Aminoglycoside-Induced Premature Stop Codon Read-Through of Mucopolysaccharidosis Type I Patient Q70X and W402X Mutations in Cultured Cells

Makoto Kamei • Karissa Kasperski • Maria Fuller •
Emma J. Parkinson-Lawrence • Litsa Karageorgos •
Valery Belakhov • Timor Baasov • John J. Hopwood •
Doug A. Brooks

Received: 19 April 2013 / Revised: 13 August 2013 / Accepted: 25 September 2013 / Published online: 6 November 2013
© SSIEM and Springer-Verlag Berlin Heidelberg 2013

Abstract The premature stop codon mutations, Q70X and W402X, are the most common α -L-iduronidase gene (IDUA) mutations in mucopolysaccharidosis type I (MPS I) patients. Read-through drugs have been used to suppress premature stop codons, and this can potentially be used to treat patients who have this type of mutation. We examined the effects of aminoglycoside treatment on the IDUA mutations Q70X and W402X in cultured cells and show that 4,5-disubstituted aminoglycosides induced more read-

through for the W402X mutation, while 4,6-disubstituted aminoglycosides promoted more read-through for the Q70X mutation: lividomycin (4,5-disubstituted) induced a 7.8-fold increase in α -L-iduronidase enzyme activity for the W402X mutation; NB54 (4,5-disubstituted) induced a 3.7 fold increase in the amount of α -L-iduronidase enzyme activity for the W402X mutation, but had less effect on the Q70X mutation, whereas gentamicin (4,6-disubstituted) had the reverse effect on read-through for both mutations. The predicted mRNA secondary structural changes for both mutations were markedly different, which may explain these different effects on read-through for these two premature stop codons.

Communicated by: Frits Wijburg, MD, PhD

Competing interests: None declared

Makoto Kamei and Karissa Kasperski are equal first authors.

This work was supported by an NHMRC project grant (NHMRC 511321; DAB and MF) and an NHMRC Senior Research Fellowship (DAB).

M. Kamei (✉) • K. Kasperski • E.J. Parkinson-Lawrence •
D.A. Brooks

Mechanisms in Cell Biology and Diseases Research Group,
School of Pharmacy and Medical Sciences, Sansom Institute for
Health Research, University of South Australia, South Australia
5001, Australia
e-mail: makoto.kamei@sahmri.com

L. Karageorgos • J.J. Hopwood
Lysosomal Diseases Research Unit, South Australian Health and
Medical Research Institute, Adelaide, South Australia 5001,
Australia

M. Fuller
SA Pathology, North Adelaide, South Australia 5006, Australia

V. Belakhov • T. Baasov
Schulich Faculty of Chemistry, Technion, Israel Institute of
Technology, Haifa 32000, Israel

Introduction

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disorder caused by a deficiency in the lysosomal exo-hydrolase, α -L-iduronidase (Neufeld and Muenzer 1995). MPS I patients exhibit clinical symptoms that include mental retardation, physical disability, short stature, skeletal deformity, somatic tissue pathology, and coarse facial features. There are three recognized MPS I clinical subgroups, which represent different points in a continuous clinical spectrum: Hurler, Hurler–Scheie, and Scheie syndromes. The majority of MPS I patients (up to 70%) present with Hurler syndrome, and have early onset and rapid disease progression (Bunge et al. 1994; Gort et al. 1998; Brooks 2002).

Molecular genetic studies show that most MPS I patients have a premature stop codon mutation in one or both alleles (Scott et al. 1992b, 1993; Hein et al. 2004;

Brooks et al. 2006). At least 17 different α -L-iduronidase premature stop codon mutations have been detected, and over 90% of Caucasian MPS I patients have at least one of these mutations (Hein et al. 2004). The association of premature stop codon mutations with early onset and rapidly progressive Hurler syndrome is the result of an inability to synthesize a full-length polypeptide and the ensuing dire consequences on enzyme activity (Scott et al. 1992b, 1993; Brooks 2002). The two α -L-iduronidase gene (IDUA) premature stop codon mutations, Q70X and W402X, are the most common mutations in MPS I patients (Scott et al. 1992a, b; Bunge et al. 1994) and this skews the clinical spectrum towards Hurler syndrome.

In people with a genetic disease caused by premature stop codon mutations, ribosomal read-through is a potential treatment strategy (Wilschanski et al. 2003; Lai et al. 2004). In a preclinical study using cultured cells, we demonstrated that gentamicin enhanced the read-through of IDUA premature stop codons and induced the synthesis of significant amounts of active α -L-iduronidase (Keeling et al. 2001; Hein et al. 2004). Aminoglycosides such as gentamicin can induce ribosomal read-through, by binding to the eukaryotic ribosome, causing a conformational change that induces faulty stop codon recognition (Francois et al. 2005). Aminoglycoside binding effectively lowers the docking energy required for the near-cognate transfer RNAs (tRNA), enabling amino acid substitution (Fourmy et al. 1998a, b; Pape et al. 2000). Glutamine and tryptophan have been reported to be the two most common amino acid insertions for mammalian stop codon read-through (Harrell et al. 2002) and these coincidentally relate to the high frequency of Q70X and W402X mutations in MPS I (Brooks et al. 2006).

