Comparative evaluation of AAV gene therapy, antisense therapy and small molecules therapy for treatment of SMA for efficacy and safety

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Summary

Spinal muscular atrophy (SMA) is a genetic disease caused by a dysfunctional SMN1 gene and is the leading cause of inherited infant death. SMA patients develop symmetric progressive muscle atrophy and weakness, eventually affecting all skeletal muscles. The most severe form of SMA, type 1, with an onset between 0-6 month of age, leads to a rapid motor neuron degeneration. This severely affects the respiratory system and usually results in a pneumonia-induced respiratory failure and death before the age of 2.

Three different drug types, small molecule (Risdiplam), antisense oligonucleotides (Spinraza™) and gene therapy (Zolgensma®) have been developed for the treatment of SMA, with Spinraza™ and Zolgensma® already approved. This thesis analyzes differences in pre-clinical and clinical development of the three treatments and compares their safety and efficacy profiles.

The pre-clinical development programs of Risdiplam and Spinraza™ are regulated under the same guidelines, which is visible in the type of pre-clinical studies conducted. In contrast, the pre-clinical development of gene therapy requires a much more flexible approach leading to a pre-clinical program for Zolgensma® that is significantly different from the other two treatment developments.

In the clinics, Risdiplam and Spinraza™ showed similarly favorable clinical safety profiles with few treatment-related adverse events, while Zolgensma® showed clear treatment-related adverse events including serious adverse reactions. The published primary and secondary endpoint data allowed an efficacy comparison of Risdiplam, Spinraza™ and Zolgensma® for SMA type 1 and SMA type 2 or 3. Overall, most efficacy endpoints favored Zolgensma®, followed by Risdiplam and Spinraza™ for treatment of type 1 SMA patients, and Zolgensma®, followed by Spinraza™ and Risdiplam for type 2 and 3 SMA patients. Consequentially, the benefit-risk ratio is likely in favor for Risdiplam for treatment of type 1 SMA patients and similar for Zolgensma® and Spinraza™ for treatment of type 2 or 3 SMA patients.

Contents

Introduction

The objective of this thesis is to compare the three classes of treatment for Spinal muscular atrophy (SMA), small molecules (Risdiplam), antisense oligonucleotides (Spinraza™) and gene therapy (Zolgensma®) with respect to their pre-clinical and clinical assessment of efficacy and safety. The thesis starts with a general introduction of SMA and the therapeutic strategies that resulted in the development of the small molecule Risdiplam, the antisense oligonucleotide Spinraza™ and the gene therapy Zolgensma® as treatments for SMA (Chapter 1).

Then, the pre-clinical and clinical development of each treatment is analyzed considering the regulatory requirements for the individual class, followed by a comparison of the differences in pre-clinical and clinical development between the three treatments (Chapter 2). Subsequentially, efficacy and safety findings of Risdiplam, Spinraza™ and Zolgensma®, pre-clinical and clinical, are compared and discussed (Chapter 3).

In addition to Risdiplam, also its predecessor RG7800 is included in the pre-clinical assessment since it illustrates the dynamic development, characteristic for small molecules.

The structure and the order of the pre-clinical analysis reflects the structure of the common technical document (CTD) Module 4 and the CTD numbering is referenced in brackets, e.g. **(CTD 4.2.4)**, to enable a structured comparison of the three unique treatment options and highlight their differences.

Chapter 1: SMA Background

1.1 Clinical background

Spinal muscular atrophy (SMA) is an autosomal recessive disease resulting from the degeneration of lower motor neurons in the spinal cord and somatic motor nuclei in the brainstem (Scoto, Finkel et al., 2017). SMA patients develop symmetric progressive muscle atrophy and weakness, starting proximally until eventually all skeletal muscles are affected (Kolb, Coffey et al., 2017).

Clinically, SMA manifests in variations in disease onset (infantile till adulthood) and difference in motor function impairment. Accordingly, SMA can be stratified into types 1 to 4 with increasing age of onset and decreasing severity (Russman, 2007). The most common form (60% of cases) is SMA1 with an onset between 0-6 month of age and rapid motor neuron degeneration that severely affects the respiratory system usually resulting in a pneumonia-induced respiratory failure and death before the age of 2. In about 10% of SMA1 cases respiratory support can prolong life into adolescence or even adulthood (Finkel, McDermott et al., 2014, Kolb et al., 2017). SMA2 (20-30 % of cases) has an onset of 6-18 month and patients are not able to walk or stand. Depending on the rate of progression the respiratory system can be affected. Life expectancy is reduced but the majority of SMA2 patients' lives well into adulthood (Zerres & Rudnik-Schoneborn, 1995). SMA3 (ca. 10 % of cases) has an onset of more than 12 month and patients are able to walk without support, although many loose this capability with disease progression. The life expectancy is normal. SMA4 (less than 5 % of cases) is the adult onset form of SMA (symptoms onset often 30 years plus) and patients experience muscle weakening impairing their mobility. Life expectancy is also unchanged in SMA4 patients.

1.2 Genetic and molecular background

The underlying genetic causes for SMA are homozygous deletions (95% of cases), 2 point mutations or one deletion and one point mutation in the SMN1 (survival motor neuron) gene, resulting in the expression of a dysfunctional SMN protein or its absence (Lefebvre, Burglen et al., 1995).

One in 50 people are carriers of a dysfunctional SMN1 gene, resulting in a disease prevalence of 1 in 11.000 live births, making it the leading cause of inherited infant death (Pearn, 1973, Sugarman, Nagan et al., 2012).

As the name implies, SMN is critical for the survival of motor neurons (Burghes & Beattie, 2009). The SMN protein together with Gemn2-8 and unrip forms a vital molecular chaperone complex necessary for the biogenesis of various ribonucleoprotein (RNP) complexes, most importantly the spliceosomal small nuclear (sn) RNPs that are responsible for regulating all aspects of gene expression (Beattie & Kolb, 2018, Gubitz, Feng et al., 2004).

SMA patient cells show a reduced capacity of snRNP assembly and the reduced assembly capacity correlates with disease severity (Gabanella, Butchbach et al., 2007, Wan, Battle et al., 2005).

Although the SMN complex is ubiquitously expressed, in SMA motor neurons are particularly affected and seem to be specifically vulnerable to SMN deficiency. A possible explanation is that due to lower SMN baseline expression levels in motor neurons, a further decrease in SMN protein leads to the gene splicing capacity falling below a critical threshold that is essential for cellular survival (Jodelka, Ebert et al., 2010, Ruggiu, McGovern et al., 2012).

The severity of the disease correlates strongly with the main genetic modifier SMN2 (Mailman, Heinz et al., 2002), albeit other modifiers can also have a profound impact on disease severity (Prior, Krainer et al., 2009). SMN2 is nearly identical to SMN1 with a single nucleotide difference (840.C to T) at an exon splice enhancer site (Monani, Lorson et al., 1999). This results in preferential splicing of exon 7 producing 85-90% dysfunctional SMNdelta7 protein that is subsequently degraded (Burnett, Munoz et al., 2009). SMN2 is usually present in multiple copies (normally two) and the 10-15% functional SMN2 protein is able to partially or fully compensate for SMN1 function. Consequentially, the number of SMN2 gene copies is positively correlated with a better prognosis (and also higher SMN type) up to a point were individuals with 5 SMN2 copies show no SMA phenotype (Elsheikh, Prior et al., 2009, Feldkotter, Schwarzer et al., 2002, McAndrew, Parsons et al., 1997).

1.3 Therapeutic strategies, targets and mechanism of action

Therapeutically the molecular disease mechanism led to the development of two main strategies (Waldrop & Kolb, 2019). The first strategy is restoration of functional SMN1 protein via expression of a transgene. The second strategy is inhibition of exon 7 splicing, thereby increasing the SMN2-to-SMNΔ7 ratio and effectively increasing the amount of functional SMN2 protein.

Other options for treatment strategies are stimulation of SMN2 gene expression to increases the amount of functional SMN2 (albeit dysfunctional SMN2 would also be increased but subsequentially degraded), neuroprotective agents and restoration of muscles (Waldrop & Kolb, 2019).

Because the lower motor neurons in the spinal cord and somatic motor nuclei in the brainstem are the primary targets of any SMA therapy (Scoto et al., 2017), the drug should either cross the blood-brain barrier (BBB) or requires intrathecal administration. Nevertheless, because SMN1 is ubiquitously expressed in healthy individuals, a systemic drug exposure, with sufficient exposure of the central nervous system (CNS), might provide additional beneficial to SMA patients.

Different approaches are being pursued with respect to the two main therapeutic strategies, which will be discussed in the following paragraph.

1.3.1 Small molecules as treatment for SMA

Drug discovery and development of small molecules is backed-up by decades of research and development in the pharmaceutical industry and experience at regulatory authorities. Additionally, analytical and bioanalytical methods exist to fully characterize the drug at every stage of the development.

However, in comparison to the inherently specific antisense oligonucleotides, developing small molecules to specifically alter splicing of a gene at one particular sequence element, as required for SMA treatments, is a daunting task. Nonetheless researcher achieved this feat with relatively few off-target effects that could have been expected due to the complexity of the ubiquitous splicing machinery (Palacino, Swalley et al., 2015, Ratni, Ebeling et al., 2018).

Members of the pyridazine class, including the small molecules Risdiplam and Branaplam, were found to stabilize the interaction between the U1/snRNP protein complex and the SMN2 pre-messenger ribonucleic acid (RNA) and thereby sequence-selectively increasing the binding affinity of U1/snRNP to the 5'ss (splicing site) of exon 7 (Figure 1). The key for achieving specificity was the high affinity of the pyridazines to the double-strand RNA-protein complex but not to the individual complex components (Palacino et al., 2015).

Treatment with the small molecules resulted in an increase of SMN protein level, improved motor function and survival in animal studies (Naryshkin, Weetall et al., 2014, Palacino et al., 2015, Sivaramakrishnan, McCarthy et al., 2017).

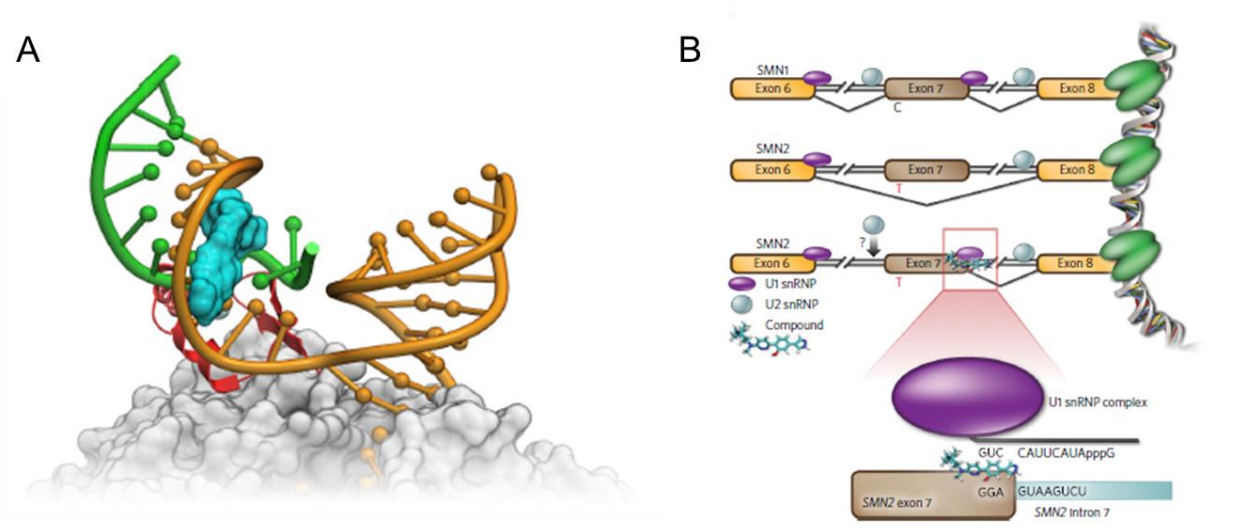

Figure 1: Modified from Figure 6 Palacino et al. 2015. "(A) Computational model illustrating the binding mode of NVS-SM2 with U1 snRNP-SMN pre-mRNA superimposed on the published U1 snRNP crystal structure. The SMN2 RNA sequence is shown in green, the U1 snRNA sequence is shown in gold, the RNA-contacting part of the U1C protein is shown in gold, the RNA-contracting part of the U1C protein is shown in red, and the compound is shown in turquoise. (B) Schematic model of mechanism of action. SMN1 and SMN2 transcripts are shown with spicing occurring through recruitment of U1 and U2 snRNP complexes flanking the exons".

1.3.2: Antisense oligonucleotides as treatment for SMA

Modification of SMN2 splicing can be achieved by targeting the deoxyribonucleic acid (DNA) or the proteins involved in the splicing process. Antisense oligonucleotides are a highly specific option to directly target DNA or RNA.

Oligonucleotides (ONs) can be synthetically produced and their properties modified with mostly well characterized site-directed chemical modifications. These include phosphorothioate backbone modifications, changes to ribose moieties (e.g. 2'-O-

methylation), changes to nucleobases (e.g. pseudouridine), and other backbone modifications (e.g. phosphorodiamidate morpholinos). Chemical modifications like phosphorothioate render the oligonucleotide resistant to endogenous nucleases, resulting in an increased *in vivo* stability and more favorable pharmacokinetic (PK) properties (Marlowe, Akopian et al., 2017)**.** Other properties that can be altered in this manner are affinity, potency or immunostimulation (Watts, Deleavey et al., 2008).

Spinraza™ (Nusinersen) is an antisense oligonucleotide targeting pre-messenger RNA splicing and thereby increasing the inclusion of exon 7. Spinraza™ is a single-stranded uniformly 2'O-methoxyethyl (2'-MOE) modified antisense oligonucleotide (18-mer) with a, phosphorothioate (PS) backbone, that targets the 15-nucleotide-long intronic splicing silencer N1 (ISS-N1) (Singh, Howell et al., 2017). ISS-N1 is located 5'ss in an intronic region of exon 7 and deletion of ISS-N1 in fibroblasts leads to a fully restored SMN2 exon 7 inclusion, suggesting ISS-N1 is the master checkpoint of exon 7 splicing (Singh, Singh et al., 2006, Singh, Androphy et al., 2004). Its intronic location also assures that targeting ISS-N1 does not affect mRNA export and protein translation. In phase I studies Spinraza™, delivered to the CNS intrathecally through lumbar puncture, increased SMN protein in the cerebrospinal fluid (CSF) and improved motor function up to 9-14 month post treatment (Chiriboga, Swoboda et al., 2016, Finkel, Chiriboga et al., 2016, Hache, Swoboda et al., 2016).

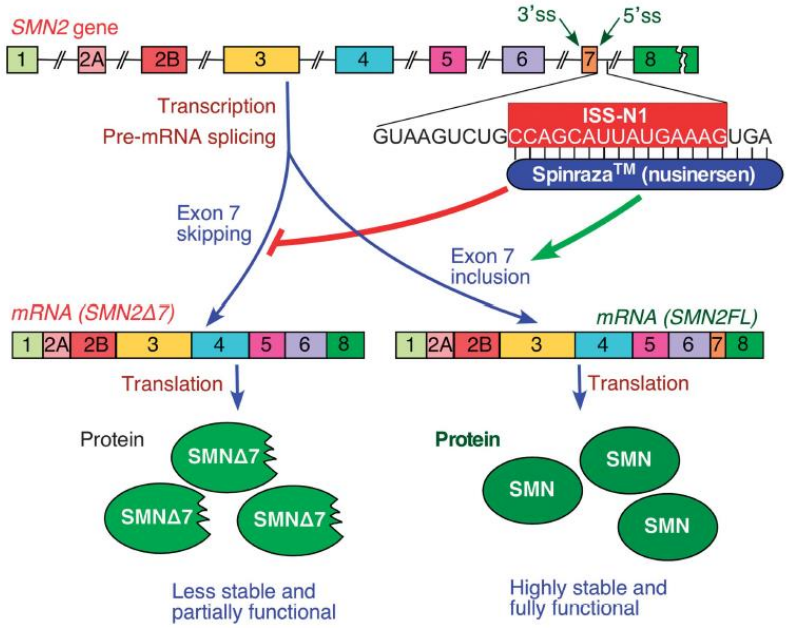

Figure 2: From Singh, Howell et. al 2017 (Figure 1). "Diagrammatic representation of SMN2 gene and Spinraza mode of action. SMN2 exons are represented by colored boxes, whereas introns are shown as broken lines. Intronic sequence immediately downstream of exon 7 is given. ISS-N1 region within this sequence is highlighted in pink box. Positions to which Spinraza anneals are indicated. SMN2 pre-RNA splicing results in exon 7-included (SMN2FL) and exon 7-skipped (SMN2Δ7) transcripts, translation of which leads to production of the full-length functional SMN protein and a truncated less stable isoform, respectively. Targeting of ISS-N1 by Spinraza prevents exon 7 skipping and as a consequence increases levels of full-length SMN."

Although, compared to small molecules ONs are a relatively recent option (approximately 20 years in clinics) in drug development (Schubert, Levin et al., 2012), their specificity and

the myriad options for chemical modifications have made them an attractive treatment choice , in particular for genetic disorders.

1.3.3. Gene therapy as treatment for SMA

Gene therapy allows to directly restore functional SMN1 protein by expression as a transgene. The gene of interest is delivered as double-stranded DNA vector genome encapsulated in an adeno-associated viral (AAV9) capside. The vector genome comprises the human SMN1 for transgene expression, regulated by a cytomegalovirus (CMV) enhancer and a chicken-β-actin hybrid (CAG) promoter. The gene therapy product is usually produced in eukaryotic cells and viral particles containing the SMN1 transgene are purified and characterized. Because living cells are employed for the production of the gene therapy, there is a relatively large degree of heterogeneity in the production process as compared to small molecules, similar to other biologics. For instance, not every virus particle might contain a functional genome and the dose needs to be defined according to an analytical method (e.g. qPCR).

With respect to CNS delivery, the adeno-associated virus AAV9 provides a suitable tropism and is able to cross the BBB and transfect target cells of SMA patients (Schuster, Dykstra et al., 2014).

1.4 Timelines for the development of SMA treatments

Figure 3 shows a brief overview of the key regulatory milestones in the development of Zolgensma®, FDA (Byrnes, 2019), Spinraza™, EMA (EMA/289068/2017, 2017) and Risdiplam, FDA (Roche, 2019, Therapeutics, 2017). The timeline from Orphan Drug Designation to Approval decision was similar for Zolgensma® and Spinraza™ and might be shorter for Risdiplam with pending approval.

