

ASX and Media release

9 May 2013

VEGF-C shown to have major role in Age-Related Macular Degeneration (AMD)

- Data presented at the Association for Research in Vision and Ophthalmology (ARVO) 2013 conference in Seattle shows that circulating blood plasma levels of VEGF-C are markedly increased in AMD patients and that VGX-300, an inhibitor of VEGF-C, works comparably to the marketed agent EYLEA™ in the laser-induced mouse model of “wet” AMD.
- The data strongly implicates VEGF-C in the pathogenesis of “wet” AMD and confirms the potential of VGX-300 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 at Harvard Medical School, publicly presented data overnight at the annual ARVO conference in Seattle showing a major link between Vascular Endothelial Growth Factor (VEGF)-C levels and AMD and that assessment of Opthea’s drug development candidate, VGX-300 (a soluble form of VEGF Receptor-3 that blocks VEGF-C and VEGF-D) in animal models of “wet” AMD confirms its potential as a new therapy for the disease.

The poster presentation entitled “Expression of VEGF-C, VEGF-D and their cognate receptors in experimental and clinical choroidal neovascularisation” (copy attached) showed for the first time, that circulating levels of VEGF-C are markedly elevated in AMD patients and that VGX-300 can inhibit the growth and leakage of new blood vessels in the retina, both hallmarks of “wet” AMD, in the internationally accepted laser-induced model of “wet” AMD disease in mice.

The efficacy of VGX-300 in the study was comparable to that of EYLEA™ which is similar in structure to VGX-300 but has a different mechanism of action and blocks VEGF-A, but not VEGF-C and VEGF-D. Sales of EYLEA™ (Regeneron/Bayer), first marketed in November 2011 for the treatment of “wet” AMD, were \$838M in 2012 and are forecast to reach \$1.3BN in 2013¹.

Opthea was created in 2012 to specifically develop Circadian’s portfolio of VEGF-C/VEGF-D inhibitors for ophthalmic disease. Opthea is currently developing VGX-300 for the treatment of “wet” AMD.

“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, “There continues to be an unmet medical need for improved treatment for “wet” AMD patients. Despite the recent advances made with the approval of agents targeting VEGF-A for this disease such as EYLEA™ and Lucentis™, at least 40-50% of these treated patients will exhibit a sub-response, indicating that this persistent angiogenesis and vascular leakage in the retinal tissue of these patients is driven by more than just one growth factor, VEGF-A.”

Dr. Megan Baldwin, CEO of Opthea, said “Our findings of the increased levels of VEGF-C in AMD patients as well as the results we have obtained with VGX-300 in an AMD disease model indicate that VEGF-C blockade could be a very important new therapeutic approach for “wet” AMD. The data highlights the potential of VGX-300 to improve vision in patients either when used alone, or as an adjunct therapy with existing anti-VEGF-A agents. We expect clinical trials in “wet” AMD patients to commence in H2 2014.”

¹. *Regeneron as reported by Reuters, Jan 8 2013.*

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About Opthea Pty Ltd

Opthea Pty Ltd is a private, 100% owned subsidiary of Circadian Technologies Limited based in Melbourne, Australia. Opthea is developing novel biologic inhibitors of angiogenesis (blood vessel growth), lymphangiogenesis (lymphatic vessel growth) and vascular leakage for the treatment of ophthalmic diseases.

Opthea’s compounds have broad utility in a range of eye diseases characterised by aberrant blood and/or lymphatic vessel growth, vascular leakage or edema, and inflammation, including wet AMD, diabetic macula edema, corneal neovascularisation and transplantation, and dry eye disease.

Opthea’s lead compound, VGX-300, is a soluble receptor that specifically and potently blocks the activity of two members of the vascular endothelial growth factor family, namely VEGF-C and VEGF-D that are involved in the progression of both retinal and corneal diseases. Opthea’s lead program is the development of VGX-300 for the treatment of “wet” (neovascular) age-related macular degeneration (wet AMD).

