



AMERICAN SOCIETY of
**GENE & CELL
THERAPY**

Committed to Research,
Education and Patient Care

Reference Standards for Cell and Gene Therapy

Boro Dropulić, PhD, MBA

Chief Science Officer and General Manager

Lentigen Technology Inc.
a Miltenyi Biotec Company

Overview

1. Introduction
2. AAV reference standards
3. Survey conducted with members of the ASGCT
 - Select ASGCT members involved in vector manufacturing/translational research
 - 10 questions. Ability to agree, disagree or undecided. Findings. Comments.
4. Summary

Introduction

- Reference standards are important tools for calibration of medicinal products
 - Types of reference standards – Internal Standards, Industry Standards
 - Purpose of reference standards
 - USP's volunteer CD34+ Cells Expert Panel
 - Standardizes a flow cytometric enumeration method to achieve reliable, consistent CD34+ cell counts
 - Definition of specific use is key to evaluating the need for, the type of, and the characterization of, reference standards
- Gene Therapy – direct injection and ex vivo gene modification of cells
 - AAV vectors (among others) have been used for direct injection gene delivery
 - Lentiviral (LV) and Retroviral vectors generally used for ex vivo gene modification of cell products
 - AAV is the active medicinal product – reference standards have been developed
 - In contrast, ex vivo LV use - is a critical raw material in the cell manufacturing process, and it is the gene modified cell that is the actual medicinal product
 - This distinction needs to guide the requirement for, and characterization of, reference standards that are being developed for the gene therapy industry

Recombinant AAV Vector Reference Standard Materials

Richard O. Snyder, Ph.D.
Philippe Moullier, MD, Ph.D.

Manufacture

Produce: Transient transfection (UF PGTC)
Purify: all-column (UF PGTC)
Formulate (UF PGTC)
Vial (ATCC)

Potter, M., Phillipsberg, G., Phillipsberg, T., Pettersen, M., Sanders, D., Korytov, I., Fife, J., Zolotukhin, S., Byrne, B.J., Muzyczka, N., Flotte, T., and Snyder, R.O. [BioProcessing J.](#) 7:8-14, 2008.

Characterize

Titer (Infectious, particle, capsid)
Purity (nucleic acid, protein)
DNA sequence
Serotype confirmation
Sterility/Myco/endo
Adventitious virus
Stability
Statistics

Titer units (Method)	Mean (95% confidence interval)
Particles per ml (ELISA)	9.18E11 (7.89E11, 1.05E12)
Vector genomes per ml (qPCR)	3.28E10 (2.70E10, 4.75E10)
Transducing units per ml (Green Cells)	5.09E08 (2.00E08, 9.60E08)
Infectious Units per ml (TCID50)	4.37E9 (2.06E9, 9.26E09)

Parameters Compared	RATIO
Particles : Vector Genomes	27.99
Vector Genomes : Infectious Units	7.51
Vector Genomes : Transducing Units	64.44
Particles : Infectious Units	210.07

Lock, M., McGorray, S., Auricchio, A., Ayuso, E., Beecham, J., Blouin-Tavel, V., Fátima Bosch-Tubert, M., Bose, M., Byrne, B., Caton, T., Chiorini, J., Chtarto, A., Clark, K.R., Conlon, T., Darmon, C., Doria, M., Douar, A., Flotte, T., Francis, J.D., Francois, A., Giacca, M., Korn, M.T., Korytov, I., Leon, X., Leuchs, B., Kroener-Lux, G., Melas, C., Mizukami, H., Moullier, P., Müller, M., Ozawa, K., Philipsberg, T., Poulard, K., Raupp, C., Rivière, C., Roosendaal, S.D., Samulski, R.J., Soltys, S.M., Surosky, R., Tenenbaum, L., Thomas, D.L., van Montfort, B., Veres, G., Wright, J.F., Xu, Y., Zelenai, O., Zentilin, L., and Snyder, R.O. Hum. Gene Ther. 21:1–13, 2010.

Availability of AAV Standards

Repository (ATCC)



Distribute (ATCC)

AAV2 (ATCC VR-1616)

AAV8 (ATCC VR-1816)

HeLa RC32 cells (ATCC CRL-2972)

pTR-UF-11 (ATCC MBA-331)

Protocols

<http://www.atcc.org>

Inter-institutional assay variability of AAV Standards

Variation between institutions for each assay despite the relatively tight correlation of assay results within an institution

