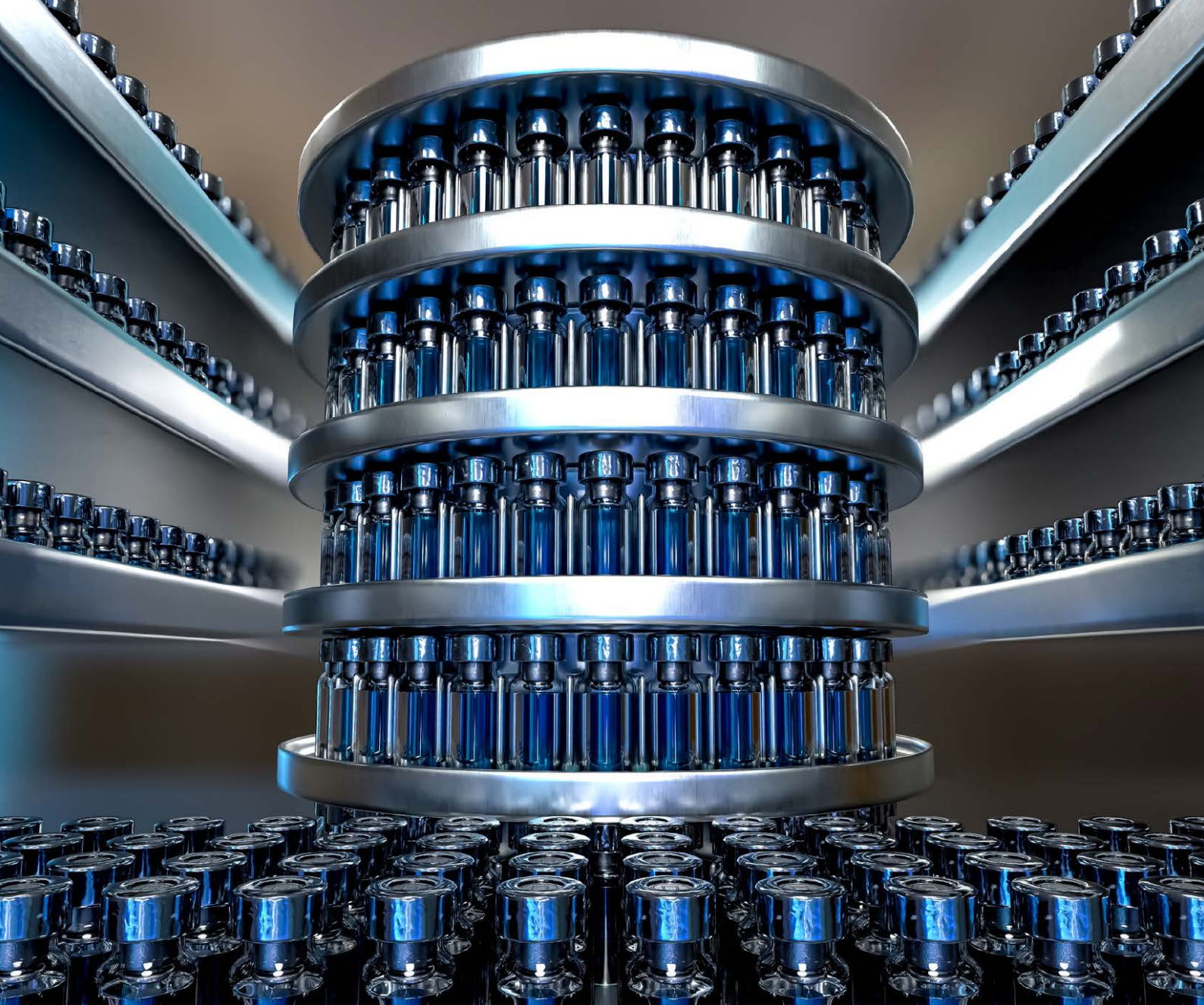




The Role of qPCR Research in the Evolution and Safety Assurance of Gene & Cell Therapies

Expert Insights

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Introduction: The Role of qPCR Research in the Evolution and Safety Assurance of Gene & Cell Therapies

Advances in cell and gene therapy over the past decade have had a profound impact on drug discovery and development in the biotechnology and biopharmaceutical industries. Innovative and future therapies will be inherently personalized, often explored for an individual's specific genetic makeup or disease profile. Previously untreatable genetic disorders and difficult-to-treat cancers are now within the realm of treatable conditions, expanding the market potential for new therapies. This evolution is driving the biotech and biopharma industries to focus more on personalized medicine approaches, moving away from the “one size fits all” model.

Adeno-associated viruses (AAVs) have played a pivotal role in this evolution. Over the last decade, they have become a crucial tool in biotechnology and biopharma, especially in exploring gene therapy, due to their unique and advantageous properties. AAVs are non-enveloped viruses that can be engineered to deliver DNA to target cells and therapeutic genes to potentially treat various genetic disorders. This approach has become popular in research due to its non-pathogenic nature, ability to infect both dividing and non-dividing cells and capacity for long-term expression of therapeutic genes without integration into the host genome.

Similarly, chimeric antigen receptor T-cell (CAR-T) therapy and T-cell receptor-engineered T-cell (TCR-T) therapy are recognized as distinct forms of immunotherapy that involve engineering and modifying T cells to potentially attack specific cancer cells. For example, in CAR-T therapy, T cells are engineered to produce artificial receptors (CARs) capable of selectively binding to specific antigens found on cancer cells. On the other hand, TCR-T therapy involves engineering T-cells to potentially express T-cell receptors that can recognize cancer-specific antigens presented by the major histocompatibility complex.

In all these approaches, qPCR (quantitative or Real-Time PCR) is the preferred molecular genetic technique. It enables fast and precise analysis of genetic information. It is an essential tool in the biotech and biopharmaceutical industries, from research and development to clinical applications and personalized medicine, aiding in the understanding and exploring potential treatment of complex diseases. Real-time PCR techniques can aid in the identification of genes linked to diseases, allowing researchers to pinpoint potential targets for the development of new therapies.

Furthermore, qPCR is used for researching and identifying mutations or genetic variations at the early stages of drug development to help support effectiveness of potential treatment options for individuals with a specific genetic profile. qPCR is also a fundamental tool in pharmacogenomics, the study of how genes affect an individual's response to drugs, which has led to the development of personalized treatments.

