

# Pills for Multiple Sclerosis

Throughout 2009, multiple sclerosis conferences were dominated by presentations, and gossip, about three big trials. And here they are, finally served up in one multiple sclerosis bonanza issue of the *New England Journal of Medicine!* These are serious trials, likely to be highly cited in the multiple sclerosis literature, and to be repeatedly lauded as “launching a new dawn in multiple sclerosis treatment” or something like that.

So what are these drugs? You may already know fingolimod as “FTY720”, an unlicensed drug that has been around the scientific and transplantation literature for a while. It acts in an entirely novel way. It antagonises the sphingosine-1-phosphate type 1 receptor on lymphocytes, which react by withdrawing the receptor from their cell surface. Normally, these receptors are needed for lymphocytes to get their cue to leave the comfort of the lymph node and do their stuff in the wide world. So fingolimod stops them leaving the lymph nodes and there is effective peripheral lymphocyte depletion. Very unusually for a drug in

multiple sclerosis, fingolimod crosses the blood-brain barrier. There is reasonable animal data that it may have neuroprotective effects, but these have not been demonstrated in humans yet. Cladribine is an old drug; it received FDA approval in the 1980s for treatment of hairy cell leukaemia. It is phosphorylated to a toxic nucleotide CdATP, which accumulates and causes DNA strand breaks, inhibition of DNA synthesis, and cell death. The selectivity of its action on lymphocytes arises from the accident that these cells have a high ratio of deoxycytidine kinase to 5'-nucleotidase. So, cladribine leads to prolonged T and B cell lymphopenia.

- The big news, of course, is that both drugs are given as tablets. This is an important advance in convenience for people with multiple sclerosis. This is particularly true of cladribine, which is so potent that as few as eight tablets a year appears to be effective! For the obsessive, I have tabulated the details of the trials below. Here are just a few thoughts.

- On the whole, the FDA likes to see two phase 3 trials of a multiple sclerosis drug. So, the fact that there are two for fingolimod but only one for cladribine probably means that fingolimod is ahead in the race to get to market. (Indeed when Merck tried to submit cladribine for licensing to the FDA last year, they were politely turned down, for undeclared reasons).
- In each trial, the drug has a useful impact on relapse rate, greater than would be expected for interferon-beta. But the key efficacy outcome in multiple sclerosis trials is disability and, strictly speaking, neither drug has shown any greater efficacy than interferon-beta on disability. This is because cladribine has only been tested against placebo, and in the fingolimod-interferon head-to-head (TRANFORMS) there was actually no statistically significant difference in disability measures. I don't want to be churlish here... the greater impact on relapse rate than interferon may translate into greater efficacy against disability for

