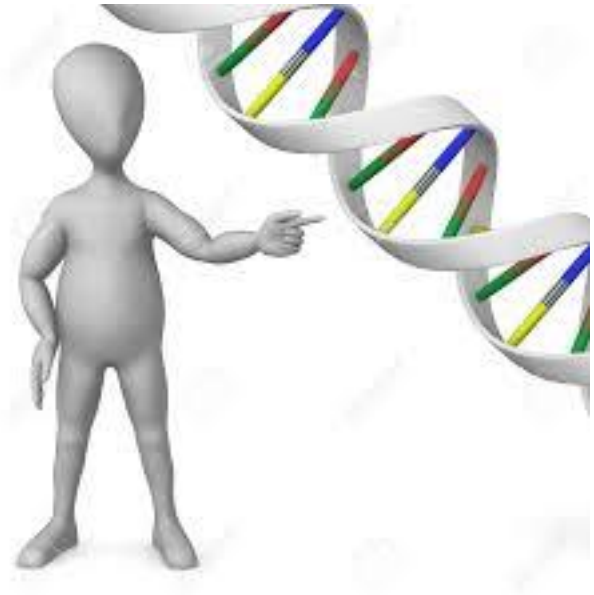
The economic advantage of in-house 1 day NGS



- *Dr. Javier Hernández Losa*

- *30th August 2021*

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Agenda

- 1) Genexus Incorporation
- 2) Experience with OPA analysis in NSCLC
- 3) Experience in other Solid Tumors

Why perfom NGS analysis?

- **Molecular Profiling in solid tumors**
  - Soft Tissue Tumors
  - Paediatric Tumors

- **Biomarker analysis**
  - NSCLC
  - CRC
  - Other Tumor Type

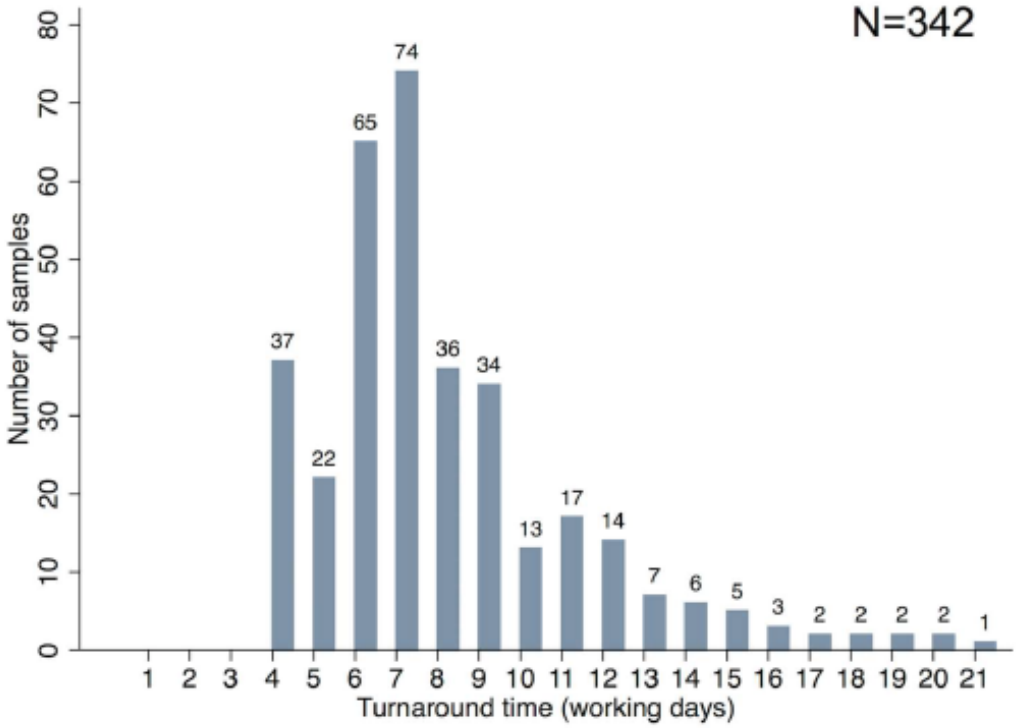
- **Translational Research**

PLOS | MEDICINE

RESEARCH ARTICLE

Clinical applicability and cost of a 46-gene panel for genomic analysis of solid tumours: Retrospective validation and prospective audit in the UK National Health Service

A



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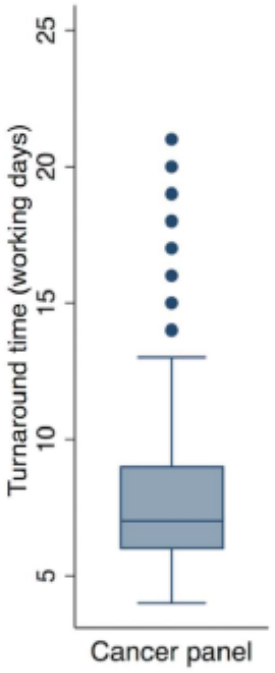
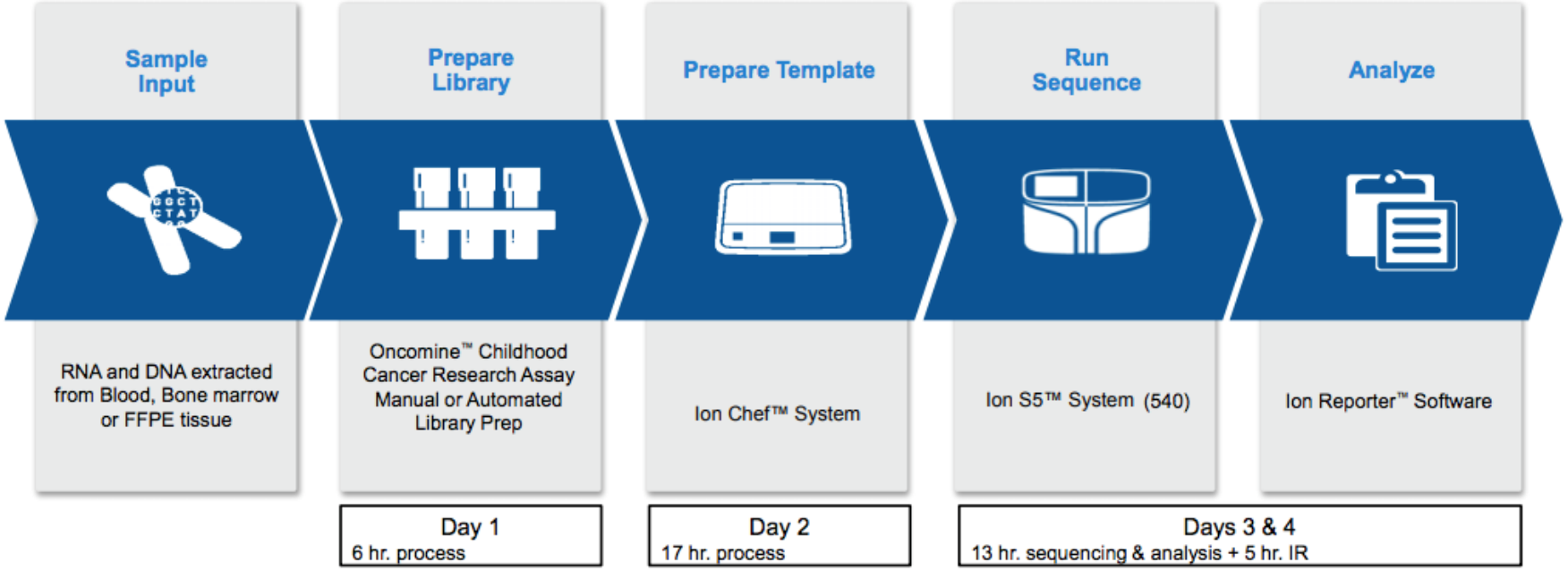


Hamblin A et al. Plos Medicine 2017.

OCCRA Workflow

- RNA and DNA from same sample
  - Seven DNA samples plus one negative control –PLUS-
  - Seven RNA samples plus one negative control per chip



Sample Selection and tumor area evaluation (1-2 days).
Sample collection for cost-effective batch sizes.(1-5 days)

TAT 7-10 days

PLOS | MEDICINE

RESEARCH ARTICLE

Clinical applicability and cost of a 46-gene panel for genomic analysis of solid tumours: Retrospective validation and prospective audit in the UK National Health Service

Table 4. Total cost per sample and type of malignancy by resource category.

Cost Item	Type of Test					Combination of Single Gene Tests for Different Malignancies		
	Ion AmpliSeq Panel	cobas *BRAF*	cobas *EGFR*	cobas *KRAS*	*KIT* (Sanger Sequencing)	NSCLC (*BRAF*, *EGFR*, *KRAS*)	Melanoma (*BRAF*, *NRAS*, *KIT*)	Colorectal Carcinoma (*KRAS*, *NRAS*)
Equipment	£18 (5%)	£0 (0%)	£0 (0%)	£0 (0%)	£0 (0%)	£1 (0%)	£1 (0%)	£0 (0%)
Consumables	£185 (55%)	£34 (48%)	£62 (59%)	£93 (66%)	£83 (60%)	£177 (64%)	£159 (57%)	£134 (65%)
Staff	£48 (14%)	£23 (33%)	£23 (22%)	£23 (16%)	£30 (21%)	£48 (17%)	£65 (23%)	£35 (17%)
Miscellaneous[a]	£31 (9%)	£2 (2%)	£2 (2%)	£2 (1%)	£2 (2%)	£5 (2%)	£5 (2%)	£3 (2%)
Overheads	£56 (17%)	£12 (17%)	£17 (17%)	£23 (17%)	£28 (17%)	£46 (17%)	£50 (18%)	£35 (17%)
Total test cost	£339 (100%)	£71 (100%)	£104 (100%)	£141 (100%)	£138 (100%)	£276 (100%)	£280 (100%)	£208 (100%)
Total test cost in US dollars	$449	$94	$138	$187	$183	$366	$371	$276

Hamblin A et al. Plos Medicine 2017.

