Proposal: Temporal variation in exhaled volatile organic compounds in response to therapeutic intervention in esophageal cancer patients

Principal investigator:
Donald E Low FACS FRCS (c) FRCSI (Hon) FRCSE (Hon)
Head Of Thoracic Surgery and Thoracic Oncology
Director Digestive Disease Institute Esophageal Center of Excellence
President Ryan Hill Research Foundation
Virginia Mason Medical Center
1100 Ninth Ave
Seattle, WA, USA 98111
Telephone: +1 (206) 223-6164
Email: Donald.Low@virginiamason.org

Co-investigator:
George B Hanna FRCS, PhD
Professor of Surgical Sciences
Head of Division of Surgery
Department of Surgery and Cancer,
Imperial College London,
St Mary's Hospital,
London, W2 1NY, UK
Telephone: +44 (0)207 886 2125
E-mail: g.hanna@imperial.ac.uk

SPECIFIC AIMS

Hypothesis: we hypothesize that cancer-associated Volatile Organic Compounds (VOCs) will vary significantly following therapeutic intervention in patients with esophageal cancer (EC) and that variation will predict treatment response.

Primary aim: to determine longitudinal variation in exhaled VOC concentrations during intended curative therapy for EC cancer.

Secondary aims:

i. To validate the previously published model for a exhaled VOC breath test of EC cancer
ii. To correlate the exhaled VOCs of EC cancer patients to important clinical parameters
iii. To determine the relationship between exhaled VOCs and response to neoadjuvant chemoradiotherapy
iv. To study changes in exhaled VOCs following tumor resection and their association with disease recurrence.
v. To correlate the exhaled and urinary VOC concentrations in EC cancer patients
vi. To longitudinal variation in the upper gastrointestinal microbiome and its relationship to exhaled and urinary VOC concentrations.

STUDY OUTCOMES

Primary outcome

i. Confirmation of exhaled and urinary VOC biomarkers of EC cancer

Secondary outcomes

i. Verification of changes in VOC concentrations that occur in response to therapeutic intervention
ii. Refine diagnostic model for the detection and monitoring of EC cancer through analysis of exhaled VOCs
iii. Linkage of longitudinal VOC data to predominant upper gastrointestinal bacterial species
iv. Patient acceptability of breath test

BACKGROUND AND SIGNIFICANCE

Background: The global prevalence of esophageal cancer (EC) in 2016 was 2.75 million cases with an estimated 1.60 million new cases and 1.25 million deaths during the same year (1, 2). Early EC cancer typically has non-specific symptoms that are commonly ascribed to benign conditions. Current guidelines recommend referral for endoscopy, only in the setting of 'red flag' symptoms that are frequently associated with inoperable disease. As a consequence, 7 out of 10 new cases of EC cancer diagnosed are considered to be at an advanced stage with less that 1 in 3 patients eligible for potentially curative therapy and less than 1 in 5 patients alive at 5 years (3).

Volatile organic compounds and EC cancer biomarkers:
Our approach to this clinical challenge is to establish a non-invasive test for the detection of EC cancer that is based upon the unique signature of volatile organic compounds (VOCs) within exhaled breath.

Volatile organic compounds (VOCs) are carbon-containing molecules that are sufficiently volatile to be detectable in a gas form at room temperature. VOCs may emanate from numerous sources within the human body as well as our local environment. Established uses of VOC measurements include the assessment of environmental contamination, the flavor and fragrance industries and in counter-terrorism. There is now established evidence that VOCs which are released from the human body in breath and urine can be used as a non-invasive method of detection and monitoring (patho)physiological processes.

The analysis of VOCs excreted within exhaled breath is a particularly attractive diagnostic prospective as it offers a potentially non-invasive window on to normal human physiology and diseases states within the body. Examples of the analysis of exhaled VOCs within clinical practice include breathalyzer devices for ethanol detection, ^{13}C urea breath testing for H. pylori, exhaled Nitric Oxide in asthma and Hydrogen/Methane testing for small bowel bacteria overgrowth (4-7). There is now an appreciation that increased production of certain VOCs may be associated with cancer. In a recent literature review, we identified 63 publication presenting data for target VOC analysis in 12 distinct cancer subtypes. Pooled analysis of these studies revealed a combined sensitivity and specificity of 79% (95% CI 77%-81%) and 89% (95% CI 88%-90%) respectively for a 'cancer breath test'. This review included six publications concerning the VOC-based breath tests for the diagnosis of EC cancers (8-13). VOCs released from the urine of EC cancer patients has also been shown to contain a unique profile, although no study has directly compared this to breath signatures (14).

