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

GENOMIC CHARACTERIZATION OF HODGKIN LYMPHOMA AND ITS INTEGRATION INTO PERSONALIZED CARE USING LIQUID BIOPSY

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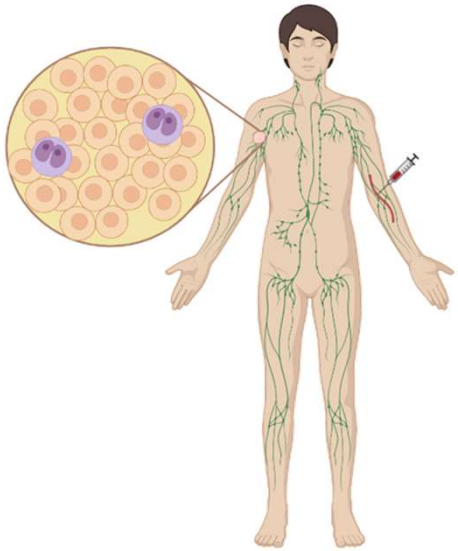
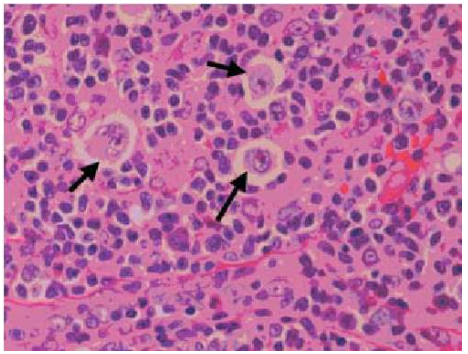
Universidad Francisco de Vitoria UFV Madrid

INTRODUCTION: HODGKIN LYMPHOMA





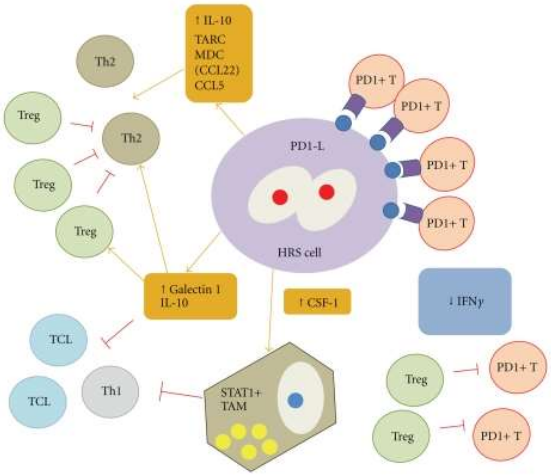
→Rare malignant disease (0.4% of all neoplasms)

→90% of HL are classical HL (cHL)

→Primarily affects young people

→Cure rate of 80%

→High mortality rate due to overtreatment



- Primary cause of death caused by treatments side effects: secondary tumours and cardiovascular events → treatment modulation based on risk of lymphoma-related death.
- Main characteristic: It features giant malignant cells, typically multinucleated, derived from B lymphocytes. They make up approximately 1% of the tumour mass → **Hodgkin Reed-Sternberg (HRS).**

Weniger MA, Küppers R. Leukemia. 2021.

Connors JM. Nature reviews Disease primers. 2020.

Montes-Moreno, S. Advances in hematology. 2011.

INTRODUCTION: HODGKIN LYMPHOMA AND LIQUID BIOPSY

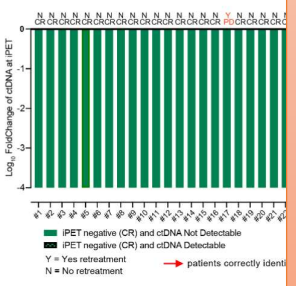
→Targeted panel CAPP-SEQ to detect ctDNA

ORIGINAL PAPER

BJHaem

Baseline circulating tumour DNA and interim PET predict response in relapsed/refractory classical Hodgkin lymphoma

Eleonora Calabretta[1,2] | Martina di Trani[2] | Francesco Corrado[1,2] | Martina Sollini[1,3] | Vanessa Cristaldi[2] | Fabrizio Marino[1,2] | Lodovico Terzi di Bergamo[4] | Alessio Bruscaggin[4] | Maria Cristina Pirosa[4,5] | Stefania Bramanti[2] | Arturo Chiti[1,3] | Davide Rossi[4,5] | Carmelo Carlo-Stella[1,2]



→Targeted panel CAPP-SEQ to characterize HRS cells and to detect ctDNA

LYMPHOID NEOPLASIA

Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma

Valeria Spina,[1,*] Alessio Bruscaggin,[1,*] Annarosa Cuccaro,[2] Maurizio Martini,[3] Martina Di Trani,[4] Gabriela Forestieri,[1] Martina Manzoni,[5] Adalgisa Condoluci,[1,6] Alberto Arribas,[1] Lodovico Terzi-Di-Bergamo,[1] Silvia Laura Locatelli,[4] Elisa Cupelli,[2] Luca Ceriani,[6] Alden A. Moccia,[6] Anastasios Stathis,[6] Luca Nassi,[7] Clara Deambrogi,[7] Fary Diop,[7] Francesca Guidetti,[1] Alessandra Cocomazzi,[3] Salvatore Annunziata,[8] Vittoria Rufini,[8] Alessandro Giordano,[8] Antonino Neri,[5,9] Renzo Boldorini,[10] Bernhard Gerber,[6] Francesco Bertoni,[1,6] Michele Ghielmini,[6] Georg Stüssi,[6] Armando Santoro,[4,11] Franco Cavalli,[1,6] Emanuele Zucca,[6] Luigi Maria Larocca,[3] Gianluca Gaidano,[7] Stefan Hohaus,[2,†] Carmelo Carlo-Stella,[4,11,†] and Davide Rossi[1,6,†]

→ Improve HRS cell isolation approaches

→ Fixed NGS panels: No extensive WGS mutational profile determination in HRS cells

→ Need for improving sensitivity in detecting ctDNA

d cell sorting

ner mutational

Seq) to detect

ps://doi.org/10.1038/s41586-023-06903-x

→Targeted panel to characterize HRS cells and ctDNA

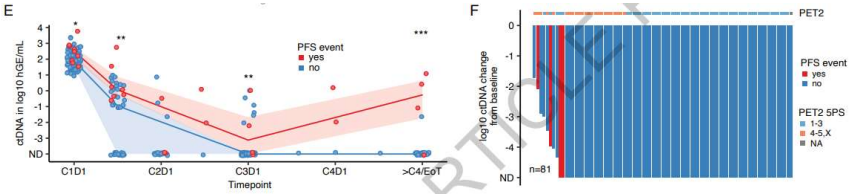
Med

CellPress

Clinical and Translational Article

In-depth cell-free DNA sequencing reveals genomic landscape of Hodgkin's lymphoma and facilitates ultrasensitive residual disease detection

Distinct Hodgkin lymphoma subtypes defined by noninvasive genomic profiling



INTRODUCTION: HODGKIN LYMPHOMA AND LIQUID BIOPSY


Jesús García-Velasco


Esperanza López

→ In-depth characterization of the genomics of HRS cells, coupled with blood biomarker detection, to monitor disease response in cHL patients.