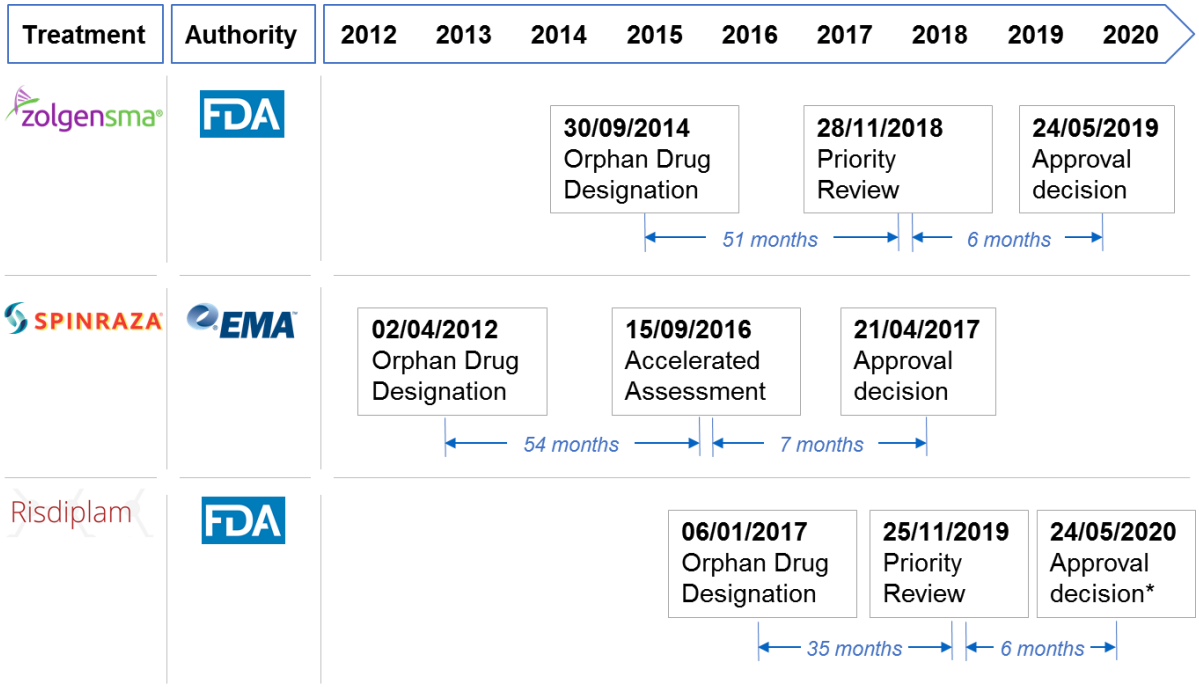

*Figure 3: Overview of Key Regulatory Milestones. * the FDA has postponed the approval decision for Risdiplam by 3 months, due additional data submission.*

Chapter 2: Assessment of safety and efficacy for SMA treatments

2.1. Risdiplam

Risdiplam belongs to the small molecules and the marketing authorization application (MAA) in the European Union (EU) has to be submitted through the Centralized Procedure (CP): Article 3(1) (new active substance for neurodegenerative disorder) and Annex of (726/2004, 2004) point 4 (orphan medicinal product). In the United States (US), new small molecule drugs are regulated according to FD&C Act, Section 505 (new drug application), PHS Act 58 Stat. 632 and CFR 21, Part 312 (21CFR312, 2019). When it comes to pre-clinical and clinical development International Council for Harmonisation (ICH) guidelines exist on most topics and they are integrated into national law in EU member states and the US. In the following section a summary of regulatory requirements for pre-clinical and clinical development is presented and their application to the case of Risdiplam is analyzed. A summary of the Risdiplam studies can be found in Supplementary Tables P1 and C1.

2.1.1 Pre-clinical Safety and Efficacy

The pre-clinical development of Risdiplam, being a small molecule, is guided by the overreaching ICH Guideline M3(R2) (EMA/CPMP/ICH/286/95, 2008) and more specific ICH guidelines on the individual topics (e.g. S7A) (EMA/CPMP/ICH/539/00, 2001).

RG7800 is the predecessor of Risdiplam and had already entered phase 1 human clinical trials for SMA treatment, before safety findings in a parallel cynomolgus monkey study (long-term chronic toxicity) triggered a stop of the trial. Subsequentially, strategic optimization of RG7800 with respect to safety and molecular properties lead to the discovery of Risdiplam (RG7916) with a much improved efficacy and safety profile (Ratni et al., 2018).

2.1.1.1 Pharmacology

One of the first pre-clinical topics is the investigation of primary pharmacodynamics (**CTD 4.2.1**) with the objective of determining the mode of action (MoA), plasma levels needed for efficacy and a dose-response curve. Also, the animal species for toxicity studies are selected.

The MoA of Risdiplam and its predecessors was investigated *in vitro*, in SMA patient-derived induced pluripotent stem cell (iPSCs) and cynomolgus iPSCs. Risdiplam promoted the inclusion of exon 7, generating full length mRNA and increasing SMN protein levels (Poirier, Weetall et al., 2018).

Because SMA is the result of a dysfunctional or absent SMN1 protein and a treatment consequentially needs to show the restoration of functional protein, mouse disease models were selected for *in vivo* Pharmacodynamic (PD) and PK. The dose response curve for three doses and two application routes were investigated in the C/C-Allele mouse model of mild SMA phenotype with three different oral daily doses (1,3, 10 mg/kg) and in the delta 7 mouse model with severe SMA phenotype administering (0.1, 0.3, 1 mg/kg) doses intraperitoneal (IP) (Feng, Ling et al., 2016). A prolongation of lifespan at

all doses and a dose dependent improvement of the neuronal pathology were observed. Toxicity studies were additionally performed in rat and cynomolgus monkeys.

Investigation of secondary pharmacodynamics (**CTD 4.2.2**) serves to determine the MoA and off-target effects of a drug. In the case of Risdiplam an *in vitro* splicing assay was used as a tool to understand the molecular mechanism underlying the observed genotoxic effects of the Risdiplam predecessor RG7800. The assay subsequentially enabled to screen for candidates with less off-target effects (genes involved in cell cycle regulation and cell death, in particular Forkhead Box M1 (FOXM1)) and a better potency for SMN2, leading to the discovery of Risdiplam.

For the safety pharmacology (**CTD 4.2.3**) investigation a dedicated ICH guideline exists, S7A (EMA/CPMP/ICH/539/00, 2001), defining the goal of safety pharmacology to "investigate […] undesirable pharmacodynamic effects […] on physiological functions, in relation to exposure in the therapeutic range and above". The investigation might also include metabolites of the finished product if concerns exist. The core battery of tests includes three vital organ systems, the cardiovascular system, the CNS and the respiratory system, each with a defined set of tests. Cardiovascular tests include measuring blood pressure, heart rate and an ECG. Most importantly, because the prolongation of the QT-interval is prognostic for arrhythmias, the *in vitro* hERG (human Ether-a-go-go Related Gene)-assay and *in vivo* electrophysiological QT investigations are routinely performed. The CNS is usually investigated with the Irwin test or the Functional Observational Battery (FOB). Additionally, neuro-histopathology can be informative. For assessment of the respiratory system parameters of the ventilatory function (respiratory rate, tidal volume) and lung function (spontaneous breathing, lung airflow & volume, hemoglobin oxygen saturation) are monitored. Depending on the drug or drug class the species for *in vivo* safety pharmacology are selected.

An effect in the hERG assay (IC_{50} 1.8µM) and on the QTC interval in cynomolgus monkeys was observed for the Risdiplam predecessor RG7800. Risdiplam was chemically designed to improve the safety pharmacology profile and consequentially showed no effect in the hERG assay (IC_{20}>5µM) and the absence of a QTC interval prolongation.

For CNS safety pharmacology no observations in relevant animal tests have been made (F. Hoffmann La Roche, data on file).

Investigation of pharmacodynamic drug interactions (**CTD 4.2.4**) are needed when drugs have the same target or a similar side effect profile and if they are likely used together. Because Risdiplam is metabolized by flavin-containing monooxygenase (FMO) and FMO is not targeted by other chemicals (Phillips & Shephard, 2017, Sturm, Gunther et al., 2019), drug-drug-interactions (DDI) through this mechanism are unlikely.

2.1.1.2 Pharmacokinetics

Pharmacokinetics (**CTD 4.3**) determines the essential parameters describing how the drug, at low pharmacological doses, is absorbed (A), distributed (D), metabolized (M) and excreted (E) from the organism (ADME). PK investigation shows if the drug stays long enough in the blood and reaches sufficient concentrations to affect the target organs.

Together with toxicokinetic data this allows to estimate the therapeutic window and calculate starting doses for clinical trials. Additionally, PK data for significant metabolites are determined. Apart from the general pre-clinical guideline ICHM3(2) (EMA/CPMP/ICH/286/95, 2008), ICH S3A describes in more detail requirements for pharmacokinetic and toxicokinetic studies (EMA/CPMP/ICH/384/95, 1995).

PK investigations for Risdiplam and its predecessor RG7800 were performed in mice, rats and cynomolgus monkeys (Poirier et al., 2018, Ratni et al., 2018). RG7800 displayed a relatively long half-life and large volume of distribution in rats and cynomolgus monkeys ($T_{1/2}$=19 & 42h; V_{ss}=29 & 20 L/kg). Additionally, the N-dealkylated metabolite was found in all tested species and reached up to 9% of the total plasma concentration distributing primarily into muscle tissue. The large V_{ss} and the unfavorable metabolism of RG7800 contributed to the safety issues observed *in vivo*. Risdiplam was specifically designed to eliminate the negative features of RG7800 and displayed much more favorable PK properties. In rats and monkey half-life ($T_{1/2}$=6.4 & 5.4h) and V_{ss} (V_{ss}=3.1 & 2.0 L/kg) decreased as compared to RG7800. The N-hydroxylated metabolite of Risdiplam is also produced at much lower levels then RG7800 as investigated *in vitro* liver microsomes (3.8%) and hepatocytes (1.7%). A safety comparison of the metabolites (RG7800 and Risdiplam) follows in the toxicity section (**4.4.7.5**). Distribution and elimination of Risdiplam were investigated with quantitative whole-body autoradiography (QWBA) in rats and monkeys (Poirier et al., 2018). Risdiplam and RG7800 have a high passive permeability which is beneficial to gastrointestinal tract (GI) and tissue uptake and both distribute from blood into multiple tissues including CNS. Risdiplam distribution and elimination in brain, CSF and other tissues correlate well with plasma and full elimination is achieved several weeks post-dose. Risdiplam is a strong *in vitro* substrate for both rodent drug efflux transporters multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP) but not human transporters. This limited the CSF concentrations in rodents but still enable sufficient SMN protein expression levels for PD assessment. In monkeys, there was no evidence for an effect of the transporters on brain distribution.

2.1.1.2 Toxicology

The pre-clinical toxicology (**CTD 4.4**) studies are performed according to the general ICH Guideline M3 (EMA/CPMP/ICH/286/95, 2008) and ICH S6 (EMA/CHMP/ICH/731268/1998, 2011). Subsections, like Carcinogenicity (**CTD 4.4.4**), additionally have their own dedicated guidelines. Toxicology investigations are performed to identify the drug-related toxicities in a concentration dependent manner. The derived no-adverse-effect-levels (NOAEL) are converted to the Human equivalent dose (HED) and upon multiplication with a safety factor determine the upper bound for the maximum recommended starting dose (MRSD) (EMEA/CHMP/SWP/28367/07, 2018, FDA/CDER/2005, 2005). Toxicology investigations are usually performed in two animal species, one rodent and one non-rodent.

The data historically obtained in the acute toxicity study (**CTD 4.4.1**) are nowadays gathered in other studies, like dose escalation. The major focus of pre-clinical toxicology is to identify organ specific toxicity after repeated administration of high doses, as

investigated in repeat dose toxicity studies (**CTD 4.4.2**). The repeat dose study duration is determined by the maximum duration of the clinical trial and the duration of the indicated treatment. Long term repeat dose studies that investigate chronic toxicity (6-12 months) can be done in parallel to the first clinical studies.

Because human SMN2 has no functional animal equivalent, investigated toxicity for Risdiplam and RG7800 is either off-target, i.e. unrelated to pre-mRNA splicing, or secondary target, i.e. interaction with the splicing machinery in general. Repeat dose toxicity was investigated in rat and cynomolgus monkeys, and the long-term chronic toxicity for RG7800 was performed in parallel to the first-in-human (FiH) trial.

Evidence of phospholipidosis (Epithelial vacuolation and foamy macrophages in rat) was observed for RG7800. This can be classified as off-target effect due to the high V_{ss} value of RG7800 and it was possible to eliminate this effect in the re-designed Risdiplam, by targeted modifications to the chemical structure. Many of the observed toxicities (Micronucleation of rat bone marrow erythroblasts, histopathological changes in gastrointestinal tract epithelia, lamina propria, exocrine pancreas epithelia in mice, rats and/or cynomolgus monkeys, Parakeratosis/hyperplasia/degeneration of skin, tongue larynx epithelia and degeneration of germ cells in testis of cynomolgus monkeys and rats), were secondary target effects on the pre-mRNA splicing machinery as determined by *in vitro* splicing assays. These effects were associated with cell cycle or apoptosis genes, in particular FOXM1. The splicing assay was also used to screen for compounds with less secondary target effects. However a linear correlation between the potency for SMN2 and FOXM1 was observed, suggesting that at least for this class of compounds the secondary target effects cannot be eliminated (Ratni et al., 2018). What could be eliminated by targeted drug-design, however, was the formation of a highly potent (secondary target) metabolite (**CTD 4.4.7.5**). In the case of RG7800, the main (N-dealkylated) metabolite was 10-fold more potent for FOXM1 and SMN2 splicing than RG7800 and did not reach the brain. Because the main (N-hydroxylated) metabolite of Risdiplam did not alter FOXM1 or SMN2 splicing, a main source of secondary target toxicity could be removed. In the repeat-dose studies the secondary target effects for Risdiplam were reversible or partially reversible and clear NOAELs could be established, which allowed the calculation of an MRSD.

The long-term chronic toxicity of RG7800 in cynomolgus monkeys revealed non-reversible histological findings in the retina. Although the parallel clinical trial was halted because of this finding, it was not possible to eliminate this toxicity hinting at a potential class effect of SMN2 splice modifiers. Since, retinal degeneration was not observed in rats this might be a species-specific toxicity. Because these findings were observed at considerably higher concentrations than human and No Observed Effect Level (NOELs) could be established, the clinical evaluation of Risdiplam was continued.

Genotoxicity studies (**CTD 4.4.3**) are performed according to ICH guideline S2 (EMA/CHMP/ICH/126642/2008, 2008) and serve to identify mutagenic or genotoxic risk (effects concerning genes or chromosomes). There is a set of standard tests that needs

to be performed *in vitro* (**CTD 4.4.3.1**) (e.g. the bacterial ames test) and *in vivo* (**CTD 4.4.3.2**) (e.g. the micronucleus assay in mice).

Risdiplam and RG7800 showed no evidence of genotoxicity *in vitro*. Risdiplam and RG7800 showed an increased frequency of micronucleated cells but the comet assay was negative indicating no direct DNA interaction. Thereby the mechanism of action is unclear, but genotoxicity is likely due to secondary splice target interaction.

Two ICH guidelines, S1A and S1B (EMA/CPMP/ICH/140/95, 1995, EMA/CPMP/ICH/299/95, 1998) exist for the pre-clinical evaluation of Carcinogenicity (**CTD 4.4.4**). The purpose of these studies is to "identify tumorigenic potential in animals and […] assess the relevant risk in humans" (EMA/CPMP/ICH/140/95, 1995) and studies are performed in two rodent species. Carcinogenicity studies are triggered by treatment duration, causes of concern (from molecular structure or class) or evidence from other pre-clinical studies (repeat dose, genotoxicity).

Reasons for studies according to the guidelines are ambiguous genotoxicity tests (unknown MoA), while reason against it are the absence of causes of concern (i.e. direct DNA interaction) and potentially low life expectancy in a subpopulation of patients. Carcinogenicity studies are often deferred to after approval for life saving rare disease indications. Suspicion may be existing for Risdiplam based on cell cycle arrest and micronuleus observation but because no evidence for tumor formation in a mouse transgenic model was observed (F. Hoffmann La Roche, data on file) no carcinogenicity studies were performed.

The pre-clinical assessment of reproductive and developmental toxicity (**CTD 4.4.5**) is guided by the ICH guideline S5(R3) (EMA/CHMP/ICH/544278/1998, 2020). Fertility is investigated in rats, embryotoxicity in rodents/rabbits/monkeys and pre-& post-natal development in rats.

Effects of Risdiplam and RG7800 are in line with toxicity findings (secondary splicing targets, e.g. FOXM1) on other rapidly dividing cells, i.e. effects on reproductive function are to be expected at least at higher doses in animal studies based on the effects seen with Risdiplam on cell cycle interaction. Effects were observed on male germ cells in rats and monkeys. The findings were fully reversible. Four studies in juvenile animals (**CTD 4.4.5.4**), three in mice and one in rats, were performed to gather PK and PD data to support the pediatric development in the main target patient populations.

Local tolerance (**CTD 4.4.6**) is investigated according to the EMA guideline (EMA/CHMP/SWP/2145/2000, 2016), usually together with general toxicity. The study should be performed prior to first human exposure and according to the clinical RoA. Additionally, accidental exposure should be investigated before clinical phase III. Tests should be performed in one relevant species.

Since Risdiplam is orally administered in the clinic, local tolerance is not necessary (EMA/CHMP/SWP/2145/2000, 2016).

Immunotoxicity (**CTD 4.4.7.2**) is investigated according to ICH guideline S8 (EMA/CHMP/167235/2004, 2006) using observations from general toxicity studies and additional studies when justified by weight of evidence. Immunogenicity is rare for small molecules when compared to biologics.

For Risdiplam no related observations in *in vitro* or animal studies have been made (F. Hoffmann La Roche, data on file).

Metabolites (**CTD 4.4.7.5**) were discussed in previous paragraphs.

Pre-clinical phototoxicity studies (**CTD 4.4.7.7**) are performed according to ICH guideline S10 (EMA/CHMP/ICH/752211/2012, 2014). Studies are triggered by certain physico-chemical properties including a Molar extinction coefficient > 1000/Lmol/cm, absorbance between 290-700nm or a positive photoreactivity test (reactive oxygen species (ROS)). Risk assessment is required before clinical phase I and experimental evaluation before clinical Phase III. The classical *in vitro* phototoxicity test is the 3T3 Neutral Red Uptake (NRU)-test. Whereas RG7800 showed phototoxicity in the 3T3 NRU test, Risdiplam had no effect even at the highest, solubility limited concentration.

2.1.2 Clinical Safety and Efficacy

2.1.2.1 Regulatory background

In the US, before the use of an investigational drug in humans, an IND (Investigational new drug) application according to 21CFR Part 312 (21CFR312, 2019) is required for a new drug or biologic without a license. 21CFR Part 312 also specifies the requirements an IND application must fulfill, including the application form 1571, a general investigational plan, investigator brochure, study protocols, chemistry, manufacturing, and control (CMC), PD and Toxicology data.

In the EU clinical trials are regulated by the Clinical Trials Directive 2001/20/EC (2001/20/EC, 2001) which will be replaced by the Clinical Trial Regulation 536/2014 (536/2014, 2014). Other international documents that are explicitly mentioned in the EU documents are the Declaration of Helsinki (Association, 2013) (in both) and the ICH Good clinical practice (GCP) Guideline E6 (EMA/CHMP/ICH/135/1995, 2017) (in the regulation).

Detailed guidance on clinical trials and specific subtopics exist from the European Medicines Agency (EMA) and the FDA. On the international level, also a set of ICH guidelines exists, the ICH Efficacy (E) Guidelines, which are implemented and references in the EMA and FDA guidances. These include ICH E6 (EMA/CHMP/ICH/135/1995, 2017) on good clinical practice and ICH E8 (CPMP/ICH/291/95, 1998) on the overall structure of clinical development and the individual phases.

The clinical development is classically divided into 3 phases. The exploratory Phase I, with the first-in-human (FiH) trial usually performed in healthy volunteers using a single ascending dose. Main parameters investigated here are tolerability, PK and if possible PD. Subsequentially, Phase I studies investigate vulnerable populations, multiple ascending doses and food interactions. The main objectives of Phase II are proof-of-concept (IIa) and dose-finding (IIb). First evaluations of safety and efficacy in patients are

performed in this phase using a limited treatment duration. In phase III pivotal trials are performed to confirm efficacy for short- and long-term treatment and correspondingly confirm short- and long-term safety using the optimal dose. The data from Phase III allows the benefit/risk evaluation of the drug and comparison with other treatments.

2.1.2.2 Phase I

The clinical development of Risdiplam started with its predecessor RG7800. The RG7800 FiH study was a placebo-controlled, double blind study to investigate safety and tolerability with a single-ascending oral dose in healthy male subjects. PK investigations revealed a slightly more than dose-proportional increase in plasma exposure, a maximum plasma concentration, C_{max}, of 5-8h and a half-life, $T_{1/2}$, of 120h. The long half-life was in line with findings in preclinical studies of RG7800. PD analysis revealed a measurable effect of RNA splicing in blood cells, with levels of full length SMN2 mRNA in whole blood increasing and SMN2Δ7 mRNA decreasing with dose and exposure. No detectable changes in SMN1 full length mRNA could be observed and all tested doses were safe and tolerated well (Ratni et al., 2018). The data from Phase I made it possible to advance into Phase IIa with 12-week, double blind, placebo controlled, multiple dose study in adult and pediatric SMA patients (Moonfish). Parameters to investigate were efficacy, safety, tolerability, PK and PD. First PD data showed an increase in full length SMN2 mRNA levels and up to 2-fold increase in protein vs. baseline after 12 weeks treatment. However, Moonfish was put on hold due to safety findings in long term (LT) chronic toxicity study in cynomolgus monkeys.