**Table: Antibody targets and associated conditions**

STUDY NAME	TRANSFORMS	FREEDOMS	CLARITY
Study drug arms	Fingolimod 1.25 mg daily	Fingolimod 1.25 mg daily	Cladribine 3.5mg/kg daily for 8-20 days a year
	Fingolimod 0.5 mg daily	Fingolimod 0.5 mg daily	Cladribine 5.25 mg/kg daily for 8-20 days a year
Comparator	Avonex	Placebo	Placebo
Trial duration	12 months	24 months	96 weeks
N	1292	1272	1326
(N completed study)	1153	1033	1184
Inclusion criteria	RRMS EDSS 0-5.5, 1 relapse in last year; may or may not have had previous disease-modifying therapy	RRMS EDSS 0-5.5, 1 relapse in last year	RRMS EDSS 0-5.5, 1 relapse in last year, may or may not have had previous disease-modifying therapy
Annualised relapse rate reduction	38-52% reduction versus interferon	54-60 % reduction versus placebo	54-58 % reduction versus placebo
Annualised relapse rate	0.2 and 0.16 (fingolimod) versus 0.33, interferon p<0.001	0.18 and 0.16 (fingolimod) versus 0.4 placebo, p<0.001	0.14 and 0.15 versus 0.33 placebo, p<0.001
% patients with confirmed disability accumulation over 3 months	6.7 % and 5.9 % versus 7.9 % p=0.5 <b>NOT SIGNIFICANT</b>	17.7 % and 16.6 % versus 24.1 % <b>p = 0.01 and 0.03.</b>	14.7 % and 15.1 % versus 20.6 % on placebo, <b>p = 0.02 and 0.03</b>
Worrying AEs	<b>INFECTION:</b> 2 deaths (herpes simplex encephalitis and disseminated primary varicella) on fingolimod; also <b>CANCER:</b> 2 breast cancers in each of the fingolimod groups. 5 basal cell carcinomas and 3 melanomas in the fingolimod groups versus 1 basal cell carcinoma and 1 squamous cell carcinoma in the interferon group.	<b>CANCER:</b> 1 breast cancer in the fingolimod groups versus 3 in the placebo group. 5 basal cell carcinomas and 1 melanoma in the fingolimod groups versus 3 basal cell carcinomas and 1 melanoma in the placebo group.	<b>INFECTION:</b> neutropenia seen in 3 patients on cladribine and 1 case of exacerbation of latent tuberculosis. 3 cases of primary varicella <b>CANCER:</b> 3 cases in study in cladribine groups (melanoma, pancreas and ovary) with one emerging after study period (choriocarcinoma).
Less worrying AEs	also non-fatal herpesvirus, macular oedema, AV block, hypertension	also non-fatal herpesvirus, macular oedema, AV block, hypertension	Herpes zoster in 20 patients on cladribine, none on placebo

these drugs... and a discernible difference in disability might emerge from longer follow-up of the TRANSFORMS patients...

- The duration of these trials is a big issue. The fingolimod-interferon head-to-head was only 12 months, which is nothing in the life of a patient with multiple sclerosis. Just as short duration compromises power for efficacy outcomes, so too does it impair our ability to understand the safety issues. And it becomes very difficult to spot dose effects. In both trials, two doses were tested, but to my mind it is not clear yet which is preferable.
- These drugs are not as safe as interferon-beta. There is a "signal" that both slightly increase the risk of cancer and infection, especially by herpes and varicella viruses. It is hard to be definite about these risks, as these are low frequency events in all the trials and there is plenty of "noise"; for instance, the skin cancer signal in TRANSFORMS is not really replicated in the FREEDOMS trial.

A glib conclusion might be the ubiquitous "more research needed". And certainly that is true: hopefully we will get to hear the long-term follow-up of these trial patients for one. But, it is very easy to say these things...and forget these are seriously large and expensive trials that have already consumed literally hundreds of millions of dollars, and dominated investigator resources at hundreds of sites around the world. I am beginning to come round to thinking that there ought to be another way of testing drugs. Perhaps both drugs could be licensed as probationary drugs for a few thousand patients and then a full license reconsidered in a few years time? Like learner plates?

#### – Alasdair Coles

Cohen JA, et al.; the TRANSFORMS Study Group. Oral Fingolimod or Intramuscular Interferon for Relapsing Multiple Sclerosis. *NEJM* – 2010 Feb 4;362(5):402-415.

Kappos L, et al.; the FREEDOMS Study Group. A Placebo-Controlled Trial of Oral Fingolimod in Relapsing Multiple Sclerosis. *NEJM* – 2010 Feb 4;362(5):387-401.

Giovannoni G, et al.; the CLARITY Study Group. A Placebo-Controlled Trial of Oral Cladribine for Relapsing Multiple Sclerosis. *NEJM* – 2010 Feb 4;362(5):416-426.

