AdAlta
next generation protein therapeutics

ASX Announcement

AdAlta to present eye fibrosis data at Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting

MELBOURNE Australia, 8 May, 2017: AdAlta Limited (ASX:1AD), the biotechnology Company advancing its lead i-body candidate towards clinical development, announces that the AdAlta, La Trobe University and The University of Melbourne team will present data on the lead compound, AD-114, for the treatment of eye fibrosis at the Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting.

The ARVO Annual Meeting to be held May 7-11 2017, in Baltimore, Maryland, is the largest gathering of eye and vision researchers in the world, attracting over 11,000 attendees from more than 75 countries. The meeting provides a platform for individuals from academia to industry to discuss novel targets and disease pathways as well as the most promising emerging therapies.

Members of the collaboration, including AdAlta Chief Scientific Officer A/Prof Mick Foley and collaborator Professor Erica Fletcher from The University of Melbourne, will present data on AdAlta's lead candidate, AD-114, for the treatment of eye fibrosis. AD-114 may provide a novel treatment for wet-AMD that has a different mechanism of action to currently-approved therapies.

Details of the poster presentation:
Presentation Number - Posterboard Number: 2259 - B0213
Presentation Type: Poster Session
Session Number: 281
Session Title: Bruch's membrane and choroid in macular disease
Session Date/Times: May 8, 2017 from 3:45 PM to 5:30PM

The poster entitled *"Inhibition of the chemokine receptor CXCR4 reduces pathology in a laser induced mouse model of choroidal neovascularization"* is attached and available on the company's website www.adalta.com.au.

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Notes to Editors

About AdAlta

AdAlta Limited is an Australian based drug development company headquartered in Melbourne. The Company is focused on using its proprietary technology platform to generate i-bodies, a new class of protein therapeutics, with applications as therapeutic drugs to treat disease.

I-bodies are a promising, novel class of drugs that offer a new and more effective approach to treating a wide range of human diseases. They are identified and developed using our proprietary technology platform.

We have pioneered a technology that mimics the shape and stability of a crucial antigen-binding domain, that was discovered initially in sharks and then developed as a human protein. The result is a range of unique compounds, now known as i-bodies, for use in treating serious diseases.

AdAlta is developing its lead i-body candidate, AD-114, for the treatment of idiopathic pulmonary fibrosis (IPF) and other human fibrotic diseases, for which current therapies are sub-optimal and there is a high-unmet medical need.

The Company also plans to continue further drug discovery and development directed towards other drug targets and diseases with its i-body technology platform.

Further information can be found at: www.adalta.com.au.

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Inhibition of the chemokine receptor CXCR4 reduces pathology in a laser induced mouse model of choroidal neovascularization

Wang, Xilun A; Foley, Michael; Venables, Gene and Fletcher, Erica
La Trobe Institute for Molecular Science, La Trobe University and School of Biosciences, University of Melbourne

Posterboard Number: 2259 - B0213

Purpose

Age related macular degeneration (AMD) is currently treated with a range of anti-VEGF inhibitors[1]. Although these treatments have had a profound effect on the acute pathology, in the longer term, vision loss continues for many patients. New drugs are needed to effectively treat wet-AMD. One approach is to target cytokine signalling, which has been implicated in the development of neovascularization pathology[2]. The central aim of this project was to evaluate the role of the chemokine receptor CXCR4 in a mouse model of choroidal neovascularization (CNV).

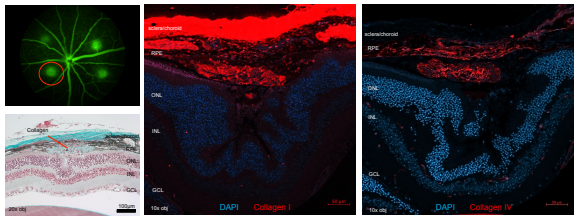
Aim

To determine if anti-CXCR4 single domain antibodies (i-bodies) reduce retinal pathology and fibrosis in a laser induced model of choroidal neovascularization.

Methods

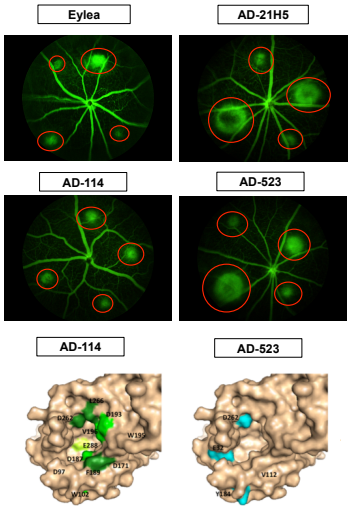
Four CNV lesions per eye were induced in 8 week old female C57BL6 mice (n= 10 eyes/group) using a continuous wave laser (Micron III, 532nm, 350 mW). Animals were intravitreally injected with either a single domain like antibody known as an i-body targeting CXCR4 (AD-114 12µg/ml), a negative control i-body (AD-21H5 12µg/ml) or vehicle (PBS). Leakage was assessed using fluorescein angiography and lesion size was quantified using image J at 7 days. The eyes were then removed, fixed and stained using Masson's trichrome stain. CNV lesion height/choroid height ratio was measured using image J. mRNA expression and a gene ontology (GO) was also undertaken. RNA was extracted from the retina and RPE, 7 days after laser. mRNA expression levels were compared using qPCR arrays (84 fibrosis associated genes). Significantly expressed genes relative to control ($p< 0.05$, fold change ±1.5) underwent overrepresentation testing on the Panther GO platform to identify any potential gene networks modified by CXCR4 inhibiting i-bodies.