Extended administration of aminoglycosides to patients can cause nephrotoxicity and ototoxicity, a complication that is not ideal for a therapeutic agent (Darlington and Smith 2003; Rougier et al. 2004; Bitner-Glindzicz and Rahman 2007; Touw et al. 2009). Developing compounds with increased read-through capacity and reduced toxicity will therefore improve therapeutic potential. The paromomycin derivatives NB30 (Rebibo-Sabbah et al. 2007) and NB54 (Nudelman et al. 2009) have been reported to have significant read-through capacity and minimal toxicity (Nudelman et al. 2009; ClinicalTrials.gov 2010b). In addition, NB30 and NB54 have recently been shown to exhibit excellent biocompatibility and low toxicity *in vivo* (Goldmann et al. 2010, 2012). Here we evaluated six aminoglycosides (Fig. 1) for their capacity to induce premature stop codon read-through for the IDUA mutations Q70X and W402X established in CHO-K1 cells. We also examined the transcript secondary structure of these mutations to gain an appreciation of the differential efficacy for read-through.

Experimental Procedures

Aminoglycoside compounds for premature stop codon read-through. Amikacin, gentamicin, lividomycin, and paromomycin were purchased as sulfate salts (Sigma-Aldrich, Sydney, Australia).

Cell culture and extract preparation. CHO-K1 cells containing the human MPS I mutations Q70X and W402X (CHO-Q70X and CHO-W402X; with UAG premature stop codons) and the wild-type CHO-IDUA were cultured and harvested as previously described (Hein et al. 2004). The CHO-IDUA, CHO-Q70X, and CHO-W402X cell lines were generated as stably transfected CHO cell lines expressing either the human cDNA coding for α -L-iduronidase or its respective mutant forms, as previously described (Hein et al. 2004). For read-through analysis, the CHO-K1 cells were cultured to confluence in T-75 flasks (Greiner Bio-One, Monroe, NC, USA), harvested and seeded (5×10^5 cells in 2 mL of F12 culture media) into six-well culture plates (Nunc, Rochester, NY, USA). The cells were cultured for 24 h, then incubated with read-through drugs for 96 h (in fresh F12 culture media), before being harvested (Hein et al. 2004).

Human MPS I fibroblasts were established from skin biopsies archived in the National Referral Laboratory for Lysosomal, Peroxisomal and Related Genetic Disorders at SA Pathology, Adelaide, Australia, with the approval of the Children, Youth and Women's Health Service Research Ethics Committee. These fibroblasts were established and maintained as previously described (Ashton et al. 1992). Triplicates of each Q70X, W402X and unaffected fibroblast cell lines were cultured to confluence in T-75 flasks before the addition of the read-through drugs (for 96 h in BME culture media). The cells were then washed twice with PBS, resuspended in 200 μ L 20 mM Tris-HCl (pH 7) containing 0.5 M NaCl, and then sonicated for 20 s to yield cell extracts (Myerowitz and Neufeld 1981). To remove cellular debris, fibroblast cell extracts were centrifuged at 17,000g for 10 min at room temperature; the supernatant was then stored at -20°C for subsequent analysis of protein and enzyme activity. The protein in cell extracts was determined using a Pierce Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Sydney, Australia); α -L-iduronidase activity was determined by a fluorometric immunobinding assay as previously described (Hein et al. 2003).

Secondary structure predictions of IDUA transcripts. The predicted secondary structures of wild-type (full-length sequence; GenBank Accession Number NM_000203) and mutant IDUA transcripts (Q70X mutation: C to T base change at position 296; W402X mutation: G to A base change at position 1294) were generated using the RNA

Fig. 1 Structure of the aminoglycosides used in this study, including 4,6-disubstituted compounds gentamicin and amikacin and the 4,5-disubstituted compounds lividomycin, paromomycin, NB30, and NB54

fold web server (<http://rna.tbi.univie.ac.at>; (Gruber et al. 2008)). The Minimum Free Energy algorithm (Zuker and Stiegler 1981) was used to predict the secondary structure; comparisons were made by overlapping the structures electronically using graphics files to identify landmark structural elements near the mutations. Putative ribozyme structures were detected visually by inspection of the altered secondary structures and by known ribozyme sequence requirements (Reymond et al. 2009).

