University of Pittsburgh

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Formula Grant Overview

The University of Pittsburgh received $8,236,620 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Cellular Systems Biology in Cancer Drug Discovery - The project focuses on the development of a research program to discover and develop small molecule anticancer therapeutics. At the University of Pittsburgh, novel chemistries are combined with cellular systems biology and computational pharmacology approaches to drug discovery. The overriding goal of this project is to further enhance the broad scientific capabilities at the University of Pittsburgh in these research areas and to deploy them for the development of novel anticancer drug candidates and, ultimately, commercial drugs.

Duration of Project

1/1/2011 - 12/31/2013

Project Overview

The pharmaceutical industry has been hampered in developing new drugs, including cancer drugs, by the complexity of biological systems that give rise to on- and off-target effects of lead compounds in living systems. It is now understood that life is based on integrated, interacting networks of genes, proteins, and metabolic processes that give rise to either normal or, in disease, abnormal functions. This complex "systems biology" must be addressed to create a safe and effective drug. The pharmaceutical industry has, instead, focused primarily on simplifying biological testing to speed up the process. The result has been a serious failure rate (>90 %) in getting drug candidates safely through the drug discovery and development process. However, academic groups are ideally positioned to investigate cancer biology and potential off-target compound toxicity by directly studying this complexity with more sophisticated cell-based model systems and computational methods. The University of Pittsburgh Drug Discovery Institute (UPDDI) has been integrating its expertise in cancer biology, chemistry, medicinal chemistry, safety assessment, and computational molecular and systems biology to pursue novel cancer drug discovery. The goal of the present project is to advance anticancer drug discovery by

integrating current activities with novel chemistry, systems biology, and computational pharmacology approaches and applying the new methodology to developing three classes of targets: (1) improved modulators of microtubule dynamics, (2) transcription factors (e.g., STAT3 [signal transducer and activator of transcription]), and (3) modulators of the interaction between two critical proteins (e.g., Myc-Max) in an important cellular signaling pathway (Myc is also a transcription factor). Functional, cell-based models using human cell lines, primary cells, and ultimately validated stem cell-derived cells will be developed and investigated using medium-throughput and high-content light microscope imaging methods, as well as mechanistic probe molecules, to define the systemic response to both commercially available and novel drug candidates. Computational methods will be developed and applied to assess the protein-protein and protein-inhibitor interactions at the molecular structural level and to relate the cellular responses to known patterns of gene and protein responses, cellular pathways, and protein-protein interaction networks. Critical attention will be given to polypharmacology effects for early safety assessment and compound prioritization. Promising compounds will be further developed by lead optimization and pharmacokinetics studies, including animal models.

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Expected Research Outcomes and Benefits

This project will harness collaborations across the University of Pittsburgh from multiple departments and institutes to create novel compounds that can be progressed toward effective therapeutics. A key outcome will be to progress one or more compounds to the point at which there is significant interest by the pharmaceutical industry to either license the candidate drug or collaborate with UPDDI to develop the candidate further. The project will increase the University's research capacity by creating new opportunities in tissue engineering, chemical synthesis, systems biology, and computational biology and should yield new technologies that can be licensed to industry partners. The University of Pittsburgh has a strong presence in cancer biology with the University of Pittsburgh Cancer Institute (UPCI) and has significant expertise and federal funding that can facilitate the drug discovery process. Together with the Departments of Pharmaceutical Sciences, Chemistry, and Computational and Systems Biology, UPDDI is also a member of the Chemical Biology Consortium (CBC) of the National Cancer Institute, and the Department of Chemistry hosts one of five National Institutes of General Medical Sciences (NIGMS)-sponsored Centers for Chemical Methodologies and Library Development. These

centers provide the researchers access to chemical libraries and broad collaborations with other academic centers of excellence working on cancer therapies. The application of a cellular systems biology approach increases the potential of a more personalized medicine outcome for sub-populations when stem cells replace the human cell lines and primary cells in the future.

Summary of Research Completed

Based on results reported last year, in the final six months of this project (7/1/13-12/31/13) we have developed an early safety-assessment panel, expanded reference-safety database content, and produced and published a computational tool to analyze heterogeneity in the distribution of cellular responses.

Further work on milestones for the period 7/1/2012-6/30/2013. (We continued to work on the following two milestones from the prior reporting period.)

1. Demonstrate the predictive capabilities of the early safety assessment panels with a reference safety database of at least 200 compounds and publish the results.

   Progress: Six "fit for purpose" models to screen for cardiotoxicity, hepatotoxicity, metabolic-liver clearance, and drug metabolites have been established in UPDDI. The low-throughput zebrafish embryo cardiotoxicity model described in last year's report has been converted to a moderate-throughput screen (in the industry standard microplate format) capable of using low power, white-light video microscopy and computational-image processing to identify potential pro-arrhythmia drugs. In addition to our pre-existing rodent liver cell and HepG2 models, we have successfully established a primary human hepatocyte plate-based assay to facilitate compound profiling. Our collaborators will test three systems to identify hepatotoxins—the zebrafish assay to identify cardiotoxins, the microsomal metabolic drug stability, and the new addition of drug clearance and drug metabolites in human hepatocytes.

