Clinical features of Ewing and chondrosarcoma

Maldegem, A.M. van

Chapter 9

General discussion and future perspectives

Sarcoma

Sarcomas are tumours that arise in tissue of mesenchymal origin. Of all new cancers diagnosed each year only 1% is a sarcoma. The incidence of sarcoma is low with less than 1,000 new cases in the Netherlands each year. Sarcoma can be further subdivided into bone and soft tissue sarcoma, including gastrointestinal stromal tumours. Ewing- and chondrosarcoma are two of the 3 most common subsets of bone sarcoma (osteosarcoma being the third).

Ewing sarcoma

Ewing sarcoma is characterized by a chromosomal translocation involving the EWSR1 gene on chromosome 22 and an *ETS* transcription factor gene, with *EWSR1-FLI1* being the most common fusion protein. With a median age of 15 years, most patients diagnosed with Ewing sarcoma are adolescents. The average 5-year overall survival (OS) for localised disease is 60–65%, for metastatic disease the outcome is poor with a 5 year OS of only 20% [1-2]. New treatment options to improve the outcome for these patients are needed. An overview of clinical studies enrolling Ewing sarcoma patients published between 1990 and 2010 is given in **chapter 2**. With a total of 42 phase I/II clinical trials conducted in a time period of 20 years the results are disappointing. Of these only nine trials were designed specifically for Ewing sarcoma patients. It is however encouraging that during the study period (till 2010), the number of trials started per year gradually increased. A general PubMed search shows that from 2010 till 2017 the results of at least 12 phase I, two phase I/II and 17 phase II studies enrolling Ewing sarcoma patients have been published. There is an increase in publications from 2012 till 2014 with an average of 6 new articles each year. From 2015 the number decreases with only 3 articles published in 2017. Treatments tested were mostly non-cytotoxic drugs like mTOR inhibitors, histone deacetylase inhibitors or insulin growth factor receptor antibodies. For most studies the results are non-superior to the standard treatment regimens and the outcome in metastatic or primary chemotherapy refractory disease remains poor.

Preclinical data

A clinical trial is ideally based on treatment models which are tested in *in vitro* and *in vivo* studies. One pathway that has been extensively studied in cancers is the insulin growth factor (IGF) signalling pathway. IGF plays an important role in normal cell development, while overexpression of the pathway components have been implicated in malignancy [3-5].

Chapter 3 gives an overview of the *in vitro* and *in vivo* work conducted in Ewing sarcoma for understanding the role of the IGF pathway. Several drugs are available for inhibition of the insulin growth factor 1 receptor (IGF-1R) and these have been tested in Ewing sarcoma patients either as monotherapy or in combination with other types of drugs. The results of these trials were not as promising as expected and the pharmaceutical companies decided to stop further development. Subgroups of patients did have a good and long response to treatment with IGF-1R inhibition and there is an urgent need for better biomarkers to select patients. Of the conventional biomarkers, patient age, male gender, tumour size and tumour location were proven to be clinically relevant for predicting prognosis [6-8]. Many other biological biomarkers for outcome prediction were tested and interesting markers, like genes and proteins of the 9p21 locus, have to be further tested for validation [9]. For clinical practice no good biological biomarkers are available. Cancer related inflammation and smouldering inflammation in the tumour microenvironment is being recognised as an important factor in genetic instability of cancer cells [10]. It contributes to proliferation, angiogenesis and development of metastases [11]. The CRP/Albumin ratio can be used as an inflammatory indicator in patients' blood and a high ratio is significantly correlated with a poor prognosis in Ewing sarcoma, but needs validation before it can be used clinically [12]. New biomarkers for predicting outcome at time of diagnosis of patient populations with comparable clinical disease stage are being studied, but predicting individual patient response to therapy is still very difficult. Obtaining tumour cells with biopsy before and after treatment of patient and comparing responders to no responders to the treatment can be used to identify novel biomarkers. These materials could be obtained by adding tumour biopsies as a part of a phase I or II trial design for new treatment strategies. Our aim would be to find biomarkers for which patients can be tested before start of treatment to predict response to therapy and hereby compile a patient specific treatment regimen.

