



application of AT1R antagonism as a therapy for patients with LDS. There is both retrospective and evolving prospective evidence that AT1R antagonism may be beneficial to patients with Marfan syndrome (6, 18, 19). Together, the findings in patients with Marfan syndrome and the results from the Gallo et al. study (12) indicate that patients with LDS may potentially benefit from AT1R antagonism. Losartan has been the ARB of choice in most ongoing trials; however, the use of an ARB with a more favorable pharmacokinetic profile and longer half-life may enhance the protective effects against TAAs. The availability of the LDS mouse described by Gallo et al. (12) provides a model to determine the relative efficacies of this class of drugs before application to humans.

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Toward postnatal reversal of ocular congenital malformations

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Aniridia is a panocular disorder that severely affects vision in early life. Most cases are caused by dominantly inherited mutations or deletions of the PAX6 gene, which encodes a transcription factor that is essential for the development of the eye and the central nervous system. In this issue of the JCI, Gregory-Evans and colleagues demonstrate that early postnatal topical administration of an ataluren-based formulation reverses congenital malformations in the postnatal mouse eye, providing evidence that manipulation of PAX6 after birth may lead to corrective tissue remodeling. These findings offer hope that ataluren administration could be a therapeutic paradigm applicable to some major congenital eye defects.

Conflict of interest: José-Alain Sahel is a founder of and consultant for GenSight and Pixium Vision and a consultant for Sanofi and Gene Signal.

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Mutations that inactivate gene function by promoting premature translational termination cause a large number of human diseases. It is thought that at least one-third of all genetic diseases and many types of can-

cer are the result of such mutations (1, 2). These mutations are referred to as nonsense mutations, premature stop mutations, or premature termination codons (PTCs). Given that PTCs often result in a complete loss of protein function, the associated diseases usually manifest as severe phenotypes. Examples of PTC-associated diseases include CF, Duchenne muscular dystrophy (DMD), and aniridia, among others.

Aniridia, a panocular disorder

Aniridia is a rare eye disease with an estimated prevalence of approximately 1 in 40,000 to 1 in 100,000 individuals. It is present at birth and characterized by a total



or partial absence of iris tissue and foveal hypoplasia that results in reduced visual acuity, with eyesight in the range of 20/100 to 20/200, and nystagmus (reviewed in ref. 3). There are multiple ocular abnormalities associated with aniridia, although these vary within and between families. The associated ocular defects usually manifest later in life and may include cataract, glaucoma, corneal opacification and vascularization, lens subluxation, strabismus, and optic nerve coloboma and hypoplasia. Patients with aniridia may have other sensory deficits, including reduced olfaction, hearing difficulties, and, rarely, behavioral problems and developmental retardation. About two-thirds of aniridia cases are familial, as the result of dominantly inherited mutations or deletions of the *PAX6* gene (3). About 90% of the reported *PAX6* mutations lead to termination of protein translation (4), which generates truncated unstable and biologically inactive proteins.

Highlights on the role of PAX6 in eye development

The *Pax6* gene is located at 11p13 chromosome and encodes a highly conserved transcriptional regulator with two DNA binding domains and a transcriptional transactivation domain. *Pax6* is expressed in all structures of the developing eye and in regions of the forebrain, hindbrain, cerebellum, and spinal cord (5–7), the olfactory system (8), and endocrine pancreas (9). Based on a very complex transcriptional regulation, *Pax6* displays highly complex spatiotemporal and quantitative expression patterns in all of these various developmental contexts (10, 11).

PAX6 plays a role at multiple stages during eye development. In early stages, *PAX6* expression in the surface ectoderm is essential for formation of the lens, while at later stages, expression in the lens vesicle is necessary for lens fiber differentiation and crystallin gene expression (reviewed in refs. 11, 12). Correct *PAX6* dosage is critical for the development of eye and brain, and both overexpression and haploinsufficiency have phenotypic consequences. In humans, *PAX6* haploinsufficiency causes aniridia (13), while in mice, *Pax6* haploinsufficiency results in the small eye (*Sey*) phenotype (heterozygous *Pax6^{Sey}/-* mice) (14). Homozygous mice (*Pax6^{Sey/Sey}* mice) die in the perinatal stage and present with multiple severe brain abnormalities, no eyes, and no nasal structures (6, 14–16). Overexpression of the *PAX6* gene also results in eye and brain malformations (17, 18), confirming

