

Therapeutic Inspiration from Great Biological Stories in Humans

*Drug discovery progress via nature's 'human experiments'
plus superb clinical observation*

“Many physicians have never lacked motivation to develop treatments for diseases. Instead of the messy work of studying sick patients, scientists now prefer experimenting with inbred mice and cultured cells. Their results accrue faster and are scientifically cleaner, but they arguably are less germane to health.”

Tom Stossel, M.D.

American Cancer Society Professor Emeritus
Harvard Medical School

The Wall Street Journal, January 2017

“The techniques have galloped ahead of the concepts. We have moved away from studying the complexity of the organism; from processes and organisation to composition.”


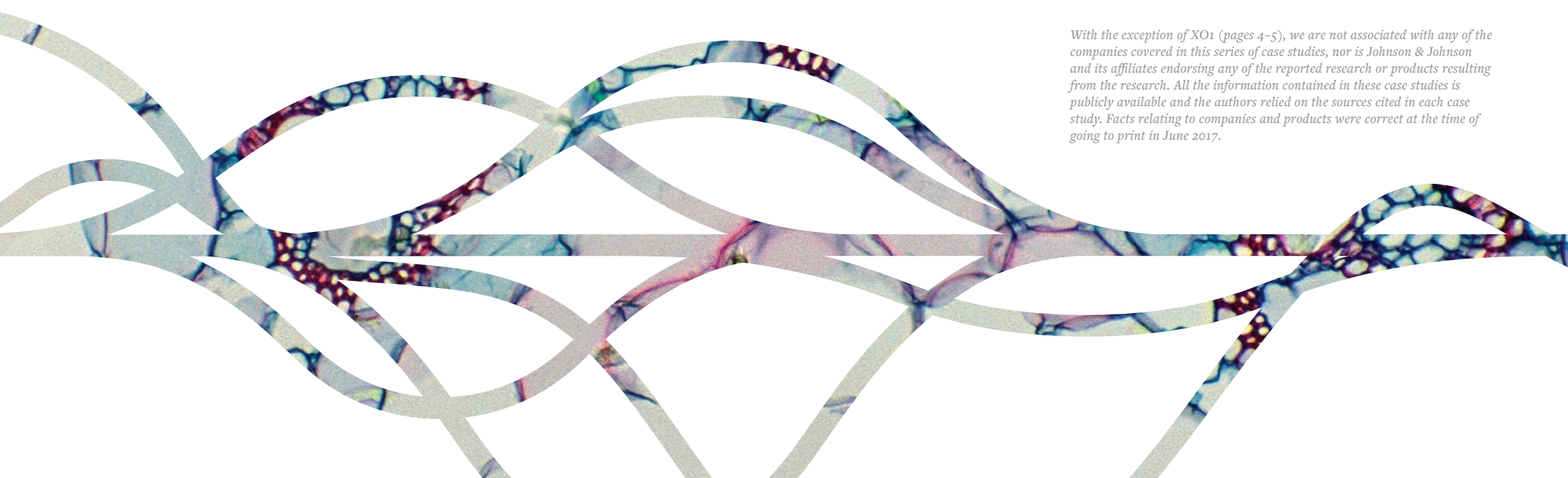
Sir James Black, OM FRS FRSE FRCP

Financial Times, February 2009

“We Icelanders are an excellent animal model for humans. This is exactly the way you find common disease genes.”

Kari Stefansson, M.D.

CEO of deCODE Genetics
Bio-IT World, February 2004

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Introduction

On 2 December 1943, the Axis powers bombarded the Italian port of Bari, which was occupied by Allied forces. Amongst the 17 ships sunk that night was the SS John Harvey. Unbeknown to her crew, she was carrying a secret cargo of mustard gas shells being deployed in Europe should the Axis powers resort to using banned chemical weapons. As a result of the sinking, large quantities of mustard gas were released, causing mass civilian and military casualties in the town. US Army physician Lieutenant Colonel Stewart F Alexander led the medical response. He observed that many of those exposed developed low white blood cell counts. As a result, the Bari incident is widely, but erroneously, cited as the inspiration that led to the use of mustard agents for the treatment of cancers.¹

In fact, the first use of a mustard (alkylating) agent in the treatment of cancer occurred in secret a year before the Bari incident, at Yale University. It had been known since the First World War that mustard agents were potent suppressors of blood cell generation as a result of direct clinical observation of casualties.² During the Second World War, Goodman and Philips at the Yale Department of Pharmacology were conducting secret research into chemical weapons. As part of this effort, the use of nitrogen mustards was investigated in haematological malignancies such as lymphoma. The first human patient was treated in May 1942. As a result the nitrogen mustard 'HN2', called mustine, became the first chemotherapy for cancer.

Vital role of clinical observation

The mustard gas story is a classic example of clinical observation leading to the development of novel therapeutics. However, in the early 21st Century we are at risk of neglecting the important role of clinical observation as a source of drug discovery in favour of basic scientific research at an ever larger scale.³ This is against a background of declining R&D productivity as measured by the expenditure on drug R&D and the number of new drug approvals, causes of which might include a 'basic research brute force bias'.⁴

There is no doubt that basic science is vital for, and contributes enormously to, progress in the development of new therapeutics. However, as the examples in this short document will show, clinical observation and the exploitation of natural or serendipitous human experiments, be they genetic in origin or otherwise, continue to be responsible for some of the most transformational innovations in medicine. Industry is belatedly re-discovering this truth, albeit new terms such as *extreme phenotyping* suggest an emerging industrialised approach that may miss the very nature of how such serendipitous discoveries are made:

- Individually rare
- Unpredictable
- Unscalable
- Undirectable

Re-asserting the role of clinical observation in patients is not at odds with the great protagonists of experimental medicine. Claude Bernard, who determined that medicine should be based upon science and primarily through laboratory experimentation, recognised the need for clinical observation to inspire and enable subsequent hypothesis generation and laboratory experiments: "*the hospital is the antechamber of scientific medicine*".

The purpose of this short collection of case studies is to guide and educate ourselves and our external technology transfer partners as to **'what good looks like'** in their search for the next great biological 'story'. In doing so, we seek to move our gaze from mice to humans in our quest for therapeutic inspiration. A recent study found that selecting drug targets with human genetic support could double success rate in clinical development.⁵

Many of these stories took a great deal of time to mature from initial biological observation to introduction of a successful treatment, such as in the case of imatinib mesylate, which took 30 years. Today's myriad of technologies and insights from basic research should enable us to radically shorten development timelines.

Richard Mason MBBS MRCP

Head of Johnson & Johnson Innovation, EMEA

1. Papac R.J. Origins of cancer therapy. *Yale Journal of Biology and Medicine*. 2001; **74**(6): 391-398. 2. Krumbhaar E.B. *et al.* The blood and bone marrow in yellow cross gas (mustard gas) poisoning. *Journal of Medical Research*. 1919; **40**(3): 497-508. 3. Tom Stossel. Don't thank big government for medical breakthroughs. *Wall Street Journal*. January 5 2017. 4. Scannell J.W. *et al.* Diagnosing the decline in pharmaceutical R&D efficiency. *Nature Review Drug Discovery*. 2012; **11**(3): 191-200. 5. Sansone P. *et al.* The support of human genetic evidence for approved drug indications. *Nature Genet*. 2015; **47**(8): 856-860.

Ichorcumab, the 'blood of the gods'

Auto-antibody against thrombin exosite-1 found in a woman with severely prolonged coagulation times, but with no bleeding tendency

Target Thrombin (exosite-1)	Institution(s) Addenbrooke's Hospital and University of Cambridge, UK
Status Development (early clinical) at Johnson & Johnson	Discoverer(s) Trevor Baglin and James Huntington
Class/indication Anticoagulant/various thrombotic disorders	Molecule Monoclonal antibody

The human story: A head injury and some unusual blood chemistry

In 2008, a 54-year-old woman arrived in the Accident & Emergency department of Addenbrooke's Hospital, Cambridge, UK. She complained of a headache that had developed over several weeks since accidentally hitting her head against a kitchen wall. A CT scan identified a traumatic subacute subdural haematoma, a serious condition where blood collects between the skull and the surface of the brain.

The patient was scheduled for neurosurgery and as part of her pre-operative work-up a coagulation screen was performed. This showed that her blood had a degree of anticoagulation consistent with severe haemophilia i.e. markedly elevated TT, APTT and PT coagulation times.

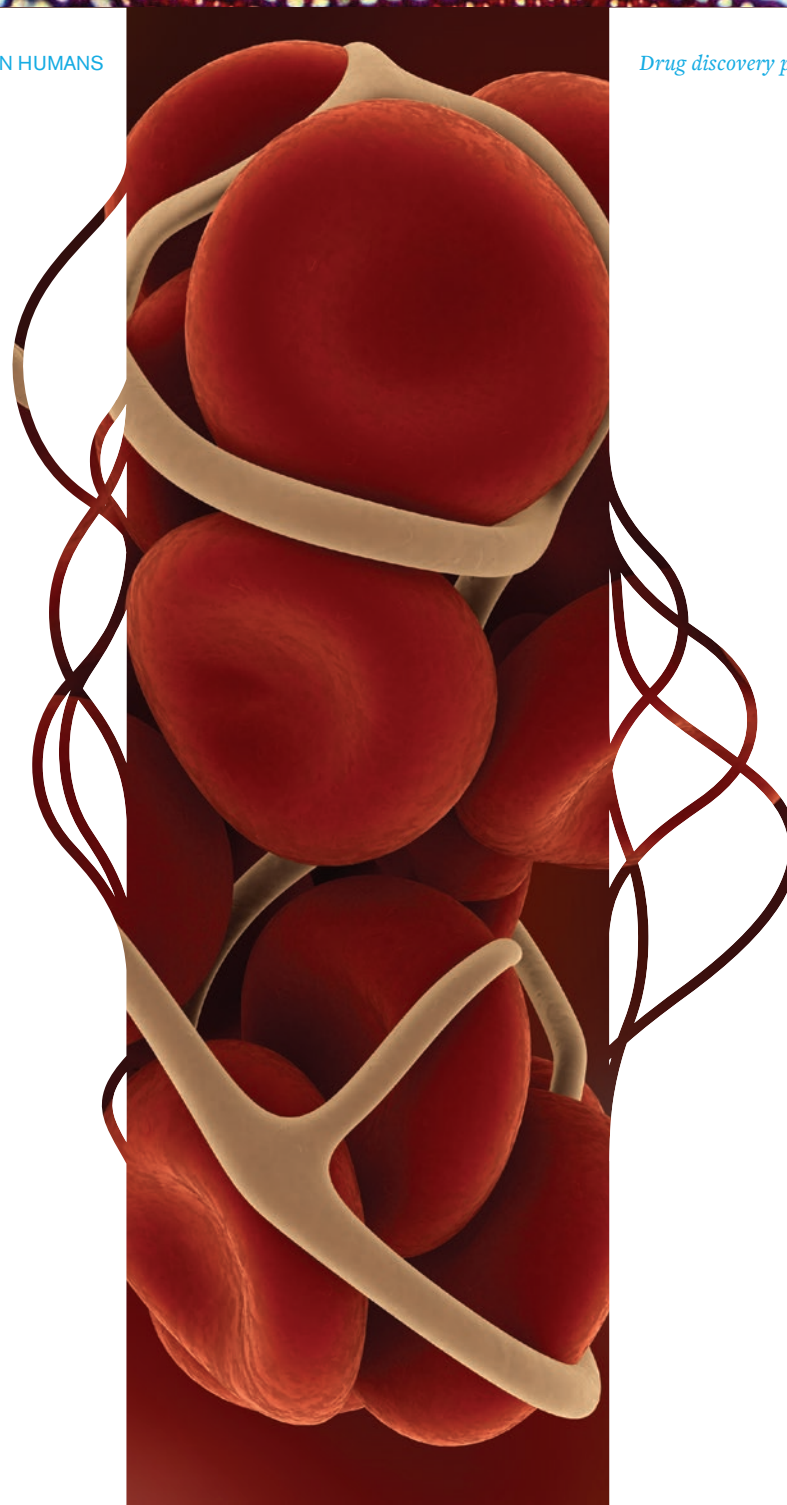
Despite this, and to the surprise of the medical team, her bleeding stopped and she made a complete recovery without intervention.

