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The oral splicing modifier RG7800 increases full length *survival of motor neuron 2* mRNA and survival of motor neuron protein: Results from trials in healthy adults and patients with spinal muscular atrophy

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Abstract

Spinal muscular atrophy (SMA) is a rare genetic and progressively debilitating neuromuscular disease. It is the leading genetic cause of death among infants. In SMA, low levels of survival of motor neuron (SMN) protein lead to motor neuron death and muscle atrophy as the SMN protein is critical to motor neuron survival. SMA is caused by mutations in, or deletion of, the *SMN1* gene. A second SMN gene, *SMN2*, produces only low levels of functional SMN protein due to alternative splicing which excludes exon 7 from most transcripts, generating truncated, rapidly degraded SMN protein. Patients with SMA rely on limited expression of functional SMN full-length protein from the *SMN2* gene, but insufficient levels are generated. RG7800 is an oral, selective SMN2 splicing modifier designed to modulate alternative splicing of *SMN2* to increase the levels of functional SMN protein. In two trials, oral administration of RG7800 increased in blood full-length *SMN2* mRNA expression in healthy adults and SMN protein levels in SMA patients by up to two-fold, which is expected to provide clinical benefit.

Keywords: Neuromuscular disease; Spinal muscular atrophy; Survival of motor neuron; SMN protein; SMN2 splicing modifier.

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease that causes muscle atrophy and weakness and devastating disease-related complications. It is the leading genetic cause of mortality in infants, with an

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incidence of 1 in 6000–11,000 live births and a carrier frequency estimated at 1 in 40–60 [1–3]. SMA has a broad range of severity and a heterogeneous patient population, and is categorized into Type 1 through 4, defined by age of onset and the most advanced motor milestone achieved. Common symptoms include hypotonia, muscle weakness and atrophy, and impaired mobility [2,4,5]. Symptoms of Type 1 SMA (Werdnig–Hoffman disease; the most severe type) manifest before 6 months of age and patients are never able to sit independently [2,4,5]. Type 1 SMA leads to progressive respiratory failure, with death from respiratory distress usually occurring within 2 years [2,4,5]. Type 2 SMA (intermediate or Dubowitz syndrome) is typically diagnosed between 6 and 18 months of age with patients unable to walk or stand independently [2,4]; approximately 93% of people with Type 2 SMA live to be 25 years of age [6]. The disease onset in patients with Type 3 SMA (Kugelberg–Welander disease) typically occurs after 18 months of age and mild cases may not be noticeable until late childhood [2,4]. Children with Type 3 SMA achieve independent ambulation but it is often diminished or lost later [2,4,5]. Symptoms of Type 4 SMA do not appear until adulthood and patients may eventually require a walking aid [4]. Although one therapy (the antisense oligonucleotide nusinersen [SPINRAZA™]) has been approved for the treatment of SMA, there is still a significant unmet need [2,7], in particular for oral therapies with systemic efficacy [4,8,9].

SMA is caused by deletion or mutation of the survival of motor neuron 1 gene (*SMN1*) [10]. As a consequence of intrachromosomal duplication, humans carry a second SMN gene, *SMN2*; chromosomal instability results in a variable *SMN2* copy number [4,11,12]. Compared with *SMN1*, *SMN2* contains a translationally silent C- to T-mutation which leads to alternative splicing of exon 7, and consequently to reduced expression of full-length SMN mRNA transcripts, and therefore protein. Exclusion of exon 7 from the *SMN2* transcript (*SMNΔ7* mRNA) results in an unstable SMNΔ7 protein that is rapidly degraded [2,11,13]. *SMN2* produces only 10–15% of full-length transcripts which are translated to functional, full-length SMN protein [2]. In patients with SMA (who lack a functional *SMN1* gene), levels of full-length SMN protein depend completely on this limited production by the *SMN2* gene. Given the essential role of the SMN protein, it is not unexpected that disease severity is inversely correlated with the number of *SMN2* copies. The *SMN2* copy number, however, does not fully predict the disease phenotype, and other phenotypic modifiers have been identified [2,14,15].

One strategy to restore SMN protein levels in SMA patients is the modulation of *SMN2* splicing to favor inclusion of exon 7. RG7800 (also known as RO6885247) is an orally administered, selective *SMN2* splicing modifier that has been studied in two Phase 1 trials. The mechanism of action of the class of molecules RG7800 belongs to (*SMN* splicing modifiers) was recently elucidated. These molecules modulate pre-mRNA splicing by directly interacting with the splicing machinery and allowing alternative splicing to occur [16,17]. Preclinical work showed that RG7800 increases SMN protein levels and leads to a significant survival benefit in mouse models of SMA. This suggests that *SMN2* splicing modifiers may offer a new oral therapy for patients with SMA [18]. We report the safety, pharmacokinetic (PK) and pharmacodynamic (PD) results from two Phase 1 clinical trials of RG7800 in healthy adults (first-in-human study, Eudract number 2013-004097-95) and in patients with SMA (first-in-patient study: MOONFISH, NCT02240355). The first-in-human study was a placebo-controlled, single-ascending-dose (SAD) study with a 3-week follow-up period to assess the safety, tolerability, PK and PD of single oral doses of RG7800 in healthy male adults. The first-in-patient study was a multicenter, placebo-controlled study of RG7800 administered orally once-daily for 12 weeks to patients with SMA. The primary objective of both trials was to assess the safety and tolerability of RG7800. Further details of the trial design and protocols can be found in the online Methods section. We report here the data obtained from the healthy subject study and the first cohort of adolescent and adult SMA patients after administration of 10 mg RG7800 once daily for 12 weeks. The study was put on hold shortly after enrollment in the second cohort had started due to safety findings in the ongoing animal toxicology studies. An improved splicing modifier and follow-up compound, risdiplam (RG7916; RO7034067), is currently under evaluation in three clinical trials.

