

Pragmatic, Multicenter, Double-Blind Evaluation of Circulating Cancer-Associated Macrophage-Like Cells for Detection of Cancer in Indeterminate Pulmonary Nodules

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ABSTRACT

PURPOSE Cancer-associated macrophage-like cells (CAMLs) are specialized myeloid polyploid cells that emanate from primary tumor masses and transit circulation in a variety of malignancies, which have the potential to track cancer progression and therapy response. Previous studies have demonstrated that larger CAMLs ($\geq 30 \mu\text{M}$ and $\geq 50 \mu\text{M}$) are particularly associated with disease progression. We hypothesized that CAMLs could provide additional cancer risk information for patients with indeterminate lung nodules.

MATERIALS AND METHODS This was a double-blind, multicenter study to evaluate CAMLs in the blood as diagnostic biomarkers for malignancy. We enrolled 203 patients with indeterminate nodules (0.8–3 cm) and no diagnosis of cancer. Blood samples were obtained at the time of the initial evaluation and before diagnostic procedures; physicians were blinded to the CAML results. We determined the prevalence of CAMLs $\geq 30 \mu\text{M}$ (CAML+) at the initial screening and calculated screening statistics including positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity of CAML+ for any malignancy detected during follow-up.

RESULTS Of the 203 enrolled patients, 200 had their samples sent for CAML analysis. For evaluable patients ($n = 191$), there were 41 with non-small cell lung cancer, four with small cell lung cancer, six with carcinoma, and five with other malignancies. For eight patients, a biopsy was either nondiagnostic or not feasible; they were treated for lung cancer with radiotherapy (and counted as cancer). CAMLs were detected in 45.0% of all patient samples (95% CI, 37.8% to 52.4%). For the detection of any malignancy, CAML PPV and NPV were 27.9% and 61.9%, respectively, and sensitivity and specificity were 37.5% and 51.2%, respectively. Defining CAML+ as larger CAML size ($\geq 50 \mu\text{M}$) did not significantly alter the results.

CONCLUSION CAMLs are neither sensitive nor specific to the presence of malignancy in indeterminate pulmonary nodules.

ACCOMPANYING CONTENT

 Appendix

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INTRODUCTION

Lung cancer remains a major cause of cancer-related death in the United States.¹ The National Lung Screening Trial (NLST) determined that low-dose computed tomography (LDCT) screening could decrease lung cancer deaths by 20%.² However, over 39% of the screened patients were determined to have positive screens with 96.4% false positives. This very high false-positive rate requires further testing, has the potential of loss to follow-up, and has resultant patient stress and anxiety. While lung nodules < 0.8 cm are considered low-risk findings and nodules > 3.0 cm high-risk, nodules between

0.8 and 3.0 cm are considered “indeterminate” and represent a management challenge.³

Several studies have evaluated methods to predict which nodules harbor malignant diseases. Location, characteristics of nodule spiculation, age, female sex, number of nodules, and carcinogen exposure have frequently been cited as predictive factors for malignancy.^{4,5} There is variability in the definitions of indeterminates.⁶ In NLST, screen positivity was defined by the greatest nodule diameter of 4 mm or larger. By contrast, a European study (Dutch-Belgian Randomized Lung Screening Trial, NELSON) based screening

CONTEXT

Key Objective

Does the presence, type, or size of cancer-associated macrophage-like cells (CAMLs) help identify the presence of malignancy in patients with indeterminate pulmonary nodules?

Knowledge Generated

Despite the presence of CAMLs in 45% of the evaluated patients, they did not identify a population at higher risk.

Relevance

Indeterminate pulmonary nodules are a common problem. Although the current study is negative, the approach used in this study can serve as a template for future studies.

interpretation on nodule volumetry using a tiered approach.⁷ They classified nodules $<50 \text{ mm}^3$ (4.6-mm diameter) as negative, nodules $>500 \text{ mm}^3$ ($>9.8\text{-mm}$ diameter) positive, and nodules $50\text{--}500 \text{ mm}^3$ indeterminate. These indeterminate nodules underwent an early (3-month) follow-up LDCT to assess growth; nodule volume doubling times were then used to distinguish between positive screens requiring additional diagnostic procedures and negative screens. An evaluation of $>12,000$ nodules from two data sets was able to develop an algorithm with excellent discrimination between benign and malignant nodules (receiver-operating characteristic [ROC] curve >0.90).⁵ However, the above-discussed and similar experiences rely on continued follow-up, significant radiologic expertise, and coordination.⁸ However, there are also potential risks. In the NLST, for those with confirmed lung cancer, 28.4% of patients experienced a complication and most (26.2%) considered “intermediate” or “major.” Of these patients, 1.5% died within 60 days of the most invasive diagnostic procedure.