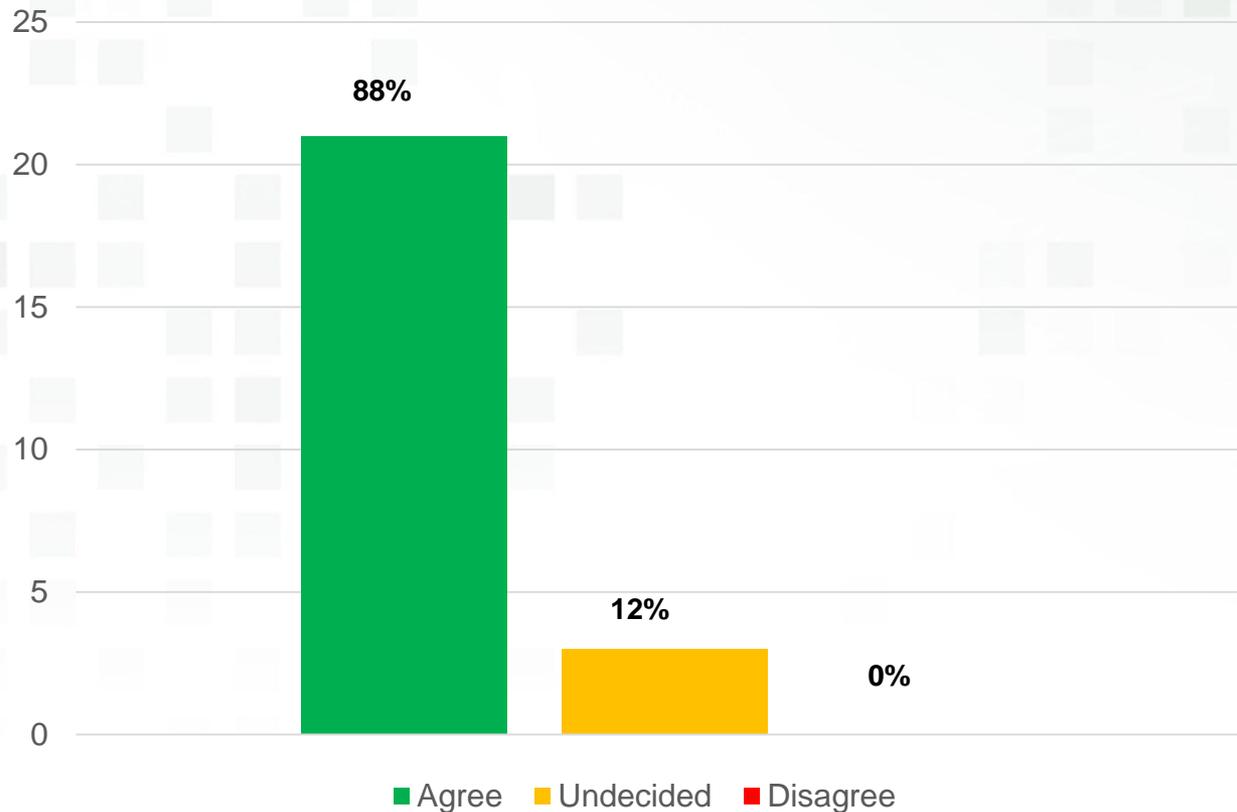
Relatively poor degree of inter-laboratory precision and accuracy was apparent even though attempts were made to standardize the assays by providing detailed protocols and common reagents

D'Costa S, Blouin V, Broucque F, Penaud-Budloo M, François A, Perez IC, Le Bec C, Moullier P, Snyder RO, Ayuso E.
Mol Ther Methods Clin Dev. Mar 30;5:16019, 2016

Survey

- ASGCT members
- Vector manufacturing, clinical translational research
- N=24
- 10 questions
- SurveyMonkey

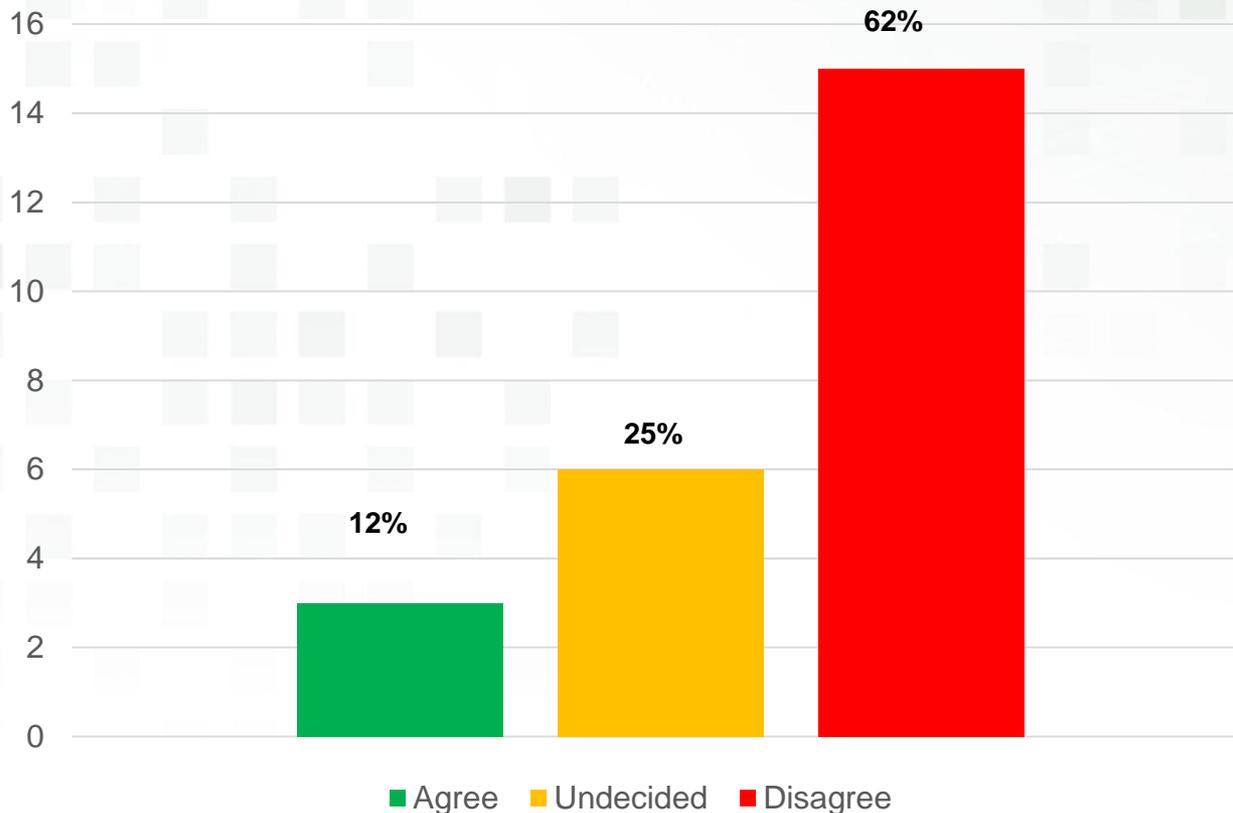
1. I think reference standards are important for the development of Gene and Cell Therapy Products



