RESEARCH ARTICLE

The Functional Mechanisms and Clinical Application of Read-Through Drugs

Alfred Zimmerman, Diana Jackson
Western State Medical School of Wisdom Department of Surgery

Abstract: According to previous reports, nearly one in 10 genetic diseases are caused by nonsense mutations around the world. Nonsense mutations lead to premature transcription terminations in cells, which in turn generate non-functional, truncated proteins. In recent years, read-through drugs are playing increasing prominent roles in the researches related to genetic diseases caused by nonsense mutations. However, due to the fact that the mechanisms lying behind translation termination still remain to be elucidated, the mechanistic research and clinical application of read-through drugs are facing new challenges. This review mainly discusses about the pathogenesis of genetic diseases caused by nonsense mutations, and then introduces the current clinical application of read-through drugs. Finally, we display some problems that remain to be solved and propose some possible coping strategies.

Keywords: Genetic disease; nonsense mutation; read-through drug; functional mechanism; clinical application

In the process of protein synthesis, the termination of codon means the end of translation. However, in some cases, such as gene mutation, the original codon encoding an amino acid is converted into a termination codon, resulting in early termination of translation and the production of nonfunctional truncated proteins. These abnormal proteins disrupt the normal function of cells, leading to genetic diseases such as Duchenne mus-cular dystrophy (DMD) and cystic fibrosis (CF). This abnormal termination codon is called a premature termination codon (PTC), and the mutation is called a nonsense mutation. According to statistics, around 1/10 of human genetic diseases (especially monogenic diseases) caused by nonsense mutations in the world is caused by[1].

In order to treat diseases caused by PTC, PTC reading therapy emerged in recent years, and has achieved encouraging results. The therapy uses a number of read-through drugs to restore protein synthesis by eliminating decoding errors caused by PTC during the translation process. Currently, the commonly used drugs for promoting reading are aminoglycosides and non aminoglycosides, the two major categories of[2]. Among them, aminoglycosides include traditional aminoglycoside antibiotics (such as amikacin, tobramycin, balomycin, gentamicin and hereditary mycin) and synthetic aminoglycoside derivatives (such as NB30, NB54, NB74, NB84 and TC007). Non-aminoglycosides are Ataluren (PTC124), Negamycin, Tylosin, Clitocine, RTC13/14, and some macrolide antibiotics[3]. This article reviews the mechanism and clinical application of reading-promoting drugs in order to provide reference for the research and treatment of genetic diseases caused by nonsense mutations.

1. The mechanism of promoting drug reading

In the process of translation, sometimes the ribosome will cause "misreading" of the genetic information of mRNA, that is, the termination codon will be used as a meaningful codon to prolong the peptide chain. This misreading mechanism is the theoretical basis of PTC reading therapy, that is, using drugs to improve the probability of ribosomal mis-

doi: 10.18063/gds.v2i1.

reading, to treat some diseases caused by PTC. In order to understand the above mechanisms, the mechanisms of extension and termination of translation, extension and termination of translation under the influence of drugs, and drug-induced PTC reading are explained.

1.1 Stop codon read through mechanism

The translation of intracellular proteins includes two processes: elongation and termination. In the process of peptide chain extension, the cognateaminoacyl tRNA (Cognateaminoacyl tRNA) first enters the ribosome A site, and then the amino terminal and the carboxyl terminal of the peptide chain undergo dehydration and condensation, thus prolonging the peptide chain. Finally, with the transposase EF-G, the tRNA dissociates from the ribosome, which moves a codon toward 3', ready to accept the next associated aminoacyl tRNA[4].

During the termination of peptide chain, the termination codon (UAA, UGA, UAG) is not recognized by aminoacyl tRNA, but by the release factor (RF). In prokaryotes, release factors include RF1, RF2 and RF3. RF1 and RF2 are responsible for terminating codon; RF3 is GTP enzyme, which plays a role in assisting the former two.

In eukaryotes, the release factors include eRF1 and eRF3, which combine to terminate the execution of the complex. ERF1, which is similar to aminoacyl RNA, can recognize and bind three termination codons and directly regulate the release of new peptide chains. ERF3 is similar to RF3 and eRF1[5].

Although cells contain many mechanisms to ensure proper translation, the efficiency of translation termination is still difficult to achieve 100%, and this efficiency is related to the competition between near-cognate aminoacyl tRNA (Near-cognate aminoacyl tRNA with only two base and codon pairs) and eRF1. If the proximally associated aminoacyl tRNA accidentally binds to the termination codon, allowing the nonsense codon to be decoded again into a meaningful codon, the extension continues until the next termination codon, a phenomenon known as termination codon reading. In general, the probability of PTC reading through is less than 1%, and the probability of normal termination of codon reading through is less than 0.1%. In addition, studies have shown that the read probabilities of the 3 stop codons are UGA > UAG > UAA[2].

1.2 Drug induced PTC reading mechanism

In 1996, Howard *et al*.[6] first used drug-induced PTC reading to correct nonsense mutations in mammalian cells. After nearly 20 years of research, it is generally believed that the key point of reading through therapy is to make near-related aminoacyl tRNA competently inhibit eRF1, promote the binding of near-related aminoacyl tRNA and PTC, thereby increasing the probability of PTC reading through. In the course of PTC reading therapy, all kinds of amino acids may be integrated into polypeptide chain[7]. If PTC is not at a critical site of a functional protein, such as a catalytic center, then PTC read-through therapy

The method can at least partially restore the function of damaged proteins and benefit patients with genetic diseases such as DMD.

