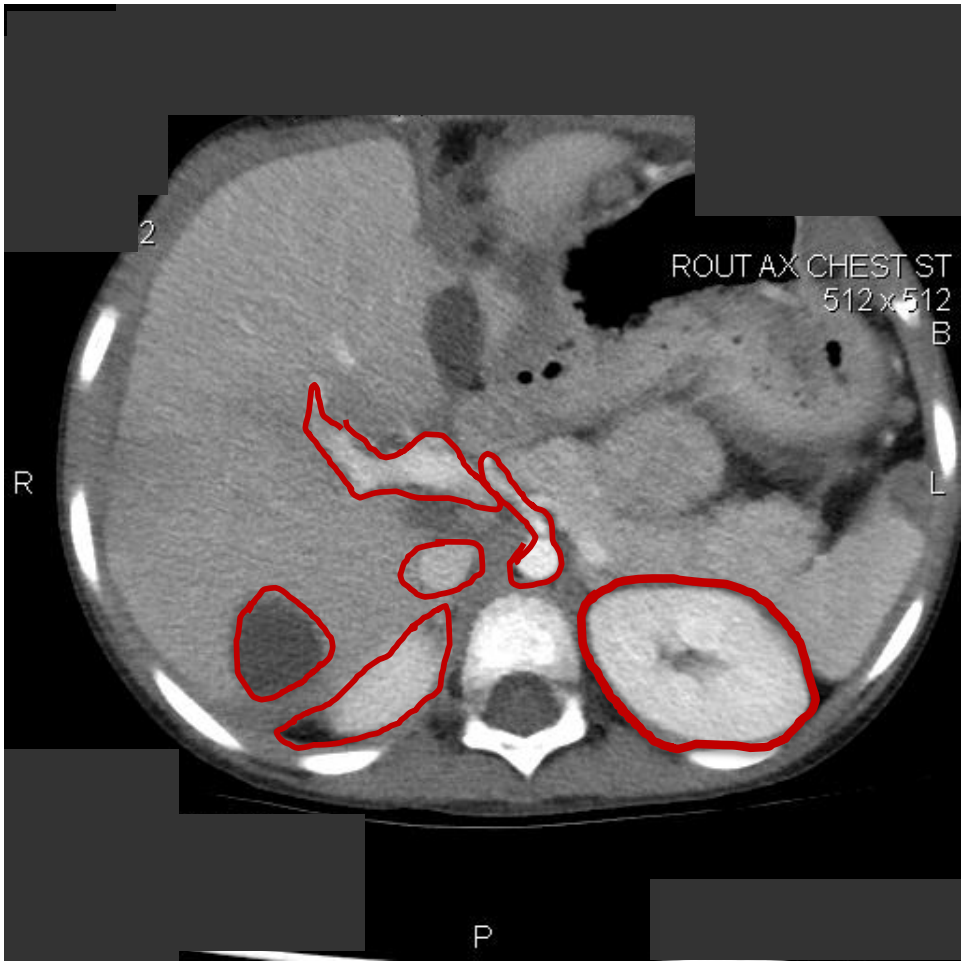




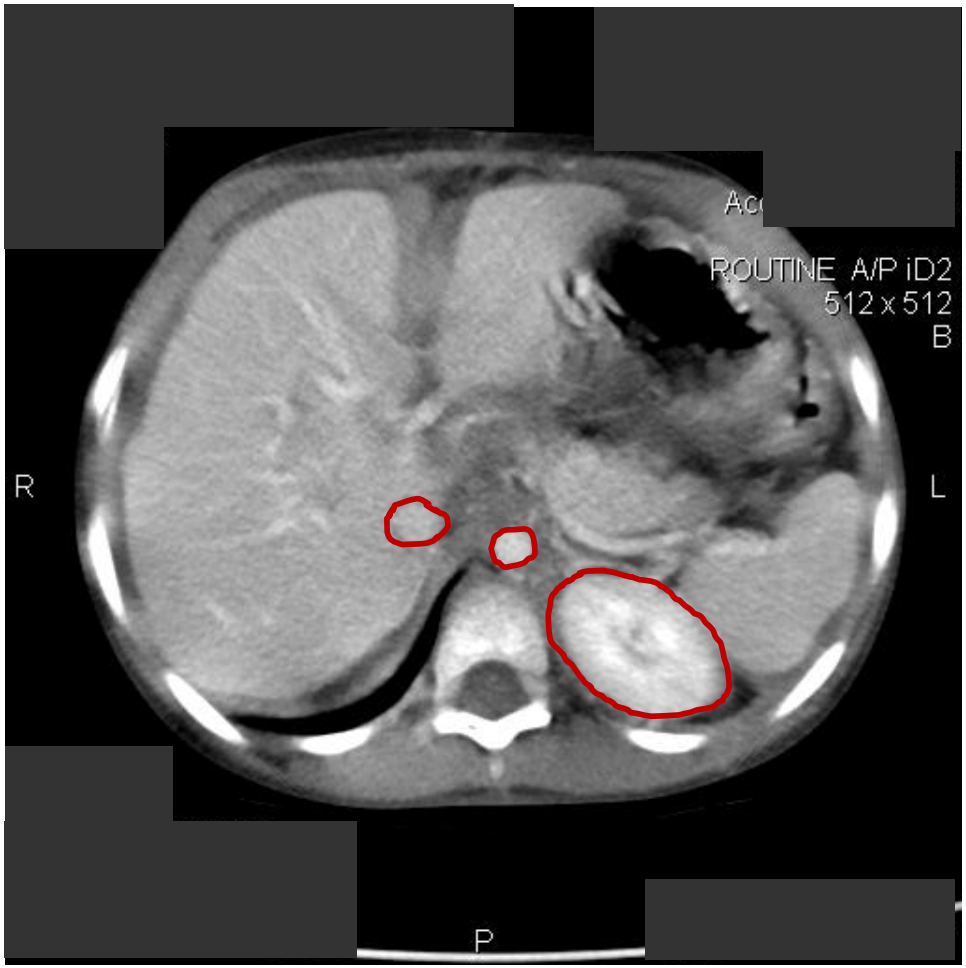


MRI Before and After Crizotinib (ALK inhibitor)

Scan date March, 2016 (Pre Treatment)



Scan date June, 2016 (Post Treatment)



Precision Genomics Program

Contact Precision Genomics Program
317.274.7308

Find A Doctor

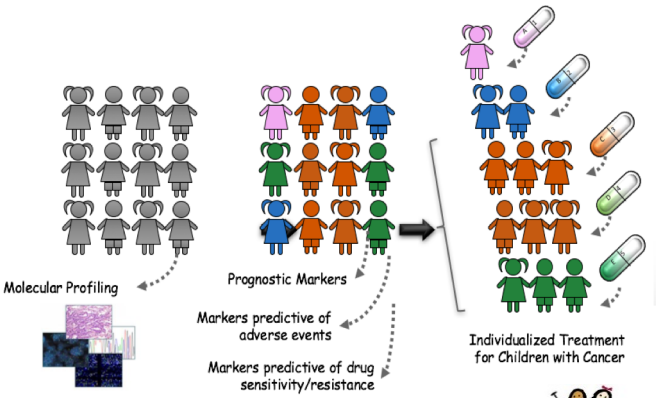
Request An Appointment

On This Page:

The Precision Genomics Program at Riley at IU Health helps children with all types of relapsed or aggressive cancer receive personalized treatment. Our innovative approach to care can help reveal new treatment options that may be effective when other treatment options no longer work.

