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Mechanistic Modeling of Joint Circulating Cell-free DNA Concentration—Tumor Size Kinetics under Immune-Checkpoint Inhibitors in Advanced Cancer

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ChISM

Size
Cell-free DNA
Immunotherapy
Signature
Monitoring

Hôpitaux Universitaires de Marseille ap-hm

adelis ADVANCED SEPARATIVE TECHNOLOGIES

amU Institut Laënnec, sciences numériques et IA pour la santé Aix Marseille Université

id solutions oncology

CRCM Centre de Recherche en Cancérologie de Marseille 50 ans de recherche et d'innovation

amU Institut cancer et immunologie Aix Marseille Université

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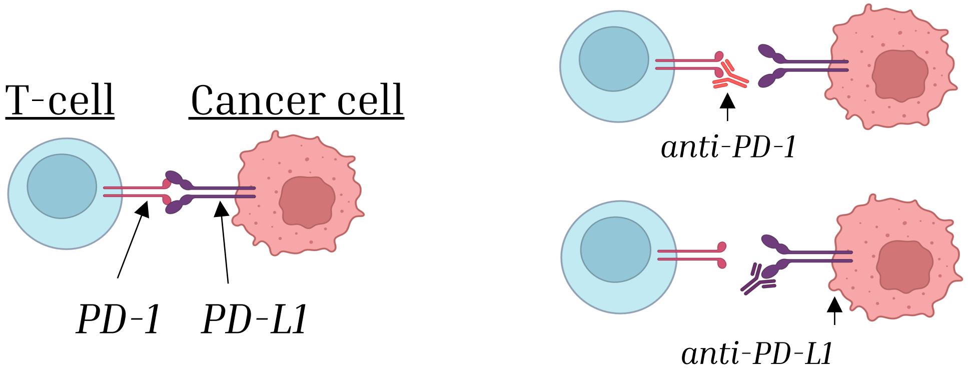
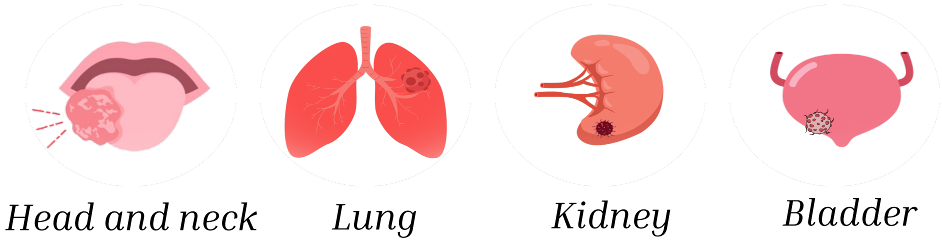
Immunotherapy in oncology : how to predict progression?

- 2011+ : FDA approval of immune-checkpoint inhibitors (ICI) targeting PD-1)
- 20-40 % long-term response[1]



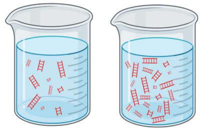


PREDICTING PROGRESSION?

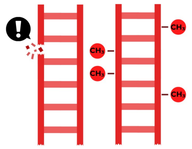
Gold standard biomarkers: PD-L1 expression (+ TMB)

New biomarker: liquid biopsy[2] → Circulating cell-free DNA (**cfDNA**)

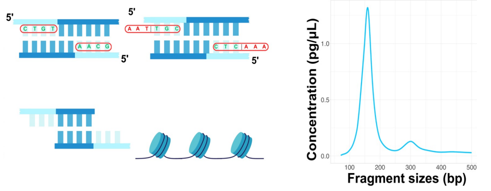
- ✓ Half-life: 15min-2h
- ✓ Systemic
- ✓ Non-invasive
- ✓ Cost-effective



Concentration[3]



Mutations



Fragmentome

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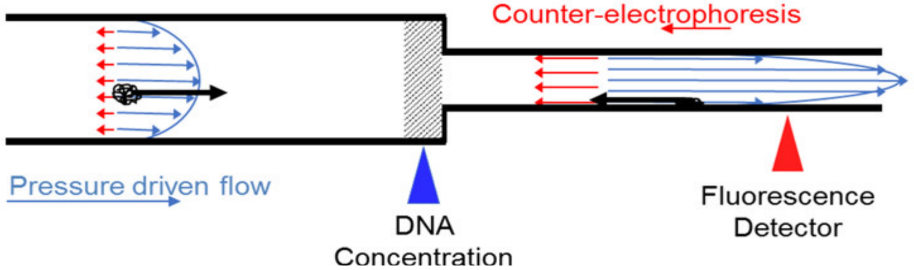
[1] Gilberto De Castro et al., J Clin Oncol, 2022; Sharma et al., Cell, 2017
[2] Siravegna et al., Nat Rev Clin Oncol, 2017
[3] Ribba et al., Front Pharmacol, 2023

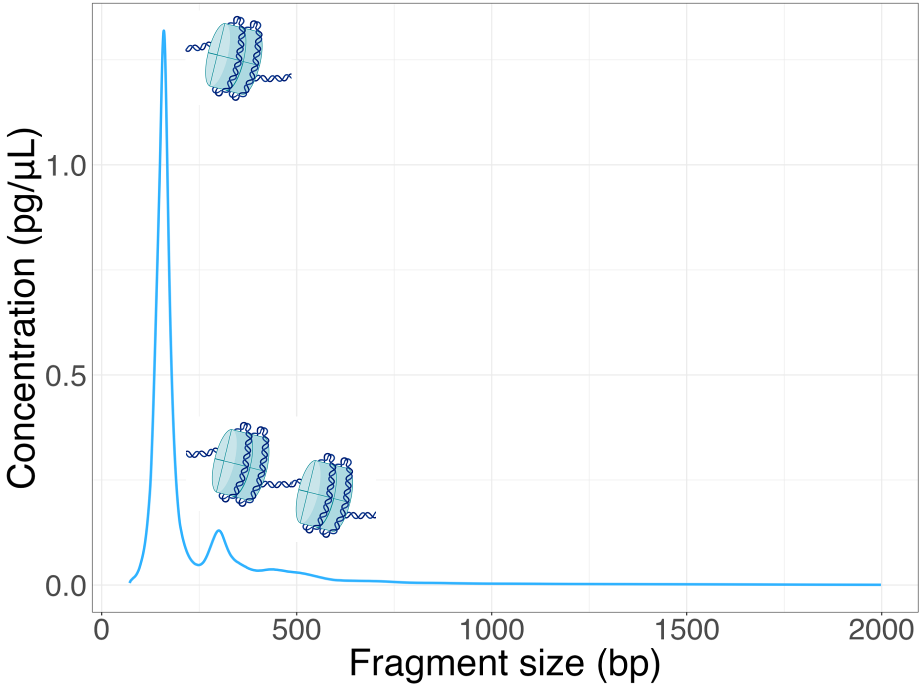
TMB = Tumor Mutational Burden

CfDNA size profile as a promising biological marker

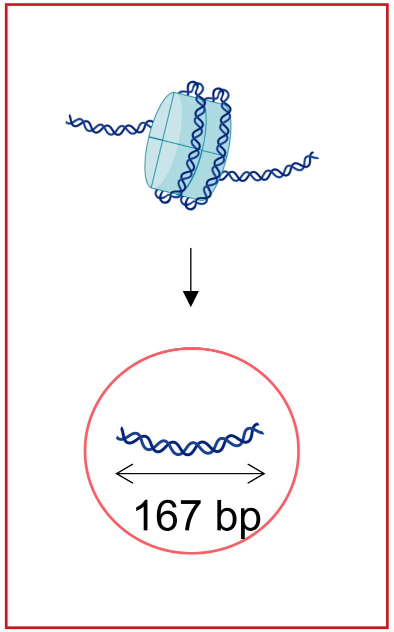
µLAS technology:



- Independent of genome position
- No need of prior DNA extraction
- Only needs 1 µL of plasma
- Cost-effective ~ 15€/sample
- 2 bp - accuracy on fragments sizes



☐ Apoptosis
☐ Necrosis
☐ Active secretion



Andriamanampisoa et al, Anal Chem, 2018; Boutonnet et al, Anal Chem, 2023 Grabuschnig et al., Int J Mol Sci, 2020 bp = base pair

Objectives

ChISM

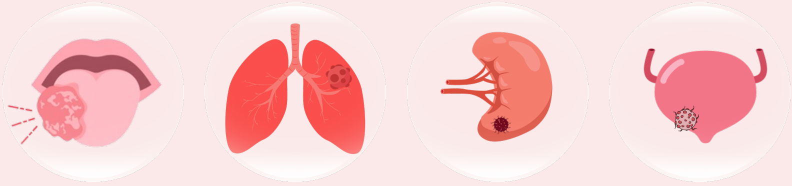
Size
Cell-free DNA
Immunotherapy
Signature
Monitoring

Develop a **mechanistic model** of the **joint cfDNA – tumor kinetics (TK)** in advanced cancer patients undergoing ICI

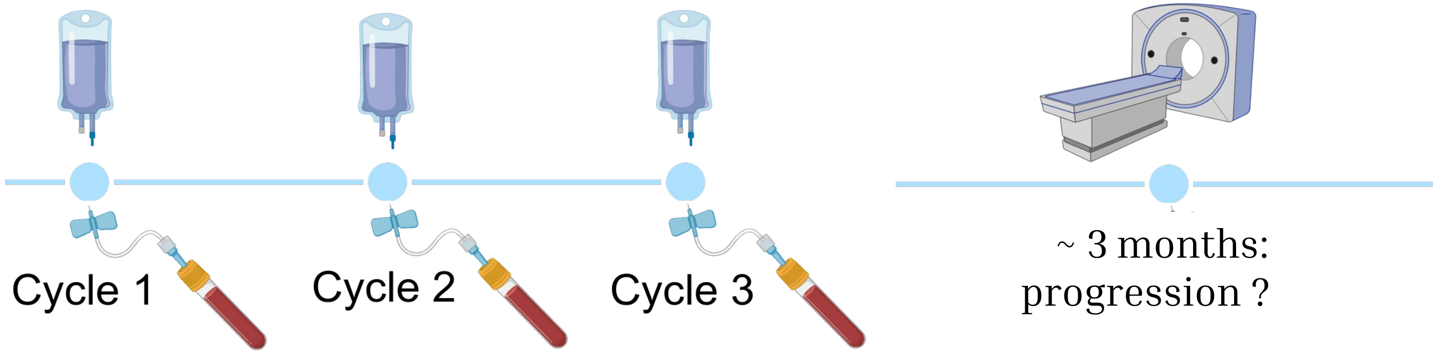
Assess pre-treatment **cfDNA size profiles** and **early, on-treatment, model-based parameters** as predictors of immunotherapy resistance

SChISM: Size CfDNA Immunotherapy Signature Response

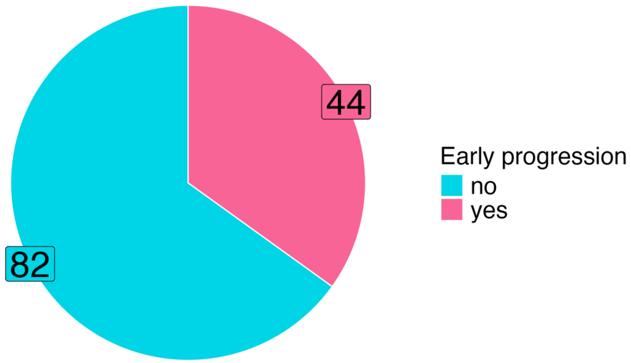
126 patients

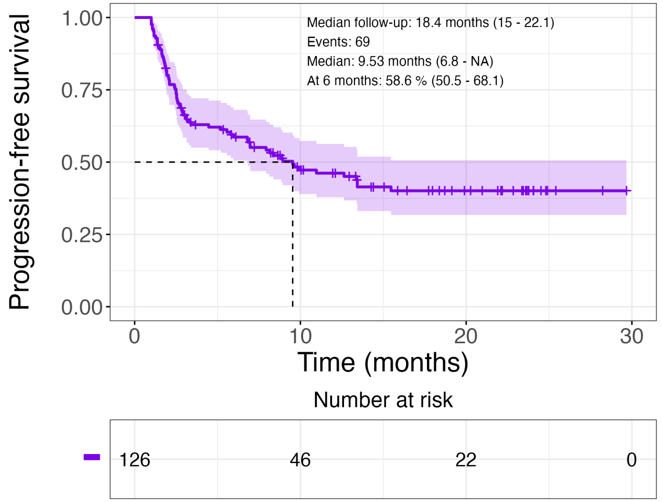


ICI



~ 3 months: progression ?





- Outcomes:
  - Early progression (EP)
  - Progression-free survival (PFS)

SChISM: Size CfDNA Immunotherapy Signature Response

Clinical variables

Age, tumor type, sex, Eastern Cooperative Oncology Group (ECOG)

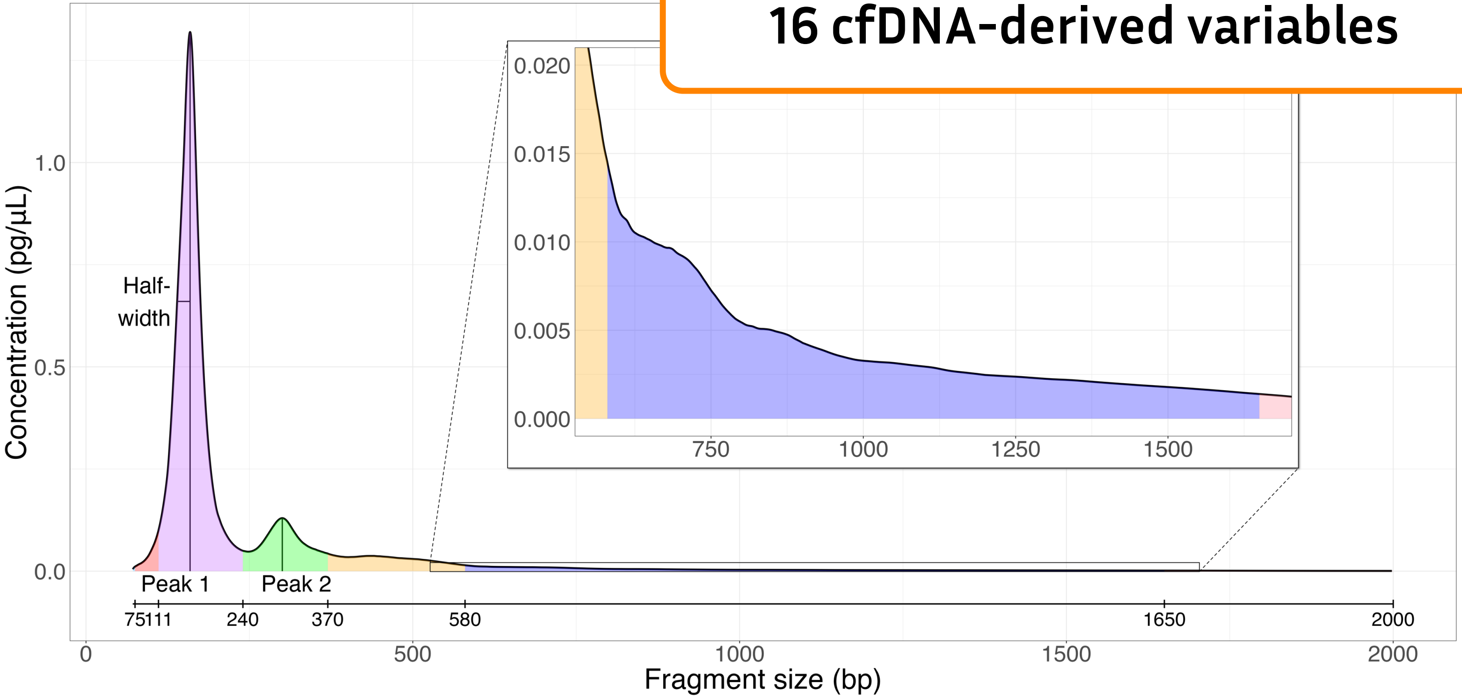
Biological variables

Neutrophil to lymphocyte ratio ($\boldsymbol{NLR}$)

