Int J Clin Exp Med 2016;9(7):12574-12583
www.ijcem.com /ISSN:1940-5901/IJCEM0014952

Original Article

Role of ifosfamide chemotherapy for patients with non-metastatic osteosarcoma: a meta-analysis with 1724 patients

Jian Tu[1*], Xianbiao Xie[1*], Yongqian Wang[1*], Lili Wen[2], Bo Wang[1], Xian Zhong[3], Xuqi Sun[3], Mengqi Wang[3], Jianqiu Kong[3], Gang Huang[1], Junqiang Yin[1], Jingnan Shen[1]

[1]Bone and Soft Tissue Tumor Center, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; [2]Department of Anesthesiology, Sun Yat-sen University Cancer Center, Guangzhou, China; [3]The Eight Year Program, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China. [*]Equal contributors.

Received December 15, 2014; Accepted March 31, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Background: Chemotherapy improves the survival rate of patients with non-metastatic osteosarcoma from 20% to 70%. However, the role of ifosfamide (IFO) in combination with other agents is still controversial. We conducted this meta-analysis to assess the efficacy of IFO in patients with non-metastatic osteosarcoma. Methods: An electronic search of PubMed, The Cochrane Library and EMBASE was performed using the search terms *osteosarcoma* and *ifosfamide* for studies published prior to Sep 6, 2014. All randomized controlled trials and observational comparative studies were included to compare the regimens of IFO to those without IFO for patients with non-metastatic osteosarcoma. Results: Eight studies with a high quality of methodology were included in the analyses, involving 1724 patients. No significant differences were demonstrated in the 5-year event free survival (EFS) (OR = 0.98, 95% CI: 0.57-1.69, P = 0.94, random effects model), overall survival (OS) (OR = 0.74, 95% CI: 0.44-1.26, P = 0.27, random effects model) or histological response rate (OR = 1.09, 95% CI: 0.88-1.34, P = 0.44, random effects model) between regimens containing IFO and those without IFO. For patients without IFO receiving neo-adjuvant chemotherapy, good histological responders had a better 5-year EFS (OR = 0.50, 95% CI: 0.29-0.83, P = 0.008, fixed effects model) than poor responders even when salvage chemotherapy including IFO was performed. The regimens with IFO caused more myelo-suppressive events, such as leukopenia, thrombocytopenia, and febrile neutropenia, than those without IFO (P < 0.005, respectively). Conclusion: The non-metastatic osteosarcoma patients treated with IFO had a similar histological response rate and 5-year EFS and OS, but more myelo-suppressive events than the patients treated without IFO. Whether IFO can be recommended as a first line therapy for patients with non-metastatic osteosarcoma should be identified in further studies.

Keywords: Osteosarcoma, ifosfamide, survival outcome, myelo-suppressive events, metastasis

Introduction

Osteosarcoma is the most common primary malignant bone tumor that typically occurs in children, adolescents and young adults [1]. A combination of neo-adjuvant chemotherapy, surgery and adjuvant chemotherapy is regarded as the standard treatment. The 5-year overall survival (OS) of non-metastatic patients has improved dramatically to 70% since the multi-agent chemotherapy was introduced in the 1970s. The use of multi-agent neo-adjuvant chemotherapy also decreases the rate of amputation surgery for osteosarcoma patients. Unfortunately, the 5-year OS decreases to 20-30% when metastasis occurs [2]. The most commonly used agents are high-dose methotrexate (HDMTX), cisplatin, doxorubicin and ifosfamide (IFO). However, it is still unclear how to combine these agents to obtain the best survival outcome and less toxic events.

IFO is the most controversial agent among these four drugs, and it was recommended as the first line therapy by the National Comprehensive Cancer Network (NCCN) in 2014. Some trials have demonstrated that IFO can increase the survival rate and histological response rate for osteosarcoma patients, while some studies have reported that IFO does not

increase the survival rate. However, the Children's Oncology Group found that the addition of IFO improved only the histological response, and not the OS among osteosarcoma patients [3].

Additionally, the toxicity of multi-agent chemotherapy is also an issue that cannot be ignored during long-time chemotherapy. The patients usually suffer from a high rate of toxicity, such as leukopenia, thrombocytopenia, nausea and vomiting, even when granulocyte colony-stimulating factor support is administered. The multi-agent chemotherapy also affects the protocol compliance of patients. However, it is still unclear whether the use of IFO will increase toxicity events or not.

We conducted this meta-analysis to assess the effect of IFO on osteosarcoma patients, and to explore whether IFO should be added to neo-adjuvant or adjuvant chemotherapy.

Method

Literature sources

A comprehensive search of databases, including PubMed, Cochrane Library, and EMBASE, was performed by searching the terms *osteosarcoma* and *ifosfamide*. The related article function was used to broaden the search. Two authors independently screened the titles and abstracts to determine potential eligibility for this study. When discrepancies occurred, a consensus was achieved after further discussion. The latest search date was August 19, 2014.

Inclusion and exclusion criteria

The inclusion criteria were defined as follows: 1. prospective or retrospective comparative studies; 2. only involved patients with non-metastatic osteosarcoma; 3. neo-adjuvant and adjuvant chemotherapies were performed; 4. having two different regimens, and IFO was involved in at least one of the regimen; and 5. the article was reported in English. Phase I and II studies or studies without data of the 5-year OS and 5-year event-free survival (EFS) were excluded. The newest and most informative article was selected when multiple studies were published by the same group during the same period.

Definition and data extraction

EFS was calculated from the time of diagnosis until tumor recurrence, occurrence of a secondary tumor, death or the last follow-up examination. OS was calculated from the time of diagnosis to death or the last follow-up examination. Histological response was analyzed by the percentage of tissue necrosis. When more than 90% of tissue necrosis was observed, the patients were classified as having a good response. Otherwise, patients were regarded as poor response. Two authors independently extracted the following data: first author, year of publication, 5-year OS, 5-year EFS, histological response rate, toxicity events (death related to chemotherapy, leukopenia, thrombocytopenia, anemia, febrile neutropenia, red blood cell (RBC) transfusion, platelet transfusion, mucositis, nausea and vomiting). We contacted authors for original data if relevant information was unclear or missing.

Quality assessment

Two authors independently assessed the quality of each included study to determine whether the selected studies were appropriate for pooling data. The methodological quality of randomized controlled trials (RCTs) by the modified Jadad scale with a score of 0-10 was assigned to each trial [4]. A study was regarded as low quality, if the score was less than 4. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of observational comparative studies with a score of 0-9 assigned to each study [5]. A study was regarded as low quality, if the score was less than 5. Additionally, the heterogeneity of each study was valued through a visual inspection of forest plots and with a standard chi^2 test and inconsistency (I^2) statistic. *P* values less than 0.05 or I^2 more than 50% indicated significant heterogeneity.

