

Novel biomarkers in bone sarcomas — diagnosis, treatment selection, and clinical trials

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Abstract

Malignant bone tumors (MBT) are a rare and heterogeneous group of tumors, arising mostly in children. Localized disease is usually treated with surgery, but prognosis worsens in advanced stages. Currently, with limited biomarkers in clinical use, prognosis depends on histological grading and clinical features. However, the use of biomarkers remains inadequate, limiting treatment efficacy and increasing the risk of recurrence and disease progression for patients. Potential biomarkers based on genomics, proteomics, and clinical characteristics are currently entering clinical use in multiple cancers. Biomarker research in MBT faces additional challenges resulting from the rarity of these entities. Emerging biomarker concepts require clinical validation to create robust frameworks for precision oncology. This review of new biomarkers is based on relevant literature from Pubmed, Scopus, and clinictrials.gov databases retrieved in November 2023. At present, the definition of prognostic markers in malignant bone tumors remains challenging. More research is needed, particularly to tailor treatments based on advanced genetic profiling and analysis of individual tumor and patient characteristics. Many newly identified biomarkers have not been clinically validated.

Keywords: biomarker, bone tumor, genetics, sarcoma

Introduction

Primary bone sarcomas [malignant bone tumors (MBT)] are a group of rare, malignant neoplasms, which account for fewer than 0.2% of all malignant neoplasms [1]. The most frequent MBT is osteosarcoma, followed by chondrosarcoma and Ewing sarcoma. Chondrosarcoma is the most common MBT occurring in adults, whereas osteosarcoma and Ewing sarcoma are more frequent in adolescents [2]. The 10-year relative survival rate is 61.9% with the best prognosis for chondrosarcoma [3–6]. In the pediatric MBT population, average 5-year survival rates of 60% to 70% have been reported [5]. Approximately 25%

of MBT patients are initially diagnosed with metastases, which significantly affects their prognosis [7]. Survival statistics change in the case of metastatic disease, as the five-year survival rate is 20–40% in a metastatic setting [2]. However, the survival of MBT patients has improved over the years, reflecting advances in management of the disease [8, 9]. Transitional research in MBT patients is ongoing, and new biomarkers have been proposed for diagnostic, prognostic, and predictive applications.

Biological markers are defined as factors that can be detected and monitored as indicators of normal biological or pathogenic processes, as well as pharmacological responses to a therapeutic intervention [10]. Cancer biomarkers can be secreted by both cancer cells and healthy tissues in response to the ongoing cancer process [11]. Biomarkers may be detected in tumor tissue, in the patient's serum, or in healthy tissues [12]. Cancer biomarkers are classified in multiple ways, regarding the origin or context in which they are

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used. These characteristics are either molecular, cellular, immunological, genetic, or imaging-based [12]. At this point, multiple definitions of biomarkers are used because of their putative applications. Furthermore, a single biomarker can meet multiple criteria for different categories. Thus, while definitions may overlap, it is important to distinguish features that specify particular purposes of their use. Diagnostic biomarkers are used to detect and confirm the presence of a condition; sometimes they can be used also to classify a disease subtype. Prognostic biomarkers are used to identify the probability of a clinical event, such as disease recurrence or disease progression [13]. Predictive markers, on the contrary, allow identifying patients who will or will not benefit from a specific medical intervention [14].

Biomarkers have been used over many years in the evaluation of cancer. Common examples include

CA-125 in ovarian cancer or CA-19.9 in pancreatic cancer as indicators for diagnosing and monitoring the disease course [15, 16]. Currently, laboratory techniques detect specific biomarkers such as hormone-receptor status in breast cancer as a predictor of response to adjuvant and neoadjuvant chemotherapy [17–20]. Recent advancements in this regard include genetic biomarkers, for instance, the Oncotype DX Breast Recurrence Score test, which measures the expression of 21 genes on breast cancer tissues, helping clinicians decide on the most beneficial treatment approach [21]. Other genetic predictive biomarkers have been established over 10 years of studies and grouped in the Pharmacogenomics Knowledgebase [22].

