

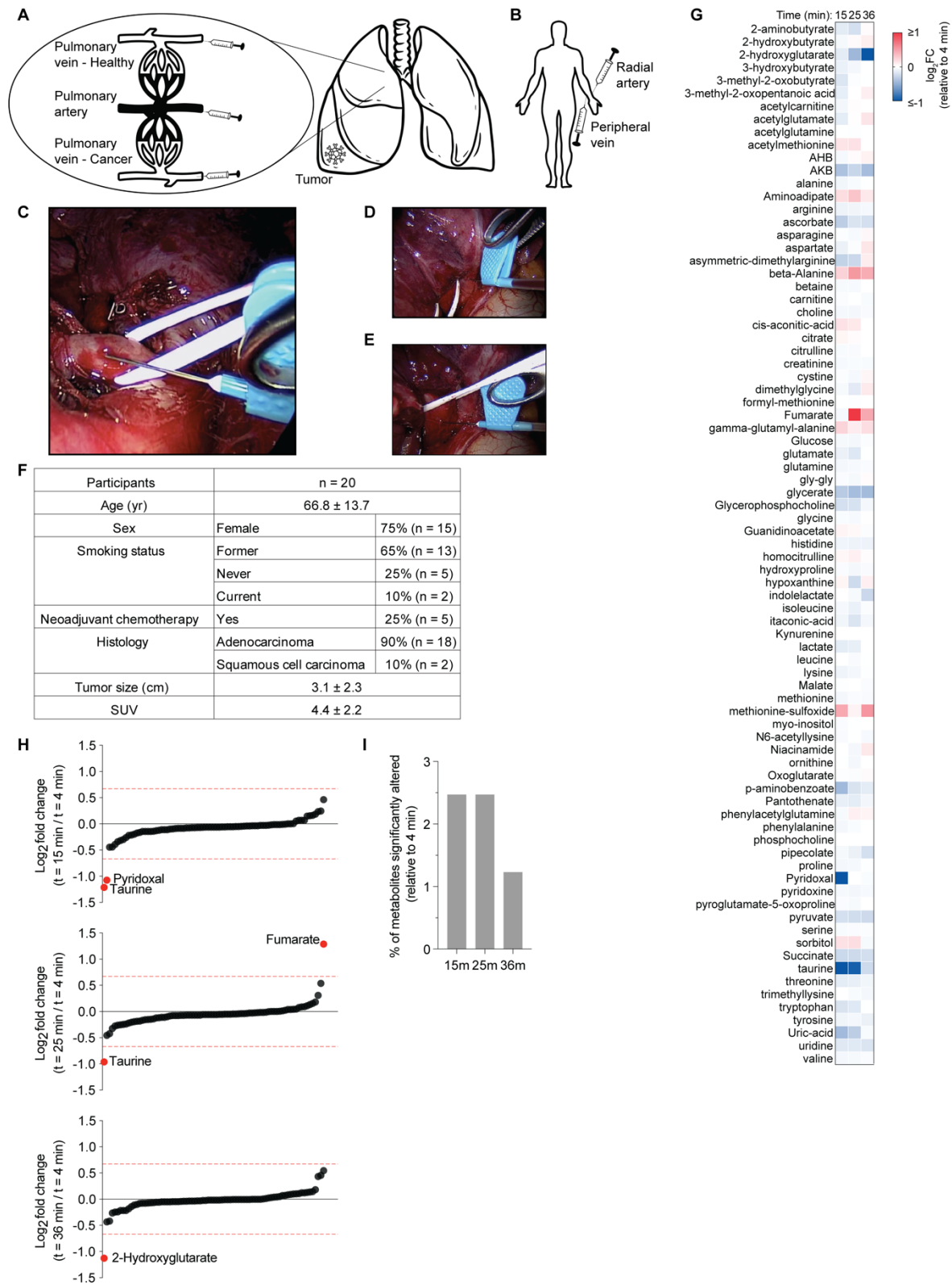
Supplementary Material

Participant	Age	Sex	Race	Tumor Location	Histology	Neoadjuvant Therapy	Pathology Tumor Size (cm)	Pathologic Stage	Comorbidities
1	66	F	Caucasian	RML	Adeno	None	2.8	pT1cN0	Breast Cancer, HLD, HTN
2	76	F	Caucasian	RLL	Adeno	Carboplatin + Pemetrexed	2.8	ypT2aN2	HLD, HTN
3	81	F	Caucasian	RUL	Adeno	None	3.1	pT1cN0	Breast Cancer, Hypothyroidism, Raynaud's disease
4	62	M	Caucasian	RUL	Adeno	None	3.5	pT2aN0	BPH
5	67	M	African American	LLL	Adeno	None	1.9	pT1bN1	Aortic stenosis, CKD, COPD, DM, Gout, HLD, HTN
6	70	F	Caucasian	RML	Adeno	None	1.8	pT1bN1	CAD, Hypothyroid, HLD, HTN, MDD, OA
7	74	F	Caucasian	LUL	Adeno	Clinical Trial: Cisplatin + Pemetrexed +/- Nivolumab	0	ypT0N0M0	DM, HLD, HTN, IBS, TIA