Our approach to addressing this clinical challenge is to establish a non-invasive breath test for early detection of EC cancer based on its unique profile of volatile organic compounds (VOCs). Our previous studies have reported higher concentrations of specific VOCs, including volatile fatty acids (butanoic-, pentanoic- and hexanoic acid), within the breath, gastric content and urine of EC cancer patients (1-4). We have also

established standardized and quality controlled protocols for breath sampling, transfer and storage that ensure accuracy and reproducibility of VOC analysis by mass spectrometry methods (5-9).

Infrastructure for research

Existing infrastructure at Virginia Mason Medical Center that will support the proposed study include: high volume center for the management of esophageal cancer; support from the Benaroya Research Institute at Virginia Mason including access to study coordinators, and; a track record for conducting high quality research in esophageal cancer.

The proposed study will be conducted in collaboration with the volatile biomarker research group, based at St Mary's Hospital (Imperial College London). The primary focus of this group's research has been the non-invasive diagnosis of gastrointestinal cancer through the detection of VOCs within exhaled breath. They have previously established diagnostic models of gastroesophageal and colorectal cancers (8, 9, 15, 16). In a recent multicenter validation study they demonstrated a diagnostic performance of a breath test for gastroesophageal cancers based on five VOCs, with an area under the receiver operator characteristic curve of 0.85 and sensitivity and specificity of 80% and 81% respectively (15). Their previous work has alluded to importance of volatile fatty acids, aldehydes and phenols as tumor markers. In parallel to these clinical trials they have sought to investigate the mechanisms whereby VOCs are deregulated in cancer by undertaking carefully designed in-vitro and in-vivo studies. Specifically they have demonstrated an association between gastroesophageal cancer, its predominant associated bacterial species (E.coli, K. pneumoniae and S. anginosus) and VOCs production (unpublished data). Whilst these findings give support to the hypothesis that cancer has associated bacteria, no study has sought to directly compare the intestinal microbiome and exhaled VOC levels.

Critical knowledge gap: Previous studies have established an important link between deregulated VOC production and EC. These studies have all assessed exhaled VOCs at a single point in time using a cross-section study design. Longitudinal variation in exhaled and urinary VOCs, the contribution of the upper gastrointestinal microbiome and the effect of therapeutic intervention in EC cancer patients remain unknown. An understanding of this variation is critically important in establishing intra-subject variability and likely mechanisms of VOC production and release. Furthermore the expected decline in target VOCs following chemoradiotherapy and/or tumor resection would both verify the link between the tumor and aberrant VOCs production as well as offering an opportunity to utilize VOCs as a means to assess treatment response including the early detection of recurrence.

RESEARCH DESIGN AND METHODS

Study design: longitudinal cohort study

Sample size: 50 curative tri-modality patients. Study sample size has been determined pragmatically based on estimated patient recruitment during the course of 1 year in a high volume center for the management of EC

Human subjects: patients undergoing potentially curative therapy for EC cancer at Virginia Mason Medical Center who meet the eligibility criteria will be invited to participate in this study.

Inclusion criteria: patients with the following characteristics will be eligible for inclusion in this study:

i. aged 18-90 years
ii. newly diagnosed, treatment naïve patients with esophageal and/or gastroesophageal junctional cancer
iii. planning to undergo curative treatment, including neoadjuvant chemoradiotherapy and surgical resection

Exclusion criteria: patients with the following characteristics will not be eligible for inclusion in this study:

i. pregnant females
ii. without malignant esophageal disease
iii. malignancy at a secondary site other than the esophagus
iv. patients undergoing palliative treatment for esophageal cancer
v. patients not receiving neoadjuvant chemoradiotherapy and surgical resection for esophageal cancer
vi. inability or unwillingness to provided written informed consent

Study time points: subjects will be asked to provide exhaled breath, urine and saliva samples at the following time points during their routine care:

i. Before commencing treatment for esophageal cancer*
ii. Following conclusion of neoadjuvant therapy (at the time of a routine outpatient appointment or on the day of surgery)
iii. Following surgical resection (after the removal of the nasogastric tube, during the inpatient hospital stay)
iv. At the time of routine postoperative follow-up at VM (between six months to one-year following surgery)

Subjects will also be asked to complete an engagement questionnaire at one of the study timepoints. See “Subject Engagement” section of the protocol.