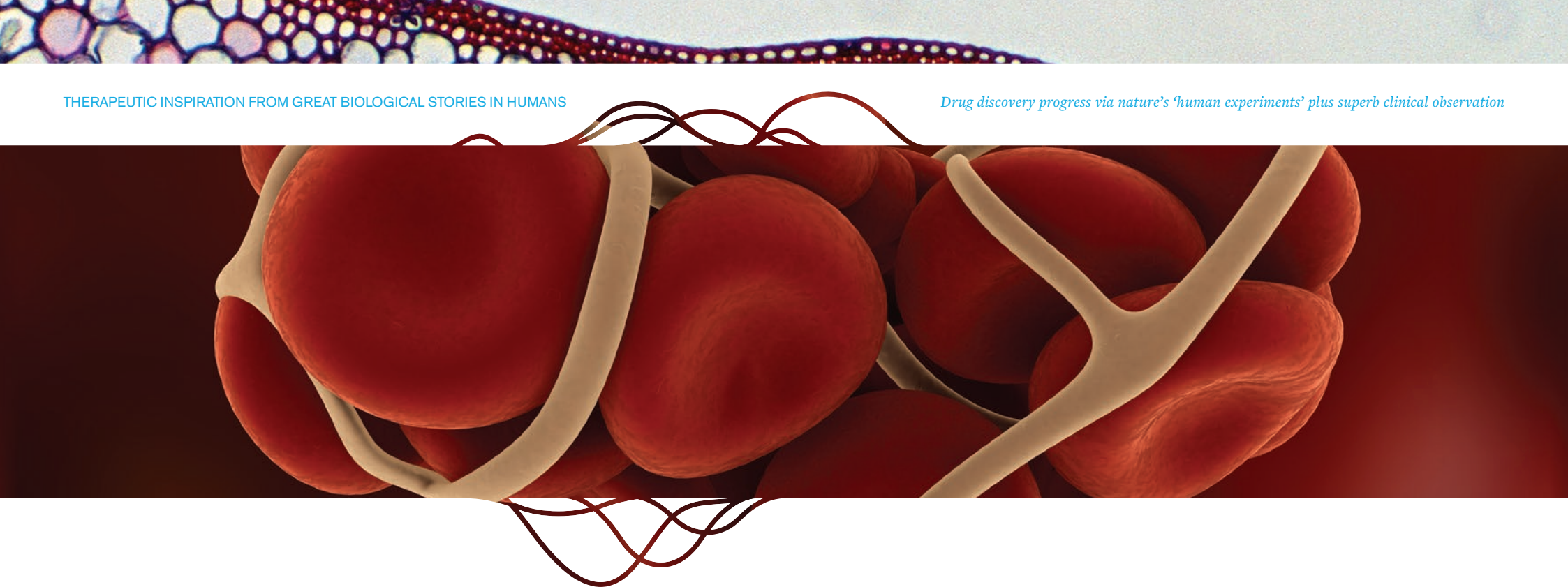
The patient had no relevant medical history and was not receiving anticoagulant drugs. A full investigation of her apparent acquired haemophilia was undertaken by Dr Trevor Baglin and Jim Huntington, Professor of Molecular Haemostasis at the University of Cambridge.

They found that the source of the patient's anticoagulation was an antibody generated by the patient. It bound to exosite-1 of thrombin – a key enzyme in the coagulation pathway.

During follow-up, the patient displayed no increased bleeding tendency and has had no abnormal bleeding in the eight years since first presentation, despite persistently high levels of this IgA autoantibody in her plasma and markedly elevated coagulation times.

Baglin and Huntington, who had previously collaborated on research into thrombin inhibitors, hypothesised that the antibody conferred unique properties – anticoagulation without causing bleeding – a combination long sought in the field of anticoagulation, but thought by many to be impossible to achieve.





The path to a drug

Baglin and Huntington recognised the tremendous therapeutic potential of a drug with this unique profile – for example in preventing heart attacks and strokes. They worked to develop a synthetic antibody designed to mimic their patient's IgA antibody. They named their new antibody *ichorcumab*, after Greek mythology, where ichor was the ethereal fluid in the blood of the gods supposed to confer immortality. It was then licensed to a small biotechnology company called XO1 Limited.

XO1 set about developing this IgG antibody as a new anticoagulant medicine. *Ichorcumab* went into manufacturing, testing began in preclinical models of thrombosis and bleeding to determine its therapeutic index in comparison to existing anticoagulants such as apixaban and warfarin.

Ichorcumab is a remarkable story of science and serendipity – an injured patient with a unique blood-clotting profile came under the care of a clinical team with profound knowledge of blood clotting mechanisms. A perfect blend of observation and research led to the discovery of an exciting new anticoagulant.

Current status

XO1 was acquired by Janssen Pharmaceuticals, a pharmaceutical company of Johnson & Johnson, in 2015 and *ichorcumab* was renamed JNJ-375. It entered Phase 1 clinical testing in late 2016.

Further reading

Baglin T. *et al.* Potential new treatment for haemophilia developed by Cambridge researchers. <https://www.cam.ac.uk/taxonomy/term/10132/feed> Last accessed: 28 April 2017.

Chintala SK. *et al.* Improving the therapeutic index with JNJ-375: a novel long acting exosite-1 thrombin inhibitor in animal models. Abstract, ISTH Meeting 2017.

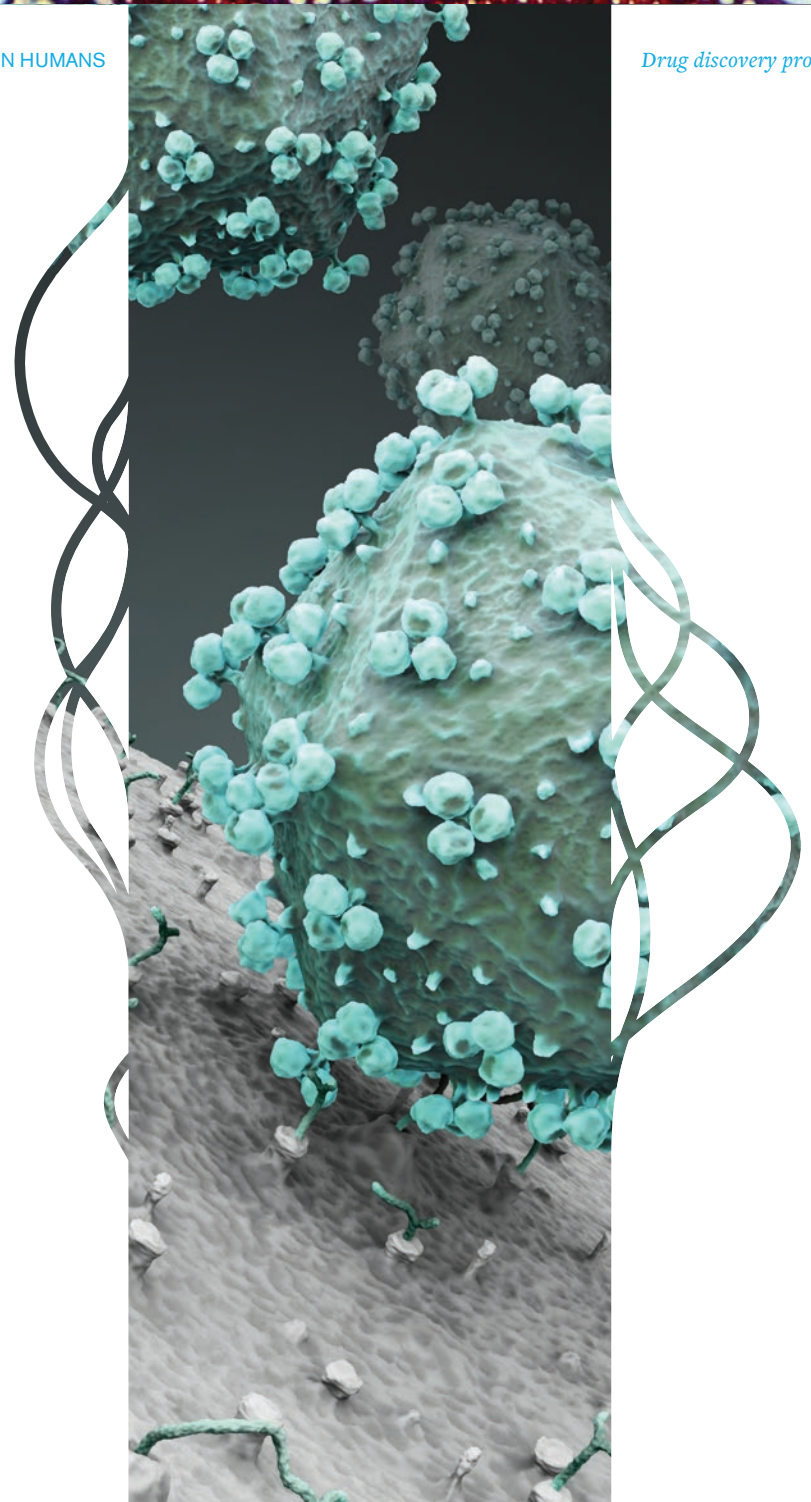
Sex workers and protection

Commercial sex workers who remained HIV-negative after years of active prostitution proved crucial to the discovery of a new class of antiretroviral medications

<i>Target</i> CCR5	<i>Institution(s)</i> Pfizer
<i>Status</i> Marketed Drug	<i>Discoverer(s)</i> Pfizer
<i>Class/indication</i> HIV	<i>Molecule</i> CCR5-receptor antagonist

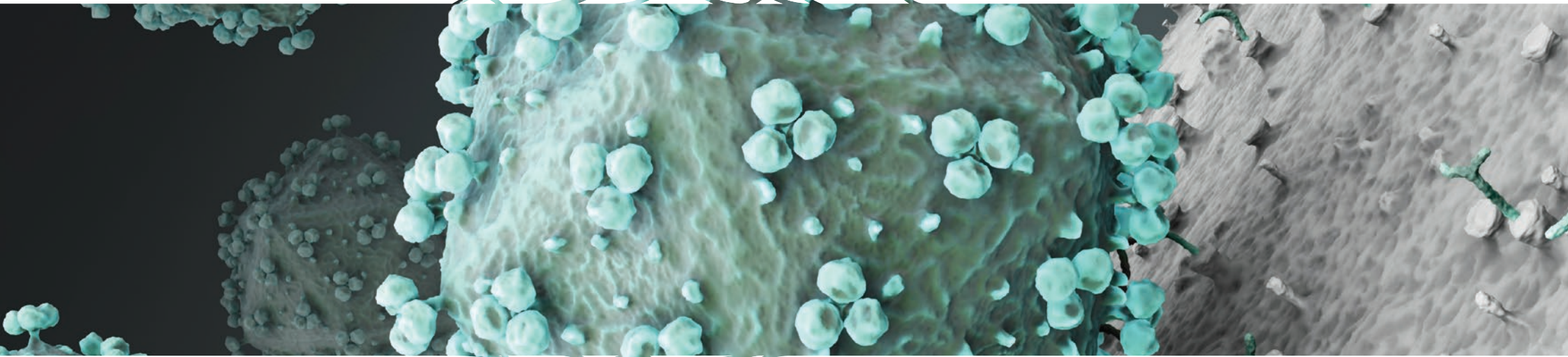
The human story: Some unusual epidemiology

In the late 80s and early 90s when the HIV 'epidemic' was at its height, it was recognised that in some geographic locations the spread of the disease was slower than expected. Among high-risk groups there appeared to be a small number of individuals who showed either resistance or delayed development of the disease despite multiple episodes of unprotected exposure. One such subpopulation was a group of female sex workers in Nigeria – but what was the source of their unusual natural immunity?



They were found to have a mutation in the gene that codes for a cell surface receptor called chemokine receptor 5 (CCR5). This proved to be a key player in the HIV infection process. It has long been known that viruses depend on cell surface proteins to capture and transport the virus into cells for replication. In the case of HIV, several groups, including Hongkui Deng at the Howard Hughes Medical Institute in New York, showed that HIV-1 entry required a 7-TM GPCR chemokine receptor, CCR5 to bind to gp120 to gain entry to the host cell.

Additionally, a related CXC chemokine receptor, CXCR4, was also shown to aid viral entry. Michel Samson at the Université Libre de Bruxelles reported that though CCR5 is highly prevalent in Caucasian populations, it was absent in some black populations in Western Africa and in some Japanese sub-populations. These protected individuals were found to have a base pair deletion (CCR5-Δ32) in the gene which encodes the CCR5 receptor. This rendered the virus unable to bind to the host cell. The population was otherwise healthy, so it was surmised that the loss of the CCR5 receptor afforded protection against viral fusion and infection.



The path to a drug

The human data were clear. A loss-of-function mutation (LOF) afforded protection against the virus. A selective inhibitor, an antagonist for the CCR5 receptor, should therefore constitute a treatment for HIV. As a drug target, CCR5 was immediately attractive as it belonged to a well-known class of GPCR's which had previously been extensively drugged. Using classical small molecule drug discovery methods, Pfizer pursued an antagonist to CCR5 and an antiretroviral drug was developed.

What is interesting about this story is that a highly effective drug was developed against a target which describes a proven 'loss-of-function' mutation in a human population.

Further reading

MacArthur R.D. *et al.* Maraviroc: The first of a new class of antiretroviral agents. *Clinical Infectious Diseases*. 2008; **47**: 236-241.

