

BMJ Open

Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-013324
Article Type:	Protocol
Date Submitted by the Author:	04-Jul-2016
Complete List of Authors:	Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Stobo, David; Queen Elizabeth University Hospital, Radiology Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Oncology, Patient-centred medicine, Research methods
Keywords:	Mesothelioma, Biomarker, Diagnosis, Prognosis

SCHOLARONE™
Manuscripts

TITLE:

DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL
ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL
OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

AUTHORS:

Selina Tsim^{1/2}, Caroline Kelly³, Laura Alexander³, Carol McCormick³, Fiona
Thomson³, Rosie Woodward⁴, John E Foster⁴, David B Stobo⁵, Jim Paul³,
Nick A Maskell⁶, Anthony Chalmers^{2/7}, Kevin G Blyth^{1/8}

Affiliations

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital,
Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University
Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow,
U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of
Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of
Glasgow, U.K.

Corresponding Author and Address

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

Keywords:

Mesothelioma

Biomarker

Diagnosis

Prognosis

WORD COUNT:

3998/4000

ABSTRACT (300/300)

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer[®] proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

METHODS AND ANALYSIS

Serum and plasma (for SOMAscan[®] and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

1
2
3 marker and their performance will be compared to serum Mesothelin. Blood
4
5 levels will be compared to paired pleural fluid levels and MPM tumour volume
6
7 (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic
8
9 value of each marker will be assessed and a large bioresource created.
10
11

12 13 14 **ETHICS AND DISSEMINATION**

15
16 The study has been approved by the West of Scotland Research Ethics
17
18 Committee (Ref: 13/WS/0240). A Trial Management Group meets on a
19
20 monthly basis. Results will be published in peer-reviewed journals, presented
21
22 at international meetings and disseminated to patient groups.
23
24

25
26
27 **TRIAL REGISTRATION NUMBER: ISRCTN10079972**

28 29 30 **STRENGTHS OF THIS STUDY**

- 31
32
- 33 • Prospective recruitment of patients at presentation with suspected pleural
34 malignancy, reflecting when blood biomarkers would be drawn in clinical
35 practice and avoiding potential confounding factors such as pleurodesis
36
37
 - 38 • Strict sampling, processing and storage methods used in all patients
39
 - 40 • Potential confounders including renal function, body weight and
41 concomitant medications recorded
42
43
 - 44 • All participants subject to rigorous diagnostics with a minimum of 12
45 months' follow-up in patients who do not have a histological or cytological
46 diagnosis of malignancy
47
48
 - 49 • A large bio-resource of serum, plasma, whole blood and pleural fluid, with
50 prospectively collected detailed clinical information will be created
51
52
53
54
55
56
57
58
59
60

LIMITATIONS OF THIS STUDY

- While the study design accounts for inherent diagnostic difficulties in Malignant Pleural Mesothelioma (MPM), with follow-up of patients with 'benign' pleural disease for a minimum of 12 months, there is likely to be a population of patients included in the study with suspected MPM who do not have a firm histological diagnosis. Every effort will be made in these cases to ascertain the post-mortem findings where available.
- Study dropout – there is likely to be a population of patients with MPM included in the study who will not be fit to return for follow-up clinic visits (or a study research visit) due to the natural history of the disease. In these patients it will not be possible to perform 3 month follow-up blood biomarker sampling (exploratory outcome). There is also likely to be a cohort of patients, e.g. who do not have a diagnosis of malignancy, who will become lost to follow-up.
- The final number of study participants who are diagnosed with MPM will not be known until the study completes recruitment. This results in a degree of uncertainty regarding the power available to test the primary hypotheses. The statistical analysis plan included in this manuscript takes into account this uncertainty. The protocol does however include an estimated final number of MPM based on audit data from several of the recruiting centres and number of MPM cases recruited during the course of the study will be kept under review by the Trial Management Group.

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [7] with diagnostic yields for malignancy >90% [6] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

1
2
3 including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15,16]
4 and osteopontin [17], are higher in patients with MPM than in asbestos-
5 exposed controls (AECs) and patients with secondary pleural malignancies.
6
7 Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM
8
9 [18,19] is the most widely studied and is associated with an MPM sensitivity of
10
11 56-77% at 95% specificity [14,16,20]. However, a recent meta-analysis (of
12
13 4491 individuals (1026 with MPM)) reported a sensitivity of only 32% at 95%
14
15 specificity. Mesothelin does not, therefore, contribute to current diagnostic
16
17 algorithms [21]. MPF offers no advantage over mesothelin [16], while the
18
19 clinical utility of osteopontin is limited by stability and reproducibility concerns
20
21 [17].
22
23
24
25
26
27
28
29

30 An ideal MPM biomarker would be measurable in blood for ease of collection
31 and offer sufficient sensitivity at high specificity in patients presenting with
32 suspected MPM. Differentiation between advanced disease patients and
33 appropriate controls is of limited value. High specificity is mandatory for a low
34 prevalence disease, and should apply to patients with asbestos exposure and
35 non-MPM pleural disease. Biomarker results should also correlate with
36 disease extent and have defined relationships with potential confounders
37 including renal function [22] and the effect of pleural interventions. The latter
38 is important because the precedent has been established in prostate [23,24]
39 and breast cancer [25], that recent sampling, resection or peri-tumoural
40 inflammation may affect biomarker expression. This is particularly relevant to
41 MPM where biopsies are frequently large and often combined with
42 pleurodesis. Several previous biomarker studies, which validated
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

inconsistently in external populations, used samples acquired at later time-points, often post-diagnosis (and post-pleurodesis) including samples taken prior to, during, or after resection surgery [17,26,27]. The aim of the DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic performance of the SOMAscan proteomic classifier [28] and fibulin-3 [26], which have demonstrated high sensitivity and specificity in recent retrospective series. The study has been designed to generate clinically meaningful results, which can be related to MPM biology and confounding factors, and applied to patients at first presentation.

SOMAmer-based Proteomic Classifier

The SOMAscan assay is a highly multiplexed proteomic platform that utilizes SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and quantify proteins [29]. A 13-protein classifier was developed by SomaLogic Inc. (Boulder, Colorado), using this novel proteomics-based biomarker detection technique [28] in a retrospective study over 800 proteins were measured in the serum of 117 MPM patients and 142 AECs, collected at surgical MPM centres in the US between 1996 and 2011. Using a panel of 13 differentially expressed proteins and a cut-point of 0.5, the classifier was able to segregate MPM from controls with an area under the curve (AUC) of 0.99 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38 cases/62 controls) [28]. The combined sensitivity for the three cohorts was 93% at 91% specificity. Based on the published ROC curve for the validation cohort, sensitivity at 95% specificity appeared to be approximately 78%,

1
2
3 although the authors did not report this value. This performance exceeds that
4
5 of any previous MPM biomarker, although the classifier's specificity appeared
6
7 lower in patients with non-MPM pleural effusion (n=32). There was a modest
8
9 correlation between classifier score and disease stage, but prognostic
10
11 significance was not assessed. The 13 classifier proteins (nine up-regulated,
12
13 four down-regulated) have not previously been associated with MPM. Their
14
15 functions fall into two broad groups; regulation of proliferation and
16
17 inflammation. Quite apart from their biological relevance to MPM, the latter is
18
19 an important potential confounder because many of the patients involved will
20
21 have previously undergone pleurodesis. In addition, several groups have
22
23 reported an independent interaction between prognosis and inflammatory
24
25 biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [30-32],
26
27 monocytosis [33] and the modified Glasgow Prognostic Score [32].
28
29 Therefore, adequate understanding of the diagnostic and prognostic utility of
30
31 this assay requires replication in a pre-pleurodesis cohort and prospective
32
33 evaluation of interactions between inflammatory biomarkers and SOMAscan
34
35 scores.
36
37
38
39
40
41
42

43 **Fibulin-3**

44
45 Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-
46
47 containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [34].
48
49 Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura
50
51 [26] and expressed and secreted by MPM cell lines [27]. Pass et al
52
53 retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136
54
55 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [26].
56
57
58
59
60

1
2
3 A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and
4 a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases.
5
6
7 However, in a blinded external validation set, sensitivity was below 40% (at
8
9
10 95% specificity), with an AUC=0.87.

11
12
13
14 Subsequent studies have revealed mixed results. In a study of 153 patients
15 (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity)
16 at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which
17 was significantly inferior to mesothelin measured in the same patients
18 (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM
19 threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay
20 and internally-defined cut-points, Agha et al reported 100% sensitivity/78%
21 specificity in differentiating MPM cases (n=25) from non-malignant pleural
22 disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from
23 secondary pleural malignancies (n=11) [35]. No combined sensitivity was
24 reported. An Italian study found no difference in Fibulin-3 levels but used
25 serum (not plasma), a control group without pleural disease (Asbestosis) and
26 contained only 14 patients with MPM [36].
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **METHODS AND ANALYSIS**

46 **Study Design**

47
48 DIAPHRAGM is a prospective, multi-centre observational study. The study
49 incorporates sampling windows that correspond to the proposed use of a
50 diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy
51 (SPM). The overall study design is summarized in Figure 1. The main impact
52
53
54
55
56
57
58
59
60

1
2
3 of this design is that biomarkers will be drawn before a diagnosis is made. In
4
5 addition to better replicating the future use of these markers, this avoids the
6
7 potential confounding effect of pleurodesis on biomarker results. The
8
9 diagnostic performance of the SOMAmer panel and Fibulin-3 will be assessed
10
11 using cut-points determined in the relevant original studies and compared to
12
13 mesothelin. Identical processing and storage protocols will be used in patients
14
15 with SPM and a group of AECs (see Figure 2). Potential confounders
16
17 including renal function, inflammatory indices and drugs will be recorded at all
18
19 visits. An exploratory, cross-sectional Magnetic Resonance Imaging (MRI)
20
21 sub-study will determine if there is any correlation between blood biomarker
22
23 levels and MPM tumour volume, as has been established for Mesothelin using
24
25 Computed Tomography-Positron Emission Tomography scanning [37].
26
27
28
29
30
31

32 **Study Objectives and Outcome Measures**

33
34 These are presented in Table 1.
35
36
37

38 **Setting**

39
40 At least 737 consecutive patients with SPM will be recruited from 21 centres
41
42 (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and
43
44 more clinically orientated units. This should make the results of the
45
46 DIAPHRAGM study generalizable to patients presenting with SPM to acute
47
48 hospital services. The principal criterion used to select centres was that they
49
50 had sufficiently evolved pleural diagnostic services to deliver a reliable
51
52 diagnosis. Specifically, access to on-site thoracoscopy (ideally including local
53
54
55
56
57
58
59
60

1
2
3 anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting
4
5 (for diagnostic review and staging) was required.
6
7
8

9 10 **Screening and Eligibility Assessment**

11 Suspected Pleural Malignancy

12
13
14 Cases will be identified on presentation to a Respiratory out-patient clinic or
15
16 acute hospital admissions unit. This will be based on the history, examination
17
18 and available investigations. Potentially eligible patients will be provided with
19
20 the study Patient Information Sheet (PIS, see Online Supplementary
21
22 Appendix 1) and eligibility assessed based on the following criteria:
23
24
25
26

27 *Inclusion Criteria:*

- 28 • SPM, defined by a unilateral pleural effusion or pleural mass lesion
- 29
- 30 • Sufficient fitness for diagnostic sampling (site investigator's clinical
- 31
- 32 judgment)
- 33
- 34 • Informed written consent
- 35
36
37
38
39

40 *Exclusion Criteria:*

- 41 • Intercostal chest drain in-situ, or inserted within the previous 3 months
- 42
43
44
45
46
47

48 Asbestos-related pleural plaques are not an inclusion criterion since these are
49
50 absent in up to 25% of MPM cases [38], and are also common in asbestos-
51
52 exposed populations without MPM [39]. Patients with lung nodules or other
53
54 visceral mass lesions are not excluded, assuming the investigator suspects
55
56 pleural malignancy. This is because of the high prevalence of lung nodules in
57
58
59
60

1
2
3 the target population (older patients, commonly smokers) and the high false
4
5 positive rate of CT imaging in this regard [40].
6
7

8
9
10 Subjects recruited to the SPM arm will generate cohorts of MPM and non-
11
12 MPM pleural disease of various aetiologies, likely including Benign Asbestos-
13
14 related Pleural Effusion and secondary pleural malignancies. These numbers
15
16 will be sufficient to address the primary objective with sufficient statistical
17
18 power to inform clinical practice (see later section).
19

20
21
22
23 Asbestos-exposed control (AEC) subjects

24
25 109 AECs will be recruited via invitations sent by Clydeside Action on
26
27 Asbestos (CAA), an advocacy body based in Glasgow with a database of over
28
29 600 clients, or by Respiratory clinics at the host centre. Individuals will be
30
31 invited to participate by letter (if identified via CAA) or given the PIS (see
32
33 Online Supplementary Appendix 2) at clinic. All subjects will be invited to a
34
35 single research clinic visit assuming the following Eligibility Criteria are met.
36
37

38
39
40
41 *Inclusion Criteria:*

- 42
43
44
45
46
47
48
49
50
51
- Documented history of asbestos exposure and associated pleural plaques, asbestosis or diffuse pleural thickening
 - Willing and able to travel to a research clinic interview in Glasgow
 - Informed written consent

52
53
54
55
56
57
58
59
60

Exclusion Criteria:

- Known MPM
- Known or suspected other thoracic malignancy under investigation

- Known pleural effusion of any cause

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

Inclusion Criteria:

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

Exclusion Criteria:

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 3) and will be asked to provide additional informed written consent.

Consent

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

Outcome Measures

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

Final Diagnosis

A specific cytological or histological pleural diagnosis will be sought in all patients according to national guidelines [21]. This will be recorded as the Final Diagnosis, which may be based on immediate repeat biopsies felt to be indicated by the site PI (see Figure 1). Any cytologically or histologically confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will be recorded without the need for any further updates. However, sites will need to provide updates for any non-MPM diagnosis that is not cytologically or histologically confirmed (e.g. parapneumonic effusion). These will be submitted on the 12-month anniversary of the original diagnosis, or as soon as any new pleural diagnosis is made. This aims to capture any false negative diagnostic tests from the initial presentation, acknowledging the major diagnostic challenges posed by pleural malignancies, particularly MPM.

Biomarker Sampling and Storage

Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate processing performed at each study centre. Samples can be taken before or after pleural aspiration. Patients with positive pleural cytology cannot be recruited (see Figure 1(a)). Duplicate samples will be collected for all measurements at all visits, ensuring redundancy in case of loss or damage to samples during transportation to the appropriate central laboratory. SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of venous blood will be collected first into a vacutainer tube containing SST clot activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of venous blood will be collected second into a vacutainer tube containing EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties only) 20 ml of pleural fluid will be also collected into a plain container if pleural fluid is being drawn for diagnostic/therapeutic purposes at the same visit.

Biomarker Processing and Storage

Serum samples will be allowed to clot for 30 minutes before centrifugation. Plasma and pleural fluid samples will be centrifuged immediately. All samples will be centrifuged at 2200g for 15 minutes at room temperature. For all samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials of at least 250µL volume, labeled and placed into a -80 freezer within 2 hours. Samples will be stored at each recruiting centre until batched transport to the appropriate study laboratory.

Biomarker Analyses

SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan proteomic analyses [28]. This utilises SOMAmer reagents to specifically bind to protein targets in blood. Relative protein concentrations will be converted to measurable nucleic acid signals that are quantified by hybridization to DNA microarrays [29].

Fibulin-3 and mesothelin levels will be measured using ELISA methods validated according to the FDA-recommended guidelines for bioanalytical methods [41]. Fibulin-3 levels will be measured using the commercially available ELISA (USCN Life Science Inc, Wuhan, China) as in the original Pass study [26]. Mesothelin will be measured using the Mesomark ELISA (Fujirebio Diagnostics, Inc, PA, USA). In parallel, we aim to develop a custom multiplex ELISA assay that has the potential to simultaneously measure multiple biomarkers (fibulin-3, mesothelin and osteopontin) with greater accuracy (U-PLEX, Meso Scale Diagnostics, Rockville, USA)

Magnetic Resonance Imaging

Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow, on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using VIBE sequences. A stack of axial slices covering the entire lung and surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA contrast (Gadovist) will be administered via a peripheral intravenous line as a 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied

1
2
3 slice positions to provide pre and post-contrast images. The total scan time
4 will be around 45 minutes. Regions of enhancing pleural tumour will be
5 defined using semi-automated signal intensity thresholding based on contrast-
6 enhanced axial slices using Myrian Intrasure™ software.
7
8
9

10 11 12 13 *Survival*

14 Survival will be recorded in days from the date of study registration to the data
15 of death, from any cause.
16
17
18
19

20 21 22 23 **Sample Size, Assumptions and Uncertainties**

24 Sample size estimations for each marker were based on published data at the
25 point of study design and a projected MPM incidence of 13-20% in the SPM
26 cohort. The power available to test the hypotheses below is therefore reported
27 as a range, based on final MPM numbers lying between 83 (13% incidence)
28 and 120 (20% incidence).
29
30
31
32
33
34
35
36
37

38 39 Primary Objective

40 41 *SOMAscan Assay*

42 We hypothesize that the MPM sensitivity and specificity exceed 90%, based
43 on previously reported performance in combined training, verification and
44 validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–
45 95.6%) [28]). Recruitment of 83-120 MPM patients will allow us to distinguish
46 a sensitivity of >90% from a sensitivity <80% with 80-93% power,
47 respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will
48 allow discrimination between a specificity <80% and a specificity >90%, with
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 80-88% power at the 5% 1-sided level of statistical significance. The standard
4
5 error in the estimated sensitivity and specificity will be less than 5%, across all
6
7 possible outcomes.
8
9

10 11 *Fibulin-3*

12
13
14 We hypothesize that the MPM sensitivity will exceed 80% and that the
15
16 specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based
17
18 on a reduced level of performance to the primary results reported by Pass et
19
20 al (97% sensitivity, 95% specificity), given lower sensitivity in the external
21
22 validation cohort studied (40% at 95% specificity) [26].
23
24
25
26

27
28 With 83-120 MPM patients the study will be able to distinguish a sensitivity of
29
30 >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-
31
32 sided level of statistical significance. The standard error in the estimated
33
34 sensitivity will be less than 5%. In order to achieve 90% power to distinguish
35
36 a specificity of >90% from a specificity <85% at the 5% 1-sided level of
37
38 statistical significance, a random sample of 378 non-MPM samples will be
39
40 analysed. The standard error in the estimated specificity will be <2.3%.
41
42
43
44

45
46 The study data will be used to estimate the AUC for the SOMAscan marker
47
48 for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming
49
50 83-120 patients in the MPM group and 83-120 in the non-MPM group the
51
52 AUC can be estimated with a 95% confidence interval of width 0.120-0.168
53
54 (assuming a cut-point exists with a reasonable sensitivity of 80% and a
55
56 modest specificity of 40%). If more sensitive/specific cut-points exist the width
57
58
59
60

1
2
3 of the 95% confidence interval will be much reduced. The study data will be
4
5 used to develop a new diagnostic signature based on Fibulin-3 and
6
7 SOMAscan results to distinguish MPM from non-MPM effusions.
8
9

10 11 12 Secondary Objectives

13
14 The study data will be used to determine whether baseline SOMAscan results
15
16 and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are
17
18 independent prognostic factors for MPM. A correlation of 0.4 between existing
19
20 prognostic factors and each marker has been assumed. For the baseline
21
22 levels, to detect an approximate doubling in median OS (from 6 month to 12
23
24 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of
25
26 statistical significance between a good/poor prognostic group based on
27
28 dichotomising these markers requires at least 83 MPM patients recruited over
29
30 three years with approximately 6 months subsequent follow-up to observe 66
31
32 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be
33
34 detected (80% power, 5% 2-sided level of statistical significance) when 49
35
36 deaths are observed in the estimated 66 out of 83 patients who survive to 3
37
38 months.
39
40
41
42
43
44

45 46 Exploratory Objectives

47
48 These will be addressed in the MRI sub-study, which will generate a sample
49
50 of at least 20 MPM patients. This will allow moderately large associations
51
52 (0.6) between the exploratory outcome measures (see Table 1) to be detected
53
54 at 80% power at the 5%, two-sided level of statistical significance. The effect
55
56 of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be
57
58
59
60

1
2
3 assessed using all 50 patients recruited. This will allow moderately small
4 differences (standardised difference of 0.4) to be detected with 80% power at
5
6
7 the 5% two-sided level of statistical significance.
8
9

10 11 12 **Statistical Analysis Plan**

13 14 **Primary Analysis**

15
16 Sensitivity and specificity at pre-specified cut-offs will be estimated using
17 standard approaches for proportions. The diagnostic performance of each
18 biomarker will be assessed using ROC curves. All patients with MPM (n=83-
19 120) will be included and compared with AECs and a random sample of non-
20 MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs
21 and 83 non-MPM cases will be randomly selected. All AECs and 378 non-
22 MPM cases will be used for Fibullin-3 analyses. Logistic regression will be
23 used to estimate a diagnostic model using biomarker results. Cross validation
24 will be used to provide robust estimates of AUC and specificity at fixed
25 sensitivity rates of 80%, 90% and 95%.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 **Secondary Analysis**

42
43 A prognostic model will be developed using Cox proportional hazard
44 techniques. The modelling process will incorporate biomarker measurements
45 (at presentation (both markers) and at 3 months (Fibulin-3 only) and other
46 known prognostic features (e.g. performance status, histology).
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Exploratory Analysis

The association between SOMAscan results/fibulin-3 in blood and tumour volume/measures of tumour angiogenesis will be estimated by Pearson or Spearman correlation, depending on the normality of the data. The same methods will be used to test the association between fibulin-3 in blood and pleural fluid. Changes in Fibulin-3 levels before and after histological sampling (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon signed rank sum test (depending on the normality of the data).

