
Article:
Heymann, D. orcid.org/0000-0001-7777-0669, Heymann, M.F. and Brown, H.K. (2015) Drugs in early clinical development for the treatment of osteosarcoma. Expert Opinion on Investigational Drugs. ISSN 1354-3784

https://doi.org/10.1080/13543784.2016.1237503

Drugs in early clinical development for the treatment of osteosarcoma

Journal:	*Expert Opinion On Investigational Drugs*
Manuscript ID	EOID-2016-0096.R1
Manuscript Type:	Review
Keywords:	Clinical trials, Immunotherapy, Macrophages, Microenvironment, Osteosarcoma, Immunomodulation, Cancer Initiating Cells

Abstract

Introduction: Osteosarcomas are the main malignant primary bone tumours found in children and young adults. Conventional treatment is based on diagnosis and resection surgery, combined with polychemotherapy. This is a protocol that was established in the 1970s. Unfortunately, this therapeutic approach has reached a plateau of efficacy and the patient survival rate has not improved in the last four decades. New therapeutic approaches are thus required to improve the prognosis for osteosarcoma patients.

Areas covered: From the databases available and published scientific literature, the present review gives an overview of the drugs currently in early clinical development for the treatment of osteosarcoma. For each drug, a short description is given of the relevant scientific data supporting its development.

Expert opinion: Multidrug targeted approaches are set to emerge, given the heterogeneity of osteosarcoma subtypes and the multitude of therapeutic responses. The key role played by the microenvironment in the disease increases the number of therapeutic targets (such as macrophages or osteoclasts), as well as the master proteins that control cell proliferation or cell death. Ongoing phase I/II trials are important steps, not only for identifying new therapies with greater safety and efficacy, but also for better defining the role played by the microenvironment in the pathogenesis of osteosarcoma.

Key words: Clinical Trials, Immunotherapy, Macrophages, Microenvironment, Osteosarcoma, Immunomodulation, Cancer-Initiating Cells

Article highlights

- Tumour microenvironment is a key modulator in osteosarcoma development and is the source of new therapeutic targets
- Immunomodulators are promising drugs for controlling refractory and recurrent osteosarcoma (e.g. anti-GD2 therapy)
- Bone cells and bone matrix are two potential new targets for osteosarcoma (e.g. the anti-RANKL antibody, radium-223)
- Nanomedicine has led to the development of a new generation of compounds from "old" drugs (e.g. Nab-paclitaxel)
- Large biological cohorts with relevant clinical annotations are essential for rare tumours and will be an important source of new therapeutic targets

1. General features of osteosarcoma

Osteosarcoma accounts for 50% of all bone sarcomas, and is the most frequent primary malignant tumour found in children and young adults. With a peak of incidence at around 18 years, the male/female sex ratio is 1:4. A second peak of incidence is described in the elderly following radiotherapy, or in conjunction with Paget disease. The metaphyses of the long bones are their preferred development site. The proximal end of the tibia or humerus, as well as the distal end of the femur, is frequently affected. Sixty per cent of all cases of osteosarcoma are detected in the knee [1,2].

Osteosarcoma is part of a large family of heterogeneous histological tumour entities of mesenchymal origin [3-9]. It expresses osteoblastic markers such as the runx2 master gene, alkaline phosphatase, osteocalcin or bone sialoprotein [10,11]. As a result, it has now been recognized that conventional osteoblastic osteosarcoma cells originate from a mesenchymal cell or committed osteoblast in which an initial oncogenic event occurs, followed by secondary genetic alterations [12]. Osteosarcomas are bone forming tumours associated with an osteolytic component which defined according its intensity: osteoblastic, osteolytic or mixed tumour entities. Osteosarcoma is thus a genetically complex disease. A recent study analysing a series of 44 osteosarcoma patients perfectly illustrates their high level of heterogeneity and complexity [13]. As expected, these authors demonstrated recurrent *TP53* and *RB1* somatic alterations and identified 84 point mutations and 4 deletions related to 82 different genes [13]. Similarly, Kovacs *et al.* studied the genetic alterations of 31 osteosarcomas and demonstrated that more than 80% of the cases could be explained by the fact that they exhibited a specific combination of single-base substitutions, a loss of heterozygosity, or large-scale genome instability. They identified alterations in 14 driver genes (*TP53, RB1*, *BRCA2*, *BAP1*, *RET*, *MUTYH*, *ATM*, *PTEN*, *WRN*, *RECQL4, ATRX*, *FANCA*, *NUMA1* and *MDC1)* with signatures characteristic of BRCA1/2-deficient tumours

[14]. They also proposed a new model for osteosarcoma development in which a *TP53* and/or *RB1* mutant cell initiated a monoclonal disease. This cell population exhibited higher chromosomic instability, leading to both the emergence of new cell clones and polyclonal disease associated with these secondary genetic events [14]. The combination of multiple genetic events and a favourable microenvironment facilitate tumour growth [15-20]. It has been hypothesised that this microenvironment may be a sanctuary that sustains cell dormancy and contributes to drug resistance [20-22].

As osteosarcomas are bone-forming tumours, one of their signatures is the presence of osteoid tissue in close contact with spindle tumour cells. The morphology and organisation of tumour cells (such as extracellular matrix components) make it possible to identify various tumour subtypes, including osteoblastic, fibroblastic, chondroblastic, and highly vascularised telangiectactic forms, as well as giant cell enriched tumours [3-11]. Osteosarcomas are particularly prone to inducing lung metastases, which occur within 36 months of diagnosis and which have a strong impact on patient survival rate. Bone metastases can also occur in osteosarcoma, and they are associated with a worse survival rate than lung metastases [23]. The survival rate is estimated at around 50-70% after 5 years for non-metastatic patients and decreases dramatically to 30% when lung metastases are detected at the time of diagnosis (around 20% of patients) [24,25]. Unfortunately, these values have not changed in the last four decades [24]. The aim of the present review is to provide an overview of the main therapeutic approaches currently in development in the treatment of osteosarcoma.

2. Conventional therapeutic approaches to osteosarcoma

The therapeutic protocol currently in use for osteosarcoma was established by Rosen *et al*. at the end of the 1970s. It is a multimodal approach that combines surgery and polychemotherapy [26]. The advantages of chemotherapy were established by Link *et al.* in a

randomised clinical trial that compared surgery with postoperative chemotherapy, and surgery alone [27]. Chemotherapy can be administered before (pre-operative, or neo-adjuvant, chemotherapy) and/or after surgery (post-operative, or adjuvant, chemotherapy). Overall, the duration of the chemotherapy is around 6 to 12 months and combines doxorubicin, cisplatin, methotrexate and ifosfamide which are among the most efficient chemotherapeutic agents that have been identified for osteosarcoma [28]. The European Osteosarcoma Intergroup carried out a retrospective study of several clinical trials analysing various drug combinations and demonstrated the advantages of combining at least three drugs (reference combination: doxorubicin + methotrexate + cisplatin), and concluded that the doxorubucin/cisplatin association should no longer be considered as the standard chemotherapy combination for patients aged under 40 years with localised resectable osteosarcoma [29]. In addition, they demonstrated that chemotherapy-induced toxicity was a prognosis for overall survival, with the presence of greater toxicity generally associated with better survival [30]. However, although the advantages of neo-adjuvant chemotherapy have not been demonstrated [31], it is beneficial in several ways in treatment: i) it makes possible better delineation of tumours due to the formation of avascular collagenous pseudocaspules and then facilitates the definition of the surgical margin, ii) it reduces local tumour recurrence rates, iii) it makes it possible to evaluate the therapeutic response by means of histology, iv) it facilitates the preparation of definitive surgery for limb-salvage procedures by gaining time [32]. The Huvos grading system defines the therapeutic response and is established for the resected tumour, scoring the percentage of residual viable tumour cells (grade I >50%; grade II from 11 to 50 %; grade III from 1 to 10%; grade IV: no viable cells detected) [33]. Patients graded III and IV are considered to be good responders, and those graded I and II to be poor responders. As with a poor histological response, inadequate surgical margins are also an additional risk factor for local recurrence. The quality of the tumour resections, as evaluated by the quality of the

