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Strategy for potency determination of gene therapy products

Overview of the BioPhorum GT Potency Strategy Workstream Discussions

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Outline

- Introduction to BioPhorum
- Potency assay selection tool
- Focus on the infectivity assay (TCID50)
- Conclusion
- Q&A

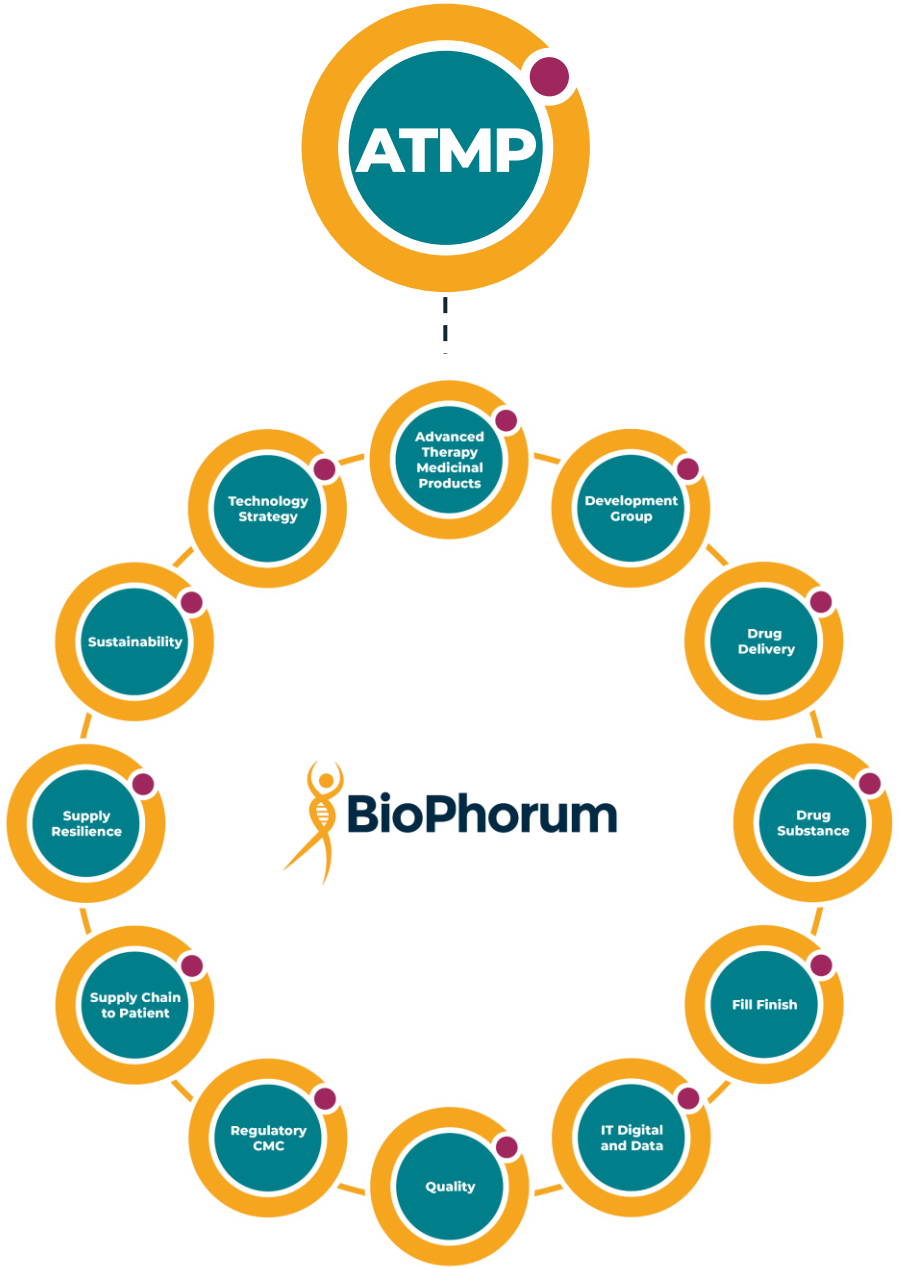
Phorums: providing benefits across the biopharmaceutical supply chain

Drug Substance, Fill Finish, Development Group, Information Technology, Advanced Therapy Medicinal Products, Sustainability, Quality and Drug Delivery
Accelerating the way the industry delivers near-term results, making best practice development and implementation faster, cheaper, and smarter.

