Circulating cell-free DNA size distribution as a prediction marker for early progression undergoing immune checkpoint inhibitors

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cfDNA : non-encapsulated fragments of DNA released, during apoptosis, necrosis or lysis, in body fluids. Its half-life turn around 15 min, making its monitoring more representative of the real-time present quantities.

circulating tumor DNA : portion of circulating cell-free DNA originating from tumor cells.

BACKGROUND

Despite a significant proportion (20 to 40%)[1] of advanced cancer patients having long-term response to treatment with immune checkpoint inhibitors, many of them do not respond and experience early progression, defined as progression at the first imaging evaluation. Establishing reliable and early predictive biological markers for guiding clinical practice is essential. Analysis of circulating cell-free DNA (cfDNA) fragments size distribution profiles (fragmentome)[2] offers a promising non-invasive method for assessing treatment response independently of a specific molecular target, cancer type, and treatment.

STUDY

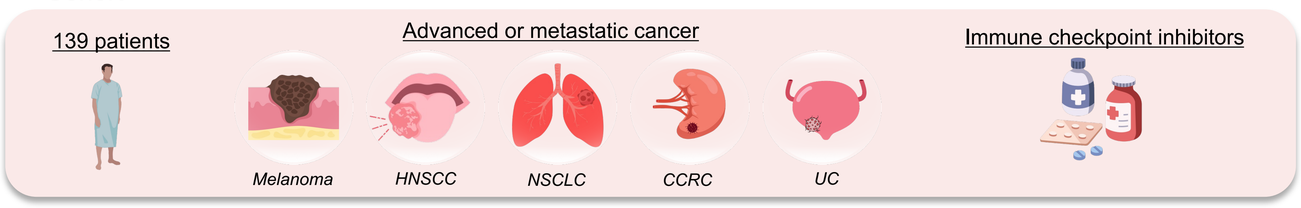
SChISM (Size CfDNA Immunotherapies Signature Monitoring): clinical study proposing an innovative approach based on patented, standardized cfDNA quantification methods (provided by **ID-Solutions and Adelis**), providing size profiles fluctuations of plasmatic cfDNA.

OBJECTIVE

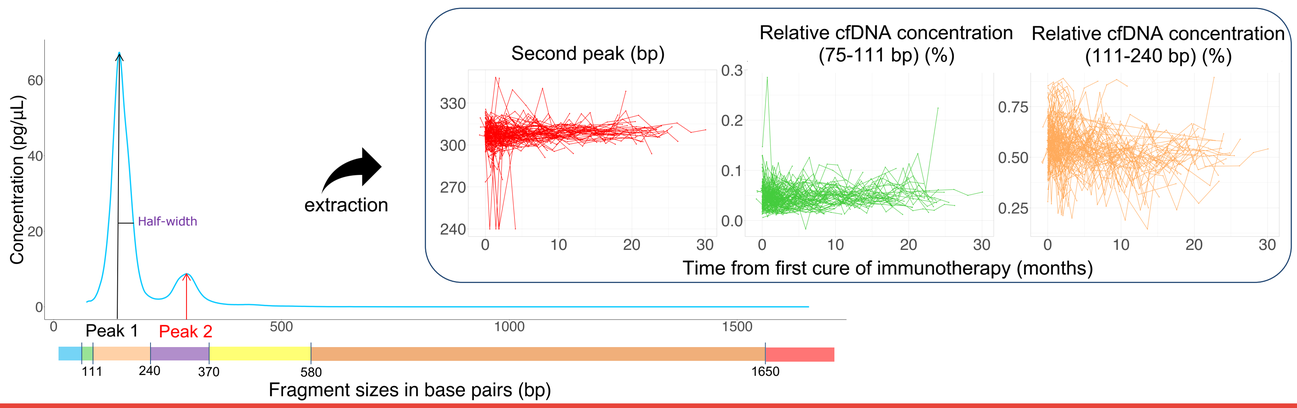
To model real-time variations in cfDNA size profile to early predict response and survival following immunotherapy for five cancer types, to early adjust therapeutic strategy and prevent ICI-related progression or toxicity.

