Present Situation of Viral Vector Manufacturing and Ways to Overcome Potential Barriers in View of the Routine Large Scale Production and Use of Viral Vectors

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Abstract

The marketing authorization of two gene therapy treatments in Europe has opened the door to routine manufacturing of viral vectors, in particular, of MLV, LV, and AAV vectors, meaning that a quantum leap in routine manufacturing technology will be necessary in order to be able to produce the required quantities. However, this quantum leap is already required for producing the vector amounts necessary for performing advanced clinical trials, in particular, with respect to neuromuscular diseases (NMDs), such as Duchene Muscular Dystrophy (DMD). This review presents briefly the actual situation of the production of the most important viral vectors (AV, MLV, LV, and AAV) and barriers to be overcome in view of the large scale manufacturing of these vectors for routine use.

Keywords: Adenoviral (AV); γ-retroviral (MLV); Lentiviral (LV); Adeno-associated viral (AAV); Vectors; Production technology; Potential improvements

Abbreviation: AV: Adenovirus; MLV: Murine leukemia Virus ; LV: Lentivirus; AAV: Adeno-associated virus

Introduction

Since the first clinical gene therapy trial in 1990 [1] this novel therapeutic concept (ATMP=advanced therapy medicinal product) has come of age and since 2012 two gene therapy treatments (in 2012: Glybera, an AAV1 vector for the in vivo gene therapy treatment of LPL deficiency [2], and in 2016: Strimvelis, MLV vector for the ex vivo gene therapy treatment of ADA-SCID [3]) have received marketing authorization in Europe with more authorizations of further gene therapy treatments coming in the near future. Both diseases are rare diseases, meaning that only few/very few patients are available. Furthermore, the localized IM administration (treatment of LPL deficiency) or the ex vivo treatment of patient cells with recombinant MLV in the case of the ADA-SCID treatment require low to moderate vector amounts which can be generated using the existing technologies; however, in the case of the treatment of non-rare diseases, such as drepanocytosis with about 50 million patients worldwide (http://www.futura-sciences.com/sante/actualites/medecine-therapie-genique-sucess-drepanocyte-60907/), or neuromuscular diseases, like Duchene Muscular Dystrophy, requiring whole body treatment (=muscle tissue represents 50% of the whole body weight) with vector doses exceeding $5 \times 10^{13}$vg/kg for reaching at least 40% of dystrophin-positive fibers in the GRM dog model [4], the present manufacturing technologies are insufficient/inappropriate and novel more efficient technologies are urgently required (=need for a quantum leap).

Today the most important vector types for gene therapy of rare and non-rare diseases are MLV/LV and AAV vectors. Up to 2016 18.2%/5.8% and 7% of all gene therapy trials, respectively, were based on the use of these vectors (http://www.abedia.com/wiley/vectors.php). MLV (mouse leukemia virus based viral) vectors in parallel to adenoviral vectors have been largely developed in the 1990s; however, severe events have been observed in a number of clinical studies of primary immune deficiencies, including SCID-X1, CGD, and WAS because of insertional activation of proto-oncogenes (=insertional mutagenesis) [5]. Nevertheless, MLV vectors are still of interest today and several CAR-T cell applications are based on the use of these viral vectors. On another hand, the interest in the use of adenoviral vectors (percentage of all gene therapy trials
performed with this vector type: 21.4%) decreased also with the end of the 1990s, first because they lead only to a transient transgene expression and second because of the severe adverse event leading to the death of a patient which had developed a systemic inflammatory response syndrome, biochemically detectable disseminated intravascular coagulation, and multiple organ system failure upon infusion of a dose of $6 \times 10^{10}$ particles/kg [6]. Thus today their field of therapeutic use is rather restrained and this type of vector is essentially developed in the field of oncolytic viruses [7].

Despite this reduced interest in the use of adenoviral and MLV vectors, I have included them in this mini-review.Obviously manufacturing issues are vector specific and the barriers to overcome are different for each of them.

