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CureFast: creating a Legacy by accelerating Childhood Cancer Research

Technical abstract

One in five children will not survive cancer. Novel therapeutic approaches are needed for this subset of patients. We propose to address gaps in basic and translational research by improving model systems for pediatric cancer. To this end, we have developed a Legacy Gift (research autopsy) program called Cancer Registry for Familial and Sporadic Tumors (CUREfast) to enable parents of children with cancer to donate tumor tissue to the research community. By focusing on rare and treatment-refractory tumors, we target disease areas most in need of cell lines and mouse models for functional studies. We will use this tissue to create cell lines and patient-derived xenograft mouse models. We will perform drug sensitivity testing for a 60-agent panel on primary cell suspensions, and will use the cell lines for DNA exome sequencing & RNA deep sequencing. Through our pilot studies we have collected Legacy Gifts and advanced disease surgical material from over 500 patients across the country, resulting in 19 patient-derived xenograft models created in collaboration with the Jackson Laboratory. The model systems and data generated in this work would represent an innovative step and should prove pivotal to pediatric preclinical research efforts for 'orphan' childhood cancers.

Lay abstract

One in five children with cancer will not survive. We need more and better models to study pediatric cancer in the laboratory so we can understand it and develop more effective treatments. We propose to collect tumor samples at the time of autopsy from children who have died of their disease ("legacy gifts") through a program called the Cancer Registry for Familial and Sporadic Tumors (CUREfast). We can grow the cancer cells from these patients in mice and use them in various experiments including genetic analysis and drug testing. By focusing on the most aggressive tumors that have not responded to treatment, we target the diseases most in need of scientific study. We will share our data with the larger research community so that many scientists can make use of it. Our hope is that the gifts these parents make will allow their child's death to benefit future children with cancer in a meaningful way.

Introduction

One in 5 children with cancer will not survive (1). We propose to address gaps in basic and translational research by improving model systems for pediatric diseases (2-4). To this end, we have developed a Legacy Gift (research autopsy) program called the Cancer Registry for Familial and Sporadic Tumors (CUREfast) to enable parents of children with cancer to donate tumor tissue to the research community. It may seem unusual to think that innovation can come from research autopsy – but we believe it can be field-changing, especially for rare childhood cancers for which cell lines and mouse models do not exist, and for which functional studies have never before been performed. Examples of these pediatric cancer orphan diseases are intracranial germ cell tumor (no cell lines or mouse models until recently), metastatic hepatoblastoma (essentially no representative cell lines), anaplastic Wilms' tumor (2 cell lines, 2 mouse models) and pediatric epithelioid sarcoma (1 cell line). By focusing on resource development for these childhood cancers, using treatment-refractory tumor samples, we open the door to true innovation. We have collected Legacy Gifts and advanced disease surgical material from over 500 patients across the country, resulting in 19 patient-derived xenograft (PDX) models created in collaboration with the Jackson Laboratory including four "first-in-world" disease models: epithelioid sarcoma, *PAX7:FOXO1+* alveolar rhabdomyosarcoma, and parameningeal rhabdomyosarcoma (5). These autopsies still represent a small sample of the children lost to cancer every year, and thus a tragically lost opportunity for the research community that faces limited availability of pediatric models. To improve pediatric cancer model systems, we propose:

Specific Aims

Aim 1: Create PDX mouse models with The Jackson Laboratory using tissue from relapsed or autopsy patients representing the "1 in 5" children. This aim will focus on sarcomas and other pediatric solid tumors.

Aim 2: Generate primary cell suspensions for direct "on the spot" drug sensitivity testing with a drug panel of 60 agents in the COG drug portfolio. This data will be paired for each case with DNA exome sequencing & RNA deep sequencing, enriching the utility and applicability of our data, which will be broadly shared before publication.

Pediatric preclinical models are few and often decades old; more representative models are needed to ensure that preclinical studies are done with predictive accuracy across different models of the same disease (6-9). New models developed in this work, both *in vitro* and PDX *in vivo*, will allow for the preclinical validation of novel treatments for critical diseases; when paired with primary culture drug screening and DNA and RNA sequencing data generated and shared readily with the research community, the materials generated in this work represent an innovative step and should prove pivotal to pediatric preclinical research efforts for 'orphan' childhood cancers.

