# Biology of Osteosarcomas

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Unlike other sarcomas, high-grade osteosarcoma (HGOS) is characterized by complex, unbalanced karyotypes and alterations in multiple genes and pathways. Due to HGOS high genetic instability, recurrent chromothripsis (a massive genomic rearrangement due to a cataclysmic event in which chromosomes are fragmented and subsequently aberrantly assembled), kataegis (high number of genetic changes due to localized hypermutation areas), and chromoplexy (a process generating chimeric chromosomes) are rather common events and lead to multiple malignant cell populations within the same tumor [1, 2].

The pathways governed by the TP53 and retinoblastoma 1 (RB1) tumor suppressor genes are those that have most consistently been found to be involved in HGOS pathogenesis. In fact, the majority (around 80%) of HGOS patients have alterations of one or both pathways [3]. The TP53 gene product plays a major role in the cell response to DNA damage and RB1 regulates cell cycle progression. Therefore, alterations of pathways governed by these two genes may allow cells to proliferate and become malignant after the acquisition of additional genetic aberrations. This is the reason why children affected by the Li–Fraumeni syndrome (carrying germline deletion/mutations of TP53) or familial retinoblastoma (carrying germline mutations of RB1) have a dramatically higher risk to develop HGOS [3].

Biologic and genetic studies of HGOS have clearly shown that during development and progression, tumor cells acquire several genetic changes, which may account for not only the aggressive behavior of this neoplasm but can also be responsible for the development of resistance to chemotherapeutic drugs [4, 5]. Taking these features into consideration, research on new drugs for novel treatment modalities of HGOS has been devoted to identify and validate agents against new candidate therapeutic targets, which have proved or appeared to be relevant for HGOS pathogenesis, treatment response, or clinical outcome. The current research goal of drug development for HGOS consists in the identification and validation of agents that can be administered as adjuvant to conventional chemotherapeutics to better control the local and metastatic disease, as well as to improve the efficacy of standard chemotherapy regimens without increasing their collateral adverse toxicity [6]. These facts offer the hope for not only an increased survival probability, but also for an improved quality of life of cured patients, which is particularly relevant for tumors mainly affecting young people like HGOS.

As a complement to these goals, the validation of predictive and prognostic markers for HGOS

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is highly needed in order to allow a patient stratification based on specific characteristics of each tumor and on a precise risk evaluation aimed to identify those subgroups of patients with the highest probability to benefit from each innovative treatment.

An information that clearly emerged from clinical studies is that the major cause of failure of the current treatment protocols for HGOS is the natural or acquired drug resistance, which occurs in 35–45% of patients. Therefore, the identification and validation of drug resistance-related markers as prognostic factors and potential new therapeutic targets are highly warranted.

Several studies have indicated that the ATP-binding cassette (ABC) transporter ABCB1 (also named as MDR1 or P-glycoprotein) plays an important role in drug resistance and treatment response of HGOS patients [7–11]. Therefore, targeting this molecule appears to be an interesting therapeutic option to improve treatment results in HGOS patients who are unresponsive to conventional regimens.

In the past 30–35 years, several ABC transporter modulators or inhibitors have been described and entered clinical Phase I-II-III trials for different human tumors. The clinical use of such modulators has however, been limited by the severe collateral toxicity that has been encountered at the concentrations required to enable these drugs to significantly inhibit the ABC transporters activity [6]. More recently, a new generation of ABC transporter inhibitors has been developed, few of which showed promising preclinical activity at significantly lower dosages also in HGOS [6, 12]. If this evidence will be further confirmed, in the next years we should have enough information about the possibility to include ABC transporter inhibitors in association with conventional chemotherapeutics in the treatment of HGOS patients unresponsive or with reduced sensitivity to conventional drugs.

The provided evidence about the clinical relevance of ABCB1 expression level in HGOS has been however, taken into account to stratify patients and modulate treatment in the Phase II-III Italian Sarcoma Group (ISG) trial ISG/OS-2 (https://ClinicalTrials.gov/show/NCT01459484). In this protocol, HGOS patients are stratified on the basis of ABCB1 expression level at diagnosis and, subsequently, of the extent of tumor necrosis after preoperative chemotherapy. Patients overexpressing ABCB1 receive a more intensified treatment regimen, which also includes mifamurtide. It is however, worthwhile noting that mifamurtide is not an inhibitor of ABCB1 but a nonspecific immunomodulator, which has successfully been used in clinical trials for metastatic and nonmetastatic HGOS patients [13–15]. In the next 2–3 years, on the basis of the results obtained by this protocol, it will be possible to estimate the actual effectiveness of this treatment strategy.

One important challenge that has recently emerged as a possibility to improve the clinical results of conventional treatments in several human cancers is to consider not only the tumor features that are directly associated with treatment unresponsiveness, but also those related to development of adverse treatment-related toxicities. This approach is aimed to potentiate the efficacy of conventional chemotherapeutic drugs without increasing their adverse collateral toxicities.

In the past decade, pharmacogenomic studies applied to HGOS have started to provide information on the understanding of how genes can affect individual drug response and susceptibility to toxic events [16, 17]. This body of evidence may be of great help to select the drugs and treatment dosages which adapt best for each patient guiding the modulation and individualization of specific therapeutic approaches. As a future perspective, it could be also predicted that the application of high-throughput genetic analyses, such as next-generation sequencing, may extend pharmacogenomics to the entire genome instead of single genes or pathways, leading to the rapid identification of new markers to be considered for improving the standard HGOS clinical treatment protocols [18].

On the basis of the information which has been reported so far, it can be predicted that, in the next 5–10 years, there is a concrete possibility to identify agents with efficacy and safety profiles superior or complementary to those of conventional drugs, which may be considered for

innovative treatment strategies for groups of HGOS patients selected by using novel validated biomarkers.

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