infantile convulsions. Cell Rep 2012; 1: 2–12.
Liao Y, Deprez L, Maljevic S, Pitsch J, Claes L, Hristova D, et al. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. Brain 2010; 133: 1403–14.
Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, et al. $Na_V1.1$ localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. J Neurosci 2007; 27: 5903–14.
Oyrer J, Maljevic S, Scheffer IE, Berkovic SF, Petrou S, Reid CA. Ion channels in genetic epilepsy: from genes and mechanisms to disease-targeted therapies. Pharmacol Rev 2018; 70: 142–73.
Schattling B, Fazeli W, Engeland B, Liu Y, Lerche H, Isbrandt D, Friese MA. Activity of $Na_V1.2$ promotes neurodegeneration in an animal model of multiple sclerosis. JCI Insight. 2016; 1: e89810.
Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. Physiol Rev 2008; 88: 1407–47.
Valtorta F, Benfenati F, Zara F, Meldolesi, J. PRRT2: from paroxysmal disorders to regulation of synaptic function. Trends Neurosci 2016; 39: 668–79.
Wagnon JL, Meisler MH. Recurrent and non-recurrent mutations of *SCN8A* in epileptic encephalopathy. Front Neurol 2015; 6: 104.
Wolff M, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in *SCN2A*-related disorders. Brain 2017; 140: 1316–36.

'Chase': in dogged pursuit of a therapy for spinal cord injury

This scientific commentary refers to 'Therapeutic efficacy of microtube-embedded chondroitinase ABC in a canine clinical model of spinal cord injury', by Hu *et al.* (doi:10.1093/brain/awy007).

New therapies for human spinal cord injuries are badly needed (Ahuja *et al.*, 2017). Developing such therapies is a challenging task because injuries are heterogeneous in terms of spinal location and severity. Ideally a therapy will work for survivors with acute injuries as well as for those with chronic injuries that happened less recently. In this issue of *Brain*, Hu and co-workers describe a successful canine clinical trial of a drug therapy for chronic spinal cord injury which led to an improvement in limb coordination during walking (Hu *et al.*, 2018).

This trial was carried out using dogs that had suffered naturally occurring spinal cord injuries; for example, as a consequence of road traffic accidents or herniated intervertebral discs, which occur commonly in Dachshunds and other dogs with disproportionally long vertebral columns. These injuries vary in terms of spinal level, severity and time since injury; as such, these dogs provide an opportunity to evaluate a therapy in a heterogeneous population of subjects prior to evaluating it in a cohort of humans with spinal cord injuries that are likely to be heterogeneous even after selection for inclusion (Fig. 1). The canine injuries have good face validity; they result from a variety of accidents rather than from controlled, defined injuries induced surgically under anaesthesia (like human injuries and unlike most other preclinical studies): the future will show if they also have good predictive validity for human spinal cord injury.

The candidate therapy assessed was chondroitinase ABC ('Chase'), which is a bacterial enzyme that removes sugar sidechains from extracellular matrix molecules including chondroitin sulphate proteoglycans. These are found within the intact and injured nervous system and are potent inhibitors of axonal growth. Degradation of chondroitin sulphate sugar sidechains with Chase has been shown to improve axon growth *in vitro* and *in vivo* in many publications in species including mice, rats, cats, squirrel monkeys (Moon *et al.*, 2001; Bradbury *et al.*, 2002; Jefferson *et al.*, 2011; Bowes *et al.*, 2012) and now dogs. After spinal cord injury, Chase has also been shown to enhance neuroplasticity in different spinal networks leading to functional improvements in breathing (Alilain *et al.*, 2011) as well as walking (Bradbury *et al.*, 2002) and grasping (Garcia-Alias *et al.*, 2009). The fact that many independent laboratories have each found benefits of Chase with few, if any, reports of side effects, is reassuring.

