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Circulating tumor DNA in advanced non-small-cell lung cancer patients with HIV is associated with shorter overall survival: Results from a Phase II trial (IFCT-1001 CHIVA)

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ABSTRACT

Introduction: HIV is an exclusion criterion for most lung cancer (LC) trials, however LC is the most common non-AIDS-defined malignancy in people living with HIV (PLHIV), poorer prognosis than the general population. Circulating tumor DNA (ctDNA) was a prognostic marker in LC patients from the general population. This study assessed ctDNA's prognostic value in PLHIV from a dedicated phase II trial.

Methods: Overall, 61 PLHIV with advanced non-squamous non-small-cell lung cancer (NSCLC) participated in the IFCT Phase II trial evaluating first-line four-cycle carboplatin (Ca) AUC5 pemetrexed (P) 500 mg/m^2 induction therapy every 3 weeks, followed by P maintenance therapy. Blood samples collected before treatment were analyzed to detect ctDNA using ultra-deep targeted next-generation-sequencing (NGS).

Results: Appropriate samples were available from 55 PLVIH and analyzed for ctDNA detection. Including 42 males (76.4 %), 52.9 years median age, 51 smokers (92.7 %), five with non-squamous NSCLC Stage III (9%), 50 Stage IV (91 %), and performance status (PS) 0−2. ctDNA was detected in 35 patients (64 %), 22 with high and 13 with low ctDNA levels. Overall, 77 % were positive for *TP53*, 29 % for *KRAS*, and 11 % for *STK11* mutations, more than one alteration was detected in 43 % of samples. Multivariate analysis showed that positive ctDNA was significantly associated with shorter PFS (HR, 4.31, 95 %CI: 2.06−8.99, $p < 0.0001$), and shorter OS (HR, 3.52, 95 %CI: 1.72−7.19, $p < 0.001$). Moreover, OS was significantly longer for patients with low ctDNA levels at diagnosis as compared to high ($p = 0.01$).

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Conclusion: We show that ctDNA detection using ultra-deep NGS is an independent prognostic factor in PLHIV with advanced NSCLC.

1. Introduction

Since the advent of highly active antiretroviral therapy (HAART), mortality rates of acquired immunodeficiency syndrome (AIDS) due to opportunistic infections or AIDS-defining cancers have markedly decreased [1]. Nevertheless, people living with HIV (PLHIV) still face higher risk of non-AIDS-related cancer, with lung cancer (LC) being the leading cause of cancer-related deaths [2–5].

Cancer chemotherapy or targeted agents have to be administered with care in PLHIV due to higher comorbidity rates in this population. Several national guidelines have recommended similar treatments for PLHIV as the general population, albeit with closer attention paid to the possible interactions or additive toxicities of antitumor drugs and HAART [6–8]. Clinical trial data on cancer treatment in PLHIV are scarce, as these patients are mostly excluded from trial participation. When our current trial was designed, no clinical study dedicated to PLHIV with non-squamous non-small cell lung cancer (NSCLC) was available. However, it was urgent to better define the therapeutic management of cancer-afflicted PLHIV. To achieve this goal, the IFCT1001-CHIVA (NCT01296113) trial was designed to optimize chemotherapeutic regimens for NSCLC in PLHIV and to identify prognostic markers [9].

Plasma circulating tumor DNA (ctDNA) is a noninvasive validated biomarker for the detection of druggable alterations such as the detection of *EGFR* mutations at diagnosis or recurrence. In 2016, we developed and published a next-generation sequencing (NGS) based approach to test for ctDNA in clinics [10]. Using this method, we demonstrated that ctDNA positivity at diagnostic was associated with a poor prognosis in patients with stage IV NSCLC independently of the type of mutation identified in plasma [11]. Moreover, ctDNA clearance under LC therapy was significantly linked to objective response, longer PFS and OS, thereby reflecting treatment efficacy [11].

In line with this study, we conducted an ancillary analysis of samples collected in the IFCT1001-CHIVA trial to better characterize molecular alterations in NSCLC in PLHIV, define potential prognostic factors, and identify targets for therapeutic approaches.

