Biochemical and imaging surveillance in germline *TP53* mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study

Anita Villani, Ari Shore, Jonathan D Wasserman, Derek Stephens, Raymond H Kim, Harriet Druker, Bailey Gallinger, Anne Naumer, Wendy Kohlmann, Ana Novokmet, Uri Tabori, Marta Tijerin, Mary-Louise C Greer, Jonathan L Finlay, Joshua D Schiffman, David Malkin

Summary

Background Carriers of a germline *TP53* pathogenic variant have a substantial lifetime risk of developing cancer. In 2011, we did a prospective observational study of members of families who chose to either undergo a comprehensive surveillance protocol for individuals with Li-Fraumeni syndrome or not. We sought to update our assessment of and modify the surveillance protocol, so in this study we report both longer follow-up of these patients and additional patients who underwent surveillance, as well as update the originally presented surveillance protocol.

Methods A clinical surveillance protocol using physical examination and frequent biochemical and imaging studies (consisting of whole-body MRI, brain MRI, breast MRI, mammography, abdominal and pelvic ultrasound, and colonoscopy) was introduced at three tertiary care centres in Canada and the USA on Jan 1, 2004, for carriers of *TP53* pathogenic variants. After confirmation of *TP53* mutation, participants either chose to undergo surveillance or chose not to undergo surveillance. Patients could cross over between groups at any time. The primary outcome measure was detection of asymptomatic tumours by surveillance investigations. The secondary outcome measure was 5 year overall survival established from a tumour diagnosed symptomatically (in the non-surveillance group) versus one diagnosed by surveillance. We completed survival analyses using an as-treated approach.

Findings Between Jan 1, 2004, and July 1, 2015, we identified 89 carriers of *TP53* pathogenic variants in 39 unrelated families, of whom 40 (45%) agreed to surveillance and 49 (55%) declined surveillance. 19 (21%) patients crossed over from the non-surveillance to the surveillance group, giving a total of 59 (66%) individuals undergoing surveillance for a median of 32 months (IQR 12–87). 40 asymptomatic tumours have been detected in 19 (32%) of 59 patients who underwent surveillance. Two additional cancers were diagnosed between surveillance assessments (false negatives) and two biopsied lesions were non-neoplastic entities on pathological review (false positives). Among the 49 individuals who initially declined surveillance, 61 symptomatic tumours were diagnosed in 43 (88%) patients. 21 (49%) of the 43 individuals not on surveillance who developed cancer were alive compared with 16 (84%) of the 19 individuals undergoing surveillance who developed cancer (p=0·012) after a median follow-up of 46 months (IQR 22–72) for those not on surveillance and 38 months (12–86) for those on surveillance. 5 year overall survival was 88·8% (95% CI 78·7–100) in the surveillance group and 59·6% (47·2–75·2) in the non-surveillance group (p=0·0132).

Interpretation Our findings show that long-term compliance with a comprehensive surveillance protocol for early tumour detection in individuals with pathogenic *TP53* variants is feasible and that early tumour detection through surveillance is associated with improved long-term survival. Incorporation of this approach into clinical management of these patients should be considered.

Funding Canadian Institutes for Heath Research, Canadian Cancer Society, Terry Fox Research Institute, SickKids Foundation, and Soccer for Hope Foundation.

Lancet Oncol 2016; 17: 1295–305

Published Online August 5, 2016 http://dx.doi.org/10.1016/S1470-2045(16)30249-2

See Comment page 1184

Department of Pediatrics (A Villani MD, J D Wasserman MD, U Tabori MD, Prof D Malkin MD), **Division of Hematology/Oncology** (A Villani, H Druker MSc, A Novokmet BA, U Tabori, Prof D Malkin), **Division of Endocrinology** (J D Wasserman), **Genetics & Genomic Biology Program** (A Villani, A Shore BScH, J D Wasserman, B Gallinger MSc, A Novokmet, U Tabori, Prof D Malkin), **Department of Genetic Counselling and Department of Molecular Genetics** (H Druker, B Gallinger), **Division of Biostatistics, Design and Analysis** (D Stephens MSc), **and Department of Medical Imaging** (M Tijerin MD, M-L C Greer MBBS), **The Hospital for Sick Children, and Department of Medicine and Division of Medical Oncology and Hematology, Princess Margaret Cancer Center** (R H Kim MD), **University of Toronto, Toronto, ON, Canada; Division of Pediatric Hematology/Oncology, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA** (A Naumer MS, W Kohlmann MS, J D Schiffman MD); **and Division of Hematology/Oncology, Nationwide Children's Hospital, The Ohio State University, Columbus, OH, USA** (Prof J L Finlay MBChB)

Correspondence to: Prof David Malkin, Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, ON M5G 1X8, Canada **david.malkin@sickkids.ca**

Introduction

Li-Fraumeni syndrome is a cancer predisposition syndrome associated with germline pathogenic variants in the tumour suppressor gene *TP53*. Traditionally characterised by various early-onset tumours, consisting of sarcoma, breast cancer, brain tumours, leukaemia, and adrenocortical carcinoma, accumulating data and next-generation sequencing efforts are expanding understanding of the cancer spectrum and risk in this population. Genetic, epigenetic, and environmental modifiers of the Li-Fraumeni phenotype remain to be fully elucidated, and clinical management of this patient population continues to be challenging. The established cancer risk estimates of carriers of germline *TP53* pathogenic variants continue to inform both patient and clinician objectives of effective pre-emptive intervention delivery to this population.

In 2011, we published what was, to our knowledge, the first prospective study[1] of comprehensive clinical surveillance for individuals with Li-Fraumeni syndrome, with the aim of early tumour detection and positive effect on treatment-related morbidity and mortality. The hypothesis of the study was that comprehensive surveillance of individuals with

Research in context

Evidence before this study

We searched PubMed for studies of surveillance and familial cancer syndromes published between Jan 1, 2000, and Aug 31, 2015, in English, using a combination of the following search terms: "familial cancer", "hereditary cancer syndrome", "Li-Fraumeni syndrome", "hereditary breast-ovarian cancer syndrome", "Lynch syndrome", "hereditary non-polyposis colorectal cancer", "tumor surveillance", "tumor screening", "breast cancer", "brain tumors", "adrenocortical carcinoma", "osteosarcoma", "soft-tissue sarcoma", "leukemia", "lymphoma", "colorectal carcinoma", "gastric carcinoma", and "whole-body MRI". We also searched the National Comprehensive Cancer Network guidelines for surveillance recommendations. To our knowledge, no studies have been published describing the effectiveness of a surveillance protocol for individuals with Li-Fraumeni syndrome since our original prospective observational study was published in 2011. Two studies describe the effectiveness of an incident ^{18}F-fluorodeoxyglucose PET-CT scan for asymptomatic tumour detection in adult patients with Li-Fraumeni syndrome, but document a high number of false positives and leave concern for radiation exposure in this susceptible population. Findings from several studies further support use of MRI for familial breast cancer screening. Use of whole-body MRI for individuals with cancer predisposition syndromes has been examined in retrospective studies; these studies have reported high sensitivity and negative predictive value. The characteristics of some Li-Fraumeni syndrome-associated tumours have also been described, revealing an age dependency of *TP53* mutation positivity in unselected patients with adrenocortical carcinoma.