STUDY OBJECTIVES

❖ Characterization of the mutational profile of isolated Hodgkin-Reed Sternberg cells by image cytometry: **DEPArray PLUS system – Menarini**.
- In-depth genomic characterization: **CNVs**, **Structural Variants**, SNVs, Indels

❖ Monitoring treatment response in these patients through the implementation of patient-specific mutation panels in liquid biopsy.

TUMOUR GENOMIC INFORMATION

ctDNA MONITORING

CLINICAL DATA

IMPROVE CLINICAL MANAGEMENT

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

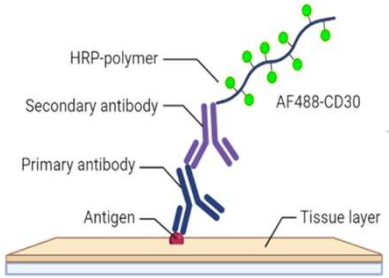
HRS cells isolation: Tissue dissociation from FFPE biopsy and cell selection

Mechanical disaggregation

1.

Staining and cells selection

2.



MENARINI silicon biosystems

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

DEPArray™ PLUS system: Technology overview

The best-in-class digital single cell technology just got even better

- **Improved workflow automation**
- **Faster digital cell sorting**
- **Increased flexibility**
- **9 fluorescent channels**

9 Fluo + Brightfield			
CH	Ex Filter	Em Filter	CellBrowser™
1	350-404 nm	447-460 nm	VIOLET
2 NEW		509-522 nm	VIO-FITC
3 NEW		603-627 nm	VIO-ORANGE
4	426-450 nm	690-730	PerCP-Cy5.5
5	453.5-486.5 nm	509-522 nm	FITC
6	541-556 nm	572-594	PE
7 NEW		754-816 nm	PE-FAR RED
8	600-630 nm	661.5-690.5	APC
4' NEW		690-730	APC-Cy5.5
9 NEW		754-816 nm	APC-FAR RED

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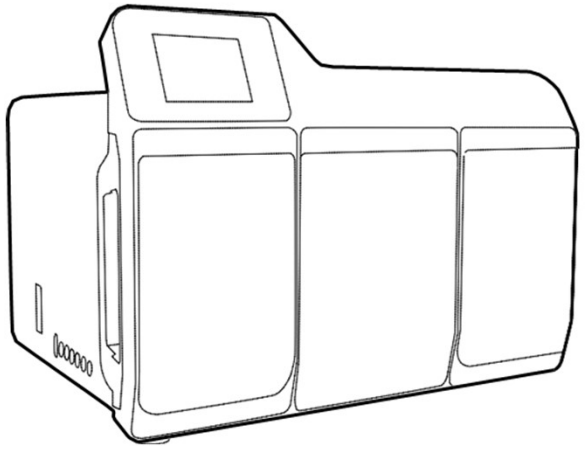
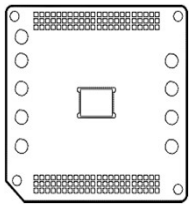
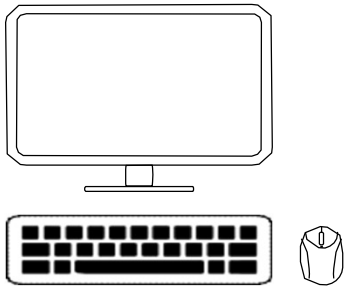
DEPArray™ PLUS system: Technology overview



DEPArray™ Control Unit



DEPArray™ Cartridge



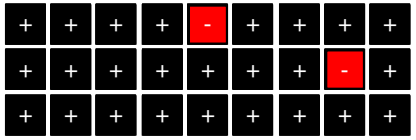
CellBrowser™ Software

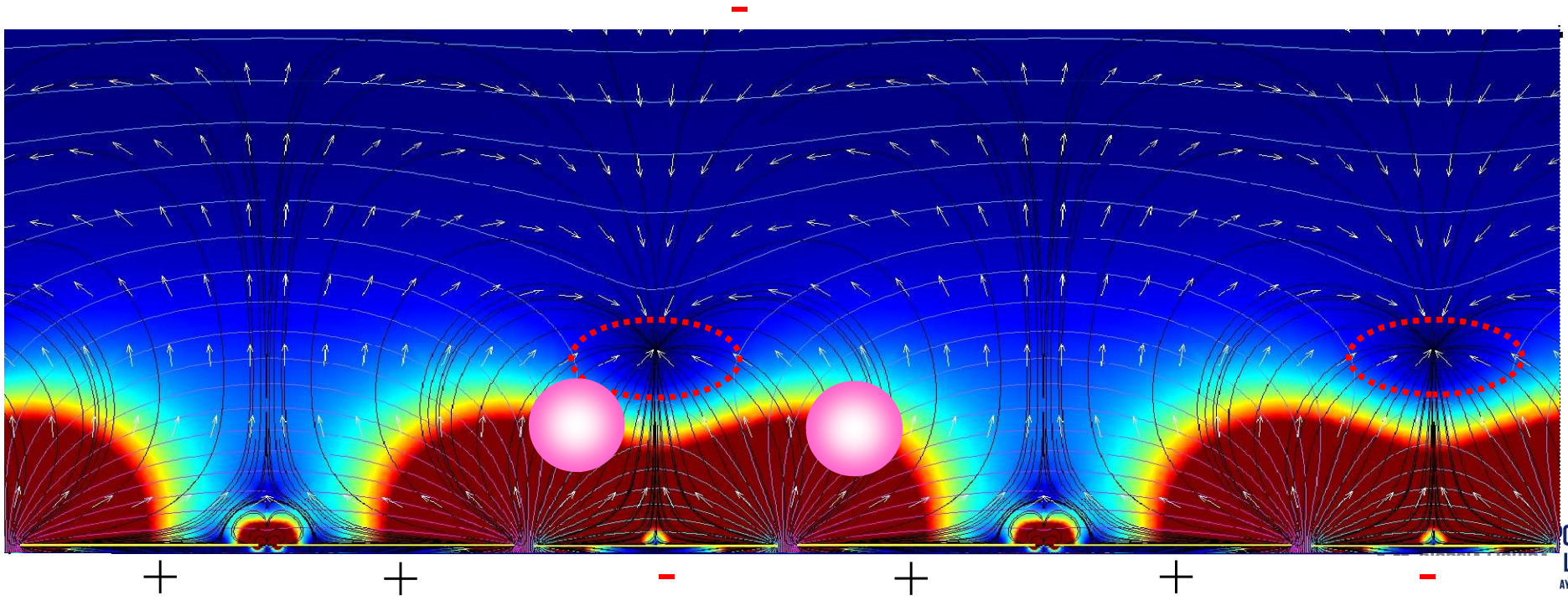
The DEPArray™ system is a semiconductor based technology for **precise isolation of pure single cells.**