As mentioned before, extensive research based on a multidisciplinary approach is conducted regarding cancer biomarkers (Fig. 1). Due to their diversity and incomplete understanding of molecular drivers of

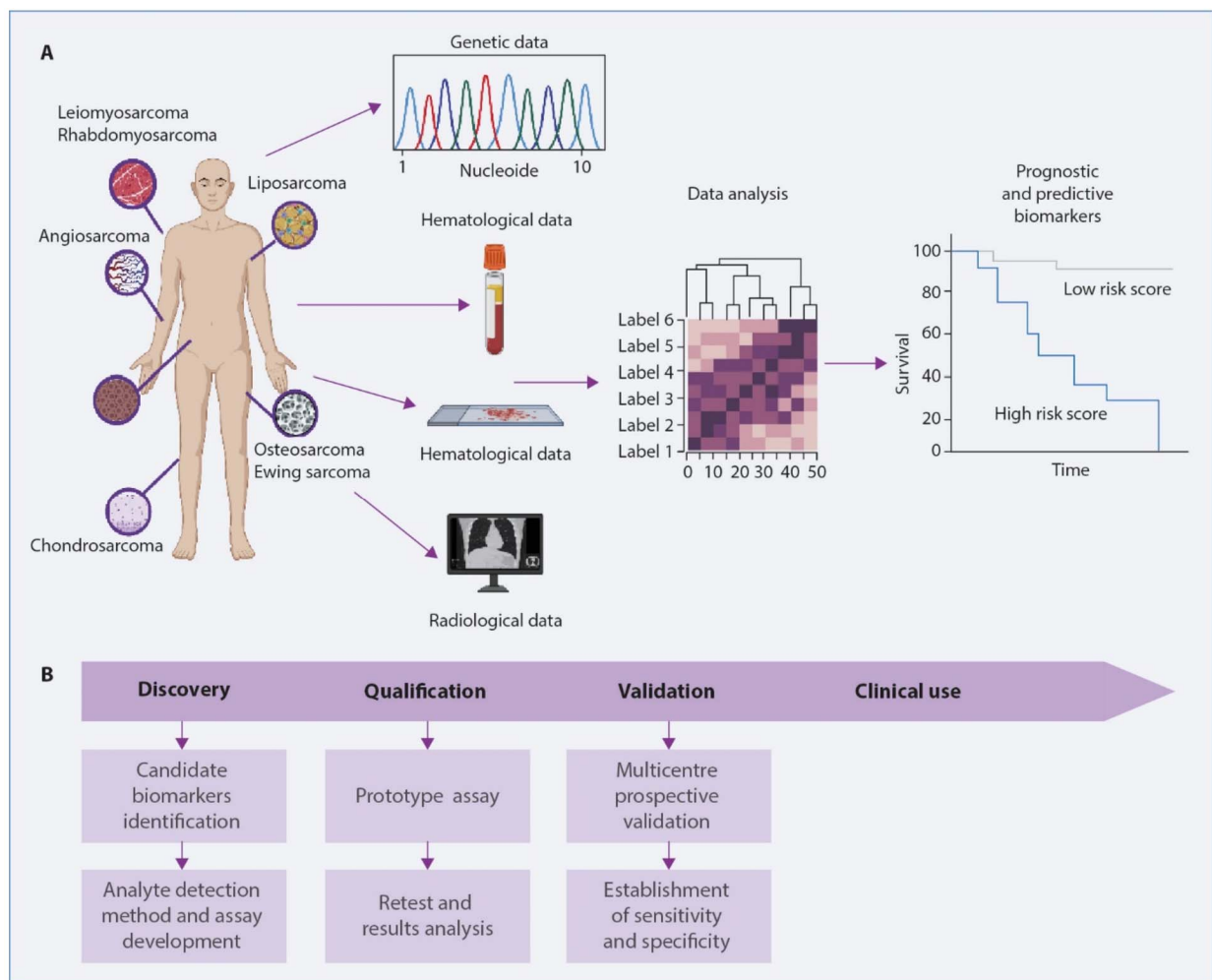


Figure 1. A. Actual usage of prognostic and predictive biomarkers in clinical background; **B.** Research in the development of new biomarkers and their clinical application. The process of establishing a novel biomarker has multiple phases including candidate identification, verification, and validation. Most of the biomarkers for sarcomas mentioned in this review are still in the early phases, and only a few of them entered clinical practice. Considering the heterogeneity of this cancer and natural biological variation, a multidisciplinary approach based on proteomics, genomics, and clinical research should be applied

genesis and progression, sarcomas pose a challenge to establishing consistent biomarkers. Compounds that have been investigated as biomarkers in MBT include, among many serum substances produced by both tumor and healthy cells, genetic features of the sarcomas, circulating tumor DNA (ctDNA), micro-RNA (miRNA), inflammation process markers, and receptors expressed on tumor cells [23]. Due to the key role of patient risk assessment in the classification for specific clinical management of sarcomas, studies validating signatures that can be used to predict disease and treatment response are intriguing. In this review, we discuss currently used and potential biomarkers in sarcomas, with a particular focus on genetic markers.