Added value of this study

This multicentre study is the first, to our knowledge, to assess the feasibility and effectiveness of a comprehensive surveillance protocol for both adults and children with Li-Fraumeni syndrome. We provide prolonged follow-up data for an expanded cohort of carriers of pathogenic *TP53* variants, showing widespread feasibility of early, asymptomatic tumour detection and a sustained survival advantage for those undergoing surveillance. We present an updated multimodality surveillance protocol based on our data and evidence for tumours that can occur in the context of Li-Fraumeni syndrome. This study also adds to the body of literature supporting use of whole-body MRI as an imaging modality for cancer predisposition syndromes with a heterogeneous tumour profile.

Implications of all the available evidence

These data raise awareness of the importance and value of surveillance strategies for early tumour detection, not only in the context of Li-Fraumeni syndrome, but also for other syndromes of cancer susceptibility. We provide evidence to support health policy changes in the insurance industry and for global implementation of surveillance protocols in multidisciplinary health-care settings. Further data from genomic studies are still required to understand the roles of genetic modifiers and low-penetrance alleles before risk stratification of individuals and provision of tailored surveillance recommendations can be attempted.

Li-Fraumeni syndrome will improve survival through early diagnosis of malignant tumours at early stages or low biological grades. The published surveillance protocol[1] has been adopted internationally, including in many centres across North and South America, Australia, Europe, Japan, and the Middle East. In this study, we update our findings after prolonged longitudinal follow-up and additional patient accrual. We also outline modifications to the surveillance recommendations on the basis of data published in the last 5 years.

Methods

Patients and study design

The surveillance protocol[1] was implemented at The Hospital for Sick Children (Toronto, ON, Canada) on Jan 1, 2004, and adopted shortly thereafter by the Primary Children's Hospital and Family Cancer Assessment Clinic at the Huntsman Cancer Institute at the University of Utah (Salt Lake City, UT, USA) and the Division of Hematology/Oncology at the Children's Hospital of Los Angeles (Los Angeles, CA, USA; appendix p 4). Families at these centres suspected of a diagnosis of Li-Fraumeni syndrome on the basis of clinical criteria were offered *TP53* testing. Mutation-positive individuals opted to either undergo surveillance or not and were thereafter divided into surveillance and non-surveillance groups. Patients could cross over between groups at any time. For the 2011 study,[1] individuals were followed up prospectively until Nov 1, 2010. In this study, we extended follow-up to July 1, 2015.

We obtained written informed consent from all adult family members and children older than the age of consent in their respective jurisdictions. Parents provided written informed consent for children younger than the age of consent. Research testing for the study was approved by research ethics boards at the participating institutions and the clinical surveillance protocol was approved in each participating institution.

See Online for appendix

Procedures

TP53 variant analysis (gene sequencing and copy number quantification) was carried out in the clinical molecular diagnostic laboratories at The Hospital for Sick Children or Clinical Laboratory Improvement Act-certified commercial laboratories. As previously

described,[1] sequencing of exons 2–11, including at least 50 bases into introns, was done in addition to multiplex ligation-dependent probe amplification analysis of gene copy number. All families received genetic counselling before and after *TP53* analysis, and carriers of germline *TP53* pathogenic variants were offered participation in the surveillance protocol, which has been updated (panel 1). The protocol used physical examination and frequent biochemical and imaging studies (consisting of whole-body MRI [appendix p 4], brain MRI, breast MRI, mammography, abdominal and pelvic ultrasound, and colonoscopy). Any medical concerns for individuals in the non-surveillance group were investigated at the discretion of the treating physician.

We reviewed results of surveillance investigations and recorded any follow-up investigations. We documented the clinical details of new cancer diagnoses in study participants and confirmed them by review of pathology reports where possible. All individuals were treated according to the standard of care for newly diagnosed tumours at their respective institutions.

Outcomes

The primary outcome measure was detection of asymptomatic tumours by surveillance investigations. To address this primary outcome, we systematically recorded and counted new cancer diagnoses. We defined a false negative as any tumour diagnosed between surveillance scans (with a preceding negative scan) and a false positive as a suspected tumour leading to biopsy that was pathologically proven to not be neoplastic. The secondary outcome was 5 year overall survival, defined from the time of tumour diagnosis symptomatically or by surveillance until death from any cause, last follow-up, or the end of the study.

Statistical analysis

To compare baseline characteristics between individuals in the surveillance and non-surveillance groups, we used Fisher's exact test for discrete variables (sex and previous malignant cancer diagnosis) and a Mann-Whitney test for the continuous variable (age). Given the underlying germline pathogenic *TP53* variant in individuals with

Panel 1: 2016 version of the surveillance protocol for individuals with germline *TP53* pathogenic variants

Children (birth to age 18 years)

Adrenocortical carcinoma
- Ultrasound of abdomen and pelvis every 3–4 months
- Blood tests every 3–4 months:* 17-OH-progesterone, total testosterone, dehydroepiandrosterone sulfate, and androstenedione
- 24 h urine cortisol, if feasible

Brain tumour
- Annual brain MRI

Soft tissue and bone sarcoma
- Annual rapid whole-body MRI

Leukaemia or lymphoma
- Blood tests every 3–4 months: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase

General assessment
- Complete physical examination every 3–4 months, including anthropometric measurements plotted on a growth curve (with particular attention to rapid acceleration in weight or height), signs of virilisation (pubic hair, axillary moisture, adult body odour, androgenic hair loss, clitoromegaly, or penile growth), and full neurological assessment
- Prompt assessment with primary care physician for any medical concerns

Adults

Adrenocortical carcinoma (age 18–40 years)
- Ultrasound of abdomen and pelvis every 3–4 months
- Blood tests every 3–4 months:* 17-OH-progesterone, total testosterone, dehydroepiandrosterone sulfate, and androstenedione
- 24 h urine cortisol, if feasible

