Research Article

Cancer Prevention Research

*Early*CDT-Lung: An Immunobiomarker Test as an Aid to Early Detection of Lung Cancer

Stephen Lam[1], Peter Boyle[2], Graham F. Healey[3], Paul Maddison[4], Laura Peek[7], Andrea Murray[3], Caroline J. Chapman[5], Jared Allen[3], William C. Wood[8], Herb F. Sewell[6], and John F.R. Robertson[5]

Abstract

Recent publications have reported the technical and clinical validation of *Early*CDT-Lung, an autoantibody test which detected elevated autoantibodies in 40% of lung cancers at diagnosis. This manuscript reports the results of *Early*CDT-Lung run on four new (postvalidation) data sets. The following four cohorts of patients ($n = 574$) with newly diagnosed lung cancer were identified: group 1 ($n = 122$), 100% small cell lung cancer (SCLC); group 2 ($n = 249$), 97% non-small cell lung cancer (NSCLC); group 3 ($n = 122$), 100% NSCLC; group 4 ($n = 81$), 62% NSCLC. Serum samples were obtained after diagnosis, prior to any anticancer treatment. Autoantibody levels were measured against a panel of six tumor-related antigens (p53, NY-ESO-1, CAGE, GBU4–5, Annexin 1, and SOX2) in the *Early*CDT-Lung panel and previously established cutoffs applied. In groups 2, 3, and 4, patients were individually matched by gender, age, and smoking history to a control individual with no history of malignant disease. Assay sensitivity was tested in relation to cancer type and stage, and in the matched normals to demographic variables. The autoantibody panel showed sensitivity/specificity of 57%/n.d (not done) for SCLC in group 1, 34%/87% for NSCLC in group 2, 31% and 84% for NSCLC in group 3, and 35%/89% for NSCLC and 43%/89% for SCLC in group 4. There was no significant difference in positivity of *Early*CDT-Lung and different lung cancer stages. These studies confirm the value of an autoantibody assay, *Early*CDT-Lung, as an aid to detecting lung cancer in patients at high risk of the disease. *Cancer Prev Res; 4(7); 1126–34. ©2011 AACR.*

Introduction

Recent publications have reported on the technical and clinical validation of an autoantibody assay for lung cancer, *Early*CDT-Lung (1, 2). The clinical manuscript reported that these immunobiomarkers detected both non-small cell (NSCLC) and Small Cell Lung Cancer (SCLC), and that there was no significant difference between different lung cancer stages, indicating that the antigens included identified early- as well as late-stage disease. As such, *Early*CDT-Lung was reported to offer a diagnostic tool and a potential system for monitoring patients at high risk of lung cancer.

The need for an aid to detect lung cancer early is undisputed. Lung cancer is the worldwide leading cause of cancer-related mortality (3). Outcomes are substantially better with early, localized disease compared with locally advanced and metastatic disease, with 5-year survival rates of 53%, 23.7%, and 3.5%, respectively (4). A recent review of SCLC, previously regarded as a disease for which the primary treatment was systemic chemotherapy, has reported excellent survival for early, localized disease that has been resected with or without adjuvant chemotherapy (5). Lim and colleagues reported a 5-year survival rate of 52% for stage 1 without adjuvant chemotherapy (6), whereas Brock and colleagues reported a survival rate of 58% overall for stage 1, rising to 87.5% for stage-1 patients who had surgery followed by platinum-based adjuvant chemotherapy (7). There is, therefore, increasing evidence that early-stage disease treated by surgery with or without (neo)adjuvant chemotherapy can have substantially better 5-year survival rates than late-stage disease.

Ongoing clinical trials are investigating the use of spiral computed tomography (CT) in "at-risk" individuals (8–17). One of the major problems with CT is the high rate of false positives (as high as 50% in a prevalence round;

Authors' Affiliations: [1]Department of Pulmonary Medicine, British Columbia Cancer Agency, Vancouver, Canada; [2]International Prevention Research Institute (iPRI), Lyon, France; [3]Oncimmune Ltd., Nottingham City Hospital; [4]Department of Neurology, Queen's Medical Centre; Divisions of [5]Breast Surgery and [6]Immunology, University of Nottingham, Nottingham, United Kingdom; [7]Oncimmune USA LLC, De Soto, Kansas; and [8]Department of Surgery, Emory University School of Medicine, Atlanta, Georgia

Corresponding Author: John F.R. Robertson, Division of Breast Surgery, University of Nottingham, Nottingham City Hospital, Nottingham, NG5 1PB, United Kingdom. Phone: 115-823-1876; Fax: 115-823-1877; E-mail: john.robertson@nottingham.ac.uk

doi: 10.1158/1940-6207.CAPR-10-0328

ref. 10), which dictates that many individuals require follow-up examinations and a substantial proportion of individuals undergo unnecessary thoracotomy (18). A recent manuscript by the Lung Screening Study reported that up to 7% of patients who were screened by CT underwent some level of invasive procedure (19). This suggests that a test with a higher specificity than CT that can identify high-risk individuals with early-stage disease would be a valuable aid to the early detection of lung cancer.

This article reports the results of *Early*CDT-Lung in 4 new (postvalidation) data sets from individuals in the United States, Canada, and the United Kingdom involving measurement of these immunobiomarkers in the serum of patients with newly diagnosed lung cancer (prior to any treatment) and matched controls.

