Proof-of-mechanism for a diagnostic probe generating D5-ethanol as an on-breath reporter molecule for lung cancer – Evolution phase 1

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At A Glance:

- The expression of metabolic pathways associated with the production of breath biomarkers have been found to be altered in lung cancer. Through administration of a so-called exogenous Volatile Organic Compound Probe (EVOC-probe) such metabolic pathways could be utilised to generate sensitive and specific on-breath signals for lung cancer.
- D5-ethyl-β-D-glucuronide may have value as such a diagnostic EVOC probe to establish the presence of extracellular β-glucuronidase as a sign of lung cancer in humans.
- The potential of this approach was substantiated in this study by demonstrating the differential expression of β-glucuronidase in the tumor microenvirnoment in human lung cancer tissue samples.
- *In vivo* administration of the EVOC probe demonstrated an excellent safety and tolerability profile.
- D5-ethanol was detected on breath providing a proof of mechanism for the cleavage of D5-ethyl-β-D-glucuronide in humans.

1. Background and Objectives

Analysis of volatile metabolites in breath represents an attractive potential diagnostic modality for lung cancer.

One of the key challenges for the realization of this potential is to optimize the signal-to-noise ratio in the fundamentally complex matrix of breath.

The Evolution study is a proof of mechanism study to evaluate whether administration of a probe compound, specific to tumour-associated extracellular β-glucuronidase, results in the production of a unique exogenous volatile organic compound (EVOC) on breath. Such an EVOC probe could prove of significant diagnostic value as a breath-based test for lung cancer.

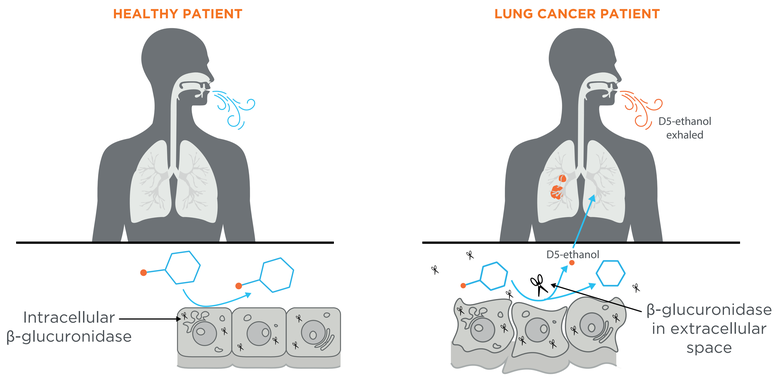

Figure 1: Mechanism of targeting β-glucuronidase with D5-ethyl-β-D-glucuronide as EVOC probe.

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/
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2. Methods

Tissue analysis

- Evaluation of β-glucuronidase activity in human lung tissues by immunostaining in 140 lung tissue core samples (Rabbit Polyclonal IgG, Abcam ab244453).
- Samples included 86 lung cancers (stage I-III) across a range of histologies as well as adjacent non-malignant tissue in lung cancer subjects (38) and benign lung tissue resection in controls (16). The latter included non-malignant lung tissue and lymph nodes.
- Percent positivity of β-glucuronidase in neoplastic cells and in the tumor microenvironment (TME) components were scored manually by an independent board-certified MD pathologist.
- Any TME component showing positivity for glucuronidase, was classified as "positive" (1), if there was no glucuronidase in the TME, this was classified as "negative" (0).

In vivo

- A phase 1a single ascending dose study in 21 healthy controls was conducted to evaluate safety and tolerability of the EVOC-probe.
- A complentary phase 1b study is ongoing to evaluate proof of mechanism by determining if D5-ethanol could be detected on breath in response to cleavage of the probe.
- For 31 patients of phase 1b (12 lung cancer patients and 19 healthy controls), D5-ethyl-β-D-glucuronide has been administered intravenously and breath samples were collected pre-dose and at variable intervals up to 180 minutes after treatment.

