Next generation sequencing solution for every laboratory: my experience

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Hospital del Mar

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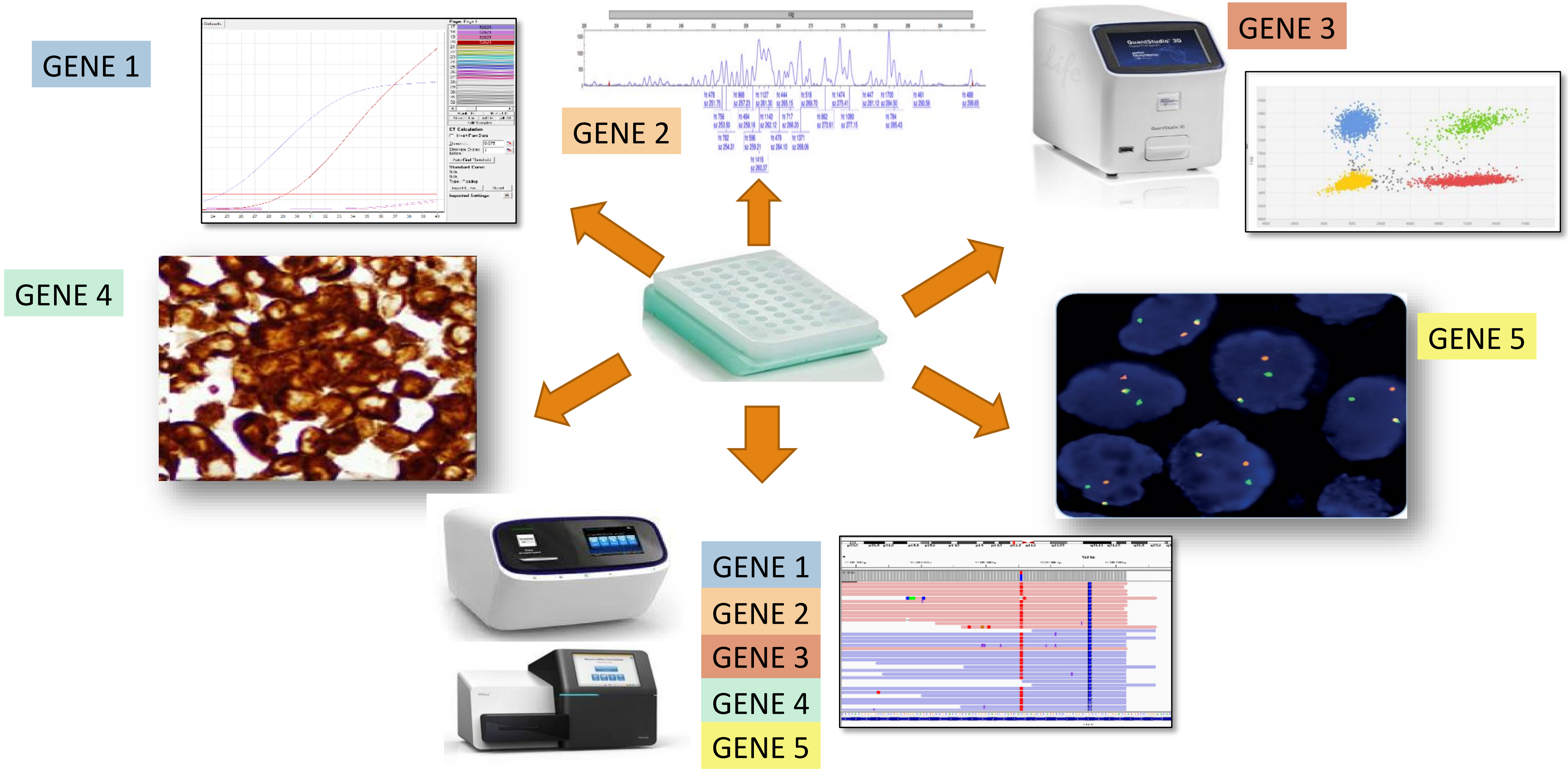
Laboratory of Molecular Diagnostics



GENE 1

GENE 2

GENE 3

GENE 4

GENE 5

GENE 1
GENE 2
GENE 3
GENE 4
GENE 5

NGS: Next Generation Sequencing

Hospital del Mar experience in NGS

BRCA1/BRCA2

Gene 1 Gene 2 Gene 4

Gene 3 Gene 5

EGFR
ALK
ERBB2
ERBB4
FGFR1
FGFR2
FGFR3
MET
DDR2
KRAS
PIK3CA
BRAF
AKT1
PTEN
NRAS
MAP2K1
STK11
NOTCH1
CTNNB1
SMAD4
FBXW7
TP53

Sequential gene testing →

Broad gene testing –
Single gene testing in urgent cases

Routine Implementation

Acreditación

ENAC
Entidad Nacional de Acreditación

ACREDITACIÓN
1246/LE2342

CONSORCI MAR PARC DE SALUT DE BARCELONA
Servei de Patologia

Biomarker Point
Punto de encuentro para la determinación de biomarcadores oncológicos