Breast cancer
- Monthly breast self-examination (age 18 years onwards)
- Clinical breast examination twice a year (age 20–25 years onwards, or 5–10 years before earliest known breast cancer in the family [whichever comes first])
- Annual mammography† and breast MRI screening‡ (age 20–75 years, or 5–10 years before earliest known breast cancer in the family [whichever comes first])
- Consider risk-reducing bilateral mastectomy

Brain tumour (age 18 years onwards)
- Annual brain MRI

Soft tissue and bone sarcoma (age 18 years onwards)
- Annual rapid whole-body MRI‡
- Ultrasound of abdomen and pelvis every 3–4 months

Colorectal cancer
- Colonoscopies every 2 years (start at age 25 years, or 10 years before earliest known colon cancer in the family [whichever comes first])

Melanoma (age 18 years onwards)
- Annual dermatological examination

Leukaemia or lymphoma (age 18 years onwards)
- Blood tests every 3–4 months: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase

General assessment
- Complete physical examination every 3–4 months
- Prompt assessment with primary care physician for any medical concerns

*Serial specimens obtained at the same time of day and processed in the same laboratory. †Breast ultrasound with mammography as indicated by breast density, but not instead of breast MRI or mammography. ‡Breast MRI to alternate with annual rapid whole-body MRI (one scan every 6 months).

Li-Fraumeni syndrome, each tumour was treated as an independent entity. For the comparison of overall survival in the surveillance and non-surveillance groups, the unit of analysis was the tumour rather than the individual. We allocated each tumour to either the surveillance or non-surveillance group according to the group status of the individual at the time of diagnosis, and the tumour's group status did not change over time. We analysed survival from each of these tumours, irrespective of whether the individual remained in their initial group or crossed over to the other group. For participants with multiple tumours, cause of death would be attributed to one of the tumours (this was usually the most recent tumour) and we censored tumours that were not the cause of death on the date of death.

We compared the number of survivors in the surveillance and non-surveillance groups with a stratified exact conditional logistic regression to account for the clustered nature of the binary data (in which a cluster represents a family). We created a family variable and assigned the same designation for measurements from the same family or same individual. We then used this newly created variable as a clustering variable in a marginal Cox proportional hazards model with a robust sandwich estimator. We used the new family variable as the identification statement to indicate that observations (ie, individuals) with the same identification are from the same cluster and thus correlated. We generated baseline survival estimates from the model described above and plotted them for the surveillance and non-surveillance groups. We also used an alternative shared frailty model to account for potential within-cluster correlation (which assumes non-independence of tumours; appendix pp 1–3). We analysed all data using SAS version 9.4.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 1, 2004, and July 1, 2015, we identified 89 carriers of a *TP53* pathogenic variant from 39 families for whom sufficient clinical data were available. 49 (55%) individuals initially declined participation in the surveillance protocol and 40 (45%) agreed to surveillance. 19 (21%) patients crossed over from the non-surveillance to the surveillance group during the course of the study, giving a total of 59 (66%) individuals who underwent surveillance. All 19 individuals who switched from non-surveillance to surveillance had developed at least one cancer before switching study groups. We placed all children who were eligible for surveillance on the surveillance protocol because no parent opted their child out of surveillance. Children in the non-surveillance group were probands and were diagnosed with Li-Fraumeni syndrome after their initial cancer diagnosis. We noted no significant differences in baseline characteristics of participants between each of the two groups (table 1).

	No surveillance (n=49)	Surveillance (n=40)	p value
Sex			0·20
Male	24 (49%)	14 (35%)	..
Female	25 (51%)	26 (65%)	..
Age (years)*	23·0 (11·6–44·5)	18·0 (10·9–30·0)	0·12
Previous malignant cancer diagnosis	10 (20%)	5 (13%)	0·40

Data are n (%) or median (IQR). *At last follow-up or death.

Table 1: **Baseline characteristics**

Compliance with the surveillance protocol was 100% for individuals participating in the Canadian publicly funded health-care system and more than 90% for patients in the USA. Although not systematically assessed for participants in the USA, the most frequently cited reason for patients declining surveillance was an absence of insurance coverage. No patients withdrew from the study. We noted occasional delays between two surveillance interventions, but these delays were no longer than 1–2 weeks, with the exception of patient 2 in family six.

Over a median period of 32 months (IQR 12–87), the clinical protocol identified 40 asymptomatic neoplasms in 19 (32%) of 59 patients who underwent surveillance, including both malignant tumours and low-grade or premalignant lesions (table 2). Two additional cancers were diagnosed between surveillance assessments (false negatives) in one patient and two biopsied lesions were non-neoplastic entities on pathological review (false positives). Since this study was initiated in 2004, 43 (88%) of 49 patients in the non-surveillance group have developed 61 symptomatic neoplasms. We confirmed 94 (93%) of the 101 symptomatic tumours by review of pathology reports. We confirmed the tumour type for the remaining seven tumours from the medical records (when surgical procedures were previously done at a referring hospital rather than one of the primary institutions) or with radiological diagnoses when a definitive surgical procedure had not yet been completed.

16 (84%) of the 19 individuals with tumours detected by surveillance were alive after a median follow-up of 38 months (IQR 12–86; table 2). 21 (49%) of the 43 individuals in the non-surveillance group who had a tumour detected symptomatically were alive after a median follow-up of 46 months (IQR 22–72), which is significantly fewer than the number in the surveillance group (p=0·012). All patients who died in the non-surveillance group died of cancer. We noted a significant survival advantage in patients who underwent surveillance: 5 year overall survival was 88·8% (95% CI 78·7–100) in the 59 patients in the