8	79	F	Caucasian	RML	Adeno	None	0.9	pT1aN0	Breast Cancer, Lymphoma, HLD, HTN, hypothyroid
9	73	F	Caucasian	RLL	Adeno	Cisplatin + Pemetrexed	2.2	ypT1cN0	HLD, HTN, OA
10	67	M	Caucasian	LUL	SCC	Clinical Trial: Cisplatin + Pemetrexed +/- Nivolumab	0.1	ypT1aN0	HLD
11	69	M	Caucasian	RUL	Adeno	None	5.7	pT3N0	DM, HTN
12	28	F	African American	LUL	Adeno	None	1.5	pT2aN1	IBS, Iron deficiency anemia, Li-Fraumeni syndrome
13	76	F	Caucasian	LUL	Adeno	None	2.6	pT1cN0	Age Related Macular Degeneration , DM, HTN, HLD,
14	77	F	African American	LLL	Adeno	None	1	pT3N0	Diverticulosis, HLD, HT
15	73	F	African American	RLL	SCC	None	3.5	pT2aN2	Anxiety, GERD, HTN, MDD, OA
16	77	M	Caucasian	LLL	Adeno	None	1.5	pT1bN0	HLD, Rectal Adenocarcinoma
17	67	F	Caucasian	LUL	Adeno	Carboplatin + Paclitaxel + Pembrolizumab	2.9	ypT3N0	CAD, GERD, HLD, Thrombocytopenia

