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# Survival implications of the relationship between tissue versus circulating tumor DNA *TP53* mutations – a perspective from a real-world precision medicine cohort

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## Abstract

Interrogating the genomics of circulating tumor DNA (ctDNA) (the liquid biopsy) has advantages in patients in whom tissue biopsy is difficult. However, the reported concordance between genomic analysis of tissue DNA and ctDNA is variable among studies. Herein, we characterized the clinical implications of the relationship between mutations in *TP53* genes in tissue DNA versus ctDNA. The molecular profiles of both liquid (Guardant Health) and tissue biopsies (Foundation Medicine) from 433 patients were analyzed (pan-cancer setting). In 71/433 (16%) cases, all same *TP53* mutations were detected in both tissue DNA and ctDNA; 18/433 (4%), same mutation plus additional mutation/mutations; 27/433 (6%), different *TP53* mutations. In 99/433 (23%) cases, *TP53* mutations were detected only in tissue DNA; 43/433 (10%), only in ctDNA; and in 175/433 (40%), no *TP53* mutations were detected in either test. When *TP53* mutations were identical in tissue and ctDNA, the alterations were enriched for nonsense mutations, and survival was significantly shorter in multivariate analysis (as compared to different mutations in ctDNA versus tissue or no mutations); this finding was independent of tumor type, time interval between tests, and the %ctDNA for *TP53* mutations. In summary, in 16% of 433 patients with diverse cancers, *TP53* mutations were identical in tissue DNA and ctDNA. In these individuals, the alterations were enriched for stop-gain (nonsense) mutations (results in a premature termination codon). Though unknown confounders cannot be ruled out, these patients fared significantly worse than those whose ctDNA and tissue DNA harbored different *TP53* mutation portfolios or no *TP53* mutations.

## Introduction

Cancer is caused by a sequence of acquired somatic genomic aberrations (1). Recent advances in cancer genome sequencing led to large-scale projects characterizing the genomic landscape of many tumor types (2, 3). These projects contributed to the identification of cancer genomic involvement in specific tumor types (4), and, in addition, it was realized that, often, each patient's tumor harbors a unique mutational profile (5, 6). In the last decade, developments in sequencing technology made tumor genomic analysis feasible for clinical application. In parallel, many new drugs targeting specific cancer genes have been developed and approved. These coupled endeavors hold promise for the application of a personalized medicine approach in cancer treatment (7-9). This approach already led to major clinical achievements such as targeting *BRAF* V600E mutations in melanoma (10) and *EGFR* mutations in lung cancer (11), among others. It was also shown, in a meta-analysis of clinical trials, that a personalized (biomarker-based, especially genomic biomarkers) approach is more beneficial compared to chemotherapy or targeted therapy of unselected patients (12).

Traditionally, genomic analysis of tumors is performed on DNA from tumor specimens obtained by surgery or biopsy. Circulating tumor DNA (ctDNA) represents tumor-derived DNA molecules that circulate in the bloodstream. These molecules can be isolated and analyzed for genomic alterations ("liquid biopsy") (13). ctDNA has the advantage of requiring only a small tube of blood and, hence, being less invasive than tissue biopsy. However, tissue biopsy still remains standard in most cases for the initial diagnosis and molecular profiling. The advantages of liquid biopsy are especially exploitable for cases in which tumor material is not sufficient for molecular profiling, to monitor response to therapy, and to identify the emergence of mutations causing resistance and/or that can be targetable by other drugs (14-17).

The concordance level between genomic analysis of tumor DNA and ctDNA when taken simultaneously can be as high as 80-90%(18-21) , though other investigators have shown lower concordance (15). It should also be kept in mind that there may be biologic reasons that underlie differences between tissue and ctDNA results: (i) ctDNA can be suppressed by therapy; (ii) tissue DNA tests the genomics in a small piece of tissue, while ctDNA may reflect shed DNA from multiple metastatic sites (hence better

representing tumor heterogeneity) (15): and (iii) shedding of DNA into the bloodstream may be limited from some sites. To our knowledge, the clinical implications of the relationship between tumor DNA and ctDNA concordance for *TP53* are not established.

