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Future Directions in the Treatment of Osteosarcoma

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Abstract

Purpose of Review—Overall survival rates for osteosarcoma have remained essentially unchanged over the past three decades despite attempts to improve outcome via dose intensification and modification based on response. This review describes recent findings from contemporary clinical trials, advances in comprehension of osteosarcoma biology and genomic complexity, and potential opportunities using targeted and immune-mediated therapies.

Recent Findings—Recent results from international collaborative trials have failed to demonstrate an ability to improve outcomes using a design in which the randomized question is dictated based on histologic response to preoperative chemotherapy. Novel prognostic markers assessable at diagnosis are vital to identifying subsets of osteosarcoma. Clinical trials focus has now shifted to serial phase II studies of novel agents to evaluate for activity in recurrent and refractory disease. In-depth analyses have revealed profound genomic instability and heterogeneity across patients, with nearly universal TP53 aberration. While driver mutational events have not clearly been established, frequent derangements in specific pathways may suggest opportunities for therapeutic exploitation. Genomic complexity may lend support to a role for immune-mediated therapies.

Summary—Rigorous preclinical investigations are potentially generating novel strategies for treatment of osteosarcoma that will inform the next generation of clinical trials, with the opportunity to identify agents that will improve survival outcomes.

Keywords

osteosarcoma; genomic complexity; TP53; targeted therapy; immunotherapy

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INTRODUCTION

Osteosarcoma is the most common primary bone malignancy of childhood and adolescence, with approximately 400 new cases each year in the United States.[1] The current management of newly diagnosed osteosarcoma includes cycles of neoadjuvant chemotherapy comprised of 3 to 4 cytotoxic agents (cisplatin, doxorubicin, methotrexate, ifosfamide), followed by surgical resection of disease and additional cycles of postoperative therapy. Patients with localized disease have a 65-70% 5-year survival rate,[2] while those who present with metastatic disease (most commonly in the lung parenchyma and distant skeletal sites) experience poor survival rates of 19-30%.[3, 4] Despite numerous attempts in large clinical trials to augment therapy via dose intensification and addition of chemotherapeutic agents, survival rates for osteosarcoma have stagnated over the past three decades.

Current efforts within the Children's Oncology Group (COG) and other independent investigators recognize the limitations of continued investigations of a small number of cytotoxic agents in varied schedule and dose to treat a tumor well known for chemotherapy resistance, and have shifted focus to identifying potential tumor vulnerabilities via genomic aberrations and enzymatic pathways that can be exploited, using novel therapies supported by robust preclinical testing and animal modeling. The purpose of this article is to review the current state of the field as well as opportunities for present and future treatment strategies in osteosarcoma.

THE USE OF STANDARD CYTOTOXIC AGENTS IN THERAPY

Recent international collaborations focused on whether survival outcomes could be changed through modification of adjuvant therapy with the randomized question for each group based on histologic response of the resected primary tumor, a well-established prognostic factor. In order to accrue a large enough patient cohort to complete an appropriately powered study, COG, the Cooperative Osteosarcoma Study Group (COSS), the European Osteosarcoma Intergroup (EOI), and the Scandinavian Sarcoma Group (SSG) developed the EURAMOS-1 (European and American Osteosarcoma Study Group) clinical trial.[5] The two primary objectives were to determine whether the addition of ifosfamide and etoposide to post-operative chemotherapy would improve outcomes for patients who demonstrated a poor response to standard neoadjuvant chemotherapy (defined as ≤90% necrosis of the resected primary tumor), and whether the addition of a 2-year maintenance treatment with pegylated interferon alfa-2b (INF-α-2b) after completion of standard treatment would improve outcomes for good responders. The trial required immense collaborative efforts to navigate regulatory and funding barriers, but proved successful in accruing 2,260 patients over a six-year period.[5]

