The clinical impact of high-profile animal-based research reported in the UK national press: a detailed discussion of articles from 1995, and full search results from the Nexis database.

[Supplement 1]


Once Bitten – *The Times*, January 10th 1995

Leishmaniasis—a parasitic disease transmitted by sand fly bites affects up to one million people annually, killing up to 30,000. Symptoms include skin lesions, destruction of mucous membranes, fever and anaemia (WHO). *The Times* reported a new therapy against the Leishmania parasite.[1] Small antisense DNA molecules selectively killed mouse macrophages *in vitro*, infected with the intracellular form of the parasite (amastigote). Live mice had been previously used to produce the infective promastigotes, which entailed significant suffering and death, including “apathy, weight loss, an ulcerated lesion at the inoculation site, and metastasis in the nose at clinical examination”, as well as brain and meninges inflammation,[2] and infection encompasses the lymph nodes, liver, spleen, kidney and bones, and often results in death when allowed to progress.[3, 4]

The researchers found that 30% of the mouse blood cells were ‘cured’ of the parasite. Based on this result, and similar, cited results against other parasites that led the authors to speculate they had found “a new class of tools and a prototype of therapeutic agents against protozoan parasites”), the news article claimed “The same strategy might be eventually used for parasites responsible for sleeping sickness, Chagas disease, or even malaria.”

There is no antisense therapy for leishmaniasis, and none in clinical trials. While a few antisense therapies for other targets have been pursued, these are not related to this ‘breakthrough’. Two 2016 reviews opined “the future is bright” with “great potential”, but reported successes that were limited to *in vitro* results and promise in animals, and while “several” were in clinical trials there remained significant challenges.[5, 6] Another suggested that more than 70 of this type of intervention were in clinical trials[7] for “various cancer states and neurodegenerative diseases”, but none were for the diseases mentioned in the media article and paper. The FDA licensed the antisense therapy Fomivirsen (Vitravene) in 1998 for cytomegalovirus-induced retinitis, which was withdrawn a few years later due to superior therapies,[7, 8] and Mipomersen (Kynamro) for hypercholesterolaemia in 2013,[9] which can only be prescribed in exceptional circumstances due to risk of liver damage.

This field is still active, however. While the chief author of this study last published related papers in 1999,[10, 11] these were cited in a 2013 paper reporting antisense regulation of gene expression in mouse macrophages containing leishmania promastigotes.[12]

2 and 22. Baboon Bone Marrow transplant for HIV/AIDS Therapy – Failed

Lifeline from a baboon; Aids victim pins hopes on monkey bone marrow – *Daily Mail*, December 18th 1995

AIDS Patient to be Given Baboon Marrow—*The Times*, July 11 1995
An AIDS patient who had been treated by ‘an array of experimental drugs for 15 years’ was given a pioneering transplant of bone marrow from a baboon. The hope was that, by augmenting the patient’s own bone marrow and immune cells with the baboon’s, the baboon’s immune cells would take over. This would be advantageous to the patient as baboons are naturally resistant to HIV, so baboon immune cells would not be depleted by the HIV and could therefore survive to help ward off other opportunistic infections that ultimately result in AIDS-related deaths of humans.

Though acknowledged as speculative and risky by some, there was still optimism from scientists and doctors involved. The main doctor caring for the patient (Assistant Professor Steven Deeks of the University of California) opined, “…we are hopeful that we have a chance to succeed.” No associated report could be found, but another was published the year before (1994) describing the very same procedure: while the transplant of baboon bone marrow was “well tolerated” in this procedure, it did not result in successful engraftment of the baboon cells, and the patient died of progressive disease two weeks after discharge. There were, therefore, few grounds for optimism. Although the patient survived (eventually dying in 2006), the procedure was unsuccessful. Professor Deeks accepted that the baboon transplant “did not take” and that tests had shown no baboon cells to be present in the patient, though this had spurred him on to be “more aggressive” with any future transplant.

Some scientists involved in the development of this research published a follow-up report of this episode in 2004. Some positive spin was put on the outcome, stating there had been “transient microchimerism” (some baboon cells had survived in the patient for up to two weeks), “clinical and virological improvements” (the amount of HIV in the patient’s blood was lower for up to 11 months (though this was probably due to the nonmyeloablative conditioning of the patient prior to the surgery)), and that the patient had survived with no adverse events, but ultimately “no long-term improvement was achieved”. It appears that this technique has not been attempted subsequently: a search of the literature with terms "Transplantation, Heterologous", "Bone Marrow", “Humans” and “Papio” (baboon) produced 29 papers at the time of searching, but none were relevant to baboon/human transplants.

Little detailed information is available on the procedures the baboon underwent, or his fate. We do know he was bred in the U.S. for research, was housed in a single cage, and was quarantined in the University of Pittsburgh after transfer there for three months. He was subjected to multiple blood draws to screen for infectious agents (which may have included stressful tranquiliser-dart knockdowns), and had bone marrow harvested from his vertebral column for the transplantation.

3. Malaria Vaccine Produced in Edible Plants – Failed

A green way to health – The Times, January 23rd 1995

The main thrust of this breakthrough was that vaccines could be produced in plants – perhaps, eventually in edible plants, which would provide a cheap means of producing huge amounts of easily administered vaccine.

The premise is that scientists can engineer viruses that infect plants to contain genes from disease-causing microorganisms. When plants are infected with this modified virus, the
introduced gene produces a protein that is found in the pathogen, which can induce an immune response to that pathogen when eaten by people, acting as a vaccine.

This breakthrough was a proof of principle, involving the production of a malaria parasite protein in tobacco plants. When engineered tobacco mosaic virus (TMV) was used to infect tobacco, the plants produced the malaria-parasite protein.\[16\] An extract of the plants was tested in mice to see if this protein elicited an immune response, which it did, though this was not reported in the associated scientific paper.

The scientists speculated that one hectare of plants could produce up to quarter of a metric tonne of vaccine per month, inexpensively. The newspaper article mentioned parallel efforts to produce plant-based hepatitis B, foot-and-mouth-disease, and cholera vaccines.

The same author co-authored a follow-up paper 4 years later, expounding the virtues of TMV-based protein production,\[17\] though it was accepted that while “The chimeric virus particles promoted a potent antigen-specific response in mice…the surface presentation needs further optimization to improve protection against disease as is often the case in carrier/epitope immunizations.” He had established that high yields could be manufactured consistently and at low cost, but still not that the antigen itself was appropriate and sufficiently functional! In a paper published 14 years afterwards, (2009), he reported general advances made in the production of “vaccine products” using the same tobacco-based platform.\[18\] No mention was made of any progression with a malaria vaccine, however, and it was acknowledged that “data on safety and immunogenicity of these vaccines in humans are urgently required to move the product concept forward”—in other words, 14 years later, there were still no human data.

Another 2009 paper reported the production of immunogenic (in mice) malaria-parasite in tobacco plants using a new TMV-related expression system, while acknowledging “limited success” of previous attempts, “particularly those from Plasmodium [the malaria parasite]”.\[19\]

More recent publications in this field are informative. A 2016 review noted, “There are several plant-based vaccines that have been produced, with some of them being currently at the clinical trial phase”—in other words, more than two decades after the 1995 TMV-malaria ‘breakthrough’, have any plant-based vaccines actually been approved for human marketing and use?\[20\] The same review reports that one had been licensed at the time of writing—producing a subunit of hepatitis B virus in Cuba—but none had been licensed elsewhere. The authors noted, “…there are still challenges that limit the rate of successful production of these third-generation vaccines”, and “…even though the production of plant-based vaccines had been initiated almost two decades since 1989, a few challenges still have to be overcome in order to develop them into highly efficacy vaccines [sic]”. A 2015 review noted that no product had been licensed for humans despite of 30 years of endeavour, though several had reached clinical trials, all for influenza, with five being produced using tobacco.\[21\]

Several other contemporary publications (and the World Health Organization\[22\]) also noted the lack of approved malarial vaccines specifically, along with the future promise of plant-based candidates (e.g.\[23, 24\]), despite “none of the malarial proteins evaluated as candidate vaccines to date fulfilling expectations”.\[25\] Presently, there is a candidate malaria vaccine (Mosquirix, GlaxoSmithKline) in pilot programmes in Africa, to test its safety and efficacy\[22, 26, 27\], but it appears to have “limited efficacy and relatively short window of protection”.\[28\] A further 16 vaccines were listed in the “Global malaria vaccine pipeline” at the time of writing\[22\] (see http://www.who.int/immunization/research/development/Global_malaria_vaccine_pipeline_2
015Sep.pdf?ua=1), with another six trials completed with “reporting overdue”. None appeared to involve TMV/tobacco as a means of production.

4. Anti-allergy vaccine – Failed

Ultimate allergy shot; Innovation: British company boasts of a vaccine with huge potential – *The Independent, 29th January 1995*

This article promised a general anti-allergy vaccine, effective against, for example, “hay fever, allergic asthma, and reactions to food, drugs, cats, dogs and bee stings”, and “potentially one of the biggest-selling drugs ever” due to almost one third of the world’s population suffering from some form of allergy.

Although allergic reactions are specific to the particular allergen, the biological molecules that mediate them have common structural elements. The company Peptide Therapeutics developed a molecule directed toward one of these common elements, designed to inhibit its generation of allergic responses.

Allergens induce IgE antibody production, which bind to cells and cause the production of histamine and other substances that mediate the allergic reaction. The researchers designed a peptide that binds to common structures in IgE, inhibiting its activity and therefore the release of histamine in allergic responses.

The vaccine had reached clinical trials, and was proposed to be available in the UK “in five years” (by 2000). This confidence was illustrated by the company (set up just two years earlier) raising £4.5 million in venture capital, and seeking flotation in London.

No obvious related, contemporary scientific paper could be found. However, the founder of Peptide Therapeutics had published a few related papers previously. In 1994, he authored a response to a Lancet article in which doubt had been expressed about vaccines for asthma, defending his company’s approach.[29] He referenced one of his previous publications in which he stated “there is every reason to believe that this vaccine should work therapeutically—i.e., after disease initiation—as well as prophylactically against all IgE-mediated allergic reactions, including extrinsic asthma”.[30] This paper reported experiments in rabbits, in which two major facets of the allergic response—IgE antibody production and histamine release—were inhibited.

This led to further research. In 1999, the researchers reported the advancement of the allergy vaccine peptide into the preliminary clinical trials noted in the media article.[31] These trials revealed that 2-3 immunisations of people with food allergies enabled them to tolerate their previously allergenic foods,[32] and consequently the vaccine was proceeding to “full-scale phase 2 clinical trials”. However, one year later it was reported that “a further clinical trial failed to confirm this”, though other peptides showed promise as anti-allergy vaccines.[33]

Nonetheless, development of this approach was ultimately unsuccessful. Peptide Therapeutics merged with Oravax to become Acambis, in turn acquired by Sanofi Pasteur in 2008. There is no drug of this type in Sanofi’s portfolio or pipeline.
In spite of the failure of this approach, a related—though crucially, different—approach to interfering with IgE’s role in allergic response has resulted in useful therapies. For example, the drug Omalizumab (Xolair)—a monoclonal antibody therapy for allergic asthma—targets IgE antibodies involved in allergy response, and prevents them binding to mast cells that are pivotal to allergic reactions.[34–36] The asthma drug Mepolizumab (Nucala) targets Interleukin-5 (IL-5), which, though a different target to IgE, is involved in IgE production (IL-5 mediates IL-4-induced IgE production by B-cells), so there is a functional link.[37, 38] However, no peptide-based vaccine for general allergies could be found.

5. Attenuated-HIV live-virus vaccine – Failed

Vaccine hope for AIDS – The Times, 30th January 1995

An attenuated form of Simian Immunodeficiency Virus (SIV) protected monkeys against infection with virulent (disease-causing) SIV, and also a hybrid SIV/HIV virus.[39, 40] It was engineered to be deficient in the nef gene and, while infectious to monkeys, appeared not to cause immunodeficiency and associated disease. Infected monkeys remained healthy for two years, compared to those with normal SIV who died of immunodeficiency-related infections within a year.[39] Such nef-deficient SIVs had previously been demonstrated to be present in the blood of infected macaques for a shorter period of time than wild-type SIVs.[41] Further, a nef-deficient HIV had been discovered in a human who had survived for 11 years despite probable multiple exposures to virulent HIV.[42]

Researchers therefore proposed nef-deficient HIV might protect humans from virulent HIV infection and from developing AIDS.

Though some caution and safety concerns are conveyed in the media article, there is significant positive speculation. Dr Jim Stott of the National Institute for Biological Standards and Control opined, “The attenuated virus induces a protection against an immunodeficiency virus that is far more potent than anything yet devised”, and “The message is that there are grounds for hope”.

These grounds for hope, based on results in monkeys, were, nonetheless, eclipsed by other disappointing results and acknowledgements of the “difficulty, even with a live virus vaccine, in protecting against an AIDS virus infection”.[43] as well as by the aforementioned safety concerns (e.g. “Extrapolation to human beings will require extensive evaluation of the safety of attenuated retroviruses”[40]. The reason for caution was that nef-deficient SIV vaccination—while inducing protection against pathogenic SIV infection in adult macaques—was pathogenic to neonatal macaques, in whom it caused AIDS-associated symptoms.[44, 45]

These safety concerns, based on fears of the attenuated virus reverting to pathogenic wild type, remain today.[46, 47]

At the time of searching, there remained, well over 20 years later, no attenuated HIV/AIDS vaccine in existence, or apparently even in development.

6. New improved mouse model of AD, for better testing of new therapies - Failed
A new genetically modified (GM) mouse was claimed to represent a better model for human Alzheimer’s disease (AD) than previous, similar types of GM mice.\cite{48}

The similarity involved the gene inserted into the mice: the human APP gene (β-amyloid precursor protein). This gene and the protein it produces have been strongly associated with AD.\cite{49} Mutations in the APP gene are linked to AD, and fragments of the APP protein form characteristic amyloid plaques in the brains of AD patients.

This prompted initial efforts to create mice genetically modified to contain the APP gene. However, none of these APP-GM mice, prior to this article in 1995, had shown pathology similar to human AD pathology (see \cite{48}). The researchers created mice modified with another form of the APP gene, containing a single mutation known as ‘Indiana’ (717 V→F)—a mutation identified in AD patients,\cite{50} in an effort to create a better model.

They reported that their GM mouse had more “Alzheimer-like neuropathology” than previous mouse models, which should be more predictive when testing prospective new human therapies, giving “a huge boost to research into Alzheimer's by providing a model in which potential therapies can be tested.”

Due to highly variable nomenclature of GM mice, it was not straightforward to follow up this mouse and its impact on AD research (see e.g. informatics.jax.org/allele/MGI:2151935, and alzforum.org/research-models/pdappline109). However, the following information was located from various sources.

The GM mouse in question may be known as the ‘PDAPP mouse’, produced by Athena Neurosciences (USA). This company was acquired by Elan Pharma just one year later (1996), and this company ceased operations in 2013, which might have suggested it was not a great commercial success. However, this mouse is still being used in research, with 147 papers associated with ‘PDAPP’ in PubMed (October 2018). It is used alongside many other GM AD mice, as “No one animal model fully replicates the pathogenesis of AD, but rather only model different aspects of the disease”.\cite{51} This continued use of multiple, different GM mice may be due to the fact that mouse and human APPs differ by 17 amino-acids (around 2-3% of the total), with 3 of these in the crucial β-amyloid region (6-8% of the total). These can have significant functional consequences, which may have manifested in the observable differences of pathology of GM mice and human AD patients, as well as the many failures in translation of new drugs to humans that appeared efficacious in animals.\cite{52,53}

PDAPP mice have been used since this ‘breakthrough’ to test various proposed human therapies. Just a few years later, anti-β-amyloid antibodies reduced the levels of β-amyloid plaques in the brains of PDAPP mice, and so were proposed to treat human AD patients.\cite{54-56}

The U.S. Alzheimer’s Association (alz.org) lists five FDA-approved therapies for AD. None are cures—all treat symptoms which, according to the UK Alzheimer’s Society (alzheimers.org.uk), “…can temporarily alleviate symptoms, or slow down their progression, in some people…but the benefits are small, and drugs should only be one part of a person's overall care.” It is estimated only about half of patients benefit, and any benefit may only last 6-12 months (alzforum.org). These five therapies are donepezil (Aricept), galantamine (Razadyne), memantine (Namenda), rivastigmine (Exelon), and a donepezil/memantine
combination (Namzaric). Rivastigmine was approved by the FDA in 2003, and too soon to have been impacted by the reported breakthrough in 1995. Three of these four individual drugs are cholinesterase inhibitors, which target the process that breaks down a key neurotransmitter, and the fourth (memantine) targets excess glutamate in the brain, resulting from brain cells damaged by the disease.

Alongside these few and partial successes, the scale of failure is great. As of mid-October 2018, almost 2,000 current and completed AD clinical trials are listed on clinicaltrials.gov, and a 2014 review stated that greater than 300 interventions had been tested at the time of writing. This review highlighted several salient clinical trial results for proposed new AD therapies alongside preclinical data from GM mice associated with them, which included some using PDAPP mice. Many examples of failure were noted, in which Phase 2 and 3 clinical trials were terminated for safety and efficacy issues, despite success in PDAPP mice. A 2015 publication, partly due to failures such as these, as well as the authors’ own experience of “unstable phenotypes” of PDAPP mice and different results in different mice (including the PDAPP mouse), opined that data “raises the thorny issue of how well APP tg [GM] mice represent the situation in AD brain”.

These numerous failures have not dampened enthusiasm and speculation from some sources. In 2010—15 years after her original PDAPP mouse paper—the lead author published a ‘Viewpoint’ on amyloid-beta immunotherapy, acknowledging that the field of AD therapy was in need of positive change, in the form of new agents that did more than “offer modest and transient symptomatic relief”. At the same time, however, she noted the results of “numerous” clinical trials were “eagerly anticipated”, as they were “supported by extraordinarily compelling data” including “several encouraging results”. One of these agents was a high-profile failure in Phase 3 trials, bapineuzumab, for which it was stated that success “would not only be a tremendous validation of preclinical modelling of a progressive neurological disease, but would represent a new era in hypothesis-driven, disease-modifying therapy for this poorly served patient population.” At the time of writing (2018), nothing had changed: clinical trials continue to fail in large numbers, and still no new drug has reached the market. The optimism from researchers continues, too: a UK national newspaper announced 12 AD drugs in advanced clinical trials, which should be available to patients within three years (e.g. Daily Express, 21 Mar 2018)—every one targeting β-amyloid; and CNN reported a β-amyloid targeting antibody therapy—BAN2401—progressing to Phase 3 clinical trials (CNN, 26 Jul 2018). CNN did, however, highlight that many drugs targeting β-amyloid had failed in recent years, including Merck’s verubecestat in 2017-18, Johnson & Johnson’s atabecestat in 2018, Pfizer’s bapineuzumab Phase 3 trial in 2013, and Eli Lilly’s solanezumab Phase 3 trial in 2016. The latter had been claimed to be the most promising candidate drug ever, but its failure was so disappointing it led to serious and unprecedented levels of concern over the entire amyloid hypothesis for AD.

There is growing concern over animal models of AD, and in particular, the hypothesis that amyloid plaques are the cause. Pharma IQ reported in “Lessons from failed Alzheimer’s research” that “Amid pressure to limit discovery losses and be more ethically responsible, a movement is building to encourage researchers to develop drug testing models that do not involve animals” Doubts about the amyloid hypothesis specifically are stronger than ever: it has been shown that many people without dementia have substantial amyloid deposition in their brains, and, conversely, that some Alzheimer’s patients have very few, or even no, amyloid plaques. And the U.S. Department of Health and Human Services, in the National
Plan to Address Alzheimer’s Disease, has urged an increase in clinical and human-specific research in efforts to understand and cure the disease.[71]

7. New Anti-Depressant Drug is Also Treatment for MS - Failed

Drug Hope for MS Sufferers– The Times, Mar 2 1995

Researchers announced that a new drug being tested for the treatment of depression, rolipram, may also be an effective treatment for multiple sclerosis (MS). This speculation was based on rats with experimental auto-immune encephalomyelitis (EAE), which some believe is a model for human MS.