Another interesting treatment target also described in chapter 3 is the Poly-ADP-ribose-polymerase (PARP) pathway. PARPs are proteins involved in DNA repair; they detect single strand breaks and activate the repair process by recruitment of the enzymatic DNA repair mechanism [13]. Ewing sarcoma cell lines were found to be very sensitive to PARP inhibition [14], and even tumour cells from heavily pre-treated and relapsed patients were extremely sensitive *in vitro*. Only one phase II clinical trial enrolling Ewing sarcoma patients has been published until now. With no complete or partial responses the results of this study were disappointing. Preclinical models suggest that better results may be found when combining PARP inhibition with chemotherapy or radiotherapy [15-17]. *In vitro* a synergistic effect was proven when combining PARP inhibition with cytotoxic drugs. Several phase I studies treating Ewing sarcoma patients with combination regimens are currently recruiting or recently finished but do not yet have published results.

Of all new developed drugs or treatment regimens with promising results in preclinical testing approximately 90% when tested in a phase II or III trials fail [18-19]. Most of the negative results are due to inadequate preclinical testing. *In vitro* screening for new drugs is done on cell lines or xenografts of immune-deficient mice and the response criteria are cell death or tumour regression. However, these techniques cannot replicate the complex alterations in tumours on cellular and tissue functions [20]. Animal models play an important role in testing pharmacokinetics of new anticancer drug. However, these animal models are not perfect with uncertainties in interpretation of the results and high costs. For testing on human cells, cell cultures are used but with limitations regarding representatively after prolonged culture. Moreover, the blood flow which delivers oxygen and nutrition cannot be mimicked. The chemical and physical stimulation from the surrounding environment is usually also absent *in vitro*. This gap between *in vitro* and *in vivo* testing needs to be solved. Organ-on-a-chip or body-on-a-chip is a new technology which may be able to fill this gap. With this technique the behaviour of different organs and their physical and chemical (micro-)environment is being reproduced in *in vitro* models by microfluidic devices.

Clinical experience

For patients diagnosed with inoperable Ewing sarcoma there are limited standard treatment regimens. When these patients fail to respond to standard treatment they are usually enrolled in a clinical trial or are treated with a drug regimen based on expert opinion. In several major European sarcoma centers oncologists treat unresectable Ewing sarcoma patients with the combination of etoposide with either cisplatin or carboplatin. The data of 107 of these patients were collected and the results are summarized in **chapter 4**. With a 5-year OS of 24.5% for the patients who received carboplatin and etoposide and 20% for those who received cisplatin and etoposide this regimen is a potential additional treatment option in this patient population. The combination of etoposide and carboplatin had a better progression free survival and fewer side effects, especially with regards to nephrotoxicity, than expected.

Epigenetic changes are increasingly thought to play an important role in the development of cancer [21]. Epigenetic changes regulate gene expression, without altering the nucleotide sequence of the DNA. It alters gene expression by histone modification and chromatin remodelling, DNA methylation, loss of imprinting, and microRNAs interference [22]. Epigenetic changes play an important role especially in Ewing sarcoma. The molecular basis of Ewing sarcoma is very homogeneous with the EWSR1-FLI1 fusion gene. But the disease behaviour is very heterogeneous between different patients. This heterogeneity might be caused by epigenetic changes. Analysing DNA methylation in a Ewing sarcoma

patient cohort showed epigenetic tumour heterogeneity between patients but also within a tumour [23]. A correlation was found between high intra-tumour heterogeneity and metastatic disease at diagnosis. The histone demethylase KDM3A has recently been found to be upregulated by the EWSR1-FLI1 fusion [24]. Activation of KDM3A *in vitro* upregulates metastatic promotor pathways and is an important factor in aggressive behaviour of Ewing sarcoma. MCAM was found to be a target of KDM3A. In non-cancer cells MCAM is involved in regulating cell adhesion, angiogenesis and extravasation [25-27]. In different cancer types expression of MCAM has been correlated with a poor prognosis and metastatic disease [28-29].

