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The challenge of gene therapy for neurological diseases: strategies and tools to achieve efficient delivery to the central nervous system

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Abstract

For more than ten years, gene therapy for neurological diseases has experienced intensive research growth and more recently therapeutic interventions for multiple indications. Beneficial results in several phase 1/2 clinical studies, together with improved vector technology have advanced gene therapy for the central nervous system (CNS) in a new era of development. While most initial strategies have focused on orphan genetic diseases, such as lysosomal storage diseases, more complex and widespread conditions like Alzheimer's disease, Parkinson's disease, epilepsy or chronic pain are increasingly targeted for gene

therapy. Increasing numbers of applications and patients to be treated will require improving and simplifying gene therapy protocols to make them accessible to the largest number of affected people. While vectors and manufacturing are a major field of academic research and industrial development, there is a growing need to improve, standardize and simplify delivery methods. Delivery is the major issue for CNS therapies in general, and particularly for gene therapy. The blood brain barrier restricts the passage of vectors; and strategies to bypass this obstacle are a central focus of research. Here, we present the different ways that can be used to deliver gene therapy products to the CNS. We focus on results obtained in large animals that have allowed the transfer of protocols to human patients and have resulted in the generation of clinical data. We discuss the different routes of administration, their advantages and their limitations. We describe techniques, equipment and protocols and how they should be selected for safe delivery and improved efficiency for the next generation of gene therapy trials for CNS diseases.

Introduction

The central nervous system (CNS) is protected by a unique microvasculature, the blood-brain-barrier (BBB), composed of endothelial cells connected by tight junctions and adherent processes. The BBB controls brain homeostasis as well as ion and molecule movements thus protecting the CNS against potential intruders. The restrictive nature of the BBB provides an obstacle for drug delivery to the CNS, and major efforts have been made to generate methods to modulate or bypass the BBB for delivery of therapeutics. Contrarily, some pathologies of the CNS including stroke, multiple sclerosis, brain traumas and neurodegenerative disorders, alter the BBB causing it to become more permeable, allowing the entry of molecules that can induce inflammatory responses and lead to neuronal damage^{1,2}.

Gene therapy has been applied to several CNS diseases, including neurodegenerative³ and neurodevelopmental disorders^{4,5}, but also increasingly for diverse conditions such as epilepsy⁶, glioblastoma⁷ and pain⁸. Increasingly, gene therapy products can be tailored to counter the pathophysiological mechanisms of particular disease mechanism, including the use of gene replacement^{9–11,12,13}, gene silencing¹⁴, transplicing¹⁵, modulation of cellular pathways to improve phenotype^{16–19} or expression of suicide gene²⁰.

Gene therapy products can be delivered by various routes of administration, using either *ex vivo* or *in vivo* strategies. *Ex vivo* gene therapy involves autologous transplantation of hematopoietic stem cells corrected by genetic modification of lentivirus (HSC-GT) outside the body and subsequent transplantation of the cells back into the patient. HSC-GT has shown Efficacy for HCS-GT has been shown in clinical trials for leukodystrophies (ALD, MLD)^{21,22}. Therapeutic action involves either production of the therapeutic protein by donor derived cells that can migrate into the CNS (permanent source of the missing enzyme), and/or modulation of the immune environment (replacement of microglial cells and/or perivascular macrophages). *In vivo* gene therapy requires direct introduction of the vector (carrying the therapeutic gene) into the patient (Figure 1). Intravenous administration is a common route of administration for *in vivo* gene therapy but for CNS diseases the the limited capacity of gene delivery systems to cross the BBB remains a significant obstacle Strategies such as disruption of the BBB integrity (by osmotic or biochemical means) or improvement of viral vector capsids continue to be developed toto enhance peripheral administration. Direct delivery into the parenchyma of the brain or the cerebrospinal fluid (CSF) bypasses the BBB and permits more targeted gene delivery . Following intrathecal or intracerebroventricular administration, the therapeutic vector enters the cerebrospinal fluid and is delivered throughout the CNS (at least to tissue adjacent to CSF spaces). Following intraparenchymal administration, the therapeutic vector enters the brain parenchyma and is delivered locally into brain cells. The diffusion of the therapeutic product is limited around the injection site; however, secretion-uptake may improve diffusion, notably the case for most lysosomal storage disease enzymes.

The vector of choice for *in vivo* CNS delivery is the adeno-associated viral (AAV) vector. Recombinant AAVs have been widely used for CNS gene therapy, demonstrating safety, stable and long-term expression and some degree of neuronal tropism relevant for many therapeutic applications. A large body of preclinical results have been obtained, particularly in large animals like dogs, cats and non-human primates that have demonstrated feasibility for clinical use²³.

Wild-type AAVs are non-enveloped parvovirus, which are characterized by an icosahedral capsid and a 4.7 kb single stranded DNA genome. To complete a replication cycle, AAVs require coinfection by a helper virus like adenovirus or herpes virus. AAVs infect humans and other species including the non-human primate (NHP)²⁴. Natural infection with AAV is not

known to lead to disease, although there is controversy concerning hepatocellular carcinoma^{25,26}. The minimal sequence needed to generate Recombinant AAVs is restricted to the 145 bp within the inverted terminal repeats (ITRs) flanking the transgene²⁷. The overall capacity of AAV to package an ITR-flanked genome productively is the approximate size of the wild-type AAV genome (i.e., 4.7 kb).

Overall AAV vectors have been used and have proven their safety and low immunogenicity tolerance in more than 200 human studies²⁸. Efficiency of an AAV administration is determined mainly by the capsid, that directs the tropism of the virion, but can be impacted by the route of administration²⁹. It is also well established that the specificity of transgene expression is dependent on both the capsid and the regulatory elements present in the vector³⁰. There are a large number of AAV serotypes based on capsid structure³¹. AAV2, the first AAV serotype to be used as a replication-defective vector, has been the most extensively characterized. Other AAV serotypes developed later as vectors, employ a cross-packaging system, in which genomes flanked by AAV2 ITRs are packaged in other AAV capsids. These serotypes have a wide variety of tissue and cell tropism³². For gene transfer to the CNS the most frequently used capsids have been AAV1³³, AAV2^{34–36}, AAV5³⁷, AAV9¹² and AAVrh.10^{9,38}. AAV9 was shown to naturally cross the BBB and allowing for widespread, but limited expression of therapeutic genes in the CNS after a single intravenous injection of vector^{39,40}. Importantly, the first clinical report of gene-replacement therapy for SMA type I has demonstrated the safety and efficacy of this approach⁴¹. However, the efficacy of brain transduction efficacy may vary with the patient age at treatment. Ongoing efforts to engineer capsids with improved capacity to cross the BBB. AAV variants^{42,43} such as AAV.PHP.B and AAV-B1^{41,44} have resulted in superior capacity for CNS targeting, at least in the animal models tested⁴⁵.

Lentiviral vectors (LV) are members of the Retroviridae family, and based either on human immunodeficiency virus (HIV), non-primate primates (SIV), or others such as equine infectious anemia virus (EIAV)⁴⁶. The viral genome contains two long terminal repeats (LTR), with elements required for gene expression, reverse transcription and integration into host chromosome⁴⁷. For safety and efficacy, third generation of LVs do not have viral genes. Gag, Pol and Rev genes are present in the packaging construct that allow the production of the recombinant vector but not in the transgene construct. Different pseudotypes of LV with different envelopes allowing for different viral tropism have been developed. The most

frequently used is the vesicular stomatitis virus glycoprotein (VSV-G), which has a broad tropism *in vitro* and neuronal and glial tropism *in vivo*^{48,49}. Lentiviruses are able to penetrate the intact nuclear membrane through nuclear pores, do not require cell division and can efficiently infect quiescent cells^{50,51}. This ability to transduce dividing and non-dividing cells, long-term stable expression through transgene integration into the chromosomes of host cells, and their large cloning capacity make LVs desirable vectors for gene therapy⁵². They also have a cloning capacity of 9.7kb.

LVs are particularly useful for ex vivo gene therapy applications. Hematopoietic stem cells (HSCs) can be stably transduced using lentiviral vectors; allowing for stable, indefinitely persisting expression within the host cell, despite repeated cell division. This characteristic has been widely applied to CNS lysosomal storage diseases or Adrenoleukodystrophy^{53,54}. Random integration in the genome of host cells is associated with a potential genotoxicity risk, as previously observed with retroviruses^{55,56}. However, long-term follow-up of gene therapy trials has not identified adverse events associated with insertional mutagenesis^{57–59}. *In vivo* use of lentivectors for CNS applications has been more limited. Because of their capacity to transduce neurons, they have been tested for the treatment of Alzheimer's disease (AD) and Parkinson's disease (PD)^{60–62}. For this review, we focused more on AAV since they have been more widely used in CNS targeting for large animals and human clinical trials.

Evaluation of administration routes in preclinical studies

Translation to clinical application after proof-of-concept in mouse models of the disease most often requires efficacy and tolerance studies in large animals.

Many species have been used⁶³ for preclinical development. Affinity for the different types of brain cell in large animal species remains a major issue. The choice of the large animal model to be used is mainly based on the use of a large animal model of the disease when available^{64–67} otherwise on anatomical aspect to mimic the best situation to be reproduced for future clinical trials.

When the gene therapy product is administered by injection in the blood stream or the CSF any suitable species of large animal can be employed. However, when delivery is based on direct intra-parenchymal injection into the brain for characterizing biodistribution, toxicity, diffusion, affinity with different types of brain cells, pigs, sheep and non-human primates (NHP) are preferred species for modeling trajectories and efficient targeting. In all cases it

1 must be emphasized that the use of large animals is strictly limited for ethical reasons and
2 should be performed in GLP or GLP-like conditions to be usable for regulatory documents.