Results for each of the primary objectives on EURAMOS-1 have now been reported. For good responders, no significant difference was observed between patients who were treated only with standard MAP therapy and those who were randomized to receive INF-α-2b. The analysis was confounded by refusal of a large proportion of randomized patients to start INF-α-2b; of those who received treatment, 39% stopped early, mostly due to toxicity or disease progression.[6] The results of the poor responder cohort were equally disappointing;

no difference in 3-year event-free survival (EFS) was observed between the two randomized arms. Those who received ifosfamide and etoposide experienced significantly greater likelihood of grade 4 non-hematologic toxicities, and were unable to receive the full cumulative doses of chemotherapy. A higher rate of secondary malignant neoplasms was observed although the difference in the arms was not statistically significant.[7]

PROGNOSTIC MARKERS

The results of the EURAMOS-1 trial provide additional evidence that diminishes the value of histologic response as a prognostic marker, with no established ability to impact survival through adjustments of post-operative treatment. As previous literature also demonstrates inability to augment survival curves through dose intensification and increased rate of histologic response, clinical trials should no longer incorporate histologic response as a reference point for randomization of post-operative therapy. An increased focus on osteosarcoma biology, pathway analysis and genetics has provided new potential biologic markers of disease. These include single nucleotide polymorphism (SNP) variants in the *NFIB* gene that affect osteosarcoma cell migration and proliferation, and are associated with metastasis in certain lineages.[8] Several microRNAs have been suggested to impact prognosis; miR-214 is upregulated in osteosarcoma tissues and independently prognostic for progression-free survival and overall survival. A locus at 14q32 associated with miR-382, miR-134 and miR-544 has demonstrated an inverse correlation between aggressive tumor behavior and residual expression of microRNAs.[9, 10] DNA methylation analysis may reveal patterns with prognostic significance.[11] Further evaluation and prospective validation of these markers in future studies will establish their role in prognostication of tumor response and survival outcomes.

A NEW PARADIGM FOR PRECLINICAL DEVELOPMENT AND CLINICAL INVESTIGATION

The EURAMOS-1 trial results provided further proof that improvement of survival outcomes for osteosarcoma would not be achieved through continued adjustments of dose and schedule of the same cytotoxic agents in use for the past thirty years. Concurrently, attempts to develop and conduct large scale clinical trials with novel therapeutic agents have been complicated by three factors: 1) rarity of diagnosis, 2) failure to understand the mechanisms of osteosarcoma biology leading to resistance to most chemotherapeutic agents, and 3) the lack of radiographic regression of bulky lesions with treatment that hinders the ability to measure response by conventional methods. The result of these three factors is the lack of novel agents with activity in the treatment of patients with osteosarcoma. Collaborative efforts have recently focused on providing rigorous preclinical data for the development of new therapeutics before consideration for clinical trials, including a comprehensive understanding of mechanism of action, validation of markers of both exposure and response, and use of animal models (e.g. murine and canine) to assess efficacy.[12] These studies have been supported by the development of the Childhood Sarcoma Biostatistics and Annotation Office, which links patient data to archived tissue samples and provide biostatistical support to researchers.[13] These investigations have begun to yield several promising agents with therapeutic potential, which will be described subsequently.

The need to retrospectively assess prior failures of novel agents was vital to interrogate the standard approach to interpreting clinical response. A pooled analysis was conducted for seven previous phase II trials conducted by COG and its preceding collaborative groups that included strata for recurrent/refractory osteosarcoma patients with measurable disease. The 4-month EFS was 12%; radiographic responses were observed in only 3 of the trials.[14] Recognizing the limitations of traditional use of radiographic response as a primary endpoint for phase II studies for osteosarcoma, current and future planned clinical investigations of novel therapeutics are incorporating evaluations of "controlled stable disease" and will be statistically powered to use this measure as a surrogate for progression-free survival. Furthermore, while osteosarcoma remains a rare diagnosis, the development of serial clinical trials of new therapeutics targeted exclusively toward recurrent and refractory osteosarcoma holds promise for accelerating investigations of drug efficacy. The first phase II trial to be developed under this paradigm included eribulin mesylate, a microtubule inhibitor that demonstrated activity in osteosarcoma cell lines and xenografts in the Pediatric Preclinical Testing Program.[15] While expected to enroll 1.3 patients per month based on prior phase II studies, the trial rapidly accrued 19 patients in 3.5 months.[16] The brief interval observed from enrollment to analysis demonstrates the potential success of such a clinical trial model to expedite the analysis of novel agents for clinical effectiveness, and the willingness of patients to continue to seek new therapies when available.