Reference site

2011 2014 2015 2016 2017 2018 2019

Pyrosequencing

Fluorescent SBS NGS

Ion semiconductor-SBS NGS

Lessons learned

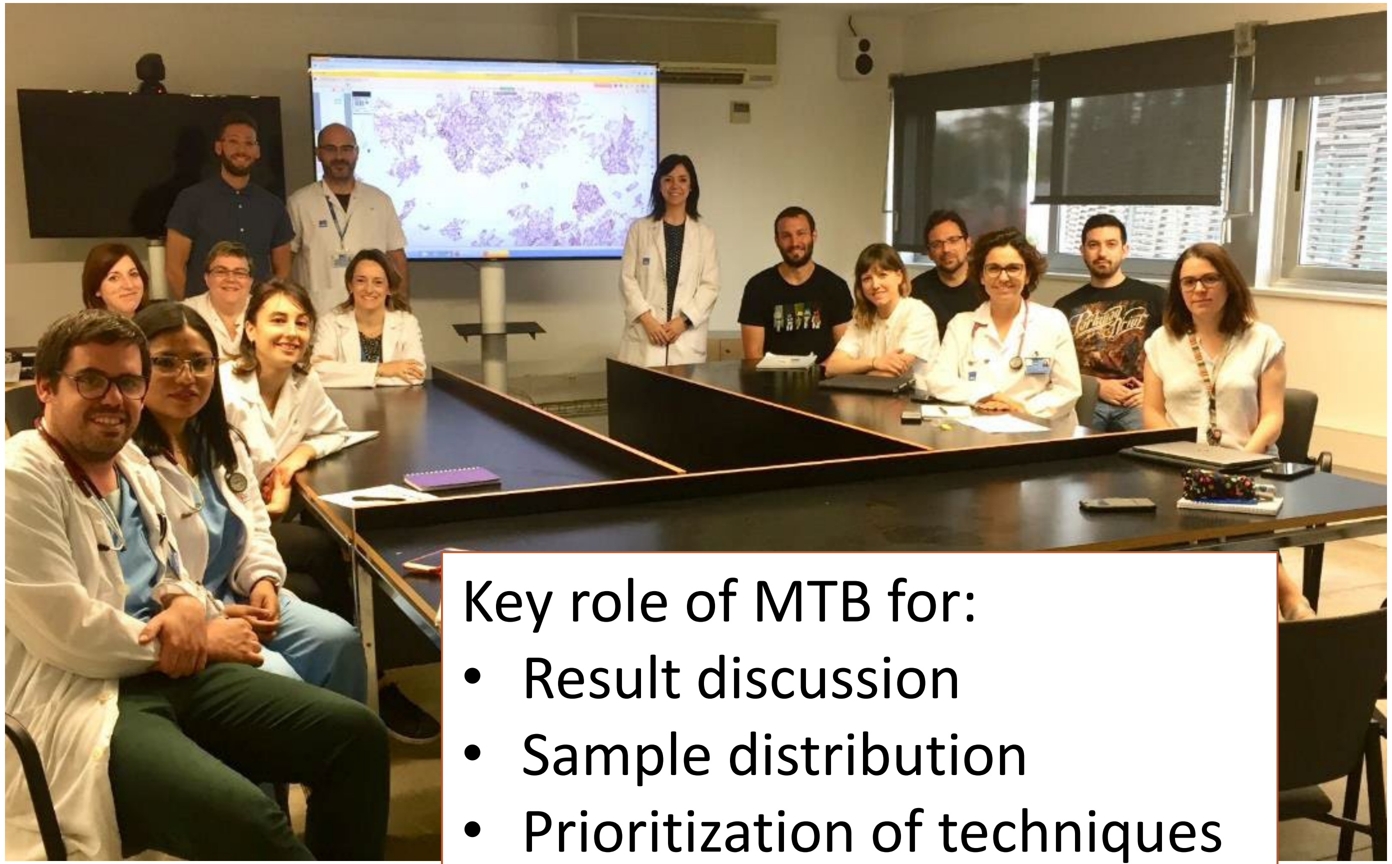
- Not all samples are valid for NGS – single gene backup maybe needed
- Sample quality is crucial for avoiding false positive results
- Importance of non-targetable driver genes –controls
- Concomitant genetic alterations
- Keep bioinformatic analysis up to date
- Participation in external quality controls

Molecular Tumor Board set-up: September 2017

Pathologists

Oncologists

Technicians



Bioinformaticians

Biologists

Residents

Pharmacist

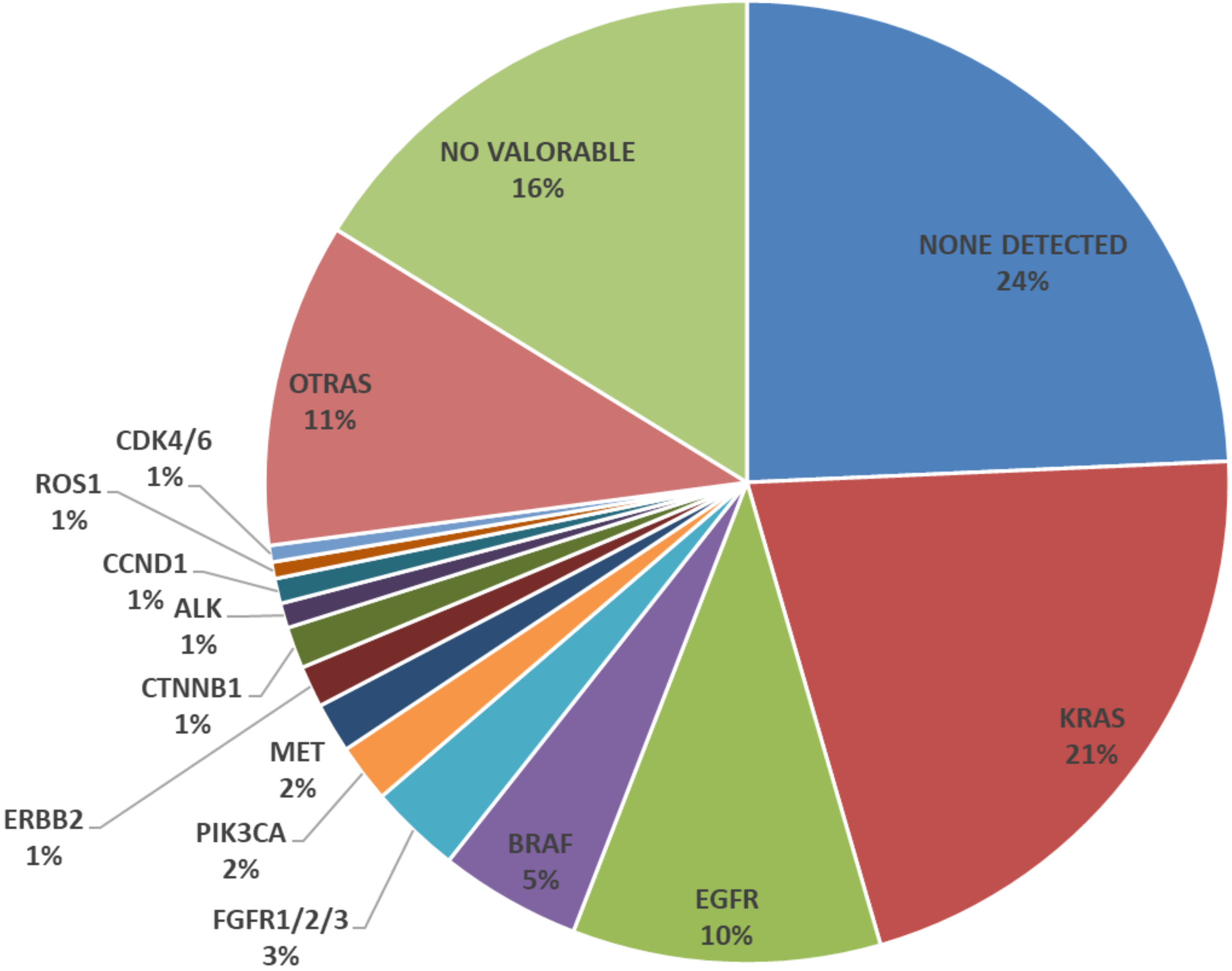
Key role of MTB for:

- Result discussion
- Sample distribution
- Prioritization of techniques
- Adjustment of TAT

