DOI: 10.17816/KMJ2021-313

Low-molecular-weight fibroblast growth factor-2 — a viable prognostic factor for gastric gastrointestinal stromal tumors

E.G. Mikheeva[1], A.M. Aukhadieva[1], A.G. Sabirov[2], S.V. Boichuk[1]*

[1]Kazan State Medical University, Kazan, Russia;
[2]Tatarstan Regional Clinical Cancer Center, Kazan, Russia

Abstract

Aim. To examine the expression of fibroblast growth factor-2 and its isoforms in gastrointestinal stromal tumors and assess the prognostic value of this marker.

Methods. The study included 44 patients with gastric gastrointestinal stromal tumors of the stomach who were prescribed surgical or combined treatment with the targeted drug imatinib (imatinib mesylate). Immunohistochemistry (IHC)-staining and immunoblotting with monoclonal antibodies were used to assess the expression of FGF-2. Statistical analysis for differences in clinical and morphological parameters was performed by using Student's, Mann–Whitney–Wilcoxon and Fisher's tests. Differences were considered significant at $p < 0.05$.

Results. Fibroblast growth factor-2 expression was assessed in tumor tissues in 39 out of 44 analyzed patients. The frequency of fibroblast growth factor-2 expression in the observed patients was 84.6% (33/39). The moderate and strong fibroblast growth factor-2 expression was detected in 21 (53.8%) patients with gastric gastrointestinal stromal tumors. High expression of low-molecular weight (18 kDa) fibroblast growth factor-2 isoform was found in all tumor samples from patients with high-risk gastrointestinal stromal tumor (prognostic group 6) (p=0.039), which indicated the active secretion of this ligand by its signalling pathway in the cancer cells. Patients with high levels of low-molecular-weight fibroblast growth factor-2 showed a higher level of Ki-67 proliferative activity (p=0.013) and tumor size (p=0.0017). Patients with increased expression of the low molecular weight isoform of fibroblast growth factor-2 in gastric gastrointestinal stromal tumor had a higher risk of recurrence, as well as larger tumor size and proliferative activity compared with patients without expression of this isoform. The level of fibroblast growth factor-2 expression in tumor samples, determined by immunohistochemistry-staining, increases after initiation of imatinib to based therapy, which may indicate the formation of resistance to this targeted drug and the progression of the disease.

Conclusion. The results of the study suggest that FGF-2 might be an independent prognostic marker of gastric gastrointestinal stromal tumor and a viable therapeutic target.

Keywords: gastrointestinal stromal tumors (GISTs), imatinib mesylate (IM), resistance, FGF-2.

For citation: Mikheeva E.G., Aukhadieva A.M., Sabirov A.G., Boichuk S.V. Low-molecular-weight fibroblast growth factor-2 — a viable prognostic factor for gastric gastrointestinal stromal tumors. *Kazan Medical Journal.* 2021; 102 (3): 313–321. DOI: 10.17816/KMJ2021-313.

Background. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract, developing from interstitial Cajal cells, having pacemaking activity, and setting the rhythm of contractions (peristalsis) of the hollow organs of the gastrointestinal tract. Before these tumors were described, they belonged to a different group owing to the lack of necessary information obtained based on the results of immunohistochemical (IHC) and molecular genetic studies. Before these data were obtained, these malignant tumors were long referred to the group of smooth muscle tumors of the gastrointestinal tract (such as leiomyomas, leiomyosarcomas, or leiomyoblastomas), and patients with GISTs received chemotherapy with extremely low efficiency (0%–27%).

After it was revealed that the main pathogenetic factor of GISTs is activating and mutually exclusive mutations *c-KIT* or *PDGFRA* [1–3], the prognosis of patients with GISTs (including those with inoperable and metastatic forms of the disease) has radically changed for the better.

For correspondence: boichuksergei@mail.ru Received 19.04.2021; accepted 12.05.2021.

Currently, the main first-line drug used for treatment of patients with GISTs is targeted drug therapy with imatinib [imatinib mesylate (IM), glivec], which inhibits the activity of the aforementioned tyrosine kinases [4]. Indeed, the administration of targeted therapy with IM leads to a significant increase in the duration of the relapse-free period in patients with GISTs and significantly slows down the disease progression, including inoperable and metastatic forms of the disease [5, 6].

