For reprint orders, please contact: reprints@futuremedicine.com

Pharmacogenomics of second-line drugs used for treatment of unresponsive or relapsed osteosarcoma patients

Second-line treatment of high-grade osteosarcoma (HGOS) patients is based on different approaches and chemotherapy protocols, which are not yet standardized. Although several drugs have been used in HGOS second-line protocols, none of them has provided fully satisfactory results and the role of rescue chemotherapy is not well defined yet. This article focuses on the drugs that have most frequently been used for second-line treatment of HGOS, highlighting the present knowledge on their mechanisms of action and resistance and on gene polymorphisms with possible impact on treatment sensitivity or toxicity. In the near future, validation of the so far identified candidate genetic biomarkers may constitute the basis for tailoring treatment by taking the patients' genetic background into account.

First draft submitted: 30 June 2016; Accepted for publication: 8 September 2016; Published online: 24 November 2016

Keywords: adverse drug reactions • chemotherapy • CYP450 • drug resistance • drug transporter genes • osteosarcoma • pharmacogenomics

Osteosarcoma is the most common tumor of bone, which can exhibit different levels of malignancy [1,2]. High-grade osteosarcoma (HGOS) is a very aggressive neoplasm that, in unselected populations, is responsible for death of approximately 40–50% of patients [2,3]. The subgroup of the so-called conventional HGOS includes patients with high-grade tumors located in the extremities, nonmetastatic at diagnosis and younger than 40 years [1]. In this subgroup of HGOS, prognosis is slightly better, reaching 60–65% when patients are treated with multiagent neoadjuvant chemotherapy [4–6].

The major clinical problem limiting the cure rate of HGOS patients is relapse, which in most cases consists in development of lung metastasis within the first 24–36 months from diagnosis [7,8]. Treatment of relapsed HGOS is based on different approaches and second-line chemotherapy protocols which, however, are not standardized and can rescue no more than 20–25% of patients [4,5].

Methods of data searching & selection

A systematic literature search was performed using 'treatment' and 'osteosarcoma' as key words in PubMed [9]. We further restricted the selection to studies performed in humans. By using all the aforementioned different combinations and selections, the literature searches revealed a total of 13,976 results. The key terms 'polymorphism', 'toxicity', 'drug response', 'drug resistance', 'pharmacogenetic', 'pharmacogenomic', 'epigenetic', 'methylation' and 'clinical trial' were then used to refine the results of the previous search, in order to select the publications which were strictly related to each section of this review. Full-length articles and reviews in English were taken into consideration.

Only drugs used in at least two different clinical trials with published results were included in this review and, consequently, we considered genes and gene polymorphisms with possible relevance for response and/or

Claudia M Hattinger¹,
Serena Vella¹, Elisa Tavanti¹,
Marilù Fanelli¹, Piero Picci²
& Massimo Serra^{*1}

¹Pharmacogenomics & Pharmacogenetics
Research Unit of the Laboratory of
Experimental Oncology, Orthopaedic
Rizzoli Institute, Via di Barbiano 1/10,
I-40136 Bologna, Italy

²Laboratory of Experimental Oncology,
Orthopaedic Rizzoli Institute, Via di
Barbiano 1/10, I-40136 Bologna, Italy

*Author for correspondence:

Tel.: +39 051 636 6762

Fax: +39 051 636 6763

massimo.serra@ior.it

Future
Medicine

metabolism of these agents. For this candidate gene selection purpose, PharmGKB pathways were consulted [10]. Information about gene functions in relation to drug metabolism and transport was also extracted by searching genes in OMIM ([11], where * indicates the gene name).

Drugs for second-line treatment of osteosarcoma patients

As stated above, nowadays, really effective drugs for HGOS second-line chemotherapy, in addition to those that are commonly used in first-line treatments, are not available. Several drugs, that are not usually included in the first-line chemotherapy protocols, have been explored for rescue treatments of relapsed HGOS patients (Table 1), showing however not fully satisfactory results.

The reason behind this can be ascribed to two main factors:

- the presence of mechanisms of resistance developed against first-line chemotherapeutic agents, which can also be responsible for unresponsiveness to the drugs used to treat relapsed patients;

- the lack of information about biomarkers and genetic determinants, which may guide the selection and the use of second-line chemotherapeutic drugs.

Current research & clinical goals

On this background, the first and absolutely important aim that needs to be achieved to improve the efficacy of second-line chemotherapy in HGOS patients, who did not benefit from first-line treatments, consists in the identification of new agents that can be considered for innovative therapeutic strategies. Unfortunately, despite there are several Phase II–III ongoing regimens which are evaluating new therapeutic approaches [5,12] new drugs have not emerged as highly effective for second-line treatment of HGOS patients.

In addition to this, it is important paying more attention to the mechanisms of resistance against first-line HGOS chemotherapeutics, which can also be responsible for unresponsiveness to second-line drugs. It is worth noting that several of the resistance mechanisms against second-line drugs are actually the same that are responsible for unresponsiveness to the agents used in first-line chemotherapy (Tables 2 & 3), which

Table 1. Drugs which have been used for second-line chemotherapy in high-grade osteosarcoma.	
Drug	Main mechanism(s) of action
Vinca alkaloids (vinorelbine, vinblastine)	Binding to β -tubulin, inhibition of microtubules formation and microtubules destabilization Cell-cycle arrest and apoptosis induction
Taxanes (docetaxel, paclitaxel)	Binding to β -tubulin, microtubules stabilization and inhibition of their disassembly during mitosis Cell-cycle arrest and apoptosis induction
Gemcitabine	Cytidine analogue that blocks DNA synthesis during the S phase of cell cycle Cell-cycle arrest and apoptosis induction
Etoposide	Binding to both DNA and topoisomerase II, leading to single- and double-strand DNA breaks Inhibition of cell proliferation and apoptosis induction
Bleomycin	Induction of single- and double-strand DNA breaks G2/M cell cycle arrest and apoptosis induction
Dactinomycin (actinomycin D)	DNA intercalation and stabilization of cleavable complexes of topoisomerases I and II with DNA Formation of DNA single-strand breaks Inhibition of DNA replication and RNA transcription Inhibition of cell proliferation and apoptosis induction
Cyclophosphamide	Alkylation of DNA and formation of intrastrand and interstrand DNA crosslinks Interference with DNA replication and induction of apoptosis
Carboplatin	Formation of intra- and interstrand DNA crosslinks Inhibition of DNA replication and transcription and induction of apoptosis
Topotecan Irinotecan	Interaction with the topoisomerase I-DNA complex, leading to DNA damage Inhibition of DNA replication and transcription Inhibition of cell proliferation and apoptosis induction

Table 2. Most relevant cross-resistance mechanisms against first-line chemotherapeutic agents and drugs used for second-line treatment in high-grade osteosarcoma.

	Mechanisms of resistance						
	ABC1-mediated increased efflux	ABCC transporters-mediated increased efflux	ABCG2-mediated increased efflux	Increased DNA damage repair	Increased GSTs-mediated detoxification	Increased CYPs-mediated detoxification	Increased UGTs-mediated detoxification
First-line drugs							
Doxorubicin							
Cisplatin							
Methotrexate							
Ifosfamide							
Second-line drugs							
Vinca alkaloids							
Taxanes							
Gemcitabine							
Etoposide							
Bleomycin							
Dactinomycin (Actinomycin D)							
Cyclophosphamide							
Carboplatin							
Topotecan							
Irinotecan							
	Moderately relevant or to be confirmed			Relevant		Highly relevant	

	ABCB1 (MDR1)	ABCC1 (MRP1)	ABCC2 (MRP2)	ABCC3 (MRP3)	ABCC4 (MRP4)	ABCC5 (MRP5)	ABCC6 (MRP6)	ABCC10 (MRP7)	ABCC11 (MRP8)	ABCG2 (BCRP)
Doxorubicin										
Methotrexate										
Cisplatin										
Ifosfamide										
Vinca alkaloids										
Taxanes										
Gemcitabine										
Etoposide										
Bleomycin										
Dactinomycin (actinomycin D)										
Cyclophosphamide										
Carboplatin										
Topotecan										
Irinotecan (SN-38)										

Information was derived from literature and from three different databases: the Human Transporter Database [13], the Pharmacogenomics Knowledgebase (PharmGKB; [14]), and the UCSF-FDA TransPortal Drug Transporter Database [15].
MDR: Multidrug resistance.

may therefore be already present in tumor cells that survived first-line treatments. This fact may significantly limit or reduce the efficacy of rescue treatment protocols.

Another limiting factor that may negatively affect the efficacy of second-line anticancer agents is the presence of genetic modifications affecting genes encoding for enzymes involved in their metabolism (Tables 2 & 4), which have been indicated to contribute to the development of resistance [16].

In addition to this, the improvement of the efficacy of the presently available agents, without increasing its collateral adverse toxicity, is highly warranted. An important contribution that appears to be of great help to achieve this aim can come from pharmacogenomics, with the identification of germline or somatic genetic polymorphisms, which can be predictive for sensitivity and toxicity of chemotherapeutic treatments.

The accomplishment of these goals will offer the hope for increasing survival and cure probabilities of unresponsive and relapsed HGOS patients and will also improve their quality of life by reducing or avoiding treatment-associated toxicities.

Drugs for rescue treatments of relapsed HGOS patients

As mentioned above, several drugs have been used for rescue treatments of relapsed HGOS patients, of

which the most frequently used are listed in Table 1 and briefly described here below. Antifolates that have been used in second-line treatment of HGOS patients (namely, trimetrexate and pemetrexed) were not considered in this manuscript since they have extensively been reviewed elsewhere [6,18].

