PTC readthrough

By Chris Cain, Senior Writer

There is ample evidence that **PTC Therapeutics Inc.**'s lead compound ataluren promotes premature stop codon readthrough *in vivo*, but at least two academic groups have found that the Duchenne muscular dystrophy and cystic fibrosis therapy has no activity in *in vitro* assays used to measure readthrough.^{1,2} Resolving the discrepancy will require a better understanding of ataluren's mechanism of action and could help inform the development of next-generation therapeutics.

Many genetically inherited diseases are caused by nonsense mutations that give rise to premature termination codons (PTCs), which reduce or eliminate the production of full-length, functional protein. These include about 5%–10% of cystic fibrosis (CF) cases and 10%–15% of Duchenne muscular dystrophy (DMD) cases. CF is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*), and DMD is caused by mutations in *dystrophin* (*DMD*).

Proof of concept for using small molecules to promote PTC readthrough was established by academic studies of the aminoglycoside antibiotic geneticin (G418), which directly binds to ribosomes and alters the fidelity of translation. However, the toxicity of that compound class led researchers to conduct extensive screens to identify nonaminoglycoside compounds with similar effects on translation.

The lead molecule to emerge from these efforts was ataluren (PTC124), a nonaminoglycoside, readthrough-promoting agent developed by PTC Therapeutics.³ The compound has completed a Phase IIb trial in DMD and a Phase III trial in CF. It is under review to treat nonsense-mediated DMD in the EU.

Although it missed the primary functional endpoints in both the Phase IIb and III trials, nonsignificant therapeutic trends were observed, and publications of data from mice or patient samples showed that ataluren significantly increased *dystrophin* and *CFTR* expression compared with controls.^{3,4}

Controversy arose in 2009 when a group led by James Inglese at the **National Human Genome Research Institute** published in the *Proceedings of the National Academy of Sciences* that ataluren can directly bind to firefly luciferase and drive an increase in luminescence in an *in vitro* reporter system.²

Because a screen initially used to identify ataluren relied on increasing firefly luciferase luminescence as a proxy for detecting PTC readthrough,³ this result raised questions about how the compound was selected for further study. In a separate experiment that used a different

luciferase protein to measure PTC readthrough, ataluren had no effect.

Inglese, who is head of the laboratory of assay development and screening technology at the **National Center for Advancing Translational Sciences** and adjunct investigator at the National Human Genome Research Institute, declined to comment specifically on ataluren or the new study.

Now, a group at the **University of Dundee** has raised additional questions about whether ataluren promotes readthrough activity *in vitro*.¹

The team used a diverse set of assays designed to measure readthrough *in vitro*, including two different luciferase assays, a β -galactosidase assay and assays measuring collagen expression. The researchers also performed experiments in which the identity of the stop codon was varied to see if nucleotide context played a role in sensitivity to readthrough agents.

In all experiments, geneticin induced dose-dependent stop codon readthrough. Ataluren had no significant effect at any dose in any assay.

Stuart McElroy, the Dundee screening scientist who led the study, told *SciBX* that he did not set out to raise questions about ataluren.

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—PTC Therapeutics Inc.

“We originally used ataluren and the aminoglycoside antibiotics as standard molecules to validate the [readthrough] assays we were developing. We felt comfortable that the assays were working and useful because of the positive results with the aminoglycosides,” he said. “We could not, however, explain the negative results for ataluren except within the context of the published off-target activity on firefly luciferase. We decided that the best

place for the data was in the public domain for the scientific community to judge.”

Results were published in *Public Library of Science Biology*.

Context matters

PTC Therapeutics said it disagrees with the general claim that ataluren does not promote readthrough *in vitro*, adding that the use of cDNA templates that lack introns could be responsible for these seemingly contradictory results.

In a statement to *SciBX*, PTC Therapeutics said ataluren has a long history of positive results in diverse model systems.

“Twelve published studies completely independent of PTC Therapeutics have demonstrated that ataluren promotes readthrough of premature translation termination codons. These results are from a large set of nonsense alleles in diverse experimental systems addressing multiple genetic disorders,” the company said.

David Bedwell, professor of microbiology, genetics and cell biology at **The University of Alabama at Birmingham**, agreed. “Nonsense suppression with aminoglycosides is very easy, so just about anyone can get them to suppress nonsense mutations. However, they’re not relevant for clinical use,” he said. Bedwell is a consultant for PTC Therapeutics and has worked on readthrough compounds including ataluren.

One key difference is that unlike aminoglycosides, the molecular target of ataluren remains unknown, which precludes a precise

understanding of how the compound promotes readthrough. “Ataluren has been shown to clearly promote readthrough, and the most interesting unresolved question about its mechanism of action is the precise nature of its target,” PTC Therapeutics said.

The company said that because the systems used by McElroy do not contain introns, they may not accurately model how the compound works *in vivo*. “One key element in our studies was the inclusion of an intron in the reporter gene. The McElroy manuscript used cDNAs lacking introns and thus did not take into account the potential impact of the pre-mRNA splicing process on the translation of nonsense-containing mRNAs,” the company said. “This difference is important because several studies have shown that mRNAs derived from intronless precursors or mRNAs otherwise deprived of specific exon-junction complex proteins are markedly deficient in translation activity.”

Olivier Namy, associate scientist at the **Centre National de la Recherche Scientifique** (CNRS) and the **University of Paris-Sud 11**, agreed that this could be important. “One possibility would be that ataluren only acts on PTCs found in endogenous mRNA and not on PTCs found in reporter systems,” he said. “The main difference between both mRNAs is the presence of introns. This leads to an important difference as endogenous mRNAs are sensitive to nonsense-mediated decay.”

In his own lab, Namy is screening for compounds that promote readthrough.

McElroy agreed that differences could be due to the DNA template used. “We and others have shown that stop codon context, including the downstream nucleotide, results in differences in the fidelity of the stop signal and apparently how much of a readthrough effect geneticin has, although geneticin was active for every sequence,” he said. “There may be more complexity to the story with sequence elements, further downstream or upstream of the stop codon, that are required to observe activity of PTC124 in the *dystrophin* or *CFTR* genes; however, this is just speculation.”

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**—Stuart McElroy,
University of Dundee**

PTC Therapeutics is continuing to work to understand precisely how ataluren acts to promote readthrough. Ataluren is currently in a Phase III open-label trial in patients with DMD, and the company plans to begin a Phase III trial in patients with CF in 2H13.

Inglese said that the controversy illustrates a larger point about the need for redundancy in screening systems. Last year, his team published work in *Nature Methods* describing a screening system that integrates readouts from two independent reporter genes.⁵

“One of the broader implications of this work, an area we have explored deeply, points to the need to more fully understand the mechanistic liabilities of technologies forming the basis of high throughput screening assays and other drug discovery and development processes. In the present work, reporter bias appears to lead to the confounding results, and this is a pervasive screening problem in general,” he said.

Cain, C. *SciBX* 6(30); doi:10.1038/scibx.2013.779
Published online Aug. 8, 2013

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