Peer Review File

Article Information: https://dx.doi.org/10.21037/jgo-22-1111

Reviewer A

Comment 1: […] weaknesses including conclusions not supported by the results […]

Reply 1: Thank you for this comment. We did adjust our meaning in the conclusion to make it more clear.

Changes in the text: Added "usually: in line 192. The conclusion was reformulated, new sentences were added and some deleted, it now reads: "As presented in this case report, they might be difficult in terms of imaging diagnostics and their genetic landscape may change in time, resulting in possible resistance to administered treatment. Therefore, there is a need to apply a non-standard diagnostic process and treatment. The case depicts the importance of complex genetic testing combined with histopathological diagnosis due to the treatment efficacy prediction. Moreover, it emphasizes the need for the establishment of unified diagnostics and treatment guidelines for pediatric gastrointestinal stromal tumors, including current possibilities of targeted treatment." (lines 192-200).

Comment 2: […] lack of key experimental details […]

Reply 2: Following Reviewer A and C suggestions, we added more information on genetic testing that was performed. We believe that imaging and histopathological diagnostics does not lack any data and Reviewer C suggested that the case report is too detailed.

Changes in the text: We reformulated section in lines 98-105, so it now reads: "A retrospective genetic assessment of the specimen was performed in a certified laboratory. Targeted Sanger sequencing on *KIT* (exons 9, 11, 13, 17, reference sequence *KIT* LRG_307t1; LRG_307p1) and *PDGFRA* (exon 12, 14, 18, codon 842 in exon 18 and D842V mutation – *PDGRFA* LRG_309t1; p.LRG_309p1) was executed. The limit of detection was 20% of heterozygous cells with the mutation. The percentage of cancer cells in the tested material was >50%. The tissue fragment for DNA isolation containing cancer cells was extracted by macrodissection. The percentage of cancer cells exceeded the detection limit of the method used. *KIT* mutation in exons 13 and 17 was found; *KIT* exons 9 and 11 were impossible to be assessed."

We added "in the same certified laboratory" and "of the same reference sequence" in lines 133-134 as well as "Germline control was not performed to date." in line 136.

Comment 3: The authors should also proofread the manuscript more carefully. Please correct typographic errors throughout the text.

Reply 2: We indeed overlooked some errors, i.e. did not italicize all genes, capitalized some things wrongly. We also made amendments in English language (we are not native English speakers).

Changes in the text: Deleted "has" (line 21), replaced "yet" to "though" (line 35), replaced "in contrast" to "compared" (line 38), added "followed by", deleted "and" in line 43. Deleted "any" in line 59. We reformulated histopathological examination description to make it more clear (lines 69-76).

We decapitalized vimentin, desmin (lines 75-76). Changed “same” into “exact (line 91). Added comma in lines 95 and 107. Deleted word “firstly” in line 108, “up” and “firstly” in line 106. Added word “the” n line 117. Deleted “also” in line 119. We replaced “ragments of the stomach” with “stomach fragments” in line 127. and “was free of the tumor” into “tumor-free” in lines 130-131. We deleted “they” in line 131. We added “antibodies against” in line 132 and “the” in line 136. We changed “be different” into “differ” and “big mass itself” into “neoplasm mass” in line 152. We reformulated the sentence in lines 154-157 so it now reads “Neuroblastoma, nephroblastoma, germinal tumors, or hepatoblastoma may be considered especially in the younger pediatric population, while in adolescents - hepatocarcinoma. Finally, pancreatoblastoma, gastrointestinal tract carcinomas, or GISTs belong to rare yet possible entities”. We reformulated the sentence In line 161 – “procedure was complicated with” was changed into “complicated the procedure.. Then in lines 162-163 we changed “with carefulness” into “carefully”. We deleted “any” in line 165 and “visible” in line 166. We changed “in the light of” into “as per” in line 168. We reformulated lines 169-170, so they now read “This case report highlights imaging diagnostic difficulties as the disease’s progression was not seen adequately in each imaging technique […] and corrected “watch and wait” into “watch-and-wait” (line 171). We added “due to” in line 177.

Comment 4: Using “c-Kit” and “KIT” interchangeably throughout the manuscript is confusing.

Reply 4: This issue has been fixed.

Changes in the text: Replaced “c-KIT” with “*KIT*” (line 31).

Comment 5: Please explain all abbreviations when they appear first. There are too many to list.

Reply 5: Abbreviations such as: EUS, PET, HPF, *KIT*, SDH, *IGF1R*, DOG1, SMA, NSE, NGS were explained.

Changes in the text: *KIT* (line 31), SDH (line 33), *IGF1R* (line 33), HPF (line 72-73), DOG1 (lines 73-74), SMA (line 74), NSE (lines 74-75), MRI (line 85), PET (line 86) abbreviations were explained. EUS replaced with “endoscopic ultrasound” in line 92. NGS explained as next-generation sequencing in line 178.

