European Reference Network
for rare or low prevalence complex diseases

Network Genetic Tumour Risk Syndromes (ERN GENTURIS)

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European Reference Network on GENetic TUmour RIsk Syndromes

Guidelines for the identification of individuals who should be tested for germline disease-causing *TP53* variants and for their subsequent clinical management

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1. SURVEILLANCE PROTOCOL IN CARRIERS OF *TP53* DISEASE-CAUSING VARIANTS – GUIDELINE SUMMARY

This guideline has been drawn from the best available evidence and the consensus of experts in this area and it is regularly updated to reflect changes in evidence. The expectation is that clinicians will follow this guideline, unless there is a compelling clinical reason specific to an individual patient not to.

Table 1. Summary of the surveillance protocol

Exam	Periodicity	Age to start	Age to end	Condition	Evidence
Clinical examination	Annual	18 years	-		Moderate
Whole-Body MRI	Annual	Birth	-	High cancer risk *TP53* variant	Moderate
		18 years	-		Strong
Breast MRI	Annual	20 years	Until 60-70 years		Strong
Brain MRI*	Annual	Birth	18 years	High cancer risk *TP53* variant	Moderate
		18 years	Until 60-70 years		Moderate
Abdominal ultrasound	Every 6 months	Birth	Until 18 years		Strong
Urine steroids	Every 6 months	Birth	-		Weak

*The first scan should be conducted with Gadolinium enhancement; brain MRI should alternate with the WBMRI, so that the brain is imaged at least every 6 months.

2. INTRODUCTION

From Li-Fraumeni syndrome to heritable *TP53*-related cancers

Germline alterations of *TP53,* encoding the p53 protein, cause inherited cancers which are diverse, in their type and age of onset. The p53 protein normally acts as a guardian of the genome, and if DNA damage occurs, p53 triggers a response based on transcription regulation of numerous genes involved in cell cycle, DNA repair, apoptosis, senescence and metabolism. Heterozygous germline *TP53* alterations were initially identified in the **Li-Fraumeni syndrome** (LFS), described in 1969 by Frederick Li and Joseph Fraumeni (Li and Fraumeni, 1969; Malkin et al.,1990; Srivastava et al., 1990). LFS is characterized by a **strong familial aggregation of cancers**, **early-onset of tumours** and **wide tumour spectrum,** including the so-called core LFS cancers: *i.e.* **soft-tissue sarcomas** (STS), **osteosarcomas** (OS), **adrenocortical carcinomas** (ACC), **central nervous system** (CNS) **tumours** and very early-onset **female breast cancers**. Germline alterations of *TP53* are mainly identified among children with cancers or among adult females with breast cancers, in both cases often **without familial history of cancer**. For this reason, the initial description of LFS has drastically changed through time, as well as our perception of cancers related to germline alterations of *TP53* (Gonzales et al., 2009; Ruijs et al., 2010; Bougeard et al., 2015). The **diversity of clinical presentations** associated with germline *TP53* alterations justifies the expansion of the LFS concept to a wider cancer predisposition syndrome designated heritable ***TP53*-related cancers (h*TP53*rc)**. Criteria for germline *TP53* variant screening named "Chompret criteria" have been adapted several times and the recently adjusted and contemporary criteria are depicted in table 2 The use of these criteria in clinical practice allows recognition of individuals at risk of developing **h*TP53*rc** (Bougeard et al., 2015). Regardless of familial history, the detection rate of disease causing germline *TP53* variants has been estimated to be: 50% in children presenting with ACC or choroid plexus carcinomas; up to 73% in children with rhabdomyosarcoma of embryonal anaplastic subtype (Hettmer et al., 2014; Wasserman et al., 2015; Bougeard et al., 2015), and; between 3.8% and 7.7% in females with breast carcinoma before 31 years of age (Fortuno et al. 2018). These data demonstrate that **familial history of cancer** in not the most important feature among *TP53* disease causing variant carriers, and therefore **should not be mandatory when considering genetic testing of *TP53***.

The contribution of ***de novo* variants** to h*TP53*rc have been estimated to be **between 7-20%** and approximately one fifth of these *de novo* mutations occur during embryonic development, resulting in **mosaics** (Gonzalez et al., 2009; Renaux-Petel et al., 2018).

Table 2. Chompret criteria for *TP53* testing (Bougeard et al., 2015)

Familial presentation: Proband with a *TP53* core tumour* before 46 years AND At least one first- or second-degree relative with a core tumour before 56 years; or **Multiple primitive tumours:** Proband with multiple tumours, including 2 *TP53* core tumours*, the first of which occurred before 46 years, *irrespective of family history;* or **Rare tumours:** Patient with adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma of embryonal anaplastic subtype*, irrespective of family history;* or **Very early-onset breast cancer:** Breast cancer before 31 years*, irrespective of family history.*

**TP53* core tumours: premenopausal breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumour, adrenocortical carcinoma

Interpretation of germline *TP53* variants

Because the *TP53* gene is currently included in several cancer gene panels broadly used in genetic testing, the number of *TP53* tests performed in non-suggestive clinical situations has exponentially increased. This leads recurrently to the detection of incidental germline *TP53* variants. As in other genetic conditions, when a germline variant is detected in a cancer patient, it is critical to demonstrate whether the variant is disease-causing and corresponds either to a class 5 (pathogenic) or a class 4 (likely pathogenic) variant, according to the international guidelines of the American College of Medical Genetics (ACMG), or not. Since the distinction between class 5 and class 4 variants is particularly subtle for *TP53* variants, these variants will be designated in the current ERN guideline as "**disease-causing variants**".

The most common consequence of germline variants causing h*TP53*rc is the functional inactivation of the protein. Whereas the interpretation of *TP53* variants predicted to result into loss of function, such as nonsense or frameshift deletions or insertions is usually obvious,

the interpretation of missense variants, representing the majority, is often challenging and requires specific expertise.

Classification of *TP53* missense variants, in agreement with the ACMG guidelines, is based on several items including *phenotypical data* (previously identified in patients fulfilling the Chompret criteria; presentation of the patient fulfilling the Chompret criteria); *frequency of the variant in the general population*, as reported the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/), *bioinformatics* and *in silico predictions* using different algorithms, and *functional analyses* of the variants performed using different *in vitro* assays performed either in yeast or cultured cells (Kato et al., 2003; Zerdoumi et al. Hum Mol Genet. 2017; Giacomelli et al. 2018; Kotler et al. Mol Cell Oncol. 2018; http://p53.iarc.fr/). The outcome of the integrative analysis of these parameters allows the allocation of *TP53* variants into the different ACMG classes.

