Peer Review File

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Reviewer Comments

Well- designed study with good methods with quality control. Manuscript was short and succinctly written. Just some minor comments

1) *This area has been well researched and reported with multiple publications previously and this study just confirmed the known finding in the field of T790M liquid biopsy about the sensitivity and specificity. However, it is interesting to note that second liquid biopsy detect T790M in several samples*

Reply 1: We thank the reviewer for highlighting the novelty of detecting T790M in a second liquid biopsy.

2) *Author reported that more than 3 positive droplets were considered 'positive' for T790M detection. This may need to be elaborated (is this based on literature review of other studies, have you done any negative control and found that the cut-off level to be 3 positive droplets as you have seen up to 3 droplets for healthy control plasma samples?). Without clarification, there is a possibility that patient samples with 1-3 positive droplets are in fact positive with low allelic frequency, and perhaps this patients should have a second sample tested.*

Reply 2: We now refer that the cut-off is used according to the recommendations of the manufacturing company.

Lines 171-174: Samples with three or more positive mutant droplets were considered positive, as recommended by the best practice guidelines for rare mutation detection (25). If one or two droplets were observed, the result was considered inconclusive and whenever possible a second sample was collected and tested.

3) *Discussion is quite brief. I couldn't find any discussion about limitation of this study eg. retrospective nature and bias associated,*

Reply 3: We have extended the Discussion as suggested.

Lines 221-222: Although this was a retrospective study potentially influenced by bias associated with patient selection, (…).

4) *Do you have more information on clinical data for these patients? if not this should be discussed in discussion as limitation. Relevant information will be when the plasma were taken (pre treatment with 3rd gen TKI or some were taken post treatment). Whether all patients only had 1 line of treatment with TKI or if they have received other treatment besides TKI eg chemo/trial. Burden of disease/absence or presence of visceral disease will be relevant as it will affect about much ctDNA will be detected in plasma.*

Reply 4: Additional clinical information is available for some, but not for all patients studied. We will share this clinical data upon request.

5. *Page 5 line 223-230 the author said T790M is seen more commonly in exon19del patients. Perhaps some reference could be put in to discuss that it is known that patients with x19del have better prognosis in general than L858R and some speculations of whether it is due to them having T790M (therefore have an additional line of treatment with 3rd gen TKI) or whether being on 1st gen TKI for longer increase the chance of T790M will be interesting for discussion.*

Reply 5: We thank the reviewer for raising this interesting point. The Discussion was modified accordingly.

Lines 235-241: A more recent literature review confirmed that detection of the T790M mutation was more frequent in del19 mutated patients (53%) than in L858R mutated patients (36%) with acquired resistance to EGFR-TKIs (28). One possibility is that patients with the del19 mutation are more sensitive to TKIs, and therefore cells with the T790M mutation are more likely to be selected and enriched (28).