that the appropriate level of expression is crucial for proper development. Inappropriate expression of *Pax6* is even able to induce ectopic eyes in flies and frogs (19, 20). Postnatally, strong expression of *Pax6* is detected in neurons of various brain regions, including the olfactory bulb (7, 21). In both embryonic and adult neurogenesis, *Pax6* is an important regulator of neural stem cell proliferation and differentiation into neurons (12, 22). Although knowledge of *PAX6* functions in differentiated cell types remains very scarce, it is now clear that *PAX6* is maintained in several cell types of the adult eye (lens epithelium, corneal limbus, pigmented ciliary body, iris) and is implicated in self-renewal and regeneration of ocular structures (reviewed in refs. 11, 23).

Ataluren-containing formulation reverses congenital eye defects

In this issue of the *JCI*, Gregory-Evans et al. (24) report exciting data on postnatal manipulation of *Pax6* dosage through a mutation-independent nonsense mutation suppression strategy in the *Pax6^{Sey}/-* mouse eye. The *Pax6^{Sey}/-* mouse is a relevant model of human aniridia, with a naturally occurring Gly194X stop codon mutation (UGA) in the *Pax6* gene (14). For in vivo studies, systemic administration of the nonsense suppression drug 3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl] benzoic acid (ataluren, also known as PTC124) (25) was used. Systemic administration of ataluren had limited benefits on the cornea and induced an incomplete functional rescue. To increase the drug concentration in the eye, a topical application of 1% aqueous ataluren suspension was used. Use of this topical formulation resulted in a partial histological rescue of the retina and lens defects; however, these beneficial effects were accompanied by marked ocular irritation in the *Pax6^{Sey}/-* mouse. In order to improve particle dispersion properties and increase suspension viscosity and ocular exposure, a unique ataluren-containing formulation was designed, referred to as START (0.9% sodium chloride, 1% Tween 80, 1% powdered ataluren, 1% carboxymethylcellulose). Importantly, START not only abolished the irritation but also reversed lens and retinal defects in the *Pax6^{Sey}/-* eyes. The effects were greater than those induced by ataluren alone, administered either topically or systemically, and the rescue of the corneal deficit achieved WT characteristics. The ELISA analysis of START-treated *Pax6^{Sey}/-*

retinal and corneal epithelium protein lysates revealed that *Pax6* protein levels increased to 90% ± 5% of WT following treatment. Impressively, START therapy improved functional deficits of the retina, as measured both by electroretinography scotopic b-wave amplitudes and a behavioral optokinetic tracking test. In both cases, there was substantial benefit from START therapy compared with the other tested treatments. Furthermore, START promoted responses that were similar to those measured in WT animals. The effects of ataluren and the START formulation were compared with those of gentamicin, an aminoglycoside antibiotic known to suppress stop codons in vitro and in vivo (26). As expected, treatment with gentamicin resulted in remarkable normalization of the eye malformation defects. Because *Sey* mice that are homozygous for *Pax6* mutations (*Pax6^{Sey-1Neu}* mice) did not respond to START therapy, the authors suggested that this treatment specifically targets nonsense mutations.

Ataluren for genetic disorders caused by nonsense mutations

The original identification of ataluren arose from a cell-based luciferase assay of nonsense codon suppression and demonstration of selective ribosomal readthrough of PTCs (25). Initially, ataluren was determined to be effective in models of DMD (25). Ataluren treatment was shown to promote production of full-length and functionally active dystrophin in primary muscle cells from humans and mice expressing dystrophin nonsense alleles. Furthermore, ataluren administration rescued striated muscle function in a mouse model of DMD. The effect of ataluren in this study was found to be superior to gentamicin. Aminoglycoside antibiotics were previously shown to promote in vivo readthrough of nonsense mutations, resulting in expression of full-length proteins and/or correction of the protein function (26–28). Encouraging results were also obtained in models of CF, in which ataluren effectively suppressed the hCFTR-G542X nonsense mutation in vivo (29). Based on these proof-of-concept studies, human clinical trials have been undertaken. The safety profile of single- and multiple-dose administration of ataluren was demonstrated in healthy adult volunteers (30). The oral administration of ataluren suppressed nonsense mutations and reduced the epithelial electrophysiological abnormalities caused by CFTR dysfunction.



tion in patients with CF (31, 32). Because ataluren is not specific to a gene but to a type of mutation, it is believed that it could be an efficient treatment for many other disorders that are associated with nonsense mutations. Indeed, correction of nonsense bone morphogenetic protein receptor (*BMP2*) and *SMAD9* mutations by ataluren in lung- and blood-derived cells from patients with pulmonary arterial hypertension has also been demonstrated (33).

