cfDNA analysis in a molecular pathology laboratory

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- Speaker was provided travel and hotel support by Thermo Fisher Scientific for this presentation, but no remuneration

Ipatimup – Porto - Portugal

Ipatimup

- Founding member of i3S
- Leading cancer research institute in Portugal
- Founding partner of **Porto Comprehensive Cancer Center**

i3S

- Biggest research in health institute in Portugal (1250 researchers);
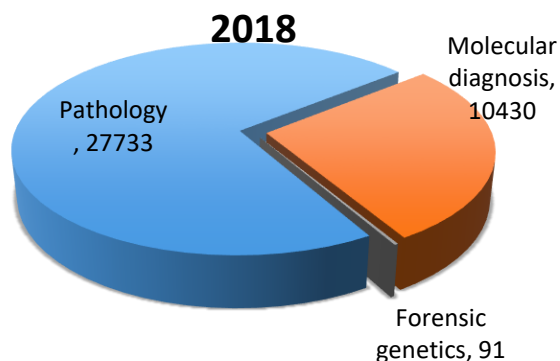- Cancer, Neurosciences and Host-Pathogen interactions research lines

IPATIMUP diagnostics

Provide services in the areas of surgical pathology and cytopathology, genetic diagnosis and genetic identification and parentage, in order to improve Portuguese citizens life quality.

Molecular diagnosis

- *Tumor mutation screening* – 4220 cases

- *Genetic diagnosis* – 2960 cases

- *Pre-natal screening* – 3260 cases

Laboratory accreditation

2018

Pathology, 27733

Molecular diagnosis, 10430

Forensic genetics, 91

NP EN ISO 15189

NP EN ISO 17025

Lung cancer clinically relevant alterations

EGFR sensitizing
- Gefitinib [4]
- Erlotinib [4]
- Afatinib [4]
- Osimertinib [4]
- Necitumumab [4]
- Rociletinib [3]

ALK
- Crizotinib [4]
- Alectinib [4]
- Ceritinib [4]
- Lorlatinib [2]
- Brigatinib [2]

ROS1
- Crizotinib [4]
- Cabozantinib [2]
- Ceritinib [2]
- Lorlatinib [2]
- DS-6051b [1]

EGFR sensitizing 17%
ALK 7%
EGFR other 4%
MET 3%
>1 3%
HER2 2%
ROS1 2%
BRAF 2%
RET 2%
NTRK1 1%
PIK3CA 1%
MEK 1%
KRAS 25%
Unknown oncogenic driver detected 30%
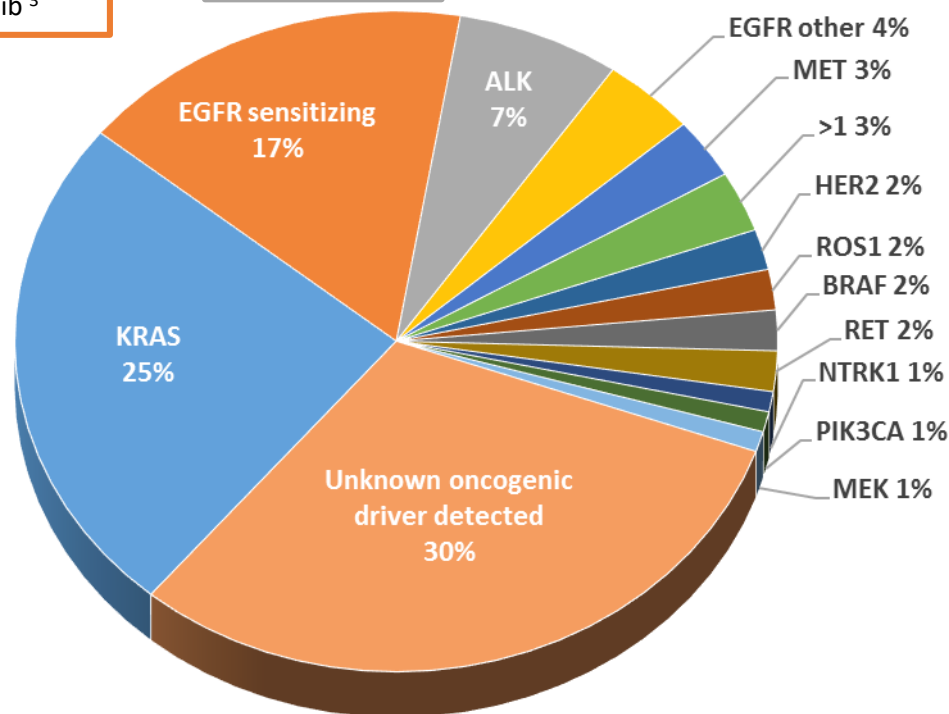
KEY
1 – Phase I   3 – Phase III
2 – Phase II   4 - Approved

Lung cancer clinically relevant alterations

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- Gefitinib [4]
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MET
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HER2
- Transtuzumab emtansine [2]
- Afatinib [2]
- Dacomitinib [2]

