

Research Article

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Consistency Analysis of Genomic Alterations Between Tumor Tissue and Circulating Cell-Free DNA in Untreated Lung Adenocarcinoma in Southern Jiangsu Region of China

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Abstract

As a novel minimally invasive biopsy method, circulating Cell-Free DNA (CFDNA) has been widely used in clinical genomic screening for targeted therapy. However, genomic analysis of tumor tissue is the standard method, especially in untreated lung adenocarcinoma, and the consistency of alterations identified in liquid biopsies and those detected in tissue specimens is not routinely being determined in untreated cancer patients. In this retrospective study, target-captured sequencing based on next-generation sequencing was performed in 252 paired of tumor Tissue DNA (TDNA) and CFDNA samples of untreated Chinese lung adenocarcinoma patients in Southern Jiangsu region of China. And the consistency of somatic alterations across frequently mutated genes and key cancer driver genes was quantified. Results showed that frequently mutated genes were EGFR, TP53, KRAS, CDKN2A, PIK3CA, etc. in tumor tissue samples, and EGFR, TP53, KRAS, CDKN2A, PIK3CA, etc. Meanwhile, the analyzed value of consistency or inconsistency would be partly influenced by patients' histopathologic stage, smoking status and VUS (variants of unknown significance) categories. The inconsistent mutation rate discovered in paired samples may be illustrated by the spatial and temporal heterogeneity in tumor. These results highlight the general utilization of cfDNA in untreated lung adenocarcinoma patients for target therapy, suggesting that TDNA and CFDNA are also complementary in untreated lung adenocarcinoma.

Keywords: Untreated lung adenocarcinoma; Consistency; Tumor tissue DNA; Circulating cell-free DNA.

Introduction

With the increasing mortality, lung adenocarcinoma has become the most common cancer and the leading cause of cancer-related death in China [1]. According to histology type, over 85% lung adenocarcinoma patients were classified into Non-Small Cell Lung Cancer (NSCLC). And more than 70% of them were in advanced stage when firstly diagnosed, and then accompanied

by a 5-year survival rate of about 15% [2,3]. Detection of gene mutation of patients with NSCLC for targeted therapy has become a clinical routine testing. Although tissue biopsy is considered to be the gold standard method for identifying DNA genomic alterations, its application remains to be limited. For instance, the major complication rates of thoracic biopsies were reported at 5.2% [4]. Besides, tumor tissue-based biopsies are defective in consideration of spatial and temporal space: intratumor

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heterogeneity may result in missing of critical DNA genomic alterations and underestimation of the genomic variability within a tumor [5-8], and in some metastatic disease instances, actionable molecular alterations may exist at multiple sites, not easily accessible via a single tissue biopsy. Therefore, both characterizing tumor mutations at baseline and real-time with treatment are necessary for the assessing of tumor evolution, response to therapy and individualized changes in treatment.

Nowadays circulating tumor Cell-Free DNA (CFDNA) assays have emerged as a less invasive method of assessing tumor genomic alterations by sampling of peripheral blood [9,10]. High consistency has been reported between tumor tissue DNA and cfDNA in studies investigating the genomic alterations in a variety of advanced cancers. For example, the presence of EGFR alterations in NSCLC, multiple genetic alterations in pancreaticobiliary cancers (KRAS, TP53, APC, FBXW7, SMAD4), exons 12-13 mutations of KRAS in colorectal cancer, BRAF V600E and KIT mutations in melanoma, and BRAF, EGFR, KRAS, and PIK3CA frequently altered in several cancers [11-14]. In addition to targeted therapy, cfDNA assays showed potential applications of early detection of metastatic disease and monitoring of minimum residual disease [15].

However, the quantity of cfDNA varies considerably among patients with different carcinomas and even tumoral pathological subtypes. In some localized disease and early-stage disease, the percentage of purified cfDNA in the blood may be low, while in advanced cancers, cfDNA is variable with tumor types such as pancreatic, ovarian, colorectal, breast, bladder, esophageal, melanoma. And hepatocellular carcinoma expresses higher percentages of cfDNA, while others, such as brain, renal, prostate, and thyroid cancers express detectable circulating DNA in less than 50% of patients [16]. As a result, Kuderer et al showed significant inconsistency in a limited number of patients with diverse solid tumors [17].