Doubts on the mechanism underlying the effect of ataluren

Recently, the effectiveness of ataluren as a nonsense suppression agent has been questioned. In their initial characterization, Welch et al. described upregulation of luciferase reporter activity in response to ataluren, which they attributed to readthrough of the PTC (25). Other studies found that ataluren can increase luciferase reporter activity independently of PTC readthrough (34, 35). McElroy et al. (36) used a panel of PTC reporter assays, including transient transfection, stable cell lines, plate-based functional enzyme assays, and direct protein detection to compare activity of ataluren to the well-characterized readthrough activity of geneticin. In all of these assays, the activity of geneticin was documented, while ataluren had no measurable effects. Dual mechanisms of action for ataluren, a molecular target other than the luciferase reporter (35), or differences in the *in vivo* and *in vitro* activity of ataluren (36) have all been suggested as the source of disparity in these controversial findings. It should be considered also that multiple factors can affect the response to readthrough treatments, including identity of the PTC and its sequence context, the chemical composition of the readthrough drug, the level of PTC-bearing transcripts, and variability in nonsense-mediated mRNA efficiency (reviewed in ref. 37). In fact, while the underlying mechanism of ataluren action remains unclear, the efficacy of this drug has been confirmed both in animal models and clinical trials, particularly those focused on CF (29, 31). Further evidence of ataluren-mediated translational readthrough efficacy was reported in Usher syndrome type 1C. For this disease, ataluren treatment of cell culture, retinal explant, and *in vivo* models has been demonstrated to recover harmonin expression and restore harmonin scaffolding function (38). Cultured cells from patients with infantile neuronal ceroid

lipofuscinosis, which is the result of nonsense mutations in palmitoyl protein thiosterase 1 (*PPT1*), exhibited a restoration of PPT1 enzyme activity following ataluren treatment that was virtually identical to the PPT1 enzyme activity restored by gen-tamicin treatment (39). Another word of caution should be expressed regarding the specificity of ataluren and its possible off-target undesirable effects.

Exon skipping versus readthrough

Theoretically, the readthrough approach is applicable to all nonsense mutations; however, an exon-skipping approach is specific to certain types of mutations, including deletion, nonsense, splice site, and duplication mutations (reviewed in ref. 40). Successful exon skipping requires specific oligos that are designed for each exon and use small synthetic modified RNA or DNA molecules (antisense oligonucleotides [AO]). The AOs must be able to recognize, bind, and mask the targeted exon from the splicing machinery and promote specific exon exclusion from the mature mRNA (41). The entire exon-skipping process leads to restoration of the genetic code and the protein function. Most nonsense mutations in the *DMD* gene can be treated either with exon skipping or the readthrough strategy discussed above, with both strategies being tested in clinical trials. A first trial in patients with DMD reported that treatment with different doses of ataluren daily for 4 weeks was well tolerated; however, ataluren failed to meet statistical significance in its primary end point, which was improvement in the six-minute walking distance (6MWD) test within the 48-week duration of the phase IIb trial. It should be noted that ataluren slowed the loss of walking ability, as measured by the time to persistent 10% worsening in 6MWD (42). Similarly, drisapersen (also known as GSK2402968 and PRO051), an investigational AO currently in late-stage development for treatment of patients with DMD, did not provide significant functional improvement (as measured by the 6MWD test) compared with placebo in a phase III clinical study (43).

Conclusion

The mechanism(s) of action underlying the effects of ataluren and its functional efficacy remain to be fully determined. Despite controversial data from animal and human studies, readthrough mutation-targeted therapies, in principle, hold promise as a common therapeutic paradigm for a

large number of genetic disorders that are caused by nonsense mutations. The study by Gregory-Evans et al. (24) demonstrates that the ataluren-containing START formulation promoted the synthesis of a full-length PAX6 protein and stably reversed congenital ocular malformations in postnatal mouse eye. These new findings offer additional experimental support for the beneficial effects of ataluren in genetic diseases caused by nonsense mutation and some basis for extending the therapeutic potential of ataluren toward congenital eye malformations.

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