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BRAF
- Vemurafinib [2]
- Dabrafenib [2]

RET
- Cabozantinib [2]
- Alectinib [2]
- Apatinib [2]
- Vandetanib [2]
- Ponatinib [2]
- Lenvatinib [2]

MEK1
- Trametinib [2]
- Selumetinib [3]
- Cobimetinib [1]

PIK3CA
- LY3023414 [2]
- PQR 309 [1]

NTRK1
- Entrectinib [2]
- LOXO-101 [2]
- Cabozantinib [2]
- DS-6051b [1]

Pie chart:
- EGFR sensitizing 17%
- ALK 7%
- EGFR other 4%
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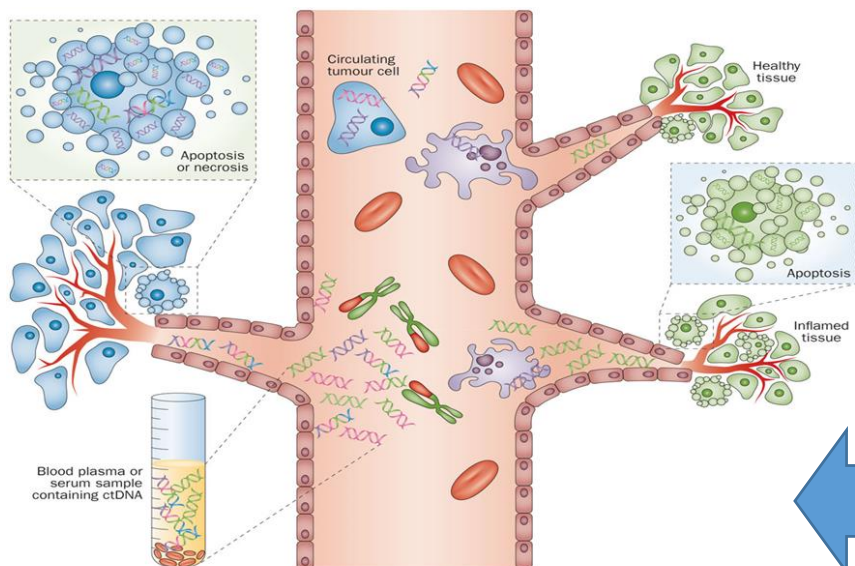UNIVERSIDADE DO PORTO