   Cardiotoxicity progress: Previously, we reported the development of a cardiotoxicity screen in zebrafish embryos as a low-throughput video microscopy method. The literature and our own results have demonstrated that the zebrafish embryo can be used to identify drug compounds that are pro-arrhythmic and inhibit the zebrafish equivalent of the human cardiac *human ether-à-go-go related gene* (hERG) channels.

   Methods/results: The cardiotoxicity screen tool developed in this project at the UPDDI uses the Arrayscan VTI (ThermoFisher) High Content Screening (HCS) instrument to acquire short video clips of zebrafish imaged at 5X in each of the wells of a 96-well plate (Fig. 1). Ten-second video clips from all 96 wells can be collected in approximately 15 minutes (Fig. 1A). An example frame captured from each well A1–A6 and B1–B6 is presented to the user for quality control (Fig. 1B). The image analysis software graphically presents the frame-by-frame change in pixel intensity (Fig. 1C). Each video clip is then analyzed using algorithms developed in UPDDI, as previously reported, to calculate heartbeat rate and periodicity between beats (Fig. 1D). This rapid screen has been tested successfully in a pilot study and has the capacity to process up to 1,000 compounds a week (per instrument, limited to the supply of zebrafish) in duplicate at a single drug dose, or more than 100 compounds a week in a full eight-point dose response.

Metabolic stability screening progress: This screening assay is designed to predict the liver clearance of compounds in early drug discovery to identify those with undesirable pharmacokinetic (PK) properties. Metabolite identification is important in drug discovery to account for toxicity caused by the generation of reactive intermediates or the production of the active pharmacophore from a pro-drug. We have tested and used the metabolic screen to measure hepatic clearance and identify major drug metabolites in a novel 3D human liver organoid model.

Methods/results: Compounds and metabolites (Table 1) were tested in the liver organoid and analyzed using the basic metabolic stability screening protocol previously reported (Fig. 2). All parent compounds were introduced into the liver organoid model. Media was collected for 24 hours. All parent compounds and metabolites were fragmented to the correct daughter ions. (Fig. 2A) Mass spectrometry of testosterone in influx media. (Fig 2B) Mass spectrometry of testosterone in efflux media. (Fig 2C) Mass spectrometry demonstrating the cytochrome P450 3A4 (Cyp3A4) signature metabolite 6-beta-hydroxytestosterone in efflux media. The clearance results of all compounds and metabolite identifications agree with published findings.

MOT-based cytotoxicity screening progress: We have previously developed and reported on the use of a two-cell-type assay designed to identify oxidative stress as the mechanism of toxicity (MOT). The HCS image-based assays use a panel of probes designed to identify general cytotoxicity, reactive oxygen species generation, and mitochondrial function. We expanded the original two-cell screen, which used HepG2 cells and primary rat hepatocytes, to include primary human hepatocytes.

Methods/results: UPDDI now offers three mammalian cell panels and the zebrafish cardiotoxicity test to our collaborators to identify liver and cardiac toxicity. Table 2 presents data from a development series of compounds profiled by the assays. Column headers list the features measured in each model system. The heat map is color-coded to show the toxicity risk indicated by each of the assays. These data are being used, along with chemical properties and efficacy data, to prioritize compounds for further development.

2. Further optimize the computational tools and publish the results

Progress: This investigation is part of a larger QSP program initiated in 2012-13. QSP integrates the best experimental and clinical methods with computational and systems biology to optimize the discovery and development of therapeutics. We extended our analysis of cellular heterogeneity in the STAT3 signaling pathway in head-and-neck and breast cancer cell lines to more robustly characterize heterogeneity in a way that can be incorporated into large-scale biology projects, including screening in drug discovery, as well as to develop improved diagnostics and cancer therapies.

Methods/results: We developed a trio of quantitative heterogeneity indices, *diversity (DIV)*, *non-normality (n-NRM)*, and *percent-outliers (%OL).* These define the degree of variation, whether the variation is normally (randomly) distributed, and whether there are significant numbers of outlier cells (Fig. 3). The histograms in Fig. 3 illustrate the general nature of the

heterogeneity indicated by each index. In each case the histograms are normalized to a total area of 1. For example, an increase in Div results from a broadening of the distribution as illustrated by the red histogram (Fig 3, top), compared with the blue histogram (Fig 3, top). The nNRM index indicates a splitting of the population into 2 or more subpopulations again indicated by the red histogram (Fig 3., middle). Finally, %OL indicates the presence or small numbers of cells which respond very differently than those in the main distribution, as indicated by the small spikes on the red histogram (Fig. 3 bottom). Thus, each of these indices captures a unique aspect of heterogeneity. Combined, they can be used to classify cellular systems into heterogeneity types that are directly related to the underlying mechanisms. Using thresholds established for each index, a binary decision tree can be used to characterize heterogeneity in a given sample (Fig. 4). The thresholds for DIV (0.03) and nNRM (0.05) were selected as the mean + 3 standard deviation (SD) for each index in replicate negative control wells for human tumor Cal33 cells. The threshold for %OL (4.5 percent) is the percent outliers expected for a normal distribution. We applied the heterogeneity indices to the inhibition of STAT3 activation by interleukin 6 (IL-6) in Cal33 cells (Fig. 5). Cells were exposed to Pyridone-6 or Stattic at the indicated concentrations for three hours prior to stimulation with 50 ng/ml of IL-6. (Fig 5A) Log-scaled distributions of inhibition by pyridone-6. (Fig. 5B) Three heterogeneities were calculated, DIV (red), nNRM (green), and %OL (blue). (Fig 5C) Log-scaled distributions of inhibition by Stattic. (D) The same three heterogeneity indices are plotted for the linear scaled distributions of Stattic inhibition. The vertical dashed lines indicate the IC50 for the well-averaged signal intensities. In late Fall 2013 we began work on a manuscript entitled: *Identifying and Quantifying Heterogeneity in High Content Analysis: Application of Heterogeneity Indices to Drug Discovery*, for submission to PLOS ONE.

