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Challenges In Assuring Quality In Advanced Therapy Medicinal Products: Cell, Gene, Tissue-Based Therapies, And Organoids With Emerging Mass Spectrometry Approaches

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ABSTRACT

Advanced Therapy Medicinal goods (ATMPs) a branch of modern biomedicine act as a boon for the treatment of previously incurable diseases via revolutionizing modern biotechnologically engineered medicine such as Cell-based therapies, gene therapies, tissue-engineered goods, and developing organoid systems. These technologies have shown extraordinary therapeutic significance, from gene replacement therapy in neurology to CAR-T cells in oncology and bioengineered grafts in regenerative medicine. Quality assurance is confronted with unprecedented challenges because to its complexity, reliance on live materials, and patient variability. Unlike conventional small-molecule pharmaceuticals, ATMPs are neither solely defined by their chemical structure or a single crucial qualitative characteristic. Dynamic biological characteristics like cell viability, differentiation potential, vector integrity, scaffold mechanics, and organoid functionality all affect how well they work as medicines. Although they offer crucial information, traditional analytical methods such as flow cytometry, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and histology are frequently reductionist, damaging, or unable to capture the multifaceted state of these treatments. This restriction emphasizes the necessity of sophisticated characterisation technologies. High- resolution and multi-omics analysis in proteomics, metabolomics, glycomics, lipidomics, and imaging applications are made possible by Mass Spectrometry (MS), which has become a potent tool. These methods provide previously unheard-of insight into molecular fingerprints that are directly related to reproducibility, safety, and potency. In this review, the types and characteristics of ATMPs are examined, key quality assurance difficulties are identified, and innovative MS techniques are discussed to close the gaps. By combining robust quality frameworks with state- of-the-art analytical tools, the field can ensure that ATMPs reach their transformative promise while maintaining the highest standards of patient safety and treatment dependability..

Keywords: Advanced Therapy Medicinal Products (ATMPs), Cell and Gene Therapy,Tissue Engineering, Organoids, Mass Spectrometry-Based Quality Control

INTRODUCTION

Advanced Therapy Medicinal Products (ATMPs) is a developing branch of biomedical innovations that is expanding beyond the potential of conventional pharmaceuticals. In comparison with conventional drugs or recombinant biologics, ATMPs gene therapies, somatic cell therapies, tissue-engineered products, or organoid systems for the treatment or prevention of genetic, metabolic, degenerative, and cancerous diseases. These therapies are designed not only to alleviate symptoms but to address underlying pathologies, offering unprecedented clinical opportunities in oncology, neurology, regenerative medicine, and rare genetic disorders. The complexity and dynamic nature of ATMPs, however, introduce significant challenges in characterization, quality assurance, and regulatory oversight. Emerging mass spectrometry approaches, including proteomics, metabolomics, glycomics, lipidomics, and imaging mass spectrometry, provide a powerful analytical platform to comprehensively profile these products. By revealing multidimensional molecular signatures, mass spectrometry enables a deeper understanding of therapeutic function, supporting reproducibility, safety, and efficacy (1).

Decades of research in tissue engineering, molecular genetics, and cell biology have led to the

development of ATMPs. The foundation for modern cellular and genetic therapies was laid by early research in the late 20th century that concentrated on hematopoietic stem cell transplantation and early gene therapy trials. Early clinical trials showed the potential of these treatments, but they also brought to light some of the hazards, such as immune-related side effects and erratic biological activity. A uniform legal framework and categorization system were established with the official recognition of ATMPs by the European Medicines Agency's Regulation (EC) No 1394/2007 (2), guaranteeing uniform oversight throughout Europe. The clinical importance of ATMPs has been materialize over the past 20 years by the approvals of biomedicine such as Luxturna® for the treatment of inherited retinal dystrophy and Chimeric Antigen Receptors (CAR-T) cell therapies for hematologic malignancies (3). Organoid technology has also become a versatile platform for both personalized disease modeling and therapeutic development as it helps in developing mini-organs that mimics the structure and function of the real organ by the utilization of stem cell invitro (4). In order to account for their various modes of action and therapeutic goals, the European Medicines Agency (EMA) divides ATMPs into four major categories.

Gene Therapy Medicinal Products (GTMPs): The most advanced type of biomedicines,

known as GTMPs, alter or manipulate gene expression or genetic material for therapeutic reasons via mediating transcription or translation. Products including viral or non-viral vectors and genetically modified cells, such as plasmids, messenger RNA (mRNA), DNA, viral vectors, or genetically engineered human cells (such CAR-T cells), might be included in this description. Examples: Zolgensm (spinal muscular atrophy),

Luxturna (hereditary retinal diseases) Kymriah and Yescarta (blood cancers) (5).

Somatic-Cell Therapy Medicinal Products (sCTMPs): It involves the utilization of modified living cells for regeneration, replacement, or immunomodulation. Examples: Holoclar (eye burns), Strimvelis (immune deficiency), and Zalmoxis (hematological cancers) (6).

Tissue-Engineered Products (TEPs): It is the type of biomedicine in which damage tissue are repaired or replaced by the use of biologically developed tissues Examples: bioengineered cartilage, skin substitutes, and corneal implants (7).

Combined ATMPs: are therapies that integrates cell therapy, tissue-engineered product, or gene therapy with one or more medical devices, forming an integral part of the final product. These products use a device to provide structural support, deliver, or contain the advanced therapeutic component, enabling the reconstruction or engineering of tissues. Examples: cell-based therapies embedded within a collagen scaffold cartilage repair or cells within a tissue-engineered matrix.