Vinca alkaloids (vinorelbine, vinblastine)

The Vinca alkaloids are natural or semisynthetic nitrogenous bases extracted from the plant *Catharanthus roseus* G, which exhibit cytotoxic effects on different cell types. Several Vinca alkaloids have been extensively studied, but only vincristine, vinblastine and vinorelbine have been approved for clinical use in human tumors.

The main mechanism of Vinca alkaloids cytotoxicity is related to the interactions with tubulin and disruption of microtubules of the mitotic spindle apparatus, leading to metaphase arrest, blockage of cell proliferation and, eventually, induction of apoptosis [19–21].

Vinca alkaloids resistance is associated with the classic pleiotropic or multidrug resistant phenotype, due to overexpression of ATP-binding cassette (ABC) transporters, such as ABCB1, ABCG2 and members of the ABCC family [22]. The multidrug resistant phenotype, and in particular the overexpression of ABCB1, also confers cross-resistance to sev-

eral other first- and second-line HGOS drugs, such as anthracyclines (doxorubicin), taxanes (docetaxel and paclitaxel), epipodophyllotoxins (etoposide) and actinomycines (Tables 2 & 3) [22–24].

Vinca alkaloids are metabolized in the liver. The CYP3A isoenzyme appears to be the major responsible for their biotransformation, but also other members of the CYPs family or the GSTM1 detoxify these drugs (Table 4) [16,25].

The main toxic effect of Vinca alkaloids is neutropenia (which is the principal dose-limiting toxicity of these drugs), but they can also induce peripheral neurotoxicity and gastrointestinal toxicities [26].

Vinca alkaloids have been used to treat several human tumors, and they have also been explored in HGOS. In a clinical trial performed between 1980 and 1983, 106 patients with primary HGOS of the extremities were treated with surgery followed by adjuvant chemotherapy with vincristine, methotrexate and doxorubicin [27]. After a follow-up of 20 years, event-free and overall survival were, respectively, 38 and

44%, being in line with the results obtained with other adjuvant treatment regimens.

In another study, vincristine was used together with high-dose methotrexate and cisplatin to treat 43 patients with metastatic HGOS [28]. Among the 29 patients who had previously received chemotherapy, responses to this drug combination included two complete and six partial remissions, and eight disease stabilizations, indicating that this rescue treatment was of some efficacy.

More recently, in a Phase II study performed by the Société Française des Cancers et leucémies de l'Enfant et de l'adolescent, efficacy of the vinorelbine plus cyclophosphamide combination was assessed in children and young adults with recurrent or refractory solid tumors [29]. This protocol recruited also ten HGOS patients, who unfortunately did not respond to this treatment approach.

Taxanes (docetaxel, paclitaxel)

Taxanes are cytotoxic agents that have been widely used to treat a range of solid malignant tumors. Docetaxel

Table 4. Drug metabolizing enzymes involved in detoxification and/or activation of second-line chemotherapeutic drugs that have been used in high-grade osteosarcoma.

Drug	Drug metabolizing enzymes involved in:	
	Detoxification/inactivation	Activation
Vinorelbine	CYP2D6; CYP3A4 GSTM1	
Vinblastine	CYP2D6; CYP3A4; CYP3A5 GSTM1	
Docetaxel	CYP1B1; CYP2A4; CYP2C8 GSTP1	
Paclitaxel	CYP1B1; CYP2C8; CYP2C9; CYP3A4; CYP3A5; CYP3A7; CYP19A GSTP1	
Gemcitabine	GSTT1	
Etoposide	CYP1A2; CYP2C8; CYP2C9; CYP2E1; CYP3A4; CYP3A5 GSTs UGTs	
Bleomycin	GSTs	
Dactinomycin (actinomycin D)	CYP1A1; CYP1A2	
Cyclophosphamide	CYP2A6; CYP2C18; CYP2C19; CYP3A4; CYP3A4; CYP3A7 GSTs	CYP2A6; CYP2B6; CYP2C8; CYP2C9; CYP2C19; CYP3A4; CYP3A5
Carboplatin	GSTP1	
Topotecan	CYP3A4	
Irinotecan	CYP2B6; CYP3A4; CYP3A5; CYP3A7 UGT1A1; UGT1A3; UGT1A4; UGT1A6; UGT1A8; UGT1A9; UGT1A10	CYP3A4; CYP3A5

Information was derived from literature and from the Pharmacogenomics Knowledgebase (PharmGKB; [14]) or the SuperCYP [17] databases.

(Taxotere®, Aventis Pharma S.A., Antony, France) and paclitaxel (Taxol®, Bristol–Myers Squibb, NJ, USA) are the most commonly used taxanes, which have been included in several neoadjuvant, adjuvant and second-line chemotherapy protocols, alone or in combination with drugs with different mechanisms of action and nonoverlapping toxicity profiles. The taxanes bind to tubulin (to binding sites that are distinct from those of the Vinca alkaloids), stabilize the microtubule and inhibit its disassembly, leading ultimately to cell death by apoptosis [19,26].

Resistance occurs through a variety of mechanisms, the most common of which is the overexpression of ABCB1 that causes the efflux of taxanes from tumor cells (Tables 2 & 3) [26]. Other described mechanisms of taxanes resistance are intrinsic and acquired mutations of tubulin or altered expression of microtubule-associated proteins, which can interfere or prevent the normal binding of tubulin with these drugs [26].

Taxanes are detoxified by several CYP isoenzymes and by GSTP1 (Table 4), and it has been reported that resistance can also be associated with increased detoxification due to GSTP1 overexpression (Table 2) [16,30].

Neutropenia is the principal toxicity related to taxanes treatment, whereas neuropathy and cardiac rhythm disturbances are less frequent events [31].

Paclitaxel was used as single drug in a Phase II trial for patients with HGOS and its variants, who were unresponsive to standard chemotherapy with doxorubicin, ifosfamide, cisplatin and high-dose methotrexate [32]. Unfortunately, despite being well tolerated, paclitaxel alone did not prove to be active for treating resistant HGOS patients. The same evidence was found in another trial for recurrent or metastatic soft tissue sarcomas and HGOS [33].

However, nab-paclitaxel (ABI-00, Abraxane; Celgene Corporation, NJ, USA), a recently developed formulation of paclitaxel designed to reduce its side effects, has provided interesting results in HGOS. In experimental models, nab-paclitaxel revealed a greater antitumor efficacy compared with paclitaxel, being also able to overcome the ABCB1-mediated paclitaxel-resistance [34–36]. A Phase I/II clinical trial [37] is presently recruiting pediatric and adult patients with different malignancies, including HGOS, to find a safe dose and to assess the clinical activity of this new drug.

Docetaxel showed some activity in HGOS when used together with gemcitabine, suggesting that combinations of gemcitabine and taxanes should be further investigated in bone sarcomas [38].

Gemcitabine

Gemcitabine is an antineoplastic antimetabolite that masquerades as purine or pyrimidine, preventing their

incorporation into DNA during the S phase of the cell cycle. As consequence, DNA synthesis and cell division are stopped [39]. Gemcitabine is a prodrug that, once transported into the cell by different membrane-located solute carrier family members (SLC28A1, SLC28A3, SLC29A1 and SLC29A2), must be phosphorylated by deoxycytidine kinase to an active form [39,40].

Resistance to gemcitabine has been described to be mainly associated with altered activities of the GSTT1 enzyme, which is responsible for its detoxification (Table 4), upregulation of the membrane transporter ABCC2 (Tables 2 & 3) and alterations in the apoptotic pathways [39,41,42]. It is worth noting that gemcitabine represents a promising candidate for combination chemotherapy, since synergism or positive interaction with conventional antineoplastic agents (e.g., cisplatin, taxanes, camptothecins, anthracyclines, antifolates) have been demonstrated in both *in vivo* and *in vitro* models, providing the rationale for translating these combined treatments in clinical trials.

Recently, the combined use of gemcitabine and docetaxel has been tested in clinical trials and it has shown activity in relapsed rhabdomyosarcoma [43] and recurrent HGOS patients [38,44,45]. In general, the combination of gemcitabine with docetaxel was found to be well tolerated and the provided evidence indicated that this treatment may be of some efficacy to rescue recurrent or refractory HGOS patients. These promising findings claim for additional evaluation of the use of this two drugs combination as second-line treatment for HGOS patients.

Etoposide

Etoposide (also known as VP-16, Etopophos® [Baxter Healthcare Corporation, IL, USA], or Vepesid® [R.P. Scherer GmbH, Eberbach/Baden, Germany]) is a cytotoxic anticancer drug, which forms a complex with DNA and the topoisomerase II enzyme, preventing religation of the DNA strands and, consequently, causing DNA strand breaks. In cancer cells, this causes proliferation arrest and promotes apoptosis.

The major mechanisms of etoposide resistance are reduced intracellular drug accumulation via increased ABCB1/ABCC1 expression and/or efflux activity, decreased expression of topoisomerase II and enhanced DNA damage repair (Tables 2 & 3) [23,46].

Etoposide is detoxified by several CYP, GST and UDP glucuronosyl transferases (UGT) enzymes (Table 4).

Etoposide has been used in combination with other drugs for both first-line and rescue chemotherapeutic treatment of several different human tumors.

A French randomized trial compared the efficacy of preoperative treatment with high-dose methotrexate

plus doxorubicin to high-dose methotrexate plus etoposide and ifosfamide in 234 children/adolescents with localized HGOS patients [47]. A good response to preoperative treatment (tumor necrosis greater than 95%) was achieved in 56% of patients in the etoposide-ifosfamide arm versus 39% in the doxorubicin arm ($p = 0.009$). However, the 5-year event-free survival was only slightly greater in the etoposide-ifosfamide than in the doxorubicin arm, whereas the 5-year overall survival was similar in both protocol arms.

