CELL & GENE THERAPY INSIGHTS

RAW & STARTING MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

Developing an understanding of the analytical landscape for testing complex biological raw materials in advanced therapy medicinal products: a CRO perspective

Alistair Michel & David Neville

Unlike antibody-based products that are generally single, highly purified proteins, ATMPs are complex products. Production involves the use of new technologies and older technologies that are adapted to ATMP production. Because of these additional complexities, the regulatory expectations for this type of product have also increased. An important aspect of the manufacture of cell and gene therapy products is the role of raw materials and the controls required to ensure consistent product quality and ultimately patient safety. Important aspects of analytical approaches to ensure raw material quality will be discussed in this White Paper.

Cell & Gene Therapy Insights 2021; 7(2), 317–326

DOI: 10.18609/cgti.2021.056

INTRODUCTION

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells and can generally be classified into three distinct categories: gene therapy, somatic-cell therapy and tissue-engineered medicines. All ATMPs function by manipulating the target biological system to treat the disease.

An important aspect of the manufacture of cell and gene therapy products is the role of raw materials and the controls required to ensure consistent product quality and, ultimately, patient safety. The fundamentals of Good Manufacturing Practices (GMP) still apply. Importantly, within the EU the terms 'starting materials' and 'raw materials' must also be distinguished. Starting materials (SM) cover the human cells and tissues used in the final product, whereas raw materials are defined within EU Directive 2001/83/EC [1].

Not all companies have the ability or resource to perform the required raw materials testing. In such instances, partnerships with CROs are considered, and the choice may be dictated by the regulatory status (EMA/FDA inspected and approved), experience and knowledge around the product area. The CRO must have an understanding of what may be required and can help to guide clients through the testing regime. This paper will not cover all of the aspects that are fundamental to ensure the quality of the product is as required but does consider the points that are important when you are forming partnerships with a CRO to perform aspects of the raw materials testing.

The characterization of materials from reliable suppliers, using developed and validated methods or pharmacopeial methods, are key elements to maintaining a dependable supply of products. Additionally, it is not just the raw materials used to manufacture the biological product, but the packaging used, possible interactions with medical devices, and impurities like sub-visible particles, leachables, extractables, which also need assessment. The identification and validation of appropriate methods, and the identification of appropriate partners to perform these analyses, are important aspects to consider.

As ATMPs are not single biomolecular entities, there may be differences in approach to what is required for antibody-based products. In certain cases, research grade chemicals may be used as part of the development process and may not be GMP-sourced. In such instances, the developer much provide evidence that the raw materials are of the highest grade/quality possible but must still provide evidence that the material is safe and does not provide a risk for the patient. For certain treatments, the donor cells are derived from the patient and it is not possible to test to defined specifications and, for tests that might be employed, greater variability might be observed, e.g. cell depletion or enrichment using monoclonal antibody-based approaches. Additionally, it is possible that starting materials may arise from a non-GMP facility. Therefore, there are additional factors that need to be considered through the product lifecycle for an ATMP, nit observed for more traditional antibody-based therapies.

Currently, the major products from the biopharmaceutical industry have been recombinant proteins and antibody-based therapies, both originator molecules and biosimilars. However, the number of cell and gene therapy medicinal products under development is increasing. Currently, there are 1109 clinical trials worldwide, with 97 in Phase 3 [2], and 154 trials ongoing in the UK as of Dec 2020 [3], which is approximately 14% of the world total. In addition, in 2019 there were 11 approved treatments within Europe [4] and 18 in the US [5].

This document does not cover all the required tests for all regulatory domains for cell and gene therapy products (e.g. FDA, EMA, MHRA) but, where referenced, will focus on the regulations for the European market in the main. The document gives a CRO perspective that may be involved in the testing

DOI: 10.18609/cgti.2021.056

process, and the awareness and expertise that the CRO must have to allow the client to produce a safe product of the desired quality. However, similar adherence to quality, testing, supply, etc. of raw materials will apply across the different agencies. For non-EU markets, the region-specific regulations must be understood and followed; further information that is specific to your proposed market can be obtained from the relevant regulatory authorities.

RAW MATERIALS

Within the EU and outlined in 'Guidelines of 22.11.2017 Good Manufacturing Practice for Advanced Therapy Medicinal Products' are the factors required to ensure product manufacture that complies with Good Manufacturing Practice (GMP). Sections 7.1 and 7.2 are applicable to the understanding of the thought processes that must be considered during the production of ATMPs. Importantly, Section 7.1 states "The quality of starting and raw materials is a key factor to consider in the production of ATMPs. Particular attention should be paid to avoiding contamination and to minimizing as much as possible the variability of the starting and raw materials".

