ProtaGene
Analytical Excellence

APPLICATION NOTE

Advanced Analytical Approaches for Improved Development of Cell and Gene Therapies

Introduction

Cell and gene therapies have the potential to provide long-term therapeutic efficacy, but their development is both risky and cost-intensive. Obtaining a better understanding of the specific structural attributes of vector proteins that influence clinical delivery and outcomes is essential for advancing both individual candidates and the platforms on which they are based.

Strategic application of advanced analytical techniques can help improve selection of candidates and guide development decisions, leading to a greater number of safer, more efficacious cell and gene therapies successfully completing development and entering the market. High-resolution LC-MS-based characterization, quantification, and *in vivo* expression profiling approaches are increasingly being applied to enable improved program understanding, controls, and clinical outcomes assessment.

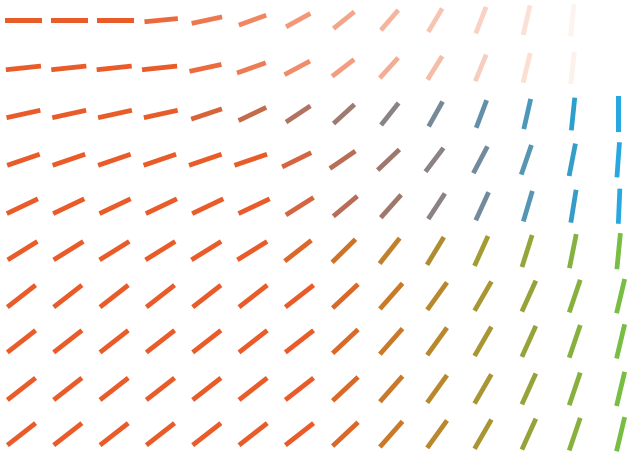
Highlights

We apply advanced analytical approaches for enhanced development of cell and gene therapies including:

- **Vector Protein Characterization and PTM Analysis; Correlations to Infectivity**
- **LC-MS HCP Profiling in Vector Production Lots**
- **Identification and Quantification of Gene-Edited Proteins in Target Cells and Tissues**
- **Quantitation and Characterization of Expression Products in Cells, Pre-Clinical and Clinical Samples**

Applications of Mass Spectrometry in Cell and Gene Therapy Product Development

LC-MS and intact MS can be used to analyze the detailed sequences of the vector proteins, as they confirm identity through complete amino acid sequence coverage, enabling analysis of sequence variants, PTMs, and splice variants. Peptide mapping by LC-MS coupled with intact protein analysis can be used to characterize the N-termini of VPs, establish VP ratios, and differentiate AAV serotypes using accurate mass measurement. Detailed LC-MS-based characterization can be used to identify and quantify key PTMs, and, more importantly, accelerate product development. These tools have increasingly become expected standards in the development of cell and gene therapy products as they may impact the potency and stability of the product.



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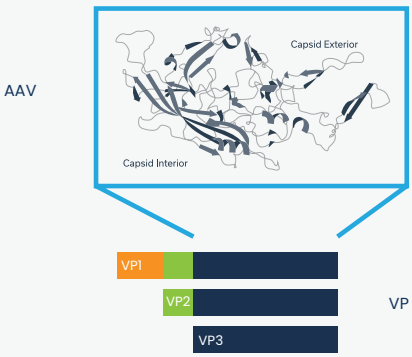
Current Approach to Analytical Development of AAV-based Cell and Gene Therapy Products

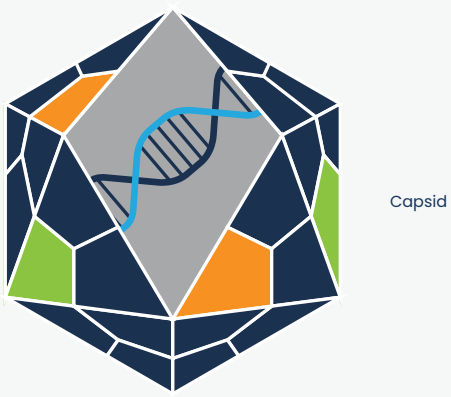
Key AAV Product Attributes

- Capsid Characterization
  - Percentage of Full Capsids (Empty to Full Ratio), Partially Full
  - Identity of Capsid Proteins (VP1, VP2, VP3, Truncated Speciel Splice Variants)
    - PTMs That Correlate with Stability/Potency (Deamidation, N-Acetylation)
  - VP Stoichiometry
  - Aggregation
- Purity
  - Residual Host Cell Proteins

Base VP Structure Unit



AAV Encapsulated Therapeutic Gene



Monitoring *In Vivo* Therapeutic Cell and Gene Expression

Quantitative analysis of gene expression products *in vivo* can be for the evaluation and optimization of the cell and gene therapy platforms. In many cases, there is a substantial background of highly similar protein already present in the tissue. MS-based analysis provides an accurate and sensitive method for quantifying and differentiating therapeutic expression from the background that can be used to track the persistence of therapeutic protein over time.

This includes profiling the expressed protein and identifying CQAs, which can guide selection and optimization of candidates and improve dosing decisions.

- Recover Representative Sample from Patient
- Quantify Therapeutic Expression Levels Over Time
- Profile Attributes of Expressed Product and Develop Correlations to Outcomes

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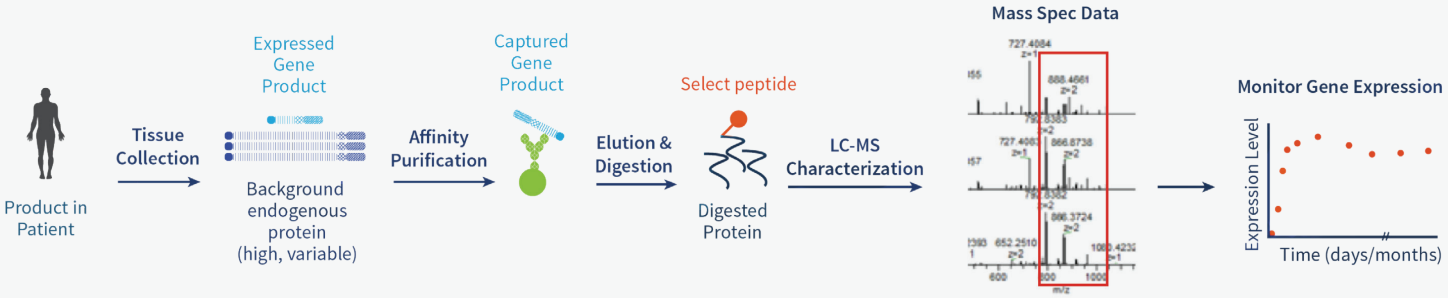
Your Strategic Development Partner

Each analytic service program is customized to suit our individual client's particular molecules and the phase-appropriate regulatory demands while keeping with excellent science and the best practices of the industry. We successfully build integrated strategies through collaboration with our clients to ensure a productive and positive experience. Some of our key established areas of work include:

- Viral Vector Characterization (AAV, LVV, others)
- Lot Comparability
- Process Development Changes
- HCP/Impurities ID and Profiling
- *In Vivo* Gene Expression Product Quantitation and Characterization
- Confirmation of Gene Editing and Evaluation of Therapeutic Expression Levels

In Vivo Workflow



Enabling Tomorrow's Therapeutics

Schedule a call today with our PhD experts to discuss how we can help you advance and accelerate the development of your cell and gene therapy products.

Contact Us

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