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Lentiviral Vectors Come of Age? Hurdles and Challenges in Scaling Up Manufacture

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Abstract

The pharmaceutical industry has been attracted to the gene therapy field and is starting to support clinical trials, establishing collaborative strategies to develop commercial products which in many cases are based on lentiviral vectors. The predictable widespread use of lentiviral vectors in next-generation gene therapy scenarios aimed at dealing with not only rare diseases raises important challenges and hurdles regarding their manufacture. The author reflects on this in the chapter on the state of the art in the manufacture of lentiviral vectors, addressing some current manufacturing processes, their achievements, and the uncertainties in ensuring a validated process capable of releasing consistent vector quality that meets global health authorities’ requirements. In summary, the proposal looks at the goals and challenges that must be addressed in manufacturing lentiviral vectors, in order to satisfy supply in the commercial stage, before we reach the next stage in gene therapy.

Keywords: lentiviral vector, large-scale manufacturing, gene therapy

1. Introduction

The practice of medicine is undergoing a revolution, moving from a focus on the treatment of symptoms, toward targeting the genetic cause of the disease. The huge development of disciplines, including but not restricted to molecular and cellular biology and genetic sciences, provides the framework for the advancement of individualized precision medicine. This new conception of medicine is based on the novel paradigm: the genes represent medicines themselves. Gene therapy is the groundbreaking strategy, which uses genes as medicines. Gene therapy is no longer an experimental approach [1, 2], and as with any novel therapy, patients’ benefits must be balanced against the nonzero risk of the therapeutical approach. Most products currently assayed in clinical trials of gene therapy are viral vectors [3], i.e., biological products that challenge both the manufacturing processes in order to guarantee the supply of adequate quantities of the active vector and the regulatory requirements from the medicine agencies of target countries. In summary, viral vector production on a large scale implies novel challenges for a multidisciplinary field, in order to accommodate such specific requirements within the industrial process.

The first gene therapy experiments took advantage of the strategy that members of the Retroviridae family of viruses evolved to spread and remain stable in
the host, with the integration of their genomes. Since those experiments we have assisted to a fast development of the viral vector field fuelled by promising data raised from early studies until the achievement of the current scenario [4, 5]. The gene therapy field is witnessing a sort of gold rush that is boosting personalized medicine by confronting many diseases as genetically treatable traits. Early vector developments focused on Gammaretrovirus as integrating entities for the delivery of a stable expression of the correctable gene, but since the last decade, they have been displaced by vectors deriving from the Lentivirus genus. As opposed to Gammaretrovirus, the Lentivirus displayed preferred integration sites in coding genomic regions rather than in transcription regulatory regions, and this has become a major safety feature to exploit. Nowadays, non-replicative self-inactivating lentiviral (SIN) vectors are used in the vast majority of novel gene therapy clinical trials using integrative vectors and are considered by now the optimal tools for ex vivo gene therapy and the safest and easiest-to-use vectors available for the delivery of genes into mammalian tissues [6].

The use of retroviral vectors in gene therapy as an emerging technology is following the Gartner hype cycle. Hope and expectation were seen when gene therapy entered the clinic in the early 1990s, but due to a lack of profound success and the unexpected death in 2000 of two patients, caused by the treatment, the expectations slowed down. In the following decade (2000–2010), two approaches coexisted: first, follow-up and ongoing clinical trials that were using an integrative type of vector (gammaretroviruses, gRV) used in those clinical trials resulting in unexpected fatal deaths and, second, intensive academic research focused on the development of new viral vectors and methodical exploration of the clinical procedure. This resulted in the advent of a novel type of vector called SIN-LV, derived from the causative agent of AIDS, the Lentivirus HIV, properly modified and engineered to render them safer (see Vectorizing HIV). In the current decade (2010 to the present), we have been recording investigative clinical trials using both vectors in several hematological and neurodegenerative rare diseases with a conclusion: the feasibility of the second-generation gene therapy approaches [4, 5]. This is mostly due to the huge development of SIN-LV vectors, due to their safer profile, in comparison with gammaretroviruses [6].