Cancer risk associated with germline *TP53* variants

A challenge when dealing with *TP53* variant carriers is to estimate **the cancer risk** or **penetrance** associated with each *TP53* variant, and this cancer risk **has recently been revisited**. Indeed, the global penetrance of germline disease-causing *TP53* variants was initially calculated using information mainly from familial cases (Chompret et al 2000). The exclusion of non-familial cases likely resulted in an ascertainment bias and an **overestimation of disease penetrance** (de Andrade et al, 2019). Furthermore, the **penetrance** of germline disease-causing *TP53* variants is **variable**. One factor explaining the variability of this penetrance is **the type of the variant** itself: Some of the p53 proteins bearing missense mutations are classified as **dominant-negative** due to their ability to complex and reduce the transcriptional activity of wild-type p53 protein, producing malfunctioning or **non-functioning p53 tetramers**. These **dominant-negative missense *TP53* variants** are usually detected in families with childhood cancers and are generally **more penetrant**. In contrast, null variants (frameshift or nonsense variants, splicing variants, large genomic rearrangements, and non-dominant-negative missense variants), are predominantly identified in families with mostly adult cancers and have a lower disease penetrance (Bougeard et al. 2015). A remarkable example of a low penetrant, but still disease-causing variant, is the non-dominant-negative

missense p. Arg337His variant, present in 0.3% of the population from Southern Brazil and associated to a founder effect (Figueiredo et al. 2006; Achatz et al., 2007; Palmero et al., 2008). The difference in the clinical severity between dominant-negative missense variants and the remaining ones is explained by a difference in their biological impact on the p53 transcriptional activity. Indeed, measurement of the transcriptional response to DNA damage in cells harbouring heterozygous *TP53* variants, has shown that dominant-negative missense variants have a more drastic impact on p53 DNA binding and transcriptional response to DNA damage, than the other types of heterozygous alterations (Zerdoumi et al., 2017). The clinical annotation of the variants and updated functional data allows, progressively, dichotomizing disease-causing *TP53* variants in "**high cancer risk"** and **"low cancer risk"** alleles. The **phenotypic variability observed within the same family** (e.g. a child affected with cancer and the parent, carrier of the same variant, being not affected in childhood) strongly supports the existence of **genetic modifying factors** and their identification represents, at the present time, a top priority in the field. It is more and more evident that phenotypic expression in carriers of *TP53* disease-causing variants is dependent on environmental factors, as germline *TP53* variants may turn p53 into a protein permissive to oncogenic stress.

<u>The impact of radio and chemotherapy in the development of second primary tumours</u>

Germline *TP53* variant carriers have a **remarkably high incidence** of **second primary tumours**, which may occur in more than 40% of *TP53* variant carriers (Bougeard et al. 2015; Mai et al., 2016). Second primary tumours often develop after the exposure of *TP53* variant carriers to radio and/or chemotherapy treatments. The demonstration of the contribution of radiotherapy and conventional chemotherapy to the development of second primary tumours in these carriers came from consistent observations of sequential development of multiple tumours after the treatment of a first one and the development of tumours within the radiotherapy field (Bougeard et al., 2015). A cause-effect was strongly supported by studies of the impact of chemotherapy and radiotherapy in mutant *TP53* lymphocytes and LFS mouse models (Kasper et al., 2018).

Surveillance protocols

Surveillance protocols for carriers bearing disease-causing *TP53* variants have recently been elaborated in the framework of an international consortium coordinated by Canadian and US teams (Villani et al., 2016; Kratz et al., 2017). These protocols indicate that such carriers should undergo **abdominal ultrasound** every 3-4 months, **annual whole-body MRI (WBMRI**) and **annual brain MRI** from **the first year of life**. Additionally, female carriers should undergo annual **breast MRI** from the age of 20 years onwards**.** After the application of these surveillance protocols, several international studies have confirmed the efficiency of WBMRI, with an overall estimated detection rate of 7% for new and localized primary cancers (Ballinger et al., 2017; Caron et al. 2017; Ruijs et al., 2017; Saya et al., 2017; Bojadzieva et al., 2018; O'Neill et al., 2018; Paixao et al., 2018).

This guideline has been put together by members of the ERN GENTURIS in order to integrate the available information with clinical utility for the management of patients with heritable *TP53*-related cancers (h*TP53*rc).

3. AIMS

The **h*TP53*rc** Guideline Development Group has prepared this guideline document to assist health care professionals in the evidence-based diagnosis and surveillance of **cancer-free individuals** and **cancer patients** who carry **germline disease-causing *TP53* variants**.

Clinical guidelines are statements to support decision making, based on systematically evaluated evidence for a specified clinical circumstance. Whilst these clinical guidelines are based on the latest published evidence, care of each individual remains first and foremost the responsibility of their treating medical professionals. Decisions for care should always be based on the individual needs, person preferences and individual circumstances of each patient. Clinical guidelines should support clinical decision making, but never replace clinical professionals. Guidelines present recommendations based on expert opinion and published evidence and are not mandates. **These guidelines do not signify nor intend to be a legal standard of care**. This is particularly critical for h*TP53*rc, considering the diversity of clinical expression related to germline *TP53* variants.

4. SCOPE & PURPOSE - OVERALL OBJECTIVES OF THE GUIDELINE

The scope of this guideline is to agree upon and define (i) the cancer patients and cancer-free individuals, who should be tested for germline *TP53* variants, and (ii) the methods and frequency for screening and surveillance of **individuals** with a **germline disease-causing *TP53* variant**.

Diagnosis of **h*TP53*rc** is mainly performed by cancer geneticists, adult and paediatric oncologists. **h*TP53*rc** is difficult to be recognized by these and other clinicians, due to the wide range of clinical presentations and the great variability in age of tumour-onset between families or within the same family. This complexity most likely supports the existence of still undefined modifier genetic, epigenetic and environmental factors. **Germline disease-causing *TP53* variants** can be detected in **cancer patients either with** or **without familial history of cancers**. As mentioned above, this is most likely explained by **incomplete penetrance** and by the fact that a significant fraction of cases is caused by ***de novo*** germline *TP53* variants.

Individuals carrying **disease-causing *TP53* variants** have a **high risk** of developing **multiple primary cancers** in their lifetime. Once these individuals develop their first tumour, treatment with radiotherapy and genotoxic chemotherapies contribute to increase their risk to develop other primary cancers. Therefore, **identification of a disease-causing *TP53* variant in a cancer patient is important before initiating the treatment.** This should lead not only to the **prioritization of surgical treatments** but also, **if possible**, to **avoid radiotherapy** and consider **the use of non-genotoxic chemotherapies, as a sensible alternative**. For instance, in young women with breast cancer occurring before 31 years of age, or in children with rhabdomyosarcoma of anaplastic subtype, *TP53* testing should be performed before the initiation of the treatment, and if a germline disease-causing ***TP53*** variant is identified, radiotherapy should, if possible, be avoided.