This clinical trial is of an unusually high methodological standard for work using animals. It is a properly controlled, randomized, clinical trial with observers blinded to intervention. The treatment was delivered using percutaneous injections into spinal cord under fluoroscopic guidance; control dogs received needle puncture of the skin to maintain blinding of assessors and owners. Relatively large volumes were injected directly into the spinal cord parenchyma and in the future, it will be important to fully evaluate the risks and safety of this approach. The trial was based on prior sample size calculations (with $\geqslant 80\%$ power to detect the effect size of interest) and a large number of dogs ($n = 60$) were randomized into the trial. The primary outcome measure was analysed on an intention-to-treat basis (i.e. involving all animals that were randomized to treatment); to enable this, statistical analyses involved a multilevel linear model that can handle

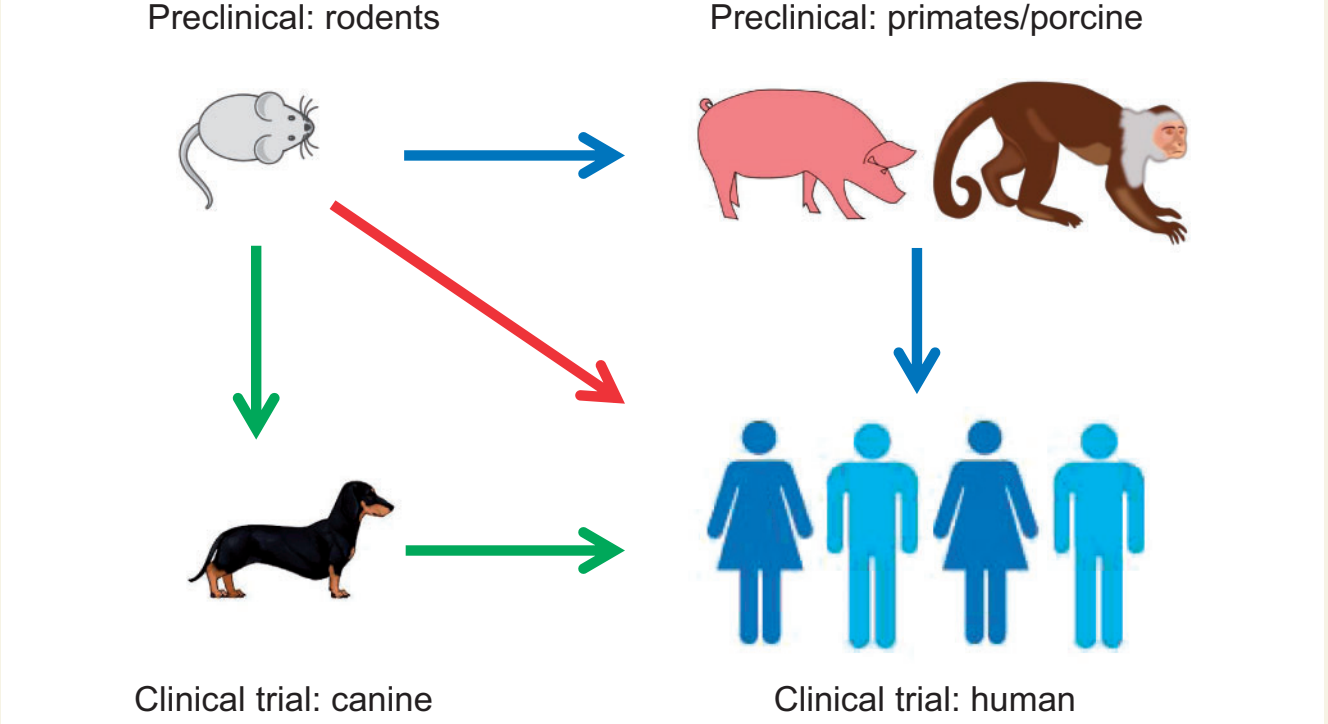

Figure 1 Route to clinical translation of Chase and other therapeutics for spinal cord injury. There is a pressing need to advance experimental therapies for spinal cord injury to the clinic. However, the clinical translation of novel therapeutics for spinal cord injury is notoriously slow. The most direct route is to advance directly from small animal (mainly rodent) preclinical studies to human trials (depicted by red arrow) and this has been the route for several stem cell and pharmacological therapies. However, to date there has been limited clinical success, possibly due to the heterogeneity of the clinical population. There is merit in using large animal models prior to human trials and current therapies such as anti-Nogo-A antibody treatment (ATI-355: NCT00406016) and autologous human Schwann cells (ahSC: NCT01739023) have followed this route (depicted by blue arrows). These preclinical models can prove valuable for dosing, biodistribution and surgical refinement studies (e.g. the porcine spinal cord is similar in size to the human spinal cord) and for evaluating efficacy in a species closer to human (e.g. primates for studies of skilled hand function). However, these studies can be precluded by prohibitive costs and ethical hurdles. Hu *et al.* used an alternative route to evaluate a promising experimental regenerative therapy (Chase). Based on preclinical basic research findings, derived largely from rodent models, they evaluated the therapy in a canine clinical population. This is a population of pet dogs that have sustained a spinal cord injury and can be enrolled in clinical trials at a veterinary research institution. This type of trial provides an opportunity to assess the potential of an experimental therapeutic in a clinical group with heterogenous injuries of different severities, sustained at different spinal levels, and whose injuries are considered chronic since they were sustained at least 3 months prior to trial recruitment, when any spontaneous recovery would have reached a plateau. Thus, this population can serve as a model for the majority of patients living with spinal injuries. In a rigorous trial design, Hu *et al.