	Sex	No surveillance					Surveillance					
		Tumour type	Age at diagnosis (years)*	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)	Tumour type	Mode of detection	Age at diagnosis (years)†	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)‡
Family one: Arg175His (c.524G>A)												
Patient 1	M	MB	14	Dead	15	13	..	..	..	..	..	..
Family two: IVS03-11 C>G												
Patient 1	M	RMS	3	Dead	5	19	..	..	..	..	..	..
Patient 2	M	Myxoid FS	45	Dead	46	22	..	..	..	..	..	..
Patient 3§	F	..	..	..	..	..	MFH	WBMRI and CE	30	Alive	43	155
Family three: Tyr163Cys (c.488A>G)												
Patient 1§	F	AA	13	Dead	20	80	MDS	CBC	17	Dead	20	30
Family four: Arg158His (c.473G>A)												
Patient 1	M	CPC	4	Dead	5	10	..	..	..	..	..	..
Patient 2	M	NBL	2 days	Dead	2 weeks	12 days	..	..	..	..	..	..
Patient 3	F	..	..	..	..	..	TA	CE and US	29	Alive	35	75
Family five: Arg248Gln (c.743G>A)												
Patient 1	F	MB	2	Dead	2	1	..	..	..	..	..	..
Patient 2	M	OS	15	Dead	18	35	..	..	..	..	..	..
Family six: Ser241Tyr (c.721T>A)												
Patient 1	M	CPC; AML	1; 3	Dead	3	30; 5						
Patient 2	F	..	..	..	..	..	CPC and LGG; ACC	Brain MRI; AUS, ABW	4; 7	Dead	11	81; 41
Family seven: His193Pro (c.578A>C)												
Patient 1	F	..	..	..	..	..	ACC and CPC; osteochondroma; OS¶ and OS¶; osteochondroma	AUS, ABW, and brain MRI; WBMRI; ..; WBMRI	1; 7; 7; 10	Dead	10	118; 37; 29
Family nine: c.783-2A>G												
Patient 1	..	..	..	..	..	..	Left DCIS; right IDC; left DCIS	CE; MG; prophylactic mastectomy	31; 36; 37	Alive	40	114; 46; 42
Family ten: Thr125Thr (c.375G>A)												
Patient 1	M	RMS	3	Alive	8	53	..	..	..	..	..	..
Family 11: c.376-1G>A												
Patient 1	M	RMS	3	Alive	11	95	Early glial lesion	Brain MRI	11	Alive	11	5
Family 12: Ser240Gly (c.718A>G)												
Patient 1	F	ACC	1	Alive	2	13	..	..	..	..	..	..
Family 15: Arg248Gln (c.743G>A)												
Patient 1§	F	PTC; AA	27; 31	Alive	33	82; 24	..	..	..	..	..	..
Family 16: Arg181Cys (c.541C>T)												
Patient 1	M	RMS	1	Alive	3	26	ACC; OS	WBMRI; brain MRI	2; 2	Alive	3	16; 16
Family 19: Arg158His (c.473G>A)												
Patient 1	F	ACC	2	Alive	8	70	..	..	..	..	..	..
Family 20: Pro128fs (c.384_385delTGinsC)												
Patient 1	F	GBM	10	Dead	16	70	..	..	..	..	..	..
Family 21: Trp146X (c.438G>A)												
Patient 1	M	OS	9	Alive	10	13	..	..	..	..	..	..
Family 22: Arg248Trp (c.742C>T)												
Patient 1	M	MM; LC	47; 48	Dead	50	36; 24	..	..	..	..	..	..
Patient 2	M	AML	39	Dead	39	6	..	..	..	..	..	..
Patient 3§	F	IDC	23	Dead	23	6	..	..	..	..	..	..

(Table 2 continues on next page)

	Sex	No surveillance					Surveillance					
		Tumour type	Age at diagnosis (years)*	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)	Tumour type	Mode of detection	Age at diagnosis (years)†	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)‡
(Continued from previous page)												
Patient 4	M	..	..	..	..	..	Diffuse fibrillary astrocytoma (grade II); compound dysplastic naevus with atypia; colonic tubular adenoma; superficial MFH; colonic tubular adenoma	Brain MRI; CE; CS; CE; CS	24; 25; 27; 29; 30	Alive	31	86; 74; 50; 22; 14
Patient 5	F	..	..	..	..	..	SSA	CS	25	Alive	28	38
Patient 6	F						Early glial lesion; diffuse astrocytoma (grade II)	Brain MRI; brain MRI	11; 16	Alive	18	79; 19
Family 24: Arg196X (c.586C>T)												
Patient 1§	F	Colonic adenoma; IDC	35; 37	Alive	39	55; 21	..	..	..	..	..	..
Family 25: Ala347Asp (c.1040C>A)												
Patient 1	F	High-grade pleomorphic sarcoma	19	Alive	23	43	..	..	..	..	..	..
Family 26: Arg110fs (c.329_330insGTTTCCG)												
Patient 1	F	IDC; PTC	40; 41	Alive	45	53; 51	Meningioma	Brain MRI	43	Alive	45	17
Patient 2§	F	PAC	42	Dead	44	24	..	..	..	..	..	..
Family 27: Ile195Thr (c.13344T>C)												
Patient 1	M	AA	33	Alive	38	51	..	..	..	..	..	..
Family 28												
Patient 1	M	LS; LMS; OC	46; 48; 51	Dead	52	67; 50; 12	..	..	..	..	..	..
Patient 2	M	Pleiomorphic LS	19	Dead	19	3	..	..	..	..	..	..
Family 29: Glu271Val (c.14481A>T)												
Patient 1	F	..	..	..	..	..	SCC; DCIS; BC chest wall	CE; prophylactic mastectomy; CE	34; 36; 40	Alive	43	108; 81; 36
Patient 2	F	ACC	1	Alive	11	131	..	..	..	..	..	..
Family 30: Pro177Arg (c.530C>G)												
Patient 1	F	..	..	..	..	..	Chordoma	WBMRI	17	Alive	18	10
Patient 2	F	..	..	..	..	..	DNET	Brain MRI	15	Alive	16	12
Patient 3	M	OS	17	Alive	21	49	Chondroma; CRC	WBMRI; WBMRI	20; 21	Alive	21	8; 1
Patient 4	M	Melanoma; giant cell tumour	40; 42	Alive	48	99; 82	..	..	..	..	..	..
Family 31: Arg267Trp (c.799C>T)												
Patient 1	F	MDS	52	Alive	58	68	Breast FA; SE; melanoma in situ; SSA	MRI breast; brain MRI; CE; CS	53; 56; 57; 58	Alive	58	58; 15; 4; 1
Family 32: E9+1 G>A at nucleotide 14755												
Patient 1§	F	LS; PAC	57; 58	Dead	59	22; 10	..	..	..	..	..	..
Family 33: Leu194Phe (c.580C>T)												
Patient 1§	M	LC	48	Alive	50	25	..	..	..	..	..	..

(Table 2 continues on next page)

surveillance group and 59·6% (47·2–75·2) in the 30 patients in the non-surveillance group (p=0·0132; 42 tumours in 19 individuals in the surveillance group *vs* 61 tumours in 43 individuals in the non-surveillance group; figure 1). We obtained similar survival estimates and p values using the frailty model (appendix pp 1–3).

