Abstract nº
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Multi institutional evaluation of a new NGS assay for mutation detection from cfDNA in lung cancer

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Background

The detection of actionable mutations in lung cancer is still a major challenge due to the lack of tissue specimens for molecular profiling of the tumor in approximately 25% of patients. The circulating cell-free tumor DNA (ctDNA) isolated from plasma of cancer patients is an alternative, minimally invasive source of tumor DNA that also allows rapid determination of the mutational status of the tumor. However, the intrinsic low abundance of mutations in cfDNA makes their detection and quantification in plasma a challenging task. Here we report a multi-institutional (Figure 1) validation of the Oncomine cfDNA Lung Cancer assay for the analyses of cfDNA in molecular pathology laboratories.

Aim

Perform a multi-institutional validation of the Oncomine cfDNA Lung Cancer assay for the analyses of cfDNA in molecular pathology laboratories

Figure 1. OncoNetwork Consortia.

Material & Methods

1. Biological material

- Multiplex I cfDNA Reference Standard (Horizon Discovery, Cambridge, United Kingdom);
  - DNA derived from human cell lines and fragmented to an average size of 160±10% (144bp–176bp) to closely resemble cfDNA extracted from human plasma;
  - reference standard covers 8 mutations in the EGFR, KRAS, NRAS and PIK3CA genes at 5%, 1%, 0.1% allelic frequencies and wild-type allele (Figure 2);
  - the same lot of reference standards was distributed to the participating laboratories within the OncoNetwork Consortium (Figure 1).

5% Multiplex

Gene	Variant	AF (%)
EGFR	L858R	5.0
EGFR	ΔE746 - A750	5.0
EGFR	T790M	5.0
EGFR	V769-D770insASV	5.9
KRAS	G12D	6.3
NRAS	Q61K	6.3
NRas	A59T	6.3
PIK3CA	E545K	6.3

1% Multiplex

Gene	Variant	AF (%)
EGFR	L858R	1.0
EGFR	ΔE746 - A750	1.0
EGFR	T790M	1.0
EGFR	V769-D770insASV	1.0
KRAS	G12D	1.3
NRAS	Q61K	1.3
NRas	A59T	1.3
PIK3CA	E545K	1.3

0.1% Multiplex

Gene	Variant	AF (%)
EGFR	L858R	0.1
EGFR	ΔE746 - A750	0.1
EGFR	T790M	0.1
EGFR	V769-D770insASV	0.1
KRAS	G12D	0.13
NRAS	Q61K	0.13
NRas	A59T	0.13
PIK3CA	E545K	0.13

Additional variants present in the cell line DNA, at approximately 20-40% AF, include:
- *EGFR* p.G719S;
- *PIK3CA* p.H1047R;
- *MAP2K1* p.Q56P;
- *BRAF* p.V600E

Figure 2. Variants with respective allelic frequencies present in the reference samples used for the validation study.

2. Library preparation for tag-sequencing

- Target amplification (Figure 3) performed using Oncomine™ Lung cfDNA Assay (ThermoFisher Scientific);
  - 35 amplicons covering 169 known hotspot SNVs & indels in 11 clinically relevant genes (*ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1* and *TP53*).

3. Template preparation and Sequencing

- Template preparation was performed using the manual Ion OT2 or the automated Ion Chef system according to laboratory availability;
- Libraries were multiplexed as four on a 318/520 chip or eight on a 530 chip;
- Samples were sequenced twice in each participating laboratory (Figure 1);
- Sequencing was performed using the Ion PGM system, Ion Proton System or Ion S5xl system according to availability.

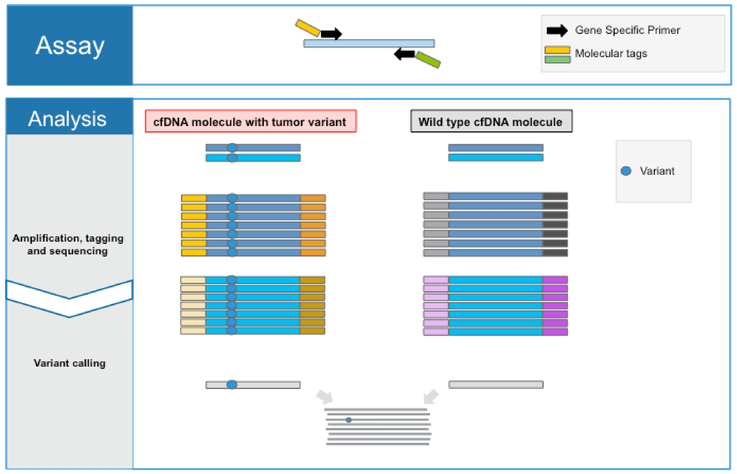

Figure 3. Illustration of the principle behind target amplification and variant calling for tag-sequencing.