Results

Aminoglycoside-induced read-through in CHO-K1 cell lines. CHO-K1 cells (no IDUA construct) had no detectable α -L-iduronidase activity either before or after treatment with aminoglycosides (data not shown); which involved detection of only human IDUA activity by specific immune capture (Hein et al. 2003). The maximum read-through response was defined for the aminoglycosides (0.5 mg/mL for gentamicin, amikacin, and paromomycin; and 2.0 mg/mL for lividomycin, NB30, and NB54), using CHO-Q70X

and CHO-W402X cells (data not shown). In CHO-Q70X cells, α -L-iduronidase activity increased 3.1-fold with gentamicin, 1.8-fold with amikacin, and 2.2-fold with paromomycin, when compared to an untreated CHO-Q70X control (Fig. 2a). In CHO-W402X cells, α -L-iduronidase activity increased 1.7-fold with gentamicin, 1.8-fold with amikacin, and 3.1-fold with paromomycin, when compared to an untreated CHO-W402X control (Fig. 2b). α -L-Iduronidase activity did not increase in CHO-IDUA cells following treatment with any of the aminoglycosides (data not shown).

There was a significant increase in α -L-iduronidase activity in CHO-Q70X cells treated with 2 mg/mL of either NB30 (1.2-fold) or NB54 (1.7-fold), when compared to untreated CHO-Q70X cells (Fig. 3a). In CHO-Q70X cells, treatment with 0.5 mg/mL gentamicin resulted in higher α -L-iduronidase activity than either NB30 or NB54 (Fig. 3a). Some experimental variability was observed for the amount of IDUA activity in response to gentamicin read-through, for different experiments (Figs. 2, 3). Treatment of CHO-W402X cells with either 0.5 mg/mL or 2 mg/mL NB30 had no significant effect on α -L-iduronidase activity (Fig. 3b)

Fig. 2 Aminoglycoside treatment of CHO-Q70X and CHO-W402X cells. CHO-Q70X (Panel a) and CHO-W402X (Panel b) expression cells were treated with either gentamicin (0.5 mg/mL), amikacin (5 mg/mL), or paromomycin (4 mg/mL) and read-through assessed by analysis of α -L-iduronidase activity relative to an untreated control. Results were expressed in pmol/min/mg of α -L-iduronidase activity, corrected for total cell protein and represented the mean $\pm$ SD for three independent replicates. * and ** represent a significant difference from the untreated control at, respectively, $p < 0.05$ and $p < 0.001$

Fig. 3 NB30 and NB54 treatment of CHO-Q70X and CHO-W402X cells. CHO-Q70X (Panel a) and CHO-W402X (Panel b) expression cells were treated with either NB30 (0.5 or 2 mg/mL) or NB54 (0.5 or 2 mg/mL) and compared with either an untreated control or a 0.5 mg/mL gentamicin-positive control (gold standard). Results were expressed in pmol/min/mg of α -L-iduronidase activity, corrected for total cell protein, and represented the mean $\pm$ SD for three independent replicates. * and ** represent a significant difference from the untreated control at, respectively, $p < 0.05$ and $p < 0.001$

but treatment with NB54 resulted in a significant increase (1.7-fold for 0.5 mg/mL and 3.7-fold for 2 mg/mL), when compared to an untreated CHO-W402X control (Fig. 3b). α -L-Iduronidase activity in CHO-W402X cells was higher with NB54 treatment when compared to gentamicin (0.5 mg/mL; Fig. 3b).

In CHO-W402X cells, lividomycin had no effect at 0.5 mg/mL but α -L-iduronidase activity increased 2.9-fold at 1 mg/mL and 7.8-fold at 2 mg/mL (Fig. 4). The lack of availability of lividomycin precluded further testing of this compound.

Treatment of human fibroblasts with gentamicin and NB54. There was little or no detectable α -L-iduronidase activity in Q70X/Q70X or W402X/W402X skin fibroblasts (UAG premature stop codons; data not shown). In Q70X/Q70X fibroblasts, α -L-iduronidase activity increased to 0.27

pmol/min/mg with 2 mg/mL NB54, and to 0.48 pmol/min/mg with 0.5 mg/mL gentamicin (Fig. 5a); in contrast, α -L-iduronidase activity in W402X/W402X fibroblasts increased to 1.04 pmol/min/mg with 2 mg/mL NB54 and to 0.26 pmol/min/mg with 0.5 mg/mL gentamicin (Fig. 5a). NB54 and gentamicin treatment did not significantly change α -L-iduronidase activity in unaffected control fibroblasts (Fig. 5b).

