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Title

Gene therapy of the central nervous system: general considerations on viral vectors for gene transfer into the brain.

Thérapie génique du système nerveux central : considérations générales sur les vecteurs viraux pour le transfert de gène dans le cerveau

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Abstract

English abstract

The last decade have nourished strong doubts on the beneficial prospects of gene therapy for curing fatal diseases. However, this climate of reservations is currently being transcended by the publication of several successful clinical protocols, restoring confidence in the opportunity of therapeutic gene transfer. A strong sign of this present enthusiasm for gene therapy by clinicians and industrials is the market approval of the therapeutic viral vector Glybera, the first commercial product in Europe of this class of drug. This new field of medicine is particularly attractive when considering therapies for a number of neurological disorders, most of which are desperately waiting for a satisfactory treatment. The central nervous system is indeed a very compliant organ where gene transfer can be stable and successful if provided through an appropriate strategy. The purpose of this review is to present the characteristics of the most efficient virus-derived vectors used by researchers and clinicians to genetically-modify particular cell types or whole regions of the brain. In addition, we discuss major issues regarding side effects such as genotoxicity and immune response associated to the use of these vectors.

Résumé en français

Suite aux récents succès de divers protocoles thérapeutiques de transfert de gène, notamment appliqués aux pathologies de la rétine, et à la mise sur le marché du Glybera, le premier produit commercial en Europe pour cette classe de médicaments, on observe un regain d'intérêts pour la thérapie génique sur les plans clinique et industriel. Ce nouveau domaine de la médecine expérimentale est particulièrement enthousiasmant si l'on considère que la plupart des maladies neurologiques, attendent désespérément l’apparition d’un traitement satisfaisant. Le système nerveux central est en effet un organe où le transfert de gène peut être stable et réussi s’il est administré selon une stratégie appropriée. L’objectif de cette revue est de présenter les qualités des vecteurs viraux les plus efficaces utilisés actuellement par les chercheurs et les cliniciens pour modifier génétiquement des cellules neurales ou des régions entières du cerveau. Nous abordons également des questions concernant les effets secondaires tels que la génotoxicité et la réponse immunitaire associées à l'utilisation de ces vecteurs.

I. Introduction

Gene therapy is a modern field of experimental medicine aiming at modifying the gene pool of cells to halt the disease progression. This specialty, first conceived in the 1960's in the imagination of eminent scientists such as JBS Haldane [1], has gained practical credibility in the past two decades with the progress of molecular biology and genetics, allowing the enrichment of both our arsenal of tools for gene transfer and our knowledge of the pathogenesis of several obscure diseases. Presently, most efficient tools for gene transfer are vectors derived from viruses, keeping their ability to introduce nucleic acids in the cell but abolishing their replication faculty. In this matter much progress has been done, putting gene transfer at the gate of current clinical practice [2-4]. This of course has also raised questions about ethical and safety issues regarding the use of virus derived vectors, the toxicology and pharmacological side effects linked to their use and the possibility for these elements to modify gametes [5]. These topics are being broached at the same time as viral vectors are being developed and have contributed in many ways in their progressive improvement.

Successful gene therapy balances the efficacy of gene transfer on one side and the knowledge of the pathological process on the other. Among all of our organs, of which none resist modern tools for gene transduction, targeting the brain possibly has most awoken our interest due to the complexity of its organization, its role in regulating bodily functions and interactions with the environment but also because it is the place of grave neuropsychiatric affections. The brain is a compact conglomerate of circuits, controlling autonomous activity, storing information and interconnecting sensory structures to effectors through complex neuronal processing of electrical influx.

Numerous types of neural cells shape this superstructure of which intimate functions are just being uncovered.

There are four rough families of brain disorders that are candidate to gene therapy treatments, and which have been recently reviewed in detail [6]. These are a) tumors (Glioblastoma), b) inflammatory affections (multiple sclerosis), c) neuronal degeneration (Parkinson's, Huntington's and Alzheimer's diseases) and d) neuronal dysfunction (storage diseases, Rett and Down syndromes), among many others. The suitability of gene therapy for each of these affections, is actually being documented in animal models and progressively scaled-up to patients. For all of them, though, the two greatest constraints to restore tissue homeostasis are functional and spatial and require combining appropriately 1) the choice of the transgene, 2) the time window of intervention, 3) the ability to target the appropriate cells and, 4) the level and stability of transgene expression.

