Project Jacob's Hope

Program Overview

Jacob's Hope is the name we have given to the degenerative and regenerative diseases program that is highly experimental and investigational.

It is names after a very brave young man called Jacob who has a disease known as Duchenne muscular dystrophy (DMD). Jacob and thousands of children like him around the world, is the reason we are running this program.

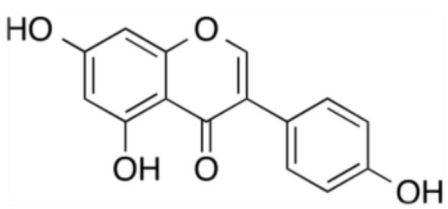
The Novogen strategy for drug development

Over the last 20 years, Novogen have developed a range of compounds, originally based on a naturally occurring plant chemical called *genistein*. The most recent generation of these compounds, designed for anti-cancer use, are called the *superbenzopyrans (SBPs)*. Genistein shows natural anti-cancer activity in cells cultured in the laboratory but is no use as a drug molecule itself because it is not very potent and 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*.

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.

Genistein



The aim of the Degenerative Disease Program is to use the same strategy to isolate compounds effective against a range of genetic and age-related illnesses. This is a process of two parts; expertise in drug design and the provision of high-quality models of disease. Novogen have 20 years experience of the former and are establishing collaborations with expert scientists from some of the leading laboratories in Australia and the world to enable the latter.

Genistein

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 key that can unlock many different doors within the corridors of our cells. When genistein is added, many of these doors are unlocked and many different cellular processes are either activated or silenced. While this makes genistein's action complicated to comprehend, it also provides multiple opportunities for new clinical applications other than treating cancer. The anti-cancer function represents only one of the doors of activity and we are now starting to understand the potential opportunities that lie behind the other doors.

It turns out that these doors lead to 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.

Cost of degenerative disease

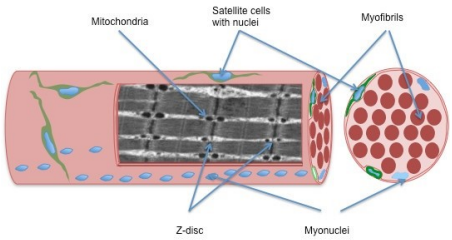
Since the beginning of recorded history, young children have outnumbered the elderly but United Nations statistics show that before we reach 2020, the percentage of people over 65 will overtake the number of people who are less than 5. In 2010 the number of people over 65 was 524 million and by 2050, that figure will have tripled to 1.5 billion, with the majority located in the China and India.

Life expectancy is increasing and expectations for individual wealth continue to rise but the economic burden of caring for the health of the elderly is a significant obstacle to continued growth. 25-30% of people over 85 have dementia; most requiring a high level of care for the basic activities of living. Alzheimer's disease is the most common form of dementia and estimates indicate that between 27-36 million people are currently living with the disease. Globalizing trends mean that more families are moving to cities leaving ageing parents unable to care for themselves. This does not take into account the enormous social and emotional burden these diseases place on those affected and their families.

Overview—5 areas of potential application

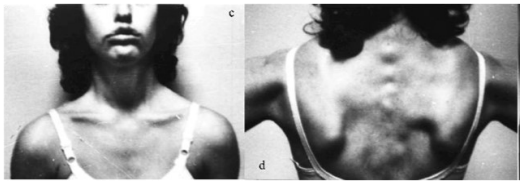
Stem Cell Regeneration

Many adult tissues, such as muscle, 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. One example is Duchenne muscular dystrophy (DMD) in which a lack of the protein dystrophin causes muscles to deteriorate leading to progressive disability. DMD affects approximately 1 in 3500 live male births throughout the world and there is no cure. Image reproduced from Thorsen et al. (2012) BMC Cancer 12:123 DOI: 10.1186/1471-2407-12-123



Neurodegenerative Diseases

Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease 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. There have been no significant advances in dementia treatment in the last ten years.



Muscular dystrophy

Unlike the other muscular dystrophies, which are mostly caused by loss of function mutations in key muscle genes, such as the Dystrophin gene that causes DMD, facioscapulohumeral dystrophy (FSHD) is caused by the activation of a gene called *DUX4* that is normally silent in muscle tissue. This gene encodes a protein 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. Image of an FSHD patient reproduced from Kazakov et al. (2013) Hereditary Genetics: Current Research DOI: 10.4172/2161-1041.S1-007

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.

