LETTER OF INTENT TO APPLY FOR [1]

THE TERRY FOX NEW FRONTIERS PROGRAM PROJECT GRANT (PPG) (2018)

Deadline: Tuesday August 8th, 2017 5:00 pm Pacific Daylight Time

Email to ppg@tfri.ca

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PROGRAM TITLE: The Terry Fox New Frontiers Program Project Grant in the Early Detection and Prevention of Cancer in Li-Fraumeni Syndrome

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PROPOSED START DATE: July 1, 2018

RESEARCH INSTITUTES: The Hospital for Sick Children, IWK Health Centre, Ontario Institute for Cancer Research, Princess Margaret Cancer Centre

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Names of the Institutes	Names of Project Investigators
The Hospital for Sick Children	David Malkin, Anna Goldenberg, Adam Shlien, Andrea Doria, Ran Kafri
IWK Health Centre	Jason Berman
Ontario Institute for Cancer Research	Trevor Pugh
Ontario Cancer Institute	Gang Zheng

[Expand table as required]

[1] The Project Leader should refer to the 2018 PPG LOI Guide before completing this proposal form.

LIST OF PROJECTS & CORES INCLUDED IN THE PROGRAM PROJECT GRANT APPLICATION

#	Short Title of Project / Core	Principal Investigator
1	The clinical implications of intra-tumoural heterogeneity in LFS	Adam SHLIEN/Anita VILLANI
2	Predictive Modeling of Age of Onset and Tumor Type in LFS	Anna GOLDENBERG
3	Early Tumor Detection by Circulating Tumor DNA Biomarkers in LFS	David MALKIN/Trevor PUGH/Adam SHLIEN
4	Novel Approaches to Molecular / Tissue Imaging and Clinical Surveillance in LFS: Multicentric Validation Study	Andrea DORIA/Gang ZHENG/Anita VILLANI
5	Using the zebrafish as a model for chemoprevention for *TP53* mutation carriers	Jason BERMAN
6	Pharmacologic Prevention of Malignancy in LFS	David MALKIN/Ran KAFRI/Anita VILLANI/Jason BERMAN
7	LFS Sequencing and Data Repository	Anna GOLDENBERG/David MALKIN

[Expand table as required]

LIST OF INVESTIGATORS INCLUDED IN THE PROGRAM PROJECT GRANT APPLICATION

#	*Full Name*	*Institutional Affiliation*
	Role in Program Project Grant Application	*Signature*
1	David MALKIN	
	Project Leader, Principal Investigator of Project #3, Co-investigator of Project #6 and CORE	
2	Adam SHLIEN	

	Principal Investigator of PROJECT #1, Co-Investigator Project #3	
3	Anna GOLDENBERG	
	Principal Investigator of PROJECT #2 and Principal Investigator of CORE	
4	Andrea DORIA	
	Principal Investigator of PROJECT #4	
5	Jason BERMAN	
	Principal Investigator of PROJECT #5, Co-Investigator Project #6	
6	Ran KAFRI	
	Principal Investigator of PROJECT #6	
7	Gang ZHENG	
	Co-Investigator of PROJECT #4	
8	Trevor PUGH	
	Co-Investigator of PROJECT #3	
9	Anita VILLANI	
	Co-Investigator of PROJECTS #1, 4 and 6	

[Expand table as required]

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1. SCIENTIFIC ABSTRACT *(max 1 page)*

Background: Li-Fraumeni syndrome (LFS) is a highly penetrant autosomal dominantly inherited cancer predisposition disorder with a population frequency of ~1:5000. Germline *TP53* mutations cause >80% of LFS, and are linked to a wide spectrum of cancers including adrenocortical carcinoma, rhabdomyosarcoma, brain tumors, and early onset breast cancer even in the absence of a family cancer history. Understanding the genetic and phenotypic heterogeneity of LFS formed the basis of our initial TFRI PPG. In the first two years of this Program, we reported an association of differential global methylation with tumor phenotype in *TP53* mutation carriers, validated our novel surveillance protocol for early tumor detection, established F1 progeny of *tp53* (LFS) mutant zebrafish to utilize for chemical screens, created an algorithm that accurately predicts age-of-onset in *TP53* mutation carriers, and generated data suggesting a unique molecular architecture in LFS-derived tumors.

Overall Goals: We now build on this progress and introduce new investigators and collaborators who bring expertise in innovative imaging and ctDNA surveillance technologies, in addition to introduction of novel biologic agents that restore wtp53 expression. This will lead us to resolve the two major challenges faced by LFS patients: 1) Improve sensitivity and specificity of early tumor detection leading to improved survival; and 2) Develop and implement rational chemoprevention strategies for *TP53* mutation carriers.

Expected Outcomes: NGS, miRNA and methylome sequencing of tumors and germline samples (**Project 1**) will reveal a molecular LFS signature to be used as a specific, sensitive ctDNA biomarker in both our *Trp53* knock-in mouse and prospective human studies (**Project 3**). Introduction of porphyrin lipoprotein-mimicking (PLP) nanoparticles will enable enhanced imaging surveillance to complement our evolving MRI approaches (**Project 4**) and correlations with molecular biomarkers (**Project 3**). Multilevel analysis of sequencing, clinical and imaging data (amalgamated in the **Core**) will define algorithms for precise predictive measures of age-of-onset and tumor type in LFS (**Project 2**). Our *tp53* murine and zebrafish models will be used for both comprehensive surveillance (**Projects 3+4**) and chemical screens (**Project 5**), respectively, and as pre-clinical platforms, together with patient-derived cell lines exhibiting a spectrum of heterozygous *TP53* mutations to study the chemopreventive and therapeutic effects of promising new agents including p53 refolders (APR246), peptides (CAP50) and modulators of mutant p53 function (statins and mTOR inhibitors) (**Project 6**). Our findings will support development and implementation of the first prospective studies of molecular surveillance for early tumor detection in

LFS and introduction of early phase clinical trials for prevention and treatment of cancer for this devastating disease.

2. OVERALL DESCRIPTION OF APPLICATION *(max 5 pages)*

BACKGROUND: Individuals with Li-Fraumeni Syndrome (LFS) (OMIM#151623) account for 17% of all patients in cancer susceptibility syndromes. Germline *TP53* mutations are the causative event in 80% of all LFS patients with a population carrier rate of at least 1:5000 (Malkin 2011). Since the initial discovery of the link between germline *TP53* mutations and LFS in 1990 (Malkin 1990), studies of this genetically and phenotypically heterogeneous disorder have led to a better understanding of the mechanisms of cancer susceptibility and p53 function, role of genomic instability in cancer predisposition, and introduction of an internationally accepted approach to clinical surveillance for early tumor detection (Villani 2016). The spectrum of malignancies in LFS is diverse, and age of onset is typically substantially younger than in their sporadic counterparts. Heterogeneity in tumor subtype and age of onset may result from coincident alterations of genes that modify the phenotypic effects of the underlying *TP53* mutation. Our group has been a long-standing leader in understanding the genetic/genomic basis of LFS, and in providing insight in the potential value that this information would have in refining cancer risk prediction in *TP53* mutation carriers. Prior work from our group demonstrated that polymorphisms in both *TP53* and *MDM2*, an integral component of p53 function, influence age of cancer onset in LFS; that accelerated telomere attrition is associated with younger age at first cancer diagnosis in *TP53* mutation carriers (Tabori 2010); that DNA copy number variation (CNV) is higher in *TP53* mutation carriers (Shlien 2008); and that chromothripsis occurs in tumors of patients with germline *TP53* mutations (Rausch 2012). Work conducted in the course of the first two years of this TFRI PPG has identified a potential role of two miRNAs, miR605 (Id Said 2015) and miR34A (Samuel 2016a), in the phenotypic heterogeneity characteristic of LFS; differential methylation in *TP53* mutation carriers (Samuel 2016b) that appears associated with tumor phenotype; creation of a predictive algorithm of age of onset in *TP53* mutation carriers based on methylation profile (Brew 2017); creation of a zebrafish model harbouring LFS-associated heterozygous mutations (Szergey 2017); and an enhanced multi-modality clinical surveillance protocol for early tumor detection in LFS (Villani 2016). Observations from this latter study demonstrated that while 100% of tumors presenting clinically in patients who did not undergo surveillance were malignant, more than 60% of those detected by surveillance were pre-malignant and low-grade, suggesting that tumors may actually have a pre-malignant or dormant phase which could be exploited both in terms of more refined early detection techniques (such as use of circulating tumor