*Where feasible at the time of initial breath sampling subjects will be asked to provide breath samples on two separate routine clinical appointments (often on consecutive days) in order to assess short-term temporal variation in exhaled VOC levels. At the

time of their initial assessment it is common for patients to have routine clinical appointments on consecutive days.

Any data that are not able to be collected with reasonable efforts will not be considered protocol deviations.

Exhaled breath collection and analysis: Subjects will be asked to fast for a minimum of 1-2 hours prior to breath sampling, with an ideal fast of 4 hours or longer. Where possible sampling will be performed on a day when subjects are fasting as part of their routine care (e.g. prior to CT-PET or endoscopy). Before breath sampling, subjects will also be asked to rinse their mouth with water. Following a period of relaxed tidal breathing subjects will be asked to exhale directly in to double thickness Nalophan® bags (Scentroid, Canada).

Breath samples will be immediately (within 30-120 mins of collection, target: 30 minutes) transferred from Nalophan sample bags to thermal desorption (TD) tubes (Markes International, Llantrisant, UK) pre-packed with 200 mg of Tenax and 100 mg of Carbograph 5. 500ml aliquots of breath will be transferred to four TD tubes via a handheld precision pump (Easy-VOC, Markes International, Llantrisant, UK). TD tubes will be securely capped and stored at -80^{o}C prior to being transferred to Imperial College London for analysis.

TD tubes containing exhaled VOCs will be analyzed by Gas Chromatography Mass Spectrometry (GC-MS). For thermal desorption GC-MS analysis, adsorbent tubes will be loaded into a sample tray (TD-100, Markes International). The tube will be dry-purged for 1 minute to remove excess moisture and air. VOCs will be desorbed from the tube by a specific combination of heat and gas flow and will be refocused on the cold-trap. The cold-trap is then rapidly heated to 290^{o}C with carrier gas flow so that VOCs are transferred to the GC column in a narrow band. For separation, the GC column will be ramped. The mass spectrometer will run in electron impact ionisation mode, scanning mass ions 20-250 m/z. An initial solvent delay will minimize interference from water. Chromatographic data will be analyzed using the Mass Hunter package (Agilent Technologies) in combination with the NIST mass spectral library (2014, Maryland, USA) for compound identification and semi-quantification.

Urine sampling and analysis: Patients will be asked to provide a clean catch urine sample (approximately 10ml) into a sterile sample container. Urine samples will be aliquoted and stored at -80^{o}C prior to being transferred on dry ice to Imperial College London for analysis.

VOCs within the headspace gas above urine samples sealed in air-tight vials will be analyzed by GC-MS. Urine samples (2ml) will be diluted in 6ml deionized pure water, acidified using HCl to pH 2.0. Urine osmolality (OsmoPRO®, Advanced Instruments, Norwoor, UK) will be analyzed for the purpose of subsequent standardization. Samples will be placed in air tight headspace vials into which a HiSorb probe (Markes International, Llantrisant, UK) will be inserted. Headspace vials including the HiSorb probe will be heated and agitated at 60^{o}C for 30 minutes. Headspace VOCs extracted

onto HiSorb probes will be analyzed using an Agilent 7890B GC with 5977 MSD (Agilent Technologies, Cheshire, UK) coupled to a Markes TD-100 device.

Saliva sampling and analysis: Subjects will be asked to fast for a minimum of 1-2 hours (Target: 4 hours) prior to saliva sampling. Where possible saliva sampling will be performed on a day when subjects are fasting as part of their routine care (e.g. prior to CT-PET or endoscopy). Saliva samples (1-3ml) will be collected by asking subjects to spit into a sample pot. Saliva samples will be aliquoted and stored at -80^{o}C prior to being transferred on dry ice to Imperial College London for analysis.

Bacterial RNA will be extracted from saliva samples and analyzed by 16S rRNA gene amplicon sequencing to identify the species composition. Bacterial community types derived from tissues biopsies will be identified by cluster analysis of compositional data from 16S rRNA sequencing. Bacterial interaction networks will be constructed to identify any keystone species.

