POINT BY POINT IN RESPONSE TO REVIEWERS for the manuscript "Molecular methods for colorectal cancer screening: progress with next-generation sequencing evolution" of Salma Abbes, Simone Baldi, Hayet Sellami, Amedeo Amedei, Leila Keskes

REVIEWER 1

I have two comments: 1. The number of references is 190, I think the authors can reduce that. It is too long for a review article. 2. The conclusion is too long. The authors need to be specific about the highlights of the review article. Congratulations for the paper.

Q/S 1: *The number of references is 190, I think the authors can reduce that. It is too long for a review article*

Q/S 1 Reply: *In agreement with the reviewer we have reduced the number of references from 191 to 129*

Q/S 2: *The conclusion is too long. The authors need to be specific about the highlights of the review article*

Q/S 2 Reply: *We thank the reviewer for the compliments on our paper, in agreement with his comments we have shortened the conclusions.*

REVIEWER 2

Q/S 1: *Introduction L27: add a parenthesis*

Q/S 1 Reply: *Thank you, we have corrected the mistake (Line 42)*

Q/S 2: *Fecal immunochemical test: remove the ": "*

L64: "Fecal immunochemical test (FIT)": Add capital letters to mark the abbreviation

L67: "sufficiently flexible to adjust cut-off concentration for positivity" : Specify how and according to which parameters the cut-off is adjusted

L71: "DNA testing": Please specify which DNA testing techniques are concerned

L72: « the low clinical sensitivity for both cancers and advanced adenomas when used at a low cut-off": Please specify the sensibility/specificity of the test in these 2 cases

Q/S 2 Reply: *Thank you, we have removed the ":" (Line 77), added capital letters for FIT (Line 80) and specified the DNA testing (line 89). Moreover, we have added the parameters for FIT' cut-off (Lines 83-85) and we have added the FIT sensitivity for both cancers and advanced adenomas (Lines 90-91).*

Q/S 3: *5.1 Single gene sequencing- L99: "influences": influence*

Q/S 3 Reply: *Thank you, we have corrected the mistake (Line 118).*

Q/S 4: *5.2 Multi-target stool DNA test- L125: "sensitivity of 66%": please specify the specificity*

"et al": italicize throughout the text

Q/S 4 Reply: *Thank you, we have added the specificity (Line 155) and we have italicized "et al" throughout the text*

Q/S 5: *Droplet Digital PCR- L141: "due to its high sensitivity in comparison to traditional standard procedures": specify the sensibility for the CRC screening in the text- L160: "although the many advantages" ➔ although there are many advantages*

Q/S 5 Reply: *Thank you, we have added the ddPCR' sensitivity for CRC (Line 173) and we have corrected the mistake in Line 191.*

Q/S 6: *The Idylla approach- L169: "CRC- related mutations" ➔ CRC-related mutations, L171: FFPE add the meaning of the abbreviation*

Q/S 6 Reply: *Thank you, we have corrected the mistake (Line 200) and we have added the meaning of FFPE (Line 202)*

Q/S 7: Methods based on NGS technologies

L188: SNP add the meaning of the abbreviation

L198: better quality management of CRC ➔ better quality of care

L265: "for many other advantages" ➔ with ?

L324: "complicated data output" ➔ please precise

L367: "In addition, the abundance of short-chain fatty acids (SCFAs) resulted altered in CRC patients compared to healthy controls" : Delete "resulted" in the sentence and specify the alteration (decrease)

L374: "the assumption of probiotics… can ➔ the supplementation with… could increase chances of therapeutic success

- *Are there data on probiotic and prebiotic supplementation in CRC patients?*
- *The bacterial richness is largely altered in many pathologies and could help to screen them. Is there any data on gene count in colorectal cancer?*

Q/S 7 Reply: *In agreement with the reviewer, we have added the meaning of SNP (Line 221), corrected the mistakes in Lines 230, 296 and 434, precised the meaning of complicated output (Lines 376-377) and discussed the effects of prebiotic and probiotic supplementation on CRC patients (please see lines 418-425)*

Q/S 8: *Conclusion- L399: "the therapeutic management of CRC patients" ➔ care*

L402 : "such circular RNA (circRNA) and Piwi-interacting RNA (piRNA) : Why not describe and discuss these biomarkers in the text?

Q/S 8 Reply: *Thank you, we have corrected the mistake in Line 463 but we have preferred to not discuss circRNA and piRNA in the text because the findings in this field are still novel and conflicting, surely we will dedicate more focus on these molecules in future works*

Q/S 9: *In the table- Sensitivity/specificity (CRC): Sensitivity/specificity (CRC screening)*

Q/S 9 Reply: *Thank you, we have corrected it in the table*

Q/S 10: Key Words-twice colorectal cancer
Q/S 10 Reply: Thank you, we have corrected it

REVIEWER 3

Q/S 1: *An abstract that summarizes the paper should be added.*

Q/S 1 Reply: *Thank you, we have added an abstract summarizing the paper (Lines 16-30)*

Q/S 2: *Figures that show the principal or clinical examples for each diagnostic method may be prepared and provided, to make it better understood for general readers.*

Q/S 2 Reply: *We thank the reviewer for the right suggestion, we have prepared 3 figures.*

Q/S 3: *lines 104-110, the principal of liquid biopsy method may be described, to provide a better understanding for readers.*

Q/S 3 Reply: *In agreement with the reviewer, we have added the requested information (please see Lines 127-130)*

Q/S 4: *The principals of different NGS approaches may be added, to provide a better background information for readers.*

Q/S 4 Reply: *Thank you, we have added some principles of NGS sequencing (please see lines 359-373)*

Q/S 5: *The paragraph in section 7 is too long, which can be divided to several paragraphs to present a better logic.*
Q/S 5 Reply: *In agreement with the reviewer, we have divided the section 7 in paragraphs*