Neural Regeneration Research

Gene therapy for Spinal Muscular Atrophy: the possibility of optimising SMN1 delivery to correct all neurological and systemic perturbations

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Figure

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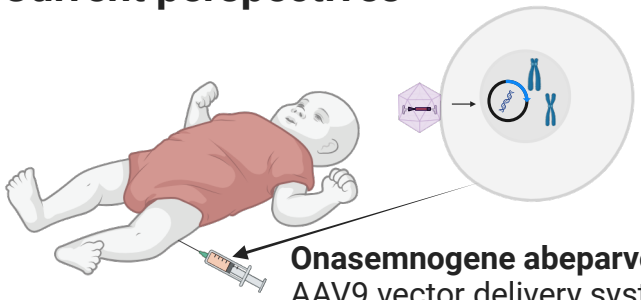
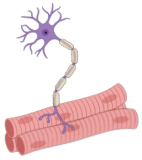
AAV9 therapy for SMA - Current & future perspectives

Current perspectives



Onasemnogene abeparvovec
AAV9 vector delivery system for the *SMN*-encoding gene

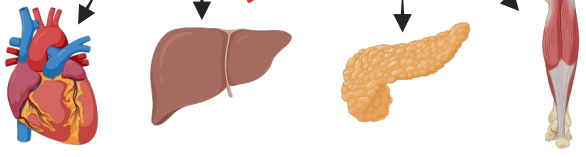


Usually good in pre-symptomatic infants, but variable outcomes for others

SMN

Transduction varies between cells, tissues & organs
Overproduction of SMN can cause toxicity

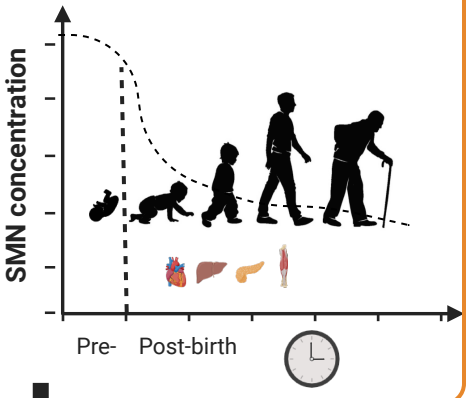
Impact variable



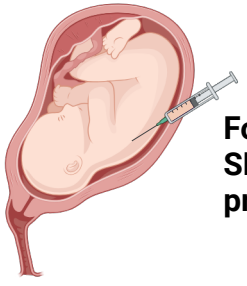
Heart **Liver** **Pancreas** **Muscle**

Research focus

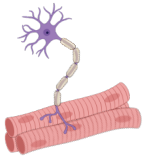
- ***Determine physiological levels of SMN during aging***
- ***Determine physiological levels of SMN in peripheral tissues***
- ***Optimise vector design, timing & dosage for AAV9 delivery of SMN gene***



Future perspectives



Foetal delivery of SMN gene for predicted severe SMA

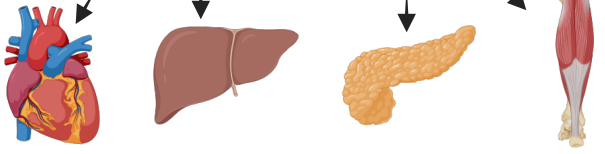


Maximise outcome in CNS

SMN

Replicate physiological levels of SMN in cells, tissues & organs

Optimise impact in peripheral tissues



Heart **Liver** **Pancreas** **Muscle**

Covering Letter

To,

The Editor

Sub: Submission of Manuscript for publication

Dear Sir,

We intend to publish an article entitled **"Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct neurological and systemic perturbations"** in your esteemed journal as a Perspective Article.