KICK-OFF: September 2017

Experience as reference site

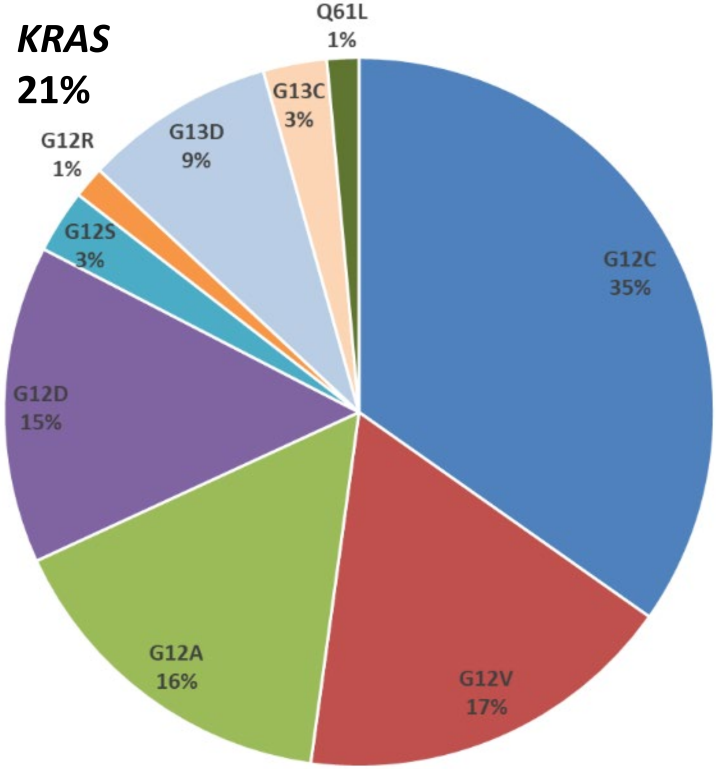
358 samples received from November 2019 to December 2021→ 300 with evaluable result (84%)



NONE DETECTED	86
KRAS	76
EGFR	37
BRAF	17
FGFR1/2/3	11
PIK3CA	7
MET	7
ERBB2	5
CTNNB1	5
ALK	3
CCND1	3
ROS1	2
CDK4/6	2
OTHER ALTERATIONS	39
NOT EVALUABLE	58

36% of samples with mutations in *KRAS*, *EGFR* or *BRAF*

KRAS
21%

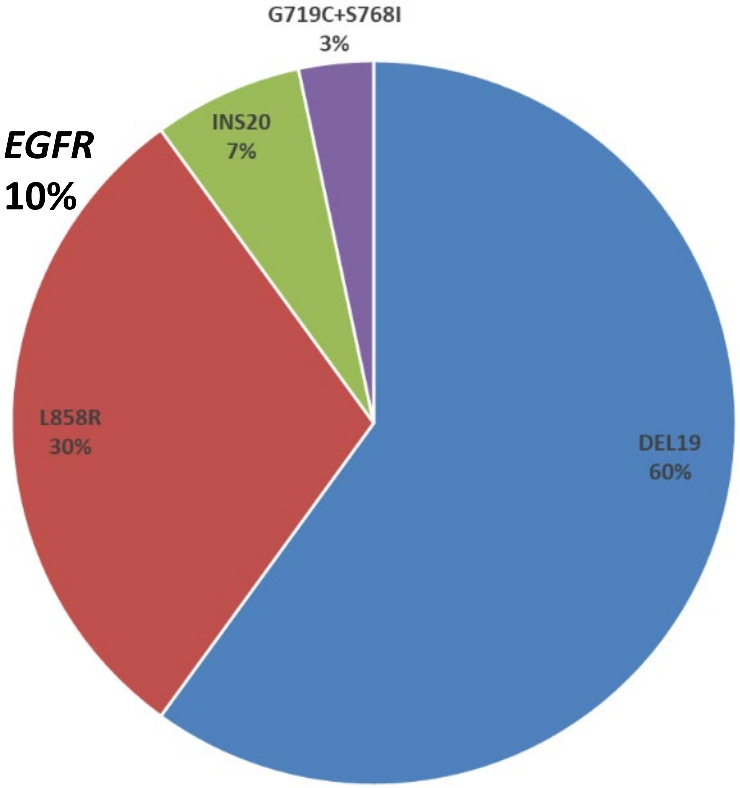


Codons 12 & 13 exon 2 *KRAS* (≈75% codon 12)

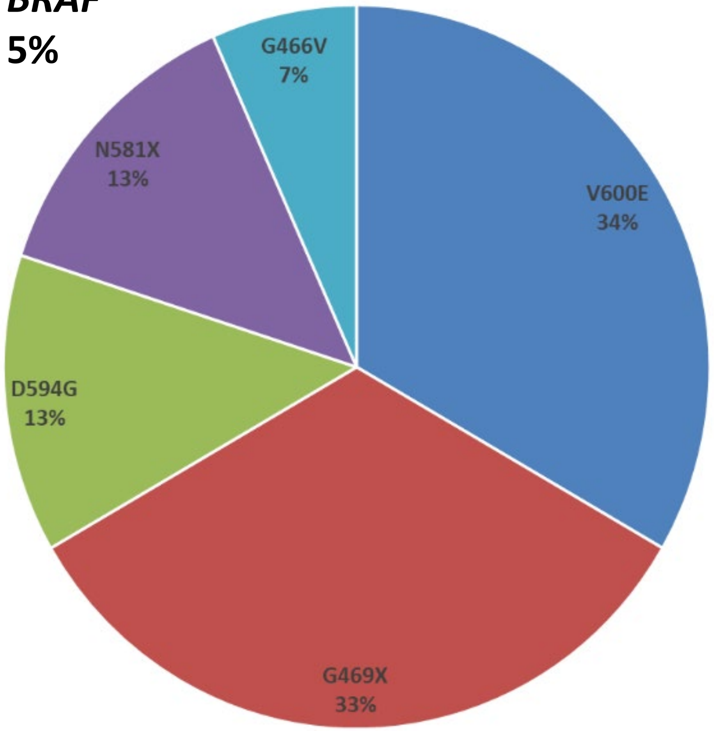
KRAS p.G12C most frequent

Comutations *STK11, TP53, KEAP1*

EGFR
10%



BRAF
5%



90% del19 or L858R

Ins20: *de novo resistance* / new generation TKIs

G719X+S768I rare composite mutations

1/3 *BRAF* V600E (aprox. 50% non-V600)

Low/High kinase activity (BRAF and/or MEK inh)