Adenoviral Vectors

The production technology of adenoviral vectors is based on ‘simple’ infection of producer cells (PerC6 or HEK293 cells) growing in suspension using serum-free media. In many studies cell growth and vector production conditions have been optimized by Altaras et al. [8]. The same authors have also extensively reviewed downstream processing and formulation issues related to adenoviral vectors. Furthermore, scale-up studies at 250L scale in view of a 10000L production scale have been performed in the frame of a project for the development of an E1 deleted Ad5 vaccine for HIV-1 vaccination [9]. In addition, Shen et al. [10] developed a process at a 500L scale for the routine production of a replication competent Ad5 (AdRG1.3) as rabies vaccine (ONRAB® oral rabies vaccine) for control of rabies in wildlife in several provinces of Canada. In both cases, volumetric productivities of $5 \times 10^{10}$ vp/mL were observed. Both examples show that there are no scale-up issues for manufacturing adenoviral vectors and that the quantities required can be produced because of an existing technology. The only drawback of the existing technology relates to the low cell density ($0.5-2 \times 10^{10}$ particles/ml) at infection because of the so-called ‘cell density effect’ in particular when using HEK293 cells [8].

Alleviation can be achieved either by using: (i) Fixed/packed bed reactor systems: Cultures of HEK293 cells or their sub clones in fixed/packed bed reactors lead to a 5 to 10 fold increase in bioreactor biomass at the time of infection and allow a perfusion regime for maintaining the cells in a metabolic state optimal for growth, infection and vector production. Using this technology, cell densities of $5 \times 10^{10}$ per packed bed reactor (14L working volume~$3.6 \times 10^{10}$ particles/ml) could be obtained. The culture was infected at this cell density in view of the production of adenoviral vectors for cancer therapy (‘Gendicine’), which had been authorized in China [8,11]. However, it should be indicated here, that a non-disposable, scale-limited Celli gen Plus reactor system was used for this purpose. Using a similar culture principle but as disposable and scalable culture system (iCELLis) [12] could produce $1.6 \times 10^{14}$ and $6.1 \times 10^{15}$ viral particles vp per batch, respectively, using the small scale Nano system (4m$^2$) and the large scale 500/100m$^2$ system. The specific vector productivities/cm$^2$ were similar at both scales. In the iCELLis 500/100m$^2$ cell numbers of 103600 in average per cm$^2$ were obtained which translates into $1.6 \times 10^{10}$/ml. These data clearly infer that even in the case of fixed bed reactor systems the optimal cell density calculated per medium volume contained in the reactor stays between 1.6 and $3.6 \times 10^{10}$/ml which is the upper limit of the ‘cell density effect’ for adenovirus infection using HEK293. (ii) Novel more efficient cell lines: A second solution to alleviate the cell density effect phenomenon is the use of other cell lines. In the case of PerC6 cells a certain cell density effect was also reported although via medium exchanges before and after infection, these cells can be infected at cell densities of $10^9$/ml, achieving high volumetric productivities despite the strong decrease in cell-specific productivity at high cell concentrations [13]. In contrast, a rather recently developed cell line (1G3), established via the immortalization of human amniocytes with the adenoviral E1A and E1B genes, in view of the production of adenoviral vectors could be cultivated at cell densities up to $9 \times 10^9$/ml in serum-free suspension culture whereas HEK293 cells reached only a cell density of $6.1 \times 10^9$/ml. Furthermore these cells showed only a minor cell density effect at infection cell density of $5 \times 10^9$/ml in comparison to $3 \times 10^9$/ml; with respect to $1 \times 10^9$/ml, the specific vector production rate was similar for 1 and $5 \times 10^9$/ml [14].

Finally, for the production of oncolytic adenoviral vectors, HeLaS3 cells have been used in a high cell density perfusion process at a 70L pilot scale (post infection cell densities: $1-2 \times 10^9$/ml) for generating vector titers of up to $3.4 \times 10^{11}$vp/ml ($3.1 \times 10^{10}$ vp/c) or $>10^{15}$vp per large scale run. The very interesting observation in this example is that no cell density effect as observed for HEK293 or PerC6 cells was observed [15].

In any case, today the main interest of adenoviral vectors resides in their use for vaccine purposes and as oncolytic viruses.

γ- Retroviral (MLV – Murineleukemia Virus) Vectors

These vectors were one of the first vectors which had been developed up to clinical trials. Their main advantage of the use of retroviral vectors is the fact that these vectors can be produced using stable producer cell lines which constitutively produce viral vectors, though for certain applications transient transfection methods have also been used. Due to safety considerations related to the use of mouse fibroblast cell lines because they carry defective MLV homologous sequences and multiple copies of murine non-homologous sequences (VL30), respectively, being able to recombine with vector components and to be packaged, reverse transcribed and integrated into the target cell genome [16,17], in the recent years they have been replaced by human cell lines with a clear preference for HEK293 cells [18], though derivatives of PG13 cells (NH3T3 based)
are still in use. The advantage of HEK293 cells is that they are widely used, that they can be cultivated in serum-free media in suspension culture [19-21], that the metabolic pathways of some sub clones in relation to the production of retroviral vectors have been well characterized [22,23] and that different clones have been developed which can produce different retroviral vector pseudo types VSVg [19], amphotrope [20,24], GalV [21,25] and RD114 [21].

