IX SIMPOSIO · SYMPOSIUM | 2024
BIOPSIA LÍQUIDA · LIQUID BIOPSY

EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE

25, 26 Y 27 DE ENERO · JANUARY 25th, 26th and 27th

INNOVATIVE STRATEGIES FOR ENHANCING DETECTION SENSITIVITY OF CIRCULATING TUMOUR COMPONENTS IN EARLY BREAST CANCER

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#SimposioBiopsiaLiquida

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Universidad Francisco de Vitoria
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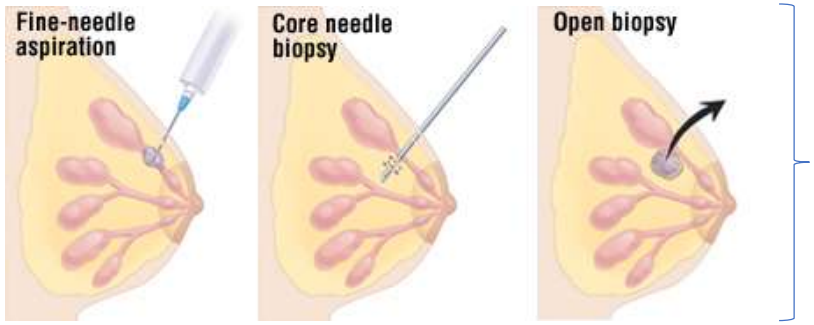
LIQUID BIOPSY AND BREAST CANCER

INTRODUCTION: BREAST CANCER

→ BREAST CANCER

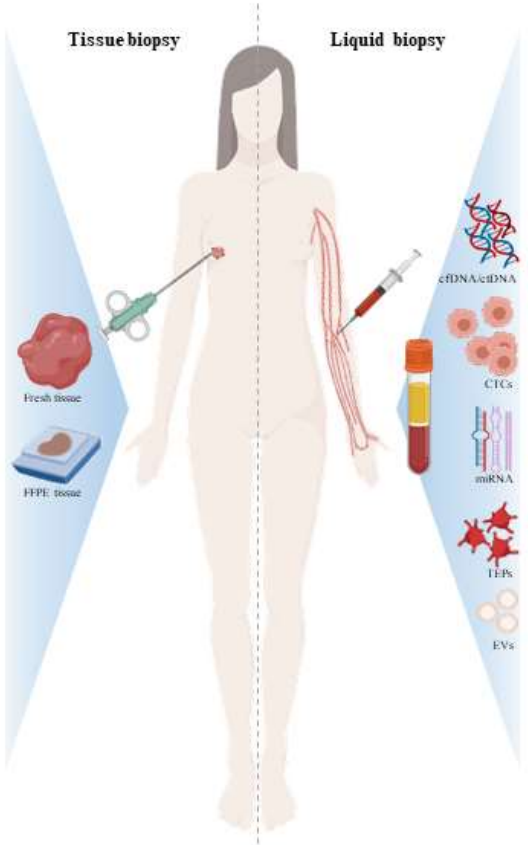
- 95% diagnosed at early stages.
- 28-65% mortality reduction by screening programs (mamography)
- 20% metastatic incurable disease.

→ TISSUE BIOPSIES



OncotypeDX
PAM50-ROR

Chemo + hormono
Tumour subtype
Risk-Of-Relapse



- Minimal Invasive
- Shorter time procedure
- Lower cost for sample obtention
- More complete tumour molecular information
- Compatible with tumour monitoring during surveillance/treatment

Alba-Bernal A. Ebiomedicine 2020

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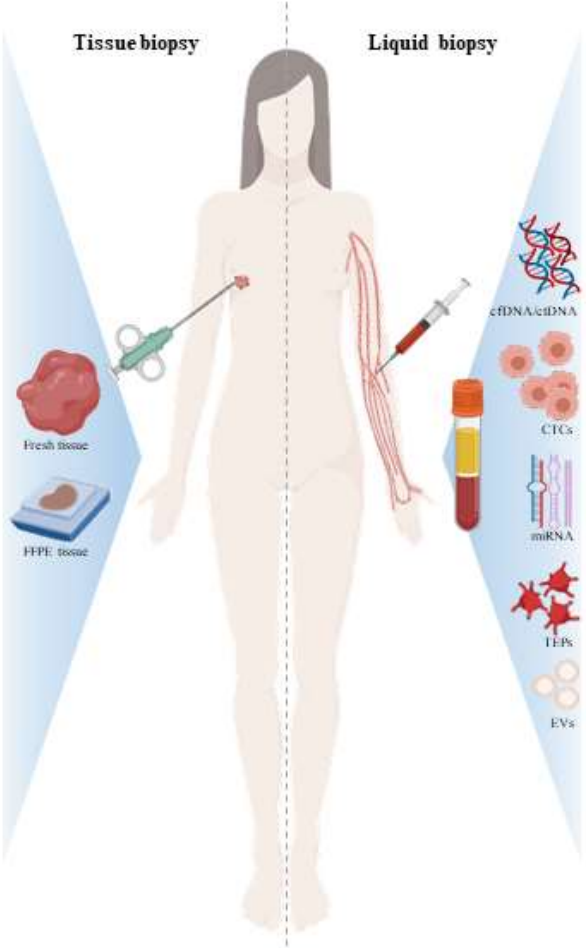
INTRODUCTION: LIQUID BIOPSY



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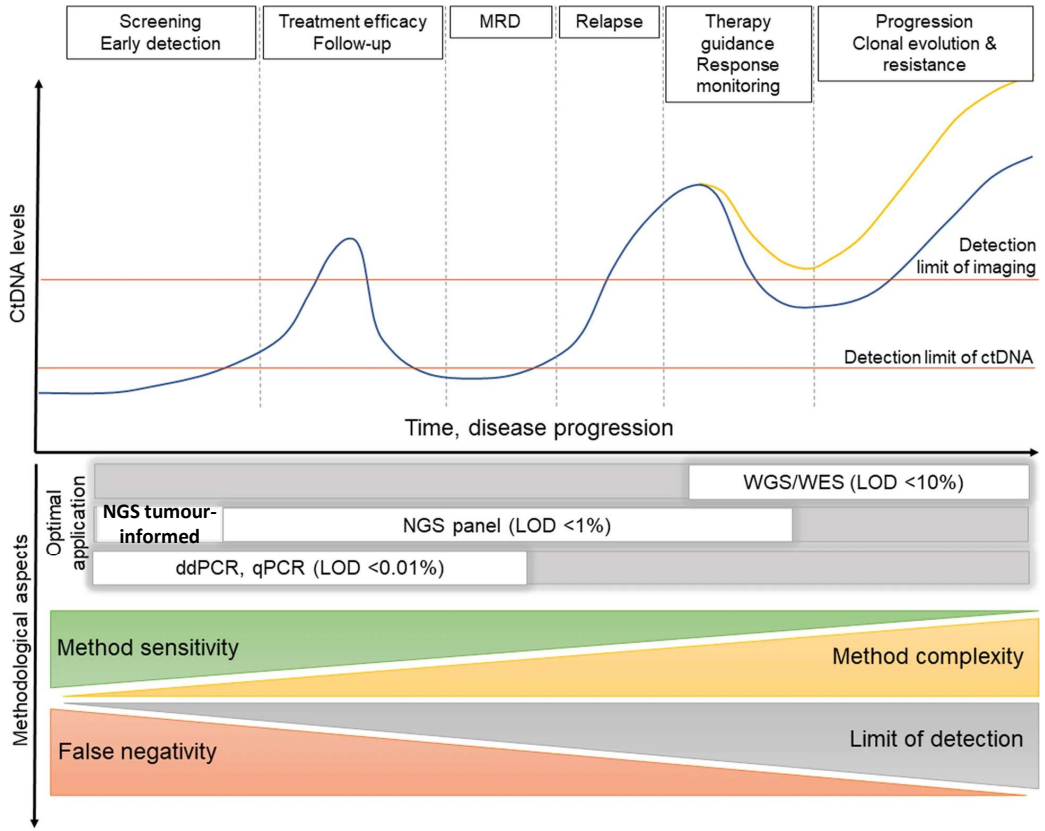
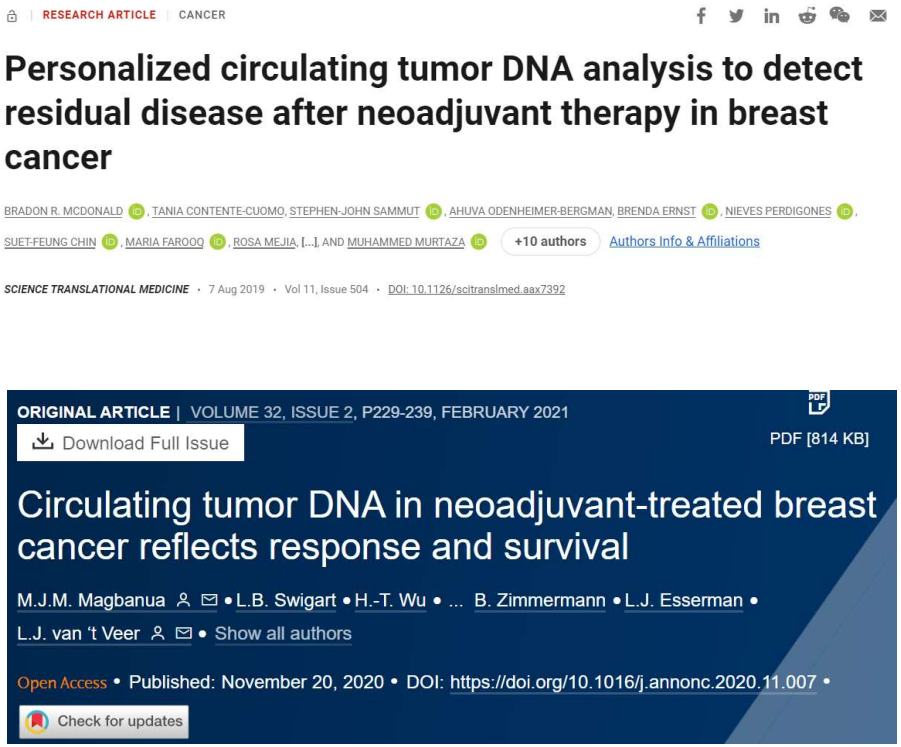
Alba-Bernal A. Ebiomedicine 2020