NGS vs Single Gene Approach

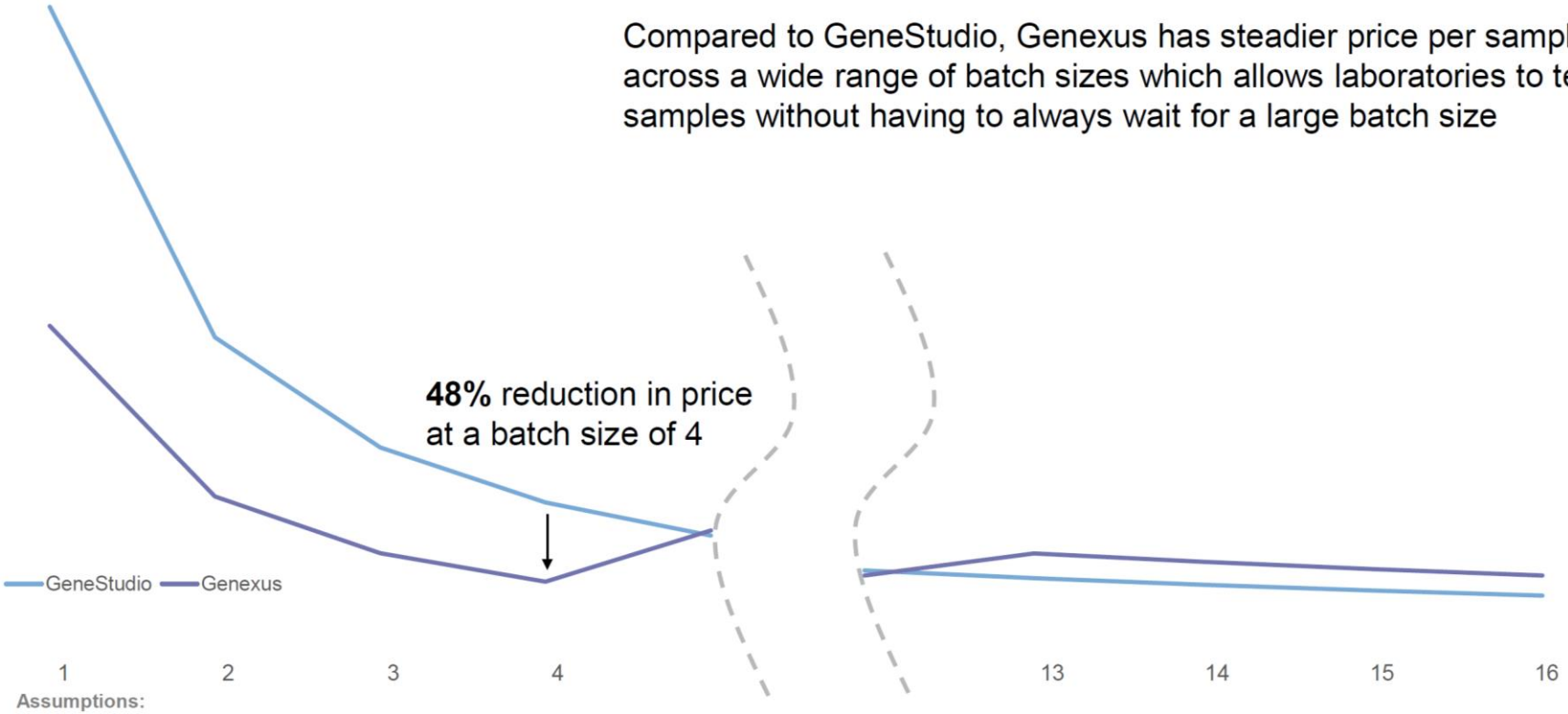
Diferencial			NSCLC							
	NGS	TOTAL (€)	EGFR MUT	ALK TRANS	ROS1 TRANS	RET TRANS	NTRK1 TRANS	MET MUT	BRAF mut	PD-L1
1505	820	2325	375	465	295	295	295	350	250	215
			CRC							
			KRAS MUT	NRAS MUT	BRAF MUT					
130		950	350	350	250					
			GIST							
			KIT MUT	PDGFRA MUT						
-170		650	300	350						

**** The prices were obtained globally in Spain by different suppliers and are expressed in euros***

Estimation from SEAP-SEOM Biomarkers Plattform report 2017

Genexus Is Cost Effective at Low Batch Sizes

Compared to GeneStudio, Genexus has steadier price per sample across a wide range of batch sizes which allows laboratories to test samples without having to always wait for a large batch size



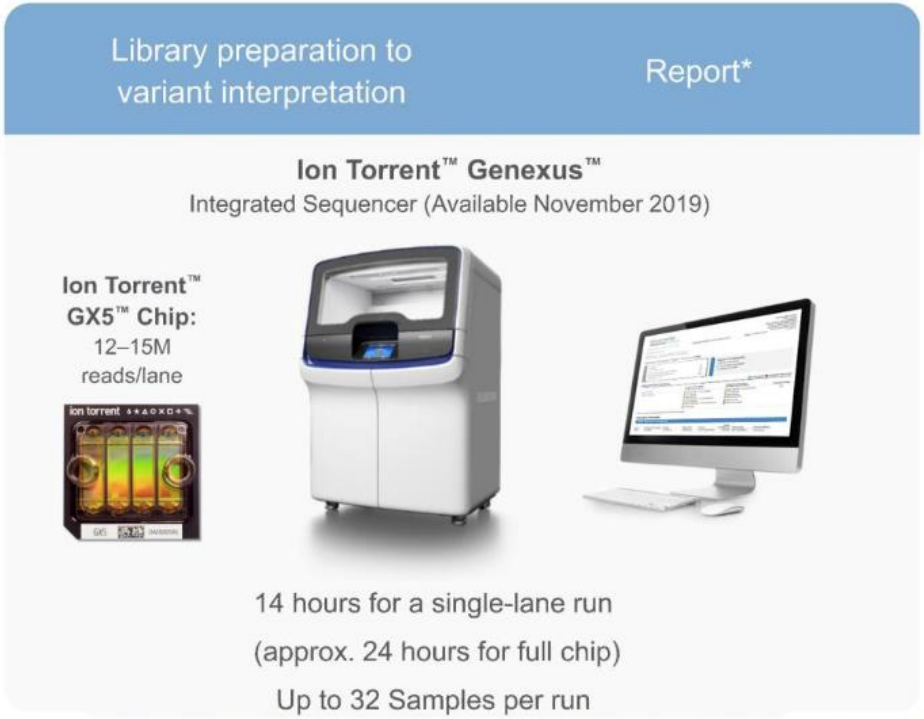
Assumptions:

- For Genexus, per sample pricing calculated using list prices for Oncomine Precision Assay with associated Genexus consumables (running FFPE samples)
- For GeneStudio, per sample pricing calculated using list prices for Oncomine Focus Assay with associated GeneStudio S5 consumables (Ion Chef automated library preparation)

Batch sizes are important

			8	7	6	5	4	3
Description	Number reactions	PRICE	PRICE	PRICE	PRICE	PRICE	PRICE	PRICE
ArcherFusionPlexSarcomaPanel(8rx)	8	2724,2	340,53	340,53	340,53	340,53	340,53	340,53
ArcherMBCAdaptersA17-A24for Illumina								
MiseqReagentkitv2(300cycles)	8	1190,4	148,80	170,06	198,40	238,08	297,60	396,80
AB0003MBC1V2300	8	3914,6						
AgencourtFormaPure	50	440,0	8,80	8,80	8,80	8,80	8,80	8,80
AgencourtAMPureXPBeads5mL	25	285,4	11,42	11,42	11,42	11,42	11,42	11,42
iTaqUniversalSYBRGreenSupermix	160	134,0	0,84	0,84	0,84	0,84	0,84	0,84
KAPAUniversalLibraryQuantificationKit	56	615,9	11,09	11,09	11,09	11,09	11,09	11,09
QUBITASSAYTUBES*SETOF500*	500	237,12	0,47	0,47	0,47	0,47	0,47	0,47
QUBITRNAHSASSAYKIT,500	500	66,5	0,13	0,13	0,13	0,13	0,13	0,13
			522,1	543,3	571,7	611,4	670,9	770,1

Why Genexus?



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- ✓ Workflow optimization batches (n=4 samples/line)
- ✓ Full automation
- ✓ Reduction in TAT (14 hours)
- ✓ Possibility to combine FFPE and Liquid Biopsy analysis

ONCOMINE PRECISION ASSAY (OPA)

DNA

Genes con cobertura Hotspot:

AKT1	ESR1	MAP2K2
AKT2	FGFR1	MET
AKT3	FGFR2	MTOR
ALK	FGFR3	NRAS
AR	FGFR4	NTRK1
ARAF	FLT3	NTRK2
BRAF	GNA11	NTRK3
CDK4	GNAQ	PDGFRA
CDKN2A	GNAS	PIK3CA
CHEK2	HRAS	PTEN
CTNNB1	IDH1	RAF1
EGFR	IDH2	RET
ERBB2	KIT	ROS1
ERBB3	KRAS	SMO
ERBB4	MAP2K1	TP53

45 genes

CNV (ganancia y pérdida de copias):

ALK
AR
CD274
CDKN2A
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
KRAS
MET
PIK3CA
PTEN

