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Adeno-associated virus and lentivirus vectors: a refined toolkit for the central nervous system

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The last two decades have witnessed the increasing instrumentalization of viruses, which have progressively evolved into highly potent gene transfer vehicles for a wide spectrum of applications. In the context of the central nervous system (CNS), their unique gene delivery features and targeting specificities have been exploited not only to improve our understanding of basic neurobiology, but also to investigate diseases or deliver therapeutic candidates. As a result, we have started moving away from the opportunistic use of recombinant vectors that are derived from naturally existing viruses toward the rational engineering of tailored lentivirus (LV) and adeno-associated virus (AAV) vectors for specific use in the CNS.

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Viral vectors

Virus-mediated gene delivery applications include fundamental neurobiological investigation [1], disease modeling [2], and gene therapy [3•], each requiring a unique set of features: that is tropism, transduction efficiency, safety, and packaging capacity [4]. This has led to the investigation of more than a dozen viral species from various families [5]. Vectors derived from lentivirus and adeno-associated viruses are currently the most frequently used for rodent and primate CNS research and in gene therapy clinical trials [5,6].

Adeno-associated viral (AAV) vectors

AAVs are non-pathogenic, single stranded DNA viruses from the *Dependovirus* genus of the *Parvoviridae* that require helper virus infection for successful replication [7]. Viruses of this genus are non-enveloped and transduce dividing and non-dividing cells. A complete review of AAV biology is beyond the scope of this article but has been reviewed thoroughly elsewhere [8]. After transduction, the 4.7 kb viral genome remains predominantly episomal, providing long-term gene expression in non-dividing cells. The viral capsid is composed of three capsid proteins (VP1, VP2, and VP3) and the amino acids of the common VP region compose the protein domains that are exposed on the surface of the assembled capsid. They are responsible for surface topology and determine tropism and specificity [9]. Artificial AAV serotypes with new characteristics and tropisms have been produced by altering the cap genes [10]. The ease with which it is possible to alter AAV enables the development of large vector libraries (Figure 1) to screen and identify vectors with specific features that are tailored for specific applications [11••].

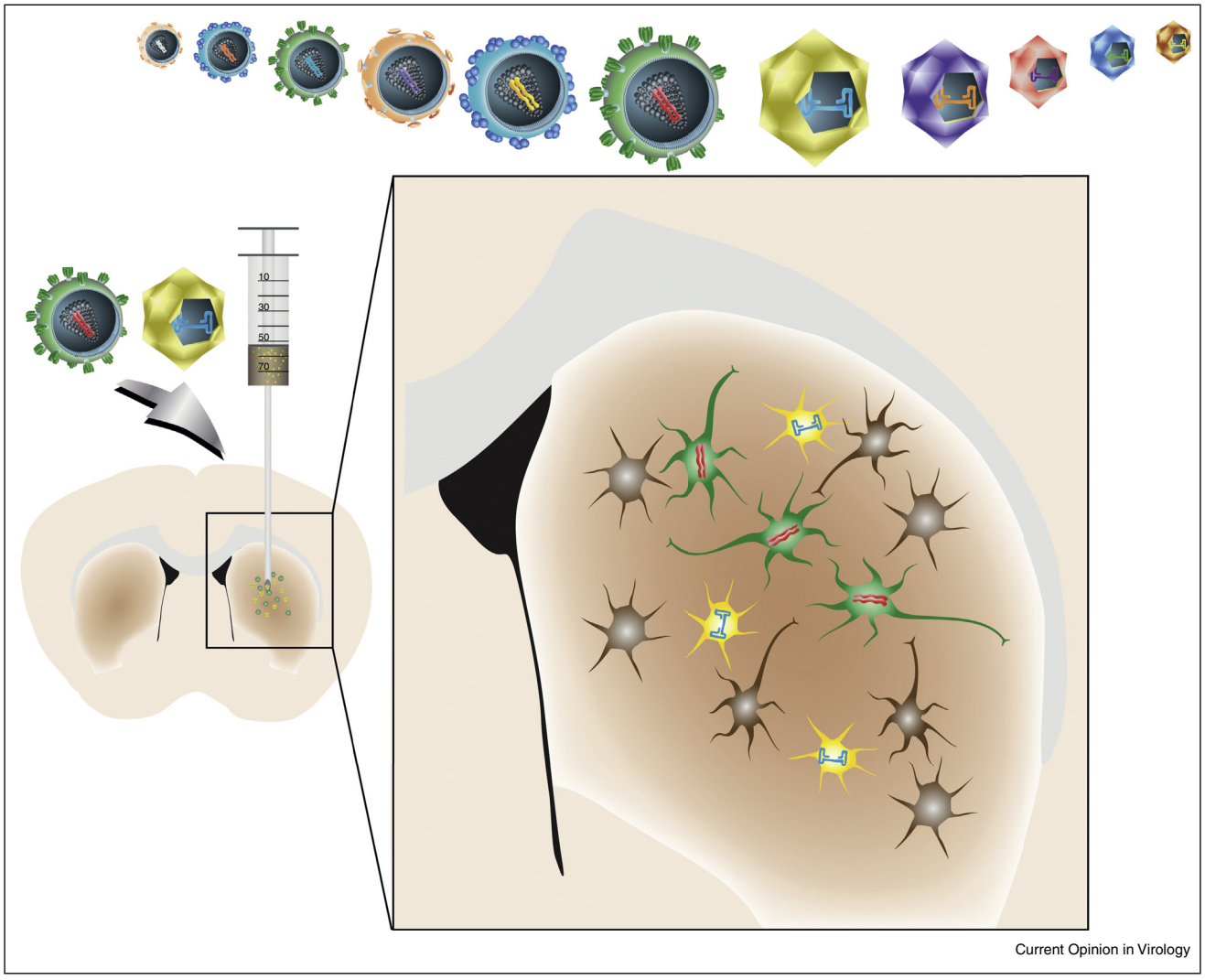
Lentiviral vectors (LV)

