COMPARING DIFFERENT METHODS OF *FGFR1* ABERRATIONS ANALYSIS IN SQUAMOUS CELL LUNG CANCER (SQCLC) POTENTIAL TARGETED THERAPY

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INTRODUCTION

Data indicate that aberrations of *Fibroblast Growth Factor Receptor 1 (FGFR1)* e.g. gene amplification, fusions or mutations are involved in pathogenesis of SqCLC. Correlation between *FGFR1* aberrations and response to anti-FGFR treatment was observed in several clinical trials of FGFR inhibitors. In targeted therapies, choosing the best patient selection method is crucial for maximum therapeutic response. Here we compare different methods of *FGFR1* aberrations analysis.

MATERIALS AND METHODS

20 formalin-fixed, paraffin embedded (FFPE) samples with the highest tumour density were selected from cohort of 204 patients with SqCLC. *FGFR1* aberrations were analysed using different methods (see Fig.1)

Patients` characteristics

Table 1. Clinicopathological characteristics of patients

Number of cases		20
Age in years : median (range)		67,27 (58-77)
Gender	female	10
	male	10
Stage	I	5
	II	4
	III	4
	IV	0
	no data	7
Grade	G1	0
	G2	13
	G3	5
	no data	2

DNA/RNA concentration in isolated samples was estimated by Nanodrop™ and quality of the samples was analyzed by Bioanalyzer system (RNA samples) or TapeStation System (DNA samples) (Agilent).

Fluorescence *In Situ* Hybridization method (FISH)

The analysis of *FGFR1* gene copy number was performed using probes specific for the 8p12 locus and the chromosome 8 centromere (CEN 8) (Zyto*Light*® SPEC FGFR1/CEN 8 Dual Color Probe and Zyto*Light*® FISH-Tissue Implementation Kit, ZytoVision GmbH, Germany). A total of sixty nuclei (20 contiguous tumour cell nuclei from three areas) were individually evaluated by counting green *FGFR1* and orange CEN 8 signals. *FGFR1* amplification criteria were previously described by Schultheis *et al.*: *FGFR1*/CEN 8 signal ratio ≥ 2.0 or the average number of *FGFR1* signals per nucleus ≥ 6 or ≥10% of tumour cells containing ≥15 *FGFR1* signals or large clusters.

Immunohistochemistry (IHC)

FGFR1 protein expression was determined by immunohistochemistry method. Tissue slides were subjected to antigen retrieval in Target Retrieval Solution, pH 9 (DAKO/Agilent Technology; Denmark) with PT Link (DAKO/Agilent Technology; Denmark). Tissues were incubated with anti-FGFR1 rabbit polyclonal antibody (Cell Signaling Technology, clone D8E4, Danvers, MA, USA). Detection was performed with EnVision TM+ system (DAKO/Agilent Technology, Denmark). Overexpression was defined as staining intensity ≥2+ (graded from 0 to 3+) in ≥10% tumour cancer cells.

Next-generation sequencing (NGS)

RNA isolated from FFPE samples preparations was sequenced by NGS (MiSeq Illumina) using RNA-FusionPlex Lung panel). The NGS results were analyzed in Archer analysis software v6.0 (Archer Dx). The *FGFR1* relative gene expression level was assessed based on the ratio of unique RNA reads originating from probes across the targeted gene and housekeeping genes (*CHMP2A, GPI, RAB7A* and *VCP*) .

MATERIALS AND METHODS

Real-time PCR

FGFR1 expression was determined by the Real-time PCR methods method (Applied Biosystems 7500 Fast Real Time PCR system (Thermo Fisher)): The Real-time PCR reactions were run in triplicates using about 10 ng of total RNA, previously subjected to reverse transcription to cDNA and TaqMan Gene Expression Assays (Thermo Fisher): *FGFR1* (Hs00241111_m1) and *POLR2A* (Hs00172187_m1) as reference gene. Obtained data were quantified using the delta Ct method for relative quantification of gene expression.

nCounter®Analysis System

The expression level of *FGFR1* gene mRNA and copy number variation (CNV) were determined on the NanoString® Platform using custom panels.
The analysis consisted of a specific determination of mRNA copy number through hybridization with the fluorescent probes. Using the custom probe panel, hybridization of 300 ng of RNA isolated from FFPE samples was performed. mRNA level ≥ 800 counts was considered elevated.
For copy number variation ratio ≥ 4 was considered elevated, compared to neoplastically unchanged lung tissue.