14 genes

RNA

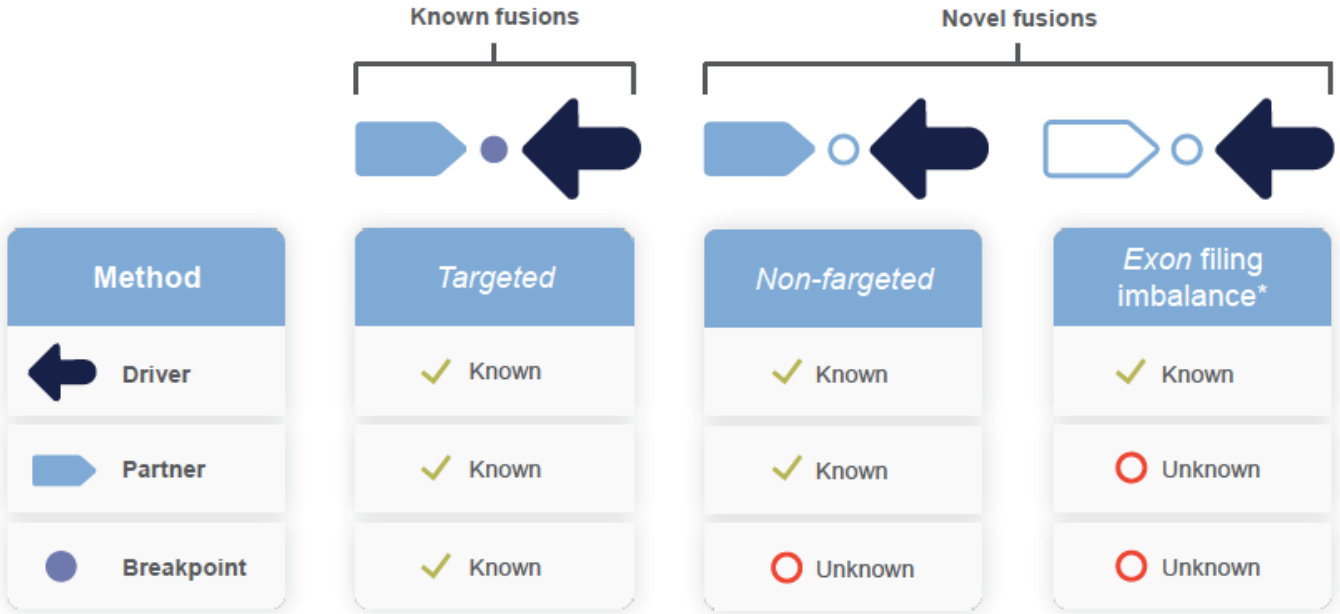
Fusiones Génicas:

ALK	RET
AR	ROS1
BRAF	RSPO2
EGFR	RSPO3
ESR1	
FGFR1	
FGFR2	
FGFR3	
MET	
NRG1	
NTRK1	
NTRK2	
NTRK3	
NUTM1	

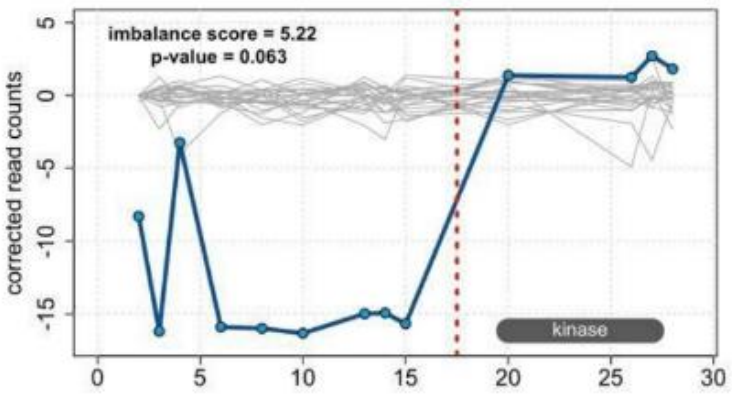
18 variants

FusionSync Detection Technology

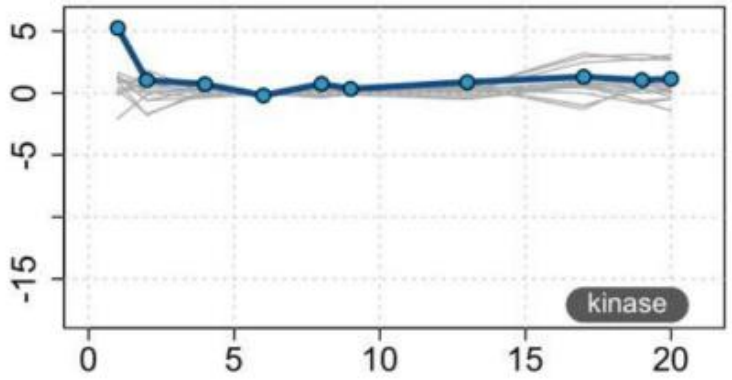
FusionSync Detection Technology is a synchronous approach that combines three methods for sensitive, specific, and broad detection of known and novel fusions



ALK FUSION



NO FUSION

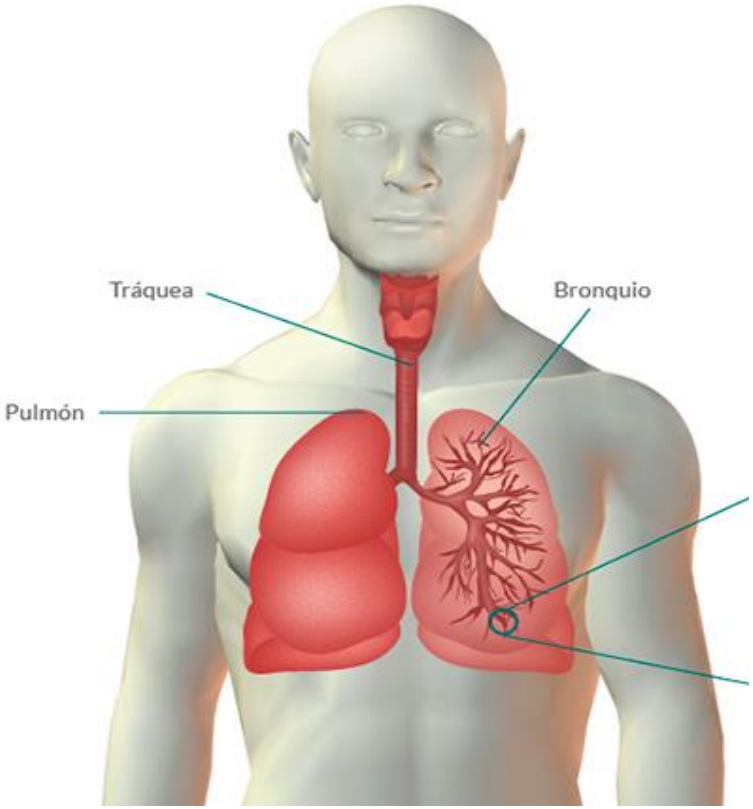


Oncomine Precision Assay Throughput on Genexus

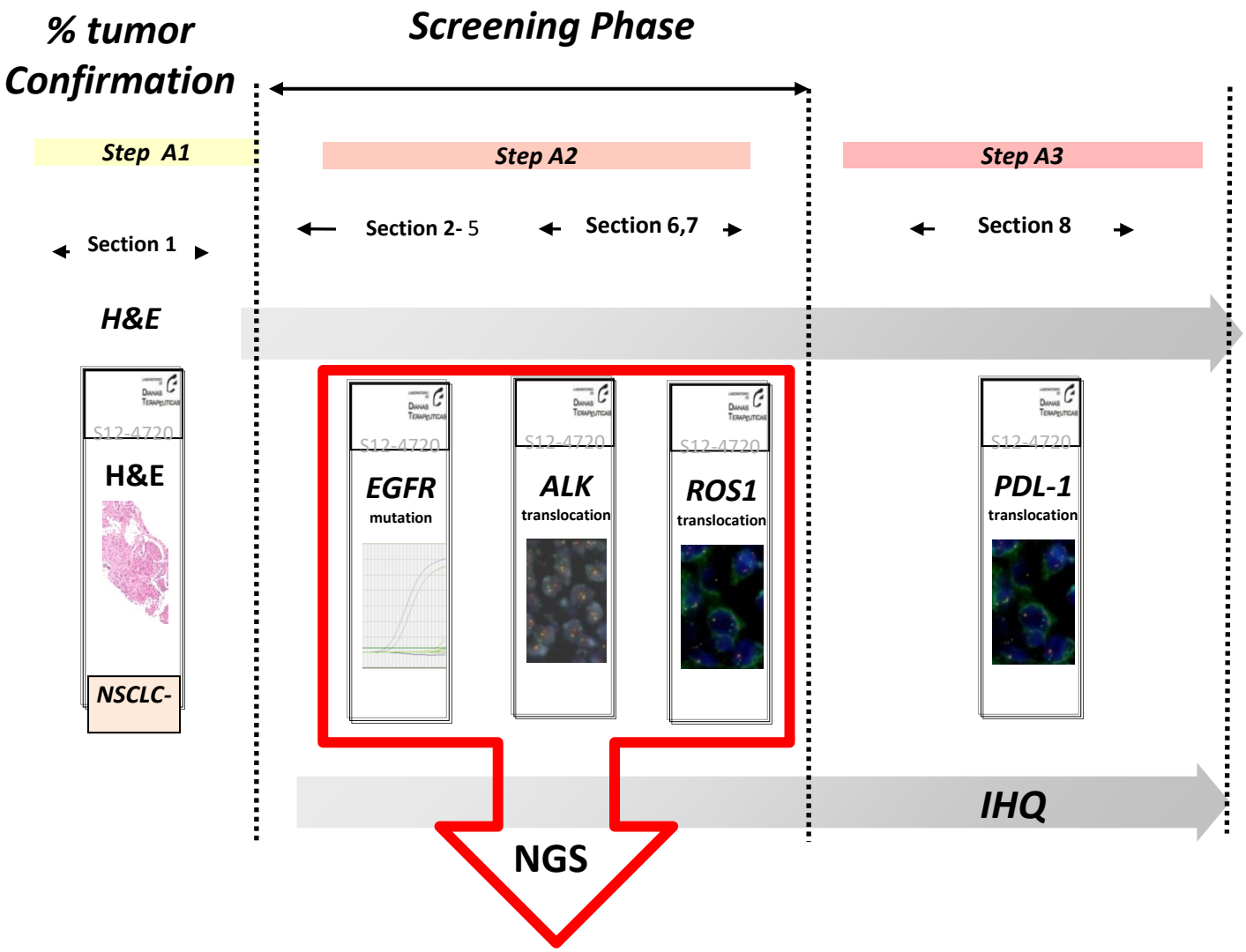
	Assay	1 Lane	2 Lanes	3 Lanes	4 Lanes
Max Number of Samples	Oncomine Precision Assay (for DNA & RNA workflow)	4	8	12	16
	Oncomine Precision Assay (for cfTNA workflow)	1	2	3	4