surgical margins, is correlated with a high risk of local recurrence [34,35]. Unfortunately, at present, there is no consensus for staging and comparing these margins between all surgical/pathological teams. Although this histological assessment is a key parameter in patient follow-up, the key challenge has been to determine whether the modification to post-operative treatment according to the therapeutic response analysed after the neo-adjuvant chemotherapy can improve the patients' therapeutic response [36]. The European and American Osteosarcoma Study Group (EURAMOS), composed of the Children's Oncology Group (COG), the Cooperative Osteosarcoma Study Group (COSS), the European Osteosarcoma Intergroup (EOI), and Scandinavian Sarcoma Group (SSG), analysed the impact of the nature of post-chemotherapy on 2,260 registered patients (good and poor responders) [37]. In a large clinical trial called EURAMOS-1, they compared the therapeutic advantages of MAP (Methotrexate/Doxorubicin/Cisplatin) and MAPIE (MAP/Ifosfamide/Etoposide) in poor responders, and MAP and MAPinf (MAP/Interferon-α) in good responders. In poor responders, MAP *vs* MAPIE therapy did not show any difference in event-free survival [38]. Similarly, in good responders, MAPInf was not statistically different from MAP alone [39]. Overall, these results do not support adaptation of post-operative chemotherapy based on histological response. Osteosarcoma tumours are notoriously radioresistant [39]. However radiotherapy is used when adequate surgery is impossible, such as when the tumour is located in a high risk area (e.g. spine, pelvis, head and neck) [41,42]. Radiotherapy can thus help to sterilize microscopic margins, and then contribute to local control of osteosarcoma growth in patients in whom surgical resection cannot lead to negative margins [43]. In addition, radiotherapy is a useful palliative tool for paediatric patients, especially when it comes to controlling bone pain [44].

3. Multi-target drugs and osteosarcoma

The poor results obtained with conventional therapeutic approaches led to the exploration of new, more effective treatments with less toxicity [45-47] (Figure 1). In this context, numerous clinical trials have been proposed, directly targeting cancer cells and/or their microenvironment. Insulin-like growth factor-1 (IGF-1) and its receptor (IGF1-R) are expressed by osteosarcoma cells [48]. IGF-1 expression has been associated with the aggressiveness of the disease [48], However, IGF-1R status had no effect on median progression-free survival [50]. Based on this observation and an abundant literature exploring the advantages of blocking IGF-1 signalling in preclinical models, clinical trials targeting IGF-1 signalling using anti-IGF-1 or anti-IGF1R were set up [45]. Anti-IGF1-R antibodies were well-tolerated, although an extremely limited number of tumour responses were reported when it was used as a single or combined therapy [51]. These results can be explained by the existence of alternative signalling pathways that control cell proliferation [52], and/or by therapeutic escape through activation of phospho-AKT [53]. However, sirolimus, an mTOR inhibitor, has been identified as being a potentially interesting compound in osteosarcoma [54]. A phase I clinical trial [NCT02517918, "Metronomic chemotherapy in patients with advanced solid tumours with bone metastasis and advanced pretreated osteosarcoma (METZOLIMOS)", 2015-2017, patients >13 years old] has been started. This study will include patients with unresectable locally advanced or metastatic osteosarcoma. The maximum tolerated dose is the primary outcome when sirolimus is administered in combination with cyclophosphamide, methotrexate and zoledronic acid.

Numerous cytokines and growth factors act through activation of receptor tyrosine kinases (RTKs) and control cell proliferation, survival and migration [55]. Therefore, most of the RTK inhibitors (e.g. imatinib mesylate, dasatinib, sunitinib) considered to be multi-target therapies were assessed, although unfortunately their efficacy was low [55-65]. Pazotinib, which targets VEGFR, PDGFR and c-KIT [61,62], and sorafenib, which targets RET and

VEGFR, show benefits in paediatric bone sarcomas by affecting angiogenesis [63,64]. Sofwat *et al*. reported significant clinical responses in three metastatic osteosarcoma patients treated with 800 mg of oral pazopanib daily [62]. Clinical trials recruiting a significant number of patients are in progress to confirm the initial data obtained (Table 1). Grignani *et al*. studied the therapeutic effects of sorafenib in relapsed and unresectable high-grade osteosarcoma (clinical trial ref. NCT00889057, 35 patients) [64]. Thirty-five young and adult patients were enrolled and treated with 400 mg of sorafenib twice daily until progression or unacceptable toxicity. Sorafenib demonstrated activity as a second- or third-line treatment in terms of progression-free survival at 4 months, however the main limitation of this study was the lack of a control group. Associating sorafenib with everolimus did not produce any significant additional benefit compared to sorafenib alone [64]. Similarly, regorafenib is an oral multikinase inhibitor of angiogenic (VEGFR1-3, TIE2), stromal (PDGFR-β, FGFR), and oncogenic kinases (KIT, RET, and RAF). A phase I clinical trial revealed preliminary evidence of antitumor activity in patients with solid tumours including osteosarcoma [65]. A phase II trial started in 2014 is currently in the recruitment phase (Table 1).

c-MET (Mesenchymal Epithelial Transition) and its ligand hepatocyte growth factor are involved in many pathophysiological processes, especially in oncology. c-MET is a tyrosine kinase receptor encoded by the MET proto-oncogene and induces signalling pathways involving PI3K/Akt, MAPK and NFκB. Its transforming activity was initially identified in osteosarcoma cells and named MNNG HOS transforming gene [66]. Both proteins are expressed by musculoskeletal tumours [67], and osteosarcomas exhibit aberrant expression of the receptor [68-70]. In a preclinical model, c-Met inhibition reduced osteosarcoma growth, dysregulated bone remodelling [71], and sensitised cancer cells to chemotherapy [72]. These observations were the justification for setting up a phase II clinical trial using cabozantinib, a c-MET inhibitor (NCT02243605, "Cabozantinib-s-malate in

treating patients with relapsed osteosarcoma or Ewing sarcoma"). Enrolment of 90 patients (> 12 years old) treated for relapsed osteosarcoma started in December 2014. The final data will be collected in June 2016 for the primary outcome measure. Dose use in sarcomas corresponds to 60 mg tablets taken orally once a day in a 28-day cycle, repeated every 28 days in the absence of disease progression or toxicity. The primary outcome will be the antitumour activity of cabozantinib, in terms of 6-month objective response (complete response, partial response) and 6-month non-progression.

