Use of Liquid Biopsies in Clinical Oncology: Pilot Experience in 168 Patients

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Statement of translational relevance:

We are currently facing many changes in traditional paradigms for cancer treatment. One of the most striking advances is a deeper understanding of genomic abnormalities that drive a variety of tumors, offering unique opportunities of new treatment options. Analysis of cell-free DNA in the plasma of patients with cancer using next-generation sequencing (NGS) is a potentially powerful tool for the detection/monitoring of alterations present in circulating tumor DNA (ctDNA). Our results demonstrate that of the 98 patients with alterations, 71.4% had ≥ 1 alteration potentially actionable by an FDA-approved drug. Further, ctDNA tests provide information complementary to that in tissue biopsies. In addition, ctDNA tests may be useful in determining prognosis (percentages of ctDNA ≥ 5% correlated with shorter survival) and treatment, as patients who were matched to drugs targeting alterations identified in their plasma achieved encouraging rates of stable disease ≥ 6 months/partial remission (42%).

ABSTRACT

Purpose: There is growing interest in using circulating tumor DNA (ctDNA) testing in patients with cancer.

Experimental Design: 168 patients with diverse cancers were analyzed. Patients had digital next-generation sequencing (54 cancer-related gene panel including amplifications in *ERBB2, EGFR*, and *MET*) performed on their plasma. Type of genomic alterations, potential actionability, concordance with tissue testing, and patient outcome were examined.

Results: Fifty-eight percent of patients (98/168) had ≥1 ctDNA alteration(s). Of the 98 patients with alterations, 71.4% had ≥ 1 alteration potentially actionable by an FDA-approved drug. The median time interval between the tissue biopsy and the blood draw was 2.7 months for patients with ≥ 1 alteration in common compared to 14.4 months (P=0.006) for the patients in whom no common alterations were identified in the tissue and plasma. Overall concordance rates for tissue and ctDNA were 70.3% for *TP53* and *EGFR*, 88.1% for *PIK3CA*, and 93.1% for *ERBB2* alterations.

There was a significant correlation between the cases with ≥ 1 alteration with ctDNA ≥ 5% and shorter survival (median = 4.03 months versus not reached at median follow up of 6.1 months; P<0.001). Finally, five of the twelve evaluable patients (42%) matched to a treatment targeting an alteration(s) detected in their ctDNA test achieved stable disease ≥ 6 months/partial remission compared to two of 28 patients (7.1%) for the unmatched patients, P=0.02.

Conclusions: Our initial study demonstrates that ctDNA tests provide information complementary to that in tissue biopsies and may be useful in determining prognosis and treatment.

INTRODUCTION

Performing tumor biopsies remains the standard practice to establish cancer diagnosis and to detect potentially actionable alterations(1,2). However, tissue biopsies have limitations as they are invasive, expensive, and can expose the patient to pain and complications. Multiple serial biopsies are also unpalatable to patients, and some tumor sites are difficult to access. In addition, a biopsy of the primary site or one metastatic site may not reflect the complete genomic makeup of the malignancy, as heterogeneity can be found both between tumor lesions and within the same tumor(3–5).

Circulating tumor DNA (ctDNA) is composed of small fragments (about 150-200 base pairs) of DNA that are released from cells undergoing apoptosis or necrosis in malignant lesions (primary or metastatic) and can be detected and sequenced in the blood of patients with cancer(6,7). Detection of cell-free DNA was first described in 1994 by Sorenson et al.(8), and numerous articles were published in the past few years, indicative of a growing interest in this non-invasive diagnostic method(9–18). Potential application scenarios include using ctDNA to supplement or substitute for tissue biopsies, especially in cases where tissue biopsies are risky or the quantity/quality of the tissue biopsied does not allow testing, and to use repeat sampling and genomic profiling to detect tumor evolution, response and resistance(6,19–21). Of interest, fluids such as urine can also be used to detect ctDNA(22,23). Finally, the use of ctDNA tests would increase chances to interrogate shed DNA from multiple metastases, perhaps better reflecting heterogeneity of the cancer burden in its entirety.

Herein, we report our clinical experience with the use of ctDNA testing in 168 patients with diverse cancers followed at UC San Diego, Moores Cancer Center.

MATERIAL AND METHODS

Patients

We retrospectively reviewed the clinicopathologic and outcomes data of 168 consecutive patients with diverse solid cancers followed at UC San Diego Moores Cancer Center, for whom molecular testing (ctDNA test) had been performed on their plasma (June 2014 until February 2015). This study (PREDICT-UCSD (Profile Related Evidence Determining Individualized Cancer Therapy); NCT02478931) was performed and consents obtained in accordance with UCSD Institutional Review Board guidelines.

Sequencing

Digital Sequencing™ was performed by Guardant Health, Inc. (Guardant360, www.guardanthealth.com/guardant360/), a CLIA-certified and College of American Pathologists (CAP)-accredited clinical laboratory. This test identifies potential tumor-related genomic point mutations within 54 cancer-related genes (**Supplemental Table 1**) as well as amplifications in *ERBB2, EGFR*, and *MET* through analysis of cell-free DNA extracted from plasma (from two 10 ml blood tubes). This circulating tumor DNA assay has high sensitivity (detects 85%+ of the single nucleotide variants detected in tissue in advanced cancer patients) and analytic specificity (>99.9999%)(24).