Methods

Patients

Findings from 4 separate groups of patients with newly diagnosed lung cancer are reported. Blood samples were obtained after diagnosis but prior to receiving any anticancer treatment. In 3 of the 4 groups (groups 2–4), patients with lung cancer were, as far as possible, individually matched by gender, age, and smoking history to control individuals with no previous history of malignant disease. These controls were taken from the normal population. Blood samples from more than 5,000 individuals were collected and were used to match with the individual cancer patients. Matching was conducted on the basis of basic demographics but without any knowledge of autoantibody data. The demographic characteristics of the control versus the study population are given in the Appendix.

Group 1 comprised 122 patients with SCLC presenting to a single center in the United Kingdom. Baseline patient characteristics are shown in Table 1. Samples from this group were run on the *Early*CDT-Lung test without matched controls as the aim was to provide further confirmation of the sensitivity of the test for SCLC in a larger group of patients. The validation data set contained 73 SCLC samples (2). Group 2 comprised 249 patients with lung cancer collected in multiple European centers. The lung cancer patients were matched for age, sex, and smoking history with samples from normal populations in Europe ($n = 237$) and the United States ($n = 246$; ref. Table 1). The normal controls do not exactly match the number of lung cancer patients, because after the studies were run it was noted that 15 of the controls had been included in other postvalidation studies reported in this article: the authors felt that any individual control sample should not be included more than once. Group 3 comprised 122 patients with lung cancer treated at a single center in Vancouver, Canada, who were matched to 114 control samples from high-risk individuals who did not have lung cancer (Appendix; Table 1). The 122 patients with lung cancer included 3 individuals who were initially designated as controls but were found to have developed lung cancer in the follow-up period. These 3 were, therefore, included in the cancer group for the sensitivity and specificity analysis. It should be noted, however, that it was only after the laboratory data had been transferred to our collaborators in Vancouver that the clinical data were made available to the laboratory researchers. Group 4 comprised 81 patients who were also matched to controls based on age, sex, and smoking history. One of the primary reasons for including the matched normals in groups 2 to 4 was to provide further confirmation of the specificity of the *Early*CDT-Lung test in high-risk individuals.

Tumor pathologic information was available for the patients with lung cancer, including TNM (Tumor, Node, Metastasis) staging, tumor-type SCLC or NSCLC, and NSCLC subtype histology (Table 2). Because this was not a CT screening trial, no CT data are available for these patients. In the clinical validation manuscript (2), early-stage disease included stage-1 or -2 NSCLC and limited disease of SCLC, and the same definition was used when analyzing these 4 new data sets to assess the sensitivity of *Early*CDT-Lung for early- and late-stage disease.

Autoantibody positivity by stage of disease and histologic subtype was not reported in the clinical validation manuscript. However, with significantly greater numbers of lung cancers, these data were analyzed by combining the 4 postvalidation data sets and the validation data set described by Boyle and colleagues (2).

Serum samples were evaluated in the *Early*CDT-Lung assay for autoantibodies against p53, NY-ESO-1, CAGE, GBU4–5 (also known as FLI3072 or TDRD12), Annexin 1, and SOX2, as previously reported (1, 2). For each group, samples from patients with cancers, matched normals, and control sera for the assay were interspersed: samples were assayed in an order so that any batch effects would be spread over all sample types. The laboratory staff, performing the assay, were blinded to the disease state of individual samples. In group 3, the samples were run, and the assay results returned to the clinician supplying the samples before any clinical data were released.

Autoantibody assay

Autoantibody levels were determined by a quality-controlled, semiautomated indirect ELISA in which samples were allowed to react with a titration series of antigen concentrations. All liquid-handling steps were carried out by using an automated system. Briefly, purified recombinant antigens were diluted to provide a semilog titration series for each antigen from 160 to 1.6 nmol/L. Control antigens consisting of the purified BirA or NusA tags were also included to allow subtraction of the signal because of nonspecific binding to bacterial contaminants. Antigen dilutions were adsorbed to the surface of microtiter plate wells in phosphate buffer at room temperature. After washing in phosphate-buffered saline containing 0.1% Tween 20, pH 7.6, microtiter plates were blocked with a gelatine-based blocking buffer. Serum samples (diluted 1 in 110 in a blocking buffer) were then added to the plates and allowed to incubate at room temperature with

Table 1. Lung cancer patient characteristics

	Group 1 ($n = 122$)	Group 2 ($n = 249$)	Group 3 ($n = 122$)	Group 4 ($n = 81$)
Median age, y (range)	65 (43–86)	62 (23–82)	70 (45–90)	70 (50–86)
Patients >60 y, *n* (%)	84 (68.9)	138 (55.4)	97 (80.2)	67 (82.7)
Gender, *n* (%)				
Male	68 (55.7)	201 (80.7)	51 (41.8)	43 (53.1)
Female	54 (44.3)	48 (19.3)	71 (58.2)	38 (46.9)
Smoking history, *n* (%)				
Current	78 (63.9)	102 (41.0)	44 (36.1)	40 (49.4)
Previous	40 (32.8)	120 (48.2)	58 (47.5)	33 (40.7)
Never	4 (3.3)	27 (10.8)	18 (14.8)	1 (1.2)
Not determined	0 (0.0)	0 (0.0)	2 (1.6)	7 (8.6)

shaking for 90 minutes. Following incubation, plates were washed, and horseradish peroxidase-conjugated rabbit anti-human IgG (Dako) was added. After a 60-minute incubation, the plates were washed and 3,3′,5,5′-tetramethylbenzidine was added. Color formation was allowed to proceed for 15 minutes before the optical density of each well was determined spectrophotometrically at 650 nm. The assay included a calibration system which utilized fluids drained from pleural or peritoneal cavities of patients with lung cancer, producing calibrated reference units (1).