Workflow	# of samples
Simultaneous DNA- and RNA-based variant analysis	up to 16 FFPE tissue samples (one DNA reaction and one RNA reaction per sample)
DNA-only or RNA-only variant analysis	up to 32 FFPE tissue samples

Experience in NSCLC



Workflow Modifications

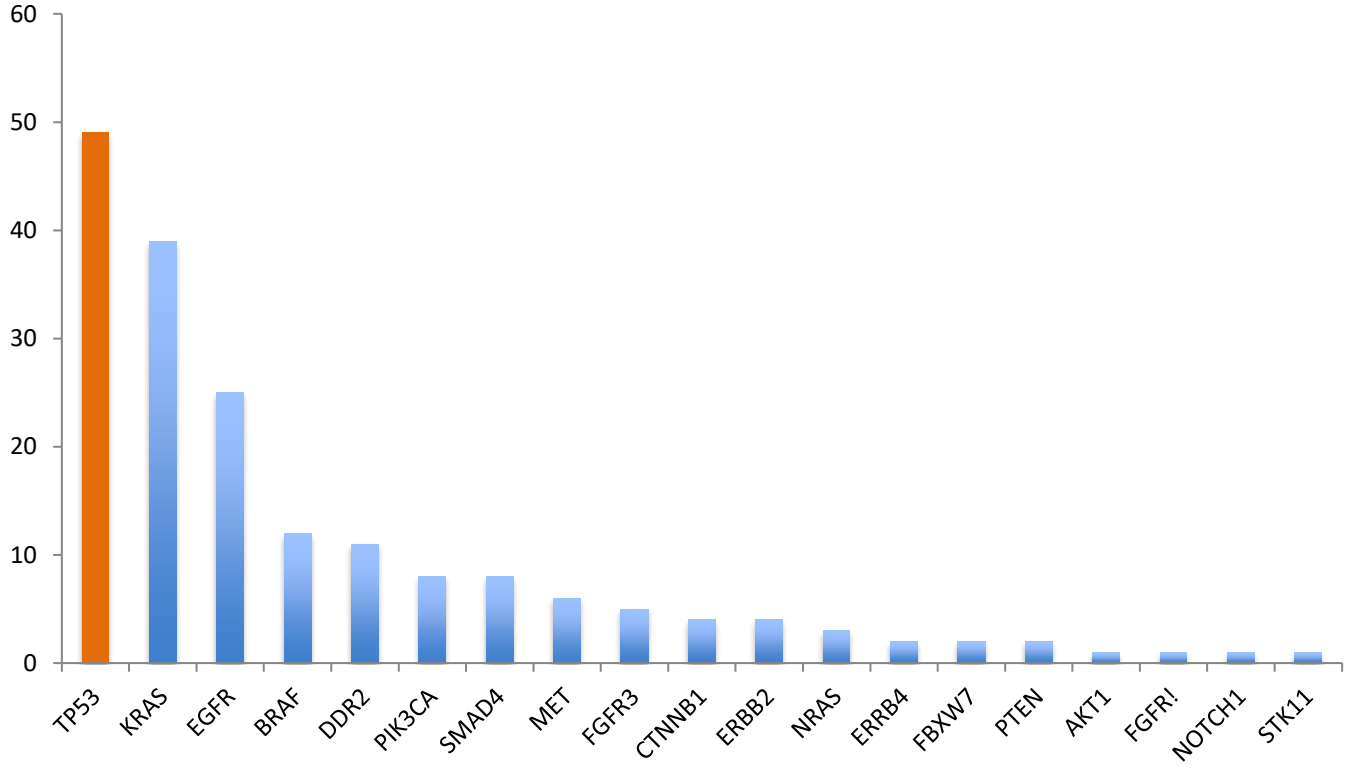


Adaptado Conde et al. Clin Transl Oncol 2013

Initially IonS5xl + IonChef

-Biomarker testing in NSCLC
-Molecular Profiling in Pediatric Tumors
-Molecular Profiling in Myeloproliferative diseases

Experience in NSCLC



N= 221 NSCLC samples

Molecular Biology Lab. Vall d´hebron

Genexus acquisition 2020

COVID-19
Coronavirus

Jaime Vicioso. EngineerThermo Fisher Scientific

Different handicaps

Hospital Accessibility
Genexus installation
Software installation
Genexus setup
Training ➔ ***Webex***

Live Lens®

Rescue®

Comparative Study in NSCLC samples

Shared Genes		Unique Genes		
AKT1	**FGFR3**	AKT2	FLT3	NTRK1
ALK	**KRAS**	AKT3	GNA11	NTRK2
BRAF	**MAP2K1**	AR	GNAQ	NTRK3
CTNNB1	**MET**	ARAF	GNAS	PDGFRA
EGFR	**NRAS**	CDK4	HRAS	RAF1
ERBB2	**PIK3CA**	CDKN2A	IDH1	RET
ERBB4	**PTEN**	CHEK2	IDH2	
FGFR1	**TP53**	ERBB3	KIT	
FGFR2		ESR1	MAP2K2	
		FGFR4	MTOR	

NSCLC Samples analyzed by reference test
n = 15 samples

Comparative Study in NSCLC samples

ID Mostra	GEN	Canvi AA	Canvi nt	Freq Al·lèlica	Profunditat
1	KRAS	p.Gly12Asp	c.35G>A	13,2	2.577
	RET	p.Cys620Arg	c.1858T>C	3,3	849
2	BRAF	p.Gly469Ala	c.1406G>C	32,4	5.023
3	CD274 Gain-of-Function Copy Number 12,05				
4	KIT	p.Val530Ile	c.1588G>A	32	571
	TP53	p.Gly245Val	c.734G>T	37,3	643
	AR LOSS 0,82				
5	EGFR	p.Leu858Arg	c.2573T>G	20,2	4.664
6	KRAS	p.Gly12Cys	c.34G>T	11,7	2.577
7	EGFR	p.E746_A750del	c.2236_2250delGAATTAAGAGAAGCA	30	5.032
8	KRAS	p.Gln61His	c.183A>C	13,7	4.159
9	KRAS	p.Gly12Val	c.35G>T	44,8	6.961
10	EGFR	p.Leu858Arg	c.2573T>G	24,1	1.563
11	Sense Alteracions				
12	CTNNB1	p.Asp32Asn	c.94G>A	39,4	1.205
	KIT	p.Val530Ile	c.1588G>A	6,7	1.179
	TP53	p.Asp281Gly	c.842A>G	46,3	721
13	Sense Alteracions				
14	P53	p.Val157Phe	c.469G>T	22,8	5.001
	KRAS AMPLIFICATION CNV Ratio 3,52				
15	ERBB3	p.Asp297Tyr	c.889G>T	20,6	1.867
	PIK3CA	p.Glu545Asp	c.1635G>C	7,7	3.522

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Results by OPA

Comparative Study in NSCLC samples

ID Mostra	GEN	Canvi AA	Canvi nt	Freq Al·lèlica	Profunditat
1	KRAS	p.Gly12Asp	c.35G>A	6,32	1.771
2	BRAF	p.Gly469Ala	c.1406G>C	28,96	2.738
3	SMAD4	p.His132Tyr	c.394C>T	34,77	3.103
	TP53	p.Glu349Ter	c.1045G>T	16,65	2.721
4	ERBB4	p.Gln346His	c.1038G>T	22,12	3.996
	TP53	p.Gly245Val	c.734G>T	34,7	3.971
5	EGFR	p.Leu858Arg	c.2573T>G	18,2	3.988
6	KRAS	p.Gly12Cys	c.34G>T	11,96	3.963
	TP53	p.Gln104Ter	c.310C>T	12,68	1.554
7	EGFR	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAGAAGCA	22,85	3.921
8	KRAS	p.Gln61His	c.183A>C	12,91	3.998
9	KRAS	p.Gly12Val	c.35G>T	41,6	3.966
	TP53	p.Arg110Pro	c.329G>C	30,4	1.589
	DDR2	p.Met441Ile	c.1323G>A	21,08	4.000
10	EGFR	p.Leu858Arg	c.2573T>G	25,35	3.988
11	Sense Alteracions				
12	CTNNB1	p.Asp32Asn	c.94G>A	52,89	2.320
	TP53	p.Arg110His	c.329G>A	74,98	2.370
	TP53	p.Asp281Gly	c.842A>G	54,29	2.387
13	TP53	p.Val217fs	c.649delG	30,21	3.969
14	TP53	p.Val157Phe	c.469G>T	35,8	3.109
	DDR2	p.Met441Ile	c.1323G>A	26,46	3.998
15	SMAD4	p.Gln256Leu	c.767A>T	5,15	1.185
	PIK3CA	p.Glu545Asp	c.1635G>C	4,9	3.960