In the process of reducing side effects of RG7800 by intelligent drug-design, Risdiplam was discovered. Pre-clinical data indicated a better safety profile and a more favorable PK profile with reduced half-life. Risdiplam was therefore selected as the new clinical candidate for SMA treatment and a new FiH study was conducted. NCT02633709 is a phase I study with healthy subjects to investigate safety, tolerability, PD (mRNA), food effect and CYP450A3A inhibition for Risdiplam. Risdiplam showed a linear PK response from 0.6-18.0mg with C_{max} & AUC increasing dose-proportionally. In comparison to RG7800 and in line with pre-clinical results the half-life was significantly reduced, 40.1-68.7h (2-18mg), 24.8h (0.6mg). No food effect and only a minor effect of itraconazole on PK (11% increase of AUC,9% C_{max} reduction) was observed. The PD data for Risdiplam showed a dose-dependent increase of SMN2FL/SMN2Δ7 mRNA ratios with a median time to max of 4-8h postdose. 18.0mg Risdiplam led to 64% increase of SMN2FL mRNA compared to baseline. No effect on SMN1 mRNA was observed and as expected in healthy subjects, no effect on the SMN protein level. The safety data for this FiH Risdiplam study reported no deaths, no moderate or severe adverse event (AE), no AE withdrawals and no serious adverse events (SAEs). No clinically significant treatment- or dose-dependent changes relative to the baseline were observed and no individual clinically significant QT changes were observed.

2.1.2.3 Phase II/III

In contrast to RG7800, Risdiplam successfully completed clinical phase I, and several phase II and III studies have been performed or are still ongoing.

A pivotal study (Sunfish/NCT02908685), oral Risdiplam administration, was performed with patients of type 2 or 3 SMA, including children and adults (age 2-25). The study was a double-blind, placebo controlled, combined phase IIb/phase III study with two parts. 51 patients participated in the dose-finding part and 180 patients in the confirmatory part of this trial. Patients were evaluated according to total score of Motor Function Measure 32 (MFM-32) at 12 months (primary endpoint). Sunfish met its primary endpoint in November 2019 (Dunant, 2020) and significant improvement of treated patients vs placebo controls was observed with 1.55 points (p=0.0156).

The following secondary endpoints were also evaluated: percentage of participants achieving stabilization (MFM32 ≥0) or improvement (MFM32 ≥3) in MFM32 total score, change from baseline in Revised Upper Limb Module (RULM) total score, change from baseline in Hammersmith Functional Motor Scale Expanded (HFMSE) total score and change from baseline in SMA Independence Scale (SMAIS) total score at Month 12.

Significant results were observed for stabilization, 70% Risdiplam vs. 54% placebo (p=0.043) and improvement, 39% Risdiplam vs 22% placebo (p=0.0469). A significant improvement of 1.59 points was also observed for the RULM endpoint (p=0.0028) at 12 months and the caregiver-reported SMAIS endpoint ca. 2.7 points (p=0.022). No significant improvement was observed for the patient-reported (≥12 years) part SMAIS endpoint, ca. 1.2 points (p=0.1778) and the HFMSE endpoint, 0.58 difference in change (ca. 1 vs 0.4) (p=0.3) at 12 months.

A second pivotal phase III study (Firefish/NCT02913482) was performed in infants (age 1-7 month), type 1 SMA, using an open-label design with oral Risdiplam administration. Part 1 of the study investigated safety in 21 patients and part 2 investigated efficacy in 41 patients. The primary endpoint was the proportion of patients sitting without support for ≥5sec at 12 months of treatment as assessed by the Bayley Scales of Infant and Toddler Development III (BSID-III). Positive results were reported on the efficacy part and no safety signals were identified in the safety part of Firefish (Dunant, 2020). 29% of infants reached the primary endpoint vs 0% natural history (p<0.0001, performance criterion=5%).

The following secondary endpoints were also evaluated: • time to death or permanent ventilation; • achievement of motor milestones measured by the Hammersmith Infant Neuromuscular Examination (HINE-2) with increase (improvement) and decrease (worsening) defined as a change of ≥2 points kick, ≥1 point head control, rolling, sitting, crawling, standing or walking; • proportion of infants who achieve an increase of ≥4 points in the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND); • the proportion of infants who achieve a score of ≥40 in the CHOP-INTEND; • the ability to swallow and feed orally and the number of nights in hospital per infant by Month 12.

93% (38/41) of patients were alive at 12 months vs ca. 30% in natural history data. Statistically significant improvements were observed for the HINE-2 endpoint, 32/41 (78%) responded (p<0.0001, performance criterion=12%), the CHOP-INTEND endpoint

(median +20 points) 90% (37/41) ≥4 points and 56% (23/41) score ≥40 at 12 months points ($p<0.0001$, performance criterion=17%). Furthermore 95% (36/38) maintained their ability to swallow at 12 months and 89% (34/38) were able to feed orally while natural history data shows that all infants older than 12 months require feeding support. 49% (20/41) of patients did not require hospitalization up to 12 months and 1.30 hospitalizations per patient-year as compared to 4.2-7.6 hospitalizations per year for natural data were recorded.

In addition to the two pivotal studies, treatment with Risdiplam is investigated in genetically diagnosed but pre-symptomatic newborn to six-week-old SMA patients (Rainbowfish/NCT03779334). The study is an open-label, single-arm, multicenter study investigating efficacy, safety, PK and PD. The recruitment phase of this study is not completed yet.

Another study with ongoing recruitment is the Phase III, open-label, exploratory study (Jewelfish/NCT03032172) which investigates Risdiplam treatment in 6 month – 60 years old SMA patients, previously treated with other SMA therapies.

2.2. Spinraza™

Spinraza™ belongs to the oligonucleotide drugs and more specifically to the antisense oligonucleotides (ASOs). ASO share properties from biologicals and small molecules. ONs exist in various forms naturally in the body, but as a drug can be synthesized, well-defined and characterized like small molecules. Due to this fact they have mostly been regulated as small molecules (Kornbrust, Cavagnaro et al., 2013), with respect to marketing authorization and guidelines (see Risdiplam regulatory background). Although no official ON specific guideline exists, a dedicated working group on Oligonucleotide safety was formed, including leaders in the field from industry and academia. The oligonucleotide safety working group (OSWG) has published recommendations based on experience with regulators, reflecting the consensus between industry and regulatory agencies on best practice to assess activity and safety of novel oligonucleotides (Schubert, Levin et al., 2012). In the following section the regulatory requirements for ONs, with focus on ASOs, in comparison to small molecules are discussed. Afterwards, it is discussed how they are followed in the case of Spinraza™, in pre-clinical and clinical part based on the European Public Assessment Report (EPAR) (EMA/289068/2017, 2017). A more detailed summary of the Spinraza™ studies can be found in Supplementary Tables P2, C2.

2.2.1 Pre-clinical Safety and Efficacy

The pre-clinical development of Spinraza™ is guided mostly by the same guidelines as small molecules including the overreaching ICH Guideline M3(R2) (EMA/CPMP/ICH/286/95, 2008) and more specific ICH guidelines (e.g. S7A) (EMA/CPMP/ICH/539/00, 2001) on the individual topics. However, the nature of ASOs MoA and the physico-chemical properties of ONs pose specific challenges for pharmacological and toxicological investigations. In the following paragraphs Spinraza™ is discussed with respect of regulatory requirements of “unformulated” ONs. For details on formulated ONs see OSWG recommendations (Marlowe et al., 2017), US FDA guidance (FDA/CDER/2018, 2018), EMA/CHMP reflection article (EMA/CHMP/806058/2009/R2, 2013) on liposomes and 2011 AFSSAPS Recommendations on Nanoparticles (AFSSAPS/DEMEB/2011, 2011).

2.2.1.1 Pharmacology

One of the key differences of ASOs in comparison with small molecules, is the intrinsically high specificity often resulting in no cross-species activity. This is particularly the case when the human target lies in the untranslated region of mRNA (low conservation). Because even one mismatch drastically reduces potency and 2 mismatches can render ASO completely ineffective, the pool of pharmacologically responsive species is often reduced. Even in non-human primates (NHP) differences in target sequences are often 1 or 2 nucleotides. On the bright side, the high specificity of ASOs makes cross-species activity predictable for matching sequences and guides the selection of the adequate species.

ISS-N1, the molecular target of Spinraza™, is located in the intronic region 7 of human SMN2 and only humans possess the SMN2 gene. Mice have only one SMN gene with 5

out of 15 nucleotide mismatches as compared to human SMN genes (Singh et al., 2006). Therefore, transgenic mouse models were employed to study the pharmacology and safety pharmacology was studied in pharmacologically unresponsive species (rodents and monkey). The OSWG also recommends the investigation of exaggerated (on-target) pharmacology (EP) (Kornbrust et al., 2013) but because of the limitation of pharmacologically active species and since the underlying cause for SMA is a loss of function, the on-target safety risk was considered minimal and EP investigation was not required (EMA/289068/2017, 2017).

The primary pharmacodynamics (**CTD 4.2.1**) of Spinraza™ were investigated in multiple transgenic mouse models including a mild SMA model expressing 4 copies of hSMN2 on a mouse SMN (-/-) background (Hsieh-Li, Chang et al., 2000) and more severe mouse models. Spinraza™ was able to modulate SMN2 splicing, producing full length SMN protein, which resulted in a significantly increased life span and improved motor function. The secondary pharmacodynamics (**CTD 4.2.2**) were not investigated because of the specific MoA.

Similar to small molecules, safety pharmacology (**CTD 4.2.3**) of ASOs is performed according to ICH guidelines S7A and S7B (EMA/CPMP/ICH/423/02, 2005, EMA/CPMP/ICH/539/00, 2001). The OSWG additionally published recommendations on safety pharmacology testing (Berman, Cannon et al., 2014). Most of the OSWG recommendations on safety pharmacology testing are based on experience with systemic administration of ASOs with PS backbone, the same class as Spinraza™. A particularity of ONs with respect to safety pharmacology is that the observations depend mostly on the class of ON and the chemical modifications and little on the actual sequence or sequence composition. Consequently, the species selection for safety pharmacology testing is influence by the ON class of the drug. For instance PS induced complement activation can only be investigated in a few species (cynomolgus monkeys, mini pig) and authorities expect safety pharmacology studies to be performed in cynomolgus monkeys (Black, Farrelly et al., 1994).

Similar to small molecule drugs, ON safety pharmacology testing includes the core battery of the three vital organ systems, the cardiovascular system, the CNS and the respiratory system, each with a defined set of tests (EMA/CPMP/ICH/539/00, 2001). However, experience with ON class effects is taken into consideration in the test selection. Traditionally, many of the *in vivo* safety pharmacology studies were conducted as stand-alone studies, but more recently the studies are combined or integrated into repeat-dose studies (Berman et al., 2014). This approach has also been chosen for Spinraza™ (EMA/289068/2017, 2017).

The hERG assay is a standard test in the core battery to investigate cardiovascular effects. However, the hERG channel is unaffected by PS ONs, like Spinraza™, in the clinically relevant dose range (Kim, Kim et al., 2014), no effects have been observed in pre-clinical studies and the access to channel in test system cells is limited. Therefore, the hERG assay is not recommended (EMA/CPMP/ICH/423/02, 2005) and *in vivo* assessments in NHP, according to ICH S7A (EMA/CPMP/ICH/539/00, 2001) are sufficient

for cardiovascular evaluation in most cases. No effects on cardiovascular parameters like blood pressure (diastolic, systolic, mean arterial) and heart rate were observed for Spinraza™.

Although, systemically administered ONs are rapidly cleared from the blood stream and no significant blood–brain barrier (BBB) crossing takes place (Boado, Tsukamoto et al., 1998) the CNS must still be evaluated in these cases according to ICH S7A guidelines (EMA/CPMP/ICH/539/00, 2001). Because Spinraza™'s route of administration (RoA) is intrathecal, the CNS exposure is drastically increased as compared to systemically administered ON and a CNS evaluation clearly relevant. Whereas no effects on neurobehavioral assessments (FOB) or learning parameters were observed, histopathology revealed mild focal neuronal vacuolation the hippocampus. Curiously, the vacuolations were only observed with formalin fixation and not with other fixation procedures, suggesting an interaction of formalin with local ON accumulations. This preparation artifact was indeed confirmed in a 6 weekly intrathecal doses study in cynomolgus monkeys (AS11). Transient changes in lower spinal reflex following intrathecal bolus application larger than 3mg were observed. Because of the reversibility and the lack of neurobehavioral findings it is suggested the changes to the lower spinal reflex are procedure related (i.e. intrathecal administration) but because the RoA is an integral part of the medicinal product, the findings must be included in the summary of product characteristics (SmPC) (5.3).

Respiratory parameters for Spinraza™ were investigated according to ICH S7A (EMA/CPMP/ICH/539/00, 2001) which requires the measurement of frequency of breathing and at least one additional respiratory parameter. No changes in function of the lung and corresponding parameters minute volume, tidal volume or respiratory rate were observed.

In addition to the core battery, PS are known to localize and subsequentially accumulate in the proximal tubules of the kidney following repeat systemic administration. Since Spinraza™ is administered intrathecal, this is less of a concern and it was detected in kidney and liver at only low concentrations (<120µg/g at the highest intrathecal dose). Concentrations are below the known toxicity thresholds (Henry, 2008) and no treatment related findings were observed. Nevertheless, a warning concerning class effects on kidney function was integrated into the SmPC.

Pharmacodynamic drug interactions (**CTD 4.2.4**) were not investigated due to the specific MoA of Spinraza™.

2.2.1.2 Pharmacokinetics

Pharmacokinetics (**CTD 4.3**) of ONs is described by the same essential parameters as small molecules (ADME) and the same guidances apply (EMA/CPMP/ICH/286/95, 2008, EMA/CPMP/ICH/384/95, 1995). PK of Spinraza™ were obtained from four separate monkey studies (APK01, AS01, AS03, AS06) (EMA/289068/2017, 2017). Because of the clinical RoA (repeated intrathecal administration), which is not feasible in rodents, cynomolgus monkeys were selected as the primary species for PK and toxicity

investigations. Intrathecal injection bypasses the BBB such that Spinraza™ reaches the target CNS tissue without initial absorption (**CTD 4.3.2**) and rapidly distributes through the CSF to CNS tissue with little metabolic clearance (**CTD 4.3.3**). Plasma exposure was generally 1-3 orders of magnitude lower than CSF exposure.

APK01 was 4-week multiple dose study in adult cynomolgus monkeys administering 4 intrathecal lumbar doses or 4 IV bolus doses at 1mg/dose once weekly. CSF and plasma concentrations for intrathecal administration showed a multiphasic disposition: fast distribution followed by a slower, sustained elimination phase (similar to single intrathecal dose in AS01). The CSF C_{max} was reached after 1h compared to 4h plasma C_{max} after intrathecal. CSF half-life was 102 days and tissue half-lifes for brain and spinal cord regions were 74-275 days (116 days median).

PK data in juvenile monkeys was gathered in the repeat-dose studies AS03 (14-week) and AS06 (53-week): CSF, plasma & tissue concentrations were consistent with APK01 and AS01 and a dose-dependent CSF & plasma concentration increase was observed with CSF $T_{1/2}$= 111 days (AS06). Plasma T_{max}: 2-5 hours after intrathecal bolus was also consistent with APK01 & AS01.

While the major route of metabolization (**CTD 4.3.4**) for small molecules is via Cytochrome P450 (CYP450) mediated oxidative metabolism, Spinraza™ is not predicted to be a CYP450 substrate from *in vitro* studies. The major way of ASO metabolization is the slow exonuclease mediated hydrolysis creating shorter ONs. A 17-mer oligonucleotide (N-1 from 3'end) was detected in relative quantities of more than 15% in monkey tissue. However, the efficiency of hybridization with the ASO target and thereby the potency decreases for shorter metabolites (reduced T_m) so the parent ASO binding should still be more effective. Indeed, a 15-mer version of Spinraza™ was synthesized and showed similar or slightly reduced activity *in vitro* and in a transgenic mouse model. Finally, the antisense orientation of Spinraza™ and its metabolites prevents duplex formation, so no reduction or antagonism is expected.

The main pathway of whole body clearance (**CTD 4.3.5**) for compounds like Spinraza™ and its metabolites is urinary excretion, so additionally studies for Spinraza™ have not been conducted (Geary, Watanabe et al., 2001).

A large fraction of Spinraza™ is bound to human plasma proteins (>94%, weak binding), but due to the different binding sites in comparison to hydrophobic small molecule there is low potential for PK drug interactions (**CTD 4.3.6**). *In vitro* studies also showed that Spinraza™ is neither inhibitor not substrate for human transporters (*in vitro* studies: BCRP, P-gp, OAT1 & 3, OCT2, OATP1B1&B3, BSEP transporters), nor inducer or inhibitor of CYP450 mediated oxidative metabolism.

2.2.1.3 Toxicology

The same guidelines that apply for small molecule pre-clinical toxicology (**CTD 4.4**), apply also to ONs. This include the general ICH Guideline M3 (EMA/CPMP/ICH/286/95, 2008) and ICH S6 (EMA/CHMP/ICH/731268/1998, 2011) and subsection specific guidelines. As it is the case for SMs, acute toxicity investigations (**CTD 4.4.1**) are integrated in other

toxicity studies. Repeat dose toxicity studies (**CTD 4.4.2**) are also the central toxicology studies for ONs.

Due to the nature of the clinical RoA and the expected ON class specific toxicities, repeat-dose investigations for Spinraza™ were carried out in cynomolgus monkeys. A mentioned above, safety pharmacology studies were integrated into the repeat-dose studies (EMA/289068/2017, 2017).

Two studies were carried out in juvenile monkeys, including a 14-week study (AS03) with 5 weekly doses of 0.3 and 1mg (loading period) followed by biweekly maintenance doses of 3 mg. The second study in juvenile monkeys was a 53-week study (AS06) with 5 weekly doses of 1,3 and 7 mg, followed by maintenance doses every 6 weeks. In repeat-dose studies in cynomolgus monkeys Spinraza™ was well tolerated with no effects on food consumption and body weight. No clinical persistent effects occurred but acute, transient deficits in lower spinal cord reflexes were observed at the highest doses. These effects occurred within several hours post-dose and were reversible within 48h post-dose. As stated above, these findings could be product related and a long-term registry study was suggested as follow up. Apart from this, no abnormal findings in systemic organ pathology, physical examinations, immune system, clinical pathology, maturation of skeletal system, cardiovascular, and ophthalmic assessment could be observed.

Genotoxicity studies (**CTD 4.4.3**) are performed according to regulatory guidelines for small molecules, including a battery of genotoxicity tests for gene mutations and chromosome damage as outlined in the ICH S2 (R1) guideline (EMA/CHMP/ICH/126642/2008, 2008). Additionally, the OSWG published recommendations on genotoxicity testing for ON (Berman, Barros et al., 2016).

In comparison with small molecules, an important point to consider when performing genotoxicity assays, is that due to their large size and chemistry, uptake of ONs cannot be assumed. Evidence for uptake in the cell types used for genotoxicity testing is therefore essential. Because of their known responsiveness to nucleoside analogs and because uptake is more likely, mammalian cell assays are generally considered more relevant than bacterial assays for ON genotoxicity studies. However, the bacterial mutagenicity (Ames) test is preferred for testing of all ONs that contain non-ON components, such as delivery formulations or conjugate/linkers (Berman et al., 2016). DNA damage can also be assessed *in vivo* through standard DNA strand break assays, such as the Comet assay (EMA/CHMP/ICH/126642/2008, 2008).

The studies on genotoxicity using the standard battery of *in vitro* and *in vivo* genotoxicity assays have been consistently negative across all the chemical ON classes so far, with over 50 compounds tested containing PS linkage and at least 23 compounds tested with 2'-MOE. Because of this consistently negative data, a lack of chemical interaction with DNA and an unlikely sequence-dependent interaction (e.g. triple helix formation), the EMA CHMP SWP considers genotoxicity testing to be no longer warranted for ONs with selected chemistries (e.g., PS linkages, 2'-MOE and 2'-O-Me modifications (EMEA/CHMP/SWP/199726/2004, 2005). Since Spinraza™ is an ON with PS linkage and 2'-MOE modifications, genotoxicity should have not been necessary. However, bacterial

reverse mutagenesis assays, *in vitro* chromosomal aberration assay, *in vivo* CD-1 mouse bone marrow micronucleus assay were still performed and, as expected, gave negative results (EMA/289068/2017, 2017).