2. Methods

2.1. Study design and patient population

All eligible patients were HIV-positive with histological/cytological confirmed NSCLC [12] tumor-node-metastasis (TNM) Stage III-IV, and no previous chemotherapy. Other eligibility criteria were: age (18–75 years), Eastern Cooperative Oncology Group performance status (ECOG-PS) 0–2, weight loss $\leq$10 % of total body weight within the previous month, and normal organ function. This ancillary clinical trial received ethics committee approval and was conducted in line with French legislation, Good Clinical Practices, and the Declaration of Helsinki principles. All patients provided written informed study-specific consent. ClinicalTrials.gov Identifier: NCT01296113 [9].

Patients received first-line intravenous chemotherapy with pemetrexed (P) at 500 mg/m^2 bolus infused over 10 min, every 3 weeks, combined with carboplatin (C) bolus infusion, with a target area under the curve (AUC) value of 5 mg ml^{-1} min^{-1}, on Day 1 for four cycles maximum [13]. Thereafter, patients with controlled disease and ECOG-PS $\leq$2 were given P maintenance.

No other anticancer therapies were permitted for first-line therapy. All patients received optimal antiretroviral therapies during all the chemotherapy regimen. Chemoprophylaxis against *Pneumocystis* pneumonia (PCP) and toxoplasmosis with trimethoprim-sulfamethoxazole (TMP-SMX) or alternative agents (pentamidine, atovaquone) in case of allergy or intolerance, was left to the investigator's appreciation but recommended if CD4 count was below 200 cells/μL.

2.2. Patients and tumor material

The Phase II IFCT-1001, involving 61 PLHIV with advanced NSCLC, reached its primary endpoint, with 31 PLHIV exhibiting adequate disease control rate (DCR) at Week 12. This first trial dedicated to NSCLC, clearly revealed first-line four-cycle CP induction followed by P maintenance to be effective and reasonably well-tolerated, with no unexpected toxicities or opportunistic infections reported [9].

The ancillary study's objectives were to evaluate the prognostic value of ctDNA at baseline with respect to progression-free survival (PFS) and overall survival (OS), prior to treatment initiation and to define a ctDNA threshold, enabling patient stratification into high and low ctDNA levels to possibly use this threshold for other studies.

Blood samples for ctDNA detection were collected at baseline, prior to chemotherapy initiation. The samples were processed within 2 h, with plasma immediately stored frozen. The clinical data prospectively collected were gender, age, PS, smoking history, TMN tumor stage, treatment drugs, dates of treatment initiation and end, and radiological evaluation using RECIST criteria, along with dates of progression, death, and last follow-up.

2.3. DNA extraction from plasma samples

The Maxwell® RSC ccfDNA Plasma Kit on a Maxwell 16 system was used to extract 1 mL of plasma according to the manufacturer's instructions (Promega, Charbonnières-les-Bains, France). DNA was measured using the Qubit™ dsDNA HS Assay Kit (Life Technologies – Thermo Fischer Scientific, Saint Aubin, France). DNA samples were kept frozen at −20 °C before use.

2.4. NGS analysis and quantification

Colon and Lung Cancer Panel V2 libraries were prepared from 6 μL of cell free DNAs corresponding to a median of 4 ng [43−0.5 ng] DNA input following the recommended protocol from Ion Ampliseq library preparation kit v2. Seven samples had <300 genomes analyzed. Libraries were normalized using the Ion Library Equalizer™ Kit, pooled, processed on a Ion Chef™ System for template preparation and chip loading (Ion PI™ Hi-Q™ Chef Kit, Ion PI™ Chip Kit v3, Thermo Fisher Scientific), and sequenced using a Ion Proton™ System. The FASTQs sequencing data were aligned to the human genome (hg19) and processed using IonTorrent Suite V5.0.4.0 that includes the Torrent Variant Caller V5.0.4.0 to generate VCF that were subsequently annotated on a galaxy plateform that uses the SAFIR2 annotation tool [14]. In parallel, samples were analyzed after BAM recalibration using a specific algorithm developed to detect allelic ratio <2% the BPER method [10] which is publicly available at https://cran.r-project.org/web/packages/PlasmaMutationDetector/index.html. Results provided by BPER method and annotated VCFs were registered blindly from clinical data. Following this, ctDNA concentration was categorized into null (0%), low (detected by BPER; <2%) and high concentrations (detection by caller >2%).