Outcome measure

The primary outcomes of this meta-analysis were comparisons of 5-year EFS and OS between different chemotherapy regimens with or without IFO. Subgroup analyses were also performed according to study types, including IFO used in neo-adjuvant or adjuvant chemotherapy and histological response to IFO. The secondary outcomes included comparisons of the histological response rate, metastasis free survival, and toxicity event rate.

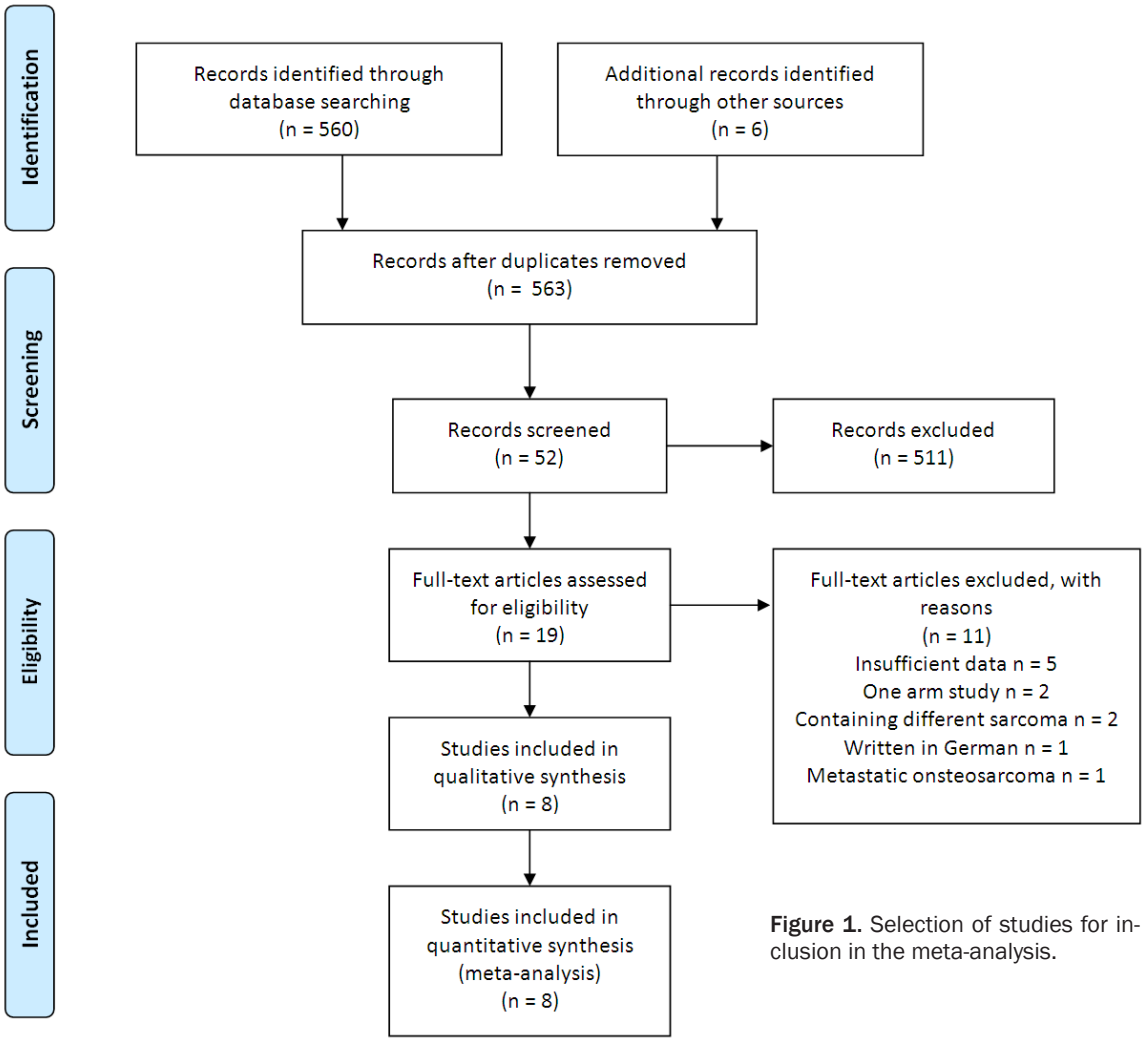


Figure 1. Selection of studies for inclusion in the meta-analysis.

Statistical analysis

The meta-analysis was performed using Review Manager (version 5.0, the Cochrane Collaboration) using two-side hypothesis testing with alpha = 0.5. The odds ratio (OR) was chosen to compare the dichotomous variables. Additionally, the heterogeneity of each study was valued through a visual inspection of forest plots and with a standard chi^2 test and inconsistency (I^2) statistic. *P* values less than 0.05 or I^2 more than 50% indicated significant heterogeneity. Statistical significance was set at a *P* value ≤ 0.05.

Result

*Overview of the included studies (**Figure 1**)*

A total of 563 articles were identified through the comprehensive search, of which 511 articles were excluded according to a screen of the titles and abstracts. Nineteen full-text articles were assessed for eligibility after further evaluation. Finally, 8 articles were included [3, 6-12], leaving 11 articles excluded. Among the excluded articles, 5 studies were excluded because of insufficient data on OS and EFS [13-17], 2 studies were excluded as one arm studies [18, 19], 1 excluded study was written in German [20], 2 excluded studies focused on different sarcomas [21, 22] and 1 excluded study compared regimens with IFO and regimens without IFO on metastatic osteosarcoma [23].

*The characteristic of the included studies (**Table 1**) and quality assessment*

Nine studies involving 1724 patients were included. The baseline characteristics of the patients were shown in the **Table 1**. Three of

Table 1. Characteristics of the included studies

Study ID	Study type	Total number of patients	Age (years) Median (range)	Female (%)	Follow-up Median (range)
Ferrari 2012	prospective	246	14 (4-39)	100 (41)	76 (31-115)
Hong 2011	retrospective	124	16 (4-59)	58 (46.8)	68.4 (4.8-200)
Meyers 2008	prospective	657	NR	301 (45)	92.4 (NR)
Deley 2007	prospective	234	13.2 (3.1-19.5)	103 (44)	77 (36-120)
Ferrari 1999	retrospective	94	15 (4-40)	46 (48)	90 (72-106)
Rha 1999	retrospective	36	16 (8-41)	16 (44.4)	23 (10-98)
Fuchs 1998	retrospective	169	NR	64 (37.9)	100 (NR)
Bacci 1993	retrospective	164	NR	75 (45.7)	54 (36-76)
No. of increased ALP patients (%)	No. of increased LDH patients (%)	Neoadjuvant chemotherapy regiments		Adjuvant chemotherapy regiments	
84 (40)	62 (31)	MTX+CDP+ADM+/-IFO		MTX+CDP+ADM+/-IFO	
92 (74.2)	NR	CDP+ADM+/-IFO		CDP+ADM+/-IFO	
267 (41)	238 (36)	MTX+CDP+ADM+/-IFO		MTX+CDP+ADM+/-IFO	
59 (25)	NR	MTX+/-ADM+/-(IFO+ETO)		MTX+/-(IFO+ETO)+/-(CDP+ADM)	
41 (43)	29 (31)	MTX+CDP+ADM		MTX+CDP+ADM+/-IFO	
12 (32.4)	NR	CDP+ADM		CDP+ADM+/-(IFO+ETO)	
NR	NR	MTX+CDP+ADM+/-IFO		MTX+CDP+ADM+/-IFO	
85	100	MTX+CDP+ADM		MTX+CDP+ADM+/-(IFO+ETO)	