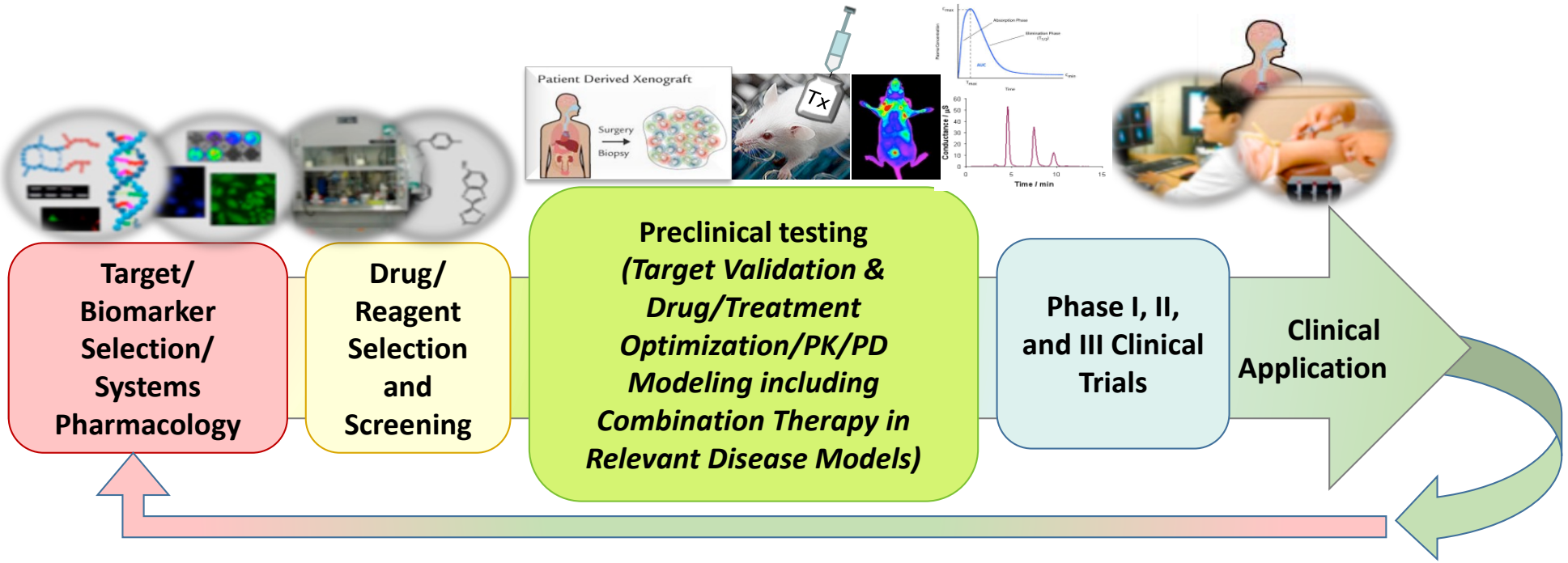
The precision genomics team uses genetic testing to identify DNA, RNA and proteins in cancer cells that can be precisely targeted with different treatments. Based on test results, the team then recommends treatment options that are specific to your child's needs. These targeted therapies may also have fewer side effects than previous treatments, helping your child have a better quality of life.

The Precision Genomics Program is the only program of its kind in Indiana to offer this comprehensive, personalized medicine service. We provide each step of care, from biopsy of cancer cells to testing to evaluation and treatment. Our multidisciplinary team includes a pediatric physician,



Riley Hospital for Children
Indiana University Health

From Bedside to Bench… From Bench to Bedside



Pediatric Hematology-Oncology Initiatives

- Aggressive Solid Tumor Disease Priorities Initially:
  - *Ewing's Sarcoma*
  - *Osteosarcoma*
  - *Rhabdomyosarcoma*
  - *Wilms tumor and other renal tumors*
  - Brain Tumors (focused on HGG and LGG)
  - Neuroblastoma
  - Germ Cell Tumors
  - Other, as identified including rare tumors
- Normal tissue (normal and/or normal adjacent) – critical to developing pediatric-specific diagnostic panel
- Liquid Tumor Initiatives
  - HSCT
  - Bone Marrow Failure
  - Leukemias

U54 (new diagnosis –AND- relapsed/refractory from same patient)

Despite our success in markedly increasing survival in pediatric cancer patients, there is much work to do

You are helping to support:

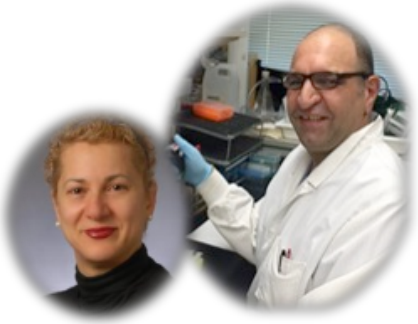
- Development of novel preclinical models for investigation of aggressive pediatric solid tumors
- Investigating targeted and combination therapies for better clinical outcomes

Your continued support will help with:

- Focused investigation of aggressive renal tumors and rhabdomyosarcoma
- Implementing novel clinical trials aimed at establishing effective strategies to improve outcomes for progressively smaller subsets of drug-resistant cases with specific genetic/molecular alterations.

Incorporate molecular biomarkers to direct therapy for children with relapsed solid tumors

The Models: *Development of patient-derived xenografts from Riley Hospital patients*

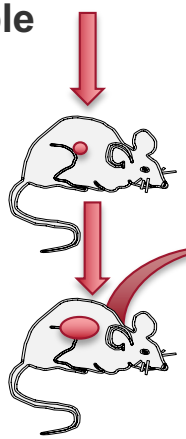


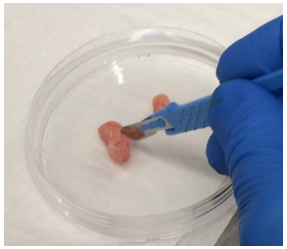
Establish a clinico-biologic repository of molecularly characterized pediatric malignancies

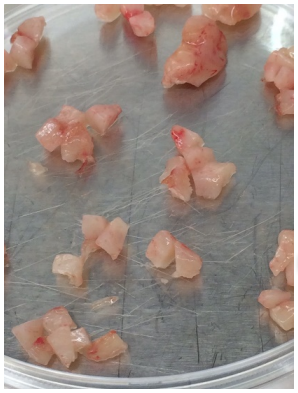
From OR: Harvest and directly/immediately implanted into mice.