	Sex	No surveillance					Surveillance					
		Tumour type	Age at diagnosis (years)*	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)	Tumour type	Mode of detection	Age at diagnosis (years)†	Status	Age at death or age at end of follow-up (years)	Follow-up time (months)‡
(Continued from previous page)												
Family 34: Ile251Leu (c.751A>C)												
Patient 1	F	BC; pleiomorphic sarcoma	42; 46	Dead	49	90; 40	..	..	..	..	..	..
Patient 2	M	..	..	..	..	..	RA; thyroid Hürthle cell adenoma	CS; WBMRI	16; 16	Alive	17	12; 12
Patient 3	M	Microinvasive CRC; colonic adenoma; GBM	18; 22; 22	Dead	25	80; 37; 33	..	..	..	..	..	..
Patient 4	M	Macroprolactinoma; schwannoma; colonic adenoma; AA	44; 50; 51; 54	Alive	55	127; 59; 47; 10	..	..	..	..	..	..
Family 35: del exon 1												
Patient 1§	M	Pleiomorphic high-grade sarcoma	40	Dead	42	31	..	..	..	..	..	..
Patient 2	F	DCIS; DCIS; meningioma	32; 33; 38	Alive	41	111; 96; 40	LC; RAC	WBMRI; CS	40; 41	Alive	41	13; 1
Patient 3	M	OS	12	Dead	15	28	..	..	..	..	..	..
Patient 4	F	PTB	12	Alive	18	72	..	..	..	..	..	..
Family 36: Arg273His (c.818G>A)												
Patient 1§	F	BC	16	Alive	19	46	..	..	..	..	..	..
Family 37: Ala347Asp (c.1040C>A)												
Patient 1§	F	BC; cardiac SC	49; 57	Dead	57	105; 9	..	..	..	..	..	..
Family 38: Arg282Trp (c.742C>T)												
Patient 1	F	ACA	1	Alive	6	64	..	..	..	..	..	..

TP53 pathogenic variant listed after each family. Tumour types separated by "and" represent concurrent diagnoses; those separated by a semicolon represent sequential diagnoses. M=male. MB=medulloblastoma. RMS=rhabdomyosarcoma. FS=fibrosarcoma. F=female. MFH=malignant fibrous histiocytoma. WBMRI=whole-body MRI. CE=clinical examination. AA=anaplastic astrocytoma. MDS=myelodysplastic syndrome. CBC=complete blood count. CPC=choroid plexus carcinoma. NBL=neuroblastoma. TA=thyroid adenoma. US=ultrasound. OS=osteosarcoma. AML=acute myeloblastic leukaemia. LGG=low-grade glioma. ACC=adrenocortical carcinoma. AUS=abdominal ultrasound. ABW=adrenal bloodwork. DCIS=ductal carcinoma in situ. IDC=invasive ductal carcinoma. MG=mammography. PTC=papillary thyroid carcinoma. GBM=glioblastoma multiforme. MM=malignant meningioma. LC=lung cancer. CS=colonoscopy. SSA=sessile serrated adenoma. PAC=pancreatic adenocarcinoma. LS=liposarcoma. LMS=leiomyosarcoma. OC=oesophageal cancer. SCC=squamous cell carcinoma. BC=breast cancer. DNET=dysembryoplastic neuroepithelial tumour. CRC=colorectal carcinoma. FA=fibroadenoma. SE=subependymoma. RA=rectal adenoma. RAC=rectal adenocarcinoma. PTB=phyllodes tumour of the breast. SC=sarcoma. ACA=adrenocortical adenoma. *Median age is 32 years (IQR 12–46). †Median age is 23 years (IQR 9–36). ‡Time from diagnosis to death or last follow-up or the end of the study. §Individuals who had other malignant diagnoses before study onset. ¶Tumours diagnosed symptomatically while on surveillance.

Table 2: **Clinical details and survival of carriers of germline *TP53* pathogenic variants with cancer from families with Li-Fraumeni syndrome, by surveillance group**

No single surveillance modality was predominately implicated for initial tumour detection; rather, various surveillance elements were instrumental in different individuals (figure 2). Panel 2 shows the types of tumours detected by surveillance and their grade. Two malignant tumours—synchronous osteosarcomas—presented symptomatically in one individual in the surveillance group (family seven, patient 1; table 2). In retrospect, a whole-body MRI (WBMRI) scan completed 3 months before showed early lesional changes at the site of at least one of the tumours. This individual ultimately died from metastatic relapse of disease (osteosarcoma). Two other individuals in the surveillance group have died, including one participant who died from transplant-related morbidities while receiving treatment for myelodysplastic syndrome after surviving both an adrenocortical carcinoma (before study initiation) and an anaplastic astrocytoma (family three, patient 1; table 2). The other individual had three neoplasms diagnosed by age 7·5 years—a choroid plexus carcinoma, a low-grade glioma, and an adrenocortical carcinoma (family six, patient 2; table 2). A delay in surveillance imaging (abdominal ultrasonography) occurred before the diagnosis of adrenocortical carcinoma, which nevertheless presented as a localised asymptomatic mass with a subsequent ultrasound examination. The tumour was gross totally resected without spillage and no adjuvant chemotherapy was initially administered. The patient subsequently developed distant pulmonary, brain, and bone metastases and died despite receiving several months of aggressive salvage therapy. This patient is the only patient who had a delay between planned imaging appointments of more than a few days.

We documented incidental findings from surveillance imaging. As expected, most of these findings were identified by WBMRI. In addition to non-specific

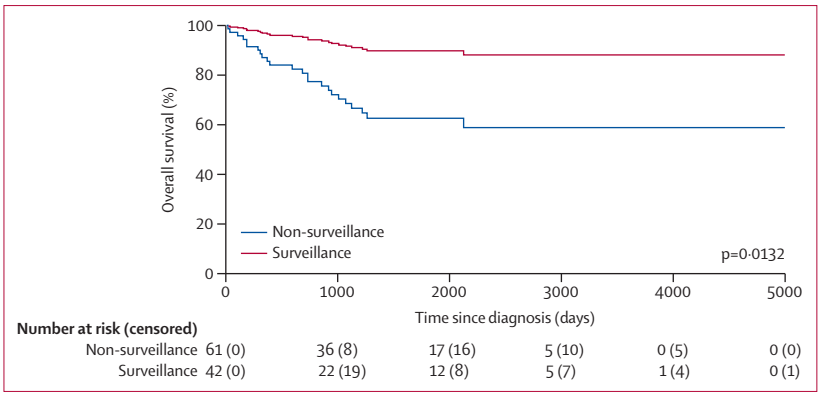

Figure 1: **Overall survival in the surveillance and non-surveillance groups**
Number at risk refers to the number of tumours, not individuals.

