ASX and Media release

6 May 2014

VEGF-C blockade with OPT-302 reduces established wet AMD lesions in the mouse

- Data presented at the Association for Research in Vision and Ophthalmology (ARVO) 2014 conference in Orlando shows that OPT-302 (formerly VGX-300), an inhibitor of VEGF-C, can prevent the formation of wet AMD lesions and regress established lesions, in the laser-induced mouse model of wet AMD.
- OPT-302 reduced wet AMD lesion size and vessel leakage to a comparable extent as the marketed agent EYLEA®.
- The data shows that VEGF-C levels are elevated in wet AMD and that OPT-302 can also reduce the expression of genes involved in blood vessel growth and inflammation in the mouse model.
- The data strongly implicates VEGF-C in the pathogenesis of wet AMD and confirms the potential of OPT-302 as a novel therapeutic for the disease either as a single agent or as an adjunct to existing agents targeting VEGF-A.

Circadian Technologies Limited (ASX: CIR, OTCQX:CKDXY), through its 100% owned subsidiary Opthea Pty Ltd, announced today that its collaborator Dr. Kameran Lashkari of The Schepens Eye Research Institute/Massachusetts Eye & Ear at Harvard Medical School, publicly presented data overnight at the annual ARVO conference in Orlando demonstrating that Opthea's drug development candidate, OPT-302 (formerly VGX-300, a soluble form of VEGF Receptor-3), inhibits hallmarks of wet AMD progression in the laser-induced model of the disease in mice, further confirming the potential of OPT-302 as a novel therapy for the disease.

The poster presentation entitled "VEGF-C and VEGF-D blockade by VGX-300 inhibits choroidal neovascularisation and leakage in a mouse model of wet AMD" (copy attached), showed that OPT-302 (VGX-300) can inhibit the formation of wet AMD lesions in this internationally accepted mouse model of the human disease when administered at the onset of the disease. Furthermore, when administered to mice with established wet AMD lesions, OPT-302 effectively reduced the size and leakage of vessels to a comparable extent as the marketed agent EYLEA® and reduced ocular inflammation.

EYLEA® (Regeneron/Bayer) has a distinct mechanism of action to OPT-302 and blocks VEGF-A, but not VEGF-C and VEGF-D. Sales of EYLEA® in the US, first marketed in November 2011 for the treatment of wet AMD, were $US1.4BN in 2013[1] and are forecast to reach $US1.7BN in 2014[2]. At least 45% of patients with wet AMD exhibit some degree of resistance to anti-VEGF-A therapy.

Wet AMD is the leading cause of blindness for people over the age of 50 in the US and Europe and is estimated to affect over 1.5 million people worldwide. Wet AMD is characterised by the growth of new blood vessels into the central region of the retina (the macula). This new vessel growth leads to severe and rapid vision loss, exacerbated by fluid and protein leakage, inflammation and scar formation in the retinal tissue.

Dr. Kameran Lashkari, M.D., PhD, stated, “Current therapies for wet AMD target VEGF-A. Although they represent an advance for the treatment of the disease, there continues to be an unmet medical need for improved treatments. The current data indicates that VEGF-C is important in wet AMD and that administration of OPT-302 (VGX-300) to mice with established lesions, a model that is applicable to the clinical treatment paradigm, can reduce disease burden.”

Dr. Megan Baldwin, CEO of Circadian, said “OPT-302 is a novel agent with the potential to improve vision in patients either when used alone, or as an adjunct therapy with existing anti-VEGF-A therapies to achieve a more complete blockade of the processes involved in wet AMD progression. Circadian remains on-track to commence clinical trials with OPT-302 in wet AMD patients in the first half of 2015.”

1. *Regeneron as reported by Reuters, Jan 14 2014.*
2. *Regeneron Press Release Feb 11 2014.*

Company and media enquiries

Megan Baldwin
CEO & Managing Director
Circadian Technologies
Tel: +61 (0) 3 9826 0399 or
megan.baldwin@circadian.com.au

About Circadian Technologies Limited

Circadian (ASX:CIR; OTCQX:CKDXY) is a biologics drug developer focusing on ophthalmic 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 focused on developing OPT-302 (formerly VGX-300, soluble VEGFR-3) for ‘back of the eye’ disease such as wet age-related macular degeneration (wet AMD). 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 anti-lymphatic antibody-based drug IMC-3C5 targeting VEGFR-3.

About wet AMD

Wet (neovascular) age-related macular degeneration, or wet AMD, is a disease characterised by the loss of vision in the middle of the visual field caused by degeneration of the central portion of the retina (the macula). Abnormal growth of blood vessels below the retina, and the leakage of fluid and protein from the vessels, causes retinal degeneration and leads to severe and rapid loss of vision.