Preclinical data prove that high expression of EWSR1-FLI1 is associated with open chromatin formation [30]. Chromatin modification is regulated by histone deacetylases (HDACs) and upregulation of HDACs are associated with silencing of tumour suppressor genes and oncogenesis [22]. In Ewing sarcoma cells the expression of EWSR1-FLI1 is associated with upregulation of HDAC and HDAC is thereby an interesting therapeutic target [31]. In **chapter 5** a patient treated with the HDAC inhibitor panobinostat is reported. This patient had a relapse after primary treatment with chemotherapy and surgery. On panobinostat treatment the patient had stable disease for a total of 18 months. In other solid tumours panobinostat is given in a combination with conventional chemotherapy and these results suggest that combination treatment is better. Combination with checkpoint-inhibitors in phase I studies is currently also being explored.

Chondrosarcoma

With an average age of 50 years chondrosarcoma affects mostly adult patients. It consists of five subtypes, the most common form being the conventional (75%) and more rare subtypes include mesenchymal, dedifferentiated, clear cell and periosteal chondrosarcoma. Conventional chondrosarcoma can be subdivided in atypical cartilaginous tumours (previously called chondrosarcoma grade 1), grade 2 or 3 depending on nuclear size, matrix composition, mitotic activity and degree of cellularity. The most important predictor of outcome is grade, with grade 3 having the worst outcome with a ten year survival of approximately 38% [32]. The only cure for patients diagnosed with chondrosarcoma is surgery as most subtypes of chondrosarcoma have always been considered chemotherapy resistant. Lately new preclinical and retrospective studies show that individual patients may benefit from non-cytotoxics, chemo- and radiotherapy depending on subtype. From 2000 till 2013 31 phase I/II or retrospective studies enrolling chondrosarcoma patients have been published, an overview of these studies can be found in **chapter 6**. In the phase I

trials mostly non-cytotoxic drugs were tested and in the retrospective studies all treatments were conventional chemotherapy based. The outcome for most enrolled patients was still poor but a small subset did seem to respond to treatment. New biomarkers need to be found to predict response before start of treatment.

Clinical data

For patients with advanced, unresectable conventional chondrosarcoma the outcome is regarded to be poor but clinical studies were so far almost lacking. In **chapter 7** the outcome of advanced chondrosarcoma patients in two major European bone sarcoma centers is being investigated. A total of 171 patients were identified. The OS data show that chemotherapy in unresectable chondrosarcoma patients may increase survival, but further studies are warranted. Radiotherapy shows a survival advantage and is common practice for locally advanced conventional central chondrosarcomas in both reference centers. There are currently no standard treatment regimens for unresectable chondrosarcoma patients. Most patients are treated according to expert opinion treatment schedules and it is unclear which schedule improves outcome significantly. To answer this question, in **chapter 8** the treatment and response data from 112 patients diagnosed with unresectable chondrosarcoma in four major sarcoma treatment centers worldwide was collected. No patient had a complete response, seven patients had a partial response and they were all treated with chemotherapy based regimens. Patients with conventional or mesenchymal chondrosarcoma had a better OS than dedifferentiated chondrosarcoma patients. Patients were treated with a diverse range of treatment regimens and the numbers were too small to do statistical analysis. Many non-cytotoxic drugs were based on preclinical data with promising results: in the clinical setting these results were not yet seen. Better patient selection before start of treatment based on biomarkers needs to be conducted.

Future perspectives

A recurring theme in this thesis on systemic therapy in Ewing and chondrosarcoma is the need to select the right patient population before start of treatment. Currently used biomarkers as age, gender, grade and subtype are not capable to distinguish between possible responders and non-responders. Several molecular markers have been identified and are being tested in the (pre-)clinical setting but no distinctive marker has been found yet [33].

Ewing- and chondrosarcoma are rare forms of cancer which makes it difficult to perform good (pre-)clinical studies. Most treatment decisions for patients with recurrent or

unresectable disease are based on expert opinion. Centers of expertise need to be established where preclinical research can be performed and patients need to be referred to these centers to increase the number of patients enrolled in clinical trials. Until now many studies, including the ones in this thesis, are retrospective which can be used to generate hypotheses but they cannot fully answer the question if a treatment regimen improves outcome and what the side-effects are. The rEECur trial is an example of an international study enrolling patients with recurrent or primary refractory Ewing sarcoma. In this trial four different chemotherapy regimens are compared and the goal is to recruit 525 patients. During the trial biospecimens are collected for preclinical research. From the start in 2015 311 patients are enrolled and the first preliminary results were presented at ASCO 2019 [34]. This trial will be used as a platform to study new prospective arms and has the potential to change clinical practice in the future. A similar trial for advanced or metastatic chondrosarcoma would be interesting, but even harder to start because of the even rarer occurrence.