DNA (ctDNA)), or molecularly targeted imaging modalities, as well as for development of preventive measures by agents that modify or abrogate mutant p53 function.

Notwithstanding the great progress that our integrated Program Team has made in understanding the role for inherited and acquired (epi)genetic events that modify the cancer phenotype in *TP53* mutation carriers, our tools to detect and predict tumor onset for a specific individual with LFS are still crude. Furthermore, it has only been recently that data has emerged demonstrating potential efficacy of p53 modifying drugs in pre-clinical models of somatic alterations in p53. We propose that the combined use of novel 'molecular' imaging and ctDNA surveillance will substantially improve the sensitivity and specificity of early tumor detection in *TP53* mutation carriers. We also propose that emerging agents designed to structurally modify mutant p53 and activate wild-type p53, some of which are already in clinical trials in the context of sporadic cancer, can be harnessed as chemopreventive agents in LFS. We have expanded our Program significantly to engage the remarkable expertise of world-leading Canadian scientists (**Pugh, Zheng, Kafri**) in the study and application of ctDNA and miRNAs (**Project 3**), porphyrin lipoprotein-mimicking (PLP) nanoparticles (**Projects 4 and 6**) and live cell/single cell imaging and functional genomics (**Project 6**) to complement the expertise of the investigators in our initial application (**Malkin, Shlien, Goldenberg, Doria, Berman**) to build on the productivity generated from our sequencing (**Project 1**), predictive modeling (**Project 2**), imaging surveillance (**Project 4**), and zebrafish (**Project 5**) work.

OBJECTIVES: The overarching objectives of this comprehensive multi-investigator Program is to use complex molecular and clinical determinants of LFS to resolve the two major challenges faced by LFS patients: 1) Improve sensitivity and specificity of early tumor detection leading to improved survival; and 2) Develop and implement rational chemoprevention strategies for *TP53* mutation carriers. These objectives will be met through the following specific aims encompassed by each of the six integrated projects and supported by a comprehensive sequencing and data/tissue repository Core.

Aim 1: Define the molecular heterogeneity of LFS tumors and utilize this information to create a molecular signature of LFS cancers to be used as a molecular biomarker of early detection (ctDNA);

Aim 2: Create a multi-level model of risk to predict age of onset and tumor type in *TP53* mutation carriers;

Aim 3: Use a murine Trp53 mutant model and prospective human studies to develop and validate a sensitive and specific ctNDA biomarker predictive of tumor onset in LFS;

Aim 4: Develop and validate novel imaging modalities utilizing novel MRI and PLP platforms to improve early tumor detection;

Aim 5: Utilize our mutant *tp53* zebrafish models to identify effective agents for tumor prevention through chemical screens and direct exposure to recently identified p53 modifying agents;

Aim 6: Create and utilize a novel LFS cancer cell observatory and *in vivo* models to evaluate the biologic effects of agents identified through the zebrafish chemical screen or modulators of mutant p53 function.

IMPORTANCE AND NOVELTY: *TP53* mutation carriers have a strikingly elevated cancer risk throughout childhood, early and late adult life. Germline *TP53* mutations are the initiating event in a cascade of molecular and epigenetic alterations that ultimately lead to malignant transformation in selected target organs. While *TP53* mutation carrier rate in the general population is cited as being ~1:5000, somatic *TP53* mutations in sporadic tumors remain the most frequently altered gene in human cancer. Murine models of p53 dysfunction (mutant p53 knock-in) or p53 deletion (p53 null), *in vitro* models to interrogate p53 function, and biochemical and biologic studies of the p53 regulatory pathway, provide major insights into human carcinogenesis. The study of LFS, and the more precise molecular profiling of *TP53* mutation carriers has led to improved survival through implementation of novel multi-modality clinical surveillance protocols (Villani 2016). Expansion of these surveillance studies through use of innovative techniques such as porphyrin lipoprotein-mimicking (PLP) nanoparticles and PET-MRI offer opportunities to improve both sensitivity and specificity of early tumor detection. Definition of LFS-specific molecular signatures, expanding from our current studies of genome and methylome to include miRNAs will provide a biomarker for detection by novel ctDNA techniques. Utilization of the *Trp53* mutant mouse (R270H) is now in place to study early tumor detection with both ctDNA and prospective imaging will complement the studies in our human LFS population. These mice, together with our novel zebrafish model, as well as the unique cancer observatory designed for live cell imaging and functional analysis, provide the ideal platforms to determine the functional and biological effects of emerging promising agents that modify mutant p53 function (statins, mTOR inhibitors, peptides and p53 refolders) and are provided to us through our international collaborators. The combination and complementarity of expertise in whole genome, exome, methylome and miRNA sequencing, computer based modeling of multi-level genetic, biologic and clinicopathologic data, emerging powerful imaging techniques and model organism and cell system platforms, and the exciting introduction of viable drugs to modify mutant p53 function in both pre-clinical and clinical settings represent a unique ‘marriage’ of

scientific disciplines that define what we believe to be a powerful and highly novel approach to define the true molecular basis of tumor formation in LFS and to alter the natural course of the disease.

COLLABORATION AND SYNERGY: The initial two years of this Program has seen a rapid and effective evolution of this creative team. Almost all projects are jointly supervised by project investigators, students work together on all projects, and manuscripts and meeting presentations demonstrate joint authorship across all labs. At the time of this submission, four new manuscripts are in preparation: creation of the zebrafish models; sequencing of p53-wild-type LFS families to identify other causal genetic events; modeling of age-of-onset predictions utilizing the methylation data and retrospective evaluation of the MRI surveillance which has demonstrated dormancy of LFS tumors. The addition of new investigators to the Program derive from direct interactions with these individuals on related projects, and extension of their studies to the p53-LFS story. The direction of the Program continues to evolve with engagement of new coinvestigators collaborators bringing opportunities particularly in adapting new imaging (PLP)(Zheng), new ctDNA platforms (Pugh), new cell biology imaging and functional analsyis techniques (Kafri) , and new drugs (collaborators Rotter, Prives, Del Sal, Wiman, Selimanova). We believe this expanded, yet focused team, represents the most comprehensive of its kind in the world.

