

Validity of an NGS-based multiple gene panel in identifying actionable mutations for patients with NSCLC in a Chinese hospital

WEI CAO¹, CHENGHAI YAN², HAILONG WANG², TOM TANG², HAIFENG WANG² and DUJUAN LIU²

¹Department of Cardiothoracic Surgery, The Second Hospital of Anhui Medical University, Hefei, Anhui 230601;

²First Dimension Biosciences (Suzhou) Co., Ltd., Industrial Park, Suzhou, Jiangsu 215126, P.R. China

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Abstract. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. A number of targeted therapies have been approved for clinical use or are in clinical trials. Next generation sequencing (NGS) is widely applied in the identification of actionable genomic alterations and enables personalized cancer therapy for patients. Several multiple-gene panels are available in China for the practice of precision medicine-based cancer therapy. However, the efficiency of these panels requires evaluation. The current study investigated 23 NSCLC samples using a custom designed panel of complete coding regions of ~180 cancer driver genes (FD-180) and whole exome sequencing for control samples, obtained from white blood cell samples. The results obtained suggested that actionable mutations with available targeted therapeutic options were identified in 69.6% of cases, including 60.9% of therapeutic targets recommended by the National Comprehensive Cancer Network guidelines. Furthermore, 8.7% of patients had a gene mutation that potentially qualified them for clinical trials or associated off-label therapies. As such, the results obtained in the current study demonstrated the reliability of the targeted NGS panel and its potential use for identifying actionable gene alterations and designing personalized therapies for patients with NSCLC.

Introduction

In 2015, cancer-associated mortalities in China were estimated at 2.8 million, with 4.3 million newly identified cases, the most common of which being lung cancer (1). Non-small cell lung cancer (NSCLC) is the most commonly histologically diagnosed lung cancer, accounting for ~85-90% of cases (2). In the past decade, genetic and genomic profiling in NSCLC

has improved considerably, and with it, the understanding of underlying molecular mechanisms of disease pathogenesis and strategies for targeted therapies (3-5). Potential inhibition targets for 60% of NSCLC cases in the form of actionable driver alterations have been identified through molecular profiling (6). Evidence supports the effectiveness of several of these targeted therapies, including, gefitinib [epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI)], erlotinib (sensitizing EGFR mutations) and crizotinib [sensitizing ALK receptor tyrosine kinase (ALK) and ROS proto-oncogene 1 receptor tyrosine kinase fusion] (7).

Next-generation sequencing (NGS) is used in the field of genomic research and clinical applications (8). For patients with NSCLC, targeted NGS provides improved drug target screening and additional potential avenues of treatment (9-12). Due to the ever-growing number of discovered predictive markers for therapies and the limited availability of submitted tissue samples, the long-standing 'one test-one drug' paradigm is gradually being replaced by multiplexed genotyping platforms (13). One such platform is the FoundationOne CDx (FICDx), which received marketing approval by the Food and Drug Administration (FDA). FICDx performs NGS-based *in vitro* diagnostics to detect genetic mutations in all solid tumor types and serves to identify potential beneficiaries for several FDA-approved targeted treatment options (14). With more NGS platforms being developed, standardization of reports from various test platforms is warranted (15).

In the present study, a total of 23 NSCLC samples, containing 21 Formalin-Fixed Paraffin-Embedded (FFPE) and two cell-free DNA (cf)DNA samples were analyzed. The coding regions of ~180 cancer driver genes were explored using The Roche Nimblegen (Roche Nimblegen, Inc.) custom-designed panel. Subsequently, the captured regions were sequenced by NGS to detect various types of mutations. Whenever available (for a total of 16 patients), the sequences were compared with exome sequences obtained from white blood cell (WBC) samples.

Materials and methods

Specimen collection. A total of 23 patients with stage III/IV NSCLC at the time of diagnosis were included in the present study. Patients were recruited from The Second Hospital of

Correspondence to: Dr Dujuan Liu, First Dimension Biosciences (Suzhou) Co., Ltd., Industrial Park, 218 Xinghu Street, Suzhou, Jiangsu 215126, P.R. China
E-mail: liudujuan@firstdimension.net

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Anhui Medical University (Hefei, China) between March 2017 and October 2017. The patients did not receive preoperative chemotherapy or radiotherapy, and those with prior TKI therapy were excluded from the study. There were 21 FFPE specimens and 2 cfDNA specimens in patients who did not have tissue samples available at presentation. All FFPE samples were independently assessed by two experienced pathologists and had a minimum tumor content of 40%. Blood samples from 16 patients were collected as controls to validate the identified germline mutations. Patient clinical data are presented in Table I. The samples were obtained from 10 males and 13 females (median age, 62 years; age range, 31-77 years). Among these patients, there was 1 smoker and 22 non-smokers.