4. Targeting the bone microenvironment

Osteosarcoma cells are able to dysregulate the bone microenvironment by activating osteoclast differentiation and resorption, which in turn stimulate tumour growth by releasing proliferative factors stored in the extracellular matrix [17]. A vicious cycle is thus established between osteosarcoma and bone cells that identify osteoclasts as a potentially interesting target in bone sarcoma [73,74]. Preclinical investigations demonstrated that nitrogen-containing bisphosphonates decreased the proliferation of osteosarcoma cell lines *in vitro* and induced cell death [75,76]. In murine models, zoledronic acid decreased the volume of the primary tumour [77,78] and also the number of lung metastases induced [79,80]. In addition, combining it with chemotherapy revealed its value with regard to improving tissue repair and preventing tumour recurrence [81]. The mechanisms of action of zoledronic acid can be explained by its pleiotropic effects on osteosarcoma, especially modulating angiogenesis, and the bone and immune environment [82]. However, in 2010, Endo-Munoz *et al*. brought into question the therapeutic advantages of zoledronic acid, showing that a blockade of osteoclastogenesis played a part in the development of osteosarcoma lung metastases [83]. A phase III clinical trial called OS2006 (NCT00470223, "Combined chemotherapy with or without zoledronic acid for patients with osteosarcoma") enrolled 318 patients (children and

adults). This clinical trial was stopped prematurely due to the absence of any significant difference between the groups with or without zoledronic acid [84]. Various hypotheses can be advanced, including: i) the development of a resistance mechanism associated with farnesyl diphosphate synthase in long-term treatment with zoledronic acid [85]; ii) the development of drug resistance due to the emergence of stemness properties in treated cancer cells [86]. A phase I clinical trial is in progress associating sirolimus with cyclophosphamide, methotrexate and zoledronic acid (NCT02517918, see paragraph 3). In addition to monocyte lineage, γ9δ2 T cells are key targets for zoledronic acid [87,88]. By inducing the release of phosphor-antigens, zoledronic acid induces the proliferation of these T lymphocytes. Interestingly, osteosarcoma cells are sensitised to zoledronic acid [89]. Using it to amplify *ex vivo* γ9δ2 T cells and sensitise osteosarcoma to the immune response may be a future treatment possibility. Based on an immonuregulatory effect, a phase I clinical trial is due to study the safety of transplantation with a haploidentical donor's peripheral blood stem cell graft depleted of $TCR\alpha\beta^+$ cells and $CD19^+$ cells, in conjunction with zoledronic acid (NCT02508038, 21 patients, 2016-220, recruiting).

RANKL (Receptor Activator of Nuclear Factor Factor kappeB) and its receptor RANK clearly control osteoclast differentiation/activation, and consequently bone remodelling [90]. RANK is not only expressed by monocyte lineage (e.g. macrophages, dendritic cells, osteoclasts) and by endothelial cells, it is also expressed by osteosarcoma cells, as revealed by RT-qPCR and immunostaining. Depending on the series published, 18 to 69% of osteosarcoma cells express RANK [91-93]. A reverse correlation between RANK expression and the overall survival of patients with osteosarcoma has been demonstrated, but not with the response to chemotherapy [92]. Similarly, Bago-Horvath *et al*. reported that RANK expression is a negative prognostic factor for disease-free survival [93]. RANKL is also expressed by osteosarcoma cells [94,95]. One recent report has ignited controversy

regarding the role of RANK/RANKL in the pathogenesis of osteosarcoma [95]. These authors did not detect the presence of RANK in osteosarcoma samples, and concluded that autocrine RANKL/RANK signalling in human osteosarcomas may not be operative, and anti-RANKL therapy may not directly affect the tumour [95]. This discrepancy may be explained by the decalcification methods used and also by the source of the antibodies. Preclinical investigations demonstrated that RANKL blockade by osteoprotegerin, or soluble RANK delivery, has a strong impact on tumour development [96-98]. In other cancer cell types, tumour-infiltrating regulatory T cells appear to be the main source of RANKL, and may be a strong regulator of local immunity [99]. Denosumab is a fully humanised antibody that blocks RANKL binding to RANK and its functional activities [100]. In 2015, in a RANKL/RANK positive tumour, Cathomas *et al*. reported complete metabolic remission for over 18 months after treatment with combined sorafenib and denosumab, in a patient with progressive osteosarcoma after two lines of chemotherapy and radiotherapy [101]. A phase II clinical trial was thus initiated in 2015 led by the Children's Oncology Group (NCT02470091, "Denosumab in Treating Patients With Recurrent or Refractory Osteosarcoma"). Ninety patients (age range: 11 to 50 years) who have relapsed or become refractory to conventional therapy with a regimen including some combination of high dose methotrexate, doxorubicin, cisplatin, ifosfamide and etoposide, will be included. Two cohorts will be formed: cohort I, patients with measurable disease according to RECIST, and cohort II, patients with complete resection of all sites of metastatic disease within 30 days prior to enrolment. Each patient will receive denosumab s.c. on day 1 (days 1, 8, and 15 in the first course of treatment). The treatment will be repeated every 4 weeks (28 days) for up to 24 months or 26 courses, whichever occurs first, in the absence of disease progression or unacceptable toxicity. At the end of the course of treatment, patients will be followed up for 3 years. The primary outcomes will be: i) the disease control rate at 4 months (cohort I), compared to historical

Children's Oncology Group experience with an objective response rate greater than 5%; ii) the disease control rate at 12 months, compared to historical Children's Oncology Group experience (cohort II); iii) and the RECIST response at 4 months, compared to historical Children's Oncology Group experience with an objective response rate greater than 5%. The final data collection date for the primary outcome measure is April 2019. Secondary objectives include: i) investigation of pharmacokinetics and pharmacodynamics; ii) description of the tolerability of denosumab; iii) a review of the disease control rate and objective response rate for patients with recurrent osteosarcoma restricted to bone; iv) investigation of the biological markers associated with the therapeutic response to denosumab.

5. Immunomodulating drugs and osteosarcoma

Several reports have underlined the therapeutic value of using immunotherapies or immunomodulatory-based therapies for osteosarcoma (see reviews [102-105]). Clinical investigations in osteosarcoma dogs gave impressive evidence of their efficacy and strengthened the interest of immunotherapies in human pathology [106-112]. In this context, the number of new drugs activating the immune system has exploded in the last 10 years and numerous phase I and II clinical trials are in progress in osteosarcoma.

5.1. Mifamurtide (L-MTP-PE)

Mifamurtide is a synthetic analogue of a bacterial cell wall component and is a potent activator of the immune response, especially macrophages. It was used alone and in combination with chemotherapy [113,114]. This immunomodulator improved overall survival from 70 to 78% (p=0.03) in combination with chemotherapy, and resulted in a one-third reduction in the risk of death from osteosarcoma [1115,116]. Mifamurtide was denied

approval by the U.S. Food and Drug Administration (FDA) in 2007 and authorised by the European Medicines Agency (EMA) in 2009. The therapeutic efficacy of Mifamurtide is not universally accepted but is included in the treatment of osteosarcoma patients in the UK, Spain, Turkey, Israel, Mexico and other countries in Europe, Asia and South America [117,118]. L-MTP-PE stimulates both the macrophages' cytotoxic function and the secretion of high numbers of soluble mediators, including TNF, IL-1, IL-6 or IL-8 which stimulate the angiogenesis and development of metastases [119]. In a phase II trial, Kleinerman et al demonstrated that Mifamurtide induced the infiltration of macrophages into patient osteosarcoma lung metastases and that these macrophages were "activated" macrophages [120]. Furthermore, there was also a significant difference in both progression-free and overall survival patient treated with Mifamurtide [121]. The density of tumour-associated macrophages seems to be a key biological parameter and is linked to poor prognosis. In osteosarcoma, Buddingh *et al.* showed that macrophages exhibit M1 and M2 phenotypes and demonstrated a link between M2 macrophages and angiogenesis [122]. Similarly, in preclinical models of osteosarcoma, the recruitment of the M2 subtype is correlated with tumour angiogenesis and lung metastasis [123]. However, these observations have not been linked with Mifamurtide and available clinical data support the therapeutic benefit of this molecule in newly diagnosed osteosarcoma patients who present metastases [124]. Overall, these studies confirm the key role played by macrophages in the pathogenesis of osteosarcoma. The clinical investigations into the clinical benefits of mifamurtide continue, with an ongoing clinical trial combining mifamurtide and ifosfamide (Table 2). In osteosarcoma, around 50% of patients are poor responders to intensive conventional chemotherapy and these poor/no responses are frequently related to the over-expression of Multi-Drug Resistance protein-1 (MDR1 or P-gp for P-Glycoprotein or ABCB1). ABCB1 is also involved in the drug resistance mechanism associated with numerous compounds,

including certain protein kinase inhibitors which increase its expression [125]. Patient stratification of high-grade osteosarcoma patients was suggested in 2006 by Serra *et al*. [126]. The effect of mifamurtide combined with chemotherapy will be re-evaluated in relation to ABCB1 expression. More than 200 non-metastatic patients will be included (ongoing recruitment, 2011-2020) in NCT014559484 trials in which overall survival will be the primary outcome (Table 2). Recently, Pahl *et al*. observed that the induction of macrophage anti-tumour activity (M1 subtype) by mifamurtide required IFN-γ [127]. This approach may be highly relevant for optimising mifamurtide therapy in osteosarcoma patients, and may open up new opportunities for this drug even if the combination of interferon and chemotherapy has not revealed any significant difference compared to conventional chemotherapy alone [55].