Cancer-associated macrophage-like cells (CAMLs) are specialized myeloid polyploid cells that emanate from primary tumor masses and transit the circulation in patients with a variety of malignancies, which can be used to track cancer response to therapy, progression, and/or relapse.^{9–14} Prior data have demonstrated that CAMLs are absent in healthy controls and are rare in persons with benign masses.¹⁵ Therefore, we hypothesized that CAMLs could provide additional information regarding the cancer risk in patients with indeterminate lung nodules.

MATERIALS AND METHODS

Study Design

Patients were recruited from pulmonary nodule clinics at the Fox Chase Cancer Center and University of Pennsylvania. The eligibility criteria were pragmatic: referral for a pulmonary nodule that had not yet been biopsied and that met the definition of an “indeterminate” nodule (ie, 0.8–3.0 cm). Patients were excluded if they had a prior diagnosis of lung cancer or

other invasive malignancy within the past 2 years; history of rheumatologic disease; or history of ongoing infectious processes, including hepatitis B or C or HIV. All participants provided written informed consent before the study. The study was approved by all relevant institutional review boards.

Basic demographic and medical data, including smoking history, occupational exposure, military history, prior diagnoses, and medications, were recorded. Radiologic information, including the location of the nodule (lobe), size, and characteristics (eg, ground glass, solid, and spiculation), was recorded. All patient evaluations and assessments were performed per the standard of care and physician discretion (Fig 1). Blood samples were drawn by standard venipuncture at the time of enrollment. Each blood sample contained 10 cc of blood in two tubes (20 cc). CAML detection was performed using the CellSieve microfiltration system at Creatv Microtech according to the established criteria. All samples were run in a Clinical Laboratory Improvement Amendments-certified laboratory under a College of American Pathologists assurance program, with designated controls and validations. Interanalyses and intra-analyses were performed by trained and certified cytologists with random quality control assessments.^{16–20} Cells captured by the CellSieve filter were stained for CK18, CK19, CD31, and CD45.⁹ CAMLs were defined as enlarged ($\geq 30 \mu\text{m}$ in diameter) multinuclear cells with diffuse cytoplasmic cyto-keratin and either CD14 or CD45. Epithelial-mesenchymal transition (EMT) circulating tumor cells were defined as diffuse/nonfilamentous cytokeratin-positive cells with pleomorphic nuclei that were negative for CD45 and CD14.^{21–23} If a patient underwent an invasive diagnostic procedure of any type (surgery, bronchoscopy, CT, or ultrasound-directed biopsy), an additional sample was drawn at the time of the procedure. Patients were followed for a minimum of 1 year from accrual (last patient accrued) to a maximum of 5 years (patient accrued at the beginning of the study).

Statistical Analysis

Patient demographics and clinical characteristics were summarized using descriptive statistics. For each blood