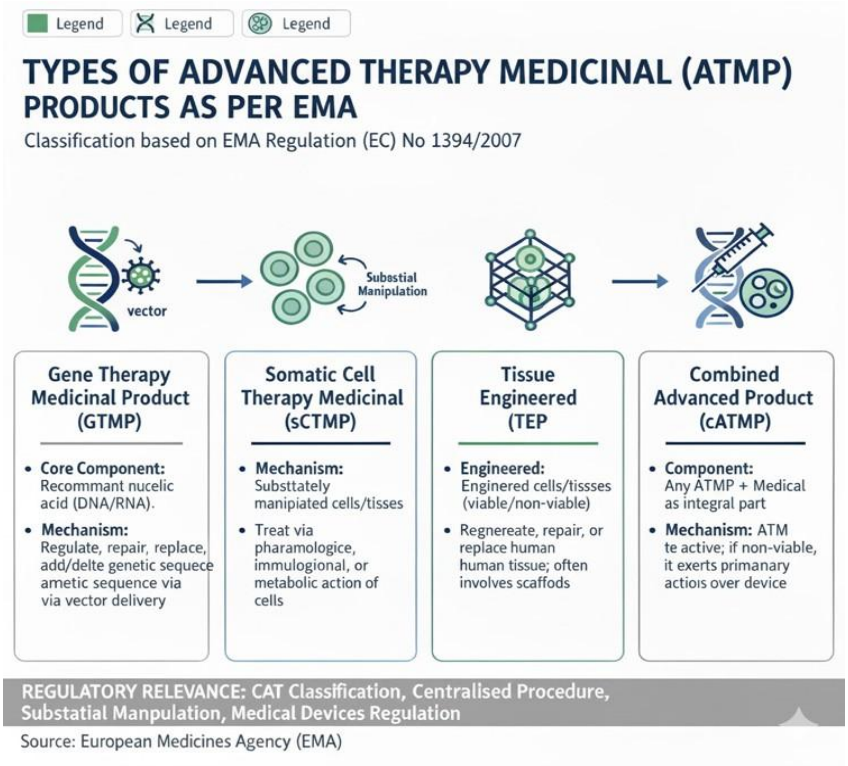

Figure:1: Advanced Therapy Medicinal goods categories as per European Medicines Agency

ATMPs has a remarkable contribution in clinical application across multiple disciplines such as oncology, neurology, regenerative medicines. In oncology CAR-T cells and oncolytic viruses therapy has overcome the limitation of traditional chemotherapies for refractory hematologic malignancies (8). Furthermore, in the field of neurology, specifically in non-curable neurodegenerative diseases like spinal cord muscular atrophy, where gene therapy (onasemnogene abeparvovec) (9) has demonstrated potential for nerve regeneration, as well as new stem-cell approaches for Parkinson's disease and stroke has shown remarkable contribution

(10). Cartilage scaffolds, corneal epithelium transplants, and tissue-engineered skin grafts are examples of regenerative medicine procedures that repair damaged tissues in both form and function. In comparison with traditional medicine ex-vivo gene therapies show promising cure in treating rare diseases like beta-thalassemia and adrenoleukodystrophy, providing transformative results in cases where no other treatments were available. Overall, these applications demonstrate ATMPs' versatility and ability to alter therapy paradigms in a range of therapeutic domains (11).

Maintaining the quality of ATMPs is essential because their effectiveness is dependent on their molecular makeup as well as their functional viability, which includes the cells' capacity to engraft, persist, differentiate, or release therapeutic chemicals in vivo. A robust quality assurance procedure for evaluation of heterogeneity, manufacturing processes, culture conditions, storage, and delivery methods of ATMPs is required as if there is any error in manufacturing of ATMPs the patient can witness risks such as immune rejection, loss of potency, contamination, or unintended biological effects. Since many ATMPs are used as one-time, irreversible interventions, it is both ethically and scientifically required to implement strict quality control procedures. Additionally, sophisticated analytical techniques are necessary to guarantee consistent potency, safety, and reproducibility throughout the product lifecycle (12).

Conventional analytical tools are essential for traditional pharmaceutical and biologic research; however, they are not sufficient for complete analysis of ATMPs. Although methods like flow

cytometry, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), and histology labeling offer insights on genetic alterations, protein expression, or cell surface indicators, they are fundamentally Simplistic and frequently fall short of capturing the complex biological state of a living therapy. Destructive assays decrease the product's availability for patient usage, and many of these techniques call for high sample quantities, which may not be possible for limited-yield ATMPs. Furthermore, traditional methods cannot incorporate molecular and functional context and lack spatial resolution, especially in tissue-engineered constructions and organoids where heterogeneity is essential to therapeutic efficacy (13).

A potent solution that makes it possible to characterize ATMPs in high-resolution, multi-omics is mass spectrometry as it overcomes all the limitations of the traditional analytical methods. Comprehensive investigation of protein expression and post-translational alterations is made possible by proteomics, which also reveals functional markers associated with differentiation and potency. While glycomics and lipidomics describe glycosylation patterns and membrane lipid composition that affect immunity and cell signalling, metabolomics offers insight into cellular metabolic states that correlate with viability and therapeutic efficacy. In order to capture heterogeneity at the microscale, imaging mass spectrometry maps the distribution of biomolecules within tissues and organoids, adding even more spatial resolution. Researchers can link molecular fingerprints to functional outcomes and develop a systems-level understanding of ATMPs by combining these complementary approaches. This will support strict quality assurance frameworks that are necessary for the safe and efficient clinical use of these cutting- edge therapies (14).

Advanced Therapy Medicinal Products: Categories and Characteristics

Advanced Therapy Medicinal Products (ATMPs), which offer medicines derived from living things and biologically complex structures, represent a major breakthrough in biomedicine. In order to preserve clarity in the face of differing mechanisms, regulatory requirements, and quality assurance issues, the EMA divides these into four categories: cell therapy, gene therapy, tissue-engineered products, and organoids. Regarding manufacturing, safety, and consistency in medicinal efficacy, each categorization offers unique opportunities and challenges (15).

Table:1 List of Advanced Therapy Medicinal Products (5-7)

ATMP Category	Characteristics	Defining Properties
Gene Therapy Medicines	Biological medicinal products containing recombinant nucleic acid sequences used to regulate, repair, replace, add, or delete a genetic sequence.	Used for treating genetic disorders, cancers, or long-term diseases; do not include vaccines against infectious diseases.
Somatic Cell Therapy	Products containing cells or tissues substantially manipulated to alter their biological characteristics, physiological functions or intended use.	Cells/tissues may be engineered or modified; used for regenerative or immune-modulatory purposes; challenging manufacturing.
Tissue-Engineered Products	Products containing engineered cells/tissues to regenerate, repair, or replace human tissue with altered functionality or structure.	Includes organoids and scaffolds; addresses tissue damage or loss; complexity in scale-up and quality control.
Combined ATMPs	Products that combine cells, genes, or tissues, possibly with medical devices, to achieve therapeutic effects.	Complexity in regulatory and manufacturing pathways, emerging technology integration like organoids and AI.