Considering the diversity of tumours caused by germline *TP53* variants, the most appropriate imaging exam in carriers appears to be the **annual WBMRI**, given the high efficiency of this strategy in early tumour detection, reported multiple times after 2016. According to the recently recommended protocols (Villani et al., 2016; Kratz et al., 2017), this surveillance should be initiated **after birth** and also include **abdominal ultrasound** every 3-4 months, **brain MRI** every year, and **breast MRI** every year in female carriers after 20 years of age. Considering the wide age-range of tumour-onset observed in *hTP53rc,* the challenge is to determine **the most appropriate age for intimating such a surveillance.**

Health care questions

It is critical to define the key clinical questions regarding genetic testing and cancer surveillance, when dealing with individuals and/or patients bearing germline *TP53* variants that are associated with increased cancer risk. These questions should address the organ(s) to be screened during surveillance, the modality to be used for cancer screening, the age at which screening for each cancer should be initiated, and the periodicity of surveillance for each cancer type.

Key clinical questions include, but are not restricted, to:

- Identify **which patients** with either sporadic or familial cancers **should be tested for germline variants in the *TP53* gene**, considering the clinical heterogeneity of h*TP53*rc and absence of specific phenotypes.
- Outline **the need of psychosocial support** in these patients and families.
- Identify geographical areas where **uncertainties** exist regarding **consensus recommendations** and gaps in evidence that are essential to be addressed in **future research**.

Main target population

All individuals with a germline disease-causing *TP53* variant. This population includes:

Cancer patients with:

- Certain types of childhood cancers;
- Certain types of multiple cancers;
- Very-early breast cancer occurring in females (before 31 years of age);
- Familial history of certain cancers.

***Cancer-free individuals* in the context of pre-symptomatic testing:**

- Unaffected adults belonging to families where a germline disease-causing *TP53* variant has been identified;
- Unaffected children, belonging to families where a germline disease-causing *TP53* variant associated to a high cancer risk has been identified;
- Prenatal testing which is implemented in certain European countries.

5. KEY FINDINGS & RECOMMENDATIONS (INCL. DIFFERENT MANAGEMENT OPTIONS)

Cancer Patient Recommendations	
Recommendation 1	All patients who meet the modified "**Chompret Criteria**" **should** be tested for germline *TP53* variants: • *Familial presentation*: proband with a *TP53* core tumour (breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumour, adrenocortical carcinoma) before 46 years AND at least one first- or second-degree relative with a core tumour before 56 years; or • *Multiple primitive tumours*: proband with multiple tumours, including 2 *TP53* core tumours, the first of which occurred before 46 years, irrespective of family history; or • *Rare tumours*: patient with adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history; or • *Very early-onset breast cancer*: Breast cancer before 31 years, irrespective of family history
Recommendation 2	Children and adolescents **should** be tested for germline *TP53* variants **if presenting with**: • **Hypodiploid acute lymphoblastic leukemia (ALL)**; or • Otherwise unexplained ***sonic hedgehog*-driven medulloblastoma***; or • **Jaw osteosarcoma***; or • **Periosteal osteosarcoma***
Recommendation 3	Patients who develop a **second primary core *TP53* tumour**, within the **radiotherapy field**, **should** be tested for germline *TP53* variants
Recommendation 4	**a**. Patients **older than 46 years** presenting with **breast cancer** without personal or familial history fulfilling the "Chompret Criteria" **should not** be tested for germline *TP53* variants
	b. Any patient presenting with **isolated breast cancer** and not fulfilling the **"Chompret Criteria"**, in whom a disease-causing *TP53* variant has been identified, **should** be referred to an **expert multi-disciplinary team** for discussion
Recommendation 5	**Children with any cancer** from **southern** and **south-eastern Brazilian** families **should be tested** for the **p.R337H Brazilian** founder germline *TP53* variant

* These parts of the recommendation should be supported by publications before the release of the guidelines.

Pre-symptomatic Testing Recommendations	
Recommendation 6	**Adult first-degree relatives** of individuals with germline disease-causing *TP53* variants **should** be offered testing for the same germline *TP53* variant
Recommendation 7	**The testing in childhood,** from birth, of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **high cancer risk *TP53* variant conferring a high cancer risk in childhood**: • The index case has developed a childhood cancer; or • Childhood cancers have been observed within the family; or • This variant has already been detected in other families with childhood cancers; or • This variant corresponds to a dominant-negative missense variant
Recommendation 8	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should not be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **low cancer risk *TP53* variant** and **does not confer a high cancer risk in childhood**: • The index case has not developed a childhood cancer; and • Childhood cancers have not been observed within the family; and • This variant has not already been reported in other families with childhood cancers; and • This variant does not correspond to a dominant-negative missense variant
Recommendation 9	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be discussed with their parents** if cancers have occurred in early adulthood (before the age of 25 years) within the family, or if there is **insufficient evidence in the databases or registries to determine the childhood cancer risk.** This discussion should address the burden, and uncertain benefits, of surveillance in childhood, before a decision is made whether to test the child for germline disease-causing *TP53* variants.

Surveillance recommendations in carriers of germline disease-causing *TP53* variants	
Recommendation 10	**In children**, **clinical examination**, with specific attention to signs of virilization or early puberty, and measurement of arterial hypertension should be performed in children **every 6 months**
	In adults, **clinical examination should** be performed **annually**
Recommendation 11	In adults, **WBMRI should** be conducted **annually**
Recommendation 12	In individuals with **high cancer risk *TP53* variants, WBMRI** without Gadolinium enhancement, **should** be conducted **annually, from birth**
Recommendation 13	In **female individuals, breast MRI** with Gadolinium enhancement, **should** be conducted **annually, from 20 years** onwards
Recommendation 14	**In children,** from **birth**, and in **adolescents** (< 18 years), **abdominal ultrasound** for the detection of adrenocortical carcinoma (ACC) **should** be conducted at least **every 6 months**
Recommendation 15	**When abdominal ultrasound does not allow a proper imaging of the adrenal glands, measurement** of urine **steroids**, for detection of ACC, should **probably be conducted** at least **every 6 months**
Recommendation 16	**In adults until 50 years, brain MRI should** be conducted **annually**
Recommendation 17	In individuals with **high cancer risk *TP53* variants, brain MRI should** be conducted **from birth**, **annually**
Recommendation 18	If surveillance includes brain MRI, at **least the first** (prevalence) scan **should** be conducted using **dedicated brain MRI** with Gadolinium enhancement
Recommendation 19	If surveillance includes annual **brain MRI,** this should alternate with the **WBMRI**, so that the brain is imaged at least every 6 months
Recommendation 20	**Colonoscopy** should be performed, **from 18 years,** every **5 years, only** if the carrier received **abdominal radiotherapy** for the treatment of a previous cancer, or if there is a **familial history of colorectal** tumours suggestive of an increased genetic risk

6. STAKEHOLDER INVOLVEMENT - TARGET USERS OF GUIDELINES:

Guideline Development Group

The **ERN Cancer Surveillance Guideline for patients with heritable *TP53*-related cancers** (h*TP53*rc) was established by molecular and clinical geneticists and clinicians with expertise in paediatrics, oncology, or radiology, as well as affected individuals and parent representatives. Although the guidelines are written primarily for geneticists and oncologists, they can also be used by other physicians, patients or other interested parties.