Changes to the Study Protocol since Trial Opening

The protocol described accurately reflects Version 5, of the protocol, dated 17/6/16. The following changes were made in previous versions:

- Version 2, dated 14/2/14:
 - Safety reporting reduced following risk assessment by study Sponsor.
 - Collection of duplicate blood samples as provision for loss or damage and for sample retention in tissue bank.
 - Greater flexibility to timing of first blood draw.
- Version 3, dated 17/10/14:
 - Addition of recruitment of Controls from Respiratory Medicine clinics
 - Addition of exclusion criteria for patients with chest drains in-situ.
 - Eligibility for the MRI sub-study extended to patients proceeding to image-guided pleural biopsy
- Version 4, dated 27/4/15:
 - Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.

- Version 5, dated 17/6/16:
 - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

Definition of End of Study

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

ETHICS AND DISSEMINATION

Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking incoming forms for compliance with the protocol, data consistency, missing data and timing. The CRUK Glasgow CTU will control data consistency and data quality by entering trial data onto CTU database. Computerised and manual consistency checks will be performed and queries issued in cases of inconsistency or missing information. An audit trail of changes to the database will be maintained.

Safety Considerations

Participants in the MRI sub-study will be asked at their 1-month follow-up visit about the occurrence of Adverse Events (AEs) related to the administration of MRI contrast (Gadolinium). These will be followed until resolution.

Dissemination

The results of the study will be presented at national and international scientific meetings and published in full in a peer-reviewed journal (authorship will be according to that journal's guidelines). A lay summary will be produced and disseminated to interested parties.

Trial Management

The trial will be coordinated from CRUK Glasgow CTU by the Trial Management Group (TMG), including the Chief Investigator, selected co-investigators, project manager, trial statistician, clinical trial co-ordinator and IT staff. The TMG will oversee the running of the trial and meet monthly.

Acknowledgements

The authors are grateful to the Chief Scientist Office (Scotland) as the study funder, cancer research networks infrastructure, the participating sites and staff, SomaLogic Inc and the patients involved. ST is part funded by the West of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS Career Research Fellowship. CR-UK and ECMC are acknowledged by FT and CMcC.

REFERENCES

- 1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *The European respiratory journal* 2011;**38**:1420–4. doi:10.1183/09031936.00000811
- 2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung cancer* 2015;**88**:344–8. doi:10.1016/j.lungcan.2015.03.005
- 3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung cancer* 2014;**83**:S28–9. doi:10.1016/S0169-5002(14)70076-5
- 4 Renshaw AA, Dean BR, Antman KH, *et al.* The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;**111**:106–9.
- 5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *The European respiratory journal*. 2010;**35**:479–95. doi:10.1183/09031936.00063109
- 6 Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. *Cancer* 1993;**72**:389–93.
- 7 Rusch VW, Giroux D. Do we need a revised staging system for malignant

- 1
2
3 pleural mesothelioma? Analysis of the IASLC database. *Ann*
4
5 *Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-
6
7 319X.2012.11.10
8
- 9
- 10
11 8 Salyer WR, Eggleston JC, Erozan YS. Efficacy of pleural needle biopsy
12 and pleural fluid cytopathology in the diagnosis of malignant neoplasm
13 involving the pleura. *Chest* 1975;**67**:536–9.
14
- 15
16
17
18 9 Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic
19 analysis for the evaluation of pleural effusion: analysis of 414 cases.
20
21 *Mayo Clin Proc* 1985;**60**:158–64.
22
23
24
- 25
26 10 Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy
27 as compared with that of pleural fluid examination. *Mod Pathol*
28 1991;**4**:320–4.
29
30
31
32
- 33
34 11 O'Rourke N, Garcia JC, Paul J, *et al*. A randomised controlled trial of
35 intervention site radiotherapy in malignant pleural mesothelioma.
36 *Radiotherapy and oncology : journal of the European Society for*
37 *Therapeutic Radiology and Oncology* 2007;**84**:18–22.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 12 Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after
invasive diagnostic procedures in patients with pleural mesothelioma. A
randomized trial of local radiotherapy. *Chest* 1995;**108**:754–8.
- 13 Robinson BWS, Creaney J, Lake R, *et al*. Mesothelin-family proteins and
diagnosis of mesothelioma. *Lancet* 2003;**362**:1612–6.
doi:10.1016/S0140-6736(03)14794-0

- 1
2
3 14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and
4 mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–
5 902. doi:10.1136/thoraxjnl-2014-205205
6
7
8
9
10
11 15 Onda M, Nagata S, Ho M, *et al.* Megakaryocyte potentiation factor
12 cleaved from mesothelin precursor is a useful tumor marker in the serum
13 of patients with mesothelioma. *Clin Cancer Res* 2006;**12**:4225–31.
14 doi:10.1158/1078-0432.CCR-06-0472
15
16
17
18
19
20
21 16 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin,
22 megakaryocyte potentiating factor, and mesothelin proteins as markers in
23 the serum of patients with malignant mesothelioma. *J Thorac Oncol*
24 2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b
25
26
27
28
29
30
31 17 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural
32 mesothelioma, and serum osteopontin levels. *The New England journal*
33 *of medicine* 2005;**353**:1564–73. doi:10.1056/NEJMoa051185
34
35
36
37
38 18 Chang K, Pai LH, Batra JK, *et al.* Characterization of the Antigen (Cak1)
39 Recognized by Monoclonal-Antibody K1 Present on Ovarian Cancers and
40 Normal Mesothelium. *Cancer research* 1992;**52**:181–6.
41
42
43
44
45
46 19 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with
47 epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg*
48 *Pathol* 1992;**16**:259–68.
49
50
51
52
53 20 Hollevoet K, Nackaerts K, Thimpont J, *et al.* Diagnostic Performance of
54 Soluble Mesothelin and Megakaryocyte Potentiating Factor in
55 Mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5.
56
57
58
59
60

1
2
3 doi:10.1164/rccm.200907-1020OC
4
5

6 21 Hooper C, Lee YCG, Maskell N, *et al.* Investigation of a unilateral pleural
7
8 effusion in adults: British Thoracic Society Pleural Disease Guideline
9
10 2010. *Thorax*. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978
11

12
13 22 Hollevoet K, Nackaerts K, Thas O, *et al.* The effect of clinical covariates
14
15 on the diagnostic and prognostic value of soluble mesothelin and
16
17 megakaryocyte potentiating factor. *Chest* 2012;**141**:477–84.
18
19 doi:10.1378/chest.11-0129
20
21

22
23 23 Bergamini S, Bellei E, Reggiani Bonetti L, *et al.* Inflammation: an
24
25 important parameter in the search of prostate cancer biomarkers.
26
27 *Proteome science* 2014;**12**:32. doi:10.1186/1477-5956-12-32
28
29

30
31 24 Yuan JJ, Coplen DE, Petros JA, *et al.* Effects of rectal examination,
32
33 prostatic massage, ultrasonography and needle biopsy on serum prostate
34
35 specific antigen levels. *The Journal of urology* 1992;**147**:810–4.
36
37

38
39 25 Wong V, Wang D-Y, Warren K, *et al.* The effects of timing of fine needle
40
41 aspiration biopsies on gene expression profiles in breast cancers. *BMC*
42
43 *cancer* 2008;**8**:277. doi:10.1186/1471-2407-8-277
44
45

46
47 26 Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion
48
49 biomarker for pleural mesothelioma. *The New England journal of*
50
51 *medicine* 2012;**367**:1417–27. doi:10.1056/NEJMoa1115050
52
53

54
55 27 Kirschner MB, Pulford E, Hoda MA, *et al.* Fibulin-3 levels in malignant
56
57 pleural mesothelioma are associated with prognosis but not diagnosis.
58
59
60

- 1
2
3 *British journal of cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286
4
5
6 28 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant
7 pleural mesothelioma in asbestos-exposed individuals with a noninvasive
8 proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091.
9 doi:10.1371/journal.pone.0046091
10
11
12
13
14
15
16 29 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic
17 technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004.
18 doi:10.1371/journal.pone.0015004
19
20
21
22
23
24 30 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-
25 lymphocyte ratio is an indicator of poor prognosis in malignant
26 mesothelioma patients undergoing systemic therapy. *Clin Cancer Res*
27 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245
28
29
30
31
32
33 31 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area
34 Mesothelioma and Pemetrexed trial: a multicentre prospective
35 observational study evaluating novel markers of chemotherapy response
36 and prognostication. *British journal of cancer* 2015;**112**:1175–82.
37 doi:10.1038/bjc.2015.62
38
39
40
41
42
43
44
45 32 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-based
46 prognostic indices in malignant pleural mesothelioma. *J Thorac Oncol*
47 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1
48
49
50
51
52
53 33 Burt BM, Rodig SJ, Tilleman TR, *et al.* Circulating and tumor-infiltrating
54 myeloid cells predict survival in human pleural mesothelioma. *Cancer*
55 2011;**117**:5234–44. doi:10.1002/cncr.26143
56
57
58
59
60

- 1
2
3 34 Zhang Y, Marmorstein LY. Focus on molecules: fibulin-3 (EFEMP1). *Exp*
4
5 *Eye Res* 2010;**90**:374–5. doi:10.1016/j.exer.2009.09.018
6
7
8
9 35 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the
10 diagnosis of malignant mesothelioma. *Egyptian Journal of Chest*
11 *Diseases and Tuberculosis* 2014;**63**:99–105.
12
13 doi:10.1016/j.ejcdt.2013.10.004
14
15
16
17
18 36 Corradi M, GOLDONI M, Alinovi R, *et al.* YKL-40 and mesothelin in the
19 blood of patients with malignant mesothelioma, lung cancer and
20 asbestosis. *Anticancer ...* 2013.
21
22
23
24
25
26 37 Creaney J, Francis RJ, Dick IM, *et al.* Serum soluble mesothelin
27 concentrations in malignant pleural mesothelioma: relationship to tumor
28 volume, clinical stage and changes in tumor burden. *Clin Cancer Res*
29
30 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929
31
32
33
34
35
36 38 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of
37 pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301.
38
39 doi:10.1093/jnci/djs513
40
41
42
43
44 39 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis:
45 dose- and time-response relationships based on HRCT data. *The*
46 *European respiratory journal* 2009;**34**:72–9.
47
48 doi:10.1183/09031936.00094008
49
50
51
52
53 40 Baldwin DR, Callister MEJ, Guideline Development Group. The British
54 Thoracic Society guidelines on the investigation and management of
55 pulmonary nodules. *Thorax* 2015;**70**:794–8. doi:10.1136/thoraxjnl-2015-

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

207221

41 Administration UFAD. *Guidance for industry, bioanalytical methods validation.*

<http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm> 2013.

For peer review only

CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

1
2
3 questions related to the accuracy or integrity of any part of the work
4
5 are appropriately investigated and resolved
6
7
8

9
10 Carol McCormick

- 11 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 12 interpretation of data for the work
- 13
- 14 ▪ Revising the work critically for important intellectual content
- 15
- 16 ▪ Final approval of the version to be published
- 17
- 18 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 19 questions related to the accuracy or integrity of any part of the work
- 20 are appropriately investigated and resolved
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28

29
30 Fiona Thomson

- 31 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 32 interpretation of data for the work
- 33
- 34 ▪ Revising the work critically for important intellectual content
- 35
- 36 ▪ Final approval of the version to be published
- 37
- 38 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 39 questions related to the accuracy or integrity of any part of the work
- 40 are appropriately investigated and resolved.
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48

49
50 Rosie Woodward

- 51 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 52 interpretation of data for the work
- 53
- 54 ▪ Revising the work critically for important intellectual content
- 55
- 56
- 57
- 58
- 59
- 60

- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

1
2
3 or interpretation of data for the work

- 4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
- Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

20 Nick A Maskell

- 21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
- Contribution to the design of the work; data analysis and interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

40 Anthony Chalmers

- 41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Contribution to the conception and design of the work; interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kevin G Blyth

- Principal contribution to the conception and design of the work; data acquisition, analysis and interpretation of data for the work
- Drafting the work
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

FUNDING

This work was supported by the Chief Scientist's Office of the Scottish Government (Project Grant ETM/285) and the West of Scotland Lung Cancer Research Group (Award September 2015). KGB is part-funded by NHS Research Scotland.

COMPETING INTERESTS STATEMENT

SomaLogic Inc. have provided funding for all SOMAscan assays.

FIGURE LEGENDS**Figure 1**

Summary of the design of the DIAPHRAGM study. Figure 1(a) relates to patients with Pleural Effusion and Figure 1(b) relates to patients with a Pleural Mass, but no significant fluid component.

