Effectiveness of multi-drug regimen chemotherapy treatment in osteosarcoma patients: a network meta-analysis of randomized controlled trials

Xiaojie Wang†, Hong Zheng†, Tao Shou¹, Chunming Tang¹, Kun Miao¹ and Ping Wang*²

Abstract

Background: Osteosarcoma is the most common malignant bone tumour. Due to the high metastasis rate and drug resistance of this disease, multi-drug regimens are necessary to control tumour cells at various stages of the cell cycle, eliminate local or distant micrometastases, and reduce the emergence of drug-resistant cells. Many adjuvant chemotherapy protocols have shown different efficacies and controversial results. Therefore, we classified the types of drugs used for adjuvant chemotherapy and evaluated the differences between single- and multi-drug chemotherapy regimens using network meta-analysis.

Methods: We searched electronic databases, including PubMed (MEDLINE), EmBase, and the Cochrane Library, through November 2016 using the keywords “osteosarcoma”, “osteogenic sarcoma”, “chemotherapy”, and “random*” without language restrictions. The major outcome in the present analysis was progression-free survival (PFS), and the secondary outcome was overall survival (OS). We used a random effect network meta-analysis for mixed multiple treatment comparisons.

Results: We included 23 articles assessing a total of 5742 patients in the present systematic review. The analysis of PFS indicated that the T12 protocol (including adriamycin, bleomycin, cyclophosphamide, dactinomycin, methotrexate, cisplatin) plays a more critical role in osteosarcoma treatment (surface under the cumulative ranking (SUCRA) probability 76.9%), with a better effect on prolonging the PFS of patients when combined with ifosfamide (94.1%) or vincristine (81.9%). For the analysis of OS, we separated the regimens to two groups, reflecting the disconnection. The T12 protocol plus vincristine (94.7%) or the removal of cisplatinum (89.4%) is most likely the best regimen.

Conclusions: We concluded that multi-drug regimens have a better effect on prolonging the PFS and OS of osteosarcoma patients, and the T12 protocol has a better effect on prolonging the PFS of osteosarcoma patients, particularly in combination with ifosfamide or vincristine. The OS analysis showed that the T12 protocol plus vincristine or the T12 protocol with the removal of cisplatinum might be a better regimen for improving the OS of patients. However, well-designed randomized controlled trials of chemotherapeutic protocols are still necessary.

Keywords: Osteosarcoma, Chemotherapy drugs, Progression-free survival, Overall survival, Meta-analysis

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Background
Osteosarcoma is the most common type of primary malignant bone tumour. It exhibits a high metastasis rate and is frequently detected in adolescents at sites of rapid bone growth [1, 2]. Although osteosarcoma is frequently treated by surgical joint amputation or disconnection, the prognosis remains poor in patients with metastatic osteosarcoma [3]. Therefore, the ultimate treatment of this disease not only depends on primary tumour control but also the removal of small metastases. Thus, adjuvant chemotherapy combined with the surgical removal of the primary tumour is needed to reduce the size of the tumour, clear the metastases, and improve progression-free survival (PFS) and overall survival (OS).

Osteosarcoma is also a relatively drug-resistant tumour, and the treatment effect of single-drug chemotherapy is not ideal [4, 5]. Thus, multi-drug regimens are necessary to control tumour cells at various stages of the cell cycle, eliminate local or distant micrometastases, and reduce the emergence of drug-resistant cells [6]. Various systematic reviews have examined osteosarcoma chemotherapy, but the results are controversial. A previous study suggested that ifosfamide-based chemotherapy could significantly improve the PFS and OS of osteosarcoma patients [7]. However, recent traditional meta-analyses have not determined whether ifosfamide application and chemotherapy have similar histological response rates and 5-year PFS and OS in non-metastatic and primary osteosarcoma patients; thus, ifosfamide is not recommended [8–10]. Additionally, in a systematic review concerning the dose of chemotherapy drugs, high-dose drugs did not significantly improve the PFS and OS of patients compared to moderate-dose drugs [11–13]. Thus, additional studies are needed to resolve these controversies.

From the 1970s to the present, many adjuvant chemotherapy protocols have shown various efficacy differences and controversial results. No definitive evidence exists regarding which treatment is more advantageous for clinical application [14, 15]. The aim of the present study was to analyse the existing chemotherapy protocol through direct and indirect comparisons to guide clinical application. However, an analysis of each type of chemotherapy protocol is too complex and cumbersome. Therefore, in the present study, we classified the types of drugs used in adjuvant chemotherapy and evaluated the differences between single or multi-drug chemotherapy regimens using a network meta-analysis.