NR: No record; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; MTX: methotrexate; CDP: cisplatin; ADM: adriamycin mycin; IFO: ifosfamide; ETO: etomidate.

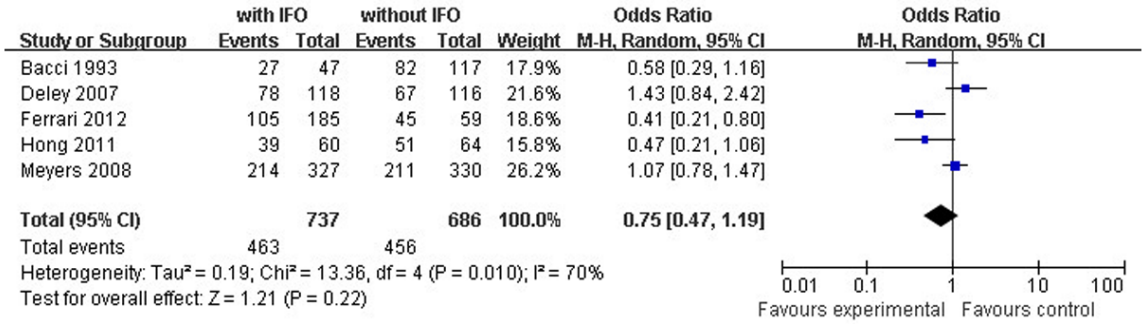


Figure 2. Effects of IFO on 5-year EFS.

the included studies were prospective, while the other 5 were retrospective. The efficacy of IFO was compared in the neo-adjuvant regimens in 5 studies, while the other 3 studies focused on the efficacy of IFO in the adjuvant regimens. All of the RCTs had a score higher than 4 (7.7 ± 1.5) and were considered high quality. The scores of the observational comparative studies were not less than 5 (6.0 ± 0.7), and were regarded as high quality. There were 5 agents administered in the included studies: methotrexate (MTX), cisplatin (CDP), Adriamycin (ADM), ifosfamide (IFO), and etomidate (ETO).

Primary outcomes

No significant differences were found in the comparison of the 5-year EFS (OR = 0.75, 95% CI: 0.47-1.19, P = 0.22, random effects model, **Figure 2**) or OS (OR = 0.73, 95% CI: 0.43-1.26, P = 0.26, random effects model, **Figure 3**) between regimens containing IFO and regimens without IFO.

As for subgroup analysis according to different study types, a significant difference was noted in the 5-year EFS for retrospective studies (OR = 0.53, 95% CI: 0.31-0.90, p = 0.02, random

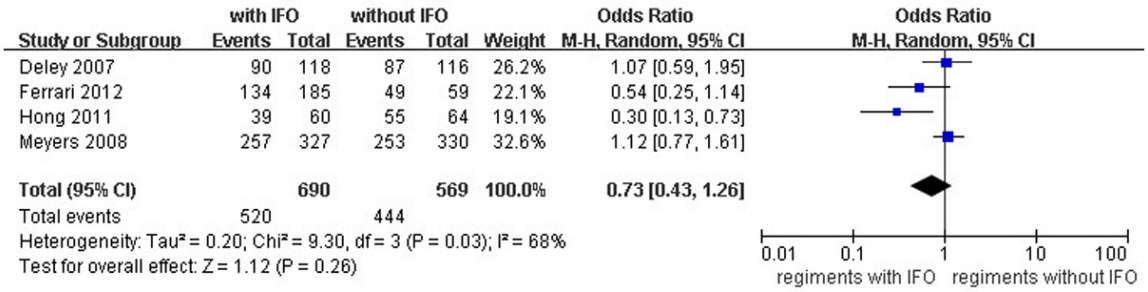


Figure 3. Effects of IFO on 5-year OS.

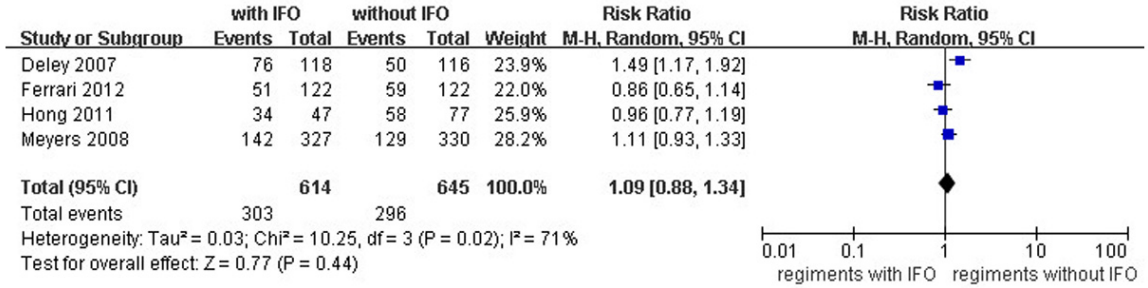


Figure 4. Effects of IFO on histological response rate.