Within the EU the terms 'starting materials' and 'raw materials' must also be distinguished. Starting materials (SM) cover the human cells and tissues used in the final product, whereas raw materials are defined within EU Directive 2001/83/EC [1]. These definitions are further clarified in a concept paper released by the industry association European Biopharmaceutical Enterprises (EBE). The paper is entitled "Management and Control of Raw Materials Used in the Manufacture of Biological Medicinal Products and ATMPs" [6] and states that for ATMPs, raw materials (RM) are process inputs that are not intended to be part of the final product. By the definitions found in the directive and concept paper, RM cover the chemically defined growth media, process buffers, cryopreservation solutions, chemical transfection agents, and also cytokines, growth factors, enzymes, etc.

An important point also mentioned within the concept paper is made regarding excipients, which although not a RM, can be managed and controlled by a similar approach. The paper defines excipients as "pharmaceutically inactive components of the final formulation that are required to maintain the activity and stability of the active pharmaceutical ingredient and bring suitable functionalities of the defined dosage form." For ATMPs, water for injections, simple buffer solutions and stabilizers such as sucrose would be defined as excipients. Excipients can also include higher risk excipients such as human serum albumin and dimethyl sulfoxide (DMSO).

RM are, therefore, subject to the full process and quality regimes of GMP regulations. For ATMPs, Annex 2 of the EMA GMP guidelines is also applicable and although not the subject of this white paper, some aspects will be more stringent than for conventional therapeutics [7].

One further point to note is that raw materials can be synthesized chemical entities or of biological origin. For raw materials of biological origin, General chapter 5.2.12 of the European Pharmacopeia – 'Raw materials of biological origin for the production of cell-based and gene therapy medicinal products' is relevant. This chapter's overall aims are the following:

1. Identify the critical quality attributes of raw materials of biological origin
2. Harmonize variable practices and make the regulatory expectations more predictable
3. Encourage raw materials manufacturers to provide consistent, predefined quality and to record and share information on the origin and quality of the raw material
4. Help users managing batch-to-batch variations and changes in raw materials.

RAW MATERIALS TESTING

As biological products, the testing of raw materials may involve the use of established pharmacopeial methods, as well as the development and validation of novel methods. RM should be produced following applicable GMP guidelines to provide documented evidence of purity, potency, consistency, stability and traceability. The quality assurance system must comprise major GMP procedures including change control, deviation, Out-of-Trend and Out-pf-Specification procedures. At all stages of manufacturing, processing, and QC, the use of Standard Operating Procedures (SOPs) by qualified and trained personnel following validated and consistent processes must be performed.

Though there are many standardized pharmacopeial tests for synthesized RM, the same does not apply for biological or complex materials. Testing for biological materials requires the use of many different techniques and many methods may be bespoke for the raw material used. Companies may not have all the facilities, or pre-requisite experience, to perform all the required testing. Therefore, the establishment of partnerships with contract research organizations (CROs) can be an important step in a new product life-cycle. In addition, the CRO must be licensed by the relevant regulatory authority (EMA, MHRA, FDA, etc.) to satisfy the requirements of both quality systems and GMP. An important consideration in a CRO partnership is data retention. A minimum of 30 years storage is required and the challenges that this entails (electronic data/computer systems/data formats/maintaining readability/ensuring the data can be removed from storage and read) are multiple. Data retention time is often based on standard small molecule drug shelf lives as based on best practice, as advised through regulatory agencies, or through specific client-contracted times. This time period may be as short as six years and can extend for longer periods. Therefore, a CRO must have the ability and processes in place to extend data retention times to meet the desired requirements (e.g. 30 years), and to ensure that the tests performed/results obtained/reports can be recovered for the client.

What are the reasons and why are the appropriate testing methods important for raw materials?

Within Europe, The European Medicines Agency develops scientific guidelines to help pharmaceutical companies and individuals to prepare marketing-authorization applications for human medicines [8]. This guidance covers testing regimes that must be applied. This testing is to ensure that processes are in control, raw materials are tested to the required standards and ensure the products are safe for human use. For raw materials, a risk-based approach must be undertaken to ensure quality (Section 2 of the GMP guidelines for ATMPs [9]). This approach is performed by the manufacturer to identify and define the criteria which must be inherent in the quality of raw materials used. To assist in identifying and prioritizing suitable criteria, a series of questions can be asked prior to any selection of raw material supplier – examples of questions that could form the beginning of a suitable framework can be found in Table 1. This list is not exhaustive, and other questions may be required. However, it forms the basis of understanding the types of issues that it is better to resolve early in the product life-cycle. This reduces the risk of non-compliance and also forms the basis of initial discussions with laboratories that may be used for raw materials testing.

