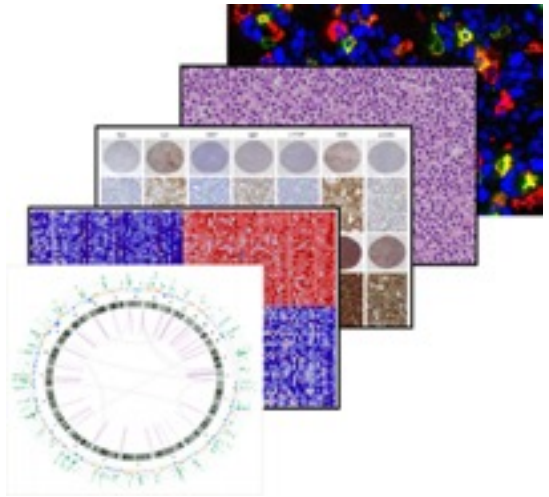



# Mesa 2 Proceso diagnóstico Biología Molecular

ELOISA JANTUS LEWINTRE, [1,2,3]

LABORATORIO ONCOLOGÍA MOLECULAR

FUNDACIÓN PARA LA INVESTIGACIÓN DEL HOSPITAL GENERAL UNIVERSITARIO DE VALENCIA

[1] Jefa del Laboratorio de Oncología Molecular, Fundación de Investigación del Hospital General Universitario de Valencia (FiHGUV)

[2] Profesor Asociado, Departamento de Biotecnología, Universidad Politécnica de Valencia

[3] Red Temática de Investigación Cooperativa en Cáncer (RTICC)

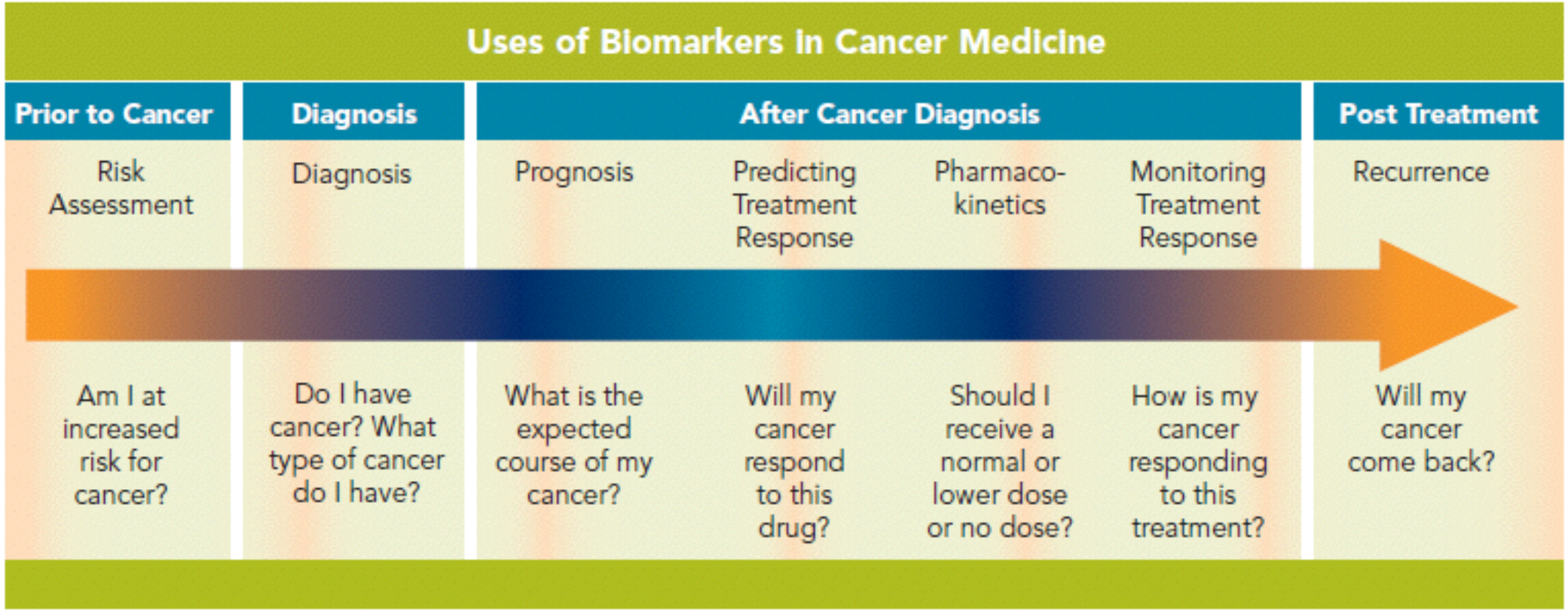
# Determinaciones moleculares en cáncer de pulmón:

-Porqué hacemos análisis moleculares?

-Qué determinaciones?

# TIPOS DE BIOMARCADORES

Característica que puede ser objetivamente medible y evaluable como un indicador de un proceso biológico normal, patológico o como respuesta farmacológica a una intervención terapéutica.



# BIOMARCADORES: Predictivos

| FÁRMACO (13) | DIANAS (12) | TUMOR (10) | BIOMARCADOR (7) |
|---|---|---|---|
| Imatinib | KIT, BCR-ABL, PDGFR | GIST | C-Kit, PDGFR |
| Trastuzumab | HER-2 | Mama | HER 2 |
| Pertuzumab | HER-2 | Mama | HER-2 |
| Lapatinib | TKI HER2 | Mama | HER-2 |
| Gefitinib<br>Afatinib | EGFR TKI | NSCLC | EGFR |
| Erlotinib | EGFR TKI | NSCLC, Páncreas | EGFR |
| Cetuximab | EGFR (Mab) | CRC, Cabeza y Cuello | K-Ras |
| Panitumumab | EGFR (Mab) | CRC | K-Ras, N-Ras |
| Temsirolimus | mTOR | RCC | No |
| Everolimus | mTOR | RCC, Mama, NETs Pancreáticos | No |
| Vandetanib | EGFR,VEGF,RET | Medular Tiróides | No |
| Vemurafenib | BRAF | Melanoma | B-Raf |
| Crizotinib | EML4-ALK | NSCLC | ALK |