Lactate dehydrogenase level (LDH)

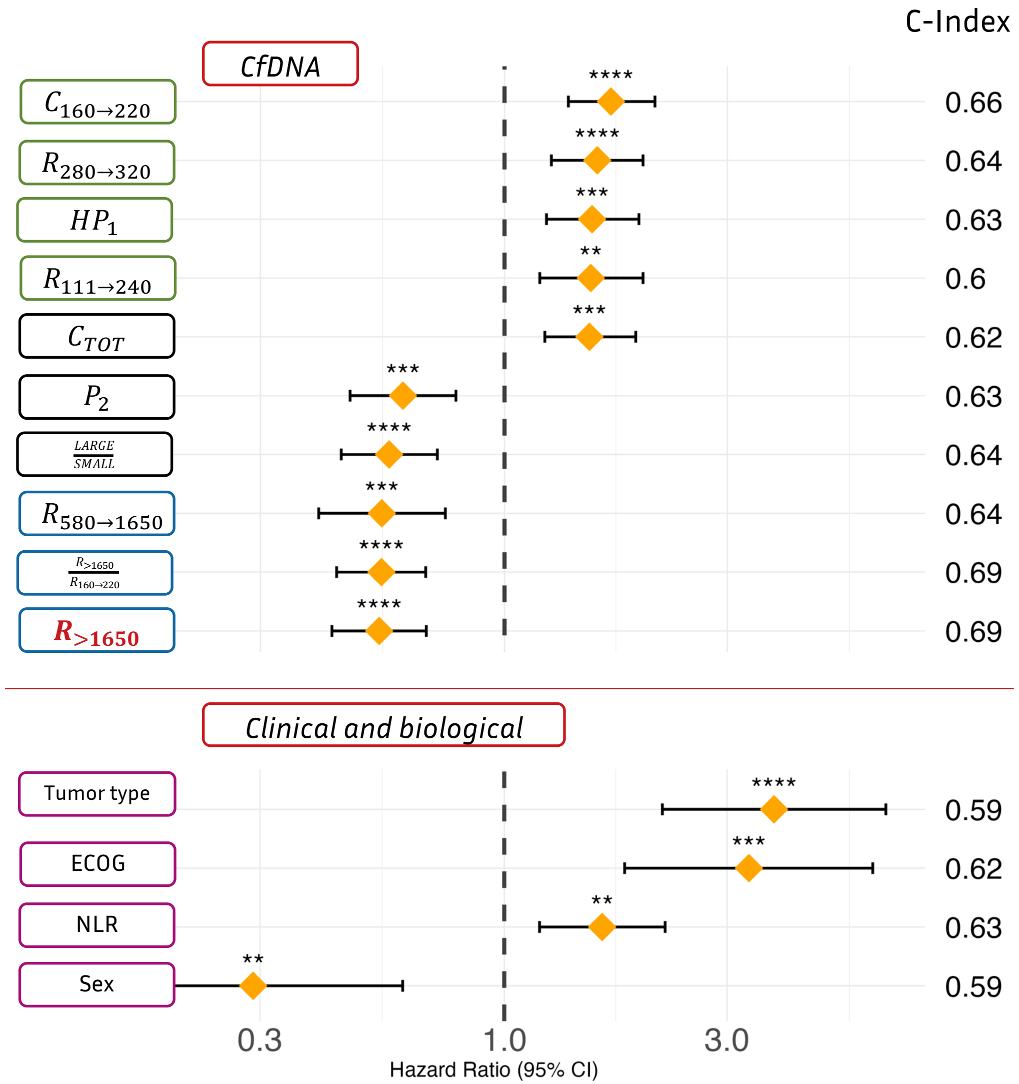
CfDNA variables

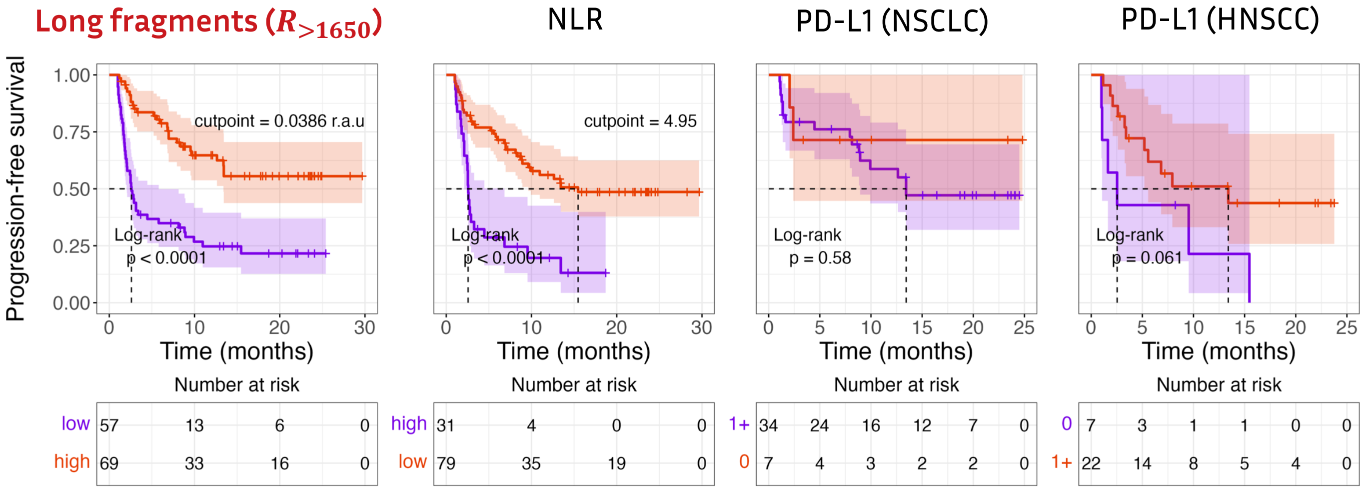
- Total concentration (pg/µl) $\boldsymbol{C_{TOT}}$
- Location of the peaks (bp) $\boldsymbol{P_1, P_2}$
- Height of the peaks (pg/µl) $\boldsymbol{HP_1, HP_2}$
- Half-width of first peak $\boldsymbol{HW_1}$
- Absolute concentrations (pg/µl): $\boldsymbol{C_{a\to b}}$
- Relative concentrations $\boldsymbol{R_{a\to b}}$ (over C_{TOT})

