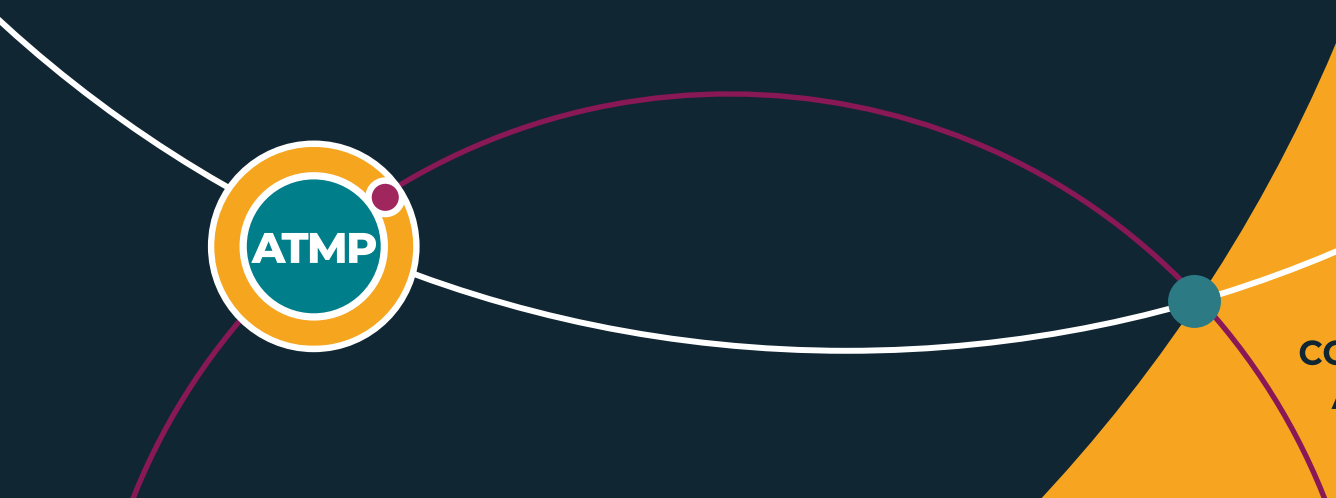
BioPhorum

GENE THERAPY POTENCY STRATEGY

Gene therapy potency strategy roadmap

A guide to navigating the complexities of potency



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About BioPhorum

We enable the global biopharmaceutical industry to connect, collaborate and accelerate progress for the benefit of all.

Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum's membership now comprises top leaders and subject matter experts from global biopharmaceutical manufacturers and suppliers, working in both long-established and new Phorums. They articulate the industry's technology roadmap, define the supply partner practices of the future, and develop and adopt best practices in drug substance, fill finish, process development and manufacturing IT.

In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

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1.0

Introduction

1.1 Purpose

It is broadly recognized that meeting regulatory expectations for phase-appropriate assessments of potency for *in vivo* gene therapies is a challenge in the advanced therapy medicinal products (ATMP) industry. Additionally, expectations from regulators differ based on geographic region, providing additional challenges for cohesive potency strategy development worldwide.

The BioPhorum gene therapy potency strategy workstream was created to bring together industry experts to form a consensus view on the complex challenge of defining potency strategy for recombinant adeno-associated virus (rAAV) gene therapy products. The gene therapy potency strategy workstream consists of 34 members from 19 companies. It has sought to understand current approaches and associated challenges through discussions, outreach and an industry benchmarking survey (conducted during March 2024 with 24 responses from 22 companies). Through this survey and twice monthly discussions, the workstream created a roadmap to help support those involved in rAAV gene therapy potency strategy development, particularly those that are new to the field. This roadmap consists of visual representations of consensus approaches and considerations that users can apply to their own specific development situations and candidates. It includes sections of text that provide detail on two specific challenges faced by the industry relating to potency assays, namely, the TCID50 assay and cell line selection for potency testing. There is a mismatch between scientific rationale and global regulatory expectations for the TCID50 assay that are difficult to reconcile. In addition, guidance that is available on use of representative or non-representative cell lines for potency assays is not always clear. Both sections in this roadmap provide an industry consensus from members of the workstream to drive further discussion and alignment in the industry. Ultimately, however, the complexity of issues means that case-by-case discussions with program teams and regulatory representatives are still required.

1.2 How to navigate

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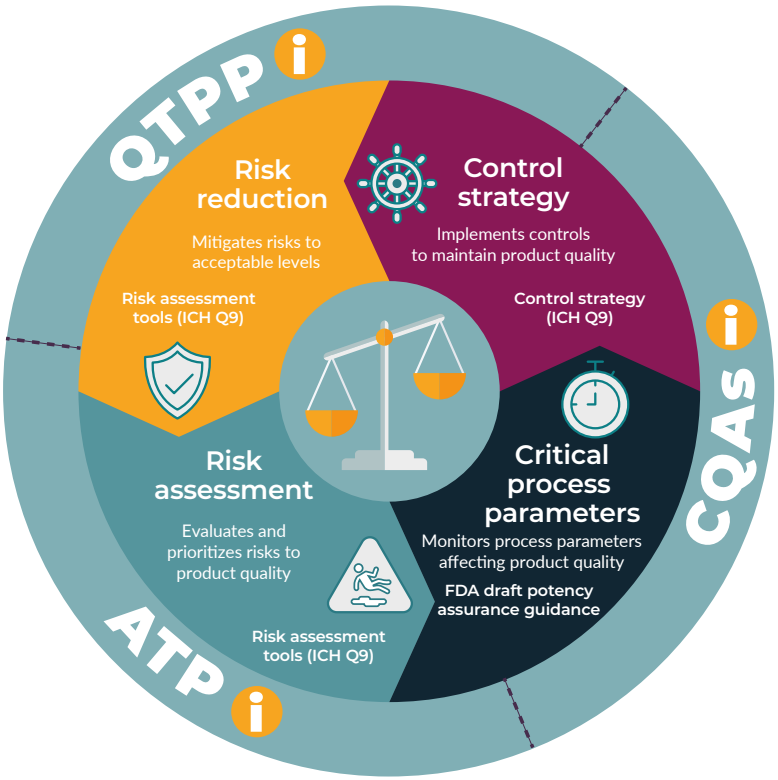
2.0

Integrated approach to product understanding



An integrated approach to product understanding, especially for potency, relies on the interplay between the quality target product profile (QTPP), critical quality attributes (CQAs), analytical target profile (ATP), risk assessment and control strategy.