2.5. Statistical analyses

Statistical analyses were performed on the intention-to-treat population, using SAS software (Version 9.4). Survival curves and rates between groups were compared using the log rank test and Kaplan Meier method, with a follow-up censure on June 30, 2017. Hazard ratios (HRs) and 95 % confidence intervals (CIs) were calculated using a Cox model. The statistical significance level was set at a two-sided P <0.05.

3. Results

3.1. Patient characteristics

From May 2011 to July 2015, 61 patients were enrolled among them 6 patients were not considered due to insufficient plasma available for ctDNA detection. The remaining 55 patients, include 42 males (76.4 %), 51 (92.7 %) smokers, and 46 patients (83.6 %) exhibiting a PS 0−1, with a median age of 52.9 years (Table 1). Patients were classified as Stage III in five (9.1 %) and Stage IV in 50 (90.9 %). The median HIV viral load was undetectable (<50 copies/mL) in 44 of the 55 documented patients (80 %) and median CD4 count was 418 cells/μL (range: 18–1230). The clinical parameters of these 55 patients did not differ from those of the full 61-patient cohort (Table 1).

3.2. ctDNA and molecular biological tumor characteristics

Of the 55 contributive samples, 35 (64 %) exhibited positive ctDNA at baseline, whereas 20 (36 %) did not (Table 2). Overall, ctDNA detection rates were similar to those reported in HIV-undetermined NSCLC patients [13]. Among the 35 exhibited positive ctDNA, *TP53* mutations were the most common, identified in 27 (77 %) patients. *KRAS* mutations were the second most common, revealed in 10 (29 %) patients. The incidence rate is similar to the HIV-undetermined population [15], despite that patients were heavy smokers. *STK11* inactivating mutations were detected in four samples (11.4 %) but only one sample was *KRAS/STK11* co-mutated. Uncommon mutations included *EGFR, NRAS and BRAF* (for detailed molecular data refer to Suppl Table S1). In line with previously reported observations, *EGFR* mutations were less common than in the general population [16]. In samples with high circulating DNA level, we identified gene amplifications in NRAS ($n = 1$), FGFR1 ($n = 1$), MET ($n = 2$), EGFR ($n = 1$) and ERBB2 ($n = 1$). Overall, 43 % of samples carried more than one molecular alteration.

Table 1
Patient characteristics.

Characteristic	*N (%)*
Sex	
Male	42 (76.4)
Female	13 (23.6)
Age	
Median	52.91
Range	38−67.5
Smoker	
Yes	51 (92.7)
No	4 (7.3)
PS at inclusion	
0−1	46 (83.6)
2	9 (16.4)
Histology	
Adenocarcinoma	50 (90.9)
Sarcomatoid	1 (1.8)
Others non squamous	4 (7.3)
TNM stage	
III	5 (9.1)
IV	50 (90.9)
CD4+ nadir	
Median	156
Range	0−822
CD4+ count cells/μL	
Median	418
Range	18−1230
Undetectable viral load	44 (88)
Cancer history	
Yes	9 (16.4)
No	46 (83.6)
ART at study entry	
Yes	49 (98)
No	1 (2)
AIDS	
Yes	18 (32.7)
No	37 (67.3)

PS, performance status; TNM, tumor-node-metastasis; ART, antiretroviral therapy.

Table 2
Molecular characteristics.

Characteristic	*N (%)*
Detection of ctDNA	
positive	35 (64)
negative	20 (36)
Detected alterations	
TP53	27 (77) / mutations
KRAS	10 (28.6) / mutations
STK11	4 (11.4) / mutations
EGFR	2 (5.7) / 1 mutation, 1 amplification
NRAS	2 (5.7) / 1 mutation, 1 amplification
BRAF	2 (5.7) / mutations
MET	2 (5.7) / amplifications
DDR2	1 (2.9) / mutation
HER2	1 (2.9) / 1 amplification
SMAD4	1 (2.9) / mutation
MAP2K1	1 (2.9) / mutation
ERBB4	2 (5.7) / mutations
FGFR1	1 (2.9) / 1 amplification
FGFR3	1 (2.9) / mutation

3.3. Link between ctDNA and patient characteristics

We observed no statistically significant associations between clinical patient characteristics and ctDNA at baseline (Table 3). The only factor that tended towards significance was the association between TNM status and ctDNA ($p = 0.05$). It must, however, be noted that this comparison lacked statistical power, owing to the few Stage III cases in our PLHIV cohort ($n = 5$).

