Appendix 1

Supplementary pathology methods

Gross pathology

A complete necropsy was performed by a board-certified veterinary pathologist to inspect the treated tissues and collateral structures for ablation lesions, macroscopically evaluate downstream organs, perform gross treatment zone measurements, and collect samples for histopathologic analysis. The entire lung was serially sectioned, and regions of expected ablation zones (based on image review) were then serially sliced perpendicular to the expected needle trajectory. For percutaneous treatments the needle insertion site was used as a basis for initial sectioning when visible.

Identified treatment sites were isolated and collected, leaving a margin (~1 cm) of unaffected tissue on each side. The slice with largest ablated zone cross section was used to measure the lesion dimensions. Slice thicknesses through the lesion were used to calculate the z-dimension (defined as along the axis of needle insertion). Each tissue sample was washed with saline and digital images with a calibrated ruler were captured. All identified treatment sites were fixed in 10% neutral buffered formalin (NBF) after measurements and were sent for histopathological sectioning and staining.

Representative tissue samples from organs downstream of the treatment sites were collected and immersed in 10% NBF. These included: the untreated lung, transverse sections of the heart, liver, kidney, spleen, brain, and treatment site draining lymph nodes (left and right tracheobronchial).

Histopathology

Histopathology was performed on grossly identified treatment site lesions, draining lymph nodes, tissues immediately collateral to the treatment sites, and select nontarget organs (R & L Tracheobronchial lymph nodes, Liver, R & L Kidneys, Brain, Spleen, Heart and Diaphragm, Caudal Vena Cava, and Thoracic Wall) in each animal depending on the treatment location.

All trimmed target and non-target tissues were processed by routine methods into paraffin. Tissue blocks were cut at approximately 5 µm and the resulting sections were stained with H&E. Additional sections of each treatment site block were cut at approximately 5 µm and stained with Masson's trichrome.

All slide sections were evaluated via light microscopy, and the results were documented as qualitative descriptions. For treatment sites, semiquantitative methods were used to score necrosis, fibrosis/fibroplasia, inflammation, hemorrhage, hemosiderosis, edema, extravascular thrombus/fibrin, intravascular thrombus/fibrin, and peribronchiolar lymphoid hyperplasia. When inflammation was present, the specific inflammatory cell types were recorded. All nontarget tissues were assessed for alterations to normal architecture, and any significant histological observations were recorded.

Blood samples were collected prior to treatment and prior to termination for all animals and were analyzed for hematology, serum biochemistry, and coagulation panels.

Supplemental pathology results

In the percutaneous group, there were focal areas of pleural thickening with white/light tan appearance due to pleural fibrosis consistent with the sites of needle insertion rather than treatment.

