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## Mutation specific therapies

### Introduction

Two therapies for Duchenne patients are currently being tested in clinical trials, which are applicable only to patients with specific mutations: PTC124 (treats only stop mutations) and exon skipping (restores the genetic code for certain deletions). Here I will explain why these approaches can not be applied to all patients.

As you can read in the [mutations section](#), a disruption of the genetic code leads to the use of aberrant protein subunits, whereas a point mutation causes a premature stop signal.

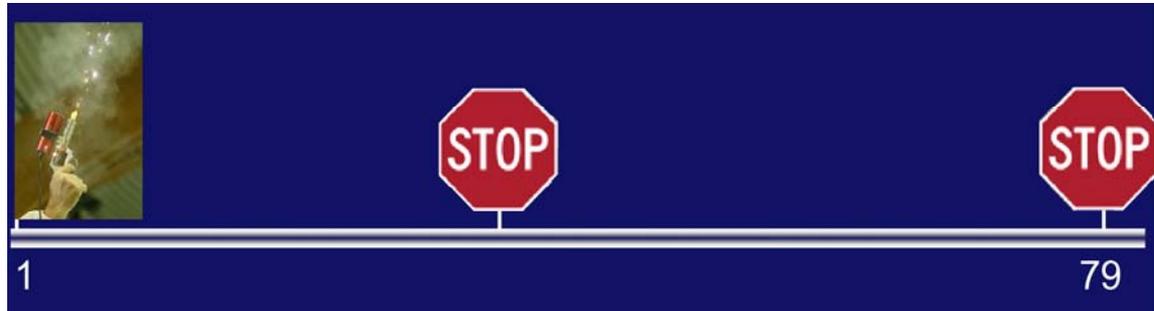
### PTC124

Point mutations are small changes in the DNA that alter a protein subunit code into a stop signal. These stop signals are normally only present at the end of an mRNA (figure 1).



*Figure 1. Messenger RNA contains the genetic code for a protein. The translation of the mRNA into protein is performed by the protein factory. This factory needs a "start" signal to initiate protein translation (in the first exon) and a stop signal (at the end of the mRNA, i.e. exon 79 for dystrophin) to indicate that the protein is complete.*

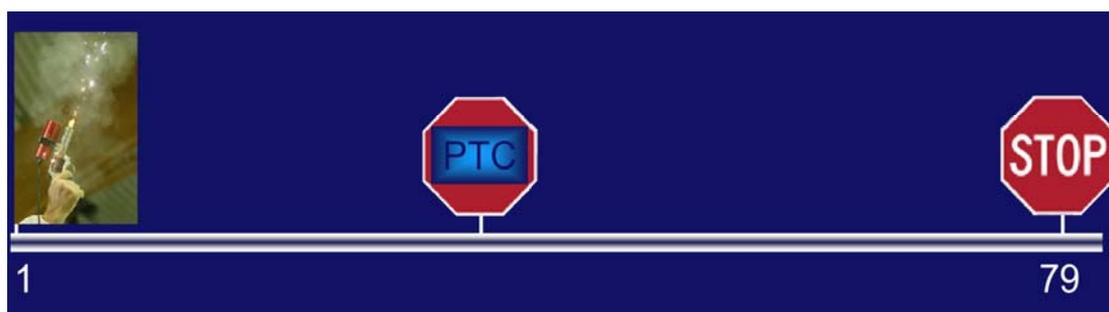
Due to stop mutations, in addition to the normal stop signal at the end of the genetic code, an extra stop signal is present in the middle of the genetic code (figure 2).



*Figure 2. A stop mutation causes the premature stop of protein translation (and thus only half a protein is made).*

Generally, there are additional signs that indicate to the protein factory that the translation into protein is almost complete (e.g. normal stop signals are located at the end of the mRNA and not in the middle). You can compare this with a stop sign in traffic: it makes sense at a very busy crossing, but not in the middle of a highway. There is thus a difference between normal and aberrant stop signals. Nevertheless, the protein factory obeys the aberrant stop signal and protein translation is stopped.