Despite the impressive clinical results of targeted therapy with IM, more than half of the patients with GISTs develop resistance to this drug 2 years after the start of treatment. At present, a large number of molecular mechanisms have been described, which determine the development of secondary GIST resistance to IM. These include secondary mutations in *c-KIT* (usually in exons 13 and 17) [7], mutation in *BRAF* V600E [8], loss of *c-KIT* expression, and activation of other types of tyrosine kinases (e.g., Fak, Axl, and c-Met) [9, 10].

If resistance to IM develops due to secondary mutations in *c-KIT*, second-line targeted drugs (sunitinib) or third-line drugs (regorafenib) are prescribed [11–13]. However, their intake does not lead to a significant therapeutic effect (compared with the effect in the same patients who had received IM) and is accompanied by severe side effects as well as the development of resistance. Attempts to use new-generation tyrosine kinase inhibitors (such as nilotinib, masatinib, sorafenib, pazopanib, and dovitinib) in the treatment of patients with IM-resistant GISTs turned out to be ineffective and did not affect significantly the disease course and prognosis.

The above-mentioned finding indicates the presence of other molecular mechanisms of the formation of secondary GIST resistance to IM. Indeed, our earlier studies have demonstrated that one of the alternative mechanisms for the development of IM-resistant GISTs may be the activation of the signaling pathway of fibroblast growth factor (FGF) [14], and its inhibition results in the restoration of GIST sensitivity to IM both *in vitro* and *in vivo* [15]. We further revealed that the main pathogenetic mechanism of the activation of the fibroblast growth factor receptor (FGFR) signaling pathway in IM-resistant GIST cell lines is the overproduction of FGF-2 [16], which is known to be a ligand that activates the FGFR signaling pathway.

Aim. In this regard, this study aimed to find evidence of the activation of the FGFR signaling pathway in patients with GISTs based on the assessment of the level of FGF-2 expression in tumor materials, as well as to analyze the prognostic significance of this marker.

Materials and methods of research. This study examined tumor materials from patients who underwent surgical or combined treatment at the Republican Clinical Oncological Dispensary of the Ministry of Health of the Republic of Tatarstan for the period from 2014 to 2020.

The diagnosis of GISTs was confirmed in study patients by histological and IHC (CD117) methods using monoclonal antibodies to c-KIT (clone A4502, DAKO) at a dilution of 1:1000. The expression level of FGF-2 in paraffin sections of tumor tissue was determined on a BenchMark Ventana GX automatic immunohistostaining device (Roche) using monoclonal antibodies to anti-FGF-2 (clone C2, Santa Cruz) at a dilution of 1:200. The expression level of this marker and its intracellular distribution were assessed on a 3-point scale: 0, no staining; 1, weak membrane or nuclear staining; 2, nuclear staining of moderate intensity; 3, bright nuclear staining).

Analysis of the expression level of various FGF-2 isoforms (18, 20, 22.5, and 34 kDa) was performed by immunoblotting. Tumor tissue lysates were obtained using a radioimmune precipitation assay containing protease and phosphatase inhibitors (Sigma, USA) with subsequent determination of the protein concentration in the analyzed samples. For the detection of FGF-2, the corresponding monoclonal antibodies to FGF-2 (clone G2, sc-365106, Santa Cruz) were used at a dilution of 1:200.

Data obtained were processed using the R computer program (Vienna, Switzerland). Qualitative attributes were compared using Fisher's exact test, since the expected values after compiling contingency tables did not exceed 5. The hypothesis of the normal distribution of quantitative data was tested using the Shapiro–Wilk test. The significance of differences between groups of data that did not follow a normal distribution was assessed using the nonparametric Mann–Whitney test. The parametric Student's *t*-test was used to compare data that followed a normal distribution. Quantitative data were presented by median and quartile range. The difference was considered significant at $p < 0.05$.

