

Shareholder Newsletter

During the month of June, BCAL Diagnostics presented at ASCO (American Society of Clinical Oncology) Annual Scientific Meeting to an international audience. Dr Gillian Lamoury (BMed FRANZCR, Radiation Oncologist), presented BCAL's second poster presentation, "Detection of localised breast cancer by lipidomic signature directly from plasma" to this prestigious meeting on June 4 in Chicago, IL, USA.

LIPIDOMIC SIGNATURE FROM PLASMA TO DETECT LOCALISED BREAST CANCER

#565

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A NOVEL BLOOD SCREENING TEST FOR BREAST CANCER DETECTION

Screening mammography has many technical, logistic and diagnostic limitations. An effective and accurate blood test should increase the screening detection rate and reduce mortality from breast cancer. BCAL Diagnostics is evaluating the potential of a plasma lipidomic biomarker signature to detect early-stage breast cancer.

PLASMA EXTRACELLULAR VESICLES (EV) LIPIDOMIC BIOMARKER SIGNATURE

Following BCAL's previous report and studies on lipid signatures derived from plasma-enriched EVs that effectively distinguished people with localised breast cancer from cancer-free controls, BCAL aimed to investigate the utility of the above lipid signature directly in plasma.

Rationale

To reduce the complexity and to advance the commercial viability of the test as they move towards clinical application, we seek to evaluate the cancer detection performance of previously identified lipid signature in plasma samples eliminating the EV enrichment step.

PREVIOUS PLASMA EV STUDY COHORT & PILOT PLASMA STUDY

Blood was collected from women with stage 0-IV breast cancer; age and BMI matched breast cancer-free controls.

Plasma samples (n = 256) corresponding to patients from Cohort 3 and 4 were selected for analysis.

■ Control ■ IDC (stages I-IV) ■ IDC (stages I-III) ■ ILC (stages I-III) ■ DCIS (stage 0)

Cohort	EV study				EV Cohort		AUC*
	Control	IDC	ILC	DCIS	Control	Cancer	
Cohort 1	44	101	100	48	1	101	0.89
Cohort 2	101	100	48	2	3	100	0.77
Cohort 3	101	100	48	4	384	100	0.88
Cohort 4	100	51	48	384	50	50	0.88

*Internally cross validated

LIPIDOMIC METHODOLOGY



A plasma lipid biomarker signature shows potential for detection of early breast cancer with IMPRESSIVE AUC

Ongoing prospective studies will evaluate the BCAL lipid biomarker-based test as an adjunct to mammographic screening and pathological diagnosis

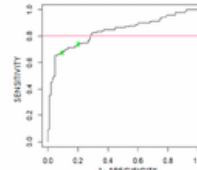
DATA ANALYSIS AND BIOMARKER SELECTION



The same data analysis and biomarker selection workflow applied on previous EV studies was employed on the pilot plasma cohort, analysing 400+ curated lipids. The classification potential of plasma samples was assessed via Partial Least Square Discriminant analysis (PLSDA) and logistic regression using leave one out cross-validation (LOOCV).

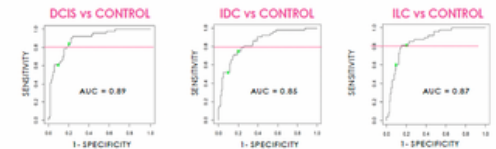
THE PLASMA LIPID SIGNATURE PERFORMANCE IS COMPARABLE TO EV'S

Performance (LOOCV)	EV 256 Samples	Plasma 256 Samples
Accuracy	0.78	0.79
Sensitivity	0.81	0.79
Specificity	0.74	0.78
Pos Pred Value	0.82	0.84
Neg Pred Value	0.73	0.73
AUC	0.85	0.85



PLASMA BIOMARKER PERFORMANCE IS STRONG IN CANCER SUBTYPES

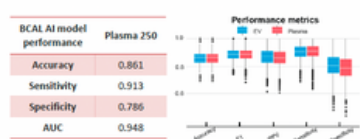
Receiver operating characteristic (ROC) curves for pilot plasma cohort data



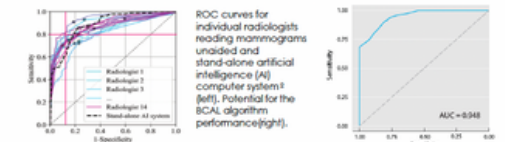
The more conservative PLSDA approach demonstrated consistent performance in EV and Plasma cohorts. This supported pursuing testing in neat plasma.

NEXT STEP

To identify lipid signatures with higher predictive power, a proprietary Artificial Intelligence (AI) biomarker discovery pipeline was employed.



