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SPOTLIGHT

Gene therapy analytics and CMC

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CONTENTS VOLUME 11 · ISSUE 3

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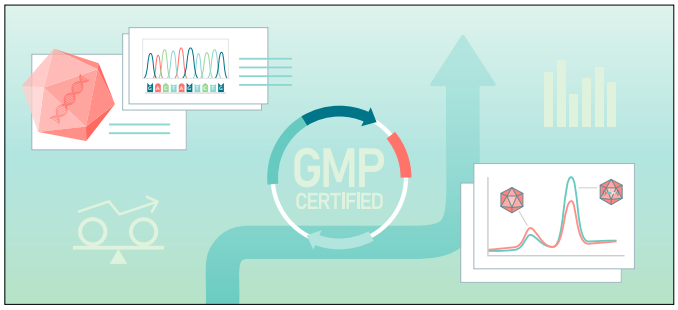

CELL & GENE THERAPY INSIGHTS

GENE THERAPY ANALYTICS AND CMC

SPOTLIGHT

EXPERT INSIGHT

A structured approach to addressing leachable risk in cell and gene therapies

Jason Creasey

The purpose of this article is to demonstrate the importance of a structured approach to the determination of leachable risk within a given cell or gene-based therapy (CGT). These drug product types offer a unique challenge in that it is impractical to make leachable measurement directly in the final drug product. A 'simulation' of leachable present needs to be made. The suitability of that simulation is dependent on knowledge and understanding, and this article will offer an opinion on how this is best achieved through a structured process beginning with process mapping and the development of a risk assessment process. In this process, leachable risk is identified, scored, evaluated. Then experiments are designed to map the risk to achieve risk mitigation via mechanism which include simulated leachable studies. Using such an approach not only is the leachable risk addressed but it provides a mechanism to better ensure the testing done best represents accurately the true risk of leachables in CGT.

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Cell and gene-based therapies (CGT) [1–4] offer a significant challenge in providing appropriate regulatory information for their approval when it comes to looking at drug product leachables. The term leachable and the associated term extractable [5] have been defined by a variety of groups and regulatory agencies. The term leachable has been defined by USP in its guidance chapter <1663> [6] as foreign organic and inorganic chemical entities that are present in a packaged drug product because they have leached into the packaged drug product from a packaging/delivery system, packaging component, or packaging material of construction under normal conditions of storage and use or during accelerated drug product stability studies. Also, the term PERL (process equipment-related leachables), is mentioned in USP chapters such as USP <665> [7,8], to add to the lexicon. The term leachable has been previously used in many recommendations and guidelines from organizations ranging from regulators such as the US FDA [9] and the EMEA [10] and by consortia such as PQRI

[11–13] and ELSIE in their whitepapers [14,15] and other output [16–20].

I would offer these definitions:

Leachables are elements or substances that are present in the packed drug product because they migrated, washed, or leached from manufacturing materials components, packaging or delivery devices during the product drug's manufacture, storage, or delivery to a patient.

Extractables are elements or substances that are detected, identified, and sometimes quantified from manufacturing materials, packaging materials or delivery materials associated with a drug product as a result of designed experiments (extractable studies). These studies are conducted under pre-defined conditions of extraction temperature, extraction time and extraction solvent, plus other relevant parameters which may vary according to purpose of the study.

Extractable study purpose should be clearly defined, designed, and matched to requirement. One such purpose being simulated leachable studies. This means that simulated leachable studies produce simulated leachables and these simulated leachables can then be safety risk assessed with the assumption that their identity and exposure levels are not significantly different from actual leachable in a CGT drug product.

Thus, these definitions and the difference between extractable and leachable study forms part of this manuscript since it should be obvious that CGT drug products do not allow for direct measurement of a leachable.

The primary reason is the potentially unique mechanism via which drug products in this category are manufactured. CGT will utilize autologous or allogeneic donation as the basis for the therapy. Particularly (but not completely) in autologous donation the formation of the drug product only occurs when the patient's own cells are harvested, treated, then re-infused. Thus, conventional leachable testing would see drug product (patient's donated cells) used not for treatment but for chemical analysis, which cannot be justified; given the likely low risk that leachables will represent a significant safety risk, and their negative influence on the accuracy of the deployed methods.

Therefore, the widely accepted alternative is the design and testing of simulated leachable samples. This paper seeks to outline how this might be best approached via a highly structured process, where risk from potential leachables is identified, assessed, scored then evaluated to determine the design of (extractable) studies including a test of simulated leachable samples [21].

LEACHABLE RISK

Let us first look at risk in general terms, then see how this might be applied to leachable risk. ICH Q9 (R1) [22,23], provides a harmonized guideline on the study of quality risk management. Within this guideline it offers both definition and principles of risk management.

It offers two guiding principles, which should be considered for leachable risk management. These are:

- The evaluation of risk should be based on scientific knowledge and ultimately linked to the protection of the patient
- The levels of effort and formality and documentation of risk management should be commensurate with the level of risk

It offers statements on the formality of the risk management which should apply and three areas where this is particularly important:

- A complex area
- An important area
- Where there is uncertainty

This suggests that, when an area has these attributes, a formality in risk management activity is increasingly important. It also offers the overall definition of risk to be a combination of a severity of harm (the consequence) and probability of occurrence of harm (likelihood). So, combining these guidelines, it can be expressed for leachables as:

Leachable Risk = (Severity of Harm/Hazard, including any uncertainty) × (Probability of Harm, including any uncertainty)

Uncertainty should be included in both terms above, and as illustrated can significantly affect the outcome in terms of risk scoring. It might be considered the detection term in a failure modes effect analysis (EMEA) [24].

Hazard identification and Harm have been linked together here, since the overall hazard is a safety hazard to patients taking the CGT, but in order to better understand and define this, it is important and necessary to identify all the sources of that risk.

Thus, the first step is risk identification (how and where the risk occurs). In this step all potential contributors to leachable risk must be surfaced. The suggestion of how to do this is linked to the tool which seems best suited to provide a structured approach to this step and the further steps of risk analysis (scoring to provide an input into risk evaluation) and risk evaluation (sorting and comparison against a risk criterion). All these terms are broadly aligned with definitions provided in ICH Q9. However, to make them applicable to leachable risk some further lower-level definitions will be required.

RISK IDENTIFICATION

This for CGT application is possibly the most complex and time-consuming aspect of the process. As mentioned above, the tool used here to best effect is FMEA. Each material in use in the manufacturing process for the drug product must be considered, this consideration should map the whole process from beginning to point of delivery to the patient. Clearly this means that it is highly linked to methods of collection, transfer, manufacturing, and delivery. Some cell-based therapies may share common approaches and processing steps, for example, the high-level steps illustrated in Figure 1.

But other systems may differ, for example gene therapies which manufacture a specific protein, or mRNA vaccines. Regardless of the process steps, these need to be clearly defined, together with the sub-process activities within them (Figure 2).

Each of the sub-process steps will have an associated set of equipment which is used to enable the step. Each of these materials, if in contact with the process stream, could be a source of potential leachables due to the materials of construction, so both the equipment and the materials of construction must be listed as part of the risk identification step.

The FMEA format can assist with this, as the failure mode is the risk identified. Each line entries will be formatted as in Table 1.

FIGURE 1

Typical process steps in cell-based therapies

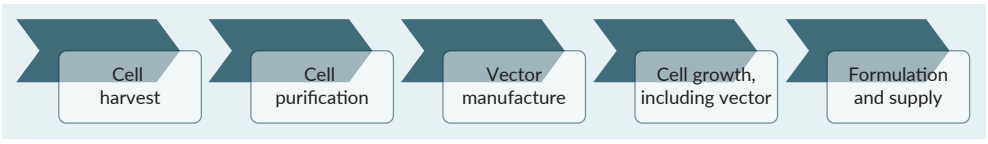
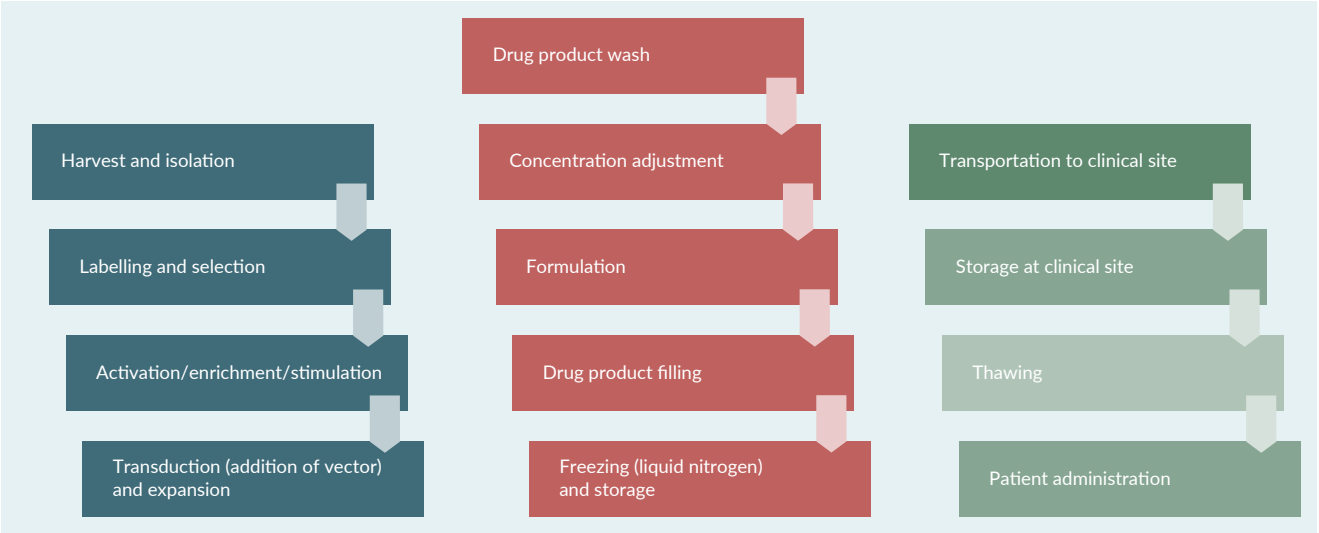


FIGURE 2

Potential sub-process steps.



Risk identification is complete when every material in contact with the process stream up to and including delivery to patient has been listed in the format illustrated in Table 1.

The next step to assess the leachable risk is risk analysis. This risk analysis takes the risk identified and assigns a two-part score. Score one is aligned with severity of harm, score two the probability of that harm.

Both these scores are linked to available knowledge. That knowledge is two tiered in nature. The higher of the tiers relates to knowledge of the material. The lower tiers are linked to substances within the material, these substances when removed from the material during studies were defined as extractable or leachables.

As mentioned in the introduction, the difference between extractables and leachables is the subject of another set of definitions. Leachables are actually a little easier to define. These substances being those present in the drug product (in the case of CGT leachables are not readily directly detected). Extractables are those substances which can be removed (extracted) from the material as a result of a designed study, as such the extractable found in a given material can vary both in identity and quantity depending on the design of the study. Designs which deploy harsher conditions (solvents, time, temperature) give rise to more extractables.

For CGT therapy, what we will eventually do is to create an extractable study (containing extractables), but one designed to simulate leachables.

SEVERITY OF HARM SCORING

Thus, to score severity of harm you assess whether the given material identified in risk identification, has a high or a low score against the criteria set (relevant to severity of harm). Table 2 illustrates the suggested traits which should be used, as mentioned above these traits are two-tiered and both should be considered [25–40]. Traits linked to level two (extractables) being given more weight, as they are more directly relevant to leachable risk.

As can be seen, this scoring requirement includes collection and establishment of various types of knowledge. Absence of these items is more likely to generate higher scores, but the highest score is only given when there is evidence of a safety concern.

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TABLE 1

Where is the risk and how does the risk occur?

Risk event ID	Process step	Process step subcategory	Material/leachable source
CH1	Cell harvest	Harvest and isolation	Bag used to collect harvested cells
Cause	**Risk event**		**Effect**
Because of ...	...there is a risk that...		...resulting in...
Process stream contact with the bag	substances migrate from the material of the bag		Substances in the bag transfer into the final DP at unsafe levels, which could then be dosed to patients

Let consider the risk outlined above, during cell harvest a bioprocessing bag is used to collect and temporarily storage cells. The attributes that direct the severity of hazard can then considered in the following manner:

One possibility is no information is provided by the vendor of the bag. As seen from the suggested scoring this would result in the default scoring of 7. When that is eventually combined with the probability score a score of this magnitude may well prompt the need for more study. Therefore, there is a vested interest in seeking out more information which is the intention of weighting in the scoring. As illustrated in Table 2, the aim is to collect information which speaks to the level of possible hazard which could be present. In this example, that links to both the characteristics of the bag which might either point to directly or indirectly to biocompatibility (e.g., bag having been tested to a recognized standard USP <87> [27], USP <88> [28], Food certification against CFR [38] or EU regulation [40]), or to the extractable found within the bag. The weighing of the information should then be related to the risk identified. Also, this would mean extractable information would be given the largest weighting alongside biocompatibility results. A failure in biocompatibility would be a typical reason to raise the hazard severity score to 10, as would identification of an extractable of safety concern when found at a level relevant to product use. The converse is the reasoning behind a lowering of the score from its default position.

PROBABILITY OF HARM SCORING

The scoring of probability of harm takes a similar approach to scoring of severity of harm (see Table 3). Four scoring levels are defined. Although not typically available, the scoring scheme illustrates how simulated leachable data would be used, and simulated leachable data may be available from other projects and justified for use. Once again, the uncertainty is a factor in the scoring and availability of simulated leachable data removes that. However, what is more typically available at the point of risk assessment is the use of conditions which influence the leaching of substances from the material within the assessment. These 'probability factors' have been defined as:

- Temperature
- Time
- Surface area contact (between leachable source and leaching media/drug production formulation)
- Nature of leaching media
- Compatibility of the source material with drug product formulation

TABLE 2

Criteria and scoring for severity of harm [25–40].

Score	Description	Example safety-based criteria
1: low severity of harm (certain)	No identified harm to patient safety	Material/component is well characterized, and extractable data concludes extractable substances are well below a concern level and biological reactivity data available; or a material considered to be low risk due to its fundamental physical characteristics (glass, ceramic or stainless steel, plus some certification)
4: low severity of harm (uncertain)	Data shows some potential harm to patient safety	Appropriate biological reactivity data available and/or substances are above the generic concern threshold, but below the associated permissible exposure for identified compounds; or biocompatibility data and physiochemical data but no extractable data; or biocompatibility and food contact data but no extractable data
7: high severity of harm (uncertain)	Limited or no information, but presumption of a harm	No data or insufficient biological reactivity or extractable/leachable data available; extractable/leachable data shows that substances are detected above generic concern threshold, but associated permission exposure data is not available either because the substances are not identified, or no toxicological data is available; default score when no information available
10: high severity of harm (certain)	Demonstrated to be a harm to patient safety	Substances detected above associated permissible exposure limits; absence statement indicates a toxic substance to be present in material; failed both biocompatibility and physicochemical test for material; failed biological reactivity test on material

- Likelihood of purging of leachable before it can give rise to patient exposure

Many of these are included because they a linked directly to diffusion from material into the process stream. Additionally, a factor linked to general compatibility has been included as if there is evidence that material in question is not generally compatible with drug product stream, then leachables are more likely to be present. Separately a consideration for purging is included, this can be very relevant for CGT systems which often include one or more steps in which the upstream process is purified/diluted/purged thus removing potential leachables reaching the patient in whole or part. A judgement/justification must be included when this factor is used. In the example given for a cell harvesting bag, there would be many potential purge points between harvest and patient administration. This then can be documented within the leachable management process.

The two sets of scores are brought together to define the risk score. Therefore, risks scores can range from 1 to 100. With individual scores 1,4,7, or 10.

Turning back to the example of the bag. Assuming there is not yet available simulated leachable data. The scoring would be decided based on the probability suggested. Higher scores being more likely when the combination of storage time, storage temperature, surface area and the nature of the contacting fluid, and its compatibility with the bag are considered together with the possibility of purging later in the process. Time, temperature, and surface area are factors linked with diffusion rates, the nature of the material of the bag also will set a diffusion coefficient [41]. The fluid composition again influences diffusion rate of any leachates, through either its effect on the contact (bag) material, or through solubility effects. As mentioned, ultimately the probability is likely low scoring due to purging points, the risk assessment should explore and document these.

RISK EVALUATION

Risk evaluation is an important step where the scoring is compared to a pre-defined scheme. A variety of risk evaluations could be considered but using the scoring outlined here, the suggested risk evaluation scheme

TABLE 3

Criteria and scoring of probability of harm.

Score	Effect	Example criteria
1: low probability (certain)	Unlikely: little or no chance of leachables	Projected level of leachables is less than below a concern level for any detected substance; modelled leachable migration indicates leachables are unlikely; in-use conditions indicates leachable migration is unlikely (from probability factors)
4: low probability (uncertain)	Possible: leachables levels are likely to be low or moderate	Projected leachables (extractable) are expected to be present, but lower than expected toxicological concern level; modelled leachable migration indicates leachables are low/moderate (lower than a toxicological concern level); in-use conditions indicates leachable migration is likely to be low (from probability factors)
7: high probability (uncertain)	Likely: represents a credible possibility of leachables at elevated levels	Projected leachables (extractables) are present and may be approaching expected toxicological concern level; modelled leachables may be approaching the expected toxicological concern level; in use conditions represent a plausible possibility of leachables (from probability factors); no prediction has been made (default)
10: high probability (certain)	Probable: high levels of leachables observed or likely to occur	Projected level of leachables (extractables) is greater than safe exposure limit for detected substances (PDE); modelled leachable is greater than safe exposure limit; in use conditions represent a high probability that leachables will be observed at high levels (from probability factors)

is based on a risk scoring matrix shown in Figure 3.

As illustrated, the risk evaluation matrix outputs two scenarios. Either the risk identified is scored green (low)—risk acceptance, or red (high). Red risks result in more activity (risk mitigation). That activity will be highly dependent on the risk which has been identified and the current status of the available knowledge. Scoring of 10 on either scale is given a red rating. This conservative approach allows you to consider how mitigation should be documented. This approach is fundamental in accepting a risk-based approach, where finite resources are targeted into activity that has used a science led process to determine relative risk. Accepting that low risk needs no further activity frees up resources to focus on areas which conservatively might offer more risk.

RISK MITIGATION IN CGT

When red risks are identified, the next stage in risk management will be to consider how to mitigate that risk. At this stage in the process, the nature of the risk will be the driver for activity. The most likely outcome is that knowledge gaps are driving the higher score, rather than a truly high risk. Indeed, an awareness of which of these two situations is present is a key outcome [42–44].

If knowledge gaps are not present, and the risk is truly high, there is little to be gained from additional studies which will only reconfirm high risk. Studies may refine the risk or may form part of a control strategy whereby the risk is accepted as high and now needs to be controlled.

A more likely outcome is that there is uncertainty. Designing an extractable study will lower uncertainty and making that study a simulated leachable study will speak directly to leachable risk (principally the likelihood of leachable exposure to patients).

The design and purpose of the extractable study becomes of particular importance. Each variable in that design needs to be well thought out in terms of both what value it brings and how it may or may not be predictive of leachable in the drug product. Keep in mind that for CGT products, it will not be possible to directly monitor for leachables.

EXTRACTABLE STUDIES (WHICH ARE NOT SIMULATED LEACHABLE STUDIES)

In these cases, it is important to distinguish between designs of extractable study.

FIGURE 3

Risk scoring matrix.

Probability				
10	10	40	70	10
7	7	28	49	70
4	4	16	28	40
1	1	4	7	10
Severity	1	4	7	10

Do something to reduce the risk, gather more information (extractable or leachable test)
No further actions, risk is judged low; document in risk assessment report

Extractable studies that deploy solvent systems, and extraction times or types which are very different from the eventual drug product formulation are fundamentally providing a different purpose. The primary value of that purpose is the discovery of the composition of the material. The value of that discovery should be set in context. Whilst it is useful to have, it is certainly different from a prediction of what a patient will be exposed to when the drug product is delivered. Thus, the attributes of extractable studies of this kind are:

- They could vary widely in their design (but still be linked to identified risks)
- An aid to the planning of simulated leachable studies
  - Can provide a sense of what substances will be important to target
  - Can provide a sense of what analytical methods will provide key information
- Should not be used directly as an input into safety assessment, since they will overestimate exposure, and they are likely to include substances not relevant to the assessment
- They can provide aid in identification of substance detected in simulation, since they would typically have higher levels which aid in providing higher quality datasets for spectroscopic data processing
- They also might provide insight into sources of simulated leachables through giving a correlation to groups of substances representing a common additive or route of degradation
- They can be used as part of the risk assessment process to plan more activities to define and accept the risk through the assembly of knowledge
- They would map to sampling points other than what represents final patient exposure, perhaps by being mapped to sampling points further upstream (see **Figure 2**)

Many aspects of extractable design have been discussed in PQRI published recommendations and summarized in USP chapters such as <USP 1663>.