Implant within 3-4 hours after harvest in OR
Tumor fragment implantation in matrigel "soft"plug

P0 = original sample







P2

P3

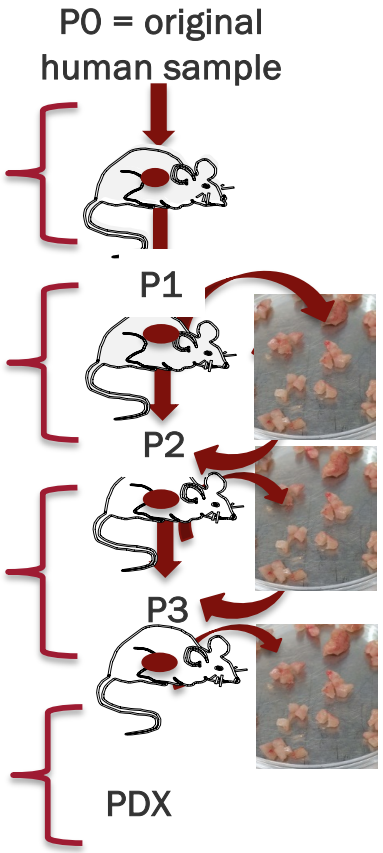
Harvest tumor = P1

- Flash freeze
- Cryopreserve
- Implant P1

The Models: *Development of patient-derived xenografts from Riley Hospital patients*

Not an overnight process:

- Osteo resection (relapsed #1) 5/2017; PDX generated 8/2017;
- Osteo resection (relapsed #2, same patient) 8/2017; PDX generated 1/2018
- Rhabdo resection 6/2017; PDX generated 8/2017



Harvest tumor = P1

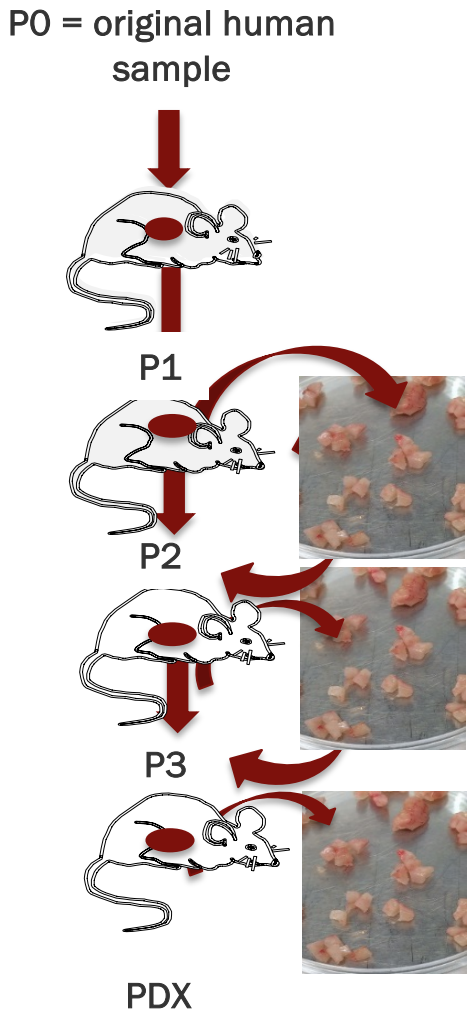
- **Flash freeze**
- **Cryopreserve**
- **Implant P1 tumor into P2 mouse**
- **Implant P2 tumor into P3 mouse**

- **IDEXX: Cell authentication and pathogen testing (P0 - P3)**
- **CellCheck16: Human 16 marker STR profile**
- **Human/mouse inter-species analysis**
- **hIMPACT Profile pathogen PCR analysis**

- **Whole Genome Sequencing – NY Genome Center**
- **PO, P1, & P2**
- **Matched blood - germline comparison**

NEW YORK GENOME CENTER

The Models: *Development of patient-derived xenografts from Wilms' Tumor patients at Riley Hospital patients*



R-HT98 (Biopsy) **4/3/2018**
Tissue amount 0.53 gram **Kidney**
Wilm's tumor
FF (I tube), flank of two NSG mice
P0 (original patient tissue sample) is available
PDX MP1 was extracted from 1st mouse on 5/14/18.
PDX MP1 was extracted from 2nd mouse on 6/25/18.
Tissue stored as FF and FM (4 vials each and also expand in vitro)
***In vitro* cells from MP1 is available**
PDX MP2 was extracted on 7/6/18

Wilms' Tumor samples collected from Riley Hospital patients

IPB#	Plasma	Buffy coat	DNA (buffy coat)	Tissue for DNA	DNA from tissue	Tissue for RNA	RNA from tissue	KP lab code	Surgery date
IPB.02.00081	Y		Y	Y		Y		R-HT69	3/8/2017
IPB.02.00179	Y	Y		Y		Y		R-HT98	4/3/2018
IPB.02.00195	Y	Y				Y			5/3/2018
IPB.02.00198									5/10/2018
IPB.02.00201	Y	Y		Y		Y			6/6/2018
IPB.02.00210				Y		Y			6/28/2018
IPB.02.00213	Y	Y		Y		Y			7/16/2018

Why research models are needed…

- These patient samples help us in the development of models (patient-derived xenografts and cell lines) so we can explore therapeutic options which are *CRITICALLY* needed to improve clinical outcomes for pediatric patients with aggressive cancers.

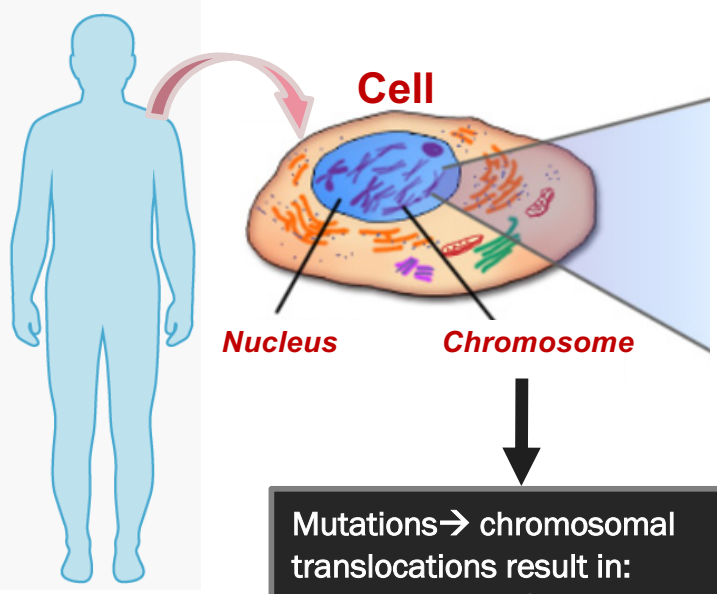
- Genetic changes contribute to the difficulty in treating some of these aggressive pediatric cancers.