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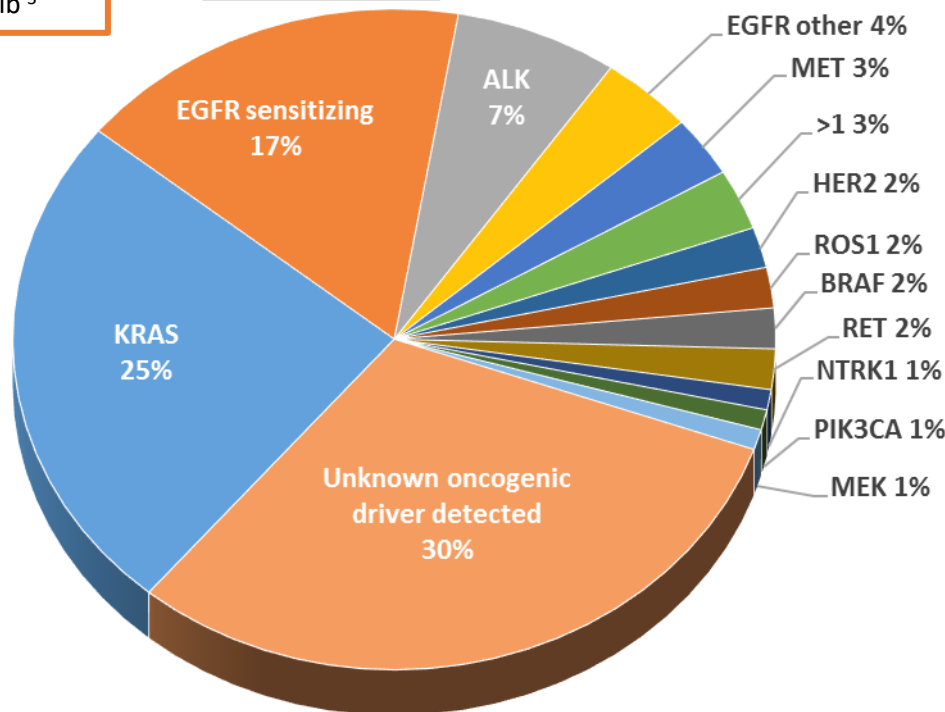
Comprehensive biomarker characterization
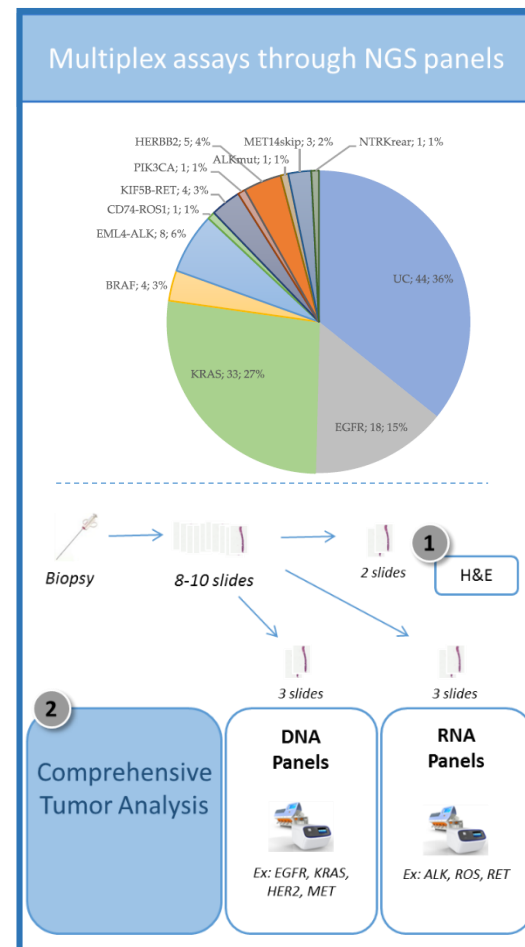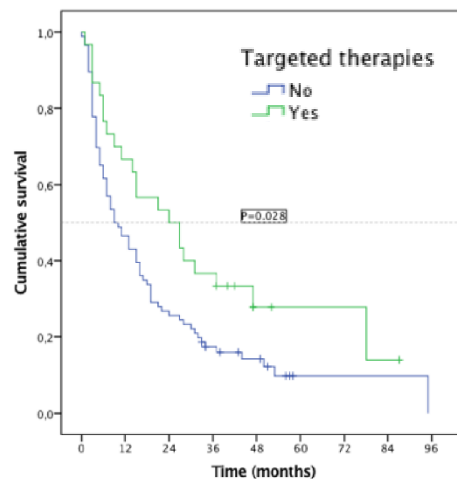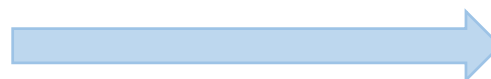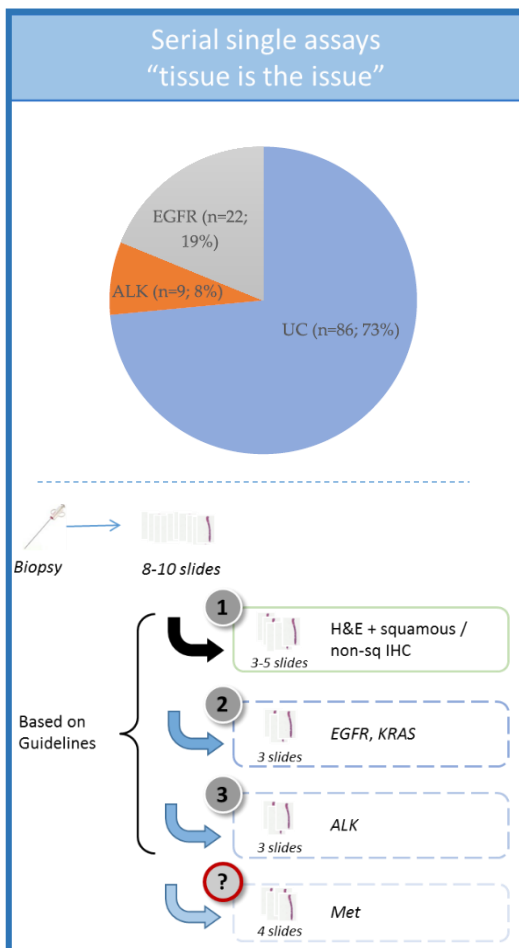
Comprehensive biomarker testing leads to better future patient care
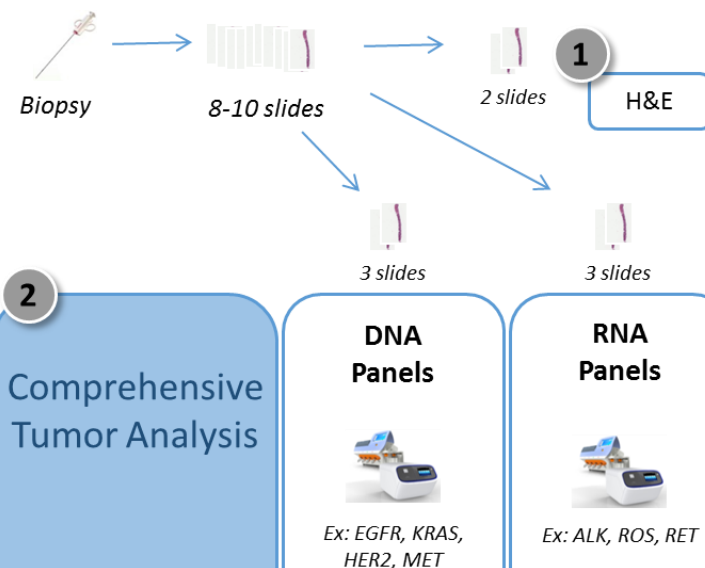
Lung cancer characterization
ctDNA analysis