All assays were conducted as 2 replicates and the mean value taken as the overall assay measurement. Samples were judged to be positive if they fulfilled 2 criteria, that is, they showed a dose–response to the antigen titration series and the measured autoantibody signal to 1 or more of the antigens in the *Early*CDT-Lung assay was above the cutoff set for that antigen in the commercial assay.

The initial data analysis to determine whether the sample was positive or negative was carried out in a completely automated system in which the sample list and raw plate data were kept separate until a final merge. The assay results (positive or negative) were then added to the different data sets with the clinical data and the sensitivity and specificity calculated.

For the statistical analysis, positivity rates were analyzed as $2 \times r$ contingency tables by using standard χ^2 tests with the respective degrees of freedom. For the forest plots, CIs for sensitivity were derived under a binomial assumption.

Assay cutoffs

In the validation studies (2), the cutoffs for the autoantibody assays to the 6 antigens in the commercial *Early*CDT-Lung assay had been set to achieve a specificity of 90% in the matched control groups, to produce a test that could be used for early detection in a high-risk population and that would be health economically viable. To accomplish this, a Monte Carlo direct search method (20) was applied to find an optimized set of antigen-specific cutoffs yielding the maximum sensitivity for the fixed specificity of 90%. In these new studies, no further optimization was carried out and the commercial cutoffs were applied, providing further confirmation of the clinical utility of the commercial cutoffs.

Results

Autoantibody expression

The sensitivity and specificity of the *Early*CDT-Lung assay in each of the 4 groups, broken down by tumor type (NSCLC and SCLC), are shown in Table 3. For comparison, the sensitivity and specificity reported for the panel of the same 6 antigens in the Clinical Validation manuscript (2) are also included in Table 3. These show that the results for the 4 new data sets, by using the commercial assay cutoffs (i.e., not optimized for each individual data set), fall within the 95% CIs of the validation data. The one exception was the specificity for group 3 where the matched normal controls had a lower than expected specificity; however, these individuals had almost double the mean pack-years compared with the validation population (45.2 compared with 26, respectively), making them a much higher risk for cancer development.

Combining all data sets where all 6 antigens were measured (Table 3) gave 1,077 patients with lung cancer plus 1,296 matched controls. The sensitivity/specificity of the *Early*CDT-Lung was 38%/88% overall, with 34%/88% for NSCLC and 50%/88% for SCLC.

In this study, positive predictive values (PPV) for *Early*CDT-Lung, along with prevalence-based accuracy values for an assumed lung cancer prevalence of 1.5% would be 4.5% and 87.0%, respectively. At a lung cancer prevalence of 2.0%, PPV would be 6.0% with an accuracy of 86.8%, and at 2.7% prevalence, PPV would be 8.0% with 86.4% accuracy.

Effect of patient and disease characteristics on autoantibody assay sensitivity and specificity

Antigen positivity by histologic subtype for the panel and also for each of the antigens is shown in Tables 4 and 5. There was a higher sensitivity for SCLC compared with NSCLC ($P \leq 0.001$) but no difference in sensitivity between the subtypes of NSCLC ($P = 0.35$). The results by tumor

Table 2. Tumor stage and histology according to gender

	Group 1 ($n = 122$)		Group 2 ($n = 249$)		Group 3 ($n = 122$)		Group 4 ($n = 81$)	
	Male ($n = 68$)	Female ($n = 54$)	Male ($n = 201$)	Female ($n = 48$)	Male ($n = 51$)	Female ($n = 71$)	Male ($n = 43$)	Female ($n = 38$)
Tumor type, *n* (%)								
NSCLC	0 (0.0)	0 (0.0)	185 (92.0)	46 (95.8)	51 (100.0)	71 (100.0)	28 (65.1)	21 (55.3)
SCLC	68 (100.0)	54 (100.0)	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (32.6)	16 (42.1)
Unknown	0 (0.0)	0 (0.0)	12 (6.0)	2 (4.2)	0 (0.0)	0 (0.0)	1 (2.3)	1 (2.6)
NSCLC stage, *n* (%)								
I	0 (0.0)	0 (0.0)	105 (56.8)	22 (47.8)	30 (58.8)	41 (57.7)	5 (17.9)	1 (4.8)
II	0 (0.0)	0 (0.0)	16 (8.6)	7 (15.2)	15 (29.4)	16 (22.5)	1 (3.6)	0 (0.0)
III	0 (0.0)	0 (0.0)	40 (21.6)	11 (23.9)	6 (11.8)	12 (16.9)	3 (10.7)	3 (14.3)
IV	0 (0.0)	0 (0.0)	16 (8.6)	0 (0.0)	0 (0.0)	2 (2.8)	3 (10.7)	5 (23.8)
Unknown	0 (0.0)	0 (0.0)	8 (4.3)	6 (13.0)	0 (0.0)	0 (0.0)	16 (57.1)	12 (57.1)
NSCLC histology, *n* (%)								
Squamous	0 (0.0)	0 (0.0)	87 (47.0)	11 (23.9)	23 (45.1)	7 (9.9)	15 (53.6)	4 (19.0)
Adenocarcinoma	0 (0.0)	0 (0.0)	77 (41.6)	30 (65.2)	25 (49.0)	58 (81.7)	4 (14.3)	10 (47.6)
Large cell	0 (0.0)	0 (0.0)	5 (2.7)	3 (6.5)	3 (5.9)	2 (2.8)	0 (0.0)	0 (0.0)
Not determined	0 (0.0)	0 (0.0)	16 (8.6)	2 (4.3)	0 (0.0)	4 (5.6)	9 (32.1)	6 (28.6)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
SCLC stage, *n* (%)								
Limited SCLC	21 (30.9)	17 (31.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (28.6)	6 (37.5)
Extensive SCLC	47 (69.1)	37 (68.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (64.3)	8 (50.0)
Not determined	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	2 (12.5)