The potential of cell and gene therapies to provide cures for previously untreatable diseases remains one of the most exciting prospects in modern medicine. However, the use of technologies such as PCR to find and validate potential drug targets, to enable the safety and efficacy of novel treatments, or to tailor drugs to each patient's unique genetic makeup, is critical to drug discovery and the development of future cell and gene therapies.

This Expert Insights eBook begins with a research paper from Tchedre *et al.* [1]. The study demonstrates that intravitreal delivery of recombinant adeno-associated virus (rAAV2) carrying multi-characteristic opsin (MCO) for gene therapy in retinal diseases is safe in dogs, showing limited off-target presence and no significant immune reaction, paving the way for potential human application. Analysis of vector DNA in live body wastes and body fluids showed a minimal increase of vector copy in urine and feces, suggesting viral vector clearance via these routes.

qPCR tools in this study serve as a sensitive technique for detecting the presence of vector DNA in targeted and non-targeted tissues, thus evaluating the specificity and safety of the gene therapy approach. This report underscores the promise of rAAV-mediated optogenetic opsin delivery for the treatment of retinal degenerative

diseases and highlights the safety of the procedure in animal models as a critical step toward human clinical trials.

The second publication by Gokemeijer *et al.* [2] discusses the development and advancement of CAR-T and TCR-T cell therapies. CAR-T therapies have demonstrated great success against hematological malignancies and are being explored for their potential use against solid tumors and autoimmune diseases. Still, the development of CAR-T and TCR-T cell therapies presents unique challenges due to factors such as patient-derived cells, intracellular expression, and *in vivo* expansion. Therefore, innovative bioanalytical and immunogenicity assessments are necessary.

A robust bioanalytical package is crucial for the advancement of these complex therapies, requiring customized analytical methods and sampling plans to accurately interpret kinetics and immunogenicity data. In this publication, the authors favor molecular measurements using PCR due to their sensitivity and correlation with CAR-T cell levels, despite the challenges of accurately measuring and interpreting cellular kinetics and dynamics. Different molecular assays, such as qPCR/dPCR and RNA-seq, offer options for monitoring CAR-T cell kinetics, with qPCR-based methods often preferred due to sensitivity, cost, and easier handling.

Overall, qPCR technology is indispensable in the biotech and biopharma sectors, where it plays a pivotal role in research, development, clinical applications, and

exploring personalized medicine. It is particularly relevant in exploring gene or cell therapies, such as AAV, CAR-T, and TCR-T treatments. Its significance in increasing our understanding and treatment of complex diseases cannot be overstated.

We hope this eBook will help researchers seeking to learn about the most recent advancements in gene and cell therapies, such as AAV, CAR-T, and TCR-T treatments, and the integral role of qPCR technology.

For more information, we encourage you to visit Thermo Fisher's page on [Real-Time PCR Solutions](#) to gain a deeper understanding of available options and best solutions for the application of qPCR tools in future drug discovery and development.

Julian Renpenning, Ph.D.

Scientific Writer at Wiley Analytical Science

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qPCR and dPCR for biopharma

QualTrak real-time PCR and digital PCR ecosystem for streamlined biologics development

Biologics—including vaccines, cell and gene therapies, monoclonal antibodies (mAbs), and their biosimilars—have great potential for the treatment and prophylaxis of a broad range of diseases. As of 2018, eight of the top ten best-selling drugs in the US were mAbs [1]. mAb biosimilars are expected to follow a comparable path, with the biosimilars market set to double by 2025 [2]. Cell and gene therapies are also predicted to increase, with a projected compound annual growth rate (CAGR) of almost 25% in the near future [3]. This growth offers tremendous opportunities, especially for manufacturers who can develop new and effective therapies quickly.

Rapid and efficient development of new and effective biologics is no easy feat. Researching, developing, and manufacturing new biologic therapies is an arduous and expensive process fraught with regulatory and supply chain challenges. It takes an average of 10 years and \$2.6 billion to develop a new treatment, and only 12% of candidates make it to market [4], while others end up in “the valley of death”—the transition from laboratory to trial where candidates often fail.

With such drastic implications for time and resources, it is imperative that biologic manufacturers choose the right commercial collaborators from the start—agile partners who can improve predictivity, speed, and consistency to bridge basic and clinical research.

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Work with a partner who offers reliable, high-quality PCR products for every step of the development process

Developing and manufacturing biologics requires compliant technologies and methods that provide excellent accuracy, consistency, and quality control (QC) with continuous automation potential to ensure the best possible product is delivered in the right time frame.

PCR methods that enable quantification of target nucleic acid sequences—namely quantitative real-time PCR (qPCR) and digital PCR (dPCR)—have become core technologies for delivering high-quality results in a range of applications throughout biologics development and biomanufacturing. qPCR and dPCR methods are the gold standards for sensitive, specific detection and quantification of nucleic acid targets, which are useful for QC testing a wide variety of biologic characteristics. Leveraging these versatile technologies is imperative to the success of biologics development.

Furthermore, the highly regulated biopharma atmosphere necessitates current good manufacturing practice (cGMP)-compliant facilities, workflows, and the utmost commitment to data integrity through the use of service-level agreements (SLAs) and laboratory information management system (LIMS)-integrated software.

A vendor who provides high-quality qPCR and dPCR products—assays, reagents, and instruments for analysis—is vital for the success of biologics development. These offerings must provide accurate and consistent data for critical decision-making in every step of the process, from discovery to commercial manufacturing.

The role of Thermo Fisher Scientific in PCR-based detection of coronavirus

“PCR” became a household term because of its common use in SARS-CoV-2 detection and surveillance; however, its applications in the coronavirus crisis do not end there. In particular, qPCR continues to play an important role in the development of SARS-CoV-2 treatments and vaccines. qPCR assays, reagents, and instruments developed by Thermo Fisher were not only cited in detection, but also in the development research for several SARS-CoV-2 vaccines, contributing on many levels to pandemic mitigation efforts.

Leverage high-throughput, high-quality products throughout the entire workflow

Central to maximizing the accuracy and efficiency of your biologics development is working with a provider that offers a complete qPCR workflow ecosystem. With Applied Biosystems™ QualTrak™ Real-Time PCR and qPCR Solutions for Biopharma, Thermo Fisher is dedicated to providing an end-to-end ecosystem for a range of applications—so you can leverage the right technologies at the right time.

