RSNN
Regulatory Science Netwerk Netherlands

SIG Expert meeting report on *in vivo* gene editing

The Regulatory Science Network Netherlands (RSNN) Special Interest Group (SIG) Advanced Therapies expert meeting on *in vivo* gene editing, held on July 15, 2025, in Utrecht, the Netherlands, brought together a multidisciplinary group of experts with the aim of examining the current European regulatory landscape for therapeutic *in vivo* gene editing. The meeting sought to identify regulatory gaps, particularly in the translational space from non-clinical to clinical development, and to formulate actionable recommendations that could inform the ongoing update of regulatory guidelines, including those under revision by the Committee for Advanced Therapies (CAT) of the European Medicines Agency (EMA). The ultimate objective was to stimulate regulatory innovation while ensuring robust safety and quality standards, facilitating patient access to these emerging therapies.

Date and time	The meeting took place on Tuesday, 15 July 2025, from 15:00 to 19:00 CEST.	
Location	Jaarbeursplein 6, Utrecht, the Netherlands (hosted by Lygature).	
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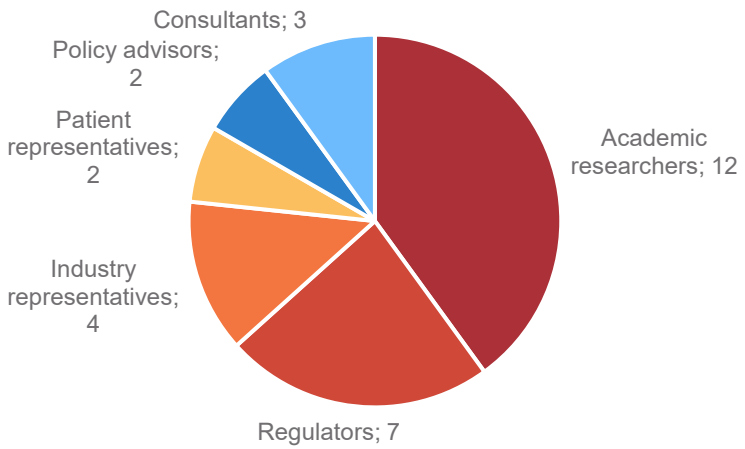
Attendees	Approximately thirty participants attended the meeting. The group was selected to reflect a diversity of perspectives across the Dutch ecosystem (Figure 1).	

Figure 1: Composition of workshop attendees



SUMMARY

The expert meeting on *in vivo* gene editing, organized by the RSNN SIG Advanced Therapies, brought together a diverse group of stakeholders to explore scientific and regulatory challenges in this rapidly evolving field. *In vivo* gene editing holds great promise, particularly for unmet medical needs, but raises complex scientific and regulatory questions. Discussions focused on three main themes: off-target effects, non-clinical model systems, and quality criteria for manufacturing. Participants agreed that there is currently no harmonized regulatory framework for off-target assessment, highlighting the need for standardized, transparent methods and patient participation in risk evaluation. For non-clinical models, the limited predictive value of traditional animal systems was emphasized, with growing support for advanced alternatives such as iPSC models, organoids, and *in silico* approaches. Case-by-case, benefit-risk-based frameworks were viewed as essential requiring early alignment and the courage to move beyond classical defaults toward justified, advanced models. On quality criteria for manufacturing, the need for robust assays was underlined to enable a critical quality attribute (CQA)-driven approach to manufacturing. Such assays are essential to monitor and control critical attributes, and to perform comparability studies when changes in raw materials (often limited in quality documentation) or platform improvements are introduced. Key recommendations of the meeting included the development of harmonized criteria for off-target effect detection, creation of a European registry for long-term monitoring of off-target effects, and adoption of parallel learning strategies where non-clinical and early clinical data are integrated. Early scientific advice and practical case-based regulatory guidance were identified as crucial for aligning expectations between developers and regulators. The meeting underscored the importance of transparency, collaboration, and patient involvement in shaping future regulatory pathways.

