## B. RESEARCH STRATEGY

## B.1. BACKGROUND AND SIGNIFICANCE

*Li-Fraumeni Syndrome definition*

LFS is an inherited cancer predisposition syndrome first described in 1969 by Li and Fraumeni[1,2]. The classical definition of LFS includes a proband diagnosed with sarcoma before the age of 45 years, with a first degree relative with any cancer before the age of 45 years, plus another first or second-degree relative with cancer under 45 years or a sarcoma at any age[15,16]. These criteria have been updated by Chompret and colleagues[17,18] and most recently summarized[19] to include:

- a proband diagnosed with a tumor belonging to the LFS spectrum (e.g., soft tissue sarcoma, osteosarcoma, brain tumor, pre-menopausal breast cancer, adrenocortical carcinoma, leukemia and lung bronchoalveolar cancer) and at least one first- or second-degree relative with an LFS tumor (except breast cancer, if the proband has breast cancer) before age of 56 years or with multiple primary tumors ***OR***
- a proband with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum and first of which occurred before age 46 years ***OR***
- a patient with adrenocortical carcinoma (ACC) or choroid plexus tumor, irrespective of family history

*TP53 gene alterations are the main cause of LFS*

In 1990, Malkin et al. showed that the underlying genetic defect in LFS involves germline mutations in the *TP53* gene[3]. *TP53*, a tumor suppressor, codes a 53-kd nuclear transcription factor that has important regulatory control over cell proliferation and homeostasis, specifically the cell cycle, DNA repair processes, and apoptosis. The gene consists of 11 exons, of which exons 2 to 11 represent coding regions. As many as 80% of LFS families harbor detectable germline *TP53* mutations[4-8]. Although diagnosis of LFS is currently based on clinical criteria[19], Birch has suggested that the diagnosis should be based on the presence of germline *TP53* mutation and that clinical criteria should be used to select families for *TP53* testing[20].

*TP53 functions and changes related to cancer*

The best characterized tumor suppressive activities of *TP53* are apoptosis and cell cycle arrest[21]. A more recently recognized function is induction of senescence through growth arrest and cellular senescence mediated by induction of microRNA miR-34a[21,22]. *TP53* also

modulates cell migration[23]. Thus, loss of *TP53* contributes in a number of ways to promote cell proliferation and migration required for tumor growth and invasiveness[24]. In addition to loss of function, compelling evidence suggests a gain-of-function role for the mutant protein[24]. Mutant protein that may therefore directly promote cancer development, including resistance to chemotherapy[25] and induction of angiogenesis[24,26].

*Known mutations and polymorphisms in TP53* ***– current knowledge and IARC database***

**Mutations:** *TP53* is one of the most frequently altered genes in human cancer; in addition to germline mutations underlying LFS, somatic mutations are observed in 10-60% of sporadic cancers[24]. Due to the wide research interest in *TP53* and LFS, the International Agency for Research on Cancer (IARC) created a database of LFS and related syndromes by compiling information on families with LFS and related syndromes and on individuals carrying germline *TP53* mutations described in the literature[14,27]. The analyses of the IARC database demonstrate that the most frequent alterations are single-nucleotide substitutions leading to a mutant protein that differs from the wild-type protein by one amino acid (missense mutations). Thus, *TP53* differs from other tumor suppressor genes, such as *APC*, *RB* or *BRCA1/2* which are inactivated frequently by deletions or nonsense mutations[14]. Another striking difference between *TP53* and these other cancer susceptibility genes is that germline mutations in *TP53* are associated with a wide cancer spectrum, while the others are associated with a narrow spectrum (e.g., *BRCA-1/2* cause mostly breast and ovarian cancer) [28].

