Guidelines for completion:

- Complete all relevant sections
- Attach any additional information e.g. images to a separate document
- Please be aware that lay sections should be targeted towards an audience with little to no scientific knowledge

Grant information	
Project reference number:	2012/05/Janes
Project title:	Infrared Spectral Biomarkers of Lung Cancer Risk
Institute:	University College London
Principal Grant Holder:	Prof. Sam Janes
Project start date:	1/10/2013
Project finish date:	30/9/2014

1. Abstract and statement of context

Lung cancer is the leading cause of cancer death worldwide, earlier detection and identification of those at highest risk will lead to improvement in the survival of lung cancer patients.

We hypothesise that infrared spectroscopy of buccal mucosal cells will detect patients with or stratify those at risk of lung cancer. Optically detected high risk patients can then enter more intensive screening programmes including radiology and autofluorescence bronchoscopy. Tobacco smoke exposure causes a host response to toxic damage resulting in a widespread 'field of injury' throughout the lung extending from the parenchyma to the nose and mouth. Cancers can arise anywhere within this field. This injured epithelium is morphologically normal but gives abnormal readings with infrared spectroscopy and extends to the oro-respiratory tract, including the buccal mucosa. We therefore propose to develop this innovation as a cheap, non-invasive, high throughput technique for detecting individuals at high risk of developing lung cancer and therefore increase the number of curable lung cancers detected.

2. Lay statement

It is known that smoking causes damage throughout the airways including the nose and mouth and that it is possible to detect this 'field of injury' using modern optical technologies. We aim to develop one such technique using infrared light shone at cells taken painlessly from the lining of

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the cheek to determine an individual's risk of developing lung cancer. Those people deemed 'high risk' would then be kept under surveillance and any lung cancers that develop cured at an early stage.

3. Relevance of your research to a person with lung cancer

The main problem with lung cancer is that there are usually no symptoms when the disease is in its earliest and curable stages- in the UK there is currently no way of detecting individuals who are likely to develop lung cancer or those who have early stage disease. We aim to develop a rapid, cost-effective and non-invasive technique for identifying these individuals and thereby improve the cure rate of those affected. We also hope those with more advanced disease will benefit by being identified and offered treatment at an earlier time point.

4. Timeline of research proposed

During the final 12 months a large number of patient samples will be tested to build and refine a statistical model to detect patterns in the data that relate to risk of lung cancer. The patterns that we find will be analysed to determine the changes within the cell causing them giving us an insight into the biology driving it.

5. Summary of progress made in the first year (maximum 2 paragraphs **which must be in laymen's terms)**

During the first year we have collected patient samples to determine the best way to process them; which chemicals to use, which types of slide and how to store and transport the samples in order to achieve the best possible results. In total approximately 50 samples have been analysed during this initial phase. More recently we have used this information to conduct the first of our pilot experiments which involved testing the cells from 20 patients- some with cancer, some smokers and other non-smokers. From this small sample we were able to demonstrate that the technique has the potential to deliver our project aims.

We have strengthened our collaboration with the statistics department who are helping us to analyse our complicated results, together we have built a software program that can automatically extract the important information and analyse the data. We have also developed another technique to help us differentiate between cancerous and non-cancerous samples using the same optical technique and software. To determine the biological cause of the changes in the cells we have developed techniques for growing both normal and cancerous cells in the laboratory.

6. Outline your main research achievements over the last year (maximum 5 bullet points **which must be in laymen's terms)**

- Development of a robust and repeatable process for sample collection, preparation, storage and analysis
- Pilot data to suggest that we are able to detect changes in the normal cheek cells of lung cancer patients compared to smokers without lung cancer and non-smokers
- Development of a software platform for automation of data pre-processing and analysis
- Use of the same system to differentiate cancerous from normal tissue in real-time.

