Molecular Therapeutic Strategies for Spinal Muscular Atrophies: Current and Future Clinical Trials

Chiara Zanetta, MD; Monica Nizzardo, PhD; Chiara Simone, PhD; Erika Monguzzi, PhD; Nereo Bresolin, MD; Giacomo P. Comi, MD; and Stefania Corti, MD, PhD

Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy

ABSTRACT

Background: Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease caused by mutations in the survival motor neuron gene (SMN1) and the leading genetic cause of infant mortality. Currently, there is no effective treatment other than supportive care.

Objective: This article provides a general overview of the main aspects that need to be taken into account to design a more efficient clinical trial and to summarize the most promising molecular trials that are currently in development or are being planned for the treatment of SMA.

Methods: A systematic review of the literature was performed, identifying key clinical trials involving novel molecular therapies in SMA. In addition, abstracts presented at the meetings of the Families of Spinal Muscular Atrophy were searched and the Families of Spinal Muscular Atrophy Web site was carefully analyzed. Finally, a selection of SMA clinical trials registered at clinicaltrials.gov has been included in the article.

Results: The past decade has seen a marked advancement in the understanding of both SMA genetics and molecular mechanisms. New molecules targeting SMN have shown promise in preclinical studies, and various clinical trials have started to test the drugs that were discovered through basic research.

Conclusions: Both preclinical and early clinical trial results involving novel molecular therapies suggest that the clinical care paradigm in SMA will soon change. (Clin Ther. 2014;36:128–140) © 2014 Elsevier HS Journals, Inc. All rights reserved.

Key words: clinical trials, gene therapy, ISIS-SMNIR, iclesoxime, oligonucleotides, small molecules, spinal muscular atrophy.

INTRODUCTION

Spinal muscular atrophies (SMAs) are a group of hereditary autosomal recessive neuromuscular diseases that are characterized by the degeneration of motor neurons in the spinal cord and brainstem, resulting in progressive proximal muscle weakness, hypostenia, and paralysis, which are usually symmetrical. SMA is the most frequent genetic cause of infant mortality, with an estimated incidence of 1 in 6000 to 1 in 10,000 live births and a carrier frequency of 1 in 40 to 1 in 60.1,2 The classical form of the disorder is caused by a genetic mutation3 in the 5q11.2-q13.3 locus, which affects the survival motor neuron (SMN) gene4 and leads to the reduction of SMN protein. SMA is clinically conventionally classified into 4 phenotypes (I, II, III, and IV) on the basis of age of onset and highest motor function achieved, with an additional phenotype (type 0) to describe the severe forms with an antenatal onset.5 Prognosis depends on the phenotypic severity, ranging from high mortality within the first year for SMA type I to no mortality for the chronic and later-onset forms. The increased attention to early diagnosis and to several aspects of management of SMA has stimulated the development of clinical guidelines and standards of care.6,7 In the past decade, many promising new therapeutic approaches have been tested in clinical trials of patients with SMA8–11 but with limited or no success. At the present, no effective therapy is available for SMA, besides supportive care. Consequently, the development of novel therapies in SMA now has strong academic, government, and industry involvement, in addition to
the interest of several parental organizations and foundations. The goal of the present article was to summarize the literature on the emerging molecular therapeutic approaches that are currently being investigated or planned to be tested in clinical trials of SMA (Figure 1).

METHODS
A systematic review of the English-language articles listed in PubMed over the past 10 years was performed by using the following key words: spinal muscular atrophy, SMA, oligonucleotides, gene therapy, molecular therapy, and small molecules. All articles found were systematically analyzed and taken into consideration when preparing the review. In addition, abstracts presented at the annual 2010 to 2013 meetings of the Families of Spinal Muscular Atrophy were searched by using the same key words, and the Families of Spinal Muscular Atrophy Web site (www.fsma.org) was carefully analyzed. Finally, a selection of SMA clinical trials registered at clinicaltrials.gov has been included in the article.

