

13 June 2014

## ASX ANNOUNCEMENT

### ACTINOGEN ISOLATES DEMONSTRATE ANTITUMORIGENIC ACTIVITY

- Proliferation of GBM Cancer Stem Cell lines (U87, U373) inhibited by Actinogen isolates
- Significant reduction of proliferation in CSC populations in GBM cell lines (A172, U138).
- CSC sphere disruption, cell anchorage, and cell death observed with different isolates for the CSCs across all 4 cell lines.

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The Board of Actinogen Limited (ASX: ACW) is pleased to announce that it has received positive initial results from its Cancer Stem Cell (CSC) project conducted by researchers at Curtin University.

11 isolates from Actinogen's library were tested on the ability to demonstrate antitumorigenic effects by affecting the viability of cancer stem cells in selected glioblastoma cell lines. Glioblastoma is considered as the most aggressive type of brain cancer. The cell lines tested included U87, U138, A172 and U373. Researchers analysed the viability of cells post treatment using MTT assay, cell morphology and gene expression profiling.

Proliferation of CSCs from U87 was inhibited by the isolate samples ac4935, ac5049, ac5084, and ac5092. Proliferation of CSCs from U373 was inhibited by the isolate samples ac4904, ac4935, ac5059 and ac5086. In both A172 and U138, the isolates ac5049, ac5073 and ac5099 were observed to cause significant reduction (10-25% and 25-30% respectively) in the proliferation of CSC populations. The variation between cell lines could be explained by different origin of these cells.

Interestingly, the proliferation of the cancer cell populations from A172 and U138 was significantly inhibited by isolates ac5059 and ac 5086. The cell populations were reduced by approximately 80%. This data is consistent with our previous internal results and supports the strong anti-cancer activity of some of the actinomycetes isolates.

Sphere formation, which is a characteristic phenotype of CSCs, was examined following treatment with isolates. Reduction in sphere size, together with restoration of anchorage and differentiation are indicators of the inhibition of CSC activity. When these parameters were thoroughly examined after the treatment with isolates, it was observed that there was sphere disruption, cell anchorage, and cell death with respective isolates for the CSCs from four cell lines.

Gene expression analysis of the glioma-specific CSC marker (CD133), cell death marker (Bax); and cell proliferation marker (Cyclin D1) was conducted by RT-PCR. Overall, the data was concurrent with cell viability assay and cell morphology characterization and favours further investigation into the mechanism of action of actinomycetes isolates on cancer stem cells viability.

“We are very excited to see the data on anti-tumour activity of our actinomycetes isolates that has elements consistent with our previous findings and will be updating the investors on our future steps in this project” said Dr Brendan de Kauwe, Executive Chairman of the Company.

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Dr Brendan de Kauwe  
Executive Chairman  
**Actinogen Limited**

#### **About Cancer Stem Cells**

Cancer stem cells have been reported in many human tumours and are classified as a highly tumorigenic subpopulation that drives tumour formation, proliferation and metastasis. CSCs share a variety of biological properties with normal stem cells such as capacity for self-renewal and propagation of differentiated progeny. However, CSCs differ from normal stem cells in their inherent resistance mechanisms against radiation- and chemotherapy-induced cancer cell death, enabling them to survive and initiate tumour recurrence. Despite their potential clinical importance, the regulation of CSCs at the molecular level is not well-understood and no drugs specifically targeting CSCs been developed to date. However, recent research in brain tumours has identified a CD133+ cell population as a cancer stem cell population, giving the way to some targeted therapeutic approaches.

#### **About Actinogen Limited**

Actinogen Limited (ASX: ACW)

Actinogen is dedicated to the discovery and isolation of a group of environmental bacteria known as the Actinomycetes. Actinomycetes have been shown to be able to use a wide range of unusual nutritional resources and often produce bioactive molecules as a by-product that have been proven to be useful to man; including well known commercial examples such as bacterial antibiotics, anti-fungal agents, anticancer agents and a variety of other chemicals that are used in the control of physiological and physical processes.

The Company's primary focus is on drug development and therapeutics, with one of its lead therapeutic programs directed towards discovering and developing drugs to treat brain cancer and potentially other oncological diseases, by the targeted killing of cancer stem cells (CSCs); with research currently being conducted in collaboration with Curtin University.

In addition, there are examples of Actinomycetes that can degrade industrial wastes such as oils, tar, domestic and industrial waste, and the rehabilitation of oil spills. The Company is currently conducting research in the breakdown of Biomasses to Bioethanols in collaboration with Leaf Energy Ltd (ASX: LER).

Actinogen has proven high-level expertise in the discovery and isolation of Actinomycetes from WA soils and in the detection of bioactive molecules they produce. The Company's strategy is to collaborate with groups nationally and internationally who are active in the development of technology that is synergistic with or that could utilise the Company's current Actinomycetes research projects and exclusive intellectual property.