https://dx.doi.org/10.21037/jtd-2024-1976

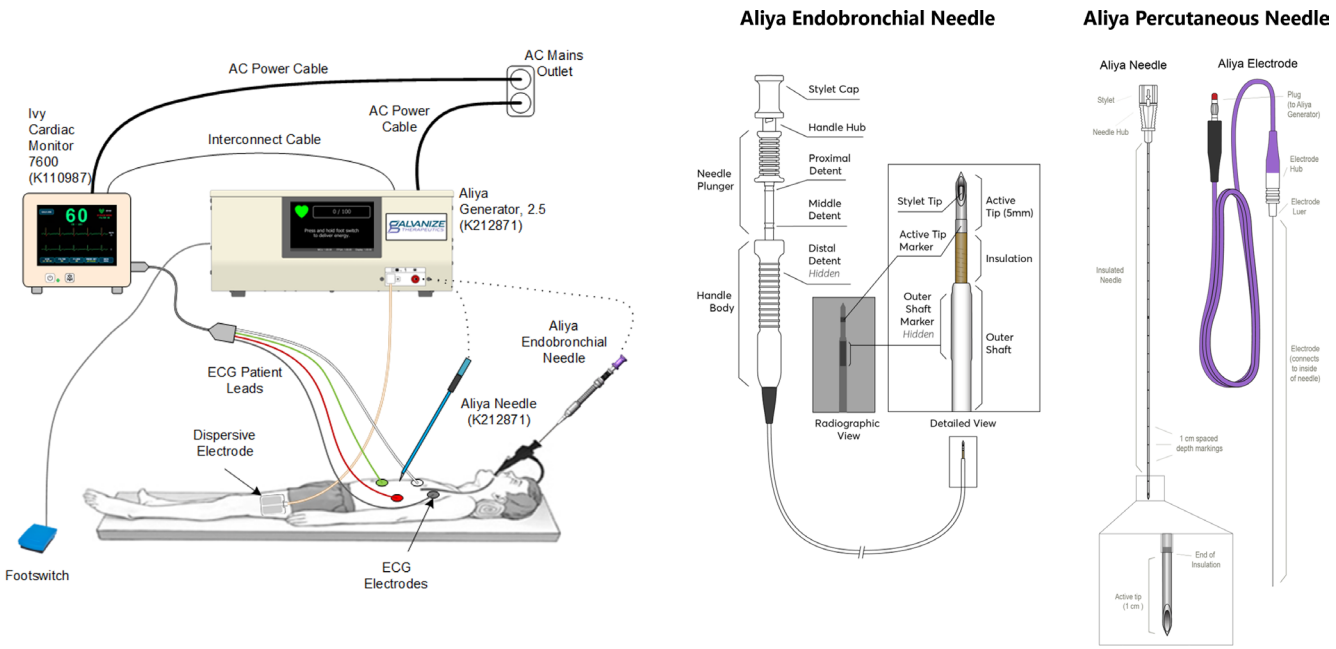
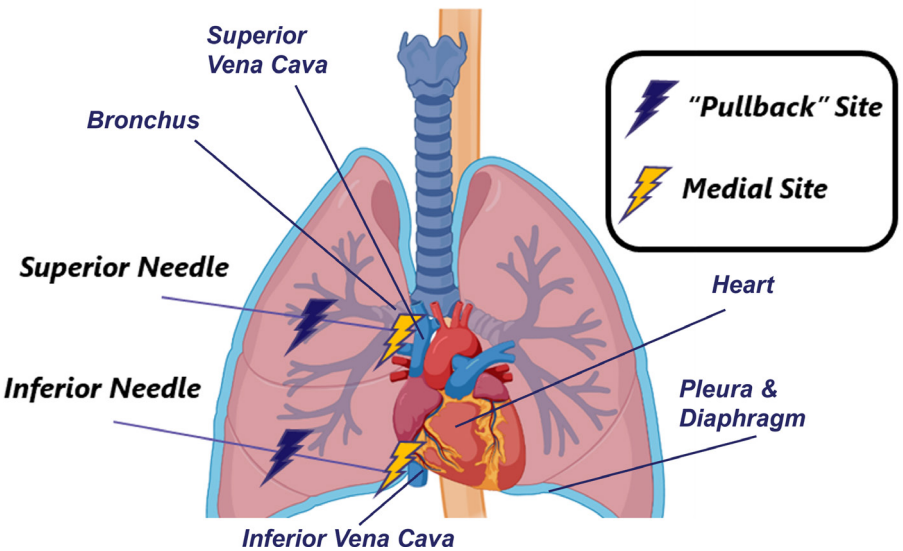


Figure S1 Aliya system components. The entire system depicting the patient interface is shown (left) with the endobronchial (middle) and percutaneous (right) needles.



Targeted Structures
Pleura
Pericardium
Diaphragm
Inferior Vena Cava
Pulmonary arteries
Bronchii

Figure S2 Representation of treatment locations and critical structures.

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Table S1 Pre-treatment clinical pathology results