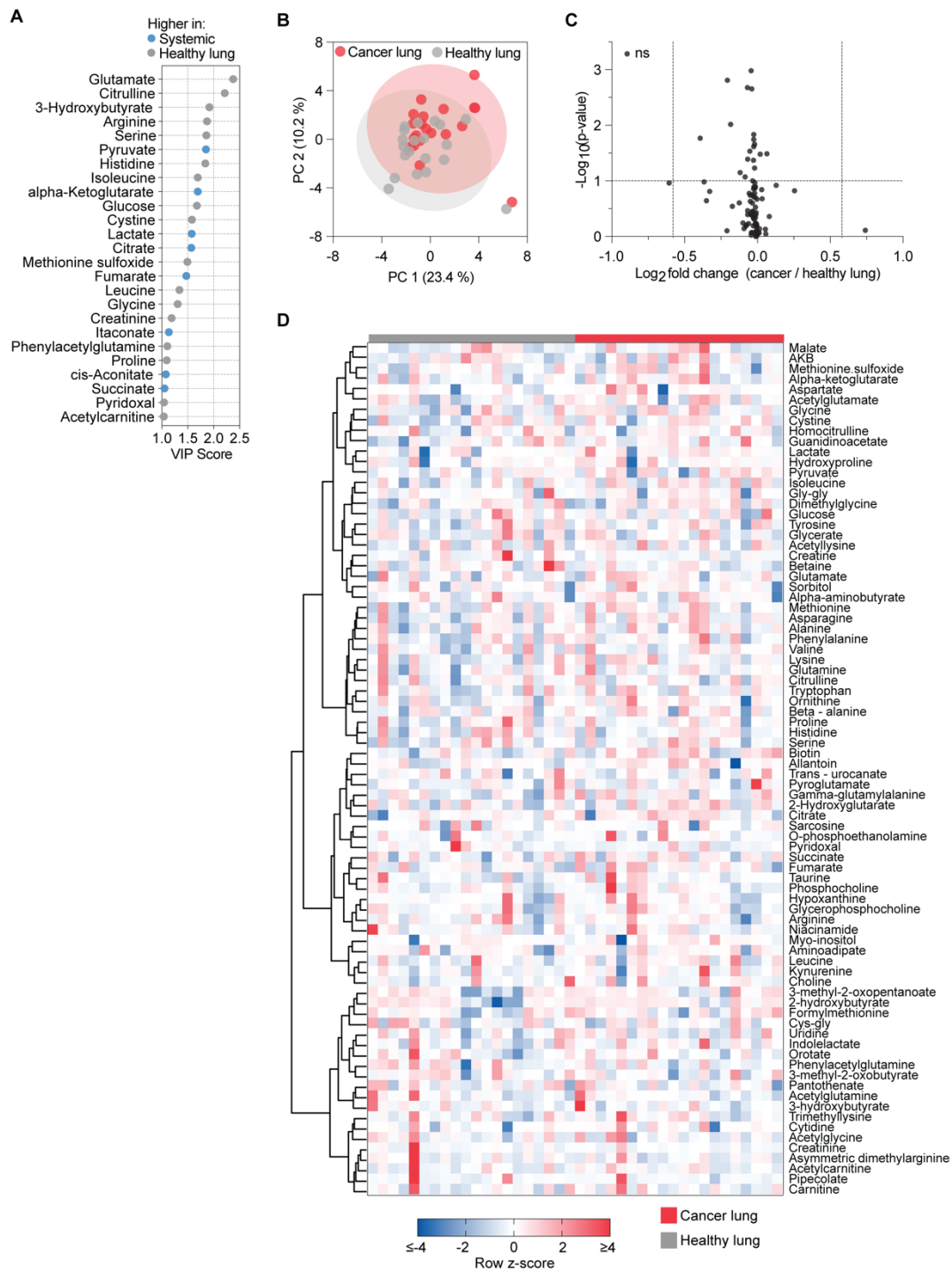
18*	47	F	Other	LUL	Adeno	None	3.2	pT1bN0	DM, HLD, HTN, OA, OSA
19*	47	F	Other	RLL	Adeno	None	3.8	pT2aN0	DM, HLD, HTN, OA, OSA
20	49	F	Caucasian	RUL	Adeno	None	2.3	pT1cN0	Asthma, GERD, HTN
Abbreviations: M – Male; F- Female; RUL – Right Upper Lobe; RML – Right Middle Lobe; RLL – Right Lower Lobe; LUL – Left Upper Lobe; LLL – Left Lower Lobe; Adeno – Adenocarcinoma; SCC – Squamous Cell Carcinoma; BPH – Benign Prostatic Hypertrophy; CAD – Coronary Artery Disease; COPD – Chronic Obstructive Pulmonary Disease; CKD – Chronic Kidney Disease; DM – Diabetes Mellitus; GERD – Gastroesophageal Reflux Disease; HLD – Hyperlipidemia; HTN – Hypertension; IBS - Irritable Bowel Syndrome; MDD – Major Depressive Disorder; OA -Osteoarthritis; OSA – Obstructive Sleep Apnea; TIA – Transient Ischemic Attack. * Subject 18/19 was the same patient with cosynchronous bilateral tumors that were resected in separate encounters									

Supplemental Table 1. Clinical and pathological characteristics of study participants.

