BREATH® BIOPSY

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Breath-based detection of lung cancer using Exogenous Volatile Organic Compound targeting β-glucuronidase in the tumour microenvironment

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Aims

- Validate published results identifying D5-ethyl-βD-glucuronide as a non-invasive probe for cancer detection in mice
- Establish the presence of extracellular βD-glucuronidase in tissue samples from human lung cancers
- Demonstrate the ability to reliably detect D5-ethanol in human breath samples using Breath Biopsy
- Perform a Phase 1a study to assess safety of administering D5-ethyl-βD-glucuronide in healthy individuals
- Assess sensitivity and evaluate baseline D5-ethanol levels in human breath

1. Background and Objectives

Lung cancer is one of the most common forms of cancer (2.21 million new cases in 2020) and is a leading cause of death worldwide (1.8 million deaths in 2020 [1]). 5 year survival is around 16% having increased gradually over the last 40 years. [2]. A key issue is late diagnosis, with over half of cases diagnosed in Stages III and IV [3] where survival can be as much as 15-fold lower than in Stage I.

Widespread public screening programmes for lung cancer targeting at risk populations represent one of the greatest opportunities to improve early detection. However, currently the only suitable methods (e.g. CT scanning) are a limited resource that requires specialist capabilities and is not easily accessible to the majority of the population. A breath test for lung cancer represents a non-invasive, preferable approach for screening that would be more affordable, easy to use and accessible than current options.

A growing body of research has demonstrated the great potential for breath as a means to detect, monitor and guide treatment for a range of illnesses. Breath is a complex sampling matrix and issues such as high background signal and inconsistent sampling methods have so far limited progress in developing a clinically-relevant breath test for cancer.

One solution to this is the use of EVOC® Probes, the administration of a molecular probe that is responsive to disease-specific metabolic pathways and results in a product that can be detected and monitored on breath. In 2019, Lange et al. reported the use of D5-ethyl-βD-glucuronide (D5-EthGlu) as an EVOC Probe in mice, measured through the release of D5-ethanol [4]. We have performed research to validate these results and to initially assess the viability of D5-EthGlu for use as an EVOC Probe in human lung cancers.

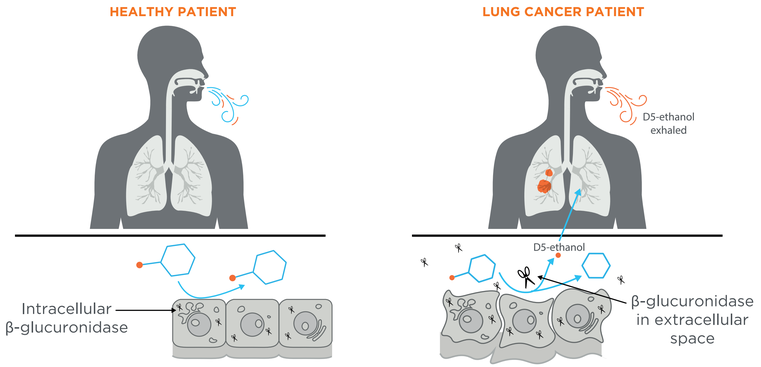


Figure 1: Mechanism of targeting β-glucuronidase with D5-EthGlu as EVOC probe.

References

1. Cancer fact sheet, World Health Organization, who.int/news-room/fact-sheets/detail/cancer
2. Lung cancer statistics, Cancer Research UK, cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer#heading-Two
3. Early diagnosis data hub, Cancer Research UK, crukcancerintelligence.shinyapps.io/EarlyDiagnosis/
4. Lange et al. (2019) Volatile Organic Compound Based Probe for Induced Volatolomics of Cancers *Angewandte Chemie International Edition* pubmed.ncbi.nlm.nih.gov/31518472/