	Animal ID						Normal range
	P1	P4	P2	P3	P5	P6	
Hematology							
WBC	14.9	16.8	21.4	19.0	18.3	16.6	5.4–22.4
RBC	5.7	5.3	5.8	6.3	6.6	6.2	4.7–7.6
HGB	10.0	9.8	10.5	10.4	11.2	11.4	7.9–12.1
HCT	30.4	29.1	31.5	31.9	34.9	34.8	25.6–40.9
MCV	53.3	55.1	54.3	50.5	52.9	55.8	45–62.6
MCH	17.6	18.5	18.1	16.5	17.0	18.3	13.6–19.1
MCHC	32.9	33.6	33.3	32.7	32.1	32.8	26.5–33.9
RDW	16.0	14.7	15.0	16.8	15.3	16.0	26.6–21.8
PLT	280.0	415.0	386.0	390.0	333.0	328.0	152–546
MPV	7.6	7.5	7.8	7.5	7.6	9.2	7.9–12.1
% NEUT	24.2	27.1	27.0	34.3	33.3	37.0	20–70
% LYMPH	67.9	61.7	62.0	58.2	56.8	57.5	35–75
% MONO	4.6	7.8	8.6	4.3	6.4	3.2	0–10
% EOS	0.3	0.8	0.3	0.7	0.4	0.1	0–15
% BASO	0.6	0.5	1.1	0.5	0.6	0.3	0–3
Biochemistry							
Globulin (g/dL)	2.6	3.7	3.9	3.0	2.8	3.2	1.8-4.2
A/G ratio	1.3	0.9	0.8	1.3	1.3	1.1	0.6-1.7
AST (U/L)	34.0	22.0	32.0	31.0	19.0	17.0	0–51
Albumin (g/dL)	3.4	3.2	3.1	3.8	3.6	3.5	3.0–4.2
Urea (mg/dL)	9.0	8.0	10.0	8.0	9.0	7.0	2.0–14.0
Creatinine (mg/dL)	1.8	1.3	1.6	1.6	1.8	1.7	0.8–1.9
Calcium (mg/dL)	9.2	9.6	9.6	10.6	11.9	10.2	9.0–11.0
Carbon Dioxide (mmol/L)	38.0	35.0	36.0	36.0	34.0	39.0	21–40
Glucose (mg/dL)	89.0	90.0	88.0	77.0	112.0	97.0	58–162
Phosphorous (mg/dL)	6.9	6.4	6.7	7.2	7.3	7.8	3.1–9.5
Total Protein (g/dL)	6.0	6.9	7.0	6.8	6.4	6.7	4.8–6.5
ISE-Na (mmol/L)	140.5	137.4	141.2	144.0	140.6	141.1	125.0–147.0
ISE-K (mmol/L)	3.8	3.9	3.9	4.1	4.1	4.1	2.90–4.60
ISE-Cl (mmol/L)	96.6	94.4	95.5	98.9	97.0	95.6	92.0–110.0
Cholesterol (mg/dL)	95.0	78.0	86.0	89.0	82.0	84.0	57–100
Creatine Kinase (U/L)	566.0	214.0	486.0	1,189.0	836.0	923.0	0–1152
GGT (U/L)	51.6	27.8	63.1	48.3	47.5	59.9	0.0–47.0

Table S1 (*continued*)

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Table S1 (*continued*)

	Animal ID						Normal range
	P1	P4	P2	P3	P5	P6	
Total Bilirubin (mg/dL)	0.1	0.1	0.1	0.1	0.1	0.0	0.0–0.4
Triglycerides (mg/dL)	20.9	12.0	25.8	27.2	33.6	26.8	0.0–0.0
ALP	149.0	67.0	98.0	102.0	209.0	125.0	0.0–0.0
ALT (U/L)	66.0	34.0	44.0	62.0	51.0	37.0	0.0–0.0
Coagulation							
Prothrombin time (s)	13.6	13.2	13.9	14.0	13.2	14.1	10.60–13.90
Activated PTT (sec)	29.7	16.1	24.0	30.8	40.1	40.6	11.20–57.20
Fibrinogen (mg/dL)	158.0	137.0	206.0	160.0	167.0	176.0	131.00–307.00

*, gray cells show out of range parameters. The reference ranges for clinical pathology parameters were established from the historical data at the animal labs. NA, not available; NM, not measured.

Table S2 Pre-termination clinical pathology results

	Animal ID						Normal range
	P1	P4	P2	P3	P5	P6	
Hematology							
WBC	16.1	10.0	22.2	20.4	14.5	13.9	5.4–22.4
RBC	5.4	3.9	5.5	6.3	6.3	5.5	4.7–7.6
HGB	9.3	7.1	9.7	10.3	10.5	10.2	7.9–12.1
HCT	29.0	22.0	3.1	31.8	32.2	30.3	25.6–40.9
MCV	54.1	56.3	55.3	50.7	51.5	55.2	45–62.6
MCH	17.5	18.1	17.9	16.3	16.8	18.5	13.6–19.1
MCHC	32.2	32.1	32.3	32.2	32.7	33.6	26.5–33.9
RDW	17.4	14.7	15.1	16.7	15.1	15.1	26.6–21.8
PLT	349.0	405.0	273.0	410.0	295.0	369.0	152–546
MPV	8.3	8.2	10.1	7.7	8.4	8.5	7.9–12.1
% NEUT	27.2	32.0	52.2	28.1	28.6	24.7	20–70
% LYMPH	63.0	63.1	38.5	65.5	65.0	68.1	35–75
% MONO	7.9	3.7	6.5	5.0	3.4	4.0	0–10
% EOS	0.5	0.3	1.7	0.6	0.2	0.1	0–15
% BASO	0.5	0.3	0.4	0.5	0.6	0.3	0–3

Table S2 (*continued*)

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Table S2 (*continued*)

