Advancing Gene Therapy Research with Trapped Ion Mobility Spectrometry

Gene therapy is a rapidly developing field of therapeutic treatment that holds the potential to treat a wide range of diseases, including cystic fibrosis, sickle cell, cancer, heart disease, diabetes, and human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS).

In 1988, the first clinical trial of human gene therapy was conducted for the treatment of Gaucher disease.[1] Since then, significant strides have been made in the field of gene therapy, propelled by enhancements in analytical technology, genomics, and molecular biology. For example, the United States Food and Drug Administration (FDA) has approved 37 cell and gene therapy products which have been licensed to treat several conditions[2] and currently, there are over 1,100 open gene therapy clinical trials and ribonucleic acid (RNA)-based therapy clinical trials globally.[3]

This article explores how trapped ion mobility spectrometry (TIMS) is advancing research into gene therapy products and progressing the development of new treatments for diseases that were previously considered untreatable.

History of Gene Therapy

Speculation about gene therapy began in the 1960s when scientists hypothesised that introducing deoxyribonucleic acid (DNA) sequences into patients' cells could hold the key to curing genetic disorders.[4] Though without protection from a carrier, the nucleic acid material was rapidly eliminated from the body.

It wasn't until the 1980s when the first notable advancements in gene therapy were made with the discovery of vectors as a delivery method and a paper was published demonstrating the use of a virus to insert genes into blood-forming stem cells in mice.[5] Then, in 1990, came the first human success story in a patient who was born with a severe combined immunodeficiency (SCID) due to lack of the enzyme adenosine deaminase (ADA).[6] Doctors delivered a healthy ADA gene into the patient's blood cells, using a disabled virus unable to spread in the body. Despite initial setbacks, including the death of a patient in 1999, subsequent trials demonstrated promising results, heralding the potential of gene therapy for treating genetic diseases.

Having acknowledged the crucial need for a delivery system to protect the nucleic acid, usually small interfering ribonucleic acid (siRNA) and on occasion DNA, as it travels through the body to reach the target cells, researchers are now investigating a variety of delivery options, including viral vectors and non-viral vectors (such as nanoparticles and liposomes) to improve gene delivery efficiency and specificity.

The Rise of Recombinant Adeno-associated Virus (rAAV) Vector

AAV is a non-enveloped virus belonging to the genus Dependoparvovirus in the family Parvoviridae that is non-pathogenic, replication-defective and packages a single-stranded viral DNA.[7] As a small virus (approximately 20–25 nanometer in diameter), rAAV can carry a small payload of single-stranded DNA (ssDNA) which can be used for therapeutic purposes. The ssDNA is encapsulated in viral proteins and transported to the site of action in the body.

rAAV has emerged as a leading gene delivery vehicle (vector) for *in vivo* gene therapy due to several advantages, namely high infectivity, efficient delivery of therapeutic genes into target cells, and low pathogenicity,[7] minimising the risk of causing disease in the host. AAV also possesses widespread tissue tropism, allowing it to target a broad range of tissues throughout the body.[8] Furthermore, rAAV-mediated gene expression demonstrates long-term persistence, even in non-dividing cells.

Recent advancements in developing clinically desirable rAAV capsids have contributed substantially to the growth of the gene therapy field. Researchers continue to refine rAAV vectors to enhance their targeting specificity and reduce unwanted immune response to further improve the efficacy of gene therapy treatments. Developments in vector design and engineering to provide reliable and robust delivery look set to advance targeted gene delivery, while minimising adverse effects. However, final preparation, formulation, and characterisation of rAAV drug substances are fundamental to support preclinical and clinical applications.

Solutions for rAAV Characterisation

To ensure the safety and efficacy of rAAV drugs for human gene therapy, the quality of rAAV vectors should be carefully monitored and controlled. Characterisation of rAAV preparations includes the determination of identity, potency, purity, stability, and safety of a batch.

The characterisation of rAAV-based gene therapy products represents significant challenges owing to their extremely large molecular sizes, structural complexity, heterogeneity, and limited sample amounts.1 Mass spectrometry (MS) is one of the key analytical tools that can overcome these challenges and serves as an important technique for the analysis of multiple attributes.

For quality control, MS is used to identify the amino acid composition and sequence of viral proteins, and to monitor the post translational modification (PTM) status of viral proteins, leading to determine the exact stoichiometry of viral proteins in rAAV.[9] MS is also used to identify the host cell protein (HCP). Typically, rAAV is composed of three different viral proteins: VP1, VP2 and VP3, each with distinct functions. Accurate determination of the stoichiometry is crucial for researchers as a higher number of VP1 and VP2 proteins usually correlates with greater efficacy.[10] Therefore, precise quantification of these proteins is essential for quality control. PTM at certain amino acids in rAAV have impact on

the transgene expression level thus quantification of PTM is also essential for rAAV quality control.[11] The viral vector output is then used by a clinical group who adds the genetic material and takes it into a clinical trial.

The TIMS Advantage

MS has become an indispensable tool for proteomic research. However, alone, its ability to comprehensively characterise protein complexes, particularly regarding complete sequence coverage and detailed analysis of molecular interactions and downstream effects, remains limited.

TIMS has emerged as a novel technique that addresses these limitations. TIMS offers significant improvements in sensitivity, selectivity, and separation speed for peptides and proteins. This enhanced capability is particularly valuable for analysing low-abundance species that are often crucial for understanding disease mechanisms. By offering a new dimension of separation based on size and shape, TIMS provides valuable insights into protein conformation and dynamics, furthering understanding of protein function in complex biological systems.

TIMS uses an electrical gradient to separate ions based on their size and shape in the gas phase. This allows for the selective manipulation of ions within the TIMS tunnel, enabling their controlled release, fragmentation, identification, and quantification through a process known as parallel accumulation-serial fragmentation (PASEF). With TIMS and PASEF, the ion mobility measurements can be used to determine ion specific collisional cross section (CCS) values. The incorporation of CCS values introduces a critical fourth dimension to proteomic analyses, transitioning the field from reliance on the traditional 3D-proteomics approach (retention time, mass-to-charge ratio (m/z), and tandem MS (MS/MS) fragment ion spectra) to more comprehensive 4D-proteomics. This additional dimension of CCS data significantly enhances the system's selectivity, leading to more reliable quantitation of proteins within complex biological samples.

Conclusion

Gene therapy presents a promising future for disease treatment across a broad spectrum of diseases. Its potential to revolutionise biopharmaceutical research offers significant hope for patients battling genetic disorders. Continued advancements, fostered by collaborative efforts and guided by ethical considerations, position gene therapy as a cornerstone of personalised and precision medicine. Using advanced characterisation methods, such as TIMS, will deliver safe and effective treatments to individuals, holding the promise of improved health outcomes and a significant enhancement in quality of life.

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