

## 2. Answering reviewers:

### Code 02497043

1. The title in the e-mail: « **Are liquid biopsies a scientific “spy” that foresees cancer before any clinical and diagnostic tools?** »

The title in the downloaded manuscript file: « **Are liquid biopsies a scientific “spy” that foresees cancer ?** » Which one is right?

#### Answer to point 1:

If respecting the Frontier manuscript Format rules, the **second one** would have to be the right one due to the restriction of the number of words. Personally, I prefer the first choice.

2. The purpose of this manuscript is neither the "summary" nor the "introduction" section. Needs to be added.

#### 3. Introduction section ; Paragraph 1:

“Lung cancer is the leading cause of cancer death worldwide in both men and women due to growth and aging of population. The global lung cancer burden of annual cases is expected to double by 2050 [1].” It is not appropriate that the first part of the introduction is related to lung cancer. Because, this manuscript describes the liquid biopsy not only the lung cancer, but the general cancers.

#### Answer to point 2 and 3:

**Abstract:** Since 1948, circulating free DNA was first identified in human blood. Circulating tumour DNA is in fact DNA shed by tumour cells from all metastatic tumour locations throughout the whole body, and is thrown into the bloodstream and can then be isolated by a standard blood draw. Using this technique scientists can obtain a wide view of tumour heterogeneity, identify different mechanisms of drug resistance, what is its predominance and the clinical rational of precision cancer medicine become a part of our daily practice. Secondly, early detection of cancer may also contribute to global decrease in cancer mortality.

#### Introduction

Cancer mortality has decreased globally in the United States and Europe, as well as the individual risk of dying from cancer, due to recent reliable data <sup>1</sup>. Although analysis confirms that decrease in cancer mortality is across all income levels, a difference has also been reported in low and middle income countries in which this decline still needs to be clarified. Preventive strategies in cancer control, specifically addressing risk factors, have been one of the measures that lead to these results. Nevertheless, early detection and intervention in high risk groups, with a non-invasive, accurate, sensitive and specific method such as analysis of circulating free DNA (cfDNA), is still the most effective measure to reduce cancer mortality <sup>2</sup>.

## Code 00608195

This is a very nice review of the importance of the circulating tumour DNA as a biomarker for the continuum of cancer. Nevertheless, the authors confound the term “liquid biopsy”, used in several titles, with the determination of the ctDNA, because liquid biopsy is much more than this – determination of the circulating tumour cells, exosomes, RNA, etc. So, and because the paper is interesting and it is very well written with a clear English, I propose that the authors change the titles, for instance, to “Are the determination of ctDNA a scientific “spy” that foresees cancer ?”.

**ANSWER:** I agree for that liquid biopsies is a much wider subject and in fact I’m specifying in circulating tumor DNA. So I do agree that the title should be changed from:

**Are liquid biopsies a scientific “spy” that foresees cancer?**

to

**Is the determination of ctDNA a scientific “spy” that foresees cancer?**

## Code 00608206

Review of the manuscript: Manuscript NO: 34314 Title: Are liquid biopsies a scientific "spy" that foresees cancer ? Invited manuscript, The article presents an updated review of the liquid biopsy (detection of free circulating DNA in blood). I consider that the review is well written, understandable and addresses the main questions related to the topic: definition, history, utility, sensitivity and specificity of the test, advantages and disadvantages and possible future use. It clearly presents many of the advantages of a liquid biopsy but I think it should further develop the reasons why it is not yet a standard clinical practice. The structure of the article is adequate and the bibliography is updated. It could be worth to include in the bibliography some relevant article of some of the authors on the subject.

**ANSWER: 1<sup>st</sup> & 2<sup>nd</sup> Point:**

It is not yet a standard of care because only in this May 2017, were the guidelines for NGS suggestions for liquid biopsies validated by the College of American Pathologists, by the Association of Molecular Pathology as well as the European Society of Pathology. For these reasons, although they have been added to the testing palette for NSCLC by NCCN, only now has validation occurred and therefore, it is not yet a standard of care. Nevertheless many clinical studies are attempting to empower the utility and benefit of liquid biopsies as an all, not only ctDNA ref.

**REF:** Circulating tumor markers: harmonizing the yin and yang of CTCs and ctDNA for precision medicine. I. S. Batth, A. Mitra, S. Manier, I. M. Ghobrial, D. Menter, S. Kopetz & S. Li. Annals of Oncology 28: 468–477, 2017

doi:10.1093/annonc/mdw619

## Code 00608210

Dear Editor, I thank you very much for giving the opportunity to review this manuscript. This topic is interesting. However, there are some major and minor comments. Besides, there are many grammar errors. The authors should consider professional and/or native English speaker advice for writing the academic manuscript. Below are my comments.

