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## **Cell-Free DNA Fragmentome Dynamics as a Biomarker for Immune-Checkpoint Inhibition in Advanced Carcinoma**

Linh Nguyen Phuong, Frederic Fina, Romain Zakrajsek, Lucie Della-Negra, Laurent Greillier, Pascale Tomasini, Jean-Laurent Deville, Audrey Boutonnet, Frédéric Ginot, Jean-Charles Garcia, et al.

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## 1. Background

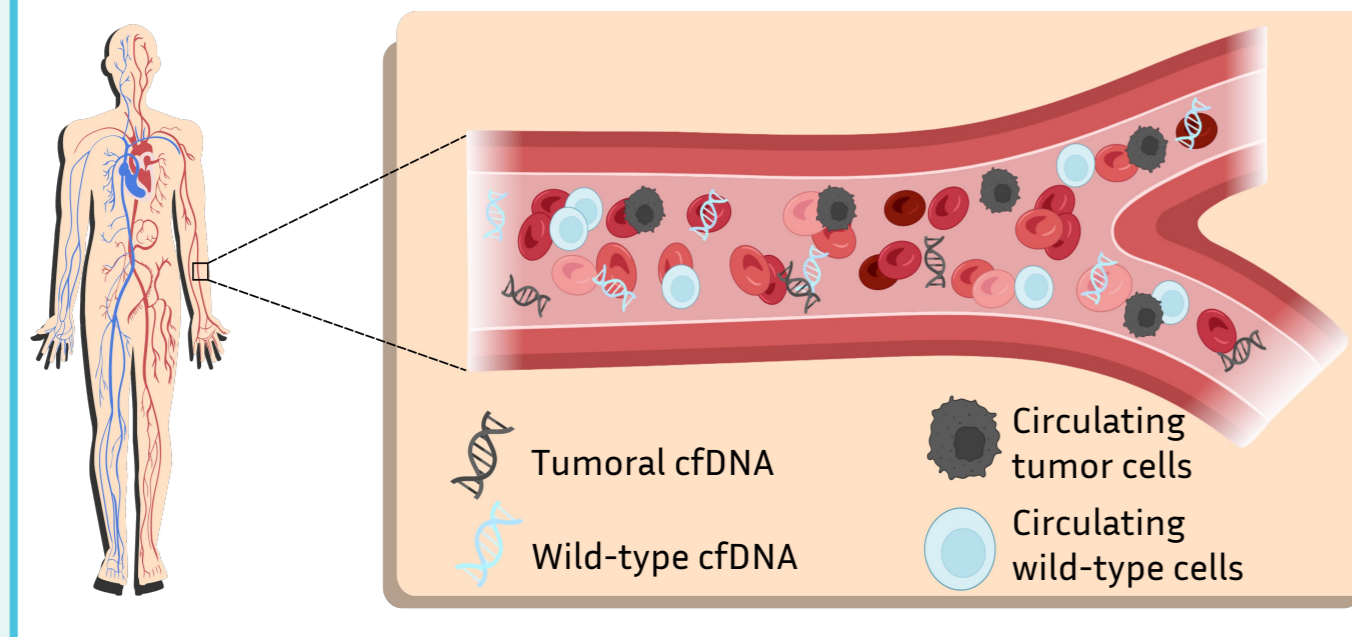


**CLINICAL CHALLENGE**

- 20-40% of advanced cancer patients show long-term response to immune checkpoint inhibitors (ICIs)
- Many experience early progression (EP, progression at first radiological assessment)
- Need for reliable early predictive biomarkers