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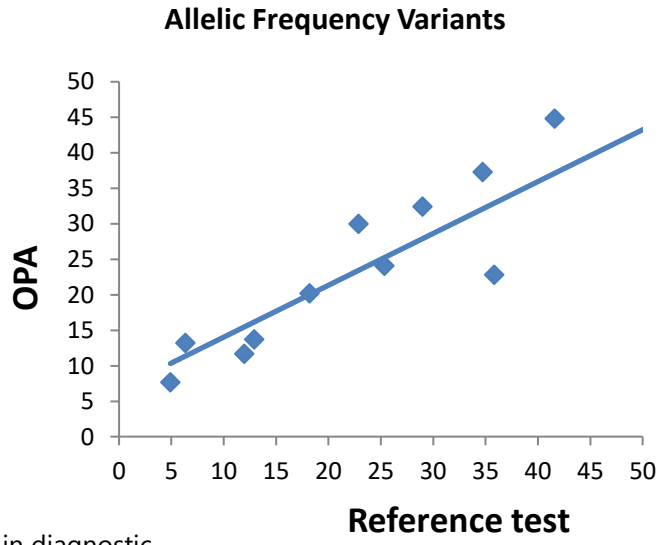
Results by reference test

Comparative Study in NSCLC samples

				Reference test		OPA	
Sample ID	GEN	Aminoacid	Nucleotide	FA	Reads	FA	Reads
1	*KRAS*	p.Gly12Asp	c.35G>A	6,32	1771	13,2	2577
2	*BRAF*	p.Gly469Ala	c.1406G>C	28,96	2738	32,4	5023
4	*TP53*	p.Gly245Val	c.734G>T	34,7	3971	37,3	643
5	*EGFR*	p.Leu858Arg	c.2573T>G	18,2	3988	20,2	4664
6	*KRAS*	p.Gly12Cys	c.34G>T	11,96	3963	11,7	2577
7	*EGFR*	p.Glu746_Ala750del	c.2236_2250delGAATTAAGAGAAGCA	22,85	3921	30	5032
8	*KRAS*	p.Gln61His	c.183A>C	12,91	3998	13,7	4159
9	*KRAS*	p.Gly12Val	c.35G>T	41,6	3966	44,8	6961
10	*EGFR*	p.Leu858Arg	c.2573T>G	25,35	3988	24,1	1563
12	*TP53*	p.Asp281Gly	c.842A>G	54,29	2387	46,3	721
12	*CTNNB1*	p.Asp32Asn	c.94G>A	52,89	2320	39,4	1205
14	*TP53*	p.Val157Phe	c.469G>T	35,8	3109	22,8	5001
15	*PIK3CA*	p.Glu545Asp	c.1635G>C	4,9	3960	7,7	3522

NSCLC retrospective cases
n = 15 samples



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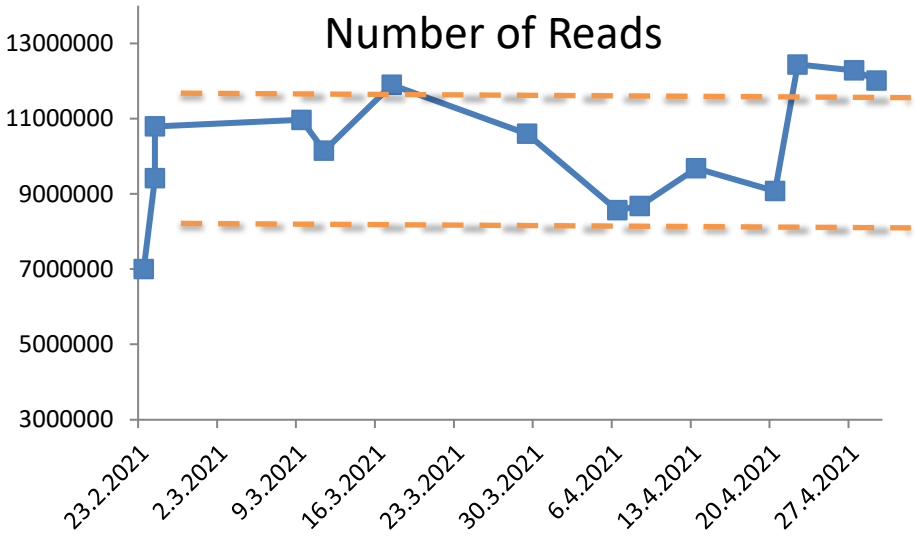
Quality Control Metrics



New Genexus Software

Genexus | Ion Torrent

Samples Runs Monitor Results Assays

Samples / Manage Samples

Filter Samples by... | Sample Name | Enter Sample Name

+ Create Sample | Import Samples | Delete | Export | + Prepare Library Batch

Sample Name	Collection Date	Created On	Disease Category	Cancer Type	Sample Type	Gender	Actions
DNA_21_1374_RNA_21_1437	2021-04-13	2021-04-13 12:51	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1492-2_RNA_21_1493	2021-04-13	2021-04-13 12:51	Cancer	Unknown Primary Origin	FFPE	Female	Edit | Audit | Notes
DNA_21_1490-2_RNA_21_1491	2021-04-13	2021-04-13 12:48	Cancer	Non-Small Cell Lung Cancer	FFPE	Female	Edit | Audit | Notes
DNA_21_1539_RNA_21_1540	2021-04-13	2021-04-13 12:47	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1537_RNA_21_1538	2021-04-13	2021-04-13 12:47	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1535_RNA_21_1536	2021-04-13	2021-04-13 12:46	Cancer	Non-Small Cell Lung Cancer	FFPE	Female	Edit | Audit | Notes
DNA_21_1529_RNA_21_1530	2021-04-13	2021-04-13 12:45	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes
DNA_21_1542_RNA_21_1543	2021-04-13	2021-04-13 12:42	Cancer	Non-Small Cell Lung Cancer	FFPE	Male	Edit | Audit | Notes

Quality Control Metrics

Genexus | Ion Torrent — Samples | Runs | Monitor | Results | Assays

Results / 2021_04_27 / OPA DNA-Fus-w2.6.0 / DNA_21_1784_RMA_21_1785

DNA_21_1784_RMA_21_1785 — Summary | QC ✓ | Variants | Plugins

Run QC

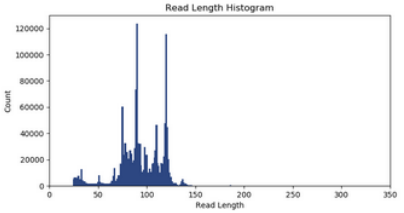
Key Signal	—	57
Not Set		
Percent Loading	—	92%
Not Set		
Raw Read Accuracy	—	98,8
Not Set		

Templating Control QC - CF-1

Average Reads Per Lane	—	69.168
Not Set		
Base Call Accuracy	—	96,7%
Not Set		
Mean AQ20 Read Length (bp)	—	73
Not Set		

Sample QC - DNA ✓

MAPD	✓	0,24
[0-0.5]		
Mapped Reads	—	1.523.989
Not Set		
Mean AQ20 Read Length (bp)	—	81
Not Set		
Mean Read Length (bp)	—	93
Not Set		
Uniformity Of Base Coverage	—	96,13
Not Set		



Sample QC - RNA ✓

Mapped Reads	—	440.090
Not Set		
Mean Read Length (bp)	—	90
Not Set		
RNA Expression Ctrls Detected	✓	7
>=5		

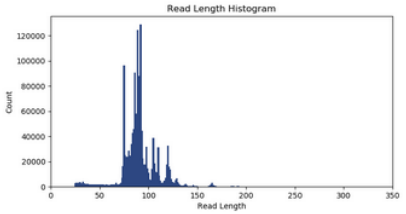
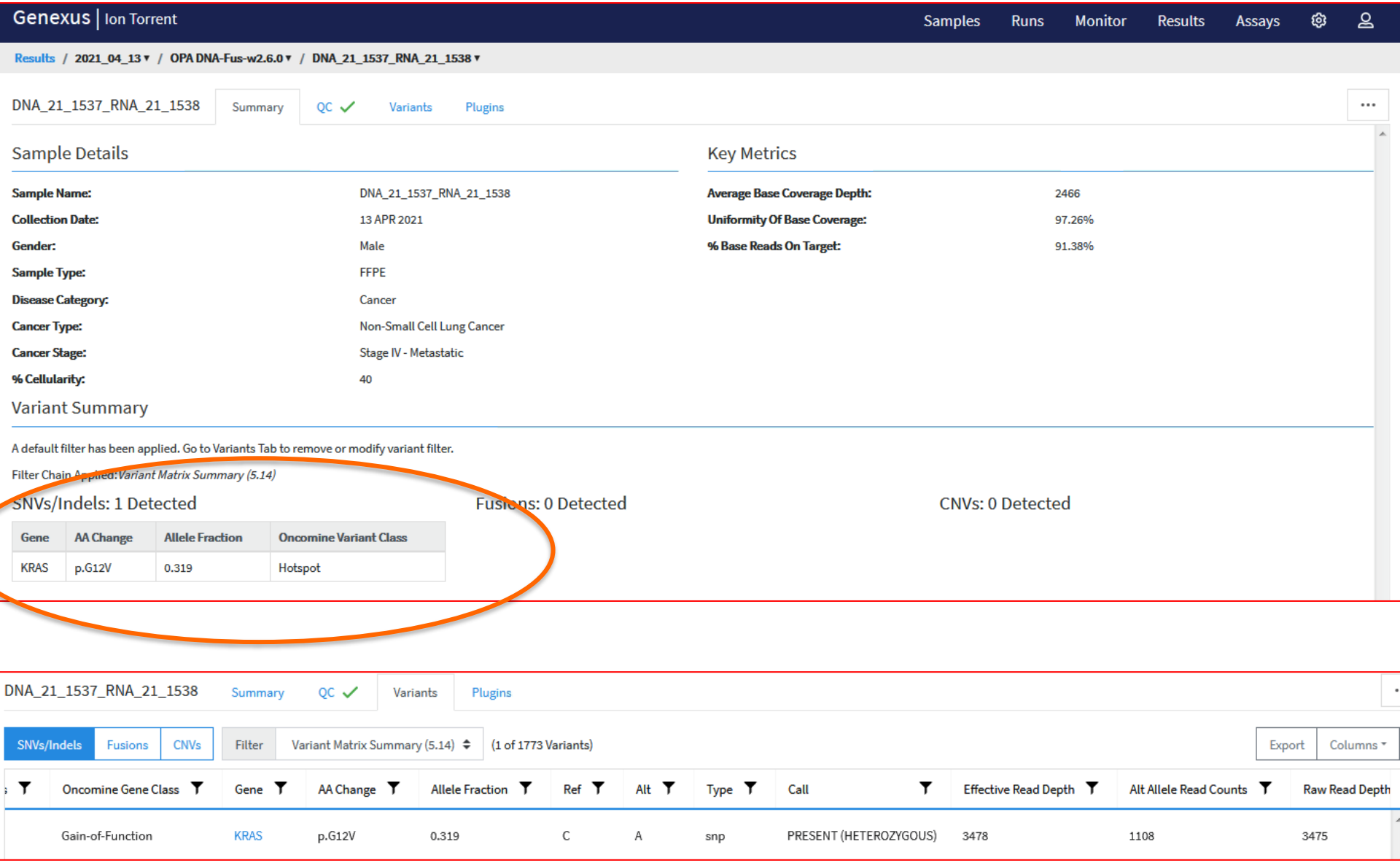


