RESEARCH ARTICLE

Editorial Process: Submission:02/08/2023 Acceptance:09/14/2023

Novel somatic Missense Mutations in Exon 11 of the *KIT* Gene are Detected in Melanoma

Nadezhda Palkina[1], Anna Tyumentseva[2], Tatiana Ruksha[1]*

Abstract

Objective: The aim of the present study was to analyze mutations of the mast/stem cell growth factor receptor Kit (*KIT*) gene in patients with melanoma from Eastern Siberia regions of the Russian Federation. **Methods:** *KIT* gene mutations in exons 11 and 13 were analyzed by Sanger sequencing in 57 tumor samples obtained from patients with *KIT*-positive melanomas localized in preferable locations. **Result:** Mutations were identified in 21% of patients. Among them, multiple mutations were identified in five patients. A total of 18 mutations were observed in the *KIT* gene, of which three were deletions and fourteen substitution mutations. Age, gender and clinicopathological characteristics of patients with cutaneous *KIT*-positive melanoma in Eastern Siberia corresponded to the European population. According to computational prediction tools, all mutations were evaluated as potentially harmful. **Conclusion:** The six novel mutations reported in the present study expand our knowledge on the molecular pathogenesis of melanoma, which can be used to further explore methods to improve disease therapeutic strategies.

Keywords: Mucous melanoma- acral-lentiginous melanoma- *KIT* mutations- targeted therapy

Asian Pac J Cancer Prev, **24 (9)**, 3015-3020

Introduction

The mast/stem cell growth factor receptor Kit (*KIT*) gene encodes type III kinase tyrosine receptor, which is involved in cell differentiation, proliferation and survival. *KIT* gene homo- and heterozygous hereditary mutations affect receptor functioning and lead to impaired pigmentation and fertility, and can cause some diseases such as mastocytosis. Somatic mutations in the *KIT* gene were revealed to cause melanocyte malignant transformation (Larue et al., 1992). The most frequent mutations occur in exons 11 and 13, and are associated with melanoma sensitivity to protein kinase inhibitors (Reddy et al., 2017). Therefore, *KIT* mutation status has clinical relevance as *KIT*-positive melanomas respond to targeted therapy.

KIT gene somatic mutations are most frequently observed in mucosal melanoma (MM) (15-40% of cases), acral-lentiginous melanoma (ALM) (18-35% of cases) and in melanomas occurring in chronically sun-damaged skin (CSD) (23-28% of cases). The prevalence of *KIT* mutations varies across different regions of the world and is not uniform within Asian and Caucasian populations. In fact, the frequency of ALM varies from 1-3% in Caucasians to 41-65% in Asians, whereas the frequency of MM ranges between 3 and 60% (Tzen et al., 2014; Abbaspour Babaei et al., 2016).

The present study investigated somatic *KIT* mutations in patients with melanoma from the Eastern Siberian region of the Russian Federation treated between 2015 and 2018. Over 90% of the individuals in this region are of Caucasian origin, and tend to exhibit a low frequency of ALM and MM (Motorina et al., 2018). Patients were selected to represent subtypes of melanoma with a higher number of *KIT* mutations: ALM, MM and CSD-sites raised melanomas. The limited sample size was due to the fact that, in general, the Eastern Siberian region is characterized by a low incidence of melanoma development from the aforementioned sites despite the increasing number of annual cases (Gyrylova et al., 2014).

In the present study, we aimed to determine the diversity of *KIT* gene mutations in patients with melanoma in Siberian regions of the Russian Federation and attempted to evaluate the pathogenicity of the mutations detected using computational tools for mutation oncogenicity prediction.

Materials and Methods

A total of 57 formalin-fixed and paraffin-embedded melanoma tissues were obtained from the Krasnoyarsk Regional Pathological Bureau and Tomsk National Research Medical Center of the Russian Academy of Science. This study was approved by the Krasnoyarsk State

*[1]Department of Pathophysiology, Krasnoyarsk State Medical University, P. Zeleznyaka str. 1, 660022, Krasnoyarsk, Russian Federation. [2]Krasnoyarsk Scientific Center of the Siberian Branch the Russian Academy of Sciences, 50, Akademgorodok Str., Krasnoyarsk, 660036, Russian Federation. *For Correspondence: tatyana_ruksha@mail.ru*

Medical University Local Ethics Committee (protocol no. 36/2011; issued on December 22, 2011). Diagnosis and clinical characteristics, including anatomical site, clinical phenotype and patient age and gender, were collected from patient and pathology case reports were obtained after written consent.