Cell-Based Therapies

Living cells are used in cell-based therapies to replace, repair, or regenerate tissues. These cells can be acquired from donors (allogeneic) or from the patient (autologous). The process of customized manufacture can be costly, time-consuming, and uneven, even while autologous techniques lower the danger of immunological rejection and give the patient specificity (16, 17). Allogeneic "off-the-shelf" alternatives, on the other hand, have the potential to cause graft- versus-host disease and host immunological rejection, but they also have the advantages of scalability and batch homogeneity (18, 19).

Cell collection (e.g., tissue biopsy or leukapheresis), isolation, growth, formulation, cryopreservation, delivery, and potential genetic modification are all steps in the production chain. From the quality of donor cells to variations in reagent batches, culture media composition, expansion techniques, and the impact of freeze-thaw cycles, variability can occur at any point (20, 21). Strict quality assurance procedures are required due to these variables. Viability, identity, purity, immunogenicity, and sterility are important characteristics to keep an eye on (22, 23).

The process of gathering, activating, transducing, expanding, washing, formulating, and reinfusing patient T cells is an example of the complexity of CAR-T treatments. To preserve potency, phenotypic, and consistency, each of these processes needs to be carefully controlled (24, 25). Furthermore, consistency in differentiation, aging, and genetic alterations throughout

passages present additional hurdles for stem cell therapies, such as mesenchymal stromal cells or derived induced pluripotent stem cells (26).

Gene Therapies

By creating functional genetic sequences or altering preexisting ones, gene treatments aim to correct or change genetic defects. Viral vectors (like AAV, lentivirus, and retrovirus) and non- viral systems (such lipid nanoparticles and electroporation) are examples of delivery techniques. The high transduction efficiency of viral vectors makes them popular, but they also pose problems with immunogenicity, vector integrity, and manufacturing process scaling. Although non-viral vectors are thought to be safer and more versatile, they frequently have problems with transfection effectiveness and transient expression (27, 28).

The vector genome's potency (efficiency in transduction and expression), integrity (whether full- length or truncated), and absence of replication-competent viruses or other contaminants must all be assessed via quality control. Off-target events, especially with gene editing methods such as CRISPR, necessitate detecting unwanted changes or chromosomal changes. Long-term stability is also essential: episomal vectors must sustain expression durability without declining, whereas integrated vectors must avoid insertional mutagenesis (29).

Prominent clinical examples include onasemnogene abeparvovec (Zolgensma®, a systemic AAV for spinal muscular atrophy) and Luxturna (an AAV-based therapy for retinal disorders). Their efficacy and safety are highly dependent on the

vector's purity, immunogenicity, biodistribution, and continuous observation throughout time (30).

Tissue-Engineered Products

Tissue-engineered products merge cells with scaffolds, biomaterials, or ECM analogs to rebuild tissue structure and function. Scaffolds, whether natural polymers, synthetic hydrogels, or decellularized ECM, are designed to guide cell behavior, support mechanical load, and degrade safely (31). Quality must address biomechanical properties, reproducibility in scaffold fabrication and cell seeding, sterility, and most importantly, functional integration (32). This last aspect is critical for the success of tissue-engineered products.

One of the critical aspects in the production of tissue-engineered products is the sterilization process. It is crucial to preserve scaffold integrity and cell viability, as any compromise in these areas can significantly affect the product's performance (33, 34). Tissue-engineered ATMPs such as bioengineered skin grafts, cartilage implants, and corneal constructs have been clinically explored. Their success is not solely determined by histological appearance, but also by mechanical robustness, vascularization, and integration with host tissue (35).

Organoids as Emerging ATMPs

Organoids are self-organizing, three-dimensional cell constructs that mimic important aspects of natural organs and are produced from pluripotent or adult stem cells. They can be used as models for individualized treatment, pharmacological screening, illness, and possibly regenerative transplantation. However, organoids provide unique challenges for quality assurance. Heterogeneity in structure, cellular composition, and function is caused by donor variability. Subtle variations in ECM scaffolds, growth factor gradients, and handling procedures make it difficult to reproduce results between batches and labs. Many organoids have foetal phenotypes instead of completely differentiated adult states, indicating that functional maturity frequently lags behind in vivo tissues. Cryopreservation is nonetheless vulnerable because interior architecture or viability is frequently harmed by freezing and thawing (33).

Organoids may be incorporated into translational contexts for regenerative medicine, specifically in the areas of liver, retina, or neurological repair. Ex vivo therapy decisions are already guided by personalized cancer organoids that are generated from patient tumor samples. However, advanced analytics must be used to prove robustness in viability, sterility, genetic stability, and functional performance before clinical deployment may take place (33, 36).

Quality Assurance in ATMP Development: Current Landscape

ATMPs are subject to separated frameworks in the European Union (EU) and United States of America (USA) in order to guaranty their quality, efficacy, safety and GMP. In the EU, ATMPs are referred to in Directive 2003/63/EC (which amends Directive 2001/83/EC) as biological products which include gene therapy products and manipulated cells. he EU operates a complex system of legislation: regulations (applicable in all Member States and directly applicable in national legal systems), directives (requiring national realization) and recommendations (non- binding practical advice) all underpinned by the European Pharmacopoeia (EP) that lays down binding quality standards (37). The legislative framework is published under EudraLex volumes 1, 4 and 5 with Good manufacturing practice (GMP) being covered under Directive 2017/1572/EU which supplements the Community Code 2001/83/EC (38). In the USA, ATMPs are mostly regulated by the US Food and Drug Administration (FDA), and references to their regulation can be found in Title 21 of the Code of Regulations (CFR) where Parts 210 and 211 outline the provision for Current Good Manufacturing Practice (cGMP), which require quality control of the drug product to ensure that each batch specifically has the identity, strength, purity, and quality it is represented to have, including its sterility, documentation, and traceability (39). Both authorities are doing GMP inspections for assurance of compliance, have quality assurance concepts for ATMPs (40).