Potency reflects the biological activity of a therapeutic based on the mechanism(s) of action (MoA) and is directly tied to its therapeutic effect. If potency fluctuates, there may be a high risk of an impact on clinical performance. The QTPP is the starting point for development. It defines the need for potency and its clinical relevance as a key product quality goal. CQAs link potency to process and materials. Identifying potency-related CQAs helps pinpoint which aspects of the product and process must be tightly controlled. By evaluating risks to potency-related CQAs, an assessment strategy that minimizes variability and degradation of the product can be designed. A robust control strategy ensures that every batch meets potency requirements. The ATP ensures that the analytical methods used to measure potency are in line with ICH Q2(R2)[1] expectations to support good manufacturing practice (GMP)-compliant product release and stability testing.

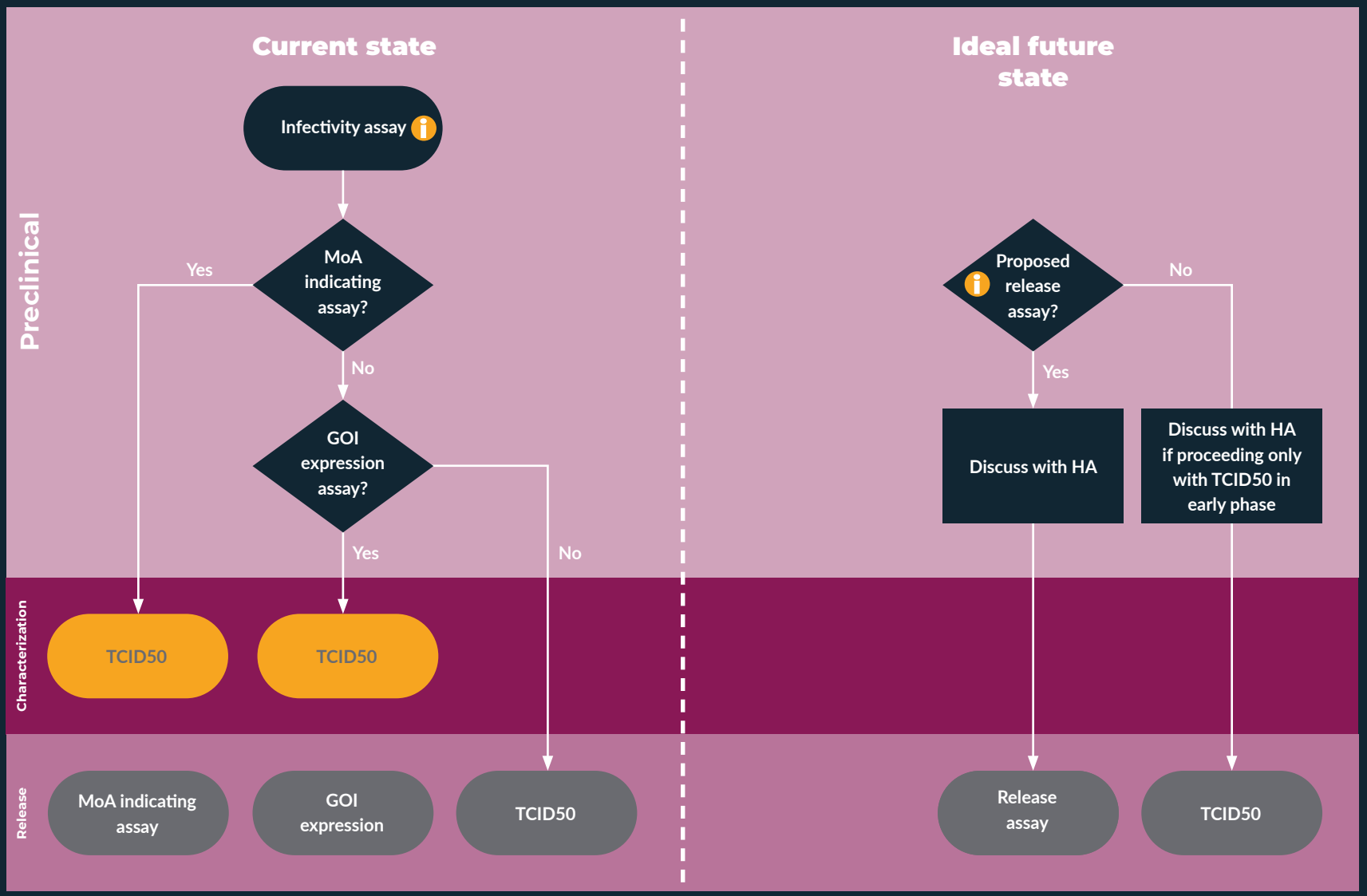
With a well-thought-out potency assurance strategy which takes into consideration the manufacturing process and a thorough understanding of how potency is assessed, regulatory submissions can be stronger and have greater chances of a positive outcome.

3.0

Roadmap visuals

3.1 Phase-appropriate potency assay implementation for release testing

This visual shows the current state and the consensus ideal future state for determining the potency release assay at the preclinical stage.