DNA was isolated from macrodessected slides of melanoma tissues containing no less than 60% of malignant cells using a AmpliSens® DNA-sorb-B kit (AmpliSens, Russia), according to the manufacturer's instructions. Sanger sequencing with separation by capillary electrophoresis was performed using primers to amplify exons 11 and 13 of the *KIT* gene synthesized by Evrogen (Moscow, Russia). The primers used were as follows: Exon 11 forward, 5'-CTCTCCAGAGTGCTCTAATGAC-3' and reverse, 5'-AGCCCCTGTTTCATACTGACC-3'; and exon 13 forward, 5'-CGGCCATGACTGTCGCTGTAA-3' and reverse, 5'-CTCCAATGGTGCAGGCTCCAA-3'.

Four computational tools were used to predict the pathogenicity of the identified mutations: Sorting Intolerant From Tolerant (SIFT) (http://sift.jcvi.org/), Polymorphism Phenotyping V-2 (PolyPhen-2.2.2; http://genetics.bwh.harvard.edu/pph2/index.shtml), MutationTaster2 (http://www.mutationtaster.org/) and MutationAssessor Release 3 (mutationassessor.org/r3). A SIFT score predicts whether an amino acid substitution affects protein function. The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). The score can be interpreted as follows: i) 0.0 to 0.05, variants with scores in this range are considered deleterious (the closer the score is to 0.0, the more confidently it can be predicted that this variant is deleterious); 0.05 to 1.0, variants with scores in this range are predicted to be tolerated (benign) (the closer the score is to 1.0, the more confidently it can be predicted that this variant is tolerated). The PolyPhen-2 score ranges from 0.0 (tolerated) to 1.0 (deleterious). Variants with scores of 0.0 are predicted to be benign. The closer the score is to 1.0, the more confidently it can be predicted that this variant is deleterious. The score can be interpreted as follows: i) 0.0 to 0.15, variants with scores in this range are predicted to be benign; ii) 0.15 to 1.0, variants with scores in this range are possibly damaging; iii) 0.85 to 1.0, variants with scores in this range are more confidently predicted to be damaging. According to the Mutation Assessor analysis, which provides a functional impact score (FI): i) ≤0.8, "neutral"; ii) 0.8≤1.9, "low"; iii) 1.9≤3.5, "medium"; and iv) >3.5, "high". MutationTaster indicator values range from 0 to 1. Values close to 1 have high predictive reliability. The value of indicator 1 is regarded as the result causing the disease. Each in silico tool uses different methods for nucleotides substitution pathogenicity, which are detailed in Table 1 according to the information on their websites. The Fast Adaptive Shrinkage Computational Tool (FASTA) format and Ensembl sequence identifiers (nucleotide, amino acid and protein) were used for queries in programs (see Table 2).

Results

In the selected samples, the most common clinical types were superficial spreading (40.5%) and nodular melanomas (27.7%). ALM and MM accounted for 17.0 and 8.5% of the cases, respectively. The median age of *KIT*-positive patients was 59 years (range, 36-67 years). Within *KIT*-positive patients, one patient was under the age of 40, six patients were in the age range of 40-60 years and three patients were older than 60 years of age (Table 3). Melanomas with less than 1.01-Breslow depths were not presented in the study; 25% melanomas were characterized by a Breslow depth of 1.01-2.0 mm; in 25% melanomas Breslow depth was 2.01-4.0 mm; 50% tumors were characterized by Breslow depth greater than 4.0 mm. Of the patients with melanomas raised from CSD-sites and ALM, 25% were male and 75% were female, whereas all patients with MM were female.

KIT gene mutations were identified in 12 patients (21%). All mutations identified were localized in exon 11 and occurred in 16.7% (2/12) of ALM, in 50% (6/12) of CSD melanomas, and in 33.4% (4/12) of MM. Among them, multiple mutations were identified in five patients. A total of 18 mutations were observed, of which six had not been registered in the Catalogue of Somatic Mutations in Cancer (COSMIC) database [c.1761C>G (p.N587K), c.1761 C>T (p. N587Y), c.1750T>A (p. F584I), c.1657T>G (p.Y553D), c.1666C>A (p.Q556K), c.1749-1750GT>TA (p.E583D)_(p.F584I)]. No mutations were detected in exon 13.