Somatic mutations in the tumor suppressor *TP53* gene are the most frequent alterations in human cancers. More than 80% of somatic and germline *TP53* alterations are missense mutations leading to the synthesis of a stable mutant protein that accumulates in the nucleus of tumour cells (22). Analysis of the data generated by the various cancer genome projects highlights the high frequency of *TP53* mutations and reveals that several *TP53* hot spot mutants are the most common oncoprotein variants expressed in several types of tumors (22). Hence, focusing on mutations in *TP53* seems an optimal starting point to investigate the clinical importance of comparison between tumor tissue DNA and ctDNA taken at different time points of the disease course.

In the current study, we hypothesized that patients with greater concordance between tissue and blood *TP53* alterations might have poorer survival since the concordance might reflect higher variant allele fraction of *TP53* in tumor. We analyse a unique clinical database of 433 patients with different tumor types. All patients underwent molecular profiling of tumor DNA and ctDNA. We focus our investigation on the relationship between both tests for *TP53* mutation status and the clinical correlates of this relationship.

# Materials and Methods

## Patients

The molecular profiles of both liquid and tissue biopsies from 433 consecutive eligible patients with different cancer types, seen at the University of California San Diego Moores Cancer Center starting in June 2014 were analyzed. We included patients who had no prior treatment as well as those with prior systemic therapies. The last ctDNA specimen was obtained on 09/2017. Eligibility implied adequate follow up and demographic data availability and patient meeting UCSD IRB guidelines for consent or waiver. Demographics of each of these patients were provided by chart review, including, but not limited to age, gender, cancer diagnosis, tumor origin, date of biopsy report or blood test, date of diagnosis, and survival time (**Figure 1).** The study was performed in accordance with the UCSD internal review board-approved protocol (NCT02478931) and for any investigational therapies for which the patient gave written consent.

## Next-Generation Sequencing

The ctDNA molecular profiles were provided by Guardant Health Inc. (http://www.guardanthealth.com/); tissue testing was performed by Foundation Medicine (https://www.foundationmedicine.com/genomic-testing#how-does-it-work). Both laboratories are Clinical Laboratory Improvement Amendment (CLIA)-accredited and Foundation Medicine test is now FDA approved for reporting pathogenic variants. Detailed Methods are previously published(23, 24).

**ctDNA testing:** As reported in Lanman and colleagues, 5–30 ng of ctDNA was isolated from plasma (two 10 ml Streck tubes drawn for each patient) and sequencing libraries were made with custom in-line barcode molecular tagging and complete sequencing at 15,000× read depth (24). The panels use hybrid capture followed by next-generation sequencing (NGS) of the crucial exons in a panel of 54–73 genes and report all four major types of genomic alterations (indels, fusions, point mutations, and copy number amplifications). Post-sequencing bioinformatics matches the complementary strands of each barcoded DNA fragment to remove false-positive results(24). The variant allele fraction (%ctDNA) is calculated as the number of mutated DNA molecules divided by the total number (mutated plus wild type) of DNA fragments at that allele. We used the

maximum %ctDNA if a patient had two different TP53 alterations, unless we referred to a specific TP53 alteration. The majority of cell-free DNA is wild type; hence, the median %ctDNA of somatic alterations is <0.5%. The analytic sensitivity reaches detection of one to two single-mutant fragments from a 10-ml blood sample (0.1% limit of detection), and the analytic specificity is greater than 99.9999%(24).