Testing is however recommended for ONs with novel chemistry where genotoxicity data is lacking and novel linkers used to conjugate molecules (e.g., PEG, peptide, or ligand). The same holds true for oligonucleotide-related impurities and any materials used in preparing an ON (or its delivery system) that are known DNA-reactive mutagens or have structural alerts for DNA-reactive mutagenicity (e.g., by in silico assessment) (EMA/CHMP/ICH/731268/1998, 2011).

For Spinraza™, oligonucleotide impurities qualification was performed in the 53-week repeat dose toxicology study in juvenile monkeys (AS06) and small molecule impurities assessed accordingly (EMA/CHMP/ICH/83812/2013, 2018). Acceptance limits were set well below the qualification levels.

The two ICH guidelines for the pre-clinical evaluation of Carcinogenicity of small molecules (**CTD 4.4.4**), S1A and S1B (EMA/CPMP/ICH/140/95, 1995, EMA/CPMP/ICH/299/95, 1998), in principle also apply to ONs. However, since the historic genotoxicity data on ONs, in particular certain chemistries (e.g., PS linkages, 2'-MOE and 2'-O-Me) has been negative and there exists no mechanism for tumor induction, carcinogenicity studies should in general not be required. In the case of Spinraza™, negative genotoxicity data was generated and a weight of evidence assessment came to the conclusion that carcinogenicity studies were not necessary (EMA/289068/2017, 2017).

The pre-clinical assessment of reproductive and developmental toxicity (**CTD 4.4.5**) is guided by the ICH guideline S5(R3) (EMA/CHMP/ICH/544278/1998, 2020). The selected species for fertility testing are rats, embryotoxicity is investigated in rodents/rabbits/monkeys and pre-& post-natal development in rats.

A combined fertility and early development study in CD-1 mice (3, 10, 25 mg/kg) (**CTD 4.4.5.1**), two studies on embryo-fetal development (**CTD 4.4.5.2**) in New Zealand White rabbits (0, 6, 12.6, 25 mg/kg) and a pre- and post-natal development study (**CTD 4.4.5.3**) on female pregnant CD-1 mice (SC, 1.4, 5.8, 17.2 mg/kg) was performed for Spinraza™ (subcutaneous). All studies were negative (EMA/289068/2017, 2017). As discussed above, AS03 & AS06 were performed in juvenile monkeys. Additionally, several studies for Zolgensma® were performed in juvenile animals (**CTD 4.4.5.4**) reflecting the important juvenile patient population. In a 13-week toxicity study in juvenile CD-1 mice, 1,10,50 mg/kg/ weekly doses were administered SC (intrathecal in mice is not technically feasible) from post-natal day 4 (PND4) till PND25. From PND25 till PND95 two doses were administered per week. Animals tolerated Spinraza™ well across all dose levels and showed no clinical findings or effects on morbidity, mortality, body weights, food consumption, ophthalmic examination, hematology, clinical chemistry, necropsy, growth, and development. At 50mg/kg kupffer cell hyperthrophy was observed in male animals and vacuolated macrophages on lymph nodes in male and female animals, increasing the corresponding organ weights. Higher spleen weights were observed in males at 10mg/kg

and at 50mg/kg in males and females. These observations were in line with adult toxicity studies; hence no juvenile specific toxicity could be observed.

As for small molecules, local tolerance (**CTD 4.4.6**) is investigated according to an EMA guideline (EMA/CHMP/SWP/2145/2000, 2016) usually in one species and together with other studies (EMA/CPMP/ICH/286/95, 2008). The RoA for Spinraza™ is intrathecal, which according to the guideline requires parenteral tolerance testing, and histopathology examinations should be considered on a case-by-case basis. For Spinraza™ several studies using subcutaneous (SC) injection have been performed, and hence local tolerance is covered.

Effects and toxicities in relation to the immune system, Antigenicity (**CTD 4.4.7.1**) and Immunotoxicity (**CTD 4.4.7.2**), are investigated according to ICH guideline S8 (EMA/CHMP/167235/2004, 2006) using observations from general toxicity studies and additional studies when justified by weight of evidence.

Although ONs are typically not immunogenic in monkeys (studies can even reutilize animals), pro-inflammatory effects in animals have been described for ON therapeutics with charged backbone and persistence in tissue. Therefore, an evaluation of the immune system may be warranted and should be conducted during repeat-dose toxicity studies (like for small molecules) via clinical observations, hematology evaluations, and tissue histopathology (Berman et al., 2014).

For Spinraza™, parameters of the immune system were investigated in 14- and 53-week repeat dose studies and no abnormal effects with respect to age were observed. More specifically, “no indication of cellular activation and cytokine production due to pro-inflammatory effects in the animal studies and none in the clinical setting” have been observed (EMA/289068/2017, 2017).

Residual solvents Permitted Daily Exposure (PDE) values were assessed according to ICH Q3(R5)(EMA/CHMP/ICH/82260/2006, 2010) and it was concluded that they are adequately purged during downstream processing (DSP). For a discussion on other impurities (**CTD 4.4.7.6**) see paragraph on genotoxicity assessment above.

2.2.2 Clinical Safety and Efficacy

2.2.2.1 Regulatory background

As discussed in the pre-clinical part, Spinraza™ belongs to the oligonucleotide drugs. Because ONs are usually synthesized and can be well defined and characterized like small molecules they have mostly been regulated as such (Kornbrust et al., 2013). Hence the regulatory background for Spinraza™ is that of the small molecule Risdiplam which was previously discussed, including the key regulations 21CFR Part 312 (21CFR312, 2019) in the US, Clinical Trials Directive 2001/20/EC (2001/20/EC, 2001) in the EU and ICH guidelines ICH E6 (EMA/CHMP/ICH/135/1995, 2017) and ICH E8 (CPMP/ICH/291/95, 1998) in ICH countries.

In the following the clinical studies for Spinraza™ are discussed based on the EPAR (EMA/289068/2017, 2017). The same clinical studies were also the foundation of the FDA assessment and approval (209531, 2016).

The clinical development plan allowed patients who completed their initial phase I studies to continue in subsequent phase II and III studies (Figure 4), as shown in Figure 3 of the EPAR.

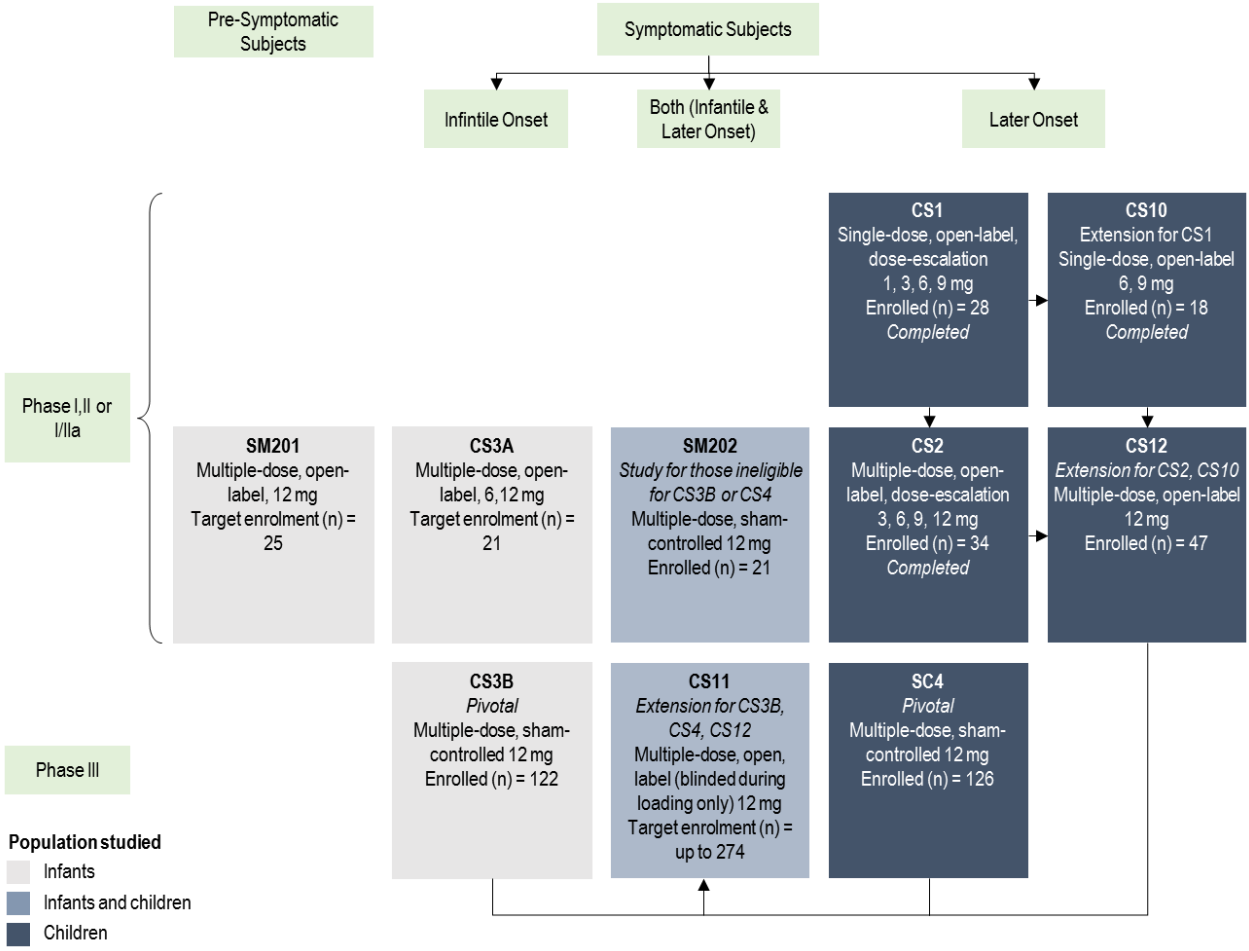

Figure 4: Clinical Development Plan for Spinraza™: Flow of Subject Population Study. Modified from Figure 3 Spinraza™ EPAR (EMA/289068/2017, 2017).

2.2.2.2 Phase I

FiH studies of phase I, investigating tolerability, PK and PD, are usually performed in healthy volunteers. However, because of the intrathecal RoA for Spinraza™ and the procedure associated risks, all clinical studies were performed in SMA patients.

Phase I started with the study CS1, an open-label, single-dose (intrathecal), dose-escalation (1,3,6,9mg) study investigating safety, tolerability and PK. The patient population consisted of stable SMA patients between 2-14 years of age. Patients then entered either the open-label studies CS2 or CS10, multiple-dose dose-escalation (3,6,9,12mg) or single-dose applications (6 or 9mg), respectively. Patients showed a dose-dependent (CS1, CS2, CS10) and time-dependent (CS2) improvement in HFMSE

total score. CS10 showed maintenance of significant improvements for 9mg patients. Patients form CS2 and CS10 continued treatments, repeat doses (12mg), in the study CS12. All patients maintained or improved their HFMSE scores.

Pharmacokinetic parameters were obtained from the combined phase I studies. A T_{max} of between 1.7 – 6.0 hours in plasma was measured in later-onset patients and infants. Spinraza™ distributed widely in CNS tissues and spinal cord and clearance happened via systemic circulation. A biphasic disposition in plasma was observed. The terminal elimination half-life in CSF (later onset) was: 135-177 days compared to 63-87 days in plasma. Dose proportionality and time dependencies were similar between later-onset patients and infants and a proportional increase in CSF from 1 to 12mg was observed. An approximately dose proportional increase in C_{max}, AUC values were observed in plasma. CSF trough concentration reached 1.4 to 3.0-fold accumulation, with a steady state after multiple loadings with 12 mg and maintenance doses at 22.5 month.

2.2.2.3 Phase II

The phase II study CS3A included 20 infantile-onset, type 1 SMA patients, with 4 patients receiving 6mg loading (intrathecal) and 12mg maintenance doses, and 16 patients receiving 12mg loading and 12mg maintenance doses. Overall, 65% of patients met the primary endpoint achievement of a new motor milestones (HINE) with earlier and greater improvement for the 12mg loading cohort compared to 6mg.

Additionally, following secondary endpoints were investigated: An increase in total CHOP-INTEND score of ≥4 points at the final examination (median 670 days) was observed in 55% (11/20). An improvement of the peroneal amplitude by ≥0.5 mV at the last examination before data cut-off date was observed in 65% (13/20) of patients. The survival rate was 75% (15/20) and 65% (13/20) of patients did not require permanent ventilation.

2.2.2.4 Phase III

CS5/SM201 investigates efficacy in pre-symptomatic SMA patients (≤ 6weeks,2 or 3 SMN2 gene copies). 69% of patients reached the primary endpoint of 2 or more motor-milestones at day 64 and 100% of patients achieved at least one motor-milestone at days 183 and 302.

Following secondary endpoints were evaluated: An increase in total CHOP-INTEND score of ≥4 points at days 64, 183, 302 was observed for 54% (7/13), 80% (8/10) and 60% (3/5) of patients, respectively. An improvement of ≥0.5 mV in peroneal amplitude at days 64, 183, 302 was observed in 64% (7/11), 90%(9/10) and 60%(3/5) of patients and 100% of patients were alive and did not requiring permanent ventilation at the end of the study.

CS3B is the pivotal study for Spinraza™ treatment of SMA investigating efficacy, safety and tolerability in Infantile-onset SMA patients (5q homozygous deletion or mutation, or compound heterozygote, 2 SMN2 copies, onset ≤ 6 month). Intrathecal administration of multiple 12mg doses of Spinraza™ were administered at days 1,15,29,64 and maintained with does once every 4 months. Dose levels and intervals were based on non-clinical toxicology and PK in monkeys. The study design was double-blind, randomized, sham-procedure controlled.

Primary endpoints were the percentage of motor milestone responders (HINE) and time to death or permanent ventilation. 51% of patients reach a motor milestone at the end of the study (≥190 days treatment) vs 0% in controls ($p<0.0001$). Compared to control patients a 47% reduction in risk of death or permanent ventilation was observed and subgroup analysis hinted that treatment early in disease reduces the risks even further (see secondary endpoints).

Several secondary endpoints were also evaluated. The percentage of CHOP-INTEND responders, patient survival rate, proportion of patients not requiring permanent ventilation, response rate with respect to compound muscle action potential (CMAP) (peroneal amplitude≥1mV), time to death or permanent ventilation in patients above and below the study median disease duration (subgroup analysis).

A 71% CHOP-INTEND improvement vs. 52% worsening in Controls and 65% ≥ 4 points improvement vs. 44% worsening in Controls was observed. Additionally, Spinraza™ treated patients had a 62.8% lower risk of death with a survival rate of 85% (68/80), a 34% lower risk of permanent ventilation and CMAP responders in the treated patients were 35% (18/51) vs 0% in controls with 20% (10/51) of patients improving by ≥1mV and 4% (2/51) improving by ≥2mV.

The subgroup below the study median disease duration showed a 76% reduced risk in death or ventilation while for the subgroup above the study median disease duration the risk was 16% reduced.

The safety comparison of Spinraza™ treated versus control patients showed AE in 96% vs 98% patients with severe or moderate event in 88% vs 95%, severe events in 56% vs 80% and SAE in 76% vs 95% of patients. All SAEs were considered non-treatment related by the investigators. No difference in AEs could be observed between loading and maintenance phases. Due to pre-clinical findings of hippocampal vacuoles a special monitoring and review of AEs suggestive of epilepsy was performed, however no epilepsy was reported.

The phase III clinical study CS4, double-blind, sham-procedure controlled, investigated efficacy and safety in later-onset, type 2 or 3 SMA patients. The primary endpoint was a change in HFMSE score at 15 months and an improved score of 4 points in Spinraza™ group vs a decrease of 1.9 points in controls ($p=0.0000002$) was observed.

Following secondary endpoints were also reached in the study: The percentage of patients who achieve ≥ 3-point HFMSE score improvement from baseline at 15 months was 57.3% in Spinraza™-treated patients vs 20.5% in controls. The proportion of subjects who achieve any new WHO (world health organization) motor milestone was 17.1% for the Spinraza™ group and 10.5% in controls and the number of WHO motor milestones achieved per patient was 0.3 points higher at 15 months. Additionally, the change from baseline in Upper Limb Module (ULM) test at 15 months was 3.7 points (Spinraza™) vs 0.3 (controls). 1 subject in both groups achieved standing alone and no patient achieved walking with assistance at 15 months.

The safety comparison in CS4 of Spinraza™ treated versus control patients showed AEs in 93% vs 100% of patients with severe or moderate AE in 42% vs 48%, severe AE in 5% vs 7% and SAE in 14 vs 26% of patients. None of the SAEs were considered related to treatment by investigators. The AE of procedural nausea was the only event considered to be “possibly related” to treatment by investigators. No Suspected Unexpected Serious Adverse Reaction (SUSARs) have been reported across the clinical trials for Spinraza™.

CS11 and SM201/CS5 are ongoing studies investigating longer term effects of Spinraza™ treatment. CS11 is a continuation of CS3B, CS4 and CS12. At the time of the report most AEs were considered related to SMA or adequate for the age of the population. Only few AE investigators considered “possibly related” to the treatment. This included ALT (Alanine aminotransferase) & AST (Aspartate transaminase) increase, pyrexia, increased eosinophil and lymphocyte counts in one subject, weight bearing difficulty and muscular weakness in one subject and hyperreflexia and tachycardia in one subject.

2.3. Zolgensma®

Gene therapy shares many properties of other biopharmaceuticals, which are regulated according to ICH Guideline S6 (EMA/CHMP/ICH/731268/1998, 2011). However, because gene therapy is a relatively recent development with relatively little clinical experience, additional and different guidelines in individual countries exist, even inside the ICH. In the EU, gene therapy is regulated as advanced therapy medicinal products (ATMP) and MAA submitted through the Centralized Procedure: Article 1a, ATMP Regulation (1394/2007, 2007) and point 4 (orphan medicinal product) Annex of (726/2004, 2004). In the US, a Biological license application (BLA) according to 21 CFR 601 (21CFR601, 2019) is required.

In the following the regulatory requirements for gene therapy are discussed and compared to how they are followed in the pre-clinical and clinical development of Zolgensma® (submitted to the FDA). A more detailed summary of the Zolgensma® studies can be found in Supplementary Tables P3 and C3.

2.3.1 Pre-clinical Safety and Efficacy

The pre-clinical development of Zolgensma® is guided by specific guidances on gene therapy products by FDA (FDA/CBER/OCTGT/2013, 2013) and EMA (EMA/CAT/80183/2014, 2018, EMEA/CHMP/GTWP/125459/2006, 2008) and the FDA considers ICH S6 Guidance (EMA/CHMP/ICH/731268/1998, 2011) and ICH M3 (EMA/CPMP/ICH/286/95, 2008) useful references for safety guidance and reproductive toxicity, respectively. Additional gene therapy specific guidances include the FDA guidance on long-term gene therapy follow-up and the EMA guidance on ERA (EMEA/CHMP/GTWP/125491/2006, 2008). The following discussion is mainly based on the FDA gene therapy guidance.

One of the major themes of the FDA gene therapy guideline is that pre-clinical studies should be specifically adapted to the gene therapy medicinal product (GTMP) under investigation (clinical investigations, product characteristics, MOA(s), indication, product delivery). Instead of a well-defined set of required studies the guidance employs a science-driven approach, incorporating basic toxicological principles. Therefore, this means studies can be designed and combined to achieve specific pre-clinical objectives, which need to be fulfilled before the clinical phase: 1. Biological plausibility, 2. Determining active dose levels, 3. Determining the starting dose level, selecting the dosing regime and devising a dose-escalation strategy for clinical trials, 4. Assessing feasibility and safety of route of administration, 5. Define patient eligibility criteria, 6. Identification of biomarkers, 7. Identification of potential public health risks. The resulting studies can be classified into proof-of-concept (POC) studies, biodistribution studies and toxicology studies, although combinations, like hybrid pharmacology-toxicology studies are encouraged.

2.3.1.1 Pharmacology

For gene therapy, POC studies are the central pre-clinical pharmacology studies. An important first objective of POC studies is to confirm the species selection with respect to disease population and safety assessment. The selection is usually based on *in vitro* screenings (including morphologic evaluation, functional assays, immunophenotyping)

and for the purpose of establishing the relevance of the species for the disease: *in vivo* pilot studies. In contrast to small molecule pre-clinical studies, animal models of disease are encouraged (morphological & functional/behavioral changes). Many factors need to be considered in the selection of animal model, including comparability and timing of the disease model, permissiveness/susceptibility to infection by the vector and immune response to vector and transgene. Indeed, multiple animal models may be required to sufficiently characterize pharmacology and toxicology.