SIMULATED LEACHABLE STUDIES

Simulation studies are by definition (see also ISO10993-18 terms and definitions,

simulated-use extraction' [45,46]) an effort to design systems that aim to predict both quantitative and qualitatively the exposure a patient receives. Thus, what they receive and how much. In particular, the how much part is unique to simulation studies, the what part might overlap with other extractable studies. Simulation studies output will be the input into a safety assessment to determine leachable risk. A full approach to safety assessment is beyond the scope of this paper but in general the aims of that assessment would be to confirm that predicted/simulated leachable offer no risk to patients since both their identity and quantity are not such that they fall outside published and calculated permitted exposures [47,48], by either falling below a generic calculated safety concern threshold or a permitted daily exposure (PDE) for a named substance. Their key features then are:

- Must be relatable to eventual patient exposure, this through choice of solvent or through mechanism of exposure such as length of time or choice of temperature
- They will be examined carefully to determine both identity and quantities of substances which they predict as leachables
- They will be used to update the risk assessment process and complete it
- They would map to a specific sampling point just prior to patient exposure, but there is an opportunity to apply them to other sampling points

CONCLUSIONS

Simulated leachable studies are key to establishing leachable risk. However, it should be noted that they need to be used within a structured leachable risk assessment for best effect. They form part of a program of work which should be seen as starting with structured risk assessment. Only when risks are clearly articulated and defined should work begin on the design of any mitigating studies which would include simulated leachable studies. They are complimentary to other kinds of extractable studies and other kinds of knowledge generation (e.g., an understanding of material composition or the supply chain for a given component, together with compliance documentation). None of these work in isolation. All of these work to the common goal of understanding leachable risk in the context of the protection of the quality and safety of CGT drug products.

REFERENCES

1. Azhagiri MKK, Babu P, Venkatesan V, Thangavel S. Homology-directed gene-editing approaches for hematopoietic stem and progenitor cell gene therapy. *Stem Cell Res. Ther.* 2021; 12(1), 500.
2. White LA, Vaask I, Härdin J, *et al.* Abstract 7242: immunostimulatory gene therapy co-expressing immune activators TMZ-CD40L and 4-1BBL with a PD-L1-targeting affibody molecule to reactivate T cell immunity. *Cancer Res*. 2024.
3. Budde D, Jurkiewicz E. Risk analysis of leachables in cell and gene therapy using a CAR-T model process. *Int. J. Pharmaceut.* 2021; 607, 121015.
4. Wang H, Li C, Obadan AO, *et al. In vivo* hematopoietic stem cell gene therapy for SARS-CoV2 infection using a decoy receptor. *Hum. Gene Ther.* 2022; 33(7–8), 389–403.

5. USP. <*1663*> *Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems'*. US Pharmacopeia. https://doi.org/10.31003/USPNF_M7126_03_01.

6. USP. <*1664*> *Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems'*. US Pharmacopeia. https://doi.org/10.31003/USPNF_M7127_03_01.

7. USP. <*665*> *Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceuticals Drug Substances and Products'.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M11135_03_01.

8. USP. <*1665*> *Characterization and Qualification of Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products'.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M11136_02_01.

9. FDA. *Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics.* May 1999; US Food and Drug Administration. https://www.fda.gov/media/70788/download.

10. EMA. *Guideline on Plastic Immediate Packaging Materials. EMEA European Medicines Agency.* May 2005; European Medicines Agency. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-plastic-immediate-packaging-materials_en.pdf.

11. PQRI. *Safety Thresholds and Best Demonstrated Practices for Extractables and Leachables in Parenteral Drug Products (Intravenous, Subcutaneous, and Intramuscular)*. 2022; The Product Quality Research Institute.

12. PQRI. *Safety thresholds and best practices for extractables and leachables in orally inhaled and nasal drug products*. 2006; The Product Quality Research Institute.

13. Norwood DL, Paskiet D, Ruberto M, *et al.* Best practices for extractables and leachables in orally inhaled and nasal drug products: an overview of the PQRI recommendations. *Pharm. Res.* 2008; 25(4), 727–739.

14. ELSIE leachable risk management. https://elsiedata.org/risk-assessment.

15. BioPhorum. *Best practices guide for evaluating leachables risk from polymeric single-use systems guidance.* 2021. https://www.biophorum.com/download/best-practices-guide-for-evaluating-leachables-risk-from-polymeric-single-use-systems.

16. Pahl, I., Pahl, A., Hauk, A, *et al.* Assessing biologic/toxicologic effects of extractables from plastic contact materials for advanced therapy manufacturing using cell painting assay and cytotoxicity screening. *Sci. Rep.* 2024; 14, 5933.

17. Budde D, Maier TV, Menzel R, *et al.* Identification and evaluation of cell-growth-inhibiting bDtBPP-analogue degradation products from phosphite antioxidants used in polyolefin bioprocessing materials. *Anal. Bioanal. Chem.* 2020; 412, 4505–4518.

18. Bossong M, Budde D, Hauk A, *et al.* Biosorption of process-equipment-related leachables (PERLs) in biomanufacturing: a quantitative approach to study partitioning of PERLs in a cell culture system. *Int. J. Pharm.* 2023; 635, 122742.

19. Arroyo A, Booij P, Woldemariam G, *et al.* Cell and gene therapies: challenges in designing extractables and leachables studies and conducting safety assessments. *J. Pharm. Sci.* 2024; 113, 513–522.

20. Wakankar AA, Wang YJ, Canova-Davis E, *et al*. On developing a process for conducting extractable-leachable assessment of components used for storage of biopharmaceuticals. *J. Pharm. Sci.* 2010; 99(5), 2209–2218.

21. Maven E&L Expert Consultancy. https://www.MavenEandL.com.

22. US FDA. *ICH Guideline Q9 on Quality Risk Management*. *Guidance FDA-20220D00705.* May 2023. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q9r1-quality-risk-management.

23. ICH Quality Guidelines. https://www.ich.org/page/quality-guidelines.

24. Rezaei F, Yarmohammadian MH, Haghshenas A, Fallah Ali, Masoud F. Revised risk priority number in failure mode and effects analysis model from the perspective of healthcare system. *Int. J. Prev. Med.* 2018; 9(1), 7.

25. FDA. *Q3D(R2): Guideline for Elemental Impurities*. *Guidance FDA-2013-D-1156.* Sep 2022. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q3dr2-guideline-elemental-impurities.

26. FDA. *Guidance for Industry: M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk*. *Guidance FDA-2022-D-0055.* May 2023. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q9r1-quality-risk-management.

27. USP. *<87> Biological Reactivity Tests,* In Vitro. US Pharmacopeia. https://doi.org/10.31003/USPNF_M98833_01_01.

28. USP. *<88> Biological Reactivity Tests,* In Vivo. US Pharmacopeia. https://doi.org/10.31003/USPNF_M98834_01_01.

29. USP. *<381> Elastomeric Components in Injectable Pharmaceutical Product Packaging/Delivery Systems*. US Pharmacopeia. https://doi.org/10.31003/USPNF_M99140_06_01.

30. USP. *<383> Cured Silicone Elastomers for Pharmaceutical Packaging and Manufacturing Components.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M15855_02_01.

31. USP. *<661> Plastic Packaging Systems and Their Materials of Construction.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M99420_05_01.

32. USP. *<661.1> Plastic Materials of Construction.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M8008_13_01.

33. USP. *<661.2> Plastic Packaging Systems for Pharmaceutical Use.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M8009_06_01.

34. USP. *<1031> The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M99754_04_01.

35. USP. *<1231> Water for Pharmaceutical Purposes.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M99956_07_01.

36. USP. *<1381> Assessment of Elastomeric Component Used in Injectable Pharmaceutical Product Packaging/Delivery Systems.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M11357_02_01.

37. USP. *<1661> Evaluation of Plastic Packaging Systems for Pharmaceutical Use and Their Materials of Construction.* US Pharmacopeia. https://doi.org/10.31003/USPNF_M9293_06_01.

38. Code of Federal Regulations. *Part 177—Indirect Food Additives: Polymers*. https://www.ecfr.gov/current/title-21/part-177.

39. Qian S, Ji H, Wu XX, *et al*. Detection and quantification analysis of chemical migrants in plastic food contact products. *PLoS ONE* 2018; 13(12), e0208467.

40. EUR-Lex. *Document 02011R0010-20250120, Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food*. http://data.europa.eu/eli/reg/2011/10/2025-01-20.

41. BPSA. *Extractable/Leachable Considerations for Cell and Gene Therapy Drug Product Development*. 2020; Bio-Process Systems Alliance. https://www2.bpsalliance.org/forms/store/ProductFormPublic/Extractables-Leachables-Considerations-for-Cell-Gene-Therapy-Drug-Production-Development.

42. Hauk A, Menzel R, Pahl I, Budde D. Process development and manufacturing: extractables and leachables from single-use systems and their assessment in cell and gene therapy applications. *Cytotherapy* 2023; 25(6), S184.

43. Li K, Rogers G, Nashed-Samuel Y, *et al.* Creating a holistic extractables and leachables (E&L) program for biotechnology products. *PDA J. Pharm. Sci. Technol*. 2015; 69(5), 590–619.

44. Hammond M, Nunn H, Rogers G, *et al.* Identification of a leachable compound detrimental to cell growth in single-use bioprocess containers. *PDA J. Pharm. Sci. Technol*. 2013; 67, 123–134.

45. ISO. *10993-18 Chemical characterisation of medical device materials within a risk management process*. 2020; International Organization for Standardization.

46. ISO. *10993-1 Biological evaluation of medical devices part 1: evaluation and testing within a risk management process.* 2018; International Organization for Standardization.

47. Ekwall B, Nordensten C, Albanus L. Toxicity of 29 plasticizers to HeLa cells in the MIT-24 system. *Toxicology* 1982; 24, 199–210.

48. Broschard TH, Glowienke S, Bruen US, *et al.* Assessing safety of extractables from materials and leachables in pharmaceuticals and biologics—current challenges and approaches. *Regul. Toxicol. Pharmacol*. 2016; 81, 201–211.

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CELL & GENE THERAPY INSIGHTS

GENE THERAPY ANALYTICS AND CMC

SPOTLIGHT

INNOVATOR INSIGHT

Salt-tolerant endonucleases, the benefits for viral vector manufacturing and a comparison of two marketed enzymes

Marc Struhalla and Svenja Michalek

Recombinantly produced *Serratia marcescens* endonucleases, such as DENARASE® and Benzonase®*, are widely used for DNA removal in viral vector production processes. To achieve higher vector yields, manufacturers are increasingly using higher salt concentrations, up to 500 mM, which adversely impacts the activity of *S. marcescens* endonucleases. Consequently, producers of viral vectors are seeking alternative, more salt-tolerant enzymes for nucleic acid digestion.

In this article, we describe the making of DENARASE® High Salt, an engineered variant of the *S. marcescens* wild-type enzyme, and its potential to streamline viral vector manufacturing processes. In addition, we present test results from an independent, not-for-profit CGT research and technology organization, comparing the new enzyme with another commercially available salt-tolerant endonuclease.

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In the manufacturing of viral vectors for cell and gene therapies, as well as vaccines, the removal of residual plasmid and host cell-derived DNA and RNA is critical to meet regulatory requirements, ensure product safety, and streamline vector purification. Endonucleases enzymatically cleaving polynucleotide chains to fragments of approximately 3–5 base pairs are commonly used for this purpose. Recombinantly produced *Serratia marcescens* endonucleases, such as DENARASE®, are the industry standard for DNA removal in viral vector manufacturing processes. These endonucleases are typically used to reduce the plasmid and host cell DNA during viral vector harvest.

It has been shown that higher salt concentrations can significantly improve viral vector solubility and reduce aggregate

formation [1]. Additionally, increased salt levels may enhance DNA accessibility for degradation, leading to higher purity and safety of the final product. However, the activity of *S. marcescens* endonucleases is inhibited at elevated salt concentrations.

This article introduces an engineered version of the wild-type *S. marcescens* endonuclease, which exhibits improved tolerance to salt, thereby offering greater flexibility in bioprocessing.

ENZYME ENGINEERING AT c-LEcta

Natural enzymes can be optimized to meet the requirements of industrial applications. Enzyme engineering allows the optimization of enzyme properties by introducing changes into the protein's amino acid sequence, utilizing directed evolution to address previously unmet needs [2].

At c-LEcta, an enzyme engineering platform (ENESYZ®) has been developed that mimics the principles of natural evolution to improve enzymes through a step-by-step cyclic process. A key element of this platform is the design and creation of enzyme variant libraries. Bioinformatics, machine learning, and artificial intelligence are applied to design smart enzyme libraries with extraordinarily high hit rates.

Another critical element is the screening process itself: enzyme libraries must be screened for the necessary properties, ideally with a high throughput. Multiparameter screens ensure the identification of enzymes that meet the needs of the targeted application.

BENEFITS OF ENDONUCLEASES AND HIGH SALT FOR VIRAL VECTOR MANUFACTURING

Endonucleases are enzymes that specifically hydrolyze the phosphodiester bonds between nucleotides, effectively breaking down all forms of nucleic acids. They are commonly used to remove contaminating DNA in the production processes of viral vectors. DNA resulting from the lysis of genetically transformed, often immortalized cell lines typically used for viral vector production, can potentially be infectious, immunogenic, and/or oncogenic, therefore posing a potential threat to patients. With significant progress in the development of viral vector-based therapeutics, the FDA has set limits for the concentration and size of residual DNA in the final drug product [3].

Because of their efficiency and specificity for DNA degradation, endonucleases from *S. marcescens* became part of the standardized workflow for viral vector manufacturing processes, in which after viral vector expression in host cells, virus particles are harvested. This may require a lysis step depending on the virus type, followed by endonuclease treatment ahead of further purification steps [4]. Efficient DNA removal not only enhances the safety profile of viral vector-based therapeutics but can improve overall process efficiency. By reducing the viscosity of cell lysates, endonuclease treatment facilitates downstream processing, resulting in overall higher vector yields [5].

To improve the safety and affordability of innovative cell and gene therapies, there is both a need and an opportunity for further production process improvements. Higher salt concentrations have been discussed as a tool for further process optimizations of viral vectors such as adeno-associated viruses (AAV). Salt concentrations between 200–400 mM have been reported to improve the solubility of viral vectors and DNA, reducing the formation of aggregates, which is especially beneficial at higher cell densities and vector concentrations [1,6,7]. Additionally, the increased solubility of DNA at elevated salt concentrations may make it more accessible for degradation, resulting in improved vector yield and higher purity of the final drug

product. However, this approach presents challenges. Wild-type *S. marcescens* endonucleases are inhibited by increasing salt concentrations [8]. Additionally, currently available salt-active enzymes exhibit low activity and suboptimal salt and pH profiles, necessitating the adaptation of the production process to the endonuclease used. This increases the activity of the endonuclease but also reduces the overall flexibility and efficiency of the process.

ENGINEERING OF A SALT-TOLERANT *SERRATIA MARCESCENS* ENDONUCLEASE

To address the unmet need for an endonuclease with robust activity at elevated salt levels, c-LEcta engineered a salt-tolerant version of the wild-type DENARASE®. Based on feedback from viral vector manufacturers, the preferred requirements for the engineered enzyme were defined as activity over a broader spectrum of process-relevant salt concentrations, combined with robust DNA removal activity at physiological pH 7.4.

By introducing a combination of amino acid substitutions, a set of DENARASE® mutants with improved activity at higher salt conditions was generated. This robust activity across the entire salt spectrum was not only observed under standard *S. marcescens* endonuclease release assay conditions at pH 8, but also at the more process-relevant pH 7.4. After thorough characterization of the salt-tolerant DENARASE® variants, the mutant with the most optimal activity profile was selected and is now available as DENARASE® High Salt. As shown in Table 1, the wild-type *S. marcescens* endonuclease DENARASE® and the engineered DENARASE® High Salt are produced using the same *Bacillus* strain as a production host and share multiple enzyme characteristics, such as molecular weight, temperature optimum and pH range. They slightly differ in their isoelectric point and DENARASE® High Salt requires higher levels of the essential co-factor magnesium for optimal performance.

In summary, the introduced modifications enable DENARASE® High Salt to remain active in a broader range of bioprocessing-relevant salt and pH conditions. At the same time, the great similarity to the wild-type enzyme allows for very similar manufacturing process and compatibility with the DENARASE® ELISA kit

TABLE 1

Comparison of DENARASE® and DENARASE® High Salt.

	DENARASE®	DENARASE® High Salt
Enzyme origin	*Serratia marcescens*	Engineered from DENARASE®
Production host	*Bacillus sp.*	
Molecular weight	27 kDa (per monomer)	
Temperature optimum	37 °C	
pH range	pH 7.0–9.0	
Isoelectric point	pH 6.2	pH 7.83
Magnesium optimum	1–5 mM	5–25 mM

Both enzymes share multiple characteristics including molecular weight, temperature optimum as well as pH range and are produced in the same production host. They differ slightly in their isoelectric point and the concentration of magnesium needed for optimal performance.

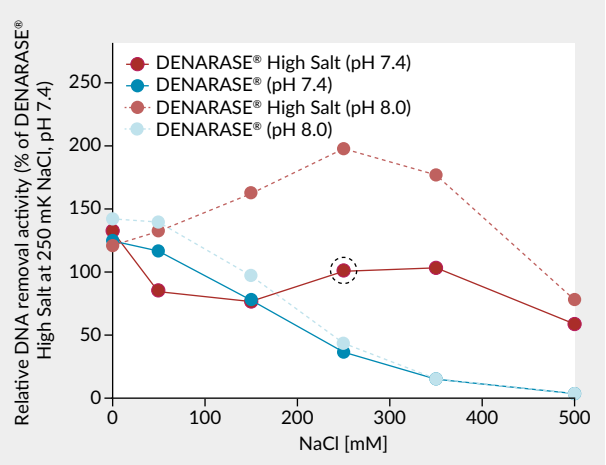
for quantifying residual enzyme could be maintained.

To determine the optimal usage/process conditions for the wild-type versus the salt-tolerant DENARASE® enzyme, a comparative analysis was conducted using c-LEcta's Salt Performance Test**. Comparing the most relevant process parameters, the effect of increasing sodium chloride concentrations at pH 7.4 (process relevant condition) and at pH 8.0 (DENARASE® release assay conditions) were analyzed (Figure 1).

DENARASE® High Salt exhibits superior performance at elevated salt concentrations, maintaining robust DNA removal activity, whereas the wild-type DENARASE® shows a decline in activity with increasing salt levels. Both enzymes exhibit substantial activity at pH 7.4, which can, but does not necessarily need to be further enhanced by adjusting the pH to 8.0. The standard DENARASE® enzyme remains the most cost-effective solution for salt concentrations from 0–150 mM. At higher salt concentrations DENARASE® High Salt is recommended. Its strong performance across a wide range of salt and pH conditions provides maximal flexibility for processes that benefit from salt addition.

FIGURE 1

Effect of increasing NaCl concentrations on enzyme activity of DENARASE® and DENARASE® High Salt at pH 7.4 (process-relevant) and pH 8.0 (QC-relevant).



DNA removal activities of the wild-type *Serratia marcescens* endonuclease (DENARASE®) and DENARASE® High Salt were tested at 0–500 mM NaCl (pH 7.4 and pH 8; 5 mM $MgCl_2$) using the c-LEcta Salt Performance Test**. Measured DNA removal activities ($Unit_{Test}$/µl) were first normalized by the applied nuclease units as specified in the respective CoAs ($Unit_{CoA}$/µl). The resulting normalized activities ($Unit_{Test}/Unit_{CoA}$) are shown relative to activity of DENARASE® High Salt at 250 mM NaCl (=100%; indicated by dotted circle).