A more complete description and strategy for the implementation of QSP was accepted in the Fall of 2013 for publication as a book chapter, *High-Content Analysis with Cellular and Tissue Systems Biology: a Bridge between Cancer Cell Biology and Tissue-Based Diagnostics* in The Molecular Basis of Cancer. J. Mendelsohn, P. M. Howley, M. A. Israel, J. W. Gray and C. Thompson, Elsevier. This chapter discusses the application of high-content cell methods to cancer cell systems, and combining computational approaches, including machine learning, modeling, and network inference, to enhance understanding of the impact of cellular heterogeneity and spatial complexity on cancer development and progression.

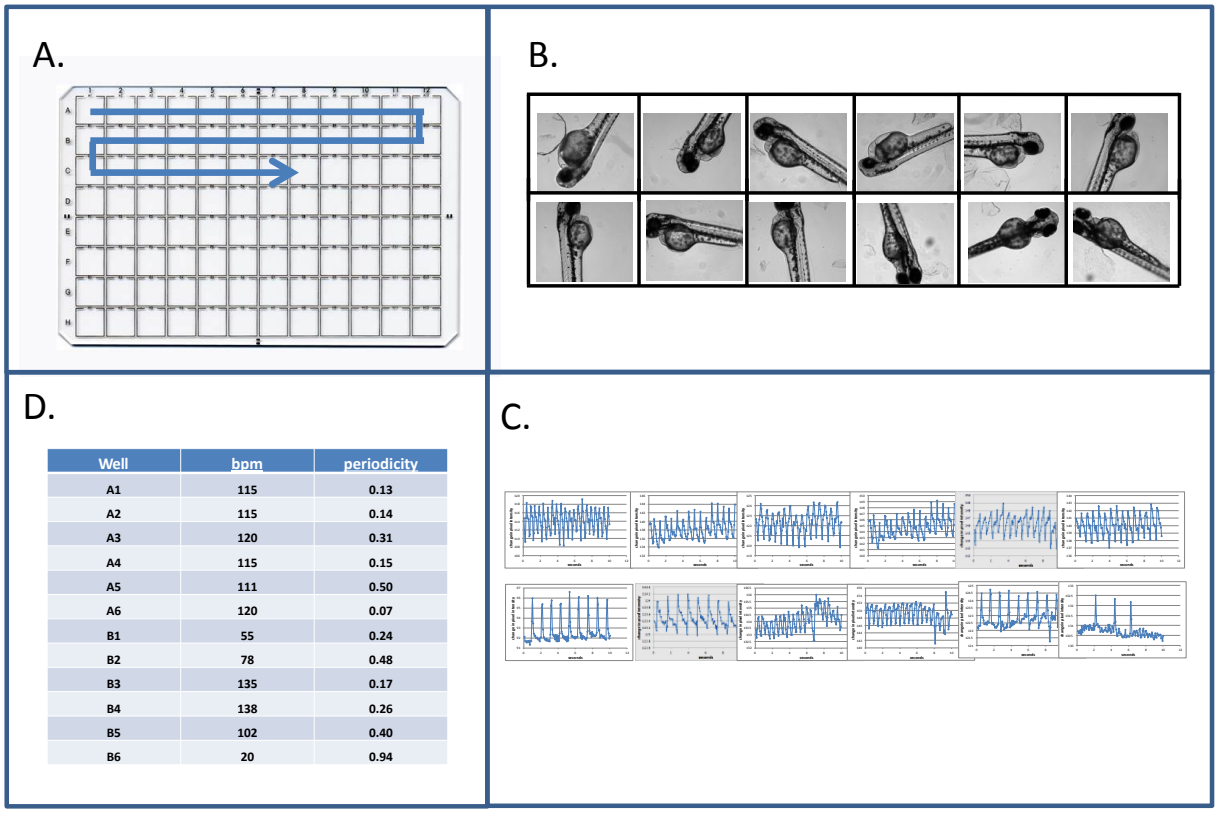
Milestone(s) for 7/1/2013-12/31/2013: (Project ended on 12/31/2013)

1. Take at least one additional drug candidate into preclinical testing
   Progress: The STAT3 head-and-neck cancer project has produced two chemical scaffolds that are the basis of lead optimization studies. Efforts are underway to identify the mechanism of action (MOA). This work has been a collaboration with Jennifer Grandis, MD, and Donna Huryn, PhD.
   Methods/results*:* Initial MOA studies suggest the blocking of the activation of the IL-6 receptor complex. Further studies are progressing.