Research—Stem Cell Regeneration

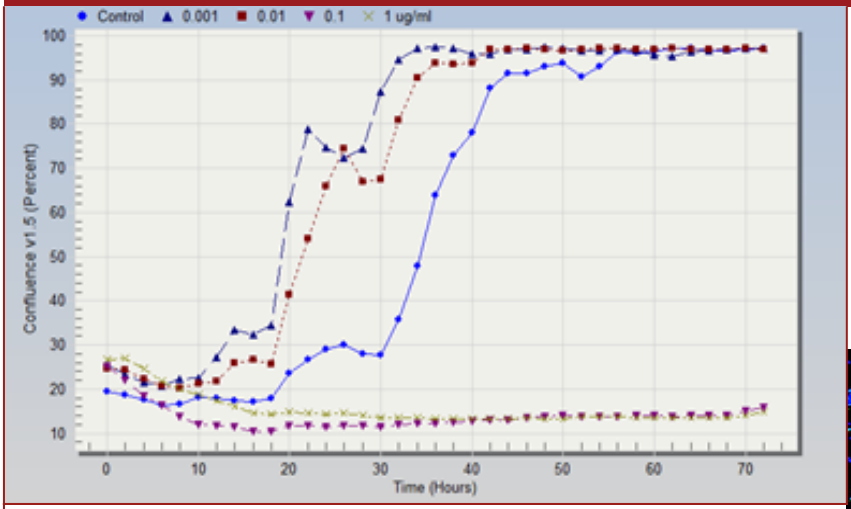
*A chance finding during work on ovarian cancer indicated that one of the main **SBP** candidates was effective at promoting proliferation and differentiation in cultured stem cells. Encouraging the body's own capacity to repair itself is an ideal form of therapy that avoids many problems such as graft rejection. Promoting the ability of our own stem cells in the brain to regenerate new nerve cells is regarded as the holy grail of potential new neurological treatments.*

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.

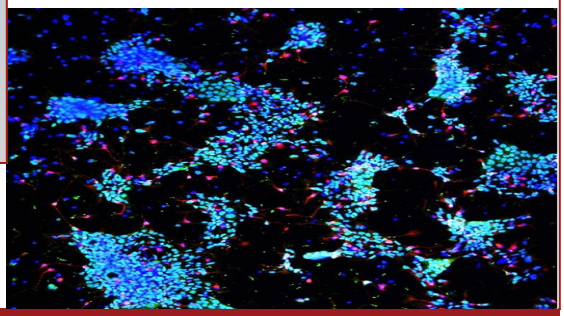
In addition to normal embryonic stem cells, Genea Biocells also have a bank of embryonic stem cells derived from early embryos diagnosed with genetic disease during IVF screening procedures. Parallel studies in neuronal differentiation with cells that have the mutation that causes Down's syndrome and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

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.

Impact of SBP on ovarian cancer stem cells



The blue line tracks growth of an ovarian cancer stem cell line with no drug treatment, yellow and purple lines indicate cells treated at effective anti-cancer SBP concentrations and the red and dark blue lines indicate cells treated at very low sub-therapeutic doses. The impact of low concentrations indicates a significant increase in cell number over time and a change in the shape of the cells also indicated that cells had become more 'normal'.

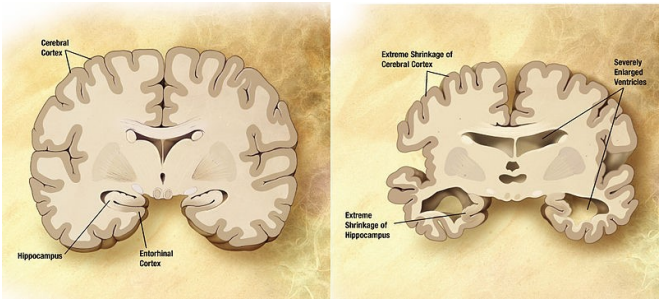


Human embryonic stem cells in culture that are being driven to form nerve cells. Blue marks the nuclei and allows us to count the total number of cells, green marks cells that contain a marker for early stem cells and red marks the newly-formed nerve cells, which can be counted and analysed for level of maturity.