TRAINING AND MENTORSHIP: Drs. Malkin, Berman, Doria and Zheng are well-established investigators with strong track records in training graduate students, post-doctoral research fellows and MD trainees. Drs. Goldenberg, Shlien, Kafri and Pugh are extraordinarily talented 'young investigators' who have already trained outstanding students. The integration of senior, mid-career and junior investigators in this Program Project lends itself especially well to mentorship among the investigators themselves. During the first 2 years of the Program, the team had monthly face-to-face meetings in the Research building at SickKids (skyping in Dr. Berman's team from Halifax). At each meeting, two or three students would present their current work which allowed for dynamic, vibrant interactive discussion. In addition, the Berman team came to Toronto for an all-day face-to-face meeting (October 2016) and a second similar meeting is planned in November 2017. In addition, all students involved in the project have had the opportunity to present their work in poster or platform format at no less than 30 individual meetings (national and international). These opportunities allows students to 'test' their ideas and results to a larger audience, and to meet many of the leaders in their respective fields. Importantly, students also have the opportunity to meet LFS families (either at the biennial LFS Symposia (next one to be hosted in

Toronto April 2018) or through Dr. Malkin's Cancer Genetics clinic. These training and mentorship activities will continue throughout the duration of this renewal grant.

INSTITUTIONAL COMMITMENTS: Each PI has committed lab space and significant infrastructure resources. Drs. Malkin, Shlien, Goldenberg, Kafri and Doria's labs are in the Peter Gilgan Centre for Research and Learning (PGCRL) at SickKids. Drs. Zheng and Pugh's labs are at the MaRS building (Ontario Institute for Cancer Research and Ontario Cancer Institute) within a 10 minute walk of SickKids and in recently renovated state-of-the-art space. Dr. Berman's recently expanded laboratory is arguably the most state-of-the-art zebrafish facility in the country. Each of the SickKids labs are in complementary research neighborhoods (Cancer and Stem Cell Biology (DM, RK), Genetics and Genome Biology (AS, AG) and Clinical Health and Evaluative Sciences (AD)) which boast extraordinary core infrastructures to support the conduct of all the proposed studies. In his capacity as Associate Director of the Molecular Diagnostics Laboratory and Director of the Translational Genomics Program, Dr. Shlien is particularly well-positioned to facilitate the sequencing elements of this Program. The diagnostic imaging department has dedicated research MRI (and research PET-MRI) facilities, and the animal studies are already underway with both housing and MRI/CT imaging facilities at the nearby Centre for Phenogeomics.

ROLE OF PARTNERS: Several key partners are engaged to facilitate this Program. Dr. Malkin is Chair of the International Li-Fraumeni Exploration (LiFE) Consortium. The consortium comprises over 25 major cancer centres worldwide, including key ones in the US (Dana-Farber Cancer Institute, MD Anderson Cancer Center, City of Hope, Huntsmann Cancer Institute at University of Utah, the NIH, St. Jude Children's Research Hospital, Children's Hospital of Philadelphia, and others), Europe (University of Manchester, Great Ormand Street, London; the French Li-Fraumeni consortium (Lyons, Paris, Rouen); DFKZ, Heidelberg and Hannover), Brazil (AC Carmago Hospital, Sao Paolo; Cancer Genetics Center, Porto Allegre), and elsewhere. Samples from patients are readily available from all these centres and several collaborative projects have been completed over the last 20 years among these institutions and investigators affiliated with them. Several are identified collaborators on this renewal application. A centralized data registry has been established at City of Hope and each centre maintains its own institutional registry. Thus, the data and material available to the Program, particularly for validation studies, is extremely rich. In adiditon to this support, we have confirmed new collaborations with several scientists who have made major contributions in the development of emerging therapies including Prof. Varda Rotter (Weizmann Institute of Science, Israel) who has developed a series of peptides (CAPs) that activate mutant p53 to wild-type function; Prof. Galina Selimanova and Klas Wiman (Karolinska Institute,

Sweden) how are the lead scientists at APREA and developed the family of p53 refolders (APR246, etc) that are now in early clinical trials in ovarian cancer, melanoma and lymphoma; and Prof. Carol Prives (Columbia University, NY) and Giannani Del Sal (Cancer Research Institute, Trieste, Italy) who have extended the use of statins in pre-clinical applications to activate p53, and (Del Sal) who has identified a unique miRNA (miR60) that is secreted exclusively by mutant p53 expressing cells. All are major, long-standing leaders in the study of p53 and have all enthusiastically provided us the agents to work with in the various respective proposed projects. In addition to this support, each project investigator maintains close collaborations with colleagues in their respective fields who will be engaged as collaborators in a judicious manner to enhance the intellectual capacity of the studies – and to generate discussion and input on novel approaches and techniques as required.

PROGRESS REPORT *(max 3 pages)*

4a. INDIVIDUAL PROJECTS – PROJECT #1: The clinical implications of intra-tumoural heterogeneity in LFS (PI: Adam SHLIEN; co-PI: Anita VILLANI)

BACKGROUND: Li-Fraumeni syndrome (LFS) cancers arise early in life through molecular mechanisms that have not been fully described. In LFS patients, *TP53*, a central, multi-functional tumour suppressor, whose role impinges on nearly every hallmark of cancer, is mutated from the earliest stage of embryogenesis. Every cell in the body is thus 'primed' with a single copy mutation. Given this, it is surprising that while the spectrum of cancers in LFS is wide, the vast majority are confined to very few cell types; namely, breast, brain, sarcoma and the adrenal cortex. We hypothesized that a detailed understanding of the somatic evolutionary dynamics of LFS tumours would help untangle this major unanswered question. Based on compelling unpublished data from our initial submission, we will now broaden our investigation into the molecular mechanisms and timing of mutational signatures underpinning LFS tumours. Then, we will initiate a tumor sequencing program for LFS patients who currently have cancer, leveraging existing pipelines and infrastructure at SickKids for precision medicine. This program will recruit from centres in the international Li-Fraumeni Exploration (LiFE) Consortium, will be the first specifically tailored for tumour-prone families, and will determine if intratumoural heterogeneity is associated with poor survival in LFS.

PRELIMINARY DATA

Multi-site sequencing of LFS and matched sporadic cancers. To determine the temporal order of somatic mutations in LFS cancers, we established a robust protocol for partitioning small tumor specimens into multiple sections (3- 8/tumour), after evaluation by an experienced pathologist, then extracting high quality DNA/RNA for deep sequencing. For every LFS tumor sequenced, we also analysed a matched sporadic tumor of the same histiotype from a patient who did not harbor a germline *TP53* mutation.

Massive intra-tumoural heterogeneity in LFS. In the sequencing data of LFS patients, we found a staggering amount of intra-tumoral heterogeneity. When comparing tumour sections less than 1cm apart, we observed large differences in mutational burden and up to 2,000 private substitutions, indicating early divergence and continuously acquired subclonal mutations.

Sudden rearrangement bursts as a signature of LFS cancer. As expected, we saw patterns of sudden and coordinated rearrangement bursts in LFS tumors ('chromothripsis'). However, to our surprise, these patterns were very common and appeared to be enriched at specific loci. For example, in one adrenocortical carcinoma from a patient with a germline *TP53* R158H mutation, we found increased

mutational load with 4909 SNVs and 1851 SVs, with a particular enrichment for inversions. Chromothriptic-like rearrangements were observed clustering in a small number of chromosomes, particularly chromosome 8. In addition, we saw mutational signatures that points to defective homologous recombination (Signature 3). Taken together, these unpublished data suggest that there exists a defined signature for LFS tumourigenesis.