4 Intraparenchymal delivery

5 The principle studies of intra-parenchymal (IP) gene delivery in large animals are
6 summarized in Table 1. Direct IP injection – either direct perfusion or convection enhanced
7 delivery (CED)- allows targeted delivery in CNS regions while bypassing the BBB. However, IP
8 injections remain more complex to perform than ICV or IT techniques for several reasons⁶⁹.
9 The choice of the target region is always a challenging balance between efficacy and safety.
10 Therefore, the specific anatomy as well as pathophysiology of the disease should be
11 considered when choosing target sites.. Targeting the white matter in multiple sites may
12 enhance vector spreading¹¹. In particular, AAV vectors are readily transported along axons
13 which facilitate the distribution of the therapeutic gene⁷⁰. The spreading and directionality of
14 AAV transport are serotype-dependent⁷¹. AAV2, which has been widely used in IP gene
15 delivery, resulted in anterograde transport⁷² of vector particles from basal ganglia to cortex
16 in NHP^{73,74}. In contrast, AAV6 is axonally transported exclusively in a retrograde
17 direction⁷² while AAV9 shows a bidirectional transport and is dose dependent⁷⁵.

18 Recent studies suggest that AAV5 and AAVrh10 have more global transduction with
19 widespread distribution in the brain^{11,76,77} and spinal cord. Lentiviral vectors are able to deliver
20 the therapeutic gene in a restricted area^{78,79}.

22 Intracerebrospinal fluid delivery

23 As an alternative to intraparenchymal delivery and to target larger brain or spinal cord
24 volumes, especially in neurodegenerative diseases involving widespread regions, an
25 alternative to intraparenchymal injection is intra-CSF delivery.

26 For this purpose, three major routes can be used: intracerebroventricular delivery into
27 the ventricles, the cisterna magna as widely used in large animal models (Table 1) or by
28 intrathecal delivery into the CSF surrounding the spinal cord (Table 2).

29 Lentiviral vectors have been used in one approach in adult sheep, but this reported
30 attempt resulted in very limited transduction close to the needle track with up to 2.5mm
31 rostro-caudal transduction⁸⁰. AAVs lead to greater transduction especially in adults. ICV
32 delivery in young animals differ, providing extensive transduction of motorneurons in some

1 cases. Using scAAV9 in 5-days old pigs⁸¹ and in 10-11 days old dog, Katz et al. reported a TPP1
2 activity increase in CSF a few days after transduction, but then loss of the transgene due to
3 immune response against the transgene product⁸². AAV9 and AAVrh10 have been
4 predominantly injected with doses ranging from 10^{12} vg to 5.10^{13} vg in dogs, NHP and cat^{70,83–}
5 ⁹¹ mainly in the *cisterna magna* with either GFP or therapeutic genes (Table 2). Diffuse
6 transgene expression was obtained in the cortex and cerebellum (Table 2). In most studies,
7 efficacy of spinal cord targeting was not analyzed; however, available studies report highly
8 variable results with large motoneuron transduction⁹².

9 As an alternative to ICV delivery or depending on the pathology to improve spinal cord
10 targeting, delivery in the CSF can be achieved through intrathecal injection. Studies have been
11 performed in dogs^{89,93,94}, NHP^{13,88,95}, pigs^{87,96} and marmosets⁹⁷ mainly using AAV9 or AAVrh10
12 vector in neonates (5 day-old) to adult animals (Table 3). Two major techniques are routinely
13 used for intrathecal delivery, either a single delivery without the use of a catheter, generally
14 performed in the L4/L5 space, or with prior insertion of a catheter to allow either single or
15 multiple deliveries^{87,98}. Intrathecal delivery leads to efficient motoneurons transduction with
16 efficiency ranging from 10-30%⁸⁷ to 80%¹³ depending on the study (Table 3), but sparse
17 transduction in the brain (Table 3). Two studies have evaluated placing the animal in the
18 Tredelenburg position with the feet elevated above the level of the head to improve the
19 upward diffusion of the vector. Variable results were reported, one showing no
20 improvement⁹⁹ and the other one a slight increase, with up to 55% targeting of cervical
21 motoneurons¹³ (Table 3).

22 Even if ICV delivery is designed to target basal ganglia (Figure 1d), intracisternally to
23 target the cortex (Figure 1c) and intrathecal delivery for the spinal cord (Figure 1b), the direct
24 relationship between the site of injection and the efficient delivery to the target has never
25 been proven, and appear to different between NHP and human in relation to the volume of
26 the respective brains and the distance between CSF pathways and the CNS targets.

27 In between intraparenchymal and intra-CSF delivery, for intraspinal targets, spinal
28 subpial delivery has been proposed in mice, rats and pigs^{100,101}. Despite the short distances
29 for spinal diseases, the primary issues remain the need for an open micro-neurosurgical
30 approach following laminectomy (or a laminotomy) and the risk of neurological deficits
31 secondary to spinal cord subpial bleeding.

Intravenous delivery

As an alternative to more invasive injections, IV delivery has recently become an option to target the CNS, especially with the SMN trial⁴¹. AAV9 and AAVrh10 are once again the major serotypes injected dosing from 10^{12} vg/kg up to 10^{14} vg/kg. In neonates (2 to 7 days-old) studies have demonstrated up to 39% transduced motoneurons in cats⁸¹ but other studies were unable to detect the transgene⁹² (Table 4). In adults differ with mild to no neuronal transduction^{85,94} either in spinal cord and brain (Table 4). Transduction was in any case much lower compared to ICV⁸⁸ or intrathecal delivery and glial cells were predominantly transduced^{90,96}, without clear explanation.. In addition, IV delivery led to an significant transduction of peripheral organs (Table 4).

Routes of administration in clinical studies

While intravenous delivery is readily transferred from animal models to human trial conditions, CSF or brain delivery techniques must be carefully adapted for human conditions (brain anatomy and volumes). ICV, cisternal and intrathecal methods, can however all be used in human subjects. There is very little intraventricular vector diffusion after cisternal or intrathecal injection; most of the product remains in the spinal compartment or reaches the peri-cerebral space. Because of the size of the brain and the distance between the ventricles and the cortex, it is impossible to achieve consequent and homogenous diffusion by any CSF route.

Intraparenchymal injection is possible in the human brain, in deep nuclei, or in the white matter (but may need multiple simultaneous injections to obtain sufficient diffusion), but there is no reliable technique for delivering to the spinal cord parenchyma.

The doses of vector delivered depends on the route of administration. While intraparenchymal delivery requires small amounts of vector ranging from 10^9 to $2.5 \cdot 10^{12}$ total vg in NHP and human, doses from 10^{12} to $5 \cdot 10^{13}$ vg are required for ICV or intrathecal delivery. Intravenous delivery dramatically increases the number of particles required; from $5 \cdot 10^{12}$ to $5 \cdot 10^{14}$ vg (10^{12} to 10^{14} vg/kg) in NHP and close to 10^{15} vg in patients in the SMA trial ($2 \cdot 10^{14}$ /kg) (see tables 1-6).

Local administration of low doses of vector limits biodistribution and the risk of immunogenicity or toxicity due to AAV capsid or expression of the transgene. The high doses

required for intra-CSF and IV administration raise issues of immunogenicity, manufacturability and final cost.

Translating gene therapy proof of concept in animal models to clinical application in patients requires adapting delivery protocols. Translation may be simple for intra-CSF or IV delivery, based on the weight of the animal or the volume of the brain tissue or of the CSF. Modeling the delivery into the brain parenchyma is more critical for designing a clinical protocol. This should take into consideration the volume of the target region determined by imaging, the sites and the number of injections, the degree of anatomical precision required, the volumes to be injected, and the flow (constant or CED). Translating these parameters from large animal brain to human brain requires specific anatomical adaptations (needle track in particular) and evaluation of feasibility, safety and efficiency in terms of biodistribution in animal models under conditions as close as possible to the human clinical procedure.

There is no limits for age, and theoretically, it is possible to treat even newborns by all routes of delivery. It is possible to use frameless stereotactic delivery using magnetic or optic neuronavigation. In addition, robotic systems are now available even in the youngest subjects. The youngest child treated by our team with intracerebral delivery (16 targets supra and infratentorial) was 9 month-old⁹.

Intra cerebro-spinal fluid delivery

To overcome the inability of the gene therapy vectors to cross the BBB, direct injection within the CNS compartment has been attempted¹⁰². All trials using these routes of administration are summarized in Table 5. Three main modalities can be discussed:

- Intrathecal lumbar administration with 2 potential modalities:

(1) Direct unique injection by a lumbar tap or through an intraspinal catheter connected to a subcutaneous reservoir. The use of a single injection by lumbar tapping is a non-surgical procedure. It could be done, in a medical environment, with local anesthesia. In difficult cases, the use of Fluoroscopy or Ultrasonography⁶⁸ can facilitate the procedure (or to guide a catheter insertion). The main inconvenience of this technique is the risk of CSF leak in the extradural space at the moment of needle removal and the one to two days post procedure. Consequently, it is impossible to precisely control the quantity of vector

administered within the intradural space. The use of atraumatic G22 needle (Sprotte) can minimize this risk⁶⁸.

