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Circulating H3K27 Nucleosomes to monitor Lung Cancer Patients During Treatment, a Universal Biomarker Quantifying the Minimal Residual Disease (MRD) in Plasma Samples

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Introduction

Lung cancer is the leading cause of cancer worldwide. In patients with stage III and IV lung cancer, a subset can be cured by radiotherapy, and/or combined treatment approaches, including chemotherapy, immunotherapy and targeted therapies. Following curative-intent first-line therapies, current routine clinical surveillance involves serial CT imaging. However, such surveillance can detect only macroscopic disease recurrence and is frequently inconclusive. Circulating tumor DNA (ctDNA) sequencing is also widely used to identify and monitor treatment plans.

Studies have demonstrated that cancer cells harbor both genetic mutations and epigenetic aberration. As those epigenetic modifications are reversible, epigenetic-based drugs have become an attractive option for cancer therapy. Among all those modifications, trimethylation of the histone H3 at lysine 27 (H3K27Me3) catalyzed by the enzyme EZH2, is a crucial epigenetic process in tumorigenesis. In this context, ctDNA molecular profiling in addition to the analysis of circulating nucleosomes and their histone PTMs before and after patient's treatment could provide a wider view of the genetic and the epigenetic landscape of a tumor. Therefore, we conducted a retrospective, non-interventional study to assess the dynamic levels of H3K27Me3-nucleosomes in NSCLC patients under treatment to evaluate the MRD, to drive the choice of the following line of treatment. This study was conducted into the routine management of NSCLC patients (treatment included chemotherapy combined or not with immunotherapy, or targeted therapy).

For the first time, we highlighted the putative role of circulating H3K27Me3-nucleosomes as blood-based biomarker for quantifying the MRD to monitor NSCLC patients during treatment

A parallel study was performed on NSCLC at diagnosis (Published Abstract Number: 1013, AACR 2023).

Patients & Methods

Study Population: Patients were recruited from the pulmonology department at Lyon University Hospital from 2015 to 2022 (Etude RNIPH 22-5065 NUCLEO_CIRCAN avis CSE n°22-5065). Inclusion criteria were: >18 years old with histologically proven NSCLC lung cancer. It was done into routine use. K2EDTA plasma samples from 304 patients during progression defined by a clinical or CT-scan modifications.

For custom-validated NGS library preparation, 10–100 ng cfDNA were used, using a custom capture-based technology provided by SOPHiA GENECTICS (Lausanne, Switzerland) and performed according to the manufacturer's instructions. The custom panel covered 78 genes involved in cancer (such as EGFR, TP53 or KRAS).

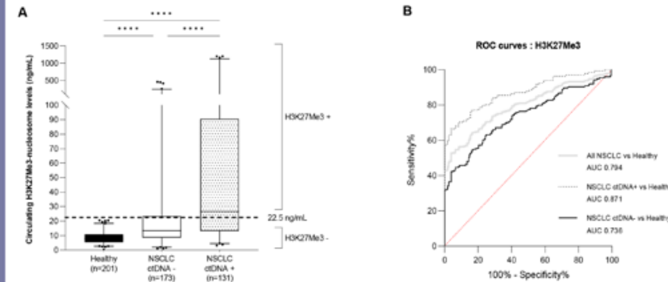
Nu.Q® assay: Quantitative measurement of circulating H3K27Me3-nucleosomes in K2-EDTA plasma samples was performed using the Nu.Q® immunoassay (Belgian Volition SRL, Isnes, Belgium) according to the manufacturer's instruction. Sandwich immunoassay was performed on an IDS-i10 automated chemiluminescence immunoanalyzer (Immunodiagnostic Systems Ltd (IDS) UK). Briefly, 50µl of plasma was mixed with an acridinium ester labeled anti-nucleosome detection antibody. Then, an H3K27Me3-antibody coated beads were added. Finally triggers solution were added and the signal emitted was measured by the luminometer.

Statistical analyses were performed using GraphPad Prism (GraphPad Prism software version 9.5.0, San Diego, CA, USA). Descriptive statistics were used, and results are reported as mean, median, 2.5th-25th-75th and 97.5th percentiles. Data were subjected to Kolmogorov–Smirnov normality test. As data are not normally distributed, non-parametric methods were used : Mann-Whitney U test and Kruskal-Wallis H test for groups comparison. Significance values are represented by *: *p-value* < 0.05; **: *p* < 0.01; ***: *p* < 0.001; ****: *p* <0.0001.

Results

High level of circulating H3K27Me3-nucleosomes observed in NSCLC samples during treatment is more pronounced in presence of mutated ctDNA.

NSCLC samples were classified based on molecular profiling of ctDNA conducted by NGS analyses and circulating H3K27Me3-nucleosomes were quantified by chemiluminescent Nu.Q® immunoassays.



A. Box plot analysis demonstrating significantly higher circulating H3K27Me3-nucleosome levels in NSCLC samples during progression (n=304) compared to healthy samples (n=201). This level is significantly higher in NSCLC samples when ctDNA was detected in blood samples ($median_{ctDNA-}$ = 13.35 ng/mL vs $median_{ctDNA+}$ = 26.05 ng/mL, respectively, *p_value<0.0001*)

B. Receiver-operating characteristic (ROC) curve analysis of circulating H3K27Me3-nucleosomes demonstrating better clinical performances on ctDNA+ (in dotted line) sub-group compared to the whole cohort (in grey) and the ctDNA- (in black) sub-group (*p_value<0.0001*).