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- Development of a technique that allows us to grow a portion of the cells to be stored for future analysis

7. Is your project running to the proposed budget? If not, how will you address this in subsequent years?

Yes

8. Please confirm that any outstanding issues outlined in the award letter have been addressed/resolved

There are no outstanding issues

9. Focus for the second year (maximum 5 bullet points)

- Session for the Diamond synchrotron facility are scheduled for next year already, work at the Imperial site with 2D FTIR image continues
- Patient recruitment for the main body of further analysis is underway, Estimate to be able to analyse samples from 150-200 patients
- Continue to refine statistical methods for analysis and build a prediction model.
- Validate findings by splitting the data into a test/ discovery and validation cohort to generate sensitivity and specificity values for the technique
- Investigate the biological cause driving the observed changes.

10. Publications:

a) List any presentations or publications, which have arisen so far from this project by completing the table below (please add extra rows as necessary).

b) Provide **electronic** draft or final copies of all publications

Impact factor	How presented (oral, poster or journal publication)	Title	Year	Authors	Journal publication name / conference venue	Status (in submission, in press or published)
n/a	Poster	Incidental discovery is the prevalent referral pathway to surgery for early stage lung cancer	2013	See attached	BTOG	Presented
n/a	Poster	Infrared spectral cytopathology of the respiratory tract a pilot study	2013		WCLC	Accepted for presentation Oct 2013 (Sydney)
n/a	Poster	Patterns of disease recurrence and modality of detection following surgery for early stage lung cancer	2013		WCLC	Accepted for presentation Oct 2013 (Sydney)

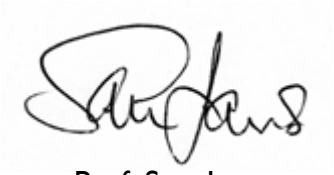
n/a	Oral presentation	The natural history of bronchial pre-invasive disease	2013		BTS	Accepted for presentation Dec 2013

11. Provide any images (not under copyright) that can be used by RCLCF for illustrative purposes along with a short description

I agree for this report to be distributed to the Roy Castle Lung Cancer Foundation's Grants Committee, the individuals and trusts that have supported this grant and RCLCF's intellectual property representatives. All data will be treated in confidence. I understand that RCLCF may distribute a summary of this report for fundraising purposes.

☐ √ By ticking this box I agree to the above and confirm that the content of this report has been approved by both the grant holder and researcher

Name:



Prof. Sam Janes

Dr. James Brown

Date: 18/09/2013

Please return the completed report to Jackie Tebbs at grants@roycastle.org

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Incidental discovery is the prevalent referral pathway to surgery for early stage lung cancer

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1. INTRODUCTION

Surgery for early stage non-small cell lung cancer offers the best prospect of cure. An obvious approach to improving the outlook for lung cancer patients is to establish clinical pathways that facilitate early detection. However, the impact of current referral strategies in identifying surgically treatable lung cancer cases and subsequent pathways to surgery is unknown.

2. METHODS

Routes of referral for patients undergoing surgical resection with curative intent for primary lung cancer at a single centre were recorded between 2007 and 2011. The primary endpoint was the percentage of patients diagnosed incidentally compared to those detected via the traditional primary care 'Target' referral system. Demographic data were recorded along with modality of investigation leading to detection of lung cancer, presence or absence of respiratory symptoms at referral, pathological stage and lung cancer specific mortality.

84 patients with non-small cell lung cancer underwent surgery during the study period

3. DEMOGRAPHICS (Table 1)

		Number (%)
Gender	Male	47 (56)
	Female	37 (44)
Age	<50	5 (6)
	50-75	60 (71)
	>75	19 (23)
Ethnicity	Caucasian	72 (86)
	South Asian	3 (4)
	East Asian	5 (6)
	African	4 (4)
Smokers		67 (80)
Non-smokers		17 (20)

4. RESULTS: Pathways of referral

The majority of patients with operable lung cancer were discovered incidentally during investigations performed by hospital specialities 61% (n=51; 95% CI 50-70%) (fig 1).