The same group also performed the multicenter OS2006 Phase III study, which was primarily aimed to assess the efficacy of zoledronic acid in HGOS [48]. In the preoperative phase, pediatric patients were treated with methotrexate, ifosfamide and etoposide whereas adult patients received ifosfamide, doxorubicin and cisplatin. In the postoperative chemotherapy, patients with a good histological response received the same drugs as in the preoperative phase. In case of poor response to preoperative chemotherapy, pediatric patients were treated with a regimen in which ifosfamide and etoposide were replaced by cisplatin and doxorubicin, whereas adult patients received etoposide plus ifosfamide. Patients were randomly assigned to receive the aforementioned standard chemotherapy with or without ten zoledronate intravenous infusions (four preoperative and six post-operative). The study, which started in 2007, closed in 2014 and enrolled 318 patients, did not show any improvement in treatment efficacy and prognosis as consequence of zoledronic acid addition to conventional chemotherapeutic drugs [48]. However, the rate of good histological response was higher in patients treated with methotrexate, ifosfamide and etoposide (73%) compared with those who received ifosfamide, doxorubicin and cisplatin confirming the results previously obtained by the same group [47].

These two studies indicated that HGOS patients may also be efficiently treated with methotrexate, ifosfamide and etoposide, especially when response to doxorubicin and cisplatin is poor.

Another important information concerning the effectiveness of etoposide in HGOS can be obtained by the largest trial that has been conducted so far, the EURAMOS-1 [49,50,51]. In this intercontinental treatment protocol, which enrolled 2260 patients with resectable HGOS from 326 centers across 17 countries, preoperative chemotherapy included doxorubicin, methotrexate and cisplatin. Poor responders to preoperative chemotherapy (patients with $\geq 10\%$ viable cells in the resected tumor tissue) were randomized and were postoperatively treated with doxorubicin, methotrexate and cisplatin with or without ifosfamide

and etoposide [51,52]. Event-free survival did not differ between the two treatment arms. Intensification of postoperative treatment with ifosfamide and etoposide was associated with more frequent grade 4 non-hematological toxicity [52]. The authors concluded that these results do not support the addition of ifosfamide and etoposide to the first-line chemotherapy in HGOS patients with poor response to preoperative treatment [52].

Etoposide was also used to treat metastatic or relapsed HGOS patients. In a French Phase II study, etoposide was used in combination with ifosfamide to treat relapsed or refractory childhood HGOS [53]. This trial showed that this drug combination had tolerable toxicity, was efficient and deserved evaluation in Phase III studies. In fact, among the 27 treated patients, six showed complete and seven partial responses, leading to a cumulative response rate of 48%.

Another evidence about etoposide effectiveness in HGOS derived from a Phase II/III trial, in which it was used together with high-dose ifosfamide in 43 newly diagnosed metastatic HGOS patients [54]. The overall response rate was 59%, with four patients (10%) showing complete response and 19 patients (49%) experiencing partial response. Unfortunately, two patients died because of therapy-associated toxicity. Authors concluded that the combination of etoposide and high-dose ifosfamide can be considered for treating patients with metastatic HGOS, despite a significant risk of toxicity.

Etoposide was also used together with ifosfamide, doxorubicin and methotrexate, in a Phase I/II clinical trial, which recruited 13 patients with metastatic or axial HGOS [55]. A good histological response was achieved in all the seven patients in which tumor necrosis was assessed, indicating a good efficacy of this four-drugs combination. However, these responses were not sustained and treatment produced substantial toxicity.

Bleomycin

Bleomycin is an antibiotic which also exerts antitumor activity. The drug binds to DNA leading to DNA single- and double-strand breaks. This yields G2/M cell cycle arrest and, eventually, apoptosis induction [56].

In human tumor cells, resistance to bleomycin has been indicated to derive from a reduced cellular drug uptake, enhanced DNA damage repair and/or increased detoxification (Table 2) [57]. Reduced drug uptake derives from decreased levels of bleomycin-binding sites at cell surface, which play a crucial role in drug internalization and, consequently, in its cytotoxicity [58]. Bleomycin is metabolized by GSTs (Table 4) and rapidly eliminated primarily by renal excretion [56]. Increased detoxification can be also

responsible for bleomycin resistance and it has been correlated with the activity of the bleomycin hydrolase enzyme [56,59], whereas the impact of GSTs-mediated inactivation still remains to be further confirmed.

The most serious complication of bleomycin treatment is pulmonary fibrosis and impaired lung function. It may also cause inflammation of the lungs that can result in lung scarring. Other common side effects of bleomycin treatment include fever, weight loss, vomiting and rash.

Bleomycin has been used in nonmetastatic HGOS to intensify standard multidrug chemotherapy protocols, in addition to doxorubicin, cisplatin, methotrexate, dactinomycin and cyclophosphamide. However, no real improvement has been obtained by this treatment intensification [60–64].

Bleomycin is presently sometimes used for second-line treatment of relapsed (frequently inoperable) HGOS patients. Moreover, the demonstration that the cytotoxicity of bleomycin can be augmented several 100-fold by electroporation [65] led to the development of rescue treatments based on the use of electrochemotherapy with bleomycin in locally advanced or metastatic soft tissue sarcomas [66]. Based on the positive results obtained by this approach, this treatment is presently taken into consideration also for treating relapsed, inoperable HGOS patients.

To further improve bleomycin efficacy, recently photochemical internalization techniques were used to enhance drug transport into tumor cells and showed promising activity in advanced sarcomas [5,67].

Dactinomycin (actinomycin D)

Dactinomycin, also known as actinomycin D, is the most significant member of a class of antitumor antibiotics and one of the oldest anticancer drugs. Dactinomycin inhibits transcription by binding DNA at the transcription initiation complex and by preventing elongation of RNA chain by RNA polymerase (Table 1) [68]. Since dactinomycin can bind DNA duplexes, it can also interfere with DNA replication, cause single-strand DNA breaks and induce apoptosis [69,70].

The major mechanism of resistance against dactinomycin is the overexpression and/or increased activity of ABCB1 (Table 2) [71,72].

The CYP1A1 and CYP1A2 are the enzymes which are mostly responsible for its detoxification (Table 4), and the main adverse effects of dactinomycin include bone marrow depression and gastrointestinal toxicity.

As mentioned above, dactinomycin has sometimes been used for first-line treatment of nonmetastatic HGOS in multiagent chemotherapy protocols including standard drugs (doxorubicin, cisplatin, methotrexate), as well as bleomycin and cyclophosphamide [60–64].

Since these protocols did not improve outcome and survival compared with those based only on standard first-line drugs, dactinomycin, bleomycin and cyclophosphamide were not further considered for treatment of newly diagnosed, nonmetastatic HGOS. However, they may be taken into consideration for second-line treatment of unresponsive, relapsed HGOS patients.

Cyclophosphamide

Cyclophosphamide is an alkylating agent with an effective anticancer activity. It is a prodrug which, in the liver, is converted into phosphoramidate mustard and acrolein (two very active compounds) by several CYP isoenzymes (Table 4). Phosphoramidate mustard introduces alkyl radicals into DNA strands, which interfere with DNA replication by forming DNA cross-linkage. Since cross-linked DNA is unable to complete normal cell division, it stops cancer cells from growing and leads to cell death.

The GST system appears to be directly involved in the detoxification of cyclophosphamide and their metabolites (Table 4), thus being related also to resistance against this drug (Table 2) [73,74]. The ability of the cell to repair cyclophosphamide-induced DNA lesions, possibly through nucleotide excision repair or other processes, can also significantly contribute to resistance [73]. Moreover, it has been demonstrated that cyclophosphamide is a substrate of ABCC4, which may play an important role in resistance against this drug (Table 2) [74].

Cyclophosphamide is used to treat different types of cancer and its activity has also been assessed in HGOS. In a Phase II trial, which recruited 26 relapsed HGOS patients, cyclophosphamide was used together with etoposide [75]. This protocol achieved a response rate of 19%, a disease stabilization rate of 35%, a progression-free survival at 4 months of 42% and an overall survival at 1 year of 50%. These data indicated that this approach may be of some help for rescue relapsed HGOS patients.

Moreover, cyclophosphamide has been used in combination with several other drugs to treat HGOS patients, as described in the sections about etoposide, bleomycin, dactinomycin, carboplatin and topotecan.

Carboplatin

Carboplatin is an analogue of cisplatin with less non-hematologic toxicity (particularly, ototoxicity and nephrotoxicity) than the parental compound. Like all the other platinum drugs, also carboplatin is metabolized by GSTP1 and resistance can derive from increased levels and/or activity of this enzyme as well as from increased DNA damage repair and ABCC2 overexpression (Tables 2 & 4) [76,77].

Carboplatin activity in HGOS has been evaluated in several studies. The Children's Cancer Group used carboplatin to treat patients with drug-resistant, recurrent lymphomas and solid tumors (including 14 HGOS patients). Among sarcomas, carboplatin showed some activity against Ewing's sarcoma and soft tissue sarcomas, but not in HGOS [78]. However, in another study, continuous-infusion of carboplatin demonstrated limited activity as an upfront agent in patients with metastatic HGOS at diagnosis [79].

A treatment protocol based on the use of high-dose chemotherapy with carboplatin plus etoposide and peripheral blood stem cell rescue enrolled 32 HGOS patients with metastatic relapse and resulted in a toxic death, a 3-year overall survival rate of 20% and a 3-year disease-free survival rate of 12% [80].

In another study, the combined treatment with carboplatin, ifosfamide and etoposide of children and adolescents with recurrent/refractory sarcomas (including 34 patients with HGOS) appeared to be of moderate efficacy, suggesting further exploration of this drug combination in relapsed patients [81].