## ALS: of mice and miR-206

Amyotrophic lateral sclerosis (ALS) kills over 1200 people each year in the UK and remains incurable. Around 10% of cases are familial. Much of what we understand about disease pathogenesis has come from models based on mutations of superoxide dismutase 1 (SOD1), which account for about 20% of familial ALS (FALS). In the December 11th issue of *Science*, Williams and a team led by Eric Olson in Dallas, Texas, describe how they have used the G93A SOD1 mouse model to implicate a specific micro-RNA, miR-206, in disease progression and survival, and have suggested that this may be a therapeutic target. Using a battery of transgenic overexpressing and knockout mice they show that miR-206 is significantly overexpressed in G93A mice, coincident with onset of an ALS phenotype. MiR-206 appears to be involved in muscle reinnervation following denervation, a possibly compensatory sequence of events also seen in ALS patients. These findings are interesting in the light of recent advances in ALS research, which have implicated RNA-processing agents, in particular TDP-43 and FUS. TDP-43 is the hallmark protein of pathological inclusions in ALS, and mutant isoforms of TDP-43 and FUS account for around 8% of familial ALS (reviewed by Lagier-Tourenne and Cleveland 2009). Intriguingly, both proteins have been implicated in mi-RNA biogenesis as they interact with the Drosha complex (Gregory et al 2004).

Micro-RNAs are short RNA sequences ~22 nucleotides in length. Rather than coding for protein, they target complementary sequences on other RNA molecules, usually inhibiting their translation by binding to the upstream 3'-untranslated region. Mi-RNAs have diverse cellular roles through modulation of gene expression. Genes encoding mi-RNAs are therefore not 'junk' DNA as previously thought. Furthermore, mi-RNAs have been implicated in cancers, and aberrant mi-RNA pathways may contribute to the pathogenesis of various neurodegenerative diseases, including frontotemporal dementia, Parkinson's disease, Alzheimer's disease and Huntington's disease (reviewed by Hébert and Strooper, 2009).

Williams et al show that miR-206 may exert its trophic effects by downregulating histone deacetylase 4 (HDAC4) expression and thus

increasing fibroblast growth factor binding protein 1 (FGFBP1) expression. Histones are proteins that package DNA, and when acetylated they release the condensed DNA allowing gene transcription to occur. This is regulated by histone acetyltransferases (HATs), and HDACs. Interestingly, histone metabolism has previously been linked with sporadic ALS risk through the genetic association of ELP3 variants. ELP3 is part of the RNA polymerase II complex and is involved in histone acetylation and RNA elongation (Simpson et al, 2008). The therapeutic potential of HDAC inhibitors is also under investigation, and although results have been promising in animal models, clinical study has so far not demonstrated any positive effects (Piepers et al, 2009). The authors state that miR-206 appears to have a 'salutary function in ALS'. It is more accurate to describe the mice as suffering a 'motor neuron phenotype' rather than ALS, which is a human disease. It will of course be important to validate these results in humans. It is also important that similar studies are conducted in other models of ALS, particularly TDP-43 transgenic animals, as SOD1 ALS and SOD1 transgenic models are not characterised by TDP-43 inclusions. Nevertheless, the findings are significant, not only in highlighting another potential mechanism for ALS pathogenesis but also in suggesting a novel therapeutic approach.

#### – Jemeen Sreedharan, Guy's and St Thomas' NHS Trust, London

Williams AH et al. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. *SCIENCE* – 2009;326(5959):1549-54.

Lagier-Tourenne C and Cleveland DW. *CELL* – 2009;136(6):1001-4.

Gregory RI et al. *NATURE* – 2004;432(7014):235-40.

Hébert SS and De Strooper B. *TRENDS NEUROSCI* – 2009;32(4):199-206.

Simpson CL et al. *HUM MOL GENET* – 2008;18(3):472-81.

Piepers S et al. *ANN NEUROL* – 2009;(2):227-34.

## EPILEPSY: here today, gone tomorrow. Back the day after?

So you have started medication for the patient's epilepsy and they are doing well. You have discharged them at the earliest opportunity, under NHS new-to-follow-up rules, patted them on the back and they have left with a jovial: "Hope I never see you again doc". Will you smile benignly or wistfully? Will it be good-bye or more likely, according to this study, *au revoir*? Of the 566 patients entering the study, 85 were excluded, leaving 481 evaluable. Of these 225 did not achieve a one year remission and 256 did. Of the 256, only 154 achieved a sustained remission, for 5 years, just over one third of the original cohort. The five year cumulative relapse rate was 40% and 25% became refractory. A handful of these later became surgical candidates, although it was considered in all the patients. The strongest factor predicting relapse was a higher number of drugs required to achieve a remission; others were the duration of pre-mission epilepsy and the frequency of seizures prior to remission. When we counsel our seizure-free patients about their illness in relation to life choices and particularly their career choices, it would be wise to consider these data.