Supply Partner and Supply Chain to Patient
Creating the supply chains the industry needs; defining, developing and implementing solutions for business processes, systems and culture.

Technology Strategy
Revolutionizing the way industry develops longer term transformational manufacturing and technology capabilities. Focusing on strategy and a ten-year time horizon, defining needs, difficult challenges, and potential solutions.

Regulatory CMC
Enable engagement and alignment with regulatory stakeholders to accelerate the adoption and successful implementation of advances in manufacturing.



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ATMP Workstream: Gene Therapy Potency Strategy

Problem:

- Demonstrating regulatory expectations for potency in GT and translating these into assay is challenging.
- Multiple MoAs which are often not fully understood

Goals:

- Benchmark experiences
- Challenge standard approaches to potency strategy
- Provide guidance on potency strategy considering phase, MoA and other variables based on industry consensus
- Position paper

Benefits:

- Reduce the risk of quality/regulatory non-compliance and associated delays
- Reduced time to market

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Acknowledgements – Gene therapy potency strategy workstream

Contributors

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Potency assay selection tool

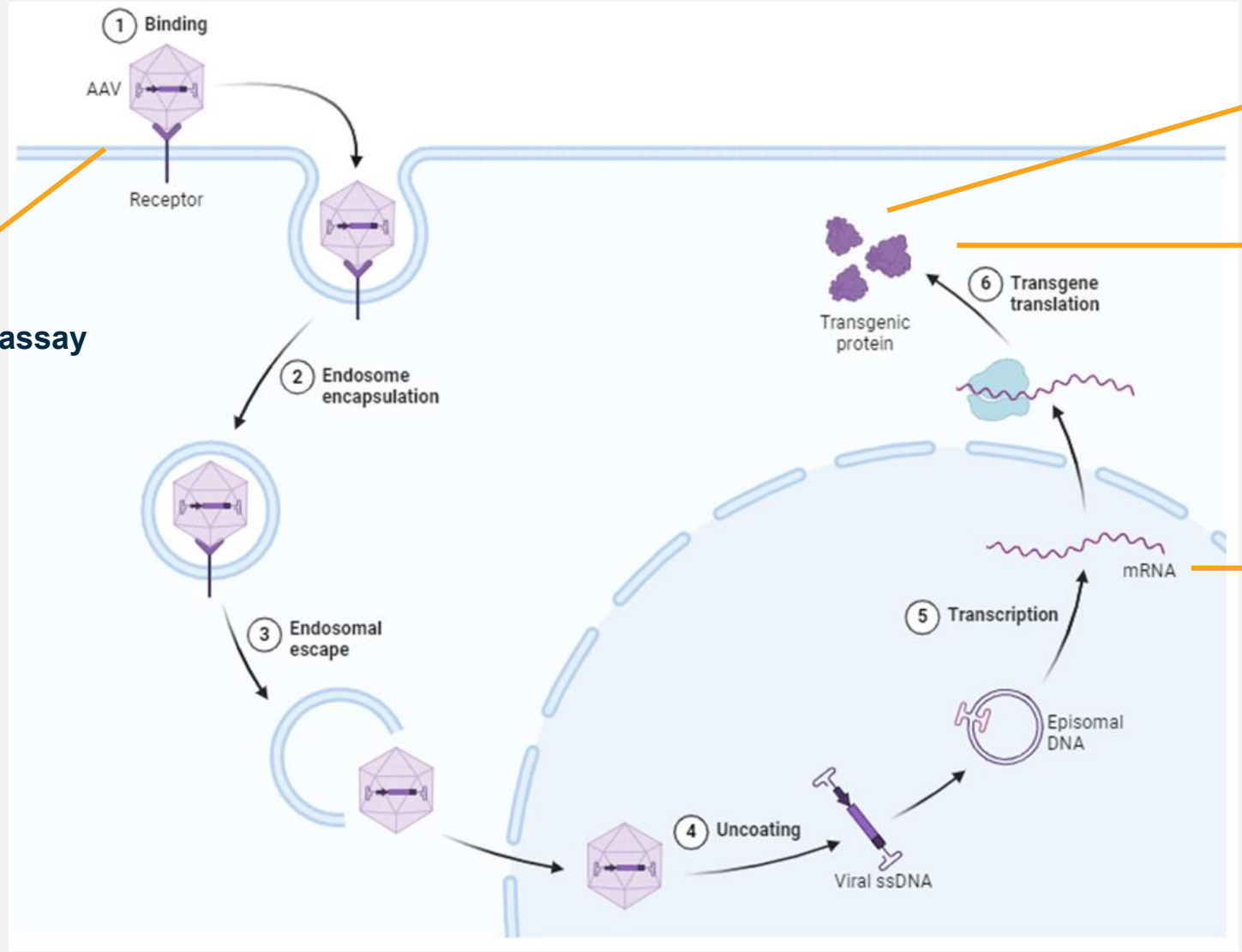
Present industry consensus view in an easy to interpret way

