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Bone sarcomas: ESMO-PaedCan-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up

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Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up

Incidence and epidemiology

Primary bone tumours are rare, accounting for <0.2% of malignant neoplasms registered in the EURO CARE database [1]. Different bone tumour subtypes have distinct patterns of incidence, and are each no more than 0.3 cases per 100,000 per year. Osteosarcoma (OS) and Ewing sarcoma (ES) have a relatively high incidence in the second decade of life, whereas chondrosarcoma is commoner in older age.

OS is the first primary cancer of bone (incidence: 0.2–0.3 per 100,000 per year^[Hans1]). The incidence is higher in adolescents (0.8–1.1 per 100,000 per year at age 15–19), where it accounts for >10% of all solid cancers. The ~~male:female~~^{male: female} ratio is 1.4:1. Most osteosarcomas of younger patients arise in an extremity, while the proportion of axial tumour sites increases with age. Risk factors for the occurrence of OS include previous radiation therapy, Paget disease of bone [2], and germ-line genetic abnormalities associated with Li–Fraumeni syndrome, Werner syndrome, Rothmund–Thomson syndrome, Bloom syndrome, and hereditary retinoblastoma [3].

Chondrosarcoma (CS) is the most frequent bone sarcoma of adulthood. The incidence is about 0.2 per 100,000 per year^[Hans2], ^{with}_[p3] a median age at diagnosis between 30 and 60 years.⁵⁷ No gender predominance has been reported.

ES is the third commonest primary malignant bone tumour. It occurs most frequently in children and adolescents, but is also seen in adults. Median age at diagnosis is 15 years and there is a male predilection (1.5:1). In white Caucasians under the age of 25 years, ES has an incidence of 0.3 per 100,000 per year [4], and it is even rarer in the African and Asian population. The genetic basis for the difference between ethnical groups has been recently linked to a common genomic germline variant, which extends a microsatellite, thereby facilitating the binding of the EWSR1-FLI1 chimeric protein to the *EGR2* gene locus, leading to higher expression of the transcription factor early growth response 2 (*EGR2*) and increased susceptibility to ES {Grünewald, T.G.P. et al. Nat. Genet. 47, 1073–1078; 2015}. The genetic basis for this difference

has been recently linked to modifier genetic loci based on GGAA repeats associated with EGR2 gene and to loci located nearby the TARDBP gene [p4]. The most common ES primary sites are the extremity bones (50% of all cases) followed by pelvis, ribs and vertebra. However, any bone can potentially be affected and a soft-tissue origin is also possible, especially in adults [p5].

Chordomas are even rarer compared to other subtypes, with an incidence of ~0.5 per million per year [5].

High-grade spindle/pleomorphic sarcomas of bone: Spindle cell sarcomas of bone are a heterogeneous group of primary malignant bone tumours that do not fulfil the histological criteria for a diagnosis of osteosarcoma, chondrosarcoma or Ewing's sarcoma (ref: 74 Pakos 2011).

[p6]-

Giant cell tumor of bone is a benign intramedullary bone tumor composed of mononuclear cells and osteoclast-like multinucleated giant cells, with a variable and unpredictable potential for aggressive growth. It represents approximately 5% of primary bone tumors and about 15-20% of benign bone tumors. (ref: Picci et al., Atlas of musculoskeletal tumors and tumorlike lesions; Klenke FM et al, Giant cell tumor of bone: risk factors for recurrence. Clin Orthop Relat Res. 2011;469(2):591–9.)
Giant cell tumor of bone [p7]?

Diagnosis and pathology / molecular biology

The medical history should focus on characteristic symptoms characteristics such as duration, intensity and timing of pain. The presence of persistent non-mechanical bone pain, predominantly at night, should ~~cause concern and~~ prompt a radiological assessment. Swelling and functional impairment can be present if the tumour has progressed through the cortex and distended the periosteum, but they are often later signs. The differential diagnoses of a bone sarcoma lesion includes s osteomyelitis, benign tumours and bone metastases, all of which outnumber primary bone sarcomas [6-8]. The diagnosis can be strongly oriented by the age of the patient. Under 5 years, a destructive bone lesion could be interpreted predominantly as either metastatic

neuroblastoma or Langerhans cell histiocytosis (LCH)~~eosinophilic granuloma~~. Above 5 years, the likelihood of a primary bone sarcoma is higher. In adult patients, after 40 years of age, bone metastases and myeloma will be the most common diagnoses [9].

Conventional radiograph in two planes is the first radiological investigation. When the diagnosis of malignancy cannot be excluded with certainty on radiographs, the next step should be magnetic resonance imaging (MRI) of the whole compartment with adjacent joints, which is regarded today as the best modality for local staging of extremity and pelvic tumours [10]. Computed tomography (CT) ~~can~~may provide additional information, by allowing a better visualization of calcifications, periosteal bone formation, ~~medullary extension~~_[p8] and cortical destruction, but is not routinely required.

All patients with a bone lesion which is likely to be a primary malignant bone tumour on a radiological basis should be referred to a bone sarcoma centre or to an institution belonging to a specialised sarcoma network [11-14]. Children and adolescents should be referred to centres which in addition provide age-specific expertise. The biopsy and the pathological diagnosis require expertise in the field and should be discussed in a multidisciplinary setting.

The biopsy of a suspected primary malignant bone tumour should be carried out at the reference centre by either the surgical team who will perform the definitive tumour resection or by a dedicated interventional radiologist [11-14]. In most patients, a core-needle biopsy, taken under imaging control, can be an appropriate alternative to open biopsy. The contamination of surrounding tissue should be minimized and adequate multiple sampling of representative areas must always be provided. To ensure that the tissue is representative of the tumour, X-rays of the biopsy location are recommended and in some circumstances ~~tumor imprints (touch preps) or~~ a frozen section may be required_[p9]. ~~Since~~When osteomyelitis is in the radiological differential diagnosis, a potential differential diagnosis, samples ~~should~~may be sent for microbiological culture_[p10] if sufficient material is available in all cases_[JB11]. If an open biopsy is needed_[p12], it should be carried out using a longitudinal incision. In aggressive and malignant tumours of bone, the biopsy tract and the channels through which drains have been placed must be considered to be potentially contaminated and must later be removed

together with the resection specimen, in an effort to minimize the risk of a local recurrence. Therefore, biopsy tracts should be clearly marked by means of a small incision or an ink tattoo to ensure that the location is recognised at the time of the definitive procedure. In cases of spinal column involvement, laminectomy or decompression should be avoided unless necessary to relieve spinal cord ~~compression,compression~~ and tissue sampling must be performed whenever a bone sarcoma is suspected.