Since in most of the cases, γ-retroviral vectors are produced using stable cell lines, production is based either on repeated batch cultures using roller bottles or Cell Factories or on perfusion cultures using fixed bed or suspension culture systems. It is obvious that the use of roller bottles or Cell Factories represent an intermediate production scale whereas fixed bed or suspension culture based reactor system are scalable culture systems. Whereas during the production phase, roller bottles (850cm² and 1700cm²) and Cell Factories (CF10) can produce 120-270ml (0.14-0.31ml/(cm²*d)) and 600-650ml (about 0.1ml/(cm²*d)), respectively, per day [26] meaning that the overall daily volume depends on the number of parallel culture devices, fixed bed or suspension culture reactor systems show more elevated production capacities. In the case of fixed bed reactors (Cell Cube) the daily perfusion rates leveled between 0.045 and 0.124ml/(cm²*d) and, for instance, between 200-400L of vector supernatant have been produced with the largest Cell Cube unit (340000cm²) within 13 days of perfusion culture [27].

However, the production phases are rather different: for the roller bottle and Cell Factory based systems, vector harvests are essentially performed during 3-4 consecutive days; in case of the Cell Cube based production processes; vector production phases beyond 13 consecutive days have been communicated. More details can be found in the review by [26].

More recently, in view of the production of retroviral vectors for the CAR-T cell approach, roller bottle [28]: use of a PG13 based producer cell line, Cell Factory [29] and fixed bed reactor [29]: use of a HEK293 based producer cell line [21] based manufacturing processes have been used. The roller bottle and Cell Factory based processes showed production limitations as presented above allowing the production of 120-240ml and of 600ml, respectively, of vector supernatant per culture unit meaning that the production lot consisted of 3100-6200ml/day (use of 26 roller bottles-[28]) or 3600ml/day (use of 6 CF10 stacks- [29]). The comparison of the CF10 based production process with a fixed bed reactor based production process (use of the disposable iCELLis Nano system) clearly indicated the superiority of the fixed bed reactor system for retroviral vector production. Whereas the production lot using 6 CF10 stacks consisted of 10.8L of supernatant produced during 3 consecutive days, the iCELL is Nano system produced 24L of vector supernatant during 10 production days, thus confirming data published by Merten et al. in 2001. This vector quantity generated with the fixed bed reactor system was sufficient for the transduction of 70 to 500 patient T-cell doses [29].

A further advantage of the use of the iCELL is Nano system is that it is characterized by reduced contamination risk (because this system is a closed system), reduced operator involvement and the fact that suspension cells as well as surface adherently growing cells can be cultured in this reactor system.

Although the production of up to 500 patient doses is sufficient for a clinical trial [29], for application in the frame of the CAR-T cell approach larger vector amounts will be required: On one side, a larger scale iCELL is system (e.g. the iCELLis 500) could be implemented which by identical behavior of the producer cells would generate 13100 – 93500 doses per run, which is a rather realistic and straightforward endeavor. However, on another side a real suspension process would also be possible because HEK293 cells can be adapted to suspension culture. That this is feasible could be shown by [21] and depending on the cell line (pseudo type), identical or 2-6 times lower vector titers have been produced in comparison to adherently grown cells. However, production cultures could be continued for up to 3 months by keeping constant vector productivities.

Furthermore in this context, [19] could show that such a process can be intensified by increasing the reactor biomass via the implementation of cell retention system (acoustic filter) allowing medium perfusion without losing active biomass. The implementation of this high density process at a 3L scale allowed a 10-fold increase in vector productivity in comparison to the adherent culture. However, for the moment, these achievements have not yet been evaluated at large scale.

In addition to technological optimization, the culture conditions, including the choice of the pH, pO₂, medium composition, such as the addition of fructose instead of glucose or the addition of cholesterol, etc., have to be optimized for increasing vector productivity. Details on the different parameters and their impact on retroviral vector productivity were published in a review by [30] and will not be further described here.