These studies will be conducted annually over a period of five years until accrual for target (orphan) pediatric cancers are met for each disease.

Significance

Overall cure rates for childhood cancer have remained nearly the same for more than a decade, necessitating an evolution of our approach. Two addressable gaps for better basic and translational research exist: **First**, the basic materials for research are rare. For instance, rhabdomyosarcoma continues to be studied using the Rh30 and RD cell lines, which were established in 1987 and 1968, respectively [1,2]; similarly, most brain tumor cell lines were established before the need for serum-free conditions was recognized. Furthermore, for certain brain tumors like intracranial germ cell tumor, no cell line has ever been reported. Finally, existing cell lines are generally taken from newly diagnosed patients – not relapsed ones. **Second**, COG disease subcommittees often design trials of new agents with only indirect evidence for activity against childhood cancer – and with a limited number of biological replicates for each disease (that is, usually only a handful of cell line or animal models have been tested – and it would be difficult to say that these select model systems are representative of the disease). Whereas the PPTC has insufficient resources to test all worthwhile ideas emerging from the COG disease committees, the requested funding here would help ensure that informative preclinical studies could be conducted by a wider range of laboratories with a more diverse set of tools.

It is a valid question to ask whether the effort to generate new *in vitro* and *in vivo* resources for pediatric cancers are truly needed? In addition to the rationale above, affirming studies now indicate that for cancer as a whole, patients die of disease that is very different from their primary tumor. This clonal selection and evolution process is evident not only in corporal tumors [3,4], but also in pediatric central nervous system (CNS) tumors [5]. Thus, the tumors we obtain from relapsed or autopsy patients will truly be different from the primary tumors collected at initial diagnosis and most representative of the disease state when the pediatric oncologist is trying to salvage a relapsed or refractory patient. In fact, serial samples for the same patient from initial diagnosis, relapse and autopsy may be the most informative (and important) types of samples to study.

Given the above rationale for creating a broader diversity of cultures from high risk patients, then which diseases should be priorities? Based upon Center for Disease Control (CDC) statistics, the leading causes of childhood cancer death are relapsed & refractory leukemias, brain tumors, bone cancers (*e.g.,* Ewing's sarcoma and osteosarcoma), neuroblastoma, and **soft tissue sarcomas** including rhabdomyosarcoma and undifferentiated sarcomas [6,7]. In this regard, the NCI Clinical Trials Evaluation Program (CTEP) reported in August 2014 that despite advances in survival outcome for other childhood cancers, progress for metastatic sarcoma remains specifically limited [7]. Furthermore, sarcomas are a major cause of cancer among adolescents & young adults with cancer, for whom improvement in outcomes remain particularly elusive [8]. Additional diseases worth noting include **hepatoblastoma** for which, until recently, no advanced stage disease cell lines or patient-derived xenograft models have been publicly available [9].

Thus, this pilot program to generate new research reagents for high risk sarcomas and other pediatric solid tumors is very much in keeping with the most under-researched pediatric cancers.

A sizable amount of work has been done by our group with respect to the logistics of autopsy-derived tissue for research. With our colleagues on the COG STS committee, we have published the position paper below:

> Spunt SL, Vargas SO, Coffin CM, Skapek SX, Parham DM, Darling J, Hawkins DS, Keller C. **The clinical, research, and social value of autopsy after any cancer death: A perspective from the Children's Oncology Group Soft Tissue Sarcoma Committee.** *Cancer*. 2012;118(12):3002-9. PMID 22006470.

We also received NCI R01 supplement funding by way of the Caroline Pryce Walker Act to conduct a survey of the barriers to research autopsy for families and caregivers, and the manuscript describing this study has been published:

Alabran JL, … Spunt SL, Keller C. **Overcoming Autopsy Barriers in Pediatric Cancer Research.** *Pediatric Blood & Cancer*, 2013 Feb;60(2):204-9. Epub 2012 Sep 26, 2013 Feb;60(2):204-9 PMID 23015377.

Of note, our publication appeared with a commentary from the Chair of the COG Pathology Committee:
Jarzembowski JA, Hicks MJ. **Pediatric autopsy consent: Helping families create hope out of despair.** *Pediatr Blood Cancer*. 2013 Feb;60(2):173-4 [PMID: 23109284].