# BIOMARCADORES: Predictivos

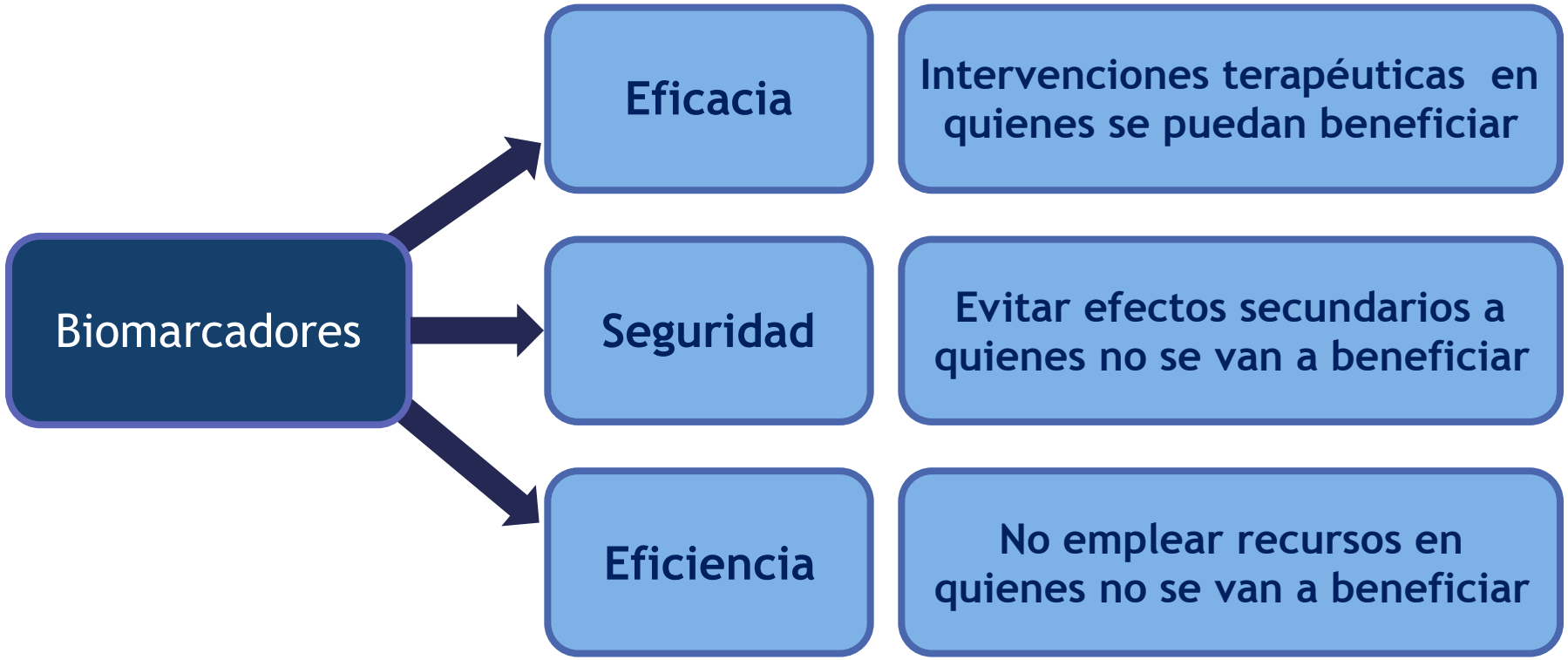
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lung | NSCLC | EGFR (HER1): mutations in tyrosine kinase domain | Sequencing, ISH | EGFR: Erlotinib, gefitinib, afatinib | 15% adenocarcinomas in USA (higher in Asians, women and nonsmokers) | In clinical use | (76) |
| | | ALK: Inversion in chromosome 2 leads to EML4-ALK fusion oncogene | FISH (IHC) | ALK: crizotinib, ceritinib (alectinib under development) | 4% (mostly adenocarcinoma) | In clinical use | (77) |
| | Lung adenocarcinoma | Multiple genes: | | | | Continued validation | (49) |
| | | BRAF (V600E and non-V600E) | Multiplex sequencing | BRAF: AZD6244 | 2% | | |
| | | EGFR (HER1): mutations in tyrosine kinase domain | | EGFR: erlotinib, gefitinib, afatinib, cetuximab | 17% | | |
| | | HER2: oncogene overexpression | | HER2: decinutubub, neratinib, lapatinib, trastuzumab | 3% | | |
| | | KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy | | KRAS: erlotinib, tivantinib, everolimus, ridaforalimus, AZD6244 | 25% | | |
| | | ALK: inversion in chromosome 2 leads to EML4-ALK fusion oncogene | | ALK: crizotinib, ceritinib | 8% | | |
| | | MET | | MET: cizotinib | <1% | | |

PDL-1 expression (10-50%)

Anti-PDL1 immunotherapy

Goossens et al, TCR

# BIOMARCADOR




Conley BA, Taube SE. Dis Markers 2004;20:35–43;
Kelloff GJ, Sigman CC. Eur J Cancer 2005;41:491–501;
President's Council of Advisors on Science and Technology (PCAST): 'Priorities for Personalized Medicine' September 2008;
Heinemann V, et al. Cancer Treat Rev 2013; 39:592-601.

# Determinaciones Moleculares en cáncer de pulmón:

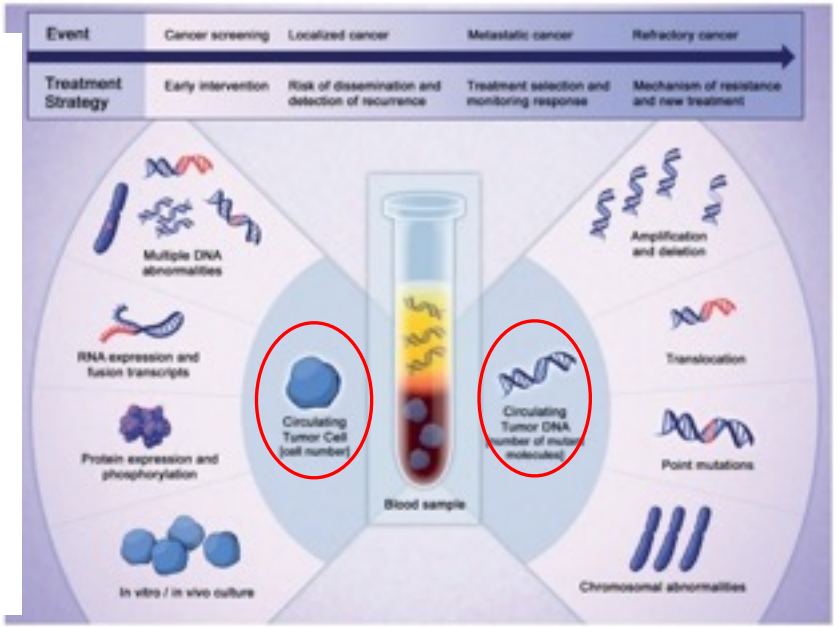
Dónde?

illumina

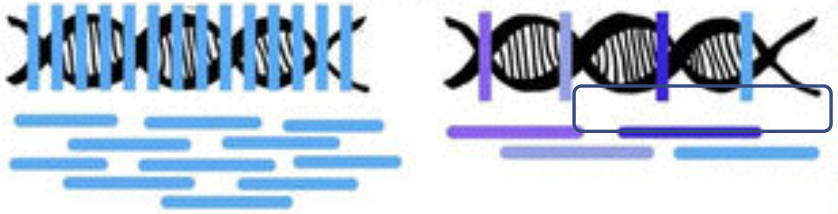
Primary tumour

Macrodissection

Isolation of tumour nuclei




Whole-exome or targeted sequencing



Point mutations

Amplifications

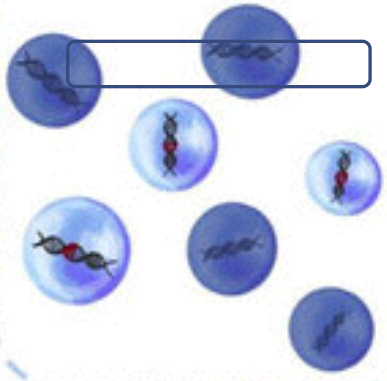
Insertions / deletions

Clinical annotation

Assessment of clinical actionability

Discovery of prognostic indicators

Digital mutation detection



Real-time detection of disease recurrence

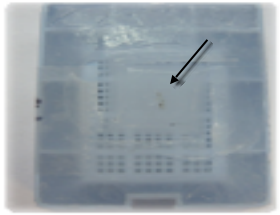

Cube 6i flow cytometer

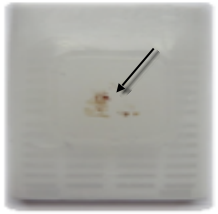
Da Haber and Velculescu; 2014, Cance

# Determinaciones Moleculares en cáncer de pulmón:

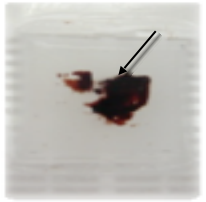
Qué información necesitamos?
Cómo la obtenemos?