Table 6 Example QC metrics for FFPE DNA+RNA isolated from tumor FFPE samples *(continued)*

QC metric	Value
Sample QC – DNA	
MAPD	0.18–0.24
Mapped Reads	525,000–1,000,000
Mean AQ20 Read Length (bp)	85–95
Mean Read Length (bp)	85–100
Uniformity Of Base Coverage	97–99%
Sample QC – RNA	
Mapped Reads	105,000–650,000
Mean Read Length (bp)	70–100
RNA Expression Ctrls Detected	5–7


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / 2021_04_13 / OPA DNA-Fus-w2.6.0 / DNA_21_1537_RNA_21_1538
DNA_21_1537_RNA_21_1538
Summary
QC
Variants
Plugins
Sample Details
Sample Name: DNA_21_1537_RNA_21_1538
Collection Date: 13 APR 2021
Gender: Male
Sample Type: FFPE
Disease Category: Cancer
Cancer Type: Non-Small Cell Lung Cancer
Cancer Stage: Stage IV - Metastatic
% Cellularity: 40
Key Metrics
Average Base Coverage Depth: 2466
Uniformity Of Base Coverage: 97.26%
% Base Reads On Target: 91.38%
Variant Summary
A default filter has been applied. Go to Variants Tab to remove or modify variant filter.
Filter Chain Applied: Variant Matrix Summary (5.14)
SNVs/Indels: 1 Detected
Fusions: 0 Detected
CNVs: 0 Detected
Gene
AA Change
Allele Fraction
Oncomine Variant Class
KRAS
p.G12V
0.319
Hotspot

DNA_21_1537_RNA_21_1538
Summary
QC
Variants
Plugins
SNVs/Indels
Fusions
CNVs
Filter
Variant Matrix Summary (5.14)
(1 of 1773 Variants)
Export
Columns
Oncomine Gene Class
Gene
AA Change
Allele Fraction
Ref
Alt
Type
Call
Effective Read Depth
Alt Allele Read Counts
Raw Read Depth
Gain-of-Function
KRAS
p.G12V
0.319
C
A
snp
PRESENT (HETEROZYGOUS)
3478
1108
3475

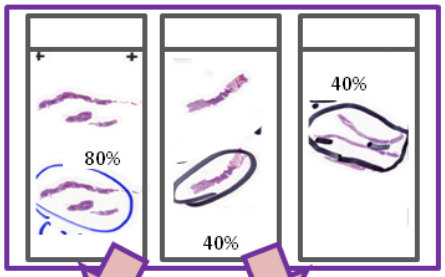
Workflow of NGS by Genexus

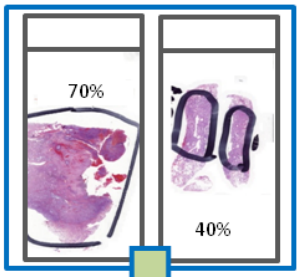
Monday	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
				
DNA +RNA extraction 2	Quantification 3	DNA +RNA extraction 2	Molecular Tumor Board	**Analysis** 5
	Genexus Load 4	**Analysis** 5	Quantification 3	**Tissue Selection** 1
	Tissue Selection 1		**Genexus Load** 4	

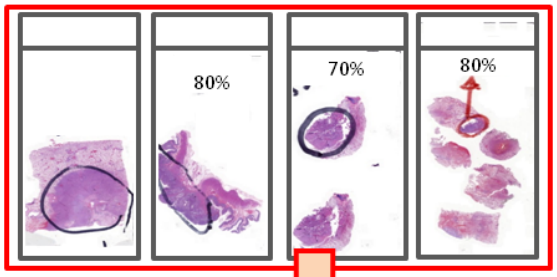
Molecular Biology Lab. Vall d´hebron

Sample Optimization (pre-analytics)

ALGORITMO MANEJO DE LA MUESTRA PARA NGS EN CÁNCER DE PULMÓN







CITOLOGÍAS

Diff-Quick:
DNA: **EZ1**
RNA: **FormaPure RNA Prep**

< 20%T

no hacer NGS

> 20% T
- **3-4** rollitos 10 µM para DNA

Extracción DNA Manual:
High Pure FFPET DNA Isolation Kit

- **3** rollitos 10 µM para RNA

Extracción RNA Manual:
FormaPure RNA Prep

> 20% T
- **2** rollitos 10 µM para DNA

Extracción DNA automática:
Qiasymphony

- **2** rollitos 10 µM para RNA

Extracción RNA Manual:
FormaPure RNA Prep

Macrodisección de la zona tumoral

> 20% T y tamaño zona grande
- **3** rollitos 10 µM para DNA

Extracción DNA automática:
Qiasymphony

- **3** rollitos 10 µM para RNA

Extracción RNA Manual:
FormaPure RNA Prep

> 20% T y tamaño zona pequeña
- **3-4** rollitos 10 µM para DNA

Extracción DNA Manual
High Pure FFPET DNA Isolation Kit

- **3-4** rollitos 10 µM para RNA

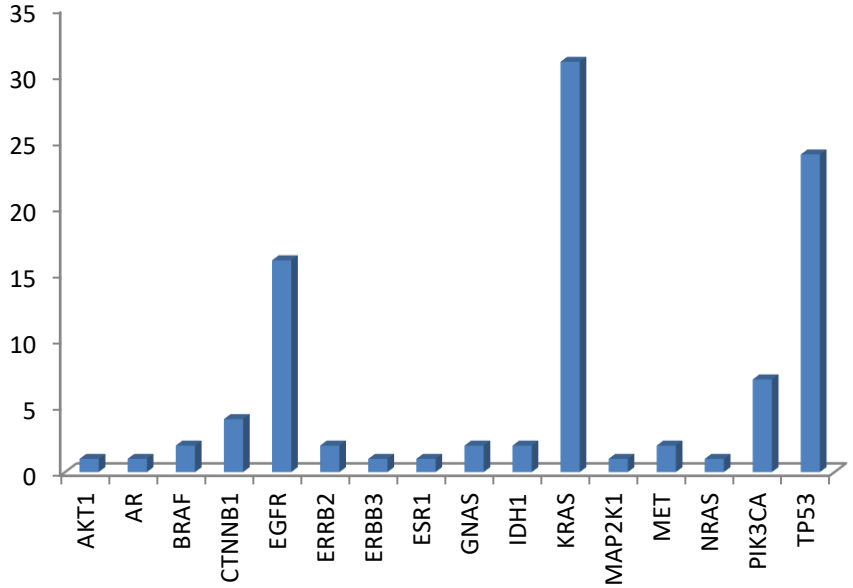
Extracción RNA Manual:
FormaPure RNA Prep

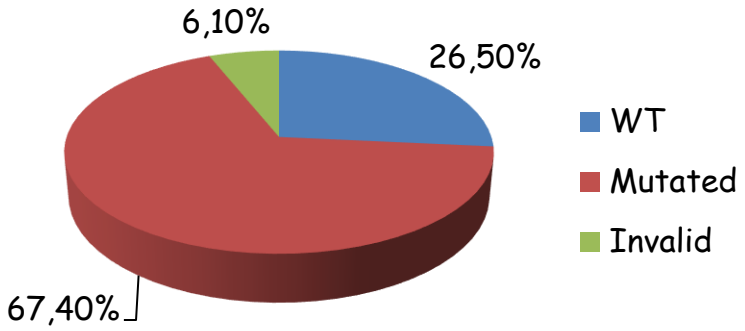
DNA
Si < [5] ng/µL
Concentrar con:
GeneJET FFPE DNA Purification kit
DNA Estandar

RNA
Si < [10] ng/µL
Concentrar con:
GeneJET RNA Purification kit

Protocolo LIBRERÍAS de DNA y RNA

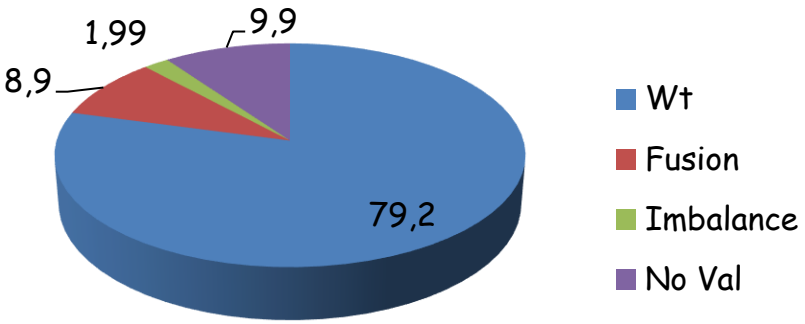
Biomarker Testing in NSCLC by OPA





DNA analysis (n=98)

- 01/03/2021 to 30/06/2021
- 4-8 samples/week



RNA analysis (n=101)

Biologia Molecular HUVH

Internal Molecular Committee

- Weekly (Thursday 8.30h) sample piorization and results discussion internally with Pathologist.
- Bibliography update
- Pathologist + Molecular Biologist