### Major

- For lung cancer, please summarize the ctDNA sequences that have been already processed for liquid biopsies in clinical practice such as EGFR mutation, ALK, KRAS, or other else and also provide the accuracy of each.

ANSWER: Concerning EGFR mutation several studies have reported a wide range of concordance rates between ctDNA and tissue samples [9-12]. T790M genetic aberrations in EGFR have also been referenced as acquired resistance to target therapies [13,14]. For example, as stated by Naygaard *et al* in plasma using the ARMS-qPCR technique KRAS mutations have also been reported in NSCLC[15]. Concerning ALK mutations such as, C1156Y and L1196M have also been identified as acquired resistance to target therapy with crizotinib[16].

**9. Wang, S. *et al*.** Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients. *Clin Chim Acta* 430C, 63–70 (2013)

**DOI:10.1016/j.cca.2013.12.026**

**10. Jing, C. W., Wang, Z., Cao, H. X., Ma, R. & Wu, J. Z.** High resolution melting analysis for epidermal growth factor receptor mutations in formalin-fixed paraffin-embedded tissue and plasma free DNA from non-small cell lung cancer patients. *Asian Pac J Cancer Prev* 14, 6619–6623 (2013)

**PMID: 24377577**

**11. Zhang, H. *et al*.** Comparison of EGFR signaling pathway somatic DNA mutations derived from peripheral blood and corresponding tumor tissue of patients with advanced non-small-cell lung cancer using liquid chip technology. *J Mol Diagn* 15, 819–826 (2013)

**12. Kim, H. R. *et al*.** Detection of EGFR mutations in circulating free DNA by PNA mediated PCR clamping. *J Exp Clin Cancer Res* 32, 50 (2013)

**DOI:10.1186/1756-9966-32-50**

**13. Yun, C. H. *et al*.** The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl Acad. Sci. USA* 105, 2070–2075 (2008)

**DOI: 10.1073**

**14. Murtaza, M. *et al*.** Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497, 108–112 (2013)

**DOI: 10.1038**

**15. Nygaard, A. D.,** Garm Spindler, K. L., Pallisgaard, N., Andersen, R. F. & Jakobsen, A. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. *Lung Cancer* 79, 312–317 (2013)

**DOI: 10.1016**

**16. Choi, Y. L. *et al.*** EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N. Engl. J. Med.* 363, 1734–1739 (2010)

**DOI:10.1056**

- Page 3 “When compared tissue samples with ctDNA, by sequencing method, the results reported showed, an overall accuracy of 87% (336/386 patients)” Which ctDNA sequences that they applied? The reference that the authors cited was only News!!

ANSWER: I’m sorry but i placed the wrong reference. It concerns the abstract presented at ASCO 2016 (ref. 6&7)

**6 Rosell R,** Karachaliou N. Lung cancer: Using ctDNA to track EGFR and KRAS mutations in advanced-stage disease. *Nature Reviews Clinical Oncology* 2016; 13,401–402

**DOI:10.1038**

7: ASCO: Abstract: **Karachaliou N.** LBA 11501, Jun 07, 2016

- Page 5 cost benefit, routine clinical practice and Table 1. Please provide the references.

ANSWER: [17]

Which ctDNA sequences that the authors recommend to check for early detection, evaluate relapse and how often? There are many questions to use liquid biopsies in these ways.

ANSWER:

First it requires detection of a specific mutation or mutations in the tumour tissue to then look for same ones in the ctDNA after surgery and during follow-up. Concerning detection of residual disease after curative surgery, 6 to 8 weeks ctDNA should be measured [18]. They were measured in this study during two to five years after surgery. ctDNA can in fact detect upfront specific genetic alterations months before clinical biomarkers or imaging studies.

**18. Diehl, F *et al.*** Circulating mutant DNA to assess tumor dynamics. *Nat Med* 14:985-990, 2008

**DOI:10.1038**

In addition, is it really cost-effective? The authors should not use their opinions without evidence-based data in this review.

ANSWER: Based on two articles, both state that tissue biopsies increase cost for patient care and an uncomfortable invasive procedure [20,21]

**20. Luis A. Diaz Jr** and Alberto Bardelli. Liquid Biopsies: Genotyping Circulating Tumor DNA. J Clin Oncol 32:579-586; 2014

DOI: 10.1200

**21. Overman M J**, Modak J, Kopetz S, *et al*: Use of research biopsies in clinical trials: Are risks and benefits adequately discussed? J Clin Oncol 31:1722, 2013

DOI: 10.1200

**Minor** • The words that have abbreviation should be used with abbreviation, such as ctDNA, cfDNA throughout the manuscript. corrected

- The authors should not cite as ASCO 2016 or News. Please show the references that the readers can further read. – corrected above
- Many grammar errors and not academic writing - corrected
- Please correct reference style- corrected.

**References:** also corrected and marked in yellow.