The second important objective is to find a pharmacologically effective dose range and the optimal schedule (also with respect to disease onset) (**CTD 4.2.1**). This also includes confirmation of target delivery to the anatomic site/tissue/cell of interest and finding the optimal RoA.

In vivo pre-clinical pharmacology of Zolgensma® was performed in neonatal SMNΔ7 mice administering a single intravenous (IV) injection (1.2x10^13 to 1.1x10^14 vg/kg). A dose dependent improvement of survival and body weight gain was observed with highest effects at PND1 or 2 dosing. Additionally, early non-clinical vectors demonstrated improved motor function, neuromuscular transmission, cardiac function and body weight gain in SMNΔ7 mice (Byrnes, 2019).

2.3.1.2 Pharmacokinetics

The gene therapy equivalent to small molecule PK studies are Biodistribution studies (**CTD 4.3.3**). Prior to administration in humans, biodistribution studies are required for a new vector class or established vectors with significant changes (vector backbone, formulation, ROA, dosing schedule, vector dose levels). Biodistribution studies investigate the localization to non-target cells/tissues and level of viral replication. Additionally, the level and persistence of vector and expressed transgene are investigated.

The biodistribution study for Zolgensma® was carried out in neonatal, 12 weeks old FVB (Friend leukemia virus B) mice. Intravenous administration of 1.5x10^14 vg/kg resulted in highest vector DNA organ concentrations in the following order: heart, lung, liver, lumbar spinal cord, quadriceps muscle, brain, ovary, spleen, testis. Human SMN mRNA levels suggested highest protein expression in the following order: heart, quadriceps, liver, lung, brain, lumbar spinal cord. Low mRNA levels were observed in gonads and spleen.

2.3.1.3 Toxicology

Similar to small molecules the objective of gene therapy toxicology studies is identification, characterization and quantification of potential local and systemic toxicities. In addition to acute and chronic toxicities as observed with small molecules, there might also be a delayed effect, for instance due to a delayed expression of the transgene. Because gene therapy toxicity studies often use disease models, the responsiveness of species and pathophysiology of the animal disease should be taken into consideration.

The set of investigated standard safety parameters includes mortality, clinical observations, body/organ weights, physical examinations, food/water consumption, clinical pathology, gross pathology and histopathology. Additionally, gene therapy specific safety parameters include neurological exams, ophthalmic exam, behavioral testing,

cardiac assessments, humoral/cellular immune responses, hyperplasia/tumors, putative biomarkers, immuno-histochemistry and imaging.

Toxicity for Zolgensma® was investigated in neonatal FVB mice using an intravenous administration (**CTD 4.4.1**). For doses of 2.4x10^14 vg/kg and above, minimal to slight perivascular and chronic inflammation in the lung was observed. For doses of 7.9x10^13 vg/kg and higher a minimal till mild dose-dependent degeneration of the myocardium was observed. For 1.5x10^14 vg/kg and above a dose-dependent increase in occurrence and severity of adverse cardiac findings (minimal to moderate atrial thrombosis, slight to marked atrial dilation, minimal to slight fibroplasia, myocardial degeneration & inflammation) was observed, which was sometimes associated with increased heart weights, enlarged heart, abnormal shape or a large atrium. Adverse findings were also observed in liver (minimal to moderate hepatocyte degeneration/necrosis, minimal to slight hepatocellular hypertrophy, perinuclear vacuolation, increased Kupffer cells). Consequentially, Zolgensma®-related mortality was due to cardiac and liver toxicities (atrial thrombosis, atrial dilation, fibroplasia, myocardial degeneration, mononuclear cell infiltration, hepatocellular degeneration) (Byrnes, 2019).

Due to gene therapy product nature (e.g. integrating viral vectors) carcinogenicity/tumorigenicity studies (**CTD 4.4.4**) during early stages of product development are usually required. The FDA Guidance Long Term Follow-Up on gene therapy contains detailed information depending on the product type in its table 1. (FDA/CBER/2020, 2020). According to the guideline, AAVs' lack of mechanism for integration or genome editing (integration into AAVS1 site in human chromosome 19 has been eliminated by rep and cap removal from vector DNA (Surosky, Urabe et al., 1997)) and cumulative preclinical and clinical evidence suggests no (or very low frequency of) integration or genome editing. Since Zolgensma® is AAV-based there should be no need for carcinogenicity studies. Along the same line of thinking, studies of gene therapy specific toxicities (**CTD 4.4.7.7**), i.e. germline transmission were also not performed.

The need to perform reproductive/developmental toxicity (**CTD 4.4.5**) depends on target patient population and product type (including the expressed transgene). Studies are required before Phase 3 clinical trials. Because for more than 90% of the SMA patient population the disease onset is before the 12 month of age and many don't reach adulthood, when untreated (Finkel et al., 2014, Kolb et al., 2017) there is reduced need of performing those studies in the majority of SMA patients.

For Zolgensma®, according to the summary basis for regulatory action (Byrnes, 2019) "Studies to evaluate the safety pharmacology, developmental and reproductive toxicity, genotoxicity, carcinogenicity/tumorigenicity were not conducted for ZOLGENSMA®. These studies were not warranted based on the product characteristics, results from the toxicology studies, and target patient population."

According to ICH guideline M3 (EMA/CPMP/ICH/286/95, 2008) local tolerance (**CTD 4.4.6**) investigation of parenterally administered drugs, vary between ICH regions. Local tolerance studies are not recommended in the US, hence for the US Zolgensma® MAA, it was not performed.

For gene therapy there is a huge potential for immunogenic/neutralization (**CTD 4.4.7.1 & CTD 4.4.7.2**) reactions against vector, the expressed transgene and/or against endogenous proteins (transgene homologues) in particular for prolonged expression. In principle this warrants the conduct of long-term preclinical studies. However, because it is difficult to translate the immune response from animal (in particular for non-NHP, e.g. mice) to human, it is more appropriate to evaluate the response *in vitro* and in FiH studies. For Zolgensma®, the immune response was studied in the clinic (see below).

In the EU GTMPs also need to undergo Environmental risk assessment (ERA) (**CTD 1.6**, region specific module) (EMEA/CHMP/GTWP/125491/2006, 2008).

2.3.2 Clinical Safety and Efficacy

2.3.2.1 Regulatory Background

Clinical trials for gene therapy is regulated by the same guidelines as small molecules, including 21CFR Part 312 (21CFR312, 2019) in the US, the Clinical Trials Directive 2001/20/EC (2001/20/EC, 2001) in the EU and ICH guidelines ICH E6 (EMA/CHMP/ICH/135/1995, 2017) and ICH E8 (CPMP/ICH/291/95, 1998) in ICH countries. In addition, dedicated guidelines from FDA (FDA/CBER/OCTGT/2015, 2015) and EMA (EMA/CAT/80183/2014, 2018) cover the particularities of gene therapy clinical trials.

2.3.2.2 Phase I

The main objective of the phase I studies for gene therapy, in particular FiH, is the safety evaluation with respect to nature and frequency of adverse reactions (ARs) and in relation to the dose (FDA/CBER/OCTGT/2015, 2015). Furthermore, it might be possible to obtain first pharmacology and efficacy data.

The initial dose selection should be based on previous clinical experience, whenever possible, because a prediction from pre-clinical data is not as reliable as for small molecules. Because gene therapy products can persist, repeated dosing is often not acceptable until preliminary understanding of the toxicity and duration of activity are obtained. The dose for most gene therapy products is based on vector titer. However, for some vector types, dose might be better captured by vector genomes (AAV) as determined by qPCR or particle number (AVs). Because Zolgensma® belongs to AAVs dose is determined by vector genomes (vg).

Due to the extended and permanent effects of the gene therapy, clinical studies on healthy volunteers, including FiH, are mostly not acceptable. Instead, the appropriate patient population is selected based on benefit-risk assessment and the quality of data that can be expected. The first clinical trial for Zolgensma® is performed on SMA Type 1 patients. This patient population is the most severely affected and are hence most likely to have a positive benefit-risk ratio. Performing pediatric before adult clinical studies is the exception and only possible under special circumstances. According to the FDA guideline (FDA/CBER/OCTGT/2015, 2015) clinical studies on pediatric patients before assessment in adults are only appropriate when the childhood forms show a rapidly deteriorating clinical course while adult-onset phenotypes may be mild. The SMA spectrum is well

described by the previous sentence and hence FiH pediatric studies are justified. Although an untreated control group for diseases with a lack of natural history is discussed in the guideline, it is acknowledged that it might not be feasible due to procedure related difficulties in blinding or ethical reasons.

For Zolgensma® the FiH study (START) was an open-label, ascending-dose study in SMA Type 1 patients. Single intravenous doses of 4.3-4.6x10^13vg/kg (low dose) and 1.1-1.4x10^14vg/kg (high dose) were administered and patients received oral corticosteroids to suppress potential immune reactions. Safety and following preliminary primary efficacy endpoints were evaluated. Survival after 24 months was 67% (low dose) vs. 100% (high dose). And 0% of low dose patients vs 75% high dose patients achieved the motor milestone sitting w/o support for ≥ 30 sec and 0% vs. 16.7% achieved the motor milestone of standing and walking.

Clinical biodistribution was investigated in two patients who died 5.7 month and 1.7 month after receiving the 1.1x10^14 vg/kg dose. Vector DNA levels were highest in liver, spleen, inguinal lymph node and heart (in this order). Additionally, Vector DNA was also found in brain, peripheral nerves, spinal cord, muscles, kidney, pancreas, lung and thymus. SMN protein expression was found in neuronal and glial cells of the brain, spinal motor neurons, skeletal muscles, heart, liver, kidney, lung, pancreas, spleen, thymus, stomach, large and small intestines, and inguinal lymph nodes.

Environmental Risk/Viral shedding was investigated in saliva, urine and stool samples. DNA shedding analysis was performed for multiple time points and in multiple patients. Vector DNA shed mostly in stool, and much lower in saliva and urine and declined to non-detectable levels by 1-2 month (stool), 1-2 weeks (urine), 3 weeks (saliva).

2.3.2.3 Phase II

The main objectives of Phase II are proof-of-concept (IIa) and dose-finding (IIb) but since both objectives were already reached in phase I, subsequent studies were phase III.

2.3.2.4 Phase III

Phase III (STR1VE-US) was an open-label, single-arm study in Type 1 SMA patients with natural history data of SMA as control. Single intravenous dose of 1.1x10^14vg/kg (high dose) were administered and patients received oral corticosteroids to suppress potential immune reactions.

Primary efficacy endpoints were sitting without support for 30 seconds or more at 18 months of age and survival at 14 months of age. 47% of treated patients vs 0 % Natural history controls reached the first primary endpoint and 67% of treated patients vs. 25% Natural history controls were alive at 14 months of age.

Additional secondary endpoints were independence of ventilatory support, which was achieved by 81% (18/22) at 18 month and 68% (15/22) patients overall. The composite endpoint Ability to thrive at 18 months of age was achieved by 41% (9/22) of patients ($p<0.0001$ vs natural history) and CHOP-INTEND score above 40 and 50 were achieved by 95% (21/22) and 64% (14/22) of patients respectively.

The overall safety evaluation for all clinical studies included 44 Type 1 SMA patients receiving intravenous infusions of 1.1x10^14 vg/kg or above (41 patients) and 4.3-4.6x10^13vg/kg (3 patients).

An increase in anti-AAV9 antibody titers from below 1:50 to more then 1:800,000 in most patients was observed which is expected to preclude the possibility of re-administration.

One death occurred due to respiratory failure secondary to disease progression. One death after seizures due to leukoencephalopathy was observed and a connection to Zolgensma® application could not be excluded. Three serious adverse reactions were observed including elevation of aminotransferase, up to 40xULN (Upper Limit of Normal) in two patients and acute serious liver injury in 1 patient. Most frequent adverse reactions (incidence >5%): were vomiting and elevated aminotransferases, transient decreases in platelet counts and transient increase in cardiac troponin-I levels.

Therefore, acute liver injury and a considerable increase in aminotransferases were major serious risks associated with Zolgensma®.

Another phase III study is currently being conducted for Zolgensma® with intrathecal administration in type 2 SMA patients using three different doses: Dose A (6x10^13vg), Dose B (1.2x10^14vg), Dose C (2.4x10^14vg). Primary endpoints are change in HFMSE scores from baseline at 12 months compared to natural history and the ability to stand without support for ≥3 seconds. Interim results for primary endpoints were 6.0 points mean increase in HFMSE scores for Dose B subgroup 1 (≥2 years and <5 years) and the ability to stand in 8% (1/13) of subgroup 2 (≥6 months to <2 years). Following interim results were observed for the secondary endpoints increase in HFMSE scores ≥3 from baseline at 12 months, Dose B (≥2 years and <5 years): 92% (11/12); Dose B (≥6 months to <2 years): 83% (5/6) and walking independently for ≥5 steps, Dose B (≥6 months to <2 years): 8% (1/13).

Chapter 3: Comparison of the treatments

3.1. General comparison

The small molecule Risdiplam, the antisense oligonucleotide Spinraza™ and the gene therapy Zolgensma® can be compared from different perspectives and depending on the point of view interesting similarities and differences appear. All three target the same disease pathway but different sites of it. With respect to the MoA, Risdiplam and Spinraza™ are similar. Whereas Risdiplam affects the SMN2 expression by stabilizing a protein-RNA interaction, Spinraza™'s target is the SMN2 gene itself, both treatments resulting in an increase of full length SMN2 protein expression. Zolgensma® in contrast replaces the dysfunctional SMN1 directly.

With respect to active substance Spinraza™ and Zolgensma®, being both nucleic acids, are similar while Risdiplam is chemically the most different of the three. This also ties into the high specificity of the nucleic acid-based drugs, because of specific Watson-crick base pairing, whereas Risdiplam has the classical off-target profile of a small molecule. When looking at the drug product, however, Risdiplam is like Spinraza™ because both are synthesized and thereby well-defined and characterized. Zolgensma® in contrast is delivered in an AAV vehicle, which introduces the high process dependence of biological drugs. For instance, the protein capsid might be differently glycosylated or otherwise post-translationally modified depending on the batch. The ratio of full and empty particles might also be different and in consequence the dose is not easily defined. Also, with the AAV capsid another known source of immunogenicity is introduced.

Table 1: General comparison of the three treatments. Grey boxes highlight similarities of treatments.

	Risdiplam (Small Molecule)	**Spinraza™ (Antisense oligonucleotides)**	**Zolgensma® (Gene therapy)**
Target	SMN2 gene	SMN2 gene	SMN1 protein
MoA	Stabilize protein-RNA interaction	Transcription modifier	Transgene expression
API	Small Molecule	Nucleic acid	Nucleic acid
Specificity	Specific	Extremely specific	Extremely specific
Drug Product	Synthesis	Synthesis	Expression in living cells
Formulation	Small molecule + Excipients	ASO + Excipients	Plasmid DNA + AAV protein capsid
Identity	Defined substance	Defined substance	Quality differences due to PTMs
Dose	Defined quantities	Defined quantities	Variance in packing ratios
Application	Continuous-dosing (?)	Multiple-doses (?)	1 dose
Safety Profile	Molecule Specific + some class effect	Class effect	Gene therapy specific
PK (Elimination)	Days	Months	Probably permanent
RoA	Oral	Intrathecal	Intravenous

With respect to the PK, the three drugs lie on a continuum, with the small molecule Risdiplam being eliminated within days ($T_{1/2}$(Plasma)= 40.1-68.7h), the ASO Spinraza™

being eliminated from CSF after many months ($T_{1/2}$(CSF)=135-177 days) and the gene therapy Zolgensma® likely being permanent. An interesting topic associated with this is the price of the treatment or per dose, as controversially discussed in the health technology assessment (HTA) field. However, this is beyond the scope of this work. The RoA is similar for Risdiplam and Zolgensma® whereas the intrathecal application of Spinraza™ is an invasive procedure that introduces RoA related risks and impacts pre-clinical and clinical study design (e.g. controls).

3.2 Comparison of performed studies

3.2.1 Pre-clinical

From the pre-clinical perspective, i.e. what studies were performed, Risdiplam and Spinraza™ are very similar. When looking at the pre-clinical comparison table, the pattern of pre-clinically performed studies is nearly the same for Risdiplam and Spinraza™ whereas that for Zolgensma® is quite different and relatively sparse. This is the consequence of Risdiplam and Spinraza™ being both synthesized and well defined, and thereby historically regulated under the same guidelines. The individual pre-clinical studies performed for Risdiplam and Spinraza™ have well defined but relatively narrow objectives usually with clear guidance on the study requirements. However, it is nowadays common to combine multiple studies when possible, due to ethical (animal welfare) and effectiveness considerations. The approach for gene therapy pre-clinical development is a more flexible approach with fewer but more comprehensive required study types due to the inherent variability between different gene therapy products and their relatively recent application as a drug. The focus of pre-clinical gene therapy development are key objectives, like determining the right dose, investigating the biodistribution with respect to delivery and expression and the associated toxicities, which are then combined in the scientifically most effective way. Therefore, three major pre-clinical study types were performed for Zolgensma®: POC studies, biodistribution and single-dose toxicity.

As discussed above, the set of performed pre-clinical study for Risdiplam and Spinraza™ is similar, however, some differences with respect to study types and design exist. Secondary pharmacodynamics is not studied for Spinraza™ because of the high specificity of ONs in general.

When it comes to safety and toxicology, one of the major differences of Risdiplam versus Spinraza™ is the relative importance of class effects. Whereas for certain small molecule types class-effects are known, the safety profile of ONs is relatively independent of the actual sequence but rather depend on the class of ONs and the chemical modifications. This also means that the safety profile is rather predictable for the common/classical ON classes and new evidence can be generalized quickly. In the case of Spinraza™ being part of the well-studied ASO class with PS backbone, there are specific recommendations as discussed in the pre-clinical paragraph. These include cardiovascular investigations in cynomolgus monkey (complement activation) and using *in vivo* assessments instead of the hERG assay. Observed accumulation in liver and kidney as a class effect for systemically administered ASOs, expected negative genotoxicity and expected pro-inflammatory effects.

Local tolerance for Risdiplam, oral administration, is not required and was not performed, whereas for Spinraza™ (intrathecal administration) this was investigated with parenteral dose testing. Local tolerance studies are not recommended for parenterally administered drugs in the US and hence they were not performed for Zolgensma®.

Table 2: Pre-clinical studies comparison: Blue highlights pre-clinical studies that were conducted, grey those that were not conducted. For white fields, no information was found.

			Risdiplam (Small Molecule)	Spinraza™ (Antisense Oligo)	Zolgensma® (Gene therapy)
4.2 (2.4.2,2.6.2/3) Pharmacology	**4.2.1** Primary Pharmacodynamics				
	4.2.2 Secondary Pharmacodynamics				
	4.2.3 Safety Pharmacology				
	4.2.4 Pharmacodynamic Drug Interactions				
4.3 (2.4.3,2.6.4/5) Pharmacokinetics	**4.3.2** Absorption				
	4.3.3 Distribution				
	4.3.4 Metabolism				
	4.3.5 Excretion				
	4.3.6 PK drug interactions				
	4.3.7 Other PK				
4.4 (2.4.4,2.6.6/7) Toxicology	**4.4.1** Acute Toxicity ("Single-Dose")				
	4.4.2 Repeat-Dose				
	4.4.3 Genotoxicity	**4.4.3.1** *In vitro*			
		4.4.3.2 *In vivo*			
	4.4.4 Carcinogenicity	**4.4.4.1** Long-term			
		4.4.4.2 Short- or medium-term			
		4.4.4.3 Other studies			
	4.4.5 Reproductive and Developmental Toxicity	**4.4.5.1** Fertility and early embryonic development			
		4.4.5.2 Embryo-fetal development			
		4.4.5.3 Prenatal and postnatal development			
		4.4.5.4 Juvenile animals			
	4.4.6 Local Tolerance				
	4.4.7 Other toxicity	**4.4.7.1** Antigenicity			
		4.4.7.2 Immunotoxicity			
		4.4.7.3 Mechanistic studies			
		4.4.7.4 Dependence			
		4.4.7.5 Metabolites			
		4.4.7.6 Impurities			
		4.4.7.7 Other (e.g. phototoxicity)			

3.2.2 Clinical

For Risdiplam and its predecessor RG7800 several studies were performed. FiH RG7800 (Phase I, Healthy subjects), FiH Risdiplam (Phase I, Healthy subjects), Rainbowfish Risdiplam (Phase I, pre-symptomatic Type 1), moonfish RG7800 (Phase I, Type 1), Sunfish Risdiplam (Phase IIb/III, Type 2 or 3), Firefish Risdiplam (Phase III, Type 1), Jewelfish (Phase III, Type 2 or 3, already treated).

