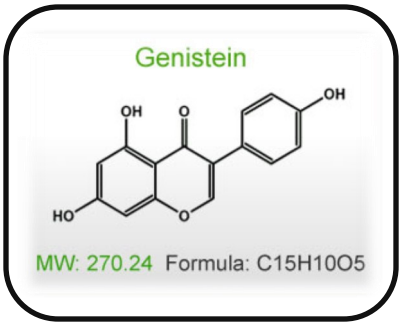
DEGENERATIVE DISEASES

Degenerative Diseases Program Overview



Genistien-related compounds and degenerative disease

Over the last 20 years, Novogen have developed a range of compounds, based on a naturally occurring plant chemical called *genistein*. The most recent generation of these compounds, designed for anti-cancer use, are called the *super-benzopyrans (SBPs)*. Genistein shows natural anti-cancer activity in cells cultured in the laboratory but is no use as a drug molecule itself because it does not survive well through the process of oral ingestion and absorption into the body.

By repeatedly modifying genistein's chemical structure, Novogen have enhanced and refined these anti-cancer properties to an extraordinary degree, ultimately resulting in the Company's main SBP drugs *Cantrixil* and *Trilexium*.

The successful development of anti-cancer compounds is a process of two parts; expertise in drug design of genistein-related molecules and high-quality models of disease involving cultured cells or animals to test the compounds on. Novogen have 20 years experience of the former and are establishing collaborations with scientists from some of the leading laboratories in Australia and the world to facilitate the latter.

Based on an up-to-date assessment of published genistein research and the data Novogen has accumulated on the properties of their own compounds, five areas of potential application are currently being researched

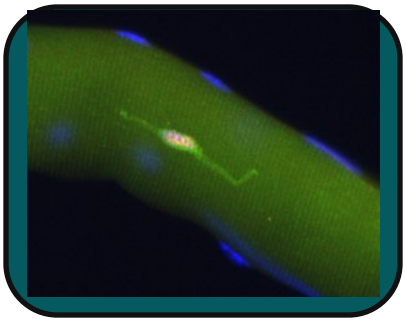
Genistein has poor drug like qualities but closely related chemicals designed by Novogen could provide novel therapies. One such chemical, Phenoxodiol, originally designed by Novogen as an anti cancer agent, also protects neuron-like cells from toxic insults in a culture dish.

An increasingly large body of current research on the properties of genistein shows that it is one of the most versatile compounds ever isolated in nature because it can trigger so many different responses from cells. These different responses are activated by binding of the molecule to a variety of different components within the cell. Imagine a cartwheel with genistein at the hub and a series of spokes radiating from it that contact different individual parts of the cellular machinery.

When genistein is added, the wheel turns and many different cellular components are all pulled at once. While this makes genistein's action complicated to unpick and comprehend, it also provides multiple opportunities for new clinical applications other than treating cancer. The anti-cancer activity represents only one spoke of the wheel and the cellular contacts of the other spokes are now starting to become clear.

It turns out that some of these spokes make contact with and exert control over critical cell functions such as cell proliferation, energy production, gene activity, stress responses and immune system activation. These are pathways that are central to the development of treatments for a variety of degenerative diseases. The challenge and the opportunity is to isolate these individual modes of action and to enhance and refine their power using the same successful strategy used to develop the anti-cancer compounds.

Overview—5 areas of potential application

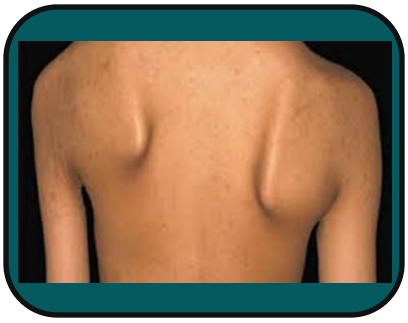


Stem Cell Regeneration

Many adult tissues contain pockets of dormant or slowly cycling stem cells that can be activated during damage and disease and will proliferate and repair the lost or diseased tissue. However, sometimes the disease process is so severe that it overwhelms the capacity for regeneration leading to progressive decline (e.g. Duchenne muscular dystrophy). In DMD lack of the protein dystrophine causes muscles to deteriorate and break down leading to progressive difficulty with walking and general mobility. Approximately 1 in 3500 live male births throughout the world. There is no cure for DMD.