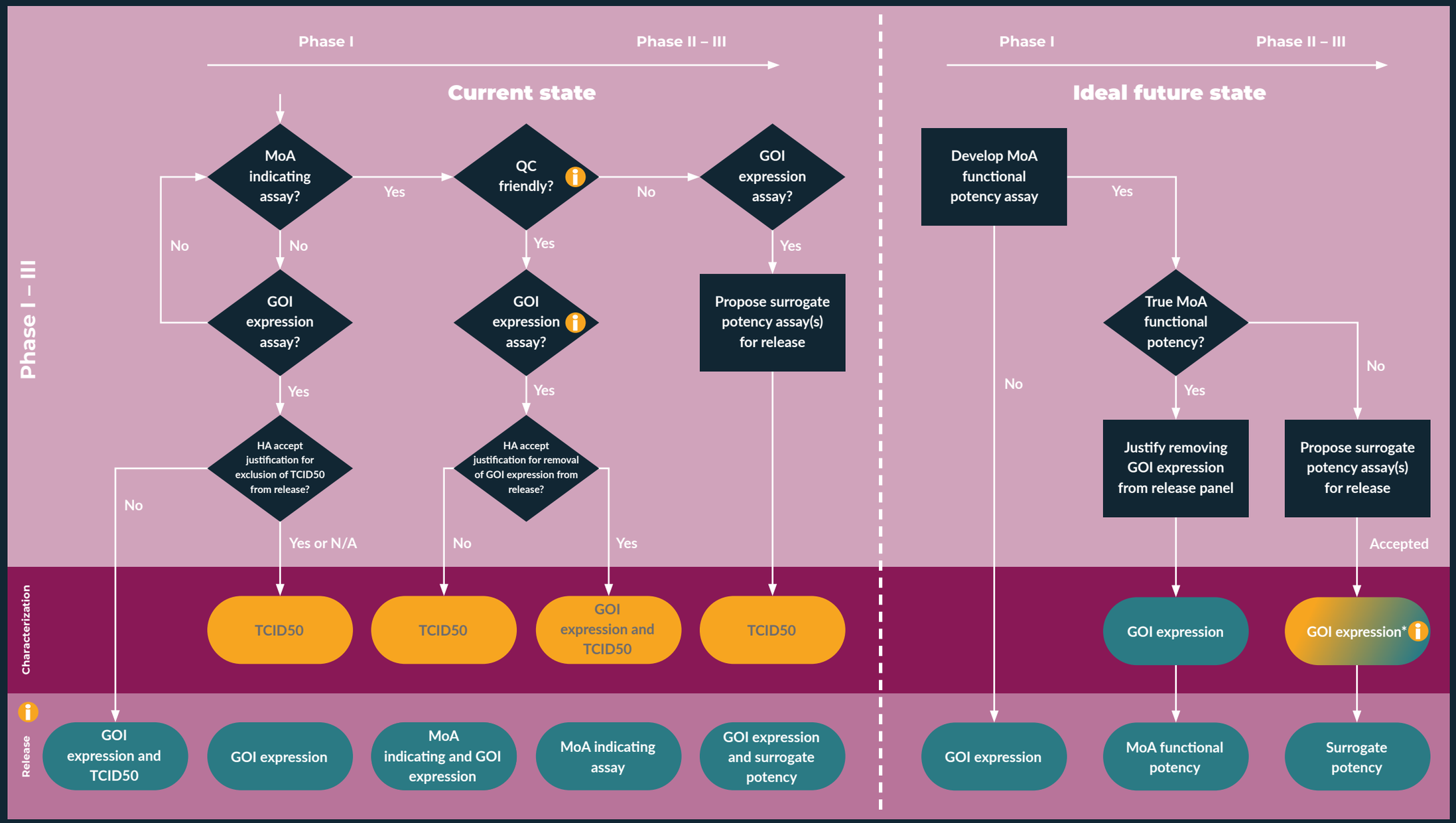


Key

Characterization | Release | 'Fit for purpose'

3.1 Phase-appropriate potency assay implementation for release testing (continued)

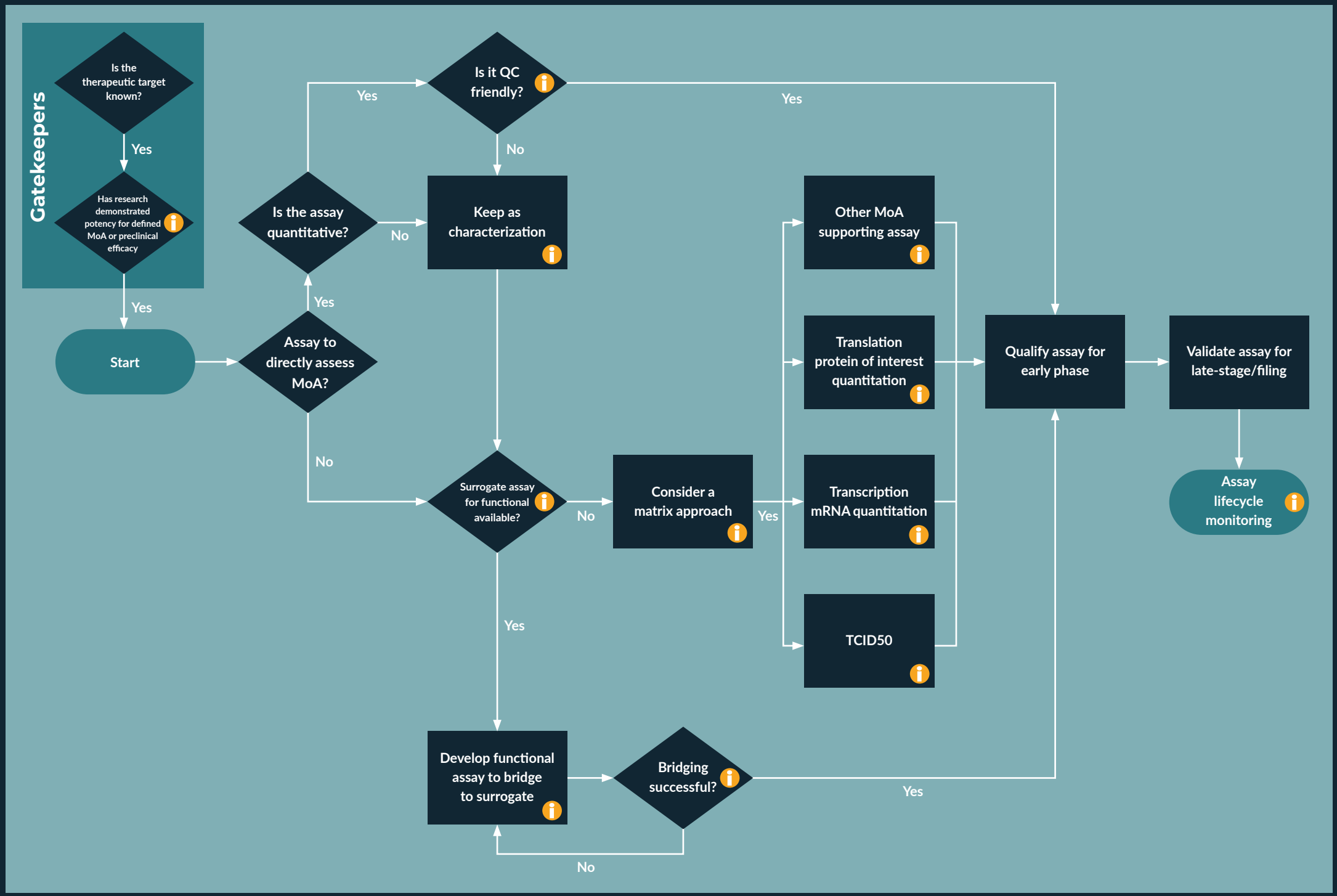
This visual shows the current state versus the consensus ideal future state for determining the potency release assay as you progress from phase I through to phase II/III stage.