staging according to the International Association for the Study of Lung Cancer (IASLC, 7th edition) are shown in Tables 6–9. When the stage of disease was looked at within NSCLC (I–IV) and SCLC (limited and extensive disease), there was no significant difference ($P = 0.54$ and $P = 0.78$, respectively). For the 4 postvalidation data sets, the sensitivity of *Early*CDT-Lung for early- and late-stage disease is shown in Figure 1.

Discussion

Irrespective of cancer type, early detection improves prognosis by allowing earlier treatment before the cancer spreads. The National Lung Screening Trial has shown that early screening, in the form of low-dose CT scans, can decrease lung cancer mortality by 20%, which highlights the value of early screening (21). However, the high

Table 3. Comparison of specificity and sensitivity of the training, validation, and postvalidation sets

Study group		Antigens in panel	Number: Ca/N	% NSCLC	Overall sensitivity/ specificity (%)	Sensitivity NSCLC	Sensitivity SCLC	Specificity for lung cancer
Training set[a]	OD	6	234/225	71	39/89	36	45	89
Validation set[a]	RU	6	269/269	76	37/90	34 (27, 41)	45 (34, 57)	90 (86, 93)
Group 1	RU	6	122/0	0	57/NA	-	57	-
Group 2	RU	6	249/483	97	34/87	34	N/D	90
Group 3	RU	6	122/114	100	31/84	31		84
Group 4	RU	6	81/205	62	38/89	35	43	89
All studies		6	1,077/1,306		38/88	34 (31, 38)	50 (44, 56)	88 (86, 90)
Validation + 1–4		6	843/1,071		38/88	33 (30, 37)	51 (44, 58)	88 (86, 90)
Groups 1–4		6	574/802		39/87	33 (29, 38)	54 (46, 62)	87 (85, 89)
Groups 2–4		6	452/802		34/87	33 (29, 38)	43 (25, 63)	87 (85, 89)

Abbreviations: NA, not applicable; N/D, not analyzed; OD, optical density; RU, reference unit.
[a]Previously published.

Table 4. Panel and individual autoantibody positivity by histologic type: panel positivity

Subtype	Number of samples	Panel positive	% positive
Adenocarcinoma	270	69	25.6
Large cell	15	5	33.3
Squamous	234	73	31.1
SCLC	220	112	50.9

$\chi^2 = 36.7$; 3df $P < 0.001$.
NSCLC versus SCLC: $\chi^2 = 34.8$; 1df $P < 0.001$.
Adenocarcinoma versus large cell versus squamous: $\chi^2 = 2.1$; 2df $P = 0.35$.

proportion of noncancerous changes detected on chest CT, and the additional expensive diagnostic procedures needed, makes a strong case for a simple biomarker test that can be used as a diagnostic tool.

This report further confirms that *Early*CDT-Lung is a validated assay for the detection of autoantibodies to selected cancer-associated antigens in the peripheral blood. The study also confirms that the assay, by using the previously validated cutoffs, gives a sensitivity up to 40% for an overall lung cancer population. In patients with lung cancer, NSCLC typically accounts for 80% to 87% of cases and SCLC accounts for 13% to 20% of all cases, the exact proportions depending on a variety of factors such as the proportion of smokers versus former smokers and the level of smoking history. A further important point is that because the cutoffs used are those previously defined, they were not optimized for any of the 4 data sets. This provides further prospective confirmation of the reproducibility and clinical utility of the test.

For all 4 study groups, the sensitivity of the test by type of lung cancer (i.e., NSCLC and SCLC) was within the 95% CI of the validation study results (Table 3). The validation data set contained 73 SCLC samples. Although this was more than 13% of the validation group (2), and therefore greater than the percentage of lung cancers which are small cell according to the Seer database, it was felt that a larger data set was warranted to more accurately assess the sensitivity of the 6-antigen *Early*CDT-Lung test in SCLC. The data also confirm that the test detects early-stage cancer (stage I/II NSCLC plus limited SCLC) as well as it detects late-stage disease (stage III/IV NSCLC plus extensive SCLC; Fig. 1). This is particularly important if these immunobiomarkers are to act effectively as an aid to early detection. The presence of such a signal in early-stage disease is precisely what would be expected of an *in vivo* amplification signal such as the humoral immune response. This is in contrast to cancer-associated antigens, which are markers of tumor burden and not useful for the early detection or screening of breast (22, 23) or colorectal cancers (24, 25).

Previous publications (1, 2, 26–36) have highlighted the potential value of a panel of autoantibodies for the early detection of cancer. This study shows the sensitivity of both the overall panel and each individual autoantibody assay (Tables 4–9), and in doing so highlights the benefit of measuring autoantibodies to a panel of cancer-associated antigens compared with only 1 autoantibody assay. Tables 4–9 highlight that the panel as currently presented has a higher sensitivity for SCLC than NSCLC. They also highlight that individual autoantibody assays have different percentage sensitivity for different subtypes of lung cancer. As more assays are run and the number of patients with lung cancers increases, it may be possible to give an estimate of what subtype of lung cancer a patient is most likely to have, based on the pattern of autoantibody results.