Before release, analysis of tissue and cell procurement directives (2004/ 23/EC and 2002/

98/EC), establish that donor material was of good quality and maintained traceability in line with EU quality assurance. In the case of starting materials outside these rules, the manufacturers or sponsors carry the responsibility to establish what is considered as compliant via marketing or through clinical trial authorization. Traditional pharmaceutical quality assurance tools have had difficulty meeting the needs of ATMPs due to their complexity; however, these issues have largely been addressed with the rise of mass spectrometry (MS) for detailed characterization of gene therapy vectors (such as capsid composition and contaminants) and cell therapies (protein expression and metabolic markers). MS technologies are recommended as complementary to flow cytometry, quantitative polymerase chain reaction (qPCR), and bioassays, with method validation adapted to development phases. PCR and quantitative PCR (qPCR) are critical for detecting and quantifying therapeutic genes in ATMPs, offering high sensitivity to confirm transgene presence, integrity, and expression levels, and to detect contamination or mutation risks. Despite their utility and advantages like automation compatibility and fast turnaround, PCR/qPCR face limitations including false positives from environmental nucleic acid contamination and inadequate detection of epigenetic changes or complex genomic rearrangements, requiring stringent standardization and validation in use (41).

Mass Spectrometry: Principles and Relevance for ATMPs

Mass Spectrometry (MS) is a vital analytical technique extensively used across biological sciences, including the field of Advanced Therapy Medicinal Products (ATMPs). Mass spectrometry identifies and quantifies molecules by ionizing chemical compounds into charged particles and measuring their mass-to-charge ratios (m/z). Mass spectrometry (MS) stands as a cornerstone analytical technique in the field of advanced therapy medicinal products (ATMPs), providing unparalleled insights into the molecular composition, structure, and functionality of complex biological therapies. At its core, MS operates by first ionizing biological molecules into charged particles through techniques such as electron ionization (EI), electrospray ionization (ESI), or matrix-assisted laser desorption/ionization (MALDI) (42).

These ionized molecules are then separated within a mass analyzer based on their mass-to-charge ratio (m/z), employing sophisticated devices like quadrupole analyzers, time-of-flight systems, or high-resolution Orbitraps. Upon detection of the separated ions, a mass spectrum an intensity plot of ions versus their m/z values is produced, which functions as a distinct molecular fingerprint. The characterization of biomolecules in ATMPs, such as gene therapies, cell therapies, and tissue-engineered products, which are known for their intrinsic complexity and heterogeneity, depends on this basic technique, which enables precise molecular identification, quantification, and structural elucidation (43). The relevance of MS to ATMPs is substantial, primarily because these advanced therapies consist of heterogeneous biomolecules with complex post-translational modifications (PTMs), non-covalent interactions, and dynamic conformational states that are often beyond the reach of conventional biochemical assays. Traditional biophysical techniques such as circular dichroism, fluorescence spectroscopy, and size-exclusion chromatography offer limited scope, often focusing on singular aspects of molecular structure or lacking molecular-level specificity. In contrast, MS can probe biomolecular conformations, detect PTMs, and even capture non-covalent complexes in solution, providing a holistic view of the therapeutic product's molecular integrity and biological function under physiologically relevant conditions. Specialized methods like hydrogen/deuterium exchange coupled with MS (HDX-MS) (44) further enable the study of protein dynamics and higher-order structures critical for understanding the stability and efficacy of biotherapeutics. This broad capability is essential for quality control, batch-to-batch consistency, tracking degradation routes, and assisting with regulatory compliance for ATMPs, since the therapeutic mechanism is frequently closely linked to molecular structure. MS provides revolutionary improvements in resolution, sensitivity, and molecular depth when compared to traditional assays. The biological activity or concentration of a single analyte is often the main focus of conventional approaches, which find it difficult to unravel molecular heterogeneity or offer thorough molecular profiling. The ultrahigh resolution of MS, on the other hand, allows it to distinguish molecules that differ by only a few Dalton in mass, allowing for the remarkably accurate delineation of complex mixtures of proteins, peptides, lipids, or metabolites. In MS, sensitivity levels can be as low as femtomoles or even attomoles, which enables the identification and measurement of trace-level elements that may affect the safety or effectiveness of treatment. Additionally, MS offers a depth that concurrently catches a broad range of molecular species and interactions, providing a systems-level understanding of the molecular composition of the therapy. Beyond simple identification, this capability includes the quantification of minute changes and structural variations that may be diagnostic or predictive of therapeutic efficacy. In ATMPs, where comprehension of the entire biochemical landscape is crucial, MS-based techniques have developed into crucial platforms for omics-level characterization (45). Four major omics fields underscore this utility:

Proteomics employs MS to identify and quantify proteins in complex biological samples,

detecting post-translational modifications like phosphorylation and glycosylation that influence protein function and therapeutic performance. Proteomic analysis, leveraging tandem MS techniques, elucidates protein expression profiles, molecular interactions, and structural variants within cellular and therapeutic systems, pivotal for optimizing ATMP design and monitoring therapeutic impact (45).

Metabolomics uses MS to profile small-molecule metabolites that reflect the biochemical activity within cell therapies or engineered tissues. Mass spectrometry coupled with chromatographic separation enables detection of diverse metabolites, providing insights into metabolic pathways, cell health, and responses to therapy, thereby guiding process optimization and patient monitoring (45, 46).

Lipidomics focuses on lipid species central to cell membrane integrity, signaling pathways, and energy storage. MS-based lipidomics identifies diverse lipid molecules, many of which serve as biomarkers for inflammation, immune response, or cell differentiation states related to ATMP efficacy and safety (45, 47).

Applies MS to characterize carbohydrates and glycoconjugates, structures crucial for cellular recognition, signaling, and the pharmacokinetics of glycoprotein therapeutics. The precise mapping and quantification of glycan structures support understanding therapeutic behavior and immunogenicity in ATMPs (48).