*Despite the overwhelming number of germline TP53 mutations described to date, it is clear that not all mutations have the same biologic or phenotypic impact*. For example, exons 5 through 8 of *TP53* gene code for the DNA-binding domain of p53 protein, and this region contains 8 “mutation hotspot” codons (175, 176, 220, 245, 248, 249, 273, and 282); approximately 30% of mutations cluster within this region[14]. In addition to these mutations common to LFS and sporadic tumors, the next most frequent mutations in LFS (codon 133, 152 and 337) are rarely mutated in sporadic tumors (<0.8% of all somatic mutations)[14]. Mutants vary also by the extent of their capacity to exert a dominant negative effect over the wild-type *TP53*; 80% of common mutants can exert dominant negative effect, compared to 45% of less common mutants[13]. Over 70% of germline *TP53* alterations are missense mutations, the next most common are deletions (10%)[14], most of which are small (1-4 bp) and induce a frameshift.

*Polymorphisms:* In addition to these mutations observed in the context of LFS, variations in *TP53* DNA sequence have been found in unaffected individuals, and such variations are considered SNPs. Thus far, 85 SNPs in *TP53* have been described[13], and several were shown to increase risk of cancer[29-31], while the PIN3 polymorphism was associated with

delayed tumor onset in *TP53* mutation carriers[32]. Thus, SNPs can affect cancer susceptibility and information about SNPs can inform future studies about their role in cancer susceptibility among LFS patients, as well as their possible role in sporadic cancer.

*Genotype-phenotype correlation in TP53 mutation carriers*

Thus far, no correlation has been found between a specific mutation and a tumor type[24]. However, when mutations were grouped based on whether they reside within or outside the DNA binding domain (Structure Groups 1-3) or confer a “p53 null” phenotype (Structure Group 4), genotype-phenotype correlations emerged[14]. Namely, brain tumors were more likely to be associated with mutations in the DNA binding domain associated with binding to the minor groove of target DNA, while missense mutations in the region outside DNA binding domain were strongly correlated to ACC[14]. In addition, it was shown that the type of mutation may influence the age of tumor onset[14], and that total loss of transactivation is associated with an earlier age of onset, compared to mutations that retain partial transactivation activity[13].

Despite these valuable genotype/phenotype insights, the correlations between genotype and phenotype in LFS remain poorly understood. Namely, variability in tumor type and ages of onset within any family harboring the same germline *TP53* mutation can be quite extreme, precluding recognition of obvious genotype-phenotype associations[28]. Further research is needed to understand the role of other potential genetic and epigenetic modifiers to account for phenotype variability. Such knowledge is essential for improvement of management strategies for LFS families, as it will help refine the criteria of low versus high risk for cancer development among members of LFS families (Fig. 1).

*Other mutated genes and genetic effect modifiers*

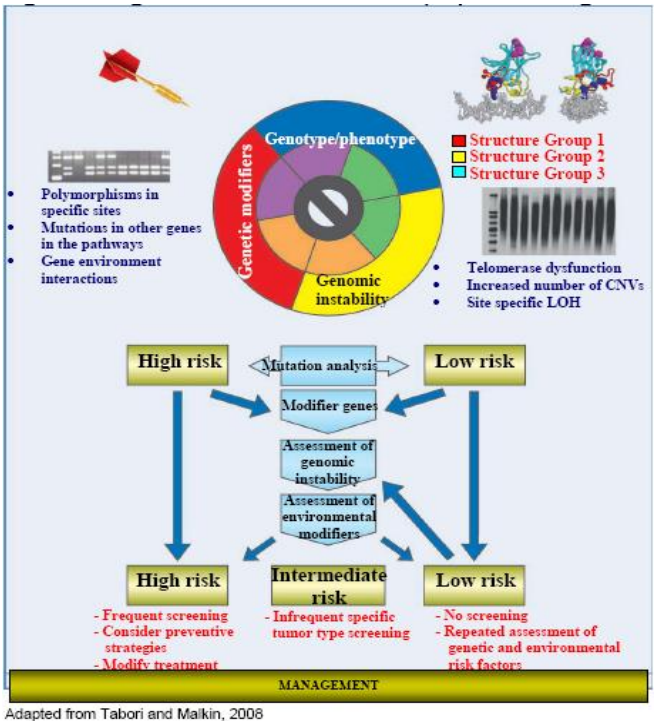
Not all LFS patients have identifiable TP53 mutations, leaving the possibility that other genes may underly LFS. Candidate genes examined, such as CHEK2 mutations identified in five LFS families who lacked TP53 mutation, failed to show that these mutations predispose to LFS per se, but rather only to the breast cancer in the context of LFS families[14]. A linkage study implicated a 4cM region on chromosome 1q23 of susceptibility to LFS, however a specific gene has not been identified yet[33]. Thus, TP53 remains the only molecular explanation for this syndrome[14] and a more comprehensive approach, such as exome sequencing, is needed to resolve whether any other genes underlie LFS in the families lacking identifiable TP53 mutations. In addition, such novel approach would lead to discovery of genes that may act as strong genetic modifiers in the families with an identified TP53 mutation. Indeed, evidence has begun to emerge that changes in other genes may modify cancer risk in germline TP53 mutation carriers, most notably MDM2 variant, SNP309[34]. Bougeard et al. confirmed such

