Journal of Bone Oncology 10 (2018) 36–40

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

Review Article

Advances in limb salvage treatment of osteosarcoma

Yichun Yang[a,b,1], Lei Han[b,1], Zewei He[b,1], Xiaojuan Li[b,1], Suping Yang[b,1], Jifei Yang[b], Ya Zhang[b], Dongqi Li[b], Yihao Yang[b,*], Zuozhang Yang[b,*]

[a] Department of Medical, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, No 16, Jichang Road, Baiyun District, Guangzhou, Guangdong 510405, People's Republic of China

[b] Bone and Soft Tissue Tumors Research Center of Yunnan Province, Department of Orthopaedics, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, Kunming, Yunnan 650118, People's Republic of China

ARTICLE INFO

Keywords:
Osteosarcoma
Limb salvage
Chemotherapy
Surgery
Infection

ABSTRACT

Osteosarcoma is the most common primary malignant bone tumor; its standard treatment includes neoadjuvant chemotherapy combined with surgery. Neoadjuvant chemotherapy has significantly improved the 5-year survival and limb salvage rates in osteosarcoma since the 1870s. The survival rate of patients with limb salvage was not inferior to that of amputees, and therefore, limb salvage has become the main surgical option for patients with osteosarcoma. The 5-year survival rate for osteosarcoma has plateaued. However, new advances in limb salvage therapy in osteosarcoma, including adjuvant chemotherapy, ablation techniques, bone transport techniques, and computer navigation techniques, are now available. This report summarizes the recent advances in limb salvage therapy for osteosarcoma over the past decade.

1. Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor of the long bones, with children and adolescents at particular risk. OS is a disorder of differentiation in bone arising from mesenchymal tissues. The prevalence ratio among males and females is 1:1.4; the annual incidence rate is 2-3/1000000. This neoplasm also frequently occurs in adults aged 40 and over [1]. The disease is closely linked to several factors, including age, gender, race, height, genetics and congenital abnormalities of bone. Secondary OS is correlated with Paget's disease and radiosensitization [2]. Prior to 1970, the treatment of OS depended primarily on surgical resection, resulting in 5-year survival rates below 20% [3]. Eighty percent of patients diagnosed with have evidence of micrometastasis; the 5-year survival rates in this population are in the range of 10–20%. Since the introduction of effective chemotherapeutic agents in the 1970s and subsequent developments in neo-adjuvant chemotherapy, the prognosis for these patients has improved significantly, with the 5-year survival rates increasing to 66–82% over the past 40 years [4]. Neoadjuvant chemotherapy combined with surgery is now the standard treatment paradigm. With recent advances in surgery, adjuvant chemotherapy, diagnostic imaging, and reconstruction materials, limb salvage has become the main treatment strategy in OS, with approximately 80–85% of patients currently willing to accept this option. This paper reviews the developments in limb salvage treatment for OS in recent years.

2. Development of adjuvant chemotherapy research

The use of drugs including adriamycin (ADM), cisplatin (DDP), high dose methotrexate (HD-MTX), ifosfamide (IFO) and epirubicin (EPI) improves the survival rate of patients with OS [4]. However, high-dose chemotherapy also results in toxicities, including myelosuppression and gastrointestinal reactions. HD-MTX is associated with serious and sometimes fatal toxicity. Chemotherapy dose reduction is an important strategy; therefore, there is a need to ensure the efficacy and simultaneously enhance the sensitivity of chemotherapy drugs. Strategies including the use of aspirin and neoadjuvant chemotherapy with shock waves are new research directions in this area.

2.1. Aspirin-adjuvant chemotherapy in OS

Aspirin is one of the most widely used non-steroidal anti-inflammatory drugs. A randomized controlled trial by Rothwell [5] et al. showed that daily aspirin could reduce the risk of developing colon cancer and metastasis. De et al. [6] observed that different concentrations of aspirin (20/100/1000 μM) could increase the apoptosis rate of osteosarcoma MG-63 cells. They also found that 1000 μM aspirin concentration increased the rate of cell death, while low concentrations of

* Corresponding authors.
E-mail addresses: 635942159@qq.com (Y. Yang), yangzuozhang@163.com (Z. Yang).
[1] These authors contributed equally to this work, and each is considered as a first author.

https://doi.org/10.1016/j.jbo.2017.11.005
Received 9 November 2017; Accepted 23 November 2017
Available online 26 November 2017