AIM 1: RECONSTRUCT THE EVOLUTIONARY HISTORY OF LFS CANCER.

Our unpublished data highlighted the association between *TP53* germline mutations and clustered rearrangement bursts (chromothripsis). Why these are less frequent when *TP53* is *somatically* mutated is unclear. In some cases, chromothripsis appeared to disrupt key oncogenes, including negative regulators of *TP53* itself – for example in one embryonal rhabdomyosarcoma we saw hundreds of rearrangements on chromosome 12 near *MDM2*, which targets *TP53* for proteosomal degradation. To answer these questions, and expand on these exciting findings, we have gathered a cohort of 60 LFS tumours for multi-site whole genome sequencing. These are SickKids patients for whom full clinical information is available. To this internal cohort we will add an additional 40 LFS tumours from our colleagues in the LiFE Consortium and other individual institutions who refer patients to our clinical program. Each of these 100 tumours will be microdissected and multi-site sequenced (average five sites per tumour) using our established protocol. In this rich dataset - which will represent the largest collection of sequenced genomes from a hereditary cancer and the only to have been multi-site sequenced - **we will determine the landscape of chromothripsis in LFS, whether it is restricted to specific tumor types, if it is always an early event and, finally, which genes it recurrently targets**.

Our unpublished data supports the notion that germline *TP53* mutated cancers acquire chromothripsis early in their evolution, that this is shared by every cell in the tumour from which clonal diversion occurs. In some cases, the late divergence involves many new structural rearrangements (such as translocations that may lead to new fusions), but not point mutations. Imagining this evolution as a phylogenetic tree, LFS cancers have a wider trunk that contains many more mutations than sporadic cancers (of the same histotype). These data indicate that multiple branches emerge, indicating widespread intratumoural heterogeneity, but when they arise and whether they contain new driver mutations is unknown. **In this sub-aim we will analyse intra-tumoural heterogeneity, construct phylogenetic trees of LFS surrounding stromal cells and tumours from different organ systems.** We will follow this up with a novel single cell RNA-Seq experiment to determine whether somatic genetic diversity leads to transcriptional heterogeneity. The molecular 'signatures' generated from these studies

will inform the ctDNA biomarker work (**Project 3**), predictive algorithm studies (**Project 2**), and emerging therapeutics (**Project 6**).

AIM 2: DEVELOP AN INTERNATIONAL TUMOR PROFILING PROGRAM FOR LFS.

We will initiate an LFS precision medicine program, making clinical-grade genomics available to every LFS patient with active cancer, no matter where they're being treated. We will use a multi-site sequencing test which will capture actionable mutations missed due to somatic heterogeneity. This test will include mutations for which there are known therapies (such as activating fusions in kinases), detection of hypermutation (for immune checkpoint inhibition) and mutational signatures (for detecting homologous repair deficient tumors that may respond to PARP inhibition). This testing program will leverage existing clinical infrastructure (co-investigator **Dr. Anita Villani** who is a co-lead of the SickKids Cancer Sequencing (KiCS) program, and will be the first to offer "universal coverage" for these patients whose lifetime cancer risk is nearly 100%. **We expect there to be massive interest and will work directly with the Li-Fraumeni Syndrome Association and Living LFS to recruit subjects.** In so doing, we expect to be able offer testing to >500 patients per year. In this cohort, we will determine which secondary mutations are most common in LFS cancers, and whether the degree of intra-tumoural heterogeneity is associated with poor survival or the presence of advanced disease.

4b. INDIVIDUAL PROJECTS – PROJECT #2: *(Predictive Modeling of Age of Onset and Tumor Type in LFS (PI: Anna GOLDENBERG)*

BACKGROUND: LFS is characterized by great heterogeneity in tumor type and age of onset. This feature complicates strategies for early tumor detection. While our studies of ongoing surveillance of *TP53* mutation carriers have demonstrated reduced mortality, the complex imaging and biochemical blood test based protocol is particularly challenging to use in very young children, and detection specificity and sensitivity are still suboptimal for standard radiologic interpretation. A predictive model of age of onset could help indicate in what age window a patient is at risk to develop cancer and determine when a patient should be subjected to more invasive surveillance. This more precise approach will ultimately decrease the cost of surveillance while simultaneously lead to better health outcomes for LFS patients.

PRELIMINARY DATA: In the last two years, we used a cohort collected at the Hospital for Sick Children (SickKids) LFS database in our predictive model of age of onset. We follow a cohort of LFS patients and their families (277 *TP53* mutation carriers and 272 non-carrier family members). Of the 277 carriers, 91 currently have or have had cancer. From published germline methylation data, consisting of ~450,000 probe sites, for 81 of those patients, we performed two types of analysis. First, we selected methylation probes that differentiated a cohort of LFS cancer patients from healthy controls. Second, we identified regions that distinguished between *TP53* mutant patients that did not have cancer from *TP53* wt controls. Our machine learning model (Elastic Net) was able to achieve 86% correlation between true and predicted values of the age of onset. Additionally, we have tested the ability of our models to predict whether an individual will be diagnosed before or after the age of 4. Our classification model on average achieved 91% accuracy. We verified that our model does not simply predict age of sample collection by using our cohort of LFS patients that do not have cancer yet (n = 40). This cohort had a matching distribution of the age of sample collection as the patients used in our model. The model has no predictive power on the age of sample collection confirming that our model is indeed highly predictive of the age of cancer onset in LFS TP53 Mut patients. In our preliminary results we have established that germline methylation profiles can aid in cancer surveillance of LFS patients and are potentially predictive of age of cancer onset. Based on our preliminary data we have multiple hypotheses and questions we would like to address as part of our prediction of predisposition and early detection of the time of onset and tissue type pipeline.

HYPOTHESES:

1) Methylation patterns may also be effective in predicting tissue type;
2) To understand the mechanism by which methylation affects age of onset, we need to combine it with other omics type data to characterize the state of the cell in more detail
3) We can aid surveillance by integrating methylation and imaging data to detect which features give the earliest indications of the tumor onset.

SPECIFIC AIMS:

Aim 1. Identify methylation regions predictive of primary tumor type

Aim 2. Integrate mRNA, miRNA, DNA-sequencing data to understand mechanisms leading to early onset, prioritizing therapeutically targetable methylation regions.

Aim 3. Identify whether methylation can aid imaging in identifying earlier onset of cancer.

RESEARCH PLAN:

To achieve **Aim 1**, we will identify differentially methylated regions in the germline of patients with different types of cancer and using these regions build a classifier to predict which patients are predisposed to which kinds of cancer. This analysis pipeline is very similar to what we have already done for detecting whether or not an LFS patient will get cancer before the age of 4 years and we thus do not anticipate any difficulties. Although we are not able to guarantee that a patient's germline methylation pattern is predictive of the cancer of onset, our preliminary data indicates that the methylation signatures in a small number of patients exhibit different patterns between different types of cancer and thus we are likely to identify regions predictive of the cancer type as well.