RESULTS
Designing a Reasonable Clinical Trial for SMA: Issues to Address
This section analyzes some of the issues and concerns that need to be taken into account to design a reasonable clinical trial for the treatment of SMA. The increasing expansion of clinical trials planning has raised several concerns, including the following: are there enough patients identified to enroll easily? What is the relative power of the study that can be obtained? In addition, it is imperative to discern if there are reliable, valid, and sensitive outcome measures available.12

Many difficulties exist in undertaking randomized, double-blind, placebo-controlled studies of rare disorders such as SMA. These difficulties include the process of enrollment and stratification, even though international registries for patients have increased the chances of identifying and recruiting patients for clinical trials. In terms of stratification, SMN2 copy number could be used as a stratification criterion because the number of copies of SMN2 correlates with motor function achieved.13 Nevertheless, even in a relatively homogeneous group of SMA type I patients, 2 cohorts of subjects can be identified: 1 characterized by an early presentation (genetic diagnosis < 6 months) and another cohort with a relatively late onset of symptoms (genetic diagnosis > 6 months). Furthermore, another aspect that makes the analysis of the data even more complicated is that the intensity of care can profoundly affect survival.14 Moreover, common standards of care, as well as consistent assessment methods and outcome measures.
measures to make data from different centers comparable, are needed when designing a clinical trial.15

Drug selection process and study design are factors that also need to be taken into consideration. Drug selection based on preclinical data may not be a reliable predictor of a therapeutic effect in patients. Thus, it is important to define how preclinical models parallel the disease in humans and which of the most predictive outcomes are for human SMA. Another important point to address is the correct evaluation of drug pharmacokinetics in humans. For instance, it is fundamental to demonstrate that the target compound induces SMN protein expression in the required cell types in vivo in humans, following what it does in preclinical models.

Furthermore, the majority of Phase I and II trials have enrolled patients with SMA types II and III; Phase III trial design is underdeveloped. Trial implementation has to contend with various challenges: information on SMA populations is not complete, natural history data are not always reliable, and the differences in incidence and survival between countries are unknown.

Treatment in late stages of the disease could partly explain past trial failures. Mouse studies found that neonatal treatment is fundamental for efficacy, suggesting that a limited therapeutic window exists, at least in animal models.16 It has been suggested that the first few months of life are the most critical time of denervation in SMA types I and II, leading to the idea that implementation of neonatal trials and the development of strategies for identifying patients at presymptomatic or early symptomatic stages would be the best option for developing an efficient clinical trial for SMA. Therefore, the timing of SMN rescue can be different in patients with SMA type I compared with SMA type II or III patients.

Another difficulty is the absence of validated markers of disease progression. For SMA type I, electrophysiologic measures such as motor unit number estimation (MUNE) and compound muscle action potential (CMAP) may be better indicators of efficacy than survival.17 Furthermore, biomarkers of treatment effects are urgently needed. Currently, numerous overlapping motor functional scales serve as clinical end points. These should be tailored for presymptomatic, early symptomatic, and chronic stages for each SMA subtype.

Overall, the main recommendations for conducting a reasonable clinical trial can be summarized as follows: (1) testing of the preclinical effectiveness of drugs in SMA animal models; (2) planning of the feasibility of each phase of the trials; (3) harmonization of the European regulatory processes for drug approval; (4) targeting of the optimal therapeutic window for SMA; and (5) analysis of biomarkers (measures of upper limb function for nonambulant patients, muscle biomarkers [MUNE/CMAP, muscle MRI, EIM] and the SMA Foundation’s biomarker panel) (Table).

The balance of this review provides information on the main clinical trials based on molecular strategies that have been conducted and are ongoing (Figure 1).

Neuroprotection Strategy for SMA: Olesoxime (TRO19622)

Olesoxime (TRO19622) is a small molecule with a cholesterol-like structure that displays strong neuroprotective properties.18 It has demonstrated effectiveness in keeping motor neurons alive in culture. Preclinical in vitro studies have shown that the compound promotes the function and survival of neurons and other cell types under disease-relevant stress conditions when interacting with the mitochondrial permeability transition pore (Figure 2A).