1 μM or 10 μM did not. Tang et al. [7] demonstrated that aspirin could inhibit the growth of osteosarcoma and increase the sensitivity to chemotherapy drugs after blocking the NF-κB pathway in vivo and in vitro; other studies confirmed that aspirin could effectively inhibit the NF-κB pathway [8,9]. Liao et al. [10] reported that aspirin controlled the metastasis of osteosarcoma by blocking the NF-κB pathway in cell and animal experiments. This confirmed that aspirin enhanced the therapeutic effect of osteosarcoma in vivo and in vitro. The standard dose of aspirin as adjuvant therapy has not been determined, but in colon cancer clinical research, 75–300 mg is considered low dose and 75–1500 mg is high dose [11]. A recent clinical study of aspirin as an adjuvant treatment in colorectal, breast, stomach, esophagus and prostate cancers at doses of 100 mg and 300 mg daily revealed a dose-dependent effect on tumor prognosis [11]. Liao et al. [10] showed that the aspirin dose for mouse was 100 mg/kg, while it was equal to 8.13 mg/kg for adult. The optimal dosage of aspirin as adjuvant therapy for osteosarcoma could be compared to the dosages for colorectal cancer patients. However, the specific dosage and clinical efficacy require further clarification in future research.

2.2. Extracorporeal shock wave assisted chemotherapy in osteosarcoma

Extracorporeal shock wave therapy has been successfully utilized in patients with kidney stones since reported by Chaussy et al. in 1980 [12]; since then, it has been widely used in the treatment of urinary calculi, with good clinical effect. Further intensive research and development in extracorporeal shock wave has produced promising results in Schleberger and Ludwig's studies of musculoskeletal conditions, such as delayed union, nonunion of long bone fractures and aseptic necrosis of the femoral head [13,14]. Shock waves are the product dramatic changes in pressure; its underlying principle is to form a positive pressure wave by a sharp rise in the medium water vaporizing expansion within a few nanoseconds, and then forming a negative wave by collapsing rapidly and falling sharply and finally causing a cavitation effect under the action of negative wave force [15]. Van Wamel et al. [16] found that the cavitation effect could breakdown the cell membrane associated with jet, microbeam and shock waves, and then form reversible or irreversible holes; this is referred to as "sonoporation". In other words, the cell membrane permeability increased in response to the shock wave, which causes entry of extracellular macromolecular substances into the cell [17]. Kato et al. [18] confirmed that the shock waves promoted bleomycin into human colon cancer cells SW480 in vivo and in vitro by autoradiography. Meanwhile, it improved the curative effect of chemotherapy by increasing cell apoptosis and decreasing the cell proliferation role in solid tumor tissues. Catalano et al. [19] showed that combined with docetaxel, shock waves could be applied in thyroid cancer cell lines ARO and CAL-62. Compared with paclitaxel monotherapy, there were higher concentrations of docetaxel in the tumor cells and a significantly higher cell apoptosis rate in the combined treatment group. Yu and his team members [20] found that low dose shockwaves acton Jurkat cells, and ATP in Jurkat cells was released to outside, therefore, a high concentration of extracellular ATP induced the changes in cell function. Puthussery et al. [21] confirmed apoptosis associated with extracellular ATP concentration. Scholars found that this increased the amount of MTX in U2OS cells and induced its apoptosis after the effect of shock waves in osteosarcoma cell lines in in vitro experiments. At the same time, they also detected a high concentration of ATP outside the cells. Although the related research mainly focused on the cellular level, it is expected to become a new direction for study in the adjuvant treatment of osteosarcoma.

3. Ablation applied in limb salvage surgery in patients with osteosarcoma

Tumor ablation refers to using physical or chemical methods for the in situ elimination of tumor cells. There two methods of ablation: temperature ablation and chemical ablation. Temperature ablation includes microwave, radio frequency (RF), laser, high intensity focused ultrasound ablation and cryoablation. Chemical ablation includes anhydrous alcohol ablation, glacial acetic acid, among others. The treatment of liver cancer is the most common current application of tumor ablation technology. In recent years, ablation has been gradually applied in the limb salvage treatment of osteosarcoma, resulting in favorable clinical curative effect.

3.1. High intensity focused ultrasound ablation

High Intensity Focused Ultrasound (HIFU) is a new non-invasive treatment method for local solid tumors. The operating principle of treatment is that the ultrasound energy is focused in the target tumor tissue; tumor tissue is thus killed by thermal effects and cavitation effects produced by ultrasonic focusing and simultaneously activating the patient's anti-tumor immune system [22,23]. Liet al. [24] found that despite local skin burn and other local complications such as skin dysesthesia when using HIFU to treat 7 patients with limb osteosarcoma, it did have effects on relieving pain and improving local joint activity function. Follow-up results show that the median survival time of the patients is 68 months, and their five-year survival rate is 71.4%. Comprehensive analysis shows that HIFU treatment of an extremity with osteosarcoma is safe and feasible. In addition to the treatment of osteosarcoma, Li and his team members [25] also extended the application of HIFU toother types of primary malignant bone tumors and metastatic bone tumors, and the overall effect on primary malignant bone tumors was 84.6%, while for metastatic bone tumors it was 75%. The 5-year survival rate of primary malignant bone tumors was 38.5%, and for metastatic bone tumors it was 0%. Obviously, the results show that the treatment effect in patients with primary malignant bone tumors is superior to that in patients with metastatic tumors. Chen [26] performed HIFU treatment in 80 patients with primary malignant bone tumors of Enneking stage IIB or phase III to prove that HIFU treatment is feasible and effective, and can be used as a new technology for limb salvage treatment of osteosarcoma in the future. Yu et al. [27] applied HIFU treatment to 27 patients with unresectable locally recurrent osteosarcoma. The statistical results show that the response rate is 51.8%, disease control rates 85.2%, and the median survival time is 21 months. Patients without lung metastasis have better disease control rates and longer time to local disease progression compared to those with lung metastasis. HIFU provides a new direction in the treatment of osteosarcoma limb salvage, but we need to strictly control the indications. At present, the number of treatments is relatively small; large-scale randomized controlled trials still need to be conducted to determine its efficacy.