Rolipram prevented rats from developing EAE, and slowed down the progression of the disease in rats who already had EAE.[72] The mechanism involved a suppression of the activity of Tumour Necrosis Factor (TNF), which has been implicated in both EAE and MS.

There was much optimism about these findings.[73] The drug was also tested in marmosets, in whom EAE had been induced by injecting them with human brain tissue, and it had prevented the development of EAE.[74] Some had also proposed it as being a treatment for other TNF-associated disorders such as arthritis, septicaemia (blood poisoning) lupus and some forms of diabetes, and wanted clinical trials of the drug in MS patients to begin immediately.

The scientific paper reporting the breakthrough discussed how many in vitro studies had prompted the experiments in EAE rats, including the association of TNF with EAE and MS, its links with demyelination,[75] its toxicity to oligodendrocytes,[76] and the anti-inflammatory activity of rolipram.[77, 78] They did not hold back in their optimism, also citing encouraging results on relapsing-remitting EAE in mice,[79] and opining that the drug should be applicable to other diseases involving inflammation or autoimmunity.

The author of an article responding to the paper reporting this breakthrough cited work published 2-6 years previously, fortifying the link between TNF and EAE and MS, which included therapeutic and preventive effects of antibodies to TNF in EAE mice, as well as localisation of TNF in MS plaques in human tissue and TNF receptor in human patients.[73]

Many publications since this work have outlined serious issues with the use of rolipram, including “There are some discrepancies between in vitro and in vivo effects of rolipram, as well as between results obtained in animal models and clinical studies. The clinical use of rolipram is limited because of its behavioural and other side effects”.[80] Twelve years later, in 2013, a review of the class of drugs of which rolipram is one (PDE4 inhibitors) discussed how rolipram was investigated for its antidepressant activity for some time, before its PDE4 inhibitory and anti-inflammatory properties were noted (inhibition of PDE4 leads to decreased TNF levels, hence its link to treatment of EAE and MS). These findings were due to human and human in vitro research. Rolipram never reached clinical use for any indication, however, due to unacceptable adverse effects in humans.[81]

Rolipram appears to be limited to experimental use only, and was never approved for the treatment of MS in humans. The WHO, Medscape, and NIHCE/BNF do not list it as an approved drug. A clinical trial was registered in 2001 to investigate it as an MS therapy (clinicaltrials.gov, NCT00011375), but no results have been posted. Other trials of rolipram
have investigated its use as a research tool only, for instance to help gauge the efficacy of new PDE4 inhibitor drugs (clinicaltrials.gov).

The Multiple Sclerosis Information Page of the NIH’s National Institute of Neurological Disorders and Stroke (updated June 2018) states, “There is as yet no cure for MS.” (Ninds.nih.gov) A number of drugs have been approved that are therapeutic. Three forms of interferon-β have been approved by the U.S. FDA, as well as a synthetic form of myelin called copolymer I (Copaxone). Various other agents are in use, including teriflunomide, dimethyl fumarate, Novantrone (mitoxantrone), dalfampridine (Ampyra), and the monoclonal antibody natalizumab (Tysabri) under strict guidelines due to adverse effects.

The mode of action of the interferons involves TNF—the target of rolipram. This has nothing to do with the results of using rolipram in EAE rats, however, as interferon-β’s effects om TNF were already known and being studied in humans, in vitro and in vivo (e.g. [82–85]).

TNF targeting remains an active area of research, but there are increasing concerns over this type of intervention. A 2017 review reported that five TNF blockers had been approved for clinical use, but cautioned that “serious side effects associated with immune suppression have been reported, including central and peripheral nervous system (CNS) demyelinating disorders”. With specific regard to MS treatment, trials in EAE animals have shown “beneficial effects”, but subsequent clinical trials have given “surprisingly unfavourable results”, with most patients’ conditions worsening. Other research suggests TNF targeting for MS therapy—as suggested by experiments involving EAE animals—can actually make things much worse, exacerbating MS in humans (e.g. [87, 88]).

8. Identification of gene causing some childhood deafness and blindness brings hope of cure - Failed

Mouse Gene Points Way to Site of Child Hearing and Sight Defect—The Guardian, Mar 3 1995

A gene associated with deafness and blindness was discovered in humans, building on previous work in mice. Mutations in the gene—known as shaker-1 (sh1)—are responsible for the most severe form of these disabilities—known as Usher syndrome type 1b (USH1B)—in thousands of children in the UK.

The article reported two ‘Letters’ in the same issue of Nature, by separate groups that had collaborated—one working with mice [89], the other with humans [90]. It was claimed that the mouse study expedited the identification and characterisation of the gene in humans.

The sh1 gene encodes myosin protein (Type VIIa), involved in the cytoskeleton. Cellular architecture is therefore affected when the gene contains certain mutations, of which three were identified in mice. The authors of the human research, also cognisant of previously observed cytoskeletal abnormalities in USH patients, hypothesised that its human homologue could be the gene defective in USH1B. This human gene is called MYO7A.

The literature, however, suggests the importance of the mouse research may have been exaggerated.
First, much was already known about the gene and its link to Usher syndrome prior to these Nature publications. For example, the human paper[90] notes that: Usher syndrome was already known to be an autosomal recessive trait; abnormal organisation of microtubules had already been associated with defective eye and ear function in Usher patients; three different genes had already been linked to USH1, largely by human studies,[91–93] and the USH1B gene had been mapped to a specific region of human chromosome 11 (11q13.5). The mouse paper[89] acknowledged that there was already evidence for myosin I involvement in hearing, and that other myosins were implicated in any case (including myosin VIIa here). Further, the USH1B locus had been mapped, in research involving humans, 2-3 years previously.[93, 94] Other contemporary reports cited prior human investigations that had mapped causal genes for USH (e.g. [95, 96]).

The authors of the mouse paper (and others) also highlighted concerns about species differences, affecting extrapolation to humans and confidence in the role of the sh1 gene in human USH. For example, "the phenotypes of the original sh1 mutant and of the five other sh1 mutants seem to be different from that of USH1 patients, because no sign of retinal degeneration has so far been reported in these mouse mutants",[89] They accepted that another myosin may be compensating for the defective myosin VIIa in the mouse, or the nature of the mutation itself could be different—in another gene entirely in humans. A 2015 review of Usher syndrome acknowledged many shortcomings of associated animal models, including: different USH1 distributions; poor replication of phenotypes in mice; inconsistent results across strains and species; and differential gene expression and protein localisation, structure and function[97]

Regardless of the debatable merits of the mouse work, almost quarter of a century later there is still no cure for USH, based on myosin VIIa or otherwise. The intervening years have seen further GM mouse models, many based on sh1 gene mutations, as well as pre-clinical and clinical trials of gene therapies targeting MYO7A (see e.g. [97–101] and clinicaltrials.gov). These are mainly focussed on treating the visual aspect of the disease, as the auditory issues appear to be addressed mainly by cochlear implants. It has recently been suggested that gene therapy for the cochlea may be 20 years away.[98]

Retinal gene therapy, specifically, has provided encouraging results in mice, correcting phenotypes resulting from mutant MYO7A in murine photoreceptor and retinal pigment epithelial cells.[102–104] Human trials are ongoing for UshStat (Sanofi), a MYO7A gene therapy (clinicaltrials.org, NCT01505062 and NCT02065011, and [99]), though the long-term safety trial is not expected to be complete until 2033. Clinical trials have been stymied by difficulties with delivering the MYO7A gene in humans: the size of the gene poses difficulties, and delivery by splitting it into two may cause other issues with safety and therapeutic efficacy. In addition, all Usher genes express multiple isoforms, and forms expressed in human and mice retinas may differ (see [101]), and the gene may be delivered only to certain parts of the retina, which may exclude photoreceptors, precluding therapeutic efficacy. Delivery methods can also pose a risk of insertional mutagenesis, and result in very variable intercellular expression levels.[99, 100]

Another complicating factor is that, in 2015, it was reported that 13 genes had been associated with USH (almost exclusively due to human investigation)—suggesting the pathology is complex, and that speculative claims of cures based on the discovery of one gene, such as MYO7A, are wildly optimistic.[97] This is further evidenced by acknowledgements that the pathology remains far from understood, and that no cure or significant treatment has yet been realised.
While one author of the mouse study cautioned, “It’s a long haul, but it’s getting faster”, it is unlikely they anticipated no success almost 25 years later. If and when any MYO7A gene therapy is successful in humans, the role of the mouse studies in the identification of the gene in humans is of little importance. Much of the work had been done in humans; the gene would have been located in humans in any case, without much delay due to increasingly powerful molecular methods and building on this previous work; many significant confounding species differences continue, and suggest mouse models are misleading; and the involvement of many other genes in the disease could mean that MYO7A gene therapy might not be sufficient in any case, even if technically successful in the future. If success is achieved, it will have been due to human-specific research.

9. Inhibitors of ras genes could be a cure for many cancers - Failed

Stopping cancer in its tracks—The Times, Mar 21 1995

This article reported the proceedings of a meeting of the American Association for Cancer Research, where some scientists had speculated that blocking the activity of mutant ras genes with ‘ras inhibitor’ drugs would revolutionise cancer treatment and cure.

There are three ras genes in humans (HRas, KRas and NRas), involved in regulating the growth of cells. Ras mutations can disrupt this regulation, and are responsible for around one third of human cancers.[105]

Merck expected to begin clinical trials within 12-18 months, leading to “safer and more effective patient therapies”. A researcher stated that, because ras was “one of the hottest targets for cancer therapy”, “Just about every pharmaceutical company has its own ras-inhibitor research programme, or is thinking of starting one.” Some scientists had targeted an enzyme in the ras pathway called farnesyltransferase (FPTase or FTase), and believed the specificity of this unprecedented approach would lead to treatments with no adverse effects. They also reported the development of more potent and effective ras inhibitors, and other inhibitors with different targets in the ras pathway, some of which may “offer greater promise.” Overall, the optimistic consensus was that animal studies had “selectively suppressed tumour growth without harmful effects”, and that, “If we can get these drugs into patients, I think the data will show the concept is right.”

No one scientific paper prompted the article. However, previously, two of the quoted researchers reported the inhibition of ras by an FTase inhibitor,[106] and subsequently the development of inhibitors of ras via the FTase, and other, strategies.[107] Notably, several contemporary papers highlighted the preceding decade-long failure of inhibiting oncogenic Ras function,[108–110] but that the discovery of FPTase activity being central to the carcinogenicity of ras “opened up a new avenue” and had “yielded potential anticancer agents”. Quickly, papers followed showing that FTase inhibitors were potent inhibitors of the growth of human tumours transplanted into mice (known as ‘xenografts’), and were not toxic to non-cancer cells (see [110]).

More recent literature, however, reveals serious problems in translating success in animals to human therapeutic efficacy, despite three decades of effort. The U.S. National Cancer Institute admits “Thus far, developing ways to block RAS gene function has been ineffective”, and
“many still consider RAS proteins as virtually “undruggable” targets for therapy”\textsuperscript{[105]}—echoed in contemporary papers.\textsuperscript{[111–113]} KRas has been described as “one of the most maddeningly difficult targets”, and a “graveyard” with “a long period of failures”.\textsuperscript{[114]} Inhibiting FTase, specifically, was said to “work like gangbusters in mice”, but all that reached human testing failed,\textsuperscript{[114, 115]} including two in Phase 3 human trials, lonafarnib and tipifarnib.\textsuperscript{[113]} Twenty-three years after The Times article, there is no effective ras-pathway based therapy for cancer in humans.

Two recent reviews discuss the long-term and widespread failure of bringing preclinical ‘success’ to the clinic. They remain optimistic that clinical benefit of one or more ras-based therapies will eventually be realised,\textsuperscript{[116, 117]} though this is not based on mouse data, but on \textit{in vitro} and \textit{in silico} methods.\textsuperscript{[112, 114]} For example, three drugs have recently been approved that inhibit molecules that can interact with ras,\textsuperscript{[118]} though it has been cautioned that “it is important to acknowledge the limitations of the current generation of compounds, which remain unsuitable for human trials and offer limited options for optimization”.\textsuperscript{[118]} Some suggest that a number of ras inhibitors may have some degree of therapeutic value for some cancers in some people, perhaps in combination, but with caution.\textsuperscript{[113]}

10. New drug for Alzheimer’s (GTS-21) - Failed

\textbf{Database – Fags for the memory—The Observer, April 9 1995}

A memory-enhancing drug—GTS-21 (also known as DMXB, DMXBA and DMXB-A)—made elderly rabbits become as alert as rabbits half their age, by stimulating a receptor in the brain in the same way as nicotine, but without nicotine’s side effects such as addiction and high blood-pressure. Because one symptom of Alzheimer’s disease is the loss of receptors in the brain that respond to nicotine, it was proposed that this drug could improve the memories of Alzheimer’s patients by stimulating remaining receptors, without side effects.

The researcher mentioned in the article had reported initial findings regarding GTS-21 on cognitive performance tests in rabbits one year previously.\textsuperscript{[119]} These experiments involved significant suffering: individually housing 60 rabbits in steel cages, restraint, and use of eyelid retractors and ‘headstages’ that delivered puffs of air to their corneas. Sessions lasted around one hour after administration of the drug, following fifteen 90-trial sessions for “conditioning”. Rabbits were killed after testing by anaesthesia and decapitation.

This ‘eyeblink classical conditioning paradigm’ makes the rabbits learn that hearing a tone means an imminent puff of air to their eye. They therefore anticipate this when they hear a tone, and blink. Cognitive decline, e.g. with age, means that the individuals take longer to learn this association, and therefore positive cognitive effects of new drugs can be measured if their learning improves. GTS-21 injections had facilitated leaning/acquisition in these rabbit experiments.

The paper that prompted The Observer article built on this, comparing two stimulants of nicotine-receptors in the brain—GTS-21 (DMXB), and another, DMAB—with nicotine itself, and their effects on cognitive function in aged rats,\textsuperscript{[120]} who displayed age-associated memory impairment (AAMI), in similar ways to ageing humans.
Again, results—obtained by making rats jump from a floor delivering electric shocks onto a pole, and navigate through mazes by memory—showed that GTS-21 (DMXB) enhanced cognition, learning and memory, though this time in rats, comparable to nicotine but without undesirable side effects, and so the authors inferred that the same could be true in humans with AAMI and/or Alzheimer’s disease.\textsuperscript{120}

Given that there was much literature reporting animal and human studies showing the cognition-enhancing effects of nicotine (the authors themselves cited many examples), and that GTS-21 (DMXB) is based on (and very similar to) a naturally occurring nicotinic agonist (anabaseine) known to bind nicotine receptors, it is puzzling why many animal behavioural experiments, some involving significant suffering, were repeated for GTS-21. Indeed, a 2012 review of GTS-21 cited experiments showing it improved cognition and learning in monkeys, too,\textsuperscript{121, 122} that it was in clinical development for Alzheimer’s treatment, that a human study showed a robust relationship between GTS-21 dose and cognitive response, and cited four Phase I clinical trials.\textsuperscript{123} clinicaltrials.gov revealed only one trial (Phase 2, Completed) of the drug specifically for Alzheimer’s disease (NCT00414622) (last updated April 2007). The UK Alzheimer’s Society (alzheimers.org.uk) noted “No new drugs have been licensed in the UK for Alzheimer’s disease since memantine in 2002”. This is the only type of licensed drug that isn’t an acetylcholinesterase/cholinesterase inhibitor. Three of these are licensed: donepezil, rivastigmine, and galantamine.

While GTS-21 is still researched, notably for schizophrenia,\textsuperscript{124, 125} and rheumatoid arthritis,\textsuperscript{126} little or nothing is being done regarding its use as a treatment for dementia.

More recent reviews have little or nothing to add. A 2010 review\textsuperscript{127} cited the aforementioned early-phase clinical studies of GTS-21/DMXB.\textsuperscript{123, 128} A 2013 review of this specific class of drugs for Alzheimer’s\textsuperscript{129} merely cited this 2010 review,\textsuperscript{127} while a similar, specific review in 2017,\textsuperscript{130} cited the aforementioned 2013 review,\textsuperscript{129} the 1995 paper that prompted the media article,\textsuperscript{120} the associated 1994 paper from the same group,\textsuperscript{119} and a 2000 extension of the their rabbit experiments;\textsuperscript{131} cognitive impairment induced by a type of anaesthesia in aged rats;\textsuperscript{132} and a Phase 2 clinical trial of GTS-21 in schizophrenia patients, concluded in 2008, which failed to improve cognition.\textsuperscript{133} In summary: GTS-21/DMXB failed to be licensed for the treatment of human dementia, Alzheimer’s or otherwise, despite efficacy in animals, and ongoing research suggests it will never be used for that or any other purpose, at least in its current form.

\textbf{11. Mending broken bones with injectable ‘Skeletal Repair System’—Succeeded (with caveats)}

\textit{Cast Away Your Plaster Cast—The Times, April 25 1995}

\textit{The Times} reported the development of an injectable paste that can be introduced—“like toothpaste”—into broken or fractured bones, or bones affected by osteoporosis.

This bone substitute—known as Skeletal Repair System (SRS)—was invented by Norian Corporation (USA). It was not the first bone substitute intended to replace metal implants, which can fail, and plaster casts, which are associated with a variety of complications and
disadvantages; but it was claimed to be a better match for real bone than anything that had gone before it, which facilitated better and more efficient replacement with actual bone during healing. This cement – and similar types – are known as calcium phosphate type cements.

The report cited a paper in Science, stating “Experiments with animals have given good results, and the first tests on human patients...have produced good repairs of broken wrists”.\[^{134}\] It did not go into detail about animal use, but rabbits and dogs were involved: bone sections were removed from the ulnas of 12 rabbits, and cement injected. The rabbits were x-rayed, and killed “at 12 weeks” for tissue examination. Human investigations involved repairing the fractured distal-radius of a 49-year-old woman, for whom x-rays showed “stabilisation” and “maintenance of correct position” following injection of SRS.

A 2003 paper discussing the background to SRS\[^{135}\] cited six references, from 1966 through to 1995, all based on human investigations. ‘Self-curing’ polymethyl-methacrylate bone cement was found to heal human fractures, and later to efficiently manage distal radial fractures, also in older patients. This type of cement was not remodelled or incorporated into the bone, however, and the process of it curing may have impaired healing.

This 2003 paper also reported human clinical investigations of SRS during the five years after The Times report, between 1996 and 2000,\[^{136–138}\] as well as the authors’ own clinical research—a large, prospective, controlled, randomised study with SRS building on this, which concluded that the use of SRS may allow for accelerated rehabilitation when used to repair distal radial fractures.\[^{135}\] They did, however—in common with some previous human data—report that “The risk of extrusion of the SRS cement into undesirable locations has been a substantial concern”, leading to a higher complication rate.

More recently (2012-2013): Ozer & Chung\[^{139}\] cited the papers above from a decade or more earlier,\[^{135–137}\] as well as uncontrolled case series from 2003\[^{140}\] and 2007\[^{141}\] that showed SRS was safe and supportive. Dorozhkin’s review\[^{142}\] reported problems with SRS use, including a high rate of infectious complications, which led some to discontinue SRS for some specific uses.\[^{143}\] SRS had shown high infection rates in other human studies,\[^{144–146}\] as well as cement fragmentation\[^{145}\] and wound dehiscence.\[^{146}\]

A 2010 meta-analysis of calcium phosphate cements, like SRS, noted they were first introduced in ceramic form in 1992\[^{147}\]—-three years prior to The Times article and the associated paper.\[^{134}\] The “paste form” became available in the “early 1990s”, again before these publications. It also notes that Norian is one of the three most popular types of cement, but cautions, “numerous authors have reported quite variable short- and long-term results”, and “recent studies have questioned their long-term results”. Their analysis reported complication rates of 13%, though they believe that addressing publication bias and incorporating longer-term follow-up of patients would increase that: increased patient follow-up terms revealed a complication rate of 31%, up from a shorter term 19%.\[^{148, 149}\] Overall, complication rates reported by scientific papers were up to 62%, and these complications could not only extend for many years after surgery, but they could also be serious: “The majority of these complications were major, necessitating a second operation or causing a failure of the reconstruction”.\[^{147}\]

One final point, further illustrating how human data indicated major issues and caveats with Norian SRS/XR (see \[^{150–153}\]). Norian was bought by Synthes in 1999, in turn acquired by Johnson & Johnson in 2012. At the time of Norian’s sale to Synthes, SRS had been approved
for use in the arm, and another version, CRS, for use in the skull. Ten years later, in 2009, Synthes was accused, by the U.S. attorney in Philadelphia, of “running illegal clinical trials—essentially, experimenting on humans”. They had mixed SRS with barium sulphate, in a new formulation known as XR, to facilitate visualisation on x-rays. Though XR had been approved by the US FDA in 2002, it had expressly not been approved for use in certain spinal surgeries, such as the treatment of vertebral compression fractures—a common consequence of osteoporosis. This was due to concerns over Norian cement leaking into blood vessels—numerous in the spine—which, it was known, could cause blood clotting with severe or lethal consequences.