2. Have at least one meeting with a pharmaceutical/biotechnology company to discuss licensing and/or collaborative development of leads

We are in discussions with a number of pharmaceutical companies to collaborate on drug development.

3. Initiate at least two new targets for investigation
   Progress: Due to continued success on the STAT3 project and early safety-assay panels, we have focused efforts on extending these projects and publishing results.



Well	bpm	periodicity
A1	115	0.13
A2	115	0.14
A3	120	0.31
A4	115	0.15
A5	111	0.50
A6	120	0.07
B1	55	0.24
B2	78	0.48
B3	135	0.17
B4	138	0.26
B5	102	0.40
B6	20	0.94

Fig. 1. 96-well Video Capture and Data Analysis Method for Zebrafish Embryo Cardiotoxicity Screening.

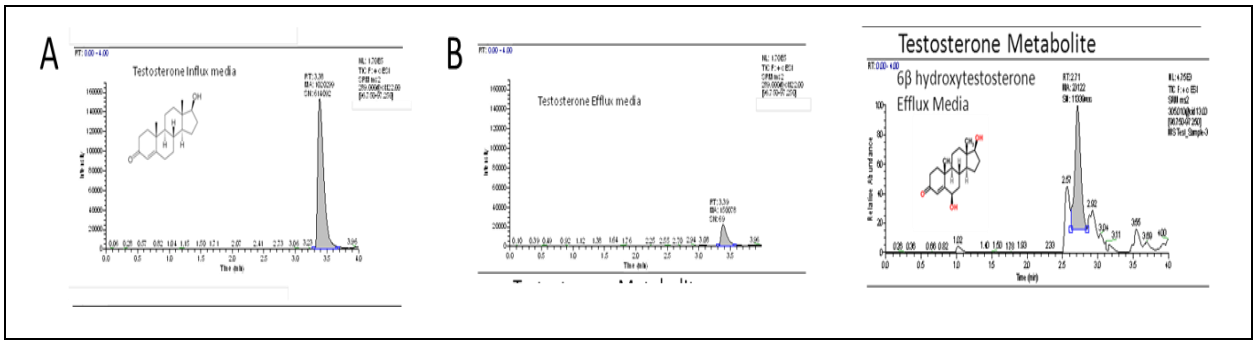


Fig. 2. Hepatic Clearance and Metabolite Identification of Selected Compounds.

Table 1. Hepatic Clearance and Metabolite Identification of Selected Compounds

Drug (concentration influx media)	Retention time (min.)	Parent → Daughter Ions	Drug mass area influx media	Drug/ metabolite mass area efflux media	% parent remaining/ clearance class
Caffeine (100uM)	1.6	195 → 138	45,426,893	36,066,715	%80 – low clearance
Phenolphthalein (10 uM)	3.0	319 → 225	1,769,425	9162	<1% High clearance
Phenolphthalein Glucuronide	2.5	495 → 319	None Detected	325,188	
Terfenadine (5 uM)	3.2	472 → 436	103,487	157	<1% High clearance
Metabolite 1– Hydroxyterfenadine	2.8	488 → 452	None Detected	14,800	
Metabolite 2– Carboxyterfenadine	2.8	502 → 466	None Detected	120,500	
Testosterone (5 uM)	3.4	289 → 97	1767523	2967	< 1% High Clearance
Metabolite-6β-hydroxytestosterone	2.7	305 → 269	None Detected	23122	

Table 2. Toxicity Profile Results For UPHD Compounds

	Human Hepatocytes (IC50 in uM)					HepG2 Cells (IC 50 in uM)			rat hepatocytes (IC50 in uM)		Zebrafish
Compound	Mito Func. donor 1 5/4/13	Mito Func. donor 2 7/2/13	ROS donor 1 5/4/13	ROS donor 2 7/2/13	Donor 1 48 hr cytotoxicity	Cyto C Release	3 day cyto	mito inhibition	rheps 5 Day cytotoxicity	Rat heps Mito func.	Cardiotoxicity heart beats/min
UPHD 25											
UPHD 49							82.2				
UPHD 36							148.3		237.8		
UPHD 79											
UPHD 190							45.9				
UPHD 77	126.5	37.9	71.8	70.0	43.3	70.5	25.0	21.1	86.6	38.7	54 @ 19 um
UPHD 186	288.8	97.4	124.8	146.0	60.7	133.8	80.7	83.2	82.4	61.2	87 @ 19 uM
UPHD 183											
UPHD 191	231.5	164.5	216.6	255.0	76.4	211.4	57.7	137.5	126.6	129.4	99 @ 19 um
UPHD 182											
UPHD 193	87.7	86.7	92.2	138.0	58.9	124.9	49.9	77.9	125.8	103.5	90 @ 19 uM
UPHD 188		164.8	244.6		110.8		69.6	221.1	94.6		
UPHD 20									107.2		
Vehicle											131
	red = high risk		yellow = mild risk			orange = moderate risk			Green = no effect (minimal risk)		

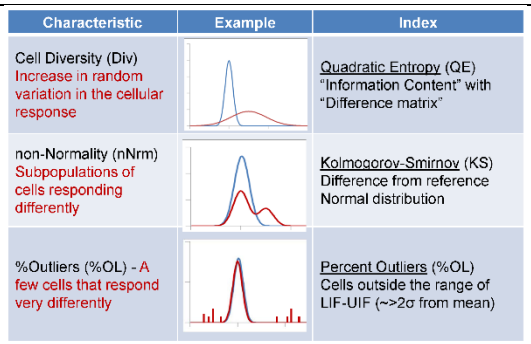


Fig. 3. Three indices for characterizing cellular heterogeneity.