Until now, more clinical researches are necessary for the determination of the optimal cohort of patients that will clinically benefit from genetic assays of cfDNA. It is also critical to testify whether cfDNA testing may be useful in untreated patients with tumor tissue unavailable for the clinical targeted therapy decisions. Therefore, in this study, we recruited matched tissue and liquid biopsies from 126 untreated lung adenocarcinoma patients in Southern Jiangsu region of China, and quantified the consistency of somatic alterations in frequently mutated genes and key cancer driver genes. The goal was to identify consistency of genomic alterations obtained from tumor tissue DNA and cfDNA analyses in untreated lung adenocarcinoma patients. As with genomic alterations contributed to tumor heterogeneity, it's necessary to assess the fidelity of cfDNA.

Methods

Patients and specimens: In this subject, totally 293 LUAD patients in Southern Jiangsu region of China were recruited and underwent in both tumor tissue and cfDNA NGS testing for precise medication between May 2023 to September 2025. All patients have provided written informed consent. All procedures and the methods were carried out in accordance with the approved guidelines. Of these patients, 41 were excluded because of insufficient or unqualified DNA sample, or lacking paired sample.

Therefore, the final study cohort was consisted of 252 patients with both qualified tumor tissue DNA and peripheral blood cfDNA. Clinical and tumor characteristics were obtained retrospectively via patient chart review.

DNA extraction and quality control: Details of samples' requirements were referred to previous study [17]. Denay Blood & Tissue kit (Qiagen, Hilden, Germany) was used for the extraction of cell free DNA from blood sourced samples and tumor tissue DNA from tissue sourced samples respectively. Then, agarose gel electrophoresis was performed to check for the degradation and protein/RNA pollution of extracted DNA. Concentration of extracted DNA was determined by Qubit dsDNA HS assay kit on Qubit Fluorometer according to the manufacturing protocol (Life Technologies, Carlsbad, CA, USA). And then, DNA quality (OD 260/280) was measured by Nanodrop (Thermo Fisher Scientific, USA). The extracted DNA sample which has a OD ratio between 1.8 and 2.0 and a mass more than 100 ng (TDNA) and 10 ng (CFDNA) was considered qualified and would be used for following library preparation.

Library preparation and target-hybrid capturing: Library preparation was performed according to the manufacturer's instructions of KAPA Hyper prep kit (illumine Co., Ltd, San Diego, USA). In short, the fragment DNA was orderly subjected to terminal repair, A-tailing adenylation, and ligation to indexed adapters. And then, it was subsequently amplified by Polymerase Chain Reaction (PCR) with respectively 10 cycles for cfDNA and 5 cycles for TDNA samples. The targeted sequencing is an improved assay based on conventional hybridization capture. The TDNA and CFDNA targeted sequencing platforms were self-developed for clinical use and it included the detection of Single-Nucleotide Variants (SNV) and small Insertions/Deletions (INDEL) with a panel of 78 clinically relevant cancer genes (Table 1). After library preparation and hybridization capture, the targeted TDNA and CFDNA fragments were amplified by PCR with 15 cycles for sequencing.

Sequencing and bioinformatic processing: The following sequencing was performed on Illumina Next Seq CN500 according to the manufacturer's instructions. The quality control of raw data was conducted and clean reads were obtained after removing adaptor sequences and reads of low mapping quality. For genetic alteration and variant analysis, sequences were aligned to the reference human genome (hg19) by BWA software. The linkage of sequencing fragments was optimized by GATK software and the information of SNV/INDEL was acquired by VarScan2 software [19,20]. The sequencing coverage of targeted positions was $\geq 90\%$ required, and the average sequencing depth for blood specimens and tissue specimens was respectively $\geq 5000\times$ and $\geq 500\times$ required. A variant with a alteration frequency $\geq 0.2\%$ for blood specimens and $\geq 1.0\%$ for tissue specimens was considered to be credible. Final alteration results were performed by non-synonymous variants filtering with 1000 genomes database for single nucleotide polymorphism (SNP) loci excluding. The variants which didn't have a definite clinical significance recorded by Clin Var database were defined as VUS (variants of unknown significance) in this study.