	Animal ID						Normal range
	P1	P4	P2	P3	P5	P6	
Biochemistry							
Globulin (g/dL)	2.6	2.5	3.6	2.6	2.4	3.3	1.8-4.2
A/G ratio	1.2	0.9	0.8	1.2	1.4	0.9	0.6-1.7
AST (U/L)	31.0	15.0	29.0	26.0	24.0	17.0	0–51
Albumin (g/dL)	3.1	2.2	2.8	3.2	3.4	2.9	3.0–4.2
Urea (mg/dL)	7.0	6.0	5.0	6.0	9.0	7.0	2.0–14.0
Creatinine (mg/dL)	1.5	1.2	1.4	1.3	1.9	1.6	0.8–1.9
Calcium (mg/dL)	11.0	9.4	10.4	11.6	10.3	10.9	9.0–11.0
Carbon dioxide (mmol/L)	38.0	33.0	38.0	43.0	34.0	35.0	21–40
Glucose (mg/dL)	86.0	56.0	89.0	63.0	104.0	79.0	58–162
Phosphorous (mg/dL)	6.7	5.5	6.4	7.9	6.4	7.0	3.1–9.5
Total Protein (g/dL)	5.7	4.7	6.4	5.8	5.8	6.2	4.8–6.5
ISE-Na (mmol/L)	142.0	136.1	139.2	140.3	138.5	140.9	125.0–147.0
ISE-K (mmol/L)	3.7	3.9	4.0	4.0	3.7	4.0	2.90–4.60
ISE-Cl (mmol/L)	97.1	94.4	97.2	96.3	94.7	95.5	92.0–110.0
Cholesterol (mg/dL)	106.0	66.0	101.0	97.0	85.0	69.0	57–100
Creatine kinase (U/L)	448.0	604.0	572.0	540.0	484.0	516.0	0–1152
GGT (U/L)	47.7	16.8	61.6	43.0	44.5	67.9	0.0–47.0
Total bilirubin (mg/dL)	0.1	0.1	0.1	0.1	0.1	0.1	0.0–0.4
Triglycerides (mg/dL)	27.6	8.5	47.5	30.3	15.7	14.9	0.0–0.0
ALP	128.0	86.0	58.0	84.0	92.0	112.0	0.0–0.0
ALT (U/L)	58.0	35.0	43.0	56.0	51.0	49.0	0.0–0.0
Coagulation							
Prothrombin time (s)	12.9	13.6	12.9	12.0	13.4	13.1	10.60–13.90
Activated PTT (s)	41.7	18.5	59.4	29.9	40.3	42.2	11.20–57.20
Fibrinogen (mg/dL)	177.0	144.0	253.0	155.0	184.0	196.0	131.00–307.00

* Gray cells show out of range parameters. NA not available NM not measured. The reference ranges for clinical pathology parameters were established from the historical data at the animal labs.

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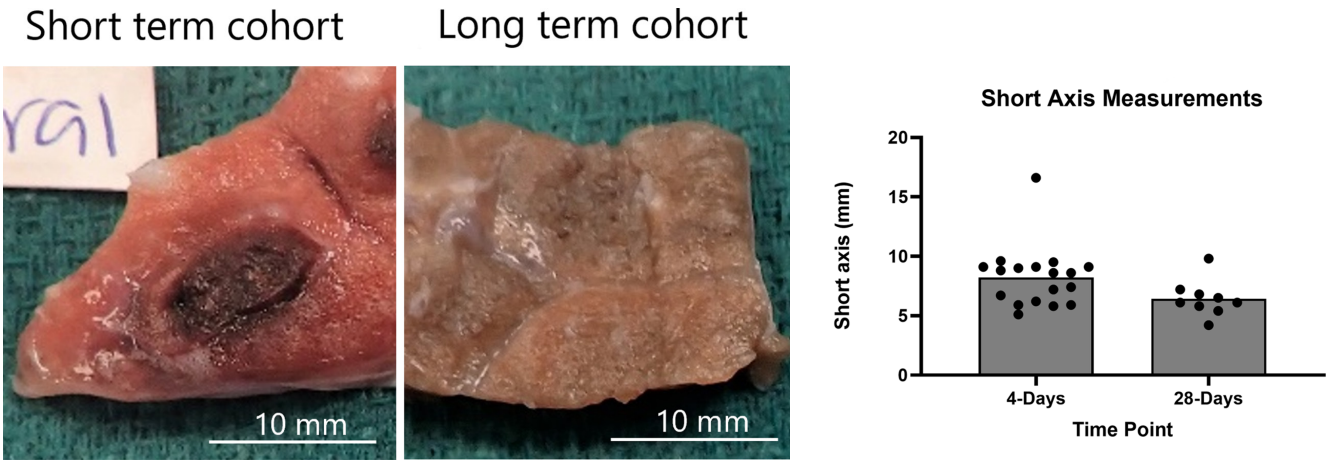

Figure S3 Representative gross images short term (left) and long term (right).