Gene therapy products have entered the commercialization phase, and a dozen treatments have been approved since 2012 in EU and the USA [1]. Up to last year, all of them were treatments for rare or ultrarare conditions, but in August 2017, a new key milestone for gene therapy development can be added to the chart, reinforcing the concept of gene therapy use in frequent pathologies in which current treatments are failing; the Novartis receives the first ever FDA approval for a CAR-T cell therapy, Kymriah® (tisagenlecleucel), for children and young adults with B-cell acute lymphoid leukemia (ALL). The goals reached in the past two decades in the gene therapy field open novel expectations offering a cure to rare genetic diseases, cancer, infectious diseases, and vaccine development, and in the short-medium term, innovations in the field will make affordable genetic intervention covering an array of diseases with a gene-defined cause [7].

The aim of this review is to recapitulate specific problems related to the manufacture of lentiviral vectors in particular during production stages, known as the upstream process (USP), focusing on the limitations that exist when scaling up, due to the nature of the virus, the particularities of the lentivector life cycle, and the producer cell line commonly used for production. There are excellent reviews [13–16] that provide a detailed description of the methodologies that can be followed in order to produce lentivectors on a large scale. There is a growing interest in the lentiviral vector field, and there are many topics worthy of a description. For those interested in more detailed, specific topics, i.e., manufacturing of CAR-T
approaches, purification strategies, or specific problems linked to the target cell, I recommend references [10, 11, 17].

2. Delivering genes with Lentivirus

The virus-derived vector as delivery system lies at the heart of most currently employed forms of gene therapy; without the viral vectors, there is no treatment. These viruses must be custom-made in specialized facilities for each treatment, but manufacturing them is costly and onerous: it requires great expertise and multidisciplinary teams and specialized facilities with stringent conditions both for safety/containment and demanding production methods, under good manufacturing practice with regard to compliance (GMP) [8].

Viral vectors are complex bioproducts with an ordered architecture and are very sensitive to handling and environmental conditions. For these reasons, there are stringent requirements aimed at preserving biological activity during all stages of the manufacturing and delivery process. Then, during the production, purification, storage, and transportation stages, it is necessary to maintain specific rigorous control aimed at minimizing the loss of biological activity, in addition to controls which are common to other bioproducts, such as sterility. This implies that the manufacture of large amounts of a viral vector cannot simply be produced by transferring the know-how and well-established procedures developed in the pharmaceutical industry for the production of monoclonal antibodies or recombinant proteins.

The large-scale manufacture of lentivectors for use in humans is becoming the bottleneck in the success of ongoing or planned gene therapy development to be launched in the near future [9]. Indeed, the manufacturing capabilities of the companies to satisfy the short-medium term markets are central to decision-making for backers and investors, who are becoming cautious with regard to biotech firms developing gene therapy products that do not have a secure virus source [9]. Several papers have recently reported the need to succeed in developing a global manufacturing process for lentiviral vectors, driven by a deep understanding of both the product and the process, in order to establish the viral vector product profile and critical quality attributes [10, 11]. In addition, lentiviral products require the creation of a worldwide accepted and adopted international standard, suitable for the standardization of trials, in particular quantitation trials related to the potency of the target product, allowing a comparison of cross-manufacturing results for any lentiviral platform [12].