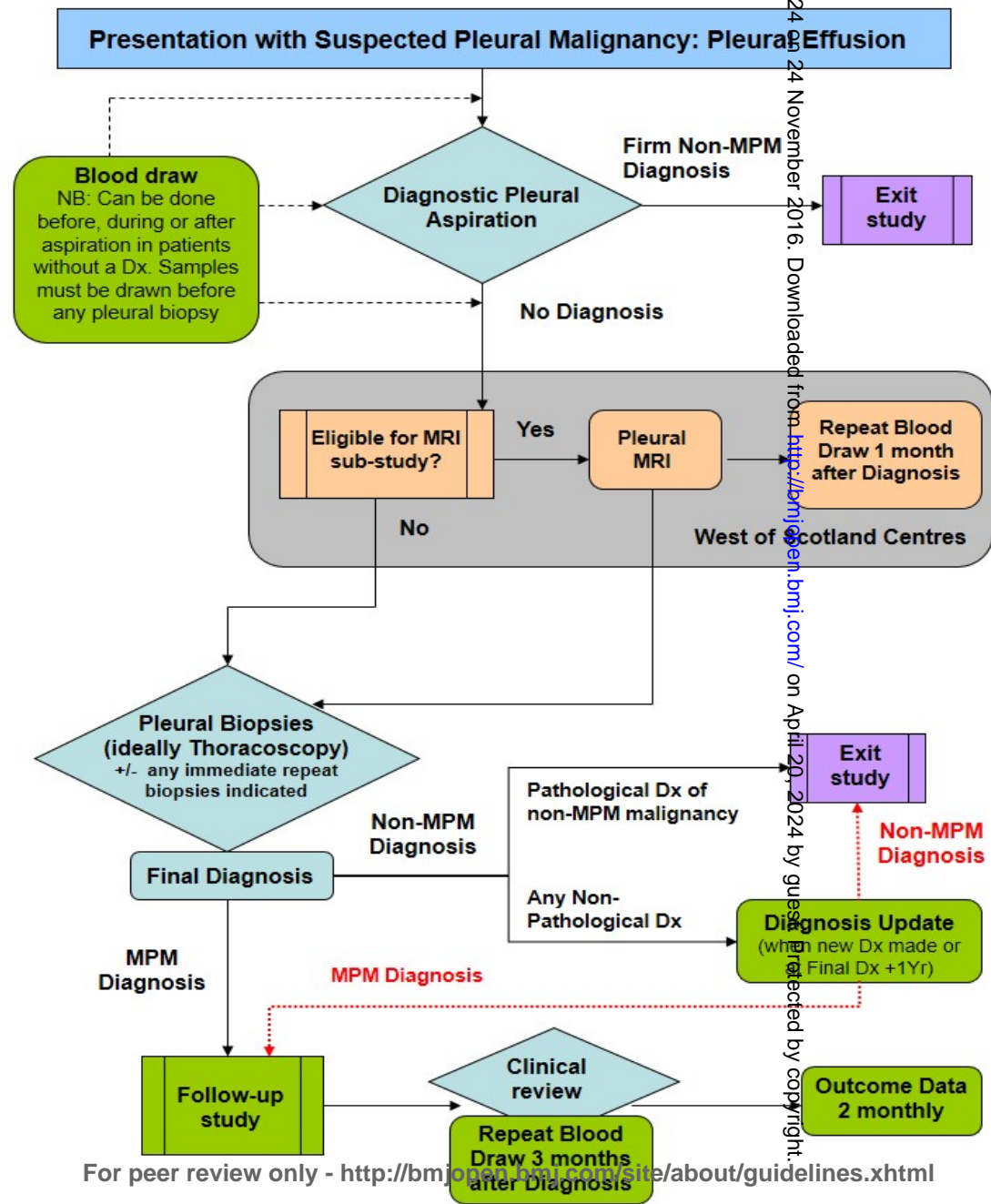
For peer review only

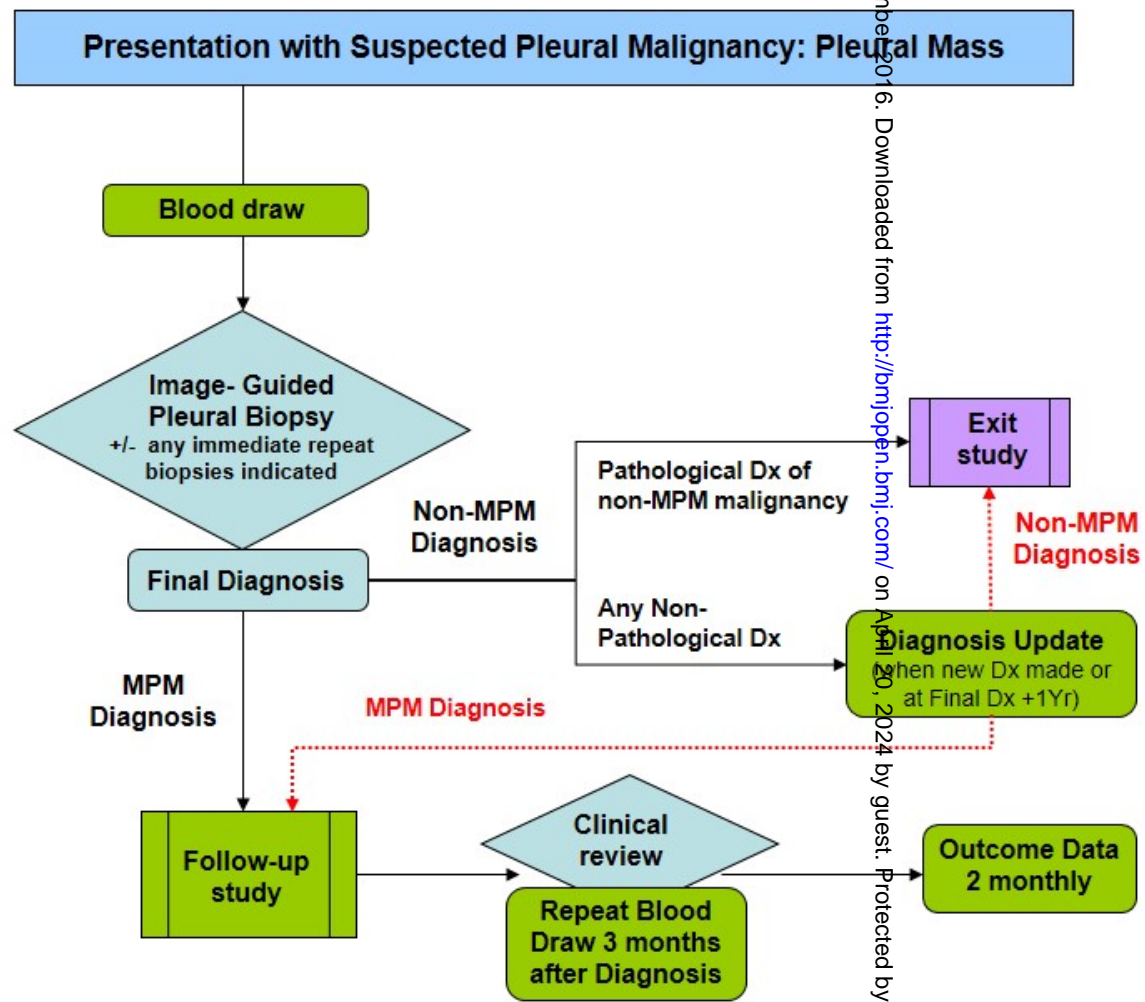
TABLES

Table 1. Outcome Measures used in the DIAPHRAGM study

Research Objective	Outcome Measures
Primary	
To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value	Serum SOMAscan Plasma Fibulin-3 Final diagnosis reached
Secondary	
To determine whether: 1. SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients 2. early changes in Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM	Serum SOMAscan & plasma Fibulin-3 at presentation Survival (from registration) Plasma Fibulin-3 3 months post-Dx Survival (from registration)
Exploratory	
To determine whether: 1. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI 2. there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) 3. there is a correlation between Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM 4. Fibulin-3 results are affected by pleural biopsy +/- fluid drainage and pleurodesis at the time of diagnosis	Serum SOMAscan Plasma Fibulin-3 MPM tumour volume at MRI, defined using Myrian Intrasure™ software Serum SOMAscan Plasma Fibulin-3 The following MRI biomarkers: • MRI-ECE • Redistribution rate constant (K_{ep}) • Elimination rate constant (K_{el}) Fibulin-3 in paired blood and pleural fluid samples Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis

013324 on 24 November 2016. Downloaded from <http://bmjopen.bmj.com/> on April 20, 2024 by guest. Protected by copyright.





013324 on 24 November 2016. Downloaded from <http://bmjopen.bmj.com/> on April 20, 2024 by guest. Protected by copyright.



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
√Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
√Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
√Protocol version	3	Date and version identifier
√Funding	4	Sources and types of financial, material, and other support
√Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
√Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
√Objectives	7	Specific objectives or hypotheses
√Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
- | | | |
|-----------------------|-----|--|
| √Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained |
| √Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) |
| √Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial |
| √Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended |
| √Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) |
| √Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations |
| √Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size |

Methods: Assignment of interventions (for controlled trials)

Allocation:

- 50
51
52
53
54
55
56
57
58
59
60
- | | | |
|------------------------|-----|--|
| NA Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions |
|------------------------|-----|--|

1			
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned
6			
7	NA	16c	Who will generate the allocation sequence, who will enrol participants,
8	Implementation		and who will assign participants to interventions
9			
10	NA Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12			how
13			
14		17b	If blinded, circumstances under which unblinding is permissible, and
15			procedure for revealing a participant's allocated intervention during
16			the trial
17			
18			

Methods: Data collection, management, and analysis

19			
20			
21	√Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
22	methods		trial data, including any related processes to promote data quality (eg,
23			duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with
25			their reliability and validity, if known. Reference to where data
26			collection forms can be found, if not in the protocol
27			
28			
29		18b	Plans to promote participant retention and complete follow-up,
30			including list of any outcome data to be collected for participants who
31			discontinue or deviate from intervention protocols
32			
33	√Data	19	Plans for data entry, coding, security, and storage, including any
34	management		related processes to promote data quality (eg, double data entry;
35			range checks for data values). Reference to where details of data
36			management procedures can be found, if not in the protocol
37			
38			
39	√Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
40	methods		Reference to where other details of the statistical analysis plan can be
41			found, if not in the protocol
42			
43		20b	Methods for any additional analyses (eg, subgroup and adjusted
44			analyses)
45			
46		20c	Definition of analysis population relating to protocol non-adherence
47			(eg, as randomised analysis), and any statistical methods to handle
48			missing data (eg, multiple imputation)
49			

Methods: Monitoring

50			
51			
52	√Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
53			and reporting structure; statement of whether it is independent from
54			the sponsor and competing interests; and reference to where further
55			details about its charter can be found, if not in the protocol.
56			Alternatively, an explanation of why a DMC is not needed
57			
58			
59			
60			

1		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
2			
3			
4			
5			
6	√Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
7			
8			
9			
10	√Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
11			
12			
13			
14			
15	Ethics and dissemination		
16			
17	√Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
18			
19			
20	√Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
21			
22			
23			
24			
25			
26	√Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
27			
28		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
29			
30			
31	√Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
32			
33			
34			
35			
36	√Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
37			
38			
39	√Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
40			
41			
42			
43	√Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
44			
45			
46	√Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
47			
48		31b	Authorship eligibility guidelines and any intended use of professional writers
49			
50		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-013324.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Sep-2016
Complete List of Authors:	Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Stobo, David; Queen Elizabeth University Hospital, Radiology Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Oncology, Patient-centred medicine, Research methods
Keywords:	Mesothelioma, Biomarker, Diagnosis, Prognosis

SCHOLARONE™
Manuscripts

TITLE:

DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

AUTHORS:

Selina Tsim^{1/2}, Caroline Kelly³, Laura Alexander³, Carol McCormick³, Fiona Thomson³, Rosie Woodward⁴, John E Foster⁴, David B Stobo⁵, Jim Paul³, Nick A Maskell⁶, Anthony Chalmers^{2/7}, Kevin G Blyth^{1/8}

Affiliations

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital, Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow, U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of Glasgow, U.K.

Corresponding Author and Address

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

Keywords:

Mesothelioma

Biomarker

Diagnosis

Prognosis

WORD COUNT:

3998/4000

ABSTRACT (300/300)

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer[®] proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

METHODS AND ANALYSIS

Serum and plasma (for SOMAscan[®] and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

1
2
3 marker and their performance will be compared to serum Mesothelin. Blood
4
5 levels will be compared to paired pleural fluid levels and MPM tumour volume
6
7 (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic
8
9 value of each marker will be assessed and a large bioresource created.
10
11

12 13 14 **ETHICS AND DISSEMINATION**

15
16 The study has been approved by the West of Scotland Research Ethics
17
18 Committee (Ref: 13/WS/0240). A Trial Management Group meets on a
19
20 monthly basis. Results will be published in peer-reviewed journals, presented
21
22 at international meetings and disseminated to patient groups.
23
24

25
26
27 **TRIAL REGISTRATION NUMBER:** ISRCTN10079972
28

29 30 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 31
32
- 33 • Prospective, multi-centre study recruiting a representative sample of
34 patients in an intention-to-diagnose population
35
 - 36 • Strict sampling, processing and storage methods used in all patients
37
 - 38 • Robust diagnostics and 12 months' follow-up
39
 - 40 • Creation of a large bio-resource annotated with detailed, prospectively
41 collected clinical information, for use in future biomarker discovery and
42 validation studies
43
 - 44 • The final number of study participants with MPM, and therefore the power
45 available to test the primary objective, will not be known until recruitment is
46 complete.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [6] with diagnostic yields for malignancy >90% [7] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

1
2
3 including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15,16]
4 and osteopontin [17], are higher in patients with MPM than in asbestos-
5 exposed controls (AECs) and patients with secondary pleural malignancies.
6
7 Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM
8
9 [18,19] is the most widely studied and is associated with an MPM sensitivity of
10
11 56-77% at 95% specificity [14,16,20]. However, a recent meta-analysis (of
12
13 4491 individuals (1026 with MPM)) reported a sensitivity of only 32% at 95%
14
15 specificity. Mesothelin does not, therefore, contribute to current diagnostic
16
17 algorithms [21]. MPF offers no advantage over mesothelin [16], while the
18
19 clinical utility of osteopontin is limited by stability and reproducibility concerns
20
21 [17].
22
23
24
25
26
27
28
29

30 An ideal MPM biomarker would be measurable in blood for ease of collection
31 and offer sufficient sensitivity at high specificity in patients presenting with
32 suspected MPM. Differentiation between advanced disease patients and
33 appropriate controls is of limited value. High specificity is mandatory for a low
34 prevalence disease, and should apply to patients with asbestos exposure and
35 non-MPM pleural disease. Biomarker results should also correlate with
36 disease extent and have defined relationships with potential confounders
37 including renal function [22] and the effect of pleural interventions. The latter
38 is important because the precedent has been established in prostate [23,24]
39 and breast cancer [25], that recent sampling, resection or peri-tumoural
40 inflammation may affect biomarker expression. This is particularly relevant to
41 MPM where biopsies are frequently large and often combined with
42 pleurodesis. Several previous biomarker studies, which validated
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 inconsistently in external populations, used samples acquired at later time-
4
5 points, often post-diagnosis (and post-pleurodesis) including samples taken
6
7 prior to, during, or after resection surgery [17,26,27]. The aim of the
8
9 DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic
10
11 performance of the SOMAscan proteomic classifier [28] and fibulin-3 [26],
12
13 which have demonstrated high sensitivity and specificity in recent
14
15 retrospective series. The study has been designed to generate clinically
16
17 meaningful results, which can be related to MPM biology and confounding
18
19 factors, and applied to patients at first presentation.
20
21
22
23
24

25 **SOMAmer-based Proteomic Classifier**

26
27
28 The SOMAscan assay is a highly multiplexed proteomic platform that utilizes
29
30 SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and
31
32 quantify proteins [29]. A 13-protein classifier was developed by SomaLogic
33
34 Inc. (Boulder, Colorado), using this novel proteomics-based biomarker
35
36 detection technique [28] in a retrospective study over 800 proteins were
37
38 measured in the serum of 117 MPM patients and 142 AECs, collected at
39
40 surgical MPM centres in the US between 1996 and 2011. Using a panel of 13
41
42 differentially expressed proteins and a cut-point of 0.5, the classifier was able
43
44 to segregate MPM from controls with an area under the curve (AUC) of 0.99
45
46 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification
47
48 (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38
49
50 cases/62 controls) [28]. The combined sensitivity for the three cohorts was
51
52 93% at 91% specificity. Based on the published ROC curve for the validation
53
54 cohort, sensitivity at 95% specificity appeared to be approximately 78%,
55
56
57
58
59
60

1
2
3 although the authors did not report this value. This performance exceeds that
4
5 of any previous MPM biomarker, although the classifier's specificity appeared
6
7 lower in patients with non-MPM pleural effusion (n=32). There was a modest
8
9 correlation between classifier score and disease stage, but prognostic
10
11 significance was not assessed. The 13 classifier proteins (nine up-regulated,
12
13 four down-regulated) have not previously been associated with MPM. Their
14
15 functions fall into two broad groups; regulation of proliferation and
16
17 inflammation. Quite apart from their biological relevance to MPM, the latter is
18
19 an important potential confounder because many of the patients involved will
20
21 have previously undergone pleurodesis. In addition, several groups have
22
23 reported an independent interaction between prognosis and inflammatory
24
25 biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [30-32],
26
27 monocytosis [33] and the modified Glasgow Prognostic Score [32].
28
29 Therefore, adequate understanding of the diagnostic and prognostic utility of
30
31 this assay requires replication in a pre-pleurodesis cohort and prospective
32
33 evaluation of interactions between inflammatory biomarkers and SOMAscan
34
35 scores.
36
37
38
39
40
41
42

43 **Fibulin-3**

44
45 Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-
46
47 containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [34].
48
49 Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura
50
51 [26] and expressed and secreted by MPM cell lines [27]. Pass et al
52
53 retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136
54
55 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [26].
56
57
58
59
60

1
2
3 A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and
4 a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases.
5
6
7 However, in a blinded external validation set, sensitivity was below 40% (at
8
9
10 95% specificity), with an AUC=0.87.

11
12
13
14 Subsequent studies have revealed mixed results. In a study of 153 patients
15
16 (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity)
17
18 at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which
19
20 was significantly inferior to mesothelin measured in the same patients
21
22 (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM
23
24 threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay
25
26 and internally-defined cut-points, Agha et al reported 100% sensitivity/78%
27
28 specificity in differentiating MPM cases (n=25) from non-malignant pleural
29
30 disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from
31
32 secondary pleural malignancies (n=11) [35]. No combined sensitivity was
33
34 reported. An Italian study found no difference in Fibulin-3 levels but used
35
36 serum (not plasma), a control group without pleural disease (Asbestosis) and
37
38 contained only 14 patients with MPM [36].
39
40
41
42
43
44

45 **METHODS AND ANALYSIS**

46 **Study Design**

47
48 DIAPHRAGM is a prospective, multi-centre observational study. The study
49
50 incorporates sampling windows that correspond to the proposed use of a
51
52 diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy
53
54 (SPM). The overall study design is summarized in Figure 1(a) and 1(b). The
55
56
57
58
59
60

1
2
3 main impact of this design is that biomarkers will be drawn before a diagnosis
4 is made. In addition to better replicating the future use of these markers, this
5 avoids the potential confounding effect of pleurodesis on biomarker results.
6
7 The diagnostic performance of the SOMAmer panel and Fibulin-3 will be
8 assessed using cut-points determined in the relevant original studies and
9 compared to mesothelin (using the MESOMARK® ELISA (Fujirebio
10 Diagnostics Inc, PA, USA). Identical processing and storage protocols will be
11 used in patients with SPM and a group of AECs. Potential confounders
12 including renal function, inflammatory indices and drugs will be recorded at all
13 visits. The timing of the biomarker blood draw in relation to pleural aspiration
14 (pre-aspiration or post-aspiration) will be recorded in order to assess the
15 effect of this intervention on biomarker results. An exploratory, cross-sectional
16 Magnetic Resonance Imaging (MRI) sub-study will determine if there is any
17 correlation between blood biomarker levels and MPM tumour volume, as has
18 been established for Mesothelin using Computed Tomography-Positron
19 Emission Tomography scanning [37].
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 **Study Objectives and Outcome Measures**

42 These are presented in Table 1.
43
44
45
46

47 **Setting**

48 At least 600 consecutive patients with SPM will be recruited from 21 centres
49 (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and
50 more clinically orientated units. This should make the results of the
51 DIAPHRAGM study generalizable to patients presenting with SPM to acute
52
53
54
55
56
57
58
59
60

1
2
3 hospital services. The principal criterion used to select centres was that they
4 had sufficiently evolved pleural diagnostic services to deliver a reliable
5 diagnosis. Specifically, access to on-site thoracoscopy (ideally including local
6 anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting
7 (for diagnostic review and staging) was required.
8
9
10
11
12

13 14 15 16 **Screening and Eligibility Assessment**

17 18 **Suspected Pleural Malignancy**

19
20 Cases will be identified on presentation to a Respiratory out-patient clinic or
21 acute hospital admissions unit. This will be based on the history, examination
22 and available investigations. Potentially eligible patients will be provided with
23 the study Patient Information Sheet (PIS, see Online Supplementary
24 Appendix 1) and eligibility assessed based on the following criteria:
25
26
27
28
29
30
31
32
33

34 *Inclusion Criteria:*

- 35 • SPM, defined by a unilateral pleural effusion or pleural mass lesion
- 36 • Sufficient fitness for diagnostic sampling (site investigator's clinical
- 37 judgment)
- 38 • Informed written consent
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46

47 *Exclusion Criteria:*

- 48 • Intercostal chest drain in-situ, or inserted within the previous 3 months
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

Asbestos-related pleural plaques are not an inclusion criterion since these are
absent in up to 25% of MPM cases [38], and are also common in asbestos-

1
2
3 exposed populations without MPM [39]. Patients with lung nodules or other
4 visceral mass lesions are not excluded, assuming the investigator suspects
5 pleural malignancy. This is because of the high prevalence of lung nodules in
6 the target population (older patients, commonly smokers) and the high false
7 positive rate of CT imaging in this regard [40].
8
9

10
11
12
13
14
15
16 Subjects recruited to the SPM arm will generate cohorts of MPM and non-
17 MPM pleural disease of various aetiologies, likely including Benign Asbestos-
18 related Pleural Effusion and secondary pleural malignancies. These numbers
19 will be sufficient to address the primary objective with sufficient statistical
20 power to inform clinical practice (see later section).
21
22
23
24
25
26
27

28 29 30 Asbestos-exposed control (AEC) subjects

31
32 109 AECs will be recruited via invitations sent by Clydeside Action on
33 Asbestos (CAA), an advocacy body based in Glasgow with a database of over
34 600 clients, or by Respiratory clinics at the host centre. Individuals will be
35 invited to participate by letter (if identified via CAA) or given the PIS (see
36 Online Supplementary Appendix 2) at clinic. All subjects will be invited to a
37 single research clinic visit assuming the following Eligibility Criteria are met.
38
39
40
41
42
43
44
45
46

47 48 *Inclusion Criteria:*

- 49 • Documented history of asbestos exposure and associated pleural
50 plaques, asbestosis or diffuse pleural thickening
51
- 52 • Willing and able to travel to a research clinic interview in Glasgow
53
- 54 • Informed written consent
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Exclusion Criteria:

- Known MPM
- Known or suspected other thoracic malignancy under investigation
- Known pleural effusion of any cause

Detailed asbestos exposure histories will be taken from all participants in both the suspected pleural malignancy cohort and the asbestos-exposed control cohort. This will be done using an asbestos exposure questionnaire derived from Health and Safety Executive asbestos survey [27] (see Online Supplementary Appendix 3). This questionnaire includes recording of the nature of occupational exposure(s), which can be correlated to likely fibre exposure. The duration and first year of exposure is also recorded. Non-occupational sources of exposure are also recorded (e.g. the washing of an occupationally exposed spouse's work clothes). Only AECs with documented imaging sequelae of asbestos exposure (e.g. pleural plaques) and an asbestos exposure history will be included.

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

Inclusion Criteria:

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

Exclusion Criteria:

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 4) and will be asked to provide additional informed written consent.