Most of the *KIT* sequence changes observed were base substitutions (83.3%) although transversions (60%) and transitions (40%) were presented as well. Besides, three deletions were found that varied in length from 9 to 70 nucleotides. One of them was located predominantly in intron *KIT* gene. Base-pair substitutions involving the replacement of a purine by a purine (p.E554K), substitutions involving the replacement of a pyrimidine by a pyrimidine (p.P585L), base-pair substitutions involving the replacement of a purine by a pyrimidine (p.Y553D) and deletions nucleotides (c.1664_1715del) are shown in Figure 1.

All detected mutations were predicted as pathogenic by the SIFT prediction algorithm (http://sift.jcvi.org/) and PolyPhen-2.2.2 (http://genetics.bwh.harvard.edu/pph2/index.shtml). MutationTaster2 evaluated novel mutations revealed as "low" or "neutral" in terms of pathogenicity, whereas the MutationAssessor database determined them as "medium" pathogenic (Table 4).

Discussion

In the present study we identified *KIT* gene mutation frequency in patients with ALM, MM and CSD from two Eastern Siberian regions of the Russian Federation. Melanoma *KIT* mutation frequencies varied within different populations. The *KIT* gene mutation rate was reported to be 6% in 134 ALM cases from a German population (Satzger et al., 2008) and 11% in patients with MM from an Italian population (Ponti et al., 2017). The frequency of *KIT* gene mutations was identified as 21.4% in ALM, 18.3% in MM and 15.6% in CSD skin melanomas in a multicenter American study of 328 patients with melanoma treated by a *KIT* small molecule inhibitor imatinib mesylate (Doma et al., 2020). In the present study,

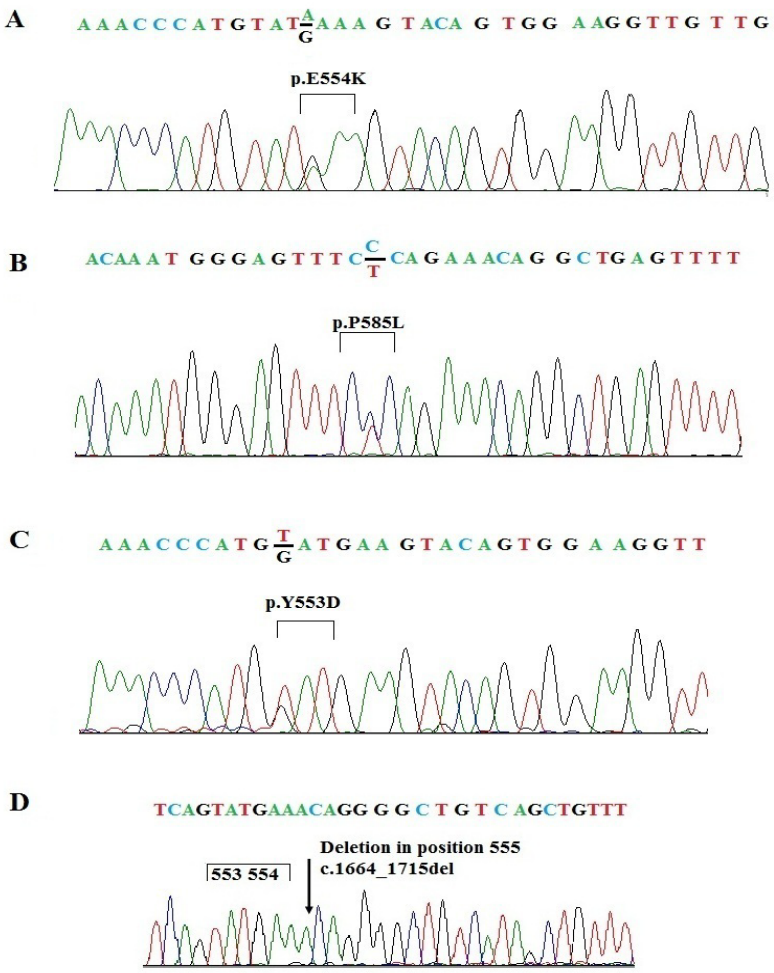

Figure 1. Results of *KIT* Mutation Analysis in Patients with Acral Lentiginous Melanoma via Sanger Sequencing. (A) Transitions nucleotide in exon 11 of the *KIT* gene c.1662 A>G (p.E554K). (B) The chromatogram shows the transitions in c.1755C>T (p.P585L). (C) Transversion of nucleotide in exon 11 of the KIT *gene* c.1657 T>G (p.Y553D). (D) Non-frameshift deletions of several base pairs of nucleotide in position 555 *KIT* gene c.1664_1715del.