Patient demographics and metadata: essential accompanying patient demographics and metadata will include: age; sex; comorbidities; height; weight; tumor histology and stage; details of (neo)adjuvant therapy; details of surgery; duration of pre-test fasting, and; radiological parameters (tumor dimensions, nodal burden and PET-standardized uptake value). Where possible, data will be obtained via the IRB approved institutional database (IRB18-076).

Statistical analysis: Both univariate and multivariate data analysis techniques will be applied to identify breath and urinary VOCs with the best discriminating ability and to develop a multivariate discriminant analysis model. Appropriate univariate statistical techniques will be used to compare intra- and inter- individual variability in exhaled VOCs in response to therapeutic intervention. Associations between predominant bacterial species and VOCs with be determined through a univariate and multivariate data analysis techniques.

Using the data collected, several machine learning algorithms will be adapted, trained and evaluated for the purpose of diagnostic inference based on the breath sample chemical analysis. We will start with an exploratory analysis of the VOC concentration data, including Principal Component Analysis (PCA) and Multi-dimensional Scaling (MDS), to reduce the feature space and develop intuitions about the data. Next, Machine Learning Algorithms (MLA) such as Support Vector Machines (SVM) and k-Nearest Neighbors (kNN) will be trained and validated to reliably separate cancer from non-cancer cases. An Information Theory based MLA will also be developed. Cross-validation algorithms such as k -fold training will be used to rigorously assess the reliability of the learning algorithms. Models will then be compared through suitable accuracy scores and ROC curves will be produced to aid the selection of appropriate clinical diagnostic measure, which balances specificity against sensitivity.

Sample storage

Breath, urine and saliva samples will be stored in a -80^{o}C freezer within the Benaroya Research Institute. Samples will be deidentified and only labeled using a unique study identifier composed of the patient's study ID and the date the sample was collected.

Data storage

Virginia Mason Medical Center subject data will be stored in a pseudonymized form within Microsoft Excel. Data will be stored on a password protected institutional (Virginia Mason Medical) computer. Any paper records, including patient consent forms, will be stored in locked filing cabinet within the department of Thoracic Surgery Virginia Mason Medical Center as well as in the Clinical Research Program offices at Benaroya Research Institute. Access to the data will be limited to the principal investigator, nominated members of the research team and any regulatory or institutional compliance team member in case of review or audit only. Any data from samples shared with Imperial College London (UK) will be in a linked anonymized form.

Subject engagement: Subjects who are recruited to this study will be asked to complete a short survey, ideally at the first study time point (but acceptable to be collected at any study time point), designed to assess their understanding and opinions regarding breath testing.

PROTECTION OF HUMAN SUBJECTS

Human Subject involvement and characteristics

Human subjects will be enrolled into this study. The purpose of recruiting human subjects is to determine the influence of EC and neoadjuvant therapy on VOC biomarkers and the upper gastrointestinal microbiome. Recruitment of human subjects is critical to achieving the intended outcomes of this study.

Patients with newly diagnosed esophageal and/or gastroesophageal junctional adenocarcinoma will be recruited from Virginia Mason Medical Center (n=50). Patients will be asked to donate an exhaled breath, urine and saliva sample at four study time points. With the exception of a subject engagement questionnaire (completed at the time of the first study visit) all other data that are utilized as part of this study originates from patients' routine clinical care and their electronic medical record.

Patients who appear to meet study eligibility may sign consent for this study during the diagnosis and staging phase of esophageal and/or gastroesophageal junctional adenocarcinoma. Initial samples will be collected for these subjects after consent is obtained. Upon completion of diagnosis and staging, it is possible that the subject may not be recommended for curative tri-modality treatment (surgical resection and chemoradiation therapy). Although these subjects are not the focal population for this study, their samples at the applicable study time points are valuable for comparison purposes. As such, these subjects will continue to have samples captured at the protocol defined study time points whenever possible, but any samples not collected (generally due to the subject not returning to VM within the appropriate timeframe) will not be considered a protocol deviation. These subjects will not count toward the goal of 50 enrolled curative tri-modality subjects. Their data will be comparative only.

We do not plan to enroll vulnerable subject groups (children, persons with mental disability, prisoners) where ability to give voluntary informed consent may be questioned or compromised.