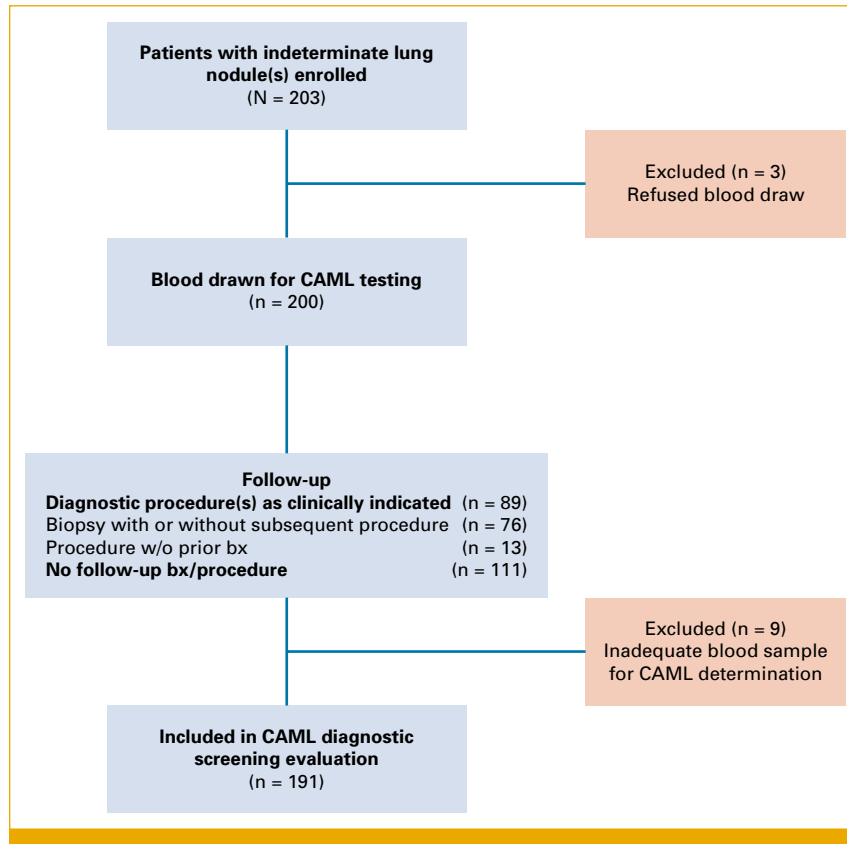


FIG 1. Flow diagram. CAML, cancer-associated macrophage-like cell.

sample, CAML results were recorded as the largest CAML cell detected, as well as the number of CAML cells detected using a 30- μ M cell diameter. Nine patients with inadequate blood samples were excluded from the study. For each patient, we determined the binary outcome (any ν none) for the presence of CAMLs, denoted as CAML+. We calculated the proportion of patients with CAML+ with a 95% two-sided CI. For diagnostic screening statistics, we used CAML+ status and malignancy status (any cancer) to determine estimates and 95% confidence intervals for positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity. Malignancy outcomes included patients treated with stereotactic body radiotherapy (SBRT) without biopsy. In the sensitivity analysis, we used a 50- μ M cell diameter to define CAML+, a threshold used in previously published studies.

We also evaluated the diagnostic accuracy for malignancy using cells demonstrating epithelial-mesenchymal transition (EMT+) and the combined CAML+ and EMT+ indicators in two ways: (1) CAML+ or EMT+ and (2) CAML+ and EMT+.

To determine whether known risk factors for lung nodules may affect the diagnostic utility of CAML+, we first identified the factors that were significantly associated with malignancy. Potential factors included age at enrollment, sex, smoking history, personal history of chronic obstructive pulmonary disease, family history of lung cancer, nodule size, location, and contour. We compared malignancy status

with categorical risk factors and EMT+ using Chi-square tests and with continuous risk factors (age and nodule size) using two-sided Wilcoxon rank-sum tests. We included patients with missing data in each comparison. Not all patients had audited nodule measurements, which limited the sample size in the multivariable models.

For risk factors that were significantly associated with malignancy, logistic regression was used to evaluate whether the risk factors influenced the association of CAML+ with malignancy outcome. These selected risk factors were smoking status (ever ν never for cigarette use), spiculation (yes/no), and nodule location (upper lobe ν not) and were included as covariates in logistic models for the malignancy outcome with CAML+ for patients with complete covariate information. The predictive accuracy of logistic regression models for “any malignancy” is shown as ROC curves and AUC. The utility of adding CAMLs to the predictive model was assessed by examining the difference in AUC values using Delong test.

As an alternative to adjusting for risk factors separately, we calculated a risk variable using the Mayo risk equation to estimate the probability of malignancy on the basis of patient’s age, smoking status, nodule size, spiculation (yes/no), and upper lobe location (yes/no).⁴ For the cancer history predictor used in the Mayo equation, we assumed that patients had not had a cancer diagnosis more than 5 years before enrollment, on the basis of the entry criteria of no

TABLE 1. Demographic and Clinical Data^a

Characteristic	N (%)
Sex	
Male	80 (40.0)
Female	120 (60.0)
Race	
White	163 (81.5)
African American	28 (14.0)
Other	9 (4.5)
Age at enrollment, years, median (range)	69.2 (37.4-93.9)
BMI at enrollment, median (range)	27.8 (16.4-61.2)
n missing	12
Smoking history	
Current	32 (16.0)
Former	89 (44.5)
Never	67 (33.5)
Unknown	12 (6.0)
Asbestos exposure?	
Yes	26 (13.0)
No	156 (78.0)
Unknown	18 (9.0)
Personal history of COPD	
Yes	59 (29.5)
No	141 (70.5)
Family history of lung cancer	
Yes	60 (30.0)
No	140 (70.0)
Nodule characteristics ^b	
Maximum dimension, mm, median (range)	14 (8-35)
Location	
RUL	40 (25.0)
RML	21 (13.1)
RLL	35 (21.9)
LUL	33 (20.6)
LLL	26 (16.3)
Lingula	5 (3.1)
Missing	40
Contour	
Smooth	43 (38.1)
Spiculated	22 (19.5)
Lobulated	19 (16.8)
Ill-defined	29 (25.7)
Missing	87
Density	
Solid	84 (72.4)
Part solid	18 (15.5)
Ground glass	14 (12.1)
No data	84