In October 2010, Trophos SA initiated a pivotal efficacy and safety study of olesoxime, in ~150 SMA patients, in a program funded by the Association Francaise contre les Myopathies in France. This efficacy and safety study is a 24-month, Phase II, multicenter, randomized, adaptive, double-blind, placebo-controlled study in nonambulant patients aged 3 to 25 years with SMA type II and type III.19 Recruitment of 165 patients with SMA was completed in September 2011; no further patients can be enrolled. Patients in the study are treated for 2 years, and results are expected before the end of 2013. Trophos has been granted orphan drug designation for olesoxime for the treatment of SMA by the US Food and Drug Administration (FDA). In the European Union, olesoxime has been granted Orphan Medicinal Product designation for SMA by the European Commission.20 Randomization was 2:1 for olesoxime or matching placebo. The subjects enrolled in this study received either a liquid suspension formulation of olesoxime (100 of 150 patients; 100 mg/mL at a dose of 10 mg/kg administered orally once a day with food at dinner) or a liquid suspension formulation of placebo (50 of 150) always administered once a day with food at
Table. Description of biomarkers, functional scales, and electromyography measures conventionally used in the spinal muscular atrophy (SMA) field. SMA-MAP biomarkers: list of top 13 SMA motor function regressor markers, called SMA-MAP, in 2 SMA populations. Twenty-seven analytes were selected for inclusion into a new biomarker panel, called SMA-MAP. The 13 analytes that regressed to motor outcomes of SMA in both the BforSMA (Biomarker for SMA) study and PNCR NHS (Pediatric Neuromuscular Clinical Research Network History Study) were included in the panel and are shown in the table. A description of the main functional scales and electromyography measures conventionally used is provided.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full Name</th>
<th>Function</th>
<th>Correlated Outcome Measures</th>
<th>Correlation to Modified HFMS</th>
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<tr>
<td></td>
<td></td>
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<td>Motor</td>
<td>PNCR NHS P</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>BforSMA P</td>
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<td>SMA-MAP biomarkers</td>
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<tr>
<td>COMP</td>
<td>Cartilage oligomeric matrix protein</td>
<td>Cell proliferation, apoptosis, cell movement and attachment; binds strongly to calcium</td>
<td>Motor MUNE Strength</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AXL</td>
<td>Receptor tyrosine kinase</td>
<td>Transduces signals from the extracellular matrix by binding growth factor GAS6 regulating: cell survival, cell proliferation, migration, and differentiation</td>
<td>Motor PQP</td>
<td>FVC</td>
</tr>
<tr>
<td>CD93</td>
<td>Cluster of differentiation 93</td>
<td>Cell to cell adhesion, clearance of apoptotic cells</td>
<td>Motor MUNE PQC Strength</td>
<td></td>
</tr>
<tr>
<td>PEPD</td>
<td>Peptidase D</td>
<td>Splits dipeptides with a prolyl or hydroxyprolyl residue in the C-terminal position; plays an important role in collagen metabolism</td>
<td>Motor MUNE Strength</td>
<td>CMAP</td>
</tr>
<tr>
<td>THBS4</td>
<td>Thrombospondin-4</td>
<td>Adhesive glycoprotein, mediates cell-to-cell and cell-to-matrix interactions</td>
<td>Motor MUNE Strength</td>
<td></td>
</tr>
<tr>
<td>LUM</td>
<td>Lumican</td>
<td>Regulates: collagen fibril organization and circumferential growth, corneal transparency, epithelial cell migration, and tissue repair</td>
<td>Motor MUNE Strength</td>
<td>CMAP</td>
</tr>
<tr>
<td>MB</td>
<td>Myoglobin</td>
<td>Myoglobin is the primary oxygen-carrying pigment of muscles</td>
<td>Motor Strength</td>
<td>FVC</td>
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Table (continued).

