ASX and Media release

9 March 2012

Positive data published on performance of Circadian's Cancers of Unknown Primary Diagnostic Test

• Data shows diagnostic test capable of detecting primary source of cancers with high level of accuracy and specificity across 15 different tumour types.
• Commercial launch expected April/May 2012.

Circadian Technologies Limited (ASX: CIR, OTCQX:CKDXY) announced today that Dr Keith Byron Scientific Director of Healthscope Advanced Pathology, publically presented data for the first time in respect of Circadian's novel diagnostic technology for "Cancers of Unknown Primary" (CUP), at the "Science and the City" meeting of the Royal College of Pathologists of Australia currently being held in Sydney.

The poster presentation entitled "Development of a Gene Expression Based Assay to Determine the Origin of Metastatic Carcinomas of Unknown Primary" (copy attached) showed that the CUP test was able to detect actual primary source of known tumour types with 93% accuracy within the first three predictions and had 98.5% specificity across 15 different cancer types.

The CUP diagnostic methodology identifies a patient's tumour type by comparing its pattern of gene expression to a database of known tumours. It is hoped that by correctly identifying a patient's tumour type, clinicians can choose the most effective treatment strategy for the cancer. CUP is generally less well known and publicised than other cancer types. However, it is actually more common than leukaemia and is the fifth most common cause of death due to cancer in Australia. In 2007 Cancer Council Australia estimated the incidence of CUP to be around 2900 cases per annum; American Cancer Society estimated USA incidence at around 32,000 per annum and Cancer Research UK estimated UK incidence at around 14,000 per annum.

The diagnostic test method has been developed in collaboration between Circadian, Healthscope, the Peter MacCallum Cancer Centre, a leading specialty cancer centre, and scientists at NICTA (National ICT Australia).

Healthscope, through its subsidiary Clinical Laboratories Pty Ltd, has rights to develop, clinically validate and market the test throughout Australia, New Zealand, Malaysia and Singapore. Circadian retains rights to market the test in the remainder of the world. Healthscope has paid Circadian an upfront fee, and will pay a royalty on sales of the test. Circadian, through its wholly owned subsidiary Cancer Therapeutics Limited, owns exclusive worldwide rights to the test through a licensing arrangement with the Peter MacCallum Cancer Centre and NICTA.

Robert Klupacs, Circadian Managing Director and CEO stated, "We are delighted that Healthscope has now been able to publically share these very promising results.. We are excited by the forthcoming launch in the next few months and to the test making a major impact on clinical diagnosis".

Dr Keith Byron, Scientific Director of Healthscope's Advanced Pathology Division said "It is very pleasing to be able to share, for the first time, the data we have generated over the past 3 years of development.

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Healthscope is excited that after the extensive development program we have undertaken with our partners that we are now on the cusp of commercialising this ground breaking diagnostic technology. The CUP test will undergo further refinement and validation in the coming months through an ongoing beta-test trial process, which allows oncologists limited early access, after which it will be launched commercially. We expect to launch in the April/May timeframe."

Prof David Bowtell, Head of the Cancer Genomics Program at the Peter MacCallum Cancer Centre and a co-inventor of the diagnostic methodology added, "The data we have published today is extremely exciting. It is very gratifying that this product of our translational research efforts will be made available to clinicians throughout the region. The concept of personalising treatments for patients based on highly specialised diagnostics is now very well accepted in oncology and has been shown to have significant patient benefit. We believe that the assay will lead to earlier diagnosis, improved treatment outcomes and enhanced quality of life for patients"

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About Circadian Technologies Limited

Circadian (ASX:CIR; OTCQX:CKDXY)) is a biologics drug developer focusing on cancer and 'front of the eye' disease therapies. It controls exclusive worldwide rights to a significant intellectual property portfolio around Vascular Endothelial Growth Factor (VEGF)-C and -D. The applications for the VEGF technology, which functions in regulating blood and lymphatic vessel growth, are substantial and broad. Circadian's internal product development programs are primarily focussed on developing VGX-100 (a human antibody against VEGF-C) as a treatment for solid tumours, in particular glioblastoma and colorectal cancer, as well as for 'front of the eye' disease such as corneal neovascularisation and/or dry eye disease applications. Circadian has also licensed rights to some parts of its intellectual property portfolio for the development of other products to ImClone Systems, a wholly-owned subsidiary of Eli Lilly and Company, including the antilymphatic antibody-based drug IMC-3C5 targeting VEGFR-3.