(continued in next column)

TABLE 1. Demographic and Clinical Data^a (continued)

Characteristic	N (%)
Number of nodules	
1	148 (74.0)
2-5	52 (26.0)

Abbreviations: COPD, chronic obstructive pulmonary disease; LLL, left lower lobe; LUL, left upper lobe; RLL, right lower lobe; RML, right middle lobe; RUL, right upper lobe.

^aAnalytic cohort of 200 patients excludes three patients who declined to provide blood samples after consenting.

^bFor patients with multiple nodules, characteristics of the nodule with the largest dimension are included.

cancer diagnosis within 2 years of enrollment. We used the Mayo risk probability variable as a covariate in a separate logistic regression model (comparing the AUC values with and without CAML). All statistical tests were two-sided with a 5% type I error, and analyses were performed using SAS software (version 9.4; Cary NC).

RESULTS

Between May 21, 2019 and November 30, 2022, 203 patients consented; three patients declined to provide blood samples after consent and were excluded from the analytic cohort. Patient demographics are listed in Table 1. Of the 200 samples sent for CAML evaluation, nine specimens were inadequate for analysis and 191 patients with adequate baseline specimens were considered evaluable. CAMLs were detected in 45.0% of the evaluable patients (95% CI, 37.8% to 52.4%). For patients in whom CAMLs were detected, the median number of CAMLs was two and the mean was 3.6 (range, 1-28; Appendix Table A1). CAMLs measured 30 to 241 μ M in diameter; the median diameter for the largest CAML per patient was 62 μ M and the mean was 80.5 μ M.

Seventy-six patients underwent biopsy procedures, with 39 patients also receiving surgery or SBRT, 13 patients on surgery or SBRT without a preceding biopsy, and 111 patients

TABLE 2. Diagnostic Testing Accuracy of CAML+ for Any Malignancy in Patients With Indeterminate Nodules

Diagnostic Accuracy Metric	CAML+, ≥ 30 μ M (n = 86 of 191)	CAML +, ≥ 50 μ M (n = 60 of 191)
Sensitivity	37.5% (25.6%-49.4%)	21.9% (11.7%-32.0%)
Specificity	51.2% (42.5%-59.9%)	63.8% (55.4%-72.1%)
Positive predictive value	27.9% (18.4%-37.4%)	23.3% (12.6%-34.0%)
Negative predictive value	61.9% (52.6%-71.2%)	61.8% (53.5%-70.2%)

Abbreviation: CAML, cancer-associated macrophage-like cell.

TABLE 3. Diagnostic Testing Accuracy of EMT+ Only and CAML+ With EMT+

Diagnostic Accuracy Metric	EMT+		CAML+ or EMT+		CAML+ and EMT+	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Positive predictive value	43.6%	28.0% to 59.2%	32.1%	23.2% to 41.0%	36.8%	15.2% to 58.5%
Negative predictive value	69.1%	61.7% to 76.4%	64.7%	54.6% to 74.9%	66.9%	59.8% to 73.9%
Sensitivity	26.6%	15.7% to 37.4%	53.1%	40.9% to 65.4%	10.9%	3.3% to 18.6%
Specificity	82.7%	76.1% to 89.3%	43.3%	34.7% to 51.9%	90.6%	85.5% to 95.6%

Abbreviations: CAML, cancer-associated macrophage-like cell; EMT, epithelial-mesenchymal transition.

with no follow-up procedures. Of those biopsied, 67 underwent biopsy within 60 days of enrollment.

There were 41 patients with non–small cell lung cancer, four with small cell lung cancer, six with carcinoid, and five with other malignancies. For eight patients, biopsy was non-diagnostic or not feasible but were treated for lung cancer with SBRT and counted as a cancer diagnosis.