Multiplex assays through NGS panels

Biopsy → *8-10 slides* → *2 slides* ① H&E

3 slides *3 slides*

② Comprehensive Tumor Analysis

DNA Panels
Ex: EGFR, KRAS, HER2, MET

RNA Panels
Ex: ALK, ROS, RET

Tissue may not be available for all assays or situations

Circulating tumour cell

Healthy tissue

Apoptosis or necrosis

Apoptosis

Inflamed tissue

Blood plasma or serum sample containing ctDNA

Diagnosis — Therapy selection

Monitoring — Therapy resistance

Relapse — Therapy selection
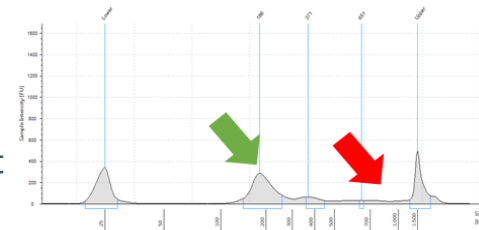
Validated strategy

1. Plasma collection

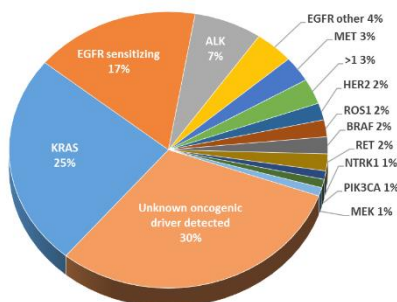BD Vacutainer PPT
(K2EDTA)

2. cfDNA isolation

MagMax
cfDNA
extraction kit

3. NGS variant identification

- Ion AmpliSeq **Colon & Lung** Cancer Research Panel

- Ion AmpliSeq **RNA Fusion Lung** Cancer Research Panel

- Oncomine **cfDNA Lung** assay

Ion S5xl System

Pie chart labels:
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4. Digital PCR validation

TaqMan Assays

QuantStudio 3D Digital PCR System

ISO 15189 accreditation

Sidebar flow:
- Plasma collection
- cfDNA isolation
- NGS Variant Discovery
- dPCR validation

OncoNetwork multicentric study
sensitivity and specificity

Lab	Sensitivity	Specificity	NPV	PPV
ARCNET	89,6%	99,6%	99,6%	87,8%
CROM	95,8%	100,0%	99,8%	100,0%
IPATIMUP	95,8%	100,0%	99,8%	100,0%
Radboud	89,6%	99,8%	99,6%	95,6%
Queens	97,9%	99,9%	99,9%	97,9%
St James	95,8%	100,0%	99,8%	100,0%
UHCW	95,8%	99,8%	99,8%	93,9%
Charite	95,8%	99,8%	99,8%	93,9%
Viollier*	97,5%	99,8%	99,9%	95,1%
HEGP	93,7%	99,7%	99,8%	91,8%
Kindai*	95,8%	99,7%	99,8%	92,0%
Total	**94,8%**	**99,8%**	**99,8%**	**95,9%**

Multiplex I cfDNA Reference
Standard Set (5%, 1%, 0,1%, 0%)

- **EGFR** p.L858R
- **EGFR** p.E746_A750delELREA
- **EGFR** p.T790M
- **EGFR** p.V769_D770insASV
- **KRAS** p.G12D
- **PIK3CA** p.E545K

horizon

OncoNetwork multicentric study

Benchmarking at 0.1% AF

		OncoNetwork study	FOUNDATION ACT	GUARDANT360
Genes (n)		11	62	73
SNVs	sensitivity	**83.9%**	67.3%	63.8%
	PPV	**99.1%**	93.6%	92.1%
InDels	sensitivity	83.9%	**86.2%**	67.8%
	PPV	99.1%	**100%**	88.4%

www.foundationmedicine.com   www.guardanthealth.com

Confident detection of variants at 0.1% AF

Liquid biopsy clinical performance study

Concordance analysis of liquid and tissue biopsy in NSCLC patients: a multi-institutional molecular pathology study

Multi-institutional study, 159 NSCLC patients who underwent tissue and plasma-NGS analysis:

1. 94 patients had concurrent tissue and plasma NGS analysis at diagnosis

2. 65 patients were monitored through plasma-NGS analysis – followed-up over time and compared to

    tissue biopsy at diagnosis

Alborelli I., Costa J.L. et al.: data from IPATIMUP (Porto) and the Institute of Pathology of Basel