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

Prior publication: **AAV9-mediated SMN gene therapy rescues cardiac desmin but not lamin A/C and elastin dysregulation in *Smn*$^{2B/-}$ spinal muscular atrophy mice**

Support: Faculty Research Fund (Faculty of Medicine & Health Science, Keele University) Career Development Award to support Dr Sharon Brown – (April 2022)

Conflicts of interest: Nil

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Thanking you,

Yours' sincerely,

Professor Heidi Fuller

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Manuscript Title: Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct neurological and systemic perturbations

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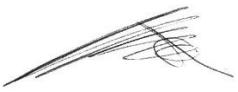
1 Professor Heidi Fuller ----------		30 April 2024
2 Professor Rafael J. Yáñez-Muñoz---------		29th April 2024
3 Dr Sharon Brown----------		30th April 2024

Type of article: Perspective Article

Title of the article: Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct all neurological and systemic perturbations

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Conflicting Interest (If present, give more details):

RJYM has filed a patent application for a novel sequence optimized *SMN1* cDNA.

Title of the article:

Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct all neurological and systemic perturbations

Main Text:

Spinal muscular atrophy (SMA) is a genetic condition that results in selective lower motor neuron loss with concomitant muscle weakness and atrophy. The genetic cause of SMA was understood in 1995 when loss or impairment of the *Survival Motor Neuron 1 (SMN1)* gene was identified as the main contributing factor (Lefebvre et al., 1995). This, in combination with the discovery that humans have a 'back-up' gene, *SMN2*, which can produce low levels (~10%) of the full-length functional SMN protein, has led to the generation of SMA-specific gene therapies. SMA was traditionally classified according to age of symptom onset and developmental milestones achieved, with life expectancy and severity varying between individuals. Now, *SMN2* copy number is used as a proxy for prediction of disease severity, with higher *SMN2* copy number typically being associated with reduced severity of SMA, although this relationship is not absolute: some individuals with low *SMN2* copy number have less severe SMA phenotypes and *vice versa.* Additionally, the aetiology of SMA is further complicated by other factors, such as non-typical nucleotide variants and *SMN2*-independent modifiers of disease severity.

Genetic therapies for SMA have generated a paradigm shift in expected outcomes, with substantial changes in survival and progression of the condition. This has led to a considerable rethink of the research needs for individuals with SMA, as the natural history of the disease has changed with the therapies, and the new phenotypes are only beginning to be understood. These therapies either target the *SMN2* gene to increase functional SMN protein levels (Nusinersen/Spinraza® and Risdiplam/Evrysdi®) or directly increase *SMN* gene levels using adeno-associated virus serotype 9 (AAV9) administration of the *SMN* gene (onasemnogene abeparvovec/Zolgensma®). In particular, the one-off intravenous viral administration of the *SMN* gene has dramatically altered the natural course of SMA, especially in individuals treated pre-symptomatically (Strauss et al., 2022). AAV9 gene therapy delivers full-length human *SMN* cDNA to peripheral organs and to the central nervous system (CNS) by crossing the blood-brain barrier and results in an increased production of functional SMN protein (Thomsen et al., 2021). Onasemnogene abeparvovec is a non-replicating self-complementary AAV9 vector controlled by the hybrid CMV enhancer/chicken beta-actin promoter that enables the transgenic *SMN* gene to reside within the nucleus as an extrachromosomal episome.

There is currently limited information regarding how well AAV9 treatment can restore CNS and systemic perturbations in SMA. Some individuals with SMA show very little or no response to treatment with gene therapy, whilst for just under half of those continuing to thrive over the course of treatment, a degree of symptomatic burden remains (STR1VE-US: NCT03306277 & STR1VE-EU: NCT03461289). Following the treatment of two symptomatic infants with SMA Type I, who died from respiratory complications unrelated to onasemnogene abeparvovec, increased SMN production was detected throughout the CNS and peripheral organs in samples from necropsies, with motor neurons having a comparable size and shape to non-SMA motor neurons (Thomsen et al., 2021). Issues such as cognitive and communicative development and early scoliosis and kyphosis have been investigated in AAV9 treated individuals with SMA Type I, with some concerns being raised (Soini et al., 2023). Multiple examples of partial rescue have also been reported in preclinical models. At the cellular level, our work investigating the proteomic alterations in heart tissues harvested from two mouse models of differing SMA severity revealed many differentially expressed