The Guideline Development Group was supported by a core writing group of ERN GENTURIS HCP Members from different Member States and who are recognized experts and specialized in molecular oncobiology and/or clinical practice and/or in the diagnosis and management of heritable *TP53*-related cancers.

Approach to secure views and preference of target population

ERN GENTURIS Heritable *TP53*-Related Cancer Guideline Development Group was supported by a Patient Advisory Group of six affected individuals and parent representatives that have experience with the heritable *TP53*-related cancer syndrome. The Core Writing Group leads had joint meetings with the Patient Advisory Group to integrate the discussions between the two groups.

Involving the patient and parent representatives in the development of these guidelines and in the Guideline Development Group helped to ensure that:

- the questions addressed are relevant to them and will make a positive impact on patient care;
- important aspects of the experience of illness are considered;
- critical clinical and patient important outcomes are identified and prioritised;
- the balance of benefits and harms of the intervention is appropriately considered, when recommendations are formulated in conjunction with patient values and preferences.

The Patient Advisory Group advised on the scope, target population and clinical questions the guideline aimed to address and rate the outcomes in terms of their importance.

The representatives also mapped the needs of children and adults living with a heritable *TP53*-related cancer along an ERN GENTURIS 'Patient Journey', which was used to inform the development of the guideline. The group also review the findings of the literature and recommendations.

Acknowledgement

The ERN GENTURIS Heritable *TP53* Related Cancer Guidelines Development Group gratefully acknowledges the assistance and general guidance provided by following leads as honorary members of the Heritable *TP53* Related Cancer Guidelines Group:

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To be confirmed	Community Representative	…
To be confirmed	Community Representative	…

7. PUBLICATION HISTORY AND SUMMARY OF CHANGES

Publication history

ERN GENTURIS first published the Cancer Surveillance Guideline for patients with heritable *TP53* related cancers in 2019.

Summary of changes

This version of the guideline has not had any further revisions since its publication in 2019.

Process for updating the guideline

Any new evidence that has been published will be updated to the Network clinical leads, on an annual basis and consideration for updating the guideline thereafter. New versions will be published on the Network's website and circulated through the ERN GENTURIS Members.

8. METHODOLOGY (RIGOUR OF DEVELOPMENT) - SEARCH METHODS

Criteria for selection of evidence

Pubmed was searched using the following terms:

> (screening[title/abstract] OR surveillance[title/abstract] OR detection[title/abstract]) AND (LFS[title] OR Li-Fraumeni[Title] OR TP53[title]) AND "humans"[MeSH Terms]

Results from the initial Pubmed search: 337 Papers

Additional papers were requested from experts in the field and references of all the papers were considered.

Strengths and limitation of evidence

The quantification of strength of evidence for a recommendation is a composite of harm and benefit. As a general note for these recommendations, the harms a recommendation seeks address are often clear, however the magnitude of the benefit of a specific recommendation are often not as clear. Meaning the published evidence for a recommendation can be often classified 'weak', even when experts are convinced that the recommendation is correct.

The evidence available to consider this guideline came from a limited number of papers, which typically reported on small samples or cohorts. Indirect evidence from analogous conditions was often needed to address the clinical questions that form this guideline.

Method for formulating recommendations.

List the papers considered in each the topic for the recommendations:

Note was made of the **Design** of each study (RCT, Observational, Systematic Review, Expert Opinion)

Note was made of the **Quality** of each study with any particular limitation with respect to the topic or recommendations

Note was made of the **Directness** of the study to the topic or recommendations

Write recommendations in one of four stylistic formats:

Should, Should Probably, Should Not, Should Probably Not

Should & Should Not, were taken to mean - most well-informed people (those who have considered the evidence) would take this action

Should Probably & Should Probably Not, were taken to mean - the majority of all informed people would take this action, but a substantial minority would not

Grade the overall evidence for that recommendation in one of four stylist formats:

Strong, Moderate, Weak, Very Weak

Strong - Further research is unlikely to change our confidence in the direction of effect between benefit or harm

Moderate - Further research is likely to change our confidence in the magnitude of benefit or harm and might change the direction

Weak - Further research is very likely to change our confidence in the magnitude of benefit or harm and is likely to change the direction

Very weak - The estimate of the balance between harm and benefit is uncertain

Guideline methodology

The ERN GENTURIS **Heritable *TP53* Related Cancer (hTP53rc)** Guidelines Development Group used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for assessment of quality of evidence and grading of recommendations. GRADE quality assessment, that is applied to the body of evidence is reported under four distinct levels - high, moderate, low, and very low – to reflect the level of confidence and certainty in the published evidence. The final quality rating of the evidence was assessed under the following areas:

- limitations in study design or implementation (risk of bias)
- imprecision of estimates (wide confidence intervals)
- inconsistency (variability in results)
- indirectness of evidence
- publication bias.

GRADE, however, is not appropriate for making guidelines recommendations when there is limited, low-quality and conflicting evidence, and consensus statements are more appropriate in these scenarios.

In day-to-day practice, clinicians will not have the time to explore the evidence as thoroughly as a guideline panel, nor devote as much thought to the trade-offs, or the possible underlying values and preferences in the population. Therefore, the Core Writing Group has made recommendations even when confidence in effect estimate is low and/or desirable and undesirable consequences are closely balanced. Such recommendations have been classified as ‘weak’ and been qualified. The recommendations have been graded on the quality of evidence; balance between benefits and harms; include the values and preferences of patients; and consider the feasibility, equity & acceptability of implementation and use.

Strength of recommendation has been determined through a consensus-based approach and through active engagement of affected individuals and parent representatives, specifically balancing the desirable and undesirable consequences of surveillance and alternative care strategies, quality of evidence, and values and preferences held by the patient representatives.

External Validation

ERN GENTURIS has actively involved external experts from different speciality areas that are relevant to the scope of the guideline to review the findings and recommendations developed in this guideline.

In addition, the Heritable *TP53* Related Cancer (hTP53rc) Guideline Development Group engaged with the European Society of Human Genetics as an independent review of the guideline.

9. EPIDEMIOLOGY AND AETIOLOGY

Epidemiology

The frequency of carriers with germline disease-causing variants in the *TP53* gene has recently been estimated, from large databases of unselected individuals, to be approximately **1/4,500 individuals** (de Andrade et al., 2019), which is in agreement with a previous estimate of 1 in 5,000 from testing of very early-onset breast cancer cases (Lalloo et al., 2003). However, this does not correspond to the prevalence of h*TP53*rc, if one considers the incomplete penetrance related to *TP53* disease-causing variants. Taking into account this incomplete penetrance, the **prevalence of h*TP53*rc can be estimated** to a magnitude of **1/10,000 individuals**. Southern and South-Eastern regions of Brazil constitute geographical exceptions, since they represent the only areas where a specific germline disease-causing *TP53* variant (c.1010G>A; p. Arg337His) has been associated with a **founder effect**. In Southern and South-Eastern regions of Brazil, the frequency of this variation is **1/300 individuals** (Palmero et al., 2008).