For Spinraza™ studies were performed, with patients continuing from one study to the next (see Picture 1). CS1 (Phase I, Type 2 or 3), CS2 (Phase I/II a, Type 2 or 3), CS10 (Phase I, Type 2 or 3), CS12 (Phase I, Type 2 or 3), CS3A (Phase II, Type 1), CS5/SM201 (Phase III, pre-symptomatic Type 1), CS11, CS3B (Phase III, Type 1), CS4 (Phase III, Type 2 or 3).

For Zolgensma® a FiH study START (Phase I, Type 1), the pivotal STR1VE study (Phase III, Type 1), the SPR1NT study (Phase III, Type 1 pre-symptomatic) were performed and the STRONG study (Phase II/III, Type 2) is ongoing.

Risdiplam and Zolgensma® (except STRONG study) were administered orally or IV, whereas Spinraza™ was administered intrathecal which needed specifically trained personal and introduced a source of AE. For Risdiplam one study (Sunfish) was placebo controlled, Spinraza™ had the pivotal studies sham-procedure controlled, whereas Zolgensma® only used comparison between doses and to natural history.

Risdiplam FiH studies were performed in healthy subjects, while Spinraza™ and Zolgensma® FiH were directly performed in patients due to the invasive intrathecal procedure and the permanent effect of gene therapy respectively.

3.3 Comparison of Efficacy and Safety

3.3.1 Efficacy

In order to compare efficacy of Risdiplam, Spinraza™ and Zolgensma® available published data for primary and secondary endpoints were sorted by SMA type and screened for overlap. For type 1 pre-symptomatic there was insufficient overlap to allow a comparison. The timing of the endpoints was not identical in many cases, which is considered in the efficacy comparison.

For Type 1 SMA patients the 5 following endpoints were identified that allowed at least pairwise comparison. 1. the percentage of infants who achieve an increase of ≥4 points in the CHOP-INTEND, 2. the proportion of infants who achieve a score of ≥40 in the CHOP-INTEND, 3. Proportion of patients sitting without support, 4. Percentage of motor milestone responders (HINE), 5. time to death or permanent ventilation. The first endpoint was shared between Risdiplam and Spinraza™. 90% of Risdiplam type 1 SMA patients reached a CHOP-INTEND increase of ≥4 points at 12 months (Firefish) while only 55% (CS3A) and 65% (CS3B) of Spinraza™ type 1 SMA patients reached this endpoint at 24 months (median duration) and 6 months treatment respectively. Although, this endpoint is not specifically mentioned for Zolgensma®, endpoint 2 suggests that a proportion of

patients similar to Risdiplam reached this endpoint. The bigger increase at an earlier time point for Risdiplam (and potentially Zolgensma®) indicates that this endpoint is in favor of Risdiplam (and potentially Zolgensma®). CS3B study might still show a bigger improvement at 12 months once data is available. The second endpoint was shared between Risdiplam and Zolgensma®. At 12 months of treatment 56% of Risdiplam patients (Firefish) met this endpoint ≥40 in the CHOP-INTEND score whereas 95% of Zolgensma® patients (STR1VE-US) met this endpoint. Assuming a similar baseline, this endpoint is in favor of Zolgensma®. The third endpoint is also shared between Risdiplam and Zolgensma®, favoring Zolgensma® with 75% (START, high dose) and 59% (STR1VE-US) of Zolgensma® patients and 29% of Risdiplam patients (Firefish) achieving this milestone after 12 months of treatment. Endpoint 4 is shared between Risdiplam and Spinraza™. 78% of Risdiplam patients at 12 months compared to 65% (CS3A) and 51% (CS3B) of Spinraza™ patients at 23 months and 6 months of treatment were motor milestone responders (HINE). This endpoint is therefore also in favor of Risdiplam. The 5th endpoint is shared between all three treatments. The survival rate is 93% for Risdiplam (Firefish) at 12 months, 75% (CS3A) at 24 months and 85% at 6 months for Zolgensma® and 100% (START) at 24 months and 81% (STR1VE-US) at 12 months of treatment. This endpoint is therefore slightly in favor of Risdiplam over Zolgensma®, followed by Spinraza™.

For Type 2 or 3 SMA patients 2 endpoints were identified that allowed at least pairwise comparison but note that for Zolgensma® the administration was intrathecal as opposed to oral as in type 1 SMA studies. Endpoints: 1. the change from baseline in HFMSE total score, 2. the proportion of subjects with at least 3 points increase in HFMSE score. The first endpoint could be compared between all three treatments. The HFMSE increase was 1 point for Risdiplam (Sunfish) at 12 months of treatment, 4 points for Spinraza™ (CS4) at 15 months of treatments and 6 points for Zolgensma® (STRONG) at 12 months. This endpoint favors Zolgensma® over Spinraza™, while for Risdiplam this endpoint was not significantly different from placebo group. Endpoint 2 is shared between Spinraza™ and Zolgensma® with the 57% of Spinraza™ patients (CS4) reaching the endpoint at 15 months as compare to 92% (≥2 years and <5 years) and 83% (≥6 months to <2 years) with Zolgensma®. This endpoint therefore favors Zolgensma®.

Table 3: Endpoint comparison for type 1 and type 2/3 SMA

	Type 1 SMA					Type 2 or 3 SMA	
Endpoints	**Infants who achieve an increase of ≥4 points in the CHOP-INTEND**	**Infants who achieve a score of ≥40 in the CHOP-INTEND**	**Infants sitting without support**	**Motor milestone responders (HINE)**	**Survival rate**	**Change from baseline in HFMSE total score**	**Subjects who achieve ≥ 3-point HFMSE score increase from baseline**
Risdiplam	**90%** (37/41) ≥4 points in CHOP-INTEND total score (median +20 points) at **12 months** of treatment (Firefish)	**56%** (23/41) score ≥40 at **12 months** (Firefish)	**29%** (12/41) **≥5sec** at **12 months** (Firefish)	HINE-2: **78%** (32/41) responded at **12 months** (Firefish)	**93%** (38/41) alive at **12 months** (Firefish)	0.58 difference in change (ca. **1 point** vs 0.4) from HFMSE baseline vs placebo (p=0.3) at **12 months** (Sunfish)	*No publicly available data*
Spinraza	**55%** (11/20) at **24 months** median treatment (CS3A) **65%** ≥ 4 points improvement **(≥6 months** treatment) (CS3B)	*No publicly available data*	*No publicly available data*	**65%** (13/20) of patients at **24 months** (CS3A) **51%** of patients reach a motor milestone at the end of the study **(≥6 months** treatment) (CS3B)	Survival rate **75%** (15/20) at **24 months** (CS3A) Survival rate **85%** (68/80) **(≥6 months** treatment) (CS3B)	Improved score of **4 points** in Spinraza group at **15 months** (CS4)	Spinraza **57.3%** at **15 months** (CS4)
Zolgensma	*No publicly available data*	**95%** (21/22) at **≥12 month of treatment** (STR1VE-US)	**0%** (0/3) of low dose patients and **75%** (9/12) high dose **≥30sec** at **24 months treatment** (START) **59%** (13/22) **≥30 seconds** at **≥12 month of treatment** (STR1VE-US)	*No publicly available data*	Survival **67%** (2/3) (low dose) vs. **100%** (12/12) (high dose) at **24 months** (START). **68% (**15/22) **anytime** and **81%** (18/22) **≥12 month of treatment** (STR1VE-US)	Dose B (≥2 years and <5 years): **6.0 points** mean increase at **12 months** (STRONG)	Dose B (≥2 years and <5 years): **92%** (11/12); Dose B (≥6 months to <2 years): **83%** (5/6) at **12 months** (STRONG).

3.3.2 Safety

Some general observations for the three treatments are compared first and then the safety of the three treatments is compared according to affected organ system (see Table 4).

For Risdiplam no deaths, no moderate or severe AE and no SAEs were observed in FiH study with healthy subjects and no safety signals were identified in the pivotal phase III study (Firefish/NCT02913482).

For Spinraza™, possibly treatment related AEs were observed but in general the ratio of AEs in treated vs. control patients is favorable for Spinraza™. In CS3B, the safety comparison of treated versus control patients showed AE in 96% vs 98% patients with severe or moderate event in 88% vs 95%, severe events in 56% vs 80% and SAE in 76% vs 95% of patients. The safety comparison in CS4 of Spinraza™ treated versus control patients showed AEs in 93% vs 100% of patients with severe or moderate AE in 42% vs 48%, severe AE in 5% vs 7% and SAE in 14 vs 26% of patients. All SAEs were considered non-treatment related by the investigators and no SUSARs have been reported during the clinical trial program of Spinraza™.

For Zolgensma®, several AE were observed that were possibly or likely treatment related and classified as adverse reactions (ARs). The details are discussed for the different organ systems.

Cardiovascular

For Risdiplam, no individual clinically significant QT changes were observed (FiH). Spinraza™ trials (CS11 and SM201/CS5) reported an AE of tachycardia in one subject which investigators considered “possibly related” to the treatment. For Zolgensma®, a transient increase in cardiac troponin-I levels was observed as one of the most frequent adverse reactions (incidence >5%).

CNS

For Risdiplam, no clinically significant changes in ophthalmological assessments were observed, but the AE of headache was observed in 20% and vomiting in 14.2% of cases. For Spinraza™, due to pre-clinical findings of hippocampal vacuoles a special monitoring and review of AEs suggestive of epilepsy was performed, however no epilepsy was reported. AE “possibly related” to treatment by investigators included nausea (CS4) and hyperreflexia in one subject (CS11 and SM201/CS5). In the Zolgensma® clinical trials one death after seizures due to leukoencephalopathy was observed and a connection to the treatment could not be excluded. Additionally, vomiting was part of the most frequent adverse reactions (incidence >5%).

Respiratory System

For Risdiplam several AE of the respiratory system were reported. Respiratory tract infections appeared in 31.7%, nasopharyngitis in 25.8% and cough in 14.2% of treated patients. Additionally, serious lower respiratory tract infections were observed in 10% of treated vs only 2% controls. However, they were classified as unrelated to the treatment. For Spinraza™ no effects on respiratory rate, tidal volume, and minute volume (pulmonary

functions) were observed. For Zolgensma®, one death occurred due to respiratory failure. This was considered secondary to disease progression.

Kidney & Liver

No kidney or liver specific safety observations were made for Risdiplam. Spinraza™ studies (CS11 and SM201/CS5) showed elevated ALT & AST levels, a potential marker for liver damage and this was considered “possibly related” to the treatment. Three serious adverse reactions were observed for Zolgensma®, including an increase of aminotransferase (up to 40xULN) in two other patients and acute serious liver damage (1 patient). Most frequent adverse reactions (incidence >5%): were elevated aminotransferases. In conclusion, major serious risks for acute liver injury and a substantial increase in aminotransferases were associated with Zolgensma® treatment.

Immune system

For Risdiplam, only pyrexia was reported in 20.8% as AE. For Spinraza™ no sign of cellular activation and cytokine production because of pro-inflammatory effects in clinical studies have been observed. However, in CS11 and SM201/CS5, pyrexia was reported as “possibly related” to the treatment, and an increased eosinophil and lymphocyte count was observed in one subject. Because the viral origin of the Zolgensma® vehicle, a huge potential for immunogenic/neutralization responses directed against vector was predicted. Indeed, an increase in anti-AAV9 antibody titers from below 1:50 to more then 1:800,000 in most patients was observed which is expected to preclude the possibility of re-administration. Additionally, a transient decrease in platelet counts was observed as one of the most frequent adverse reactions (incidence >5%).

Other organs

For Risdiplam, abdominal pain (25%) and diarrhea (16.7%) were observed as additional AEs and for Spinraza™ muscular weakness and weight bearing difficulty in one subject (CS11 and SM201/CS5) was considered “possibly related” to the treatment.

Table 4: Comparison of safety findings

	Risdiplam	Spinraza™	Zolgensma®
General	No safety signals in healthy subjects and Type 1 SMA patients	CS3B Treated vs- untreated: AE 96% vs 98%; severe or moderate 88% vs 95%, severe 56% vs 80%, SAE 76% vs 95%. CS4 Treated vs- untreated: AEs 93% vs 100%; severe or moderate 42% vs 48%, severe 5% vs 7%, SAE 14% vs 26%. SAEs considered non-treatment related No SUSARs in clinical trials.	
Cardiovascular	No clinically significant QT changes in healthy subjects (NCT02633709)	No effects on cardiovascular parameters; CS11 and SM201/CS5: tachycardia in one subject considered "possibly treatment related"	Transient increase in cardiac troponin-I levels (AR incidence >5%)
CNS	No clinically significant changes in ophthalmological assessments; headache (AE incidence 20%); vomiting (AE incidence 14.2%)	Special monitoring and review of AEs suggestive of epilepsy: no epilepsy was reported; CS4: AE procedural nausea "possibly treatment related"; CS11 and SM201/CS5: AE hyperreflexia, (one subject) "possibly treatment related"	One death after seizures due to leukoencephalopathy ("possibly treatment related"); Vomiting (AR incidence >5%)
Respiratory	Respiratory tract infection (AE incidence 31.7%), nasopharyngitis (AE incidence 25.8%), cough (AE incidence 14.2%); serious lower respiratory tract infections (10% treated vs 2% Ctrl)("reported unrelated")	No effects on pulmonary function (respiratory rate, tidal volume, minute volume)	One death due to respiratory failure secondary to disease progression
Kidney & Liver		CS11 and SM201/CS5: AE elevated aminotransferases ALT & AST (liver damage) "possibly treatment related"	3 SARs: acute serious liver injury (n=1) and elevation of aminotransferase (up to 40xULN) (n=2); elevated aminotransferases (AE incidence >5%) → major serious risks: acute liver injury and substantial increase in aminotransferases
Immune system	Pyrexia (AE incidence 20.8%)	No cellular activation and cytokine production due to pro-inflammatory effects; CS11 and SM201/CS5: AE pyrexia, increased eosinophil and lymphocyte count (n=1) "possibly treatment related"	Increase in anti-AAV9 antibody titers from <1:50 to >1:800,000 → expected to preclude possibility of re-administration; transient decreases in platelet counts (AR incidence >5%)
Other organs	Abdominal pain (three subjects), diarrhea (AE incidence 16.7%)	CS11 and SM201/CS5: AE muscular weakness and weight bearing difficulty (n=1) "possibly treatment related"	

Conclusion & Outlook

The existence of three different drug types, small molecule (Risdiplam), antisense oligonucleotide (Spinraza™) and gene therapy (Zolgensma®) for the treatment of same disease, Spinal muscular atrophy (SMA), provided a unique opportunity to analyze differences in pre-clinical and clinical development and compare their safety and efficacy profiles.

The similarity of Risdiplam and Spinraza™ pre-clinical development programs is driven by their regulation under the same (small molecule) guidelines, which is a consequence of their well-defined and -characterized physicochemical properties (Kornbrust et al., 2013). This includes typical studies like pharmacodynamic, pharmacokinetics, safety pharmacology and repeat-dose. In contrast, the inherent variability in its production and the permanence of gene therapy imposed a much more flexible and objective-driven approach for Zolgensma® with three major pre-clinical study types: the POC study, biodistribution and single-dose toxicity.

Risdiplam pre-clinical side effects are due to off-target effects, which cannot be 100% eliminated in small molecules. Although optimization of RG7800, which eventually led to the discovery of Risdiplam, eliminated several side effects, the toxicity due to secondary splicing targets involved in cell cycle regulation, like FOMX1, could not be eliminated and was responsible for the main toxicity findings (fully reversible effects on rapidly dividing cells, like male germ cells). In contrast, the nature of oligonucleotide hybridization makes Spinraza™ a molecule with no side-effects due to secondary pharmacodynamics. For the ON Spinraza™ class-effects are of much higher importance as compared to small molecules and dominate its safety and toxicology profile. Because Spinraza™ belongs to the PS class of ONs with extensive pre-clinical and clinical experience, accumulation in kidney and associated toxicities can be expected. The intrathecal RoA reduced this accumulation to subtoxic concentrations (Henry, 2008) but was likely responsible for the observed changes to the lower spinal reflex. For Zolgensma® cardiac and liver toxicities were observed.

First clinical data for orally administered Risdiplam was generated in healthy subjects, while for Spinraza™ surgery required for intrathecal administration precluded this. The permanent gene expression made studies of Zolgensma® in healthy subjects not acceptable. The pivotal study for Zolgensma® was only compared to natural history data, while the pivotal Risdiplam study was placebo controlled and the pivotal Spinraza™ study used a sham-procedure in the control group. In particular for Spinraza™ the control group was important to verify procedure-introduced adverse events.

Overall, Risdiplam showed the most favorable clinical safety profile with no treatment related SAEs and most AEs related to the disease. Spinraza™ showed relative few treatment related AEs and most of them only in single individuals. Only Zolgensma® showed clear treatment related AEs including acute serious liver injury, which is in line with pre-clinical findings, and one possibly related death after seizures due to leukoencephalopathy. Additionally, an increase in anti-AAV9 antibodies of several orders of magnitude was observed upon Zolgensma® administration, which is expected to

interfere with repeated administration. Zolgensma® is currently investigated in a phase III using intrathecal administration. This might positively affect liver toxicity but bears the risks of introducing procedure-related AEs.

Efficacy of Risdiplam, Spinraza™ and Zolgensma® was compared according to shared endpoints (primary and secondary) for SMA type 1 and SMA type 2 or 3. Published data was not sufficient to compare treatments for pre-symptomatic SMA type 1. Overall, most efficacy endpoints favor Zolgensma® (2 out of 3= 67%) followed by Risdiplam (3 out of 5 = 60%) and Spinraza™ (0/3= 0%) for treatment of type 1 SMA patients, as assessed by 5 common endpoints. For the treatment of type 2 SMA patients most efficacy endpoints favor Zolgensma® (2 out of 2= 100%) followed by Spinraza™ (0 out of 2 but 1 out of 1 versus Risdiplam) and then Risdiplam, as assessed by 2 shared endpoints. Because in the type 2 and 3 SMA treatment comparison several significant endpoints (including primary endpoints) did not overlap, the outcome of the analysis is somewhat limited.

Taking clinical safety and efficacy data together, even though Zolgensma® wins the efficacy comparison for type 1,2 and 3 SMA, the safety profile favors Risdiplam and Spinraza™. Therefore, the benefit-risk ratio is likely in favor for Risdiplam for treatment of type 1 SMA patients and similar for Zolgensma® and Spinraza™ for treatment of type 2 or 3 SMA patients.

Once more clinical data on Risdiplam becomes publicly available, a better comparison of endpoints will be possible, including pre-symptomatic type 1 SMA patients and a more comprehensive comparison for type 2 or 3 SMA patients. It will be interesting if this shifts the comparison in favor of Risdiplam, because here none of its significant endpoints could be used. Furthermore, it will be interesting if the change in RoA for Zolgensma® from IV to intrathecal will positively affect its benefit-risk ratio.