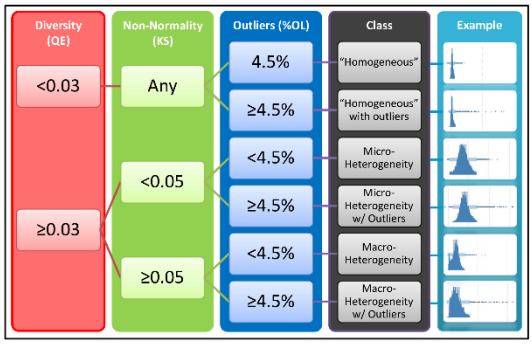


Fig. 4. Decision tree for interpreting the Heterogeneity Indices.

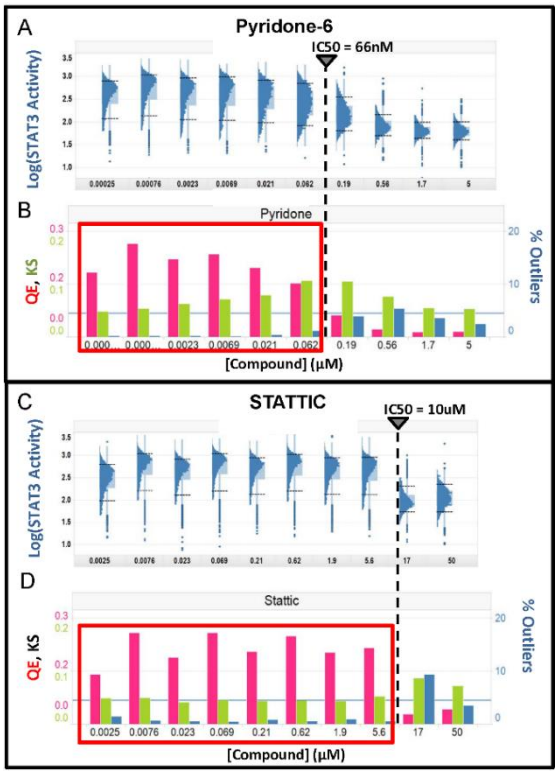


Fig. 5. Heterogeneity in response to inhibitors of STAT3 activation.

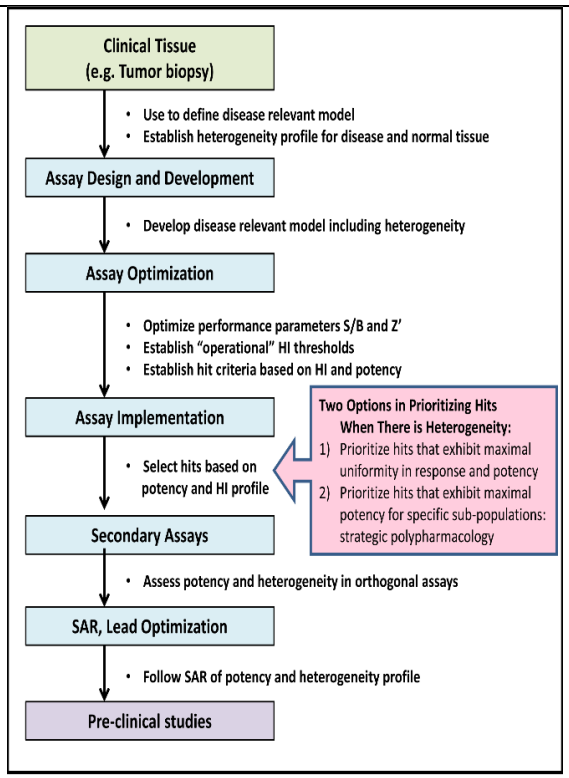


Fig. 6. Heterogeneity analysis applied throughout the early drug discovery process.

Research Project 2: Project Title and Purpose

Complex Genetics of Congenital Heart Disease - This study will examine the complex genetics of congenital heart disease (CHD), specifically, the role of genetic variants of genes encoding ciliary proteins (the ciliome) in the pathogenesis of CHD. Previous studies have suggested an association between ciliary dysfunction and CHD with heterotaxy, a birth defect characterized by discordant cardiac, lung, and visceral organ situs resulting from aberrant embryonic left-right patterning. This study will use next-generation sequencing technologies to analyze whole exomes of CHD patients to identify disease-causing polymorphisms, specifically in the genes comprising the ciliome. These findings will likely shed light on the complex genetics of CHD and may lead to new diagnostic genetic tests that can help identify patients, specifically infants and children, at high risk for ciliary disorders.