Samples must be interpreted by an experienced bone sarcoma pathologist, in collaboration with the radiologist, and discussed in a multidisciplinary team. The request form should be filled with all the details that might be relevant for diagnosis, including patient's age, the site of the tumour, radiological findings, if there are multiple lesions, family history, and, for surgical specimens, ~~eventual~~ preoperative treatments.

With the increasing capability for accurate molecular diagnosis and next generation ~~DNA-deep~~ sequencing technologies of DNA or RNA, samples should be quickly submitted for pathological assessment. The collection of fresh frozen tissue is strongly encouraged, to enable molecular diagnostics. As an alternative, decalcification in EDTA instead of formic acid can be considered. Upon arrival^[JB13], and before formalin fixation, T tumour imprints (touch preps) can be considered taken^[p14] ^[JB15] (might be useful for tumour-specific translocation by fluorescence in situ hybridisation, FISH in some institutions), ~~and tissue/cell suspensions should be kept frozen in cryomolds for later DNA and RNA extraction~~. A further option is to establish primary cell cultures for cytogenetics and other studies, such as patient-derived xenografts within research protocols^[p16] with informed consent. ~~The collection of fresh frozen tissue and tumour imprints (touch preps) is encouraged, because future current molecular pathology assessments might be performed in the patient's interest.~~ Informed consent for tumour banking should be routinely sought, enabling later analyses for research, depending on local regulations.

The nature of the bone specimen received for pathology reporting should be recorded, i.e. needle biopsy, curettage or excision (e.g. segmental resection, limb salvage amputation, or other complex resection, such as a hemi-pelvectomy). It is usually necessary to decalcify the bone tumour biopsy using specific standard operating

procedures. The histological features of the tumour should be described and the tumour type (and subtype) specified according to the 2013-most recent version of the World Health Organization (WHO) Classification^[15]. The results of relevant ancillary investigations (e.g. immunohistochemistry or molecular assessments) should be accurately recorded^[16]. Molecular diagnostics techniques currently used include FISH, RT-PCR, and next generation sequencing technologies. Examples include translocation detection in ES and mesenchymal CS. The latter have revealed diagnostic information for OS, ES translocation types, histone subtype mutations in giant cell tumour of bone isocitrate dehydrogenase (IDH1 and IDH2) mutations in and driver mutation frequency in conventional chondrosarcoma, and MDM2 amplification in parosteal and intramedullary low grade osteosarcoma and are strongly recommended in the diagnostic work-up of any small round cell bone sarcoma.^[JB18]

For surgical specimens, the size (measured in three dimensions in mm) of the tumour in the resected bone should be recorded. The pathology report should also describe the extent of local tumour spread, including involvement of specific anatomical soft tissue and bone compartments. It should be recorded whether the resection margins are either clear or infiltrated and the distance (in mm) of tumour from the nearest resection margin measured. Photographs should be taken of the intact specimen and of the tumour slabs after sawing. A complete, representative slab of the tumour, usually in the longitudinal axis as guided by the radiological images, should be embedded for microscopy in a grid-manner. This is especially relevant after neoadjuvant chemotherapy to assess response. The tumour should be coded using Systematic Nomenclature of Medicine (SNOMED) or International Classification of Diseases for Oncology (ICD-O) codes^[p19].

Staging and risk assessment

All cases of suspected bone tumours should be formally discussed ~~o~~ⁱn a multidisciplinary teambasis with the radiologist who has interpreted the imaging, the pathologist who has reviewed the biopsy material, the surgeon, the radiation and the medical and/or pediatric oncologist. The output of the multidisciplinary discussion must be recorded, in order to minimise the risk of errors in diagnosis, staging, risk assessment and treatment.

Several staging systems for bone tumours are in use [17, 18]_[p20]. However, none of them is perfect or generally accepted. Generally, tumour burden (volume) and the presence of detectable metastases are the two main factors that are taken into consideration in the clinical staging of these diseases. General staging should be carried out to assess the extent of distant disease, including bone scintigraphy, chest radiographs and CT [19]. Whole-body MRI and positron emission tomography (PET)/CT or PET/MRI are increasingly utilised for staging (including skip bone lesions) [20]_[p21]. Additional appropriate imaging studies and biopsies can be taken from suspicious sites, as the exact staging of the disease has an impact on treatment and outcome. No specific laboratory tests for the diagnosis of bone sarcoma are routinely available. Baseline serum analysis in ES and OS should include alkaline phosphatase (AP) and lactate dehydrogenase (LDH) given their proven prognostic value [21, 22].

Next_[p22] generation sequencing technologies performed on tumor tissue have revealed diagnostic information for OS_[JB23]-ES and other round cell sarcomas (translocations), mesenchymal chondrosarcoma (Hes related family BHLH transcription factor with YRPW motif 1 - nuclear receptor coactivator 2 HEY1-NCOA2), types, histone subtype mutations in giant cell tumour of bone and conventional driver mutation frequency in chondrosarcoma (IDH) and giant cell tumor of bone (H3 histone family member 3A (H3F3A)).

A pathological fracture may lead to the dissemination of tumour cells into surrounding tissues and increase the risk of local recurrence. In cases of fracture, internal fixation is contraindicated as it disseminates the tumour further into both bone and soft tissues and increases the risk of local recurrence. External splintage is recommended_[p24].

Chemotherapy can result in renal, cardiac and auditory dysfunction. Before starting the treatment, baseline renal function testing, assessment of cardiac function and audiogram (in the case of platinum derivatives) should be performed. Sperm storage is recommended for male patients of reproductive age. For female patients, a fertility physician should be consulted about potential for_[p25] ovarian sampling and cryopreservation.

Treatment (locoregional and advanced disease)

Given the rarity of the disease and the complexity of management, the accepted standard for bone sarcomas is treatment at reference centres and/or within reference networks able to provide access to the full spectrum of care and age-specific expertise. In these centres/networks, therapy is usually given within either the framework of prospective, often collaborative, clinical studies or established treatment protocols. In the case of high-grade osteosarcoma, ES or pleomorphic sarcoma, following biopsy proven-diagnosis, primary dose intense ^[JW26] chemotherapy ^[p27] is indicated.

osteosarcoma

Osteosarcoma usually arises in the metaphysis of a long bone, most commonly around the knee in children and adolescents. Involvement of the axial skeleton and craniofacial bones is primarily observed in older patients. High-grade osteosarcoma frequently metastasizes, the lung being the most frequent metastatic site by far, followed by distant bones.