3.2. Microwave ablation

Microwave ablation is a type of thermal ablation, utilizing 900–2450 MHz radio waves, which can cause intense oscillation of water molecules to create thermogenesis. This method can induce coagulation necrosis of tumor cells [28,29]. The main sequence of microwave treatment of osteosarcoma is: first, separating tumors in vivisection, and at the same time protecting the normal tissue around the tumor after the stripping, to prevent excessive heat damage; second, inactivating the tumor in situ by microwave ablation array with many microwave antennae and eliminating all inactive tissues; finally, using bone cement, allogeneic bone and artificial bone and other biological materials together with strong internal fixation to fill the defect and rebuild mechanical support [30]. Fan et al. [30] used microwave ablation to treat 153 patients with osteosarcoma of extremities; follow-up statistics indicate that the 5-year survival rate was 73.9% and the activity function of the affected limb improved significantly; therefore, microwave ablation is a novel effective treatment for extremities with osteosarcoma. Li and his team members [31] used the navigation

assistance of microwave ablation combined with reconstruction of bone with fibula flap transfer to treat 11 cases in which the proximal tibia bone violated the epiphysis in osteosarcoma patients; on average, at 48 months of follow-up, no local recurrence was observed, and there were 2 complications of deep infection and partial epiphyseal chemical necrosis. Postoperative limb MSTS score an average of 29 points. This strategy of osteosarcoma joint limb-salvage surgery provides a new treatment method, however, more experience and longer follow-up are necessary. Yu and his team members [32] found that microwave ablation treatment of osteosarcoma can be used to produce specific antitumor effects; Den et al. [33] experimental results also support this conclusion. Ghahremani and his team members [34] found that in the 40 nm nano gold, microwave ablation and chemotherapy combined with osteogenesis Saos-2 cells have a stronger exterminative effect. Qunand his team members' [35] conclusions also support that microwave thermal therapy can enhance the sensitivity of tumors to radiation and chemotherapy. With advancements in microwave ablation treatment research in osteosarcoma, additional advantages of this technique are constantly being discovered, and the treatment approaches will be refined.

3.3. Cryoablation

Treatment of cryoablation involves rapid cooling to the appropriate temperature, slow thawing, and repeat ice-thaw cycles [36]. The mechanism of killing the tumor is to directly damage tumor cells through temperature changes, microvascular damage causing hypoxia and ischemia to tumor cells, and the release of antigen after repeated freezing and thawing of tumor cells, so that tumor cells relieve body immune suppression, and tumor necrosis ultimately leads to the immune killing effect on tumor cells. In 1969, Marcove et al. applied cryoablation in patients with osteosarcoma [37]. Since that time, cryotherapy systems and surgical techniques have been greatly improved. The widely used third-generation argon-helium cryotherapy system can precisely control the freezing temperature, time and region, while it is also equipped with rapid temperature drop devices and different types of cryoablation needles [38]. Li et al. [39] applied cryosurgery in 12 patients with osteosarcoma near the joint, the application of the technology retained the patient's epiphyseal, articular cartilage, meniscus, cruciate ligament and other autologous tissue, thereby preserving the integrity and continuity of joints, and resulting in improved stability and activity function. Good tumor control, fewer complications, and excellent postoperative joint function were observed in follow-up. Meller et al. [40] collected and analyzed 440 patients with bone tumors who were treated with cryotherapy. After 3–18 years of follow-up, 84.5% of the patients had an excellent limb function assessment. The local recurrence rate was 8%, with only 6.3% of patients experiencing complications. These clinical studies have shown that limb salvage therapy with cryoablation is safe and effective in patients with osteosarcoma. Application of this technology can effectively retain the limbs, joints and even epiphysis; moreover, it can avoid surgical resection temporarily or permanently. Nishida and his team members [41] demonstrated that murine osteosarcoma cells after freezing in liquid nitrogen can induce a systemic anti-tumor immune response. Osteosarcoma cells after cryoablation combined with dendritic cells can enhance anti-tumor and inhibit metastatic tumor growth immune responses. The treatment of tumor segment reconstruction after liquid nitrogen freezing can induce systemic anti-tumor immune response. Although the cryoablation has advantages of no toxicity, no radioactivity, and an analgesic effect, the therapeutic effect of limb salvage therapy in patients with near-joint osteosarcoma still needs a long-term follow-up; further clinical study of ablation temperature, duration and cycle is needed.