Anticipated Duration of Project

1/1/2011 - 12/31/2014

Project Overview

Heterotaxy is a birth defect characterized by discordant cardiac, lung, and visceral organ situs resulting from aberrant embryonic left-right patterning. Patients with heterotaxy exhibit some of the most complex congenital heart diseases (CHD). Such patients usually require risky surgical repairs, with high rates of postsurgical morbidity, and often develop postsurgical respiratory complications, sometimes becoming ventilator dependent. As mucociliary clearance in the airway requires ciliary motion, respiratory complications and poor outcome could arise from undiagnosed ciliary dysfunction, such as that associated with primary ciliary dyskinesia (PCD). This possibility is particularly compelling for heterotaxy patients, as motile cilia are also required for the specification of laterality during embryonic development. To investigate the role of cilia defects in CHD, we recently initiated a clinical study that recruited patients with complex CHD associated with heterotaxy. From a cohort of 42 patients, 41 percent were observed to exhibit ciliary dysfunction. These results strongly suggest a role for the cilium in human CHD and heterotaxy. We hypothesize that sequence variants in genes required for cilia structure and function (i.e., genes of the ciliome) may play a central role in CHD pathogenesis. To test this hypothesis, we will use whole exome capture and massively parallel sequencing to scan for mutations in the coding exons of the human genome, with particular focus on the ciliome genes. The enriched genomic deoxyribonucleic acid (DNA) recovered from sequence capture will be amplified and sequenced with 100 X coverage using an AB SOLiD™ sequencing instrument. Of particular interest are nonsynonymous variants leading to missense or nonsense mutations and splicing defect variants or small insertions/deletions that may cause frameshifts. Mutations causing CHD are expected to be rare in the unaffected population, with allele frequencies < 1 percent. High priority single nucleotide polymorphisms (SNPs) identified from this analysis will be validated by direct sequencing of patient genomic DNA and further interrogated across datasets available in the public domain, such as from the 1,000 Human Genomes Project. The findings from this first patient cohort will be validated with similar analysis of a replication cohort of an additional 200 patients to be recruited from Children's Hospital of Pittsburgh of UPMC. Overall, these studies may yield novel insights into the complex genetics of CHD. Results from this study may suggest changes in the standard of care that may improve the long-term prognosis of heterotaxy patients with complex CHD.

Specific Aims:

Aim 1. We will identify possible disease-causing sequence variants using whole exome capture and next-generation sequencing in 50 patients with complex CHD and heterotaxy.

Aim 2. Sequence variants recovered in the ciliome and CHD genes will be interrogated using online repositories, such as dbSNP or HapMap, to identify those that are rare variants or mutations. These sequence variants will be validated by capillary sequencing of the patient DNA and further interrogated against sequencing data from the 1,000 Human Genome Project and other publically available human exome/whole genome datasets.

Aim 3. We will validate rare disease-causing sequence variants recovered in the CHD patients. Validation will occur by conducting exome capture and SOLiD sequencing of a replication cohort of 200 CHD patients to be recruited from Children's Hospital of Pittsburgh of UPMC.

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Expected Research Outcomes and Benefits

Our study seeks to demonstrate a link between ciliary dysfunction, mutations involving genes essential for cilia structure and function, and CHD. These findings may suggest a broad role for the cilium in a wide spectrum of CHD previously thought to have no unifying etiology. This hypothesis, if validated, may suggest changes in the standard of care that could improve the postsurgical outcomes and long-term prognosis of patients with complex CHD. In addition, results from our DNA analyses may provide the basis for the development of diagnostic genetic tests that can help identify patients at high risk for PCD among patients with complex CHD. These data could also suggest changes in the standard of care to include presurgical screening for PCD and instituting appropriate preoperative and postoperative pulmonary therapies, which could improve the long-term prognosis of heterotaxy patients with complex CHD. Overall, these studies may yield novel insights into the complex genetics of CHD and provide strategies for improving the long-term outcomes for our most vulnerable patient population—infants and young children with complex CHD.

Summary of Research Completed

Our study remains focused on interrogating the genetic etiology of CHD. Our initial study focused on patients with CHD associated with heterotaxy (the randomization of left-right patterning of visceral organ asymmetry). CHD patients with heterotaxy exhibit high morbidity and mortality associated with increased postsurgical respiratory complications. Because motile cilia are required for left-right patterning and airway clearance, we hypothesized that motile cilia defects may underlie the CHD/laterality phenotype and increased respiratory complications in heterotaxy patients. Hence, our goal has been to investigate the prevalence of motile cilia defects in the airways of CHD/heterotaxy patients and determine whether those with airway ciliary dysfunction may have increased burden for mutations known to cause primary ciliary dyskinesia (PCD). PCD is a sinopulmonary disease elicited by mucociliary clearance defects caused by dyskinetic or immotile cilia in the airway. Indeed, our exome sequencing analysis revealed an enrichment for PCD gene mutations in heterotaxy patients with airway ciliary dysfunction. These studies suggest that worse postsurgical respiratory outcomes in hetertoaxy patients may be related to cilia mutations causing airway cilia dysfunction.