(2) To avoid this major inconvenience, the alternative is to install a subcutaneous reservoir connected to an intraspinal catheter⁶⁸. This procedure must be done in the operative room, under general anesthesia. Injection must be performed one or two weeks after the initial surgery and the removal of the system at least one week after. This technic guaranties control of the injected volume, but needs two general anesthetics and increases the risk of infection. The main incertitudes of this technique is determining the exact distribution of the product between the different compartments; intraspinal CSF, intraventricular and intracranial subarachnoidal spaces (Figure 1). The main disadvantage of the ITL access results from the natural flow of CSF from the intraventricular choroid plexus to the subarachnoid space around the spinal cord and finally to the pericerebral spaces for resorption through the venous system. Reaching the brain target through the CSF stream requires a large dose volume. Other associated strategies can be used to improve the efficacy such as a buffer flush or a Tredelenburg position. Complications due to the device and its implantation also have to be considered. Meningitis can be a severe infectious complication that needs removal of the system and adapted antibiotics treatment. Other mechanical complication can be encountered such as migration, rupture or disconnection, kinked or obstructed catheter. A CSF leak around the catheter to the subcutaneous space can lead to an artificial meningocele that can impair the ability to infuse the treatment. To overcome this problem, two other modes of administration have been proposed.

- Intracisternal administration^{103,104}:

In most cases, it is possible to inject within the cisterna magna at the level of the craniovertebral junction. Even if it is possible to do it while the patient is awake, general anesthesia will be preferred, especially in the pediatric population. The risk of leakage is very low through this route and in most cases the reservoir is useless.

- Intraventricular administration

A third way route to the CSF space is to inject directly within the cerebral ventricles. In most cases, it will be done in the frontal horn by direct puncture under stereotactic guidance or more frequently now under frameless neuronavigation. In adults, it is

easily doable under local anesthesia and light sedation, in the pediatric population general anesthesia is preferable. Installation of an intraventricular catheter connected to a reservoir may be preferred, however for a single injection, direct injection is safe and efficient. It is also better for controlling the volume of injection and its distribution. However, because of the risk of parenchymal bleeding, most authors prefer the cisternal approach.

Intraparenchymal delivery

Few human trials using the intracerebral route are reported (Table 6); Both in adults (Huntington, Parkinson and Alzheimer diseases) and children (SMA, Canavan, Batten, mucopolysaccharidosis, MLD). The vast majority of the trials have employed AAV vectors (from 10^7 to 10^{13} vg) with injections in the deep grey nuclei or in the white matter (only for metabolic diseases in children). Most published trials are Phase 1/2 and one is phase 3. Several studies obtained promising results even if partial or preliminary. Importantly, few severe adverse events have been reported.

Technical aspects: state of the art

Regarding delivery technique, we focus here on intracerebral delivery, indeed, other routes of delivery have been largely described previously and have not been researched as intensively.

Devices for intraparenchymal delivery of therapeutics agents

Intraparenchymal drug delivery systems offer a practical method for bypassing the BBB to deliver gene therapy. Direct access to parenchyma allows delivery at doses and concentrations that would otherwise correspond to very high levels and volumes systemically. However, this method still has constraints and limitations. For more than ten years, considerable research has focused on developing methods to enhance drug delivery¹⁰⁵, through dedicated intraparenchymal devices.

Principles: Intraparenchymal delivery has two principle challenges : minimize backflow along the injection device and promote optimal drug distribution¹⁰⁶, often in a spherical tissue volume. The distribution volume depends on infusion flow rate, infusion volume and number of injection sites and the type of vector that is injected¹⁰⁷. The occurrence of backflow depends on several variables including cannula radius, infuse flow rate, and tip location¹⁰⁸. Indeed,

many parameters influence the safety and efficiency of infusion: infusion flow rate, cannula size, infusion volume, and drug molecular size/charge¹⁰⁹. Variations in flow rate impact the location of infusate distribution. Lower infusion rates (under 1 $\mu\text{l}/\text{min}$) are associated with distribution localized primarily to the target tissue (they are used for focal injection in specific areas, mainly in grey matter nuclei) whereas higher infusion rates result in increased distribution into the surrounding parenchyma («overflow»)^{106,109}. Several techniques have been proposed to increase infusion rates (up to 10 $\mu\text{l}/\text{min}$); among them the use of CED¹¹⁰ (convection enhanced delivery) which appears to provide the best compromise for extensive diffusion with minimal local damage. They are used combined with multiple injections (up to 16)⁹ when the whole brain must be treated (lysosomal diseases)¹¹. Increases in infusion rates raise the local pressure around the infusion site and also increases the extent of backflow. Cannula size seems to have no effect on distribution ; however, larger cannulas cause more tissue damages and therefore produce decreased resistance pathways along the brain parenchyma-catheter interface that are associated with increased rates of backflow¹⁰⁹.

Catheters subtypes: Three kind of delivery devices can be distinguished : catheters derived from another use (especially intraventricular devices), «homemade» designed catheters^{11,38,105,111} from teams experimenting with pre-clinical intracerebral drug delivery or for early clinical studies³⁸, and more recently commercialized catheter for specific intraparenchymal use (often developed from the homemade devices, and for intracerebral chemotherapy). Principal available devices are described in Table 7.

Ventricular catheters (2–3 mm outer diameter) that have been implanted in clinical trials to treat glioma have failed to distribute effectively and have been linked to poor distributions^{112,113}. Microcatheters (less than 1mm outer diameter) seem more reliable, and all FDA approved catheters for CED belong to this category.

Design: Recent catheters have been designed to reach the goals of parenchyma delivery : minimize invasiveness through a minimal diameter tubing (Casanova), optimize infusion parameters to maximize distribution volume, and a reflux inhibiting feature is required to halt backflow along the catheter entry track¹¹². All these characteristics are summarized in Table 7. Means to minimize backflow are the following : polymer-impregnated tips^{105,114}; stepped-design cannula^{108,115,116} Recessed-design cannula^{112,117} (Bristol). Means to enhance delivery are: multiporous cannula, multiport catheters, mobile-tip catheter, balloon-type cannula. A ‘valve tip’ has been proposed to prevent blockage by occluding the inner bore

of the cannula with a stylet during insertion¹⁰⁶. Another new advance is a multi-site delivery catheter with one single tube¹¹⁸ (CMC, IMI) that allows multiple targeting points and more homogenous delivery in a three dimensional array.

Pumps and syringes: To enable injection of small volumes at precise, low speeds, specific pumps and syringes must be employed. For pumps, most are manufactured for research use (Harvard Apparatus, Holliston Massachusetts, USA), and special authorizations must be obtained for clinical use.

Injection systems: Combining precision delivery and limited procedure time.

Classical stereotactic technics are well adapted for injection in a unique site of brain parenchyma - however, new techniques allowing multiple brain injections of the gene therapy for many indications are needed. Specific 3D MR sequences allow precisetargeting in white matter or in deep grey matter nuclei (striatum, thalamus, caudate nucleus...). Preplanning using neuronavigation software (Medtronic® or Brainlab®) facilitates the choice of trajectories, modeling delivery and rehearsingthe surgical procedure. Optic or magnetic neuronavigation system with or without robotic tools (Rosa®, Renishaw®, Medtronic®, Brainlab®) allows frameless insertion of multiple cannulas, supra or infra-tentorially according to the preplanning. Altogether these systems allow the delivery of therapeutic product reproducibly with high precision (around 1mm). Intra-operative Real-time MRI has also been proposed as an additive tool to verify the position of the cannula and to check the diffusion of the product with simultaneous gadolinium injection^{119,120}, albeit with the risk of long term toxicity.

Risks assessment

Linked to the delivery procedure

Risks due to anesthesia are mainly correlated to the disease itself and related comorbidities. All injections or catheter placement can result in a CNS injury or hemorrhagic complications. Lumbar puncture is at very low risk of complication if done at the low lumbar level without spinal cord anomalies such as low tethered cord. Epidural hematoma can occur, especially with repeated punctures. Spinal epidural hematoma can remain asymptomatic but sometimes cause radicular pain and even rare motor impairment or sphincter dysfunction in case of cauda equina syndrome. A motor impairment requires a surgical evacuation. Lumbar

puncture can cause subarachnoid bleeding leading to radiculitis, pain and headache. In case of spinal deformity, fluoroscopy or ultrasonography may help to guide the puncture.

Intrathecal catheter placement needs the use of a guide wire to conduct the catheter to the thoracic level or higher and can result in a spinal cord injury and cause motor or sensitive (transient in the vast majority of the cases) dysfunction. The risk of a hemorrhagic complication in the epidural or intradural space is higher in relation to the larger size of the needle.

Intracranial hypotension symptoms can occur secondary to a lumbar puncture and cause severe orthostatic headaches which can be managed with supine position, painkillers and if needed, blood transfusion.

Intracisternal puncture requires an adequate cisternal space (verified by MRI), a motionless situation and an experienced operator. The size of the cisterna magna and the shape of the skull, a Chiari anomaly or a foramen magnum stenosis increase the risk of neurological complication (Bulbomedullary junction injury responsible for cardiorespiratory arrest).

Intraventricular access is performed through a right frontal of the brain. Bleeding can occur along the trajectory at each level: sub cutaneous hematoma or epidural hematoma are rare. Subdural hematoma can occur by direct bleeding or an intracranial hypotension secondary to a CSF loss, intraparenchymal hematoma can be asymptomatic but may, if extended, lead to motor or cognitive impairment. Intraventricular bleeding may be responsible for headaches and sometimes secondary hydrocephalus.

