Feasibility study:
comparing tissue and blood profiles at baseline for the identification of genomic alterations

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ThermoFisher SCIENTIFIC

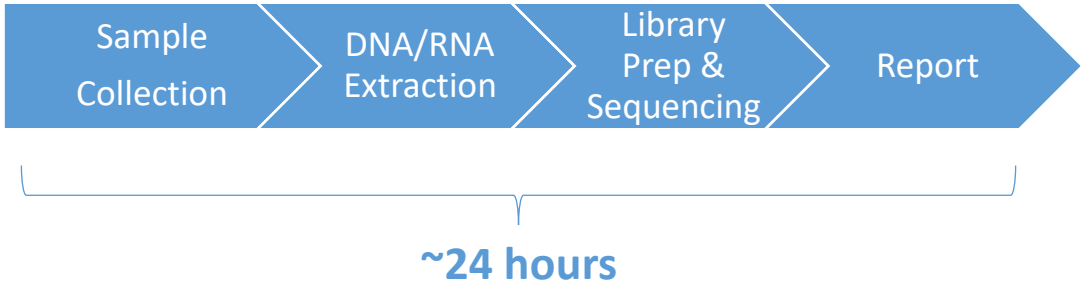
IRE Molecular diagnostics: NSCLC

- Lung Cancer: >5000 cases analyzed since 2016 (840 cases 2022)
- Comprehensive molecular characterization
- High number of weekly cases to sequence
- Timely access to testing is a critical first step for best cancer management