SUPERIOR DNA CLEARANCE: BENCHMARKING OF DENARASE® HIGH SALT AGAINST OTHER SALT-ACTIVE ENDONUCLEASES

In an internal benchmarking study using the c-LEcta Salt Performance Test, the DNA removal activity of DENARASE® High Salt was evaluated at various salt concentrations and compared to several other commercially available salt-active endonucleases (Salt-E1 to Salt-E4). As illustrated in Figure 2, DENARASE® High Salt consistently demonstrated superior DNA clearance activity across all tested salt concentrations (0–500 mM NaCl), outperforming all other enzymes in the study at pH 7.4. Most of the tested salt-active nucleases require elevated sodium chloride concentrations to function effectively. In contrast, DENARASE® High Salt maintains robust activity across a broad range of salt concentrations, from 0–500 mM. This unique characteristic allows DENARASE® High Salt to remove DNA efficiently without the need for additional buffer adjustments or salt supplementation.

The ability of DENARASE® High Salt to remain active across such a wide range of salt concentrations offers significant operational advantages. It simplifies the bioprocessing workflow by eliminating the need for precise salt concentration adjustments. Additionally, this flexibility allows for the full utilization of bioreactor capacities, enhancing overall process efficiency and productivity.

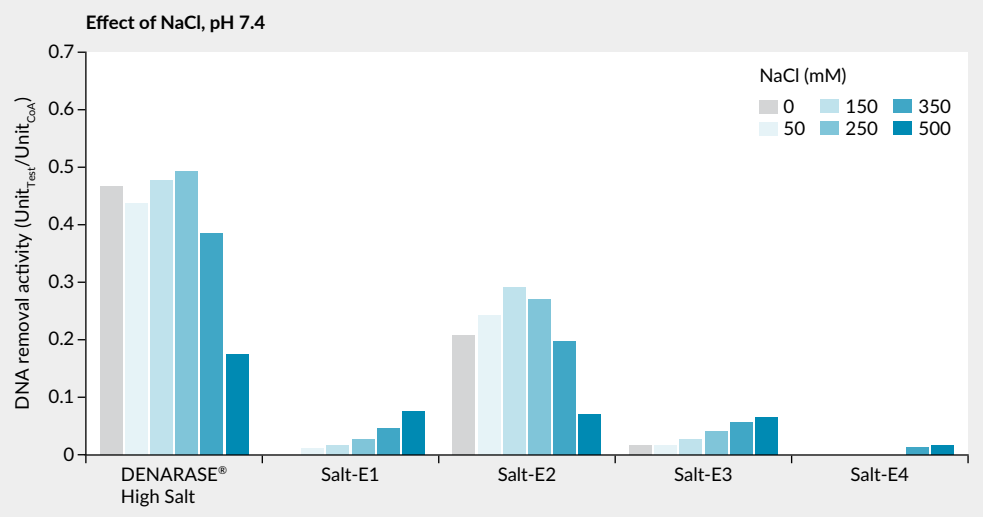
Since *in vitro* activity assays cannot fully replicate viral vector processing

conditions, the DNA removal efficiency of DENARASE® High Salt in viral lysates was analyzed in collaboration with Cell and Gene Therapy Catapult (CGT Catapult).

In a comparative study, DENARASE® High Salt was benchmarked against Salt-E1 for the removal of DNA from a proprietary CGT Catapult AAV2 lysate. Identical amounts of both enzymes were used across all experimental conditions (50 U/mL). DNA removal efficiencies were monitored at pH 7.4, without additional salt or with final concentrations of 200, 350, and 500 mM sodium chloride. To represent optimal conditions for Salt-E1, an additional experimental condition of pH 8.0 with 500 mM salt was included. Analyses of remaining total and host cell-derived DNA levels were

FIGURE 2

The effect of increasing NaCl concentrations on the enzyme activity of DENARASE® High Salt and several commercially available salt-active nucleases: Salt-E1 to Salt-E4.



DNA removal activities of commercial salt-active endonucleases (Salt-E) were tested at NaCl concentrations of 0, 50, 150, 250, 350, and 500 mM (pH 7.4; 15 mM $MgCl_2$) using the c-LEcta Salt Performance Test**. Measured DNA removal activities [Unit$_{Test}$/µl] were normalized by the applied nuclease units as specified in the manufacturer's CoAs [Unit$_{CoA}$/µl] and are depicted as [Unit$_{Test}$/Unit$_{CoA}$]. Measured DNA removal activities and CoA activities are listed in Table 2.

TABLE 2

DNA removal activities of salt-active endonucleases (Salt-E) per µl sample as A: measured using the c-LEcta Salt Performance Test** (U$_{Test}$/µL) and B: outlined in suppliers' CoAs (U$_{CoA}$/µL).

	DNA removal activities						
	A: Measured with c-LEcta Test (U$_{Test}$/µl]						B: CoA
Nuclease NaCl [mM]	0	50	150	250	350	500	acc. to CoA
DENARASE® High Salt	168.8	158.1	172.5	177.0	137.3	61.4	358.0
Salt-E1	0.2	0.2	0.4	0.7	1.1	1.9	26.0
Salt-E2	5.9	6.8	8.3	7.7	5.6	1.9	28.2
Salt-E3	4.0	5.2	8.3	12.6	16.1	19.7	306.0
Salt-E4	0.4	0.6	1.0	1.5	2.4	4.1	250.0

performed directly post-lysis of host cells and after an affinity capture step.

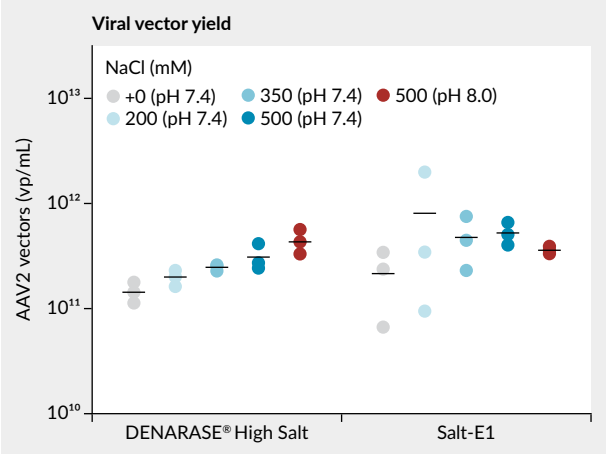
Before examining DNA removal, the effect of different salt conditions on viral vector yield was quantified using a capsid enzyme-linked immunosorbent assay for AAV2 (Figure 3). Consistent with previous findings, the addition of salt increased viral particle titers depending on the salt concentration. This indicates that high salt levels improve the solubility of AAV particles and DNA or prevent aggregate formation. While individual DENARASE® High Salt treated samples exhibited consistent AAV2 vector titers at the different experimental conditions, there was an unexpected variance in the Salt-E1 results.

These fluctuations might be attributed to sub-optimal process conditions for Salt-E1 such as insufficient salt concentration, pH level, or magnesium concentration.

In the next step, DNA clearance from AAV2 lysates was quantified using Picogreen, a widely used method for the unselective detection of double stranded DNA, allowing for the quantification of both residual plasmid and host cell derived DNA impurities (Figure 4). DENARASE® High Salt demonstrated a consistent DNA reduction across all tested salt and pH conditions post-lysis, achieving DNA levels below the quantification limit in post-capture material.

FIGURE 3

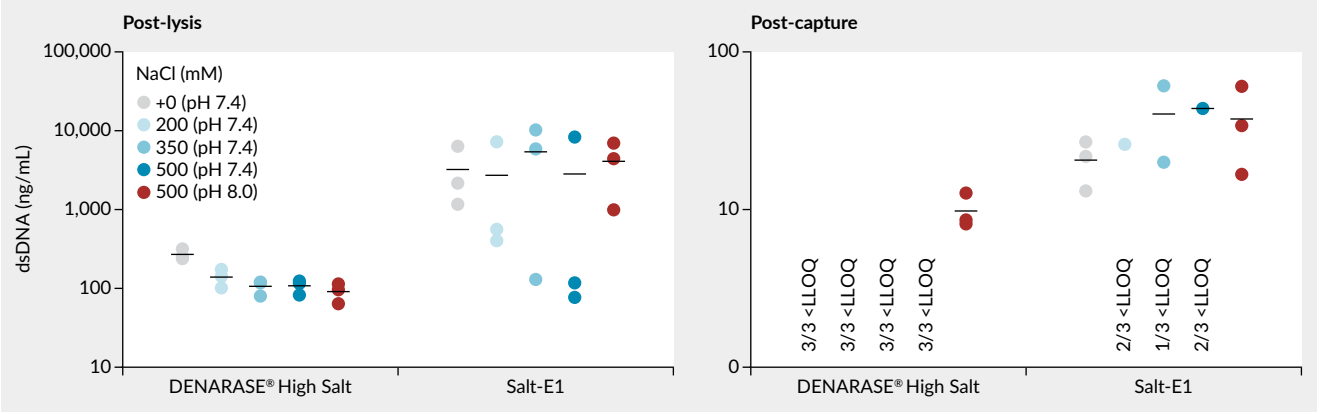
High Salt concentrations increase viral vector yield.



After cell lysis, the effect of different salt and pH conditions on the yield of assembled AAV2 vectors was quantified via an automated enzyme-linked immunosorbent assay (ELLA, Simple Plex™ Assay; Bio-Techne) according to the manufacturer's protocol. Experiments were conducted at 200, 350, and 500 mM NaCl or without salt addition (+0) and pH 7.4 as well as at 500 mM NaCl and pH 8.0. 50 U/mL of the respective endonuclease was applied for 60 min.

FIGURE 4

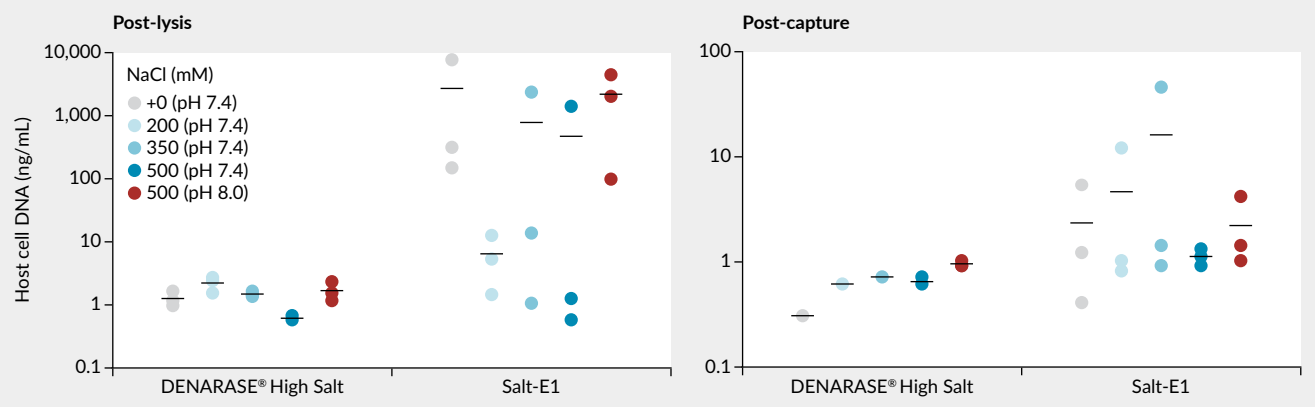
Comparison of total DNA clearance by DENARASE® High Salt and Salt-E1.



Total DNA (dsDNA) removal activities of DENARASE® High Salt and Salt-E1 from AAV2 lysates were analyzed using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen™) according to the manufacturer's protocol. Experiments were conducted at 200, 350, and 500 mM NaCl or without salt addition (+0) and pH 7.4 as well as at 500 mM NaCl and pH 8.0. 50 U/mL of the respective endonuclease was applied for 60 min and samples were taken directly after endonuclease treatment (post-lysis) and after a capture step using affinity resins loaded onto filter plates (post-capture). Black lines indicate mean values of triplicate measurements.

FIGURE 5

Comparison of host cell DNA clearance by DENARASE® High Salt and Salt-E1.



Host cell DNA removal activities of DENARASE® High Salt and Salt-E1 from AAV2 lysates were analyzed via quantitative PCR detecting remaining fragments of human genomic host cell DNA derived from the producer cell line HEK 293. Experiments were conducted at 200, 350, and 500 mM NaCl or without salt addition (+0) and pH 7.4 as well as at 500 mM NaCl and pH 8.0 (empty dots). 50 U/mL of the respective endonuclease was applied for 60 min and samples were taken directly after endonuclease treatment (post-lysis) and after a capture step using affinity resins loaded onto filter plates (post-capture). Black lines indicate mean values of triplicate measurement.

The application of DENARASE® High Salt consistently resulted in approximately 10-fold lower DNA levels compared to Salt-E1 in post-lysis samples. This superior performance was observed irrespective of the salt concentration or pH level. While Salt-E1 also achieved DNA removal, it exhibited much higher variability and less efficiency, especially at lower salt concentrations and pH.

Since the amount of residual DNA in most post-capture samples was below the limit of detection for the Picogreen analysis, a more sensitive qPCR-based method was used to specifically quantify host cell-derived DNA (Figure 5). It could be demonstrated that the residual DNA consistently reached a level of approximately 1 ng/mL for all DENARASE® High Salt treated samples. Overall similar host cell-derived DNA levels were obtained in post-capture samples after treatment with Salt-E1. However, the qPCR method also confirmed the variability in DNA clearance when using Salt-E1. These findings highlight the robustness and reliability of DENARASE® High Salt in maintaining low DNA impurity levels under varying process conditions.

Based on the analysis of DNA removal efficiencies on proprietary AAV2 lysates from CGT Catapult, it can be concluded that DENARASE® High Salt removes DNA from AAV lysates with the highest efficiency. It outperforms the competitor enzyme regarding both the extent and reproducibility of DNA reduction, demonstrating robust performance under the tested process-relevant pH and high salt conditions.

SUMMARY

Enzyme engineering of the *S. marcescens* endonuclease, the current industry standard for DNA removal in bioprocessing, has proven to be a successful approach for developing a new enzyme with improved process characteristics. DENARASE® High Salt remains active across a broader range of salt and pH conditions, which enhances yield and reduces the costs associated with viral vector production.

DENARASE® High Salt exhibits high activity under a wide spectrum of process-relevant conditions, establishing it as an industry-leading enzyme for DNA removal. Its robust performance ensures

seamless integration into preferred process workflows and it is fully compatible with the DENARASE® ELISA Kit. This compatibility further simplifies the monitoring and validation of DNA removal processes, making DENARASE® High Salt an optimal choice for bioprocessing applications that benefit from high salt concentrations.

**c-LEcta's Salt Performance Test (see also DENARASE® High Salt Validation Guide, section 3.1). Nuclease products were tested at different NaCl concentrations in reactions conducted for 60 min at 30 °C in 50 mM Tris-HCl (pH 7.4), 5 or 15 mM MgCl2, 0.8 mg/ml salmon sperm DNA, and 0.1 mg/ml bovine serum albumin. Reactions were stopped with perchloric acid, incubated on ice for 30 min, centrifuged, and the absorbance of the supernatants was measured at 260 nm and corrected against blanks without nuclease (ΔA260). One test Unit (UnitTest) of enzyme activity was defined as the amount of enzyme that produces a change in absorbance at 260 nm of 1.0 in the time of 30 min.

Q&A

Marc Struhalla and **Svenja Michalek**

Q How much magnesium should be used for DENARASE® and DENARASE® High Salt enzymes?

SM Magnesium is an essential cofactor for *S. marcescens* endonucleases. Typically, 1–2 mM magnesium is sufficient for optimal activity of the original DENARASE®. Due to the increased salt concentrations, 5–25 mM of magnesium is needed for optimal activity of the DENARASE® High Salt enzyme. For initial testing, we recommend starting with a concentration of 15 mM magnesium.

Q How many amino acids substitutions were made to generate DENARASE® High Salt?

MS The number of amino acid substitutions we made was relatively small. It required only four mutations to the wild-type DENARASE® to achieve the desired shift in the salt profile. These targeted changes were sufficient to enhance the enzyme's performance under high salt conditions, aligning it with our specific process requirements.

Q How compatible is the DENARASE® ELISA Kit with DENARASE® High Salt?

SM As DENARASE® High Salt only differs in a few amino acids from the wild-type, the ELISA Kit can be used for detection and quantification of DENARASE® High Salt. DENARASE® High Salt shows slightly different affinity for the antibodies being used. Since the kit only contains a standard solution of the wild-type enzyme, it is necessary to multiply ELISA kit readings of DENARASE® High Salt by a correction factor to obtain accurate protein concentrations. This factor was determined in a study performed by c-LEcta's contract manufacturer that also developed the ELISA Kit. Alternatively, one can also prepare a DENARASE® High Salt standard solution. In the future, we plan to set up the kit with both the wild-type and the High Salt standard.

Q Where is the production site of the DENARASE® High Salt located?

MS DENARASE® High Salt, like the standard DENARASE®, is produced in Germany. This is also where the headquarter of c-LEcta is located.

Q How is the DENARASE® High Salt performance affected by the presence of different detergents?

SM Given that detergents are often used in lysis buffers, we conducted an internal characterization to assess the effect of commonly used detergents such as Triton-X 100 and its alternatives, Tween 20, Tween 80. Our findings indicate that none of these detergents negatively affect the enzyme activity of DENARASE® High Salt, when applied under typical conditions.

Q Are there any different quality grades of DENARASE® High Salt available?

MS Since we launched the product a few months ago, different package sizes for R&D-grade quality are available. This grade is suitable for process development work. Additionally, we are planning to launch the DENARASE® High Salt GMP-grade, intended for use in commercial manufacturing processes, in Q2 2025.

Q How well does DENARASE® High Salt perform at different temperatures?

SM The temperature range for the wild-type DENARASE® and DENARASE® High Salt is similar. Both enzymes have a temperature optimum at 37 °C. At

temperatures ranging from 20–50 °C, they retain more than 40% of their activity. We have temperature curves available in our validation guides, so anyone interested can get in touch to review the data.

Q Are there any plans for further engineered versions of DENARASE®?

MS Currently, we are satisfied with the existing versions of DENARASE®. So far, we have not identified any gaps or process conditions that our current enzymes cannot address. If there are any specific needs or ideas for a new type of endonuclease, we are confident in our ability to develop it. Please feel free to get in touch to discuss any potential opportunities.

Q How can the DENARASE® enzyme be inactivated?

SM DENARASE® is used in the bioprocessing of viral vectors where it is being physically removed during downstream processing. In most processes, the enzyme is removed in the downstream process, so no inactivation is required. In addition, over-digestion is not a concern as viral DNA is protected by the assembled capsids. Enzyme removal for standard *S. marcescens*-derived endonucleases is achieved through multiple chromatography steps as part of viral vector downstream processing, where the enzyme simply flows through. By the final polishing step, the enzyme is completely removed. So far, we have not observed any differences when customers tested DENARASE® High Salt.

For other applications, the addition of magnesium-chelating agents like EDTA can be used for reversible enzyme inhibition. Alternatively, a significant reduction in enzyme activity could be achieved through very high temperature or the addition of a proteinase.

REFERENCES

1. Wright JF, Le T, Prado J, *et al.* Identification of factors that contribute to recombinant AAV2 particle aggregation and methods to prevent its occurrence during vector purification and formulation. *Mol Ther.* 2005; 12(1), 171–178.
2. Cherry JR, Fidantsef AL. Directed evolution of industrial enzymes: an update. *Curr. Opin. Biotechnol.* 2003; 14(4), 438–443.
3. Yang H. Establishing acceptable limits of residual DNA. *PDA J. Pharm. Sci. Technol.* 2013; 67(2), 155–163.
4. Srivastava A, Mallela KMG, Deorkar N, Brophy G. Manufacturing challenges and rational formulation development for AAV viral vectors. *J. Pharm. Sci.* 2021; 110(7), 2609–2624.
5. Fernandes RP, Göbel S, Reiter M, *et al.* Streamlining the purification of a clinical-grade oncolytic virus for therapeutic applications. *Sep. Purif. Technol.* 2025, 354; 128769.
6. Adamson-Small L, Potter M, Byrne BJ, Clément N. Sodium chloride enhances recombinant adeno-associated virus production in a serum-free suspension manufacturing platform using the herpes simplex virus system. *Hum. Gene Ther. Methods* 2017; 28(1), 1–14.