**CIRCULATING CELL-FREE DNA (cfDNA): AN INNOVATIVE APPROACH?**

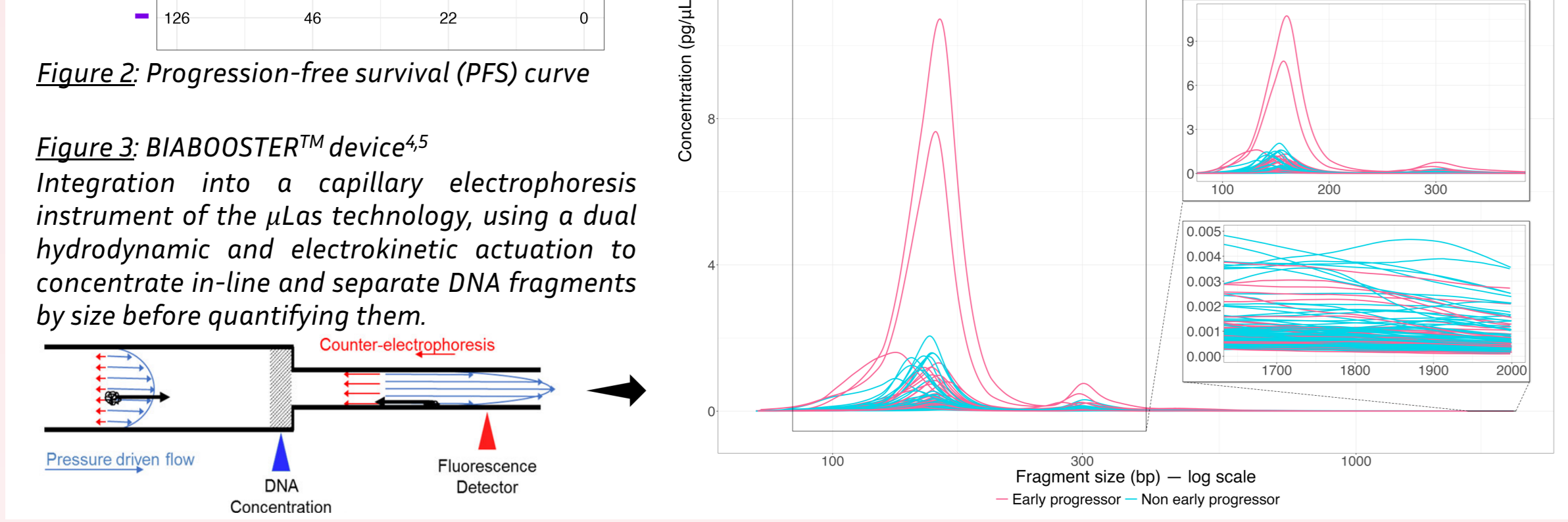
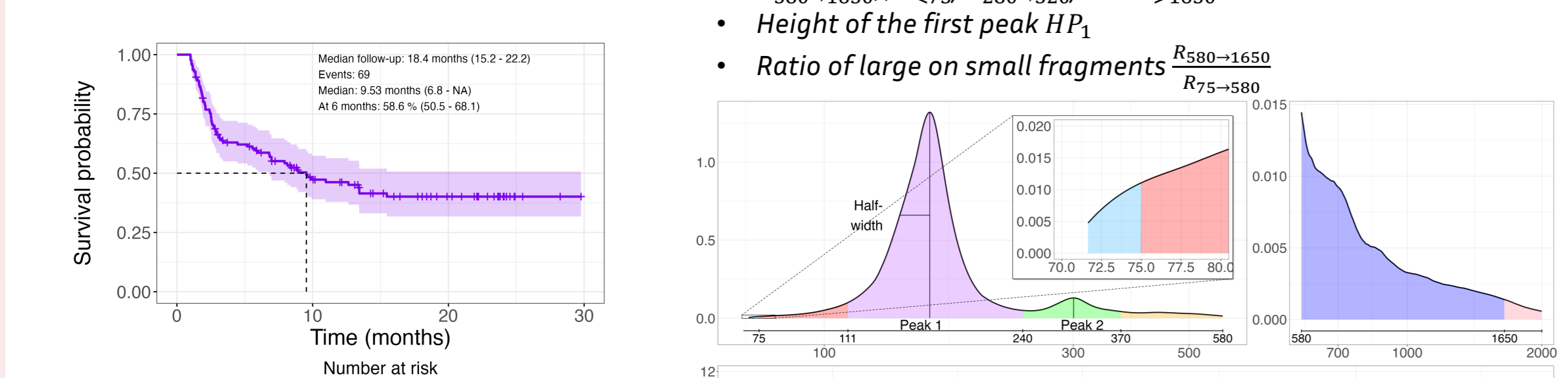
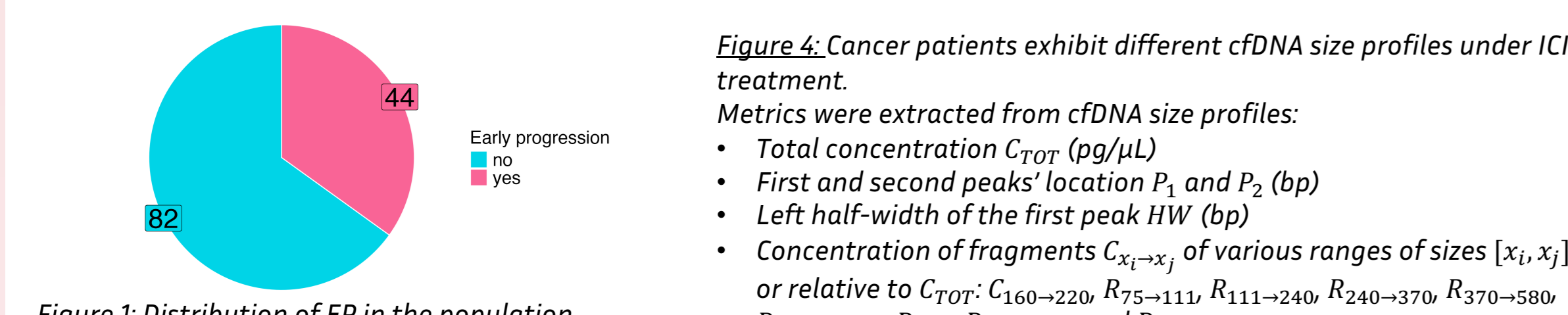
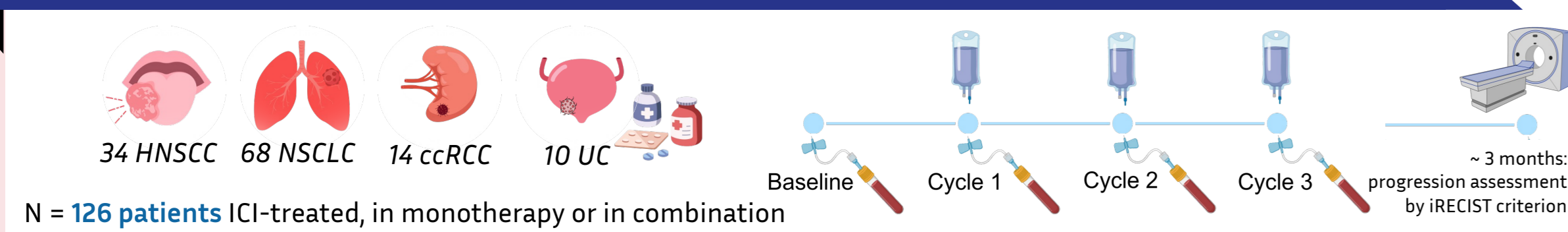
- Non-encapsulated fragments of DNA released by cells
- Contains both tumoral and wild-type—mainly immune—cells
- Released under apoptosis, necrosis, active secretion
- Half-life ~15 min – 2h
- Non-invasive and high-frequency monitoring
- Plasmatic cfDNA size profile<sup>2</sup> vary under clinical conditions