NEDERLANDSE SAMENVATTING

De expertmeeting over *in vivo* gene editing, georganiseerd door RSNN, bracht verschillende stakeholders samen om de regulatoire uitdagingen in dit snelgroeiende veld te bespreken. Therapeutische *in vivo* gene editing biedt veelbelovende mogelijkheden, vooral voor onvervulde medische behoeften, maar roept complexe wetenschappelijke en regulatoire vragen op. De discussie richtte zich op drie hoofdthema's: off-targeteffecten, non-klinische modelsystemen en kwaliteitscriteria voor productie. Deelnemers constateerden dat er momenteel geen geharmoniseerd kader bestaat voor de beoordeling van off-targeteffecten en benadrukten de noodzaak van gestandaardiseerde methoden en transparantie. Voor non-klinische modellen werd de beperkte voorspellende waarde van klassieke diermodellen benadrukt, met toenemende aandacht voor alternatieve benaderingen zoals humane iPSC-modellen, organoïden en *in silico* modellering. Een casus-specifieke, nut-risico aanpak werd als essentieel gezien, waarbij vroege afstemming en durf nodig zijn om voorbij gebaande paden te gaan en te kiezen voor onderbouwde, geavanceerde modellen. Wat betreft kwaliteitscriteria voor productie werd het belang van robuuste assays benadrukt om een 'critical quality attribute' (CQA)-gestuurde benadering van productie mogelijk te maken. Dergelijke assays zijn essentieel om kritieke attributen te monitoren en te beheersen, en om vergelijkbaarheidsstudies uit te voeren wanneer er veranderingen plaatsvinden in grondstoffen (vaak met beperkte kwaliteitsdocumentatie) of bij verbeteringen van het productieplatform. Belangrijke aanbevelingen voortkomend uit de meeting waren onder meer het ontwikkelen van geharmoniseerde criteria voor off-target effectdetectie, de oprichting van een Europees register voor lange termijn monitoring van off-target effecten en het toepassen van parallelle strategieën waarin het ophalen van non-klinische en vroege klinische gegevens worden gecombineerd. Vroegtijdig wetenschappelijk advies en richtlijnen gebaseerd op praktijkvoorbeelden (casuïstiek) werden gezien als cruciaal voor het afstemmen van verwachtingen. De bijeenkomst benadrukte de waarde van transparantie, samenwerking en patiëntbetrokkenheid in de toekomstige regulatoire ontwikkeling.

INTRODUCTION

In vivo gene editing represents a transformative therapeutic modality that allows for the direct correction of disease-causing mutations within the human body, often through a single intervention. Whereas *ex vivo* gene editing has already achieved notable success in the treatment of hematological malignancies and hemoglobinopathies, *in vivo* approaches are still at an earlier stage of clinical development. Nevertheless, their potential to deliver curative therapies, particularly for rare monogenic diseases for which no alternative treatments exist, is increasingly recognized.

Despite this promise, it remains unclear how therapeutic *in vivo* gene editing fits within the current European regulatory framework. Existing guidelines, such as those governing gene therapy medicinal products and the application of Good Manufacturing Practice (GMP) standards to advanced therapies, provide an important foundation (annex 1). However, questions remain as to how these principles translate into practice for novel gene editing-based therapeutic products. The inherent scientific complexity of gene editing systems and lipid nanoparticle (LNP) or adeno-associated virus (AAV)-based delivery methods complicates regulatory assessment, underscoring the need for greater clarity regarding acceptable non-clinical evidence, definitions of risk thresholds for off-target effects, and the establishment of robust quality standards.

To address these challenges, the Regulatory Science Network Netherlands (RSNN) Special Interest Group (SIG) Advanced Therapies, with the support of Lygature, organized an expert meeting on the topic of *in vivo* gene editing. The organizing committee comprised Bas Blits, Lourens Bloem, Thilo Buck, Martina Cornel, Emmely de Vries, Mouraya Hussein, Chantal van Litsenburg, and Daniël Warmerdam.