The study was approved by local ethical committee.

Statistical analysis

Spearman test was performed to assess correlation between: mRNA level and protein expression; CNV and FISH results (GraphPad Prism 8).

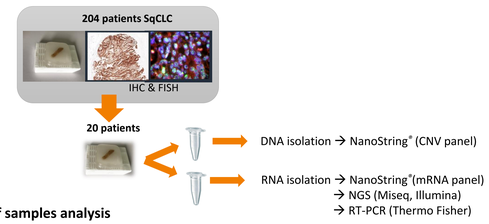


Figure 1. Scheme of samples analysis

RESULTS

The range of mRNA level (NanoString®) varied between 289.05 and 8902.82 counts (mean=1667.85). *FGFR1* amplification determined by FISH was observed in 11/20 (55%) samples. Overexpression of FGFR1 protein was observed in 10/20 (50%) specimens. FISH and CNV NanoString® results were consistent in 75% of SqCLC samples (15/20). High-level amplification evaluated by FISH correlated with CNV assessed with NanoString® (p=0.0154; r=0.5). mRNA (NanoString®) and protein overexpression level for FGFR1 was consistent in 18/19 (94.70%) and correlated (p<0.0001; r=0.9) for 19 SqCLC tumour samples.

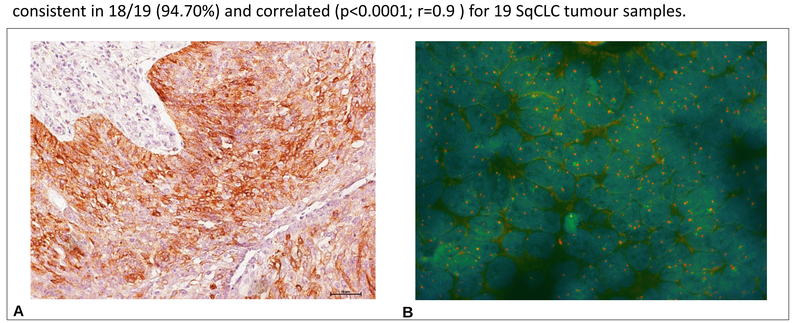
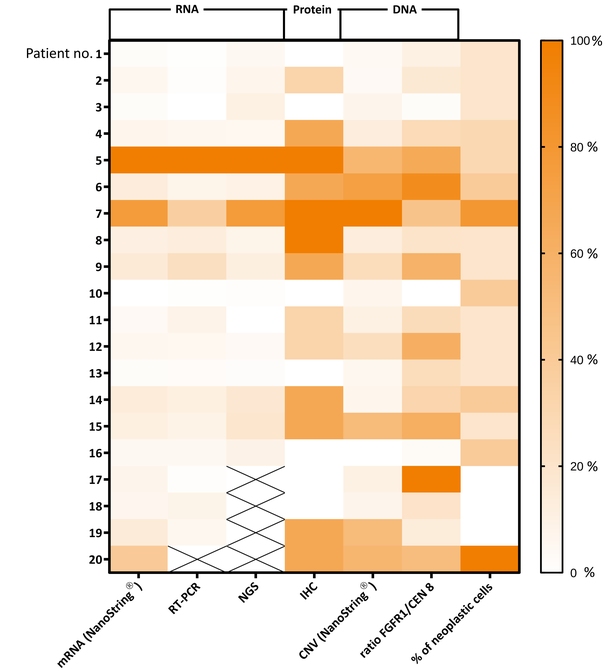


Figure 2. Representative examples of FGFR1 expression (A) and amplification (B) in SqCLC samples; green signal -*FGFR1*; orange - CEN 8

RESULTS

Figure 3. Comparing different methods of *FGFR1* aberration detection.
Normalized results; the lowest value is 0% ( ) and the highest value is 100% ( )



CONCLUSIONS

Multiplexed analysis of FGFR1 aberrations may contribute to broader patient population eligible for FGFR inhibitor therapy, which may increase their overall clinical feasibility and potential therapeutic benefits.

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