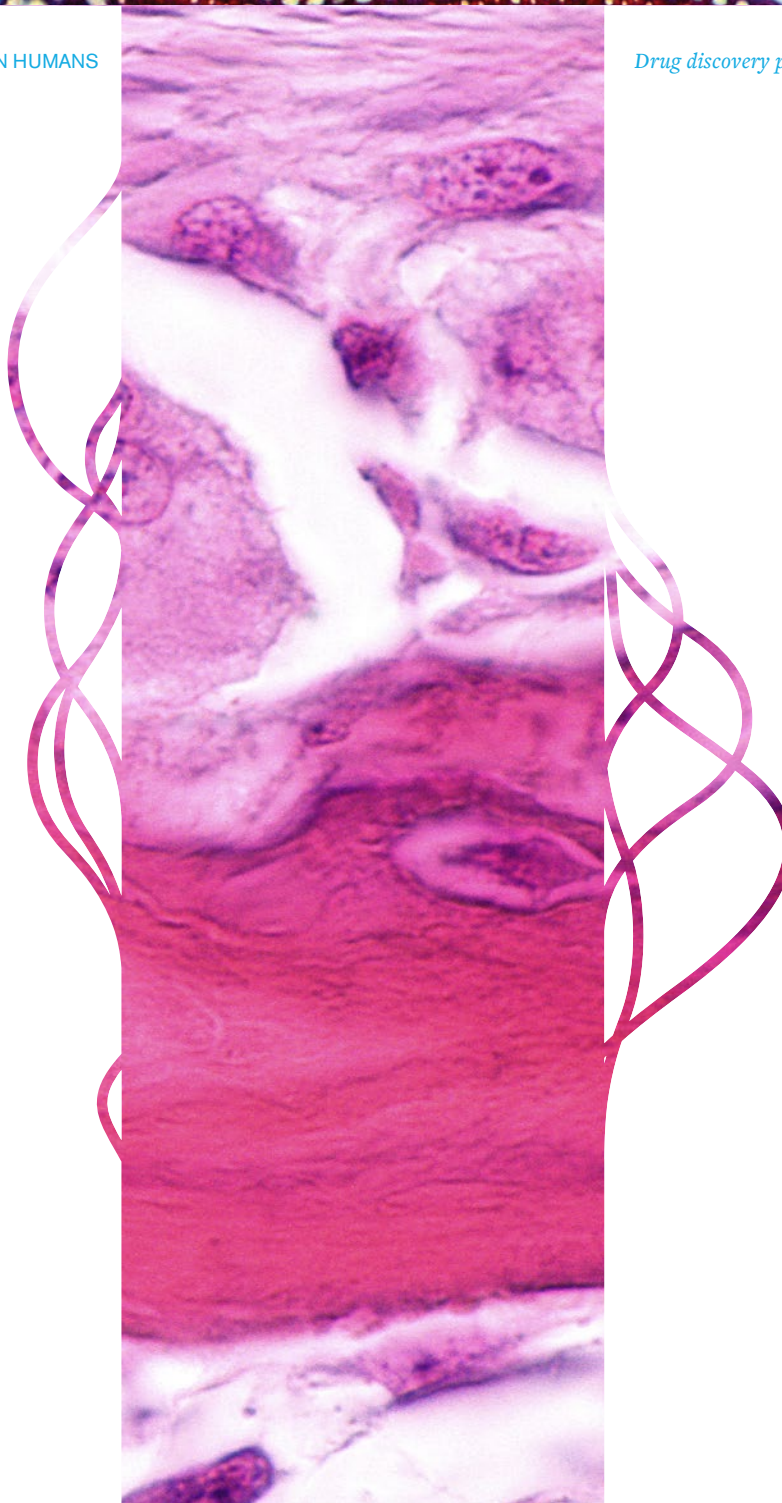
Viking bones for the elderly?

Studying people with rare bone disorders identified important signalling pathways that regulate bone formation – and opened fresh avenues for drug development

Target Sclerostin	Institution(s) Amgen, Eli Lilly, Novartis, OsteoGeneX
Status Amgen Phase 3 trial complete	Discoverer(s) Celltech (now UCB)
Class/indication Osteoporosis	Molecule Sclerostin monoclonal antibody

The human story

Egill Skallagrímsson, a tenth-century Viking, was a colourful warrior, poet and an early anti-hero. The thickness and strength of his skull and his very ugly facial features with a prominent mandible spurred the theory that Egill suffered from Paget's disease of bone. However, Paget's bone, while thickened, lacks structural integrity, infrequently involves the mandible and is prone to fractures. More recent discoveries of sclerosing bone diseases suggest Egill may have been the first documented sufferer of van Buchem disease, although this remains conjecture in the absence of his remains.

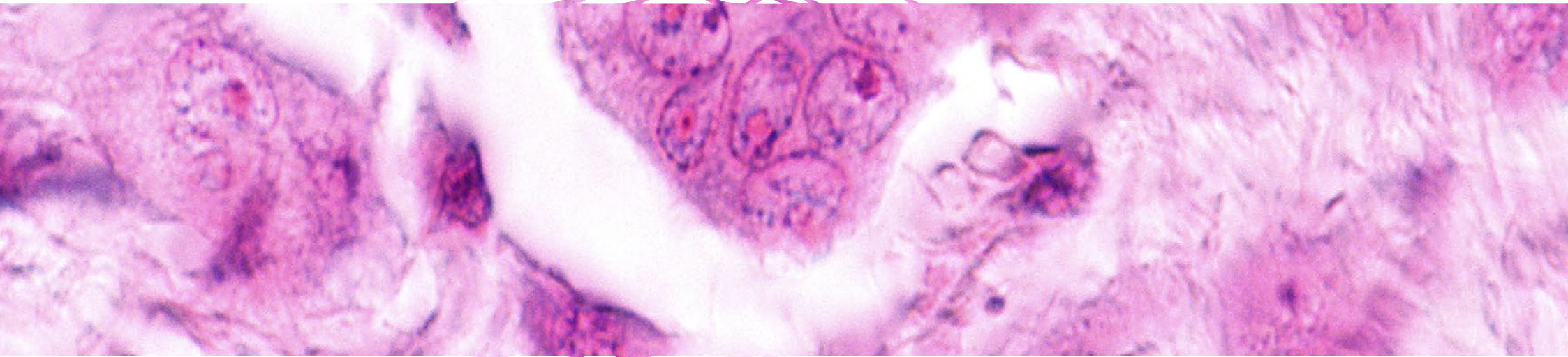


Sclerosteosis and van Buchem disease are two rare sclerosing bone disorders with closely related phenotypes. Sclerosteosis had been described predominantly in Afrikaners of Dutch descent in South Africa, while most patients diagnosed with van Buchem disease came from a small fishing village in the Netherlands.

The skeletal manifestations of these diseases are characterised by progressive generalised osteosclerosis, an abnormal hardening of bone and increased bone density. This is most pronounced in the mandible and skull, with enlargement of the jaw and facial bones, leading to facial distortion, increased intracranial pressure and cranial nerve entrapment, often associated with facial nerve palsy and hearing loss.

The genetic defect that leads to sclerosteosis was identified in a newly cloned gene called SOST, which encodes for sclerostin – a protein which is produced in bone cells called osteocytes. The function of sclerostin is to inhibit bone formation as part of the normal regulation of bone growth. SOST gene mutations stall production of functional sclerostin, leading to excessive bone growth.

Five mutations of this gene have been identified in patients with sclerosteosis, of which three introduce a premature termination codon and the others interfere with splicing of the gene. No mutations within this gene could be found in patients with van Buchem disease, but a 52-kb deletion 35-kb downstream of the SOST gene was identified. The deleted region was later found to contain regulatory elements for SOST transcription, explaining its ability to induce a phenotype closely resembling that of patients with sclerosteosis.



The path to a drug

The identification of sclerostin deficiency as the cause of sclerosteosis and van Buchem disease opened a new area in bone therapeutics. Furthermore, a treatment capable of stimulating bone formation would clearly be useful in common conditions such as osteoporosis.

Both Amgen with UCB and Eli Lilly are developing an antibody for sclerostin. In a Phase III study of Amgen's anti-sclerostin antibody, romosozumab, one year of treatment in post-menopausal women reduced the risk of vertebral fractures compared to the placebo group. It also increased the bone mineral density in the lumbar spine, femoral neck and total hip compared with placebo. Adverse events were balanced between the groups (Cosman, *et al. NEJM* 2016; 375: 1532–1543). Romosozumab was originally discovered by Celltech (now owned by UCB). Celltech entered in a partnership with Amgen in 2002 for the product's development.

Novartis has also developed an antibody to sclerostin (BPS804) and OsteoGeneX is developing small molecule inhibitors.

There have been concerns that stimulation of bone formation by increasing Wnt signalling may lead to unwanted skeletal effects. The Wnt inhibitor factor 1 (WIF1), for example, has been identified as a candidate tumour-suppressor gene in human osteosarcoma, suggesting that the susceptibility to osteosarcoma may be increased in patients receiving novel anabolic treatments targeting Wnt antagonists. These issues need further investigation.

The current status

In May 2017, Amgen announced that romosozumab had met primary and secondary endpoints in their ARCH phase III trial, but that 'an imbalance in positively adjudicated cardiovascular serious adverse events was observed as a new safety signal'; this 'will have to be assessed as part of the overall benefit:risk profile' of the drug.

Further reading

Moester M. J. C. *et al.* Sclerostin: Current knowledge and future perspectives. *Calcif Tissue Int* 2010; **87**: 99–107.

Hamersma H *et al.* The natural history of sclerosteosis. *Clin Genet* 2003; **63**: 192–197.

Stride P. Egill Skallagrímsson: The first case of Van Buchem disease? *J R Coll Physicians Edinb* 2011; **41**: 169–173.

Boy with autism opens pathway to new treatments

How a child's genetic mutation led to the identification of a simple molecular defect subsequently found in Fragile X syndrome patients

Target BK _{Ca} channel	Institution(s) University of Orléans and the Regional Hospital of Orléans (France)
Status Preclinical development	Discoverer(s) Sylvain Briault, M.D., Ph.D. Olivier Perche
Class/indication Cognition and behaviour enhancer in Fragile X syndrome	Molecule Fluoro-oxindole potassium channel opener

The human story

In 2002, a six-year-old boy underwent genetic counselling at Tours Hospital in France as part of the investigation of his severe intellectual disability and autism. The boy's growth was normal and he had no abnormal physical features.

Chromosomal analysis revealed a spontaneous (not-inherited) breakage in a gene coding for physiological processes essential for the regulation of smooth muscle tone and neuronal excitability (a *de novo* chromosomal translocation [9q23/10q22] disrupting the *KCNMA1*

gene which codes for the channel-forming alpha subunit of BK_{Ca} channels ['big potassium' channels] which are characterised by their large conductance for potassium ions through cell membranes). The boy's mutation resulted in a 50% reduction of *KCNMA1* protein production.

The scientific team had therefore identified a novel pathophysiological pathway directly involving BK_{Ca} channels that appeared to be associated with severe intellectual difficulty and autism. They wanted to know if patients suffering from Fragile X syndrome – a condition with similar mental deficiencies and behavioural problems – might also have decreased overall BK_{Ca} channel activity.

Biochemical analysis of cell lines from Fragile X patients indeed demonstrated a decrease in BK_{Ca} channel activity similar to that measured in the boy with the *KCNMA1* gene defect. Decreases in BK_{Ca} channel activity were also seen in an animal model of Fragile X syndrome (*FMR1* knock-out mice). Decreased BK_{Ca} channel activity also reduced the release of glutamate into the synapses of neurons.

A link between mutations in the *FMR-1* gene and BK_{Ca} channel activity was recently published showing that a R138Q mutation in the *FMR1* gene in a patient with intellectual disability disrupted FMRP's interaction with BK_{Ca} channels.

Based on the observation of decreased BK_{Ca} channel activity in the boy with the *KCNMA1* gene defect, Fragile X syndrome patients and *FMR1* knock-out mice, the team set out to determine whether this decreased activity is the cause of the intellectual disability and autism seen in Fragile X syndrome, or merely an association or secondary issue.



The path to a drug

Dr Briault started looking for a pharmacological agent that could restore BK_{Ca} channel activity. He found a fluoro-oxindole potassium channel opener, that was discovered and tested by Bristol-Myers Squibb (BMS) as a potential neuroprotectant for the treatment of acute ischaemic stroke. The molecule did not show efficacy in a Phase III clinical trial in stroke and BMS discontinued development. The company gave contractual permission to CNRS and Dr Briault to use the molecule for research and development. They also provided access to the complete documentation of the molecule's preclinical development.

Dr Briault and his team subsequently showed that BMS-204352 restored BK_{Ca} channel activity to normal in cell lines from the patient with the *KCNMA1* mutation and in cell lines from patients with Fragile X syndrome. BMS-204352 also restored BK_{Ca} channel activity, synaptic glutamate levels and cognitive and behavioural functions in *FMR1* knock-out mice to the same as wild type mice.

Current status

Dr Briault and his team are now hoping to be able to develop BMS-204352 as a treatment for Fragile X syndrome patients, at some point proceeding into clinical studies (<http://www.fraxadev.fr/en/>).

Further reading

Laumonier F. *et al.* Association of a functional deficit of the BKCa channel, a synaptic regulator of neuronal excitability, with autism and mental retardation. *Am J Psychiatry*. 2006; **163**(9): 1622–1629.

Klyachko *et al.* Independent role for presynaptic FMRP revealed by an *FMR1* missense mutation associated with intellectual disability and seizures. *PNAS*. January 27 2015; **112**:949-956.

The woman who was always hungry

New treatments for diabetes and obesity may emerge from research that began with a woman who was always hungry. She was always snacking to avoid lethargy – but why was her blood sugar level crashing?

Target Fibrilin/Asprosin	Institution(s) Baylor College of Medicine, Houston, TX
Status Target validation	Discoverer(s) Atul Chopra
Class/indication Diabetes/obesity	Molecule TBD

The human story: Snacks all day

Dr Atul Chopra, a geneticist from Baylor College of Medicine in Texas, was presented with an unusual case. His patient, a 23-year-old woman told him that she needed constant small meals in order to avoid becoming hypoglycaemic. She could only eat tiny amounts due to a rapid sensation of fullness.

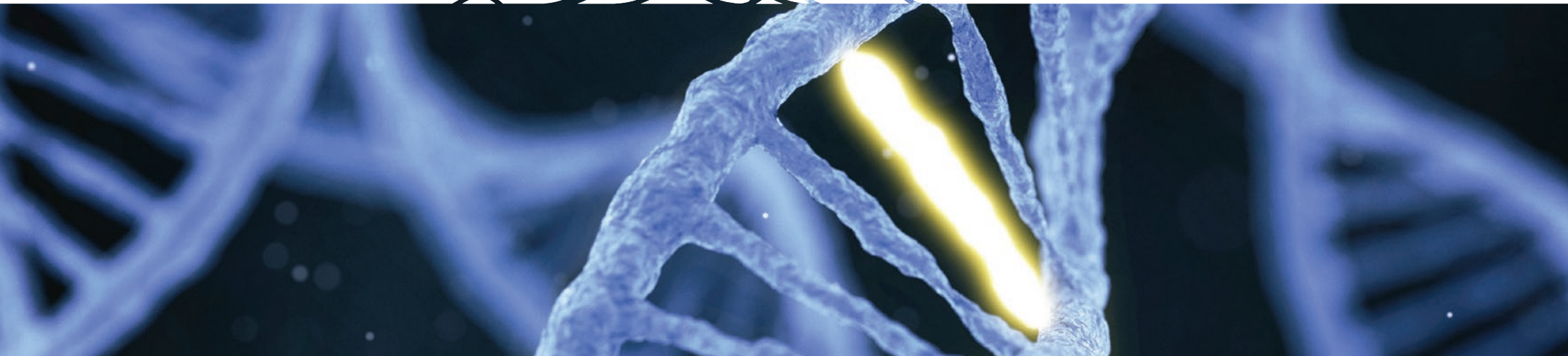
Her condition resembled a rare disease called neonatal progeroid syndrome (NPS) – in which individuals present with congenital, partial lipodystrophy. Unlike other forms of lipodystrophy, Dr Chopra's patient had normal fasting glycaemia with a two-fold decrease in her insulin levels.