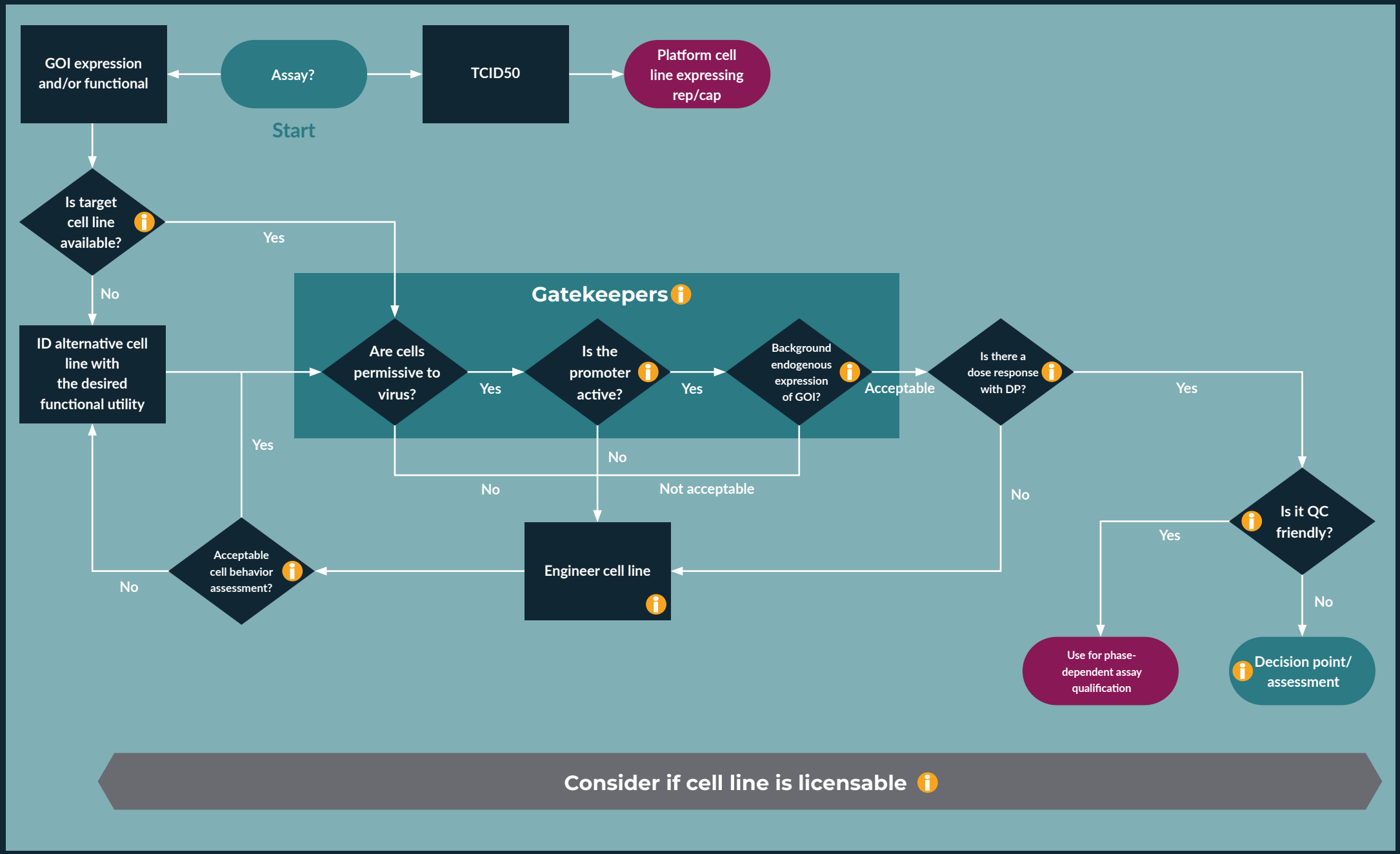
Key

Characterization | Release | 'Fit for purpose'

3.2 Potency assay strategy based on MoA



3.3 Cell line selection



4.0

Justification for non-representative cell line selection

4.1 Definitions

Representative cell lines: These are disease relevant cells derived from a specific tissue that is targeted by the viral gene therapy. They can be either primary cells, immortalized cell lines and/or induced pluripotent stem cell (iPSC)-derived cells and can be non-human if no relevant human model is available.

Non-representative cell lines: Cell lines that may not be inherently tissue-specific nor representative of the targeted disease but may be engineered to adequately show the dose response of the product and are appropriate for a QC assessment of potency.