proteins pertinent to cardiovascular development and function, in particular the intermediate filament proteins, lamin A/C and desmin, and elastin (Brown et al., 2023). Treatment of *Smn*$^{2B/-}$ mice with either a Zolgensma-like AAV9-*SMN1* vector or an AAV9 encoding a novel sequence-optimized *hSMN1* (Nafchi et al., 2023) increased SMN levels, albeit to differing degrees (sub- and supraphysiological compared to WT levels, respectively). These treatments increased desmin to levels found in WT animals, but neither intervention restored lamin A/C or elastin abundance towards WT levels. Similarly, other studies have noted that AAV9 treatment does not address all phenotypic effects of low SMN. In SMA mice, neither distal ear nor tail necrosis nor spleen size were rescued following AAV9 treatment, suggesting some degree of cardiovascular impairment (Deguise et al., 2020). In human SMA astrocytes, glutamate transporter activity remained abnormal, which subsequently impacted motor neuron activity and synaptic health (Welby & Ebert, 2023). A caveat of the treatment is that over time, non-dividing cells appear to maintain their SMN levels, but in cells/tissues/organs undergoing turnover, *SMN* expression declines, possibly returning to the original levels (Chaytow et al., 2021).

SMN protein is ubiquitously expressed throughout the body and SMA is considered a multisystem condition, but there is limited knowledge regarding both the function of SMN beyond the CNS (Thomsen et al., 2021) and the level of SMN production required in peripheral organs and tissues (Xie et al., 2024). SMN is essential for many neurodevelopmental processes, but decreased SMN levels also impact the liver, muscle, heart, and pancreas in SMA mice models (Reilly et al., 2023), although for humans, the pathogenic implications are unclear (Thomsen et al., 2021) (Fig. 1). Since gene therapy can extend life expectancy for individuals with SMA, organs including the liver, pancreas and heart may need to be clinically monitored. In addition, SMN levels are much higher in tissues during gestation compared to postnatally suggesting a need during human prenatal development (Reilly et al., 2023). It follows that postnatal AAV9 administration may be too late to rescue all downstream effects of low SMN in severe SMA. This may include structural problems in the neuromuscular junction (Reilly et al., 2023) and increased glial activation due to loss of motor neurons and replacement with astrocytes (Thomson et al., 2021), and may also explain the continued dysregulation of lamin A/C and elastin that we observed in *Smn*$^{2B/-}$ mice following AAV9 treatment (Brown et al., 2023).

Current postnatal clinical delivery of AAV9-*SMN* has dose-dependent safety concerns of liver damage and is done with prophylactic prednisolone to minimise serum aminotransferase elevation. Also, pre-clinical studies have highlighted cases of neurodegeneration of the dorsal root ganglia (DRG) in nonhuman primates following high AAV doses, and in SMA mice overexpression of *hSMN* also led to loss of proprioceptive neurons in DRGs and motor neurons (as discussed in Xie et al., 2024). Other possible complications include transient thrombocytopenia, elevated troponin levels and fatal thrombotic microangiopathy. Similar dose-dependent effects have been reported in clinical and preclinical work using AAV vectors of several serotypes targeting other diseases (Servais et al., 2023). Therefore, key to addressing general complications with AAV treatment is the optimisation of the vector dose, regardless of cargo.

In the case of SMA, AAV9 delivery can lead to overproduction of SMN in some tissues compared to endogenous levels, and this may cause toxic effects (Zwartkruis & Groen, 2024). Work focussing on optimisation of *SMN1* delivery to replicate endogenous tissue levels is ongoing. A recent open-label, multicentre, single-arm, non-randomized, single-dose escalation clinical trial in China with EXG001-307, an AAV9-*SMN* therapy injected intravascularly at similar doses as Zolgensma but favouring neuronal transgene expression, has reported initial positive results (Wu et al., 2023). A more efficient, sequence optimized human *SMN1* cDNA has been recently described by Nafchi et al. (2023) and used with

integration-deficient lentiviral vectors (IDLVs) and AAV9 vectors in cell culture and in mice, demonstrating significantly higher expression levels than wild-type *SMN1* cDNA. By combining another codon-optimized version of the human *SMN1* cDNA with a derivative of the endogenous *SMN1* gene promotor, Xie et al. (2024) have shown better safety and improved efficacy, a broader therapeutic window, longer life span, increased cardiac, respiratory and motor function and minimal peripheral disease manifestations in a mouse model of SMA, compared to a Zolgensma-like vector. The challenge is how to evaluate these possible alternative therapies in clinical trials, considering that there are three effective marketed treatments for SMA, and therefore any use of experimental treatments could raise ethical concerns.