Nucleic acid preparation. Deparaffinization of 40 μ m tissue sections was performed with 100% xylene (Sigma Aldrich; Merck KGaA, Darmstadt, Germany) followed by 100% ethanol as described previously (16). Deparaffinized samples were suspended in proteinase K-contained buffer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Following extraction using phenol-chloroform (Sigma Aldrich; Merck KGaA), DNA samples were treated with ethanol (Sigma Aldrich; Merck KGaA) precipitation and resuspended in deionized water. cfDNA blood collection tubes (Streck, Inc., Omaha, NE, USA) were used for the collection of 10 ml blood samples. Procurement of 1.2 ml of plasma from every patient was performed prior to cfDNA extraction using centrifugation twice (4°C; 1,600 x g for 10 min). cfDNA was isolated with the QIAamp Circulating Nucleic Acid kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. Validation of adopted protocols for the present study was obtained from previous findings (17). A total of 2 ml of total peripheral blood was used to process WBC DNA as a control, using a FlexiGene DNA kit (Qiagen, Inc.). Quantification of isolated DNA samples was performed using a NanoDrop spectrophotometer (Thermo-Fisher Scientific, Inc.), and by fluorimetry, which was conducted using Qubit dsDNA high-sensitivity and/or broad-range assay kits (Thermo Fisher Scientific, Inc.).

Configuration of the somatic subpanel. The somatic cancer gene list was selected based on the following cancer-associated databases such as the Catalogue of Somatic Mutations in Cancer (COSMIC; cancer.sanger.ac.uk/cosmic), MyCancerGenome (www.mycancergenome.org), CancerIndex (www.cancerindex.org), FDA (www.fda.gov), European Medicines Agency (www.ema.europa.eu/ema), National Comprehensive Cancer Network (NCCN; www.nccn.org), PHARMGKB (www.pharmgkb.org/), MDAnderson Cancer Center (pct.mdanderson.org), DRUGBACK (www.drugbank.ca), ClinicalTrials (clinicaltrials.gov), Google Scholar (scholar.google.com) and PubMed (www.ncbi.nlm.nih.gov/pubmed) databases, studies published between 2000 and 2018.

Configuration of the germline subpanel. A preliminary cancer gene list was built based on germline alterations linked with predisposition for hereditary cancer and sourced from the Human Gene Mutation Database (www.hgmd.cf.ac.uk), ClinVar (www.ncbi.nlm.nih.gov/clinvar), Online Mendelian Inheritance in Man (www.omim.org), Genetics Home Reference (ghr.nlm.

Table I. Overview of patient and tumor characteristics in the present study.

Characteristics	n (%)
Sex	
Male	10 (43.5)
Female	13 (54.5)
Age	
>60	13 (54.5)
≤60	10 (43.5)
Smoking status	
Smoker	1 (4.3)
Non-smoker	22 (95.7)
Stage	
I	9 (39.1)
II	1 (4.3)
III	6 (26.1)
IV	3 (13.0)
Unknown	4 (17.4)
Type of specimen	
FFPE tissue samples	21 (91.3)
Cell-free DNA (cfDNA)	2 (8.7)
Genomic DNA (gDNA)	16 (69.6)

FFPE, Formalin-fixed, Paraffin-embedded; cfDNA, cell-free DNA; gDNA, genomic DNA.

nih.gov) and Orphanet (www.orpha.net) databases. Filtering of the list was performed and genes were removed based on a number of criteria, including a lack of scientific evidence supporting their association with cancer. The tumor-associated genes in the FD-180 panel were listed in Table II.

Library construction and sequencing. The Whole Exome Sequencing (WES) library was constructed using the Illumina TruSeq DNA Sample Prep kit (Illumina, Inc., San Diego, CA, USA), and targeted exome enrichment was performed using the SeqCap EZ Human Exome Library v2.0 kit (Roche Nimblegen Inc.). Library construction and targeted region enrichment for FD-180 were completed with the KAPA Hyper Prep kit for Illumina platforms (Kapa Biosystems; Roche Diagnostics, Indianapolis, IN, USA) and the SeqCap EZ Choice Library (Roche Nimblegen, Inc.), respectively. The FD-180 panel was used for the simultaneous sequencing of all exons of ~180 tumor-associated genes (data not shown).

Paired-end sequencing was performed with the Illumina X Ten or NEXT SEQ 500 sequencing instrument (Illumina, Inc.) according to the manufacturer's protocol, yielding ~150 bp short sequence reads. An average of ~15G of data was generated for each WES sample, accounting for 100-200X coverage of the entire exome. The average data for FD-180 target samples was 3G.

Data analysis. Raw data were processed into a clean FASTQ output with flexbar (v3.3; <https://github.com/seqan/flexbar>)

Table II. The gene list of FD-180 panel.

