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GUIDELINES FOR THE LI-FRAUMENI AND HERITABLE *TP53*-RELATED CANCER 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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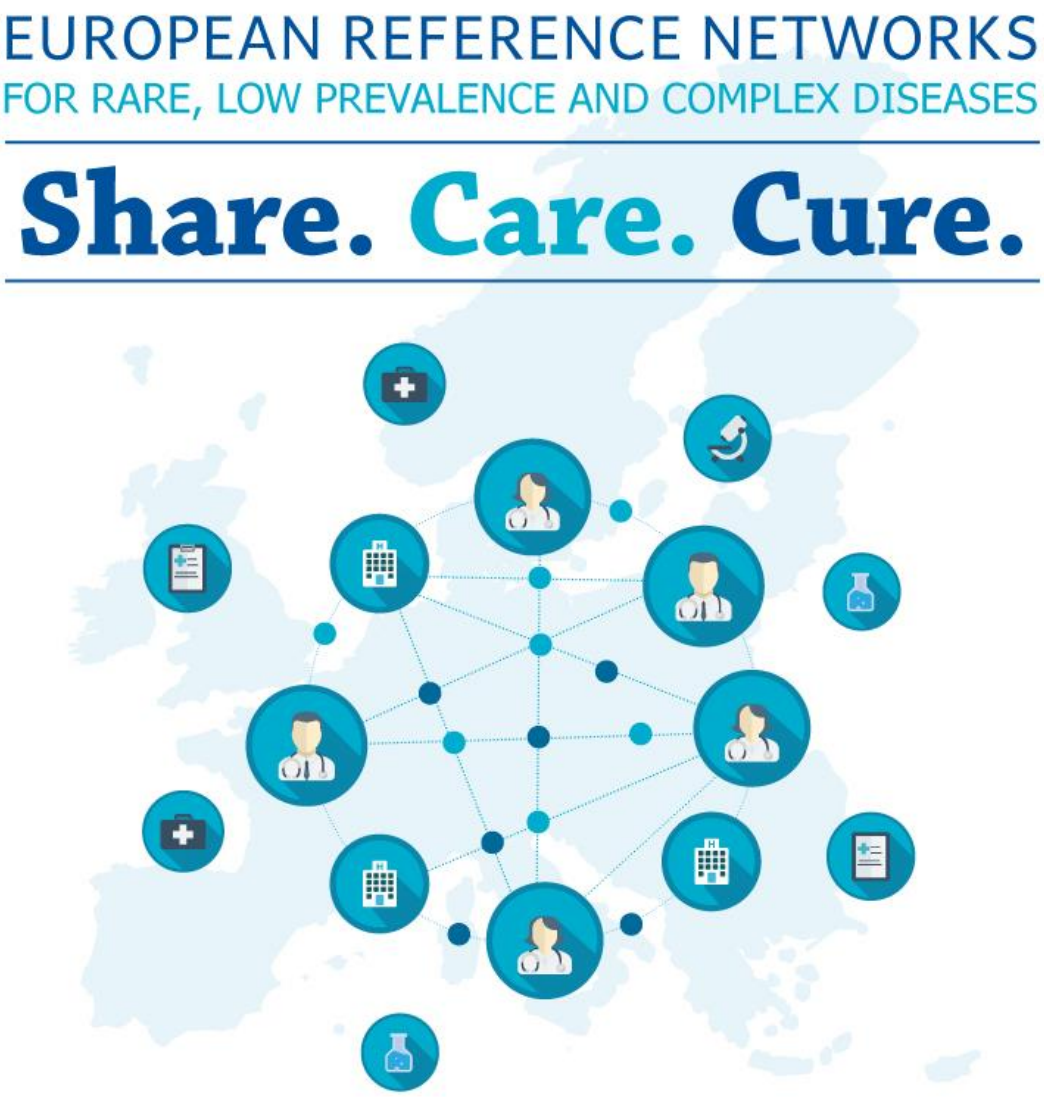