Norian, however, wanted to begin using XR to treat this condition as they considered it would be lucrative, but the US FDA ordered them to proceed through the proper channels to test its safety, and obtain an Investigational Device Exemption, or IDE, to permit clinical trials. Such trials would involve many patients, and be lengthy and expensive. The company, instead, persuaded “a few sites” to perform 60-80 human procedures, and publish the results—a quicker and cheaper approach. Unfortunately, between 2002-2004, at least five people died undergoing these procedures, and more almost did so. This had taken place despite data highlighting its risk: small amounts of XR had caused human blood to form clots in test tubes, suggesting blood vessels in patients’ hearts or lungs could also be blocked. In addition, injection of XR into a pig’s vein had caused clots in its lungs that killed it within seconds. Yet, their unauthorised trials proceeded, and they failed to report the deaths and adverse events. Consequently, in 2009, the company pleaded guilty to dozens of charges of felonies and misdemeanours, was fined $23 million, and four of its executives were imprisoned.

It must be concluded, therefore, that even though SRS was approved for human use, the animal data did not predict many of the major complications of SRS use that were revealed by research with humans. While SRS remains in use, it is used with caution and only for particular purposes, and certain caveats must be borne in mind—all as a result of human studies.

12. Curing cancer by p53 gene therapy—Failed

Why Our Cells Must Perish—The Times, May 1 1995

The ‘p53’ gene is—among many normal cellular processes—inolved in apoptosis: the controlled, programmed death of cells, which often takes place when they are damaged, or when they become cancerous.

When normal apoptosis is suppressed, damaged cells can grow and multiply uncontrollably, leading to cancer. Because the p53 gene is intimately involved in one of the major pathways of apoptosis, if this gene is inactivated in any way, cancer can ensue. P53 is therefore one of a class of genes known as ‘tumour suppressors’, because their normal function suppresses tumour formation—and, as a corollary, their abnormal function can lead to tumour formation. p53 is the most commonly inactivated tumour suppressor gene in human cancer, with p53 mutations present in around half of human cancers.[154]

p53 has, therefore, been a target of gene therapy, in efforts to develop a cure for cancers involving abnormal p53 function. An article in The Times reported “Experiments carried out on animals are encouraging” with a view to p53 gene therapy specifically in lung cancer patients.

The associated paper discusses a safety evaluation of p53-gene delivery to cultured human lung cells and to mice, using an adenovirus vector.[155] Adenoviruses cause several infections in
humans, including colds, chest infections, and gastrointestinal infections. These tend to be mild to moderate in most people, though can be serious, especially in individuals with compromised immune systems and/or existing diseases.\textsuperscript{1156} The authors reported that the recombinant adenovirus-p53 efficiently infected human cells and produced p53 protein, and that mice who had the recombinant virus injected into their tracheas showed a “relatively low degree of acute toxicity”, that none of the injections proved to be lethal, and that the p53 gene was successfully expressed and p53 protein produced. It is worth noting that what the researchers inferred as low toxicity comprised “shortness of breath”, “cough”, “burning sensation in the chest”, and “Symptoms which chronic pneumonitis may lead to: fatigue, weight loss, exercise intolerance, cyanosis and finger clubbing.”

Four years later (1999) a review by the same principal investigator noted that restoring p53 function \textit{in vitro} and \textit{in vivo} could induce apoptosis, and that Phase 1 clinical trials had shown p53 gene therapy to be safe and feasible, as well as inducing tumour regression in patients with non-small-cell lung cancer (NSCLC).\textsuperscript{1157} NSCLC was deemed to be a logical therapeutic target because it often is associated with mutant p53; it is one of the leading causes of cancer deaths in the USA; survival when detected at a late stage is very short; and standard therapies were not very effective. Studies in human tumours \textit{in vitro}, as well as in mice, showed therapeutic effects. The authors cited their own study (the paper associated with the media article, discussed above\textsuperscript{1155}), as well as their 1996 publication reporting their initial human study of NSCLC patients.\textsuperscript{1158} This was “promising” enough to lead to Phase 2 trials in NSCLC, as well as other, patients.

This promise continued to be expressed a decade later. A 2004 review of gene therapy for NSCLC, while highlighting important gaps in knowledge and in technology, conveyed optimism that this approach would eventually be successful, based on clinical trial data demonstrating that p53 gene therapy increased sensitivity to established radiotherapy and chemotherapy interventions.\textsuperscript{1159} Similar p53-enhanced sensitivity to other anti-cancer agents was also shown in cultured human cells,\textsuperscript{1160} in a 2006 paper that noted adenovirus delivery of p53 continued to be investigated in several types of human tumours, including NSCLC,\textsuperscript{1161} ovarian cancer,\textsuperscript{1162} and breast cancer.\textsuperscript{1163} With specific regard to NSCLC, a 2005 review noted seven clinical trials (five Phase 1, two Phase 2) of p53 gene therapy, all but one involving recombinant adenovirus. Results had been mixed, with varying numbers of tumour regression and stable disease outcomes, though the authors noted effects on tumour metastases had been “limited”.\textsuperscript{1164} Another, 2004, review noted that various strategies for gene therapy for lung cancer had been trialled, but problems with the delivery of the therapeutic constructs had been experienced, leading to disappointing results.\textsuperscript{1165}

In 2013, a review noted that development of adenovirus-p53 anti-cancer therapy had continued at pace.\textsuperscript{1166} Clinical studies showed it to be well tolerated via different methods of administration, as therapies on their own or in combination with other therapies, and for different types of cancers, including many for NSCLC. Multiple Phase 1, Phase 1/2, and Phase 2 clinical studies specific to NSCLC with different types of adenovirus vectors were reported, with similar encouraging outcomes, in which “some patients demonstrated tumour regression” and the most common adverse events were only transient fevers.

One issue is that the main vectors tested have been ‘replication deficient’ adenovirus vectors, as these are believed to be safer than non-replication deficient viruses.\textsuperscript{1167} However, these vectors have drawbacks: they may have a lower rate of introducing the p53 gene into tumour cells, and lead to a lower level of p53 expression, which appears to have limited the anti-tumour efficacy of this type of therapy. Therefore, some believe that new, tumour-specific, replication-competent vectors must be developed and tested in order to improve outcomes.\textsuperscript{1166} A 2017 review discussing the use of these replication-competent (‘oncolytic’) adenoviruses noted “the therapeutic effects of p53 gene therapy have been limited”, and “no such therapeutic approach
has been approved in the United States”,[168] despite 31 clinical trials, 11 of which were for lung cancer. This review cited two Phase 4 clinical trials as “ongoing” (neither for lung cancer: NCT00902083 and NCT00902122), but the clinicaltrials.gov database notes that both trials are past their completion date of 2012, and that neither has been updated or verified since then. The authors also highlight that these failures may be due to the nature of the vectors used. The failure of p53-directed therapies for cancer is not limited to gene therapy, however. A 2018 review noted “there is still no effective p53-targeted therapy available in the clinical setting to date”[169] and, while many authors continue to speculate of promise and eventual success, some admit that most p53 drugs are “still at early stages of development and many of them are progressing slowly toward clinical implementation”, while acknowledging how many potential drugs targeting mutant p53 have failed.[169]

One important caveat to the lack of approval of any p53 gene therapy in the USA, Europe and almost worldwide, is that a recombinant adenovirus-p53 gene therapy has been in clinical use for some time, albeit approved only in China, at least to date. This therapy—Gendicine—was approved by the Chinese FDA in 2003 for the treatment of head and neck squamous cell carcinoma, to be used in combination with radiotherapy (HNSCC).[170] Between this approval in 2003 and a review of Gendicine in 2015, 16 clinical trials involving it were conducted, all in China, in patients with various cancers, including for NSCLC.[171, 172, 173] Notably, in the 15-16 years since Gendicine’s approval in China, neither it, nor any other similar type of recombinant adenovirus-p53 gene therapy, has been approved anywhere else in the world.[174] Perhaps one of the most high-profile alternatives—Advexin—“failed to file” with the US FDA in 2007/8, with the company responsible—Introgen—bankrupt just one year later, accused of “repeatedly misleading investors” about its results and promise.[175] A 2017 review, specifically discussing adenovirus-p53 gene therapy for cancer, notes that “no active clinical trials can currently be found in databases suggesting severe problems associated with this strategy”.[176] The authors echo opinions elsewhere, that various issues associated with previous approaches may be overcome via the use of newly engineered viruses and/or the use of CRISPR/Cas9 genetic-engineering technology. Other researchers have opined that there have been “a multitude of challenges”, including the possibility that even if p53 is successfully and sufficiently reinstated in p53-mutant cells, the mutant p53 protein therein may negate its effects.[177] Nevertheless, a 2018 Opinion Paper proposes a new system based on CRISPR/Cas9, in which, instead of introducing a new p53 gene into cancerous cells, CRISPR would replace the mutant p53 locus in host-cell DNA[178]—although the authors admit the concept needs careful validation.

In summary: p53 gene therapy has seen very limited success to date, on the basis of the approval of the drug Gendicine in China in 2003. It must be of concern, however, that the 15-16 years since this approval has not seen any further approval repeated elsewhere in the world; that there are many and varied acknowledged issues with p53 gene therapy, especially for lung cancer, which many authors believe need to be overcome for success in this area; that these issues have been reflected in the failure of many clinical trials of p53 therapies, both for gene therapy and other therapeutic areas; and that the field has moved on, for example, developing different viral vectors, delivery with porous microparticles,[179] etc. This breakthrough, therefore, could only be considered a partial success at best, but with regard to the lung cancer mentioned in the media article, it remains, at least for now, a failure.

As summarised in 2015 review,[173] this is underpinned by human research: p53 gene therapy was first shown to be feasible in 1993 in human cells in vitro,[180] and the first recombinant adenovirus-p53 introduced p53 into four different human cell lines, showing “promising tumorigenicity inhibiting effects” in 1994[181]—both prior to the media article and associated paper in 1995. While mouse studies were also conducted prior to the first human trial, reported
in 1996, which involved patients with NSCLC,[158] given the amount of human-specific work in vitro and in vivo that preceded it, as well as the known and acknowledged failure of preclinical trials to correctly predict human outcomes, it must be concluded that any successes of this technique, and all caveats associated with it, are a result of human research and data.

13 and 15. Injection to treat obesity/“end the need to slim or diet”—Failed

Anti-obesity jab may end need to diet or exercise—The Times, May 16 1995
Can this injection get rid of your fat forever—Daily Mail, May 23 1995

Based on “a decade of research into antibodies that destroy fat cells in pigs and sheep” (as well as mice, rats, chickens and rabbits[182]), scientists hoped to conduct human trials of an injection for obesity within 2-3 years, and to have the injection available soon after, to “end the need to slim or diet”.

The injection, described as “essentially biological liposuction”, took two forms. One takes human fat cells (adipocytes), and injects them into a mouse. The mouse’s immune system raises antibodies to those cells, which are harvested, cultured, and injected back into an obese person with the aim of destroying adipocytes. The researchers planned to humanise the mouse antibodies, to avoid them raising an immune response when injected into humans—something the scientists involved viewed as “quite a straightforward process these days.”

The other form involved screening human fat tissue for existing antibodies to fat cells. People have antibodies to many if not all of their tissues and organs, though these are normally ‘turned off’. The researchers planned to identify these, and ‘turn them back on’ to attack fat cells.

This idea had been mooted by one of the quoted scientists a few years earlier (1992),[183] prompted by a ban of the use of hormones to regulate adiposity in animals farmed for food. Notably, the author cautioned that immunisation for the same purpose involved immune responses that were “typically difficult to evoke and virtually impossible to regulate.” The year following the Times article (1996), he published an article with the same title, discussing the same issue, describing experiments in various species in which antibodies were tested for their fat-removing properties.[184] The effects differed between species. Indeed, specific concerns were noted regarding human applicability, including antibody specificity, and adverse effects including inflammatory issues and locomotor function. Two years on (1998), he reported the successful treatment of obese rats with anti-adipocyte antibodies “with minimal side effects”,[182] and the following year (1999) opined optimism that this type of intervention could reach clinical use, while cautioning “the requirements for such antibodies in terms of specificity and toxicity will be much more demanding and this remains the biggest obstacle to development of cytotoxic antibodies to adipocytes for the control of obesity”.[185]

More recent papers show this caution was well founded. A 2012 review noted, “Drugs that target pathways in metabolic tissues, such as adipocytes...have shown potential in preclinical studies, but none has yet reached clinical development”.[186] A 2018 review listed six FDA-approved anti-obesity medications, noting “Most of these drugs work through CNS pathways that either reduce appetite or enhance satiety, with the exception of orlistat, which decreases the absorption of fat”. In other words, none of these is an antibody targeting the destruction of adipocytes, as reported in the original article. Further, the same authors reviewed new anti-obesity drugs “on the horizon” that did not contain any mention of this type (antibody-targeting
of adipocytes) of approach.[188] Another (2017) review notes “Several anti-obesity drugs have been approved in the USA, European Union, Australia, and Japan including sympathomimetics, pancreatic lipase inhibitors, GABA_\text{A} receptor activators, a serotonin 2C receptor agonist, opioid antagonist, dopamine-norepinephrine reuptake inhibitor, and glucagon-like peptide-1 (GLP-1) receptor agonists”—i.e. no drug of the type in the *Times* article.[189]

Part of the reason for the low number of approved anti-obesity drugs, for “troubling safety concerns” with at least three of them,[190] and possibly also for the failure of antibody-mediated adipocyte destruction, may be “limits in translating preclinical data to human studies”. [190] These translational difficulties are also evidenced by drug failures: fenfluramine, dexfenfluramine and sibutramine were withdrawn due to cardiovascular issues; rimonabant because of serious psychiatric problems;[191] and others cite high attrition rates.[192]

Targeting adiposity with antibodies is not entirely abandoned, though it has taken a different path. A 2017 paper reported antibody-based targeting of Follicle-Stimulating Hormone (FSH) (i.e. adipocytes are not directly targeted), building on previous work to elucidate hormonal regulation of bone mass and possible treatments for osteoporosis.[193] One serendipitous observation of related experiments in mice showed that blocking FSH using antibodies didn’t only result in increased bone mass, but also in decreased adiposity—and the latter now appears to be the main focus of this research. One researcher cautioned, however, “whether it works in humans is another matter”.[194]

Just one week after *The Times* article (Breakthrough #13), the *Daily Mail* carried a similar report. Though the investigators are different, the two articles and breakthroughs are the same. The *Daily Mail* article was even more optimistic of human success, claiming “Stubborn fat and unsightly bulges could vanish overnight, according to British researchers who say they have found the ultimate weapon for fighting the flab”; “In the not-too-distant future, a swift and painless injection could end obesity for good…the treatment would cost no more, and possibly considerably less, than liposuction…these fat-destroying antibodies might end up in the beauty creams of the future”; “There seems no doubt that this new technology will appear: the only question is when”; and “Slim hips on demand are at least five years away”, and “The fact that it has been seen to work in several animal species shows that it is very likely to work in humans, too.”

Nevertheless, Springer Nature’s ‘Adis Insight’ platform noted that pre-clinical development of this therapy for obesity was discontinued in 1998-99, and ‘discontinued’ generally in 1999.

14. Cure for cancer (solid tumours)—Failed

Trials begin on pill that could control cancer—*The Independent*, May 19 1995

*The Independent* reported the “exciting potential” of an anti-cancer drug, BB-2516 (Marimastat).

Preliminary, early-stage clinical trials were ongoing in the UK and the US, involving ten patients with colon, ovarian, prostate or pancreatic cancer. The drug was intended to treat “patients with secondary cancers for whom no standard treatment is available”; and clinical trials were based on successful mouse data, as well as from 30 human volunteers.
BB-2516/Marimastat blocked matrix metalloproteinases (MMPs), which are over-produced in solid tumours, aiming to prevent metastasis and angiogenesis. The developers (British Biotech, UK) were optimistic, though cautioned, “Until we complete these preliminary human trials, we can't say anything positive”, “If you can take a pill every day for the rest of your life that keeps the cancer under control, it will transform people's expectations of cancer,” and, “It is very early days, but it is exciting.”

Initially, optimism continued. One year later (1996), Nature Biotechnology reported that encouraging Phase 2 clinical trials had made British Biotech one of the four most valuable biotechnology companies in the world; the chief executive opined that “The side effects [joint and muscle pain] are benign, easily recognised, and manageable”; and various market analysts were confident, claiming probability of it becoming a major cancer drug from 50% to “definitely”. [196]

Just a few years later, however, (1998-99), British Biotech’s dismissed Head of Clinical Research alleged the company was guilty of “extreme and unfounded optimism”, and a UK House of Commons Select Committee concluded the company had wilfully misled the public about marimastat’s progress. [197, 198] This was borne out by scientific papers and clinical trials results. In 2001, a clinical trial for pancreatic cancer showed Marimastat to be no better than an established chemotherapy drug, gemcitabine. [199] In 2003, a ‘Drugs in R&D’ article stated, “Marimastat has been in pivotal phase 3 trials in glioblastoma, breast, ovarian and small and non-small cell lung cancer, but these trials have all been discontinued because marimastat failed to show superior efficacy over either standard chemotherapy or placebo. Altogether, seven Phase 3 studies have failed to meet their primary end-points”. [200] Marimastat (also known as TA-2516 or TA 2516) failed to prolong progression-free survival in patients with metastatic breast cancer in Phase 3 clinical trials, and caused musculoskeletal toxicity at higher doses, which led to inferior survival. [201]

In 2007, it was reported that two Phase 3 trials for small cell lung cancer failed to show a beneficial effect over placebo, in which 18% of patients experienced severe musculoskeletal pain that led to significantly worse quality of life. Other Phase 3 trials for advanced ovarian cancer, inoperable colorectal cancer liver metastases, glioblastoma multiforme and gliosarcoma, and metastatic breast cancer (mentioned above) showed no difference over placebo for survival. They did describe a Phase 3 study for advanced gastric cancer, which suggested it could be useful as a maintenance treatment following chemotherapy, [202] but this was not realised. In 2008, a book on anti-cancer drugs noted, “Marimastat is orally active and it has also undergone several Phase 3 assays, showing poor performance. Results from clinical trials with this first generation of MMP inhibitors [the class of drugs of which marimastat is one] have been disappointing and have led many investigators to conclude that MMPs are not suitable targets for the treatment of human cancer”. [203]

More recently (2013), a review of novel anti-metastatic agents stated, “Unfortunately, these drugs [marimastat and related compounds] produced serious adverse effects that led to the premature termination of their development”, and “the disappointing results of MMPIs (e.g. marimastat, prinomastat, tanomastat) in clinical trials have forced a re-evaluation of MMP inhibition strategies”. [204] Reasons for these failures included that they interrupted beneficial processes mediated by MMPs, leading to long-term side-effects; interfered with other important cellular events with similar consequences; and that they could even promote cancer progression. [204] In 2014, a chapter on lung cancer in clinical oncology book stated, “treatment
with marimastat after induction therapy for SCLC [small cell lung cancer] did not improve survival and imparted a negative effect on quality of life\textsuperscript{205}

Despite all the hype and PR, based on “encouraging trials in animals”, the drug was a complete human failure, suffering from both lack of efficacy and serious side effects.