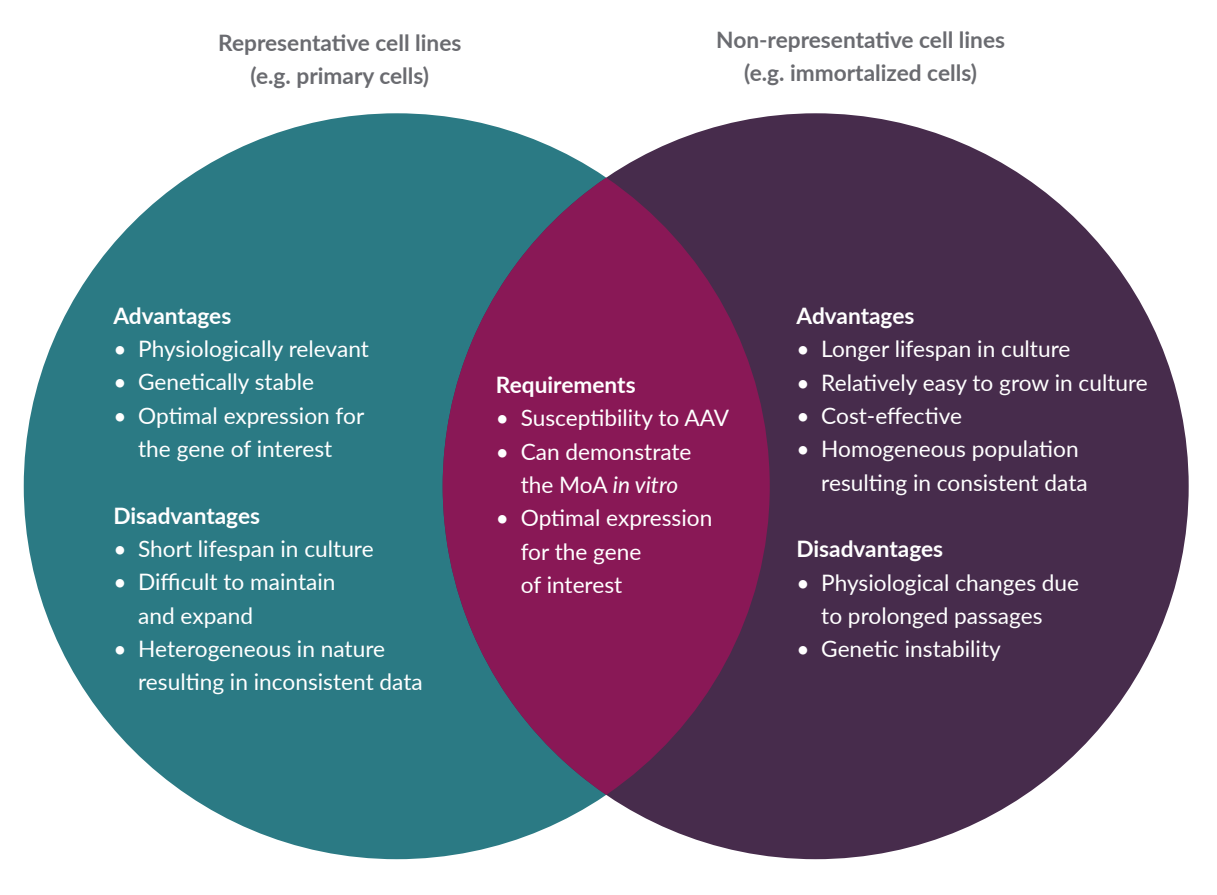
4.2 Challenges with representative cell lines

Potency assays must be robust and reproducible. A representative cell line may not express the target receptors or have the intracellular machinery required for the efficient uptake of the rAAV serotype and/or expression or function of the transgene product. Due to the challenges with the cell biology of some representative cell lines, these lines may be difficult to work with and lead to inconsistency in potency results. Over time and with multiple passages, some cell lines may lose consistency in gene expression and responsiveness, leading to cell line instability and variability in assay performance[3]. Some cell lines may not be sensitive enough to detect small changes in potency when the MoA involves a subtle effect on the intracellular signaling or downstream gene expression. Especially for rare diseases, human-derived representative cell lines are limited.

Some relevant primary cell lines or engineered primary cell lines require complex culture conditions, have a short lifespan or are difficult to expand. Representative cell lines may have biological limitations, such as changes in phenotype or differentiation in cell culture or genetic instability, all of which can cause the cells to be less representative of the target disease tissue, and in some cases contribute to misleading potency results. These challenges can complicate the use of representative cell lines for release testing in the GMP lab. Heterogeneity in cell lines can lead to high intra- and inter-assay variability which may present challenges with assay qualification and validation strategy.

Generating a representative cell line can be time-consuming, necessitating the use of an existing cell line for early phases. This varies on a case-by-case basis (Figure 1).

Figure 1: Comparison between representative and non-representative cell lines in cell-based assays



4.3 Benefits of using non-representative cell lines

4.3.1 Preliminary information and early-phase use

Non-representative cell lines are valuable for collecting preliminary information about the MoA of a drug under development and evaluating potential readouts. Non-representative cell lines are particularly useful when representative cells are difficult to handle or are not readily available. Their use is often limited to early-phase studies due to the lack of immortalized representative lines that are commercially available. In the best-case scenario, the final cell type would have biological relevance to the disease phenotype.

Engineering commercially available immortalized non-representative cell lines is an alternative approach to generating representative cell lines to improve AAV uptake, expression of cell type-specific transgene and/or subsequent demonstration of the functional

activity of the transgene[4]. Engineered cell lines with high permissiveness to viruses have been developed to ensure more consistent and reliable measurement of AAV transduction and functional activity. Improving permissivity to infection by viruses can be achieved by either adding transduction enhancers to the cultures or engineering cell lines to introduce components to enhance or augment viral entry and/or transgene expression. Some options that have been shown to improve the transduction of adeno-associated virus serotype 9 include hyaluronidase and neuraminidase during transduction[5, 6], which are known to be glycan-modifying enzymes.

Non-representative cell lines are often more cost-effective and suitable for high-throughput applications, making the processes of method development and qualification more efficient. These lines can help standardize assays across multiple programs, improving the understanding and reproducibility of results.