Collaborating sites

This work will be conducted as part of a collaboration between Virginia Mason Medical Center (Seattle, USA) and Imperial College London (London, UK).

Sources of Materials

Patients will be required to provide written informed consent prior to enrollment in this study. Participants will be provided with an approved informed consent form and will receive careful explanation by the investigator or approved delegate of the study team consisting of what is involved by taking part in the study, including potential risks and benefits.

i. Patient demographics and meta-data will be recorded for all patients (please see data collection section above). There is very little risk of breach of confidentiality.
ii. Breath samples: breath sampling is entirely non-invasive and poses negligible risk of harm to patients. Patients will be asked to provide a breath sample by blowing directly into a disposable (single use) sample bag. The sample is then transferred and stored within a Thermal Desorption (TD) tube. Samples will be analyzed at Imperial College London. Breath samples are destroyed during the analysis process.
iii. Urine samples: urine sampling is entirely non-invasive and poses negligible risk of harm to patients. Patients will be asked to provide a urine sample (10ml) by urinating into a sample pot that they will be provided with. The sample will be analyzed at Imperial College London. Urine samples will be destroyed following the analysis process.
iv. Saliva samples: saliva sampling is entirely non-invasive and poses negligible risk of harm to patients. Patients will be asked to provide a saliva sample (1-3ml) by expectorating into a sample pot that they will be provided with. The sample will be analyzed at Imperial College London. Saliva samples will be destroyed following the analysis process.

Potential Risks

Risks and benefits

The principal risks of this study relate to the preservation of patient confidentiality in a multicenter study.

All study related documents and patient information for subjects enrolled in this study will be stored securely within a password protected networked computer at Virginia Mason. Hard copies of consent forms and paper questionnaires will be stored in a locked filing cabinet within the Benaroya Research Institute at Virginia Mason. The same standards regarding data and sample storage are practiced at Imperial College London.

Patients recruited to this study are being considered for curative treatment of esophageal cancer involving chemoradiotherapy and surgery. During the course of their treatment patients will be regularly required to fast and undergo invasive investigations and treatments. The risk of additional non-invasive interventions including breath, urine and saliva sampling are therefore considered to be negligible. Breath, urine and saliva sampling will be performed at the time of a routine clinical visit to avoid patients having to make additional visits to the hospital.

It is intended that the current study should have minimal impact on patient's routine clinical care. No extra financial costs will be passed to patients or their insurance provider.

Whilst no direct benefit is intended for individuals who agree to take part in this study, some patients may receive altruistic reward through participation in medical research that seeks to help other patients in the future.

Benefit is intended for future patients by establishing a greater understanding of methods of assessing and monitoring nutritional status in the setting of EC and surgery.

Adequacy of Protection Against Risks

Recruitment and Informed Consent

Informed written consent will be sought from all patients recruited to this study.

Patient identification: patients eligible for inclusion will be identified from the surgical practice of the local Principal Investigator (Dr Low, Virginia Mason Medical Center). Members of the research team will meet regularly with the multidisciplinary clinical team to ensure suitable identification and recruitment of patients.

Patients will be first approached by an appropriately trained member of the research team prior to receiving any treatment for EC either at the time of a routine outpatient appointment or by telephone. Patients will be provided with details of the study in an Informed Consent Form (ICF). When patients are initially approached, the details and requirements of the study will be explained to them and all questions answered. They will be allowed adequate time to decide whether they wish to take part in this study. If initial contact is via telephone, patients will be offered the opportunity to have the ICF sent to them either in hard copy or electronic form. Written informed consent will be obtained in person prior to the completion of any study specific procedures at either a routine outpatient appointment or on the day of sample collection.

In case of non English speaking patients, a certified, impartial translator will be included in the informed consent process to ensure the patient has a good understanding of the study and his/her requirements and that all questions are answered.

Patients will be made aware that any personal and confidential data related to their participation in this study will not be shared with anyone outside of their routine clinical care team, designated members of the research team or compliance oversight entities as required. Similarly it will be explained to patients that data from collected questionnaires will be pseudonymized before being analyzed.

It will be explained to the patient that a decision not to take part will have no influence on the standard of care they receive. Likewise patients will be informed that their consent for participation can be withdrawn at any time without need for explanation and without affecting the standard of care they receive. In the event of a patient withdrawing from the study, any samples that have been collected will be retained for analysis.