Lentiviruses belong to the family of *Retroviridae*. The most extensively studied lentivirus is HIV-1 which possesses two copies of a positive sense RNA genome of approximately 9 kb [12]. In contrast to gamma retroviruses, LV retroviruses have the unique ability to translocate across the nuclear membrane and infect non-dividing cells. The genome contains nine genes from which only the gag, pol, and rev genes are co-expressed for viral vector production in HEK293 T host cells [13,14••]. In this system, all virulence factors have been deleted to generate non-pathogenic and replication-deficient LV vectors [14••]. The envelope gene of HIV-1 is, in most cases, replaced by a heterologous gene to alter tropism and specificity [15]. The most commonly used envelope is the vesicular stomatitis virus glycoprotein (VSV-G). LVs pseudotyped with the VSV-G efficiently transduce neurons (Figure 1) and are highly mechanically resistant, facilitating their concentration and purification [14••].

Application of AAV and LV vectors for gene therapy

The field of gene therapy has been the major driver for the research and development of viral vector technologies. The efforts are reflected by the 2356 clinical trials that have been conducted to date. Only 43 of these trials have targeted brain diseases (February 2016, http://www.wiley.com/legacy/wileychi/genmed/clinical/). The low number of CNS trials relative to other indications (cancer

Figure 1



Cell-type specific targeting of CNS cells with various AAV serotypes and lentiviral vectors. The scheme illustrates the large collection of AAV and lentiviral vectors available for CNS applications. Transduction of subpopulations of cells is made possible (neurons, astrocytes, microglial cells, plexus choroïd, progenitor cells, etc.) by modifying the capsids (AAV) or envelopes (LV), or by integrating specific regulatory elements (promoter, microRNA-based detargeting strategies).

or immunodeficiency) highlights the difficulty of vector delivery to the CNS but also our only partial understanding of these diseases. The blood-brain barrier (BBB) limits entry of molecules to the brain. Thus, vectors are delivered directly to their site of action, most commonly via intracranial or intrathecal injection. Specific viral vectors are capable of crossing the BBB, but intravenous injection exposes the vectors to circulating antibodies, leads to widespread transduction of various tissues, and therefore requires very high doses of vector [16–18].

Because of the increased need of vectors for translating gene therapy applications into the clinic, the development of new protocols for viral vector production is becoming central as manufacturing protocols must be compliant with good manufacturing practice (GMP) and suitable for the production and qualification of large batches. For a detailed review on this topic, please refer to the following publications [19,20].

Most clinical trials for treatments of neurodegenerative diseases used AAV or LV vectors (http://www.wiley.com/legacy/wileychi/genmed/clinical/). This includes AAV gene therapy for Canavan disease, a pediatric leukodystrophy [21], Parkinson's and Alzheimer's disease [22] [23•], AADC deficiency [24], and LV gene therapy for X-linked adrenoleukodystrophy [25] and metachromatic leukodystrophy [26]. For a detailed review on gene