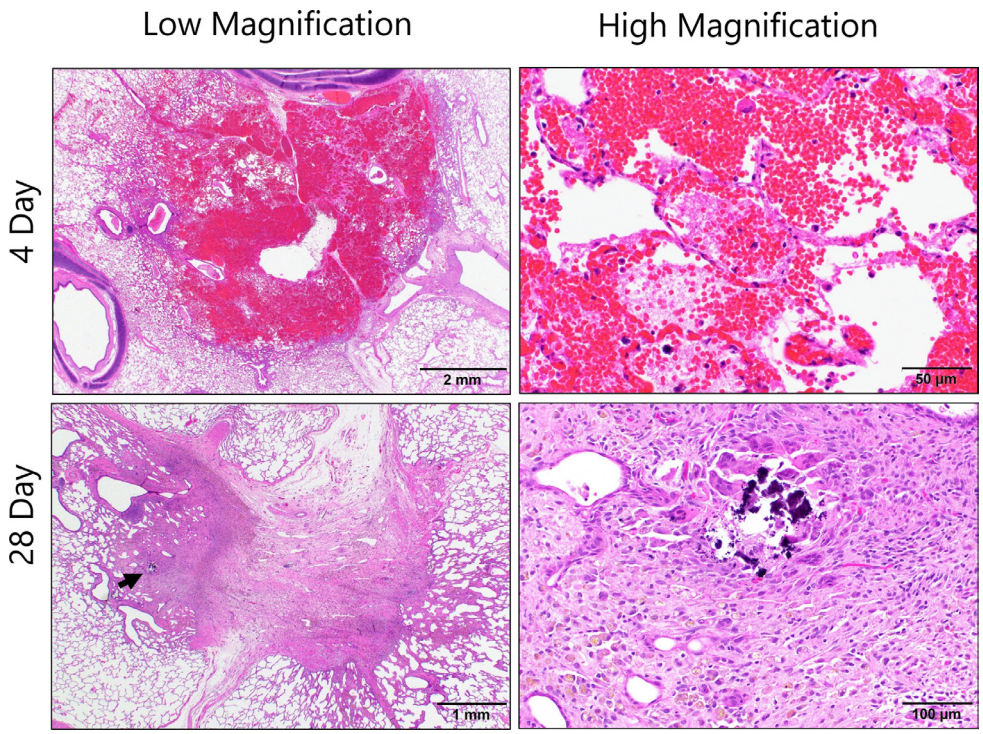

Figure S4 Representative H&E-stained ablation zone at low magnification (left) and high magnification (right) for both short (top) and long (bottom) term timepoints. At 4 days the zone is well demarcated and consists of necrosis, hemorrhage, and hyperemia. At 28 days a well demarcated treatment site lesion composed of fibrosis within the pulmonary parenchyma; The arrow denotes a focus of mineralization which is shown at higher magnification. The mineralizaed material is associated with multinucleated giant cells, and hemosiderin-laden macrophages are common within the zone of fibrosis.

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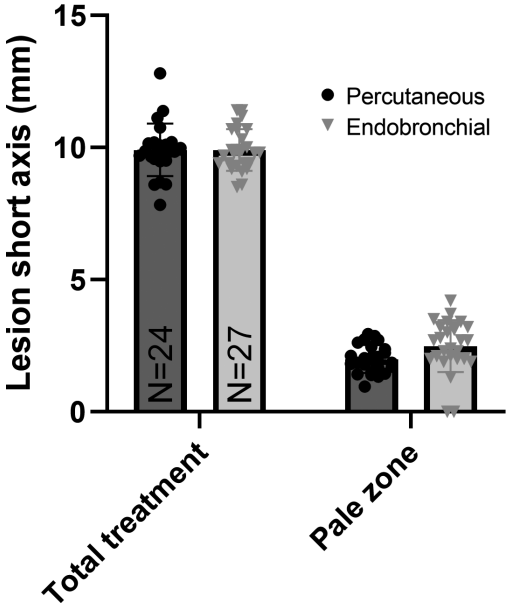

Figure S5 Comparison between lesion size created by percutaneous *vs.* endobronchial needles in liver.

https://dx.doi.org/10.21037/jtd-2024-1976