PTC124 is a drug that can make the protein factory ignore the stop signals that do not make much sense (in the middle of a highway), so that protein translation can continue and a complete protein can be generated (figure 3). The real stop signal (at the end of mRNA (or at a busy crossing)) are not ignored.



*Figure 3. PTC hides the aberrant stop signals and protein translation can continue until the end of the mRNA (the real stop signal).*

PTC does not affect mutations that disrupt the genetic code (deletions or duplications of one or more exons, small deletions or duplications within an exon, or small mutations that disrupt exon definition during splicing (splice site mutations)). For these mutations, there is not a single stop signal at an unusual place, but rather aberrant protein subunits are included into the protein. PTC will not change this (even with PTC the parts that make a model airplane, still cannot make a model car).

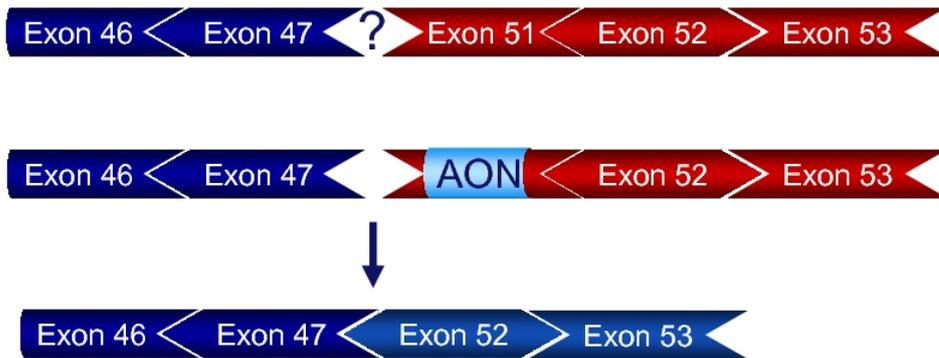
## Exon skipping

The aim of exon skipping is to restore the genetic code, so that the correct protein subunits are used (instead of the aberrant ones), to allow the synthesis of a partially functional proteins (a model car that has some parts missing is more useful than one with aberrant airplane parts). The reasoning for this approach is explained [here](#) in detail. Exon skipping can be used to restore the genetic code for different mutations types.

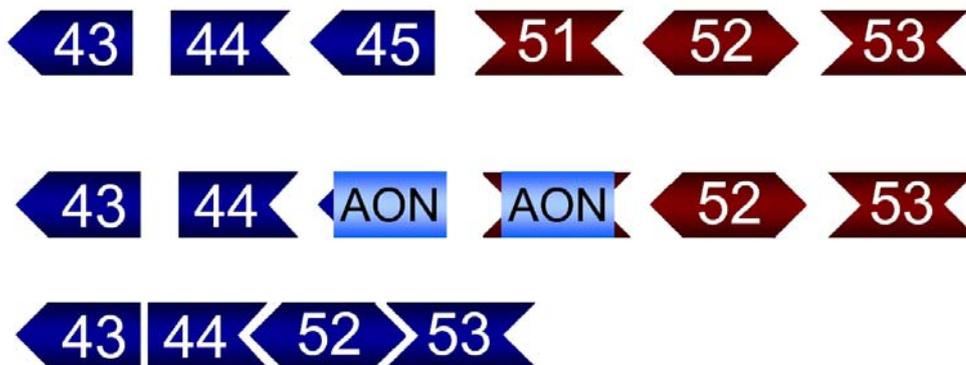
The following figures contain three parts: at the top the situation at DNA level (so the level of the gene) is depicted, in the middle the RNA copy is shown (occasionally one of the exons is hidden by an AON). At the bottom the resulting mRNA (so only the genetic code (the exons) is shown.

## Deletions

For most deletions of one or more exons, the genetic code can be restored by the skipping of one (figure 4) or two (figure 5) exons.