For outreach, we held an educational session entitled, "**Legacy Gift (Autopsy) Workshop for care providers on postmortem donations for research**" in parallel to the Fall 2011 COG meeting. Our presentation was done by a social worker in collaboration with two mothers, with first-hand experience with pediatric cancer legacy gifts done for their sons. The message conveyed was that, if timing is right, approaching a family about an autopsy for research is not only feasible but gives the family a positive experience from the loss.

Generation of primary cell cultures and patient-derived xenografts is of increasing interest to the cancer research field, but is a process that takes time to develop. We began an institutional protocol in May 2011 at our previous lab's institution (OHSU) to create genetically- and pharmacologically-characterized primary cell cultures of all-comers pediatric cancers– as well as select young adult cancers (brain tumors and sarcomas that are found to also occur in childhood). Representative chemical screen results are given in Figure 1.

We are also developing Patient-Derived Xenografts (PDXs) of pediatric cancers in a partnership with The Jackson Laboratory. For these models, human tumor samples are directly engrafted into NOD-SCID-gamma (NSG) mice. Models can take 4-7+ months to establish.

Preliminary Data

In our pilot study period, we made significant efforts facilitating Legacy Gifts (Research Autopsies) for the purpose of establishing putative primary cell cultures and obtaining cryopreserved tumor tissue obtained at autopsy for distribution to researchers across the nation. For example, we have received dozens of legacy gifts (see examples in Table 1).

In order to share our samples with the scientific community, we have prepared and shipped **more than 1,590** en bloc pediatric and adult tumors, matched primary cell cultures and/or cryo-viable tumors for patient-derived xenograft development to Nationwide Children's Hospital Biopathology Center (the BPC was selected as an independent site for depositing samples because the BPC hosts the COG Biorepository). This includes primary, relapsed, and autopsy derived sarcomas and brain tumor samples representing the breadth of cancer histology.

In addition to the samples sent to Nationwide Children's Hospital Biopathology Center, our samples have been available to researchers around the globe directly from our laboratory as well. Recipients include Washington University at St. Louis, Virginia Tech University, the National Cancer Institute, Vanderbilt University the University of Ottawa, the University of Iowa, the Ohio State University, Cincinnati Children's Hospital Medical Center, the University of Minnesota, and the NCI Patient-Derived Models Repository at the Frederick National Laboratory for Cancer Research.