Aetiology

h*TP53*rc result from germline deleterious alterations of **one of the two copies** of the *TP53* gene. Deleterious variants **inactivate** the p53 protein, which **normally acts** as a **guardian of the genome** when DNA damages occur, and regulates the transcription of numerous genes involved in cell cycle, DNA repair, apoptosis, senescence and metabolism. In a carrier of a germline *TP53* deleterious variant, the level of p53 functional protein is insufficient to ensure appropriate biological response to DNA damage and this contributes to the malignant transformation of the cell. Therefore, germline deleterious variants act as **permissive events**. The tumour spectrum associated with germline *TP53* disease-causing variants is probably explained by the fact that these *TP53* variants have a "**truncal" effect** on **progenitor/stem cells** originated from the **mesoderm** and **ectoderm**, which increase their survival and allow their expansion (Amadou et al., 2018; Levin et al., 2019). Some germline missense variants, not only inactivate one of the parental alleles, but also produce a mutant protein able to interact with and inactivate the protein encoded by the remaining wild-type allele. These variants are called **dominant-negative missense variants,** are often more penetrant than

other *TP53* variants, and are usually associated with a more severe clinical expression in terms of age of tumour onset.

10. HERITABLE *TP53* RELATED CANCER (hTP53rc) SURVEILLANCE

Recommendations in this guideline are divided into three sections.

1. The ***first* set** of recommendations regards to **cancer patients**, that should be offered *TP53* testing.
2. The ***second* set** of recommendations regards **first-degree relatives of patients carrying** a confirmed germline disease-causing *TP53* variant (pre-symptomatic testing).
3. The ***third* set** of recommendations regards **all confirmed carriers** of germline disease-causing *TP53* variants that should undergo cancer surveillance.

SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS FOR INDEX CASE

There are many described variants in *TP53*, but not all are associated with an increased risk of cancer development. The following recommendations highlight the circumstances that should be considered when guiding at risk unaffected individuals, or cancer patients for genetic testing of the *TP53* gene. The "Chompret Criteria" are well recognized and supported by strong evidence. The present recommendations build on those criteria and highlight specific and current evidence-based circumstances, supporting or dismissing *TP53* germline genetic testing.

Cancer Patient Recommendations		
Recommendation 1	All patients who meet the modified "**Chompret Criteria**" **should** be tested for germline *TP53* variants: • *Familial presentation*: proband with a *TP53* core tumour (breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumour, adrenocortical carcinoma) before 46 years AND at least one first- or second-degree relative with a core tumour before 56 years; or • *Multiple primitive tumours*: proband with multiple tumours, including 2 *TP53* core	**Strong Evidence**

	tumours, the first of which occurred before 46 years, irrespective of family history; <u>or</u> • *Rare tumours*: patient with adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history; <u>or</u> • *Very early-onset breast cancer*: Breast cancer before 31 years, irrespective of family history	
Recommendation 2	Children and adolescents **should** be tested for germline *TP53* variants **if presenting with**: • **Hypodiploid acute lymphoblastic leukemia (ALL)**; <u>or</u> • Otherwise unexplained ***sonic hedgehog*-driven medulloblastoma***; <u>or</u> • **Jaw osteosarcoma***; <u>or</u> • **Periosteal osteosarcoma***	**Moderate Evidence**
Recommendation 3	Patients who develop **a second primary core *TP53* tumour** within the **radiotherapy field should be tested** for germline *TP53* variants	**Moderate Evidence**
Recommendation 4	**a.** Patients **older than 46 years** presenting with **breast cancer without** personal or familial history fulfilling the "Chompret Criteria" **should not** be tested for germline *TP53* variants	**Strong Evidence**
	b. Any patient presenting with **isolated breast cancer** not **fulfilling the "Chompret Criteria"** and in whom a germline disease-causing *TP53* variant has been identified **should** be referred to an **expert multi-disciplinary team** for discussion	**Strong Evidence**
Recommendation 5	**Children with any cancer** from **southern** and **south-eastern Brazilian** families **should be tested** for the **p.R337H Brazilian** founder germline *TP53* variant	**Strong Evidence**

SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS FOR PRE-SYMPTOMATIC TESTING

The following set of recommendations highlights the available evidences that trigger genetic testing of *TP53* in first-degree relatives of individuals carrying disease-causing germline *TP53* variants. This summary reflects already available strong evidence of the benefit of early identification of some cancers, but as yet weak evidence regarding wider benefits in germline *TP53* variant carriers.

Pre-symptomatic Testing Recommendations		
Recommendation 6	**Adult first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be offered testing** for the same germline *TP53* variant	**Strong Evidence**
Recommendation 7	**The testing in childhood,** from birth, of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **high cancer risk *TP53* variant conferring a high cancer risk in childhood**: • the index case has developed a childhood cancer; or • childhood cancers have been observed within the family; or • this variant has already been detected in other families with childhood cancers; or • this variant corresponds to a dominant-negative missense variant	**Strong Evidence (increased childhood cancer risk)** **Moderate Evidence (absolute risk)** **Strong Evidence (benefit of early detection of ACC)** **Weak Evidence (detection of other tumours)**
Recommendation 8	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should not be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **low cancer risk *TP53* variant** and **not conferring a high cancer risk in childhood**: • the index case has not developed a childhood cancer; and	**Moderate Evidence**

	• childhood cancers have not been observed within the family; and • this variant has not already been reported in other families with childhood cancers; and • this variant does not correspond to a dominant-negative missense variant	
Recommendation 9	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be discussed with their parents** • if cancers have occurred in **early adulthood** (before the age of 25 years) within the family, • or if there is **insufficient evidence in the databases or registries to determine the childhood cancer risk.** This discussion should address the burden, and uncertain benefits, of surveillance in childhood, before a decision is made whether to test the child for germline disease-causing *TP53* variants.	**Moderate Evidence**

RECOMMENDATIONS FOR SURVEILLANCE OF GERMLINE DISEASE-CAUSING VARIANT CARRIERS

This section presents recommendations regarding the method, type and frequency of surveillance for carriers of germline disease-causing *TP53* variants. There is not yet sufficient evidence that evaluates or qualifies the balance of the benefits and risks of any given surveillance method.