2022

solution

fully automatic platform



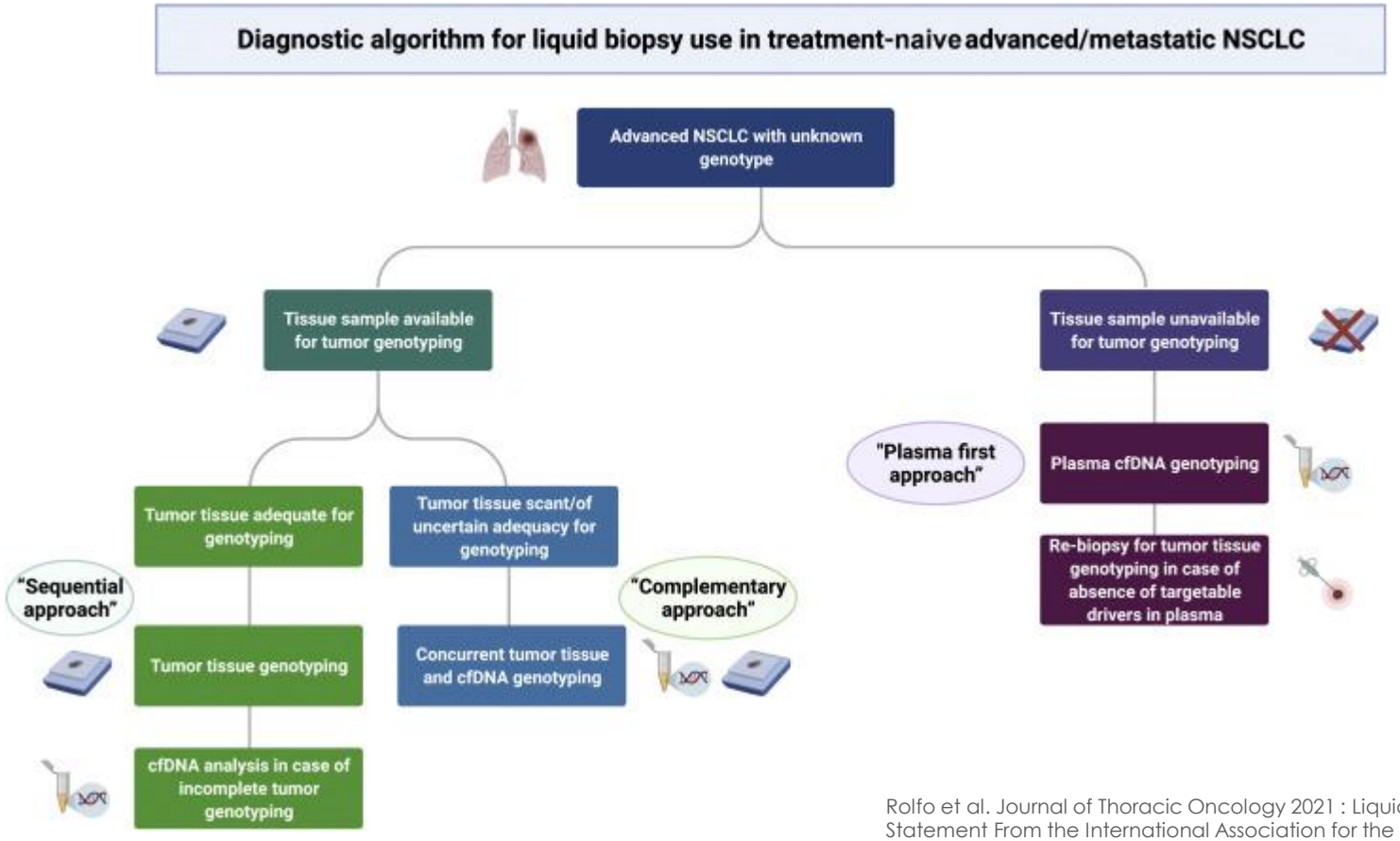
WE HAVE MADE A NUMBER OF IMPROVEMENTS

1) Reduction in TAT: single day work flow
2) A new 50 gene panel includes all relevant and emerging targets in precision oncology research
3) A higher **reproducibility** and **efficiency**
4) Liquid biopsy analysis

DNA hotspots					CNVs		Inter-genetic fusions		Intra-genetic fusions
AKT1	CHEK2	FGFR3	KIT	NTRK3	ALK	FGFR1	ALK	NTRK1	AR
AKT2	CTNNB1	FGFR4	KRAS	PDGFRA	AR	FGFR2	BRAF	NTRK2	EGFR
AKT3	EGFR	FLT3	MAP2K1	PIK3CA	CD274	FGFR3	ESR1	NTRK3	MET
ALK	ERBB2	GNA11	MAP2K2	PTEN	CDKN2A	KRAS	FGFR1	NUTM1	
AR	ERBB3	GNAQ	MET	RAF1	EGFR	MET	FGFR2	RET	
ARAF	ERBB4	GNAS	MTOR	RET	ERBB2	PIK3CA	FGFR3	ROS1	
BRAF	ESR1	HRAS	NRAS	ROS1	ERBB3	PTEN	MET	RSPO2	
CDK4	FGFR1	IDH1	NTRK1	SMO			NRG1	RSPO3	
CDKN2A	FGFR2	IDH2	NTRK2	TP53					

Background of the feasibility study

minimally invasive liquid biopsy and rapid genomic profiling are expected to be in future potential options for initial genomic screening