POTENTIAL OF THE BCAL TEST TO BE ADJUNCT TO MAMMOGRAPHY



FUTURE DIRECTIONS

Clinical studies are underway to confirm and validate BCAL test performance. Development and validation of BCAL methodology in a NATA-accredited clinical lab. Upcoming clinical study will investigate the test as an adjunct to mammography.

References

1. Ekao EU et al. Errors in Mammography Cannot be Solved Through Technology Alone. Asian Pac J Cancer Prev (2018);
2. Kraljicova-Rud et al. Detection of Breast Cancer with Mammography: Effect of an Artificial Intelligence Support System (2019).

Acknowledgements

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As an Australian owned and operated company developing a blood-based test for the Australian community, we're proud to work closely with leading Australian clinicians, surgeons and oncologists.

In June BCAL Diagnostics was invited to present at the Melbourne **Breast surgeons journal club**. With 36 breast surgeons in attendance, we were able to share important updates to the BCAL Diagnostics science. As we continue to gather samples for our research, BCAL has engaged the breast cancer management community to assist in the collection of patient samples. We are now looking to expand our sample collection to Melbourne and this group of surgeons will help us in this endeavour.

Breast Cancer Trials Conference - BCAL Diagnostics is delighted to be a Bronze sponsor at the 2023 BCT Conference in Auckland. Leading breast surgeon Dr Sanjay Warriar will be presenting on behalf of BCAL Diagnostics at the BCT Conference. This is the most significant conference for breast cancer surgeons and oncologists in Australia and New Zealand. BCAL continues to build clinical credibility by presenting our results and rationale from the blood test for breast cancer development and clinical studies. This is important step as we will be launching our test first in Australia and New Zealand. Dr Warriar will be providing a scientific and clinical update based on the presentation at ASCO in Chicago in June 2023.

This month we have seen BCAL Diagnostics featured in **The Australian, Forbes Australia** and **10 News**.

The continued extensive interest from the Australian media has helped build awareness of BCAL Diagnostics, driving hundreds of people to the BCAL website and register their interest in providing much needed blood samples.



Research and Development update

Installation and Qualification of the Vanquish/Orbitrap Exploris 240 LCMS instrument. This will be the research workhorse Mass Spectrometer in our new laboratory, which is being established as the location for the first entry of BCAL's breast cancer diagnostic to the market.

The Science Team, after training by suppliers Thermo Fisher Scientific and optimisation of the system have run the first BCAL plasma samples to verify performance. The Team has commenced our first in-house stability study to inform optimal and acceptable sample collection and transport conditions for when the test is launched commercially.

Other recent milestones include:

Installation and Qualification of the TSQ Altis Plus Triple Quad Mass Spectrometry instrument in partnership with Thermo Fisher Scientific. This system has been placed by Thermo Fisher as a seed instrument in the BCAL lab to explore the viability of the platform as the clinical diagnostic workhorse, offering enhanced performance for the BCAL markers. Since this is the Triple Quad mass spectrometer is most commonly used by diagnostic laboratories worldwide, our studies will confirm the transfer of the BCAL test from the research phase to the product development phase on a widely available and commonly used clinical laboratory instrument. This will facilitate the adoption of our test into routine clinical laboratories once we receive regulatory approval.

In other news

We would also like to welcome a number of new additions to the BCAL Diagnostics team:

Hardikkumar (Hardik) Patel - Quality Co-ordinator

Hardik will be responsible for the implementation and ongoing management of the Quality Management System, compliant to all standards relevant to our current and future needs.

Hardik is an experienced Quality Assurance Associate with knowledge of Quality Management Systems (QMS), including Quality Assurance & Regulatory Compliance, Quality Auditing and Inspection, Good Documentation and Management, and Good Manufacturing Practice (GMP).

Dr Ashley Miller, PhD - Research Scientist

Ashley joins R&D in the Science Team, to progress current studies and work on future discovery and development projects.

Ashley brings extensive LC-MS/MS experience across multiple platforms using various specialised techniques. He has extensive experience in method development and method validation in commercial laboratories NATA accredited to ISO 17025.

Our development partner in the US, Precion Inc. have completed their development work and are currently carrying out a 656 patient clinical study to confirm the analytical assay they developed for BCAL and to confirm the transferability of the BCAL lipidomic test for breast cancer between laboratories and instrument platforms. Technology transfer from Precion to BCAL is scheduled for late July which is on the schedule for product launch in late 2024.

Our next newsletter will provide an update on the technology transfer and the outcome of the 656 patient clinical study presently underway.

This announcement has been approved for release by the board.