GENOMIC COMPLEXITY OF OSTEOSARCOMA

As part of efforts to identify targets for novel therapeutic agents, several groups have taken advantage of increasing access to a variety of methods of genomic analysis to analyze osteosarcoma samples via whole genome and exome sequencing, transcriptome evaluation of gene expression, and epigenetic modifications. These investigations have revealed striking genomic complexity, as well as profound interpatient heterogeneity (see **Figure 1** and **Figure 2**). This includes distinct chromosomal regions of hypermutation referred to as "kataegis" and a vast number of structural variations.[17] While p53 aberrancy has long been associated with osteosarcoma via germline mutations in Li-Fraumeni syndrome, recent comprehensive genomic analyses have revealed that nearly all osteosarcomas have alterations of *TP53* or associated pathway genes such as *MDM2;* mutations in *RB1* and associated pathway genes are also frequently present.[17, 18] Whole genome sequencing analysis of 19 osteosarcoma tumors revealed that 55% of patients demonstrated a structural variant inactivating *TP53*, most commonly as a translocation involving intron 1.[17] Other common alterations revealed from these studies include mutations of *DLG2* and *ATRX*[17] and deletions of *CDKN2A/B*; alterations of members of the PI3K/mTOR pathways were also identified in 24% of samples in one cohort.[18] Forward genetic screening using Sleeping Beauty transposon mutagenesis enriched for genes in the PI3K/mTOR, ErbB, and MAPK pathways, as well as previously unknown oncogenes related to osteosarcoma such as *Sema4d* and *Sema6d*, genes associated with axon guidance.[19] Other methods including genome-wide association studies have revealed SNPs within a locus at the *GRM4* gene associated with susceptibility to osteosarcoma,[20] as well as the aforementioned SNP within *NFIB* which may increase risk of metastasis.[8]

While p53 and RB1 are suspected of acting as major oncogenic drivers, the lack of intron 1 rearrangements in other p53-associated tumors suggests pre-existing genomic instability in osteosarcoma that predisposes to structural variations; subsequent events including PTEN loss may accelerate tumorigenesis.[19] Recent data has also suggested a role for DNA methylation patterns in osteosarcoma relapse; analysis of 17 diagnostic biopsy samples revealed increased methylation at more than 17% of tested loci, with a strong association between methylation at the Toll-like receptor 4 gene (*TLR4*) and 5-year EFS.[11] Further understanding of the diverse effects of osteosarcoma's genetic complexity is anticipated from the National Cancer Institute's Therapeutically Applicable Research to Generate Effective Treatments (TARGET) program, which is actively performing integrated multi-platform analyses of osteosarcoma samples.[21] Generation of sufficient genetic and epigenetic data is hoped to enable correlations between genomic changes, tumor biology, and clinical behavior.

TARGETED THERAPIES

Preclinical studies of osteosarcoma biology, genomic and pathway analyses, and drug screening have now provided several therapeutic opportunities to be evaluated in clinical studies (**Table 1**). This includes denosumab, a fully human monoclonal antibody targeting the receptor activator of nuclear factor κB ligand (RANKL). RANK signaling promotes motility and anchorage-independent growth of osteosarcoma cells;[22] transgenic mouse models with alterations of RANKL have been observed to develop osteosarcoma. Preclinical drug screening has also suggested activity of glembatumumab vedotin, which targets the transmembrane glycoprotein NMB (GPNMB; osteoactivin). In PPTP testing, glembaumumab demonstrated maintained complete responses in three of six osteosarcoma xenografts.[23] Further testing demonstrated expression of GPNMB in 92.5% of 67 human osteosarcoma tissue samples and correlation between GPNMB protein expression and *in vitro* cytotoxicity.[24] A third potential target for treatment is the disialoganglioside GD2. Anti-GD2 therapy has improved survival outcomes for patients with high-risk neuroblastoma who have minimal residual disease; based on this success, the chimeric anti-GD2 antibody dinutuximab (ch14.18) was recently approved by the Food and Drug Administration. Nearly all osteosarcomas tumors expressed GD2 by immunohistochemistry in a recent investigation;[25] subsequent analysis showed that in relapsed patients, GD2 expression was universally maintained upon recurrence.[26] Several trials utilizing varied forms of anti-GD2 therapy are currently open (NCT02173093; NCT00743496; NCT02107963; NCT02502786). Clinical trials are currently in development for the use of denosumab, glembatumumab vedotin, and dinutuximab for the treatment of recurrent and refractory osteosarcoma.