1. I think reference standards are important for the development of Gene and Cell Therapy Products

- For any given (external/universal) reference standard, manufacturers should agree on a need/ use for the standard. specifically a set of tests that are important to compare results across labs.
- Reference standards have proven to be of limited value but a discussion of possible standard in light of current progress in CGT products would be useful.
- Early establishment of references is very important. It is likely that bridging to new reference materials will be required over the course of clinical development, but never too early to establish the first iteration.
- The major caveat is that until, the field is settled down the standards can rapidly become irrelevant, or even constricting. An example is the AAV2 standard, which at the second attempt was put together correctly, but I'm not sure that it has much relevancy today.
- High priority
- But only for agents that are beyond Phase I

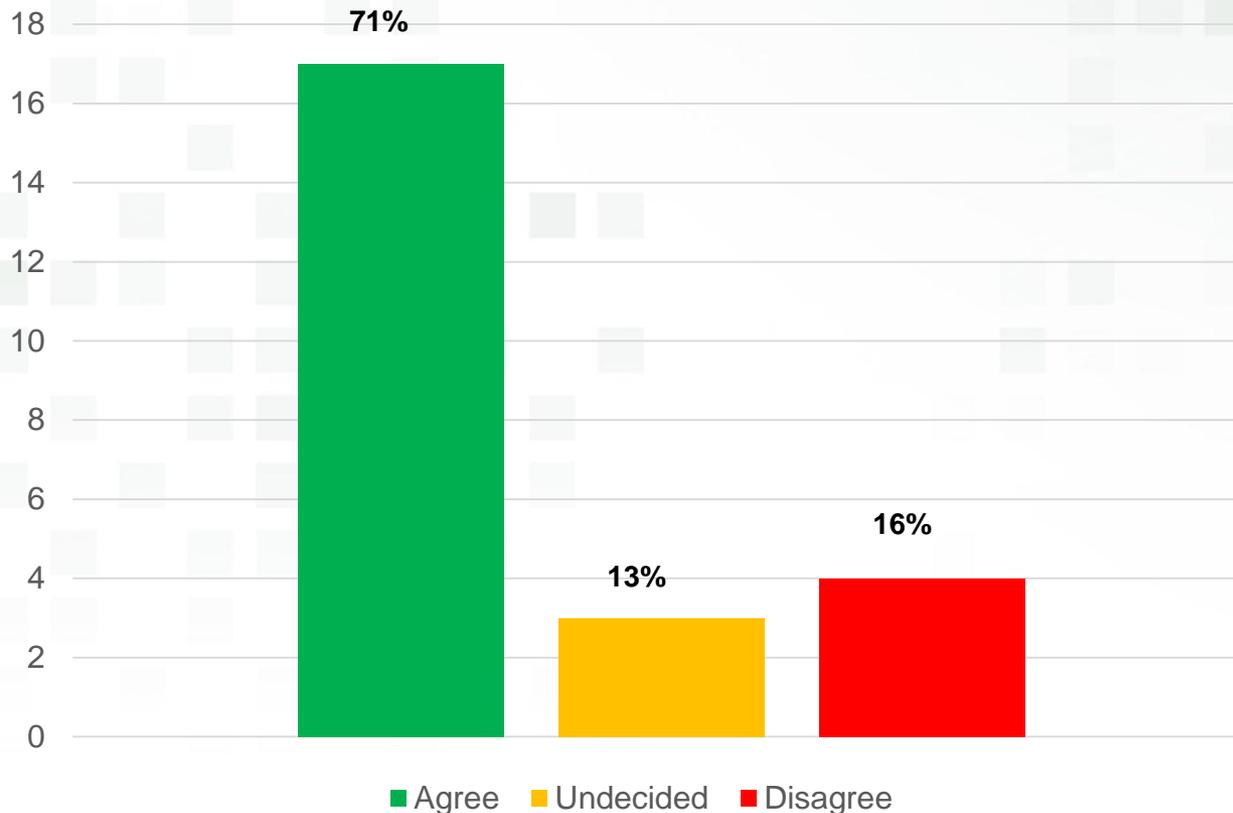
2. I think reference standards are a potential hindrance to the development of the Gene and Cell therapy field