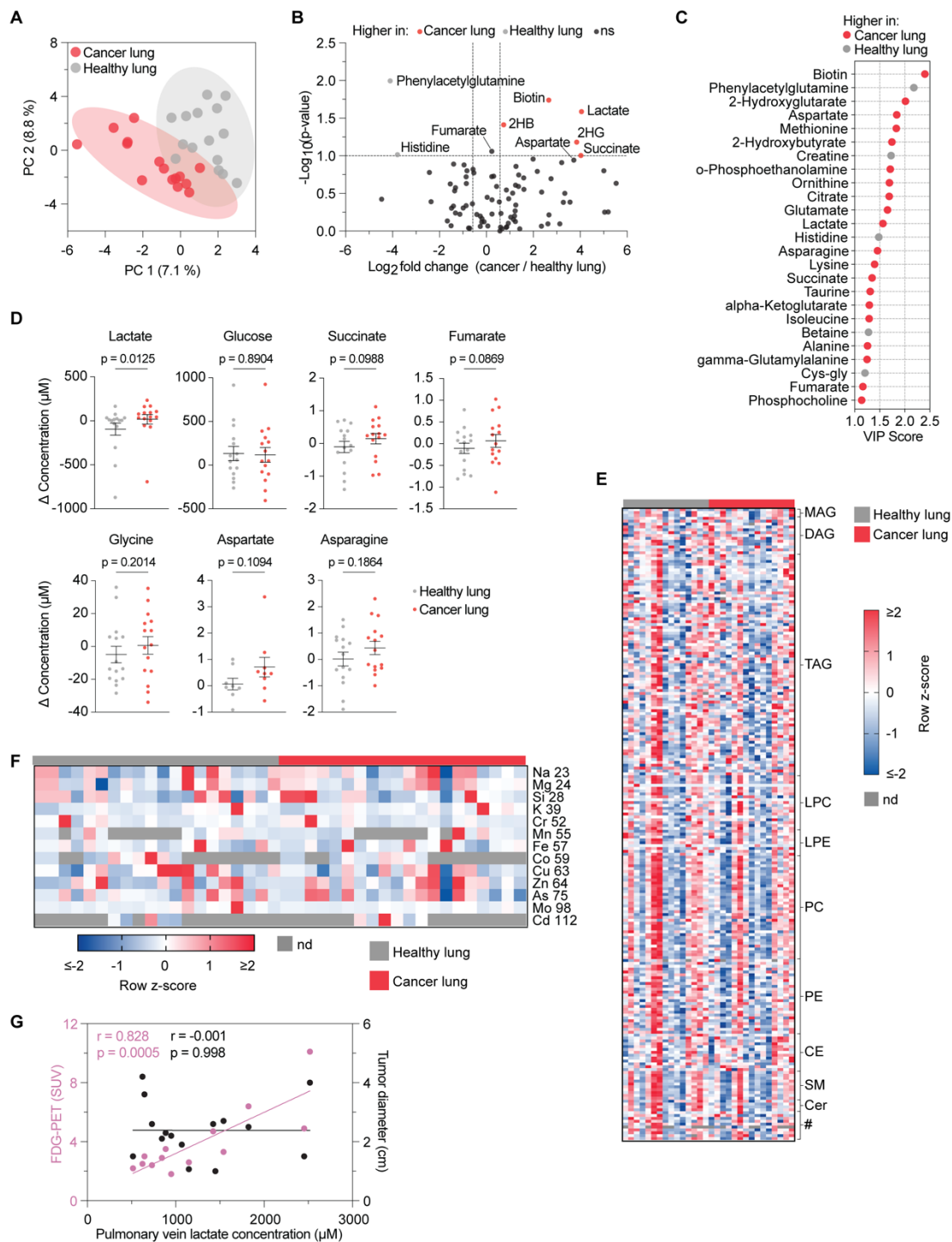
Summary of demographic, clinical and pathological data of 20 included non-small cell lung cancer resections from 19 patients.



Supplemental Figure 1. Validation of intraoperative blood sampling and processing conditions. (A-B) Schematic illustrating sampling sites in the study. (A), Pulmonary vasculature including tumor-bearing and non-tumor-bearing (healthy) lung lobes. (B), Systemic sites including the radial artery and a peripheral vein. **(C-E)** Intraoperative imaging during a video assisted thoracoscopic right upper lobectomy. Sampling of the pulmonary artery (C), pulmonary vein draining the cancer containing lobe (right superior pulmonary vein) (D); and healthy lobe (right middle pulmonary vein) (E) is shown. **(F)** Summary of clinical characteristics for the 20 NSCLC participants included in the study. **(G)** Heatmap showing average $\log_2$ fold changes in plasma metabolite concentrations over the sample processing time, measured relative to baseline ($t = 4$ min). AHB: alpha-hydroxybutyrate; AKB: alpha-ketobutyrate; gly-gly: glycyl-glycine. **(H)** Plot showing ranked metabolites based on absolute $\log_2$ fold change at different times during sample processing as indicated. Metabolites exceeding the significance threshold ($|\log_2 \text{ fold change}| > 0.58$) at each time point are highlighted in red. **(I)** Bar plot showing the percentage of metabolites significantly altered ($|\log_2 \text{ fold change}| > 0.58$) at each time point during sample processing relative to $t = 4$ min.