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

DEPArray™ PLUS system: Technology overview

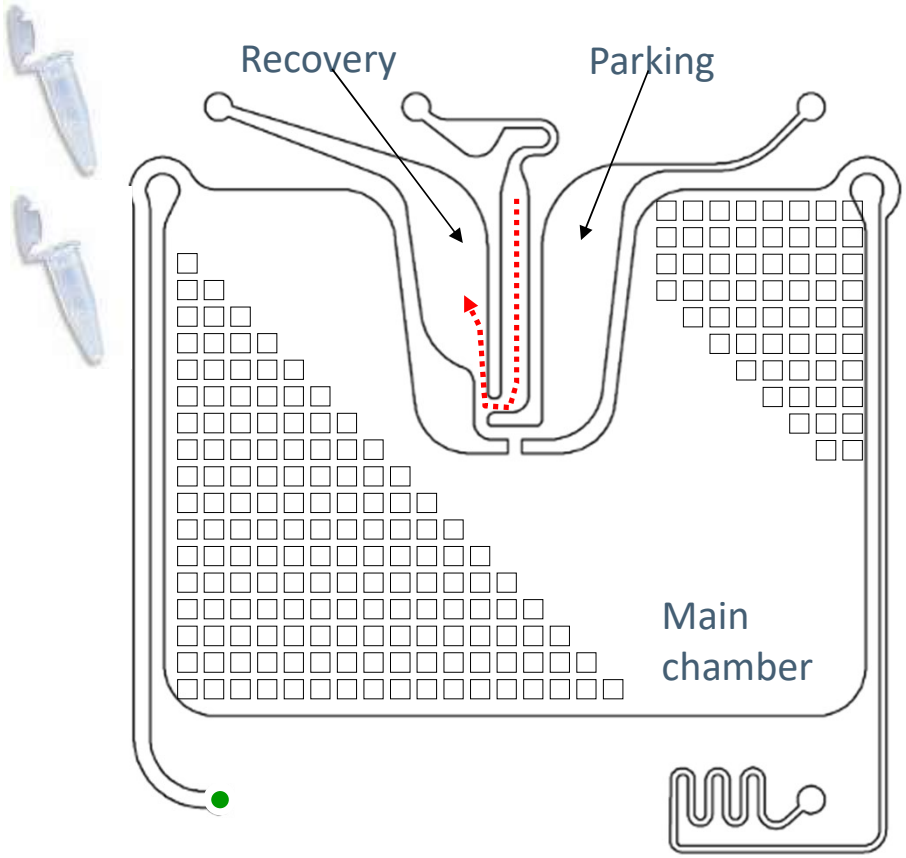
300,000 microchip controlled electrodes to create DEP cages
Changing polarity of each electrode allows cells to be moved from cage to cage over the microchip.





METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

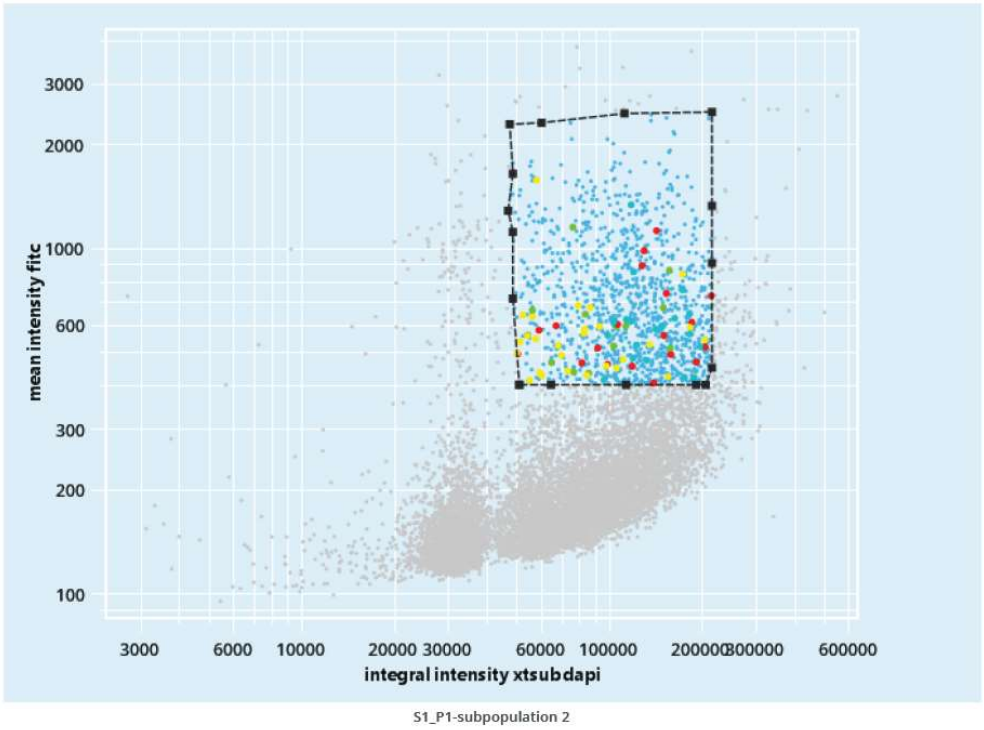
DEPArray™ PLUS system: Technology overview



1. Inject, trap and image all cells
2. Move all cells of interest into Parking chamber
3. Move separately to Recovery chamber and flush

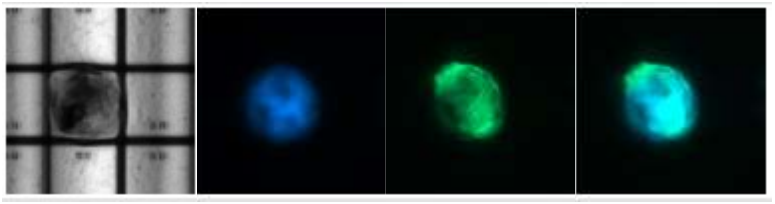
METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

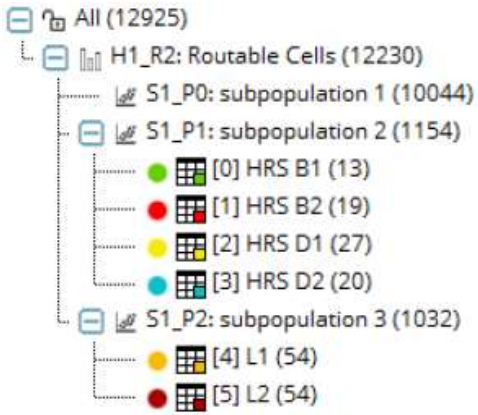
DEPArray™ PLUS system: Scan settings and HRS cell selection