Single base mutations are predicted to cause significant changes in the secondary structure of IDUA transcripts. The Q70X mutation (CAG to TAG) caused changes in the predicted mRNA secondary structure 20 base pairs before and 2 base pairs after the premature stop codon. This resulted in a change from a Q70 stem (Fig. 6a) to a Q70X loop structure (Fig. 6b). In addition, at base positions 413 to 475 there were changes from a relatively simple loop and stem structure to a more complex double stem-loop

Fig. 4 Lividomycin treatment of CHO-W402X cells. CHO-W402X cells were treated with 0.5, 1.0, or 2 mg/mL of lividomycin (*solid bars*) and read-through assessed by α -L-iduronidase activity relative to an untreated control (*open bars*). Results were expressed in pmol/min/mg of α -L-iduronidase activity, corrected for total cell protein, and represented the mean of duplicate analyses

structure (Fig. 6a and b). The W402X mutation (TGG to TAG) resulted in mRNA changes 74 base pairs before and 10 base pairs after the premature stop codon (Fig. 6d), causing a predicted shift in the secondary structure from a W402 stem (Fig. 6c) to a W402X stem-loop structure (Fig. 6d). In addition, a stem-loop (base positions 1457 to 1479) was extended (base positions 1470 to 1637) and created a putative hammerhead self-cleaving ribozyme structure (base positions 1607 to 1612 and 1614; Fig. 6b). In addition, “landmark” secondary structures surrounding the area were also significantly changed (Fig. 6a and b). The predicted structural changes were greater for the W402 to W402X transition than the Q70 to Q70X transition.

Discussion

MPS I is a common mucopolysaccharidosis disorder with most patients exhibiting mental retardation. Current therapies for MPS I include enzyme replacement therapy and bone marrow transplantation (Brooks 2002; Moore et al. 2008; D'Aco et al. 2012). While these therapies are effective for somatic tissue pathology, there are limitations with regard to the treatment of neuropathology. Drug-induced premature stop codon read-through is a potential alternative therapy; these drugs can potentially cross the blood–brain barrier from circulation, override premature stop codons, and provide full-length functional protein.

Gentamicin has been investigated as a read-through agent for MPS I (Keeling et al. 2001; Hein et al. 2004), and this aminoglycoside has the capacity to cross the blood–brain barrier. Aminoglycosides are 2-deoxystreptamine derivatives, which can be modified by links at the 4, 5, or 6 positions. These molecules contain at least two ring

Fig. 5 NB54 and gentamicin treatment of MPS I patient skin fibroblasts. **(a)** MPS I patient skin fibroblasts with either W402X/W402X or Q70X/Q70X genotype (UAG premature stop codons; *open bar*) were treated with either NB54 (*shaded bar*) or gentamicin (*closed bar*). **(b)** Unaffected control fibroblasts (*open bar*) were treated with either NB54 (*shaded bar*) or gentamicin (*closed bar*). Results for **a** and **b** were expressed as pmol/min/mg of α -L-iduronidase activity, corrected for total cell protein and represented the mean $\pm$ SD for three independent replicates. * represented a significant difference from the untreated control at $p < 0.05$

structures and, depending on modifications, may contain up to 5 rings: rings I and II have been shown to interact with RNA molecules, while rings III and above appear to contribute less to this binding (Carter et al. 2000; Kotra et al. 2000; Cashman et al. 2001; Vacas et al. 2010). The mechanism for aminoglycoside-mediated stop codon read-through is not fully understood: the binding of aminoglycosides to eukaryotic ribosomal RNA is thought to cause a conformation change in the ribosome (Francois et al. 2005), enabling tRNA binding and amino acid substitution (Carter et al. 2000) but aminoglycosides can also stabilize the mRNA of nonsense transcripts (Floquet et al. 2011).

Here, we examined six aminoglycosides for their capacity to induce read-through of the mutations Q70X and W402X. Lividomycin generated the highest W402X

Fig. 6 Predicted secondary structures of wild-type and mutant IDUA transcripts. Predicted secondary structures of both wild-type and mutant transcripts (both Q70X and W402X) were generated electronically using the Minimum Free Energy model and the local differences in the vicinity of the premature stop codon mutations (PTCs) were examined for their alterations. All PTCs and their precursors are indicated by red circles (Q70, Q70X, W402, and W402X). The

orientation of the mRNA is indicated (5' and 3'). The first common base between Q70/Q70X (a and b) and W402/W402X (c and d) are indicated by *pale blue* and *cyan circles*, respectively. Both Q70X (b) and W402X (d) mutations cause alterations in mRNA secondary structures compared to their precursors Q70 (a) and W402 (c), respectively. The putative hammerhead self-cleaving ribozyme structure of the W402X transcript (d) is indicated by *green circles* (RZ)

read-through but drug availability limited these studies. Of the other drugs investigated, gentamicin had the most effect on Q70X, while NB54 had a greater effect on the W402X mutation. From the literature, the apparent sequence context dependency of aminoglycosides on the efficacy of read-through induction is not clear. We concluded that 4,6-disubstituted 2-deoxystreptamine molecules were more effective at inducing read-through for Q70X, whereas 4,5-disubstituted 2-deoxystreptamine molecules were more effective for W402X.