For discovery, qPCR and dPCR products developed by Thermo Fisher provide highly sensitive, specific, and accurate technologies for investigating pathogen and disease biology, measuring therapeutic efficacy, and evaluating host response. They can also be used to validate transgene and vector assembly, quantify mRNA expression, identify cell culture contaminations, and more.

Our qPCR and dPCR tools also aid process development, preclinical research, and clinical research by validating key nucleic acid sequences; measuring safety, efficacy, and toxicity; and collecting QC data for establishing specifications for regulatory requirements. Among a wide variety of additional applications, qPCR and dPCR can be used to maintain genetic consistency, reliability, and regulatory conformity in cell line development.

Once regulatory bodies have approved a biologic in the context of an explicit bioprocess, qPCR or dPCR can be leveraged to maintain product performance through manufacturing quality control and adherence to established lot-release criteria.

Table 1. Suggested applications for qPCR and dPCR in biopharma discovery, development, and manufacturing.

Phase	Goal	mAbs applications	Vaccine applications	Cell and gene therapy applications
Discovery	Identify new potential drug targets and candidates	- Antibody screening and selection confirmation - Gene identification - Biomarker identification	- Antigen identification - Antibody screening - Biomarker identification	- Gene identification - Gene replacement strategy screening - Receptor screening - Biomarker identification
Process development	Produce therapeutics for use in preclinical studies and beyond	- Detection of impurity or contamination - Viral clearance checks - Sterility testing - Bioburden checks - Host cell DNA identification - Verification of accurate plasmid construction - Verification of accurate gene integration - Cell culture monitoring	- Confirmation of accurate plasmid construction - Verification of accurate gene integration - Cell culture monitoring - Viral and particle characterization	- Confirmation of accurate plasmid construction - Measurement of viral titers, vector copy numbers, and percent modified cells
Preclinical and clinical research	Confirm safety, efficacy, toxicity, biodistribution, and biomarker analysis <i>in vitro</i> and <i>in vivo</i>	- Patient screening and identification - Infection detection - Biomarker detection and tracking - Molecular pathology analysis	- Viral testing - Safety and efficacy testing - Patient screening - Adaptive immune response assessments	- Evaluation of biodistribution, vector shedding, and transgene expression - Monitoring of cell therapy frequencies in patients
Manufacturing	Increase confidence in the manufacturing quality of vaccines, mAbs, and their biosimilars to ensure safety and efficacy	- In-process testing and lot release - Adventitious agent testing - Testing for residual host DNA - Viral clearance screening - Bioburden checks - Sterility testing	- In-process testing and lot release - Adventitious agent testing - Testing for residual host DNA - Testing for purity and efficacy	- Measurement of vector copy numbers - In-process testing and lot release - Adventitious agent testing - Testing for residual host DNA - Testing for purity and efficacy

qPCR and dPCR offer more consistent results due to innovative instrumentation, optimized master mixes, and predesigned and custom assays

Instrumentation

High-throughput, user-friendly, and automation-ready qPCR instrumentation can provide consistency and speed by reducing error-prone manual tasks and making 24/7 operations possible. Applied Biosystems™ QuantStudio™ Real-Time PCR instruments offer continuous automation and improved user experience for laboratory innovation and smarter workflows.

The quality of a PCR instrument's thermal cycling and blocks is a core aspect to effectively pairing excellent assay consistency and throughput. To this end, most QuantStudio real-time PCR systems incorporate our industry-leading Applied Biosystems™ VeriFlex™ temperature control technology to give users the utmost command over thermal cycling and allow highly precise temperature zones across the block. Importantly, VeriFlex blocks offer high accuracy (set point vs. actual temperature) and precise temperature zones for reliable and rapid determination of optimal annealing temperatures and data collection, especially when paired with our reaction plates specifically designed for increased uniform thermal contact.

The Applied Biosystems™ QuantStudio™ 6 and 7 Pro Real-Time PCR Systems offer voice commands and hands-free operation, remote instrument access, push-button help access, and automatic facial authentication for greater utilization, more efficient troubleshooting, and increased user control. The QuantStudio 7 Pro system also boasts built-in automation compatibility and 21 CFR Part 11–compliant software, which further digitizes and secures your data collection.

QuantStudio 6 and 7 Pro systems increase throughput and assay flexibility with their interchangeable block feature, accommodating 96-well 0.2 mL blocks, 96-well 0.1 mL blocks, and 384-well plate formats. The QuantStudio 7 Pro system is also compatible with Applied Biosystems™ TaqMan™ Array cards. QuantStudio Pro instruments also offer exceptional reproducibility, demonstrating minimal well-to-well and instrument-to-instrument variation (Figure 1). Together, these advanced features prime our qPCR instruments to revolutionize critical assays in your biologics program, all while minimizing labor-intensive steps and maximizing user efficiency.

