Challenges of analytical method validation for ATMPs

Quality Control of medicinal products is a fundamental aspect for assuring the quality, efficacy and safety of the product. Testing of the product at intermediate manufacturing stages, such as testing of Drug Substance (DS), and on the final Drug Product (DP) are performed to confirm the product meets the established specifications as defined in the Marketing Authorisation. This does not differ for Advanced Therapy Medicinal Products (ATMPs). Because of the novelty and complexity in both molecule characteristics and manufacturing processes, analytical method validation for ATMPs can be of particular challenge.

Advanced Therapy Medicinal Products

ATMPs represent a novel class of complex biological products that are at the forefront of scientific innovation and hold great potential to improve health care. This heterogeneous group of pharmaceuticals are classified into three main types, known as Gene Therapy Medicinal Products (GTMPs), Tissue-Engineered Products (TEPs) and somatic Cell Therapy Medicinal Products (CTMPs) [1]. The sub-category of somatic CTMPs refers to a biological medicine that contains or consists of cells that have been subject to substantial manipulation to change their biological characteristics, or cells that are not intended to be used for their original function.

Many of the ATMPs are in development for (ultra) orphan indications and originating from academic Research and Development (R&D) groups. Transitioning manufacturing and testing activities from an academic setting to a Good Manufacturing Practice (GMP) environment can be challenging. The primary focus in non-GMP research and development institutions often lies more with cell manipulation than with Regulatory quality and compliance aspects. This because GMP regulations are not applicable at the pre-clinical R&D phase and as such, there is a lack of regulatory and GMP expertise.

Analytical Target Profile

The attributes of the DS or DP that are to be tested to obtain the necessary information about product quality safety and efficacy are determined during the Drug Development stage. The information gathered from the test results supports the establishment of the Analytical Target Profile (ATP) that provide a required range in the quality criteria for key performance characteristics of the product. Ranges defined in the ATP create a direct line from the product's Critical Quality Attributes (CQAs) and the release specifications. Establishing the ATP for each assay in relation to the CQAs in the early phases of product development should be a priority for any drug developer to provide clarity and direction for the ongoing analytical and process development. A timely start at the early development phase also avoids non-productive development, saving time and money. Establishing these product and analytical characteristics, and measuring them consistently, helps to support regulatory submissions and claims of comparability at later phases. As the product develops and is characterized, the associated analytical methods also move from the Research and Development (R&D) stage into Good Manufacturing Practices (GMP) manufacturing. From the Clinical Trial phases onward, analytical methods are routinely used, monitored and subject to continual improvement that are managed via Change Management. This is also known as the Analytical Product Lifecycle, and guidance in the analytical procedure development and its lifecycle is given in a new draft document from the International Council for Harmonisation (ICH); ICH Q14 "Analytical Procedure Development" [2].

Analytical Method validation

Once a product is manufactured in accordance with GMP, the analytical test methods are required to be validated and described in the product's Marketing Authorisation or Technical Dossier. Validation of these methods can be a complicated venture, depending on the type of analytical method and product. International Council for Harmonisation (ICH) guideline ICH Q2R1 "Validation of Analytical Procedures: Text and Methodology" provides guidance that Quality Control Laboratories can avail of in consideration of which

analytical method characteristics are applicable for method validation. Despite the information available, validation of analytical methods for Advanced Therapy Medicinal Products (ATMPs) to can be even more challenging. This is due to the inherent variability in starting materials, complex biological features and associated manufacturing process. Other factors are the limited batch history and sample availability due to low batch sizes and high manufacturing costs, lack of available assay references and assay controls.

Facing challenges for ATMPs

Unlike more mature biological pharmaceutical products such as monoclonal antibodies, which like ATMPs such as AAVs, are highly heterogeneous, analytical techniques and process manufacturing workflows are still the subject of intense development optimization and change. The analytical methods used to support ATMP drug development and release can be thought of in terms of three categories related to their level of maturity and expected analytical development and validation time costs. Firstly, "fully mature" drug characteristics that are analogous to those seen in more established molecule types can be analysed using established methodologies used for other biological molecules. Examples include excipient testing for antifoam clearance, Host Cell Protein (HCP) and Host Cell (HC) DNA impurity. This makes them relatively simple to implement from the earliest phases of development, often with kit-based assays and fully GMP analytical systems and software.

A second group of assays include those which are commonly found in in GMP QC release laboratories. Therefore, they can take advantage of the fully developed analytical platforms and software used for this purpose, but with some additional considerations which may require further analytical development effort and special attention during validation. Examples in this second group include the measurement of characteristics such as Post Translational Modifications (PTMs) of capsid proteins by peptide mapping, analysis and relative quantification of capsid proteins by LC-UV/FLD or CE UV/FLD, SEC for aggregate analysis and protein impurity determinations can be expected to require further development. This second group does use many of the techniques commonly found in established GMP QC release laboratories over the world. When using established technologies a Company can take advantage of the fully developed analytical platforms and software packages used for this purpose Some additional considerations may require further analytical development effort and special attention during validation if based on equivalent assays such as those used in mAb products. PTMs measured at the peptide or protein level must consider the likely impact of sample preparations used to disrupt the capsid. SEC and other particle sizing methods have to be suitable for larger size monomers and aggregates, with AAV molecule being >30 times larger than monoclonal antibodies and protein concentrations are also likely to be significantly lower and thus sensitivity may be a consideration. Monoclonal antibodies are often manufactured to concentrations greater than 100mg/mL whereas AAVs are likely to have protein concentrations of <0.05mg/mL and are manufactured at significantly lower batch volumes. Protein impurity assessments are also likely to be required to perform at protein concentrations significantly less than those seen for antibodies methods and may be required to perform at a level too low for many existing procedures.

