

Commentary

Commentary on: SMARCB1 as a novel diagnostic and prognostic biomarker for osteosarcoma

 **Consolato M. Sergi**^{1,2,3}

¹AP Division/Pathology Laboratories, Children's Hospital of Eastern Ontario, University of Ottawa, 401 Smyth Rd, Ottawa, Ontario K1H 8L1, Canada; ²Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada; ³Department of Orthopedics, Tianyou Hospital, Wuhan University of Science and Technology, Wuhan, Hubei, China

Correspondence: Consolato M. Sergi (csergi@cheo.on.ca)



In the last couple of decades, biomarkers have been on the rise for diagnostic and predictive value. There has been a rush to identify new markers using new technologies and drug repurposing approaches. SMARCB1 acronym arises from the SWI/SNF (SWItch/Sucrose Non-Fermentable)-related **M**atrix-associated **A**ctin-dependent **R**egulator of **C**hromatin subfamily **B** member **1** (*SMARCB1*). It is a molecule, whose role is associated with the sucrose metabolism. *SMARCB1* is also called *INI1* (Integrase Interactor 1). The molecule was discovered in the mid-1990s. Its role as a loss-of-function marker for malignant rhabdoid tumors (MRT) of renal and extrarenal origin has enormously expanded the spectrum of involved neoplasms since that time. Several tumors have been characterized by genetic aberrations in the *SMARCB1* gene. They include reduction in expression, loss of expression, and mosaic expression. Most of the tumors are sarcomas, but a variegated group of tumors with mixed phenotypes has also been delineated. It is well known that the outcome of patients harboring genetic aberrations in the *SMARCB1* gene has been poor. Guo et al. reported that reduced *SMARCB1* expression occurred in 70% of osteosarcomas. Their data significantly correlated with poor neoadjuvant response. These authors emphasize a shorter progression-free and overall survival of the patients demonstrating an altered expression of this gene. Interestingly, mRNA *in silico* analysis established that *SMARCB1* expression correlates with the response to chemotherapy of osteosarcoma patients, but there was no reliable correlation between *SMARCB1* expression level and metastasis, response to neoadjuvant therapy, overall survival, and progression-free survival. The study involved a tissue microarray (TMA) on bone tumors that may limit the full evaluation of the gene expression. Nevertheless, Guo et al.'s study is remarkable. It expands the list of the tumors harboring an altered *SMARCB1* gene expression and suggests that this marker should be investigated in every pathology workup for potential predictive value. On the other side, much work needs to be done if we hope that we strive to provide additional therapeutic strategies for osteosarcoma patients with altered *SMARCB1* gene expression.

SWI/SNF (SWItch/Sucrose Non-Fermentable)-related Matrix-linked Actin-dependent Regulator of Chromatin subfamily B member 1 (*SMARCB1*) expression has been a marker, increasing its spread in oncology and pathology as a diagnostic and predictive outcome marker in the last three decades. The gene is located on chromosome 22q11.23 and contains nine exons spanning approximately 50 kb. The *SMARCB1* gene is also called *INI1* (Integrase Interactor 1). It translates for a subunit of the SWI/SNF chromatin-remodeling complex, which is ATP-dependent [1–7].

In 2001, Turelli et al. demonstrated that incoming retroviral preintegration multiplexes intriguingly trigger the exportin-interceded cytoplasmic transfer of the SWI/SNF component INI1 and the nuclear body constituent PML (promyelocytic leukemia), a tumor-suppressor protein [8]. PML is essential for developing a macromolecular nuclear structure called the PML-associated nuclear body (PML-NB), which