Methods
This network meta-analysis was performed in accordance with Preferred Reporting Items for Systematic Reviews (PRISMA) statement [16].

Data search strategy and selection criteria
Two authors independently performed the literature search through November 2016 using electronic databases, including PubMed (MEDLINE), EmBase, and the Cochrane Library, with the keywords “osteosarcoma”, “osteogenic sarcoma”, “chemotherapy”, and “random*”, without language restriction. The bibliographies of the obtained publications and relevant reviews were also assessed to ensure that no relevant studies were inadvertently omitted. The publications included in the present study met the following criteria: (1) randomized controlled trial (RCT) design; (2) inclusion of osteosarcoma patients; (3) examination of two or more groups using different single- or multi-drug regimens; and (4) inclusion of PFS or OS as an outcome.

The exclusion criteria consisted of the following: (1) non-RCT studies; (2) studies including patients with other types of sarcomas, such as Ewing sarcoma; (3) non-chemotherapy controlled studies, such as surgery or radiotherapy controlled studies; (4) studies comparing the same chemotherapeutic drug type, such as a drug dose-related study; and (5) non-desired outcome studies. Additionally, reviews, comments, case reports, basic studies, and conference reports were also excluded.

Data extraction
Two authors independently extracted the following information from eligible studies: first author’s name, publication year, location, research time, study register or abbreviation, sample size, average age, ratio of males, type of disease, experimental intervention, control, and follow-up. In the present analysis, the major outcome was PFS, and the secondary outcome was OS, as some patients changed the initial randomized treatment after disease progression. We assessed the methodological quality of the included trials using the Cochrane Collaboration’s tool, which assigns grades of “high risk”, “unclear risk”, or “unclear risk” of bias across the seven specified domains [17].