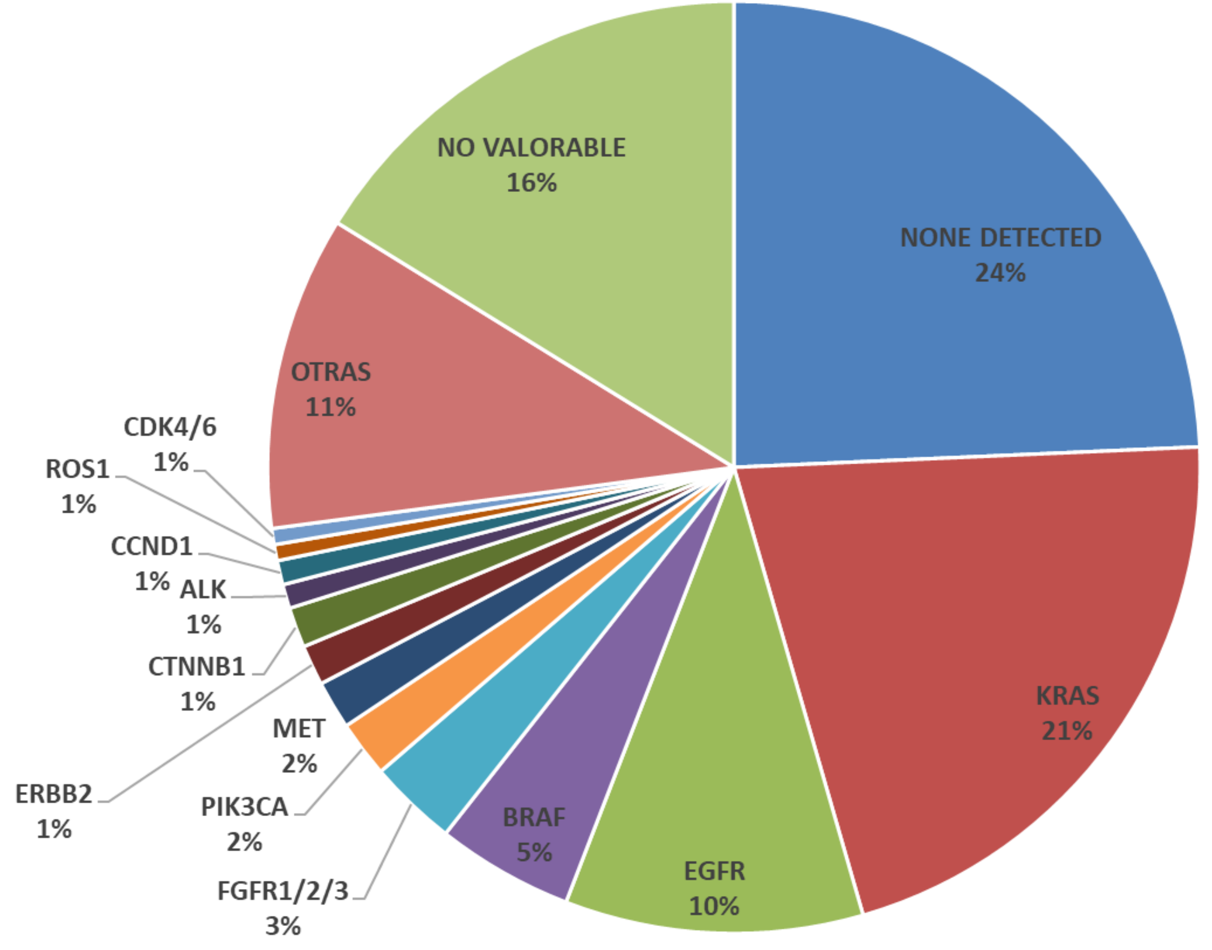
Skoulidis F, Goldberg ME, Greenawalt DM, et al. Cancer Discov. 2018
Canon J, Rex K, Saiki AY, et al. Nature. 2019
Jeanson A, Tomasini P, Souquet-Bressand M, et al. J Thorac Oncol. 2019
Liu C, Zheng S, Jin R, et al. Cancer Lett. 2020

Dankner M, Rose AN, Rajkumar S, et al. Oncogene. 2018
Vyse S and Huang PH. Signal Transduct Target Ther. 2019
Cai Y, Wang Y, Sun J, et al. J Int Med Res. 2020
Mazieres J, Cropet C, Montané L, et al. Ann Oncol. 2020

Reference site : Non evaluable samples (16% of cases)

Insufficient material : limited sample, <20% tumoral cells and/or <10ng DNA/RNA

Material with artifacts : overfixation , necrotic areas



Single gene testing

New sample

Plasma sample

Review of quality parameters

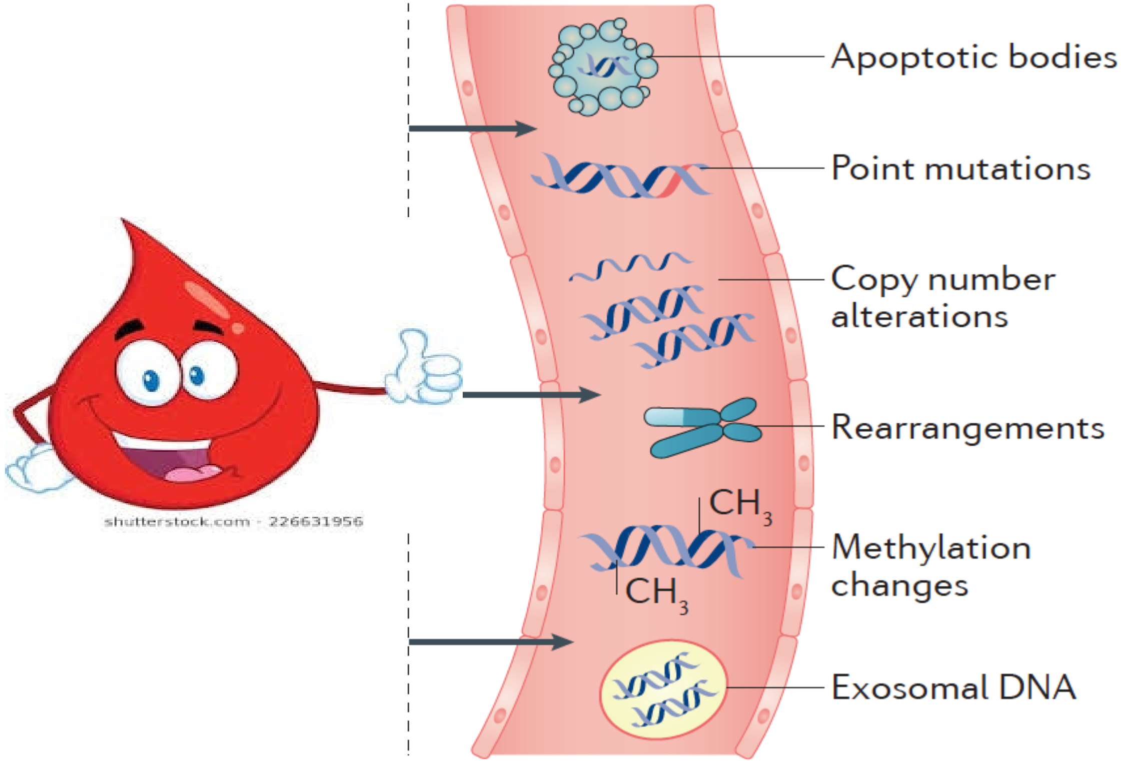
Review of tumoral percentage

Correlate with patient's clinic

Limitations

Samples may be:

- Small
- Old
- Low number of tumoral cells
- Limited material to obtain DNA & RNA