Stejskal P. et al. Molecular Cancer 2023

LIQUID BIOPSY AND BREAST CANCER: DIFFERENT APPROACHES

DIFFERENT NOVEL STRATEGIES



RESEARCH ARTICLE | CANCER

Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer

BRADON R. MCDONALD, TANIA CONTENTE-CUOMO, STEPHEN-JOHN SAMMUT, AHUVA ODENHEIMER-BERGMAN, BRENDA ERNST, NIEVES PERDIGONES, SUET-FEUNG CHIN, MARIA FAROOQ, ROSA MEJIA, [...], AND MUHAMMED MURTAZA +10 authors Authors Info & Affiliations

SCIENCE TRANSLATIONAL MEDICINE · 7 Aug 2019 · Vol 11, Issue 504 · DOI: 10.1126/scitranslmed.aax7392

ORIGINAL ARTICLE | VOLUME 32, ISSUE 2, P229-239, FEBRUARY 2021

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PDF [814 KB]

Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival

M.J.M. Magbanua • L.B. Swigart • H.-T. Wu • ... B. Zimmermann • L.J. Esserman • L.J. van 't Veer • Show all authors

Open Access • Published: November 20, 2020 • DOI: https://doi.org/10.1016/j.annonc.2020.11.007 •

Check for updates

- **NGS ULTRA-DEEP SEQUENCING: DESIGN AND OPTIMISATION OF A SEQUENCING PANEL FOCUSED ON BREAST CANCER SCREENING**

→ **Fixed panel + Ultradeep sequencing**

- **BLOOD VOLUME INCREASE: DEVELOPMENT OF A NOVEL METHODOLOGY FOR THE DETECTION OF CIRCULATING TUMOUR COMPONENTS (ctDNA and CTCs) IN BREAST CANCER**

→ **Elevated blood volumes + easy-to-implement methodology (ddPCR)**

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

OBJECTIVES



Alfonso Alba Bernal Maria Elena Quirós Esperanza López

Primary Objectives:

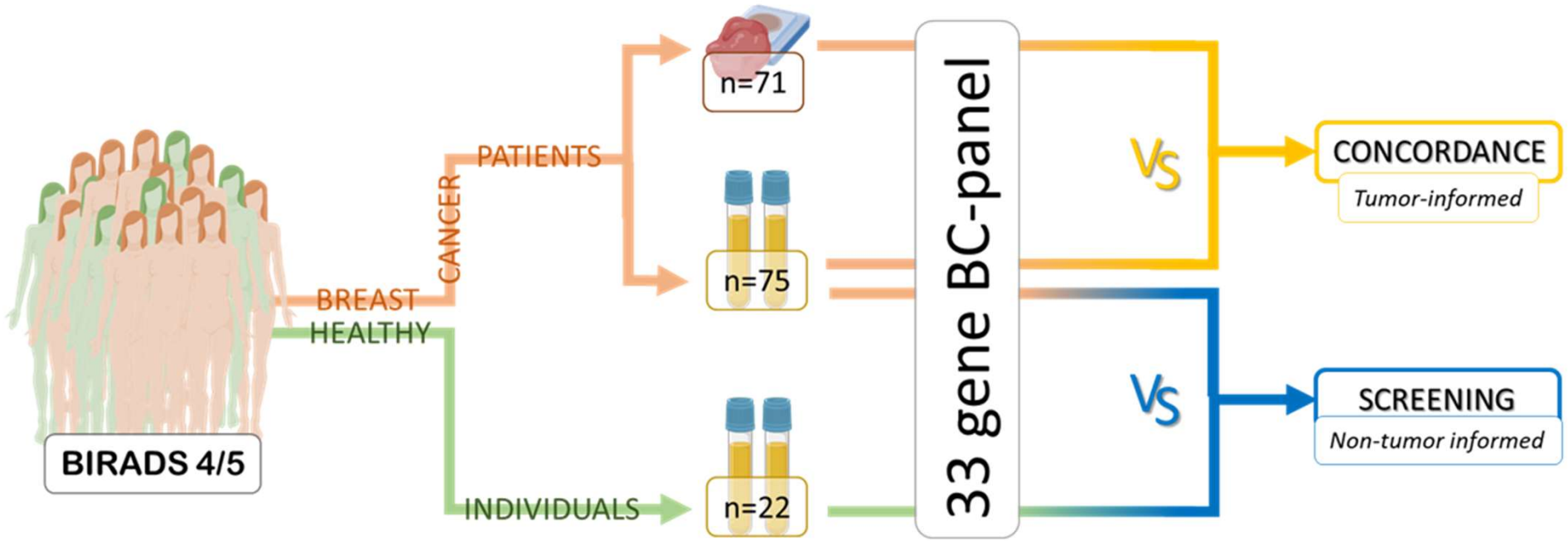
- To detect and characterize ctDNA in patients with BIRADS 4c and 5 mammographic lesions before any invasive diagnostic or therapeutic procedure.
- To compare the mutational profiles identified in plasma with those observed in solid biopsy samples.
- To develop a liquid biopsy methodology, independent of information from solid biopsy, for identifying breast cancer in women at high risk.

Secondary Objectives:

- To establish a correlation between plasma sequencing and the clinicopathological characteristics of patients.

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

STUDY DESIGN



Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

METHODOLOGY

- Simultaneous extraction of plasma and fresh-frozen tumour biopsy guided by ultrasound.

- An in-house designed panel was employed for the extraction, covering the following genes: *AKT1, ARID1A, ATM, BAP1, BRAF, BRCA1, BRCA2, CBFB, CDH1, CDKN1B, CTCF, ERBB2, ESR1, GATA3, HRAS, KDM6A, KRAS, MAP2K4, MAP3K1, MEN1, NCOR1, NF1, PBRM1, PIK3CA, PIK3R1, PTEN, RB1, RUNX1, SF3B1, SMAD4, TBX3, TP53, USP9X - Patented*

- SureSelectXT-HS (Agilent) was employed for library generation, incorporating unique molecular identifiers (UMIs).