Statistics analysis
We initially conducted a pairwise meta-analysis using a random effect model, as this model is likely the most appropriate and conservative methodology accounting for between-trial heterogeneity within each comparison [18]. For dichotomous outcomes, odds ratios (ORs) or logarithm transformation with 95% confidence intervals (CIs) were calculated to determine the sizes of the effects. We also used a random effect network meta-analysis for mixed multiple treatment comparisons because this analysis fully preserves the within-trial randomized treatment comparisons in each trial [19]. To rank the treatments for each outcome, we used the surface under the cumulative ranking (SUCRA) probabilities [20]. Comparison-adjusted funnel plots were used to determine whether small-study effects
| Author                  | Year | Location | Research time | Study register/ abbreviation | Sample size | Average age | Male/Female | Type of disease | Follow-up |
|------------------------|------|----------|---------------|------------------------------|-------------|-------------|-------------|-----------------|-----------|
| Yan Zhang [22]         | 2013 | China    | 2007–2008     | NA                           | 76          | 24.4 ± 1.7  | 44/32       | Enneking II-III | 5 years   |
| Neyssa M. Marina [23]  | 2016 | International | 2005–2011   | EURAMOS-1                     | 2260 (618)  | 4–40        | 365/253     | High grade      | 62–63 months|
| Sophie Piperno-Neumann  | 2016 | France   | 2007–2014     | OS2006                       | 318         | 15.4 (5.8–50.9) | 179/136     | High grade      | 3.9 years |
| Stefan S. Bielack [25] | 2015 | International | 2005–2011   | EURAMOS-1                     | 2260 (1041) | 14 (11–16)  | 421/295     | High grade      | 44 months |
| Alessandra Longhi [26] | 2014 | Italy    | 2007–2011     | EudraCT: 2006-002676-18      | 20          | 34 (11–65)  | 11/9        | Postrelapse     | 73 months |
| J.S. Whelan [27]       | 2012 | Europe   | 1982–2000     | EOI (BO02/80831)             | 179         | 3–40        | 102/77      | High grade      | 9.4 years |
|                        |      |          |               | EOI (BO03/80861)             | 391         | 3–38        | 261/130     | High grade      | 9.4 years |
| Hui Zhao [28]          | 2010 | China    | 2002–2007     | NA                           | 32          | 18.5 (7–68) | 16/16       | Lung metastasis | 60 months |
| Alexander J. Chou [29] | 2009 | USA      | 2001–2005     | CCG/POG (INT-0133)           | 91          | <30         | 56/35       | High-grade intramedullary metastasis | 89 months |
| Paul A. Meyers [30]    | 2008 | USA      | 2001–2005     | CCG/POG (INT-0133)           | 662         | 13 (1–30)   | 361/301     | High grade, Non-metastasis | 7.7 years |
| Marie-Cecile Le Deley [31] | 2007 | France | 1994–2001     | SFP–OS94 (NCT00180908)       | 234         | 13.2 (3.1–19.5) | 131/103     | High grade      | 77 months |
| Paul A. Meyers [32]    | 1998 | USA      | 1986–1993     | MSKCC (T12) protocol         | 73          | 15.8 (4.6–36.4) | 42/31       | High grade      | 91.4 months |
| Robert L. Souhami [33] | 1997 | International | 1986–1991 | EOI (T10) protocol           | 407         | NA          | 261/130     | High grade, Non-metastasis | 5.6 years |
| Michael P. Link [34]   | 1993 | International | 1982–1984 | MIOS                         | 36          | NA          | NA          | High grade, Non-metastasis | 4–8 years |
| John H. Edmonson [35]  | 1984 | USA      | 1976–1980     | Mayo Clinic                  | 38          | 17 (9–62)   | 24/14       | Postoperation    | 31–74 months|
| K. Winkler [36]        | 1984 | Germany  | 1979–1982     | COSS-80                      | 116         | 14 (5–24)   | 69/47       | High grade      | 30 months |
| F. Eilber [37]         | 1987 | USA      | 1981–1984     | NA                           | 112         | 15 (4–75)   | 44/15       | Non-metastasis  | 2 years   |
| D.R. Sweetnam [38]     | 1986 | UK       | 1975–1981     | NA                           | 194         | 1–40        | 111/83      | Lung metastasis | 26–94 months|
| K. Winkler [39]        | 1988 | Germany  | 1982–1984     | COSS-82                      | 125         | 14          | 73/52       | Osteosarcoma     | 6 years   |
| Vivien H.C. Bramwell [40] | 1992 | Canada   | 1983–1986     | EOI                          | 198         | NA          | 114/84      | High grade      | 5 years   |
| John C. Irv [41]       | 1976 | USA      | 1974–1975     | Mayo Clinic                  | 26          | NA          | NA          | Osteosarcoma     | 15 months |
| C. Jasmin [42]         | 1978 | France   | 1976–1978     | EORTC                        | 27          | 18 (9–28)   | 13/14       | Osteosarcoma     | 2 years   |
| Gilchrist GS [43]      | 1978 | USA      | NA            | NA                           | 32          | NA          | NA          | Osteosarcoma     | 753 days |
| J.M.V. Burgers [44]    | 1988 | Netherlands | 1978–1983 | EORTC-SIOP-03 (20781)       | 140         | 1–30        | 87/53       | Osteosarcoma     | 5 years   |

Abbreviations: CCG Children’s Cancer Group, COSS Cooperative Osteosarcoma Study Group, EOI the European Osteosarcoma Intergroup, EORTC European Organization for Research on Treatment of Cancer, EURAMOS-1 The European and American Osteosarcoma Study Group, MIOS the Multi-institutional Osteosarcoma Study, MSKCC Memorial Sloan-Kettering Cancer Center, SFOP Societe Francaise d’Oncologie Pediatric, SSG the Scandinavian Sarcoma Group, NA not available

*aMean ± standardization; median (minimum-maximum); minimum-maximum
were present in the analysis conducted in the present study [21]. All tests were two-tailed, and a p value of less than 0.05 was considered statistically significant. Data analyses were performed using STATA software (version 14.0; Stata Corporation, College Station, TX, USA).

**Results**

**Literature search**

In the present study, 747 articles were identified after the duplicates were removed. A total of 678 articles were excluded after the titles and abstracts were screened. The full texts of the remaining 69 articles were assessed, and the following types of studies were removed: non-randomized design (19); comparisons of the same type of chemotherapeutic drug (12); duplications or secondary studies (9); non-controlled studies (2); no desired outcomes (2); and other sarcoma studies (2). Eventually, 23 articles assessing a total of 5742 patients were included in the present systematic review [22–44] (Fig. 1, Table 1).

