Identification of Fibroblast Growth Factor Receptors (FGFRs) Alterations (alts) at DNA and RNA-level by One-Step Next-Generation Sequencing

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INTRODUCTION

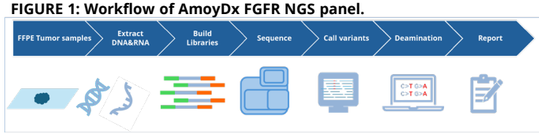
- FGFR inhibitors are currently in clinical development[1] or approved for locally advanced/metastatic urothelial cancer (e.g., erdafitinib)[2] and cholangiocarcinoma with FGFR fusion or/and mutation[3].
- AmoyDx FGFR NGS Panel was developed for FGFR alteration detection based on both DNA and RNA.
- The goal of this study is to assess and validate the performance of AmoyDx FGFR NGS Panel in pan-cancer FGFR alteration detection by comparing with a comprehensive genomic profiling test (AmoyDx master panel) and a health authority (HA) approved DNA-based NGS panel.

Assay type	Panel size	Alteration type	
		DNA	RNA
AmoyDx FGFR NGS panel	FGFR 1-4	mutations	Fusions
AmoyDx master panel	Broad panel	mutations	Fusions
HA approved DNA-based NGS panel	Broad panel	mutations, fusions	NA

METHODS

- DNA&RNA co-capture process was developed for AmoyDx FGFR NGS panel (Figure 1). Libraries was built using "one-step" method (Reverse transcription of RNA and PCR of cDNA were done in one-step operation) . LOD of SNV/Indel was 5% VAF, 250 copies/100ng.
- Both AmoyDx FGFR NGS Panel and AmoyDx master panel are DNA/RNA based for detecting FGFR alteration with optimized bioinformatics pipeline to eliminate baseline noise caused by deamination events, which is commonly found in aged FFPE samples.
- Cell lines, cell line/patient derived xenografts (CDXs/PDXs) and 397 samples of 26 cancer types (90 samples >10 years) were used for validation of AmoyDx FGFR NGS Panel.
- Total 382 and 50 pan-cancer samples were respectively tested to compare AmoyDx FGFR NGS Panel with AmoyDx master panel and HA approved DNA-based NGS panel.
- Break-apart FISH (Fluorescence In Situ Hybridization) assay was carried out on FFPE slides using AmoyDx FGFR2 Fusion Analysis Kit.

FIGURE 1: Workflow of AmoyDx FGFR NGS panel.



RESULTS

100% accuracy of AmoyDx FGFR NGS panel in reference alterations detection

- 36 FFPE samples from cell lines and CDXs/PDXs with known FGFR status (25 fusions, 5 mutations, 6 wild-types) were tested by AmoyDx FGFR NGS Panel with concordance rate 100% (Table 1).

TABLE 1: 100% assay accuracy achieved by AmoyDx FGFR NGS panel

	Reference alterations			
AmoyDx FGFR NGS Panel	**Mutation**	Positive	Negative	Total
	Positive	5	0	5
	Negative	0	31	31
	Total	5	31	36
	Fusion	Positive	Negative	Total
	Positive	25	0	25
	Negative	0	11	11
	Total	25	11	36
The overall percent agreement		100%		

Reference alterations	
Fusions	**Mutations**
FGFR2-AFF3	FGFR3 R248C
FGFR2-BICC1	FGFR3 S249C
FGFR2-CASP7	FGFR3 Y373C
FGFR2-CCDC6	FGFR3 C382R
FGFR2-MCU	
FGFR2-OFD1	
COL25A1-FGFR2	
FGFR3-BAI1AP2L1	
FGFR3-TACC3	

Solid Tumors

RESULTS

High concordance between AmoyDx FGFR NGS panel and reference assays

- In clinical sample testing, AmoyDx FGFR NGS Panel showed high agreement with AmoyDx master panel (99.5%, Table 2) and HA approved DNA-based NGS panel (98.0%, Table 3).

TABLE 2: High concordance between AmoyDx FGFR NGS Panel and AmoyDx master panel

	AmoyDx master panel			
AmoyDx FGFR NGS Panel	**Mutation**	Positive	Negative	Total
	Positive	29	0	29
	Negative	1	194	195
	Total	30	194	224
	Fusion	Positive	Negative	Total
	Positive	20	1	21
	Negative	0	137	137
	Total	20	138	158
The overall percent agreement		99.50%		

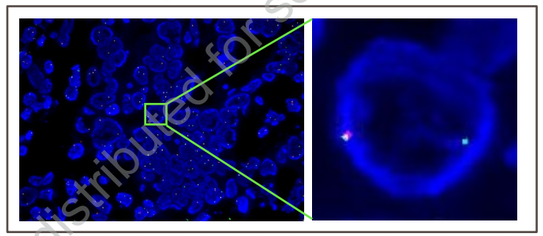
TABLE 3: High concordance between AmoyDx FGFR NGS Panel and HA approved DNA-based NGS panel.