Supplemental Figure 2. Direct comparison of pulmonary vein metabolite levels lacks sensitivity to distinguish cancer from healthy lung. **(A)** Variable importance in projection (VIP) plot of the top 25 metabolites distinguishing non-tumor-bearing (healthy) lung circulation (pulmonary vein of a non-tumor lobe minus pulmonary artery) from systemic circulation (peripheral vein minus radial artery). Metabolites in blue are elevated in systemic circulation; those in grey are elevated in healthy lung. Derived from data shown in Figure 1B. **(B)** Partial least squares discriminant analysis (PLS-DA) comparing metabolite profiles from the pulmonary vein of a healthy lobe versus that of a cancer-bearing lobe (n = 20). **(C)** Volcano plot depicting metabolites significantly altered between cancer-bearing and healthy pulmonary veins. Significance was defined as a fold change >1.5 and a raw p-value <0.1 (paired two-tailed t-test, n = 20). **(D)** Hierarchical clustering of z-score-normalized metabolite concentrations in blood from cancer-bearing versus healthy lung lobes (both calculated as pulmonary vein minus pulmonary artery). Rows represent individual metabolites; patient groups were not clustered (n = 20). AKB: alpha-ketobutyrate; cys-gly: cysteinylglycine; gly-gly: glycyl-glycine.



Supplemental Figure 3. Tumor-associated metabolic alterations are also found in patients who did not receive neoadjuvant therapy. (A) Partial least squares discriminant analysis (PLS-DA) comparing metabolite profiles from cancer-bearing versus non-cancer bearing (healthy) lung tissue (both calculated as pulmonary vein minus pulmonary artery) in patients who did not receive neoadjuvant therapy (n = 15). **(B)** Volcano plot showing metabolites significantly altered between cancer-bearing and healthy lung in this subset of patients. Significance defined as fold change >1.5 and raw p < 0.1 (paired two-tailed t-test, n = 15). 2HB: 2-Hydroxybutyrate; 2HG: 2-Hydroxyglutarate. **(C)** Variable importance in projection (VIP) plot of the top 25 metabolites distinguishing cancer from healthy lung in patients without neoadjuvant therapy. Metabolites in red are elevated in cancer; those in grey are elevated in healthy lung. Based on data in panel (A). **(D)** Selected metabolites differing between cancer-bearing and healthy lung in patients who did not receive neoadjuvant therapy. Data are mean $\pm$ SEM; p-values from Wilcoxon matched-pairs signed rank test (n = 15). **(E)** Heatmap of lipid species in the peripheral vein draining healthy (grey) or cancer-bearing (red) lung, normalized to the pulmonary artery. Each row is z-score normalized across samples (n = 15). Lipid classes: MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CE, cholesteryl ester; SM, sphingomyelin; Cer, ceramide. @ denotes the following metabolites (top to bottom): sphingosine, palmitoylethanolamide, cholesterol, campesterol, piperine, coenzyme Q9, coenzyme Q10, alpha-tocopherol, delta-tocopherol, gamma-tocopherol. **(F)** Heatmap of metal ion concentrations in the pulmonary vein draining healthy (grey) and cancer-bearing (red) lung lobes, normalized to the pulmonary artery. Data represent all participants (n = 20). Each row is z-score normalized across samples. **(G)** Scatter plot showing lactate levels in the cancer-draining pulmonary vein plotted against FDG-PET SUV (magenta, n = 13) or tumor size size (black, n = 15) in patients that did not receive neoadjuvant therapy. Pearson correlation coefficients and p-values are indicated.