Lo Frecuente





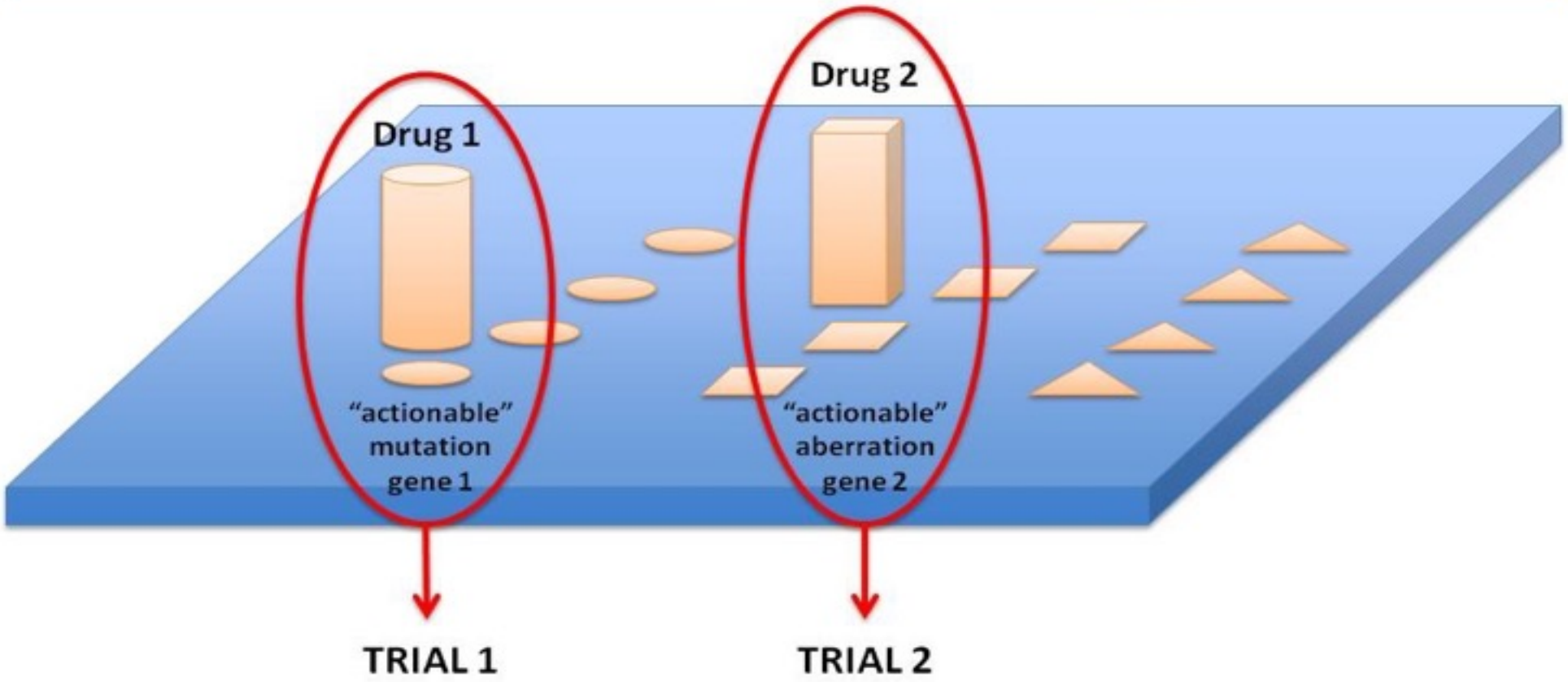
Lo Raro



Haciendo **MÁS** con **menos**

# CAMBIAR LA ESTRATEGIA….

One actionable aberration ---> One targeted drug ---> One trial

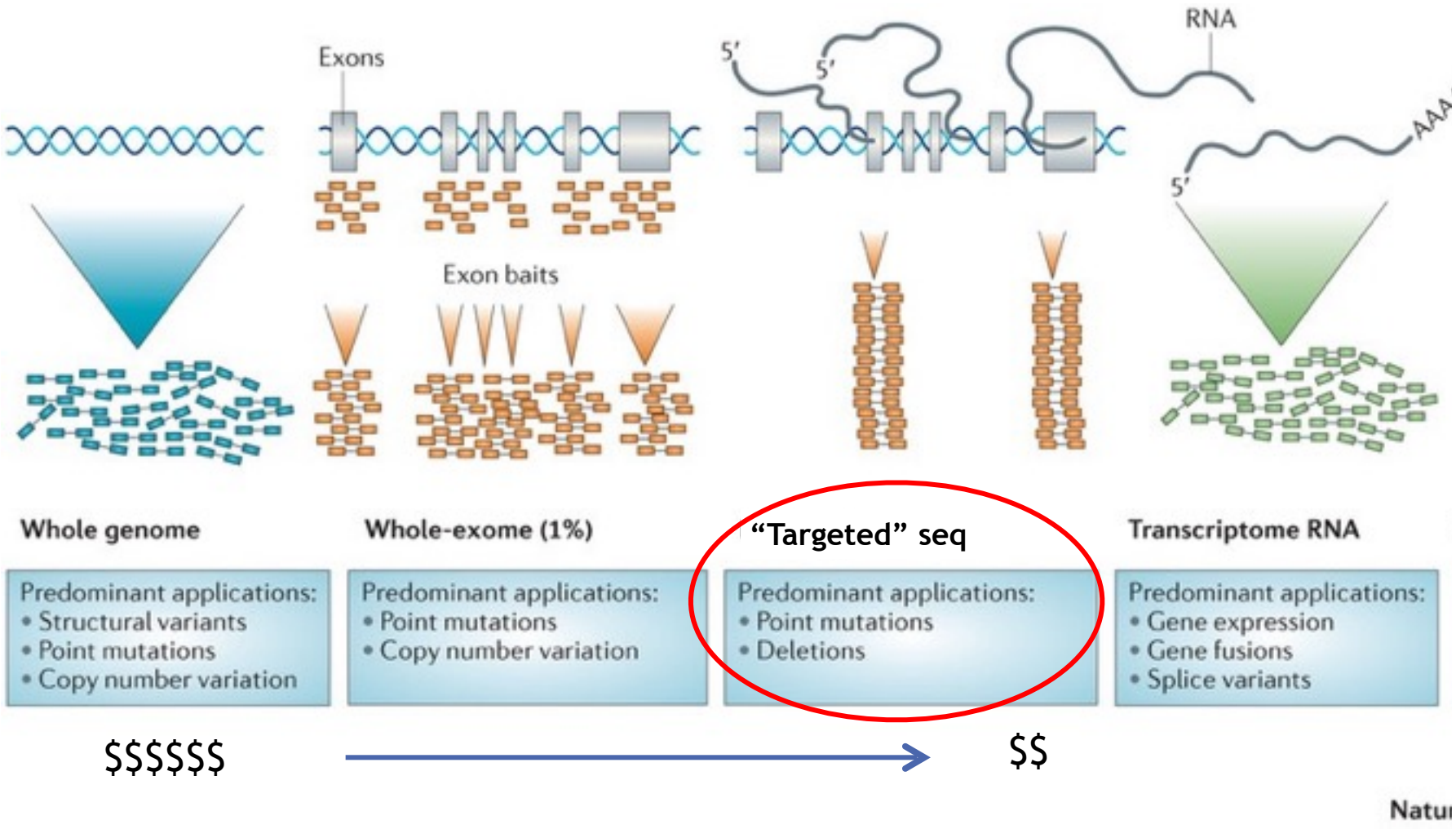



= an extremely time/energy consuming experience with low efficiency!!!