Results. We analyzed tumor samples from 44 patients with GISTs, including samples from 12 (27.3%) male and 32 (72.7%) female patients who received standard treatment. The median age of the patients was 64 (33–78) years. Moreover, 59.5% of the patients with gastric GISTs had a spindle cell tumor.

The main clinical and morphological characteristics of patients with GISTs are presented in Table 1.

The level of FGF-2 expression by IHC method was evaluated in 39 patients with gastric GISTs. The microscopic presentation when assessing the degree of FGF-2 expression is shown in Fig. 1.

Table 1. Clinical and morphological characteristics of patients with gastrointestinal stromal tumors

Case no.	Age, years	Gender	Tumor location	Tumor size, cm	Risk of recurrence	Histological type	Ki-67, %	FGF-2	Treatment with IM
1	55	m	stomach	8	not available	spindle cell	not available	3	+
2	63	f	ovary (m)	not available	not available	not available	not available	1	+
3	64	m	greater omentum (m)	3.4	high	epithelioid	10	1	+
4	65	f	stomach	7.3	low	epithelioid	2	1	+
5	61	f	stomach	7.3	high	spindle cell	5	0	–
6	59	m	stomach	22.5	high	epithelioid	6	0	–
7	60	f	stomach	2	low	spindle cell	5	0	–
8	59	f	stomach	17	high	combined	10	0	–
9	74	m	stomach	5	high	epithelioid	3	0	–
10	67	f	stomach	5	high	epithelioid	10	1	–
11	53	f	stomach	3	low	combined	12	2	–
12	67	f	stomach	12.3	high	combined	4	1	–
13	69	f	stomach	1.5	low	epithelioid	7	2	–
14	69	f	stomach	1.5	low	spindle cell	3	2	–
15	65	f	stomach	0.8	low	combined	4	1	–
16	56	f	stomach	3	low	spindle cell	4	2	–
17	57	f	stomach	4.4	low	spindle cell	7	3	–
18	61	f	stomach	13	high	spindle cell	4	1	–
19	65	f	stomach	1.5	low	combined	4	not available	–
20	54	m	stomach	6	high	epithelioid	13	not available	–
21	60	m	stomach	4	low	epithelioid	2	0	–
22	53	f	stomach	7	low	spindle cell	7	3	–
23	69	f	stomach	3.4	low	spindle cell	2–3	3	–
24	68	f	stomach	3.3	low	spindle cell	6–7	not available	–
25	71	f	stomach	5.5	high	spindle cell	5–6	1	–
26	72	f	stomach with mts	10	high	combined	25–30	3	–
27	78	m	stomach	14.6	intermediate	spindle cell	8–10	not available	–
28	64	f	stomach	2.8	intermediate	spindle cell	4–5	2	–
29	50	f	stomach	3.3	low	spindle cell	1–2	1	–
30	58	f	stomach	3.3	low	spindle cell	4	3	–
32	70	f	stomach	4.25	low	spindle cell	3	1	–
31	73	m	stomach	11	high	spindle cell	10–12	3	–
33	71	m	stomach	5.5	high	spindle cell	5	3	–
34	56	m	stomach	2.25	low	spindle cell	3	2	–
35	45	f	stomach	5.3	high	spindle cell	up to 10	3	–
36	69	f	stomach with mts	9	high	spindle cell	12–14	3	–

37	47	f	stomach	3	low	spindle cell	4–5	2	–
38	63	f	stomach	6	intermediate	epithelioid	5–6	1	–
39	63	f	stomach (recurrence) with mts	10	high	combined	not available	2	+
40	73	f	stomach	4.3	high	spindle cell	less than 5	3	–
41	65	m	stomach	5.6	low	not available	1–2	not available	–
42	33	f	stomach (recurrence)	3.5	high	epithelioid	5	2	+
43	68	f	stomach	5.9	high	spindle cell	not available	3	-
44	41	m	stomach	9.3	high	spindle cell	5–6	3	–

Note: FGF, fibroblast growth factor; IM, imatinib; m, male; f, female; mts, metastases.