The included studies were published from 1976 to 2016 and were researched from 1974 to 2014. The analysis contained several multicentre large-scale studies, such as The European and American Osteosarcoma Study Group-1 (EURAMOS-1), Osteosarcoma 2006 (OS2006), and the Symposium of the Cooperative Osteosarcoma Study Group (COSS-80). Many studies contained duplicate reports. Thus, we included relatively recently published studies and referred to the outcomes of the duplicate reports. All age groups of patients were included, and slightly more men than women were included. All studies included patients with osteosarcoma defined according to a pathological diagnosis. In addition, four studies included osteosarcoma patients without metastasis, two studies included metastasis patients, and one study included relapse patients. Most studies initiated chemotherapy prior to surgery. The longest median follow-up period was 9.4 years (Table 1). All included studies had an RCT design without blinding, and most randomizations were not rigorous. However, the assessed outcome was objective; thus, the overall quality of the included studies was not ideal but was acceptable (Additional file 1: Figure S1).

For chemotherapeutic drug application, we investigated all types of drugs used in the intervention arms and classified each of the drugs of the experimental arms

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**Fig. 1** PRISMA flowchart illustrating the selection of studies included in the present analysis
by alphabetical order. The present study did not include a comprehensive analysis, reflecting the characteristics of applied chemotherapeutic protocols, as most application stages, durations, and dosages of drugs were different in different protocols (Table 2). Drugs showing no chemotherapeutic effect, such as granulocyte colony-stimulating factor (G-CSF) and muramyl tripeptide, were excluded. Drugs that may be included in chemotherapy, such as mistletoe, were included in the present analysis.

For the PFS analysis, we extracted all studies of 5-year PFS or the longest follow-up period for PFS. In the present study, we analysed 16 types of multi-drug regimens. Four multi-drug regimens were directly compared to a blank control, which indicated treatment without chemotherapy. In this analysis, the nodes were weighted according to the number of studies evaluated for each treatment, and the edges were weighted according to the precision of the direct estimate for each pairwise comparison (Fig. 2a). In network pairwise comparisons, the ABCDM (all protocol abbreviations are defined in Table 2) regimen was superior to the ACML (logOR, 1.38; 95% CI, 0.09–2.68) and Blank (logOR, 1.30; 95% CI, 0.19–2.41) regimens for the PFS outcome. The ABCDMP regimen was superior to the ACML (logOR, 2.14; 95% CI, 0.45–3.84), AML (logOR, 2.13; 95% CI, 0.00–4.26), Blank (logOR, 2.06; 95% CI, 0.50–3.62), and ML regimens (logOR, 2.24; 95% CI, 0.22–4.27), and the ABCDMI regimen, combining ABCDMP with ifosfamide, was superior to the ABCDMP regimen alone (logOR, 0.84; 95% CI, 0.09–1.59). The ABCDPMI regimen was also superior to the ACML (logOR, 2.98; 95% CI, 1.13–4.84), AML (logOR, 2.97; 95% CI, 0.71–5.23), Blank (logOR, 2.90; 95% CI, 1.17–4.63), ML (logOR, 3.08; 95% CI, 0.92–5.24), and N (logOR, 2.75; 95% CI, 0.22–5.28) regimens in the network comparisons. Moreover, the ABCDMP regimen in combination with vincristine (ABCDMPL) was superior to the ACML (logOR, 1.89; 95% CI, 0.14–3.64), AMP (logOR, 0.46; 95% CI,
0.01–0.90), AMPF (logOR, 0.64; 95% CI, 0.11–1.18), and Blank (logOR, 1.80; 95% CI, 0.19–3.42) regimens. No other significant differences were found among these regimens (Additional file 2: Table S1). Based on the SUCRA rank, the ABCDMP regimen was the most likely treatment to improve PFS in osteosarcoma patients (94.1%), followed by the ABCDMP (81.9%) and ABCDMP (76.9%) regimens. Additionally, the comparison-adjusted funnel plot used to assess publication bias and determine the presence of small-study effects did not suggest any publication bias (Additional file 3: Figure S2a). In addition, some regimens were not included in the network meta-analysis, reflecting a disconnection, and a traditional meta-analysis showed no significant difference between interventions, except for APIZ compared to MIE (OR, 2.27; 95% CI, 1.02–5.04) (Fig. 3).