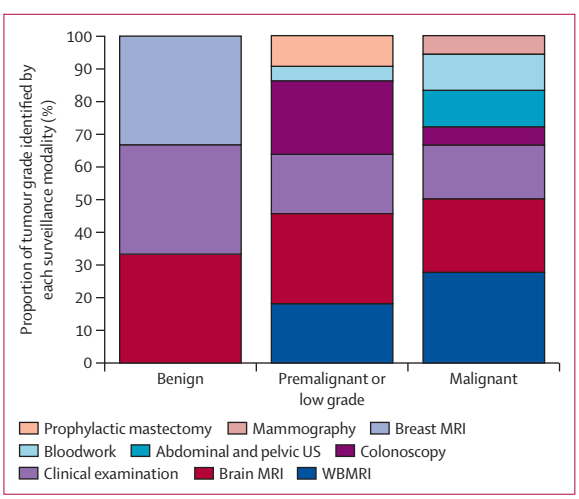

Figure 2: **Proportion of each tumour grade diagnosed by various surveillance modalities**
US=ultrasound. WBMRI=whole-body MRI.

T2-weighted hyperintensities in osseous structures and liver lesions (usually consistent with focal nodular hyperplasia), we also documented visceral (ovarian and renal) cysts. In almost all cases, we used specific dedicated interval imaging to further assess findings. A false-positive result was noted twice, both in the context of persistent abnormal musculoskeletal T2-weighted hyperintensities. Histological findings of these false-positives were consistent with a bone cyst and inflammatory changes probably attributable to local trauma. Other modalities, including brain MRI and abdominal ultrasound, yielded infrequent incidental findings.

Discussion

In this prospective observational follow-up study of a comprehensive clinical surveillance protocol (known as the Toronto Protocol) for carriers of a germline *TP53* pathogenic variant, we show persistent feasibility and effectiveness for asymptomatic tumour detection. With respect to feasibility, we observe not only that almost all patients who were in the surveillance group of the study followed the protocol closely despite the number of outpatient contacts and interventions, which, for some patients, extended over several years, but also that this compliance was maintained across the multiple centres in which patients were followed up and surveillance delays were infrequent and short. Thus, compliance with the protocol in the context of this study was good. The study findings also show the durability of enhanced long-term survival for patients whose tumours were detected by surveillance. 5 year overall survival was significantly better for individuals in the surveillance group than in the non-surveillance group. Furthermore, the results show an acceptably low number of false-positive findings in that only two patients underwent surgical procedures that subsequently revealed pathologically non-neoplastic lesions; in both cases, the patients' perioperative course was unremarkable and their recovery rapid. Only two tumours diagnosed in individuals on surveillance presented symptomatically between assessments, rendering a low proportion of false negatives.

Over an 11 year period, 40 asymptomatic tumours have been detected in 19 individuals by surveillance. Among

Panel 2: **Tumours detected by the surveillance protocol, classified by grade**

Benign
- Thyroid adenoma
- Breast fibroadenoma
- Meningioma

Premalignant or low grade
- Myelodysplastic syndrome
- Osteochondroma (three patients)
- Ductal carcinoma in situ (three patients)
- Low-grade glioma (six patients)
- Colonic or rectal adenoma (five patients)
- Dysplastic naevus
- Melanoma in situ
- Squamous cell carcinoma
- Thyroid Hürthle cell adenoma

Malignant
- Malignant fibrous histiocytoma (two patients)
- Osteosarcoma
- Adrenocortical carcinoma (three patients)
- Invasive ductal carcinoma
- Breast cancer (chest wall)
- Choroid plexus carcinoma (two patients)
- Chordoma
- Ependymoma
- Colorectal carcinoma (two patients)
- Lung carcinoma

Tumours are in one patient unless otherwise stated. Does not include two interval tumours missed by the surveillance protocol.

these tumours are aggressive, malignant lesions whose early identification afforded definitive surgical resection and less exposure to systemic cytotoxic therapies than without surveillance. Sparing of carriers of pathogenic *TP53* variants to exposure to adjuvant treatments is an important management goal because of the increased likelihood of multiple independent tumours arising over the individuals' lifetimes and because of the potential of such therapies to cause accelerated tumour onset. A substantial proportion of tumours identified by surveillance were low-grade or premalignant lesions. Although if and when these lesions would transform is impossible to predict, this latency cannot be assumed to be similar to that observed for sporadic cases in non-carriers. Given the potential for accelerated transformation in the setting of an underlying *TP53* germline pathogenic variant, we recommend early identification, close monitoring, and rapid management of such tumours. We posit that by doing surveillance in this unique patient population over time, we will identify malignant tumours in their early stages or early biological grades, which stands to affect survival. This notion is supported by the extensive number of low-grade or premalignant lesions detected by surveillance and indicates that inclusion of these tumours is in fact a reflection of the benefits of the surveillance intervention.

Over the course of this extended follow-up, three patients in the surveillance group died. Each had three tumour diagnoses in childhood; in carriers of highly penetrant *TP53* pathogenic variants, early death might be inevitable. Still, important circumstances surrounding each of these cases require specific consideration. In two of the individuals, adherence to the surveillance protocol was compromised: in patient 2 from family six, the size of the adrenocortical carcinoma would have probably been smaller at diagnosis had it been detected earlier. This individual ultimately died of a metastatic relapse of adrenocortical carcinoma. Notwithstanding this outcome, this case underscores the complex medical and psychosocial issues that frequently dissuade patients, families, and practitioners from starting aggressive therapy as each malignancy arises. Although failure of protocol execution might compromise its effectiveness for early tumour identification and thus definitive tumour management, the ability to maintain protocol compliance over many years can be perceived as daunting or lead to so-called burnout.[2] In patient 1 from family seven, an early lesion at the site of an aggressive osteosarcoma was not reported with the preceding WBMRI. This finding highlights the importance of exercising a high index of suspicion for any lesion identified during the course of surveillance, while acknowledging the lower sensitivity of WBMRI compared with targeted MRI for detection of small lesions. Management of such lesions is challenging, as false-positive findings for which aggressive intervention is pursued could cause harm. However, short-interval follow-up with dedicated imaging is a prudent approach. The third participant who died in the surveillance group (family three, patient 1) died from allogeneic bone marrow transplant treatment-related complications.