- What do we mean when we say “Genetic Changes” ?

What Are Some Of These Genetic Changes That Occur In Pediatric Sarcomas?

Mutation: **"the changing of the structure of a gene, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes."**

******Different layers at which genetic mutations can occur.***



Mutations→ chromosomal translocations result in:

- expression of novel genes or expression of other genes that result in increase/decrease of downstream targets.

Mutations in histones or genes cause genes to be shut down or expressed.→ Epigenetic changes

Mutations in the protein:

- Protein does not perform its normal function.
- Protein excessively performs its function – dysregulated (out of control).
- Protein not expressed due to genetic changes

Wilms' Tumors have overlap with mutations found in other aggressive, pediatric non-CNS solid tumors

One Example of Biological Pathway Overlap:

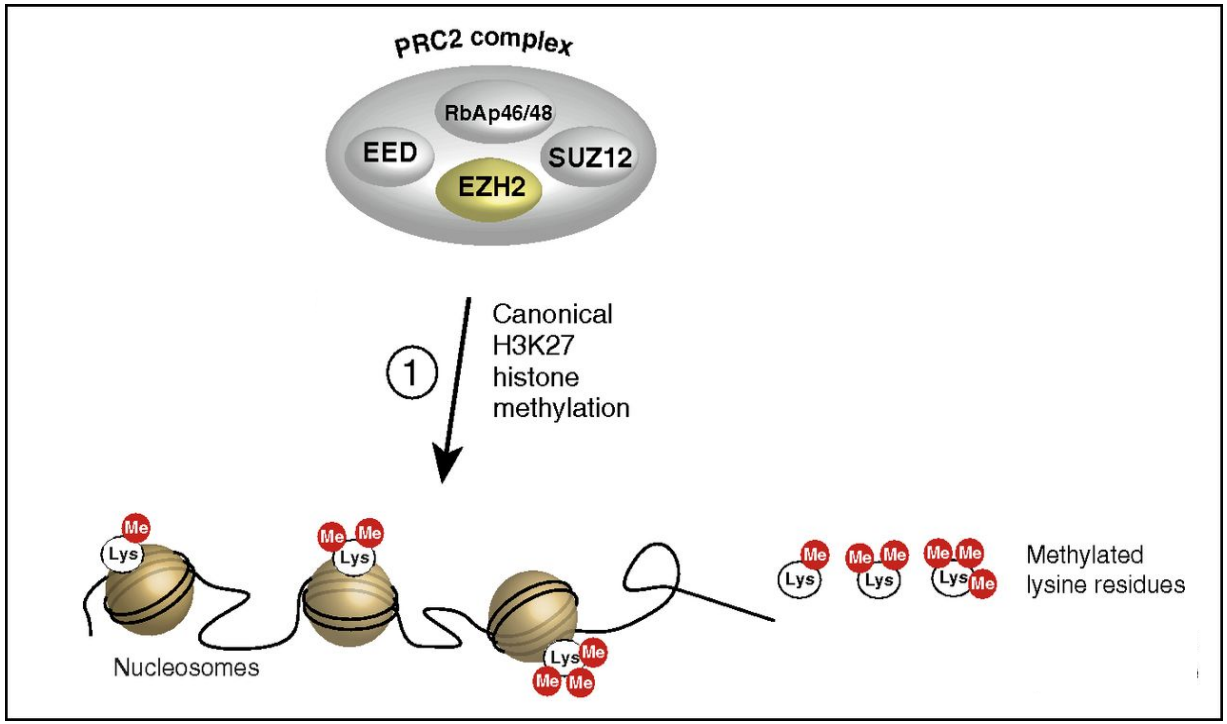
- **Polycomb genes (EZH2, BMI-1 and SUZ12) are upregulated in WT xenografts and reduced when tumor cells undergo differentiation, suggesting that they may maintain malignant renal progenitor cells**

Using models you've help support, we can investigate new therapeutic options, often with drugs already available, for pediatric patients with Wilms' tumor

Next, we will provide you an example of a similar project in Ewing's Sarcoma where we did just that……

Preclinical Validation of EZH2 as a Therapeutic Targets In Pediatric Ewing's Sarcoma Family of Tumors (ESFTs)

EZH2 In ESFTs

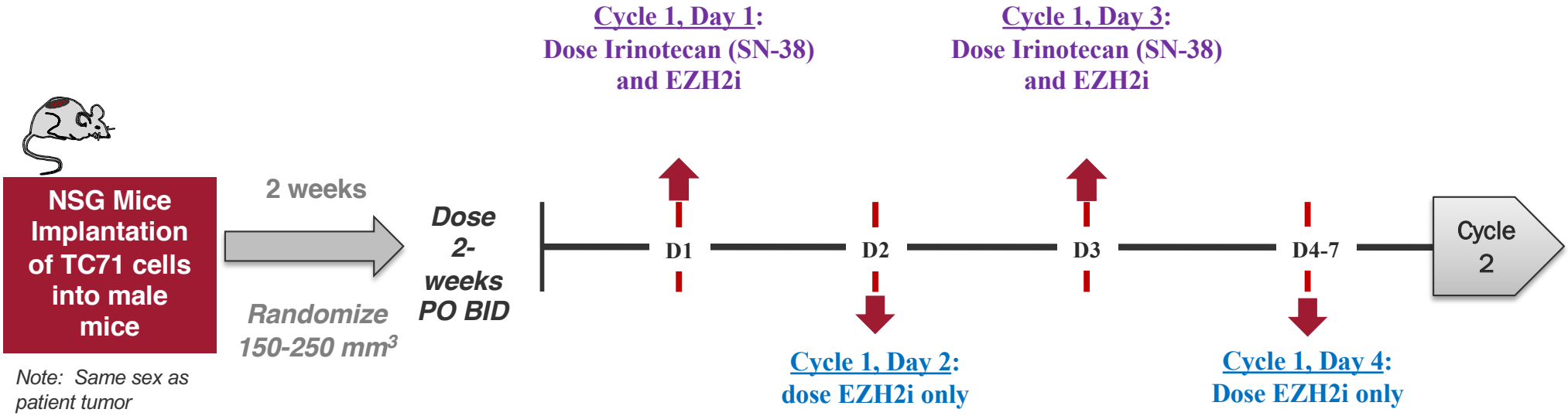


Blood. 2016, 128(7), 888-889.

Hypothesis
EZH2 contributes to chemotherapeutic resistance in pediatric ESFTs.

Through EZH2 inhibition ESFTs can be sensitized to cytotoxic effects of current standard-of-care agents.