\textbf{16a. Cannabis to treat chronic pain—Inconclusive}

Try a little flower power; Long dismissed as unscientific, plants are making a pharmaceutical comeback, says Roger Dobson—\textit{The Independent}, May 23 1995

This article discussed research into natural compounds in plants, and their potential to be sources of new medicines, for “HIV, Aids, cancers, dementia, heart disease, liver disorders, chronic pain and leukaemia”, as well as morning sickness and asthma.

One of the two main focuses was the treatment of patients with chronic nerve pain at a London hospital, using cannabis—“the first time that cannabis has been used in the NHS for 40 years”—but also cited animal experiments showing that cannabis “is 357 times more effective than aspirin in controlling pain in a peripheral nerve damage as a result of viral infections.” The treatments were part of a clinical trial, in which the patients could no longer proceed with conventional analgesics due to side effects.

The researchers involved published a report of pain relief two years later (1997), showing that administration of tertahydrocannabinol (THC, the principal psychoactive constituent of cannabis) led to “a highly significant reduction in additional analgesic requirements” for chronic inflammatory pain of gastrointestinal origin.\textsuperscript{206}

What happened subsequently is complex and contradictory, as efforts tried to demonstrate whether cannabis/cannabis-related medicines could have significant pain-relieving effects in humans.

Four years later, a 1999 report by the US Institute of Medicine concluded that cannabis was an effective treatment for pain, among other indications.\textsuperscript{207} Just a year later (2000), however, a review of “cannabinoids in clinical practice” cautioned that human reports with regard to analgesia had been conflicting, even reporting increased pain sensitivity. All its references were published shortly after, or long prior to, the 1995 media article.\textsuperscript{208} The following year (2001), another review of cannabinoids and pain relief noted that cannabinoids have been “used across the world for thousands of years”, and that science was currently working on the molecular mechanisms of, rather than establishing, those effects.\textsuperscript{209} It also stated that “plant-derived and synthetic cannabinoids are available for therapeutic use”, that “small clinical trials have demonstrated analgesic potential in acute and chronic pain”, and that related pain research in humans had been hindered by regulatory issues. It referred to papers published just 2-3 years following the media ‘breakthrough’ report in 1995, describing the use of cannabis “in the community” to treat chronic pain,\textsuperscript{210} as well as that synthetic cannabinoids such as THC and nabilone (licensed as antiemetics or appetite stimulants) had been demonstrated in clinical trials to provide pain relief.\textsuperscript{207, 211, 212}

A systematic review of nine clinical trials (2001) added to the controversy, concluding that cannabinoids were no more effective than codeine in controlling pain, and that depressant
effects on the central nervous system meant clinical use was “undesirable”.\[213\] Regarding neuropathic pain, it concluded that further studies were needed, though, in contrast to nociceptive pain, there were “suggestions of efficacy”. However, several comments on this article were highly critical. One noted that this review was neither systematic nor qualitative, was based on two “clinically questionable synthetic cannabinoids as well as oral THC, provided no consideration of the synergistic effects of herbal cannabis, and only broadly addressed their effects on general pain.\[214\]

In 2004, a randomised controlled trial showed statistically significant improvements by cannabis extracts for neuropathic pain and quality of life, with only mild to moderate adverse events, which resolved.\[215\] A 2005 review noted “anecdotal reports” of cannabis use for neuropathy, and that that cannabis improved, among other symptoms, muscle pain, nerve pain, and paraesthesia in HIV/AIDS patients.\[216\] It continued, “There have been several anecdotal and clinical trial reports that cannabis plant extract and synthetic THC and related analogues produce pain relief in humans”, the references for which predated the media-reported animal ‘breakthrough’ (1975-1981), and another almost contemporary in 1997.\[206\]

One investigator from the media article published a clinical study in 2006, concluding that an oral cannabis extract known as Cannador provided dose-related pain relief for post-operative patients, without frequent adverse effects.\[220\] He cited ten clinical trials conducted from 1997-2004, six of which were for neuropathic pain. The following year (2007), a quantitative systematic review of the effects of cannabis-based treatment on neuropathic pain concluded they were effective.\[221\]

In 2009, a meta-analysis of 18 clinical trials concluded that cannabis is moderately efficacious for chronic pain, but that any beneficial effects may be offset by potentially serious harms, while a subsequent (2013), double-blind, placebo-controlled, crossover study determined that vaporized cannabis significantly improved neuropathic pain.\[223\]

More recently, a 2016 systematic review of randomized controlled studies of cannabinoids for chronic neuropathic pain concluded, overall, that cannabinoids were “marginally superior to placebo” in terms of efficacy; and a 2017 review noted evidence that cannabis reduces neuropathic pain in advanced cancer patients, but that clinical trials of greater scale and quality were needed to establish this.\[225\]

Recent (2018) reviews of cannabis and cannabinoids discussed many issues of their use. While it is reported that the European Medicines Agency (EMA) and the US FDA have not approved the use of herbal cannabis or its extracts (NB: the EMA approved the cannabidiol-based Epidiolex to treat seizures in 2019), many countries have approved some cannabinoid-based drugs (including the UK), and several countries have authorised the medical use of herbal cannabis. Clinical applications included “multiple sclerosis to epilepsy, neuropathic pain, arthritis, nausea and vomiting due to chemotherapy, appetite stimulation in HIV/AIDS, depression, anxiety disorders, sleep disorders, psychosis, glaucoma, and Tourette syndrome”, with references spanning 2000-2014. As it stood at the time of writing, three cannabinoid medicines were marketed in several countries: Nabiximols (Sativex), a mixture of THC and cannabidiol (with small amounts of other compounds, including minor CBs, terpenoids, flavonoids and sterols), most commonly prescribed for multiple sclerosis(MS)-associated spasticity, though there remain claims of some efficacy for MS-associated neuropathic pain (http://sativex.co.uk); Nabilone (Cesamet/Canemes), for chemotherapy-associated nausea; and
Dronabinol (Marinol/Syndros), for anorexia associated with weight loss in AIDS patients, and persistent nausea due to chemotherapy.

Although Sativex/nabiximols failed to show efficacy in a small clinical trial for painful polyneuropathy in 2010,[229] smoked cannabis relieved neuropathic pain in two other studies in the late 2000s.[230, 231] Two Phase 2/Phase 3 studies revealed analgesic effects for pain related to cancer not been alleviated by opioids[232, 233] although a later (2018) report describing two Phase 3 randomised placebo-controlled studies had mixed results: it failed to demonstrate superiority to placebo in advanced cancer patients, but in the US alone it showed a statistically significant effect.[234]

Of interest in assessing the role of animal studies in predicting human responses to cannabis/cannabinoids, two recent papers reported important species differences: “Pre-clinical studies provide robust evidence of the opioid-sparing effect of cannabinoids, whereas [only] one of the nine clinical studies identified provided very-low-quality evidence of such an effect”. [235, 236]

A 2018 review concluded that (among other things), specifically for the treatment of chronic pain, including neuropathic pain, “there was conclusive or substantial evidence that Cannabis or cannabinoids are effective”,[237] based on five fair-to-good quality systematic reviews. Another, contemporary, systematic review, however, concluded there was “lack of good evidence that any cannabis-derived product works for any chronic neuropathic pain”, and that no studies contained “high quality” evidence. While it noted that all cannabis-based medicines were better than placebo for pain relief, it also concluded “The potential benefits of cannabis-based medicine (herbal cannabis, plant-derived or synthetic THC, THC/CBD oromucosal spray) in chronic neuropathic pain might be outweighed by their potential harms”.[238]

To conclude: despite cannabis-based medications being licensed in some countries, the evidence supporting their use for the relief of neuropathic pain at the very least, is mixed and contradictory. For this reason alone, this ‘breakthrough’ cannot be considered a success. Even if it had been successful, however, the impact of the cited animal studies must be doubtful. There is extensive literature of cannabis use in humans, going back many years, both recreationally and medically. For instance: “Humans have utilized cannabis products in various forms throughout recorded history”, including examples of its use for the relief of pain in ancient Egypt, and specifically neuropathic pain in the 9th century, and in the mid 19th century in the UK.[239] Other reviews noted the human use of cannabis for thousands of years;[208, 213, 240, 241] its use medically, not just in ancient times or, for example, in the 13th century, but also in 19th and early 20th centuries, including for “a myriad of painful conditions”,[208, 214, 216, 240] and that it has been able to be prescribed by UK doctors since 1971.[213] It was described in 2003 as “the third most commonly used drug after tobacco and alcohol”, with “an estimated 3 million frequent users in the UK alone”[240]—something that has not changed much.[242]

The plethora of human data and availability of people for study is augmented by significant non-animal research. THC was first isolated and characterised back in 1964, and at least 90 cannabinoids have been isolated since the 1940s.[226] Any claimed value of animal studies is also questioned by acknowledged species differences regarding analgesic efficacy,[235, 236] and it is human studies that have highlighted and informed ADRs and the cost/benefit ratio of cannabis/cannabinoid use.
Try a little flower power; Long dismissed as unscientific, plants are making a pharmaceutical comeback, says Roger Dobson—The Independent, May 23 1995

The Brain’s Messenger—The Times, June 12 1995

Drug Hopes Rest on a Host of Daffodils; A Bulb Extract May Alleviate Alzheimer’s - and Boost East Anglia Growers—The Independent, September 3 1995

In the same article on the medicinal value of plant-based compounds, which reported the pain-relieving effects of cannabis (see #16a), another salient claim was that an extract of daffodil and snowdrop bulbs could slow the progress of AD. Such was its promise, an Edinburgh pharmaceutical company aimed to extract this compound (galanthamine, also known as galantamine), from up to 20,000 tonnes of bulbs annually.

Another article in The Times reported that brain-damaged rats, manipulated to be deficient in the neurotransmitter acetylcholine (ACh) in their brains, showed a slower rate of learning to navigate their way through water mazes to find submerged platforms. This is consistent with poorer memory in AD patients, whose brains also show a deficiency in ACh, though there remains, after decades of research, controversy over whether this is cause or effect. The researchers discovered that rats who had been genetically modified (GM) with cells that replaced the lost acetylcholine could navigate their way through water mazes better than those who had not: ACh replacement appeared to restore memory function and learning deficits previously caused by its loss. It was hoped that this would lead to drugs designed to halt the decline of ACh associated with AD. However, a drug to do this was already in clinical trials (galanthamine, extracted from daffodil bulbs), and another—Tacrine—had already been licensed in some countries. A subsequent article in The Independent focussed mainly on the growing of the bulbs and the potential boost to the local economy, but also briefly discussed the progression of galanthamine into clinical trials, which had reached Phase 3, involving 560 patients across Europe.

The paper described how rats had their brains damaged at the nucleus basalis magnocellularis (NBM), via direct injections of ibotenic acid, causing “permanently and selectively damaged learning and memory”.[243] The ‘ACh-replaced’ rats had GM cells grafted/infused into their brains, and four weeks later were killed for analysis by decapitation. The need for this harmful study is open to question, given the weight of evidence implicating the cholinergic system in memory and learning. The authors themselves cited previous research that did this, including “lesions of cholinergic nuclei, pharmacological manipulations of cholinergic systems, intracerebral transplantation of fetal [sic] tissue, and anatomical changes in cholinergic pathways during ageing”. These included not just rat experiments, but also research using necropsy brain tissue from ‘normal’ individuals and dementia patients, post mortem studies of humans, and experiments involving blocking of cholinergic mechanisms in people (e.g.[244–246]). The stated value of their study was that it “had not been proved that regional acetylcholine is causally required for learning and memory”. Further, drugs to address this issue were already in use and in clinical development, so in no way could have depended on these particular animal experiments. This was tacitly acknowledged by the authors: “Our results are consistent with previous observations that transplants of cholinergic-rich fetal [sic] tissue in the neocortex reverse deficits in learning and memory following excitotoxic lesions of the NBM”.

16b, 19 and 30. Treatment for Alzheimer’s Disease with Extract of Daffodils (Galanthamine)—Partly successful, with caveats
It is worth examining the development of galanthamine—the drug in clinical trials cited in the 1995 paper—in particular for the contribution (or lack of) of animal experiments to its discovery and progress to the clinic. Also known as Reminyl, it is isolated from various bulbs of the Amaryllidaceae family, such as daffodils and snowdrops, and was discovered accidentally in the early 1950s, and used for various purposes since then, including nerve pain, polio, and in anaesthesiology.\textsuperscript{247} This 2006 review citing the above also noted it had been extensively investigated in humans, showing memory enhancement properties, though with some adverse effects; and that derivatives were being sought and tested to overcome these effects.

There was extensive human research preceding the 1995 ‘breakthrough’. An 8-week pilot study in dementia patients reported promising results in 1989,\textsuperscript{248} and a 1990 paper described the “long-term administration of galanthamine hydrobromide, a cholinesterase inhibitor, in Alzheimer’s disease”, which showed clinical improvement, albeit at high doses.\textsuperscript{249} This also examined the drug’s tolerance, its effects in a healthy volunteer, and pharmacodynamic and pharmacokinetic effects. A contemporary review of AD therapeutics (1996) cited the administration of galanthamine to AD patients in papers published 1989-1994—all prior to the claimed rat-based ‘breakthrough’.\textsuperscript{250}

A 1997 review also outlined the historic use of galanthamine to treat various painful ailments, but lamented that much related “seminal research” was conducted just after the second world war and is hard to obtain.\textsuperscript{251} Nonetheless, it noted significant human use during the early 1970s to early 1990s (again, preceding the ‘breakthrough’), and cites human pharmacokinetic and other data from that period. Notably, it stated the drug was first suggested for AD treatment in 1986 (possibly earlier), and that various clinical studies were conducted from 1987 to 1996.

A 2004 review of the development of galanthamine showed it had been used for many years in Eastern Europe, prior to its pre-clinical testing in Western Europe in the 1980s.\textsuperscript{252} In the 1950s, it was used to ease nerve pain, and to treat polio; pre-clinical experiments continued throughout the 1980s, but some salient research involved \textit{ex vivo} muscles from frogs, leeches and rabbits, rather than experiments involving live animals, to investigate its inhibitory properties for acetylcholinesterase; clinical development progressed throughout the 1990s; and it was first licensed for AD treatment in 2000 in Iceland, Ireland, Sweden and the UK, followed by the US and other countries in Europe and Asia in the early 2000s.

There have been significant (and confounding) issues, however. While multi-centre randomised trials showed it to be well tolerated and to improve cognitive function in AD patients,\textsuperscript{253, 254} two large clinical trials reported in 2005 did not show a significant difference from the effects of the placebo, with regard to rate of progression of AD over a two-year period.\textsuperscript{255} One 2018 review noted that clinical trials were “still ongoing”.\textsuperscript{256} Other major caveats, including with other cholinesterase inhibitors, donepezil and rivastigmine, included that they are effective for a maximum of about three years, and also that they treated only AD symptoms, not the disease itself.\textsuperscript{257} Other caveats are still being reported: galanthamine treatment is “still saddled with numerous side-effects”.\textsuperscript{258}

In summary: there was substantial, significant weight of evidence of the role and ACh in AD prior to the 1995 rat experiments; much of it was human specific; and much of this was acknowledged by the authors themselves. Drugs targeting this pathway were already in clinical development, and galanthamine development can’t be based on animal research—and certainly not on this particular research—due to the extensive human data relating to it, which go back
hundreds of years, and which include detailed pharmacodynamic and pharmacokinetic data preceding 1995. Human trials are still ongoing, and it is they that will clarify issues regarding safety and efficacy.

18. Sugar on the tongue can ease pain (in place of pain-relieving drugs) in young babies—Partly successful, with caveats

Sugar ‘Eases Pain in babies’—The Independent, June 9 1995

Scientists argued that placing concentrated sugar solution on babies’ tongues could replace the need for pharmaceutical analgesics, used to relieve pain associated with perinatal procedures such as blood draws, injections, heel pricks etc.

In a trial, 30 new-born babies were given sugar syrup prior to a routine blood test involving a heel prick. The babies cried for a shorter period of time compared to others who were given only water, and stronger sugar solutions had a greater effect.[259] However, the newspaper article stated “this is the first time it has been shown in humans”, and that the research was based on previous experiments on new-born rats.

This was echoed in the scientific paper in the BMJ. The authors noted that, even though “most of the anatomical pathways and neurotransmitter function necessary for pain perception are fully or nearly fully developed in the neonatal period” (citing papers from the late 1980s), “many people are still reluctant to believe that pain felt by neonates may be as severe as that felt by older children or adults.” Support for their study was provided in the form of cited research using rats in the 1980s: one showed that rats could stand on a hotplate for longer after being fed sucrose solution,[260] while similar effects had been seen with other, non-sugar sweet substances.

Many (though not all) subsequent human studies and systematic reviews found a statistically significant effect (e.g.[261–263]). A 2010 systematic review showed a moderate analgesic effect of oral sucrose solution prior to immunisation, citing publications recommending oral sucrose administration to new-borns for pain relief from 2001.[264] A 2016 Cochrane review examined 74 studies involving more than 7,000 infants, concluding orally administered sucrose was an effective analgesic for new-borns, for single painful events.[265]

However: the 2016 Cochrane review, while supporting the efficacy of sucrose for some procedures, found that others were not affected, and that evidence for other minor painful procedures was “conflicting” and needed additional investigation. Despite numerous human studies, they could not determine an optimal dose (in common with other studies, e.g.[266]), and recommended further investigation for repeated doses. Clinical nursing guidelines from 2018 also supported oral sucrose for single, minor procedures, but noted that breast milk was as effective as sucrose solution, and that breast feeding, which includes maternal contact, should be used in preference, along with other interventions such as “kangaroo care, facilitated tucking, swaddling, warmth, non-nutritional sucking and distraction”.[267] Other publications supported the equivalent efficacy of these alternatives.[268] A primary health care information officer opined, “before drug manufacturers seize a new opportunity to market prepacked phials of sterile sucrose solution, complete with dropper”—they should ask “whether simply cuddling a crying infant after a heel prick is as effective in reducing crying as 50% sucrose”. [269]
Finally, the prior weight of evidence must be considered. While the newspaper article may have been prompted by the 1995 BMJ study of babies given heel pricks,[259] this was not “the first time it has been shown in humans”. Similar findings were four years previously (1991),[262] by the same researcher who, in the 1980s, published similar experiments on rats, measuring the effects of sucrose on how much heat they could stand applied to their feet, and its effect on “distress vocalisations” (e.g.[260,270]). But these rat experiments cannot have been crucial in the development of sucrose-based analgesia for infants (which, to repeat, is not superior to the effect of cuddles, skin-to-skin contact and breast feeding). One review recorded (among other things) that, “numerous historical references pertaining to the analgesic benefits of sweet substances dating back to 632 AD”; sugar solutions, alone or in combination with other substances, had been used for infant analgesia in the late 1840s and early 1900s; sugar solution was recommended to calm infants during surgical procedures, and that the underlying mechanism (involving orally mediated release of endogenous opioids) was proposed in 1994—prior to the ‘breakthrough’ report.[271]

20. Discovery of genetic mutations that result in life extension for nematode worms, touted as a lead in anti-ageing interventions for humans—Failed

Clue to Long Life Unearthed—Daily Mail, June 19 1995

This article reported the discovery of a mutation in Caenorhabditis elegans (C. elegans) which, the authors believed, “could hold the key to prolonging human life”.

Worms carrying this mutation, in the age-1 gene, lived up to 65% longer than worms without it. One author of the associated paper opined, “I hope we will be able to use this knowledge to postpone the diseases of old age”, alongside optimism from “experts on cancer and Alzheimer’s disease”.[272] However, seven years prior to this (in 1988), a paper was published reporting another mutation in the C. elegans age-1 gene that significantly increased life span.[273]

Reviews in recent years show that the pathway in which the human homologue of the age-1 gene (PI3K) is involved, is one of the most intensely researched in this field.[274] However, many publications note the poor translation of animal data to humans in this field, and accept that, in spite of substantial research and the creation of vast amounts of knowledge, “the cause of ageing remains unclear”. Moreover, this is in spite of (or perhaps, partly due to) the fact that around one thousand life-extending genes had been discovered in research involving “yeast, worms, flies and mice” by 2013;[276] a number that will have undoubtedly grown further in the past few years. One of the theories of ageing that receives the most support is known as the free radical theory of ageing (FRTA), in which reactive oxygen species created by normal metabolic processes cause cumulative damage, and therefore dysfunction and ageing.[277] Notably, the age-1 mutations in the 1995 C. elegans paper were found to also confer resistance to increasing levels of reactive oxygen species; however, “accumulating evidence indicates that oxidative damage can be experimentally dissociated from lifespan…increasing oxidative damage does not necessarily decrease lifespan…and that having increased oxidative damage is compatible with long life” (see[275]). Another review cautioned that, while an “intake of exogenous antioxidants can reduce the progression of ageing-related pathologies such as Parkinson disease, amyotrophic lateral sclerosis and heart failure” in animals, “Applying that idea to humans, however, appeared rather problematic. Most clinical trials failed to show any
clinical benefits of antioxidant therapy for aging-related disorders such as cardiovascular disease, stroke, and type 2 diabetes” (see[278]).

