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Latest results validate biomarkers for PromarkerEso blood test for oesophageal adenocarcinoma

- **Proteomics International's novel PromarkerEso blood test for oesophageal adenocarcinoma advances with biomarker panel clinically validated in second independent patient group**
- **Test targets both oesophageal adenocarcinoma and patients with pre-malignant condition Barrett's oesophagus which affects 1-2% of adults and can arise from chronic acid reflux**
- **Oesophageal cancer is the 6th leading cause of cancer-related mortality, the 7th most common cancer globally and has a less than 20% five-year survival rate**
- **Latest results presented at the 29th Annual Lorne Proteomics Symposium, Victoria, Australia**

Proteomics International Laboratories Ltd (Proteomics International; ASX: PIQ), a pioneer in predictive diagnostics is pleased to announce a milestone for its novel blood test for oesophageal adenocarcinoma, PromarkerEso, with the clinical validation of the biomarker panel in a second independent patient group. The results are being presented at the 29th Annual Lorne Proteomics Symposium, the annual conference of the Australasian Proteomics Society, held 31 January - 3 February 2024.

Proteomics International's simple blood test utilises biomarkers—protein 'fingerprints' in the blood—to screen for oesophageal adenocarcinoma. The Company's prototype diagnostic test previously identified 89% of patients with oesophageal adenocarcinoma and 92% of patients without the disease in studies of 300 patients across two patient cohorts [ASX: 8 September 2023].

The aim of the current study was to confirm that the panel of previously identified glycoprotein biomarkers change in concentration in patients with oesophageal adenocarcinoma, and this was successfully achieved. The study analysed a second independent clinical cohort comprising patients obtained from the Victorian Cancer Biobank [ASX: 23 July 2023], with clinically diagnosed oesophageal adenocarcinoma (N=66) or healthy controls (N=99). The results demonstrated excellent statistical significance of multiple biomarkers in diagnosing oesophageal cancer. Due to missing clinical variables and small sample size however, analysis using the previously developed prototype diagnostic models was not possible. See the attached presentation for further information.

Proteomics International's Managing Director Dr Richard Lipscombe said, *"The results presented at the conference are exciting, and further strengthen the diagnostic performance of PromarkerEso. Confirming the clinical performance of the biomarkers in a second independent patient group was a critical milestone in the development of our potential breakthrough blood test. The next step in bringing PromarkerEso to the clinic is a larger validation study to confirm the diagnostic accuracy of the test, which is already progressing. Of equal significance, we are delighted to see our Promarker™ platform performing so well and mirroring the success we see with the PromarkerEndo diagnostic test for endometriosis [ASX: 1 February 2024]."*

Oesophageal adenocarcinoma is the most common form of oesophageal cancer and is an area of significant

unmet medical need. The overall five-year survival rate for this cancer is less than 20 per cent, and 1 in 20 cancer deaths worldwide in 2018 were attributed to oesophageal cancer[1]. An estimated 10-15% of patients with chronic acid reflux develop Barrett's oesophagus, a condition which is asymptomatic and affects 1-2% of Western populations[2].

Screening for oesophageal adenocarcinoma currently requires a specialist endoscopy procedure that costs US$2,750 per patient in the United States[3], where the total expenditure on treating oesophageal cancer was $2.9 billion in 2018[4]. In particular, people with Barrett's oesophagus are much more likely to get oesophageal adenocarcinoma, and are advised to get regular endoscopies to screen for oesophageal cancer. PromarkerEso could provide such early screening, both for early diagnosis of the disease, and to closely monitor at-risk patients and rule in or rule out the need for surgical procedures.

Presentation details: 29th Annual Lorne Proteomics Symposium; poster presentation [copy attached]
Title: *Validation of Biomarkers for Oesophageal cancer.*

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Authorised by the Board of Proteomics International Laboratories Ltd (ASX: PIQ).

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About Proteomics International Laboratories (PILL) (www.proteomicsinternational.com)
Proteomics International (Perth, Western Australia) is a wholly owned subsidiary and trading name of PILL (ASX: PIQ), a medical technology company at the forefront of predictive diagnostics and bio-analytical services. The Company specialises in the area of proteomics – the industrial scale study of the structure and function of proteins. Proteomics International's mission is to improve the quality of lives by the creation and application of innovative tools that enable the improved treatment of disease.

About the Promarker™ Platform
Proteomics International's diagnostics development is made possible by the Company's proprietary biomarker discovery platform called Promarker, which searches for protein 'fingerprints' in a sample. This disruptive technology can identify proteins that distinguish between people who have a disease and people who do not, using only a simple blood test. It is a powerful alternative to genetic testing. The technology is so versatile it can be used to identify fingerprints from any biological source, from wheat seeds to human serum. The Promarker platform was previously used to develop PromarkerD, a world-first predictive test for diabetic kidney disease, that is currently being commercialised. Other tests in development include for endometriosis, asthma & COPD, oesophageal cancer, diabetic retinopathy and oxidative stress.