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ABSTRACT

Fifty years after the initial recognition of the Li-Fraumeni syndrome (LFS), our perception of cancers related to germline alterations of *TP53* has drastically changed: (i) germline alterations of *TP53* are often identified among children with cancers, in particular soft-tissue sarcomas, adrenocortical carcinomas, central nervous system tumours or among adult females with early breast cancers, without familial history. This justifies the expansion of the LFS concept to a wider cancer predisposition syndrome designated heritable *TP53*-related cancer (h*TP53*rc) syndrome; (ii) the interpretation of germline *TP53* variants, corresponding mainly to missense variants, remains challenging and should integrate epidemiological, phenotypical, bioinformatics prediction and functional data; (iii) the penetrance of germline disease-causing *TP53* variants is variable, depending both on the type of variant (dominant negative variants being associated with a higher cancer risk) and on modifying factors; (iv) whole-body MRI (WBMRI) allows early detection of tumours in *TP53* variant carriers and (v) in cancer patients with germline disease-causing *TP53* variants, radiotherapy and conventional genotoxic chemotherapy contribute to the development of subsequent primary tumours. Therefore, it is critical to perform *TP53* germline testing before the initiation of treatment in order to avoid in carriers, if possible, radiotherapy and genotoxic chemotherapies. The aim of these guidelines is to assist healthcare professionals in (i) the identification of cancer patients and unaffected potential carriers, who should be tested for germline *TP53* variants, and (ii) the surveillance of carriers harbouring likely pathogenic or pathogenic *TP53* variants. In children, the recommendations are to perform clinical examination and abdominal ultrasound every 6 months, annual WBMRI and brain MRI from the first year of life, in case they harbour a *TP53* variant known to be associated with childhood cancers. In adults, the surveillance should include every year clinical examination, WBMRI, breast MRI in females from 20 until 65 years and brain MRI until 50 years.

GUIDELINE SUMMARY: SURVEILLANCE PROTOCOL IN CARRIERS OF GERMLINE DISEASE-CAUSING *TP53* VARIANTS

Exam	Periodicity	Age to start	Age to end	Condition	Evidence*
Clinical examination with, in children, specific attention to signs of virilisation or early puberty and measurement of blood pressure and, in patients who received radiotherapy, to occurrence of basal cell carcinomas within the radiotherapy field	Every 6 months	Birth	17 years		Moderate
	Annual	18 years	-		Moderate
Whole-Body MRI without gadolinium enhancement	Annual	Birth	-	High cancer risk *TP53* variant** or patient previously treated by chemotherapy or radiotherapy	Moderate
		18 years	-		Strong
Breast MRI	Annual	20 years	65 years		Strong
Brain MRI***	Annual	Birth	18 years	High cancer risk *TP53* variant	Moderate
		18 years	50 years		Moderate
Abdominal ultrasound	Every 6 months	Birth	18 years		Strong
Urine steroids	Every 6 months	Birth	18 years	When abdominal ultrasound does not allow a proper imaging of the adrenal glands	Weak
Colonoscopy***	Every 5 years	18 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

*This grading is based on published articles and expert consensus.
**A germline disease-causing *TP53* variant should be considered as "high risk" if 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 first scan should be conducted with I.V. Gadolinium enhancement; in children, brain MRI should alternate with the Whole-Body MRI, so that the brain is imaged at least every 6 months.

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1. 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**. Fifty years after the initial clinical recognition of the syndrome, 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, our perception of cancers related to germline alterations of TP53 has drastically changed through time (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 cancer (h*TP53*rc) syndrome**. 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 (Bougeard et al., 2015). Regardless of familial history, the detection rate of disease causing germline *TP53* variants has been estimated to be: 50-80% in children presenting with ACC or choroid plexus carcinomas; up to 73% in children with rhabdomyosarcoma of embryonal anaplastic subtype (Varley et al., 1999; 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 should not be mandatory when considering genetic testing of *TP53***.