For the evaluation of CAML+ (one or more detected CAMLs) and any malignancy, the sensitivity and specificity were

37.5% and 51.2%, respectively (Table 2). Positive and negative predictive values were 27.9% and 61.9%, respectively. Restricting the analysis to larger CAML sizes ($\geq 50 \mu\text{M}$) did not significantly alter the results.

We also analyzed the presence or absence of cells demonstrating EMT and whether they either had independent values or enhanced the value of CAMLs (Table 3). Of the 191 patients with adequate blood specimens, 39 (20%) had at least 1 cell with EMT+. Among those with EMT+, the range for the number of EMTs was 1–69 and median 3. Of the

TABLE 4. Association of Risk Factors and Any Malignancy Outcome

Characteristic	No Malignancy		Any Malignancy		<i>P</i> ^a
	N (%)	Median (range)	N (%)	Median (range)	
Age at enrollment, years	n = 129	69.3 (37.4–89.7)	n = 71	68.9 (43.8–93.9)	.77
Sex					.43
Male	49 (38.0)		31 (43.7)		
Female	80 (62.0)		40 (56.3)		
Smoking history ^b					.009
Current/former	71 (57.7)		50 (76.9)		
Never	52 (42.3)		15 (23.1)		
Personal history of COPD					.51
Yes	36 (27.9)		23 (32.4)		
No	93 (72.1)		48 (67.6)		
Family history of lung cancer					.58
Yes	37 (28.7)		23 (32.4)		
No	92 (71.3)		48 (67.6)		
Nodule dimension, mm	n = 74	12 (8–35)	n = 42	16 (8–32)	<.001
Nodule location					.10
Upper lobe	43 (41.0)		30 (70.9)		
Not upper lobe	62 (59.0)		25 (29.1)		
Nodule contour					.031
Spiculated	9 (13.0)		13 (33.3)		
Not spiculated	60 (87.0)		31 (66.7)		

NOTE. Percentages shown are column percentages.

Abbreviation: COPD, chronic obstructive pulmonary disease.

^a*P* value reflects Wilcoxon rank-sum tests for age and maximum nodule dimension and Chi-square tests for sex, smoking history, COPD history, family history of lung cancer, nodule location, and spiculation.

^bData not recorded for 12 subjects.