5.2. Disialoganglioside (GD2)

In 1987, Heiner *et al*. described the preferential accumulation of an anti-GD2 monoclonal antibody (3F8, a murine IgG3) at the tumour site in a preclinical model of osteosarcoma similar to previous observations made in neuroblastoma [128]. Ten years later, a phase I clinical trial revealed that a human-mouse chimeric monoclonal antibody (mAb) ch 14.18 directed against disialoganglioside (GD2) appeared to be clinically safe and effective with no specific toxicity after repeated administration [129]. An immunohistochemical study demonstrated that all the osteosarcoma tumours analysed were positive for GD2 in a series composed of 44 patients [130], and persisted upon recurrence [131]. *In vitro*, GD2 was suspected of enhancing the aggressiveness of the osteosarcoma [132]. Based on these observations, several clinical trials have been activated very recently (Table 2). Of them, one phase II trial (NCT02502786, sponsor: Memorial Sloan Kettering Cancer Center) will investigate the therapeutic advantages of the corresponding humanised form of the 3F8

antibody at a dose of 2.4mg/kg/dose for 3 days (days 1, 3, and 5) in the presence of GM-CSF. Patients (age range: 13 months-40 years) with recurrent high-grade osteosarcoma will be enrolled and the primary outcome will be event-free survival (time frame: 12 months) (Table 2). Another phase II protocol referenced NCT02484443 (sponsor: National Cancer Institute; Children's Oncology Group) is in progress and is studying the effects of a human-mouse anti-GD2 monoclonal antibody ch14.18 in combination with sargramostim (GM-CSF) in patients with recurrent osteosarcoma (Table 2). Patients up to the age of 29 years will receive sargramostim s.c. on days 1-14 and dinutuximab i.v. over 20 hours on days 4 and 5 (the dinutuximab infusion can be extended for an additional 2 days for anticipated toxicities). The treatment will repeat every 28 days for up to 5 courses in the absence of disease progression or unacceptable toxicity. The primary outcome will be disease control after 12 months. The second type of clinical trial is based on T cell therapy. Activated T cells are armed with the OKT3/3F8 bispecific antibody and will be administered in combination with IL-2 and GM-CSF (NCT02173093). GM-CSF is known to enhance the tumour antigen presentation by recruited mononuclear phagocytes and the functional coordination of $CD4^+$ and $CD8^+$ T cells. IL-2 participates to the maintenance of peripheral regulatory T cells ($CD4^+CD25^+$) and induces the proliferation of cytotoxic lymphocytes. The combination of GM-CSF, IL-2 and T cell activation by OKT3/3F8 which redirects T-cell cytolytic activity to cancer cells should improve the antitumour immune response. The first objective is to determine the maximum tolerated dose and to analyse its efficacy and side effects (Table 2). Interestingly, endothelin A receptor, which has been implicated in osteosarcoma progression and the metastatic process, potentiates the inhibitory effects of the anti-GD2 antibody on invasiveness and tumour cell viability, opening up new potential clinical investigations [133].

5.3. Nivolumab and Pembrozilumab

Nivolumab and Pembrozilumab are immunomodulators which acts by blocking the activation of programmed cell death-1 (PD-1), induced by its ligand on subset activated T and pro-B lymphocytes [134]. PD-1 is part of the immunoglobulin superfamily that interacts with programmed cell death ligand 1 (PDL1), which is a cell-surface protein expressed in numerous cancer cells including osteosarcoma [135]. By interacting with PD-1, PDL-1 induces inhibitory signalling and suppresses cytotoxic T-cell-mediated tumour responses [136,137]. PD-1 has a dual effect, promoting apoptosis in antigen-specific T lymphocytes located in lymph nodes, and decreasing apoptosis in regulatory T cells. Consequently, PD-1 can be considered to be an immune checkpoint, down-regulating the immune system by preventing T lymphocyte activation. The inflammatory process in the tumour microenvironment is the source of many soluble factors such as IFN-γ, which may increase PDL-1 expression in cancer cells and suppress local immune responses [138]. Numerous preclinical investigations have demonstrated that inhibition of the interaction between PD-1 and PD-L1 enhances the T-cell response, resulting in increased antitumour activity. However, the role of PD-L1 has not been formally demonstrated in patients even in diseases wherein the involvement of check-point inhibitors has been established (e.g. melanoma, non-small-cell lung cancer). Indeed, clinical benefits were described in patients whose cancer cells were PDL-1 negative, which raises new questions regarding the mechanism of action of this molecule [139]. A phase II study (Sarc028 trial) analysing the objective response rate in patients suffering from solid tumours including bone sarcomas and treated with pembrolizumab is in currently in progress (Table 2). A phase I/II trial will be concluded in 2016 on refractory solid tumours and sarcomas, including osteosarcoma. 242 patients will be enrolled and treated with nivolumab IV over 60 minutes on days 1 and 15. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity (Table 2).

5.4. Immunity and dendritic cell vaccine

Dendritic cells have the specific ability to initiate and modulate adaptive immune responses [140]. This specificity, associated with their role in antigen presentation, has led to their use in vaccine approaches to cancer. Matured autologous dendritic cells loaded with tumour lysates derived from tumour tissue were used as the vaccine product. In a pre-clinical model of osteosarcoma, it has been demonstrated that killer dendritic cells were able to induce an adaptive antitumour immune response with a decrease in tumour development after cross-presentation of the tumour cell-derived antigen [141]. A phase I clinical trial demonstrated the feasibility and good tolerance of dendritic cells pulsed with MAGE-A1, MAGE-A3 and NY-ESO-1 full length peptides in combination with decitabine. Antitumor activity was observed in some patients [142]. In 2012, 12 osteosarcoma patients were vaccinated with tumour lysate pulsed dendritic cells, but evidence of a clinical benefit was observed in only 2 of these patients [143]. These authors concluded that osteosarcoma patients may be relatively insensitive to DC-based vaccine treatments. A new clinical trial was initiated, enrolling 56 patients (>1 year) with confirmed sarcoma, either relapsed or without known curative therapies, and treated with autologous dendritic cells pulsed with tumour lysate (Table 2). NCT02409576 is a pilot trial ("Pilot Study of Expanded, Activated Haploidentical Natural Killer Cell Infusions for Sarcomas (NKEXPSARC)") analysing the effect of donor NK cells on clinical response determined by imaging. Twenty patients (aged 6 months to 80 years) will be included between 2015 and 2016. The patients will receive lymphodepleting chemotherapy with cyclophosphamide (1 day) followed by fludarabine (5 days) and each patient will receive IL-2 1 day before infusion of the NK cell (total 6 doses).