Aim 2 will center on harnessing the power of the newly collected data in this project to analyze the biological mechanism by which methylation or regulation in general might be affecting the age and type of cancer of each patient. For this purpose, once the data is collected and processed as part of the **Data Core Facility**, we will utilize the novel regulatory annotation that the Goldenberg lab is building. Based on a per-gene *in silico* analysis of 25 cancer types, we have discovered that each gene may fall into a different type of regulation, which we termed 'mode of regulation'. For example, methylation of the promoters explains much more variance of the gene expression of master regulators, than cytokines. Thus, we will collect miRNA and mRNA-seq data to identify the cellular mechanisms by which genes implicated by our methylation profiles are regulated. We will then prioritize resulting mechanisms for potential therapeutic targets for **Projects 5 and 6**.

Aim 3 will incorporate imaging data (generated from **Project 4**) into the predictive pipeline. We will work closely with Dr. Andrea Doria to establish features that are clinically considered predictive. In addition, we will take whole images and use established deep learning classifiers to extract *in silico* features that are early detectors of the tumor. Finally, we will integrate features of the cellular mechanisms identified in **Aim 2** together with the imaging features to build the earliest possible detector of the of the tumor given the available data.

CHALLENGES AND MITIGATING STRATEGIES: The biggest challenge in this work is to adequately control for potential confounders. For example, the blood for LFS patients may sometimes be available only at tumor detection, i.e. the germline methylation signature may be contaminated with tumor cells. We control for this confounder by testing whether our predictive system predicts age of collection in mutant *TP53* carriers who do not yet have cancer. In our preliminary work, the predictive system failed to predict the age of data collection boosting our confidence in the utility of our models.

Our **DELIVERABLES** are three fold. First, we aim to produce several deployable predictive systems that take methylation and other types of germline omics data and predict age of onset and tumor type. We will also produce a list of therapeutic targets that can be tested in **Projects 5 and 6**. Finally, we will help to identify in-silico features of earlier detection in the imaging data (**Project 4**).

4c. INDIVIDUAL PROJECTS – PROJECT #3: (Early tumor detection by circulating tumor DNA (ctDNA) in LFS) (PI: David MALKIN; co-PI: Trevor PUGH; co-PI: Adam SHLIEN)

PRELIMINARY DATA:

PROPOSED STUDIES: In this Activity, we will tailor a cell-free DNA sequencing assay to query blood samples for the presence of tumour-derived fragments harbouring somatic mutations and epigenetic marks. To tailor such a panel for LFS, we will leverage whole genome sequence data as well as methylation profiles generated by the original TFRI-funded LFS program. For the mutation detection portion of the assay, we will bait frequently mutated genes in LFS tumours (all exons of *TP53* being the most obvious) as well as sequence contexts that are more likely to be mutated in LFS tumours. For the epigenetic profiling arm of the test, we will leverage an antibody-based pull-down method to isolate methylcytosines followed by bioinformatics analysis of LFS-specific CpG islands. To enable sufficient material available to conduct both of these assays at maximum sensitivity, we will collect 20 mL of blood from each patient in 2x 10 mL Strek tubes.

Mutation detection using ctDNA sequencing

To enable full-length sequence analysis of all exons in genes of interest or any other arbitrary genomic region, the Pugh Lab has developed a circulating tumour DNA (ctDNA) sequencing assay, termed *Liquid Biopsy Sequencing* (LB-Seq) that combines a hybrid-capture method with a novel bioinformatics algorithm (Kis et al. *Nature Communications* 2017). Initially, we developed LB-Seq as part of MYELSTONE ("MYELoma STOp NEedle"), a study of patients with multiple myeloma to assess whether sequencing of cfDNA was comparable to the clinical standard of analyzing cells from painful bone marrow needle aspirates. By sequencing all exons from 5 genes in 48 patients, we found 96% mutation concordance between ctDNA versus matched bone-marrow. We also found and verified 4 mutations in blood that were missed by bone-marrow testing (>98% specificity). We have been funded to translate this method into a 38-gene clinical test within the UHN CAP/CLIA-certified molecular diagnostics laboratory. This methodology is highly flexible and we currently maintain custom panels ranging from 16-300 kb for ongoing studies of clonal monitoring and early detection of >10 different cancer types.

cfMeDIP-seq: Epigenetic analysis of circulating tumour DNA

Recently, the De Carvalho lab developed methodology to detect circulating DNA containing epigenetic marks indicative of cancers arising from specific tissues using cfMeDIP-seq (cell-free Methylated DNA Immunoprecipitation and high-throughput sequencing). By surveying hundreds of

thousands of differentially methylated regions simultaneously, this highly sensitive technique can differentiate tumours arising from multiple tissues simultaneously as well as detect ctDNA in early-stage disease. This method has been robust in over 100 samples that have been tested and will be used to find global DNA demethylation models that are suitable for monitoring LFS tumours. Distinguishing the site of origin of tumourigenesis would help guide further targeted organ-specific follow-up investigations. Many LFS patients are at risk of tumours in multiple organs, some of which, do not have effective screening or cancer preventative options. Identifying the potential organ site of tumourigenesis would be a tremendous advance in the personalized surveillance of these patients.

Molecular barcoding: Ultrasensitive detection of low frequency mutations in ctDNA

To further improve the sensitivity of both mutational and epigenetic profiling methods, Drs. Pugh and De Carvalho are collaborating with Dr. Scott Bratman to adapt a Duplex Consensus Sequence molecular barcoding method for error suppression in ctDNA analysis (Kennedy et al. *Nature Protocols* 2014) This enables correction for PCR bias and strand-specific errors arising from DNA damage introduced during extraction or library construction. We have demonstrated that this approach successfully suppresses background sequencer error, enabling mutation detection down to $1:10^4$ molecules and potentially beyond. This approach represents a significant advance for molecular profiling of patients with LFS, particularly for detection of low concentrations of ctDNA in early disease.

4d. INDIVIDUAL PROJECTS – PROJECT #4: (Novel Approaches to Molecular / Tissue Imaging and Clinical Surveillance in LFS: Multicentre Validation Study) (PI: Andrea DORIA; co-PI: Gang ZHENG; co-PI: Anita VILLANI)

BACKGROUND: Li-Fraumeni Syndrome (LFS) is characterized by a high frequency of sarcomas, premenopausal breast cancer, brain tumors, adrenocortical carcinoma, and other malignancies, which typically occur at an earlier age in affected individuals than in the general population. Unfortunately, it is as yet not possible to predict the age of onset, site or type of cancer in these patients. We recently reported the first clinical surveillance protocol for early tumor detection in *TP53* mutation carriers and demonstrated it to be feasible and associated with decreased mortality and treatment-related morbidity (Villani et al 2011, 2016). In our previous submission, we took two complementary approaches to enhance both the sensitivity and specificity of tumor detection. First (***primary aim***), we hypothesized that ^{18}FDG-positron emission tomography (PET) MRI would be more sensitive than whole body (WB)-diffusion weighted (DW) MRI and WB-short tau inversion recovery (STIR) MRI for diagnosis of hypermetabolic tumors. However, if necrosis was present early in the tumor (highly aggressive tumors) DW-MRI would be more sensitive and the combination of WB-STIR and DW would be more diagnostic (higher sensitivity and specificity) than ^{18}FDG-PET MRI. Second (***secondary aim***), we hypothesized that detection of circulating DNA (cfDNA) would accurately reflect very early tumor detection that predates both clinical and imaging surveillance. This ctDNA work is now encompassed in **Project 3**, and results will be correlated and linked to new imaging approaches proposed here.