Consistency and calculation description: Consistency was defined at locus level. There were four different types of

consistency: positive consistency, was defined as matched samples with the same identified alterations; negative consistency, was defined as matched samples without alterations in screened genes; partial consistency, was defined as having consistent alterations and at least one inconsistent alteration; inconsistency, was defined as having totally inconsistent alteration in matched samples. Besides, overall consistency was calculated as the patients of positive consistency and the negative consistency, out of the total number of patients; general consistency was calculated as the patients of positive consistency, the negative consistency and the partial consistency, out of the total number of patients.

The calculation of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were also defined at locus level and performed by comparing alterations of CFDNA with TDNA (the current gold standard). Sensitivity was calculated

as the number of patients positive in both tissue and plasma out of the positive tissue samples, whereas specificity was calculated from the number of plasma- and tissue-negative patients out of the total negative tissue samples. Positive predictive value was the tissue- and plasma-positive rate in positive plasma samples, and negative predictive value was the tissue- and plasma-negative rate in the negative plasma samples.

Statistical analysis

All statistical analyses were conducted by statistical software SPSS 20.0. Measurement data was exhibited as mean standard deviation (axis). The comparison of alteration spectrum between tissue and blood samples was measured by chi-square and fisher's exact tests as appropriate. The comparison of variants' frequency between different samples was also measured by chi-square and fisher's exact tests as appropriate. Two-sided $p < 0.05$ were considered statistically significant.

Table 1: Targeted panel used in this study.

Detection	Genes									
SNV & INDEL	ABL1	AKT1	ALK	APC	ARID1A	ATM	BRAF	BRCA1	BRCA2	CDK6
	CDKN2A	CTNNB1	DDR2	EGFR	ERBB2	ERBB3	ERBB4	ESR1	FBXW7	FGFR1
	FGFR2	FGFR3	FLT3	GNA11	GNAQ	HNF1A	HRAS	IDH1	IDH2	JAK1
	JAK2	KDR	KIT	KRAS	MAP2K1	MET	MLH1	MPL	MTOR	NF1
	NOTCH1	NPM1	NRAS	NTRK3	PDGFRA	PIK3CA	PTEN	RAF1	RET	RICTOR
	ROS1	SMAD4	SMO	SRC	TP53	TSC2	CAPN10	CDA	CMPK1	CYP19A1
	MTHFR	CYP1B1	CYP2D6	CYP3A5	DHFR	DPYD	DYNC2H1	ERCC1	GSTM1	GSTP1
	MDR1	NQO2	MTR	RRM1	SEMA3C	TYMS	UGT1A1	XRCC1		

Table 2: Characteristics of patients with both tissue and cell-free DNA NGS testing.

Variable	Number	Percentage (%)
Age (Years)		
Mean ($\pm$ SD)	55.9 $\pm$ 11.5	
Median (Range)	58(28-80)	
Sex		
Female	146	57.9
Male	106	42.1
Pathologic stage		
1	208	82.5
2	18	7.1
3	22	8.7
4	4	1.6
Family history of cancers	18	7.1
Smoking History		
Yes	60	23.8
No	192	76.2

Results

Patient characteristics: Clinical characteristics of patients and tumors are summarized in Table 2. In total, 252 patients who had qualified paired tissue DNA and cfDNA samples were recruited in this study, including 146 males and 106 females with a median age of 58 years, with histopathology confirmed LUAD. According to the staging classification of lung cancer published by World Health Organization, 208 patients were diagnosed into stage I, 18 into stage II, 22 into stage III, 4 into stage IV. 60 patients had smoking history and 18 patients had family history of cancer. According to the pathologic classification, early-stage patients (stage I and II) accounted for up to 89.6%.