As noted above, multiple complementary analyses have implicated PI3K/mTOR as a targetable pathway in osteosarcoma,[18, 19, 27] and that dual inhibition of PI3K and mTOR may abrogate proliferation and induce apoptosis.[27] Several dual PI3K/mTOR inhibitors are in clinical development; combinations of novel PI3K inhibitors such as buparlisib[28] with existing mTOR inhibitors such as everolimus and temsirolimus warrant investigation. The tyrosine kinase inhibitor (TKI) sorafenib inhibits cell growth, angiogenesis and metastasis through inhibition of VEGF and MAPK/ERK pathways[29] and has been utilized

in recent studies as a single agent or in combination.[30, 31] Combination of sorafenib with everolimus increased anti-tumor activity *in vitro* and *in vivo* via abrogation of upregulation of mTORC2.[32] A recent clinical trial of combined sorafenib and everolimus yielded a 6-month progression-free survival (PFS) of 45%,[33] generating increasing interest in development of trials incorporating sorafenib or an alternative TKI with an mTOR inhibitor.

Other druggable targets requiring further preclinical evaluation include cell cycle checkpoints Wee1 and Chk. Use of a Wee1 inhibitor (AZD1775) increases osteosarcoma sensitivity to radiation and enhances cytotoxicity of gemcitabine in cell lines,[34, 35] not only via dysregulation of cell cycle progression and mitotic catastrophe but also through enhancement of replicative stress via reduction of ATR/Chk1.[36] Inhibition of ATR is another novel approach to therapy. Osteosarcoma cells employ alternative lengthening of telomeres to overcome replicative senescence,[37] a process not infrequently associated with ATRX loss, as seen in a number of osteosarcoma samples.[17] ATR inhibition disrupts alternative lengthening and results in chromosomal fragmentation and apoptosis; osteosarcoma cell lines positive for ALT (U2OS, SAOS2) treated with the ATR inhibitor VE-821 demonstrated hypersensitivity and increased cell death compared to cell lines with telomerase activity.[38]

IMMUNOTHERAPY

Immune-based therapy has long been considered to have therapeutic potential for osteosarcoma, as far back as 1891;[39] as the field of immunotherapy has expanded, a greater number of treatment approaches have surfaced that hold potential for treatment of sarcomas via expansion of anti-tumor immunity or induction of durable anti-tumor immune response.[40] Immunomodulatory agents have previously been investigated for osteosarcoma, including INF-α-2b,[6] interleukin-2,[41] and liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE).[2, 42] Efficacy of L-MTP-PE has been difficult to interpret due to potential interaction but was suggested to improve 5-year survival;[42] presently L-MTP-PE is approved for use and available in Europe, Mexico, Turkey and Israel.[39]