Consent

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

Outcome Measures

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

Final Diagnosis

A specific cytological or histological pleural diagnosis will be sought in all patients according to national guidelines [21]. This will be recorded as the

1
2
3 Final Diagnosis, which may be based on immediate repeat biopsies felt to be
4 indicated by the site PI (see Figure 1). Any cytologically or histologically
5 confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will
6 be recorded without the need for any further updates. However, sites will need
7 to provide updates for any non-MPM diagnosis that is not cytologically or
8 histologically confirmed (e.g. parapneumonic effusion). These will be
9 submitted on the 12-month anniversary of the original diagnosis, or as soon
10 as any new pleural diagnosis is made. This aims to capture any false
11 negative diagnostic tests from the initial presentation, acknowledging the
12 major diagnostic challenges posed by pleural malignancies, particularly MPM.
13
14
15
16
17
18
19
20
21
22
23
24
25
26

27 *Biomarker Sampling and Storage*

28
29 Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate
30 processing performed at each study centre. Samples can be taken before or
31 after pleural aspiration. Patients with positive pleural cytology cannot be
32 recruited (see Figure 1(a)). Duplicate samples will be collected for all
33 measurements at all visits, ensuring redundancy in case of loss or damage to
34 samples during transportation to the appropriate central laboratory.
35
36 SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of
37 venous blood will be collected first into a vacutainer tube containing SST clot
38 activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of
39 venous blood will be collected second into a vacutainer tube containing
40 EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties
41 only) 20 ml of pleural fluid will be also collected into a plain container if pleural
42 fluid is being drawn for diagnostic/therapeutic purposes at the same visit. If
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 not done at this first opportunity, pre-diagnosis pleural fluid can also be
4 collected during local anaesthetic or general anaesthetic thoracoscopy, prior
5 to any biopsy or pleurodesis being performed.
6
7
8
9

10 11 *Biomarker Processing and Storage*

12 Serum samples will be allowed to clot for 30 minutes before centrifugation.
13 Plasma and pleural fluid samples will be centrifuged immediately. All samples
14 will be centrifuged at 2200g for 15 minutes at room temperature. For all
15 samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials
16 of at least 250µL volume, labeled and placed into a -80 freezer within 2 hours.
17 Samples will be stored at each recruiting centre until batched transport to the
18 appropriate study laboratory. Samples from WoS recruiting centres will be
19 used to create a bioresource. The bioresource will be stored as a satellite
20 collection of the NHS Greater Glasgow & Clyde Biorepository, a Health
21 Improvement Scotland (HIS)-approved tissue bank. Data will be stored in the
22 secure Cancer Research UK Clinical Trials Unit database. On study
23 completion, investigators will be invited to apply for access to data and
24 samples appropriate to their research questions. Access will be granted after
25 peer review of each proposal by a scientific board comprising members of the
26 DIAPHRAGM TMG and senior Biorepository staff. An annual update on this
27 activity will be submitted to the West of Scotland Research Ethics Committee.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 51 *Biomarker Analyses*

52 SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan
53 proteomic analyses [28]. This utilises SOMAmer reagents to specifically bind
54
55
56
57
58
59
60

1
2
3 to protein targets in blood. Relative protein concentrations will be converted to
4
5 measurable nucleic acid signals that are quantified by hybridization to DNA
6
7 microarrays [29].
8
9

10
11 Fibulin-3 and mesothelin levels will be measured by the Translational
12
13 Pharmacology Unit, Wolfson Wohl Cancer Research Centre, UK, using ELISA
14
15 methods validated according to the FDA-recommended guidelines for
16
17 bioanalytical methods [41]. Fibulin-3 levels in plasma and pleural fluid will be
18
19 measured using the commercially available ELISA (Cloud-Clone Corp.,
20
21 formerly USCN Life Science Inc, Houston, Texas, USA) as in the original
22
23 Pass study [26]. Mesothelin will be measured using the MESOMARK® ELISA
24
25 (Fujirebio Diagnostics, Inc, PA, USA). In parallel, we aim to develop a custom
26
27 multiplex ELISA assay that has the potential to simultaneously measure
28
29 multiple biomarkers (fibulin-3, mesothelin and osteopontin) with greater
30
31 accuracy (U-PLEX, Meso Scale Diagnostics, Rockville, USA)
32
33
34
35
36
37
38
39

40 *Magnetic Resonance Imaging*

41 Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow,
42
43 on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected
44
45 thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using
46
47 VIBE sequences. A stack of axial slices covering the entire lung and
48
49 surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA
50
51 contrast (Gadovist) will be administered via a peripheral intravenous line as a
52
53 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied
54
55 slice positions to provide pre and post-contrast images. The total scan time
56
57
58
59
60

1
2
3 will be around 45 minutes. Regions of enhancing pleural tumour will be
4 defined using semi-automated signal intensity thresholding based on contrast-
5 enhanced axial slices using Myrian Intrasure™ software, which has
6 previously been used to assess tumour volume in MPM. [42] MRI volumetry
7 analyses will be validated using imaging phantoms.
8
9

16 *Survival*

17 Survival will be recorded in days from the date of study registration to the data
18 of death, from any cause.
19
20
21
22
23

24 **Sample Size, Assumptions and Uncertainties**

25 Sample size estimations for each marker were based on published data at the
26 point of study design and a projected MPM incidence of 13-20% in the SPM
27 cohort. The power available to test the hypotheses below is therefore reported
28 as a range, based on final MPM numbers lying between 83 (13% incidence)
29 and 120 (20% incidence).
30
31
32
33
34
35
36
37
38
39

40 *Primary Objective*

41 *SOMAscan Assay*

42 We hypothesize that the MPM sensitivity and specificity exceed 90%, based
43 on previously reported performance in combined training, verification and
44 validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–
45 95.6%) [28]). Recruitment of 83-120 MPM patients will allow us to distinguish
46 a sensitivity of >90% from a sensitivity <80% with 80-93% power,
47 respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 allow discrimination between a specificity <80% and a specificity >90%, with
4
5 80-88% power at the 5% 1-sided level of statistical significance. The standard
6
7 error in the estimated sensitivity and specificity will be less than 5%, across all
8
9 possible outcomes.
10

11 12 13 *Fibulin-3*

14
15 We hypothesize that the MPM sensitivity will exceed 80% and that the
16
17 specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based
18
19 on a reduced level of performance to the primary results reported by Pass et
20
21 al (97% sensitivity, 95% specificity), given lower sensitivity in the external
22
23 validation cohort studied (40% at 95% specificity) [26].
24
25
26
27
28

29
30 With 83-120 MPM patients the study will be able to distinguish a sensitivity of
31
32 >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-
33
34 sided level of statistical significance. The standard error in the estimated
35
36 sensitivity will be less than 5%. In order to achieve 90% power to distinguish
37
38 a specificity of >90% from a specificity <85% at the 5% 1-sided level of
39
40 statistical significance, a random sample of 378 non-MPM samples will be
41
42 analysed. The standard error in the estimated specificity will be <2.3%.
43
44
45
46
47

48
49 The study data will be used to estimate the AUC for the SOMAscan marker
50
51 for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming
52
53 83-120 patients in the MPM group and 83-120 in the non-MPM group the
54
55 AUC can be estimated with a 95% confidence interval of width 0.120-0.168
56
57 (assuming a cut-point exists with a reasonable sensitivity of 80% and a
58
59
60

1
2
3 modest specificity of 40%). If more sensitive/specific cut-points exist the width
4 of the 95% confidence interval will be much reduced. The study data will be
5 used to develop a new diagnostic signature based on Fibulin-3 and
6 SOMAscan results to distinguish MPM from non-MPM effusions.
7
8
9
10

11 12 13 14 Secondary Objectives

15
16 The study data will be used to determine whether baseline SOMAscan results
17 and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are
18 independent prognostic factors for MPM. A correlation of 0.4 between existing
19 prognostic factors and each marker has been assumed. For the baseline
20 levels, to detect an approximate doubling in median OS (from 6 month to 12
21 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of
22 statistical significance between a good/poor prognostic group based on
23 dichotomising these markers requires at least 83 MPM patients recruited over
24 three years with approximately 6 months subsequent follow-up to observe 66
25 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be
26 detected (80% power, 5% 2-sided level of statistical significance) when 49
27 deaths are observed in the estimated 66 out of 83 patients who survive to 3
28 months.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 Exploratory Objectives

47
48 These will be addressed in the MRI sub-study, which will generate a sample
49 of at least 20 MPM patients. This will allow moderately large associations
50 (0.6) between the exploratory outcome measures (see Table 1) to be detected
51 at 80% power at the 5%, two-sided level of statistical significance. The effect
52
53
54
55
56
57
58
59
60

1
2
3 of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be
4
5 assessed using all 50 patients recruited. This will allow moderately small
6
7 differences (standardised difference of 0.4) to be detected with 80% power at
8
9 the 5% two-sided level of statistical significance.
10

11 12 13 14 **Statistical Analysis Plan**

15 16 **Primary Analysis**

17
18 Sensitivity and specificity at pre-specified cut-offs will be estimated using
19
20 standard approaches for proportions. The diagnostic performance of each
21
22 biomarker will be assessed using ROC curves. All patients with MPM (n=83-
23
24 120) will be included and compared with AECs and a random sample of non-
25
26 MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs
27
28 and 83 non-MPM cases will be randomly selected. All AECs and 378 non-
29
30 MPM cases will be used for Fibullin-3 analyses. Logistic regression will be
31
32 used to estimate a diagnostic model using biomarker results. Cross validation
33
34 will be used to provide robust estimates of AUC and specificity at fixed
35
36 sensitivity rates of 80%, 90% and 95%.
37
38
39
40

41 42 **Secondary Analysis**

43
44
45 A prognostic model will be developed using Cox proportional hazard
46
47 techniques. The modelling process will incorporate biomarker measurements
48
49 (at presentation (both markers) and at 3 months (Fibulin-3 only) and other
50
51 known prognostic features (e.g. performance status, histology).
52
53
54

55 56 **Exploratory Analysis**

57
58
59
60

1
2
3 The association between SOMAscan results/fibulin-3 in blood and tumour
4 volume/measures of tumour angiogenesis will be estimated by Pearson or
5 Spearman correlation, depending on the normality of the data. The same
6 methods will be used to test the association between fibulin-3 in blood and
7 pleural fluid. Changes in Fibulin-3 levels before and after histological sampling
8 (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon
9 signed rank sum test (depending on the normality of the data). Due to cost
10 constraints, exploratory end-points involving pleural fluid SOMAscan results
11 will be analysed at a later date.
12
13
14
15
16
17
18
19
20
21
22
23
24

25 **Changes to the Study Protocol since Trial Opening**

26 The protocol described accurately reflects Version 5, of the protocol, dated
27 17/6/16. The following changes were made in previous versions:
28
29

- 30
- 31 • Version 2, dated 14/2/14:
 - 32 ▪ Safety reporting reduced following risk assessment by study Sponsor.
 - 33 ▪ Collection of duplicate blood samples as provision for loss or damage
 - 34 and for sample retention in tissue bank.
 - 35 ▪ Greater flexibility to timing of first blood draw.
 - 36 • Version 3, dated 17/10/14:
 - 37 ▪ Addition of recruitment of Controls from Respiratory Medicine clinics
 - 38 ▪ Addition of exclusion criteria for patients with chest drains in-situ.
 - 39 ▪ Eligibility for the MRI sub-study extended to patients proceeding to
 - 40 image-guided pleural biopsy
 - 41 • Version 4, dated 27/4/15:
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60

- Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.
- Version 5, dated 17/6/16:
 - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

Definition of End of Study

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

ETHICS AND DISSEMINATION

Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking incoming forms for compliance with the protocol, data consistency, missing data and timing. The CRUK Glasgow CTU will control data consistency and data quality by entering trial data onto CTU database. Computerised and manual consistency checks will be performed and queries issued in cases of

1
2
3 inconsistency or missing information. An audit trail of changes to the database
4
5 will be maintained.
6
7
8

9 10 **Safety Considerations**

11 Participants in the MRI sub-study will be asked at their 1-month follow-up visit
12
13 about the occurrence of Adverse Events (AEs) related to the administration of
14
15 MRI contrast (Gadolinium). These will be followed until resolution.
16
17
18

19 20 **Dissemination**

21 The results of the study will be presented at national and international
22
23 scientific meetings and published in full in a peer-reviewed journal (authorship
24
25 will be according to that journal's guidelines). A lay summary will be produced
26
27 and disseminated to interested parties.
28
29
30
31
32
33

34 35 **Trial Management**

36 The trial will be coordinated from CRUK Glasgow CTU by the Trial
37
38 Management Group (TMG), including the Chief Investigator, selected co-
39
40 investigators, project manager, trial statistician, clinical trial co-ordinator and
41
42 IT staff. The TMG will oversee the running of the trial and meet monthly.
43
44
45
46
47

48 49 **Acknowledgements**

50 The authors are grateful to the Chief Scientist Office (Scotland) as the study
51
52 funder, cancer research networks infrastructure, the participating sites and
53
54 staff, SomaLogic Inc and the patients involved. ST is part funded by the West
55
56 of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Career Research Fellowship. CR-UK and ECMC are acknowledged by FT and CMcC.

For peer review only

REFERENCES

- 1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *Eur Respir J* 2011;**38**:1420–4.
doi:10.1183/09031936.00000811
- 2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;**88**:344–8.
doi:10.1016/j.lungcan.2015.03.005
- 3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung Cancer* 2014;**Supplement 1**:S28–9. doi:10.1016/S0169-5002(14)70076-5
- 4 The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. 1997;**111**:106–
9.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8996002&retmode=ref&cmd=prlinks>
- 5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *Eur. Respir. J.* 2010;**35**:479–95. doi:10.1183/09031936.00063109
- 6 Rusch VW, Giroux D. Do we need a revised staging system for malignant pleural mesothelioma? Analysis of the IASLC database. *Ann Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-319X.2012.11.10

- 1
2
3 7 Thoracoscopy in pleural malignant mesothelioma: a prospective study of
4 188 consecutive patients. Part 1: Diagnosis. 1993;**72**:389–
5
6 93.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8319170&retmode=ref&cmd=prlinks>
7
8
9
10
11
12
13 8 Efficacy of pleural needle biopsy and pleural fluid cytopathology in the
14 diagnosis of malignant neoplasm involving the pleura. 1975;**67**:536–
15
16 9.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=1126189&retmode=ref&cmd=prlinks>
17
18
19
20
21
22
23 9 Comparison of needle biopsy with cytologic analysis for the evaluation of
24 pleural effusion: analysis of 414 cases. 1985;**60**:158–
25
26 64.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=3974296&retmode=ref&cmd=prlinks>
27
28
29
30
31
32
33 10 Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid
34 examination. 1991;**4**:320–
35
36 4.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=2068057&retmode=ref&cmd=prlinks>
37
38
39
40
41
42
43 11 A randomised controlled trial of intervention site radiotherapy in malignant
44 pleural mesothelioma. 2007;**84**:18–22. doi:10.1016/j.radonc.2007.05.022
45
46
47
48 12 Prevention of malignant seeding after invasive diagnostic procedures in
49 patients with pleural mesothelioma. A randomized trial of local
50 radiotherapy. 1995;**108**:754–
51
52 8.<http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7656629&retmode=ref&cmd=prlinks>
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 13 Robinson BW, Creaney J, Lake R, *et al.* Mesothelin-family proteins and diagnosis of mesothelioma. *The Lancet* 2003;**362**:1612–6.
doi:10.1016/S0140-6736(03)14794-0
- 14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–902. doi:10.1136/thoraxjnl-2014-205205
- 15 Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. 2006;**12**:4225–31. doi:10.1158/1078-0432.CCR-06-0472
- 16 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. *J Thorac Oncol* 2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b
- 17 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med* 2005;**353**:1564–73. doi:10.1056/NEJMoa051185
- 18 Chang K, Pai LH, Batra JK, *et al.* Characterization of the antigen (CAK1) recognized by monoclonal antibody K1 present on ovarian cancers and normal mesothelium. *Cancer Res* 1992;**52**:181–6.
- 19 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg Pathol* 1992;**16**:259–68.

- 1
2
3 20 Hollevoet K, Nackaerts K, Thimpont J, *et al.* Diagnostic performance of
4 soluble mesothelin and megakaryocyte potentiating factor in
5 mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5.
6
7 doi:10.1164/rccm.200907-1020OC
8
9
10
11
12
13 21 Hooper C, Lee YCG, Maskell N, *et al.* Investigation of a unilateral pleural
14 effusion in adults: British Thoracic Society Pleural Disease Guideline
15 2010. *Thorax*. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978
16
17
18
19
20
21 22 The effect of clinical covariates on the diagnostic and prognostic value of
22 soluble mesothelin and megakaryocyte potentiating factor.
23
24 2012;**141**:477–84. doi:10.1378/chest.11-0129
25
26
27
28 23 Inflammation: an important parameter in the search of prostate cancer
29 biomarkers. 2014;**12**:32. doi:10.1186/1477-5956-12-32
30
31
32
33 24 Effects of rectal examination, prostatic massage, ultrasonography and
34 needle biopsy on serum prostate specific antigen levels. 1992;**147**:810–4.
35
36
37
38 25 The effects of timing of fine needle aspiration biopsies on gene
39 expression profiles in breast cancers. 2008;**8**:277. doi:10.1186/1471-
40 2407-8-277
41
42
43
44
45
46 26 Pass HI, Levin SM, Harbut MR, *et al.* Fibulin-3 as a blood and effusion
47 biomarker for pleural mesothelioma. *N Engl J Med* 2012;**367**:1417–27.
48
49 doi:10.1056/NEJMoa1115050
50
51
52
53
54 27 Kirschner MB, Pulford E, Hoda MA, *et al.* Fibulin-3 levels in malignant
55 pleural mesothelioma are associated with prognosis but not diagnosis. *Br*
56
57
58
59
60

- 1
2
3 *J Cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286
- 4
5
6 28 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant
7
8 pleural mesothelioma in asbestos-exposed individuals with a noninvasive
9
10 proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091.
11
12 doi:10.1371/journal.pone.0046091
- 13
14
15 29 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic
16
17 technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004.
18
19 doi:10.1371/journal.pone.0015004
- 20
21
22 30 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-
23
24 lymphocyte ratio is an indicator of poor prognosis in malignant
25
26 mesothelioma patients undergoing systemic therapy. *Clin Cancer Res*
27
28 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245
- 29
30
31 31 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area
32
33 Mesothelioma and Pemetrexed trial: a multicentre prospective
34
35 observational study evaluating novel markers of chemotherapy response
36
37 and prognostication. *Br J Cancer* 2015;**112**:1175–82.
38
39 doi:10.1038/bjc.2015.62
- 40
41
42 32 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-Based
43
44 Prognostic Indices in Malignant Pleural Mesothelioma. *Journal of*
45
46 *Thoracic Oncology* 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1
- 47
48
49 33 Circulating and tumor-infiltrating myeloid cells predict survival in human
50
51 pleural mesothelioma. 2011;**117**:5234–44. doi:10.1002/cncr.26143
52
53
54
55
56
57
58
59
60

- 1
2
3 34 Zhang Y, Marmorstein LY. Focus on molecules: Fibulin-3 (EFEMP1).
4
5 *Experimental Eye Research* 2010;**90**:374–5.
6
7 doi:10.1016/j.exer.2009.09.018
8
9
10
11 35 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the
12 diagnosis of malignant mesothelioma. *Egyptian Journal of Chest*
13 *Diseases and Tuberculosis* 2014;**63**:99–105.
14
15 doi:10.1016/j.ejcdt.2013.10.004
16
17
18
19
20
21 36 Corradi M, Goldoni M, Alinovi R, *et al.* YKL-40 and mesothelin in the
22 blood of patients with malignant mesothelioma, lung cancer and
23 asbestosis. *Anticancer Res* 2013;**33**:5517–24.
24
25
26
27
28 37 Creaney J, Francis RJ, Dick IM, *et al.* Serum Soluble Mesothelin
29 Concentrations in Malignant Pleural Mesothelioma: Relationship to
30 Tumor Volume, Clinical Stage and Changes in Tumor Burden. *Clin*
31 *Cancer Res* 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929
32
33
34
35
36
37
38 38 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of
39 pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301.
40
41 doi:10.1093/jnci/djs513
42
43
44
45
46 39 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis:
47 dose- and time-response relationships based on HRCT data. *Eur Respir*
48 *J* 2009;**34**:72–9. doi:10.1183/09031936.00094008
49
50
51
52
53 40 The British Thoracic Society guidelines on the investigation and
54 management of pulmonary nodules. 2015;**70**:794–8.
55
56 doi:10.1136/thoraxjnl-2015-207221
57
58
59
60