Several new therapeutic options with non-chemotherapy regimens are also being explored. In different cancer types mutations in *IDH1* or *-2* genes lead to tumorigenesis by altered DNA methylation and subsequently gene expression [35]. More than 50% of conventional chondrosarcomas harbour an IDH mutation [36]. These mutations are also found in solitary enchondromas as well as in enchondromas in Ollier disease and Maffucci syndrome, which are precursor lesions for chondrosarcoma, suggesting that the *IDH* gene mutation is an early event. Moreover, inhibition of mutant *IDH1* in chondrosarcoma cell lines does not affect tumorigenic properties: no effect is seen on viability, colony formation or migration [39]. This raises the question whether inhibition of mutant IDH is a therapeutic option for chondrosarcoma, or that it is non drug able as it is an early event and not important anymore after malignant progression. In other forms of cancer, especially acute myeloid leukaemia (AML), inhibition of mutant IDH seems more promising. In the last years two inhibitors, Enasidenib and Ivosidenib, are approved by the FDA for relapsed of refractory AML [38]. The mutant IDH inhibitors Ivosidenib and Olutasidenib are currently being tested in solid tumours, including chondrosarcoma. The results from these and future trails will answer the question if mutant IDH inhibition is a possible treatment regimen for chondrosarcoma.

A way to find new therapeutic options is to combine registered drugs with newly developed therapeutic options. The pharmaceutical companies could be more interested in these kind of trials if they see new treatment options for their already registered drugs or when it can lead to a registration for orphan disease treatment. An example is the COSYMO study which enrols patients with metastatic or unresectable chondrosarcoma or myxoid liposarcoma. The treatment regimen is a combination of cyclophosphamide with the mTOR

inhibitor sirolimus. This study, the retrospective studies in this thesis and the previously mentioned rEECur study were financially supported by the European Commission granted FP7 clinical trials network (278742).

In the last years immune modulating therapies are being explored as a possibility for cancer treatment. The concept of the working mechanism is that an antitumor immune response is activated, which was suppressed by the tumour adaptive mechanisms [39-40]. In several malignancies, like melanoma and lung carcinoma, impressive results were found after treatment with check-point inhibitors. The most frequently used checkpoint inhibitors are antibodies against programmed cell death receptor-1 (PD-1) and its ligand (PD-L1). In various subtypes of bone sarcomas high expression of PD-1 and PD-L1 was observed [41-42]. The level of expression is found to be correlated with recurrence in chondrosarcoma and metastatic disease in Ewing sarcoma [43-44]. A phase II trial including 13 Ewing sarcoma patients treated with the PD-1 inhibitor pembrolizumab showed no objective response and only 2 patients had stable disease [45]. Thoughtful patient selection is needed and biomarkers to make this selection have to be found. A possible biomarker for immune modulating therapies is mutational load. This is a measure of the number of mutations in a tumor genome [46]. In melanoma and non-small cell lung cancer treated with PD-1 inhibitors, patients with higher mutational loads showed better response rates [47-48]. However, in sarcoma mutational load is often low [49-50].

Altogether, finding new systemic therapies for Ewing and chondrosarcoma remains a challenging field, but international collaborations between centers of excellence, pharmaceutical industry and public grants may lead to much needed progress in the future.

References

1. Leavey P.J., Collier A.B. Ewing sarcoma: prognostic criteria, outcomes and future treatment. Expert Rev Anticancer Ther. 2008;8:617-624.
2. Rodriguez-Galindo C. Pharmacological management of Ewing sarcoma family of tumours. Expert Opin Pharmacother. 2004;5:1257-1270.
3. Chitnis M.M., Yuen J.S., Protheroe A.S., et al. The type 1 insulin-like growth factor receptor pathway. Clin Cancer Res. 2008;14:6364-6370.
4. Werner H. For debate: the pathophysiological significance of IGF-I receptor overexpression: new insights. Pediatr Endocrinol Rev. 2009;7:2-5.
5. Werner H. Tumor suppressors govern insulin-like growth factor signaling pathways: implications in metabolism and cancer. Oncogene. 2012;31:2703-2714.
6. Duchman K.R., Gao Y., Miller B.J. Prognostic factors for survival in patients with Ewing's Sarcoma using the Surveillance, Epidemiology, End Results (SEER) program database. Cancer Epidemiol. 2015;39:189-195.
7. Oberlin O.M., Deley M.C., Bui B.N., et al. Prognostic factors in localized Ewing's tumours and peripheral neuroectodermal tumours: the third study of the French Society of Paediatric Oncology (EW88 Study). Br J Cancer. 2001;85:1646-1654.