In Vivo Combination Study of Irinotecan (SN-38) and EZH2 Inhibitor (Tazemetostat) In TC-71 Tumor Xenografts

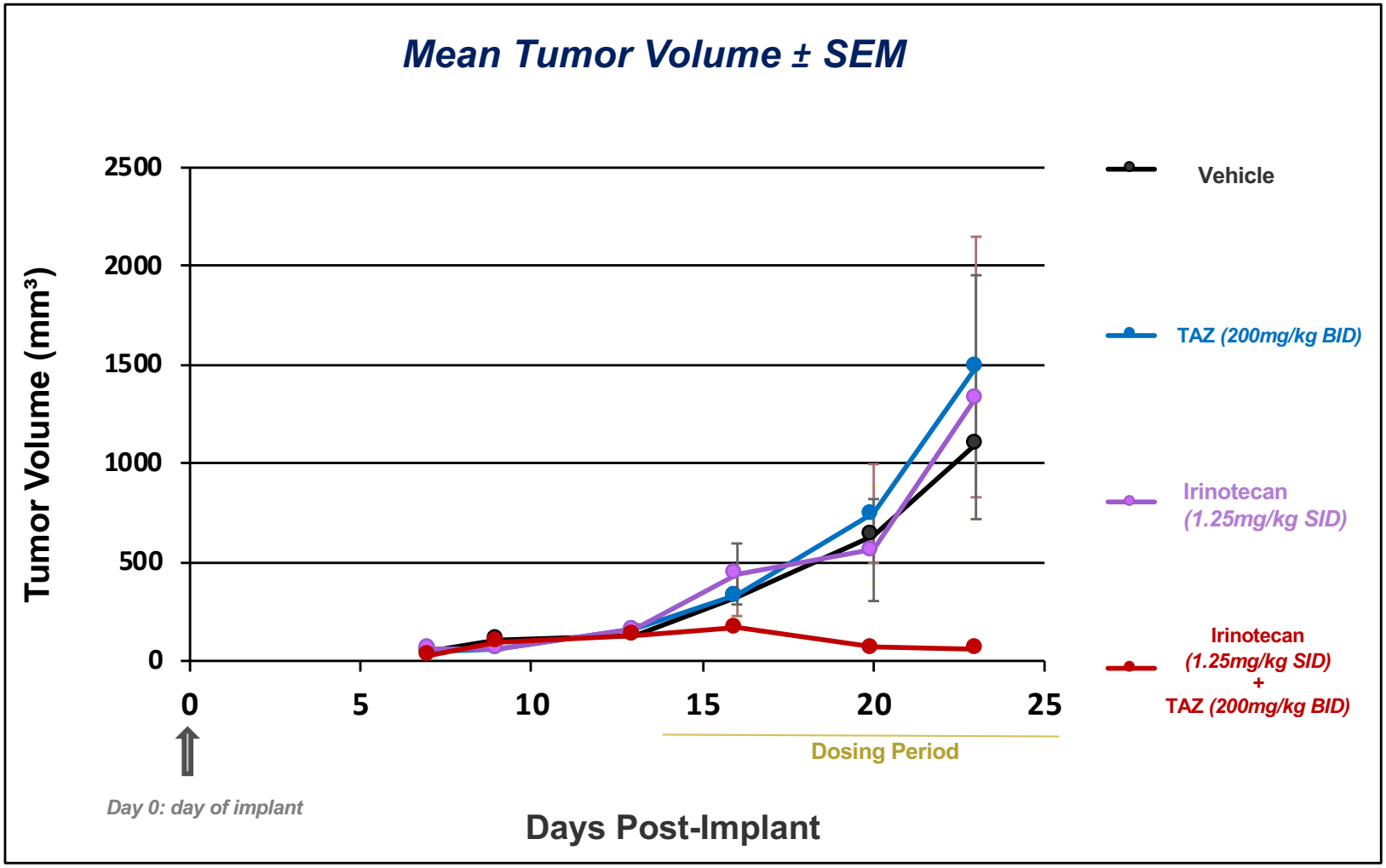


Note: Same sex as patient tumor

Groups:

- **Vehicle**
- **Irinotecan (SN-38) 1.25mg/kg , SID**
- **EZH2i/Tazemetostat 200 mg/kg, BID**
- **Combination SN38 and TAZ**

In Vivo Pharmacodynamic Study of EZH2i (Tazemetostat) +/- Irinotecan In TC-71 Tumor Xenografts



Precision Medicine in Pediatric Cancer – Primitive Neuroectodermal Tumor

- Six yr old girl, diagnosed in September 2015 with metastatic Primitive neuroectodermal tumor (PNET)
  - Surgical resection of ovarian mass
  - Started chemotherapy AEWS0031 regimen B in October 2015.
  - Radiation from February to April 2016

- Relapsed disease detected in March 2016
  - Irinotecan plus temozolomide was offered and declined due to quality of life concerns at end of life

- Entered Precision Genomics program May 2016
  - Overexpression of ALK protein was detected leading to recommendation of oral Crizotinib; parental consent granted
  - After one month of Crizotinib (June-July 2016), lesions appeared to be decreasing in size
  - Disease continues to be stable after 24 months with continued compliance with Crizotinib

Wilms Tumors

- Survival rate for WT is nearly 90%. Nevertheless, approximately 10% of patients with intermediate-risk histology and up to 25% of patients with high-risk tumors will relapse
- WT aged over 6 months receive pre-operative chemotherapy with actinomycin D (ACT-D) and vincristine (VCR). DOX is added in metastatic cases
- high-dose melphalan and autologous stem cell rescue are recommended as consolidation in both the high and very high-risk groups

Wilms Tumor Targets

- Despite the excellent prognosis for most children with WT, just under 15% of patients will relapse, usually within 2 years of diagnosis
- proportion of patients will experience severe early and late treatment-related adverse events, e.g. cardiotoxicity secondary to doxorubicin (DOX) or radiotherapy-induced organ dysfunction, musculoskeletal abnormalities, infertility and secondary malignancies
- The current aims of treatment optimization are to standardize diagnosis, to improve risk stratification, to minimize side-effects of treatment and to improve relapse monitoring according to clinical, molecular, histopathological and imaging data.

Actionable mutations in Wilms Tumors

- WT1, a zinc finger DNA-binding transcription factor
- WT1 negatively regulates the WNT pathway and mutations in the CTNNB1 gene, coding for the crucial protein beta-catenin, often occur alongside WT1 loss
- Tp53 (mutations in 70% of anaplasic tumors; some GOF mutations that can drive migration and invasion) – mutations are an indicator of poor prognosis
- Gain-of-Function MYCN is associated with poor outcome

Linking biomarkers to the best therapy

- prognostic impact of somatic genetic biomarkers (including 1q gain, TP53 mutation and MYCN aberrations).
- Encouraging results have recently been reported for WT with inhibitors of JAK1/2, topoisomerase II and exportin 1 [88e90]. The exportin 1 inhibitor selinexor demonstrated additional cytotoxic activity with rhabdoid cell lines, although all rhabdoid xenografts had progressive disease [90].