A 2017 review noted that many different ageing mechanisms had been implicated over time, including “dysfunctions of mitochondria, impaired proteostasis and stem cell function and maintenance, deregulated sensing of cell energy status and growth pathways, cellular senescence, age-related decrease in stress resistance, as well as oxidative and inflammatory stress”.[279] It listed many substances that had extended lifespan in animals by up to 25-30%, but cautioned “there is only limited evidence to demonstrate overall health benefits of using such substances so far. Findings from epidemiological studies reporting the long-term health impacts of these agents are rather inconsistent. Moreover, evidence from several studies indicates that uncontrolled consumption of some medications considered as potential anti-aging drugs may be useless or even detrimental.” A contemporary review noted that 212 compounds had shown anti-ageing activity in C. elegans, involving many biological mechanisms/pathways. Of more than one hundred “natural” substances, just five regulated the IIS pathway, implicated in the 1995 ‘breakthrough’ paper.[280]

A 2017 report “The business of anti-ageing science” also highlighted concerns over extrapolating anti-ageing data from animals to humans, under the heading, “Humans are not huge worms or big mice”. [281] It illustrated how more than 2,000 genes have been associated with longevity in nonhuman organisms.[276] but only seven genes in humans—leading to the conclusion that, in spite of huge amounts of research and publications, animals’ lives and money, “our understanding of the genetic basis of human longevity remains largely unknown” [emphasis added], as well as, “Therefore, it is plausible that most findings from short-lived model organisms will not be relevant to human beings. Briefly, not only may the pathways necessary to extend lifespan in model systems be often irrelevant to the comparatively long-lived human species, but to make matters worse, studies in model systems are mostly performed on genetically homogeneous laboratory strains that may not be representative of human populations”. [282] Furthermore, over 400 substances are listed in the ‘DrugAge’ database that can increase longevity in model organisms, yet, at the time of writing, only one had entered clinical trials (metformin—see below).[283]

One further issue with the type of finding described in the 1995 paper was discussed in a 2016 review, which considered not only increasing lifespan, but that healthspan—increasing the proportion of life in which humans are healthy—is as, if not even more, important.[284] It emphasised that studies of the type of the 1995 paper “typically focus on a single gene, which, when mutant, increases lifespan…raise more questions than answers”. They exemplify this with two reports of the same mutant gene (daf-2) in C. elegans that conclude it decreases, and increases, lifespan and healthspan respectively.[285, 286] This may be of concern with respect to the 1995 paper/media article on the age-1 gene, because another 1995 report claimed that, “These findings suggest that age-1 and daf-2 mutations do act in the same lifespan pathway and extend lifespan by triggering similar if not identical processes”. [287]

One anti-ageing drug in human trials is metformin. Used for the treatment of type 2 diabetes mellitus for more than 60 years, it has a very good safety record. Human studies have associated its use over time with lower rates of cancer and cardiovascular disease, less all-cause mortality and also lower cognitive decline, and it is currently, the subject of the “Metformin in Longevity Study (MILES—Clinicaltrials.gov identifier NCT02432287)”, and “TAME” (Targeting aging [sic] with metformin) clinical studies, involving around 3,000 elderly people.[288–290] Though metformin has been tested in animals, there is a substantial human evidence base, as well as in
vitro studies of its cellular effects, showing that it delays “a variety of age-related morbidities”. More recent human studies have shown that it significantly influences various metabolic and non-metabolic pathways linked to ageing, in muscle and fat tissue. If it is eventually approved, it “would be the first demonstration that a particular drug may delay the onset of various aging-associated human diseases.”

The value of ageing research with regard to human benefit has been widely questioned, in addition to the above caveats. A 2016 review stated, “Most of the reported life-extension mechanisms have been observed in simpler organisms and these have still to be demonstrated as viable anti-aging therapies in humans. Additionally, these do not curtail one of the hallmarks of aging, cognitive impairment… Though providing some key insights into longevity, invertebrates are, nevertheless, distant animal models and are likely unrepresentative of human biology and physiology… studies carried out in animal models may contribute in a very limited way in our understanding of aging in humans, as senescence pathways vary significantly among cells from different species.” Examples in support of the latter include telomere and telomerase differences in mouse and human fibroblasts, and discrepancies in senescence pathways even within different cells in the same species (see [294]).

Other notable differences and causes for concern include a 2014 review noting that, “According to the GenAge database of aging-related genes, >1,000 genes have been associated with longevity and/or aging in model organisms, including >100 in mice of which 51 have life-extending effects. None of these was associated with longevity in this latest, large study (referring to a genome-wide association study for human longevity [295]); and examples of longevity genes in animals not translating to human longevity that include growth hormone receptor (GHR), which, when disrupted, extends lifespan by more than 40% in mice, but when deficient in humans fails to lead to reduced mortality; and also low IGF1 levels and signalling likewise (see [282]).

Twenty-four years after the media report and associated paper, no therapy resulting from the discovery of the age-1 mutations are in clinical use, or trials, and none seems to be in development as a result. Further, the only intervention in human trials—metformin—even if it is eventually approved, is a result of voluminous human data form more than six decades of human use as a drug for type 2 diabetes mellitus. Though animal data exist, they can only be considered incidental—especially given the demonstrably poor translation of data from animals to humans in this field.

21. New, improved vaccines based on DNA (e.g. for flu immunisations)—Failed

New Vaccine Made to Order—The Times, June 19 1995

The Times reported a breakthrough in vaccinations, in which DNA was used to induce an immune response—in this case, to the flu virus.

Five years earlier (1990), a biotechnology company (Vical) had been investigating ways to make cultured cells take up foreign DNA. They also noticed that mice injected with DNA manufactured the proteins it encoded, and Merck Laboratories then hypothesised that this could be a means of vaccination. Three years later (1993), they reported success for influenza virus, and in 1995 they reported a vaccine ready for human testing.
The article was optimistic about success of this approach, claiming, “When you call at your GP's surgery for a flu jab in a few years, the injection you get may be of DNA. It is a prospect that is enthusing scientists around the world”. A scientist working on a DNA-based TB vaccine opined, “It is very exciting. The results so far have really been far better than anyone expected”; that a new DNA TB-vaccine could be ready for human trials in the next few years; and that the technology could make vaccines more effective. The associated article claimed, “The promise, then, is for more effective, and cheaper vaccines, which are stable and easier to store. There are some hopes for protection against diseases for which we have no effective vaccine, including Aids-related conditions. Finally, there is the tantalising possibility of one-shot vaccinations for combinations of diseases.” It did, however, caution that human tolerability and safety were unknown, and more work was necessary to establish long-term human safety.

The paper prompting the newspaper piece reported the immunogenicity of DNA-based flu vaccines in African green monkeys and ferrets, and that those vaccines had protected ferrets against flu infection more effectively than the licensed vaccine—concluding that “DNA vaccines may be more effective” than types of vaccines currently used.[296] It also cited previous work, some of it involving the same authors, involving the testing of DNA vaccines in mice and cows.[297–301]

This did not translate to successes in humans, in whom significant problems have been experienced. Perhaps the major difficulty was poor immunogenicity. In 2012, a paper reporting a clinical trial of a DNA HIV-vaccine acknowledged that, while some adjuvants were effective in augmenting immune response in small animals and macaques, they failed to do so in human trials.[302] They concluded that new “formulations or methods of delivery will be required.” The following year (2013), a review noted, “In humans as well as in large outbred animals, the efficiency of this [DNA] vaccination has not been so encouraging. It continues to remain an immunological problem that has to be overcome”. [303] It also noted several disadvantages that had become apparent over time, including potential carcinogenicity, the induction of antibodies to DNA itself; and that limited types of vaccines could take this form.[303]

A 2014 review highlighted that “several problems persist”, including “weak immunogenicity in humans”. [304] In reviewing attempted strategies to overcome this, it concluded that even though, “Methods such as in vivo electroporation have improved the efficacy of these vaccines”, “an optimal strategy for safe, reproducible, and pain-free DNA vaccination is yet to be developed”. Further, even though animal experiments had shown some promise in this area, “more clinical trials are needed to prove that DNA vaccination can induce the satisfactory level of effective immune responses in humans.” Crucially, they concluded, “The immune responses measured thus far were not as robust as anticipated from the preclinical studies.”

A 2015 review of flu vaccines noted the widespread acknowledgement of the need to improve vaccination, given their relatively poor protection against emerging variants, and much debate about how that could be achieved (e.g.[305]). It presented evidence of the success of DNA flu vaccines in animal tests, including the paper associated with the media article,[296] and the 2008 study using mice, ferrets and monkeys.[306] It also, however, noted that DNA vaccines had not realised their promise in humans: “Clinical trials have so far been only moderately successful, since plasmid DNA was poorly immunogenic in humans…regarding safety of DNA vaccines, concerns have been raised about integration of constructs into the vaccine recipient's genome as well as the possibility of inducing tolerance”. [305] The examples provided of “moderately successful” clinical trials were published between 2006 and 2010, and were all Phase 1.[307–309] Another 2015 review noted that clinical trials, predicated on small-animal data, had involved
cancer, human papillomavirus, hepatitis, malaria, influenza, and HIV. Yet, “Nonetheless, the results of these early clinical trials were thwarting… they turned out to be inadequately immunogenic”.[310]

Reports of cross-strain protection against flu virus challenge in macaques in 2017 still cautioned, “It is still early days in the world of DNA vaccine research, with no real human, commercial results to be expected for at least five or 10 years”.[311] The authors acknowledged that “vaccines that work well in mice are often less effective in humans”, and, while poor human immunogenicity was being boosted with alternative vaccine delivery systems, they remained less immunogenic than currently licensed vaccines based on the same antigens, and so more needed to be done to realise human success.[312]

This disparity in immunogenicity means that—while a few DNA vaccines have been licensed to date in animals (such as for Salmon Pancreas Disease,[313] as well as for West Nile virus, melanoma,[314] and H5N1 in chickens in the US[315])—there remains no human DNA vaccine licensed for use at the time of writing.[315–317] Interest remains high, however: as of December 13th 2018, the US clinical trials registry (clinicaltrials.gov) listed 263 trials (search term “DNA vaccination”). Of these, 161 had been completed, and 32 were recruiting. Further, some 20,000 scientific articles were published up to and including 2017 (PubMed and Google Scholar).[316, 318] This suggests that some human DNA vaccines might be expected by now. Yet, 23-24 years after the media report and numerous clinical trials, there are none. Most accept that no human vaccine is imminent. It is notable that DNA vaccination research plateaued between 2001 and 2010, and has decreased throughout the past decade—attributed to human immunogenicity issues.[316]

Recent (2018) reviews continue to convey caution, for instance: “The immunogenicity of DNA vaccines in humans is still too low to yield therapeutically convincing results”;[319] “DNA vaccines against influenza have been in development since the 1990s, but the initial excitement over success in murine model trials has been tempered by comparatively poor performance in larger animal models”, and, “Promising approaches have arisen from the numerous studies evaluating different DNA vaccine formulations and delivery systems, but a strategy that consistently elicits protection against influenza in large animal models has not yet emerged”. [315]

23a, 25, 26, 27, 29, 34. Daily injections of the hormone leptin could be a cure for obesity—Failed

Could These Mice Help Women to Look Like This; Forget That Diet: A Daily Jab May Soon Be Enough—Daily Mail, July 28 1995
Drug Firms ‘Hyping Research on Obesity’; Steve Connor on the Row Over Tests for an Injectable ‘Cure’ for Fatness—The Independent, July 28 1995
‘Miracle’ Cure for Fatness has Slim Chance of Success—The Independent, July 30 1995
Diet Hormone Makes Mice Thin, but Not Humans; Research Suggests Hopes of Obesity Cure Were Premature—The Independent, August 1 1995
The Mouse That Could Make Us Thin—The Times, August 29 1995
How to Lose Weight Without Trying—The Independent, September 19 1995

Within five days, four related articles reported an ‘anti-obesity jab’, which could even cure obesity-associated type 2 diabetes. Another, subsequent article reported an interview with the
scientist who discovered the ‘obesity’ gene in mice, associated with the development of this injection.

It was suggested the injection would be a “wonder cure for obesity”, and that “biotechnology analysts say the results seem to be a watershed in the scientific understanding of weight control, since the new hormone acts through a powerful pathway involving the brain's regulation of the body's weight and metabolism.” It was stated that human tests could begin within a year; the director of the US National Institute of Diabetes and Digestive and Kidney Diseases claimed the mouse data to be “a major breakthrough in obesity research”, and claimed “We have every reason to believe this could become a treatment for obesity in humans. It should be effective independent of the cause of obesity, except maybe in certain rare cases.”

Mice had been bred with genetic deficiencies that made them fat. By injecting these obese mice with the hormone leptin (which helps the body regulate its own weight, by regulating hunger/satiety, energy expenditure, and affecting adipose cells), they lost 30 percent of their body weight in two weeks. Other mice had been overfed to make them fat, and similar injections of leptin caused them to lose up to 12 percent of their body weight. Those who had symptoms of diabetes lost those symptoms; they burned calories at a greater rate; and there appeared to be no side effects. Share prices in associated companies “soared”, and the pharmaceutical company Amgen paid the researchers involved £15 million for the right to sell any drug that resulted—with hundreds of millions more to come if this were realised.

Three associated scientific papers were published in Science.[320–322] Overall, the researchers had purified leptin—also known as OB protein, a product of the ob gene—from genetically modified bacteria, injected it intravenously, intraperitoneally, or intracerebroventricularly into mice, and noted the effects on body mass/obesity. The mice were handled frequently, sometimes involving restraint, and many had cannulas implanted to facilitate injections into the brain.

Four years later (1999), the effects of injected leptin with regard to weight loss were reported in humans.[323] In a randomised, controlled trial, subcutaneous injections of leptin led to some obese subjects experiencing weight loss, though additional research was warranted, because differences between dose groups were not detectable given the study design.

The 2015 review “20 years of leptin” acknowledged, “Overall, the opportunity for leptin as a therapeutic in unselected patients with obesity and T2D [type 2 diabetes] has not been substantiated in clinical trials”.[324] A 2017 review stated, “levels of biologically active leptin are elevated in most obese subjects, and these patients do not respond to leptin treatment”. [325] This publication cited papers from 1999 and 2000, just 4-5 years following the media ‘breakthrough’ report, proving this point: that obese humans are refractory to leptin therapy.[323, 326]

The apparent paradox of elevated leptin levels with increased body mass is known as leptin resistance, which has multifactorial causes.[327] In the words of the authors, “It is more than 20 years ago that leptin and its receptor have been identified as key regulators of body weight and energy homeostasis. However, the hormone mostly failed in the clinic to treat obesity due to the fact that obese people are almost always hyperleptinemic and resistant to leptin.” Another, contemporary review cited six studies to support its statement that, “treatment with exogenous leptin resulted in no or only modest effects on body weight” in several clinical trials, concluding that, “leptin administration is likely a poor therapy for obesity treatment”. [328]
However, it seems that “giving leptin only helps in a few extremely rare cases...for the vast majority of people, the treatment won’t work”. This paper also reported that “the promise of leptin as a stand-alone magic bullet for the treatment of obesity was short lived”, with effects highly variable (even resulting in weight gain in some individuals), as well as significant adverse effects at higher doses, and that, as early as 1999, “The hope that leptin therapy would be the cure for obesity disappeared after this trial despite numerous attempts by Amgen Pharmaceuticals to pursue further studies on the hormone that many investigators thought would be a panacea for obesity treatment.” One of these involved trials of a combination of leptin with pramlintide (a synthetic form of amylin used by diabetics to lower blood sugar and reduce need for insulin), but “despite very promising initial data” in rat experiments, the trial was “stopped due to potential safety concerns”, and was not placebo controlled in any case.

Another, contemporary review noted that, though there are some similarities in leptin physiology between species, there are also “substantial differences” between humans and rodents; “unlike rodents, “there is no clear relationship between circulating leptin levels and energy expenditure in humans”, and “in general...the weight loss response to subcutaneously injected leptin in humans with obesity has been quite modest, in part reflecting leptin resistance”. Meanwhile, a 2017 study of obese children confirmed that “BMI and leptin levels had a positive relationship”, in other words that leptin resistance was associated with obesity, in which leptin, produced by adipose tissue, fails to effectively signal to the brain to decrease food intake when fat stores are adequate.

Most recently, one review conveyed surprise that leptin research was so limited, especially as leptin resistance and biology generally remain so poorly understood. At the time of writing (late 2018) the same author opined that leptin could have been one of the most important therapeutics of all time—if it had been effective in anybody other than “exceedingly rare” patients with genetically determined complete leptin deficiency—meaning that only around 25 individuals thus far have been helped by it. Their optimism was largely based on the speculative assumption that leptin efficacy could be enhanced, perhaps by co-administration with other hormones. For now, however, leptin remains an unsuccessful intervention for the treatment of obesity.

Postscript
Alongside the optimism, which continued for some time, even to date, there have been significant doubts about the promise of leptin to treat obesity.

At the time of the positive article in the Daily Mail, The Independent published three articles (see above for details). In them, one obesity researcher cautioned that success was a “distant prospect”, and, “there is an enormous leap from something that works on an obese mouse with a recognised genetic problem and something that works in people, where most obesity has nothing to do with genetic defects.” Another noted “preliminary research indicates it will fail to work in humans”, and “But humans do not always react in the same way as mice”. Another noted the human research suggesting it would fail in people, stating that the “defect in human obesity lies elsewhere”. It appears this human research was ignored, in favour of the animal research—leading to more animal research, alongside subsequent human investigations. A search of the PubMed literature database with the MeSH term ‘Leptin’, removing the MeSH term ‘Humans’, shows that 189 papers were published in 1996, the year following the media report, and this increased year on year to 1600 animal/leptin papers published in 2017.
Research on dieting people and GM mice suggests new target for weight-loss drug development—Partial success (with caveats)

Brain Chemical May Hold Secrets of Why Diets Fail to Work—The Times, August 17 1995

A study of 12 healthy women on a calorie-restricted diet showed that they had lower levels of blood tryptophan when dieting. This correlated with findings in mice, genetically modified to lack brain receptors for serotonin (involved in appetite regulation). These mice were unable to regulate their food intake, and became very fat. Tryptophan, elevated in the women’s blood, is a precursor of serotonin; so the researchers proposed that the diet-induced reduction in tryptophan in humans would lead to lower levels of serotonin. This, in turn, leads to fewer serotonin receptors in the brain, which become hyperactive in an effort to overcome the reduced serotonin levels. Theoretically, this results in intense hunger, so people overeat, leading to weight gain. This research was to elucidate biological mechanisms underpinning appetite and regulation of food intake, leading to drugs to prevent diet-induced feelings of hunger that lead to calorie-controlled diets failing.

The media article also described findings that supported this theory of tryptophan/serotonin involvement in appetite regulation, diet failure and obesity. For example, known drugs that interact with serotonin receptors, such as mianserin and clozapine, can result in weight gain; dieting is associated with disordered eating that can lead to clinical disorders such as bulimia nervosa; the withdrawn drug fenfluramine was prescribed for weight loss, and acted by increasing serotonin levels; and low-fat diets are thought to cause depression by reducing serotonin absorption by brain cells.