Wet AMD typically affects individuals aged 50 years or older, and is the leading cause of blindness in the developed world. The prevalence of AMD is increasing annually as the population ages. By year 2020, it is estimated that total number of AMD cases in the US will be approximately 20 million. Sales of the drug Lucentis® (Roche/Novartis), which targets VEGF-A but not VEGF-C, were over $US3BN in 2012. Sales of EYLEA™ (Regeneron/Bayer), which also targets VEGF-A but not VEGF-C first marketed in November 2011 for the treatment of wet AMD, were $US1.4BN in 2013 and are forecast to reach $US1.7BN in 2014. Approximately half of the people receiving Lucentis®/Eylea® are classified as non-responders or 'poor' responders and experience no significant gain in vision and/or have persistent retinal vascular leakage. There is great opportunity to improve patient responses by targeting more than one factor involved in disease progression. Existing therapies, such as Lucentis®/Eylea®, target VEGF-A that promotes blood vessel growth and leakage through its receptor VEGFR-2. VEGF-C can also induce angiogenesis and vessel leakage through the same receptor. Combined inhibition of VEGF-A and VEGF-C, has the potential to improve patient response by more effective inhibition of the pathways involved in disease progression.

A preclinical model of wet AMD has demonstrated that VEGF-C blockade can significantly inhibit disease progression and that low levels of VEGF-C during development affects formation of the retinal vessels. Like VEGF-A, VEGF-C can also induce vessel permeability that leads to vascular fluid and protein leakage. Inflammatory cytokines associated with wet AMD upregulate VEGF-C levels, and increased levels of the receptors for VEGF-C are detected in AMD tissue. VEGF-C is strongly implicated in the progression of wet AMD.

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.

VEGF-C and VEGF-D Blockade by VGX-300 Inhibits Choroidal Neovascularization and Leakage in a Mouse Model of Wet AMD

Kameran Lashkari[1], Jie Ma[1], Gianna Teague[1], Chenying Guo[1], Megan E. Baldwin[2]
1 Schepens Eye Research Institute, Massachusetts Eye & Ear, Department of Ophthalmology, Harvard Medical School, Boston, 02114 MA
2 Opthea Pty Ltd, Circadian Technologies, Level 4, 650 Chapel Street, South Yarra, Victoria 3141, Australia

Schepens Eye Research Institute
Massachusetts Eye and Ear

Harvard Medical School

PURPOSE and METHODS

Choroidal neovascularization (CNV) is the major cause of severe visual loss in subjects with AMD. At least 45% of subjects with wet AMD exhibit some degree of resistance to anti-VEGF-A monotherapy. Resistance to therapy may be related to activity of other proangiogenic factors such as VEGF-C and VEGF-D. VEGF-C and VEGF-D can participate in CNV formation by promoting angiogenesis by binding and activating VEGFR-2 and VEGFR-3. VGX-300 is a soluble form of VEGFR-3 expressed as an Fc-fusion protein that potently binds and inhibits the activity of VEGF-C and VEGF-D but not VEGF-A.

We investigated the efficacy of VGX-300 to inhibit laser-induced CNV formation and vascular leakage and compared it with Eylea® (aflibercept), a 'Trap' for VEGF-A. Laser-induced CNV was created in C57BL/6 mice using a 532 nm laser under direct visualization using a Micron III® fundus camera (4 - 9 spots/eye; 50 µm size, 50 ms, 550 Mw). On day 0 or day 7 post-laser injury, mice were administered a single intravitreal (IVT) injection of a negative isotype antibody control IgG, Eylea®, VGX-300 or the combination of VGX-300 and Eylea®. For each injection, a total of 80 µg protein was administered in a 2 µl injection (for single-agent groups, 40 µg of IgG was added to each 40 µg dose of Eylea® or VGX-300). Extent of leakage and CNV areas were determined by fluorescein angiography followed by intracardiac perfusion of FITC-dextran in gelatin (10%) on day 7 or 14 post-laser burn. Mouse ocular tissue and clinical specimens from AMD patients were histologically evaluated to identify expression and localization of VEGFs and VEGFRs. The modulation of expression of a panel of angiogenesis and inflammatory genes in mouse CNV following VGX-300 administration was evaluated by quantitative RT-PCR (qRT-PCR).

RESULTS
VGX-300 and Eylea® inhibit CNV to a comparable extent in the mouse

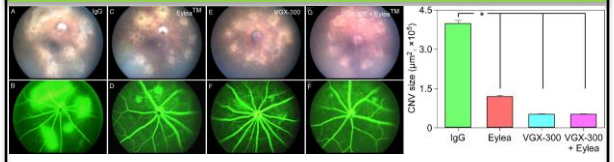

FIGURE 1. Laser injury was induced on day 0 and was followed immediately by injection of IgG, Eylea®, VGX-300 or their combination. Fundus images and angiography were taken on day 14. Both Eylea® and VGX-300 significantly reduced CNV area compared to the IgG control treated group. In this study, the single-agent activity of VGX-300 was highly significant, therefore an additive effect in the VGX-300 + Eylea® group could not be observed in this study. (n=15 mice/group).