Since publication of our initial surveillance study,[1] other surveillance research protocols have been initiated, consisting of the French LIFSCREEN project (NCT01464086), the SIGNIFY study in the UK (NCT01737255), the Dana-Farber Cancer Institute study in Boston (MA, USA),[3] the Australian Surveillance Study in Multi-Organ Cancer Prone Syndromes (ACTRN12613000987763), and a surveillance programme at the US National Institutes of Health (NCT01443468). Although the outcomes of these studies are highly anticipated, notable shortfalls, which will limit their accuracy and generalisability, should be noted. First, most exclude paediatric participants. Feasibility and effectiveness of surveillance need to be assessed in this age group given that 30–40% of all Li-Fraumeni syndrome-associated tumours occur in childhood,[4,5] with the associated greatest potential life-years gained from improved cancer outcomes. Failure to detect early disease in this age group would also prolong their exposure to the burden of late effects related to aggressive therapy. Second, the short duration of some of these studies will preclude a valid assessment of the efficacy of the planned intervention since yearly cancer incidence is low. Finally, many of these trials are using WBMRI in isolation to assess participants. Findings from our study show that WBMRI is probably insufficient on its own as a surveillance modality; indeed, the multimodal nature of this protocol underlies its effectiveness for asymptomatic tumour detection.

Nonetheless, the anatomically diverse nature of tumours associated with Li-Fraumeni syndrome suggests whole-body imaging as an important component of surveillance. WBMRI has been explored for tumour surveillance in other cancer predisposition syndromes, including *SDH*-associated hereditary paraganglioma and hereditary retinoblastoma.[6,7] A retrospective study[8] reviewed 50 surveillance WBMRI scans obtained over a 5 year period from 24 individuals, including ten children with Li-Fraumeni syndrome. Although the study numbers are small, the authors show a high sensitivity and negative predictive value of WBMRI. However, their WBMRI technique was more complex than the short WBMRI technique used in this study (appendix p 4). In our experience to date, WBMRI has successfully identified asymptomatic aggressive carcinomas and sarcomas, as well as premalignant lesions. Furthermore, most incidental findings were resolved by clinical correlation or dedicated imaging, whereas only two lesions were biopsied, resulting in a low number of false positives.

Other whole-body modalities, namely ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET-CT, have been assessed for surveillance of patients with Li-Fraumeni syndrome.[9,10] The studies documented a 10–20% detection of malignant disease; however, the number of false positives documented

in one study,[9] and the repeated irradiation exposure associated with PET-CT scanning, limit the applicability of this modality in the Li-Fraumeni syndrome population.

This study does have certain limitations that deserve consideration. The design is non-randomised, and participant self-selection could be a source of bias. Although asymptomatic tumour detection should not be affected by participant behaviour, we cannot exclude the possibility that individuals opting out of surveillance might have delayed medical attention, even once symptoms arose, or might have been less adherent to surveillance recommendations than might have been those who agreed to surveillance. This important behavioural uncertainty is worthy of exploration in that it could identify personality traits and point to behaviour modification strategies to engage at-risk patients with the value of surveillance and attention to potential symptoms. Although lead-time bias inherent to many observational screening studies could skew the results of our survival estimates, we included mortality ratios, which should be unaffected by this bias. Although we have not assessed the potential psychological effect of surveillance, investigators of one study[11] report that individuals with Li-Fraumeni syndrome see value in surveillance and feel equipped with a sense of control and security. Investigators of another study[12] have reviewed the balance of psychological benefit and burden across various cancer predisposition syndromes and reported more variable results, indicating a need for further investigation.

We have updated our original surveillance recommendations[1] on the basis of evidence accrued since its publication and in this study present an updated 2016 version (panel 1). We reported a 50% prevalence of germline *TP53* pathogenic variants in children with adrenocortical carcinoma[13] and a notable age dependency of *TP53* positivity. On this basis, we suggest that surveillance for adrenocortical carcinoma can be discontinued at age 40 years. On the basis of the fact that concentrations of biomarkers of adrenocortical dysfunction do typically rise with increased tumour burden, blood tests are used for early adrenocortical carcinoma detection. We also suggest addition of a urinary cortisol measurement, where feasible, given that 5% of adrenocortical carcinomas present with Cushing's syndrome alone and approximately 30% present with a mixed profile.[14]

The recommendations for breast cancer surveillance in susceptible groups continue to evolve. One study[15] compared the diagnostic performance of breast MRI, mammography, and breast ultrasound (alone and in combination) in women with *BRCA1* or *BRCA2* germline pathogenic variants or a high (>20%) familial risk of breast cancer. The study's findings showed a better sensitivity of breast MRI (90%) than either mammography or ultrasound (each <40%). The specificity of MRI (89%) was worse than that of both mammography and ultrasound (both 97%), and the positive predictive value of MRI (20%) was also worse than that of both mammography (28%) and ultrasound (27%). Findings from other studies[16,17] showed MRI to be twice as sensitive as mammography, with slightly less specificity and possibly detection at earlier-stage breast cancer. Taking these findings into account, we recommend annual breast MRI and mammography, alternating with annual WBMRI, for surveillance in women with Li-Fraumeni syndrome. Breast ultrasound can be added as clinically indicated, but should not be used for screening purposes. Further prospective studies in Li-Fraumeni syndrome patients might better refine the most effective method of breast cancer screening.

Finally, screening for colorectal cancer remains an important component of the management of individuals with Li-Fraumeni syndrome; almost 3% of patients meeting classic Li-Fraumeni syndrome criteria were diagnosed with early-onset (age <50 years) colorectal cancer in a registry study.[18] Although the National Comprehensive Cancer Network advises consideration of colonoscopy every 2–5 years,[19] we suggest a prudent approach of biennial colonoscopies until data for colon cancer pathogenesis and progression in the setting of germline *TP53* pathogenic variants become clearer. Although early-onset gastric cancer is also reported in Li-Fraumeni syndrome families,[20] particularly in Asian patients,[21] an absence of efficacy data for endoscopic detection precludes its incorporation into these surveillance recommendations. Notwithstanding the modifications of the protocol to improve sensitivity and specificity of early tumour detection, attention to financial and safety costs should continue to be considered. As we noted in our 2011 report,[1] further study of the balance of risks associated with anaesthesia for young children having an MRI should be considered against the risk of missing a tumour; quantifying these risk comparisons is challenging, but we highlight the need to use anaesthesia as judiciously as possible. An economic effect of surveillance will be important to establish the costs saved by elimination of the need for multimodal treatment of malignant cancers. The reduction in cost and increase in life-years gained associated with reduced therapy and increased survival from a surveillance-detected low-grade or low-stage tumour would seem intuitive; however, a detailed financial cost-benefit analysis would be helpful to illustrate this point.