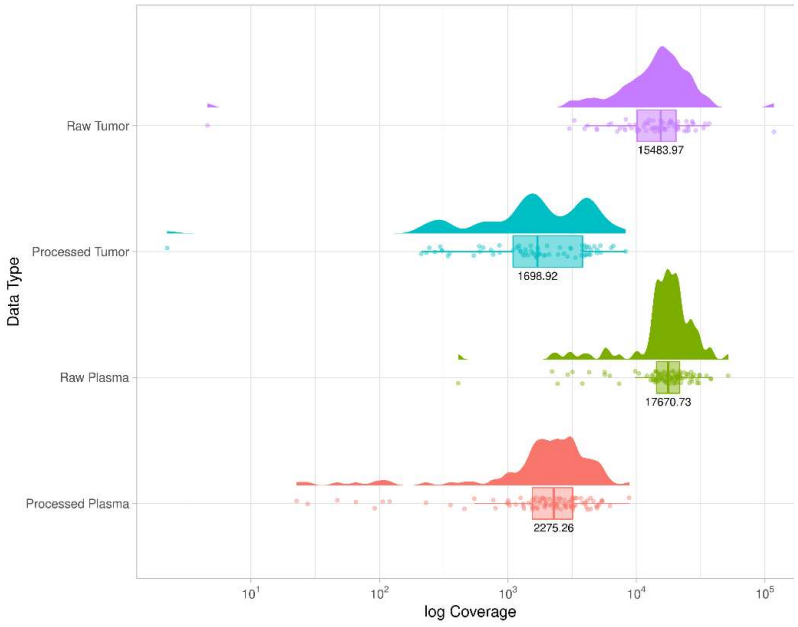
- High-depth sequencing was performed with a target of approximately 20,000X.

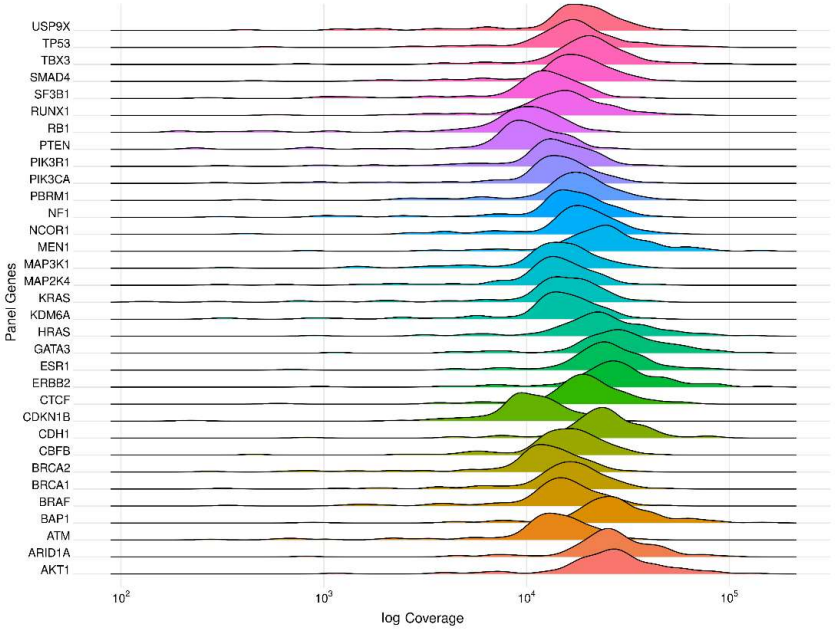
Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

RESULTS

- A remarkably high sequencing depth was achieved post-deduplication and UMI processing: tissue ~1600X and plasma ~2200X.
- The coverage demonstrated uniformity across all studied genes.





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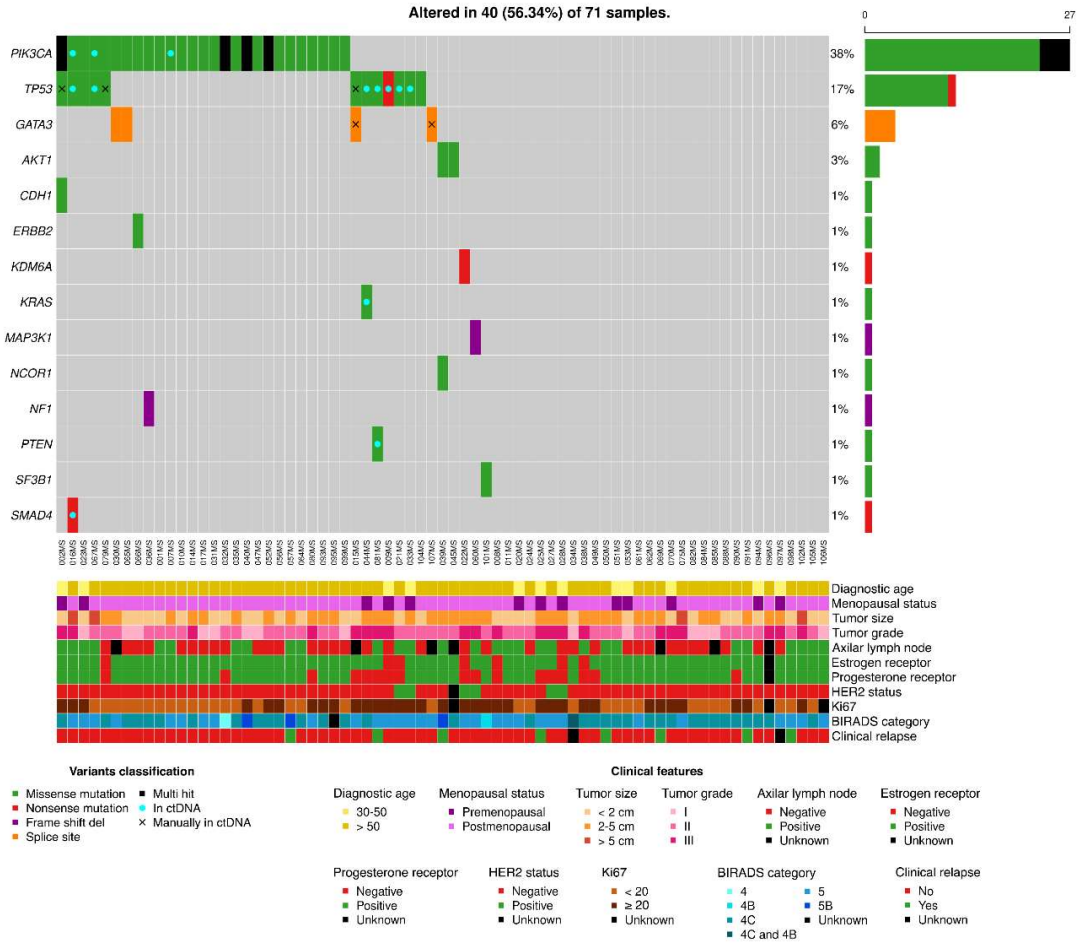
RESULTS