About Cancers of Unknown Primaries

In spite of the increasing sophistication in the diagnostic workup for malignancies, detailed investigations fail to reveal a primary site of origin for a subset of patients with metastatic cancer. This is often referred to as Carcinoma of Unknown Primary (CUP) origin or occult primary malignancy. Usually, when cancer spreads, the secondary cancer cells look like abnormal versions of the primary cancer cells (in the tissue where the cancer began). For example, if breast cancer spreads to the lungs, the metastatic tumour in the lung is made up of cancerous breast cells (not lung cells) and is then described as metastatic breast cancer (not lung cancer). If it is not possible to identify the type of cancer cells, the diagnosis is CUP. The inability to identify a primary site of cancer poses many challenges. The primary site of cancer usually dictates the treatment, expected outcome and overall prognosis. The diagnosis of carcinoma of unknown primary thus generates anxiety among patients and caregivers, who may feel that the evaluation has been incomplete.

About Healthscope

Healthscope is a leading private health care provider within Australia that uniquely operates in every State and Territory, as well as in Asia. Our reach of facilities around the country firmly places Healthscope as the second largest private hospital provider operating 44 private hospitals. The company includes a leading pathology business with facilities in Australia, New Zealand, Singapore and Malaysia; a growing medical centres division with over 45 clinics; and a diagnostic imaging division centred in major hospitals.

Healthscope 's Advanced Pathology division develops new clinical diagnostics and actively collaborates with pre-eminent research groups by validating their translational research for clinical application.

As well as providing specialised molecular pathology services, Healthscope Advanced Pathology has a strong research and development commitment. Current research programs focus on the clinical utility of pharmacogenomics, an area of great promise for improving individualised drug selection and dosing. Our other development program centres on the clinical application of genomics, with reference to cancer diagnosis.

About Peter MacCallum Cancer Centre

The Peter MacCallum Cancer Centre is Australia's only public hospital solely dedicated to cancer and one of an elite group of hospitals worldwide to have its own integrated cancer research program and laboratories. It treats more cancer patients each year than any other hospital in Australia and its highly skilled medical, nursing and allied health team is backed by Australia's largest cancer research group.

Peter Macallum Cancer Centre's Cancer Genomics Program is led by Professor David Bowtell and seeks to use sophisticated high throughput genomic technologies to improve understanding of the biology of cancer and to progress the clinical management of cancer patients through the development of individualised approaches to treatment. Research in the program focuses primarily on breast, upper gastrointestinal and ovarian cancers and sarcomas, as well as Cancers of Unknown Primary and involves some of the largest familial and population-based cancer cohorts in the world. These studies address questions of general importance to solid cancers, including inherited susceptibility to cancer and genome-wide changes in gene expression, as well as more specific questions such as prediction of response to therapy and the use of gene expression profiling to inform more accurate cancer diagnosis. Professor Bowtell is Principal Investigator for the Australian Ovarian Cancer Study, a national molecular epidemiological study of ovarian cancer, creating the largest linked biospecimen/clinical database in the world for ovarian cancer.

About NICTA

National ICT Australia Ltd (NICTA) is Australia's Information and Communications Technology Research Centre of Excellence, developing technologies to generate economic, social and environmental benefits for Australia. It's primary goal is to build and deliver excellence in ICT research and commercial outcomes for Australia. NICTA aspires to be one of the world's top ten ICT research centres by 2020. Since NICTA was founded in 2002, it has created six new companies, developed a substantial technology and intellectual property portfolio and continues to supply new talent to the ICT industry through a NICTA-supported PhD program. NICTA has five laboratories around the country. With over 700 people, NICTA is the largest organisation in Australia dedicated to ICT research.

