Timing of Gene Therapy Interventions: The Earlier, the Better

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The concept of gene replacement therapy is quite simple: If you replace a defective gene in a sufficient number of cells and express it at the right level, it will address the root cause of the disease leading to an effective treatment or cure. This somewhat simplistic view does not take into account the secondary pathologies that arise because of the genetic defect, which can cause potentially irreversible damage. Often, early preclinical studies test a gene therapy approach administered very early in the disease progression, before the onset of symptoms. However, because diagnosis for most patients comes after the onset of symptoms, a critical question is whether the proposed treatment can be effective at later stages. Previous studies showed encouraging preclinical data on the efficacy of a gene therapy approach to treat mucopolysaccharidosis type IIIA (MPS IIIA, or Sanfilippo A).[1,2]

In a recent issue of *Molecular Therapy—Methods & Clinical Development*, Fu and colleagues reported the results of a detailed investigation to address the effectiveness of the treatment at different stages of the disease.[3] Specifically, they dosed MPS IIIA mice intravenously with self-complementary adeno-associated virus serotype 9 (AAV9) vectors carrying the sulfoglucosamine sulfohydrolase (SGSH) gene at the ages of 1, 2, 3, 6, or 9 months old. At an early stage of the disease the treatment was highly effective, and at the middle stage of the disease the treatment was capable of halting the disease progression, but at the later stage of the disease (9 months) the treatment had minimal benefit to the mice. These results suggest that gene replacement in MPS IIIA has some potential to benefit a relevant population of patients, namely those whose early symptoms permitted diagnosis but who have not reached the stage of complete incapacitation. However, the results also suggest that there is a point of no return at which the disease pathology has moved beyond the primary defects related to the absence of SGSH. This raises two important points: (i) the question of how the ages and symptoms in mice relate to those of human patients and (ii) assuming these results translate effectively to human patients, early diagnosis is paramount.

MPS IIIA is a relatively typical example of a broader group of lysosomal storage diseases (LSDs). In this LSD, autosomal recessive mutations in the SGSH gene lead to toxic accumulation of glycosaminoglycans (GAGs) in lysosomes.[4,5] The natural progression of MPS IIIA is death in the second decade, with diagnosis coming around 4–6 years of age after the onset of significant neurological symptoms. Although the GAGs accumulate throughout the body, the nervous system is particularly sensitive to their accumulation and the most devastating aspects of the disease manifest there. Concurrent with the toxic accumulation of GAGs, eventually neuroinflammation develops along with neurodegeneration.[6] The mouse model for MPS IIIA has a naturally occurring missense mutation that leaves them with 3–4% residual SGSH activity. These mice develop disease similarly to human patients, although arguably more slowly given their median life span of 13 months.[7] This slow pace raises the question of whether these mice might instead model a milder form of the disease, given the residual SGSH activity. At earlier ages the mice have detectable GAG accumulations, and at 6–7 months old they reportedly have a rough coat, hunched posture, and reduced activity. Although it is problematic to match ages in mice to ages in humans, it might be fair to correlate this 6- to 7-month age in mice with a time when significant symptoms would be apparent in a human patient. This timing and rating of disease severity become quite important given Fu and colleagues' findings, to predict accurately which patients might expect benefit in a translational setting.

Fu *et al.*[3] found an age-dependent response to SGSH gene transfer. Importantly, the persistence and level of SGSH expression, as well as the clearance of GAGs, did not appear to be greatly influenced by the age at dosing up to 6 months old, allowing the authors to attribute any differences in efficacy to secondary (presumably irreversible) pathologies in the mice. When mice were dosed at 1 month old (described as presymptomatic), the rescue was nearly complete and the treated MPS IIIA mice tracked similarly to wild-type mice in terms of behavior, pathology, and survival. Wild-type mice had a median life span of 24 months, whereas untreated MPS IIIA mice lived 13 months. The survival of treated mice decreased according to the age at which they were dosed. Treatment at 1, 2, 3, or 6 months led to median survivals of 22, 20, 20, and 17 months, respectively. Mice treated at 9 months showed no survival benefit, even though their GAGs were reduced to levels similar to those at other ages of treatment.