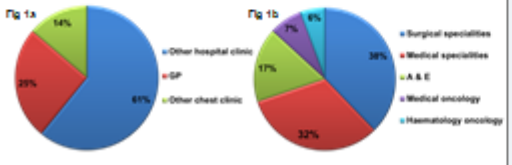

Figure 1. a. Chart showing pathway routes of referral b. Chart showing the internal hospital speciality referring patients with suspected lung cancer.

5. RESULTS: Modality of investigation

All patients referred under the 2 week wait scheme from primary care had a chest x-ray as their initial modality of investigation suggesting lung cancer and prompting referral. Patients referred from hospital clinics were most likely to have had their lesion detected serendipitously on a CT scan performed as part of their routine clinical care (fig 2).

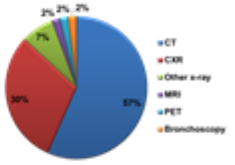

Fig 2. Initial modality of investigation suggesting lung cancer in patients referred from hospital specialities

6. RESULTS: Lung cancer specific mortality

Patients who presented with symptoms related to the cancer had a significantly higher lung cancer specific mortality (fig. 3a; p=0.0196). There was also a trend towards increased mortality in those who had a chest x-ray as their initial investigation (fig. 3b).

Fig 3a.

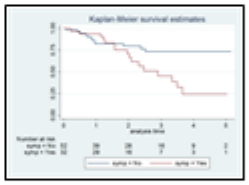

Fig 3b.

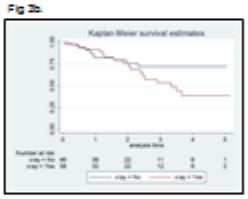

Figure 3. a. Kaplan-Meier survival estimate showing lung cancer specific mortality in patients with symptoms at referral vs. those without (p=0.0196). b. Kaplan-Meier survival estimate showing lung cancer specific mortality in patients whose initial modality was chest x-ray vs. patients whose lung cancer was detected by another form of radiological investigation (did not reach statistical significance).

7. SUMMARY

- Operable lung cancer is more often found by chance rather than design.
- Only 25% of patients with operable disease were referred by GP's.
- There is a trend towards poorer survival in patients who have chest x-ray detectable lesions and symptoms related to their cancer at referral. Figure 4 demonstrates the overlap in this group.
- Further data from multiple centres is being collected to examine this complex interaction.

Figure 4. The intersection of primary care referrals, those with symptoms at referral and patients whose initial modality of investigation was with chest x-ray.

8. CONCLUSIONS

This study suggests that asymptomatic lung cancer patients who are diagnosed serendipitously with chest CT are likely to have the best prospects of cure with surgery. Symptomatic patients were more likely to have a chest x-ray as their initial modality of investigation and overall this group have a worse prognosis. We believe these findings lend support to the case for CT screening of asymptomatic high risk individuals.

This work was funded by the Roy Castle Lung Cancer Foundation (JB). SMJ is a Wellcome Trust Senior Fellow in Clinical Science. This study was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme (SMJ, SMJ). JB/ SN received a travel bursary from Lilly Oncology to attend the conference.

Infrared Spectral Cytopathology of the Respiratory Tract : A Pilot Study

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Email: jim.brown@ucl.ac.uk

1. INTRODUCTION

Vibrational infrared (IR) spectroscopy is a powerful chemical analytical tool that can be used to detect and analyse many types of chemicals and materials including complex mixtures. Absorptions in this region arise from molecular vibrational properties and most molecules have characteristic IR spectra.

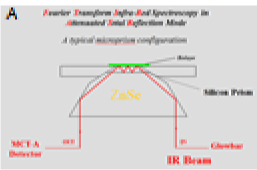
There is a growing literature on its possible medical diagnostic[1-7] use to distinguish cell types and states by characterising their IR 'molecular fingerprints' in the 1800 - 900 cm^{-1} range. Such spectra are complex since they represent an overlapping mixture of proteins, lipids, carbohydrates, DNA and cellular metabolites.