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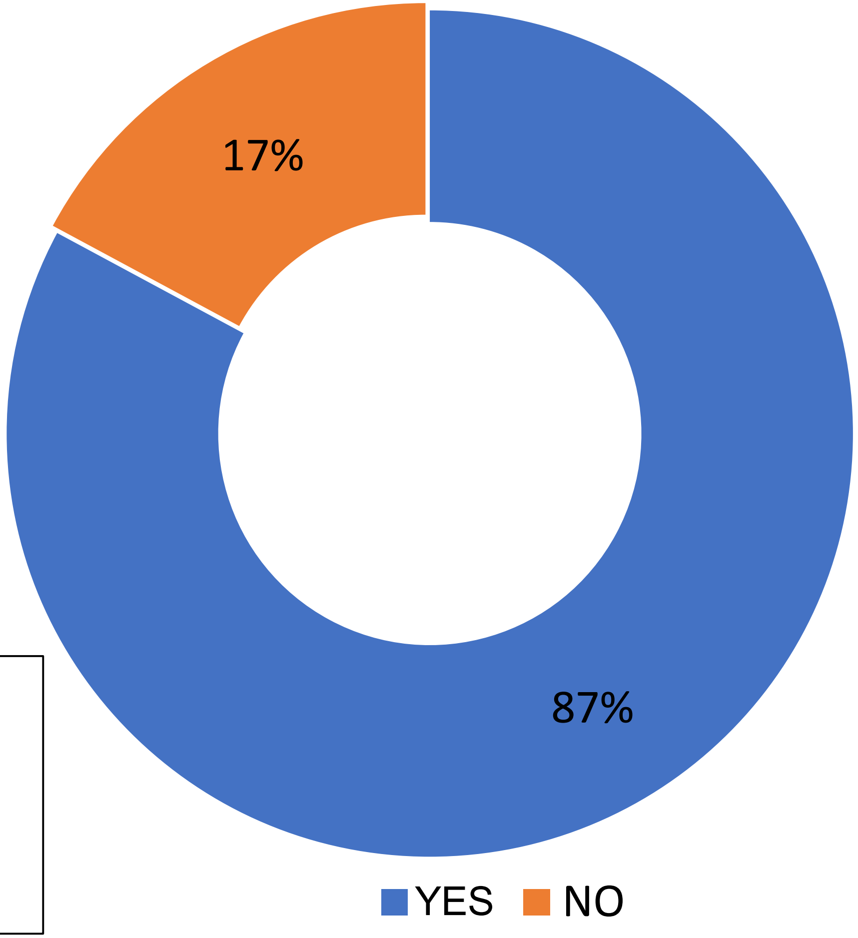
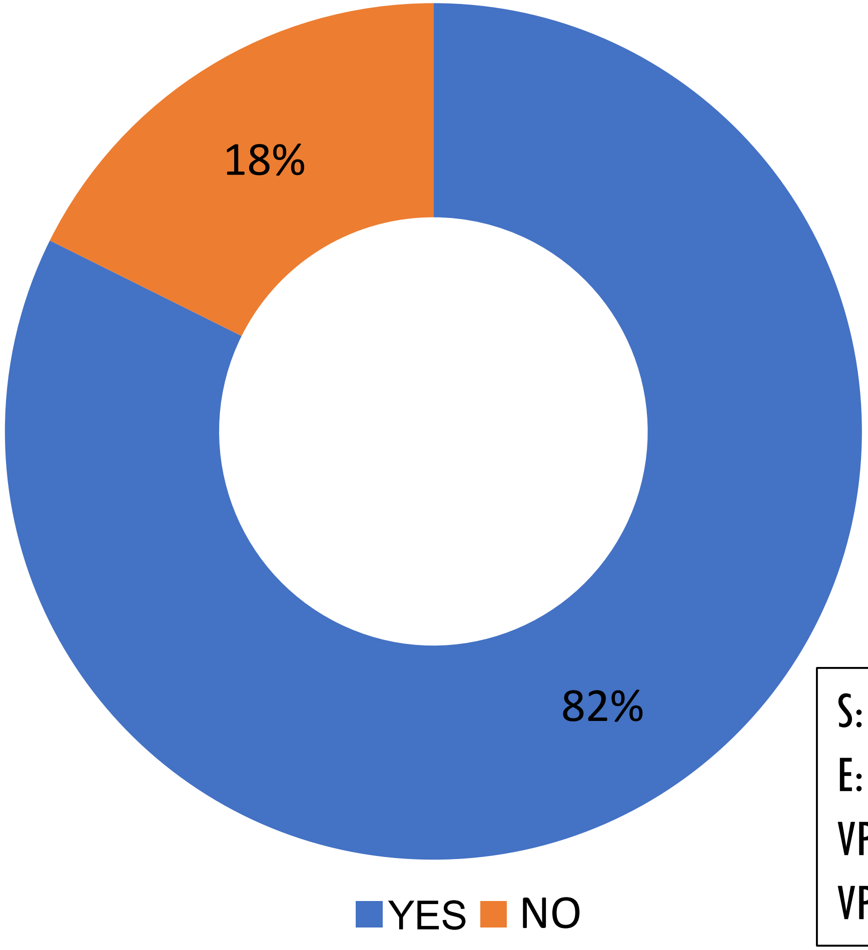
Concordance tissue / ctDNA

