Author’s response to reviews

Title: Emerging concepts in liquid biopsies

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Author’s response to reviews:

We would like to thank you for considering our manuscript entitled "Emerging concepts in liquid biopsies” (manuscript#: BMED-D-16-01701) and we are grateful to the reviewers for their remarks. Enclosed please find our revised manuscript.

As requested, we have revised our manuscript and here we provide our point-by-point response to issues raised by your referees and to your editorial points. Furthermore, we tried everything to ensure that our manuscript complies with your journal style for a “review” according to the Instructions for Authors on your journal homepage.

Reviewer #1:

This review introduces a current topic of cancer research, i.e., liquid biopsy. Although the review covers a substantial body of literature, it includes only a limited number of literatures from journals on clinical studies, and thus, it is not an appropriate source of information.

Our response: This point is well taken. However, our aim was not to write a review about clinical studies involving plasma DNA. At the same time, this reviewer has a point: on page 14, we refer to the “cobas EGFR Mutation Test v2” and its role in patients with non-small lung cancer (NSCLC). The New England Journal of Medicine just published (online on the 6th of December 2016; hard-copy on the 16th of February 2017) a beautiful study about treatment with osimertinib in EGFR T790M positive lung cancer (Mok et al. N Engl J Med 376:629-40). This study employed the cobas kit instead of tissue biopsies to identify patients eligible for treatment with osimertinib. We added this novel information to our text (on page 14) as it represents a truly progressive example as to how liquid biopsies impact clinical studies.

The following two points need specific attention:

1. In the treatment of lung cancer, the clinical application of liquid biopsy is more advanced than is recognized by the author.
Osimertinib, a third-generation epithelial growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), should only be administered to patients with T790M mutation-positive lung cancer. T790M is a mutation that confers resistance to first-generation EGFR-TKIs, namely gefitinib and erlotinib. Detection of the T790M mutation requires re-biopsy, but owing to the invasive nature of lung biopsy, liquid biopsies would be highly beneficial. The cobas EGFR Mutation test version 2 for T790M has been approved in US and Japan for detecting this mutation, despite the lack of evidence.

Our response: The revised version addresses this issue now (on page 14) and the aforementioned paper by Mok et al. represents a particularly apt example for T790M mutation testing.

The geographical location of the researchers may also affect their recognition of the clinical application. EGFR-mutation positive lung cancer accounts for ~40% of lung adenocarcinoma in the Asian population, but only ~10% in the Caucasian population. Thus, EGFR-TKIs are an important treatment option for advanced lung cancer in Japan, but not necessarily in other countries. A next-generation sequence-based detection system (Uchida et al. Clin Chem. 2015 61(9):1191-6) is already commercially available in Japan (although still not approved). On the other hand, AstraZeneca withdrew osimertinib from the German market as a result of cost negotiations.

Our response: We also included information on the different geographic distribution of EGFR mutations in lung cancer and furthermore, we discuss the interesting NGS-based detection system by Uchida et al. in the revised version. We do not address AstraZeneca and the cost issues in Germany. The study by Mok et al. demonstrating significantly greater efficacy of osimertinib as compared to platinum therapy may change the attitude towards financing this promising regimen, but this topic is in our opinion beyond the scope of this review.

2. In addition to spontaneous somatic mutations in normal cells, DNA damage during the experimental process should be considered as a cause of background variants, i.e., low frequency variants in normal individuals. This is a serious problem in laboratories, but is rarely described in literature. Some description is found in the second paper of CAPP-seq.

Our response: We really appreciate this remark by this reviewer, as it addresses a very important issue. We already referred to the second CAPP-seq paper by Newman et al.; however, we did not address background errors in the context of low-allele mutation detection. We added this to the part where we discuss detection of very low allele detection of TP53 mutations on pages 8-9.

Reviewer #3:

Reviewer #3: In the first part, the manuscript presents an overview on current liquid biopsy technologies and applications with a focus on cancer. ctDNA, CTCs, and exosomes are discussed, and examples from recent literature are given. The second part deals with novel and emerging technologies for the detection of low numbers of DNA variants, epigenetic alterations and enrichment of tumor-specific exosomes. Finally, the authors provide an outlook for the potential of liquid biopsies as diagnostic tools.
Our response: This is a very nice summary of our paper.

The chapter on mutation baseline in healthy individuals is interesting, but my feeling was that it is slightly deviating from the common theme, especially in the section where the evolution of cancer mutations is discussed.

Our response: As you certainly remember, in your invitation letter to write this review you wrote: “The issue of a baseline value - what number of mutations is normal for a healthy 20/30/40/50/60/70/80-year old person? What is normal/detectable level?” That was the main reason to include this section. We still believe that discussion of mutation baseline is very important. As outlined in our manuscript, there are tremendous efforts to increase resolution of blood-based assays and to identify with high confidence low-allele frequency variants in the blood. We are still in the process of learning what these low-allele variants actually mean from a medical/biological perspective and an appropriate discussion has to involve the issue of mutation baseline values.

A similar logic applies for the paragraph on the functional CTC studies. These include the potential for early detection, the necessity to increase technical sensitivities and specificities despite the fact that low levels of mutated alleles can already be detected quite well with current techniques, and theoretical considerations such as the demand for a minimum of genome equivalents for ultra-sensitive detection of mutant alleles in liquid biopsies.

Our response: All these points are very well taken and we hope that the issues of technical sensitivities and specificities, detection of low level mutated alleles and ultra-sensitive detection were appropriately addressed.

Editorial Requests

We have decided that the best format for your article is ‘review’. Please add the list of abbreviation and adapt the formatting according to our guidelines for reviews available here: http://bmcmedicine.biomedcentral.com/submission-guidelines/preparing-your-manuscript/reviews

Our response: We appreciate this decision and formatted the text according to the Instructions for Authors for a review article.

We have noticed that there’s some text overlap with other sources in a ‘Mutation baseline value in healthy individuals’ section, namely paragraphs 3 and 4 (ref. 56 - 10.1016/j.tig.2010.05.003, ref. 62 - 10.1073/pnas.1607794113, respectively)

Our response: We carefully rephrased the respective paragraphs.

Further changes
On page 9, discussing the efforts by GRAIL, we added the recent publication by Aravanis et al. (Cell 168:571-574) as a reference.

I hope that all points were addressed satisfactorily. Thank you for your assistance and I look forward to hearing from you.