S1_P1-subpopulation 2

Scan settings	Chip scan					Image analysis			
	Fluorophore	Exposure (ms)	Camera Gain	Lamp intensity (%)	Offset (um)	HDR	Shading Correction	Detection	Duplicate removal
CHIP SCAN 1									
DAPI	dapi	40	1X	20%	36		☑	Bright	☑
BRIGHTFIELD		2	1X	5%	25		☑	Faint	
FITC	af488	200	2X	100%	30		☑	Disable	



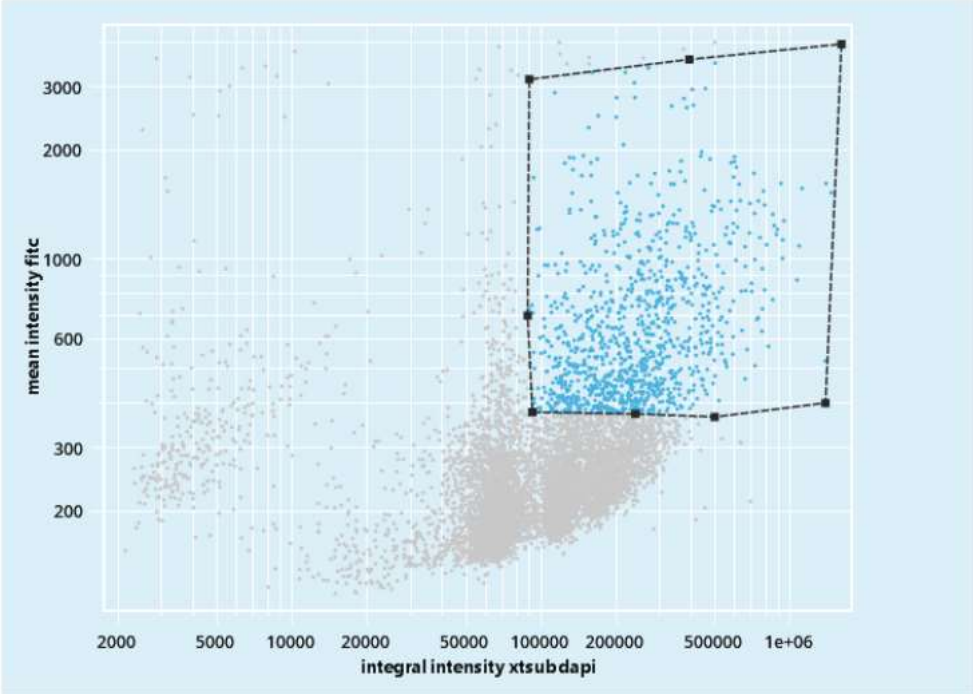


Mean intensity fitc: Minimum ≈ 400; Maximum ≈ 3000
Integral intensity xtsubdapi: Minimum ≈ 100000; Maximum ≈ 1000000

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

DEPArray™ PLUS system: Scan settings and HRS cell selection

Isolation of HRS cells based on size and staining intensity for CD30 (AF488):

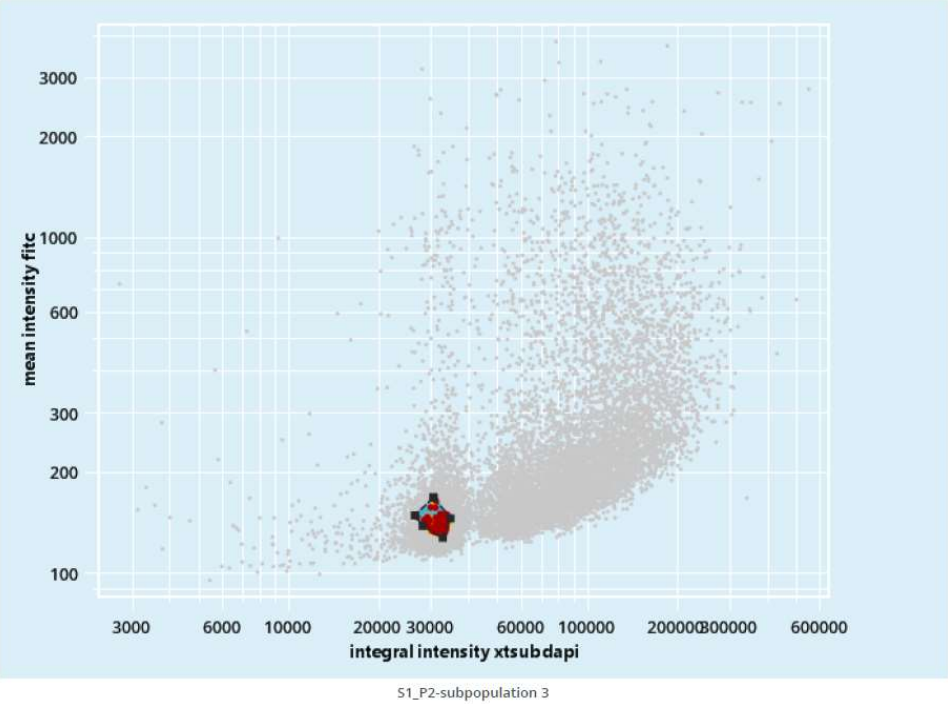


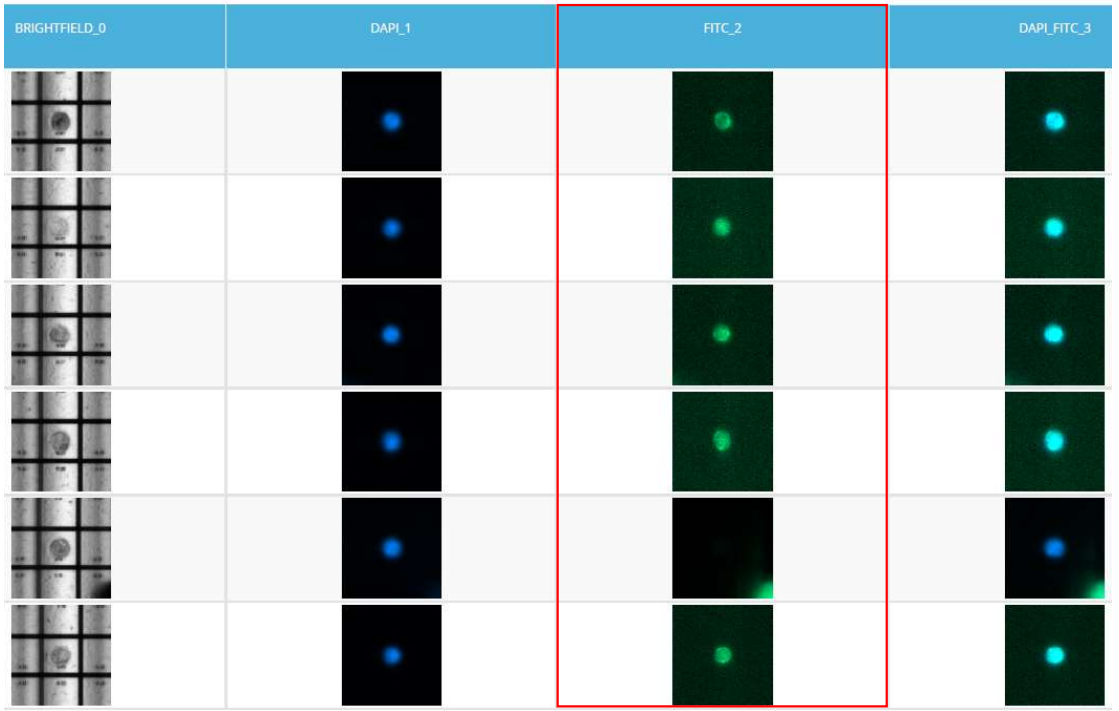
Mean intensity fitc: Minimum ≈ 400; Maximum ≈ 3000
Integral intensity xtsubdapi: Minimum ≈ 100000; Maximum ≈ 1000000