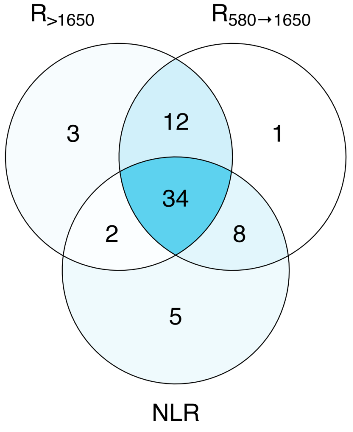


16 cfDNA-derived variables

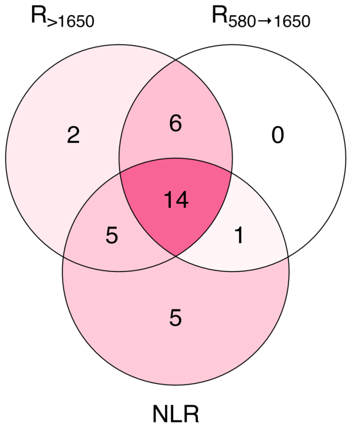
High proportion of long fragments at baseline is associated with response







True responders



True progressors

- Multi-cancer $R_{>1650}$:
  - AUC test: **0.74** [0.65—0.83]
  - PPV test: **0.56** [0.45—0.68]

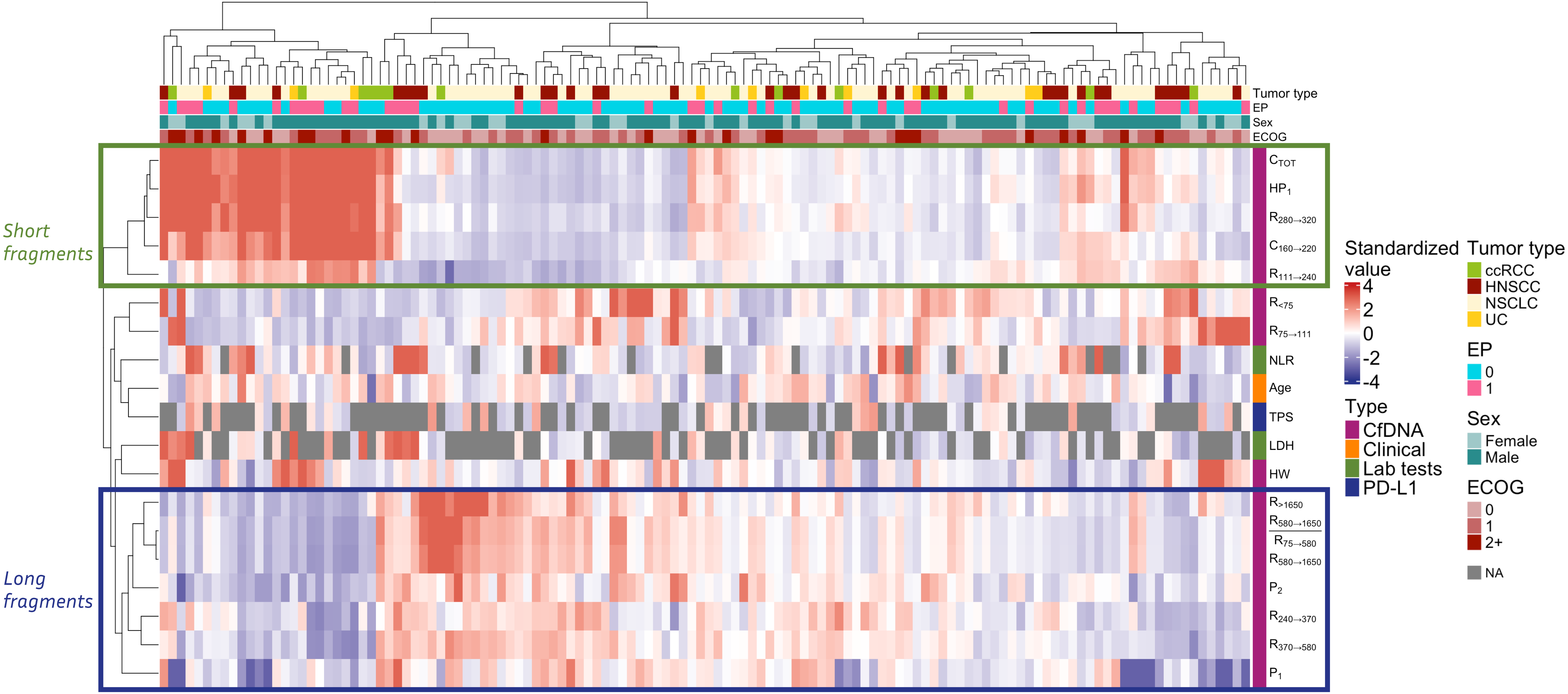
- Multi-cancer PD-L1[1]:
  - AUC: **0.65**
  - PPV: **0.34**
- Multi-cancer TMB[1]:
  - AUC: **0.69**
  - PPV: **0.42**

Salas, Nguyen-Phuong et al, ASCO, 2024; ESMO 2024

[1] Lu et al., JAMA Oncol., 2019

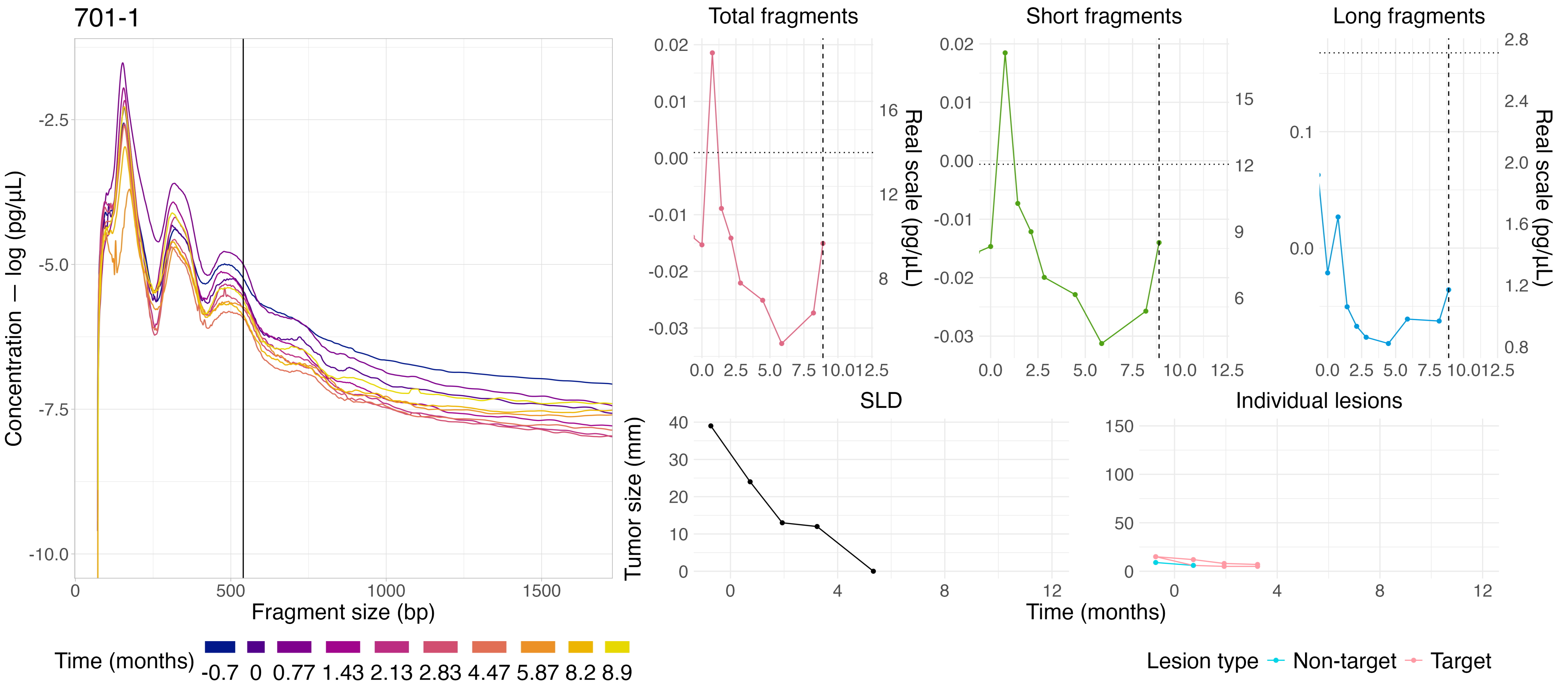
AUC = Area Under the ROC Curve
PPV = Positive Predictive Value