Alteration spectrum of lung adenocarcinoma depicted by TDNA and CFDNA: Based on the 78-gene-targeted sequencing data of paired tissue and cfDNA samples, somatic alteration spectrum of patients with untreated lung adenocarcinoma in Southern Jiangsu region of China was respectively depicted via tissue and blood samples. In tissue samples cohort (Figure 1), alterations were totally detected in 14 genes in 200 (79.4%) patients, and frequently mutated genes were EGFR (51.6%), KRAS (10.3%), TP53 (8.7%), ERBB2 (8.7%), BRAF (5.6%), and CTNNB1 (4.8%). In blood samples cohort (Figure 1), the number of mutated gene was 23, and the positive rate of patients with mutations was 130 (51.6%). TP53 was also the most frequently mutated gene with the frequency of 28.6%, and other mutated genes included EGFR (25%), KRAS (6.4%), PTEN (5.6%), BRCA1 (5.6%), and GNAS (4.8%).

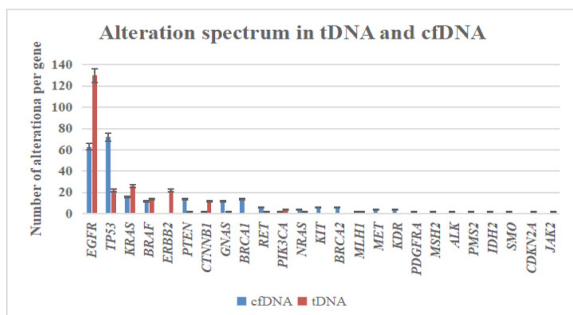


Figure 1: Alteration spectrum of untreated lung adenocarcinoma depicted by paired TDNA and CFDNA samples (n=252).

The differences of alteration spectrum of untreated lung adenocarcinoma in Southern Jiangsu region of China between tissue and blood cohorts was further compared (Figure 1). When compared to tissue cohort, frequency of aberrations in gene EGFR ($P<0.05$), KRAS ($P<0.05$), ERBB2 ($P<0.05$), BRAF ($P<0.05$) in blood cohort were significantly lower, whereas aberrations in TP53 ($P<0.05$), PTEN ($P<0.05$), BRCA1 ($P<0.05$), GNAS ($P<0.05$) were notably higher.

Consistency of genomic analyses between TDNA and CFDNA:

Of the 252 analyzed paired samples, EGFR gene mutation was positive in 130 cases (51.6%) in TDNA, and was 63 (25%) in CFDNA of untreated patients with lung adenocarcinoma. Results with VUS excluded showed that 27 (10.7%) paired sample contained the same mutations in one or more of frequent altered genes screened by the 78-gene panel (positive consistency), 34(13.4%) paired samples had no mutations in any of these screened genes in either sample type (negative consistency), yielding an overall consistency of 24.2% (61 of 252). Besides, 10 patients had partial same mutations in paired samples, yielding a partial consistency of 3.4% (10 of 252). For the rest 181 patients, 62 had TDNA mutations only, and 17 had cfDNA mutations only, and the others had inconsistent mutations in plasma and tumor tissue. Positive consistency, negative consistency, partial consistency, overall consistency, general consistency and inconsistency were analyzed according to histopathologic stages and smoking status in 252 paired TDNA and CFDNA samples (Table 3,4,5,6) with VUS excluded and included. Analysis values were slightly higher when VUS were excluded with an overall consistency of 24.2%, and general consistency of 28.2%. The same trend was also found in almost every histopathologic stage subset and smoking status subsets.

Table 3: Composite statistics of consistency according to histopathologic stage (VUS excluded).

	Histopathologic stage				
	1	2	3	4	Total
Positive consistency	23	3	1		27
Negative consistency	30		4		34
Partial consistency	8			2	10
Inconsistency	147	15	17	2	181
Overall consistency	25.5%	16.7%	22.7%	0	24.2%
General consistency	29.3%	16.7%	22.7%	50%	28.2%

Table 4: Composite statistics of consistency according to histopathologic stage (VUS included).