Designed to aid those new to the field

Interactive – able to consume at different levels of detail

Outline considerations when building a potency strategy

Monitoring of rAAV potency



Infectious titer assay
(TCID50)

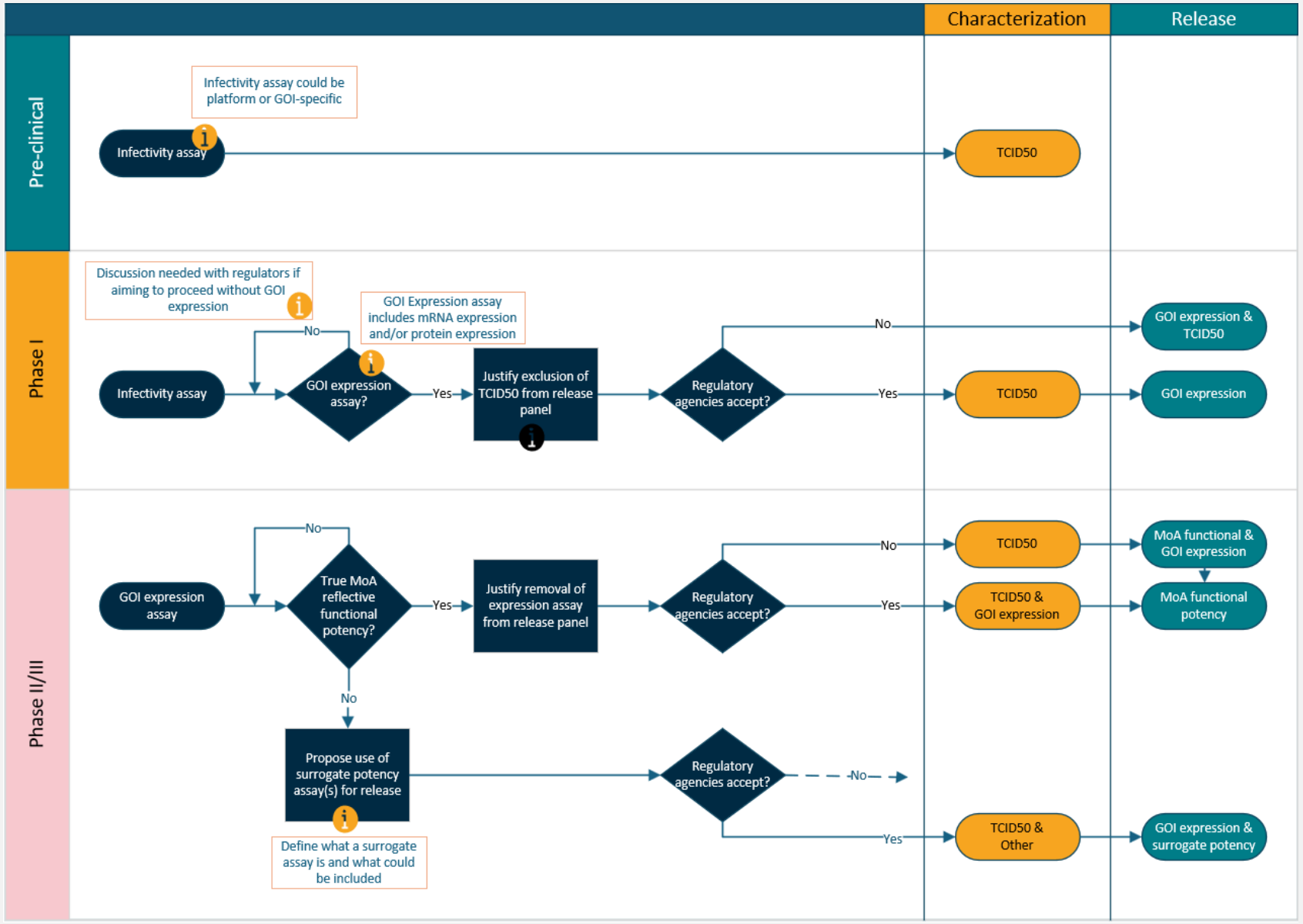
Functionality assay (MoA)
(Reporter gene, enzymatic activity,
proliferation, cell death, …)