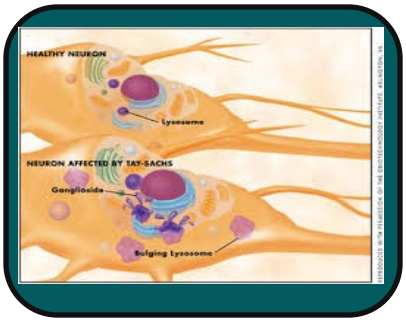
Neurodegenerative Diseases

Neurodegenerative diseases such as Alzheimer's and Parkinson's involve a lack of function in affected mature nerve cells while they are still alive. Current research shows that abnormal proteins accumulate in the cell, the energy production system becomes profoundly unbalanced and the crucial synapses with other nerve cells are blocked, thus preventing normal function. Eventually the brain cell dies and localised inflammatory processes are triggered that worsen the damage process.



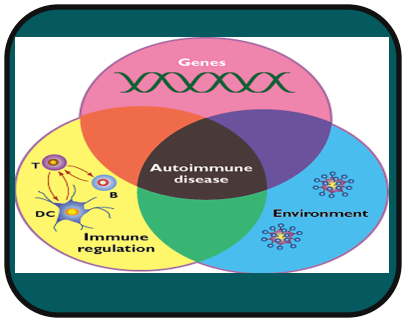
Muscular dystrophy

Unlike the other muscular dystrophies, which are mostly caused by loss of function mutations in key muscle genes, FSHD is caused by the activation of a gene called *DUX4* that is normally silent in muscle tissue. This gene encodes a transcription factor that regulates other genes and causes the inappropriate activation of abnormal cellular processes. This causes progressive muscle weakness and extreme pain starting in the muscles of the face, the shoulders and the upper arms.



Lysosomal storage disorders

This class of diseases are caused by genetic mutations in essential cellular enzymes that are part of a production line processing large chemical components of cells. When the proteins are missing, the production line starts to back up and the cell accumulates large amounts of chemical intermediates in spherical bodies called lysosomes where these production lines exist.



Autoimmune disease

Autoimmune disease occur when the defence system of the body attacks itself and leads to a range of disorders like diabetes type I, multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease.

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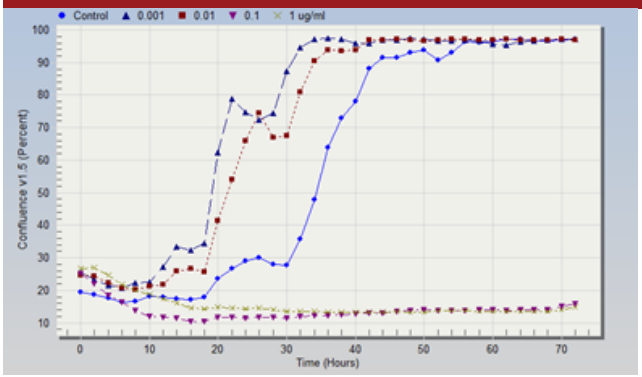
Research—Stem Cell Regeneration



The blue line indicates growth of an ovarian cancer stem cell line with no drug treatment, yellow and purple lines indicate cells treated at effective anti-cancer CS-6 concentrations and the red and dark blue lines indicate cells treat at very low sub-therapeutic doses. The impact of low concentrations indicates a significant increase in cell number over time.

A chance finding during work on ovarian cancer indicated that one of the main SBP candidates, CS -6, was effective at promoting proliferation and differentiation in cultured stem cells. Encouraging the body's own capacity to repair itself is a an ideal form of therapy and the regeneration of brain or spinal cord nerve tissue is regarded as the holy grail of neuroscience research.