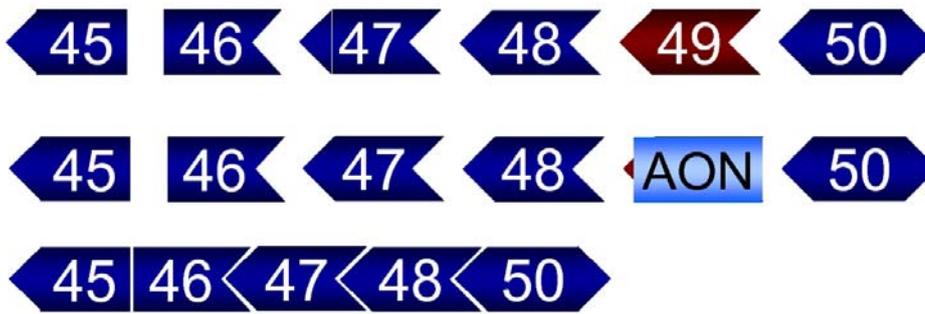
*Figure 4. In this example exon 48-50 are missing (due to a deletion). As exon 47 and 51 do not fit, the genetic code is broken. Since exon 47 and 52 do fit, the genetic code can be restored by skipping of exon 51.*



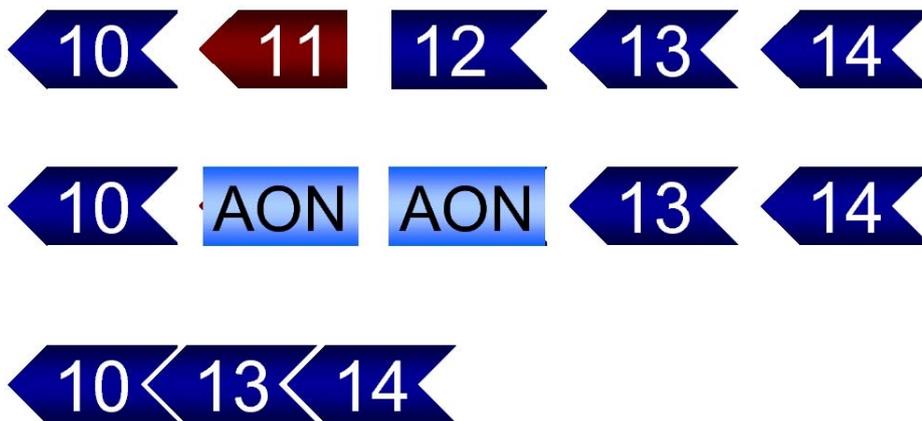
*Figure 5. In this example exon 46-50 are deleted. Exon 45 and exon 51 do not fit, so the genetic code is broken. However, skipping of a single additional exon will not restore the genetic code (exon 45 skipping does not improve things, as exon 45 and 52 do not fit either, and exon 51 skipping also does not work, as exon 45 and 52 do not fit). Only when both exon 45 and exon 51 are skipped, is the genetic code restored (exon 44 fits to exon 52).*

### Small mutations

Small mutations can result in a premature stop signal (stop mutations), or they can disrupt the genetic code (a small deletion or duplication within an exon). Using exon skipping these mutations can be bypassed (figure 6 and 7).



*Figure 6. Due to a small mutations, a premature stop signal has arisen in exon 49. Because exon 48 and exon 50 fit, skipping exon 49 will bypass the stop signal without disrupting the genetic code.*

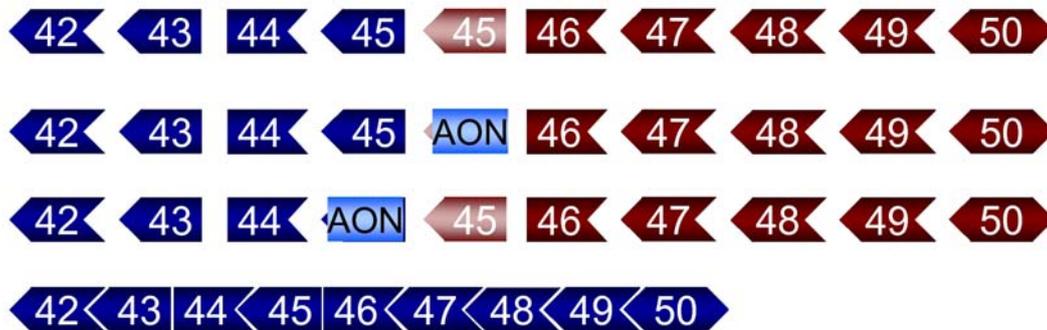


*Figure 7. Due to a small mutation, a stop signal occurs in exon 11. Exon 10 and 12 do not fit, so skipping exon 11 will bypass the stop signal, but also disrupt the genetic code. Exon 10 does fit to exon 13, so skipping both exon 11 and exon 12 will bypass the stop signal, while maintaining the genetic code.*