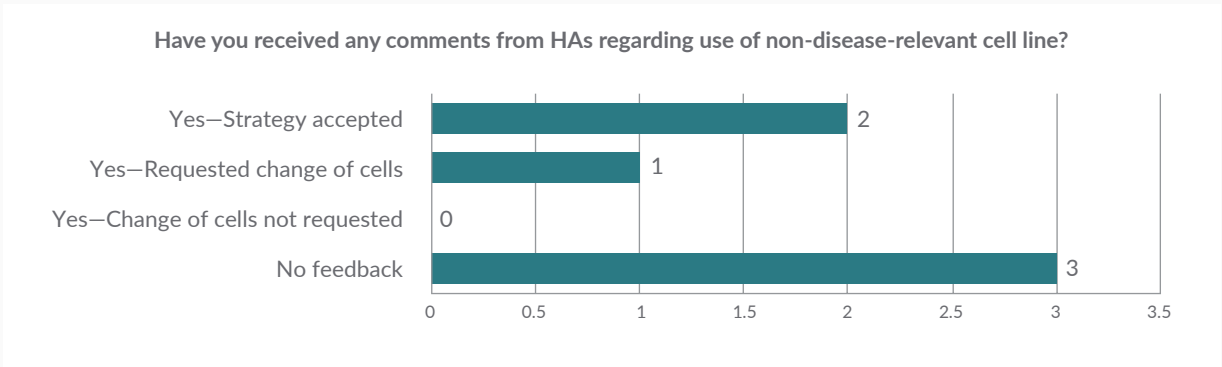
4.3.2 Ease of cultivation and maintenance

Non-representative cell lines are generally easier to cultivate and maintain. The cell lines can be well established and provide a robust and reproducible system for QC assays, ensuring feasibility during long-term use of the lifecycle of the product and large-scale screenings. With non-representative cells there can be a wider window of passages that maintain conformity, the cells may not drift as much over time compared to primary cells during the potency assay lifecycle, improving assay performance and reducing variability. Deriving the line into a single clone also adds to the stability of the line. Generic or platform cell lines are particularly useful if they can be used for multiple gene therapy programs. Regardless of the cells used, efficient transduction by the virus when working with non-representative cells is essential.

4.4 Conclusion

It can be challenging to justify to regulatory authorities that non-representative cell lines are biologically relevant. Agencies may require extensive validation and bridging studies to demonstrate that the results are applicable to the intended therapeutic context. Despite these challenges, BioPhorum member experience (Figure 2) has shown that non-representative cell lines have been accepted by regulatory bodies when consistent and reliable results that support the MoA are available to convince regulatory agencies. It should be remembered that the purpose of the potency assay is not only to demonstrate the MoA but also to ensure, with its measurement, the consistency of the batches and assess comparability during changes. It may be important to correlate results from non-representative cell lines with those obtained from more physiologically relevant cell lines or primary cells to ensure the cell model reflects the intended therapeutic context.

Figure 2: BioPhorum benchmarking survey results (March 2024)



From 18 responses, six indicate that non-disease relevant cells are used. From these six responses, the results above show health authority feedback on the approach.

5.0

Justification for exclusion of TCID50 from release panel

5.1 Historical background regarding infectious titer and regulatory expectation

The first mention of controlling infectivity in regulatory guidance for gene therapy products was in the 1995 The European Medicines Agency (EMA) guideline: "Gene therapy product quality aspects in the production of vectors and genetically modified somatic cells"[7] under Section 7.2.3. "Where possible, the particle: infectivity ratio of replication-deficient viruses should be determined and when this is excessively high rejection of the batch should be considered."

The expectation to measure infectivity was carried into the 2001 EMA[8] "Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products," the 2010 EMA[9] "Reflection paper on quality, non-clinical and clinical issues related to the development of recombinant adeno-associated viral vectors" and the 2018 EMA[10] "Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products." However, there had been inconsistency in whether infectivity of AAV is considered a measure of activity (classified under efficacy/potency) or physical content (classified under product content/titer).

The measurement or control of a 'particle: infectivity ratio' implies that there is an actual physical number of AAV particles within a sample that is capable of "infection" independent of the cell substrate or system. The concept of infectivity and infectious titer as a measure of physical content is consistent with classical virology and is only applicable for viruses that can replicate in permissive cell substrates under specific prescribed conditions.

As a member of the *Dependoparvovirus* genus, AAV is not capable of productive replication in the absence of a helper virus. In addition, rAAV products have been engineered to lack the rep and cap genes that are required for replication even in the presence of helper virus. To accommodate the traditional measure of 'infectivity' using the TCID50 method, an engineered cell line[11] is typically used to supply AAV rep and cap genes in trans and artificially support replication of recombinant AAV upon coinfection with a helper virus, usually wild-type adenovirus. However, the cell line is not likely to be universally permissive for all AAV products, since the necessary co-receptors, attachment and entry factors are specific to each AAV serotype (i.e. cell tropism differences). On the other hand, permissivity challenges can be resolved or improved by overexpression of host entry and transduction factors such as AAV Receptor (AAVR) and/or carboxypeptidase D (CPD or AAVR2) in this cell line[12].

Due to these limitations in measuring AAV uptake in cultured cells, there is no *in vitro* system that can accurately model *in vivo* infection for AAV products. Therefore, it is not possible to provide an absolutely accurate count of rAAV particles within a given sample that are capable of *in vivo* infection[13]. The ability of the AAV to 'infect' depends not only on the AAV itself, but also the chosen cell substrate which may or may not express the tropic factors necessary for the given AAV serotype, as well as the specific experimental conditions used (i.e. cell passage, infection duration, volumes/geometries, etc.). For these reasons, it is most appropriate to consider 'infectivity' as a measurement of the ability of the AAV product to deliver the vector genomic DNA to the cell substrate. Because successful delivery of the vector genomic DNA to the cell substrate is necessary for the expression of the transgene and activity of the expressed transgene product, potency methods that measure expression and/or activity of the transgene product provide evidence that the AAV is capable of 'infectivity'.