2. I think reference standards are a potential hindrance to the development of the Gene and Cell therapy field

- If developed poorly they may restrict innovation or cause hardship for start-up or academic investigators
- For the most part, reference standards have neither broadly helped or hindered the development of the field. 10 years ago, Ad standards benefited emerging manufacturing sites (mostly academic), but most such [competent] facilities have developed their own standard.
- I think it is worth a detailed and thoughtful discussion, about what needs to be in place before the effort and \$\$ to make a standard are worthwhile.
- Appropriate standards would not be a hindrance but they will have to have specifics for different gene and cell therapies.
- I think reference standards make sense for agents beyond Phase I, but standards would hinder innovation in pilot and phase I studies
-

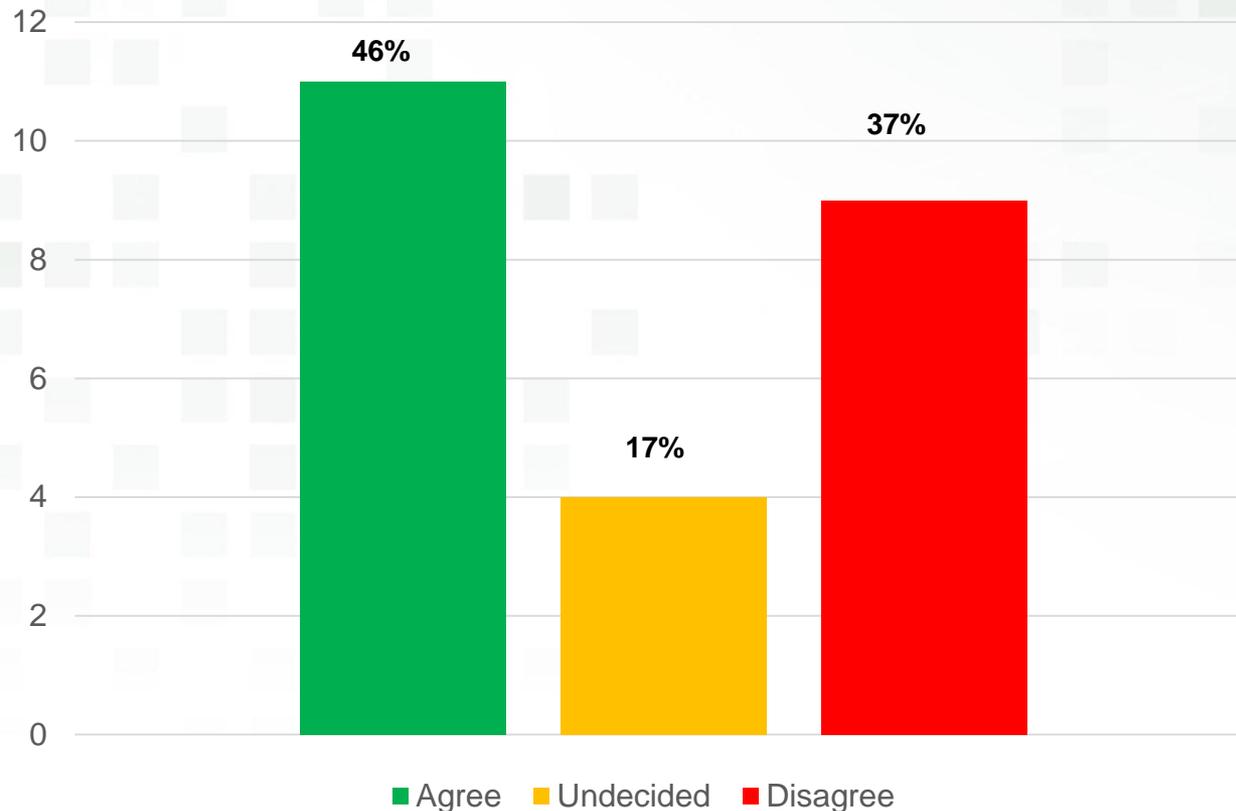
3. I think reference standards are essential for the commercial development of gene and cell therapy products



3. I think reference standards are essential for the commercial development of gene and cell therapy products

- Each manufacturer must create their own product specific internal reference standard for product development and commercialization. **It can be helpful to create a universal reference material to compare certain tests across laboratories/manufacturers, particularly for tests more standardized.**
- We have not had these standards as of yet and are doing well. this is more pressure from big pharma and from (eventually) payors for these therapies. So they are not "essential" but they are coming and we need to play a leading role.
- **Not at all. Each product is regulated on it's own merits, based on the developers own reference standards.** Different manufacturing processes and different applications of "common" technologies, such as CAR-T or lenti, are not readily compared between developers.
- In the long run they will be useful, but they are not currently essential, and see comments above
- Not for commercial development, but commercial approval.