Protein expression assay
(ELISA, Flow cytometry, WB, MS)

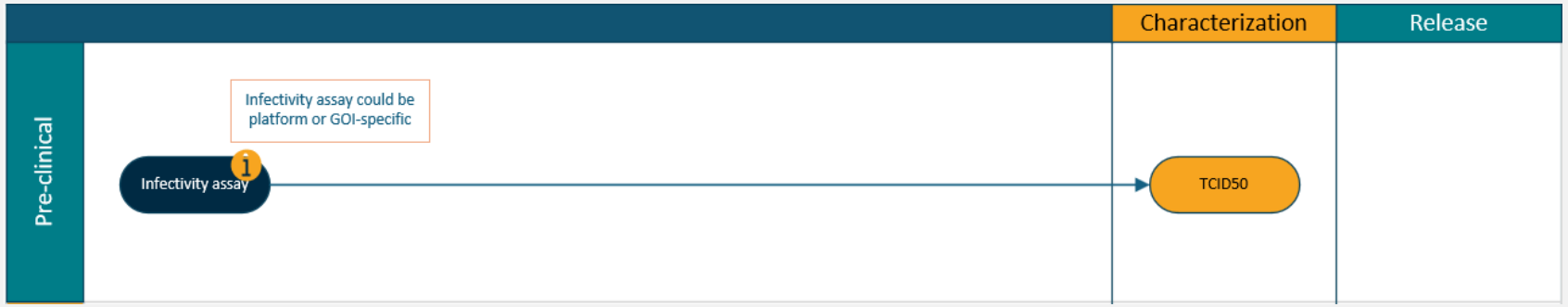
mRNA expression assay
(RT-q/dPCR, NGS)