Patients with moderate/high (groups 2 and 3) and negative/weakly positive (groups 0 and 1) levels of FGF-2 expression were comparable with each other in terms of age and gender (Table 2). No significant difference was found between the markers traditionally used in assessing risk groups of the disease (tumor size, proliferative index, etc.) in the groups with moderate/high (groups 2 and 3) and negative/weakly positive (groups 0 and 1) levels of FGF-2 expression according to the results of the IHC staining (Table 2).

Considering that analysis of the expression levels of various isoforms of FGF-2 is not possible by IHC staining, we subsequently analyzed the expression in tumors of all four known isoforms of this ligand. We analyzed 24 tumor samples obtained from patients with GISTs having different risk groups for recurrence. Eleven patients with a low risk of GIST recurrence were included, and the remaining patients (n = 13) were at a high risk of GIST recurrence.

The immunoblotting results presented in Fig. 2 show that a high level of expression of the low-molecular-weight (18 kDa) form of this ligand was noted in all GIST samples of the risk group 6 without exception, while the expression level of other isoforms (22, 22.5, and 34 kDa) did not differ significantly between the study groups of patients.

The level of expression of the low-molecular-weight form of FGF-2 in the tumor was significantly higher in patients with high Ki-67 proliferation index in tumor tissue (Fig. 3, C) and in those with a large tumor (Fig. 3, B). The recurrence risk was also higher in the group with a high level of expression of this FGF-2 isoform in the tumor (Fig. 3, A) [maximum expression was registered in patients of prognostic groups 5 and 6 (high risk of recurrence)], which can be of great prognostic value.

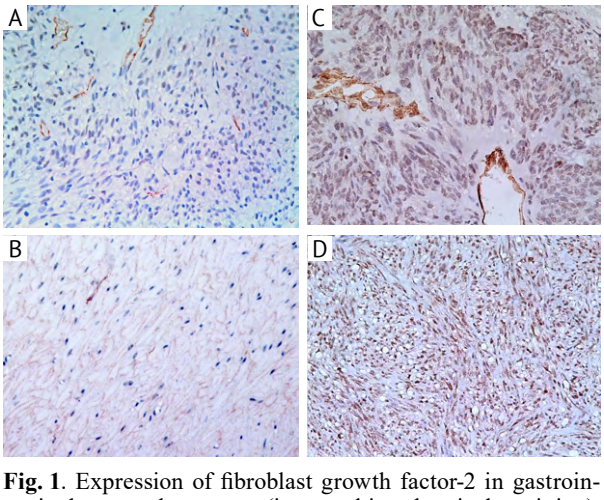

Fig. 1. Expression of fibroblast growth factor-2 in gastrointestinal stromal tumors (immunohistochemical staining): negative reaction (A); membrane reaction (B); nuclear reaction of moderate intensity (C); intense nuclear reaction (D).

Discussion. Several clinical and morphological criteria are currently used to assess the prognosis of GISTs, including the main ones with the same tumor size and mitotic index. In addition, the localization and histological type (i.e., epithelioid, spindle cell, and mixed) of the tumor have a certain prognostic value. Despite the current consensus on the assessment of GIST risk groups and the classifications proposed by Fletcher et al. [17] and the Institute of Pathology of the US Armed Forces [18], the criteria described above do not always correlate well with each other and, in some cases, can be used as independent criteria for assessing the GIST risk group. This fact is certainly a reflection of the diversity of the existing molecular mechanisms of the pathogenesis of GISTs, but at the same time, it bears certain difficulties in assessing disease prognosis, which is sometimes characterized by its unpredictability.

Moreover, the molecular genetic characteristics of GISTs can be one of the main factors that deter-

Table 2. Relationship between the expression level of fibroblast growth factor-2 (FGF-2) in the tumor and the clinical and morphological characteristics of gastrointestinal stromal tumors

Parameters	Patients with a moderate/high (groups 2 and 3) level of FGF-2 expression (n = 22)	Patients with a negative/weakly positive (groups 0 and 1) level of FGF-2 expression (n = 17)	p
Age (median, Q_1–Q_3), years	60.5 (53.5–69)	63 (60–67)	0.419
Gender (n): male female	 5 17	 4 13	1
Tumor size (median, Q_1–Q_3), cm	4.35 (3.0–7.75)	5.25 (3.85–8.55)	0.315
Proliferative Ki-67 index (median, Q_1–Q_3), %	5.0 (4.5–8.5)	4.5 (3.0–6.0)	0.12
Risk of recurrence (n): high intermediate low	 10 1 10	 9 1 6	0.866