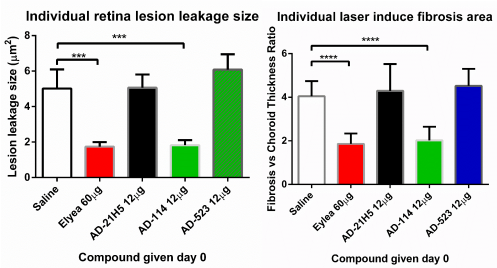
1. Day 7 is the optimum time point to study leakage and fibrosis in the mouse eye after CW laser induced lesions



Lesion leakage and fibrosis growth was present 7 days following laser induced injury. Photoreceptor and RPE disruption was observed within lesions. Significant vascularisation and detachment of the outer nuclear layer was present, and fibrosis identified by Collagen I & IV labelling noted.

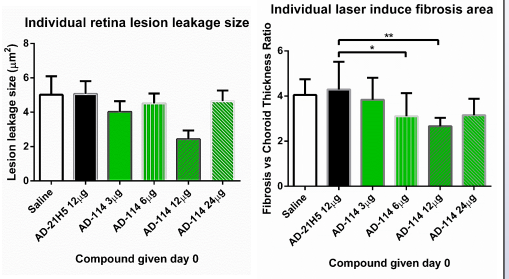
2. A CXCR4 antagonist i-body AD-114 prevents laser induced lesion leakage and fibrosis





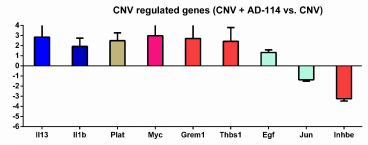
i-bodies were injected at day 0 immediately following laser injury and lesion leakage and area of fibrosis were measured following 7 days. VEGF trap (Eylea) and CXCR4 antagonist i-body AD-114 both reduced leakage by ~50% ($p<0.001$). Another anti-CXCR4 i-body AD-523 showed no effect on either leakage or fibrosis as did a negative control i-body AD-21H5. Epitope mapping of AD-114 demonstrated that it bound to residues deep in the CXCR4 binding pocket while AD-523 bound predominantly wtihin an extracellular domain.

3. Dose dependant effects of i-body on leakage and fibrosis



Differing i-body concentrations (3 µg/ml, 6 µg/ml, 12 µg/ml and 24 µg/ml) were assessed at day 0 (n = 10/group) for lesion leakage and fibrosis prevention. A concentration of 12 µg/ml was found to have the optimal effect on reducing fibrosis. The effective doses of AD-114 was between 6-24 µg/ml.

4. Differential expression of genes identified by fibrosis PCR-Array analysis following treatment with AD-114

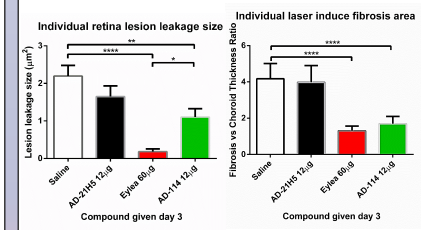


AD-114 vs Control

Description	Gene Symbol	Fold Regulation	P	Gene ontology Groups
Interleukin 13	Il13	2.8356	*	Inflammatory Cytokines and Chemokines
Interleukin 1 beta	Il1b	1.9296	*	Inflammatory Cytokines and Chemokines, Proinflammatory cytokine production
Plasminogen activator, tissue	Plat	2.484	**	Blood coagulation and fibrin degradation
Myelocytomatosis oncogene	Myc	2.9787	*	Cell cycle progression, apoptosis and cellular transformation
Gremlin 1	Grem1	2.7069	*	Signal Transduction:TGFß Superfamily
Thrombospondin 1	Thbs1	2.4172	*	Signal Transduction:TGFß Superfamily
Epidermal growth factor	Egf	1.3177	*	Cell growth, proliferation and differentiation
Jun oncogene	Jun	-1.3774	*	Cell growth, proliferation and differentiation
Inhibin beta E	Inhbe	-3.2468	*	Signal Transduction:TGFß Superfamily

A laser induced injury causes a number of genes to be modulated that are attenuated by treatment with AD-114. Of note there was significant downregulation of INHBE which is a gene in the TGFβ pathway and a previously reported fibrotic gene[4].

5. The CXCR4 antagonist i-body AD-114 is effective even when administered 3 days after laser induced injury



i-bodies were injected 3 days after laser induced injury and lesion leakage and fibrosis assessed 4 days later (ie 7 days after laser induced injury). Both VEGF-trap (Eylea) and CXCR4 antagonist i-body AD-114 treatment significantly reduced leakage and fibrosis. AD-114 was less effective in reducing lesion leakage than VEGF-trap (Eylea), but had a similar effect in reducing fibrosis. This is consistent with a different mode of action for an anti-CXCR4 antagonist.

Conclusions

- Treatment with an anti-CXCR4 i-body AD-114 reduced lesion leakage and fibrosis when evaluated 7 days are laser induced injury.
- RT-PCR analysis demonstrated that AD-114 significantly altered TGF-β signaling, cytokine signaling pathways and regulation of fibroblast proliferation.
- Overall, treatment with anti-CXCR4 i-body AD-114 may offer an alternative treatment mechanism than currently available with anti-VEGF agents.

References

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Conflicts of interest statement:

Wang, Venables, Fletcher:Code N- None; Michael Foley is the Chief Scientific Officer for Adalta: Code E Employment

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