In addition, N=101/168 patients (60%) who had ctDNA results also had next generation sequencing (NGS) performed on their tissue. For these 101 patients, tissue testing was done by Foundation Medicine (FoundationOne™, Cambridge, Massachusetts, http://www.foundationone.com). Hybridization-based capture from 315 cancer-related genes plus introns from 28 genes often rearranged or altered in cancer (N = 63 patients) and from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer (N = 38 patients) was performed. In a study using 249 FFPE cancer specimens characterized by established assays, the FoundationOne test

sensitivity achieved was 95-99% across alteration types, with high specificity (positive predictive value >99%)(25).

Concordance Rate

For the N=101 who had both types of tests (plasma and tissue), we aimed to assess the concordance. Since the tissue and plasma tests sequenced different gene panels, we only considered alterations in common for both tests (i.e. alterations tested and which could be detected by both tests (N = 54 genes)). Of the 101 patients, 63 patients had ≥ 1 alteration(s) detected in the tissue which could have been detected by the ctDNA test. We looked at specific concordance rates for *TP53, EGFR, PIK3CA* and *ERBB2* and computed the corresponding kappa statistics, which is a conservative measurement of relative agreement that takes into account agreement by chance. Kappa ranges from $\kappa = 1$ (perfect agreement) to $\kappa = 0$ (no agreement other than would be expected by chance).

Therapy and Actionability

Treatment was considered "matched" if at least one agent in the treatment regimen targeted at least one aberration or pathway component aberrant in a patient's molecular profile or a functionally active protein preferentially expressed in the tumor (as assessed by any standard of care testing, e.g. estrogen receptor (ER) or HER2 as well as NGS performed on tissue and/or ctDNA).

Statistical Analysis

When appropriate, median and 95% confidence intervals (95% CI) or range were reported. The following clinical endpoints were considered: (i) rate of [stable disease (SD) ≥ 6 months/partial response (PR)/complete response (CR)]; (ii) progression-free survival (PFS) of the first line of therapy given after ctDNA results

(PFS2); and (iii) overall survival (OS). SD, PR, or CR was determined per assessment of the treating physician. PFS was defined as the time from the beginning of therapy to progression or the time to last follow up for patients that were progression-free (patients that were progression-free on the date of last follow up were censored on that date). OS was defined as the time from the ctDNA test results date to death or last follow-up date for patients who were alive (the latter were censored on that date). The cut-off date of the analysis was June 30, 2015; all patients who were progression-free (for PFS) or alive (for OS) as of the date of analysis were censored on that date unless their date of last follow up was earlier, in which case that was the date of censoring.

Whenever appropriate, Chi-Square tests were used to compare categorical variables and the non-parametric Mann Whitney-U test to compare two groups on one continuous variable. Binary logistic regressions were performed for categorical endpoints and multiple linear regressions for continuous variables. PFS and OS were analyzed by the Kaplan-Meier method(26) and the log-rank test was used to compare variables. Statistical analysis was performed by MS with IBM Statistics SPSS version 22.0.

RESULTS

Patient's characteristics

One hundred and sixty-eight consecutive patients who were seen at UCSD Moores Cancer Center and had ctDNA molecular testing performed were reviewed and analyzed. There was a slight preponderance of women over men (58%). The median age at diagnosis was 54.5 years (95%CI: 51-59 years). The majority of our patient population was Caucasian (67%), followed by Asian (15.5%) and other (8.9%). The most common primary tumor sites were brain (33.3%), followed by lung (28%), and breast (21.4%). The majority of patients (85.1%) had metastatic/recurrent/advanced unresectable disease at the time of ctDNA testing, **Table 1 and Supplemental Table 2**. Many patients that were tested and did not have metastatic/recurrent/advanced unresectable disease had brain tumors (which comprised 56 of our 168 patients tested (18 of whom did not have metastatic/recurrent disease at the time of testing).

Description of alterations and actionability of the detected alterations

Excluding variants of unknown significance, patients had a median of one alteration (range, 0-19). Fifty-eight percent of patients (98/168) had ≥1 alteration(s) identified in their plasma. The most common alterations were in *TP53* (31.5%), followed by *EGFR* (17.3%), and *MET* (10.1%), **Figure 1A**. In total, 244 alterations (215 mutations and 29 amplifications) were identified in 168 patients. Of the 98 patients with alterations, N=77 (78.6%) had mutations only, N=17 (17.3%) had both mutation(s) and amplification(s), and N=4 (4.1%) had only amplification(s).

We then examined if we could identify variables correlating with the number of alterations. In a univariable analysis, patients with gastrointestinal cancers, as well as with lymph node, bone, lung, and liver metastasis had a significantly higher number of alterations, while patients with brain tumors had significantly less detectable alterations.

Even so, 16 of 56 patients (28.6%) with brain tumors had an alteration, with a median number of alterations in all brain tumor patients of 0 (range, 0 to 2). In the multivariable analysis, only gastrointestinal cancers (P=0.001) was an independent predictor of a higher number of alterations while brain tumors correlated with fewer alterations (P=0.019) detected in the plasma, **Table 2.**

Of the 98 patients with alterations, 74 (75.5%) had at least one alteration that could potentially be targeted by an experimental drug or an FDA-approved drug (**Figure 1B**). Of interest, 71.4% of patients with alterations had ≥ 1 alteration potentially actionable by an FDA-approved drug. However, a limited percentage of patients (12%) had alterations potentially actionable by a drug approved by the FDA for their disease (on-label use).

