High-dose AAV gene therapy deaths

The US Food and Drug Administration placed [Audentes Therapeutics'](#) phase 2 gene therapy trial for a rare neuromuscular disease [on hold](#) following the deaths of two patients receiving the higher dose of the investigational treatment AT132. Both deaths were caused by progressive liver dysfunction followed by sepsis in patients who had pre-existing liver disease. The deaths add to emerging [safety concerns](#) surrounding the use of AAV vectors. The AT132 therapy treats X-linked myotubular myopathy, a life-threatening condition characterized by profound muscle weakness from birth. The disorder is caused by a mutation in the myotubularin 1 gene, the product of which is needed for normal skeletal muscle development.

Audentes, acquired by Astellas Pharma for \$3 billion, developed AT132 to deliver a working copy of the myotubularin-1 gene to patients using an adeno-associated virus-8 (AAV8) vector. Initial results were promising. Six patients who received a low dose (1×10^{14} vector genomes (vg) per kilogram body weight), as well as one patient on the high dose (3×10^{14} vg per kg), no longer needed ventilator support and could sit, stand or walk.

The high AT132 dose delivered in the phase 2 trial was the highest for any AAV-based gene therapy to date. None of the patients who received the lower doses had liver-related adverse events, despite underlying liver disease, suggesting that toxicity was related to the higher dose or the patients' characteristics rather than to a systemic safety issue. In an [editorial](#), gene therapy pioneer James Wilson and coauthor Terence R. Flotte noted that toxicity of AT132 mirrors that seen in a [non-human primate](#) model, in which AAV directly damaged liver cells.

Toxicities have also emerged with other high-dose (at least 2×10^{14} vg per kg) AAV gene therapies, such as AveXis's Zolgensma (onasemnogene apearvovec) for spinal muscular atrophy, as well as Solid Biosciences' SGT-001 and Pfizer's PF-06939926, both in trials for Duchenne muscular dystrophy. In those studies, [adverse events](#) were not related to altered liver function; toxicity in Duchenne muscular dystrophy trials largely resulted from an immune response to AAV.

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approved in only a few indications, have limited efficacy and are generally quite toxic.

The first wave of epigenetic small-molecule drugs included the DNA methylation inhibitor Vidaza (5-azacytidine) and Dacogen (decitabine), approved for myelodysplastic syndrome and some leukemias in 2004 and 2006, respectively. Soon after, two so-called global 'histone eraser' drugs targeting the histone deacetylase (HDAC) inhibitors — Zolinza (vorinostat) and Istodax (romidepsin) — were approved for cutaneous T-cell lymphoma. But Zolinza and Istodax are not very specific epigenetic drugs. "They really target thousands of proteins," says Melnick. "There's no evidence that HDAC inhibitors carry out their antitumor function through any effect on epigenetic regulation."

The second wave of approvals included three new HDAC inhibitors. The FDA approved Beleodaq (belinostat) and Farydak (panobinostat) in 2014 and 2015, for peripheral T cell lymphoma (PTCL) and myeloma, respectively, and the Chinese FDA approved Epidaza (chidamide) for PTCL in 2015. These agents were generally more potent than their predecessors, but not necessarily more specific.

The third and newest wave of epigenetic drugs targets lysine and arginine residues on histones and is exemplified by EZH2 inhibitors like Tazverik. Post-translational modifications of specific lysines in the exposed tails of certain histones have dramatic effects on cell fate. For example, the histone mark targeted by Tazverik is crucial for the proper timing of cell differentiation, and [too much of this mark](#) can lead to cancer. "Compared with HDAC inhibitors, for example, [EZH2] is a much cleaner target," says Melnick. "And that's exciting per se, because we can really get a sense of how impactful epigenetic therapy can be."

Companies first directed their attention to EZH2 because it's overexpressed in several solid tumors, mainly prostate cancer. But in 2010 heterozygous EZH2 mutations were identified in germinal center lymphomas, including [follicular lymphomas](#). Epizyme found that the EZH2-activating mutations and the wild-type allele [cooperatively](#) drive these tumors by together adding three methyl groups to a particular histone lysine residue. "The lymphoma data seemed solid as a rock, and that's what we pursued," says Copeland.

Other companies, including GlaxoSmithKline, Constellation, Daiichi Sankyo and Pfizer, jumped in with their own EZH2 inhibitors (Table 1). Epizyme remains the leader and is now testing Tazverik in a variety of combinations in both solid

tumors and blood cancers. "Tazemetostat holds potential as a 'pipeline in a molecule,'" says Epizyme chief medical officer Shefali Agarwal.

The June FDA approval only hints at the drug's usefulness, Melnick suggests. The optimal duration and number of cycles for the therapy have not been defined, and many combinations are possible. Other epigenetic marks "might need to be targeted as well to maximally yield a therapeutic effect," says Melnick.

Epizyme seized another completely different EZH2 inhibitor opportunity in 2010 to treat rhabdoid tumors. These rare and lethal childhood cancers lose *INI1* gene expression. The INI1 protein is part of a protein complex whose function is to slide nucleosomes apart (a nucleosome is a length of DNA wrapped around histones so transcription factors can bind DNA and transcription can take place. In that year, Charles Roberts, then at the Dana-Farber Cancer Institute, reported that when INI1 is lost, cells respond by boosting EZH2, which suppresses the expression of genes vital for cell differentiation. With hyperactive EZH2, cells are unable to differentiate and remain in a stem-cell-like state. Rhabdoid cancer is the result. Knocking down EZH2 completely [blocks tumor formation](#). This is an example of synthetic lethality — a genetic deletion setting the conditions for an otherwise innocuous intervention to kill cells.

Epizyme immediately noticed. "As soon as we published it, they [Epizyme] tried it in their [rhabdoid tumor cell lines](#)," recalls Roberts. "Works great. Then they called, and I went over and gave a lecture ... that's how they got interested." (Roberts says he has never taken any funds from Epizyme.) An Epizyme trial in adults with rhabdoid-like tumors lacking INI1 showed good results in epithelioid sarcoma, an aggressive soft tissue cancer. (Pediatric rhabdoid tumor trials are ongoing.) Epizyme later launched a pivotal phase 2 adult epithelioid sarcoma trial. In it, 9 of 62 patients taking Tazverik achieved an [objective response](#), including one complete response. FDA approval followed. Pediatric trial data reported at the American Society of Clinical Oncology annual meeting in June were similar (17% of patients responded). The many non-responders puzzle Roberts, now at St. Jude Children's Research Hospital, because those patients' tumors, too, lack INI1 mutations and have hyperactive EZH2. "Why doesn't every single tumor just melt away?" Roberts asks. He is now looking into possible resistance mechanisms, which may have to do with the negative consequences of knocking down EZH2 at [certain sites](#) in the genome.