Methods

Patient selection

Patients with resectable biopsy-proven non-small cell lung cancer with tumors greater than 1cm on pre-operative imaging were approached for participation in the general thoracic surgery clinic at University of Chicago Medical Center from July 2021 – May 2023. Patients were excluded if they had a history of inherited metabolic disorders, were currently taking oral steroid medications, or were planned to undergo a sub-lobar resection. Informed consent was obtained in clinic during a pre-operative appointment (IRB: UCMC 20-1696). Patient demographics including age, sex, race/ethnicity, comorbidities, frailty status, and pathologic and radiologic evaluation of tumors were recorded (**Supplemental Figure 1F; Supplemental Table 1**).

Intraoperative sampling

At time of oncologic resection, after the dissection of the hilar vessels, blood samples were obtained by the thoracic surgery team from the pulmonary vasculature and by the anesthesiology team from the systemic circulation. Using a 25 gauge needle, arterial inflow to the lung (the pulmonary artery) and venous outflow from the cancer containing lobe's pulmonary vein and an adjacent healthy lobe's pulmonary vein were sampled (**Supplemental Figure 1A-B**). Simultaneously, the anesthesiology team obtained blood samples from a radial arterial line and a peripheral vein to provide an approximation of the concentration gradient change of metabolites across the systemic circulation.

Validation of sample processing

A single healthy volunteer provided plasma samples for evaluation of whether plasma metabolite composition is altered by time spent on ice prior to sample processing. A 20 gauge butterfly needle was used to collect 5 mL of blood from the antecubital vein of the volunteer. Four 1cc aliquots

were placed into heparin coated cryovials. Samples were left on ice for 4, 15, 25 and 36 min prior to being centrifuged at 850 x g for 10 min at 4 °C. Plasma was isolated and partitioned into 50 µL aliquots in uncoated eppendorf tubes prior to flash freezing in liquid nitrogen and stored at -80 °C prior to analysis by liquid chromatography/mass spectrometry (LC/MS).

Initial processing of intraoperative samples

In the operating room, blood samples were partitioned into 1 mL aliquots in EDTA coated cryovials before being centrifuged at 800 x g for 10 min at 4 °C. Plasma was partitioned into aliquots of 100 µL into 1.5 mL uncoated eppendorf tubes and flash frozen with dry ice prior to transport and storage at -80 °C prior to analysis by LC/MS.

LC/MS analysis of polar metabolites

Metabolite quantification in human fluid samples was performed as described previously (1). In brief, 5 µL of sample or external chemical standard pool (ranging from ~5 mM to ~1 µM) was mixed with 45 µL of acetonitrile:methanol:formic acid (75:25:0.1) extraction mix including isotopically labeled internal standards. All solvents used in the extraction mix were HPLC grade. Samples were vortexed for 15 min at 4 °C and insoluble material was sedimented by centrifugation at 16,000 x g for 10 min at 4 °C. 20 µL of the soluble polar metabolite extract was taken for analysis of polar metabolites by LC/MS.

Metabolite profiling by LC/MS was conducted on a QExactive benchtop orbitrap mass spectrometer equipped with an Ion Max source and a HESI II probe, which was coupled to a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific). External mass calibration was performed using the standard calibration mixture every 7 days. An additional custom mass calibration was performed weekly alongside standard mass calibrations to calibrate the lower end of the spectrum (m/z 70-1050 positive mode and m/z 60-900 negative mode) using the standard

calibration mixtures spiked with glycine (positive mode) and aspartate (negative mode). 2 μ L of each sample was injected onto a SeQuant® ZIC®-pHILIC 150 x 2.1 mm analytical column equipped with a 2.1 x 20 mm guard column (both 5 mm particle size; EMD Millipore). Buffer A was 20 mM ammonium carbonate, 0.1% ammonium hydroxide; Buffer B was acetonitrile. The column oven and autosampler tray were held at 25 °C and 4 °C, respectively. The chromatographic gradient was run at a flow rate of 0.150 mL min⁻¹ as follows: 0-20 min: linear gradient from 80-20% B; 20-20.5 min: linear gradient from 20-80% B; 20.5-28 min: hold at 80% B. The mass spectrometer was operated in full-scan, polarity-switching mode, with the spray voltage set to 3.0 kV, the heated capillary held at 275 °C, and the HESI probe held at 350 °C. The sheath gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow was set to 1 unit. MS data acquisition was performed in a range of m/z = 70–1000, with the resolution set at 70,000, the AGC target at 1×10^6 , and the maximum injection time at 20 msec.