A very important contribution to estimate carboplatin efficacy in HGOS derived from the comparison between two different carboplatin-based multi-agent chemotherapy protocols, which were used to treat metastatic HGOS patients [82]. The first protocol (OS-86, 12 patients) used ifosfamide, cisplatin, doxorubicin and high-dose methotrexate, whereas the subsequent regimen (OS-91, 17 patients) used the same agents at similar doses, but carboplatin was substituted for cisplatin. The worse outcome of patients treated with the OS-91 protocol indicated that cisplatin should be preferred to carboplatin to treat patients with high-risk disease, even if it is more toxic.

More recently, 71 patients with metastatic HGOS at diagnosis or axial primary tumors were treated with high-dose chemotherapy consisting in the administration of methotrexate, doxorubicin, cisplatin and ifosfamide in the preoperative treatment, followed by doxorubicin, cyclophosphamide, etoposide and carboplatin in the postoperative chemotherapy [83]. This very aggressive protocol demonstrated that administration of high-dose chemotherapy with stem cell rescue was feasible, but that it was also associated with significant toxicity. Unfortunately, patients' outcome was comparable to that achievable with conventional chemotherapy and authors concluded that this regimen was not to be considered as an effective treatment strategy to rescue high-risk HGOS patients.

Topotecan & irinotecan

Topotecan and irinotecan are the two most successful analogs of camptothecin, which were approved

for the treatment of a variety of human malignant tumors [84,85]. Differently from topotecan, irinotecan is a prodrug that is converted to its active compound SN-38 by plasma and cellular carboxylesterases [85].

Both drugs are active in the S-phase and target topoisomerase I, stabilizing the normally transient complexes between this enzyme and DNA. This interaction prevents DNA synthesis, produces DNA breaks and subsequently induces apoptosis (Table 1) [85].

Based on evidence obtained in preclinical studies, it is likely that clinical resistance to these drugs might result from inadequate drug accumulation in the tumor due to the increased efflux activity of some ABC transporters (Tables 2 & 3) [85–87], or from alterations (mutations) of topoisomerase I, which greatly influence the binding to their target [84,85]. The active irinotecan metabolite SN-38 is detoxified by several UGT isoenzymes in the liver (Table 4) [85]. Since the UGT-catalized glucuronidation of SN-38 may also be associated with an increased efflux of the drug from cancer cells, an increased UGT-mediated detoxification appears to be an important mechanism of resistance against irinotecan (Table 2) [85]. However, the actual clinical relevance in human tumors of all these mechanisms of resistance still needs to be confirmed, in particular in rare malignancies as HGOS.

When used alone, topotecan did not prove to be active in recurrent or refractory HGOS [88,89]. However, the use of topotecan together with cyclophosphamide appeared to be an attractive choice for aggressive rescue protocols aimed at curing patients with recurrent or refractory pediatric solid tumors, including HGOS [90–92].

In the Children's Cancer Group 7943 protocol, newly diagnosed patients with metastatic HGOS were treated with topotecan followed by chemotherapy with ifosfamide, carboplatin and etoposide, alternating with cisplatin and doxorubicin. Unfortunately, insufficient activity was seen with topotecan in this protocol to warrant further studies in HGOS [93].

Irinotecan was also used to treat relapsed HGOS patients. In two Phase I trials, irinotecan was used as single drug to treat children with refractory solid tumors, including HGOS, producing disease stabilization and encouraging clinical responses [94,95].

Irinotecan has also been evaluated in recurrent, metastatic HGOS patients in combination with gemcitabine showing a promising antitumor activity [96].

More recently, a Phase I study was conducted to estimate the maximum tolerated dose and the dose-limiting toxicities of oral irinotecan with gefitinib (also known as Iressa, a tyrosine kinase inhibitor against epidermal growth factor) in children with refractory solid tumors [97]. This study, which also recruited

five patients with HGOS, showed that this drug combination had acceptable toxicity and antitumor activity in pediatric patients with refractory solid tumors. Moreover, pharmacokinetic analyses confirmed that co-administration of gefitinib also increased irinotecan bioavailability, leading to an increased SN-38 lactone systemic exposure.

In another Phase I trial, irinotecan was used together with the mTOR inhibitor temsirolimus and temozolomide to treat children with recurrent/refractory solid tumors, including seven patients with HGOS [98]. This study showed that this drug combination was well tolerated, demanding for Phase II trials.

Pharmacogenomics of markers involved in activity or response of HGOS second-line drugs
 Only few (if any) reports have been published for HGOS about genetic variations involving genes which can be relevant for response to second-line drugs. Gene polymorphisms affecting drug metabolizing enzymes involved in detoxification and/or activation of several second-line HGOS drugs have been recently reviewed [16]. There is, however, other evidence in the literature of pharmacogenomic and pharmacogenetic variations that have been suggested to have clinical impact in other human tumors (Table 5), which may be taken into account also for HGOS, despite second-

line drugs have been very rarely used alone in HGOS treatment (Table 6).

Polymorphisms of drug transporter genes
 ABCB1 is known to be responsible for the efflux and resistance of several first- and second-line HGOS drugs (Tables 2 & 3). Because of *in vitro* evidence that polymorphisms of this gene could impact on dactinomycin exposure in a clinical setting, 117 patients younger than 21 years with solid tumors including bone tumors (Ewing sarcoma) and treated with dactinomycin as part of their standard therapy were genotyped for three polymorphisms of the *ABCB1* gene (1236C/T rs1128503, 2677G/T/A rs2032582 and 3435C/T rs1045642). No significant associations were found between *ABCB1* genotypes and dactinomycin pharmacokinetics or treatment-related toxicities [104].
 The *ABCB1*-3435C>T (rs1045642) and -2677G>T/A (rs2032582) polymorphisms were reported to be associated with gastrointestinal, hematological toxicity or neurotoxicity after treatment with taxanes, but data were inconsistent and must be confirmed (Table 5). Since frequency and functional consequence of genetic variants is often ethnicity, and/or tissue-specific, it is not surprising that no protective or risk variant valid for all the analyzed cancer types and ethnicities was

Table 5. Gene polymorphisms with relevant clinical impact in human tumors which may also influence second-line drugs response and toxicity in high-grade osteosarcoma.			
Genes	Polymorphism	Main findings associated with polymorphisms	Ref.
<i>ABCB1</i>	-3435C>T_rs1045642 -2677G>T/A_rs2032582	Possible associations with gastrointestinal, hematological toxicity or neurotoxicity after treatment with taxanes	[99]
<i>ABCC1ABCC2</i>	IVS11 -48C>T rs3765129 -24C>T rs717620	Associations with neutropenia in 85 advanced colorectal cancer patients treated with irinotecan as single agent	[100]
<i>ABCC4</i>	G>T rs9561778	Associations of T allele with hematological and gastrointestinal toxicity in 256 Bangladeshi breast cancer patients treated with cyclophosphamide, epirubicin and 5-fluorouracil Associations of T allele with leukopenia/neutropenia and gastrointestinal toxicity in a total of 403 Japanese breast cancer patients treated with cyclophosphamide-based combination therapy	[74] [101]
<i>CYP2C8CYP3A5</i>	*3 rs11572080 + rs10509681 *3 rs776746	<i>CYP2C8</i> *3 and <i>CYP3A5</i> *3 polymorphisms have been described as potentially predictive for hematological toxicity and neurotoxicity after taxanes treatment	[99]
<i>UGT1A1</i>	*28 rs8175347	Patients homozygous for the <i>UGT1A1</i> *28 allele have increased risk to develop hematological and/or digestive toxicities after irinotecan therapy	[102]
<i>UGT1A1</i>	*93 rs1092302	Association with neutropenia in 85 advanced colorectal cancer patients treated with irinotecan as single-agent	[100]
<i>ERCC2 (XPD)</i> and <i>GSTT1</i>	rs13181 and GSTT1 null	Associations between both polymorphisms and bleomycin-induced DNA damage	[103]

Table 6. Second-line drug combinations which have been explored in high-grade osteosarcoma.

Drug combination	Ref.
Vincristine + methotrexate + doxorubicin	[27]
Vincristine + high-dose methotrexate + cisplatin	[28]
Vinorelbine + cyclophosphamide	[29]
Paclitaxel (used as single drug)	[32]
Docetaxel + gemcitabine	[38,44,45]
Etoposide + high-dose methotrexate + ifosfamide	[47]
Etoposide + high-dose methotrexate + doxorubicin + cisplatin + ifosfamide	[50,51]
Etoposide + ifosfamide	[53]
Etoposide + cyclophosphamide	[75]
Etoposide + high-dose ifosfamide	[54]
Etoposide + carboplatin	[80]
Etoposide + ifosfamide + doxorubicin + methotrexate	[55]
Bleomycin + doxorubicin + cisplatin + methotrexate + dactinomycin + cyclophosphamide	[60–64]
Bleomycin (used as single drug) with electroporation	[65]
Carboplatin (used as single drug)	[78,79]
Carboplatin + ifosfamide + etoposide	[81]
Carboplatin + high-dose methotrexate + doxorubicin + ifosfamide	[82]
Carboplatin + doxorubicin + cyclophosphamide + etoposide	[83]
Topotecan (used as single drug)	[88,89]
Topotecan + cyclophosphamide	[90–92]
Topotecan + ifosfamide + carboplatin + etoposide , alternating with cisplatin and doxorubicin	[93]
Irinotecan (used as single drug)	[94,95]
Irinotecan + gemcitabine	[96]
Irinotecan + gefitinib (EGF inhibitor)	[97]
Irinotecan + temsirolimus (mTOR inhibitor) + temozolomide	[98]

Second-line drugs are in bold.

identified. Other genetic variants in *ABCB1* as well as in *ABCC2*, *ABCC6*, *ABCC10*, *ABCG1*, *SLCO1A2* and *SLCO1B3* genes have been reported as risk factors for taxane-induced toxicity [99].

