Measuring pharmacodynamics in early clinical drug studies in multiple sclerosis

Kanhai, K.M.S.

VIII

SUMMARY AND GENERAL DISCUSSION

This thesis describes six clinical studies: two studies that investigate new compounds to treat symptoms of multiple sclerosis (MS) (**chapters 2 and 3**), one study that investigates a new compound to treat MS (**chapter 4**) and three studies about the development of new methods to determine effects of a new class of compounds to treat MS (**chapters 5, 6 and 7**). When reflecting on the results of the studies, two major issues stand out: First, despite the range of treatments available (**chapter 1**), research into novel compounds is needed to improve the treatment and prognosis of patient with MS. Second, although we show it is feasible to integrate biomarker development into early phase clinical trials, it is not yet common practice.

MAIN OUTCOMES

Chapter 1 starts with a brief description of the disease multiple sclerosis (MS): the most common autoimmune disorder of the central nervous system, and primarily an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin (demyelination) and axons. In the introduction, we divide current treatment of MS into disease-modifying treatments (DMDS) and symptomatic treatments. We indicate that – despite its increasing efficacy and discovery of these compounds over the years – most registered DMDS are associated with a high disease burden due to side effects. For that reason, there is still a need for an effective treatment to halt the progression of disability by developing remyelinating and neuroprotective therapies. As the current frequently used biomarkers in MS diagnosis and treatment management are not yet capable of accurately measuring treatment effects of this group of compounds, trials with potential neuroreparative or neuroregenerative agents will need improved and appropriate biomarkers.

Chapter 2 describes a study to evaluate the efficacy of a novel oral formulation of Δ9-THC (ECP002A) in patients with progressive MS. Δ9-THC is one of the cannabinoids in the Cannabis sativa plant and a direct partial agonist of the cannabinoid receptors CB1 and CB2. Cannabinoids have previously shown to improve symptoms of MS including muscle spasticity and pain through the modulation of neuronal excitability via presynaptic cannabinoid receptors. The study described in chapter 2 was a proof-of-concept study with two phases: a crossover challenge ('dose-finding') phase and a 4-week parallel randomized, placebo-controlled treatment phase. Twenty-four patients with progressive MS and moderate spasticity were enrolled. Pain and spasticity were significantly reduced when measured directly after administration of ECP002A in the clinic, but not when measured in a daily diary. Nevertheless, despite the complex interplay of psycho-active effects and analgesia, the current oral formulation of Δ9-THC may play a role in the treatment of spasticity and pain associated with MS, as it was well-tolerated and showed a stable pharmacokinetic profile. Additionally, this study specifically underlined the potential added value of thorough investigation of PK/PD relationships in the target population.

In the study reported in **chapter 3** we tested a new formulation of methylprednisolone (MP), which is commonly used for the treatment of MS relapses. Both dose and dosing frequency might be reduced by incorporating free MP in glutathione (GSH) PEGylated liposomes, creating a slow-release formulation with reduced toxicity and prolonged efficacy. This first-in-human study was designed to assess the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of GSH-PEGylated liposomes containing MP (2B3-201). 2B3-201 led to a high occurrence of infusion related reactions (89%), but was otherwise considered safe, with no clinically relevant changes in (CNS) safety parameters and no serious adverse events. 2B3-201 was shown to have a long plasma half-life between 24 and 37 hours and led to prolonged immunosuppressive effects.

Chapter 4 reports a first-in-human single ascending dose study (SAD), followed by a 21-days multiple ascending dose (MAD) study, performed to assess the safety and PK of CNM-AU8. CNM-AU8 is a solution of clean-surface gold nanocrystals and being developed for its potentially immunomodulating and remyelinative effects, while leading to fewer side effects than registered gold-containing compounds. In vitro inflammatory challenge experiments were initiated to investigate possible PD effects. CNM-AU8 showed a good safety profile, which appeared to be more favorable than existing gold formulations, with the most frequently reported adverse event related to CNM-AU8 being abdominal pain (20%) CNM-AU8 had a plasma half-life between 11.5 to 26.2 days, and a first dose t_{max} between 3.3 and 3.5 hours. The low concentrations and unusual pharmacokinetic profile corresponded to the reported data on gold nanoparticles. No anti-inflammatory effects of CNM-AU8 were observed in the in vitro challenge, and therefore no ex vivo PD measurements were implemented in the clinical study. Next studies should determine whether CNM-AU8 possesses anti-inflammatory effects and remyelinating effects.