MEETING STRUCTURE AND METHODOLOGY

The meeting was designed as an interactive dialogue, structured around three critical regulatory themes:

- Off-target effects
- Non-clinical model systems
- Quality criteria for manufacturing

All participants received background materials ahead of the event, including a summary of the European regulatory framework (annex 1) and a detailed overview of therapeutic products currently in development (annex 2 and DeFrancesco 2025). The meeting featured three parallel breakout sessions, each addressing one of the above-mentioned themes. In each session, participants were grouped into multidisciplinary teams to accommodate cross-sector discussions and alignment. Discussions were guided by targeted questions derived from regulatory guidelines and real-world case

studies. The breakout sessions were followed by a plenary discussion about key outcomes and recommendations.

SESSION SUMMARIES

Session on the evaluation of off-target effects

Unintended genomic modifications pose significant safety concerns, including potential oncogenesis or genotoxicity. The discussion centered around the lack of a harmonized and predictive framework for off-target assessment. Participants emphasized the need for context-specific benefit-risk analyses tailored to individual guide RNAs, factoring in the tissue/cell environment and therapeutic indication. Techniques such as *in silico* and *in vitro* off-target prediction followed by targeted sequencing, as well as off-target analysis via whole genome sequencing (WGS) were discussed, with recognition that current methodologies still leave uncertainties. The case of k-ABE (a base editing therapy for CPS1 deficiency) was used to illustrate the challenges of defining acceptable thresholds and validating off-target effect profiles (Musunuru *et al.* 2025). There was broad consensus on the importance of public data sharing, standardized assessment protocols, and establishing European registries for long-term safety monitoring.

Session on the choice of non-clinical model systems

A central consideration was the limited translational value of classical animal models for *in vivo* gene editing. Participants noted that no single model can reliably predict human safety or efficacy. Instead, a layered, case-by-case approach is necessary. Two cases, including k-ABE, served as a useful reference, illustrating a progression from *in vitro* human cell models to transgenic mice and non-human primates (NHPs), supplemented by *in silico* simulations (Musunuru *et al. 2025*). Newer technologies, such as humanized organoids and organ-on-chip systems, were highlighted for their promise in improving predictability while reducing reliance on NHPs. The group stressed that regulators and developers must move beyond default pathways and instead use risk-based assessments to align model choice with the specific questions being asked. Supportive data from literature, platform technologies, and related products can complement non-clinical studies. Real-world and historical clinical data may also help fill gaps in non-clinical data or serve as alternatives in specific cases.

Session on quality criteria for manufacturing

The session on quality criteria for manufacturing explored which Critical Quality Attributes (CQAs) are essential for *in vivo* gene editing products and where key gaps exist. The discussion highlighted that CQAs are difficult to define consistently for novel gene editing strategies, as they are strongly context dependent. Methods and analytics were identified as the most pressing bottleneck, since reliable assays are

crucial for implementing CQAs in manufacturing. These assays are critical to support comparability assessments when implementing changes to raw materials, often accompanied by limited quality documentation (such as CoA/CoC), or when upgrading platforms. For LNP-based systems, editing efficiency, target specificity, guide RNA quality, particle size, encapsulation efficiency, sterility, and stability were emphasized, while the discussion about AAV-based systems focused on vector genome integrity, promoter specificity, persistence of expression, and risks of insertional mutagenesis. An Ishikawa analysis revealed gaps in methods (comparability studies for manufacturing changes), materials with variable levels of quality characterization, knowledge, regulatory frameworks, and alignment with EMA guidelines. Participants agreed that analytics should be a top priority, regulatory clarity is urgently needed, and patient trust depends on product consistency. Defining CQAs is essential, but significant knowledge and regulatory gaps must first be addressed.

OUTCOMES

A key outcome of the meeting was the identification of several gaps within the current European regulatory framework. These included the absence of standardized off-target effect assessment methods and guidance on the use of non-clinical models. Participants emphasized the importance of developing transparent, reproducible methodologies and promoting systematic data sharing to accommodate scientific and regulatory progress. There was broad support for parallel development strategies, whereby early-phase clinical trials continue to generate evidence that complements and strengthens the initial non-clinical work. The discussion on quality criteria underscored analytics as a critical bottleneck, as without standardized assays, the effective application of CQAs remains out of reach, which in particular causes difficulty to perform comparability studies when manufacturing changes are needed for optimization. Distinct challenges were identified for different delivery platforms (e.g. LNP, AAV), reflecting their diverse risk profiles. There was broad consensus that regulatory clarity, together with better-defined CQAs, is essential to ensure product consistency, predictability, and patient trust. The meeting further emphasized the importance of early scientific advice to guide developers through areas of uncertainty, alongside meaningful patient involvement in defining acceptable levels of risk and therapeutic outcomes. Historical datasets, natural history studies, and real-world data (RWD) were recognized as increasingly valuable in supplementing non-clinical data. Finally, participants highlighted the urgent need for practical case-based regulatory guidance to help both developers and regulators translate these principles into practice.