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[1] Nature Reviews Gastroenterology & Hepatology, 2021, doi.org/10.1038/s41575-021-00419-3
[2] American Society for Gastrointestinal Endoscopy, www.asge.org

[3] www.newchoicehealth.com/endoscopy
[4] JAMA Network Open, 2021, doi:10.1001/jamanetworkopen.2021.27784

Validation of Biomarkers for Oesophageal Cancer

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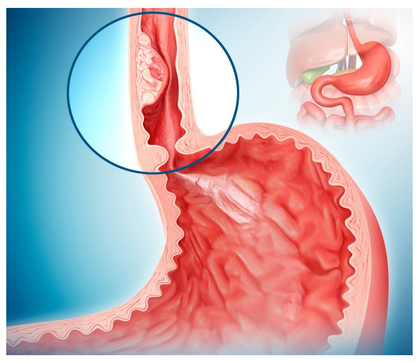
Background

- Oesophageal Adenocarcinoma (OAC) is the most common form of oesophageal cancer in Australia, usually detected in advanced stages.
- Despite advances in medical treatment, oesophageal cancer remains a lethal condition with a 5-year survival rate of around 15%[1].
- During 2023 in Australia, 1,742 people were diagnosed with oesophageal cancer and it claimed the lives of 1,419 people[2].



- **PromarkerEso**, a targeted mass spectrometry-based assay was developed to analyse serum samples for OAC.

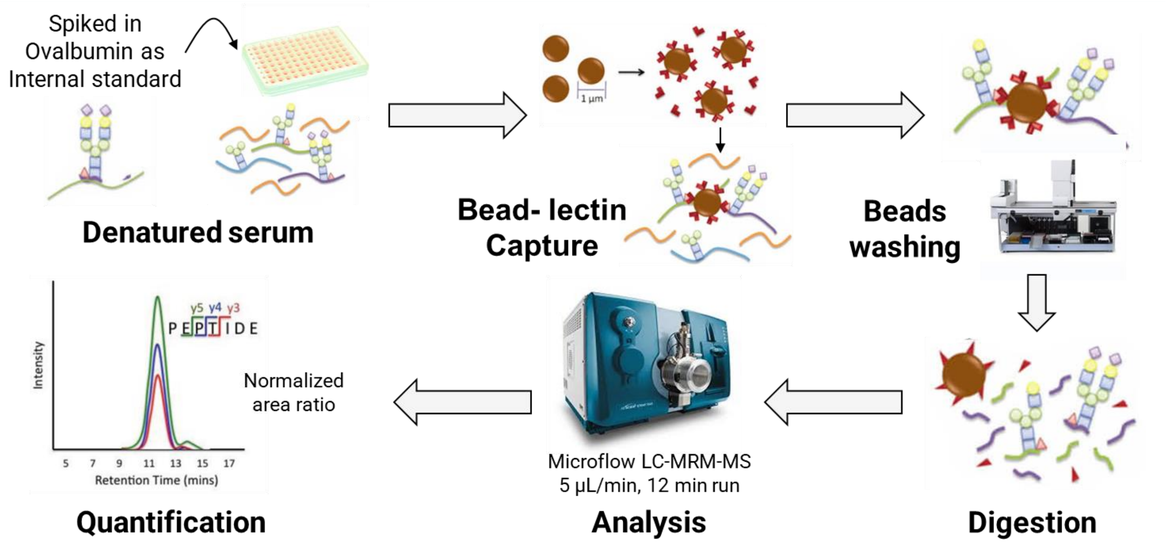
Aim

- To optimise the assay, for a simpler streamlined workflow for easier commercialisation.
- To validate a panel of previously identified protein biomarkers in an independent cohort, for use in OAC diagnosis.

Participants

- Serum samples (n=165) were collected at Victorian Cancer Biobank centre and analysed across two clinical groups:
  - **Group 1**: Healthy controls (n=99),
  - **Group 2:** Oesophageal Adenocarcinoma (n=66)
- 82% of the healthy group is female, 8% of the OAC group is female.
- Mean age for the healthy group is 37.9 years of age.
- Mean age for the OAC group is 64.6 years of age.

Methods



- The method is based on lectin pulldown of specific proteoglycans on a magnetic bead.
- The assay includes 10 proteins represented by 32 peptides.
- Compared with the method used in the discovery phase, the following improvements were made:
  - Simplified buffers with less detergent to reduce mass spectrometry interference.
  - Conversion to 96 well plate format.
  - Rapid 1 hr trypsin digestion, previously 16 hr.
  - Microflow LC-MS 12 min run, previously 45 min highflow.
- Data was analysed with Skyline and ratios against an internal standard ovalbumin peptide were calculated.
- All statistical analysis was performed in STATAv17.0, box-whisker plots were generated with an unpaired two-tailed t-test with Welch's correction.