* demonstrated improvements in forelimb-hindlimb coordination on a treadmill in the treated group and a small percentage of the treated dogs even recovered independent ambulation. Although these effects may appear modest, detection of benefit in real-life heterogenous spinal injuries, with no observable adverse effects, represents a significant advance. The potential pathway from rodent basic research, to canine clinical trials and then human trials is depicted by green arrows and represents the possibility of reduced cost and accelerated progression of experimental therapies such as Chase to phase I clinical trials in humans.

missing data. As the subjects were pet dogs, their tissues were, quite understandably, not available for anatomical, molecular and biochemical assessment of mechanism of recovery, although neurophysiology was used to look for changes in connectivity. There were no differences between groups in improvement in bladder compliance. Adverse events were minor and there was no evidence for increased limb withdrawal in response to pressure applied using Von Frey filaments. Although it is stated in the paper that the primary outcome measure was pre-specified in a grant application, in the future it will be even better if veterinary clinical and preclinical trials of this kind are pre-specified publicly in a date-stamped immutable repository (e.g. in a Registered Report; https://cos.io/rr/).

The magnitude of the improvements in coordination in this paper might seem modest if one looks at the average difference between the groups but several Chase-treated dogs (3 out of 30, reflecting 10% of the treated population) recovered independent ambulation. Moreover, this effect size is likely to be a reasonable estimate of the 'true' population effect size because this is a study involving a reasonably large number of dogs, which is of very high methodological quality. Furthermore, given that Chase was able to induce recovery in dogs treated many months after spinal cord injury (i.e. after the phase of cell death is largely complete), it is encouraging that, additionally, Chase can increase recovery after

Glossary

Chase (Chondroitinase ABC): A bacterial enzyme that degrades chondroitin sulphates.
Chondroitin sulphate: Growth inhibitory glycosaminoglycan sugar side chains that extend from a proteoglycan core protein.
Neuroplasticity: The ability of axonal projections to sprout and form new synaptic connections following injury.

acute spinal cord injury via neuroprotective mechanisms (Bartus *et al.*, 2014); accordingly, the magnitude of improvements in larger animals might yet be increased if the intervention can be given earlier.