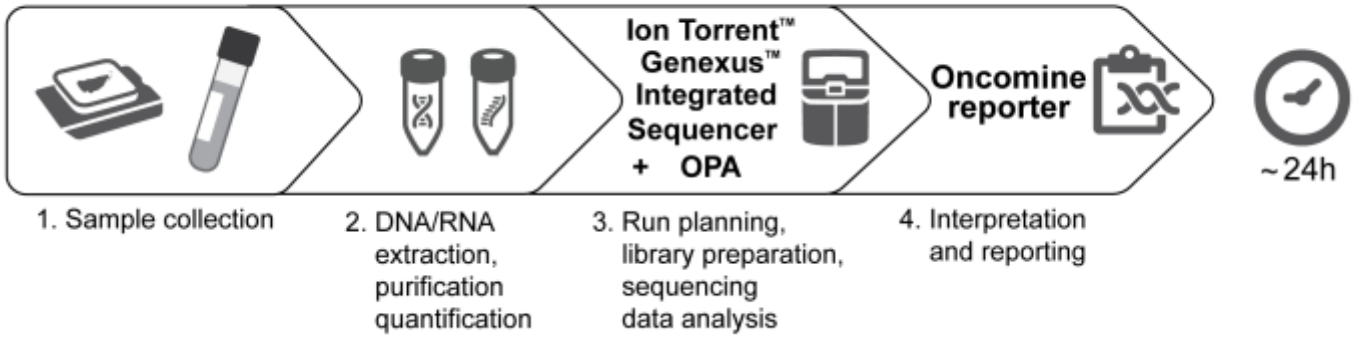


Rolfo et al. Journal of Thoracic Oncology 2021 : Liquid Biopsy for NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer (IASLC)

Aims and study design

Validation study by comparing tissue and blood profiles at baseline for the identification of variants

- ❑ To setup the preanalytical phase
- ❑ Selection of 20 positive cases (focusing on targetable alterations: *EGFR, ALK, ROS1, BRAF, RET, KRAS, NTRK1/2/3, MET* ex 14)
- ❑ Tissue and liquid biopsy samples were tested using the same NGS platform and gene panel
- ❑ To assess the concordance between genomic alterations identified through liquid biopsy and those observed in the corresponding tissue

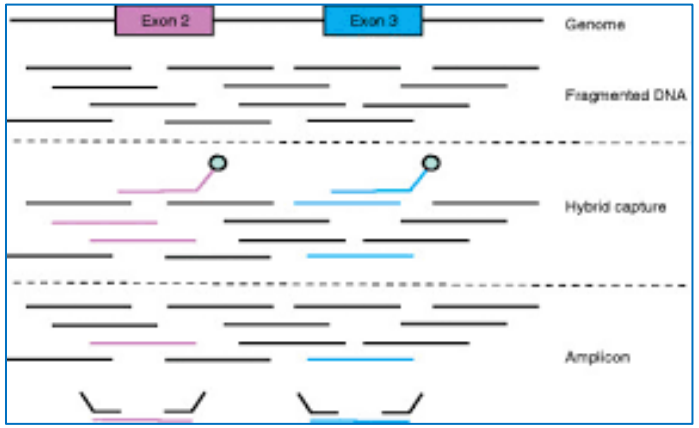


ctDNA *vs* cfTNA

❑ To date, only circulating tumor DNA (ctDNA), extracted from plasma samples and analyzed by NGS assays with a hybrid-capture approach, has obtained FDA approval in clinical practice

❑ Only two studies (Genexus-OPA)* - NGS assays with amplicon-based approach which involves analysis from cfTNA (which include both cfDNA and cfRNA)

❑ We need something new: validate the NGS assays with amplicon-based approach for use in clinical practice



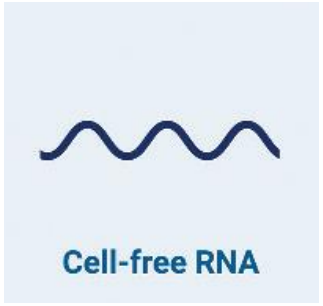
*
- *SK Low et al. Transl Lung Cancer Res 2022*
- *Gumà J et al. Pathobiology. 2023*

Liquid biopsy: Preanalytical Phase

Tumor genotyping through liquid biopsy is influenced by a large number of preanalytical variables including: sample collection, handling, processing and storage procedures

BUT if the amplicon-based approach is to be used for analysis
Careful attention should be paid to preanalytics

short half-life (only few minutes) and low concentration of the cfRNA



Liquid biopsy: Preanalytical Phase

Tumor genotyping through liquid biopsy is influenced by a large number of preanalytical variables including: sample collection, handling, processing and storage procedures

Organization of a well defined «liquid biopsy path»

What we did:

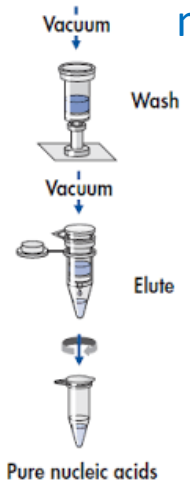
- One day per week dedicated to liquid biopsy analysis
- Booking (by the oncologist on a prepared and shared file)
- Two biologists dedicated to the preanalytical phase

as quickly as possible

Preanalytical phase

- Collection: 14 ml of peripheral blood samples in K_2EDTA tubes and processed within 15 min
- Centrifugation: Plasma was isolated by two successive rounds of centrifugation at 4°C (2000 x *g* for 20 min, and 13000 x *g* for 10 min), and stored at -80° in single-use 2 ml aliquots
- Extraction: 2 different methods:

1) QIAmp Circulating Nucleic Acid kit (Qiagen)

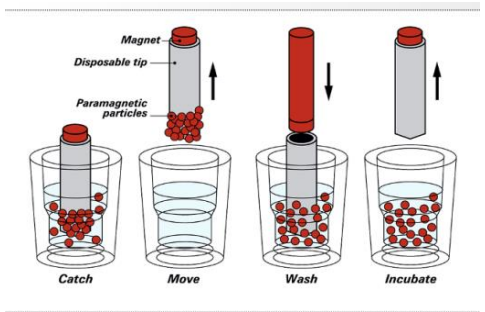


manual silica-based

2) Genexus Cell-Free Total Nucleic Acid Purification workflow

automated magnetic bead-based

Quick TAT
(less than 4 hours)



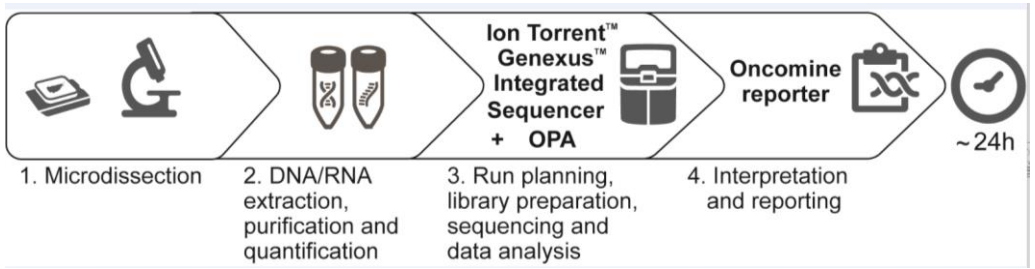
cfTNAs were fluorimetrically quantified with the Qubit dsDNA HS assay kit