Direct aminoglycoside-to-RNA interaction depends upon the RNA secondary structure and therefore on the sequence. This was initially shown in short RNA molecules (aptamers) (Wang and Rando 1995) and subsequently in messenger RNA (Voeller et al. 1995; Tok et al. 1999; Walter et al. 1999). Aminoglycoside-RNA interaction has been implicated in gene expression regulation (Suess et al. 2003). Here, lividomycin induced the most efficient read-through for

W402X. Lividomycin RNA aptamers can be dissociated using other 4,5-disubstituted aminoglycosides such as paromomycin but not readily with 4,6-disubstituted aminoglycosides such as kanamycin (Lato et al. 1995). This suggested that class-specific aminoglycoside binding might be responsible for the different amounts of read-through that we observed. The predicted secondary structure of wild-type and mutant IDUA transcripts was used to determine whether RNA structure correlated with the capacity to induce read-through. Large differences were apparent in the predicted secondary structures of the mutant transcripts in the vicinity of the altered bases, which may explain the differences in aminoglycoside binding. The W402X G to A base change altered the predicted secondary and tertiary structure, with a Uracil Turn Motif (CTGANGA) being exposed in a loop. To date five ribozymes have been characterized, which catalyze the sequence-specific intramolecular cleavage of RNA: the hammerhead (Prody et al. 1986; Forster and

Table 1 Descriptive data

Measure $N = 25$ for all measures	Mean (standard deviation)
Age: mean (S.D.)	75.0 months (53.4) range 13–220 months
Cognitive age-equivalent (BSID or KABC)	23.0 months (12.9) range 7–58 months
Developmental Quotient (mean age-equivalent of BSID or KABC/ chronological age)	44.6 (28.4) range 3–91
Vineland age-equivalent (mean of subscales except motor domain)	23.8 months (12.8) range 6–61 months
Vineland developmental quotient (mean of age equivalents except motor domain/chronological age)	44.4 (25.3) range 3–95
Standard score using normative data on the Vineland	63.0 (16.8) range 25–95

Symons 1987), hairpin (Buzayan et al. 1986), hepatitis delta virus (HDV) (Sharmeen et al. 1988), Varkud satellite (VS) (Saville and Collins 1990), and *glmS* (Winkler et al. 2004) ribozymes. The Uridine Turn Motif that we observed for the W402X mutation was characteristic of a hammerhead ribozyme.

RNA self-cleavage may help to remove some of the W402X mutant transcript, which is also degraded by nonsense-mediated decay, resulting in the very low mutant mRNA observed in MPS I cells (Menon and Neufeld 1994; Hein et al. 2004). Aminoglycosides can inhibit ribozymes when bound to messenger RNA (Walter et al. 1999; Schroeder et al. 2000) and could potentially act on the W402X-associated ribozyme activity. Novel approaches could be used to address this loss of transcript (e.g., antisense oligonucleotide technology: LNA (Obika et al. 1997; Koshkin et al. 1998); PNA (Egholm et al. 1993) and morpholino (Summerton 1999); or mutant RNA editing (Woolf et al. 1995; Morabito and Emeson 2009).

Extended administration of aminoglycosides is known to cause vestibulotoxicity (Halmagyi et al. 1994; Darlington and Smith 2003) and nephrotoxicity (Rougier et al. 2004) in humans. This has led to the development of less toxic aminoglycoside derivatives and other compounds such as NB30 and NB54 which exhibited read-through for both W402X and Q70X mutations and may be more suitable read-through agents (Hirawat et al. 2007; Du et al. 2009; Nudelman et al. 2009). Potential read-through compounds have been evaluated in preclinical trials; for example, the aminoglycoside G418 was used to prevent proximal renal tubular acidosis and proximal spinal muscular atrophy (Azimov et al. 2008; Heier and DiDonato 2009); NB30 and NB54 have been evaluated for biocompatibility and read-through potential in an animal model of Usher syndrome (Goldmann et al. 2010, 2012) and patients with cystic fibrosis have been treated with PTC124 (Wilschanski et al. 2003; Welch et al. 2007; Du et al. 2008; Kerem et al. 2008; Goodier and Mayer 2009; Peltz et al. 2009; Sermet-Gaudelus et al. 2010); and this drug has been evaluated in a Phase III clinical trial (ClinicalTrials.gov 2010b) in cystic fibrosis patients. Duchenne muscular dystrophy patients

have been treated with gentamicin (Malik et al. 2010) and PTC124 (Wilton 2007), although the PTC124 clinical trial was suspended due to lack of efficacy in these patients (ClinicalTrials.gov 2010a).

In this study, different responses to read-through drugs were observed for Q70X and W402X mutation. This may relate to the observed structural changes in these transcripts resulting in either different amounts of residual mutant mRNA and/or different transcript read-through potential. The mechanism controlling the efficacy of aminoglycoside action is not fully clear but may involve a combination of mutant transcript stabilization, modulation of the fidelity of ribosome tRNA selection/amino acid substitution, or even reduced ribozyme activity for certain mutations. In MPS I, premature stop codon read-through therapy may therefore require drugs that are tailored to each patient's specific mutations in order to deliver an optimal therapeutic outcome. The compounds tested here produced relatively small increases in IDUA activity, and it is yet to be determined if this is sufficient read-through for clinical applications.