# Metodología

| Technique | Sensitivity | Optimal Application |
|---|---|---|
| Sanger sequencing | > 10% | Tumor tissue |
| Pyrosequencing | 10% | Tumor tissue |
| Next-generation seqeuncing | 2% | Tumor tissue |
| Quantative PCR | 1% | Tumor tissue |
| ARMS | 0.10% | Tumor tissue |
| BEAMing, PAP, Digital PCR, TAM-Seq | 0.01% or lower | ctDNA, rare variants in tumor tissue |

# NGS: que tipo de información genera??




Richard Simon & Sameek Roychowdhury; Nature Reviews Drug Discovery 12, 358-369 (2013)

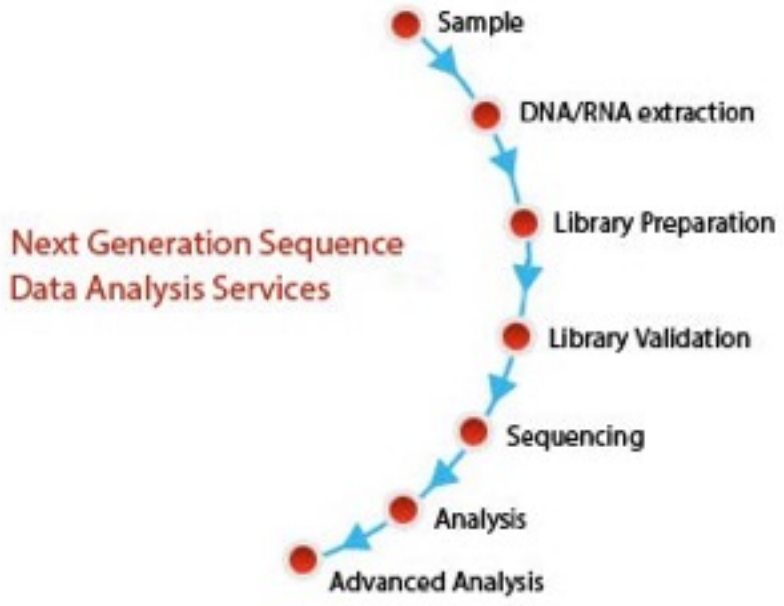



**Table 1: Gene Content on TruSight Tumor 15**

| | | |
|---|---|---|
| *AKT1* | *GNA11* | *NRAS* |
| *BRAF* | *GNAQ* | *PDGFRA* |
| *EGFR* | *KIT* | *PIK3CA* |
| *ERBB2* | *KRAS* | *RET* |
| *FOXL2* | *MET* | *TP53* |

**Table 1: TSACP Cancer-Related Genes**

| | | | | |
|---|---|---|---|---|
| *ABL1* | *EGFR* | *GNAS* | *MLH1* | *RET* |
| *AKT1* | *ERBB2* | *HNF1A* | *MPL* | *SMAD4* |
| *ALK* | *ERBB4* | *HRAS* | *NOTCH1* | *SMARCB1* |
| *APC* | *FBXW7* | *IDH1* | *NPM1* | *SMO* |
| *ATM* | *FGFR1* | *JAK2* | *NRAS* | *SRC* |
| *BRAF* | *FGFR2* | *JAK3* | *PDGFRA* | *STK11* |
| *CDH1* | *FGFR3* | *KDR* | *PIK3CA* | *TP53* |
| *CDKN2A* | *FLT3* | *KIT* | *PTEN* | *VHL* |
| *CSF1R* | *GNA11* | *KRAS* | *PTPN11* | |
| *CTNNB1* | *GNAQ* | *MET* | *RB1* | |

Cancer-related genes represented in the TSACP. For a full list of 'target regions, see the manifest file' (Myllumina login required).

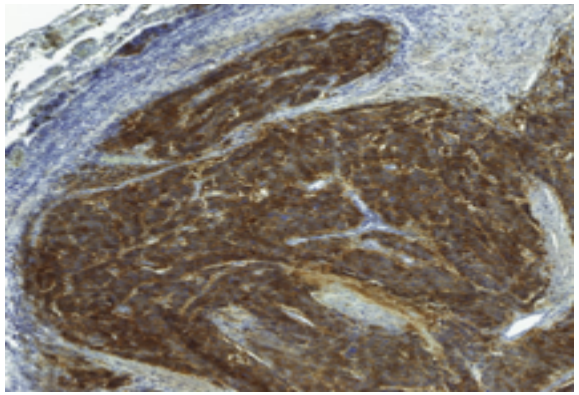
## Ampli Seq Cancer

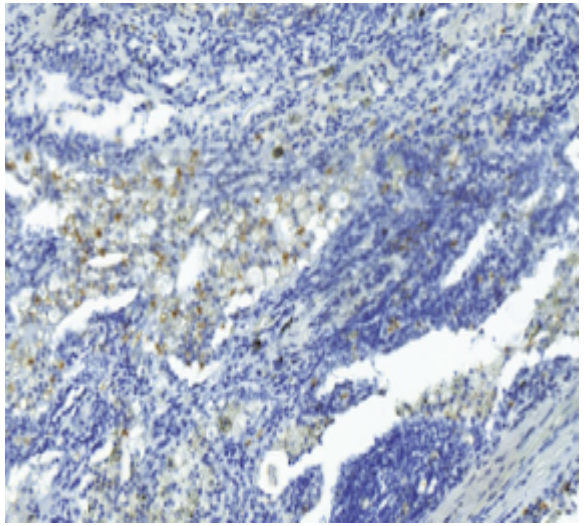
**The 50 targeted genes**

| | | | |
|---|---|---|---|
| *ABL1* | *EZH2* | *JAK3* | *PTEN* |
| *AKT1* | *FBXW7* | *IDH2* | *PTPN11* |
| *ALK* | *FGFR1* | *KDR* | *RB1* |
| *APC* | *FGFR2* | *KIT* | *RET* |
| *ATM* | *FGFR3* | *KRAS* | *SMAD4* |
| *BRAF* | *FLT3* | *MET* | *SMARCB1* |
| *CDH1* | *GNA11* | *MLH1* | *SMO* |
| *CDKN2A* | *GNAS* | *MPL* | *SRC* |
| *CSF1R* | *GNAQ* | *NOTCH1* | *STK11* |
| *CTNNB1* | *HNF1A* | *NPM1* | *TP53* |
| *EGFR* | *HRAS* | *NRAS* | *VHL* |
| *ERBB2* | *IDH1* | *PDGFRA* | |
| *ERBB4* | *JAK2* | *PIK3CA* | |