Conventional osteosarcoma is always a high-grade ~~malignancy, and accounts for 75% of all high-grade osteosarcomas~~. Low-grade central and parosteal osteosarcoma are low ^[p28] grade malignancies though may grow large and invade the medulla of bone, and transform to high grade sarcoma, whereas periosteal osteosarcoma is an intermediate-grade chondroblastic osteosarcoma, sometimes difficult to distinguish from high grade surface osteosarcoma. Adverse prognostic or predictive factors for conventional osteosarcoma include detectable primary metastases, axial or proximal extremity tumour site, large tumour size, elevated serum AP or LDH and older age [21] ^[II] ^[AF29] I, B. As mentioned above, sStaging should include local imaging studies, specifically plain radiographs and MRI of the whole affected bone ^[p30] extremity ^[p31].

Curative treatment of high-grade osteosarcoma consists of chemotherapy and surgery. Compared with surgery alone, multimodal chemotherapy treatment of high-grade localised osteosarcoma increases disease-free survival probability from 10%–20% to >60%. In general, chemotherapy is administered before and after surgery, although a formal proof that giving chemotherapy preoperatively improves survival is lacking. The extent of histological response to preoperative chemotherapy predicts

survival [21, 23, 24]. ~~Altering postoperative chemotherapy in poor responders to preoperative systemic therapy proved ineffective, yet was associated with increased acute and late toxicity in a large prospective randomized trial.~~ [JW32]

Low-grade central and parosteal osteosarcoma are malignancies with a lower metastatic potential, which are treated by surgery alone [LOE IIIIV, GOR B]. Although chemotherapy has been used for periosteal osteosarcomas, no benefit for chemotherapy was shown in two retrospective analyses [25, 26], and its use should be discouraged in this setting (LOE IV, GOR D).

Surgery should be carried out by a surgical team familiar with the wide range of surgical reconstructive options. Pediatric and adolescent patients need to be treated by surgeons with great experience in the field of pediatric bone tumors, including age-specific reconstruction challenges, such as the reconstruction of growing bones. The goal of surgery is to safely remove the tumour and yet preserve as much function as possible, striving to obtain microscopically clear surgical margins [24]. Most patients should be considered candidates for limb salvage. Either intra-lesional or marginal margins increase the local relapse rate, which is associated with reduced overall survival (OS). Thus, clear margins are the first goal of surgery [III_[AF33], B]. Areas where there is suspicion of close margins should be marked on the surgical specimen sent to pathology.

Pathological fracture does not necessarily necessitate an amputation. In chemo-sensitive tumours, primary neoadjuvant chemotherapy can be used with the expectation that a good response it [p34] will allow the fracture haematoma to contract and allow subsequent resection of the tumour and the involved soft tissues [27].

Doxorubicin, cisplatin, high-dose methotrexate, and ifosfamide, the latter usually combined with etoposide [JW35][p36], have anti-tumour activity in osteosarcoma [28-31] [I, A]. [AF37] Doxorubicin, cisplatin and high-dose methotrexate (MAP) are most frequently used as the basis of treatment [31] [II, A]. These drugs should be administered with adequate supportive care by experienced paediatric oncologists or medical oncologists at reference institutions with appropriate infrastructure and a multidisciplinary treatment approach [29]. Most current protocols include a period of pre-operative chemotherapy,

to facilitate local surgical treatment and to allow the assessment of tumour response [32, 33] ~~although this has not been proven to entail a survival benefit per se over postoperative chemotherapy alone~~ [JW38]. Treatment is commonly given over periods of 6–10 months [31]. The EURAMOS 1 prospective trial aimed to establish whether pegylated interferon $\alpha 2b$, in addition to standard MAP chemotherapy given postoperatively, could improve outcome in patients with good histological response to preoperative MAP. The results showed that many patients failed to start and complete interferon treatment, and there was overall no significant survival advantage [LOE I, GOR 4C][34]. Also, the study evaluated if altering postoperative chemotherapy in poor responders to preoperative systemic therapy might have any impact on outcome, and again no survival benefit was proven. In case of poor pathological response to preoperative MAP regimen, the postoperative addition of ifosfamide and etoposide to MAP failed to improve the survival and increased the risk of secondary malignancy compared to those patients treated with MAP regimen only (LOE I, GOR D)[34]. Whenever possible, patients with osteosarcoma should receive chemotherapy in the context of prospective studies.

Innate immune-modulation has been attempted in OS with some agents, e.g. interferon [35] and muramyl tripeptide. The use of interferon failed to show a survival advantage in patients ~~good responders with a good histological response~~ to a MAP preoperative regimen [p39]. Muramyl tripeptide added to postoperative chemotherapy was associated with a ~~significant~~ substantial advantage in overall survival OS and a non-significant trend in event-free survival in one large randomised trial [36, 37] [LOE II, GOR C] [36, 37]. Muramyl tripeptide has been approved in Europe for patients <30 years of age with completely resected localised osteosarcoma, ~~but it is not reimbursed in all European countries~~. There is no consensus in the sarcoma community on the use of this drug, because of weaknesses of the only trial available [36]. Further studies are definitely needed to identify any subgroup of patients who could benefit from immune modifying agents.

Dynamic MRI is reliable to evaluate changes in tumour vascularity and to give additional information on tumor response to primary chemotherapy [38, 39] [III, AF40] B. Similarly, the value of diffusion MRI is currently under evaluation [38].

Radiation^[p41] therapy should not be considered in cases where surgery with clear margins can be performed. When surgery cannot achieve wide margins, radiation therapy is recommended to try to extend the progression-free interval This should preferably be discussed in a multidisciplinary team beforehand and with the patient, it should be clear at the time of surgery that the goal is not an R0 resection. New radiation therapy techniques (e.g. proton beam and carbon ion therapy) should be considered particularly for unresectable primary tumours.