2. Materials and methods

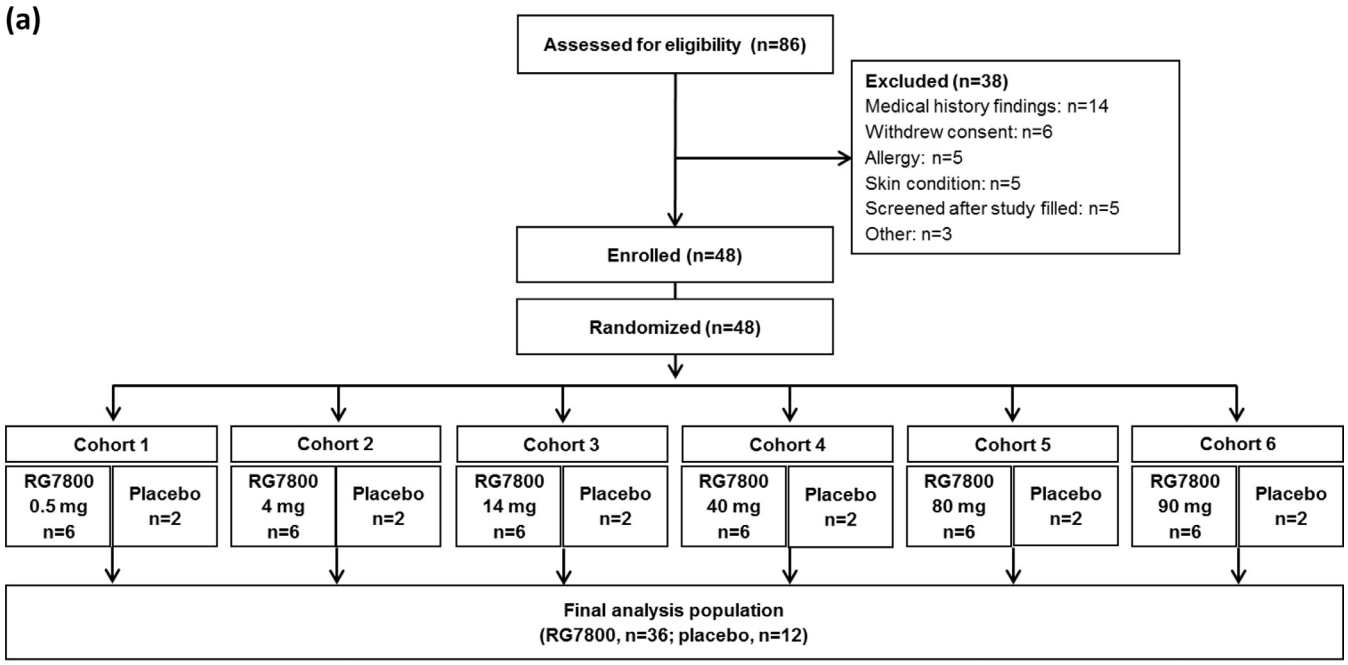
2.1. First-in-human study

2.1.1. Study design and randomization

This single-center (Centre for Human Drug Research [CHDR], the Netherlands), double-blind, randomized, placebo-controlled, SAD study used a parallel group design with six cohorts of healthy, male subjects aged 23–45 years (see table S4 for full eligibility criteria). The primary objective of the study was to assess safety and tolerability of RG7800 in healthy subjects, with secondary objectives to investigate PK and PD.

The study was conducted in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki [19,20]. The protocol and all study materials were approved by an institutional review board (IRB)/ethics committee (EC), and all subjects provided written informed consent before participation.

A list of randomized treatment assignments was generated by the study sponsor and allocated sequentially to subjects in the order in which they enrolled; both investigators and subjects were blinded to treatment allocation. Within each cohort of eight subjects, six received a single oral dose of RG7800, and two received a single oral dose of placebo (Fig. 1a). This sample size was based on practical clinical judgment with six subjects on active treatment per dose level considered sufficient to detect major tolerability and safety issues.