Dr Chopra realised that her constant need for food was related to a defect in glucose metabolism. He conducted a full metabolic assessment and sequenced her DNA. The latter identified a mutation in the profibrilin gene. Profibrilin cleavage results in fibrilin (involved in the regulation of quality of connective tissue and believed to have a role in adipose tissue biology) and a protein hormone he named asprosin (the Greek word for 'white').

Asprosin had no known function at the time of its discovery. Dr Chopra's team has spent the last few years working to clarify the role of this hormone. A recent publication in *Cell* (2016) has shed some light on the function of asprosin. Utilising antibodies against the protein, it was found that asprosin levels are reduced in patients with NPS. In addition, obese people were found to have higher levels of the hormone.

Asprosin was mainly expressed in adipose tissue and was found to have a circadian regulation, being higher during fasting, with levels dropping immediately after feeding. It was also determined that asprosin was signalling the liver to control glucose levels.

Data from animal models have shown that acute administration of recombinant asprosin leads to acute increase in glucose levels and at the same time administration of an asprosin antibody to animals results in reductions in glucose levels.



The path to a drug

This example shows how a novel mechanism can be identified by studying a patient with an extreme phenotype. Although this patient's story has excited interest, there are still many questions to be addressed.

Pre-clinical data suggest that use of an antibody to block asprosin could be beneficial in the treatment of diabetes. However, open questions still exist with respect to the effects of this protein in weight loss and appetite suppression. Further confirmation of its role and therapeutic potential are eagerly awaited.

Current status

It is still early days for the asprosin story and only time will tell if this really can be translated into a successful drug. Current efforts are aimed at reproducing some of the initial findings and conducting further validation before this can be considered a solid candidate for drug development.

Further reading

The Atul Chopra Lab
<https://www.bcm.edu/research/labs/atul-chopra>

Rare mutation led to potent new cholesterol-lowering treatments

Three generations of a French family were pivotal in the PCSK9 story

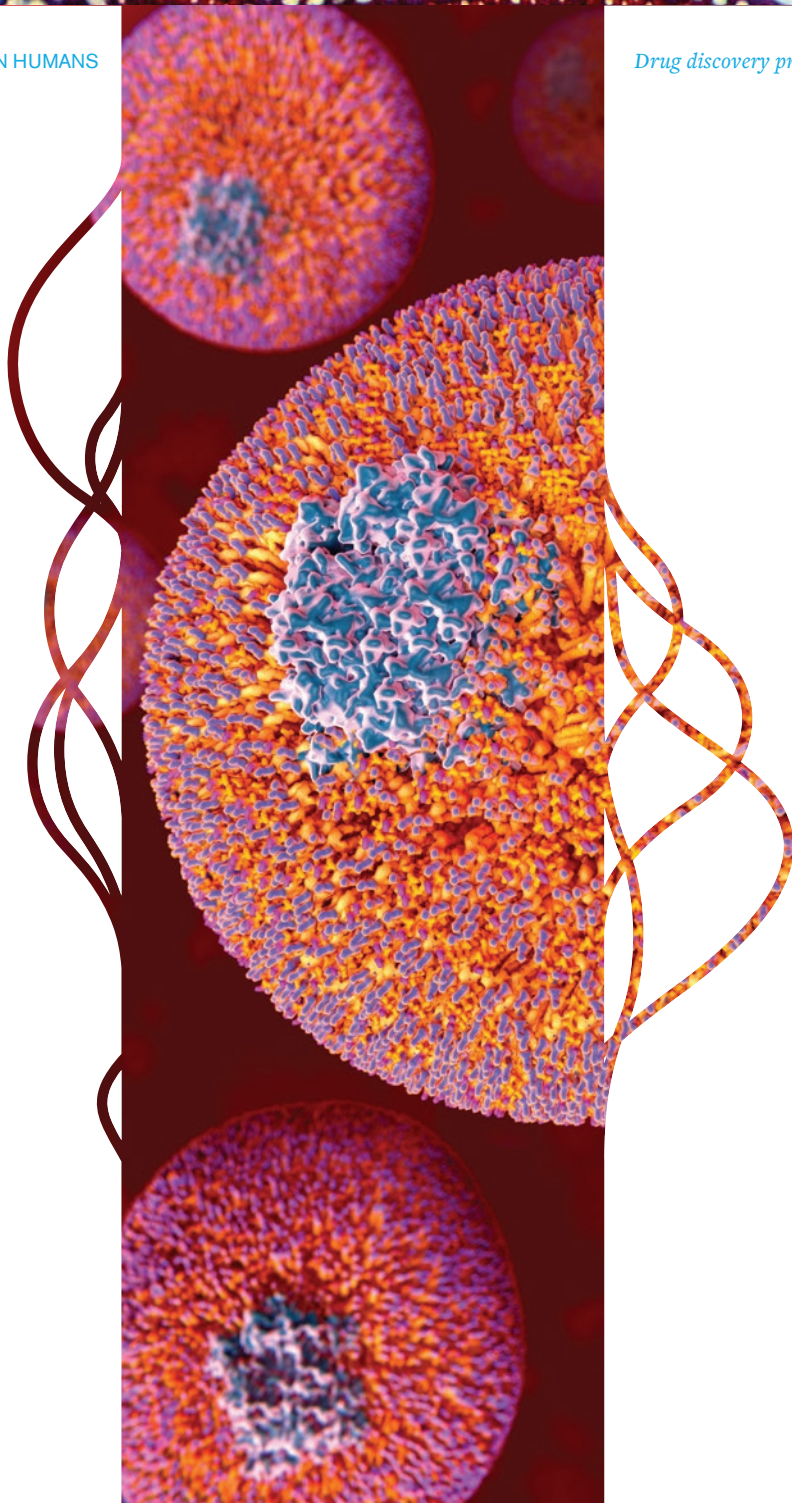
<p>Target PCSK9 (Proprotein convertase subtilisin/kexin type 9)</p>	<p>Institution(s) INSERM U383, Hôpital Necker-Enfants Malades, UTSW, Amgen, Regeneron</p>
<p>Status Marketed</p>	<p>Discoverer(s) Catherine Boileau, Helen Hobbs</p>
<p>Class/indication PCSK9 inhibitors for familial hypercholesterolemia</p>	<p>Molecule Allirocumab and evolocumab</p>

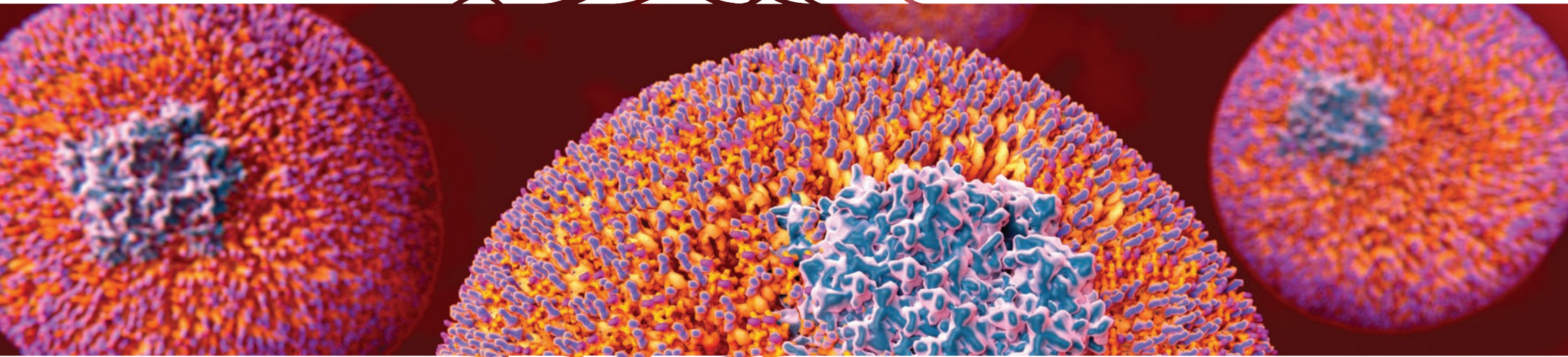
The human story: A most unusual family history

In 2003, French researchers reported on three generations of a family with astoundingly high levels of LDL cholesterol and a strong history of heart disease. The research team, led by Professor Catherine Boileau at INSERM in Paris, found that this family's stratospheric cholesterol levels were associated with a mutation in a gene called PCSK9, whose function was, at that time, unknown.

Two years later, loss-of-function mutations in the PCSK9 gene were reported by Professor Helen Hobbs from University of Texas Southwestern Medical Center – adding clinical proof of concept to the role of PCSK9 in familial hypercholesterolaemia.

It soon became clear that gain-of-function mutations in PCSK9 were genetic causes of hypercholesterolaemia and conversely, loss-of-function mutations were associated with lower concentrations of LDL cholesterol and reduced coronary heart disease. This flagged PCSK9 inhibition is an attractive new strategy for reducing levels of LDL-cholesterol.





The path to a drug

The race to identify a means to inhibit PCSK9 was started by several pharmaceutical companies. Some teams attempted to develop small molecule inhibitors; however, this proved a challenge. In a field where several oral drugs were already available and where statins were established as standard of care, it took considerable effort to believe that an injectable could be commercially viable.

The R&D teams of Amgen and Regeneron emerged as leaders in the race to develop an antibody against PCSK9. In addition, companies such as Alnylam Pharmaceuticals are attempting to reduce the protein levels using siRNA, while others are pursuing antisense oligonucleotide approach. Other companies are trying to develop vaccines.

Clinical trials with monoclonal antibodies both from Amgen or Regeneron demonstrated very powerful cholesterol lowering in the range of 60% or more, significantly better than that seen with statins. This has been associated with substantial reductions in cardiovascular events.

Current status

PCSK9 antibodies were approved in 2015 by the FDA, and two are currently marketed (alirocumab and evolocumab).

Further reading

Hall, Stephen S. Genetics: A gene of rare effect. *Nature*. 9 April 2013. <http://www.nature.com/news/genetics-a-gene-of-rare-effect-1.12773>

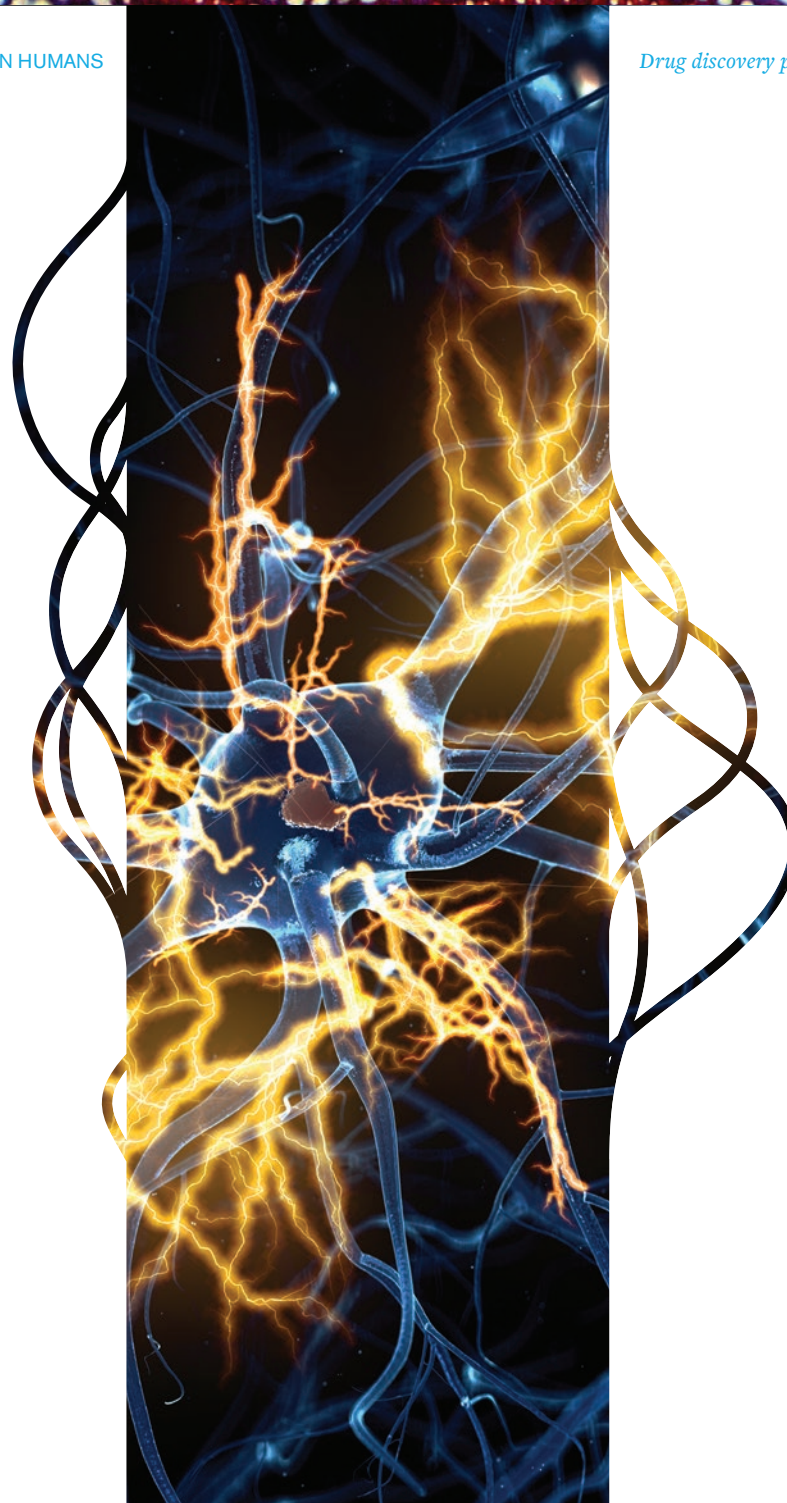
Potential gain from lack of pain?