### OBJECTIVES

- Assess pre- and on-treatment cfDNA fragment size profiles as predictors of EP after ICI
- Improve understanding of the biological processes involved in cfDNA shedding

## 2. Data



**Figure 4: Cancer patients exhibit different cfDNA size profiles under ICI treatment.**

Metrics were extracted from cfDNA size profiles:

- Total concentration  $C_{TOT}$  (pg/ $\mu$ L)
- First and second peaks' location  $P_1$  and  $P_2$  (bp)
- Left half-width of the first peak HW (bp)
- Concentration of fragments  $C_{x_1-x_j}$  of various ranges of sizes  $[x_i, x_j]$ , or relative to  $C_{TOT}$ :  $C_{160-220}$ ,  $R_{75-111}$ ,  $R_{111-240}$ ,  $R_{240-370}$ ,  $R_{370-580}$ ,  $R_{580-1650}$ ,  $R_{C75}$ ,  $R_{280-320}$ , and  $R_{>1650}$
- Height of the first peak  $HP_1$
- Ratio of large on small fragments  $\frac{R_{580-1650}}{R_{75-580}}$

## 3. Unsupervised analysis

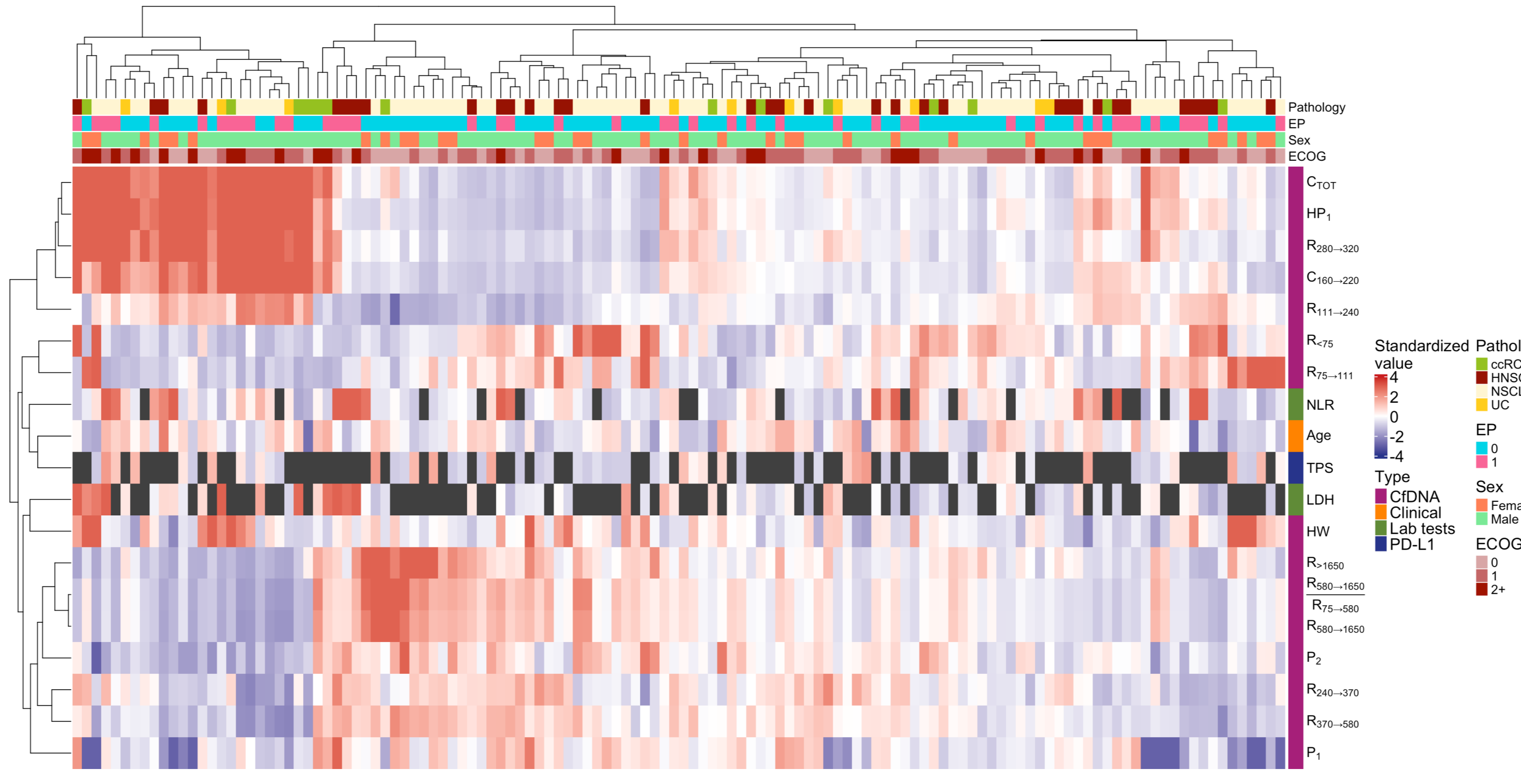


Figure 5: Hierarchical clustering of clinical, biological, and cfDNA features across the population

## 4. Supervised analysis

**Table 1: Logistic and Cox regressions results: high-molecular-weight fragments ratio as the best predictor of no EP and long PFS**

(AUC: Area Under the ROC curve; HR: Hazard Ratio; NLR: Neutrophil-to-lymphocyte ratio; MV: Multivariate (adjustment with confounding features: age, sex, ECOG, NLR, pathology); OR: Odds Ratio; UV: univariate)

(Significance: \*\*\*\*: p-value < 0.0001; \*\*\*: p-value < 0.001; \*\*: p-value < 0.01; \*: p-value < 0.05)