Although it may be argued that if the control samples used were not matched to the patient samples by time in storage, this could lead to differences in antibody levels between the groups. The controls, we describe here, were collected around the same time as the cancer cases (started in 2007 or 2008, depending on sample sets, and finished in 2010). In addition, our sample stability studies over 2 to 3 years do not indicate any decreases in signal when the blood samples are properly stored (unpublished data).

Individual autoantibodies such as p53 autoantibodies have been detected prior to diagnosis of lung cancer in smokers with chronic obstructive pulmonary disease (37) or in patients with asbestosis (38). In the latter publication, the average lead time (time from first positive sample to diagnosis) was 3.5 years (range 1–12 years). Similar publications on other single autoantibodies (39–41) also indicate the induction of autoantibodies happening relatively early in the process of carcinogenesis. Autoantibodies to a panel of cancer-associated antigetns have been reported up

Table 5. Panel and individual autoantibody positivity by histologic type: individual antigen positivity

Subtype	p53 positive (%)	SOX2 positive (%)	CAGE positive (%)	NY-ESO-1 positive (%)	GBU4–5 positive (%)	ANNEXIN1 positive (%)
Adenocarcinoma	7.4	5.6	7.8	7.8	4.1	4.8
Large cell	6.7	0.0	6.7	13.3	0.0	13.3
Squamous	11.1	6.4	6.0	9.8	3.0	3.8
SCLC	14.5	28.2	10.0	7.7	5.0	7.7

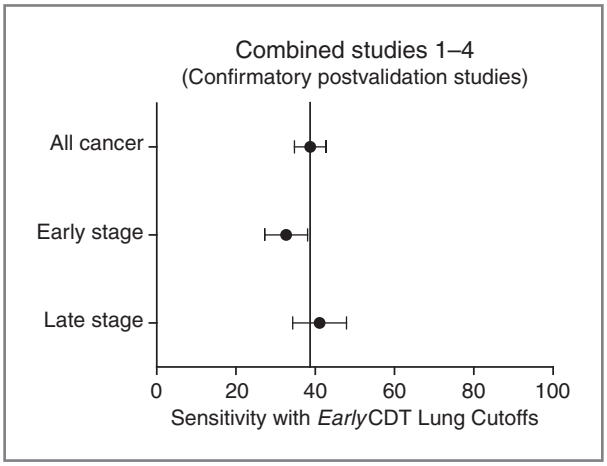

Figure 1. Forest plot showing the assay sensitivity by lung cancer stage (combined studies 1–4; see Table 3 for study details).

to 5 years before screening CT scans (32) in lung cancer and up to 4 years before screening mammography detected breast cancers in young women at increased risk (31, 33). A recent presentation on SCLC has shown that *Early*CDT-Lung was positive in prediagnostic samples between 1 and 49 months prior to diagnosis of SCLC (42).

The study also confirms that the test has good specificity. In groups 2 to 4, matched normals were run and the specificity lay within the previously reported 95% CI of the validation data (Table 3). In group 3, the specificity was 84%, which was just below the lower margin of the 95% CI. In a group of high-risk smokers or ex-smokers, there will always be some individuals who are harboring an occult lung cancer. The specificity will vary somewhat if the risk profile of a group were to be higher or lower than the validation group. The matched normals in group 3 had almost double the mean pack-years compared with the validation population (45.2 compared with 26, respectively) or the matched normals in groups 2 and 4 (20.3 and 20.4 pack-years, respectively), and it is therefore not surprising that the specificity was slightly lower in this group.

Table 6. Panel and individual autoantibody positivity by tumor stage (according to the IASLC, 7th edition): panel positivity by stage–SCLC samples

Group	Number of samples	Panel positive	% positive
Limited			
Stage IA	0	0	
Stage IB	7	4	57.1
Stage IIA	5	4	80.0
Stage IIB	2	1	50.0
Stage IIIA	27	14	51.9
Stage IIIB	6	2	33.3
Extensive	101	54	53.5

$\chi^2 = 2.5$; 5df $P = 0.78$.

Other researchers have developed risk models based on demographics from large population-based studies (43). This approach may be useful for the initial identification of a cohort at high risk of lung cancer over a defined period but does not allow repeated reassessment of the risk as many of the demographic factors in the models do not change significantly over the time period. The integration of immunobiomarkers in the blood with established demographic models may provide additive information and also provide a dynamic system for monitoring whether an individual at high risk seems to be developing a lung cancer.

In summary, these studies confirm the findings of the assay validation study (2) that *Early*CDT-Lung can detect up to 40% of lung cancers and that these immunobiomarkers detect early-stage disease as well as they detect late-stage disease. Furthermore, the pattern of autoantibody results varies between individuals and in future may provide an estimate as to what subtype of lung cancer an individual has developed. The study also confirmed that the specificity of the test is good, which is a prerequisite for it to be useful as an aid to early detection. The robust specificity of the *Early*CDT-Lung test indicates that it should make a major contribution to the diagnosis and monitoring of lung cancer patients.

It would also be important to examine the validity and utility of this test in populations with noncancer pulmonary pathologies (e.g., Chronic obstructive pulmonary disease and pneumonia). We have prospectively gathered information on concomitant benign autoimmune diseases, but not on other disorders. Data on benign lung conditions have been published in our previous validation paper which included 63 patients with benign lung conditions (2). The specificity of *Early*CDT-Lung was 89% for this group.