4. Data analysis

- Sequencing data was imported into Torrent Suite;
- Variant detection was performed using a dedicate bioinformatics cfDNA analysis pipeline within Variantcaller version 5.2.

Results

1. Reproducibility of the methodology

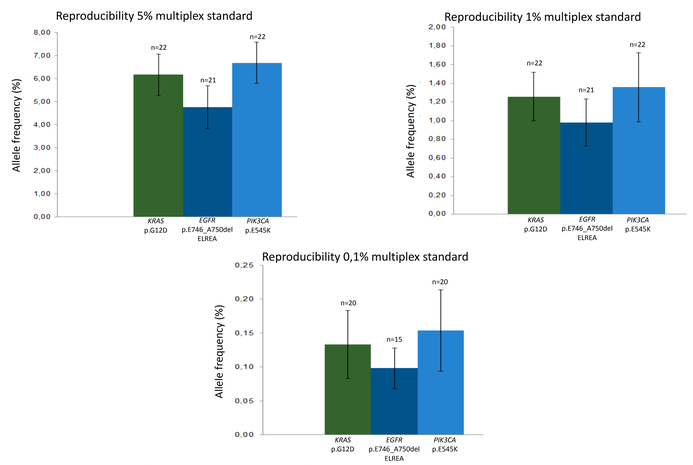

Figure 4. Results reproducibility for selected variants at different allelic frequencies.

3. Overall analytical performance of the methodology.

Table1. Analytical performance of the assay in the eleven participating laboratories.

Lab	TP	Total TP	TN	Total TN	sensitivity	PPV	NPV	specificity
ARCNET	43	48	1266	1272	89.58%	87.76%	99.61%	99.53%
CROM	46	48	1272	1272	95.83%	100.00%	99.84%	100.00%
IPATIMUP	46	48	1272	1272	95.83%	100.00%	99.84%	100.00%
Radboud	43	48	1270	1272	89.58%	95.56%	99.61%	99.84%
Queens	47	48	1271	1272	97.92%	97.92%	99.92%	99.92%
St James	46	48	1272	1272	95.83%	100.00%	99.84%	100.00%
UHCW	46	48	1269	1272	95.83%	93.88%	99.84%	99.76%
Charite	46	48	1270	1272	95.83%	95.83%	99.84%	99.84%
Viollier*	39	40	1113	1115	97.50%	95.12%	99.91%	99.82%
HEGP	45	48	1268	1272	93.75%	91.84%	99.76%	99.69%
Kindai	46	48	1268	1272	95.83%	92.00%	99.84%	99.69%
Total	493	520	13811	13835	94.81%	95.93%	99.80%	99.83%

* Single replicate of 0.1% control run

3. Detection of the *EGFR* p.T790M mutation

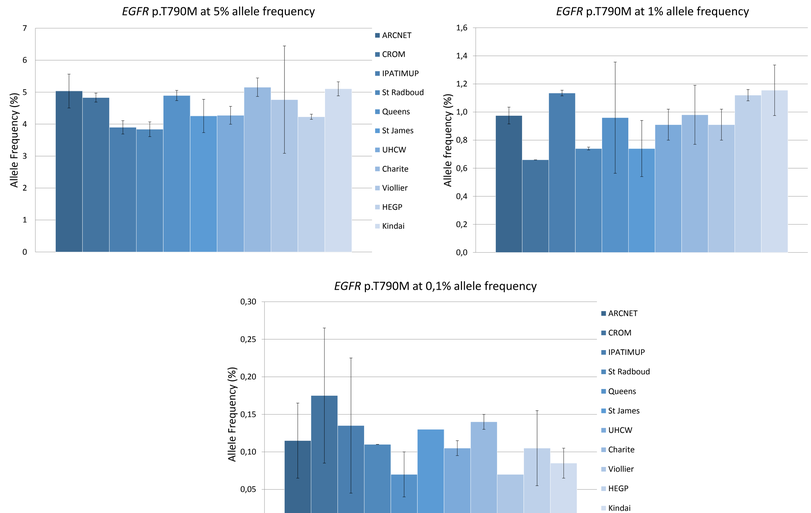

Figure 5. Detection of *EGFR* p.T790M mutation by the participating laboratories.

Conclusion

- All variants present in the reference standards were detected at the different laboratories;
- Elevated reproducibility was obtained amongst the different laboratories (Figure 4);
- An overall sensitivity of 94,81% and specificity of 99,83% was obtained for the methodology (Table 1);
- At 0.1% allelic frequency, all the participating laboratories were able to detect the challenging *EGFR* p.T790M mutation (Figure 5);
- These results confirm the potential use of the Oncomine cfDNA lung assay for plasma genotyping to allow for the noninvasive, multiplexed detection of complex, targetable genomic alterations in lung cancer.

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