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Abbreviations

2’-MOE – 2’ O-methoxyethyl
3T3 NRU – Neutral Red Uptake
AAV – adeno-associated virus
ADME – absorption, distribution, metabolism, and excretion
AE – adverse event
AFSSAPS – Agence française de sécurité sanitaire des produits de santé
ALT – Alanine aminotransferase
API – Active pharmaceutical ingredient
AR – adverse reaction
AST – Aspartate transaminase
ASO – antisense oligonucleotides
ATMP - advanced therapy medicinal products
AUC – area under the curve
AV – adeno virus
BBB – blood-brain barrier
BCRP – breast cancer resistance protein
BLA – Biological license application
BSEP – Bile Salt Export Pump
BSID – Bayley Scales of Infant and Toddler Development
CAG – chicken-β-actin hybrid
CHMP – Committee for Medicinal Products for Human Use
CHO – Chinese hamster ovarian
CHOP-INTEND – Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CMAP – compound muscle action potential
Cmax – maximal concentration
CMC – chemistry, manufacturing, and control
CMV – cytomegalovirus
CNS – central nervous system
CP – Centralized procedure
CSF – cerebrospinal fluid
CTD – common technical document
CTRL – control
CYP450 – Cytochrome P450
DDI – drug-drug-interactions
DNA – deoxyribonucleic acid
DSP – downstream processing
ELISA – enzyme-linked immunosorbent assay
EMA – European Medicines Agency
ERA – Environmental risk assessment
EU – European Union
EP – exaggerated (on-target) pharmacology
EPAR – European Public Assessment Report

FDA – Food and Drug Administration
FiH – first-in-human
FMO – flavin-containing monooxygenase
FOB – Functional Observational Battery
FOXM1 – Forkhead Box M1
FVB mouse – Friend leukemia virus B mouse
GCP – Good clinical practice
GI – gastrointestinal tract
GTMP – gene therapy medicinal product
h – hours
HED – Human equivalent dose
HFMSE – Hammersmith Functional Motor Scale Expanded
hERG – human Ether-a-go-go Related Gene
HINE – Hammersmith Infant Neuromuscular Examination
HTA – health technology assessment
IC50 – half maximal inhibitory concentration
ICH – International Council for Harmonisation
IND – Investigational new drug
IP injection – intraperitoneal (body cavity)
iPSCs – Induced pluripotent stem cell
ISS-N1 – intronic splicing silencer N1
IV injection – intravenous
JAR – Joint Assessment Report
kg – kilogram
Kpuu – Unbound partition coefficient
LooI – List of outstanding issues
LoQ – List of Questions
LT – long term
MAA – marketing authorization application
MDR1 – multidrug resistance protein 1
MFM-32 – Motor Function Measure 32
mg – milligram
MoA – Mode of Action (functional changes at cellular level)
MOA – Mechanism of action (biochemical interaction with molecular target)
MRI – Magnetic resonance imaging
mRNA – messenger ribonucleic acid
MRSD – maximum recommended starting dose
mV – milli volt
NHP – non-human primates
NOAEL – no-adverse-effect-levels
NOEL – No Observed Effect Level
OAT – Organic anion transporter
OATP – organic anion transporting polypeptides
OCT – Organic cation transporter
ON – oligonucleotide
OSWG – oligonucleotide safety working group

p – p-value
PEG – Polyethylene glycol
PD – Pharmacodynamic
PDE – Permitted Daily Exposure
PDUFA – Prescription Drug User Fee Act
P-gp – permeability glycoprotein
PIP – paediatric investigation plan
PK – Pharmacokinetic
PND – post-natal day
PRAC – Pharmacovigilance Risk Assessment Committee
PS – phosphorothioate
PTM – post-translational modification
POC – proof-of-concept
qPCR – quantitative polymerase chain reaction
QTC – corrected QT interval
QWBA – quantitative whole-body autoradiography
RNA – ribonucleic acid
RNP – ribonucleoprotein
RoA – route of administration
RULM – Revised Upper Limb Module
ROS – reactive oxygen species
SAE – serious adverse event
SC injection – subcutaneous injection
SMA – Spinal muscular atrophy
SMAIS – SMA Independence Scale
SMN – survival motor neuron
SmPC – summary of product characteristics
snRNP – small nuclear ribonucleoprotein
ss – splicing site
SUSAR – Suspected Unexpected Serious Adverse Reaction
$T_{1/2}$ – half-life
T_m – melting temperature
T_{max} – time of maximal concentration
ULM – Upper Limb Module
ULN – Upper Limit of Normal
µM – micro mol
US – United States
Vd – Volume of distribution: Vd=drug amount/concentration
Vg – viral genomes/vector genomes
V_{ss} – Vd at steady state
WHO – world health organization

Annex
Regulatory Milestones

Table M1: Regulatory Milestones Risdiplam (Roche, 2019, Therapeutics, 2017)

Date (dd/mm/yyyy)	**Milestones**
06/01/2017	**Orphan Drug Designation by FDA**
??/04/2017	Fast Track Designation by FDA
25/11/2019	**Priority Review by FDA**
24/05/2020	**Expected FDA approval decision**

Table M2: Regulatory Milestones Spinraza™, EMA (EMA/289068/2017, 2017)

Date (dd/mm/yyyy)	Milestones
02/04/2012	Orphan designation granted (5q SMA)
13/12/2012	Protocol Assistance from CHMP
15/09/2016	Accelerated Assessment granted
07/10/2016	Marketing authorization application through centralized procedure
27/10/2016	Start of CP
23/12/2016	Co-Rapporteurs first Assessment Report
26/12/2016	Rapporteurs first Assessment Report to CHMP
04/01/2017	PRAC Rapporteurs first Assessment Report to CHMP
12/01/2017	PRAC Assessment sent to applicant
24/01/2017	CHMP List of Questions (LoQ) sent to applicant
16/02/2017	Applicant response to CHMP LoQ
10/03/2017	Joint Assessment Report (JAR) to CHMP
22/03/2017	Oral explanation of applicant before CHMP
23/03/2017	List of outstanding issues (LooI) by CHMP
29/03/2017	Applicant response to LooI
07/04/2017	JAR on applicant's response to LooI
21/04/2017	Positive Opinion of CHMP
25/04/2017	Orphan designation confirmed for MA
11/04/2018	PIP Deferral

Table M3: Regulatory Milestones Zolgensma®, FDA (Byrnes, 2019)

Date (dd/mm/yyyy)	Milestones
20/12/2011	PreIND meeting
08/08/2013	IND submission
27/09/2013	Fast Track designation granted
30/09/2014	**Orphan Drug designation granted**
15/07/2016	Breakthrough Therapy designation granted
14/06/2018	Pre-BLA meeting
21/08/2018	Rare Pediatric Disease designation granted
01/10/2018	BLA 125694 submission
28/11/2018	**BLA filed, priority review**
06/02/2019	120-day safety and efficacy update received
30/04/2019	Additional efficacy and safety update for the ongoing Phase 3 trial received
24/05/2019	**Approval Letter**
01/06/2019	PDUFA* Action Due Date

Pre-clinical tables

Numbering according to ICH Module 4 (Module 2)(Bode, 2019, M4E(R2), 2016)

Table P1: Pre-clinical Studies – Compound 2 (RG7800, RO6885247) & Compound 1 Risdiplam (RG7916, RO7034067) Based on (Poirier et al., 2018, Ratni et al., 2018)

<table>
<tr><td rowspan="4">4.2 (2.4.2,2.6.2/3)
Pharmacology</td><td>4.2.1 Primary Pharmacodynamics</td><td>In vitro PD (RISDIPLAM): Active in patient-derived iPSCs (SMA type 1): promoting inclusion of exon 7, generate full length mRNA and increase SMN protein levels. IC50, Delta A2 of 113nM and 155nM in human and cynomolgus monkey iPSCs. Half-life 5-6 hours.
In vivo PD (RISDIPLAM): Evaluated in C/C-Allele mice with mild SMA phenotype: 10 days, once a day orally, 3 different doses (1,3,10 mg/kg). Evaluated in delta 7 mice with severe SMA: intraperitoneal (IP), once daily, postnatal day 3-9. Protein levels assessed in brain and quadriceps: max increase at 1mg/kg (free $AUC_{0\text{-}24h}$: 73ng.h/ml). All delta 7 ctrl animals died before PND21 (Median survival time: 10.5 days) in contrast to only two. Prolongation of lifespan at all doses (lowest dose MST 26days) and decrease in body weight loss. Neuromuscular pathology: delta 7, PND3-14, ip 0.1,0.3,1 mg/kg/day. Dose dependent decrease of vGlu1 input loss, motor neuron loss, increase of fully innervated neuromuscular junctions, muscle size compared to vehicle
(RG7800): lower increase of protein level at 3mg/kg: $AUC_{0\text{-}24h}$:118ng.h/ml</td></tr>
<tr><td>4.2.2 Secondary Pharmacodynamics</td><td>In vitro splicing assay (RG7800): In patient-derived cells; Affected genes in cell cycle regulation and cell death signaling: a.o. FOXM1 (cell cycle regulator, high expression in rapidly dividing cells) => explains in vitro & in vivo genotoxicity observations, explain reversibility of germ cell effects.
In vitro splicing assay (RISDIPLAM): Improved specificity and potency on SMN2</td></tr>
<tr><td>4.2.3 Safety Pharmacology</td><td>hERG assay (RG7800): IC50 1,8uM, IC20 0,5uM at 37oC.
Telemetry in cynomolgus monkey(RG7800): Mild QTC interval prolongation (max 10 ms), exposure reached IC20.
(RISDIPLAM): hERG channel at 37oC: no effect on hERG K+ current (IC20>5uM).
Telemetry in cynomolgus monkey (RISDIPLAM): Absence of QTC interval prolongation CNS: No observation in relevant animal tests (F. Hoffmann La Roche, data on file)</td></tr>
<tr><td>4.2.4 Pharmacodynamic Drug Interactions</td><td>(RISDIPLAM): mainly metabolized by flavin-containing monooxygenase (FMO), FMOs are not induced or inhibited by chemicals. →DDI are not readily expected.</td></tr>
<tr><td rowspan="4">4.3 (2.4.3,2.6.4/5)
Pharmacokinetics</td><td>4.3.2 Absorption</td><td rowspan="4">In vivo PK (RG7800): long half live and larger Vss in rats and cynomolgus monkeys after oral dosing.
In vivo PK (RISDIPLAM): Favorable DMPK in rat and cynomolgus monkey after i.v. and oral administration.

<table><tr><td></td><td colspan="4">rat</td><td colspan="4">Cynomolgus monkey</td></tr><tr><td></td><td>Cl[a] (mL min^{-1} kg^{-1})</td><td>V_{ss}[a] (L/kg)</td><td>$T_{1/2}$[b](h)</td><td>F[b](%)</td><td>Cl[a] (mL min^{-1} kg^{-1})</td><td>V_{ss}[a] (L/kg)</td><td>$T_{1/2}$[b](h)</td><td>F[b](%)</td></tr><tr><td>Risdiplam</td><td>8.9</td><td>3.1</td><td>6.4</td><td>~100</td><td>5.7</td><td>2.0</td><td>5.4</td><td>43</td></tr><tr><td>RG7800</td><td>25</td><td>29</td><td>19</td><td>~100</td><td>5</td><td>20</td><td>42</td><td>52</td></tr></table>Table 3 from (Ratni et al., 2018): Risdiplam: (a) iv, 1.9 mg/kg; (b) po, 5.5 mg/kg; (c) iv, 0.1 mg/kg; (d) po, 0.5 mg/kg. RG7800: (a) iv, 2 mg/kg; (b) po, 5 mg/kg; (c) iv, 0.3 mg/kg; (d) po, 1.3 mg/kg.

PK Studies 1-13 (Poirier et al., 2018) (RISDIPLAM, RG7800): Mouse, Rat, Cynomolgus Monkey.
RISDIPLAM and RG7800 have high passive permeability →beneficial for GI and tissue uptake and freely distribute from blood into CNS and multiple tissues.

RISDIPLAM distribution and elimination in plasma correlates with brain, CSF, muscle other tissues in mice, rats and monkey. Rat and monkey quantitative whole-body autoradiography (QWBA) confirmed wide tissue distribution and elimination (bone, mucosa, GI tract, pancreas, liver, lung, heart, kidney, spleen) in parallel to plasma. Full elimination from brain, CSF and plasma after several weeks post-dose.</td></tr>
<tr><td>4.3.3 Distribution</td></tr>
<tr><td>4.3.4 Metabolism</td></tr>
<tr><td>4.3.5 Excretion</td></tr>
</table>

			RISDIPLAM and **RG7800** are no MDR1 substrate in humans →beneficial for brain distribution. *In vivo* observed Kpuu for Mdr1a in rodents was 0.28 → major contributor to lower CSF levels. But did not limit SMN protein increase in brain. **RISDIPLAM** strong rodent Bcrp substrate *in vitro* was not apparent *in vivo*. **RISDIPLAM** is only a weak human Bcrp substrate. Monkey CSF **RISDIPLAM** levels reflected free plasma levels indicating no relevance of drug efflux transporters in humans (>95% homology of BCRP and MDR1). Similar penetration in monkey brain stem and cortex. SMN protein levels increased dose-proportionally in brain of SMNdelta7 and C/C-allele mice after **RISDIPLAM** and **RG7800** treatment. Protein levels stay elevated while drug is administered →no attenuation ***In vivo* metabolization (RG7800):** N-dealkylated metabolite in all species tested (human, rat, dog, minipig, cynomolgus monkey, mouse, rabbit), up to 9% plasma concentration of RG7800 in rodents and monkey; accumulates in muscle not in brain (mouse) => only off target effect!
	4.3.6 PK drug interactions		
	4.3.7 Other PK		
4.4 (2.4.4,2.6.6/7) Toxicology	**4.4.1** Acute Toxicity ("Single-Dose")		Integrated into other toxicity studies (repeat-dose)
	4.4.2 Repeat-Dose		***In vivo* toxicity (RG7800):** Epithelial vaculation and foamy macrophages in rat => suggests phospholipidosis (several tissues) <= large volume of distribution (Vss=29 L/kg in rat). ***In vivo* nonclinical safety studies (RISDIPLAM):** Chronically daily oral dose up to 26 weeks in rats and up to 39 weeks in cynomolgus monkeys: No evidence of phospholipidosis **Repeat-dose toxicity (RISDIPLAM):** Rats & cynomologus monkeys: no SMN2-> effects are off-target (unrelated to pre-mRNA splicing) or secondary targets (interaction with splicing machinery in general). Secondary target effects apparent soon after study start, no change in severity with chronic dosing. Mainly in organs with rapidly dividing cells. Associated with effects on genes like FOXM1 or MADD (cell cycle or apoptosis). Findings were reversible or partially reversible: - Micronucleation (rat bone marrow erythroblasts) & decreased cellularity in bone marrow - Histopathological changes in gastrointestinal tract epithelia, lamina propria, exocrine pancreas epithelia in mice, rats and/or cynomolgus monkeys - Parakeratosis/hyperplasia/degeneration of ski, tongue larynx epithelia - Degeneration of germ cells in testis of cynomolgus monkeys and rats Clear no observed adverse effect levels (NOAELs) for all findings. Transient effect on splice variants were reversible upon cessation of treatment. Adverse effects observed only significantly above predicted exposure desired for treatment of SMA patients (full PD effect on SMN2 target). => allowed healthy volunteer and SMA patients studies. Secondary targets identified in patient cells, iPSCs (human, monkey), rat tissue, cynomolgus **LT chronic toxicity (RG7800):** 39 weeks, In parallel to FiH (RG7800). Non-reversible histological findings in retina at considerably higher concentrations then human. **Chronic dosing studies (RISDIPLAM) & (RG7800)**: Chronic daily treatment cynomolgus monkeys: Retinal degeneration at end of oral dosing for 39 weeks for both compounds => class effect of SMN2 splice modifiers? => NOEL (no observed effect level) were established. Retinal degeneration not observed in rats (pigmented and albino) although accumulation in melanin-containing retinal tissue was similar to monkeys => suggests monkey specific effect (human relevance cannot be excluded) and toxicity not due to melanin binding.
	4.4.3 Genotoxicity	**4.4.3.1** *in vitro*	**(RG7800):** Negative bacterial reverse mutation assay **(RISDIPLAM):** no induction of gamma H2AX (dsDNA break marker) in L5178Y mouse lymphoma cells or TK6 human lymphoblastoid cells. Neither cell cycle perturbations, phosphorylation of histone H3 or polyploidy were observed.
		4.4.3.2 *In vivo*	**(RG7800):** increased frequency of micronucleated rat cells **(RISDIPLAM): Acute treatment** of rats 3 days (up to 25mg/kg/day) in a combined MN/comet study did not induce primary DNA damage (comet assay in liver) but dose dependent increase of MN erythrocytes was observed (MoA unknown, no direct DNA-

			reactivity). => studies with young and adult rats used to derive Cmax and AUC0-24h that would not induce erythrocytes' MN.
	4.4.4 Carcinogenicity	**4.4.4.1** Long-term	Reasons for studies: Ambiguous genotoxicity tests. Reasons against studies: no direct DNA interaction, low life-expectancy of patients?
		4.4.4.2 Short- or medium-term	
		4.4.4.3 Other studies	
	4.4.5 Reproductive and Developmental Toxicity	**4.4.5.1** Fertility and early embryonic development	***In vivo* germ cell toxicity (RG7800):** Rats & cynomolgus monkeys; Observed changes in male germ cell after at least 7 once-daily doses, monkeys: testes in male, decrease sperm count, increased abnormal sperms and reduced testes, full reversable after treatment stop. ***In vivo* germ cell toxicity (RISDIPLAM):** Rats & cynomolgus monkeys; Similar as for RG7800
		4.4.5.2 Embryo-fetal development	
		4.4.5.3 Prenatal and postnatal development	
		4.4.5.4 Juvenile animals	13-week study in young rats **(RG7800)** PK, PD studies in rats and mice (**Risdiplam**)
	4.4.6 Local Tolerance		Oral administration → not required → not conducted
	4.4.7 Other toxicity	**4.4.7.1** Antigenicity	
		4.4.7.2 Immunotoxicity	No related observations in in vitro or animal studies (F. Hoffmann La Roche, data on file)
		4.4.7.3 Mechanistic studies	
		4.4.7.4 Dependence	
		4.4.7.5 Metabolites	***In vivo* & *in vitro* metabolization (RG7800):** N-dealkylated metabolite in all species tested (human,rat,dog,minipig,cynomolgus monkey, mouse, rabbit), up to 9% plasma concentration of RG7800 in rodents and monkey, 10-fold more potent then RG7800 in SMN2 & FOXM1 splicing assays & in human and monkey iPSCs, strong P-gp substrate ***in vivo* metabolization (RG7800):** accumulates in muscle not in brain (mouse) => only off target effect! ***In vitro* metabolization (RISDIPLAM):** Lipophilicity 2.5 (by design) => no human P-gp ***In vitro* metabolization (RISDIPLAM):** N-hydroxylated derivative main metabolite in human liver microsomes and human hepatocytes (3-8% and 1.7% respectively). Inactive with respect for FOXM1 and SMN2 splicing. Also no effect on splicing in human and cynomolgus monkey iPSCs.
		4.4.7.6 Impurities	
		4.4.7.7 Other	***In vitro* phototoxicity (RG7800):** IC50 330nM (vs >10uM in dark) in 3T3 NRU Rats **phototox (RG7800):** confirmed *in vitro* phototox ***In vitro* phototoxicity (RISDIPLAM):** 3T3 neutral red uptake: no effect at highest (solubility limited) concentration: 9uM (ca. 3600ng/ml)

Table P2: Pre-clinical Studies – Spinraza™® (Nusinersen), Based on (EMA/289068/2017, 2017)