7. Yu C, Trivedi PD, Chaudhuri P, *et al.* NaCl and KCl mediate log increase in AAV vector particles and infectious titers in a specific/timely manner with the HSV platform. *Mol. Ther. Methods Clin. Dev.* 2021; 21, 1–13.

8. Zhu Y, Li M, Chen W, Peters A. The smart solution for DNA removal in biopharmaceutical production by benzonase endonuclease. *J. Appl. Virol.* 2013; 2, 25–33.

BIOGRAPHIES

Marc Struhalla is Co-founder, shareholder, and Managing Director of c-LEcta. He earned a PhD at the University of Hamburg, Hamburg, Germany following an undergraduate study in Biochemistry at the University of Leipzig, Leipzig, Germany. On completion of his PhD, he returned to the University of Leipzig before founding c-LEcta. Struhalla has more than 15 years of experience in product development and commercialization in the industrial biotech field and has managed the closure of numerous strategic alliances with industry partners.

Marc Struhalla, CEO, c-LEcta GmbH, Leipzig, Germany

Svenja Michalek is a Product Manager at c-LEcta. She is responsible for the biopharma portfolio, including the recently launched DENARASE® High Salt. Michalek is dedicated to helping manufacturers alleviate pain points in their viral vector production process to facilitate development and commercialization of cell and gene therapies. Prior to this Michalek was working as a post-doctoral scientist in leukemia research at the University of Konstanz, Konstanz, Germany. There she also earned her PhD following an MSc and BSc in Biological Sciences.

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AUTHORSHIP & CONFLICT OF INTEREST

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

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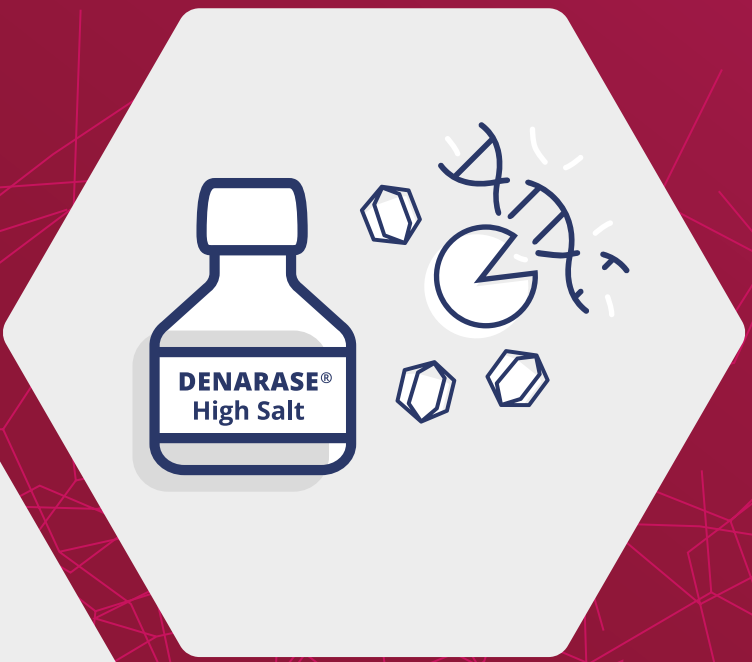
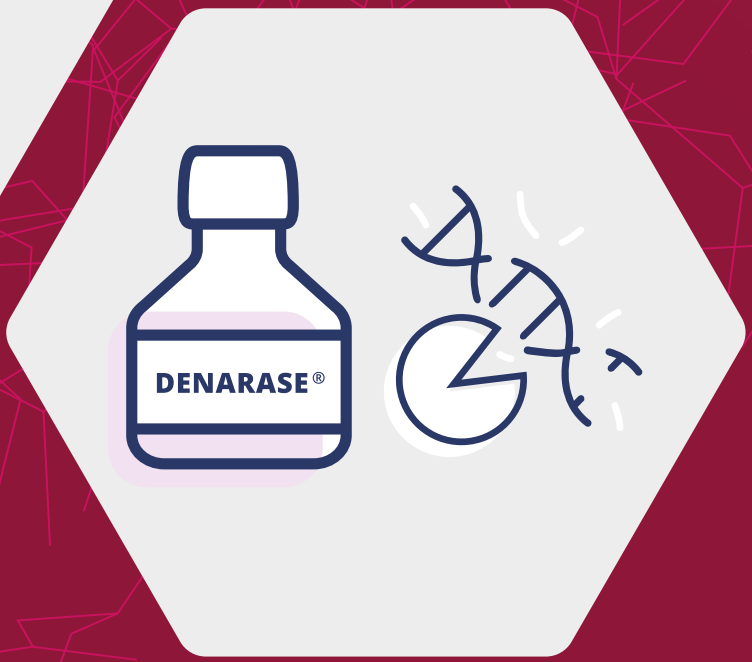
DENARASE®

High efficiency DNA removal
from no to high salt





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CELL & GENE THERAPY INSIGHTS

GENE THERAPY ANALYTICS AND CMC

SPOTLIGHT

Harnessing AI and omics for next-generation gene therapy manufacturing

INTERVIEW

"To make AI and ML truly effective, it is essential to integrate domain-specific knowledge."

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Richard D Braatz**, Chief Scientist and Co-founder of BioCurie, and **Irene Rombel**, CEO and Co-founder of BioCurie, about their work in AI-driven software and omics technologies to optimize gene therapy production. They discuss the untapped opportunities in integrating omics data, improving efficiencies, and leveraging AI to enhance therapeutic accessibility.

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Q What are you working on right now?

RB We are currently developing an AI-driven software platform designed for cell, gene, and nucleic acid therapy process development and manufacturing.

Q In your recent publication [1], you discussed the promise of omics to optimize HEK293 cells for rAAV production. Could you summarize the need for optimization and the role of omics technologies in enhancing their efficiency?

RB The primary challenge in making gene therapy accessible to patients has been the high cost of production, particularly for rAAV, as discussed in the paper. Significant improvements are required to achieve commercially viable timelines, yields, and costs that allow for widespread patient access.

IR Omics technologies offer valuable orthogonal information that can be integrated with other data sources, such as process data, to optimize both cell lines and production processes, ultimately increasing productivity. These technologies are detailed in the publication, which BioCurie wrote with several coauthors. The idea for the publication originated a few years ago when we recognized the growth in the quantity of omics data being produced and the significant opportunity that it presented.

Q What were the most unexpected insights that emerged from your research in terms of improving rAAV production using omics?

IR When conducting our analysis, we reviewed hundreds of papers, and several surprising insights emerged. One of the most striking was the sheer number of untapped opportunities and low-hanging fruit that are readily available for improvement. A key finding is that multi-omics approaches should be exploited more. Many studies have been conducted in isolation, either in academic or industrial settings, often providing only limited insights. However, these individual studies rarely integrate the data holistically. It is only recently that multi-omics approaches are being utilized more widely, and this is where meaningful synergies from a mechanistic perspective can be gained.

Another unexpected insight relates specifically to AAV production for gene therapies. The gene of interest (GOI) should not be expressed in the host cell, as it results in a useless product and, more critically, consumes energy and resources from the cell. Despite widespread awareness of this issue, surprisingly little effort has been dedicated to suppressing GOI expression in the host. The goal should be to maximize rAAV production, not GOI expression. The GOI belongs in the patient, not in the host cell.

While some studies have hinted at the significant energy drain caused by this issue, very few have addressed it systematically. A notable example is a study where researchers used a microRNA to suppress a toxic GOI, which led to a 240-fold improvement in rAAV productivity. This is a great illustration of the potential for optimization.

"It is only recently that multi-omics approaches are being utilized more widely, and this is where meaningful synergies from a mechanistic perspective can be gained."

Additionally, single-cell analysis remains largely underexplored in the field. A limitation of most omics approaches is that they provide an average view of a cell population, which has been the traditional method. However, because cell populations are heterogeneous and exhibit significant stochasticity, this approach overlooks critical information. As the field moves toward more affordable and high-throughput single-cell analysis, this will become an incredibly powerful tool that should not be overlooked. The traditional population-based approach is like using a sledgehammer—it lacks the nuance needed for more precise optimization.

There are other important factors to consider, such as cell density effects and cell line stability. These are physiological challenges that impede cost-effective production and could potentially be improved through omics-based approaches. Several such opportunities are discussed throughout the paper.

Q How do the different branches of omics contribute to optimizing the cell line for AAV gene therapy manufacturing?

IR There are numerous examples where transcriptomics, proteomics, and metabolomics—three of the most prominently exploited omics—have been effectively applied. Typically, each of these approaches has been used separately, employing one or two methodologies. However, greater value emerges when they are integrated. The individual omics approaches provide distinct insights and, in many cases, their complementary nature enhances the overall understanding, delivering more substantial results when combined.

Surprisingly, however, is how little epigenomics has been utilized. This may be due to the tools not being as mature in terms of throughput and cost compared to other omics approaches, yet there is considerable potential in this area. There are some preliminary indications of the importance of epigenomics, such as when transitioning from an adherent cell line to a suspension cell line. We postulate that epigenomics could be exploited to address challenges like the cell density effect. While further study is needed, there are many exciting opportunities to explore this further.

Two further areas that have not been fully explored are glycomics, a relatively new field, and lipidomics, which similarly remains underutilized. There are already promising signs that these fields could provide valuable insights, such as understanding cholesterol composition and its potential impact on production efficiency. Many opportunities in these areas have yet to be fully tapped.

One of the strengths of omics approaches, particularly transcriptomics, is their application in chemical library screening. This can be used to identify inhibitors or enhancers to address bottlenecks in critical pathways, such as secretion, or to target alternative pathways. Additionally, omics can be applied to understanding innate immunity in the AAV-producing host cell, including overcoming antiviral and inflammatory responses. The field has seen rapid growth in recent years, and it holds significant promise for providing actionable insights.

Q Do you see AI and machine learning (ML) playing a significant role in optimizing gene therapy manufacturing processes? How much of the so-called AI 'hype' is likely to be realized tangibly, and how might these technologies help to scale production and reduce costs in the future?

RB AI and ML are certainly playing a major role, which is why we created a company dedicated to this purpose. However, there has been skepticism around the impact of AI in healthcare. There are many published articles highlighting the overhyped expectations for AI in healthcare, followed by its failure to deliver. It is important to note that AI and ML are not one-size-fits-all solutions.

The reality is that there is not enough high-quality or high-quantity data available to achieve meaningful results with simple applications of AI. To truly harness the power of AI, more sophisticated, advanced techniques are needed to incorporate specific insights into the problem that is trying to be solved. AI's promise can be realized with the right technology—specifically by using AI to build predictive models and feed those models into optimization processes. This approach can help scale production and reduce costs effectively.

IR In regards to the 'hype', I have been invited to speak at several events to educate stakeholders on how to leverage AI for decision-making in cell and gene therapy production, as well as in the broader field of biotherapeutic drug development. While there is certainly some hype surrounding AI, there are also many pitfalls. I make it a point to educate people about these challenges, and the most important one is the concept of 'garbage in, garbage out' (GIGO). As Richard mentioned, AI and ML are only as good as the data fed to them.

A key way to overcome this issue is by assembling diverse, multidisciplinary teams that include subject matter experts in biology. AI and data experts cannot be relied on alone and with the expectation of them to solve biological problems without a deep understanding of the science. A lot of success in this field comes down to understanding the fundamentals of biology. For example, when writing the aforementioned paper, I had to revisit concepts which I had learned during my postdoctoral work, such as the post-translational modification of transcription factors. These are the kinds of insights that are critical, but that may not be immediately apparent unless you have a background in biology.

Additionally, when reviewing papers, we encountered a mix of excellent and subpar quality. It is essential to think critically when evaluating the literature and apply solid subject matter expertise throughout the process.

Q As gene therapies become more personalized, how can AI and omics work together to develop treatments that are more tailored to individual patients?

RB When considering the biggest potential for AI and omics in gene therapy, I believe it lies in optimizing process development and manufacturing. This includes cell line engineering, vector construct engineering, and the development of new

"Looking further into the future, there is also the potential of cell-free production systems."

production systems and processes. This could involve changes to plasmid systems, among other innovations. These areas represent a broader scope of improvement compared to some of the more focused approaches.

Looking further into the future, there is also the potential of cell-free production systems. In these systems, the process feedstocks are combined chemically to create the drug product. The advantage of this approach is its scalability—it can easily be adjusted from large-scale production to small-scale processes for individual patients. By designing continuous, small-scale systems, scaling production up and down can be carried out much more efficiently than with traditional biological systems. While this technology is still in the developmental phase and there are no commercial products yet, it could be key to enabling individualized treatments.

IR On the other side of the coin, considering how gene therapies can be applied to larger populations should be taken into account. Currently, there is a struggle to produce enough therapies for medium-sized populations, let alone for large-scale applications like treating Alzheimer's disease. This is another major challenge—balancing cost with the ability to produce high-quality, reproducible quantities of therapy.

At BioCurie, AI modeling and multi-omics data are being leveraged to address this entire spectrum. It is not a zero-sum game, where you have to choose between reducing costs or improving quantity. With the right approach, both can be achieved, which ultimately benefits patients. This is the driving motivation for us—especially personally, as my mother and grandmother both passed away from Alzheimer's and it has become a disease that I am particularly interested in. At the same time, I meet families with children who suffer from rare diseases, or who have tragically lost their children because these therapies could not be produced in a timely, cost-effective manner.

Q Where do you see the biggest potential for AI and omics in gene therapies, and what are you most excited about in terms of future applications?

IR The beauty of both AI and omics is that they are agnostic to the specific system used. Whether it is a pure AAV viral vector production system, a semi-synthetic system, or other systems mentioned in our paper—such as Sf9 insect cells, HeLa cells, or even recent studies on AAV production in CHO cells—the potential is broad. Ultimately, there is no confinement to a single system, and this gives flexibility to adapt and evolve with the science.

The concept of 'degrees of freedom' is often discussed in scientific research. The more flexibility that there is in tools and platforms, the more that can be explored and the boundaries of the science pushed. This is one of the most exciting aspects of AI and omics in gene therapy: their ability to provide fundamental tools and platforms that can evolve alongside scientific advancements.

However, it is important to note that some omics tools remain expensive, although they are becoming increasingly affordable and high-throughput over time. The challenge is to adopt these tools, integrate them into research, and ensure that experts can fully understand and leverage their potential. For example, the power of single-cell analysis and the understanding of stochasticity are just a few areas where AI and omics can have a significant impact.

RB When it comes to AAV production, the process ultimately depends on the cells that are making the product—at least for now. The key challenge is whether they are being produced for larger doses for fewer patients or smaller doses for many people. There is extensive literature on personalized medicine, where AI and omics can be used to guide the production of therapies tailored to individual patients. While the application of AI in this context is still emerging, omics studies can certainly help us understand the specifics of individual patients.

AI's role is less clear at this stage, but with the right technology, ML can be applied to any scale—whether it's for a single patient or millions. Technologies such as cell-free production systems may allow for greater scalability and flexibility in the future. While the field is not yet at the point where cell-free production is commercially viable, AI and ML will play an important role in refining these systems and optimizing production.

IR From a more philosophical perspective, the power of HEK-omics and multi-omics—whether or not AI and ML are involved—is that they enable an unprecedented amount of sequence and design space to be explored. This gives the ability to identify optimal solutions, whether that be a process, a part of a process, or a unique cell-line design. Additionally, these approaches can uncover non-obvious or counterintuitive insights that help us overcome cognitive biases and preconceived ideas.

A good example of this is Richard's work in mechanistic modeling of AAV production. Despite his background in chemical engineering and computer science, not biology, he used modeling to develop a novel continuous AAV production process that improved productivity nearly tenfold in his first experiment. This approach would have been counterintuitive to someone like me, with a background in biochemistry and molecular biology. It illustrates the power of using AI and omics, even when the insights that they provide are unexpected.

RB It is important to note that simply applying off-the-shelf AI and ML models is often not enough. There is a large body of published articles that discuss the limitations of AI, particularly when there is insufficient data. AI models cannot extrapolate reliably beyond the data that they are trained on, which is a principle learned very early in our education: do not extrapolate data too far beyond its context.

To make AI and ML truly effective, it is essential to integrate domain-specific knowledge. This domain knowledge becomes crucial for making meaningful extrapolations. Omics plays a critical role in providing that additional knowledge. It offers deep insights into biological systems at various scales, which helps understand what is truly happening at every step of the process. This is especially important in gene therapy, where we do not have infinite data and must rely on the knowledge we can derive from the available information.

Q Finally, what are both of your priorities for your work over the next few years?

RB Our focus is on ensuring that the company is set up for long-term success. The current priority is preparing for commercial launch of our first software products for gene therapy process development and manufacturing. In parallel, we are also laying the groundwork for the next software products for additional therapeutic modalities.

IR We are developing the first AI-driven, scalable software platform for cell, gene, and nucleic acid therapy production. We are also engaged in some exciting collaborations, though we cannot disclose details publicly at this time. These partnerships involve leading entities in the field of cell, gene, and RNA therapy. These collaborations are important as we work to further enhance our platform.

Our primary goal for the foreseeable future is to develop a scalable, AI-driven platform for decision-making in cell and gene therapy production. We are progressing towards achieving our mission to transform process development and manufacturing of advanced therapies.

REFERENCE

1. Gurazada SGR, Kennedy HM, Braatz RD, Mehrman SJ, Polson SW, Rombel IT. HEK-omics: the promise of omics to optimize HEK293 for recombinant adeno-associated virus (rAAV) gene therapy manufacturing. *Biotechnol. Adv.* 2024; 19, 108506.

BIOGRAPHIES

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Richard D Braatz is the Edwin R Gilliland Professor of Chemical Engineering at MIT and the Director of the Center for Continuous mRNA Manufacturing. Professor Braatz conducts research into advanced biomanufacturing systems, and leads process data analytics,

mechanistic modeling, and control systems for projects spanning a range of therapeutic modalities, including nucleic acid and gene therapy. Professor Braatz has collaborated with more than 20 biopharmaceutical companies. He has published over 350 papers and recently published two books on AI in manufacturing, and has released more than 40 software packages. Professor Braatz is a Fellow of IEEE, IFAC, AIChE, and AAAS and a member of the US National Academy of Engineering. Professor Braatz received an MS and PhD from the California Institute of Technology, Pasadena, CA, USA and did postdoctoral research at Dupont, Wilmington, DE, USA. He was the Millennium Chair and Professor at the University of Illinois at Urbana-Champaign and a Visiting Scholar at Harvard University before moving to MIT in 2010.

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CELL & GENE THERAPY INSIGHTS

GENE THERAPY ANALYTICS AND CMC

SPOTLIGHT

Advancing quality standards for gene therapies: a USP perspective

INTERVIEW

"Our goal is to ensure that both large and small companies... have access to the analytical tools and reference standards needed for consistent, quality production."

In this interview, Abi Pinchbeck, Editor, BioInsights, speaks to Fouad Atouf, Senior Vice President, Global Biologics, US Pharmacopeia (USP), who provides insights into USP's evolving role in supporting the cell and gene therapy industry. He discusses USP's efforts to establish and refine standards to ensure the quality, safety, and efficacy of CGT products, in addition to the future of regulatory convergence in gene therapy development.

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Q What are you working on right now?

FA At USP, our rigorous scientific work on standards involves engaging with stakeholders and initiating collaborations with a broad range of partners, including developers, industry leaders and regulators. My role is centered around ensuring the quality and safety of medicines, with a specific emphasis on biologics. I work on creating solutions that address the quality of biological medicines such as cell and gene therapies (CGTs). These solutions aim to enhance the consistency and reliability of biologic medicines, supporting their growth in the market.

Q Can you discuss the evolving role of USP in supporting the CGT industry?

A USP's work in the field of CGT began over two decades ago, when we launched the first set of documentary standards specifically for CGT. Currently, there are compendial standards that apply broadly to pharmaceutical products, including biotech products and CGTs. Examples of these compendial standards apply to sterility assurance, particulate matter, and any other crosscutting topics like impurities that are part of a pharmaceutical manufacturing process.

The approach both back then and today is to enable solutions that ensure product quality, safety, and efficacy. The goal is to build and enhance the trust that we have in medicines within the marketplace, including CGTs. With respect to gene therapy, the field has evolved tremendously, and manufacturing uses a variety of platform technologies and raw materials.