In a study of 85 advanced cancer patients treated with irinotecan as single-agent, the *ABCC2* -24C>T rs717620 was associated with the variability of irinotecan plasma concentration [100], which might be explained by the fact that this SNP leads to lower mRNA levels in normal tissues (Table 5). It is worthwhile noting that the *ABCC2* rs717620 and rs17222723 have been reported to be associated with poor histological response or leukopenia [105], and *ABCC2* rs2273697 with survival and hematological and hepatic toxicities after first-line chemotherapy treatment in HGOS patients [106].

The rs4148 polymorphisms of *ABCC3* has been described to be associated with poor response to first-line chemotherapy in three studies, but no associations

were described in association with second-line drugs used in HGOS [105].

A recent pharmacogenetic study conducted in Bangladeshi breast cancer patients treated with cyclophosphamide, epirubicin and 5-fluorouracil revealed statistically significant associations between the variant allele of *ABCC4* rs9561778 (GT+TT genotypes) and anemia, neutropenia, leukopenia and gastrointestinal toxicities (all grade III or IV) (Table 5) [74]. This observation was in line with a study on Japanese breast cancer patients (184 cases who developed adverse drug reactions and 219 cases without) suggesting that *ABCC4* rs9561778 may serve as predictor for treatment-related hematological and gastrointestinal toxicity in patients treated with cyclophosphamide-based combination therapy (Table 5) [101].

The nonsynonymous polymorphism of *ABCG2* 421C>A (rs2231142) has been functionally evaluated in relation to topoisomerase inhibitors topotecan and

irinotecan as well as to docetaxel but no conclusive results were obtained [107].

Drug metabolizing enzymes

The pharmacogenomic role of drug metabolizing enzymes in the treatment of HGOS has recently been reviewed [16]. Here below other polymorphisms, which may be relevant for second-line drug response in HGOS, have been summarized.

*CYP2C8**3 and *CYP3A5**3 polymorphisms were described as potentially predictive for hematological toxicity and neurotoxicity after taxanes treatment (Table 5), but data were not confirmed ([99] and references therein).

Recently, the variant allele of *CYP3A4* rs4646437 has been reported to be associated with better survival in HGOS patients treated with a standard methotrexate-doxorubicin-cisplatin regimen [108]. Authors suggested that this variant could lead to low *CYP3A4* expression in HGOS cells, thus better response to therapy due to less inactivation of anticancer drugs. Therapy-associated toxicity was not reported in this study. However, since this isoenzyme is also involved in the metabolisms of several second-line drugs, it appears to be a good candidate for further analysis in HGOS patients treated with rescue therapies.

The *GSTP1* rs1695 AG+GG genotypes were indicated to be significantly associated with good therapy response in breast cancer patients treated with cyclophosphamide-including therapy [74]. However, *GSTP1* rs1695 AG+GG genotypes were found to be associated with poor histological response and worse survival in HGOS patients treated with neoadjuvant chemotherapy using methotrexate, doxorubicin, cisplatin with or without ifosfamide and, in some, studies with addition of dactinomycin and vincristine in the postoperative regimen (for review see [105]).

The UGT isoenzymes are essential for detoxification of irinotecan, and the *UGT1A1**28 variant proved to play a crucial role for successful treatment with this drug (reviewed in [102]). Furthermore, the *UGT1A1**93 was suggested to be a better predictor for neutropenia based on a multivariate genetic model including polymorphisms of several drug-metabolizing enzymes and drug transporters applied to the pharmacokinetic, genetic and clinical data obtained on 85 patients with advanced cancers treated with irinotecan (Table 5, [100]). Therefore, these recommendations should be considered also for HGOS patients prior to start irinotecan therapy.

DNA repair genes

A pilot study performed on lymphocytes of 200 healthy Caucasian individuals revealed clear associations

between the *ERCC2* (XPD) rs13181 and *GSTT1*-null polymorphisms and both spontaneous and bleomycin-induced micronuclei formation, a reliable measure for DNA damage (Table 5, [103]). It is noteworthy that both polymorphisms have been reported also in HGOS patients with partially contradictory impact on survival or first-line chemotherapy-associated toxicity (for review see [16,105]).

Conclusion

HGOS is a highly malignant bone tumor, for which the major clinical factor limiting the cure rate is the occurrence of relapse, most commonly consisting in lung metastasis. Relapse rate ranges from 30 to 35% in conventional HGOS and from 60 to 65% for axial osteosarcoma (which is mainly located in pelvis and sacrum).

In case of relapse, the survival probability significantly decreases in relation to time and pattern of recurrence and possibility of achieving a second surgical remission, whereas the role of rescue chemotherapy treatment is not well defined yet [109–111]. In addition to the fact that the role of chemotherapy to rescue recurrent HGOS patients is still under discussion, there is no evidence about the best second-line drugs that should be used in this situation.

As discussed in this review, some of the drugs or drug combinations that have been used for treating relapsed HGOS patients actually showed promising or encouraging results and may, therefore, be taken into account for second-line treatment of refractory HGOS patients.

The treatment with docetaxel and gemcitabine showed promising results, suggesting that this drug combination should be further investigated in bone sarcoma patients [38,44,45].

HGOS patients with poor response to doxorubicin and cisplatin may be treated with etoposide, methotrexate and ifosfamide [47,48]. Etoposide together with ifosfamide may also be considered for treating patients with metastatic or relapsed HGOS, despite a significant risk of toxicity [53,54]. Moreover, the combined treatment with etoposide and cyclophosphamide appeared to be of some help for rescue relapsed HGOS patients [75].

Treatment with bleomycin and electroporation is presently taken into consideration for relapsed, inoperable HGOS patients [66]. The use of topotecan together with cyclophosphamide appeared to be an attractive choice for rescue treatment of patients with recurrent or refractory pediatric solid tumors, including HGOS [90–92].

Targeted drugs may also represent a new possible perspective for improving second-line treatment of HGOS. However, in the last years, several studies have

been performed to investigate new so-called target agents as well as alternative therapeutic approaches for treating HGOS relapsed patients, but none of them has emerged as highly effective so far [4–6,12].

The knowledge of the major mechanisms of resistance against first- and second-line drugs is, however, of key importance to drive the treatment choice, in order to avoid possible and unnecessary cross-resistance which may significantly limit the therapy efficacy.

Another problem related to the extensive and aggressive treatment addressed to recurrent HGOS patients is that survivors may develop long-term and permanent adverse effects due to toxicity of chemotherapeutic drugs, which is a relevant individual and social problem due to the long life expectancy of cured HGOS patients. However, it should be underlined that some of the polymorphisms described to be associated either with treatment-related toxicity or to impact on therapy response after standard HGOS first-line chemotherapy [105] might also influence responsiveness to second-line drugs. Therefore, the new frontier for improving prognosis of relapsed HGOS is the optimization of the use of the available drugs by tailoring chemotherapy treatment and the parallel development of new treatment modalities, more effective and less toxic, aimed to cure higher numbers of patients and decrease chemotherapy-associated toxicity. Under this perspective, the optimization of the use of the available drugs is based on the concept that, when HGOS patients are stratified according to a validated pharmacogenomic biomarker, chemotherapy may be tailored by modulating drug dosages and administration schedules on the basis of the patients' genetic makeup, in order to achieve the best benefit with the lowest risk for adverse toxicity. Among the pharmacogenomic markers that have emerged so far, those which appear to be of higher relevance for second-line drugs explored in HGOS are genetic variations affecting genes coding for drug transporters (ABCB1, ABCC1, ABCC2 and ABCC4), drug metabolizing enzymes (CYP2C8, CYP3A5, UGT1A1 and GSTT1) and DNA repair factors (ERCC2/XPD). These genetic variations need, however, to be further studied and validated in HGOS before being translated to the clinic.

However, it is clear that new agents and therapeutic approaches must be developed concomitantly to the identification and validation of pharmacogenomic markers to allow a more efficient treatment tailoring.

Future perspective

Pharmacogenomics is expected to significantly contribute in the near future to optimize both first-line and rescue chemotherapy treatment in HGOS patients. Several gene polymorphisms and alterations predictive of chemotherapy sensitivity and treatment-associated

toxicity have been identified in the past decades. Once validated, these genetic biomarkers may constitute the basis for tailoring treatment in newly diagnosed HGOS and, even more, in relapsed HGOS patients, both increasing their cure probability and improving their quality of life.

It must, however, be taken into consideration that HGOS is a highly heterogeneous tumor. This heterogeneity may be a considerable therapeutic challenge because biomarkers revealed in a single biopsy specimen may not be representative of the whole primary tumor as well as of its metastases or distant recurrences. This highlights the need of performing pharmacogenomic evaluations on different samples from both primary and relapsed tumors, in addition to the pharmacogenetic analysis of normal cells of the same patient, in order to get a reliable picture of the genetic background of each patient, which may drive the treatment choices more efficiently.

Although few pharmacogenetic markers related to second-line drugs used in HGOS patients have already been extensively reviewed and validated (e.g., the UGT family) [112] and guidelines have been written how to implement clinical routine for patients' characterization (e.g., *UGT1A1* genotyping prior to irinotecan therapy) [102], there is still not enough knowledge to individualize chemotherapy drug regimens for HGOS patients.

Albeit candidate gene-driven studies focusing mainly on drug metabolizing enzymes, transporter, DNA repair and apoptosis-related genes have identified several polymorphisms with possible clinical impact for HGOS, data are hardly conclusive. For the future, there is the hope that data-driven biomedical research could partially overcome time consuming *in vitro* testing of pharmacogenomic markers and drugs. As recently reviewed [113], elaborating the increasing body of large-scale genomic profiling data that has been generated on many diseases and a number of drugs and compounds by network-based approaches will hopefully provide useful information also for HGOS patients. First results, which might be helpful to direct new therapeutic approaches, have recently been obtained by exome sequencing of 123 HGOS samples [114]. This study revealed 14 driver genes for HGOS, some of which were unknown so far. In a subset of tumors, a characteristic pattern of substitution mutations, which were strongly associated with *BRCA1* and *BRCA2* mutations in breast and pancreatic cancer types, were also identified. The authors concluded that this BRCA-like phenotype warrants further testing of PARP inhibitors in HGOS.