Alborelli et al. 2019

Liquid biopsy clinical performance study

Cohort Characteristics

Characteristics	No. (%)
Patients	159 (100)
Age, y	
Median (Range)	67.5 (38 - 89)
Sex	
Male	77 (48.4)
Female	82 (51.6)
Histology	
Adenocarcinoma	158 (99.4)
Squamous cell carcinoma	1 (0.6)
Matched tissue biopsy	
Primary tumor	145 (91.2)
Metastasis	14 (8.8)
Metastatic status at blood draw	
Confirmed metastatic	136 (85.5)
Confirmed non-metastatic	17 (10.7)
Unknown metastatic status	6 (3.8)
Smoking status	
Never smoker	31 (19.5)
Smoker	31 (19.5)
Ex-smoker	29 (18.2)
Passive smoker	3 (1.9)
Unknown	65 (40.9)

Alborelli et al. 2019

Liquid biopsy clinical performance study

Benchmarking

High concordance of plasma and tissue-based molecular analysis using three different commercial assays.

Study	Total	Discordant	Tissue⁺ AND Plasma⁺ (TP)	Tissue⁻ AND Plasma⁻ (TN)	Concordance %
Aggarwal et al.	128	24	31	73	81%
Li et al.	110	23	68	19	79%
Alborelli et al.	94	11	40	43	88%

Study	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	Cohen Kappa
Aggarwal et al.	31	73	8	16	66%	90%	79%	82%	0.58
Li et al.	68	19	0	23	75%	100%	100%	45%	0.51
Alborelli et al.	40	43	2	9	82%	96%	95%	83%	0.77

Study	Genes (n)	Depth of Sequencing	cfDNA input	In-house test
Aggarwal et al.	73	10'000X	5-30 ng	no
Li et al.	37	50'000X	Up to 100 ng	not yet
Alborelli et al.	11	25'000X	1-50 ng	**yes**

Our results highlight the feasibility of using an **in-house** plasma-NGS assay for routine molecular characterization.
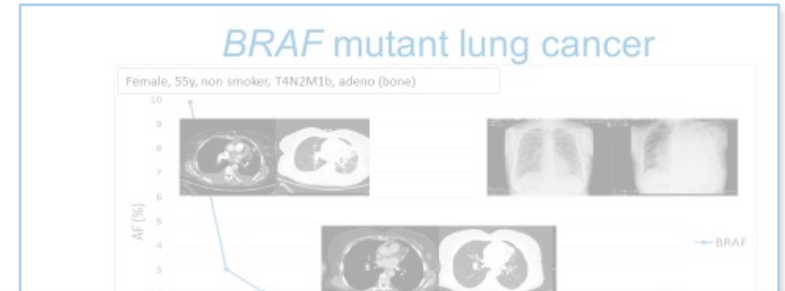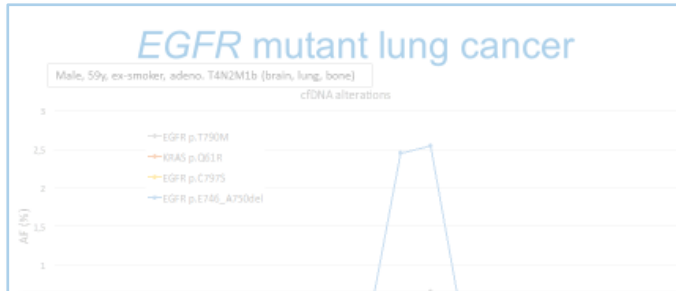
*Alborelli et al. 2019*