Synopsis

Examination of aminoglycoside-mediated read-through of premature stop codons present in the Q70X and the W402X mutations in the human α -iduronidase gene provided insights into the difference in efficacies seen in read-through drugs in use.

References

- Ashton LJ, Brooks DA, McCourt PA, Muller VJ, Clements PR, Hopwood JJ (1992) Immunoquantification and enzyme kinetics of alpha-L-iduronidase in cultured fibroblasts from normal controls and mucopolysaccharidosis type I patients. *Am J Hum Genet* 50(4):787–794
- Azimov R, Abuladze N, Sassani P et al (2008) G418-mediated ribosomal read-through of a nonsense mutation causing autosomal recessive proximal renal tubular acidosis. *Am J Physiol Renal Physiol* 295(3):F633–641

- Bitner-Glindzicz M, Rahman S (2007) Ototoxicity caused by aminoglycosides. *BMJ* 335(7624):784–785
- Brooks DA (2002) Alpha-L-iduronidase and enzyme replacement therapy for mucopolysaccharidosis I. *Expert Opin Biol Ther* 2(8):967–976
- Brooks DA, Muller VJ, Hopwood JJ (2006) Stop-codon read-through for patients affected by a lysosomal storage disorder. *Trends Mol Med* 12(8):367–373
- Bunge S, Kleijer WJ, Steglich C et al (1994) Mucopolysaccharidosis type I: identification of 8 novel mutations and determination of the frequency of the two common alpha-L-iduronidase mutations (W402X and Q70X) among European patients. *Hum Mol Genet* 3(6):861–866
- Buzayan JM, Gerlach WL, Bruening G (1986) Non-enzymatic cleavage and ligation of RNAs complementary to a plant virus satellite RNA. *Nature* 323(6086):349–353
- Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V (2000) Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* 407(6802):340–348
- Cashman DJ, Rife JP, Kellogg GE (2001) Which aminoglycoside ring is most important for binding? A hydrophobic analysis of gentamicin, paromomycin, and analogues. *Bioorg Med Chem Lett* 11(2):119–122
- ClinicalTrials.gov (2010a) Study of Ataluren (PTC124®) in non-ambulatory patients with nonsense-mutation-mediated Duchenne/Becker muscular dystrophy (nmDMD/BMD). In: Study of Ataluren (PTC124®) in nonambulatory patients with nonsense-mutation-mediated Duchenne/Becker muscular dystrophy (nmDMD/BMD). <http://clinicaltrials.gov/ct2/show/NCT01009294?term=study+of+ataluren&rank=4>
- ClinicalTrials.gov (2010b) Study of Ataluren (PTC124™) in cystic fibrosis. In: Study of Ataluren (PTC124™) in Cystic Fibrosis. <http://clinicaltrials.gov/ct2/show/NCT00803205?term=study+of+ataluren&rank=6>
- D'Aco K, Underhill L, Rangachari L et al (2012) Diagnosis and treatment trends in mucopolysaccharidosis I: findings from the MPS I Registry. *Eur J Pediatr* 171(6):911–919
- Darlington CL, Smith PF (2003) Vestibulotoxicity following aminoglycoside antibiotics and its prevention. *Curr Opin Investig Drugs* 4(7):841–846
- Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM (2008) PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc Natl Acad Sci U S A* 105(6):2064–2069
- Du L, Damoiseaux R, Nahas S et al (2009) Nonaminoglycoside compounds induce readthrough of nonsense mutations. *J Exp Med* 206(10):2285–2297
- Egholm M, Buchardt O, Christensen L et al (1993) PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature* 365(6446):566–568
- Floquet C, Deforges J, Rousset JP, Bidou L (2011) Rescue of non-sense mutated p53 tumor suppressor gene by aminoglycosides. *Nucleic Acids Res* 39(8):3350–3362
- Forster AC, Symons RH (1987) Self-cleavage of plus and minus RNAs of a virusoid and a structural model for the active sites. *Cell* 49(2):211–220