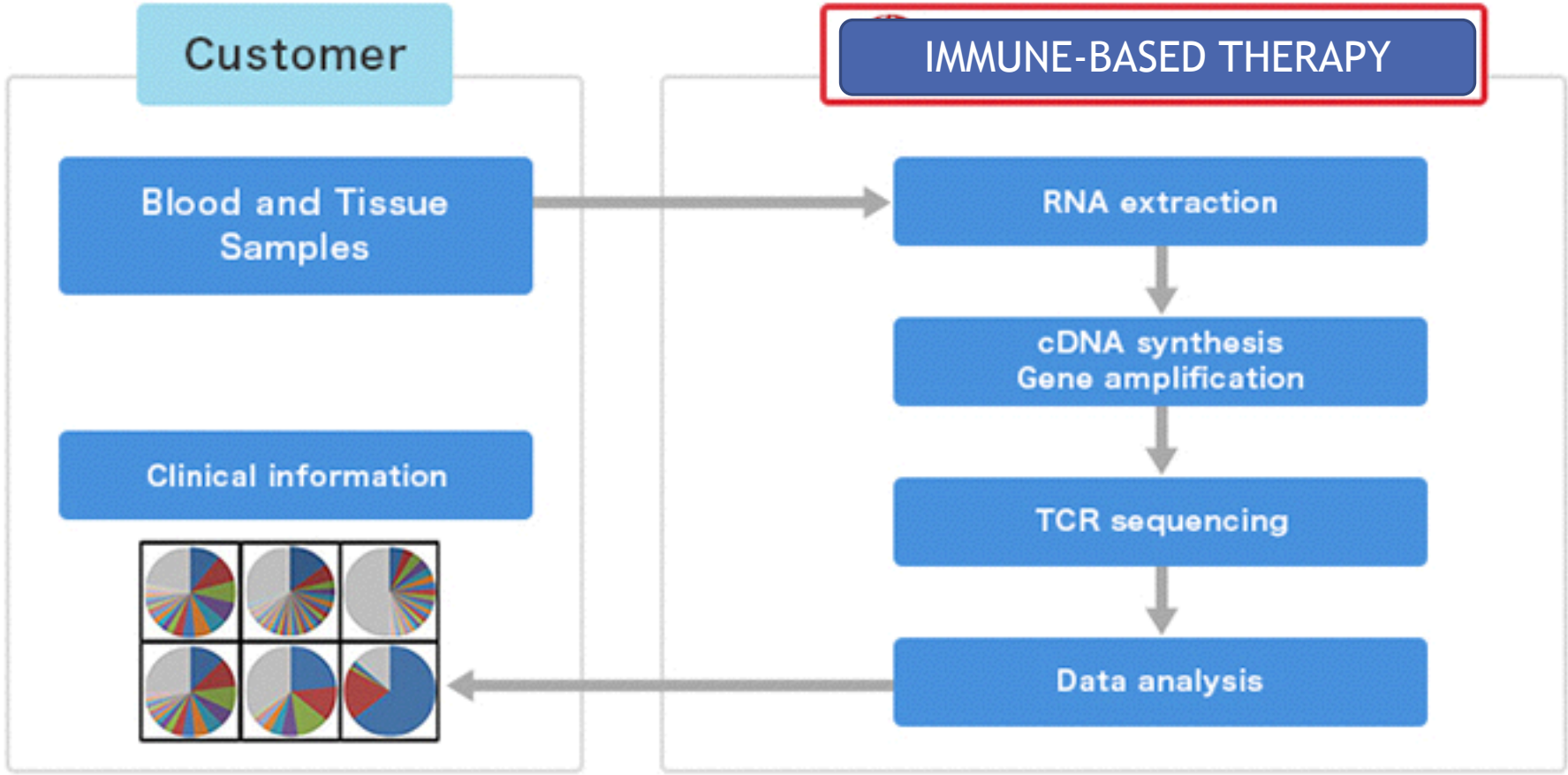
# BIOPSIA TEJIDO: INMUNOTERAPIA

PDL-1 : IHC



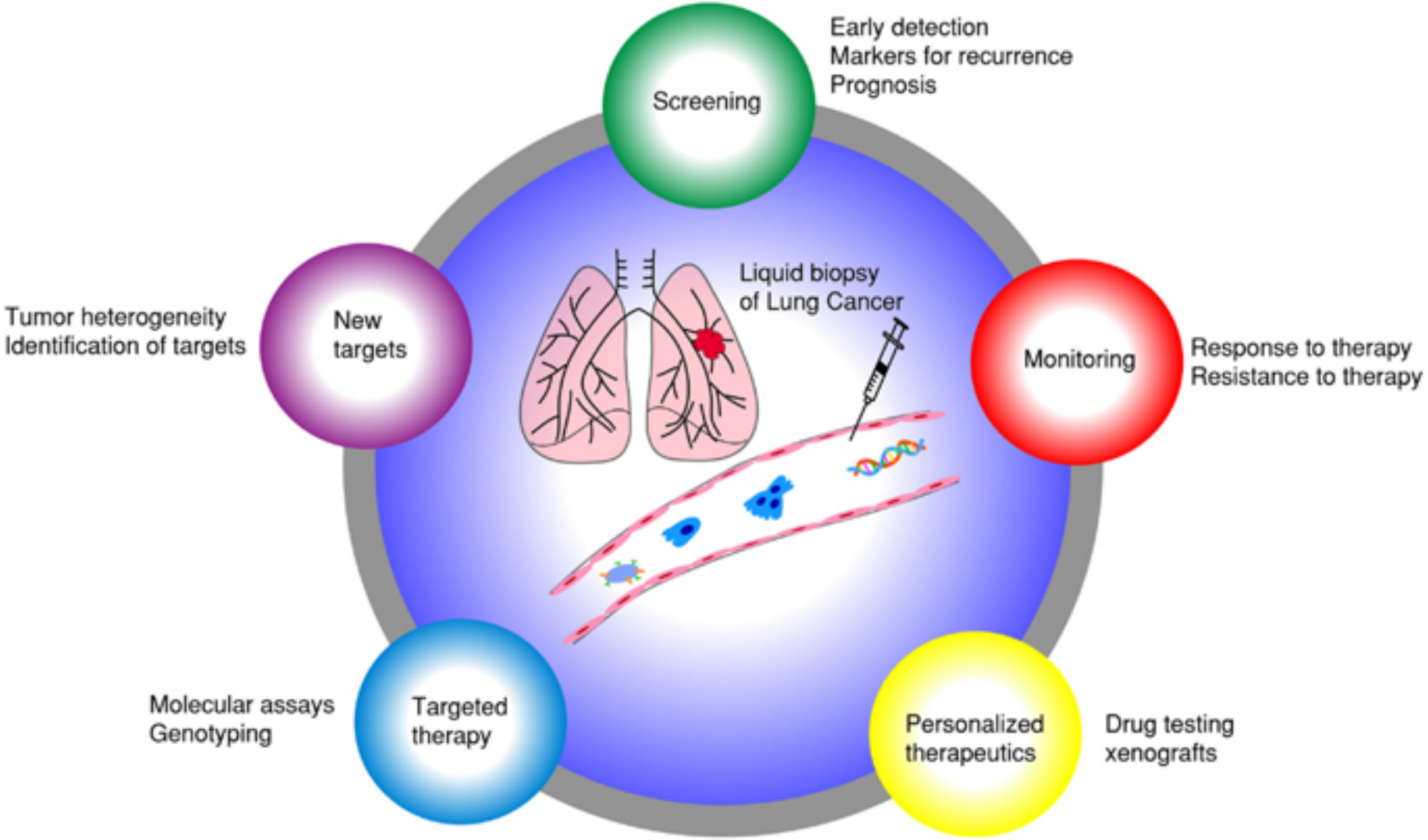


TCR - NGS : tissue/ blood

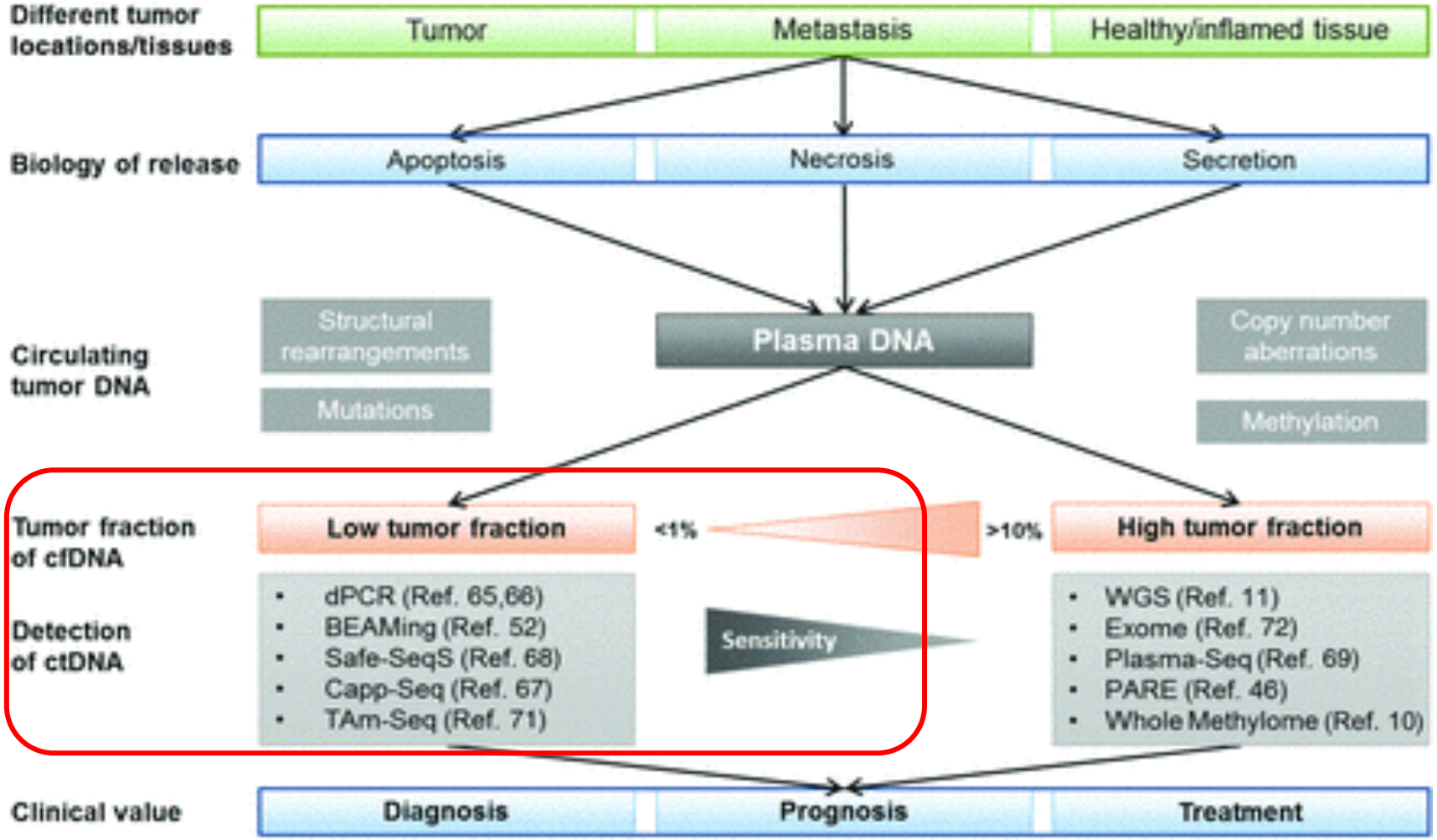



Usó et al, 2015 (Camps Lab)

# Biopsia líquida: qué información necesitamos??



# Biopsia líquida (cfDNA): que tipo de información me proporciona??




Taly et al., 2013, Vogelstein and Kinsler 1999, Diehl et al. 2008, Kinde et al. 2011, Newman et al. 2014, Forshew et al. 2012, Chan et al. 2013, Murtaza et al. 2013, Heitzer et al. 2013, Leary et al. 2012, Chan et al. 2013

# Metodología

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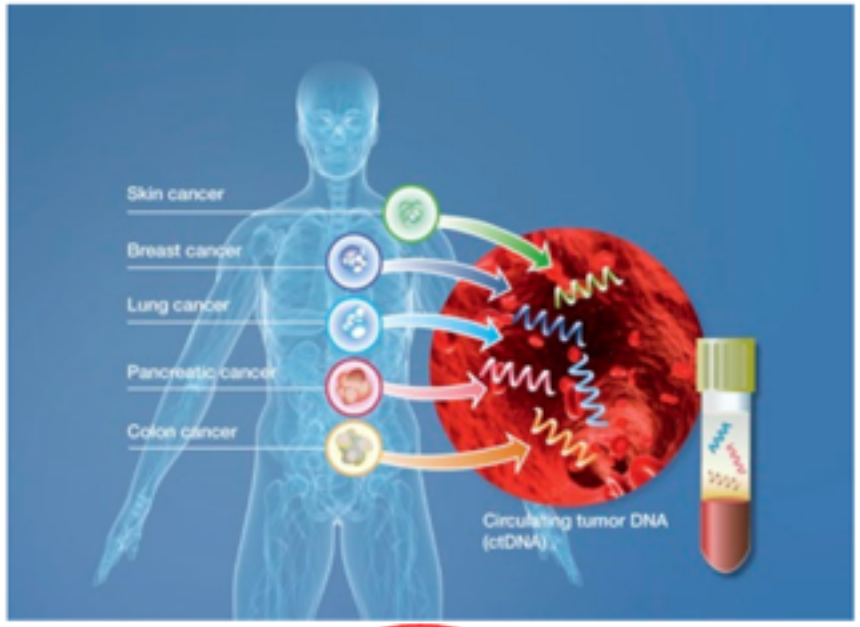
| BEAMing, PAP, Digital PCR, TAM-Seq | 0.01% or lower | ctDNA, rare variants in tumor tissue |