Concordance of the ctDNA test with tissue genomic testing

Of the 168 patients who had ctDNA test results, 101 (60%) also had tissue genomic testing (FoundationOne, see Methods). However, only 63 patients had alterations in the tissue that also were part of the ctDNA panel used. Twenty-two of the sixty-three patients (35%) had ≥1 alteration in common between the tissue and ctDNA. In these 22 patients, the median time interval between the tissue biopsy and the blood draw was 2.7 months compared to 14.4 months (P=0.006) in the 41/63 (65%) patients in whom no common alterations were identified in the tissue and plasma, **Figure 2A**.

Lastly, in addition to comparing the molecular profiles in their entirety, we studied concordance for the most frequent alterations (**Table 3 and Figure 2B**). We found that the overall concordance rates (both tests positive/both tests negative in the 101 patients who had both tissue and ctDNA testings) were 70.3% for *TP53* and *EGFR* alterations, 88.1% for *PIK3CA*, and 93.1% for *ERBB2*.

We also compared the concordance rates between tissue and ctDNA profiling in patients for whom the time interval between the tissue biopsy and blood draw used for testing was ≤ 6 months (N=39 patients) versus those in whom the time interval was > 6 months (N = 62) and found that the concordance rate for *TP53* alterations was 82.1% versus 63% (P=0.046); for *EGFR* alterations, the concordance rate of ≤ 6 months versus > 6 months was 69% versus 71%; for *PIK3CA*, 85% versus 90%; and for *ERBB2*, 97% versus 87% (not significant P-values) (**Table 3 and Figure 2B**). Of note, we observed that *MET* alterations were only detected in ctDNA tests in the 101 patients who had both ctDNA and tissue testings (9/101 = 9% in ctDNA versus 0% in tissue).

Analysis of patient outcome

Patients had a median of one prior line of therapy (range, 0-11) before ctDNA testing. Of the 168 patients, 33 (19.6%) were treated with a "matched" therapy following molecular profile results, and 39 (23.2%) with an "unmatched" therapy; the remaining patients were not evaluable for treatment after test results, mainly because they died before treatment, were only treated with surgery and adjuvant therapy, were still on prior therapy, or were lost to follow up before treatment, **Supplemental Figure 1**.

The median time from tests results until treatment initiation was two months (95%CI, 1.4-2.7 months), often because physicians ordered testing before patients had failed their prior therapy in order to have a plan available in case of failure(2). The median follow up time from ctDNA results was 6.1 months (95%CI 5.6-6.5). The median therapy line in the advanced/metastatic setting was 2 (95%CI 1-3, range 1-8) for the unmatched patients, versus 2 (95%CI 2-3, range 1-10) for the matched patients (P=0.238).

Of the 33 patients who received a matched therapy, the drug was targeting an alteration(s) detected by the ctDNA test for fifteen patients (45.5%). Of the twelve

patients evaluable for response (three patients, too early to assess), five (42%) achieved stable disease (SD) ≥ 6 months/partial response (PR), **Supplemental Table 3**. In three of the five responders, the relevant actionable alteration was found first in ctDNA and later confirmed by tissue NGS. Of the 39 unmatched patients, 28 were evaluable for response. Of these 28 patients, 2 (7.1%) achieved SD ≥6 months/PR (P = 0.02 comparing 5 of 12 versus 2 of 28). For these 40 evaluable patients, the median line of therapy for the 12 matched patients was 2.5 (95%CI 2-4) versus 2 (95%CI 1-3) for the 28 unmatched patients (P=0.291). Matched patients had their ctDNA results after a median of 30.7 months after diagnosis versus 16.0 months for the unmatched patients, though this difference was not statistically significant (P=0.637).

Percentage of ctDNA detected

The median percentage of ctDNA detected for each mutation was 0.45% (range 0.1-75) (95%CI 0.3-0.6). We investigated whether or not the percentage of ctDNA correlated with clinical outcome parameters. We observed a strong correlation between the cases with at least one gene altered with a percentage of ctDNA ≥ 5% and shorter overall survival (median overall survival = 4.03 months versus not reached at a median follow up of 6.1 months; P=0.0001 in multivariable analysis), **Figure 3**. Patients with at least one alteration with ctDNA ≥ 5% had received a statistically higher median number of prior lines of therapy before ctDNA testing compared to patients with ctDNA < 5% (2 (95%CI 1-3) versus 1 (95%CI 0-1), P=0.009). However, multivariate analysis of prognosis revealed that ctDNA only was significant for overall survival.

Regardless of treatment, patients with at least one alteration with ≥5% ctDNA also had a shorter progression-free survival (PFS) (2.1 months (95%CI 1.2-3.0) versus 4.0 months (95%CI 2.5-5.5)) for the next immediate therapy following ctDNA testing, that did not reach statistical significance (P=0.124). No difference was noted for the

rate of SD ≥6 months/PR/CR (18.2% versus 17.5%, P=1.0). Finally, we observed that patients with ≥ 5% ctDNA for at least one alteration also had a higher median number of alterations (3 (95%CI 2-4) versus 1 (95%CI 1-1), P<0.0001).

DISCUSSION

This study describes our initial experience with using ctDNA molecular diagnostic tests, including the types of alterations detected and their actionability, the concordance with tissue testing, and the outcomes of patients treated with a matched therapy. Overall, 98 of our patients (58%) showed at least one alteration in their ctDNA. In tumors other than those of the brain, the percentage was 73%, while in brain tumors it was 29%. In a multivariable analysis, only gastrointestinal cancers (P=0.001) were independent predictors of a higher number of alterations detected in the plasma**, Table 2**. It is conceivable that the high vascularization of these type of tumors might have increased the accessibility and detection rate of alterations(27–29). In contrast, patients with brain tumors had significantly fewer alterations (even though about 29% of patients with brain tumors had detectable alterations). Bettegowda et al.(30) demonstrated that ctDNA was detectable in > 75% of patients with various cancers, and in less than 50% of primary brain cancers (N = 640 patients). Therefore, based on the study above and our current study, the blood brain barrier does not preclude primary brain tumors from shedding ctDNA into the circulation(31). For patients with primary brain cancers, cerebral spinal fluid may also serve as an alternative "liquid biopsy"(32).