PRELIMINARY DATA: Extensive regulatory constraints delayed initiation of the novel imaging aspects of the study. Health Canada approval for use of FDG in PET-MRI in this study was obtained in April 2017. Recruitment of patients for WB-STIR and DW MRI studies started in September 2016. To date, 14 patients consented to participate in the study, and 12 underwent STIR and DW MR imaging studies. No areas of abnormal signal intensity (true-positives) were noted in any of the WB-STIR or DW MRI examinations performed. The criteria for utilization of PET-MRI in this study were: (1) discrepancy of results between the other two MRI techniques: or (2) a peak on the cfDNA plasma results at the time of the WB-STIR and DW MRI examination. No recruited patients have as yet fulfilled the criteria for having a PET-MRI. However, we were able to test the feasibility and value of comparing cfDNA levels and imaging phenotypes in a small cohort of patients (**Project 3**). Regarding possible associations between peaks of cfDNA and abnormal signal intensity on MRI we concurrently evaluated a prospectively

followed cohort of LFS patients (N=43) and identified cases in which peak levels of cfDNA corresponded to abnormal imaging features (phenotypic expression). WB MR imaging was crucial for diagnosis of tumors in two LFS patients since corresponding cfDNA levels did not show any peak at the time of diagnosis or only started to peak by the time the tumor was much larger in size. These constitute examples of the important role of imaging in the surveillance of LFS patients and support the need for further investigation on optimized imaging methods for diagnosis of early tumors in this population.

PROPOSED STUDIES: We realized that a larger number of subjects should be followed over time in order to allow for detection of the relatively small number of new tumors expected during the time period. We will address our primary hypotheses that these enhanced imaging approaches will increase sensitivity and specificity of imaging surveillance through the following specific Aims:

AIM 1: We will expand the initial pilot study into a multicentre North American study to determine the sensitivity (false-negative rate) and specificity (false-positive rate) of WB-STIR MRI, WB-DW, and ^{18}F-FDG PET-MRI for detection of early tumor as compared with corresponding histology (reference standard) in order to establish an optimal imaging surveillance program for LFS individuals. To date, we have confirmed participation of the imaging centres at Children's Hospital of Philadelphia, Packard Children's Hospital at Stanford University, St. Jude Children's Research Hospital (Memphis) and Texas Children's Hospital. Several dozen patients would be anticipated to be accrued to the study with this multicenter cohort.

AIM 2

1. We will create a descriptive algorithm for cases where one or more tumors are detected as a result of the surveillance program associating six proposed imaging traits (STIR signal heterogeneity, mass effect, neurovascular bundle involvement, necrosis, signal:necrosis ratio, mean ^{18}F-FDG uptake) with corresponding gene expression patterns generated from **Project 1**.
2. We will determine if an association exists between cfDNA levels (and ctDNA levels where biomarkers are validated from **Project 3**), imaging diagnosis and cancer onset.

Improved detection of incipient tumors by non-invasive diagnostic methods in a surveillance program is key for LFS individuals. The use of noninvasive imaging for early diagnosis of tumor and gene expression profiling is a fast and reliable technique which has the potential to replace high-risk invasive biopsy procedures. Because of the widespread occurrence of *TP53* mutations in sporadic cancers, there is also potentially a general benefit in ultimately being able to "marrying" functional and molecular imaging of

this project to genomic platforms ("radiogenomic paradigm") of other projects of this Grant in order to refine the signature of molecular modifiers in the future. This strategy could provide targets which could ultimately generate radiogenomic tags for even more precise and sensitive imaging surveillance.

AIM 3: To improve the imaging surveillance for early tumor detection in LFS patients and to develop novel therapeutic strategies (PROJECT 6) to ultimately improve cure rates, we plan to introduce the porphysome nanotechnology into our TFRI Program. Porphysomes are 1st-in-class organic nanoparticles with intrinsic multifunctional imaging and therapeutic properties stemming from a single, nontoxic, porphyrin-lipid building block. These diverse properties include the ability to bind metals to enable MRI and PET imaging, transform locally delivered light energy into heat, sound waves, reactive oxygen species to kill cancer cells or fluorescence to illuminate them, and efficiently ferry toxic drugs into cancer cells. In this Aim, we will build upon porphysomes' inherent multifunctionality to develop a novel image-guided drug delivery strategy that will allow us to take advantage of progress made to date in our initial program by integrating biomarkers (identifying cancer signatures (**Project 1**) to direct porphysome targeting), drug screen (identifying drug candidates as porphysome payload (**Project 6**), and surveillance (imaging methods that can be augmented by porphysomes as PET and/or MRI contrast agents (**Project 4**).

4d. INDIVIDUAL PROJECTS – PROJECT #5: *(*Using the zebrafish as a model for chemoprevention for *TP53* mutation carriers) *(*PI: Jason BERMAN*)*

BACKGROUND: The zebrafish is a powerful *in vivo* system for modeling genetic diseases and cancer due to conserved genetics and imaging facility in transparent embryos. Zebrafish surpass rodents as a vertebrate model with respect to generation of large numbers of offspring, external fertilization, rapid embryonic development and optical clarity, which together allow for the observation and manipulation of gene networks and developmental processes not easily performed in mammals. Loss-of-function (LOF) mutations, such as those that occur in tumor suppressor genes, have traditionally been more challenging to induce in zebrafish. The advent of clustered regularly interspaced short palindromic repeats (CRISPR)-based genome editing strategies now enables gene insertions of precise point mutations, providing a robust tool for the study of cancer predisposition syndromes, like LFS. Using an innovative approach employing anti-sense asymmetric single-stranded oligodeoxynucleotides (ssODNs), we introduced R143H and R217H mutations in the zebrafish *tp53* gene corresponding to R175H and R248H, respectively, the most frequent *TP53* mutations in human LFS patients. We also employed a novel allele specific PCR (AS-PCR) strategy, with a greater sensitivity than restriction enzyme-based approaches, to identify founder fish for both R143H and R217H mutations and their mutation-carrying F1 progeny. We then validated the presence of these mutations in adult F1 zebrafish at both genomic and mRNA levels. F1 generation "LFS fish" containing these mutations are now poised to be phenotyped and used to identify genetic and chemical modifiers. An apoptosis phenotype will be evaluated in embryos at 30 hours post-fertilization (hpf) 6 hours after they are subjected to DNA damage treatment by 30 Gray of γ -irradiation using acridine orange and activated caspase 3 staining with *tp53* null TALEN mutants and wild type embryos on hand in the laboratory serving as controls.

Aim 1: Genetic modifiers of LFS

The zebrafish provides a robust *in vivo* platform for evaluating the contribution of secondary lesions to malignant tumour progression. To date, we have generated a knockout zebrafish of the *microRNA 34a* (*miR34a*) gene that we been found to be epigenetically silenced in a subset of *TP53* tumours (Samuel 2016a,b). Using qRT-PCR, we observed that p53 induction using the DNA-damaging agent, camptothecin, increased levels of mir34a but not mir34b or mir34c in wild type zebrafish embryos. Double *TP53* R143H or R217H and *mir34a* mutants are now being generated. While these fish may prove to be informative in elucidating the relative role of mir34a in tumorigenesis, secondary mutations

are usually somatically acquired in a particular tissue leading to a cancer of that tissue origin. More recent advances in CRISPR-based technology in the zebrafish, such as the ability to drive Cas9 enzyme expression exclusively in specific cell populations now permits spatial control over where given mutations are expressed. We will take advantage of this technology to functionally interrogate the series of LFS-tumor specific mutations and signatures that are generated from **Project 1** and our **Core** LFS germline sequencing data.