	Histopathologic stage				
	1	2	3	4	Total
Positive consistency	5	2	1		8
Negative consistency	8				8
Partial consistency	33	1	2	4	40
Inconsistency	162	15	19		196
Overall consistency	6.3%	11.1%	4.5%	0	6.3%
General consistency	22.1%	11.1%	4.5%	100%	22.2%

Table 5: Composite statistics of consistency according to smoking status (VUS excluded).

	Smoking status		
	Yes	No	Total
Positive consistency	6	21	27
Negative consistency	6	28	34
Partial consistency	3	7	10
Inconsistency	45	136	181
Overall consistency	20%	25.5%	24.2%
General consistency	15%	29.2%	28.2%

Table 6: Composite statistics of consistency according to smoking status (VUS Included).

	Smoking status		
	Yes	No	Total
Positive consistency	3	5	8
Negative consistency	2	6	8
Partial consistency	8	32	40
Inconsistency	47	149	196
Overall consistency	8.3%	6%	6.3%
General consistency	21.7%	23.6%	22.2%

Consistency of frequent alterations in untreated lung adenocarcinoma patients between TDNA and CFDNA: The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and consistency of locus-level and gene-level were all analyzed across the three representative genes - TP53, EGFR and KRAS in lung adenocarcinoma patients (Table 4). Tissue-based NGS was considered as the gold standard for this analysis. Totally, the detection rates of EGFR mutation based on TDNA and CFDNA were 51.6% and 25%, with a overall consistency of 70.2% (VUS excluded). Compared with tumor tissue, the sensitivity, specificity, PPV and NPV of EGFR mutation in cfDNA were 45.4%, 96.7%, 93.7% and 62.4% respectively. Across the three frequently mutated genes, KRAS with an overall consistency of 84.9% ranked firstly. The significant differences (P value from χ^2 test) were also showed in genes EGFR ($P<0.05$), TP53 ($P<0.05$) and KRAS ($P<0.05$).

Table 4: CConsistency of 3 frequently altered genes (VUS excluded) between TDNA and CFDNA.

Gene	Plasma biopsy		Tissue biopsy		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's J Index	Overall consistency (%) (Diagnostic accuracy)
			+	-						
TP53	G245D	+	0	20						
		-	0	232						
	R175H	+	0	16						
		-	2	234						
	R273C	+	2	14						
		-	4	232						
	TP53 gene	+	6	66	27.3	71.3	8.3	91.1		67.5
		-	16	164						
EGFR	L858R	+	26	6						
		-	32	188						
	Exon19 in/del	+	31	0						
		-	37	184						
	EGFR gene	+	59	4	45.4	96.7	93.7	62.4		70.2
		-	71	118						
KRAS	Codon 12	+	2	4						
		-	24	222						
	KRAS gene	+	2	14	7.7	93.8	12.5	89.8		84.9
		-	24	212						

Discussion

Personalized treatment in oncology was based on their genomic profile with effective molecular targeted drugs to malignancies. Except for precision medication in the clinic, the goal of precision medicine is also to detect the emergence of genetic alterations that predicting recurrence and resistance to treatment prior to clinical symptoms or radiological evidence of disease progression [21]. The current gold standards with tissue biopsy used for clinically genomic detection, remains risky and application limited [4-8], and genomic analysis of CFDNA may hold promise to making up for the deficiency in certain circumstances. However, the clinical data in the consistency of tumor tissue DNA with CFDNA is still insufficient, especially in newly diagnosed cancer patients and patients in early stages, to determine the alternative utility of CFDNA biopsy for patients with tumor tissue unavailable in clinical treatment decisions. Herein, we present a systematic consistency analysis of comprehensive liquid biopsy and tissue-based testing in a couple of untreated lung adenocarcinoma patients in Southern Jiangsu region of China.

As with patients in histopathological stage I accounted for 82.5% in this study, the alteration spectrum of untreated lung adenocarcinoma depicted by TDNA and CFDNA both also showed

that the frequently mutated genes in lung adenocarcinoma patients were EGFR, TP53 and KARS. However, significant differences of mutation frequencies of gene EGFR, TP53, KRAS were discovered in two kinds of assays. And the consistency of alteration spectrum between TDNA and CFDNA was somewhat below expectation in untreated patients with early-stage lung cancer, which was significantly different from that with advanced cancer and metastases. But for frequently altered genes in lung adenocarcinoma patients, the consistency between TDNA and CFDNA was improved significantly to 70.2% for EGFR and 84.9% for KRAS.