Linked to the treatment

Gene therapy using viral vectors is mainly hampered by immunogenicity^{121–125} particularly with AAV. Indeed, many people have already been infected by wild-type AAV once in their life, inducing anti-capsid neutralizing antibodies (NAb) spread among various serotypes^{126–129}. Moreover, cross-reactive immunologic material (CRIM) status is also important to predict clinical response. CRIM-negative patients with null mutations or out of frame stop codons are completely unable to produce protein [that has to be supplied/involved in their disease] and are therefore more predisposed to develop an anti-transgene response^{130,131}. Such host immune responses, particularly happening when intravenous and intramuscular injections are performed, significantly impair delivery of the therapeutic protein and be possibly deleterious

for patients^{132–134}. Delivery of AAV vectors directly into the brain induce low or absent anti-capsid or anti-transgene NAb titers in serum^{119,135–138}. Thus, it must be considered for patient inclusion criteria and study design in clinical trials. Conventional strategies to prevent immunogenicity include corticosteroids or immunosuppressive drugs. Corticosteroids (e.g. methylprednisolone, prednisone) and immunosuppressive drugs (e.g. sirolimus, tacrolimus, rituximab, bortezomib, mycophenolate, cyclosporine) are administered post-injection and eventually in pretreatment in several clinical trials^{41,139,140}, though their effects are controversial^{141,142}. Other approaches are emerging such as plasmapheresis^{141,142}, editing AAV capsids to eradicate epitopes that induce NAb generation¹⁴³, using tolerogenic nanoparticles^{144,145}, and the incorporation in the transgene of microRNAs (miR) target sequences integrated in the expression cassette that specifically repress translation in antigen-presenting cells (APCs)^{146,147} or oligonucleotide sequences that inhibit toll-like receptor 9 (TLR9) activation (in development by George Church's laboratory, Harvard Medical School, patent WO2017214378A1).

Future development and perspectives

New AAV serotypes with broad CNS transduction after IV delivery

Over the last decade, significant efforts have been expended in updating the natural repertoire of viral vectors as well as engineering new serotypes¹⁴⁸. Using the Cre recombination-based AAV targeted evolution (CREATE) technique, new AAV variants have been isolated, some able to homogeneously transduce the central nervous system, especially neurons and astrocytes, after intravenous injection in mice^{149,150}, and potentially in NHP although clear evidence of efficacy remain to be demonstrated. Improved CNS transduction is linked to Ly6 which is not expressed in all mouse lines and not in NHP^{151,152}. Indeed a major challenge is to identify capsids that will be able to efficiently pass the BBB and in mice, NHPs and human subjects. In addition, the selected capsids should be compatible for large scale production.

Blood Brain barrier opening as a solution to enhance CNS targeting

Temporary disruption of the BBB might help delivery in the brain parenchyma. Osmotic disruption of the BBB with intra-arterial injection of mannitol has been widely studied and allows for delivery of a variety of drugs and agents into the CNS, including viral vectors¹⁵³.

1 However, this technique induces a diffuse opening of the BBB, precluding for targeted delivery
2 of drugs and is potentially associated with significant neurological side effects.

3 Ultrasound-induced transitory disruption of the BBB is another technique that has gained
4 increasing interest since Hynynen et al. demonstrated that the IV injection of preformed gas
5 bubbles prior to low intensity pulsed ultrasound (US) sonications allowed for a reduction of
6 the acoustic pressure necessary to safely open the BBB in rabbits¹⁵⁴. The interaction between
7 US and injected microbubbles is essential for opening of the BBB, and mechanisms of BBB
8 disruption may include transcytosis, cell fenestrations, and opening of tight junctions¹⁵⁵. US-
9 induced BBB disruption can be monitored with MRI, as a contrast enhancement in T1-
10 weighted sequences after gadolinium injection¹⁵⁴ and is limited to the US beam¹⁵⁶.

11 The safety of the technic has been assessed through pre-clinical studies. With optimized
12 parameters, histological side effects are limited to red blood cell extravasation and petechial
13 bleeding¹⁵⁷ after both single or multiple US sessions¹⁵⁸. BBB disruption may induce a transitory
14 sterile inflammatory response¹⁵⁹. Recent studies have confirmed that the technic was clinically
15 well-tolerated in non-human primates¹⁶⁰.

16 This technic was used to deliver an AAV1/2 viral vector in a targeted manner to the striatum
17 of rats. Transduction observed was mainly restricted to neurons and stable during more than
18 one year¹⁶¹. Two main approaches have been developed in order to bypass the skull interface
19 that induces attenuation and distortion of the ultrasound beam. In one case, InSightec (Haifa,
20 Israel) developed a 512-element phased-array transducer, the ExAblate® 4000 system, that
21 allows a transcranial and noninvasive opening of the BBB (McDannold et al. 2010). On-going
22 clinical trials are currently evaluating the safety of the ExAblate® system for drug delivery after
23 BBB disruption (NCT02343991, NCT02253212). In another case, CarThera SAS (Paris, France)
24 designed an implantable device, the SonoCloud® device, which can be plugged into the skull,
25 and activated through a transdermal needle¹⁵⁶. Interim results of the first clinical trial
26 (SONOCLOUD, NCT02253212) evaluating the safety of this system in adult patients treated for
27 recurrent glioblastoma with systemic carboplatin have shown no dose limiting toxicities and
28 no treatment-related serious adverse events¹⁶². A phase I trial (SONOKID) assessing the safety
29 of repeated BBB disruptions by the SonoCloud® device in association with intravenous
30 chemotherapy in recurrent supra-tentorial malignant primitive tumors in the pediatric
31 population should begin during 2020¹⁶³. More recent studies have been reported with
32 percutaneous ultrasound but with no additional value¹⁶⁴.

Encapsulated cells and Optogenetics

Encapsulated cell technology (ECT) eventually combined with optogenetics allows the delivery of treatment in a continuous or in a controlled/discontinuous ways, respectively.

ECT is a concept based on the confinement of the grafted cells within a permeable device.

ECT has already been shown to be well tolerated in large animals such as dogs for the treatment of intervertebral disc degeneration¹⁶⁵, in pigs¹⁶⁶ and NHPs¹⁶⁷ for Alzheimer's disease. Moreover, ECT has already been translated to clinical studies where the safety, feasibility, and tolerability of procedure has been shown e.g the delivery of neurotrophic factor in disorders of eye (^{168–170}; clinicalTrial.gov NCT00447980, NCT00447993, NCT02228304, NCT00447954), in Alzheimer's disease (¹⁷¹; NCT01163825), in Huntington's disease^{172,173} and in amyotrophic lateral sclerosis¹⁷⁴. Moreover delivery of anti-amyloid immunotherapy by ECT is described in AD patients^{175,176}. Thus, ECT provides an innovative approach for the local and systemic delivery of a recombinant protein in the CNS.

The optogenetics approach uses optical methods to modulate the cellular expression of molecules, which are activated by irradiation with light energy by genetic engineering to control/regulate cellular function or intracellular signal transduction¹⁷⁷. One of the challenges of optogenetics is its translatability to the clinic. Many improvements in optogenetic technologies in NHPs have been performed to exert precise control of specific cells or brain regions at the millisecond timescale and to reliably transduce cells and readout the optically induced neural modulation^{178,179}. A new optogenetic approach has been described by Ruiz et al., 2013 in which the native dura is replaced with optically transparent artificial dura allowing visual monitoring of the expression of the optogenetic agent over time.

The first optogenetic study of NHP was performed in 2009¹⁸⁰ and followed by numerous studies performed to study the link between brain function and behavior using optogenetic stimulation^{181,182}. None of the optogenetic studies allowing the delivery of treatment was performed in NPH. However, many applications of optogenetics in CNS diseases (Stroke, Epilepsy, MS, AD, PD) were described by Ordaz et al., 2017. One of the major obstacles to widely use optogenetic tools in patients remains the delivery to the brain. For this reason, it is essential to reduce brain tissue damage inflicted by probe penetrations and light-induced heating for optogenetic procedures. Once these problems are overcome, optogenetics can be an advantageous tool to treat neurological diseases providing an alternative treatment with

less side effects than current therapies¹⁸³. The first patient dosed with optogenetics was treated for retinitis pigmentosa in a clinical trial conducted by RetroSense Therapeutic in 2016. More recently, one Phase I/II clinical trial of optogenetics for vision restoration is registered (NCT02556736, sponsor Allergan; NCT03326336, sponsor GenSight), but no results have been published to date.

Hematopoietic Stem cell gene therapy to treat CNS diseases

Hematopoietic stem cell transplantation (HSCT) is the best example of stem-cell therapy and is currently an established treatment for several neurologically devastating inherited metabolic diseases, including adrenoleukodystrophy and LSDs^{21,22}. In LSDs, donor-derived microglia cells of myeloid origin are thought to be the source of enzyme after HSCT, cross-correcting the metabolic defect in affected host cells¹⁸⁴. In addition, engrafted donor-derived cells may potentially help in reducing accumulated toxic substrates in the brain. Of importance, there is evidence that stem cells are not only replacing dying cells, but are also regulating inflammation and immune responses and have pro-neurogenic effects¹⁸⁵.

Numerous studies support the notion of using stem cells as a treatment for inherited diseases like LSDs¹⁸⁴, HD, but also for complex diseases like AD, PD, ALS^{186,187}. Further pre-clinical and clinical studies are needed to ensure the safety and efficacy of these treatment options¹⁸⁷.