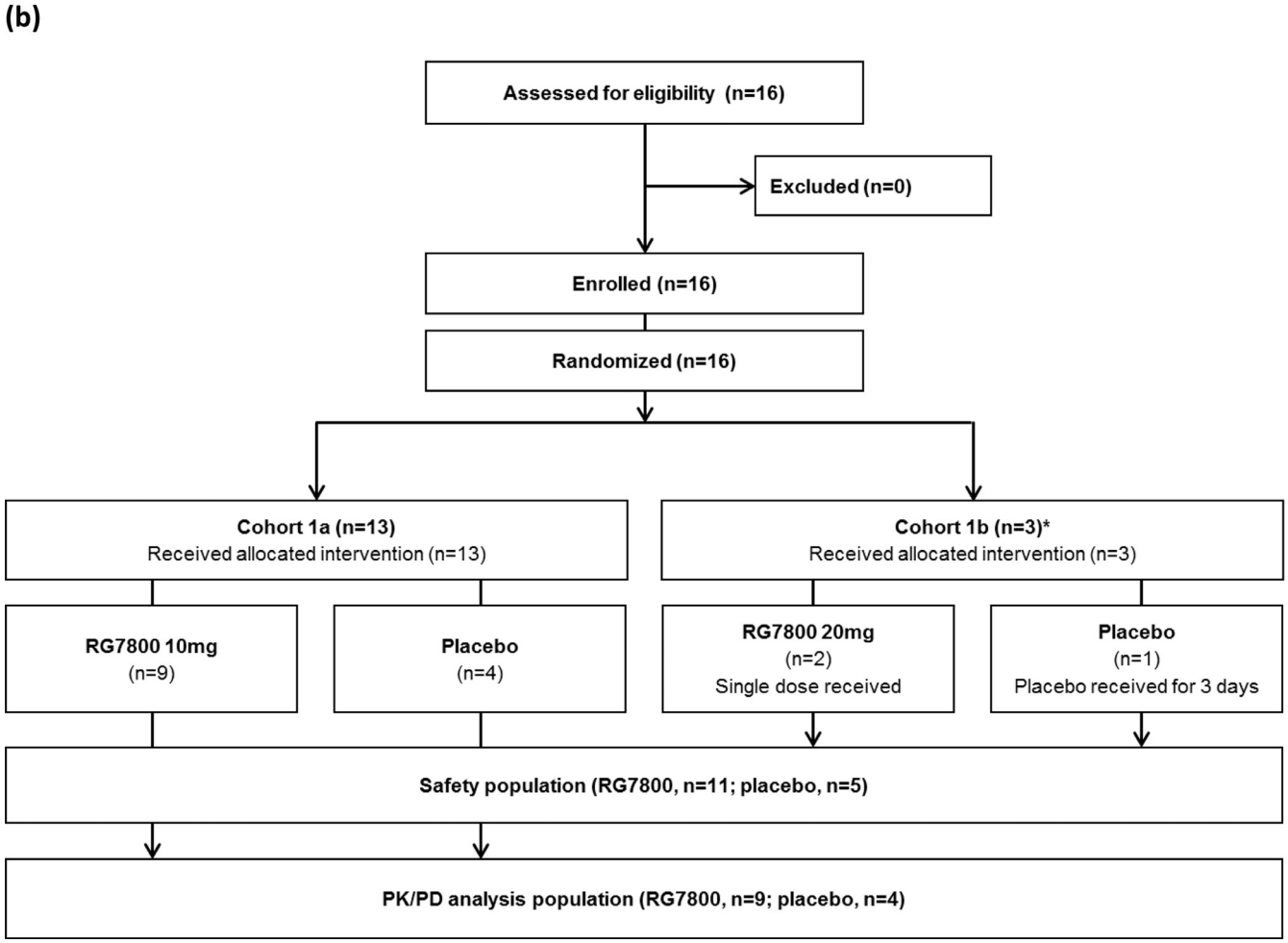


Fig. 1. (a) Study design and randomization: Phase 1 clinical trial of RG7800 in healthy adults. (b) Study design and randomization: Phase 1 clinical trial of RG7800 in patients with SMA.
PD, pharmacodynamics; PK, pharmacokinetics.
*Intervention terminated early due to study discontinuation.

2.1.2. Treatment administration

Oral solutions containing RG7800 at a dose between 0.5 mg and 90 mg and placebo were administered under fasting conditions, sequentially in ascending order: 0.5 mg (Cohort 1), 4 mg (Cohort 2), 14 mg (Cohort 3), 40 mg (Cohort 4), 80 mg (Cohort 5), and 90 mg (Cohort 6). Dose escalation proceeded in the absence of safety concerns after reviewing safety and tolerability data, PK, and available PD data from all previous cohorts. Doses administered differed from the initially planned dose range (0.5–120 mg) to meet expected exposure levels, and dose escalation was stopped at 90 mg to adhere to the pre-specified exposure cap of 1500 h.ng/mL for plasma area under the curve from 0 to 24 h ($AUC_{0-24\,h}$) which had been chosen in order to stay for all individual subjects below the exposures at which any adverse findings were observed in the animal toxicology studies.

The study was divided into a screening period of up to 4 weeks (Day −28 to Day −2), an in-clinic period of 6 days (Day −1 to Day 5) for drug administration and assessment, and a safety follow-up up to 3 weeks (conducted between Day 14 and Day 21).

2.1.3. Safety, pharmacokinetic and pharmacodynamic assessments

Safety outcome measures assessed were the incidence and severity of adverse events (AEs), incidence of laboratory abnormalities (based on hematology, clinical chemistry, and urinalysis test results), electrocardiograms (ECGs) and vital signs. PK and PD outcome measures comprised the plasma and urine concentrations of RG7800, and *SMN* mRNA levels as a marker of drug effect, respectively. Plasma and urine concentrations of RG7800 were measured using validated liquid chromatography- tandem mass spectrometry (LC-MS/MS) assays in compliance with regulatory guidelines. *SMN* mRNA (*SMN1*, *SMN2* full length, and *SMNΔ7*) were measured in whole blood samples taken before and after dosing using quantitative reverse transcription polymerase chain reaction (RT-qPCR), as previously described by Czech et al. [15].