In our study, when examining the concordance in patients who had both a tissue and ctDNA testing, 22/63 (35%) of patients had ≥1 alteration in common (**Figure 2A**). These 22 patients had a median time interval between the tissue biopsy and blood draw (used for the ctDNA test) of 2.7 months compared to 14.4 for patients in whom no common alterations were found (P = 0.006), consistent with the concept that the genomic background of tumors changes over time(33,34). When examining the percentage agreement for specific alterations (*TP53, EGFR, PIK3CA,* and *ERBB2*), the concordance rates between ctDNA and tissue NGS were in the same range (70% to

93%) of those previously reported (about 67 to 99%)(11,17,35,36). Although the number of patients was low, we observed that *MET* alterations were only detected in ctDNA (not in tissue in the 101 patients who had both tested), consistent with prior results showing higher rates of *MET* alterations in ctDNA(37); this conceivably could be due to the propensity of DNA bearing *MET*-related alterations to be shed into the blood.

Recently, Rothé et al.(38) investigated whether or not plasma could be used as an alternative to biopsies from metastatic sites for detection of molecular alterations. They analyzed and compared 69 tumor samples and 31 plasma samples originating from 17 patients with metastatic breast cancer and found that, in 13 of 17 (76%) patients, tumor and plasma provided concordant results when tumor and plasma were collected at the same time-point. In comparison, we had 39 patients who had tumor and blood collected at a close time point (≤ 6 months apart), and there was between 69% and 87% concordance for specific alterations (**Table 3**).

Of interest, a focused analysis on the positive cases (**Figure 2B**) revealed that both tests could independently detect alterations not found in the other test, stressing the clinical value and complementary nature of the techniques. This observation is perhaps not surprising considering the distinct advantages and disadvantages of each technique. For instance, ctDNA tests can theoretically detect shed DNA from multiple metastatic sites, whereas tissue biopsy DNA tests would discern only alterations in the small piece of tissue evaluated. On the other hand, ctDNA tests may not be sensitive enough to detect alterations that are important and easily identified in a tissue test, and not all sites of disease may shed DNA into the circulation. From a logistical standpoint, the median turn-over time (from blood sample collection until results) for the ctDNA test was 14 days. In comparison, our previous report demonstrated that the median time from tissue assay order until results is about a month (including approximately 15 days

necessary for pathologic specimen retrieval) (2). In addition, a blood draw is less invasive and less costly than a tissue biopsy, thus allowing for repeat testing. In an exploratory analysis, more patients achieved a SD ≥ 6 months/PR/CR in the matched group according to ctDNA results compared to the unmatched patients, (5 of 12 patients (42%) versus 2 of 28 patients (7.1%); P=0.02). The main reasons that patients were treated with an unmatched therapy were that they did not have any detectable alterations, no alterations were targetable, matching drug(s) were unavailable (e.g., clinical trial(s) too far away, no insurance coverage), and patient or physician choice(2,39). Although it was not statistically significant, we observed that the median line of therapy for the evaluable matched patients was higher, as well as the time from diagnosis to molecular results, possibly suggesting that oncologists resort to treating patients with a matched therapy later in the disease course.

When we compared patients with a percentage of ctDNA ≥5% versus <5%, we found that patients with at least one altered gene detected at levels ≥ 5% in the plasma had a significantly shorter survival (median survival =4.03 months versus not reached; P<0.0001, **Figure 3**) and PFS (for first therapy after ctDNA test) (2.1 months versus 4.0 months, P=0.124), consistent with prior studies describing prognostic value of ctDNA(6,30,40,41).

Our observations have several limitations. First, our study was retrospective. Hence, there could be unknown biases that influenced our analysis, despite the inclusion of multivariate analysis. Furthermore, when examining matched versus unmatched patients, the numbers of patients were small. Prospective studies are needed to further define the value of ctDNA tests for treatment. In addition, some of our patients did not have concurrent tissue and ctDNA biopsies and only the genes in common to both tissue and ctDNA testing panels were included, making comparison of

these modalities a challenge. On the other hand, the range of intervals between tissue and ctDNA tests allowed us to examine the influence of temporal separation of tissue biopsy and ctDNA test on molecular results and to observe that the longer the time interval between these tests, the more disparate the results. Finally, our patient population was heterogeneous, which could imply that the results may be generalizable across cancers, but it limited our understanding of individual tumor types.

To conclude, 58% of our patients had a molecular alteration and in 71% of the patients with alterations (42% of the total patients), there was an alteration that was potentially pharmacologically tractable by an FDA-approved drug (albeit often off-label) (**Figure 1B**). Unexpectedly, 28.6 percent (16 of 56) patients with brain tumors had a molecular alteration in their ctDNA test, suggesting that liquid biopsies may supplement the assessment of this difficult-to-biopsy site. In addition, 5 of 12 patients (42%) treated according to their ctDNA test results achieved a SD ≥ 6 months/PR. The degree to which tissue biopsy and ctDNA molecular results were concordant was related to the length of the time interval between the acquisition of the tissue versus blood sample. Taken together, these data suggest that ctCNA tests may have clinical utility that merits additional investigation.