4. Application of bone transfer technology in limb salvage operation of patients with osteosarcoma

Bone transfer technology is also known as external fixation bone callus lengthening surgery. It is based on Ilizarov's distraction-osteogenesis principle [42,43]; it involves osteotomy in the proximal or distal bone defect with the removal of the free bone segment to the bone defect with the help of an external fixator. In the process of transfer, new bone tissue will grow in the osteotomy area, and muscles, fascia, blood vessels, nerves that attached to bone will grow synchronously. At present, the external fixation clinical applications include Ilizarov ring external fixation extension and Orthofix single arm external extension. Bone transfer technology is mainly used in nonunions, large bone defects, short limbs caused by bone dysplasia and dwarfism. In limb salvage operations in patients with osteosarcoma after resection of large bone segments, bone defects appear; some scholars propose that the bone transfer technology as a biological reconstruction of bone defect can obtain a therapeutic effect. In 1997, Tsuchiya et al. [44] applied bone transfer technology, shortening extension technology, distraction osteogenesis combined with intramedullary nail technology in the treatment of 5, 2 and 3 cases of osteosarcoma patients, respectively; the operations were successful, and according to the position of the bone defect after resection divided into Type 5: Type I Diaphysis, Type II Metaphyseal, Type III Epiphysis, Type IV subarticular reconstruction, and Type IV joint fusion. Shalaby and his team members [45] applied Ilizarov technology combined with autogenous fibular grafting in 6 patients with distal tibial osteosarcoma who refused to undergo amputation. Five patients had stable joints after 8–20 months' fusion time, with an average time of 13.2 months. According to the MSTS scoring system, the ankle function score was 63–73%, with an average of 70%. One patient relapsed 1 year after surgery because of insensitivity to preoperative chemotherapy, and ultimately required amputation. McCoy et al. [46] believe that the Ilizarov method is an effective limb bone tumor reconstruction technology. Through a clinical application study, the reported 20 patients after resection of bone defect 1.2–18 cm, an average of 7.9 cm; postoperative growth of 3.5–18 cm, the average 7.1 cm; external fixation parameters of 9.5–58.3 d/cm, an average of 33.5 days. The postoperative MSTS score was 87% for upper extremity and 93% for lower extremity. Although the application of distraction osteogenesis for bone tumor retention of epiphyseal biomechanical reconstruction requires a great deal of time and effort, Watanabe et al. [47], performed a follow-up of more than 10 years in 22 bone tumor patients treated with distraction osteogenesis technology had satisfactory long-term therapeutic effects. Bone transfer technology in osteosarcoma limb salvage surgery has a great deal of promise for future application. However, its shortcomings in clinical application including prolonged stretch treatment, needle-prone infection and end-to-end anastomosis prone to dislocation still need to be improved.

5. Application of computer assisted navigation system (CANS) in limb salvage surgery for osteosarcoma

CANS is a new digital surgical technique, which integrates image processing, stereo navigation and clinical operation, and has the advantages of preoperative planning, simulation surgery, navigation aids and postoperative evaluation. It is widely used in departments of orthopedics, where it is called computer aided orthopedic surgery (CAOS). With the development CAOS technology, the new generation of navigation systems is mainly based on active optical navigation technology. In recent years, the navigation software module of bone and soft tissue surgery is successfully developed and applied in clinical practice, which allows operations in sites with complex anatomy and joint-preserving surgery under the guidance of a precise, safe and fast navigation system. Furthermore, it promotes the application of CAOS technology in bone tumor surgery. In the surgical planning for bone

tumors, the resection of the whole tumor, cutting of the allogeneic bone, fixation of the prosthesis and implantation of the prosthesis, the CAOS technique offer has more advantages over traditional methods [48]. Wong et al. [49] reported that through the navigation system, the preoperative CT and MRI images were fused to create a precise three-dimensional anatomical model of the tumor, which was used to treat 5 cases of pelvic and limb bone tumors, including 2 cases of osteosarcoma, with a median follow-up of 6.9 months; the postoperative MSTS score was 24–27, with an average of 25.3 points. They also found that during the operation, the preoperative planning was performed more easily and accurately with the guidance of navigation technology. To preserve the joints of the 6 patients with near-articular osteosarcoma, Li et al [50]. used CANS to perform the irregular osteotomy. The distance between the osteotomy line and the tumor is at least 6 mm; the joints of 3 patients were completely retained, while in the other 3 patients were partially preserved. The bone defects after tumor resection were reconstructed with allograft bone or vascularized fibular flap. Patients were followed for 17.5 months on average, with no local recurrence, and only 1 patient had no bone graft during the follow-up period; the average MSTS score was 88.8%. With the guidance of navigation, Cho et al. [51] successfully performed limb salvage surgery with joint preservation for 3 patients with pelvic or limb malignant bone tumors. After a mean follow-up of 28 months, the postoperative functional score was satisfactory and no tumor recurrence or metastasis occurred.