- 1
2
3 41 *Guidance for industry, bioanalytical methods validation.*
4
5 [http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Gu](http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm)
6
7 [idances/default.htm](http://ci.nii.ac.jp/naid/10016982382/) 2013. <http://ci.nii.ac.jp/naid/10016982382/>
8
9
10
11 42 Frauenfelder T, Tutic M, Weder W, *et al.* Volumetry: an alternative to
12 assess therapy response for malignant pleural mesothelioma? *Eur Respir*
13 *J* 2011;**38**:162–8. doi:10.1183/09031936.00146110
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

1
2
3 questions related to the accuracy or integrity of any part of the work
4
5 are appropriately investigated and resolved
6
7
8

9
10 Carol McCormick

- 11 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 12 interpretation of data for the work
- 13
- 14 ▪ Revising the work critically for important intellectual content
- 15
- 16 ▪ Final approval of the version to be published
- 17
- 18 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 19 questions related to the accuracy or integrity of any part of the work
- 20 are appropriately investigated and resolved
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29

30 Fiona Thomson

- 31 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 32 interpretation of data for the work
- 33
- 34 ▪ Revising the work critically for important intellectual content
- 35
- 36 ▪ Final approval of the version to be published
- 37
- 38 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 39 questions related to the accuracy or integrity of any part of the work
- 40 are appropriately investigated and resolved.
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49

50 Rosie Woodward

- 51 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 52 interpretation of data for the work
- 53
- 54 ▪ Revising the work critically for important intellectual content
- 55
- 56
- 57
- 58
- 59
- 60

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
- Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

1
2
3 or interpretation of data for the work

- 4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
- Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

20 Nick A Maskell

- 21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
- Contribution to the design of the work; data analysis and interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

40 Anthony Chalmers

- 41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Contribution to the conception and design of the work; interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

1
2
3 Kevin G Blyth
4

- 5 ▪ Principal contribution to the conception and design of the work; data
6 acquisition, analysis and interpretation of data for the work
7
- 8 ▪ Drafting the work
9
- 10 ▪ Final approval of the version to be published
11
- 12 ▪ Agrees to be accountable for all aspects of the work in ensuring that
13 questions related to the accuracy or integrity of any part of the work
14 are appropriately investigated and resolved
15
16
17
18
19

20 21 22 23 **FUNDING**

24 This work was supported by the Chief Scientist's Office of the Scottish
25 Government (Project Grant ETM/285) and the West of Scotland Lung Cancer
26 Research Group (Award September 2015). KGB is part-funded by NHS
27 Research Scotland.
28
29
30
31
32
33

34 35 36 **COMPETING INTERESTS STATEMENT**

37 SomaLogic Inc. have provided funding for all SOMAscan assays.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FIGURE LEGENDS

Figure 1

Summary of the design of the DIAPHRAGM study. Figure 1(a) is intended to describe the optimal diagnostic pathway for the majority of patients who present with significant Pleural Effusion +/- pleural thickening or a pleural mass. Figure 1(b) describes the optimal diagnostic pathway for the minority of patients who present with an isolated Pleural Mass, but no significant fluid component. The pathway chosen is ultimately at the discretion of the investigating physician.

TABLES

Table 1. Outcome Measures used in the DIAPHRAGM study

Research Objective	Outcome Measures
<p>Primary To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value</p>	<p>Serum SOMAscan Plasma Fibulin-3 Final diagnosis reached</p>
<p>Secondary To determine whether:</p> <ol style="list-style-type: none"> SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients early changes in SOMAscan and/or Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM 	<p>Serum SOMAscan & plasma Fibulin-3 at presentation Survival (from registration)</p> <p>Serum SOMAscan & plasma Fibulin-3 3 months post-Dx Survival (from registration)</p>
<p>Exploratory To determine whether:</p> <ol style="list-style-type: none"> there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM 	<p>Serum SOMAscan Plasma Fibulin-3 MPM tumour volume at MRI, defined using Myrian intrasense™ software</p> <p>Serum SOMAscan Plasma Fibulin-3 The following MRI biomarkers:</p> <ul style="list-style-type: none"> MRI-ECE Redistribution rate constant (K_{ep}) Elimination rate constant (K_{el}) <p>SOMAscan and Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis</p>

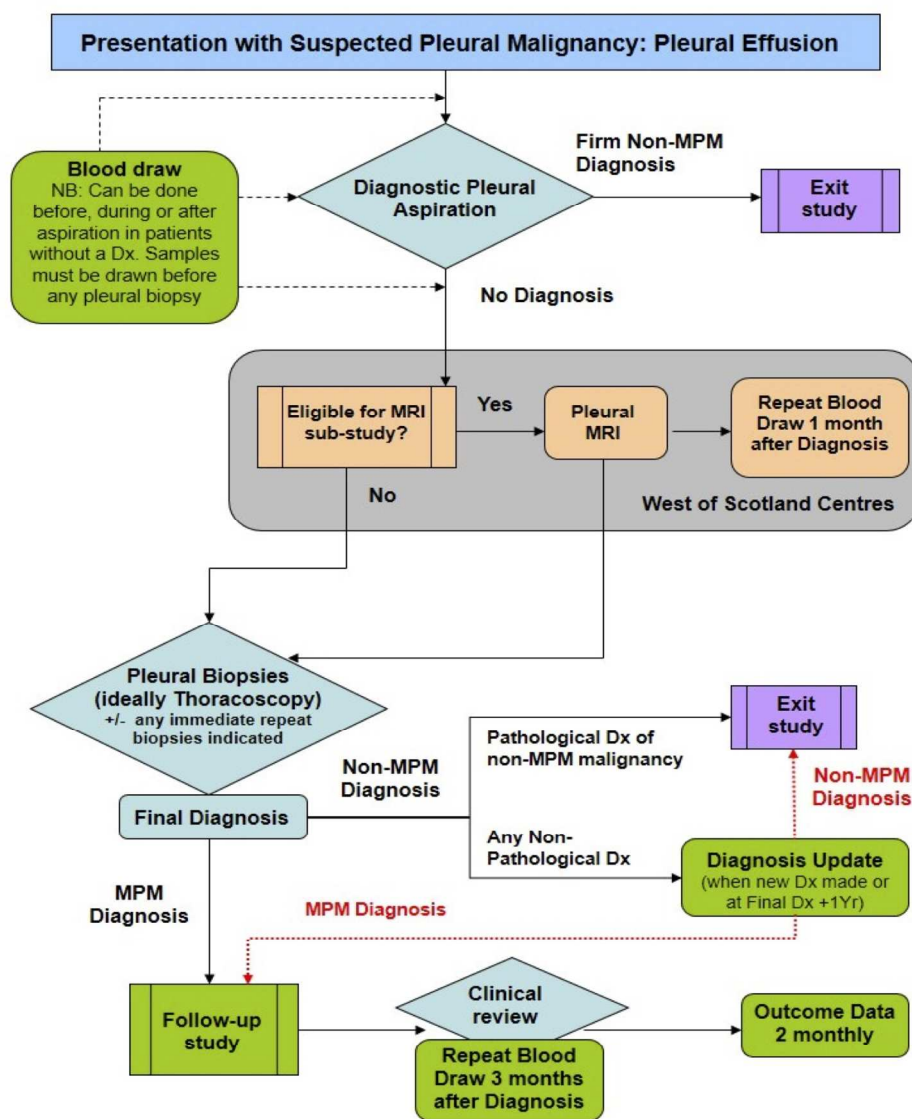


Figure 1(a)

156x180mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

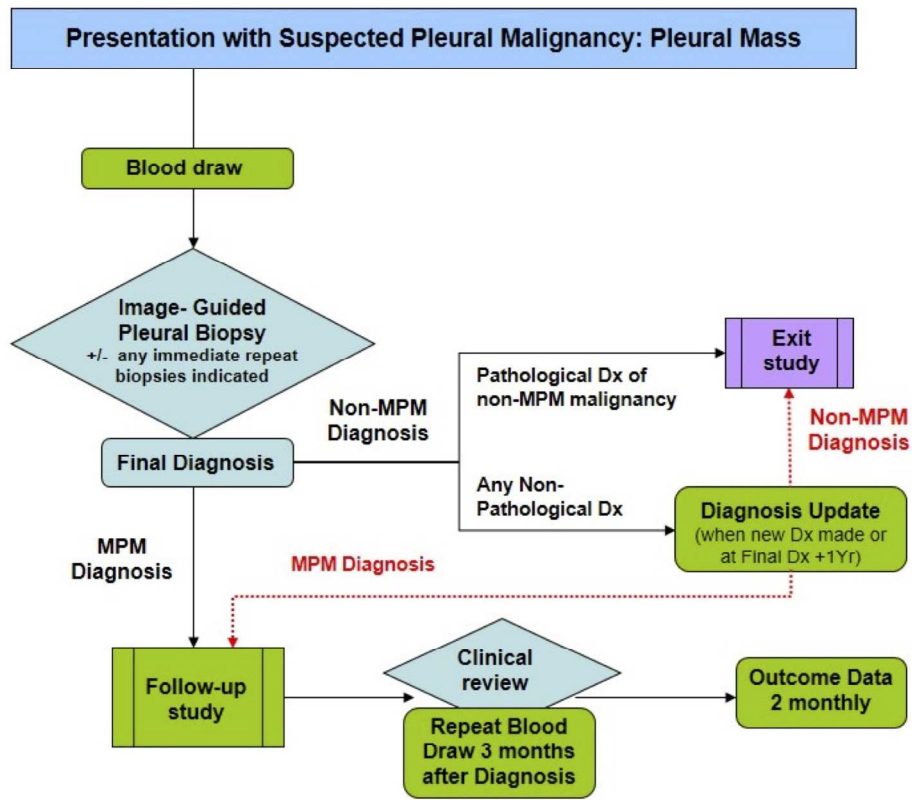



Figure 1(b)

166x144mm (300 x 300 DPI)

only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

 <p>CANCER RESEARCH UK Clinical Trials Unit, Glasgow</p>		<h2 style="margin: 0;">ASBESTOS EXPOSURE QUESTIONNAIRE</h2> <p style="margin: 0;">(to be completed for all patients on study)</p>							
<h3 style="margin: 0;">DIAPHRAGM: Diagnostic and Prognostic biomarkers in the Rational Assessment of Mesothelioma</h3>									
PATIENT INITIALS: (f) _____ (s) _____				DATE of BIRTH: <u>DD</u> / <u>MON</u> / <u>YYYY</u>					
INVESTIGATOR:				REGISTRATION DATE: <u>DD</u> / <u>MON</u> / <u>YYYY</u>					
SITE:				PATIENT TRIAL IDENTIFIER:					
DATE OF COMPLETION OF QUESTIONNAIRE: <u>DD</u> / <u>MON</u> / <u>YYYY</u>									
<h3 style="margin: 0;">Employment History No known asbestos exposure <input type="checkbox"/></h3>									
Industry / Occupation e.g. joiner, shipyard worker	Period of Employment			No. of Hours per day	Days per week	Airway protection?	Job Type <small>Please refer to codes below</small>	Job Code <small>Please refer to Page 2</small>	Indirect Exposure Details <small>e.g. via husband, via father</small>
	Start Year	No. of Years	No. of Months						
1.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
2.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
3.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
4.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
5.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			

JOB TYPE: M = Manufacturing asbestos products S = Asbestos Stripping/Removal O = Something else I = Indirect exposure

DATE: DD / MON / YYYY

INVESTIGATOR'S SIGNATURE: _____

Version 2.1, 1st July 2015

Please return completed form to: Clinical Trial Coordinator – DIAPHRAGM Study
CRUK Clinical Trials Unit, Level 0, The Beatson West of Scotland Cancer Centre, 1053 Great Western Road, Glasgow, G12 0YN

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

Job Codes (Taken from Appendix 5 of the HSE Asbestos Survey)

CODE LIST M

Textile manufacture

01 Raw material store
02 Raw material & finished product transport
03 Disintegrating/heating/opening/fibrising
04 Hopper feeding
05 Carding
06 Weaving
07 Spinning
08 Doubling/twisting
09 Braiding
10 Warping
11 Detritus handling
12 Inspecting
13 Supervising
14 Finished product store & dispatch
15 Other exposed workers
95 'Fortex' process

Asbestos cement mixture board and pipe manufacture

16 Supervising
17 Raw material store
18 Raw material transport
19 Disintegrating
20 Mixing/beatng
21 Wet board or pipe manufacture
22 Wet board or pipe handling
23 Drying
24 Dry board handling
25 Machining or cutting
26 Sanding
27 Inspecting
28 Finished product store/packing/dispatch/transport
29 Detritus handling wet
30 Detritus handling dry
31 Other exposed worker

Manufacture of asbestos/rubber/resin bitumen mixtures

32 Raw material store
33 Transporting raw materials
34 Disintegrating
35 Handling raw fibre (bag tipping/weighting/mixing)
36 Pressing/moulding
37 Cutting/finishing/machining
38 Transporting finished product
39 Finished product storage/packing/dispatching
40 Inspecting
41 Supervising

42 Other exposed worker

Asbestos board and paper manufacture

43 Raw material store
44 Raw material transport
45 Disintegrating/opening/fibrising
46 Mixing/beatng
47 Handling wet mixture
48 Drying
49 Handling dry mixture
50 Cutting/machining
51 Store/transport/packing/dispatching products
52 Supervising
53 Detritus handling
54 Inspecting
55 Other exposed workers

Garment manufacture

56 Cloth store
57 Cutting out
58 Stitching
59 Transport of materials
60 Storing/packing/dispatching
61 Inspecting
62 Supervising
63 Other exposed workers

Manufacture of dry mixes for insulation & plastering

70 Raw material stores
71 Raw material handling/bag tipping/weighting/mixing
72 Packaging
73 Stores and dispatch
74 Other exposed workers

Maintenance workers all manufacturing sectors

75 Supervising
76 Fault finder/machine fitter/installation engineer/plant engineer
77 Labour to plant engineers etc
78 Carpenters/joiners
79 Electrician
80 Plumber
81 Other building trade craftsmen eg painter
82 Labourer to building trade craftsman
83 Ventilation plant servicing
84 Factory cleaning

CODE LIST S

Asbestos stripping/removal

64 Supervising

65 Stripping/encapsulating

69 Other exposed workers (eg sampler, cleaner, scaffolder)

CODE LIST O

Ship building, repair, & breaking

85 Asbestos storeman
86 Lagging
87 Boilermakers and installers
88 Carpenters/joiners
89 Plumber
90 Engine fitter
91 Other exposed workers
92 Asbestos stripping
93 Cleaner
94 Shipbreaking

Building & construction

96 Heating engineer

97 Asbestos board cutting/fitting

98 Asbestos roofing construction and maintenance

99 Demolition

104 Other building trade craftsmen eg painter (81)[†]

105 Labourer to building trade craftsman (82)

106 Plumber (80)

107 Carpenters/joiners (78)

Miscellaneous processes

101 Use of asbestos string/rope/felt (65)

102 Fitting clutch and brake pads (37)

108 Machining/cutting asbestos/resin board (50)

109 Other exposed workers (69)

Version 2.1, 1st July 2015

Please return completed form to: Clinical Trial Coordinator – DIAPHRAGM Study
CRUK Clinical Trials Unit, Level 0, The Beatson West of Scotland Cancer Centre,
1053 Great Western Road, Glasgow, G12 0YN

BMJ Open

Diagnostic and prognostic biomarkers in the rational assessment of mesothelioma (DIAPHRAGM) study: protocol of a prospective, multi-centre, observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-013324.R2
Article Type:	Protocol
Date Submitted by the Author:	24-Oct-2016
Complete List of Authors:	Tsim, Selina; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Cancer Sciences Kelly, Caroline; Cancer Research UK Glasgow Clinical Trials Unit Alexander, Laura; Cancer Research UK Glasgow Clinical Trials Unit McCormick, Carol; Cancer Research UK Glasgow Clinical Trials Unit Thomson, Fiona; Cancer Research UK Glasgow Clinical Trials Unit Woodward, Rosemary; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Foster, John; Queen Elizabeth University Hospital, Glasgow Clinical Research Imaging Facility Stobo, David; Queen Elizabeth University Hospital, Radiology Paul, Jim; Cancer Research UK Glasgow Clinical Trials Unit Maskell, Nick; University of Bristol, Academic Respiratory Unit, School of Clinical Sciences; North Bristol NHS Trust, Respiratory Research Unit Chalmers, Anthony; University of Glasgow, Institute of Cancer Sciences; Beatson West of Scotland Cancer Centre Blyth, Kevin; Queen Elizabeth University Hospital, Respiratory Medicine; University of Glasgow, Institute of Infection, Immunity and Inflammation
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Oncology, Patient-centred medicine, Research methods
Keywords:	Mesothelioma, Biomarker, Diagnosis, Prognosis

SCHOLARONE™
Manuscripts

TITLE:

DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN THE RATIONAL
ASSESSMENT OF MESOTHELIOMA (DIAPHRAGM) STUDY: PROTOCOL
OF A PROSPECTIVE, MULTI-CENTRE, OBSERVATIONAL STUDY

AUTHORS:

Selina Tsim^{1/2}, Caroline Kelly³, Laura Alexander³, Carol McCormick³, Fiona
Thomson³, Rosie Woodward⁴, John E Foster⁴, David B Stobo⁵, Jim Paul³,
Nick A Maskell⁶, Anthony Chalmers^{2/7}, Kevin G Blyth^{1/8}

Affiliations

1. Department of Respiratory Medicine, Queen Elizabeth University Hospital,
Glasgow, U.K.
2. Institute of Cancer Sciences, University of Glasgow, U.K.
3. Cancer Research UK Glasgow Clinical Trials Unit, UK
4. Glasgow Clinical Research Imaging Facility, Queen Elizabeth University
Hospital, Glasgow, U.K.
5. Department of Radiology, Queen Elizabeth University Hospital, Glasgow,
U.K.
6. Academic Respiratory Unit, School of Clinical Sciences, University of
Bristol, UK
7. Beatson West of Scotland Cancer Centre, Glasgow, UK
8. Institute of Infection, Immunology and Inflammation, University of
Glasgow, U.K.

Corresponding Author and Address

Kevin G. Blyth

Consultant Respiratory Physician and NRS Career Research Fellow

Department of Respiratory Medicine, Administration Building,

Queen Elizabeth University Hospital,

Glasgow, G51 4TF

kevin.blyth@ggc.scot.nhs.uk

Tel: +44 141 451 6099

Keywords:

Mesothelioma

Biomarker

Diagnosis

Prognosis

WORD COUNT:

3998/4000

ABSTRACT (300/300)

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an asbestos-related cancer, which is difficult to diagnose. Thoracoscopy is frequently required but is not widely available. An accurate, non-invasive diagnostic biomarker would allow early specialist referral, limit diagnostic delays and maximize clinical trial access. Current markers offer insufficient sensitivity and are not routinely used. The SOMAmer[®] proteomic classifier and Fibulin-3 have recently demonstrated sensitivity and specificity exceeding 90% in retrospective studies. DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma) is a suitably powered, multi-centre, prospective observational study designed to determine whether these markers provide clinically useful diagnostic and prognostic information.

