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Clonal hematopoiesis detection by simultaneous assessment of peripheral blood mononuclear cells, blood plasma, and saliva

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Conflict of Interest:

JRW is owner of Resphera Biosciences. RLL is on the board of Qiagen and Ajax Therapeutics. He is or has recently been a scientific advisor to Imago, Mission Bio, Syndax. Zentalis, Ajax, Bakx, Auron, Prelude, C4 Therapeutics and Isoplexis. He has research support from Ajax and AbbVie, consulted for Janssen, and received honoraria from Astra Zeneca and Kura for invited lectures. MBF has acted as a paid consultant for Abbott, Genzyme, and Bristol Meyers Squibb for unrelated work. LAD is a member of the board of directors of Quest Diagnostics and Epitope. He is a compensated consultant to Absci, GSK, Innovatus CP, Se'er, Delfi, Blackstone and Neophore. All other authors have declared that no conflict of interest exists.

MAIN TEXT

Most genomic analyses of biofluids rely on DNA from a single source, like peripheral blood, which delivers reproducible results when reporting genomic alterations highly represented in the specimen. However, there may be value from simultaneously measuring somatic mutations in multiple biofluids from the same individual. We posited that observation of the same mutation across biospecimens could increase confidence in positive mutation calls, improve the limit of detection and perhaps reveal important clinical relationships.

To test this, we used clonal hematopoiesis (CH), a mutational profile commonly found in hematopoietic cells. CH results from the expansion of hematopoietic stem/progenitor cells and their differentiated progeny which harbor $\geq$1 somatic mutation and tracks with age (1). Many of these mutations are identical to those in acute myeloid leukemia (AML) and are associated with increased risk of cardiovascular disease, as well as AML itself (1), and can be tracked in peripheral blood mononuclear cell (PBMC) DNA or in cell-free DNA (cfDNA) from plasma.

We derived DNA from PBMCs (buffy coat) and saliva, and cfDNA from plasma, designed a high coverage capture panel to detect CH mutations and performed an analytical validation study of the assay targeting the coding regions of 19 genes associated with CH (1). Our assay identified 94.71% of variants at standard depth (1,000X), and 100% of cases at high depth (10,000X) and was highly concordant to matched buffy coat sequencing data using a targeted tissue panel (MSK-IMPACT) (2). (Supplementary Figure S1A and B, and Table S1) (2). The limits of detection were 1% VAF for standard depth and 0.3% VAF for high depth (Supplementary Figure S1 C and D and Table S2).

From 60 individuals with CH, there was high concordance between mutations detected in the buffy coat and saliva ($R^2 = 0.95$) and a similar level of concordance ($R^2 = 0.86$) in cfDNA and

the buffy coat (Figure 1A). There were no meaningful differences in VAFs between the three compartments: buffy coat (mean VAF 8.16%), cfDNA (mean VAF 7.78%), and saliva (mean VAF 7.00%, Figure 1B).

Given the relationship of CH to AML, we sequenced the buffy coat and cfDNA from 5 Myelodysplastic Syndromes (MDS) and 6 AML patients for CH variants (Figure 1C and D). In both AML and MDS, there was high concordance in the mutations detected between the buffy coat and cfDNA. VAFs between buffy coat and cfDNA in AML were also concordant. However, in the cases of MDS, the VAFs in cfDNA were significantly higher than in the buffy coat, which was not explained by an increase in peripheral circulating blasts (Supplementary Table S3). In AML, malignant blasts were present in the circulation of our patients, whereas in MDS they were confined to the bone marrow and absent in the circulation, which may explain the relative lower VAF in the buffy coat in MDS.

We next evaluated whether combining sequencing data from multiple biospecimen types would increase confidence of variant calls. We found higher confidence scores and higher sensitivity for calling mutations at low VAFs when assessing the combination of two and three biospecimens (Figure 1E) albeit at a slightly lower specificity (Supplementary Figure S1I).

Using CH as a model, simultaneous assessment of somatic mutations from multiple biofluids identified practical clinical and technical applications that are likely applicable to other biologic and clinical scenarios where mutations are measured in biofluids. Integration of the differences in mutations in DNA, and potentially other biomolecules, from different biofluid compartments will likely demonstrate differences that are measurable and actionable. In the case of hematologic malignancies, differences in VAF and diversity in circulating myeloblasts when compared to cfDNA, provides insights into disease progression, and can help distinguish

malignant from benign clonal events in the blood, in essence providing the ratio between mutant cfDNA (plasma DNA) and circulating tumor cells (buffy coat). Whether simultaneous assessment of somatic mutations from multiple biofluids is applicable to other biologic and clinical scenarios remains to be seen.

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Figure 1 – Concordance of mutations in CH, MDS and AML between biofluids and sensitivity/specificity of multiple biospecimen calls

(A) Scatterplot (L) and Boxplot (R) of VAFs in buffy coat vs saliva for CH showing saliva has lower VAFs than buffy coat (p = 4.9E-05, 95% CI (-4.21, 1.90)). **(B)** Scatterplot (L) and Boxplot (R) of VAFs in buffy coat vs cfDNA in CH showing nonsignificant differences in buffy coat and cfDNA VAFs (p = 0.11, 95% CI (-2.99, 3.13)). (**C**) Scatterplots of each AML and MDS sample comparing the VAFs buffy coat and cfDNA. **(D)** Dotplots of the VAFs in buffy coat vs cfDNA in AML, CH and MDS**. (E**) Observed sensitivity when incorporating one (single – yellow), two (duo – green) or three (trio – blue) biospecimens to establish a mutation call. *Grey line represents x=y. Comparisons were done using paired t-tests. Box – interquartile range, middle bar – median, whiskers – minimum and maximum values, dots – outliers.*