The frequency of presentations without familial cancer history is explained both by the contribution of **de *novo* variants** to h*TP53*rc syndrome, which has been estimated to be **between 7-20%** - approximately one fifth of these *de novo* mutations occur during embryonic development, resulting in **mosaics** -

(Gonzalez et al., 2009; Renaux-Petel et al., 2018) and the incomplete penetrance of germline *TP53* variants.

Table 2. Chompret criteria for *TP53* testing (updated from 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

Beside the Chompret criteria, recent reports and experience of certain centres justify to extend *TP53* testing to other clinical presentations suggestive of a germline TP53 alteration: Children and adolescents with hypodiploid acute lymphoblastic leukemia (Holmfeldt et al., 2013; Qian et al., 2018), otherwise unexplained sonic hedgehog-driven medulloblastoma (Waszak et al., 2018), jaw osteosarcoma and patients who develop a second primary tumour within the radiotherapy field of a first core *TP53* tumour which occurred before 46 years.

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. The 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/AMP guidelines, is based on several items including *phenotypical data* (identified in patients fulfilling the Chompret criteria); *frequency of the variant in the general population*, as reported the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/), *bioinformatics predictions* of the variant impact on protein or RNA splicing 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/). Optimized and stringent ACMG/AMP criteria for a specific classification of germline *TP53* variants, integrating the above considerations, are being developed by a *TP53* variant curation expert panel, under the umbrella of ClinGen. This will allow a progressive allocation or re-classification of *TP53* variants into the different ACMG/AMP classes. Since the distinction between class 5 (pathogenic) and class 4 (likely pathogenic) variants is particularly subtle for *TP53* variants, these variants are designated in the current ERN guideline as "**disease-causing**" variants.

The question about mosaicism

Like in other genetic conditions, Next Generation Sequencing has unmasked mosaic alterations due to *de novo* mutational events occurring during embryonic development. The presence of mosaic *TP53* alterations should be considered in patients with sporadic cancers strongly suggestive of a disease-causing *TP53* variant, such as childhood adrenocortical carcinoma, choroid plexus carcinoma, breast cancer before 31 years of age and in patients with multiple primary tumours belonging to the *TP53* core tumour spectrum (Renaux-Pettel et al., 2018). The absence of detectable *TP53* variants after analysis of blood DNA using NGS, even performed at a high depth, does not guarantee the absence of mosaic alterations which can be restricted to other tissues than blood. Therefore, a complete screening for *TP53* disease causing variants **in highly suggestive situations** should include the **tumour analysis**, which is so far not systematically performed. In contrast, the detection in a small fraction of NGS reads from blood DNA of a *TP53* variant does not always correspond to a mosaic alteration (Combs et al., 2017; Weber-Lassalle et al., 2018; Weitzel et al., 2018) and molecular geneticists should be aware of two pitfalls: the first corresponds to **circulating tumour DNA**, commonly observed in patients with metastatic cancers. For instance, the detection of a *TP53* variant in the blood from a patient with metastatic high grade serous

ovary carcinoma is likely to correspond to circulating tumour DNA, considering the very high frequency of somatic *TP53* alterations in these malignancies (>95%); the second is due to **clonal hematopoiesis,** corresponding to the occurrence in hematopoietic stem cells of somatic *TP53* alterations cells conferring a growth advantage. Clonal hematopoiesis was initially reported in patients over 70 years of age, but can be detected from 30 years of age. The frequency of clonal hematopoiesis is increasing with age, tobacco use and exposure to chemotherapy or radiotherapy (Coombs et al., 2017; Chen and Liu, 2019). Therefore, when a *TP53* variant is detected in a small fraction of NGS reads from blood, it is critical to respect the following rules, before concluding to the presence of a mosaic *TP53* alteration: (i) **consider the clinical presentation** (suggestive or not of the presence of a disease-causing *TP53* variant) and medical history (treatments, metastases…) and (ii) **confirm the presence of the variant** in the tissue from which the tumour originated. Further confirmation in an unaffected tissue with no lymphocyte content, such as a hair follicle, skin biopsy or nail clippings, should also be considered if circulating tumour DNA is suspected from metastatic disease.