DATA

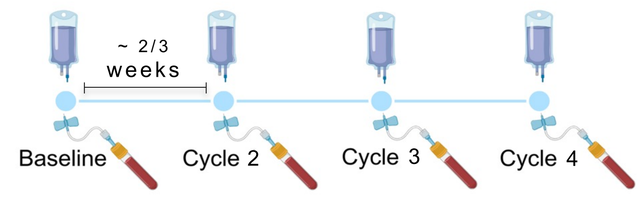
- Cohort



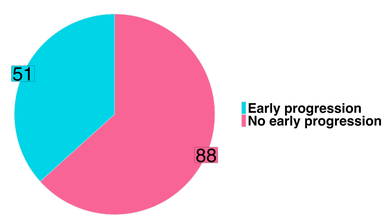
- CfDNA size profiles quantified before each immunotherapy infusion, from which 11 cfDNA metrics are extracted

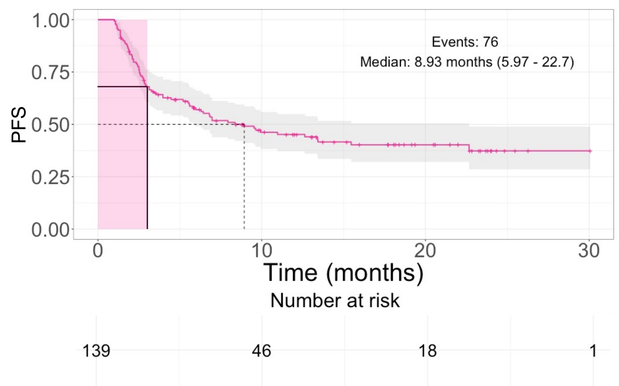


- Clinical and biological data, at baseline and during treatment



- Two outcomes
  - Early-progression
  - Progression-free survival (PFS)

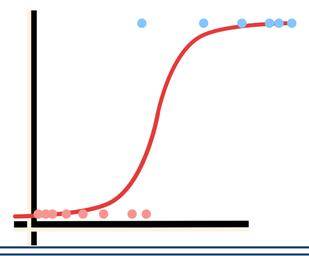




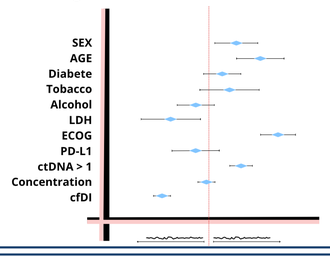
METHODS

Classification and survival analyses at baseline

Statistical analysis
- Boxplots and barplots
- Logistic regression
- Student t-test or Chi-2 test



Survival analysis
- Kaplan-Meier estimator
- Proportional hazard Cox regression
- Log-rank test



- Non-linear mixed effects modeling

Population approach:

Non-linear mixed effects modeling

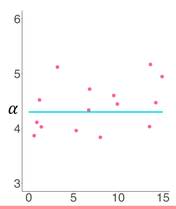
$$y_j^i = \mathcal{M}(t_j^i;\theta^i) + \varepsilon_j^i$$
$$\ln(\theta^i) = \ln(\theta_{pop}) + \eta_i$$

Where
- $\eta_i \sim \mathcal{N}(0,\omega^2)$
- ε_j^i is proportional, $\varepsilon_j^i \sim \mathcal{N}\left(0,\left(b\mathcal{M}(t_i^i;\theta^i)\right)^2\right)$