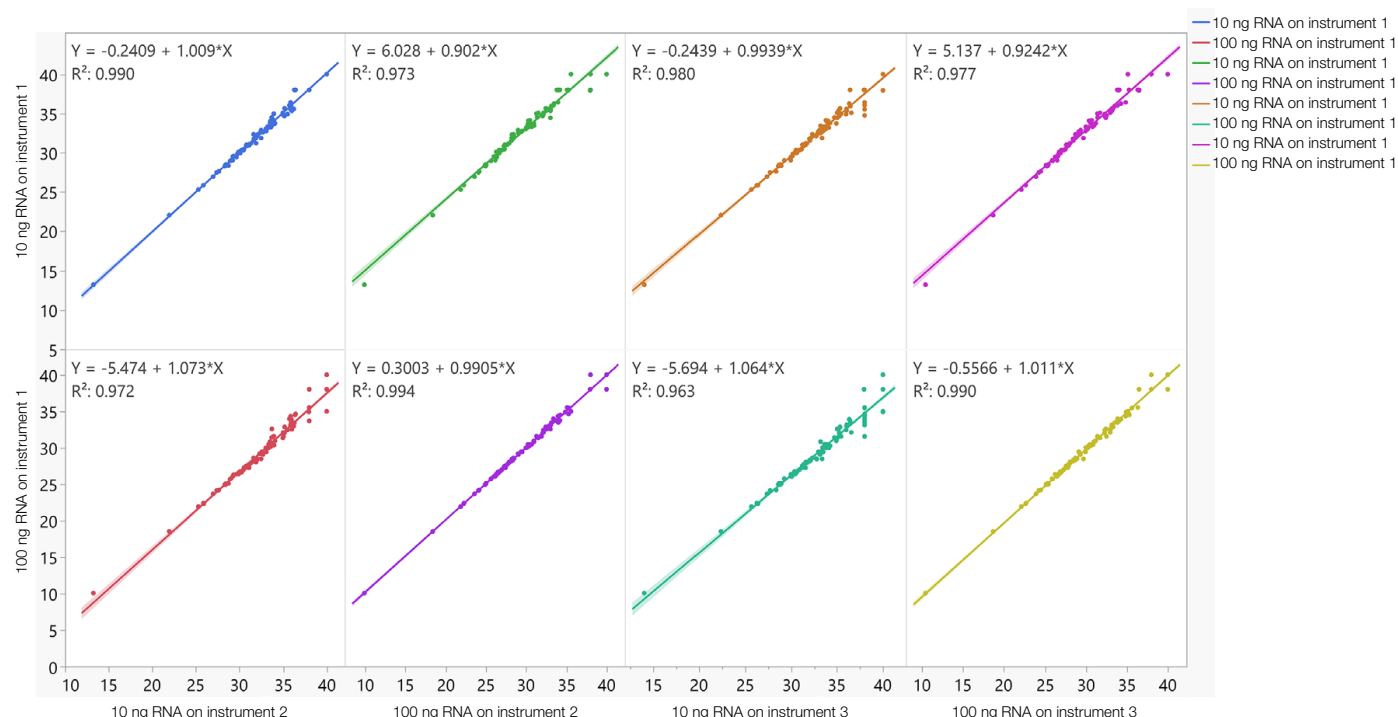


Figure 1. Reproducibility of gene expression measurements using Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plates on different instruments. Pairwise comparisons of cDNA data from 10 ng or 100 ng universal human reference (UHR) RNA on 3 different instruments show high correlations from instrument to instrument.

With respect to dPCR, the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System is easy to use and powerful by way of its unique [microfluidic array plate \(MAP\) technology](#). This proprietary technology enables consistent and automated compartmentalization of a sample into 20,000 microchambers with a best-in-class coefficient of variation (CV) of <1%. Unlike other digital PCR systems, MAP technology does not use emulsion or other droplet-based methods to compartmentalize reactions. Instead, the reagent is delivered to the microchambers with great precision and consistency using distribution channels.

Additionally, the QuantStudio Absolute Q system analyzes more than 95% of the loaded sample, ensuring highly accurate digital PCR results with less wasted sample and reagents. dPCR has emerged as a powerful tool for absolute quantitation of nucleic acids, offering accurate and precise quantification of AAV vectors without the need for reference samples or standard curves.

Master mixes

The Applied Biosystems suite of TaqMan and TaqPath real-time PCR master mixes is designed to control for common sources of variability by including passive references to compensate for small fluorescence fluctuations, inhibitor tolerance for sample-to-sample variability, and carryover contamination control methods (e.g., [UNG](#)). Our TaqPath-branded master mixes have the most rigorous analytical and functional lot release quality control in the industry, detailed in the lot-specific Certificate of Analysis (COA), which makes for exceptional lot-to-lot consistency.

For example, [Applied Biosystems™ TaqPath™ ProAmp™ Multiplex Master Mix](#) delivers sensitive and reproducible detection of up to four DNA targets in a single multiplex qPCR reaction in high-throughput genotyping and copy number variation (CNV) assays. CNV confirmed by qPCR or dPCR can be used for many applications in vaccine, mAbs, and cell and gene therapy development. These applications include antigen identification, selection, and screening; biomarker identification; vector and plasmid construction and mRNA packaging for vaccines; QC; and delivering uncompromising reliability and accuracy even in the presence of inhibitors commonly found in clinical samples. Figure 2 displays genotyping results from the TaqPath ProAmp master mix compared with mixes from other suppliers.

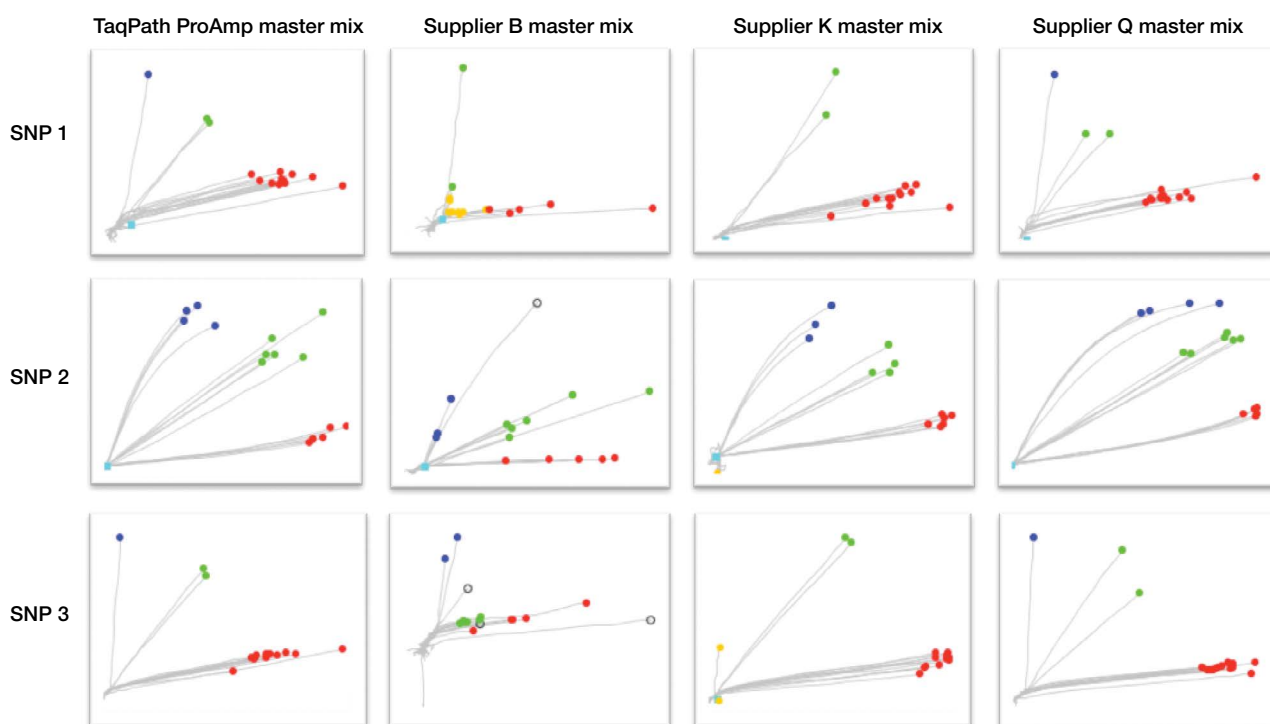


Figure 2. Genotyping results from TaqPath ProAmp Multiplex Master Mix and master mixes from other suppliers. In three TaqMan™ genotyping assays performed under standard conditions, TaqPath ProAmp master mix consistently produced accurate genotype calls and excellent cluster resolution across multiple assays, demonstrating higher data precision compared to other suppliers' mixes.