We understand and acknowledge that no cancer marker is 100% tumor-site specific and that some false-positives for lung cancer may in fact have another type of cancer. In this respect, we have preliminary data that show that the core antigens (e.g., p53 and NY-ESO-1) are also elevated in other types of cancer, such as breast or ovarian cancer. Nonetheless, in the population we are targeting, the principal demographic risk is that of lung cancer (around 2 per 100) whereas, for example, the risk of ovarian cancer is an order of magnitude lower. For this reason, we anticipate that the proportion of patients with a non–lung derived cancer will be very small. Furthermore, patients with a positive test but no detectable lung cancer should check with their physician that they have had any screening tests for other cancers (as advised by the American Cancer Society).

This study has shown that the *Early*CDT-Lung antibody panel has clinical utility for detecting lung cancer in clinical samples. There are ongoing studies testing the sensitivity and specificity of *Early*CDT-Lung in prediagnostic samples

Table 7. Panel and individual autoantibody positivity by tumor stage (according to the IASLC, 7th edition): individual antigen positivity–SCLC samples

Group	p53 positive (%)	SOX2 positive (%)	CAGE positive (%)	NYESO positive (%)	GBU4–5 positive (%)	ANNEXIN1 positive (%)
Limited						
Stage IA						
Stage IB	14.3	42.9	0.0	0.0	0.0	14.3
Stage IIA	0.0	60.0	0.0	0.0	0.0	40.0
Stage IIB	0.0	0.0	50.0	50.0	0.0	0.0
Stage IIIA	14.8	37.0	0.0	7.4	3.7	3.7
Stage IIIB	33.3	33.3	0.0	0.0	0.0	0.0
Extensive	18.8	33.7	12.9	8.9	5.9	9.9

to fully assess the utility of the panel in monitoring asymptomatic patients for lung cancer. Future work is already ongoing to look for ways to increase the sensitivity and/or specificity. This includes investigating new antigens that are additive to the current panel and also looking at using not only cutoffs for each assay based on a high-risk control population but also assessing sequential changes in an individual's results or profile compared with their own baseline test results. In addition, combining these immunobiomarkers with demographic risk models (41) to assess if they are additive is ongoing.

Appendix

Demographic characteristics of the control versus the study population

A total of 574 lung cancer sera (402 were from patients with NSCLC, 156 with SCLC, and 16 of unknown histology) were compared directly with 802 normal sera, which were analyzed as controls. Samples were obtained, with full informed consent, at different sites.

Table 8. Panel and individual autoantibody positivity by tumor stage (according to the IASLC, 7th edition): panel positivity by stage–NSCLC samples

Stage	Number of samples	Panel positive	% positive
IA	100	28	28.0
IB	119	31	26.1
IIA	11	1	9.1
IIB	52	19	36.5
IIIA	40	10	25.0
IIIB	40	10	25.0
IV	29	10	34.5

$X^2 = 5.0$; 6df $P = 0.54$

Group 1 comprised 122 patients with SCLC presenting to a single center in the United Kingdom. There were 68 males and 54 females, and the median age was 65 years (range 43–86). Group 2 comprised 249 patients with lung cancer collected in multiple European centers. The lung cancer patients were matched for age, sex, and smoking history with samples from normal populations in Europe ($n = 237$) and the United States ($n = 246$). In group 2, there were 201 males and 48 females. Controls for group 2 were selected from a prospective collection of blood samples taken from a larger sample set of normal populations in the Midlands of England and the Midwest of America. Controls for patients in group 2 were matched on the basis of gender and age ($+/-$ 4 years). As all subjects in this group were smokers, pack-year matching was attempted but a tight match was prohibited by lack of information. The normal controls do not exactly match the number of lung cancer patients, because after the studies were run it was noted that 15 of the controls had been included in other postvalidation studies reported in this article: the authors felt that any individual control sample should not be included more than once. The median age (range) of the lung cancer patients and controls was 62 (23–82) and 62 (23–82) years, respectively.

Group 3 ($n = 240$) comprised 120 patients with lung cancer treated at a single center in Vancouver and Canada, who had been matched to 120 control samples from high-risk individuals who did not have lung cancer. The gender distribution was female ($n = 63$ and 69), male ($n = 48$ and 51), and unknown ($n = 9$ and 0) for cancers and controls, respectively. The median age (range) was 69 years (± 10) for cancer patients and 62 years (± 6) for controls. Pack-years smoked were 39 ± 24 for the cancers and 45 ± 16 for the controls. *Early*CDT-Lung results were available on 236 of the 240 samples which were returned to the Vancouver center blind of any clinical data. The mean follow-up on these patients was 57 ± 13 months. There were initially 119 patients who had lung cancer and 117 controls with *Early*CDT-Lung results. Three controls with *Early*CDT-Lung results were diagnosed with lung cancer during the follow-up period (1 male and 2 female

Table 9. Panel and individual autoantibody positivity by tumor stage (according to the IASLC, 7th edition): individual antigen positivity–NSCLC samples

Stage	p53 positive (%)	SOX2 positive (%)	CAGE positive (%)	NYESO positive (%)	GBU4–5 positive (%)	ANNEXIN1 positive (%)
IA	7.0	12.0	6.0	3.0	3.0	2.0
IB	5.9	9.2	4.2	7.6	4.2	4.2
IIA	0.0	0.0	0.0	9.1	0.0	0.0
IIB	13.5	5.8	9.6	15.4	7.7	5.8
IIIA	15.0	0.0	10.0	2.5	5.0	0.0
IIIB	15.0	5.0	5.0	10.0	10.0	0.0
IV	3.4	10.3	13.8	13.8	0.0	0.0

ex-smokers 5, 30, and 40 months after the blood sample had been taken): these were placed in the cancer group for the sensitivity and specificity analysis. This gave 122 with cancer and 114 controls. Group 4 comprised 81 patients (43 males and 38 females) who were also matched to controls based on age, sex, and smoking history. One of the reasons for including the matched normals in groups 2 to 4 was to provide further confirmation of the specificity of the *Early*CDT-Lung test in high-risk individuals.