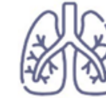
High circulating H3K27Me3-nucleosome levels are preferentially associated with TP53 mutations under treatment

The concentration of circulating H3K27Me3-nucleosomes were measured on NSCLC plasma samples sub-groups defined by absence (minus (-) sub-group) or presence (plus (+) sub-group) of a mutation in one of the 3 targeted oncodrivers (EGFR, KRAS or TP53).

Only TP53- and TP53+ sub-groups show significant difference in circulating H3K27Me3-nucleosome median levels (*p-value<0,5*).

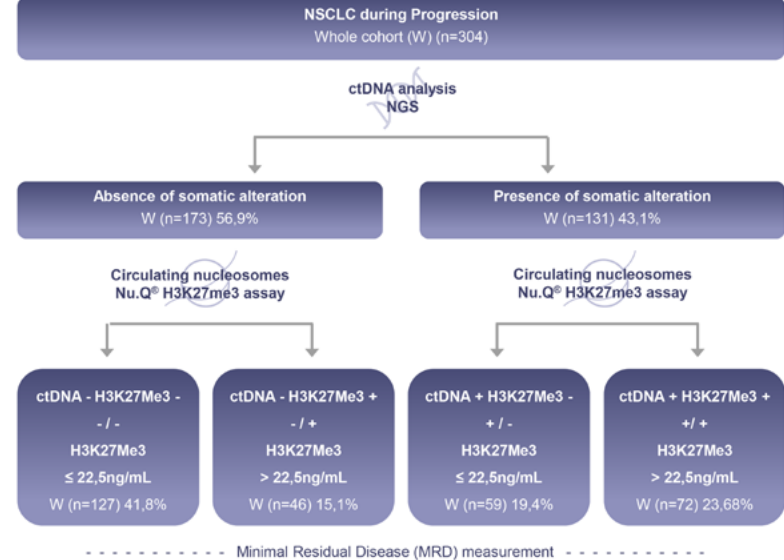
	n=	H3K27me3 (ng/mL) mean	median
NSCLC during Progression	304	67,29	16,89
ctDNA-	173	31,98	13,35
ctDNA+	131	113,92	26,05
EGFR-	53	101,91	34,08
EGFR+	78	122,09	20,69
KRAS-	124	115,30	26,70
KRAS+	7	90,00	14,80
TP53-	72	97,50	19,20
TP53+	59	134,00	36,90

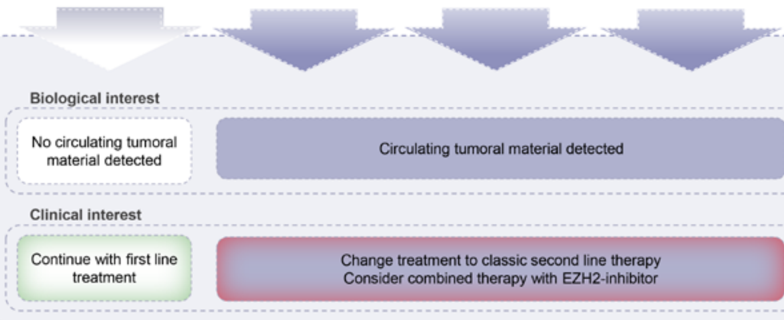
* (TP53- vs TP53+)



Non-Small Cell Lung Cancer during Progression

After or during treatment





Decision tree proposed for the classification of NSCLC samples during or after treatment progression.
After sub-grouping of NSCLC samples into ctDNA- and ctDNA+ classification; samples were classified regarding the level of circulating H3K27Me3-nucleosomes and finally organized in four classes.

The upper limit of the reference interval of circulating H3K27Me3 nucleosome levels including 95% of the healthy population was calculated at 18.4 ng/mL (SD = 4.07). A cut-off was defined at **22.5 ng/mL** (18.4+1SD) ensuring 100% specificity in this study.

Discussion

- 41.8% of samples ctDNA - / H3K27Me3 -: suggesting molecular indicator of response to treatment
- 58.2% of samples are positive for at least one or both biomarkers (ctDNA + / H3K27Me3 +): **s**uggesting molecular indicator of tumoral progression
- **Nu.Q® H3K27Me3 measurement increased the identification of disease progression from around 43% to around 58% over conventional methods, thereby increasing the identification of disease progression by over 15%.**
- **Circulating H3K27Me3-nucleosome level is a promising biomarker for MRD monitoring in NSCLC during or after treatment**
- We propose H3K27Me3-nucleosome levels could help in guiding patient management

Potential use cases of Nu.Q® H3K27Me3 combined to ctDNA are presented on time-course through NSCLC treatment (Right panel - Adapted from Natera).

Screening Diagnosis — Treatment Monitoring — Surveillance MRD — Treatment Response Monitoring

TREATMENT — RECURENCE

Levels of ctDNA — Time

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Institutional Review Board Statement: Medical data were collected through a mandatory prescription sheet and edited by the prescribing physician.
The patients were orally informed by physician about the testing useful for their clinical management.
Informed Consent Statement: All included patients were fully orally informed.

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