The clinical success of immunologic checkpoint inhibitors targeting T cell function via cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 pathway (PD-1, PD-L1) for the treatment of adult tumors such as metastatic melanoma[43] has generated great interest for their use in pediatric tumors. Increased mutational burden has been associated with response in these tumors, with tumor neoantigens considered to be the primary target of efficacious response.[44, 45] While it is unclear whether the types of genetic aberrations frequently seen in osteosarcoma (point mutations, structural variations) will predict response to these agents, the overall high mutation rate (1.2 per megabase)[17, 18] suggests that osteosarcoma is a potentially attractive target for immunotherapy. In addition, recent studies have demonstrated expression of PD-L1 on osteosarcoma cells; blockade of PD-1/PD-L1 improved cytotoxic T lymphocyte function.[46, 47] When evaluated in a K7M2 mouse model of osteosarcoma, antibody blockade of CTLA-4 and PD-L1 produced complete control of tumors and immunity to further tumor inoculation.[48] Trials are currently open via COG using the anti-

PD-1 antibody nivolumab with and without the anti-CTLA-4 antibody ipilimumab (NCT02304458) and the Sarcoma Alliance for Research through Collaboration (SARC) with the anti-PD1 antibody pembrolizumab (NCT02301039).

CONCLUSION

Recent efforts to expand opportunities for treatment of osteosarcoma through rigorous preclinical drug development, comprehensive genomic analyses, and the implementation of a histology-exclusive clinical trial model for investigation of new agents are now beginning to generate a number of therapeutic strategies to be implemented in upcoming studies. We are optimistic that this new paradigm for preclinical and clinical investigation will identify agents with a signal of activity that can be moved forward to new trials in combination with standard chemotherapy, with the goal of improving long-stagnant survival outcomes.

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KEY POINTS

- Recent collaborative efforts to study osteosarcoma in a randomized fashion failed to demonstrate improvement in outcomes by augmenting post-operative therapy based on histologic response to pre-operative chemotherapy
- A new paradigm for osteosarcoma includes rigorous preclinical drug development, understanding of mechanism of action and animal models, paired with a clinical trial model focused exclusively on osteosarcoma with both response and survival endpoints
- Osteosarcoma harbors striking genetic complexity and interpatient heterogeneity; nearly all osteosarcomas have aberrations of the *TP53* pathway
- Several biologic targeted therapies have been identified from recent comprehensive analyses and are being developed for phase II trials to determine efficacy
- Based on the high mutational load, osteosarcoma may be an attractive target for immunotherapy using anti-CTLA-4 and anti-PD-1/PD-L1 therapies