The multimodal treatment principles detailed above were generated in children, adolescents and young adults with high-grade central osteosarcoma, but also relate to adults ~~at least up to the age of 60~~^[p42] [40] [LOE III, GOR B]. ~~Adult Older patients (>40 years)~~ may require tailored regimens, especially as far as high-dose methotrexate is concerned, in particular for those aged >40. Some studies have put a threshold of 25 to remove HDMTX from the induction regimen (40b^[p43]). Doxorubicin plus cisplatin and/or ifosfamide are commonly used with age-adapted doses. Recently the addition of zoledronic acid was tested in a randomized setting and -failed to demonstrate an improvement for -relapse-free or overall survivalRFS, OS^[p44] or histological response. Its use is therefore not recommended outside of a clinical trial (LOE I, GOR D).

~~High-grade craniofacial osteosarcoma should be treated the same way as high-grade osteosarcoma of other locations, although evidence is lacking due to the absence of selective clinical studies in this patient population [IV, C]. Radiation therapy, if available proton beam/carbon ion radiation therapy, should be considered, preferably within clinical studies, when complete surgery is unfeasible.~~ Primary metastatic osteosarcoma patients are treated with a curative intent following the same principles of non-metastatic osteosarcomas^[41]. In fact, there are subsets of patients who can have a very similar prognosis to that of localised disease, provided surgical removal of all known metastatic deposits is achievable^[42] [III, B]. Approximately 25% of all patients with primary metastatic osteosarcoma and >40% of those who achieve a complete surgical remission may become long-term survivors.

High-grade craniofacial osteosarcoma should be treated the same way as high-grade osteosarcoma of other locations, although prospective evidence is lacking due to the

absence of selective clinical studies in this patient population [LOE IV, GOR C]. The delivery of all chemotherapy before surgery, using PET monitoring to ensure sustained response^[p45], has possible advantage in this population and others in who the recovery from very complex or morbid surgery may not allow timely reintroduction of post-operative chemotherapy^[43]. Radiation therapy, if available proton beam/carbon ion radiation therapy,^[p46] should be considered, preferably within clinical studies, when complete surgery is not unfeasible. The value of proton beam/carbon ion radiation therapy in this setting is currently under study.

The management of recurrent osteosarcoma needs to take into account the timing of recurrences/metastases, the number of metastases and the metastatic sites. CT scan can over- and under-estimate the number of pulmonary metastases, but the recent results have improved with spiral CT. The treatment of recurrent osteosarcoma is primarily surgical in the case of isolated lung metastases. Complete removal of all metastases must be attempted [LOE III, GOR B], as the disease is otherwise almost universally fatal, while more than a third of patients with a complete second surgical remission survive for >5 years ^[44]. Even patients with subsequent recurrences may be cured as long as recurrences are resectable, and repeated thoracotomies are often warranted ^[44]. For lung metastases, sStereotactic radiotherapy, radiofrequency ablation or cryotherapy for lung metastases might be used as an alternative options in patients unfit for surgery. Some groups also consider radiofrequency ablation (Debaere 2015, Saumet 2015) and stereotactic radiotherapy (Yu 2017) potential alternative local treatment options for small size lung or bone metastases.

The role of second-line chemotherapy for recurrent osteosarcoma is much less well defined. Treatment choice may take into account the prior disease-free interval, and often includes ifosfamide or cyclophosphamide, possibly in association with etoposide and/or carboplatin (LOE III, GOR B), and other active drugs including gemcitabine and docetaxel (LOE IV, GOR C) and sorafenib if available (LOE III, GOR B)^[p47]. In the two largest reported series, the use of second-line chemotherapy correlated with limited prolongation of survival in patients with inoperable metastatic recurrences, while a positive correlation in operable disease was observed in only one of the two ^[42, 44]. However radiologic responses and clinical benefit are commonly witnessed so that its use should always be considered^[p48]. Treatment choice may take into account the prior

disease-free interval, and often includes ifosfamide ± etoposide ± carboplatin, and other active drugs (e.g. gemcitabine and docetaxel; sorafenib).

Radiation therapy, including internal radiotherapy targeting areas of increased bone turnover, may have a role in palliation [45]. In general, despite second-line treatment, the prognosis of recurrent unresectable disease has remained poor, with a long-term post-relapse survival of <20%.

Ewing sarcoma

ES is a small, blue, round-cell tumour, periodic acid-Schiff positive and CD99 (MIC2)-positive. All ES are high-grade tumours. They can arise both from bone and soft tissues, displaying the same behaviour in principle.

The definitive diagnosis is made by biopsy, providing a sufficient material for conventional histology, immunohistochemistry, molecular pathology and biobanking. Molecular biology studies have shown that almost all of these tumours share a common TET-ETS gene rearrangement involving the EWSR1 gene on chromosome 22 [46, 47]. In most cases, this involves a reciprocal translocation t(11;22)(q24;q12) [48], but t(21;22)(q22;q12) [49, 50] and others may also occur [t(7;22), t(17;22), and t(2;22)]. In the last years, new small round cell sarcoma entities have been recognized to have a Ewing-like histopathology pattern, with novel translocations, among which such as BCOR-rearranged sarcoma preferentially affects the bone CCNB3 (in the bone), and CIC-FOXO4 or CIC-DUX4 (mainly in the soft tissues). EWS RNA binding protein 1 - nuclear factor of activated T-cells 2 (EWSR1-NFATc2), FUS RNA binding protein - nuclear factor of activated T-cells 2 (FUS-NFATc2), capicua transcriptional repressor – forkhead box O4 (CIC-FOXO4) or capicua transcriptional repressor – double homeobox 4 (CIC-DUX4). EWSR1-NFATc2, FUS-NFATc2, CIC-FOXO4 or CIC-DUX4 translocations are other examples of recurrent molecular alterations found in these malignancies. Current investigations have shown that tumour biology and prognosis actually differs from that of classical ES, making the diagnosis and identification of these cases mandatory. In a minority of tumours with Ewing-like histopathology pattern, novel translocations have been identified such as BCOR-CCNB3, EWSR1-NFATc2, FUS-NFATc2, CIC-FOXO4 or CIC-DUX4. Further investigations implicate that tumour biology may differ from classical ES. However,

given the low incidence of these rare tumors, ~~no final conclusion is possible as to how their approach should be superimposable~~ currently there is little evidence to support different approaches to their management. Inclusion in prospective registries is worthwhile (EURACAN G1^[p51]^[p52]?).