2.1.4. Statistical methods

As an exploratory study, no formal hypothesis testing was performed. A one-way ANOVA model was used to test for possible deviations of the primary PK variables (area under the curve for time 0 to infinity [AUC_{inf}] and maximum plasma concentration [C_{max}] of RG7800) from dose-proportionality.

2.2. First-in-patient study

2.2.1. Study design and randomization

This first-in-patient study was a multicenter, randomized, double-blind, 12-week, placebo-controlled, multiple dose study with three parts. Part 1 was a multiple-ascending dose study in adult and adolescent patients with SMA (Cohorts 1a and 1b; Fig. 1b), and parts 2 and 3 were designed to enroll patients from 2–11 years of age (Cohort 2a), and ≤7 months of age (Cohort 3a), respectively. Only Cohort 1a completed the study prior to the study being placed on clinical hold due to unexpected findings in animal studies; methods and results for this cohort are reported in this paper. Patients were eligible for inclusion in Cohort 1a if they were 12–55 years of age and had a confirmed diagnosis of 5q-autosomal recessive SMA, with clinical symptoms attributable to SMA Types 1–3 (see table S4 for full eligibility criteria). Patients in Cohort 1a were enrolled at sites in Italy, Switzerland and UK.

The primary objective of the study was to evaluate the safety and tolerability of 12 weeks of treatment with RG7800 in adult and pediatric patients with SMA. Secondary objectives were to investigate the multiple-dose PK of RG7800 and its metabolites, potential food effects, and PD outcomes in terms of *SMN2* splicing modification, SMN protein levels, and their correlation with PK, muscle electrophysiology, and electrical impedance myography (not reported here).

The study was conducted in accordance with Good Clinical Practice and the principles of the Declaration of Helsinki [19,20]. The protocol and all study materials were approved by IRB/ECs at: the Policlinico Agostino Gemelli, Rome, Italy; the Royal Victoria Infirmary, Newcastle, UK; and the Universitäts-Kinderspital beider Basel, Basel, Switzerland. All patients provided written informed consent before participation.

Patients were randomized 2:1 to receive RG7800 or placebo, stratified by age group (12–17 years and 18–55 years of age). A list of randomized treatment assignments was generated by the study sponsor and allocated sequentially to patients in the order in which they enrolled; investigators and subjects were blinded to treatment allocation. In line with the primary objective, a sample size of 12 patients (eight patients receiving RG7800; four patients receiving placebo) was considered sufficient to detect major tolerability and safety issues: with eight patients receiving active drug, the probability of observing at least one AE with an incidence rate of 20% in the population is >80%.

2.2.2. Treatment administration

Thirteen patients received either 10 mg of RG7800 ($n=9$) or placebo ($n=4$) as oral solutions, taken once daily with the regular morning meal for 12 weeks. The total study duration for each patient was up to 24 weeks, with the screening period extending for up to 4 weeks prior to the 12-week treatment period, and subsequently an 8-week follow-up period.

2.2.3. Safety, pharmacokinetic and pharmacodynamic assessments

Safety outcome measures assessed were the incidence, nature and severity of AEs, treatment discontinuations due to AEs, incidence of laboratory abnormalities (based on hematology, clinical chemistry, coagulation and urinalysis test results), ECG abnormalities, changes in vital signs, physical and ophthalmic examinations and any additional follow-up investigations by specialists upon emergence of specific events (including skin and ophthalmic events).

PK and PD outcome measures comprised the plasma and urine concentrations of RG7800, and *SMN* mRNA and

protein levels in blood, respectively. Plasma and urine concentrations of RG7800 were measured using validated LC-MS/MS assays. Plasma samples were taken before dosing (trough values) throughout the study, and additional samples on days 1, 14, 15, 28 and 114 (1-month follow-up visit). *SMN* mRNA (*SMN2* full length, and *SMNΔ7*) levels were measured in whole blood samples taken before and after dosing using the RT-qPCR assay described by Czech et al. [15]. SMN protein levels in blood were assessed using the validated SMN protein immunoassay previously described by Czech et al. [15].

2.2.4. Statistical methods

Results are presented with descriptive and summary statistics; analyses were exploratory with no formal statistical hypothesis testing performed.

3. Results

3.1. Participants

3.1.1. First-in-human study

Forty-eight healthy male subjects aged 23–45 years were sequentially randomized to six cohorts of eight subjects each, between January and March 2014. There were no withdrawals, and all 48 subjects received a single dose of either RG7800 or placebo (36 received 0.5–90 mg RG7800; 12 received placebo) and completed the study.

Baseline characteristics were similar across cohorts and treatments (Table S1).

3.1.2. First-in-patient study

Thirteen patients with SMA, aged 13–53 years were enrolled and randomized in Cohort 1a of this study, between November 2014 and January 2015. All 13 patients received 12 weeks of once-daily drug treatment (nine received 10 mg RG7800; four received placebo) and completed the study. There were no withdrawals and all 13 patients are included in the safety, PK and PD analyses.

