KPCRTF State Funded Projects Reporting Template

University of Kentucky – Project #1
Circulating tumor DNA as a prognostic indicator of minimal residual disease and central nervous system relapse in Acute Lymphoblastic Leukemia
Program Director: Jessica Blackburn

Reporting Period: _____Final_____

Below please provide a brief summary of the status of the Project listed as well as for each Aim listed below. Include any barriers, how and if they were overcome, and successes achieved.

Project #1: Circulating tumor DNA as a prognostic indicator of minimal residual disease and central nervous system relapse in Acute Lymphoblastic Leukemia

We have met all of the original goals of this project. Of note, we have developed a rapid and inexpensive assay to detect ctDNA from patient blood and cerebrospinal fluid using 4th generation nanopore sequencing. This assay can correctly identify minimal residual disease and central nervous system disease in ALL, in comparison to standard clinical diagnosis methods. This assay is a major step forward from the current standard clinical assay for MRD (FACS based cell detection) and the latest next gen sequencing assay currently in clinical trial for detecting MRD (Clonoseq NGS assay) because it does not require the presence of actual cells in the patient sample.

Aim 1: Create a biobank of primary leukemia samples from Kentucky pediatric ALL patients
We have banked viable samples from 18 KY pediatric ALL patients, 10 from the Appalachian region, and are in process of expanding these in mice. They will be used by our laboratory in follow-up research studies, and shared with other researchers whenever requested.

Aim 2: Determine the extent to which ctDNA in blood and CSF of ALL patients predicts or correlates with clinical diagnosis of minimal residual disease and relapse
We have created a new method to use nanopore DNA sequencing to track cfDNA in patient blood and CSF samples and showed that these methods can detect leukemia associated cfDNA in minimal residual disease and central nervous system disease. Our project is the first to show that cfDNA can be detected in ALL, and the first to use 4th generation nanopore sequencing for detection of cfDNA. We have a manuscript in submission to Journal of Clinical Investigation, and a grant submitted to the National Cancer Institute to continue this study to determine the specificity and sensitivity of this assay.

Aim 3: Develop a universal ddPCR assay to detect ctDNA in ALL patients
We have selected recurrent sites of methylation in ALL, compared to normal lymphoctyes, and are in process of validating these sites for use in clinical assays.

Timeline:

Aims **(check when completed)**	**Timetable (mths)**				
	6	**12**	**18**	**24**	√

Approve sample collection protocols with UofL IRB					X
Begin patient sample collection, expanding, banking, and processing (8 patients)					x
Optimize ctDNA collection protocols and ddPCR assays					x
Develop SOPs for sample processing, expansion and banking, TCR/BCR PCR and MiSeq, primer design, CtDNA isolation, ddPCR, and retrospective data analysis					x
Continue patient sample collection, expansion, banking, and processing (18 patients)					x
Submit samples for whole genome sequencing and methylation profiling					x
Bioinformatic data analysis					x
Continue patient sample collection, expansion, banking, and processing (18 patients)					x
Begin assays to test primers against common mutations and methylation sites					x
Write up initial results for publication					x
Present at the American Association of Cancer Research Annual Meeting and American Society for Hematology					x
Discuss findings with Cincinnati Children's Hospital to develop potential collaboration					x
Continue patient sample collection, expansion, banking, and processing (18 patients)					x
Continue development of universal assay for ctDNA in T-ALL and B-ALL patients					x
Submit grant to NIH for additional funding for patient follow-up (5 years), study expansion to NCI centers, and CLIA certification so that the ddPCR can be used in subsequent clinical trials (Blackburn Lab has only ddPCR instrument in Kentucky)					x

Deliverables:

Check when deliverable is completed:	√
Develop a large biobank in the UK research lab of de-identified patient samples that can be used for further pediatric leukemia research by June 30, 2019	x
Develop a de-identified database of methylation sequencing profiling data that can be linked with treatment outcomes for ALL patients in the state of Kentucky which can be used to compare to xnational pediatric cancer databases by December 31, 2019	X
Identify and quantify ctDNA in the blood and CSF of ALL patients and to determine which clones persist and which respond best to treatment by December 31, 2019	X
Identify a few common methylation changes that are recurrent in ALL samples by June 30, 2020	X
Develop a ddPCR primer panel that will be able to identify ctDNA in a large subset (ideally >50%) of ALL samples by June 30, 2020	x