**Tissue NGS:** Tissue NGS was performed at Foundation Medicine (CLIA lab) with assay panels of 236 or 315 genes, with detailed methods as previously described (Cambridge, MA, www.foundationmedicine.com)(23). Average depth of sequencing was greater than 250x, with 100x at > 99% of exons. This method of sequencing allows for detection of copy number alterations, gene rearrangements, and somatic mutations with 99% specificity and >99% sensitivity for base substitutions at ≥5 mutant allele frequency and >95% sensitivity for copy number alterations. A threshold of ≥8 copies for gene amplification was used. All tissue samples (Formalin-fixed, paraffin-embedded (FFPE)) were reviewed by a pathologist to ensure a sample volume of ≥1 $mm^3$, nucleated cellularity ≥80% or ≥30,000 cells and that ≥20% of the nuclei in the sample were derived from the tumor.

**Variants of Unknown Significance:** Synonymous alterations and other variants of unknown significance (VUS) were excluded and only characterized alterations were included in the analysis(23).

**Characterized alterations**: Characterized alterations refer to pathogenic alterations that are not VUSs.

### Definition of *TP53* mutations concordance type

For each patient, the specific *TP53* alterations (or their absence) were compared between ctDNA and tissue DNA. We classified the concordance patterns into six types (**Supplementary Table 1 and Figure 2**): (i) Exactly the same *TP53* mutation in tissue DNA and ctDNA; (ii) Different *TP53* mutations in tissue DNA and ctDNA; (iii) *TP53* mutation/s only in tissue DNA, (iv) *TP53* mutation/s only in ctDNA; (v) No *TP53* mutation in both tissue DNA and ctDNA; (vi) Exactly the same + others *TP53* mutations in tissue DNA and ctDNA.

## Statistical analysis

Statistical analysis was performed using the R programming language version 3.5.1 (25). Cox proportional hazards regression model and multivariable analysis was performed using the survival(26) R package. Pairwise comparisons for the continuous time interval variable were performed by pairwise Wilcoxon test with false discovery rate (FDR) correction for multiple comparisons. Survival was examined by Cox regression; patients still alive at the last follow up were censored at that time. Survival time was calculated from time of diagnosis and the defined event was death. Quartile thresholds were created using R ntile command; in case of quartile borders value overlap, the algorithm assigns the first samples to the lower quartiles. Mutations type annotation and description of their landscape of *TP53* gene were performed using MutationMapper tool in cBioPortal (https://www.cbioportal.org/mutation_mapper ) (27) and Seshat (https://p53.fr/tp53-database/seshat ) (28). We defined T125M , Q331R and Q331H as splice mutations despite the fact that cBioPortal defined them as missense mutations because despite the fact that they appear in exons , they influence splicing (29). (However, even if defined as missense mutations, the statistical relationships do not change.) Visualization was performed by the R packages of ggplot2 (29), forest plots by the R package of forestmodel(30). Balloon plots representing the residuals of chi square test were performed using the R package of corrplot(31).
Data availability: Dataset is available as **Supplementary Table 2**.

## Results

Our cohort included 433 individuals with several different tumor types (**Figure 1**). All but 28 patients had advanced disease that was surgically unresectable and/or metastatic. For each patient, genomic analysis was carried out twice: (i) for DNA obtained from tissue biopsy (FoundationOne test) and (ii) for ctDNA (Guardant360 test). The median time between the tissue biopsy date and the blood draw for ctDNA was 3.7 months (range: -14.6, 241.4). The median age at the time of ctDNA liquid biopsy was 62 years (range, 19–93 years) and the median age at diagnosis was 58.6 years (range 2.2 to 90.5 years). Approximately 55% of patients were women (**Supplementary Table 3**). Mutations in *TP53* gene were found in 215 (49.7%) tissue biopsies and in 159 (36.7%) ctDNA analysis. The landscape of the mutations in both tissue and ctDNA are given in **Supplementary Figure 1 and Supplementary Table 4.** In both tissue DNA and ctDNA, most of the mutations are located in the active domain of *TP53.*