Table 1. The Description of the *in silico* Tools

Program	SIFT	PolyPhen-2	MutationTaster2	MutationAssessor Release 3
Algorithm	Evolutionary conservation	Protein structure/ function and evolutionary conservation	Protein structure/ function and evolutionary conservation	Evolutionary conservation
Method	Compilation of a data set of functionally linked protein sequences using BLAST/ PSIBLAST	Statistical method of weighting and profiling sequences from subsets of identical sequences in several alignments using PSIC	Integration of information from various biomedical databases (Ensembl, UniProt, ClinVar, ExAC, 1000 Genomes Project, phyloP, phastCons)	Provides data from other databases, such as COSMIC, UniProt and Pfam, as well as its own "functional point of influence" on mutations
Computational tools	Matrix Dirichlet	Naive Bayesian classifier	Naive Bayesian classifier	Cross-Entropy Method
Effect	Effect of amino acid substitution on structure/ function of protein	Effect of amino acid substitution on structure/ function of protein	Cause of disease	Effect of amino acid substitution on structure/ function of protein
Score	0.00 - 1	0.00 - 1	0.0 - 215 (does not affect forecast)	-5.76 - 5.76
Score thresholds	0.05	0.432	Score does not affect forecast	1-Sep
Prediction	<0.05=	0.0 - 0.15 = "benign"	Indicator values range from 0 to 1 "D", "disease causing";	≤0.8 = "neutral"; 0.8≤1.9 = "low"; 1.9≤3.5 = "medium";
	"Damaging";	0.15 - 1.0 = "possibly damaging"	"A", "disease causing automatic" ;	>3.5 = "high"
	>0.05= "Tolerated"	0.85 - 1.0 = "probably damaging"	"N", polymorphism"; "P", "polymorphism automatic"	

Table 2. Sequence Identifiers

Database	NCBI Reference Sequence	Ensembl ID
	https://www.ncbi.nlm.nih.gov/refseq/	https://www.ensembl.org/Homo_sapiens/Gene/
Gene	*KIT*	*KIT*
Gene ID	NM_000222.3	ENSG00000157404
Protein ID	NP_000213.1	ENSP00000288135.6
Transcript ID	NC_000004.12	ENST00000288135.6

Table 3. Demographics and Baseline Characteristics of Patients with *KIT*-Positive Melanoma in the Present Study

Patient	Age (years)	Sex	Type	Anatomic site	Breslow thickness (mm)	Sanger sequencing results	
						Exon 11	Exon 13
1	62	Female	NM	Heel	4	c.1764G>A (p.R588K)	wt
2	60	Female	NM	Forearm	3	c.1660G>A (p.E554K) c.1754C>T (p.P585L)	wt
3	59	Male	NM	Shoulder	7	c.1651_1721 del70	wt
4	36	Female	Metastatic melanoma	Back	3.62	c.1657T>G (p.Y553D)	wt
5	59	Female	SSM	Forearm	1	c.1666C>A (p.Q556K)	wt
6	67	Female	SSM	Shin	1.5	c.1648–19 T>A	wt
7	46	Male	ALM	Phalanx of finger	5.8	c.1761C>G (p.N587K)	wt
8	43	Female	ALM	Phalanx of thumb	5.6	c.1755C>G (p.P585P) c.1761C>T (p.N587Y) c.1750T>A (p. F584I)	wt
9	64	Female	MM	Nasal mucosa	*	c.1687A>T (p.I563L) c.1690 A>T (p. N564H) c.1693.del.A (p.G565_D574 del)	wt
10	55	Female	MM	Nasal mucosa	*	c.1765 C>T (p.L589L) c.I687A>T (p.I563L)	wt
11	Unknown	Unknown	MM	Nasal mucosa	*	c.1664_1715 del51	wt
12	Unknown	Unknown	MM	Nasal mucosa	*	c.1749-1750GT>TA (p.E583D)_ (p.F584I)	wt

*, This parameter is not applicable for mucosal melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma; ALM, acral-lentiginous melanoma; MM, mucosal melanoma; wt, wildtype.

the *KIT* gene mutation frequency was 21% in patients with ALM, MM and CSD-raised melanomas. Previous data revealed that *KIT*-mutant melanomas were associated with patients of an increased age, clinical melanoma subtype, anatomic location and CSD, but not with gender, histological type, Breslow thickness, ulceration, mitotic rate or tumor stage (Carvajal et al., 2011). In the present study, we observed clinical characteristics in patients with *KIT* mutations similar to the European population. At the same time *KIT*-positive patients were observed, characterized by a relatively young age as the median age was 59 years.