Specific outcomes

Off-target effects

- No harmonized or predictable regulatory framework currently exists for off-target assessment in *in vivo* gene editing.
- Need for context-specific, benefit–risk analyses for each guide RNA, informed by disease severity, patient characteristics, and availability of alternative treatments.
- Off-target analysis strategies depend on the CRISPR system used, tissue/cell context, and patient genetic diversity.
- Deep sequencing is valuable but limited by cost, depth, and interpretability. Also, personalized reference genomes are needed, since standard reference genomes do not fully capture an individual's unique genetic variation.
- Animal models remain limited in predictive value for off-target effects but are informative for biodistribution studies.
- Patient participation/representation is essential in determining acceptable levels of off-target effect risks and uncertainty.

Non-clinical models

- Regulatory guidance currently provides limited direction on the choice of non-clinical models.
- Traditional animal models (rodents, NHPs) have limited translational value, as their predictive capacity for human outcomes of *in vivo* gene editing is limited.
- Newer model systems such as human iPSC and organoids, organ-on-chip platforms, and *in silico* simulations are becoming increasingly important.
- Current clinical studies illustrate the value of layered approaches combining *in silico*, *in vitro* and *in vivo* models (annex 2).

Quality criteria for manufacturing

- CQAs are difficult to define consistently for novel gene editing strategies and are strongly context-dependent.
- Methods and analytics remain the largest bottleneck; without reliable assays, CQAs cannot be applied effectively.
- For LNP-based systems, key CQAs include editing efficiency, targeting specificity, gRNA integrity, particle size, encapsulation efficiency, sterility, and stability (minimum 6 months).
- For AAV-based systems, important CQAs include empty/full capsid ratio, vector genome integrity, promoter specificity, persistence of expression, and risks of insertional mutagenesis.
- Variability in receptor integration for targeted LNPs poses potential safety concerns and should be considered as a future CQA.
- Regulatory frameworks are still incomplete and differ between EMA and FDA expectations.

RECOMMENDATIONS

The expert group formulated several recommendations to strengthen the regulatory pathway for *in vivo* gene editing. First, they called for a concerted effort to establish harmonized criteria for the detection, validation, and interpretation of off-target effects, with particular attention to context-specific risk profiles. Second, they recommended the adoption of a structured non-clinical model selection framework grounded in benefit–risk matrices. Such an approach would enable more systematic use of advanced non-clinical models alongside historical, natural history, and real-world data, thereby improving translational relevance. Third, participants strongly supported the creation of a European registry or shared data platform to capture off-target effect outcomes. This would promote transparency, enable long-term monitoring, and facilitate collective learning across therapies and technologies. Fourth, the group emphasized the critical role of early scientific advice to align expectations between developers and regulators, reduce uncertainty, and accelerate responsible clinical translation. Participants noted that, given the characteristics of advanced therapies, most non-clinical data should be available before first-in-human use, in line with the recent EMA guidance on investigational advanced therapy medicinal products. Still, carefully designed parallel learning strategies (i.e., allowing collection of some non-clinical data during early trials), combined with thorough risk assessment and mitigation, can be applied. The development of standardized, reliable assays is essential for defining and applying CQAs, which should serve as a central anchor point for ensuring consistency, predictability, and patient trust across different *in vivo* gene editing delivery platforms. Clearer regulatory guidance, combined with platform-specific CQAs, will be necessary to establish a future-proof quality framework. Finally, the group urged the development of practical case-based regulatory guidance to help developers and regulators operationalize these recommendations in day-to-day practice.