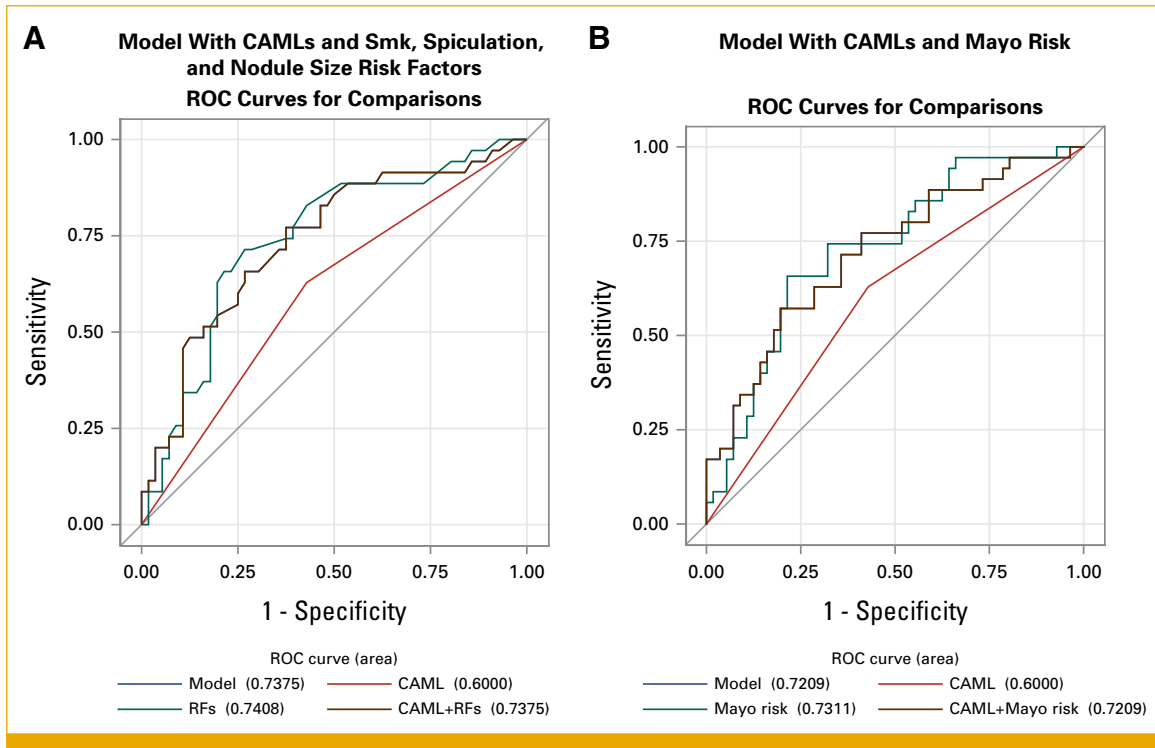


FIG 2. Logistic regression ROC results. The predictive accuracy of logistic regression models for “any malignancy” is shown with ROC curves and AUC. Each model includes 91 patients (35 had malignancy) who had complete data for the risk factors. The left panel shows ROC curves for models with (1) any CAML (yes/no) only, (2) risk factors only (smoker status, spiculation, and nodule size), and (3) CAML and risk factors. The difference in AUC for RFs versus RFs plus CAML is not statistically significant (red curve v green curve, $P = .73$). Similarly, the right panel compares ROC curves with the Mayo risk estimate (on the basis of age, nodule dimension, any nodules in upper lobes, any spiculation, and any calcification) and the addition of any CAML (yes/no) to the model. The difference in AUC is not significant ($P = .91$). CAML, cancer-associated macrophage-like cell; RF, risk factors; ROC, receiver-operating characteristic.

patients with EMT+, 44% (17/39) had any malignancy, compared with 31% (47/152) of those with no EMTs, but this difference was not statistically significant ($P = .14$). Using a combination of EMT and CAMLs did not enhance the PPV, NPV, sensitivity, or specificity. The staining characteristics of the CAMLs and EMT cells are presented in Appendix Figure A1.

We evaluated the known associations between the nodules and malignancy (Table 4). For 116 patients, nodule size was independently audited and was associated with malignancy status (Appendix Fig A2). The mean nodule size was 13.9 mm for those without malignancy, compared with 17.4 mm for those with any malignancy ($P < .001$). There was no significant improvement in the predictive accuracy for malignancy when the presence or absence of CAMLs was considered, in addition to smoking status, nodule size, and spiculation (Fig 2). There was no significant improvement when CAMLs were combined with the Mayo risk score versus the risk score alone.

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DISCUSSION

A number of blood-based biomarkers have been proposed to improve the screening and/or evaluation of indeterminate lung nodules, including evaluation of cell-free DNA, “hot-spot mutations,” and circulating tumor DNA.^{24–26} A study demonstrated that a blood-based proteomic panel is potentially useful for detecting the presence of malignancy in nodules with a low pretest probability of cancer and could reduce the number of procedures.²⁷ The population in this trial was distinctly different from that in the current report.

Preliminary studies of CAMLs have indicated their potential use in this and other settings. Unfortunately, our hypothesis that CAMLs could help differentiate between benign and malignant nodules of indeterminate size has not been confirmed. Although our hypothesis was not supported, the pragmatic, double-blind study design for a biomarker should serve as a model for future trials.

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DISCLAIMER

The content is the sole responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2017. *CA Cancer J Clin* 67:7-30, 2017
2. Aberle DR, DeMello S, Berg CD, et al: Results of the two incidence screenings in the National Lung Screening Trial. *N Engl J Med* 369:920-931, 2013
3. Detterbeck FC, Lewis SZ, Diekemper R, et al: Executive summary: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 143:7S-37S, 2013 (5 suppl)
4. Swensen SJ, Silverstein MD, Ilstrup DM, et al: The probability of malignancy in solitary pulmonary nodules: Application to small radiologically indeterminate nodules. *Arch Intern Med* 157:849-855, 1997
5. McWilliams A, Tammemagi MC, Mayo JR, et al: Probability of cancer in pulmonary nodules detected on first screening CT. *N Engl J Med* 369:910-919, 2013
6. Field JK, Smith RA, Aberle DR, et al: International Association for the Study of Lung Cancer Computed Tomography Screening Workshop 2011 report. *J Thorac Oncol* 7:10-19, 2012
7. van Klaveren RJ, Oudkerk M, Prokop M, et al: Management of lung nodules detected by volume CT scanning. *N Engl J Med* 361:2221-2229, 2009
8. Walter JE, Heuvelmans MA, de Jong PA, et al: Occurrence and lung cancer probability of new solid nodules at incidence screening with low-dose CT: Analysis of data from the randomised, controlled NELSON trial. *Lancet Oncol* 17:907-916, 2016
9. Adams DL, Martin SS, Alpaugh RK, et al: Circulating giant macrophages as a potential biomarker of solid tumors. *Proc Natl Acad Sci USA* 111:3514-3519, 2014
10. Adams DL, Bergan RC, Martin SS, et al: Correlation of cancer-associated macrophage-like cells with systemic therapy and pathological stage in numerous malignancies. *J Clin Oncol* 33, 2015 (suppl 15; abstr 11095)