Opthea has the option to expand its pipeline and preclinical and clinical programs through the development of VGX-300 for indications in addition to “wet” AMD and by progressing VGX-100, a fully human antibody targeting VEGF-C, for the treatment of ocular disorders.

About Circadian Technologies Limited

Circadian (ASX:CIR; OTCQX:CKDXY) is a biologics drug developer focusing on cancer, cancer related and ophthalmic disease therapies. It controls exclusive worldwide rights to a significant intellectual property portfolio around Vascular Endothelial Growth Factor (VEGF)-C and -D and VEGFR-3. 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 VGX-100 (a human antibody against VEGF-C) as a treatment for lymphedema resulting from breast cancer treatment and solid tumours, in particular glioblastoma and colorectal cancer, as well as developing VGX-300 (soluble VEGFR-3) for ‘back of the eye’ disease such as “wet” Age Related Macular Degeneration through its subsidiary Opthea. 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. Sales of the drug Lucentis® (Roche), which targets VEGF-A but not VEGF-C, were over \$US3B 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 \$US838M in 2012 and are forecast to reach \$1.3BN in 2013. Approximately half of the people receiving Lucentis®/Eylea® are classified as non-responders or ‘poor’ responders and experience no 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

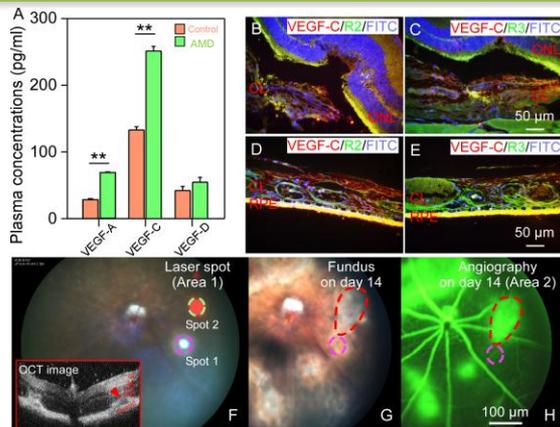
Certain statements in this ASX announcement may contain forward-looking statements regarding Company business and the therapeutic and commercial potential of its technologies and products in development. Any statement describing Company goals, expectations, intentions or beliefs is a forward-looking 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.

PURPOSE and METHODS: Choroidal neovascularization (CNV) is the major cause of severe visual loss in subjects with age-related macular degeneration (AMD). At least 40% of subjects with wet AMD exhibit some degree of resistance to anti-VEGF-A monotherapy. Persistent angiogenesis and vascular leakage in these sub-responsive subjects may be mediated by other members of the VEGF family. VEGF-C is implicated in mediating sub-response to anti-VEGF-A therapies because it can promote angiogenesis and vascular leakage through VEGFR-2 and VEGFR-3. We sought to investigate if circulating and tissue levels of VEGF-C are elevated in AMD subjects and determine if VEGF-C/D blockade can inhibit choroidal neovascularization and leakage in a mouse model of wet AMD.

Plasma VEGF-A, VEGF-C and VEGF-D levels in subjects with AMD and controls were quantitated using multiplex assay (Biorad). VEGF-C, VEGFR-2 and VEGFR-3 were localized in retinal tissues of C57BL/6 mice and AMD subjects by immunofluorescence using commercially available antibodies (Abcam). To determine whether VEGF-C and VEGF-D participate in choroidal neovascularization, we employed a mouse model of laser-induced CNV in which photocoagulation was induced at 8 – 9 positions of each mouse retina (50 μm, 700 mW, 100 ms). Mice (5/group) were administered non-specific IgG (20 mg/kg IP every 2 days), Eylea™ (aflibercept, 20 mg/kg IP every 2 days), or VGX-300 (VEGFR-3 Trap for VEGF-C & VEGF-D; single-intravitreal injection of 40 μg on day 1). CNV areas and extent of leakage were determined by fluorescein angiography (FA) followed by intracardiac perfusion of FITC-dextran in gelatin (10%) on day 14 after photocoagulation.