Disclosure of Potential Conflicts of Interest

Caroline J. Chapman and John F.R. Robertson are consultants to Oncimmune Ltd., a University of Nottingham spinout company. JFRR is also a company shareholder.

Acknowledgments

The article was drafted by the authors. However, the authors acknowledge and thank Sandra Cuscó, PhD, of Complete Medical Communications, who assisted in formatting the manuscript and submitting it, which was funded by Oncimmune Ltd.

Grant Support

The research reported was supported by Oncimmune Ltd. and the University of Nottingham.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 11, 2010; revised March 14, 2011; accepted March 31, 2011; published online July 6, 2011.

References

1. Murray A, Chapman CJ, Healey G, Peek LJ, Parsons G, Baldwin D, et al. Technical validation of an autoantibody test for lung cancer. Ann Oncol 2010;21:1687–93.
2. Boyle P, Chapman CJ, Holdenrieder S, Murray A, Robertson C, Wood WC, et al. Clinical validation of an autoantibody test for lung cancer. Ann Oncol 2011;22:383–9.
3. Boyle P, Levin B. (Eds). World Cancer Report 2001. IARC Press, Lyon, France.
4. Horner MJ, Ries LAG, Krapcho M, Neyman N, Aminou R, Howlader N, et al. (Eds). SEER Cancer Statistics Review 1975–2006. National Cancer Institute. Bethesda, MD. Available from http://seer.cancer.gov/csr/1975_2006/based on November 2008 SEER data submission, posted to the SEER website 2009.
5. Koletsis EN, Prokakis C, Karanikolas M, Apostolakis E, Dougenis D. Current role of surgery in small cell lung carcinoma. J Cardiothorac Surg 2009;4:30.
6. Lim E, Belcher E, Yap YK, Nicholson AG, Goldstraw P. The role of surgery in the treatment of limited disease small cell lung cancer: time to reevaluate. J Thorac Oncol 2008;3:1267–71.
7. Brock MV, Hooker CM, Syphard JE, Westra W, Xu L, Alberg AJ, et al. Surgical resection of limited disease small cell lung cancer in the new era of platinum chemotherapy: Its time has come. J Thorac Cardiovasc Surg 2005;129:64–72.
8. Sone S, Takashima S, Li F, Yang Z, Honda T, Maruyama Y, et al. Mass screening for lung cancer with mobile spiral computed tomography scanner. Lancet 1998;351:1242–5.
9. Henschke CI, McCauley DI, Yankelevitz DF, Naidich DP, McGuinness G, Miettinen OS, et al. Early Lung Cancer Action Project: overall design and findings from baseline screening. Lancet 1999;354:99–105.
10. Swensen SJ, Jett JR, Hartman TE, Midthun DE, Mandrekar SJ, Hillman SL, et al. CT screening for lung cancer: five-year prospective experience. Radiology 2005;235:259–65.
11. Sobue T, Moriyama N, Kaneko M, Kusumoto M, Kobayashi T, Tsuchiya R, et al. Screening for lung cancer with low-dose helical computed tomography: anti-lung cancer association project. J Clin Oncol 2002;20:911–20.
12. Henschke CI, Naidich DP, Yankelevitz DF, McGuinness G, McCauley DI, Smith JP, et al. Early lung cancer action project: initial findings on repeat screenings. Cancer 2001;92:153–9.
13. Diederich S, Wormanns D, Semik M, Thomas M, Lenzen H, Roos N, et al. Screening for early lung cancer with low-dose spiral CT: prevalence in 817 asymptomatic smokers. Radiology 2002;222:773–81.
14. Nawa T, Nakagawa T, Kusano S, Kawasaki Y, Sugawara Y, Nakata H. Lung cancer screening using low-dose spiral CT: results of baseline and 1-year follow-up studies. Chest 2002;122:15–20.
15. Gohagan J, Marcus P, Fagerstrom R, Pinsky P, Kramer B, Prorok P. Baseline findings of a randomized feasibility trial of lung cancer screening with spiral CT scan vs chest radiograph: the Lung Screening Study of the National Cancer Institute. Chest 2004;126:114–21.
16. McWilliams A, Mayo J, MacDonald S, leRiche JC, Palcic B, Szabo E, et al. Lung cancer screening: a different paradigm. Am J Respir Crit Care Med 2003;168:1167–73.
17. Kaneko M, Eguchi K, Ohmatsu H, Kakinuma R, Naruke T, Suemasu K, et al. Peripheral lung cancer: screening and detection with low-dose spiral CT versus radiography. Radiology 1996;201:798–802.
18. Sone S, Li F, Yang ZG, Honda T, Maruyama Y, Takashima S, et al. Results of three-year mass screening programme for lung cancer using mobile low-dose spiral computed tomography scanner. Br J Cancer 2001;84:25–32.