APC	ATM	ATR	AXIN2	RECQL4	RET	RNF43	RUNX1
BAP1	BLM	BMPR1A	BRCA1	SBDS	SDHA	SDHAF2	SDHB
BRCA2	BRIP1	CDC73	CDH1	SDHC	SDHD	SETBP1	SLC2A2
CDK4	CDKN1B	CDKN2A	CHEK2	SLX4	SMAD4	SMARCA4	SMARCB1
CLCN7	CYLD	DDB2	DICER1	SMARCE1	SPTA1	STK11	SUFU
EPCAM	ERCC2	ERCC3	ERCC4	TERT	TGFBR2	TP53	TP63
ERCC5	EXT1	EXT2	FANCA	TSC1	TSC2	VHL	WAS
FANCB	FANCC	FANCD2	FANCE	WRN	WT1	XPA	XPC
FANCF	FANCG	FANCI	FANCL	ABL1	ABL2	AKT1	ALK
FANCM	FH	FLCN	GALNT12	AR	ARAF	ATM	ATR
GLA	GPC3	HNF1A	MAD2L2	BRAF	BRCA1	BRCA2	CDK12
MAX	MEN1	MLH1	MLH3	CDK4	CDK6	CRKL	CDKN1A
MPL	MSH2	MSH3	MSH6	CDKN2A	CHD4	CHEK2	CSF1R
MUTYH	NBN	NF1	NF2	CTNNB1	DDR2	EGFR	ERBB2
PALB2	PHOX2B	PIK3CA	PMS1	ERBB3	ERBB4	ESR1	FANCA
PMS2	POLD1	POLE	PRF1	FANCB	FANCI	FANCL	FANCC
PRKAR1A	PRKCD	PTCH1	PTEN	FANCD2	FANCE	FANCF	FANCG
RAD50	RAD51C	RAD51D	RB1	FANCM	FBXW7	FGFR1	FGFR2
TSC1	TSC2	XRCC2	SPOP	FGFR3	FGFR4	FRK	FYN
MAPK1	MAPK3	MET	mTOR	GNA11	GNAQ	GRM3	HRAS
NF1	NOTCH1	NRAS	PDGFRA	IDH1	IDH2	KDR	KIT
PIK3CA	PIK3CD	PIK3R1	PTCH1	KRAS	LYN	MAP2K1	MAP2K2
PTEN	PTPN11	RAC1	RAF1	RET	ROS1	SMO	

through trimming of adapter sequences and removing reads of insufficient quality (average quality score <15). Raw reads were checked for the data quality using FASTQC (v0.11.5) (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Plots of quality scores across all bases in reads indicated that the majority of positions exhibited a quality score $Q \geq 20$. Raw reads were subsequently trimmed for adapter contamination using Trimmomatic (v0.32; www.usadellab.org). Leading and trailing low-quality bases (<3) were removed. Reads were scanned with a four-base-wide sliding window and the following bases of reads were cut when the average quality per base dropped below 15. Reads >50 bases were selected for subsequent analysis. Paired clean reads following Trimmomatic treatment were aligned against the human genome (hg19) using Burrows-Wheelers Aligner (18). Genome Analysis Toolkit (19) was used to do base quality score recalibration, a process in which machine learning is applied to model quality score errors empirically, and the quality scores are adjusted accordingly. Analysis of the realigned BAM files, and detection of somatic single-nucleotide variants and insertions/deletions was performed using MuTect (20). Normal germline variants were filtered out using the Single Nucleotide Polymorphism Database (dsSNP) (21) or the Exome Aggregation Consortium (ExAC; exac.broadinstitute.org). Default parameter settings were used for all programs. In-house developed scripts were used to eliminate erroneous base calls and generate final mutations based on variant frequency (>0.5%). The frequency of variation is equal to the ratio of the depth of the variant allele to the total depth of all alleles at the locus. Remaining variants were

annotated using the ExAC and InterPro database (<http://www.ebi.ac.uk/interpro/>). Gene ontology enrichment was analyzed on the website (www.geneontology.org), focusing on the genes which were mutated in >4 primary tumor samples.

Results

In order to explore the therapeutic relevance of FD-180 and improve the survival rate of patients with NSCLC, 2 cfDNA and 21 FFPE samples were studied to detect driver mutations in tumor DNA with (16 samples) or without matched WBC DNA as a control (mean sequencing depth, 98.96x WES). The average sequencing depth on the 180 target genes for the cfDNA and FFPE samples was 1,747.7X and 398.4X respectively. Q30 for all data was 86.4%, where Q scores are defined as a property that is logarithmically associated with the base calling error probabilities. Q30 means that the accuracy of base calling is 99.9% (22).

Somatic variations in primary tumor. The mutational signature of NSCLC samples was dissected through inspection of the spectrum of base substitutions. All 23 samples exhibited similar point mutation spectra. Cytosine to thymine was the most frequent transversion among somatic mutations, which accounted for 45.6% in primary tumor tissues (Fig. 1A and B).