The solutions provided by USP aim to maintain uniform quality of raw materials, ensure reliable performance of analytical methods, and achieve consistency in manufacturing process. These solutions include documented standards, reference material, and reference standards that are designed to meet the needs of the developers and other stakeholders. The USP's efforts to address the challenges related to characterization, validation, and quality assurance are designed to meet the industry's needs in scaling and meeting global regulatory expectations.

We continuously adapt to address the critical needs in the field. For example, in recent years, we identified AAV-based gene therapies as a front runner in the field of gene therapy and focused some of our efforts there. While we continue to work in other areas, AAV has become a focal point, and we are developing both best practice documents and physical reference materials to support the industry's needs.

Q What are the key considerations that developers should keep in mind when developing new reference standards and quality control measures for gene therapies?

A From USP's perspective, we leverage decades of experience developing reference materials for large molecules and complex biological substances. However, gene therapy presents unique challenges. The selection of reference materials is critical. It often requires testing batches from different suppliers and multiple samples to identify the right material. This process must align with regulatory frameworks like ICH Q10 and Q11 and local regulations in different geographies.

Once a reference material is chosen, extensive characterization is essential. The goal is to fully understand the quality attributes of the material due to the complexity and inherent variability. Understanding the suitability of a reference standard requires a

"AAV has become a focal point, and we are developing both best practice documents and physical reference materials to support the industry's needs."

"Developers and manufacturers need to ensure that reference materials are consistently available for quality control purposes..."

demonstration that the selected material is stable, reliable, and functional for the intended use—is it being used as a calibrator across different testing platforms, for example?

Another key consideration is availability. Developers and manufacturers need to ensure that reference materials are consistently available for quality control purposes, as the continuity of these materials is crucial for ensuring product consistency and regulatory compliance.

Q How do regulatory expectations shape the analysis of full, empty, and partially full capsids in gene therapy products, and what challenges do manufacturers face here?

FA There are several challenges manufacturers face when analyzing empty, full, and partially full capsids in gene therapy products. Analytical ultracentrifugation (AUC) has been considered a 'gold standard' method for measuring full and empty capsids, but it is difficult to reliably implement for routine release testing. Secondly, reference materials to help develop sensitive and reproducible assays are lacking. The third challenge relates to developing and validating methods to measure empty/full capsids and the increasingly emphasized partial capsids population, which can only be analyzed with certain methods. These three buckets of challenges are further amplified when considering product purity and consistency as manufacturing processes scale up.

Regulatory requirements continue to evolve to align with the scientific community's growing understanding of AAV gene therapies. We have known that the full viral capsid is essential for product efficacy and we now appreciate that empty and partially full capsids are non-functional and immunogenic impurities that also impact efficacy. Reducing them is incredibly important, as the amount of product that can be safely dosed is often constrained by the immunogenicity risk profile. Likewise, it is critical to the success of a product to have reliable analytical methods to quantitate empty and partial capsid impurities. Current best practice is to develop, validate, and bridge multiple orthogonal methods. Bridging between multiple methods is difficult because they are based on different biophysical properties. AUC measures a combination of particle size, shape, and density. Other methods are based on optical properties such as multi-angle light scattering, UV absorbance, or interferometry, which can also be coupled with separation techniques like chromatography. One of the most routine release tests is a combination of capsid protein immunoassays, such as ELISA, and PCR-based genome quantitation. Reference standards are important tools that help bridge between different methods.

Product consistency and quality control are also critical. Regulatory agencies expect consistency in the ratio of full to empty capsids, as this has an implication on the therapeutic dose and can influence product quality and safety. Manufacturers must also ensure rigorous in-process controls and release testing to monitor the capsid CQAs throughout the production of the gene therapy product.

Q What advice would you give to developers aiming to create an early-stage Target Product Profile (TPP) to guide product development and CMC compliance?

FA Developing a successful product development strategy requires holistic consideration of the product goals and current industry and regulatory best practices. It is important for developers to meet with the regulatory agency early in development, e.g., at the preclinical stage, and seek guidance. These conversations can be very impactful in determining how to advance with product development.

Q What are the key areas of global regulatory divergence impacting gene therapy today, and how can organizations like USP help?

FA Regulatory divergence is a risk in gene therapy, especially as the field expands globally. The key areas of divergence include differences in regulatory requirements, approval processes, and quality standards across regions.

Early engagement across stakeholders is key, and industry working groups and consortia can play an important role in raising awareness around the need for more regulatory convergence. We should not underestimate the power of those working groups, such as the ICH, and regional regulatory platforms like the Asia-Pacific Economic Cooperation (APEC), which covers a big portion of the globe. These collaborative networks can also be used as a mechanism to bring stakeholders together to address commonly identified challenges. Pharmacopoeia like USP also work in that context.

At USP, we collaborate and leverage those mechanisms to create a framework of internationally recognized standards. If there is a consensus on a method that is suitable and everyone agrees upfront, we can address convergence from the beginning. We develop internationally recognized standards with that in mind and provide education on how to use them.

USP is a Center of Excellence in the APEC region, and we work collaboratively with regulators in the region, including US FDA, Health Canada, Korea Ministry of Food and Drug Safety (MFDS), and others, to provide training for regulators. To deploy a best practice document or standard, we sometimes take the work that has been done by an industry group, such as a white paper, and use it as a starting point to create a standard. This ensures harmonization and convergence from the very beginning.

Q How do you foresee regulatory agencies addressing long-term safety and monitoring of gene therapies post-market?

FA Post-approval changes and overall product lifecycle management are some of the biggest challenges in this context. The ability to control potential product drift and an occurrence like a change in a raw material supplier are potential concerns to address. Ensuring that comparability protocols and strategies are in place to help create consistent manufacturing is important. The key word is comparability here. The US FDA's

2023 guidance on comparability is a useful resource, focusing on manufacturing changes and comparability for human cellular and gene therapy products [1].

The field is constantly evolving with new technologies. As a community, and as a group of stakeholders, we will learn more about the challenges we are facing and how to tackle them, and those lessons can be incorporated into future regulatory guidance.

Q What are your own key goal goals and priorities in your own work over the next 1–2 years?

FA Our strategy to support gene therapy product development is in line with the overall strategy for biologics and pharmaceuticals, though there are some unique aspects to be addressed. For example, we have existing documentary standards supporting CGT to update and expand upon. As gene therapy continues to evolve, USP's focus will be on developing standards for viral vectors like AAV and lentivirus, cell-based products such as CAR-T and CAR-NK gene-modified cell therapies, and addressing new challenges in manufacturing and testing [2,3]. Additionally, we will be launching new solutions to support microbial contamination control strategies for medicines including CGT.

Our goal is to ensure that both large and small companies, especially those developing therapies for rare diseases, have access to the analytical tools and reference standards needed for consistent, quality production. I believe the only way to make an impact is for our work to be executed in collaboration with the stakeholders who will use those best practices and standards themselves, including those from small startup companies, academic groups, manufacturers, and regulators, to ensure that what we develop meets the needs of the entire CGT industry. Additionally, promoting regulatory convergence remains a priority, as it will facilitate global adoption of these therapies and help ensure their widespread availability to patients in need.

REFERENCES

1. ISPE. *Guide: ATMPs—Recombinant AAV Comparability and Lifecycle Management*. Jan 2024. https://ispe.org/publications/guidance-documents/guide-atmps-raav-lifecycle-mgmt.
2. Blaszczyk A, Carreras González A. Overcoming challenges in gene therapy analytics. *Cell & Gene Therapy Insights* 2024; 10(6), 889–902.
3. Kim M, Rautela N, Khedkar S. Solving bioassay challenges for cell and gene therapies. *Cell and Gene* Jul 1, 2022. https://www.cellandgene.com/doc/solving-bioassay-challenges-for-cell-gene-therapies-0001.

BIOGRAPHY

Fouad Atouf is Senior Vice President, and Head of the Biologics Program at the US Pharmacopeia (USP) where he oversees standards development, stakeholder engagement,

and industry collaborations, all in support of ensuring the quality and safety of biological medicines. Dr Atouf has been at USP for over 15 years and served in multiple leadership roles developing quality standards for biologics and establishing relevant reference material programs. In addition to leading the work on compendial standards, Dr Atouf launched and implemented the biologics strategy focusing the implementation of technologies and standardization to support manufacturing and testing biological medicines. He has implemented new engagement models and collaboration approaches with academia, biopharma industry, and global government agencies. Dr Atouf has a strong background in the regulations and standards for pharmaceutical products including biologics, and advanced therapies. Dr Atouf is the author of numerous publications and is a frequent speaker at national and international pharmaceutical and regulatory scientific events. He holds a PhD in Cell Biology from the Pierre & Marie Curie University, Paris, France.

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CELL & GENE THERAPY INSIGHTS

GENE THERAPY ANALYTICS AND CMC

SPOTLIGHT

A path toward universal agreement on fulfilling the need for rapid and reliable sterility assays in gene therapy

Qin Xiang

VIEWPOINT

"By fostering collaboration among manufacturers, regulators, and technology developers, the gene therapy sector can work toward universal agreement..."

Cell & Gene Therapy Insights 2025; 11(3), 399–403 · DOI: 10.18609/cgti.2025.048

Gene therapy has emerged as one of the most promising medical advancements, with the potential to cure previously untreatable diseases. Since the approval of Gendicine in China in 2004 for head and neck cancer, the field has gained momentum, with 33 gene therapies approved globally by Q4 2024. Over 2,100 gene therapy products are currently in development, underscoring a transformative shift in disease treatment [1]. However, this rapid growth highlights the critical need for innovation in sterility testing to ensure the safety and efficacy of these therapies delivered timely to patients.

THE CHALLENGE OF STERILITY TESTING IN GENE THERAPY

Gene therapy products are highly diverse, including viral and non-viral vectors, engineered vectors, and patient-derived cellular therapies. Manufacturing these products is inherently complex, with each step in the process carrying a potential risk for contamination. Moreover, many gene therapy products have short shelf-lives or must be administered urgently, necessitating timely sterility testing to ensure patient safety. Traditional sterility testing methods, such as compendial culture-based assays, take several weeks to yield results, which is inadequate for rapidly emerging gene therapies.

As gene therapy products become more complex, the need for faster and more reliable sterility testing is critical. The increasing diversity of gene therapy products, coupled with manufacturing complexities, demands sterility assays that can be applied universally across various matrices.

EMERGENCE OF RAPID MICROBIAL TESTING METHODS

In response to these challenges, the industry has increasingly turned to rapid microbial testing methods (RMTMs) that deliver results within hours or days. These technologies, including ATP bioluminescence, CO_2 or respiration-based methods, PCR-based testing, Raman spectroscopy, and solid-phase cytometry, offer significant advantages over traditional methods in speed and reliability [2].

For example, ATP bioluminescence assays can detect microbial contamination in 24–48 h by measuring ATP in living cells. Similarly, PCR-based methods can provide results within hours, offering high specificity and sensitivity by amplifying specific microbial DNA sequences [2]. Next-generation sequencing (NGS), which combines long-read nanopore sequencing and machine learning, holds the potential to detect low-abundance microbial contaminants in under 24 h. While still emerging, the NGS-based technology could revolutionize contamination detection and identification, enabling more efficient manufacturing processes and reducing product validation times [3].

RMTMs not only promise faster results but also allow for earlier detection of contamination during manufacturing, thus mitigating contamination risks before final product release. These methods align with regulatory guidelines, including those from the US FDA and USP, which advocate for sterility testing at multiple stages of manufacturing to ensure early detection [4].

BARRIERS TO RMTM ADOPTION

Despite their clear advantages, RMTMs have not been widely adopted in gene therapy manufacturing. Several factors contribute to this hesitation [2]:

- Uncertainty and validation: RMTMs require rigorous validation to demonstrate equivalency to traditional methods. This process is resource-intensive and may require specialized reference materials and interlaboratory studies;
- Cost and resources: the initial costs of acquiring and validating RMTMs can be high, particularly for smaller manufacturers and research labs;
- Regulatory acceptance: while regulatory agencies such as the FDA, EMA, and PMDA have approved RMTMs for use, manufacturers often perceive regulatory hurdles, particularly regarding the global standardization of these methods.

These challenges have slowed the uptake of RMTMs in gene therapy

manufacturing, despite the fact that guidelines from organizations like the USP, PDA, and European Pharmacopeia are available to help manufacturers navigate the validation process.

PATH FORWARD: COLLABORATION IS KEY

Collaboration among stakeholders is essential to overcoming these barriers. Initiatives like the NIST RMTM Consortium have played a vital role in advancing RMTM adoption by bringing together regulators, technology developers, and manufacturers to work on standardization, validation, and reference material development [2].

The recent inclusion of ATP-based and CO_2-based RMTMs in the updated USP Chapters <73> and <72>, effective August 1, 2025 [5,6], marks a significant step toward the standardization of these methods in the industry. This move signals growing regulatory acceptance of rapid sterility assays and sets a precedent for future developments in the field.

Additionally, the ongoing efforts by the NIST RMTM Consortium to support the development of reference materials and facilitate interlaboratory studies will help build confidence in other promising RMTM technologies, such as the PCR-based methods [2].

MOVING TOWARD UNIVERSAL ADOPTION

The path to universal adoption of RMTMs in gene therapy requires continued collaboration between regulatory agencies, technology developers, and manufacturers. As the gene therapy field continues to expand, manufacturers must collaborate with regulators to develop tailored validation plans that address the specific risks associated with their products. These plans should consider factors like risk assessments, organism-specific validation, and method comparability. Regulatory agencies may further support adoption by offering clearer guidelines and faster approval pathways for RMTMs.

With ongoing support from initiatives like the NIST RMTM Consortium, the gene therapy industry can develop a consensus on the next most effective and reliable sterility testing methods.

CONCLUSION

The rapid growth of gene therapy underscores the increasing need for fast, reliable sterility testing. RMTMs offer a promising solution, enabling quicker detection of microbial contamination and reducing the time needed for product release. However, for these methods to be widely adopted, the industry must overcome challenges related to validation, cost, and regulatory acceptance. The recent inclusion of RMTMs in USP chapters provides a strong foundation for the standardization and adoption of these methods. By fostering collaboration among manufacturers, regulators, and technology developers, the gene therapy sector can work toward universal agreement on rapid sterility assays, ensuring the safe and timely release of these life-saving therapies.

With continued efforts from initiatives like the NIST RMTM Consortium and growing regulatory support, the future of sterility testing in gene therapy looks increasingly aligned with the adoption of RMTMs, paving the way for safer, more efficient gene therapy manufacturing.

REFERENCES

1. American Society of Gene & Cell Therapy. *Gene, Cell, and RNA Therapy Landscape Report, Q4 2024 Quarterly Data Report*. Jan 2025. https://www.asgct.org/global/documents/asgct-citeline-q4-2024-report.aspx.
2. Lin NJ, Servetas SL, Chowdhury NN, *et al*. *Report from the 2022 NIST Rapid Microbial Testing Methods (RMTM) Workshop*. Aug 2023; National Institute of Standards and Technology. https://doi.org/10.6028/NIST.SP.1292.
3. Trutt JPB, Natarajan M, Lee E, *et al*. Machine-learning driven detection of adventitious agent detection in T cell cultures using long read sequencing. *Microbiol. Spectr*. 2023; 11(5), e0135023.
4. USP. *<1071> Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach*. US Pharmacopeia.
5. USP. *<73> ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products*. US Pharmacopeia.
6. USP. *<72> Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products*. US Pharmacopeia.

BIOGRAPHY

Qin Xiang has over 20 years of experience in the biotechnology and pharmaceutical industries, specializing in Analytical Development and Quality Control (QC). At Excision BioTherapeutics, Dr Xiang led analytical and bioanalytical development supporting CMC and clinical activities for CRISPR-based gene therapy, utilizing diverse methodologies, including ddPCR and NGS. She played a key role in successful FDA submissions and the on-time delivery of clinical trial materials and clinical sample testing results. At Sio/Axovant Gene Therapies, Dr Xiang helped facilitate the company's transition from small molecules to gene therapies, overseeing analytical/CMC activities and supporting the first gene therapy IND filing and FDA approval by addressing analytical related clinical hold and non-hold items. Previously, Dr Xiang held roles at Pfizer, AbbVie, and BMS, supporting complex analytical/CMC activities and regulatory filings for small molecules. Dr Xiang holds a PhD in Environmental Science and MS and BS degrees in Chemistry, with multiple awards from the companies for her contributions to drug development and commercialization. Currently, as Founder and Independent Consultant at QX Bio & Pharma Consulting, LLC, she provides strategic support in Analytical and QC activities for clients across various drug development stages.

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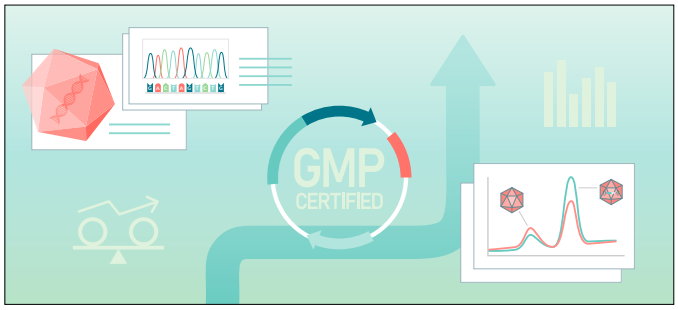
CELL & GENE THERAPY INSIGHTS

CONTENTS VOLUME 11 · ISSUE 3



CELL & GENE THERAPY INSIGHTS

TRANSLATIONAL R&D

Overcoming translational challenges to advance cell therapy for neonatal brain injury

INTERVIEW

"...the conversation is shifting towards how we can provide broad and equitable patient access to these therapies..."

HAON Life Sciences, which leads the CanVas consortium and is focused on developing a cell therapy targeting infant brain injury, has recently received a €10.7 million non-dilutive grant through the Disruptive Technologies Innovation Fund, which is managed by the Department of Enterprise, Trade and Employment in Ireland and administered by Enterprise Ireland. In this interview, Jokūbas Leikauskas, Editor, BioInsights, speaks to Mikey Creane, Translational Research Manager, HAON Life Sciences, about the progress of the company's cell therapy for neonatal hypoxic-ischemic encephalopathy (HIE) and strategies to overcome translational challenges and accelerate clinical development.

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Q What are you working on right now, and what are the key focus areas at HAON Life Sciences?

MC HAON Life Sciences is a biotech company based in Dublin, Ireland, developing the CanVas Cell therapy Platform, which is a new class of endothelial

colony-forming cell (ECFC) therapies. These cells are a rare cell population with robust regenerative potential. The company's key focus areas over the next few years are brain injuries, neurodegenerative diseases, and rare blood disorders.

We have already generated promising preclinical data in early brain injury for a rare indication called HIE, which occurs when a baby's brain does not receive enough oxygen or blood flow just before or shortly after birth. HIE is one of the leading causes of infant mortality and long-term neurological disability. There are currently no approved therapies on the market to treat this disease, so there is a significant unmet medical need.

According to our data, a single dose of CanVas-001 can promote blood vessel repair and reduce blood-brain barrier permeability. Additionally, we have observed lower levels of brain inflammation, along with an increase in neuroprotection and repair. For these reasons, we are also exploring the feasibility of using CanVas-001 to treat other debilitating adult neurodegenerative diseases.

Q Can you tell us more about your role in the International Society for Cell & Gene Therapy (ISCT)?

MC ISCT is a global society with over 4,000 members worldwide focused on the preclinical and translational aspects of cell and gene therapy (CGT). I have been an active member of ISCT since 2022, and I have to say that it's been wonderful for me from a professional point of view. I have learned so much more about the scientific, regulatory, ethical, and industry challenges within the field, and my network has increased exponentially. What I really love about ISCT is the community's willingness to share their wisdom and experience with early-stage professionals like me.

I am also an active member of the Early-Stage Professionals (ESP) Committee, where I work with my colleagues to run a mentoring program. In this program, ESPs are mentored by experts in the CGT field over the course of a year. We match mentors and mentees with each other based on their subject matter expertise and training. I have benefited so much from this mentoring program. I have been mentored by some wonderful people who have helped me grow both personally and professionally. To further increase ESP engagement within the field, we also run a series of educational webinars and workshops throughout the year focused on career guidance and important CGT topics.

