ASX and Media release

7 June 2011

VEGF-C and VEGF-D identified as biomarkers for Avastin resistance in colorectal cancer patients

- Data presented at American Society for Clinical Oncology ("ASCO") Annual meeting in Chicago
- Data obtained from 42 patients treated with chemotherapy (5-flourouracil and irinotecan) plus bevacizumab (Avastin®) enrolled in a Phase 2 clinical trial
- VEGF-C shown to be significantly raised just prior to and at the time of disease progression (treatment resistance), while VEGF-D shown to be significantly increased after disease progression
- Data supports concept of targeting additional VEGF family proteins to improve treatment outcomes

Melbourne, Australia June 7, 2011– Circadian Technologies Limited (ASX: CIR) announced today the presentation of data at the American Society of Clinical Oncology (ASCO) Annual Meeting in Chicago USA associating resistance to Avastin® with increases in plasma VEGF-C and D concentrations.

The poster presentation entitled "The Association of Alternate VEGF Ligands with Resistance to Anti-VEGF Therapy in Metastatic Colorectal Cancer (mCRC)" is contained in the Appendix and is also available on Circadian's website at www.circadian.com.au.

Resistance to Avastin® is a frequent occurrence in the treatment of certain cancers with resulting loss of response and disease progression. The study, which was led by Drs Lieu and Kopetz at The University of Texas MD Anderson Cancer Center, showed that increases in VEGF-family markers in patients with metastatic colorectal cancer are associated with Avastin® resistance. In particular, VEGF-C increases were seen in patients prior to and at the time of disease progression while receiving Avastin® and chemotherapy.

Mr. Mark Sullivan, Head of Development for Circadian, said: "The clinical data provide an important insight into the role that VEGF-C plays in the development of Avastin® resistance. The findings are supportive of our strategy for combining our VEGF-C antibody (VGX-100) with Avastin® to seek better outcomes for patients. The data also provide further rationale for the development of VEGF-C and/or VEGF-D based biomarker tests to monitor cancer therapy".

Scott Kopetz, M.D.,Ph.D. of MD Anderson said: "Our data show that the VEGF-family ligands, other than VEGF itself, are associated with Avastin®-containing chemotherapy resistance in patients with metastatic colorectal cancer. We are planning prospective confirmatory studies to further evaluate and validate these findings."

Mr. Robert Klupacs, CEO of Circadian Technologies, said: "We are excited to see the ever increasing amount of clinical data validating VEGF-C and D as important therapeutic targets.

Level 1 10 Wallace Avenue Toorak Victoria 3142 Australia

T +61 (3) 9826 0399 F +61 (3) 9824 0083 www.circadian.com.au

ABN 32 006 340 567

We look forward to continuing our excellent collaboration with Dr Kopetz and his team at MD Anderson. We remain on track to commence clinical trials in cancer patients with our VEGF-C antibody VGX-100."

Circadian's wholly owned subsidiary, Vegenics, owns worldwide rights to an extensive intellectual property portfolio covering angiogenesis targets VEGF-D, VEGF-C and the receptor protein VEGFR-3.

Company enquiries

Robert Klupacs
Managing Director - Circadian
Tel: +61 (0) 3 9826 0399 or
robert.klupacs@circadian.com.au

Media enquiries

Kyahn Williamson
Buchan Consulting
Tel: +61 (0) 3 9866 4722
kwilliamson@bcg.com.au

Media Enquiries – International

Lauren Glaser
The Trout Group LLC
251 Post Street, Suite 412
San Francisco, CA 94108
Tel +1 215 740 8468
lglaser@troutgroup.com

About Circadian Technologies Limited

Circadian (ASX:CIR) is a biologics drug developer focusing on cancer 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 focussed on novel anti-cancer therapeutics for large unmet needs. 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 antibody-based drug IMC-3C5 targeting VEGFR-3.

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. Avastin®, which is sold by Genentech, now part of Roche, had U.S. sales in 2009 of US$5.7 billion and worldwide sales in excess of US$8.6 billion. Avastin® is approved by the US FDA in the following indications: metastatic colorectal cancer, non-squamous-cell lung cancer, metastatic breast cancer, glioblastoma, metastatic renal cell carcinoma.