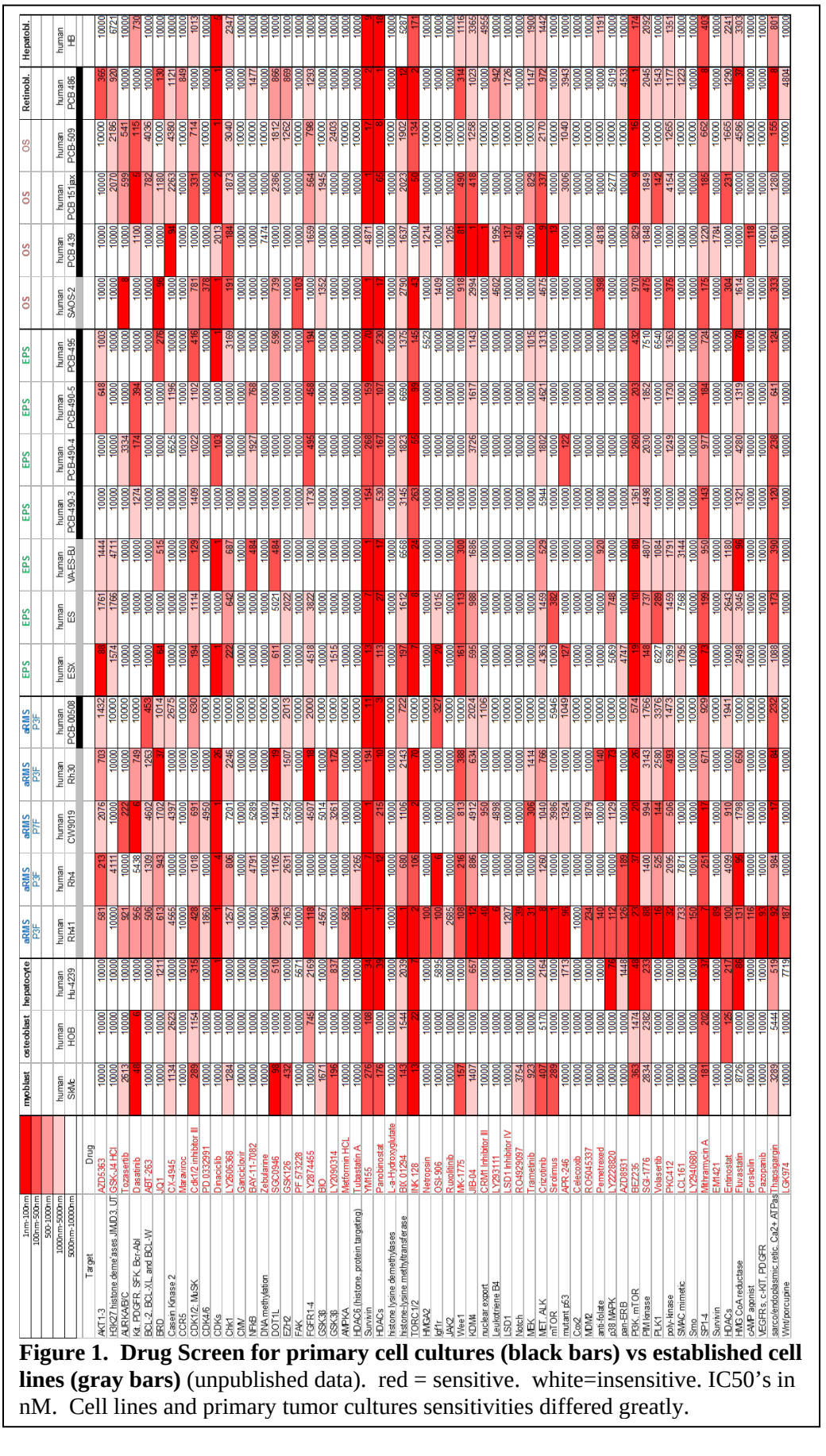


Figure 1. Drug Screen for primary cell cultures (black bars) vs established cell lines (gray bars) (unpublished data). red = sensitive. white=insensitive. IC50's in nM. Cell lines and primary tumor cultures sensitivities differed greatly.

We have also sent fresh tumor samples to The Jackson Laboratory (JAX), from which 23 patient-derived xenograft (PDX) models from 18 patients have been established. Three of these models are "first-in-world" to our knowledge (epitheliod sarcoma; Pax7:Foxo1+ alveolar rhabdomyosarcoma, and parameningial rhabdomyosarcoma). Additional models