Aim 2a: Chemical modifiers of LFS

The facility with which zebrafish embryos can be subjected to high-throughput chemical screens provides an unprecedented opportunity to evaluate compounds that restore apoptosis *in vivo*, thereby representing potential therapeutic compounds. There has been dramatic progress in development of small-molecule compounds capable of re-activating several missense mutant *TP53* variants. These compounds are capable of restoring normal TP53 function of loss-of-function *TP53* mutants leading to induction of apoptosis and cell-cycle arrest after DNA damage. In addition, it is conceivable that other rescue mechanisms of apoptosis after DNA damage exist in tp53 mutant cells. Another advantage of performing such a screen in zebrafish is that compounds can undergo *in vivo* modifications and can be simultaneously assessed for effectiveness and toxicity in the whole animal context of zebrafish larvae. We will use zebrafish to identify compounds, which can rescue apoptosis in the LFS *tp53* mutant fish and serve as lead compounds for treatment of LFS and other p53-related cancers.

Experimental approach: Using a unique small particle biosorter ideally suited to zebrafish embryos, we will employ known bioactive compounds from Sigma Lopac 1280 and Biomol ICCB (Enzo) libraries, which comprise a total of 1760 chemical agents and are readily available in the Berman laboratory. Many of these compounds represent previously approved Federal Drug Agency (FDA)/Health Canada drugs, which will enable the rapid translation of promising “hits” to Phase I clinical trials as a re-purposed therapy. In addition, we can obtain and employ the National Cancer Institute (NCI) Diversity library consisting of almost 2000 compounds. This library was used in several successful drug screens aimed at identifying p53-reactivating compounds (Bykov and Wiman, 2014).Thus, in employing these particular libraries, we have the opportunity to discover truly new prospective agents, as well as repurpose known medications. LFS *tp53* mutants, *tp53*-null and wild type embryos will be arrayed at 24 hpf, 3 embryos to a well in 96-well plates. Compounds will be transferred from library to embryo plates and irradiated embryos will be analysed to identify compounds that restore cellular apoptosis and other measures of

p53 reactivation in a p53-mutant background. In addition, the specific compounds (statin, APR246, CAP250 peptides and mTOR inhibitors) (**Project 6)** will also be tested in this system.

Aim 2b: Toxicity screening of promising compounds

Since detailed toxicity screening is not feasible in the context of a high-throughput chemical screen, we will perform in parallel toxicity and efficacy screening of hit compounds found in **Aim 2a**. The zebrafish provides a unique whole organism context with conserved organ systems and metabolism in which to effectively and efficiently conduct these studies. Such additional screening is important for establishing the lowest effective concentrations of compounds that do not cause significant toxicities that may prohibit their translation to individuals with LFS. We can examine overall morphological abnormalities, cardiac phenotypes (structural abnormalities, alterations in heart rate), glomerular filtration rate and lateral line neuromast phenotypes, the latter serving as a proxy for ototoxicity in humans.

4d. INDIVIDUAL PROJECTS – PROJECT #6: (Pharmacologic prevention of Malignancy in LFS) (PI: Ran Kafri; co-PI: David Malkin)

BACKGROUND: Pharmacological treatment of solid malignant tumours has proven a difficult task. Optimistically, recent studies suggest that an attractive alternative may exist – the pharmacological prevention of tumour onset. In recent years, cumulative evidence has indicated that lifespan may be regulated by activity in the mTOR signal transduction pathway[1–3]– a pathway that is also the central regulator of cell size[4]. Surprisingly, mTOR inhibition also caused lifespan extension in mice that are genetically predisposed to cancer, including mice with oncogenic mutations in *TP53* and *Her2*, the former being the cause of LFS.

Evidence relating mTOR inhibition to lifespan extension was first observed 2003 in C. elegans [PMID: 14668850]. Since then, drugs that suppress mTOR activity (including metformin) were shown to result in a 20%-50% increase in the lifespan of yeast[5,6], worms[7], flies[3] and mice[2,8,9]. Caloric restriction, an intervention that increases lifespan in several species, functions by suppression of mTOR activity[10]. Last, genetic depletion of mTOR pathway components results in longer lifespans in fruit flies and mice[10–13].

Consistent with the influence of mTOR on lifespan, mTOR inhibition delays the onset of many forms of cancer[18]. Rapamycin, a drug that inhibits mTOR, significantly delays onset of spontaneously formed cancers in normal inbred mice[9]. More surprisingly, rapamycin also delays onset of cancers in mice that are genetically predisposed to form tumours, including transgenic HER-2/neu cancer-prone mice[19] and mice with oncogenic p53[20]. In fact, in some cases, mTOR inhibition seems to have completely prevented the onset of cancer, even in transgenic mice carrying mutations in *TP53* and *KRAS* [PMID: 16166381, & PIMD 27869650].

In this proposal, we will investigate the possibility of extending lifespan of LFS patients by inhibiting mTOR. We will also explore the mechanisms by which mTOR inhibition mediates its influence on tumorigenesis in LFS patients. One factor that makes this proposal particularly promising is that, in mice models, mTOR inhibition was shown to postpone or eliminate the onset of p53 driven tumorigenesis [PMID: 27869650]. Thus, this proposal marks a different approach to cancer therapy – instead of seeking pharmacological treatments of malignancy, we seek the pharmacological prevention of its initial onset.

The onset of a tumour is a single transformation event – a highly rare event encompassing a genetic change that takes place in single, random, somatic cell. Despite the importance of this very rare process,

it has remained one of the least investigated aspects of cancer. The reason for this is simple: cell transformation events are highly infrequent and, therefore, hard to study. To overcome this research challenge, we propose to establish the cancer cell observatory in the context of LFS in which the 'single rare' event is the *TP53* mutation – an experimental system to continuously observe millions of single live cells over extended periods of time, seeking and investigating the rare events that are the seeds of cancer.

Aim 1: Establishment of the cancer cell observatory. We will combine a unique resource of the Malkin lab with a unique technology of the Kafri lab to establish the ***cancer cell observatory***. The Malkin lab has established a comprehensive library (Table 1) of skin-derived fibroblast lines collected from patients with Li-Fraumeni syndrome (LFS). In addition, the Malkin lab has access to both healthy and tumour cells derived from patient biopsies from a variety of organs. As observed by the Malkin Lab, cultured non-cancerous cells derived from LFS patients typically accumulate mutations, lose the wild-type *TP53* allele, and demonstrate morphologic and biologic properties consistent with evasion of senescence and cellular transformation. Following such cells with continuous time lapse microscopy will provide a window onto the transformation process and the factors that influence it (including mTOR inhibition). The library of cell lines collected by the Malkin Lab is unique not only in its magnitude but also by the fact that each specific cell line is linked with detained clinical data on one specific patient. In addition, cell lines have been or are currently characterized for genome-wide methylation patterns, RNAseq and whole genome sequencing. Such information will provide an important window into the molecular landscape of LFS patients and provides a baseline on which to compare changes acquired during passage and transition through senescence to transformation both with or without mTOR treatment.