For the OS analysis, we separated the regimens into two groups, reflecting the disconnection. The first group included AMP, AMPF, AMPI, AMPIE, AP, and ABCDMP. Four regimens were directly compared to AMP, and we directly compared AP and ABCDMP (Fig. 2b). In the network comparisons, the ABCDMP regimen showed a significant advantage compared to the AMP (logOR, 0.47; 95% CI, 0.02–0.92), AMPF (logOR, 0.65; 95% CI, 0.01–1.29), and AP (logOR, 0.31; 95% CI, 0.04–0.57) regimens (Additional file 4: Table S2). The results showed that ABCDMP was most likely the best regimen for improving the OS (94.7%) of osteosarcoma patients, followed by the AP (58.3%) and AMPF (56.8%) regimens. The second group included ABCDM, ABCDMP, ACML, BCDM, ML, and N. Four regimens were directly compared to the Blank condition (Fig. 2c). In the network comparison, the ABCDMP regimen was superior to the AML (logOR, 1.99; 95% CI, 0.14–3.84), Blank (logOR, 1.54; 95% CI, 0.37–2.70), and ML (logOR, 1.76; 95% CI, 0.03–3.49) regimens, and no other significant difference was found among comparisons (Additional file 5: Table S3). Regarding rank, ABCDMP (89.4%) was most likely to be the best regimen, followed by N (70.1%) and BCDM (60.9%). The comparison-adjusted funnel plot showed no obvious publication bias (Additional file 3: Figure S2b and c). A comparison of regimens not included in the network meta-analysis revealed that APIR had a significant advantage over API in improving the OS (OR, 3.48; 95% CI, 1.17–10.32) of the patients (Fig. 3). However, this result was based on a single study and lacked precision and robustness.