Genea BIOCELLS

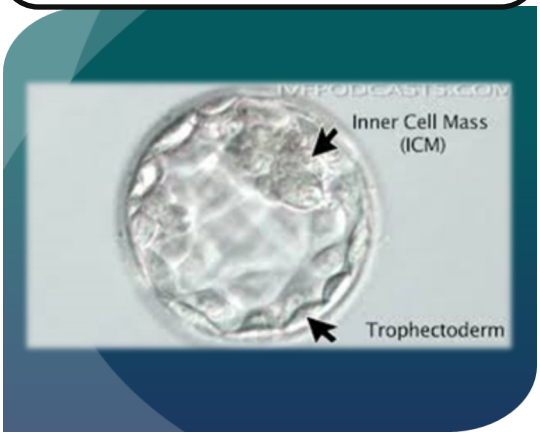
Novogen have formed a *partnership with Genea Biocells* to screen its library of compounds on models of stem cell repair in adult tissues. Genea Biocells have established cell culture systems that drive the differentiation of human embryonic stem cells to form neurons or muscle cells in culture and the impact of the Company's compound library is being tested using markers of proliferation and differentiation.

In addition to normal embryonic stem cells, Genea Biocells also have a bank of embryonic stem cells derived from early embryos diagnosed with genetic disease during IVF screening procedures. Parallel studies in neuronal differentiation with cells that have the mutation that causes Down's syndrome and infantile neuroaxonal dystrophy (IND) will also explore the potential to enhance and normalize these cells. Preliminary studies have already indicated that CS-6 enhances the proportion of differentiated neuronal precursors in IND cells

Similarly, cells with the muscle diseases facioscapulohumeral dystrophy, myotonic dystrophy and nemaline myopathy are being used to test the impact of the compounds on the differentiation of these abnormal cells in the context of muscle disease.

Work with Genea Biocells commenced September 2014 and results are now beginning to emerge (November 2014)

An early human embryo (blastocyst) at the stage at which embryonic stem cells are isolated (1st week of pregnancy). Using embryos created by IVF, the embryonic stem cells are taken from the inner cell mass, which would normally go on to produce the body of the embryo. At this stage, it is just a tiny ball of cells.



Research—Neurodegenerative diseases

Late stage Alzheimer's disease: causes shrinkage of the brain as a result of cell death. A large amount of the remaining cells have ceased to work because of the presence of intracellular 'tangles' and extracellular 'plaques'. The extracellular plaques are made up of the abnormal protein amyloid-beta.

Genistein-related molecules

Several lines of evidence indicate that genistein-related molecules are strong candidates to slow or halt the progress of the disease process. Work on cell culture models of Alzheimer's disease indicate that genistein can protect cells from death induced by amyloid-beta peptides (thought to be the primary toxin in Alzheimer's disease) and provides protection from the oxidative stress and mitochondrial dysfunction that seems to be a universal part of neurodegeneration. Phenoxodiol, one of the early anti-cancer drugs produced by Novogen, also shows neuroprotective properties.

One of the contentious issues is how to model these complex diseases 'in a dish' in a way that is truly predictive of efficacy against neurodegeneration in humans. In recent years, many drugs that looked promising in cell culture and animal models have failed to meet the primary outcome measures in clinical trials. There has been a strong desire in the neurodegenerative disease research community to focus on protection of cell function rather than the much easier, but probably misleading, assay for protection from cell death.

It is not currently clear what are the spanners that are thrown into the works to stop the cogs inside the neuron from turning or how that leads to a chain of consequences ending in shutdown of the brain cell. Large resources are being invested in addressing these questions. However, it is becoming obvious that while the initiating problem is different in each of the neurodegenerative diseases (e.g. Alzheimer's, Parkinson's, motor neuron disease, Huntington's and frontotemporal dementia), the consequences usually converge on the same set of cellular pathways.