3.4. Progression-free survival

Statistical analysis results are summarized in Table 4. On univariate analysis, statistically significant correlations were observed between ECOG PS 0−1 vs. PS 2 status and PFS (HR, 2.97; 95 %CI: 1.51−5.86; p = 0.002), as well as between ctDNA absence vs. presence and PFS (HR, 2.87; 95 %CI: 1.52−5.44, p = 0.001). Multivariate analysis revealed AIDS status to be significantly linked to PFS, the former being a negative prognostic factor (HR, 2.17; 95 %CI: 1.09−4.32, p = 0.03). ctDNA presence was shown to be significantly associated with a shorter PFS (HR, 4.31; 95 %CI: 2.06−8.99, $p < 0.0001$), with a PFS difference of approximately 3 months (5.9 mo. ; 95 %CI: 2.6–20.5 for ctDNA absence vs. 2.9 mo.; 95 %CI: 1.6–4.1 for ctDNA presence).

3.5. Overall survival

Statistical analysis results are summarized in Table 4. On univariate analysis, parameters linked to OS were ECOG PS 0−1 vs. PS 2 status (HR, 5.82; 95 %CI: 2.73−12.38; $p < 0.0001$), HAART (HR, 0.20; 95 %CI: 0.04−0.87; $p = 0.03$), and ctDNA absence vs. presence (HR, 2.79; 95 % CI: 1.43−5.45, $p = 0.003$). Multivariate analysis revealed ECOG PS 2 status to be significantly linked to OS, the former being a negative prognosticator (HR, 4.10; 95 %CI: 1.62−10.36, $p = 0.003$). Similarly, in

Table 3
ctDNA levels and demographic characteristics.

	ctDNA – (n = 20)	ctDNA + (n = 35)	*p*-value	Low ctDNA level (n = 13)	High ctDNA level (n = 22)	*p*-value
Sex						
Male	17 (85)	25 (71.4)	0.33	9 (69.2)	16 (72.7)	1.00
Female	3 (15)	10 (28.6)		4 (30.8)	6 (27.3)	
Age						
Median	53.64	52.57	0.10	52.91	52.02	0.68
Range	46.1–67.5	38–66.5		44.9–66.5	38–63.8	
Smoker						
Yes	18 (90.0)	33 (94.3)	0.62	12 (92.3)	21 (95.5)	1.00
No	2 (10.0)	2 (5.7)		1 (7.7)	1 (4.5)	
PS at inclusion						
0–1	17 (85)	29 (82.9)	0.41	12 (92.3)	17 (72.7)	0.38
2	3 (15.0)	6 (17.1)		1 (7.7)	5 (22.7)	
Histology						
Adenocarcinoma	19 (95)	31 (88.6)	0.79	13 (100)	18 (81.8)	0.23
Sarcomatoid	0	1 (2.9)		0	1 (4.5)	
Others non squamous	1 (5.0)	3 (8.6)		0	3 (13.6)	
TNM stage						
III	4 (20.0)	1 (2.9)	**0.05**	0	1 (4.5)	1.00
IV	16 (80.0)	34 (97.1)		13 (100)	21 (95.5)	
Cancer history			0.71			0.63
Yes	4 (20.0)	5 (14.3)		1 (7.7)	4 (18.2)	
No	16 (80.0)	30 (85.7)		12 (92.3)	18 (81.8)	
ART						
Yes	17 (100.0)	32 (97.0)	1.00	12 (100.0)	20 (95.2)	1.00
No	0	1 (3.0)		0	1 (4.8)	
AIDS						
Yes	8 (40.0)	10 (28.6)	0.38	4 (30.8)	6 (27.3)	1.00
No	12 (60.0)	25 (71.4)		9 (69.2)	16 (72.7)	
Virological status						
CD4 rate	383.5 [58–954]	456 [18–1230]	0.56	456 [18–1230]	430.5 [34–1079]	0.87
CD4 nadir	150.5 [0–631]	160 [0–822]	0.40	181 [30–822]	136 [0–800]	0.46
Viral load	20 [0–95499]	40 [0–6598]	**0.06**	40 [0–6598]	40 [0–5924]	0.61

TNM, tumor-node-metastasis; ctDNA, circulating tumor DNA; PS, performance status. ctDNA-: no ctDNA detected; ctDNA+: ctDNA detected.
Bold formatting highlights p-values that reach significance (or are limit to).