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TABLES

Table 1. Patient characteristics

Characteristics	Total patients, N = 168
Age at diagnosis (years) (Median, CI 95%)	54.5 (50.5-58.7)
Gender (N, %)	
Women	98 (58%)
Men	70 (42%)
Race (N, %)	
Caucasian	113 (67.3%)
Asian	26 (15.5%)
Other	15 (8.9%)
African American	5 (3%)
Hispanic	5 (3%)
Unknown	4 (2.4%)
Type of cancer (N, %)	
Brain	56 (33.3%)
Lung	47 (28%)
Breast	36 (21.4%)
Gastrointestinal	13 (7.7%)
Genitourinary	8 (4.8%)
Gynecologic	2 (1.2%)
Other*	6 (3.6%)
No. of patients who also had tissue testing	N =101 (60%)
Median time (95%CI) from ctDNA blood draw until results (N=168)	14 days (14-15)
Median time (95% CI) from ctDNA blood draw and time of tissue biopsy for specimen used for tissue NGS test† (N = 101)	10.5 months (6.1-14.8)

*Other: lymphoma (n=2), nerve sheath tumor, sarcoma, thymoma, and melanoma (each n=1). †All tested with FoundationOne assay (see Methods).

Table 2. Variables correlating with the number of alterations

		Univariable	Multivariable		
Variables	**No. alterations median, (95% CI)**	**P-value***	**B coef. (95%CI)**	**t-statistic†**	**P-value[a]**
TUMOR TYPES:					
Brain‡		<0.001	-0.90 (-1.7; -0.15)	-2.37	**0.019**
YES (N=56)	0 (0-0)				
NO (N=112)	1.5 (1-2)				
Gastrointestinal		<0.001	1.93 (0.77; 3.08)	3.30	**0.001**
YES (N=13)	2 (1-6)				
NO (N=155)	1 (0-1)				
METASTATIC SITES:		0.001	0.55 (-0.25; 1.36)	1.36	0.176
Lymph node					
YES (N=32)	2 (1-3)				
NO (N=136)	1 (0-1)				
Bone		0.016	0.09 (-0.66; 0.84)	0.244	0.807
YES (N=50)	2 (1-3)				
NO (N=118)	0 (0-1)				
Lung		<0.001	0.45 (-0.32; 1.22)	1.15	0.252
YES (N=38)	2 (1-3)				
NO (N=130)	1 (0-1)				
Liver		<0.001	0.78 (-0.03; 1.60)	1.89	0.060
YES (N=34)	2 (1-3)				
NO (N=134)	1 (0-1)				

Only variables with N > 10 and P-values ≤ 0.05 in the univariable analysis are represented. *P-values were calculated using linear regression models (univariable and multivariable analyses). All the variables were included in the multivariable model. †The t-statistic is the ratio of the B coefficient and the Standard Error; the higher the value, the greater is the importance of the variable in the model. ‡Note: brain tumors were associated with less alterations.

Table 3. Concordance for specific alterations (tissue versus ctDNA)

	Overall concordance, N=101				Concordance for N=62 with tissue biopsy to blood draw time interval > 6 months				Concordance for N=39 with tissue biopsy to blood draw time interval ≤ 6 months				P-value* overall concordant	P-value† positive concordant
	Negative	**Positive**	**Overall %**	**Kappa (SE)**	**Negative**	**Positive**	**Overall %**	**Kappa (SE)**	**Negative**	**Positive**	**Overall %**	**Kappa (SE)**		
TP53	N=49	N=22	70.3%	0.367 (0.093)	N=30	N=9	63.0%	0.174 (0.125)	N=19	N=13	82.1%	0.635 (0.123)	**0.046**	**0.011**
EGFR	N=65	N=6	70.3%	0.099 (0.107)	N=44	N=0	71.0%	-0.167 (0.040)	N=21	N=6	69.2%	0.287 (0.158)	1.0	**0.019**
PIK3CA	N=85	N=4	88.1%	0.335 (0.148)	N=55	N=1	90.3%	0.202 (0.209)	N=30	N=3	84.6%	0.409 (0.199)	0.529	0.585
ERBB2	N=92	N=2	93.1%	0.335 (0.186)	N=60	N=0	96.8%	-0.016 (0.012)	N=32	N=2	87.2%	0.396 (0.201)	0.105	1.0

Negative concordance: alteration was not detected in both tests. Positive concordance: alteration was detected in both tests. Overall concordance percentages included negative and positive concordant cases, i.e. when both the tissue and the ctDNA were negative or positive. *2-sided Chi-Square test, compares the % of overall concordance between N=62 with biopsy interval time > 6 months and N=39 with biopsy interval time ≤ 6 months. †2-sided Chi-Square test, compares the % of positive concordance (both tissue and blood were positive) between N=62 with biopsy interval time > 6 months and N=39 with biopsy interval time ≤ 6 months. Only alterations with ≥ 10 patients with the anomaly are represented (except for *ERBB2* which was added because of its actionability). Alterations were examined at the gene level (i.e., did not distinguished the location of alteration within gene). All the genes examined (*TP53*, *EGFR*, *PIK3CA*, and *ERBB2*) were present in the tissue and ctDNA panels. SE = Standard Error.