METHODS AND ANALYSIS

Serum and plasma (for SOMAscan[®] and Fibulin-3, respectively) will be collected at presentation, prior to pleural biopsy/pleurodesis, from 83-120 MPM patients, 634-724 patients with non-MPM pleural disease and 109 asbestos-exposed controls. Final numbers of MPM/non-MPM cases will depend on the incidence of MPM in the study population (estimated at 13-20%). Identical sampling and storage protocols will be used in 22 recruiting centres and histological confirmation sought in all cases. Markers will be measured using the SOMAscan proteomic assay (SomaLogic Inc.) and a commercially available Fibulin-3 ELISA (USCN Life Science Inc.). The standard error in the estimated sensitivity and specificity will be <5% for each

1
2
3 marker and their performance will be compared to serum Mesothelin. Blood
4
5 levels will be compared to paired pleural fluid levels and MPM tumour volume
6
7 (using Magnetic Resonance Imaging) in a nested sub-study. The prognostic
8
9 value of each marker will be assessed and a large bioresource created.
10
11

12 13 14 **ETHICS AND DISSEMINATION**

15
16 The study has been approved by the West of Scotland Research Ethics
17
18 Committee (Ref: 13/WS/0240). A Trial Management Group meets on a
19
20 monthly basis. Results will be published in peer-reviewed journals, presented
21
22 at international meetings and disseminated to patient groups.
23
24

25
26
27 **TRIAL REGISTRATION NUMBER: ISRCTN10079972**

28 29 30 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 31
32
- 33 • Prospective, multi-centre study recruiting a representative sample of
34 patients in an intention-to-diagnose population
 - 35 • Strict sampling, processing and storage methods used in all patients
 - 36 • Robust diagnostics and 12 months' follow-up
 - 37 • Creation of a large bio-resource annotated with detailed, prospectively
38 collected clinical information, for use in future biomarker discovery and
39 validation studies
 - 40 • The final number of study participants with MPM, and therefore the power
41 available to test the primary objective, will not be known until recruitment is
42 complete.
- 43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is an invasive thoracic malignancy, strongly associated with prior asbestos exposure. The median survival for patients with MPM is poor at 9-10 months [1,2]. However, the prognosis of individuals is highly variable and largely determined by histological subtype [2]. MPM frequently presents as an emergency with a large, symptomatic pleural effusion [3]. Early specialist referral is frequently required because pleural fluid aspiration cytology is unreliable [4] and histological confirmation is recommended in all patients [5]. Thoracoscopy (under local or general anaesthesia) [5], enables widespread tissue sampling [6] with diagnostic yields for malignancy >90% [7] but is not available in all centres. Thoracoscopy also allows pleurodesis or indwelling pleural catheter placement.

A reliable, non-invasive diagnostic biomarker for MPM would be a major clinical advance. This would allow clinicians to reliably differentiate likely MPM from secondary pleural malignancies (e.g. lung or breast cancer), which may present with similar clinical and imaging features but require less evolved diagnostic pathways. This reflects the improved sensitivity of pleural cytology in these diseases [8-10] and the frequent option of alternative sites for tissue biopsy. A positive MPM biomarker test could facilitate early referral to a thoracoscopy centre and avoid unnecessary diagnostic delay (e.g. due to repeated pleural aspirations), minimising the risk of subsequent needle-tract metastases [11,12] and maximizing opportunity for clinical trial enrolment. Previous studies have demonstrated that blood levels of single proteins,

1
2
3 including mesothelin [13,14], megakaryocyte potentiating factor (MPF) [15]
4 and osteopontin [16], are higher in patients with MPM than in asbestos-
5 exposed controls (AECs) and patients with secondary pleural malignancies.
6
7 Mesothelin, a cell-adhesion glycoprotein that is over-expressed in MPM
8
9 [17,18] is the most widely studied and is associated with an MPM sensitivity of
10
11 56-77% at 95% specificity [14,15,19] but much reduced performance in
12
13 patients with non-epithelioid MPM. A recent meta-analysis (of 4491 individuals
14
15 (1026 with MPM)) reported a sensitivity of only 32% at 95% specificity.
16
17 Mesothelin does not, therefore, contribute to current diagnostic algorithms
18
19 [20]. MPF offers no advantage over mesothelin [15], while the clinical utility of
20
21 osteopontin is limited by stability and reproducibility concerns [16].
22
23
24
25
26
27
28
29

30 An ideal MPM biomarker would be measurable in blood for ease of collection
31 and offer sufficient sensitivity at high specificity in patients presenting with
32 suspected MPM. Differentiation between advanced disease patients and
33 appropriate controls is of limited value. High specificity is mandatory for a low
34 prevalence disease, and should apply to patients with asbestos exposure and
35 non-MPM pleural disease. Biomarker results should also correlate with
36 disease extent and have defined relationships with potential confounders
37 including renal function [21] and the effect of pleural interventions. The latter
38 is important because the precedent has been established in prostate [22,23]
39 and breast cancer [24], that recent sampling, resection or peri-tumoural
40 inflammation may affect biomarker expression. This is particularly relevant to
41 MPM where biopsies are frequently large and often combined with
42 pleurodesis. Several previous biomarker studies, which validated
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 inconsistently in external populations, used samples acquired at later time-
4
5 points, often post-diagnosis (and post-pleurodesis) including samples taken
6
7 prior to, during, or after resection surgery [16,25,26]. The aim of the
8
9 DIAPHRAGM study is to prospectively evaluate the diagnostic and prognostic
10
11 performance of the SOMAscan proteomic classifier [27] and fibulin-3 [25],
12
13 which have demonstrated high sensitivity and specificity in recent
14
15 retrospective series. The study has been designed to generate clinically
16
17 meaningful results, which can be related to MPM biology and confounding
18
19 factors, and applied to patients at first presentation.
20
21
22
23
24

25 **SOMAmer-based Proteomic Classifier**

26
27
28 The SOMAscan assay is a highly multiplexed proteomic platform that utilizes
29
30 SOMAmer (Slow Off-rate Modified Aptamers) reagents to selectively bind and
31
32 quantify proteins [28]. A 13-protein classifier was developed by SomaLogic
33
34 Inc. (Boulder, Colorado), using this novel proteomics-based biomarker
35
36 detection technique [27] in a retrospective study over 800 proteins were
37
38 measured in the serum of 117 MPM patients and 142 AECs, collected at
39
40 surgical MPM centres in the US between 1996 and 2011. Using a panel of 13
41
42 differentially expressed proteins and a cut-point of 0.5, the classifier was able
43
44 to segregate MPM from controls with an area under the curve (AUC) of 0.99
45
46 +/- 0.01 in training (60 MPM/60 controls), 0.98 +/- 0.04 in blinded verification
47
48 (19 MPM/20 controls) and 0.95 +/- 0.04 in blinded validation sets (38
49
50 cases/62 controls) [27]. The combined sensitivity for the three cohorts was
51
52 93% at 91% specificity. Based on the published ROC curve for the validation
53
54 cohort, sensitivity at 95% specificity appeared to be approximately 78%,
55
56
57
58
59
60

1
2
3 although the authors did not report this value. This performance exceeds that
4
5 of any previous MPM biomarker, although the classifier's specificity appeared
6
7 lower in patients with non-MPM pleural effusion (n=32). There was a modest
8
9 correlation between classifier score and disease stage, but prognostic
10
11 significance was not assessed. The 13 classifier proteins (nine up-regulated,
12
13 four down-regulated) have not previously been associated with MPM. Their
14
15 functions fall into two broad groups; regulation of proliferation and
16
17 inflammation. Quite apart from their biological relevance to MPM, the latter is
18
19 an important potential confounder because many of the patients involved will
20
21 have previously undergone pleurodesis. In addition, several groups have
22
23 reported an independent interaction between prognosis and inflammatory
24
25 biomarkers in MPM, including neutrophil-to-lymphocyte ratio (NLR) [29-31],
26
27 monocytosis [32] and the modified Glasgow Prognostic Score [31].
28
29 Therefore, adequate understanding of the diagnostic and prognostic utility of
30
31 this assay requires replication in a pre-pleurodesis cohort and prospective
32
33 evaluation of interactions between inflammatory biomarkers and SOMAscan
34
35 scores.
36
37
38
39
40
41
42

43 **Fibulin-3**

44
45 Fibulin-3 is a secreted glycoprotein, encoded by the epidermal growth factor-
46
47 containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene [33].
48
49 Fibulin-3 is over-expressed in MPM tumours relative to adjacent benign pleura
50
51 [25] and expressed and secreted by MPM cell lines [26]. Pass et al
52
53 retrospectively measured fibulin-3 in the plasma of 92 MPM patients, 136
54
55 AECs, 93 patients with non-MPM pleural effusion and 43 healthy controls [25].
56
57
58
59
60

1
2
3 A plasma cut-point of 52 ng/ml provided 97% sensitivity at 95% specificity and
4 a 95% CI of the AUC of 0.97-0.99 in differentiating MPM from all other cases.
5
6
7 However, in a blinded external validation set, sensitivity was below 40% (at
8
9
10 95% specificity), with an AUC=0.87.

11
12
13
14 Subsequent studies have revealed mixed results. In a study of 153 patients
15
16 (82 with MPM), Creaney et al reported a sensitivity of 22% (at 95% specificity)
17
18 at the same 52 ng/ml cut-point and an AUC of 0.671 (0.606 to 0.732), which
19
20 was significantly inferior to mesothelin measured in the same patients
21
22 (sensitivity 56% (at 95% specificity); AUC 0.816 (0.755 to 0.867)) at a 2.5 nM
23
24 threshold [14]). In a small Egyptian study using an unspecified Fibulin-3 assay
25
26 and internally-defined cut-points, Agha et al reported 100% sensitivity/78%
27
28 specificity in differentiating MPM cases (n=25) from non-malignant pleural
29
30 disease (n=9), and 88% sensitivity/82% specificity in differentiating MPM from
31
32 secondary pleural malignancies (n=11) [34]. No combined sensitivity was
33
34 reported. An Italian study found no difference in Fibulin-3 levels but used
35
36 serum (not plasma), a control group without pleural disease (Asbestosis) and
37
38 contained only 14 patients with MPM [35].
39
40
41
42
43
44

45 **METHODS AND ANALYSIS**

46 **Study Design**

47
48 DIAPHRAGM is a prospective, multi-centre observational study. The study
49
50 incorporates sampling windows that correspond to the proposed use of a
51
52 diagnostic biomarker, i.e. at presentation with Suspected Pleural Malignancy
53
54 (SPM). The overall study design is summarized in Figure 1(a) and 1(b). The
55
56
57
58
59
60

1
2
3 main impact of this design is that biomarkers will be drawn before a diagnosis
4 is made. In addition to better replicating the future use of these markers, this
5 avoids the potential confounding effect of pleurodesis on biomarker results.
6
7 The diagnostic performance of the SOMAmer panel and Fibulin-3 will be
8 assessed using cut-points determined in the relevant original studies and
9 compared to mesothelin (using the MESOMARK® ELISA (Fujirebio
10 Diagnostics Inc, PA, USA). Identical processing and storage protocols will be
11 used in patients with SPM and a group of AECs. Potential confounders
12 including renal function, inflammatory indices and drugs will be recorded at all
13 visits. The timing of the biomarker blood draw in relation to pleural aspiration
14 (pre-aspiration or post-aspiration) will be recorded in order to assess the
15 effect of this intervention on biomarker results. An exploratory, cross-sectional
16 Magnetic Resonance Imaging (MRI) sub-study will determine if there is any
17 correlation between blood biomarker levels and MPM tumour volume, as has
18 been established for Mesothelin using Computed Tomography-Positron
19 Emission Tomography scanning [36].
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

41 **Study Objectives and Outcome Measures**

42 These are presented in Table 1.
43
44
45
46

47 **Setting**

48 At least 600 consecutive patients with SPM will be recruited from 21 centres
49 (20 in the UK, 1 in Republic of Ireland). These are a mixture of academic and
50 more clinically orientated units. This should make the results of the
51 DIAPHRAGM study generalizable to patients presenting with SPM to acute
52
53
54
55
56
57
58
59
60

1
2
3 hospital services. The principal criterion used to select centres was that they
4 had sufficiently evolved pleural diagnostic services to deliver a reliable
5 diagnosis. Specifically, access to on-site thoracoscopy (ideally including local
6 anaesthetic thoracoscopy (LAT)) and a regional mesothelioma MDT meeting
7 (for diagnostic review and staging) was required.
8
9
10
11
12

13 14 15 16 **Screening and Eligibility Assessment**

17 **Suspected Pleural Malignancy**

18
19 Cases will be identified on presentation to a Respiratory out-patient clinic or
20 acute hospital admissions unit. This will be based on the history, examination
21 and available investigations. Potentially eligible patients will be provided with
22 the study Patient Information Sheet (PIS, see Online Supplementary
23 Appendix 1) and eligibility assessed based on the following criteria:
24
25
26
27
28
29
30
31
32
33

34 *Inclusion Criteria:*

- 35 • SPM, defined by a unilateral pleural effusion or pleural mass lesion
- 36 • Sufficient fitness for diagnostic sampling (site investigator's clinical
- 37 judgment)
- 38 • Informed written consent
- 39
- 40
- 41
- 42
- 43
- 44
- 45
- 46
- 47

48 *Exclusion Criteria:*

- 49 • Intercostal chest drain in-situ, or inserted within the previous 3 months
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

Asbestos-related pleural plaques are not an inclusion criterion since these are
absent in up to 25% of MPM cases [37], and are also common in asbestos-

1
2
3 exposed populations without MPM [38]. Patients with lung nodules or other
4 visceral mass lesions are not excluded, assuming the investigator suspects
5 pleural malignancy. This is because of the high prevalence of lung nodules in
6 the target population (older patients, commonly smokers) and the high false
7 positive rate of CT imaging in this regard [39].
8
9

10
11
12
13
14
15
16 Subjects recruited to the SPM arm will generate cohorts of MPM and non-
17 MPM pleural disease of various aetiologies, likely including Benign Asbestos-
18 related Pleural Effusion and secondary pleural malignancies. These numbers
19 will be sufficient to address the primary objective with sufficient statistical
20 power to inform clinical practice (see later section).
21
22
23
24
25
26
27

28 29 30 Asbestos-exposed control (AEC) subjects

31
32 109 AECs will be recruited via invitations sent by Clydeside Action on
33 Asbestos (CAA), an advocacy body based in Glasgow with a database of over
34 600 clients, or by Respiratory clinics at the host centre. Individuals will be
35 invited to participate by letter (if identified via CAA) or given the PIS (see
36 Online Supplementary Appendix 2) at clinic. All subjects will be invited to a
37 single research clinic visit assuming the following Eligibility Criteria are met.
38
39
40
41
42
43
44
45
46

47 48 *Inclusion Criteria:*

- 49 • Documented history of asbestos exposure and associated pleural
50 plaques, asbestosis or diffuse pleural thickening
51
- 52 • Willing and able to travel to a research clinic interview in Glasgow
53
- 54 • Informed written consent
55
56
57
58
59
60

Exclusion Criteria:

- Known MPM
- Known or suspected other thoracic malignancy under investigation
- Known pleural effusion of any cause

Detailed asbestos exposure histories will be taken from all participants in both the suspected pleural malignancy cohort and the asbestos-exposed control cohort. This will be done using an asbestos exposure questionnaire derived from Health and Safety Executive asbestos survey [40] (see Online Supplementary Appendix 3). This questionnaire includes recording of the nature of occupational exposure(s), which can be correlated to likely fibre exposure. The duration and first year of exposure is also recorded. Non-occupational sources of exposure are also recorded (e.g. the washing of an occupationally exposed spouse's work clothes). Only AECs with documented imaging sequelae of asbestos exposure (e.g. pleural plaques) and an asbestos exposure history will be included.

Cross-sectional MRI sub-study

50 patients will be recruited to address the study's exploratory objectives (see Table 1). Eligibility will be determined based on the following criteria.

Inclusion Criteria:

- Pleural histological sampling (by LAT/image-guided biopsy) indicated to investigate SPM following a non-diagnostic pleural aspiration
- Recruited in a West of Scotland centre

Exclusion Criteria:

- Unable to undergo MRI (claustrophobia or known contraindications such as pacemaker, ferrous metal implants or foreign body)
- Allergy to Gadolinium contrast
- Renal impairment (eGFR <30ml/min)
- Pregnancy

Based on previous audit data from the host centre we expect at least 40% (n=20) of patients in the sub-study to have MPM. Eligible subjects will be approached at the clinical visit during which non-diagnostic pleural aspiration results, and the need for further investigation, are discussed. Subjects will be provided with a separate PIS (see Online Supplementary Appendix 4) and will be asked to provide additional informed written consent.

Consent

All subjects will be given sufficient time (as judged by themselves) to provide written informed consent after reading the relevant PIS and having the opportunity to ask questions.

Outcome Measures

The outcome measures associated with each of the trial's objectives are detailed in Table 1.

Final Diagnosis

1
2
3 A specific cytological or histological pleural diagnosis will be sought in all
4 patients according to national guidelines [20]. This will be recorded as the
5 Final Diagnosis, which may be based on immediate repeat biopsies felt to be
6 indicated by the site PI (see Figure 1). Any cytologically or histologically
7 confirmed non-MPM diagnosis (e.g. pleural metastases from lung cancer) will
8 be recorded without the need for any further updates. However, sites will need
9 to provide updates for any non-MPM diagnosis that is not cytologically or
10 histologically confirmed (e.g. parapneumonic effusion). These will be
11 submitted on the 12-month anniversary of the original diagnosis, or as soon
12 as any new pleural diagnosis is made. This aims to capture any false
13 negative diagnostic tests from the initial presentation, acknowledging the
14 major diagnostic challenges posed by pleural malignancies, particularly MPM.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 *Biomarker Sampling and Storage*

33
34 Blood samples (+/- pleural fluid in WoS centres) will be drawn and immediate
35 processing performed at each study centre. Samples can be taken before or
36 after pleural aspiration. Patients with positive pleural cytology cannot be
37 recruited (see Figure 1(a)). Duplicate samples will be collected for all
38 measurements at all visits, ensuring redundancy in case of loss or damage to
39 samples during transportation to the appropriate central laboratory.
40 SOMAmer biomarker levels will be measured in serum; therefore, 9 ml of
41 venous blood will be collected first into a vacutainer tube containing SST clot
42 activator. Fibulin-3 levels will be measured in plasma; therefore, 9 ml of
43 venous blood will be collected second into a vacutainer tube containing
44 EDTA. In centres contributing to the exploratory MRI sub-study (WoS sties
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 only) 20 ml of pleural fluid will be also collected into a plain container if pleural
4
5 fluid is being drawn for diagnostic/therapeutic purposes at the same visit. If
6
7 not done at this first opportunity, pre-diagnosis pleural fluid can also be
8
9 collected during local anaesthetic or general anaesthetic thoracoscopy, prior
10
11 to any biopsy or pleurodesis being performed.
12
13

14 15 16 *Biomarker Processing and Storage* 17

18 Serum samples will be allowed to clot for 30 minutes before centrifugation.
19
20 Plasma and pleural fluid samples will be centrifuged immediately. All samples
21
22 will be centrifuged at 2200g for 15 minutes at room temperature. For all
23
24 samples, the supernatant will be withdrawn by pipette, aliquoted into cryovials
25
26 of at least 250µL volume, labeled and placed into a -80 freezer within 2 hours.
27
28 Samples will be stored at each recruiting centre until batched transport to the
29
30 appropriate study laboratory. Samples from WoS recruiting centres will be
31
32 used to create a bioresource. The bioresource will be stored as a satellite
33
34 collection of the NHS Greater Glasgow & Clyde Biorepository, a Health
35
36 Improvement Scotland (HIS)-approved tissue bank. Data will be stored in the
37
38 secure Cancer Research UK Clinical Trials Unit database. On study
39
40 completion, investigators will be invited to apply for access to data and
41
42 samples appropriate to their research questions. This will allow external
43
44 validation of new markers, including those reported since the study's design
45
46 (such as High Mobility Group Box-1 (HMGB-1)) [41], in an intention to
47
48 diagnose population, Access will be granted after peer review of each
49
50 proposal by a scientific board comprising members of the DIAPHRAGM TMG
51
52
53
54
55
56
57
58
59
60