Conclusion

The recent beneficial results demonstrated in phase I-II studies in human patients together with improved vector technology have placed gene therapy for CNS diseases in a new development paradigm. These approaches are no longer restricted to rare genetic diseases, but are being applied to common disease indications and pathways significantly expanding the scope of gene therapy for CNS indications. This expansion requires simplifying delivery protocols and anticipating the increasing need of vector production, particularly for IV targeting. Treating an increasing number of patients requires standardized delivery protocols suitable for adaptation in multiple centers around the world. Further, manufacturability is a central component in expanding the GT field; not only the production of high quality and high quantity of vectors to meet future clinical demand, but also the cost and our capacity to make new gene therapy products accessible to all patients.

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Legends

Figure 1:

(A) Circulation of the CSF. Secretion by the choroid plexus and circulation within the ventricles to the foramen of Magendie and Lushka (black arrows) to the extraventricular subarachnoid spaces. Then circulation around the brain and the spinal cord (blue arrows), to the resorption spaces (mainly the superior sagittal sinus)

(B) Intrathecal injection targeting the spinal cord

(C) Injection within the cisterna magna targeting the more superficial area of the brain (mainly the cortical zone)

(D) Intraventricular injection targeting the deepest regions of the brain (mainly the basal ganglia and the subventricular nucleus. Notice that the hippocampus region can be theoretically reached by cisternal or intraventricular injection

(E) Following the CSF flow, a part of the vectors will reach the resorption areas and join the cerebral blood stream and then the cerebral circulation to the peripheral organs

Figure 2: Intracerebral injection in NHP and humans

(A) Personal data of intracerebral gene therapy with 12 intracerebral delivery in white matter for San Filippo A and MLD. (B) Neuronavigation software for preplanning. (C) Delivery improvement with MRI Smartflow catheters; (D) Immediate Post-operative MRI and (E) Brain delivery in NHP with the same MRI smartflow catheters.

1 **Figure 1:**

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1 **Figure 2: Intracerebral injection in NHP and humans**

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Table 1: Intraparenchymal delivery in large animal models

Transgene / Pathology	Target	Specie	Age	Number	Vector / Buffer	Dose	Volume	Speed	Results	Article
ARSA for MLD	3 areas of the centrum semiovale white matter or in the deep gray nuclei (caudate nucleus, putamen, thalamus)	NHP	2–3-year-old	6	AAV 2 - 5	$1.9 \cdot 10^{12}$ vg to $3.8 \cdot 10^{11}$ vg	40 μ l/deposit	3 μ l/min	AAV vector was detected in a brain volume of 12–15 cm ³ that corresponded to 37–46% of the injected hemisphere. ARSA enzyme was expressed in multiple interconnected brain areas over a distance of 22–33 mm. ARSA activity was increased by 12–38% in a brain volume that corresponded to 50–65% of injected hemisphere	37
GDNF for PD	striatum, substantia nigra, caudate nucleus	NHP, rhesus	8 aged (25years) + 5 young adults	8+5	Lentiviral vector				Extensive GDNF expression with anterograde and retrograde transport was seen in all animals. In aged monkeys, lenti-GDNF augmented dopaminergic function. In MPTP-treated monkeys, lenti-GDNF reversed functional deficits and completely prevented nigrostriatal degeneration. Additionally, lenti-GDNF injections to intact rhesus monkeys revealed long-term gene expression (8 months). In MPTP-treated monkeys, lenti-GDNF treatment reversed motor deficits in a hand-reach task	78
APOE2-HA for AD	intrahippocampal	NHP	4- to 7-year-old	2	Recombinant AAVrh.10	$5 \cdot 10^{12}$ vg (vg; 0.7– $1.2 \cdot 10^{12}$ vg/kg	15 μ L per injection site	1 μ L/min	AAVrh.10hAPOE2-HA directly into the hippocampus/entorhinal cortex achieved easily detectable, diffuse ApoE2 expression in targeted regions using this route of delivery compared to the non-treated controls	188
NAGLU for lysosomal disease	white matter	dog		25	AAV2.5 AAV 5.5	$5 \cdot 10^{11}$ vg, $1.5 \cdot 10^{12}$ vg/ml; $8 \cdot 10^{11}$ vg, $2.5 \cdot 10^{12}$ vg/ml; $20 \cdot 10^{11}$ vg, $6.5 \cdot 10^{12}$ vg/ml	8 x 40 μ l	2 μ L/min)	In immunosuppressed dogs, vector was efficiently delivered throughout the brain, induced α -N-acetyl-glucosaminidase production, cleared stored compounds and storage lesions	189
CLN2 for Late infantile neuronal ceroid lipofuscinosis	head and body of the caudate nucleus, hippocampus and overlying cerebral cortex	NHP			AAV 2	3.6×10^{11} pu	180 μ l	1 μ l/min	Assessment at 5 and 13 weeks demonstrated widespread detection of TPP-I in neurons, but not glial cells, at all regions of injection. The distribution of TPP-I-positive cells was similar between the two time points at all injection sites	190
α -iduronidase	putamen and centrum semiovale	NHP		6	(rAAV2/1, rAAV2/2, and rAAV2/5	$1.4 \cdot 10^{10}$ vg	2x50 μ l		global diffusion throughout the brain was not significantly different between the three serotypes. However, rAAV2/1 and rAAV2/5 resulted in higher vector copy numbers per cell than did rAAV2/2, respectively, in the brain and the distal neuronal structures	191
CMV-hrGFP	corona radiata, striatum, and basal forebrain	NHP cynomolgus		8	AAV1	2.3 to $6.9 \cdot 10^{11}$ vg	10 to 150 μ l	0.2 to 3 μ l/min	AAV1 is actively trafficked to regions distal from the infusion site. In addition to neuronal transduction, a significant nonneuronal cell population was transduced by AAV1 vector	192
AAV-TK ,AAV2-AADC		NHP			AAV2				at least 75% of the putamen could be covered by a single infusion of the vector	193
human acidic sphingomyelinase For Nieman Pick disease	brainstem (1 site) and thalamus (bilateral)	NHP		4	AAV2	$1 \cdot 10^{12}$ vg/ml	33 to 199 μ l	0,1 increased at 10-min intervals to 0.2, 0.5, 0.8, 1.0, and 2.0 μ l/min	We found that enzymatic augmentation in brainstem, thalamic, cortical, as well subcortical areas provided convincing evidence that much of the large NHP brain can be transduced with as few as three injection sites.	194
hARSA	external capsule and thalamus	NHP	2-3 months	9	Lentivirus	$5 \cdot 10^7$ TU/injection site	80 μ l	NA	favorable safety profile and consistent pattern of LV transduction and enzyme biodistribution. Efficient gene transfer in neurons, astrocytes, and oligodendrocytes close to the injection sites resulted in robust production and extensive spreading of transgenic enzymes in the whole CNS	195
<u>heparan sulfate</u>	thalamus + ICV	NHP			AAV2				The combination of thalamic and intracerebroventricular delivery resulted in transduction of oligodendrocytes in superficial cortical layers and neurons in deeper cortical layers	196

<u>proteoglycan receptor</u>										
hARSA	White matter, 6 sites	NHP		14	AAVrh10	$5.5 \cdot 10^{11}$ vg/hemisphere	30 μ l for each deposit, 0.5 μ l/min	0.5 μ l/min	After injection of the 1 st dose, AAVrh.10- hARSA vector was detected in a large part of the injected hemisphere, while ARSA activity exceeded the normal endogenous activity level by 14–31%	11
hASM for NP-A	12 sites : motor cortex, occipital cortex, striatum and thalamus, hippocampus, cerebellum	NHP	2Y/o	2	AAV1	$2.6 \cdot 10^{12}$ gc	520 μ l total	NA	a combination cortical, subcortical, and cerebellar injection protocol could provide therapeutic levels of hASM to regions of the NHP brain	197
ARSA	6 sites deep gray matter + white matter	NHP	3- to 6-year-old	11	AAVrh.10	$1.5 \cdot 10^{12}$ vg	50- μ l/deposit	1-3 microl/min	Of the five routes studied, administration to the white matter generated the broadest distribution of ARSA, with 80% of the brain displaying more than a therapeutic (10%) increase in ARSA activity above PBS controls	85
SGSH	Subcortical white matter	Dogs		3	AAVrh.10	$1 \cdot 10^{12}$ vg and $2 \cdot 10^{12}$ vg	500 μ l/deposit (2 or 4 deposits)	10 μ l/min	extensive distribution into both rostral and caudal brain regions. significant amounts of vector DNA were found in only 37% of brain punches, increases of SGSH activity of 20% or greater relative to vehicle-treated animals were found in 78% of the brain punches tested 4 weeks after injection.	77
SGSH	Subcortical white matter	NHP	4 years	2	AAVrh.10	$7.2 \cdot 10^{11}$ vg	50 μ l/deposit (4 deposits)	5 μ l/min	presence of vector DNA in a limited proportion (11%) of brain punches, but a wide distribution of SGSH enzymatic activity of 20% or more of control levels in the near totality (97%) of the NHP brain 6 weeks after injection.	77
	striatum	NHP			tricystronic lentiviral vector				restoration of extracellular concentrations of dopamine and corrected the motor deficits for 12 months without associated dyskinesias	198