Protection against Risk

Risk of breach of confidentially
This study will involve the handling, storage and transportation of sensitive patient data including: consent forms; demographic data; meta-data; questionnaire responses, and results of physiological and biochemical analysis.

With the exception of consent forms, all data will be stored in a pseudonymized form using patients' unique study ID. Electronic data will be stored within password protected institutional computers. Paper records and clinical samples will be stored within appropriate institutional facilities protected by mechanical and/or electronic security controls. Only the principal investigator will have access to the code linking patient identities to pseudonymized data and the link will not be broken.

Patient demographics and meta-data, will be collected either in paper or electronic (Microsoft Office Excel) form. Where data are collected in paper form they will be subsequently transcribed to the electronic database.

Breath, urine and saliva samples will be labelled using a unique study identifier composed of patient's study ID and date sample was collected. It will not contain PHI.

All samples transferred to Imperial College London for the purpose of analysis will done so in a linked anonymized from, such that patients cannot be identified by outside parties. The link will never be provided in the future.

Once data analysis is complete, the data obtained in this study will be added to the IRB approved institutional database (IRB18-076). All research specific paper records associated with this protocol will be securely archived and stored for a minimum of 10 years after the study closes. The data will be stored long-term under IRB18-076 and any archival will follow as indicated in that protocol.

Risk of physical harm
It is considered that the risk of physical harm associated with this study is negligible. All interventions are non-invasive.

Identifying and reporting adverse events

Reporting Procedures

All adverse events (serious or non-serious) that are directly related to study activities will be reported. Adverse events (serious or non-serious) that occur as a result of patient's disease process or routine care, and any AEs that are not directly related to study procedures, will not be reported.

Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the local Principal Investigator.

Non serious adverse events

All unexpected non-serious adverse events which are related to study procedures should be recorded.

Serious adverse events

All serious adverse events related to a study procedure will be considered unexpected and thus will be reported. A report including all pertinent details related to a serious adverse event (including but not limited to: start date, stop date, thorough description of the event, treatment, and outcome) should be reported to the local Principal Investigator (Virginia Mason Medical Center, Dr Low) within 24 hours. Additionally, all such serious adverse events will be reported to the IRB.

Contact details for reporting SAEs
USA
Dr Donald Low MD
Donald.Low@virginiamason.org
Tel: (206) 223-6164

Data Monitoring

The Clinical Research Program will assess the risk of this trial and will devise and implement an internal monitoring and/or auditing plan for this trial. This plan will be revised as necessary during the life of the trial based upon a variety of factors, including but not limited to: protocol amendments, staff turnover, enrolment metrics, and compliance issues noted.

Potential Benefits Of The Proposed Research To The Subjects and Others.

There are no immediate benefits for the research participants recruited in to this study. Benefit is intended for future patients through aiding in surgical research. Specifically this will be through an improved understanding of the assessment of nutritional status in patients with EC. Patients may nevertheless gain altruistic reward through participation.

It is considered that the proposed study does not pose unreasonable risk to patients. Participation in this study is intended to have no adverse impact of patient's routine standard of care and quality of life. Enrollment to the study will be conducted in accordance with Good Clinical Practice and patients will be requested to provide informed written consent.

Potential impact: This research is intended to verify VOC biomarkers of EC cancer and to establish new understanding regarding response to therapeutic intervention. It is intended that this work will be conducted in parallel with other planned studies that are designed to establish the both the underlying mechanism for deregulate VOC production in EC cancer and methods for optimization diagnostic performance. The collective knowledge drawn from these studies will build a platform for future research by establishing: (i) proof of principal; (ii) mechanism; (iii) methodology for use in clinical practice, and; (iii) acceptability and feasibility. Using this evidence we will seek further funding to conduct a phase 4 biomarker development study (17). If we are successful in achieving these outcomes, we will have a clear line-of-sight to the introduction of our innovation into clinical practice. This work will benefit from our existing links to industry and the NIHR London in vitro diagnostic co-operative.

Research team:
Dr Donald Low is an expert EC cancer surgeon practicing at Virginia Mason Medical Center, a high volume center for the management of this disease. Virginia Mason has a long history of collaborative and innovative translational research designed to enhance patient experience. Existing infrastructure within Virginia Mason will support the conduct of this study through a dedicated team of research coordinators.