Surveillance recommendations in carriers of germline disease-causing *TP53* variants		
Recommendation 10	**Clinical examination,** with specific attention to signs of virilization or early puberty, and measurement of arterial hypertension should be performed, in children **every 6 months**	**Moderate Evidence**
	In adults, **clinical examination** should be performed **annually**	**Moderate Evidence**
Recommendation 11	In **adults, WBMRI should** be conducted **annually**	**Strong Evidence**
Recommendation 12	In individuals with **high cancer risk *TP53* variants, WBMRI** without Gadolinium enhancement, **should** be conducted **annually,** from **birth**	**Moderate Evidence**
Recommendation 13	In **female individuals from 20 years** onwards, **breast MRI,** with Gadolinium enhancement, **should** be conducted **annually**	**Strong Evidence**
Recommendation 14	In **children from birth**, and **adolescents** (< 18 years), **abdominal ultrasound** for the detection of adrenocortical carcinoma (ACC), **should** be conducted at least **every 6 months**	**Strong Evidence**
Recommendation 15	**When abdominal ultrasound does not allow a proper imaging of the adrenal glands, measurement** of **urine steroids** for detection of ACC, **should probably** be conducted at least **every 6 months**	**Weak Evidence**
Recommendation 16	In **adults until 50 years, brain MRI should** be conducted **annually**	**Moderate Evidence**

Recommendation 17	In individuals with **high cancer risk *TP53* variants, brain MRI should** be conducted **annually** from **birth**	**Moderate Evidence**
Recommendation 18	If surveillance includes brain MRI, at least the **first (prevalence) scan should** be conducted using **dedicated brain MRI** with Gadolinium enhancement	**Moderate Evidence**
Recommendation 19	If surveillance includes annual **brain MRI**, this **should** probably alternate with the **WBMR**I, so that the brain is imaged at least every 6 months.	**Weak Evidence**
Recommendation 20	**Colonoscopy** should be performed, **from 18 years,** every **5 years, only** if the carrier received **abdominal radiotherapy** for the treatment of a previous cancer or if there is a **familial history of colorectal** tumours suggestive of an increased genetic risk	**Weak Evidence**

THE QUESTION OF COLORECTAL CANCER

There is a perception that colorectal cancer is associated with *TP53* germline pathogenic variants, based mainly on a single case described in a single study from 2015 (Yurgelun et al 2015). In that report, six *TP53* variants were found, with none occurring in families meeting Chompret criteria. **Examination of these *TP53* variants, based on the current classification criteria used in 2019, shows that only one of the six variants meets criteria for being classified as a class 4 variant (likely pathogenic)**, with three being clearly benign (class 2) and one being classified as variant of uncertain significance (class 3). On the basis of only one pathogenic variant amongst 457 colorectal cancers, this does not confirm an increased risk for colorectal cancer. Observational data from 205 *TP53* carriers in Manchester and over 600 from France has respectively shown 0 and 6 cases of colorectal cancer. As such, there is no convincing evidence of increased risk for colorectal cancer in carriers of germline *TP53* disease causing variants. Therefore, a high risk of colorectal cancer can be confidently excluded.

11. PSYCHOLOGICAL NEEDS

There are several psychological issues to consider when engaging with patients and families with a cancer related syndrome, where h*TP53*rc stands out as it causes an increased risk in children and young adults for cancer, and screening and prevention programs means a high burden for both the individual and the family.

In contrast to sporadic cancers, when the initial focus commonly is on treatment and survival, the diagnosis in families with inherited cancer risks often precedes with a long-term awareness of cancer risk, experiences of illness, and reduced anticipation of survival. They have often witnessed the death of loved ones, and have seen several family members suffer of cancer simultaneously, resulting in a severe emotional burden. There is still a need to develop and evaluate the psychological, social and behavioral impact of h*TP53*rc and to elaborate evidence-based counseling strategies addressing family communication, coping strategies, family planning, as well as cancer prevention. Since h*TP53*rc entails a high risk for cancer during childhood and early adulthood, it may be of importance with longitudinal care that is made available recurrently as these individuals reach developmental milestones that intersect with risk management, risk perception and family formation (Shepherd et al, 2018). Services that deliver these diagnoses, and the subsequent surveillance, are encouraged to facilitate the formation and continuation of support groups, whether face-to-face or online, for the facilitation of peer-support.

12. OUTCOMES AND DEFINITIONS

Germline disease-causing variants in *TP53* are overall rare events, but have a high impact on cancer risk and quality of life in affected families. Therefore, it is of great importance to join forces and collect knowledge on a European level. By establishing European guidelines and databases, we will be able to collect more data and gain proof for further clinical handling of these families.

- We will outline the impact of whole-body MRI as a surveillance tool
- Gain knowledge in the wide variety of genotype-phenotype correlations presented in different families.
- Follow-up of prophylactic measures such as mastectomy.
- Stimulate disease awareness, psychosocial handling and Quality of life measures

- Improve genetic counselling

13. ALTERNATIVE MANAGEMENT

The ERN guidelines propose to adapt the US/Canadian protocols to each germline disease-causing *TP53* variant carrier. The **heavy alternative** is to offer in Europe the US/Canadian protocol to each germline disease-causing *TP53* variant carrier, independently of the personal and medical history and type of *TP53* variant. The **light alternative** is to limit the medical follow-up, in children, to abdominal ultrasound which is a simple and accessible imaging exam able to detect adrenocortical carcinoma and, in adult premenopausal females, to breast MRI since breast cancers represent the main cancer risk in adults.

14. IMPLEMENTATION - ADVICE & TOOLS, FACILITATORS / BARRIERS, RECOURSE IMPLICATIONS AND MONITORING & AUDIT

Implementation of these guidelines will require their progressive diffusion to the different stakeholders. For a faster and more efficient implementation, these European-adapted guidelines should be adopted and diffused by the General Direction of Health of each European Country in their native language. A more fragmented, but rather more tangible approach, will be the diffusion to medical societies potentially involved in the management of carriers of germline *TP53* variants: geneticists, oncologists, paediatricians and radiologists. This can be achieved by presentations at annual meetings organized by these societies and patient associations.

The main barriers will be the **unequal geographical and financial access to whole-body MRI** in the different European countries, the **financial cost** of annual imaging exams, **the acceptance**, in terms of costs and organization, by health professionals of a surveillance protocol including **annual whole-body MRI** and **the acceptance**, in terms of quality of life, **by patients** and **families** of annual screening requiring several imaging exams. The acceptance and cost-efficiency of the ERN guidelines should be monitored and evaluated by an European prospective study.

15. RESEARCH RECOMMENDATIONS

The evidence base for screening and surveillance for some organ systems in this guideline are, as always when is concerns rare disorders, limited. Some of the quality of the evidence regarding baseline risk has been rated as weak.

The evidence base for screening and surveillance for some organ systems in this guideline are also limited and some of the quality of the evidence regarding baseline risk has been rated as weak.

In 2019, the priorities of research in the field of heritable *TP53*-related cancers include:

- **Evaluation of the tumour detection rate and efficiency of brain MRI in germline disease-causing *TP53* variant carriers.** Whereas numerous studies have confirmed the efficiency of whole-body MRI, in terms of tumour detection, data concerning the utility of brain MRI are insufficient.
- **Evaluation of the impact of the surveillance protocols on patients' survival.**
- **Characterization of biomarkers and functional tests able to predict the cancer risk in germline disease-causing *TP53* variant carriers**. The biomarkers can include genetic variants acting as modifier factors or epigenetic alterations predictive of the tumour risk. Functional tests can correspond to high-throughput assays testing all the possible *TP53* variants or to reliable personalized assays, able to quantify in medical practice the biological impact of the variant. The identification of such biomarkers is crucial to ensure, in the future, a personalized and appropriate medical management of germline disease-causing *TP53* variant carriers, considering the heterogeneity of the penetrance and diversity of associated clinical presentations.
- **Identification of environmental factors that could modify cancer risk in germline disease-causing *TP53* variant carriers.** Since pathogenic germline *TP53* act as permissive alterations, results obtained with radiotherapy and genotoxic chemotherapies suggest that other physical agents, or molecules with a potential genotoxicity activity, might increase cancer risk in germline disease-causing *TP53* variant carriers.
- **Development of simple blood tests, complementary to imaging, to improve earlier tumour detection in germline disease-causing *TP53* variant carriers**. These markers can correspond to DNA (somatic genetic or epigenetic alterations) or non-DNA markers detectable in circulating blood or other biological fluids. Early-tumour detection is critical

for the prognosis in most of the tumours associated to germline disease-causing *TP53* variants. Surveillance protocols are based on several annual MRI and may be heavy for the patients, families as well as the health professionals. Development of validated blood markers would facilitate clinical management.