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). Carriers of a germline *TP53* variant who are identified in this clinical context have **a cancer risk of 80% at age 70** (Amadou et al., 2018). The exclusion of non-familial cases likely resulted in an ascertainment bias and an **overestimation of disease penetrance** (de Andrade et al., 2019).

Indeed, the cumulative cancer incidence of germline disease-causing *TP53* variants was initially calculated using information mainly from familial cases and was estimated to 73-100% by age 70, with risks close to 100% in women (Chompret et al., 2000; Mai et al., 2016; Amadou et al., 2018). The predominance of familial cases likely results in an **ascertainment bias** and an **overestimation of disease penetrance**. This should be regarded in perspective with **the prevalence** in **the general population** of germline disease-causing *TP53* variants, which was recently estimated, based on a conservative approach, to be in the magnitude of **1 among 4500 individuals** (de Andrade et al. 2019). In c childhood, the main tumour risks are ACC, STS, osteosarcomas and CNS tumours whereas the main tumour risk in adults corresponds to female breast cancers, female *TP53* variant carriers have an excessively high risk of developing breast

cancer before 31. There is no known elevated risk of male breast cancer (Chompret et al., 2000; Gonzalez et al., 2009; Ruijs et al., 2010; Bougeard et al., 2015; Mai et al., 2016; Amadou et al., 2018; Shin et al., 2020). There is a perception that colorectal cancer is associated with germline pathogenic TP53 variants [31-33]. (Wong et al., 2006; Yurgelun et al 2015; McFarland et al., 2019). However, the corresponding studies suffer from methodological limitations and interpretation of some reported TP53 variants is problematic. Families with a germline *TP53* variant and an additional history of colorectal cancer in the pedigree may have increased risk of colorectal cancer. This increased risk is, however, not associated with the *TP53* variant itself and, on the basis of the published studies, a **high risk of colorectal cancer can be confidently excluded in carriers of disease-causing *TP53* variants**.

As it will be discussed in the following chapter, **carriers previously treated by radiotherapy** or **chemotherapy** for a first cancer, have **a very high risk of second primary tumours**, estimated at least to 40%.

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 should allow, progressively, dichotomizing disease-causing *TP53* variants in "**high cancer risk"** and **"low cancer risk"**

alleles. It should be noticed that this distinction, such as the classification of the variant into the different ACMG/AMP classes, is a dynamic process based on the current knowledge. In this context, the ERN GENTURIS is considering, as a next task, the creation of a curated and updated germline *TP53* variant database. 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.

The impact of radio and chemotherapy in the development of second primary tumours

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). Subsequent 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). Therefore, in cancer patients, testing for disease-causing *TP53* variants must absolutely take place before starting treatment and if a disease-causing *TP53* variant is found, priority should be given to surgical or ablative treatments, avoiding radiotherapy when possible and using preferably non-genotoxic chemotherapies.

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** (the first with gadolinium enhancement) from **the first year of life**. Additionally, female carriers should undergo annual **breast MRI** from the age of 20 years onwards. The option of **risk-reducing mastectomy** may be discussed on a case-

by-case basis (Kratz et al., 2017).Several international studies, mostly performed without gadolinium-based contrast agents (GBCAs), have **confirmed the efficiency of WBMRI**, with an **overall estimated detection rate of 7%** for **new** and **localized** primary cancers on a first prevalent screen (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). Given that GBCAs may be retained for months or years in several organs, multiple GBCAs administrations should probably be avoided in germline *TP53* variant carriers and only macrocyclic GBCAs, which are apparently less retained in the body (Layne et al., 2018), should be used.

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 germline disease-causing *TP53* variants causing heritable *TP53*-related cancer (h*TP53*rc) syndrome.

2. COMPOSITION OF THE 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 onco-biology 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.

3. CONFLICT OF INTERESTS

All members of the ERN GENTURIS h*TP53*rc 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.