FIGURE LEGENDS

Figure 1. Frequent alterations identified and potential actionability

Panel A: Bar graph representing the most frequent alterations identified in the overall population (N=168) and per the tumor types with at least 10 patients: brain (N=56); lung (N=47); breast (N=36); and gastrointestinal (N=13; of whom three had colorectal cancer). Patients with brain tumors had significantly less alterations identified (P<0.001). In non-brain cancers (N=112), we identified alterations in 82/112 (73%) patients. Only the five most frequent genes are represented (for brain and gastrointestinal cancers we only represented the genes altered in ≥ 1 patients and for lung cancers *ALK* and *NOTCH1* had the same frequency). **Panel B**: Pie chart representing the frequency of patients with actionable alterations in patients with alterations identified, overall and in the four cancer types with at least 10 patients.

Figure 2. Alteration detection by tissue and ctDNA tests

Panel A: Diagram showing the percentage of common alterations between the tissue and ctDNA tests. **Panel B:** Venn diagrams representing the proportion of patients for whom the alteration was only detected in the tissue, detected in both the tissue and ctDNA (positive concordance), and in whom the alteration was only detected in ctDNA. See also **Table 3** for additional information.

Figure 3. Overall survival according to the percentage of ctDNA

N=154/168 patients evaluable for OS analysis (N=14 patients died or were lost to follow up before ctDNA results). Median overall survival for patients with ctDNA ≥ 5% = 4.03 months versus not reached for patients with ctDNA < 5% (P=0.0001 in multivariable analysis, median follow up time = 6.1 months.

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 1. Frequent alterations identified and potential actionability

Figure 1A. Frequent alterations: overall and per tumor type

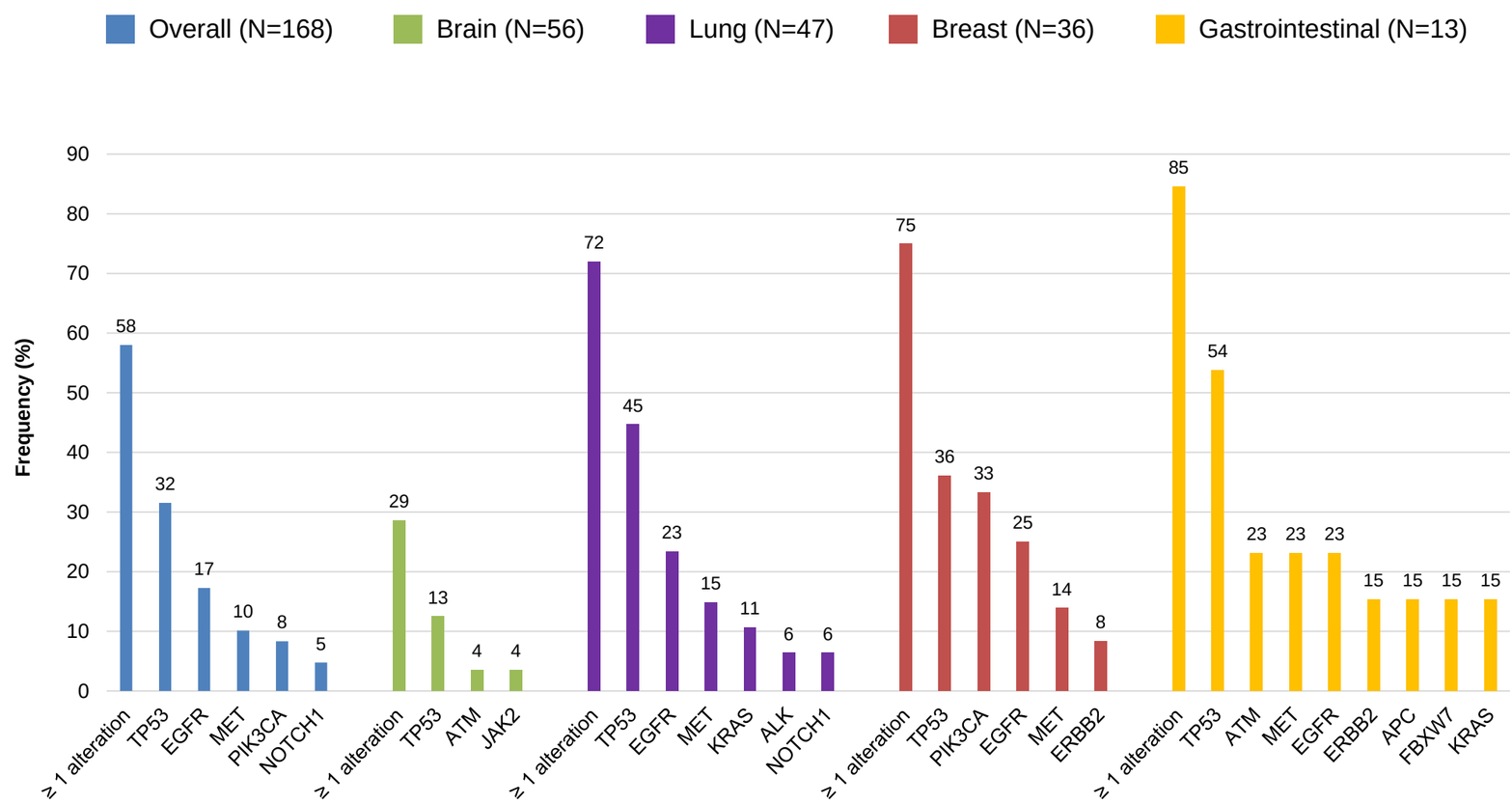

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Figure 1B. Potential actionability overview of the alterations identified