Table 4
ctDNA levels and progression-free survival/overall survival.

	Progression-free survival						Overall survival					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95 % CI	*p*	HR	95 % CI	*p*	HR	95 % CI	*p*	HR	95 % CI	*p*
Age												
≤50 years	1	–	–				1	–	–			
>50 years	0.87	0.51–1.48	0.61				0.74	0.43–1.27	0.27			
Cancer history												
No	1	–	–	1	–	–	1	–	–	1		
Yes	1.78	0.86–3.67	0.12	1.92	0.88–4.21	0.10	1.80	0.84–3.86	0.13	1.59	0.67–3.75	0.29
ECOG PS												
0–1	1	–	–	1	–	–	1	–	–	1	–	–
2	2.97	1.51–5.86	**0.002**	1.99	0.91–4.38	0.09	5.82	2.73–12.38	**<0.0001**	4.10	1.62–10.36	**0.003**
CD4+												
≤200	1	–	–				1	–	–			
>200	1.13	0.58–2.20	0.71				0.87	0.45–1.69	0.68			
Viral load HIV												
≤50	1	–	–				1	–	–			
>50	1.10	0.57–2.13	0.78				0.95	0.47–1.91	0.89			
Antiretroviral therapy												
No	1	–	–				1	–	–	1	–	–
Yes	0.50	0.12–2.10	0.34				0.20	0.04–0.87	**0.03**	0.61	0.06–5.72	0.66
AIDS												
No	1	–	–	1	–	–	1	–	–	1	–	–
Yes	1.48	0.84–2.60	0.17	2.17	1.09–4.32	0.03	1.59	0.89–2.84	0.12	1.75	0.89–3.42	0.10
ctDNA												
-	1	–	–	1	–	–	1	–	–	1	–	–
+	2.87	1.52–5.44	**0.001**	4.31	2.06–8.99	**<0.0001**	2.79	1.43–5.45	**0.003**	3.52	1.72–7.19	**0.0006**

ctDNA, circulating tumor DNA; HR, hazard ratio; 95 % CI, 95 % confidence interval; PS, performance status; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.
Bold formatting highlights p-values that reach significance (or are limit to).

multivariate analysis, ctDNA presence was shown to be significantly associated with a shorter OS (HR, 3.52; 95 %CI: 1.72–7.99, $p = 0.0006$).

3.6. Impact of ctDNA levels

Our multivariate analysis revealed that not only ctDNA presence but also its level impacted PFS and OS (Figs. 1 and 2). In multivariate analysis, markedly elevated ctDNA vs. absent ctDNA levels were associated with both a shorter PFS (median, 2.3 mo.; 95 %CI: 1.3–3.3 vs. 5.9 mo.; 95 %CI: 2.6–20.9, $p = 0.0003$) and a shorter OS (median 4.3 mo.; 95 %CI: 1.9–6.8 vs. 13.7 mo.; 95 %CI: 7.2–30.3, $p = 0.0001$). However, for patients with only slightly-elevated ctDNA levels, the PFS was only superior (although non-significant $p = 0.053$) to those observed in patients exhibiting highly-elevated ctDNA levels.

4. Discussion

Non-small-cell lung cancer (NSCLC) is the most common non-AIDS-related malignancy responsible for death in PLHIV. Mutational status is known to be mandatory to choose and optimize treatment in LC patients. Tumors from PLHIV have not been extensively analyzed and little is known concerning specificities of mutation profiles in this population. The widespread use of NGS technology enables broad testing of genetic alterations of NSCLC patients and was optimized for tissues and plasma DNA testing [10,11,17]. Using this tool, we reported herein the first prospective trial investigating the prognostic impact of ctDNA based on NGS technology in advanced-staged NSCLC in PLHIV. Whereas other studies only explored a small number of mutant genes in NSCLC, such as *EGFR* and *KRAS* [18,19], we conducted comprehensive molecular profiling in plasma with large-scale screening to refine the predictive value of ctDNA.