3. Vectorizing HIV

Human immunodeficiency virus (HIV) belongs to the family Retroviridae, subfamily Orthoretrovirinae, and genus Lentivirus of animal viruses. According to Baltimore’s classification, it is an RNA reverse-transcribing virus (group VI). Retroviridae members are among the more exclusive entities in the virus taxon, and research in the field has provided outstanding insights into key concepts on biology, which were the basis demonstrating that dogmas do not stand up in science (the central dogma on molecular biology), providing one of the most useful tools in molecular biology (retrotranscriptase), supporting the concept of the existence of genes that provoke cancer (viral oncogenes), and finally, demonstrating that viral-genome integration also evolved in animal viruses as an efficient method of transmission. This breaking concept drove the original idea of gene therapy.
The HIV pandemic in the 1980s alerted humanity to the silent spreading of a deadly disease. HIV, a Lentivirus, was revealed to be a highly sophisticated virus with fine-tuned regulation, and it was mostly deciphered a decade after its discovery [5]. Scientists took advantage of the impressive knowledge gained about the virus in a decade transforming a dangerous virus into a safe viral vector. It is worth mentioning that HIV was described as a new virus in 1987, and barely 10 years later, a safe version of HIV-derived vectors was demonstrated as efficient in animal models [23].

Taking advantage of the previous studies with Gammaretrovirus vectors, the HIV-derived vectors were engineered to contain a mere 15–20% of the original virus, allowing plenty of room for transgenes/regulatory sequences. A method for production was established, and subsequent improvements ensure that the currently available format of the third generation of self-inactivating (SIN) vector [18] is generated as the safest and easiest-to-use vector available. It has multipurpose uses, from research and preclinical studies to clinical trials as there is a commercial product based on this type of vector. In all cases the procedure followed for production is almost the same, representing one of the main drawbacks, as manufacturing is not fully established, and a series of major concerns must be resolved in order to cover large mid- to long-term market requirements. Below is a brief summary of several relevant factors which must be addressed.

Production of HIV-derived vectors is a poorly optimized process, and a major hurdle to large-scale manufacturing is due in part to a deficient production of fully biologically active virions recovered in the culture media [19]. This is a dynamic process involving both production and inactivation rates, which ultimately renders on average 3–10 virus per producer cell [20], whereas during natural lentiviral infection, the number is close to $10^3$/cell [21] and far removed from other vector systems such as AAV or Adenovirus, which render $10^4$–$10^5$ viral particles per producer cell. There are at least four major issues during production that can result in this poor yield: (a) transfection robustness, (b) protein interactions during morphogenesis, (c) the nature of the cell system used for production, and (d) extremely labile essential components within the viral particles that lose activity during the production testing [22, 23].

### 3.1 DNA transfection

Production of HIV-derived lentivectors, and likewise other retroviral vectors derived from feline or equine Lentivirus or from gammaretroviruses, is based on DNA transfection of producer cells. The overall method was firstly demonstrated as feasible in pioneering research using poliovirus [24, 25], and it is based on the concept that the viral genome cloned in plasmids can recapitulate the genetic and morphogenetic instructions upon introduction in a eukaryotic cell in order to produce viral progeny. Early gene therapy studies developed a further step by splitting viral components in different plasmids, allowing the generation of non-replicative viral vectors as nonstructural/replication instructions which were no longer packaged in the progeny. These systems are currently also used to produce AAV-derived vectors. The basis is that packaging signals acting in cis are encoded in discrete regions of the viral genome. By including those sequences in the transfer plasmid bearing the therapeutic/reporter gene, transgenes are encapsidated in the virions. All the accessory functions are expressed during production from the so-called helper plasmids but are not licensed for encapsidation, as they do not carry the packaging signals, nor are they encoded in the transfer plasmid. The current model of production on large or small scales is based on DNA transfection of three or four plasmids. For a full, detailed description of the plasmid used in the production of
HIV-derived vectors, see [8]. As a result, efficiency is compromised by the proportion of cells transfected with the proper combination of plasmids, and indeed earlier second-generation production systems that use just three plasmids are more efficient in production [6].