Table 1. OHSU Pilot Phase PDX models developed with JAX

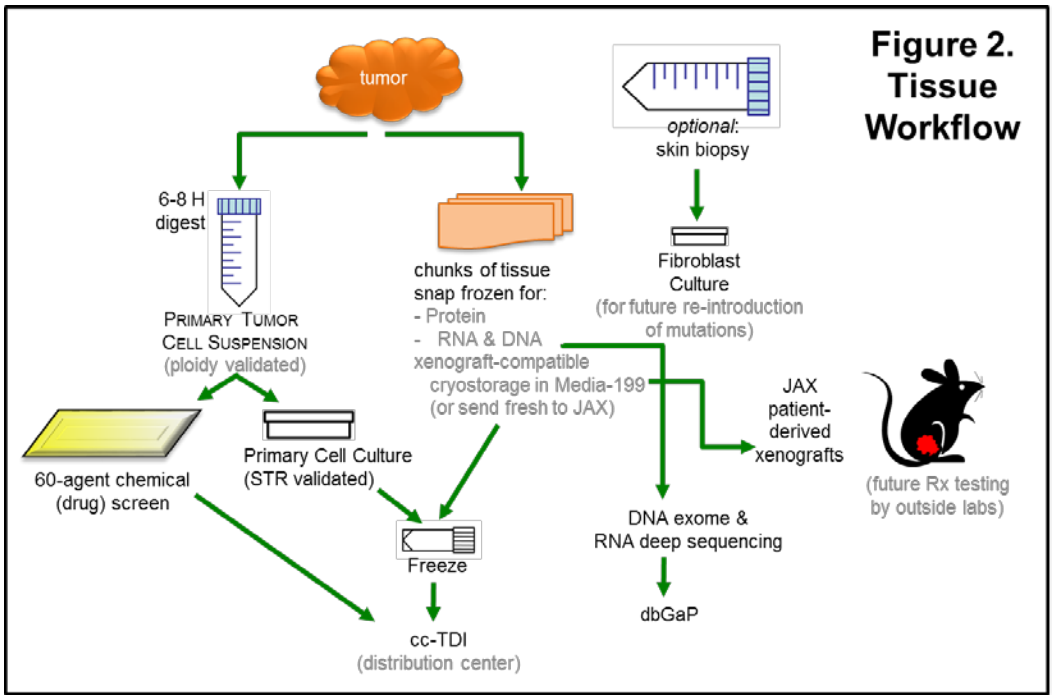
JAX ID	KellerLab ID	Gender	Age	Diagnosis	world first PDX?	Tumor Type	Source
J000078604	PCB-00490	Female	22	epithelioid sarcoma	YES	Metastatic	surgical/ deceased
TM01571	PCB-00430	Female	54	Ewing's sarcoma		Primary	surgical
TM01616	PCB-00469.1	Male	23	Ewing's sarcoma		Metastatic	autopsy
TM01617	PCB-00469.2	Male	23	Ewing's sarcoma		Metastatic	autopsy
TM01618	PCB-00469.3	Male	23	Ewing's sarcoma		Metastatic	autopsy
TM01619	PCB-00469.4	Male	23	Ewing's sarcoma		Metastatic	autopsy
J000093555	PCB-00509	Male	12	osteosarcoma		Metastatic	surgical
TM00039	PCB-00151	Male	11	osteosarcoma		Primary	surgical
TM01569	PCB-00429	Male	23	osteosarcoma		Primary	surgical
TM01632	PCB-00476	Male	20	rhabdomyosarcoma (hereditary Rb)	YES	Primary	surgical
J000077591	PCB-00481b	Male	13	rhabdomyosarcoma, alveolar		Metastatic	autopsy
J000077608	PCB-00481c	Male	13	rhabdomyosarcoma, alveolar		Metastatic	autopsy
J000077636	PCB0-0481e	Male	13	rhabdomyosarcoma, alveolar		Metastatic	autopsy
TM01165	PCB-00380	Female	2	rhabdomyosarcoma, alveolar		Primary	surgical
TM00360	PCB-00082	Female	14	rhabdomyosarcoma, embr. (parameningeal)	YES	Primary	autopsy
TM01634	PCB-00477	Male	34	synovial sarcoma		Primary	surgical