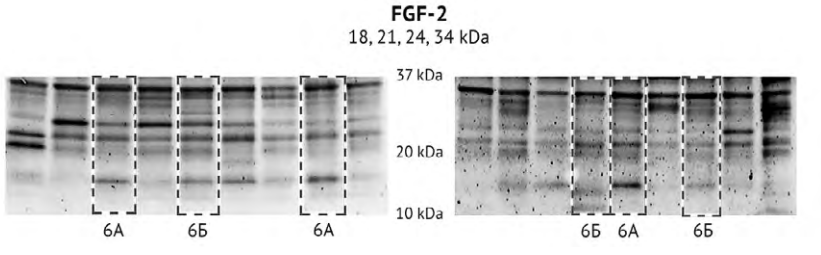

Fig. 2. Level of expression of isoforms of fibroblast growth factor (FGF-2) (18, 22, 22.5, and 34 kDa) in gastrointestinal stromal tumors of the stomach determined by immunoblotting. Samples of tumors with a high risk of recurrence (risk group 6) are highlighted with a dashed line.

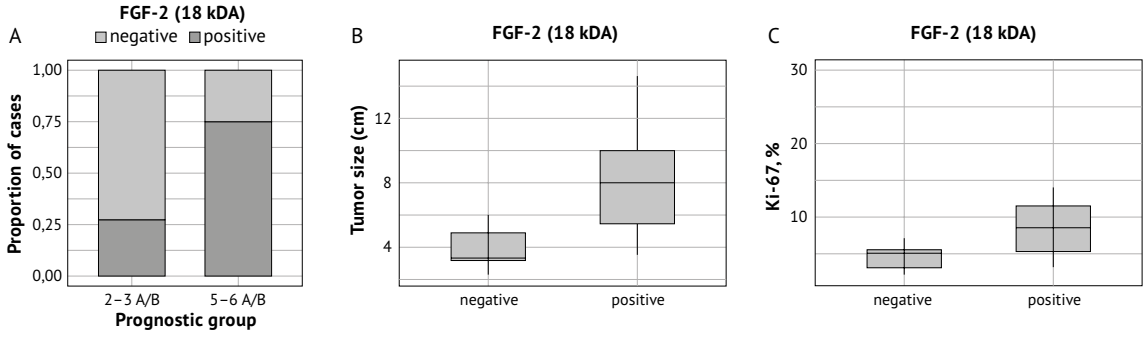

Fig. 3. A Level of the low-molecular-weight (18 kDa) isoform of fibroblast growth factor-2 (FGF-2) in patients of prognostic groups 5 and 6 (high risk of recurrence) and patients of prognostic groups 2 and 3 (low recurrence risk), p = 0.039 (Fisher's exact test). B, C. Comparison of sizes (p = 0.002, Mann–Whitney test) and proliferative activity index (p = 0.013, Mann–Whitney test) in patients with gastric gastrointestinal stromal tumors, positive and negative for the low-molecular-weight (18 kDa) isoform of FGF-2, according to the immunoblotting results. Data are presented by median and quartile range.

mine the sensitivity of GISTs to targeted IM therapy, and the development of secondary mutations in receptor tyrosine kinase *c-KIT/PDGFRA* is traditionally considered the main mechanism of their resistance to the above targeted drug. Nevertheless, the low efficacy of second (sunitinib)- and third (regorafenib)-lines of targeted drug therapy indicates the presence of alternative (i.e., not associated with secondary mutations of the above tyrosine kinase genes) molecular mechanisms of secondary GIST resistance to IM [7, 11, 12]. Therefore, studies aimed at identifying new effective prognostic markers of GISTs, as well as their resistance to IM, appear relevant from both scientific and practical points of view.

Our results indicate signs of autocrine activation of the FGFR signaling pathway in primary GISTs, as evidenced by an increased level of FGF-2 expression in most primary gastric GISTs (84.6%, 33/39). Given the well-known role of the FGFR signaling

pathway in maintaining a high proliferative potential of cells and their viability, this factor may be one of the mechanisms of disease progression.