4. PURPOSE AND SCOPE OF THIS GUIDELINE

4.1. WHY WAS THIS GUIDELINE PRODUCED?

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 surveillance.**

These guidelines take into account **the diversity of clinical presentations** associated with **germline *TP53* variants**, the **variability** of the ***TP53* variant penetrance**, the role of **radiotherapy** and **chemotherapy** in the development of **subsequent primary tumours** and the **medical benefit** of **surveillance protocols**.

4.2. WHO IS THE GUIDELINE FOR?

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**.

Diagnosis of **hT*P53*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.

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.

4.3. WHAT IS THE GUIDELINE ABOUT?

4.3.1 SCOPE

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**.

4.3.2 HEALTH 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**

4.3.3 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.

4.3.4 CARE SETTING

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 a European prospective study.

4.3.5 EPIDEMIOLOGY & 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 variant is **1/300 individuals** (Palmero et al., 2008).

Aetiology: h*TP53*rc syndrome results 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.

5. KEY FINDINGS & RECOMMENDATIONS

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.

5.1. CANCER PATIENTS WHO SHOULD BE TESTED FOR GERMLINE DISEASE-CAUSING *TP53**

Cancer Patient Recommendations	
Rec. 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
Rec. 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**
Rec. 3	Patients who develop a **second primary-tumour**, within the **radiotherapy field of a first core *TP53* tumour which occurred before 46 years, should** be tested for germline *TP53* variants

Rec. 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
Rec. 5	**Children with any cancer** from **southern** and **south-eastern Brazilian** families **should be tested** for the **p.R337H Brazilian** founder germline *TP53* variant

*Testing for disease-causing TP53 variants should be performed before starting treatment in order to avoid in variant carriers, if possible, radiotherapy and genotoxic chemotherapy and to prioritize surgical treatments

5.2. PRE-SYMPTOMATIC TESTING RECOMMENDATIONS

Pre-symptomatic Testing Recommendations	
Rec. 6	**Adult first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be systematically offered** testing for the same germline *TP53* variant
Rec. 7	**The testing in childhood,** from birth, of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be systematically 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
Rec. 8	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should *not* be systematically 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
Rec. 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 31 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.

5.3. SURVEILLANCE RECOMMENDATIONS IN CARRIERS OF GERMLINE DISEASE-CAUSING *TP53* VARIANTS

Surveillance recommendations in carriers of germline disease-causing *TP53* variants	
Rec. 10	**In children, clinical examination** should be performed **every 6 months,** with specific attention to signs of virilization or early puberty, and measurement of arterial hypertension
	In adults, **clinical examination** should be performed **annually** with specific attention, in patients who received radiotherapy, to occurrence **of basal cell carcinomas** within the radiotherapy field
Rec. 11	In adults, **WBMRI without gadolinium enhancement** should be conducted **annually**
Rec. 12	In individuals with **high cancer risk *TP53* variants or previously treated by chemotherapy or radiotherapy, WBMRI** without Gadolinium enhancement, **should** be conducted **annually, from birth**
Rec. 13	In **female individuals, breast MRI** should be conducted **annually, from 20 years** until 65 years
Rec. 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**
Rec. 15	**In children,** from **birth**, and in **adolescents** (< 18 years), **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**
Rec. 16	**In adults until 50 years, brain MRI should** be conducted **annually**
Rec. 17	In individuals with **high cancer risk *TP53* variants, brain MRI should** be conducted **from birth, annually**
Rec. 18	If surveillance includes brain MRI, at **least the first** (prevalence) scan **should** be conducted using **dedicated brain MRI** with gadolinium enhancement

Rec. 19	If surveillance includes annual **brain MRI,** this should alternate with the **WBMRI**, so that the brain is imaged at least every 6 months
Rec. 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. METHODS FOR GUIDELINE DEVELOPMENT

6.1. ESTABLISHMENT OF THE GUIDELINE DEVELOPMENT GROUP

The ERN GENTURIS h*TP53*rc syndrome guideline was established by molecular and clinical geneticists and clinicians with expertise in paediatrics, oncology, or radiology, as well as affected individuals and parent representatives. The Guideline Development Group was supported by a Core Writing Group composed 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 h*TP53*rc. The Core Writing Group leads had joint meetings with a Patient Advisory Group composed of affected individuals and parent representatives that have experience with h*TP53*rc syndrome. The elaboration of these guidelines has then actively involved external experts from different speciality areas that are relevant to the scope of the guideline.