Clinical biomarkers

Multiple biomarkers can be classified as “clinical”; cost efficiency and high specificity enable their application as guides for clinical management. The actual list of clinical biomarkers, used in various cancers as well as sarcomas can be found in the National Comprehensive Cancer Network Compendium [24].

In MBT patients, the leading clinical prognostic factor is the extent of the disease, which is defined using one of the staging systems for bone sarcoma [25–27], as well as histological subtype and grade assessed according to the World Health Organization (WHO) guidelines [28]. These factors have shown a significant prognostic value [3, 6, 29] and constitute the basis of therapeutic decisions [30, 31]. The necessity to combine these factors led to the development of nomograms for MBT. Nomograms are prognostic instruments that integrate determinant variables and yield a numerical likelihood of a clinical result [32]. Numerous nomograms have been established also for the prognosis of patients with chondrosarcoma [33], osteosarcoma, and Ewing sarcoma [34–36]. These nomograms also utilize such factors as the occurrence of surgery, chemotherapy, or the patient’s age. The age at diagnosis is also an independent predictor of increased survival — patients with Ewing sarcoma, aged less than 28 years have a favorable prognosis [36], as well as patients with osteosarcoma, younger than 24 years [37].

Basic treatment for patients with Ewing sarcoma and high-grade osteosarcoma often consists of surgery, preceded by neoadjuvant chemotherapy [30, 31]; radiologic and pathologic responses of the tumor to the neoadjuvant treatment may constitute prognostic factors. There are various systems for outcome prediction by histological response evaluation in patients with Ewing sarcoma, of which the Bologna system [38] and 100% tumor necrosis threshold [39] showed the highest prognostic values [40]. In a study investigating radiological and histological predictors of the

clinical outcome, the maximal standardized uptake value (SUV) less than 2.5, obtained after neoadjuvant treatment of patients with the Ewing sarcoma family of tumors, was a predictor of an increase in 4-year progression-free survival (PFS) (80% vs. 33%, $p = 0.036$) [41]. So was the favorable histological response, defined as 10% or less viable tumor cells: 4-year PFS was 71% vs. 38% in patients without favorable histological response [41]. Similarly, patients with high-grade osteosarcoma achieving poor histological response ($\geq 10\%$ viable tumor) after neoadjuvant treatment had decreased event-free survival (EFS) [hazard ratio (HR) = 2.13; $p < 0.001$] [42]. In the case of recurrent disease, patients with a longer disease-free interval between surgery and relapse, have a more favorable prognosis. Patients with Ewing sarcoma who experienced recurrence less than 1 year after the surgery, had 6% 2-year post-relapse survival, whereas those, who experienced recurrence later, achieved 88% 2-year post-relapse survival ($p = 0.001$) [43]. Similarly, patients with osteosarcoma, enrolled in three different randomized controlled trials, had increased post-relapse survival if the relapse occurred later than 2 years after the randomization (HR = 0.58; $p = 0.003$) [44].

Currently, there are no specific laboratory tests available in clinical practice for diagnosis and staging of MBT [30, 31]. Serum lactate dehydrogenase (LDH) level has been shown in a meta-analysis to have a significant prognostic value for overall survival (OS) in osteosarcoma patients with pooled HR = 1.87 (1.58–2.2) [45]; as well as alkaline phosphatase (ALP) level, with pooled HR ranging from 1.60 (1.38–1.86) [46] to 1.78 (1.52–2.07) [47]. Similarly, in a meta-analysis including patients with Ewing sarcoma, abnormally elevated LDH level was shown to be a strong prognostic factor for decreased OS — pooled HR = 2.9 (2.09–4.04), and 5-year PFS — pooled HR = 2.4 (1.93–2.98) [48]. Determination of ALP and LDH levels is recommended by clinical practice guidelines in patients with Ewing sarcoma or osteosarcoma during diagnostic investigation, treatment, and surveillance [30, 31]. Moreover, other studies included other characteristics such as the neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), and the lymphocyte-to-monocyte ratio (LMR). A meta-analysis evaluated NLR, LMR, and serum CRP levels [49–53] in patients with different bone sarcoma subtypes and indicated that increased NLR is associated with shorter OS — HR = 1.76 (1.29–2.41) and PFS — HR = 1.77 (1.09–2.88), while increased LMR predicts longer OS — HR: 0.73 (0.57–0.92), but not PFS [51]. On the contrary, platelet-to-lymphocyte ratio (PLR) had no significant prognostic value [51]. In osteosarcoma patients, CPR and erythrocyte sedimentation rate (ESR) are associated with poorer prognosis [53].

