General discussion

Chapter 8

8 General discussion

During the past years major steps forward have been made in developing antisense-mediated exon skipping, a potential therapy for DMD, which hopefully can turn the very severe progressive disease in a milder disease course and improve the quality of life. In addition, the knowledge about disease pathology and AONs is increasing. The antisense compounds are continuously developed further. However, there is still a lot to discover and hurdles to overcome to make the therapy successful. There are several strategies to try to improve its efficacy. One way is to improve the AONs itself or their administration. Another way is to enhance the therapeutic outcome by improving the amount/quality of their target, *i.e.* muscle.

Towards the first approach in **chapter 3 and 4** more detailed studies into the pharmacokinetic and pharmacodynamic properties of AONs in *mdx* mice are described. In **chapter 3** the effect of different dosage schemes in *mdx* mice at several levels was tested. Compared to single weekly injections, dividing the same total amount of AON over multiple injections showed to enhance its effects. Furthermore this chapter covered the influence of the frequency of repetition of injections on the maintenance of these effects after initial treatment. In both cases it revealed that increasing the desired effects, *i.e.* exon skipping and dystrophin restoration also increases the AON load on non-target organs, *i.e.* the liver, kidney and spleen, thereby increasing the risk of side effects on the long term. Therefore a balance between both effects has to be found. Towards this aim, in **chapter 4** a more detailed analysis of the pharmacokinetic and pharmacodynamic profile of AONs in *mdx* mice was performed by studying the turnover of the compound, skipped transcripts and newly formed dystrophin protein. This highlighted in particular the long half-life of the dystrophin protein. These studies may have implications for optimising AON administration schemes.

In the second part of this thesis several compounds were tested to directly or indirectly influence antisense-mediated exon skipping effects. In **chapter 5** an agent described in the literature to directly enhance AON-mediated exon skipping, 6-thioguanine (6TG), was tested in cultured cells and locally in the *mdx* mouse. *In vitro* a large number of undesired skipping events were observed, underlining that caution must be taken when using such compounds, since the chance of side effects is high. *In vitro* enhancement was only observed with suboptimally designed AONs or at suboptimal AON-concentrations, while *in vivo* no differences were observed. Here only 6TG has been tested, but other small molecules, like the recently described dantrolene, could be more effective and specific.

In **chapter 6 and 7** pharmaceutical compounds, which could potentially improve muscle quality, were tested in combination with AONs. In **chapter 6** prednisolone was used. At the moment, since a therapy targeting the underlying genetic defect is lacking, corticosteroids (predniso(lo)ne or deflazacort) are the main (pharmacological) treatment for DMD patients. Therefore most participants in clinical trials are using these compounds, which might influence the uptake and/or efficiency of AONs. Results showed that both therapies did not negatively influence each other and that prednisolone might even slightly enhance the therapeutic outcome of AON treatment. In **chapter 7** another pharmaceutical compound that showed promising results in literature on preserving muscle quality and functionality in *mdx* mice, losartan, was tested in combination with AONs to see if it could enhance efficacy. However these experiments were stopped prematurely, since interim analyses did not show any beneficial effects of losartan itself or on the working of AONs. This was supported by new literature. The results of these losartan experiments are an example of how sometimes published results can be difficult to reproduce, but at the same time underline the value of

trying to reproduce other (or your own) results and the importance of publishing negative outcomes, to avoid unnecessary repetition or clinical development of suboptimal drugs. In addition to the compounds described in these chapters, several compounds have been tested in cell models for their effect on exon skipping or *in vivo* for their effect on muscle quality in the *mdx* mouse, but unfortunately no compound has been found so far that can enhance exon skipping itself or improve the therapeutic outcome *in vivo* by improving muscle quality.

The hypothesis behind combining AONs with muscle quality improving agents was that AONs can only be effective if their target, dystrophin RNA, is present, which is only produced by muscle cells. First clinical trials had shown that the amount of dystrophin production and functional outcome depended on the amount of muscle tissue left, *i.e.* the muscle quality. However, improving muscle quality could also have a negative effect on AON efficiency. It is known from studies comparing the uptake of AONs between wild type and *mdx* mice that the uptake is much lower in wild type mice, since the AON uptake is facilitated by the leakiness of the dystrophic muscle fibres. If this permeability decreases by improving muscle quality, the uptake might be hampered. Therefore the reason no enhancement of AON effects could be found, can either be that there is no effect or that the positive and negative effects are neutralizing each other. Furthermore large interindividual variation causes that only large improvements will be picked up and longer treatment time might be needed, before differences will be found. This also raises the question how meaningful very small differences would be in the clinic at the end.

Probably larger improvements can be made by improving the effectiveness of the AON therapy itself. One strategy is to use viral vectors to get a higher and longer lasting expression of AONs. However, this approach has many drawbacks as viral vectors can evoke immune responses, as a result of which repetition of treatment is not possible. At the moment, a better strategy seems to be to modify the AONs themselves. Preclinical studies *in vitro* and *in vivo* have shown that large improvements can be made by for example conjugating muscle-targeting peptides to them. Although also here prudence is called for, since some of these peptides have shown to be toxic in higher animals, like primates.

An issue that appears in every study is the difficulty of targeting the heart, where observed exon skipping levels and restored dystrophin protein are much lower compared to skeletal muscle. This will become more and more important once the therapy becomes more effective, since higher activity levels will result in a higher workload on the heart. Also here conjugation of the AON with a specific heart-targeting peptide might be an option.

Nevertheless results from recent clinical trials have shown the importance of muscle quality. It indicates that starting treatment as early as possible would be recommended. The therapy can only put a hold on or slow down muscle degeneration, not bring back muscle that is already lost. Reaching a level of dystrophin restoration high enough to do so, is needed. Therefore more detailed studies on the effect of different levels of dystrophin restoration on muscle overall muscle functioning, but also on differences between individual muscles are very helpful.

All these matters require further investigation and optimisation of the AON-mediated exon skipping therapy. Especially the clinical trials will give more insights on the translation from animal models to humans and on the problems that have to be tackled in order to make it an effective therapy.