Liquid biopsy clinical performance study

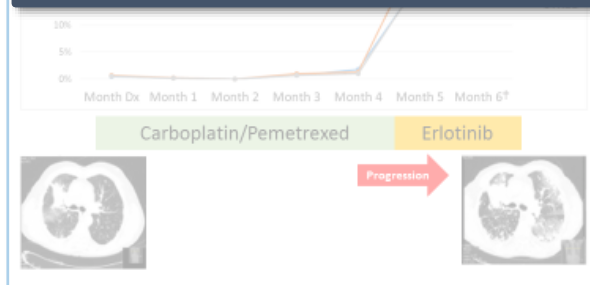*Alborelli et al. 2019*
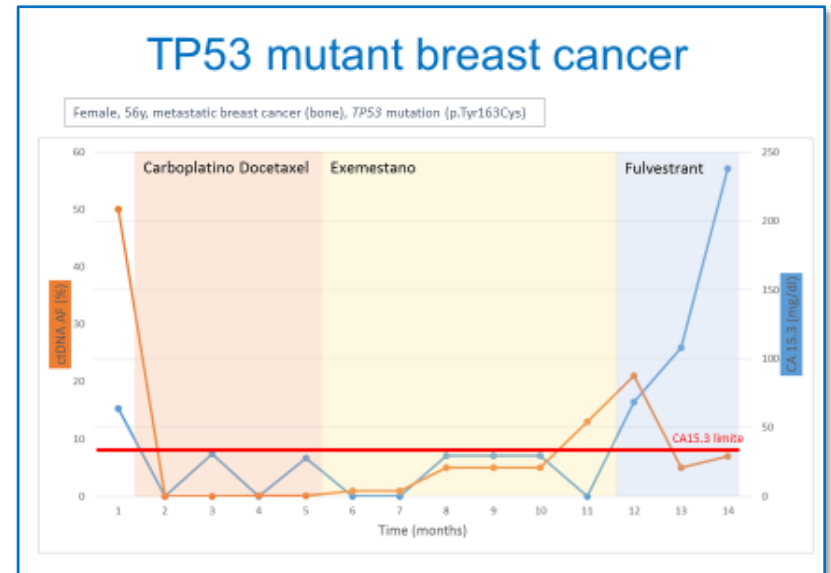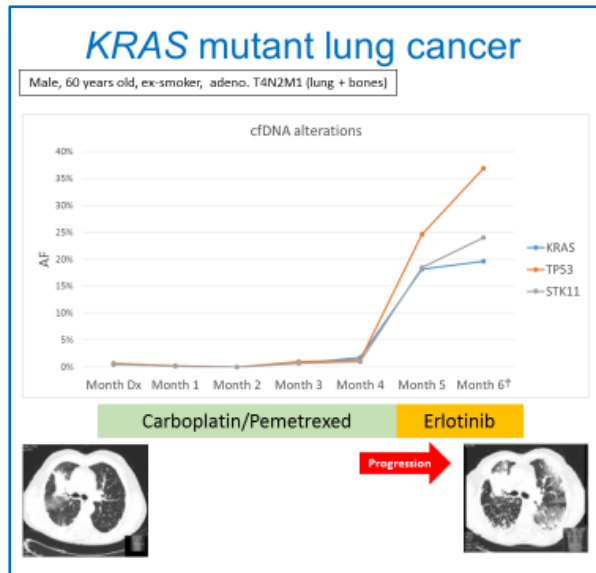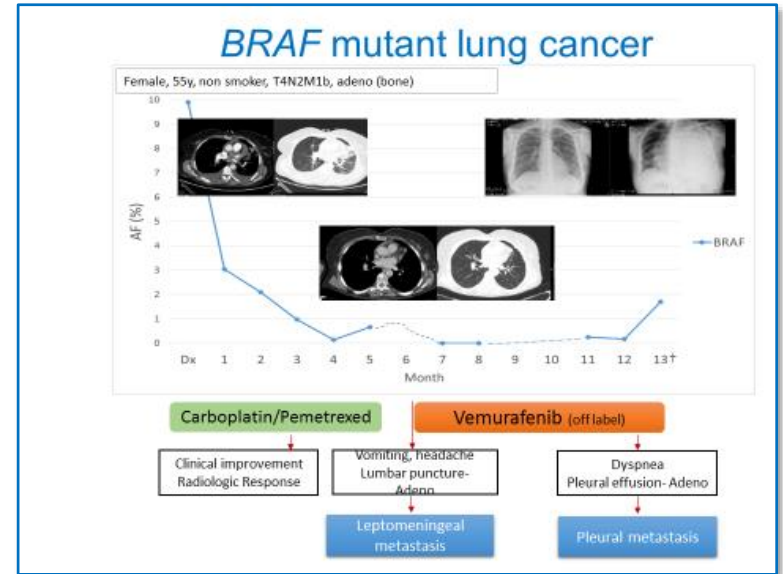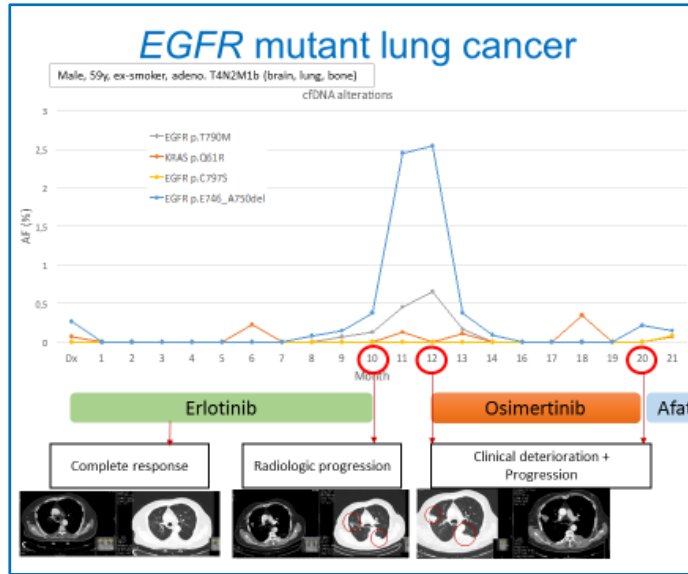
Liquid biopsy clinical performance study