1.2.1 Drug induced codon misreading mechanism induced by

In prokaryotes, ribosome screening mechanism for associated aminoacyl tRNA has been well understood. First, aminoacyl tRNA enters the A site of the ribosome in the form of tRNA/EFTu.GTP trimer (eukaryotic corresponding to tRNA/eEF1A.GTP). Then G530, A1492 and A1493 bases in the ribosomal Helix44 region were screened for aminoacyl tRNA[8], which is the stage in which aminoglycosides work. Normally, when an A site associated with aminoacyl tRNA binds to 16S RNA, A1492 and A1493 flip out of the interior of Helix44, interacting directly with the microchannel of the cis-anticodon helix, and G530 converts from cis-conformation to trans-conformation. This conformational change will accelerate the hydrolysis of GTP, promote the dissociation of EF-Tu, and lead to a series of cascade reactions, so that the synthetic peptide chain can be extended. After entering the A site, the proximally associated aminoacyl tRNA could not induce conformational changes in A1492, A1493 and G530, and was eventually excluded. Structural biology studies have shown that aminoglycosides can bind to two bases A1408 and G1491 on Helix44 to form hydrogen bonds and alter the conformation of Helix44, thereby inducing structural effects similar to those associated with aminoacyl tRNAs, i.e. the release of A1492 and A1493 from the helix, forcing them to act as near-related amino groups.

Acyl tRNA. Although G530 does not undergo significant conformational changes due to the action of aminoglycosides, the changes in A1492 and A1493 are sufficient to provide appropriate free energy and greatly increase the probability of misreading[9].

Some related studies have also proposed different views on the process of the interaction between proximally related aminoacyl tRNA and ribosomes. Ogle *et al*.[10] suggested that the binding of related tRNA to ribosomes could change the 30S subunit from open to closed. Conversely, the closure of the 30S subunit was not significant when the near-related tRNA entered the A site, and the binding of aminoglycosides to the 30S subunit caused the 30S subgene to rotate and shut down, allowing for meaningful codon misreading. However, Natalia and other[11] think that the research of Ogle has certain limitations. They proposed that both the amino-tRNA and the near-related amino-tRNA could shut down their domains once they bound to the 30S subunit.

1.2.2 Drug induced stop codon read through mechanism

The terminating processes of prokaryotes and eukaryotes are different. Laurberg *et al*.[12] suggested that when prokaryotic translation terminates, RF1 or RF2 binds to site A and the ribosome conformation changes. This change enables RF1 or RF2 to accurately decode ribosomal 30S.

The heart extends to the 50S subunit of the peptidyl transferase center. Like aminoacyl tRNA, RF and codon interactions involve three bases G530, A1492 and A1493, but these three bases are not directly involved in terminating codon recognition, but help stabilize RF's catalytic center, promote the hydrolysis of peptidoacyl tRNA on the ribosome and release the peptide chain. Although the mechanism of aminoglycosides inducing stop codon reading is not fully understood, the effect of aminoglycosides on A1492 and A1493 may be one of its mechanisms. The conformational changes of the two bases result in the competitive binding termination codon of the near-related aminoacyl tRNA to RF, resulting in plasmid identity and relatively weak binding of aminoglycosides[13]. This also explains the loss of the effect of the reading-promoting drugs on the termination codon in eukaryotes. In eukaryotes, the 18S subunit of ribosome has a slightly less PTC effect than the 16S subunit of prokaryotes, while in prokaryotes it interferes with the synthesis of whole proteins.

1.3 Drug selection mechanism for PTC and stop codon.

Although PTC-induced diseases have been greatly improved by reading-through drugs, the mechanism of their action has also brought some worries to clinical application: whether reading-through drugs will affect the translation process of cells; whether some peptide chains will be prolonged and induced by stopping codon reading-through.

Cell unfolded protein response? The researchers selected Hsp70 as an indicator of unfolded protein response. According to Keeling *et al*, the concentration of Hsp70 was only slightly elevated when large doses of reading-promoting drugs were administered to primary fibroblasts. It can be seen that PTC

Reading therapy has neither significantly affected the rate of translation nor seriously affected the normal stop codon recognition. Why are drugs that promote reading more sensitive to PTC? Amrani *et al*.[15] suggested that the residence time of ribosomes at normal termination codons was longer than that at PTC, suggesting that the translation termination efficiency at normal termination codons was higher than that at PTC, which created the conditions for promoting the role of the drug. Hoshino *et al*.[16] showed that eRF3 interacts with the polymeric A-binding protein (PABP) at the 3'end of mRNA to improve the efficiency of translation termination. If eRF3 binds to the normal termination codon, it is closer to PABP and facilitates the interaction; if it binds to PTC, it is farther away from PABP and the interaction is weaker. In practice, PABP abundance, the relative length of 3'UTR and the nucleotide sequence around the termination codon also affect the read-through[9].