Depending on the ATMP, more questions than listed in Table 1 may be required to implement the correct risk-based approach. The EBE concept paper previously described [6] expands on the questions asked in Table 1 and provides guidance, based on EBE member companies, on how to establish a suitable framework. As it has been written from a commercial understanding of the regulatory requirements, it is advised reading for anyone wanting to establish raw material management.

Where raw materials are of biological origin, the General Chapter 5.2.12 of the European Pharmacopeia is relevant. The general

DOI: 10.18609/cgti.2021.056

requirements refer to: Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Material batch), Storage, and Labelling.

These general quality requirements apply for tests, which include, but are not limited to the following:

- Appearance
- Solubility
- Osmolality
- pH
- Elemental impurities
- Total protein
- Related substances
- Microbiological control
- Viral contaminants
- Bacterial endotoxins
- Mycoplasma
- Stabilizer
- Water

Many biological-derived materials may be complex mixtures, where it is not always possible to characterize completely the components of the mix. It is important that the testing regime can define the consistency,

TABLE 1

Choosing a raw material of suitable quality.

Supplier quality	▶ Is the supplier GMP-qualified for the raw material? ▶ Can they consistently produce the material to the desired quality? ▶ Can they consistently produce the material in the desired quantities for production needs? ▶ Is there sufficient stability data on the RM?
Multiple suppliers and Qualification of suppliers	▶ Is there more than one supplier? ▶ Do the raw materials have the same effect in your process? ▶ How similar/different are the raw materials? ▶ Are differences a risk? ▶ Qualify all suppliers and ensure that the quality criteria that you define can be met by the suppliers ▶ Try to avoid single, unique suppliers if possible
GMP materials	▶ The earlier the raw materials, produced under GMP, are used in the development process, the easier the subsequent processes of lab, pilot to production scale to generate clinical material
Partner contracts	▶ Have the appropriate defined contracts in place with your supplier to ensure robust quality and supply
Understand and monitor the material source	▶ How consistent is the product quality? ▶ Is the company stable financially? ▶ Where does the company source its raw materials, and is this secure? ▶ What happens if the company is bought out by a larger company? ▶ You may want to consider/explore whether the supplier has a business continuity plan in place (to cover natural disasters, hacking of systems, etc.)
Understand the testing regime	▶ Are the tests robust? ▶ What happens as regulatory requirements change? ▶ What happens if tests are not available due to kit availability, end-of-life of equipment, etc. ▶ As testing/equipment advances, will the requirements for impurities, protein purity, etc. change?
How variable is the raw material?	▶ Understand the important aspects of your raw material – purity, bioactivity, source, etc.

TABLE 2

Examples of impurities that can be introduced during the production process.

Impurity	Process or product related	Method that could be used
Aggregates	Product	Sub-visible particles, SEC, DLS
Degradation products	Product	HPLC methods, cIEF, cSDS, WB
Host cell proteins	Process	ELISA, LC-MS/MS
Host cell DNA	Process	qPCR
Vector-derived DNA	Product	qPCR

performance and safety of the material being used.

This is undertaken by a combination of testing and, where required, bioassays. The risks that were identified in initial scoping of raw material quality (using the risk-based approach) act to guide as to the combination of tests required to ensure quality of the raw material.

Where cell growth, expansion and/or maintenance is an aspect of the manufacturing process, for example during the manufacture of chimeric antigen receptor T-cells (CAR-T), raw materials can include:

- Sera and serum replacement
- Proteins produced by recombinant DNA technology
- Proteins extracted from biological materials
- Cell growth factors
- Cytokines required for cell differentiation
- Vectors

For many raw materials used in the manufacture of cell-based therapies, it is important that they are sterile, and if not, of known biological contamination with full justification for the non-sterile status. Therefore, sterility testing is a prerequisite prior to use. Within Europe, ICH Q4B [10], and, in particular, ICH Q4B Annexes 4A, B and C are relevant and define the relevant considerations for sterility testing and refer to the desired pharmacopeial chapters for the EU, US and Japan.

