Large cfDNA fragments (≥ 1.5 kb) carry clinically and biologically relevant information for liquid biopsy

Frédéric Ginot[1]; Linh Nguyen Phuong[2]; Audrey Boutonnet[1]; Sébastien Benzekry[2]; Pascal Pedini[3]; Alizée Sébastian[3]; Frédéric Fina[1]; Sébastien Salas[2,4].

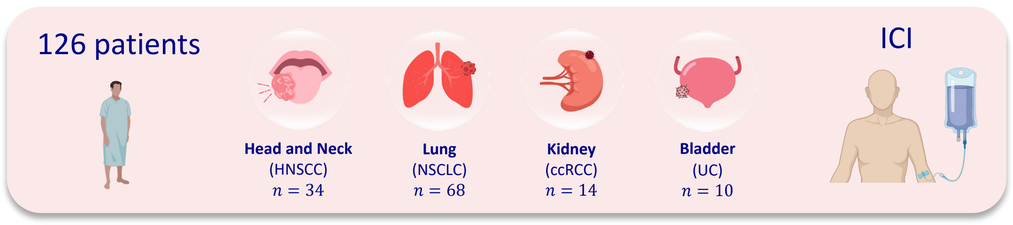
1. Adelis SAS, Labège, France | 2. COMPO, Inria, CRCM, Marseille | 3. Immunogénétique, EFS, Marseille | 4. AP-HM, Marseille, France

Introduction – the SChISM study

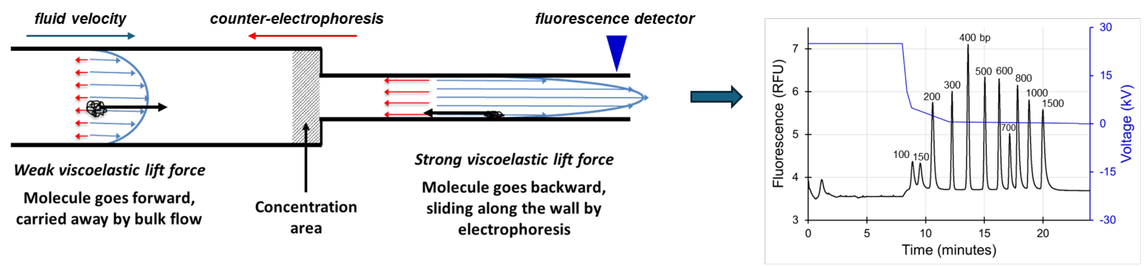
Immune-checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but a large proportion of patients is resistant to the treatment. The SChISM study (SI 20.07.23.62212) was designed to monitor and predict response by measuring the size distribution of cfDNA in patients ICI-treated in monotherapy or combination.

CLINICAL CHALLENGE

- 20-40%[1] of advanced cancer patients show long-term response to ICIs
- Many experience early progression (EP), progression at 1st radiological assessment
- Need for reliable early predictive biomarkers

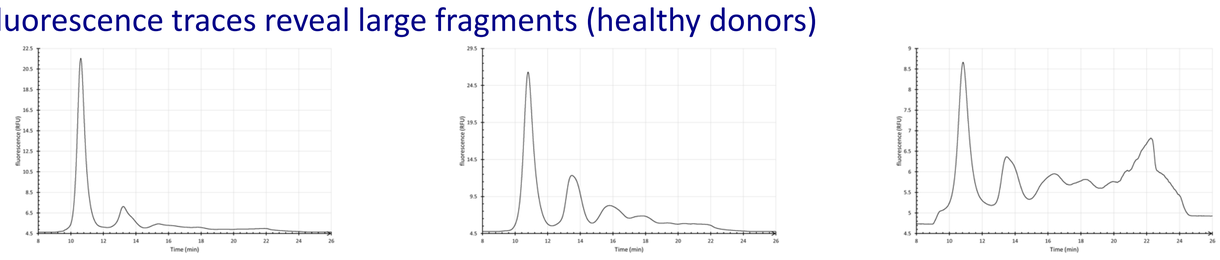


BIABooster System

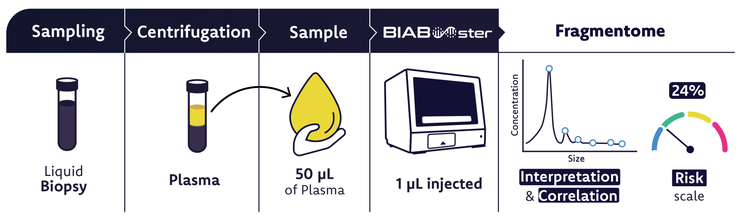


BIABooster system is based on a dual hydro- and electro-kinetic actuation, which pushes DNA towards walls, where flow rate is reduced. It gives an electrophoresis-like view of the sample, with a ~1,000-fold increase in sensitivity over state-of-the-art CGE, thanks to in-line DNA concentration. It can also operate without DNA extraction from plasma[2,3].