# Clinical Applications of ctDNA Testing




Screening & Diagnosis → Prognosis → Targeted Therapy → Response Prediction → Monitoring Resistance/ Recurrence

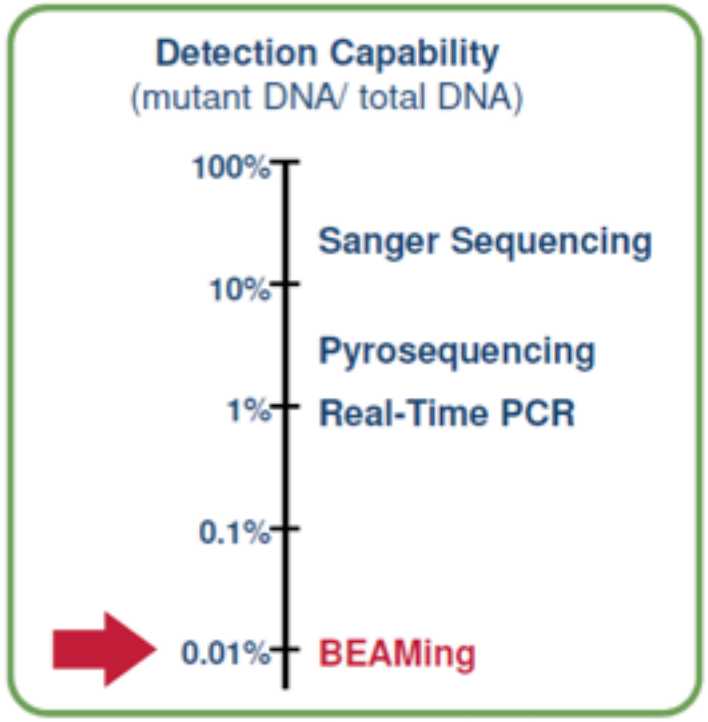
- ↑ Monitor tumour burden (surrogate marker)
- ↑ ↓ Detection of minimal residual disease,
- ↑ Mutational status
- ↑ Assessment of molecular heterogeneity
- ↑ Early detection of therapy resistance
- ↓ ↑ Early detection of disease.

| Technique | |
|---|---|
| PCR-based approaches | |
| Nested real-time PCR | |
| PARE | |
| → ARMS-Scorpion PCR | EGFR |
| PAP-A amplification | |
| → BEAMing | |
| Microfluidic digital PCR | |
| → Droplet-based digital PCR | |
| Targeted deep sequencing | |
| TAm-Seq | |
| Safe-SeqS | |
| CAPP-Seq | |
| Ion-AmpliSeq™ | |
| Whole exome sequencing | |
| Whole-genome sequencing | |


Ignatiadis M & Dawson SJ. Ann Oncol (2014)