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>DPP4</td>
<td>Dipeptidyl peptidase-4</td>
<td>Antigenic enzyme expressed on the surface of most cell types associated with: immune regulation, signal transduction, and apoptosis</td>
<td>Motor MUNE PQP Strength</td>
<td>0.001 &lt; 0.001</td>
</tr>
<tr>
<td>SPP1</td>
<td>Secreted phosphoprotein 1, also known as osteopontin</td>
<td>Has a role in: biomineralization, bone remodeling, immune response, chemotaxis, apoptosis, and cell activation</td>
<td>Motor</td>
<td>0.002 &lt; 0.001</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>Chitinase-3-like protein 1</td>
<td>Catalyzes the hydrolysis of chitin; has a role in: inflammation and tissue remodeling</td>
<td>Motor</td>
<td>0.01 &lt; 0.001</td>
</tr>
<tr>
<td>CDH13</td>
<td>Cadherin 13, also known as H-cadherin (heart)</td>
<td>Roles: mediation of intracellular signaling in vascular cells, regulation of cell growth, guiding molecule in vascular and nervous system</td>
<td>Motor</td>
<td>0.039 0.001</td>
</tr>
<tr>
<td>APCS</td>
<td>Amyloid P component</td>
<td>Interacts with DNA and histones, scavenges nuclear material released from damaged circulating cells</td>
<td>Motor</td>
<td>0.041 &lt; 0.001</td>
</tr>
<tr>
<td>LEP</td>
<td>Leptin</td>
<td>Inhibits appetite, acts as adiposity signal, interacts with amylin, mediates the feeling of satiety, promotes angiogenesis, promotes the activity of lung surfactant, acts on bone metabolism</td>
<td>Motor</td>
<td>0.058 &lt; 0.001</td>
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**Functional scales**

| MFM       | Motor function measure | Quantitative scale that makes it possible to measure the functional motor abilities of patients affected by neuromuscular diseases; whatever the diagnosis and the extent of motor deficiencies, MFM allows us to: specify the | — | — |

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<td></td>
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<td>symptoms and the evolution of neuromuscular diseases; objectivize the repercussion of the therapeutic measures; direct the rehabilitation and adaptation measures; facilitate communication between the various persons in charge of care; and select homogeneous groups of patients in view of therapeutic trials</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HFMS</td>
<td>Hammersmith Functional Motor Scale</td>
<td>Functional motor scale that can be used to: assess gross motor abilities of nonambulant children with SMA, monitor progression and gross motor abilities over time</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Electromyography measures</td>
<td></td>
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<td>CMAP</td>
<td>Compound muscle action potential</td>
<td>The size of the evoked CMAP reflects the number of muscle fibers accessible from the stimulated nerve and will be reduced by atrophy (from whatever cause) by conduction block in the peripheral nerve or at the neuromuscular junction</td>
<td>—</td>
<td>—</td>
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<tr>
<td>MUNE</td>
<td>Motor unit number estimation</td>
<td>They have been used to study the rate of motor unit loss in patients affected by neuromuscular disorders</td>
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FVC = forced vital capacity; PQP = PedQL quality of life, parent score; PQC = PedQL quality of life, child score.
The primary end point of the study is the change from baseline in the Motor Function Measure (MFM) functional scale. Secondary end points include the Hammersmith Functional Motor Scale (HFMS) and electromyography (CMAP and MUNE) as well as measures of safety, tolerance, and quality of life. The study is being conducted at 22 centers in 7 European countries. Inclusion criteria include weakness and hypotonia consistent with a clinical diagnosis of SMA type II or III and laboratory documentation of homozygous absence of SMN1 exon 7 and/or deletion and mutation on other alleles. To be included, subjects had to have an MFM relative score (percentage of the maximum sum of both dimensions) \( \geq 15\% \) (D1 + D2 score) and HFMS score at baseline \( \geq 3 \). The study enrolled nonambulant patients, defined as patients with an HFMS score \( \leq 38 \), aged 3 to 26 years at time of enrollment. The patients’ age at onset of dinner.
symptoms had to be $\leq 3$ years. Additional inclusion criteria were normal laboratory test results and the ability to take the study treatment. Exclusion criteria were significant comorbidities and administration of other medications intended for SMA treatment. The ongoing study period was divided into 3 stages: stage 1, three-month safety assessment to confirm the adequate dose after 1 month of treatment (an independent data monitoring committee [DMC] has the goal of assessing the safety of olesoxime every 3 months); stage 2, efficacy/futility analyses at 1 year (a first-interim efficacy analysis was planned; after that, all patients will be treated for 1 year [52 weeks] to assess the need to continue the study to reach the planned objectives); and stage 3, efficacy and safety analysis at 2 years.