Certain mutations identified in the present study had not been previously identified in melanoma, but mutations in the observed regions were described in Gastrointestinal Tumor (GIST) and identified both as pathogenic and negative prognostic indicator (Incorvaia et al., 2021; Liang et al., 2021; Yamauchi et al., 2021). Driver mutations of

Table 4. Prediction Scores from SIFT, Polyphen-2, Mutation Assessor and Mutation Taster of *KIT* Gene Novel Mutations

Mutation	SIFT score/ functional impact	PolyPhen2 PSIC score/ functional impact	Mutation assessor,FIS score/ functional Impact	MutationTaster, functional impact
N587K	0.050/ deleterious	1/ damaging	2.045/ medium	“Disease”
N587Y	0.050/ deleterious	1/ damaging	2.595/ medium	“Disease”
F584I	0.020/ deleterious	0.994/ damaging	2.775/ medium	“Disease”
Y553D	0.050/ deleterious	1/ damaging	3.19/ medium	“Disease”
Q556K	0.025/ deleterious	0.998/ damaging	2.65/ medium	“Disease”
E583D	0.050/ deleterious	1/ damaging	3.22/ medium	“Disease”

the *KIT* gene in various types of cancers are characterized by specific hotspot regions. At the same time L576P and K642E mutations were described as the most frequent mutations in melanoma (Reddy et al., 2017). In the present study we found substitutions in regions 553-564 and 583-589, and deletions that were previously described as characteristic of GIST. It can be assumed that signaling pathway cascades in different types of cancer are the same, and therefore, the pathogenic mutations of *KIT* in GIST have the same effect in melanoma (Liang et al., 2013).

Six mutations were subjected to bioinformatics analysis: N587K, N587Y, F584I, Y553D, Q556K and E583D. The results of the analysis are presented in Table 4. Score values obtained using computational prediction tools allow for the consideration of nucleotide substitutions as negative and disease causing. However, the sole use of bioinformatics analysis is a limitation of the present study. An expanded in vitro study may increase and unveil the clinical significance of gene alterations revealed in patients with melanoma.

Mutations in different regions of the *KIT* gene can cause activation of different signaling pathways (Chauvot de Beauchêne et al., 2014). Activating mutations in the juxtamembrane domain coded by exon 11 lead to a more prominent activation of the PI3K/mTOR, MAPK and p38 signaling pathways compared with mutations in exon 13 (Sanlorenzo et al., 2016). The function of the juxtamembrane domain is to inhibit receptor 1 activation as a result of phosphorylation, which can occur in several tyrosine residues (Y553, Y568, Y570 and Y578). Mutations in exon 11, including point substitutions and deletions, can lead to a decrease in the autoinhibitory function of the receptor and can be considered as pathogenic if not investigated experimentally or clinically.

The present study identified two synonymous mutations: P585P (c.1755C>G) in ALM and L589L (c.1765C>T) in MM. Synonymous mutations are often disregarded because they do not affect the final amino acid sequences of proteins. However, codon biases and the resulting changes to the mRNA secondary structure can alter mRNA stability and ribosomal translation rates, and can lead to alternative final conformations of proteins with distinct biological outcomes (Sauna and Kimchi-Sarfaty, 2011).

Based on the data obtained, the identified mutations should be known as harmful and regarded as an option with clinical significance. We consider it unlikely that these genetic variants are pathogenic. The lack of clinical information related to these mutations requires further study.

In summary, we revealed a relatively high prevalence of *KIT* gene mutations in patients with melanoma from the Eastern Siberia region. Mutation variants detected in the present study do not correspond with the *KIT* gene mutation profile described previously for melanoma, but are more commonly associated with GIST. While the present study examined *KIT* gene mutations in patients with melanoma from Eastern Siberia, the results may contribute to the development of targeted therapy for patients with melanoma worldwide. Therefore, the novel mutations described in the present study warrant further investigation.