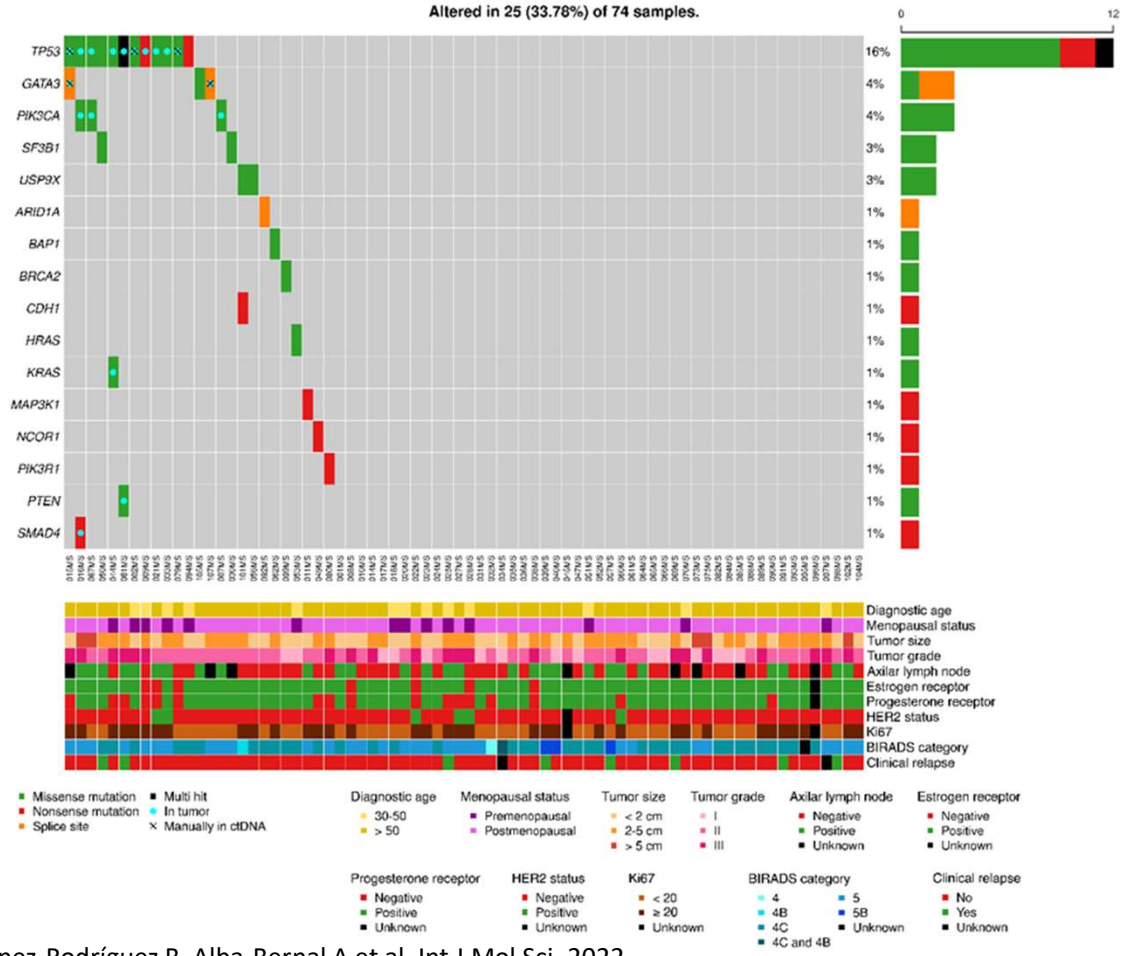


- A total of 71 tissue samples were sequenced.
- Sixty-one distinct variants were identified in the tissue analysis.
- Mutations were observed in 40 out of 71 (56.33%) individuals.
- The most frequently mutated genes in the tumor were *PIK3CA*, *TP53*, and *GATA3*.

Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

RESULTS

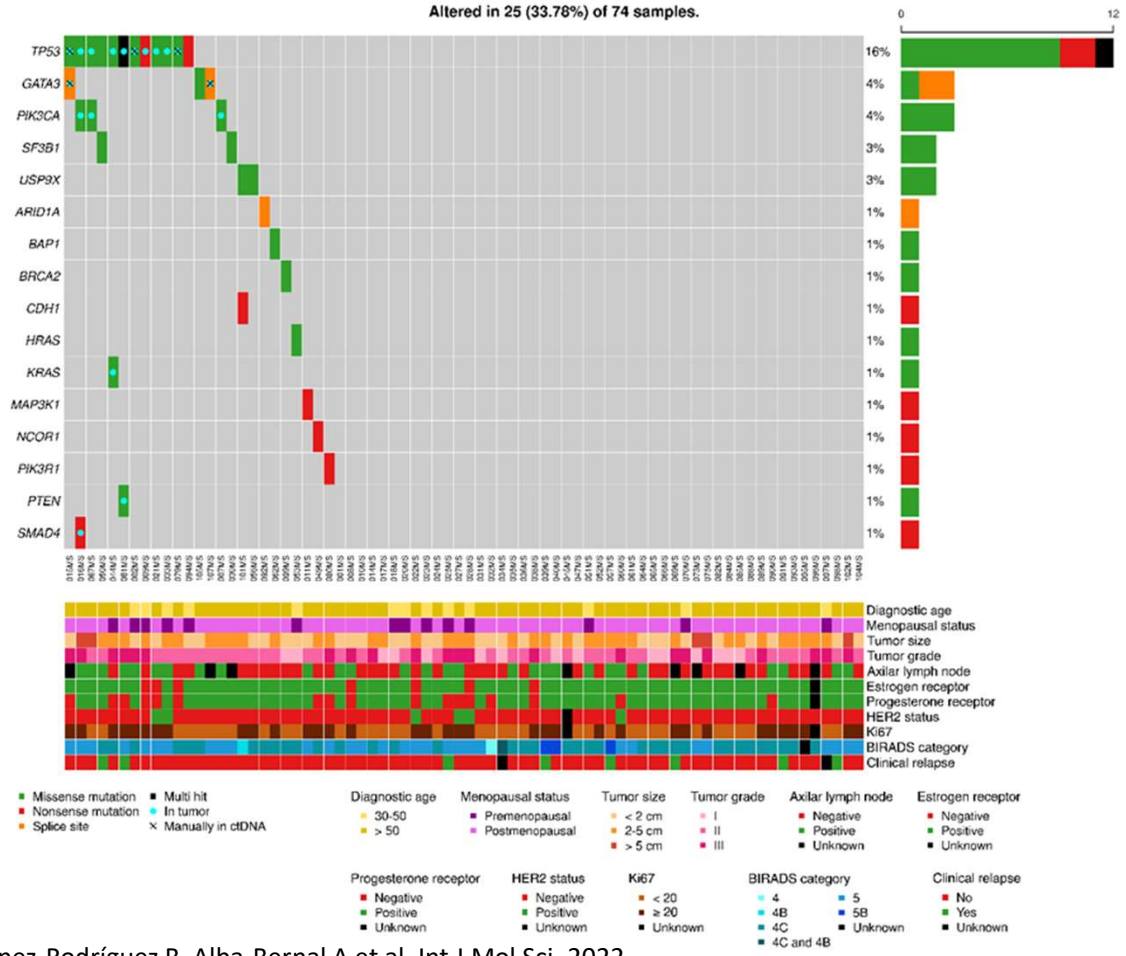


- Sequencing was conducted on 75 plasma samples.
- One sample resulted in sequencing failure.
- Both automated and manual variant detection methods were employed.
- The most mutated genes in plasma were *TP53*, *GATA3*, and *PIK3CA*.
- Mutations were observed in 25 out of 74 (33.78%) individuals.
- Sixteen mutations were not found in the pre-treatment biopsy BAG analysis.
- **Eight patients** exhibited mutations detected in plasma but not in their corresponding tumour.
- Eighteen out of 61 variants detected in the tumour were also identified in plasma, accounting for **29.50%**.

Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

RESULTS



- Bioinformatic workflow for detecting mutations in plasma based on their presence in both COSMIC and TCGA.
- 25/74 (33.78%) individuals presented mutations in plasma.
- As a control group, 22 healthy individuals were sequenced.
- **3 mutations** were found in 3 plasma samples out of 22 sequenced healthy individuals (13.63%).
- **Sensitivity: 31.08%, Specificity: 86.36%, PPV: 88.46%**
- Significant associations:
  - Elevated median VAF - tumour grade
  - More than 1 mutation in plasma – relapse
  - TP53 mutations - ER+

Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: NGS ULTRA-DEEP SEQUENCING

CONCLUSIONS

- We detected ctDNA at a lower allelic frequency than reported with similar technologies—> >0.075%.

- The concordance between mutations found in plasma and tumour was comparable to previous publications in early breast cancer, despite our population being studied in the initial diagnostic manoeuvre.

- We observed high specificity and a high positive predictive value for breast cancer detection without tumour genetic information. While sensitivity was low, it surpassed previous studies.

Jiménez-Rodríguez B, Alba-Bernal A et al. Int J Mol Sci. 2022

LIQUID BIOPSY AND BREAST CANCER: INCREASED BLOOD VOLUMES

OBJECTIVES



Alfonso Alba Bernal Maria Elena Quirós Esperanza López

Primary Objectives:

- Develop an ultrasensitive, novel, and easily implementable methodology for the dual detection of CTCs and ctDNA.
- Assess the response to NAC before surgery by detecting ctDNA and CTCs.
- Detect post-surgery MRD and monitor disease response during adjuvant chemotherapy.

Secondary Objective:

- Establish a predictive model using the detection of ctDNA and/or CTCs to anticipate the response to NAC before surgery.