GOI expression assays

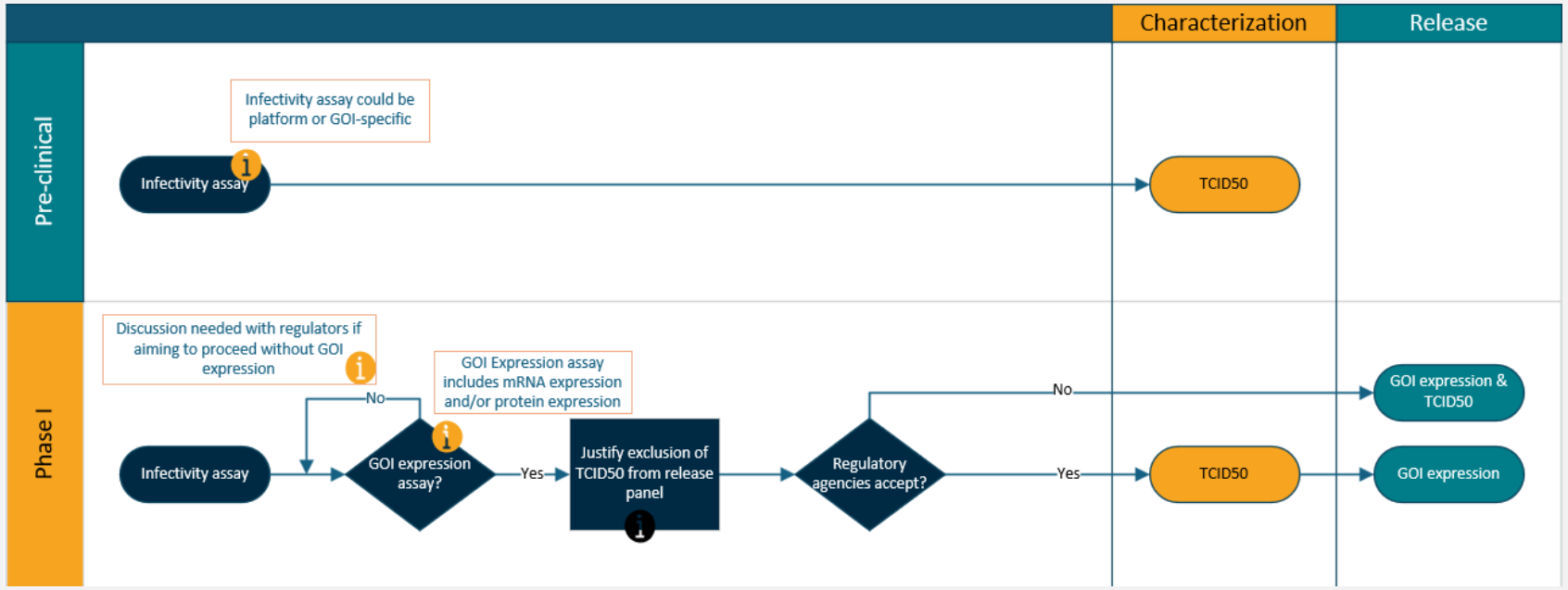
Phase appropriate potency assay selection – tool