Overall, 525 somatic mutations were detected in the 23 NSCLC samples. The primary type of mutation identified was missense mutations (82.1%; Fig. 1C and D). Notably all the mutations reported were within the protein coding regions, as

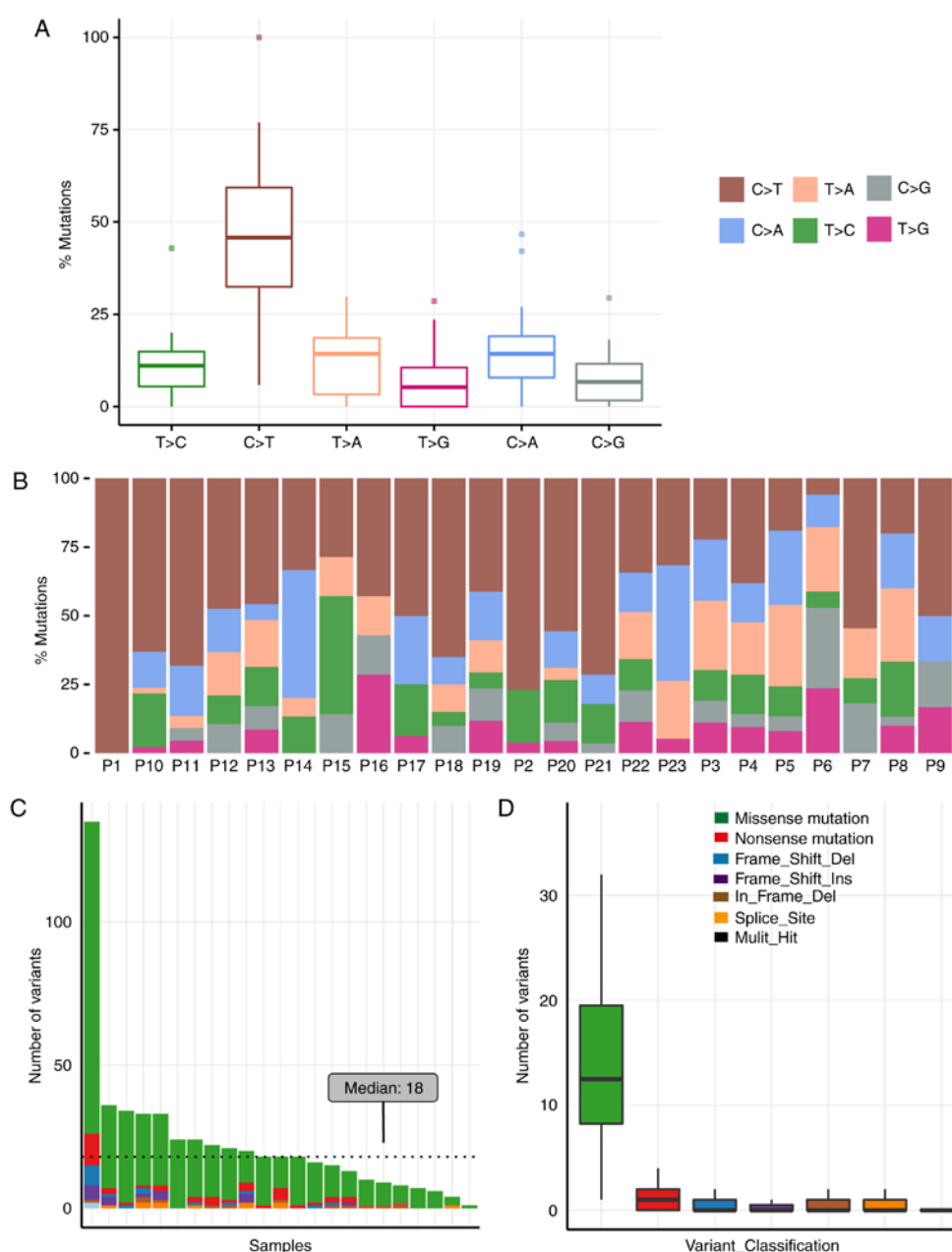


Figure 1. Statistical analyses of somatic mutations in 23 patients with NSCLC. (A) A breakdown of the observed substitution mutations. (B) Distribution of different variation types among all samples. The different colors present the base substitutions in the samples. (C) An overview of the types of mutations observed. (D) Distribution of different variation types among all samples. The different types of mutations are presented using different colors. P1-P23 present the patient ID. T, tyrosine; C, cytosine; A, adenine; G, guanine; Del, deletion; Ins, insertion.

the capturing probes used in the current study were restricted to coding sequences. Variations with >5% frequency, as reported in either the dbSNP or the ExAC database, were excluded. Mutation types and numbers were compared with the normal tissue datasets, and the median variant count among the 23 samples was 18 (Fig. 1C and D). The high variant count in patient 1 remains unclear. Possible causes include poor living conditions and long cancer progression time prior to diagnosis.