Table 1. Selection of emerging blood markers with prognostic value in the context of malignant bone tumors

Study	Reference	Biomarker	Histological subtype	HR	Endpoint
Aggerholm-Pedersen et al.	[50]	ACBS = 1 ACBS = 2	Osteosarcoma, Ewing sarcoma, Chondrosarcoma	2.7 (1.3–5.6) 3.6 (1.8–7.2)	Disease-specific mortality
Yang et al.	[52]	NPS Group 2 NPS Group 3	Osteosarcoma	5.9 (1–6.4) 6.5 (1.2–13.6)	OS
Ouyang, Wang	[54]	SII \geq 565	Osteosarcoma	1.001 (1–1.002)	Cancer-specific survival
De Angulo et al.	[55]	ALC > 500 cells/ μ L after 15 days from 1 st chemotherapy cycle	Ewing sarcoma family of tumors	0.1 (0.02–0.5)	OS

ACBS — Aarhus composite biomarker score; ALC — absolute lymphocyte count; HR — hazard ratio; NPS — Naples prognostic score; OS — overall survival; SII — systemic immune-inflammation index

Table 2. Selection of emerging molecular biomarkers with prognostic value in the sarcomas

Study	Reference	Biomarkers	Biomarker assessment	Histological subtype	HR	Endpoint
Mahmoud et al.	[60]	Survivin expression \geq 50%	IHC	Ewing sarcoma	1.86 (1–3.46)	Event-free survival
Shulman et al.	[61]	Loss of STAG2 expression	IHC	Ewing sarcoma	HR = N/a 5-year EFS: 58.5% (expression) vs. 16.7% (loss)	5-year OS
Abrahamo-Machado et al.	[62]	Brachyury negative staining	IHC	Ewing sarcoma	2.23 (1.24–4)	OS
Ge et al.	[63]	High TIM-3 level	ELISA (obtained from serum)	Osteosarcoma	2.12 (2.01–6.11)	OS
Jiang et al.	[64]	High WNT6 expression	ELISA (obtained from serum)	Osteosarcoma	2.23 (1.06–10.84)	OS

EFS — event-free survival; ELISA — enzyme-linked immunosorbent assay; HR — hazard ratio; IHC — immunohistochemistry; OS — overall survival; TIM-3 — T-cell Immunoglobulin and mucin domain 3; WNT6 — WNT family member 6;

Less frequently used potential hematological biomarkers for MBT are summarized in Table 1.

Molecular biomarkers

Molecular biomarkers are a broad group of compounds ranging from small molecules and nucleic acids to cells. Their common feature is that they might be discovered using genomics or proteomics [56]. This section will mainly discuss biomarkers accessed by proteomics methods, detected in patients' serum or tissues [57, 58]. Genomic features will be mentioned in the following sections.

Currently, there is no molecular biomarker for bone sarcoma that is recommended in routine clinical practice, most potential biomarkers are at the stage of retrospective studies and still need validation for this indication [30, 59] although molecular studies should be considered [31]. The most extensive research includes protein expression such as p53, Ki-67, SOX2, and markers of hypoxia and angiogenesis. Less applicable biomarkers and future research directions are summarized in Table 2 [60–64].

Moreover, in MBT, certain molecular biomarkers can be identified within tumor tissue thus potentially enhancing pathological assessments. The prognostic significance of such biomarker — VEGF expression — has been demonstrated in patients with osteosarcoma, indicating worse outcomes if the VEGF expression was higher — HR = 1.75 [95% confidence interval (CI) 1.21–2.28] [65]. This can be explained by the key role of VEGF in mediating angiogenesis and, therefore, facilitating tumor progression [66]. Similarly, shorter OS and disease-free survival (DFS) in patients with Ewing sarcoma and osteosarcoma have been associated with high expression of p53 in the tumor tissue [47]. In osteosarcoma patients, it was also found, that high expression of Ki-67 not only indicates poor prognosis but also predicts the development of distant metastases — HR = 3.04 (1.51–6.12) [48]. In patients with Ewing sarcoma, high expression of SOX2 (determined by immunohistochemistry) was — independently of other prognostic factors — associated with decreased overall survival and correlated with increased risk of tumor relapse [49]. High