One important information from this ancillary study is that ctDNA detection rates using the BPER method were similar in PLHIV with NSCLC to those reported in HIV-undetermined patients [13]. Our data are in line with that described by Pécuchet et al. in an HIV-undetermined NSCLC population, reporting a 71 % ctDNA detection rate [11]. In our series, 7 samples had a very limited amount of total DNA in plasma, the estimated number of genome used for NGS was <300 that limited rare mutant allele detection (data not shown). For those samples 3/7 were positive for ctDNA. So, we can estimate that we could have missed the presence of low ctDNA fractions for only 4 samples. Although most plasma studies supported a high concordance between tumor and plasma, one limitation of this study is that we had no tissues available to match tumor and plasma profiles. Mutation profiles identified in plasma samples could therefore be a biased surrogate of tumor profiles and reflect those of a group of patients with a poor prognosis. However, the profiles found in plasma from PLHIV are more or less consistent with the expected distribution in NSCLC. *EGFR* mutations were less common in PLHIV than in the general population, as previously reported by some of us [19]. This may be due to these subjects being heavy smokers, as increased smoking has formerly been linked to significantly lower *EGFR* mutation rates [19]. *KRAS* mutations, which are thought to be refractory to standard chemotherapy and targeted treatments like tyrosine kinase inhibitors (EGFR-TKIs), were found in 29 % of our patients, roughly in line with formerly published data [18,20]. The high prevalence of *TP53* mutations (77 % of positive ctDNA samples) may be PLHIV specific and would need confirmation, but it is possibly linked to the cohort's heavy smoking habit. Looking at our cohort's overall mutation profile, these patients are not deemed likely to benefit from TKI treatment. This is exactly where immune checkpoint blockade directed against the programmed cell death 1 (PD-1)/programmed death ligand 1 (PD-L1) axis may step in. The clinical impact of these agents has already been confirmed in several randomized trials [21], with proven superiority in terms of response rate, PFS, and OS, along with a more favorable safety profile compared to standard chemotherapy.

A few gene amplifications (*EGFR, HER2, FGFR1* and *MET*) were detected in samples with high tumor DNA fraction in blood. Such alterations could be targeted with specific inhibitors and represent alternative therapeutic approaches for the patients. We found that, in PLHIV with NSCLC, ctDNA positivity was an independent marker of poor prognosis with significant shorter PFS and OS. Consequently, ctDNA detection at baseline via high-throughput NGS technology is strongly linked to treatment benefits in terms of PFS and OS. We showed that not only ctDNA presence, but its amount was shown a potential outcome predictor. Similar observations had previously been made by Zhu et al. [22], concerning circulating *EGFR* concentrations, with high circulating *EGFR* at baseline proven to be linked with shorter survival under TKI-therapy. According to the authors, and in line with our own thoughts, these *EGFR* levels were most likely related to be superior tumor mutational burden and higher *EGFR* levels within pulmonary tissues.

Similar observations have previously been revealed for other cancer types, such as melanoma patients [23] and colorectal carcinoma (CRC) patients. In patients with advanced colorectal cancer, patients with high