Obviously, in addition to the optimization of the culture conditions impacting the cellular metabolism, the improvement of the cell metabolism via genetic means is a supplementary option for boosting vector production. In this context, [31] could show that the down-regulation of either hypoxia inducible factor 1 (HIF1) alone or together with pyruvate dehydrogenase kinase (PDK) in HEK293 based retroviral producer cells led to a more than 20-fold and 30-fold, respectively, increase in specific productivity of infectious viral titers. The joint knock-down of both genes had an activating effect on many metabolic pathways.

In summary, it can be estimated that the implementation of these different improvements-improvement of culture conditions, use of high cell density production process, metabolic modification of the cells via genetic manipulation-will lead to an overall estimated improvement in vector production levels by 300-fold or more per given reactor volume.
Lentiviral (LV) Vectors

The development of the third generation of lentiviral vectors [33-36] which are characterized by the fact that no replication competent virus can be generated through recombination events has opened the door to their use in humans for gene therapy purposes. Very rapidly these vectors have replaced MLV vectors to a large extent for treatment of rare diseases, in particular of primary immune deficiencies (PIDs), because of their improved safety features, but also because lentiviral vectors can transduce non-dividing cells, and they can be produced at higher vector titers than MLV vectors. In addition, to their use for the treatment of PIDs, the LV vector system is also developed in the frame of the CAR-T cell approach and in vivo applications for the treatment of diseases of the central nervous system and the retina.

a) Production issues: Lentiviral vector production is essentially done using a transfection based approach of HEK293 (T) cells grown in 10-stack Cell Factories (CF - 10). Vector containing supernatant is harvested once or twice after transfection. The largest scale consists in the production of supernatant using 120 CF-10 units producing 120L of supernatant which then is purified leading to about 500ml of vector preparation (total vector quantity:~10^12ig) which is sufficient for the ex vivo gene therapy treatment of about 15 patients with a PID. Most of the developed purification protocols are based on chromatography and membrane based methods and are thus scalable. For details the reader is referred to the article by [37].

The main drawback of this production mode is the rather limited scalability. Even the use of 120 Cell Factories (CF-10) is a small scale production system because it is based on several production cycles of each cycle consisting of 10 CF-10 units per week followed by pooling of purified sub-batches [38].

In principle, there are two approaches for at least partially solving these drawbacks: The first approach is to perform transient transfection in suspension culture of HEK293T cells by using PEI. The feasibility of this approach could be shown by [39,40] at a 3L and 50L scale, respectively. In this context, the implementation of a perfusion protocol would allow multiplying the harvest volume by 2 or 3 while keeping constant the plasmid quantities. Though this might be a solution for medium scale productions with a potential maximal scale of 200L (with a potential vector output of 7x10^12ig), there are several shortcomings, which reside in the variability of a transfection based production process, the enormous costs associated with the use of 4 different plasmids (for HIV-1 LV vectors) e.g. for a 200L culture 750mg plasmids are required [37] and the open question is whether PEI is separated from the vectors during purification or whether it is co-purified because for the moment no analytical methods are available.

The second approach consists in the development and implementation of stable producer cell lines. Though this is probably the real solution for the present problem there have been many attempts to develop such cell lines, however, for the moment, only one stable cell line has been developed up to the production of clinical grade vector preparations. The main difficulties are related first to the cytotoxicity of some of the viral gene products such as the lentiviral protease as well as the VSV-g envelope protein largely used for this type of vectors. A solution is the development of an inducible cell line which has the advantage that the cells can be amplified without the metabolic burden related to vector production (which probably leads to a genetic stabilization of the cell line) and after having obtained the cell density optimal for vector production, LV vector production is induced. Though this is feasible such cell lines generally show a production window of about 1 week after induction [41,42] with vector titers beyond 10^7TU/ml. Amongst other induction systems, the tet system is largely used. In principle, tet-off and tet-on induction systems are available which are based on the induction of expression either upon removal of doxycycline or upon the addition of doxycycline, respectively. Though the tet-on induction system is the system which can be readily used for large scale production in suspension culture [41] because the inducer is added for inducing the expression, for the moment only the tet-off system has found its way to GMP production of lentiviral vectors [42,43]. Scale-up of this induction system is not straightforward because a medium change as well as a washing step of the producer cells is required for inducing vector production. In single cell suspension culture this is rather difficult to perform wherefore [43] immobilized the producer cells in FibraCel disks which are then cultured in WAVE reactors (working volume: 25L). As such the cells can be washed relatively easily. During vector production, medium is repeatedly changed for 6-8 days for harvesting vector containing supernatant. Though such a process is feasible a real scale-up to several 1000L runs cannot be imaginary.