Furthermore, continued efforts are being made to optimize this therapy. To prolong activity of the enzyme, the Chase used in this study was buffered in trehalose (which stabilizes proteins and helps retain the activity of enzymes) and embedded in lipid microtubes (which enable sustained release). Prior work in rodents indicates sustained local delivery for 6 weeks with this preparation, which is an advance from previous protocols that involved multiple repeat injections (e.g. Bradbury *et al.*, 2002). However, even longer-term delivery may be necessary to achieve more significant functional improvements, as suggested by recent gene therapy studies in rodents, in which viral vector delivery of Chase enabled prolonged administration over many spinal segments (Bartus *et al.*, 2014). Other efforts are focused on generating mutated variants of Chase with improved thermal stability and mammalian compatibility, as well as pharmacological approaches to mimic the action of Chase, for example by inhibiting proteoglycan sulphation. It will be interesting to evaluate the efficacy of these emerging therapeutics, as potentially they may have a faster route to gaining regulatory approval than the native Chase enzyme.

Of note, however, there are several clinical trials, either active or completed, that have used a clinical grade preparation of bacterial Chase (SI-6603, generic name condoliase) for the treatment of patients with lumbar disc herniation involving nerve root compression. A recent randomized, double-blind, multicentre phase III trial successfully met its primary end point with significantly greater reductions in worst leg pain within 13 weeks in patients that received condoliase injected into the intervertebral disc compared to patients with control injections; their 1 year follow-up suggests that condoliase, at least when injected into a disc, is safe and well tolerated in this patient group (Chiba *et al.*, 2017). This is an important step towards first-in-human use of Chase in spinal cord injury. Nevertheless, concerns remain over potential immunogenicity of this bacterial protein when injected into the CNS. De-immunization of the protein may be required, and/or rigorous preclinical testing to prove it is non-immunogenic, before regulatory approval is granted for clinical trials involving people with spinal cord injuries who are often immunocompromised.

In conclusion, this work confirms that it is feasible, sensitive and effective to evaluate candidate therapies for spinal cord injury in well-powered, blinded, randomized, controlled trials using a heterogeneous and relatively large cohort of naturally-injured large animals. These data show that Chase is safe and effective in improving gait in another large species. Together with the positive human phase III data for herniated lumbar discs, the Chase is on!

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References

Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, et al. Traumatic spinal cord injury. Nat Rev Dis Primers 2017; 3: 17018.

Alilain WJ, Horn KP, Hu H, Dick TE, Silver J. Functional regeneration of respiratory pathways after spinal cord injury. Nature 2011; 475: 196–200.

Bartus K, James ND, Didangelos A, Bosch KD, Verhaagen J, Yanez-Munoz RJ, et al. Large-scale chondroitin sulfate proteoglycan digestion with chondroitinase gene therapy leads to reduced pathology and modulates macrophage phenotype following spinal cord contusion injury. J Neurosci 2014; 34: 4822–36.

Bowes C, Massey JM, Burish M, Cerkevich CM, Kaas JH. Chondroitinase ABC promotes selective reactivation of somatosensory cortex in squirrel monkeys after a cervical dorsal column lesion. Proc Natl Acad Sci USA 2012; 109: 2595–600.

Bradbury E, Moon L, Popat R, King V, Bennett G, Patel P, et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 2002; 416: 636–40.

Chiba K, Matsuyama Y, Seo T, Toyama Y. Condoliase for the treatment of lumbar disc herniation: a randomized controlled trial. Spine (Phila Pa 1976) 2017, in press. [Epub ahead of print]. doi: 10.1097/BRS.0000000000002528.

Garcia-Alias G, Barkhuysen S, Buckle M, Fawcett JW. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat Neurosci 2009; 12: 1145–51.

Hu H, Granger N, Pai B, Bellamkonda R, Jeffery N. Therapeutic efficacy of microtubule-embedded chondroitinase ABC in a canine clinical model of spinal cord injury. Brain 2018; 141: 1017–27.

Jefferson SC, Tester NJ, Howland DR. Chondroitinase ABC promotes recovery of adaptive limb movements and enhances axonal growth caudal to a spinal hemisection. J Neurosci 2011; 31: 5710–20.

Moon L, Asher R, Rhodes K, Fawcett J. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci 2001; 4: 465–6.