2. HYPOTHESIS

IR spectroscopy will differentiate histological grade of bronchial biopsies by identifying spectral changes corresponding to the stepwise progression from dysplasia to invasive cancer.

3. METHODS- INFRARED SPECTROSCOPY

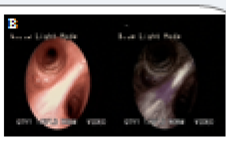
ATR-FTIR spectra were recorded at room temperature with a Bruker IFS 66/S spectrometer (A), fitted with a liquid nitrogen-cooled MCT-A detector and equipped with an Attenuated Total Reflection microprism (SensIR 3 mm silicon crystal; 3 internal reflections). All frequencies quoted have an accuracy to ± 1cm^{-1}. Typically, 1000 interferograms at 4 cm^{-1} resolution were averaged over 60s before Fourier Transformation. In all cases, a background spectrum of the clean prism surface in air was first recorded.



IR Data Manipulation

Spectra of individual biopsies were normalised on the basis of intensities of their amide II protein band before averaging. Where necessary, corrections were made for residual contributions of suspending media and for variations in atmospheric water vapour. These averaged spectra are shown both as absorbance versus frequency plots and as the second derivatives (17 smoothing points) of these same plots.

4. SAMPLES



- Following ethical committee review patients attending UCLH bronchoscopy service were asked to participate in the study
- Normal and abnormal epithelia were differentiated using autofluoresence bronchoscopy (B)
- Biopsy samples were placed in 0.9% saline (FTIR spectrally inert) and analysed fresh.
- Matched specimens were sent for routine histopathological examination for clinical classification
- In total 55 biopsies were taken from 33 patients representing 21 normal sites, 9 with low grade dysplasia, 18 high grade dysplasias and 7 invasive squamous cell carcinomas

5. RESULTS

Fig. 1: Averaged absolute FTIR spectra of biopsy samples: normal biopsies (n=21), low grade dysplasia (n=9), high grade dysplasia (n=18), invasive squamous cell carcinoma (n=7)

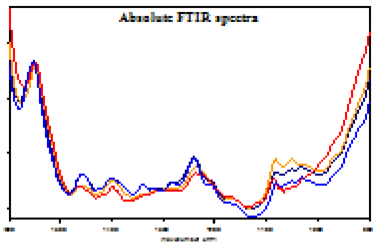
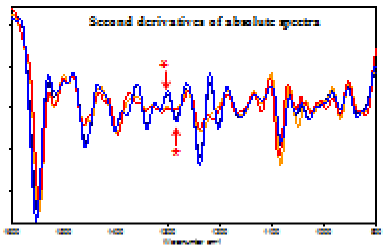


Fig. 2: Averaged second derivative of absolute spectra: normal biopsies (n=21), low grade dysplasia (n=9), high grade dysplasia (n=18), invasive squamous cell carcinoma (n=7)



6. DISCUSSION

It is relatively quick, easy and non-destructive to obtain high quality mid-IR spectra of tissues and cells using the FTIR/ATR technique and has the additional advantage that no chemical manipulations of samples are necessary.

In this study the IR spectra are, as expected, dominated by contributions from the principal cellular components and so have the same overall general pattern. Differences will be small in absolute spectra but can be accentuated in second derivative plots of the same data (a method that narrows and sharpens multiple overlapping bands).

We have shown clear differences between the normal/ low grade and high grade/ cancer samples; two groups that are clinically relevant to distinguish. A simple algorithm comparing the relative peak heights (Fig 2 *) in the 1270-1290 cm^{-1} range differentiates between individual biopsies with 100% diagnostic accuracy in this cohort.

The observed changes suggest possible alterations in one or more major protein components (1200-1500 cm^{-1}) as well as changes indicative of glycoproteins, lipids and/or DNA/RNA level changes (1000-1100 cm^{-1} range [8,9]).