- **Adaptation of conventional therapies and development of new therapeutic strategies for h*TP53*rc**. Conventional genotoxic chemotherapies and radiotherapy contribute to the development of tumours secondary to treatment, in germline disease-causing *TP53* variant carriers. Experimental data suggest that there is a dose effect. The clinical utility of the dosage in conventional chemotherapy regimen, especially in childhood cancers may need to be re-evaluated in these cases. When there is no alternative to conventional treatments, adaption of the drug or radiotherapy doses, and the use of proton therapy that ensures a more focused delivery of radiations than photonic therapy, might constitute therapeutic options in germline disease-causing *TP53* variant carriers. The efficiency of non-genotoxic therapies, such as combined targeted therapies or immunotherapies and of molecules able to interact or modify wild-type or mutant p53 protein should be evaluated.
- **Research into active risk-reducing therapies**. Some drugs such as metformin, aspirin may have some impact in reducing the risk of cancer initiation. Research to investigate this potential mitigating strategy is urgently required.
- **Evaluation of the psychological, social and behavioural impact of hTP53rc**.
- **Elaboration of evidence-based counselling strategies** addressing family communication, coping strategies, family planning, as well as cancer prevention.

APPENDIX – 1

EDITORIAL INDEPENDENCE - FUNDING BODY; COMPETING INTERESTS RECORDED AND ADDRESSED.

All members of ERN GENTURIS PTEN Core Writing Group have provided disclosure statements on all relationships that they have that might be perceived to be a potential source of a conflict of interest. This information is publicly accessible through the ERN GENTURIS website.

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Funding Summary

Lead	Role	Funding Organisation
Prof. Thierry Frebourg	Core Writing Group Chair	Rouen University Hospital, Rouen, France • To be added other funding agencies
Prof. D. Gareth Evans	Core Writing Group Clinical Member	Manchester Universities Foundation Trust, Manchester, U.K. • To be added other funding agencies
Ass. Prof. Svetlana Bajalica Lagercrantz	Core Writing Group Clinical Member	Hereditary Cancer Unit, Karolinska University Hospital, Stockholm, Sweden • To be added other funding agencies
Prof. Carla Oliveira	Core Writing Group Clinical Member	Porto Comprehensive Cancer Center, Porto, Portugal & i3S/Ipatmup, Porto, Portugal • To be added other funding agencies

APPENDIX 2 – EXPLICIT LINK BETWEEN EVIDENCE AND RECOMMENDATIONS

Paper	Design	Quality	Directness
(Giacomazzi et al., 2013)	Observational	No significant methodological issues	Direct: Southern and south-eastern Brazilian
(Achatz et al., 2007)	Observational	No significant methodological issues	Direct: Southern and south-eastern Brazilian
(Curtin et al., 2013)	Observational	Small effect size	Direct: First degree relative of paediatric cases presenting before age 19yrs
(Curtin et al., 2013)	Observational	Small effect size	Direct: Second- and third-degree relatives of paediatric cases presenting before age 5yrs
(Ruijs et al., 2010)	Observational	Small sample: Huge effect size	Direct: "Chompret Criteria"
(Yurgelun et al., 2015)	Observational	Large sample Over estimates *TP53* relevance	Direct: Colorectal cancer
(Ballinger et al., 2017).	Observational	Large sample	Direct: WBMRI
(Saya et al., 2017)	Case – Control	Moderate sample	Direct: WBMRI
(Paixao et al., 2018)	Observational	No significant methodological issues	Direct: WBMRI
(O'Neill et al., 2018)	Observational	Small sample Feasibility	Direct: WBMRI
(Villani et al., 2011)	Observational	Small sample Longitudinal	Direct: Surveillance
(Villani et al., 2016).	Observational	Small sample Longitudinal	Direct: Surveillance

APPENDIX 3 – PLAIN LANGUAGE SUMMARY

European Reference Network
for rare or low prevalence complex diseases
Network Genetic Tumour Risk Syndromes (ERN GENTURIS)

www.genturis.eu

ERN GENTURIS Plain Language Summary:

Guidelines for the identification of individuals who should be tested for germline disease-causing TP53 variants and for their subsequent clinical management

INTRODUCTION

The *TP53* gene is susceptible to genetic spelling changes, often called mutations or genetic variants. Some inherited changes to the *TP53* gene can mean people who have them have a high chance of developing certain cancers, especially early in life. Historically the clustering of these cancers was known as **Li-Fraumeni syndrome** (LFS), but because there are lots of other ways these changes to *TP53* can cause cancers, in the guideline they are called "**hereditary *TP53*-related cancers (h*TP53*rc)**". Not all changes to *TP53* are harmful, in the guideline the changes to the *TP53* gene that are known to increase a person's cancer risk are called "***TP53* disease-causing variants**". The guideline builds on the internationally recognised approach to testing for *TP53* changes, known as the "Chompret criteria".

When to test for *TP53* changes (Known as "Chompret Criteria")

Familial presentation:
Proband (first person affected in a family) with a *TP53* core tumour*before 46 years
AND
At least one **first- or ***second-degree relative with a core tumour before 56 years;

or

Multiple primitive tumours:
Proband with multiple tumours, including 2 *TP53* core tumours*, the first of which occurred before 46 years, *irrespective of family history;*

or

Rare tumours:
Patient with adrenal cancer, choroid plexus carcinoma, or rhabdomyosarcoma of embryonal anaplastic subtype (muscle tumour)*, irrespective of family history;*

or

Very early-onset breast cancer:
Breast cancer before 31 years*, irrespective of family history.*

**TP53* core tumours are the most common tumours with LFS: premenopausal breast cancer, soft-tissue sarcoma, osteosarcoma (bone tumour), brain tumour, adrenal cancer
**First degree relative: parent, sibling, child

***Second degree relative: aunt, uncle, grandparent, grandchild, nieces, nephew, half-sibling

Diagnosis of **h*TP53*rc** is mainly performed by cancer geneticists, oncologists and paediatric oncologists. Diagnosis of **h*TP53*rc** is difficult, due to the wide range of clinical presentations (i.e. clinical symptoms) and great variability in age of tumour-onset between families or within the same family. ***TP53* disease-causing variants** can be detected in cancer patients either with or without familial history of cancers.