2. Clinical application of drugs for promoting reading

In recent years, many experiments have confirmed the reading-promoting activity of reading-promoting drugs at different levels[9]. Gentamicin, hereditary mycin (G418) in aminoglycosides, a series of synthetic balomycin derivatives (NB30, NB54, NB74, NB84), and PTC124[3] in non-aminoglycosides have been studied extensively. . Hereditary diseases used in clinical trials include CF, DMD, Ataxia telangiectasia (AT), Rett syndrome (RTT), hemophilia A and B

(HA and HB), mucopolysaccharidosis type I (MPS-I), spinal muscular atrophy. Spinal muscular atrophy type I (SMA I), Usher syndrome type I (USH1), X-linked nephrogenic diabetes insipidus (XNDI), congenital muscular dystrophy (CMD), colorectal cancer (CRC), familial diabetes insipidus Adenomatous polyposis, Limbgirdle muscular dystrophy type 2B (Miyoshi/LGMD2B) and so on.

Among them, CF and DMD genetic research is the most in-depth and the most clinical value. Gentamicin and PTC 124 are the only drugs that have entered the clinical trial phase, and the derivatives of balomycin (NB54, NB84) are considered to be a potential new generation of drugs. The following is an illustration of the progress in the study of drugs for promoting reading.

2.1 Study on the application of gentamycin

Gentamicin is one of the most widely studied drugs. Although some clinical trials have confirmed its efficacy in some PTC-related diseases, its wide application in clinic is limited because of the toxic and side effects of aminoglycoside antibiotics.

2.1.1 Treatment of DMD

In 1999, Barton-Davis *et al*.[34] confirmed for the first time that gentamicin could promote PTC reading in animals by using Mdx (3185C T) mouse model. In 2001, Wagner *et al*.[35] administered gentamicin sulfate intravenously for 14 consecutive days to 4 patients with nonsense mutation of DMD / BMD (nmDBMD), but no complete expression of Dystrophin was found before and after administration. In 2003, Ploitano *et al*.[36] carried out similar clinical trials in 4 patients with nonsense mutation of DMD. Results Immunohistochemical staining showed intact expression of full-length atrophic protein in 3 patients with nonsense mutation of UGA. Meanwhile, plasma creatine kinase (CK) level and cardiopulmonary function were improved. Therefore, Ploitano *et al*. speculated that gentamicin might only be effective in some nonsense mutant DMD patients. In 2010, Malik and other[20] conducted a longer clinical trial. The results showed that there were no significant ototoxicity, vestibular toxicity and nephrotoxicity in the subjects 6 months after receiving the appropriate concentration (7.5 mg/kg). It was also found that the increased expression of myotrophic protein after gentamicin treatment may be partly related to the dosage of gentamicin and the basal expression of myotrophic protein before treatment. It is noteworthy that although the expression of amyotrophic protein increased after treatment, clinical efficacy showed that, in addition to plasma CK levels were significantly reduced, patients with muscle strength, maximal voluntary isometric contraction test (MVICT), forced vital capacity (FVC), 6-minute walking distance (6 MWD) and other clinical evaluation indicators were only slight. Improvement has no significant clinical significance. Accordingly, Malik *et al*[20] thinks that this may be related to gentamicin concentration. If the dose is increased, there may be better clinical results, but it will increase the risk of toxic side effects.

2.1.2 Treat CF

In 2000, Wilschanski *et al*.[37] validated the efficacy of gentamicin in nonsense mutant CF (nmCF) patients under nasal administration. They used Nasal potential difference (NPD) as a therapeutic evaluation standard. The results showed that 7 of the 9 subjects with W1282 * nonsense mutation showed signs of partial Cl_channel recovery after 14 days of treatment. However, after 1 weeks of withdrawal, the NPD of the subjects returned to the pre drug status. In 2007, Sermet-Gaudelus *et al*.[21] selected the Y122* mutant with the best response to gentamicin as the entry point on the basis of cell experiments, and treated 9 CF patients with this mutant. After 15 days of continuous intravenous administration, the clinical symptoms (including cough, sputum production, respiratory status, vital capacity and other physiological indicators) of the experimental group were significantly improved, while those with other mutations (G542 *, R1162 *, W1282 * and R553 *) had no significant clinical effect. It also suggests that gentamycin may only promote the reading of specific nonsense mutations.

In the same year, Clancy *et al*.[38] also carried out nasal administration of 11 patients with nmCF, the results showed that no significant changes in the function and localization of CFTR were detected. This is obviously in conflict with the experimental results of Wilschanski *et al*. Clancy *et al*.[38] suggested that this might be related to the difference in genome composition of the study subjects --- most of the genotypes selected by the former were W1282 * / W1282 * or W1282 * / G542 *; while Clancy's subjects were mostly heterozygotes with nonsense mutations and deletion mutations

(such as F508 / G542 *) and basically did not contain W1282 *. . In addition, the differences in the efficiency of nonsense-mediated RNA decay (NMD) pathways and the slight differences in the way of administration may also influence the experimental results.