Nevertheless, the results of the IHC study of gastric GISTs revealed no differences in tumor size ($p = 0.3145$), proliferative index ($p = 0.1203$), and disease risk group ($p = 0.8657$) between the groups with high and low levels of FGF-2 expression in the tumor.

Considering that the analysis of the expression levels of individual FGF-2 isoforms, which differ from each other not only in molecular weight, but also in the mechanism of action, cannot be performed by IHC staining, the next stage of the study was to determine the expression levels of all four known FGF-2 isoforms in gastric GISTs. Immunoblotting results demonstrated that in all (without exception) cases of gastric GISTs with a high risk of recurrence (risk groups 5 and 6), the low-molecular-weight (18 kDa) isoform of FGF-2 is expressed (Fig. 3, A), which is also a characteristic of larger tumors (Fig. 3, B) and proliferative index (Fig. 3, C).

Four FGF-2 isoforms are known in humans; one of them has a low-molecular-weight (18 kDa) and three have a high-molecular-weight (22, 22.5, and 34 kDa), differing from each other not only in their molecular weight, but also intracellular localization and molecular mechanisms of action [19]. The low-molecular-weight isoform is cytoplasmic and functions in an autocrine manner, binding on the cell surface with one of the four types of FGFRs in combination with heparin sulfate proteoglycans, which induces the activation of downstream signaling pathways that regulate the proliferation and survival of tumor cells [20]. Moreover, high-molecular-weight isoforms of FGF-2 are entirely nuclear and exhibit activity through an intracrine mechanism, binding to specific intracellular receptors. This indicates that the activity of high-molecular-weight isoforms of FGF-2 does not depend on their binding to FGFR [21].

Thus, the overexpression of the low-molecular-weight form of FGF-2 in patients with gastric GISTs at a high risk of recurrence reveals the molecular mechanisms of the disease progression, indicating the active secretion of FGF-2 by tumor cells; therefore, we can consider this FGF-2 isoform as a promising prognostic marker.

The data presented are consistent with the results of our earlier studies, which testified the IM-induced activation of the FGFR signaling pathway in GIST cell lines, which is implemented through the active secretion of FGF-2 by tumor cells and serves as a ligand for FGFR1 and FGFR2 [14, 16]. Indeed, the presence of neutralizing anti-FGF-2 antibodies in GIST cell cultures abolishes completely the effect of the targeted drug IM and induces their apoptosis [16]. This, in turn, testifies the potential prospects of using inhibitors of the FGFR signaling pathway to enhance the effect of the targeted drug IM and to re-sensitize GISTs to IM in cases with existing resistance to the first-line drug. The validity of this provision is confirmed by the research results by our scientific group [15] and other scientific groups [22, 23].

Thus, the results of these studies complement the current concept of the mechanisms of the development of secondary resistance of GIST to the targeted drug IM, caused by the activation of alternative tyrosine kinase-mediated signaling pathways in the absence of secondary mutations of *c-KIT* and *PDGFRA*.

CONCLUSION

Evaluation of the level of expression of the low-molecular-weight form of FGF-2 in GISTs can be considered a promising prognostic marker of a high risk of recurrence of gastric GISTs and the development of its resistance to IM during the course of targeted therapy. This, in turn, predetermines expansion of the range of targeted drugs and the introduction of inhibitors of the FGF signaling pathway in the regimens of combined targeted therapy of GISTs.

Author contributions. E.G.M. conducted the immunohistochemical studies, immunoblotting, and systematization of the characteristics of patients with gastric GIST; A.M.A. performed statistical processing of the results obtained; A.G.S. obtained and characterized the tumor material; S.V.B. was the work supervisor, created the research design, and wrote the article.

Funding. This work was supported by the Russian Foundation for Basic Research (Grant No. 20-015-00034).

Conflict of interest. The authors declare no conflict of interest.