VARIABLE	EARLY PROGRESSION		PROGRESSION-FREE SURVIVAL			
	AUC	OR UV	OR MV	C-INDEX	HR UV	HR MV
$R_{>1650}$	0.73	0.39 (0.25 – 0.62)****	0.3 (0.14 – 0.63)**	0.69	0.54 (0.43 – 0.68)****	0.43 (0.32 – 0.58)****
$C_{160-220}$	0.70	2.1 (1.4 – 3.2)***	2.2 (1.2 – 4)**	0.66	1.7 (1.4 – 2.1)****	1.9 (1.5 – 2.5)****
$\frac{R_{580-1650}}{R_{75-580}}$	0.69	0.54 (0.36 – 0.8)**	0.46 (0.25 – 0.83)*	0.66	0.6 (0.48 – 0.75)****	0.49 (0.37 – 0.64)****
$R_{280-320}$	0.67	1.8 (1.2 – 2.7)**	2.3 (1.2 – 4.3)**	0.64	1.6 (1.3 – 2)****	1.8 (1.4 – 2.4)****
$R_{380-1650}$	0.65	0.51 (0.32 – 0.82)**	0.53 (0.25 – 1.1)	0.64	0.55 (0.4 – 0.75)****	0.51 (0.34 – 0.75)****
$P_2$	0.64	0.56 (0.36 – 0.86)**	0.39 (0.19 – 0.79)**	0.63	0.61 (0.47 – 0.79)****	0.54 (0.39 – 0.73)****
$HP_1$	0.66	1.7 (1.1 – 2.5)**	2 (1.1 – 3.6)*	0.63	1.5 (1.2 – 1.9)****	1.9 (1.4 – 2.5)****
$C_{TOT}$	0.65	1.7 (1.1 – 2.5)**	2.2 (1.2 – 3.9)**	0.62	1.5 (1.2 – 1.9)****	1.9 (1.4 – 2.5)****
NLR	0.70	2.5 (1.5 – 4.2)***		0.63	1.6 (1.2 – 2.2)**	

