Genetic therapy for spinal muscular atrophy

Alex MacKenzie

A severe inherited neuromuscular disease is corrected in mice by intravenous gene delivery.

In the world of inherited pediatric disorders, the case of spinal muscular atrophy is particularly poignant. Most afflicted infants and children, while largely neurologically and completely cognitively intact, grow progressively weaker over time, with many ultimately succumbing to respiratory failure at a young age. Thus, research in this issue by Kaspar and colleagues[1] reporting a gene therapy rescue of the disease phenotype in a mouse model of spinal muscular atrophy is welcome news—all the more so given the authors' preliminary data suggesting that the approach could work in primates. This study, combined with the possibility of disease screening in newborns, raises, for the first time, hope of real therapeutic progress against this as yet untreatable disorder.

The autosomal recessive 5q spinal muscular atrophies (so called as the disease gene maps to chromosome 5q13.1) are characterized by a loss of motor neurons, resulting in weakness of all volitional muscles and often an ultimately unsustainable respiratory failure[2]. Although the outlook for patients with spinal muscular atrophy type I, the most common and severe form of the disease, has improved with better nutritional and particularly better respiratory care (at least in the developed world)[3], it is still one of the leading inherited causes of infant mortality.

The genetics of 5q spinal muscular atrophy are complex. Instead of the discrete disabling intragenic mutations that underlie most recessive disorders, a portion or, most frequently, the entirety of the survival motor neuron (SMN)-1 gene is usually homozygously deleted. The ubiquitously expressed and evolutionarily conserved SMN protein is involved with many aspects of RNA metabolism.

Alex MacKenzie is at the Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario, Canada.
e-mail: mackenzie@cheo.on.ca

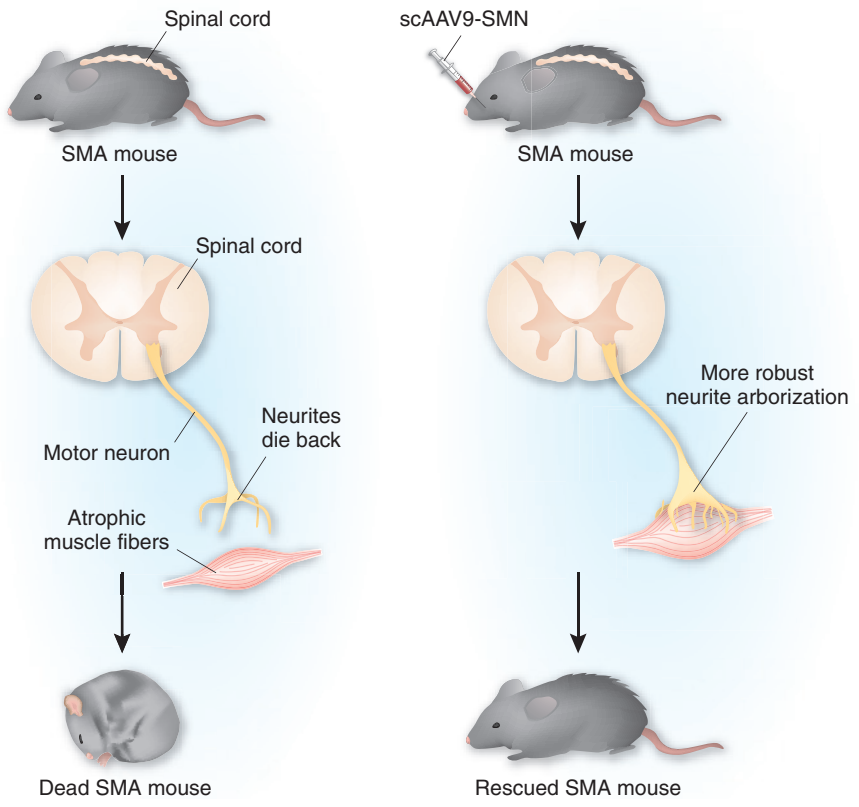

Figure 1 Gene therapy for mice with spinal muscular atrophy (SMA). SMA mice (null for the murine SMN gene and homozygous for variants of human SMN transgenes) are born with a normal motor neuron complement. However, the motor neurons undergo rapid attrition, likely a result of synaptic failure and denervation with attendant muscular atrophy. The mice become wasted and succumb at two weeks of age (left), analogous to an untreated mild human type I SMA. Injection of scAAV9-SMN into the facial vein of day-old SMA pups results in SMN expression in ~40% of motor neurons, normalization of synaptic electrophysiology and an extension of life span to >250 days, albeit at half the size of unaffected mice (right).

