Author’s response to reviews

Title: Exome sequencing of primary breast cancers with paired metastatic lesions reveals metastasis-enriched mutations in the A-kinase anchoring protein family (AKAPs)

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Response to comments:

We want to thank the reviewer Dr Callari for important and insightful comments.
I will try to meet them in the order they are brought up.

1) Data from Lefebvre et. al. PLOS 2016 (1) was suggested in order to compare our results with other data sets. This paper presents mutations from 216 metastatic tumors without paired primary tumors. Instead a subset of primary tumors from the TCGA data set are selected and used as reference. This is a drawback and limits the clinical importance of the data.
Nevertheless, AKAP mutations were found in 30 out of 216 patients (14%).

In total 42 AKAP mutations were found in ten AKAP genes (3, 4, 6, 8, 8L, 9, 11, 12, 13, 14). Even after removing "MILD impact" mutations 27/216 = 12.5% of patients carry mutations. Since the study lacks primary tumors it is impossible to know the background mutation status of these genes and they have to be approximated with data from TCGA via www.cbioportal.org (TCGA provisional data set) in which 6.7% of the tumors carry AKAP mutations, but probably lower if considering low impact and neutral mutations. It should be noted that this number includes all 14 AKAP genes. On the other hand AKAP mutations are more numerous than the most enriched gene ESR1 (lowest FDR) that is mutated in 21 out of 216 patients (9.7%).

Since Lefebvre et al. do not list the mutations called from the bam. files of the TCGA data we cannot know the background rate of the 8 listed enriched genes but it is generally <1-2% using cbioportal.

Although we cannot state the statistical significance of the AKAP mutations from this paper it is clear that a high proportion of the metastatic tumors contains AKAP mutations to a higher degree than expected from primary tumor data.

We therefore believe that this study support our findings.

2) We have clarified that fnac were not FFPE, all where fresh frozen directly after aspiration. Please see page 8.

3) It is true that there seem to be a high degree of heterogeneity in the first cohort with a low number of shared mutations. We have added some comments in the discussion on this topic. New reference added (2).
4) Regarding using different bioinformatic pipelines. We have changed some formulations according to the comments. However, even if not identical bioinformatic platforms, we still think this is a valid approach since no direct conclusions or comparisons are made between the different data sets.

References