Clinical Trials

- MYCN proteins have been considered undruggable until the recent introduction of a class of inhibitors of AuroraA [30]. These inhibitors destabilise interactions between Aurora A and MYCN and are being tested in several adult phase I and II studies.
- The sole pediatric phase II study using an Aurora A kinase inhibitor alisertib showed an objective response in only 1 in 10 WT patients [118].
- Current trials include erlotinib, an epidermal growth factor receptor (EGFR) inhibitor, ramucirumab, a vascular endothelial growth factor receptor (VEGF) inhibitor, talazoparib, a PARP inhibitor and selinexor [119].

Models of Wilms Tumors

- A second murine model arose from the finding that Lin28 overexpression during kidney development prevents the final stage of differentiation, leading to WT formation in mice-82

- The WiT49 anaplastic cell line, from a xenograft of a human WT lung metastasis, is stable with biphasic histology. It carries a TP53 mutation, overexpresses IGF2 and has wild-type WT1, with a high tumour occurrence rate when transplanted into recipient mice [85].

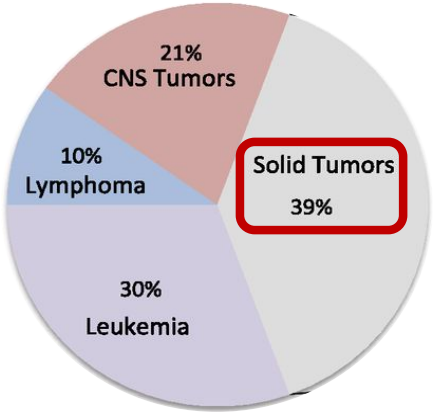
- identifying better prognostic molecular markers for WT
- intra-tumoural heterogeneity
- intensified (the addition of etoposide [ETO]/cyclophosphamide [CYC] to VCR/ACT-D and DOX) treatment for stage III/IV WT with loss of heterozygosity (LOH) of 16q and 1p (5% of WT), which is associated with reduced OS-COG trial
- Compared with a historical control group, they found that the new 5 drug regimen significantly improved event-free survival (EFS) and the intensified treatment is likely to continue as standard for favourable histology WT displaying LOH of 16q and 1p

Long-term side effects of therapy

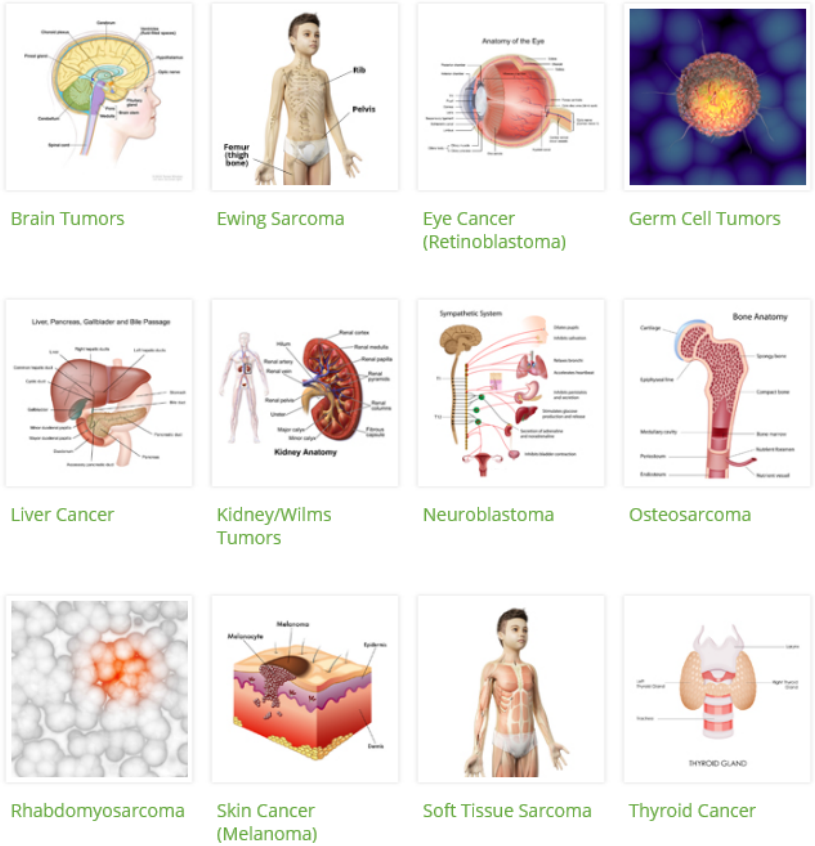
- The risk of congestive heart failure increases with the cumulative dose of DOX administered, with a critical threshold of 240 mg/m2.
- Cardiotoxicity is potentiated by the concurrent use of radiotherapy, with females and infants more susceptible [109]. Similarly, DOX seems to potentiate the adverse effects related to radiotherapy, likely due to its radiosensitisation of cells. These effects include abnormal tissue growth within the target area and secondary malignancies.

- In this context, omission of agents likely to cause late effects is important to consider in treatment optimisation. As mentioned previously, the last trial, to demonstrate that a reduction in intensity was acceptable, found DOX does not need to be added in the treatment of stage II-III intermediate-risk WT

Pediatric Solid Tumors



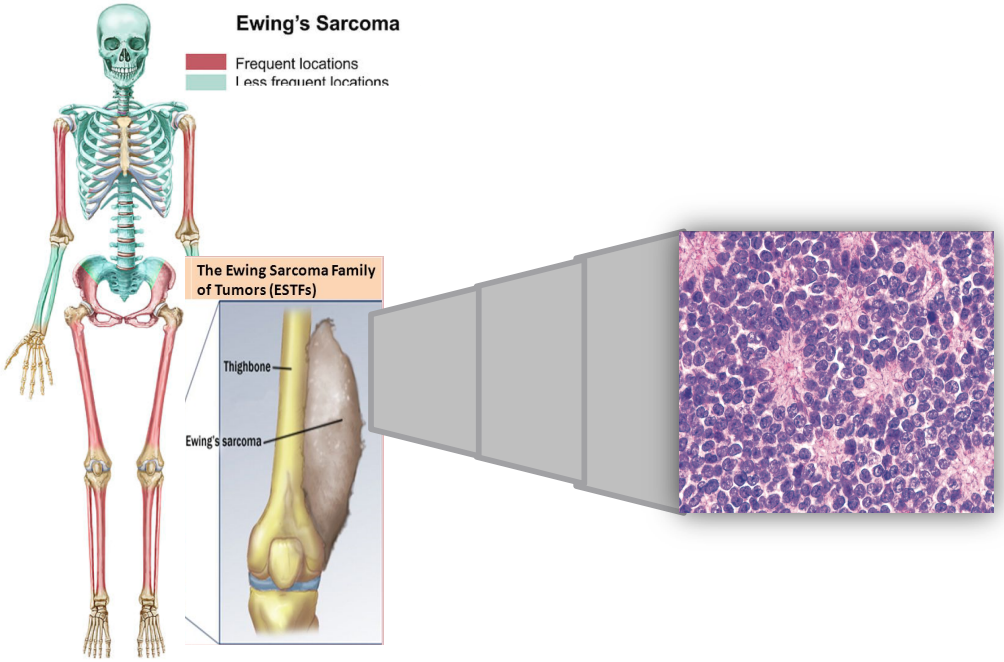
Pediatrics in Review
February 2018, VOLUME 39 / ISSUE 2
From the American Academy of Pediatrics