- storage: stored at -80°C until analysis

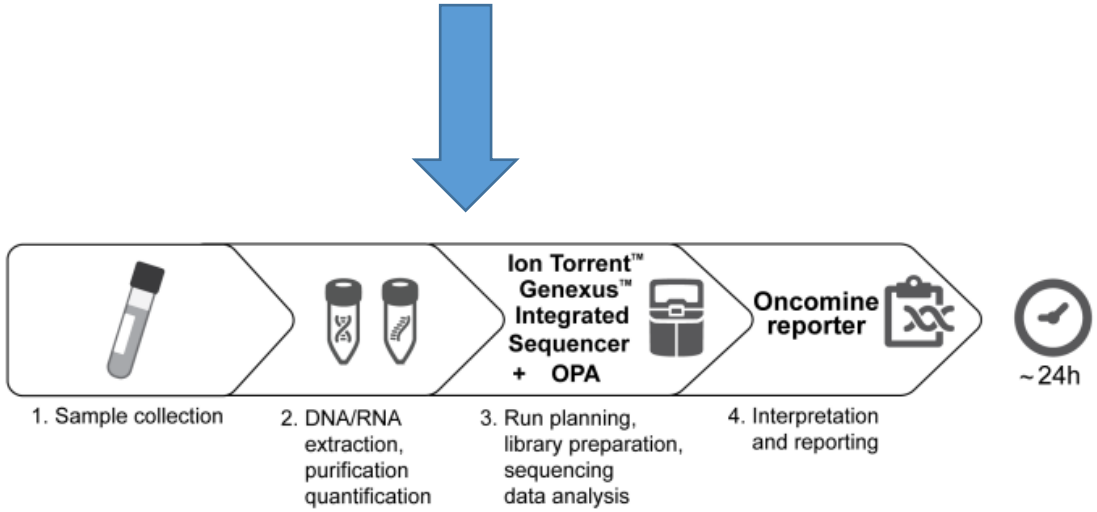
FFPE Tissue samples

A1	EGFR p.L858R	TP53 p. H179R
A2	EGFR p.L858R	
A3	EGFR p.E746_A750del	
A4	EGFR p.E746_A750del	
A5	EGFR p.L861Q	EGFR p.G719A
A6	EGFR p.H773_V774insY	TP53 p.V216L
A7	BRAF p.V600E	
A8	ERBB2 p. Y772_A775dup	
A9	KRAS p.G12C	TP53 p.Y220C
A10	KRAS p.G12D	
A11	ALK fusion: :EML4(13)-ALK(20)	
A12	ALK fusion: TPM3(7)-ALK(20)	
A13	ROS1 fusion: CD74(6)-ROS1(34)	TP53 p.L194R
A14	NTRK3 fusion: ETV6(4)-NTRK3(14)	
A15	RET fusion: CCDC6(1)-RET(12)	TP53 pG266V
A16	RET fusion: CCDC6(1)-RET(12)	
A17	RET fusion KIF5B(15)-RET(12)	
A18	MET exon 14 skipping	MET c.2942_1G>A
A19	MET exon 14 skipping	
A20	MET amplification	

- ❑ Selection of 20 positive tissue samples
- ❑ Molecular characterization with OPA-Genexus



- ❑ Plasma at baseline stored at -80



RESULTS: extraction and sequencing

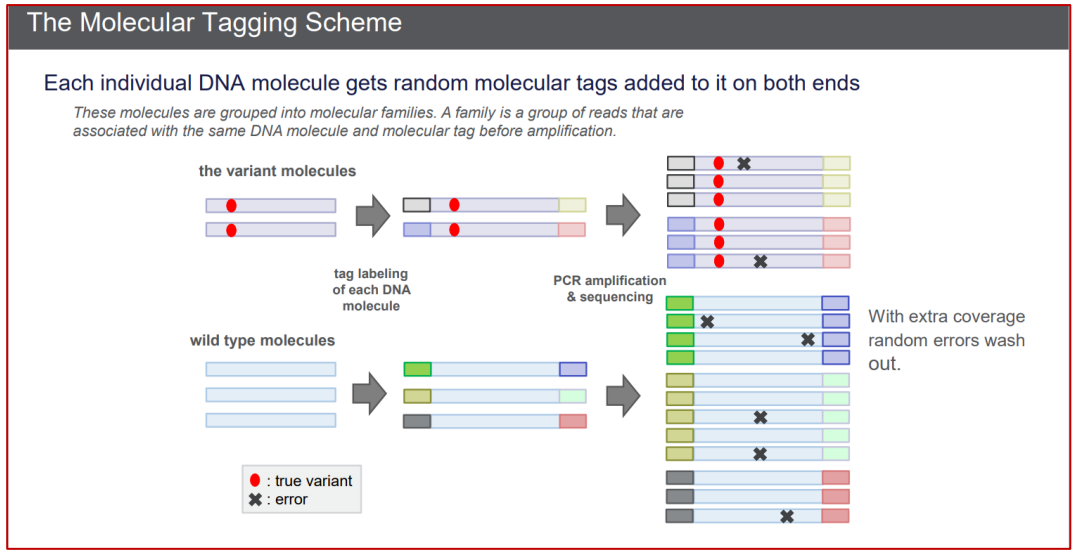
- An average of 50 ng (range 10-1200 ng, median: 40ng) of cfTNA were extracted from the plasma samples
- No statistically significant difference was observed in terms of yield (ng of extracted cfTNA) between the two extraction methods
- Almost all samples (90%) achieved the optimal input at 20 ng for Genexus-OPA sequencing
- The success rate for library construction and sequencing was 100%
- TAT: 2 days (from sampling to report)