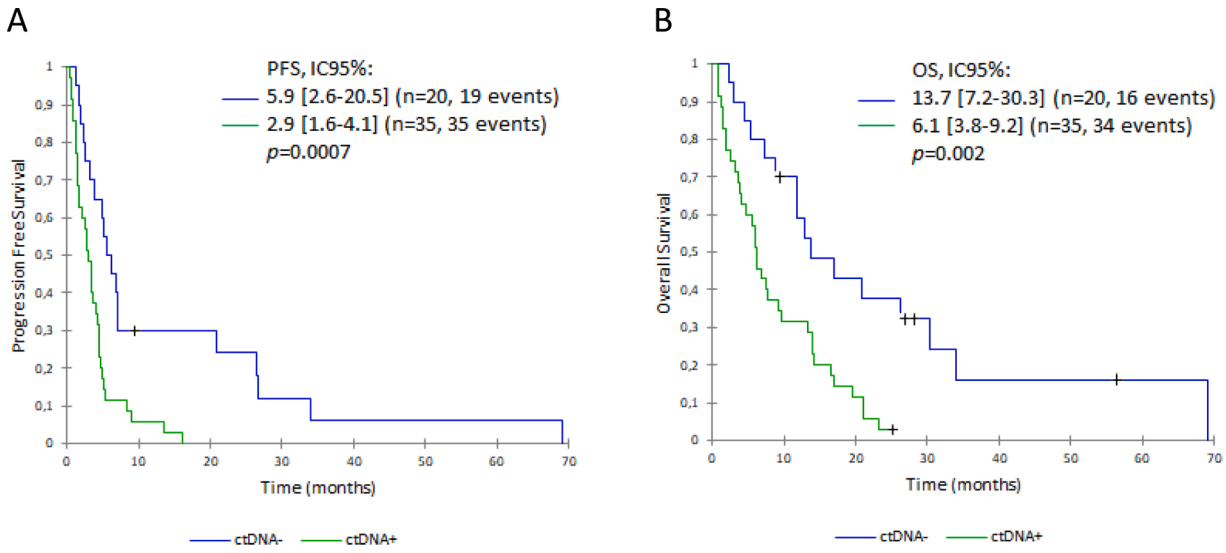

Fig. 1. Impact of ctDNA presence on A) progression-free survival and B) overall survival. OS, overall survival; PFS, progression-free survival; ctDNA, circulating tumor DNA; 95 %CI, 95 % confidence interval.

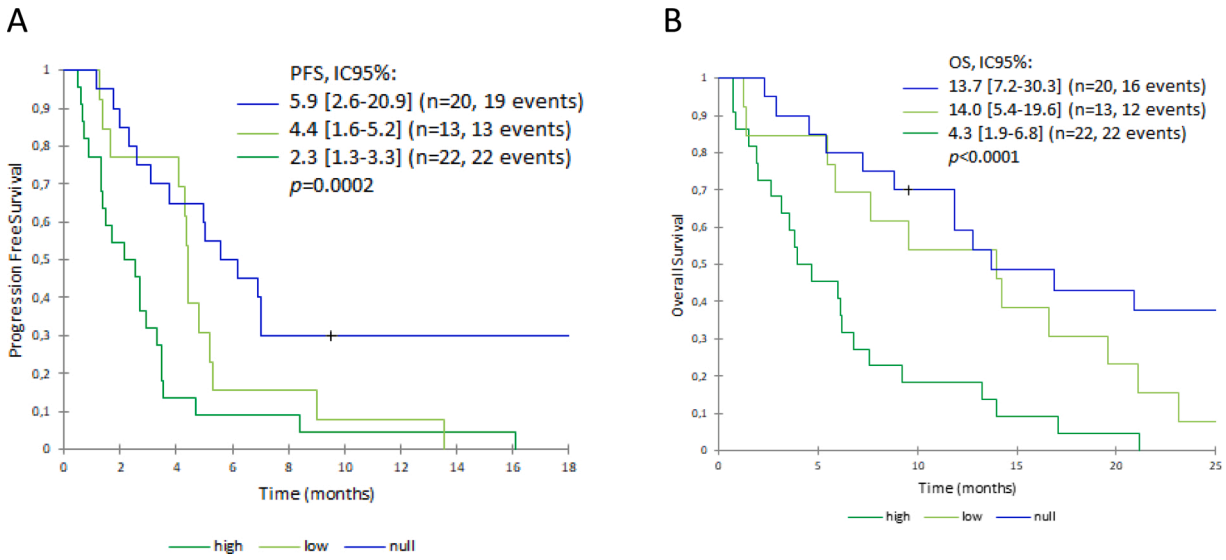

Fig. 2. Impact of ctDNA level amplitude on A) progression-free survival and B) overall survival. PFS, progression-free survival; OS, overall survival; ctDNA, circulating tumor DNA; 95 %CI, 95 % confidence interval; high, low and null. Null (0%), low (detected by BPER; <2%) and high concentrations (>2%).

ctDNA had a shorter overall survival as compared to low ctDNA, suggesting that quantification of ctDNA could be used to classify good and bad prognosis patients [24]. This information is in line with what we show in PLHIV with advanced NSCLC. We could not analyze, in our series, the evolution of ctDNA concentration during treatment as the only available sample was at baseline. This information could also be of interest in evaluation response to treatment as shown in colorectal, melanoma and lung cancer [24–26].