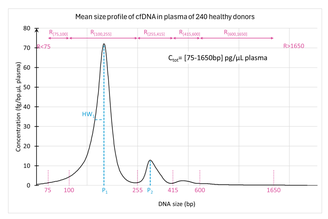
Fluorescence traces reveal large fragments (healthy donors)



Easy workflow

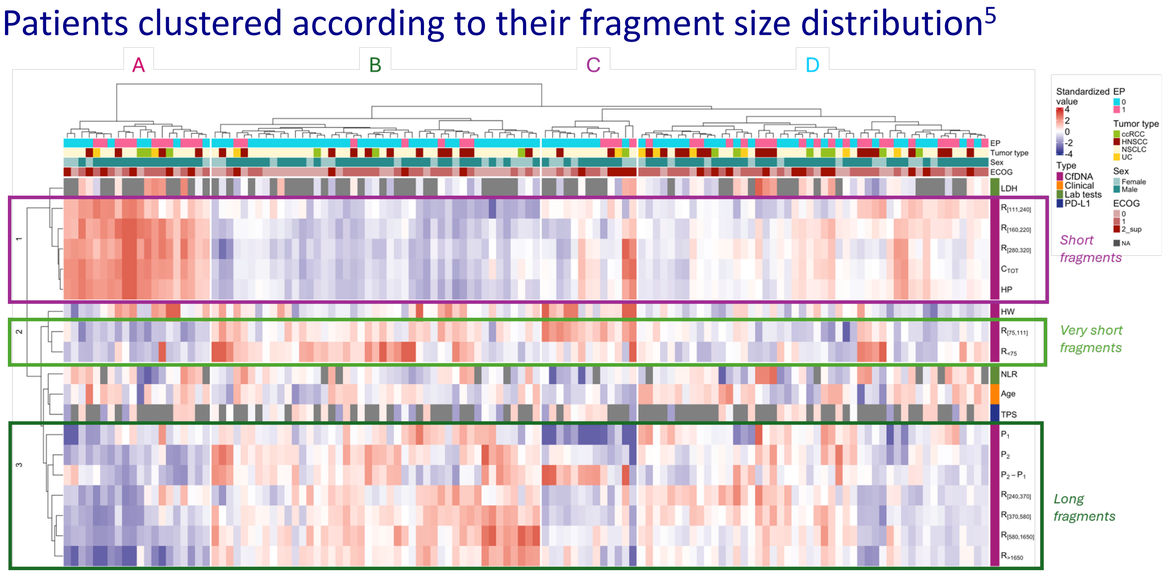


Size distribution descriptors

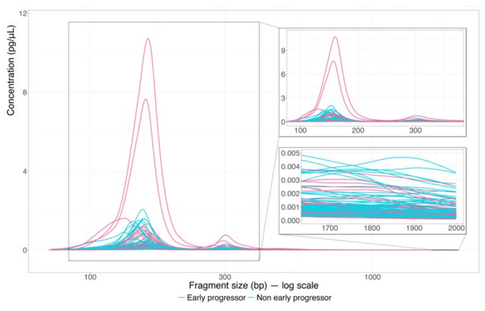


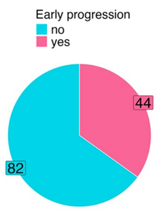
Results

Patients clustered according to their fragment size distribution[5]

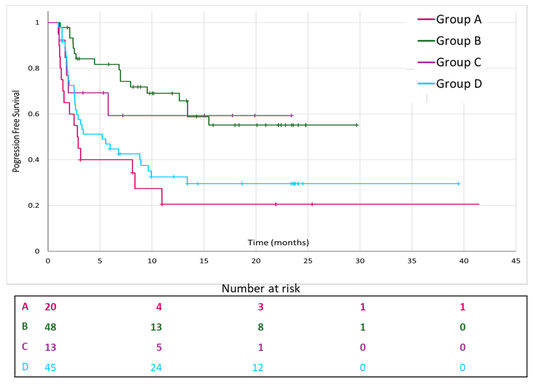


Unsupervised hierarchical clustering of clinical, biological, and cfDNA features across patients





Distribution of EP in patients



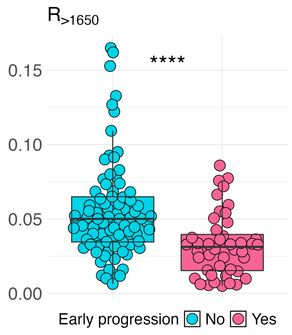
Progression-free survival (PFS) curves according to clusters