**Discussion**

In the present study, we analysed single- or multi-drug regimens of chemotherapy for the treatment of osteosarcoma using a network meta-analysis. We did
| Author                              | Year | Study register/short name | Intervention | Abbr. | Control | Abbr. |
|-------------------------------------|------|----------------------------|--------------|-------|---------|-------|
| Yan Zhang [22]                     | 2013 | NA                         | Adriamycin; cisplatin; ifosfamide; recombinant human endostatin | APIR  | Adriamycin; cisplatin; ifosfamide | API   |
| Neyssa M. Marina [23]              | 2016 | EURAMOS-1                  | Adriamycin; methotrexate; cisplatin | AMP   | Adriamycin; methotrexate; cisplatin; ifosfamide; etoposide | AMPIE |
| Sophie Piperno-Neumann [24]        | 2016 | OS2006                     | Methotrexate; ifosfamide; etoposide; zoledronate | MIEZ  | Methotrexate; ifosfamide; etoposide | MIE   |
| Stefan S. Bielack [25]             | 2015 | EURAMOS-1                  | Adriamycin; methotrexate; cisplatin; zoledronate | AMPIZ | Adriamycin; cisplatin; ifosfamide | API   |
| Alessandra Longhi [26]             | 2014 | EudraCT: 2006-002676-18    | Viscum album | V     | Etoposide | E     |
| J.S. Whelan [27]                   | 2012 | EOI (BO02/80831)           | Adriamycin; methotrexate; cisplatin | AMP   | Adriamycin; cisplatin | AP    |
| Hui Zhao [28]                      | 2010 | NA                         | Cisplatin; pirarubicin | PT    | Ifosfamide; pirarubicin | IT    |
| Alexander J. Chou [29]             | 2009 | CCG/POG (INT-0133)        | Adriamycin; methotrexate; cisplatin | AMP   | Adriamycin; cisplatin; methotrexate; ifosfamide | AMPI  |
| Paul A. Meyers [30]                | 2008 | CCG/POG (INT-0133)        | Adriamycin; methotrexate; cisplatin | AMP   | Adriamycin; cisplatin; methotrexate; ifosfamide | AMPI  |
| Marie-Cecile Le Deley [31]        | 2007 | SFOP-OS94 (NCT00180908)   | Adriamycin; methotrexate | AM    | Methotrexate; ifosfamide; etoposide | MIE   |
| Paul A. Meyers [32]                | 1998 | MSKCC (T12) protocol      | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; cisplatin | ABCDMP| Bleomycin; cyclophosphamide; dactinomycin; methotrexate; | BCDM  |
| Robert L. Souhami [33]             | 1997 | EOI (T10) protocol        | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; vincristine | ABCDML| Adriamycin; cisplatin | AP    |
| Michael P. Link [34]               | 1993 | MIOS                       | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; cisplatin | ABCDMP| Blank | Blank |
| John H. Edmonson [35]              | 1984 | Mayo Clinic               | Methotrexate; vincristine | ML    | Blank | Blank |
| K. Winkler [36]                    | 1984 | COSS-80                   | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; interferon | ABCDMF| Adriamycin; methotrexate; cisplatin; interferon | AMPF  |
| F. Eilber [37]                     | 1987 | NA                         | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; | ABCD  | Adriamycin; methotrexate; cisplatin | AP    |
| D.R. Sweetnam [38]                 | 1986 | NA                         | Adriamycin; methotrexate; vincristine | AML   | Methotrexate; vincristine | ML    |
| K. Winkler [39]                    | 1988 | COSS-82                   | Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; cisplatin; ifosfamide | ABCDMP| Adriamycin; bleomycin; cyclophosphamide; dactinomycin; methotrexate; cisplatin; | ABCDMP |
| Vivien H.C. Bramwell [40]          | 1992 | EOI                       | Adriamycin; methotrexate; cisplatin | AMP   | Adriamycin; cisplatin | AP    |
| John C. Ivins [41]                 | 1976 | Mayo Clinic               | Transfer factor | N     | Adriamycin; methotrexate; vincristine | AML  |
not analyse the chemotherapeutic effect according to protocols because the application stage, duration, and dosage of each drug varied. The PFS analysis showed that the ABCDMP, ABCDML, and ABCDM regimens were most likely to improve PFS in osteosarcoma patients. In the present study, the ABCDMP regimen played a critical role in a treatment involving the T12 protocol (including adriamycin, bleomycin, cyclophosphamide, dactinomycin, methotrexate, cisplatin) used at the Memorial Sloan Kettering Cancer Center (MSKCC) between 1986 and 1993. This more intensive preoperative regimen comprised two courses of cisplatinum and doxorubicin in addition to a high dose of methotrexate and bleomycin, cyclophosphamide, and dactinomycin [32]; it showed a better effect on prolonging the PFS of patients when combined with ifosfamide or vincristine. However, these results are partially supported by a previous view that ifosfamide-based chemotherapy significantly improves the PFS of osteosarcoma patients [7]. In the secondary outcome analysis, we also observed that the regimens with more types of drugs showed better results, but use of a transfer factor also showed advantages. However, these results should be considered with caution, as most studies changed the initial protocol and required more active chemotherapy with metastasis or progression. Therefore, the effective gap between interventions could be reduced, resulting in bias. The practice of changing the chemotherapy regimen is common, correct, and ethical in clinical practice.

Despite the present results, it is undeniable that when the number of different types of chemotherapeutic drugs increases, the cytotoxicity and adverse effects will also simultaneously increase. Thus, a balance exists, suggesting that multi-drug regimens could significantly prolong the PFS of osteosarcoma patients but lead to more serious adverse effects. Adverse effects are common in chemotherapy and include nephrotoxicity, ototoxicity, and bone marrow suppression. Serious adverse effects will affect the application of the chemotherapy programme and even the quality of life of patients.

Thus, in clinical practice, cytoprotective agents, such as muramyl tripeptide, are also frequently and simultaneously used for chemotherapy. However, this agent is not widely used, and the literature did not show that cytoprotective agents significantly improved the PFS and OS of patients [29]. Therefore, in the present study, we did not analyse the use of cytoprotective agents. In addition, Viscum album, transfer factor, and recombinant human endostatin are non-traditional chemotherapy drugs that show a cytotoxicity effect. Although they are controversial, we still included these types of drugs in the present analysis.

In the present study, neoadjuvant chemotherapy was used in most included studies. Neoadjuvant chemotherapy includes the administration of chemotherapeutic agents prior to the main treatment, and this regimen has several advantages: (1) It can eliminate micrometastases early to avoid metastases caused by delayed surgery or low resistance. (2) It can control the primary tumour and reduce the chance of surgical tumour spread. (3) It can assess the chemotherapeutic effect and guide the postoperative chemotherapy. (4) It can assess the prognosis earlier. Although the results of RCTs suggested no significant effect on the outcome of patients when comparing preoperative chemotherapy to postoperative chemotherapy [45], neoadjuvant chemotherapy for limb salvage and the surgical process is still worthy of clinical application.