Patients cluster according to their fragment size distribution



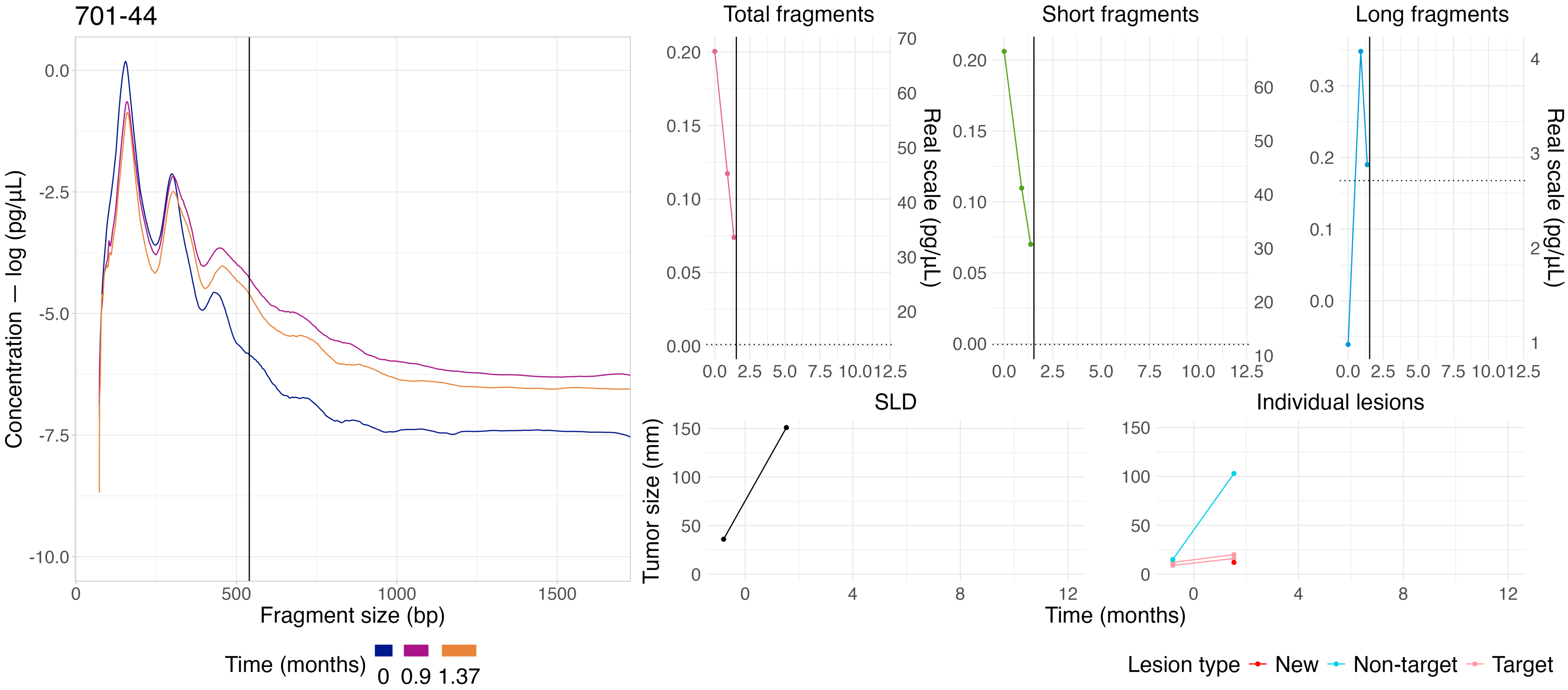
Responder

Short and long fragments showed different kinetics

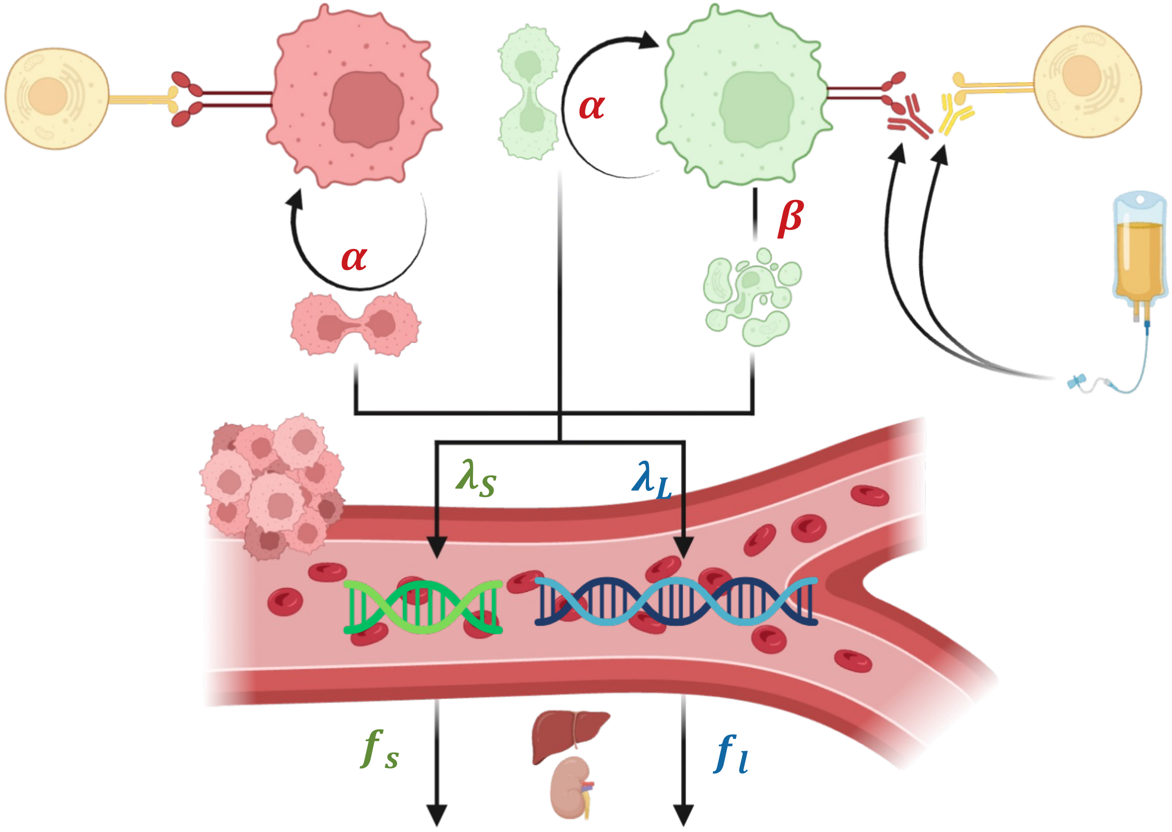


Progressor

Short and long fragments showed different kinetics



Joint modeling of tumor and size-dependent cfDNA kinetics



1. Tumor cells (Sum of Largest Diameters, SLD, T) comprise two sub-populations: treatment-**resistant** cells T_R and treatment-**sensitive** T_S ones.
2. Short fragments D_S 540-75) bp) are **proportionally** released through:
   - T **growth**, through **active secretion** during **proliferation**
   - T_S **death** through **apoptosis**
3. Long fragments D_L 1650-540) bp) are **proportionally** released through:
   - T **growth**, through **active secretion** and/or **necrosis** of the tumor microenvironment
   - T_S **death** through **necrosis**
4. CfDNA is **cleared** from the circulation by liver and kidneys, **depending on fragment size** $f_s(D_s)$, $f_l(D_l)$.