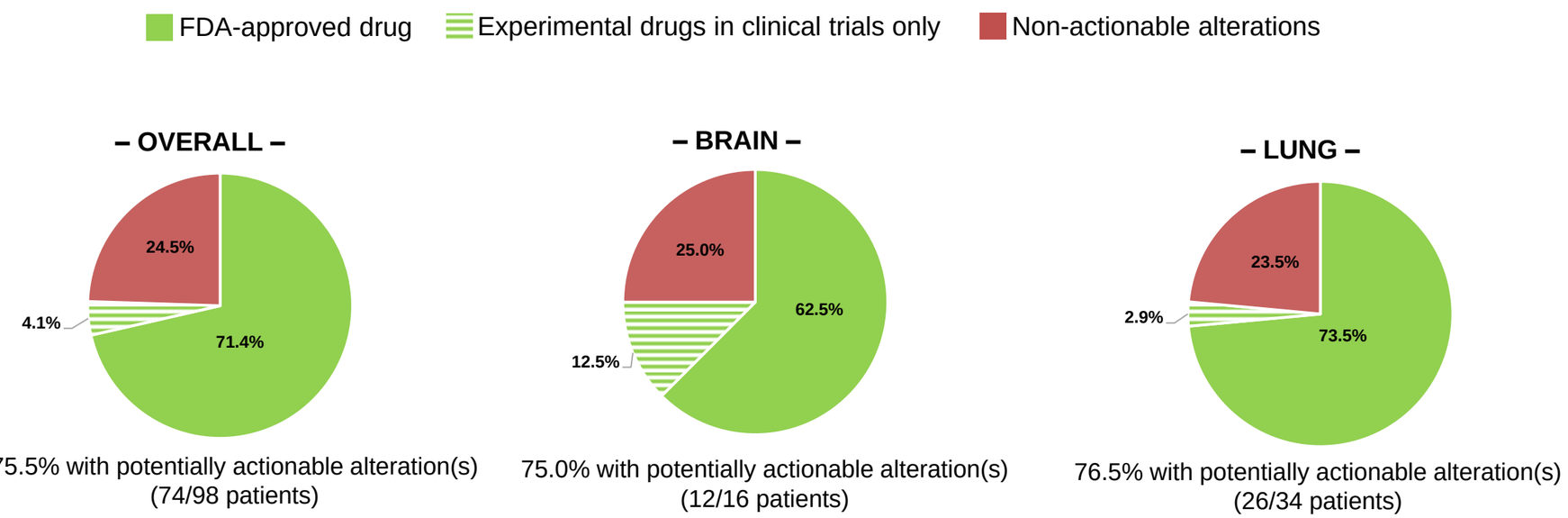

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Figure 2. Alteration detection by tissue and ctDNA tests