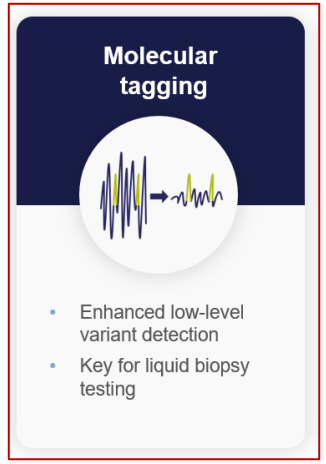
RESULTS

Summary of NGS parameters

Parameters	Average	Range
Total reads	11.668.314	4,222,995–14,139,983
Mapped reads	11,344,432	3,322,939–13,737,486
Mean read length (bp)	97	94–101
Mean depth (×)	41,054	27,789–57,595
Median molecular coverage (×)	2,117	472–2,770
Molecular uniformity	94%	58%–98%

- MMC refers to the molecular families that are associated with the same DNA molecule
- molecular tagging is carried out to increase sensitivity and render it applicable to liquid biopsy.





RESULTS: analysis of concordance rate

Comparison of genomic profiling between tumor tissue and liquid biopsy

85.2% (23/27) of genomic alterations detected from tumor tissue were concordantly detected from plasma cfTNA

20 cases:

Tissue & cfTNA	n=16
Tissue only	n=4
cfTNA only	n=0

Detected
Not detected

Tissue only:
1 EGFR DEL19
1 MET AMPLIFICATION
1 MET EXON 14 SKIPPING
1 ALK FUSION

❑ SNV and INDEL:
VAF average: 3.5% (0.6-42)

❑ FUSIONS:
Reads count per million: 400 (50-1000)

A1	EGFR p.L858R	TP53 p. H179R	
A2	EGFR p.L858R		
A3	EGFR p.E746_A750del		
A4	EGFR p.E746_A750del		
A5	EGFR p.L861Q	EGFR p.G719A	
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A16	RET fusion: CCDC6(1)-RET(12)		
A17	RET fusion KIF5B(15)-RET(12)		
A18	MET exon 14 skipping	MET c.2942_1G>A	
A19	MET exon 14 skipping		
A20	MET amplification		

FALSE NEGATIVE RESULTS

Not detected in cfTNA

Tissue only:
1 *EGFR* DEL19
1 *MET* AMPLIFICATION
1 *MET* EXON 14 SKIPPING
1 *ALK* FUSION

The 4 non-detected variants in cfTNA could be associated with a low percentage of mutated fragments below the LOD of the assay used:

False negative results in LB are generally caused by:

- A low tumor burden of disease (could be the case of *EGFR* deletion)
- Low or no ctDNA shedding due to the tumor site
- Vascularization or histology of the cancer lesions (such as isolated brain metastasis) (could be the case of *ALK* fusion)
- nucleic acid degradation in the preanalytical phase

RESULTS

MET exon 14 skipping: NGS tissue (DNA + RNA) *vs* liquid biopsy (cfTNA)

Results / 25_Mag_22_OPA_Enzo_LB_MTB ▾ / OPA DNA w3.2.0 ▾ / NL357_22 ▾

NL357_22 QC ✓ Key Findings **Variants** Plugins Reports

All | SNVs/Indels | CNVs Gene List Only

Variant Matrix Summary (5.16) Filter Chain Applied
1 of 2,138 SNVs/Indels Edit Filters

Locus	Oncomine Variant Class	Gene	AA Change	Allele Frequency	Coverage	Nuc Change	Raw Read Depth	Effective Read Depth
chr7:116411902	METExon14Skipping	MET	p.?	0.227	12651	c.2942-1G>A	13214	12651

Genexus Assay Development | Ion Torrent Dashboard Samples Runs Results Assays

Results / LUNG_31_05_22 ▾ / OPA Fusions w3.1.0 ▾ / NL357_22_RNA ▾

NL357_22_RNA QC ✓ Key Findings **Variants** Plugins Reports

Fusions Gene List Only

Variant Matrix Summary (5.16) Filter Chain Applied
1 of 1,007 Fusions Edit Filters Export Columns ▾