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are often also called Kremer bodies, nuclear domains-10, and, of course, PML oncogenic domains. A few months later, Wu et al. identified that GADD34 and SNF5 can share a trimeric complex with chimeric fusion proteins [9]. It leads to inhibition of GADD34-arbitrated programmed cell death. The same authors suggested that GADD34 intercedes growth suppression through its interface with SNF5. A few years later, Vries et al. detected that loss of SNF5 function in malignant rhabdoid tumor (MRT)-originated cells headed to the instability of chromosomes and polyploidy [10]. Interestingly, the re-expression of SNF5 reinstated the coupling between cell cycle progression and ploidy checkpoints. In contrast, neoplasm-associated SNF5 mutations aggravated poly- and aneuploidy by abolishing chromosome segregation. Subsequently, loss of SNF5 function causes increased levels of MAD2 due to unregulated E2F1 activity, as identified by Vries et al. [10]. This results in causing a defective spindle checkpoint. They determined that SNF5 exerts ploidy control over a pathway that comprises cyclin D, CDK4, E2F, p16 (INK4a; CDKN2A), and RB1. Jagani et al. established that SNF5 is a negative controller of GLI-hedgehog signaling [11]. A few years ago, Wang et al. established that SMARCB1 increased the number of SWI-SNF complexes by experiments leading to the re-expression of SMARCB1 in brain and kidney rhabdoid cell lines and in *Smarchb1*-null mouse embryonic fibroblasts [12]. They also found increased protein levels of SWI/SNF subunits, predominantly ARID1A and ARID1B [13]. Chromatin immunoprecipitation analysis was key in determining that re-expression of SMARCB1 also augmented chromatin habitation by SMARCA4 and SMARCC1 [14–16]. SWI/SNF complexes chiefly targeted enhancers located distally to transcriptional start sites, no matter SMARCB1 was present or not. It also leads to the conclusion that SMARCB1 let stabilize SWI/SNF complexes at enhancers. Moreover, SMARCB1 has also been demonstrated to play a role in the down-regulation of the Wnt signaling pathway gene expression. It represses retinoblastoma (RB) target genes, including E2F factors and CCND1, promoting *c-Avian Myelocytomatosis Viral Oncogene Homolog Hemoglobin (MYC)* oncogene-mediated transactivation. In addition, it induces aberrant activation of hedgehog signaling by networking GLI1 and concentrating at GLI1-regulated promoters [11]. It also epigenetically influences the action of the polycomb complex.

Loss of *SMARCB1/INI1* expression is characteristically found in atypical teratoid/rhabdoid tumor of the central nervous system [17], renal rhabdoid tumors (pediatric and adult), and renal medullary carcinoma [18]. The list grew and it now includes epithelioid malignant peripheral nerve sheath tumor or malignant schwannoma [19], sino-nasal carcinoma [20,21], epithelioid sarcoma [22], extra-skeletal myxoid chondrosarcomas [23], pediatric poorly differentiated chordoma [24], and myoepithelial carcinoma [5,25–28]. Thus, both mesenchymal and epithelial malignant neoplasms are characterized by a genetic aberration of this key component of the chromatin arrangement.

Recently, Guo et al. investigated *SMARCB1* gene expression in osteosarcoma and its clinical significance with regard to chemotherapeutic sensitivity and outcome of their patients [29]. They examined 114 specimens from 70 osteosarcoma patients to build a tissue microarray (TMA) and assess the staining of SMARCB1 protein using a straightforward Avidin–Biotin Complex-based immunohistochemically run assay or immunofluorescence on paraffin blocks. The mRNA expression of this gene was investigated *in silico* using open-access RNA sequencing. They found a weak *SMARCB1* expression in about two-thirds of the osteosarcoma specimens in the TMA. Their data significantly correlated with poor neoadjuvant response and shorter progression-free and overall survival. The study of the mRNA by *in silico* analysis confirmed that *SMARCB1* expression correlates somehow with the response to chemotherapy and prognosis in osteosarcoma patients. However, it seems that there was no reliable correlation between SMARCB1 staining and metastasis, response to neoadjuvant therapy, overall survival, and progression-free survival. The reliability would be improved by detailed analysis of *SMARCB1* expression in different types of osteosarcomas.

The primary concern of the present study is the use of TMA [30]. Not to be misunderstood, TMA is terrific and beneficial to research, but limitations may apply and should be taken into account [31]. In Figure 1, a composite of three microphotographs of an osteosarcoma with variable *SMARCB1/INI1* expression is presented. To construct the TMA, the authors studied 114 formalin-fixed, paraffin-embedded (FFPE) osteosarcoma tissue blocks obtained from 70 enrolled patients. TMA has been a cost-effective choice for most pathology institutions and research groups. Still, the limited distribution of staining to small cores instead of the whole section has raised doubts on the validity of studies involving microarrays. Over the years, it has been concluded that an important and satisfactory number of cores needs to be present in the TMA block to be relevant. There is probably no safe rule because it also depends on the epitopes and on the retrieval techniques adopted. One major consideration is that the fixation of some of the bony tissue specimens relies on multiple hours of immersion with formaldehyde and subsequent decalcification, which may hamper the reproducibility of some data. Nevertheless, the study raises important aspects, and the increased number of cores used by Guo et al. in their study may consolidate the conviction that further studies are necessary to evaluate properly *SMARCB1* expression with the response to chemotherapy and prognosis in osteosarcoma patients. Children's Oncology Group biorepositories and International Society of Pediatric Oncology (SIOP)'s databanks may