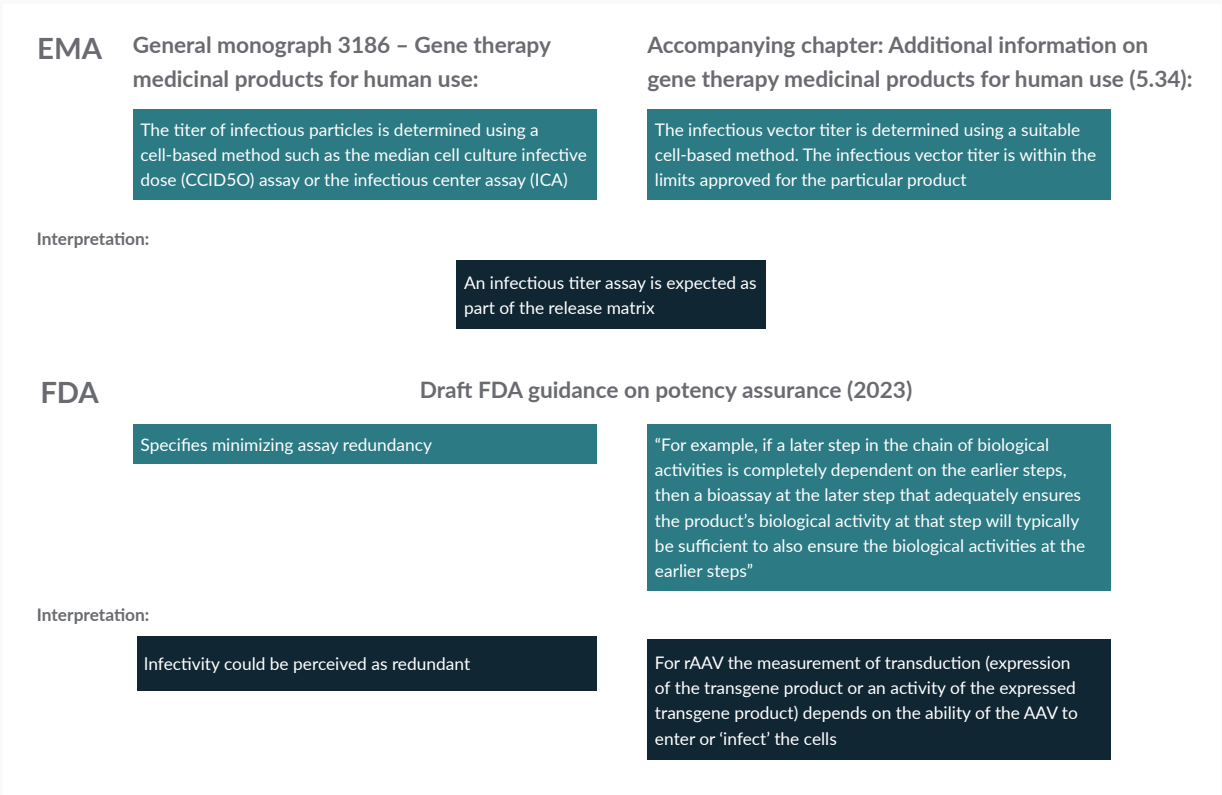
5.2 Differences in FDA and EMA guidance regarding the use of TCID50

The draft US Food & Drug Administration (FDA) guidance[14] on potency assurance specifies minimizing assay redundancy: "[…] if a later step in the chain of biological activities is completely dependent on the earlier steps, then a bioassay at the later step that adequately ensures the product's biological activity at that step will typically be sufficient to also ensure the biological activities at the earlier steps." For AAV, the measurement of transduction (expression of the transgene product or an activity of the expressed transgene product) depends on the ability of the AAV to enter or 'infect' the cells. This is also reflected in the new US Pharmacopeia (USP) draft chapter, USP <1067>[15], stating that "Vector infectivity, also known as infectious titer, can be confirmed through assays that comprise the potency matrix (e.g. gene and protein expression and functional activity)".

European Pharmacopeia (Ph. Eur.) specifically mentions the need to have an infectious titer assay, namely in *Ph. Eur. monograph – Gene therapy medicinal products for human use (3186)*: "Infectious particle titer. The titer of infectious particles is determined using a cell-based method such as the median cell culture infective dose (CCID50) assay or the infectious center assay (ICA)" and in the accompanying chapter: *Additional information on gene therapy medicinal products for human use (5.34)*: "Infectious vector titer. It is determined using a suitable cell-based method. The infectious vector titer is within the limits approved for the particular product" and "Ratio of vector particle titer to infectious vector titer: within the limits approved for the particular product."

The difference in expectations between FDA and EMA regarding the use of TCID50 poses a challenge for developing a coherent risk-based potency assurance strategy that can be accepted globally, which has the potential to negatively impact patients' access to life-changing gene therapy products in certain markets.

Figure 3: Comparison of key parts of guidance relevant to EMA and FDA highlighting differences regarding use of TCID50



5.3 Limitations of the TCID50

5.3.1 Background

The TCID50 assay is an end-point dilution method measuring the infectious titer of a virus. A permissive cell line is exposed to serial virus dilutions (10–12 replicates per dilution level) and wells are scored for infection based on cytopathic effect (CPE) (presence of CPE = positive or 1; no CPE = negative or 0). The ratio of positive wells over total wells per dilution level is used with the Spearman-Kärber statistical method to find the dilution at which 50% of the cells are infected and expressed in infectious units per mL (IU/mL).

5.3.2 rAAV vectors do not produce CPE

As rAAV vectors do not produce any CPE and are incapable of self-replication, the TCID50 assay was adapted to be able to measure their so-called 'infectivity'. Viral replication is assessed via vector genome amplification by quantitative polymerase chain reaction (qPCR) or digital polymerase chain reaction (dPCR). Wells are scored 0 (negative) or 1 (positive) based on whether their signal exceeds an infectivity threshold roughly corresponding to the background in control wells (i.e. cells infected with helper wild-type adenovirus only).

5.3.3 High variability

The TCID50 method exhibits a high degree of variability, with reported inter-assay coefficients of variation (CV) ≥35%, even when the assay precision is optimized with dPCR[16]. Due to the high variability, specifications may be too broad to be considered meaningful for controlling batch consistency and stability of rAAV products. Variability arises from a number of sources, for example, the operational consistency of performing large sample dilutions necessary for the limiting dilution assay, variability in cell culturing and assay conditions, as well as the variability of measuring vector genome amplification signals that are close to the threshold or background signal at the lowest vector concentrations. An additional source of complexity comes from assay-related reagents, such as the helper virus, adenovirus 5, the supply of which must be carefully secured to prevent variability that may arise from using different batches or supply sources.

5.3.4 Does not predict *in vivo* efficacy

The assay does not accurately predict *in vivo* efficacy since viral replication is not part of the rAAV mechanism of action.