A complete inability to feel pain is very rare, which made individuals with this characteristic invaluable to pain researchers

Target Na _v 1.7 ion channel	Institution(s) Cambridge University and others Convergence/Biogen Xenon/Genentech
Status Development	Discoverer(s) Numerous. James Cox
Class/indication Pain	Molecule Small molecule antagonist

The human story

You may think that life without pain would be fantastic. A trip to the gym would be easy, a marathon could be completed in three hours, a dentist or surgeon would have no need for risky anaesthesiology. At home, DIY efforts would result in cleanly drilled holes, beautifully hit nails and pulling a well-baked cake out of the oven would not require gloves.

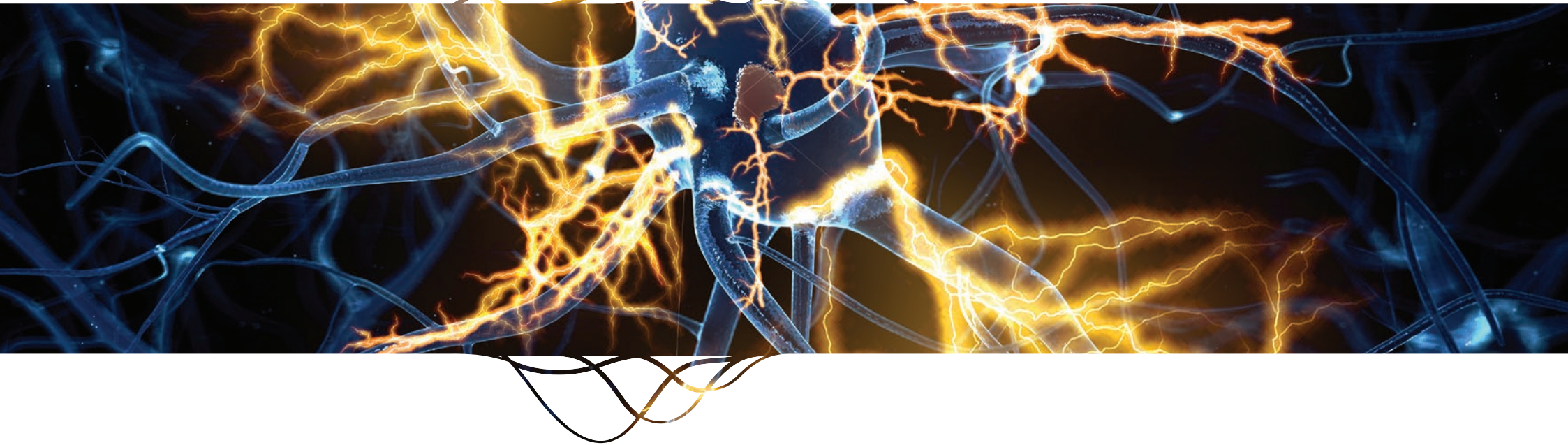


As you reflect on these comfortable scenarios you may rapidly come to realise that pain is actually rather helpful and protective. Pain allows us to minimise tissue damage, and pain encourages us to remove ourselves from a dangerous environment; just look at the crippling injuries suffered by people with leprosy.

Living a life without pain can be fatal. However, there are now several families across the world in Brazil, Italy and Pakistan who have varying reductions in pain sensitivity from a dulled sense to a complete absence. Those with a complete absence live a life of trepidation with frequent fractures, contusions and burns.

Despite these risks, there are some pains we can definitely do without. The search for new analgesics has led to careful genetic investigation into these rare human phenotypes who feel little or no pain.

James Cox and his co-workers at the Cambridge Institute for Medical Research at Addenbrooke's Hospital investigated some of these families and published their findings in *Nature* (2006). They investigated three related families from northern Pakistan. They found that the SCN9A gene which encodes the subunit of the voltage-gated sodium channel, Na_v1.7, was affected. Three mutations S459X, I767X and W897X were shown to cause varying degrees of loss-of-function of the Na_v1.7 ion channel. They concluded that SCN9A is an essential requirement for nociception (the activity of the sensory nerve cells of the pain pathway) in humans, and that their findings should stimulate the search for novel analgesics that selectively target this sodium channel subunit.



The path to a drug

Sadly these findings will not help the affected individuals in the human phenotype because they are suffering from a crucial loss of function in a key ion channel. However, ion channels are well described as drug targets and several groups, notably Biogen/Convergence and Xenon/Genentech are at the forefront of the identification of antagonists to the Na_v1.7 ion channel. The gain of a selective Na_v1.7 antagonist in a healthy individual should help depress nociceptive pain.

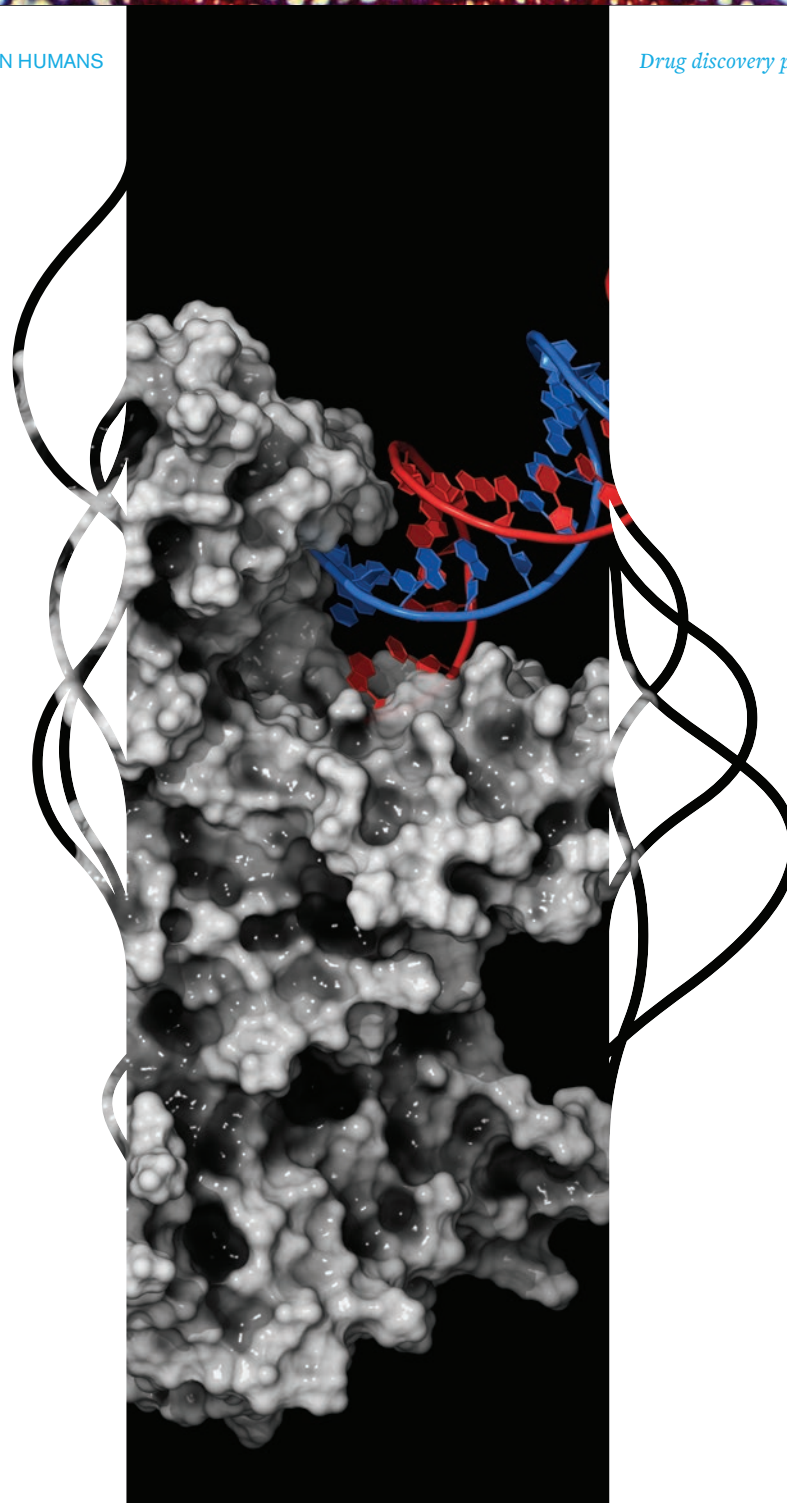
Further reading

Cox J. *et al.* An SCN9A channelopathy causes congenital inability to experience pain. *Nature*. 2006; **444**(7121): 894–898.

A family affair: On the trail of genetic faults

Many decades of research and clinical observation led to drugs that are highly targeted to cancers linked to specific genetic faults

<p>Target</p> <p>Poly (adenosine diphosphate-ribose) polymerase (PARP)</p>	<p>Institution(s)</p> <p>Cancer Research UK, Cambridge University KuDOS Pharmaceuticals, The Institute of Cancer Research, and AstraZeneca</p>
<p>Status</p> <p>Olaparib (Lynparza) approved for use in women with ovarian cancer</p>	<p>Discoverer(s)/ Researchers</p> <p>Professor Steve Jackson, Alan Ashworth, Professor Jonathan A. Ledermann and many others</p>
<p>Class/indication</p> <p>Ovarian cancer and investigation in other malignancies</p>	<p>Molecule</p> <p>Olaparib</p>



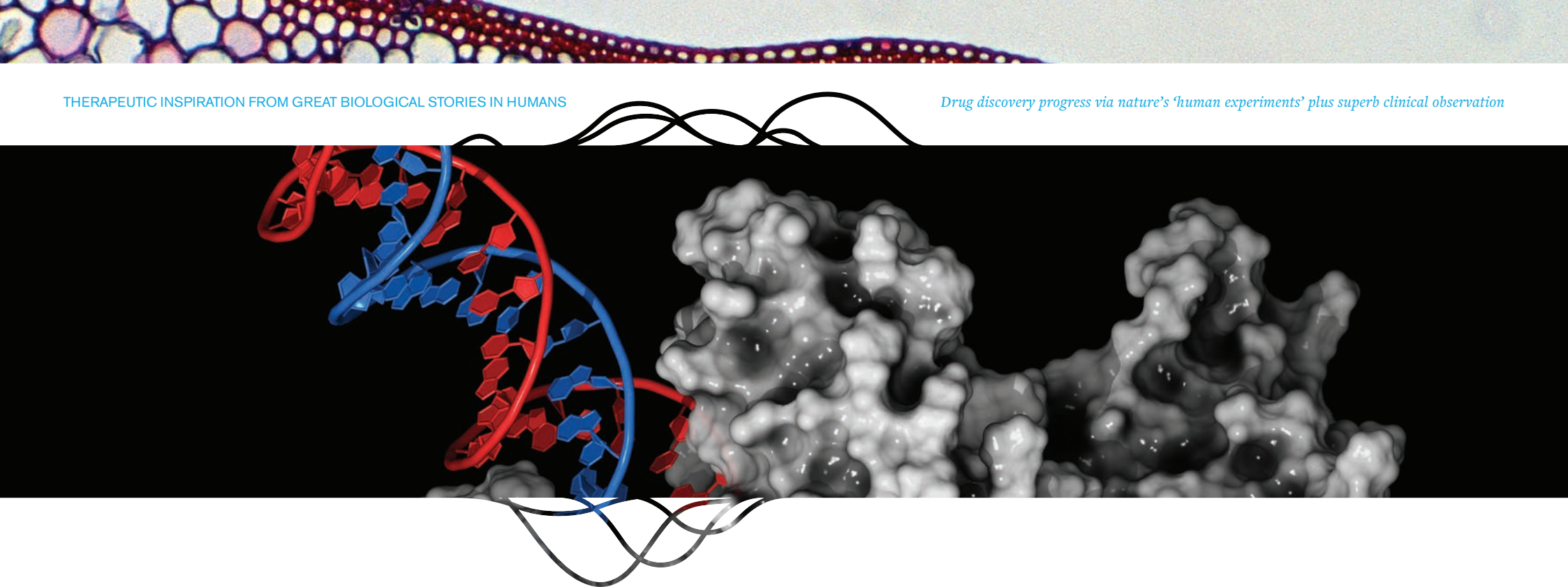
The human story: Following the family line

Family history is still one of the strongest predictors of a woman's chance to develop breast cancer. Back in the 1940s, evidence was emerging that breast cancer could run in families. A natural place for researchers to study these findings was the genetics of cancer prone families.