- Fourmy D, Recht MI, Puglisi JD (1998a) Binding of neomycin-class aminoglycoside antibiotics to the A-site of 16 S rRNA. *J Mol Biol* 277(2):347–362
- Fourmy D, Yoshizawa S, Puglisi JD (1998b) Paromomycin binding induces a local conformational change in the A-site of 16 S rRNA. *J Mol Biol* 277(2):333–345
- Francois B, Russell RJ, Murray JB et al (2005) Crystal structures of complexes between aminoglycosides and decoding A site oligonucleotides: role of the number of rings and positive charges in the specific binding leading to miscoding. *Nucleic Acids Res* 33(17):5677–5690
- Goldmann T, Rebibo-Sabbah A, Overlack N et al (2010) Beneficial read-through of a USH1C nonsense mutation by designed aminoglycoside NB30 in the retina. *Invest Ophthalmol Vis Sci* 51(12):6671–6680
- Goldmann T, Overlack N, Moller F et al (2012) A comparative evaluation of NB30, NB54 and PTC124 in translational read-through efficacy for treatment of an USH1C nonsense mutation. *EMBO Mol Med* 4(11):1186–1199
- Goodier JL, Mayer J (2009) PTC124 for cystic fibrosis. *Lancet* 373(9673):1426; author reply 1426–1427
- Gort L, Chabas A, Coll MJ (1998) Analysis of five mutations in 20 mucopolysaccharidosis type I patients: high prevalence of the W402X mutation. *Mutations in brief no. 121*. Online. *Hum Mutat* 11(4):332–333
- Gruber AR, Lorenz R, Bernhart SH, Neubock R, Hofacker IL (2008) The Vienna RNA websuite. *Nucleic Acids Res* 36(Web Server issue): W70–74
- Halmagyi GM, Fattore CM, Curthoys IS, Wade S (1994) Gentamicin vestibulotoxicity. *Otolaryngol Head Neck Surg* 111(5):571–574
- Harrell L, Melcher U, Atkins JF (2002) Predominance of six different hexanucleotide recoding signals 3' of read-through stop codons. *Nucleic Acids Res* 30(9):2011–2017
- Heier CR, DiDonato CJ (2009) Translational readthrough by the aminoglycoside geneticin (G418) modulates SMN stability in vitro and improves motor function in SMA mice in vivo. *Hum Mol Genet* 18(7):1310–1322
- Hein LK, Hopwood JJ, Clements PR, Brooks DA (2003) The alpha-L-iduronidase mutations R89Q and R89W result in an attenuated mucopolysaccharidosis type I clinical presentation. *Biochim Biophys Acta* 1639(2):95–103
- Hein LK, Bawden M, Muller VJ, Sillence D, Hopwood JJ, Brooks DA (2004) Alpha-L-iduronidase premature stop codons and potential read-through in mucopolysaccharidosis type I patients. *J Mol Biol* 338(3):453–462
- Hirawat S, Welch EM, Elfring GL et al (2007) Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J Clin Pharmacol* 47(4):430–444
- Keeling KM, Brooks DA, Hopwood JJ, Li P, Thompson JN, Bedwell DM (2001) Gentamicin-mediated suppression of Hurler syndrome stop mutations restores a low level of alpha-L-iduronidase activity and reduces lysosomal glycosaminoglycan accumulation. *Hum Mol Genet* 10(3):291–299
- Kerem E, Hirawat S, Armoni S et al (2008) Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet* 372(9640):719–727
- Koshkin AA, Singh SK, Nielsen P et al (1998) LNA (Locked Nucleic Acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* 54(14):3607–3630
- Kotra LP, Haddad J, Mobashery S (2000) Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother* 44(12):3249–3256
- Lai CH, Chun HH, Nahas SA et al (2004) Correction of ATM gene function by aminoglycoside-induced read-through of premature termination codons. *Proc Natl Acad Sci U S A* 101(44):15676–15681
- Lato SM, Boles AR, Ellington AD (1995) In vitro selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution. *Chem Biol* 2(5):291–303