Table:2: Omics-Level Characterization

Omics Field	Role of Mass Spectrometry (MS)	Applications in ATMPs
Proteomics	Identification and quantification of proteins; detection of post-translational modifications (phosphorylation, glycosylation) using tandem MS	Elucidates protein expression profiles, molecular interactions, structural variants; optimizes ATMP design and monitors therapeutic impact
Metabolomics	Profiling small-molecule metabolites via MS coupled with chromatographic separation	Provides insights into metabolic pathways, cell health, therapy response, guides process optimization and patient monitoring
Lipidomic	Identification of diverse lipid species using MS	Focuses on lipids involved in membrane integrity, signalling, energy storage; biomarkers for inflammation, immune response, cell differentiation; relates to ATMP efficacy and safety
Glycomics	Characterizes carbohydrates and glycoconjugates using MS	Maps and quantifies glycan structures crucial for cellular recognition, signalling, pharmacokinetics of glycoprotein therapeutics; supports understanding therapeutic behaviour and immunogenicity in ATMPs

Application of Mass Spectrometry in Quality Assurance of ATMPs

Advanced Therapy Medicinal Products (ATMPs) are an emerging class of therapeutics of genes, cells or through tissue engineering, which depend on advanced analytical methods for comprehensive quality assurance. With high sensitivity and molecular specificity to various biomolecular species, MS is a powerful technology for the safety, efficacy and consistency of ATMPs in drug development and manufacturing.

Cell-Based Therapies

Cell therapies are treatments in which living cells are used as therapeutic agents, and thus characterization is required to elucidate cell identity, potency, function and safety. MS has 3 major applications:

Proteomic profiling of cell identity, and potency MS-based proteomics reveals the complete protein content of a cell and can identify signature proteins that defines the cell and correlates to its therapeutic potency. This profiling is very important to validate the identity and the uniformity of the cell line in batch to batch, both are essential for Regulatory (13, 49, 50).

Metabolomics for culture monitoring: Culturing cells for therapy requires monitoring of

metabolic states to preserve cell viability and therapeutic function. MS-driven metabolomics enables rapid detection and quantification of small molecules and metabolic intermediates in culture media, providing real-time insights into cell health, nutrient utilization, and excretion profiles (51).

Secretome analysis for functional assessment: Cells secrete bioactive molecules that

contribute to therapeutic effects. MS-based Secretome analysis identifies and quantifies secreted proteins and peptides, assessing functionality and quality of the cell product. This approach informs batch release criteria and potency assays by linking secreted factor profiles to clinical performance (52).

Gene Therapies

Gene therapies generally involve delivering genetic material into patients' cells, frequently via viral vectors (53). MS applications here focus on molecular characterization of vector components and verification of gene expression:

MS-based lipidomics for viral vector formulation: Viral vectors have lipid components critical for stability and delivery efficiency. MS lipidomics characterizes lipid composition, aiding formulation optimization and quality control to ensure vector integrity and function (54).

Peptide mapping to confirm transgene expression: MS peptide mapping identifies and quantifies peptides derived from the therapeutic transgene product, confirming correct expression and post-translational modifications. This quality attribute ensures the therapeutic gene is producing the desired protein product accurately (55).

MS detection of impurities and residual host-cell proteins: Manufacturing of viral vectors can introduce process-related impurities such as host cell proteins and nucleic acids. MS- based methods provide sensitive and specific detection, crucial for ensuring product purity and safety prior to clinical application (56).

Tissue-Engineered Products

Tissue-engineered products (TEPs) combine cells with scaffolds or matrices to restore, maintain, or improve tissue function (57). MS contributes to understanding and assuring biomaterial quality:

Extracellular matrix (ECM) characterization using MS: MS analyzes the composition and modifications of ECM proteins critical for scaffold function and cellular interaction. Understanding ECM components facilitates the design of biomimetic structures that support cell growth and integration (58).

Collagen cross-linking and scaffold stability: Collagen, a major ECM protein, undergoes enzymatic cross-linking affecting mechanical properties. MS quantifies cross-link density and types, informing scaffold stability and durability assessments imperative for clinical efficacy (59).

Glycosylation analysis of biomaterials: Glycosylation modulates ECM protein interactions and immunogenicity. MS glycomics profiles glycosylation patterns on scaffold materials, critical for batch consistency and biocompatibility (60).

Organoids

Organoids are three-dimensional cellular structures that model organ function, demanding analytical precision to characterize heterogeneous and dynamic systems (61):

Single-cell proteomics for heterogeneity mapping: MS-based single-cell proteomics enables high-resolution analysis of protein expression at the individual cell level within organoids. This technique maps cellular heterogeneity and identifies distinct cell populations, critical for understanding organoid development and therapeutic potential (62, 63).

Metabolomic signatures of organoid maturation: MS metabolomics monitors biochemical changes during organoid growth and differentiation, providing signatures associated with maturation stages. These markers serve as quality metrics to assess the developmental progress and functional readiness of organoids (64, 65).

Imaging MS to map drug distribution and biomarker localization: Imaging mass spectrometry spatially resolves molecular distributions within organoids, visualizing drug penetration and localization of biomarkers. This application supports pharmacodynamic studies and safety assessments in organoid-based drug testing platforms (66, 67).

Table:3: Application of Mass Spectrometry (13,49,51-67)

ATMP Category	MS Application	Purpose / Quality Attribute Assessed	Outcome / Significance
Cell-Based Therapies	Proteomic profiling	Identify cell-specific protein signatures; assess potency and identity	Confirms cell identity and uniformity across batches for regulatory validation
	Metabolomics for culture monitoring	Analyse metabolites and small molecules in culture media	Monitors cell viability, nutrient use, and metabolic health in real-time
	Secretome analysis	Quantify secreted proteins and peptides	Evaluates functional quality; informs batch release and potency assays
Gene Therapies	MS-based lipidomics	Characterize lipid components of viral vectors	Optimizes vector formulation, stability, and delivery efficiency
	Peptide mapping	Identify peptides from therapeutic transgene product	Confirms correct expression and post-translational modifications
	Detection of impurities	Identify host-cell proteins and nucleic acids	Ensures product purity and safety before clinical application
Tissue-Engineered Products (TEPs)	ECM characterization	Analyse protein composition and modifications of scaffolds	Supports biomimetic scaffold design for optimal cell integration
	Collagen cross-linking analysis	Quantify cross-link density and types	Assesses scaffold stability, mechanical integrity, and durability
	Glycosylation profiling	Analyse glycosylation of ECM proteins or biomaterials	Ensures batch consistency, biocompatibility, and reduced immunogenicity
Organoids	Single-cell proteomics	Measure protein expression at single-cell resolution	Maps cellular heterogeneity; identifies subpopulations and development states
	Metabolomic signatures	Track biochemical changes during growth/differentiation	Defines quality metrics for organoid maturation and functionality
	Imaging mass spectrometry	Visualize molecular and drug distribution	Maps drug penetration and biomarker localization for pharmacodynamic assessment