7. CONCLUSIONS

- High quality mid-IR spectra of biopsy specimens reveal differences between pathological groups.
- This technology has the potential to provide real-time near patient cytopathological diagnosis for the bronchoscopist or thoracic surgeon for instance to confirm clear resection margins at surgery.
- Identification of the chemical changes occurring at a cellular level may lead to reverse translation and better understanding of the processes driving progression to malignant transformation.
- Future work will refine and validate the observed changes.

8. REFERENCES

[illegible]

Patterns of disease recurrence and modality of detection following surgery for early stage lung cancer

Jim Brown, Stephanie Brazil, Dorcas McAsey, Shahideh Safavi, Ceri Morgan, Sakib Rokadiya, Mamta Ruparel, Dipak Mukherjee, Sarah Doffman, Sara Lock, Amit Patel, Alistair Reinhardt, Jeremy George, Sam Janes, Neal Navani

Background
There is a significant risk of disease recurrence following surgery with curative intent for non-small cell lung cancer (NSCLC). However, limited data is available on the patterns of recurrence and best practice for follow up imaging after lung cancer surgery. Current practice in the United Kingdom (UK) is to perform interval chest x-ray for 5 year following surgery. We aimed to determine the incidence, anatomical site, modality of detection and percentage of patient requiring acute admission as a harbinger of disease recurrence post thoracotomy for NSCLC

Methods
Records of consecutive patients with NSCLC who underwent thoracotomy and resection of early stage lung cancer at 5 institutions situated in the South East of the UK between October 2007 and September 2012 were interrogated. Data collection was completed in Jan 2013.

Results
A total of 314 patients were included; 59 (18.8%) patients died from disease recurrence during the study period, the site of recurrence was lung, central nervous system, bone/ soft tissue, abdominal and lymph node respectively in 24 (40.7%), 17 (28.8%), 10 (16.9%), 4 (6.8%) and 4 (6.8%) cases. In 45 (76.2%) patients disease recurrence was detected during outpatient consultation, modality of detection for routine chest x-ray, CT and other modalities were respectively 7 (15.5%), 28 (62.2%) and 10 (22.3%) in every case CT was prompted by change in symptoms, a clinically palpable mass lesion or clinical suspicion. Other modalities used were MRI, ultrasound and lymph node aspiration in 4, 3 and 3 cases respectively. Emergency admission accounted for 14 (23.8%) patients pathway to detection of recurrence, of these 9 (64.3%) were admitted with symptoms relating to cerebral metastases, 4 (28.6%) with symptomatic breathlessness and 1 (7.1%) with a pathological fracture.

Conclusions
Almost a quarter of patients with relapsed lung cancer following surgery present with acute symptoms requiring emergency admission. Standard chest x-ray follow-up detects very few recurrences with most cases being detected once reported symptoms direct further investigation. It is currently unknown whether earlier detection of recurrence may offer symptomatic or survival gains however avoidance of emergency admissions is likely to have a positive impact on quality of life. Further studies to investigate which patients are at highest risk of recurrence and the most appropriate post-surgical follow-up strategies are required.

The natural history of bronchial pre-invasive disease

Jim Brown, Georgia Hardavella, Bernadette Carroll, Mary Falzon, Neal Navani, Jeremy George, Sam Janes