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

DEPArray™ PLUS system: Scan settings and HRS cell selection

Isolation of lymphocytes based on circularity, small size and no signal for CD30





Mean intensity fitc: Minimum ≈ 130; Maximum ≈ 180
Integral intensity xtsubdapi: Minimum ≈ 25000; Maximum ≈ 35000

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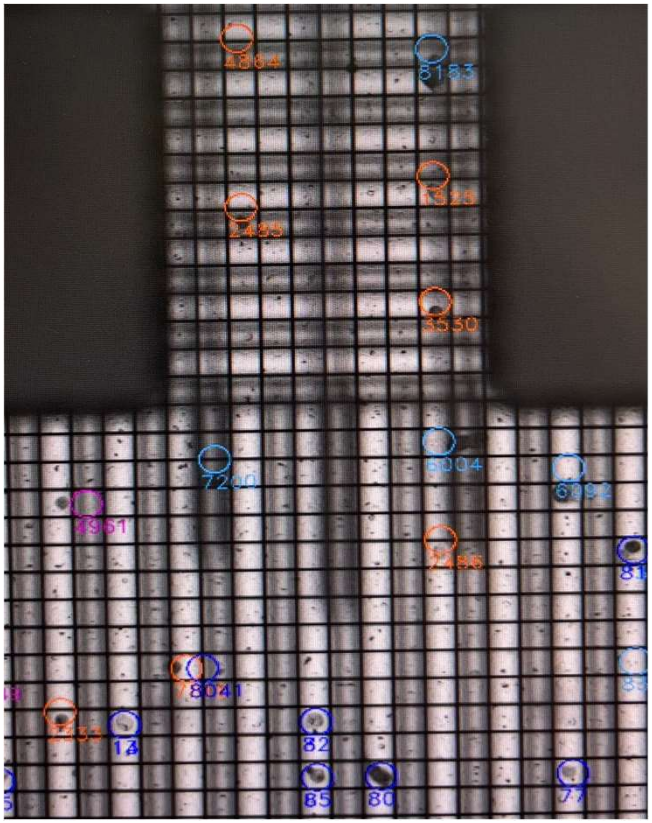
DEPArray™ PLUS system: Scan settings and HRS cell selection



Cell routing

Cell parking

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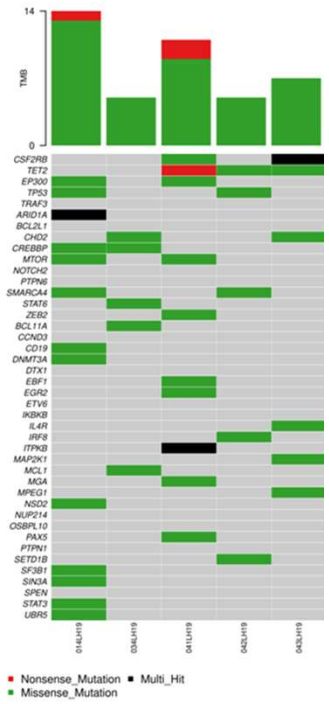
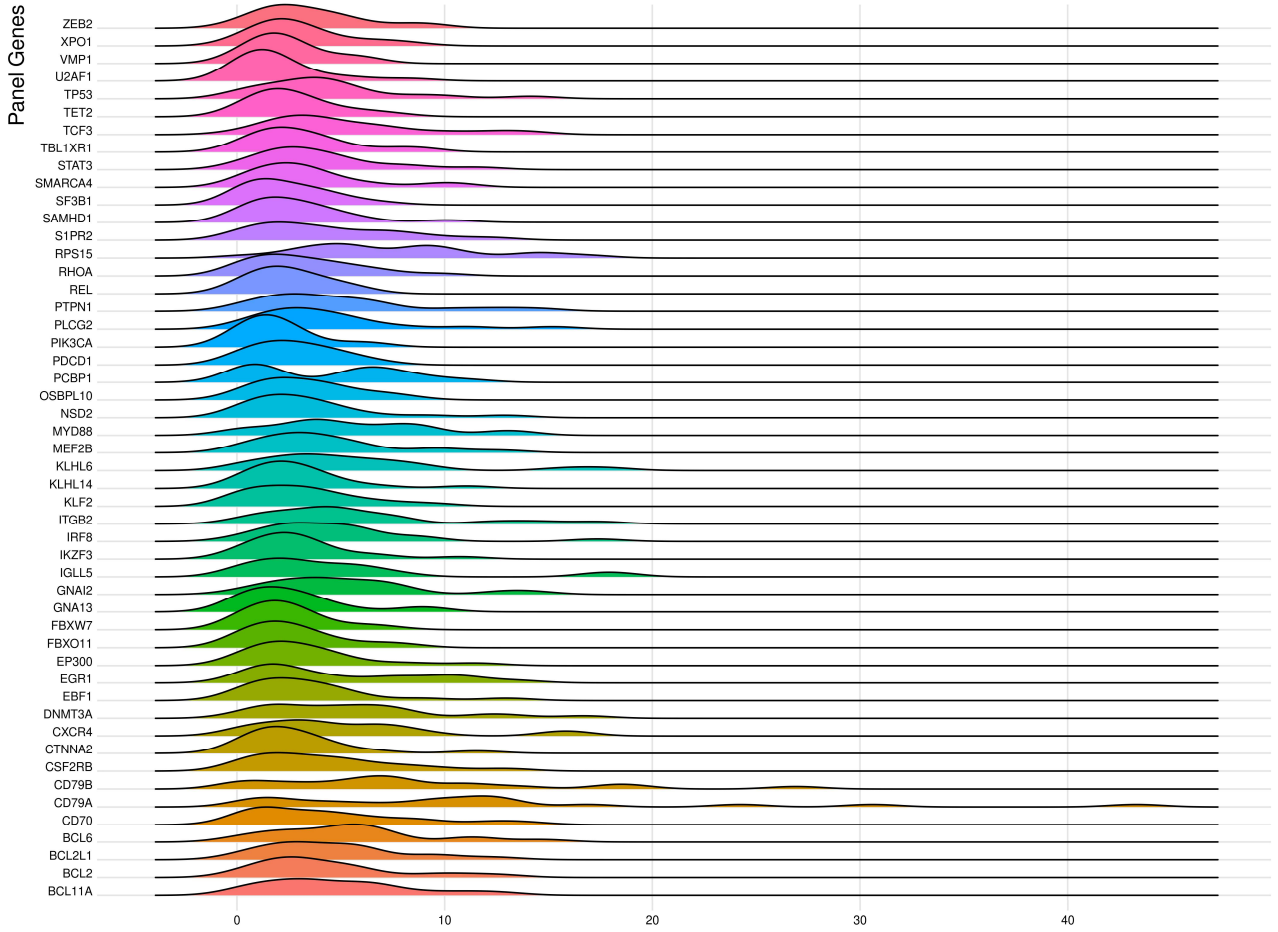
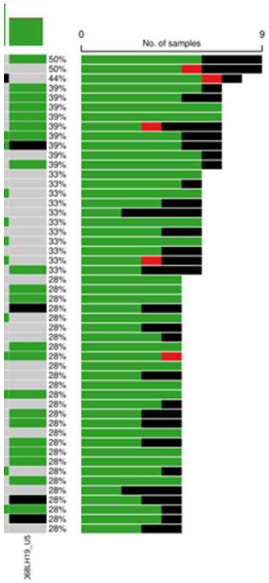
HRS cells characte