Individuals carrying ***TP53* disease-causing variants** have a **high risk** of developing **multiple primary cancers** in their lifetime. Once individuals develop their first tumour, treatment with radiotherapy and certain chemotherapies may increase their risk of developing other cancers. Therefore, testing for *TP53* disease-causing variants should take place before starting treatment. And if a *TP53* disease-causing variant is found, priority should be given to surgical or ablative treatments, avoiding radiotherapy when possible and using only non-genotoxic chemotherapies.

GUIDELINE AIMS

The **h*TP53*rc** Guideline has been created to assist healthcare professionals provide the most up-to-date approaches to diagnosis and surveillance of **cancer-free individuals with TP53 disease-causing variants** and **cancer patients** who carry ***TP53* disease-causing variants**. The guideline was based on the best evidence and the consensus of experts in caring for people with **hTP53rc**. It presents recommendations to support care, but a clinician, in discussion with an affected individual, may tailor the exact care to the person's preferences and needs.

SCOPE & PURPOSE OF THE GUIDELINE

The scope of this guideline is for surveillance (screening for cancer) of individuals with a ***TP53* disease-causing variant** and testing of their first degree-relatives.

KEY RECOMMENDATIONS

Recommendations for cancer patients
All patients who meet the modified "**Chompret Criteria**" should be tested for *TP53* disease-causing variants
Children and adolescents **should** be tested for germline *TP53* variants **if presenting with**: **Hypodiploid acute lymphoblastic leukemia (ALL)**; or Otherwise unexplained ***sonic hedgehog*-driven medulloblastoma***; or **Jaw osteosarcoma***; or **Periosteal osteosarcoma***
Patients who develop a **second primary core *TP53* tumour**, within the **radiotherapy field**, **should** be tested for germline *TP53* variants
a. Patients **older than 46 years** presenting with **breast cancer** without personal or familial history fulfilling the "Chompret Criteria" **should not** be tested for germline *TP53* variants

b. Any patient presenting with **isolated breast cancer** and not fulfilling the **"Chompret Criteria"**, in whom a disease-causing *TP53* variant has been identified, **should** be referred to an **expert multi-disciplinary team** for discussion
Children with any cancer from **southern** and **south-eastern Brazilian** families **should be tested** for the **p.R337H Brazilian** founder germline *TP53* variant

* These parts of the recommendation should be supported by publications before the release of the guidelines.

Pre-symptomatic Testing Recommendations for people without cancer
Adult first-degree relatives of individuals with germline disease-causing *TP53* variants **should** be offered testing for the same germline *TP53* variant
The testing in childhood, from birth, of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **high cancer risk *TP53* variant conferring a high cancer risk in childhood**: • The index case has developed a childhood cancer; or • Childhood cancers have been observed within the family; or • This variant has already been detected in other families with childhood cancers; or This variant corresponds to a dominant-negative missense variant
The testing in childhood of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should not be offered**, if updated knowledge, based on databases and registries, shows that the variant can be considered as a **low cancer risk *TP53* variant** and **does not confer a high cancer risk in childhood**: • The index case has not developed a childhood cancer; and • Childhood cancers have not been observed within the family; and • This variant has not already been reported in other families with childhood cancers; and This variant does not correspond to a dominant-negative missense variant
The testing in childhood of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be discussed with their parents** if cancers have occurred in early adulthood (before the age of 25 years) within the family, or if there is **insufficient evidence in the databases or registries to determine the childhood cancer risk.** This discussion should address the burden, and uncertain benefits, of surveillance in childhood, before a decision is made whether to test the child for germline disease-causing *TP53* variants.

Surveillance for people with **germline disease-causing TP53 variants**					
Exam	**Periodicity**	**Age to start**	**Age to end**	**Condition**	**Evidence**
Clinical examination	Annual	18 years	-		Moderate
Whole-Body MRI	Annual	Birth	-	High cancer risk *TP53* variant	Moderate
		18 years	-		Strong
Breast MRI	Annual	20 years	Until 60-70 years		Strong
Brain MRI*	Annual	Birth	18 years	High cancer risk *TP53* variant	Moderate
		18 years	Until 60-70 years		Moderate
Abdominal ultrasound	Every 6 months	Birth	Until 18 years		Strong
Urine steroids	Every 6 months	Birth	-		Weak

*The first scan should be conducted with Gadolinium enhancement; brain MRI should alternate with the WBMRI, so that the brain is imaged at least every 6 months.

Whole-body MRI: This examination should include the whole body including the complete extremities.

Psychological Needs

***TP53* disease-causing variants** cause an increased risk in children and young adults of cancer, screening and prevention programs means a high burden both for the individual and their family. Diagnosis, in a family, of an inherited cancer predisposition comes with a long-term awareness of cancer, experiences of illness, and anticipation of reduced life expectancy. Those families have often witnessed the death of loved ones, and seen several family members suffer from cancer simultaneously, which can result in a severe emotional burden. Services that deliver these diagnoses, and the surveillance that follows, are encouraged to support the formation and continuation of support groups, whether face-to-face or online, for affected people to support each other.

APPENDIX 4 – GADOLINIUM PASS

Estimated 450 million doses with gadolinium based contrast agents (GBCAs) has been administered to patients worldwide with very limited number of side effects.
It is known that gadolinium is retained for months or years in several organs. The highest concentrations (nanomoles per gram of tissue) have been identified in the bone, followed by other organs (brain, skin, kidney, liver, and spleen). Linear GBCAs cause more retention than macrocyclic GBCAs. Clinical consequences of gadolinium retention have not been established in patients with normal renal function.
There is very limited information about safety of multiple GBCAs administrations (>30-50 times), so it is advisable to document the GBCAs administration in patients who are expected to receive multiple doses, like people with genetic tumor risk syndromes.

Gadolinium Pass

Number:

Last name: .....

First name: .....

Date of birth: .....

Name of the GBCA	Structure		Dose ml/kg	Comments
Magnevist	linear	ionic	0,2	Restricted to intra-articular use in the EU
MultiHance		ionic	0,2	Restricted to liver use in the EU
Omniscan		non-ionic	0,2 0,1 kidney	Not allowed in the EU
Primovist Eovist (USA)		ionic	0,1	Only for liver imaging
Dotarem	macro-cyclic	ionic	0,2	
ProHance		non-ionic	0,2	
Gadovist (EU) Gadavist (US)		non-ionic	**0,1**	

The following gadolinium based contrast agents (GBCAs) were given:

	Date	MRI type*	*Name of the GBCA*	*L/M***	*Weight (kg)*	*Dosage (ml)*	*Comments, complications*	*Signature and/or stamp of the physician*
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
Number of received GBCAs altogether:								

*MRI type: breast, whole-body (WB), brain, abdomen, thorax, extremities

** Please indicate whether the contrast agent is linear or macrocyclic.

APPENDIX 5 - REFERENCES

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