Regulatory and Standardization Challenges

Current EMA and FDA Guidelines for ATMPs

The regulatory landscape for advanced therapy medicinal products (ATMPs) has developed considerably over the past decade, primarily through the frameworks laid out by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA). The EMA regulates ATMPs under Regulation (EC) No. 1394/2007 (68), which integrates cell therapy, gene therapy, and tissue-engineered products into a centralized approval pathway. This regulation is complemented by a series of guidance documents from the Committee for Advanced Therapies (CAT), covering quality, safety, and efficacy considerations. These documents stress the importance of demonstrating comparability between manufacturing batches, ensuring robust potency assays, and establishing appropriate long-term follow-up for treated patients. More recently, the EMA has introduced specific guidelines for investigational ATMPs, acknowledging the challenges posed by small batch sizes and individualized therapies, while also piloting initiatives to support academic and nonprofit developers by reducing regulatory and financial barriers (69-71).

In the U.S., the FDA regulates ATMPs under the biologics framework, particularly through the

Center for Biologics Evaluation and Research (CBER) (72). The agency has published multiple guidance documents on chemistry, manufacturing, and control (CMC) (73) for gene and cell therapy products, emphasizing the need for comprehensive characterization of vectors, cells, and final products. Importantly, the 21st Century Cures Act introduced the Regenerative Medicine Advanced Therapy (RMAT) (74) designation, which provides accelerated pathways for promising therapies addressing unmet needs. Together, these frameworks have laid a foundation for the development of ATMPs, but their adaptability is continuously tested by the complexity and variability inherent in cutting-edge therapies (75).

Lack of Harmonized Frameworks for Organoid-Based Therapies

While ATMP regulation has gained momentum, organoid-based therapies remain largely outside these well-defined frameworks. Organoids three-dimensional structures derived from stem cells that mimic native tissue architecture hold enormous potential for regenerative medicine and personalized therapy. However, their complex composition, donor-specific variability, and diverse manufacturing approaches make them difficult to classify within existing categories of medicinal products. Currently, neither EMA nor FDA provides specific guidance dedicated to organoid therapeutics. Instead, they are often evaluated on a case-by-case basis, creating regulatory uncertainty for developers and delaying clinical translation (76).

This lack of harmonization has profound implications. For example, tumor organoids used for drug testing or personalized oncology could revolutionize treatment strategies, yet their pathway to clinical adoption remains unclear. Without standardized definitions, potency criteria, or comparability requirements, organoid developers face uncertainty regarding regulatory expectations. Moreover, inconsistent oversight across jurisdictions hinders global collaboration and commercialization. Addressing these gaps will require international cooperation, with regulators, industry, and standard-setting bodies working together to create harmonized frameworks that recognize the unique biological and manufacturing features of organoids (77- 79).

Validation and Reproducibility Hurdles for MS-Based Assays

Mass spectrometry (MS) has emerged as a powerful analytical tool for ATMPs, enabling detailed characterization of proteins, metabolites, and lipids. Despite its potential, MS-based assays face significant challenges in terms of validation and reproducibility. The high sensitivity of MS can generate variability across instruments and laboratories, making it difficult to establish

universally accepted reference standards. Furthermore, the absence of consensus on calibration protocols and data interpretation creates barriers to consistent application. These limitations are particularly problematic for ATMPs, where lot-to-lot consistency and reproducibility of potency assays are critical for regulatory approval.

Regulatory agencies expect assays to comply with principles of validation similar to those outlined in ICH Q2(R2) (80), which covers analytical validation for conventional drugs. However, applying these principles to MS-based assays in the context of complex biological products is not straightforward. For example, defining specificity, linearity, and reproducibility is more challenging when the analyte is a heterogeneous population of biomolecules rather than a single compound. This gap between regulatory expectations and technical capabilities underscores the urgent need for harmonized guidelines on MS assay validation tailored to ATMPs. Collaborative consortia and cross-laboratory studies will be essential to generate reproducible protocols and build regulatory confidence in MS as a reliable tool for ATMP characterization (81) (82).

Standard-Setting Initiatives (ICH, ISO, USP)

Several international standard-setting bodies have attempted to address these challenges, albeit in fragmented ways. The International Council for Harmonization (ICH) provides foundational quality frameworks such as Q9 (83) on quality risk management and Q10 (84) on pharmaceutical quality systems, which are now being integrated into updated GMP guidance for ATMPs. These frameworks promote a risk-based approach, urging manufacturers to anticipate potential product and

process failures. However, while ICH standards are globally influential, they do not yet provide detailed guidance specific to MS-based assays or organoid therapeutics.

Similarly, organizations such as the International Organization for Standardization (ISO) and the United States Pharmacopeia (USP) have issued standards relevant to ATMP manufacturing, such as cleanroom classifications, equipment calibration, and reference materials for biologics. Nonetheless, gaps remain in covering novel modalities like organoid-based therapies or omics- driven potency assays. USP, for instance, has begun to publish reference standards for viral vectors and culture media, but it has not yet defined dedicated chapters for organoid or MS-based potency testing. Bridging these gaps will require closer alignment between ICH, ISO, and USP to create a unified regulatory ecosystem that reduces uncertainty for developers and ensures consistent quality standards across regions.

Case Studies of Regulatory Approvals: CAR-T Products

Perhaps the most illustrative examples of regulatory success within the ATMP space are chimeric antigen receptor T-cell (CAR-T) therapies. The approval of Kymriah (tisagenlecleucel) (85) by the FDA in 2017 marked a turning point, followed closely by the authorization of Yescarta (axicabtagene ciloleucel) (86). Since then, multiple CAR-T products such as Tecartus, Breyanzi, Abecma, and Carvykti (87) have gained approval across the U.S. and Europe. These cases highlight how regulators adapted existing frameworks to accommodate the unique challenges of autologous therapies, including complex manufacturing logistics, limited shelf life, and patient- specific variability (88).

Despite these achievements, CAR-T approvals highlight continued regulatory challenges. The requirement for strict post-market safety monitoring, validated potency assays, and strict in- process controls has led to a large increase in development costs and durations. Moreover, organoid-based and other next-generation ATMPs are likely to confront the same difficulties as CAR-T products, including scalability, patient access, and manufacturing reproducibility. Regulators and developers can find ways to expedite the development of novel therapies by examining CAR-T case studies. They can also see the need for new standards that take into account the unique complexity of various product kinds (89-92).