- **Pediatric Solid Tumors Account for 30-40% of all childhood cancers.**
- **Prognosis of aggressive pediatric solid tumors (i.e sarcomas, Wilms tumors) still remain dismal.**
- **Critical need for investigating other therapeutic options.**
- **Difficult to treat because of various genetic complexities (genetic changes).**

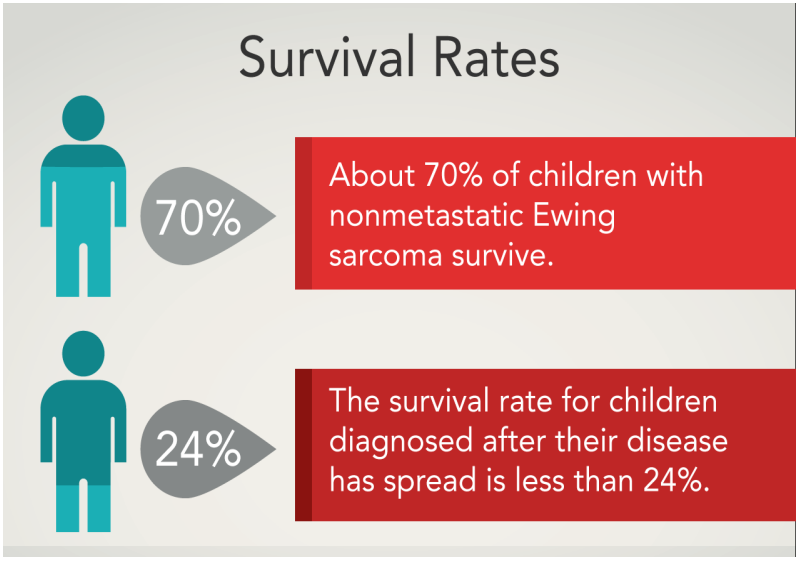
Ewing's Sarcoma Family Of Tumors (ESFTs)

2nd most common pediatric bone and soft tissue tumor.



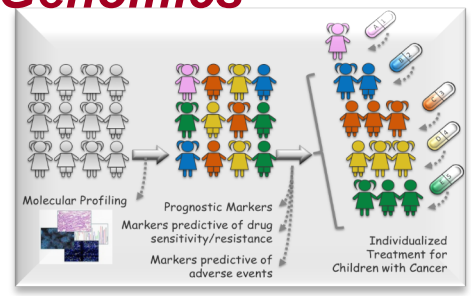
Source: http://fineartamerica.com/featured/ewings-sarcoma-locations-trifocal-communications.html

Chromosomal translocation: EWS-FLI1 protein is seen in 85% of pediatric Ewing's Sarcoma patients.

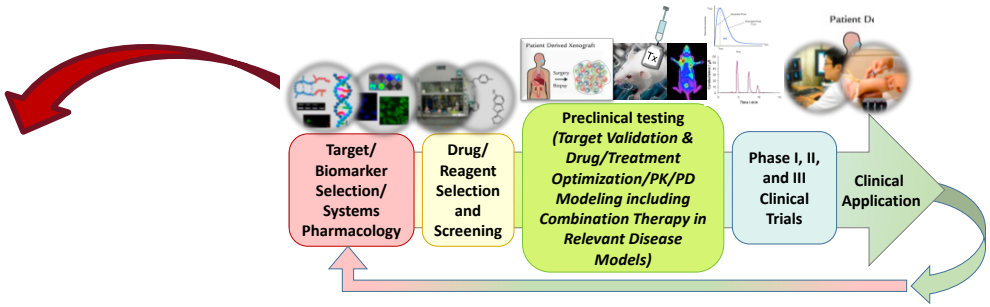


One reason is genetic changes that occur which could cause resistance to therapies. This needs to be investigated in ESFTs to improve clinical outcomes.

Significantly enhance the volume, quality, impact, and reputation of research at IU: *Research in Parallel to Pediatric Precision Genomics*

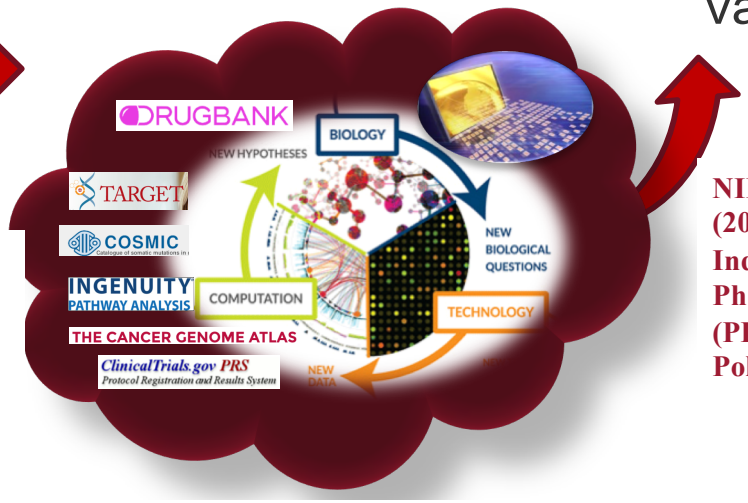


Clinical Care *(Precision Genomics)*



Preclinical Research and Target/Biomarker Validation

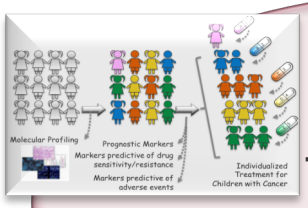
NIH/NCI P50 (2015-2020)
Developmental HyperActive Ras Tumor SPORE (DHART)
(MPI: Clapp, IU and Shannon, UCSF)
1st Pediatric SPORE funded by NCI



NIH/NICHD U54 (2016-2021)
Indiana University Center for Pediatric Pharmacology and Precision Medicine (ICP^{3}M)
(PI: Renbarger; Co-PI Project 1: Renbarger and Pollok)

Systems Pharmacology / Bioinformatics

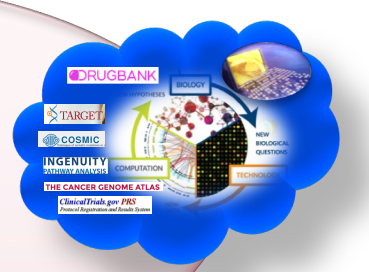
Clinical Input To Help Prioritize Targets And Drug Selection



Riley Hospital Precision Genome & Transcriptome Sequencing Data (Omics Clinical Diagnostic Partners)

Balancing 'innovative' scientific findings while still helping the patients

Public Data (Genome/ Transcriptome/ Survival Outcomes) (NCI/TARGET, etc.)



