Citations Summary for Corning Cell Expansion Cultureware Used for Cell, Immune, and Gene Therapy Applications

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GENE THERAPY

1. **Long-term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B**
   Nathwani AC, et al. N Engl J Med 371(21):1994-2004, 2014.

   In this study, the stability of transgene expression and long-term safety in 10 patients (men enrolled in an initial Phase 1 dose-escalation trial) with severe hemophilia B were evaluated. A single intravenous infusion of AAV8 (scAAV2/8-LP1-hFIXco) vector in all 10 patients resulted in long-term therapeutic factor IX expression associated with clinical improvement. AAV8 vector used for this study were manufactured using Corning® CellSTACK® (10-layer) culture chambers and were characterized as described (Hum Gene Ther 22(5):595-604, 2011). With a follow-up period of up to 3 years, no late toxic effects from the therapy were reported (ClinicalTrials.gov number, NCT00979238).

2. **AAV2 Gene Therapy Readministration in Three Adults with Congenital Blindness**
   Bennett J, et al. Sci Transl Med 4(120):120ra15, 2012.

   Through comparison of pre- and post-surgical testing in patients with Leber congenital amaurosis (LCA2), this study demonstrated that delivery of recombinant adeno-associated virus (AAV) carrying the RPE65 gene (AAV2-hRPE65v2) to the contralateral eye is safe even if years have passed since the initial treatment. Data indicate that each patient showed improvement in multiple measures of retinal and visual function in the injected eye. Furthermore, before and after comparisons of psychophysical data and functional magnetic resonance imaging (fMRI) results support effectiveness of gene therapy readministration in LCA2 patients. Results in two patients receiving different doses in each eye suggest a possible dose-response effect of the gene therapy vector. AAV2-hRPE65v2 in HEK293 cells were amplified in roller bottles (Corning) as described (Wright JF, et al. Current Gene Therapy, 10:341-349, 2010).

3. **RNA-based Gene Therapy for HIV with Lentiviral Vector-modified CD34(+) Cells in Patients Undergoing Transplantation for AIDS-related Lymphoma**
   DiGiusto DL, et al. Sci Transl Med 2(36):36ra43, 2010.

   In this study researchers designed and conducted a clinical trial to assess the safety and feasibility of hematopoietic progenitor cell-based lentiviral gene therapy for HIV in the context of treatment for AIDS-related lymphoma. Stem cell gene therapy for HIV resulted in sustained RNA expression in the blood of patients for up to 2 years following transplant. Results support feasibility for the development of an RNA-based cell therapy platform for HIV. The lentiviral vector used in this study was manufactured using Corning CellSTACK culture chambers according to cGMP requirements and was fully released tested according to FDA guidelines prior to enrolling patients as described (Bioprocess Int 10(2):32-43, 2012).

4. **CNTF Gene Therapy Confers Lifelong Neuroprotection in a Mouse Model of Human Retinitis Pigmentosa**
   Lipinski DM, et al. Mol Ther 23(8):1308-1319, 2015.

   Employing a murine model of retinal disease, this study demonstrates that ciliary neurotrophic factor (CNTF) confers life-long protection against photoreceptor degeneration. Recombinant AAV serotype 2 (rAAV2/2) virus packaging the CNTF construct for this study was produced by transient co-transfection of HEK293 cells seeded in Corning HYPER*Flask*® cell culture vessels. Results suggest that robust cell survival is directly linked to the dose of CNTF. This application provides valuable insights into potential novel therapeutic avenues for diseases such as retinitis pigmentosa and amyotrophic lateral sclerosis, for which CNTF has been evaluated unsuccessfully in clinical trials.

5. **Manufacturing of Recombinant Adeno-associated Viral Vectors for Clinical Trials**
   Clément N, and Grieger JC. Mol Ther Methods Clin Dev 3:16002, 2016.

   This review summarizes manufacturing and characterization of 'AAV2-hRPE65v2' vector used in one completed Phase I/II clinical trial. Regulatory challenges and strategies that were successfully used for this groundbreaking trial are described. For the generation of AAV2-hRPE65v2 in HEK293 cell culture using roller bottles (Corning), a consistent, high, specific vector productivity was achieved as described (Wright JF, et al. Curr Gene Ther 10:341-349, 2010). Results obtained in clinical trials using AAV expressing RPE65 for Leber Congenital Amaurosis (LCA) support the enormous potential of therapeutic gene transfer for serious human diseases.

6. **Phase I/II Trial of Adeno-associated Virus-mediated Alpha-glucosidase Gene Therapy to the Diaphragm for Chronic Respiratory Failure in Pompe Disease: Initial Safety and Ventilatory Outcomes**
   Smith BK, et al. Hum Gene Ther 24(6):630-640, 2013.

   This study describes 180-day safety and ventilatory outcomes for five ventilator-dependent children in a Phase I/II clinical trial of AAV-mediated GAA gene therapy (rAAV1-hGAA) following intradiaphragmatic delivery. Data support that intramuscular AAV-mediated GAA gene replacement therapy to the diaphragm is feasible and safe in children with chronic ventilator dependence because of Pompe disease. rAAV1-hGAA vector were produced using a 10-layer Corning® CellSTACK® culture chamber by the standard $CaPO_4$ cotransfection method.