Table 2. cc-TDI Pilot Phase samples and PDX models developed with JAX

Patient CF#	Diagnosis	Sample Type	Sent for PDX	PDX Status	# Snap Frozen	# Cryo-Preserved	Primary Cultures	Drug Screened	exome seq?	RNA seq?
CF-00001	rhabdomyosarcoma, alveolar	autopsy	Yes	established	26	2		yes	1	1
CF-00002	rhabdomyosarcoma, alveolar	autopsy	Yes	established	22	12		no	1	1
CF-00003	colon cancer	surgery (relapse)	No	N/A	5	5		yes	-	-
CF-00004	rhabdomyosarcoma, alveolar	surgery (relapse)	Yes	established	4	5		no	1	1
CF-00005	rhabdomyosarcoma, alveolar	PDX	No	established	N/A	N/A	N/A	no	-	-
CF-00006	rhabdomyosarcoma,embryonal	PDX	No	established	N/A	N/A	N/A	no	-	-
CF-00007	rhabdomyosarcoma,NOS	PDX	No	established	N/A	N/A	N/A	yes	-	-
CF-00008	breast cancer (triple negative)	surgery (relapse)	No	N/A	N/A	N/A	N/A	yes	-	-
CF-00009	--	No sample received	No - no sample	N/A	N/A	N/A	N/A	no	-	-
CF-00010	alveolar rhabodomyosarcoma	No sample received	No - no sample	N/A	N/A	N/A	N/A	no	-	-
CF-00011	desmoplastic small round cell tumor	autopsy	Yes	no engraftment	23	24	1	no	-	-
CF-00012	juvenile pilocytic astrocytoma, NF1-associated	surgery	No	N/A	0	0	2	no	-	-
CF-00013	metastatic alveolar rhabdomyosarcoma	autopsy	Yes	established	11	24	2	no	1	1
CF-00014	metastatic alveolar rhabdomyosarcoma	autopsy	Yes	no engraftment	0	2	2	no	1	1
CF-00015	Diffuse Intrinsic Pontine Glioma	autopsy	Yes	in progress	34	21	1	no	1	1
CF-00016	metastatic alveolar rhabdomyosarcoma	autopsy	Yes	no engraftment	7	8	5	no	2	2
CF-00017	metastatic Ewing's sarcoma	autopsy	initiating	N/A	26	30	4	no	1	1
CF-00018	metastatic Ewing's sarcoma	autopsy	initiating	N/A	30	45	7	no	-	-
CF-00019	MPNST	surgery	initiating	N/A	6	6	1	yes	-	-
CF-00020	Ewing's Sarcoma? (unknown)	surgery	no	N/A	0	0	1	no	-	-
CF-00021	desmoplastic small round cell tumor	autopsy	Yes	in progress	82	38	8	no	-	-
CF-00022	alveolar soft parts sarcoma	autopsy	no	N/A	0	1	2	no	-	-
CF-00023	teratoma	autopsy	initiating	N/A	39	23	2	no	-	-
CF-00024	Ewing's Sarcoma	autopsy	initiating	N/A	118	37	8	no	-	-
CF-00025	pleuropulmonary blastoma	surgery (relapse)	no	N/A	0	0	0	no	-	-
CF-00027	Anaplastic Wilm's Tumor	autopsy	no	N/A	1	0	N/A	no	-	-
CF-00028	Anaplastic Wilm's Tumor	surgery	no	N/A	0	0	N/A	no	-	-
CF-00029	Anaplastic ependymoma	autopsy	initiating	N/A	39	6	2	no	-	-
CF-00030	brain tumor	autopsy	not yet	N/A	72	26	4	no	-	-
CF-00031	anaplastic Wilm's tumor	surgery	no	N/A	0	0	N/A	no	-	-
CF-00036	rhabdoid tumor	autopsy	no	N/A	0	0	N/A	no	-	-
CF-00070	angiosarcoma	Right lower quadrant	not yet	N/A	46	18	6	no	-	-
CF-00083	hepatoblastoma - metastatic	autopsy	Yes	in progress	24	15	3	no	-	-
CF-00086	sarcoma, intimal, metastatic	autopsy	initiating	N/A	6	4	1	no	-	-
CF-00096	hepatoblastoma	surgery	not yet	N/A	N/A	N/A	N/A	no	-	-

Furthermore, these samples have become novel research tools. As evidence of this, an autopsy-derived embryonal rhabdomyosarcoma sample was included in a publication from a group at Harvard Medical School: Chen et al. "Glycogen synthase kinase 3 inhibitors induce the canonical WNT/β-catenin pathway to suppress growth and self-renewal in embryonal rhabdomyosarcoma." *Proceedings of the National Academy of Sciences* 111, no. 14 (2014): 5349-5354. In addition, we have recently reported PCB-00082. a Legacy Gift/research autopsy model: Hooper JE, … Bult CJ, Airhart SD, Keller C. *A Patient-Derived Xenograft Model of Parameningeal Embryonal Rhabdomyosarcoma for Preclinical Studies*. Sarcoma. 2015;2015:826124. doi: 10.1155/2015/826124. Epub 2015 Nov 30.

Experimental Design
An overview of the experimental design is presented in Figure 2.



Methods

(Aim 1) Create PDX mouse models with The Jackson Laboratory using tissue from relapsed or autopsy patients representing the "1 in 5" children.

Experimental Set #1: Sample collection. This protocol when piloted at cc-TDI was based on regional and national referrals by oncologists or the patients themselves (for example, see the patient-driven initiative at http://focusonrhabdo.org/bio-specimen-donation/#.VKDO3F4AAA). The Consortium proposal here significantly increases the scope of samples that can be collected. Primary recruitment centers are the Medical College of Wisconsin, University of Oklahoma Health Science Center, Connecticut Children's Medical Center and Johns Hopkins Medical Center. When a surgical tissue sample or autopsy tissue is available at a consortium or outside surgical pathology department, *whatever tumor tissue is left over from surgery* or any/all autopsy tissue collected will be placed aseptically into available standard media without serum (RPMI). Normal tissue (blood or other tissue) will be collected as a genetic reference of normal DNA. Whenever possible (mostly autopsies), skin biopsies will be taken for later establishment of paired normal fibroblast cultures (in case future researchers would like to use these fibroblasts to introduce and study mutated genes found in the patient's tumors).