Following LC/MS analysis, metabolite identification was performed with XCalibur 2.2 software (Thermo Fisher Scientific) using a 5 ppm mass accuracy and a 0.5 min retention time window. For metabolite identification, external standard pools were used for assignment of metabolites to peaks at given m/z and retention time, and absolute metabolite concentrations determined as previously described (1). To account for individual patient differences, metabolite concentrations in the pulmonary veins (cancer-bearing lobe draining pulmonary vein: PVC; non-cancer-bearing lobe (healthy)-draining pulmonary vein: PVH) were normalized to the patient-specific pulmonary artery (PA) concentration. This was achieved by calculating the difference between each vein's concentration and the corresponding pulmonary artery concentration before comparing the relative consumption and production of individual metabolites.

LC/MS lipidomics

Positive ion mode analyses of polar and nonpolar lipids were conducted using an LC/MS system composed of a Shimadzu Nexera X2 U-HPLC (Shimadzu) coupled to an Exactive Plus orbitrap mass spectrometer (ThermoFisher Scientific). 10 μ L of human fluid sample was precipitated with 190 μ L of isopropanol containing 1,2-didodecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids) as an internal standard. After centrifugation, 2 μ L of supernatant was injected directly onto a 100 $\times$ 2.1 mm, 1.7- μ m ACQUITY BEH C8 column (Waters). The column was eluted isocratically with 80% mobile phase A (95:5:0.1 v/v/v 10 mM ammonium acetate/methanol/formic acid) for 1 min followed by a linear gradient to 80% mobile phase B (99.9:0.1 v/v methanol/ formic acid) over 2 min, a linear gradient to 100% mobile phase B over 7 min, then 3 min at 100% mobile phase B. Mass spectrometry analyses were performed using electrospray ionization in the positive ion mode using full scan analysis over 220 to 1,100 m/z at 70,000 resolution and 3 Hz data acquisition rate. Other mass spectrometry settings were as follows: sheath gas 50, in source collision-induced dissociation 5 eV, sweep gas 5, spray voltage 3 kV, capillary temperature 300°C, S-lens RF 60, heater temperature 300°C, microscans 1, automatic gain control target 1×10^6 , and maximum ion time 100 ms. Lipid identities were determined on the basis of comparison to reference standards and reference plasma extracts and were denoted by the total number of carbons in the lipid acyl chain(s) and total number of double bonds in the lipid acyl chain(s). Ion counts normalized to an internal standard were reported.

Inductively coupled plasma mass spectrometry (ICP-MS) for metal analysis

Samples were digested with 2 mL/g total wet weight nitric acid (Trace metal grade; Fisher) for 24 h at room temperature (RT), followed by treatment with 1 mL/g total wet weight hydrogen peroxide (Trace metal grade; Fisher) for another 24 h at RT. Samples were diluted with ultrapure water (VWR Chemicals ARISTAR ULTRA) followed by ICP-MS (Perkin Elmer Nexion 2000) using 50 ppb Bismuth as internal standard.

Statistics

Initial descriptive statistical analysis including comparison of individual metabolite concentrations, and linear regression comparing metabolite concentration to patient characteristics including tumor size and PET avidity was performed in R version 4.2.0 (Foundation for Statistical Computing, Vienna, Austria). Metaboanalyst 6.0 (2) was used to perform pairwise comparisons of metabolite concentrations, partial least squares determinant analysis, create metabolite heat maps, and identify variables of importance.

Study approval

Written informed consent was obtained from all patients. This study was initially approved on 5/10/2021 and was overseen for the duration of the study protocol by the University of Chicago IRB (IRB#20-1696). All Intraoperative images were obtained with patient consent at time of their procedure and consent has been retained.

Data availability

Values for all data points in graphs are reported in the Supporting Data Values file. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

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