Thus the most straightforward way, is either the use of tet-on inducible cell lines or cell lines which produce the lentiviral vector constitutively, which obviously is only possible when other envelope proteins than VSV-g and a mutated and less toxic version of the lentiviral protease are used. More detailed information on the development of stable producer cell lines can be found in the review by [37].

b) Further improvements: Though the VSV-g envelope protein has its advantages, in particular, with respect to the...
very large tropism as well as the stability towards harsh environmental conditions during downstream processing, other pseudo types are of high interest because of their cell and tissue specific tropism. Often such pseudo types show a much higher transduction efficiency of their target cells/tissues than when using VSV-g pseudo typed lentiviral vectors and have also a much better potential for in vivo use than the VSV-g pseudo types. For instance, by comparing purified GaLV-TR LV vector pseudo types with VSV-g LV vector pseudo types we could show that the GaLV-TR pseudo types could transduce up to 70% of hematopoietic stem cells at an MOI of 20, whereas when using a VSV-g pseudo typed LV vectors only 20% of the cells were transduced when using an MOI of 200 [44]. This represents an estimated improvement of a factor about 30 fold by taking into account the reduced MOI and the improved transduction efficiency. From this example it is obvious that the use of very specific envelope proteins will allow a considerable reduction in the vector quantity for transduction purposes and thus reduced vector amount administered directly or indirectly to the patient, and thus represents also an increase in the number of doses which can be produced with one production run.

In principle, the replacement of VSV-g pseudotype by LV vectors pseudotype with other more specific env proteins is an important endeavor, however, due to the lack of scalable purification means for most of these pseudo types these developments have not been done up to now. In this context, [44] presented a purification protocol for GaLV-TR enveloped LV vectors which could be the base on one side for the establishment of a large scale purification protocol of this pseudo type and on the other side for development of purification protocols of other LV-pseudo types.

Further improvements concern the replacement of Retronectin by Vectofusin-1 [45] or the Enhancing Factor C (EF-C=Protransduzin®) [46,47] for enhancing cell transduction without the need for coating of plates as required for Retronectin which will lead to a streamlining of the transduction process as well as to a reduction of the vector quantity required for transduction of the target cells.

**AAV Vectors**

The adeno-associated virus belongs to the Parvoviridae family and is classified in the Dependovirus genus, because it requires replication of a helper virus (adenovirus, herpesvirus) for its own replication and production. Thus for producing AAV vectors, viral helper functions have to be provided either via co-replication of a helper virus or the presence of the helper function sequences.

**Different production modes/required vector dose**

The first production method is based on the transient transfection of HEK293 cells using either a three or a two plasmid system. Three days after transfection the cells are lysed for recovering the AAV which is then further purified using at a small scale precipitation and gradient centrifugation based methods [48] or at a larger scale by chromatography and membrane based methods [49]. As for the lentiviral vectors, the initial scale up was performed via the use of roller bottles or Cell Factories in order to produce vector quantities required for phase I/II clinical trials. In this context, we have developed a process based on 10 Cell Factories (CF-10) generating 2x10^{12}vg of AAV1 after purification. The size of such a vector lot is sufficient for a phase I clinical trial in the frame of the treatment of γ-sarcoglycanopathy (via intramuscular (IM) administration) for which altogether 2x10^{12}vg of AAV1 were required (this trial consisted in three equal groups which have received three escalating doses of the vector) [50]. However, gene therapy of muscle diseases reasonably cannot be performed via intramuscular (IM) administration and only systemic treatments are reasonable. Such a treatment would require minimal doses of >5x10^{12}vg/kg of patient weight as established in a dog model of DMD [4]. This signifies that 5x10^{12}vg AAV would be required for the treatment of 10 patients at 3 different doses. In the case of using the Cell Factory approach, about 2500 CF-10 units would be required; however, this is not realistic.

**Advanced production systems**

The scale up of AAV vectors can be performed by using different biological systems:

a) Transfection is suspension: the transfection process can be intensified via the transfection of HEK293 cells cultured in suspension in stirred tank or WAVE reactors. Using a selected HEK293 cell clone producing >10^{13}vg/c, [51] have established a suspension process at a 20L scale (WAVE reactor) which allows the generation of 2x10^{12}vg per run. The implementation of a perfusion system, taking advantage of the release of AAV vectors from the producer cells during prolonged culture phase, will lead to a further production increase by a factor of 5-10fold [51]. Though it can be estimated that the largest scale will probably be 200L or 250L with the possibility to produce 2x10^{12}-2.5x10^{13}vg per run, this might still be too little for the routine treatment of DMD patients.

b) Based on this situation, other production systems should be considered. There are essential three different biological systems available which can be used for the large scale production of AAV vectors:

(i) HeLa cells based stable producer cells containing the rep-cap and the recombinant vector sequence (transgene cassette flanked by the two ITRs) which after having obtained the optimal infection cell density are infected with adenovirus for inducing AAV vector production.