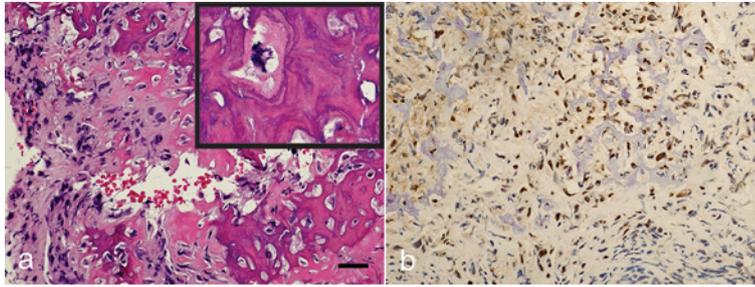


Figure 1. Osteogenic Sarcoma (Osteosarcoma) and SMARCB1 Expression

(a) Microphotograph of an osteosarcoma showing osteoid production and anaplastic tumor cells. Bar is 20 μm . Anaplasia, which derives from the Greek words $\acute{\alpha}\nu\alpha$ (backward) and $\pi\lambda\alpha\sigma\iota\varsigma$ (formation), is a biological condition of cells exhibiting poor cellular differentiation, loss of the morphological features of maturity, and poor orientation with respect to each other. Anaplastic cells demonstrate nuclear pleomorphism, abnormal nucleus-cytoplasm ratio, prominence of nucleoli, and increased proliferation index. In the microphotograph inset, a multipolar mitosis of a neoplastic cell (hematoxylin and eosin staining, 200x original magnification) is seen. (b) Microphotograph of an osteosarcoma showing variable nuclear staining for SMARCB1/INI1 (anti-SMARCB1/INI1 immunohistochemistry on formalin-fixed and paraffin-embedded tissue, Avidin-Biotin Complex, 200x original magnification).

be key for the research to further concretize the role of *SMARCB1/INI1* in future protocols of the College of American Pathologists [21,32].

The cumulative data on the activity of SMARCB1 in epigenetic regulation have provided an ideal platform to trial biologically targeted therapies. Currently, some agents include: (1) EZH2 inhibitors, which prevent *SMARCB1/INI1*-deficient neoplasms progression and have been proved to sensitize neoplastic cells to the effects of radiation therapy; (2) CDK4 inhibitors, which inhibit tumor cell growth by G1 arrest; (3) aurora-A-kinase inhibitors, inhibits aurora A; (4) histone deacetylase inhibitors (HDACi), with a similar mechanism of action to the EZH2 inhibitors; and (5) DNA methyltransferase inhibitors [33,34].

Overall, although the study involved a TMA, which may limit the full evaluation of the gene expression, Guo et al.'s study expand the list of the tumors harboring an altered *SMARCB1* gene expression and suggests that this marker should be investigated in every pathology workup for potential predictive value [29]. On the other side, much work needs to be done if we hope to provide additional therapeutic strategies for patients affected with osteosarcoma with or without altered *SMARCB1* gene expression [35–42].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

FFPE, formalin-fixed, paraffin-embedded; INI1, Integrase Interactor 1; MRT, malignant rhabdoid tumors; NB, nuclear body; PML, pro-myelocytic leukemia; RB, retinoblastoma; SMARCB1, SWI/SNF-related Matrix-associated Actin-dependent Regulator of Chromatin subfamily B member 1; SWI/SNF, SWI/SNF/Sucrose Non-Fermentable; TMA, tissue microarray.