8. Bacci G., Ferrari S., Bertoni F., et al. Prognostic factors in non-metastatic Ewing's sarcoma of bone treated with adjuvant chemotherapy: Analysis of 359 patients at the Istituto Ortopedico Rizzoli. J Clin Oncol. 2000;18:4-11.
9. van Maldegem A.M., Hogendoorn P.C.W., Hassan A.B. The clinical use of biomarkers as prognostic factors in Ewing sarcoma. Clin Sarcoma Res. 2012;8:7.
10. Mantovani A., Allavena P., Sica A., et al. Cancer-related inflammation. Nature. 2008;454:436-444.
11. Diakos C.I., Charles K.A., McMillan D.C., et al. Cancer-related inflammation and treatment effectiveness. Lancet Oncol. 2014;15:493-503.
12. Li Y.J., Yang X., Zhang W.B., et al. Clinical implications of six inflammatory biomarkers as prognostic indicators in Ewing sarcoma. Cancer Manag Res. 2017;9:443-451.
13. Ame J.C., Spenlehauer C., de Murcia G. The PARP superfamily. Bioessays. 2004;26:882-893.
14. Brenner J.C., Feng F.Y., Han S., et al. PARP-1 inhibition as a targeted strategy to treat Ewing's sarcoma. Cancer Res. 2012;72:1608-1613.
15. Norris R.E., Adamson P.C., Nguyen V.T., et al. Preclinical evaluation of the PARP inhibitor, olaparib, in combination with cytotoxic chemotherapy in pediatric solid tumors. Pediatr Blood Cancer. 2014;61:145-150.
16. Lee H.J., Yoon C., Schmidt B., et al. Combining PARP-1 inhibition and radiation in ewing sarcoma results in lethal DNA damage. Mol Cancer Ther. 2013;12:2591-2600.
17. Tentori L., Muzi A., Dorio A.S., et al. Pharmacological inhibition of poly(ADP-ribose) polymerase (PARP) activity in PARP-1 silenced tumour cells increases chemosensitivity to temozolomide and to a N3-adenine selective methylating agent. Curr Cancer Drug Targets. 2010;10:368-383.
18. Arrowsmith J. Biobusiness Briefs: Trial watch: Phase II failures: 2008–2010. Nat Rev Drug Discov. 2011;10:328-329.
19. Amiri-Kordestani L., Fojo T. Why Do Phase III Clinical Trials in Oncology Fail so Often? J Natl Cancer Inst. 2012;104:568-569.
20. Lane A.N., Higashi R.M., Fan T.W. Preclinical models for interrogating drug action in human cancers using Stable Isotope Resolved Metabolomics (SIRM). Metabolomics. 2016;12.
21. Owen L.A., Kowalewski A.A., Lessnick S.L. EWS/FLI mediates transcriptional repression via NKX2.2 during oncogenic transformation in Ewing's sarcoma. PLoS One. 2008;3:1965.
22. Heerboth S., Lapinska K., Snyder N., et al. Use of epigenetic drugs in disease: An overview. Genet Epigenet. 2014;6:9-19.
23. Sheffield N.C., Pierron G., Klughammer J., et al. DNA methylation heterogeneity defines a disease spectrum in Ewing sarcoma. Nat Med. 2017;23:386-395.
24. Parrish J.K., Sechler M., Winn R.A., et al. The histone demethylase KDM3A is a microRNA-22-regulated tumor promoter in Ewing Sarcoma. Oncogene. 2015;34:257-262.
25. Shih I.M., Elder D.E., Speicher D., et al. Isolation and functional characterization of the A32 melanoma-associated antigen. Cancer Res. 1994;54:2514-2520.
26. Lin Y., Wu X., Shen Y., et al. A novel antibody AA98 V(H)/L directed against CD146 efficiently inhibits angiogenesis. Anticancer Res. 2007;27:4219-4224.
27. Bardin N., Blot-Chabaud M., Despoix N., et al. CD146 and its soluble form regulate monocyte transendothelial migration. Arterioscler Thromb Vasc Biol. 2009;29:746-753.
28. Wang Z., Yan X. CD146, a multi-functional molecule beyond adhesion. Cancer Lett. 2013;330: 150-162.
29. Zhang X., Wang Z., Kang Y., et al. MCAM expression is associated with poor prognosis in non-small cell lung cancer. Clin Transl Oncol. 2014;16:178-183.
30. Sand L.G., Szuhai K., Hogendoorn P.C. Sequencing overview of Ewing sarcoma: A journey across genomic, epigenomic and transcriptomic landscapes. Int J Mol Sci. 2015;16:16176-16215.
31. Sakimura R., Tanaka K., Nakatani F., et al. Antitumor effects of histone deacetylase inhibitor on Ewing's family tumors. Int J Cancer. 2005;116:784-792.
32. Fiorenza, F., Abudu A., Grimer R.J. et al., Risk factors for survival and local control in chondrosarcoma of bone. J Bone Joint Surg Br. 2002;84:93-99.
33. Evola F.R., Costarella L., Pavone V., et al. Biomarkers of Osteosarcoma, Chondrosarcoma, and Ewing Sarcoma. Front Pharmacol. 2017;7:150.
34. https://abstracts.asco.org/239/AbstView_239_253971.html.