Table 2: Intracerebroventricular administration in large animal models

Transgene / Pathology	Target	Specie	Age	Number	Vector / Buffer	Dose	Volume	Speed	Results	Article
shRNA SMN +GFP or SMN	Cisterna Magna	Pig	5 days	14	scAAV9	6.5.10 ¹² vg/kg (shSMN)		slow and constant	reduction of SMN mRNA levels by 73% in motoneurons postnatally	81
GFP	Cisterna Magna	NHP	adult	3	AAV9	1.1.10 ¹³ vg	2ml		Presence of serious adverse side effects with a non- pronounced cellular loss in the Purkinje layer. No side effect in animal with more restricted GFP expression.	84
hAADC	Cisterna Magna	NHP	adult	1	AAV9	3.10 ¹³ vg	2ml		broad transduction throughout cerebellum and brain cortex similar to what was obtained with GFP but no cerebellar cell dysfunction	
GFP	Cisterna Magna	Sheep	adult	3	LV	1.3.10 ⁸ TU (N=2) and 1.10 ⁹ TU	10 and 100ul	1min	GFP expression in cells along the needle track and in the brain parenchyma up to 2.5 mm rostral-caudal and lateral to the injection site. Both neurons and astrocytes were similarly transduced	80
VEGF	Cisterna Magna	cat	adult	3	ssAAV1	10 ¹² /kg	1ml/kg		strong expression of the transgene in the spinal cord, even at the lowest dose.	199
VEGF	Cisterna Magna	cat	adult	4	scAAV9	10 ¹² /kg and 10 ¹³ /kg	1ml/kg		numerous GFP-positive neurons in the cortex, thalamus and cerebellum	
CAG-GFP	Cisterna Magna	NHP	adult	2	AAV7 (saline, 5% sorbitol, 0.001% pluronic F-68)	2.10 ¹³ vg	2ml	0.5ul/min	cells throughout the NHP brain from prefrontal to occipital cortex and cerebellum, sparsely in striatum. GFP-positive cells clustered around blood vessels weak transduction in periphery. GFP-positive cells at all spinal cord levels, in motor neurons and some astrocytes DRG of the cervical region, proximal to the site of injection and some satellite glial cells surrounding DRG neurons	70
CAG-GFP	Cisterna Magna	NHP	adult	2	AAV9 (saline, 5% sorbitol, 0.001% pluronic F-68)	1.8.10 ¹³ vg	2ml	0.5ul/min	cells throughout the brain from prefrontal to occipital cortex and cerebellum, sparsely in striatum. GFP-positive cells clustered around blood vessels. GFP-positive cells at all spinal cord levels, in motor neurons and some astrocytes. DRG of the cervical region, proximal to the site of injection and some satellite glial cells surrounding DRG neurons	
GFP	Cisterna Magna	NHP	2-3 years	3	sc AAV9	1.25.10 ¹³ vg	3+3ml		15–50% motor neuron transduction throughout the cervical, thoracic, and lumbar segments. GFP-positive pyramidal neurons; Purkinje cells throughout the cerebellar cortex were also efficiently transduced. not detected in the liver and spleen of the monkeys	87
GFP	Cisterna Magna	Dog	adult	7	AAV9	2.10 ¹³ vg	1ml	100ul/min or bolus in 15s	widespread CNS transduction	89
human SGSH	Cisterna Magna	Dog	adult	2	AAV9	2.10 ¹³ vg	1ml	bolus in 15s	the loss of transgene expression was due to the use of a nonspecies-specific transgene	
canine SGSH	Cisterna Magna	Dog	adult	3	AAV9	2.10 ¹³ vg	1ml	bolus in 15s	activity peaked 2–4 weeks, but persistent at high levels for 3 months	
eGFP	Cisterna Magna	NHP	adult	4	ssAAV9	5.10 ¹² /kg and 2.5.10 ¹² /kg			substantial vector deposition in the brain and spinal cord (1VGC) Vector copy numbers quite high in liver and spleen.	88
ApoE2/HA	Cisterna Magna	NHP	adult	3	AAVrh10	5.10 ¹³ vg	1-1.3ml	0.5ml/min	CM: 92.0% of cubes had vector levels >1,000 copies/ig DNA, heavy staining of the ependymal cells of the choroid plexus, but also in areas around the frontal and mid-brain, including the hippocampal region, as well as areas around the posterior of the brain and spinal cord.	86
CAG-GFP	Cisterna Magna	NHP	adult	2	AAV9 sc	1.8.10 ¹³ vg	2ml	0.5ul/min	prefrontal to occipital cortex and mainly cerebellum. number and intensity of GFP-positive cells were much greater after CM infusion than ICA delivery. greater astrocytic than neuronal tropism via both routes. not shield against AAV antibodies. Sparse GFP expression was observed in the spleen and liver.	83
CMV-hAADC	Cisterna Magna	NHP	adult	5	AAV9	0.3 / 1 / 3.10 ¹³ vg	2ml	0.5ul/min	prefrontal to occipital cortex and mainly cerebellum. number and intensity of GFP-positive cells were much greater after CM infusion than ICA delivery, greater astrocytic than neuronal tropism via both routes. not shield against AAV antibodies. Sparse GFP expression was observed in the spleen and liver	
GUSB	Cisterna Magna	Dogs	adult and 2months	7	AAV9	1.8.10 ¹³ vg	1ml	1-2 min	ICV and IC vector administration resulted in similarly efficient transduction throughout the brain and spinal cord. ICV cohort developed encephalitis associated with a T-cell response to the transgene product, a phenomenon that was not observed in the IC cohort	99
GFP	Cisterna Magna	NHP	adult	2	AAV9	1.83.10 ¹² vg	1.5ml		substantial transduction was found in the hypothalamus along the third ventricle and in the central gray surrounding the Sylvian aqueduct. extensive transduction was found in the subcommissural organ, located within the dorsal third ventricle, whereas some (GFP)-positive vestibular neurons were found near the fourth ventricle.	90

GFP	Cisterna Magna	NHP	adult	2	AAV2,5	2.10 ¹² vg	1.5ml		very strong transduction of the ependymal structure	
GFP	Cisterna Magna	Cat	50 days	6	scAAV9	10 ¹² vg/kg	1-1,5ml		more efficiently targeting of MNs of the lumbar (99%) than of the cervical regions (84%) spinal cord. numerous oligodendrocytes were also transduced in the brain and in the spinal cord white matter of young cats, but not of neonates	92
CAG-ARSA-FLAG / MLD	lateral ventricle	NHP	adult	2	AAVrh10 in PBS	1,5.10 ¹² vg	75ul	15ul/min	almost no copies and no ARSA expression (11/83=13%) whereas gold standard: white matter 82%	85
ApoE2/HA	left or right ventricle	NHP	adult	3	AAVrh10	5.10 ¹³ vg	1-1.3ml	0.2ml/min	ICV: 90.0% of cubes had vector levels >1,000 copies/lg DNA, heavy staining of the ependymal cells of the choroid plexus, but also in areas around the frontal and mid-brain, including the hippocampal region, as well as areas around the posterior of the brain and spinal cord.	86
CAG-TPP1 /CLN2	lateral ventricles +/- CM	Dog (TPP deficient)	10-11 days	7	AAV2	1.1.10 ¹² vg	1,5ml		TPP1 activity in CSF detectable at 5 days but no more detectable at 2 months	82
IDUA / MPS I	suboccipital	Cat	adult	5	AAV9	10 ¹² vg/kg	1-2ml	bolus	complete correction of biochemical and histological manifestations throughout the CNS. antibody responses against IDUA which reduced detectable enzyme without substantially reducing efficacy. no evidence of toxicity	88
GFP	ICV	Dog	adult	2	AAV9	2.10 ¹³ vg	1ml	bolus in 15s	Comparable with CM administration	89
GFP	ICV	NHP			AAV2 - HBKO	1.8.10 ¹³ vg	1.5ml	1-10ul/min	widespread cortical transduction, with oligodendrocytes transduced. Robust motor neuron transduction observed in all levels of the spinal cord	200
hASM-HA	CM	NHP	adult	6	AAV9	1.32.10 ¹³ vg	6ml	1ml/min (n=3) or 1ml/h (n=3)	Infusion of the vector in brain and spinal cord after MRI but only with High speed delivery	201
hASM-HA	ICV	NHP	adult	2	AAV9	1.32.10 ¹³ vg	6ml	1ml/min (n=3) or 1ml/h (n=3)	much larger cortical distribution at the end of the acquisitions, including occipital cortical regions that were not covered by any other routes of delivery	201
ciDUA	Suboccipital	Dogs	28 days	3	AAV9	10 ¹² vg/kg	0.5ml	bolus	vector distributed throughout the CNS. supraphysiologic expression of IDUA in CSF, which declined to the normal range	94