Since our initial studies on heterotaxy patients, we have expanded our patient recruitment and analysis to CHD patients without heterotaxy. From the recruitment and analysis of nearly 400 CHD patients without heterotaxy (and 60 with heterotaxy), we have observed that CHD patients without heterotaxy also exhibit a high prevalence of ciliary dysfunction. Particularly exciting is that in this past year, we have begun to conduct postsurgical outcome studies of the CHD patients undergoing palliative cardiac surgeries; these studies have shown that CHD patients with ciliary dysfunction have significantly more postsurgical respiratory complications with increased morbidity (see Table 1). We are now conducting whole exome sequencing analysis on a subset of these patients exhibiting a wide range of CHD, approximately half of whom exhibit ciliary dysfunction and half whom do not (Table 2). Disease controls comprised of patients suspected to have PCD were included in this exome sequencing analysis. Using the exome sequencing data from these patients, we will be able to determine whether there is increased burden for PCD and other cilia mutations in CHD patients with ciliary dysfunction versus those without ciliary dysfunction.

During the past year, we also began a collaboration with Ashok Panigrahy, MD, to explore the potential association of ciliary dysfunction with worse neurodevelopment outcomes in CHD patients. These studies are motivated by the fact that CHD patients are known to have high risk for poor neurodevelopmental outcomes; both nonmotile primary cilia and motile cilia are known to play important roles in brain development and function. While Dr. Panigrahy's group has been conducting brain magnetic resonance imaging (MRI) on CHD patients for detection of structural brain defects, we have examined airway ciliary function on these same CHD patients.

Airway Cilia Defects and Respiratory Complications in Jeune Syndrome Patients

In our patient recruitment effort, we recruited a patient with *situs inversus totalis* who was diagnosed with Jeune syndrome. Our whole-exome sequencing analysis revealed compound heterozygous pathogenic *WDR35* mutation in this patient. *WDR35* encodes an intraflagellar transport (IFT) A component (IFT121) is essential for primary cilia assembly and has recently been shown to cause ciliary skeletal disorders like Sensenbrenner, and short-rib polydactyly syndromes, both of which have significant phenotypic overlap with Jeune syndrome. This patient exhibited severe pulmonary complications, which in such ciliopathy patients is usually attributed to the restrictive rib cage associated with the skeletal dysplasia. However, this patient exhibited obstructive airway disease and atelectasis, which are not usually observed with Jeune syndrome. This observation led us to hypothesize that the *WDR35* mutations may also disrupt motile cilia function in the airway required for mucociliary clearance.

To test this hypothesis, we conducted two tests usually used for PCD diagnosis: videomicroscopy of ciliary motion in nasal epithelial cells and measurement of nasal nitric oxide (nNO) level. We observed immotile/dyskinetic airway cilia and very low nNO, phenotypes typically seen with PCD. *WDR35* siRNA gene knockdown in *ex vivo* cultures of reciliating human respiratory epithelia disrupted ciliogenesis and motile cilia function, demonstrating WDR35 is also required for motile cilia function in the airway. Together, these findings suggest that patients with non-PCD ciliopathy can also suffer pulmonary complications arising from motile cilia defects in the airway. The findings from this study are clinically significant, as it suggests that ciliopathy patients can have motile cilia defects that put them at risk for PCD-like

pulmonary complications. Evaluation for PCD in these patients may allow early aggressive pulmonary therapy to improve their clinical outcomes. This study has been submitted for publication.

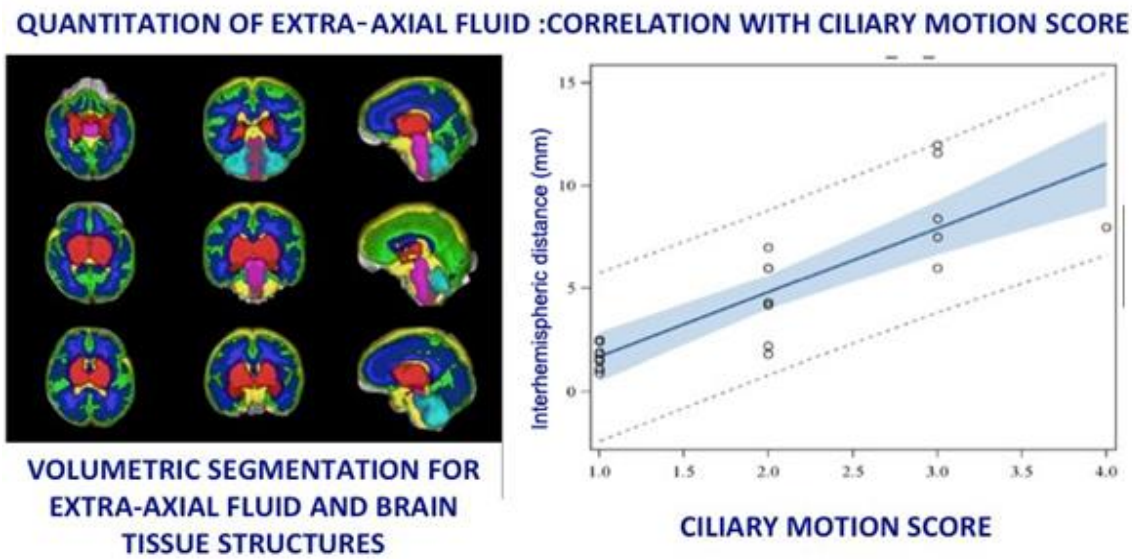
Table 1. Postoperative Outcomes and Ciliary Dysfunction in CHD Patients*