One future possibility for consideration would be the administration of AAV9 therapy into the developing foetus. Since the foetus is smaller than a neonate, the treatment dose required would be reduced, it may result in better biodistribution within the developing tissues, and immune tolerance for the treatment might be present. Neonatal screening for SMA is not universally available, however, and screening for SMA in unborn children would present additional challenges and may carry a risk of pregnancy loss. *In utero* delivery using IDLVs, with much reduced insertional mutagenesis risk compared to standard integrating vectors, has shown them to be efficient for spinal cord transduction in mice (Peluffo et al., 2012). An attempt at *in utero* treatment of SMA with AAV9 in mice, with vector delivery by intracerebroventricular injection yielded partial phenotypic rescue, but there was significantly lower survival to term of injected SMA foetuses than of injected wild-type or heterozygous mice (discussed in Waddington et al., 2024). Additionally, ultrasound-guided, foetal injections of AAV9 in pigs led to premature delivery, unlike saline injections, regardless of route of administration (intracerebroventricular, umbilical hepatic vein, intraperitoneal (reviewed by Waddington et al., 2024)). A number of hurdles will therefore need to be overcome before this type of prenatal treatment is ready for clinical testing, including ethical and technical issues.

In conclusion, it is becoming apparent that, at least in their current form, AAV9 gene therapy and *SMN2*-modifying treatments may not fully address all effects of low SMN levels and so there remains a need to develop optimised gene therapy methods and possibly treatments to complement gene therapies. As research efforts continue towards these goals, many fundamental questions will need to be addressed including establishing: the optimal time to provide gene therapy; whether this should start during gestation, at least in the most severe cases; how much SMN is required to benefit all cells/tissues/organs that are affected in SMA; whether differential expression of *SMN* in cells/tissues/organs can be obtained from one treatment or whether combining systemic and locally delivered transgene will help ensure that *SMN* expression is optimal in each relevant location; whether other treatments are needed in tandem or following gene therapy; and whether severity-specific and / or age-specific therapies are required to tackle different downstream consequences of SMA reduction throughout the disease. Core to fully addressing these questions is also a need for a comprehensive understanding of the natural history of SMA and how it evolves with the existing treatments at the molecular, cellular, and whole system level.

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LEGEND

Figure 1.

AAV9 therapy for SMA - Current & future perspectives (created with BioRender.com). Schematic illustrating the variability of outcome for SMA patients and impact on peripheral tissues following current AAV9 therapy (Current perspectives); unanswered questions regarding SMN levels during aging and in peripheral tissues, and the need for optimisation of AAV9 therapy (Research focus); 'Future perspectives' for SMA research to address timing and dosage of AAV9 delivery to optimise impact for SMA patients. AAV9: adeno-associated virus vector serotype 9; CNS: central nervous system; SMA: Spinal Muscular Atrophy; SMN: survival motor neuron; ↑ indicates an increase.

Covering Letter

To,

The Editor

Sub: Submission of Manuscript for publication

Dear Sir,

We intend to publish an article entitled **"Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct neurological and systemic perturbations"** in your esteemed journal as a Perspective Article.

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

Prior publication: **AAV9-mediated SMN gene therapy rescues cardiac desmin but not lamin A/C and elastin dysregulation in *Smn*$^{2B/-}$ spinal muscular atrophy mice**

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Conflicts of interest: Nil

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Manuscript Title: Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct neurological and systemic perturbations

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3 Dr Sharon Brown----------		30th April 2024

Title Page

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RJYM has filed a patent application for a novel sequence optimized *SMN1* cDNA.