The associated scientific paper detailed the human study referred to in the article, and referred to the authors’ previous work studying mice and humans. Interestingly, another paper was published in the same journal four months earlier, describing GM mice lacking functional ‘2C’ serotonin receptors (5-HT2C), who exhibited abnormal feeding and poor appetite control—as well as a predisposition to “spontaneous death from seizures”. It also noted that some drugs that enhance serotonin transmission, such as Prozac (fluoxetine) and dexfenfluramine, can control overeating in certain circumstances.

Five years previously (1990), the principal investigator published a report of human blood tryptophan levels before and after a calorie-restricted diet. They concluded “dieting reduces the availability of circulating tryptophan for brain 5-HT [aka serotonin] synthesis”, supporting the hypothesis that “Altered brain 5-HT function may play a part in some of the psychological consequences of dieting, including the development of clinical eating disorders”. It also contained a summary of previous human (mostly) and animal studies pointing towards the importance of serotonin in appetite and food-intake regulation, published 8-11 years beforehand; and to tryptophan being a precursor of serotonin from 1970, as well as reduced tryptophan altering serotonin levels in the brain from the early 1970s. Subsequent human research had implicated diet-altered tryptophan levels in associated neuroendocrine responses in women, including prolactin secretion, which is a measure of serotonin function. Other references were provided from the mid-1980s, showing that brain serotonin activity and pathways influence satiety mechanisms, and impaired function can lead to binge eating, poor impulse control, depression, and eating disorders.
It is clear that this area of research goes back some time— with the exception of one or two specifics, the results in the media article and associated paper are not significantly ‘new’. To further illustrate: a 2015 review noted that serotonin “has been implicated in the control of satiety for almost four decades” (i.e. around 1975, 20 years prior to the 1995 ‘breakthrough’); and that the association of serotonin and the appetite-suppressant effect of fenfluramine stimulated research and interest in this field (fenfluramine was developed in the 1960s, and approved by the FDA for appetite suppression/the treatment of obesity in the early 1970s).\textsuperscript{[339]} A 2009 review of serotonin receptor agonism for the treatment of weight loss cited nine studies that investigated its effects on metabolic rate: eight were human studies, and six of these were published prior to, or concurrently with, the 1995 reports.\textsuperscript{[340]} Direct knowledge of the role of specific serotonin receptors— often associated with mouse research— had also been investigated prior to, and around the time of, the 1995 ‘breakthrough’ reports. For example, the aforementioned 2009 review also stated that “the established efficacy of these 5-HT receptor agonists [fenfluramine and dexfenfluramine] was thought to be largely due to stimulation of the 5-HT\textsubscript{2c} receptor”, and cardiac toxicity seen with these drugs in humans was thought to be via the 5-HT\textsubscript{2b} receptor, and so drug development efforts were directed towards the former.\textsuperscript{[340]} Further, the 5-HT\textsubscript{2c} receptor gene and its protein product had been studied \textit{in vitro} in 1994,\textsuperscript{[341, 342]} and had been preferentially activated in human volunteers, demonstrating that it reduced food intake (e.g.\textsuperscript{[343–345]}) and was “instrumental in the generation of clinical hypotheses for involvement of the 5-HT\textsubscript{2c} receptor in human disease”.\textsuperscript{[346]}

Research is still very active. A 2017 review\textsuperscript{[347]} noted that human obesity is associated with a reduced plasma-level of tryptophan, independent of dietary intake or weight reduction.\textsuperscript{[348, 349]} It cited recent research (2014-2015) describing associations of low tryptophan levels or disturbed tryptophan metabolism with obesity, and that sudden dietary shortages of tryptophan results in a dramatic shortage of blood serotonin— so serotonin synthesis in the brain is highly dependent on plasma availability of tryptophan.\textsuperscript{[350]} It also showed that research investigating tryptophan supplementation has been increasing for some time: from around 20 reports in 2007 to more than 40 in 2016. The authors could only conclude, however, that “it might be very useful in the treatment of obesity to consider the supplementation of Trp [tryptophan] during caloric restriction diet”,\textsuperscript{[351]} and “Trp supplementation could prove very useful in the treatment of uncontrolled weight gain or prevent neuropsychiatric symptoms”.\textsuperscript{[352]}

The contribution of animal research is controversial in any case. A 2018 review stated, “Whereas animal studies have greatly enhanced our understanding of the neuronal pathophysiology of obesity… it remains largely unknown how these paradigms translate to human obesity”.\textsuperscript{[353]} If this is the case, it appears that animal studies have “greatly enhanced our understanding of the neuronal pathophysiology of obesity” …in animals, not humans. Species differences are acknowledged: the expression of serotonin receptors depends on species,\textsuperscript{[354, 355]} and some authors acknowledge “sometimes conflicting data” in transgenic mice, and that “such genetic defects, however, do not account for the obesity epidemic in humans”.\textsuperscript{[339]} “Interspecies heterogeneity in 5-HT\textsubscript{2} receptor pharmacology” was acknowledged in the mid 1980s and early 1990s.\textsuperscript{[342]}

Nevertheless, a selective 5-HT\textsubscript{2c} agonist drug \textit{has} been approved for appetite control and weight loss, by the name of lorcaserin (marketed as Belviq, Arena Pharmaceuticals; previously known as APD356). It was approved by the US FDA in 2012, following rejection in 2010.\textsuperscript{[356, 357]} It was identified as being potent and selective \textit{in vitro}, before the usual animal tests for safety and efficacy.\textsuperscript{[356]} It is acknowledged as “the most well tolerated anti-obesity drug available”,\textsuperscript{[189]} and recent research has confirmed this, via a double-blind, placebo-controlled,
randomised controlled trial showing no significantly higher risk of cardiovascular events.\[358\] Its efficacy, however, is debatable: compared to placebo, those who took the drug alongside other measures such as diet and exercise only lost an extra 2.8kg over 40 months; at a cost of up to £2,700 per year. It remains available in the US only.

In summary, this may be regarded as a partial breakthrough, with significant caveats. There is a drug (lorcaserin/Belviq), though approved only in the US and with caveats regarding efficacy. This drug is based on research into the tryptophan/serotonin pathway, but it cannot have depended on the 1995 breakthroughs, given the volume of prior, productive research. It may be difficult to assess the contribution of animal and human research, but this summary shows two important considerations: human research has been substantial and informative, and the impact of animal research must be questionable anyway, given acknowledged species differences and difficulties in extrapolation. The pathway had been the subject of human research for years; serotonergic drugs had already been approved for some time before the 1995 ‘breakthrough’, and pointed towards the promise of serotonergic pathway interventions for weight loss/treatment of obesity; human weight of evidence had underlined the importance of serotonin receptors, and the 5-HT2C receptor specifically, for years; and animal data were pursued even though many cautioned they could not be relied upon. Finally, tryptophan supplementation itself still being researched and, though some optimism is conveyed, is only recommended for further research and is not used.

23b, 31, 32, 33. Genetically modified pig organs will successfully address the shortage of organs for human transplant—Failed

Could these mice help women to look like this; forget that diet: a daily jab may soon be enough—Daily Mail, July 28th 1995 [Brief mention of animal organ transplants in article focusing on obesity jabs]
Pig Hearts Could End Fatal Lack of Transplant Organs—The Times, September 14 1995

The moral implications of animal transplants will disturb many. But an eminent Cambridge don says we should rejoice: 'Pigs will be tailored for each of us so we have organs for emergencies. Godparents may give children their own pigs, bred on a scientific farm’—Daily Mail, September 14 1995
Pioneer spurned by Britain—The Independent, September 17 1995

In the main article of four, The Times reported the expected commencement of clinical trials of pig-to-human organ transplants—xenotransplantation (XTP).

These trials would be based on experiments in which hearts from GM pigs survived for up to 60 days in monkeys who had received transplants, supported by immunosuppressive drugs to help prevent rejection—a perennial issue with organ transplantation, but particularly with transplantation between different species. The article was optimistic: the director of the company directing the experiments, Imutran, claimed “a big hurdle in the development of transplants between species known as xenotransplantation had been overcome”; “the rejection problems involved in xenotransplantation are being solved”; and that they had “found a way to trick the immune system of a primate into accepting a pig organ”, while the director of transplant services at Papworth Hospital stated, “If progress continues the way it is, we intend to start human clinical trials in 1996”, and that it would be at least five years before animal transplants were generally available. At the same time, the article noted the urgent need for
organs for transplantation, stating that “The first organ transplants from pigs to humans are expected to begin next year in a move that could signal an end to the global shortage of human donors”, and “If successful, the technique could open up the prospect of animal transplants to thousands more patients who are denied treatment because of a shortage of human organs.”

A paper in Nature carried an associated report.\textsuperscript{[359]} The medical director of Imutran (David White) was quoted again, reporting that 10 monkeys with pig hearts had survived an average of 40 days, with two surviving for more than 60 days. The basis for this improved survival was that the new GM pigs, providing donor hearts for the monkeys, had been genetically engineered in an attempt to overcome hyper-acute rejection (HAR). This is the almost immediate rejection of an organ following transplantation, which can occur within minutes. There are other types of rejection that may occur subsequently: acute/acute vascular rejection, which can take several days, and chronic rejection, which can take years. However, White was dismissive of concerns about such levels of confidence being premature, and that much more understanding of the mechanisms of transplant rejection was needed, stating, “As far as we can see, the other hurdles have not raised their head of [sic] the timeframe of our experiments.”

A few months later, White expanded on the strategy Imutran was using to overcome immune rejection of pig organs. Broadly, this involved the regulation of complement: GM pigs had been created into whom genes for two regulators of complement activity had been inserted.\textsuperscript{[360]}

The following year did not see any human trials commence, and transplantations were not taking place within five years, as promised. In fact, while research has progressed, the intervening quarter of a century has revealed numerous and unforeseen challenges, and human trials still seem distant. First, Imutran was shut down by its parent company Novartis in 2000, which moved its XTP research to the US to take advantage of a “relaxed regulatory climate” and “defined regulatory criteria that would allow human trials”, though amid allegations that they were avoiding bad publicity surrounding Imutran’s conduct, and negative public sentiment in the EU.\textsuperscript{[361]} Just three years later, the company to which the research was transferred—BioTransplant—filed bankruptcy papers and was delisted from Nasdaq.\textsuperscript{[362, 363]} Imutran, it was reported in 2003\textsuperscript{[364]} had some of its XTP experiments conducted at Huntingdon Life Sciences in Cambridgeshire, UK, during which the company was accused of working with the Home Office to underestimate the suffering caused by their research; breaches of the law went unpunished; monkeys and baboons died of vomiting, fits, and diarrhoea; suffered spasms, paralysis, and strokes, and some died en route to the lab from Africa, while being transported in cramped crates that breached regulations for longer than was legally permitted; and all the while Imutran was claiming it was on the cusp of clinical trials, ready to begin human transplants of pig organs within a year (in 1995).

Other companies and researchers pressed ahead, despite more problems, and a wholesale failure to deliver on earlier promises. To illustrate: 12 patients with transplanted chimpanzee kidneys died within two months of surgery, and another with a chimpanzee heart died within two hours, as long ago as 1964;\textsuperscript{[365, 366]} in 1984, was the well-known transplant of a baboon heart into “Baby Fae”, who had been born with a serious heart problem, and who died 20 days after surgery.\textsuperscript{[367]} …In 2001, six years after the reported breakthrough, the “previously enthusiastic” United Kingdom Xenotransplantation Interim Regulatory Authority commented that the “evidence of efficacy has not advanced at the rate predicted” and the “likelihood of whole-organ xenotransplantation being available within a worthwhile time frame may be starting to recede”.\textsuperscript{[368]}
In 2004, a combination of GM pig hearts and an intervention that blocked host antibodies that would have attacked them, extended survival times in baboons, thought the average was only 37 days.\textsuperscript{370} Yet, despite such setbacks, the XTP market for organs was still postulated, in the late 1990s, to be worth up to $11 billion per year by 2010.\textsuperscript{370, 371}

Authors of a 2005 review cautioned, “Complete prevention of organ rejection, transmission of infectious agents and setting appropriate ethical boundaries remain issues to be resolved.” In 2008, as many researchers were lauding progress made with interventions in alpha-Gal mediated organ rejection, others appreciated the importance of other rejection mechanisms: “non-Gal antibodies to the xenograft--and disordered thromboregulation represent major immunological barriers to long-term xenograft survival”.\textsuperscript{372}

These barriers have clearly manifested, with recent publications confirming that organ rejection is still a major issue in XTP. For example, though one researcher considered the survival times of 90 days “impressive”,\textsuperscript{373} orthotopic heart transplants from pigs to baboons were associated with a maximum survival of 195 days, though this particular animal had to be killed due to signs of heart and liver dysfunction.\textsuperscript{374} The International Society of Heart Lung Transplantation suggested that clinical trials of heart XTP should be considered when pig hearts could be transplanted into nonhuman primates (NHPs), with predefined immunosuppression, with “60% survival at 3 months and a minimum of 10 animals surviving for this period”,\textsuperscript{375, 376} but this has still not happened, even though some claim that this goal may be attainable.\textsuperscript{376} Further, most experiments have involved heterotopic, rather than orthotopic, transplants, in which the transplanted organ is placed away from its normal site in the abdomen, which is non-life supporting; orthotopic transplantation, where the organ is placed in its usual site, in order to support life, will be required by the regulatory authorities.\textsuperscript{377}

Regarding kidney XTP, a 2017 paper reported that, even despite GM pigs engineered to have one of the most important genes that induce rejection ‘knocked out’, survival has increased to just “several months”,\textsuperscript{378} and even then, attempts to taper the administration of multiple immunosuppressive drugs have failed.\textsuperscript{379} This removal of immunosuppression is important, because the level needed to prolong survival of the organ is associated with “prohibitive morbidity and mortality”,\textsuperscript{378} i.e., if the recipients don’t die from organ rejection, they eventually die from infections due to immunosuppression. Another 2017 review noted the longest orthotopic liver XTP survived for less than one month, and the longest orthotopic lung transplant less than five days.\textsuperscript{380}

Additionally, issues with the transfer of pathogenic microorganisms from the donor pigs to organ recipients continue. Significant efforts to combat these have taken place, including detection methods, elimination programmes, animal selection, vaccination, treatment, and others, and have been applied to various microorganisms such as the porcine cytomegalovirus (PCMV), hepatitis E virus (HEV), porcine lymphotropic herpesviruses, and porcine circoviruses, which can, among other things, reduce survival time following XTP and cause liver disease (see\textsuperscript{374}). Porcine endogenous retroviruses (PERVs) have also been an issue, which can result in tumours and/or immunodeficiency to transplant recipients, and which are still considered to be a transmissible risk, and a risk that is difficult to evaluate experimentally, with only the long-term follow-up of XTP recipients providing the answer.\textsuperscript{374, 381} Efforts are underway to derive PERV-free pigs as a source of donor organs via breeding, selection, vaccination, and genetic modification (see\textsuperscript{374}). It has been reported that PERVs in the genomes of two immortalised cell-lines have been removed by genetic engineering (CRISPR/Cas9-mediated), which could eliminate the risk from PERVs in XTP.\textsuperscript{382} However, new, more
Sensitive, analytical methods have revealed “numerous new viruses” in the DNA of pigs not detected by other approaches, all with unknown impact on XTP.[382]

Other variables affecting XTP survival include immune suppression, donor genetics, recipient species (i.e. humans will probably react differently to NHPs), viral status, the level of pre-existing anti-pig antibody, prophylactic antiviral and antibacterial therapy, and postoperative care.[376] Even now (2019-2020), immune suppression and donor genetics are “incompletely understood in a field undergoing rapidly evolving experimental changes in both components”.[376] Further, a 2018 review noted that, while survival had increased over the years (decades) “from days to months”, “additional barriers due to antigenic and physiologic differences in cross-species transplantation continue to remain a challenge”. [383] These include significant post-transplant proteinuria (protein in urine, indicating kidney damage), growth problems (organs continue to grow following transplant—an issue that also affects heart XTP) that can lead to organ compression and dysfunction, and the high level of immunosuppression required.

Ongoing work towards human trials centres around increasing ‘tolerance’ via multiple genetic modifications of pigs, targeting the many (and increasing) antigens involved in organ rejection. The current level of immunosuppression required to prolong survival post-XTP is still unacceptably high, and so even greater genetic modification of pig donors is necessary.[378] This is already high: multi-transgenic pig kidneys containing five modified genes have been tested in baboons: one combination allowed survival of six months or more, while another still resulted in serious problems, leading to the conclusion that “the exact responsible genes have yet to be identified”.[383]

It therefore must be asked; how much genetic modification might permit an adequate level of survival? And, even if it were possible, could it ever be enough? If so, will the adverse and off-target effects of the GM process have been properly taken into account? This may be illustrated by the identification of another crucial antigen involved in rejection, B4GALNT2.[384] The authors noted that one of the initial ‘successes’ of GM pigs to address the rejection was the knockout of the Gal gene—this helped resolve Gal-mediated rejection, but “did not eliminate antibody-mediated rejection and instead highlighted the importance of antibody directed to non-Gal pig antigens.” They noted the discovery of several other antigens, including Neu5Gc-modified glycans and the SDa antigen in humans, as well as their recently discovered B4GALNT2 pig antigen (analogous to human SDa); as well as noting that confirming the expected impact of Neu5Gc-modified glycans to XTP organ rejection would remain difficult because of the absence of antibodies to them in NHPs...though not in humans. They also noted the remaining “possibility of additional immunogenic glycans and proteins” that could mediate XTP organ rejection. Reviews from 2017-18 detail the complexity of XTP organ rejection, and the numerous genetic modifications created in attempts to overcome it: 26-30 different modifications in pigs, involving genes associated with Gal, complement regulation, cellular immune response, anticoagulation, anti-inflammatory, anti-apoptotic, and other pathways, but also noted that other, new antigens were being discovered that may require further genetic modifications.[385, 386]

The science media remain optimistic, but also caution that there remain serious issues. For example, “the struggle to overcome host immune response and fears that organs could transmit pig viruses to humans [that] scared off pharmaceutical funders”, following “a flood of optimism and investment in the early 1990s”; “For all the optimism, researchers are far from being able to offer patients an organ with a lifetime warranty. They are still discovering new
mechanisms of immune rejection and debating which genetic changes to pigs are best”; “…other organs pose bigger challenges. The lung, for example, has proved highly sensitive to inflammation, and experimental animals have survived only a handful of days”; and “any whole organ transplant will for now require a cocktail of immunosuppressant drugs that could leave patients vulnerable to infections. That’s a big obstacle to commercial success”.[387]

Within a few days of the article in The Times, two other pieces on the subject were printed in the Daily Mail and The Independent.

The article in the Daily Mail was not science-based, but an overly speculative positive spin on the results from Imutran. It is worth quoting from it, as it illustrated comprehensively the issue of exaggeration and embellishment that, many agree, permeates scientific research and the reporting of it. It claimed that the experiments constituted, “the most wonderful news to have emerged from the world of medicine for more than a decade”, and were, “a cause for rejoicing.” It stated, “The implications are stunning. We are witnessing the beginning of a major step forward in human happiness”; and, “The chance now exists that hundreds of thousands of people who are waiting for heart, liver and kidney transplants, and would die because of the lack of donors, will now live. Sufferers from many other diseases also face hope.” The author—a university lecturer and consultant in clinical biochemistry—went on, “Not only will all those who need these major organ transplants be treated, but whole new therapies will emerge. Many cancers, for example, are now treated by chemotherapy or radiotherapy, but in future it might be kinder, safer and more effective to just transplant the affected organ. Other patients suffer from inherited metabolic defects such as the porphyria which afflicted the late King George III. A cure in the future will be a liver transplant. Diabetes might be treated with a pancreas transplant. Psoriasis might be treated with a skin cell transplant. Cystic fibrosis might be treated with multiple organ transplants. The possibilities are nearly endless and truly amazing. A whole new era of health and longevity is now dawning.” He concluded that, “In 100 years’ time, it may well be routine for godparents to present each child with his or her own pig family, specially bred on a scientific farm.”

The article in The Independent was in the Business section, and focussed on why British venture capitalists failed to back Imutran’s British scientists, who instead had to turn to the US. Positive spin again was replete in the piece: it stated that, “Imutran's method could turn into an enormous money-spinner - possibly one of the richest in the world.” One of the major financial backers appreciated that “These things take between six and ten years to mature”—but we now know that, a quarter of a century later, we are still waiting.