Table 3. Selection of emerging genetic biomarkers of malignant bone tumors

Study	Reference	Histological Subtype	Biomarker	Biomarker assessment	HR	Comments
Bennani-Baiti et al.	[71]	Ewing sarcoma	<i>CXCR4</i> and <i>CXCR7</i>	qRT-PCR	8.0 for OS, $p = 0.02$	High expression of both <i>CXCR4</i> and <i>CXCR7</i> correlates with the shortest OS
Ohali et al.	[72]	Ewing sarcoma	High and low-risk subsets of genes	cRNA oligonucleotide microarray; qRT-PCR	N/A	Higher expression correlates with poor prognosis 72-month PFS = 100 vs. 12.5%; $p = 0.002$
Liu et al.	[73]	Ewing sarcoma	> 1 OncoPanel gene (standard panel of cancer genes)/ <i>TP53</i> / <i>STAG2</i> mutation	Whole genome sequencing	2.51 (1.01–6.26) (subhazard ratio for time to progression)	Trend toward significant impact on OS was also observed — HR = 2.32 (0.93–5.83); $p = 0.07$

FISH — fluorescence *in situ* hybridization; HR — hazard ratio; N/A — not applicable; OS — overall survival; PFS — progression-free survival; RT-PCR — reverse transcription polymerase chain reaction

CCT6A expression was also proposed as a prognostic biomarker in patients with Ewing sarcoma [67]. Also, circulating molecular biomarkers (which are easily accessible, given their non-invasive character) were proposed, including high baseline serum levels of IGF-1 and IGF-BP3, which were related to shorter EFS in patients with Ewing sarcoma [68].

Genetic biomarkers

The development of tumor genomic analyses has changed sarcoma classification and currently, on this basis, additional rare subtypes of sarcomas are distinguished. Bone sarcomas encompass disorders such as translocations or activating mutations with simple karyotypes, as well as tumors with multiple genomic rearrangements and large chromosomal gains and losses, commonly involving cell cycle genes e.g. *TP53*, *MDM2*, *RBI*, and *CDK4* [69, 70]. The use of these features in diagnostics and differential diagnosis is known, and work on the prognostic and predictive value of genetic disorders is ongoing, but only some have entered clinical practice. Herein we summarize the most important potential genetic biomarkers in bone sarcomas. Furthermore, other less commonly used or presumed genetic biomarkers in sarcoma are summarized in Table 3 [71–73].

Cytogenetic alterations result in patterns of gene expression, often involving products that control cancer cell proliferation, apoptosis, and metastatic potential, which can determine the prognosis and response to therapy [74]. Alterations that showed biomarker potential can be found in Ewing sarcoma, where nearly 90% of tumors harbor a recurring translocation t(11;22) (q24;q12). That joins the *FLII* and *EWSR1* genes, and the rest of the Ewing family tumors have an alternative translocation involving the *EWSR1* gene [75]. Due to the great heterogeneity of *EWSR1* gene fusions with *FLII* and other genes, multiple

studies accessed variants of chromosomal breaking points and less common fusions such as with *ERG*, *ETV1*, and *ETV4* genes to establish their clinical significance. They concluded the most common mutation covering *EWSR1* exon 7 to *FLII* exon 6 was a significant positive predictor of OS [76–78].

Considering that only some sarcomas have characteristic karyotype abnormalities, research focuses on single-gene mutations and the expression of the proteins they encode as potential future prognostic biomarkers. In MBT genes involved in two main tumor-suppressor pathways showed an impact on patients' survival. *TP53* and *RB* both serve as crucial genes in cancer pathogenesis and progression. Two large meta-analyses indicated their prognostic value in osteosarcoma. Patients with *TP53* mutations showed shorter 2-year OS when compared to the patients with WT *TP53* — relative risk (RR) = 1.79 (1.12–2.84) [79] while mutations resulting in loss of *RB1* function resulted in a higher mortality rate in osteosarcoma — RR = 1.62 (1.23–2.1), a significant increase in metastasis — OR = 3.95 (1.86–8.38), and a reduction in the response to chemotherapy — OR = 0.35 (0.13–0.94) [80]. Genomic analyses of chondrosarcoma pointed out DNA mutations in isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*), yet their prognostic impact is disputed. It was stated that OS for patients with *IDH1/2* mutations was significantly lower than in patients without mutations (93% vs. 64%; $p < 0.001$). Conversely, other studies suggested no influence of those mutations on prognosis [81, 82]. Taking these implications into account, clinical trials with IDH inhibitors have begun, but to date, there have been no consistent results and the predictive role of this mutation remains unknown [83].