Although most ES can be recognised with classical haematoxylin and eosin (H&E) stain and immunohistochemistry, ~~CD99 expression, molecular confirmation is mandatory. When EWSR1 and FUS are not rearranged, further workup for other, rare round cell sarcomas including BCL6 corepressor (BCOR) rearranged round cell sarcoma is required~~^[p53]. ~~CIC, and BCOR gene rearrangement detection is mandatory~~^[JB54] in all patients with small round cell sarcomas including^[p55] CD99, EWS translocation detection is recommended in all patients and mandatory when the clinical-pathological presentation is unusual, or the histological diagnosis is doubtful [II, B]. A reference laboratory for ES diagnosis should have access to different techniques including both FISH, and reverse transcription polymerase chain reaction (RT-PCR) or as well as next generation sequencing^[JB56] [50]. The laboratory should be enrolled in an external quality assurance programme. ~~RT-PCR is the investigation of choice~~^[JB57] When frozen tissue is available, techniques that identify both fusion partners, i.e. RT-PCR or anchored multiplex PCR based targeted NGS are the techniques of choice, although RNASeq. The latter can also be applied to non-decalcified or EDTA decalcified FFPE. ~~is increasingly being utilised, and~~ FISH is a good choice ~~only~~ when ~~only~~ formalin-fixed paraffin-embedded tissue (or touch preps (imprints)) are available. There are several commercial sources for EWSR1 EWS break-apart probes. Assays using EWSR1 break-apart probes do not detect EWS-FLI1 fusions, but only EWSR1 EWS rearrangements, which should not be a problem when interpreted in the appropriate clinical and pathological context. Next generation sequencing should be considered when no typical translocation has been detected by conventional methods. Detection of rare translocations is important especially in the context of other rare round cell Ewing like small round blue cell sarcomas with more aggressive clinical course carrying CIC or BCOR rearrangement the CIC-DUX4 fusion gene. However, differential diagnosis versus other sarcomas carrying EWSR rearrangements may be challenging^[p58].

Bone marrow biopsies and aspirates (from sites distant to the primary or known metastatic lesions) may be considered in the staging, in the face of a very low incidence of bone marrow metastases in localised disease, especially if PET scan ~~was carried out~~ is negative^[p59]. The added prognostic value of molecular positivity over light microscopic evaluation has not yet been proven [**LOE IV, GOR C**].

Between 20% and 25% of patients are diagnosed with metastatic disease (10%: lung—10%: bones/bone marrow—5%: combinations, or others)^[51, 52]. Staging must be oriented to detect lung, bone and bone marrow metastases^[p60]. Multiple bone metastases confer a poorer outcome than lung/pleural metastases (<20% compared with 20%–40% 5-year survival). Other known prognostic factors are tumour size or volume, serum LDH levels, axial localisation or older age (>15 years). A poor histological response to preoperative chemotherapy and incomplete or no surgery for local therapy are further adverse prognostic factors^[22, 53-57] ~~[III, AF61, B]~~. Molecular structure of fusion transcripts has not been shown to be of prognostic value with current treatment protocols, ~~with the exception of~~ CLC-DUX4 translocation^[EdA62] ~~[BB(CM63)[p64][JB65]]~~. (59new) Genomic analysis with the assessment of copy number variation has been shown to be of prognostic value^[58, 59]. In addition Stag2, TP53 and CDKN2A mutations confer poorer outcomes. With surgery or radiotherapy alone, 5-year survival was <10%. With the currently recommended multimodal approaches including chemotherapy, survival is ~60%–75% in localised and ~20%–40% in metastatic disease, respectively, depending on metastatic sites and burden.

Current trials employ 3–6 cycles of initial combination chemotherapy after biopsy, followed by local therapy, and another 6–10 cycles of chemotherapy usually applied at 2- to 3-week intervals. Treatment duration is thus 10–12 months. Agents considered to be most active include doxorubicin, cyclophosphamide, ifosfamide, vincristine, dactinomycin and etoposide^[60-64]. Almost all active protocols are based on five- to six-drug combinations of these substances [**LOE I, GOR A**]. Chemotherapy intensity (interval compression) ~~is~~ was positively associated with outcome in pediatric and adolescent (<18 years) patients^[p66] in a prospective North American study.

~~High-dose chemotherapy with haematopoietic stem-cell transplantation...~~ The use of high dose chemotherapy with escalated alkylating agent dose and blood stem cell

rescue has attracted much attention in ES since the 1970s [65]. Only recently have the results of randomised studies with busulfan and melphalan indicated that this approach results in a survival advantage for tightly defined and highly selected patients with poor response to induction [Hans67] chemotherapy (LOE II, GOR A)[66]. No such advantage was evident for patients presenting with pulmonary metastases (LOE II, GOR D).

IIA

In contrast to OS, ES_[p68] is a more radiosensitive tumour than OS. -The goal of local therapy for the primary tumour is to ensure that the entire volume of tissue involved at diagnosis is treated. Complete surgical excision, where feasible, is regarded as the best modality of local control, given the higher risk of local recurrence when radiotherapy is used as the sole treatment of the primary tumour. Surgery must involve excision of all tissues originally involved with tumour, not just the tissue that is left after chemotherapy shrinkage or be supplemented by radiotherapy. Radiotherapy alone (in the range of 45–60 Gy, depending on location) should be applied if complete surgical excision is impossible. Postoperative radiotherapy should be given in cases of inadequate surgical margins and discussed when histological response in the surgical specimen was poor (i.e. >10% viable tumour cells) [55] [LOE IV, GOR B]. The dose of postoperative radiation therapy is also 45–60 Gy, depending on margins, response and location. Intralesional surgery must be avoided, as there is no benefit when compared with radiation therapy alone [55]. Change in the size of the soft tissue mass is easily evaluated on MRI and is a good predictor of tumour response [38, 39]. Dynamic MRI is not as reliable as in osteosarcoma [39], as remaining small tumour foci may not be detected. Sequential FDG-PET evaluation might be of additional value [67].

The treatment of adult patients follows the same principles as for ES in typical age groups. However, tolerability of therapies in older patients needs to be taken into account when transferring treatment protocols conceived for children and patients of age ≤40–50 years. Treatment of patients with extraskeletal ES follows the same principles as for bone ES, thus incorporating chemotherapy in all cases as well as postoperative radiation therapy in most cases, with the possible exception of

superficial lesions. For extraskkeletal ES, postoperative radiation therapy is generally used, with the exception of good prognosis, superficial ES.

Patients with metastases at diagnosis treated with the same treatment approach as patients with localised disease, have a worse prognosis. In patients with lung metastases, whole-lung irradiation may confer a survival advantage [LOE II, GOR C] [56]. [II, B]. The role of surgical resection of residual metastases is less well defined when used alone without chemotherapy.