References

- 1 Parker, N.A., Al-Obaidi, A. and Deutsch, J.M. (2020) SMARCB1/INI1-deficient tumors of adulthood. *F1000 Res.* **9**, 662, <https://doi.org/10.12688/f1000research.24808.2>
- 2 Treece, A.L. (2020) Pediatric renal tumors: updates in the molecular era. *Surg. Pathol. Clin.* **13**, 695–718, <https://doi.org/10.1016/j.path.2020.08.003>
- 3 Pawel, B.R. (2018) SMARCB1-deficient tumors of childhood: a practical guide. *Pediatr. Dev. Pathol.* **21**, 6–28, <https://doi.org/10.1177/1093526617749671>
- 4 Dong, C., Zhang, R., Xu, L., Liu, B. and Chu, X. (2022) Assembly and interaction of core subunits of BAF complexes and crystal study of the SMARCC1/SMARCE1 binary complex. *Biochem. Biophys. Res. Commun.* **599**, 9–16, <https://doi.org/10.1016/j.bbrc.2022.02.007>
- 5 Schaefer, I.M. and Hornick, J.L. (2021) SWI/SNF complex-deficient soft tissue neoplasms: an update. *Semin. Diagn. Pathol.* **38**, 222–231, <https://doi.org/10.1053/j.semmp.2020.05.005>
- 6 Choi, S.K., Kim, M.J. and You, J.S. (2020) SMARCB1 acts as a quiescent gatekeeper for cell cycle and immune response in human cells. *Int. J. Mol. Sci.* **21**, 3969, <https://doi.org/10.3390/ijms21113969>
- 7 He, S., Wu, Z., Tian, Y., Yu, Z., Yu, J., Wang, X. et al. (2020) Structure of nucleosome-bound human BAF complex. *Science* **367**, 875–881, <https://doi.org/10.1126/science.aaz9761>
- 8 Turelli, P., Doucas, V., Craig, E., Mangeat, B., Klages, N., Evans, R. et al. (2001) Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. *Mol. Cell* **7**, 1245–1254, [https://doi.org/10.1016/S1097-2765\(01\)00255-6](https://doi.org/10.1016/S1097-2765(01)00255-6)
- 9 Wu, D.Y., Tkachuck, D.C., Roberson, R.S. and Schubach, W.H. (2002) The human SNF5/INI1 protein facilitates the function of the growth arrest and DNA damage-inducible protein (GADD34) and modulates GADD34-bound protein phosphatase-1 activity. *J. Biol. Chem.* **277**, 27706–27715, <https://doi.org/10.1074/jbc.M200955200>
- 10 Vries, R.G., Bezrukove, V., Zijderduijn, L.M., Kia, S.K., Houweling, A., Oruetxebarria, I. et al. (2005) Cancer-associated mutations in chromatin remodeler hSNF5 promote chromosomal instability by compromising the mitotic checkpoint. *Genes Dev.* **19**, 665–670, <https://doi.org/10.1101/gad.335805>
- 11 Jagani, Z., Mora-Blanco, E.L., Sansam, C.G., McKenna, E.S., Wilson, B., Chen, D. et al. (2010) Loss of the tumor suppressor Snf5 leads to aberrant activation of the Hedgehog-Gli pathway. *Nat. Med.* **16**, 1429–1433, <https://doi.org/10.1038/nm.2251>
- 12 Wang, X., Lee, R.S., Alver, B.H., Haswell, J.R., Wang, S., Mieczkowski, J. et al. (2017) SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. *Nat. Genet.* **49**, 289–295, <https://doi.org/10.1038/ng.3746>
- 13 Kang, E.Y., Tessier-Cloutier, B., Duggan, M.A., Stewart, C.J.R., Lee, C.H. and Kobel, M. (2021) Loss of ARID1B and SMARCB1 expression are specific for the diagnosis of dedifferentiated/undifferentiated carcinoma in tumours of the upper gynaecological tract and cervix. *Histopathology* **79**, 160–167, <https://doi.org/10.1111/his.14333>
- 14 Nemes, K., Johann, P.D., Tuchert, S., Melchior, P., Vokuhl, C., Siebert, R. et al. (2022) Current and emerging therapeutic approaches for extracranial malignant rhabdoid tumors. *Cancer Manag. Res.* **14**, 479–498, <https://doi.org/10.2147/CMAR.S289544>