Specific recommendations

Off-target effects

- Provide clarity about off-target detection approaches, method validation, and rules for interpretation.
- Establish a shared platform or registry (organized by therapeutic modality rather than indication) for long-term monitoring of off-target effects.
- Apply stepwise dosing strategies in early-phase clinical studies to mitigate potential off-target effects.

Non-clinical models

- Encourage the development and use of fit-for-purpose and validated advanced human-relevant models (organoids, iPSC, AI-supported data integration) to reduce reliance on traditional 'safe harbour' models like NHPs.
- Provide developers with trusted, practice-oriented guidance beyond formal guidelines by leveraging case-based examples concerning non-clinical model choice, performance and regulatory acceptance.
- Promote the use of supportive data — such as published literature and platform data — as valid elements of the non-clinical package when robustly substantiated. Clinical data (e.g. RWD and historical data) can also support the overall benefit-risk assessment and the design of the non-clinical program if justified.

Quality criteria for manufacturing

- Prioritize the development of standardized assays for potency, comparability, and off-target assessment.
- Define platform-specific CQAs for LNP and AAV systems, acknowledging their distinct risk profiles.
- Strengthen regulatory frameworks to explicitly address gene editing–specific quality challenges.
- Encourage the development of new analytical methods to evaluate the potential for receptor-mediated internalization and therefore specific distribution of LNPs.
- Promote alignment between EMA and FDA to reduce regulatory discrepancies and enhance global consistency.

REFERENCES

- Musunuru K *et al*. Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease. N Engl J Med. 2025;392(22):2235-2243. doi: 10.1056/NEJMoa2504747.
- DeFrancesco L. Precision gene editing medicine makes history, and it's just getting started. Nat Biotechnol. 2025 Jul;43(7):1019-1022. doi: 10.1038/s41587-025-02741-6.

ANNEX 1: EUROPEAN REGULATORY FRAMEWORK

Gene therapy-specific guidelines

In vivo gene editing products are classified as Advanced Therapy Medicinal Products (ATMPs). Relevant regulatory guidelines and perspectives include:

- Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products
- Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products
- Genome-editing medicinal products: the EMA perspective
- Other: European Medicines Agency (EMA)
- Ph. Eur. General chapter 5.34 Additional information on gene therapy medicinal products for human use. Monograph 3186 Gene therapy medicinal products for human use.

Non-clinical safety and biodistribution

Key concerns include off-target effects, genotoxicity, immunogenicity, and germline transmission. To address these concerns developers must present a comprehensive biodistribution and toxicology profile, supported by *in silico*, *in vitro*, and *in vivo* data. Applicable guidelines include:

- Preclinical safety evaluation of biotechnology-derived pharmaceuticals
- Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials
- Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products
- Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells

GMP-manufacturing

All components of an *in vivo* gene editing product (e.g., gRNA, nuclease, LNPs) must be produced under Good Manufacturing Practice (GMP) conditions. Upcoming revisions to the GMP guidelines may impact specific requirements for sterility, stability, and batch consistency. Key references include:

- Guidelines on Good Manufacturing Practice for Advanced Therapy Medicinal Products
- Concept of upcoming revision

Clinical trial application

Clinical trials in Europe require approval via national competent authorities under the Clinical Trials Regulation. *In vivo* gene editing may be classified as Genetically Modified Organism (GMO) therapy, leading EU countries to require an environmental risk assessment, which requires separate national approvals.