In 2005, James *et al*.[39] treated 5 patients with severe hemophilia with nonsense mutations (3 HA, 2 HB) by intravenous gentamicin (7 mg/kg) for 3 consecutive days. Results Activated partial thromboplastin time (APTT) and plasma coagulation factor activity (F_or F_) were slightly improved in 2 patients. In addition, there was a slight increase in the F IX antigen in 1 subjects (2%~5.5%), but no active F IX was detected. This indicates that the F IX induced by the patient does not play an effective role in procoagulant action. In addition, even in two patients who responded to gentamicin, there was no complete correlation between shortened APTT and increased coagulation factor activity. James *et al*.[39] believed that this was due to the limitations of detection methods, because slight changes in the activity of F_or F_ (1.6% ~ 2.0%) were difficult to pass general methods.

It is measured. Only by fluorescence thrombin generation test (FTGT) can we get better correlation. In general, gentamicin has many limitations on the treatment of severe hemophilia caused by nonsense mutations.

2.2 Study on the application of Barone mycin derivatives (NB54 and NB84)

Because of the side effects of traditional aminoglycoside antibiotics, Nudelman *et al*.[3] designed and synthesized a series of derivatives (NB30, NB54, NB74 and NB84) with lower toxicity and higher efficiency. Among them, NB30 and NB54 are the first and second generation of PAM derivatives respectively, and NB74 and NB84 are the third generation of this series. Compared with the first two generations, the third generation had higher efficiency and less toxicity. Current studies at the cellular level in vitro have confirmed that these derivatives have read-through activity for nonsense mutations in CF, DMD, MPS-I, RTT, USH1 and other genetic diseases, but there are few reports in vivo. In 2011, Rowe *et al*.[24] used the CF mouse model (Cftr/hCFTR-G542*) to verify that NB54 also had a good dose-dependent read-through activity in vivo. At the same time, compared with gentamicin, mice were better tolerated under high dose of NB54. In 2014, Gunn *et al*.[25] reported NB84 long-term treatment of Iduatm 1 Kmke MPS I mice (IDUA-W402*). The results showed that after 28 weeks of treatment, the abnormal phenotypes in brain, heart, bone and other tissues were significantly reduced, and the GAG storage in all tissues was significantly reduced compared with the control group. In addition, the activity of mice returned to the wild-type level, and no significant toxic side effects were observed. This indicates that the series of drugs may have better clinical application prospects.

2.3 Application Research of PTC124

PTC124, the most widely studied non-aminoglycoside reading-promoting drug, is currently in the third phase of clinical trials in the United States and has been approved for sale in some European countries[41]. Compared with aminoglycosides, this drug has the advantages of low toxicity, high efficiency and oral administration, so it is widely used in the treatment of many nonsense mutation genetic diseases. In addition to DMD and C F, PTC124 enhancers were confirmed in nonsense mutations such as Miyoshi/LGMD2B, USH I C, and carnitine palmityltransferase 1A deficiency[31,32,42].

2.3.1 treatment of DMD

In 2007, Welch *et al*.[30] reported that PTC124 had high reading-promoting activity on primary myocytes of Mdx mice cultured in vitro. They found the ratio of amyotrophic protein to myosin in DMD patients treated with low concentrations of PTC124 (5 ug/mL)

The value reached 40%~60%, and the ratio did not increase after increasing the concentration of the drug. Moreover, animal experiments on Mdx mice further confirmed that PTC124 was low toxic and effective in vivo (the atrophic protein of experimental mice returned to normal levels of 20% - 25%). In the same year, Phase 1 clinical trials on the safety and pharmacokinetics of PTC124[43] confirmed its safety in humans. Studies have shown that the maximum effective concentration (2-10 ug/mL) can be achieved by three doses daily (10 mg/kg, 10 mg/kg, 20 mg/kg), and there are no obvious side effects and accumulation in vivo.

The subsequent 2 clinical trials showed that PTC124 is safe and effective in the treatment of nmDMD. In a phase 2

clinical trial, 38 boys with nmDMD underwent 28 days of treatment, and muscle biopsy showed a significant increase in the expression of amyotrophic protein in 34% of the subjects[44]. Quantitative analysis showed that 61% of the subjects had an increased expression of myotrophic protein and 84% had a decreased serum CK level. This indicates that the damaged muscle cells have improved with the increase of the content of the dystrophin. But Finkel *et al*.[44] also pointed out that only the expression of myotrophic protein in biopsy tissues and the change of serum CK level as the evaluation index of pharmacodynamics is not enough (because biopsy tissues can not represent the overall situation of the muscle, CK levels fluctuate relatively large, but also affected by the patient's activity time), should be used. A more scientific evaluation item is used as an index to judge the curative effect of DMD patients.

To this end, the United States conducted a longer phase 2B clinical trial (double-blind trial). They recruited 174 boys from 11 countries with nmDBMD who were able to walk (6 MWD (> 75 m) and divided them into three groups: low-dose (40 mg / kg / d), high-dose (80 mg / kg / d) PTC 124, and placebo for 48 weeks. The results showed that the treatment effect of the low dose group was obvious. The mean difference of 6 MWD between the two groups was 31.3 m, reaching the minimum clinical significance change (MCID) of 28.5-31.7 m[45]. Timed functional tests (TFTs) such as climbing stairs and lying upright also showed that muscle function declined more slowly in the low-dose group than in the placebo group. After 48 weeks of treatment, 74% of the low-dose group had no deterioration, compared with 56% of the placebo group. However, it should be noted that the progress of the disease in the high-dose group was not as good as that in the low-dose group, but was similar to that in the placebo group, especially in the high-dose group with high concentration (>19.3 ug/mL), and there was no significant difference in the evaluation indexes between the high-dose group and the placebo group. It should be closer to the low dose group[41]. The researchers believe that this is consistent with the bell shaped dose effect curve hypothesis proposed by PTC124. In addition, after data analysis, the researchers concluded that children older than 7 years of age with a certain walking ability might be more stable. Phase 3 clinical trials and phase 3 extended trials on PTC124 for nmDBMD are under way to further verify the efficacy of the drug. Significant clinical significance.