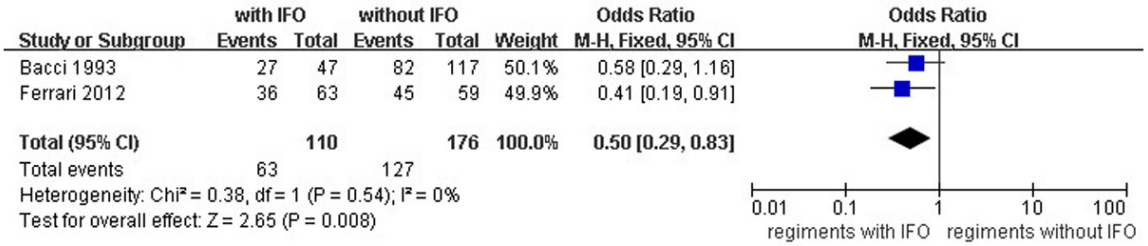


Figure 5. 5-year EFS of IFO on poor histological responsers without IFO in the neoadjuvant.

effects model, Supplementary Figure 1). However, no difference was found in the 5-year EFS for prospective studies (OR = 0.89, 95% CI: 0.49-1.63, P = 0.71, random effects model, Supplementary Figure 1) or the 5-year OS for retrospective studies (OR = 0.98, 95% CI: 0.74-1.31, P = 0.90, random effects model, Supplementary Figure 2). When the studies with the same regimens (HDMTX-CDP-ADM vs. HDMTX-CDP-ADM-IFO) were selected to perform subgroup analysis, there was no difference in the 5-year EFS (OR = 0.69, 95% CI: 0.27-1.77, P = 0.44, random effects model, Supplementary Figure 3) or OS (OR = 0.84, 95% CI: 0.41-1.69, P = 0.62, random effects model, Supplementary Figure 4).

Secondary outcomes

The pooled OR of the histological response rate between regimens containing IFO in the neo-adjuvant chemotherapy and those without IFO was 1.09 (95% CI: 0.88-1.34, P = 0.44, random effects model, **Figure 4**), based on 1259 patients from 4 studies. Interestingly, when the poor responders receiving neo-adjuvant chemotherapy without IFO were administered salvage adjuvant chemotherapy containing IFO, the 5-year EFS was worse than the good responders receiving chemotherapy without IFO (OR = 0.50, 95% CI: 0.29-0.83, P = 0.008, fixed effects model, **Figure 5**). Additionally, there was a significant difference in the 3-year

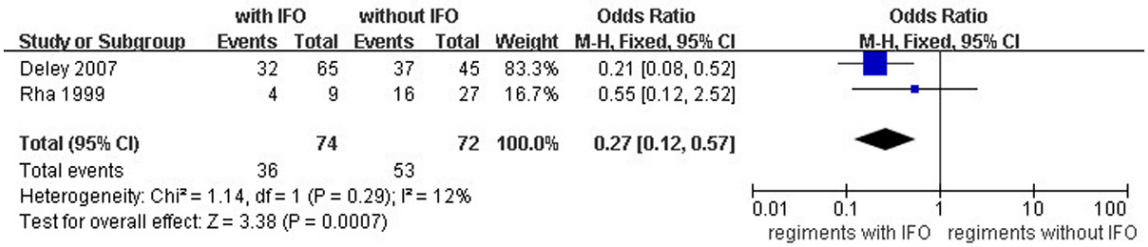


Figure 6. 3-year EFS of IFO on poor histological responsers without IFO in the neoadjuvant.

Table 2. Summary of Secondary Outcomes

Clinical Outcome	No. Studies	Odds Ratio(95% CI, P)	*P* value for Chi²	I^2 (95% CI) for OR (%)
Death related to chemotherapy	2	3.05 (0.31-29.73, 0.34)	NA	NA
Leukopenia	3	5.91 (1.28-27.33, 0.02)	0.002	84
Thrombocytopenia	2	5.85 (3.36-10.17, < 0.00001)	0.48	0
Anemia	1	NA	NA	NA
Febrile neutropenia	3	3.32 (2.32-4.74, < 0.00001)	0.08	52
RBC transfusion	2	2.15 (1.71-2.70, < 0.00001)	0	64
PLT transfusion	2	2.59 (1.89-3.54, < 0.00001)	0	85
Nausea and vomiting	1	NA	NA	NA
Mucositis	1	NA	NA	NA
Renal toxin	1	NA	NA	NA

NA: not applicable.

EFS compared the poor responders with the good responders without IFO in the neo-adjuvant chemotherapy (OR = 0.27, 95% CI: 0.12-0.57, P = 0.0007, fixed effects model, **Figure 6**).

Table 2 summarized all of the toxic event rates between regimens with IFO and those without IFO. The incidence of anemia, mucositis, nausea and vomiting between regimens with IFO and those without IFO was not significantly different. However, the addition of IFO led to significantly higher incidences of leukopenia (OR = 5.91, 95% CI: 1.28-27.33, P = 0.02, random effects model, Supplementary Figure 6), thrombocytopenia (OR = 5.85, 95% CI: 3.36-10.17, P < 0.00001, random effects model, Supplementary Figure 7), febrile neutropenia (OR = 3.32, 95% CI: 2.32-4.74, P < 0.00001, random effects model, Supplementary Figure 8), RBC transfusion (OR = 2.15, 95% CI: 1.89-3.54, P < 0.00001, random effects model, Supplementary Figure 9) and PLT transfusion (OR = 2.59, 95% CI: 1.89-3.54, P < 0.00001, random effects model, Supplementary Figure 10) compared to those without IFO. No difference was found in the death related to chemotherapy between regimens with or without IFO (Supplementary Figure 5).

Discussion

Our meta-analysis summarized all eligible studies comparing the effect of IFO for osteosarcoma patients. Three RCTs and five observational comparative studies were collected, involving 1724 patients with non-metastatic osteosarcoma. The pooled data revealed that the chemotherapy regimens with IFO had similar rates of 5-year OS, EFS and histological response than those without IFO. As for patients treated without IFO in the neo-adjuvant chemotherapy, poor responders had a worse survival rate than good responders even using salvage chemotherapy with IFO. However, more toxic events occurred with the use of IFO.

IFO is a typical nitrogen mustard alkylating agent, containing the ethylene immonium ion, which can combine with the double bonds of DNA. Therefore IFO can interfere with the replication and transcription of cancer cells. It is widely used in lung cancer, breast cancer, and

sarcoma. However our results did not support the addition of IFO to first-line chemotherapy for non-metastatic osteosarcoma. Additionally, according to the INT-0133 study, the regimens containing IFO were not better than those without IFO for patients with metastatic osteosarcoma [23]. Recently, Judson reported a randomized controlled phase 3 trial that demonstrated that the combination of IFO and doxorubicin was not superior to doxorubicin alone for first-line treatment of advanced soft-tissue sarcoma [24]. The underlying mechanism is still unknown. These results may be due to a plateau that was reached by these three or four agents for patients with non-metastatic osteosarcoma. Therefore it was very important to identify as less as few drugs as possible to reach the plateau with less toxic events.

Table 1 indicated that different regimens were compared in different studies, so we performed subgroup analyses by distinguishing between different chemotherapy regimens containing IFO. A significant difference in the 5-year EFS in retrospective studies may result from data bias in case selection. Because IFO may interact with other drugs, studies using the same regimens (MTX+CDP+ADM+/-IFO) were selected for subgroup analysis. Nevertheless, no differences were found. These findings were consistent with the previous studies concentrating on the effect of IFO on non-metastatic Ewing's sarcoma [25].