Research—Neurodegenerative diseases

Protecting and restoring brain cell function

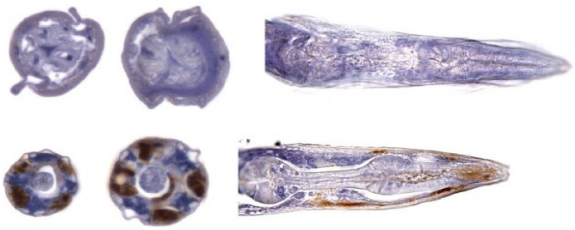
It is not completely clear what are the spanners that are thrown into the works to stop the cogs inside the neuron from turning or how that leads to a chain of consequences ending in shutdown of the brain cell. Large resources are being invested in addressing these questions. However, it is becoming 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. Therefore, effective treatments for one will almost certainly be useful for all.



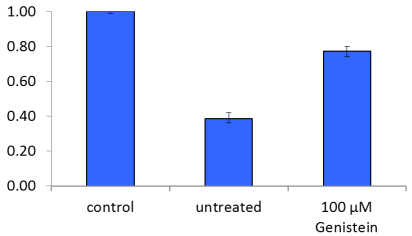
Late stage Alzheimer's disease: causes shrinkage of the brain as a result of cell death. A large amount of the remaining cells have ceased to work because of the presence of intracellular 'tangles' and extracellular 'plaques'. The extracellular plaques are made up of the abnormal protein amyloid-beta.

Sophisticated tools for drug discovery

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.



The transgenic *Caenorhabditis elegans* amyloid-beta model system. Sections through a normal worm (top) and a transgenic worm (bottom) stained for the human amyloid-beta peptide. Image reproduced from McColl et al (2012) BMC Molecular Neurodegeneration 7:57 DOI: 10.1186/1471-2431-11-97



A preliminary investigation to test whether genistein can protect cell function in the *Caenorhabditis elegans* model system clearly showed a powerful ability to inhibit the paralysing effect of the amyloid-beta peptides made in these transgenic worms. (control) normal worms that have no amyloid-beta, (untreated) transgenic worms producing human amyloid-beta peptides, (100um Genistein) transgenic worms treated with genistein showing effective protection from the paralysing effect of amyloid-beta.

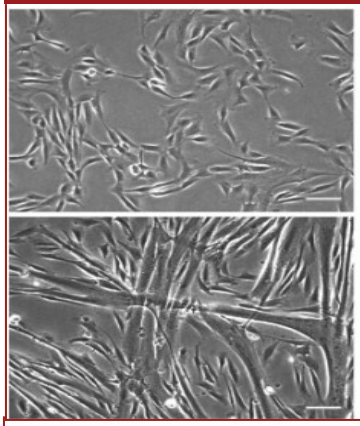
In what is, virtually, a vacuum of therapeutic options for neurodegenerative disease, any treatment that halts or slows the progress of the disease process would be an unprecedented leap forward of major significance.

Research—Drug discovery in dementia

Evidence shows that genistein-related molecules are strong candidates to slow or halt the progress of dementing illnesses such as Alzheimer's disease. Amyloid-beta, which is thought to be the primary toxin in Alzheimer's disease, will kill cells in culture in a way that is similar to the cell death found in Alzheimer's disease. The addition of genistein provides protection from this cell death by relieving the oxidative stress and mitochondrial dysfunction that seems to be a universal part of many forms of neurodegeneration, not just Alzheimer's.

However, effective drug discovery in Alzheimer's has proved to be particularly elusive, probably because Alzheimer's is more complex than we originally thought. One of the most contentious issues is how to model complex neurological diseases, 'in a dish', in a way that is truly predictive of efficacy against neurodegeneration in ageing people. 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 using more sophisticated tools for drug discovery.

Research—Muscular dystrophy



Modelling abnormal muscle in a dish

Human embryonic cells can be made to develop into early muscle fibres, called myotubes. The upper photo shows individual myoblast cells and the bottom shows the same set of cells after they have been induced to fuse together to form myotubes. Image reproduced from Trenerry et al. (2011) BMC Physiology 11:6 DOI: 10.1186/1472-6793-11-6

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.