2.3.2 Treatment of CF

In 2005, two phase 2A clinical trials of PTC124 for CF were conducted in the United States and Israel in parallel. The results of the Israeli group showed that PTC124 had a significant therapeutic effect on CF. After two rounds of 14-day oral administration of PTC124 (16 mg/kg/d, 40 mg/kg/d, with a 14-day stoppage interval), the average NPD of 23 subjects decreased by 7.1 mV and 3.7 mV[46]. Total chlorine transport values of 57% and 43% of the patients reached the normal range (> 5 mV) in the first and second rounds of trials, respectively, but returned to abnormal state after withdrawal. In addition, some subjects had slight improvements in vital capacity, lung symptoms, body weight, and neutrophil count. At the same time, Kerem *et al*.[46] also found that in subjects with the same nonsense mutation type (W1282*), the change of total chlorine transport value (CFTR expression) was positively correlated with the amount of intracellular mRNA template, and the type of PTC and the upstream and downstream sequence of PTC may also be factors affecting the reading efficiency.

In 2006, researchers conducted a 12-week phase 2B clinical trial on 19 patients who had participated in the 2-year trial. The results showed a significant improvement in chloride secretion in 64% (7/11) low-dose group (16 mg/kg/d) and 57% (4/7) high-dose group (40 mg/kg/d) (total chlorine transport value < 5 mv)[47]. At the same time, the results also showed that the recovery of CFTR function was time-dependent and returned to the state before treatment after withdrawal. The following year, 30 CF patients under 18 years of age in France and Belgium were also treated with PTC124 in clinical trials[48]. About 50% of the subjects changed their total chlorine transport value to or above 5 mV after two rounds of treatment, and the improvement of the high dose group was relatively greater. This indicates that PTC124 is equally effective in treating children with nmCF.

In 2009, a phase 3 clinical trial involving 238 nmCF patients was initiated with the support of the above clinical trials. In a double-blind trial conducted by 36 centers, subjects were treated for 48 consecutive weeks (40 mg/kg/d). The researchers used "FEV1% predicted" and "rate of deterioration of lung symptoms" as primary endpoints and secondary endpoints, respectively. The results showed that there was no significant difference in the above two indexes between the experimental group and the placebo group, and there was no significant difference in the results of chloride concen-

tration and NPD in sweat between the two groups. This is clearly inconsistent with previous clinical trials; however, further studies have found that this may be due to long-term use of tobramycin in CF patients interfering with the treatment of PTC124. There was a significant difference between the results of patients who had not been treated with tobramycin for a long time and those of the control group ($P < 0.05$), and the results of cytological studies on luciferase also confirmed that aminoglycoside antibiotics such as tobramycin weakened the ability of PTC124 to promote reading comprehension. Therefore, another phase 3 trial is under way to further confirm the efficacy and safety of PTC124 in NMCF patients without tobramycin.

3. Existing problems and coping strategies

Although data have shown that reading-promoting drugs are effective in the treatment of DMD and CF, there are still many problems to be solved.

3.1 The effect of promoting drug reading

In the clinical trials of DMD and CF, either aminoglycosides or PTC124, only some of the subjects had better efficacy, and a considerable number of subjects did not respond significantly to reading-promoting drugs[21,38,44]. According to the previous study on the mechanism of promoting reading drugs, the mechanism of many new drugs is still unclear. In order to further prove the specificity and sensitivity of reading-promoting drugs, more data on the mechanism of action of new drugs are needed.

3.2 Incorporation of non functional amino acids

Because of the addition of near aminoacyl tRNA at the PTC site, the newly synthesized peptide chains are not all active. If the mutation site happens to be in the functional region of the protein, or is a special amino acid indispensable to the function of the protein, then the vast majority of the translated proteins may not be active, thus affecting the efficacy. Since different drugs may induce different proximally related tRNA to enter the ribosomal A site corresponding to PTC, it is recommended to try other drugs for patients who are insensitive to one drug.

3.3 The interference of NMD channels

NMD pathway often reduces the abundance of PTC-carrying mRNA in cells, thus reducing the target of drug-mediated reading (mRNA template) and the amount of protein synthesized, which directly affects the efficacy of drug-mediated reading[2]. For this reason, researchers can try to use NMD pathway inhibitors to partially restore the content of mRNA template.

3.4 Drug delivery and side effects

Most aminoglycoside read-through drugs are administered intravenously, which is inconvenient for patients requiring long-term treatment, compared with oral PTC124 preparations. More importantly, long-term use of aminoglycoside antibiotics such as gentamicin increases the risk of ototoxicity and nephrotoxicity[23]. In contrast, PTC124 has more advantages in this respect, and new aminoglycosides with low toxicity and high efficiency such as NB54, NB84 also have application prospects.