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:

Name	Speciality / Role	Hospital, Member State
Prof. Thierry Frebourg	CWG Chair	Rouen University Hospital and Inserm U1245, Rouen, France
Prof. D. Gareth Evans	CWG Team	Manchester Universities Foundation Trust, Manchester, UK
Ass. Prof. Svetlana Bajalica Lagercrantz	CWG Team	Hereditary Cancer Unit, Karolinska University Hospital, Stockholm, Sweden
Prof. Carla Oliveira	CWG Team	Porto.Comprehensive Cancer Center, Porto, Portugal & i3S/Ipatimup, Porto, Portugal
Rita Magenheim	CWG Team	Germany / Hungry
Dr Emma Woodward	GENTURIS	St Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
Prof. Elke Holinski Feder	GENTURIS	Medizinisch Genetisches Zentrum, München, Germany
Prof. Stefan Aretz	GENTURIS	University Hospital, Bonn, Germany

Prof. Maurizio Genuardi	GENTURIS	HCP Fondazione Policlinico Universitario A. Gemelli, Roma, Italy
Dr. Ignacio Blanco	GENTURIS	Institut Català de la Salut, Barcelona, Spain
Prof. Jan Lubinski	GENTURIS	Pomeranian Medical University - University Clinical Hospital, Szczecin, Poland
Dr. Hector Salvador	GENTURIS	Hospital Sant Joan de Déu, Barcelona, Spain
Dr. Laurence Brugières	Paediatric Oncologist	Institut Gustave Roussy, Villejuif, France
Dr. Christian Kratz	Paediatric Oncologist	Hannover Medical School, Hannover, Germany
Dr. Suzette Delaloge	Breast Cancer Oncologist	Institut Gustave Roussy, Villejuif, France
Prof. Lennart Blomqvist	Radiologist	Diagnostic Imaging Unit, Karolinska University Hospital, Stockholm, Sweden
Pan Pantziarka	Community Representative	The George Pantziarka TP53 Trust, U.K.

6.2. RATING THE QUALITY OF THE EVIDENCE FOR EACH OUTCOME ACROSS STUDIES (IN ACCORDANCE WITH GRADE)

The ERN GENTURIS **Heritable *TP53* Related Cancer (h*TP53*rc) syndrome** 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.

6.3. FORMULATING AND GRADING STATEMENTS

The guidelines were elaborated on the basis of 337 published articles extracted from Pubmed, 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]. Additional articles were requested from experts in the field and references of all the articles were considered. As is typical for many rare diseases, the volume of peer-reviewed evidence available to consider for these guidelines was small and came from a limited number of articles, which typically reported on small samples or series. To balance the weight of both published evidence and quantify the wealth of expert experience and knowledge, we have used for evidence grading the following scale: (i) *strong evidence*: consistent evidence and new evidence unlikely to change recommendation and expert consensus; (ii) *moderate evidence*: expert consensus or majority decision but with inconsistent evidence or significant new evidence expected and (iii) *weak evidence*: inconsistent evidence AND expert agreement.

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 to address are often clear, however the magnitude of the benefit of a specific recommendation are often not as clear. Therefore, 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 Probably Not, Should 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 informed people would take this action, but a substantial minority would not

6.4. INTERNAL AND EXTERNAL REVIEW

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) Syndrome Guideline Development Group engaged with the European Journal of Human Genetics as an independent review of the guideline.

ERN GENTURIS first published the Guidelines for the identification of individuals who should be tested for germline disease-causing *TP53* variants and for their subsequent clinical management in 2019.