Although CAOS makes the operation more accurate, it also has some disadvantages including that the navigation facilities are heavy, experienced operators are required, the operation is complex and lacks flexibility. In the study of Wong [52], computer aided design and computer aided manufacturing of surgical fixtures were used to solve these problems. To verify the accuracy of the method, he performed cadaveric experiments. The experiment took 1 min to set the position of the fixture, and another 3 min to cut the gap osteotomy, through the fixture osteotomy length error is less than 1 mm. After the success of the cadaver experiment, the surgical fixture was successfully applied to 1 patient with low-grade osteosarcoma of the femur. However, further evaluation to determine the clinical efficacy of surgical fixture will require more clinical cases. The successful application of CAOS makes the accuracy of the operation much higher than that of traditional orthopedic procedures, and the resection and reconstruction of limb salvage surgery will be more accurate, safer and more convenient with the further improvement of navigation systems.

6. Advances in infection prevention after limb salvage surgery for osteosarcoma

Postoperative infection is a major complication of limb salvage in the treatment of osteosarcoma. Because patients' health status is disturbed and high doses of chemotherapy often lead to the severe decline of leukocytes and platelets, which attenuate their anti-infection ability, they are prone to postoperative infection. It has been reported that the infection rate has reached 30% [53]. Peri-prosthetic infection (PPI) can easily occur after prosthesis reconstruction. In a prospective study conducted by Hardes et al. [54], 51 cases were reconstructed with a silver-plated prosthesis after tumor segment resection, while 74 cases received a no-surface-coating titanium alloy prosthesis. When followed for more than 5 years, the postoperative infection rate of the former group was 5.9%, compared to 17.6% in the latter group. Among the group using the titanium alloy prosthesis, 38.5% of patients required amputation due to peri-prosthetic infection. This study suggests that the silver-plated prosthesis can reduce the infection rate, while its side effects and clinical efficacy need further study with long-term follow up and larger sample statistics. At the Sixteenth International Conference on Limb Salvage (ISOLS), Nisichenko from Russia and Wafa from Britain reported that applying a silver-plated prosthesis during prosthesis revision, tibial prosthesis, radiotherapy and other surgeries associated with high infection risks has obtained good prophylactic effect. Furthermore, Tsuchiya from Japan reported that an iodine - plated titanium alloy prosthesis applied for infection prevention and immunosuppression also produces satisfactory effects. Other scholars used prostheses coated with antibiotics to treat osteosarcoma patients and to control infection. Li et al. [55] confirmed in vitro that chitosan – methotrexate - titanium substrate not only suppressed adherence and proliferation of oncocytes of giant cell tumor(GCT), promote its apoptosis, and also prevent bacterial adherence and consequently forming bacterial biofilm to protect from infection. This material may solve the problem of infection after tumorous artificial prosthesis operation but also need further experiments conducted in animals and in vivo.

7. Expectations

With the further development and clinical application of basic research, reconstruction, reconstruction materials, and limb salvage treatments in osteosarcoma change with each passing day. Advancements of new adjuvant chemotherapies have greatly increased the survival time of patients with osteosarcoma; however, there are many problems associated with a prolonged survival time, such as the limb-salvage surgery limb length discrepancy, implant infection, loosening, and breakage; the emergence of these problems has accelerated the improvement and development of surgical technology for limb salvage treatment of osteosarcoma. The biologic reconstruction concepts of retaining the epiphysis and joint, melting technology, handling technology and computer-assisted navigation technology for improving resection and reconstruction techniques for limb salvage treatment of osteosarcoma are hot topics. However, new theories and new technologies lack long-term follow-up, and their clinical efficacy remains to be verified. I believe that, in the near future, these shortcomings and insufficiencies can be improved and future breakthroughs in the treatment of osteosarcoma limb-salvage will also be more mature, more optimized and more effective.

Acknowledgments

This research was supported in part by grants (no. 81372322 and no. 81460440) from the National Natural Science Foundation of China, a grant (no. 2014FB059) from the Joint Special Funds for the Department of Science and Technology of Yunnan Province-Kunming Medical University, grants (No. 2017NS196, 2017NS197) from the Scientific Research Projects of Internal Research Institutions of Medical and Health Units in Yunnan Province, and a grant (No. BSJJ201406) from the Doctor Scientific Research Startup funds of Tumor Hospital of Yunnan Province which the Third Affiliated Hospital of Kunming Medical University.

Conflicts of interest

The authors declare that they have no conflicts of interests.