Table 3 : Intrathecal administration in large animal models

Transgene / Pathology	Target	Specie	Age	Number	Vector / Buffer	Dose	Volume	Speed	Results	Article
GUSB / MPS VII	IT	Dog	18-20 days	3	AAV9	5.10 ¹² vg/kg	1-2ml	1-2min	~50-fold higher expression and a more profound effect on markers of disease than IV delivery	93
GUSB / MPS VII	IT	Dog	18-20 days	2	AAV10	5.10 ¹² vg/kg	1-2ml	1-2min	~50-fold higher expression and a more profound effect on markers of disease than IV delivery	
GUSB	Lumbar L4/L5	NHP	adult	6	AAV9	2.10 ¹³ vg	5ml		transduction efficiency was not improved by placing animals in the Trendelenburg position after injection.	99
GFP	Lumbar cistern	NHP	adult	8	AAV9	1.83.10 ¹² vg (N=6) and 5.5.10 ¹² vg (N=2)	1ml		very strong transduction of the ependymal structure	202
GFP	L4/L5	NHP	adult	7	scAAV9	1.10 ¹³ vg/kg	1ml	Bolus	73% of motor neurons targeted in the lumbar region, 53% of motor neurons targeted in thoracic region, and 29% in the cervical spinal cord; tilting for 10 minutes was sufficient to increase motor neuron transduction to 55, 62, and 80% in the cervical, thoracic, and lumbar region. GFP in all brain regions with particularly strong signals in the hippocampus and in the motor cortex. In the brainstem, high transduction of the hypoglossal and trigeminal nuclei, with more than 60 and 75% motor neurons. In the cerebellum, both Purkinje cells and cells of the nuclear layer demonstrated high GFP expression	13
miRNA anti SOD1	L5 and cathether	NHP	adult	32	AAV10		2.5ml	7.5ml/hour	preimplantation of a catheter and placement of the subject with head down at 30° during intrathecal infusion. efficient delivery and effective silencing of the SOD1 gene in motor neurons	98
GFP	L5/L6	Pig	5 days	1	AAV9	5.2.10 ¹² vg/kg	0.25ml	bolus	Extensive motor neuron transduction at all levels of the spinal cord. The brain regions with the highest levels of GFP expression were cerebellar Purkinje cells, nerve fibers within the medulla as well as discrete nuclei, such as the olivary nucleus. Expression within the rest of the brain was restricted to scattered cells near the meningeal surfaces. No obvious expression in periphery	96
miRNA anti SOD1	Lumbar	Marmosets	less than 4 years	9	AAV10	6.10 ¹² vg/kg	300ul	bolus	high transduction of lumbar spinal cord, than thoracic and cervical . High liver transduction. In brain, mild to good transduction, depending on the regions. Robust GFP staining was seen at the injection site at LSC level all the way to CSC level.	95
GFP	L5 with cathethers	Pigs	2 months	2	scAAV9	3 x 1.10 ¹² vg	3x0.5ml	Bolus	GFP expression in 10–30% of the motor neurons, and one segment (L2) showed GFP expression in 35% of the motor neurons.	87
eGFP	Lumbar punctura	NHP	adult	2	ssAAV9	2.5.10 ¹² /kg			10x lower gene transfer throughout the spinal cord, and up to 100-fold less in the brain compare to CM. Vector copy numbers quite high in liver and spleen.	88
hASM-HA	Lumbar	NHP	adult	3	scAAV9	1.32.10 ¹³ vg	6ml	1ml/min or 1ml/hour	Leakage of the infusion in musculature	201
GFP	Lumbar	Sheep	adult	2	scAAV9		15ml	1ml/min	the frontal, occipital, and parietal cortices exhibited extensive neuronal and glial cell transduction, whereas in the motor cortex glial transduction was primarily observed. Scarce positive neurons were present in the caudate, putamen, and thalamus. Strong GFP staining was noted in the cerebellum, including Purkinje cells, deep cerebellar nuclei and adjacent	203

									axons in the white matter, cerebellar peduncles, and brainstem. Robust neuronal transduction was observed along the entire length of the spinal cord, from cervical to lumbar	
miRNA onto SOD1	Lumbar L4/L5	Marmoset	adult	1	AAV10	2.7.10 ¹² vg	250ul	slow bolus	robust expression in motor neurons along the full length of the spinal cord	204

Table 4 : Intravenous administration in large animal models

Transgene / Pathology	Target	Specie	Age	Number	Vector / Buffer	Dose	Volume	Speed	Results	Article
CAG-GFP	Internal carotid artery	NHP		3	AAV9 sc	3.10 ¹³ vg	11 / 21 / 40ml	4ml/min	broader transgene distribution throughout the CNS, extending through several cortical regions. Scattered GFP-expressing cells were found from the pre- frontal to occipital cortex, and in the cerebellum. widespread peripheral organ transduction (Liver and Spleen)	83
CAG-ARSA-FLAG / MLD	Intra-arterial (right middle cerebral artery)	NHP	adult	1	AAVrh10 in PBS	1,5.10 ¹² vg	12ml	bolus	almost no copies and no ARSA expression (6/77=7,8%) whereas gold standard: white matter 82%	85
GUSB / MPS VII	IV	Dog	3 days	1	AAV9	2.10 ¹³ vg/kg	1-2ml	1-2min	limited GUSB expression in cortical and hippocampal neurons, and Purkinje cells, while average expression levels in other brain tissues were only ~1 to 6% of normal levels Similar to AAV10 injected animals. Clear motoneurons transduction	93
GUSB / MPS VII	IV	Dog	3 days	1	AAV10	2.10 ¹³ vg/kg	1-2ml	1-2min	limited GUSB expression in cortical and hippocampal neurons, and Purkinje cells, while average expression levels in other brain tissues were only ~1 to 6% of normal levels Similar to AAV9 injected animals. Clear motoneurons transduction	
NAGLU	IV	NHP	adult	8	ssAAV9	1 (N=2) or 2.10 ¹³ vg/kg (N=4)	5ml	bolus	global CNS and broad somatic transduction. evident vector transduction throughout the brain. Low levels of preexisting anti-AAV9 antibodies did not diminish vector transduction but high-level of preexisting anti-AAV9 Abs lead to reduced transduction in liver and other somatic tissues, but had no detectable impact on transgene expression in the brain.	40
GFP	carotid artery	Cat	2 months	1	AAV-B1	3.4.10 ¹² vg		Bolus	sparse but widespread neuronal gene transfer throughout the brain. AAV-B1 transduced neurons in the cerebral cortex, striatum, hippocampus, thalamus and Purkinje neurons in the cerebellum and motor neurons throughout the midbrain. no indication that AAV-B1 transduced endothelial cells. negligible transduction of liver was observed with AAV-B1, while strong gene transfer to skeletal and cardiac muscle could be detected	205
VEGF	jugular vein	Cat	2 days	3	scAAV9	10 ¹² vg/kg	1ml/kg	bolus	no detectable protein	92
GFP	jugular vein	Cat LIX1	7 days	2	scAAV9	1.5.10 ¹² vg	1ml	Bolus	GFP was detected from the cervical part of the spinal cord to the cauda equina in a number of cells in the ventral spinal cord. In neonates, up to 39 and 34% of the MNs. Nerve fibers of the fasciculi gracilis and cuneatus dorsal sensory tracts also contained large amounts of GFP.	91
GFP	jugular vein	Cat LIX1	7 weeks	2	scAAV9	1.2.10 ¹² vg	3.6ml	Bolus	up to 15% of MNs transduction observed	
cIDUA	jugular vein	Dogs	90 days	4	AAV8	3.10 ¹² vg/kg	0.5-1ml	bolus	mild in serum IDUA activity	94
GFP	saphenous or intracarotid	NHP	adult	4	sc AAV9	0.9- 1.10 ¹³ vg/kg	10ml/kg	2.5ml/min	predominant transduction of glia in NHPs after both intravenous and intra-arterial administration	90

VEGF	jugular vein	cat	adult	6	scAAV9	10 ¹² /kg (N=2) and 10 ¹³ /kg (N=4)	1-2ml		transduction of the spinal cord occurs in neonatal and adult cats but the level of transgene expression remained low in both adult and neonate-injected animals. transduction was detected essentially in liver.	199
GFP	saphenous vein	NHP	adult	1	AAV9	1-3.10 ¹⁴ vg/kg		bolus	only MN and cells with glial morphology that were sparsely scattered. most abundant number of GFP-expressing cells in all cortical regions lateral geniculate midbrain, pons and medulla. Subcortical structures such as thalamus and putamen were also GFP+ but at a lower cell density. mostly glial transduction with microglia and astrocytes. high levels of vector in liver and also other peripheral tissues	96
GFP	catheter through the brachial artery until aorta	NHP	adult	1	AAV9	2.7.10 ¹³ vg/kg		bolus	Motor neuron targeting extensive transgene expression throughout the entire brain. most abundant number of GFP-expressing cells in all cortical regions lateral geniculate, midbrain, pons and medulla. Subcortical structures such as thalamus and putamen were also GFP+ but at a lower cell density. glial transduction with microglia and astrocytes. Liver, heart, testis and largely peripheral transduction	
IDUA	IV	NHP	adult	2	AAV9	2.5.10 ¹³ vg/kg 7.5.10 ¹³ vg/kg	1ml	Bolus	vector well tolerated. GFP expression was detected in most non-CNS tissues, including liver and muscle, kidney, pancreas, heart, spleen, and pituitary. very low transduction was observed the frontal cortex and spinal cord and hippocampus and cerebellum but transduction in DRG. Good tolerance of the virus	45
IDUA	IV	NHP	adult	2	AAVPHP.B	2.5.10 ¹³ vg/kg 7.5.10 ¹³ vg/kg	1ml	Bolus	vector well tolerated. GFP expression was detected in most non-CNS tissues, including liver and muscle, kidney, pancreas, heart, spleen, and pituitary equivalent to AAV9 except for in skeletal muscle, where transduction was higher for PHP.B vector well tolerated. Immune response against the virus	45
eGFP	IV	NHP		1	ssAAV9	2.10 ¹³ vg/kg			vector distribution to the CNS was substantially lower than that achieved at four- to eightfold lower doses via CM	88