Experimental Set #2: Creation of new, publicly-available patient-derived xenografts. For this aim, 10 high-risk, relapsed or autopsy pediatric solid tumor specimen collected we will attempt to generate a patient-derived xenograft model each year. Samples would initially be sent to JAX by way of cc-TDI (a workflow that has worked in the past). In some instances, tumor samples in media will be sent directly to the JAX Sacramento, CA, location.

Experimental Set #3: Genetic characterization of primary tumor and PDX model. All models will be validated by comparative patient-xenograft histology. Models created will also be validated by exome sequencing (normal tissue or peripheral mononuclear cells, primary tumor and PDX tumor trios). From this exome data (100x, paired end 150 bp reads), we will assess somatic mutations, gene copy number amplifications, and insertions & deletions (indels). We will also perform RNA deep sequencing (polyA library preparation for RNA-seq quantification, 20M reads/sample, single-end 50 bp reads) to affirm that mutated and/or amplified genes are also expressed or over-expressed. This data would then be deposited available from the Jackson Laboratory by request, mirrored at cc-TDI so that sample availability and de-identified sample features (mutations, RNA expression, drug sensitivity) for each sample can be viewed online, as well as in a public repository (*i.e.*, dbGaP).

To make DNA sequencing data available from the cc-TDI website portal, 2 networked secure computing multicore servers (Xeon E5 family servers (Ivy Bridge EP 48 cores with 128GB main memory) and 30TB disk space) and storage configured with RAID 6 will serve to process sequencing data. These servers have the optimized software pipelines to assemble and analyze DNA whole genome, DNA whole exome or RNA deep sequencing in a matter of a few hours. Human pipelines are based on the Broad Institute's GATK best practices (https://software.broadinstitute.org/gatk/best-practices/). For variant discovery in DNA, this standard includes mapping to reference using BWA-mem, marking duplicates with Picard and then BaseRecalibration with GATK 3.6. Thereafter, the pipeline uses HaplotypeCaller for germline, and MuTect2 for somatic. For human sequence data we use the hg38 references. STAR is used to map RNA-seq reads, and we use RSEM to quantify expression of transcripts and genes. The pipelines and tools are available to run through Galaxy and the command line. Finally, HTcondor job scheduler distributes the tasks amongst the servers.

Experimental Set #4. Model distribution. All models would be publicly (commercially)-available through The Jackson Laboratory catalog. This program is well established and the team at JAX Cancer Services is experienced at establishing hundreds of solid tumor and hematological patient-derived cancer xenografts.

(Aim 2) Generate primary cell suspensions for direct "on the spot" drug sensitivity testing with a drug panel of 60 agents in the COG drug portfolio.

Experimental Set #5: Characterization of primary tumor cells & tissues for 26 autopsy & refractory surgical cases. As stated above, once specimens are received at cc-TDI, part of the tumor will be immediately sent in media to The Jackson Laboratory in Sacramento, CA, for the generation of a patient-derived xenograft model. The remainder (0.2 – 1gm) will be digested with collagenase or similar proteolytic enzymes used with the Miltenyi gentleMACS Dissociator to create a single-cell suspension that is either (i) directly aliquoted onto drug screen plates by our Wellmate robot as described below in Exp. Set#3, or (ii) cultured in basal stem cell media with supplemental growth factors (PDGF-AB, PDGF-CC, bFGF, EGF, B27) to generate a primary cell culture. Primary cell cultures will be validated by STR genetic marker analysis versus the original tumor chunk to validate the culture. Once a primary tumor cell culture is established & validated, this culture will be cryopreserved for future investigator study, with a goal of >10 vials of > 1 million cells each. Leftover tumor received by cc-TDI will be snap frozen for protein, RNA or DNA extraction (for future studies of tumor proteins, RNA and/or DNA) and/or frozen as a Media199-cryopreserved chunk to be compatible with later generation as an *unpassaged* primary cell culture or implantation to make a patient-derived xenograft (for those labs who take a different PDX generation approach than The Jackson Laboratory). Aliquots of all these materials archived at cc-TDI for storage and widespread distribution to any qualified requester. If a large number of samples are requested that would deplete the bank, we will consult the COG disease committee biology subcommittees (RST/STS, CNS, RARE or other) whether the request is appropriate. We intentionally will have a lower threshold to share materials than the COG Biorepository, in part because the quantity of material from autopsy cases is significantly more than what COG receives for biopsies. We have a proven track record for sharing and distributing materials, as outlined earlier in this application. A very simple MTA boilerplate is already in place and is frequently used for distributing our samples.