These results very clearly show the benefit of earlier intervention. Although treatment at 1–3 months would probably correlate with an age in humans before typical diagnosis, at 6 months old the mice are clearly symptomatic and might correlate with an age at around the time human children are diagnosed. This result is encouraging because it shows a potential benefit of the most common patient population. However, the complete lack of functional benefit for mice with advanced symptoms (9 months old) suggests that if human patients do not receive treatment soon after diagnosis, the likelihood of any benefit is low. The study did not directly address whether any reversal of existing symptoms or pathology occurred. The data imply that the treatment essentially halts the progression of the

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disease at whatever stage it has reached, at least up to treatment at 6 months old in mice.

The age dependence of treatment intervention is a recurring theme in gene therapy studies. AAV-mediated gene transfer studies for late infantile neuronal ceroid lipofuscinosis (Batten disease) showed a very similar pattern of survival benefit, whereby treatment at pre- or early-symptomatic ages resulted in considerably better outcomes compared with treatment after the emergence of symptoms.[8,9] In a mouse model of severe spinal muscular atrophy in which the mice survived approximately 14 days, treatment at postnatal day 1 (P1) provided the most pronounced benefit, P5 resulted in a partial rescue, and P10 provided minimal, if any, benefit.[10] Another example is Canavan's disease, in which a knockout mouse model typically had an onset of symptoms at approximately 13 days and lived 27 days. Intravenous injection at P0, P7, P13, and P20 with very high doses (~4×10^{14} vg/kg) of AAV/*ASPA* vectors showed an age-dependent rescue assessed by functional measures, with all treatment ages showing some survival benefit.[11] These studies all similarly suggest that restoration of gene function in these diseases can halt disease progression but has only a limited ability to reverse secondary pathology that is a downstream consequence of the gene mutation.

The field of gene therapy has had a recent renaissance spurred by an increasing number of highly successful preclinical studies using approaches with feasible translation to humans. Improved vector technology is having an enormous impact.[12] Manufacturing technology and the capacity to readily produce human doses is increasing. Clinical trials based on those encouraging preclinical studies are starting.[13] Hopes are high, but the results of studies such as that by Fu *et al*. should perhaps temper those expectations. Most patients with genetic diseases are diagnosed after the onset of symptoms, and these are going to constitute the most common patient population. In all the studies cited above, these are the patients who are likely to receive a relatively modest benefit instead of the more dramatic rescue seen at earlier ages. This benefit could still be significantly and meaningfully better than the absence of treatment, but nowhere near a cure. Investigators involved with gene transfer trials aiming to treat degenerative diseases will need to be careful communicating the realistic potential for benefit in symptomatic patients, especially when preclinical studies are conducted at the more ideal, presymptomatic age in animal models.

The gene therapy community may not recognize this yet, but it is going to become a very strong advocate of newborn screening. The field of hematopoietic stem cell transplantation has experienced this necessity for early intervention, in particular for LSDs. An example is globoid cell leukodystrophy (Krabbe disease), which is an LSD caused by lack of the GALC gene. Hematopoietic stem cell transplantation for Krabbe disease is very beneficial—but not curative—when administered very early in life but wholly ineffective when given after the onset of symptoms.[14] These findings prompted some states, led by New York, to adopt newborn screening for Krabbe disease.[15] Most, if not all, gene transfer studies that examine the age dependence of treatment effects have concluded that earlier treatment maximizes the therapeutic effects. Assuming that clinical experience will mirror these preclinical findings, the logical progression in treating younger patients is the development and implementation of better diagnostic procedures. The optimal approach to identifying patients before the onset of symptoms, when a treatment will be maximally effective, is with newborn screening.

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