In a similar fashion to antibody-based therapies, if manufacture of the ATMP generates process-related and product-related impurities (Table 2), it is up to the manufacturer to ensure that all potential impurities have been identified and limits defined.

There are many possible tests that can be performed in the biological world to define raw material characteristics that are important to ensure product consistency and to define the quality attributes of the raw materials. The tests must meet pre-defined quality requirements for identity, purity and biological activity. No one test can define the total quality attributes of a raw material, and the desired quality attributes must be defined to guide the testing regime. The tests are orthogonal to each other and ensure that a consistent product is used in the manufacturing process.

The method(s) for each test must give consistent performance and undergo a validation process, in accordance with ICH Q2 [11] guidelines before being used for routine testing. In addition, the supply of reference materials, where possible and the evaluation of the stability of representative batches of raw materials to ensure that the test is in control, must form part of any testing plan.

Testing of chemical raw materials for ATMPs will generally be defined within the pharmacopeial compendia. In terms of biological raw materials, examples of experimental approaches can be found in Table 3. As well as developing bespoke methods, commercial kits designed to detect common cytokines, cell proteins and other common biomolecules are available and can be used to create GMP validated raw material methods. However, these kits often require additional expertise in adapting them to ensure that either the kit can detect the raw material within the sample matrix, or if interference is observed, a suitable sample preparation procedure is developed to allow the raw material

DOI: 10.18609/cgti.2021.056

TABLE 3

Potential testing approaches for common categories of ATMP raw materials.

Sample type	Testing required	Suitable technique that can be implemented	Comments
Serum	ID	SDS-PAGE/Western Blot	SDS-PAGE provides a protein profile that can be used to confirm serum type. When combined with a species-specific western blot, both species and serum type can be suitably identified
Serum free cell culture media	ID	CE-SDS for protein profiling can be implemented if available sample volume is restricted	
Defined protein components	ID and/or Quantitative	ELISA	ELISA methods can form the linchpin to any raw material testing regime as they are versatile, and a well-developed method can be created to be cost effective, robust and quick to run A qualitative ELISA can be developed to confirm ID of component by a positive/negative result. Alternatively, a fully quantitative ELISA can be developed to confirm ID and concentration of component
	Functionality	Bioassays	Ability of RM to selectively activate cells can be demonstrated by a bioassay
		Flow cytometry	Ability of a RM to bind to specific cells can be demonstrated by flow cytometry
		Occasionally a bespoke ELISA	For certain components, a bespoke ELISA could demonstrate depletion of specific cells by a RM, creating a method that is more cost effective than a cell-based method
	ID and characterization	HPLC – mass spectrometry	Intact mass – ID test for purified or expressed proteins Protein sequencing following tryptic and/or chymotryptic digestion Small molecule analysis/screening Leachables and Extractables Identification of post-translational/in-process/storage modifications
		HPLC – protein	Purity – Reverse Phase or SEC Aggregates/Oligomers – SEC, DLS
		HPLC – N-linked Oligosaccharides, following release using PNGase F	Fingerprinting/sequencing to confirm correct glycosylation
	Microbiological assays	Sterility, bioburden, endotoxin, and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing. Virological detection and Baceriophage testing may be necessary, and should be additionally considered
Buffers	Presence/absence of: sugars vitamins amino acids other chemically synthesized small molecules Ions	HPLC – small molecule May be coupled to UV, fluorescence, CAD, ELSD, PED/PAD, MS) or combinations of detectors	Usually defined pharmacopeial methods
	Ions or heavy metals	ICP-MS	Can be used to detect ions or heavy metals
	Microbiological assays	Sterility, Bioburden and microbiological testing	Not required for all reagents but need to be considered when planning raw material testing

to be tested using the commercial kit. In addition, these assays need to be validated with the appropriate matrix.

In addition, a CRO such as RSSL can also provide clients with the ability to extend beyond raw materials testing (both biological and chemical) and enable testing through other aspects of the product life cycle. This may include:

- Extractables and leachables from single-use processing components/fill vials/culture bags/etc
- Stability testing using GMP testing regimes of temperature and relative humidity, and photostability may be required for some light-sensitive RM (Media) according to ICH Q1B [12]
- Dissolution testing of raw materials into conditions similar to process conditions
- Emergency Testing of raw materials

Additionally, your CRO should have the expertise to develop and validate methods to ICH standards and additionally, stand up to scrutiny by the commissioning company and regulatory authorities.

