Supplementary

Study design and methods

CT-detected lung cancer cases

Tumor histology was coded according to ICD-O-3 version 2003 or 2013 as appropriate. For this article, the morphology codes were combined to the following groups: small-cell lung cancers (ICD-O-3: 8041/3, 8042/3, 8045/3, 8044/3); non–small-cell lung cancers, subdivided into squamous cell carcinomas (8070/3, 8072/3, 8071/3, 8083/3, 8076/3, 8078/3), adenocarcinomas (8140/3, 8255/3, 8250/3, 8480/3, 8550/3, 8260/3, 8310/3, 8490/3, 8046/3, 8250/3, 8253/3), (8250/3, 8253/3) large cell carcinomas (8013/3), carcinoids (8240/3, 8246/3, 8249/3), unspecified carcinomas (8010/3) and malignant neoplasms (8000/3).

Participant selection within the LUSI trial: nested case-control design

Participants with screen-detected lung cancer are represented by the blood sample taken at the time of the suspicious LDCT scan that led to further diagnostic work up. Participants in RS1 are represented by the sample taken at the baseline examination, and in RS2 by the sample taken at the time of their first suspicious LDCT scan.

Sample processing and laboratory assays

Reagents were brought to room temperature 2 hours before the start of the assay. Control A was used on plate one with patient samples one through five run on the same plate. Control B was measured with patient samples six through ten on plate two. All controls and samples were pipetted in duplicate on their respective plate. 100 µL of control or patient samples were added to their wells, the wells sealed and incubated at room temperature for 90 minutes with shaking (400 rpm). Samples were tapped out on absorbent paper and the wells washed four times with wash buffer with tapping out of buffer between each wash cycle. 100 µL of secondary antibody was then added to each well followed by covered incubation with shaking for a further 60 minutes. The four step wash cycle was then repeated and 100 µL of substrate added to each well. Color development was carried out for no more than 15 minutes in the dark at room temperature with intermittent shaking per hand. 100 µL of stop solution was added to each well and the plates read at a wavelength of 650 nm within 10 minutes (Victor III, Perkin Elmer).

The resulting optical density values were copied and pasted into the provided Excel calculation table from Oncimmune. The provided table indicated the Control Pass/Fail status along with a result status for the patient samples [non-significant level (NSL), moderate or high].

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Table S1 LDCT evaluation algorithm applied in the German randomized lung cancer screening trial LUSI

Newly observed nodules (first screening round or new in subsequent rounds)		Known nodules (early recalls or subsequent screening rounds)	
Outcome by nodule size	Action	Outcome by nodule growth	Action
Without abnormality or nodules <5 mm	Back to routine screening (12 months)	–	–
Nodules ≥5 and <8 mm	Early recall (6 months)	>600 VDT	Back to routine screening
		400–600 VDT D <7.5 mm	Early recall (6 months)
Nodules ≥8 and ≤10 mm	Early recall (3 months)	D ≥7.5–10 mm	Early recall (3 months)
Nodules >10 mm/not highly suspicious		≤400 VDT or D >10 mm	Immediate recall
Highly suspicious	Immediate recall	Malignant	Treatment
		Non-malignant	Back to routine screening

VDT, volume doubling time; mm: millimeters; D, diameter.

Table S2 Blood collection and LDCT-scan results

Blood sample	LDCT-scan result					
	Non-suspicious	Early recall 6 months	Early recall 3 months	Immediate recall	Not available	Total
Baseline						
Available samples	1,362	285	62	44	1[‡]	1,754
Unavailable samples[†]	215	45	6	9		275[§]
Round 1 total (T0)	1,577	330	68	53	1[‡]	2,029
Rounds 2 to 5						
Available samples	206[¶]	16	5	8		235
Unavailable samples	1,598	20	11	28		1,657
Round 2 total (T1)	1,804	36	16	36		1,892
Available samples	5	21	19	15		60
Unavailable samples	1,770	5	4	10		1,789
Round 3 total (T2)	1,775	26	23	25		1,849
Available samples	2	43	24	23		92
Unavailable samples	1,720	3	1	10		1,734
Round 4 total (T3)	1,722	46	25	33		1,826
Available samples	1	43	22	21		87
Unavailable samples	1,710	6	1	6		1,723
Round 5 (T4)	1,711	49	23	27		1,810

LDCT, low-dose computed tomography; T0, T1, T2, T4: time points zero until four. [†], blood draw not carried out (in case of unsuspicious findings after T0) or unsuccessful blood draw; [‡], one subject could not be scanned because of overweight. The subject was excluded from the study. [§], out of 275 unavailable samples: 234 were not carried out because the freezer was not available, 38 patient-related, 1 blood-draw technique-related; [¶], red cells indicate samples taken as replacement for unsuccessful baseline blood draws. These were taken even if the LDCT-Scan results were non-suspicious.

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Table S3 Performance of EarlyCDT®-Lung amongst subjects with suspicious nodules, by nodule size

Largest diameter (mm)	EarlyCDT®-Lung result									
	HIGH		MOD		NS		OR (95% CI)		LR+	
	LC	No LC	LC	No LC	LC	No LC	Positive (high)	Positive (high or moderate)	Positive (high)	Positive (high or moderate)
<10	1	4	0	3	10	78	2.03 (0.20–19.95)	1.11 (0.12–10.02)	1.93 (0.24–15.77)	1.10 (0.16–7.53)
≥10	5	0	0	1	29	4	–	0.69 (0.06–7.51)	1.17 (1.02–1.35)	0.95 (0.65–1.39)
Overall[†]	6	4	0	4	39	82	3.31 (0.88–12.39)	1.58 (0.51–4.86)	1.92 (1.09–3.40)	1.50 (0.55–4.06)

[†], for one subject, the CT scan evaluation at round 2 was deemed suspicious (with immediate recall) even in the absence of pulmonary nodules, due to the identification of atelectasis (collapsed lung) in the scan images. That subject was excluded for these analyses. MOD, moderate level result; NS, no significant level results; OR, odds ratio; LR+, positive likelihood ratio; LC, lung cancer; mm, millimeters.

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