Predesigned and custom assay formats

In addition to reliable and efficient instrumentation and master mixes, predesigned and custom assay formats can also provide a way to increase the reproducibility and speed of your qPCR analysis. By working with a proven vendor of customized assays used in biologics development, you can be sure that consistency is inherent to the assay—reducing or eliminating assay development and validation time.

Our TaqPath assays are manufactured to highly rigorous standards in full compliance with ISO13485 and 21 CFR Part 820 quality system requirements. Formulations of your specific probe and primer sequences will be provided at a guaranteed yield. When used with QuantStudio 6 and 7 Pro systems, [Applied Biosystems™ TaqMan™ Array Plates with RFID](#) enable automatic upload of your plate layout and assay information. The instrument automatically reads the RFID tag when the plate is loaded, seamlessly transferring the assay data to your EDS file. This means that manual download and transfer of TaqMan files is no longer necessary, saving valuable time and delivering your results faster.

Together, innovative and user-friendly qPCR and dPCR instrumentation, predesigned and custom assays, and master mixes encompass a complete ecosystem that can help you overcome hurdles in biologics development and move an investigational candidate to the finish line.

Ensure quality assurance (QA), control, and compliance with advanced technology and compliant software, documentation, and facilities

Biologics manufacturing requires ongoing quality control and adherence to established lot-release criteria. While QC assay consistency remains a key challenge to ensure low lot-to-lot variability, these assays must also provide strengthened data integrity.

Cell and gene therapies, mAbs, and vaccines require many of the same QC analyses, but often come with increasing compliance and safety expectations due to the complex nature of these therapeutics and a challenging regulatory environment. Biomanufacturers need reliable, efficient assays for therapeutic potency, critical quality attributes, bioburden analysis, monitoring, sterility testing, detection and identification of impurities (e.g., host DNA) and contamination events, confirmation of viral clearance, and other measures for QA and QC.

Incorporating high-quality qPCR into your product analyses can fast-track your biologics manufacturing by maximizing uptime and workflow efficiency. A comprehensive ecosystem of qPCR and dPCR technology designed to work synergistically helps your product analysis program thrive. The Thermo Fisher ecosystem

of qPCR and dPCR instruments, assays, reagents, and software is designed to work together for simpler, more flexible, and highly efficient workflows. Further, the qPCR ecosystem was built to specifically support cGMP-compliant workflows in post-discovery stages of development.

Strong quality management systems, from raw materials to final manufacturing, are essential to establish operations in cGMP-compliant workflows from the very beginning and ensure appropriate design, monitoring, and control of manufacturing processes and facilities.

In addition to employing quality, cGMP-compliant qPCR instruments and assays, supporting software should also be CFR 21 Part 11-compliant. The qPCR ecosystem is built by Thermo Fisher to support cGMP-compliant workflows with automated, compact systems uniquely designed with innovative, time-saving features that increase productivity and uptime. These instruments and their software are built to seamlessly integrate with SLAs and LIMS.

A partner that is up-to-date and can provide you with the most current compliance documentation is key to a streamlined manufacturing process. Thermo Fisher is proactive about supplying its partners with the most current compliance documentation for consistent, ongoing regulatory support throughout the PCR workflow.

Leverage a robust supply chain to ensure uninterrupted PCR operations

Supply chain insecurity can compound existing manufacturing challenges, so ensuring supply chain continuity is a critical component of building a successful biologics program. A global and complex supply chain ensures goods and services are sourced from geographically diverse sites around the world, building resilience against damaging vulnerabilities such as cyber attacks, adverse weather events, and pandemics, and ensuring your organization has strategic options when such issues arise.

Working with a supplier that leverages a sophisticated and resilient supply chain will optimize the development and manufacturing of your vaccines, cell and gene therapies, mAbs, and their biosimilars. Our team has risk management programs in place to map, monitor, and lower supply chain risk based on supplier performance, geographic exposure, and environmental practices.

Working with a complex partner like [Thermo Fisher Scientific](#) can ensure your biologics manufacturing is resilient and scales appropriately with demand.

Conclusions

Choosing to adopt better PCR technology and the right partner offers tremendous benefits for discovery, development, and manufacturing of biologics, including vaccines, cell and gene therapies, mAbs, and their biosimilars. To unlock the most effective instruments, master mixes, assays, and compliant software for your biologics program, find a vendor with a complete and thriving qPCR ecosystem.

Thermo Fisher is a long-established, trusted leader that can provide the leading cGMP-compliant QualTrak Real-Time PCR technologies and support to guide you through every PCR step of biologics development. Thermo Fisher guarantees continuous automation, accuracy, consistency, and compliance to its partners—setting them up for success from the first phase of discovery through widespread distribution of their biologic.

Visit thermofisher.com/qpcr/biopharma to get started with Thermo Fisher Scientific as your full-service biopharmaceutical PCR products provider. Our Applied Biosystems QualTrak biopharma-specific qPCR and dPCR products and workflows are designed to fast-track your biologics development pipeline, allowing you to develop the highest quality mAbs, biosimilars, vaccines, and other therapeutics in the shortest amount of time.

