Title: To Fibroblast or Not to Fibroblast: Outcomes and Clinical Features of Patients Pursuing Confirmatory Germline Testing Following *TP53*-Positive Results

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Abstract: Multigene panel testing (MPT) via next generation sequencing (NGS) is standard practice for providers ordering hereditary cancer testing. However, due to the nature of NGS read depth and frequency of somatic variants in genetic testing specimens, a realized limitation is the identification of genetic variants of unknown origin, often in TP53. This poses a challenge for providers to delineate if a TP53 pathogenic/likely pathogenic variant (PV) is of germline origin (diagnostic of Li Fraumeni Syndrome [LFS]) or somatic origin. Often, confirmation testing of an alternative specimen, such as cultured fibroblasts (CF) is utilized to rule-in germline origin. Though this approach can inform cancer risk and management, most results are noninformative and a burdensome process for patients, providers, and the testing laboratory. To help guide best practices and resource utilization following TP53 PV identification on MPT, we investigated and compared clinical features of patients who pursued confirmatory CF testing (CCFT) and whether testing was informative (positive) or non-informative (negative). Our hypothesis is that individuals with informative CCFT results will demonstrate characteristics more similar to LFS than those with non-informative results.

Patients with a TP53 PV on MPT were identified from a large diagnostic laboratory between January 1, 2018, and August 15, 2024. Cancer history (excluding non-melanoma skin cancer) of those who pursued CCFT were described and compared based on result outcome (informative = positive; non-informative = negative). Number of cancer diagnoses, age at first diagnosis, and age at testing were compared between informative and noninformative CCFT using statistical analyses.

A total of 1,665 individuals were identified to carry a TP53 PV on MPT. Only about 1% were known to meet Chompret criteria or the PV was known in the family at the time of testing. Of the remaining patients, CCFT was pursued by 152 in an attempt to delineate the origin of the TP53 PV. Of those, 26 patients (17%) were informative (positive), and 126 patients (83%) were noninformative (negative). Individuals with informative CCFT were more likely to have a younger age at initial cancer diagnosis and testing (39.5 years and 41.9 years, respectively), compared to those with non-informative results (55.8 years and 61.5 years, respectively; $p<0.0001$). There was no statistical difference in the total number of cancer diagnoses per patient between the two groups ($p=0.38$).

Breast cancer was the most common diagnosis reported in both CF-outcome groups. Notably, the CF-positive group reported 3 sarcomas (1 leiomyosarcoma, NOS; 1

osteosarcoma; 1 soft tissue sarcoma) of 32 total cancer diagnoses (9.4%). The CF-negative group reported 2 sarcomas (1 uterine leiomyosarcoma; 1 Kaposi sarcoma) of 156 cancer diagnoses (1.3%). There were no other LFS-specific cancers reported in either group. In this study, we assessed characteristics of individuals pursuing CCFT following TP53 PV identification on MPT in an attempt to determine if particular clinical characteristics are more likely to produce results informative of germline origin. We identified that patients with a younger age of cancer onset and testing were more likely to have informative CCFT, resulting in a diagnosis of LFS. Patients with a sarcoma diagnosis were more likely to have informative CCFT, however these numbers were too small to power a statical analysis. Further, the sarcoma types in the non-informative CCFT group are not typically known to be associated with LFS, unlike the sarcomas reported in the informative group. Based on these data, age at diagnosis and/or sarcoma history should be considered when determining the utility of CCFT following identification of a TP53 PV on MPT.