#### – Mark Manford, Neurology Unit, Addenbrooke's Hospital, Cambridge, UK.

Schiller Y. Seizure relapse and development of drug resistance following long term seizure remission. *ARCHIVES OF NEUROLOGY* – 2009;66:1233-39.

## Channels under scrutiny

Louis Ptáček at the University of California, San Francisco, and an international team have uncovered a novel inwardly rectifying potassium channel gene, KCNJ18, which encodes Kir2.6, and in the process have identified a possible mechanism for some cases of thyrotoxic hypokalaemic periodic paralysis (TPP). Kir gene mutations have had disease implications before, in Andersen syndrome (periodic paralysis, cardiac arrhythmias, and dysmorphic features, Kir2.1, Ptáček again, *Cell*, 2001). But this paper showcases a triumph of discovery. The problem with KCNJ18 (Kir2.6) is that it is so similar to KCNJ12 (Kir2.2), with 98-99% homology at the coding region, and as such has been interpreted previously as a polymorphism of KCNJ12.

The find is arrived at beautifully, first by fishing out channels with putative thyroid-response elements in their promoter regions, and then by applying some lateral thinking to PCR to overcome the *doppelgänger* problem. Then, 6 mutations were found in patients with TPP, but not controls, with varying prevalence in different ethnic groups; the highest at 33% in a group of TPP patients from Brazil, the US and France. The functional studies of each of the mutations are ongoing, but some are shown to alter conductance. The proposed model is that triiodothyronine (T3) enhances transcription of KCNJ18 in order to help stabilise the muscle membrane, which is already affected in a number of ways by thyrotoxicosis – in the cases with a mutant, the rectifying channel is a false friend.

A small study from Barcelona provides some support for the intuitive notion of genetic mutations that, rather than defining a disease, shape the subtleties of phenotype and modulate the details. The paper by Serra et al examines the electrophysiological function of a mutation in CACNA1A found in two members of a large familial hemiplegic migraine family; the pair conspicuous by an absence of face/tongue paraesthesiae or hemiplegia, though visual aura was retained. Such a mutation seems to impair interaction of the channel with vesicle exocytosis machinery, perhaps through the structural alteration in the I-II loop. A proposed model is that such a reticence to engage synaptic machinery may reduce cortical spreading depression, in non-visual cortex areas at least.

– **Mike Zandi**

Ryan DP, et al. Mutations in potassium channel Kir2.6 cause susceptibility to thyrotoxic hypokalemic periodic paralysis. *CELL* – 2010 Jan 8;140(1):88-98.  
Serra SA, et al. A mutation in the first intracellular loop of CACNA1A prevents P/Q channel modulation by SNARE proteins and lowers exocytosis. *PNAS* – 2010;107:1672-7. Epub 2010 Jan 8.

## REPAIR: the tale of making new neurons!

The discovery a few years ago that somatic cells could be reprogrammed back into pluripotent stem cells (iPS cells) caused a great deal of excitement, especially when the initial work in mice was extended to human skin cells. The way to do this was surprisingly not that complicated and over the years refinements in the necessary factors have been made along with an increase in the efficiency of the process. Now Vierbuchen et al have gone a stage further by reprogramming murine fibroblasts directly into neurons. The authors argued that committed fibroblasts could be made into neurons by using neural-lineage specific transcription factors of which 19 were chosen for starters. Whilst the possible combinations of 19 different factors to find the right recipe is enormous, the authors discovered that one single factor (*Ascl1*) was sufficient to induce immature neuronal features and that two additional factors (*Brn2* and *Myt1l*) could turn these cells into mature iN (induced neuronal) cells. These latter cells had all the hallmarks of neurons- they could generate action potentials; they expressed a whole range of neuronal markers and could even make synapses. The iN so generated were interestingly mainly glutamatergic and thus excitatory with markers of cortical identity- which is rather different to that seen with many other neurons derived from other sources of stem cells which tend to be GABAergic.