Text

Title of the article:

Gene therapy for Spinal Muscular Atrophy: perspectives on the possibility of optimising *SMN1* delivery to correct all neurological and systemic perturbations

Main Text:

Spinal muscular atrophy (SMA) is a genetic condition that results in selective lower motor neuron loss with concomitant muscle weakness and atrophy. The genetic cause of SMA was understood in 1995 when loss or impairment of the *Survival Motor Neuron 1 (SMN1)* gene was identified as the main contributing factor (Lefebvre et al., 1995). This, in combination with the discovery that humans have a 'back-up' gene, *SMN2*, which can produce low levels (~10%) of the full-length functional SMN protein, has led to the generation of SMA-specific gene therapies. SMA was traditionally classified according to age of symptom onset and developmental milestones achieved, with life expectancy and severity varying between individuals. Now, *SMN2* copy number is used as a proxy for prediction of disease severity, with ~~a~~ higher *SMN2* copy number typically being associated with ~~a~~ reduced severity of SMA, ~~even~~ although this~~e~~ relationship is not absolute: ~~with~~ some individuals with low *SMN2* copy number having~~e~~ less severe SMA phenotypes and *vice versa.* ~~Thus~~Additionally, the aetiology of SMA is further complicated by other factors, such as~~,~~ non-typical nucleotide variants and *SMN2*-independent modifiers of disease severity. ~~.~~

Genetic therapies for SMA have generated a paradigm shift in expected outcomes, with substantial changes in survival and progression of the condition. This has ~~caused~~ led to a considerable ~~shift~~ rethink ~~in~~ of the research needs for individuals with SMA, as the natural history of the disease has changed with the therapies, and the new phenotypes are only beginning to be understood. These therapies either target the *SMN2* gene to increase functional SMN protein levels (Nusinersen/Spinraza® and Risdiplam/Evrysdi®) or directly increase *SMN* gene levels using adeno-associated virus serotype 9 (AAV9) administration of the *SMN* gene (onasemnogene abeparvovec/Zolgensma®). In particular, the one-off intravenous viral administration of the *SMN* gene has dramatically altered the natural course of SMA, especially in individuals treated pre-symptomatically (Strauss et al., 2022~~Paul et al., 2020~~). AAV9 gene therapy delivers full-length human *SMN* cDNA to peripheral organs and to the central nervous system (CNS) by crossing the blood-brain barrier and results in an increased production of functional SMN protein (Thomsen et al., 2021). Onasemnogene abeparvovec is a non-replicating self-complementary AAV9 vector controlled by the hybrid CMV enhancer/chicken beta-actin promoter that enables the transgenic *SMN* gene to reside within the nucleus as an extrachromosomal episome. ~~Following the treatment of two symptomatic infants with SMA Type I, who died from respiratory complications unrelated to onasemnogene abeparvovec, increased SMN production was detected throughout the CNS and peripheral organs in samples from necropsies, with motor neurons having a comparable size and shape to non-SMA motor neurons (Thomsen et al., 2021).~~

There is currently limited information regarding how well AAV9 treatment can restore CNS and systemic perturbations in SMA. Some individuals with SMA show very little or no response to treatment with gene therapy, whilst for just under half of those continuing to thrive over the course of treatment, a degree of symptomatic burden remains (STR1VE-US: NCT03306277 & STR1VE-EU: NCT03461289). Following the treatment of two symptomatic infants with SMA Type I, who died from respiratory complications unrelated to onasemnogene abeparvovec, increased SMN production was detected throughout the CNS and peripheral organs in samples from necropsies, with motor neurons having a comparable size and shape to non-SMA motor neurons (Thomsen et al., 2021). Issues such as cognitive and communicative development ~~(Ngawa et al., 2023)~~ and early scoliosis and kyphosis have