Novogen have initiated a preliminary compound screen in these worms at the Florey Institue of Neuroscience Research , Melbourne and the initial findings clearly show that genestein provides functional protection from the paralysing effect of amyloid-beta

A powerful drug screening system using a nematode worm called *Caenorhabditis elegans* has emerged that can easily assess the functional consequences of neurodegenerative insults (such as amyloid-beta). As the neurodegenerative toxin accumulates and cell function begins to slow, the mobile worms become paralysed; an effect that is easily visible under the microscope. Agents that can protect the worm's neuromuscular system from paralysis are potentially predictive of similar benefits against neurodegeneration in the human brain.

In what is, virtually, a vacuum of therapeutic options for neurodegenerative disease, any treatment that halts or slows the progress of the disease process would be an unprecedented leap forward of major significance.

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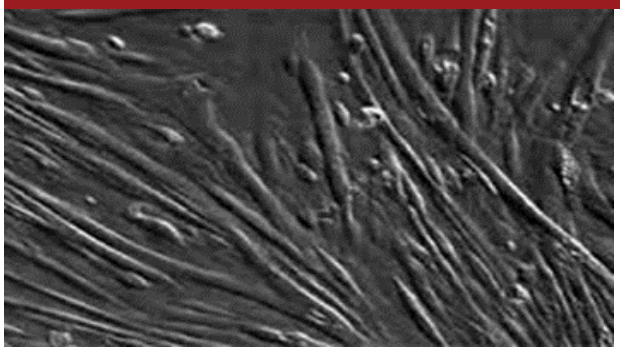
Research—Muscular dystrophy



Human embryonic stem cells can be made to develop into early muscle fibres, called myotubes (photo shows a microscope image of myotubes in a culture dish). FSHD myotubes are smaller and thinner than normal.

Genistein-related compounds

Modulation of the epigenetic machinery turns out to be another spoke of the genistein activity wheel, particularly for the silencing of repetitive DNA regions such as those where *DUX4* is found. Therefore, the aim of this research project is to isolate genistein-related compounds that are effective at silencing *DUX4*.

This work is made possible through an excellent model of early events in the FSHD disease process developed by Genea Biocells. By isolating embryonic stem cells from embryos diagnosed with FSHD and developing methods that allow them to drive such cells into the skeletal muscle pathway, Genea have shown that early FSHD muscle fibres grown in a tissue culture dish switch on *DUX4,* just like in the disease, resulting in smaller and thinner muscle cells with intracellular abnormalities that match the disease process.

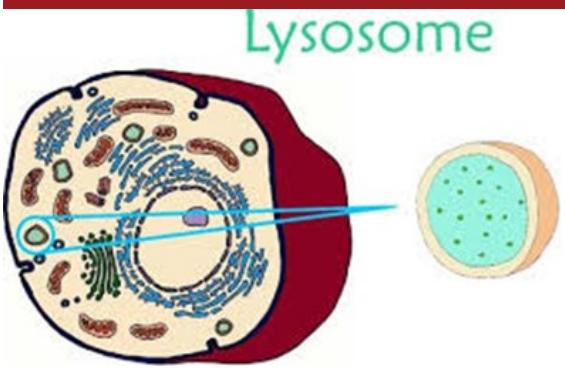
The first screen for compounds that might shut down DUX4 and rescue the cells from these abnormalities began November, 2014

Dux 4

DUX4 is activated in muscle through the failure of *epigenetic* control mechanisms. Epigenetic mechanisms can control gene activity by changing the density of the packing material in direct contact with our DNA and are normally responsible for keeping genes such as *DUX4* locked away from accidental activation. However, changes in the organization of the DNA around the *DUX4* gene in FSHD patients means that the usual signals for the epigenetic machinery are not present, the gene is not packaged properly and becomes available for sporadic activation.

In another joint venture with Genea Biocells, Novogen have begun a research program to investigate the potential use of genistein-related compounds for the treatment of the debilitating muscle disease, facioscapulohumeral dystrophy (FSHD), which is supported by a New Concept Grant from the charitable foundation, FSHD Global, awarded in July 2014.