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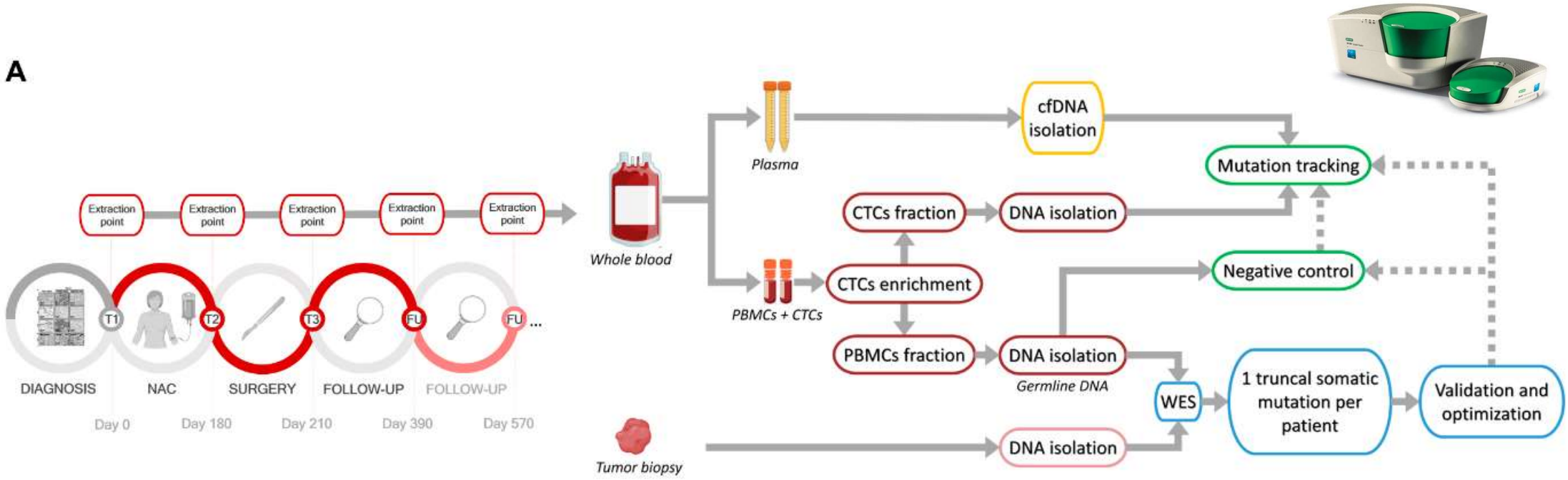
METHODOLOGY



21 EARLY BREAST CANCER PATIENTS – NAC TREATED

FOLLOW-UP FOR PATIENTS:

- **GRADE 3**
- **AFFECTED AXILLARY LYMPH NODES**
- **HER2-POSITIVE/TNBC TUMOURS**

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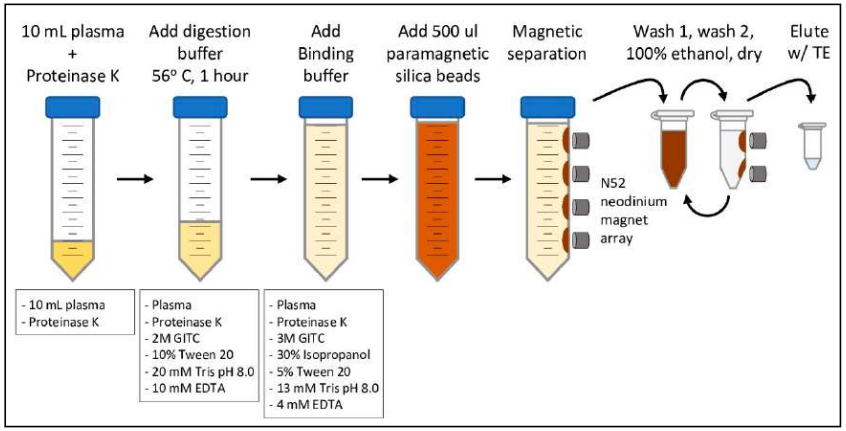
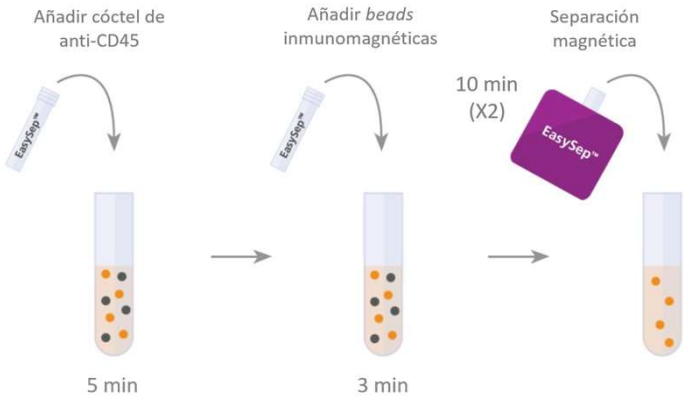
LIQUID BIOPSY AND BREAST CANCER: INCREASED BLOOD VOLUMES

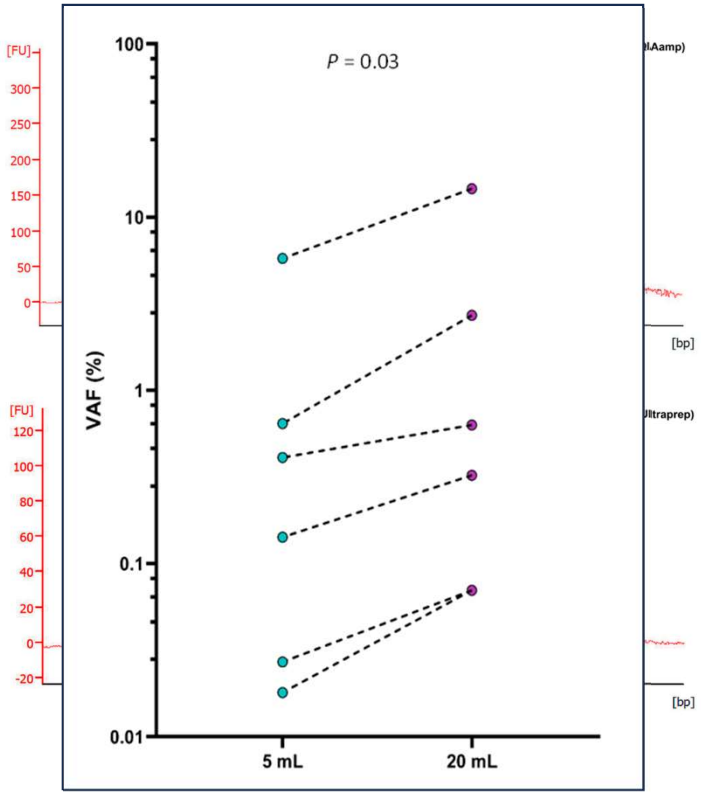
METHODOLOGY

ctDNA



CTCs





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Raymond CK, Raymond FC, Hill K. PLoS One. 2020