been investigated in AAV9 treated individuals with SMA Type I, with some concerns being raised (Soini et al., 2023). Multiple examples of partial rescue have also been reported in preclinical models. At the cellular level, our work investigating the proteomic alterations in heart tissues harvested from two mouse models of differing SMA severity revealed many differentially expressed proteins pertinent to cardiovascular development and function, in particular the intermediate filament proteins, lamin A/C and desmin, and elastin (Brown et al., 2023). Treatment of *Smn*$^{2B/-}$ mice with either a Zolgensma-like AAV9-*SMN1* vector or an AAV9 encoding a novel sequence-optimized *hSMN1* (Nafchi et al., 2023) increased SMN levels, albeit to differing degrees (sub- and supraphysiological compared to WT levels, respectively). These treatments increased desmin to levels found in WT animals, but neither intervention restored lamin A/C or elastin abundance towards WT levels. Similarly, other studies have noted that AAV9 treatment does not address all phenotypic effects of low SMN. In SMA mice, neither distal ear nor tail necrosis nor spleen size were rescued following AAV9 treatment, suggesting some degree of cardiovascular impairment (Deguise et al., 2020)~~;~~. ~~Other examples include the morphology of directly induced neurons produced from SMA patient fibroblasts being only partially rescued by AAV treatment (Sierra-Delgado et al., 2023), and i~~In human SMA astrocytes, ~~astrocytic~~ glutamate transporter activity ~~remaining~~ remained abnormal, which subsequently impacted motor neuron activity and synaptic health (Welby & Ebert, 2023). ~~One particular~~A caveat of the treatment is that over time, non-dividing cells appear to maintain their SMN levels, but in cells/tissues/organs undergoing turnover, *SMN* expression declines, possibly returning to the original levels (Chaytow et al., 2021).

SMN protein is ubiquitously expressed throughout the body and SMA is considered a multisystem condition, but there is limited knowledge regarding both the function of SMN beyond the CNS (Thomsen et al., 2021) and the level of SMN production required in peripheral organs and tissues (Xie et al., 2024). SMN is essential for many neurodevelopmental processes, but decreased SMN levels also impact the liver, muscle, heart, and pancreas in SMA mice models (Reilly et al., 2023), although for humans, the pathogenic implications are unclear (Thomsen et al., 2021) (Fig. 1). Since gene therapy can extend life expectancy for individuals with SMA, organs ~~like~~ including the liver, pancreas and heart may need to be clinically monitored. In addition, SMN levels are much higher in tissues during gestation compared to postnatally suggesting a need during human prenatal development (~~Burlet et al., 1998~~Reilly et al., 2023)~~. and a lack of SMN during embryonic development resultings in phenotypic and molecular perturbations, further supportingindicating a crucial role for SMN during pre-symptomatic embryonic development (Motyl et al., 2020). This ~~suggesting~~It follows~~s~~ that postnatal AAV9 administration may be too late to rescue all downstream effects of low SMN in severe SMA. This may include~~, for example, such as~~ structural problems in the neuromuscular junction (Reilly et al., 2023) ~~or~~ and increased glial activation due to loss of motor neurons and replacement with astrocytes (Thomson et al., 2021), and may also explain the continued dysregulation of lamin A/C and elastin that we observed in *Smn*$^{2B/-}$ mice following AAV9 treatment (Brown et al., 2023).

Current postnatal clinical delivery of AAV9-*SMN* has dose-dependent safety concerns of liver damage and is done with prophylactic prednisolone to minimise serum aminotransferase elevation. Also, pre-clinical studies have highlighted cases of neurodegeneration of the dorsal root ganglia (DRG) in nonhuman primates following high AAV doses ~~(Tukov et al., 2022)~~, and in SMA mice overexpression of *hSMN* also led to loss of proprioceptive neurons in DRGs and motor neurons (as discussed in Xie et al., 2024~~Van Alstyne et al, 2021~~). Other possible complications include transient thrombocytopenia, elevated troponin levels and fatal thrombotic microangiopathy. Similar dose-dependent effects have been reported in clinical and preclinical work using AAV vectors of several serotypes targeting other diseases (Servais et al., 2023). Therefore, key to addressing general complications with AAV treatment is the optimisation of the vector dose, regardless of cargo.