Recently, a completely different approach applying targeted exon sequencing to 1162 patients with sar-

coma, including 124 patients with HGOS, revealed an excess of functionally pathogenic variants in *TP53*, *BRCA2*, *ATM*, *ATR* and *ERCC2* [115]. In addition, they showed a measurable contribution of polygenic effects to sarcoma risk, but even more important, that one of four patients carried genetic germline variants with potential therapeutic significance.

Therefore, based on this evidence, it is reasonable thinking that in the near future high-throughput sequencing may provide new genetic information with relevant implications for risk management and treatment.

A new perspective for improving second-line treatment efficacy in HGOS may derive from the nanomedicine field of research. In other words, a recently developed formulation of paclitaxel designed to reduce its side effects, the nab-paclitaxel, has provided interesting results in HGOS experimental models showing a greater antitumor efficacy compared with paclitaxel [34–36], also if a more recent report did not confirm some of the results previously obtained in HGOS preclinical models [116].

Nab-paclitaxel is a solvent-free, nanoparticle albumin bound (nab) formulation of paclitaxel, which increases the intratumoral concentration of the native drug because its intracellular incorporation is facilitated by transporters with high affinity for albumin. By following this strategy, additional similar formulations may be developed in the near future also for other drugs of possible value for second-line HGOS treatment.

Financial & competing interests disclosure

The authors' studies cited in this manuscript were supported by grants from the Italian Association for Cancer Research (Associazione Italiana per la Ricerca sul Cancro, A.I.R.C.; grants to M Serra) and Istituto Ortopedico Rizzoli (5x mille contributions to the Rizzoli Institute). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Although several drugs have been used for high-grade osteosarcoma (HGOS) second-line treatment, none of them has provided fully satisfactory results and the role of rescue chemotherapy is not well defined yet. The major factors limiting the efficacy of rescue treatments in HGOS appear to be the presence of mechanisms of resistance developed against first-line chemotherapeutic agents, which can also be responsible for unresponsiveness to second-line drugs, and the lack of information about biomarkers and genetic determinants which may guide the protocol planning.
- The heterogeneous response of HGOS patients to second-line treatments derives not only from a clinicopathological variability, but also from the different patients' genetic background. Although several gene polymorphisms and alterations predictive of both first-line and second-line chemotherapy sensitivity and treatment-associated toxicity have been identified in the past decade in HGOS, all of them need to be further validated to be effectively transferred to clinical practice. Once validated, these genetic biomarkers may constitute the basis for tailoring treatment in HGOS patients, both improving their cure probability and quality of life.
- In the future, the way for improving prognosis of relapsed HGOS patients, as well as of patients who are unresponsive to first-line chemotherapy treatment, will be to optimize the use of the available drugs by tailoring treatment on the basis of tumor and patients' pharmacogenomics characteristics. This approach is expected to stimulate the development of new treatment modalities aimed to cure higher numbers of patients and, concomitantly, decrease chemotherapy-associated toxicity.

References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

- 1 Rosenberg AE, Cleton-Jansen A-M, de Pinieux G *et al.* Conventional osteosarcoma. In: *WHO classification of tumours of soft tissue and bone*. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F (Eds), IARC, Lyon, 282–288 (2013).
- 2 Picci P. Classic Osteosarcoma. In: *Atlas of Musculoskeletal Tumors and Tumorlike Lesions*. Picci P, Manfrini M, Fabbri N, Gamberotti M, Vanel D (Eds). Springer International Publishing, Cham, Switzerland, 147–152 (2014).
- 3 Bruland OS, Pihl A. On the current management of osteosarcoma. A critical evaluation and a proposal for a modified treatment strategy. *Eur. J. Cancer* 33(11), 1725–1731 (1997).
- 4 Ferrari S, Serra M. An update on chemotherapy for osteosarcoma. *Expert Opin. Pharmacother.* 16(18), 2727–2736 (2015).
- 5 Hattinger CM, Fanelli M, Tavanti E *et al.* Advances in emerging drugs for osteosarcoma. *Expert Opin. Emerg. Drugs* 1–20 (2015).
- Interesting overview about the present and the future treatment approaches for high-grade osteosarcoma (HGOS).
- 6 Hattinger CM, Pasello M, Ferrari S, Picci P, Serra M. Emerging drugs for high-grade osteosarcoma. *Expert Opin. Emerg. Drugs* 15(4), 615–634 (2010).
- 7 Anninga JK, Gelderblom H, Fiocco M *et al.* Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? *Eur. J. Cancer* 47(16), 2431–2445 (2011).

- **Key report about the evolution of chemotherapy treatment of HGOS.**
- 8 Ferrari S, Briccoli A, Mercuri M *et al.* Late relapse in osteosarcoma. *J. Pediatr. Hematol. Oncol.* 28(7), 418–422 (2006).
- 9 National Center for Biotechnology Information. www.ncbi.nlm.nih.gov/entrez/query.fcgi
- 10 PharmGKB. www.pharmgkb.org/pathway/
- 11 OMIM. www.ncbi.nlm.nih.gov/omim/?term=*
- 12 Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. *J. Clin. Oncol.* 33(27), 3029–3035 (2015).
- **Updated report about the future perspectives for HGOS treatment.**
- 13 Human Transporter Database. <http://htd.cbi.pku.edu.cn/>
- 14 PharmGKB. www.pharmgkb.org/index.jsp
- 15 University of California, San Francisco. <http://dbts.ucsf.edu/fdatransportal/>
- 16 Hattinger CM, Serra M. Role of pharmacogenetics of drug-metabolizing enzymes in treating osteosarcoma. *Expert Opin. Drug Metab. Toxicol.* 1–15 (2015).
- 17 Preissner S, Kroll K, Dunkel M *et al.* SuperCYP: a comprehensive database on Cytochrome P450 enzymes including a tool for analysis of CYP-drug interactions. *Nucleic Acids Res.* 38(Database issue), D237–D243 (2010). <http://bioinformatics.charite.de/>
- 18 Hattinger CM, Tavanti E, Fanelli M, Vella S, Picci P, Serra M. Pharmacogenomics of genes involved in antifolate drug response and toxicity in osteosarcoma. *Expert Opin. Drug Metab. Toxicol.* [dx.doi.org/10.1080/17425255.2017.1246532](https://doi.org/10.1080/17425255.2017.1246532) (2016) (In Press).
- 19 Downing KH. Structural basis for the interaction of tubulin with proteins and drugs that affect microtubule dynamics. *Annu. Rev. Cell Dev. Biol.* 16, 89–111 (2000).
- 20 Himes RH. Interactions of the catharanthus (Vinca) alkaloids with tubulin and microtubules. *Pharmacol. Ther.* 51(2), 257–267 (1991).
- 21 Wang LG, Liu XM, Kreis W, Budman DR. The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. *Cancer Chemother. Pharmacol.* 44(5), 355–361 (1999).
- 22 Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer* 2(1), 48–58 (2002).
- 23 He SM, Li R, Kanwar JR, Zhou SF. Structural and functional properties of human multidrug resistance protein 1 (MRP1/ABCC1). *Curr. Med. Chem.* 18(3), 439–481 (2011).
- 24 Nobili S, Landini I, Mazzei T, Mini E. Overcoming tumor multidrug resistance using drugs able to evade P-glycoprotein or to exploit its expression. *Med. Res. Rev.* 32(6), 1220–1262 (2012).
- 25 Attaoua C, Vincent LA, Abdel Jaoued A *et al.* Differential involvement of glutathione S-transferase μ 1 and multidrug resistance protein 1 in melanoma acquired resistance to vinca alkaloids. *Fundam. Clin. Pharmacol.* 29(1), 62–71 (2015).
- 26 Morris PG, Fornier MN. Microtubule active agents: beyond the taxane frontier. *Clin. Cancer Res.* 14(22), 7167–7172 (2008).
- 27 Longhi A, Pasini E, Bertoni F, Pignotti E, Ferrari C, Bacci G. Twenty-year follow-up of osteosarcoma of the extremity treated with adjuvant chemotherapy. *J. Chemother.* 16(6), 582–588 (2004).
- 28 Morgan E, Baum E, Bleyer WA *et al.* Treatment of patients with metastatic osteogenic sarcoma: a report from the Children's Cancer Study Group. *Cancer Treat Rep.* 68(4), 661–664 (1984).
- 29 Minard-Colin V, Ichante JL, Nguyen L *et al.* Phase II study of vinorelbine and continuous low doses cyclophosphamide in children and young adults with a relapsed or refractory malignant solid tumour: good tolerance profile and efficacy in rhabdomyosarcoma – a report from the Societe Francaise des Cancers et leucemies de l'Enfant et de l'adolescent (SFCE). *Eur. J. Cancer* 48(15), 2409–2416 (2012).
- 30 Arai T, Miyoshi Y, Kim SJ *et al.* Association of GSTP1 expression with resistance to docetaxel and paclitaxel in human breast cancers. *Eur. J. Surg. Oncol.* 34(7), 734–738 (2008).
- 31 Rowinsky EK. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu. Rev. Med.* 48, 353–374 (1997).
- 32 Patel SR, Papadopoulos NE, Plager C *et al.* Phase II study of paclitaxel in patients with previously treated osteosarcoma and its variants. *Cancer* 78(4), 741–744 (1996).
- 33 Gian VG, Johnson TJ, Marsh RW, Schuhmacher C, Lynch JW. A Phase II trial of paclitaxel in the treatment of recurrent or metastatic soft tissue sarcomas or bone sarcomas. *J. Exp. Ther. Oncol.* 1(3), 186–190 (1996).
- 34 Wagner LM, Yin H, Eaves D, Currier M, Cripe TP. Preclinical evaluation of nanoparticle albumin-bound paclitaxel for treatment of pediatric bone sarcoma. *Pediatr. Blood Cancer* 61(11), 2096–2098 (2014).
- 35 Yang Y, Niu X, Zhang Q, Hao L, Ding Y, Xu H. The efficacy of abraxane on osteosarcoma xenografts in nude mice and expression of secreted protein, acidic and rich in cysteine. *Am. J. Med. Sci.* 344(3), 199–205 (2012).
- 36 Zhang L, Marrano P, Kumar S *et al.* Nab-paclitaxel is an active drug in preclinical model of pediatric solid tumors. *Clin. Cancer Res.* 19(21), 5972–5983 (2013).
- 37 ClinicalTrials.gov. <https://ClinicalTrials.gov/show/NCT01962103>
- 38 Song BS, Seo J, Kim DH, Lim JS, Yoo JY, Lee JA. Gemcitabine and docetaxel for the treatment of children and adolescents with recurrent or refractory osteosarcoma: Korea Cancer Center Hospital experience. *Pediatr. Blood Cancer* 61(8), 1376–1381 (2014).
- 39 Mini E, Nobili S, Caciagli B, Landini I, Mazzei T. Cellular pharmacology of gemcitabine. *Ann. Oncol.* 17(Suppl. 5), v7–v12 (2006).