- Informative in different tumor models

- Informative for different future therapeutic strategies

- Allows real-time study of the disease

- Allows study of future clinical relapse anticipation

- Identification of resistance mechanism
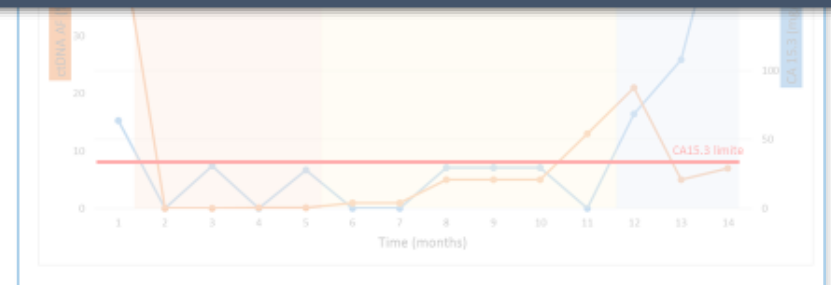
3s — INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE — UNIVERSIDADE DO PORTO

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First Experience from the "New World"

Thermo Fisher Scientific workshop – AMP 2019

6th November 2019

José Luis Costa

(jcosta@ipatimup.pt)

NGS clinical research timeline….
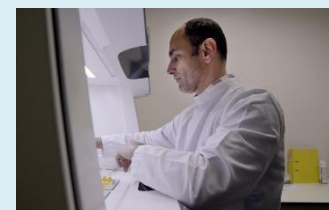
Ion PGM installation

BRCA1/2 panel (77 amplicons)

RASopathies (123 amplicons)

Ampliseq Colon & Lung panel

Ampliseq Fusion panel

Oncomine cfDNA lung assay

2011	2012	2013	2014	2015	2016	2017	2018	2019

Ion OT2

Ion Chef

Ion Chef

Ion Chef

Ion PGM

Ion Proton

Ion S5XL

Ion S5

Ion Reporter

Genexus System

First Experience from IPATIMUP, Portugal

Commercial control samples

- Horizon and SeraCare FFPE and cfDNA reference material

Clinical research samples

- Lung cancer tissue and liquid biopsy research samples
- Previously characterize on an Ion S5XL system

First Experience from IPATIMUP, Portugal

First Experience from IPATIMUP, Portugal
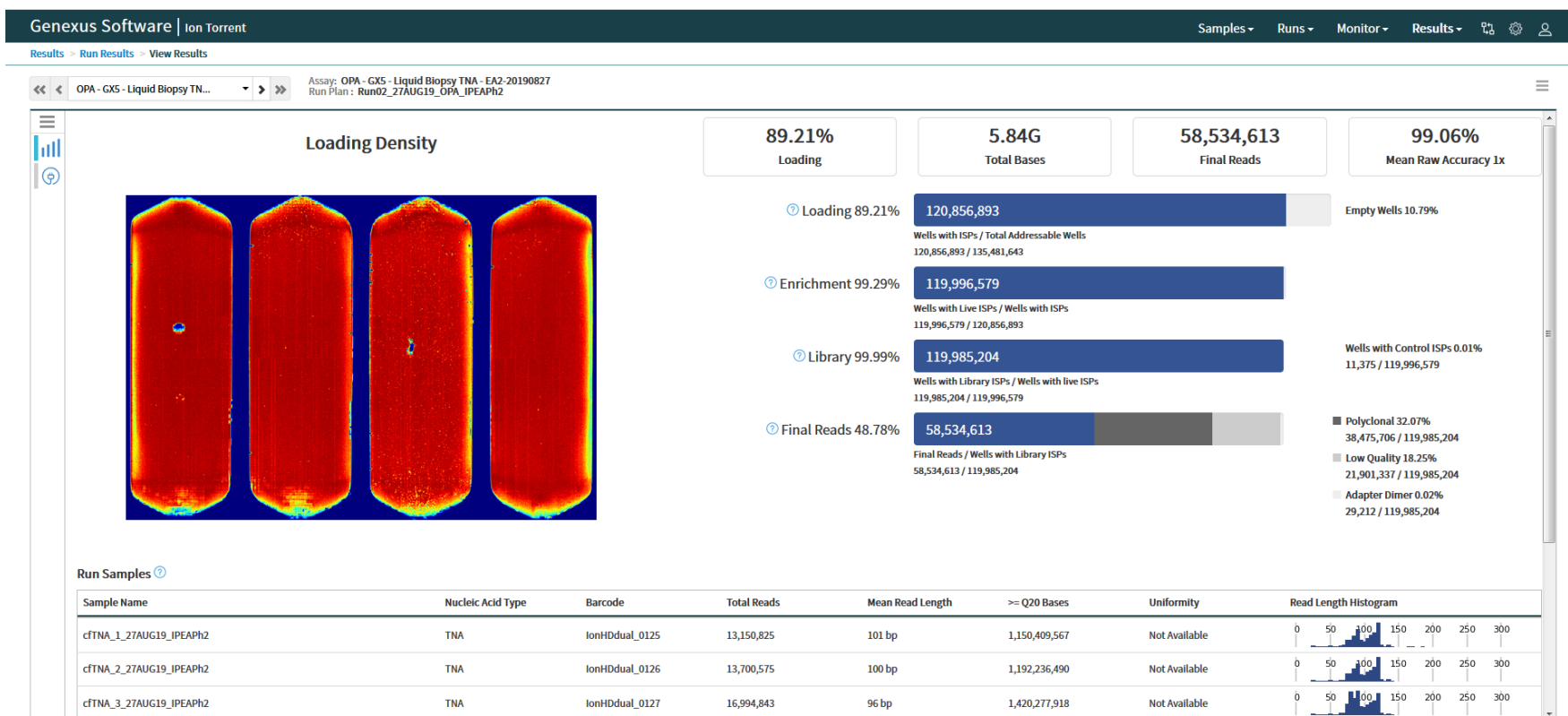
First Experience from IPATIMUP, Portugal