Detection and quantification of tumor DNA in plasma was shown to be a valuable approach to predict prognosis and to monitor treatment in many cancer types. We show here that this is also true for PLHIV with NSCLC. ctDNA testing clearly opens up an opportunity for precision treatment in cancer patients. However, at present, we do not know whether ctDNA is likely to become a tool for stratifying patients in clinics or in future cancer trials.

The major strength of our study was its prospective design, along with the use of a dedicated NGS targeted panel that tested most commonly encountered mutations in NSCLC. The additional value of our validated ultra-deep targeted NGS technology is that this method, in contrast to others, could be applied in the absence of available tumor tissue.

In conclusion, this prospective ancillary trial is one of the first to focus on PLHIV with advanced-staged NSCLC and demonstrated that ctDNA at baseline, prior to anticancer therapy implementation, was a marker of prognosis. We do not know if ctDNA will be proven able to monitor therapeutic responses in this population, and thus be a therapeutic decision-making marker. Further prospective interventional trials are now warranted to corroborate these encouraging findings.

Statement of translational relevance

Lung cancer has become the leading cause of cancer death in people living with HIV (PLHIV). However, there are few clinical trials dedicated to PLHIV. The CHIVA IFCT-1001 study, the first trial assessing the efficacy of a platinum-doublet chemotherapy in HIV-infected patients with advanced non-squamous cell non-small-cell carcinoma (NSCLC), showed that chemotherapy is effective and well-tolerated. We conducted here an ancillary study from CHIVA trial to characterize molecular profile of NSCLC in 61 PLHIV using circulating tumor DNA (ctDNA). ctDNA detection rates were similar to those observed in HIV-undetermined population. We showed that etection of ctDNA at baseline was an independent prognostic factor on overall survival with a 3 to 4-fold higher risk of death. *EGFR* mutations were less common, whereas *KRAS* mutations were similar and *TP53* higher than reported in literature in the HIV-undetermined population. Further prospective trials are needed to validate this marker in routine.

Author contributions

Marie Wislez: Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Writing - Original Draft. **Charlotte Domblides:** Investigation, Data Curation, Formal analysis, Writing - Original Draft. **Laurent Greillier:** Resources, Writing - Original Draft. **Julien Mazières:** Resources, Writing - Original Draft. **Isabelle Monnet:** Resources, Writing - Original Draft. **Lize Kiakouama-Maleka:** Resources, Writing - Original Draft. **Xavier Quantin:** Resources, Writing - Original Draft. **Jean Philippe Spano:** Resources, Writing - Original Draft. **Charles Ricordel:** Writing - Original Draft. **Philippe Fraisse:** Resources, Writing - Original Draft. **Henri Janicot:** Resources, Writing - Original Draft. **Clarisse Audigier-Valette:** Resources, Writing - Original Draft. **Elodie Amour:** Data Curation, Formal analysis, Writing - Original Draft. **Alexandra Langlais:** Investigation, Data Curation, Formal analysis, Writing - Original Draft. **Nathalie Rabbe:** Conceptualization, Methodology, Project administration, Resources, Investigation, Writing - Original Draft. **Alain Makinson:** Resources, Writing - Original Draft. **Jacques Cadranel:** Resources, Writing - Original Draft. **Pierre Laurent-Puig:** Conceptualization, Methodology, Data Curation, Formal analysis, Writing - Original Draft. **Armelle Lavolé:** Resources, Data Curation, Formal analysis, Writing - Original Draft. **Hélène Blons:** Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Writing - Original Draft.

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Role of the funding source

The funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

Charlotte Domblides: Speaker: Amgen, Astra-Zeneca, Bristol-Myers Squibb, and MSD. Travel, accomodations, expenses: Amgen, Astra-Zeneca, Bristol-Myers Squibb, MSD, Pfizer, Pierre Fabre, Roche. Boards: Astra-Zeneca.

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Appendix A. Supplementary data

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