Results

- The optimised assay shortened the original 3-day process to 1 day.
- All 10 proteins assayed were significantly associated with disease status.
- Of the 32 peptides, 25 (78%) showed significant association.
- Box plots with significance bars were generated for each peptide where significant comparisons were identified. Figure 1 shows 10 of the peptides that had significant differences between healthy controls and OAC samples.
- Due to small sample size, external validation of previously developed multivariate models was not possible.

Results

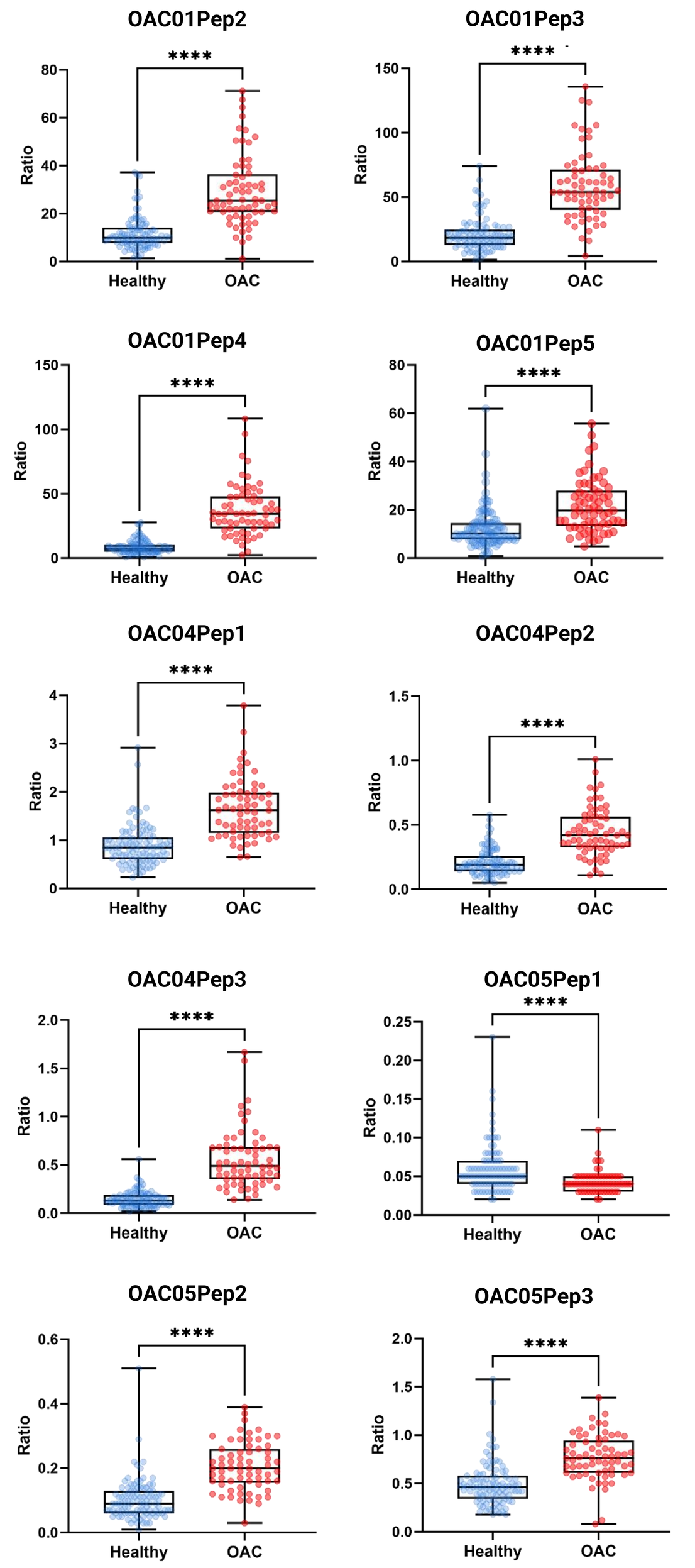


Figure 1: Box plots comparing the data distribution of Ovalbumin normalized ratios for 10 peptides that showed a consistent significant correlation for healthy control vs Oesophageal Adenocarcinoma. Individual data points are shown as blue dots (Healthy, n=99) and red dots (OAC, n=66). The horizontal line in the middle of the box is the median value of the scores and the lower and upper boundaries indicate the 25th and 75th percentiles, respectively. ****represents p-value < 0.001, unpaired two-tailed t-test, Welch's correction.

Conclusions

- The optimised **PromarkerEso** assay was developed for the detection of 10 proteins (32 peptides) identified as biomarkers for OAC.
- All 10 proteins were significantly associated with OAC in this independent clinical cohort, providing robust evidence for inclusion in a diagnostic test for detection of OAC.

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[1] Robert *et. al.* (2023). Oesophageal cancer 2023. Surgery (Oxford), 11(41), [2] Gi Cancer Organisation. (2024). https://gicancer.org.au/cancer/oesophageal-cancer/.