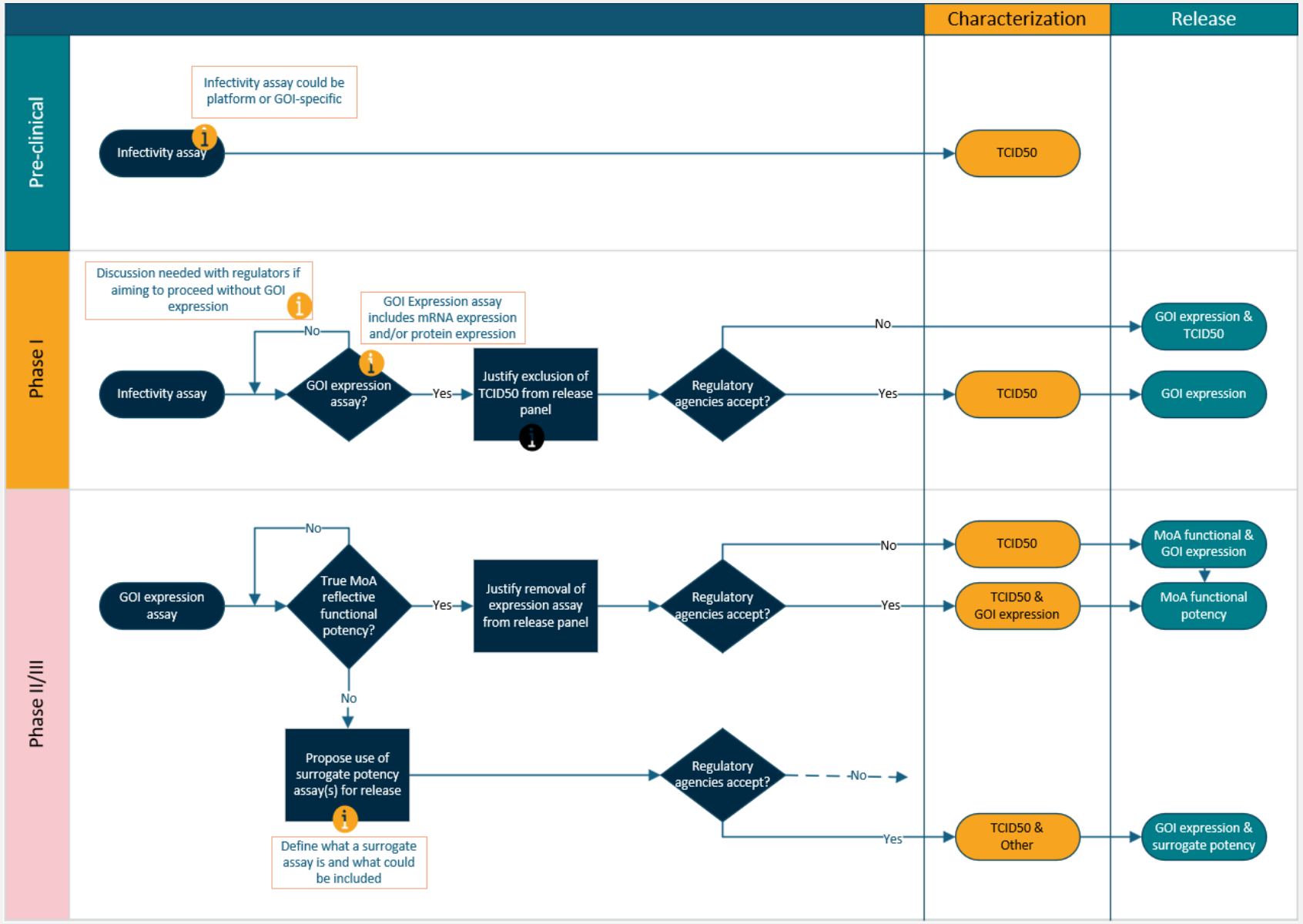
Phase appropriate potency assay selection – tool



Phase appropriate potency assay selection – tool



Phase appropriate potency assay selection – tool



Regulatory expectations regarding the infectious titer (TCID50)

European pharmacopeia 11.7:

General monograph 3186 - Gene therapy medicinal products for human use

- *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)*

Accompanying chapter: Additional information on gene therapy medicinal products for human use (5.34).

- *The infectious vector titer is determined using a suitable cell-based method. The infectious vector titer is within the limits approved for the particular product.*

- ***Ratio of vector particle titer/infectious particle titer** is within the limits approved for the particular product.*