- VAF>= 50% in tumour for C > T variants (FFPE artifacts)

Alig, S.K., Shahrokh Esfahani, M., Garofalo, A. et al. Nature (2023)

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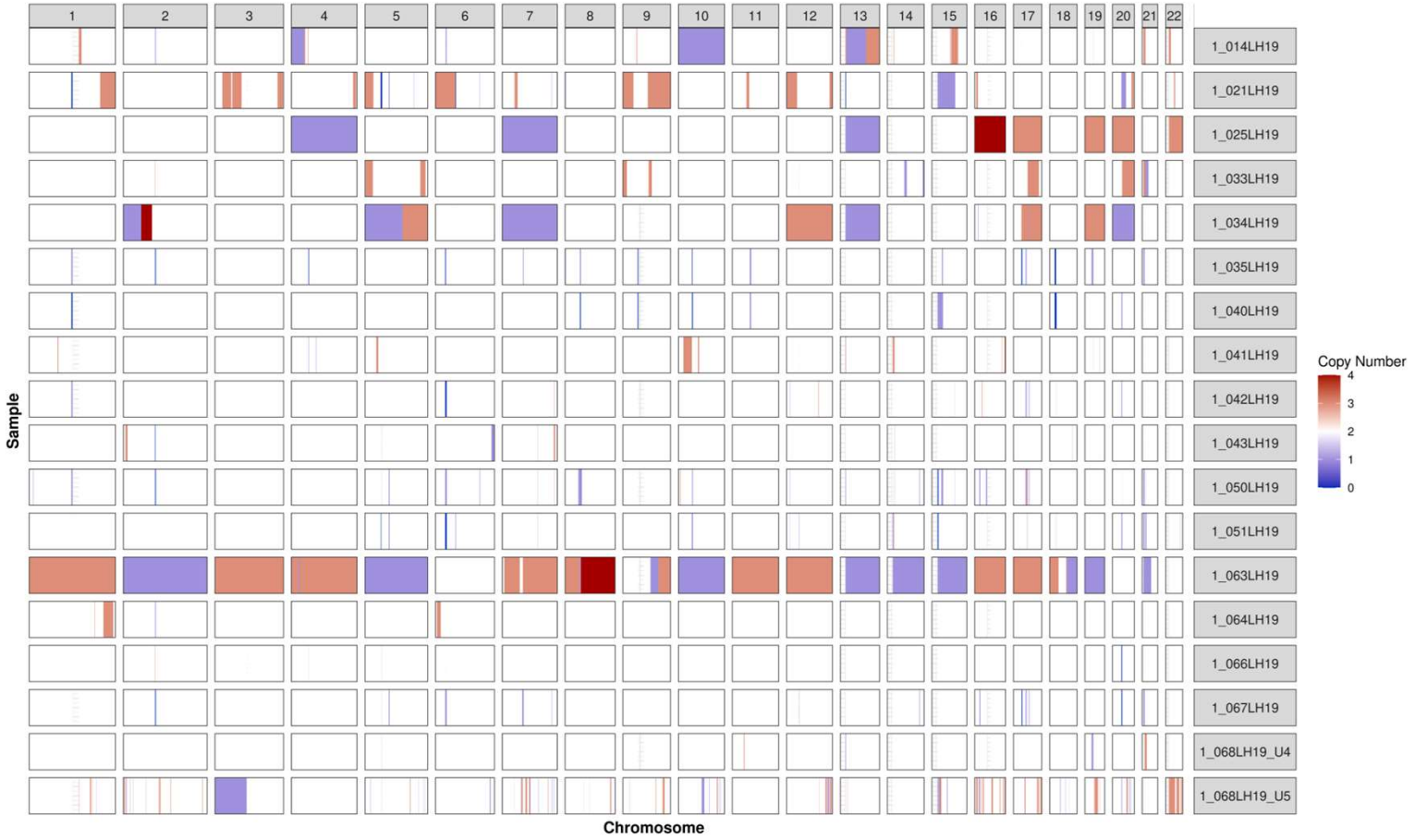
HRS cells characterization