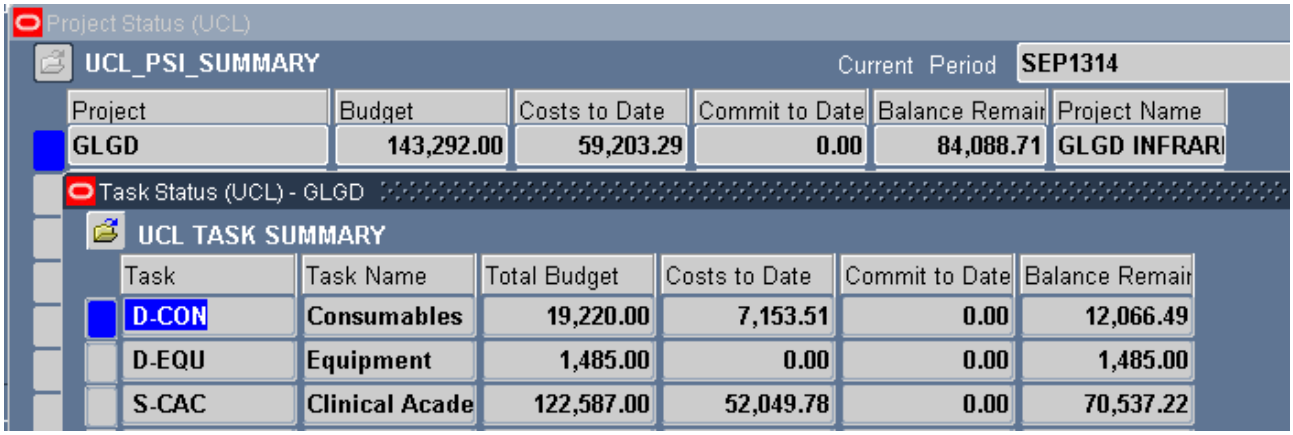
Background
Bronchial pre-invasive lesions represent the earliest stages of the stepwise progression of squamous carcinogenesis, they predominantly affect the large airways and are readily detectable using autofluoresence bronchoscopy (AFB) however very little is known about the natural history of these lesions and no randomised data exists to determine whether intervention before progression to invasion improves outcome.

Methods
A total of 94 patients with bronchial dysplasia were enrolled into an on-going surveillance cohort at University College London Hospital running prospectively since 1999. Lesions were biopsied longitudinally and kept under regular surveillance with AFB and low dose annual CT scanning until resolution or progression to invasive disease occurred. Retrospective analysis of lesional destiny was undertaken to determine the proportions of progressive vs. regressive lesions that occur in low grade dysplasia (LGD- squamous metaplasia, mild and moderate dysplasia) vs. high grade dysplasia (HGD- severe dysplasia (SD) and carcinoma-in-situ). A lesion was considered to have progressed/ regressed if it crossed between groups (LGD, HGD, invasive cancer).

Results
A total of 117 separate lesions that were biopsied on more than one occasion were identified of which 61 were HGD and 56 LGD. Of the low grade lesions 54/56 (96%) regressed or remained static, 1 (2%) progressed to CIS and 1 (2%) to invasive carcinoma both of these lesions progressed from moderate dysplasia. Of the high grade lesions there were 13 SD and 48 CIS, overall 35/61 (57%) of HGD progressed to invasive cancer 9/61 (15%) regressed and 17/61 (28%) remained static. There was a trend toward higher progression to cancer (62% vs 56%) and lower rates of regression (8% vs. 17%) for SD versus CIS in the HGD cohort although the numbers are too small to be statistically significant (see fig. 1). In the HGD group median time to invasion was 9.5 months (range 3-49), static lesions were documented to have remained as such for a median of 17 months (range 4-60).

Expenditure summary:

A full report will be prepared by the finance department in early October, below is a screenshot summarising the expenditure so far:



Project Status (UCL)

UCL_PSI_SUMMARY — Current Period: SEP1314

Project	Budget	Costs to Date	Commit to Date	Balance Remair	Project Name
GLGD	143,292.00	59,203.29	0.00	84,088.71	GLGD INFRARI

Task Status (UCL) - GLGD

UCL TASK SUMMARY

Task	Task Name	Total Budget	Costs to Date	Commit to Date	Balance Remair
D-CON	Consumables	19,220.00	7,153.51	0.00	12,066.49
D-EQU	Equipment	1,485.00	0.00	0.00	1,485.00
S-CAC	Clinical Acade	122,587.00	52,049.78	0.00	70,537.22

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