As part of the Leadership Development Program that ISCT offers, I am also involved in multiple other ISCT committees. For instance, I recently completed a 2-year term on the Mesenchymal Stromal Cell Committee, and I am also a member of the Ethics of Cell and Gene Therapy Committee and the European Regional Executive Committee. This opportunity has enabled me to learn and obtain guidance from Key Opinion Leaders within the field. The practical experience that I am getting on these committees has been a real immersive learning experience for me from a scientific, ethical, and leadership point of view.

The ISCT Annual Meeting, which is the largest translational CGT conference, will take place in New Orleans, USA, this year from 7 to 10 May. I am always excited to attend this meeting as I see it as a place where I can go to put my finger on the pulse to learn not only what is happening today in the field but also what future developments are in the pipeline. The session I am looking forward to attending the most at ISCT 2025 is the Launch and Patient Access Plenary titled *Therapies Without Borders: Getting Therapies to Patients in*

"Along with establishing a robust supply chain, it is essential to find suppliers who truly understand the process and what you, as the developer, are aiming to achieve."

Need Around the World. This session is being chaired by industry veteran Phil Vanek and has an amazing lineup of speakers.

Q What are the critical key challenges in advancing the clinical development of allogeneic cell therapy candidates? How can these hurdles be addressed?

MC First and foremost, securing high-quality starting materials is a key challenge. Along with establishing a robust supply chain, it is essential to find suppliers who truly understand the process and what you, as the developer, are aiming to achieve. It is crucial to analyze and risk-assess each element of the supply chain and ensure that you are partnered with reliable suppliers who can match your needs and demands. For example, can your supplier provide materials such as cell culture medium and antibodies at GMP-grade to support your transition to larger-scale clinical production?

Manufacturing at scale is one challenge, but doing so at a reduced cost is another. The field has made great progress in developing innovative automated technologies that enable developers to scale up quicker and more cost-effectively. However, I do think there is room for refinement. It is important to take time to choose the cell processing and manufacturing technology that best suits your cell type, as we know it is not a one-size-fits-all model. Ensure that the technology enables you to scale up as well as scale down as needed. As CGT developers, when we are picking manufacturing technologies, we want to ensure that the technology will enable the lower-scale process development experiments but also provide us with assurance that it will support the larger processes that will be needed when we want to scale up. Getting this right at an early stage can be difficult.

Another key challenge is the lack of trained personnel in the field, especially in the area of CGT process development and manufacturing. Training in the manufacturing of CGT can be very intense, especially for individuals who do not possess a background in this area. To address this, I think we need to develop more specialized courses like those offered by the University of Galway in Ireland, including MSc in Regenerative Medicine and MSc in Cellular Manufacturing and Therapy courses. In addition to this, great training programs are being developed by the ISCT Institute of Training and Development. These accredited courses are delivered by Key Opinion Leaders in the field and are focused on the manufacturing of novel CGTs and cover all elements of the translational process. Increases in the development of courses like these will be important for the training of the future CGT workforce. But it is not just about training in theory—real-life experience is just as important. If we can increase the number of practical training programs at manufacturing facilities and CDMOs to help these graduates/trainees get real-life experience, it would be incredibly beneficial.

Q Why is it important to consider translational aspects early in the development of cell therapies?

MC The development of cell therapies should start with a good preclinical *in vivo* design. This is crucial because the results of *in vivo* preclinical studies set the foundation for your work as you move towards clinical translation. Early considerations for your proof-of-concept studies include choosing a suitable animal model, animal sex (important for assessing any sex-related differences), and ensuring that these animals are exposed to the same environmental factors, such as housing, bedding, food, water, and handling techniques. If your experiments require a novel surgical procedure, which often can be the case, these procedures must be carried out consistently by the same surgeon or surgical team. If any changes or refinements are required to the surgery or cell delivery process, the surgeon must be given enough time to hone their skills prior to starting these preclinical studies. It is all about minimizing variability.

Ideally, all procedures and processes should be carried out as close to good laboratory practice (GLP) principles as possible. I know this can be challenging for academic institutions that have small research teams and limited resources, but as GLP-like as possible, the better.

Other crucial aspects that are often overlooked include randomization and blinding. The block randomization method is a must to ensure a balanced treatment assignment over a period of days. Blinding is critical at every stage of the study execution—from delivering the CGT to data capture at each timepoint, and most importantly, during data processing and analysis. In essence, every investigator involved *in vivo* preclinical studies, where possible (acknowledging that this may not be possible for small teams), should be blinded to minimize any potential bias. At the end of the day, we are translating therapies to patients, hence, we want to ensure that we are removing all elements of bias so that we are reliably able to reproduce the study results consistently.

It is important to define the critical quality attributes (CQAs) of your cell therapy as early as possible in order to develop a strong manufacturing process. Essentially, it is fundamental to examine all the physical, chemical, and biological characterization aspects of the therapy. This allows developers to really understand their therapy and enables them to develop a robust manufacturing process. It is important to know your process inside and out. You need to continually test it until you break it. Knowing where the breaking points on your manufacturing process are really helps identify the critical points and timeframes for measuring CQAs. In addition, investing early in the development of a matrix of quantitative assays that measure the biological activity or potency of your cell therapy is so important. These assays will help you monitor, throughout your product development, the impact of any changes you make to your manufacturing process (for example, change of culture vessel, cell seeding density, culture medium) and will help identify any donor-to-donor variability in your starting material.

"...investing early in the development of a matrix of quantitative assays that measure the biological activity or potency of your cell therapy is so important."

Q How has the translational cell therapy field changed in the past 10 years?

MC When I first started, there was a lack of resources and published information, particularly on toxicology testing of cell therapies and potency assay development. However, over the last 10 years, we have seen an immense increase in these publications, meaning there are far more resources available now.

During that time, the science has also made tremendous progress. For example, we have gained a deeper understanding of cell biology, and more specifically, how cells interact with the immune system. There have also been vast improvements in lentiviral and AAV vector development. On top of that, CRISPR-Cas9 technology has helped enhance the development of next-generation CGTs.

Previously, the field was primarily focused on getting therapies to first-in-human testing and then aiming for market approval. But now we are seeing many more late-stage clinical trials and actual approvals. For example, we have seen approvals for multiple different CAR-T cell therapies and mesenchymal stromal cell (MSC) therapies, such as Takeda's Alofisel® (although that was recently withdrawn) in Europe and Mesoblast's RYONCIL® in the USA.

Over the last 10–15 years, there has been a lot of attention on CGT development, manufacturing, and the path to regulatory approval. However, I think we are now entering a new phase. With more therapies approved, the conversation is shifting towards how we can provide broad and equitable patient access to these therapies and how reimbursement strategies can be integrated earlier in the development process.

Q What are your goals and priorities over the next 1–2 years, both for yourself and for HAON Life Science as a whole?

MC HAON, which leads the Can-Vas Consortium, has just received a €10.7 million non-dilutive grant fund via the Disruptive Technology Innovation Fund, which is a fund managed by the Department of Enterprise, Trade and Employment and administered by Enterprise Ireland.

This is an extremely exciting project that brings together industry, clinicians, and research institutions to develop a new class of cell therapy with ECFCs and MSCs. It is a 3-year project that will allow HAON to develop Can-Vas 001 to a Phase 1b first-in-human study in infants with HIE, as well as progress the CanVas platform to Phase 1 for adults.

As Translational Research Manager, my goal for the next 3 years is to coordinate all the project's activities to ensure we meet our clinical timelines. In addition to this, I will lead the preclinical GLP toxicology and biodistribution studies.

It is an exciting time for me, and I am relishing the challenge. I will draw on the experience that I obtained from my time at the Centre for Cell Manufacturing Ireland (academic GMP facility) and the Regenerative Medicine Institute (REMEDI) at the University of Galway, where I worked previously before joining HAON Life Sciences. Here I was involved in both programme management and preclinical package development for two MSC clinical trials: CLI trial [1] and NEPHSTROM trial [2]. The CLI trial was a Phase 1b clinical trial testing autologous MSCs in no option patients with critical limb ischemia. NEPHSTROM

was a Phase 1a/2b placebo-controlled clinical trial testing MSCs in patients with Diabetic Kidney Disease. During my time in REMEDI, I also worked on multiple preclinical projects that investigated the use of ECFCs and MSCs as a combinational treatment for CLI. Overall, it is an exciting time for me, and I cannot wait to see what the next few years bring.

REFERENCES

1. Mohamed SA, Howard L, McInerney V, *et al.* Autologous bone marrow mesenchymal stromal cell therapy for 'no-option' critical limb ischemia is limited by karyotype abnormalities. *Cytotherapy* 2020; 22(6), 313–321.
2. Remuzzi P, Remuzzi G, Griffin MD, *et al.* Safety and preliminary efficacy of mesenchymal stromal cell (ORBCEL-M) therapy in diabetic kidney disease: a randomized clinical trial (NEPHSTROM). *J. Am. Soc. Nephrol.* 2023; 34(10), 1733–1751.

BIOGRAPHY

Mikey Creane obtained his BSc in Physiology, MSc in Regenerative Medicine, and PhD in Translational Cell Therapy at the University of Galway, Galway, Ireland between the years of 2005 and 2016. After his Postdoctoral research training, Dr Creane worked for many years as a Research Fellow and Programme Manager in the Regenerative Medicine Institute (REMEDI) at the University of Galway, where he worked on translating stromal cell therapies from laboratory studies to first-in-human testing. Dr Creane has also worked as Strategic Lead for the Centre of Cell Manufacturing Ireland, which is an academic Good Manufacturing Practices facility located at the University of Galway. In September 2024, Dr Creane joined HAON Lifesciences as Translational Research Manager, where he is leading the preclinical development of novel cell therapies for rare diseases. Dr Creane is an active member of the International Society for Cell and Gene Therapy (ISCT) and serves on the Early Stage Professionals Committee, Committee on the Ethics of Cell and Gene Therapy and European Regional Executive Committee.

Mikey Creane, Translational Research Manager, HAON Life Sciences, Dublin, Ireland

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CELL & GENE THERAPY INSIGHTS

TRANSLATIONAL R&D

Exploring translational challenges and strategies for advancing gene therapies in neurodegenerative diseases

INTERVIEW

"...the advanced therapy community is working to develop faster, more cost-effective manufacturing processes..."

Jokūbas Leikauskas, Editor, BioInsights, speaks to Joseph Scarrott, Process Development/R&D Lead, Gene Therapy Innovation and Manufacturing Centre (GTIMC) at the University of Sheffield, about translational gene therapies for neurodegenerative diseases, focusing on efforts to improve safety, efficacy, and cost–effectiveness, and accelerate research to clinical-grade manufacturing.

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Q What are you working on right now?

JS We officially opened as a brand-new facility on October 2 last year. We are mainly focusing our efforts on validating our clean rooms, personnel, and instrumentation, as well as getting our quality management systems up and running. This is all in preparation for an MHRA licensing submission later this year.

> "[the advanced therapy community] needs to show that AAV-based gene therapy products have greater efficacy compared to existing treatment options."

In terms of process development, which falls under my purview, we are working through our existing small-scale AAV manufacturing processes to see where we can make the most impactful improvements before transferring them to our large-scale manufacturing space. We are taking a holistic approach, evaluating all components of the process with respect to quality, safety, and efficacy, while refining the small-scale process and carrying out any necessary scale-up activities.

Q What are the key focus areas at the GTIMC? What role does GTIMC play as part of the Innovation Hubs for Gene Therapies (IHfGT)?

JS The IHfGT is a Medical Research Council/LifeArc-funded initiative designed to advance the clinical development of new genetic treatments through the production of GMP viral vectors, alongside translational support and regulatory advice. The GTIMC (based in Sheffield, UK) is one of the Hub members, together with NHS Blood and Transplant in Bristol, and the London Hub—which is a collaboration between King's College London, Royal Free NHS Foundation Trust, and UCL

At the GTIMC, we have four key focus areas: manufacturing, process innovation, translational support, as well as training and skills. These four pillars of our centre enable us to support gene therapy research in the UK as broadly as possible.

Additionally, as part of the IHfGT, we can leverage our strong links to academia. Being part of the University of Sheffield, we have direct connections to the academic side of gene therapy research and translational work. As we are within the Division of Neuroscience at the university, we understand the challenges researchers face in bridging the translational gap—moving from promising laboratory results to safe and effective treatments in the clinic.

Q What are the critical key challenges in advancing the clinical development of AAV-based gene therapy products?

JS From our perspective, there are three main challenges. Firstly, we need to show that AAV-based gene therapy products have greater efficacy compared to existing treatment options. This can be particularly challenging in cases of extremely rare diseases with very small patient populations, where conducting controlled trials or clinical studies is difficult.

The second challenge is safety. We need to assure both the public and patients that our treatments are safe and that the medicines meet rigorous safety standards.

The third major barrier is cost. No matter how safe or effective we can demonstrate these medicines to be, it becomes almost irrelevant if the cost of production is so high that

it makes them unaffordable to patients or healthcare systems. The lack of accessibility to potentially life-saving treatments is a major challenge in translation, raising ethical concerns if people cannot access the care they need. Additionally, high costs pose a barrier to the broader adoption of gene therapies across the healthcare spectrum.

In response to this, the advanced therapy community is working to develop faster, more cost-effective manufacturing processes—improving efficiency, yield, and potency to ultimately reduce the cost per dose.

Q Regarding partial AAV capsid formation and removal, what are the key strategies you are implementing to address this hurdle?

JS Our quality control laboratory is equipped with a mass photometer, which will allow us to resolve and quantify the proportion of partial capsids in our purified viral preparations. We intend to use this not only for release assays but also to identify points in the manufacturing process where we can reduce the number of empty and partial capsids in the final product.

Currently, we expect most of that removal to occur during downstream purification steps, but we are also exploring ways to prevent the formation of partials at the upstream stage. Instead of simply removing them, we aim to stop their formation altogether by optimizing transfection parameters and evaluating different plasmid concentrations and compositions.

At GTIMC, mass photometry is the most appropriate method because it is relatively easy to use and requires a much smaller sample volume compared to other techniques. It still provides high resolution but does not have the same barriers to entry compared to methods such as analytical ultracentrifugation, where limited product availability can be a constraint, or charge detection mass spectrometry, which requires highly specialized equipment and expertise.

In the future, I believe the field will continue to rely on orthogonal approaches to quantify partials in manufactured material. However, I am hopeful that as we gain a deeper understanding of the dynamics of AAV packaging, we can prevent partial formation in the first place rather than relying heavily on downstream optimization. Any effort to filter out partial capsids in downstream processes risks reducing overall yield, so improving our biological understanding of partial capsid formation in the first place could lead to better control over their removal and ultimately make them less of a concern in final product formulations.

Q How do you approach scaling up the production of AAVs, while maintaining high quality and consistency? What are some of the process innovations GTIMC is exploring in this area?

JS As part of our scale-up process, we aim to align as closely as possible with quality-by-design (QbD) principles, particularly in terms of how process changes impact the product's critical quality attributes. In particular, we are very interested in exploring how digital modelling could potentially accelerate scale-up, starting from the smaller R&D scale.

"...our focus is on creating a reliable process that consistently produces high viral titers with the lowest possible number of impurities."

Unlike scaling up pharmaceutical drugs, where each production cycle yields the same product, every gene therapy client we work with will essentially require a new drug, whether that involves a different serotype or a transgene. For instance, there may be aspects of a specific serotype or transgene that could introduce variability into the process, which presents additional challenges. To address this hurdle, our focus is to identify potential sticking points early and implement rigorous controls during development. By thoroughly understanding how elements of the small-scale process affect downstream outcomes, we can proactively integrate those insights into our scale-up strategy.

Q How does GTIMC ensure the safety and long-term efficacy of gene therapies, particularly for rare and complex neurodegenerative diseases?

JS As part of our MHRA licensing requirements, we need to demonstrate control over both our processes and products. All of the development activities for clinical-grade material are conducted with patient safety as the primary goal, in line with QbD principles. We have a strong quality assurance and quality control team, ensuring that any clinical-grade material undergoes stringent characterization before release to confirm it meets all required medicinal standards.

From a process development perspective, our focus is on creating a reliable process that consistently produces high viral titers with the lowest possible number of impurities, including empty and partial capsids. We are also continuously working to maximize the potency of our products. The hope is that in future clinical trials, we can avoid the toxicities observed in patients treated with extremely high doses. By increasing potency, we aim to reduce the number of vectors required per dose, ultimately making the therapy safer.

Q GTIMC is driving experimental science into global clinical trials to combat devastating neurodegenerative diseases and change millions of patient lives. What do you think the future holds for gene therapies in treating these diseases?

JS From my perspective, the future of gene therapies for neurodegenerative diseases looks very bright. We have already seen impressive results in treating monogenic diseases, such as spinal muscular atrophy with Zolgensma®. This progress is likely to continue, particularly as we advance in vector design, vector targeting, and improved gene editing techniques. However, the treatment of polygenic diseases, especially those with unknown etiology, will continue to be very challenging.

Our colleagues at the Sheffield Institute for Translational Neuroscience and other academic groups in the UK have made significant strides in identifying disease-causing

pathways and generating preclinical models of these complex diseases. This opens up a major possibility for using gene therapies as disease modifiers, rather than outright cures. In essence, these therapies may not cure diseases but could positively modify their progression, particularly for diseases that share common pathways.

Q What are your goals and priorities over the next 1–2 years, both for yourself and for GTIMC as a whole?

JS We aim to achieve MHRA licensing and establish fully GMP-compliant AAV manufacturing at a 50-liter scale. The goal of my team in particular is to successfully develop processes for two to three different serotypes at a small scale within the R&D laboratory and be able to offer these to clients in both R&D and GMP-like grades. Ultimately, we want to accelerate the products from the academic laboratory to clinical-grade manufacturing as quickly as possible. We hope to have developed a solid pipeline for this within the next few years.

BIOGRAPHY

Joseph Scarrott has been working with adeno-associated virus (AAV) since 2012, both as a tool for translational gene therapy research and as a manufacturing target for process development. He completed his doctorate degree in Neuroscience at the University of Sheffield investigating off-target effects of an AAV-mediated gene therapy for SOD1-linked amyotrophic lateral sclerosis and is a co-inventor on patents for both a gene therapy treatment for hereditary spastic paraplegia 47 and an improved manufacturing process for AAV. Currently, Joseph is responsible for AAV manufacturing process development and research activities at the Gene Therapy Innovation and Manufacturing Centre (GTIMC) at the University of Sheffield.

Joseph Scarrott, Process Development/R&D Lead, Gene Therapy Innovation and Manufacturing Centre (GTIMC), University of Sheffield, Sheffield, UK

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CELL & GENE THERAPY INSIGHTS

REGULATORY INSIGHTS

INTERVIEW

Advancing optogenetic therapies for retinal diseases: a regulatory vision

INTERVIEW

"We are at an exciting stage in our drug development program, and I am committed to advancing Nanoscope's mission, driving regulatory success, and supporting our pipeline's growth."

Jokūbas Leikauskas, Editor, *Cell & Gene Therapy Insights*, speaks to Ananta Ayyagari, Director of Regulatory and Intellectual Property, Nanoscope Therapeutics Inc. about the regulatory landscape of optogenetic therapies for retinal degenerative diseases, and clinical trial progress. They also discuss the evolving patent landscape for gene therapies, and the regulatory challenges in developing and commercializing such transformative treatments.

Cell & Gene Therapy Insights 2025; 11(3), 321–326 · DOI: 10.18609/cgti.2025.036

Q Can you tell us a little about your background and current role?

A I hold a doctorate in Chemistry with a background primarily focused on synthesizing novel cationic lipids for gene transfection applications. During my postdoctoral research, I developed expertise in liposome-based drug and gene delivery systems. As part of my doctoral training, I gained valuable experience in patent

"Our gene therapy delivers light-sensitive MCOs into retinal cells with the potential to detect ambient light and improve activities of daily living."

drafting, having served as an inventor on multiple patents. This exposure sparked my deep interest in the field of intellectual property (IP).

At Nanoscope Therapeutics, I contribute to both IP and Regulatory Affairs. In my role as the Director of Regulatory Affairs, I am actively involved in authoring and submitting key documents for IND and biologics license applications, regulatory strategy development, orphan drug designation requests, fast track designation submissions, and initiation of clinical trials. I am also involved in interactions with regulatory authorities, including the preparation of briefing packages and related tasks. As the director of IP, I oversee all aspects of patent drafting and prosecution, as well as trademark filings, in collaboration with our patent attorneys.