Our results revealed that IFO cannot increase the histological response rate when added to neo-adjuvant chemotherapy. This finding was not consistent with previous studies in which regimens with 4 drugs (MTX+CDP+ADM+IFO) were able to increase the histological response rate. This result may be due to different neo-adjuvant regimens that were used in the included four studies on histological response rate. Two studies compared MTX+CDP+ADM with MTX+CDP+ADM+IFO, while one study compared CDP+ADM with CDP+ADM+IFO, and one study compared MTX+ADM with MTX+IFO+ETO. Furthermore, previous studies demonstrated that an increased histological response rate could translate into a better survival outcome [26]. In the current study, the link between histological response and survival outcome was not explored, because insufficient original data were extracted from the published paper and bias caused by different regimens cannot be ignored. This link should be further studied by well-designed RCTs in the future.

However, for patients receiving neo-adjuvant chemotherapy without IFO, many centers prefer to add IFO to the patients with a poor histological response as a salvage chemotherapy. Unfortunately, our study demonstrated that these patients with poor histological response had a significantly lower survival rate than those with good response, even using the salvage chemotherapy. On one hand, the worse outcomes were not caused by IFO. These patients with a poor response may develop resistance after neo-adjuvant chemotherapy. If the dose of IFO was increased, it may overcome the resistance. However, increased toxic events may be accompanied by the increased dose. On the other hand, our results demonstrated that IFO would increase toxicity events, which may weaken the patient's status and immune system. As Issels's study demonstrated IFO sensitively targeted human lymphocytes through metabolic stress during treatment [27]. Therefore, the addition of IFO to the patients with a poor response would be detrimental to the survival outcome. It was still unclear whether an intensified dose of IFO in the salvage chemotherapy can improve the survival outcome of patients with poor histological response. How to increase the survival outcomes of these patients with poor histological response should be analyzed in further studies.

Toxicity events caused by chemotherapy are not uncommon and affect the quality of life of patients, as most of them are young and suffer from the long therapy period. Based on our analyses, regimens containing IFO were significantly more myelosuppressive than regimens without IFO. This finding is consistent with studies concentrating on effects of IFO on metastatic soft tissue sarcoma and Ewing's sarcoma, both of which found that regimens with IFO caused more toxic events [28]. IFO can irreversibly inhibit the proliferative response to interleukin-2 in a dose-dependent manner and also induce phosphorylation of HSP27 by depleting glutathione [29]. However, the incidence of mucosa reactions cannot be pooled because of insufficient data. Based on the only study that reported data on mucosa reactions, the incidence of mucosa reactions was quite similar between regimens with IFO and those with-

out IFO. As prior review reported that high incidence of grade 3 or 4 mucositis was associated with longer survival [26]. The survival rate in the study was not significantly different. This finding demonstrates the internal integrity of our study. However, the incidences of encephalopathy and cardiotoxicity were not reported in the included studies, which are regarded as specific toxic events caused by IFO.

The current meta-analysis had some limitations that must be considered. First, there were only 3 RCTs and 5 observational studies included in this meta-analysis. Second, between-study heterogeneity was significant for chemotherapy regimens. The chemotherapy regimens used at the different cancer centers may be slightly different, such as different agents, different ways to receive the drugs and different doses of one agent. It would be ideal to pool data from different RCTs using the same chemotherapy regimens. However, it is extremely difficult to conduct RCTs to compare chemotherapy regimens. This issue highlights the importance of conducting meta-analyses.

Conclusion

This meta-analysis provides some evidences that the chemotherapy regimens with IFO have similar 5-year EFS, OS and histological response rates compared to regimens without IFO, but cause more myelosuppressive events. Even when IFO was used as salvage chemotherapy, the poor responders had a lower survival rate than the good responders. Whether IFO can be recommended as a first line therapy for patients with non-metastatic osteosarcoma should be identified in further studies.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (No. 81202118); Specialized Research Fund for the Doctoral Program of Higher Education (No. 20120171120088); and The Young Teachers Training Plan of Sun Yat-sen University (No. 13ykpy22).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Jingnan Shen and Junqiang Yin, Bone and Soft Tissue Tumor Center, First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China. Tel: +86 20 87335039; Fax: +86 20 87332150; E-mail: shenjn01@hotmail.com (JNS); yinjunqiang77@163.com (JQY)

References

[1] Biermann JS, Adkins D, Benjamin R, Brigman B, Chow W, Conrad ER, Frassica D, Frassica FJ, George S, Healey JH, Heck RJ, Letson GD, Mayerson J, McGarry SV, O'Donnell RJ, Patt J, Randall RL, Santana V, Satcher RL, Schmidt RG, Siegel HJ, Wong MK, Yasko AW. Bone cancer. J Natl Compr Canc Netw 2007; 5: 420-37.

[2] Sun L, Li Y, Li H, Zhang J, Li B, Ye Z. Analysis of chemotherapy dosage and dosage intensity and survival outcomes of high-grade osteosarcoma patients younger than 40 years. Clin Ther 2014; 36: 567-78.

[3] Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, Ferguson WS, Gebhardt MC, Goorin AM, Harris M, Kleinerman E, Link MP, Nadel H, Nieder M, Siegal GP, Weiner MA, Wells RJ, Womer RB, Grier HE. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival--a report from the Children's Oncology Group. J Clin Oncol 2008; 26: 633-8.

[4] Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996; 17: 1-12.

[5] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-5.

[6] Ferrari S, Ruggieri P, Cefalo G, Tamburini A, Capanna R, Fagioli F, Comandone A, Bertulli R, Bisogno G, Palmerini E, Alberghini M, Parafioriti A, Linari A, Picci P, Bacci G. Neoadjuvant chemotherapy with methotrexate, cisplatin, and doxorubicin with or without ifosfamide in non-metastatic osteosarcoma of the extremity: an Italian sarcoma group trial ISG/OS-1. J Clin Oncol 2012; 30: 2112-8.

[7] Hong S, Shin SJ, Jung M, Jeong J, Lee YJ, Shin KH, Roh JK, Rha SY. Comparison of long-term outcome between doublet and triplet neoadjuvant chemotherapy in non-metastatic osteosarcoma of the extremity. Oncology 2011; 80: 107-17.

[8] Le Deley MC, Guinebretiere JM, Gentet JC, Pacquement H, Pichon F, Marec-Berard P, Entz-Werle N, Schmitt C, Brugieres L, Vanel D, Dupouy N, Tabone MD, Kalifa C. SFOP OS94: a randomised trial comparing preoperative high-dose methotrexate plus doxorubicin to high-dose methotrexate plus etoposide and ifosfamide in osteosarcoma patients. Eur J Cancer 2007; 43: 752-61.