3.2 Pseudotyping

Lentiviral vectors can be designed to carry heterologous envelope proteins. This pseudotyping allows the selective targeting of specific cells, conferring broader uses on the vector. Thus, lentivectors bearing RD114 [26], CD105 [27], and more recently measles virus [28] envelope glycoproteins, among others, have been described as conferring specific targeting in B cells, T cells, and hematopoietic stem cells, respectively. However, most of the lentivectors that have entered into the clinic and are commonly being used in research are pseudotyped with Vesicular stomatitis virus g protein (VSVg). There are two reasons for this. First, a wide number of different cell types are targeted by such an envelope, and second, it confers robustness on the viral particle during the purification stages [29].

However, the presence of the VSV receptor in the producer cell line contributes to diminishing the viral burden in the harvest [30]. Envelope proteins are membrane proteins that pass through the secretory pathway involving the endoplasmic reticulum cisternae and the Golgi apparatus, before they reach the plasma membrane, a system also used for the synthesis and recycling of the membrane receptors. Prevention of a premature encounter between ligand and receptor is mandatory, in order to increase the env protein available for the morphogenetic program. Indeed, the impact of this phenomenon has evolved in the natural infection of HIV. Vpu, an HIV accessory protein (see below), plays a dual role in the viral cycle, firstly by promoting egress in a cell-type-dependent manner and secondly by controlling the recircularization of envelope proteins during the synthesis and preventing premature binding of the HIV gp160 env protein with the CD4 natural receptor during T-cell infection [31, 32]. To our knowledge no data has been published which accounts for the impact of such a process (autotransduction) during HIV-derived VSV-pseudotyped processes, but data obtained in our company indicate that this phenomenon is actually taking place in the producer cell. By specific quantitation of retrotranscribed RNA and integrated copies of cDNA in the producer cell, we have been able to quantitate that 30–50% loss of the actual viral particles produced are lost by reentering in the producer cell. Different lines are currently being developed at VIVEbiotech to minimize or fully prevent this phenomenon.

3.3 Helper functions

In the case of HIV, unlike to Gammaretrovirus, there is an array of six viral products collectively named accessory proteins. Their roles are different throughout the viral life cycle. Some are related to controlling the innate/cellular immune response (vif), others modulate the adaptive immune response (nef), while others are mandatory for a fully regulated genetic program (tat, rev), morphogenesis and the egress (vpu), or viral infectivity (vif, nef).

HIV-derived vectors are produced in the absence of five of the six accessory proteins encoded by the wild-type virus: with the exception of rev, the other five are not expressed in helper plasmids. The rationale is to minimize the presence of viral sequences in the transfer plasmid, enabling safer vectors and minimizing the recombination between viral sequences in order to limit the risk of rescue of wild-type virus during production. Tat protein was unnecessary within the design of
third-generation vectors, as no viral promoter was used in production. However, as has been demonstrated, the effect of those other accessory proteins is not negligible, and their function during vector production is controversial [31, 32].

3.4 Manufacturing virus from DNA

As described above DNA transfection is the current and unique manner to produce lentiviral vectors. Three major concerns must be considered about this approach:

- **Efficiency.** Transfection of four plasmids raises a few concerns that affect the reproducibility and efficacy [6]. Alternative procedures rely on the generation of stable producer cells bearing helper functions, limiting the transfection to just the transfer plasmid, which aids robustness and production yields. Different approaches for either constitutive or inducible systems have been designed [33], but in most cases low titers have been achieved. Toxicity of the VSV proteins has been cited as limiting more efficient systems.

- **Quantity/quality.** DNA, just like any other reagent employed in the manufacturing process, requires identical strict compliance with GMP. The production of batches requires a certification of analysis that includes protein contamination, sterility, and the sequencing of all plasmids, both helper and transfer plasmids. These tests and the large amount of DNA required to scale-up the process require an improvement in cost-effective methods regarding quantity.