$$
\begin{cases}
\dfrac{dT_R}{dt} = \alpha \cdot T_R \\
\dfrac{dT_S}{dt} = \begin{cases} \alpha \cdot T_S & if\ t < 0 \\ (\alpha - \beta) \cdot T_S & if\ t \geq 0 \end{cases} \\
T = T_R + T_S \\
\dfrac{dD_s}{dt} = \lambda_s \cdot (\alpha \cdot T + \beta \cdot T_S) - f_s(D_s) \\
\dfrac{dD_l}{dt} = \lambda_l \cdot (\alpha \cdot T + \beta \cdot T_S) - f_l(D_l)
\end{cases}
$$

Initial conditions:

$$
\begin{cases}
T_S(t = 0) = T_{S_0} \\
T_R(t = 0) = T_{R_0} \\
D_S(t = 0) = D_{S_0} \\
D_L(t = 0) = D_{L_0}
\end{cases}
$$

Nguyen et al., AACR 2025

Population approach

Non-linear mixed-effects

$$\theta = \{T_{R_0}, T_{S_0}, \alpha, \beta, D_{s_0}, \lambda_s, k_{D_s}, D_{l_0}, \lambda_l, k_{D_l}\}$$

$$\forall \theta_k \in \theta, \log(\theta_k) \sim \mathcal{N}\left(\log\left(\theta_{k_{pop}}\right), \omega^2_{\theta_k}\right)$$

1) Tumor size parameter identification independently of the cfDNA data

Tumor error model: constant

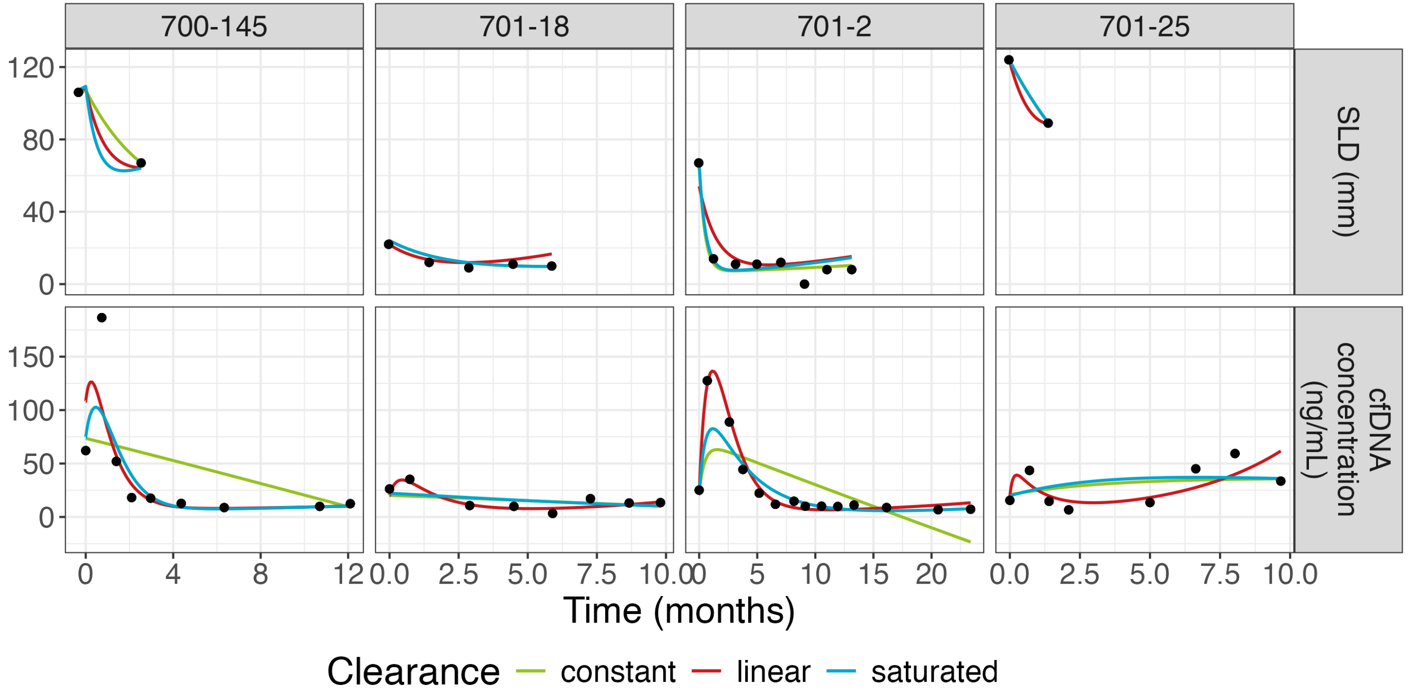
2) Joint tumor—cfDNA parameters identification with tumor population parameters fixed

cfDNA error models: proportional

n = 109

Number of samples per patient	
	median (min-max)
Tumor imaging	2 (1—10)
cfDNA samples	7 (1—20)

Best clearance function: linear



$$\begin{cases} \dfrac{dD_s}{dt} = \lambda_s \cdot (\alpha \cdot T + \beta \cdot T_S) - kD_s \cdot D_s \\ \dfrac{dD_l}{dt} = \lambda_l \cdot (\alpha \cdot T + \beta \cdot T_S) - kD_l \cdot D_l \end{cases}$$

