SUMMARY

There are an estimated 5,000 to 8,000 rare diseases, affecting between 27 and 36 million people in the EU. For many rare diseases, current treatment options are limited. There are various regulatory incentives on European and national levels to stimulate and facilitate the development of treatments for patients with a rare disease, but availability and accessibility to treatment may be limited by an absence of high quality evidence of treatment efficacy and safety. The gold-standard research design to obtain this evidence is the parallel-group randomised controlled trial (RCT), but this type of trial design usually requires large numbers of patients. This limits its usefulness to rare disease research, where the small number of patients available, as well as geographical and logistical barriers, may make it difficult to set up such trials.

An n-of-one trial is a double blind, randomised, controlled, multiple crossover trial in an individual patient. It thus shares characteristics with the group-randomised controlled trial, except that the unit of randomisation is not the patient over the treatments, but the order of the treatments over time. Due to this crossover nature, n-of-one trials are suitable to test the effectiveness of symptomatic treatments with a short onset and offset of biological action, for relatively stable diseases. N-of-one trials can be used to test the effect of treatment in an individual patient, but when conducted according to the same protocol, results from n-of-one trials can be combined to provide population effect estimates. Because each patient contributes multiple cycles of data and acts as his or her own control, the sample size needed to obtain population effect estimates is much reduced compared with a parallel-group randomised controlled trial. Thus, n-of-one trials may be particularly suited to doing research in small patient populations.

To date, hardly any research has been done to examine whether n-of-one trials would be feasible and acceptable to patients with a rare disease, clinicians, healthcare regulators deciding about market approval and reimbursement of treatment, and other stakeholders. The aim of this dissertation was therefore to examine the feasibility, clinical utility, and acceptability to various stakeholders of n-of-one trials in patients with a rare disease.

Part I of this dissertation addresses the research question whether n-of-one trials have a role in obtaining evidence of treatment effectiveness in a single patient with a rare disease.

Chapter 2 presents the results of a study in a patient with an unusual presentation of the rare genetic disease hereditary neuropathy with liability to pressure palsies (HNPP) who responded well to treatment with intravenous immunoglobulins (IVlg). The genetic confirmation of her diagnosis raised doubts about the appropriateness of continued long-term treatment with IVlg, which has a high production cost and limited availability. However, an n-of-one trial clearly showed that this patient derived benefit from this treatment. This study shows that it
is feasible to set up an n-of-one trial for a single patient with a rare disease in regular clinical practice.

**Part II** of this dissertation is a hypothetical exploration of the question whether results from (series of) n-of-one trials are acceptable to healthcare regulators deciding about market approval and reimbursement of treatment.

**Chapter 3** presents the results of a multidisciplinary stakeholder meeting with 21 representatives from four groups of stakeholders: pharmaceutical industry, healthcare regulators, clinical and academic researchers, and patients and consumers. Qualitative analysis of this meeting shows the important role of regulators: the pharmaceutical industry indicated that clarity was needed regarding the acceptability of evidence from n-of-one trials for regulatory purposes. Additionally, clinicians highlighted a need for regulatory incentives to conduct n-of-one trials for rare diseases in clinical practice to reflect the time investment needed. Regulators emphasised the importance of obtaining population effect estimates for regulatory purposes, and raised concerns about heterogeneity of treatment effects and information about treatment safety. These findings informed the other studies of this dissertation.

**Part III** of this dissertation presents the results of a second clinical study. Based on the results of the stakeholder meeting, we designed and performed a pilot-implementation of a series of n-of-one trials to assess whether its results can be used to determine treatment effectiveness in a group of patients with a rare disease. We chose the topic of our study after consulting patients with rare neuromuscular disorders (via their patient organisation) and neuromuscular disease specialists (via the Dutch interuniversity platform for neuromuscular disease specialists and researchers) about medicines that posed reimbursement problems. We collated their answers and compared them with the suitability criteria for n-of-one trials described above. From the resulting disease-drug combinations, we chose ephedrine for myasthenia gravis as our second study topic.

Myasthenia gravis, a rare disease of the neuromuscular junction, is often initially treated with acetylcholinesterase inhibitors. Patients who do not respond well to this medication may be treated with corticosteroids or other immunosuppressive medication, but these may have serious side effects. Clinical observations suggest that some patients who do not adequately respond to acetylcholinesterase inhibitors alone may benefit from add-on treatment with ephedrine. However, this is an off-label treatment in the Netherlands and reimbursement is therefore not guaranteed. We explored the existing evidence in the medical literature on ephedrine for myasthenia gravis, and developed a trial protocol to investigate the effectiveness of ephedrine for myasthenia gravis using n-of-one trials.
Chapter 4 describes the results of a Cochrane systematic review of ephedrine for the treatment of myasthenia gravis, neonatal myasthenia, and the congenital myasthenic syndromes. This review shows that despite being used in clinical practice, there is no high quality evidence from RCTs or quasi-RCTs to support the use of ephedrine to control the symptoms of myasthenia gravis. The only evidence that ephedrine may be beneficial came from five case reports.