11. Gironde DJ, Bergan RC, Alpaugh RK, et al: Cancer associated macrophage-like cells are prognostic for highly aggressive prostate cancer in both the non-metastatic and metastatic settings. *Cancers (Basel)* 15:3725, 2023
12. Ali A, Adams DL, Kasabwala DM, et al: Cancer associated macrophage-like cells in metastatic renal cell carcinoma predicts for poor prognosis and tracks treatment response in real time. *Sci Rep* 13:10544, 2023
13. Augustyn A, Adams DL, He J, et al: Giant circulating cancer-associated macrophage-like cells are associated with disease recurrence and survival in non-small-cell lung cancer treated with chemoradiation and atezolizumab. *Clin Lung Cancer* 22:e451-e465, 2021
14. Moran JA, Adams DL, Edelman MJ, et al: Monitoring PD-L1 expression on circulating tumor-associated cells in recurrent metastatic non-small-cell lung carcinoma predicts response to immunotherapy with radiation therapy. *JCO Precis Oncol* [10.1200/PO.22.00457](https://doi.org/10.1200/PO.22.00457)
15. Adams DL, Adams DK, He J, et al: Sequential tracking of PD-L1 expression and RAD50 induction in circulating tumor and stromal cells of lung cancer patients undergoing radiotherapy. *Clin Cancer Res* 23:5948-5958, 2017
16. Adams DL, Cristofanilli M, Lin SH, et al: Phenotyping and clinical utility of phagocytic polyploid giant cancer macrophages in blood. *Cancer Lett* 631:218007, 2025
17. Tang CM, Zhu P, Li S, et al: Blood-based biopsies-clinical utility beyond circulating tumor cells. *Cytometry A* 93:1246-1250, 2018
18. Manjunath Y, Mitchem JB, Suvilesh KN, et al: Circulating giant tumor-macrophage fusion cells are independent prognosticators in patients with NSCLC. *J Thorac Oncol* 15:1460-1471, 2020
19. Nitschke C, Markmann B, Konczalla L, et al: Circulating cancer associated macrophage-like cells as a potential new prognostic marker in pancreatic ductal adenocarcinoma. *Biomedicines* 10:2955, 2022
20. Gardner KP, Aldakkak M, Tang CM, et al: Circulating stromal cells in resectable pancreatic cancer correlates to pathological stage and predicts for poor clinical outcomes. *NPJ Precis Oncol* 5:25, 2021
21. Adams DL, Alpaugh RK, Tsai S, et al: Multi-phenotypic subtyping of circulating tumor cells using sequential fluorescent quenching and restaining. *Sci Rep* 6:33488, 2016
22. Genna A, Vanwynsberghe AM, Villard AV, et al: EMT-associated heterogeneity in circulating tumor cells: Sticky friends on the road to metastasis. *Cancers (Basel)* 12:1632, 2020
23. Mego M, Mani SA, Cristofanilli M: Molecular mechanisms of metastasis in breast cancer—clinical applications. *Nat Rev Clin Oncol* 7:693-701, 2010
24. Zhao M, Xue G, He B, et al: Integrated multiomics signatures to optimize the accurate diagnosis of lung cancer. *Nat Commun* 16:84, 2025
25. Nguyen THH, Vu GH, Nguyen TT, et al: Combination of hotspot mutations with methylation and fragmentomic profiles to enhance multi-cancer early detection. *Cancer Med* 14:e70575, 2025
26. He L, Zhang B, Zhou C, et al: A combined model of circulating tumor DNA methylated SHOX2/SCT/HOXA7 and clinical features facilitates the discrimination of malignant from benign pulmonary nodules. *Lung Cancer* 199:108064, 2024
27. Silvestri GA, Tanner NT, Kearney P, et al: Assessment of plasma proteomics biomarker's ability to distinguish benign from malignant lung nodules: Results of the PANOPTIC (Pulmonary Nodule Plasma Proteomic Classifier) trial. *Chest* 154:491-500, 2018

APPENDIX

TABLE A1. Numerical Cell Distributions and Ranges per 15-cc Sample

Variable	EMT CTC	CAML	Largest CAML
Average	1.85	2.29	61.00
Median	0	1	36.50
Standard deviation	7.22	5.44	83.53
High	78	39	480
Low	0	0	0

Abbreviations: CAML, cancer-associated macrophage-like cell; CTC, circulating tumor cells; EMT, epithelial-mesenchymal transition.