Unsurprisingly, in all metazoan species save humans, complete ablation of *SMN* is embryonically lethal. In humans, however, loss of *SMN1* is offset by the presence of a variable number of copies of the human-specific gene *SMN2*, which makes a small amount of SMN protein, permitting initial survival of the organism but not of all motor neurons[4].

Why motor neurons are the cell type most severely affected by loss of SMN has been a central conundrum in the field. Although many aspects of the molecular pathogenesis are still unclear, recent evidence points to a synaptopathy possibly devolving from a deficient presynaptic transcriptome, resulting in denervation and early motor neuron

attrition[4]. In distinction to the better known amyotrophic lateral sclerosis, spinal muscular atrophy seems to be a truly cell autonomous disorder: antenatal transgenic motor neuron SMN repletion is essentially curative in spinal muscular atrophy mice[5] (and presumably in humans). Whether postnatal SMN restoration would have a similar benefit was unknown until the present work.

The greater the *SMN2* copy number—both in infants and children with spinal muscular atrophy and in mouse models—the milder the disease. This observation has made robust pharmacologic inducers of *SMN2* a holy grail of translational researchers. The best result reported so far was achieved using trichostatin, a potent histone deacetylase inhibitor (a perennial drug class favorite for transcript modulation in monogenic disease treatment), combined with nutritional supplementation (also a popular choice when optimizing treatment of pediatric disease). The approach roughly tripled the 15-day lifespan of genetically engineered mice with severe spinal muscular atrophy[3].

This comparatively modest success made welcome a 2009 report from the Kaspar group[6] describing a self-complementary adeno-associated virus (scAAV)-9 vector that crosses the blood-brain barrier after systemic administration. Because scAAV9 transduced motor neurons in neonatal mice (although not in adult mice), it seemed well suited to gene therapy for spinal muscular atrophy. The self-complementarity of the scAAV genome is a crucial aspect of its potency, halving the AAV payload (happily, SMN is only 300 amino acids) but, by virtue of the dimeric inverted-repeat genomic structure, often allowing for a marked increase in transgene expression[7].

Now, the Kaspar group, working with spinal muscular atrophy pioneer Arthur Burghes, details by far the most successful rescue yet reported of a genetically and physiologically faithful mouse model of severe spinal muscular atrophy[1]. To achieve this landmark, the authors injected scAAV9 carrying *SMN1* into the facial vein of mice pups on postnatal day 1 (**Fig. 1**). The result: transduction of 40% of motor neurons and an extension of longevity from 2 weeks to 250 days and counting, combined with normal motor function and almost normalized neuromuscular electrophysiology. Intriguingly, the mice are roughly half the size of their wild-type siblings. The diminutive stature of humans with spinal muscular atrophy is well known; whether the small size of the mice results from rescue of less than half of the motor neurons and/or from a missing 'extra-motor-neuron' growth-determining role of SMN is unclear.

An obvious question with such work is whether it can be extended to primates. Anticipating this issue, the authors studied systemic injection of scAAV9-GFP in a cynomolgus macaque at 1 day of age. Four weeks later, the level of GFP transduction in spinal motor neurons was similar to that seen in mice, auguring well for application to humans.

This is heartening news on several levels. Along with recent encouraging reports of AAV gene therapy of retinal disease, it marks the further rehabilitation of gene therapy as a credible therapeutic alternative for neurologic diseases. In addition, a significant worry confronting spinal muscular atrophy researchers—given reports of 'fetal' disease, rapid postnatal motor neuron attrition observed even in the condition's milder forms and the rather limited success achieved with pharmacologic *SMN2* induction—was that a postnatal intervention may just be too late and that motor neurons were doomed irrespective of any rapid *SMN* repletion in newborns. Although extrapolation from mouse data to humans is never certain, the dramatic results reported here put pay to concerns that postnatal therapy shall be ineffective.