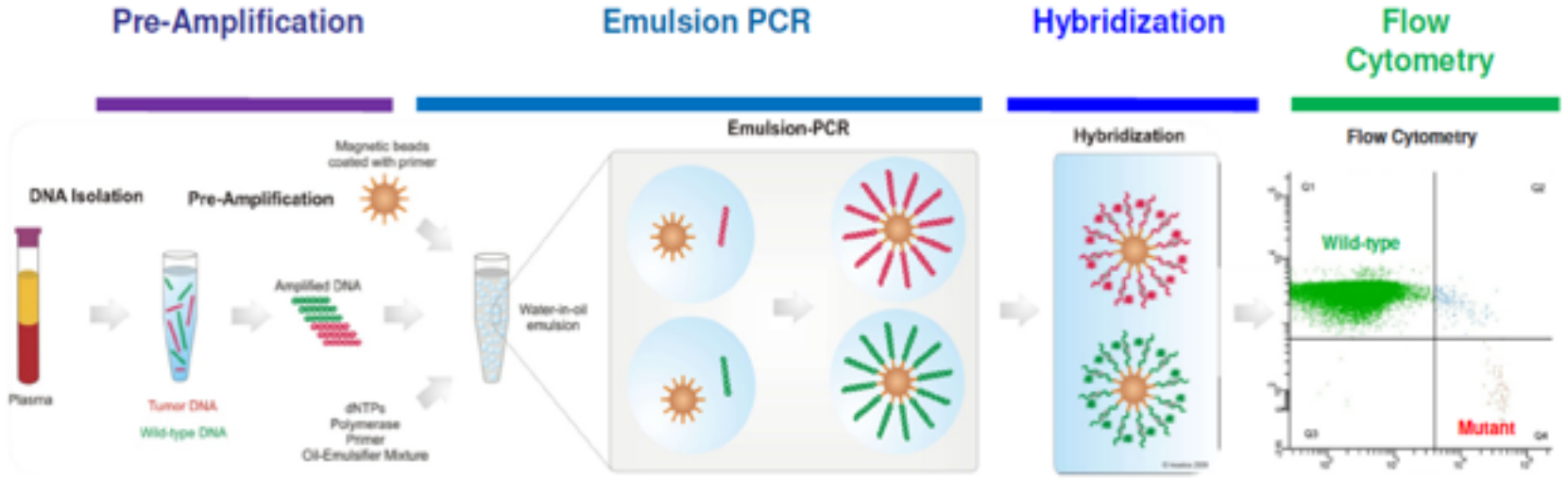
# BEAMing

Named after four of its principal components: **B**eads, **E**mulsion, **A**mplification, **M**agnetics




## BEAMing Digital PCR Technology

Highly sensitive quantitative digital PCR technology that employs bead-based amplification in water-in-oil emulsions and allele-specific hybridization followed by flow cytometry for detecting small amounts of mutated DNA released by tumors into the blood circulation




We must be SCRUPULOUS in the analytical phase to avoid erroneous results

Clinically irrelevant molecular changes due to the high sensitivity

Li et al, Nature Methods, 2006

# Biopsia líquida (cfDNA): que tipo de información me

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 201

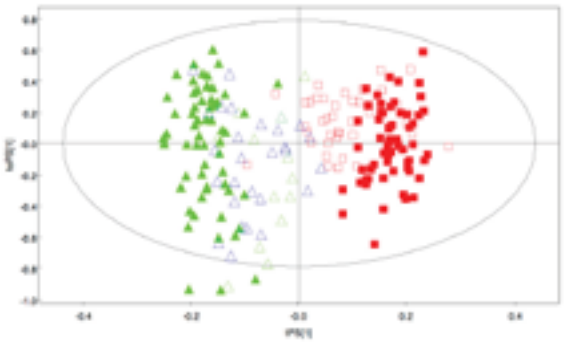
## Serum metabolomic profiling facilitates the non-invasive identification of metabolic biomarkers associated with the onset and progression of non-small cell lung cancer

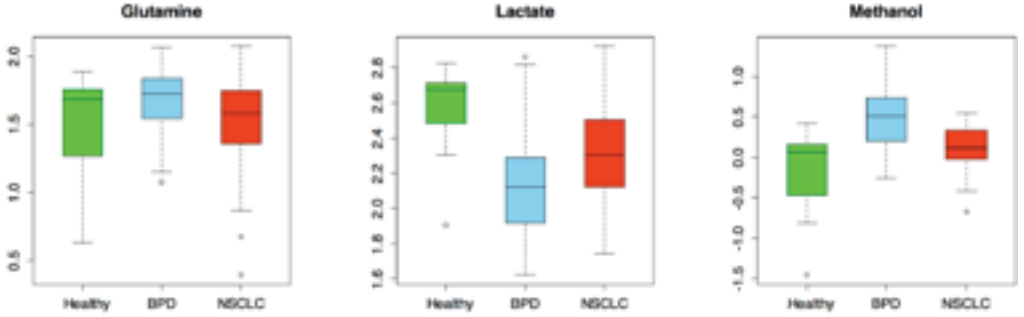
Leonor Puchades-Carrasco[1], Eloisa Jantus-Lewintre[2], Clara Pérez-Rambla[1,2,3], Francisco García-García[4], Rut Lucas[2], Silvia Calabuig[2], Ana Blasco[5], Joaquín Dopazo[4,6,7], Carlos Camps[2,5,8] and Antonio Pineda-Lucena[1,3]

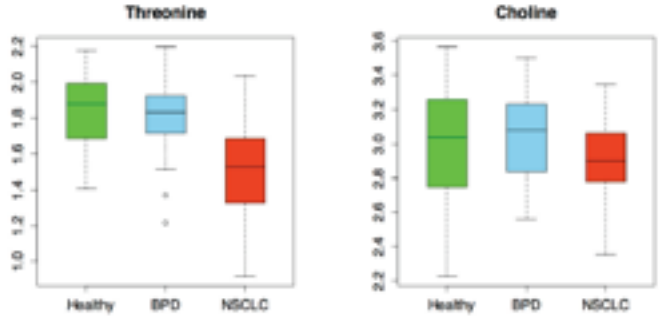
A

| Validation set | Predicted as NSCLC | Predicted as healthy | Correctly classified |
|---|---|---|---|
| NSCLC patients (40) | 38 | 2 | 95% |
| Healthy group (13) | 1 | 12 | 92.31% |
| BPD group (27) | 4 | 23 | |

B






| Validation set | Predicted as NSCLC | Predicted as healthy | Correctly classified |
|---|---|---|---|
| NSCLC patients (40) | **31** | 9 | 77.50% |
| Healthy group (13) | 3 | **10** | 76.90% |
| BPD group (27) | 9 | 18 | |

Puchades et al, Oncotarget 2016 (Camps Lab

# Determinaciones Moleculares en cáncer de pulmón

Quién?