Therapeutics promoting myelin synthesis may enhance recovery in demyelinating diseases such as MS. However, no suitable method exists to quantify myelination. The turnover of β-galactosylceramide (β-GALC, a myelin component) is indicative of myelination in mice, but its turnover has not been determined in humans.

Chapter 5 describes a study in which six healthy subjects consumed 120 mL 70% D_2O daily for 70 days to label β-GALC. We subsequently used mass spectrometry and compartmental modeling to quantify the turnover rate of β-GALC in cerebrospinal fluid. Maximum deuterium enrichment of body water ranged from 1.5 to 3.9%, while that of β-GALC was much lower: 0.05–0.14%. This suggested a slow turnover rate, which was confirmed by the model-estimated β-GALC turnover rate of 0.00168 day^{-1}, corresponding with a half-life of 413 days. Based on the results, we suggested that additional studies in patients with MS are needed to investigate whether β-GALC turnover could be used as an outcome measure in clinical trials with remyelination therapies.

Chapter 6 describes the follow-up study wherein we labeled myelin with deuterium in 5 MS patients, and besides β-GALC also modeled the myelin breakdown product N-Octadecanoyl-sulfatide (NO-Sulf). The turnover rate constants of β-GALC and NO-Sulf with non-negligible turnover were 0.00186 and 0.00714 day^{-1} in MS patients, corresponding with a turnover half-life of 373 days and 96.5 days, respectively. The effect of MS on the NO-Sulf (49.4% lower fraction) was more pronounced compared to the effect on β-GALC turnover (18.3% lower fraction). The kinetics of myelin breakdown products in the CSF were different in patients with MS compared with healthy subjects. This could be caused by slower myelin production in these patients, a higher level of degradation of a more

stable component of myelin, or, most likely, by a combination of these two processes. Deuterium labelling in combination with lumbar punctures would be a useful method for the quantification of metabolic processes of the central nervous system and myelin turnover in patients with progressive MS and can therefore be used in proof of concept studies with remyelination therapies.

Chapter 7 reports a randomized, double-blind, placebo-controlled, cross-over trial with fampridine in patients with MS and internuclear ophthalmoparesis (INO). We examined if the velocity of saccadic eye movements in INO improved with fampridine treatment in patients with MS. Main outcome measures were the change of peak velocity versional dysconjugacy index (PV-VDI) and first-pass amplitude VDI (FPA-VDI). Fampridine significantly reduced both PV-VDI (-17.4%, 95% CI: -22.4%, -12.1%; p<0.0001) and FPA-VDI (-12.5%, 95% CI: -18.9%, -5.5%; p<0.01) and therefore clearly improves saccadic eye movements due to INO in MS patients. According to the results of this study, treatment response to fampridine may be used in patient selection for enrolment in studies of compounds enhancing remyelination in MS using the velocity of saccadic eye movements as a primary outcome measure.

EARLY IMPLEMENTATION OF BIOMARKERS IN CLINICAL STUDIES

When a new compound is tested in humans for the first time (First-in-Human studies, or FIH-studies), traditional study objectives concern the exploration of safety, tolerability and pharmacokinetics. This commonly used phased approach can accordingly support decisions to continue or discontinue with a new compound (go/no-go decision) and to plan dosage and dosage schedules[1]. Despite this phased approach, there is no reason to exclude the additional evaluation of the mechanism of action or efficacy of the compound in FIH studies[2].This might save time, costs and it could support decisions in early stage of the development.

Saving costs and time during the developmental pipeline of new compounds is one of the largest challenges in early drug development. The current drug development time is over ten years[3]. Only one out of ten drug development projects make it all the way from FIH until approval, and this is even lower for neurological compounds, according to the industry lobby group BIO[4]. During drug development studies in large patient populations are often unsuccessful due to the their inability to proof efficacy, as was shown by a study in four large pharmaceutical companies[5]. This report also showed that development programs that include a biomarker have increased chances of success. There is reason to believe that early implementation of biomarkers in clinical studies might shorten drug development timelines, Therefore, the early implementation of biomarkers in clinical studies is highly welcomed.