Experimental Set #6: Drug screen characterization of primary cell suspensions. Our drug screen of 60 targeted small molecule inhibitors will be performed using our laboratory's SciClone G3 liquid handling robot. All of these agents are purchased commercially, so that no MTA is required and experiments for combinations are not restrained. For IC50 determinations, 60 drugs are plated in 4 different concentrations from 1nM to 10 μM in triplicate using two 384-well plates. Each well is seeded with 3,000 – 10,000 cells as determined by a pre-screen of cell culture specific detectability by a luminescence-based cell viability assay. Drugs are incubated for 72 hours after which the CellTiterGlo® viability assay is read by our Biotek plate reader. Absolute IC50 values are calculated as previously described [10]. A drug will be considered a 'hit' if the IC50 is below the known achievable serum concentration (C_{sss}) for pediatric or adult patients. In cases where C_{sss} is not known, we will use the known human peak drug concentration 5-30 minutes after administration (C_{max}). If C_{max} is not known, we will estimate the C_{max} ($C_{max\text{-}EST}$) based upon the maximal tolerated dose of the drug from pediatric or adult Phase I trials and the total serum volume of an average child or adult, factoring the degree of serum protein drug binding. These results are available to anyone receiving the specimen from the cc-TDI archive, as well as via the online cc-TDI CureFast portal. If any of the molecule shows promising results, we would also seek additional, outside funding to test the compound in the matched PDX models that are generated from the same tissue. This would then complete the loop of preclinical drug screening using the resources that we have created through the consortium.

DRUG SCREEN (custom)

drug name	target
marizomib (salinosporamide A, NPI-0052)	20S proteasome
LDN-212854	ACVR1/BMPRs
AZD5363	AKT1-3
LDN193189	ALK2/ACVR1
dorsomorphin	ALK2/ACVR1
brefeldin A	ATPase inhibitor
AMG900	AURK-A, B, C
ABT-263(Navitoclax)	BCL-2, BCL-XL, and BCL-W inhibitor
GSK525762 (I-BET-762)	BRD
JQ1	BRD
dinaciclib (SCH727965)	CDKs
GSK-923295(GSK-923295A)	CENP-E inhibitor
LY2606368	Chk1
PF670462	CK1d/CK1e
naproxen	Cox inhibitor (non-selective)
laccaic acid	DNMT
pinometostat (EPZ5676)	DOT1L
thapsigargin	endoplasmic reticulum
GSK126	EZH2
PF 573228	FAK
LY2874455	FGFR1-4
pemetrexed	folate metabolism
UNC0642	G9a
CHIR-99021	GSK3β
GSK-J4 HCl	H3K27 histone demethylases JMJD3,UTX
CUDC101	HDAC I/II and EGFR, HER2
CUDC907	HDAC1/2/3/10 and PI3K
panobinostat (LBH-589)	HDACs
entinostat	HDACs
BIX 01294 HCl	Histone-lysine methyltransferase
CUDC-305	hsp90
CUDC-427	IAP inhibitor
OSI-906 (linsitinib)	Igf1r
BMS-754807	Igf1r, AURK
JIB-04 (NSC693627)	KDM4
masitinib	Kit and PDGFRα/β
dasatinib	Kit, PDGFR, SFK, Bcr-Abl
toceranib	KIT, VEGF 2, PDGFRβ
LSD1 Inhibitor IV	LSD1
trametinib (Mekinist, GSK1120212)	MEK
crizotinib (PF-02341066)	MET, ALK
sirolimus (rapamycin)	mTOR
APR-246 (PRIMA-1)	mutant p53
aprepitant	NK1R
DAPT	Notch
CRM1 Inhibitor III	nuclear export
AZD8931	Pan-ERB
BKM120 (Buparlisib)	PI3K, mTOR

Table 1. Custom chemical (drug) screen.

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