1
2
3 and senior Biorepository staff. An annual update on this activity will be
4
5 submitted to the West of Scotland Research Ethics Committee.
6
7
8

9 10 *Biomarker Analyses*

11 SomaLogic Inc. (Boulder, Colorado, USA) will perform all SOMAscan
12 proteomic analyses [27]. This utilises SOMAmer reagents to specifically bind
13
14 to protein targets in blood. Relative protein concentrations will be converted to
15
16 measurable nucleic acid signals that are quantified by hybridization to DNA
17
18 microarrays [28].
19
20
21
22
23
24

25 Fibulin-3 and mesothelin levels will be measured by the Translational
26
27 Pharmacology Unit, Wolfson Wohl Cancer Research Centre, UK, using ELISA
28
29 methods validated according to the FDA-recommended guidelines for
30
31 bioanalytical methods [42]. Fibulin-3 levels in plasma and pleural fluid will be
32
33 measured using the commercially available ELISA (Cloud-Clone Corp.,
34
35 formerly USCN Life Science Inc, Houston, Texas, USA) as in the original
36
37 Pass study [25]. Mesothelin will be measured using the MESOMARK® ELISA
38
39 (Fujirebio Diagnostics, Inc, PA, USA).
40
41
42
43
44

45 *Magnetic Resonance Imaging*

46
47 Patients will be scanned at the Queen Elizabeth University Hospital, Glasgow,
48
49 on a 3.0T Siemens Verio MRI Scanner. After localisation of the affected
50
51 thoracic cavity, an isotropic 3D T1-weighted volume will be acquired using
52
53 VIBE sequences. A stack of axial slices covering the entire lung and
54
55 surrounding pleura will be acquired as a set of short breath-holds. Gd-DTPA
56
57
58
59
60

1
2
3 contrast (Gadovist) will be administered via a peripheral intravenous line as a
4
5 15-40 ml bolus (0.05 mmol/kg). VIBE sequences will be reacquired at copied
6
7 slice positions to provide pre and post-contrast images. The total scan time
8
9 will be around 45 minutes. Regions of enhancing pleural tumour will be
10
11 defined using semi-automated signal intensity thresholding based on contrast-
12
13 enhanced axial slices using Myrian Intrasure™ software, which has
14
15 previously been used to assess tumour volume in MPM. [43] MRI volumetry
16
17 analyses will be validated using imaging phantoms.
18
19

20 21 22 23 *Survival*

24
25 Survival will be recorded in days from the date of study registration to the data
26
27 of death, from any cause.
28
29

30 31 32 **Sample Size, Assumptions and Uncertainties**

33
34 Sample size estimations for each marker were based on published data at the
35
36 point of study design and a projected MPM incidence of 13-20% in the SPM
37
38 cohort. The power available to test the hypotheses below is therefore reported
39
40 as a range, based on final MPM numbers lying between 83 (13% incidence)
41
42 and 120 (20% incidence).
43
44
45

46 47 *Primary Objective*

48 49 *SOMAscan Assay*

50
51 We hypothesize that the MPM sensitivity and specificity exceed 90%, based
52
53 on previously reported performance in combined training, verification and
54
55 validation sets (sensitivity 93.2% (88.6–97.7%), specificity (90.8% (86.1–
56
57
58
59
60

1
2
3 95.6%) [27]). Recruitment of 83-120 MPM patients will allow us to distinguish
4
5 a sensitivity of >90% from a sensitivity <80% with 80-93% power,
6
7 respectively, at the 5% 1-sided level of significance. 83-120 MPM patients will
8
9 allow discrimination between a specificity <80% and a specificity >90%, with
10
11 80-88% power at the 5% 1-sided level of statistical significance. The standard
12
13 error in the estimated sensitivity and specificity will be less than 5%, across all
14
15 possible outcomes.
16
17
18
19

20 21 *Fibulin-3*

22
23 We hypothesize that the MPM sensitivity will exceed 80% and that the
24
25 specificity will exceed 90% (at the 52 ng/ml cutoff). These figures are based
26
27 on a reduced level of performance to the primary results reported by Pass et
28
29 al (97% sensitivity, 95% specificity), given lower sensitivity in the external
30
31 validation cohort studied (40% at 95% specificity) [25].
32
33
34
35

36
37 With 83-120 MPM patients the study will be able to distinguish a sensitivity of
38
39 >80% from a sensitivity <70% with 65-80% power, respectively, at the 5% 1-
40
41 sided level of statistical significance. The standard error in the estimated
42
43 sensitivity will be less than 5%. In order to achieve 90% power to distinguish
44
45 a specificity of >90% from a specificity <85% at the 5% 1-sided level of
46
47 statistical significance, a random sample of 378 non-MPM samples will be
48
49 analysed. The standard error in the estimated specificity will be <2.3%.
50
51
52
53

54
55 The study data will be used to estimate the AUC for the SOMAscan marker
56
57 for distinguishing MPM from non-MPM patients in the SPM cohort. Assuming
58
59
60

1
2
3 83-120 patients in the MPM group and 83-120 in the non-MPM group the
4
5 AUC can be estimated with a 95% confidence interval of width 0.120-0.168
6
7 (assuming a cut-point exists with a reasonable sensitivity of 80% and a
8
9 modest specificity of 40%). If more sensitive/specific cut-points exist the width
10
11 of the 95% confidence interval will be much reduced. The study data will be
12
13 used to develop a new diagnostic signature based on Fibulin-3 and
14
15 SOMAscan results to distinguish MPM from non-MPM effusions.
16
17
18
19

20 21 Secondary Objectives

22
23 The study data will be used to determine whether baseline SOMAscan results
24
25 and/or fibulin-3 levels, or a change in levels at 3 months (Fibulin-3 only), are
26
27 independent prognostic factors for MPM. A correlation of 0.4 between existing
28
29 prognostic factors and each marker has been assumed. For the baseline
30
31 levels, to detect an approximate doubling in median OS (from 6 month to 12
32
33 months - a hazard ratio of 2) with 80% power and 5% 2-sided level of
34
35 statistical significance between a good/poor prognostic group based on
36
37 dichotomising these markers requires at least 83 MPM patients recruited over
38
39 three years with approximately 6 months subsequent follow-up to observe 66
40
41 deaths. For the 3-month change levels, a hazard ratio of 2.38 can be
42
43 detected (80% power, 5% 2-sided level of statistical significance) when 49
44
45 deaths are observed in the estimated 66 out of 83 patients who survive to 3
46
47 months.
48
49
50

51 52 Exploratory Objectives

53
54
55
56
57
58
59
60

1
2
3 These will be addressed in the MRI sub-study, which will generate a sample
4 of at least 20 MPM patients. This will allow moderately large associations
5 (0.6) between the exploratory outcome measures (see Table 1) to be detected
6
7
8
9
10 at 80% power at the 5%, two-sided level of statistical significance. The effect
11 of pleural biopsies +/- drainage/pleurodesis on Fibulin-3 levels will be
12 assessed using all 50 patients recruited. This will allow moderately small
13 differences (standardised difference of 0.4) to be detected with 80% power at
14 the 5% two-sided level of statistical significance.
15
16
17
18
19

20 21 22 23 **Statistical Analysis Plan**

24 25 **Primary Analysis**

26
27 Sensitivity and specificity at pre-specified cut-offs will be estimated using
28 standard approaches for proportions. The diagnostic performance of each
29 biomarker will be assessed using ROC curves. All patients with MPM (n=83-
30 120) will be included and compared with AECs and a random sample of non-
31 MPM cases. Due to cost constraints related to SOMAscan analyses 83 AECs
32 and 83 non-MPM cases will be randomly selected. All AECs and 378 non-
33 MPM cases will be used for Fibullin-3 analyses and for comparison with
34 Mesothelin. Logistic regression will be used to estimate a diagnostic model
35 using biomarker results and clinical or radiological variables. Cross validation
36 will be used to provide robust estimates of AUC and specificity at fixed
37 sensitivity rates of 80%, 90% and 95%.
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 53 54 **Secondary Analysis**

55
56
57
58
59
60

1
2
3 A prognostic model will be developed using Cox proportional hazard
4 techniques. The modelling process will incorporate biomarker measurements
5 (at presentation (both markers) and at 3 months (Fibulin-3 only) and other
6 known prognostic features (e.g. performance status, histology).
7
8
9
10

11 Exploratory Analysis

12 The association between SOMAscan results/fibulin-3 in blood and tumour
13 volume/measures of tumour angiogenesis will be estimated by Pearson or
14 Spearman correlation, depending on the normality of the data. The same
15 methods will be used to test the association between fibulin-3 in blood and
16 pleural fluid. Changes in Fibulin-3 levels before and after histological sampling
17 (at 1 month follow-up) will be compared using a paired t-test or Wilcoxon
18 signed rank sum test (depending on the normality of the data). Due to cost
19 constraints, exploratory end-points involving pleural fluid SOMAscan results
20 will be analysed at a later date.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 Changes to the Study Protocol since Trial Opening

40 The protocol described accurately reflects Version 5, of the protocol, dated
41 17/6/16. The following changes were made in previous versions:
42
43
44

- 45 • Version 2, dated 14/2/14:
 - 46 ▪ Safety reporting reduced following risk assessment by study Sponsor.
 - 47 ▪ Collection of duplicate blood samples as provision for loss or damage
 - 48 and for sample retention in tissue bank.
 - 49 ▪ Greater flexibility to timing of first blood draw.
- 50 • Version 3, dated 17/10/14:
51
52
53
54
55
56
57
58
59
60

- Addition of recruitment of Controls from Respiratory Medicine clinics
 - Addition of exclusion criteria for patients with chest drains in-situ.
 - Eligibility for the MRI sub-study extended to patients proceeding to image-guided pleural biopsy
- Version 4, dated 27/4/15:
 - Update to the exclusion criteria for the AECs to include known or suspected thoracic malignancy under investigation.
 - Version 5, dated 17/6/16:
 - Power projections adjusted based on interim reporting of MPM incidence from recruiting centres.

Definition of End of Study

The trial will end 2 years after the last patient with confirmed MPM is recruited or whenever all patients with MPM have died (whichever occurs first).

ETHICS AND DISSEMINATION

Ethics

The study protocol, all documents and amendments have been approved by the West of Scotland Research Ethics Service (Ref: 13/WS/0240).

Monitoring, Data Management and Quality Assurance

No on-site monitoring will be undertaken. Two telephone-monitoring calls will be conducted by a CRUK Glasgow CTU Monitor to carry out process, compliance and documentation checks. Central monitoring of trial data will be performed by the Trial Statistician and Clinical Trial Co-ordinator by checking

1
2
3 incoming forms for compliance with the protocol, data consistency, missing
4 data and timing. The CRUK Glasgow CTU will control data consistency and
5 data quality by entering trial data onto CTU database. Computerised and
6 manual consistency checks will be performed and queries issued in cases of
7 inconsistency or missing information. An audit trail of changes to the database
8 will be maintained.
9
10
11
12
13
14
15
16
17
18

19 **Safety Considerations**

20 Participants in the MRI sub-study will be asked at their 1-month follow-up visit
21 about the occurrence of Adverse Events (AEs) related to the administration of
22 MRI contrast (Gadolinium). These will be followed until resolution.
23
24
25
26
27
28

29 **Dissemination**

30 The results of the study will be presented at national and international
31 scientific meetings and published in full in a peer-reviewed journal (authorship
32 will be according to that journal's guidelines). A lay summary will be produced
33 and disseminated to interested parties.
34
35
36
37
38
39
40
41
42

43 **Trial Management**

44 The trial will be coordinated from CRUK Glasgow CTU by the Trial
45 Management Group (TMG), including the Chief Investigator, selected co-
46 investigators, project manager, trial statistician, clinical trial co-ordinator and
47 IT staff. The TMG will oversee the running of the trial and meet monthly.
48
49
50
51
52
53
54
55

56 **Acknowledgements**

57
58
59
60

1
2
3 The authors are grateful to the Chief Scientist Office (Scotland) as the study
4 funder, cancer research networks infrastructure, the participating sites and
5 staff, SomaLogic Inc and the patients involved. ST is part funded by the West
6 of Scotland Lung Cancer Research Fund. KGB is part funded by the NHS
7 Career Research Fellowship. CR-UK and ECMC are acknowledged by FT
8 and CMcC.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

- 1 Musk AW, Olsen N, Alfonso H, *et al.* Predicting survival in malignant mesothelioma. *Eur Respir J* 2011;**38**:1420–4.
doi:10.1183/09031936.00000811
- 2 Beckett P, Edwards J, Fennell D, *et al.* Demographics, management and survival of patients with malignant pleural mesothelioma in the National Lung Cancer Audit in England and Wales. *Lung Cancer* 2015;**88**:344–8.
doi:10.1016/j.lungcan.2015.03.005
- 3 Tsim S, Dick C, Roberts F, *et al.* 76 Early experience of a regional mesothelioma MDT in the West of Scotland. *Lung Cancer* 2014;**Supplement 1**:S28–9. doi:10.1016/S0169-5002(14)70076-5
- 4 Renshaw AA, Dean BR, Antman KH, *et al.* The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;**111**:106–9.
- 5 Scherpereel A, Astoul P, Baas P, *et al.* Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *Eur. Respir. J.* 2010;**35**:479–95. doi:10.1183/09031936.00063109
- 6 Rusch VW, Giroux D. Do we need a revised staging system for malignant pleural mesothelioma? Analysis of the IASLC database. *Ann Cardiothorac Surg* 2012;**1**:438–48. doi:10.3978/j.issn.2225-319X.2012.11.10
- 7 Boutin C, Rey F. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. *Cancer* 1993;**72**:389–93.
- 8 Salyer WR, Eggleston JC, Erozan YS. Efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of malignant neoplasm involving the pleura. *Chest* 1975;**67**:536–9.
- 9 Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic

- 1
2
3 analysis for the evaluation of pleural effusion: analysis of 414 cases.
4 *Mayo Clin Proc* 1985;**60**:158–64.
5
6
7
8
9
10 Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy
11 as compared with that of pleural fluid examination. *Mod Pathol*
12 1991;**4**:320–4.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 11 O'Rourke N, Garcia JC, Paul J, *et al.* A randomised controlled trial of
intervention site radiotherapy in malignant pleural mesothelioma.
Radiother Oncol 2007;**84**:18–22. doi:10.1016/j.radonc.2007.05.022
- 12 Boutin C, Rey F, Viallat JR. Prevention of malignant seeding after
invasive diagnostic procedures in patients with pleural mesothelioma. A
randomized trial of local radiotherapy. *Chest* 1995;**108**:754–8.
- 13 Robinson BW, Creaney J, Lake R, *et al.* Mesothelin-family proteins and
diagnosis of mesothelioma. *The Lancet* 2003;**362**:1612–6.
doi:10.1016/S0140-6736(03)14794-0
- 14 Creaney J, Dick IM, Meniawy TM, *et al.* Comparison of fibulin-3 and
mesothelin as markers in malignant mesothelioma. *Thorax* 2014;**69**:895–
902. doi:10.1136/thoraxjnl-2014-205205
- 15 Creaney J, Yeoman D, Demelker Y, *et al.* Comparison of osteopontin,
megakaryocyte potentiating factor, and mesothelin proteins as markers in
the serum of patients with malignant mesothelioma. *J Thorac Oncol*
2008;**3**:851–7. doi:10.1097/JTO.0b013e318180477b
- 16 Pass HI, Lott D, Lonardo F, *et al.* Asbestos exposure, pleural
mesothelioma, and serum osteopontin levels. *N Engl J Med*
2005;**353**:1564–73. doi:10.1056/NEJMoa051185
- 17 Chang K, Pai LH, Batra JK, *et al.* Characterization of the antigen (CAK1)
recognized by monoclonal antibody K1 present on ovarian cancers and
normal mesothelium. *Cancer Res* 1992;**52**:181–6.
- 18 Chang K, Pai LH, Pass H, *et al.* Monoclonal antibody K1 reacts with

- 1
2
3 epithelial mesothelioma but not with lung adenocarcinoma. *Am J Surg*
4 *Pathol* 1992;**16**:259–68.
5
6
7 19 Hollevoet K, Nackaerts K, Thimpont J, *et al*. Diagnostic performance of
8 soluble mesothelin and megakaryocyte potentiating factor in
9 mesothelioma. *Am J Respir Crit Care Med* 2010;**181**:620–5.
10 doi:10.1164/rccm.200907-1020OC
11
12
13
14 20 Hooper C, Lee YCG, Maskell N, *et al*. Investigation of a unilateral pleural
15 effusion in adults: British Thoracic Society Pleural Disease Guideline
16 2010. *Thorax*. 2010;**65 Suppl 2**:ii4–17. doi:10.1136/thx.2010.136978
17
18
19
20
21 21 Hollevoet K, Nackaerts K, Thas O, *et al*. The effect of clinical covariates
22 on the diagnostic and prognostic value of soluble mesothelin and
23 megakaryocyte potentiating factor. *Chest* 2012;**141**:477–84.
24 doi:10.1378/chest.11-0129
25
26
27
28 22 Bergamini S, Bellei E, Bonetti LR, *et al*. Inflammation: an important
29 parameter in the search of prostate cancer biomarkers. *Proteome*
30 *Science* 2014 *12:1* 2014;**12**:32. doi:10.1186/1477-5956-12-32
31
32
33
34 23 Yuan JJ, Coplen DE, Petros JA, *et al*. Effects of rectal examination,
35 prostatic massage, ultrasonography and needle biopsy on serum prostate
36 specific antigen levels. *J Urol* 1992;**147**:810–4.
37
38
39
40 24 Wong V, Wang D-Y, Warren K, *et al*. The effects of timing of fine needle
41 aspiration biopsies on gene expression profiles in breast cancers. *BMC*
42 *Cancer* 2008;**8**:277. doi:10.1186/1471-2407-8-277
43
44
45
46 25 Pass HI, Levin SM, Harbut MR, *et al*. Fibulin-3 as a blood and effusion
47 biomarker for pleural mesothelioma. *N Engl J Med* 2012;**367**:1417–27.
48 doi:10.1056/NEJMoa1115050
49
50
51
52 26 Kirschner MB, Pulford E, Hoda MA, *et al*. Fibulin-3 levels in malignant
53 pleural mesothelioma are associated with prognosis but not diagnosis. *Br*
54 *J Cancer* 2015;**113**:963–9. doi:10.1038/bjc.2015.286
55
56
57
58
59
60