Locus	Genes (Exons)	Read Counts	Type	Gene Isoform	Mol Cov. Mutant	Read Counts Per Million
chr7:116411708 - chr7:116414935	MET(13) - MET(15)	2532	RNAExonVariant	MET-MET.M13M15.1	562	8117

Genexus Assay Development | Ion Torrent Dashboard Samples Runs Results Assa

Results / BIORETMET_05_05_22 ▾ / OPA cfTNA w3.2.0 ▾ / ctTNA_ptCG_t0 ▾

ctTNA_ptCG_t0 QC ✓ Key Findings **Variants** Plugins Reports

All | SNVs/Indels | Fusions | CNVs

Oncomine Variants (5.16) Filter Chain Applied
2 of 3,159 Variants Edit Filters

Type	Gene	Allele Frequency (%)	Coverage	Nuc Change	Effective Read Depth	Genes (Exons)	Read Counts
snp	MET	7.9	1615	c.2942-1G>A	1615		
RNAExonVariant	MET					MET(13) - MET(15)	217

RESULTS

Reporting fusion in LB from cfRNA is quite challenging given its instability

A complete overlap (100%) in RET fusion calling was observed in 3 cases
by comparing tissue and blood profile at baseline

NGS tissue report

Materiale inviato:
Perviene inclusione n 21/8051 relativa a biopsia pleurica sede di adenocarcinoma

ESAMI RICHIESTI: analisi dello stato mutazionale dei seguenti geni: ***ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK1,2,3, RET, ROS1***

METODICA UTILIZZATA: test NGS (Next Generation Sequencing); piattaforma Ion Torrent Genexus;

pannello Oncomine Precision Assay (OPA) (DNA+RNA)

Cellularità tumorale presente nel campione: 30%

RISULTATO

E' STATA RILEVATA UNA FUSIONE DEL **GENE *RET: CCDC6* (1)-*RET* (12)**

(livello di significatività clinico-terapeutica: 1*; IA**)

FCCDC6 esone 1 - *RET* esone 12; fusione gain of function; numero di reads 764

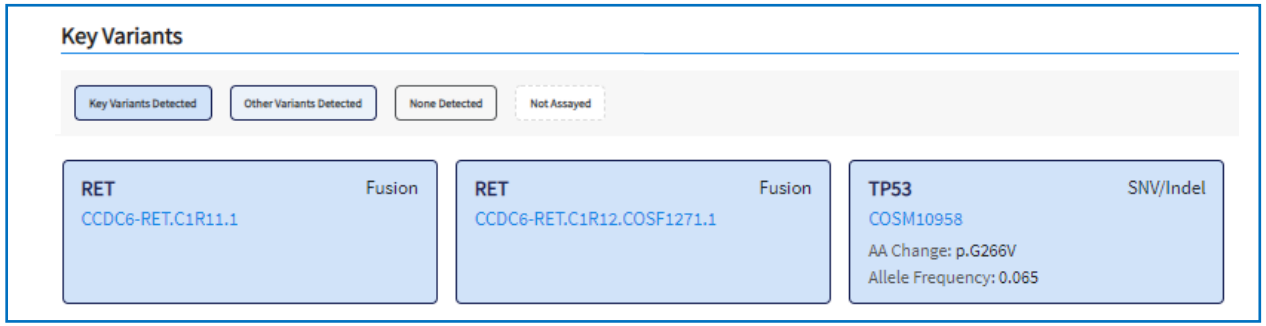
- E' stata rilevata inoltre la fusione: ***CCDC6* (1)-*RET* (11)** (livello 1*; IA**); *CCDC6* esone 1 - *RET* esone 11; fusione gain of function; numero di reads 178

Livelli di significatività clinico terapeutica:

1*/Tier IA marcatore predittivo di risposta a farmaco approvato in questo tipo di neoplasia**