# TECNOLOGIA


Cube 6i flow cytometer

illumina

ion torrent

Roche

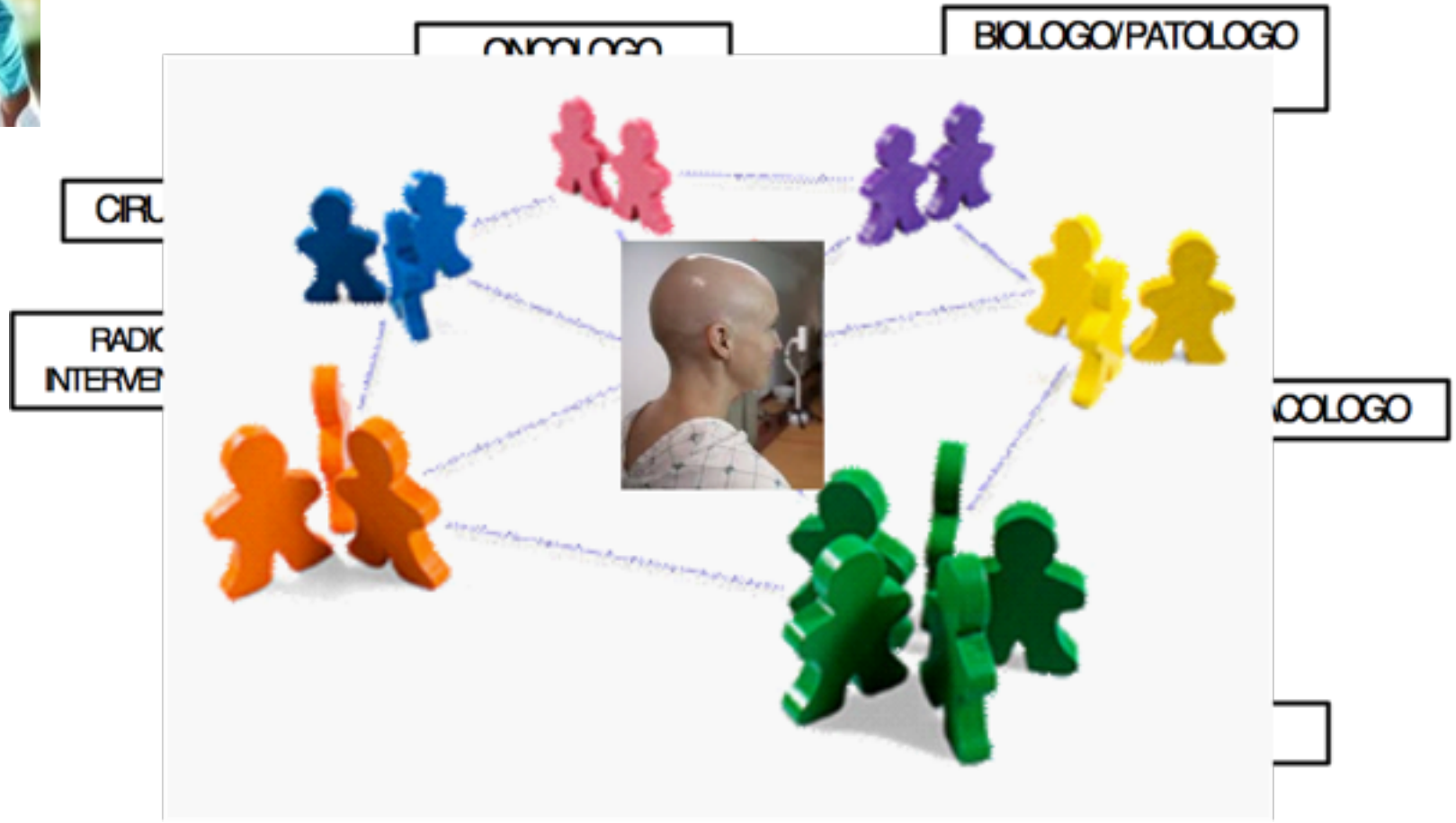
# EXPERTISE

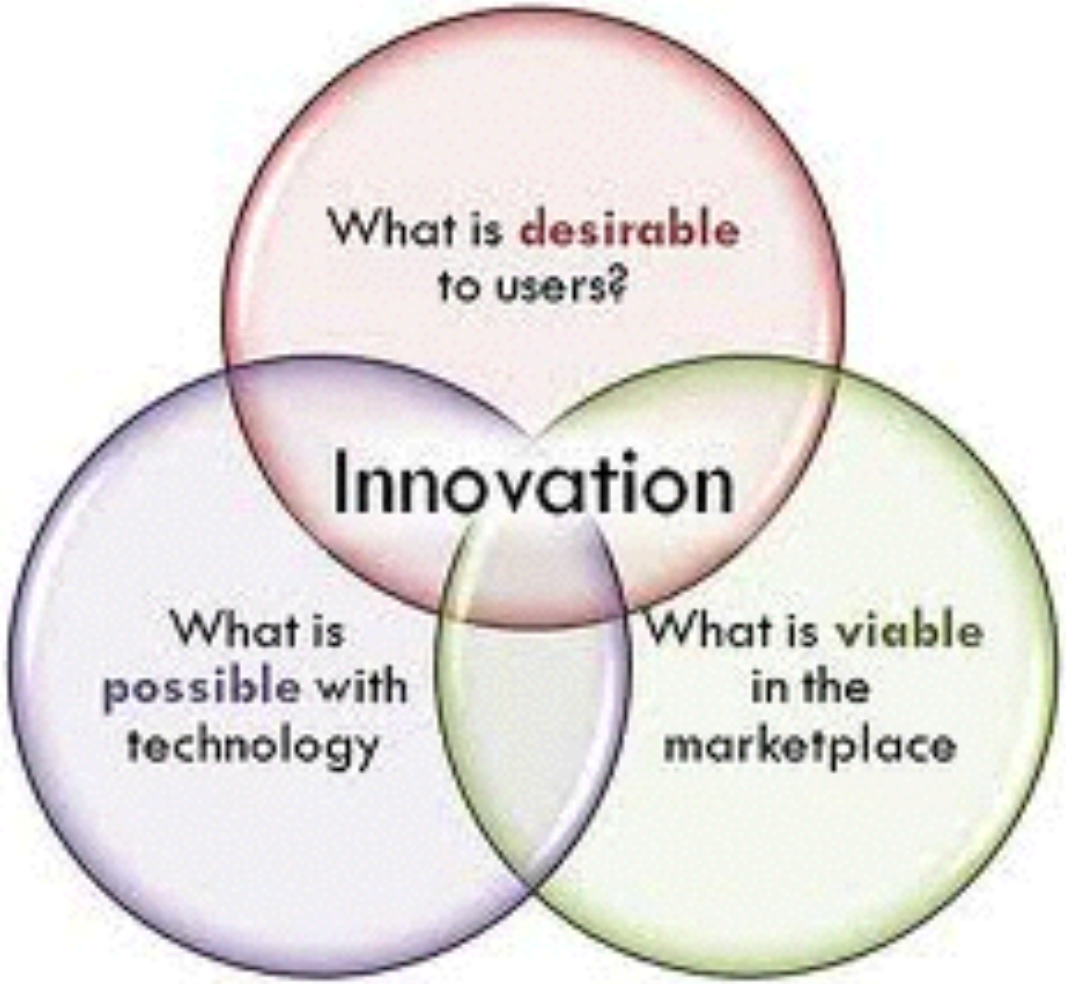

# CALIDAD



EMQN
The European Molecular Genetics Quality Network

# EQUIPOS MULTIDISCIPLINARES




What is desirable to users?
Innovation
What is possible with technology
What is viable in the marketplace

**LAB. ONCOLOGIA MOLECULAR**

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Sandra Gallach
Marta Usó  E. Sanmartin
Eva Escorihuela

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Alfonso Berrocal
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Ma. José Safont
Cristina Caballero
Ma. José Godes
Vega Iranzo

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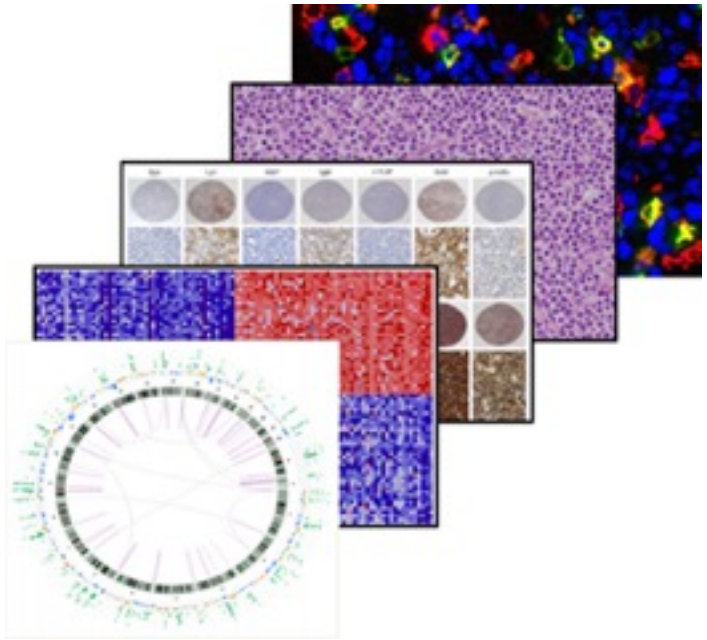
Antonio Arnau
Enrique  Pastor
Eva García del Olmo
Aminta Martinez

Antonio Pineda  Jerónimo Forteza
María Jesús Vicent  Rafael Sirera  Rosa Farràs  María Campos Segura  Rut Lucas


# Eloisa Jantus Lewintre

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