[9] Ferrari S, Mercuri M, Picci P, Bertoni F, Brach DPA, Tienghi A, Mancini A, Longhi A, Rimondini S, Donati D, Manfrini M, Ruggieri P, Biagini R, Bacci G. Nonmetastatic osteosarcoma of the extremity: results of a neoadjuvant chemotherapy protocol (IOR/OS-3) with high-dose methotrexate, intraarterial or intravenous cisplatin, doxorubicin, and salvage chemotherapy based on histologic tumor response. Tumori 1999; 85: 458-64.

[10] Rha SY, Chung HC, Gong SJ, Shim KY, Ahn JB, Yang WI, Shin KH, Yoo NC, Kim JH, Roh JK, Lee CI, Kim BS. Combined pre-operative chemotherapy with intra-arterial cisplatin and continuous intravenous adriamycin for high grade osteosarcoma. Oncol Rep 1999; 6: 631-7.

[11] Fuchs N, Bielack SS, Epler D, Bieling P, Delling G, Korholz D, Graf N, Heise U, Jurgens H, Kotz R, Salzer-Kuntschik M, Weinel P, Werner M, Winkler K. Long-term results of the co-operative German-Austrian-Swiss osteosarcoma study group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. Ann Oncol 1998; 9: 893-9.

[12] Bacci G, Picci P, Ferrari S, Ruggieri P, Casadei R, Tienghi A, Brach del Prever A, Gherlinzoni F, Mercuri M, Monti C. Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremities. Results in 164 patients preoperatively treated with high doses of methotrexate followed by cisplatin and doxorubicin. Cancer 1993; 72: 3227-38.

[13] Oberlin O, Fawaz O, Rey A, Niaudet P, Ridola V, Orbach D, Bergeron C, Defachelles AS, Gentet JC, Schmitt C, Rubie H, Munzer M, Plantaz D, Deville A, Minard V, Corradini N, Leverger G, de Vathaire F. Long-term evaluation of Ifosfamide-related nephrotoxicity in children. J Clin Oncol 2009; 27: 5350-5.

[14] Marina NM, Poquette CA, Cain AM, Jones D, Pratt CB, Meyer WH. Comparative renal tubular toxicity of chemotherapy regimens including ifosfamide in patients with newly diagnosed sarcomas. J Pediatr Hematol Oncol 2000; 22: 112-8.

[15] Winkler K, Beron G, Delling G, Heise U, Kabisch H, Purfurst C, Berger J, Ritter J, Jurgens H, Gerein V, Et A. Neoadjuvant chemotherapy of osteosarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response. J Clin Oncol 1988; 6: 329-37.

[16] Smeland S, Muller C, Alvegard TA, Wiklund T, Wiebe T, Bjork O, Stenwig AE, Willen H, Holmstrom T, Folleras G, Brosjo O, Kivioja A, Jonsson K, Monge O, Saeter G. Scandinavian Sarcoma Group Osteosarcoma Study SSG VIII: prognostic factors for outcome and the role of replacement salvage chemotherapy for poor histological responders. Eur J Cancer 2003; 39: 488-94.

[17] Smeland S, Bruland OS, Hjorth L, Brosjo O, Bjerkehagen B, Osterlundh G, Jakobson A, Hall KS, Monge OR, Bjork O, Alvegaard TA. Results of the Scandinavian Sarcoma Group XIV protocol for classical osteosarcoma: 63 patients with a minimum follow-up of 4 years. Acta Orthop 2011; 82: 211-6.

[18] Bacci G, Ferrari S, Longhi A, Picci P, Mercuri M, Alvegard TA, Saeter G, Donati D, Manfrini M, Lari S, Briccoli A, Forni C. High dose ifosfamide in combination with high dose methotrexate, adriamycin and cisplatin in the neoadjuvant treatment of extremity osteosarcoma: preliminary results of an Italian Sarcoma Group/ Scandinavian Sarcoma Group pilot study. J Chemother 2002; 14: 198-206.

[19] Ferrari S, Smeland S, Mercuri M, Bertoni F, Longhi A, Ruggieri P, Alvegard TA, Picci P, Capanna R, Bernini G, Muller C, Tienghi A, Wiebe T, Comandone A, Bohling T, Del PA, Brosjo O, Bacci G, Saeter G. Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol 2005; 23: 8845-52.

[20] Bielack S, Kempf-Bielack B, Schwenzer D, Birkfellner T, Delling G, Ewerbeck V, Exner GU, Fuchs N, Gobel U, Graf N, Heise U, Helmke K, von Hochstetter AR, Jurgens H, Maas R, Munchow N, Salzer-Kuntschik M, Treuner J, Veltmann U, Werner M, Winkelmann W, Zoubek A, Kotz R. [Neoadjuvant therapy for localized osteosarcoma of extremities. Results from the Cooperative osteosarcoma study group COSS of 925 patients]. Klin Padiatr 1999; 211: 260-70.

[21] Van Winkle P, Angiolillo A, Krailo M, Cheung YK, Anderson B, Davenport V, Reaman G, Cairo MS. Ifosfamide, carboplatin, and etoposide (ICE) reinduction chemotherapy in a large cohort of children and adolescents with recurrent/refractory sarcoma: the Children's Cancer Group (CCG) experience. Pediatr Blood Cancer 2005; 44: 338-47.

[22] Antman K, Crowley J, Balcerzak SP, Rivkin SE, Weiss GR, Elias A, Natale RB, Cooper RM, Barlogie B, Trump DL, Et A. An intergroup phase III randomized study of doxorubicin and dacarbazine with or without ifosfamide and mesna in advanced soft tissue and bone sarcomas. J Clin Oncol 1993; 11: 1276-85.

[23] Chou AJ, Kleinerman ES, Krailo MD, Chen Z, Betcher DL, Healey JH, Conrad ER, Nieder ML, Weiner MA, Wells RJ, Womer RB, Meyers PA.

Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children's Oncology Group. Cancer 2009; 115: 5339-48.

[24] Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, Kerst JM, Sufliarsky J, Whelan J, Hohenberger P, Krarup-Hansen A, Alcindor T, Marreaud S, Litiere S, Hermans C, Fisher C, Hogendoorn PC, Dei TA, van der Graaf WT. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol 2014; 15: 415-23.