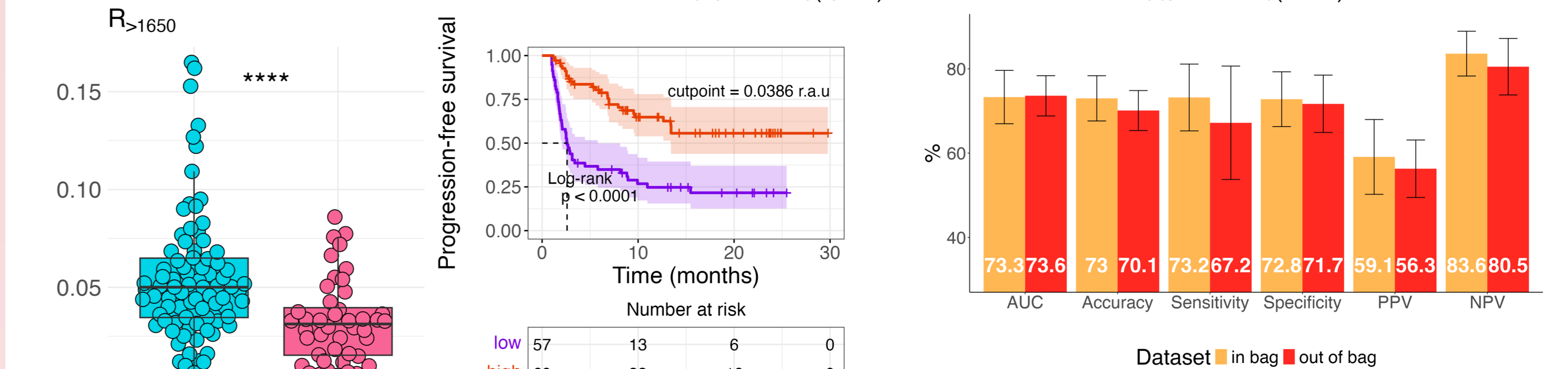
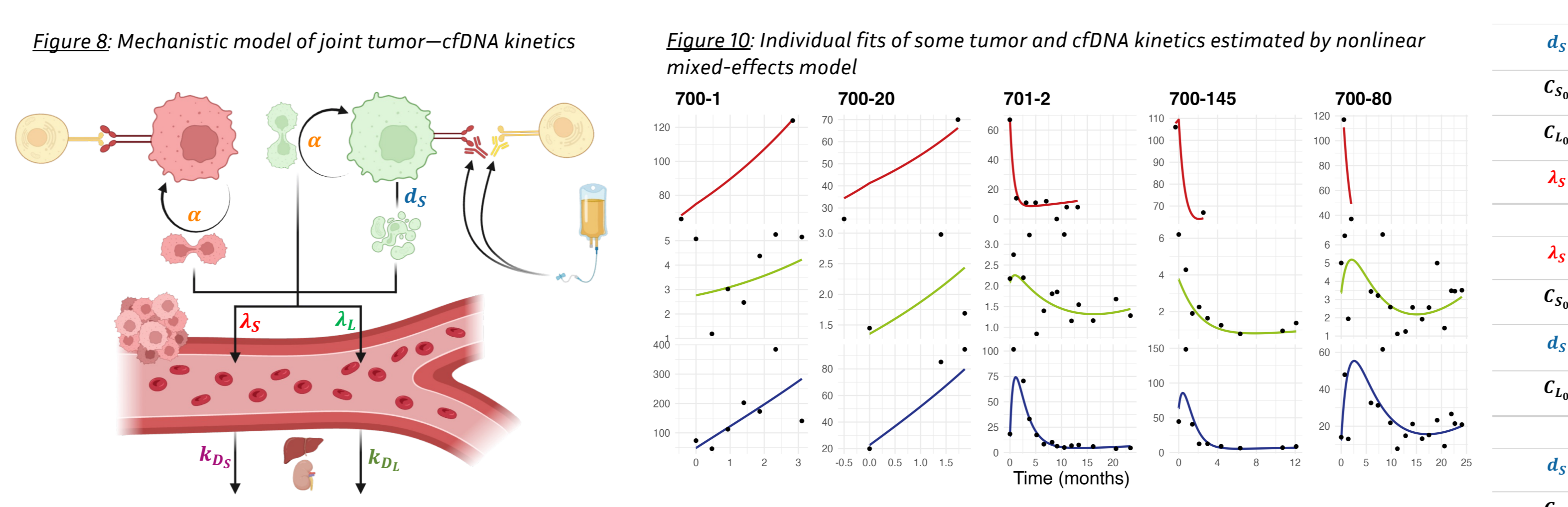


Figure 6: Higher ratio of long cfDNA fragments is associated with extended PFS and no EP under ICIs

## 5. Mechanistic modeling

**Biological hypothesis:**

- Tumor cells (assimilated to the sum of largest diameters  $S(t)$ ) comprise two sub-populations: cells sensitive to treatment  $S_S$  and resistant ones  $S_R$ , governed by first ordered kinetics driven by a shrinkage parameter  $d_S$  and a regrowth parameter  $\alpha$ .
- Cancer cells induce the release of DNA fragments:
  - Short fragments (assimilated to the log of the concentration  $C_S(t)$  of fragments ranging from 75 to 540 bp) due to their chaotic apoptosis-proliferation cycle characteristics<sup>6</sup> and ICIs-sensitive cells death by apoptosis, with a rate  $\lambda_S$
  - Long fragments (assimilated to the log of the concentration  $C_L(t)$  of fragments ranging from 540 to 1650 bp) due to their chaotic proliferation cycle characteristics<sup>6</sup> and treatment-sensitive cells death by necrosis, with a rate  $\lambda_L$
- CFDNA is continuously cleared from the circulation by liver and kidneys, in a linear process depending only on its concentration: with a rate  $k_{D_S}$  for short fragments and  $k_{D_L}$  for long ones.



**Table 2: Population parameters estimation through nonlinear mixed-effects approach**

Parameter	$C_{S_0}$	$C_{L_0}$	$d_S$	$\alpha$	$\lambda_S$	$\lambda_L$	$k_{D_S}$	$k_{D_L}$
Population parameter	2.4	0.35	0.3	0.047	3.8e-4	0.092	0.013	3.2
Random effect	0.2	0.62	0.76	1.1	2.5	0.52	2.1	1.3

**Table 3: Survival analysis of the individual parameters on full and truncated kinetics.**

Only parameters significant in univariate analysis are displayed. Tumor kinetics parameters,  $\alpha$  and  $d_S$ , are highly associated with long and short PFS, respectively, as well as the estimation of short and long fragments  $D_{S_0}$  and  $D_{L_0}$  at time  $t = 0$ .  $D_{S_0}$  and  $D_{L_0}$  remain significant when truncating data at 1.5, 2, 2.5, and 3 months.