References

[1] S. Bielack, D. Carrle, P.G. Casali, Osteosarcoma: esmo clinical recommendations for diagnosis, treatment and follow-up, Ann. Oncol. 20 (4) (2009) 137–139.
[2] G. Ottaviani, N. Jaffe, The etiology of osteosarcoma, Cancer Treat. Res. 152 (2009) 15–32.
[3] I.K. Dhammi, S. Kumar, Osteosarcoma: a journey from amputation to limb salvage, Indian J. Orthop. 48 (3) (2014) 233–234.
[4] S. Ferrari, P. Ruggieri, G. Cefalo, A. Tamburini, R. Capanna, F. Fagioli, A. Comandone, R. Bertulli, G. Bisogno, E. Palmerini, M. Alberghini, A. Parafioriti, A. Linari, P. Picci, G. Bacci, Neoadjuvant chemotherapy with methotrexate, cisplatin, and doxorubicin with or without ifosfamide in nonmetastatic osteosarcoma of the extremity: an italian sarcoma group trial isg/os-1, J. Clin. Oncol. 30 (17) (2012) 2112–2118.
[5] P.M. Rothwell, M. Wilson, J.F. Price, J.F. Belch, T.W. Meade, Z. Mehta, Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials, Lancet 379 (9826) (2012) 1591–1601.