35. Extending tamoxifen prescribing for breast cancer patients could increase survival; but would liver cancer be an issue, as suggested by rodent studies?—Failed

Drug Test on Women with Breast cancer—The Guardian, October 13 1995

This wasn’t precisely an animal-based breakthrough, but cited animal tests of a drug, the results of which had the potential to adversely affect human trials and the use of the drug.

The drug was tamoxifen, used to treat breast cancer, often in conjunction with other interventions. The article reported the commencement of one of the biggest human drug trials, involving 20,000 women with breast cancer, which aimed to assess the drug’s long-term benefits and risks. This was prompted by uncertainty about how long patients should take
tamoxifen, and evidence suggesting some women could benefit by taking it for longer than the usual two years. Researchers wanted to assess benefits with respect to cancer survival, heart disease and osteoporosis, and risks of liver and womb cancers—the former suggested by rodent experiments.

Trial organisers expected results in around 10-15 years (2005-2010), and hoped the extended five-year period on tamoxifen would lead to two lives saved for every 100 patients.

The associated papers reported evidence from rats, suggesting an increase in liver cancer with tamoxifen administration. The authors were also examining liver samples from patients treated with tamoxifen, for DNA damage that can lead to tumour formation, but noted, “A carcinogen which is genotoxic in the rat liver may not necessarily express its carcinogenicity in the same target organ in humans”, and that even mice show a significant difference to rats in this respect; mice “have been found to be refractory to the induction of liver tumours by tamoxifen”.[388, 389]

Human evidence, however, already existed that tamoxifen was not a risk for liver cancer, from pooled data from large studies in Sweden and Denmark in the early 1990s involving almost 5,000 women.[391, 392] Data suggested that uterine and gastrointestinal cancers in humans, instead, would be good targets for a “thorough examination”. Another contemporary paper (1995) noted that tamoxifen had been effectively ‘tested’ in humans for 20 years, and that long-term tamoxifen therapy was already commonplace: some 6 million women-years of clinical experience already existed.[393] Further, the author noted that, if evidence of liver carcinogenicity in rats had existed 20 years previously, especially alongside the then scant evidence of therapeutic efficacy, then “clinical testing would have stopped”, and tamoxifen might not have been approved.

In the 24 years since this report, no evidence supports the suggested link (from rat experiments) between tamoxifen and liver cancer in humans. ToxNet (https://toxnet.nlm.nih.gov) noted that some adverse hepatic effects of the drug had been recorded in humans upon “rechallenge” with tamoxifen, but that, crucially, a literature review showed that “no conclusive evidence linked tamoxifen with an increased risk [of hepatocellular cancer] in humans.” Of interest, but not directly relevant to the rat studies and concern over liver carcinogenicity, the data generally support the prescription of tamoxifen long-term, showing that extending treatment to five or even ten years continues suppression of recurrence of breast cancer, improves survival, and reduces mortality.[394–396]

36. Mouse experiments suggested the natural hormone melatonin could reverse the ageing process, and promote rejuvenation and extend healthspan, also in humans—Failed

Melatonin; It Has Been Hailed as a Wonder Drug that can Cure Insomnia and Reverse Ageing. So Why is it Being Banned?—Daily Mail, October 17 1995

In 1995, the UK Medicines Control Agency (now the Medicines and Healthcare Products Regulatory Agency, MHRA) banned over-the-counter sales of the synthetic hormone melatonin, making it prescription only. The reason given was that melatonin was receiving significant coverage in the press, with many claims of its efficacy in treating insomnia,
mitigating jet-lag, slowing ageing, and other beneficial effects. They felt obliged to investigate, and so sought control over its availability while they conducted research.

Melatonin is naturally produced by the pineal gland, which, in humans, begins to decline at around the age of 50. Because it affects many biological processes, such as “sleep, reproduction, immunity, body temperature, growth, development and the ageing process”; these may be adversely affected when melatonin levels decrease. Many people who use the supplement claim it is helpful.

Two of its chief advocates, scientists Walter Pierpaoli and William Regelson, have published widely on melatonin and how it could, reverse the ageing process. Some of Regelson’s experiments involved giving melatonin to elderly mice. He claimed this prevented their ageing compared to controls, and they also remained “clear-eyed, sprouted thick, glossy coats, retained their muscular strength and bounded about their cages with youthful vigour. They also lived six months longer than the untreated mice and demonstrated all the sexual prowess of much younger mice - right up until they died.” In spite of various cautions regarding a lack of long-term safety evidence and issues with adrenal and pineal gland exhaustion, the article stated, “So far there is no evidence that melatonin is unsafe.”

Pierpaoli’s paper most recent to the media article notes how the ageing pineal gland “results in a negative chain reaction of events that profoundly affect all basic neuroendocrine functions, resulting in deregulation of sexual, adrenal, thyroid, and immunologic feedback control” – leading to deranged “ultradian, circadian, and seasonal adaptive processes”[398] Because melatonin regulates these processes, it follows, supplementation can stop and even reverse them. Further, because melatonin is a potent scavenger of reactive oxygen species, which can damage cells and lead them to degenerate and die, decreasing levels of melatonin with age leads to less successful scavenging, in turn leading to greater damage and ageing—something melatonin supplementation could potentially reverse.

A quarter of a century later, the issue is unresolved, with much conflicting data, conclusions and opinion meaning that more human-based research is needed. In 2004, a review noted that while melatonin could not be considered to extend longevity, there was evidence to suggest it may be a “rejuvenating agent” and beneficial to the ageing process—though more research was required.[399] The author also noted that melatonin had received much attention particularly in the last decade (1995-2005), and that recent publications had proposed it as a therapeutic agent for improving quality of life in the elderly. They detailed evidence for melatonin’s value as an agent to delay ageing, including positive effects on immune function and ability to sustain levels of immunocompetence, the suppression of which has been linked with accelerated ageing (in both humans and animals); and its role in sleep, which is rejuvenating and restorative; in maintaining circadian rhythms; and as a free-radical scavenger, preventing cellular damage leading to ageing. They concluded, however, that it could not yet be recognised as a rejuvenating agent. This was echoed in 2005: “Melatonin has also occasionally been claimed to confer other medical benefits e.g. preventing such age-related diseases as atherosclerosis, cancer, and Alzheimer’s disease. The evidence in such claims is sparse”.[400] A 2007 paper noted that the only widely accepted indications for its therapeutic use were sleep disorders, and circadian rhythm disorders such as jet lag;[401] other uses remained “not definitively proved”, and melatonin still could not be thought of as a rejuvenating or longevity-extending agent in humans.
A more positive review, in 2010, summarised the biological activities of melatonin linked to physiological outcomes that should positively affect ageing.[402] For example, its antioxidant activity involves scavenging various different reactive oxygen species, and has indirect effects via regulation of antioxidant enzymes. In clinical trials, it has been shown to prevent cell damage under acute and chronic conditions, and is considered to be ripe for further research. The paper cited studies showing positive effects on arthritis, type 2 diabetes, hypertension, and female infertility. A 2011 review cautioned, however, that if one accepts positive results in mice, one must also accept negative data: multiple studies have shown an increase in tumours in these mice, which may not be explained by their increased longevity (see[403]). These authors (and others) recommended, based on all available evidence, that the “missing experimental evidence” on melatonin’s safety and efficacy can only be answered by studying many people taking melatonin regularly, e.g. in the US where it is freely available.

A 2012 review noted that, while “healthier ageing” had been demonstrated in “numerous publications” reporting rodent research, “the application to humans has, in this field, more or less remained at a stage of discussion or a suggestion”. [404] The following year (2013), a review noted that extension of life-span was mainly confined to senescence-accelerated mice, and that it wouldn’t be possible to determine whether melatonin could extend human lifespan in the foreseeable future. [405] With regard to healthy ageing effects, these had “been documented in several mouse strains”, but human effects were still unknown.

While a 2014 paper noted that melatonin is protective against ischemia-reperfusion injury in various organs, and has “substantial” ability for myocardial protection,[406] a subsequent (2016) paper accepted that “research is extremely limited on the association of endogenous melatonin levels...with important aspects of healthy aging”, and concluded there was, at best, a weak association of melatonin with cognitive or physical performance.[407] Indeed, another 2016 review of ageing theories and potential therapies discussed many interventions, including caloric restriction, stem cells, antioxidants, and hormonal therapies, but failed to mention melatonin,[294] as did another which concluded that best approaches to remain healthy in older age involved physical activity and food choices, and which included a discussion of various “nutraceuticals” such as resveratrol, omega-3 fatty acids, curcumin, and vitamin D, though no mention was made of melatonin.[408]

Another review noted, “evidence remains limited regarding the overall health benefits of these substances, including epidemiological studies exploring the consequences of their long-term intake on human health”.[293] However, a contemporary systematic review of melatonin levels in older people revealed that, over a period of one day, total melatonin production seems not to change with age, but the maximum nocturnal peak might decline—though great inter-individual variability existed.[409] It cited two reviews to support a statement that “higher melatonin levels are suggested to play a major positive role in healthy ageing and longevity”, published 20 years apart, in 1993[410] and 2013,[405] showing that there was still evidence and opinion on the side of melatonin supplementation for healthy ageing. In addition, the authors stated that there were still no known side effects of melatonin supplementation, so it “could be a safe alternative or additive to current therapy.”

One, recent (2018) review stopped short of citing melatonin as an effective human therapy, calling for further experiments and clinical trials.[411] However, a contemporary review noted the huge amount of research and publications on melatonin and its effects over time: 96% of reviews had been published post-2000, and they concluded that their “present review does [emphasis added] lend support to the notion that endogenous and exogenous MLT [melatonin]
is associated with improved health outcomes".[412] They warned that there were some conditions in which melatonin supplementation should be considered with caution, such as various immune-related conditions, and also that there was “less evidence connecting MLT with specific diseases in a systematic way”, which needed to be research more comprehensively. They also stated that several important confounding factors were difficult to take into account. Overall, therefore, even in 2018 and following more than four decades of research, the researchers concluded that much more systematic research was needed to connect melatonin with health.

With regard to melatonin as a therapy for specific diseases, evidence from GM mice suggested it could be beneficial for Alzheimer’s disease (AD) patients, if results could be extrapolated to humans.[413] A subsequent (2017) publication cited clinical research suggesting that melatonin could retard the progression of mild cognitive impairment in AD, but also stated that supplementation must be very early in the pathological process (before symptoms appear, if possible) for any benefit, and, that many clinical trials would be necessary.[414] A contemporary meta-analysis of randomised controlled trials however, showed that, although melatonin supplementation improved sleep time, it failed to improve cognitive abilities.[415]

It is notable that melatonin was being studied in humans long before the 1995 ‘breakthrough’. In the mid-to-late 1970s, scientists were reporting human melatonin levels, and how they varied with the time of day and with age (e.g.[416]). One should question why so much focus was dedicated to mouse experiments, when so much human experimentation, without confounding interspecies differences, was being conducted—often with contrasting results. Some 24 years later, the evidence base remains inconclusive with regard to human efficacy and beneficial effects on ageing. The BNF still lists the only indication for which melatonin can be prescribed as ‘insomnia’, [417] and MedScape echoes this, though also includes circadian rhythm disorders as having FDA orphan drug status. Medscape notes “Research is continuing” for other uses.[418] The only current clinical trial explicitly resulting from a search for ‘melatonin’ and ‘aging’ (at the time of writing) was “‘Aging Program Project Grant 6 (PPG6), posted in 2018, examining melatonin supplementation and potential improvement of cardiometabolic functions.[419] PubMed also suggests interest in melatonin supplements may be declining. A 2017 publication cited almost 300 references, and discussed many proposed effects and mechanisms of melatonin at length.[420] A PubMed search in January 2019 for ‘melatonin’, ‘aging’ and ‘human’ produced almost 400 papers, but only 25 were published in the last three years (2016 to date), with just six in 2018. Overall, it cannot be concluded that the findings reported in the mid-1990s have resulted in a therapeutic breakthrough for human ageing, and indeed there seems to be waning interest to resolve the matter of melatonin’s postulated benefits for ageing humans.

37, 38, 39, 40. Growing tissues in the lab for transplantation: the famous/infamous ‘mouse with an ear on its back’ experiment—Partial success

Is This a Breakthrough to Benefit Mankind . . . or Science Running Amok?—Daily Mail, October 24 1995
Of Mice, Men and Wacky Medicine; Off-the-Shelf Skin Cloned from the Human Body?—The Guardian, October 28 1995
Genetic Engineering: Tinkering with the Destinies of Mice and Men—The Observer, October 29 1995
S & N's $ 1bn Science Fiction Adventure—Daily Mail, October 30 1995
The image accompanying this ‘breakthrough’ is perhaps one of the most famous/infamous that represent animal experimentation—a mouse with a human ear growing on its back.

The breakthrough involved growing a human ear (external ear only) in a dish, by culturing human cells around an ear-shaped scaffold. After around four weeks, the ear was grafted onto the back of a mouse, whose immune system had been engineered to not reject the transplanted human cells. The main purpose was to determine whether the ear could continue to live after grafting, with a view to growing tissues and organs in the lab and transplanting them into people. The article suggested ears created in the lab could be transplanted to humans within two years, and the technology—tissue engineering—could eventually be used as a source of organs for transplant. One of the scientists involved claimed, “Tissue engineering, if ultimately successful, will not only totally eliminate the need for donor organs, but also the stress associated with being on a donor list…”, while others were optimistic for its use in reconstructive and plastic surgery, but were cautious about transplantation.

An associated paper by the two main scientists proposed that tissue engineering/cell transplantation techniques could be superior to reconstructive surgery using prosthetic materials or tissue transplants. Cells from the patients themselves, or a donor, are seeded onto biodegradable polymers, and retain intrinsic tissue or organ structures and functions as they grow.[421] The authors referenced eight studies demonstrating that “implantation of donor cells and biomaterial scaffolds promotes in vivo tissue regeneration”, published 1991-1994—i.e., this had been done before, with some variations. Notably, a clinical investigation along similar lines, with human skin grafted onto burn wounds, had been published three years earlier (1992), and proceeded to clinical trials.[422] This preceded six of the eight referenced animal studies, and was contemporary with another.

Whatever the foundation of this technology and the degree of contribution of animal studies, recent publications illustrate how it has been developed and is used clinically. Some 18 years on from the ‘breakthrough’, publications still reported significant issues with tissue engineering. A 2013 paper stated, “a major challenge in traditional tissue engineering approaches is the generation of cell-seeded implants with structures that mimic native tissue, both in anatomic geometries and intra-tissue cellular distributions…existing techniques are still incapable of easily creating organ or tissue parts with the required spatial heterogeneities and accurate anatomical geometries to meet the shortage of donor organs for transplantation”. With respect to the ear, the authors continued, “total external ear reconstruction with autogenous cartilage—with the goal of re-creating an ear that is similar in appearance to the contralateral auricle—remains one of the most difficult problems in the field of plastic and reconstructive surgery”. [423] The US National Institute of Biomedical Imaging and Bioengineering authored an informative web page, also in 2013, on the subject, which, in common with many publications, conveyed promise and optimism of further successes in humans, but couched in cautious language. It noted “Artificial skin and cartilage are examples of engineered tissues that have been approved by the FDA; however, currently they have limited use in human patients”, and “Currently, tissue engineering plays a relatively small role in patient treatment. Supplemental bladders, small arteries, skin grafts, cartilage, and even a full trachea have been implanted in patients, but the procedures are still experimental and very costly. While more complex organ tissues like heart, lung, and liver tissue have been successfully recreated in the lab, they are a long way from being fully reproducible and ready to implant into a patient”. [424]
A 2015 review noted that advances had been made in the building of tissues and parts of organs that can be functional *in vivo*, and replace a defective or diseased tissue or organ. Amid “promising results” clinically, it cautioned that “there are many challenges that still need to be addressed in building composite tissues and organs and these include not optimal procedures for scaffold fabrication, limited biomaterial availability, and methods for growing different cell types at precise locations in a given bioscaffold to facilitate blood vessel or a neuronal generation following implantation…the precise microarchitecture—both external and internal, and the pore structure that determines the transport oxygen and nutrients for cell survival, has not yet been achieved”. It speculated that the advent of 3D printing could solve these difficulties (though not for fully functional organs, at least in the near future), but that “several hurdles” remained regarding the fabrication of 3D biostructures with high resolution.

Several 2016 publications are noteworthy. A review of 3D biomaterial printing acknowledged, “Although there has been recent progress in the field, on-demand fabrication of functional and transplantable tissues and organs is still a distant reality”. The authors cited two major challenges that must be overcome, but, if overcome, they would make the technique “an indispensable tool for both investigating complex tissue and organ morphogenesis and for developing functional devices for a variety of diagnostic and regenerative medicine applications.” They reiterated “a significant amount of research still needs to be performed to identify the…governing mechanisms that lead to optimal tissue growth and function, including relevant-size, vascularized, multi- tissue structures”, and “Vascularization is cited as the most significant challenge in advanced tissue and organ engineering, and current strategies have achieved limited success. Without adequate and rapid vascularization, interior cells and tissue will become necrotic in large structures, and this may lead to loss of graft function and infection.” One encouraging caveat was that pre-seeding with endothelial and progenitor cells led to the self-assembly of capillary-like structures that could join up with host vasculature, but that this was limited to very small vessels. Similar issues, involving integration with host tissues and post-implantation functionality and integration, were noted by a 2018 review. A contemporary review noted that they had started on this road almost 30 years previously, in 1988. They noted the degree of interest in the technology, with, at the time of writing, more than 100 companies involved, already generating almost $4 billion in sales. But what has been realised? With regard to the liver: in 1995, the implantation of engineered liver cells in dogs led to results that “were not adequate to produce a device for human testing”, and the authors cited developments with 3D bioprinting and increased resolution, but no eventual success. Regarding the spinal cord, the authors reported the implantation of engineered neural stem cells into the deliberately damaged spines of monkeys that caused hind-limb paralysis and lack of sensation. They noted that efficacy and safety matched those of rat studies, enabling them to proceed to human trials, with “no negative consequences and initial promising results”. At the time of writing, nothing could be found in PubMed, nor on clinicaltrials.gov, to follow up on this. One tangible human success was the creation of human skin, engineered to treat burns and diabetic skin ulcers, which had been received by one million people by 2016. Successes in other areas, such as the development of scaffolding materials, use of stem cells, improvement of culture conditions, and research into other tissues, had all been reported as progressing toward the ultimate goal of human implantation, but these had not happened yet.

Another 2016 paper cited “several challenges” on advancing the ‘engineered ear’ into clinical practice, more than twenty years after the ‘breakthrough’. It described how “The complex, largely unsupported, three-dimensional auricular neocartilage structure is difficult to maintain”; that inflammatory and immunological responses were an issue for neocartilage formation in immunocompetent hosts; and that de-differentiation of specialised cells was a
They did, however, report that they had managed to create a stable, ear-shaped elastic cartilage from sheep chondrocytes, around a titanium wire frame, which kept its shape for around 20 weeks—amounting to progress, at least. Another review described progress made in more ‘challenging’ areas, namely renewable cell sources, biomaterial scaffolds with appropriate properties, mitigation of host responses, and vascularisation. It described progress in many areas, resulting in “Several tissue-engineering products [that] have shown potential for clinical application over the past decade.” However, the authors accepted that “successful clinical application of engineered tissues has been very limited largely because of the persisting challenges in achieving biological functions of cellularized constructs and their host compatibility.” While they listed several approaches anticipated to boost clinical translation by addressing these persistent issues, almost quarter of a century after the ‘breakthrough’, progress against persistent difficulties was still proving difficult.

Most recently: a search of clinicaltrials.gov with the term “tissue engineering” revealed 73 recorded clinical trials, of which 27 were active; and PubMed, from the same search term, showed almost 5,000 papers, of which more than 350 had been published in the first 16 days of 2019. In spite of ongoing significant issues, therefore, this is still a very active area of research, though there remains some way to go clinically. To illustrate: recent (2019) papers have reported “promising preclinical results with cartilage growth” in craniofacial reconstruction, another, on bone and cartilage tissue engineering, cautioned, “Up till now, a mechanically competent vascularized osteoinductive/conductive construct remains to be documented” and “The most important and considerable fact is safety for short and long-term with several challenges”. Finally, a paper on strategies to address cell senescence and optimise cell immortalisation, combined with optimisation of the matrix on which they grow, noted, “Cellular senescence is a major hurdle for primary cell-based tissue engineering and regenerative medicine”, which must be overcome “In order to acquire an abundant number of cells for functional tissue engineering”. It cited “numerous attempts” to deal with this problem, and warned that genetic modification attempts to achieve this were inadvisable and dangerous, due to “the potential risks of malignant transformation and tumorogenesis”.