6. Targeted alpha radiotherapy: Radium-223

The principle of alpha radiotherapy is to induce double strand breaks in DNA [144]. Radium-

223 (^{223}Ra) is a bone-seeking alpha-emitter which has been studied extensively in preclinical models [145]. Its half-life is 11·4 days. Its biodistribution in mice revealed that bone matrix is its preferred location of retention. Radium-233 is well tolerated, with doses of 50–250 kBq/kg, and has antitumour effects in preclinical murine models [146]. A first phase I clinical trial confirmed its potential clinical interest in skeletal metastases [147]. A recent phase III (NCT00699751) clinical trial in 921 patients with symptomatic castration-resistant prostate cancer with two or more bone metastases demonstrated the clinical benefit of radium-233 therapy [148]. In light of the marked retention of radium-233 in the bone matrix, a phase I trial has been set up for osteosarcoma to determine the maximum tolerated dose (NCT018335201, "Phase I Dose Escalation of Monthly Intravenous Ra-223 Dichloride in Osteosarcoma", 2013-2017, ongoing but not recruiting) in 15 patients (> 15 years). The phase I starting dose was 50 kBq/kg Ra-223 dichloride i.v. over several minutes on day 1 of each 4-week cycle.

7. Alternative compounds for the treatment of osteosarcoma

Numerous targeted therapies are due to be assessed in clinical trials (Table 3). Of these drugs, those using the signalling pathways or enzymes involved in the cell cycle appear particularly interesting.

7.1. CC-115: a dual mTOR-DNA protein-dependent protein kinase inhibitor

Optimisation of a series of triazoles led to the discovery of CC-115, which is able to both bind to mTOR and the DNA-protein dependent protein kinases involved in DNA repair mechanisms, and inhibit both of them [55,149]. CC-1115 inhibits both raptor-mTOR (TOR complex 1 or TORC1) and rictor-mTOR (TOR complex 2 or TORC2), and decreases the proliferation of cancer cells. DNA-PK is a serine/threonine kinase and from the PI3K-related

kinase family of protein kinases. DNA-PK is activated following DNA damage and is involved in repairing breaks in double-stranded DNA via the DNA nonhomologous end joining (NHEJ) pathway [150]. By inhibiting DNA-PK, CC-115 impacts the DNA-repair mechanisms of tumour cells, inhibits the proliferation of numerous cancer cell lines, and increases cell apoptosis [151]. CC-115 has an anti-tumour effect *in vivo* as demonstrated by the inhibition of solid tumour growth in pre-clinical models of prostate cancer [149]. Interestingly, targeting DNA-PK increased the sensitivity of osteosarcoma cells to chemotherapeutic agents [152]. Treating cancer cells with CC-115 increases sensitivity to both chemo- and radiotherapy. A phase I trial has been set up (NCT01353625) in which 144 patients will receive increasing doses of oral CC-115 (starting with 0.5mg daily, in cycles of 28 days) (Table 3).

7.2. Abmaciclib: a CDK4 and CDK6 inhibitor

Cell cycle progression is controlled by cyclin-dependent kinases (CDK), which are dysregulated in numerous cancer cells, leading to uncontrolled cell proliferation. Of the various kinases identified, CDK4 and related CDK6 play a part in the progression of cells into the DNA synthetic phase of the cell-division cycle. CDK4 and CDK6 act more specifically in the first gap phase (G_1) of the cell cycle and they assemble with D-type cyclins (D1-D3) in response to various extracellular signals (i.e. mitigen activities and cytokine-induced signalling) to constitute enzymatically-active holoenzyme complexes [153]. Abmaciclib (LY2835219) is a CDK4 and CDK6 inhibitor capable of blocking the growth of cancer cells. Abemaciclib specifically inhibits CDK4/6 and related associated phosphorylation cascades such as Rb phosphorylation in early G1. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, blocking the cell cycle in the G1 phase, suppressing DNA synthesis and reducing cancer cell proliferation. This drug is currently being assessed in a phase I trial in children with recurrent or refractory solid tumours (NCT02644460) (Table 3).

7.3. Glembatumumab vedotin: an anti-gpNMB therapy

Glycoprotein non-metastatic melanoma protein B(gpNMB)/osteoactivin is a transmembrane glycoprotein that is highly expressed in various types of cancer. gpNMB is known to promote the invasion, migration and metastatic progression of cancer cells by modulating matrix metalloproteinase expression, but also by inhibiting the activation of tumour-reactive T lymphocytes via its binding to syndecan-4. gpNMB is also expressed by immune cells, including antigen-presenting cells, and may promote their adhesion to endothelial cells in an integrin-dependent manner. Furthermore, gpNMB decreases cell apoptosis and increases vascular density [154]. Recently, Roth *et al*. demonstrated that osteosarcoma gpNMB and its targeting by the antibody-drug conjugate glembatumumab vedotin resulted in cytotoxic activity [155]. A phase I/II trial has been initiated (NCT02487979) in 38 recurrent or refractory patients (Table 3).

7.4. Nanomedicine: Nab-paclitaxel and MM-398

Nanoparticles offer the possibility of encapsulating poorly soluble drugs and improving their half-life, bioavailability and efficacy [156]. Nab-paclitaxel is a new formulation of conventional paclitaxel. It is solvent free, and comes in a nanoparticle albumin-bound (Nab) form. Nab-paclitaxel was designed to reduce the side effects of paclitaxel and docetaxel. Its activity is similar to paclitaxel, and it blocks the cell cycle in G2/M by stabilising the microtubules and consequently blocking chromosome duplication. Nab-paclitaxel has demonstrated its therapeutic advantages over paclitaxel in preclinical models, and combining it with gemcitabine in osteosarcoma may be of great interest [157]. A phase I/II trial was initiated in 2013 in paediatric patients with recurrent/refractory solid tumours, including osteosarcoma (NCT01962103, Table 3).

Based on similar technology, MM-398 is a stable nanoliposomal irinotecan with

higher cytotoxicity than the original drug. The drug was assessed successfully in a preclinical model of Ewing sarcoma [158] and the results provoked the initiation of a phase I trial (NCT02013336) in paediatric solid tumours (Table 3).

8. Radiotherapy, miscellaneous trials and preparation for future investigations

In parallel to the ongoing clinical trials centred on new drugs, complementary approaches has been proposed for treating high-risk located osteosarcoma and recurrent disease. Although osteosarcoma is considered to be a radioresistant form of cancer, radiotherapy is used in the treatment of osteosarcoma in high-risk locations (such as the spine) to control local and recurrent development of tumours, and reduce pain, especially in a palliative context [44,159]. Several clinical trials are currently in progress to evaluate its efficacy in controlling bone pain and/or its therapeutic impact (Table 4). Recently, carbon ion radiotherapy was shown to be of interest in the management of unresectable osteosarcomas by providing good local control of the tumour without unacceptable morbidity [160,161]. Complementary investigations are required to validate carbon ion radiotherapy as a curative option in these patients.

Establishing biological cohorts for rare tumours takes a very long time. Such cohorts are nevertheless one of the key points for studying the pathogenesis of a specific disease, especially heterogeneous pathologies when they are associated with clinical annotations. Several trials have been initiated to collect biological samples from osteosarcoma patients (e.g. tissue, blood) and will be open until 2100, enrolling 1000 patients (trials NCT02132182, NCT00580385, NCT00954473, NCT00899275, Table 4). These biological cohorts are and will be useful for helping define various differential diagnoses (trial NCT01336803, Table 4).