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Biodistribution of adeno-associated virus type 2 carrying multi-characteristic opsin in dogs following intravitreal injection

Adapted from Tchedre, K.T. *et al.*

Retinitis pigmentosa (RP) refers to disorders characterized by the degeneration of photoreceptors in the eye, which hinders visual ability by the absence of photoactivated electrical signals in the retina, and non-cortex transmission of those signals to the visual cortex [1]. The prevalence of RP is 80,000 patients in the US, out of which 20,000 patients have 20/200 vision or less. Further, vision loss increases with aging [2], which is a major concern since our population is living longer. There is no FDA-approved therapy to halt degeneration, and no clinically meaningful therapies can restore vision once vision loss has ensued.

Recently, the authors developed an optogenetic therapy using multi-characteristic opsin (MCO), which effectively re-photosensitizes photoreceptor-degenerated retina in mice leading to vision restoration in an ambient light environment [1]. The development of recombinant adeno-associated viral (rAAV) vectors provides excellent vehicles for efficacious delivery and long-term expression of gene therapy molecules, such as MCO. The MCO-encoding gene, with distal CMV promoter and ON-bipolar cell-specific mGluR6-enhancer along with mCherry as a reporter and enhancer, is packaged in an adeno-associated virus type 2 (AAV2) vector for transducing retinal cells, and then drives retinal circuitry functions and visually guided behavior after intravitreal injection. Here, this study reports the biodistribution of the vMCO-I in live samples, targeted retina, and non-targeted organs following intraocular delivery. The results indicate that, after the intraocular delivery of the vMCO-I, the off-target presence of vMCO-I is limited. AAV2-based delivery of MCO to the retina and evaluation of dose-dependent MCO expression in targeted tissue (i.e. retina) is the key to establishing a safer therapeutic dose.

Methods

Multi-characteristic opsin (MCO) gene under metabotropic glutamate receptor mGluR6, with reporter mCherry, was cloned into the pAAV-MCS vector. The viral titer was determined by qPCR with a standard curve generated by linearized plasmid DNA, and intravitreal

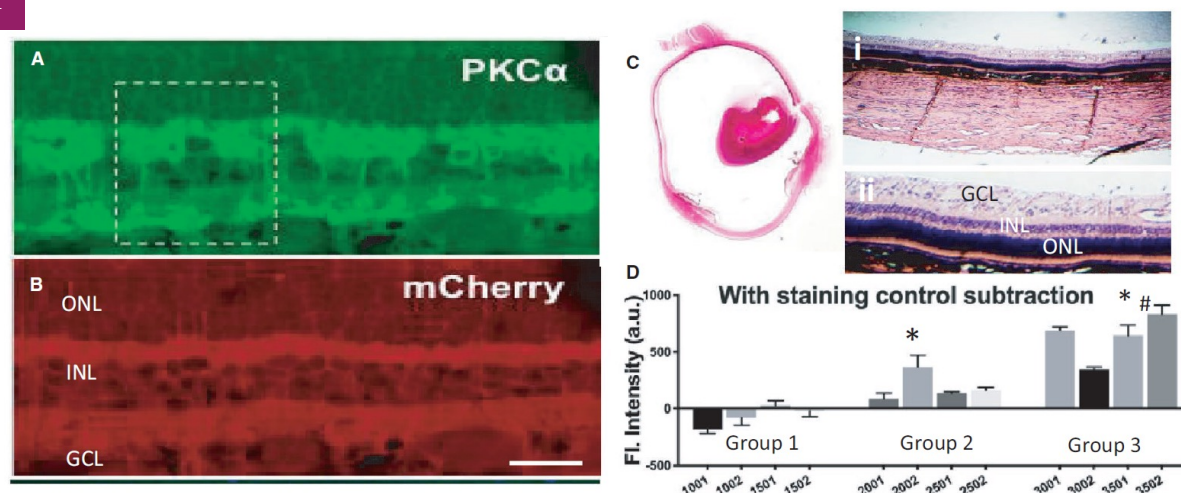
injections were performed with the control vehicle (Group 1), the high dose (Group 2), and the low dose (Group 3). Organ tissue (heart, liver, spleen, kidney, mesenteric, mandibular, testis/ovary), as well as feces, saliva, and nasal secretions, were collected and analyzed using immunofluorescence microscopy or qPCR. For qPCR, DNA from feces, saliva, nasal secretion, and urine was collected using the Thermo Fisher Scientific GeneJET Genomic DNA purification kit, and qPCR was performed using Thermo Fisher Scientific's Master Mix (Applied Biosystems) and Power Up SYBR Green Master Mix (Thermo Fisher, A25776) on an Applied Biosystems QuantStudio 3 real-time PCR System.

Results

The main objective of this study was to determine the biodistribution of the vMCO-I in live samples, targeted retina, and non-targeted tissues after intravitreal injections. Transgene expression in retinal cells (bipolar cells) was assessed using mCherry fluorescence and was significantly increased in Groups 2 and 3 (Fig. 1).

One major hurdle in using viral vectors for *in vivo* gene therapy is the development of host cellular immune responses to the vector, which may lead to the elimination of transgene expression [3]. The absence of a dose-correlated or significant increase in IFN- γ (green) or cellular CD45 (red) signals suggests that the intravitreal vMCO-I injection did not lead to inflammatory cytokine or immune cell infiltration.

Figure 1



Immunohistochemical analysis of MCO expression in the retina. (A) Immunostained images of Bipolar cell marker, (B) mCherry expression, (C) H7E stained eye section, (D) mCherry fluorescence quantification.