As regards to the central nervous system (CNS), practical feasibility of gene therapy was acquired in the 1980s and 1990s with several experiments demonstrating the possibility to transfer genes into mammalian brain cells either through direct gene transfer into the parenchyma [7-10] or through *ex vivo* gene transfer [11-13]. Since then, developments of gene therapy for brain diseases have been sporadic, hampered by the extensive media coverage in the scientific community of few clinical trials that have resulted in the occurrence of serious side effects [14], but also by the slow progress in our comprehension of disease pathology and often to the lack of appropriate animal models. Nevertheless, hundreds of approaches have been explored in animals, with disparate results but often raising hopeful medical expectations. This, notably, led to significant clinical achievements in humans that although concerning few patients and despite variable therapeutic efficacies, indicated that genetically engineered cells can remain functional for years in human organisms. It is the case for several rare genetic disorders such as X-linked adrenoleukodystrophy [15] and metachromatic leukodystrophy [16] both treated by hematopoietic stem cells complementation with a functional cDNA replacing the affected gene. Following these recent achievements, and considering the fact that a great amount of neurologic and psychiatric diseases are currently in a therapeutic deadlock, gene therapy appears today as a promising treatment for diverse brain affections. In principle it allows: (i) delivery of therapeutic factors directly into the CNS, bypassing the blood-brain barrier; (ii) long term effects with a one-shot treatment and (iii) the implementation of curative treatments.

Practically, gene therapy proceeds empirically with strategies of variable levels of precision regarding the cause of the disease that may or may not have an identified genetic origin. The most obvious indications concerning well-characterized genetic anomalies will be approached through straightforward replacement or shutdown of gene expression, requiring a rather technological setup. Instead, idiopathic diseases will be arduous to handle, as they will require acting on general aspects of affections, such as cell death or proliferation, ignoring the actual dysfunction.

Two emblematic examples of approaches to counteract neurodegenerative processes of idiopathic or genetic origin regard strategies developed in animal models of Parkinson's and Huntington's diseases, respectively [6]. Animal models of idiopathic Parkinson's disease have been extensively treated by protection of dopaminergic neurons through over expression of trophic factors (GDNF) in the substantia nigra [17-19] or alternatively through expression of enzymes for dopamine synthesis in surviving cells of the striatum [3] or GABA in the subthalamic nucleus [20]. In Huntington's diseases models, although a trophic approach has also been extensively explored [21] a more precise line aimed at silencing the mutant Huntingtin gene in the GABAergic medium spiny neurons of the striatum was explored [22-24]. As for these and other prototypical gene therapy approaches the modeled disease could be slowed they subsequently have been, or are being, progressively scaled-up for translational therapies in humans [3,20].

This however, remains experimental as several factors significantly break the transit from bench to bedside. A major hurdle to the growth of human gene therapy concerns the standardization of vectors for efficient and safe gene transfer. As most efficient vectors are derived from viruses, they raise justified concerns from the community. As an alternative, much effort is undertaken to develop non-viral vectors, to transfer nucleic acids naked or with liposomes or nanoparticles. Although attractive in terms of cloning space, ease of production and control of inflammatory and immune response, the effectiveness of these synthetic particles remains disappointing allowing only limited expression of the therapeutic gene *in vivo* [25,26]. These non-viral vectors are in fact largely out performed by virus-derived vectors that take advantage of viral tactics to introduce their genomes into host cells and are thus largely preferred to reach therapeutic-efficient gene transfer.

The purpose of this review is to provide an update on the different viral vectors currently available for clinical or preclinical research for gene transfer into the brain. In the first part we will discuss the characteristics and constraints of gene transfer applied to the CNS. Then we will describe the characteristics of the different viral vectors that are currently available to target the brain. Finally, in a last part, we will discuss the potential side effects that can be caused by these vectors and mention the envisaged solutions to overcome them.