HOW TO INTEGRATE BIOMARKERS IN EARLY CLINICAL TRIALS

There are several ways to integrate biomarkers in early clinical trials. This thesis shows examples of how to successfully plan and combine biomarker development with FIH-studies. In order to select appropriate biomarkers for a clinical development program, one should use knowledge of the pharmacology of the compound and the target patient population, and biomarkers should be fit-for-purpose, and capable of showing essential drug properties and effects[6]. The chapters of this thesis include studies that illustrate different ways of integrating biomarkers into early clinical trials:

1 a biomarker study prior to a FIH study,
2 a combination of a biomarker and FIH study, and
3 a biomarker study performed after a FIH study.

A biomarker study prior to a FIH study

The studies performed to measure myelin kinetics **(chapters 5 and 6**) describe biomarker studies that were initiated before the start of a FIH study. We used animal data and literature to design a study aiming for the measurement of deuterium-labeled myelin breakdown products. The first part of this biomarker study was performed in healthy volunteers, as we were unconfident whether this biomarker would be measurable in humans. Including a cohort of healthy volunteers would enable us to observe how subjects adhered to the study protocol, e.g. drinking heavy water daily for a long period and 4 lumbar punctures within 6 months. Additionally, it would enable us to calculate kinetics of myelin breakdown products in healthy volunteers and to use this information for the development of future biomarker studies[7]. As this first study in healthy volunteers showed positive results, and the confirmation that myelin breakdown products were a measurable biomarker, a similar study with the same design in patients with MS was initiated[8].

The testing of a biomarker in a study before the start of a FIH study has several advantages. It can be seen as proof-of-mechanism testing, before the FIH study has even started. In phase 1b or 2 studies, biomarkers can be useful to support clinical endpoints[9]. Biomarkers can be specifically present in the target patient group, for example MS patients with an internuclear ophthalmoplegia (INO)[10,11], but can also be tested by the NeuroCart in healthy volunteers (**chapter 2**)[12]. Performing a biomarker study in patients prior to a FIH study allows us to have a subsequent patient cohort, but also investigational staff already in place for a phase 1 or 2 follow-up study. This can decrease timelines, while patient studies are generally more time (and therefore cost) consuming when it comes to the recruitment of patients and staff. Finally, biomarker studies provide novel insights into the (background of) diseases and pharmacological processes and therefore is a relevant field wherein scientists from industry and academia can interact.

Chapter 7 illustrates how biomarker studies provide novel insights in the clinical pharmacology of diseases. We developed a biomarker that uses a pharmaceutical compound (fampridine) to show possible improvement in horizontal eye movements of patients

with MS and an INO (**chapter 7**). Fampridine is a potassium channel blocker that improves nerve conduction, and thereby function of demyelinated nerve fibers. It does not improve eye movements if demyelination of the medial longitudinal fasciculus (MLF) is absent. The fact that fampridine improved eye movements in these patients meant that the MLF was demyelinated, but also that axonal degeneration had not occurred yet. This population therefore appeared to be very useful to demonstrate effects of new compounds that are expected to accelerate (or improve) remyelination (**chapter 7**). For this reason, a subsequent study in healthy volunteers was not considered as INO is only present in a patient population. Because of the short half-life of fampridine, we chose a cross-over design to limit the duration of the study for patients (3-6 weeks). An additional advantage of this study design was that it yielded a database of INO patients who can be contacted as soon as this biomarker will be used in a study with a remyelinating compound. This study also added information about the efficacy of the compound used. Fampridine is not indicated for treatment of an INO yet, but this study showed that it might be worthwhile exploring fampridine as a treatment option for these patients. And if an INO patient would like to be treated with fampridine, an acute fampridine challenge (as described in **chapter 7**) could show if the patient is likely to respond to the treatment. Furthermore, the INO severity can be tracked with the same eye-tracking method to monitor long-term treatment response.

Prior to the FIH study with CNM-AU8 (**chapter 4**) we performed a small biomarker study. This was an in vitro study that was designed to show possible inflammatory effects of the compound that was being tested (gold nano-particles)[13,14]. Results of this in vitro biomarker study did not demonstrate inflammatory effects of gold nanoparticles. However, the specific inflammatory assay was based on previously reported animal studies[14]. One could speculate whether the use of other in vitro assays would have led to more insight into the role of gold in the immune system. In this specific example, the negative results from the biomarker study were a valid reason to not include the biomarker assay in the subsequent phase I clinical trial. Planning a biomarker study before start of the phase I clinical study did require higher initial costs, but eventually prevented the use of an unsuitable biomarker in the following phase I trials.