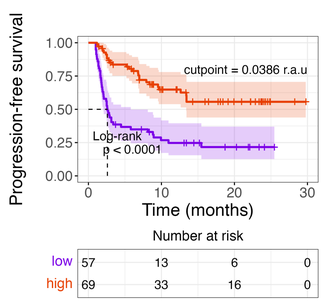
Presence of long fragments predict no early progression[4,5]

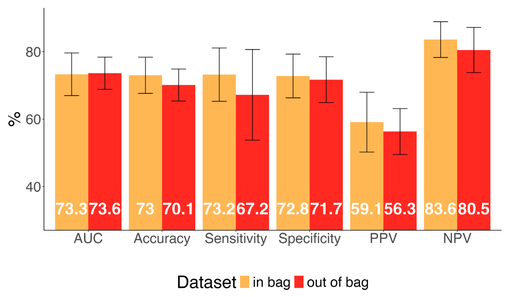
Logistic and Cox regressions results: high-molecular-weight fragments ratio as the best predictor of no EP and long PFS.

	EARLY PROGRESSION			PROGRESSION-FREE SURVIVAL		
VARIABLE	AUC	OR UV	OR MV	C INDEX	HR UV	HR MV
	CFDNA METRICS					
$R_{>1650}$	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) ****
$C_{160\to220}$	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) ****
$\frac{C_{580\to1650}}{C_{75\to580}}$	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) ****
$R_{280\to320}$	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) ****
$R_{580\to1650}$	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) ****
	CLINICAL AND BIOLOGICAL VARIABLES					
NLR	0.70	2.5 (1.5 — 4.2) ***		0.63	1.6 (1.2 — 2.2) **	

(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; ***:< 0.001; **: < 0.01; *: < 0.05)*







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

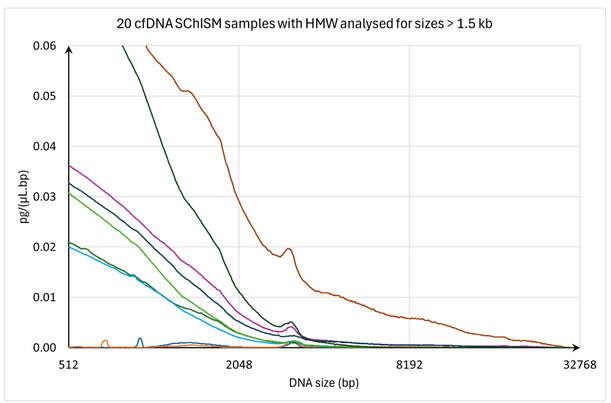
Mean ± SD of performance metrics computed over 100 bootstraps iterations

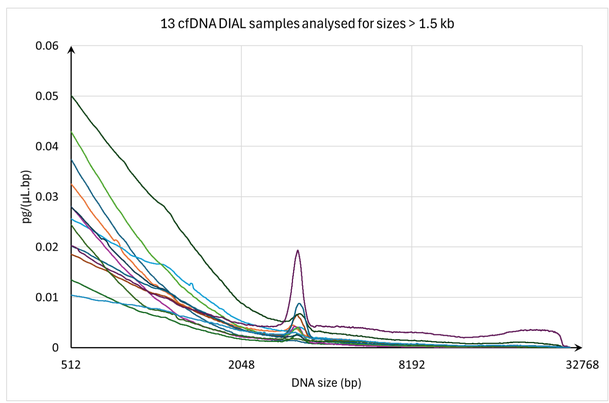
(70% train/in bag, orange; remaining out-of-bag patients, red)
(PPV: positive predictive value; NPV: negative positive value)

Conclusions

- ICI-treated patients with lower cfDNA fragmentation tend to not early progress and to have longer PFS.
- Our best baseline 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.
- An intriguing 3.1 kb fragment is often found, which needs to be identified.
- The BIABooster system enables studying full cfDNA size distribution in a straightforward manner.

An intriguing ~3.1 kb fragment is often found in size profiles





cfDNA was extracted and analysed for fragments greater than 1.5 kb:
Left : ID-Xtract extraction (ID-Solutions) – SChiSM Patients
Right : QIASymphony extraction (Qiagen) – Healthy Donors from DIAL study (SI 22.01274.000075)

Presence of the ~3.1 kb fragment :

- Healthy donors : 11 / 13 samples
- Cancer patients : 10/27 samples – no link with EP
- Working hypotheses :
  - Artefact caused by the fixative included in the blood collection tubes (Roche BCT)
  - If not, NETOSIS or mitochondrial derived cfDNA

BIABooster µLAS Technology

For more information contact: **fginot@adelis-tech.com**

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