Important to have our own patient population with clinical outcomes to enrich public data:

1. Public datasets are limited (i.e. clinical outcome, disease progression); and
2. More importantly, it is where we find innovative oncogenes and paths forward

Actionable target selection: ***Many factors to consider for prioritization at the bench***

Where are the gaps in the knowledge base?
Status of current clinical trials / standard of care
PK and Toxicity profile for pediatric tolerability of the drug
Drug availability and insurance coverage
What do funding agencies want (i.e. peer-reviewed science)

R-HT98 (Biopsy) **4/3/2018**

Tissue amount 0.53 gram **Kidney Wilm's tumor**

FF (I tube), flank of two NSG mice

P0 (original patient tissue sample) is available

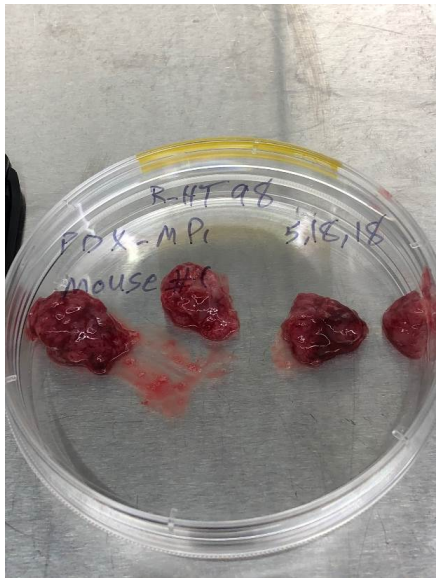
PDX MP1 was extracted from 1st mouse on 5/14/18.

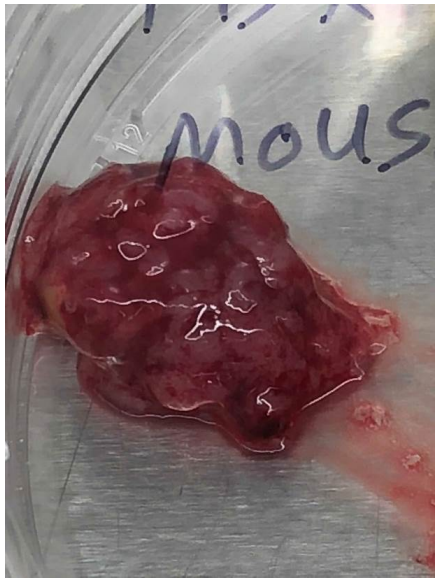
PDX MP1 was extracted from 2nd mouse on 6/25/18.

Tissue stored as FF and FM (4 vials each and also grow on culture medium for in vitro growth).

***In vitro* cells from MP1 is available**

PDX MP2 was extracted on 7/6/18





Pediatric Precision Genomics- Bench-to-Bedside Sarcoma Development Team: Project 1 ESFTs: Evaluating Actionable Clinical Findings- EZH2

Biomarkers Identified by Riley Pediatric Precision Genomics

Biomarkers	Drug Recommendations
ABL	dasatinib, sunitinib, ...
ALK	ceritinib, alectinib, crizotinib
CDK4/6	palbociclib, ribociclib
EGFR	erlotinib
ERCC1	cisplatin
EZH2	**tazemetostat**
FGFR 1-3	ponatinib, nintedanib
FLT3	cabozantinib, Ponatinib, ...
IGF1R	ceritinib, ganitumab
JAK1,2	ruxolitinib,
JAK3	tofacitinib
KIT	cabozantinib, pazopanib, ...
MEK	trametinib, cobimetinib
MET	cabozantinib, crizotinib
mTOR	sirolimus, temsirolimus, ...
PDGFR	pazopanib, imatinib
RAF	vemurafenib, dabrafenib
TOP1	topotecan, irinotecan
TOP2A	doxorubicin
TP53	AZD1775, prexasertib

Balancing 'innovative' scientific findings while still helping the patients

Preliminary Data:

- Retrospective Cohort Pilot Study: Target Selections from Pediatric Cancer Patients' Diagnostic Reports from Paradigm (Gene Expression, Copy Number Variation, Mutations, and 24 Proteins)
- Public data and Riley data compared to survival
- Pathways enrichment and network analysis (Ingenuity)
- Pathways, targets and mechanistic literature searches
- Integration of analysis of gene expression and CNV to uncover molecular signatures linked to sarcoma progression

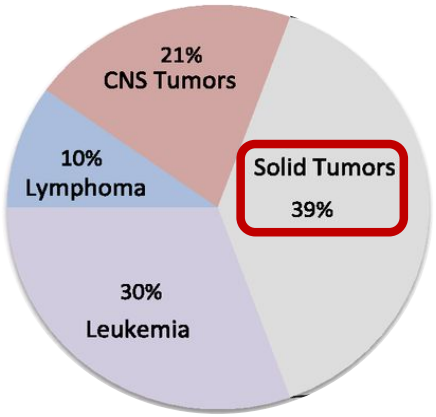
Ewing's Sarcoma

Target of interest: EZH2

PK-PD to optimize target engagement-

Building upon frontline therapy & dual targeting strategies

Pediatric Solid Tumors



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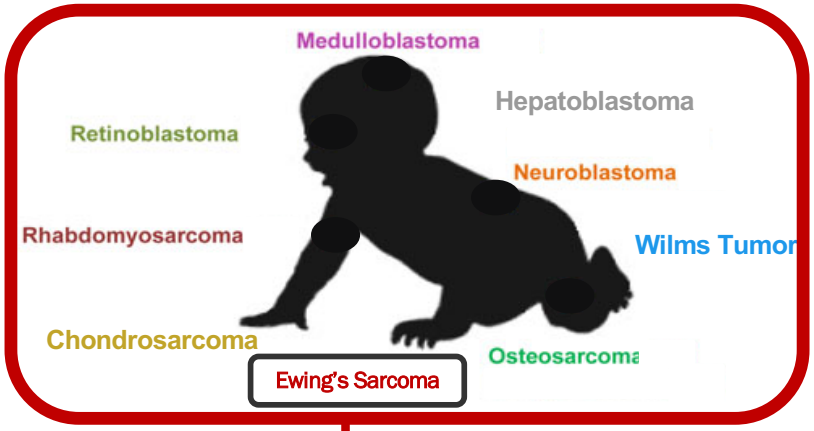

Today's focus on Ewing's Sarcoma