Supplementary material

Materials and Methods

The patient signed consent forms to be registered in the neuroendocrine neoplasm database of the *Hospices Civils de Lyon* (approved by the national data protection commission (*Commission nationale de l'informatique et des libertés*, CNIL) on the 6th November 2015 (n°15-111), and for the utilization of its tumour samples by the tissue bank '*Tumorothèque des Hospices Civils de Lyon*', recognized by the French Ministry of Health (DC 2008–2072 and AC 2008–2073). Blood samples were collected as part of the CIRCAN program, a prospective translational program for the evaluation of tumour biomarkers in liquid biopsy.

Biochemistry

All biochemistry biomarkers were analysed at the Multi-Site Medical Biology Laboratory of the *Hospices Civils de Lyon*. The determination of UFC was carried out using immunochemiluminescence technique kit (Architect C16000, Abbott), ACTH assay was performed using immunometric technique kit (Cobas, Roche). LDH (ref 2P56-21) and ALP (ref 7D55-21) were carried out using spectrophotometric technique kits (Architect C16000, Abbott).

cfDNA molecular profile

Plasma was prepared from 20-30 mL of blood collected in K2 EDTA tubes (BD, 367525, 18 mg). All blood samples were delivered to the laboratory within 5 hours after collection. cfDNA was extracted from 4 mL of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Cat No 55114, Valencia, CA, USA), with a Qiagen vacuum manifold following the manufacturers' instructions. cfDNA was then eluted in a final volume of 60 µL of elution buffer (AVE). cfDNA libraries were created using the capture technology provided by in-

house panel. The assay covered mutations on 78 genes. Fastq files, obtained by the demultiplexing of Base-Call Files (BCL), were analysed using the Sophia DDM Platform version 5.1.9 (Sophia Genetics, Saint-Sulpice, Switzerland). The human genome Hg19 (GRCh37.p5) was used as the reference genome. Detailed analytical considerations have been previously published (Garcia *et al.* 2021).

Circulating atypical cell enumeration

Enrichment and labelling of circulating atypical cells were carried out using an in-house optimized immunostaining assay (Garcia *et al.* 2019). For performing circulating atypical cell enrichment, a volume of 7.5 mL of blood was lysed in 22.5 mL of red blood cell lysis buffer (CBB-F016003, Biolidics limited®), mixed gently by inverting and incubated stand at room temperature for approximately 10 minutes. Then, cells were centrifuged at 500 g for 10 minutes and the supernatant was removed. The pellet was resuspended in 4 mL of resuspension buffer (786-650, BIOSCIENCES®) and loaded onto the CTC Chip™ FR1 of the microfluidic ClearCell® FX1 system. Cell enrichment was collected and centrifuged at 500 g for 10 minutes prior to cyto-centrifuging on the polylysin sample glass slide for performing immunofluorescence assay.

After incubation with 200 µL of paraformaldehyde 4% for 10 minutes, the cytospot was incubated for 30 minutes in 200 µL of in-house saturation mix (Fetal Bovine Serum 5%, Fc Receptor Blocking Reagent 5%, Bovine serum albumin 1%, and PBS) for blocking non-specific protein binding. Then, it was incubated overnight at 4°C in primary antibody against CD45 (rat anti-human monoclonal antibody, MA5-17687, ThermoFisher) diluted at 1:500 in saturation mix. It was then washed three times in PBS and incubated for 1 hour at room temperature in labelled anti-rat AlexaFluor-647 secondary antibody (dilution 1:500) (VB296618, ThermoFisher) and DAPI (dilution 1:1000) for cell nuclei visualization. The

stained cytospot was washed four times in PBS and air-dried during 5 minutes. Slide was mounted with Fluoromount medium (Fluoromount™ Aqueous Mounting F4680, Sigma®) to prevent photo-bleaching and preserve the fluorescent labelled molecules for long-term storage. Slide was scanned by an automated microscope (LionHeart FX Automated microscope, Biotek, USA) and was analysed by microscopy software (Gen5™ version 3.09, Biotek, USA). The circulating atypical cells were DAPI+ and CD45- and had malignancy characteristic pattern (size, shape, mononuclear, nucleo-cytoplasmic ratio).