- 15 Ravindra, N., Rao, S., Arivazhagan, A. and Santosh, V. (2021) Maternal embryonal leucine zipper kinase immunoreactivity in atypical teratoid/rhabdoid tumors: a study of 50 cases. *Childs Nerv. Syst.* **37**, 3769–3775, <https://doi.org/10.1007/s00381-021-05335-0>
- 16 Duan, H., Gao, W., Wang, L., Cao, F. and Teng, L. (2021) Undifferentiated colonic neoplasm with SMARCA4 germline gene mutation and loss of SMARCA4 protein expression: a case report and literature review. *Diagn. Pathol.* **16**, 30, <https://doi.org/10.1186/s13000-021-01091-6>
- 17 Hoot, A.C., Russo, P., Judkins, A.R., Perlman, E.J. and Biegel, J.A. (2004) Immunohistochemical analysis of hSNF5/INI1 distinguishes renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. *Am. J. Surg. Pathol.* **28**, 1485–1491, <https://doi.org/10.1097/01.pas.0000141390.14548.34>
- 18 Cheng, J.X., Tretiakova, M., Gong, C., Mandal, S., Krausz, T. and Taxy, J.B. (2008) Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior. *Mod. Pathol.* **21**, 647–652, <https://doi.org/10.1038/modpathol.2008.44>
- 19 Schaefer, I.M., Dong, F., Garcia, E.P., Fletcher, C.D.M. and Jo, V.Y. (2019) Recurrent SMARCB1 inactivation in epithelioid malignant peripheral nerve sheath tumors. *Am. J. Surg. Pathol.* **43**, 835–843, <https://doi.org/10.1097/PAS.0000000000001242>
- 20 Ayyanar, P., Mishra, P., Preetam, C. and Adhya, A.K. (2021) SMARCB1/INI1 deficient sino-nasal carcinoma: extending the histomorphological features. *Head Neck Pathol.* **15**, 555–565, <https://doi.org/10.1007/s12105-020-01246-9>
- 21 Agaimy, A. (2022) Proceedings of the North American Society of Head and Neck Pathology, Los Angeles, CA, March 20, 2022, SWI/SNF-deficient sinonasal neoplasms: an overview. *Head Neck Pathol.* **16**, 168–178, <https://doi.org/10.1007/s12105-022-01416-x>
- 22 Needs, T. and Fillman, E.P. (2022) *Epithelioid Sarcoma*, StatPearls, Treasure Island (FL, USA)
- 23 Kohashi, K., Oda, Y., Yamamoto, H., Tamiya, S., Oshiro, Y., Izumi, T. et al. (2008) SMARCB1/INI1 protein expression in round cell soft tissue sarcomas associated with chromosomal translocations involving EWS: a special reference to SMARCB1/INI1 negative variant extraskeletal myxoid chondrosarcoma. *Am. J. Surg. Pathol.* **32**, 1168–1174, <https://doi.org/10.1097/PAS.0b013e318161781a>
- 24 Zhao, J., Feng, J., Chen, L., Ma, Y., Ding, D. and Wang, H. (2021) Poorly differentiated SMARCB1/INI1-negative chordomas. *Clin. Neuropathol.* **40**, 36–45, <https://doi.org/10.5414/NP301277>
- 25 Wang, L., Yee-Chang, M., Sun, W., Melamed, J., Sims, A. and Shi, Y. (2022) Myoepithelial carcinoma of soft tissue is a diagnostic challenge on fine-needle aspiration: case report and review of literature. *Diagn. Cytopathol.*, <https://doi.org/10.1002/dc.24950>
- 26 Khazeni, K., LaBove, H., Wilky, B., Rosenberg, A.E., Paulus, E., Chim, H. et al. (2018) Myoepithelial carcinoma or epithelioid sarcoma - a rare diagnosis with poor prognosis. A case report and review of literature. *Int. J. Surg. Case Rep.* **49**, 239–243, <https://doi.org/10.1016/j.ijscr.2018.06.016>
- 27 Le Loarer, F., Zhang, L., Fletcher, C.D., Ribeiro, A., Singer, S., Italiano, A. et al. (2014) Consistent SMARCB1 homozygous deletions in epithelioid sarcoma and in a subset of myoepithelial carcinomas can be reliably detected by FISH in archival material. *Genes Chromosomes Cancer* **53**, 475–486, <https://doi.org/10.1002/gcc.22159>