Of the 13 patients, eight were male, five were female, and all had a primary diagnosis of SMA. There were four patients with Type 2 SMA and five patients with Type 3 SMA in the RG7800 treatment group, whereas all four patients in the placebo group presented with Type 3 SMA. In line with this, the age of diagnosis was lower and the duration of disease was longer in the RG7800 treatment group, whilst the two groups were balanced in terms of age at baseline of the study. There were three ambulatory patients in the RG7800 treatment group and three in the placebo group. *SMN2* gene copy number ranged from three to four in all patients for whom copy number could be determined ($n = 12$). Other baseline characteristics were similar across treatment groups; further details are provided in Table S2.

After completion of the first cohort of patients (Cohort 1a) and soon after enrollment in the second cohort of patients (Cohort 1b) had begun, Roche took the decision to suspend dosing in this study as a precautionary measure due to an eye finding in a chronic animal toxicology study, which was observed at a higher exposure of the investigational medicine RG7800 than that used in clinical studies.

At this point, three patients had been enrolled in Cohort 1b: two patients had received a single dose of 20 mg RG7800, and one had received placebo for 3 days.

Further dosing of patients in this study was suspended to allow detailed investigation of this toxicology finding, and the study was placed on clinical hold and finally terminated. We report here the data for the primary and secondary outcomes of Cohort 1a. Given the very limited exposure to RG7800 of the patients enrolled in Cohort 1b, they were excluded from PK/PD analysis but included in the safety analyses.

3.2. Safety and tolerability

No safety concerns associated with RG7800 administration in healthy adults or patients with SMA were identified at any dose level in either study. No serious AEs were reported, and there were no withdrawals due to AEs. No clinically significant changes from baseline in vital signs (systolic and diastolic blood pressure, heart rate, body temperature), ECGs, or ophthalmological examinations were observed in either healthy subjects or SMA patients.

3.2.1. First-in-human study

A total of 45 AEs were reported by 28 subjects in the healthy adult population; 42 were rated as mild, and three were rated as moderate (gastroenteritis, headache, influenza-like illness; all reported by patients who received 4 mg RG7800). Forty AEs were considered treatment related; they were all of limited duration and resolved spontaneously without sequelae. The most frequently reported AEs (across all treatment groups including placebo) were headache ($n = 10$), influenza-like illness ($n = 3$), back pain ($n = 3$), musculoskeletal discomfort ($n = 3$) and rhinitis ($n = 3$); no other AEs were reported by more than one subject. There was no increase in incidence or severity of AEs with increased dose or exposure to RG7800. A summary of AEs is provided in table S3.

3.2.2. First-in-patient study

Safety profiles were similar between treatment groups after 12 weeks of once-daily treatment with 10 mg RG7800 or placebo. All patients, including those who received placebo, reported at least one AE with a total of 42 AEs reported in the RG7800 treatment group ($n = 9$) and 21 AEs in the placebo group ($n = 4$). The most commonly reported AEs were nasopharyngitis ($n = 4$), influenza ($n = 3$), diarrhea ($n = 2$), dry mouth ($n = 2$), erythema ($n = 2$), pyrexia ($n = 2$), and headache ($n = 2$); no other AEs were reported by more than one patient. A summary of AEs is provided in Table 1.

All AEs reported in the RG7800 treatment group were mild or moderate in severity, except one case of elevated liver enzymes (grade 4 AE), which was reported only at the second follow-up visit approximately 2 months after the end of treatment; analysis of a repeat sample obtained 6 days later showed that all values had returned to normal ranges except

Table 1
Summary of treatment-related AEs in patients with SMA.

Safety parameter	Treatment group		
	10 mg RG7800 $N=9$	20 mg RG7800 $N=2$*	Placebo $N=5$†
Subjects reporting ≥ 1 AE (% of subjects)	9 (100%)	1 (50%)	5 (100%)
Number of AEs	42	1	24
Number of treatment-related AEs	9	0	15
Treatment-related AEs reported (number of subjects if reported by >1)	Coagulation test abnormal Corneal disorder Dry mouth Increased lacrimation Involuntary muscle contractions Liver function test abnormal Nasopharyngitis	0	Dry mouth Dry skin Dry throat Erythema Headache Limb discomfort Nasopharyngitis (2) Otitis media Pyrexia Rash Skin fissures Upper abdominal pain
Number of SAEs	0	0	0
Number of withdrawals due to AEs	0	0	0

AE, adverse event; SAE, serious adverse event; SMA, spinal muscular atrophy.
* Patients received a single dose of 20 mg RG7800 before the study was discontinued.
† One patient received placebo for 3 days before the study was discontinued.

alanine aminotransferase and gamma-glutamyl transpeptidase, which however had decreased significantly too; the event was reported as resolving at the final visit.

3.3. Pharmacokinetics

3.3.1. First-in-human study

Analysis of RG7800 PK after a single oral dose (0.5–90 mg) in healthy adult subjects showed that plasma exposure increased in a slightly more than dose-proportional manner. C_{max} was reached at 5–8 h post-dose (time to maximum plasma concentration [T_{max}]), and the mean terminal half-life was approximately 120 h (Table S5). Plasma concentrations of RG7800 after a single dose of 0.5 mg were below or near the lower limit of quantification (0.1 ng/mL), and therefore were excluded from the analysis. As a result of the long half-life, subjects were exposed to RG7800 for over 2 weeks after receiving a single dose. A pre-specified exposure cap of 1500 h.ng/mL for plasma area under the curve from time zero to 24 h after dosing ($AUC_{0-24\,h}$) was applied to the trial, and with exposure somewhat higher than initially predicted, dose escalation was stopped at 90 mg instead of the planned 120 mg, although no safety concerns were noted. The highest individual plasma exposure over the first 24 h post-dose ($AUC_{0-24\,h}$) was 1190 h.ng/mL at 90 mg.