5.3.5 Labor intensive

Finally, the assay's low throughput (one rAAV batch per 96-well plate), high degree of complexity and high failure rate which necessitates thorough analyst training, risks ergonomic injury and questions the relevance of keeping it in the release panel for rAAV-based gene therapy products.

5.3.6 Development and validation of TCID50 distracts resource from where it is most needed

There is limited incentive to improve the TCID50 method due to uncertainty about agency acceptance. A robust cell-based potency assay that reflects the MoA is better able to demonstrate consistency of potency across GMP batches compared to the TCID50 assay. Development efforts to improve the TCID50 method and to maintain its lifecycle for GMP use may distract sponsors from allocating sufficient time and resources to developing robust potency methods that are reflective of biological activity or MoA, which is of paramount importance from the standpoint of assuring product potency and stability.

5.4 Justification for removal/ exclusion of TCID50

5.4.1 There are other methods that are more beneficial from a patient-centric perspective

The therapeutic effect of the product is not dependent on infectious titer of the rAAV itself, and TCID50 assay results do not accurately represent clinically relevant activity. For rAAV capsids and serotypes that are designed to target specific cell types, the TCID50 assay does not mirror the differences in vector tropism.

Even if the TCID50 assay or other infectivity assay were developed with improved precision, the measurement of infectivity for rAAV products does not provide additional benefit when a functional assay is available that correlates with efficacy in the patient and/or is indicative of safety.

5.4.2 Ratio of vector particle titer to infectious vector titer is only one way to measure vector purity

Since the infectivity of rAAV is highly dependent on the *in vitro* system and the relative permissivity of the cells to the specific serotype, the concept of infectious vector titer as an absolute measure of physical content is inaccurate and inappropriate for rAAV. The control of non-functional vector-related impurities (e.g. empty capsids) is better achieved using other analytical techniques such as analytical ultracentrifugation (AUC), mass photometry, optical density (OD), polymerase chain reaction (PCR)/ enzyme-linked immunosorbent assay (ELISA), etc. The product's manufacturing consistency and key CQAs can be effectively characterized and controlled using a comprehensive set of analytical methods, minimizing the added value of TCID50 assay results.

5.4.3 TCID50 is redundant with other potency methods

Potency methods that measure transduction (expression or biological activity) of the transgene are indicative of successful infection. It is possible for there to be a vector that is infectious yet incapable of supporting the expression of the transgene product. However, it is not possible for transgene products to be expressed if the vector is not infectious. (Transduction means infection occurred, while infection does not necessarily mean transduction can occur.) Therefore, it is most desirable to have potency methods that provide a measure of the biological activity of the expressed transgene product.

Removal of TCID50 from the release and stability panel would be in line with new FDA draft guidance on potency assurance[14].

5.4.4 Conclusion

As part of a control strategy, when the CQAs are adequately controlled by other validated methods, the removal of the TCID50 assay does not increase product risk.

5.5 Recommendations

1. Start with the end in mind: Develop a potency assay that is fit for a release assay from early phase, which minimizes reliance on TCID50.
2. Avoid redundancy: In line with the wording of the draft FDA guidance[14], once there is a method that measures transduction or activity of the transgene, reliance on TCID50 is not necessary and this can be moved to a characterization method as a minimum.
3. Advocate for harmonization between EMA and FDA (and other regulatory bodies, e.g. Medicines and Healthcare products Regulatory Agency (MHRA,) Japan): Harmonization of regulatory requirements regarding the necessity of the TCID50 assay for product release could help drug developers in managing resources efficiently. This alignment would enable a focus on developing more reliable potency methods (reflective of biological activity or MoA) to support product quality measurement and establish manufacturing consistency.

6.0

Summary

This roadmap addresses a critical industry challenge: demonstrating appropriate potency for *in vivo* gene therapies across development phases while navigating diverse global regulatory expectations. Developed through extensive industry collaboration involving 34 members from 19 companies and informed by comprehensive benchmarking, this roadmap provides practical guidance for potency strategy development.

The roadmap establishes a framework integrating product understanding, analytical methodology and regulatory considerations. It emphasizes a phase-appropriate approach where early development may utilize simpler assays focused on mechanism demonstration, while later phases require more comprehensive functional testing aligned with clinical outcomes.

Key strategic recommendations include:

1. **Integrated approach** linking the QTPP, ATP and CQAs to ensure potency reflects the product's MoA
2. **Matrix approach** utilizing complementary methods when a single assay cannot capture all relevant attributes
3. **Pragmatic cell line selection** with justified use of non-representative cell lines when they demonstrate suitable performance and correlation to therapeutic effect
4. **Critical evaluation of traditional methods** such as TCID50, with recommendations to avoid redundant testing when more clinically relevant methods are available
5. **Regulatory strategy** focused on scientific justification and harmonization across global authorities.

This document serves as both a practical guide for implementation and a foundation for continued industry alignment on potency strategy development for gene therapies. By following this roadmap, developers can create scientifically sound, regulatorily acceptable potency strategies that ultimately support the delivery of safe and effective gene therapies to patients.

Glossary

Term	Definition
AAV	Adeno-associated virus
AAVR	Adeno-associated virus receptor
ATMP	Advanced therapy medicinal product
ATP	Analytical target profile
AUC	Analytical ultracentrifugation
CPD	Carboxypeptidase D
CPE	Cytopathic effect
CQAs	Critical quality attributes
CRO	Contract research organization
DP	Drug product
dPCR	Digital polymerase chain reaction
DS	Drug substance
ELISA	Enzyme-linked immunosorbent assay
EMA	The European Medicines Agency
FDA	US Food & Drug Administration
GMP	Good manufacturing practice

Term	Definition
GOI	Gene of interest
HA	Health authority
ICH	International Council for Harmonisation
iPSC	Induced pluripotent stem cells
MHRA	Medicines and Healthcare products Regulatory Agency
MoA	Mechanism of action
mRNA	Messenger RNA
OD	Optical density
QC	Quality control
qPCR	Quantitative polymerase chain reaction
QTPP	Quality target product profile
rAAV	Recombinant adeno-associated virus
TCID50	Tissue culture infectious dose 50%
USP	US Pharmacopeia

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