Implicated biological processes and molecular function. Gene Ontology (www.geneontology.org) enrichment analysis was performed with 40 frequently mutated genes (genes which were mutated in >4 primary tumor samples). 'Peptidyl-tyrosine phosphorylation', 'peptidyl-tyrosine

modification', 'phosphatidylinositol-mediated signaling', 'inositol lipid-mediated signaling' and 'protein autophosphorylation' were the top five biological process of these high frequency genes in the 23 NSCLC samples (Fig. 2A). Notably, the molecular functions of these genes included 'protein tyrosine kinase activity', 'transmembrane receptor protein tyrosine kinase activity', 'transmembrane receptor protein kinase activity', 'phosphatidylinositol 3-kinase activity' and 'phosphatidylinositol-4,5-bisphosphate-3-kinase activity' (Fig. 2B).

Actionable mutations with available targeted therapies. The top 20 mutated genes in the 23 tumor samples were identified by counting the frequency of mutations in different genes in the

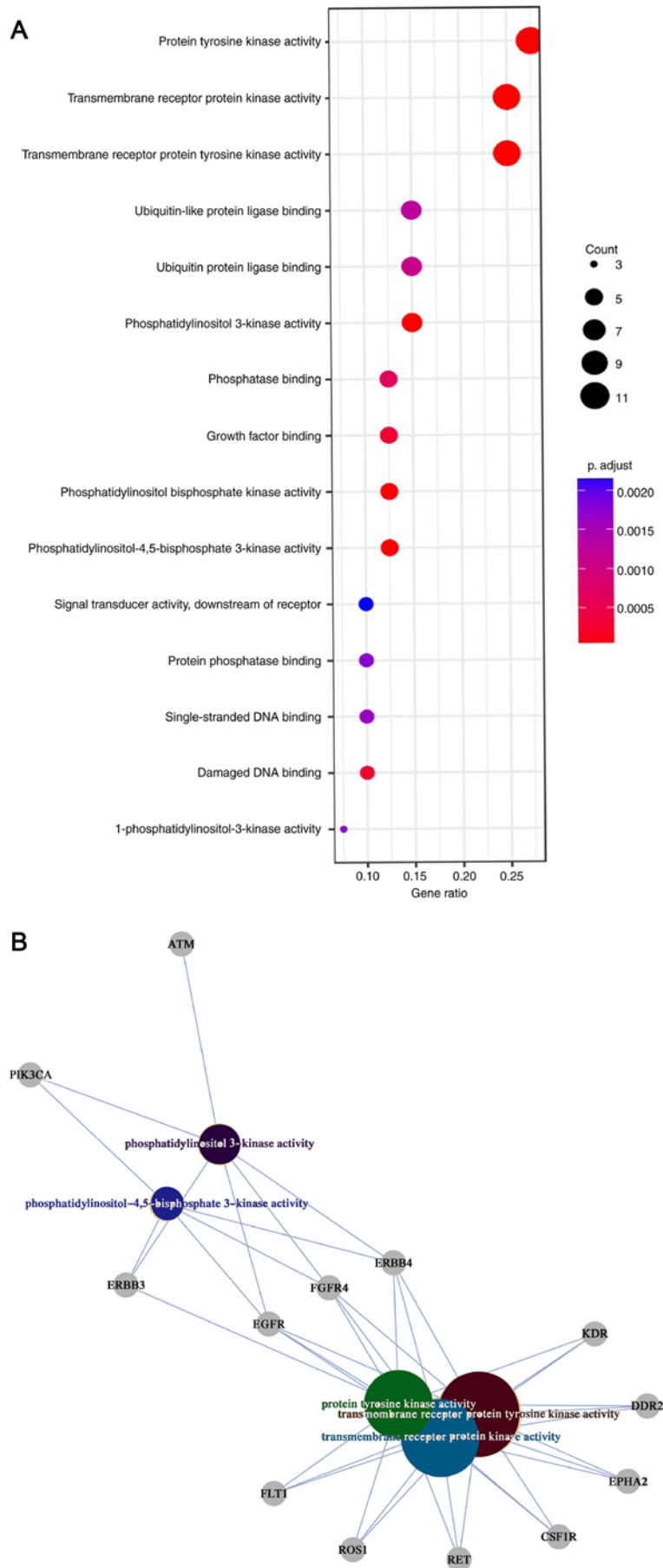


Figure 2. The biological processes and molecular function for the mutational genes in the 23 patients with NSCLC. (A) Main biological processes and gene ratio for frequently mutated genes. (B) Association between frequently mutated genes and their molecular function. Associated genes are present in the grey circles. P adjust, adjusted P-value. NSCLC, non-small cell lung cancer.