ANNEX 2: OVERVIEW IN VIVO GENE EDITING CLINICAL TRIALS

Trial / Product	Indication	Off-targets	Nonclinical models	Quality control	References
NTLA-2001 ***Intellia Therapeutics***	Hereditaire transthyretine-amyloïdose (hATTR)	*In silico* evaluation using Cas-OFFinder *In vitro* screening using GUIDE-Seq and SITE-Seq Validation in primary human hepatocytes exposed to saturating doses of the gene editing therapy product using AMP-Seq and long-read sequencing for structural variations	*In vitro* using primary human hepatocytes *In vivo* using humanized *TTR* mouse and rat model and non-human primate	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Cas9, Lipid nanoparticle (LNP) delivery Safety and efficacy testing (*rigorous testing / risk assessment / contamination control*): No data	Gillmore et al. NEJM 2021 Finn et al. Cell Reports 2018
NTLA-2002 ***Intellia Therapeutics***	Hereditary angioedema (HAE)	*In silico* evaluation using Cas-OFFinder *In vitro* screening using SITE-Seq Validation in primary human hepatocytes exposed to saturating doses of the gene editing therapy product using AMP-Seq and long-read sequencing for structural variations	*In vitro* using primary human hepatocytes *In vivo* using humanized KLB1 mice and non-human primates	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Cas9, LNP	Walsh et al. J Allergy Clin Immunol 2022 Longhurst et al. NEJM 2024 Cohn et al. NEJM 2025
VERVE-101 ***Verve Therapeutics***	Heterozygous familial hypercholesterolemia (HeFH)		*In vitro* using primary human hepatocytes *In vivo* using mouse and NHP model	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Adenine base editor, LNP	Press release 2022 Lee et al. Circulation 2023 Musunuru et al. Nature (2021)

Trial / Product	Indication	Off-targets	Nonclinical models	Quality control	References
BEAM-302 ***Beam Therapeutics***	Alfa-1-antitrypsinedeficiëntie (AATD)		*In vivo* using rodent models for AATD: NOD-SCID-gamma chain knockout-PiZ mouse model carrying multiple copies of the human PiZ allele and a humanized PiZ rat model *In vitro:* PiZZ fibroblast	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Adenine base editor, LNP	Press release 2023 ASGCT Poster 2020
EDIT-101 ***Editas Medicine***	Leber congenitale amaurosis type 10 (LCA10)	*In silico* evaluation using Cas-OFFinder *In vitro* screening using GUIDE-Seq and Digenome-Seq Validation in U2OS and ARPE-19 cells and human retinal explant tissue	*In vitro* using human patient fibroblasts and retinal explants *In vivo* using humanized CEP290 mouse model and non-human primates	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Cas9, AAV5	Maeder et al. Nat Med 2019 Pierce et al. NEJM 2024
EBT-101† ***Excision BioTherapeutics***	HIV-infectie	*In silico* evaluation using Cas-OFFinder Validation using whole genome sequencing in non-human primates	*In vitro* using human primary T-cells *In vivo* using humanized mice and non-human primates	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): SaCas9, AAV9	Press release 2022 Burdo et al. Gene Therapy 2023
PM359 (*ex vivo*) ***Prime Medicine***	Chronic Granulomatous Disease (CGD)	Validation using Prime Edited CGD patient CD34+ cells in engrafted in bone marrow of mice	*In vitro* using CGD patient CD34+ cells *In vivo* using engraftment in mice		Press release 2024
K-abe (n=1) ***NIH***	Carbamoyl-phosphate synthetase 1 deficiency (CPS1)	*In silico* evaluation using GuideScan2 v2.2.1 *In vitro* screening using ONE-Seq, CHANGE-Seq-BE and UNCOVER-Seq/GUIDE-Seq Validation of 21 potential off-targets in Huh-7 cells exposed to high concentrations of the gene editing therapy product	*In vitro* using Huh-7 cells *In vivo* using humanized mouse model and non-human primates	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): Base editor, LNP	Musunuru et al. NEJM 2025

Trial / Product	Indication	Off-targets	Nonclinical models	Quality control	References
HELP ***China***	Severe refractory herpetic stromal keratitis (HSK)	*In vitro* screening using Guide-Seq	*In vivo* using mouse models *Ex vivo* using human corneas	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): SpCas9, Lentiviral	Wei et al. Mol Ther 2023 Yin et al. Nat Biotechnol 2021
SB-318; SB-913; SB-FIX-1501‡ ***Sangamo therapeutics***	Mucopolysaccharidosis I (MPS I) and II (MPS II) and hemophilia B		*In vivo* using mouse models	Product characterization (*identity, purity, and potency / analytical methods / potency assays*): ZFN, AAV	Harmatz et al. Mol Ther 2022 Ou et al. Mol Ther 2019

†Primaire endpoints (safety, immunogenicity and biodistribution) met, but not the efficacy measures: Letchumanan et al (2025) Epigenetics Chromatin.

‡Programs stopped