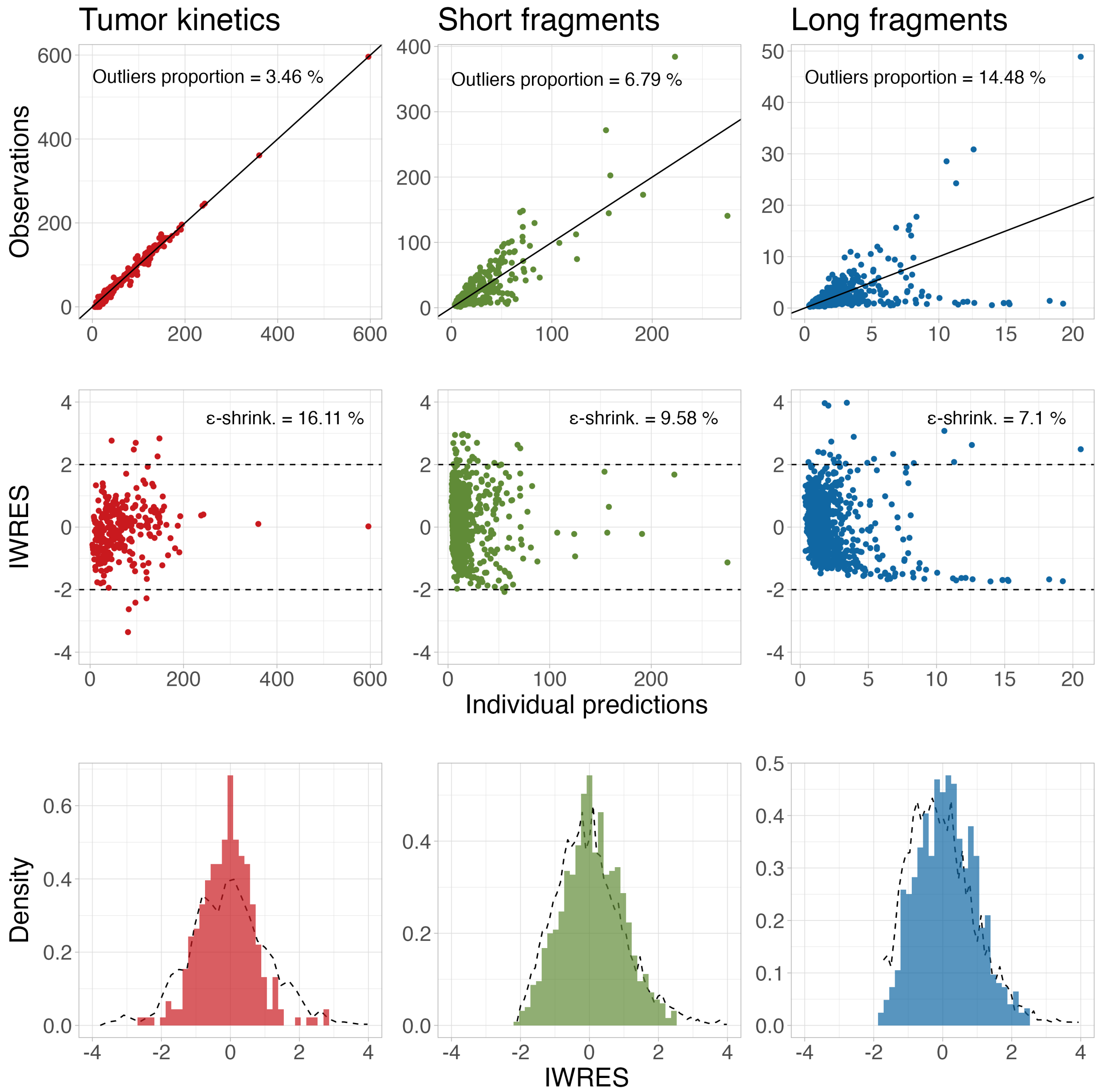
Model diagnostics

TK model			STOCHASTIC APPROXIMATION	
	VALUE	C.V. (%)	S.E.	R.S.E.(%)
FIXED EFFECTS				
α_{pop}	0.065		0.015	23
β_{pop}	0.36		0.045	12
$TR_{0_{pop}}$	7.9		1.7	22
$TS_{0_{pop}}$	43		4.1	9.7
STANDARD DEVIATION OF THE RANDOM EFFECTS				
$\omega_{\alpha_{pop}}$	0.9	110	0.13	14
$\omega_{\beta_{pop}}$	0.83	99	0.12	14
$\omega_{TR_{0_{pop}}}$	0.88	110	0.15	17
$\omega_{TS_{0_{pop}}}$	0.86	100	0.073	8.5
ERROR MODEL PARAMETERS				
a	8.7		0.74	8.5

Joint model			STOCHASTIC APPROXIMATION	
	VALUE	C.V. (%)	S.E.	R.S.E.(%)
FIXED EFFECTS				
$D_{s_{0_{pop}}}$	11		0.94	8.9
$D_{l_{0_{pop}}}$	1.4		0.1	7.4
$\lambda_{s_{pop}}$	0.38		0.047	12
$\lambda_{l_{pop}}$	0.086		0.013	15
$kD_{s_{pop}}$	0.29		0.038	13
$kD_{l_{pop}}$	0.53		0.089	17
STANDARD DEVIATION OF THE RANDOM EFFECTS				
$\omega_{D_{s_{0_{pop}}}}$	0.71	82	0.07	9.9
$\omega_{D_{l_{0_{pop}}}}$	0.51	54	0.062	12
$\omega_{\lambda_{s_{pop}}}$	0.94	120	0.095	10
$\omega_{\lambda_{l_{pop}}}$	0.96	120	0.094	9.8
$\omega_{kD_{s_{pop}}}$	0.95	120	0.11	11
$\omega_{kD_{l_{pop}}}$	1.2	170	0.12	11
ERROR MODEL PARAMETERS				
b_{SHORT}	0.43		0.013	3
a_{TK}	8.7			
b_{LONG}	0.55		0.018	3.2

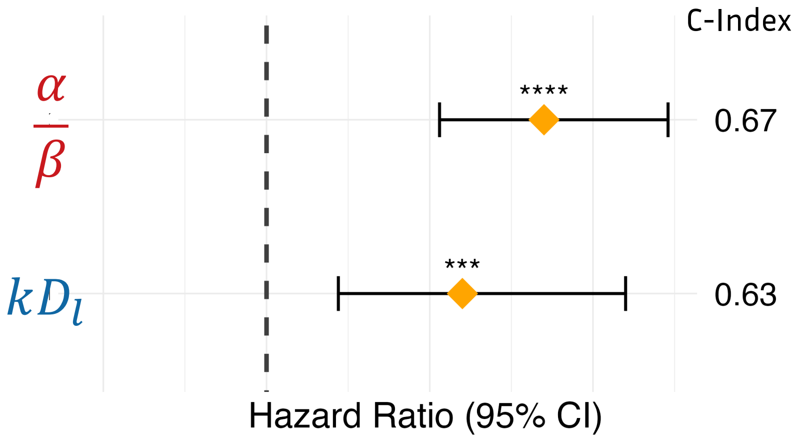
Correlation of the estimates $\in [-0.12, 0.44]$

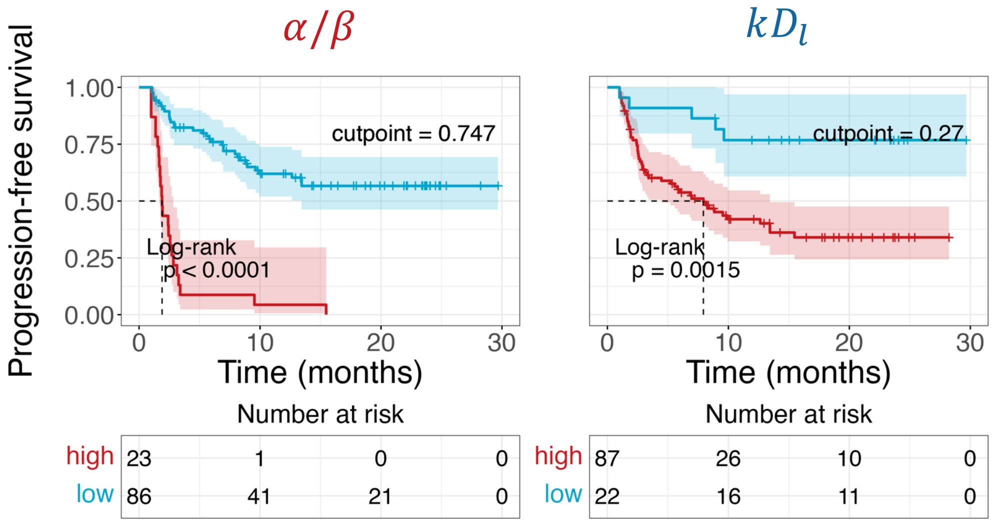
Condition number = **4.74**



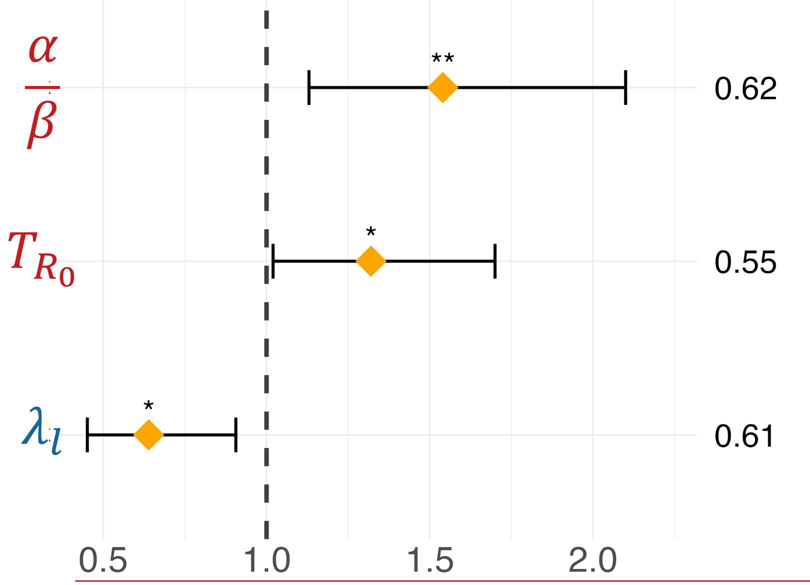
Parameters of the dynamic modeling are predictive of the PFS

