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Retracing events

More definitive answers about the death of a woman in an adeno-associated virus (AAV) gene therapy trial for arthritis must await a meeting of the Recombinant DNA Advisory Committee (RAC) later this month.

Eight years ago, the field of gene therapy was devastated by the death of Jesse Gelsinger, who suffered multiple organ failure after receiving a single injection of an adenoviral vector. In the aftermath, clinical studies were halted at the Institute for Human Gene Therapy at the University of Pennsylvania, 70 other gene trials were reevaluated by the US Food and Drug Administration and serious flaws were uncovered in the reporting of adverse events. Now the death of another human volunteer, three weeks after receiving a second injection of an AAV vector in a gene therapy trial for arthritis, is prompting comparisons. This time around, though, the link between the gene therapy and the patient's demise appears much less clear cut.

Since the first AAV gene therapy trial in 1994, the virus has been administered to ~600 people in 48 clinical trials worldwide, predominantly in single-gene disorders but also in complex diseases of the central nervous system, cancer and heart. According to the US National Institutes of Health RAC, a total of 29 adverse events have been reported from 12 US trials, none of them deemed remarkable or serious.

It is difficult to think of a more ubiquitous or innocuous virus—roughly 80–90% of the population is infected with AAV during childhood. The viral vector has a tremendously safe record in animal studies in a diverse number of species. What's more, AAV vectors in clinical use are gutted and incapable of replication, the only remnant of the AAV single-stranded (ss)DNA genome being the inverted terminal repeats flanking the transgene of interest. For these vectors to replicate *in vitro*, the additional presence of helper plasmids is required: one containing adenovirus genes, such as E1A, the other AAV replication and capsid (Cap) genes. *In vivo*, replication would require a unique condition of co-infections with adenovirus, wild-type AAV and recombinant AAV vector.

Last year, transient, self-limited and asymptomatic liver toxicity was reported in two of seven subjects receiving higher doses of a recombinant AAV serotype 2 (AAV2) vector expressing human factor IX for the treatment of hemophilia (*Nat. Med.* **12**, 342–347, 2006). Similarly, another human trial carried out by Amsterdam Molecular Therapeutics in which an AAV1 vector was administered intramuscularly to treat lipoprotein lipase deficiency also found evidence of low toxicity, with transiently elevated levels of creatine phosphokinase, a marker associated with muscle damage.

At its meeting this June, the RAC set aside an entire day to discuss these reports. What is clear is that the transient and delayed toxicity observed in these subjects originated from T cell–mediated destruction of AAV vector–transduced cells. As most people have already been exposed to AAV, memory T cells are likely be mobilized in response to challenge with higher doses of AAV vector. At present, however, it is unknown whether AAV capsid peptides processed and presented by the major histocompatibility complex class I pathway originate from the replication-incompetent vector initially administered, from contaminants generated during AAV vector preparation (e.g., capsids containing ssDNA encoding Cap rather than the transgene) or even from endogenous AAV reactivated by vector transfection. A completely different explanation is that cryptic alternative open reading frames within transgenes encode peptides containing T-cell epitopes.

Whatever the case, these events bear little resemblance to the catastrophic symptoms of Jolee Mohr. Which leads us to the Targeted Genetics trial in which she was enrolled.

The phase 1/2 dose-escalation study was designed to assess the safety of an AAV2 vector encoding a fusion of IgG1 Fc and tumor necrosis factor (TNF)-α receptor (TNFR; aka Enbrel) in adults with inflammatory arthritis and persistent moderate or severe swelling in one or more joints. Subjects concurrently on disease-modifying antirheumatic drugs or biologic TNF-α antagonists (e.g., Enbrel, Remicade or Humira) were eligible for enrollment. The rationale appears to have been that delivery of the gene therapy directly to the affected joint would result in local expression of TNFR directly at the lesion, which might be inaccessible to systemic biologic therapies.

According to Targeted Genetics, since the trial began in October 2005, none of the trial's other 126 participants had experienced "serious side effects"; 70 subjects had no ill effects whatsoever. In the case of Mohr, however, preliminary postmortem findings indicate she was sufficiently immunocompromised to contract massive infections of the opportunistic fungus *Histoplasma capsulatum* and herpes simplex virus.

Of particular note, Humira, one of the conventional antiarthritis drugs Mohr was already taking, is well known to increase susceptibility to histoplasmosis. In addition, Humira's label counterindicates its use in combination with other TNF-α blocking therapies. In hindsight, therefore, the combination of a TNFR gene therapy in people already on TNF-α blockers might have been a disaster waiting to happen. However, it is important to note preclinical data suggested that localized delivery of TNFR gene did not lead to significant increases in serum TNFR. Thus, it is plausible that the opportunistic infections were solely due to Humira.

A final point is that in the event that TNFR gene therapy is implicated in the death of Jolee Mohr, it should be viewed in the context of serious adverse events in medicine as a whole. And here the statistics are humbling. According to the *Journal of the American Medical Association* (**279**, 1200–1205, 1998), 2 million US citizens suffer drug interactions each year. Of these, 106,000 leave the hospital in body bags. This means drug toxicities are the fourth largest cause of death in the United States.

In that context, two deaths in seven years—one of which might not even be connected to gene therapy—is no reason for a regulatory crackdown. Further research on the AAV platform should be encouraged and carefully monitored clinical trials continued. If any lesson can be taken away from the events of recent weeks, it is that many more questions remain about gene therapies than answers.