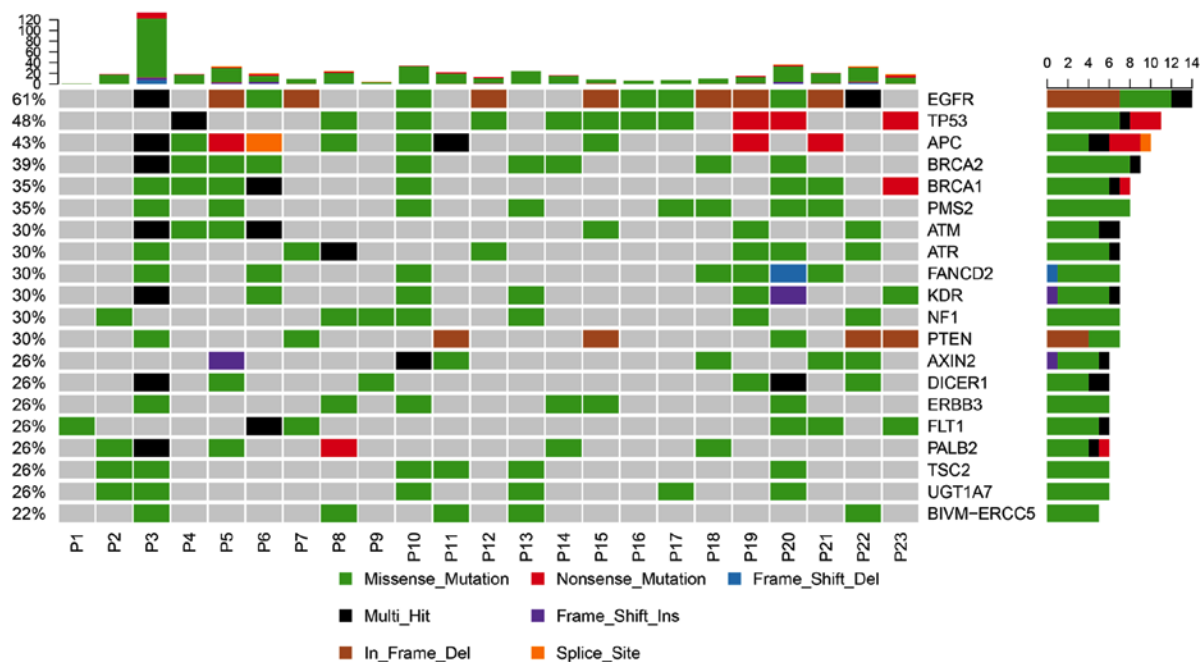


Figure 3. Top 20 mutated genes in the 23 patients with NSCLC. A total of 20 genes' name are listed. P1-P23 present the patient ID. Each type of mutation is presented by a different colour. The values (0-120) on top of the figure present the total number of all the different mutations identified in each patient. The values (22-61%) on the left present the frequency of each gene mutation among the 23 patients. The values (0-14) on the right present patient number who harbored the mutations of the listed genes. NSCLC, non-small cell lung cancer.

samples (Fig. 3). EGFR, TP53, and APC were the most common mutated gene with a frequency of 61, 48 and 43% respectively. Among the 23 NSCLC samples, 69.57% (16/23) of the patients carried the mutations with available matched targeted therapeutic options (Table III). This proportion is consistent with the previous study (23). In this study, EGFR mutations which occurred in exon 18-21 were the most common mutations carried by 14 patients. A total of 13 patients harbored the EGFR sensitizing mutations, which were recommended for first-line treatment with gefitinib, erlotinib, afatinib, dacomitinib, Icotinib or osimertinib according to the NCCN, FDA or China Food and Drug Administration (CFDA) guidelines (24-26). One of these 13 patients harbored the acquired resistance mutation EGFR p.T790M, and was amenable to third-generation EGFR inhibitors, including osimertinib, for first-line or subsequent treatment (27). Patient 21 carried EGFR p.746_750del and PIK3CA p.R108H mutations simultaneously (Table III). According to previous studies, approximately 4-5% of EGFR-mutated lung cancer types with acquired resistance to EGFR-TKI therapy harbor PIK3CA mutations (28-30). A total of 2 patients harbored a KRAS p.G12C mutation, which is a common variant in NSCLC tumors (31). CDKN2A p.W110X was detected simultaneously with EGFR p.E746_A750del in patient 12 (Table III).

Germline mutations with clinical implications. A number of cancer types manifest as a result of inherited defects in key genes within the genome. These genes are a subclass among the cancer driver genes. Their function may involve tumor suppressor genes, oncogenes, genes involved in DNA repair, cell cycle control, apoptosis and angiogenesis factors (32). In the present study, germline mutations were identified through sequencing of the WBC DNA from patients and validated with

paired tumor sample sequencing for 16 patients. The cancerous germline mutations identified in the 15 patients are presented in Table IV. A total of 15/16 patients (93.8%) exhibited cancer driver gene mutations. A total of 57.1% of the mutations were previously reported in the ExAC database and were described as 'Uncertain significance or not provided' in the NCBI ClinVar database, whereas 42.9% were identified as *de novo* mutations.