- 28 Hollmann, T.J. and Hornick, J.L. (2011) INI1-deficient tumors: diagnostic features and molecular genetics. *Am. J. Surg. Pathol.* **35**, e47–e63, <https://doi.org/10.1097/PAS.0b013e31822b325b>
- 29 Guo, T., Wei, R., Dean, D.C., Hornicek, F. and Duan, Z. (2022) SMARCB1 expression is a novel diagnostic and prognostic biomarker for osteosarcoma. *Biosci. Rep.* **42**, <https://doi.org/10.1042/BSR20212446>
- 30 Khouja, M.H., Baekelandt, M., Sarab, A., Nesland, J.M. and Holm, R. (2010) Limitations of tissue microarrays compared with whole tissue sections in survival analysis. *Oncol. Lett.* **1**, 827–831, <https://doi.org/10.3892/ol.00000145>
- 31 Jantuan, E., Chiu, B., Chiu, B., Shen, F., Oudit, G.Y. and Sergi, C. (2021) The tumor microenvironment may trigger lymphoproliferation in cardiac myxoma. *Transl. Oncol.* **14**, 100911, <https://doi.org/10.1016/j.tranon.2020.100911>
- 32 Sergi, C.M. (2022) Biorepository - a key component of research studies. *Contemp. Clin. Trials* **112**, 106655, <https://doi.org/10.1016/j.cct.2021.106655>
- 33 Nemes, K. and Fruhwald, M.C. (2018) Emerging therapeutic targets for the treatment of malignant rhabdoid tumors. *Expert Opin. Ther. Targets* **22**, 365–379, <https://doi.org/10.1080/14728222.2018.1451839>
- 34 Fruhwald, M.C., Biegel, J.A., Bourdeaut, F., Roberts, C.W. and Chi, S.N. (2016) Atypical teratoid/rhabdoid tumors-current concepts, advances in biology, and potential future therapies. *Neuro Oncol* **18**, 764–778, <https://doi.org/10.1093/neuonc/nov264>
- 35 Sergi, C.M. (2021) Targeting the 'garbage-bin' to fight cancer: HDAC6 inhibitor WT161 has an anti-tumor effect on osteosarcoma and synergistically interacts with 5-FU. *Biosci. Rep.* **41**, <https://doi.org/10.1042/BSR20210952>
- 36 Burnett, M., Abuetabh, Y., Wronski, A., Shen, F., Persad, S., Leng, R. et al. (2020) Graphene oxide nanoparticles induce apoptosis in wild-type and CRISPR/Cas9-IGF/IGFBP3 knocked-out Osteosarcoma Cells. *J. Cancer* **11**, 5007–5023, <https://doi.org/10.7150/jca.46464>
- 37 Ali, N., Venkateswaran, G., Garcia, E., Landry, T., McColl, H., Sergi, C. et al. (2019) Osteosarcoma progression is associated with increased nuclear levels and transcriptional activity of activated beta-Catenin. *Genes Cancer* **10**, 63–79, <https://doi.org/10.18632/genesandcancer.191>
- 38 Sergi, C., Shen, F. and Liu, S.M. (2019) Insulin/IGF-1R, SIRT1, and FOXOs pathways-an intriguing interaction platform for bone and osteosarcoma. *Front. Endocrinol. (Lausanne)* **10**, 93, <https://doi.org/10.3389/fendo.2019.00093>
- 39 Osasan, S., Zhang, M., Shen, F., Paul, P.J., Persad, S. and Sergi, C. (2016) Osteogenic sarcoma: a 21st century review. *Anticancer Res.* **36**, 4391–4398, <https://doi.org/10.21873/anticancer.10982>
- 40 Ressler, S., Radhi, J., Aigner, T., Loo, C., Zwerschke, W. and Sergi, C. (2009) Insulin-like growth factor-binding protein-3 in osteosarcomas and normal bone tissues. *Anticancer Res.* **29**, 2579–2587
- 41 Sergi, C. and Zwerschke, W. (2008) Osteogenic sarcoma (osteosarcoma) in the elderly: tumor delineation and predisposing conditions. *Exp. Gerontol.* **43**, 1039–1043, <https://doi.org/10.1016/j.exger.2008.09.009>
- 42 Santer, F.R., Bacher, N., Moser, B., Morandell, D., Ressler, S., Firth, S.M. et al. (2006) Nuclear insulin-like growth factor binding protein-3 induces apoptosis and is targeted to ubiquitin/proteasome-dependent proteolysis. *Cancer Res.* **66**, 3024–3033, <https://doi.org/10.1158/0008-5472.CAN-05-2013>