19. Croswell JM, Baker SG, Marcus PM, Clapp JD, Kramer BS. Cumulative incidence of false-positive test results in lung cancer screening: a randomized trial. Ann Intern Med 2010;152:505–80.
20. Geyer CJ. Estimation and optimization of functions. In: Markov Chain Monte Carlo in Practice (Eds: WR Gilks, S Richardson and DJ Spiegelhalter). 1996; pp 241–58. Chapman and Hall, London.
21. NCI. Lung cancer trial results show mortality benefit with low-dose CT. http://www.cancer.gov/newscenter/pressreleases/2010/NLSTresultsRelease. Accessed 11 April 2010.
22. Robertson JF, Pearson D, Price MR, Selby C, Badley RA, Pearson J, et al. Assessment of four monoclonal antibodies as serum markers in breast cancer. Eur J Cancer 1990;26:1127–32.
23. Robertson JF, Pearson D, Price MR, Selby C, Pearson J, Blamey RW, et al. Prospective assessment of the role of five tumour markers in breast cancer. Cancer Immunol Immunother 1991;33:403–10.
24. Laurence DJ, Stevens U, Bettelheim R, Darcy D, Leese C, Turberville C, et al. Role of plasma carcinoembryonic antigen in diagnosis of gastrointestinal, mammary, and bronchial carcinoma. Br Med J 1972;3:605–9.
25. Thomas WM, Robertson JF, Price MR, Hardcastle JD. Failure of CA19–9 to detect asymptomatic colorectal carcinoma. Br J Cancer 1991;63:975–6.
26. Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. J Clin Invest 2001;108:1411–5.
27. Houghton AN. Cancer antigens: immune recognition of self and altered self. J Exp Med 1994;180:1–4.
28. Brichory FM, Misek DE, Yim AM, Krause MC, Giordano TJ, Beer DG, et al. An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. Proc Natl Acad Sci U S A 2001;98:9824–9.
29. Zhang JY, Casiano CA, Peng XX, Koziol JA, Chan EK, Tan EM. Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epidemiol Biomarkers Prev 2003;12:136–43.
30. Nesterova M, Johnson N, Cheadle C, Cho-Chung YS. Autoantibody biomarker opens a new gateway for cancer diagnosis. Biochim Biophys Acta 2006;1762:398–403.
31. Robertson JFR, Chapman C, Cheung K-L, Murray A, Pinder SE, Price MR, et al. Autoantibodies in early breast cancer. J Clin Oncol 2005;23:549.
32. Zhong L, Coe SP, Stromberg AJ, Khattar NH, Jett JR, Hirschowitz EA. Profiling tumor-associated antibodies for early detection of non-small cell lung cancer. J Thorac Oncol 2006;1:513–9.
33. Chapman C, Murray A, Chakrabarti J, Thorpe A, Woolston C, Sahin U, et al. Autoantibodies in breast cancer: their use as an aid to early diagnosis. Ann Oncol 2007;18:868–73.
34. Chapman CJ, Murray A, McElveen JE, Sahin U, Luxemburger U, Tureci O, et al. Autoantibodies in lung cancer: possibilities for early detection and subsequent cure. Thorax 2008;63:228–33.
35. Robertson JFR, Graves CRL, Price MR. Tumour Markers US 7,402,403,B1. Nottingham: Onclmmune Ltd. 1999.
36. Chapman CJ, Thorpe AJ, Murray A, Parsky-Kowalska C, Allen J, Stafford K, et al. Immuno-biomarkers in small cell lung cancer: potential early cancer signals. Clin Cancer Res 2011;17:1474–80.
37. Trivers GE, De B V, Cawley HL, Caron G, Harrington AM, Bennett WP, et al. Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. Clin Cancer Res 1996;2:1767–75.
38. Li Y, Karjalainen A, Koskinen H, Hemminki K, Vainio H, Shnaidman M, et al. p53 autoantibodies predict subsequent development of cancer. Int J Cancer 2005;114:157–60.
39. Pereira-Faca SR, Kuick R, Puravs E, Zhang Q, Krasnoselsky AL, Phanstiel D, et al. Identification of 14–3-3 theta as an antigen that induces a humoral response in lung cancer. Cancer Res 2007;67:12000–6.
40. Rohayem J, Diestelkoetter P, Weigle B, Oehmichen A, Schmitz M, Mehlhorn J, et al. Antibody response to the tumor-associated inhibitor of apoptosis protein surviving in cancer patients. Cancer Res 2000;60:1815–7.
41. Suzuki H, Graziano DF, McKolanis J, Finn OJ. T cell-dependent antibody responses against aberrantly expressed cyclin B1 protein in patients with cancer and premalignant disease. Clin Cancer Res 2005;11:1521–6.
42. Chapman CJ, Robertson JF, Murray A, Titulaer M, Lang B, Thorpe A, et al. The presence of autoantibodies to tumour-associated antigens can predate clinical diagnosis of small cell lung cancer. Chest 2010;138:775A.
43. Tammemagi CM, Pinsky PF, Caporaso NE, Kvale PA, Hocking WG, Church TR, et al. Lung cancer risk prediction–prostate, lung, colorectal and ovarian cancer screening trial models and validation. J Natl Cancer Inst 2011; In press.

Cancer Prevention Research

*Early*CDT-Lung: An Immunobiomarker Test as an Aid to Early Detection of Lung Cancer

Stephen Lam, Peter Boyle, Graham F. Healey, et al.

Cancer Prev Res 2011;4:1126-1134.