3.5 The problem of immune response

For patients with nonsense mutations that do not express the target protein at all before treatment, the protein produced by the read-through drug is likely to be a new antigen for the body. And this new antigen will lead to the production of specific immune cells in vivo, and react with them, and eventually be cleared by the immune system. Although this is only a theoretical hypothesis, tests have found that T cells with strong reactivity to amyotrophic protein[20] have been detected in a patient with DMD who did not respond to gentamicin treatment. Accordingly, in the specific treatment process, it is suggested that weigh the pros and cons and use immunosuppressive agents for treatment.

3.6 The mechanism of action of new drugs for promoting reading, such as PTC124

Auld *et al*.[50] suggested that there were loopholes in the design of luciferase reporter gene experiments for screening PTC124. Their experiments show that PTC124 is a competitive inhibitor of luciferase, which can bind and stabilize luciferase in cells and prolong its half-life. After cell lysis, the activity of firefly luciferase increased. In other words, the luciferase gene containing the early termination codon may not increase its expression due to the read-through effect of PTC124. To solve this problem, more structural biological evidence is needed to support the interaction between PTC124 and ribosomes.

References

1. Keeling KM, Bedwell DM. Suppression of nonsensemutations as a therapeutic approach to treat geneticdiseases. Wiley Interdiscip Rev RNA, 2011, 2(6): 837–852.
2. Bidou L, Allamand V, Rousset JP, Namy O. Sense fromnonsense: therapies for premature stop codon diseases.Trends Mol Med, 2012, 18(11): 679–688.
3. Lee HLR, Dougherty JP. Pharmaceutical therapies to recode nonsense mutations in inherited diseases. Pharmacol Ther,2012, 136(2): 227–266.
4. Joe Middle East. Molecular biology. Beijing: Military Medical Science Press, 2012.
5. the zhouravleva g, frolova, goff (x, s, r) - vechtomov, kisselev, philip m. terminationof translation in eukaryotes is governed by two interactingpolypeptide chain release factors, erf1 and erf3. emboj, 1995, 14 (16) 4065–4072.
6. - m, frizzell ra, bedwell dm. aminoglycosideantibiotics restore cftr function by overcoming premature stop mutations. nat med. 1996, 2 (4): 467–469.
7. a review mcclendon fearon k, v, b, premature translation termination mutations bedwell. dm is efficiently suppressed in a highly conserved region of the yeast ste6p, amember of the atp binding cassette (abc) terfamily carriers. j biol chem, 1994, 17802–17808 269 (27).
8. mittelstaet wohlgemuth, pohl c j, konevega al, rodnina mv. evolutionary optimization of speed and accuracy of decoding on the ribosome. philos trans r soc b biol sci through 2011, 366 (1580), 2979–2986.
9. keeling km, wang d, asshole, bedwell dm. reg - ression of premature termination codons as a therapeutic approach. crit rev biochem mol biol, 2012, 47 (5): 444–463.
10. ogle jm, murphy, fv, tarry mj, ramakrishnan. sele - trna by the ribosome, and requires a transition from an open to a closed form. cell, 2002, 111 (5): 721–732
11. demeshkina, jenner, westhof, yusupov, yusu - pova g. a new understanding of the decoding site on the ribosome. nature, 256–259 484 (7393).
12. laurberg, korostelev asahara h, zhu jy, trakhanovs, noller hf. structural basis for translation terminationon the 70s ribosome. nature, 2008, 454 (7206): 852–857.
13. fan - minogue, bedwell dm. eukaryotic ribosomal rnadeterminants of aminoglycoside resistance and their role in translational fidelity. nas, (2008), 14 (1): 148–157.
14. keling km, brooks da, hopwood jj, li, c, thompson jn, bedwell dm. gentamicin mediated suppression of shout stop syndrome mutations restores a low level of alpha activity and reduces the iduronidase gly cosaminoglycan lysosomal accumulation. hum mol genet, 2001,10: 291–299 (3).
15. amrani, ganesan, kervestin, mangus da, ghosh, a. he (3 '- utr promotes aberrant termination and triggers nonsense mediated mrna decay. nature, 2004, 432 (7013): 112–118.
16. hoshino, imai m, kobayashi t, uchida, katada t. the eukaryotic polypeptide chain releasing factor (erf3 / gspt) carrying the translation termination signal to the3 '- poly (a) tail of mrna. direct association of erf3 / gspt with polyadenylate binding protein. j biol chem, 1999274 16677–16680 (24).
17. the 450 km, fan m, lm, liu xl, kovacs, sorscher, bedwell dm. clinical doses of mycobacterial provide more effective suppression of the human cftr - g542x sto mutation than gentamicin in a transgenic cf mouse model j mol med (berl), 2006, 84 (7): 573–582.
18. howard mt anderson cb, - u, khatri s, gesteland rf, atkins jf, flanigan km. readthrough of dystrophin stop codon mutation induced by aminoglycosides. annneurol, 2004, 55 (3): 422–426
19. wang d, belakhov v, kandasamy j, baasov t, li, sc, li
yt, bedwell dm, keeling km. the designer aminog - lycoside nb84 significantly reduces glycosaminoglycan accumulation associated with mps i - h in the idua - w392x mouse. mol genet metab, 2012, 105 (1): 116–125.
20. malik v, rodino - klapac lr, viollet l, wall c, king w, al dahhak r, lewis, bob cj, kota j, serrano - munuera c, hayes j, mahan jd, campbell kj, banwell b, dasouki m watts v, sivakumar k, bien - german language literature r, flanigan km, sahenk z, barohn rj, walker cm, mendell jr. gentamicin - induced readthrough o f stop codons in metabolic on metabolic syndrome. ann neurol, 2010, 67 (6): 771–780.
21. sermet - gaudelus i, renouil m, fajac a, bidou l, parbaille b, pierrot, davy, bismuth, reinert p, lenoir g, lesure jf, rousset jp, edelman a. in vitro prediction of stop codon suppression by intravenous gentamicin in patients with cystic fibrosis: a pilot study.bmc med in 2007, 5: 5.
22. sangkuhl k, schulz a, römpler h, yun j, wess j, beautiful mountain t. aminoglycosides mediated rescue of a disease - cau - sing nonsense mutation in the v2 vasopressin receptor gene in vitro and in vivo. hum mol genet, 2004, 13