6.5. TIMELINE AND PROCEDURE 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.

6.6. FUNDING AND FINANCIAL SUPPORT

This guidelines document was developed with the financial support of the European Commission. No external sources of funding and support have been involved. ERN GENTURIS is one of the 24 European Reference Networks (ERNs) approved by the ERN Board of Member States. The ERNs are co-funded by the European Commission. EU funding is limited to administrative assistance and travel and meeting expenses. No honoraria or other reimbursements have been provided. For more information about the ERNs and the EU health strategy, please visit http://ec.europa.eu/health/ern

Below is a summary of the funding organisations for the members of the Core Writing Group.

LEAD	ROLE	FUNDING ORGANISATION
Prof. Thierry Frebourg	Core Writing Group Chair	Rouen University Hospital, Rouen, France Rouen University and Inserm, France
Prof. D. Gareth Evans	Core Writing Group Clinical Member	Manchester Universities Foundation Trust, Manchester, U.K. Some consultancy advise for Astrazeneca.
Ass. Prof. Svetlana Bajalica Lagercrantz	Core Writing Group Clinical Member	Hereditary Cancer Unit, Karolinska University Hospital, Stockholm, Sweden
Prof. Carla Oliveira	Core Writing Group Clinical Member	Porto Comprehensive Cancer Center, Porto, Portugal & i3S/Ipatmup, Porto, Portugal

7. RECOMMENDATIONS

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		
Rec. 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	**Strong Evidence**

Rec. 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**	**Moderate Evidence**
Rec. 3	Patients who develop a **second primary tumour,** within the **radiotherapy field of a first core *TP53* tumour which occurred before 46 years, should** be tested for germline *TP53* variants	**Moderate Evidence**
Rec. 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**
Rec. 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		
Rec. 6	**Adult first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be systematically offered testing** for the same germline *TP53* variant	**Strong Evidence**
Rec. 7	**The testing in childhood,** from birth, of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should be systematically 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)**

Rec. 8	**The testing in childhood** of **first-degree relatives** of individuals with germline disease-causing *TP53* variants **should *not* be systematically 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 • 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	**Moderate Evidence**
Rec. 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 31 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		
Rec. 10	**In children, clinical examination** should be performed **every 6 months,** with specific attention to signs of virilization or early puberty, and measurement of arterial hypertension	**Moderate Evidence**
	In adults, **clinical examination** should be performed **annually** with specific attention, in patients who received radiotherapy, to occurrence **of basal cell carcinomas** within the radiotherapy field	**Moderate Evidence**
Rec. 11	In **adults, WBMRI without gadolinium enhancement** should be conducted **annually**	**Strong Evidence**
Rec. 12	In individuals with **high cancer risk *TP53* variants** or **previously treated by chemotherapy or radiotherapy, WBMRI** without gadolinium enhancement, should be conducted **annually,** from **birth**	**Moderate Evidence**
Rec. 13	In **female individuals from 20 years** onwards, **breast MRI,** should be conducted **annually**	**Strong Evidence**
Rec. 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**

Rec. 15	In **children from birth,** and **adolescents** (< 18 years), **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**
Rec. 16	In **adults until 50 years, brain MRI** should be conducted **annually**	**Moderate Evidence**
Rec. 17	In individuals with **high cancer risk *TP53* variants, brain MRI** should be conducted **annually** from **birth**	**Moderate Evidence**
Rec. 18	If surveillance includes brain MRI, at least the **first (prevalence) scan** should be conducted using **dedicated brain MRI** with gadolinium enhancement	**Moderate Evidence**
Rec. 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**
Rec. 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**

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 germline *TP53* variants cause 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 the identification in individuals of germline disease-causing *TP53* variants and of the recognition of h*TP53*rc and to elaborate evidence-based counseling strategies addressing family communication, coping strategies, family planning, as well as cancer prevention. Since carriers of germline disease-causing *TP53* variants entail 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. 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.

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

8. WHAT DO OTHER GUIDELINES STATE?

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.