Longitudinal modeling

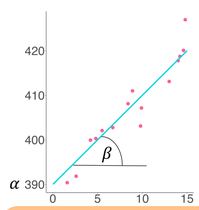
- Empirical longitudinal modeling

Constant



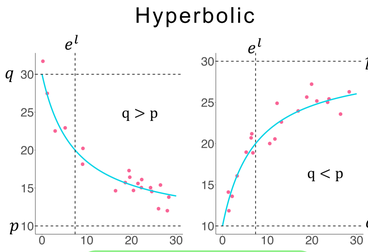
$y_j^i = \alpha^i, \quad \forall j$

Linear



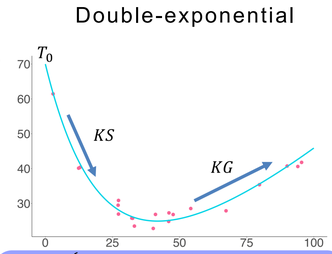
$y_j^i = \alpha^i + \beta^i t_j^i, \quad \forall j$

Hyperbolic



$$y_j^i = p^i + \frac{e^{l^i}(q^i - p^i)}{t_j^i + e^{l^i}}$$

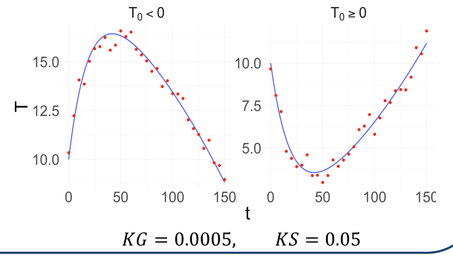
Double-exponential



$$y_j^i = \begin{cases} S_0^i e^{KG^i t} & t \le 0 \\ T_0^i\left(e^{KG^i t} + e^{-KS^i t} - 1\right) & t > 0 \end{cases}$$

- Integration of 'decline following growth' modeling (concave curve)

$$\begin{cases} \frac{dS}{dt} = -KS.S \\ \frac{dR}{dt} = KG.R \\ T = S + R \\ T(t=0) = |T_0| \end{cases} \Rightarrow T(t) = \begin{cases} T_0(e^{KG.t} + e^{-KS.t} - 1), & T_0 \ge 0 \\ T_0(e^{KG.t} + e^{-KS.t} - 3), & T_0 < 0 \end{cases}$$