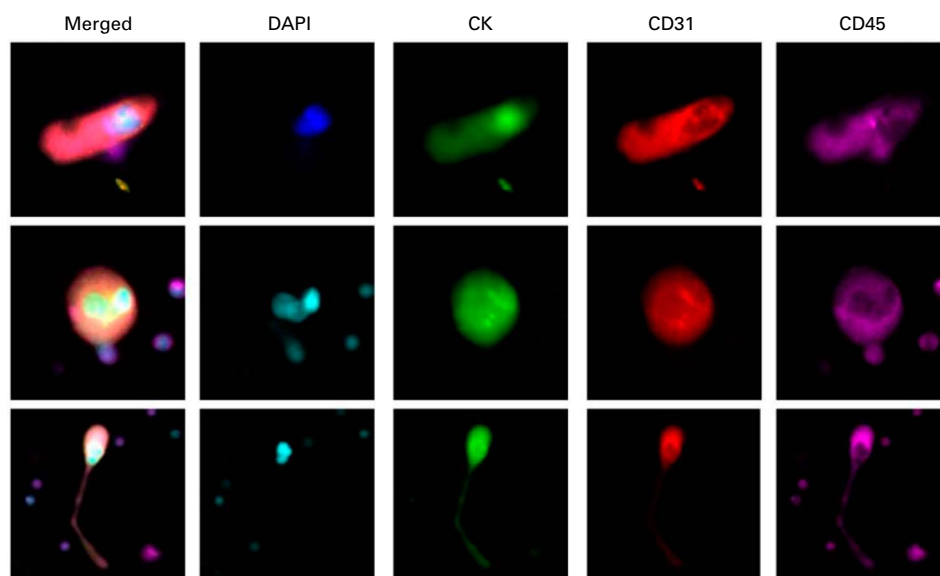
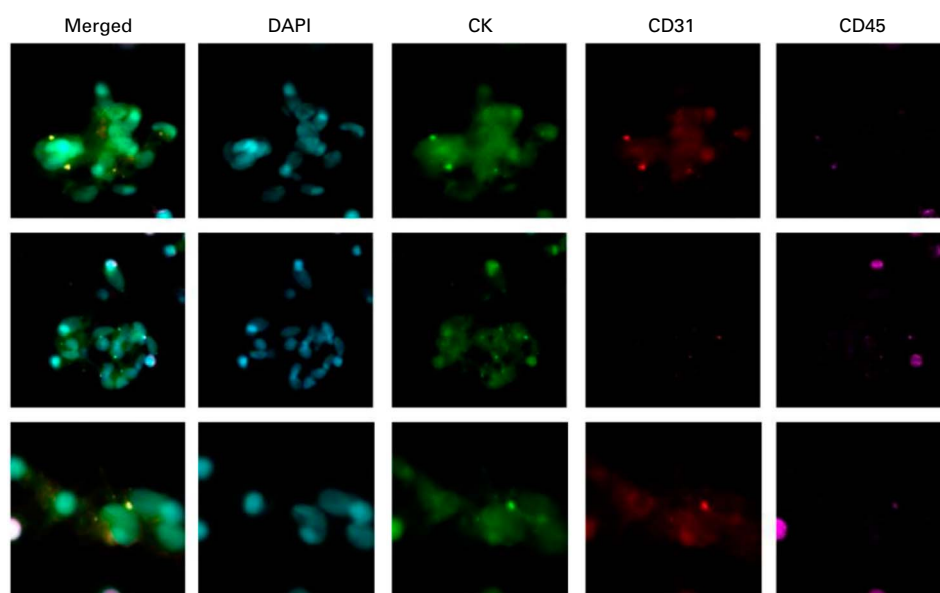
A CAML Examples**B** EMT CTC Examples

FIG A1. Representative images of (A) CAMLs and (B) EMT CTCs. CAML, cancer-associated macrophage-like cell; CTC, circulating tumor cells; EMT, epithelial-mesenchymal transition.



FIG A2. Association between nodule size and malignancy.