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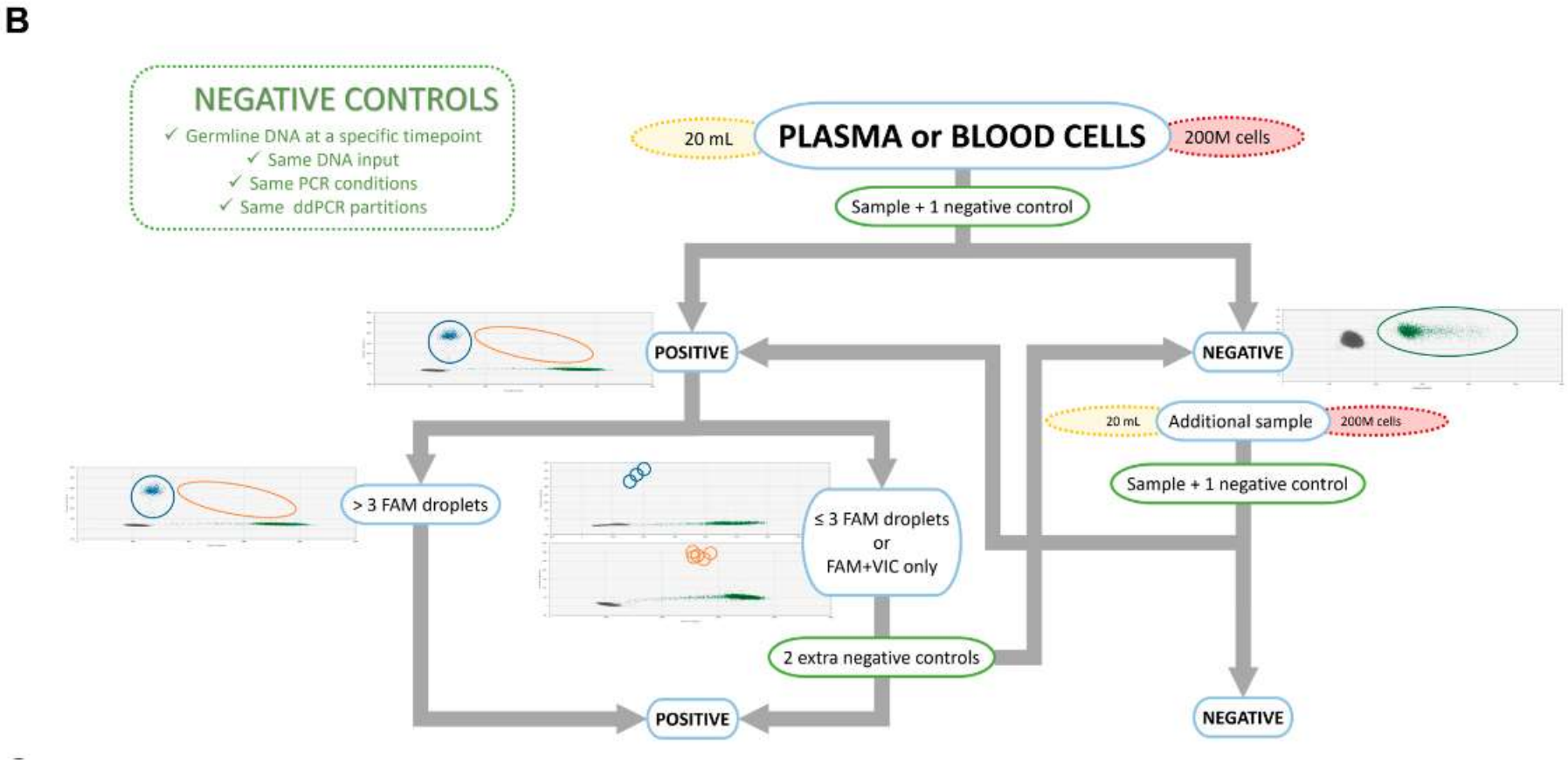
METHODOLOGY



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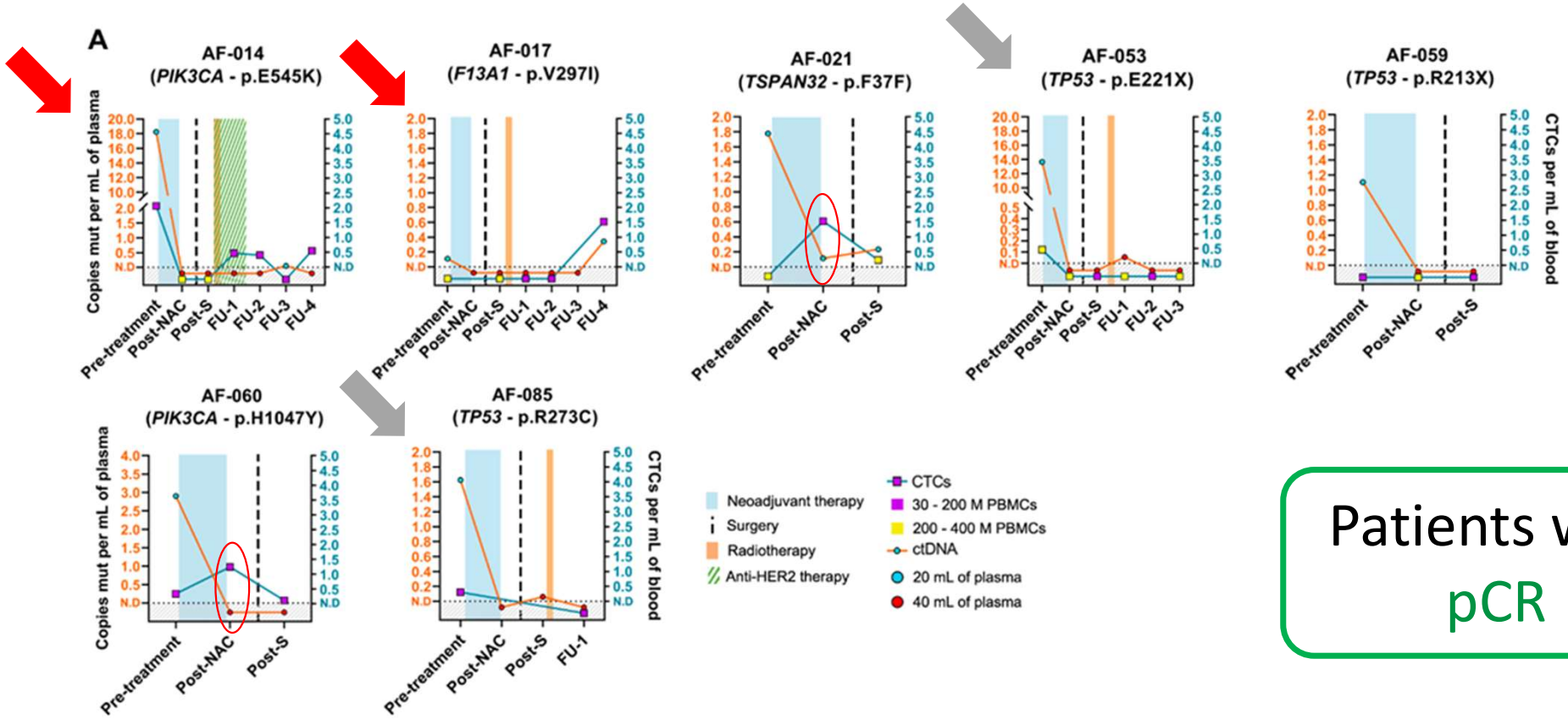
RESULTS



Patients with pCR

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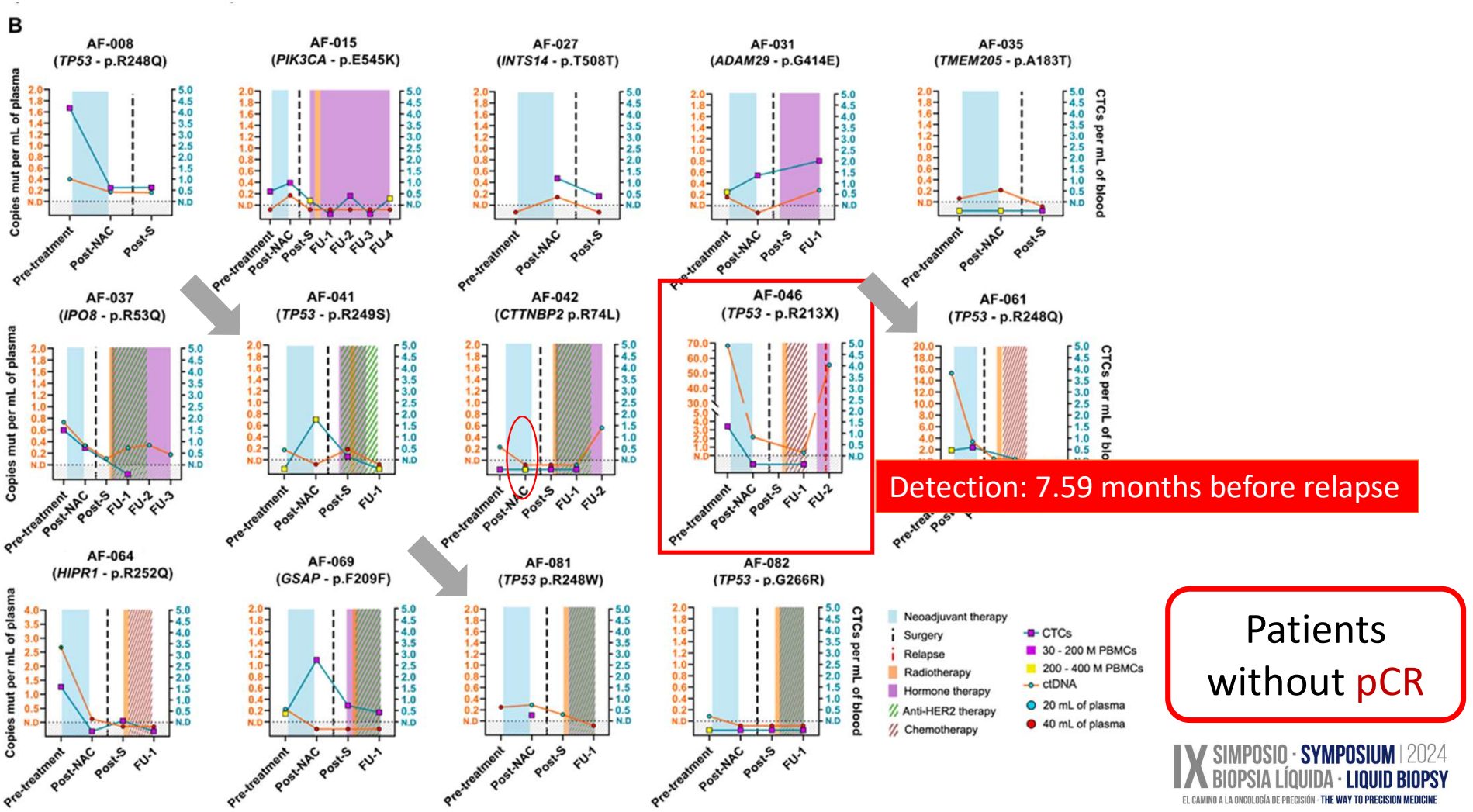
RESULT