As large-scale genomic studies are completed, insights will be gained into the contribution of genetic modifiers and low-penetrance alleles to further tailor surveillance approaches. Studies of unselected patients with adrenocortical carcinoma and early-onset colorectal cancer give support to the notion that low-penetrance alleles and attenuated personal and family cancer histories might change the definition and management of individuals with germline *TP53* pathogenic variants;[13,22] in fact, this notion has been suggested by some groups.[23] Looking forward, new approaches to surveillance in cancer-susceptible

populations using emerging biomarkers, such as circulating tumour DNA, will be an exciting area of research. At present, however, we advocate regular, comprehensive surveillance of carriers of germline *TP53* pathogenic variants on the basis of these data showing early tumour detection and sustained survival benefit.

Contributors

AV and DM designed the study; collected, analysed, and interpreted data; and drafted and critically revised the manuscript. JDS, JDW, RHK, UT, JLF, and WK collected, analysed, and interpreted data and critically revised the manuscript. M-LCG developed the whole-body MRI (WBMRI) technique, interpreted the WBMRI data, and critically revised the manuscript. MT interpreted the WBMRI data and critically reviewed the manuscript. BG, HD, ANo, ANa, and AS collected data and critically revised the manuscript. DS did the statistical analysis and interpreted data.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

This work is supported by grants from the Canadian Institutes for Heath Research, Canadian Cancer Society, Terry Fox Research Institute, and SickKids Foundation to DM and from the Soccer for Hope Foundation to JDS and JLF. AV was funded, in part, by a scholarship from the Canadian Institutes for Health Research. We would like to thank Shiyi Chen for her valuable additional statistical support.

References

1 Villani A, Tabori U, Schiffman J, et al. Biochemical and imaging surveillance in germline *TP53* mutation carriers with Li-Fraumeni syndrome: a prospective observational study. *Lancet Oncol* 2011; **12:** 559–67.

2 Jhaveri AP, Bale A, Lovick N, et al. The benefit and burden of cancer screening in Li-Fraumeni syndrome: a case report. *Yale J Biol Med* 2015; **88:** 181–85.

3 Li-Fraumeni whole-body MRI study. http://www.dana-farber.org/uploadedFiles/Library/adult-care/treatment-and-support/centers-and-programs/cancer-genetics-and-prevention/li-fraumeni-whole-body.pdf (accessed Aug 17, 2015).

4 Nichols KE, Malkin D, Garber JE, Fraumeni JF Jr, Li FP. Germ-line *p53* mutations predispose to a wide spectrum of early-onset cancers. *Cancer Epidemiol Biomarkers Prev* 2001; **10:** 83–87.

5 Le Bihan C, Moutou C, Brugières L, Feunteun J, Bonaïti-Pellié C. ARCAD: a method for estimating age-dependent disease risk associated with mutation carrier status from family data. *Genet Epidemiol* 1995; **12:** 13–25.

6 Jasperson KW, Kohlmann W, Gammon A, et al. Role of rapid sequence whole-body MRI screening in *SDH*-associated hereditary paraganglioma families. *Fam Cancer* 2014; **13:** 257–65.

7 Friedman DN, Lis E, Sklar CA, et al. Whole-body magnetic resonance imaging (WB-MRI) as surveillance for subsequent malignancies in survivors of hereditary retinoblastoma: a pilot study. *Pediatr Blood Cancer* 2014; **61:** 1440–44.

8 Anupindi SA, Bedoya MA, Lindell RB, et al. Diagnostic performance of whole-body MRI as a tool for cancer screening in children with genetic cancer-predisposing conditions. *AJR Am J Roentgenol* 2015; **205:** 400–08.

9 Nogueira ST, Lima EN, Nobrega AF, et al. (18)F-FDG PET-CT for surveillance of Brazilian patients with Li-Fraumeni syndrome. *Front Oncol* 2015; **5:** 38.

10 Masciari S, Van den Abbeele AD, Diller LR, et al. F18-fluorodeoxyglucose-positron emission tomography/computed tomography screening in Li-Fraumeni syndrome. *JAMA* 2008; **299:** 1315–19.

11 Lammens CR, Bleiker EM, Aaronson NK, et al. Regular surveillance for Li-Fraumeni syndrome: advice, adherence and perceived benefits. *Fam Cancer* 2010; **9:** 647–54.

12 Gopie JP, Vasen HF, Tibben A. Surveillance for hereditary cancer: does the benefit outweigh the psychological burden?—a systematic review. *Crit Rev Oncol Hematol* 2012; **83:** 329–40.

13 Wasserman JD, Novokmet A, Eichler-Jonsson C, et al. Prevalence and functional consequence of *TP53* mutations in pediatric adrenocortical carcinoma: a children's oncology group study. *J Clin Oncol* 2015; **33:** 602–09.

14 Michalkiewicz E, Sandrini R, Figueiredo B, et al. Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. *J Clin Oncol* 2004; **22:** 838–45.

15 Riedl CC, Luft N, Bernhart C, et al. Triple-modality screening trial for familial breast cancer underlines the importance of magnetic resonance imaging and questions the role of mammography and ultrasound regardless of patient mutation status, age, and breast density. *J Clin Oncol* 2015; **33:** 1128–35.

16 Lauby-Secretan B, Scoccianti C, Loomis D, et al. Breast-cancer screening—viewpoint of the IARC Working Group. *N Engl J Med* 2015; **372:** 2353–58.

17 Warner E, Hill K, Causer P, et al. Prospective study of breast cancer incidence in women with a *BRCA1* or *BRCA2* mutation under surveillance with and without magnetic resonance imaging. *J Clin Oncol* 2011; **29:** 1664–69.

18 Wong P, Verselis SJ, Garber JE, et al. Prevalence of early onset colorectal cancer in 397 patients with classic Li-Fraumeni syndrome. *Gastroenterology* 2006; **130:** 73–79.

19 National Comprehensive Cancer Network. Genetic/familial high-risk assessment: breast and ovarian. March 15, 2016. http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf (accessed March 18, 2016).

20 Masciari S, Dewanwala A, Stoffel EM, et al. Gastric cancer in individuals with Li-Fraumeni syndrome. *Genet Med* 2011; **13:** 651–57.

21 Ariffin H, Chan AS, Oh L, et al. Frequent occurrence of gastric cancer in Asian kindreds with Li-Fraumeni syndrome. *Clin Genet* 2015; **88:** 450–55.

22 Yurgelun MB, Masciari S, Joshi VA, et al. Germline mutations in patients with early-onset colorectal cancer in the Colon Cancer Family Registry. *JAMA Oncol* 2015; **1:** 214–21.

23 Bougeard G, Renaux-Petel M, Flaman JM, et al. Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. *J Clin Oncol* 2015; **33:** 2345–52.