Trophos announced the completion of the interim analysis of the pivotal efficacy study of olesoxime. The independent DMC has reviewed the treatment effects at 1 year, taking into account the primary outcome measures of efficacy and changes in motor function (MFM scale), along with the latest safety report including ECG tracings, periodic laboratory findings, hemostatic parameters, and serious adverse event listings for all participants. Based on the trial-stopping criteria as defined in the protocol, as well as the absence of safety concerns related to olesoxime treatment, the DMC recommendation is to continue the study as planned. Following the recommendations of the DMC, the study will continue until all participants are treated for 2 years, with finalization of the last patient scheduled for September 2013. Top-line results are expected by the end of 2013.20,21

**Oligonucleotides Therapy for SMA: ISIS-SMNRx**

SMA is caused by the loss of the SMN1 gene, leading to reduced SMN protein levels. The human genome harbors an additional SMN2 gene (or genes) that produces low levels of full-length SMN but cannot adequately compensate for the loss of SMN1 due to an aberrant splicing. The majority of SMN2 gene transcript lacks exon 7 and the resultant SMNΔ7 mRNA is translated into an unstable but partially functional protein. Therapeutic strategies with the goal of promoting exon 7 retention, thus increasing full-length SMN2 transcript, are promising treatments for patients with SMA. Different splice-silencing sequences in SMN2 have been identified as potential targets for splice modifications directed by antisense oligonucleotides. A particularly strong splice silencer is found in SMN2 intron 7 downstream of exon 7. Antisense oligonucleotides designed against this motif promote SMN2 exon 7 retention in the mature SMN2 transcripts, increasing SMN protein expression in SMA cells (Figure 2B).22

Isis Pharmaceuticals is developing a specific oligonucleotide called ISIS-SMNRx designed to act against this strong splicing silencer to promote the inclusion of the missing exon (exon 7); the goal is to create an mRNA that codes for the production of the full-length functional SMN protein for proper motor neuron function. The FDA granted orphan drug status and fast track designation to ISIS-SMNRx for the treatment of patients with SMA. Isis is currently in collaboration with Biogen Idec to develop and potentially commercialize this compound to treat all types of SMA. Under the terms of the January 2012 agreement, Isis is responsible for global development and Biogen Idec has the option to license the compound until completion of the first successful Phase II/III study. Several nonprofit organizations (including Families of Spinal Muscular Atrophy) also support this study. ISIS-SMNRx is a uniformly 2′-O-methoxyethyl modified antisense drug that corrects the splicing disorder in SMN2, resulting in the production of fully functional SMN protein in model systems. In mild and severe SMA mouse models, it is able to provide both a phenotypic and a pathologic benefit when delivered centrally23,24 and has a long half-life in central nervous system tissue (> 6 months in animal models). The results in preclinical studies were very promising, with rescue of the SMA phenotype with early systemic treatment.25