The VEGF-C inhibitor, VGX-100, a key therapeutic in Circadian's portfolio, block this alternative stimulator for VEGFR-2. As such, it has the potential to block blood vessel growth in tumours resistant to anti-VEGF-A therapy and, when used in combination with drugs like Avastin®, may completely shut down angiogenesis (the growth of blood vessels) mediated by VEGFR-2, resulting in greater clinical efficacy.

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 endeavor 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.

Level 1 10 Wallace Avenue Toorak Victoria 3142 Australia

T +61 (3) 9826 0399 F +61 (3) 9824 0083 www.circadian.com.au

ABN 32 006 340 567

The Association of Alternate VEGF Ligands With Resistance to Anti-VEGF Therapy in Metastatic Colorectal Cancer (mCRC)

C Lieu, H Tran, Z Jiang, M Mao, M Overman, C Eng, J. Morris, L Ellis, J Heymach, S Kopetz

Departments of Gastrointestinal Medical Oncology, Surgical Oncology, and Thoracic Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Updated Abstract

Background: Circulating angiogenic factors have been previously shown to be altered in patients (pts) with mCRC on bevacizumab (BV)-containing regimens. However, a systematic evaluation of alterations in levels of the VEGF family of ligands may provide insights into resistance mechanisms.

Methods: 42 pts with mCRC were treated on a single arm phase II study with 5-FU, irinotecan, and BV (FOLFIRI+BV), and plasma was collected for cytokine levels. VEGF-C, VEGF-D, and PlGF were measured using ELISA (R&D) or suspension bead multiplex assay (BioRad). Plasma samples were obtained at baseline, prior to radiographic progression, and at the time of radiographic progression. In a retrospective larger cohort used for validation, plasma from 403 matched pts with mCRC were obtained prior to any chemotherapy or after progression on a regimen with or without bevacizumab. Comparisons were done by the two-sided, nonparametric Wilcoxon paired test.

Results: Following FOLFIRI+BV treatment, VEGF-C was increased prior to progression and at the time of progression (+43% [p=0.045] and +72% [p=0.004], respectively), similar to previously reported changes in PlGF (+67%, p<0.001). Levels of VEGF-D were increased at the time of progression (+39%, p=0.04). In a second cohort, compared with pre-chemotherapy plasma, samples obtained a median of 3 weeks after progression on a regimen with BV had lower levels of VEGF-C but higher levels of VEGF-D (only +6%, p=0.018) and PlGF (+32%, p<0.0001). When compared to pts receiving chemotherapy alone, pts receiving prior BV had significantly elevated levels of PlGF (+72%, p<0.0001) but not VEGF-C and VEGF-D (+5% and +7%, p=NS). Levels of PlGF and VEGF-D were negatively correlated with the time from last chemotherapy dose to sample collection (p<0.0001) suggesting that changes at the time of progression are transient.

Conclusions: Increases in VEGF-D and PlGF were observed after progression on chemotherapy and BV. VEGF-C changes require validation in a prospective cohort. These changes may be reversible after discontinuing therapy. VEGF family ligands other than VEGF itself are associated with BV-containing chemotherapy resistance in mCRC.

Background

- Therapies incorporating the monoclonal antibody bevacizumab, an inhibitor of vascular endothelial growth factor (VEGF), have demonstrated efficacy in metastatic colorectal cancer (mCRC).[1]
- Despite the benefit provided by bevacizumab-based regimens, clinical resistance develops, and preclinical work has suggested that alternate proangiogenic factors, such as placental growth factor (PlGF), VEGF-C, and VEGF-D may modulate sensitivity to anti-VEGF therapy and allow regrowth of tumor-associated vasculature.[2]
- Cytokine and angiogenic factors (CAFs) have previously been shown to be modulated in patients with mCRC after receiving bevacizumab-containing chemotherapy, but validation of these findings is warranted.[3]
- The relative impact of standard chemotherapy with bevacizumab on circulating levels of PlGF, VEGF-C, and VEGF-D has not been previously described in CRC.