NICTA is funded by the Australian Government as represented by the Department of Broadband, Communications and the Digital Economy, and the Australian Research Council through the ICT Centre of Excellence program. The NICTA laboratories are also funded by their respective Victorian, Australian Capital Territory, New South Wales, and Queensland Governments. In addition, NICTA is supported by The University of Melbourne, Monash University, RMIT University, University of Ballarat, Deakin University, The Australian National University, Griffith University, University of New South Wales, University of Queensland, Queensland University of Technology and The University of Sydney.

The Diagnostic Genomics Team at NICTA brings expertise in "Signal Processing and Pattern Recognition" to the CUP project.

About Circadian's pipeline of treatments for cancer

The clinical and commercial success of Avastin®, an antibody that blocks the activity of VEGF-A, clinically validated anti-angiogenic drugs as an effective means of inhibiting solid tumour growth. By blocking the interaction of VEGF-A with its receptors, primarily VEGFR-2, the multi-billion dollar cancer therapeutic slows tumour growth by inhibiting blood vessel recruitment into the tumour, effectively starving tumours of essential nutrients and oxygen required for growth. However after a short period of time tumors can begin to grow again in the presence of Avastin®. Avastin® is approved by the US FDA in the following indications: metastatic colorectal cancer, non-squamous-cell lung cancer, metastatic breast cancer, glioblastoma, and metastatic renal cell carcinoma.

The angiogenic receptor VEGFR-2 can also be stimulated by VEGF-C and hence an inhibitor such as VGX-100, a key therapeutic in Circadian's portfolio, can produce greater blockade of this receptor pathway. As such, VGX-100 has the potential to block blood vessel growth in tumours which grow in the presence of Avastin® therapy and hence may completely shut down angiogenesis (the growth of blood vessels) mediated by VEGFR-2.

VEGF-C along with the molecule VEGF-D are also the only known proteins to bind and activate VEGFR-3 which drives lymphatic vessel and tumour-associated blood vessel growth. Inhibitors of VEGF-C thus have therapeutic potential to inhibit not only primary tumour growth through their anti-angiogenic activities, but to also inhibit tumour spread or metastasis via the lymphatic vessels - a mechanism of tumour dissemination that is often the deadliest aspect of many tumour types and a mechanism that is not effectively blocked by anti-VEGF-A or anti-VEGFR-2 therapeutics.

Inherent risks of Investment in Biotechnology Companies

There are a number of inherent risks associated with the development of pharmaceutical products to a marketable stage. The lengthy clinical trial process is designed to assess the safety and efficacy of a drug prior to commercialisation and a significant proportion of drugs fail one or both of these criteria. Other risks include uncertainty of patent protection and proprietary rights, whether patent applications and issued patents will offer adequate protection to enable product development, the obtaining of necessary drug regulatory authority approvals and difficulties caused by the rapid advancements in technology. Companies such as Circadian are dependent on the success of their research and development projects and on the ability to attract funding to support these activities. Investment in research and development projects cannot be assessed on the same fundamentals as trading and manufacturing enterprises. Thus investment in companies specialising in drug development must be regarded as highly speculative. Circadian strongly recommends that professional investment advice be sought prior to such investments.

Forward-looking statements

Certain statements in this ASX announcement may contain forward-looking statements regarding Company business and the therapeutic and commercial potential of its technologies and products in development. Any statement describing Company goals, expectations, intentions or beliefs is a forward-looking statement and should be considered an at-risk statement. Such statements are subject to certain risks and uncertainties, particularly those risks or uncertainties inherent in the process of developing technology and in the process of discovering, developing and commercialising drugs that can be proven to be safe and effective for use as human therapeutics, and in the endeavour of building a business around such products and services. Circadian undertakes no obligation to publicly update any forward-looking statement, whether as a result of new information, future events, or otherwise. Actual results could differ materially from those discussed in this ASX announcement.