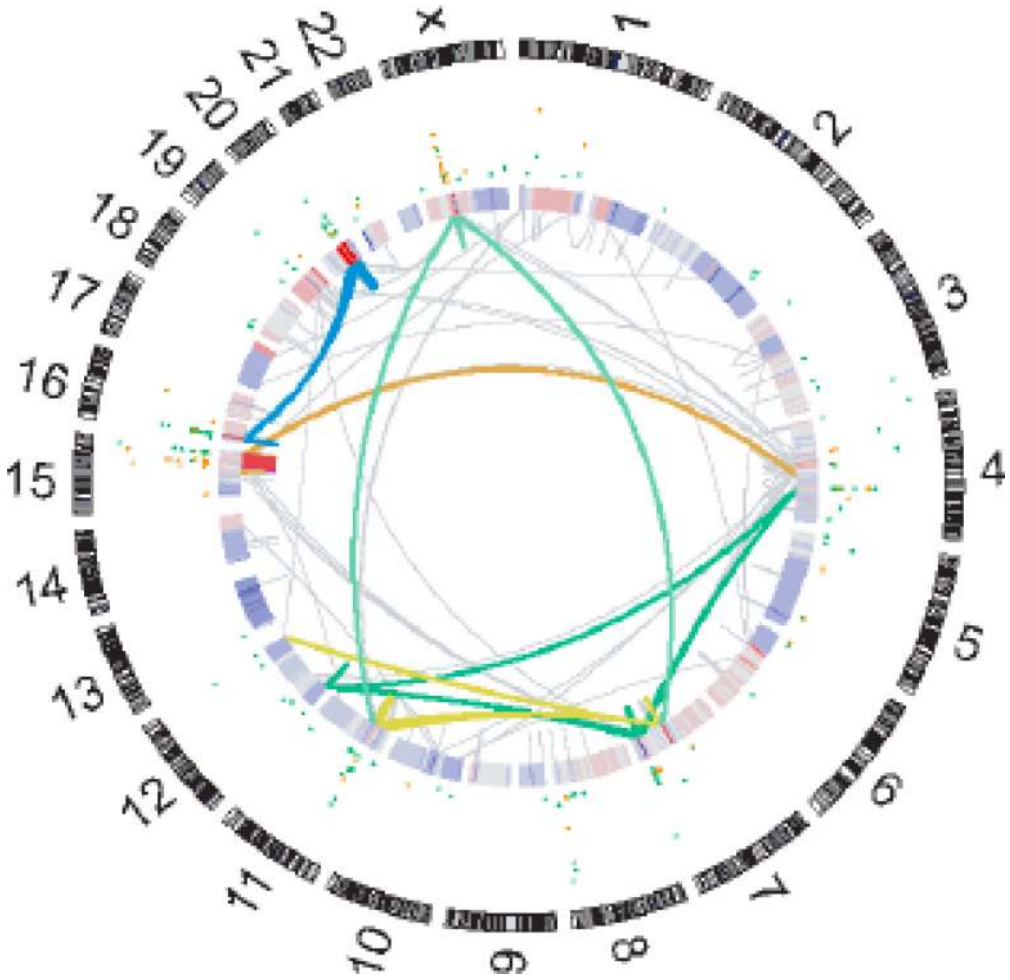

Figure 1.

A CIRCOS plot demonstrates genomic complexity within a single osteosarcoma tumor sample. Chromosomes are depicted on the outer most track. A blue-red heatmap circle indicates copy number ratio estimates from whole genome sequencing. Interchromosomal and intrachromosomal rearrangements are represented by lines and arcs within the heat-map circle. C>T (green) and C>G (yellow) mutations are plotted outside the heat-map circle. An area of numerous C>T and C>G mutations in concert with significant intrachromosomal rearrangements at chromosome 15 suggests a focal area of hypermutation or “kataegis”.