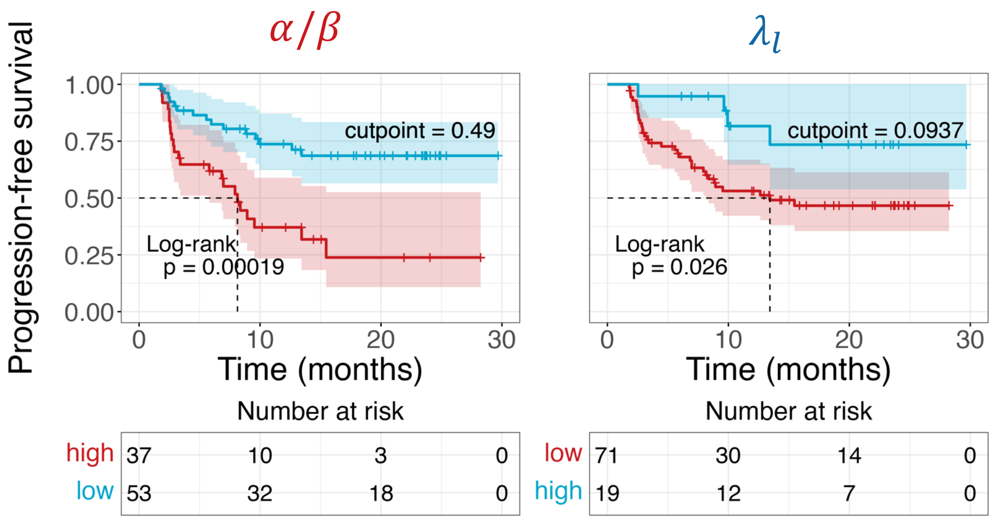
Full kinetics
(n = 109)





Early-on treatment
(6 weeks, n = 90)



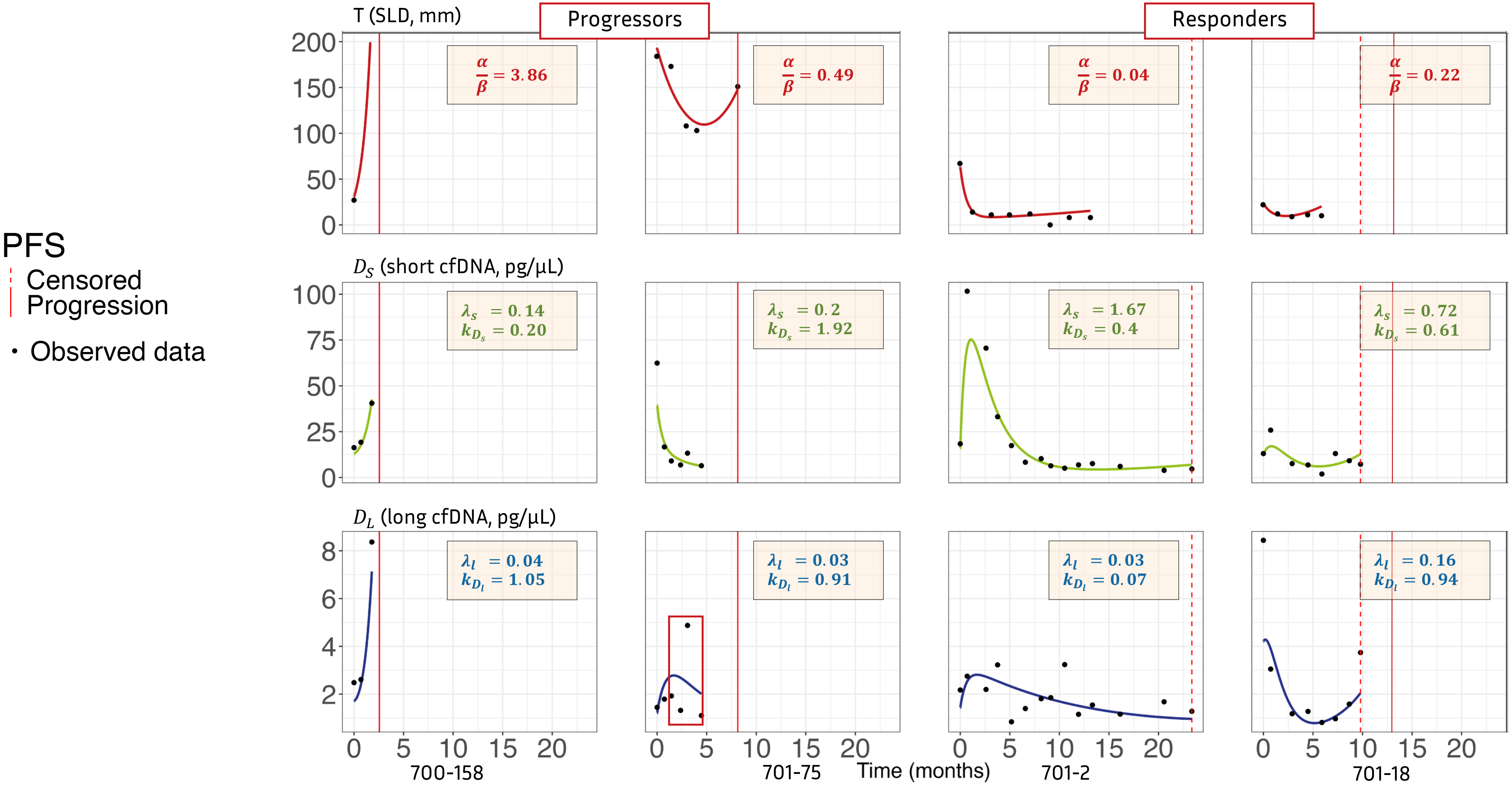


Nguyen et al., AACR 2025

α: tumor growth rate
β: tumor decay rate

kD_l: long fragments clearance rate
λ_l: long fragments release rate

The model describes different size-dependent cfDNA kinetics



Nguyen et al., AACR 2025

Conclusions and perspectives

- ICI-treated patients **with lower fragmentation** of cfDNA **before** treatment tend to **respond better** and to have **longer PFS**.
- Mechanistic modeling offers biological insights to explain the **interplay between cfDNA and tumor kinetics**.

Agnostic statistical modeling → Mechanistic modeling of joint cfDNA-tumor kinetics → **Mechanistic modeling of joint size-dependent cfDNA-tumor kinetics**

Did not fit

Fitted but not predictive

Fitted and predictive

- Assess results on **validation** cohort (150 patients to come)
- Improve mechanistic modeling, exploring **cfDNA from hematopoietic origin**
- Joint **TK-cfDNA-PFS** modeling
- **Integrate the model** parameters into multivariable **machine learning**

Acknowledgement

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Thanks for your attention!