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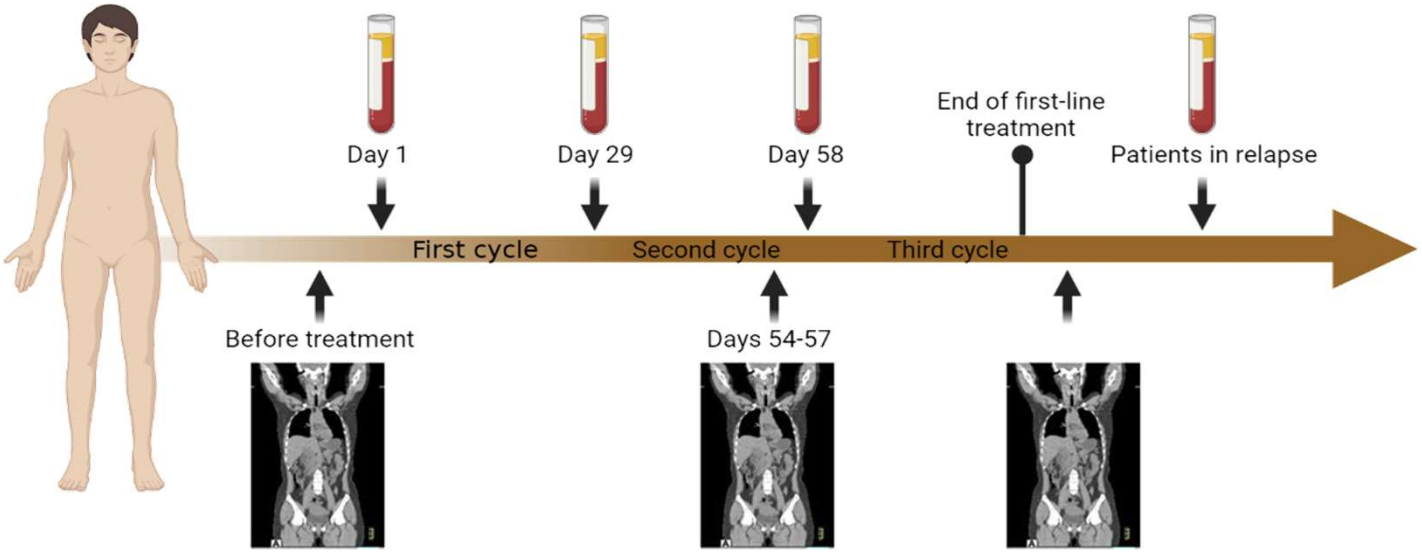
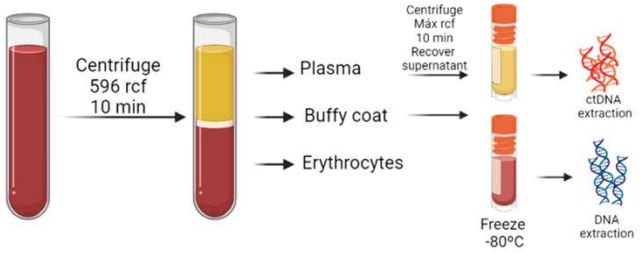
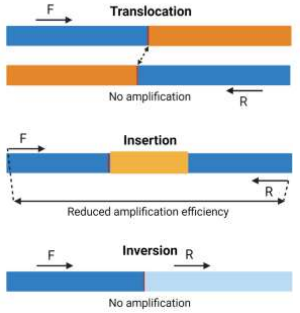
Personalized treatment monitoring using liquid biopsy





Blood Biomarkers:

- Structural variations



- Mid-size indels
- SNVs

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

Personalized treatment monitoring using liquid biopsy

Yanira Heredia
Alejandro Martín

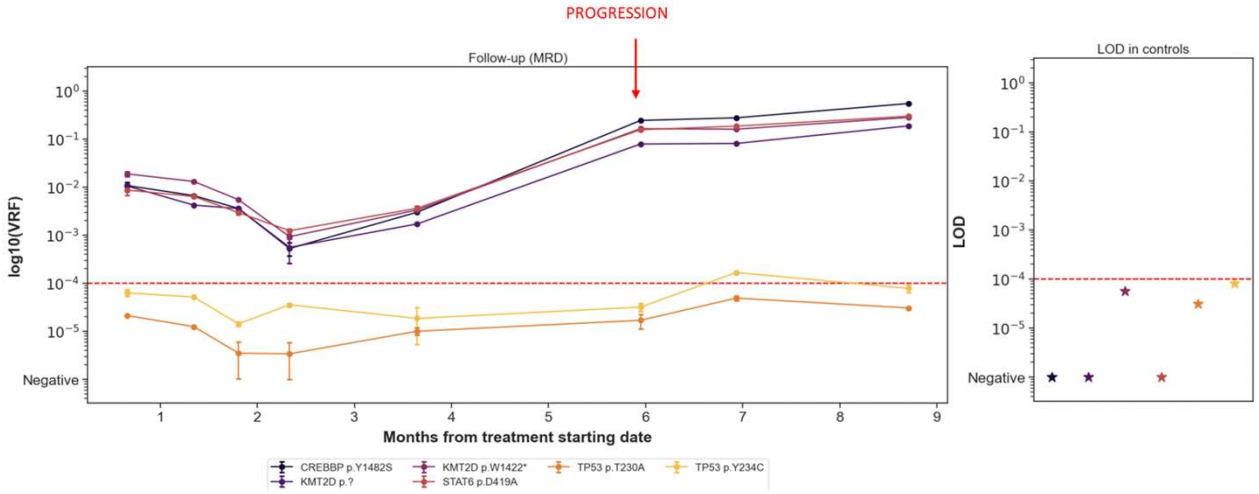
Altum sequencing

Article | Published: 03 January 2023

LYMPHOMA

Real-life disease monitoring in follicular lymphoma patients using liquid biopsy ultra-deep sequencing and PET/CT

Ana Jiménez-Ubieto, María Poza, Alejandro Martin-Muñoz, Yanira Ruiz-Heredia, Sara Dorado, Gloria Figaredo, Juan Manuel Rosa-Rosa, Antonia Rodriguez, Carmen Barcena, Laura Parrilla Navamuel, Jaime Carrillo, Ricardo Sanchez, Laura Rufian, Alexandra Juárez, Margarita Rodriguez, Chongwu Wang, Paula de Toledo, Carlos Grande, Manuela Mollejo, Luis-Felipe Casado, María Calbacho, Tycho Baumann, Inmaculada Rapado, Miguel Gallardo, Pilar Sarandeses, Rosa Ayala, Joaquín Martínez-López & Santiago Barrio