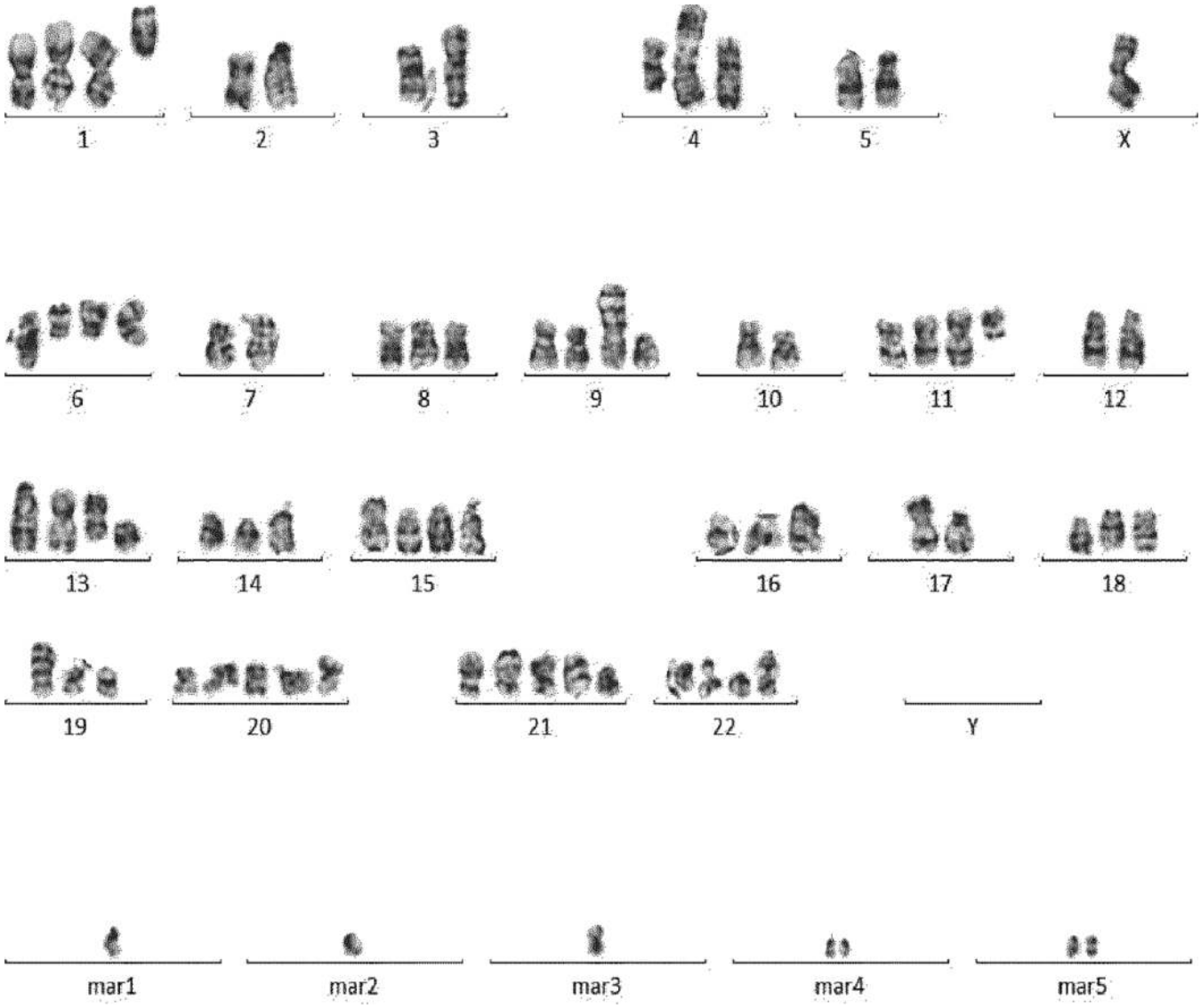

Figure 2.

A karyotype of a patient with osteosarcoma demonstrates multiple structural and numeric abnormalities.

Table 1

A selected list of novel therapeutic agents actively or recently under investigation that have included enrollment for patients with recurrent or refractory osteosarcoma. Clinical trials are listed on www.clinicaltrials.gov.

Therapeutic	Target	Clinical Trial
Eribulin mesylate	Microtubule inhibition	NCT02097238
Inhaled lipid cisplatin	Cytotoxic agent	NCT01650090
Denosumab	Receptor activator of nuclear factor κB ligand (RANKL)	NCT02470091
Glembatumumab vedotin	Glycoprotein NMB (osteoactivin)	NCT02487979
Dinutuximab (ch14.18)	GD2	NCT02484443
Hu3F8	GD2	NCT02502786
Hu14.18K322A	GD2	NCT00743496
GD2-bispecific activated T cells	GD2	NCT02173093
Anti-GD2 chimeric antigen receptor T cells (GD2-CAR.OX40.28.z.ICD9)	GD2	NCT02107963
Bevacizumab	VEGF	NCT00667342
Sorafenib	VEGFR2-3, PDGFR-β, CRAF, BRAF, c-Kit, FLT3	NCT00889057; NCT01804374
Regorafenib	VEGFR1-3, TIE2, PDGFR-β, FGFR, KIT, RET, RAF	NCI02048371
Pazopanib	VEGFR1-3, PDGFR-α, PDGFR-β, FGFR, c-Kit, CSF-1	NCT01956669; NCT01759303
Cabozantinib	MET, VEGFR2, RET, KIT, TIE2	NCT02243605
Sirolimus	FKBP12/mTORC1	NCT02517918
Everolimus (RAD001)	FKBP12/mTORC1	NCT01804374
Liposomal muramyl tripeptide phosphatidylethanolamine (L-MTP-PE)	Immunomodulatory agent	NCT00631631; NCT02441309
Pegylated interferon α-2b (INF-α-2b)	Immunomodulatory agent	NCT00134030
Nivolumab (± ipilimumab)	PD-1 (± CTLA-4)	NCT02304458
Pembrolizumab	PD-1	NCT02301039

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