Targeting Tumor Associated AKR Activity with EVOC Probes to Detect Lung Cancer

BREATH® BIOPSY

Cancer metabolism represents a promising and largely untapped focus for diagnostic testing. Whilst cancer genomics can vary extensively, these changes converge onto key metabolic pathways...

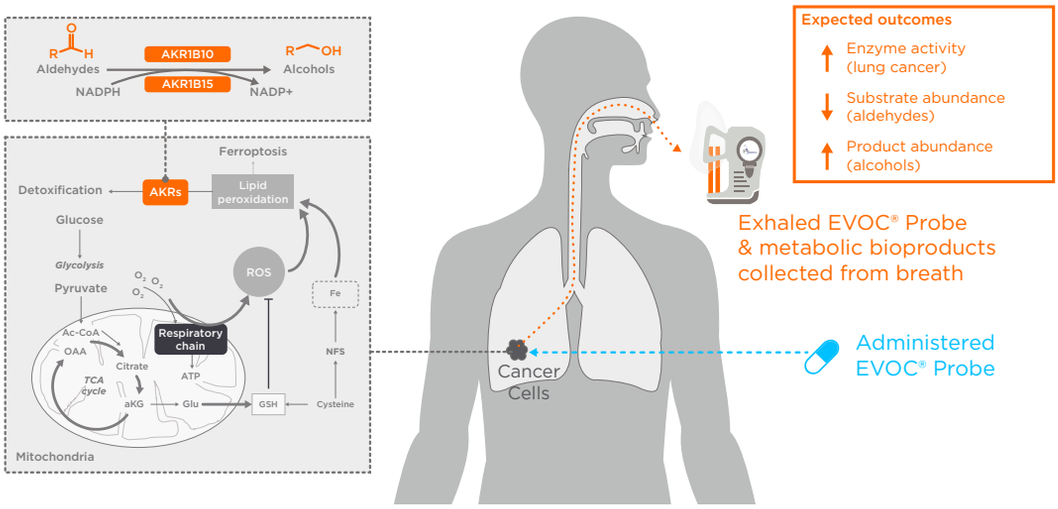

Figure 1: The EVOC Probe approach. An administered EVOC Probe is introduced and monitored non-invasively on breath along with its metabolic products. Aldo-keto reductases (AKRs) are a potential EVOC Probe target that are upregulated in some cancers as an adaptation to oxidative stress and lipid peroxidation.

Lung cancer is an area where there is significant unmet need to improve early detection through screening of at-risk populations. The location of lung cancer makes them well suited for detection via breath sampling.

Rapid growth, poor blood flow and persistent genetic mutations in cancer cells contribute to a high level of oxidative stress characterized by an increase in reactive oxygen species (ROS). In turn, ROS promote destructive processes such as lipid peroxidation which produce aldehydes. Human lung cancers upregulate the expression of aldo-ketoreductase (AKRs) enzymes to help process these excess aldehydes and reduce them to alcohols.

We are seeking to investigate whether AKRs can be targeted using an exogenous volatile organic compound probe (EVOC® Probe), the metabolism of which can be monitored on breath using Breath Biopsy®.

...the use of agents such as UDP-glucose in PET scans serves to illustrate how we can target metabolic pathways with exogenous compounds to provide high sensitivity cancer detection...

To inhibit the catalytic activity of AKR1B10/B15, two lung cancer cell lines were treated for 24h with two AKR inhibitors (Tolrestat: IC50 and/or IC75 based on AKR activity assay; JF0064: 10μM).

For permanently abrogated AKR1B10 or AKR1B15 expression, three clones of AKR1B10 knockout and three clones of AKR1B15 knockout in the background of A549 cells were developed using the CRISPR-Cas9 system. A Mock-Cas9 was also used as a control (wild type/WT cells). Cells were treated with 10μM of aldehydes (30 μM for Alkanal 2), and aliquots of cell culture media were collected at 3 timepoints for headspace analysis.

50μL of aqueous samples containing the volatiles were used for headspace analysis. Quality control mix (deuterated standards and a panel of aldehydes and alcohols) were injected onto sorbent tubes and ran throughout the sequence.

The mass spectrometry method was selected based on the higher sensitivity and specificity for each pair of aldehydes and alcohol products, either TD-GC-MS or TOF-MS.