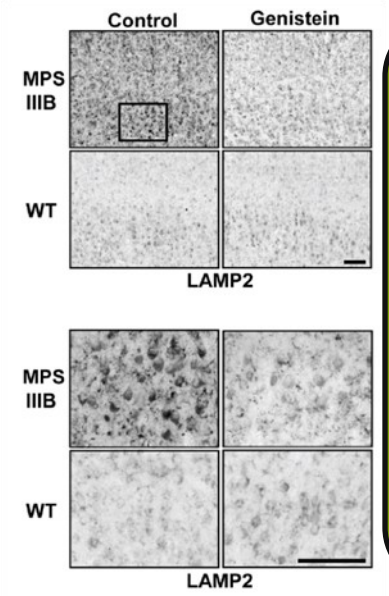
Research—Lysosomal Storage Disorders (LSDs)



Lysosomes are small spherical bodies found in all cells of the body, which are responsible for degradation and recycling of intracellular materials.

An experimental pipeline for drug development has been planned and Novogen are currently seeking funding support to begin work on this project.

In cultured cells taken from patients with this disease, genistein has been shown to reduce lysosomal accumulation of the damaging mucopolysaccharides and this has formed the basis of a new therapeutic strategy called *substrate reduction therapy*. The major advantage of this system is it would utilise small molecule drugs that can cross the blood-brain barrier. Genistein is currently being tested as a treatment for Sanfilippo syndrome in a clinical trial in the UK.



Sections through the brain tissue of mice that have the disease MPSIII (Sanfilippo syndrome) stained to show the presence of lysosomes by detecting lysosomal associated membrane protein (LAMP2). Mice treated for 11 months with genistein have much less accumulation of abnormal lysososmes than mice that receive no treatment (Control) and look similar to the levels found in normal (wild type) mice (WT). The image at the bottom is a high-power image of the area marked by the box in the upper panel.

Genistein is unlikely to form the basis of a truly effective drug because it is rapidly altered and degraded when it is taken orally and thus only a small fraction survives intact to be exposed to the abnormal brain cells. Therefore, Novogen have established a collaboration with Professor Grzegorz Wegrzyn in Poland who is responsible for the original concept of treating MPSIII in this way and has high-quality cell models that can be used to test new genistein-related compounds.

Research—Autoimmune disease

Autoimmune disease occur when the defence system of the body attacks itself and leads to a range of disorders like diabetes type I, multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease.

Many experiments show that genistein-related compounds can selectively suppress the immune system so that levels of activation are reduced without blocking its activity altogether. This is the key desirable outcome that forms the basis of most current treatments for these diseases.

Novogen have previously generated genistein-related compounds that show an ability to suppress T cell responses, which are critical players in autoimmune disease and others that will reduce pathology on rodent models of inflammatory bowel disease.

Accumulating evidence also shows that genistein has the potential to block specific processes in cell culture and rodent models of rheumatoid arthritis and multiple sclerosis that is not directly dependent on direct immunosuppression. Therefore, there are several spokes of genistein action that are applicable within this disease category alone.

Novogen is currently seeking advice from leading experts in the field in how to convert these observations into experimental strategies for drug development.

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Clinical Strategy and Market Advantage

* Destroys the cancer stem cells that cause tumor re-growth
* Destroys the cancer cells that have become resistant to chemotherapy
* Can administer the drug to the site of the tumor

Cost of Ovarian Cancer

Projected Clinical Use

Market Opportunity

Market research group GBI Research forecast the global ovarian cancer therapeutics market will grow to US1.9B in 2020 at a CAGR (Compound Annual Growth Rate) of 3.4%

About Novogen Ltd

Novogen has two main drug technology platforms: super-benzopyrans (SBPs) and anti-tropomyosins (ATMs). SBP compounds have been created to kill the full range of cells within a tumor, but particularly the cancer stem cells. The ATM compounds target the microfilament component of the cancer cell and when used in conjunction with standard anti-microtubular drugs, result in comprehensive and fatal destruction of the cancer cell's cytoskeleton. Ovarian cancer, colorectal cancer, malignant ascites, prostate cancer, neural cancers (glioblastoma, neuroblastoma in children) and melanoma are the key clinical indications being pursued, with the ultimate objective of employing both technologies as a unified approach to first-line therapy.