Biodistribution of vMCO-I in live samples

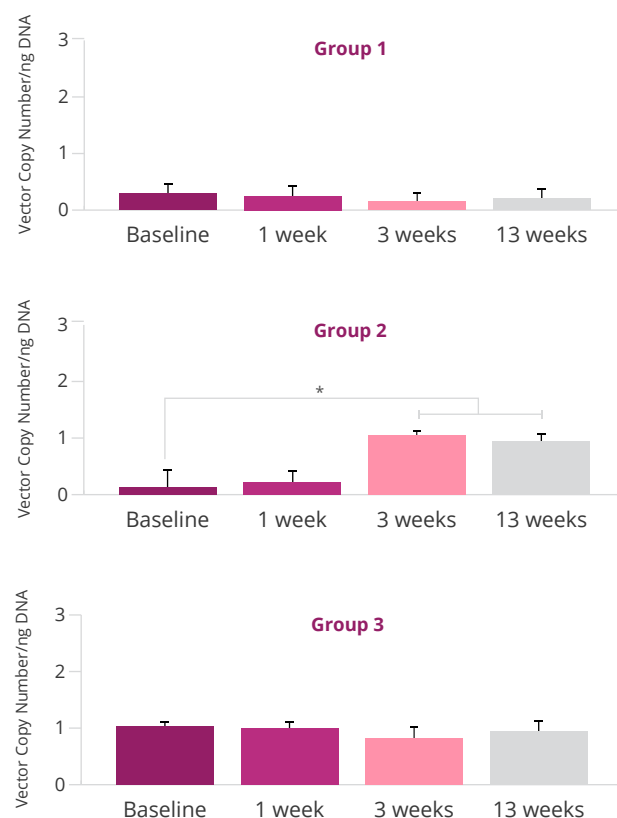
To understand the biodistribution of vMCO-I and its clearance, its presence in body wastes (urine and feces) and body fluids (saliva and nasal secretions) was assayed. Analysis of Groups 1, 2, and 3 feces samples showed no significant pre-to post-injection variation over time for AAV vehicle-injected or low-dose vMCO-I-injected group (Fig. 2). Additionally, no viral DNA was detected in the feces sample compared to the positive control. Similarly, the saliva samples of all groups showed a non-significant increase in the qPCR signal of the vMCO-I ITR from pre-(baseline) to post-injection (weeks 1, 3, 13). This analysis was performed using a qPCR tool from Thermo Fisher (Applied Biosystems QuantStudio 3 real-time PCR System).

Biodistribution of intravitreally injected AAV2 in non-targeted tissues

Biodistribution of vMCO-I was assayed using genomic DNA from non-targeted tissues of distant organs (lung, liver, kidney, spleen, mesenteric/mandibular lymph node, heart, and testis/ovary). In the negative control (Group 1), qPCR results did not show any significant presence of vector genomes. In Groups 2 and 3, signals in various tissues were detected in individual subjects. However, since there was no consistent +ve amplification in any organ across all animals in each group, an average (Av.) with standard deviation (SD) was plotted. The data was then subjected to a one-way ANOVA test. Finally, to confirm the presence of the vector, PCR was performed on all tissue samples that showed positive results from the qPCR analysis (Fig. 3), and this resulted in no detectable bands. Furthermore, PCR analysis did not

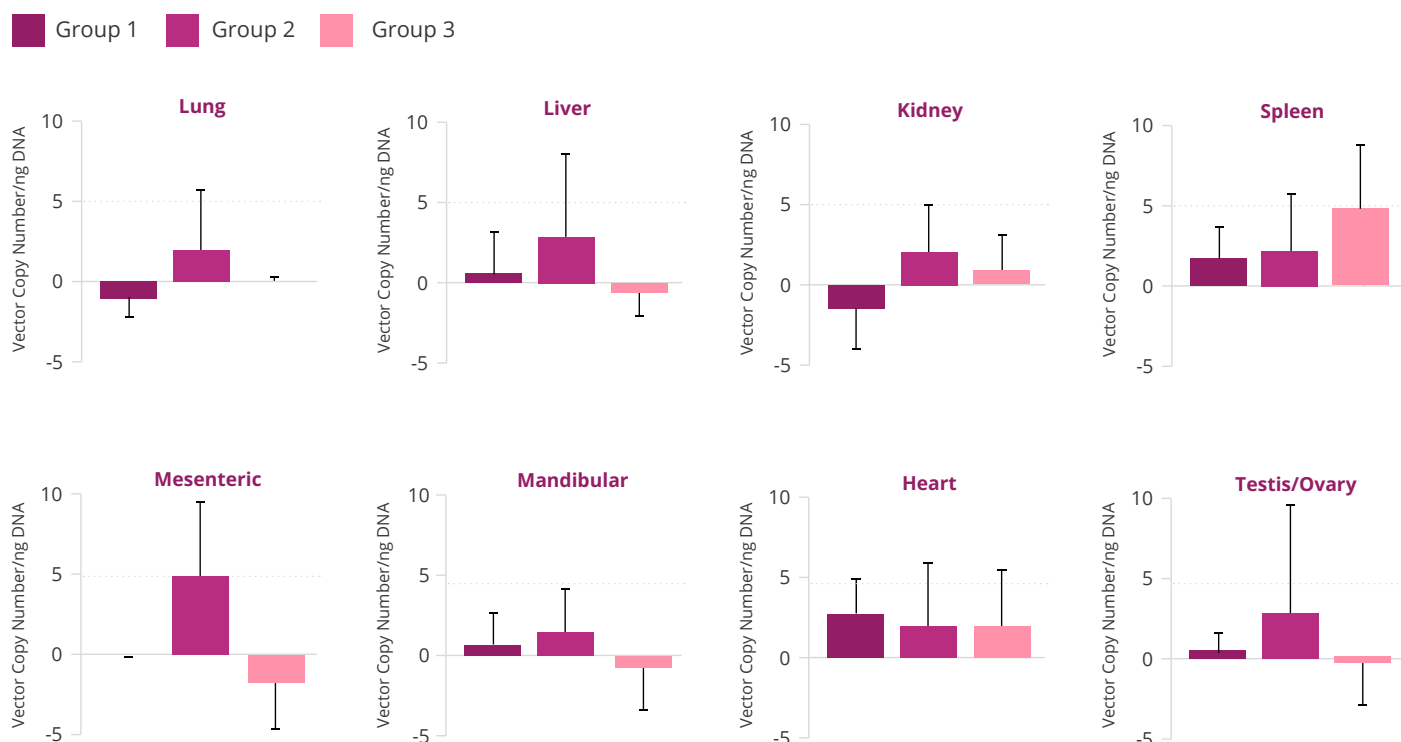
detect vector genomes in the gonad or ovaries samples, indicating that the risk of germ-line transmission of vector DNA is very low.

Figure 2



Time course quantification of AAV2 vector sequences in feces samples using qPCR, before and after intraocular injection.

Figure 3



Summary of qPCR detection of vector copy number in different tissues. Data are expressed as the mean of the vector copy number; Av. $\pm$ SD. Except for mesenteric tissue, one-way ANOVA analysis is not significant between the groups. However, the average values are within the error range of the qPCR assay, which is attributed to sensitivity and variation in sample handling. The limit of detection (LOD = 5 vector copy) is shown by dotted horizontal lines.