REFERENCES

1. Hirota S., Isozaki K., Moriyama Y., Hashimoto K., Nishida T., Ishiguro S., Kawano K., Hanada M., Kurata A., Takeda M., Muhammad Tunio G., Matsuzawa Y., Kanakura Y., Shinomura Y., Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998; 279: 577–580. DOI: 10.1126/science.279.5350.577.

2. Rubin B.P., Singer S., Tsao C., Duensing A., Lux M.L., Ruiz R., Hibbard M.K., Chen C.J., Xiao S., Tuveson D.A., Demetri G.D., Fletcher C.D., Fletcher J.A. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res*. 2001; 61: 8118–8121.

3. Heinrich M.C., Corless C.L., Duensing A., McGreevey L., Chen C.-J., Joseph N., Singer S., Griffith D.J., Haley A., Town A., Demetri G.D., Fletcher C.D., Fletcher J.A. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science*. 2003; 299: 708–710. DOI: 10.1126/science.1079666.

4. Joensuu H., Roberts P.J., Sarlomo-Rikkala M., Andersson L.C., Tervahartiala P., Tuveson D., Silberman S., Capdeville R., Dimitrijevic S., Druker B., Demetri G.D. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N. Engl. J. Med*. 2001; 344: 1052–1056. DOI: 10.1056/NEJM200104053441404.

5. Demetri G.D., Mehren von M., Blanke C.D., Van den Abbeele A.D., Eisenberg B., Roberts P.J., Heinrich M.C., Tuveson D.A., Singer S., Janicek M., Fletcher J.A., Silverman S.G., Silberman S.L., Capdeville R., Kiese B., Peng B., Dimitrijevic S., Druker B.J., Corless C., Fletcher C.D., Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med*. 2002; 347: 472–480. DOI: 10.1056/NEJMoa020461.

6. Verweij J., Casali P.G., Zalcberg J., LeCesne A., Reichardt P., Blay J.-Y., Issels R., van Oosterom A., Hogendoorn P.C., Van Glabbeke M., Bertulli R., Judson I. Progression-free survival in gastrointestinal stromal tumors with high-dose imatinib: Randomized trial. *Lancet*. 2004; 364: 1127–1132. DOI: 10.1016/S0140-6736(04)17098-0.

7. Gramza A.W., Christopher L.C., Michael C.H. Resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumors. *Clin. Cancer Res*. 2009; 15 (24): 7510–7518. DOI: 10.1158/1078-0432.CCR-09-0190.

8. Agaram N.P., Wong G.C., Guo T., Maki R.G., Singer S., Dematteo R.P., Besmer P., Antonescu C.R. Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. *Genes Chromosomes Cancer*. 2008; 47: 853–859. DOI: 10.1002/gcc.20589.

9. Sakurama K., Noma K., Takaoka M., Tomono Y., Watanabe N., Hatakeyama S., Ohmori O., Hirota S., Motoki T., Shirakawa Y., Yamatsuji T., Haisa M., Matsuoka J., Tanaka N., Naomoto Y. Inhibition of focal adhesion kinase as a potential therapeutic strategy for imatinib-resistant gastrointestinal stromal tumor. *Mol. Cancer Ther*. 2009; 8: 127–134. DOI: 10.1158/1535-7163.MCT-08-0884.

10. Mahadevan D., Cooke L., Riley C., Swart R., Simons B., Della Croce K., Wisner L., Iorio M., Shakalya K., Garewal H., Nagle R., Bearss D. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. *Oncogene*. 2007; 26: 3909–3919. DOI: 10.1038/sj.onc.1210173.

11. Rock E.P., Goodman V., Jiang J.X., Mahjoob K., Verbois S.L., Morse D., Dagher R., Justice R., Pazdur R. Food and Drug Administration drug approval summary: sunitinib malate for the treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma. *Oncologist*. 2007; 12: 107–113. DOI: 10.1634/theoncologist.12-1-107.

12. Demetri G.D., Reichardt P., Kang Y.-K., Blay J.-Y., Rutkowski P., Gelderblom H., Hohenberger P., Leahy M., von Mehren M., Joensuu H., Badalamenti G., Blackstein M., Le Cesne A., Schöffski P., Maki R.G., Bauer S., Nguyen B.B., Xu J., Nishida T., Chung J., Kappeler C., Kuss I., Laurent D., Casali P.G.; GRID study investigators. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013; 381: 295–302. DOI: 10.1016/S0140-6736(12)61857-1.