EGFR mutations



S: 0.82
E: 1
VPP: 1
VPN: 0.88

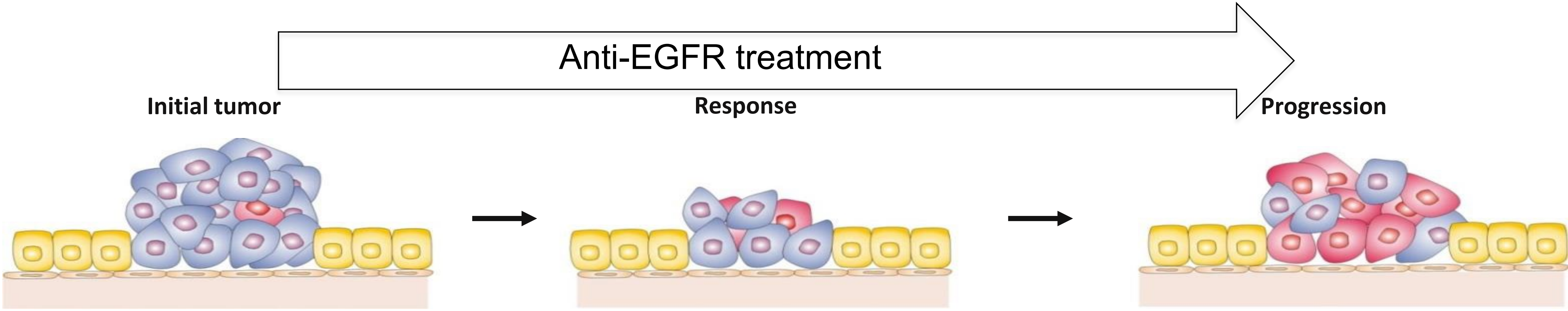
KRAS mutations



S: 0.82
E: 1
VPP: 1
VPN: 0.62

Taus *et al,* Clin Lung Cancer 2018; Taus *et al,* Arch Bronconeumol 2021

Temporal heterogeneity - Clonal selection

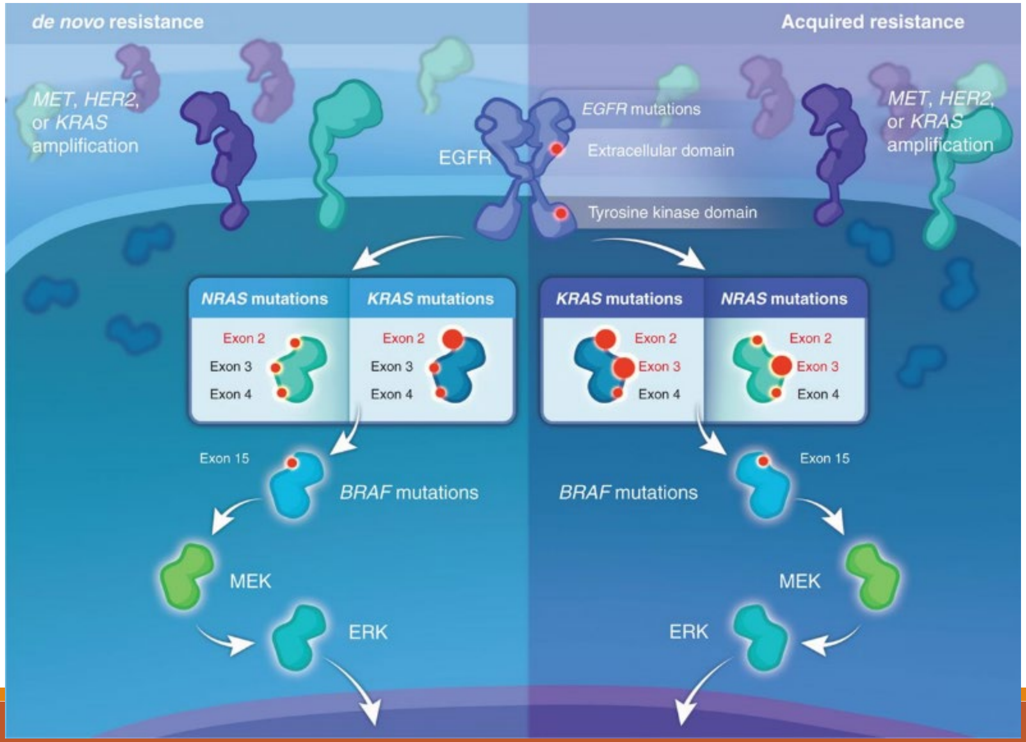


Anti-EGFR treatment

Initial tumor

Response

Progression



Dienstmann R et al. Am Soc Clin Oncol Educ Book. 2015:e149-56

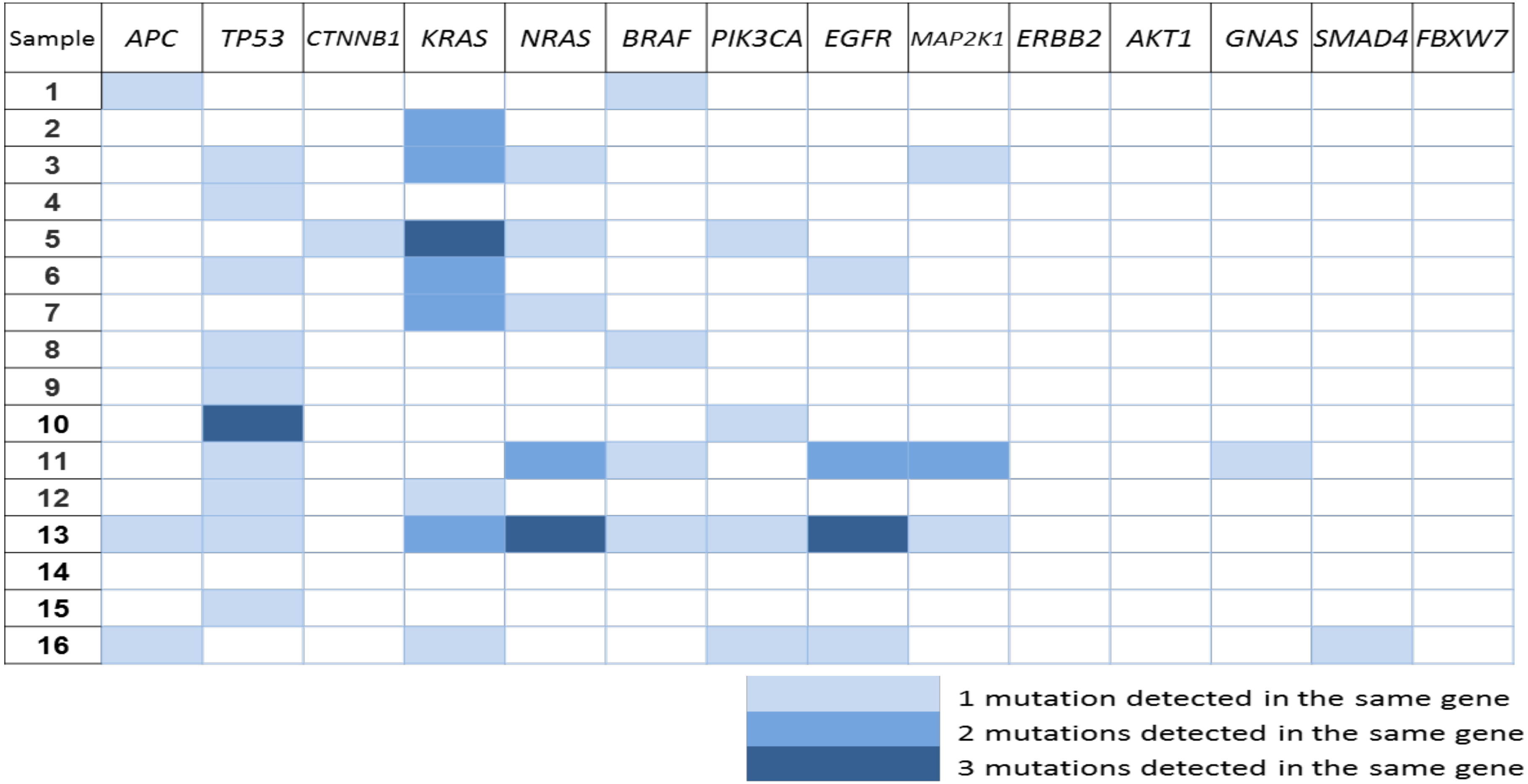
Sandra Misale et al. Cancer Discovery 2014;4:1269-1280