Urinary excretion of unchanged RG7800 was 6–13% of the dose administered (at 40–90 mg) over the sampling interval of 72 h post-dose.

3.3.2. First-in-patient study

A summary of plasma PK in patients with SMA ($n=9$) is provided in Table S6. Similar to the PK in healthy adults, RG7800 was slowly absorbed in patients, with a median T_{max} of 8 h on Day 1 and at 6 h on Day 28 (Table S6). As expected with the long half-life, accumulation of RG7800 was observed, with a mean accumulation ratio of 9 (Table S6), and steady-state was reached after approximately 4 weeks of once daily dosing of RG7800 (Fig. 2). Plasma concentrations slowly declined when treatment stopped, with RG7800 concentrations close to the lower limit of quantification in all patients at 4 weeks after the last dose (Fig. 2).

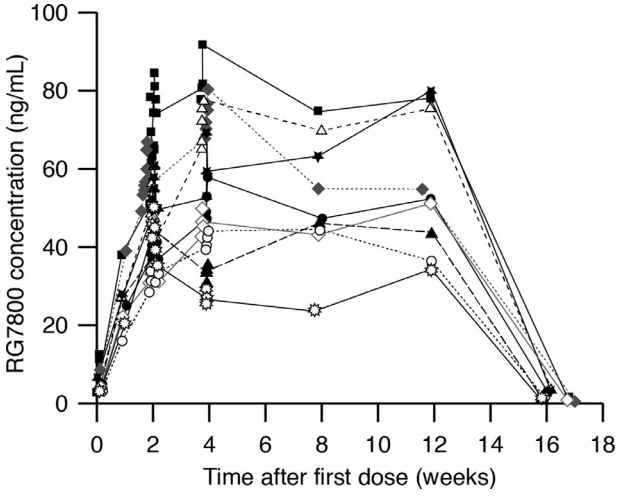


Fig. 2. RG7800 plasma concentrations over 12 weeks of once-daily 10 mg dosing in individual patients with SMA ($n=9$).
SMA, spinal muscular atrophy.

The effect of food on the PK of RG7800 was investigated on Days 14 and 15 in a randomized sequence: each patient received once a dose of 10 mg RG7800 after a 10-h overnight

fast, and once within 30 min of starting a high-fat, high-calorie breakfast. The PK of RG7800 was unaffected by food intake (Table S6).

3.4. Pharmacodynamics

3.4.1. First-in-human study

SMN1, full-length *SMN2* (*SMN2 FL*) and *SMN2Δ7* mRNA levels were measured by RT-qPCR in blood samples collected at Days 1, 2, 3 and 5, according to methods previously described by Czech et al. [15]. RG7800 showed a dose- and exposure-dependent effect on *SMN2* exon 7 splicing in healthy adult subjects: RG7800 increased *SMN2 FL* mRNA and decreased *SMNΔ7* mRNA levels, and individual increases correlated well with the individual exposure (Fig. 3). One subject in the 14 mg RG7800 treatment group was found to have zero copies of the *SMN2* gene, and was therefore excluded from the analysis of *SMN2* mRNA.

3.4.2. First-in-patient study

As in healthy adult subjects, *SMN2 FL* increased and *SMN2Δ7* mRNA levels decreased in blood of patients with SMA treated with RG7800 ($n = 9$), demonstrating a shift in *SMN2* splicing towards inclusion of exon 7. A mean increase of up to two-fold in the ratio of *SMN2 FL: SMNΔ7* mRNA compared with baseline (Fig. 4) was observed. Ratios had reverted to baseline by 1 month after the end of treatment with RG7800.

In line with the *SMN2 FL* mRNA increase, SMN protein concentrations in blood in patients treated with RG7800 increased by up to two-fold versus baseline (Fig. S1). The individual SMN protein increase correlated well with individual drug exposure (Fig. 5).

4. Discussion

The results reported from these early Phase 1 trials in healthy adult subjects and patients with SMA show that treatment with the oral *SMN2* splicing modulator RG7800 for up to 3 months duration was well tolerated and did not raise any safety concerns. Administration of RG7800 in these studies led to a dose- and exposure-dependent effect on *SMN2* mRNA splicing, promoting the generation of *SMN2 FL* mRNA while *SMNΔ7* mRNA decreased. SMN protein in blood of patients with SMA increased up to two-fold versus baseline for the duration of the 3 months treatment with 10 mg RG7800.

The primary objective of both studies was to assess the safety and tolerability of RG7800. AEs were reported for most participants in the studies; they were all mild or moderate (with the exception of one isolated finding of elevated liver enzymes observed 2 months after the end of treatment) and distributed across the RG7800 and placebo groups. Dose escalation in the first-in-human SAD study was stopped at 90 mg to comply with the predefined exposure cap based on the animal toxicology data. There was no safety concern and no increase in adverse events up to the highest dose tested.