- 1
2
3 27 Ostroff RM, Mehan MR, Stewart A, *et al.* Early detection of malignant
4 pleural mesothelioma in asbestos-exposed individuals with a noninvasive
5 proteomics-based surveillance tool. *PLoS ONE* 2012;**7**:e46091.
6 doi:10.1371/journal.pone.0046091
7
8
9
10 28 Gold L, Ayers D, Bertino J, *et al.* Aptamer-based multiplexed proteomic
11 technology for biomarker discovery. *PLoS ONE* 2010;**5**:e15004.
12 doi:10.1371/journal.pone.0015004
13
14
15 29 Kao SCH, Pavlakis N, Harvie R, *et al.* High blood neutrophil-to-
16 lymphocyte ratio is an indicator of poor prognosis in malignant
17 mesothelioma patients undergoing systemic therapy. *Clin Cancer Res*
18 2010;**16**:5805–13. doi:10.1158/1078-0432.CCR-10-2245
19
20
21
22
23 30 Hooper CE, Lyburn ID, Searle J, *et al.* The South West Area
24 Mesothelioma and Pemetrexed trial: a multicentre prospective
25 observational study evaluating novel markers of chemotherapy response
26 and prognostication. *Br J Cancer* 2015;**112**:1175–82.
27 doi:10.1038/bjc.2015.62
28
29
30
31 31 Pinato DJ, Mauri FA, Ramakrishnan R, *et al.* Inflammation-Based
32 Prognostic Indices in Malignant Pleural Mesothelioma. *Journal of*
33 *Thoracic Oncology* 2012;**7**:587–94. doi:10.1097/JTO.0b013e31823f45c1
34
35
36
37 32 Burt BM, Rodig SJ, Tilleman TR, *et al.* Circulating and tumor-infiltrating
38 myeloid cells predict survival in human pleural mesothelioma. *Cancer*
39 2011;**117**:5234–44. doi:10.1002/cncr.26143
40
41
42
43 33 Zhang Y, Marmorstein LY. Focus on molecules: Fibulin-3 (EFEMP1).
44 *Experimental Eye Research* 2010;**90**:374–5.
45 doi:10.1016/j.exer.2009.09.018
46
47
48
49 34 Agha MA, El-Habashy MM, El-Shazly RA. Role of fibulin-3 in the
50 diagnosis of malignant mesothelioma. *Egyptian Journal of Chest*
51 *Diseases and Tuberculosis* 2014;**63**:99–105.
52 doi:10.1016/j.ejcdt.2013.10.004
53
54
55
56
57
58
59
60

- 1
2
3 35 Corradi M, Goldoni M, Alinovi R, *et al.* YKL-40 and mesothelin in the
4 blood of patients with malignant mesothelioma, lung cancer and
5 asbestosis. *Anticancer Res* 2013;**33**:5517–24.
6
7
8
9 36 Creaney J, Francis RJ, Dick IM, *et al.* Serum Soluble Mesothelin
10 Concentrations in Malignant Pleural Mesothelioma: Relationship to
11 Tumor Volume, Clinical Stage and Changes in Tumor Burden. *Clin*
12 *Cancer Res* 2011;**17**:1181–9. doi:10.1158/1078-0432.CCR-10-1929
13
14
15 37 Pairon J-C, Laurent F, Rinaldo M, *et al.* Pleural plaques and the risk of
16 pleural mesothelioma. *J Natl Cancer Inst* 2013;**105**:293–301.
17 doi:10.1093/jnci/djs513
18
19
20 38 Paris C, Thierry S, Brochard P, *et al.* Pleural plaques and asbestosis:
21 dose- and time-response relationships based on HRCT data. *Eur Respir*
22 *J* 2009;**34**:72–9. doi:10.1183/09031936.00094008
23
24
25 39 Baldwin DR, Callister MEJ, Guideline Development Group. The British
26 Thoracic Society guidelines on the investigation and management of
27 pulmonary nodules. *Thorax* 2015;**70**:794–8. doi:10.1136/thoraxjnl-2015-
28 207221
29
30
31 40 HSE. The Asbestos Survey. <http://www.hse.gov.uk> 2016;:1–150.
32
33
34 41 Napolitano A, Antoine DJ, Pellegrini L, *et al.* HMGB1 and Its
35 Hyperacetylated Isoform are Sensitive and Specific Serum Biomarkers to
36 Detect Asbestos Exposure and to Identify Mesothelioma Patients. *Clin*
37 *Cancer Res* Published Online First: 5 January 2016. doi:10.1158/1078-
38 0432.CCR-15-1130
39
40
41 42 Services UDOHAH. *Guidance for Industry Bioanalytical Method*
42 *Validation (2001)* [http://www.fda.](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation)
43 [gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation.](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation)
44
45
46 43 Frauenfelder T, Tutic M, Weder W, *et al.* Volumetry: an alternative to
47 assess therapy response for malignant pleural mesothelioma? *Eur Respir*
48 *J* 2011;**38**:162–8. doi:10.1183/09031936.00146110
49
50
51
52
53
54
55
56
57
58
59
60

CONTRIBUTIONS

Selina Tsim

- Contribution to the conception or design of the work; data acquisition, analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Caroline Kelly

- Contribution to the design of the work; analysis and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Laura Alexander

- Contribution to the design of the work and interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that

1
2
3 questions related to the accuracy or integrity of any part of the work
4
5 are appropriately investigated and resolved
6
7
8

9
10 Carol McCormick

- 11 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 12 interpretation of data for the work
- 13
- 14 ▪ Revising the work critically for important intellectual content
- 15
- 16 ▪ Final approval of the version to be published
- 17
- 18 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 19 questions related to the accuracy or integrity of any part of the work
- 20 are appropriately investigated and resolved
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28
- 29

30 Fiona Thomson

- 31 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 32 interpretation of data for the work
- 33
- 34 ▪ Revising the work critically for important intellectual content
- 35
- 36 ▪ Final approval of the version to be published
- 37
- 38 ▪ Agrees to be accountable for all aspects of the work in ensuring that
- 39 questions related to the accuracy or integrity of any part of the work
- 40 are appropriately investigated and resolved.
- 41
- 42
- 43
- 44
- 45
- 46
- 47
- 48
- 49

50 Rosie Woodward

- 51 ▪ Contribution to the design of the work; data acquisition, analysis, or
- 52 interpretation of data for the work
- 53
- 54 ▪ Revising the work critically for important intellectual content
- 55
- 56
- 57
- 58
- 59
- 60

- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

John E Foster

- Contribution to the conception and design of the work; data acquisition, analysis, or interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

David B Stobo

- Contribution to the design of the work; interpretation of data for the work
- Revising the work critically for important intellectual content
- Final approval of the version to be published
- Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Jim Paul

- Contribution to the conception and design of the work; data analysis

1
2
3 or interpretation of data for the work

- 4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
- Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

20 Nick A Maskell

- 21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
- Contribution to the design of the work; data analysis and interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

40 Anthony Chalmers

- 41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Contribution to the conception and design of the work; interpretation of data for the work
 - Revising the work critically for important intellectual content
 - Final approval of the version to be published
 - Agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

1
2
3 Kevin G Blyth
4

- 5 ▪ Principal contribution to the conception and design of the work; data
6 acquisition, analysis and interpretation of data for the work
7
- 8 ▪ Drafting the work
9
- 10 ▪ Final approval of the version to be published
11
- 12 ▪ Agrees to be accountable for all aspects of the work in ensuring that
13 questions related to the accuracy or integrity of any part of the work
14 are appropriately investigated and resolved
15
16
17
18
19

20 21 22 **FUNDING** 23

24 This work was supported by the Chief Scientist's Office of the Scottish
25 Government (Project Grant ETM/285) and the West of Scotland Lung Cancer
26 Research Group (Award September 2015). KGB is part-funded by NHS
27 Research Scotland.
28
29
30
31
32
33
34
35

36 **COMPETING INTERESTS STATEMENT** 37

38 SomaLogic Inc. have provided funding for all SOMAscan assays.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FIGURE LEGENDS

Figure 1

Summary of the design of the DIAPHRAGM study. Figure 1(a) is intended to describe the optimal diagnostic pathway for the majority of patients who present with significant Pleural Effusion +/- pleural thickening or a pleural mass. Figure 1(b) describes the optimal diagnostic pathway for the minority of patients who present with an isolated Pleural Mass, but no significant fluid component. The pathway chosen is ultimately at the discretion of the investigating physician.

TABLES

Table 1. Outcome Measures used in the DIAPHRAGM study

Research Objective	Outcome Measures
<p>Primary To determine whether SOMAscan results and/or Fibulin-3 levels in blood at presentation can differentiate MPM from asbestos-exposed controls and patients with other causes of pleural effusion with a sufficient degree of sensitivity and specificity to be of routine clinical value</p>	<p>Serum SOMAscan Plasma Fibulin-3 Final diagnosis reached</p>
<p>Secondary To determine whether:</p> <ol style="list-style-type: none"> SOMAscan results and/or Fibulin-3 levels at presentation provide clinically useful prognostic information in MPM patients early changes in SOMAscan and/or Fibulin-3 levels after diagnosis (at 3 months) are associated with a poorer prognosis in MPM 	<p>Serum SOMAscan & plasma Fibulin-3 at presentation Survival (from registration)</p> <p>Serum SOMAscan & plasma Fibulin-3 3 months post-Dx Survival (from registration)</p>
<p>Exploratory To determine whether:</p> <ol style="list-style-type: none"> there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour volume, defined by MRI there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and tumour angiogenesis (as defined by perfusion-based MRI biomarkers) there is a correlation between SOMAscan and/or Fibulin-3 levels in blood and pleural fluid at presentation in patients with MPM 	<p>Serum SOMAscan Plasma Fibulin-3 MPM tumour volume at MRI, defined using Myrian intrasense™ software</p> <p>Serum SOMAscan Plasma Fibulin-3 The following MRI biomarkers:</p> <ul style="list-style-type: none"> MRI-ECE Redistribution rate constant (K_{ep}) Elimination rate constant (K_{el}) <p>SOMAscan and Fibulin-3 at presentation and at 1 month post-biopsy +/- drainage and pleurodesis</p>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

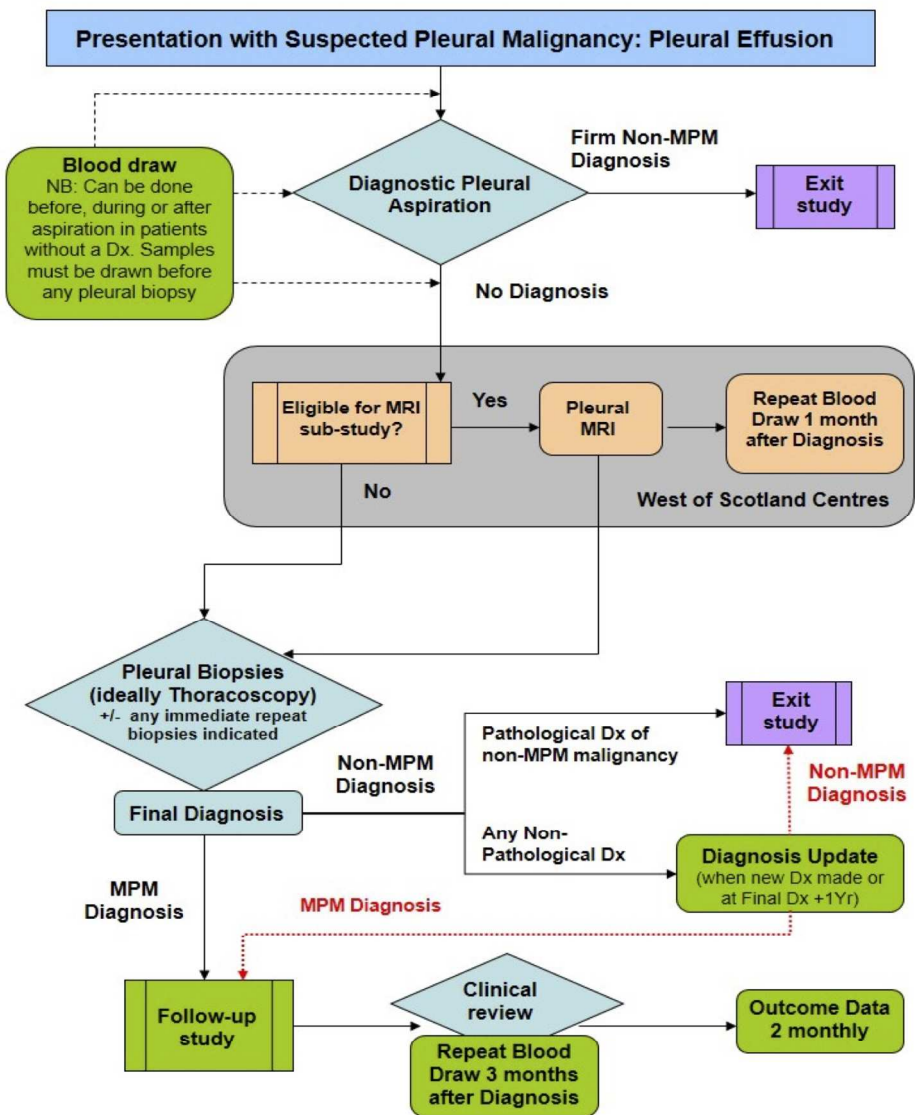


Figure 1(a)

156x180mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

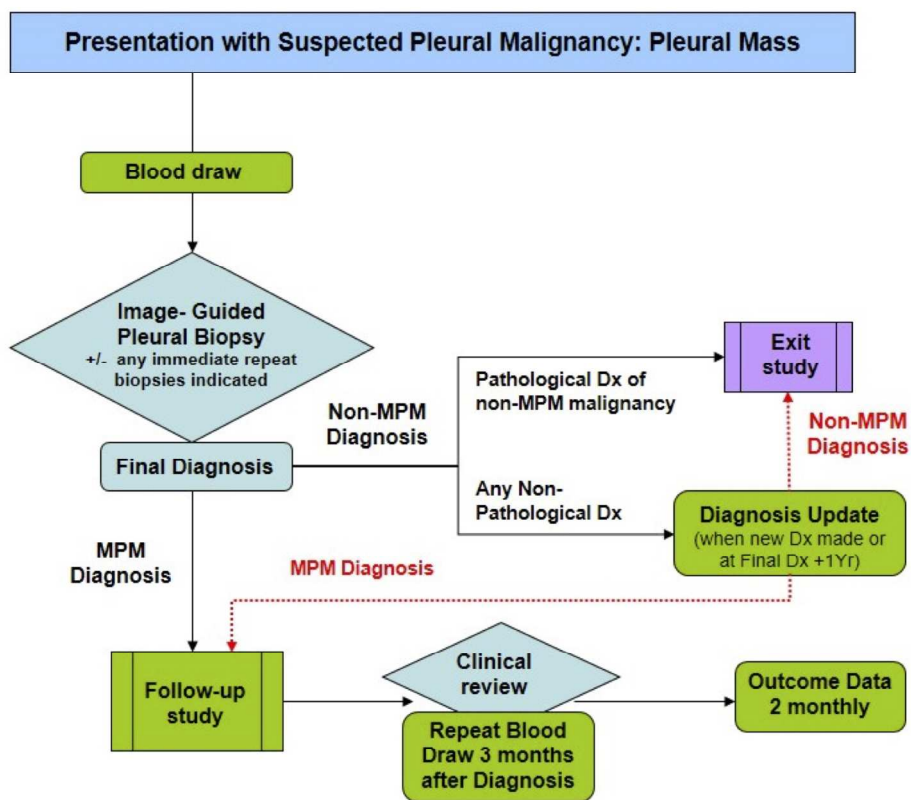


Figure 1(b)

166x144mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



ASBESTOS EXPOSURE QUESTIONNAIRE

(to be completed for all patients on study)

DIAPHRAGM: Diagnostic and Prognostic biomarkers in the Rational Assessment of Mesothelioma

PATIENT INITIALS: (f) _____ (s) _____

DATE of BIRTH: DD / MON / YYYY

INVESTIGATOR:

REGISTRATION DATE: DD / MON / YYYY

SITE:

PATIENT TRIAL IDENTIFIER:

DATE OF COMPLETION OF QUESTIONNAIRE: DD / MON / YYYY

Employment History

No known asbestos exposure

Industry / Occupation e.g. joiner, shipyard worker	Period of Employment			No. of Hours per day	Days per week	Airway protection?	Job Type <small>Please refer to codes below</small>	Job Code <small>Please refer to Page 2</small>	Indirect Exposure Details <small>e.g. via husband, via father</small>
	Start Year	No. of Years	No. of Months						
1.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
2.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
3.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
4.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			
5.	YYYY			_____	_____	Yes <input type="checkbox"/> No <input type="checkbox"/>			

JOB TYPE: **M** = Manufacturing asbestos products **S** = Asbestos Stripping/Removal **O** = Something else **I** = Indirect exposure

DATE: DD / MON / YYYY

INVESTIGATOR'S SIGNATURE: _____

Version 2.1, 1st July 2015

Please return completed form to: Clinical Trial Coordinator – DIAPHRAGM Study
CRUK Clinical Trials Unit, Level 0, The Beatson West of Scotland Cancer Centre, 1053 Great Western Road, Glasgow, G12 0YN

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>

Job Codes (Taken from Appendix 5 of the HSE Asbestos Survey)

CODE LIST M

Textile manufacture

01 Raw material store
02 Raw material & finished product transport
03 Disintegrating/heating/opening/fibrising
04 Hopper feeding
05 Carding
06 Weaving
07 Spinning
08 Doubling/twisting
09 Braiding
10 Warping
11 Detritus handling
12 Inspecting
13 Supervising
14 Finished product store & dispatch
15 Other exposed workers
95 'Fortex' process

Asbestos cement mixture board and pipe manufacture

16 Supervising
17 Raw material store
18 Raw material transport
19 Disintegrating
20 Mixing/beatng
21 Wet board or pipe manufacture
22 Wet board or pipe handling
23 Drying
24 Dry board handling
25 Machining or cutting
26 Sanding
27 Inspecting
28 Finished product store/packing/dispatch/transport
29 Detritus handling wet
30 Detritus handling dry
31 Other exposed worker

Manufacture of asbestos/rubber/resin bitumen mixtures

32 Raw material store
33 Transporting raw materials
34 Disintegrating
35 Handling raw fibre (bag tipping/weighting/mixing)
36 Pressing/moulding
37 Cutting/finishing/machining
38 Transporting finished product
39 Finished product storage/packing/dispatching
40 Inspecting
41 Supervising

42 Other exposed worker

Asbestos board and paper manufacture

43 Raw material store
44 Raw material transport
45 Disintegrating/opening/fibrising
46 Mixing/beatng
47 Handling wet mixture
48 Drying
49 Handling dry mixture
50 Cutting/machining
51 Store/transport/packing/dispatching products
52 Supervising
53 Detritus handling
54 Inspecting
55 Other exposed workers

Garment manufacture

56 Cloth store
57 Cutting out
58 Stitching
59 Transport of materials
60 Storing/packing/dispatching
61 Inspecting
62 Supervising
63 Other exposed workers

Manufacture of dry mixes for insulation & plastering

70 Raw material stores
71 Raw material handling/bag tipping/weighting/mixing
72 Packaging
73 Stores and dispatch
74 Other exposed workers

Maintenance workers all manufacturing sectors

75 Supervising
76 Fault finder/machine fitter/installation engineer/plant engineer
77 Labour to plant engineers etc
78 Carpenters/joiners
79 Electrician
80 Plumber
81 Other building trade craftsmen eg painter
82 Labourer to building trade craftsman
83 Ventilation plant servicing
84 Factory cleaning

CODE LIST S

Asbestos stripping/removal

64 Supervising

65 Stripping/encapsulating

69 Other exposed workers (eg sampler, cleaner, scaffolder)

CODE LIST O

Ship building, repair, & breaking

85 Asbestos storeman
86 Lagging
87 Boilermakers and installers
88 Carpenters/joiners
89 Plumber
90 Engine fitter
91 Other exposed workers
92 Asbestos stripping
93 Cleaner
94 Shipbreaking

Building & construction

96 Heating engineer

97 Asbestos board cutting/fitting

98 Asbestos roofing construction and maintenance

99 Demolition

104 Other building trade craftsmen eg painter (81)[†]

105 Labourer to building trade craftsman (82)

106 Plumber (80)

107 Carpenters/joiners (78)

Miscellaneous processes

101 Use of asbestos string/rope/felt (65)

102 Fitting clutch and brake pads (37)

108 Machining/cutting asbestos/resin board (50)

109 Other exposed workers (69)

Version 2.1, 1st July 2015

Please return completed form to: Clinical Trial Coordinator – DIAPHRAGM Study
CRUK Clinical Trials Unit, Level 0, The Beatson West of Scotland Cancer Centre,
1053 Great Western Road, Glasgow, G12 0YN