Figure 2A

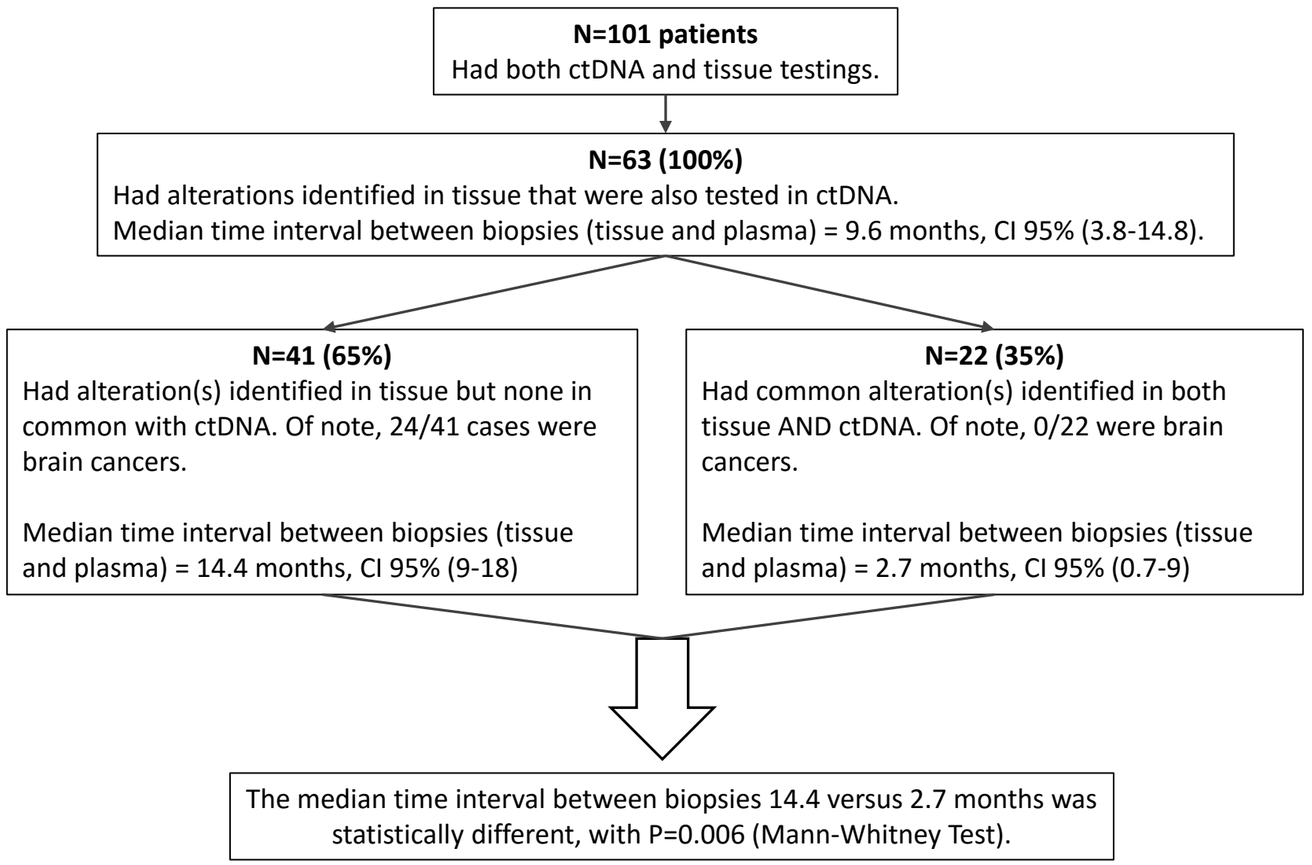

Figure 2B. Detection of alterations in *TP53, EGFR, PIK3CA,* and *ERBB2* genes in tissue and ctDNA.

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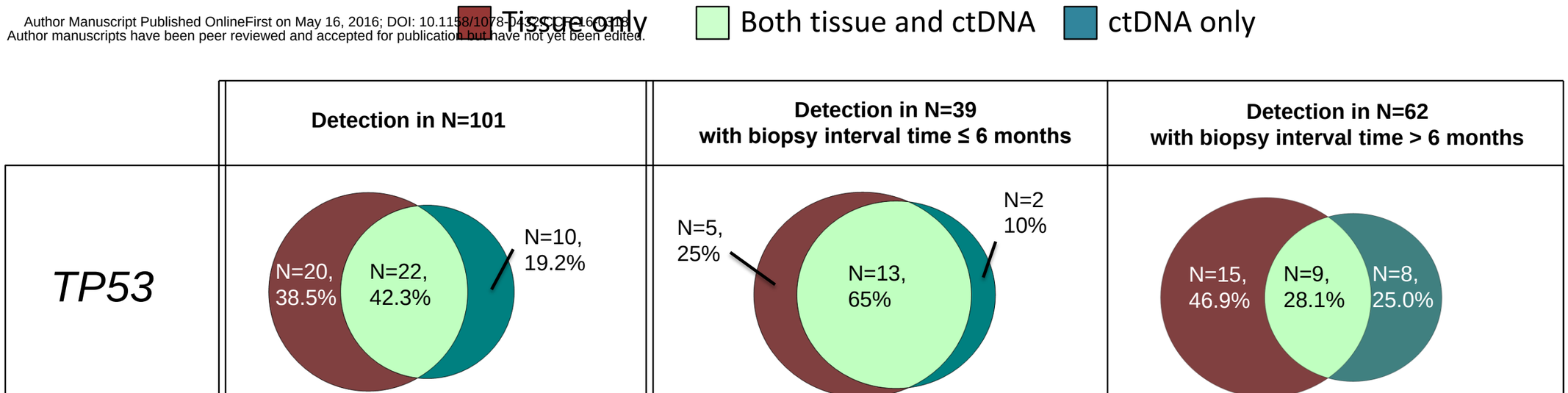

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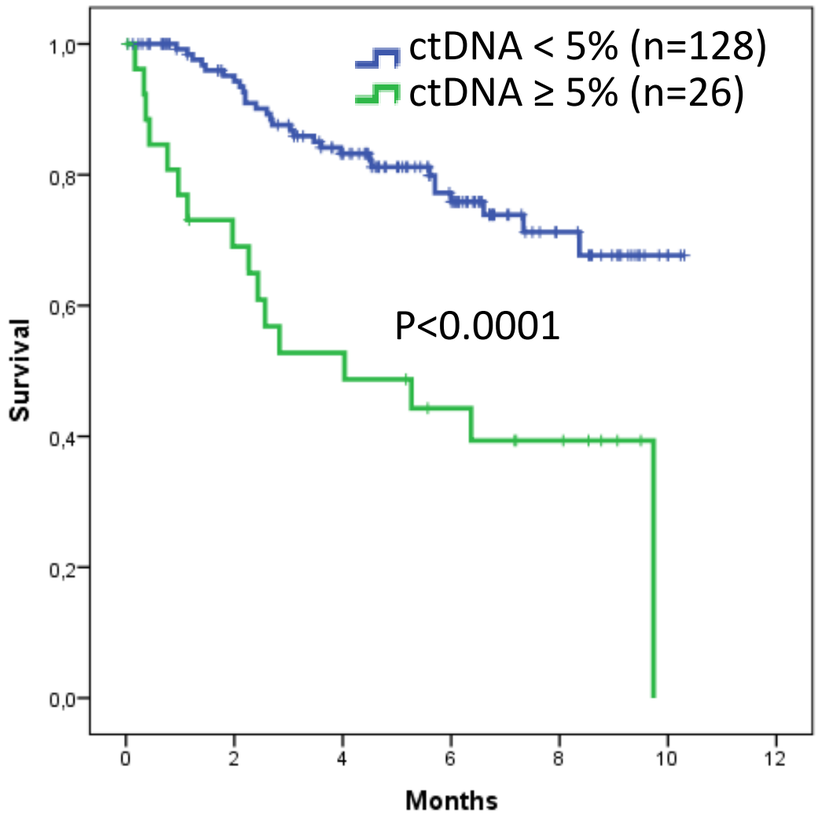
Figure 3. Overall survival according to the percentage of ctDNA



AACR American Association for Cancer Research

Clinical Cancer Research

Use of Liquid Biopsies in Clinical Oncology: Pilot Experience in 168 Patients

Maria Schwaederle, Hatim Husain, Paul T Fanta, et al.

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