	HA approved DNA-based NGS panel			
AmoyDx FGFR NGS Panel	**Alteration**	Positive	Negative	Total
	Positive	12	1	13
	Negative	0	37	37
	Total	12	38	50
The overall percent agreement		98.00%		

Conflicting result between AmoyDx FGFR NGS panel and HA approved DNA-based NGS panel was confirmed by FISH

- Conflicting result confirmed by FISH revealed 1 fusion missed by HA approved DNA-based NGS panel but detected by AmoyDx FGFR NGS Panel (Figure 2 and 3), which demonstrated advantage of FGFR alteration detection by DNA+RNA.

FIGURE 2: Conflicting result confirmed Positive by FISH



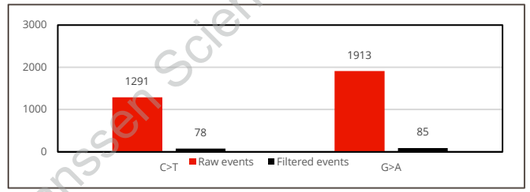
Note: Analysis was performed using 100 × oil objective for left image. Representative image of positive signal was enlarged on the right. FGFR2 dual color break-apart probe consist of 5' end green and 3' end red signals. Either single green dot or separate green and red dots denote FGFR2 fusion positive signal, reflecting FGFR2 break apart.

FIGURE 3: Detailed FGFR breakpoints and fusion form of the conflicting result sample.

Using Algorithm to filter false C:G>T:A substitutions (Figure 4)

- An optimized bioinformatics pipeline for identifying deamination events could effectively filtered out C:G>T:A false positive signals[4]. Through the filtering process, the number of C>T substitutions in the raw data was reduced from 1291 to 78, and the number of G>A substitutions was reduced from 1913 to 85.

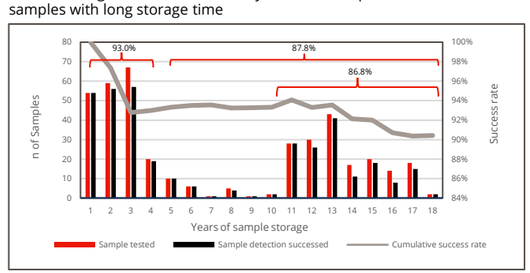
FIGURE 4: Identified C>T and G>A substitution events between raw data and filtered data.



High success rate of AmoyDx FGFR NGS panel detection in samples with long storage time (Figure 5)

- The testing success rates of samples less than 5 years old, more than 5 years old and more than 10 years old were 93.3%, 87.8% and 86.8%, respectively.

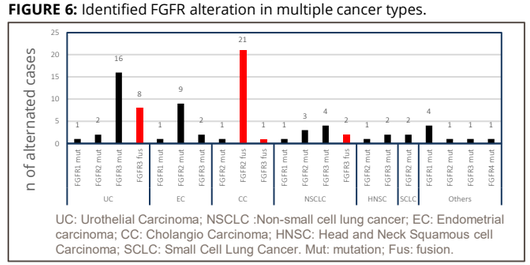
FIGURE 5: High success rate of AmoyDx FGFR NGS panel detection in samples with long storage time



Detected FGFR alterations in different cancer types (Figure 6)

- FGFR alteration differs between cancer types. FGFR3 SNV/Indel frequently occurred in Urothelial Carcinoma and Non-small cell lung cancer. In Endometrial carcinoma, FGFR2 mutations commonly occurred. FGFR2 fusions were predominant FGFR alteration in cholangiocarcinoma.

FIGURE 6: Identified FGFR alteration in multiple cancer types.



UC: Urothelial Carcinoma; NSCLC :Non-small cell lung cancer; EC: Endometrial carcinoma; CC: Cholangio Carcinoma; HNSC: Head and Neck Squamous cell Carcinoma; SCLC: Small Cell Lung Cancer. Mut: mutation; Fus: fusion.

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Presented by Changbin Zhu at AACR; April 7, 2024; San Diego, California.

KEY TAKEAWAY

AmoyDx FGFR NGS Panel provides a novel opportunity to identify FGFR altered pan-cancer patients using a robust DNA+RNA NGS platform, which shows high success rate even in aged samples and may be a potent tool for sensitive and reliable detection of FGFR alts for clinical diagnostics.

FGFR alteration differs between cancer types. Comprehensive platform using RNA and DNA for sequencing may fully identify patients with FGFR alteration to guide treatment.

CONCLUSIONS

AmoyDx FGFR NGS panel (DNA/RNA based) showed high concordance with AmoyDx master panel and HA approved DNA-based NGS panel in FGFR alteration testing, including mutations and fusions.

An optimized bioinformatics pipeline for identifying deamination events could effectively filtered out C:G>T:A false positive signals.

AmoyDx FGFR NGS panel yielded similar success rates from samples new and older than 10 years old.

DISCLOSURES

Min Qing, Xiaofang Zhuo, Ting Shen, Chengjuan Xiong, Xuesong Lyu, Renee Tate, Qibiao Wu, Longen Zhou, and Shibu Thomas are full employee of Janssen Research & Development.
Xuejun Chen, Wenqing Su, Wangwang Ning, Jianqing Wang, Huihui Yan, Ziqiang Yin, Zhan Huang, Yaxin Xue, and Changbin Zhu are full employee of AmoyDX.

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