Development of a Gene Expression Based Assay to Determine the Origin of Metastatic Carcinomas of Unknown Primary.

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Introduction

Carcinomas of unknown primary (CUP) account for 3–5% of all malignancies and are thus among the ten most-frequent cancers worldwide. The prognosis for patients with CUP is poor, with median survival of eleven months from the time of diagnosis. In light of the poor prognosis, morbidity and patient anxiety associated with extensive clinical investigations, the oncologist must decide how far to pursue identification of the primary tumour. However, when a primary tumour has been identified and specific treatment initiated, improved response rates and overall survival has been demonstrated.

Gene expression profiling using microarray technology has been demonstrated to be effective for the classification of cancer. A tumour's gene expression profile is believed to reflect the normal differentiated state of the cell of origin combined with the aberrant gene expression changes associated with disease transformation. It has also been shown that a tumour's gene expression signature is maintained even if the tumour has metastasised to a distant site and closely resembles that of the primary tumour.

Here we present stage one, of the development of a gene expression assay and tumour class prediction model to identify the site of origin of metastatic carcinomas of unknown primary.

Methods

RNA extraction and processing: Seven micron sections of formalin fixed paraffin embedded (FFPE) tissue were first reviewed by a pathologist. Areas on the tissue containing the greatest amounts of tumour were macro-dissected to enrich to at least 80-90% tumour content. RNA from this tissue was then extracted using a modification of the Qiagen RNA Easy FFPE RNA extraction kit. Quantitation of RNA was then determined using the Quant-iT Ribo Green kit (Invitrogen) on a Nanodrop 3300 fluorimeter. RNA quality was assessed by amplifying a fragment (90 bp) of the highly expressed RPL13A ribosomal protein gene by reverse transcription PCR using an ABI7900.

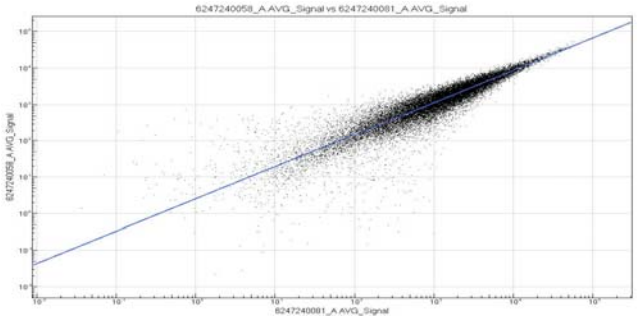
Gene expression determination: Whole genome expression analysis was performed using the Illumina DASL humanHT-12-V4 BeadChip that is able to detect 29,285 coding and non-coding transcripts .

Data analysis: A binary Support Vector Machine was used as the basic classification method together with Recursive Feature Elimination as the feature selection method. To build a prediction model, cross validation on the training expression data was conducted, and the optimal number of genes selected, based on the greatest accuracy achieved.

Results

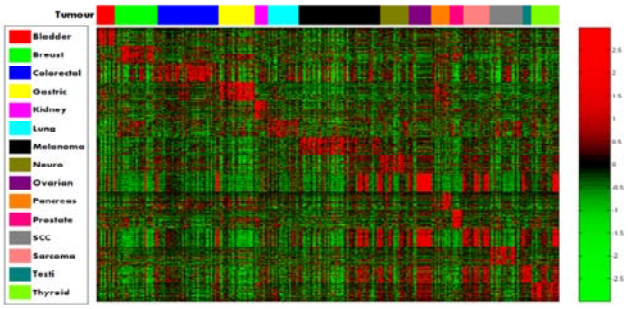
RNA was extracted from formalin fixed paraffin embedded samples containing known metastatic and primary tumours of various classes and varying degrees of differentiation. This set of samples comprised three hundred and ninety nine tumours of fifteen classes. All were analysed as described. To ensure reproducibility of gene expression detection, a control tumour sample was extracted then assayed on several occasions and on different days throughout the project. Figure 2 demonstrates representative raw intensity scatter plots of the control sample RNA run on separate days demonstrating good correlation.