A combination of a biomarker and FIH study

The use of biomarkers in a FIH study has several advantages. Treatment effects in such a combined study can easily be compared to the placebo arm, corrected for individual baseline levels. In **chapter 3** such a FIH study is described, wherein liposomal methylprednisolone was studied, and both blood biomarkers and cognitive testing were tested in the first three cohorts of healthy volunteers. In the subsequent three cohorts of the study, we decided to only measure the 'fit-for-purpose' biomarkers showing a clear effect. It should be noted that in this specific study the pharmacodynamics (PD) of the active part of the compound (methylprednisolone) had already been reported in previous studies: inhibition of the hypothalamic-pituitary-adrenal (HPA) axis[15], glucose homeostasis disturbances, and depletion of osteocalcin[16,17] and lymphocytes[18].

A proof-of-pharmacology study can be performed in healthy volunteers if the new compound affects the biomarker in healthy persons. If this is not feasible, it is a good option to do a FIH study in a patient population. A healthy volunteer study can generally be performed on a shorter term and is cheaper than a patient study, which is beneficial for planning purposes, i.e. early availability of safety and pharmacokinetic (PK) data allows for the timely adjustment of the development program. Nevertheless, PK and safety data can be different in patients and healthy subjects, which might cause modification of the existing drug development plan. Additionally, recruitment and enrollment of a specific patient population in this early stage can lead to increased awareness of patients as well as study sites, which can lead to a time and cost-effective planning of phase 2 studies. It should be noted though that patients in these studies are often less representative for the eventual target patient population: patients in such a study are usually less severely affected.

A biomarker study after a FIH study.

Although the regulatory agencies encourage the use of efficacy parameters in early stage drug development[1], it does have many advantages to wait for the first FIH-results. First of all, there will be more information available on safety, tolerability and the pharmacokinetic profile of the compound. Reported PK parameters of the compound be supportive when tracking and implementing the 'fit-for-purpose' biomarker in the most efficient way.

The phase 2 study with an oral formulation of Δ9-tetrahydrocannabinol described in **chapter 2** has an interesting design, being a hybrid between a typical multiple-dose study to investigate safety profiles, PK and PD parameters, and a FIH study to establish proof-of-pharmacology[19]. The study consisted of a challenge phase and treatment phase, and involved a variety of biomarkers: the objective measurement of spasticity (H/M ratio)[20,21], postural instability, visual perception test, attention test and working-memory test. The challenge phase in the study design proved to be an elegant way to investigate individual PK and safety profiles, before continuing to the treatment phase, in which patients received the compound three times a day for four weeks.The cross-over design of this part of the study intended to support the interpretation of the different biomarkers tested, thereby reducing the risk of bias. The starting dose in the treatment phase was adjusted to the individual patient, reflecting clinical practice better than in other studies.

THE INTEGRATION OF BIOMARKERS IN THE DEVELOPMENT OF TREATMENTS IN MS

This discussion summarizes the three approaches of how to integrate biomarker studies into early-phase clinical studies and its pros and cons. The studies performed and described in this thesis illustrate how these approaches can accordingly lead to a time and cost-efficient process of drug development. Looking back on the wide range of studies already performed to evaluate potential future treatments of MS, this knowledge allows us to reflect on the methodology used and the degree of biomarker integration in these studies.

The first clinical study with *opicinumab* (reported in 2014) consisted of two parts: single-dose administration in healthy volunteers and multiple dosing in participants with MS (both RRMS and SPMS). Although the main focus was safety, efficacy and pharmacokinetics, the study did contain exploratory efficacy markers: imaging biomarkers Magnetization Transfer Ratio (MTR) and Diffusion Tensor Imaging (DTI) of pre-existing lesions and non-lesional normal-appearing white matter (NAWM)[22]. These did not reveal any clear treatment effects, which may have been due to the small sample size (46 participants with MS). The addition of a patient cohort in this stage of development gave opportunities for the addition of proof-of-concept measurements. **Chapters 5, 6** and **7** describe methods that could have been of interest in a clinical study with a new, potentially remyelinating compound. Especially the method described in **chapter 7**, where we used video-oculography in 23 MS patients with an INO, showed significant results. This method will most likely not need a large cohort to show (significant) difference between treatment and placebo.