For patients presenting with extrapulmonary metastases, survival is even worse, less than 20% [68]. Chemotherapy is similar as for localised disease but responses are less durable. Treatment of the primary tumour is often appropriate, especially in the presence of responding metastatic disease. There is no formal evidence either for or against Observational studies indicate no advantage for high dose chemotherapy in this situation, so that its uptake differs between centers. Nand[^{p69}] no randomised studies have been reported for this approach.

Several non-randomised trials have assessed the value of more intensive, time-compressed or high-dose chemotherapy approaches, followed by autologous stem cell rescue. Ongoing [JW70]

~~In patients with lung metastases, whole lung irradiation may confer a survival advantage [II, B]. The role of surgical resection of residual metastases is less well defined when used alone without chemotherapy.~~

~~Patients with multiple bone or bone marrow metastases and those with recurrent disease still fare poorly, with 5-year survival rates of ~20%. Despite this, local control of bone metastases with either surgery or radiation therapy is recommended.~~

Recurrent ES, whether local or with distant metastases is almost always fatal even though further responses to chemotherapy are frequent and valuable. The only prognostic factor identified in relapsed patients seems to be time to relapse: patients relapsing later than 2 years from initial diagnosis have a better outcome [69] [III][AF71], B].

Doxorubicin therapy is usually no longer feasible due to previously achieved cumulative doses. Chemotherapy regimens in relapse situations are not standardised and includes alkylating agents (cyclophosphamide and high-dose ifosfamide) ^[70] in combination with topoisomerase inhibitors (etoposide and topotecan), irinotecan with temozolomide ^[LOE III, GOR B] ^[71, 72] or gemcitabine and docetaxel, or high-dose ifosfamide or carboplatin with etoposide ^[71, 72] ^[BJ72]. The relative advantages of these different regimens are currently being tested in an international randomised study ^[p73]. Observational studies of high dose chemotherapy indicate that for selected patients (those with a disease free interval of 18-24 ^[p74] months, with no extra-pulmonary disease and a complete remission before high-dose chemotherapy), use of high-dose chemotherapy can be associated with prolonged survival ^[p75] ^[Hans76]

high-grade spindle/pleomorphic sarcomas of bone

Pleomorphic sarcomas of bone comprise a diagnostically heterogeneous group of malignant tumours including undifferentiated pleomorphic sarcoma ^[16, 73, 74] ^[BJ77]. They arise in a similar age group to chondrosarcoma, but the skeletal distribution is more like osteosarcoma. They typically present with pain and have a high incidence of fractures at presentation. They represent between 2% and 5% of primary bone malignancies. Males are more frequently affected than females. An association with pre-existing disease (Paget's disease or bone infarct) or history of previous irradiation has been reported. It is not unusual for a spindle cell sarcoma to be found to be either a dedifferentiated chondrosarcoma or osteosarcoma after examining further different sections of the resection. Therefore, the diagnosis should be established in a multidisciplinary setting and IDH mutation analysis should be considered when the radiological images suggest a chondrosarcoma.

Pleomorphic sarcomas typically present in older patients with a lytic lesion in bone. In many, the differential diagnosis will be against metastases. Full staging and biopsy are required to reach a diagnosis. Pathological fractures are common and should not undergo internal fixation before biopsy ^[p78]. Treatment strategies mimic those of osteosarcoma, with chemotherapy and complete en bloc resection including any soft tissue component. Their sensitivity to chemotherapy is poorly known and studies on specific histologies, as currently defined (especially after reappraisal of histologies previously known as MFH), are highly required ^[p79]. Radiation therapy may be considered

in inoperable lesions. A global effort to collect these cases would be helpful to establish diagnostic and prognostic criteria as well as recommended treatments.

Histology-driven?

Chondrosarcoma

Most chondrosarcomas arise as primary malignant tumours. The majority are low grade, locally aggressive, non-metastasising tumours (atypical cartilaginous tumour / chondrosarcoma grade I), rather than high grade (grades II–III) [75]. Grade 1 chondrosarcomas can be labelled atypical cartilaginous ~~hondrogenic~~ tumours, as it is currently defined by the WHO 2013, [Hans80] since they usually do not metastasise (ref to be added 78new). ~~One should be aware that grade~~ Grade I chondrosarcomas may be treated with radiation therapy when located at critical sites such as the skull base. Most chondrosarcomas arise centrally in the metaphyseal region of long bones, but they can also develop in flat bones such as pelvis, rib and scapula. High-grade chondrosarcoma frequently arises in the axial skeleton and long bones. Chondrosarcoma can arise in pre-existing benign lesions such as enchondroma and osteochondroma. In these circumstances, they are referred to as secondary central chondrosarcoma and secondary peripheral chondrosarcomas, respectively. The majority of chondrosarcomas are of the conventional subtype, but rarer subtypes include mesenchymal and clear-cell chondrosarcoma [76, 77]. In rare circumstances, conventional chondrosarcomas can ‘dedifferentiate’ into a very high-grade tumour with a dismal prognosis: the so-called de-differentiated chondrosarcoma [76, 77]. Most chondrosarcomas are solitary, but they can occur as multiple lesions in syndromic patients with multiple osteochondromas and enchondromatosis.

~~Most chondrosarcomas present with a painless mass.~~ Pain at the site of a cartilaginous lesion may be an indicator of malignancy. In the case of chondrosarcoma, a contrast-enhanced MRI can reveal high-grade areas. This provides a useful guide to the site of biopsy [78]. For large axial and pelvic chondrosarcoma, heterogeneity is common and most lesions contain high grade elements. The differentiation between benign enchondroma or osteochondroma and malignant [JB81] atypical cartilaginous tumor / grade I chondrosarcoma grade I can be difficult, but dynamic contrast-enhanced MRI can give hints [p82]. In the phalanges of the hands and feet, malignancy is extremely rare, but in the other long bones central

cartilaginous lesions should be considered atypical cartilaginous tumour low-grade chondrosarcoma unless proven otherwise [76].

Inoperable, locally advanced and metastatic high-grade chondrosarcomas have a poor prognosis [76, 77]. Prognosis depends on histological grade. However, histological classification is subject to variability in interpretation, with grade II and III chondrosarcomas often grouped together even though there is a wide spectrum of outcome and heterogeneity of grade elements within tumours [75]. Also, grade I tumours (atypical cartilaginous chondrogenic tumours) are not necessarily curable in all cases, mainly due to problematic local recurrence or progression to high grade. In particular, dedifferentiated chondrosarcomas are aggressive and frequently metastasise [76].