### Duplications

Duplications disrupt the genetic code because one or more exons are duplicated. This complicates exon skipping, as the AONs can not distinguish the duplicated and the original exon (they are completely similar). For duplications involving a single exon, exon skipping can be used to restore the genetic code (figures 8, 9 and 10). However, for

larger duplications, exon skipping is currently too complicated (figures 11, 12 and 13).



*Figure 8. A duplication of exon 45. As the additional exon 45 (white/red) does not fit to the original exon 45 (blue) the genetic code is broken. However, skipping either the duplicated or the original exon will restore the genetic code completely (this is the normal genetic code: no exons are missing). This is the most ideal result that can be obtained through exon skipping. However, sometimes exon skipping is too efficient (figure 9). The efficiency of exon skipping is difficult to predict and only becomes apparent when AONs are tested in cultured muscle cells from individual patients with a single exon duplication.*



*Figure 9. When exon 45 skipping is too efficient for a patient with an exon 45 duplication, both the original and the duplicated exon will be skipped. As exon 44 does not fit to exon 46 this will disrupt the genetic code.*



*Figure 10. If skipping a single exon is not feasible (figure 9) because exon skipping is too efficient, the genetic code can still be restored by skipping an additional (third) exon (exon 44 in this example). Since exons 43 and 46 fit, this will restore the genetic code. (In this example it*

*is also feasible to skip exon 46 in addition to both exons 45, since exon 44 and 47 also fit).*



*Figure 11. Exon skipping for larger duplications is complicated. In this example, exons 44-48 are duplicated. This disrupts the genetic code, as exon 44 and exon 48 do not fit. The genetic code can be restored through skipping of the duplicated exon 44. However, an AON targeting exon 44 will also recognize the original exon 44 (figure 12 and 13), which will disrupt the genetic code (regardless of whether the duplicated exon is skipped).*



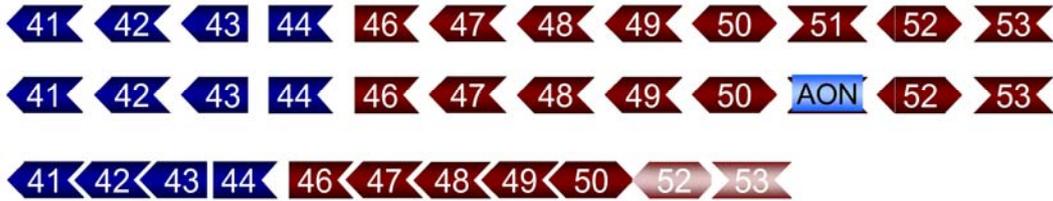
*Figure 12. Skipping the original exon 44 disrupts the genetic code, as exon 43 and 45 do not fit.*



*Figure 13. When both the original and the duplicated exon are skipped the genetic code is broken as well. There are three possibilities (skipping both exons 44, skipping the original exon 44 and skipping the duplicated exon 44) and only one of them (skipping the duplicated exon 44) is beneficial. Therefore, the efficiency of AON treatment will be very low.*

### **Applicability of exon 51 skipping**

Skipping exon 51 is applicable to the largest group of Duchenne patients (13% of all patients). Figure 4 shows how exon 51 skipping can restore the genetic code. Figure 14 explains why exon 51 skipping does not work for mutations that require the skipping of other exons.



*Figure 14. Exon 51 skipping for a patient with an exon 45 deletion. A deletion of exon 45 disrupts the genetic code (exon 44 and exon 46 do not fit). This deletion can NOT be restored by exon 51 skipping. When exon 51 AONs are used anyway this will result in a disruption of the genetic code at two positions: at the location of exon 44-46 (does not fit) and at the location of exon 50-52 (also does not fit). So exon 51 does not restore the genetic code for this mutation, but instead disrupts it at an additional position.*