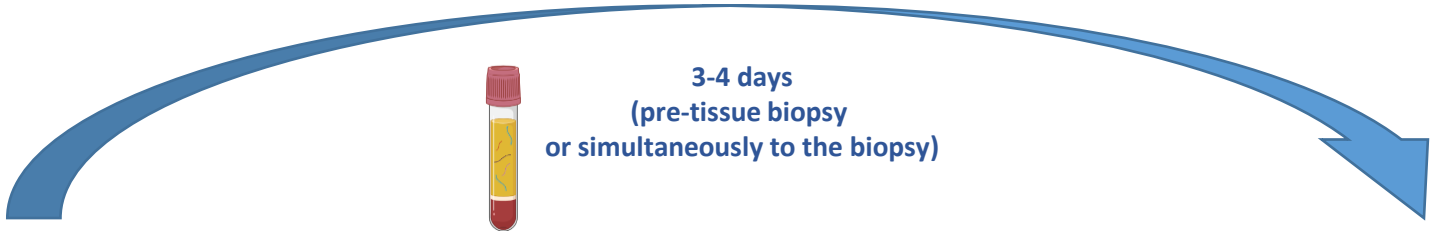
-E' stata rilevata la presenza di una mutazione a carico del gene ***TP53* : G266V;** NM_000546.5 : c.797GGGGG>T; p.(Gly266Val); frequenza allelica della variante: 19%; oncogenica

cfTNA



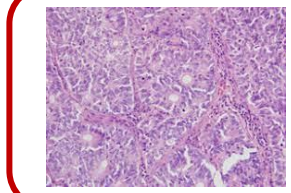
Future outlook

Integrate liquid biopsy earlier into diagnostic pathway



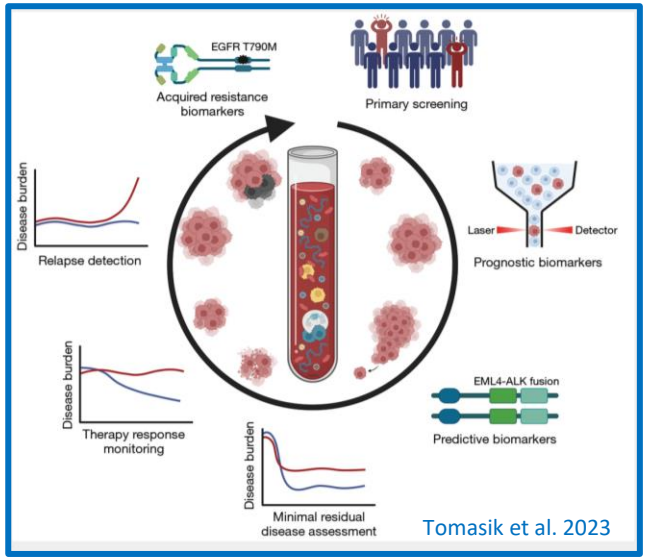
Scan, staging

Tissue Biopsy and Diagnosis



Molecular Diagnosis and Treatment

Current and potential applications of liquid biopsy in NSCLC



Tomasik et al. 2023

Conclusions

- ✓ High concordance in identifying actionable mutations between tissue and cfTNA
- ✓ Gene fusion detection from cfRNA can be achieved through amplicon-based NGS analysis provided there is a well-organized liquid biopsy path
- ✓ Liquid biopsy (cfTNA) could be used as complementary to tumor tissue biopsies to identify actionable mutations
- ✓ The shorter TAT approach provided by Genexus-OPA suggested the possibility of performing cfTNA genomic profile in advance
- ✓ This enables the detection of actionable mutations while waiting for the results of tumor biopsy or when tissue quantity is not sufficient for testing

Acknowledgments

Edoardo Pescarmona, Pathologist
Paolo Visca, Pathologist

Francesca Rollo, Biologist
Enzo Gallo, Biologist
Livia Ronchetti, Biologist
Cristiana Ercolani, Biologist

The whole team at Pathology Unit
Molecular Diagnostics Laboratory
Regina Elena National Cancer Institute, Rome, Italy