- 40 Plunkett W, Huang P, Xu YZ, Heinemann V, Grunewald R, Gandhi V. Gemcitabine: metabolism, mechanisms of action, and self-potential. *Semin. Oncol.* 22, 4(Suppl. 11), 3–10 (1995).
- 41 Bai J, Sata N, Nagai H. Gene expression analysis for predicting gemcitabine sensitivity in pancreatic cancer patients. *HPB (Oxford)* 9(2), 150–155 (2007).
- 42 Bergman AM, Pinedo HM, Peters GJ. Determinants of resistance to 2',2'-difluorodeoxycytidine (gemcitabine). *Drug Resist. Updat.* 5(1), 19–33 (2002).
- 43 Rapkin L, Qayed M, Brill P *et al.* Gemcitabine and docetaxel (GEMDOX) for the treatment of relapsed and refractory pediatric sarcomas. *Pediatr. Blood Cancer* 59(5), 854–858 (2012).
- 44 Gosiengfiao Y, Reichel J, Woodman J, Ben-Ami T, Walterhouse D. Gemcitabine with or without docetaxel and resection for recurrent osteosarcoma: the experience at Children's Memorial Hospital. *J. Pediatr. Hematol. Oncol.* 34(2), e63–e65 (2012).
- 45 Navid F, Willert JR, Mccarville MB *et al.* Combination of gemcitabine and docetaxel in the treatment of children and young adults with refractory bone sarcoma. *Cancer* 113(2), 419–425 (2008).
- 46 Hansen LT, Lundin C, Helleday T *et al.* DNA repair rate and etoposide (VP16) resistance of tumor cell subpopulations derived from a single human small cell lung cancer. *Lung Cancer* 40(2), 157–164 (2003).
- 47 Le Deley MC, Guinebretiere JM, Gentet JC *et al.* 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* 43(4), 752–761 (2007).
- 48 Piperno-Neumann S, Le Deley MC, Redini F *et al.* Zoledronate in combination with chemotherapy and surgery to treat osteosarcoma (OS2006): a randomised, multicentre, open-label, Phase III trial. *Lancet Oncol.* 17(8), 1070–1080 (2016).
- 49 ClinicalTrials.gov.
<https://ClinicalTrials.gov/show/NCT00134030>
- 50 Marina N, Bielack S, Whelan J *et al.* International collaboration is feasible in trials for rare conditions: the EURAMOS experience. *Cancer Treat Res.* 152, 339–353 (2009).
- 51 Whelan JS, Bielack SS, Marina N *et al.* EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. *Ann. Oncol.* 26(2), 407–414 (2015).
- **Interesting report about the results obtained in this unique European–American trial for HGOS.**
- 52 Marina NM, Smeland S, Bielack SS *et al.* Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. *Lancet Oncol.* 17(10), 1396–1408 (2016).
- 53 Gentet JC, Brunat-Mentigny M, Demaille MC *et al.* Ifosfamide and etoposide in childhood osteosarcoma. A Phase II study of the French Society of Paediatric Oncology. *Eur. J. Cancer* 33(2), 232–237 (1997).
- 54 Goorin AM, Harris MB, Bernstein M *et al.* Phase II/III trial of etoposide and high-dose ifosfamide in newly diagnosed metastatic osteosarcoma: a pediatric oncology group trial. *J. Clin. Oncol.* 20(2), 426–433 (2002).
- 55 Mctiernan A, Meyer T, Michelagnoli MP, Lewis I, Whelan JS. A Phase I/II study of doxorubicin, ifosfamide, etoposide and interval methotrexate in patients with poor prognosis osteosarcoma. *Pediatr. Blood Cancer* 46(3), 345–350 (2006).
- 56 Dorr RT. Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. *Semin. Oncol.* 19, 2(Suppl. 5), 3–8 (1992).
- 57 Wang Q, Cui K, Espin-Garcia O *et al.* Resistance to bleomycin in cancer cell lines is characterized by prolonged doubling time, reduced DNA damage and evasion of G2/M arrest and apoptosis. *PLoS ONE* 8(12), e82363 (2013).
- 58 Pron G, Belehradek J Jr, Orłowski S, Mir LM. Involvement of membrane bleomycin-binding sites in bleomycin cytotoxicity. *Biochem. Pharmacol.* 48(2), 301–310 (1994).
- 59 Sebt SM, Jani JP, Mistry JS, Gorelik E, Lazo JS. Metabolic inactivation: a mechanism of human tumor resistance to bleomycin. *Cancer Res.* 51(1), 227–232 (1991).
- 60 Aparicio J, Segura A, Montalar J *et al.* Long-term results after combined modality treatment for non-metastatic osteosarcoma. *Med. Oncol.* 16(4), 255–260 (1999).
- 61 Goorin AM, Schwartzentruber DJ, Devidas M *et al.* Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651. *J. Clin. Oncol.* 21(8), 1574–1580 (2003).
- 62 Meyers PA, Gorlick R, Heller G *et al.* Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan–Kettering (T12) protocol. *J. Clin. Oncol.* 16(7), 2452–2458 (1998).
- 63 Provisor AJ, Ettinger LJ, Nachman JB *et al.* Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. *J. Clin. Oncol.* 15(1), 76–84 (1997).
- 64 Souhami RL, Craft AW, Van Der Eijken JW *et al.* Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. *Lancet* 350(9082), 911–917 (1997).
- 65 Gothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat Rev.* 29(5), 371–387 (2003).
- 66 Campana LG, Bianchi G, Mocellin S *et al.* Electrochemotherapy treatment of locally advanced and metastatic soft tissue sarcomas: results of a non-comparative Phase II study. *World J. Surg.* 38(4), 813–822 (2014).
- 67 Jerjes W, Tan HB, Hopper C, Giannoudis PV. Spinal metastasis subjected to photodynamic therapy: an update. *Hard Tissue* 1(1), 8 (2012).
- 68 Sobell HM. Actinomycin and DNA transcription. *Proc. Natl Acad. Sci. USA* 82(16), 5328–5331 (1985).