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So, in addition to the obvious issues of clinical safety and cross-species efficacy, what are the hurdles to clinical introduction of this mode of gene therapy? Three come to mind immediately.

First, can sufficient quantities of GMP-grade, unrecombined AAV9-SMN be generated, especially as 10^{14} viral genomes are likely to be needed to treat one infant? It seems that encouraging progress has been made on this front through work by several academic and biotech groups. The accepted wisdom is that large-scale GMP AAV production is now a tractable goal[8,9]. Clearly, the substantial cost of a single treatment (on the order of several tens of thousands of dollars) would pale in comparison to the psychosocial and monetary costs of untreated spinal muscular atrophy.

Second, might there be an immune response to the AAV capsid, effectively neutralizing its impact? (A response to SMN itself should not be a concern given that all infants with spinal muscular atrophy have low levels of SMN protein encoded by *SMN2*.) This is perhaps the most imponderable and possibly insuperable of the potential barriers. It may be that we get lucky and this is not an issue; certainly, a highly efficient expression cassette permitting therapeutic levels of expression at relatively low vector doses may allow nonimmunogenic long-term expression after systemic administration. If an immune response does occur, induction of tolerance to the capsid, genetic modification of capsid antigen, transient immunosuppression, or, as recently shown, proteasome inhibition to block presentation of capsid antigen[10] would all be credible approaches to circumventing the problem.

Third, is the presymptomatic identification of infants with spinal muscular atrophy possible and likely to be undertaken? Kaspar and colleagues[6] found that scAAV9 transduction of motor neurons is most effective in neonates, and, obviously, this is when the proverbial horse is still in the barn as regards motor neuron loss in spinal muscular atrophy. The efficacy of AAV9-SMN diminishes if mice are treated on postnatal day 5, and administration at postnatal day 10 has no effect[1]. Comparable metrics will have to be determined in primates, but clearly the testing, diagnosis and treatment must happen in rapid succession, in keeping with the 2 weeks that often elapse between birth and treatment in other newborn disorders now being screened.

The arcane genetics of spinal muscular atrophy work in our favor in this regard. In distinction to most other mutationally heterogeneous monogenic disorders, the homozygous absence of a single *SMN1* exon-7 cytosine is found in most instances of classic 5q spinal muscular atrophy. Progress in detecting this single-nucleotide polymorphism in the high-throughput fashion required for a diagnostic screen has been reported from several groups[11, 12]. We are now on the cusp of combining DNA-based universal newborn screening with mass spectrometry–based organic and protein analyte detection methods. The general expectation is that when the first such DNA-based screens are launched, spinal muscular atrophy will be among the disorders included.

Despite these issues, we seem to have a perfect storm: a relatively common (at least in the rarefied world of orphan diseases of childhood) and at present untreatable disorder; a tractable means of presymptomatic diagnosis; and, with this report, the possibility of correcting the causative deficiency. Spinal muscular atrophy, which has been a proving ground for several advances in orphan disease research, including

therapeutic pharmacologic modulation of gene expression, high-throughput drug discovery and large-scale government–academic collaborations, seems poised to again serve as an instructive case study—one that may actually cure infants of a fatal disorder.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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Targeting leukemia stem cells

Hanna K A Mikkola, Caius G Radu & Owen N Witte

Acute myeloid leukemia stem cells can be made susceptible to chemotherapy by inducing them to divide.

The goal in leukemia treatment is to permanently eradicate all leukemic cells while preserving the body's reservoir of hematopoietic stem cells. In many cases, however, a seemingly successful treatment ends in disease relapse owing to the survival of a small population of dormant leukemia stem cells that are resistant to chemotherapy. In this issue, Ishikawa and colleagues[1] describe a strategy for targeting such stem cells in a mouse model of human acute myeloid leukemia. Treatment with granulocyte colony-stimulating factor (G-CSF), a cytokine that induces mobilization and cell cycle entry of hematopoietic stem cells, causes the transplanted human leukemia stem cells to proliferate and makes them susceptible to the chemotherapeutic cytarabine (**Fig. 1**). This finding supports the hypothesis that leukemia could be eradicated at its roots by developing treatments focused on the unique properties of the rare leukemia stem cells.