- **Scalability.** As mentioned above, several transfection procedures are currently available, but only two are relevant to large-scale manufacturing, whether using calcium phosphate and/or PEI (polyethylenimine) as matrixes to accomplish stable DNA complexes (currently proposed as methods for scale-up manufacturing). It is worth pointing out that to our knowledge there are no systematic studies addressing the relative efficiency of the two methods, taking into account not just vector yields but also cost-effectiveness and the impact on the cost of the production process.

4. Manufacturing Lentivirus: the VIVEbiotech approach

There are several excellent reviews on the specific steps during the manufacture of lentiviral vectors, the approaches to consider when scaling up and the critical points to consider for decision-making [13, 14, 34]. However, all of the processes must conciliate at least the next three considerations: (a) potency, meaning the capability of producing large quantities of vector; (b) robustness, i.e., highly reproducible; and (c) versatility, as demand changes according to project needs.

Manufacturing follows a process of production, purification, and concentration. At every step, specific features of the lentiviral vectors must be taken into account. Critical concerns to be considered include:

1. **Production.** It is worth remembering that lentiviral vectors are enveloped, and both cellular proteins and lipid content can vary depending on the culture conditions [22]. As previously mentioned, pseudotyping is of major relevance, as it can affect the fate of the produced virions and also contribute to the
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physical properties of the particles [35] and interaction with the solid phase in bioreactors.

2. Harvesting. It requires a consideration of the fact of interaction of the virions with the solid phase in bioreactors, in addition to the highly unstable nature of lentivectors, with their very short half-life [19], and a high dependence of biological activity on physical conditions and particle content [36–38].

3. Purification. It is critical for maintenance of the bona fide conditions of the genome and capsid, and unfortunately lentivectors are currently purified by chemically based procedures, such as ion-exchange chromatography, which significantly affect particle viability. To the author’s knowledge, there is no current industrial process for purification using affinity chromatography.

Herein is an overview of the manufacturing process developed at VIVEbiotech, which focuses on some of the critical steps. VIVEbiotech has obtained authorization from the European Medicines Agency (EMA) to provide cGMP batches of lentiviral vectors under the manufacturing process the company has fully developed (see Figure 1). VIVEbiotech is currently releasing batches of lentiviral vectors produced under these conditions to clients in EU and the USA. VIVEbiotech’s manufacture of lentivectors is based on the fixed-bed bioreactor iCELLis™ supplied by Pall. High compaction in the solid phase allows for a large culture surface, ranging from 0.53 to 2.6 m$^2$ (Nano™ configuration) in 1 liter disposable bioreactor, and is further scalable up to approximately 300 m$^2$ (500 + ™ configuration) based on chromatography principles. In the 1 liter small configuration, the carriers made of PET are fixed in a 40 mL chamber, and the process is monitored by probes controlling cell growth, and physical–chemical conditions are monitored by in-process BioXpert software. The harvest is collected by perfusion and purified by ion-exchange chromatography. It is then concentrated by tangential flow filtration, rendering a final concentration factor close to 300-fold and reaching a yield on a per-surface basis in accordance with market standards [39]. This process has been fully optimized in two remarkable steps:

Figure 1.  
Manufacturing process of lentiviral vectors optimized at VIVEbiotech.
• The seed train of producer HEK293T cells has been optimized in order to minimize the cell density of the seed. The conditions are the lowest standard achieved for other cell types which (i) allow growth with almost no operator intervention; minimizing the risk contamination, (ii) growth is fully monitored by the BioXpert program, ensuring constant, reliable growth conditions, and (iii) this has allowed us to shorten the process by a third.

• DNA transfection is based on DNA-calcium-phosphate precipitation, making our process highly reliable (close to 90% efficiency) and cost-effective, even under cGMP.

5. Challenging by numbers

As mentioned above, vector production is becoming a roadblock which is hitting gene therapy capabilities. Let us examine a few numbers in order to understand the size of the problem. So, what does lentivector-based gene therapy need to do in order to ensure it can be applied in the future? Lentiviral vectors for what? In their current design, lentivectors are capable of being used in the treatment of blood disorders, central nervous system disorders, immune therapy for certain cancers, and neurological conditions that can be treated with stem cells delivering a cargo of corrector genes. How can this be transformed into numbers? Certain statements require understanding, before setting out the main points which need to be addressed.