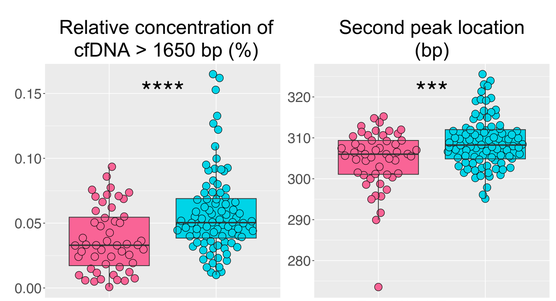


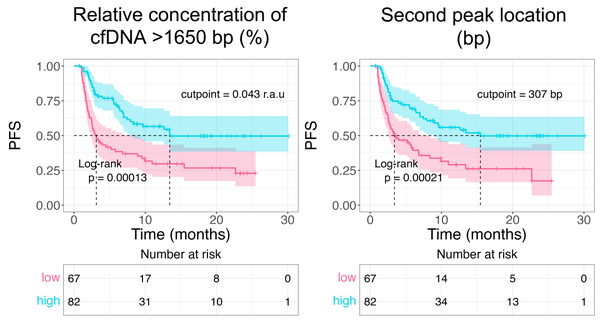
$KG = 0.0005, \quad KS = 0.05$

RESULTS

Results at baseline

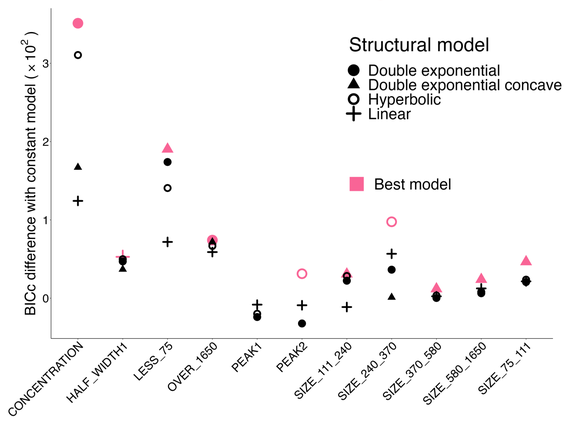
	Classification analysis						Survival analysis					
	AUC	NA	OR uv	Signif UV	OR mv	Signif MV	C Index	HR uv	Signif UV	HR mv	Signif MV
Relative concentration of cfDNA (> 1650 bp)	**0.691**	0.0%	**0.41 (0.25 - 0.67)**	***	**0.48 (0.28 - 0.83)**	**	**0.667**	**0.49 (0.36 - 0.69)**	****	**0.55 (0.40 - 0.77)**	***
Second peak	**0.649**	0.0%	**0.49 (0.32 - 0.76)**	**	**0.52 (0.31 - 0.87)**	*	**0.639**	**0.59 (0.46 - 0.75)**	****	**0.67 (0.52 - 0.87)**	**
Relative concentration of cfDNA (580 - 1650 bp)	0.621	0.0%	**0.57 (0.37 - 0.86)**	**	**0.69 (0.43 - 1.1)**	n.s.	**0.608**	**0.61 (0.46 - 0.82)**	***	**0.70 (0.53 - 0.94)**	*
Age	0.512	0.0%	0.93 (0.66 - 1.32)	n.s.	0.78 (0.53 - 1.17)	n.s.	0.505	1 (0.79 - 1.26)	n.s.	0.97 (0.76 - 1.24)	n.s.
LDH	0.359	36.7%	**3.3 (1.01 - 10.7)**	*	**6.34 (1.43 - 28.1)**	*	0.593	**1.27 (1.04 - 1.54)**	*	1.11 (0.91 - 1.35)	n.s.
Ratio neutrophiles/lymphocyte	**0.342**	18.0%	**2.46 (1.47 - 4.1)**	***	**2.08 (1.16 - 3.72)**	*	**0.627**	**1.63 (1.32 - 2)**	****	1.23 (0.94 - 1.6)	n.s.



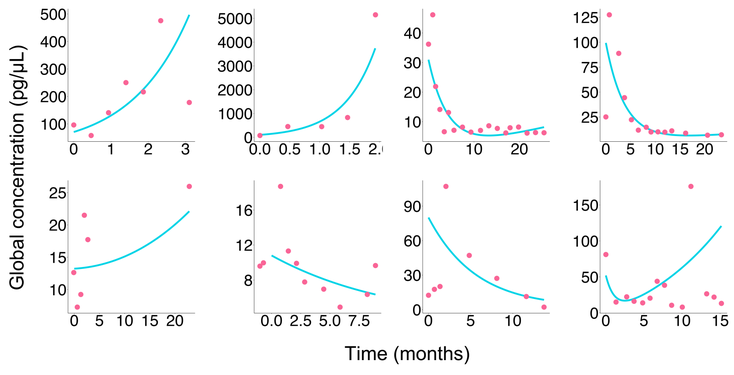


Longitudinal results

- 10/11 of the cfDNA metrics showed **significant kinetics over time**.



Individual fits estimated by the double-exponential model



FUTURE PERSPECTIVES

- **Refine population modeling**
  - Search for clinical covariates associated with model-derived parameters
  - **Mechanistic modeling** of the joint tumor kinetics and longitudinal cfDNA metrics, by integrating biological assumptions on the cfDNA release process
- **Predict early progression**
  - From early on-treatment metrics through Bayesian estimation
  - Using machine learning to combine multiple metrics into an **integrative algorithm**

1. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017;168(4):707-723. doi:10.1016/j.cell.2017.01.017
2. Qi T, Pan M, Shi H, Wang L, Bai Y, Ge Q. Cell-Free DNA Fragmentomics: The Novel Promising Biomarker. Int J Mol Sci. 2023;24(2):1503. doi:10.3390/ijms24021503