Discussion

Several single-institution studies revealed that targeted NGS permits the detection of actionable mutations in clinical lung cancer tumor samples, enabling patient selection for genotype-based therapies (10-12). The present study demonstrated the clinical utility of FD-180 profiling in NSCLC. A total of 69.6% (16/23) NSCLC samples in the present study were identified to harbor mutations with available matched targeted therapeutic options. Notably, 13 patients harbored actionable EGFR mutations, with treatment recommended. However, 3 patients may benefit from drug agents, which have not yet been approved for lung cancer. The available data indicating PIK3CA mutations that predict the response of solid tumors to phosphoinositide 3-kinase (PI3K) signaling pathway inhibitors, including everolimus (33) and temsirolimus (34,35), remain controversial. Multiple PI3K inhibitors, including BYL719, buparlisib (BKM120), taselisib (GDC0032), and GSK2636771, are under investigation in patients with PIK3CA-mutated or PTEN-mutated solid tumors (36).

KRAS p.G12C has been negatively associated with progression-free survival (37) and decreased sensitivity of EGFR-TKIs, including erlotinib and gefitinib (38,39). Currently, the role KRAS mutations serve in the selection of anticancer treatment remains unclear; however, clinical evidence suggests

Table III. Actionable genomic alterations and implications for targeted therapeutics.

Patient ID	Genomic alterations	Allele frequency in current study	Associated drugs	Implication for targeted therapeutics
P3	EGFR p.747_753del	0.249	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P5	EGFR p.746_750del	0.205	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P6	EGFR p.L858R	0.382	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P7	EGFR p.745_750del	0.378	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P8	KRAS p.G12C	0.156	Trametinib, Everolimus, Temsirolimus	Possibly sensitive
			Gefitinib, Erlotinib, Afatinib, Icotinib	Decreased EGFR-TKI sensitivity
P10	EGFR p.L861Q	0.206	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
	EGFR p.G719C	0.252	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P12	CDKN2A p.W110X	0.246	Palbociclib	Possibly sensitive
	EGFR p.E746_A750del	0.102	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P15	EGFR p.746_750del	0.248	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P16	EGFR p.L858R	0.297	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P17	EGFR p.L858R	0.287	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P18	EGFR p.747_752del	0.14	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P19	EGFR p.E746_A750del	0.367	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P20	EGFR p.L858R	0.139	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
P21	EGFR p.746_750del	0.367	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	Decreased EGFR-TKI sensitivity
	PIK3CA p.R108H	0.061	Everolimus, Temsirolimus	Possibly sensitive
P22	EGFR p.L747_T751del	0.157	Gefitinib, Erlotinib, Afatinib, Icotinib, Osimertinib	EGFR-TKI sensitive
	EGFR p.T790M	0.208	Osimertinib	EGFR-TKI sensitive
P23	KRAS p.G12C	0.464	Trametinib, Everolimus, Temsirolimus	Possibly sensitive
			Gefitinib, Erlotinib, Afatinib, Icotinib	Decreased EGFR-TKI sensitivity

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; KRAS, KRAS proto-oncogene, GTPase.

that KRAS-mutant tumors, specifically in lung adenocarcinoma, respond positively to selumetinib and trametinib (40). A phase II randomized trial indicated that a selumetinib and docetaxel combined therapy increased overall response rates and progression free survival in patients with KRAS-mutant NSCLC compared with the placebo control group (41).

A mouse model indicated that serine/threonine kinase 11-deficient KRAS mutant NSCLC tumors were resistant to mitogen-activated protein kinase (MEK) inhibition, which may be clinically relevant (42,43). In a phase I dose escalation trial with the MEK inhibitor trametinib, reductions in tumor size of 6-52% were observed in 8/22 patients with KRAS

Table IV. Germline mutations in cancer susceptibility genes in 15 patients.