Molecular profiling at the time of progression to anti-EGFR therapy

Oncomine™ Colon cfDNA Assay

Gene List

AKT1
BRAF
CTNNB1
EGFR
ERBB2
FBXW7
MAP2K1
NRAS
PIK3CA
SMAD4
TP53
APC
GNAS
KRAS



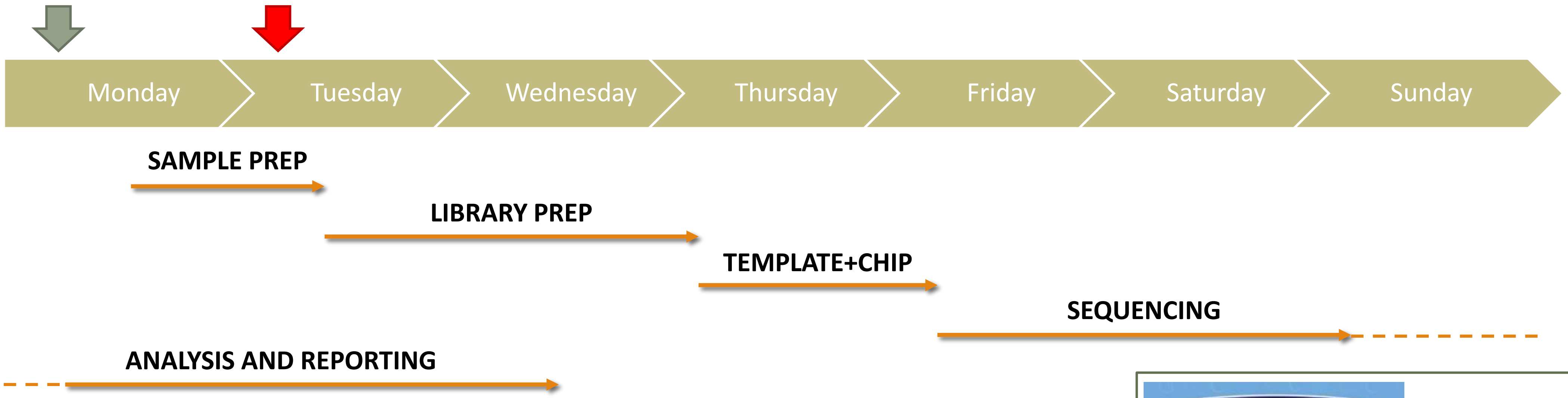
Sample	*APC*	*TP53*	*CTNNB1*	*KRAS*	*NRAS*	*BRAF*	*PIK3CA*	*EGFR*	*MAP2K1*	*ERBB2*	*AKT1*	*GNAS*	*SMAD4*	*FBXW7*
1	░					░								
2				▒										
3		░		▒	░				░					
4		░												
5			░	▓	░		░							
6		░		▒				░						
7				▒	░									
8		░				░								
9		░												
10		▓					░							
11		░			▒	░		▒	▒			░		
12		░		░										
13	░	░		▒	▓	░	░	▓	░					
14														
15		░												
16	░			░			░	░					░	

░ 1 mutation detected in the same gene
▒ 2 mutations detected in the same gene
▓ 3 mutations detected in the same gene

- At least one mutation was detected in 94% of pts (15/16)
- Median mutations per sample was 2.5 (range 1 -13)

Vidal *et al*, ESMO 2017

Workflow at Molecular Diagnostics Lab in Hospital del Mar



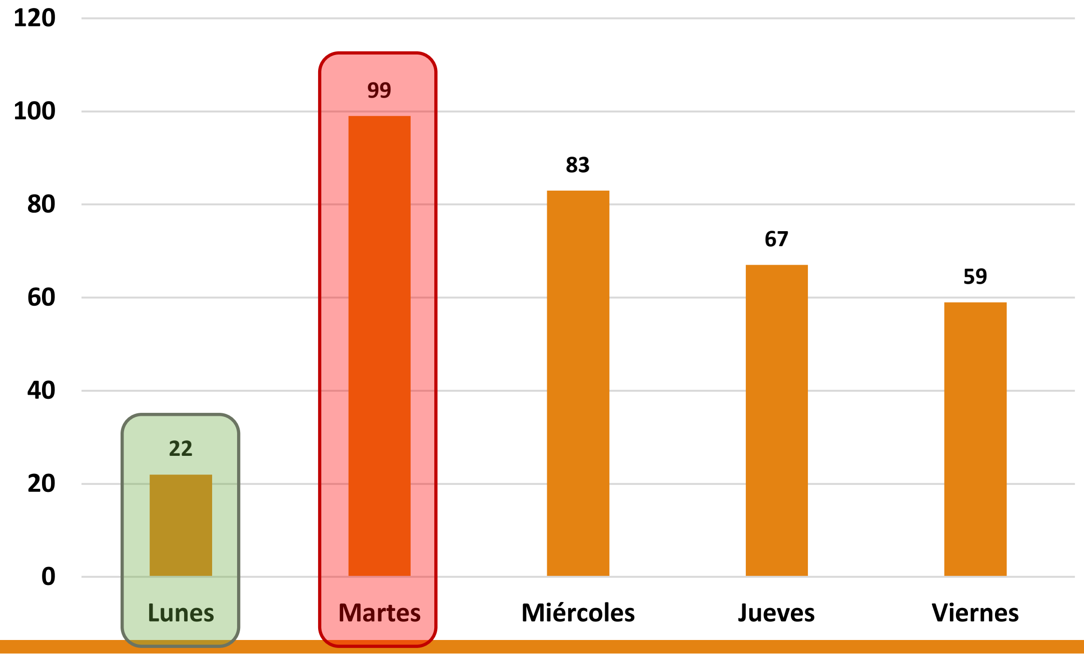
SAMPLE PREP

LIBRARY PREP

TEMPLATE+CHIP

SEQUENCING

ANALYSIS AND REPORTING



Day of reception:

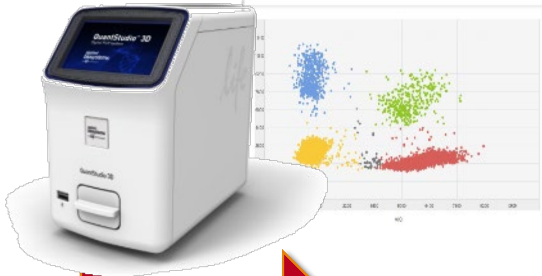
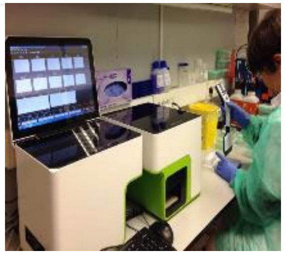
Monday: 7-9 days

Tuesday: 14-16 days

n= 358 samples received

Flexibility to accommodate small sample batches—on-instrument reagent and chip stability supports sample intake variability