(9): 893–903.
23. nudelman i, glikin d, smolkin b, hainrichson m, bela - khov v, baasov t. repairing faulty genes by aminoglycos - ides: development of new derivatives of geneticin (g418) with enhanced suppression of diseases causing nonsense mutations. bioorg med chem, 2010, 18 (11): 3735–3746.
24. rowe sm, sloane p, tang lp, backer k, mazur m, buckley lamer j, nudelman i, belakhov v, bebok z, schwiebert e, baasov t, bedwell dm. suppression of cftr premature termination codons and rescue of cftr protein and function by the synthetic aminoglycosides nb54. j mol med (amsterdam barcelona is not only fas), 2011, 89 (11): 1149–1161.
25. gunn g, dai yy, you m, belakhov v, kandasamy j, schoeb tr, baasov t, bedwell dm, keeling km. long term nonsense suppression therapy moderate mps i - h disease progression. mol genet metab, 2014, 111 (3): 374–381.
26. - vb, ebert ad, fosso my, chang cw, lorson ci. delivery of a read through inducing compound tc007, lessens the severity of a spinal atrophy on animalmodel. hum mol genet, 2009, 18 (20): 3906–3913.
27. allamand v, bidou l m, arakawa, floquet c, shiozuka m
paturneau - jouas m, gartioux c butler browne gs, mouly v, rousset jp, matsuda t, ikeda t, guicheney p.drug-induced readthrough of premature stop codon leads to the stabilization of laminin α2 chain mrna in cmd myotubes. j gene med, 2008, 10 (2): 217–224.
28. zilberberg a, lahav l, rosin - arbesfeld r. restoration of apc gene function in colorectal cancer cells by aminog - lycoside and macrolides an read through of premature termination codons. well, 2010, 59 (4): 496–507.
29. you m, liu xl, the em, hirawat, peltz sw, bedwell dm. ptc124 is orally bioavailable compound that promotes suppression of the human cftr g542x nons - ense alleles in a cf mouse model. proc natl acad sci usa, 2008, 105 (6): 2064–2069.
30. the em, barton, zhuo j, tomizawa y, friesen wj, trifillis p, paushkin s, patel m, trotta cr, hwang s wild rg, karp g, takasugi j, chen gm, jones s, ren hy, moon yc, corson d, turpoff aa, campbell, conn mm, khan a, almstead ng, hedrick j, mollin a, risher n, weetall m, yeh, branstro m aa, colacino jm, babiak j, ju wd, hirawat, northcutt vj, miller ll, spatrick p, - f, kawana m, feng hs, jacobson, peltz sw, sweeney). ptc124 targets genetic disorders cau - sed by nonsense mutations. nature, 2007, 447 (7140): 87–91.
31. overlack goldmann t, n, wolfrum u, nail wolfrum k. ptc124 mediated translational readthrough of a nonsense mutation causing usher syndrome type 1c. hum gene ther, 2011, 22 (5): 537–547.
32. wang b, yang zh, brisson bk, feng hs, zhang zq the em, peltz sw, barton, brown rh, jr., sweeney).
brane blebbing as an assessment of functional rescue of dysferlin - deficient human myotubes via nonsense suppression. j appl physiol (1985), 2010, 109 (3): 901–905.
33. you lt, damoiseaux r, nahas, gao k, hu hl, pollard jm, goldstine j, young me, henning sm, bertoni c, gatti ra. nonaminoglycoside compounds induce readthrough of nonsense mutations. j exp med, 2008, 206 (10): 2285–2297.
34. barton davis er, cordier, shoturma, leland, sweeney hl. aminoglycoside antibiotics stay dystro - phin function to skeletal muscles of mdx mice. j clinton invest, 1999, 104 (4): 375–381.
35. wagner kr, hamed d, hadley dw, gropman al, burstein ah, escolar, dm, hoffman ep, fischbeck kh. gentamicin treatment of duchenne and becker muscular dystrophy to nonsense mutations. ann neurol, 2001,49 (6): 706–711.
36. politano l, for g, for v, piluso g, papparella s, paciello or, as li. gentamicin administration in duchenne patients with premature stop codon. preliminary results. acta myol, 2003, 22 (1): 15–21.
37. wilschanski m, famini c, blau h, rivlin j, augarten, avital, kerem b, kerem and. a pilot study of the effect of gentamicin on nasal potential difference measurement in cystic fibrosis patients carrying stop mutations. am j in crit care med, 2000, 161 (3 pt 1): 860–865.
38. clancy jp, rowe sm, bebok z, aitken ml, gibson r, zeitlin p, berclaz p, moss r, knowles, mr. oster ra, mayer - hamblett n, ramsey b. no detectable improve - ments in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. am j cell mol in biol, 2007, 37 (1): 57–66.
39. james pd, raut s, rivard ge, poon mc, warner m, mckenna's, read j, lillicrap d. aminoglycoside suppression of nonsense mutations in strict hemophilia. blood, 2005, 106 (9): 3043–3048.
40. nudelman, rebibo - sabbah, cherniavsky m, belakhov
v, hainrichson m, chen play fq all the time back, schacht j, pilch sd.
ben yosef t, baasov t. development of novel aminogly - coside (nb54) with reduced toxicity and enhanced suppression of disease - causing premature stop mutations. j med chem, 2009, 52 (9): 2836–2845
41. haas m, vlcek v, balabanov p, salmonson t, bakchine s, markey g, weise m, weber brohmann article g, h, yerro cp, mendizabal mr. stoyanova - beninska v, hillegehl. the european medicines agency review of ataluren for the treatment of ambulant patients aged 5 years and older with duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. neuromuscul disord, 2015, 25 (1): 5–13.
42. tan l, narayan sb, chen j, meyers gd, bennett mj.ptc124 improves readthrough and increases enzymatic activity of the cpt1a r160x nonsense mutation. j inherit metab dis, 2011, 34 (2): 443–447.
43. hirawat s. welch em, elfring gl, northcutt vj, paushkin s, hwang s, leonard em, almstead ng, ju w, peltz sw, miller. safety, tolerability, and pharmacokinetics of ptc124, nonaminoglycoside nonsense mutation suppre - ssor, following single - and multiple dose administration to healthy harm and female adult volunteers. j clinton pharmacol, 2007, 47 (4): 430–444