- 69 Kleeff J, Kornmann M, Sawhney H, Korc M. Actinomycin D induces apoptosis and inhibits growth of pancreatic cancer cells. *Int. J. Cancer* 86(3), 399–407 (2000).
- 70 Mischo HE, Hemmerich P, Grosse F, Zhang S. Actinomycin D induces histone gamma-H2AX foci and complex formation of gamma-H2AX with Ku70 and nuclear DNA helicase II. *J. Biol. Chem.* 280(10), 9586–9594 (2005).
- 71 Biedler JL. Genetic aspects of multidrug resistance. *Cancer* 70, 6(Suppl.), 1799–1809 (1992).
- 72 Malonne H, Atassi G. DNA topoisomerase targeting drugs: mechanisms of action and perspectives. *Anticancer Drugs* 8(9), 811–822 (1997).
- 73 Gamcsik MP, Dolan ME, Andersson BS, Murray D. Mechanisms of resistance to the toxicity of cyclophosphamide. *Curr. Pharm. Des.* 5(8), 587–605 (1999).
- 74 Islam MS, Parvin S, Ahmed MU *et al.* Effect of *GSTP1* and *ABCC4* gene polymorphisms on response and toxicity of cyclophosphamide-epirubicin-5-fluorouracil-based chemotherapy in Bangladeshi breast cancer patients. *Tumour Biol.* 36(7), 5451–5457 (2015).
- 75 Berger M, Grignani G, Ferrari S *et al.* Phase 2 trial of two courses of cyclophosphamide and etoposide for relapsed high-risk osteosarcoma patients. *Cancer* 115(13), 2980–2987 (2009).
- 76 De Sousa GF, Włodarczyk SR, Monteiro G. Carboplatin: molecular mechanisms of action associated with chemoresistance. *Braz. J. Pharm. Sci.* 50(4), (2014).
- 77 Sawers L, Ferguson MJ, Ihrig BR *et al.* Glutathione S-transferase P1 (GSTP1) directly influences platinum drug chemosensitivity in ovarian tumour cell lines. *Br. J. Cancer* 111(6), 1150–1158 (2014).
- 78 Ettinger LJ, Gaynon PS, Krailo MD *et al.* A Phase II study of carboplatin in children with recurrent or progressive solid tumors. A report from the Children's Cancer Group. *Cancer* 73(4), 1297–1301 (1994).
- 79 Ferguson WS, Harris MB, Goorin AM *et al.* Presurgical window of carboplatin and surgery and multidrug chemotherapy for the treatment of newly diagnosed metastatic or unresectable osteosarcoma: Pediatric Oncology Group Trial. *J. Pediatr. Hematol. Oncol.* 23(6), 340–348 (2001).
- 80 Fagioli F, Aglietta M, Tienghi A *et al.* High-dose chemotherapy in the treatment of relapsed osteosarcoma: an Italian Sarcoma Group study. *J. Clin. Oncol.* 20(8), 2150–2156 (2002).
- 81 Van Winkle P, Angiolillo A, Krailo M *et al.* 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* 44(4), 338–347 (2005).
- 82 Daw NC, Billups CA, Rodriguez-Galindo C *et al.* Metastatic osteosarcoma. *Cancer* 106(2), 403–412 (2006).
- 83 Boye K, Del Prever AB, Eriksson M *et al.* High-dose chemotherapy with stem cell rescue in the primary treatment of metastatic and pelvic osteosarcoma: final results of the ISG/SSG II study. *Pediatr. Blood Cancer* 61(5), 840–845 (2014).
- 84 Pan P, Li Y, Yu H, Sun H, Hou T. Molecular principle of topotecan resistance by topoisomerase I mutations through molecular modeling approaches. *J. Chem. Inf. Model* 53(4), 997–1006 (2013).
- 85 Rasheed ZA, Rubin EH. Mechanisms of resistance to topoisomerase I-targeting drugs. *Oncogene* 22(47), 7296–7304 (2003).
- 86 Jia P, Wu S, Li F *et al.* Breast cancer resistance protein-mediated topotecan resistance in ovarian cancer cells. *Int. J. Gynecol. Cancer* 15(6), 1042–1048 (2005).
- 87 Leggas M, Adachi M, Scheffer GL *et al.* MRP4 confers resistance to topotecan and protects the brain from chemotherapy. *Mol. Cell Biol.* 24(17), 7612–7621 (2004).
- 88 Blaney SM, Needle MN, Gillespie A *et al.* Phase II trial of topotecan administered as 72-hour continuous infusion in children with refractory solid tumors: a collaborative Pediatric Branch, National Cancer Institute, and Children's Cancer Group Study. *Clin. Cancer Res.* 4(2), 357–360 (1998).
- 89 Hawkins DS, Bradfield S, Whitlock JA *et al.* Topotecan by 21 day continuous infusion in children with relapsed or refractory solid tumors: a Children's Oncology Group study. *Pediatr. Blood Cancer* 47(6), 790–794 (2006).
- 90 Kushner BH, Kramer K, Meyers PA, Wollner N, Cheung NK. Pilot study of topotecan and high-dose cyclophosphamide for resistant pediatric solid tumors. *Med. Pediatr. Oncol.* 35(5), 468–474 (2000).
- 91 Saylor RL 3rd, Stewart CF, Zamboni WC *et al.* Phase I study of topotecan in combination with cyclophosphamide in pediatric patients with malignant solid tumors: a Pediatric Oncology Group Study. *J. Clin. Oncol.* 16(3), 945–952 (1998).
- 92 Saylor RL 3rd, Stine KC, Sullivan J *et al.* Cyclophosphamide plus topotecan in children with recurrent or refractory solid tumors: a Pediatric Oncology Group Phase II study. *J. Clin. Oncol.* 19(15), 3463–3469 (2001).
- 93 Seibel NL, Krailo M, Chen Z *et al.* Upfront window trial of topotecan in previously untreated children and adolescents with poor prognosis metastatic osteosarcoma: children's Cancer Group (CCG) 7943. *Cancer* 109(8), 1646–1653 (2007).
- 94 Blaney S, Berg SL, Pratt C *et al.* A Phase I study of irinotecan in pediatric patients: a pediatric oncology group study. *Clin. Cancer Res.* 7(1), 32–37 (2001).
- 95 Furman WL, Stewart CF, Poquette CA *et al.* Direct translation of a protracted irinotecan schedule from a xenograft model to a Phase I trial in children. *J. Clin. Oncol.* 17(6), 1815–1824 (1999).
- 96 Zak D, Styler MJ, Rosenbluth JZ, Brodsky I. Combination of gemcitabine and irinotecan for recurrent metastatic osteogenic sarcoma. *Clin. Adv. Hematol. Oncol.* 3(4), 297–299; discussion 300–302 (2005).
- 97 Brennan RC, Furman W, Mao S *et al.* Phase I dose escalation and pharmacokinetic study of oral gefitinib and irinotecan in children with refractory solid tumors. *Cancer Chemother. Pharmacol.* 74(6), 1191–1198 (2014).
- 98 Bagatell R, Norris R, Ingle AM *et al.* Phase I trial of temsirolimus in combination with irinotecan and

- temozolomide in children, adolescents and young adults with relapsed or refractory solid tumors: a Children's Oncology Group Study. *Pediatr. Blood Cancer* 61(5), 833–839 (2014).
- 99 Frederiks CN, Lam SW, Guchelaar HJ, Boven E. Genetic polymorphisms and paclitaxel- or docetaxel-induced toxicities: a systematic review. *Cancer Treat Rev.* 41(10), 935–950 (2015).
- **Comprehensive review on polymorphisms with possible relevance for taxanes-based treatments.**
- 100 Innocenti F, Kroetz DL, Schuetz E *et al.* Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. *J. Clin. Oncol.* 27(16), 2604–2614 (2009).
- 101 Low SK, Kiyotani K, Mushiroda T, Daigo Y, Nakamura Y, Zembutsu H. Association study of genetic polymorphism in *ABCC4* with cyclophosphamide-induced adverse drug reactions in breast cancer patients. *J. Hum. Genet.* 54(10), 564–571 (2009).
- 102 Etienne-Grimaldi MC, Boyer JC, Thomas F *et al.* *UGT1A1* genotype and irinotecan therapy: general review and implementation in routine practice. *Fundam. Clin. Pharmacol.* 29(3), 219–237 (2015).
- **Interesting overview about *UGT1A1* polymorphisms in irinotecan therapy in view of facilitating their translation into clinical practice.**
- 103 Angelini S, Kumar R, Carbone F *et al.* Inherited susceptibility to bleomycin-induced micronuclei: correlating polymorphisms in *GSTT1*, *GSTM1* and DNA repair genes with mutagen sensitivity. *Mutat. Res.* 638(1–2), 90–97 (2008).
- 104 Hill CR, Cole M, Errington J, Malik G, Boddy AV, Veal GJ. Characterisation of the clinical pharmacokinetics of actinomycin D and the influence of ABCB1 pharmacogenetic variation on actinomycin D disposition in children with cancer. *Clin. Pharmacokinet.* 53(8), 741–751 (2014).
- 105 Serra M, Hattinger CM. The pharmacogenomics of osteosarcoma. *Pharmacogenomics J.* doi:10.1038/tpj.2016.45. (Epub ahead of print) (2016).
- 106 Hattinger CM, Biondi P, Iacoboni E *et al.* Candidate germline polymorphisms of genes belonging to the pathways of four drugs used in osteosarcoma standard chemotherapy associated with risk, survival and toxicity in non-metastatic high-grade osteosarcoma. *Oncotarget* (2016).
- 107 Bruhn O, Cascorbi I. Polymorphisms of the drug transporters ABCB1, ABCG2, ABCC2 and ABCC3 and their impact on drug bioavailability and clinical relevance. *Expert Opin. Drug Metab. Toxicol.* 10(10), 1337–1354 (2014).
- **Comprehensive review on clinical relevance of ABC-transporters polymorphisms in human tumors.**
- 108 Hagleitner MM, Coenen MJ, Gelderblom H *et al.* A first step toward personalized medicine in osteosarcoma: pharmacogenetics as predictive marker of outcome after chemotherapy-based treatment. *Clin. Cancer Res.* 21(15), 3436–3441 (2015).
- 109 Ferrari S, Briccoli A, Mercuri M *et al.* Postrelapse survival in osteosarcoma of the extremities: prognostic factors for long-term survival. *J. Clin. Oncol.* 21(4), 710–715 (2003).
- 110 Gelderblom H, Jinks RC, Sydes M *et al.* Survival after recurrent osteosarcoma: data from 3 European Osteosarcoma Intergroup (EOI) randomized controlled trials. *Eur. J. Cancer* 47(6), 895–902 (2011).
- 111 Kempf-Bielack B, Bielack SS, Jurgens H *et al.* Osteosarcoma relapse after combined modality therapy: an analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS). *J. Clin. Oncol.* 23(3), 559–568 (2005).
- 112 Stingl JC, Bartels H, Viviani R, Lehmann ML, Brockmoller J. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: a quantitative systematic review. *Pharmacol. Ther.* 141(1), 92–116 (2014).
- 113 Li F, Zhan M. Data-driven biomarker and drug discovery using network-based approach. *J. Genet. Genome Res.* 2(020), (2015).
- **A very interesting approach for drug discovery based on network analyses.**
- 114 Kovac M, Blattmann C, Ribi S *et al.* Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat. Commun.* 6, 8940 (2015).
- **Updated report about exome sequencing results obtained in HGOS.**
- 115 Ballinger ML, Goode DL, Ray-Coquard I *et al.* Monogenic and polygenic determinants of sarcoma risk: an international genetic study. *Lancet Oncol.* 17(9), 1261–1271 (2016).
- 116 Houghton PJ, Kurmasheva RT, Kolb EA *et al.* Initial testing (stage 1) of the tubulin binding agent nanoparticle albumin-bound (nab) paclitaxel (Abraxane®) by the Pediatric Preclinical Testing Program (PPTP). *Pediatr. Blood Cancer* 62(7), 1214–1221 (2015).