Established CNV lesions regress following VGX-300 treatment

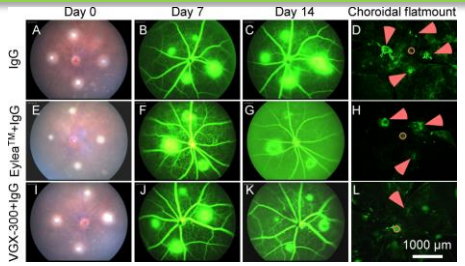

FIGURE 2. Representative fundus images, angiography and choroidal flatmounts of laser-induced CNV membranes 7 and 14 days after laser-burn and administration, on day 7, of **(A – D)** IgG (80 µg), **(E – H)** Eylea® (40 µg) + IgG (40 µg) , or **(I – L)** VGX-300 (40 µg) + IgG (40 µg) (n=10 mice/group). Red circles indicate the optic heads and arrows point to the CNV lesions.

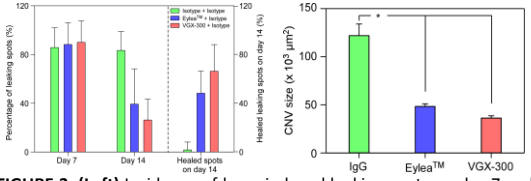

FIGURE 3. (Left) Incidence of laser-induced leaking spots on day 7 and on day 14 post-laser injury (leaking spots/photocoagulated spots × 100%) (n=10 mice/group). Treatments were administered on day 7. **(Right)** Mean size of laser-induced CNV membranes at day 14 following administration of IgG, Eylea® and VGX-300 on day 7 (n=10 mice/group).

Expression of VEGF-C and VEGFR-2/3 in CNV and clinical AMD

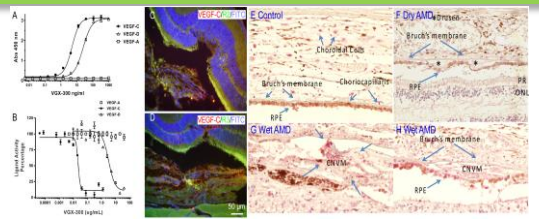

FIGURE 4. (A) VGX-300 binds VEGF-C and VEGF-D but not VEGF-A in a direct binding ELISA and **(B)** VEGFR-2 cell-based bioassay. **(C,D)** Expression of VEGF-C, VEGFR-2 and VEGFR-3 in laser-induced CNV membranes. **(E,F)** Low expression of VEGF-C in control and dry AMD. **(G,H)** Increased expression of VEGF-C around CNV membranes (arrows).

Modulation of gene expression post-laser injury and following VGX-300 treatment

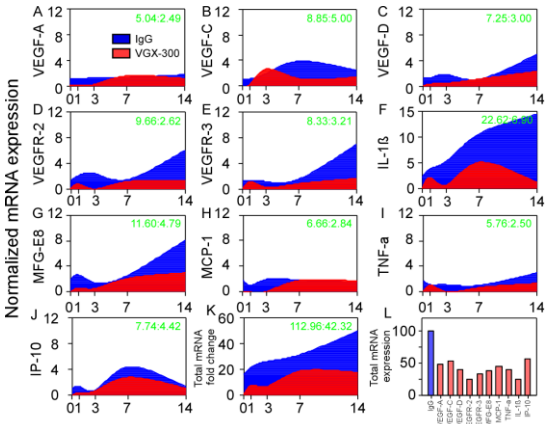

FIGURE 5. (A – J) The dynamic change in mRNA levels of a panel of angiogenic and inflammatory genes on day 0, 1, 3, 7 and 14 post-laser injury in the ocular tissue of mice treated with IgG (**blue**) or VGX-300 (**red**). IgG or VGX-300 was administered on day 0 post-laser injury by IVT injection. The **green** value in each plot is the area ratio of mRNA levels between IgG and VGX-300 treated groups. **(K)** The accumulated expression of this gene panel for each treatment group. **(L)** The normalized accumulated expression of each gene in the VGX-300 compared to IgG group (n=10 mice/group on each sampling day).

CONCLUSIONS

1. VGX-300 mediated blockade of VEGF-C/-D significantly inhibits choroidal neovascularization and vascular leakage comparably to Eylea® in the laser-induced mouse model of wet AMD.

2. Established CNV lesions in the mouse regress following treatment with VGX-300 on day 7 post-laser injury.

3. VEGF-C expression is higher in wet AMD and lower in control and dry AMD clinical specimens.

4. VGX-300 reduces the expression of genes associated with angiogenesis and inflammation following laser-induced injury.

5. Administration of single-agent VGX-300 may be an effective therapy for wet AMD. Administered in combination with anti-VEGF-A therapies, VGX-300 may have the potential to improve clinical responses, particularly in patients that are sub-responsive to anti-VEGF-A therapies.

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