Assessing the grade of chondrosarcomas is difficult and discrepant diagnoses are common even among experts [75]. Atypical cartilaginous Low-grade cartilage tumours are unlikely to metastasise, but may recur locally. Atypical cartilaginous tumours Grade I central chondrosarcomas in the long bones of the limbs can be managed by curettage with or without local adjuvant (e.g. phenol, cement and cryotherapy), with a high chance of success. Low-grade peripheral chondrosarcomas (arising from osteochondromas) should be surgically excised, aiming to excise the tumour with a covering of normal tissue over it. Higher grade chondrosarcomas (Grade II and III) and all chondrosarcomas of the pelvis or axial skeleton should be surgically excised with wide margins.

Evidence suggests that mesenchymal chondrosarcoma is more sensitive to chemotherapy and therefore are usually considered for adjuvant or neoadjuvant therapy (LOE IV, GOR B) [79, 80] [V, B]. Most authors suggest a Ewing-type chemotherapy regimen.

Dedifferentiated chondrosarcoma is often treated as a high-grade bone sarcoma, with therapies that need to be adapted to patient's age [81, 82] [LOE V, GOR C]. There is a very high risk of local recurrence following excision of dedifferentiated chondrosarcoma, particularly in the presence of a pathological fracture. If wide

margins cannot be reliably achieved with limb salvage, then amputation should be considered.

The role of radiotherapy in chondrosarcoma is limited, but may be appropriate in highly selected cases or for palliation. Excellent outcomes have been reported for skull base chondrosarcomas with high-dose radiation therapy, including proton beam or carbon ion radiotherapy, achieving 80%–90% local control rates [83].

With regard to chemotherapy, drugs active in sarcomas, [doxorubicin](#), [ifosfamide](#), may prove active in chondrosarcoma, especially [in](#) high-grade lesions [\(81, 87new\)](#). The activity of gemcitabine in combination with taxotere has been reported [84], and a potential role for mTOR inhibitors in combination with cyclophosphamide has been suggested [IVC].

giant cell tumour of bone

Giant cell tumour (GCT) of bone is a [relatively](#) rare, locally [aggressive progressive](#) tumour of the skeleton. [GCT is Although](#) classified [in the intermediate category as](#) [benign as](#), GCT can be aggressive and recurs locally in up to 50% of cases. [Soft tissue extension is significantly associated with the risk of local recurrence.](#) Up to 5% of GCTs metastasise to the lungs and [spontaneous](#) transformation to a high-grade malignancy occurs in 1%–3% of patients. [Giant cell tumors of bone contain mutations in the H3F3A gene \(predominantly at the G34 position\) which can be detected using mutation analysis or immunohistochemistry using mutation specific antibodies.](#) [The molecular driver of GCT are mutations in histone H3.](#)

Treatment options include intralesional curettage with or without adjuvant or en bloc excision. ~~Radiotherapy can provide a satisfactory local control in GCT (5-yr control rate 80%)[85]. However, the use of radiotherapy can be associated with a risk of GCT transformation into a high-grade sarcoma and can make surgical resection challenging if required. Therefore, the use of radiation therapy in GCTs should always be discussed in a multidisciplinary setting and limited to cases in which surgery leads to unacceptable morbidity and denosumab is ineffective or contra-indicated (LOE IV, COR D).~~ Denosumab, a human monoclonal antibody to RANKL, known to be overexpressed in GCT, is standard treatment in large or unresectable or metastatic

GCT ([LOE III, GOR A](#)). There is increasing evidence that, if being used [preoperatively](#)^[p83], prior to curettage, surgery is best carried out after a few months treatment, as otherwise extensive ossification may take place, making it difficult to define the extent of the lesion. It can also be used in unresectable disease and rare metastatic disease. In this setting treatment interruption is usually followed by progression, so that treatment needs to be maintained^[86]. Potential [dental](#)^{[Hans84][p85]} and skeletal side effects need to be monitored (osteonecrosis of the jaw, atypical fractures) ^[IIA]. ^[AF86] ~~There are currently no ongoing trials to define the optimum use of Denosumab~~ The optimal schedule and duration of treatment with denosumab in surgically unsalvageable GCTs is still to be settled, and the possible long-term side effects are still largely unknown.

~~Radiation in selected cases (risk of high-grade sarcoma to be mentioned)~~ Radiotherapy can provide a satisfactory local control in GCT (5-yr control rate 80%)^[85]. However, the use of radiotherapy can be associated with a risk of GCT transformation into a high-grade sarcoma and can make surgical resection challenging if required. Therefore, the use of radiation therapy in GCTs should always be discussed in a multidisciplinary setting and limited to cases in which surgery leads to unacceptable morbidity and denosumab is ineffective or contra-indicated ([LOE IV, GOR D](#)).

[Chordoma](#)^{[JW87][p88]}

Chordomas are very rare tumours, arising from the remnants of the notochord into the sacrum (50%), skull base (30%) and mobile spine (20%). Extrasketal cases have also been reported, but are extremely rare.

Median age is 60 years, but skull base presentations can also affect a younger population, including children and adolescents. ~~Chordoma~~ Chordoma is a low-grade but locally-invasive malignancy. Dedifferentiated cases are observed in 5% of patients. The metastatic potential of chordoma is ~30%. Metastases usually appear late in the natural history of disease, mostly after local recurrence.

Chordoma prognosis is more related to local aggressiveness than to metastases. Chordoma is a tumour showing notochordal differentiation. Brachyury is a transcription factor involved in notochord differentiation and [is it is expression is](#) the diagnostic

hallmark for conventional chordoma [87]. Immunohistochemistry positivity for brachyury is strongly recommended to confirm diagnosis. Dedifferentiated chordomas may lose brachyury expression. [Genomic alterations of brachyury genes \(duplications\) are also frequent in familial and sporadic forms along with activating mutations of the PI3K gene activated mutations \(92new\).](#)

Due to the rarity and long natural history of the disease, the quality of evidence available for more common tumour types is currently beyond reach for chordoma. In fact, only a few phase II trials are available and most published data are from case series and/or retrospective. Chordoma management needs to be carried out at referral centres and/or referral networks, with a multidisciplinary team including expert pathologists and radiologists, surgeons familiar with musculoskeletal tumours and site of surgery, expert radiation oncologists with access to hadron facilities, dedicated medical oncologists and a palliative care team. All diagnostic and therapeutic procedures should be discussed in a multidisciplinary expert team.