9. Conclusion

Despite numerous preclinical investigations underlining the involvement of the

microenvironment in cancer cell proliferation and migration, the role of this tissue compartment in the pathogenesis of osteosarcoma is not fully understood. Based on these observations, several phase II trials have been proposed such as the use of Denosumab or zoledronic acid targeting bone niche. In fact, most of therapies described in this review such regulators of the immune response and consequently the immune niche should be also considered as clinical approaches targeting the tumour microenvironment. The heterogeneity of cells and molecules which composed the microenvironment of osteosarcoma, enrich the list of the potential therapeutic targets (e.g. blood vessels, T cells, macrophages, and bone cells) in addition to the master proteins that control cell proliferation or cell death. Finally, most of the current phase II clinical trials are based on biological processes affecting directly or indirectly the tumour microenvironment and will provide very useful information on the clinical relevance of tumour microenvironment targeting in the near future. However, the key to success probably lies in better characterization of the disease, as this leads to better patient stratification and, consequently, to personalised medicine. Better understanding of how to control cancer-initiating cells, characterising their genotype, and identifying their functional links with their close environment are the scientific/medical challenges of the next few years. Biological cohorts will play a part in this challenge. Ongoing phase I/II trials are important steps, not only for identifying new therapies with greater safety and efficacy, but also for better defining the role of the microenvironment in the pathogenesis of osteosarcoma and more specifically in the initiation of metastases.

10. Expert opinion

Osteosarcoma is the most common malignant bone tumour. Although osteosarcomas are chemosensitive tumours, cancer cells can become drug resistant and have a tendency to form distant metastases (principally in the lungs). However, despite progress in multidrug

chemotherapy protocols and conservative limb salvage surgery, osteosarcoma survival rates have not improved for more than 30 years. Transcriptomic and phosphoproteomic assessments have identified key intracellular signalling pathways that are activated by cytokines/growth factors and sustain cancer cell proliferation. These data led to the development of a large panel and several generations of tyrosine-kinase inhibitors, which were initially promising multi-target drugs. Unfortunately, most of the drugs considered had low efficacy in osteosarcoma patients due to the development of resistance mechanisms [55-65]. However, many clinical trials failed to clearly evaluate their therapeutic value in the context of osteosarcoma with very high levels of heterogeneity. It is necessary to revisit their efficacy in view of the full expression profile of the tyrosine kinases of each patient. Sorafenib showed interesting clinical advantages, although unfortunately they remain difficult to analyse in the absence of an adequate control group. Complementary clinical trials are thus required [64]. Pazopanib [61,62], regorafenib [66] and cabozantinib (NCT02243605) may also be interesting therapeutic options.

Using the tumour microenvironment as a potential therapeutic target indicates the start of a new era for osteosarcoma patients. Immune modulators are some of the promising drugs in development in osteosarcoma (see section 5). A recently set up clinical trial is studying whether or not to associate ipilimumab, a fully human monoclonal antibody that binds CTLA-4 and blocks its interaction with CD80 and CD86 [162]. However, it is too early to conclude on any therapeutic advantages to this approach (Table 2). Mifamurtide is the frontrunner in the immunoregulator family, and it has been authorised after much debate in the Europe, but not in USA. This controversial drug was nevertheless the first to produce a significant improvement in survival rates in osteosarcoma. Although the effect was modest, this observation nevertheless identifies the concept of macrophage modulation as therapeutic option. In the last decade, several authors demonstrated the key role played by macrophages

in the pathogenesis of osteosarcoma, and, more specifically, the key point seems to be the balance in the M2/M1 macrophage subtype [122,123]. Since the development of mifamurtide [106-119], the anti-GD2 antibody [128-133], and genetically-modified T cells, vaccines have been proposed and are currently undergoing clinical trials. Conventional chemotherapies target mainly proliferating cancer cells for decreasing or slowing down the tumour development, and the most recent clinical approaches aimed also to control the behaviour of quiescent cells (e.g. cell reactivation). This is a significant modification to the philosophical approach used in oncology: associating "curative aspects" (e.g. killing of proliferating cells) and "control" of a disease *via* the immune system (e.g. control of quiescent cancer cell reactivation). Radium-233 is also a promising new therapeutic agent that is retained preferentially in the bone matrix (tumour environment) close to the cancer cells [144-148]. The clinical benefits shown in the bone metastases of prostatic cancers heighten its clinical value. Clinical trials in progress will soon provide us with the answer.

Identifying and characterising early tumour recurrence and metastasis dissemination remains necessary for proposing better adapted therapeutic strategies. These early events could be characterized by biomarkers including all the biological parameters that reflect the recurrent disease. More specifically, they reflect all the specific signatures at the transcriptional and/or protein level, as well as the isolated circulating tumour cells characterised by a specific phenotype. Metastatic spread to specific target sites (the lungs and/or bones) is a clinically intractable feature of osteosarcoma's state of dormancy (quiescence), evading detection whilst remaining primed to colonise the target metastatic organ upon induction of the right environmental cues [163-165].

Circulating tumour cells have also been isolated from osteosarcoma patients [166,167] and new technologies (e.g. microfluidic) provide an opportunity to both isolate tumour cells and "cancer initiating cells" from fixed paraffin embedded samples at the single cell level,

and better define tumour heterogeneity [168,169]. Based on the heterogeneity of osteosarcoma subtypes and therapeutic response, new patient stratification may be proposed and new multidrug targeted approaches adapted to each patient (personalised medicine) will emerge. The biological cohorts established will be one of the key factors in these developments. The gap between the new generation of drugs and conventional chemotherapy will be filled by new formulations of "old" drugs (such as Nab-paclitaxel) thanks to nanomedicine, thus improving their bioavailability, efficacy, and safety, and reducing their side effects [166,167].

Acknowledgements

This study was supported by the Ligue Nationale Contre le Cancer, FR (research project: Equipe LIGUE 2012) and the Bone Cancer Research Trust, UK (research project number 144681).

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Figure Legend

Figure 1: Main therapeutic approaches to osteosarcoma studied in clinical trials in the last three decades. The tumour microenvironment plays a key role in the pathogenesis of osteosarcoma: it facilitates the transport of gas and nutriments to cancer cells and extravasation to their metastatic location (vascular niche), induces a tolerant environment (immune niche), and dysregulates bone remodelling (bone niche). These niches play a part in cancer cell proliferation, the development of quiescent/dormant subpopulations, and drug resistance, as well as facilitating the metastatic process. Tumour niches are a source of therapeutic targets both for single therapies and those combined with direct targeting of cancer cells.

Table 1: Regorafenib and pazopanib in osteosarcoma : ongoing studies

Drug	Reference	Title	Phase	Doses	Primary outcome	Patients	Status
Regorafenib	NCT02048371	A blanket protocol to study oral regorafenib in patients with refractory liposarcoma, osteogenic sarcoma, and Ewing/Ewing-like sarcomas	II	160 mg daily	Progression-free survival	126	(2014-2017) Recruiting
	NCT02389244	A phase II study evaluating efficacy and safety of regorafenib in patients with metastatic bone sarcomas (REGOBONE)		160 mg once daily for the 3 weeks on / 1 week off	Primary efficacy endpoint is progression free survival	108	(2014-2019) Recruiting
Pazopanib	NCT01956669	Pazopanib paediatric phase II trial children's oncology group (COG) in solid tumours	II	Tablets at a dose of 450 mg/m^2/dose or as a powder in suspension at a dose of 225 mg/m^2/dose	Objective response rate in subjects' with tumours of primary interest	154	(2014-2019) Recruiting
	NCT01759303	Study of pazopanib in the treatment of osteosarcoma metastatic to the lung	II	600 mg or 800 mg once daily will be started on Cycle 1 Day 1 and will be administered continuously for each 28-day cycle	4-month Progression free survival	35	(2013-2017) recruiting
	NCT02357810	Pazopanib hydrochloride and topotecan hydrochloride in treating patients with metastatic soft tissue and bone sarcomas	II	Tablets at a dose of 450 mg/m^2/dose or as a powder in suspension at a dose of 225 mg/m^2/dose	Time from enrolment to progression	136	(2015-2017) Recuiting