It wasn't until the mid 1990s that two genes were discovered to be altered in many families with hereditary breast cancer. The first, BRCA1 (for BR_east CAncer gene), was discovered in 1994, and the second, BRCA2, in 1995. Additionally, it was shown that a particular ethnic group may be prone to specific genetic alteration. For example, it was shown that three high-risk Ashkenazi families who were unrelated, carried an identical alteration in BRCA1 (185delAG). This observation led to the study which found that 1% of the Jewish population has this alteration.

Both BRCA1 and BRCA2 are normally expressed in the cells of breast and other tissue and their function is to help repair damaged double stranded DNA breaks. Mutations in BRCA1 and BRCA2 render these genes faulty. People who inherit a fault in a copy of one of their BRCA1 or BRCA2 genes are highly likely to get breast, ovarian and prostate cancer at some stage in their lives.

In fact, women with an abnormal BRCA1 gene have a staggering 75–90% lifetime risk of developing breast cancer and around a 55% increased risk of developing ovarian cancer.



The path to a drug

As Cancer Research UK succinctly explains, cancers caused by a faulty BRCA1 or BRCA2 genes have problems with one of the mechanisms used to repair damage to their DNA. Despite this flaw they can still struggle along, botching together DNA repairs using an alternative mechanism that depends on a protein called PARP – short for poly-ADP-ribose polymerase.

Nearly two decades ago, Cancer Research UK-funded scientist Professor Steve Jackson and his team in Cambridge embarked on a mission to find drugs that can block DNA repair pathways, including PARP inhibitors. As their name suggests, these drugs target PARP and prevent it working. Without this 'back-up system', cancer cells have no way of repairing damaged DNA, so they die.

In 2005, together with Alan Ashworth and his colleagues at The Institute of Cancer Research, researchers clearly demonstrated that PARP inhibitors can halt cancer cells in their tracks, as long as they also carry BRCA1 or BRCA2 mutations.

Olaparib (AZD-2281) is the best-studied PARP inhibitor to date. It was developed by KUDOS Pharmaceuticals and later by AstraZeneca. It is now both FDA and EMA approved for germline BRCA mutated advanced ovarian cancer that has received three or more prior lines of chemotherapy.

Further reading

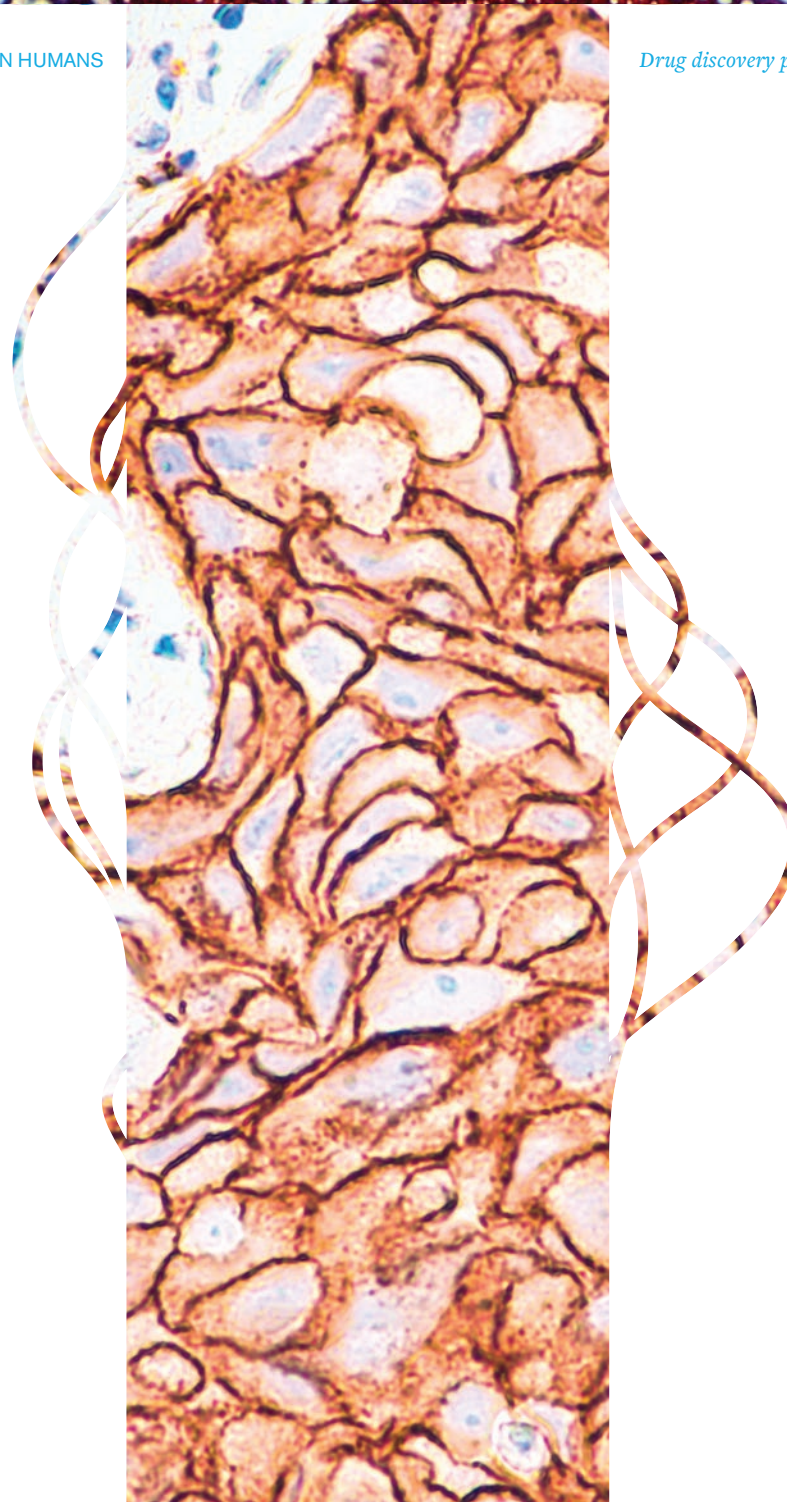
Jackson SE, Chester JD. Personalised cancer medicine. *Int J Cancer*. 2015; **137**(2):262–266.

Munroe M, Kolesar J. Olaparib for the treatment of BRCA-mutated advanced ovarian cancer. *Am J Health Syst Pharm*. 2016; **73**(14): 1037–1041.

The 'aggressive gene' that revolutionised breast cancer treatment

Why were some breast cancer patients living half as long as others? It took three decades to turn the answer into a gold standard treatment

<p>Target</p> <p>Human epidermal growth factor receptor 2 or HER2/neu</p>	<p>Institution(s)</p> <p>Johnson Comprehensive Cancer centre, UCLA Genentech Inc. and others</p>
<p>Status</p> <p>Marketed</p>	<p>Discoverer(s)</p> <p>Dr Dennis Slamon, Michael Shepard, Axel Ullrich and many colleagues</p>
<p>Class/indication</p> <p>A monoclonal antibody drug used to prevent the recurrence of/and or treatment of breast cancer that has spread beyond the breast</p>	<p>Molecule</p> <p>Trastuzumab – monoclonal antibody to HER2</p>

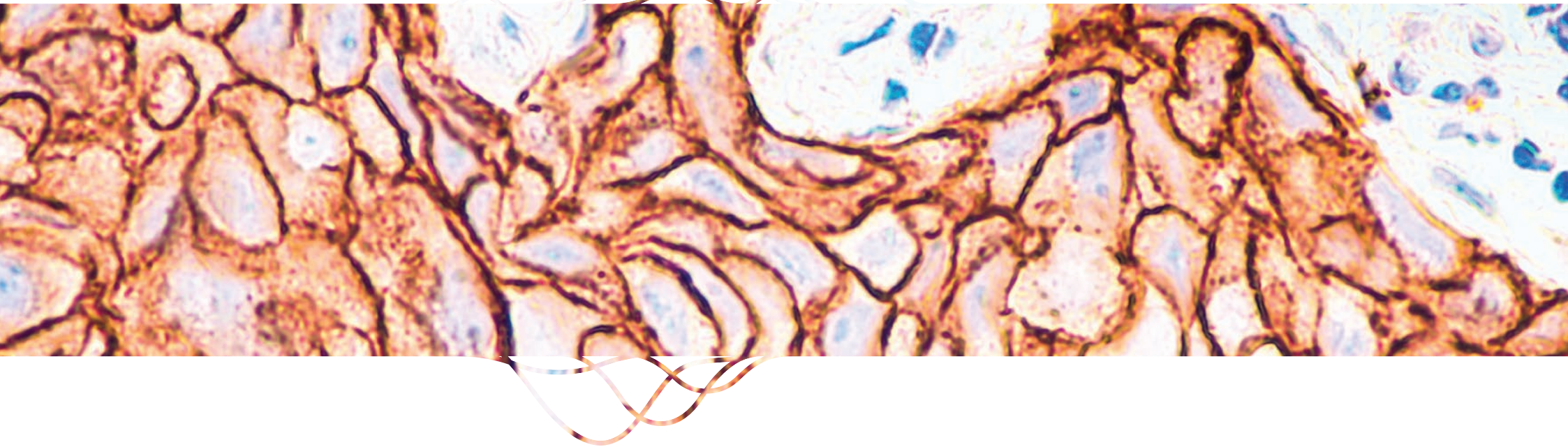


The human story

Medals and awards – far too many to list here – have been showered on Dennis J. Slamon, M.D., Ph.D. For 12 years, Slamon and his colleagues conducted the laboratory and clinical research that led to the development of the breast cancer drug trastuzumab which targets HER2 – a specific genetic alteration found in about one in four breast cancer patients.

Although many individuals and research groups have been involved in the trastuzumab story, arguably the key observation that kick-started drug development was that of Slamon's team, which for the first time linked HER2 over-expression with a more aggressive type of breast cancer.

Today trastuzumab is listed on the WHO essential list of medicines. The drug, a monoclonal antibody to HER2 was approved by the FDA in 1998 and more than 420,000 women with HER2 positive breast cancer have been treated with this drug to date.



The path to a drug

The journey to this medicine started in the mid 1980s in Dr Slamon's University labs at Johnson Comprehensive Cancer Centre, UCLA where a discovery of a mutated gene which increased cellular growth was identified as HER2, a growth factor.

These data caused a stir in the research community and further data emerged to link HER2 with cancers and in particular, breast cancer. High expression levels of HER2 were found in 30% of clinical breast cancer samples and HER2 was correlated to metastatic disease, relapse and poor survival. However, correlation alone did not mean that HER2 was a culprit fuelling breast cancer.

Solid discoveries are often readily reproduced in other labs, and indeed other groups not only corroborated the earlier work, but also began to show that HER2 could transform normal healthy cells to grow aggressively like cancer cells in an uncontrolled manner. The hypothesis presented to the oncology research community, that reducing HER2 growth factor activity could slow the growth of cancer, became a focus for research activity.

Collaborative programmes starting in academia sparked Genentech to work with UCLA to jumpstart the discovery and the development of antibodies to HER2. They invested in developing mouse models implanted with human tumours expressing HER2 to test the antibodies in the discovery phase and led to the identification of trastuzumab, which effectively binds to the HER2 receptor expressed on tumours, prevents HER2 cell signalling and flags the tumour cell for antibody dependent cell cytotoxicity (ADCC) and in doing so caused tumour regression.

Today, HER2 is targeted not only with trastuzumab, but also with another monoclonal antibody treatment, such as pertuzumab, which complements the mode of action of trastuzumab. Pertuzumab, works by binding the extracellular domain of HER2 and preventing ligand dependent dimerization of HER2 and HER3 receptors. Women faced with a poor prognosis with stage 1 to 3 breast cancer expressing HER2 now have a good prognosis.

Further reading

Perseverance – The inside story of a breast cancer breakthrough, 30 years in the making
<https://www.gene.com/stories/her2/>

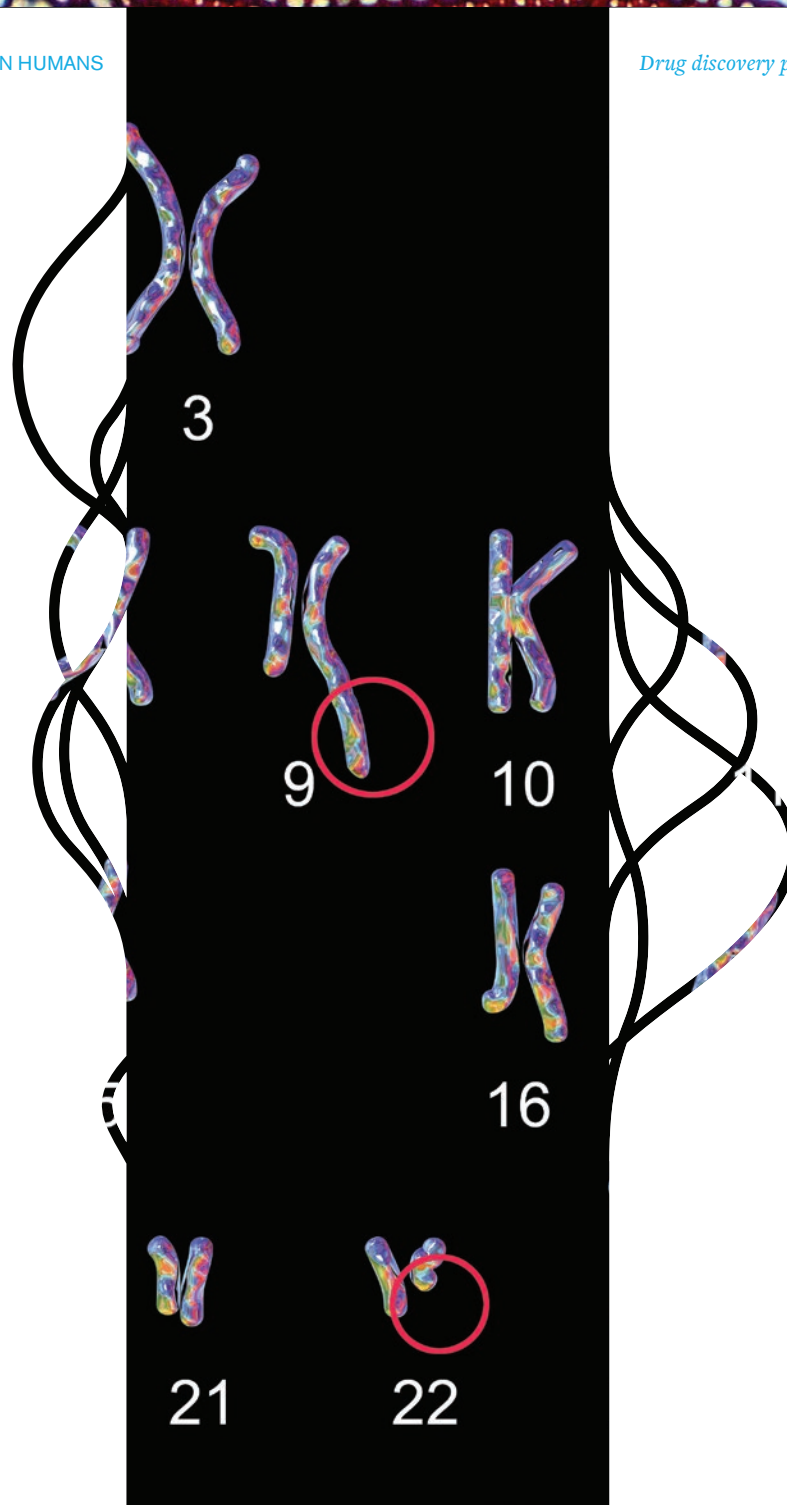
On the whole, I'd rather be in Philadelphia