II. Constraints and characteristics of gene transfer applied to the central nervous system

All organs can be genetically modified using gene transfer and gene therapy, and the brain is no exception. However, the brain possesses unique features leading to number of constraints. The first is its enclosure in the skull that considerably restricts access into it, as well as limiting organ expansion. The second is the existence of a vascular structure called the Blood-Brain Barrier (BBB) that prevents entry of most circulating cells, microorganisms and molecules giving the brain an immune-privileged status. With regard to gene transfer, this barrier, unless immature or disrupted, blocks the entry of most-types of circulating vectors from the blood compartment to the brain parenchyma, with a notable exception of some serotypes of adeno-associated virus able to naturally cross this barrier (discussed below). However, in most strategies to target brain cells, it is necessary to dispense vector particles directly into the targeted site, which involves the introduction of a catheter through the skull and intra-parenchymal or intrathecal administration. Depending on the location and volume of affected tissue areas, this procedure can be relatively simple or on the contrary quite difficult as it may damage vital circuits or nuclei.

A third constraint concerns the amount and quality of the injected particles: to avoid damaging the brain, infusion of large volumes is not possible or can only be envisaged across a long lapse of time. The vector particles thus need to be concentrated as much as possible so that the therapeutic dose is administered in a minimum time and volume. The vector stock must also be free of pathogens and inflammatory or toxic components.

Consequently an important aspect of vector development is to set-up production systems allowing the criteria of both concentration and grade of purity to be met.

III.Viral vectors for gene transfer into the brain

Engineering a viral vector consists of modifying a virus so that it can transfer nucleic acids into target cells while remaining harmless. To that effect, key elements of the virus genome are deleted, rendering it innocuous and making room for genes of interest. Consequently, classical virus-derived vectors are non-replicating, and thus require the implementation of a specific trans-complementation production system specific to each vector type. A wide variety of viruses have been used to develop virus-derived vectors for gene transfer. The most established ones are those derived from adenoviruses, adeno-associated viruses and lentiviruses. Their principal characteristics are summarized in Table 1. In this review, we shall limit our analysis to the description of these three classes of vectors, but keeping in mind that there are several others, more or less exotic including oncoretrovirus [27], Herpes-simplex virus-derived vectors [9], Sendai virus-derived vectors [28], vesicular stomatitis virus-derived vectors [29] of which use for gene therapy protocols shall, in the coming years, remain marginal.

III.1. Adenoviral vectors (Adv)

The adenovirus is part of the adenoviridae family. The virion has a size of 70 to 100 nm and is composed of an iscosaedral proteic capsid formed by three subunits, the hexon, the penton and the fiber. The hexon is the dominant subunit constituting the capsid's facets, while the penton and fiber subunits are forming spines that extend at the angles of the capsid. Among more than fifty serotypes described and classified [30], human Ad-5 is the most commonly used as a vector for gene transfer [31]. The adenoviral genome is a linear double-stranded DNA of 36 kb flanked by inverted terminal repeat sequences (ITR). The first generation of Adv has a cloning capacity of about 10 kb, and retained a significant proportion of the viral coding genome [32-35]. The last generation Adv, namely "Gutless" Adv, are completely devoid of viral coding sequences, bringing their cloning capacity to 36 kb, but require sophisticated production systems involving a helper virus capable of providing in *trans* all necessary elements for encapsidation [36-38]. Adv were the first vectors showing efficient transduction of neurons and glial cells after injection into the CNS, establishing gene transfer as a potential therapeutic option for neurological disorders [7,8]. Adv can target neurons as well as astrocytes not only in rodents [7,39], but also in dogs [40] and non-human primates [41]. They enter into the target cell via clathrin-coated vesicles following the interaction of the fiber with the coxsachie-adenovirus receptor (CAR), a member of the immunoglobulin superfamily, which is present at the surface of many cell types of different organs, including the CNS [42,43].

However, it soon became clear that administration of these vectors resulted in a significant host immune response directed against transduced cells. In fact, residual expression of viral genes from first and second generation Adv leads in just a few weeks to the clearance of the transduced cells by the immune system, in a more or less rapid process depending on their central or peripheral localization [44-46]. Gutless Adv, which are devoid of all viral genes, have a better immunogenic profile and enable more sustained expression of the transgene in the transduced cells [47]. However, they still cause an inflammatory response of the host to the capsid proteins at the time of administration, and are often contaminated with the helper virus, required to produce the viral particles [48].

Thus Adv seem appropriate vectors for transient expression of a transgene but it is generally admitted that they should be avoided for stable transgene expression over the long term. Moreover, the inflammation they trigger, even transient upon vector administration, is also a major hurdle to their use. Neuro-inflammatory processes are indeed already at work in many diseases of the CNS, so it will not appear realistic to use a therapeutic agent that could further increase this inflammation as a side effect. For this reason, implementation of Adv is relatively neglected in clinical trials for neurodegenerative diseases or neural dysfunctions. Despite these limitations, Adv have found a niche in gene therapy, their high efficiency for gene transfer and their pro-inflammatory attributes has led to them being reserved in the CNS to target incurable brain tumors [49,50].