So there we have it, three transcription factors seem to be able to drive mouse fibroblasts (including post-natal cells) to functional neurons, and according to this paper the technique seems robust and relatively efficient. If this approach can be readily replicated in other labs in much the same way as was seen for iPS, then there are exciting times ahead. Because, as the authors of this paper conclude "...iN cells could provide a novel and powerful system for studying cellular identity and plasticity, neurological disease modelling, drug discovery and regenerative medicine".

– **Roger Barker**

Vierbuchen T et al. Direct conversion of fibroblasts to functional neurons by defined factors. *NATURE* – 2010 [Epub ahead of print].

## REHABILITATION: Stroke and Mirrors

Most physiotherapy gyms will have a full-length mirror, often arranged at the end of a set of parallel bars, so that patients beginning to mobilise can monitor their own position and alignment. Although the research base for this intervention is somewhat limited, there is a growing literature on the use of mirrors in upper limb rehabilitation. In the 90's, this became associated with the work of Ramachandran and the use of a "mirror box" in the management of phantom limb pain in upper limb amputees. Although his original study has some significant methodological limitations (in sample size and selection), it gained prominence in the popular scientific literature of the time and prompted debate about the interaction between vision and proprioception as well as the potential for utilising alternative neural pathways as part of the recovery process from neurological disease. This, of course, prompted further investigation.

This meta-analysis of the effectiveness of mirror therapy in upper limb function identifies that research has been performed using mirror therapy in the recovery period following hand surgery as well as amputation, stroke and CRPS. There are only 15 published studies and the majority are methodologically weak. Of the 5 studies looking, specifically, at stroke rehabilitation, there are 27 different outcome measures and 6 different standard functional scales to assess baseline function. All of the studies demonstrated a positive effect on arm function as a result of mirror therapy, but 2 did not have a control group, making it difficult to draw conclusions about the effectiveness or otherwise of mirror therapy.

The authors speculate that the effectiveness of mirror therapy in upper limb rehabilitation may be due to restoration of sensorimotor coupling through augmented sensory feedback. It is known that the primary motor cortex (M1) is modulated by ipsilateral limb movement as well as observation of the contralateral limb and mirror therapy could facilitate this by increasing feedback from an (apparently) active limb on the affected side. Given that mirror therapy is a relatively inexpensive and safe treatment strategy, further supporting evidence from larger methodologically sound studies would be extremely welcome in providing justification for its use in upper limb rehabilitation.

– **Lloyd Bradley, Western Sussex Hospitals Trust**

Ezendam D, Bongers RM, Jannick MJA. Systematic review of the effectiveness of mirror therapy in upper extremity function. *DISABILITY AND REHABILITATION* – 2009; 31(26):2135-49.

## What is in a picture?

Just occasionally I come across a paper that captures my imagination, even if I struggle to understand the details of what has been done (I am sure my clinical fellows and PhD students would say that this is the "norm"). So it is with a recent paper in *PNAS* by Hughes et al, who have undertaken a study of the drawings of the great Flemish artist Pieter Bruegel the Elder (1525-1569). The approach they have adopted is stylometry, which is defined as "The use of mathematical and statistical techniques for the analysis of artwork". Thus in this study they have essentially modelled (using a sparse coding model if that helps) some drawings of Bruegel using a novel technique, which then allows them to show that pictures imitating those of Bruegel are just that- not originals. The modelling is all too complex to me, but the paper is a fascinating read in an evolving area which may help art historians probably attribute paintings and drawings. As such, techniques such as this could impact on verification of pieces of art, although in this paper the authors are cautious in making such claims and see their technique as helping, not replacing, those individuals charged with ascribing authorship to paintings and drawings especially those of historical value.

– **Roger Barker**

Hughes JM et al. Quantification of artistic style through the sparse coding analysis in the drawings of Pieter Bruegel the Elder. *PNAS* – 2010;107:1279-83.