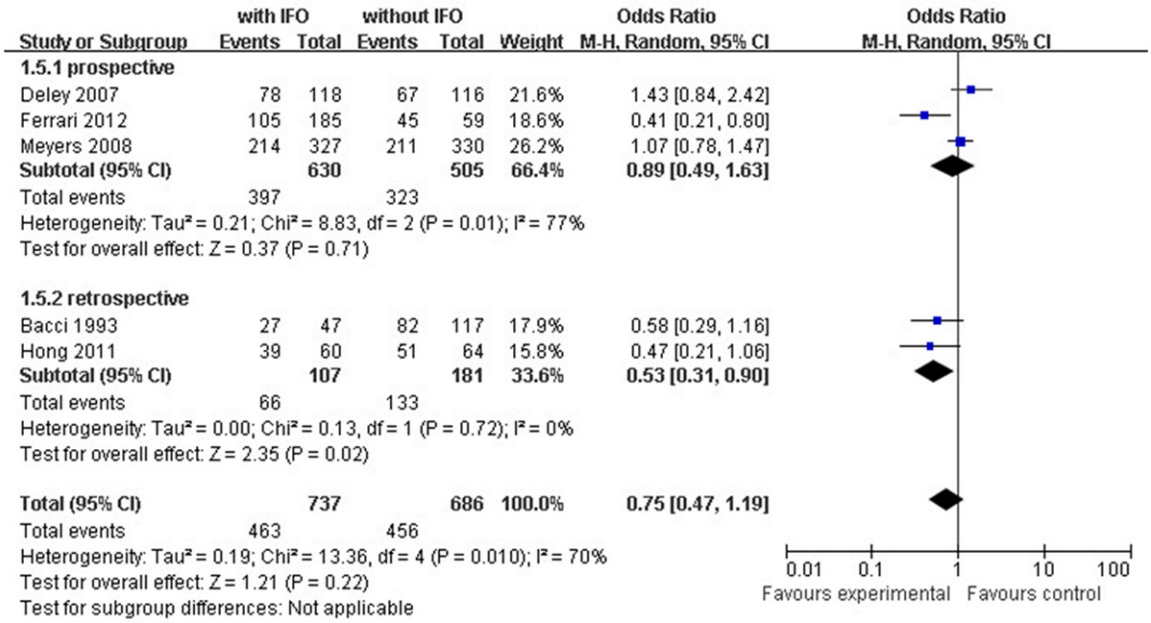
[25] Paulussen M, Craft AW, Lewis I, Hackshaw A, Douglas C, Dunst J, Schuck A, Winkelmann W, Köhler G, Poremba C, Zoubek A, Ladenstein R, van den Berg H, Hunold A, Cassoni A, Spooner D, Grimer R, Whelan J, McTiernan A, Jürgens H; European Intergroup Cooperative Ewing's Sarcoma Study-92. Results of the EICESS-92 Study: two randomized trials of Ewing's sarcoma treatment-cyclophosphamide compared with ifosfamide in standard-risk patients and assessment of benefi t of etoposide added to standard treatment in high-risk patients. J Clin Oncol 2008; 26: 4385-93.

[26] Collins M, Wilhelm M, Conyers R, Herschtal A, Whelan J, Bielack S, Kager L, Kuhne T, Sydes M, Gelderblom H, Ferrari S, Picci P, Smeland S, Eriksson M, Petrilli AS, Bleyer A, Thomas DM. Benefits and adverse events in younger versus older patients receiving neoadjuvant chemotherapy for osteosarcoma: findings from a meta-analysis. J Clin Oncol 2013; 31: 2303-12.

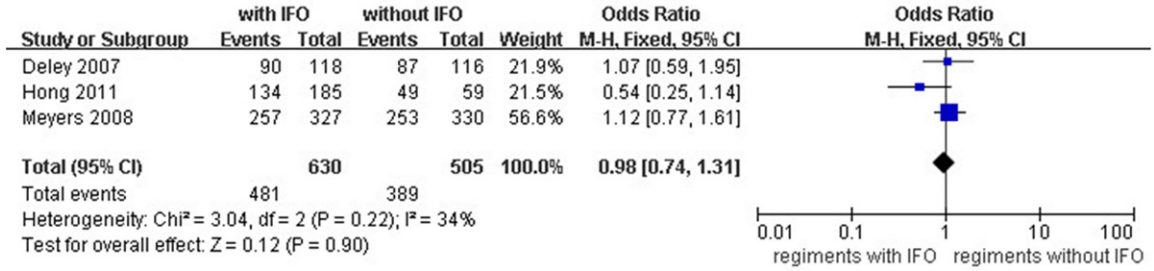
[27] Issels RD, Meier TH, Muller E, Multhoff G, Wilmanns W. Ifosfamide induced stress response in human lymphocytes. Mol Aspects Med 1993; 14: 281-6.

[28] Judson I, Verweij J, Gelderblom H, Hartmann JT, Schoffski P, Blay JY, Kerst JM, Sufliarsky J, Whelan J, Hohenberger P, Krarup-Hansen A, Alcindor T, Marreaud S, Litiere S, Hermans C, Fisher C, Hogendoorn PC, Dei TA, van der Graaf WT. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol 2014; 15: 415-23.

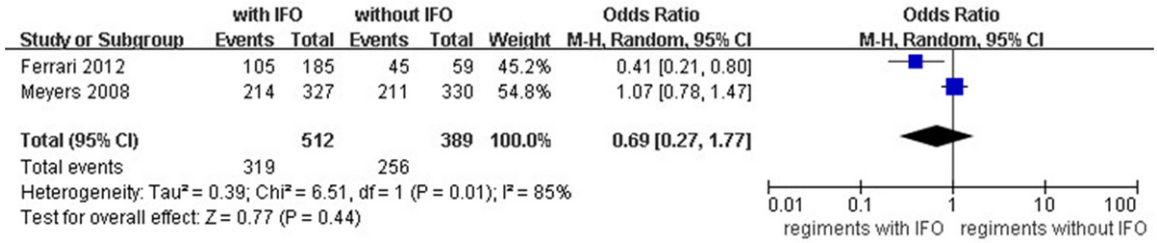
[29] Binotto G, Trentin L, Semenzato G. Ifosfamide and cyclophosphamide: effects on immunosurveillance. Oncology 2003; 65 Suppl 2: 17-20.



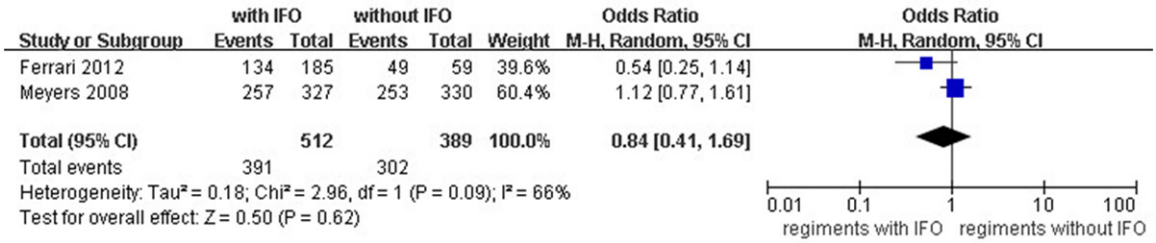
Supplementary Figure 1. Effects of IFO on 5-year EFS in different types of studies.