Table 2: Immunomodulating drugs in osteosarcoma : ongoing studies

Drug	Reference	Title	Phase	Doses	Primary outcome	Patients	Status
Mifamurtide	NCT02441309	A Eurosarc study of mifamurtide in advanced osteosarcoma (MEMOS)	II	Mifamurtide alone Ifosfamide followed by mifamurtide Reference doses : Mifamurtide : 2mg/m^2, IV infusion, once or twice/week Ifosfamide: 12-15mg/m^2	- Biological response data based on pharmacodynamic endpoints on tumour biopsy material - Radiological response defined as complete or partial response and assessed using RECIST criteria	40	(2014-2017) Recruiting
	NCT01459484	ABCB1/P-glycoprotein Expression as biologic stratification factor for patients with non metastatic osteosarcoma (ISG/OS-2)	II/III	2 mg/m^2 twice a week	Overall survival in patient with non metastatic osteosarcoma of the extremities treated with chemotherapies according to the expression of ABCB1/P-glycoprotein	225	(2011-2020) Recruiting
Anti-GD2 therapies	NCT02159443	Pretreatment anti-therapeutic antibodies (PATA) in patients treated With hu14.18K322A antibody			-Characterization of pretreatment anti-therapeutic antibodies -Number of samples with increased anti-tumour efficacy	100	(2014-2019) Recruiting
	NCT00743496	A Phase I trial of the humanized anti-GD2 antibody In children And adolescents With neuroblastoma, osteosarcoma, Ewing sarcoma and melanoma	I	From 2 mg/m^2 daily for 4 consecutive days every 28 days (1 course)], tof 60 mg/m^2 daily for 4 consecutive days every 28 days	Determine maximum tolerated dose and dose-limiting toxicity of the humanized monoclonal anti-GD2 antibody, hu14.18K322A,	75	(2008-2018) Recruiting
+ GM-CSF	NCT02502786	Humanized monoclonal Antibody 3F8 (Hu3F8) with granulocyte-macrophage colony	II	One cycle consists of treatment with hu3F8 (humanized anti-GD2 antibody) at a dose of 2.4mg/kg/dose for 3 days	Event free survival	39	(2015-2018) Recruiting

		stimulating factor (GM-CSF) in the treatment of recurrent osteosarcoma		(day 1, 3, and 5) in the presence of sc GM-CSF (day -4 through 5). These 3 doses of hu3F8 and 10 days of GM-CSF constitute a treatment cycle. Cycles are repeated at ~2-4 week intervals between first days of hu3F8, through 5 cycles. A maximum of 5 cycles will be administered on protocol.			
+ GM-CSF	NCT02484443	Dinutuximab in combination with sargramostim in treating patients with recurrent osteosarcoma	II	sargramostim SC on days 1-14 and dinutuximab IV over 20 hours on days 4 and 5 -- Treatment repeats every 28 days for up to 5 courses in the absence of disease progression or unacceptable toxicity.	Disease control	44 (up to 29 years)	(2015-2018) Recruiting
Loaded T cells	NCT02173093	Activated T cells armed with GD2 bispecific antibody in children and young adults with neuroblastoma and osteosarcoma	I	Patients receive IL-2 (300,000 IU/m^2/day) SC daily on days -2 to 35, GM-CSF (250 ug/m^2 twice per week) SC twice weekly x 5 weeks, and GD2Bi-aATC IV over 30 minutes twice weekly x 4 weeks for a total of 8 infusions. + 40, 80, and 160 x 10^6 cells/kg/infusion dose levels.	Dose-escalation study in patients with recurrent or refractory neuroblastoma (NB) and other GD2-positive tumors to evaluate the safety and tolerability and to determine the maximum tolerated dose for anti-CD3 x hu3F8 bispecific antibody (GD2Bi)-armed activated T cells	40	(2014-2018) Temporarily suspended
Loaded T cells	NCT02107963	A phase I trial of T cells expressing an anti-GD2 chimeric antigen receptor in children and young Adults with GD2+ solid tumours	I	Lymphodepletion by cyclofosfamide followed by inoculation of anti-GD2 CAR T cells from 1 x 10^5 to 1 x 10^7 transduced T cells/kg	To determine feasibility of producing anti GD2-CAR cells meeting the established release criteria and to assess the safety of administering escalating doses of anti-GD2-CAR engineered T cells in children and young adults with GD2+ solid tumours	74	(2014-2018)

+ Vaccination	NCT01953900	iC9-GD2-CAR-VZV-CTLs/refractory or metastatic GD2-positive sarcoma/VEGAS	I	From 1 x 10^6 GD2 T cells in combination with VZV vaccination	Number of subjects with a dose limiting toxicity	26	(2014-2018) Recruiting currently only Patients with osteosarcoma (Feb. 2016)
Dendritic cell vaccine	NCT01803152	A phase I trial of dendritic cell vaccination with and without Inhibition of myeloid derived suppressor cells by gemcitabine pre-treatment for children and adults with sarcoma	I	3 x 10^6, 6 x 10^6, and 12 x 10^6 dendritic cells per treatment	Number of participants with adverse events as a measure of safety and tolerability	56	(2012-2016) Recruiting
Pembrolizumab	NCT02301039	SARC028: A phase II study of the anti-PD1 antibody pembrolizumab (MK-3475) in patients with advanced sarcomas	II		Objective response rate (Assessments at 8 weeks, up to 5 years)	80 (> 12 years)	(2015-2018) Follow up ongoing
Nivolumab	NCT02304458	Nivolumab with or without ipilimumab in treating younger patients with recurrent or refractory solid tumors or sarcomas	I/II	Patients with recurrent or refractory solid tumors receive nivolumab IV over 60 minutes on days 1 and 15. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity.	Maximum tolerated dose and response to the drug	242 (12 months – 30 years)	(2015-2016) Recruiting

Table 3: Alternative therapeutic approaches of osteosarcoma: targeting of cell signalling, DNA repair, cell cycle check points, and nanomedicine

Drug	Reference	Title	Phase	Doses	Primary outcome	Patients	Status
CC-115	NCT01353625	Study to assess safety and tolerability of oral CC-115 for patients with advanced solid tumors, and hematologic malignancies	I	From 0.5mg daily, oral, in cycles of 28 days	-Dose limiting toxicity -Pharmakocineticand pharmacodynanic parameters	144 (> 18 years)	(2011-2016) Active, not Recruiting
Thiotepa	NCT00978471	Adjuvant high-dose thiotepa and stem cell rescue associated with conventional chemotherapy in relapsed osteosarcoma (OSII-TTP)	II	8-12mg/m²/day/injection Total dose for one cure:15-50mg.	Overall survival rate	66 (1- 50 years)	(2009-2018) Recruiting
Glembatumumab and vedotin	NCT02487979	Glembatumumab vedotin in treating patients with recurrent or refractory osteosarcoma	I/II	IV over 90 minutes on day 1. Treatment repeats every 90 days for up to 18 courses in the absence of disease progression or unacceptable toxicity.	Disease control rate	38 (12-49 years)	(2016-2018) Recruiting
Nab-paclitaxel	NCT01962103	To find a safe dose and show early clinical activity of weekly nab-paclitaxel in pediatric patients with recurrent/ refractory solid tumors	I/II	100-240 mg/m2 IV on Days 1, 8 and 15 of a 28-day cycle	Incidence of dose limiting toxicities	134 (6 months – 21 years)	(2013-2020) Recruiting
Abemaciclib	NCT02644460	Abemaciclib in children with DIPG or recurrent/refractory solid tumors (AflacST1501)	I		Maximum tolerated dose	50 (2 - 21 years)	(2015-220) Recruiting
MM-398	NCT02013336	Phase 1 study of MM-398 plus cyclophosphamide in pediatric solid tumors	I		Maximum tolerated dose	30 (12 months – 20 years)	(2013-2015) Data collection