### Relationship between tissue and ctDNA *TP53* mutations

**The type of *TP53* mutation correlated with whether or not it was found in both tissue and ctDNA**: Characterization of the relation of specific *TP53* mutations in tissue DNA as compared to ctDNA resulted in six mutation concordance categories (see **Methods, Supplementary Table 1**, **Figure 2**). There was an association between mutation type and the mutation concordance categories. Thus, testing the three major mutations concordance categories of: (i) “all same TP53 mutations”, (ii) “only tissue DNA TP53 mutations” and (iii) “only ctDNA TP53 mutations” concordance categories - there was strong association (p=2.6 e-10, chi-square test) between the mutation type and mutation concordance category. There were more (1) frameshift, (2) splice site, (3) in-frame deletion, and (4) "others" mutations in "only tissue DNA TP53 mutations" category whereas there were more nonsense mutations (“stop gain”) in “all same TP53 mutations” category (**Figure 3, Supplementary Table 5**).

**Same *TP53* mutations were more likely to be found in tissue and ctDNA when there were shorter time intervals between the two tests**: There was a significant association between mutation concordance category and the absolute time interval between the tests (p=0.038, chi-square test). As shown in **Figure 4a,** and

**Supplementary Table 6** – "all same TP53 mutations" category is associated with the shorter time interval of <2 months and "only ctDNA TP53 mutations" category is associated with the longer time interval of >6 months between tissue biopsy and blood draw. A time dependent linear analysis (instead of using the 2 month and 6 month cut offs between ctDNA and tissue sampling) showed no substantial change in the conclusions (see **Supplementary Table 7**).

**Colorectal cancer was more likely to have the same *TP53* mutations in tissue DNA and ctDNA than other tumor types:** There was a significant association between tumor types and mutations concordance categories (p=4.534e-07, chi-square test). As shown in **Figure 4b** and **Supplementary Table 8** – "all same TP53 mutations" category was positively associated with colorectal tumors and negatively associated with brain tumors. "Only tissue DNA TP53 mutations" category was associated with brain tumors. In addition – "Same + other TP53 mutations" category was overrepresented in head and neck tumor type.

## Relationship between tissue and ctDNA *TP53* mutations and survival

**Detecting the same *TP53* mutations in blood and tissue was associated with shorter survival:** We tested for the association between mutation concordance category and survival from diagnosis (**Figure 5**). Testing for all six mutation concordance categories was significant (p=0.014, cox regression test). The shortest survival was for patients in "all *TP53* same mutations" category. This result was significant after multivariate cox regression controlling for age, sex, %ctDNA, number of mutations in ctDNA, tumor type and time interval between the two genomic tests (OR 1.57, p=0.03, cox regression test). In contrast, patients with the other mutation concordance categories had longer survival, which was comparable to patients with "no *TP53* mutations in both" (odds ratio range: 0.85-1.14, not significant). We also analyzed survival from ctDNA blood test and found that all same *TP53* mutations versus other mutation categories (i.e. discordant ctDNA versus tissue DNA *TP53* mutation status) had significantly shorter survival: HR 2.05, 95% CI (7-15.7months); p=3 e-05.

**Higher *TP53* %ctDNA was associated with shorter survival from diagnosis:** There was a significant association between higher %ctDNA in the blood for *TP53* mutations and shorter survival from diagnosis (p=0.0003, cox regression test) (**Figure**

**6a**). This factor was not significant in the multivariate analysis controlling for mutation concordance category, age, sex, tumor type and time interval between the genomic testing (**Figure 5b**).

**Higher number of characterized mutations (pathogenic alterations that are not VUS – in *TP53* together with other genes) was associated with shorter survival from diagnosis:** There was a significant association between mutation number in tissue DNA (p=0.0004, cox regression test) or in ctDNA (p=1.1E-6, cox regression test) and survival (**Figure 6b,c,** respectively). This was also confirmed for ctDNA in multivariate analysis controlling for mutation concordance category, age, sex, tumor type and time interval between the genomic testing (**Figure 5b**).