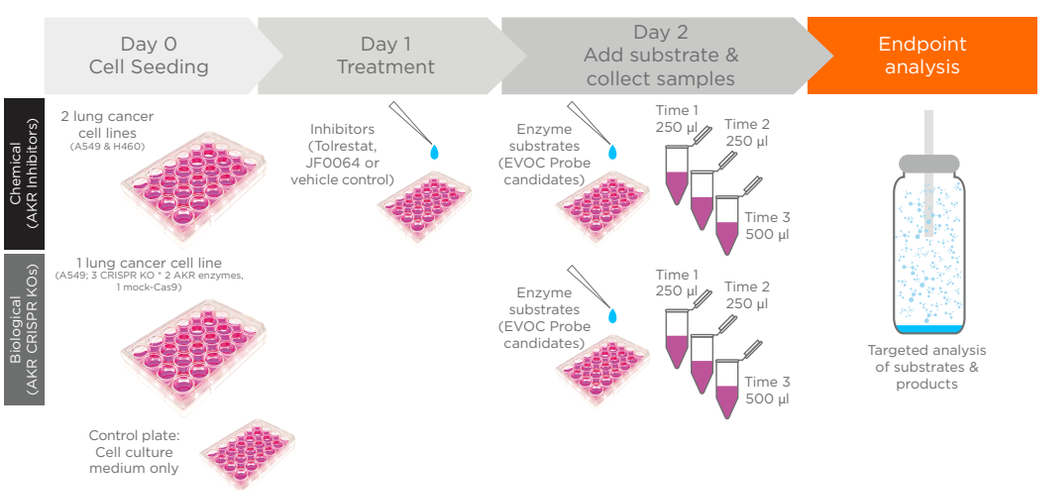

Figure 2: Overview of Cell Culture Method. The biological and chemical *in vitro* methods used to investigate the effect of AKR activity modulation on released headspace volatile organic compounds (VOCs).

Find out more about Owlstone Medical's Headspace VOC Analysis

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...our hypothesis is that a similar approach can be used in combination with non-invasive breath sampling in a form that is well suited for screening programs, to provide reliable early detection of lung cancer...

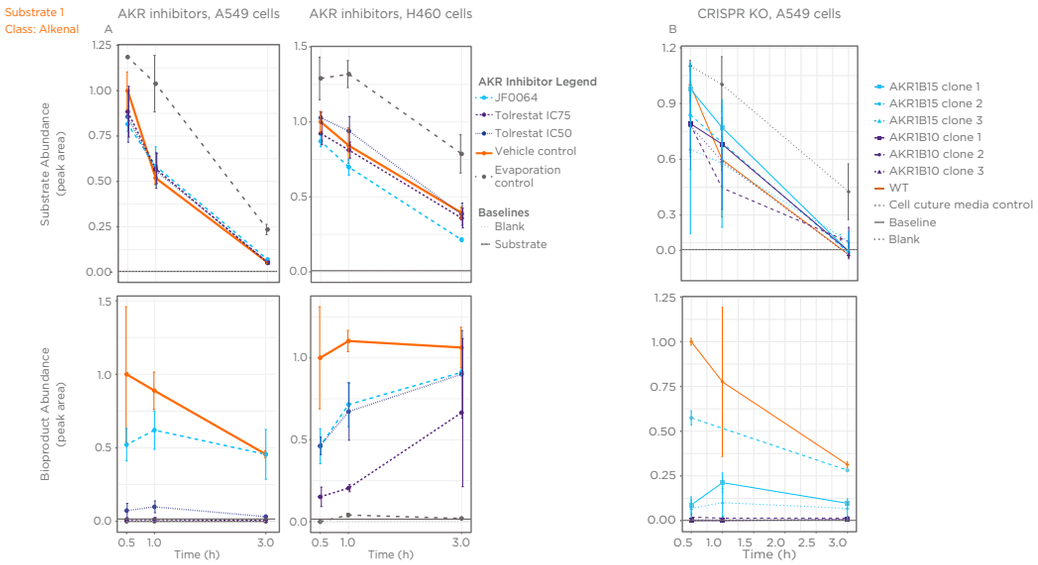

Figure 3: AKR1B10 and AKR1B15 are responsible for the reduction of an alkenal to its alkenol bioproducts in lung cancer cells. A) The AKR inhibitor JF0064 reduced about 50% of the production of alkenol and a dose-dependence response was observed between Tolrestat and the inhibition of alkenol production in A549 and H460 cells treated with alkenal 10μM. B) Knockout of AKR1B10 minimized the production of alkenol under alkenal administration (10μM). AKR1B15 knockout also reduced the production of alkenol compared to wild-type cells. Blank: cell culture media only (no aldehydes added). Baseline: mean of analysis using cell culture media (no aldehydes added) from cell lines with/without inhibitor (A) or different clones (B). Data normalized by vehicle/WT group (first timepoint).

