Response to Reviewer 1.

<Reviewer's comment>

The paper, similar to other reviews already published in the literature, must be improved with some major and minor revisions.

MAJOR REVISIONS

Q1. The review is mainly focused on the role of ctDNA in the management of NSCLC patients; experiences conducted in this field with circulating tumor cells (CTCs) and, more recently, with exosomes should also be reported by the authors.

➔ A1. Thank you for your comment. Liquid biopsy covers many biologic elements other than circulating tumor DNA (ctDNA) as the reviewer commented. However, as you can see in 'A2', most of current clinical trials use ctDNA as selected biomarker, and since the purpose of this review is to help clinicians to understand and utilize liquid biopsy in clinics, we deemed it would be more practical if our review primarily focused on the clinical application of analysis using ctDNA. We revised the last sentence of the introduction as follows: "We here review the clinical applications of liquid biopsy, focusing on ctDNA analaysis, in non-small cell lung cancer patients and discuss the challenges and future directions for this methodology."

Q2. The authors reported only historical literature data, the paper should also include a paragraph and a table dedicated to ongoing clinical trials using liquid biopsy in NSCLC patients.

➔ A2. Thank you for your comment. We added the following sentence in conclusion: "Several clinical trials are currently active trying to define clinical relevance and utility of liquid biopsy in advanced lung cancer (Table 2)."

We also added Table 2. in the revised manuscript that summarizes currently active clinical trials on liquid biopsy in advanced NSCLC.

Table 2. Active clinical trials on liquid biopsy in advanced NSCLC.

NCT	Study type	No of patients	Title	M
NCT02511288	Observational	900	LIquid BIopsies in Patients Presenting Non-small Cell Lung Cancer (LIBIL)	ct
NCT03721120	Randomized	286	Evaluation of the Feasibility and Clinical Relevance of Liquid Biopsy in Patients With Suspicious Metastatic Lung Cancer (LIBELULE)	ct
NCT03865511	Interventional	66	MEchanisms of Resistance in EGFR Mutated Nonpretreated Advanced Lung Cancer Receiving OSimErtib (MELROSE)	ct
NCT04258137	Interventional	332	Circulating DNA to Improve Outcome of Oncology PatiEnt. A Randomized Study (COPE)	ct
NCT04265534	Randomized	120	KEAPSAKE: A Study of Telaglenastat (CB-839) With Standard-of-Care Chemoimmunotherapy in 1L KEAP1/NRF2-Mutated, Nonsquamous NSCLC (KEAPSAKE)	ct
NCT04698681	Interventional	200	NGS Screening Protocol to Detect Mutation of KEAP1 or NRF2/NFE2L2 Genes for the KEAPSAKE (CX-839-014) Trial	ct

NCT04703153	Observational	200	LIQUIK: LIQUId Biopsy for Detection of Actionable Genomic BiomarKers in Patients With Advanced Non-small Cell Lung Cancer (NSCLC) (LIQUIK)	cf
NCT04790682	Interventional	300	LIquid Biopsy to prEdict Responses To First-line immunotherapY in Metastatic Non-small Cell LUNG Cancer. (LIBERTYLUNG)	ct

Q3. About technologies for ctDNA detection, pros and cons regarding the different methods for ctDNA analysis should be added.

➔ A3. Thank you for the comment. We added the following sentence in the first paragraph of section '2.1. Technologies for ctDNA analysis and their clinical application': "The pros and cons of ctDNA analysis methods covered below are summarized in Table 1."
We also added Table 1. in the revised manuscript which outlines advantages and disadvantages of different ctDNA analysis methods.

Table 1. Comparison of ctDNA analysis methods.

	Coverage	Advantage	
Targeted Assays	Pre-defined genetic mutations	Highly specific and sensitive Quantatitive	Can m
NGS Whole genome/exome sequencing	Sequencing of full genome/exome	Identification of new targets Investigation of the mechanism of resistance	Ri Cor
NGS Panels	Capture or PCR of pre-determined region (gene/exon), then sequencing	Simutaneous detection of known and unknown mutations Low cost Less complex bioinformatics	Better but Imp

MINOR REVISIONS

Q4. In the text should be reported another example of clinical application of liquid biopsy in NSCLC represented by patients with MET exon 14 skipping mutations treated with tepotinib such as demonstrated by the VISION study recently published in the NEJM.

➔ A4. Thank you for the comment. We revised our manuscript to cover MET exon 14 skipping mutation and the following paragraph were added in section '3.1. Initial genotyping and drug resistance monitoring in advanced NSCLC': "Other oncogenic driver mutations can be detected with ctDNA and used as therapeutic targets. Recently, *mesenchymal epithelial transition (MET)* protooncogene emerged as a novel targetable genetic alteration. Drilion et al. studied efficacy of crizotinib in 69 patients with NSCLC harbouring a *MET* 14 exon alteration and showed plasma-derived ctDNA assays can also detect *MET* exon 14 alterations. Among 37 patients with *MET*-exon-14-altered tumors and available plasma samples, 18 (49%) was ctDNA positive in plasma samples. Recondo and colleagues tracked genetic alterations during MET TKI therapy using plasma and tissue NGS in 20 patients with advanced *MET* exon 14-

mutant NSCLC. In this study, genomic alterations during the treatment course can successfully be traced with tissue and/or plasma NGS, however, the concordance rate between plasma and tissue NGS in 11 paired samples was only 27.3%. The VISION study evaluated efficacy of tepotinib in 152 NSCLC patients with *MET* exon 14 skipping mutations with serial tissue biopsy and ctDNA analysis. The response rate was similar in tissue and liquid biopsy groups; 48% and 50%, respectively. Among the patients with molecular ctDNA reponse, tepotinib achieved a disease control rate of 88% according to independent radiologic review. Another emerging actionable target is *RET* gene fusions. Solomon and colleagues performed analysis of ctDNA and tissue in patients with RET fusion-positive NSCLC who showed disease progression after an initial response to selpercatinib. Interstingly, *RET* solvent front and gatekeeper mutations associated with resistance was detected in ctDNA before disease progression."

Q5. The authors should specify that the role of TMB in clinical practice is still unclear. As known, pitfalls regarding TMB for its clinical application include cut-off and reproducibility, differences in gene panel platform, sample storage time and turnaround time.

→A5. Thank you for your insightful comment. We added the following paragraph in last part of the section '3.2. Use of the tumor mutation burden (TMB) to guide immunotherapy': "Despite promising data from several clinical trials in immuno-oncology, there are some challenges to overcome before TMB can be used in clinical practice: Further evaluation of tissue-plasma concordance should be carried out; Different methods measuring TMB, e.g. WES and various kinds of targeted NGS, needs to be standardized; A definitive cut-off point of TMB should be agreed upon."