Molecular Tumor Board

- Weekly (Thursday 8.00h) Molecular analysis results, with other sample results.
- Pathologist + Molecular Biologist + Oncologist.
- Next steps (invite external Pathologists + Medical Oncologists)

OPA Applications in Cancer

Melanoma

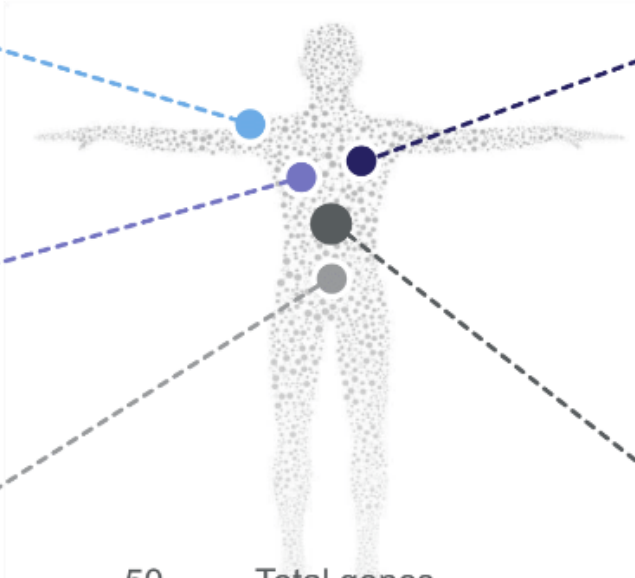
- *BRAF* mutations
- *KIT* mutations

Breast, Gastric, Esophageal, Head and Neck, and Endometrial

- *ERBB2 (HER2)* amplification
- *PIK3CA* mutations

Colon

- *BRAF* mutations
- *NRAS* mutations
- *KRAS* mutations



NSCLC

- *EGFR* mutations
- *BRAF* mutations
- *KRAS* mutations
- *ALK* fusions
- *ERBB2 (HER2)* mutations
- *MET* amplification
- *ROS1* fusions
- ***RET fusions****
- MET Exon 14 skip

All solid tumor

- *NTRK1* fusions
- *NTRK2* fusions
- *NTRK3* fusions

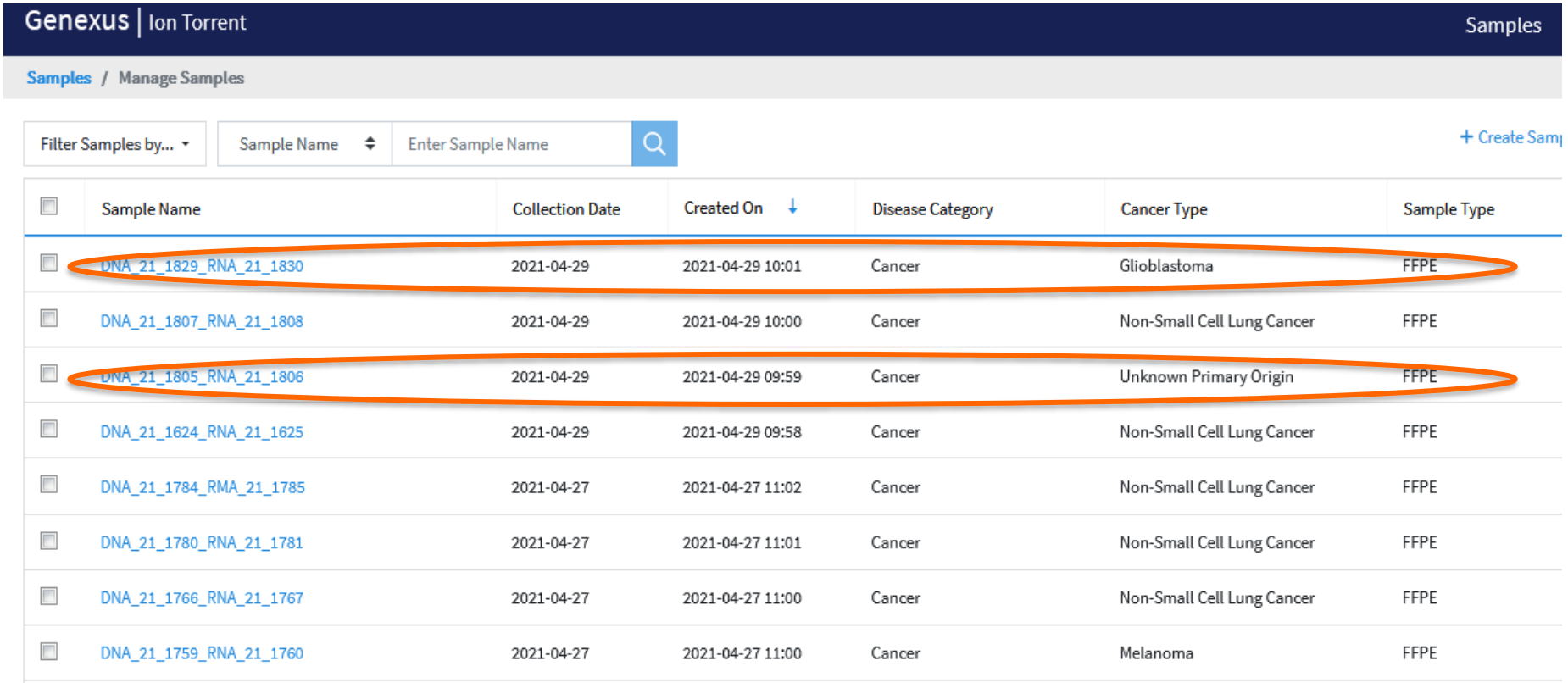
50	Total genes
45	DNA hotspot genes
14	CNV genes
16	Inter-genetic fusions
3	Intra-genetic fusions

Evidence generated using Oncomine Reporter

For Research Use Only. Not for use in diagnostic procedures.

OPA Analysis beyond NSCLC profiling

- ***Unknowm Primary Tumors (UPT)***
- ***Gliomas***
- ***Soft Tissue Tumors***



Sample Name	Collection Date	Created On	Disease Category	Cancer Type	Sample Type
DNA_21_1829_RNA_21_1830	2021-04-29	2021-04-29 10:01	Cancer	Glioblastoma	FFPE
DNA_21_1807_RNA_21_1808	2021-04-29	2021-04-29 10:00	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1805_RNA_21_1806	2021-04-29	2021-04-29 09:59	Cancer	Unknown Primary Origin	FFPE
DNA_21_1624_RNA_21_1625	2021-04-29	2021-04-29 09:58	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1784_RMA_21_1785	2021-04-27	2021-04-27 11:02	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1780_RNA_21_1781	2021-04-27	2021-04-27 11:01	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1766_RNA_21_1767	2021-04-27	2021-04-27 11:00	Cancer	Non-Small Cell Lung Cancer	FFPE
DNA_21_1759_RNA_21_1760	2021-04-27	2021-04-27 11:00	Cancer	Melanoma	FFPE