Professor George Hanna is the head of the VOC research laboratory based at St Mary's Hospital (Imperial College London). The group has over a decade of experience in the field of clinical breath testing and VOC research that is evidenced by their high impact publications within the field.

Dr Piers Boshier, a visiting scholar at Virginia Mason and NIHR clinical lecturer at Imperial College London, has extensive experience in clinical breath analysis. He is ideally placed to coordinate activities between US and UK centers.

Existing funds
This study is supported by funds from the Salgi Foundation.

Gantt chart

Year	2018				2019												2020											
Months	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Application for funding																												
IRB application																												
Study recruitment																												
Data analysis																												
Drafting manuscript																												
Application for further funding																												

References

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211-59.
2. Collaborators GBDCoD. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1151-210.
3. Cancer Research UK: liver cancer statistics: http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/liver-cancer - heading-One Accessed June 3rd 2018 2018 [
4. Harding P, Field PH. Breathalyzer accuracy in actual law enforcement practice: a comparison of blood- and breath-alcohol results in Wisconsin drivers. J Forensic Sci. 1987;32(5):1235-40.
5. Atreja A, Fu AZ, Sanaka MR, Vargo JJ. Non-invasive testing for Helicobacter pylori in patients hospitalized with peptic ulcer hemorrhage: a cost-effectiveness analysis. Dig Dis Sci. 2010;55(5):1356-63.
6. Petrone P, Sarkisyan G, Fernandez M, Coloma E, Akopian G, Ortega A, et al. Small intestinal bacterial overgrowth in patients with lower gastrointestinal symptoms and a history of previous abdominal surgery. Arch Surg. 2011;146(4):444-7.
7. Robroeks CM, van Berkel JJ, Jobsis Q, van Schooten FJ, Dallinga JW, Wouters EF, et al. Exhaled volatile organic compounds predict exacerbations of childhood asthma in a 1-year prospective study. Eur Respir J. 2013;42(1):98-106.
8. Kumar S, Huang J, Abbassi-Ghadi N, Spanel P, Smith D, Hanna GB. Selected ion flow tube mass spectrometry analysis of exhaled breath for volatile organic compound profiling of esophago-gastric cancer. Anal Chem. 2013;85(12):6121-8.
9. Kumar S, Huang J, Abbassi-Ghadi N, Mackenzie HA, Veselkov KA, Hoare JM, et al. Mass Spectrometric Analysis of Exhaled Breath for the Identification of Volatile Organic Compound Biomarkers in Esophageal and Gastric Adenocarcinoma. Ann Surg. 2015;262(6):981-90.
10. Abela JE, Skeldon KD, Stuart RC, Padgett MJ. Exhaled ethane concentration in patients with cancer of the upper gastrointestinal tract - a proof of concept study. Biosci Trends. 2009;3(3):110-4.
11. Amal H, Leja M, Funka K, Skapars R, Sivins A, Ancans G, et al. Detection of precancerous gastric lesions and gastric cancer through exhaled breath. Gut. 2016;65(3):400-7.
12. Xu ZQ, Broza YY, Ionsecu R, Tisch U, Ding L, Liu H, et al. A nanomaterial-based breath test for distinguishing gastric cancer from benign gastric conditions. British journal of cancer. 2013;108(4):941-50.
13. Amal H, Leja M, Broza YY, Tisch U, Funka K, Liepniece-Karele I, et al. Geographical variation in the exhaled volatile organic compounds. J Breath Res. 2013;7(4):047102.
14. Huang J, Kumar S, Abbassi-Ghadi N, Spanel P, Smith D, Hanna GB. Selected ion flow tube mass spectrometry analysis of volatile metabolites in urine headspace for the profiling of gastro-esophageal cancer. Anal Chem. 2013;85(6):3409-16.

15. Markar SR, Wiggins T, Antonowicz S, Chin ST, Romano A, Nikolic K, et al. Assessment of a Noninvasive Exhaled Breath Test for the Diagnosis of Oesophagogastric Cancer. JAMA Oncol. 2018.
16. Markar SR, Chin ST, Romano A, Wiggins T, Antonowicz S, Paraskeva P, et al. Breath Volatile Organic Compound Profiling of Colorectal Cancer Using Selected Ion Flow-Tube Mass Spectrometry. Ann Surg. 2017.
17. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93(14):1054-61.