4. I think reference standards need to be product specific and it would be difficult to create reference standards suitable for the industry as a whole



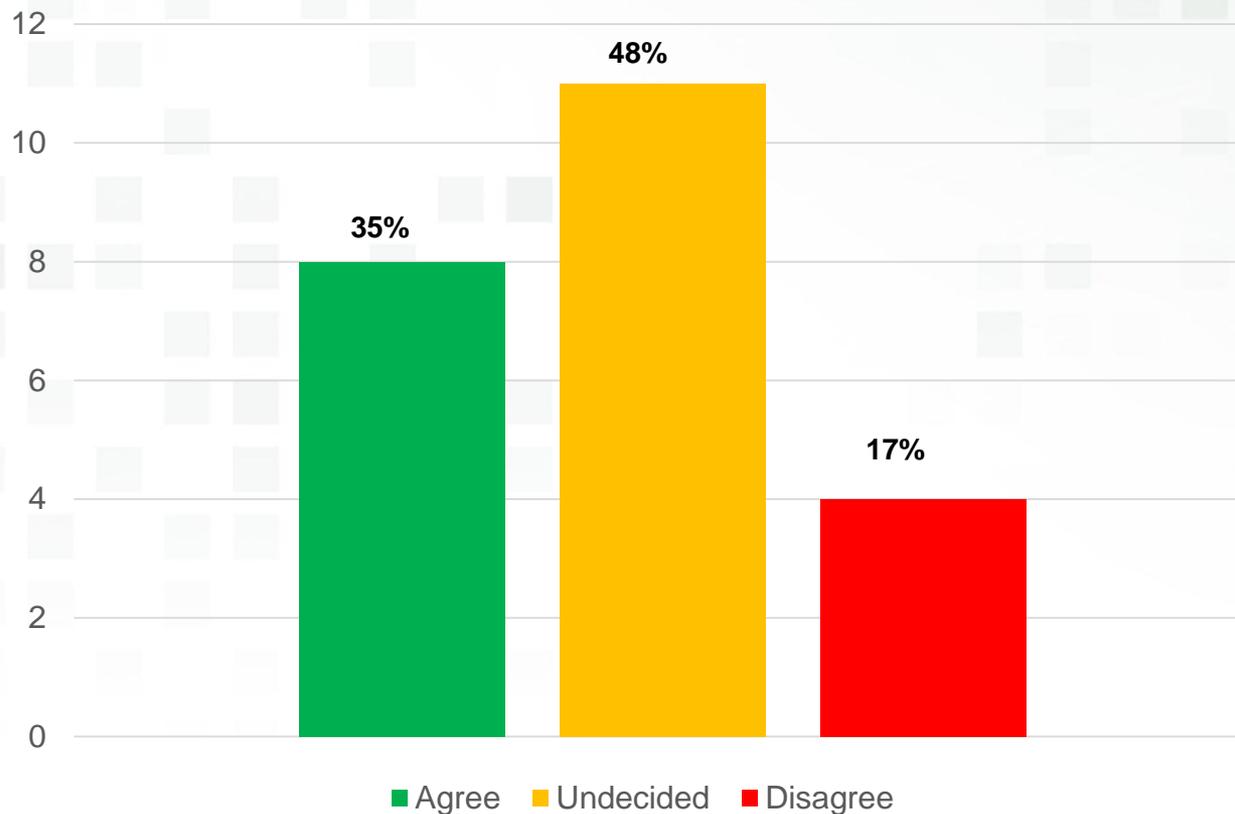
4. I think reference standards need to be product specific and it would be difficult to create reference standards suitable for the industry as a whole

- In many instances, product specific
- I think they are vector-specific, but not product specific. For example, a standard for AAV vectors would be very useful regardless of the transgene being delivered. This may not be true for all therapies though.
- Each manufacturer must create their own product specific internal reference standard for product development and commercialization. it can be helpful to create a universal reference material to compare certain tests across laboratories/manufacturers, particularly for tests more standardized. the key is for labs to agree on specific tests that really can benefit from a standard. such a standard may not be a vector, but might be a critical reagent used in a assay that is problematic for the field.
- Reference standards should be crafted specifically to be general.
- Absolutely.