Author Contribution Statement

Conceived and supervised the study: Tatiana Ruksha. Designed the experiments: Anna Tyumentseva, Nadezhda Palkina, Tatiana Ruksha. Performed the experiments: Anna Tyumentseva, Nadezhda Palkina. Analyzed the data: Anna Tyumentseva, Nadezhda Palkina, Tatiana Ruksha. Wrote the manuscript: Nadezhda Palkina, Anna Tyumentseva, Tatiana Ruksha. Made manuscript revisions: Nadezhda Palkina, Tatiana Ruksha. Data Availability: The data and materials used in the study are available on request.

Acknowledgements

Authors thank Nikolaeva Elena and Aksenenko Mariya for technical support of this work.

Ethical Declaration

This study was approved by the Krasnoyarsk State Medical University Local Ethics Committee (protocol no. 36/2011; issued on December 22, 2011).

Conflict of Interest

Conflict of interest relevant to this article was not reported.

References

Abbaspour Babaei M, Kamalidehghan B, Saleem M, Huri HZ, Ahmadipour F (2016). Receptor tyrosine kinase (c-Kit) inhibitors: a potential therapeutic target in cancer cells. *Drug Des Devel Ther*, **10**, 2443-59.

Carvajal RD, Antonescu CR, Wolchok JD, et al (2011). *KIT* as a therapeutic target in metastatic melanoma. *JAMA*, **305**, 2327-34.

Chauvot de Beauchêne I, Allain A, Panel N, et al (2014). Hotspot mutations in *KIT* receptor differentially modulate its allosterically coupled conformational dynamics: impact on activation and drug sensitivity. *PLoS Comput Biol*, **10**, e1003749.

Doma V, Barbai T, Beleaua MA, et al (2020). *KIT* Mutation Incidence and Pattern of Melanoma in Central Europe. *Pathol Oncol Res*, **26**, 17-22.

Gyrylova SN, Aksenenko MB, Gavrilyuk DV, et al (2014). Melanoma incidence mortality rates and clinico-pathological types in the Siberian area of the Russian Federation. *Asian Pac J Cancer Prev*, **15**, 2201-4.

Incorvaia L, Fanale D, Vincenzi B, et al (2021). Type and Gene Location of *KIT* Mutations Predict Progression-Free Survival to First-Line Imatinib in Gastrointestinal Stromal Tumors: A Look into the Exon. *Cancers (Basel)*, **13**, 993.

Larue L, Dougherty N, Porter S, Mintz B (1992). Spontaneous malignant transformation of melanocytes explanted from Wf/Wf mice with a Kit kinase-domain mutation. *Proc Natl Acad Sci U S A*, **89**, 7816-20.

Liang J, Wu YL, Chen BJ, et al (2013). The C-kit receptor-mediated signal transduction and tumor-related diseases. *Int J Biol Sci*, **9**, 435-43.

Liang L, Li X, Li D, et al (2021). Mutational characteristics of gastrointestinal stromal tumors: A single-center analysis of 302 patients. *Oncol Lett*, **21**, 174.

Motorina AV, Palkina NV, Komina AV, et al (2018). Genetic

analysis of melanocortin 1 receptor red hair color variants in a Russian population of Eastern Siberia. *Eur J Cancer Prev*, **27**, 192-6.
Ponti G, Manfredini M, Greco S, et al (2017). BRAF, NRAS and C-*KIT* Advanced Melanoma: Clinico-pathological Features, Targeted-Therapy Strategies and Survival. *Anticancer Res*, **37**, 7043-8.
Reddy BY, Miller DM, Tsao H (2017). Somatic driver mutations in melanoma. *Cancer*, **123**, 2104-17.
Sanlorenzo M, Vujic I, Posch C, et al (2016). Oncogenic *KIT* mutations in different exons lead to specific changes in melanocyte phospho-proteome. *J Proteomics*, **144**, 140-7.
Satzger I, Schaefer T, Kuettler U, et al (2008). Analysis of c-*KIT* expression and *KIT* gene mutation in human mucosal melanomas. *Br J Cancer*, **99**, 2065-9.
Sauna ZE, Kimchi-Sarfaty C (2011). Understanding the contribution of synonymous mutations to human disease. *Nat Rev Genet*, **12**, 683-91.
Tzen CY, Wu YH, Tzen CY (2014). Characterization of *KIT* mutation in melanoma. *Dermatol Sci*, **32**, 7-12.
Yamauchi A, Chinen Y, Chihara T, et al (2021). A case of planar-type gastrointestinal stromal tumor of the transverse colon with perforation. *Clin J Gastroenterol*, **14**, 1157-62.