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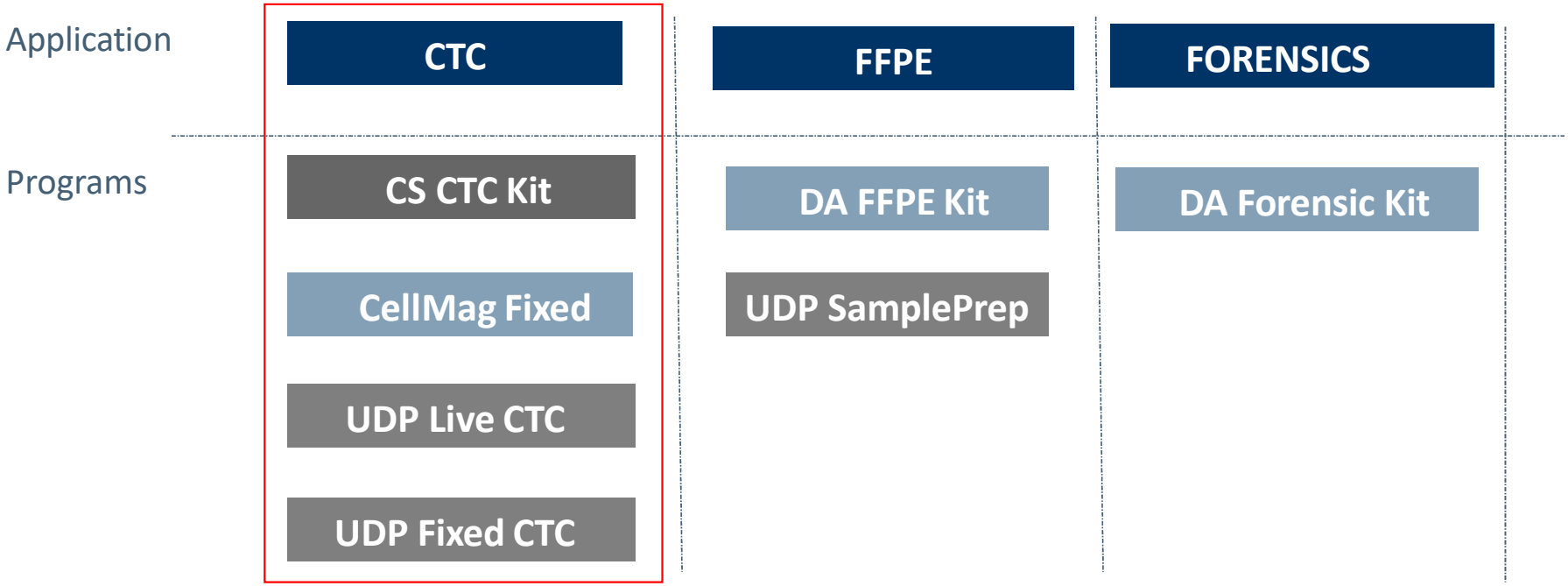
DEPArray™ PLUS system: CTCs detection

3 different applications available



CTC / FFPE applications available in 5 and 9 colours

Applications optimized with preparation kits

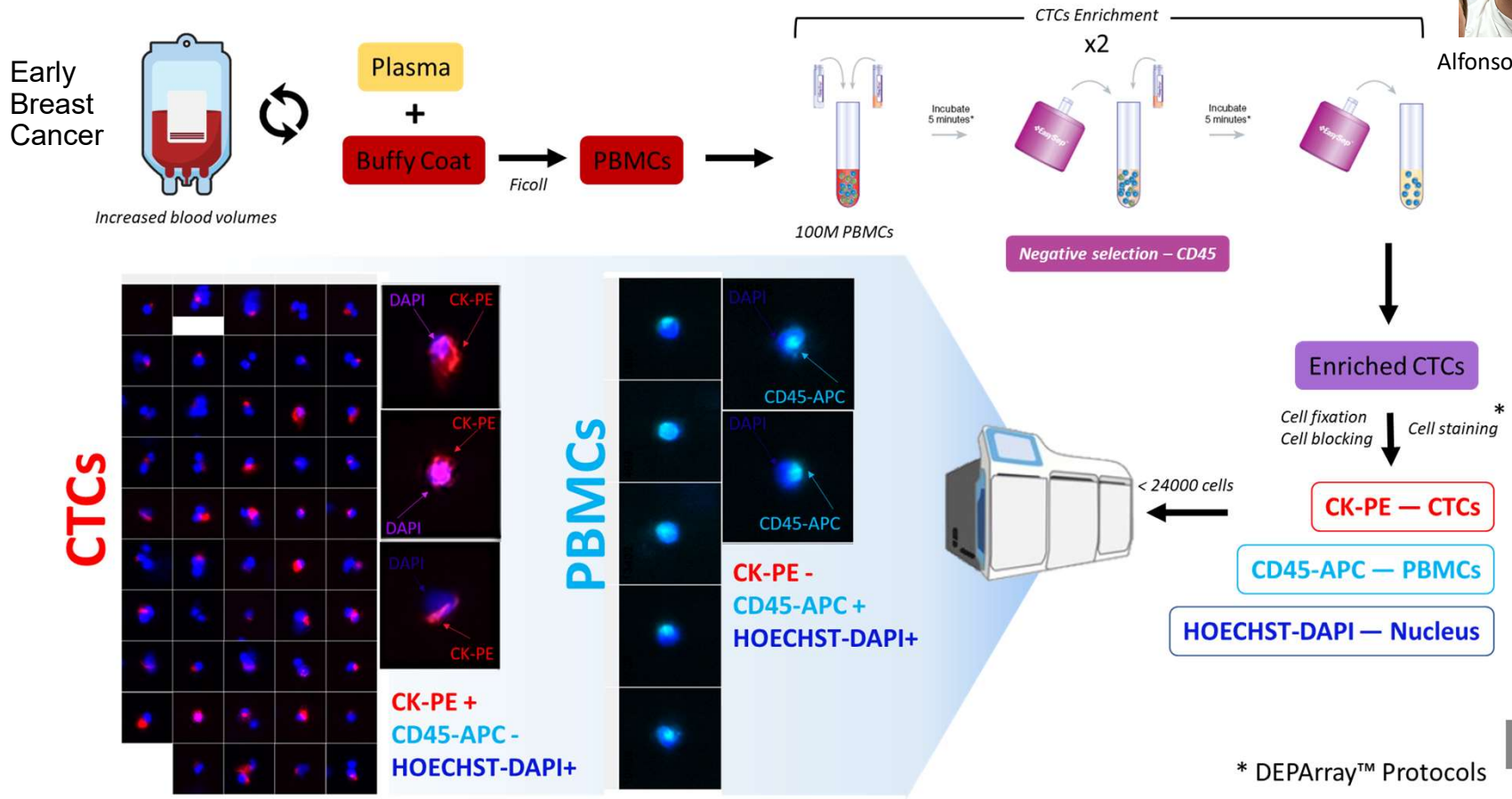
Open protocols (User Defined Protocol)

METHODOLOGY: CHARACTERIZATION OF THE MUTATIONAL PROFILE OF ISOLATED HODGKIN-REED STERNBERG CELLS USING IMAGE CYTOMETRY

DEPArray™ PLUS system: CTCs detection

Alfonso Alba Bernal

Maria Elena Quirós



* DEPArray™ Protocols

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CONCLUSIONS

1. The **DEPArray PLUS** system proves to be an exceptional platform for isolating cells at the single-cell level.

2. It facilitates the isolation of various cell types through immunofluorescence, offering up to 9 channels.

3. Successful isolation of HRS cells enabled the generation of WGS libraries in all 16 tissues examined.

4. The acquired sequencing data provided insights into CNVs, SNVs, and indels.

5. Ongoing efforts are directed towards identifying structural variants

6. Collaboration with Altum Sequencing is underway to develop ultrasensitive, patient-specific panels. These panels aim to track ctDNA and monitor treatment responses.

7. Furthermore, the **DEPArray PLUS** system has been seamlessly integrated into other projects, including the isolation of CTCs from high-volume blood samples.

THANK YOU!

MENARINI
silicon biosystems

GOTEL
Grupo Oncológico para el Tratamiento y Estudio de los Linfomas

asociación española contra el cáncer

FundaciónUnicaja

Junta de Andalucía

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