44. * * * * rs, flanigan km, wong b, bonnemann c, sampson j, sweeney hl, reha, northcutt vj, elfring g, barth j, peltz sw. phase ii study of ataluren - mediated dystrophin production in patients with nonsense mutation duchenne muscular dystrophy. plos one, 2013, 8 (12): e81302.
45. bushby k, * * * * r, wang b, barohn r, campbell c, (gp, connolly am, day jw, flanigan km, goemans n, jones kj, mercuri, and quinlivan r, renfroe jb, russman b, ryan mm, tulinius m? t, moore, lee sweeney h abresch rt, coleman kl, eagle m, florence j., gappmaier and glanzman am, henricso n, barth, j, elfring gl, reha, spiegel rj, o'donnell m w, peltz sw, mcdonald cm, the ptc124 - gd - 007 - dmd study group. ataluren treatment of patients with nonsense mutation dystrophinopathy. muscle nerve, 2014, 50 (4): 477–487.
46. kerem and hirawat s, armoni s, yaakov y, shoseyov d, cohen m, nissim - rafinia m, blau h, rivlin j, aviram m, elfring gl, northcutt vj, miller a, kerem b, wilschanski m. effectiveness of ptc124 treatment of cystic fibrosis caused by nonsense mutations: prospec phase ii trial - ing. lancet, 2008, 372 (9640): 719–727.
47. Wilschanski M, Miller LL, Shoseyov D, Blau H, Rivlin J, Aviram M, Cohen M, Armoni S, Yaakov Y, Pugatsch T, Cohen-Cymberknoh M, Miller NL, Reha A, Northcutt VJ, Hirawat S, Donnelly K, Elfring GL, Ajayi T, Kerem E.Chronic ataluren (PTC124) treatment of nonsense mutation cystic fibrosis. Eur Respir J, 2011, 38(1): 59–69
48. Sermet-Gaudelus I, Boeck KD, Casimir GJ, Vermeulen F, Leal T, Mogenet A, Roussel D, Fritsch J, Hanssens L, Hirawat S, Miller NL, Constantine S, Reha A, Ajayi T, Elfring GL, Miller LL. 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, 2010,182(10): 1262–1272.
49. Kerem E, Konstan MW, De Boeck K, Accurso FJ, Sermet-Gaudelus I, Wilschanski M, Elborn JS, Melotti P, Bronsveld I, Fajac I, Malfroot A, Rosenbluth DB, Walker PA, McColley SA, Knoop C, Quattrucci S, Rietschel E, Zeitlin PL, Barth J, Elfring GL, Welch EM, Branstrom A, Spiegel RJ, Peltz SW, Ajayi T, Rowe SM, The Cystic Fibrosis Ataluren Study Group. Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Respir Med, 2014, 2(7): 539–547.
50. Auld DS, Thorne N, Maguire WF, Inglese J. Mechanism of PTC124 activity in cell-based luciferase assays of non- sense codon suppression. Proc Natl Acad Sci USA, 2009, 106(9): 3585–3590.
51. Sheridan C. Doubts raised over 'read-through' Duchenne drug mechanism. Nat Biotechnol, 2013, 31(9): 771–773.