The *c-Myc* gene belongs to the *Myc* gene family and encodes a protein that acts as a transcription

Table 4. miRNAs with significant prognostic value in osteosarcoma patients, assessable in the tumor specimen or the patient's serum

	High expression is associated with poor prognosis (HR > 1)	High expression is associated with a favorable prognosis (HR < 1)	Reference
Tumour tissue-derived miRNAs	miRNA-21; miRNA-214; miRNA-29a; miRNA-9; miRNA-148a	miRNA-382; miRNA-26a; miRNA-126; miRNA-195; miRNA-124	[91]
Serum-derived miRNAs	miRNA-152; miRNA-29a; miRNA-29b; miRNA-196a; miRNA-196b; miRNA-221; miRNA-27a; miRNA-191; miRNA-300; miRNA-542-3p; miRNA-194	miRNA-133b; miRNA-206; miRNA-195; miRNA-223; miRNA-326; miRNA-95-3p; miRNA-497; miRNA-491; miRNA-124; miRNA-491-5p; miRNA-101; miRNA-139-5p	[95]

HR — hazard ratio

factor. It regulates cell-cycle regulation and cellular differentiation. In cancer, it is involved in invasiveness and metastatic potential. Aberrations in this gene may include amplification and overexpression, leading to tumor development. Its presence has been documented in angiosarcoma, osteosarcoma, and leiomyosarcoma. The presence of expression detected in IHC and detection of gene amplification are unequivocally associated with poorer prognosis for patients [84]. Early studies have shown that patients with *Myc* amplification have significantly worse OS and DFS compared to those without it [85]. In a study of patients with osteosarcoma, it was confirmed that *Myc* amplification was associated with a worse 3-year OS rate ($p = 0.015$) [86]. Several reports confirm the usefulness of this parameter as a prognostic factor in various subtypes of sarcomas [87, 88]. Also, the *GLI1* gene (zinc finger protein *GLI1* also known as glioma-associated oncogene) encodes the glioma-associated oncogene 1, which is a transcription factor downstream of the sonic hedgehog signaling pathway (*Shh*). The *Shh* pathway is involved in various regulatory processes within the cell. *GLI1* up-regulates target genes, particularly proliferative oncogenes that promote malignant transformation. Although its role in tumorigenesis among sarcomas has been recently discovered, a meta-analysis has shown that overexpression of *GLI1* in solid tumors is associated with worse 3-, 5-, and 10-year OS, as well as DFS [89]. The overexpression of *GLI1* in the Ewing sarcoma vincristine-resistant cell line was demonstrated in the preclinical model indicating possible predictive value [90].

Moreover, the value of several miRNAs, lncRNAs, and ctDNA as biomarkers have been investigated.

Two meta-analyses investigated a prognostic value of tumor tissue specimen-derived miRNA [91, 92] in osteosarcoma patients. However, one of them [92] has been criticized for its methodology [91]. The other one provided information on the prognostic value of 12 distinct miRNAs, of which 10 were statistically significant prognostic factors (Tab. 4) [91]. Expression of 5 distinct miRNAs (miRNA-382, miRNA-26a, miRNA-126, miRNA-195, miRNA-124) was associated with a favorable prognosis, whereas other miRNA's expression indicated a poor prognosis [91]. Similarly, in patients with Ewing sarcoma, several dysregulated miRNAs were identified, however, only one of them — miRNA-34a — yielded prognostic value as an independent factor [93, 94]. Interestingly, one meta-analysis evaluated serum-derived miRNAs as prognostic biomarkers in osteosarcoma patients [95]. It was found that 23 serum-derived miRNAs had a significant impact on patients' prognosis. Twelve of them were associated with a favorable prognosis, while others — with poor prognosis (Tab. 4). Also, several long non-coding RNAs (lncRNAs), detectable in serum, were found to yield a prognostic value. High expression of 91H, TUG1, MALAT1, ATB, and UCA1 lncRNAs correlated with poor prognosis in osteosarcoma patients [96–100]. Serum-derived miRNA, lncRNA, ctDNA, and circulating tumor cells (CTCs) may become valuable sources of information in routine clinical practice, as the liquid biopsy emerges as a promising diagnostic tool, which is non-invasive and easier than collecting additional tumor tissue samples [101]. The prognostic value of quantitative circulating tumor DNA (ctDNA) assessment (namely: personalized — with patient-specific *EWSR1::FLI1* or *EWSR1::ERG* or *EWSR1::CREM*