Table 4: Imaging, genomic and miscellaneous ongoing studies

Type	Reference	Title	Objective	Patients	Status
Bone imaging Methionine	NCT00840047	Methionine PET/CT studies In patients with cancer	The purpose of this study is to test the usefulness of imaging with radiolabeled methionine in the evaluation of children and young adults with tumor(s).	650	(2009-2018) Recruiting
Imaging biomarkers	NCT01882231	Quantitative imaging biomarkers of treatment response in osteosarcoma and Ewing sarcoma	To use changes in 3 Tesla MRI measurements of tumor protein content, cell density, and microvessel perfusion, obtained before and after a single cycle of NAC, to predict eventual tumor response	24 (> 13 years)	(2013-2017) Recruiting
	NCT01336803	Differentiation of bone sarcomas and osteomyelitis with ferumoxytol-enhanced MRI	To distinguish cancer and infection or inflammation using MRI and ferumoxytol, a new contrast agent	50 (1 – 40 years)	(2011-2016) Recruiting
Radiotherapy	NCT02520128	A phase II study of IMRT in primary bone and soft tissue sarcoma (IMRiS)	To assess the feasibility, efficacy and toxicity of Intensity Modulated Radiotherapy (IMRT)	143 (> 16 years)	(2015-2020) Not yet recruiting
	NCT02107664	The palliative radiotherapy and inflammation study - PRAIS (PRAIS)	Pain reponse	1000 (> 18 Years)	(2013-2016) Recruiting
	NCT01886105	Combination of external beam radiotherapy with ^{153}Sm-EDTMP to treat high risk osteosarcoma	Progression free survival	20 (13-65 years)	(2013-2018) Recruiting
	NCT01005043	Therapy trial to determine the safety and efficacy of heavy ion radiotherapy in patients with osteosarcoma	Feasability, toxicity	20 (> 6 years)	(2010-2020) Recruiting
Neuropsy-chological assessment MRI	NCT02309242	Long-term neurotoxic effects of chemotherapy in survivors of bone and soft tissue sarcomas. A retrospective study	Neuropsychological functioning (time frame 4 years)	60 (7-25 years)	(2014-2019) Recruiting
Genomic	NCT01047878	Genomic analysis of pediatric bone tumors	To determine whether gene expression analysis of primary tumor samples before and after chemotherapy are predictive of long-term survival in pediatric patients with bone sarcomas (Ewing sarcoma and Osteosarcoma	150 (> 18 years)	(2007-2016) Recruiting

			□ % necrosis post chemotherapy □ overall survival and event free survival		
Hearing loss	NCT02094625	N-acetylcysteine (NAC) to prevent cisplatin-induced hearing loss	Cisplatin is a key chemotherapy agent for the treatment of multiple childhood cancers but causes permanent hearing loss. This study investigates the drug N-acetylcysteine (NAC) to determine the dose necessary to protect hearing and also how well tolerated NAC is when combined with chemotherapy.	30 (1-21 years)	(2016-2019) Not yet open
Biomarkers	NCT01807052	Biomarker expression in tissue samples from patients with bone sarcomas	This trial studies biomarker expression in tissue samples from patients with bone sarcomas.	34 (up to 39 years)	From 2009 Recruiting
Molecular mapping	NCT02162732	Molecular-guided therapy for childhood cancer	Experimental technologies to determine a tumor's molecular makeup.	56 (13 months – 21 years)	(2014-2021) Recruiting
Monocyte phenotype	NCT02132182	Monocyte phenotypic and functional differences	To identify phenotypic (cell surface receptor expression) and functional differences in monocyte populations in humans with osteosarcoma as compared to published historical data on normal human monocyte values.	90 (> 6 years)	(2014-2017) Recruiting
Tissue sampling	NCT00580385	Chemotherapy resistance in osteogenic sarcoma and other solid tumors	To investigate tumors in the laboratory to determine how and why they respond, or fail to respond to different drug therapies.	750	(1997-2016) Recruiting
Blood sampling	NCT00954473	Study of blood samples from patients with osteosarcoma	Blood samples undergo polymorphism analysis of common single-nucleotide polymorphisms and haplotypes to examine genetic variation, gene-gene interactions, and the population structure.	1000	(2009-2100) Recruiting
Blood and tissue sampling	NCT00899275	Collecting and storing samples of blood and tumor tissue from patients with osteosarcoma	Blood and tissue sampling	1000	(2008-2100) Recruiting

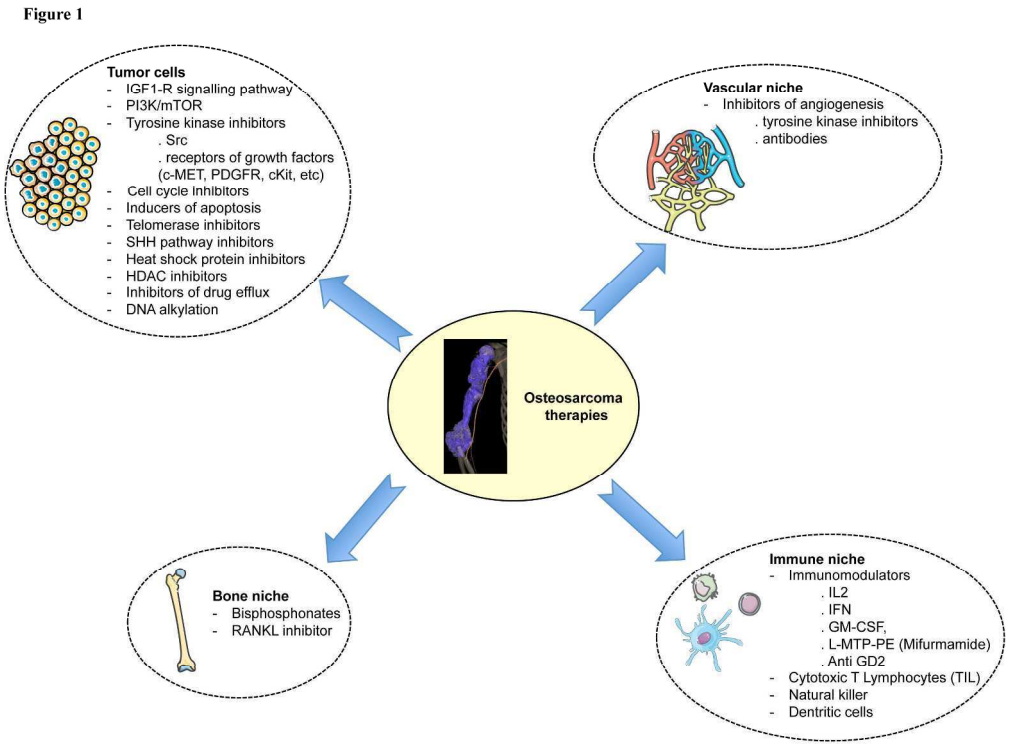

Figure 1: Main therapeutic approaches to osteosarcoma studied in clinical trials in the last three decades. The tumour microenvironment plays a key role in the pathogenesis of osteosarcoma: it facilitates the transport of gas and nutriments to cancer cells and extravasation to their metastatic location (vascular niche), induces a tolerant environment (immune niche), and dysregulates bone remodelling (bone niche). These niches play a part in cancer cell proliferation, the development of quiescent/dormant subpopulations, and drug resistance, as well as facilitating the metastatic process. Tumour niches are a source of therapeutic targets both for single therapies and those combined with direct targeting of cancer cells.

248x190mm (300 x 300 DPI)