The Philadelphia Chromosome and Chronic Myeloid Leukaemia

Target ABL tyrosine kinase	Institution(s) Novartis University of Oregon
Status Marketed drug	Discoverer(s) Nicholas Lydon Juerg Zimmermann Brian Druker
Class/indication Kinase inhibitor/Chronic Myelogenous Leukaemia and Gastrointestinal Stromal Tumours	Molecule Imatinib mesylate

The human story: Microscopy plus excellent powers of observation

"On the whole, I'd rather be in Philadelphia" was famously coined by W. C. Fields as an epitaph to his future gravestone. Ronald Reagan is said to have used the quip whilst being wheeled from surgery after his attempted assassination in 1981. However, to cancer biologists, Philadelphia will forever be linked to a very special chromosome.

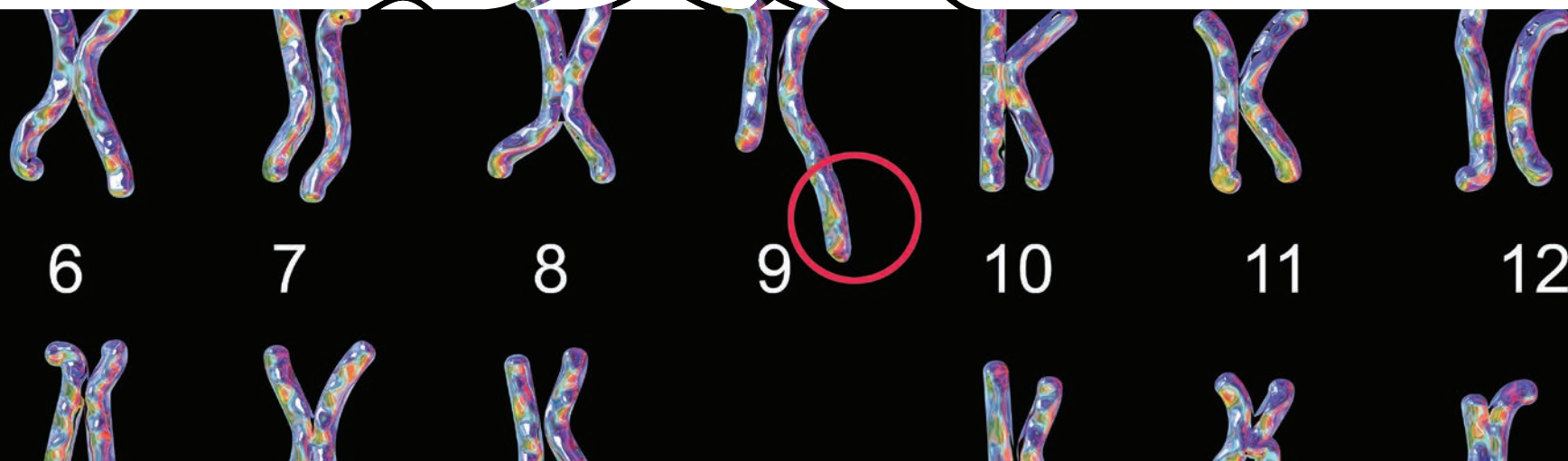


The story began more than a half-century ago in a Philadelphia laboratory, where two young researchers, David Hungerford and Peter Nowell, noticed an abnormally short chromosome in bone marrow cells from patients with chronic myelogenous leukaemia (CML). The 'Philadelphia Chromosome' was thus born and linked clearly to CML. The discovery lay somewhat dormant until 1973 when DNA-staining techniques had developed sufficiently for Rowley to discover that chromosome 22 and chromosome 9 had exchanged parts of DNA, a chromosomal translocation, to form the short Philadelphia chromosome. A specific genetic change was thus linked to a human cancer.

The path to a drug

The next step was to determine the function of this chromosome. Another decade passed until it was recognised that the translocation and fusion resulted in a new gene known as BCR-ABL. The gene was shown to encode a protein with elevated tyrosine kinase activity. Further, in mouse studies it was shown that BCR-ABL could induce leukaemia.

Drug discovery ideally needs *in vitro* methods for rapid structure-activity relationships (SAR) and cell lines derived from human leukaemia cells containing the chromosomal abnormality could be readily engineered. By the early 90s, all the pieces were in place to develop a drug; the biochemical and molecular biology had clarified how a single aberration in signalling pathway leads to the disease.



Targeting the kinase

Whilst this appeared a dream scenario, it was far from plain sailing. Kinases were well known; they are the catalysts in the energy process of cells. The challenge was to find a molecule which specifically inhibits BCR-ABL kinase. Labs in Ciba-Geigy set about doing this despite concerns that targeting the fundamental energy mechanism of cells may be a toxic event. Tens of kinases were known at the time; the human kinase gene is now thought to comprise about 500 kinases. Would it be possible to specifically inhibit a single discrete kinase?

An international collaborative programme between industry and academia got underway. By 1992, STI1571 had been synthesised and in 1996 the compound entered pre-clinical development. Clinical development was spectacularly rapid. The first CML patient was

treated in June 1998 and it took only until May 2001 to achieve FDA approval. Imatinib mesylate makes a compelling success story; CML is monogenetic so translational linkage is clear and the assay systems can be discrete and specific; however, the drug discovery team in Ciba-Geigy (Novartis from 1996) needed belief and persistence to overcome the dogma that kinases may not be 'druggable'.

Academic relationships were key in continuing to reinforce the basic biology, develop the tools for the drug discoverers and continuing to reinforce the translation. This is a story that continues to evolve; in later stages of CML, mechanisms of resistance are seen so new ligands continue to be developed but *on the whole*, the Philadelphia chromosome has proved a good place for drug discoverers to be.

Further reading

Nowell PC. Discovery of the Philadelphia chromosome: a personal perspective. *J Clin Invest.* 2007; **117**(8): 2033–2035.

The telltale signs of risky lipid levels

Target 3-Hydroxy 3-methyl CoA reductase	Institution(s) Sankyo, Merck Research Laboratories
Status Standard of care for dyslipidemia	Discoverer(s) Endo Akira
Class/indication Statins/prevention of coronary artery disease	Molecule Compactin

The human story: The 'face' of lipid chemistry

A suspicion of high cholesterol can sometimes be spurred by just looking at a patient. Indeed, actor David Suchet's high levels were diagnosed after a sharp-eyed doctor spotted arcus senilis (white rings around the iris that are a tell-tale sign of high cholesterol) during a close up shot of the actor's face on television.

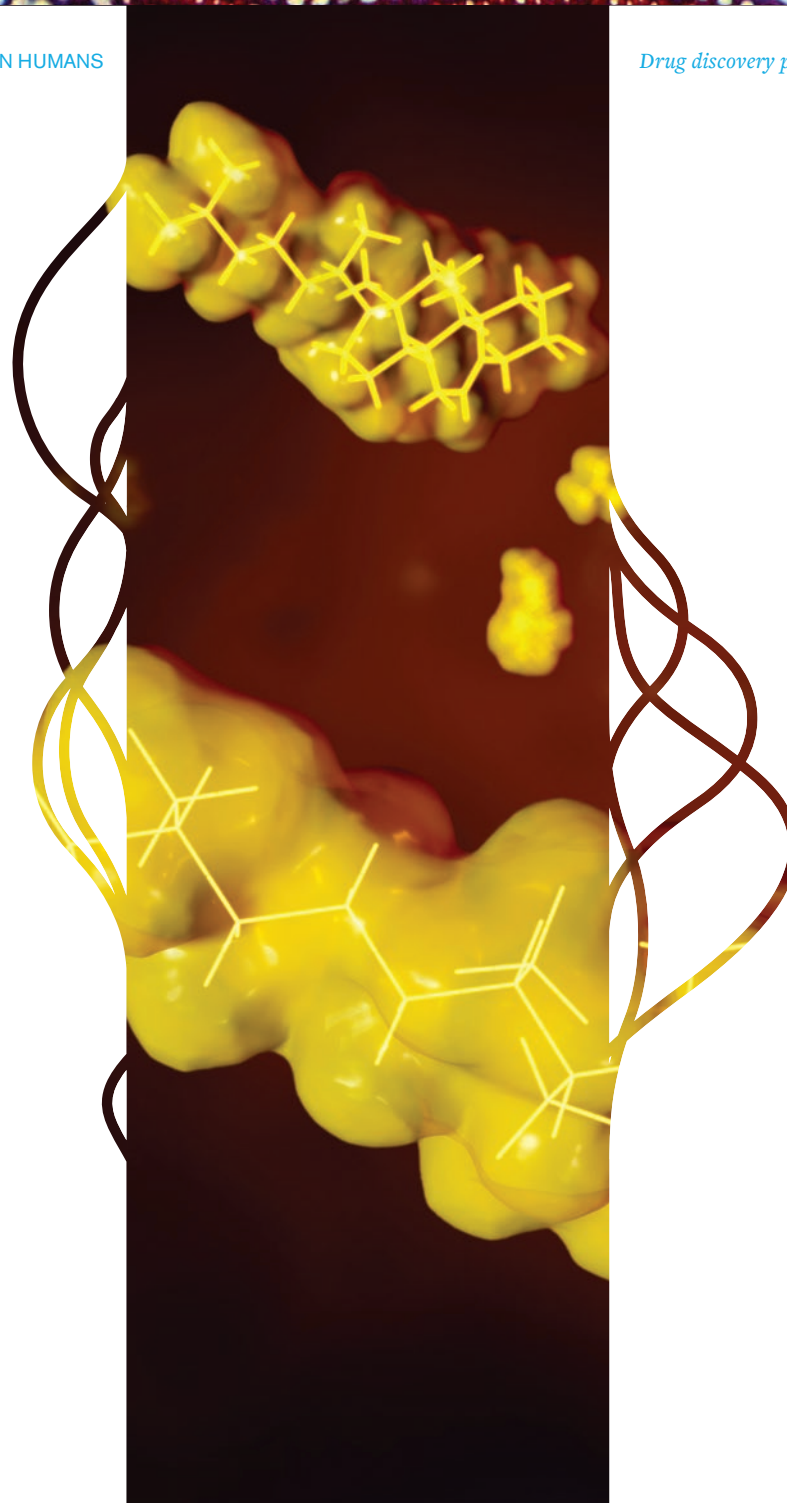
Equally, the presence of xanthomas (high-cholesterol-associated nodules of fat under the skin) have been documented since the 18th century; however, it was not until 1925 that Carl Muller from Oslo made the connection between these lesions and familial hypercholesterolaemia (FH).