4. I think reference standards need to be product specific and it would be difficult to create reference standards suitable for the industry as a whole

- I would say it is generally the case that certain reference materials are more product specific, some can be more generic (e.g. a generic rAAV2 reference material may be useful for vector purity assessment, but not potency assessment).
- they do not need to be product specific.
- For a commercial product yes, but does anyone know how to make and reproduce such standards? I think that is unlikely to be the case
- There are some standards that maybe product specific but there are clearly standards that can be broadly applied
- This is somewhat true, but there are assays that would benefit from including a reference standard without having to be product specific.
- Strongly agree.
- The gene therapy field is too diverse for single reference standard

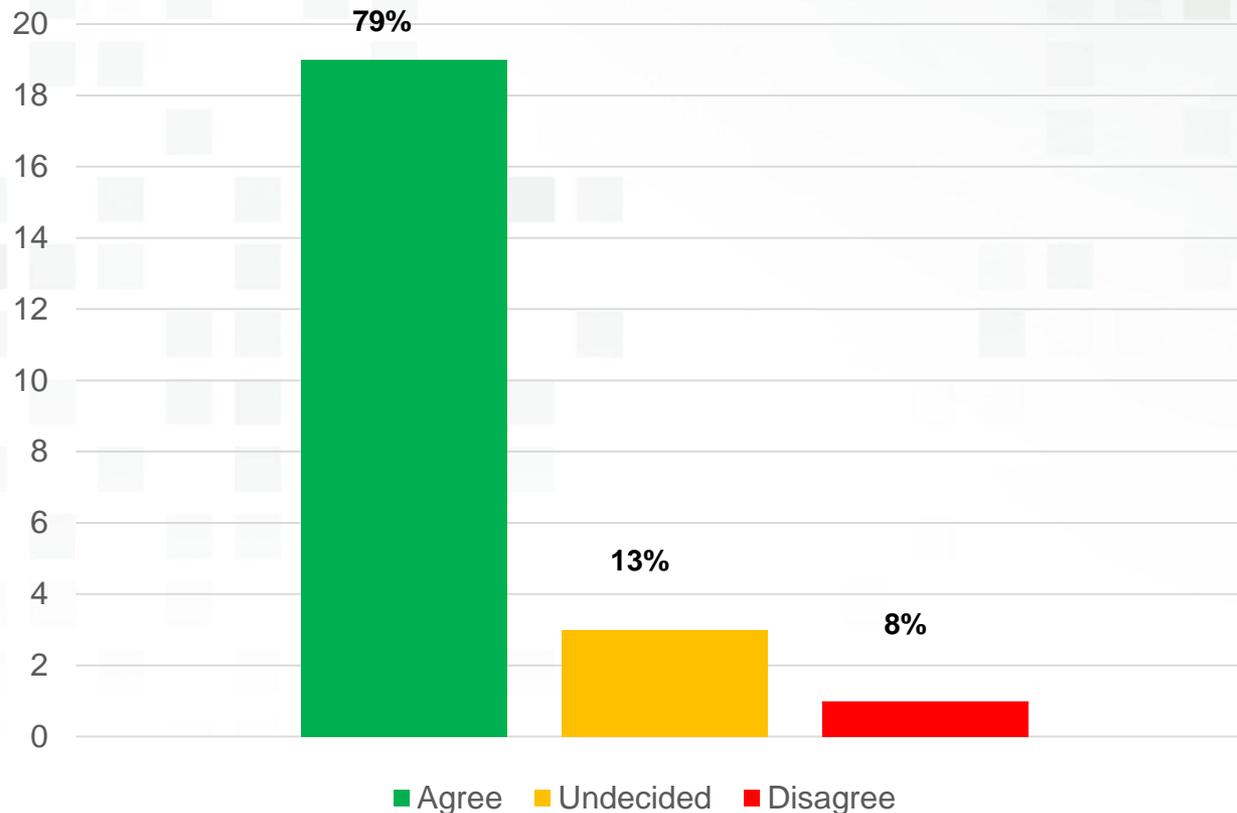
5. I think the AAV reference standards have been useful for product development



5. I think the AAV reference standards have been useful for product development

- They simply have not been used that much, and probably need to be prated. Protocols are also needed.
- very few labs are using this material. challenging for AAV to create a useful standard as serotypes vary in their in vitro and in vivo properties. for example tittering in cells lines will be different between AAV2 vs other serotypes. really need to define tests that can benefit from a universal standard.
- I have not directly used them
- Not for me but I don't make AAV products. Ask about the retroviral and Adenovirus standards as well.
- To some extent. However, the AAV2 RS was unfortunately tainted with Myco positive results that prevented is dissemination. Plus, it is difficult to obtain it in large quantitates from ATCC.