Future Perspectives

Integration of Mass Spectrometry with AI and Machine Learning for Predictive Quality Assurance

As advanced therapy medicinal products (ATMPs) become increasingly complex, the need for predictive quality assurance has grown substantially. Mass spectrometry (MS), with its ability to generate high-dimensional molecular profiles, is a natural candidate for integration with artificial intelligence (AI) and machine learning (ML). AI-driven approaches can analyze vast proteomic, metabolomic, and lipidomic datasets generated by MS, uncovering hidden correlations between molecular fingerprints and clinical outcomes. For example, ML algorithms can identify early warning signs of batch drift, forecast therapeutic potency, and flag subtle deviations that traditional assays might overlook. This predictive power not only enhances batch consistency but also reduces the risk of late-stage failures that increase costs and delay patient access (93, 94).

The integration of MS and AI also enables a shift from reactive to proactive quality assurance. Instead of relying solely on end-product release testing, predictive analytics can guide real-time decision-making during manufacturing. By continuously feeding MS data into AI models, developers can adapt bioprocesses dynamically to maintain critical quality attributes (CQAs). Furthermore, federated learning models could allow manufacturers across different facilities to share knowledge without compromising proprietary data, fostering broader improvements in quality assurance practices. In this way, AI-enhanced MS platforms have the potential to become regulatory gold standards for ensuring product integrity in the next generation of ATMPs (95,

96).

Development of Standardized Potency Assays Based on Omics Signatures

For ATMPs, potency is still one of the hardest qualities to identify and quantify, particularly for treatments with diverse or patient-specific components. The tests used today are frequently labor-intensive, cell-based, and difficult to transfer between labs. A potential remedy going ahead is the creation of standardized potency tests based on omics signatures that capture transcriptomic, proteomic, or metabolomic profiles. Because MS can identify intricate molecular patterns linked to therapeutic action, it is especially well-suited for this use and offers a repeatable and measurable indicator of potency.

International cooperation will be necessary to establish consensus biomarkers, validate reference standards, and guarantee platform-to-platform repeatability when developing omics-driven potency assays. Multi-center research, for example, may be able to find universal proteomic signatures that are associated with the effectiveness of CAR-T or organoid-based therapies. After being verified, these signatures could serve as the foundation for standardized tests that are included in regulatory frameworks. In addition to standardizing quality control worldwide, this strategy would lessen the workload associated with creating tests tailored to individual products, facilitating quicker regulatory approvals and more accurate patient results (97).

Real-Time and In-Line MS for Bioprocess Monitoring

Traditional quality assurance approaches often rely on offline assays, which are time-consuming and provide only a snapshot of product quality. By contrast, real-time and in-line MS technologies offer continuous monitoring of bioprocesses, enabling immediate detection of deviations and dynamic control of critical parameters. For example, MS can be coupled with microfluidic sampling systems to measure metabolite concentrations, cell-secreted factors, or vector integrity directly within bioreactors. This allows manufacturers to track Critical Quality Attribute (CQAs) in real time and make adjustments before the product is compromised.

The implementation of real-time MS also aligns with the principles of Process Analytical Technology (PAT) and Quality by Design (QbD), both of which emphasize proactive process control. With regulators increasingly supportive of advanced monitoring tools, real-time MS could eventually become a standard requirement for ATMP manufacturing. The ability to detect contamination, monitor nutrient dynamics, or evaluate therapeutic potency during production would greatly enhance reproducibility and reduce the risk of batch failures. Ultimately, the adoption of in-line MS could shift quality assurance from retrospective evaluation to continuous assurance, marking a paradigm shift in ATMP manufacturing (98, 99).

Expansion of Single-Cell MS to Address Heterogeneity in ATMPs

One of the defining challenges of ATMPs is cellular heterogeneity, particularly in autologous products where each batch is derived from a different patient. Bulk assays often obscure this variability, making it difficult to fully understand product composition and predict clinical outcomes. Single-cell MS technologies, such as imaging MS or mass cytometry, are poised to address this gap by providing molecular insights at the individual cell level. These tools can reveal functional differences between subpopulations of cells, identify rare but clinically significant phenotypes, and link molecular states to therapeutic performance (100).

As single-cell MS becomes more sophisticated, its role in regulatory science will expand. Detailed profiling of heterogeneity could help define potency criteria, refine release specifications, and reduce batch-to-batch variability. Moreover, integrating single-cell MS with spatial omics technologies could provide unprecedented insight into tissue-like structures such as organoids, where cell-to-cell interactions drive therapeutic function. By enabling a deeper understanding of heterogeneity, single-cell MS will not only improve quality assurance but also pave the way for safer and more effective personalized therapies (101).

Personalized quality assurance Approaches for Individualized Organoid/ATMP Products

The conventional paradigm of standardized quality assurance is under threat from the emergence of patient-specific treatments like autologous CAR-T cells and patient-derived organoids. Individualized ATMPs may necessitate customized quality assurance strategies catered to each patient or donor, in contrast to standard biologics, which are tested against predetermined criteria in large, homogeneous batches. This may entail using digital twins' computational models that forecast product behavior based on patient data and manufacturing conditions, or it could entail setting patient-specific reference ranges for potency. Personalized quality assurance would take into account the inherent diversity of customized products while guaranteeing safety and effectiveness.

Implementing personalized quality assurance frameworks will demand innovative regulatory models that balance flexibility with rigor. Regulators may need to accept adaptive specifications that vary between patients while ensuring comparability and long-term safety. Advanced analytics, including MS-based omics profiling and AI-driven predictive models, will be central to these approaches. By shifting from rigid standardization to adaptive personalization, the field can unlock the full potential of individualized therapies without compromising patient safety (102).

Need for International Regulatory Harmonization

As ATMP development accelerates globally, divergent regulatory frameworks pose a significant

barrier to innovation and patient access. While the EMA and FDA have taken important steps, inconsistencies in definitions, potency requirements, and assay validation standards complicate multinational trials and product launches. The lack of harmonized pathways for emerging modalities such as organoids and MS-based assays further exacerbates this problem. Without alignment, developers face duplicative requirements, higher costs, and slower timelines, ultimately delaying access for patients in need.