Comment 6: Why authors compared wild type GIST and KIT mutant GIST? How about PDGFRA mutant GIST? Please provide a possible explanation.

Reply 6: This comparison was made based on the fact that the treatment and approach differs in *KIT*-mut/*PDGFRA*-mut vs wild-type GIST. Regarding *PDGFRA*-mut GIST, this genetic aberration was examined in this patient and D842V mutation provides opportunity for administration of targeted treatment and that is why it was mentioned in the text. We are not exactly sure what Reviewer A means by this comment/questions though.

Changes in the text: In lines 180-184 we added rationale and short description of available targeted treatment “While there is no registered treatment for *SDH*-deficient GIST available, *PDGFRA* mutations allow for avapritinib administration. Moreover, other kinase inhibitors, such as sunitinib and regorafenib have been successfully used in pediatric population, providing disease stabilization, both in *KIT*-mutated and wild-type tumors”. Thus we deleted the following sentence in lines 184-185. We also reformulated sentence in lines 186-190 to make it more clear what we meant.

Comment 7: Please provide scale bar in Figure 1 and Figure 2.

Reply 7: Scale bar in Figure 1 has been provided. However, we decided not to provide a scale bar in Figure 2, as this would lower the clarity of the Figure. This was consulted with experienced pediatric radiologist.

Changes in the text: None. Scale bar in Figure 1 provided.

Comment 8: Please provide the number of IRB for this study.

Reply 8: IRB number for the administration of imatinib was provided in the text.

Changes in the text: We added IRB number, adding text in lines 108-109 and reformulating subsequent sentence "(Institutional Bioethics Committee Agreement no. 996/19 on 30.10.2019). The disease stabilized for a year."

Reviewer B

Comment 1: The authors should italicize gene names (e.g. line 32, PDGFRA; 91, KIT,…). Verify throughout the manuscript.

Reply 1: We italicized gene names.

Changes in the text: Italicization of gene names: *KIT, IGF1R* – lines 31, 33.

Comment 2: There are some parts with missing text (53, 173, and 174, "available at:…")

Reply 2: This was made based on the Journal, as all case reports published have an established link to the scan of CARE checklist as well as COI disclosures. We believe these links are then pasted by the Editorial Office. However, we did delete these parts.

Changes in the text: Deleted "available at: …" in lines 56, 202, 203-204.

Comment 3: Figure 1 legend is duplicated (lines 74 and 249).

Reply 3: This has been fixed and was editorial error.

Changes in the text: Deletion of the duplication (formerly line 249).

Comment 4: Figure 1 should have a scale bar in each image.

Reply 4: Scale bar in Figure 1 has been provided.

Changes in the text: None. Scale bars added.

Comment 5: Figure 1 and 2: the authors should avoid using symbols in the legend, even more so when they are not identical to the one represented in the figure. The symbols can be kept in the figure, but explained in the legend, with terms such as "arrow", "white arrow", and "arrowhead".

Reply 5: We did amendments to the Figure 1 legend. Concerning Figure 2, we changed the style and color of arrows. We believe it is now more visible and clear.

Changes in the text: The legend of Figure 1 (lines 79-83) now reads "Histopathological examination results. A: Cross section of the stomach with the tumor (black arrow) anterior vascular invasion

(arrowhead), hematoxylin&eosin staining, magnification 12x. B: A mixture of epithelioid and elongated tumor cells arranged in fascicles and nests, hematoxylin&eosin staining, magnification 40x. C: The tumor cells stained positive for CD117, magnification 200x. D: The tumor cells showed positive staining for DOG1, magnification 200x."

Comment 6: In the Figure 2 the white arrow is difficult to be identified. The authors should try to find a better option.

Reply 6: We changed white to yellow color.

Changes in the text: Change to Figure 2 – new figure sent.

Comment 7: Figure 2 legend is confusing. Please reformulate.

Reply 7: The legend has been completely reformulated by an experienced radiologist.

Changes in the text: In lines 120-124 changes have been made. The legend was shortened. Figure 2 legend now reads: "Figure 2 A-C: Coronal (A), axial (B), and sagittal (C) view of the contrast-enhanced CT abdominal scans, showing intensely enhanced solid mass (marked with yellow arrow)."

Comment 8: The authors should use a formal text throughout the manuscript: Line 89 "… demonstrated a 7 mm big tumor…".

Reply 8: We have proofread the whole manuscript once again and hopefully, all informal phrases were replaced.

Changes in the text: Deleted the word "big" in line 96.

Reviewer C

Comment 1: […] additional discussion about the genetics of pediatric vs adult GIST, and differences in management, are needed to make a compelling addition to literature.