## Discussion

In this study, we analyzed a data set of 433 cancer patients with various tumor types. The patients underwent genomic testing of tumor DNA and ctDNA at different points of time in their disease course. Mutations in *TP53* gene were found in 49.7% tissue biopsies, and in 36.7% ctDNA analysis. The lower frequency of *TP53* mutations in ctDNA is expected since the yield of ctDNA is lower compared to tissue DNA. We focused our analysis on characterized mutations in the *TP53* gene (those that are no VUS). Specifically, we investigated the relationship between *TP53* mutation state in the tumor DNA and ctDNA. Accordingly – we defined 6 mutational categories describing this relationship: (i) exactly the same *TP53* mutation in tissue DNA and ctDNA; (ii) different *TP53* mutations in tissue DNA and ctDNA; (iii) *TP53* mutation/s only in tissue DNA, (iv) *TP53* mutation/s only in ctDNA; (v) no *TP53* mutation in both tissue DNA and ctDNA; and (vi) exactly the same + others*TP53* mutations in tissue DNA and ctDNA.

We noted that there were several genomic and clinical associations with mutation concordance categories. First, mutation concordance category was associated with the mutations type. Specifically, there were more frameshift, splice site, in-frame deletion, and "others" mutations in "only tissue DNA *TP53* mutations" category whereas there were more nonsense mutations (“stop_gain”) in “all same *TP53* mutations” category. This may reflect increased selective advantage of nonsense mutations compared to missense mutations(32) that cause them to have higher variant allele frequency and

thus higher detection rates in ctDNA(33). An alternative explanation might be the possible presence of nonsense mutations in more aggressive parts of the tumor that more easily release DNA to the blood. Nevertheless, it is unclear why frameshift mutation are more abundant in “only tissue DNA *TP53* mutations” category as they should have comparable selective advantage to nonsense mutations.

Second, as expected, the same mutation in *TP53* appeared more frequently in both tissue DNA and ctDNA in cases for which the time interval between the tests was shorter, possibly reflecting bigger genomic distance at greater time intervals(34) due to tumor evolution. By contrast, there was higher frequency of “only cDNA *TP53* mutations” in patients for which there were more than six months between tissue DNA and ctDNA tests. This could to be due to tumor evolution (including possible treatment-related selective pressure) that is more marked in the group with largest time interval between the tissue DNA and ctDNA test.

The third association was with tumor type. The yield of ctDNA testing in brain tumors is among the lowest among tumor types(35), probably due to lower exposure of brain tumor to the circulation due to the blood brain barrier(14). By contrast, higher rates of “all same TP53 mutations” categories were observed for colorectal cancer, perhaps reflecting higher frequency of ctDNA detection in this tumor type(14). Interestingly, we previously found colorectal cancers (versus other malignancies) to also have higher concordance between blood and tissue results for *KRAS* alterations(36).

The fourth finding was the association of mutation concordance category with survival. Specifically, “all same *TP53* mutations” category was associated with lower overall survival (OS) whereas the other mutations concordance categories had OS comparable to the category of “no *TP53* mutation in both”. In multivariate analysis, this finding was independent of other potentially confounding factors including the time interval between tests and the %ctDNA (which is believed to reflect tumor burden). The reason for this finding is unclear, but could be hypothesized to be related to a higher variant allelic fraction of *TP53* mutation in the tumor (either primary or metastatic), which merits investigation in further studies where such data is available