fusion breakpoint — digital droplet PCR) was demonstrated in patients with Ewing sarcoma, enrolled in the EWING2008 clinical trial [102]. The assessment of the level of ctDNA enabled risk stratification for EFS and OS — the survival outcomes were worse when the level of ctDNA was higher [102]. These findings were in line with previous studies, demonstrating that the detection of ctDNA is associated with worse outcomes in patients with osteosarcoma and Ewing sarcoma [103]. Moreover, promising results were obtained from a study conducted on a small group of 30 osteosarcoma patients, in which the CTC count was found not only to be a prognostic factor, but it also correlated with response to chemotherapy [104]. Notably, a novel method of ctDNA assessment — the analysis of fragmentation patterns — has been investigated in samples from patients with different sarcoma subtypes, mostly Ewing sarcoma [105]. Apart from demonstrating the prognostic significance of the analysis of fragmentation patterns, the authors also postulated, that this method improves the reliability of liquid biopsy, as it enables precise quantification of ctDNA, tumor detection, and classification, independently of genetic aberrations [105]. The other genetic aberration-independent approach to ctDNA analysis — a methylation-based assay — has also been tested in osteosarcoma patients, corroborating the correlation of high ctDNA level with poor prognosis [106]. The great potential strength of genetic aberrations-independent methods for ctDNA detection is their wide applicability in tumors, which do not have specific, recurrent genetic aberrations [105, 106].

Another genetic biomarker — tumor mutational burden (TMB) — is defined as the number of somatic mutations per megabase (mut/Mb). High TMB is associated with a generation of neoantigens, which play an immunogenic role and elicit an antitumor immunological response [107]. In clinical practice, this can be illustrated by frequent responses to immunotherapy seen in patients with TMB-high tumors, leading to Food and Drug Administration (FDA) approval of pembrolizumab (anti-PD-1 immunotherapy) in patients with a high TMB (defined as > 10 mut/Mb) [108–110]. Bone sarcomas, however, were rarely found to exhibit high TMB: for osteosarcoma, the reported median TMB was 2.5 mut/Mb, while only 0.4% had TMB higher than 20 mut/Mb [111]. Overall, in another study in sarcoma patients, the median TMB was 2.4 mut/Mb, with 3.9% of patients having TMB greater than 10 mut/Mb (which would allow for pembrolizumab administration). Interestingly, in this same study, osteosarcoma and chondrosarcoma patients had higher TMB when compared to the majority of patients with other sarcomas, whereas Ewing sarcoma was associated with one of the lowest TMBs

[69]. The TMB was also significantly higher in adult patients, compared to adolescents and young adults [69]. Of note, higher TMB was found to be a negative prognostic factor, indicating a higher risk of relapse, for patients with localized Ewing sarcoma [73]. Another genetic biomarker, predictive for response to immunotherapy, is mismatch-repair deficiency. The dysfunction of mismatch-repair machinery leads to somatic hypermutation and generation of neoantigens, which then are recognized by the immune system, leading to antitumor immunological response [112, 113]. Current evidence does not support clinically significant occurrences of mismatch-repair deficiencies in bone sarcomas. In a recent study, a large cohort of patients with chondrosarcoma (206 patients), osteosarcoma (67 patients), and Ewing sarcoma (19 patients) has been assessed for mismatch-repair deficiency, but it was not found in any of the patients [112].

Biomarkers in clinical trials

Despite the improvement in survival of MBT patients, the efficacy of therapy in MBT patients is still unsatisfactory, as approximately 30% of all MBT patients will die within 5 years from the diagnosis [9]. A promising possibility of improving patients' outcomes is application of precision oncology in routine clinical practice. This approach assumes that each patient is treated with personalized, biomarker-matched therapy to achieve maximum efficacy. In a recent study, patients treated in biomarker-matched, early phase, clinical trials were compared with those treated in unmatched trials. In MBT patients, no statistically significant difference has been observed between patients receiving biomarker-matched and unmatched treatments (unlike in patients with soft tissue sarcomas). However, there was a trend towards improved progression-free survival and OS in biomarker-matched group [114].