There are several potential reasons contributing to the differences: First, the biopsy detection techniques are quite different. Mutations in CFDNA that were released into peripheral blood was captured and analyzed according to differential detection threshold (0.25% for liquid biopsy and 1% for tissue biopsy in this study). As with tumor cells outgrowing the blood supply, become hypoxic, and undergo apoptosis or necrosis, tumor DNA release into the peripheral blood, and then join together to CFDNA. It was a combination of DNA from the whole-body cells. Therefore, the assay platform and the origin of tumor DNA resulted in intrinsic differences in specificity and sensitivity. Second, the effects of the sampling time may also be significant.

Tumor metabolism is highly dynamic, higher consistency would be associated with shorter sampling intervals, especially shorter time between blood sampling firstly and tissue biopsy followed immediately. Third, site of biopsy is important. For patients with advanced cancer, there were more sufficient tumor DNA joined into CFDNA in the blood. A previous study also suggested that sub clonal diversification might resulted in missed driver mutations, indicating that multiple tissue biopsies in different locations would be optimal to best characterize tumor heterogeneity [22]. In this study, all the tissue biopsies were sampled at in-situ sites without considerations of histopathological stage, which possibly leading to a missing of tumor heterogeneity, especially the accumulated mutations promoting metastasis. In contrast, CFDNA may play an important role in the detection of tumor genomic heterogeneity of metastatic cancer and revealed a more comprehensive genomic profile.

In this study, to explore the consistency between tissue and CFDNA biopsies, the same sequencing alteration present in both biopsies was analyzed. And correlation analysis of both VUS included and excluded was conducted with the influences of histopathologic subtypes and smoking status. Results showed that in untreated lung adenocarcinoma in Southern Jiangsu region of China, a consistency of 45.4%, a specificity of 96.7% and PPV (positive predictive value) of 93.7% was achieved in EGFR. However, the results for all 78 genes in panel were significantly different, it showed an overall consistency of 24.2%, and general consistency of 28.2% when VUS were excluded. While the recent study examining 50 hotspot genes in tumor DNA and CFDNA reported a sensitivity of 49.9% and a specificity of 99.8% for patients with advanced or metastatic solid tumors [23]. In the study, a higher consistency was exhibited with VUS was excluded in almost all the subsets, including in different histopathologic subtypes and smoking status. Besides, the consistency displayed a gradually increasing trend associated with the developing histopathologic stage, indicating that patients with advanced stage might get a higher consistency and thus would be more beneficial from CFDNA biopsy. Potential explanations for the lack of consistency include differences in assay platform (detection techniques), spatial and sampling time factors, tumor heterogeneity, interval treatment, subclones, and potential germline DNA contamination.

It's a pity that the chromosomal structural variants, such as gene amplification and fusion, were not identified in both liquid biopsies and tissue biopsies, and the consistency analysis was not provided.

Conclusion

In conclusion, our findings indicate relatively high specificity and consistency between tumor tissue DNA and CFDNA in variants with definite significance and frequent alteration in untreated lung adenocarcinomas in Southern Jiangsu region of China. It indicates CFDNA testing to be useful in patients with tumor tissue unavailable to support clinical treatment decisions based on genomic alterations, especially in drug-related gene mutations in targeted therapy. However, there were also a variety of variants detected in tumor DNA or CFDNA biopsy a lonely, showed significantly different in early-stage lung adenocarcinomas. These indicates that tumor heterogeneity cannot be fully detected in one biopsy alone, and highlights that TDNA and CFDNA are

complementary in genomic profile, especially in early-stage lung adenocarcinomas. Due to the constraints of detect content, further exploration should include the structural variation, such as the fusion, exon skipping and CNV, synonymous alterations and alterations in untranslated regions. In addition, future studies should take more patients with multiple cancer types in different parts of China into account.