4.2 (2.4.2,2.6.2/3) Pharmacology	**4.2.1** Primary Pharmacodynamics	Multiple modified mouse models: **PK/PD relationship** mild model expressing 4 copies of hSMN2. Mouse models with more severe phenotypes were uses for efficacy assessment. Results: Spinraza™ can modulate SMN2 splicing to produce full length SMN protein resulting in significant increased life span and improved motor function.
	4.2.2 Secondary Pharmacodynamics	Not conducted: "Considering the specific binding and unique mechanism of action of Spinraza™, no secondary pharmacodynamic studies […] were performed, which was considered acceptable."
	4.2.3 Safety Pharmacology	**Safety pharmacology:** "It was not feasible to evaluate the toxicity of Spinraza™ in a pharmacologically responsive species." **Sub-chronic and chronic repeat-dose toxicology** in rodents was not technically feasible with intrathecal (intrathecal) bolus. Instead **SC (subcutaneous) was used for 13 week repeat-dose in juvenile CD-1** mice and intrathecal infusion for rat safety pharmacology. **14-week (AS03) & 53-week (AS06) toxicity** study in **cynomolgus monkeys** (0.3,1,3 and 1,3,7 mg) and 25 days in rats (0,0.02,0.06,0.2 mg/day). **Cardiovascular:** no effect on blood pressure (systolic, diastolic, mean arterial), heart rate. **CNS:** No effect on neurobehavioral assessments and learning parameters. Mild focal neuronal vacuolation (hippocampus) in presence of formalin (preparation artifact: confirmed in *AS11*, 6 weekly intrathecal doses in cynomolgus monkeys). Only transient changes in lower spinal reflex following intrathecal bolus (>= 3mg) **Respiratory:** No effects on pulmonary function (respiratory rate, tidal volume, minute volume); **Kidney & Liver:** detection in kidney and liver but at low concentrations (<120ug/g at the highest intrathecal dose), below toxicity threshold. Absence of treatment related findings. →no sustained effect of Spinraza™ on **safety pharmacology** parameters in cynomolgus monkeys.
	4.2.4 Pharmacodynamic Drug Interactions	Not conducted: "Considering the specific binding and unique mechanism of action of Spinraza™, no […] pharmacodynamic drug-drug interaction studies were performed, which was considered acceptable."
4.3 (2.4.3,2.6.4/5) Pharmacokinetics	**4.3.2** Absorption	All monkey studies: intrathecal injection bypasses the BBB such that Spinraza™ is fully available without initial absorption. Rapid distribution through CSF to CNS tissue with little metabolic clearance. Plasma exposure 1-3 orders of magnitude lower than CSF exposure.
	4.3.3 Distribution	***APK01:*** Adult cynomolgus monkeys intrathecal & IV, 4-week multiple dose study: 4 intrathecal lumbar doses or 4 IV bolus doses at 1mg/dose once weekly. CSF & plasma: multiphasic disposition (intrathecal), rapid distribution phase, slower & prolonged elimination phase (similar to single intrathecal dose *AS01*). CSF half-life 102 days. Peak CSF 1h after intrathecal (first time point) and plasma 4h after intrathecal. Comparison of intrathecal and IV plasma exposure suggests minimal metabolic clearance form CNS. Tissue half-lifes for brain and spinal cord regions 74-275 days (116 days median). ***AS03 & AS06:*** CSF, plasma & tissue concentrations consistent with *APK01*. Dose-dependent CSF & plasma concentration increase. CSF half-life study 4: 111 days. CSF day 7 consistent with *AS01*. Plasma Tmax: 2-5 hours after intrathecal bolus (consistent with previous studies).
	4.3.4 Metabolism	Exonuclease mediated hydrolysis. 17-mer oligonucleotide (N-1 form 3'end) detected in relative quantities exceeding 15%. 17-mer: less efficient hybridization. antisense orientation prevents duplex formation with parent substance => no reduction or antagonism No substrate for CYP450mediated oxidative metabolism. A 15-mer ASO version of Spinraza™ showed similar or slightly less activity *in vitro* and a transgenic mouse model.
	4.3.5 Excretion	Not investigated because excretion via urine is expected
	4.3.6 PK drug interactions	Spinraza™ highly bound (weak binding) to human plasma proteins (>94%) but different binding sites for plasma proteins then hydrophobic small molecule. => Low potential. No substrate or inhibitor for human transporters (***in vitro* studies:** BCRP, P-gp, OAT1 & 3, OCT1 & 2, OATP1B1 & 3, BSEP transporters). No inducer or inhibitor of CYP450mediated oxidative metabolism (***in vitro* studies:** 7 major enzymes).
	4.3.7 Other PK	
4	**4.4.1** Acute Toxicity ("Single-Dose")	Integrated into other toxicity studies (repeat-dose)

	4.4.2 Repeat-Dose		Repeated intrathecal administration is not feasible in rodents → repeat-dose investigations were performed in cynomolgus monkeys 14-week study (*AS03*): 0.3 and 1mg 5 weekly doses (loading period) followed by biweekly maintenance, 3 mg: 15 weekly intrathecal doses. 53-week study (*AS06*): 5 weekly doses (1,3,7 mg) followed by maintenance doses every 6 weeks. Single and Repeat-dose studies in monkeys showed Spinraza™ was well tolerated: no change in body weight, food consumption, no clinical persistent effects but acute, transient deficits in lower spinal cord reflexes at highest doses (7mg single-dose, 3mg 14-weeks study (*AS03*): cutaneous, sensory, tail, 4mg 53-week study (*AS06*): patellar, grip, anal, 5mg 6-week investigational study(*AS11*)) within several hours post-dose, reversible within 48h post-dose. Findings could be product related **(=> long term registry study?**) No abnormal findings in physical examinations, clinical pathology, ophthalmic, cardiovascular, skeletal system maturation, immune system, systemic organ pathology.
	4.4.3 Genotoxicity	**4.4.3.1** *in vitro*	Negative bacterial reverse mutagenesis assay, *in vitro* chromosomal aberration assay (CHO cells)
		4.4.3.2 *In vivo*	Negative *in vivo* CD-1 mouse bone marrow micronucleus assay
	4.4.4 Carcinogenicity	**4.4.4.1** Long-term	Not performed because Spinraza™ is non-genotoxic and no mechanism for tumor induction (weight of evidence assessment).
		4.4.4.2 Short- or medium-term	
		4.4.4.3 Other studies	
	4.4.5 Reproductive and Developmental Toxicity	**4.4.5.1** Fertility and early embryonic development	Combined fertility and early development study in CD-1 mice (3, 10, 25 mg/kg). negative
		4.4.5.2 Embryo-fetal development	Two studies on embryo-fetal development in New Zealand White rabbits (0, 6, 12.6, 25 mg/kg). negative
		4.4.5.3 Prenatal and postnatal development	Pre-and post-natal development on female pregnant CD-1 mice (SC, 1.4, 5.8, 17.2 mg/kg). negative
		4.4.5.4 Juvenile animals	**13-week toxicity** study in juvenile CD-1 mice (for **pediatric** patients), SC dose, Post-natal day (PND) 4 till PND 25: one weekly dose, PND25 till PND95: every two weeks. 1,10,50 mg/kg/dose. Results: Spinraza™ tolerance at all dose levels. No change in body weights, food consumption, morbidity, mortality, growth, development, clinical findings, ophthalmic examination, hematology, clinical chemistry, necropsy. At 50mg/kg Kupffer cell hyperthrophy (liver, male), vacuolated macrophages on lymph nodes (male & female) and higher organ weight. Higher spleen weight at 10mg/kg (males) & 50mg/kg (males and females) **AS03 & AS06** performed in juvenile monkeys
	4.4.6 Local Tolerance		Clinical RoA: Intrathecal→local tolerance: parenteral; covered by SC studies in mice
	4.4.7 Other toxicity	**4.4.7.1** Antigenicity	No evidence for cellular activation and cytokine production triggered by pro-inflammatory response in animal studies and none in clinical studies
		4.4.7.2 Immunotoxicity	
		4.4.7.3 Mechanistic studies	
		4.4.7.4 Dependence	
		4.4.7.5 Metabolites	See **4.3.4**
		4.4.7.6 Impurities	Oligonucleotide impurities qualification: 53-week **repeat dose toxicology study** (intrathecal) in juvenile monkeys (AS06). Potential small molecule impurities: **genotoxic risk** according to ICH M7 Residual solvents: **PDE values** of ICH Q3(R5)
		4.4.7.7 Other	

Table P3: Pre-clinical Studies – Zolgensma® (onasemnogene abeparvovec), based on (Byrnes, 2019)

4.2 (2.4.2,2.6.2/3) Pharmacology	**4.2.1** Primary Pharmacodynamics		***In vivo* (pre-clinical) pharmacology (Zolgensma®):** Neonatal SMNdelta7 mice, single intravenous administration, 1.2x10^13 to 1.1x10^14 vg/kg: Dose dependent improvement of survival and body weight gain. Highest at PND 1 or 2 dosing. Early non-clinical vectors demonstrated improved motorfunction, neuromuscular transmission, body weight gain and cardiac function in SMNdelta7 mice.
	4.2.2 Secondary Pharmacodynamics		
	4.2.3 Safety Pharmacology		Not conducted*
	4.2.4 Pharmacodynamic Drug Interactions		
4.3 (2.4.3,2.6.4/5) Pharmacokinetics	**4.3.2** Absorption		
	4.3.3 Distribution		**Biodistribution study (pre-clinical) (Zolgensma®):** Neonatal FVB mice, 12 weeks. Intravenous administration of **1.5x10^14 vg/kg** highest vector DNA concentration in heart, then lung, liver, lumbar spinal cord, quadriceps muscle, brain, ovary, spleen, testis. Human SMN mRNA highest expression in heart, then quadriceps muscle, liver, lung, brain, and lumbar spinal cord. Low mRNA levels in spleen and gonades.
	4.3.4 Metabolism		
	4.3.5 Excretion		
	4.3.6 PK drug interactions		
	4.3.7 Other PK		
4.4 (2.4.4,2.6.6/7) Toxicology	**4.4.1** Acute Toxicity ("Single-Dose")		Neonatal FVB mice, intravenous administration: **7.9x10^13 vg/kg** and higher => minimal till mild dose-dependent degeneration of myocardium. **1.5x10^14 vg/kg** and higher: dose-dependent increase in incident and severity of adverse **cardiac findings** (minimal to moderate atrial thrombosis, slight to marked atrial dilation, minimal to slight fibroplasia, myocardial degeneration & inflammation), sometimes associated with increased heart weights, enlarged heart, abnormal shape, large atrium. Adverse **findings in liver**: minimal to moderate hepatocyte degeneration/necrosis, minimal to slight hepatocellular hypertrophy, perinuclear vacuolation, increased Kupffer cells. **2.4x10^14 vg/kg** and higher: minimal to slight perivascular and chronic inflammation in the **lung**. Zolgensma®-related mortality due to cardiac and liver toxicities (atrial thrombosis, atrial dilation,fibroplasia, myocardial degeneration, mononuclear cell infiltration, hepatocellular degeneration).
	4.4.2 Repeat-Dose		
	4.4.3 Genotoxicity	**4.4.3.1** *in vitro*	Not conducted*
		4.4.3.2 *In vivo*	
	4.4.4 Carcinogenicity	**4.4.4.1** Long-term	Not conducted*
		4.4.4.2 Short- or medium-term	
		4.4.4.3 Other studies	
	4.4.5 Reproductive and Developmental Toxicity	**4.4.5.1** Fertility and early embryonic development	Not conducted*
		4.4.5.2 Embryo-fetal development	
		4.4.5.3 Prenatal and postnatal development	
		4.4.5.4 Juvenile animals	
	4.4.6 Local Tolerance		Not conducted in the US

	4.4.7 Other toxicity	**4.4.7.1** Antigenicity	Expected → Evaluated in clinical trials.
		4.4.7.2 Immunotoxicity	
		4.4.7.3 Mechanistic studies	
		4.4.7.4 Dependence	
		4.4.7.5 Metabolites	
		4.4.7.6 Impurities	
		4.4.7.7 Other	

* Not warranted based on product characteristics, results from toxicology studies and target patient population

Clinical Tables

Table C1: Risdiplam endpoints

Study ID	Patients	Dose	Primary Endpoints	Outcome (primary)	Secondary Endpoints & Results	Outcome (secondary & results)
Sunfish (NCT02908685)	Type 2 or 3 SMA n=51 + 180	Oral; placebo-controlled	**1)** Change from baseline in Total MFM-32 score at 12 months	**1)** Significant (p=0.0156) improvement vs placebo (1.55 points)	**2)** Percentage of participants who achieve stabilization (MFM32 ≥0) or improvement (MFM32 ≥3) in MFM32 total score at Month 12 **3)** Change from baseline in RULM total score at Month 12 **4)** Change from baseline in HFMSE total score at Month 12 **5)** Change from baseline in SMAIS total score at Month 12	**2)** Stabilization: 70% Risdiplam vs. 54% placebo (p=0.043); improvement: 39% Risdiplam vs 22% placebo (p=0.0469) **3)** 1.59 difference in change from RULM baseline vs placebo (p=0.0028) at 12 months **4)** 0.58 difference in change (ca. 1 vs 0.4) from HFMSE baseline vs placebo (p=0.3) at 12 months **5)** Ca. 2.7 (caregiver-reported) (p=0.022) and ca. 1.2 (patient-reported ≥12 years) (p=0.1778) difference in change from SMAIS baseline vs placebo at 12 months
Firefish (NCT02913482)	Type 1 SMA n=21 + 41	Oral; Open-label	**1)** Patients sitting without support for ≥5sec at 12 months of treatment (BSID-III)	**1)** 12/41 (29%) vs 0% natural history (p<0.0001, performance criterion=5%)	**2)** Time to death or permanent ventilation **3)** Achievement of motor milestones at Month 12 as measured by the HINE-2; increase(improvement)/decrease(worsening): ≥2 points kick, ≥1 point head control, rolling, sitting, crawling, standing or walking **4)** Proportion of infants who achieve an increase of ≥4 points in the CHOP-INTEND at Month 12 **5)** Proportion of infants who achieve a score of ≥40 in the CHOP-INTEND at Month 12 **6)** Ability to swallow and feed orally at Month 12 **7)** Number of nights in hospital per infant by Month 12	**2)** 93% (38/41) alive at 12 months vs ca. 30% in natural history data **3)** HINE-2: 32/41 (78%) responded (p<0.0001, performance criterion=12%) **4)** 90% (37/41) ≥4 points in CHOP-INTEND total score (median +20 points) (p<0.0001, performance criterion=17%) **5)** 56% (23/41) score ≥40 at 12 months vs. Natural History rarely reach 40 points (p<0.0001, performance criterion=17%) **6)** 95% (36/38) maintained ability to swallow at 12 months; 89% (34/38) able to feed orally vs natural history: all infants older than 12 months require feeding support **7)** 49% (20/41) did not require hospitalization up to 12 months; 1.30 hospitalizations per patient-year vs. natural data: 4.2-7.6 hospitalizations per year

Table C2: Spinraza™ endpoints

Study ID	Patients	Dose	Primary Endpoints	Outcome (primary)	Secondary Endpoints & Results	Outcome (secondary & results)
CS3A	Type 1 SMA n=21	Multiple-dose (intrathecal); open-label; 6, 12mg	**1)** New motor milestones (HINE)	**1)** 65% (13/20) of patients	**2)** Increase in total CHOP-INTEND score of ≥4 points at the time of data cut-off (median 670 days) **3)** Improvement of ≥0.5 mV in peroneal amplitude as of the last visit prior to the data cut-off date **4)** Survival rate **5)** Percent of subjects not requiring permanent ventilation.	**2)** 55% (11/20) **3)** 65% (13/20) **4)** 75% (15/20) **5)** 65% (13/20)
CS5/SM201	Type 1 SMA (≤6 weeks, Pre-symptomatic) n=13 (D64),10(D183),5(302)	Multiple-dose (intrathecal); open-label; 12 mg	**1)** Proportion of motor milestone responders (HINE)	**1)** 92% (12/13), 100%(10/10),100%(5/5) ≥1 motor-milestone; 69%(9/13), 100%(10/10),100%(10/10) ≥2 motor-milestones	**2)** Increase in total CHOP-INTEND score of ≥4 points at D64,D183,D302 **3)** Improvement of ≥0.5 mV in peroneal amplitude at D64,D183,D302 **4)** Survival rate **5)** Percent of subjects not requiring permanent ventilation.	**2)** 54% (7/13), 80% (8/10), 60% (3/5) **3)** 64% (7/11),90%(9/10), 60%(3/5) **4)** 100% **5)** 100%
CS3B	Type 1 SMA n=122	Multiple-dose (intrathecal); sham-controlled 12 mg	**1)** Proportion of motor milestone responders (HINE); **2)** Time to death or permanent ventilation (≥16 hours ventilation/ day continuously for >21 days in the absence of an acute reversible event OR tracheostomy).	**1)** 51% of patients reach a motor milestone at the end of the study (≥190 days treatment) vs 0% in controls (p<0.0001); **2)** 47% reduction in risk of death or permanent ventilation	**3)** Proportion of Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease (CHOP-INTEND) responders. **4)** Survival rate. **5)** Percent of subjects not requiring permanent ventilation. **6)** Proportion of compound muscle action potential (CMAP) responders (peroneal amplitude≥1mV). **7)** Time to death or permanent ventilation in the subgroups of subjects below the study median disease duration. **8)** Time to death or permanent ventilation in the subgroups of subjects above the study median disease duration.	**3)** Spinraza™: 71% CHOP-INTEND improvement, 65% ≥ 4 points improvement, 61% ≥ 6 points vs. Controls 52% worsening and 44% worsening ≥ 4 points **4)** 62.8% lower risk of death, survival rate 85% (68/80) **5)** 34% lower risk of permanent ventilation **6)** CAMP responders: 35% (18/51) vs 0% in controls, 20% (10/51) improved ≥1mV, 4% (2/51) improved by ≥2mV **7)** 76% reduced risk **8)** 16% reduced risk
CS4	Type 2 or 3 SMA n=126	Multiple-dose (intrathecal); sham-controlled 12mg	**1)** Change in HFMSE score at 15 months	**1)** Improved score of 4 points in Spinraza™ group vs a decrease of 1.9 points in controls (p=0.0000002)	**2)** Proportion of subjects who achieve ≥ 3-point HFMSE score increase from baseline at 15 months **3)** Proportion of subjects who achieve any new WHO motor milestone at 15 months **4)** Number of WHO motor milestones achieved per subject at 15 months **5)** Change from baseline in Upper Limb Module (ULM) Test at 15 months **6)** Proportion of subjects who achieve standing alone at 15 months **7)** Proportion of subjects who achieve walking with assistance at 15 months	**2)** Spinraza™ 57.3% vs controls 20.5% **3)** Spinraza™ 17.1% and controls 10.5%, **4)** Least squares mean difference 0.3 higher between Spinraza™ and control group **5)** Least squares mean change of 3.7 (Spinraza™) vs 0.3 (controls) **6)** Spinraza™ 1 subject vs. Control 1 subject **7)** None

Table C3: Zolgensma® endpoints

Study ID	Patients	Dose	Primary Endpoints	Outcome (primary)	Secondary Endpoints & Results	Outcome (secondary & results)
START	Type 1 SMA n=15	Single-dose (iv); Open-label; 4.3-4.6x10^13vg/kg; 1.1-1.4x10^14vg/kg; oral corticosteroids	**1)** Survival after 24 months; **2)** Sitting w/o support for ≥ 30 sec; **3)** Standing and walking;	**1)** Survival 67% (2/3) (low dose) vs. 100%(12/12) (high dose). **2)** 0%(0/3) of low dose patients vs 75%(9/12) high dose **3)** 0% (0/3) vs. 16.7% (2/12)		
SPR1NT	Type 1 SMA (≤6 weeks, Pre-symptomatic) n=29 (14:2 SMN2,15:3 SMN2)	Single-dose (iv); open-label;	**1)** CHOP-INTEND score ≥ 50 **2)** Sitting w/o support for ≥30 sec; **3)** Standing and walking	**1)** 100% (14/14) and x/15 **2)** 57% (8/14) and x/15 **3)** Standing x/14 and 27% (4/15), walking 29% (4/14) and 20% (3/15)	**4)** CHOP-INTEND score ≥ 58	**4)** 93% (13/14) and (x/x)
STR1VE-US	Type 1 SMA n=22	Single-dose (iv); Open-label; 1.10x10^14vg/kg; oral corticosteroids	**1)** Sitting without support for ≥30 seconds at 18 months of age; **2)** Survival at 14 months of age	**1)** 59% (13/22) treated vs 0% (0/23) Natural history controls; **2)** 91% (20/22) treated patients vs. 25% (6/23) Natural history controls;	**3)** No non-invasive ventilatory support (anytime, 18 month of age) **4)** "Ability to thrive" composite endpoint at 18 month of age **5)** CHOP-INTEND score ≥ 40 **6)** CHOP-INTEND score ≥ 50	**3)** 68% **(**15/22) and 81% (18/22) **4)** 41% **(**9/22) (p<0.0001 vs natural history) **5)** 95% (21/22) **6)** 64% (14/22)
STRONG	Type 2 SMA n=32 (Doses n=3 (A), n=25 (B), n=4 (C))	Single-dose (intrathecal); Dose A(6x10^13vg), Dose B (1.2x10^14vg), Dose C(2.4x10^14vg)	**1)** Change in HFMSE scores from baseline at 12 months compared to natural history **2)** Stand without support for ≥3 seconds	**1)** Dose B (≥2 years and <5 years): 6.0 points mean increase **2)** Dose B (≥6 months to <2 years): 8% (1/13)	**3)** Increase in HFMSE scores ≥3 from baseline at 12 months **4)** Walk independently for ≥5 steps	**3)** Dose B (≥2 years and <5 years): 92% (11/12); Dose B (≥6 months to <2 years): 83% (5/6) **4)** Dose B (≥6 months to <2 years): 8% (1/13)

Erklärung

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

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