Looking ahead, international regulatory harmonization will be essential to unlock the full promise of ATMPs. Efforts should build on existing collaborative initiatives, such as EMA-FDA joint workshops and ICH quality guidelines, but expand to include novel modalities. Multi- stakeholder consortia that bring together regulators, academia, and industry can accelerate consensus on standards for MS-based potency assays, organoid frameworks, and AI-enhanced quality assurance. By converging on shared expectations, regulators can reduce uncertainty, promote global trials, and ensure equitable access to life-saving therapies across regions. Harmonization, therefore, represents both a scientific necessity and a moral imperative in the era of advanced therapies (103).

CONCLUSION

Advanced Therapy Medicinal Products (ATMPs) are an innovative class of drugs that aim to cure or restore damaged organs and tissues by using novel strategies such as gene therapy, somatic cell therapy, tissue engineering, and combined therapies. In spite of their therapeutic potential for devastating and multifactorial diseases, the production and clinical use of ATMPs encounter several important challenges. The scale-up from preclinical Good Laboratory Practice (GLP) studies to Good Manufacturing Practice (GMP)-scale production is especially challenging, necessitating rigorous validation to guarantee consistent quality, safety, and efficacy. Production of such complicated biological products involves the challenge of overcoming risks of contamination during aseptic processing, with limited sterilization means due to the fragility of live cells, and reducing tumorigenic potential, particularly in stem cell-based therapies, through extensive safety assays. Evidence of therapeutic effectiveness is compromised by limited patient numbers, orphan conditions, and lack of standardization of potency and clinical outcomes. Large-scale manufacture is beset by technical, regulatory, and financial challenges such as ensuring batch-to-batch consistency, sterility, and coping with the high production costs affecting product availability. Storage and supply also require specialist solutions for cell viability preservation in freezing, thawing, and transport, where cryopreservation media and handling protocol optimization is critical. Packaging needs to be biocompatible and preserve cell integrity; the absence of standard stability data makes shelf-life determinations problematic. Preclinical studies are hindered by non-predictive models of human biology, affecting the translation of efficacy and safety to clinical success. Low-quality preclinical data, ethical concerns regarding trial design and selective reporting, uncertainty in dosing regimens, and the necessity for harmonized clinical guidelines are clinical challenges. Regulatory complexities are a result of changing and regionally diverse regimes that exact strict documentation, approvals, and compliance demands, usually onerous for educational and small-scale developers. Effective governance must direct ethical issues, guard against unauthorized treatments, and control public expectations in the face of hype and misinformation. New technologies like mass spectrometry, organoids, artificial intelligence, and automated culture systems hold great promise to improve product characterization, manufacturing uniformity, and real-time quality assurance. Collaborative work between regulators, industry, academia, and clinicians and investment in education and infrastructure will be necessary to overcome these highly complex challenges. In the end, a harmonized approach using innovation, regulatory coordination, and ethical regulation will be critical to ensuring the fulfillment of the potential of ATMPs, making safe, effective, and accessible regenerative therapies available to patients globally. This exhaustive perspective sheds light on the present scenario and dictates future course for the development of ATMP therapies for regenerative medicine. Advanced Therapy Medicinal Products are a pioneering class of drugs that utilize genes, cells, or tissues to treat diseases with unmet medical needs, such as genetic disorders, cancer, and chronic serious conditions. The enormous potential of these therapies has created unparalleled innovation, while breaking down these advances into broadly available, safe, and effective treatments presents complex challenges. Current regulatory changes reflect attempts to improve and harmonize the quality and manufacturing requirements that are ATMP- specific. In 2025, the European Medicines Agency (EMA) put forward updates to its guidance on Good Manufacturing Practice (GMP) for ATMPs in a move to align ATMP-specific standards with more general revisions like the updated Annex 1 for manufacturing sterile products. These changes highlight integration of modern quality risk management concepts from ICH guidelines, integration of innovative manufacturing technologies (e.g., automated and closed single-use systems), and explicit expectations regarding cleanroom classification and barrier systems. This modernization of the regulations is a reflection of acknowledgment that ATMP manufacture requires specialized strategies beyond traditional pharmaceuticals because of the sensitivity and complexity of the biological material. Concurrently, leading conferences and science meetings in 2025 have supported the "Renaissance of Medicine" theme under which developing scientific understanding, clinical expertise, and regulatory guidelines come together to improve ATMP development. Talks highlight emerging developments in the application of organoids and

artificial intelligence to improve preclinical modeling, optimize manufacturing controls, and tailor therapies. Such technologies hold the promise of enhanced reproducibility, scalability, and quality control. Nonetheless, their uptake necessitates validation and regulatory approval in addition to overcoming logistical hurdles pertaining to decentralized manufacturing and supply chains. Other main ongoing challenges are still at the core of the ATMP lifecycle. Producing consistently sustainable products in quantity is limited by variability in biological starting material, the requirement for sterility without harsher sterilization procedures, and the need to reduce tumorigenic and immune rejection risks. Distribution and cryopreservation logistics must be stringently optimized because cell-based therapies are delicate. Clinical trial design remains complicated with requirements for new endpoints and adaptive strategies appropriate for small patient populations and tailor-made therapies. Even with evolving regulatory frameworks, the patchwork nature of regulation around the world means there must be early and continued dialogue between regulators and developers to best manage approvals and post-market monitoring. Economic and access issues are built into the future prospect. The expensive nature of developing, manufacturing, and bringing ATMPs to market creates access barriers for patients, particularly in less resourced health systems. Initiatives like focused innovation funds and reimbursement routes are central to overcoming this divide but need balanced policy that supports sustainable innovation with affordability.

In brief, the present situation of ATMPs in 2025 represents a shift in paradigm in the field of

medicine due to revolutionary science and facilitated by evolving regulatory and manufacturing capacity. The prospects of ATMPs are critically reliant on cohesive strategies that promote technological innovation, regulatory harmonization, clinical validation, and economic feasibility. Integrated ecosystems comprising regulators, academia, industry, healthcare providers, and patients will play a pivotal role in surmounting the multifactorial problems. This synchronized strategy has the potential to realize the full clinical value of ATMPs, providing revolutionary therapeutic choices that enhance treatment outcomes for individuals with severe illness across the globe.

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