RESULTS

I. Circulating VEGF-A, -C and -D levels and tissue localization in CNV/AMD



II. Effects of VEGF-A and VEGF-C Inhibition on Laser-induced CNV

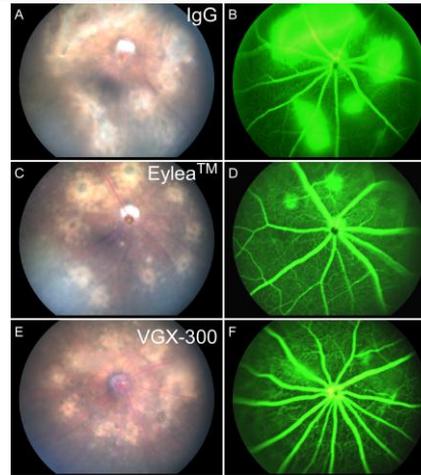


FIG 2. Fundus images and angiography of laser-induced CNV membranes 14 days after photocoagulation and administration of (A – B) IgG, (C – D) Eylea™, or (E – F) VGX-300.

III. CNV lesion sizes in choroidal flatmounts



FIG 3. Anatomical areas of CNV lesions 14 days after photocoagulation in IgG-treated eyes (A) was much larger than those of Eylea™ and VGX-300-treated eyes (B – C). Red circle, photocoagulation area; red arrow head, optic nerve head; white arrow, CNV leakage.

FIG 1. (A) Plasma concentrations of VEGF-A (n = 142), VEGF-C (n = 125) and VEGF-D (n = 70) in controls and AMD subjects. (B) Co-localization of VEGF-C, VEGFR-2, & (C) VEGFR-3 in mouse retina 14 days after laser injury. (D) Co-localization of VEGF-C, VEGFR-2 & (E) VEGFR-3 in clinical specimens of wet AMD. (F, inset) OCT image of laser-induced CNV in choroidal layer. CNV area, as shown in Fig.5A, was determined from FA as shown in (G or H) (Area 2). Percentage of relative increase of CNV area, as shown in Fig. 5B, was determined by calculating [(Area 2 – Area 1)/Area 1] x 100%. CL: choroidal layer.

IV. Incidence of CNV and intensity of leakage

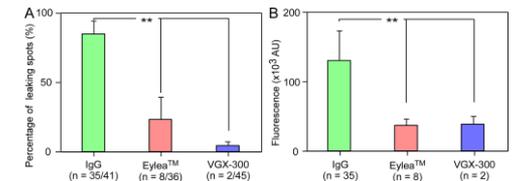


FIG 4. (A) Incidence of laser-induced leaking spots (leakage spots/photocoagulated spots × 100%). (B) Comparison of leakage intensity after FA.

V. Effects of treatment on CNV area

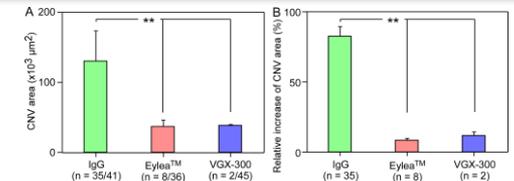


FIG 5. (A) Mean size of laser-induced CNV membranes (total area of leakage spots/total photocoagulated spots). (B) Percentage of relative increase in CNV area (refer to Fig. 1 for calculation method).

CONCLUSIONS

1. VEGF-C levels are significantly elevated in the plasma of AMD subjects compared to healthy volunteers (controls).
2. VEGF-C and its cognate receptors are expressed and co-localized in CNV membranes.
3. 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.
4. VGX-300 may be an effective monotherapy for the treatment of wet AMD by blocking VEGF-C/D.
5. Used in combination with anti-VEGF-A agents, VGX-300 may have the potential to improve clinical responses in wet AMD by more effective inhibition of the pathways involved in disease progression.

ACKNOWLEDGEMENTS

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