There was a second phase I study with opicinumab that evaluated whether the anti-LINGO-1 antibody had immunomodulatory effects[23]. Although this is worth evaluating in an auto-immune disease, the reason for this second phase I study was not clearly reported. And this did raise the question why to invest in a study evaluating immunomodulatory effects and not in a proof-of-concept study, or in the development of additional methods to measure possible remyelination? The first attempt to measure the efficacy of opicinumab was in a phase 2 study, wherein Visual Evoked Potential (VEP) latency was used as a measure of remyelination. VEP has shown to be a measure of remyelination in optic neuritis in a rat model[24]. It inferred remyelination and proved to be a practical but is an indirect measurement of remyelination[25,26].

The phase I FIH study with *RHIGM22* was performed in patients with MS (72 in total) and included biomarkers comparable to the myelin turnover method described in **chapters 5** and **6**. In this study, 21 patients received 100 mL heavy water (70% D_2O) daily for two periods: during two weeks prior to dosing (2 different doses and placebo) and between days 15 and 29. Fractional synthesis rates of specific myelin biomarkers were determined by mass spectroscopic methods from blood and CSF[27]. It is unclear why the researchers chose to give 100 mL daily in two separate periods of 14 days. Our labelling protocol, described in **Chapter 5** of this thesis, was based on animal data and the maximum amount of heavy water safely tested in previous clinical studies (120 mL, 10 weeks). Interim analyses allowed us to adapt the sampling period. Data in **chapters 5** and **6** of this thesis showed that labelling of myelin breakdown products was limited after 5 weeks of 120 mL daily heavy water administration. According to our analyses, the amount of D_2O and period of exposure used in the RHIGM22 study was probably too short to show difference in labelling. We also found that turnover of octadecanoyl-sulfatide is a useful biomarker. This molecule has not been reported as a useful biomarker to measure myelin turnover, and therefore not yet being used in studies like the phase I study with RHIGM22. Our data was not published at the time the researchers designed the study. However, the limited literature on human myelin turnover suggest that the turnover is multiple weeks to months[28,29]. This information did not support the decision to label for a limited time (2 weeks in the RHIGM22 study). Since the turnover is much longer, labelled myelin in the post-dose sample might be due to labelling in the pre-dose period.

Olesoxime is already in development as a possible treatment agent for Amyotrophic Lateral Sclerosis (ALS)[30], Spinal Muscular Atrophy (SMA)[31] and Huntington's disease[32] years before a trial in MS patients was initiated[33]. This means that FIH studies were already performed before olesoxime was explored as potential treatment for MS. Development of the compound was halted for ALS and SMA in a late phase: only after negative phase 3 results for ALS and after negative phase 2 results for SMA. Results from the study in MS patients have not been published yet. However, we know that the study enrolled 44 patients and aimed to measure potential remyelination with imaging biomarkers (secondary endpoint). The MRI scans were performed at baseline and after 12 and 24 weeks. Literature shows that a treatment response can only be observable between three to six months after the start of treatment and that a second MRI between six and twelve months is advisable[34]. Lack of a response or trend based on MRI data can therefore be due to this shorter period, especially since the use of quantitative MRI measures for the measurement of remyelination still limited in a lengthy process like the demyelination and remyelination of a MS lesion[35,36].

Amiloride is a licenced diuretic with a proven safety record. The first study to explore the neuroprotective effect of amiloride was an open-label pilot study in 14 patients with PPMS, using MRI markers of neurodegeneration as outcome measures of neuroprotection[37]. A significant reduction in normalized annual rate of whole-brain volume was observed during the treatment phase compared with the pre-treatment phase. And although changes in whole-brain atrophy rate lack pathological specificity and are relatively indiscriminate with regard to effects of neuroaxonal and myelin loss on brain volume, the results suggest that amiloride may exert neuroprotective effects.

Next, a randomised, double blind, placebo-controlled phase 2 trial in 46 patients with optic neuritis was set up[38]. The main objectives of this study included biomarkers, like retinal nerve fibre thickness, imaging biomarkers, visual function, visual electrophysiology and quality of life questionnaires. Participants in this study received five months of treatment after optic neuritis, and biomarkers were measured at six and twelve months. Results could not show amiloride protected retinal nerve fibre layer thickness in optic neuritis[39]. One of the reasons the researchers reported is the possible lack of central nervous system (CNS) penetration of amiloride. The question regarding CNS penetration, however, could have already been answered in a healthy volunteer cohort with cerebrospinal fluid (CSF) sampling, or else by including lumbar punctures in the phase 2 trial.