Although not all malignancies fit into the cancer stem cell model[2], acute myeloid leukemia is one of the prototype diseases for which there is solid evidence of stem cell–like behavior[3]. Acute myeloid leukemia stem cells and normal hematopoietic stem cells share many surface molecules, use common self-renewal mechanisms and are predominantly in a quiescent state. Hematopoietic stem cells must be quiescent to avoid exhaustion and to minimize the risk of oncogenic events. Similarly, as shown in studies of acute promyelocytic leukemia, a subtype of acute myeloid leukemia, quiescence protects leukemia stem cells from excessive DNA damage and exhaustion, increasing their self-renewal capacity and survival[4].

> "This finding supports the hypothesis that leukemia could be eradicated at its roots by developing treatments focused on the unique properties of rare leukemia stem cells."

To maintain quiescence, both hematopoietic and leukemia stem cells interact with components of the bone marrow niche. Building on their previous work[5], Ishikawa and colleagues[1] now show that leukemia cells residing near the endosteal surface are quiescent, in contrast to those located more centrally in the bone marrow cavity. Chemotherapy with cytarabine alone killed the bulk of the leukemic cells but left the leukemia stem cells adjacent to the endosteum intact. After treatment with G-CSF, however, these latter cells began to proliferate and became susceptible to cytarabine. Although the response was variable between the mice engrafted with cells from seven different patient samples, the increased sensitivity was observed in every case, causing apoptosis and an average 100-fold drop in leukemia stem cell frequency. Although the inherent toxicity of the chemotherapy made it impossible to follow the survival of the primary transplant recipients, mice that received leukemia cells from animals treated with a combination of G-CSF and cytarabine were much less likely to develop the disease and die of it than mice receiving leukemia cells from animals treated with chemotherapy alone.

A similar approach has been shown in one clinical trial to improve disease-free survival[6], whereas little benefit was observed in others[7,8]. This difference might be explained by the fact that in the trials where no improvement was seen, the patients had a more unfavorable prognosis based on age, cytogenetic abnormalities or response to previous treatment. The study by Löwenberg *et al.*[6] indicates that the standard risk group, which excludes individuals with unfavorable prognoses, is most likely to benefit from the therapy. Future studies will better define the subgroup of patients that will respond and whether the failure to respond relates to cytogenetic differences, acquired mechanisms of drug resistance or perhaps differences in bone marrow microenvironments. Notably, the behavior of leukemia stem cells was not assessed directly in the clinical studies.

Not all individuals respond equally to G-CSF during hematopoietic stem cell mobilization, and the response is especially weak in individuals with prior chemo- or radiotherapy. Alternative approaches for hematopoietic stem cell mobilization are being investigated. For example, interferon-α induces cycling of hematopoietic stem cells, rendering them more susceptible to cytotoxic agents[9]. It would be interesting to determine whether such approaches would also induce cycling of leukemia stem cells in patients who respond poorly to G-CSF.

In addition to 'nonspecific' treatments to manipulate leukemia stem cell behavior, it may be possible to affect leukemia stem cells directly by targeting molecules required for their self-renewal and interactions with the bone marrow niche. Hematopoietic stem cells are attracted to the niche by the chemokine CXCL12 (SDF1) and its receptor CXCR4 and by the adhesion molecules VCAM-VLA4 and angiopoietin-Tie2 (ref. 10). The same molecules are also involved in leukemia stem cell–niche interactions, and elevated levels of CXCR4 and VLA4 have been associated with poor response to

Hanna K A Mikkola is in the Department of Molecular, Cell and Developmental Biology, Caius G Raduis is in the Department of Medical Molecular Pharmacology and Owen Witte is an investigator with the Howard Hughes Medical Institute, University of California, Los Angeles, California.
e-mail:owenwitte@mednet.ucla.edu