Leaving aside the fact that for every condition treatable by gene therapy, the number of patients is highly variable; estimations can be made using a highly prevalent disease under phase III by bluebird bio (www.bluebird.com) such as beta-thalassemia/sickle-cell trait (SCT). According to NIH data, 1100 infants are born every year among the African American community, and more than 100,000 individuals are estimated to have SCD in the USA; in Africa 15 million Africans are estimated to have SCD, and there are 200–300,000 affected births per year worldwide (https://www.ncbi.nlm.nih.gov/books/NBK1377/). Current conditions for transduction efficiently into hematopoietic stem cells require around $>1 \times 10^{10}$ biologically active particles per vector dose per infant patient though these data can vary depending on specific features of each treatment. According to current standards of the manufacturing process to achieve such production, harvest produced from 2 square meters and equivalent to 4–6 liters of harvest per patient would need to be produced, representing a huge quantity of 10,000 liters per year to treat new infant cases in the USA for SCD. Current platforms of production and, significantly, purification procedures are not capable of addressing this situation.

6. Conclusions

Gene therapy is no longer an experimental approach to treat genetic diseases. Several medicine agencies worldwide have approved the commercialization of medicinal products based on viral vectors as intermediate medicinal products. This raises the need to manufacture large quantities of viral vectors under costly cGMP manufacturing environment. There are a limited number of pharmaceutical and biotechnology companies capable to manufacture and release lentiviral vectors of defined composition and quality control in quantities to attend the foreseeable market needs. Challenges for the development of more controlled and cost-effective manufacturing process have yet to be overcome. The complicated manufacturing
process needs to be simplified to promote standardization and yield products of increased defined composition. However, there are still open questions that arise from the system employed for production, principally related to the model of production based on DNA-transfected produced cells. Automatization of the manufacturing process is also required, in order to increase capabilities leading to an industrialization process. This will contribute to developing global manufacturing processes for lentiviral vectors and help to establish the target product profile and quality attributes. In summary, efforts in modifying the current manufacturing model of lentivectors are needed to facilitate the entry into commercialization stages.

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Conflict of interest

The author declares no conflict of interest.

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References

[1] BioPharma Dealmakers. Gene therapy ready to deliver. Nature Reviews Drug Discovery. 2017;16:B24-B25

[2] Kumar SRP, Markus KM, Biswas M, High KA, Herzog RW. Clinical development of gene therapy: Results and lessons from recent successes. Molecular Therapy. Methods & Clinical Development. 2016;3:16034:1-11. DOI: 10.1038/mtm.2016.34

[3] Kotterman MA, Chalberg TW, Schaffer DV. Viral vectors for gene therapy: Translational and clinical outlook. Viral Vectors for Gene Therapy. 2015;17:63-89. DOI: 10.1146/annurev-bioeng-071813-104938

[4] Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. Science. 2018;359:1-10. DOI: 10.1126/science.aan4672

[5] Naldini L, Trono D, Verma IM. Lentiviral vectors, two decades later. Science. 2016;353:1101-1102. DOI: 10.1126/science.aah6192

[6] White M, Whittaker R, Gandara C, Stoll EA. A guide to approaching regulatory considerations for lentiviral-mediated gene therapies. Human Gene Therapy Methods. 2017;28:163-176. DOI: 10.1089/hgtb.2017.096

[7] Mavilio F. Developing gene and cell therapies for rare diseases: An opportunity for synergy between academia and industry. Gene Therapy. 2017;24:590–592. DOI: 10.1038/gt.2017.36