In the case of SMA, AAV9 delivery can lead to overproduction of SMN in some tissues compared to endogenous levels, and this may cause toxic effects (Zwartkruis & Groen, 2024). Work focussing on optimisation of *SMN1* delivery to replicate endogenous tissue levels is ongoing. A recent open-label, multicentre, single-arm, non-randomized, single-dose escalation clinical trial in China with EXG001-307, an AAV9-*SMN* therapy injected intravascularly at similar doses as Zolgensma but favouring neuronal transgene expression, has reported initial positive results (Wu et al., 2023). A more efficient, sequence optimized human *SMN1* cDNA has been recently described by Nafchi et al. (2023) and used with integration-deficient lentiviral vectors (IDLVs) and AAV9 vectors in cell culture and in mice, demonstrating significantly higher expression levels than wild-type *SMN1* cDNA. By combining another codon-optimized version of the human *SMN1* cDNA with a derivative of the endogenous *SMN1* gene promotor, Xie et al. (2024) have shown better safety and improved efficacy, a broader therapeutic window, longer life span, increased cardiac, respiratory and motor function and minimal peripheral disease manifestations in a mouse model of SMA, compared to a Zolgensma-like vector. The challenge is how to evaluate these possible alternative therapies in clinical trials, considering that there are three effective marketed treatments for SMA, and therefore any use of experimental treatments ~~would~~ could raise ethical concerns.

One future possibility for consideration would be the administration of AAV9 therapy into the developing foetus. Since the foetus is smaller than a neonate, the treatment dose required would be reduced, it may result in better biodistribution within the developing tissues, and immune tolerance for the treatment might be present. Neonatal screening for SMA is not universally available, however, and screening for SMA in unborn children would present additional challenges and may carry a risk of pregnancy loss. *In utero* delivery using ~~integration-deficient lentiviral vectors (~~IDLVs~~)~~, with much reduced insertional mutagenesis risk compared to standard integrating vectors, has shown them to be efficient for spinal cord transduction in mice (Peluffo et al., 2012). An attempt at *in utero* treatment of SMA with AAV9 in mice, with vector delivery by intracerebroventricular injection~~, has~~ yielded partial phenotypic rescue, but ~~(Rashnonejad et al., 2019). O~~of ~~concern~~there was ~~the~~ significantly lower~~,~~ survival to term of injected SMA foetuses ~~to term was significantly lower~~ than ~~that~~ of injected wild-type or heterozygous mice (discussed in Waddington et al., 2024). Additionally, ultrasound-guided, foetal injections of AAV9 in pigs ~~have~~ led to premature delivery, unlike saline injections, regardless of route of administration (intracerebroventricular, umbilical hepatic vein, intraperitoneal~~;~~ (reviewed by Waddington et al., 2024~~Rich et al., 2022~~)). A number of hurdles will therefore need to be overcome before this type of prenatal treatment is ready for clinical testing, including ethical and technical issues.

In conclusion, it is becoming apparent that, at least in their current form, AAV9 gene therapy ~~or~~ and *SMN2*-modifying treatments may not fully address all effects of low SMN levels and so there remains a need to develop optimised gene therapy ~~delivery~~ methods and possibly treatments to complement gene therapies. As research efforts continue towards these goals, many fundamental questions will need to be addressed including establishing: the optimal time to provide gene therapy; whether this should start during gestation, at least in the most severe cases; how much SMN is required to benefit all cells/tissues/organs that are affected in SMA; whether differential expression of *SMN* in cells/tissues/organs can be obtained from one treatment or whether combining systemic and locally delivered transgene will help ensure that *SMN* expression is optimal in each ~~cell/tissue/organ~~relevant location; whether other treatments are needed in tandem or following gene therapy; and whether severity-specific and / or age-specific therapies are required to tackle different downstream consequences of SMA reduction throughout the ~~natural history of~~ disease. Core to fully addressing these questions is also a need ~~also~~ for a comprehensive understanding of the

natural history of SMA and how it evolves with the existing treatments at the molecular, cellular, and whole system level.

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LEGEND

Figure 1.

AAV9 therapy for SMA - Current & future perspectives (created with BioRender.com). Schematic illustrating the variability of outcome for SMA patients and impact on peripheral tissues following current AAV9 therapy (Current perspectives); unanswered questions regarding SMN levels during aging and in peripheral tissues, and the need for optimisation of AAV9 therapy (Research focus); 'Future perspectives' for SMA research to address timing and dosage of AAV9 delivery to optimise impact for SMA patients. AAV9: adeno-associated virus vector serotype 9; CNS: central nervous system; SMA: Spinal Muscular Atrophy; SMN: survival motor neuron; ↑ indicates an increase.

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