Currently, no systemic targeted therapy is approved by the FDA for treatment of patients with malignant bone tumors. Several agents have been tested, including ivosidenib, which is an inhibitor of oncogenic protein called IDH1 that underwent clinical investigation in patients with advanced chondrosarcoma, harboring a gain-of-function *IDH1* mutation [115]. Durable disease control was achieved in several patients treated with ivosidenib, illustrating the benefit coming from the administration of biomarker-matched personalized therapy [115]. Also, cabozantinib, which is an inhibitor of VEGFR2 and MET, was investigated, and it showed antitumor activity in patients with advanced osteosarcoma and Ewing sarcoma [116]. In this study, a biomarker analysis was performed and showed improved OS prognosis in patients with low baseline VEGF-A concentrations (which is in line

Table 5. Selected ongoing prospective biomarker studies in patients with malignant bone tumors

Clinicaltrials.gov identifier	Sarcoma subtypes allowed in participants	Aim of the study
NCT04132895 [118]	Osteosarcoma	Multi-center, prospective, observational study, which collects longitudinal samples from osteosarcoma patients to search for potential tumor tissue-derived and circulating biomarkers
NCT05942456 [119]	Osteosarcoma	Evaluation of soluble B7-H3 as a biomarker of response to the treatment and as a prognostic factor
NCT06068075 [120]	Ewing sarcoma or osteosarcoma	Evaluation of the prognostic potential of liquid biopsy (and ctDNA assessment)

with previous reports [65]), whereas higher baseline concentration of soluble MET was related to improved PFS on cabozantinib [116]. At the same time, none of the investigated circulating biomarkers had prognostic significance in Ewing sarcoma patients [116]. Interestingly, an early metabolic response (assessed by FDG-PET/CT) at the end of the first cycle of cabozantinib also had prognostic significance for patients with osteosarcoma, as well as Ewing sarcoma [116].

A recent pilot clinical trial of Vigil (patient-specific cancer vaccine) plus temozolomide and irinotecan in recurrent Ewing sarcoma patients, besides demonstrating activity of this regimen, supported the application of quantitative ctDNA assessment in response monitoring [117]. The correlation between a decrease in tumor burden and the ctDNA level was observed; however, it was unclear whether an increase in ctDNA level is strictly related to disease progression, as in 2 patients (of 10 enrolled) an increase in ctDNA level was detected without evidence of disease progression [117]. Further research is warranted, as ctDNA is a promising, minimally invasive biomarker for response monitoring. Currently, there are ongoing prospective biomarker studies in patients with malignant bone tumors; selected studies are listed in Table 5 [118–120].

Conclusions

Currently, the care of patients with primary bone sarcomas is facing challenges related to introducing personalized, precision oncology to the routine clinical practice. In the era of biomarker-driven personalization of treatment, the prognostic and predictive factors in bone sarcoma patients represent an unmet need. Recent advances in the field of translational medicine identified several promising biomarkers. Nomograms are predictive tools that combine determinant variables and return a numerical probability of a clinical outcome. Routine clinical ALP and LDH levels are biomarkers used in patients with Ewing sarcoma or osteosarcoma during diagnostic investigation, treatment, and surveillance. Molecular tumor

tissue-derived and circulating biomarkers are emerging as reliable, non-invasive factors for risk assessment, prediction, and monitoring of the response to treatment. Serum-derived miRNA, lncRNA, ctDNA, and CTCs may become valuable sources of information in routine clinical practice, as the liquid biopsy emerges as a promising diagnostic tool. Novel methods of circulating biomarkers, such as ctDNA fragmentation patterns, are expected to improve the applicability of minimally invasive assays. Bone sarcomas harbor targetable genetic aberrations, creating opportunities for personalized targeted therapy, which currently has limited availability outside clinical trials.

Article Information and Declarations

Author contributions

A.M.C.: conceived and designed the analysis, collected the data, wrote the paper, edited the paper, coordinated the project, supervision; P.B., P.C.: collected the data, wrote the paper, edited the paper; P.R.: conceived and designed the analysis, edited the paper, supervision.

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Conflict of interest

All authors declare no conflict of interest.

Supplementary material

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