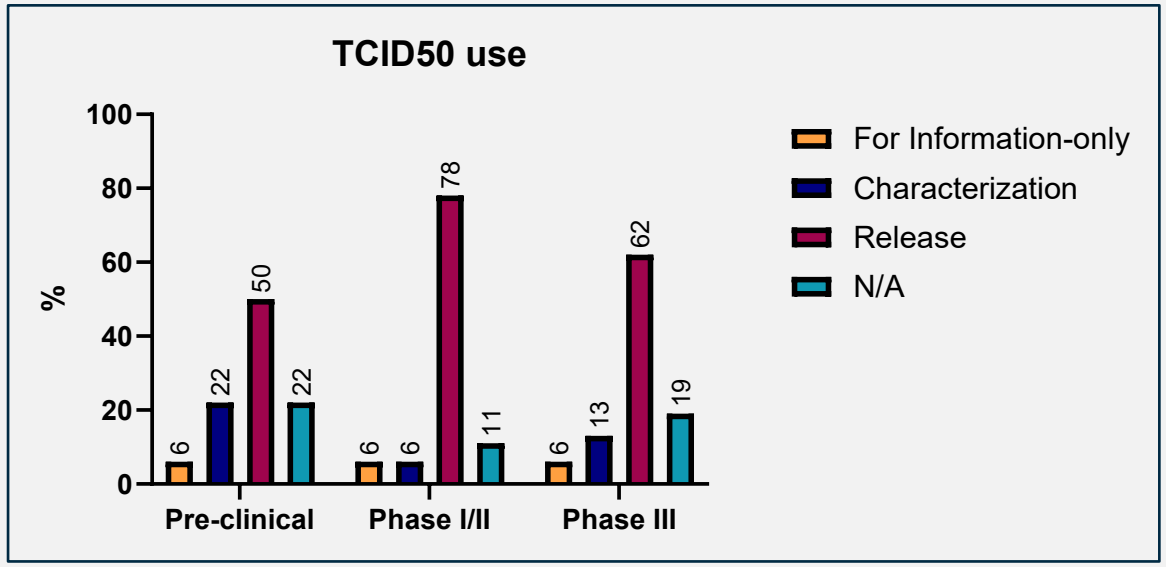
If Vg/IU = 1 = "perfect" vector (Zeltner et al. 2010)

An infectious titer assay is expected as part of the release matrix

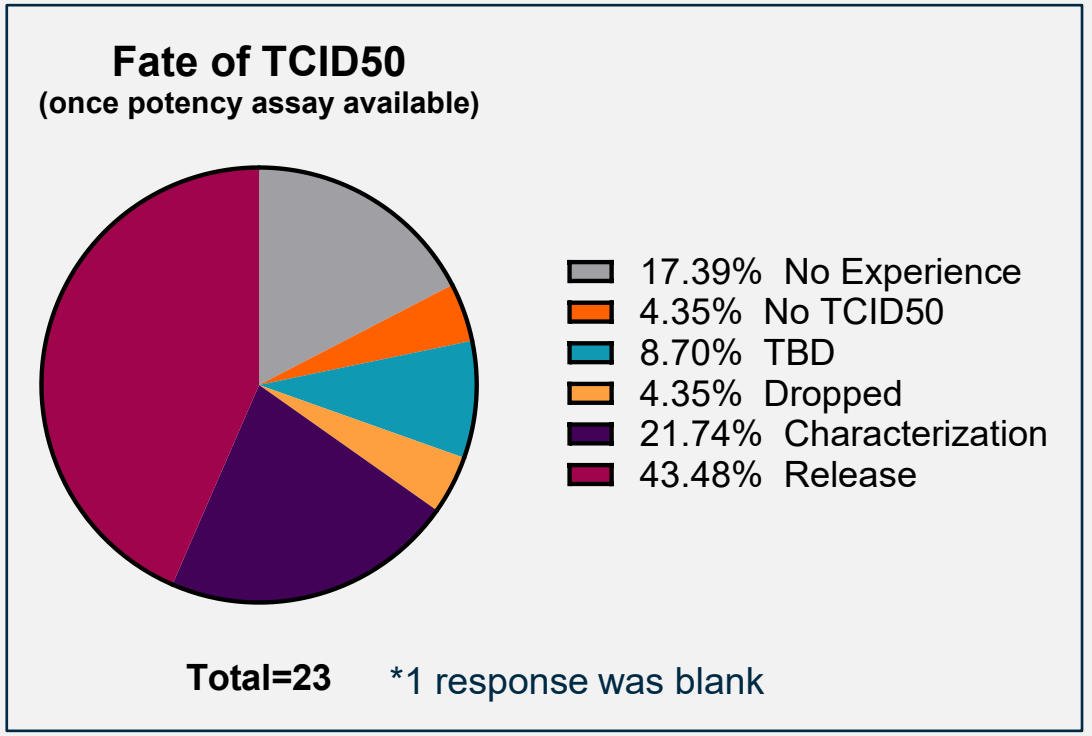
Zeltner, Nadja, et al. "Near-perfect infectivity of wild-type AAV as benchmark for infectivity of recombinant AAV vectors." *Gene therapy* 17.7 (2010)