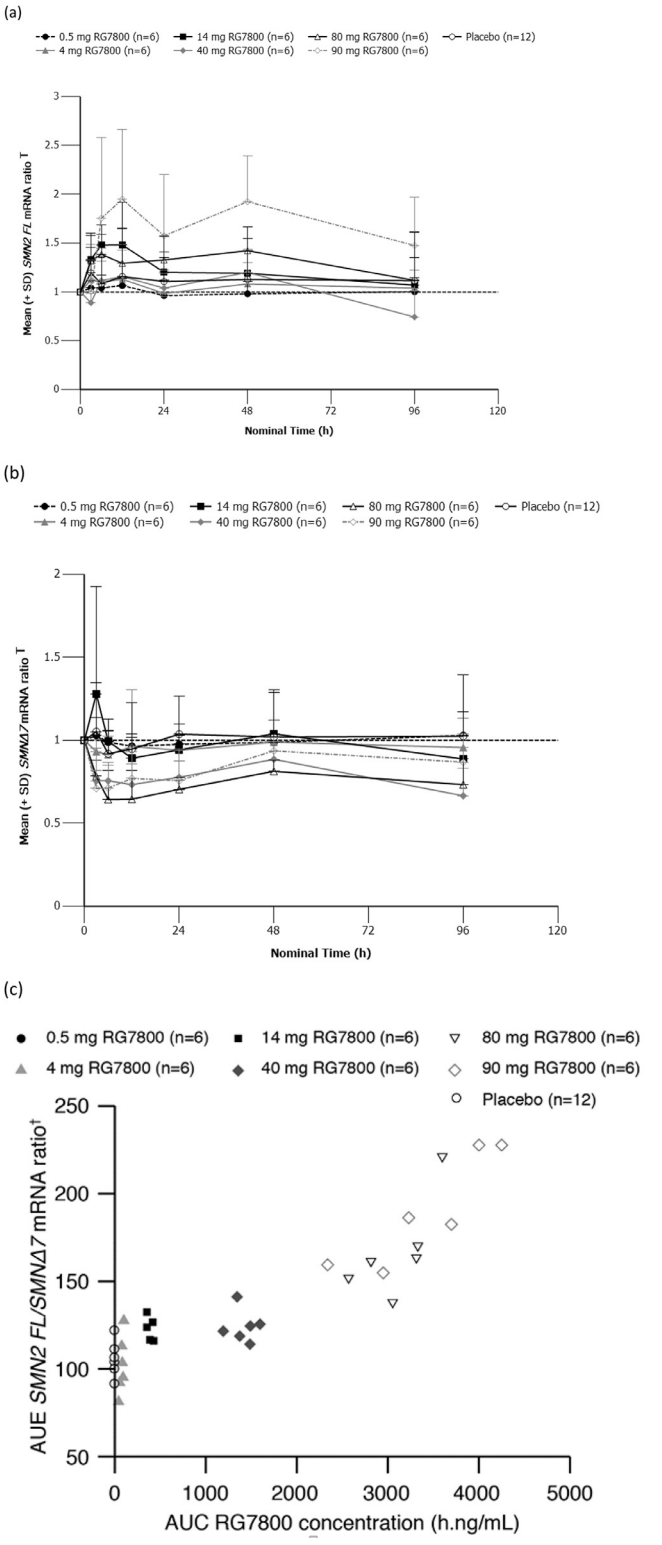


Fig. 3. (a) *SMN2 FL* mRNA and (b) *SMNΔ7* mRNA (ratio post-/pre-treatment) in healthy adult subjects after a single dose of RG7800 or placebo, and (c) *SMN2 FL/Δ7* mRNA expression ratios versus RG7800 exposure in individual healthy adult subjects.

†*SMN2 FL* or *SMNΔ7* mRNA ratio = individual subject's *SMN2 FL* or *SMNΔ7* (respectively) at time x/*SMN2 FL* or *SMNΔ7* (respectively) at baseline.

AUC, area under curve from 0–96 h post-dose; AUE, area under effect curve from 0–96 h post-dose; *SMN FL,* full-length *SMN* mRNA; SMN, survival of motor neuron.

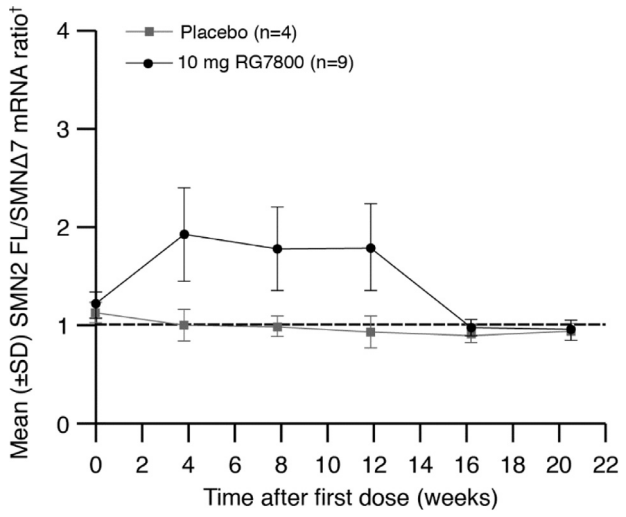


Fig. 4. *SMN2 FL/SMNΔ7* mRNA expression ratios (corrected for baseline) in patients with SMA over 12 weeks of once-daily treatment with RG7800 or placebo.
†*SMN2 FL/SMNΔ7* mRNA ratio = individual subject (*SMN2 FL/SMNΔ7* at time *x*)/(*SMN2 FL/SMNΔ7* at baseline).
SD, standard deviation; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