Three other related pieces were published in the UK national press, contemporarily. One, in The Guardian, revisited the ‘ear mouse’ and used emotive language, speculating “Because of research in tissue engineering, a small boy in America will - with luck - not have to face a series of painful operations to shape a stiff and inert ear graft from his own ribs…Heart patients could soon have their valves replaced with new ones grown from their own tissue. One day, people with spinal injuries might literally recover their nerves.” The Observer claimed, “The ‘ear-mouse’…could revolutionise transplant surgery”, but also tackled the ethical debate, citing examples of animal experiments troubling to the public. The Daily Mail published a follow-up to its main article, discussed here, stating, “If it works - experts rate the chances as 50/50 - that could open a massive market”, and, “Patients could be treated at an earlier stage of joint degeneration, and the need for joint replacements might be postponed or eliminated”; “Initially, research is concentrating on the knee, where success could open a worldwide market worth $ 1billion a year. Spinal discs could follow”, and, “Clinical trials could begin next year.”

In conclusion: After 24 years, this can be regarded as a partial success, with evidence of promise for future successes. It is clear that the production of organs for transplantation is far away, if it will ever be realised using this approach. Those whose optimism was more cautious in 1995, have been vindicated: while transplantation/growing organs is not likely, some progress has been made in some types of surgery, such as reconstructive and plastic. There remain significant and enduring problems that limit its use, even with the infamous ‘ear on the
mouse’s back’, never mind producing liver tissue, repairing spinal cord injuries, and so on. Tissue engineering is used clinically, but its use is relatively limited. Finally, the contribution of animal research is questionable, if not redundant: human investigation has been significant, and is growing—and given the issues that persist in this field, it seems clear it must be the way forward.

41. Newly discovered antigens impact success of organ transplants between sexes: new drugs targeting them will help—Failed

Gender Transplants—The Sunday Times, November 9 1995

Otherwise-identical mice consistently reject organ transplants from mice of the opposite sex. Research had identified a gene responsible for this, which the article claimed should allow the design and development of a drug to combat such rejection, reducing the need for high levels of immunosuppression post-transplant.

The associated paper described the identification, in mice, of an antigen known as ‘H-Y’, causing male transplants to be rejected by female recipients.[434] It noted that the similar human antigen (from the Smcy gene) contributed to transplant rejection in humans, similarly. While this type of rejection was not new, first reported four decades earlier, the antigens responsible had been difficult and time-consuming to identify.

The H-Y minor transplantation antigen was discovered, associated with the Y chromosome, and linked with the rejection of male-female skin grafts in mice, in the mid-1950s; 40 years prior to the 1995 ‘breakthrough’.[435, 436] The human homologue, SMCY, was reported in 1995, concurrent with the mouse ‘breakthrough’ article.[437] Notably, while this paper cites the mouse work from the mid-1950s, it also cites considerable human-based work from 1960 through to the mid-1990s, related to sex mismatch and rejection or graft-versus-host disease in recipients of bone marrow transplants, characterisation of its expression profile and immune responses involving it, and so on.

More recent publications help to elucidate what is a poorly understood and slightly impenetrable topic (due to differences and changes in nomenclature over time). The field is still rather inconclusive regarding the roles of H-Y antigens in transplant tolerance/survival/rejection. With regard to the kidney, a 2011 review concluded, “the male donor to female recipient gender discordance has minimal if any impact on immunologic kidney graft outcomes in the modern era of immunosuppression. Female recipients of male donor kidneys had neither an increased rate of acute rejection in the first year after transplant nor increased risk of graft failure related to rejection”.[438] A 2012 review speculated “Further improvements in transplantation outcomes will likely be achieved through incremental small steps, and further examination of miHA [minor histocompatibility antigens] such as H-Y may [emphasis added] provide measurable improvements in transplantation outcomes”. In other words, no tangible benefit from the discovery and characterisation of this/these antigen(s) had been realised 17 years after the 1995 ‘breakthrough’. Some progress had been made, with reported decrease of T-cell immune responses by H-Y gene therapy in vitro,[440] but with the caveat that, “Although a few studies have found a possible role of H-Y antigen in rejection, direct pathogenic evidence has not been reported. Until such data are available, the role of H-Y antigens in solid organ transplantation will remain controversial and largely unproved.” The author showed that results of studies attempting to determine the effect of H-Y antigen on
transplant outcomes were variable, and that those that had concluded there could be a benefit to receiving a male kidney had based this on several factors, including the number of nephrons.

A 2013 systematic review concluded that, while liver transplants were likely to be affected by gender with respect to graft survival/failure (though a study of 1355 patients showed no statistical difference of survival, or postoperative complications, for gender mismatched transplants), those of other organs, such as kidney, heart and lung “remained controversial”. The authors also noted, “Results of animal [emphasis added] studies support the negative impact of gender mismatch on allograft function”, that many non-immunologic factors impacted transplant outcome, such as mismatch of race, size, age and weight, and that, “the role of H-Y epitope as a minor histocompatibility antigen is still disputed”. A 2014 review noted that kidney grafts from male to female humans showed increased rates of graft rejection, though the involvement of the H-Y antigens in this could only be described as a “rationale” (see[442]). It noted that H-Y antigens were “first discovered in the 1970s”, but that efforts “to identify clinically important T- and B-cell epitopes” were ongoing, which had the potential to lead to “more effective immune modulation therapies” for transplantation.

The HUGO human-gene nomenclature database contains details of human mHA antigens identified to date, and listed 51 at the time of writing.[443] Of these, Dby (now known as DDX3Y), was found to induce—in mice—immune tolerance of bone marrow grafts when injected into them coupled with polymer microparticles.[444] Notably, this was not humans, and it was not the original H-Y antigen mentioned in the 1995 ‘breakthrough’ article (now known as KDM5D).

A 2017 review noted that, even now, “little is known concerning the real number of human mHAg [minor histocompatibility antigens] nor their clinical relevance in the allogeneic transplant setting.[445] A recent (2018) paper on the role of H-Y antigen in corneal transplantation (primary penetrating keratoplasty) revealed that it did not significantly influence graft rejection and failure, but that it could help reduce graft rejection in high-risk patients.[446] It also noted that “conflicting results” existed between mice and humans regarding H-Y antigen compatibility, further confounding inconclusive results in humans—and so more research was necessary to establish the effects of H-Y in corneal grafts.

Another recent paper examined “tolerogenic nanoparticles” to help induce antigen-specific immune tolerance (similar to those in the Dby antigen 2016 paper, above[444]), though which had only seen positive results at the time of writing in animals (one had progressed to clinical trials).[447] The authors cautioned “Animal models of autoimmunity are poorly predictive of human disease. In addition to obvious differences in the immune system, genetic diversity, lifespan, and environmental factors between humans and laboratory mice, there are a number of limitations of mouse models specific to autoimmune diseases”.

It seems that some progress has been made with further identification and characterisation of minor histocompatibility antigens, and their association with tissue/organ transplant survival. However, it is evident that much still needs to be discovered and understood; there are confounding issues with the extrapolation of data from animals to humans; the original antigen reported in the ‘breakthrough’ is one of many (at the time of writing, 51); the original antigen (then known as H-Y, now designated KDM5D) has not been instrumental in any direct progress with regard to any suggested gender discordance in transplant survival/tolerance; and immunosuppression is still the means of preventing/delaying the rejection of transplants generally, with many different therapies used clinically. For example, Medscape published a
page on immunosuppression in prevention of transplant rejection in 2015,[448] and the BNF also listed various agents. Current immunosuppressant drugs were: Immunophilin-binding agents, cyclosporine and tacrolimus (calcineurin inhibitors); the rapamycin (mTOR) inhibitor, sirolimus; antiproliferative agents, most commonly azathioprine and mycophenolate; antibodies, such as basiliximab and daclizumab; and corticosteroids. A number of future therapies were also listed, including many new antibodies targeting diverse antigens and molecules. Available information suggests that none of these appear to be based on KDM5D.

44. Naturally occurring hormone (DHEA) promises to reverse the adverse effects of ageing—Failed

Natural Hormone May Soften the Blows of Age—The Times, January 9 1995

The Times described the anti-ageing/rejuvenating effects, in animals, of the natural hormone dehydroepiandrosterone (DHEA). The article was optimistic about its prospects as an ageing therapy, declaring, “In a development that recalls The Picture of Dorian Gray, Oscar Wilde's fable of eternal youth, researchers have discovered a natural hormone produced by the body that could delay the effects of ageing... the hormone could help to defer such characteristic problems of old age as wrinkles, muscle fatigue, rheumatism, bone fragility, memory loss and some cancers.”

DHEA level declines from the age of about 25, and, at the age of 70, is typically at just one tenth of its peak level—and this decline has been linked to health issues. The article reported a human study, in which people “reported a feeling of wellbeing after a few weeks' treatment” with DHEA, but noted that this study was based on animal tests, which had been “spectacular”. The scientists sought approval for a bigger human trial, the results of which would be available in 3-4 years (1998-99).

The associated paper[449] summarised known properties of DHEA, including positive effects on memory and cognitive performance in rats (suggesting it could protect against neurodegenerative processes), but also clinical studies examining its role in, for instance, sleep and age-related dementia. It concluded, “definitive conclusions about the neuroprotective and memory-enhancing properties of DHEA in humans will rely on ongoing clinical trials”, and, “The physiologic and pathophysiologic roles of DHEA as a neuroactive molecule, however, remain speculative.” They optimistically noted, however, “Experimental data on the control of mood changes and reinforcement of memory storage, combined with clinical evidence of DHEA activity on the nervous system in vivo in humans based on electroencephalographic data, are very encouraging.”

Evidence doubting the human relevance of animal research involving DHEA supplementation, and that the positive effects of DHEA stemmed from epidemiological studies, was being reported shortly after the 1995 article and papers. DHEA’s positive effects on “numerous age-related illnesses...have been suggested by epidemiological studies in humans” reported a 1997 paper, adding, “Animal studies support a protective effect of DHEA on these age-related diseases. However, it remains unknown whether these results in animals can be transposed in humans, because adrenal secretion of DHEA seems to be particular to primates... the study of DHEA is difficult because of no known cellular receptor and no satisfactory animal model. The adrenal secretion of DHEAS seems to be specific to humans: the values are lower in the great apes and the adrenals of small laboratory animals do not secrete it”.[450]
Five years after the ‘breakthrough’, a 2000 paper by the same quoted author described the “safe” administration of DHEA to elderly subjects (aged 60-79), but did not examine beneficial effects, which were described as “potentially effective”. A contemporary paper reported the “DHEAge Study” involving 280 healthy people, showing beneficial effects of DHEA for skin condition, libido, bone health, and a lack of adverse effects. The authors echoed the aforementioned 1997 paper, stating “relevant animal data…will never be obtained because no endogenous circulating DHEA(S) in laboratory animals, allowing its role in aging to be examined, has been described”.

A 2003 double-blind placebo-controlled trial demonstrated that oral administration of DHEA for one year to people aged 60-80 failed to induce beneficial effects on muscle state and function. This paper cited previous “discordant results” concerning muscle strength, and noted that a prior study showing benefit involved fewer subjects, a higher dose, and younger people. A 2006 Cochrane review concluded there was no evidence to support any beneficial effect of DHEA on cognitive function on middle-age or older adults without dementia.

More recently: a 2012 paper described DHEA-mediated amelioration of chronic obstructive pulmonary disease (COPD), albeit in a proof-of-concept study with a limited number of patients and a 2013 paper examined the available human evidence for DHEA supplementation, concluding that its acknowledged controversial and contradictory nature was due to the short-duration and limited aspects of many human studies. Nevertheless, they added that the positive nature of some of its effects was “rather robust”, such as those on muscle, bone, cardiovascular disease and sexual function. A 2014 systematic review and meta-analysis of DHEA in postmenopausal women incorporated 23 randomised controlled trials and almost 1200 women. It concluded (with “low confidence”) that DHEA was not associated with any significant improvement in libido, sexual function, weight/BMI, bone mineral density, etc. In contrast, another 2014 review stated that the administration of DHEA had shown positive effects on muscle mass and strength, physical performance, bone mineral density, mood, sexual function, menopause symptoms, and skin, all with a good safety profile. Contrary to animal studies, however, no beneficial effects were evident on tumours of the breast and prostate. The authors concluded that DHEA had “positive effects on several age-related diseases”, but cautioned that long-term safety and efficacy were “still uncertain.”

A contemporary review agreed on many points, finding “A growing body of evidence challenges the notion that DHEA and its metabolites are merely a worthless dietary supplement with no proven health benefits. It is becoming evident that DHEA might be of value in gynaecology, endocrinology, rheumatology, dermatology and allergy”. The authors cautioned, “more large-scale, well-designed RCT [randomised controlled trial] studies are warranted before it enters routine clinical practice”, and also conveyed negative views of the contribution of animal studies: for example, “Many factors are responsible for the inconsistent/negative results of some studies. Overreliance on animal models (DHEA is essentially a human molecule), different dosing protocols with non-pharmacological doses often unachievable in humans, rapid metabolism of DHEA, co-morbidities and organ-specific differences render data interpretation difficult…Rodent tissues respond to DHEA differently due to a lack of physiologically high levels of serum DHEA; laboratory/domestic species’ low plasma DHEA levels are mainly or exclusively gonadal in origin; in contrast, human DHEA is synthesised almost entirely by the adrenals…DHEA is a ‘human’ molecule as its adrenal secretion is minimal/absent in laboratory animals (including rodents). Studies on animal models are therefore not entirely reliable and most have been excluded from our review.”
Another systematic review/meta-analysis in 2015 examined twelve interventions for ageing.[460] With regard to DHEA, it cited the aforementioned Cochrane review of 2006,[454] and a clinical trial that showed no cognitive benefit in midlife.[461] A contemporary paper examined effects on the ageing ovary, concluding, despite ongoing controversy in this area, that its use was supported by retrospective analyses, prospective self-controlled studies, and case-control studies, with regard to egg yield, egg and embryo quality, pregnancy rate, birth rate.[462] Still, the authors cautioned that efficacy could only be proved, and DHEA use recommended, following successful, “large-scale, well-designed confirmatory studies.” Another 2015 publication examined DHEA’s effects on the cardiovascular system,[463] concluding that the evidence to date of clear benefits in humans, at least for cardiovascular diseases, was “still controversial.” This is despite many positive results in animals, such as for cardiomyopathies and other cardiovascular disorders, which have been induced experimentally via high-fat diets in rabbits and rats. The authors could only conclude that, for humans, “supplementation studies were much less conclusive than expected.”

Another 2015 paper investigated DHEA’s effects on cognitive function, reviewing animal and human studies between 1994-2013.[464] Human studies had been overwhelmingly negative: 8 trials were negative, with one inconclusive and 2 positive. They concluded, “The relevant beneficial effects of DHEA treatment in animals have not confirmed in humans”, despite being “tested in a large number of animal models.” Further, “the current data do not provide clear evidence for the usefulness of DHEA treatment to improve cognitive function in adult–older subjects”, and, crucially, “This should be not surprising, given the significant differences in terms of adrenal physiology and DHEAS circulating levels, existing between rodents and humans, making the translation of the animal results such difficult…we have to acknowledge significant differences between rodent and human adrenal endocrine systems. Most of DHEA/S in humans is synthesized by the adrenal glands, while in rodents active DHEA/S is locally produced in different tissues. Moreover, rodents do not experience the age-related decrease in DHEA/S production observed in humans. In addition, the circulating DHEA/S levels in mice and rats are often unmeasurable, making any exogenous dose of DHEA/S supraphysiological. Thus, the rodent model cannot be considered the ideal one to be translated in primates or humans.”

Most recently: a 2018 paper concluded that DHEA was safe and effective for menopausal vulvovaginal atrophy and dyspareunia (painful sexual intercourse) in most women, but that further studies were needed for other conditions.[465] Another concluded that DHEA use in IVF was effective in improving clinical pregnancy, live birth rate, endometrial thickness, and retrieved eggs, though not, for example, miscarriage rate.[466]

Various medical websites provide up-to-date information surrounding DHEA and its uses. Medscape mentions its availability as an over-the-counter supplement in the US, and that it has been used for the treatment of adrenal insufficiency, lupus, fibromyalgia and depression, but that this use is not evidence based. Although it is used to improve athletic performance, this is illegal, and prohibited by professional sporting bodies.[467] The UK National Institute for Health and Care Excellence (NICE) contains just one entry for DHEA, in guidance for fertility problems, and cautions, “Do not use growth hormone or dehydroepiandrosterone (DHEA) as adjuvant treatment in IVF protocols”.[467] Medical News Today, in a 2018 article, acknowledged “Some research supports its use…However, DHEA’s effectiveness is controversial and rarely supported by evidence…DHEA has been tested for use in many diseases, including depression, osteoporosis, and lupus, but there is little evidence to confirm its benefits…People with heart disease, diabetes, anxiety, and other conditions should not use
DHEA.” Summarising variable success rates for different diseases and conditions, the website noted DHEA had produced mixed results for bone density; might improve depression; may help reduce weight in some patients to a small degree; that a short-term study for anorexia nervosa looked encouraging; that some research suggested it could improve lupus symptoms; some studies showed it could improve sexual functions, though others were inconclusive; there were contradictory results for HIV/AIDS; encouraging data for cervical cancer; and weak evidence for muscle strength. Crucially, however, it concluded that evidence for the amelioration of age-related conditions—the focus of the ‘breakthrough’—was “weak or unproven”. The website WebMD classified DHEA as being “likely effective” for vaginal thinning; “possibly effective” for ageing skin and depression; but “possibly ineffective” for ageing, physical performance, psoriasis, rheumatoid arthritis, and cocaine or heroin withdrawal; “likely ineffective” for mental function and Sjögren’s syndrome; and there was insufficient evidence for a further 25+ conditions. Finally, the Mayo Clinic noted that DHEA might be more effective at treating depression than placebo; might improve bone mineral density in elderly people (though improvements were smaller than with approved medications); limited research suggested DHEA could improve vaginal atrophy; wellbeing and body composition showed mixed results; cognitive function and muscle size and strength show no improvement in most studies; and there might be some future evidence showing improvement of adrenal insufficiency and lupus; and, crucially for our considerations here, with regard to slowing the ageing process, “research hasn’t proved this to be true”.

In summary, there remains, almost 25 years after the reported ‘breakthrough’, considerable controversy regarding the anti-ageing/rejuvenating effects of DHEA in humans. On balance, while there appears to be some consensus regarding its beneficial effects for some conditions, including some that are age-related, there is also consensus that more research needs to be done, involving longer duration studies and more human participants. Animal research cannot be considered to have played any sort of role in this, other than being a source of speculative claims for DHEA’s efficacy for many diseases and conditions, for several reasons: human studies were being conducted in any case, with people being supplemented with DHEA prior to the 1995 ‘breakthrough’; these human studies are acknowledged to have underpinned subsequent, more detailed human trials, and that more of the same are needed going forward; and there is a widespread criticism of animal studies in this field, seen as unhelpful—or even impossible, given the almost unique human-specific nature and levels of DHEA, as well as due to the existence of contradictory data from animal and human investigations. Either way, there exists no real breakthrough at this time, as DHEA appears not to be licensed for any indication, and is listed as being “likely effective” only for vaginal thinning. This may change in the future with further human research.
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**Nexis dataset from published search strategy**

Download Request: All Documents: (1-212)
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Terms: (Animal OR mouse OR mice OR rodent OR rat OR dog OR cat OR monkey OR primate OR guinea pig OR rabbit)
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Natural hormone may soften the blows of age

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Are you brewing up for a healthier heart

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The bourgeois Borderer and the Clearances; THORNS FROM THE THISTLE

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Secret dangers in the womb

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