Similar to amiloride, *quetiapine* is a registered drug, with psychosis as main treatment indication. Its potential neuroprotective and remyelinative properties have been well described[40]. Patients with MS are expected to benefit from quetiapine in multiple ways: besides possible remyelinative effects, quetiapine has shown to improve sleep[41] and might improve pain[42], both common problems in patients with MS. But because patients with MS may be more susceptible to AEs due to the brain injury that accompanies the disease, a safety, tolerability and dosing study was advised in this novel patient population.

Thirty-six RRMS patients were enrolled to find the appropriate dose for patients with MS[43]. This study unfortunately did not include biomarkers. The researchers did include clinical scales in the study, but these scales were no biomarkers related to remyelination. As quetiapine is expected to benefit patient in multiple ways, improvement in clinical scales is most likely due to multiple factors.

In an earlier article[40], the researchers advised to add imaging biomarkers to examine if quetiapine could promote remyelination. However, quetiapine has shown to have an effect on multiple systems in the body, including the immune system[44]. Improvement on MRI might be due to remyelination, due to a less active immune system, or both. Ideally, a biomarker should measure the remyelinative effect more directly. From the two methods described in this thesis, the measurement of changes in turnover of myelin breakdown products (**chapters 5 and 6**) and of improvements in horizontal eye movements in MS patients with INO (**chapter 7**), the first method is most suitable as a biomarker in a clinical study which quetiapine. Although both methods are probably able to measure remyelinating effects, the eye movements in MS patients with INO were sensitive to fatigue of the patient. Sleep improvement caused by quetiapine might improve these measurements, without even improving myelination.[41]

Guanabenz is a registered antihypertensive drug. A phase I study with oral guanabenz in patients with MS was performed[45] and supposedly has been completed, but no results have been reported yet. The main objective of the study was dose-finding: they planned to explore five different doses (28 days of treatment) in six patients with MS. The study design included MRI scans, and (although it was not specifically described) this probably included imaging biomarkers for remyelination as well.
It is not clear why they chose to use imaging biomarkers in a study with such a small sample size, and it follow the patients for a limited time period (3,5 months). Animal studies that tested guanabenz in the EAE model in mice showed the first difference with placebo ten days after the start, and this continued until day 30[46]. In **chapter 6** we describe a slower myelin turnover than reported in rodents[28], which makes the measurement of an effect after 28 days of treatment unlikely. A small sample size can be a challenge in a pilot study like the study mentioned above. A sensitive method, like the method described in **chapter 7** (eye movements in MS patients with an INO), might be a suitable method. Such study could for example contain baseline measurements and monthly measurements. It does require a specific population, namely MS patients with an INO that responds to fampridine. Recruitment of these patients in a mono-centre study is achievable: we were able to recruit 23 patients in 16 months.

Lastly, we discuss the earlier-mentioned FIH study with *gold nanoparticles* (**chapter 4**). A compound that could enhance remyelination *and* influence the immune system at the same time would be ideal in the treatment of MS: a disease that is characterized by an autoimmune response against myelin, and possibly inefficient remyelination[47]. As registered gold-containing compounds are infamous for their side effects[48,49], it was decided to perform the FIH study in healthy volunteers. However, improvement of remyelination in healthy volunteers cannot be measured, so the hypothesis that gold nanoparticles stimulate remyelination could not be tested in this study. The study did include in vitro inflammatory challenge experiments to investigate possible pharmacodynamic effects, based on reports that gold nanoparticles influence inflammatory responses[13]. However, no anti-inflammatory effects of gold nanoparticles were observed in the in vitro challenge, and therefore the planned ex vivo PD measurements were not added to the clinical study. Currently, a phase 2 study in 150 MS patients with chronic optic neuropathy is ongoing[50]. Biomarkers planned in this study include a 24-week VEP measurement as a primary endpoint.

Just like in **chapter 2**, this example shows it might be necessary to wait for a study in patients to measure a specific effect (in this case: improvement in remyelination). However, the effect on the immune system probably could have been measured in healthy volunteers. In hindsight biomarker development well before start of the first study was needed, as it proved to be more difficult than expected.

In summary, early phase drug development could benefit from more and better biomarkers that can quantify pharmacological effects of treatments for MS.