Objectives

Primary Objective

- To determine alterations in VEGF-C and VEGF-D in patients receiving FOLFIRI in combination with bevacizumab.

Secondary Objective

- To evaluate alterations in alternate VEGF ligands attributable to bevacizumab in a large retrospective cohort.

Methods

Cohort 1:

- Plasma was obtained from 42 patients with mCRC treated on a single-arm phase II study with FOLFIRI and bevacizumab.
  - Samples were obtained at baseline, following one dose of bevacizumab, following the first cycle of FOLFIRI, and at each restaging until progression of disease
- Levels of PlGF were measured by suspension bead multiplex assays (BioRad) and were analyzed per manufacturers' directions.
- Levels of VEGF-C and VEGF-D were measured by ELISA (R&D Systems)

Cohort 2:

- A single plasma sample from 403 patients with metastatic colorectal cancer was collected between 2002-2008 by the Texas Genetic Consortium.
- Patients had plasma samples obtained prior to any chemotherapy or after chemotherapy regimen. Prior treatment regimens were heterogeneous, with the most common being FOLFOX and FOLFIRI with and without bevacizumab.
- Patient information was collected and separated into 3 groups:
  - Patients receiving no prior chemotherapy (Group A)
  - Patients treated with a chemotherapy regimen without bevacizumab (Group B)
  - Patients treated with a chemotherapy regimen with bevacizumab (Group C)
  - Patients were matched for number of metastatic disease sites (Groups A, B, C) and for prior chemotherapy duration and time from last chemotherapy dose to sample collection (Groups B,C).[4]
- Comparisons were by the two-sided, nonparametric Wilcoxon paired test, with p<0.05 significance.
- Spearman correlation method was applied to assess the correlation between the time to last chemotherapy dose to sample collection and specific cytokine levels, and data was fit to a single-phase log decay to identify half-life of the elevations.

Results

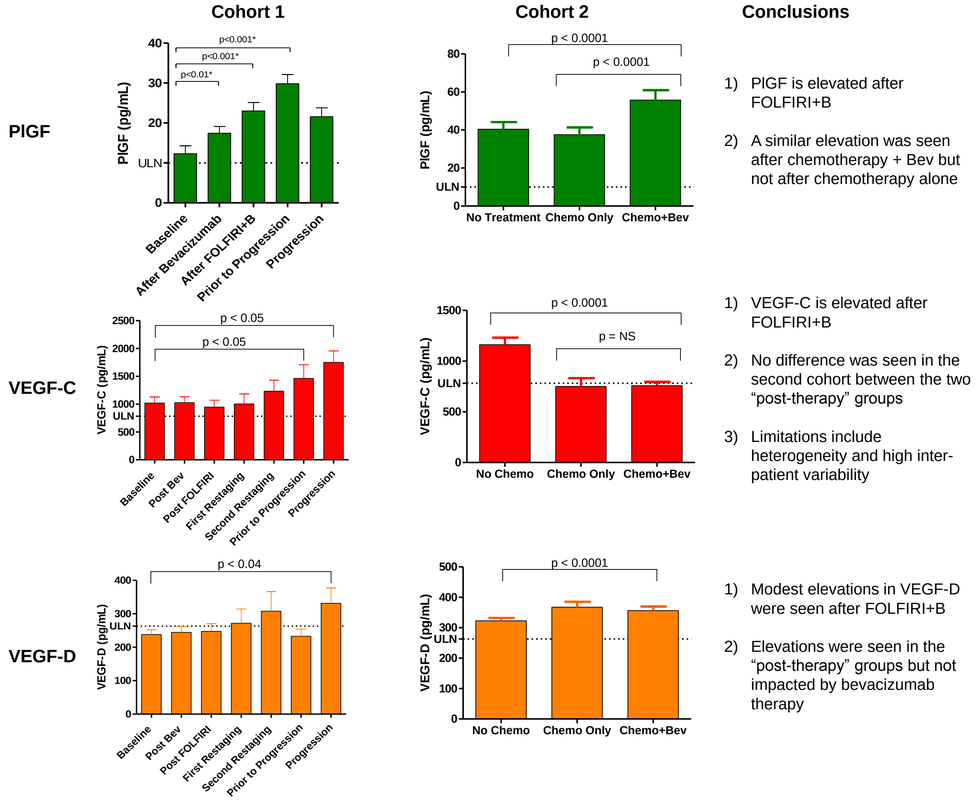
Cohort 1:

- Following FOLFIRI+BV treatment, VEGF-C was increased prior to progression and at the time of progression (+43% [p=0.045] and +72% [p=0.004], respectively), similar to previously reported changes in PlGF (+67%, p<0.001).[3]
- Levels of VEGF-D were increased at the time of progression (+39%, p=0.04).

Cohort 2:

- In the retrospective cohort, clinical characteristics were well-matched (Table 1).
- Compared with pre-chemotherapy plasma (Group A), samples obtained a median of 3 weeks after treatment with a regimen with BV (Group C) had lower levels of VEGF-C but higher levels of VEGF-D (only +6%, p=0.018) and PlGF (+32%, p<0.0001).

Figure 1. Levels of alternate VEGF ligands in cohort 1 and cohort 2



Results (cont)

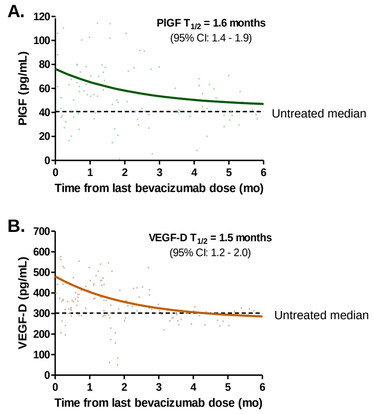
Table 1: Patient Characteristics (Cohort 2)	No chemo (Group A)	Chemo w/ Bev (Group C)	Chemo w/o Bev (Group B)	Chemo w/Bev (Group C)
Number of samples	169	169	65	65
Age, years (median)	58	53	53	53
Number of metastatic sites	1.7	1.7	1.4	1.4
Duration of prior chemotherapy (months)	N/A	N/A	5	6
Last chemotherapy to same collection (days)	N/A	N/A	35	29

- When compared to pts receiving chemotherapy alone (Group B), pts receiving prior BV (Group C) had significantly elevated levels of PlGF (+72%, p<0.0001) but not VEGF-C and VEGF-D (+5% and +7%, p=NS).

Results (cont)

- Levels of PlGF and VEGF-D were negatively correlated with the time from last bevacizumab dose to sample collection (p<0.0001) suggesting that changes at the time of progression are transient. (Figure 2)

Figure 2. Levels of alternate VEGF ligands decrease following discontinuation of bevacizumab A) PlGF, B) VEGF-D



- Limitations in the second cohort included less rigorous collection methodology, inability to confirm treatment and radiographic progression from primary source documents in all patients, and heterogeneous patient population.

Conclusions

- Increases in PlGF and VEGF-D were consistently observed after progression on chemotherapy and BV.
- PlGF and VEGF-D are negatively correlated with the length of time from last bevacizumab to plasma collection, highlighting the transient nature of these cytokine changes after discontinuation of treatment.
- VEGF-C elevation is evident in the first cohort. Results from the second cohort may be confounded by sample processing. Validation in a prospective cohort is required.
- VEGF family ligands other than VEGF itself are associated with BV-containing chemotherapy resistance in mCRC.
- Further study is required to determine if these changes are causative for antiangiogenic resistance.

References

1. Hurwitz H, et al. *N Engl J Med* 350:2335-2342
2. Ellis L, et al. *Clin Cancer Res* 14:6371-6375
3. Kopetz S, et al. *J Clin Oncol* 28:453-459
4. Hansen BB et al. JASA 99:609-18

Research support from The ASCO Foundation Young Investigators Award (CL)
And Circadian Technologies Limited, Victoria, Australia. Trial support from Genentech, San Francisco, CA. Statistical assistance from E Lin