[8] Gandara C, Affleck V, Stoll EA. Manufacture of third-generation lentivirus for preclinical use, with process development considerations for translation to good manufacturing practice. Human Gene Therapy Methods. 2018;29:1-15. DOI: 10.1089/hhtb.2017.098

[9] Kolata G. Gene therapy’s strange roadblock. The New York Times. 2017:D12

[10] Levine BL, Miskin J, Wonnacott K, Keir C. Global manufacturing of CAR T cell therapy. Molecular Therapy. Methods & Clinical Development. 2017;4:92-101. DOI: 10.1016/j.omtm.2016.12.006

[11] Wang X, Isabelle Rivière J. Clinical manufacturing of CAR T cells: Foundation of a promising therapy. Molecular Therapy—Oncolytics. 2016;3:16015. DOI: 10.1038/mto.2016.15

[12] Zhao Y, Stepto H, Schneider CK. Development of the first World Health Organization lentiviral vector standard: Toward the production control and standardization of lentivirus-based gene therapy products. Human Gene Therapy Methods. 2017;205-214. DOI: 10.1089/hgtb.2017.078

[13] Merten OW, Scheweizer M, Chahal P, Karmen AA. Manufacturing of viral vectors: Part I. Upstream processing. Pharmaceutical Bioprocessing. 2014;2:183-202. DOI: 10.4155/PBP.1416

[14] Merten OW, Scheweizer M, Chahal P, Karmen AA. Manufacturing of viral vectors: Part II. Downstream processing and safety aspects. Pharmaceutical Bioprocessing. 2014;2:237-251. DOI: 10.4155/PBP.1415

[15] Schweizernd M, Merten OW. Large-scale production means for the manufacturing of lentiviral vectors. Current Gene Therapy. 2010;10:474–486

[16] Merten OW, Charrier S, Laroudie N, Fauchille S, Dugue S, Jenny C, et al. Large-scale manufacture and characterization of a lentiviral vector produced for clinical ex vivo gene therapy application. Human Gene Therapy. 2011;22:343–356. DOI: 10.1089/hum.2010.060
[17] Bandeira V, Peixoto C, Rodrigues AF, Cruz PE, Alves PM, Coroadinha AS, et al. Downstream processing of lentiviral vectors: Releasing bottlenecks. Human Gene Therapy Methods. 2011;23:255-263. DOI: 10.1089/hgtb.2012.059

[18] Zufferey R, Dull T, Mandel RJ, et al. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. Journal of Virology. 1998;72:9873-9880

[19] Thomas JA, Ott DE, RJ G. Efficiency of HIV post-entry infection process: Evidence against disproportionate numbers of defective virus. Journal of Virology. 2007;4367-4370

[20] Andreadis S, Lavery T, Davis H, Le Doux JM, Yarmush ML, Morgan JR. Toward a more accurate quantitation of the activity of recombinant retroviruses: Alternatives to titer and multiplicity of infection. Journal of Virology. 2000;74:1258-1266

[21] Chen YH, Di Mascio M, Perelson AS, Ho DD, Zhang L. Determination of virus burst size in vivo using a single-cycle SIV in rhesus macaques. PNAS. 2007;104:19079-19084. DOI: 10.107/nas.0707449104

[22] Carmo M, Alves A, Rodrigues AF, Coroadinha AS, Carrondo MJT, Alves PM, et al. Stabilization of gammaretroviral and lentiviral vectors: From production to gene transfer. The Journal of Gene Medicine. 2009;11:670-678. DOI: 10.1002/jgm.1353

[23] Coroadinha AS, Ribeiro J, Rolda A, Cruz PE, Alves PM, Merten OW, et al. Effect of medium sugar source on the production of retroviral vectors for gene therapy. Biotechnology and Bioengineering. 2006;94:24-36. DOI: 10.1002/bit.20778