CONCLUSIONS AND FUTURE DIRECTIONS

Using biomarkers in early stage drug development should be the new standard. As long as new pharmacological treatments for MS are developed, there will be a need for more accurate biomarkers to demonstrate target engagement of the drug. There is a strong need for better biomarkers to study the effects of new drugs intended to enhance remyelination. This thesis provides examples of such biomarkers and how these can be easily integrated in early phase of drug development. Although a number of phase I studies have used exploratory efficacy objectives, it should be more common to use proof-of-pharmacology or proof-of-mechanism endpoints in these studies. Phase I studies can provide more information than safety and tolerability only.

1 EMA. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. 2018.
2 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? Nature Reviews Drug Discovery. 2010;9:856.
3 Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature Reviews Drug Discovery. 2010;9:203.
4 Mullard A. Parsing clinical success rates. Nat Rev Drug Discov. 2016;15(7):447-.
5 Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov. 2015;14(7):475-86.
6 Cohen AF, Burggraaf J, van Gerven JMA, Moerland M, Groeneveld GJ. The Use of Biomarkers in Human Pharmacology (Phase I) Studies. Annual Review of Pharmacology and Toxicology. 2015;55(1):55-74.
7 Kanhai K, Goulooze SC, Stevens J, Hay JL, Dent G, Verma A, et al. Quantifying Beta-Galactosylceramide Kinetics in Cerebrospinal Fluid of Healthy Subjects Using Deuterium Labeling. Clinical and translational science. 2016;9(6):321-7.
8 Kanhai KMS, Goulooze SC, van der Grond J, Hankemeier T, Verma A, Chavez J, et al. Kinetics of myelin breakdown products: a labeling study in patients with progressive multiple sclerosis. MS and demyelinating disorders. 2019;submitted.
9 Strimbu K, Tavel JA. What are biomarkers? Current opinion in HIV and AIDS. 2010;5(6):463-6.
10 Kanhai KMS, Nij Bijvank JA, Wagenaar YL, Klaassen ES, Lim KS, Bergheanu SC, et al. Treatment of internuclear ophthalmoparesis in multiple sclerosis with fampridine: a randomized double blind, placebo-controlled crossover trial. CNS Neuroscience & Therpaeutics. 2019.
11 Kanhai KMS, Nij Bijvank JA, Wagenaar YL, Klaassen ES, Lim K, Bergheanu SC, et al. Treatment of internuclear ophthalmoparesis in multiple sclerosis with fampridine: A randomized double-blind, placebo-controlled cross-over trial. CNS neuroscience & therapeutics. 2019.
12 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood-brain barrier penetration using the NeuroCart, a CNS test battery. Drug discovery today Technologies. 2016;20:27-34.
13 Dykman LA, Khlebtsov NG. Immunological properties of gold nanoparticles. Chemical science. 2017;8(3):1719-35.
14 Tsai CY, Lu SL, Hu CW, Yeh CS, Lee GB, Lei HY. Size-dependent attenuation of TLR9 signaling by gold nanoparticles in macrophages. Journal of immunology (Baltimore, Md : 1950). 2012;188(1):68-76.
15 Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(17):6106-15.
16 Heuck C, Wolthers OD. A placebo-controlled study of three osteocalcin assays for assessment of prednisolone-induced suppression of bone turnover. The Journal of endocrinology. 1998;159(1):127-31.
17 Kuroki Y, Kaji H, Kawano S, Kanda F, Takai Y, Kajikawa M, et al. Short-term effects of glucocorticoid therapy on biochemical markers of bone metabolism in Japanese patients: a prospective study. Journal of bone and mineral metabolism. 2008;26(3):271-8.
18 Tornatore KM, Venuto RC, Logue G, Davis PJ. CD4+ and CD8+ lymphocyte and cortisol response patterns in elderly and young males after methylprednisolone exposure. Journal of medicine. 1998;29(3-4):159-83.
19 van Amerongen G, Kanhai KMS, Baakman AC, Heuberger J, Klaassen E, Beumer TL, et al. Effects on Spasticity and Neuropathic Pain of an Oral Formulation of Delta9-tetrahydrocannabinol in Patients WithProgressive Multiple Sclerosis. Clinical therapeutics. 2017;40(9):1467-82.
20 Eisen A. Electromyography in disorders of muscle tone. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques. 1987;14(3 Suppl):501-5.