To compliment this resource, the Kafri lab has established an experimental system for high throughput monitoring of single cell dynamics over time (Figure 4). Specifically, incubated-chamber time lapse microscopy is used to image live fibroblasts. Software that is continuously being developed in the Kafri lab is then used to track thousands of single live cells and quantify morphological properties and protein expression over real time[25]. Importantly, it has been observed that transformation in LFS derived fibroblasts correlates with identifiable morphological changes that can be detected with our software. This ability to computationally monitor millions of patient derived fibroblasts over long periods of time will allow detection and characterization of the very rare events of malignant transformation, which are the seeds of tumour formation. Our software will automatically classify fibroblasts into distinct, computationally characterized morphologies. Morphological distinctions are known to represent distinctions in cell function. For example, the epithelial to mesenchymal transition is accompanied by

distinct changes in morphology[26]. Similarly, morphological features distinguish senescent cells from non-senescent cells[27] or differentiated cells from cancer cells that become dedifferentiated. **The cancer cell observatory will provide a first of a kind dataset – mapping morphological changes of single cells with molecular characterization and clinical phenotypes**.

Aim 2: Identify the downstream effectors of mTOR that delay or inhibit tumorigenesis. While direct inhibition of mTOR with rapamycin delays tumour onset, the indirect inhibition of mTOR by blocking mitochondrial respiration seems to have a more profound affect – eliminating tumorigenesis altogether (in LFS mouse models) [PMID: 27869650]. This result may not be entirely surprizing. mTORC1 has two central downstream effectors, S6Ks and 4EBPs. Rapamycin is a weak selective mTORC1 inhibitor, inhibiting activation of S6Ks but not of the 4EBPs. To better realize which of the mTORC1 effectors mediates the delay of tumorigenesis, we will rely on mice models that are knocked out for different downstream effectors of mTOR. Transgenic mice that are deficient in either S6K or 4EBP have been previously constructed [PMID: 20508131 PMID: 16166381 PMID: 15723049], as well as mice that have non-phosphorylatable rpS6 [PMID: 20508131 PMID: 16166381 PMID: 15723049]. We will introduce these knockouts into mice models of LFS and test rates of tumour formation. Similarly, to we will construct mice with organ specific knockout of mTOR components to test whether certain organs generate systemic signals that influence tumour progression.

Aim 3: Determine the effects of p53 reactivation by known p53 refolders and activators in the context of heterozygous *TP53* mutations associated with LFS. We have access to several promising agents that have been functionally validated to re-activate mutant p53 in the context of the *Trp53*$^{173m/173m}$ mouse. APR246 (Klas Wiman), CAP250 (Varda Rotter) and Lovostatin (Carol Prives) have never been previously tested in the context of heterozygous mutations. We will use the cancer cell observatory to examine the biological effects, and activation of p53 function (apoptosis, growth arrest) across our entire LFS fibroblast library – which encompasses missense, frameshift and splice-site TP53 mutations to determine how wide-ranging physiologic effects of these agents are to different mutations. This data will be of great value in determining how to utilize these agents in early clinical trial development as chemopreventive strategies and will complement the *in vivo* work outlined in **Project 3** in the murine LFS model.

5a. INDIVIDUAL CORE TECHNOLOGY PLATFORM – CORE #1: *(max 2 pages) (optional)*

The goal of establishing a data core of our project is to provide a unified easily and efficiently accessible repository containing comprehensive phenotypic and omic characterization of LFS patients. The core will serve as the end point for the data collection projects, where all of the data will be quality controlled and stored and as the starting point for all the analyses and inference projects, where any subset of the data can be efficiently retrieved and analyzed. The core is thus characterized by the data that will be stored and the services that it will provide as described below.

Data elements will include (David, please put in the numbers of projects that will be depositing, accessing the data):

- Clinical data
    - Demographic
    - TP53 mutation status
    - Family history
- Imaging data
    - Types?
- Omic data
    - DNA sequencing
        - Tumor
        - Germline (blood)
    - Methylation (850K Epic array)
        - ctDNA
        - Germline (blood)
        - Tumor (?)
    - mRNA-sequencing
        - Germline (blood)
        - ctDNA
        - Tumor
    - miRNA-sequencing
        - Germline
        - Tumor

The data will provide a resource project wide and potentially, upon completion, to the whole LFS community. To process and curate the data we will need the expertise of a

- Technician that can extract samples from patients
- Clinical data manager/coordinator - to ensure the completeness and provenance of all the clinical and sample data
- Bioinformatician to establish and perform QC on the sequencing and other types of omic datasets as they come off the biotechnological machines
- Database analyst to develop a specialized database that can store all of the diverse types of data that need to be collected and ensure efficient retrieval of the subsets necessary for various projects
- Biostatistician/data scientist to appropriately curate and QC data for the great variety of queries that will be asked of this data

This team will establish a resource unprecedented in the heterogeneity of the types of data that it stores, paving the way for many projects that aim to fully characterize patients and streamline storage and data retrieval for the integrative analyses.

6. LIST OF KEY REFERENCES

7. HIGH-LEVEL BUDGET REQUEST

[Refer to 2018 PPG LOI Guide before completing this section.]

(i) Request to Terry Fox Research Institute

PPG Component	Year 1	Year 2	Year 3	Year 4	Year 5	TOTAL
Program Overview (Program Coordinator, Travel)	$110000	$110000	$110000	$110000	$110000	$550000
Project 1: [Intra-tumoral heterogeneity in LFS] Operating	$225000	$225000	$225000	$225000	$225000	$1125000
Project 1: Equipment	$100000					$100000
Project 2: [Predictive Modeling in LFS] Operating	$90000	$90000	$90000	$90000	$90000	$450000
Project 2: Equipment	$0					$0
Project 3: [Early Tumor Detection in LFS] Operating	$165000	$165000	$165000	$165000	$165000	$825000
Project 3: Equipment	$0					$0
Project 4: [Imaging Surveillance in LFS] Operating	$205000	$205000	$205000	$205000	$205000	$1025000
Project 3: Equipment	$0					$0
Project 5: [Zebrafish Chemoprevention Model of LFS] Operating	$216000	$216000	$216000	$216000	$216000	$1080000
Project 5:	$0					$0

Equipment						
Project 6: [Pharmacologic prevention of Cancer in LFS] Operating	$175000	$175000	$175000	$175000	$175000	$875000
Project 6: Equipment	$0					$0
Core 1: [LFS Sequencing/Data Bank] Operating	$155000	$155000	$155000	$155000	$155000	$775000
Core 1: Equipment	$150000					
Operating Total	$1105000	$1105000	$1105000	$1105000	$1105000	$5525000
Equipment Total	$250000					$250000
ANNUAL TOTAL						

[Expand table as required]

(ii) Short Budget Justification

8. LIST OF SUGGESTED REVIEWERS

Please suggest a minimum of three scientific peers who would be able to evaluate your whole program. Please also suggest a minimum of two scientific peers per component (project/core) in the table below to review your application.

Full Name	*Email Address*	*Expertise Keywords*
Affiliation	*Telephone Number*	*Component of PPG to review*

[Expand table as required]

9. LIST OF REVIEWERS TO EXCLUDE

Please provide a list of reviewers who you feel would not provide an objective review of your application and a brief rationale for exclusion for each.

Full Name	*Affiliation*	*Rationale for Exclusion*

[Expand table as required]

APPENDICES – CVs

[In the table below provide a list of all the brief CVs supplied with this Letter of Intent]

	Curricula Vitae Provided	*Role*
1		Project Leader
2		Principal Investigator
3		Principal Investigator
4		Co-Investigator

[Expand table as required]