III.2. Lentiviral vectors (LV)

Lentiviruses conform one of the 7 genera of the retrovirus family, and in the biotechnological genealogy of vectors, lentivirus-derived vectors (LV) such as HIV [51] are modeled on earlier developments of retroviral vectors (RV) based on alpha, beta or gammaretroviruses [31,52].

Retroviruses are enveloped diploid particles carrying two copies of a non-translated plus strand RNA genome enclosed in a protein capsid core. They enter into cells through specific interaction between the viral envelope and a cellular receptor, which often restricts viral entry into particular cell types [53]. Upon entry into the cell, a singular hallmark of retroviruses is the reverse transcription of their viral RNA genome into a double strand DNA provirus that integrates into the cell chromatin. These events are mediated by the viral enzymes reverse transcriptase (RT) and integrase (IN) through coordinated interactions with viral and cellular factors [54] and allow the perennial introduction of genetic material into cells.

Genomes of the different retroviruses range from 8 to 12 kb and display a gradient of complexity with more or less genes and *cis*-acting sequences. Common to all retroviruses are the genes gag, pro, pol and env, always retrieved in this same order, that encode the structural elements of the viral core, the viral enzymes and the envelop. More complex lentiviruses such as HIV express additional proteins involved in the transcription and export of the viral mRNA or favoring virulence [53].

Retroviral genomes also contain common *cis*-acting sequences such as the Long Terminal Repeat (LTR) for proviral integration and contain the signals of initiation and termination of transcription; the sequence psi (Ψ) allowing encapsidation of the viral RNA and the primer binding site (PBS) and the polypurine tract (PPT) required during reverse transcription. The lentiviruses have additional *cis*-acting sequences, i.e. the central polypurine tract (cPPT) and the central termination sequence (CTS) that lead to the formation of a central DNA triplex following reverse transcription, favoring nuclear entry of the viral DNA genome [55]. Moreover, lentiviruses possess a sequence regulating the cytoplasmic export of the viral RNA genome, the Rev Responsive Element (RRE). Both RV and LV are entirely devoid of viral coding sequences, conserving only *cis*-acting elements necessary for vector RNA encapsidation, reverse transcription and integration.

RV and LV also display plenty of particularities that distinguish them. A major one concerns their divergent route towards the nucleus; RV requires cell division and nuclear membrane disruption while LV DNA enters through the nuclear pore and can then be used to modify quiescent cells [53]. At the moment of their invention [51], LV therefore represented a real progress towards genetic modification of the brain and a serious alternative to Adv.

Across the years several generations of LV have been engineered to improve their biosafety and efficiency, which have been reviewed recently [56]. Most significant contributions improving LV safety concerned the removal of the enhancer sequences from the LTR giving rise to the so-called self-inactivating (SIN) vector, with reduced interference over the internal promoter or that of surrounding host genes, but also reducing the risk of recombination with a wild type HIV genome [57]. The main changes empowering LV efficiency consisted of i) enhancing the nuclear translocation of the viral DNA genome through adding the cPPT-CTS sequence of HIV-1 in the derived vector [55,58] and ii) enhancing and stabilizing transgene mRNA by adding post-translational regulatory sequences of viral or cellular genes [59]. These improvements act synergistically to increase transgene expression by 5 to 30 times in all kinds of cells by combining the central DNA triplex [58] and the woodchuck post-translational regulatory element [59]. For specific improvements of transgene expression in neural cells the use of the 3' and 5' UTR of neuronal mRNA also prove valuable [60].