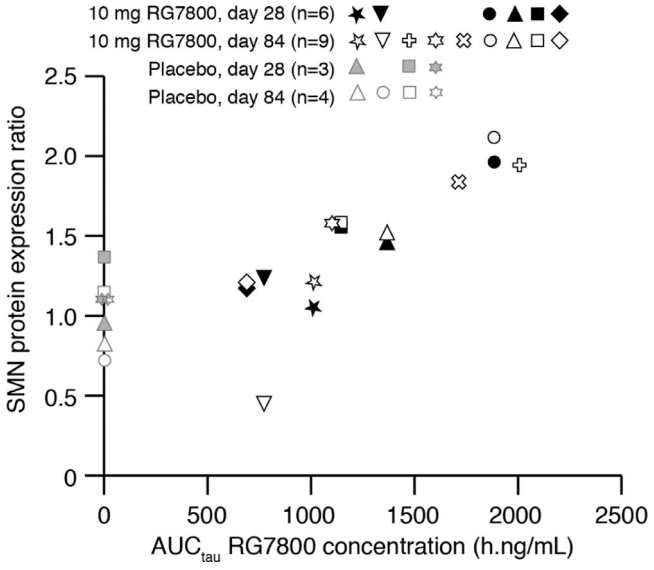


Fig. 5. SMN protein increase upon treatment (ratio post-dose/pre-dose) at Days 28 and 84 vs RG7800 exposure (AUC 0–24 h, Day 28) in individual patients with SMA ($n = 13$).
AUC, area under curve; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

Plasma pharmacokinetics in healthy adult subjects showed a greater than dose proportional increase in RG7800 exposure between 0.5 and 90 mg. Based on the estimated half-life of approximately 120 h, accumulation of RG7800 was expected upon multiple dosing, which was confirmed in the patient study with the once-daily dosing of 10 mg RG7800 over 12 weeks. Steady-state exposure was reached after 4 weeks of treatment.

The results from these two studies demonstrate proof of mechanism for the oral *SMN2* splicing modifier RG7800 in humans: *SMN2 FL* mRNA increased, while *SMN2Δ7* mRNA decreased, and SMN protein increased up to two-fold versus baseline and correlated well with individual patient exposure. This two-fold increase of SMN protein is expected to provide clinical benefit: two-fold differences in SMN protein have been observed between patients with different SMA types and *SMN2* copy numbers [15].

Since the genetic basis of SMA was elucidated 20 years ago [10], there has been considerable research to identify effective therapies. Initially promising strategies to upregulate *SMN2* gene expression, such as the use of histone deacetylase inhibitors and quinazoline derivatives, failed to provide meaningful benefits in clinical settings [4]. More recently there have been promising results with alternative approaches, including gene therapy using viral-mediated delivery (scAAV9-SMN), and anti-sense oligonucleotides (ASOs) targeting a splicing silencer within intron 7 of *SMN2* to upregulate *SMN2 FL* mRNA and SMN protein [21]. Nusinersen (SPINRAZA™), an *SMN2*-directed ASO indicated for the treatment of SMA in pediatric and adult patients, was approved by the US Food and Drug Administration in 2016 and the European Medicines Agency and other jurisdictions in 2017. However, there is still a significant unmet need for patients with SMA, particularly among patients with Type 2 or 3 SMA, who represent the largest population affected by SMA [22]. Nusinersen must be given intrathecally, while an oral therapy would significantly ease the administration of the medicine to patients.

The results we report with RG7800 are the first to show that levels of systemic SMN protein can be significantly increased in patients with SMA with an orally administered *SMN2* splicing modifier. While the results reported are promising, they are derived from two Phase 1 studies of RG7800 in a limited number of healthy adults ($n = 48$) and patients with SMA ($n = 13$). Statistical power is therefore limited, given the exploratory nature of these trials, and the relationship between *SMN2* mRNA and SMN protein levels in blood with the SMA disease state needs to be investigated. Safety and tolerability of continued treatment with this novel compound was assessed first in older and more stable patients, before exposing the youngest and most vulnerable patient group with Type 1 SMA, the most severe form of the disease. Investigations in mouse models of SMA suggest there is a critical treatment window for SMN upregulation early in disease progression [21].

In conclusion, the results of the two studies have shown that the SMN splicing modifier RG7800 has been well tolerated at the dose levels tested for up to 3 months treatment duration. PD data from healthy adults and SMA patients demonstrate proof of mechanism that oral SMN splicing modifiers are able to upregulate *SMN2 FL* mRNA and increase systemic SMN protein levels, and could potentially become the first oral treatment option for SMA. Development of RG7800 was stopped, but the optimized follow-up compound risdiplam is currently under clinical investigation. The safety and efficacy

of risdiplam is currently being assessed in three ongoing clinical trials in patients with SMA: FIREFISH [23], SUNFISH [24] and JEWELFISH [25].

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2018.10.001.

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