Detection: 7.59 months before relapse

Patients without pCR

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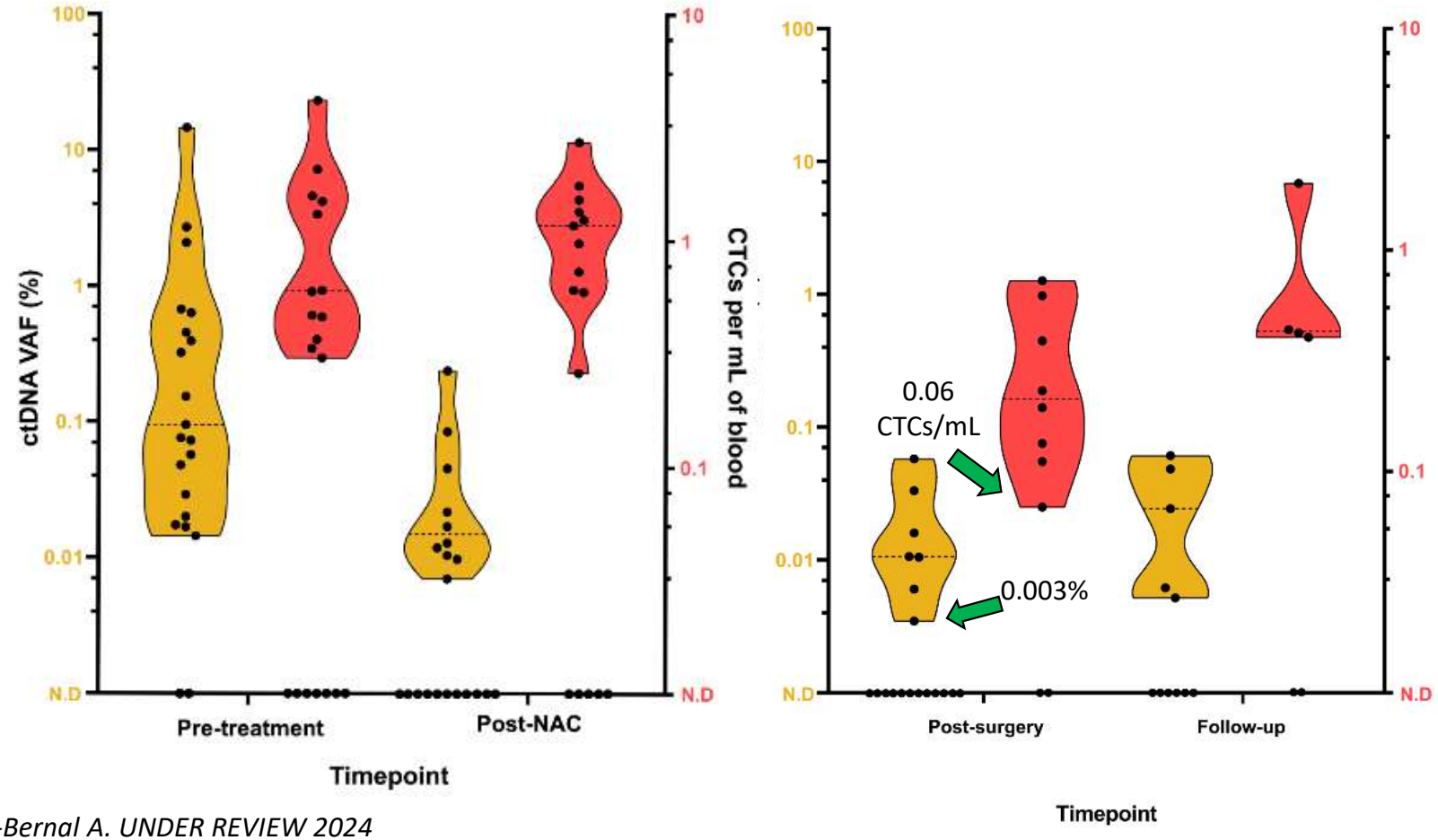
RESULTS



	ctDNA	CTCs	ctDNA and/or CTCs
Pre-treatment	90%	63%	**100%**
Post-NAC	47%	68%	66%
Post-surgery	36%	80%	63%

→ **Positivity of circulating tumour components at each timepoint.**

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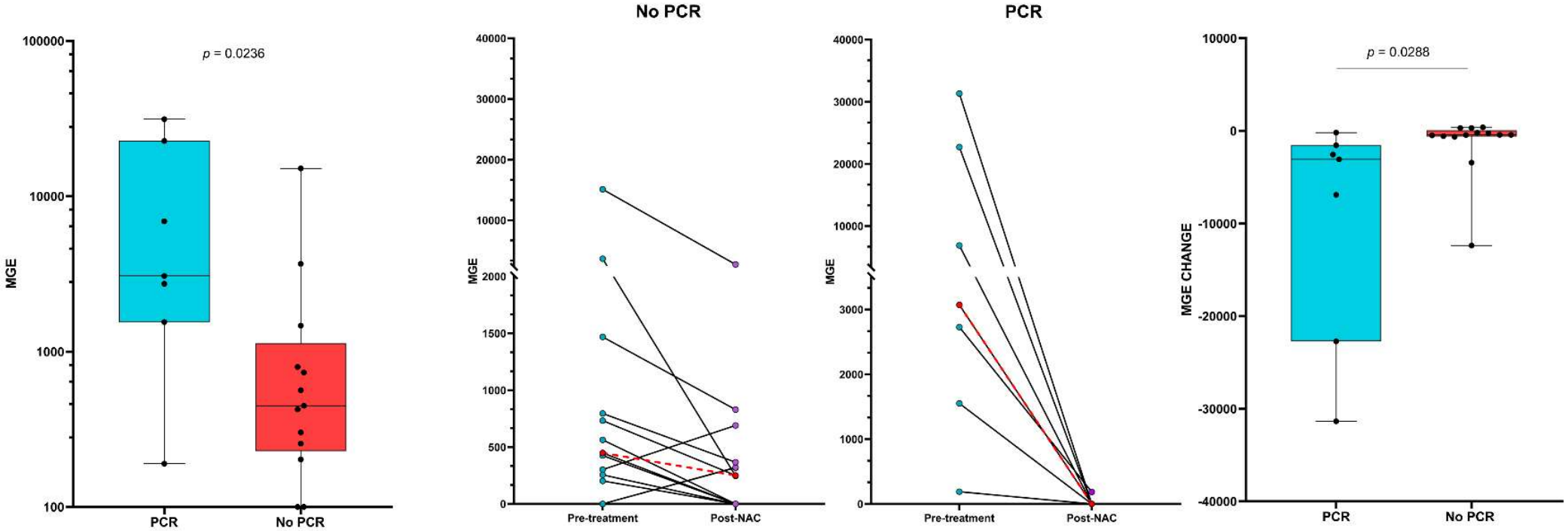
RESULTS