7. **Good Manufacturing Practice Production of Self-complementary Serotype 8 Adeno-associated Viral Vector for a Hemophilia B Clinical Trial**
   Allay JA, et al. Hum Gene Ther 2(5):595-604, 2011.

   Using Corning CellSTACK (10-layer) culture chambers this study developed a large-scale, good manufacturing practice (GMP)-compatible method for vector production and purification, and established a protocol for a Phase I/II clinical trial of AAV-mediated FIX gene transfer in subjects with hemophilia B. A 293T cell-based two-plasmid transient transfection system coupled with a three-column chromatography purification process was used to produce high quality self-complementary AAV2/8 FIX clinical-grade vector. Studies confirmed the long-term stability of the vector at -80°C (for at least 24 months) and for at least 24 hours formulated in the clinical diluent and stored at room temperature within intravenous bags. This material has been approved for use in clinical trials in the United States and the United Kingdom.

8. **Simplified Production and Concentration of HIV-1-based Lentiviral Vectors Using Corning HYPER*Flask*® Vessels and Anion Exchange Membrane Chromatography**
   Kutner RH, et al. BMC Biotechnol 9:10, 2009.

   To simplify the production and concentration of lentiviral (LV) vectors and make this approach more reproducible and cost-effective for preclinical studies in animals, this work describes a facile LV vector production system based on Corning HYPER*Flask* cell culture vessels. HYPER*Flask* vessels are high-yield, high-performance flasks that utilize a multi-layered gas permeable growth surface for efficient gas exchange, allowing convenient production of high-titer LV vectors. Results demonstrate that 293T cells used for production appeared healthier and titers obtained using the HYPER*Flask* cell culture vessels were as high or higher compared to those obtained using 150 cm^2 dishes or other multi-layer systems. Overall, this work facilitates high-titer LV vector production for preclinical studies in animal models without the need for multiple tissue culture dishes and ultracentrifugation-based concentration protocols.

9. **Downstream Processing of Lentiviral Vectors: Releasing Bottlenecks**
   Bandeira V, et al. Hum Gene Ther Methods 23(4):255-263 2012.

   Lentiviral (LV) vectors hold great promise as gene delivery vehicles. This study shows the establishment of fast, scalable, and robust downstream protocol for lentiviral production using Corning HYPER*Flask* cell culture vessels, and proposes progress in downstream processing will contribute to improve LV purification toward clinical-scale for gene therapy trials.

10. **Production of CGMP-Grade Lentiviral Vectors**
    Ausubel LJ, et al. Bioprocess Int 10(2):32-43, 2012.

    In this study, researchers developed a process that allows efficient production of large quantities of lentivirus required for Phase I/II clinical trials. 293T cells were expanded in culture and transfected in sub-batches using Corning CellSTACK 10-layer culture chambers. Results demonstrate ten of each CellSTACK (10-layer) culture chambers produced approximately 20L lentiviral supernatant each week. Using this process, all their viral products have been fully released for *ex vivo* gene therapy trials and at least one of which is complete (DiGiusto DL, et al. Sci Transl Med 2(36):36ra43, 2010).

IMMUNOTHERAPY

11. Corning KBM581: Nutritious, Serum-free Media for CAR-T Cell Expansion
Hubei W. from Bioraid Biotech Co., Ltd. (Corning Application Note CLS-CG-AN-423).

The study demonstrates that Corning lymphocyte serum-free medium (KBM581) could efficiently expand CAR-T cells to a fold rate that was sufficiently high to transfuse B-ALL (acute lymphoid leukemia of B cells) patients for clinical trial. A high proportion of CD3+CD8+ T cell subsets (>80%) was observed, which indicated the cytotoxicity of the cultured cells. Performance of this medium in a CAR-T cell culture indicate that KBM581 may be a better alternative tool for researchers conducting T cell therapy.

12. Preparation of Mesothelin Chimeric Antigen Receptor Modified T cells and Application of T cells in Pancreatic Cancer Treatment
Liu M, Wu D, Wan L, Jin H, Feng J, Qiang B, Ma H. Publication Date: 2017-03-29.

To overcome the challenges associated with immune tolerance to self-antigens in cancer patients that limits the effect of immunotherapy, a novel approach was used for the expression of tumor specific chimeric antigen receptor (CAR-mesothelin) of T cells capable of specific binding to achieve the purpose of direct tumor killing. For this study T cells were expanded in Corning lymphocyte serum-free medium (KBM581). Results support that dose administered chimeric antigen receptor (CAR) to an animal or patient may have a potential therapeutic or prophylactic effect.

13. Human T Cells Expansion Using 88-581-CM Serum-free Medium
Wang X. Cytotherapy 21(5), S35, 2019.

Immune cell therapy improves the effectiveness of chemotherapy, enhancing patient immunity, and improving the patient's overall quality of life. Adoptive immunotherapy with *in vitro*-derived T cells is emerging as a powerful tool to treat cancer in a range of clinical settings. In this study, researchers demonstrate the application of Corning® lymphocyte serum-free medium (KBM581) for the activation and expansion of human lymphocyte T cells (with or without autologous plasma) using two activation methods, CD3 antibody coating and CD3/CD28 Dynabeads activation. Data support that Corning lymphocyte serum-free medium can successfully activate and expand T cells under serum-free condition, providing high expression of CD8+ T cells and naive T cells.

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