Isis has completed a Phase I, open-label, safety, tolerability, and dose range–finding study with the purpose of testing the safety, tolerability, and pharmacokinetics of a single dose of ISIS-SMNRx administered into the cerebrospinal fluid (CSF) as a single injection in patients with SMA.26 This study was started in November 2011 and was completed in January 2013. A total of 28 subjects were enrolled in this trial, and all of them completed the study; no healthy volunteers were accepted. The inclusion criteria of this study were: a documented SMN1 homozygous gene deletion and clinical signs attributable to SMA, age 2 to 14 years at time of screening, the ability to complete all study procedures, and the demonstration that parents/patients have adequate supportive psychosocial
The major exclusion criteria were: respiratory insufficiency defined by the need for invasive or noninvasive ventilation during a 24-hour period, presence of a gastric feeding tube, previous scoliosis surgery or scoliosis surgery planned during the duration of the study that would interfere with the lumbar puncture injection procedure, conditions that interfere with intrathecal administration of the drugs, severe comorbidities, or taking medications aimed at treating SMA. Four dose levels have been sequentially evaluated. Each dose level was studied in a cohort of 6 or 10 patients; all patients received active drug. Primary end points of the study were safety/tolerability and CSF and plasma drug level pharmacokinetics. Safety and tolerability assessments were obtained through analysis of adverse events, neurologic examinations, CSF laboratory tests, vital signs, clinical laboratory tests, physical examinations and weight reports, ECGs, and the analysis of concomitant medication use. Pharmacokinetic measures used were plasma levels of the drug (over 24 hours’ postdosing and at 7 days’ postdose) and CSF levels of drug (at 7 days’ postdose).

Exploratory end points of this study of ISIS-SMNRx were HFMS and neuromuscular electrophysiology CMAP/MUNE. Safety and tolerability results can be summarized as follows: (1) ISIS-SMNRx was well tolerated, with no significant safety finding when given as a single dose up to 9 mg; (2) the injection procedure was well tolerated and was shown to be feasible; and (3) data suggest that the tolerability of the injection procedure is improved with the use of a smaller needle (ie, 24 or 25 gauge). In terms of drug concentrations, CSF and plasma drug levels were measurable, and they were dose dependent and only moderately variable. Through the use of HFMS, it was evaluated that at day 85, there was a mean change in muscle function from baseline of 3.1 points (P = 0.02) and a percentage of change of 17.6%. Moreover, 6 of 10 subjects had a change from baseline major of 4 points from their basal level score at the HFMS (3 of 6 of these subjects were aged >5 years). CMAP and multipoint incremental MUNE were performed in the highest-dose group at baseline and at day 85, with the primary purpose of determine the feasibility of using this method in patients with SMA. These examinations were performed on the right ulnar nerve that innervates the abductor digiti minimi. Electrophysiology measurements demonstrated stable CMAP, with a potential increase in MUNE. The conclusions and implications of this Phase I study are that ISIS-SMNRx was well tolerated at all dose levels, and no safety or tolerability concerns were identified. The injection procedure used was shown to be feasible in children with SMA. CSF and plasma drug concentrations observed were dose dependent and consistent with preclinical data, supporting infrequent administration (ie, every 6–9 months). An improvement in HFMS scores was observed in the group injected with the highest dose. Electrophysiology measurements in the highest-dose cohort at 3 months demonstrated an increase in MUNE with stable CMAP.