3. Results

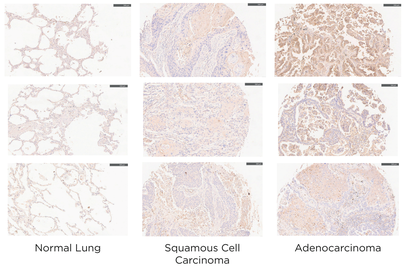

Figure 2: Representative tissue immunostaining for β-glucuronidase. A clear accumulation of β-glucuronidase in the TME can be observed for squamous cell and adenocarcinoma tissue samples. Staining of macrophages was observed across sample types.

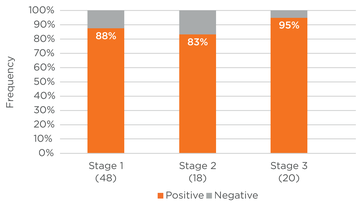

Figure 3: Immunostaining of 140 lung resection specimens. Positive indicated presence of β-glucuronidase in TME components.

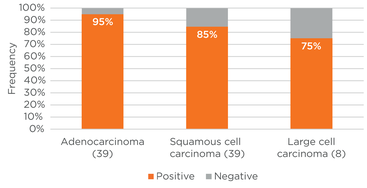

Figure 4: Positive staining of the TME happened across main lung cancer histologies.

Table 1: Age and gender distribution in Evolution Phase 1

Population	Phase 1a	Phase 1b	
	Healthy (N=21)	Healthy (N=19)	Lung cancer (N=12)
Gender [female; N(%)]	12 (57)	12 (63)	7 (58)
Age (years; Mean ±)	48 ± 15	59 ± 10	67 ± 9

Table 2: Safety and tolerability of OWL-EVO1 in Evolution Phase 1

Adverse Event	Relatedness	Intensity	SAE	Number of Occurences
Hypoglycemia	Possible	Mild	No	2
White Blood Cell Disease	Possible	Mild	No	1
Hypokalemia	Possible	Mild	No	1
Headache	Possible	Mild	No	1
	Related	Moderate	No	1
Nausea	Related	Mild	No	1

No serious adverse events (SAE) were reported during the phase 1a study and, to date, no SAE has been reported in 1b. A single individual reported a self-limiting case of nausea and a headache when dosed at 2mg/kg (highest dose).

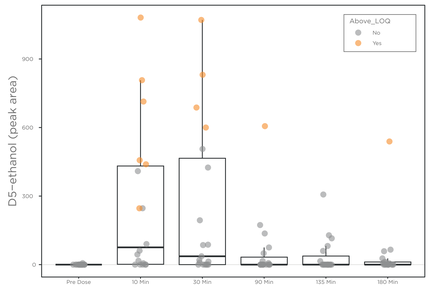

Figure 5: Preliminary results showing detection of D5-Ethanol in breath samples of 7 patients and 12 healthy individuals from Evolution Phase 1B. All individuals received 2 mg/kg probe. Breath samples were collected in 6 timepoints, including before administration of eVOC probe. ReCIVA breath sampler was used. Data from samples analysed with optimized analytical method for D5-ethanol detection is shown. D5-ethanol released by the probe cleavage was detected in a subset of individuals. However, D5-ethanol was possible to be quantified in only 13% of breath samples collected after EVOC probe treatment. LOD: limit of quantification.

4. Conclusions

- β-glucuronidase is upregulated in the TME of lung cancer cells across histological subtypes and cancer stages.
- Administration of D5-ethyl-β-D-glucuronide as EVOC probe for the evaluation of β-glucuronidase had no SAEs and was well tolerated.
- In a subset of individuals D5-ethanol can be detected on breath of humans after intravenous administration of D5-ethyl-β-D-glucuronide.
- The successful phase 1 study lays the foundation for a phase 2 study designed to explore diagnostic performance of this innovative breath test approach for lung cancer.

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