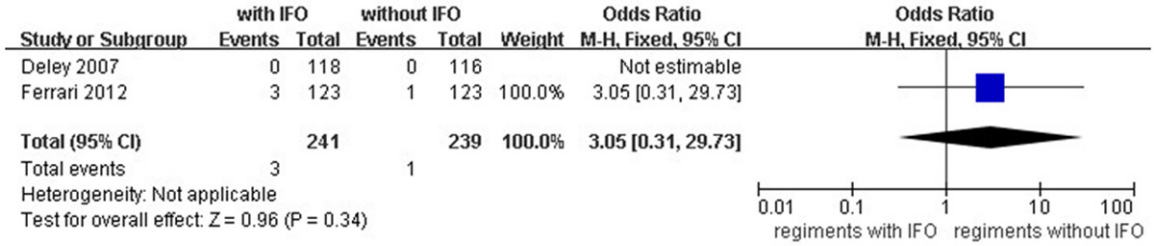
Supplementary Figure 2. Effects of IFO on 5-year OS in different types of studies.



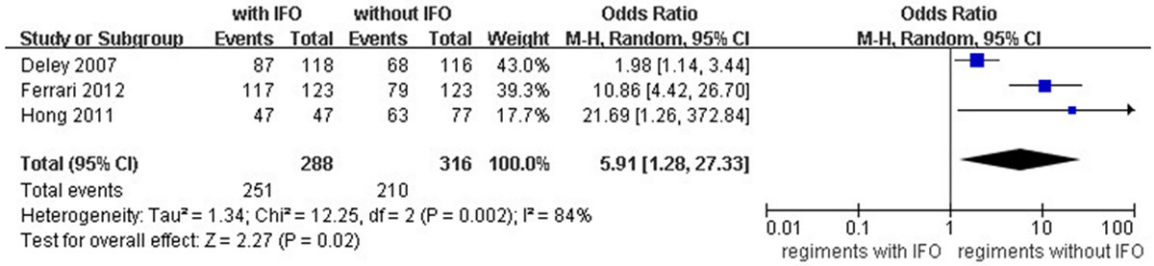
Supplementary Figure 3. Effects of IFO on 5-year EFS with same regiments studies.



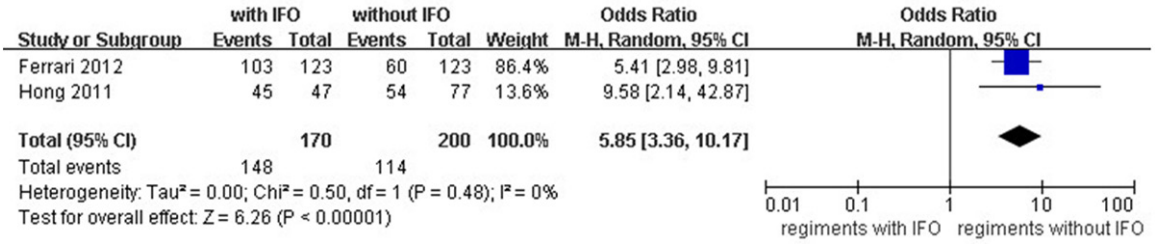
Supplementary Figure 4. Effects of IFO on 5-year OS with same regiments studies.



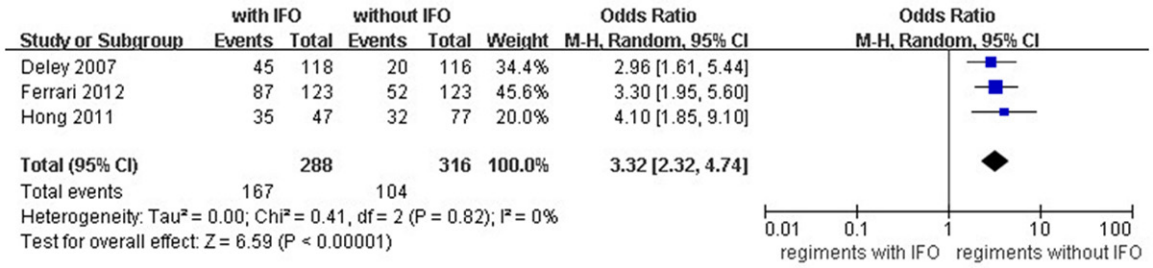
Supplementary Figure 5. Toxic effects of IFO on death.



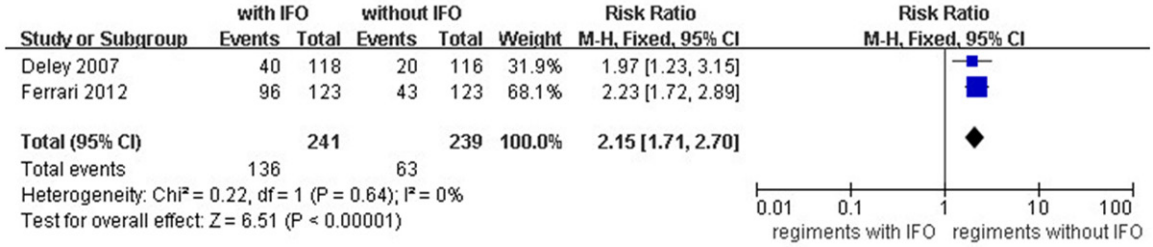
Supplementary Figure 6. Toxic effects of IFO on leukopenia.



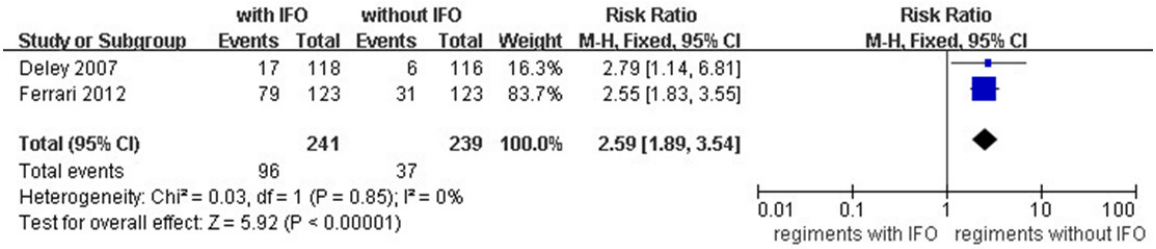
Supplementary Figure 7. Toxic effects of IFO on thrombocytopenia.



Supplementary Figure 8. Toxic effects of IFO on febrile neutropenia.



Supplementary Figure 9. Toxic effects of IFO on RBC transfusion.



Supplementary Figure 10. Toxic effects of IFO on PLT transfusion.