In September 2013, the preliminary results of the open-label Phase I study of ISIS-SMNRx in children with SMA (24 subjects) were reported; they highlighted that the majority of children with SMA receiving the 2 highest doses of the drug (6 and 9 mg) continued to show improvements in muscle function tests (mean HFMS improvement with a dose of 9 mg of ISIS-SMNRx: 5.75) up to 14 months after a single injection of the drug. The amelioration was dose dependent, and no decline was observed. Although the data are encouraging, it must be emphasized that no placebo was included in the study. Based on this evidence, the investigators have amended the infant study to increase the dose from 9 to 12 mg. In addition to the infant pilot study, Isis is also completing a multiple-dose, dose-escalating Phase Ib/IIa study of ISIS-SMNRx in children with SMA type II and III. Isis has completed dosing in all 3 dose cohorts (3, 6, and 9 mg) and is now considering adding a 12-mg dose cohort to this study. ISIS-SMNRx is now being studied in a multiple-dose Phase II study that is designed with the goal of testing the safety, tolerability, and pharmacokinetics of multiple doses of the drug administered into the spinal fluid 3 times over the duration of the trial in patients with infantile-onset SMA. Two dose levels will be evaluated sequentially. Each dose level will be studied in a cohort of 4 patients; all patients will receive active drug. The estimated number of subjects enrolled in the study is 8, and the eligible age is up to 210 days. No healthy volunteers have been accepted. The inclusion criteria are: genetic documentation of 5q SMA (homozygous gene deletion or mutation), onset of clinical signs and symptoms consistent with SMA at ≥21 days and <6 months.
(180 days) of age at study entry, receiving adequate nutrition and hydration, body weight > 5th percentile for age according to Centers for Disease Control and Prevention guidelines, medical care that meets and is expected to continue meeting guidelines set out in the Consensus Statement for Standard of Care in Spinal Muscular Atrophy in the opinion of the site investigator, gestational age of 35 to 42 weeks, and gestational body weight ≥ 2 kg. Moreover, patients need to reside within ~9 hours’ ground-travel distance from a participating study center for the duration of the study (residence > 2 hours’ ground-travel distance from a study center can require special clearance). Major exclusion criteria are: hypoxemia (oxygen saturation awake < 96% or oxygen saturation asleep < 96%, without ventilation support) and significant comorbidities, in particular those interfering with CSF administration, or treatment with another investigational drug. The main primary outcome measure of the study is the number of participants with adverse events (participants will be followed up for the duration of the study). Secondary outcome measures are: (1) plasma pharmacokinetics (assessments at 1, 2, 4, and 24 hours after dosing); (2) Cmax; (3) Tmax; and (4) the AUC from the time of the intrathecal dose to the last collected sample (20 hours after dosing). The infantile-onset study might provide important information related to the therapeutic windows of SMN rescue.

Small Molecules for SMA: Quinazolines

Quinazolines are a family of compounds that inhibit RNA decapping enzyme DcpS (involved in RNA turnover). This inhibition can consequently increase SMN2 expression (Figure 2C). A high-throughput screening identified a few quinazolines as lead compounds that increase full-length SMN2 transcript and SMN protein. These compounds had poor blood–brain barrier (BBB) penetration and required high doses to achieve a sufficient upregulation of SMN. Based on a lead quinazoline compound, derivatives were developed to overcome the obstacles associated with the low BBB penetration. Further optimization of this compound led to the identification of D157495, also called RG3039, which ameliorates the phenotype of SMA mice. Currently, RG3039 is undergoing a Phase I clinical trial in which the safety and efficacy of various doses will be evaluated. This compound is included in the Pfizer SMA Drug Development Programs as PF-06687859. A Phase I, first-in-man, double-blind, placebo-controlled, ascending single-dose, safety and pharmacokinetics study in healthy volunteers has been conducted. Two of 4 cohorts of a multidose Phase I study in 16 healthy volunteers have been completed, with no further patient enrollment. A biomarker and optimal dosing plan is being generated, as well as backup compounds, and enabling technologies have been developed.

Planned Clinical Trials

Gene Therapy

Gene therapy promises a permanent solution for SMA through viral delivery and insertion of the entire SMN1 gene or cDNA sequence into the genome of patients with SMA. This process is generally irreversible, and there are apparent risks if the exogenously delivered gene is inserted at a wrong location and/or overexpressed. Overall, the results of gene therapy aiming at SMN1 delivery by scAAV9 in SMA mice and large animals seem promising and may offer one of the best therapeutic alternatives to a specific group of SMA patients who are too weak to receive frequent invasive treatments.