Chapter 5 presents the study protocol that we subsequently developed with the input from regulatory stakeholders (see also Chapter 8 below) to determine the effect of add-on treatment with ephedrine for myasthenia gravis. The trial consisted of a small series of double blind, placebo-controlled, block-randomised n-of-one trials, followed by an optional 6-month open-label extension phase. Each n-of-one trial consisted of three cycles of two 5-day treatment periods with either ephedrine (25 mg, twice daily) or placebo. The treatment periods were block-randomised (e.g. AB-BA-BA) and each treatment period was followed by a 2-day washout period to reduce carryover effects of treatment. The main outcome measure for a clinical effect was the Quantitative Myasthenia Gravis (QMG) score. Secondary clinical effect measures were the MG-Composite and MG-Activities of Daily Living scores, VAS scores for muscle strength, and adverse effects. Because, to our knowledge, this type of trial had never been done before to obtain evidence of treatment effect for rare diseases, we also assessed feasibility of the trial based on the number of patients eligible for the trial and enrolled, the number of cycles completed, and qualitative data obtained by interviewing patients and caregivers.

Chapter 6 describes the results of the n-of-one trials that were introduced in Chapter 5. Add-on treatment with ephedrine improved the QMG score by 1.0 point (95% confidence interval 0.21-1.79) compared with placebo, which was statistically significant for the group of trial patients (n=4) as well as when extrapolated to the population treatment effect. There was also a significant average improvement in secondary outcomes measures for the trial patients (MG-Composite 2.7 (95% CI 0.68-4.65), MG-ADL 1.0 (95% CI 0.19-1.81), and VAS score for muscle strength 1.1 (95% CI 0.10-2.07)). Adverse events were mild and included palpitations, tremor, and restlessness, which are all known side effects of ephedrine. From these results, we conclude that ephedrine as add-on treatment for myasthenia gravis results in a modest improvement in symptoms and muscle weakness in patients with moderate disease severity, and that series of n-of-one trials can be used in small patient populations to determine population treatment effects.

Chapter 7 presents the results of the 6-month optional open-label extension phase of the abovementioned study. Of the patients enrolled in the n-of-one trials (n=4), three patients chose to continue with the open-label extension. The improvements observed in the n-of-one trials were not maintained during the 6-month follow-up, with no significant differences in any
of the outcome measures compared with baseline. This finding highlights the importance of long-term follow-up studies of positive randomised controlled trials.

**Part IV** of this dissertation is an empirical exploration of the question whether results from series of n-of-one trials are acceptable to healthcare regulators.

Chapter 8 presents an evaluation of the ephedrine trial. It describes the perspectives of patients, trialists and two regulatory stakeholders (for market approval and reimbursement of treatment) on the ephedrine trial. The evaluation of participants’ and trialists’ views confirmed the feasibility of conducting series of n-of-one trials in populations of patients with a rare disease. The findings also reiterated the time investment involved.

In addition, scientific advice on the trial results was sought from the Dutch organisations tasked with market approval of medicines (Medicines Evaluation Board; MEB) and coverage of treatment from the national healthcare insurance package (National Health Care Institute; NHCI). Both organisations were in principle willing to consider evidence from series of n-of-one trials, although the MEB stated that this would be a last resort option and safety of treatment would have to be substantiated in some other way. With respect to the ephedrine trial, however, neither organisation was convinced that the evidence presented by the series of n-of-one trials would have been sufficient to make decisions on licensing or reimbursement of treatment, mainly because of the small study sample, the clinical significance of the improvement in QMG score, and the variability in individual treatment responses.

Finally, **Part V** (Chapter 9) discusses the main findings of this dissertation, as well as its implications for practice, and gives recommendations for future research.

To conclude, recent incentives to develop treatments for rare diseases have necessitated new trial designs suitable to small patient populations. Series of n-of-one trials, in which each patient contributes multiple cycles of data and acts as his or her own control, share characteristics with the gold-standard randomised, controlled trial. N-of-one trials can produce high quality evidence of treatment effectiveness in a single patient, but can also produce population effect estimates if conducted in a series according to the same protocol. This greatly reduces the number of participants needed to obtain population effect estimates. However, questions remain regarding how to ensure generalisability of the evidence to the patient population and whether clinical utility, for the purposes of regulatory decisions, should be defined in terms of individual patient benefit or the average benefit to the patient population, since n-of-one trials can provide both types of evidence. External factors that may encourage the use of n-of-one trials in the future include the shift towards personalised medicine and pay-for-performance healthcare models, but barriers such as the time and financial investment
needed to run these trials may limit their uptake. Future research should focus on forming a suitable business case for n-of-one trials, including who would be best suited to drive these trials forward and how they should be paid for, because n-of-one trials are a useful tool to bring rational pharmacotherapy to patients with a rare disease.