An important feature of LV is that they remain functional as they carry heterologous viral envelopes, which provides them with new tropism properties [56]. These particles are called pseudotypes. The most commonly used envelope to pseudotype LV is the vesicular stomatitis virus glycoprotein (VSVG) that allows a wide tropism in mammalian tissues [61,62]. VSVG is stable and provides extra benefit as it withstands ultra-centrifugation allowing vector concentration to high titers [51,63]. When administered in mammalian's brain, VSVG pseudotypes are rather neurotropic but also allows transduction of glial cells [64-67]. Although large, the tropism of VSVG-pseudotyped LV seem somehow restricted *in vivo* as they preferentially transduce excitatory rather than inhibitory neurons [68]. LV pseudotyped with envelopes of neurotropic rabies (RVG) and Mokola virus (MKG) also permits transduction of non-dividing cells [69] with MKG-envelop restricting transduction to astrocytes [70]. Moreover, few reports have shown that in rodents and primates LV, either derived from HIV-1 or Equine Infectious Anemia Virus (EIAV), pseudotyped RVG, but not VSVG, allow retrograde axonal transport within the CNS or permit access to central neurons after peripheral delivery [71-74]. This is exciting and though very promising for future clinical applications, it needs further confirmation in models of disease to correlate vectors transport efficiency to therapeutic benefits in the target cells.

III.3. Adeno-associated viral vectors (rAAV)

Adeno-associated virus-derived vectors, are a matter of increasing interest in gene therapy especially concerning their use to target the CNS. They have a strong potential to transduce neurons, and enjoy a particularly safe biosecurity profile as they are derived form a poorly immunogenic and non-pathogenic virus. The vector particle consists in an icosahedral capsid of roughly 20 nm of diameter and made of 60 copies of VP1, VP2 and VP3 proteins (encoded by the AAV *cap* gene) in a ratio of 1:1:10. This capsid contains a single-stranded genomic DNA, which only retains the non-coding inverted terminal repeats (ITR) of the original virus, i.e. slightly less than 300 bp of DNA with a theoretical cloning capacity of 4.7 kb. Although in cell culture, AAV serotype 2 is known to integrate into a specific site on chromosome 19 in humans [75], the derived vector is mainly non-integrative, that is to say the vast majority of vector genomes persists in an extra-chromosomic form in the nucleus of the target cell, thereby excluding the risk of insertional mutagenesis [76-78]. Consequently, rAAV can provide a long-term expression in non-dividing target cells as CNS neurons, for which we can assume that transgene expression will persist during the cell life time, as it was demonstrated in animal models [79,80].

There is a wide variety of AAV serotypes each displaying particular tropism properties [81]. Moreover, the recombinant genome of a given seroytpe can be easily packaged into the capsid of another serotype i.e. (rAAV2/5 consists of the AAV2 recombinant genome cross-packaged in the capsid proteins encoded by the *cap* gene of AAV5) [82]. Some of these numerous serotypes have been used across laboratories to engineer vectors for use in experimental gene transfer. Several serotypes proved very effective in transducing brain neurons. This is particularly the case concerning serotypes 2/1, 2/5, 2/8, 2/9 and 2/rh10 to name only the most studied [81,83-85]. Although it seems difficult to extend a consensus from all these studies given that the effectiveness of a serotype may depend on the brain region and the species that are targeted, it remains that AAV2/5 appears to be a relatively safe choice for targeting CNS neurons. The situation is less favorable when glial cells and particularly astrocytes need to be transduced [79,81,83]. Although some of the serotypes allow the transduction of astrocytes, they require the implementation of cell-specific promoters in order to restrict expression to these cells [86-89]. In this case, the solution could come from alternative serotypes still unexplored, such as those isolated by PCR using degenerate primers from primates or other mammals [90,91].

Another very interesting feature of rAAV for CNS applications is the ability of certain serotypes, such as rAAV2/9, to transduce brain cells after intravenous administration [92-94]. Although promising, this method will require optimization before giving rise to a clinical application because it currently requires a very large vector dose and a disrupted or immature brain barrier to be effective.

The ease of rAAV production has enabled a large number of laboratories to easily access this technology and apply it in experimental gene therapy. Consequently, the therapeutic efficacy of rAAV has been demonstrated in many experimental models of CNS diseases (reviewed by Weinberg *et al.* [95], and Terzi *et al.* [96]). Finally, AAV has been - and still is - the subject of many developments and improvements that have increased significantly its efficiency. We may in particular mention: (i) double-stranded genome or self-complementary rAAV, which have a cloning capacity reduced by 50%, but that, by skipping the step of complementary strand replication upon transduction of the target cell, have a higher gene transfer efficiency [97-99]; (ii) point mutations of tyrosine residues exposed on the surface of the capsid, which can prevent viral particle ubiquitination in the cell [100-103]; (iii) the methods of capsid shuffling [104,105] and directed evolution [106,107] which, by mixing the sequences of several serotypes, provides new artificial capsids with completely new properties especially concerning their tropism and intracellular processing.