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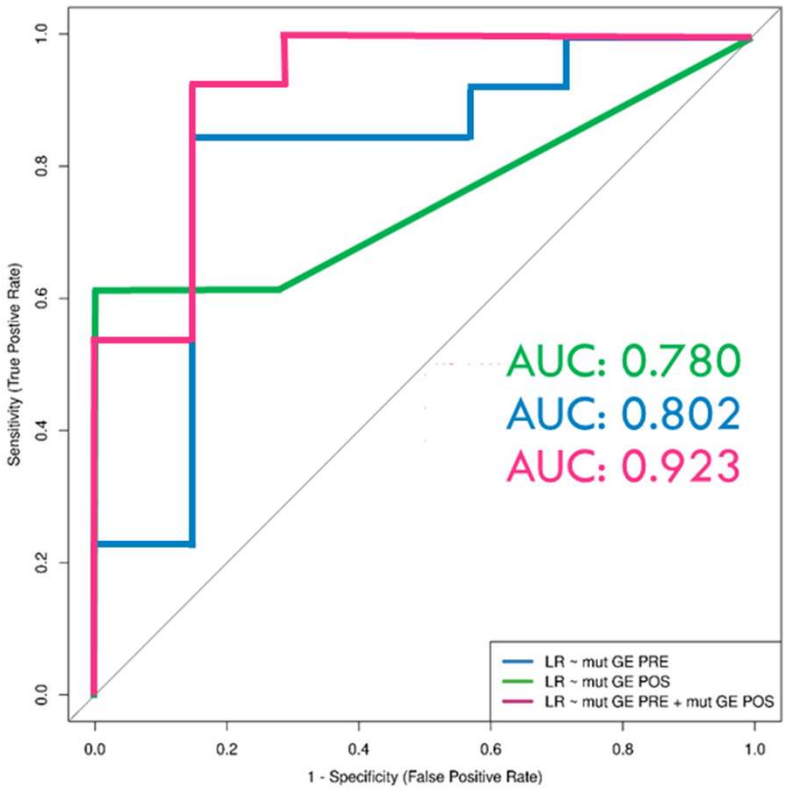

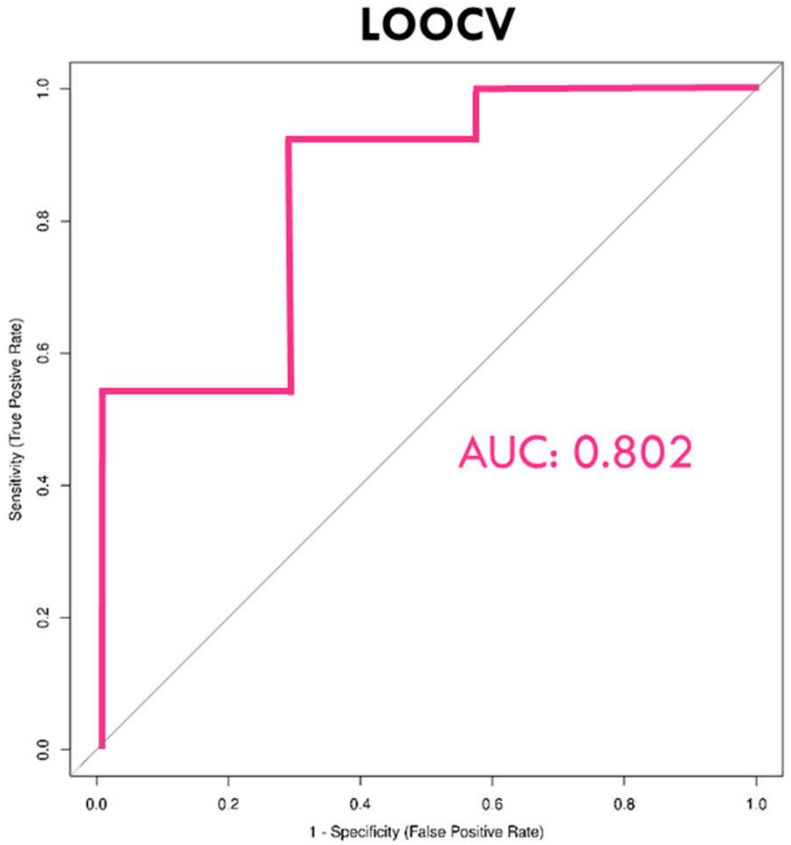

→ Prediction model development for clinical PCR. ROC curves using MGE from pre, post-treatment and both componentes (left). Curve obtained after cross-validation (right).

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RESULTS

BLOOD VOLUME REQUIRED:

- **BASELINE (PRE-TREATMENT) → 70 mL**
- **POST-NAC → 70 mL**
- **POST-SURGERY AND FOLLOW-UP SAMPLES → 95 mL**

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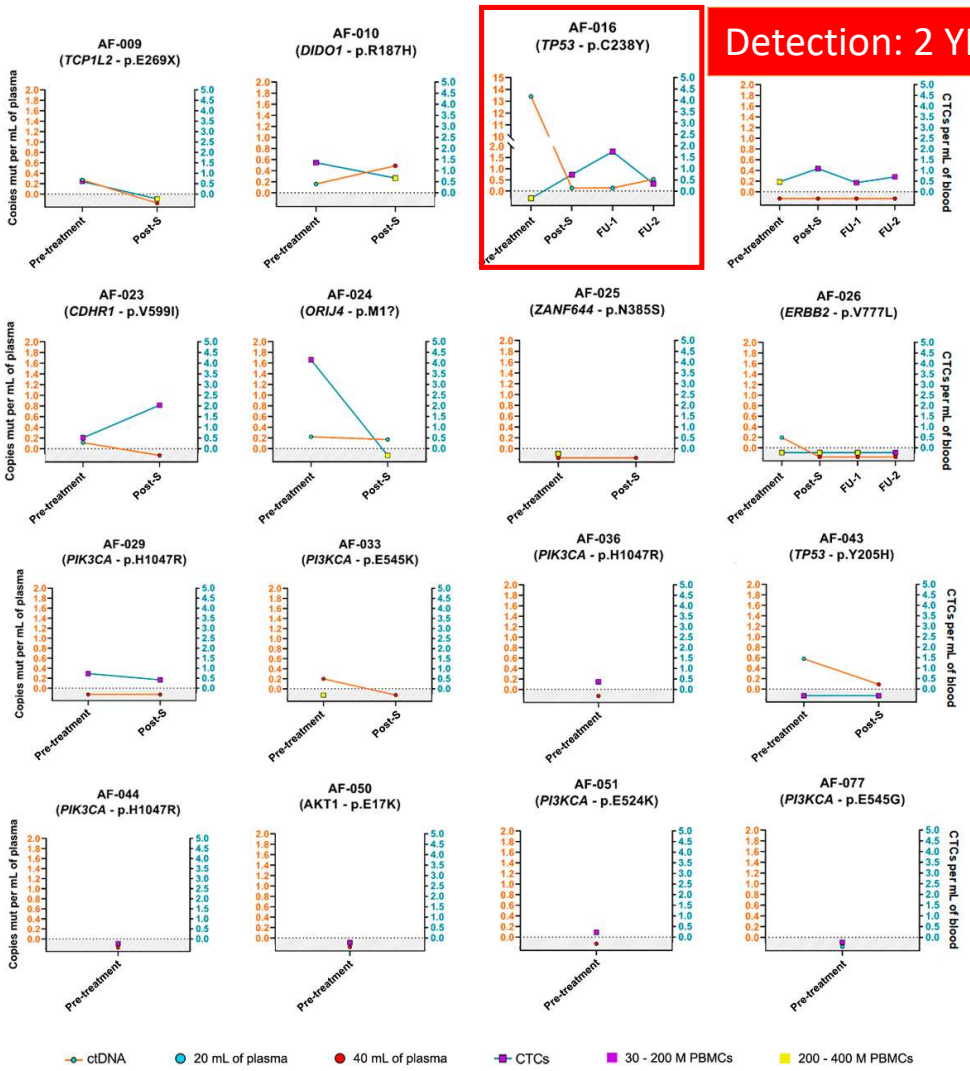
RESULTS



Detection: 2 YEARS before relapse

LIQUID BIOPSY AND BREAST CANCER: INCREASED BLOOD VOLUMES

CONCLUSIONS

- A new methodology easily implementable in clinical practice (1 mutation and ddPCR) allows for dual monitoring with ctDNA and CTCs.
- Detection was achieved in 100% of pre-treatment samples.
- Prediction of neoadjuvant therapy response: A predictive model using ctDNA with ROC values of 0.92.
- Minimal residual disease (MRD) detection in the first post-surgery sample of the only patient who has relapsed to date.
- Sensitivity of 0.003% for ctDNA detection and 0.069 CTCs/mL.

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THANK YOU!

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