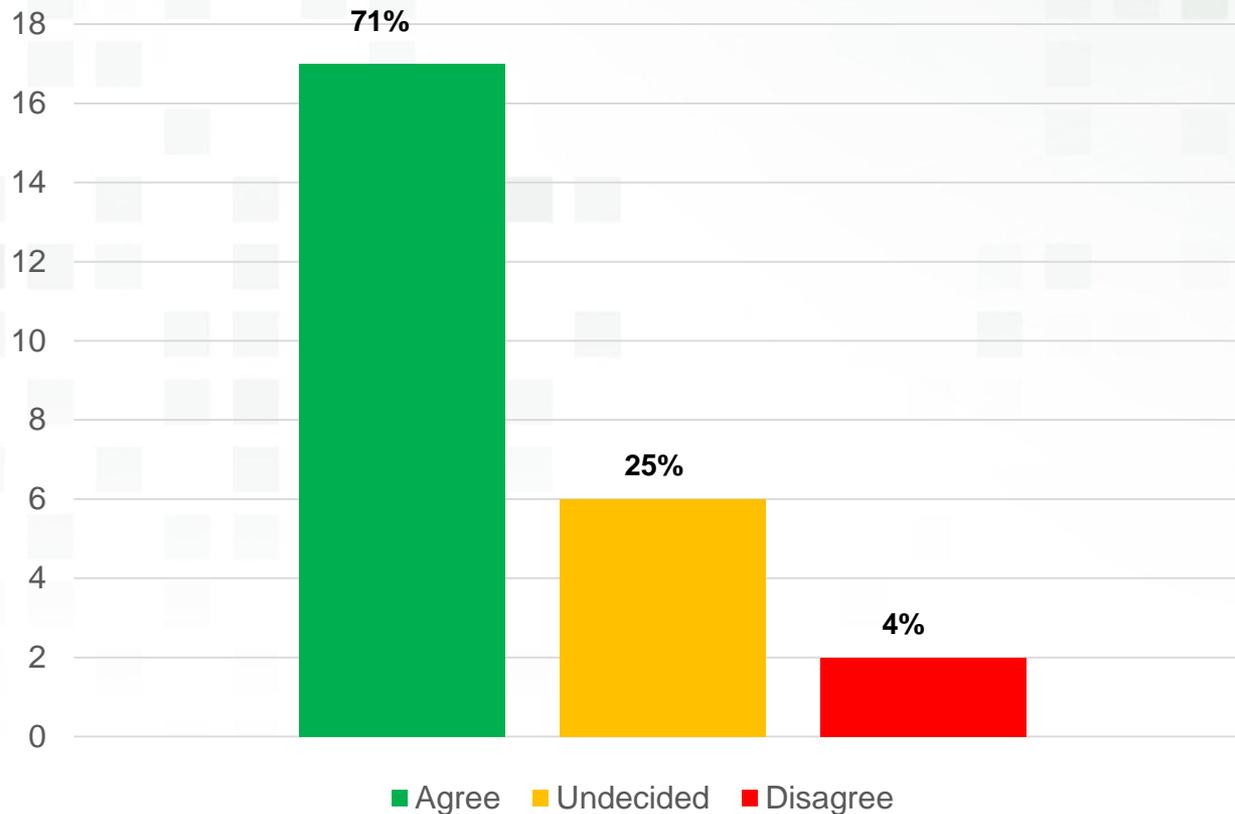
6. I think it would be useful to develop a Lentiviral vector (LV) reference standard



6. I think it would be useful to develop a Lentiviral vector (LV) reference standard

- if you want to propose a standard, you should identify tests that will benefit from having this standard. for example, the gammaretroviral standard is used for RCR assay qualification.
- The call from pharma and payors is clear, LV needs some type of standardization
- As a leading manufacturer of lentiviruses, I see no value in a reference standard.
- Probably too soon still. The manufacturing process is still pretty primitive.
- yes, especially if acceptable titer levels and shorter versions of RCL testing and/or foregoing RCL testing become possible.

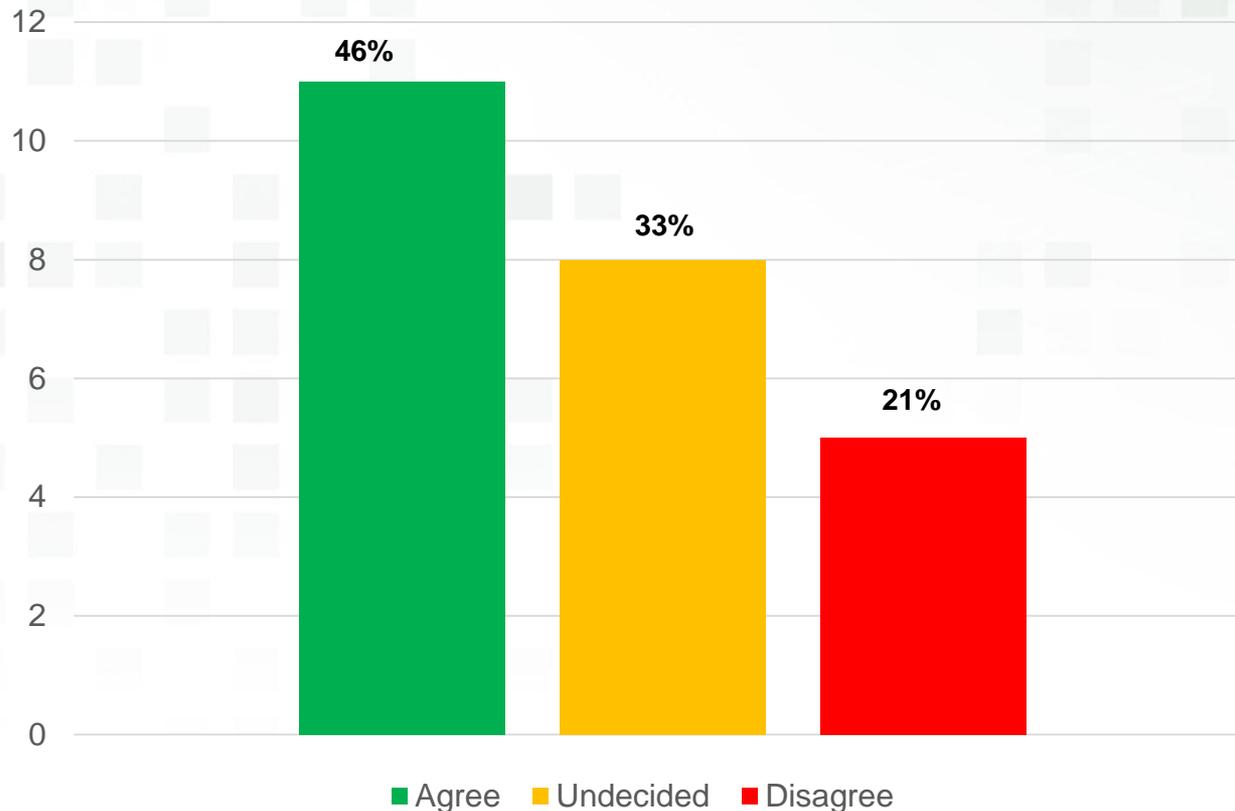
7. I think it would be useful to develop cell line standards with defined vector copy numbers