Figure 1. Comparison of gene expression for the same sample extracted and analysed on separate days



To develop a classifier to predict tumour classes, we employed a one-versus-all classification strategy. For each of the 15 tumour classes considered, we built a classifier to distinguish each tumour class from all others. The discriminating probes for each of these 15 classifiers were selected by fitting the training data with a Support Vector Machine (SVM) method. In the testing phase, gene expression data from every tumour sample was tested in all of the 15 classifiers, and accordingly, one score that indicates the similarity of the test sample to a specific tumour class is produced. By comparing the similarity of the test sample to all tumour types, we are able to rank the probability of the test sample belonging to each tumour class.

Figure 2.Heat map of gene expression



In order to evaluate the accuracy of predictions made by the classifier, we employed a cross validation strategy. To do this, the training data was split randomly into five equal sized subsets, four of which were used to develop the classifier and one is used for testing. The training and testing process strictly follow the method described above, so that we obtain the ranked predictions for each test sample. The process is repeated five times, so that each subset is used for testing once. The accuracy of the predictions was then determined by comparing them with the known tumour types. The results of this strategy are demonstrated in table 1, where both overall accuracies and class-specific accuracies can be seen for the classifiers first prediction. Also demonstrated are the accuracies of the classifier correctly predicting the samples true tumour class in the first two and three predictions.

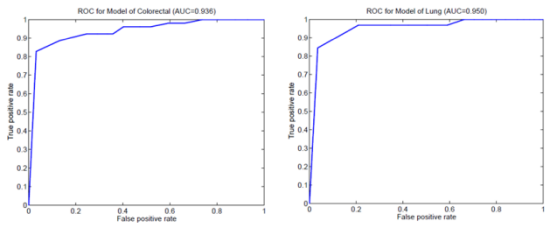
Table 1 : Classifier accuracies and specificities.

Tumour Class	N	Correct call with first prediction	Correct call within first two predictions	Correct call within first three predictions	Specificity
Overall Average		**83%**	**89%**	**93%**	**98.5%**
Bladder	13	85%	92%	92%	99.7%
Breast	56	79%	93%	96%	96.7%
Colorectal	48	85%	92%	96%	96.4%
Gastric	26	81%	85%	89%	99.2%
Kidney	14	93%	100%	100%	99.7%
Lung	30	83%	97%	97%	97.6%
Melanoma	50	78%	84%	88%	98.8%
Neuroendocrine	18	83%	89%	89%	98.7%
Ovarian	22	77%	96%	100%	98.4%
Pancreas	18	72%	78%	83%	97.7%
Prostate	19	89%	95%	95%	99.7%
Sarcoma	29	76%	83%	86%	96.6%
SCC	27	93%	93%	93%	99.2%
Testi	8	88%	88%	88%	99.7%
Thyroid	21	91%	91%	100%	100%

Area under ROC

The calculated area under the receiver operating characteristic (ROC) curve is a fundamental tool for diagnostic test evaluation. Here we have calculated the average area under the ROC curve across all 15 tumour classes to be 0.95 indicating a test of high diagnostic value. Class examples of ROC curve for both Colorectal and Lung can be seen in figure 3.

Figure 3. ROC curves for Colorectal and Lung tumour classes



Reproducibility of Predictions

To evaluate the reproducibility of the classifier, a single colorectal adenocarcinoma was analysed on four separate occasions. The gene expression data was then submitted to the classifier for prediction scoring. For all four samples submitted, the classifier correctly predicted the class to be "colorectal".

Conclusion

Although further validation is required using a independent test cohort of known metastatic tumours, as well as a cohort of samples from patients diagnosed with CUP, our data suggests that an expression-based diagnostic test could be effective in identifying the tumour of origin in patients presenting with CUP.