35. Heerboth S., Lapinska K., Snyder N., et al. Use of epigenetic drugs in disease: an overview. Genet Epigenet. 2014;6:9-19.
36. Schaap F.G., French P.J., Bovee J.V. Mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 in tumors. Adv Anat Pathol. 2013;20:32-38.
37. Suijker J., Oosting J., Koornneef A., et al. Inhibition of mutant IDH1 decreases D-2-HG levels without affecting tumorigenic properties of chondrosarcoma cell lines. Oncotarget. 2015;6:12505-12519.
38. Golub D., Iyengar N., Dogra S., et al. Mutant Isocitrate Dehydrogenase Inhibitors as Targeted Cancer Therapeutics. Front Oncol. 2019;9:417.
39. Burugu S., Dancsok A.R., Nielsen T.O. Emerging targets in cancer immunotherapy. Semin Cancer Biol. 2018;52:39-52.
40. Dempke W.C.M., Fenchel K., Uciechowski P., et al. Second and third generation drugs for immune-oncology treatment- The more the better? Eur J Cancer. 2017;74:55-72.
41. Boxberg M., Steiger K., Lenze U., et al. PD-L1 and PD-1 and characterization of tumor-infiltrating lymphocytes in high grade sarcomas of soft tissue – prognostic implications and rationale for immunotherapy. Oncoimmunology. 2018;7.
42. Torabi A., Amaya C.N., Wians F.H., et al. PD-1 and PD-L1 expression in bone and soft tissue sarcomas. Pathology. 2017;49:506-513.
43. Yang X., Zhu G., Yang Z., et al. Expression of PD-L1/PD-L2 is associated with high proliferation index of Ki-67 but not with TP53 overexpression in chondrosarcoma. Int J Biol Markers. 2018;33:507-513.
44. Machado I., Lopez-Guerrero J.A., Scotlandi K., et al. Immunohistochemical analysis and prognostic significance of PD-L1, PD-1, and CD8+ tumor-infiltrating lymphocytes in Ewing's sarcoma family of tumors (ESFT). Virchows Arch. 2018;472:815-824.
45. Tawbi H.A., Burgess M., Bolejack V., et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017;18:1493-1501.
46. https://oncologypro.esmo.org/Education-Library/Factsheets-on-Biomarkers/Tumour-Mutational-Load
47. Johnson D.B., Frampton G.M., Rioth M.J., et al. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. Cancer Immunol Res. 2016;4:959-967.
48. Rizvi N.A., Hellmann M.D., Snyder A., et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124-128.
49. Veenstra R., Kostine M., Cleon-Jansen A.M., et al. Immune checkpoint inhibitors in sarcomas: in quest of predictive biomarkers. Lab Invest. 2018;98:41-50.
50. Cancer Genome Atlas Research Network. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171:950-965.