In addition, several studies compared intra-arterial or intravenous chemotherapeutic infusion. When the same regimens were applied, no significant differences were observed in the chemotherapy response between intra-arterial and intravenous infusion [46, 47]. However, some studies suggested that intra-arterial infusion has a more active effect [48, 49]. Regarding the dosage of chemotherapeutic agents, comparisons of a high or moderate dose of methotrexate have primarily been described. A high dose of methotrexate was more widely used in patients who could tolerate this drug. However, in small-sample RCTs of children with osteosarcoma, a significant difference in outcome was not observed between different dosages [50–52].

We systematically analysed chemotherapeutic regimens for osteosarcoma patients using a network
meta-analysis, although individual chemotherapeutic protocols could not be analysed. In the present study, multi-drug regimens, such as the T12 protocol plus ifosfamide or vincristine, had a better effect on prolonging the PFS and OS of osteosarcoma patients. Further research with well-designed, double-blinded RCTs is still necessary, as the psychological evidence might also influence patient outcomes. In addition, further trials using relatively well-developed chemotherapeutic protocols would be beneficial to analyse the differences among multiple chemotherapeutic protocols.

Limitations
There are several limitations to the present study. First, the present analysis was performed at a study level, not at an individual level. Second, for chemotherapy, cytoprotective agents might also improve the survival time of patients by reducing the chemotherapy-induced damage to normal tissue, but these drugs were not analysed in this study. Third, we did not perform the Grading of Recommendations Assessment, Development and Evaluation in the present analysis, as all included studies had an RCT design without blind concealment, and most of the results showed a low risk of imprecision.

Conclusions
In conclusion, the T12 protocol has a better effect on prolonging the PFS of osteosarcoma patients when combined with ifosfamide or vincristine. For the OS, the T12 protocol plus vincristine or the removal of cisplatinum also represents the best regimen. Further RCTs of chemotherapeutic protocols are still necessary.

Additional files

Additional file 1: Figure S1. Risk of bias graph of each included study. (EPS 2203 kb)
Additional file 2: Table S1. The league table of the network for the progression-free survival estimates the treatments according to their relative effects. (DOCX 19 kb)
Additional file 3: Figure S2. Comparison-adjusted funnel plots for assessing all outcomes. a: Progression-free survival; b: Overall survival, part one; c: Overall survival, part two. (EPS 888 kb)
Additional file 4: Table S2. The league table of the network for the overall survival estimates the treatments according to their relative effects for first part. (DOCX 14 kb)
Additional file 5: Table S3. The league table of the network for the overall survival estimates the treatments according to their relative effects for second part. (DOCX 14 kb)

Abbreviations
A: Adriamycin; B: Bleomycin; C: Cyclophosphamide; CCG: Children’s Cancer Group; COSS: Cooperative Osteosarcoma Study Group; CTs: Confidence intervals; D: Dactinomycin; E: Etoposide; EOI: The European Osteosarcoma Intergroup; EORTC: European Organization for Research on Treatment of Cancer; EURAMOS-1: The European and American Osteosarcoma Study Group; F: Interferon; G-CSF: Granulocyte colony stimulating factor; I: Ifosfamide; K: Alkeran; L: Vincristine; M: Methotrexate; MIOs: The Multi-institutional Osteosarcoma Study; MSKCC: Memorial Sloan Kettering Cancer Center; N: Transfer factor; NA: Not available; ORs: Odds ratios; OS: Overall survival; P: Cisplatin; PFS: Progression-free survival; PRISMA: Preferred Reporting Items for Systematic Reviews; R: Recombinant human endostatin; RCT: Randomized controlled trial; SFOPE: Societe Francaise d’Oncologie Pediaetrique; SSG: The Scandinavian Sarcoma Group; SUCLA: Surface Under the Cumulative Ranking; T: Pirarubicin; V: Viscum album; Z: Zoledronate

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Availability of data and materials
All the data of the manuscript are presented in the paper or additional supporting files.

Authors’ contributions
XjW conceived the study. XjW and HZ searched the literature and collected the data. XjW, TS, CmT, and KM performed the statistical analysis. XjW and HZ drafted the manuscript. PW reviewed the manuscript. All authors have read and approved the final paper.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
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