Dear reviewers,

We would first like to thank you for taking the time to review and provide us with feedback on this project. The comments of both reviewers have been addressed in the main text of the manuscript file. We will be addressing each comment below.

For example, in a certain section described "recent development of "ultra-sensitive" assays", there was no description of how it is performed, the clinical use, or any benefits about this technique.

I have improved the manuscript by including an example of an ultra-sensitive assay (CAPP-Seq). I have also summarized how this technology works and what are the main benefits of utilizing this technique. See below.

- Given the presence of both healthy cells circulating cell-free DNA and ctDNA, the isolation of ctDNA continues to be a diagnostic challenge, as only approximately 0.01% of all circulating DNA is tumor-derived 14. This limitation has been overcome by the recent development of "ultra-sensitive" assays that allow differentiating ctDNA from cfDNA, which are being used not only for the detection of genetic mutations but also for the early detection of disease recurrence and monitoring for therapy response 6. One example of an ultrasensitive assay is the Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq); this technology consists of a capture-based ctDNA detection method which can detect most of the main types of mutations: copy number alterations, rearrangement, indels, and single nucleotide variants, by the evaluation of large segments of the genome utilizing enriched genomic regions that have been selected before sequencing [15,16]. This method allows for the detection of various mutations, increasing the sensitivity of the test, when compared to other NGS based assays, and aids the evaluation of intratumor heterogeneity 16. This technological advancement has led to the development of liquid biopsy, which provides a genetic characterization of tumors from blood, bronchial alveolar lavage (BAL), or

CSF sample. This technology brings many clinical utilities, more so in patients with solid tumors that are not amenable to repeat biopsies, including the measurement of disease burden, detection of emerging mutations, among others.

In another part, the order of the content showed illogical flow, because liquid biopsy should be described first before ctDNA detection. In some parts, the author seems to be confused, the analysis of circulating tumor cells (CTC) is different from circulating tumor DNA analysis.

Thank you for this observation. We have reviewed the flow in the mentioned paragraph to improve the flow and also described CTC and compared it to ctDNA which we hope improves the understanding of the manuscript. See below.

- *Circulating tumor cells (CTC) have been observed in patients' bloodstream. CTC are believed to reach a patient's plasma by migration from the principal or metastatic tumor site secondary to either tumor invasion, shedding, or after the tumor site experiences mechanical stress after surgery4. Analysis of both CTC and ctDNA is the backbone of the development of liquid biopsy.*

1. **Some typos have been noted: exones, nivolumamb, 1 ng mg-1:** This typos have been addressed within the main manuscript text.
2. **I suggest using the terms circulating cell-free DNA instead of circulating free DNA or cell circulating free DNA. Please, use the same definition for cfDNA in the text:** Thank you again for this suggestion, we have addressed this within the main manuscript text.
3. **"Opening he possibility for a new possible pharmacological approach to a disease, which is often associated with a poor survival." There is something no clear in this phrase….please control it.** This has been controlled. Please see below.
    a. These observations have led to a new possible pharmacological approach to a disease that often carries a dismal survival.

4. **"Correspondingly, Xu et al. developed and validated a combined prognosis score (cp-score) using 8 methylation markers found on ctDNA in addition to clinical, demographic and the American Joint Committee on Cancer (AJCC) stage. In their research, a cp-score <= 0.24 was determined to be low risk while a cp-score > 0.24 was classified as high risk, with a statistically significant median survival (p < 0.0001) (18)." The authors must give more details for this study such as the number of patients enrolled and the type of cancer.**

   Thank you again for this suggestion. I have given more details about the original study. Please see below.

   Correspondingly, Xu et al. developed and validated a combined prognosis score (cp-score) using eight methylation markers found on ctDNA in addition to clinical, demographic, and the American Joint Committee on Cancer (AJCC) stage. In their research among 377 hepatocellular carcinomas (HCC) samples, a cp-score <= 0.24 was determined to be low risk while a cp-score > 0.24 was classified as high risk, with a statistically significant median survival (p < 0.0001) 21. This research showed that cp-score, in combination with TNM staging, increased the prognostic prediction accuracy for patients with HCC.

5. **and limitations on the use of ctDNA analysis for cancer patients, as well as by giving information about the use of ctDNA in accepted clinical protocols and in clinical trials worldwide. A table containing this information will be very useful for readers. Future perspective should be introduced too.**

   Thank you one more time for this suggestion. We have included a table with all the current registered clinical trials utilizing liquid biopsy/ctDNA analysis and provided with information about the purpose of those researches.

Thank you again for helping us improve our manuscript.

Sincerely,

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Kathrin Dvir, MD