Manufacturing, quality control and supply of high-quality virus vectors for gene therapy

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Abstract

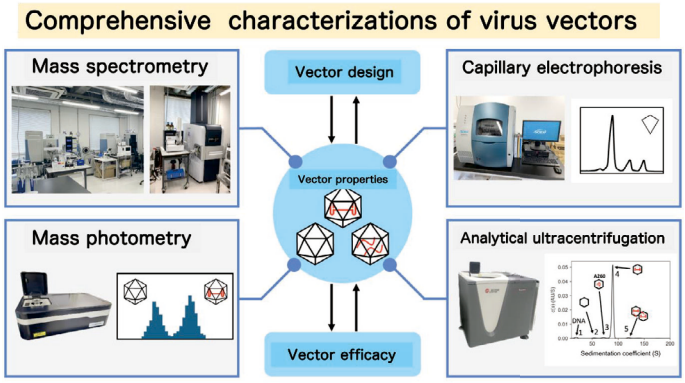
In recent years, gene therapy has entered the practical application stage, with multiple viral vector drugs being approved, mainly in Europe and the United States. Related clinical development is also progressing, and as the safety and efficacy of gene therapy becomes clearer, it is attracting a great deal of attention as the next-generation medical product. In this research, we aim to build a system that can stably provide high-quality vectors to researchers, medical doctors leading clinical trials, and companies that need them, by manufacturing high-quality vectors with our world-class vector quality analysis.

Background & Results

Our research focuses on adeno-associated virus (AAV) vectors, which are considered as a leading platform for gene therapy. AAV vectors are protein-nucleic acid complexes consisting of up to 5 kb of single-stranded DNA encapsidated in an icosahedral outer shell (capsid) consisting of three different proteins. In our project, vector production is being carried out efficiently at a high technical level, based on our world-leading analysis technology. In particular, in the size distribution analysis by analytical ultracentrifugation, we have developed an analysis method using multi-wavelength detection, and succeeded for the first time in the world in the quantitative analysis of impurity components including empty particles, which is an issue in viral vectors. Furthermore, we succeeded in evaluating vector quality in the presence of cell debris, and is being used in the development of vector production processes. Other methods include the development of a charge detection mass spectrometry and an evaluation method using deep learning of vector images acquired by cryo-electron microscopy, each of which is being used for high-quality vector production and quality control even for clinical trials. The developed advanced quality analysis has also led to the development of novel vectors. For example, the introduction of mutations in a capsid protein has led to the generation of AAV vectors with different protein stoichiometric ratios compared to the wild type, resulting in higher activity than the wild type.

Significance of the research and Future perspective

Gene therapy has enabled the treatment of genetic diseases for which there was previously no cure. Starting with Osaka University, Japan has achieved world-leading outcomes in basic research on gene therapy. On the other hand, clinical development leading to industry in Japan has not been easy. This is due to delays in the development of technology and infrastructure for the production and quality control of gene therapy vectors in Japan, which has led to the worrying situation where 'even if we want to conduct clinical trials, we cannot obtain the viral vector preparations for the trials'. This research is based on reliable and high-level science and technology with a view to industrialization, and will contribute to the promotion and development of gene therapy in Japan.



Related publications : 1. Takino R., et al. , *Mol Ther Methods Clin Dev.* in press; 2. Nakatsuka R, et al., *Analytical Chemistry.* in press.; 3. Soth S, et al. *Mol Ther Methods Clin Dev.* 2024 32(3):101291.; 4. Yamaguchi Y, et al. *Mol Ther Methods Clin Dev.* 2024 32(2):101256.; 5. Ikeda T, et al. *Viruses.* 2024 16(4):585. 6. Nishiumi H, et al. *J Pharm Sci.* 2024 113(7):1804-1815.; 7. Hirohata K, et al. *Anal Chem.* 2024. 96(2):642-651.; 8. Onishi T, et al. *Mol Ther Methods Clin Dev.* 2023 31:101142. 9. Maruno T, et al. *J Pharm Sci.* 2023 112(4):937-946.; 10. Maruno T, et al. *J Pharm Sci.* 2021 110(10):3375-3384. 11. Oyama H, et al. *Hum Gene Ther.* 2021 32(21-22):1403-1416.

Figure 1

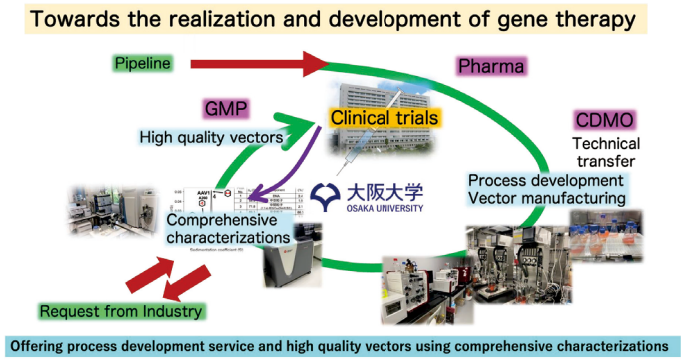


Figure 2

Patent	Japanese Patent No. 7454883, Japanese Patent Application No. 2024-182587, PCT/JP2024/018340
Treatise	Yamaguchi, Yuki; Ishii, Kentaro; Koizumi, Sachiko et al. Glycosylation of recombinant adeno-associated virus serotype 6. Mol Ther Methods Clin Dev. 2024, 32(2), 101256. doi: 10.1016/j.omtm.2024.101256 Onishi, Takayuki; Nonaka, Michika; Maruno, Takahiro et al. Enhancement of recombinant adeno-associated virus activity by improved stoichiometry and homogeneity of capsid protein assembly. Mol Ther Methods Clin Dev. 2023, 31, 101142. doi: 10.1016/j.omtm.2023.101142 Maruno, Takahiro; Usami, Kaede, Ishii, Kentaro et al. Comprehensive size distribution and composition analysis of adeno-associated virus vector by multiwavelength sedimentation velocity analytical ultracentrifugation. J Pharm Sci. 2021, 110(10), 3375-3384. doi: 10.1016/j.xphs.2021.06.031 Oyama, Hiroaki; Ishii, Kentaro; Maruno, Takahiro et al. Characterization of adeno-associated virus capsid proteins with two types of VP3-related components by capillary gel electrophoresis and mass spectrometry. Hum Gene Ther. 2021, 32(21-22), 1403-1416. doi: 10.1089/hum.2021.009
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