2. Results and Discussion

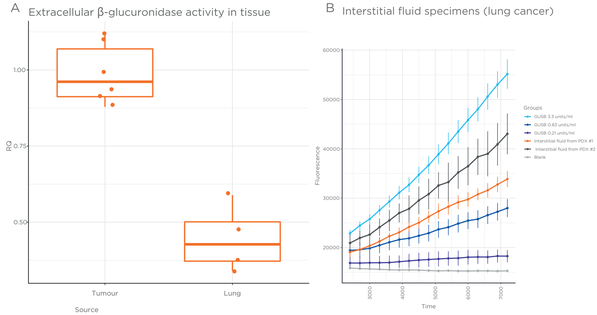


Figure 2: Extracellular β-glucuronidase activity in patient derived xenograft tissue (PDX). (A) PDX samples and lung tissue sample were placed in PBS for 10 minutes before collecting PBS for β-glucuronidase activity measurement using fluorescent activity assay. (B) Interstitial fluid samples of lung cancer PDX models showing β-glucuronidase activity. GUSB: b-glucuronidase purified from limpets

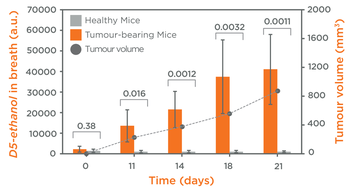


Figure 3: D5-ethanol levels in breath of tumour bearing mice compared to healthy mice. Tumours were xenografted subcutaneously on mice and probes were administered at indicated times post tumour initiation followed by D5-ethanol measurement released from mice. Tumour volumes were measured at same timepoints [4].

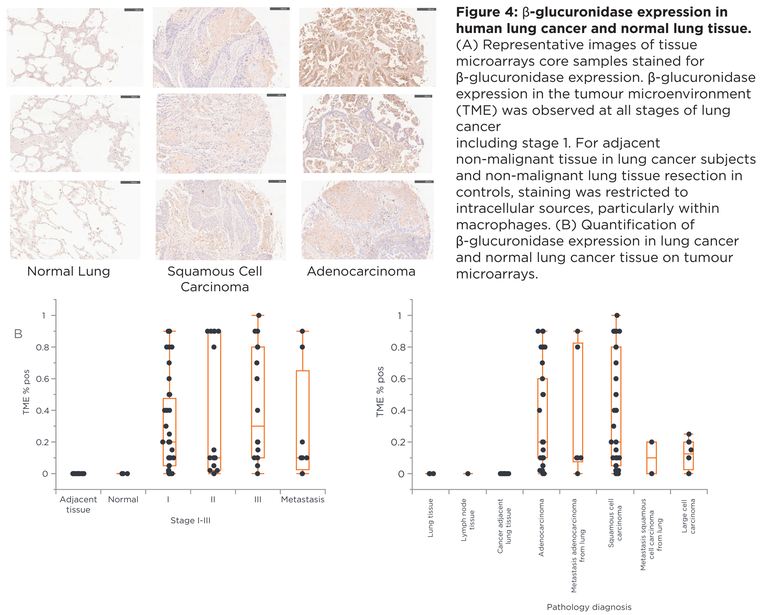
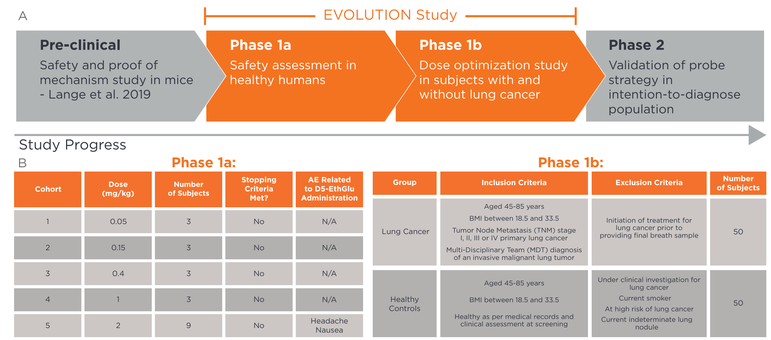


Figure 4: β-glucuronidase expression in human lung cancer and normal lung tissue. (A) Representative images of tissue microarrays core samples stained for β-glucuronidase expression. β-glucuronidase expression in the tumour microenvironment (TME) was observed at all stages of lung cancer including stage 1. For adjacent non-malignant tissue in lung cancer subjects and non-malignant lung tissue resection in controls, staining was restricted to intracellular sources, particularly within macrophages. (B) Quantification of β-glucuronidase expression in lung cancer and normal lung cancer tissue on tumour microarrays.