21 Matthews WB. Ratio of maximum H reflex to maximum M response as a measure of spasticity. Journal of Neurology, Neurosurgery, and Psychiatry. 1966;29(3):201-4.
22 Tran JQ, Rana J, Barkhof F, Melamed I, Gevorkyan H, Wattjes MP, et al. Randomized phase I trials of the safety/tolerability of anti-lingo-1 monoclonal antibody BIiB033. Neurology-Neuroimmunology Neuroinflammation. 2014;1(2):e18.
23 Ranger A, Ray S, Szak S, Dearth A, Allaire N, Murray R, et al. Anti-LINGO-1 has no detectable immunomodulatory effects in preclinical and phase 1 studies. Neurology-Neuroimmunology Neuroinflammation. 2018;5(1):e417.
24 You Y, Klistorner A, Thie J, Graham SL. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. Investigative ophthalmology & visual science. 2011;52(9):6911-8.
25 Leocani L, Guerrieri S, Comi G. Visual Evoked Potentials as a Biomarker in Multiple Sclerosis and Associated Optic Neuritis. Journal of neuro-ophthalmology : the official journal of the North American Neuro-Ophthalmology Society. 2018;38(3):350-7.
26 Mallik S, Samson RS, Wheeler-Kingshott CA, Miller DH. Imaging outcomes for trials of remyelination in multiple sclerosis. Journal of Neurology, Neurosurgery, and Psychiatry. 2014;85(12):1396-404.
27 Eisen A, Greenberg BM, Bowen JD, Arnold DL, Caggiano AO. A double-blind, placebo-controlled, single ascending-dose study of remyelinating antibody RHIGM22 in people with multiple sclerosis. Multiple sclerosis journal-experimental, translational and clinical. 2017;3(4):2055217317743097.
28 Ando S, Tanaka Y, Toyoda Y, Kon K. Turnover of myelin lipids in aging brain. Neurochemical research. 2003;28(1):5-13.
29 Morell P, H. QR. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. . In: Siegel GJ AB, Albers RW, et al, editor. 6th ed: Philadelphia: Lippincott-Raven; 1999.
30 Lenglet T, Lacomblez L, Abitbol JL, Ludolph A, Mora JS, Robberecht W, et al. A phase II-III trial of olesoxime in subjects with amyotrophic lateral sclerosis. European journal of neurology. 2014;21(3):529-36.
31 Bertini E, Dessaud E, Mercuri E, Muntoni F, Kirschner J, Reid C, et al. Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular atrophy: a randomised, double-blind, placebo-controlled phase 2 trial. The Lancet Neurology. 2017;16(7):513-22.
32 Eckmann J, Clemens LE, Eckert SH, Hagl S, Yu-Taeger L, Bordet T, et al. Mitochondrial membrane fluidity is consistently increased in different models of Huntington disease: restorative effects of olesoxime. Molecular neurobiology. 2014;50(1):107-18.
33 NCT01808885 Cg. Safety Study of Olesoxime in Patients With Stable Relapsing Remitting Multiple Sclerosis Treated With Interferon Beta. (MSREPAIR) Clinicaltrials.gov: U.S. National Library of Medicine;[Available from: https://www.clinicaltrials.gov/ct2/show/record/NCT01808885?term=olesoxime&cond=Multiple+Sclerosis&rank=1.
34 Kaunzner UW, Gauthier SA. MRI in the assessment and monitoring of multiple sclerosis: an update on best practice. Therapeutic advances in neurological disorders. 2017;10(6):247-61.
35 Chen JT, Collins DL, Atkins HL, Freedman MS, Arnold DL. Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. Annals of neurology. 2008;63(2):254-62.
36 Wattjes MP, Steenwijk MD, Stangel M. MRI in the Diagnosis and Monitoring of Multiple Sclerosis: An Update. Clinical Neuroradiology. 2015;25(2):157-65.
37 Arun T, Tomassini V, Sbardella E, de Ruiter MB, Matthews L, Leite MI, et al. Targeting ASIC1 in primary progressive multiple sclerosis: evidence of neuroprotection with amiloride. Brain : a journa of neurology. 2013;136(Pt 1):106-15.
38 McKee JB, Elston J, Evangelou N, Gerry S, Fugger L, Kennard C, et al. Amiloride Clinical Trial In Optic Neuritis (ACTION) protocol: a randomised, double blind, placebo controlled trial. BMJ open. 2015;5(11):e009200.
39 McKee JB, Cottriall CL, Elston J, Epps S, Evangelou N, Gerry S, et al. Amiloride does not protect retinal nerve fibre layer thickness in optic neuritis in a phase 2 randomised controlled trial. Multiple sclerosis (Houndmills, Basingstoke, England). 2019;25(2):246-55.