[6] E. De Luna-Bertos, J. Ramos-Torrecillas, O. Garcia-Martinez, L. Diaz-Rodriguez, C. Ruiz, Effect of aspirin on cell growth of human mg-63 osteosarcoma line, Sci. World J. (2012) 834246.
[7] Q.L. Tang, X.B. Xie, J. Wang, Q. Chen, A.J. Han, C.Y. Zou, J.Q. Yin, D.W. Liu, Y. Liang, Z.Q. Zhao, B.C. Yong, R.H. Zhang, Q.S. Feng, W.G. Deng, X.F. Zhu, B.P. Zhou, Y.X. Zeng, J.N. Shen, T. Kang, Glycogen synthase kinase-3beta, NF-kappaB signaling, and tumorigenesis of human osteosarcoma, J. Natl. Cancer Inst. 104 (10) (2012) 749–763.
[8] E. Kopp, S. Ghosh, Inhibition of NF-kappa B by sodium salicylate and aspirin, Science 265 (5174) (1994) 956–959.
[9] M.J. Yin, Y. Yamamoto, R.B. Gaynor, The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta, Nature 396 (6706) (1998) 77–80.
[10] D. Liao, L. Zhong, T. Duan, R.H. Zhang, X. Wang, G. Wang, K. Hu, X. Lv, T. Kang, Aspirin suppresses the growth and metastasis of osteosarcoma through the NF-kappaB pathway, Clin. Cancer Res. 21 (23) (2015) 5349–5359.
[11] R.E. Langley, P.M. Rothwell, Aspirin in gastrointestinal oncology: new data on an old friend, Curr. Opin. Oncol. 26 (4) (2014) 441–447.
[12] C. Chaussy, W. Brendel, E. Schmiedt, Extracorporeally induced destruction of kidney stones by shock waves, Lancet 2 (8207) (1980) 1265–1268.
[13] R. Schleberger, T. Senge, Non-invasive treatment of long-bone pseudarthrosis by shock waves (eswl), Arch. Orthop. Trauma Surg. 111 (4) (1992) 224–227.
[14] J. Ludwig, S. Lauber, H.J. Lauber, U. Dreisilker, R. Raedel, H. Hotzinger, High-energy shock wave treatment of femoral head necrosis in adults, Clin. Orthop. Relat. Res. 387 (2001) 119–126.
[15] A.J. Coleman, J.E. Saunders, L.A. Crum, M. Dyson, Acoustic cavitation generated by an extracorporeal shockwave lithotripter, Ultrasound Med. Biol. 13 (2) (1987) 69–76.
[16] Bouakz van Wanmel, Versltrs micromani polation of end othelial cells: ultmsound-midrobubble-cell interaction, Ultrasound Medbiol. 30 (9) (2004) 255–258.
[17] C.R. Byron, B.M. Benson, A.A. Stewart, M.C. Stewart, Effects of radial shock waves on membrane permeability and viability of chondrocytes and structure of articular cartilage in equine cartilage explants, Am. J. Vet. Res. 66 (10) (2005) 1757–1763.
[18] M. Kato, N. Ioritani, T. Suzuki, M. Kambe, Y. Inaba, R. Watanabe, H. Sasano, S. Orikasa, Mechanism of anti-tumor effect of combination of bleomycin and shock waves, Jpn. J. Cancer Res. 91 (10) (2000) 1065–1072.
[19] M.G. Catalano, L. Costantino, N. Fortunati, O. Bosco, M. Pugliese, G. Boccuzzi, L. Berta, R. Frairia, High energy shock waves activate 5′-aminolevulinic acid and increase permeability to paclitaxel: antitumor effects of a new combined treatment on anaplastic thyroid cancer cells, Thyroid 17 (2) (2007) 91–99.
[20] K. Tschoep, G. Hartmann, R. Jox, S. Thompson, A. Eigler, A. Krug, S. Erhardt, G. Adams, S. Endres, M. Delius, Shock waves: a novel method for cytoplasmic delivery of antisense oligonucleotides, Mol. Med. 79 (5–6) (2001) 306–313.
[21] T. Puthussery, E. Fletcher, Extracellular ATP induces retinal photoreceptor apoptosis through activation of purinoceptors in rodents, Comp. Neurol. 513 (4) (2009) 430–440.
[22] G. ter Haar, High intensity ultrasound, Semin. Laparosc. Surg. 8 (1) (2001) 77–89.
[23] F. Wu, Z.B. Wang, W.Z. Chen, J.Z. Zou, J. Bai, H. Zhu, K.Q. Li, F.L. Xie, C.B. Jin, H.B. Su, G.W. Gao, Extracorporeal focused ultrasound surgery for treatment of human solid carcinomas: early Chinese clinical experience, Ultrasound Med. Biol. 30 (2) (2004) 245–260.
[24] C. Li, P. Wu, L. Zhang, W. Fan, J. Huang, F. Zhang, Osteosarcoma: limb salvaging treatment by ultrasonographically guided high-intensity focused ultrasound, Cancer Biol. Ther. 8 (12) (2009) 1102–1108.
[25] C. Li, W. Zhang, W. Fan, J. Huang, F. Zhang, P. Wu, Noninvasive treatment of malignant bone tumors using high-intensity focused ultrasound, Cancer 116 (16) (2010) 3934–3942.
[26] W. Chen, H. Zhu, L. Zhang, K. Li, H. Su, C. Jin, K. Zhou, J. Bai, F. Wu, Z. Wang, Primary bone malignancy: effective treatment with high-intensity focused ultrasound ablation, Radiology 255 (3) (2010) 967–978.
[27] W. Yu, L. Tang, F. Lin, Y. Yao, Z. Shen, X. Zhou, High-intensity focused ultrasound: noninvasive treatment for local unresectable recurrence of osteosarcoma, Surg. Oncol. 24 (1) (2015) 9–15.
[28] G. Carrafiello, D. Lagana, M. Mangini, F. Fontana, G. Dionigi, L. Boni, F. Rovera, S. Cuffari, C. Fugazzola, Microwave tumors ablation: principles, clinical applications and review of preliminary experiences, Int. J. Surg. 6 (1) (2008) 65–69.
[29] C.J. Simon, D.E. Dupuy, W.W. Mayo-Smith, Microwave ablation: principles and applications, Radiographics 25 (1) (2005) 69–83.
[30] Q.Y. Fan, B.A. Ma, Y. Zhou, M.H. Zhang, X.B. Hao, Bone tumors of the extremities or pelvis treated by microwave-induced hyperthermia, Clin. Orthop. Relat. Res. 406 (2003) 165–175.
[31] J. Li, Z. Guo, Z. Wang, H. Fan, J. Fu, Does microwave ablation of the tumor edge allow for joint-sparing surgery in patients with osteosarcoma of the proximal tibia? Clin. Orthop. Relat. Res. 473 (10) (2015) 3204–3211.