IV. Side effects of gene transfer

IV.1. Genotoxicity

Because viral vectors are used to modify the gene content of a cell, gene transfer may generate genotoxic side effects compromising cellular homeostasis. In fact adequate cell function is determined by tight control of gene expression and protein localization and concentration. This is regulated through complex mechanisms at transcriptional, translational and/or post-translational levels but can in turn be disturbed by inappropriate transgene expression either causing protein accumulation or miss regulation of cellular biochemistry [108-111]. In addition, the use of strong viral or chimeric promoters may provoke sequestration of transcription factors and alter side genetic pathways of the cell. Thus transgene overexpression may with time exhaust transduced cells and at best compromise its function within tissues but also cause its death. This correlation between regulation of transgene expression and success of gene therapy is often underestimated with practitioners frequently opting for promoters with ubiquitous steady activity, converting genetically modified cells in 24/7 recombinant factories. An effort to regulate transgene expression is then achieved for certain diseases where a therapeutic success is strictly linked to balanced transgene expression as in hemoglobinopathies [112] or to prevent off-target suicide gene expression [113] but usually not for the majority of conditions. This issue is particularly sensitive regarding genetic modification of the brain which is composed of hundreds of cell phenotypes with tightly regulated genetic programs, therefore necessitating targeting and regulating transgene expression to a high degree of precision. To this aim the exploitation of bioinformatics resources presently allows high throughput design of mini promoters with restricted activity in diverse neural cell populations [114-116], which shall contribute to the design of coming gene therapy protocols and most probably improve their therapeutic outcomes.

Integration pattern of LV is an important genotoxic issue when considering their use for *in vivo* and *ex vivo* gene therapy. Indeed, HIV and derived vectors preferentially integrate within the core of transcribed genes of the host cell [54], which presents a risk of insertion mutagenesis. This is due to the interaction between integrase and specific cellular factors such as Lens Epithelium-Derived Growth Factor (LEDGF/p75) or the karyopherin transportin 3 (TNPO3) that aid viral nuclear entry and integration within transcribed genes [54,117]. This may lead to transformation through oncogene activation, especially when the vector carries a strong internal promoter, or through disruption of tumor suppressor genes [118-120]. This though, is significantly reduced in neural cells where integration appears to be more random, presumably due to a reduced expression of LEDGF/p75 [121]. This mutagenic adverse effect thus rather concerns other tissues featuring a more prominent gene-targeted integration such as the blood or the liver [118-120]. However, to prevent insertional mutagenesis associated to LV integration, some groups, including ours, have undertaken the development of non-integrating LV, carrying a defective integrase (IDLV), that remain as nuclear DNA circles and that are suitable to transduce brain cells [122,123]. Thus, even though transcriptional efficacy of IDLV is slightly lower than that of LV, their use to treat neurological diseases should be preferred to that of integrating vectors.

IV.2. Inflammatory / immune response

Although the brain is considered to be an immune-privileged tissue due to the BBB, an immune response induced by direct gene transfer into the CNS must be considered when designing clinical or preclinical studies. This immune response may be directed against the vector particles but also against the product of the transgene, especially when it corresponds to a protein expressed for the first time. A stronger immune response can also be directed against the transduced cells when the vector expresses remaining viral genes. This is the case with first generations of Adv, resulting in rapid clearance of transduced cells by the immune system [46,124,125], which is exploited to clear tumor cells [49,50] or for vaccination [126]. However, the latest generations of vectors, Adv, LV or rAAV, carry genomes that are completely devoid of viral coding sequences and therefore have a much-reduced propensity to generate inflammation. For this reason these vectors are preferred when a long-term expression of the transgene is required.

The different virus-derived vectors do not equally elicit an immune response. In fact, even when depleted of the entire viral coding genome, Adv can still cause cytotoxicity due to immunity against capsids, which usually result, depending on the dose, the tissue and the immune fitness, in a more or less acute cell loss [127-129]. In the case of LV and rAAV, this cytotoxicity is much less pronounced and an immune response against these particles rather depends on previous immunization, especially for rAAV, the amount of vector or the expressed transgene [130,131].