- Malik V, Rodino-Klapac LR, Viollet L, et al (2010) Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. *Annals Neurol* 9999(999A): NA
- Menon KP, Neufeld EF (1994) Evidence for degradation of mRNA encoding alpha-L-iduronidase in Hurler fibroblasts with premature termination alleles. *Cell Mol Biol (Noisy-le-grand)* 40(7):999–1005
- Moore D, Connock MJ, Wraith E, Lavery C (2008) The prevalence of and survival in Mucopolysaccharidosis I: Hurler, Hurler-Scheie and Scheie syndromes in the UK. *Orphanet J Rare Dis* 3:24
- Morabito MV, Emeson RB (2009) RNA editing as a therapeutic target for CNS disorders. *Neuropsychopharmacology* 34(1):246
- Myerowitz R, Neufeld EF (1981) Maturation of alpha-L-iduronidase in cultured human fibroblasts. *J Biol Chem* 256(6):3044–3048
- Neufeld EF, Muenzer J (1995) The mucopolysaccharidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The metabolic and molecular bases of inherited disease*. McGraw-Hill, New York
- Nudelman I, Rebibo-Sabbah A, Cherniavsky M et al (2009) Development of novel aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. *J Med Chem* 52(9):2836–2845
- Obika S, Nanbu D, Hari Y et al (1997) Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar puckering. *Tetrahedron Lett* 38(50):8735–8738
- Pape T, Wintermeyer W, Rodnina MV (2000) Conformational switch in the decoding region of 16S rRNA during aminoacyl-tRNA selection on the ribosome. *Nat Struct Biol* 7(2):104–107
- Peltz SW, Welch EM, Jacobson A, et al (2009) Nonsense suppression activity of PTC124 (ataluren). *Proc Natl Acad Sci USA* 106(25):E64; author reply E65
- Prody GA, Bakos JT, Buzayan JM, Schneider IR, Bruening G (1986) Autolytic processing of dimeric plant virus satellite RNA. *Science* 231(4745):1577–1580
- Rebibo-Sabbah A, Nudelman I, Ahmed ZM, Baasov T, Ben-Yosef T (2007) In vitro and ex vivo suppression by aminoglycosides of PCDH15 nonsense mutations underlying type 1 Usher syndrome. *Hum Genet* 122(3–4):373–381
- Reymond C, Beaudoin JD, Perreault JP (2009) Modulating RNA structure and catalysis: lessons from small cleaving ribozymes. *Cell Mol Life Sci* 66(24):3937–3950
- Rougier F, Claude D, Maurin M, Maire P (2004) Aminoglycoside nephrotoxicity. *Curr Drug Targets Infect Disord* 4(2):153–162
- Saville BJ, Collins RA (1990) A site-specific self-cleavage reaction performed by a novel RNA in *Neurospora* mitochondria. *Cell* 61(4):685–696
- Schroeder R, Waldsich C, Wank H (2000) Modulation of RNA function by aminoglycoside antibiotics. *EMBO J* 19(1):1–9
- Scott HS, Litjens T, Hopwood JJ, Morris CP (1992a) A common mutation for mucopolysaccharidosis type I associated with a severe Hurler syndrome phenotype. *Hum Mutat* 1(2):103–108
- Scott HS, Litjens T, Nelson PV, Brooks DA, Hopwood JJ, Morris CP (1992b) Alpha-L-iduronidase mutations (Q70X and P533R) associate with a severe Hurler phenotype. *Hum Mutat* 1(4):333–339
- Scott HS, Litjens T, Nelson PV et al (1993) Identification of mutations in the alpha-L-iduronidase gene (IDUA) that cause Hurler and Scheie syndromes. *Am J Hum Genet* 53(5):973–986
- Sermet-Gaudelus I, Boeck KD, Casimir GJ et al (2010) Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. *Am J Respir Crit Care Med* 182(10):1262–1272
- Sharmeen L, Kuo MY, Dinter-Gottlieb G, Taylor J (1988) Antigenomic RNA of human hepatitis delta virus can undergo self-cleavage. *J Virol* 62(8):2674–2679
- Suess B, Hanson S, Berens C, Fink B, Schroeder R, Hillen W (2003) Conditional gene expression by controlling translation with tetracycline-binding aptamers. *Nucleic Acids Res* 31(7):1853–1858
- Summerton J (1999) Morpholino antisense oligomers: the case for an RNase H-independent structural type. *Biochim Biophys Acta* 1489(1):141–158
- Tok JB, Cho J, Rando RR (1999) Aminoglycoside antibiotics are able to specifically bind the 5'-untranslated region of thymidylate synthase messenger RNA. *Biochemistry* 38(1):199–206
- Touw DJ, Westerman EM, Sprij AJ (2009) Therapeutic drug monitoring of aminoglycosides in neonates. *Clinical Pharmacokinetics* 48(2):71–88. doi:10.2165/00003088-200948020-200900001
- Vacas T, Corzana F, Jimenez-Oses G et al (2010) Role of aromatic rings in the molecular recognition of aminoglycoside antibiotics: implications for drug design. *J Am Chem Soc* 132(34):12074–12090
- Voeller DM, Changchien LM, Maley GF et al (1995) Characterization of a specific interaction between *Escherichia coli* thymidylate synthase and *Escherichia coli* thymidylate synthase mRNA. *Nucleic Acids Res* 23(5):869–875
- Walter F, Vicens Q, Westhof E (1999) Aminoglycoside-RNA interactions. *Curr Opin Chem Biol* 3(6):694–704
- Wang Y, Rando RR (1995) Specific binding of aminoglycoside antibiotics to RNA. *Chem Biol* 2(5):281–290
- Welch EM, Barton ER, Zhuo J et al (2007) PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 447(7140):87–91
- Wilschanski M, Yahav Y, Yaacov Y et al (2003) Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* 349(15):1433–1441
- Wilton S (2007) PTC124, nonsense mutations and Duchenne muscular dystrophy. *Neuromuscul Disord* 17(9–10):719–720
- Winkler WC, Nahvi A, Roth A, Collins JA, Breaker RR (2004) Control of gene expression by a natural metabolite-responsive ribozyme. *Nature* 428(6980):281–286
- Woolf TM, Chase JM, Stinchcomb DT (1995) Toward the therapeutic editing of mutated RNA sequences. *Proc Natl Acad Sci USA* 92(18):8298–8302
- Zuker M, Stiegler P (1981) Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res* 9(1):133–148