Reply 1: Thank you for this comment. We added few sentences regarding the targeted treatment of pediatric GIST. However, we believe we should not review the genetics and differences in management in this manuscript, as we last year published a review article that describes these matters. This could be perceived as self-plagiarism/reproducement of the same data.

Changes in the text: In lines 180-184 we added rationale and short description of available targeted treatment "While there is no registered treatment for *SDH*-deficient GIST available, *PDGFRA* mutations allow for avapritinib administration. Moreover, other kinase inhibitors, such as sunitinib and regorafenib have been successfully used in pediatric population, providing disease stabilization, both in *KIT*-mutated and wild-type tumors". Thus we deleted the following sentence in lines 184-185. We also reformulated sentence in lines 186-190 to make it more clear what we meant.

Comment 2: Abstract and case report differ on age, patient is 10 yrs old in abstract and 15 yrs old in body of manuscript

Reply 2: This was made to highlight the time that lasted from the first diagnosis to the time of manuscript submission (currently 15 yo). However, as Reviewer C believes it is confusing, we changed this.

Changes in the text: In line 53, we deleted “currently” and changed the text into “10” from “15”.

Comment 3: Introduction to case report states this case is metastatic, although not clear from case report areas of distant metastasis or if this just refers to regional spread with satellite lesions

Reply 3: “Metastatic” was changed into “locoregional spread” and deleted from abstract.

Changes in the text: Deleted “metastatic” (line 20), deleted metastatic (line 53) and added “with subsequent regional spread” in line 54.

Comment 4: Section on imaging of GIST (line 39) in introduction does not appear to be necessary and authors do not clearly connect this to overall message of case report.

Reply 4: This section was a rationale for differing diagnostic techniques that are described in case report, however, if the Reviewer C does not believe it is necessary, we deleted it.

Changes in the text: This section was deleted (lines 40-42).

Comment 5: Line 44, “unpleasant prognosis” should be changed to clarify meaning, does this mean worse prognosis? How much worse?

Reply 5: This is difficult to say with specific numbers. Current data does not provide specific answers. We only know the patient has worse (than other patients) prognosis, as the tumor was damaged. Hopefully, reformulation makes it more clear.

Changes in the text: Changed “unpleasant prognosis” into “recurrences” (line 45). We also developed subsequent sentences that read “In case of unfavorable prognosis and predicted sensitivity to imatinib, such treatment might be introduced” into “Imatinib treatment may be introduced in case of tumor rupture, incomplete tumor resection or disease progression when there is predicted sensitivity to this agent” (lines 48-50).

Comment 6: Overall, there is unnecessary detail in case report, for example line 81 regarding EBV/CMV status is not germane to the case.

Reply 6: We prepared this case report in accordance with CARE checklist guidelines. This makes the manuscript detailed. The viral status sentence was deleted. We trust other details are important as per the above mentioned CARE guidelines.

Changes in the text: Sentence “besides imaging diagnostics and routine complete blood count tests, Epstein Barr Virus and Cytomegalovirus IgG tests were positive” (lines 88-89) was deleted.

Comment 7: It is touched upon in discussion, but more detail is needed regarding genetic studies. Was initial sequencing targeted or whole exome sequencing? What was initial allelic fraction of mutations and was a germline control performed? Did sequencing at relapse differ from modality at diagnosis? What QC was performed to ensure tumor was sequenced and not normal tissue? Is there data on % malignant cells at initial sequencing sample and relapse? Was SDH work-up performed either by staining or germline sequencing? If not, why?

Reply 7: We added more detailed description of genetic studies. Targeted Sanger sequencing was performed in a certified laboratory that executes genetic testing for oncology nationally. The same method and the same laboratory made this sequencing twice in this patient’s tumor sample. The results did not include information about the allelic fraction, quality control, only % threshold of cells was

included. Nor germline control, neither *SDH* mutational profile/immunohistochemistry examinations were performed to date. This is due to limited funding. Additional information regarding the genetic testing was included in the manuscript.

Changes in the text: We reformulated section in lines 98-105, so it now reads: “A retrospective genetic assessment of the specimen was performed in a certified laboratory. Targeted Sanger sequencing on *KIT* (exons 9, 11, 13, 17, reference sequence *KIT* LRG_307t1; LRG_307p1) and *PDGFRA* (exon 12, 14, 18, codon 842 in exon 18 and D842V mutation – *PDGRFA* LRG_309t1; p.LRG_309p1) was executed. The limit of detection was 20% of heterozygous cells with the mutation. The percentage of cancer cells in the tested material was >50%. The tissue fragment for DNA isolation containing cancer cells was extracted by macrodissection. The percentage of cancer cells exceeded the detection limit of the method used. *KIT* mutation in exons 13 and 17 was found; *KIT* exons 9 and 11 were impossible to be assessed.”

We added “in the same certified laboratory” and “of the same reference sequence” in line 134 as well as “Germline control was not performed to date.” in line 136.