Table 5 : Clinical trials with Intra CSF or intravenous administration

Transgene / Pathology	Target	Age	Number	Vector / Buffer	Dose	Volume	Speed	Identification of the trial and results if available	Article
CLN6 / Batten	IT		13	AAV9	1.5.10 ¹³ vg			NCT02725580, Phase I/II	NA
JeT-GAN / Giant Axonal Neuropathy	IT			AAV9				NCT02362438	206
NAGLU / MPSIIIB				scAAV9					NA
SGSH / MPSIIIA	IV		9 ?	AAV sc9	5.10 ¹² vg/kg (n=3) and 1.10 ¹³ vg/kg (n=1)			NCT02716246, Phase I/II	NA
SMN type 1 / SMA	IV	child	15 (cohort 1 n=3, cohort 2 n=12)	AAV9	6.7.10 ¹³ vg/kg cohort 1 and 2.10 ¹⁴ vg/kg cohort 2			NCT02122952, Phase I/II , longer survival, motor improvement	41
SMN	IV	child	6	AAV9				NCT03837184, Phase III	NA
SGSH / MPSIIIA	IV		12	scAAV9	3.10 ¹³ vg/kg			NCT04088734	NA
GLB1 / GM1 type I and II	IV	6 months – 1 year for GM1 type I and 2-12 for GM1 type II	45	AAV9	1.5 to 4.5.10 ¹³ vg/kg			NCT03952637	NA
hTERT/ AD	IV /IT	45 years and older	5					NCT04133454	NA
HEXA / HEXB Tay sachs	IT	7 and 30 months old	2	AAVrh8	HEXA vector (0.5 mL, 9.9. 10 ¹² vg/mL) mixed with HEXB (0.413 mL, 1.2 .10 ¹³	12ml	1ml/min		203

					vg/mL) to generate a 1:1 equimolar formulation				
GBA / Parkinson	ICM	40 to 75 years	16		Low and high dose			NCT04127578	NA
GRN / Fronto temporal dementia	ICM	30 to 80 years	15		Low, medium and high dose			NCT04408625	NA
IDUA / MPS1	ICM	4 months and older	5	AAV9	1 and 5.10 ¹⁰ gc/kg			NCT03580083	NA
IDS / MPS2	ICM	4 months to 5 years	6	AAV9	1.3 and 6.5.10 ¹⁰ gc/kg			NCT03566043	NA
CLN3	IT	children	7	scAAV9	Low or high dose			NCT03770572, Phase I/IIa	NA

Table 6 : Clinical trials with Intra parenchymal administration

Transgene / Pathology	Target	Age	Number	Vector / Buffer	Dose	Volume	Speed	Identification of the trial and results if available	Article
AADC/ParkD	Striatum	adult		AAV2				NCT00229736, Increased risk of intracranial hemorrhage, Phase I motor improvement	207
TPP-1/LINCL	12 sites in WM	Children		AAV2				Unsuccessful but suggestion of slowing progression of disease	207
NTN (CERE-120) / Parkinson	Putamen		12	AAV2	1.4.10 ¹¹ vg/mL and 5.7.10 ¹¹ vg/mL	40 µl		well tolerated, significant motor score improvement 6 months after infusion	208
NTN (CERE-120) / Parkinson	Putamen	Adult 35-75 years	38 /20 sham	AAV2	5.7.10 ¹¹ vg/mL	40 µl/ hemisphere		NCT00252850, CERE 120, double blinded clinical trial, afetr 1 year : 13/38, 4/20 sham reacted adversely to the injection, no clinically significant improvement versus placebo at 12 months	208
NTN (CERE-120) / Parkinson	Putamen + Subst Nigra, bilateral	Adult 35-70 years	60	AAV2	2.4.10 ¹² vg			NCT00985517, Phase I, double blinded, safely tolerated, 5 years f-up, no significant improvement	208
AADC / Parkinson	Putamen		10	AAV2	9.10 ¹⁰ vg and 3.10 ¹¹ vg			Low and high vector dose, well tolerated, dose dependent improvement in dopamine synthesis Elevated PET signal persisted over 4 years in both groups	208
ProSavin	Striatum	Adult 48-65 years	15	Lentiviral EIAV	1.9.10 ⁷ ; 4.10 ⁷ and 1.10 ⁸ TU			NCT01856439, Phase I /II, safely tolerated, modest effects but patients with higher dose of ProSavin required lower dose of dopamine (enhanced dopamine production ?)	208
NGF (CERE-110) / Alzheimer		Adult 55-80 years	49	AAV2	2.10 ¹¹ vg			NCT00876863, Phase II, inefficient, safely tolerated	209
CLN2 / Batten	(x12 by 6 bur holes)	Children	10	AAV2	2.5.10 ¹² vg	150 µl	2 µl/min	NCT00151216, Phase I, Radiographical changes in 65% of 60 injections sites	209
GAD65 and GAD67 / Parkinson	SubThalamic Nucleus (unilateral)	Adult 25-75 years	12	AAV2	1.10 ¹¹ vg/mL; 3.10 ¹¹ vg/mL and 1.10 ¹² vg/mL	50 µl		NCT00195143, Phase I/II, safe; neuroimaging improvement, clinical improvement at 12 months post infusion, decrease of STN activity	209
NTN (CERE-120) / Parkinson			58	AAV2	5.4.10 ¹¹ vg			NCT00400634, Phase II, not effective	208
GAD65 and GAD67/ Parkinson	SubThalamic Nucleus	Adult 30-75 years	44	AAV2	1.10 ¹² vg			NCT00643890, Phase II, well tolerated, improvement at 6 months (not greater than DBS)	206
ASPA / Canavan			21	AAV2	1.10 ⁸ vg				206
ASPA Canavan	(X6)	4-83 years	13	AAV2	9.10e11vg			Phase I/II, No longterm adverse event, slowing of disease	206

CLN2 / Batten	WM		16	AAV rh.10	9.10 ¹¹ vg (n=6) and 2.85.10 ¹¹ vg (n=10)			NCT01161576, Phase I/II	?
CLN2 / Batten	WM		8	AAV rh.10	9.10 ¹¹ vg and 2.85.10 ¹¹ vg			NCT01414985, Phase I/II	?
NAGLU / MPSIIIB		Child (20-53 months)	4	rAAV2/5	4.10 ¹² vg			ISRCTN19853672, Phase I/II, well tolerated, induced sustained enzyme production in the brain, best results in the youngest patients	210
AADC / Parkinson			5	AAV2	9.10 ¹⁰ vg			Phase I, Safely tolerated, modest improvement in motor coordination at 6 months (placebo effect ?)	211
AADC / Parkinson			10	AAV2	3.10 ¹¹ vg			Phase I, safe possibly effective	212
SHSH-IRES-SUMF1 / MPSIIIA	12 sites in WM	Child (32-70 months)	4	AAV rh.10	7.2.10 ¹¹ vg	60 µl	2h	NCT01474343, good tolerance, possible but moderate clinical improvement,	38
AADC / AADC deficiency	Putamen	Child (1.67-8.42 years)	10	AAV2	1.81.10 ¹¹ vg	80 µl /target	3 µl /min	NCT01395641, Phase I/II, motor development improvement in children	35
AADC/AADC deficiency	SN + VTA							NCT02852213, Phase I	NA
CAG-NGF / Alzheimer	Nucleus Basalis Meynert	Adult 50-80 years	10	AAV2				NCT00087789, Phase I, Safe, biologically effective	212
NGF / Alzheimer		Adult >50 years	8					NCT00017940	NA
GDNF	putamen	adult	12	AAV2				NCT04167540 / Phase 1B	NA
GDNF / Parkinson	(x16, 4 in cerebellum)	Adult >18 years	24	AAV2	9.10 ¹⁰ vg; 3.10 ¹¹ vg; 9.10 ¹¹ vg and 3.10 ¹² vg			NCT01621581, Phase I	NA
ARSA / MLD	12 sites in WM	Child 6-60 months	4	AAV rh.10	1.10 ¹² vg/kg (n=2) and 4.10 ¹³ vg/kg (n=2)	60 µl / site	0,5 µl /min	NCT01801709, Phase I/II	NA
SGSH / MPS IIIA	6 sites in WM	Child	20	AAVrh10				NCT03612869, Phase III	NA
miHTT/Huntington	Intra striatal	adult	26	AAV5	6.10 ¹² vg and 6.10 ¹³ vg			NCT04120493, Phase I/II	NA
AADC		Adults 40 to 75 years	42	AAV2	2,5.10 ¹² vg			NCT03562494, Phase II	NA

Table 7 : Devices for intracerebral gene therapy delivery

Intracerebral catheter	Developing team/ company		back flow reduction	design	Article
Hamilton Syringe	Hamilton	rigid, steel	no	endport	
UCSF « homemade »	UCSF, Medgenesis Therapeutics and Brainlab AG	rigid, fused silica, then flexible canna with rigid tip ;	stepped profile	end port, step design	213
Smartflow	MRI Interventions, Inc / UCSF	rigid, ceramic, fused silica liner and a polymer sheath	stepped profile	endport, step design	214
MEMS	Alycone, Inc, Cornell	microfabricated silicon cannula	stepped profile	dual lumen, coupled to multiple proximal tubing	215
Neuroinfuse	Renishaw / Univ Bristol Renishaw Medical Solutions, Neurological Applications Department, New Mills, Wotton-Under-Edge, Gloucestershire GL12 8JR, UK	carbothane	stepped profile / recesses stepped	Sub 1 mm diameter catheters and guide tubes	216,217
glasite / emory University	IsoRay / Emory University	inflatable	no	balloon tipped, 2, 3, and 4 cm with corresponding full volumes of 5, 15 and 35 cc.	218
hollow fiber	Twin Star Medical			micro-porous	

Cleveland Multiport Catheter	Cleveland Clinic Infuseon Therapeutics	rigid	yes	Multichannel	212
Custom Made.Bristol University	Bristol University	rigid	No	One port	219
Barium – impregnated one port infusion catheter	Vygon Valley Forge, PA, USA			one port	212
Barium – impregnated one port infusion catheter	Medtronic		barium impregnated	one port	212