(ii) The Herpes simplex expression system, in which BHK21 cells adapted to grow in serum-free medium in suspension are infected with two different recombinant herpes simplex
viruses (ICP27 deficient) bringing in the rep-cap functions and the recombinant AAV vector sequence (transgene cassette flanked by the two ITRs) for inducing AAV vector production,

(iii) The Baculovirus/insect cell system, in which Sf9 cells growing in serum-free medium in suspension are infected with two different recombinant baculoviruses bringing in the rep-cap functions and the recombinant vector sequence (trans gene cassette flanked by the two ITRs) for inducing AAV vector production. These three systems producing up to 10^10vg/g are thus relatively comparable. The largest established production scales are 2000L (Thorne 2015) for use of stable cell lines, 100L for the herpes simplex based expression system and 200L for the baculovirus/insect cell system. The main disadvantage of the use of stable cell lines is the fact that the development of producer cell lines is a time consuming process requiring an efficient selection for identifying the best producer cell line process (for more details, see, [52]). The two other expression systems are not characterized by such problems, however, in both cases, the recombinant viruses (herpes simplex virus, baculovirus) have to be developed, amplified, and characterized. Improvements for increasing AAV vector productivity can be achieved on one side by improvement of the constructs used in context of the respective expression systems; and on the other side by improvement of the manufacturing process. As an example, in the case of the baculovirus/insect cell expression system, the implementation of a mono baculovirus system, the use of wild type ITRs for replacing the frequently used truncated ITRs (SUB201), or the implementation of a delta-chitinase/cathepsine baculovirus system can improve vector quality, potency and vector titre by up to 32 fold [53].

For all expression systems (except the transfection based production system), the improvement of the manufacturing process passes first by the implementation of a high cell density production process which can lead to a 10 fold increase in the reactor specific productivity as it could be shown by [54] for the baculovirus system. Furthermore the optimization of the purification protocol (e.g. replacement of the AVB column (=affinity chromatography system developed for AAV1/AAV2) by a specific support for a given AAV serotype (e.g. for AAV8: POROS Capture Select AAV8 Affinity Matrix) leads also to a 2-3 fold increase in the purification yield. The implementation of both improvements can lead to 20 fold increase in vector productivity. More details on potential improvements on AAV manufacturing can be found in the review by [53].

Finally, vector potency can be improved on one side by the treatment of AAV vectors with human serum albumin [55] which seems to improve the interaction between the vector and the target cell during the transduction event, and on the other side by the optimization of the vector caps id in view of the modification of the vector tropism (improved targeting of the target tissue and in the same time a detargeting of the liver, the natural target of AAV). Although no improvement factors can be given here, it is expected that they represent the most important lever for improving vector potency and thus reducing the vector dose required for treatment of the patients.

It is probable that the implementation of all improvements in the context of the baculovirus system will provide the possibility to reduce the reactor volume required for the production of a patient dose, e.g. for the treatment of NMDs, by 200, meaning that gene therapy of rare diseases requiring high/very high vector doses for treatment of a patient will be feasible from a technical but also from an economic stand point [56].

### Conclusion and perspectives

Each vector system has its specific characteristics meaning that in most of the cases specific optimisations can only be performed for a given vector system. Although the presently available vector systems have shown their efficiency in many clinical trials some of which have conducted to their authorization for routine use, it is obvious that all vector systems require improvements in order to increase vector productivity (cell specific as well as reactor productivity) for increasing the size of the vector amount per reactor run (lot) and in parallel reducing production costs. In particular, more improvements with respect to vector potency, are urgently needed which can be obtained by the amelioration of the viral vector itself, improvement of its interaction with and of its incorporation by the target cell, and finally the efficient transfer of the vector genome into the nucleus of the target cell=better understanding and improvement of the vector biology and the interaction of a given vector with its target tissue. At the end of the day this will lead to novel routine treatments for the benefit to mankind available to everybody in need.

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