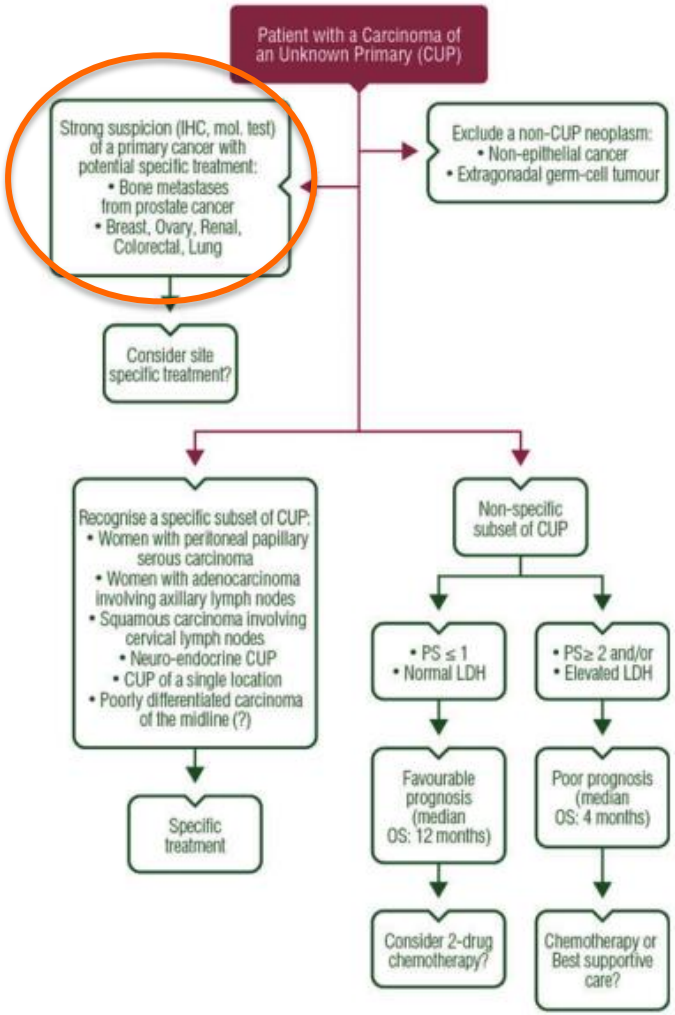
clinical practice guidelines

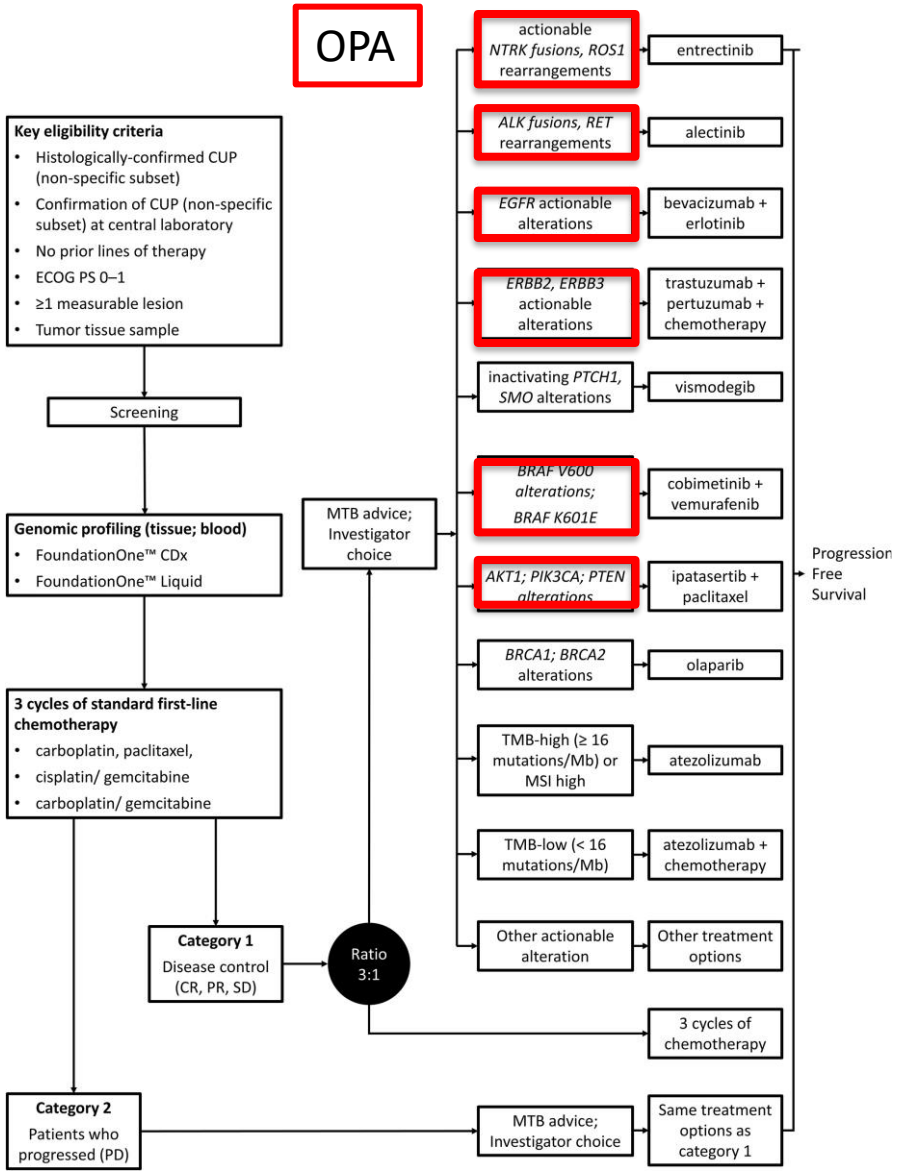
Annals of Oncology 26 (Supplement 5): v133–v138, 2015
doi:10.1093/annonc/mdv305

Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

K. Fizazi[1], F. A. Greco[2], N. Pavlidis[3], G. Daugaard[4], K. Oien[5] & G. Pentheroudakis[3], on behalf of the ESMO Guidelines Committee*

[1]Department of Cancer Medicine, Institut Gustave Roussy, University of Paris Sud, Villejuif, France; [2]Tennessee Oncology, Centennial Medical Center, Nashville, USA; [3]Department of Medical Oncology, University of Ioannina, Ioannina, Greece; [4]Department of Oncology 5073, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [5]University of Glasgow, Institute of Cancer Sciences, Glasgow, UK





Annals of Oncology 2015 26v133-v138
Pauli C et al. Oncologist 2021

Other Solid Tumor Types

	Sample	Gene	Protein	DNA	FA	Cosmic
1	CNS	TP53	p.R273H	c.818G>A	42	COSM10660
2	CUP	MET	p.R988C	c.2962C>T	50,9	COSM1666978
3	CUP	KRAS	p.G12C	c.34G>T	69,5	COSM516
4	CNS	CDKN2A		CNV 0,25		
5	Soft Tissue	TP53	p.R248Q	c.743G>A	42	COSM10662
6	CNS	KRAS	p.G12V	c.35G>T	31,9	COSM520
7	Soft Tissue	TP53	p.R213*	c.637C>T	96	COSM10654
8	CNS	EGFR	p.G598V	c.1793G>T	77,2	COSM21690
9	CNS	EGFR		CNV 2,61		
10	CNS	CDKN2A		CNV 0,09		
11	CNS	IDH1	p.Arg132His	c.395G>A	42,6	COSM27846
		TP53	p.Arg248Gln	c.743G>A	3,6	COSM10662
12	CNS	TP53	p.Arg273Leu	c.818G>T	63,9	COSM10779
13	CCR	ERBB2		CNV 18,18		

DNA-analysis

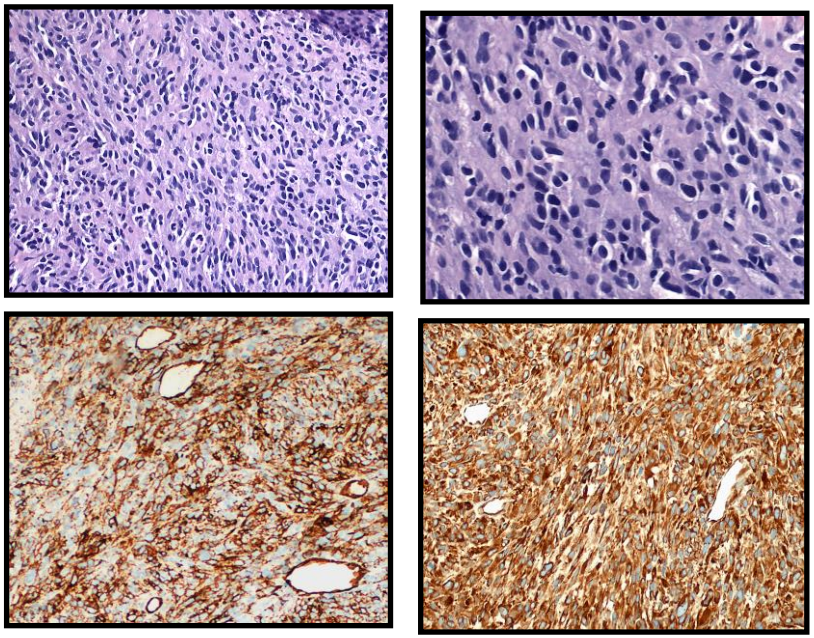
	Sample	Gene	Fusion	Reads
1	CNS	BRAF	GTF2I(6)-BRAF(10)	1.637
2	CNS	NTRK2	KANK1(3)-NTRK2(12)	3.433
3	CNS	MET	PTPRZ1(1)-MET(2)	3.197
4	Melanoma	ALK	TPM3(7)-ALK(20)	107
5	Thyroid	RET	NCOA4(7)-RET(12)	255
6	CNS	MET	CAPZA2-MET	1245

RNA-analysis

CNS: Central Nervous System Tumors.
CUP: Carcinoma of Unknown Primary Tumor.

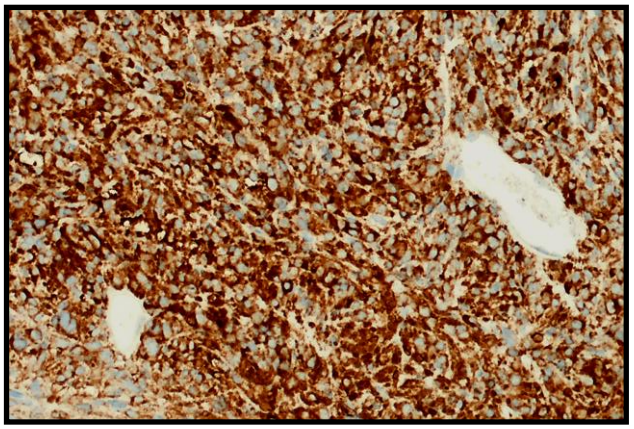
Molecular Biology HUVH

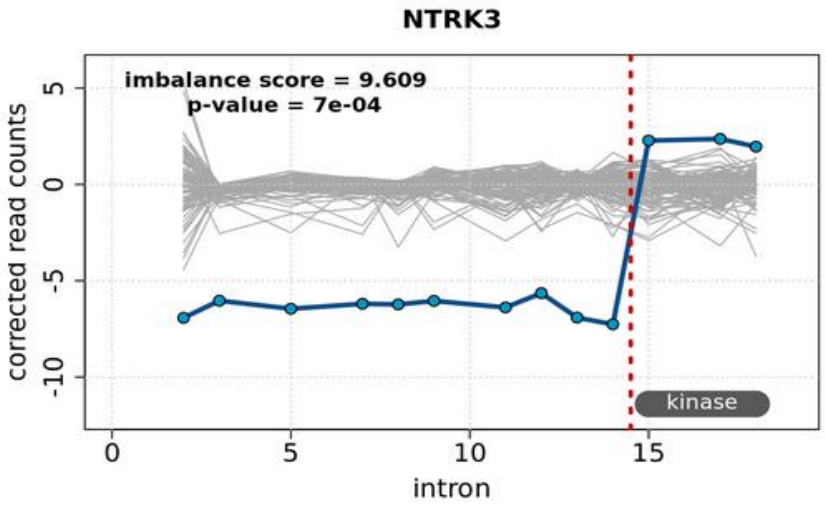
Malignant Mesenquimal Tumour
MNPST??



CD34 Vimentin

NTRK





Take Home Messages

1. Optimal OPA NGS analysis Workflow with Genexus in FFPE samples (4 samples batch)

2. TAT to Complete NGS analysis from samples arrival is currently done in less than 5 working days

3. All cases analyzed by NGS are discussed in the internal Comitee, and some of them in the MTB

4 OPA analysis could be extended to other Solid Tumor Types

Thank You

Multidisciplinar Team

Molecular Biology. Vall d´hebron