References

1. Chen W, Zheng R, Baade P D, et al. Cancer statistics in China, 2015 [J]. CA: A cancer journal for clinicians. 2016; 66(2): 115-32.
2. Travis W D, Brambilla E, Riely G J. New pathologic classification of lung cancer: Relevance for clinical practice and clinical trials [J]. Journal of clinical oncology: Official journal of the American Society of Clinical Oncology. 2013; 31(8): 992-1001.
3. Miller K D, Siegel R L, Lin C C, et al. Cancer treatment and survivorship statistics, 2016 [J]. CA: A cancer journal for clinicians. 2016; 66(4): 271-89.
4. Overman MJ, Modak J, Kopetz S, et al. Use of research biopsies in clinical trials: Are risks and benefits adequately discussed? J Clin Oncol. 2013; 31: 17-22.
5. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. NEJM. 2012; 366: 883-892.
6. Hiley C, de Bruin EC, McGranahan N, et al. Deciphering intratumor heterogeneity and temporal acquisition of driver events to refine precision medicine. Genome Biology. 2014; 15: 453.
7. Nik-Zainal S, Van Loo P, Wedge DC, et al. The life history of 21 breast cancers. Cell. 2012; 149: 994-1007.
8. Wang Y, Waters J, Leung ML, et al. Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature. 2014; 512: 155-160.
9. De Mattos-Arruda L, Cortes J, Santarpia L, et al. Circulating tumor cells and cell-free DNA as tools for managing breast cancer. Nat Rev Clin Oncol. 2013; 10: 377-389.
10. Elshimali YI, Khaddour H, Sarkissyan M, et al. The clinical utilization of circulating cell free DNA (CCFDNA) in blood of cancer patients. Int J Mol Sci. 2013; 14: 18925-18958.
11. Mok T, Wu YL, Lee JS, et al. Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy. Clin Cancer Res. 2015; 21: 3196-3203.
12. Zill OA, Greene C, Sebisano D, et al. Cell-Free DNA Next Generation Sequencing in Pancreatobiliary Carcinomas. Cancer Discov. 2015; 5: 1040-1048.
13. Kim ST, Lee WS, Lanman RB, et al. Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients. Onstage. 2015; 6: 40360-40369. doi: 10.18632/oncotarget.5465.
14. Janku F, Angenendt P, Tsimberidou AM, et al. Actionable mutations in plasma cell-free DNA in patients with advanced cancers referred for experimental targeted therapies. Onstage. 2015; 6: 12809-12821. doi: 10.18632/oncotarget.3373

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15. Diaz LA, Jr, Bardelli A. Liquid biopsies: Genotyping circulating tumor DNA. *J Clin Oncol*. 2014; 32: 579-586.
 16. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014; 6: 224-224.
 17. Kuderer NM, Burton KA, Blau S et al. Comparison of 2 Commercially Available ext-Generation Sequencing Platforms in Oncology. *JAMA Oncol* 2017; 3(7): 996-998.
 18. Lin X, Dong W, Lai X, et al. The clinical value of circulating tumor DNA detection in advanced non-small cell lung cancer [J]. *Translational Cancer Research*. 2019; 8: 170-9.
 19. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data [J]. *Genome Res*. 2010; 20(9): 1297-303.
 20. Koboldt D C, Zhang Q, Larson D E, et al. Var Scan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing [J]. *Genome Res*. 2012; 22(3): 568-76.
 21. Chae YK, Davis AA, Carneiro BA, et al. consistency between genomic alterations assessed by next-generation sequencing in tumor tissue or circulating cell-free DNA. *Onstage*. 2016; 7(40): 65364-65373.
 22. Jovelet C, Ileana E, Le Deley MC, et al. Circulating Cell-Free Tumor DNA Analysis of 50 Genes by Next Generation Sequencing in the Prospective MOSCATO Trial. *Clin Cancer Res*. 2016; 22: 2960-2968.
 23. De Bruin EC, Mc Granahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science*. 2014; 346: 251-256.