Patient ID	Gene	Amino acid change	InterPro domain	Reference single nucleotide polymorphism ID number	Allele frequency in the Exome Aggregation Consortium	Allele frequency in current study
P1	FANCD2	p.D662V	-	-	-	0.37
	SLX4	p.D1425N	-	rs766448056	9.05x10 ⁻⁰⁶	0.58
P11	POLE	p.F2258L	-	-	-	0.51
	TSC2	p.P1158S	-	-	-	0.47
P14	FANCM	p.H1103P	-	rs191339110	8.28x10 ⁻⁰⁶	0.39
P15	PTCH1	p.T265S	-	rs201174718	0.0001	0.51
	WRN	p.K577T	DEAD/DEAH box helicase domain; Helicase superfamily 1/2 ATP-binding domain; P-loop containing nucleoside triphosphate hydrolase	-	-	0.49
P16	EXT1	p.V356L	-	-	-	0.47
P19	ATM	p.A1315V	Armadillo-type fold	-	-	0.55
	AXIN2	p.V619A	-	-	-	0.45
P22	FANCA	p.D694N	-	rs201589909	0.0001	0.40
	FANCG	p.P590A	-	rs541868979	9.07x10 ⁻⁰⁵	0.33
	PMS1	p.R202K	Histidine kinase like ATPase C-terminal domain; Ribosomal protein S5 domain 2-type fold; Ribosomal protein S5 domain 2-type fold subgroup	rs2066459	0.012	0.58
P23	MSH3	p.R574Q	DNA mismatch repair protein MutS core	rs776668872	8.72x10 ⁻⁰⁶	0.46
P3	CLCN7	p.N104K	Chloride channel core	-	-	0.58
	MLH3	p.V741F	-	rs28756990	0.0145	0.44
	PTEN	c.-326_-324del	-	-	-	0.18
	SPTA1	p.N2057S	-	rs761106571	5.80x10 ⁻⁰⁵	0.46
P4	FANCM	p.I552T	Helicase C-terminal; P-loop containing nucleoside triphosphate hydrolase	rs200240871	8.25x10 ⁻⁰⁶	0.58
P5	BLM	p.R1139Q	RQC domain; Winged helix turn helix DNA binding domain	rs771776126	8.24x10 ⁻⁰⁶	0.5
P6	STK11	p.P411L	-	-	-	0.65
P7	APC	p.A1753P	-	rs587781350	1.66x10 ⁻⁰⁵	0.49
	ATM	p.D841Y	-	-	-	0.41
	FANCA	p.T1161K	-	rs142833057	3.30x10 ⁻⁰⁵	0.55
	SPTA1	p.D607H	-	rs534906145	8.28x10 ⁻⁰⁶	0.58
P8	POLD1	p.R322H	DNA-directed DNA polymerase family B exonuclease domain; Ribonuclease H like domain	-	-	0.39
P9	CLCN7	p.R775H	CBS domain	rs534953229	0.0002	0.33
	SLC2A2	p.G519E	-	rs147959014	0.0018	0.39

-, information not available.

mutation-positive tumors (44). However, the aforementioned studies require further validation with larger sample sizes and inclusion of KRAS wild-type tumors for comparison to enhance

the evidence for clinical efficacy. Therefore, the use of MEK inhibitors for treating KRAS mutation-positive tumors is only recommended in a clinical trial setting.

Somatic mutations of CDKN2A are present in various tumor types (45-47). However, currently there are no existing standard therapy options to treat tumors harboring somatic CDKN2A alterations. The loss of function mutation of CDKN2A leads to activation of CDK2/4/6, suggesting that CDK inhibitors may be beneficial for these patients (48). Palbociclib is a highly selective CDK4/6 inhibitor, which has demonstrated sensitivity in *in vitro* studies of renal cell carcinoma (49), melanoma (50), ovarian cancer (51) and breast cancer (52) cell lines, suggesting that this drug may be effective for the treatment of patients included in the present study.

Even though actionable genomic alterations are primarily somatically acquired, it has been reported that inherited cancer predisposition is predominantly associated with germline mutations (32). In this study a total of 93.8% of patients (15/16) carried germline mutations which have been previously associated with cancer. Among these 28 mutations, known and *de novo* germline mutations were identified in 57.1% (16/28) and 42.9% (12/28), respectively. Only a small proportion of NSCLC cases may be attributed to inheritance of genetic mutations (53). However, the results obtained in the present study suggested otherwise, indicating that the association between the identified mutations and lung cancer requires further investigation with a larger sample size.

Notably, the current study does not provide information on the long-term impact of the FD-180 gene panel on treatment outcomes, including morbidity, quality of life, disease-free survival and overall mortality. A limitation of the present study is therefore the lack of long-term follow-up to monitor these parameters. In conclusion, the present study demonstrated that the FD-180 gene panel is a robust diagnostic tool which may be used to identify targeted therapeutics for patients with NSCLC.

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Availability of data and material

The raw sequencing data are not publicly available due to information that could compromise the privacy of the research participants.

Authors' contributions

WC and TT designed the study. HFW collected the data. CY and HLW analyzed the data. DL designed and wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The Anhui Medical University Ethics Committee approved the study and all patients have given written informed consent prior to inclusion in the project.

Patient consent for publication

All patients provided a comprehensive consent form stating that personal data may be used for academic presentation or paper presentation, while ensuring complete anonymity prior to receiving treatment.

Competing interests

The authors declare that they have no competing interests.

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