After submission of an Investigational New Drug application, the FDA has given its approval to physician/scientists at Nationwide Children’s Hospital in Columbus, Ohio, to begin a Phase I clinical trial of a systemic AAV9-delivered human SMN gene. The clinical trial is expected to begin in early 2014 and will be limited to patients with type I SMA, with an age range of 0 to 9 months. Previous research from Nationwide Children’s Hospital Principal Investigator Brian Kaspar, PhD, demonstrated that the AAV9 viral vector is able to cross the BBB. Based on these findings and additional preclinical studies, the SMN gene will be delivered by injection into the bloodstream as part of this Phase I trial. Neurologist Jerry Mendell, MD, director of the Center for Gene Therapy at Nationwide Children’s Hospital, will lead the study. They plan to recruit 9 subjects, with an age of ≤ 6 months with proven SMN mutation and retaining 2 copies of SMN2. Exclusion criteria are the use of ventilatory support or pulse oximetry saturation < 95% and high levels of antibody directed against AAV9.

Physician/scientists are also planning a dose escalation study (low and high dose). The improvement
function will be determined by SAFETY/CHOP-IN-TEND (Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders). The planned follow-up is at 1, 2, 4, 8, and 12 weeks and then every 3 months until 2 years of age. An additional study is planned using the AAV9-delivered SMN gene, with the aim of examining a different route of administration (injecting the virus into the CSF). The safety study has been completed in large mammals, and the study is proceeding toward the pre-Investigational New Drug stage at the FDA.

Moreover, Genzyme/Sanofi are planning a SMN1 gene replacement therapy program. They are preparing to nominate a vector candidate for preclinical toxicology studies and for studies in nonhuman primates to optimize delivery protocol and dose. Overall, gene therapy is one of the most promising new approaches, given its potential effectiveness in resolving the SMA molecular defect.21

Small Molecules

Small molecule compounds aimed at increasing SMN levels offer several advantages, including an easy transport across biological barriers. Considering that SMA is a neurodegenerative disease, compounds that are transported across the BBB would be best suited for an effective therapy.

Small molecule SMN2 splicing modifiers, which increase full-length SMN mRNA and protein in cells isolated from SMA patients, are being developed by Hoffmann-La Roche AG/PTC Therapeutics, Inc/SMA Foundation.21 In August 2013, PTC Therapeutics announced the selection of a development candidate. This approach could be advantageous if an easy administration route (eg, oral administration) is possible. However, this strategy may be limited by the possibility of off-target effects.

CONCLUSIONS

Currently, no effective treatment is available for SMA and other connected motor neuron disorders. The main therapeutic strategies that are now in use are based on symptomatic treatment and supportive care. At a preclinical level, many therapeutic strategies have been investigated, and some of them have been tested and continue to be tested in clinical trials both in Europe and in the United States. This review outlined the principal aspects that should be taken into account when designing a reasonable clinical trial. Moreover, we also provided a description of the most promising clinical trials that are currently ongoing and/or are planned for the near future.

The overall evaluation is that an increasing number of clinical studies over the next few years are expected for SMA. One issue that is noteworthy and unique for SMA is that the phenotype of patients varies significantly among different SMA types (I, II, and III). Moreover, even in a relatively homogenous group of SMA type I patients, 2 cohorts of subjects can be identified: 1 characterized by an early presentation (genetic diagnosis <6 months) and another 1 with a relatively late onset of symptoms (genetic diagnosis >6 months). This aspect cannot be ignored when planning a reasonable clinical trial for SMA. SMN2 copy number directly correlates with both clinical phenotype and highest motor function achieved by patients and could therefore be used as a stratification criterion. Therefore, a powerful strategy could be to design different clinical trials according to each SMA subtype. Although many therapeutic strategies for SMA (eg, stem cell therapy) are being developed, what now seems certain is that more preclinical studies are needed before those strategies could be investigated in clinical trials and therefore used for the treatment of SMA in human patients.

Overall, the results of both preclinical and early clinical trials involving novel molecular therapies suggest that the clinical care paradigm in SMA will soon change.

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CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.
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Address correspondence to: Stefania Corti, MD, PhD, IRCCS Foundation Ca’ Granda Ospedale Maggiore Policlinico, via Francesco Sforza 35, 20122 Milan, Italy. E-mail: stefania.corti@unimi.it