The TCID50 – results from the BioPhorum survey

Potency strategy survey had 24 responses from 22 BioPhorum member companies



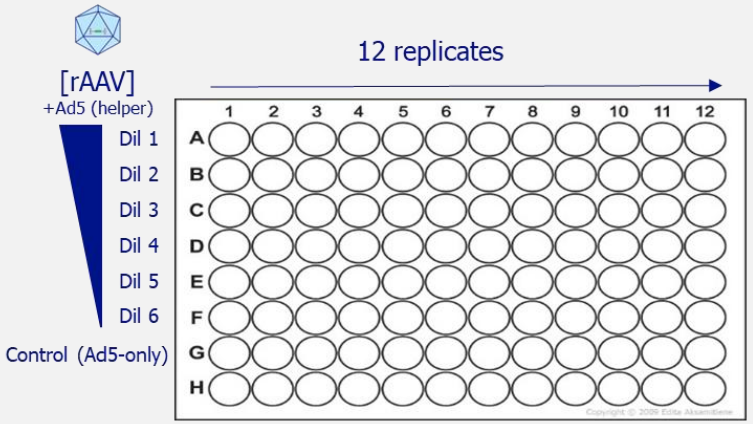
Data shows the 18 gene therapy responses



In majority of companies, TCID50 is part of the release matrix throughout clinical development

Challenges and limitations of the TCID50

Assay principle



- **Fixed choice of target cell line** (platform cell line expressing Rep/Cap) not representative of rAAV serotype tropism
- **Not representative of *in vivo* MoA**: the assay measures an artificial replication mechanism that is only induced in specific *in vitro* conditions and is not part of the product therapeutic effect
- **Low throughput** (one vector batch per 96-well plate)
- **High inter-assay variability**: extensive assay optimization and thorough operator training is required. However, even in best case scenario, inter-assay CV is still ≥35% (Duong *et al.,* Hum Gene Ther, 2023)

Inter-assay variability is the principal limitation

Justifications for removal of the TCID50 from the release panel

- High inter-assay variability: TCID50 unreliable for assessing quality, stability and manufacturing process consistency
- Accurate physical-to-infectious particle ratio challenging due to variability, so TCID50 results less reliable for product characterization
- Clinical dose is defined based on vg titer rather than infectious titer

  → Although the TCID50 assay can highlight potential concerns about a product, there are other methods that are more beneficial from a patient-centric perspective.

  → Control of non-functional vector-related impurities (e.g., empty capsids) is better achieved using other analytical techniques (e.g., AUC, MP, PCR/ELISA)

- Potency methods that measure expression or biological activity of the transgene are indicative of successful infection

  → New FDA draft guidance on potency assurance states that "(…) *if a later step in the chain of biological activities is completely dependent on the earlier steps, then a bioassay at the later step (…) will be sufficient"*

  → Supports the reduced reliance on the TCID50 assay for potency measurement and maintaining manufacturing consistency.

- Harmonizing requirements among different regulatory bodies (e.g. EMA vs FDA) regarding the necessity of the TCID50 assay for product release could help drug developers in efficiently managing resources.
- 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.

TCID50 may not be fit for intended purpose

Conclusion

- Development of a consistent and MoA-reflective potency testing strategy remains one of the key challenge of rAAV-based gene therapies.

- Members of the BioPhorum GT potency strategy workstream have benchmarked their experiences over the past months and discussed the challenges associated to the TCID50 and cell line selection.

- The upcoming position paper will summarize their consensus, offering a guidance from industry perspective on potency strategy considering phase, MoA, while challenging the need for an infectivity assay if a consistent and reliable GOI expression assay is available, as well as other assays to characterize non-functional vector related impurities.

Thank you

If you would like further information on any of the content discussed today, or are interested in joining the workstream, please get in touch:

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Q&A