B

Phase 1a:

Cohort	Dose (mg/kg)	Number of Subjects	Stopping Criteria Met?	AE Related to D5-EthGlu Administration
1	0.05	3	No	N/A
2	0.15	3	No	N/A
3	0.4	3	No	N/A
4	1	3	No	N/A
5	2	9	No	Headache Nausea

Phase 1b:

Group	Inclusion Criteria	Exclusion Criteria	Number of Subjects
Lung Cancer	Aged 45-85 years BMI between 18.5 and 33.5 Tumor Node Metastasis (TNM) stage I, II, III or IV primary lung cancer Multi-Disciplinary Team (MDT) diagnosis of an invasive malignant lung tumor	Initiation of treatment for lung cancer prior to providing final breath sample	50
Healthy Controls	Aged 45-85 years BMI between 18.5 and 33.5 Healthy as per medical records and clinical assessment at screening	Under clinical investigation for lung cancer Current smoker At high risk of lung cancer Current indeterminate lung nodule	50

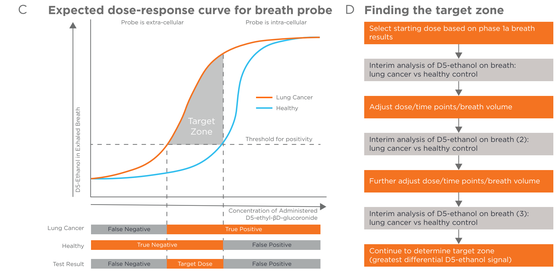


Figure 5: Design of Evolution clinical trial. (A) Study progress (B) Phase 1a design and results from safety assessment and phase 1b trial design. (C) Expected dose-response in lung cancer patients compared to healthy controls. (D) Adaptive design to find the target zone with the highest differential D5-ethanol signal on breath between lung cancer patients and heathy controls.

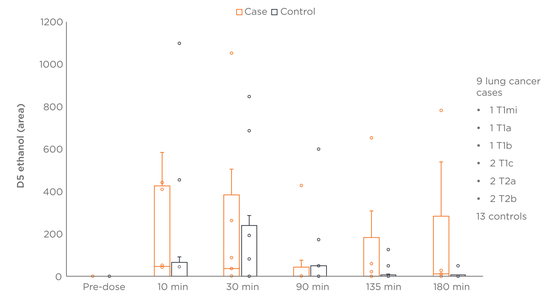


Figure 6: Preliminary results. D5-Ethanol level in breath samples of 9 patients with lung cancer and 13 healthy controls that received 2 mg/kg of eVOC probe. Breath samples collected in 6 timepoints, including before administration of eVOC probe (pre-dose). The cleavage product D5-ethanol could be detected on breath in a subset of participants.

3. Conclusions

Through a combination of *in vitro*, *in vivo* and clinical studies we have produced evidence to support the application of D5-EthGlu as an EVOC Probe to enable non-invasive breath testing as a means for early detection of lung cancer. We have produced initial data showing that D5-EthGlu is safe and acceptable for use in human patients. Our preliminary analysis demonstrates proof of mechanism for the in human cleavage of the volatile reporter molecule D5-ethanol from D5-ethyl-β D-glucuronide. Such an EVOC probe approach is attractive as it has the potential to optimise the signal to noise ratio of a breath test. These results provide a promising foundation for a phase 2 dose-finding study designed to explore diagnostic performance of this innovative breath test approach for lung cancer.