VARIABLE	N	PROGRESSION-FREE SURVIVAL		
		C-INDEX	HR UV	HR MV
FULL KINETICS				
$d_S$	109	0.65	0.61 (0.48 – 0.78)****	0.63 (0.42 – 0.93)*
$k_{D_S}$	109	0.63	0.65 (0.48 – 0.89)**	0.68 (0.48 – 0.96)*
$\alpha$	109	0.62	1.6 (1.3 – 2.1)***	1.4 (1 – 1.9)*
$D_{S_0}$	109	0.61	1.4 (1.1 – 1.8)*	1.7 (1.2 – 2.4)**
$S_{R_0}$	109	0.57	1.3 (1 – 1.7)*	2.1 (1.5 – 2.9)****
TRUNCATED KINETICS: 1.5 MONTHS				
$d_S$	90	0.70	0.58 (0.44 – 0.76)***	0.48 (0.29 – 0.79)**
$C_{S_0}$	90	0.70	1.7 (1.3 – 2.3)***	1.1 (0.71 – 1.6)
$C_{L_0}$	90	0.67	1.8 (1.3 – 2.6)***	1.2 (0.78 – 1.9)
$\lambda_S$	90	0.67	1.8 (1.3 – 2.5)***	1.2 (0.66 – 2)
TRUNCATED KINETICS: 2 MONTHS				
$\lambda_S$	84	0.69	1.8 (1.3 – 2.6)**	1.1 (0.61 – 1.8)
$C_{S_0}$	84	0.67	1.7 (1.2 – 2.3)**	1.1 (0.74 – 1.7)
$d_S$	84	0.66	0.65 (0.48 – 0.89)**	0.74 (0.46 – 1.2)
$C_{L_0}$	84	0.65	1.6 (1.2 – 2.3)**	1 (0.64 – 1.6)
TRUNCATED KINETICS: 2.5 MONTHS				
$d_S$	73	0.67	0.66 (0.45 – 0.95)*	0.74 (0.44 – 1.2)
$C_{S_0}$	73	0.66	1.6 (1.1 – 2.3)*	1.2 (0.73 – 1.9)
$C_{L_0}$	73	0.63	1.7 (1.1 – 2.5)*	1.3 (0.74 – 2.3)
TRUNCATED KINETICS: 3 MONTHS				
$C_{S_0}$	69	0.64	1.6 (1.1 – 2.5)*	1.5 (0.84 – 2.5)
$C_{L_0}$	69	0.62	1.6 (1 – 2.5)*	1.5 (0.81 – 2.7)

## 6. Conclusions & Perspectives

**Conclusions**

- ICI-treated patients with more high-molecular-weight cfDNA fragments tend to not early progress and to have longer PFS.
  - Can be explained by the activation of the cGAS-STING pathway by long fragments, boosting anti-tumor immunity through interferon production<sup>7</sup>.
- Our cfDNA biomarker is a strong predictor and outperforms the Tumor Mutational Burden (AUC = 0.69, PPV = 0.42) and PD-L1 immunohistochemistry (AUC = 0.65, PPV = 0.34) in the prediction anti-PD-1/PD-L1 response<sup>8</sup>.
- Mechanistic modeling offers biological insights to explain the interplay between cfDNA and tumor kinetics.
  - Short and long fragments are released differently depending on tumor cell sensitivity to ICIs and reflect distinct biological processes like apoptosis, necrosis, or secretion.
  - Clearance mechanisms are size-dependent, as shown by the different clearance rates  $k_{D_S}$  and  $k_{D_L}$ .
- Parameters extracted from cfDNA kinetics on-treatment are predictive of PFS.

**Perspectives**

- Assess metrics on a validation cohort (150 more patients to come).
- Subgroups analyses stratified by pathology and treatment.
- Improve mechanistic modeling, exploring fragment size-dependent release and fragmentation mechanisms
- Integrate the mechanistic modeling parameters into a machine learning framework
- Develop a medical device to individually predict the ICI resistance of new patients.