There are several limitations in this study. The cohort is heterogonous and included diverse tumor types;  future analyses of larger more homogenous cohorts are required to validate our findings. On the other hand, our observations may speak to the generalizability of the results across tumor types.  In addition, the time intervals between the tissue DNA test and the ctDNA were variable. Nevertheless, multivariable analysis showed that the association between mutation concordance category and OS was independent of tumor type and time interval between tests.  Also, it is conceivable that some of the different mutations in ctDNA represent clonal hematopoiesis (37).  Another issue is that, though we found that when *TP53* mutations were the same in tissue and ctDNA, survival was significantly shorter in multivariable analysis, statistical significance does not necessarily imply clinical significance, as some of the actual survival times were not dramatically different; furthermore, it is conceivable that there were unknown confounders in the analysis.  Finally, we did not consider the effect of therapy on detection of ctDNA alterations, and we limited our analysis to the most common gene—*TP53*; future analyses might consider including other genes, though they occur at significantly lower frequencies, and might interrogate the relationship with therapy. Notably, this is a real-world cohort, rather than a prospective analysis with pre-specified analyses.  The above mentioned heterogeneity of the cohort represents real-world application of tissue DNA and ctDNA analysis and enables interesting insights into the clinical meaning of the relation of genomic findings in tissue DNA and ctDNA.  However, verifying the true clinical significance of testing for *TP53* or any other component of ctDNA will require prospective studies with more focused clinical questions.

To summarize, in this study we characterized the relationship between genomic testing of *TP53* in tissue DNA and ctDNA.  Patients with the same *TP53* mutation portfolio in tissue and liquid biopsy had tumors enriched for premature termination codons (nonsense mutations).  Further, we have shown that the relationship between *TP53* mutations in tissue DNA and ctDNA has clinical meaning. Specifically, patients in whom specific characterized *TP53* mutations appear unchanged between tissue DNA and ctDNA had shorter survival.

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## Figure Legends

**Figure 1**. **Pie chart of cancer types.**

**Figure 2. Pie chart of TP53 mutations concordance category.**

**Figure 3. Association of mutation concordance categories and mutation type.** **a.** in a table, **b.** in a balloon plot that represents the residuals of the chi square test. Red implies positive correlation between factors and blue implies negative correlation. Intensity of the color reflects the magnitude of the correlation (greater intensity = greater degree of correlation)

**Figure 4. Balloon plots representing the residuals of the chi square tests of**: **a.** mutation concordance categories vs. time interval between the two genomic testing; **b.** mutations concordance categories and tumor types. Red implies positive correlation between factors and blue implies negative correlation. Intensity of the color reflects the degree of correlation (greater intensity = greater degree of correlation). **Abbreviations**: ctDNA= circulating tumor DNA, GI = gastrointestinal.

**Figure 5**. **Survival association between mutation concordance category as characterized by finding similar or different *TP53* mutations in tissue DNA and ctDNA. a.** Kaplan Meyer curves for survival from diagnosis; **b.** Forest plot of the multivariate cox regression. All 433 patients were included. Mutation concordance category refers just to *TP53* mutations; ctDNA percent is for *TP53* only and refers to the *TP53* mutation with maximum %ctDNA; ctDNA mutations number refers to all characterized (pathogenic) mutations, not just *TP53*; VUS excluded throughout; age, ctDNA percent, ctDNA mutations number and time between tests were analyzed as continuous variables.

**Figure 6. Survival plots for variables segmented into quartiles. a.** survival plot of ctDNA percent for *TP53* mutations (refers to alteration with maximum %ctDNA in each patient). Quartiles: 0 – (0), 1 –(0.1-0.32) , 2 – (0.33-1.5), 3-(1.5-6.7) ,4-(6.90-75). **b.** survival plot for mutations number in tissue DNA (includes all characterized pathogenic mutations that are not VUS, not just for *TP53* gene). Quartiles: 1- (1-2), 2- ( 2-4), 3 – (4-6), 4 – (6-31). **c.** survival plot for mutations number in ctDNA (includes all characterized

pathogenic alterations that are not VUS, not just for TP53 gene). Quartiles: 1- (1), 2- ( 1), 3 – (1-2), 4 – (2-17). It should be noted that mutations number in tissue DNA and ctDNA does not stand for the more general measure of tumor mutation burden (TMB), but rather for all characterized pathogenic mutations reported.