CONCLUSIONS

The prime objective of any medicinal product is to provide the patient with a product that is meets the defined quality, safety and efficacy requirements. Therefore, the processes for manufacture, process control, testing, release and adverse-reaction reporting are heavily regulated. For all active ingredients, raw materials, excipients, cell lines, media, DNA, etc. the principles of GMP apply. This is to ensure that control of all materials used are within the limits defined by the manufacturer, are acceptable to the regulatory authorities and maintain absolute product quality. Raw materials for cell and gene therapies are more diverse and often bespoke tests are required to demonstrate quality. The approaches outlined in this White Paper will help in the decision process to define the questions that need to asked about raw material supply, quality, a testing regime, types of tests and start the process of choosing where testing may be performed. Not all companies will possess the required expertise, and partnerships with suppliers and CROs may be required. When choosing a CRO, such as RSSL, the regulatory status of the CRO must be ascertained, in addition to whether they have the experience and knowledge to help with the testing required. Often this will require consultancy, ability to perform pharmacopeial testing, develop and validate methods to GMP standards, and have the required quality management systems in place. No one test will define your total process. A combination of testing will be required to confirm identification, quantity and quality of raw materials.

REFERENCES

1. US FDA Approved Cellular and Gene Therapy Products
2. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2001_83_consol_2012/dir_2001_83_cons_2012_en.pdf
3. Alliance for Regenerative Medicine
4. The Cell and Gene Therapy Catapult UK clinical trials database
5. Why Are There Only 11 Cell and Gene Therapies in Europe?
6. Management and Control of Raw Materials Used in the Manufacture of Biological Medicinal Products and ATMPs
7. EudraLex, The Rules Governing Medicinal Products in the European Union
8. EMA Guidelines relevant for advanced therapy medicinal products
9. EudraLex, The Rules Governing Medicinal Products in the European Union
10. ICH Q4B Evaluation and recommendation of pharmacopoeial texts for use in the ICH regions
11. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology
12. ICH Topic Q1B Photostability Testing of New Active Substances and Medicinal Products

DOI: 10.18609/cgti.2021.056

BIOGRAPHIES

David Neville
Senior Associate Principal Scientist, Biomolecular Analysis, RSSL

David is an Industrial Chemist turned Biochemist and has a BSc (Ind Chem) from the University of Limerick and a PhD from the University of Dundee. He has over 30 years' experience in biomedical research, specialising in oligosaccharide and protein purification and analysis. He has a wealth of technical experience including, but not limited to, HPLC, mass spectrometry, cell culture, methods development and validation, and low-pressure liquid chromatographies for protein purification. He has a mixture of industrial and academic experience and has published over thirty papers/book chapters. He has extensive client interaction/management experience, which includes planning, co-ordination with clients, experimental choice to guide work to meet set timeframes, and obtain the required information.

Alistair Michel
Senior Scientist I, Biomolecular Analysis, RSSL

Alistair is an immunologist and has a BSc (Hons) from the University of Edinburgh and an MSc from Imperial College London. He is a member of the British Society of Immunologists. He has over 15 years' experience developing immunoassays, ten of which have been spent developing and validating/verifying ELISAs (both sandwich & competitive) to ICH and ISO13485 standards. He has experience of Western Blot, T-Cell proliferation assays, SDS PAGE, Molecular Biology (DNA/RNA extraction from whole blood and performance of assays utilising PCR & RT-PCR), Bacterial and Mammalian Cell Culture, Microscopy & Histology. He has extensive project management experience, which includes planning, co-ordination with clients, organisation and execution of work to meet set timeframes.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are both employees of Reading Scientific Services Ltd. The authors declare that they have no other conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Jan 26 2021;* ***Revised manuscript received:*** *Mar 16 2021;* ***Publication date:*** *Mar 26 2021.*

CELL & GENE ANALYSIS

Accelerating your testing.
Powering your innovation.

Agile, flexible materials testing. Conveniently located.

- COMPENDIAL TESTING
- METHOD DEVELOPMENT, TRANSFER & VALIDATION
- QC RELEASE TESTING
- COMPLEX MATERIAL - VIRAL VECTORS, CULTURE MEDIA & EXCIPIENT ANALYSIS
- CELL BASED ASSAYS & POTENCY TESTING
- PATHWAY & CONTACT MATERIALS
- ICH STABILITY STORAGE & TESTING
- STERILITY TESTING
- 24/7 EMERGENCY RESPONSE SERVICE

+44 (0)118 918 4000 • enquiries@rssl.com • www.rssl.com

rssl
science with service