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

Faciosacpulohumeral Dystophy (FSHD)

FSHD is caused by the inappropriate activation of a gene called *DUX4* that is normally silent in muscle tissue. This gene encodes a protein that regulates other genes and causes the 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.

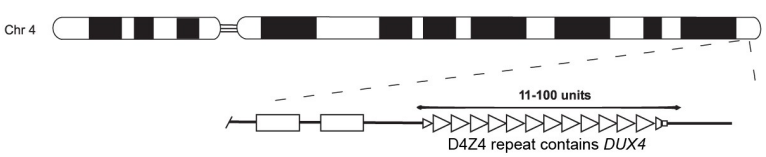
***DUX4* activation**

DUX4 is activated in muscle through the failure of *epigenetic* control mechanisms. Epigenetic mechanisms 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. 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.

FSHD drug discovery

Experiments in mice show that modulation of the epigenetic machinery turns out to be another door unlocked by genistein. This is particularly true for the silencing of repetitive DNA regions such as those surrounding *DUX4*. Therefore, the aim of this research project is to isolate genistein-related compounds that are effective at silencing *DUX4*, which would block the progress of FSHD.

This work is made possible through an accurate 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.

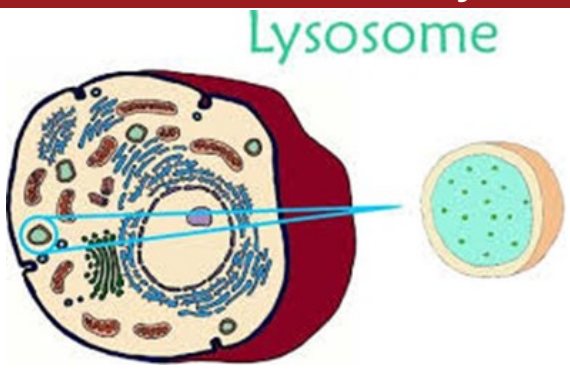


The D4Z4 DNA repeat at the end of human chromosome 4 (Chr 4) is normally approximately 100 units long but in FSHD, this is shortened to less than 10, leading to incorrect epigenetic silencing and activation of the DUX4 gene. Image reproduced from Pearson (2010) PLoS Genetics 6(10):e1001180. DOI: 10.1371

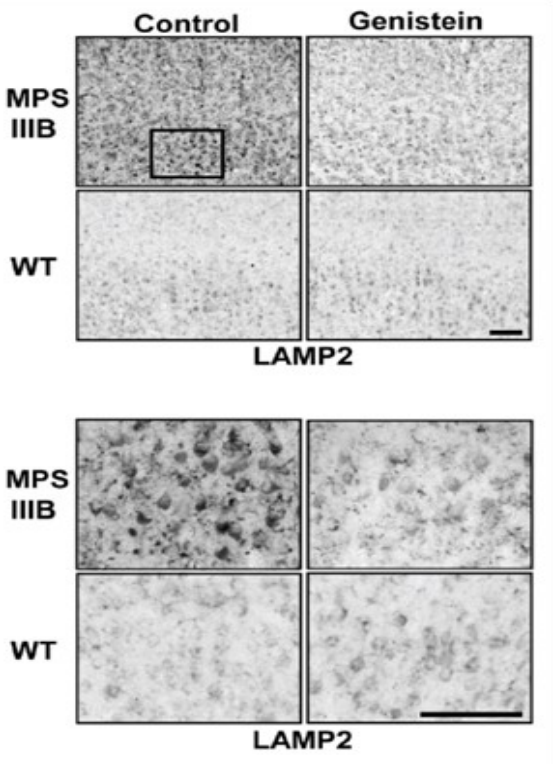
The first screen for compounds that might shut down DUX4 and rescue the cells from these abnormalities began November, 2014

NOVOGEN

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.



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 inside lysosomes. When the proteins are missing, the production line starts to back up and the cell accumulates large amounts of chemical intermediates inside the lysosomes.

Substrate Reduction Therapy

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.

Drug discovery for Substrate Reduction Therapy

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.

Genistein is an effective treatment in mice.

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. Image reproduced from Malinowska et al. (2010) PLoS ONE 5(12): e14192. DOI:10.1371/journal.pone.0014192

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.