Q Can you give us a background to Nanoscope's pipeline?

A Nanoscope is bringing transforming mutation-agnostic therapies to patients suffering from retinal degenerative diseases. Cofounded by Samarendra Kumar Mohanty and Sulagna Bhattacharya, Nanoscope is developing optogenetic gene therapies using multi-characteristic opsin (MCO) to re-sensitize the retina to detect low light levels [1]. These therapies have the potential to restore vision in millions of visually impaired individuals suffering from retinal degenerative diseases, including retinitis pigmentosa (RP), Stargardt macular degeneration (SMD), and geographic atrophies (GA), secondary to age-related macular degeneration.

Our gene therapy delivers light-sensitive MCOs into retinal cells with the potential to detect ambient light and improve activities of daily living. Our pipelines include both viral and non-viral delivery platforms.

Our initial proof of concept in a human study came from a Phase 1/2a open label trial outside the US in 11 patients with various mutations of RP causing severe vision loss [2]. After ascertaining the safety and efficacy of the therapy, Nanoscope successfully completed a randomized, double-blinded, multi-center 100-week clinical trial with the novel mutation-agnostic optogenetic product MCO-010 in patients suffering from RP [3].

We have also successfully completed a Phase 2, open-label, multi-center, 48-week trial with MCO-010 in patients with SMD [4]. We are preparing to initiate a Phase 3, randomized, double-masked, multi-center trial in the first half of 2025.

Nanoscope's mutation-agnostic pipeline includes another novel optogenetic therapy utilizing non-viral delivery of an ambient light activatable drug called MCO-020 in patients with GA [5]. This is another transformative therapy as this uses a novel image-guided near-infrared laser device to deliver the MCO-020 plasmids.

Nanoscope had several productive meetings with the US FDA to discuss the path to approval of its fast-tracked MCO-010 program for the treatment of severe vision loss due to RP. Based on these regulatory interactions, Nanoscope plans to commence the submission of a BLA in the first quarter of 2025.

Q What is the current patent landscape for cell and gene therapies (CGTs), particularly the optogenetic therapy to restore vision in people suffering from various inherited retinal degenerative diseases?

AA The patent landscape for the CGT field is experiencing enormous growth. Though optogenetics and related patents have been around for two decades, recent patent filings are focused on improving the translational use. The recent filings include novel opsin proteins with improved light sensitivities, light spectrum, novel promoters targeting special pathways or cell types, different methods of delivery (viral versus non-viral), and target disease indications. Despite the surge in IP, only few companies have progressed to clinical applications, highlighting the novelty of this space. Nanoscope is the first to conduct late-phase clinical trials demonstrating the safety and efficacy of MCO-010 in retinal degenerative diseases. However, there is substantial potential for continued evolution in this field, considering optogenetics can be applied to a broader range of disease modalities.

Q What are the key regulatory challenges that the CGT developers currently face, and how can these hurdles be addressed?

AA Some of the key challenges include developing a scalable manufacturing process, validating appropriate analytical methods, establishing comparability after process change, and reaching alignment with regulators on potency assays and primary clinical endpoint. Furthermore, seeking the regulatory authorities' alignment on the selection of the biomarker/surrogate endpoint for accelerated approval is also challenging.

With an endgame-focused mindset, CGT developers must prioritize early understanding of their drug and its mechanism of action to establish the potency assay and manufacturing process. This approach also requires anticipating potential challenges, particularly those related to manufacturing process changes during late-stage clinical studies. In such cases, CGT developers should develop a scale-down model and collaborate with regulators to design analytical comparability studies that demonstrate consistency between pre- and post-change production lots. Additionally, early-phase companies can gain significant advantages by investing in robust analytics from the outset. Conducting thorough analyses at different stages of the process enhances product understanding and strengthens process characterization, ultimately supporting faster development and regulatory alignment.

"With an endgame-focused mindset, CGT developers must prioritize early understanding of their drug and its mechanism of action to establish the potency assay and manufacturing process."

Q Nanoscope recently announced that the company will soon be initiating a Phase 3 clinical trial of MCO-010 to treat SMD. How is the regulatory landscape looking for this potentially transformative therapy?

AA The regulatory interaction for the transformative MCO-010 therapy in the SMD study has been incredibly positive, supported by significant non-clinical, CMC, and clinical data available for this platform across various retinal degenerative diseases. The FDA provided clear input on the proposed randomized 1:1 to a control cohort receiving a sham injection. Phase 3 registrational trial in patients with SMD is intended to establish the effectiveness of MCO-010, and it will be the first randomized, controlled gene therapy trial for SMD. For the proposed Phase 3 trial, we have alignment with the FDA on the primary efficacy endpoint being the change in best corrected visual acuity from baseline at week 52. This alignment was achieved on the enrolment of legally blind patients with vision worse than 20/200, and as young as 12 years old, for the upcoming study. This extends the population compared to the one studied in Phase 2. The FDA agreed on the sufficiency of the current nonclinical package to support a future BLA submission on SMD.

Q How do you see the regulatory affairs and IP field developing in the next couple of years?

AA With the recent growth in approvals within the CGT space, regulators are gaining a deeper understanding of the complexities involved in CGT drug development. As a result, they are increasingly collaborative, working closely with sponsors to guide the development programs, paving a clear path for timely patient access to these innovative therapies. Regulators are particularly receptive to accelerated approvals for drugs targeting rare degenerative diseases with high unmet needs, offering a significant advantage for patients in urgent need of such treatments.

IP is equally critical in drug development. A strong IP portfolio provides companies with a competitive edge, enabling them to leverage their patents for licensing opportunities and further development deals. Numerous patents have been filed—and continue to be filed—covering innovations such as novel proteins, treatment methods, viral vector design, and engineering for specific targetability and high yields, as well as novel non-viral vectors and methodologies. Furthermore, major pharmaceutical companies actively seek to license these groundbreaking technologies, creating mutually beneficial opportunities for CGT drug developers and the industry. This collaborative dynamic accelerates drug development, ensuring that advanced therapies reach patients more efficiently.

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Q What are your own goals and priorities for your work over the next 1–2 years?

AA I have been with Nanoscope since our first-in-human Phase 1/2a study. The journey to late-stage clinical settings in both RP and SMD has been electrifying. We are at an exciting stage in our drug development program, and I am committed to advancing Nanoscope's mission, driving regulatory success, and supporting our pipeline's growth. I look forward to seeing this transformative, mutation-agnostic optogenetics therapy become a first-line standard of care in clinics in the near future.

REFERENCES

1 Batabyal S, Gajjeraman S, Pradhan S, Bhattacharya S, Wright W, Mohanty S. Sensitization of ON-bipolar cells with ambient light activatable multi-characteristic opsin rescues vision in mice. *Gene Ther*. 2021; 28(3–4), 162–176.

2 Nanoscope Therapeutics Inc. Dose-escalation study to evaluate the safety and tolerability of intravitreal vMCO-I in patients with advanced retinitis pigmentosa. Jun 9, 2021. https://clinicaltrials.gov/study/NCT04919473.

3 Nanoscope Therapeutics Inc. Efficacy and safety of MCO-010 optogenetic therapy in adults with retinitis pigmentosa. Mar 22, 2024. https://clinicaltrials.gov/study/NCT04945772.

4 Nanoscope Therapeutics Inc. Safety and effects of a single intravitreal injection of vMCO-010 optogenetic therapy in subjects with Stargardt disease. Nov 9, 2023. https://clinicaltrials.gov/study/NCT05417126.

5 Batabyal S, Kim S, Wright W, Mohanty S. Layer-specific nanophotonic delivery of therapeutic opsin-encoding genes into retina. *Exp Eye Res*. 2021; 205, 108444.

BIOGRAPHY

Ananta Ayyagari is currently the Director of Regulatory Affairs and Intellectual Property at Nanoscope, supporting in regulatory and intellectual property development and managing vision restoration projects working closely with preclinical, clinical, CMC, and regulatory teams. At Nanoscope, Ayyagari has successfully contributed to regulatory strategy, IND filings for initiation of clinical trials, orphan and fast track designations, meeting packages for regulatory authorities, and applications for patents and trademarks. After completing her PhD in Chemistry in 2003, Ayyagari carried out postdoctoral fellowships at the Georgia Institute of Technology, University of Wisconsin-Madison and University of Oklahoma, Norman. Her expertise includes development of methods for drug and gene delivery. She has authored several papers and patents.

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CELL & GENE THERAPY INSIGHTS

BUSINESS INSIGHTS

Exploring the challenges and innovations in CGT product commercialization

INTERVIEW

"We need to…make sure that more high-value CGTs continue to move forward towards launch and serving patient communities…"

Jokūbas Leikauskas, Editor, *Cell & Gene Therapy Insights*, speaks to **Ted Slocomb**, Global Commercial Biopharma Executive, about the challenges and strategies in cell and gene therapy (CGT) product commercialization, focusing on market access, pricing, and health system readiness. They also discuss practices, such as early value demonstration and outcomes-based agreements (OBAs), while exploring future innovations to expand CGT adoption.

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Q What excites you the most about the current CGT market?

TS First and foremost, over the past decade, we have seen the development and launch of some truly transformative and even curative treatments for previously untreatable, devastating genetic diseases, later-stage cancers, and other high-burden diseases including first-wave gene therapies, genomic medicines, and

engineered cell therapies. Diseases such as spinal muscular atrophy, certain lymphomas and leukemias, sickle cell disease, hemophilia, genetic forms of blindness, and selected other serious diseases now have dramatically better treatment options, and in many cases, treatments for the first time.

Despite variability in health system acceptance and uptake, the profound and durable clinical and quality of life (QOL) improvements for affected patients from this new wave of innovative medicines are becoming increasingly established and accepted by the health system and other key stakeholders every day. While the commercial successes have been variable, the field is still in an early stage of evolution, analogous to the state of monoclonal antibodies in the early 90s. We still have a lot to learn and a long way to go as a field in terms of how to best select programs for development and launch, and I am excited to be among a select group of folks who have had the opportunity to help shape the commercialization strategies and to have gained experience and insights from this first wave of launches to apply to the new waves to come.

Wherever possible, I am grateful to have the opportunity to share insights and strategic recommendations with others in the field, especially those outside the commercial function, to help ensure future therapies achieve successful launches and most importantly, reach the broadest group of patients who can benefit from them.

Q What do you see as the critical key challenges in the commercialization of CGT products for rare diseases?

TS A lot of the fundamentals of effective commercialization of new products also apply to CGTs. These include building relationships with the patient, provider, payer, policy, and medical expert communities, knowing your target stakeholders and their unmet needs, understanding how well your product addresses those needs and where it will fit into the future competitive and treatment environment, characterizing the patient journey and understanding the barriers to uptake, early and effective disease and product message development and stakeholder education and engagement. Tailoring the organization's launch strategies, operating models and capabilities, governance, staffing, and investment levels to the opportunities and barriers faced in new product launches are all critical. Particularly for CGTs targeting rare diseases, the areas of patient community engagement and patient support, engaging treatment centers of excellence, driving referrals, and addressing regional and local payer and health system dynamics and barriers are critical. Getting the fundamentals right, starting launch planning early, and resourcing the launch properly, or in many cases, finding the right commercialization partner is critical. But on top of that, there are several unique aspects of preparing new CGTs for launch.

In the first wave of CGT products, the key challenges included educating patients and caregivers about these advanced therapies, how they differ from the standard of care (SOC), and the potential benefits and risks. Furthermore, it was challenging to prepare the payers to accept significantly higher one-time price points than those they were accustomed to. While CGT pricing is generally well justified from a price-to-value standpoint, given the long-term or curative, QOL, economic, and societal benefits, a lot of time and energy of the industry has been dedicated to making that case to health technology assessment (HTA) organizations and other policymakers. Additionally, it has become more common to invest in manufacturing early and aggressively to ensure supply for both clinical trials

"...sometimes there are considerable barriers to adoption when it comes to patient and caregiver acceptance of CGTs over alternative treatments..."

and commercial launches, often requiring the pioneering of complex and unprecedented manufacturing processes, facility development, and supply chain strategies.

Nowadays, in my opinion, assuming the fundamentals and value story development/pricing and access work are solid in the launch strategy, the key challenges in CGT adoption center around anticipating and having an approach to shape health system readiness and understanding how the new product will fit well into or need to significantly disrupt the current referral, treatment and financial patterns of the indications being targeted. The indications targeted by innovators in this space generally address areas of significant unmet clinical needs. In most cases, these therapies aim to treat serious, high-burden diseases where either no current SOC exists, or the available options are poor, or they could be significantly improved by more directly targeting or reversing the disease mechanism.

Despite high disease burden and limited options, sometimes there are considerable barriers to adoption when it comes to patient and caregiver acceptance of CGTs over alternative treatments, especially for those products that need to be manufactured individually for each patient (e.g., autologous cell therapies, *ex vivo* gene therapies). These may include logistical barriers: patients may need to travel, undergo pre-treatment, receive therapy at a specialized center, and commit to long-term follow-up, including adverse event management, which can be disruptive to patient and caregiver lives, even to the degree of offsetting choosing a therapy that offers substantial and unprecedented benefits, in some cases.

From a US health system perspective, behavioral and financial incentives to maintain the status quo have been underappreciated in launch planning for new CGTs. At the provider, prescriber-referrer, and health facility levels, there are sometimes strong short-term financial incentives to maintain the status quo, even if the adoption of a CGT could result in better long-term health outcomes and even long-term cost savings to payers and the health system overall. In some cases, existing chronic treatments generate substantial revenue for providers, creating resistance to therapies that seek to displace current standards of care. In my view, these health system and ecosystem barriers remain one of the biggest sources of inertia to overcome in ensuring that CGTs reach as many patients as possible that will benefit.

Q What approaches are you taking/do you see being taken to address the existing challenges in CGT commercialization?

TS In addition to getting the fundamentals mentioned previously, some of the best practices in CGT commercialization start with selecting the right patient population, aligning the technology, and anticipating product configuration with the unaddressed clinical needs. In many cases, this has happened organically, as advanced gene and genomic medicine therapies typically target rare genetic or genetically driven diseases with limited or no effective treatments. In other cases, when the disease does not have a genetic cause and may have other options for treatment, understanding the relative value and burden of a cell therapy option versus the existing SOC is crucial to address in your launch plan evidence generation and stakeholder communication efforts.

"Companies need to identify key stakeholders within health systems and develop new tactics to help overcome the inertia caused by misaligned practice and financial incentives..."

With some product-specific exceptions, challenges around price and access have generally been mitigated through strong value narratives, proactive evidence collection (for the burden of illness, QOL and health utility impact, proactive cost–effectiveness modeling and messaging during clinical development), HTA preparedness, and innovative payment models (such as OBAs and installment-based payments and their hybrids).

The Joint Clinical Assessment (JCA) process is currently being introduced in the EU, focusing first on oncology and ATMPs, represents a significant area of both new challenges and opportunities, requiring rapid adaptation, flexibility, and internal collaboration on the part of CGT innovators. While in theory, the introduction of the JCA pathway represents the potential for efficiency in gaining early advice to shape clinical strategy and more rapid access approvals at launch, there are certain to be added complexities and costs for CGT manufacturers due to the new process, which will be especially challenging for smaller and mid-clinical stage companies with limited commercialization resources and expertise. In particular, early to mid-clinical stage CGT innovators without internal commercial expertise or leadership will need to rely on external consultants and new ways of thinking from their clinical leaders to develop products not just for regulatory approval but for market access and commercial acceptance as well. I think being savvy on these topics will continue to be critical for leaders of not only late or commercial-stage CGT companies, but leaders of early to mid-stage companies as well, as they engage in partnership and fundraising discussions.

However, what I see remaining as more 'hit-or-miss' is the industry's collective effort to drive health policy change and educate health systems about the long-term benefits of CGTs. Companies need to identify key stakeholders within health systems and develop new tactics to help overcome the inertia caused by misaligned practice and financial incentives—where existing, less cost-effective, lower-value treatments may continue to be used simply because they generate revenue for hospitals and prescribers in the near term. There are sometimes resourcing as well as legal and regulatory barriers to innovator companies being able to fully address these challenges effectively.

This issue deserves more attention and investment by our industry sector as a whole. While certain individual companies have shaped limited changes benefitting their product or product class and organizations such as the Alliance for Regenerative Medicine have made good strides in achieving incremental policy changes and awareness of the structural barriers to broader CGT uptake, achieving more tangible impact on health system policy as an industry sector will be essential for faster and broader adoption of new therapies and sustained long-term growth in the space.

Q Given the high costs associated with CGTs, what pricing and reimbursement strategies have proven most effective?

TS Instead of using the subjective and relative words 'expensive or high priced' when referring to CGT products, I prefer using the term 'high value'. For those

of us in the CGT space, let's remove the words 'expensive', 'high cost', or 'high priced' from our vocabulary when talking about these advanced therapies. There are many well-known examples of chronic therapies such as enzyme replacement therapy (ERT) for certain rare diseases or factor replacement therapy (FRT) for which the lifetime costs of these therapies would far exceed the price of a one-time gene therapy that could obviate the need for a lifetime of ERT or FRT. While it is important to acknowledge that in some health systems, a higher than typical one-time price may represent a budget impact forecasting challenge, it is important to emphasize the broader context for the value delivered by the one-time cost, as well as, when appropriate, offer novel pricing and contracting strategies to address the uncertainty of budget impact and product outcome at the time of launch. The 'list' price that gets the headlines is hardly ever the net price paid by the payer or health system, which is often significantly less.

The CGT companies I have been involved with strongly believe in the need to invest in and establish a solid foundation for the payer/health system value story early in clinical development. This may include collaborating with the patient community to generate data on patient QOL, and positive caregiver impact. It is important to build a comprehensive value story across all pillars of product value including clinical, QOL, economic, and societal.

In essence, developing and articulating the value of your product early and often is critical. For the most part, aside from some more challenging European HTAs and payers, this approach has led to payers understanding and accepting the value of these therapies across the many dimensions of value I highlighted previously. However, two key issues that have emerged are uncertainty around real-world long-term outcomes underpinning the value story as well as pricing at launch and the unpredictability of initial budget impacts due to uncertain adoption. To address this, manufacturers have successfully implemented OBAs, which include payment-over-time models, and agreements tied to outcomes (rebates or payments linked to performance). For the most part, these strategies have been effective in mitigating concerns about pricing and access, with some notable exceptions where deep discounts from the list have been required for initial access. Paired with these programs is the requirement for innovators to support the collection of long-term outcomes data, typically through patient registries or other post-launch real-world evidence mechanisms.

While OBAs, sometimes combined with payment-over-time arrangements to mitigate the upfront budget impact, were certainly the right strategy at launch for many first-wave products, the degree to which they will be needed going forward depends on the product-indication pairing, strength of value story, and the maturity of evidence in that space, including comparison with the existing SOC or competitive in-class CGTs. Certain cell therapies have proven their long-term durability and effectiveness, and their budget impact is becoming more predictable.

Q How do you assess the commercial viability of early-stage CGT products?

TS The commercial attractiveness of advanced therapies has many layers. Firstly, it is crucial to assess the fundamentals as with any other type of new therapeutic approach or new biopharma product. Is there a significant unmet need? How burdensome

is the disease from clinical, family, patient, cost, and societal perspectives? How directly does the therapy address the mechanism of disease? Does the therapy have a reversal of disease or curative potential? What is the degree of benefit it is likely to provide regarding patient survival, QOL, and the economics of care in the short and long term? How will the new product fit into the current referral, treatment, and follow-up patterns for patients, providers, and payers? What will need to change for the adoption of the new CGT product and is it feasible to expect the benefits will outweigh the costs to drive that adoption?

All these assessment factors serve as proxies for building a compelling value story and suitable pricing model, differentiating the therapy from the SOC, and driving adoption by patients and providers and, ultimately, the financial value of the program for an innovator company. If it is a follow-on product, the potential for competitive differentiation within the class of product becomes important, although this has been the case for only a handful of CGT products thus far.

Another key factor to consider is the product configuration—whether it is autologous, allogeneic, or a biologic drug-in-vial. I believe that many areas in our space will evolve from complex autologous processes to more streamlined biological drug-in-vial models, even leapfrogging allogeneic approaches as a stopgap solution. This shift could circumvent many of the health system barriers I mentioned earlier, as these products could become easier to distribute and be reimbursed within current health system paradigms for 'off the shelf' biologic drugs.