[32] Z. Yu, J. Geng, M. Zhang, Y. Zhou, Q. Fan, J. Chen, Treatment of osteosarcoma with microwave thermal ablation to induce immunogenic cell death, Oncotarget 5 (15) (2014) 6526–6539.
[33] M.H. den Brok, R.P. Sutmuller, R. van der Voort, E.J. Bennink, C.G. Figdor, T.J. Ruers, G.J. Adema, In situ tumor ablation creates an antigen source for the Generation of antitumor immunity, Cancer Res. 64 (11) (2004) 4024–4029.
[34] F.H. Ghahremani, A. Sazgarnia, M.H. Bahreyni-Toosi, O. Rajabi, A. Aledavood, Efficacy of microwave hyperthermia and chemotherapy in the presence of gold nanoparticles: an in vitro study on osteosarcoma, Int. J. Hyperth. 27 (6) (2011) 625–636.
[35] N. Qun, X.Y. Yang, L. Li, Y.J. Liu, D.M. Hao, Numerical simulation on microwave ablation with a water-cooled antenna, Bioinform. Biomed. Eng. (2007) 698–701.
[36] A.A. Gage, J. Baust, Mechanisms of tissue injury in cryosurgery, Cryobiology 37 (3) (1998) 171–186.
[37] R.C. Marcove, T.R. Miller, The treatment of primary and metastatic localized bone tumors by cryosurgery, Surg. Clin. N. Am. 49 (2) (1969) 421–430.
[38] J. Bickels, Y. Kollender, O. Merimsky, J. Isaakov, R. Petyan-Brand, I. Meller, Closed argon-based cryoablation of bone tumours, J. Bone Jt. Surg. Br. 86 (5) (2004) 714–718.
[39] J. Li, Z. Guo, Q. Yang, C. Ji, Z. Wang, Adjuvant argon-based cryoablation for joint-preserving tumor resection in patients with juxta-articular osteosarcoma around the knee, Cryobiology 71 (2) (2015) 236–243.
[40] I. Meller, A. Weinbroum, J. Bickels, S. Dadia, A. Nirkin, O. Merimsky, J. Issakov, G. Flusser, N. Marouani, N. Cohen, Y. Kollender, Fifteen years of bone tumor cryosurgery: a single-center experience of 440 procedures and long-term follow-up, Eur. J. Surg. Oncol. 34 (8) (2008) 921–927.
[41] H. Nishida, N. Yamamoto, Y. Tanzawa, H. Tsuchiya, Cryoimmunology for malignant bone and soft-tissue tumors, Int. J. Clin. Oncol. 16 (2) (2011) 109–117.
[42] G.A. Ilizarov, The tension-stress effect on the genesis and growth of tissues: part I. The influence of stability of fixation and soft-tissue preservation, Clin. Orthop. Relat. Res. 238 (1989) 249–281.
[43] G.A. Ilizarov, The tension-stress effect on the genesis and growth of tissues: part II. The influence of the rate and frequency of distraction, Clin. Orthop. Relat. Res. 239 (1989) 263–285.
[44] H. Tsuchiya, K. Tomita, K. Minematsu, Y. Mori, N. Asada, S. Kitano, Limb salvage using distraction osteogenesis. a classification of the technique, J. Bone Jt. Surg. Br. 79 (3) (1997) 403–411.
[45] S. Shalaby, H. Shalaby, A. Bassiony, Limb salvage for osteosarcoma of the distal tibia with resection arthrodesis, autogenous fibular graft and ilizarov external fixator, J. Bone Jt. Surg. Br. 88 (12) (2006) 1642–1646.
[46] T.H. McCoy Jr, H.J. Kim, M.B. Cross, A.T. Fragomen, J.H. Healey, E.A. Athanasian, S.R. Rozbruch, Bone tumor reconstruction with the ilizarov method, J. Surg. Oncol. 107 (4) (2013) 343–352.
[47] K. Watanabe, H. Tsuchiya, N. Yamamoto, T. Shirai, H. Nishida, K. Hayashi, A. Takeuchi, H. Matsubara, I. Nomura, Over 10-year follow-up of functional outcome in patients with bone tumors reconstructed using distraction osteogenesis, J. Orthop. Sci. 18 (1) (2013) 101–109.
[48] T. Hufner, M. Kfuri Jr, M. Galanski, L. Bastian, M. Loss, T. Pohlemann, C. Krettek, New indications for computer-assisted surgery: tumor resection in the pelvis, Clin. Orthop. Relat. Res. 426 (2004) 219–225.
[49] K.C. Wong, S.M. Kumta, K.H. Chiu, G.E. Antonio, P. Unwin, K.S. Leung, Precision tumour resection and reconstruction using image-guided computer navigation, J. Bone Jt. Surg. 89 (7) (2007) 943–947.
[50] J. Li, Z. Wang, Z. Guo, G.J. Chen, M. Yang, G.X. Pei, Irregular osteotomy in limb salvage for juxta-articular osteosarcoma under computer-assisted navigation, J. Surg. Oncol. 106 (4) (2012) 411–416.
[51] H.S. Cho, J.H. Oh, I. Han, H.S. Kim, Joint-preserving limb salvage surgery under navigation guidance, J. Surg. Oncol. 100 (3) (2009) 227–232.
[52] K.C. Wong, S.M. Kumta, K.Y. Sze, C.M. Wong, Use of a patient-specific cad/cam surgical jig in extremity bone tumor resection and custom prosthetic reconstruction, Comput. Aided Surg. 17 (6) (2012) 284–293.
[53] L.M. Jeys, R.J. Grimer, S.R. Carter, R.M. Tillman, Periprosthetic infection in patients treated for an orthopaedic oncological condition, J. Bone Jt. Surg. Am. 87 (4) (2005) 842–849.
[54] J. Hardes, C. von Eiff, A. Streitbuerger, M. Balke, T. Budny, M.P. Henrichs, G. Hauschild, H. Ahrens, Reduction of periprosthetic infection with silver-coated megaprostheses in patients with bone sarcoma, J. Surg. Oncol. 101 (5) (2010) 389–395.
[55] L.H. Li, M. Li, D. Li, P. He, H. Xia, Y. Zhang, C. Mao, Chemical functionalization of bone implants with nanoparticle-stabilized chitosan and methotrexate for inhibiting both osteoclastoma formation and bacterial infection, J. Mater. Chem. B Mater. Biol. Med. 2 (36) (2014) 5952–5961.