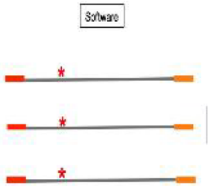
Hospital del Mar experience in NGS

2012 2013 2015 2016

Research Grant

Instituto de Salud Carlos III



UMI based NGS DNA panels

TNA based NGS panels

2011 2014 2015 2016 2018 2021





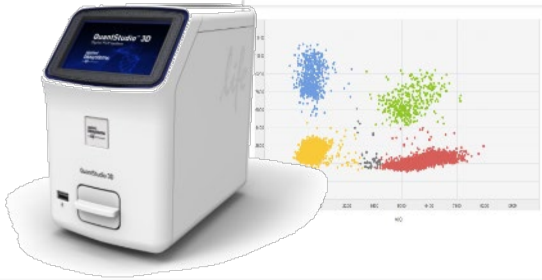

Pyrosequencing

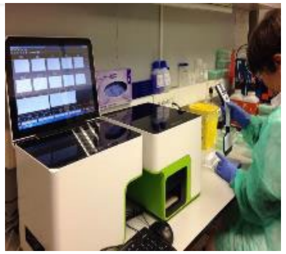
Fluorescent SBS NGS

Ion semiconductor-SBS NGS

Integrated sequencer

Hospital del Mar experience in NGS





2012 2013 2015 2016

Research Grant

ThermoFisher SCIENTIFIC

Oncomine Clinical Research Grant Program

2011 2014 2015 2016 2018 2021

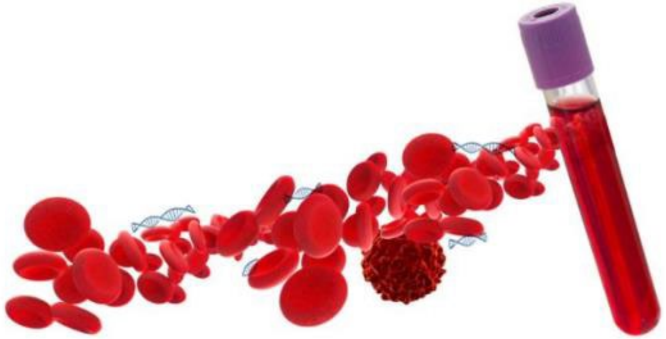
Pyrosequencing

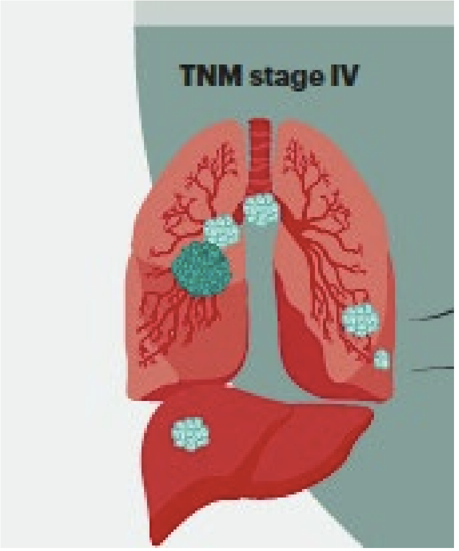
Fluorescent SBS NGS

Ion semiconductor-SBS NGS

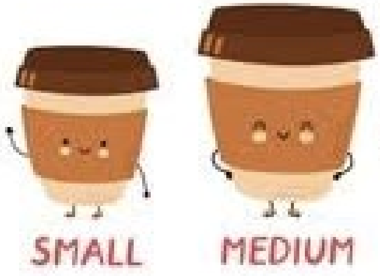
Integrated sequencer

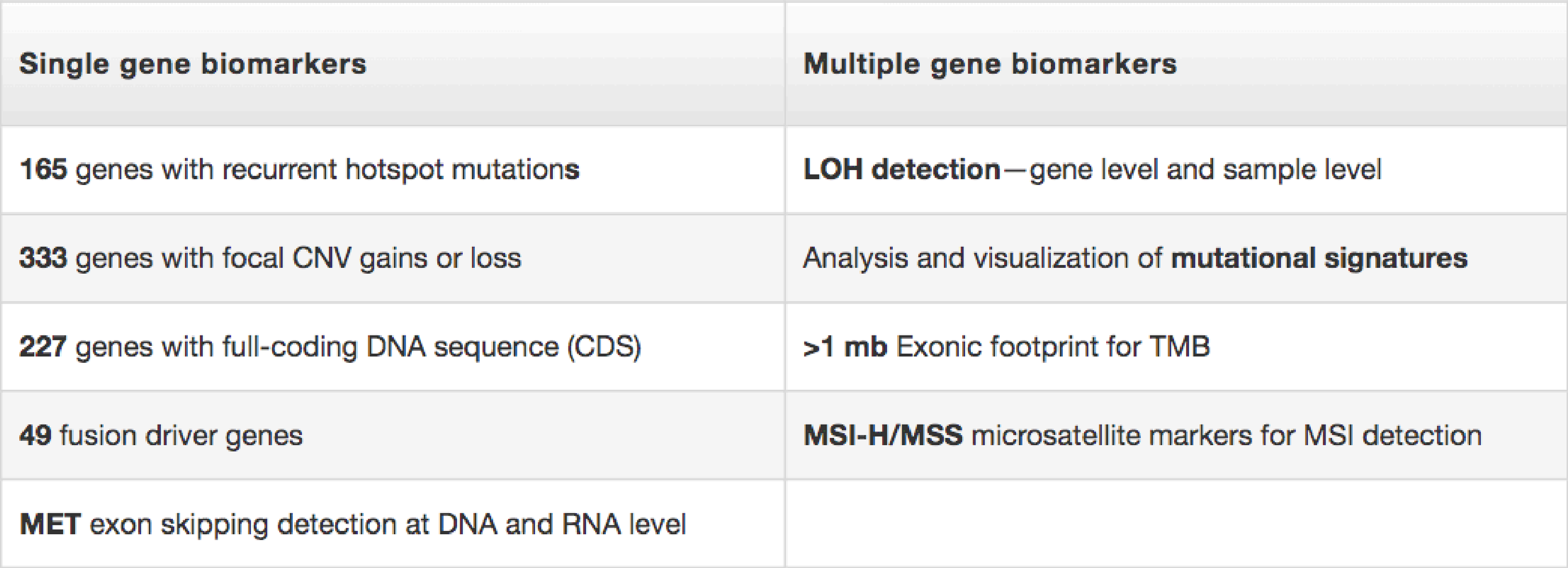
Oncomine Research Grant – Characterization of SCLC samples





Comprehensive genomic profiling panel (>500 genes)





Single gene biomarkers	Multiple gene biomarkers
165 genes with recurrent hotspot mutations	**LOH detection**—gene level and sample level
333 genes with focal CNV gains or loss	Analysis and visualization of **mutational signatures**
227 genes with full-coding DNA sequence (CDS)	**>1 mb** Exonic footprint for TMB
49 fusion driver genes	**MSI-H/MSS** microsatellite markers for MSI detection
MET exon skipping detection at DNA and RNA level	

Conclusions