Commercial control samples

- Horizon and SeraCare FFPE and cfDNA reference material

Clinical research samples

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Tissue biopsies – RNA and DNA

Lung cancer FFPE sample were sequenced using Colon and Lung or Lung Fusion panel on Ion S5XL system and using Oncomine Precision Assay on Genexus instruments

Variants covered by both panels were detected in both systems at similar allelic frequencies

Additional variants were detected using the Oncomine Precision Assay

Sample 1 presented additional p.T790M mutation that had not been previously identified (intra-sample heterogeneity?)

FFPE	Gene	AA Change	Genexus	Ion S5XL
Sample 1	**EGFR**	**p.L858R**	**43,8**	**31,3**
	TP53	p.R248Q	1,8	nd
	EGFR	**p.T790M**	**1,6**	**nd**
	ALK	p.A1200V	1,5	nd
Sample 2	EGFR	p.L747_P753delinsS	28,6	20,1
	TP53	p.C176R	3,9	nd
	RET	p.G810S	1,6	nd
	EGFR	p.P848L	1,6	nd
	FGFR3	p.R399C	0,3	nd
Sample 3	ALK	fusion	detected	detected
Sample 4	BRAF	p.V600E	47,8	36,3
	TP53	p.R175C	13,8	nd
	FGFR2	p.A648T	6,9	nd
	EGFR	p.R836C	6,2	nd
	ERBB3	p.V104M	4,4	nd
	PDGFRA	p.T849C	4,2	nd
	PIK3CA	p.V344M	3,7	nd
	MAP2K1	p.K57N	2,8	nd
	GNAQ	p.R183Q	2,1	nd
	EGFR	p.V769M	1,8	nd
Sample 5	**KRAS**	**p.G12D**	**58,3**	**44,7**
	TP53	p.C176Y	31,4	nd
	FGFR3	p.R399C	3,6	nd
	CDKN2A	p.R58Q	3,1	nd
	RET	p.R912Q	2,5	nd
	BRAF	p.D594N	2,3	nd

Liquid biopsies – Lung cancer plasma sample at recurrence

9AM
10min hands on

Plasma collection

11AM
90min hands on

cfTNA isolation

4PM
10min hands on

Variant detection

9AM
10min hands on

Report

1. Plasma collection

BD Vacutainer PPT (K2EDTA)

2. cfTNA isolation

MagMax cfTNA extraction kit

3. NGS variant identification

Genexus

+

Oncomine Precision Assay

Gene	AA Change	Mol. Freq. %
EGFR	p.E746_A750del	1,59
EGFR	p.T790M	1,78

4. OKR Report

ipatimup

IPATIMUP
Rua Julio Amaral de Carvalho 45
4200-135 Porto
Portugal

Patient ID: Tracking No: Date: 31 May 2019   1 of 2

Name: Primary Tumor Site:
Date of Birth: Sample Type:
Gender: Sample ID:
Case Number: MDV/Hospital:
Entrance Date:

Sample Cancer Type: Melanoma

Report Highlights
1 Clinically Significant Biomarkers
7 Therapies Available
27 Clinical Trials

Clinically Significant Biomarkers   Indicated Contraindicated

Relevant Therapies   Relevant Therapies

- Variant interpretation
- Informed decision

Sample

< 24h

Report

Summary

- ALL-IN-ONE solution from nucleic acids to variants;
- ACCESSIBLE: no *a priori* NGS expertize needed;
- SIMPLE: all I needed was a pipette to add my samples to be sequenced;
- ROBUST: vision-system checks all your steps;
- FAST: allowed a turn around time of 24h from plasma to report.

- This system allows a wider implementation of NGS for genomic profiling, potentially bringing precision medicine closer to clinical practice.

First Experience From
New World

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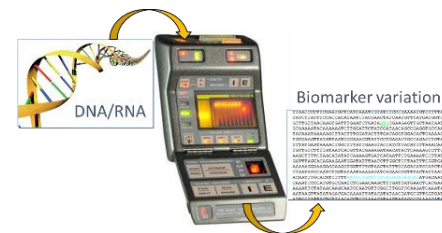
Joana Reis
José Carlos Machado

SÃO JOÃO

Venceslau Hespanhol
Gabriela Fernandes
Fátima Carneiro
Conceição Souto Moura
nurses

Thermo Fisher
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