modifier effects, showing that the G allele is associated with earlier age of tumor onset in germline TP53 mutation carriers[35]. Finally, sex and race/ethnicity may play a role as effect modifiers. For example, females have higher risk of osteosarcoma among carriers, in contrast to sporadic osteosarcoma. Race/ethnicity can play a role in disparity, however, most data is available for Caucasians only and this has not been studied thus far.

*Environmental factors*

Environmental factors, such as toxins and nutrients, play an important role in cancer development, yet they have not been effectively addressed in LFS primarily due to the relative rarity of the syndrome and its high penetrance leading to often lethal cancers. Higher life-time risk of cancer among female compared to male germline mutant *TP53* carriers (93% vs 75%, respectively) raises the possibility for the role of hormonal factors in cancer initiation in LFS[28], while animal studies suggest a possible role of nutrition as having a modifier effect showing that calorie restriction delays tumor development in *TP53* null mice[36,37]. Tobacco consumption has been associated with early onset lung and laryngeal cancer in some LFS families [38], while excess of gastric cancer in some families has been observed in high-risk Asian populations where H. pylori infection is endemic[39,40]. It has been also shown that radiation therapy induces tumors in patients with germline inactivating *TP53* mutation[41]. The implications of these findings are that such additional hits on either the *TP53* gene itself, or on the modifiers, can disturb p53 regulated cellular growth and predispose to higher risk of cancer[28].

**Figure 1. Diagram of risk assessment and proposed management.**




Adapted from Tabori and Malkin, 2008

*Current model for risk assessment*

The model below is presented as an illustration of how knowledge of genetic and environmental risk factors that can affect cancer development among LFS members can lead to better management of their health. Namely, people at immediate risk, based on demonstration of high genomic instability, would be screened more frequently for specific tumor types in hopes of earlier identification of cancers and enhancement of survival

advantages, while people perceived to be at a lower risk would be less exposed to these potentially invasive surveillance modalities. Therefore, increase in knowledge about LFS biology, one of the major goals of the proposed registry, will lead to further refinement of this model, improving risk estimates and targeting interventions according to the individual risks of cancer development (see C13). In addition to the impact on diagnosis, prognosis, screening, treatment and outcome, better understanding of the risk may help families whose child has been diagnosed with cancer make more informed decisions about the health of the index child, as well as that of other affected family members and empower them to be more proactive in managing their health.

Significance: Improved understanding of genotype-phenotype correlation and non-genetic risk modifiers is essential for individual risk stratification and thereby improved screening and treatment of LFS members. Below we describe preliminary data and methods to establish a LFS registry with close to 1,000 participants with complete genetic…