We also need to consider the cost of manufacturing and the COGs. Can the product evolve during its clinical development to a point where its manufacturing can be scaled to commercial viability? This would allow for attractive margins at a price-to-value level that we can defend with a strong value story. Some of my colleagues in clinical development and other functions sometimes might think that the lower we can drive COGs, the better to lower price and drive adoption. However, pricing is not simply based on COGs—the industry generally prices high-value, innovative new products based on the value they deliver, not what they cost to make. The ability to manufacture at a commercial scale with reasonable COGs that deliver attractive margins is critical for the reinvestment and sustainability of the entire sector and much progress has been made towards achieving more traditional 'biologics-like' gross-margins by industry leaders.

Additionally, aligning the product configuration with health system needs is crucial for the adoption of these advanced therapies. Assuming the clinical value is there, it is important to interrogate whether the therapy will fit into the existing system, and how much effort it will take to disrupt patient flow, financial flows, and the institutional attitudes toward adopting a CGT at that institution, whether it is a center of excellence or a community clinic. Understanding how the product will fit into these dynamics is critical. This consideration is not just for assessing the commercial viability once an early-stage product has been chosen. It also plays a role in evaluating business development opportunities and deciding whether to advance certain products in the clinic based on early proof of principle data. Proof of product is a term I like to use that encompasses not only insights on approvability based on early clinical results about clinical effects but insights on likely dosing levels and product configuration that indicate the likelihood of product uptake and commercial viability at a price-to-value pricing strategy.

Luckily, looking back and learning from the first wave of CGTs, we as a field are becoming smarter about where the next wave of investment should go, where the money will be well-spent on clinical development, and which new technologies and product profiles will have the most potential for success.

Q What innovations in market access or pricing models do you think will gain traction in the future?

TS Over the past decade, there has already been so much innovation in market access and pricing models related to CGTs, that it is hard to imagine the pace of innovation keeping up in the next decade.

There has been innovation in devising the models themselves, which thematically generally involve future rebates to payers from the manufacturer based on the lack of a pre-agreed outcome or future payments to the manufacturer from payers based on achieving a pre-agreed outcome—often decided by tracking an individual patient or population health outcome. This has required innovation in post-approval patient data collection and sharing—to confirm outcome with payers and determine payment terms while preserving patient confidentiality. Layered on top of this have been hybrids of outcome-based payment and payment over time models with all or part of payments spread over time becoming payable or rebated based on product performance. In addition, some new therapies with weight-based dosing have achieved flat pricing across all patients regardless of weight, spreading payer risk of outcome versus cost more evenly across all covered patients. These developments have collectively required innovations too numerous to mention here in coding, invoicing and reimbursement policy, methods of patient data collection and reporting, financial modelling, provider service models, and distribution/financial intermediary model evolution in both highly fragmented and complex systems such as the USA as well as single-payer markets. I commend those individuals, companies and coalitions who have been at the forefront of developing and advocating for implementing these models, as this has been a herculean task to date and has paved the way for the many products to come. In recent years, the piloting of an OBA payment model by the Center for Medicare and Medicaid Innovation to adapt at their option, known as the CGT Access model, which focuses first on sickle cell disease is an encouraging example of the progress being made in the adoption of new payment models.

With respect to innovative CGT payment models in the future, I think there are two key themes that will be important for innovator companies and their partners to keep in mind as they develop their launch strategies. The first is that beyond the themes and progress I talked about previously, the specific terms of innovative, outcome-based payment models will always need to be developed and tailored to the individual disease, product, market, and payer dynamics. There are no one-size fits all model by product type, market type, or payer type and there is unlikely to be one in the future. Developing a novel payment model for a new product that balances access and uptake goals with acceptable net pricing targets requires significant forethought and preparation and should be started as early as possible before launch, in parallel with and informed by value evidence and pricing strategy development. The second theme is that in more complex payer systems such as the USA or in other systems where current provider revenue streams and hospital budgets sometimes play a significant role in whether a new CGT product is adopted to its full potential to help patients, new policy and contracting solutions will need to be developed, bridging provider, payer, and manufacturers, often with significant legal and regulatory constraints to innovation that will need to be addressed. I expect to see a lot of focus and action here in the coming years from the CGT sector.

Q What are your goals and priorities over the next 1–2 years?

TS As a commercial leader in the CGT space, I would like to help those of us focused on advanced therapies learn from both the successful and unsuccessful launches to do better with the next-wave portfolio program choices and product launches. By sharing these learnings and insights into the ongoing work and refining the programs in clinical development and their launch strategies, especially with colleagues from other functions that may have had limited exposure to commercialization strategies and concepts, I hope to help the field optimize the commercial success of those programs within the existing constraints of the possible portfolio and development choices. This way, we can ensure the field continues to thrive. We need to keep attracting investment as well as building regulator and health system confidence in the potential of our products, and most importantly, make sure that more high-value CGTs continue to move forward towards launch and serving patient communities, that in many cases, are key collaborators in the development of these new therapies.

I, along with many people in my CGT network and my industry peers, strongly believe that CGT has a very bright future. Of course, there will continue to be challenges along the way, but we are still in the early days and my priority is to continue learning from these first-generation launch experiences so we can contribute meaningfully to the success of the next generation of therapies.

BIOGRAPHY

Ted Slocomb is a global commercial and market access biopharma executive with deep experience leading the commercialization of high value rare disease, gene therapy, and cell therapy products including leadership roles in new product planning, global market access/pricing, HEOR, and global marketing during his over 30-year career. He is currently Managing Partner of TSBioAdvisors, LLC, providing commercialization and corporate strategy advisory services to cell and gene therapy innovators. Most recently, Mr Slocomb was the Vice President, Global Commercial Strategy, Access and Value at Kite Pharma, leading Kite's integrated global commercial, market access, HEOR, and Opinion Leader strategy team. At Kite he also co-chaired the commercial governance for Kite's alliance with Arcellx for anitocel CAR T therapy in multiple myeloma. Prior to Kite, Mr Slocomb was the Vice President, Global Market Access—Gene Therapies for Astellas and Vice President, Commercial Planning for Audentes Therapeutics (acquired by Astellas for $3.0 billion in 2020). Prior roles included Global Commercial Leader for MYALEPT® at Amylin Pharmaceuticals, Principal at Bear Stearns Health Innoventures, LP and Senior Director, Corporate Development at Fate Therapeutics. Mr Slocomb is a former Co-Chair of the Value and Access Committee of the Alliance for Regenerative Medicine and the Corporate Alliance of Global Genes. Mr Slocomb earned an MBA in Finance from New York University, New York City, NY, USA and a BA in Genetics from the University of California, Berkeley, CA, USA.

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A novel PCR-free hybridization method for titering gene therapy vectors in crude cell lysates and various in-process matrices

Wai Choi PhD, Senior Scientist and R&D Leader, Gator Bio

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Maintaining the accuracy and efficiency of AAV genome titer quantification is crucial in gene therapy manufacturing. This poster explores a novel PCR-free assay that enhances speed, reliability, and flexibility. By utilizing DNA hybridization, immunochemistry, and bio-layer interferometry (BLI), this assay simplifies titer quantification and improves workflow efficiency.

Eliminating PCR for improved turnaround and efficiency: compatibility with crude lysates and complex matrices

GeneSwift, a PCR-free assay that combines DNA hybridization, immunochemistry, and BLI, has the ability to quantify AAV genome titers without requiring PCR amplification. Traditional PCR-based assays are not only time-consuming but also susceptible to PCR inhibitors present in crude or complex samples. By leveraging DNA hybridization and BLI technology, GeneSwift eliminates these challenges, offering a faster and more robust alternative while ensuring high sensitivity and accuracy. It is designed for use across a wide range of sample conditions, including crude lysates and in-process matrices.

In validation studies to determine robustness in crude lysates and complex matrices, AAV capsids were spiked into various in-process buffers, including those with high protein and salt concentrations. The undiluted samples were tested for their ability to generate a robust standard curve, as seen in Figure 1. Even with complex matrices such as cell lysates and high-salt buffers, the assay maintained its precision, demonstrating adaptability across different analytical steps in AAV processing.

Assay correlation with ddPCR

Quantifying AAV genome titers requires precision, and traditional methods such as droplet digital PCR (ddPCR) have been the industry standard. However, GeneSwift presents as a highly correlated alternative. In a comparative study, AAV titer samples at different concentrations in a crude cell lysate were measured and analyzed with both GeneSwift and ddPCR. This exhibited a significant correlation coefficient (R^2=0.99) as seen in Figure 2.

Following this, 16 AAV samples were prepared and the titers measured for comparison. The coefficient of variation (CV) for GeneSwift was measured at 10.1%, comparable to the 9.1% CV observed with ddPCR, confirming accuracy and reliability for titer quantification.

Adaptability beyond DNA-based AAV quantification

Beyond DNA-based AAV quantification, GeneSwift also extends its application to RNA detection. The assay has been successfully used to measure both single- and double-stranded RNA, broadening its utility across different gene therapy platforms. By simply designing specific probe sequences, researchers can customize GeneSwift for various genomic targets, further enhancing its flexibility.

Further, GeneSwift eliminates the lengthy sample preparation and multiple serial dilution steps, allowing for faster turnaround times. This streamlined workflow enables results to be obtained in under 30 min, significantly reducing processing time.

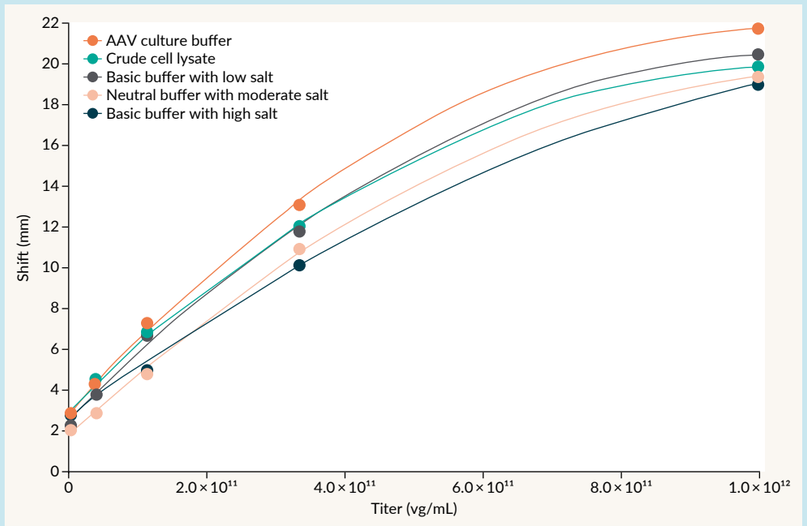

Figure 1. Standard curves of different in-process matrices to determine robustness and assay compatibility for AAV samples.

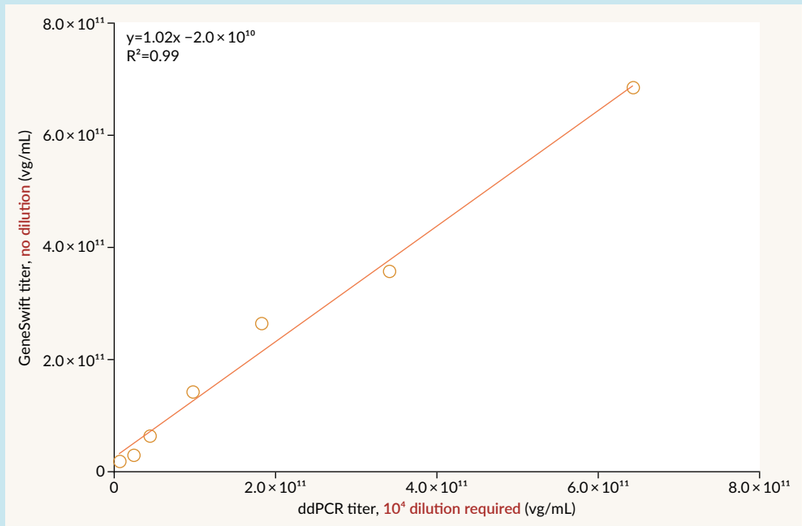

Figure 2: Correlation plot comparing the quantification of AAV titers using GeneSwift and ddPCR

Wai Choi PhD, Senior Scientist and R&D Leader, Gator Bio

With over 15 years of experience in pharmaceutical, biotechnology, and biochemical assay development, Choi currently advances gene therapy research by developing biochemical assays utilizing hybridization, immunoassay, and BLI technology at Gator Bio, Palo Alto, CA, USA. He was a postdoctoral scholar in Stanford University's Cancer Biology Program, researching a cancer biomarker with support from the California Breast Cancer Research Program. He later developed small-molecule drugs for tuberculosis in a Bill & Melinda Gates Foundation-funded program.

Essential equipment for advancing cell and gene therapy: innovations in incubation, monitoring, and cold storage

Tia Harmon, Technical Product Sales Specialist, PHC Corporation of North America

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Maintaining the quality and safety of cell and gene therapy (CGT) products requires precise control of environmental and metabolic conditions. This poster explores a live cell metabolic analyzer LiCellMo®*, which enables real-time monitoring of glucose and lactate levels, providing insights into metabolic processes in cells. The data obtained from LiCellMo can be used to evaluate the conditions of the cells, which can be applied in both metabolic research and CGT manufacturing.

Ensuring CGT product stability via metabolic monitoring

CGT products are highly sensitive and require strict environmental control during cell culture to maintain their stability, viability, and efficacy. For instance, utilizing CO_2 and multi-gas incubators to create an optimal cell culture environment, and storing the cells at ultra-low temperatures can help ensure process control, safety, and product quality.

Another increasingly important consideration is monitoring the metabolic attributes of CGT products to gain insights into the state of the cells. Visualization of cell conditions can be achieved by analyzing processes such as glycolysis and oxidative phosphorylation, which are the two primary pathways that supply energy to cells.

In-line metabolic analyzers such as LiCellMo track glucose and lactate levels in real time, calculating metabolic rates to visualize changes in cell activity during growth. This approach offers faster, continuous, and more accurate insights compared to traditional sampling methods.

Operational workflow of LiCellMo for metabolic monitoring

The LiCellMo workflow begins with a calibration to ensure accurate sensor responses for concentration measurements. This involves preparing media with two distinct concentrations of glucose and lactate, which are measured for approximately 20 hours in turn. Subsequently, cells are prepared in a 24-well plate, and the sensor module is installed. The cells are cultured, and the concentrations of glucose and lactate are continuously monitored for up to 10 days. Finally, the metabolic rate is calculated using the concentration data.

Case study: validating the accuracy of metabolic monitoring in Jurkat cells

In a proprietary study, LiCellMo was utilized to monitor glucose and lactate levels in Jurkat cells incubated in RPMI 1640 Medium over a 6-day period. On day 3, a colorimetric method was used to seed the cells and evaluate the concentrations in the culture medium, serving as reference data. As illustrated in **Figure 1**, the maximum discrepancy between the LiCellMo measurements and the reference data was 10%, demonstrating the analyzer's high accuracy.

Applications of LiCellMo in research and manufacturing

LiCellMo can be used in metabolic research to visualize the glycolytic changes, providing insights into metabolic shift and varied timing. As shown in **Figure 2A**, adding the inhibitor can help better understand the changes in glycolysis, which vary by cell type.

The device can also be employed in CGT manufacturing by monitoring glucose as a nutrient and lactate as a toxic metabolite, which enables the development of better-quality culture protocols. For example, as shown in **Figure 2B**, monitoring glucose consumption with LiCellMo can be used to optimize protocols for induced pluripotent stem cell (iPSC) manufacturing.

*Disclaimer: LiCellMo is available for purchase in the US, Canada, and select other geographies globally. For research and education use only, not for use in diagnostic procedures in the US or Canada. This product has not been approved or cleared as a medical device by the US Food and Drug Administration or Health Canada.

Figure 1. Glucose (blue line) and lactate (green line) monitoring with LiCellMo in Jurkat cells over a 6-day period.

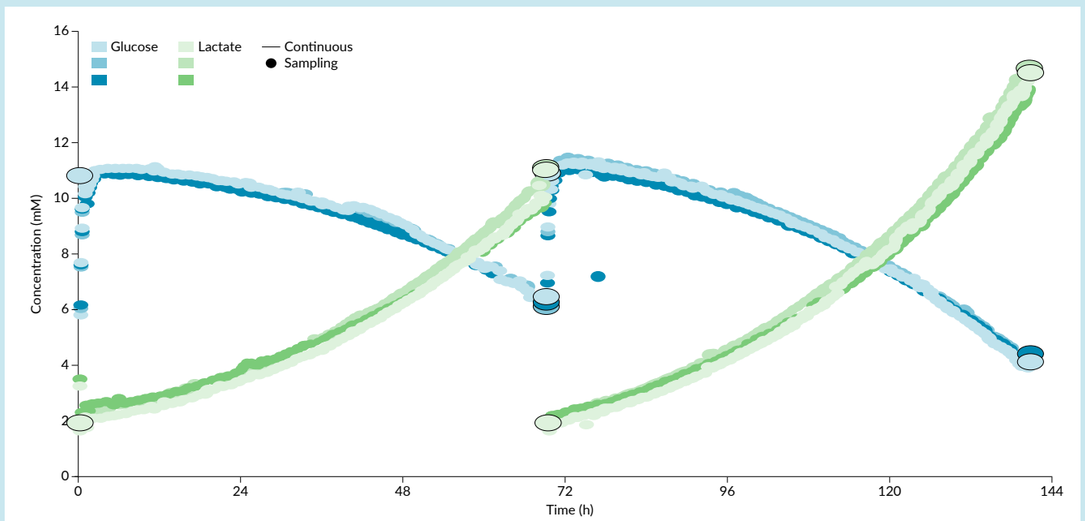
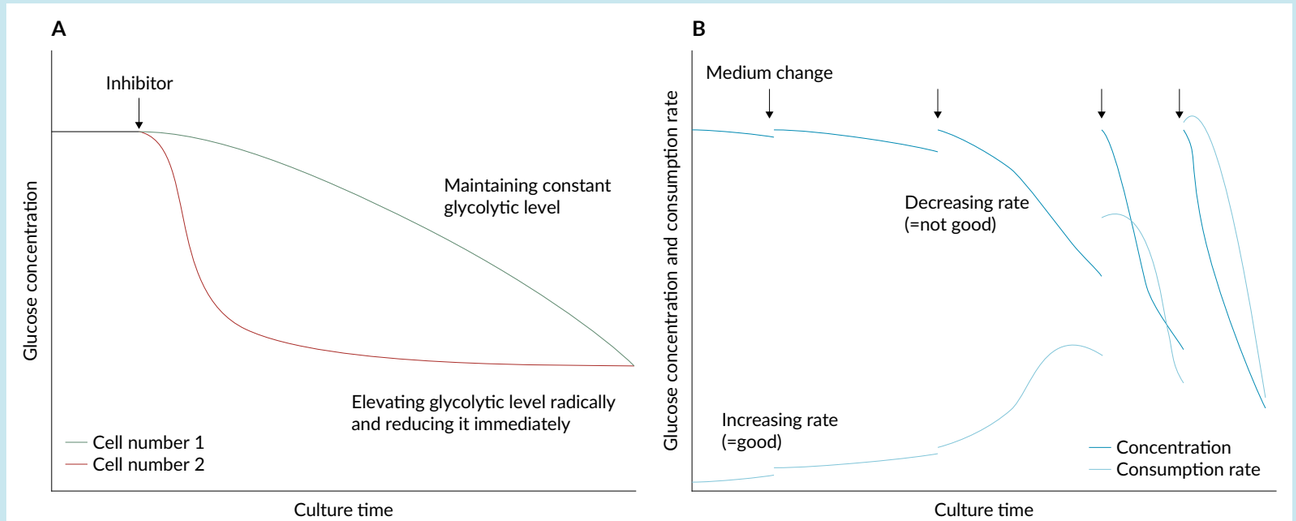


Figure 2. A: an example of detecting metabolic shifts when an inhibitor is applied, B: an example demonstrating the utility of LiCellMo data in iPSC protocol optimization.



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Tia Harmon is a Cell and Gene Therapy, Technical Product Sales Specialist for PHC Corporation of North America (PHCNA). She is committed to providing solutions for cell metabolism research that aid in the development of disease treatments. Tia has worked in the industry for 20 years in the government, academic, and biotechnology sectors. She holds a PhD in Biomedical Sciences from Morehouse School of Medicine.