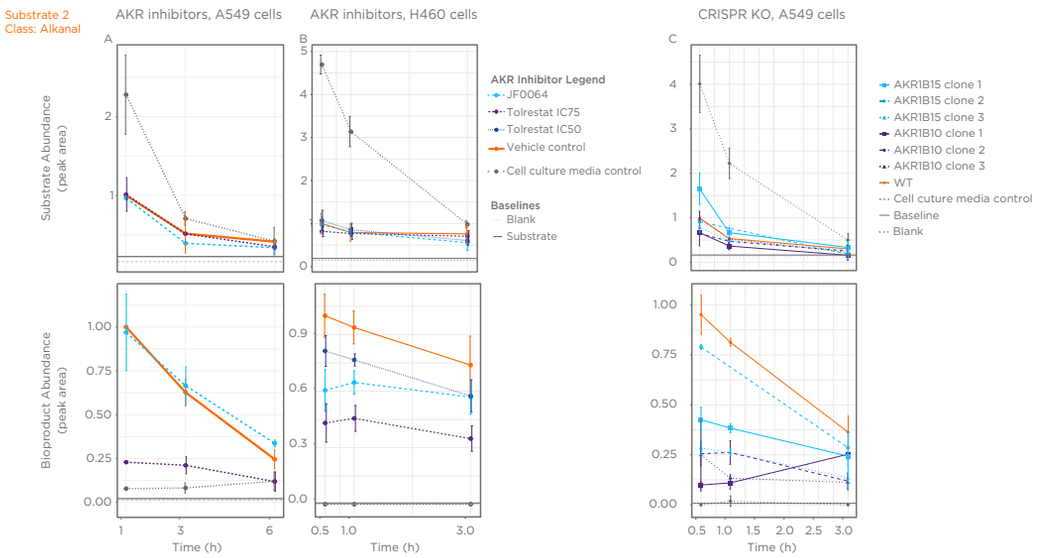

Figure 4: AKR1B10 and AKR1B15 are responsible for the reduction of an alkanal to its alkanol bioproducts in lung cancer cells. A) Tolrestat inhibited the alkanol production in A549 cells treated with alkanal 10μM, but no effect of JF0064 was detected. B) The AKR Inhibitor JF0064 reduced the production of alkanol by about 40%. A dose-dependant response was observed between tolerestat and the inhibition of alkanol production in H460 cells treated with alkanal 10μM. C) AKR1B10 knockouts and AKR1B15 knockouts with reduced expression of AKR1B10 show reduced production of alkanol under alkanal administration (10μM). AKR1B15 knockout without AKR1B10 downregulation showed 20% reduction in the production of alkanol compared to wild-type cells similar to the overall reduction in AKR activity detected by colorimetric assay. Data normalized by vehicle/WT group (first timepoint).

...incidence and mortality in these cancers are high and the majority of cases are diagnosed in the latter stages, indicating a clear clinical need to improve early detection.

Overall, headspace from A549 and H460 samples showed lower levels of aldehydes and higher levels of alcohol products than those observed in evaporation controls confirming that AKRs are active in the studied lung cancer cells. Although no significant difference is observed in the analysis of substrate abundance, our study shows that AKR1B10 and AKR1B15 are responsible for the reduction of multiple aldehydes to alcohol products in lung cancer cells.

Using an *in vitro* study of human lung cancer cells, we have demonstrated the potential to monitor the metabolic conversion of administered EVOC Probe aldehydes into alcohols by AKRs, and have used AKR inhibition/knockout to show that this relationship is specific and sensitive to manipulation.

We are now seeking to investigate the same relationships through *in vivo* and *ex vivo* sampling to further establish the potential of using EVOC Probes to target AKR metabolism as a tool for early detection of lung cancer.

Contact us to find out more about collaborating with Owlstone Medical and to discuss incorporating Breath Biopsy in your biomarker research.

breathbiopsy@owlstone.co.uk

Owlstone Medical Ltd, 183 Cambridge Science Park,
Milton Road, Cambridge, CB4 0GJ, UK

@owlstonemedical

Company Number 04955647 | VAT Number 260449214

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