[24] http://www.sciencemag.org/news/2002/07/poliiovirus-baked-scratch

[25] Cello J, Paul AV, Wimmer E. Chemical synthesis of poliovirus cDNA: Generation of infectious virus in the absence of natural template. Science. 2002;297:1016-1018. DOI: 10.1126/science.1072266

[26] Bell AJ Jr, Fegen D, Ward M, Bank RD114 envelope proteins provide an effective and versatile approach to pseudotype lentiviral vectors. Experimental Biology and Medicine. 2010;235:1269-1276. DOI: 10.1258/ebm.2010.010053

[27] Vanden Driessche T, Chuah MK. Targeting endothelial cells by gene therapy. Blood. 2013;122:1993-1994. DOI: 10.1182/blood-2013-08-518266

[28] Levy C, Amirache F, Girard-Gagnepain A, Frecha C, Roman-Rodriguez FJ, Bernadin O, et al. Measles virus envelope pseudotyped lentiviral vectors transduce quiescent human HSCs at an efficiency without precedent. Blood Advances. 2017;1:2088-2104. DOI: 10.1182/bloodadvances.2017007773

[29] Frecha C, Lévy C, Cosset F-L, Verhoeyen E. Advances in the field of lentivector-based transduction of T and B lymphocytes for gene therapy. Molecular Therapy. 2010;18:1748-1757. DOI: 10.1038/mt.2010.178

[30] Ohishi M, Shioda T, Sakuragi J-i. Retro-transduction by virus pseudotyped with glycoprotein of vesicular stomatitis virus. Virology. 2007;362:131-138. DOI: 10.1016/j.virol.2006.12.030

[31] Damme NV, Guatelli J. HIV-1 Vpu inhibits accumulation of the envelope glycoprotein within clathrin-coated, Gag-containing endosomes. Cellular Microbiology. 2008;10:1040-1057. DOI: 10.1111/j.1462-5822.2007.0101

[32] Neil SJD, Eastman SW, Jouvenet N, Bieniasz PD. HIV-1 Vpu promotes release and prevents endocytosis of
nascent retrovirus particles from the plasma membrane. PLoS Pathogens. 2006;2:354-367. DOI: 10.1371/journal.ppat.0020039.g011

[33] Manceur AP, Kim H, Misic V, Andreev N, Dorion-Thibaudeau J, Lanthier S, et al. Scalable lentiviral vector production using stable HEK293SF producer cell lines. Human Gene Therapy Methods. 2017;28:330-339. DOI: 10.1089/hgtb.2017.086

[34] Ausubel LJ, Hall C, Sharma A, Shakeley R, Lopez P, Quezada V, et al. Production of cGMP-grade lentiviral vectors. Bioprocess International. 2012;10(2):32-43

[35] Richieri SP, Bartholomew R, Aloia RC, Savary J, Gore R, Halt J, et al. Characterization of highly purified, inactivated HIV-1 particles isolated by anion exchange chromatography. Vaccine. 1998;16:119-129

[36] Le Doux JM, Davis HE, Morgan JR, Yarmush ML. Kinetics of retrovirus production and decay. Biotechnology and Bioengineering. 1999;63:654-662

[37] Carmo M, Panet A, Carrondo MJT, Alves PM, Cruz PE. From retroviral vector production to gene transfer: Spontaneous inactivation is caused by loss of reverse transcription capacity. The Journal of Gene Medicine. 2008;10:383-391. DOI: 10.1002/jgm.1163

[38] Carmo M, Dias JD, Panet A, Coroadinha AS, Carrondo MJT, Alves PM, et al. Thermosensitivity of the reverse transcription process as an inactivation mechanism of lentiviral vectors. Human Gene Therapy. 2009;20:1168-1176. DOI: 10.1089/hum.2009.068

[39] Valkama AJ, Leinonen HM, Lipponen EM, Turkki V, Malinnen J, Heikura T, et al. Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor. Gene Therapy. 2018;25:39-46. DOI: 10.1038/gt.2017.91