MRI is the best modality for local staging. CT scan should be used in the case of diagnostic doubt. Chordoma should be differentiated from benign notochordal lesions and, if radiological appearance is typical for these, biopsy is not recommended unless the lesion changes over time [88]. Preoperative core-needle biopsy is recommended. The biopsy track needs to be included in the surgical resection. In the case of skull base chordoma, preoperative biopsy can be avoided in selected cases [p89].

Tumour location is the most relevant variable to define the primary tumour treatment. The quality of surgical margins is the most important prognostic factor. En bloc R0 resection is standard treatment, when it is feasible and sequelae are acceptable/accepted by the patient, with an expected 5-year recurrence-free survival of 50%. If en bloc R0 resection is not feasible, definitive radiation therapy alone should always be considered as a valid alternative. Local relapse has extremely poor survival rates and local control is rarely achievable. Supportive care should be incorporated into the treatment from the beginning.

For skull base and upper cervical tract chordoma, R1–R2 surgery plus high-dose radiation therapy is the treatment of choice [89-91].

For sacral chordoma, surgery should definitely be offered as the first choice in case chordoma arises from S4 and below. Surgery should always be discussed in the context of other alternatives for tumours originating above S3, since surgery is always followed by important neurological sequelae. Surgery is the primary standard choice for tumours originating from S3, especially if the preservation of S2 roots is possible, as it may result in some neurological recovery (40% of cases) [92-94].

Hadrons, i.e. high-dose protons or carbon ions, are superior to photons physically and in terms of irradiation of non-target lesions, although no randomised trials are available to assess the benefit of hadrons compared with photons in chordoma. Since hadrons allow lower doses to be given to normal tissues, they should be considered the treatment of choice. Advanced technology photons could be used in the case of unavailability or non-accessibility of protons and ions, and every time they show similar dose distribution to the target and critical structures. Due to the relative radiation resistance of chordomas, a high-dose up to at least 74 GyE in conventional fractionation (1.8–2 GyE) for photon and proton therapy is required [83, 95, 96].

Indications for definitive radiation therapy are: unresectable disease; inoperable patients; neurological impairment not accepted by the patient. Radiation therapy should be considered in the case of R2 or R1 resections. The use of adjuvant/neoadjuvant radiation therapy needs to be discussed with the single patient and prospective studies encouraged.

Patients who have local recurrences are unlikely to be cured by any local salvage treatment [90]. In the case of local relapse, the choice of treatment can include surgery and/or radiation therapy and/or systemic treatment, balancing morbidity and quality of life.

For oligometastatic disease, surgery/radiofrequency ablations/stereotactic radiation of metastases can be considered in selected cases. Chemotherapy is inactive. An exception can be high-grade dedifferentiated chordoma (anecdotal responses to chemotherapy have been reported). There is ~~uncontrolled~~ phase II evidence that imatinib can be beneficial in advanced chordoma in terms of progression-free survival

and mainly non-dimensional tumour responses [97]. Its role within the treatment strategy deserves further evaluation. However when other treatments are no longer possible it could certainly be considered if available. There are data on the activity of epidermal growth factor receptor and vascular endothelial growth factor receptor inhibitors. Prospective studies are ongoing.

Sorafenib in chordoma [Hans91].

Link

Personalised medicine synopsis

Biomarker	Method	Use	LOE	GOR
Genomic characterisation	PCR, FISH, NGS	Small round cell sarcoma	III	A

Follow-up, long term implications and survivorship

Follow-up is designed to detect either local recurrence or metastatic disease at a time when early treatment is still possible and might be effective. Follow-up of high-grade tumours should include both a physical examination of the tumour site and assessment of the function and possible complications of any reconstruction. Local imaging and chest X-ray/CT should be the norm. Though strict rules cannot be provided in the absence of any formal validation, a recommended follow-up policy may foresee intervals between checks after the completion of chemotherapy approximately every 3–6 months for the first 2 years; every 6 months for years 3–5; every 6-12 months for years 5–10, and thereafter every 0,5-1-2 years according to local practice and other factors [p92]. Chest-CT, if used instead of chest X-rays, should be performed with low-dose, radiation sparing techniques, particularly in younger patients who will have a higher lifetime risk to experience second, radiation induced malignancies [BB(CM93)].

In the case of low-grade bone sarcoma, the frequency of follow-up visits may be lower (e.g. 6 months for 2 years and then annually^[p94]). Late metastases as well as local recurrences and functional deficits may occur >10 years after diagnosis and there is no universally accepted stopping point for tumour surveillance^[p95].

In ES, where osseous metastases are likely, isotope bone scanning can be used in addition, but its use needs to be weighed against the additional radiation exposure, particularly in younger patients. More modern techniques (e.g. PET or whole-body MRI) are increasingly being adopted into routine practice, but are likely to require further evaluation in clinical trials^[p96].

It is important to evaluate the long-term toxic effects of chemotherapy and radiotherapy^[p97], if appropriate. Monitoring for late effects should be continued for >10 years after treatment, depending on the chemotherapy protocol and radiation used and in conjunction with late effects services when available. Long term cardiac evaluation is of major importance since it has been shown that deterioration of cardiac function can still occur decades after anthracycline treatment (ref).^[p98]

Secondary cancers may arise in survivors of bone sarcomas, either related to, or independent of, irradiation. Secondary leukaemia, particularly acute myeloid leukaemia, may rarely be observed following chemotherapy, as early as 2–5 years after treatment. -Developments in genetic understanding of bone sarcoma point to the importance of ~~may require obtaining a detailed~~ family history and ~~of~~ genetic evaluation in high-risk families^[p99]. Patients with cancer predisposition syndromes (e.g., Li-Fraumeni or Rothmund-Thomson syndromes) require special care and follow-up.

Table 1. Levels of evidence and grades of recommendation (adapted from the Infectious Diseases Society of America-United States Public Health Service Grading System^a)

Levels of evidence

I	Evidence from at least one large randomised, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomised trials without heterogeneity
II	Small randomised trials or large randomised trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case–control studies
V	Studies without control group, case reports, experts opinions

Grades of recommendation

A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, ...), optional
D	Moderate evidence against efficacy or for adverse outcome, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

^a By permission of the Infectious Diseases Society of America[98]

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