To continue with AAV molecules the third group of assays are for characteristics such as empty/full assessment, infectivity and potency which may rely on techniques that are uncommon in pharmaceutical release settings. By their nature AAV molecules are highly complex and therefore, methods to measure this complexity may require significant development and optimization in order for them to be appropriate for use in a GMP setting. As a result, this type of method analysis could likely change the most during the development process, leading to significant extra work in proving comparability of the analytical method over the course of the assay lifecycle. It can also lead to uncertainty during specification setting in early phases. Techniques such as AUC and cryoEM would be more than capable of evaluating empty, full and partial AAV species, but are not routinely used in a GMP setting. Therefore, these methods do not have commercially available Annex 11 or 21 CFR 11 compliant software that could be used as part of the system's qualification in a GMP environment. This can lead to significant time investments for later validation activities closer to the commercialization stages of the product.

Potency

Due to the complex mechanism of action which typically characterise ATMPs, special attention should be given to the development of a suitable potency assay as early as possible in the program. Recognising the difficulties associated to the development of analytical methods that can describe the elements of an ATMP Mechanism of Action (MoA) in a quantitative manner, regulatory agencies suggest adopting a "phase-appropriate" [3] approach to potency assay method development. It is often difficult to develop a potency assay that is relevant to the intended MoAs, able to quantify biological activity while being tolerant to heterogeneity in both the product and assay components, and with the potential of being qualified or validated according to the stage of the program. Potency assays must generate quantifiable results that reflect the different elements of the MoA, they are ideally predictive of clinical efficacy, and be able to be performed in a useful timeframe. Qualifying these types of assays before first in human and validating before pivotal clinical trials alongside the establishment of appropriate and well justified release limits will likely be a major challenge in the development of any ATMP, but especially difficult with Cell therapies.

Product Availability and Reference Standards

As described previously, the amount of material that is manufactured is often in very small quantities. In some cases, these bespoke medicines are limited to just the amount that serves as the dose given to the patient. Due to the novelty and complexity of ATMPs, reference materials are not always available, thus making it more difficult to demonstrate that the procedures and the tests developed are suitable for their intended use. The use of interim references can be an option in these circumstances and their use could be recommended to provide a level of continuity and confidence in the analytical methods applied. In particular, as these interim references are developed through their analytical lifecycle where this is a requirement. Reference standards should be representative of the manufacturing process as much as is possible and may need to be replaced with associated bridging studies as the process develops. Where reference standards are not available, in assay controls can help to demonstrate assay consistency and support in proving representativeness during the drug development lifecycle.

Data from reference standards and analytical controls should be used to set assay acceptance criteria. These criteria may also be wide compared to other modalities at the clinical phase stage. However, as the drug development progresses the results from these assays should also provide assurance of manufacturing consistency. Trending results over time should confirm release criteria and, where assay variability is present, studies should be performed to ascertain the cause and establish understanding of inter and intra assay variability. In line with ICH Q14, Design of Experiment (DoE) studies could be useful in designing these studies and can help conserve limited sample amounts.

Regulatory Bodies

Throughout the development process, it is recommended that continued and frequent dialogue with regulatory agencies is maintained. Communications should include details of draft analytical strategies. This should help focus efforts for continued analytical development and validation. It also builds an understanding and justification for areas where sample and data availability may be limited. With agreement from regulatory agencies, it may also allow for the gradual reduction of release criteria once it is clear that these criteria are either not relevant to safety and efficacy or not required due to process robustness such as with process excipients.

Assay Comparability Studies

The storage and prudent use of retain samples from all key process lots could likely be critical in establishing assay comparability or for analytical bridging studies through the development process. Notably, where assays required significant development and processes have undergone significant change as is likely with these ATMP

product types. In addition to these studies, the development of platform approaches to all analytics should be considered. Although these technologies are emerging, data from similar molecules, for example of other serotypes in gene therapy, can be leveraged to support method development, validation and specification setting activities where appropriate.

Despite the fact that release analytics for new modalities may have unique challenges due to the use of new techniques, a lack of historic data and an increasingly complex mode of actions, this is not the case for the majority of assays used for release of these potential new products. A significant proportion of the required analytics are either compendial or based on well established procedures. Time and effort can more easily be focused by following a systematic approach as defined in ICH Q14. Appropriate utilization of experience and understanding of both technical capabilities of an analytical method and GMP requirements will allow the generation of a suitable analytical strategic plan to develop methods appropriately during the process development that will be able to ensure the quality, safety and efficacy of the product, meeting regulatory expectations.

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