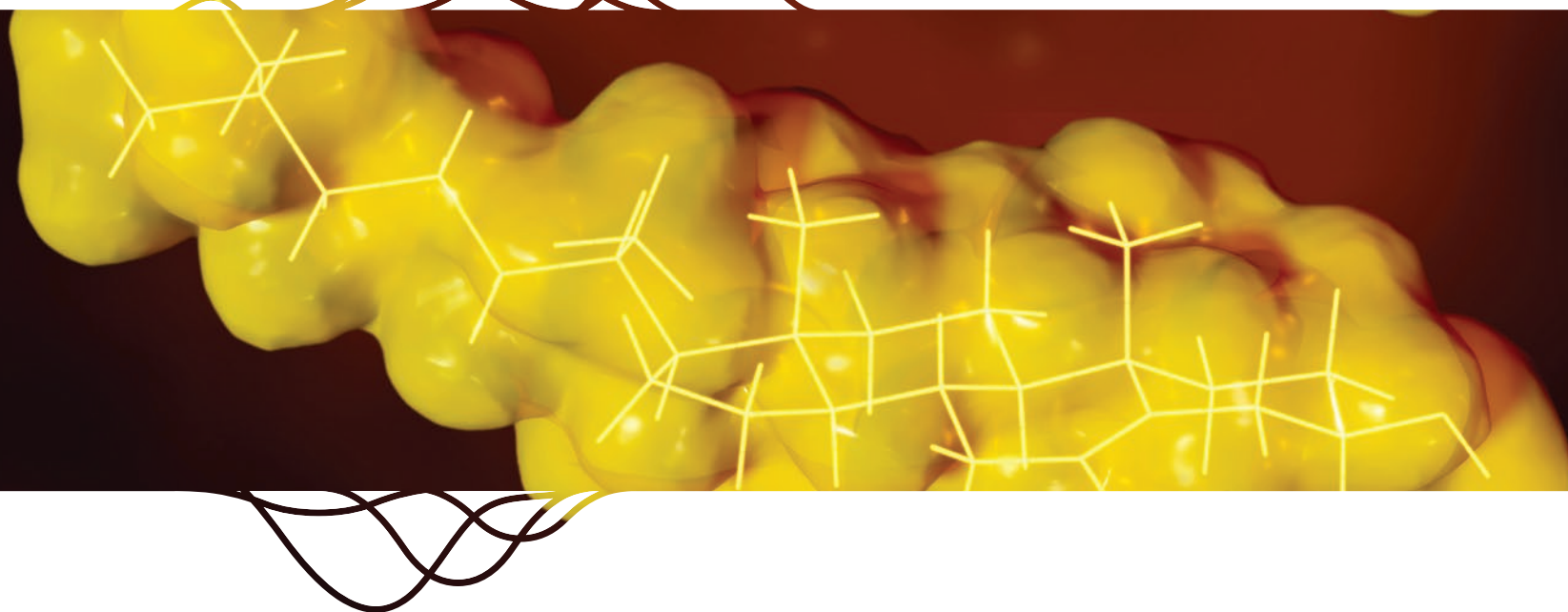
In the 1950s a correlation between cardiovascular disease and cholesterol levels was established and in 1973 Joseph Goldstein and Michael Brown identified a significant increase in the HMG-CoA reductase (the rate limiting enzyme in cholesterol synthesis) activity in skin cells of FH patients. This observation led to the discovery of the low density lipoprotein receptor (LDL-r) as a key regulator of cholesterol metabolism and earned the duo the 1985 Nobel Prize for Medicine.

The path to a drug

Merck Research Laboratories pursued the metabolism of cholesterol as a means to reduce its levels. Key enzymes in the cholesterol synthetic pathway were considered as targets. In the mid 1960s, Merck brought to the market an inhibitor of 24-dehydrocholesterol reductase, the last step in the synthesis of cholesterol. This product was soon withdrawn due to significant side effects. In a similar time frame, Akira Endo, a Japanese microbiologist identified an antimicrobial derived from *Penicillium citrinum* that had inhibitory activity on HMG-CoA reductase, the rate limiting enzyme in cholesterol synthesis. This molecule (compactin) was further developed by Sankyo, but clinical development was halted in 1980.

In 1976, Merck Research Laboratories signed an agreement with Sankyo and obtained samples of compactin and related experimental data. Under the direction of Alfred Albert, Merck set out to find its own statins and in February 1979 isolated a statin called mevinolin from the fungus *Aspergillus terreus*.





This proved very similar to compactin in chemical structure and was subsequently developed as lovastatin. It was launched by Merck in 1988 following successful clinical trials demonstrating significant decrease in LDL-cholesterol levels in hypercholesterolaemic patients.

Statins are structural analogues of HMG-CoA enzyme. They reduce LDL-cholesterol by inhibiting HMG-CoA reductase – a key enzyme involved in cholesterol synthesis. This is why statins are also referred to HMG-CoA reductase inhibitors.

Current status

Lovastatin success was followed by a raft of further statins, including simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin and pitavastatin. By 2008, an estimated 30 million people worldwide were taking a statin, generating sales of \$34 billion a year with total sales of quarter of a trillion dollars since they were introduced. This success story built on decades of careful clinical observation and research by far more groups than can be acknowledged in this short article (at least 13 Nobel Prizes have been awarded to scientists who devoted major parts of their careers to cholesterol research).

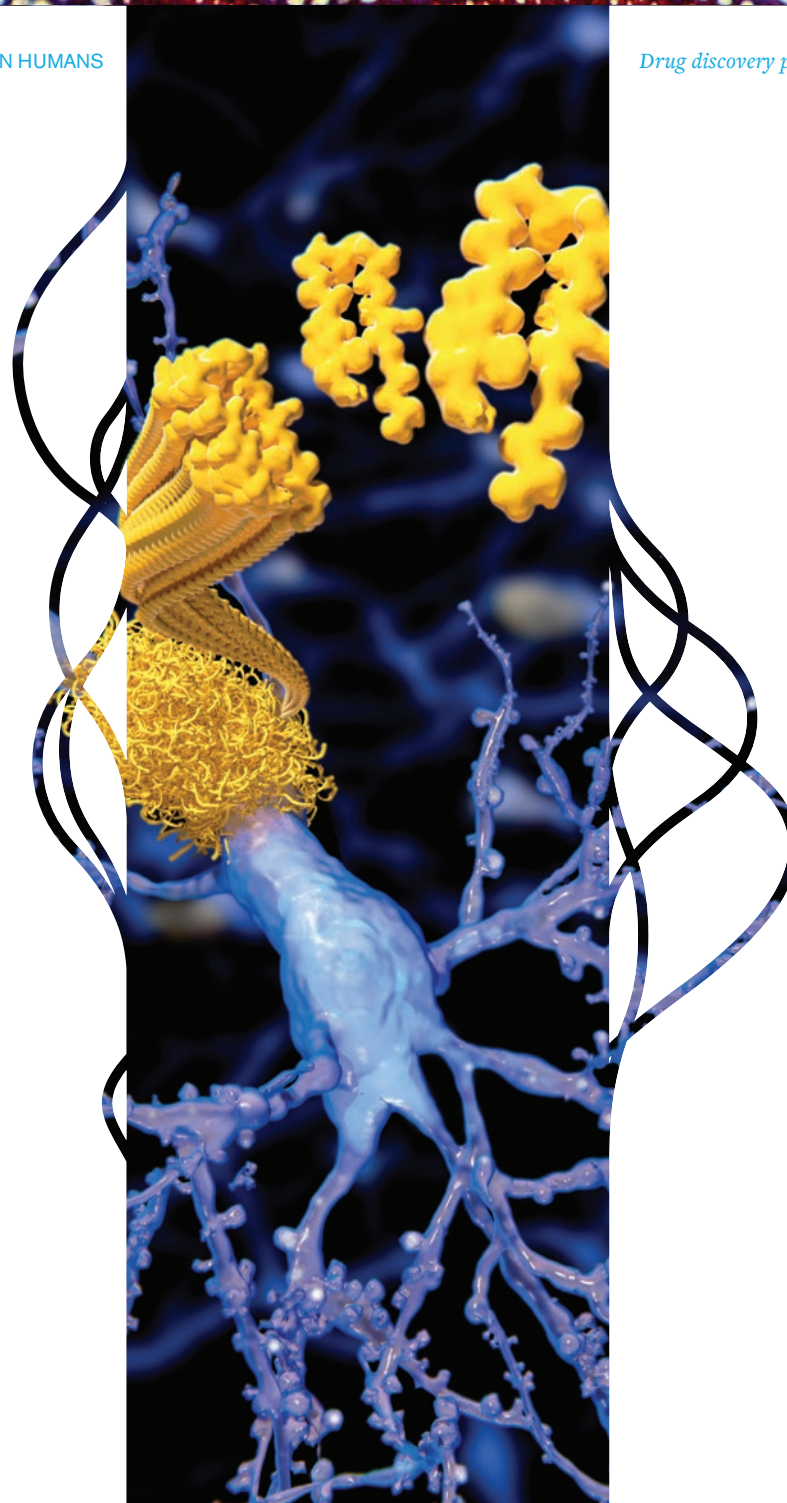
Further reading:

Akira E. A historical perspective on the discovery of statins. *Proc Jpn Acad Ser B Phys Biol Sci.* 2010; **86**(5): 484–493.

Gene mutation that applies brakes to Alzheimer's disease

BACE inhibitors and the genetics of cognitive decline: How genetic insights from reading the genomes of the Icelandic population injected fresh impetus to a drug discovery story

<p>Target</p> <p>The BACE gene/amyloid-beta</p>	<p>Institution(s)</p> <p>deCODE Genetics, Iceland, Massachusetts General Hospital, Boston and many pharmaceutical companies</p>
<p>Status</p> <p>Several small molecule BACE1 inhibitors are currently in Phase 1 – Phase 3 clinical trials</p>	<p>Discoverer(s)/ Researchers</p> <p>Professor Rudolph Tanzi Dr Kari Stefansson and the deCODE team</p>
<p>Class/indication</p> <p>BACE1 inhibitor/ Alzheimer's disease</p>	<p>Molecule</p> <p>At least ten small molecule BACE1 inhibitors are being developed</p>



The human story: Why him and not me?

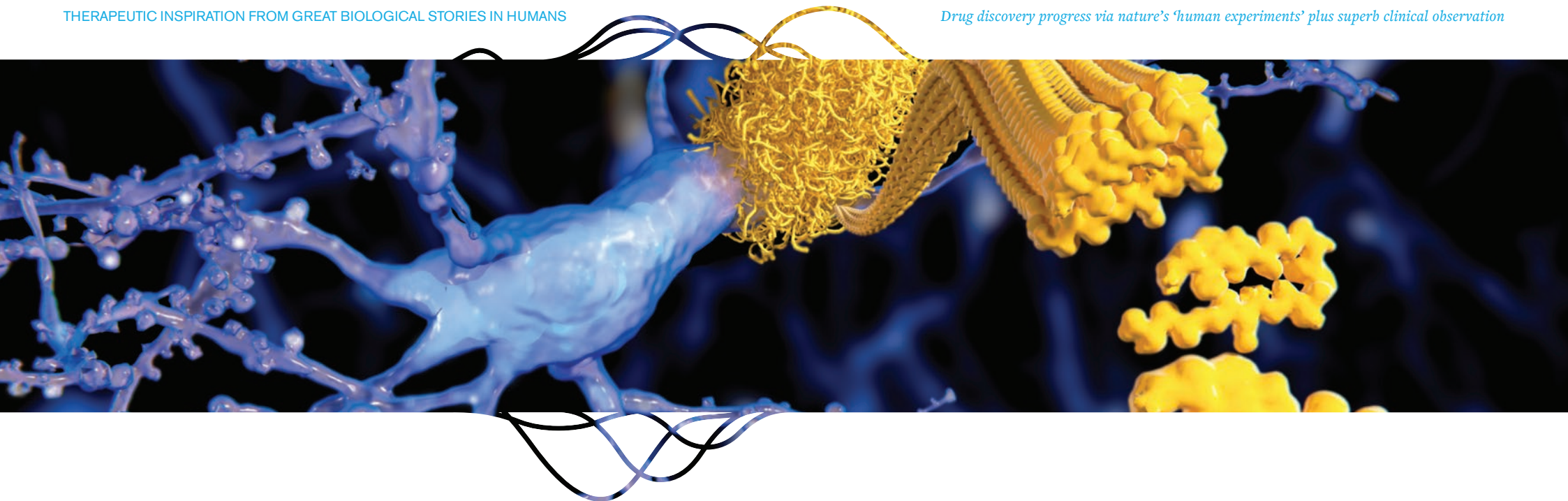
As populations age, the world faces a looming global epidemic of Alzheimer's disease – it has been estimated that by 2050, one person in 85 will be living with this debilitating disease presenting a staggering healthcare burden.

But whilst some succumb to rapid cognitive decline in their sixties, others become centenarians with their mental faculties still functioning well.

What distinguishes one group from the other? More than 25 years ago, clinicians and researchers discovered amyloid precursor protein (APP) in patients with a rare form of familial Alzheimer's that strikes in middle age. Subsequent work showed that APP is broken down to form amyloid-beta – a core component of the plaques that have become a hallmark of Alzheimer's, when found in the brains of patients at autopsy.

Fast forward to the year 2012 and publication of an intriguing genetic study showing that about 0.5% of Icelanders have a gene mutation that appears to prevent Alzheimer's. Compared with those lacking the mutation, carriers are more than five times more likely to reach 85 without being diagnosed with Alzheimer's.

Unsurprisingly for many, the mutation in question lies within the gene that codes for APP, refocusing attention on amyloid-beta as a target for drug discovery.



As Dr Kari Stefansson and colleagues in the deCODE team (authors of the Icelandic Study) related in *Nature*: 'We found a coding mutation (A673T) in the APP gene that protects against Alzheimer's disease and cognitive decline in the elderly without Alzheimer's disease. This substitution is adjacent to the aspartyl protease β -site in APP, and results in an approximately 40% reduction in the formation of amyloidogenic peptides *in vitro*. The strong protective effect of the A673T substitution against Alzheimer's disease provides proof of principle for the hypothesis that reducing the β -cleavage of APP may protect against the disease. Furthermore, as the A673T allele also protects against cognitive decline in the elderly without Alzheimer's disease, the two may be mediated through the same or similar mechanisms.'

At the heart of the drug development story is the enzyme Beta-secretase 1 (BACE1) known to be instrumental in cleaving APP to amyloid beta. Blocking BACE1 cleavage of APP may be the key to protecting against Alzheimer's.

Many large pharmaceutical companies now have BACE inhibitors in clinical development for the treatment of Alzheimer's and are investing many millions of dollars in these programmes due to a strong belief that this is a target mechanism with a high likelihood of success if patients receive treatment during the early stages of Alzheimer's when amyloid plaques start accumulating.

Further reading

Evin G. Future therapeutics in Alzheimer's disease: Development status of BACE inhibitors. *BioDrugs*. 2016; **30**: 173.