13. Boichuk S., Rausch J., Duensing A. New developments in management of gastrointestinal stromal tumors: regorafenib, the new player in the team. *Gastrointest. Cancer: Targets and Therapy*. 2014; 4: 1–10. DOI: 10.2147/GICTT.S20679.

14. Boichuk S., Galembikova A., Dunaev P., Valeeva E., Shagimardanova E., Gusev O., Khaiboullina S. A novel receptor tyrosine kinase switch promotes gastrointestinal stromal tumor drug resistance. *Molecules*. 2017; 22 (12): E2152. DOI: 10.3390/molecules22122152.

15. Boichuk S.V., Galembikova A., Dunaev P., Micheeva E., Valeeva E., Novikova M., Khromova N., Kopnin P. Targeting of FGF-Signaling re-sensitizes Gastrointestinal Stromal Tumors (GIST) to Imatinib *in vitro* and *in vivo*. *Molecules*. 2018; 23: 2643. DOI: 10.3390/molecules 23102643.

16. Boichuk S., Galembikova A., Mikheeva E., Bikinieva F., Aukhadieva A., Dunaev P., Khalikov D., Petrov S., Kurtasanov R., Valeeva E., Kireev I., Dugina V., Lushnikova A., Novikova M., Kopnin P. Inhibition of FGF2-mediated signaling in GIST — promising approach for overcoming resistance to Imatinib. *Cancers*. 2020; 12 (6): 1674. DOI: 10.3390/cancers12061674.

17. Fletcher C.D., Berman J., Corless C., Gorstein F., Lasota J., Longley B.J., Miettinen M., O'Leary T.J., Remotti H., Rubin B.P., Shmookler B., Sobin L.H., Weiss S.W. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Int. J. Surg. Pathol*. 2002; 2: 81–89. DOI: 10.1053/hupa.2002.123545.

18. Miettinen M., Lasota J. Gastrointestinal stromal tumors: Pathology and prognosis at different sites. *Semin. Diagn. Pathol*. 2006; 2: 70–83. DOI: 10.1053/j.semdp.2006.09.001.

19. Akl M., Nagpal P., Ayoub N.M., Tai B., Prabhu S.A., Capac C.M., Gliksman M., Goy A., Suh K.S. Molecular and clinical significance of fibroblast growth factor 2 (FGF2/bFGF) in malignancies of solid and hematological cancers for personalized therapies. *Oncotarget*. 2016; 7 (28): 44 735–44 762. DOI: 10.18632/oncotarget.8203.

20. Ibrahimi O.A., Zhang F., Hrstka S.C., Mohammadi M., Linhardt R.J. Kinetic model for FGF, FGFR, and proteoglycan signal transduction complex assembly. *Biochemistry*. 2004; 43: 4724–4730. DOI: 10.1021/bi0352320.

21. Yu P.J., Ferrari G., Galloway A.C., Mignatti P., Pintucci G. Basic fibroblast growth factor (FGF-2): the high molecular weight forms come of age. *J. Cell Biochem*. 2007; 100: 1100–1108. DOI: 10.1002/jcb.21116.

22. Li F., Huynh H., Li X., Ruddy D.A., Wang Y., Ong R., Chow P., Qiu S., Tam A., Rakiec D.P., Schlegel R., Monahan J., Huang A. FGFR-mediated reactivation of MAPK signaling attenuates antitumor effects of imatinib in gastrointestinal stromal tumors. *Cancer Discov*. 2015; 5: 438–451. DOI: 10.1158/2159-8290.CD-14-0763.

23. Javidi-Sharifi N., Traer E., Martinez J., Gupta A., Taguchi T., Dunlap J., Heinrich M.C., Corless C.L., Rubin B.P., Druker B.J., Tyner J.W. Crosstalk between KIT and FGFR3 promotes gastrointestinal stromal tumor cell growth and drug resistance. *Cancer Res*. 2014; 75: 880–891. DOI: 10.1158/0008-5472.CAN-14-0573.