	CHD patients without CD	CHD patients with CD	P value§
Number of Patients	34/55 (62%)	21/55 (38%)	
RACHS†	3.5 ± 1.6	3.1 ± 0.9	0.696
ARISTOTLE†	9.4 ± 3.1	9.1 ± 3.0	0.857
CICU Length of Stay	8.2 ± 6.1	7.9 ± 5.6	0.756
Postoperative Length of Stay	20.7 ± 18.1	18.8 ± 16.6	0.637
Mechanical Ventilation	4.3 ± 2.9	3.9 ± 2.6	0.626
Supportive Ventilation	7.2 ± 7.2	6.1 ± 6.1	0.362
CPAP	0.5 ± 1.6	1.0± 1.4	**0.008**
Reintubation‡	5/45 (11%)	7/26 (27%)	0.087‖
ECMO	0.2 ± 0.5	0.3 ± 0.7	0.577
Albuterol	0.5 ± 1.4	1.3 ± 2.8	**0.006**
Respiratory Medications#	5/45 (11%)	13/26 (50%)	**0.0001**‖

*All measurements in days except as noted; CICU = cardiac intensive care unit; supportive ventilation includes CPAP, high flow nasal cannula, and nasal cannula. Respiratory medications include beta-agonists, inhaled corticosteroids, mucolytics, and lung surfactant.
†Surgical complexity score; ‡Number of events of reintubation per surgery/total number of surgical events; §Number of events where respiratory medications were used/total number of surgical events; ‖P values by Pearson chi-squared test; #P values by Wilcoxon rank-sum test.

Table 2. Whole Exome Sequencing of CHD Patients

CHD Type	No. Patients	Ciliary Dysfunction
Ebstein	9	5 (56%)
HLHS	20	8 (40%)
DORV	6	4 (67%)
TOF	14	9 (64%)
HTX	39	18 (46%)
TGA	53	28 (53%)
PCD Suspects and Others	17	12 (71%)



Comparison of the CHD patients with different levels of ciliary motion defects (as assessed based on a ciliary motion score of 1 to 4, 1 being completely normal and 4 being very abnormal ciliary motion) showed a surprisingly strong correlation between airway ciliary dysfunction and brain anomalies, including an increase in extra-axial fluid (see figure above), as well as other brain anomalies, suggesting airway ciliary dysfunction is a proxy for cilia defects that may perturb brain development. The exome sequencing analysis we currently have in progress will help further elucidate whether specific ciliome mutations may contribute to both the airway ciliary dysfunction and brain anomalies in the CHD patients. Such analysis will proceed in the coming months as exome sequencing data is obtained and further analyzed and validated.

Research Project 3: Project Title and Purpose

Breaking Metabolic Symbiosis in Tumors: A New Cancer Treatment Paradigm - As cancer cells grow and evolve into tumors, they need specific nutrients and oxygen to fuel cellular metabolism, namely, through mitochondrial oxidative phosphorylation and glycolysis. This project will test the hypothesis that those cancer cells with the highest metabolic flexibility are most successful at developing tumors. In a tumor, the populations of cells farthest from an oxygen supply produce energy through glycolysis and create lactate, a waste product, which is used by the more oxygenated cell population to produce energy by oxidative phosphorylation. This project will examine how this metabolic symbiosis drives rapid tumor growth. Understanding how this interdependent metabolic sharing of carbon sources occurs in a growing tumor and learning how to break the cycle will enable improved therapeutic approaches to cancer.

Duration of Project

1/1/2011 - 12/31/2012

Summary of Research Progress

This project ended during a prior state fiscal year. For additional information, please refer to the

Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act77 Web page at http://www.health.state.pa.us/cure.

Research Project 4: Project Title and Purpose

Prostate Cancer Vaccine Clinical Trials - This project encompasses investigator-initiated clinical trials that explore the safety and efficacy of novel therapeutic cancer vaccines, with the overarching goal of improving outcomes of patients with cancer. Aim 1 includes therapeutic trials of novel cancer vaccines based on polarized alpha dendritic cells (αDC1s) for the treatment of prostate cancer, and Aim 2 examines the efficacy of novel tumor peptide-based cancer vaccines for the treatment of prostate cancer.

Duration of Project

1/1/2011 - 12/31/2012

Summary of Research Progress

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act77 Web page at http://www.health.state.pa.us/cure.

Research Project 5: Project Title and Purpose

Melanoma Vaccine Clinical Trial - This project is an investigator-initiated clinical trial that explores the safety and efficacy of novel therapeutic cancer vaccines based on advances made at the University of Pittsburgh in the generation of durably polarized alpha dendritic cells (αDC1s) for the treatment of melanoma.

Duration of Project

1/1/2011 – 12/31/2012

Summary of Research Progress

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act77 Web page at http://www.health.state.pa.us/cure.

Research Project 6: Project Title and Purpose

Glioma Vaccine Clinical Trials - The University of Pittsburgh Cancer Institute's Clinical Research Service (CRS) provides valuable resources for researchers seeking to improve patient care standards and treatment efficacy. This project encompasses investigator-initiated clinical trials that explore the safety and efficacy of novel tumor peptide-based cancer vaccines for the

treatment of low-grade glioma (LGG).

Duration of Project

1/1/2011 – 12/31/2012

Summary of Research Progress

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act77 Web page at http://www.health.state.pa.us/cure.