A pre-existing immunity to the vector prior to its administration is of particular importance for rAAV, for which a majority of the human population is seropositive. In many cases the presence of circulating antibodies is capable of neutralizing several serotypes, including 1 and 2, which strongly questions the clinical utility of these serotypes [132,133]. It is therefore necessary to continue the search for new naive serotypes that do not infect humans but display appropriate tropisms as vectors. To that aim, researchers have at their disposal many different serotypes naturally existing in nature [90,134] of which properties can be further improved with capsid-shuffling and directed evolution [106,135,136]. The possibility of an immune response against the vector particles also raises the question of the possible repeatability of vector administration. When the procedure must be repeated, the immune memory induced by the first administration may obliterate the effectiveness of successive ones. It has indeed been shown by several teams that a peripheral AAV2 infusion in rats compromises gene transfer with the same vector in the CNS [137,138]. However, a recent study, also performed on rats, showed that pre-immunization is less effective when the first dose is administered in the CNS [139]. In addition, it was demonstrated in large animals that subretinal administration of rAAV can be repeated without decreasing efficiency, even when it generates an increase of circulating antibody against the vector [140]. On this basis, patients with Leber congenital amaurosis that had an eye treated with gene therapy could have the same treatment for the second eye after 2-3 years, without significant side effects [141].

As mentioned, the immune response may be directed against the transgene product. This is the case when the transgene encodes a factor that is not recognized as a self-antigen by the immune system, either because it is an exogenous factor, or because it is a protein that is not expressed postnatally. As demonstrated by the recent study of Ciesielska *et al.*, it appears that the phenotype of the transduced cells is a key factor in generating this immune response. They compared the stability of expression over a period of eight weeks of GFP and AADC (aromatic acid decarboxylase - a candidate for gene supplementation in Parkinson's disease) supplied into the striatum of non-human primates by rAAV serotype 2 or 9 [142]. They observed that although rAAV2/9 can transduce a larger region of the striatum, expression is more stable with rAAV2/2. They speculated that this is due to a far better transduction of microglial cells by rAAV2/9 than rAAV2/2 on the one hand, and on the other, that these cells co-express markers of antigen-presenting cells. In fact the transduction of cells expressing class I or class II MHC that are able to present antigens and capable of priming adaptive immunity to the transgene, reduces the efficiency of transgene expression. Instead, the prevention of transgene expression in intravascular or extravascular hematopoietic cells with tissue specific promoters or through a miRNA detargeting strategy prevents transgene-epitopes presentation and allows persistence of transduced cells and long-term expression of the transgene [143].

Thus, when setting up a gene therapy procedure, either pre-clinical or clinical, it is essential to consider the indivisible trio, vector / transgene / target(s) cell(s) to anticipate and overcome a possible immune response compromising the cure.

V. Conclusion: further developments to obtain stereotypic vectors.

Idealness of viral vectors is a concept at the confluence of pharmacological, clinical and ethical expectancies. This concept is defined by different properties that are ethically and pharmacologically general to all vectors but clinically particular to each medical condition. In consequences, specific vectors are developed for different situations by conjugating vectors properties to the constraints imposed by each disease.

For stable gene transfer in the brain, vector particles should, in principle, be innocuous but provide efficient gene transfer. They should be used to correct a precise physiopathological process to reverse a cellular defect. To this aim, sought vectors should be engineered to target particular populations of cells and express a transgene from a physiological promoter corresponding, if applicable, to the replaced gene. The development of effective vectors shall moreover be accompanied by advances in administration procedures that should be minimally invasive and that permit vector diffusion, if needed. Consequently to reach the brain, it will be important to develop strategies to transiently disrupt the BBB, but also to create vectors that can cross the BBB or that are efficiently transported along nerve terminals so they can be administered peripherally. To treat a number of monogenic diseases with gene therapy, it will also be necessary to associate vector administration with protocols of induction of immune tolerance to the transgene product to ensure long term acceptance of genetically modified cells within the body. Hence, the future of gene therapy is tightly linked to that of other branches of biotechnology and medicine. For instance, in addition to classical engineering of vectors, much is expected from progresses in the development of new materials and nanoparticles that can be associated with viral-derived vectors, providing additional properties of immune escape, enabling BBB crossing, cell specific entry, directed integration, gene repair or other, thus far, unsuspected functions. The ongoing revolution in biology and medicine foresees that such technological advances are within reach. Slower, though, goes the progress of disease comprehension, which should always be more heavily weighted before modifying the human's genome through an irreversible procedure.

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VII. Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article

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