9. SUGGESTIONS FOR FUTURE RESEARCH

The evidence base for screening and surveillance for some organ systems in this guideline are, as always when it 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 2020, the priorities of research in the field of heritable *TP53*-related cancer syndrome include:

- **Identification of genetic variants that could modify cancer risk in germline disease-causing *TP53* variant carriers.** This is critical to understand the genetic bases of the penetrance variability of germline disease-causing *TP53* variants, to predict the cancer risk in germline disease-causing *TP53* variant carriers and to personalize in the future their follow-up. Biomarkers of tumour risks can also include epigenetic alterations.
- **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 functional assays** able to **quantify in medical practice the biological impact of the *TP53* variant**. These functional assays can correspond to high-throughput assays testing all the possible *TP53* variants or to reliable personalized assays, 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.
- **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 efficiency of brain MRI are insufficient.
- **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.

- **Evaluation of the impact of the surveillance protocols on patients' survival.**
- **Adaptation of conventional therapies and development of new therapeutic strategies for h*TP53*rc syndrome**. 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 might 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 behavioral impact of h*TP53*rc syndrome**.
- **Elaboration of evidence-based counselling strategies** addressing family communication, coping strategies, family planning, as well as cancer prevention.

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APPENDIX – PLAIN LANGUAGE SUMMARY

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. If these variants are present in all body cells, they are called "**germline variants**". This is different from somatic variants which are only present in tumour tissues. Some germline variants in 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 "**heritable *TP53*-related cancers (h*TP53*rc) syndrome**". 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 "**germline disease-causing *TP53* variants**". The guideline builds on the internationally recognised approach to testing for *TP53* changes, known as the "Chompret criteria".

Diagnosis of **h*TP53*rc syndrome** is mainly performed by cancer geneticists, adult or paediatric oncologists. Diagnosis of **h*TP53*rc syndrome** 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. **Germline disease-causing *TP53* variants** can be detected in cancer patients either with or without familial history of cancers.

Individuals carrying **germline *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 disease-causing *TP53* variants should take place before starting treatment**. And if a disease-causing *TP53* 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 syndrome guideline** has been created to assist healthcare professionals provide the most up-to-date approaches to diagnosis and surveillance of **cancer-free individuals** and **cancer patients** who carry **disease-causing *TP53* 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 **the identification of individuals who should be tested for germline disease-causing *TP53* variants**, testing of their **first degree-relatives** and for **surveillance (**screening for cancer) **of individuals** with a germline **disease-causing *TP53* variant.**

GUIDELINE SUMMARY

Surveillance for people with germline disease-causing *TP53* variants					
Exam	Periodicity	Age to start	Age to end	Condition	Evidence*
Clinical examination with, in children, specific attention to signs of virilisation or early puberty and measurement of blood pressure and, in patients who received radiotherapy, to occurrence of basal cell carcinomas within the radiotherapy field	Every 6 months	Birth	17 years		Moderate
	Annual	18 years	-		Moderate
Whole-Body MRI without gadolinium enhancement	Annual	Birth	-	High cancer risk *TP53* variant** or patient previously treated by chemotherapy or radiotherapy	Moderate
		18 years	-		Strong
Breast MRI	Annual	20 years	65 years		Strong
Brain MRI***	Annual	Birth	18 years	High cancer risk *TP53* variant	Moderate
		18 years	50 years		Moderate
Abdominal ultrasound	Every 6 months	Birth	18 years		Strong
Urine steroids	Every 6 months	Birth	18 years	When abdominal ultrasound does not allow a proper imaging of the adrenal glands	Weak
Colonoscopy	Every 5 years	18 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

*This grading is based on published articles and expert consensus.
**A germline disease-causing *TP53* variant should be considered as "high risk" if 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 first scan should be conducted with I.V. Gadolinium enhancement; in children, brain MRI should alternate with the Whole-Body MRI, so that the brain is imaged at least every 6 months.

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 medulloblastom**; or**Jaw 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

Pre-symptomatic Testing Recommendations for people without cancer
Adult first-degree relatives of individuals with germline disease-causing *TP53* variants **should be systematically** 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 systematically 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 systematically 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 31 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.

PSYCHOLOGICAL NEEDS

Germline disease-causing *TP53* 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.

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European Reference Networks

https://ec.europa.eu/health/ern_en

European Reference Network
for rare or low prevalence complex diseases
Network
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