7. I think it would be useful to develop cell line standards with defined vector copy numbers

- Yes, for lentiviral transduced lines.
- A validated reference standard would allow for the level of gene introduction, and the process by which it is measured to be standardized
- I think drug developers need to consider establishing their own relevant reference standards for their products. I would be less interested in another groups standards, or what the "consensus" standard might be from select group of "users".
- Yes. Tricky project, could end up pretty product specific

8. I think specific cell product reference standards would be useful e.g. a T cell line expressing an anti-CD19 CAR



8. I think specific cell product reference standards would be useful e.g. a T cell line expressing an anti-CD19 CAR

- The challenge is even if a cell line is developed, is it stable and is it applicable amongst different institutions and companies?
- I think you need to specify how this standard would be used. what data will be generated that can be used to help the field.
- This is too product specific. And the real test for a CD19 CAR is potency. A more general reference standard would contain integrated copies of features common to many LV. If however, the desire is to standardize the expression of CD19 on the surface of transduced T cells, we need a universally available flow cytometry reagent.
- Manufacturers and developers need to control internal processes, not compare to other groups with similar but not identical processes.

9. Are there any standards not listed that you would like to see developed?

- What would be of value is USP-like assay or specification standards. While such specifications and methodologies might benefit from some reference standards, the key is in normalizing measures of potency, purity, titer, etc.
- I would say that references for common rAAV and rLenti pseudotypes are justified.
- Mesenchymal Stem Cells
- A common data platform for Raw Material Specifications, Key Intermediates and Final Product Specifications to be shared (generically) would be quite helpful to the industry at large.
- More virus reference standards such as HSV1 and VSV.
- Cell lines for TCID50 assays for AAV of various serotypes
- clinical administration standards and medical management of toxicities (administration of anti-cytokine, which ones, use of steroids, use of drugs that trigger suicide gene).
- AAV, manufacturing standards for intermediates.

10. Do you have any other comments regarding the utility of reference standards for product development and commercialization?

- just need to come to consensus/ be specific about how the standard should be used.
- I think this is a very worthy topic for a broadly inclusive working group discussion, and I would be open to hearing others' opinions and suggestions, but my experience with previous standards (having sat on several working groups) is that they are difficult to define and used much less than the proponents of the standards anticipated. As an example: in over 15 years of cell and virus production my group never once felt obtaining some of the available Ad or AAV standards was useful.
- The questions here seem to present the need yes or no of a single national / international reference standard. I would recommend clear definition of International Reference Standard vs in laboratory reference materials.

10. Do you have any other comments regarding the utility of reference standards for product development and commercialization?

- I think reference materials depositing would be useful, but unless required by regulation unlikely to happen for innovative products initially. I looked in the NIBSC catalogue, and there are significant commercial holes (e.g. no MMR deposit for example as far as I can tell).
- Reference standards for use comparing transgene expression - for standard curve generation, assay protocols
- If the goal of "Quality" is to minimize variation then these standards are a reasonable effort (in part). But, the more significant variable is the biology of the recipient system - beyond the production cell line, vector, plasmid or infectious viral construct. For example, we need to focus our efforts on understanding the predictive variables (and assays) of an effective immune response and or side effects for each CAR-T patient as those dwarf cell product "variation".
- Not at this time. This requires a longer discussion with key players and sponsors.

Summary

- In general, members agree that reference standards are a good idea
- Development of such reference standards and their standardization with different laboratories is difficult
- Wide opinion on types of reference standard needed
- Reference standards need to have a clearly defined and limited purpose
- Development of reference standards that are widely acceptable among all stakeholders is very important
- There will need to be significant discussion among multiple stakeholders to gain consensus on the development of reference standards

Acknowledgements

Richard Snyder – AAV reference standard slides
ASGCT members that participated in the survey – Thank you!