Discussion

Recombinant adeno-associated virus 2 (rAAV2) vectors have been used extensively as efficient gene delivery vehicles for the treatment of retinal diseases. AAV2 has been the most extensively examined and has natural tropism towards retinal cells, skeletal muscles, neurons, vascular smooth muscle cells, and hepatocytes, and in humans, AAVs are not found to be pathogenic. To date, there are very few published studies of biodistribution following intravitreal injection of AAV2. Quantitative PCR assays are a recommended tool for the assessment of vector biodistribution, as demonstrated by this study, as qPCR is a sensitive method to demonstrate gene transfer. The results of this study indicate that there was limited systemic exposure to the vector, or that the injected vector was cleared before the time of analysis, while intravitreal injection did lead to cell-specific multi-characteristic opsin MCO expression.

The Applied Biosystems family of most-cited qPCR solutions is designed to simplify your process, expedite your results, streamline development, and simplify

compliance. It enables you to optimize biopharmaceutical drug development with efficient workflows supported by consistent [assays & reagents](#) manufactured under GMP manufacturing principles. Offerings include [automated instruments](#), premium service and support, and a vertically integrated supply chain.

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An IQ consortium perspective on best practices for bioanalytical and immunogenicity assessment aspects of CAR-T and TCR-T cellular therapies development

Adapted from Gokemeijer, J. *et al.*

Cell therapies, such as those using chimeric antigen receptor (CAR) T-cells, are delivering tremendous value to patients. Significant challenges remain in understanding the disposition of these novel modalities with respect to their *in vivo* pharmacology, unique biology-driven pharmacokinetics (PKs), immunogenicity, and unique manufacturing process. With advancements in gene editing technologies, identification of new targets in both liquid and solid tumors, and introduction of engineered domains that can persist in cells both *ex* and *in vivo*, increasingly complex CARs continue to be designed for autologous and allogeneic settings.

Generating a robust bioanalytical package is key to advancing these complex therapies in a data-driven manner as a choice of analytical methods and sampling plan can significantly impact downstream kinetics and immunogenicity data interpretation. Members of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) consortia companies compiled the following suggestions to help advance the field and accelerate treatments to broader populations of patients with cancer.

Bioanalytical assays

Cellular kinetics (CK) refers to the study of the *in vivo* fate of administered adoptive cell therapies (ACTs) following infusion. [1]. Like PKs, measuring and understanding the CKs of ACTs is important for establishing appropriate dose administration levels, building correlations with patient response, and potentially evaluating treatment outcomes. With ACTs, each patient receives a slightly different product, and CKs can therefore be extremely varied between individual patients and disease states. Another challenge regarding cellular therapeutics is that they utilize living cells. Therefore, unlike small molecule or protein therapeutics, the possibility of a living cell changing from the time of isolation from a patient to the time of analysis is not only possible but likely.

Two classes of techniques have evolved to measure the living ACTs: phenotypic and molecular (also referred to as genotypic). Phenotypic methods use the principle of direct measurement of a unique receptor or receptors present on the cell by flow

cytometry. Molecular methods infer the presence of a transformed cell by measuring the genetic profiles by quantitative polymerase chain reaction (qPCR). The two methodologies give potentially similar information, and both have advantages and disadvantages in terms of information provided and technical feasibility (Table 1).

Cellular kinetics: Flow cytometry-based phenotypic measurement

A standard methodology for phenotypic measurement of cellular therapeutics is flow cytometry [2]. However, developing a flow-based quantitative method has several technical challenges, such as i) the need for specific antibodies, ii) an inability to identify CAR-T cells in the presence of normal cells in the blood, iii) requiring unfrozen, fresh samples, iv) higher limit of detection. Generally speaking, the limit of detection is typically higher than that obtained with polymerase chain reaction methodologies, such as quantitative PCR (qPCR), digital PCR (dPCR), or ribonucleic acid sequencing (RNAseq). Hence, the transgene may be detected in a PCR-based gene amplification assay, but no transformed cells are detected by flow cytometry.

Cellular kinetics: PCR-based molecular assessments

Different molecular (genotypic) assays, such as qPCR/dPCR, Nanostring, or next-generation sequencing (NGS) DNA or RNA-seq, could be used to monitor CAR-T CK in cell therapy studies. PCR-based methods, for example, using TaqMan technology (Applied Biosystems,

Thermo Fisher) [3] and the QuantStudio 3D digital PCR instrument (Life Technologies, Thermo Fisher) [4], are widely used to monitor CAR-T CK and are preferred over NGS methodologies [4]. dPCR may provide higher precision which can be important when quantifying low copy numbers, such as during the persistence phase of a given cell therapy, however, the expansion phase will likely require a wider dynamic range which can be provided by qPCR. Quantitation by PCR relies on the detection and amplification of the target sequence in extracted DNA from cell pellets obtained from whole blood or enriched cells. Reagents used to prepare the

standard curve and quality controls for PCR should be evaluated for both sensitivity and specificity that meet the assay needs as well as efficiency and linearity. Efficiency and linearity of the curve, extraction recovery efficiency, and specificity are evaluated when using a qPCR method [5]. An appropriate lower limit of quantitation and limit of detection (as needed) for the qPCR assay should be defined to support validation. The use of dPCR, which applies absolute quantitation, and therefore eliminates the need for a standard curve, is an alternative approach to quantifying vector copy numbers of CAR-Ts.

Table 1

Overview of advantages and disadvantages of phenotypic and molecular CK measurements using flow cytometry or PCR-based methods.

qPCR/PCR molecular-genotypic measurement		
Parameter	Advantages	Disadvantages
Sensitivity	High sensitivity (up to 3 copies of transgene).	Can be slightly more time consuming. This might limit real-time analysis.
Range of samples	Can be applied to (a) freshly collected blood samples, (b) stored DNA samples, and (c) detection of DNA even in CAR downregulated cells.	Does not distinguish between cells that express the CAR and T cells that harbor 1 or more CAR transgene copies but are incapable of expressing the receptor.
Cell free DNA	Useful in analyzing the cfDNA: CAR-Ts contribute to the pool of cfDNA in peripheral blood. Seventy-seven copies of the CAR transgene per mL plasma could be detected in a study by Mika et al., 2021.	N/A
Liquid biopsies	Liquid biopsies are considered as modern tools to examine genetic features and treatment responses. cfDNA analysis in liquid biopsies represents a modern tool to routinely examine genetic features and treatment responses in several solid tumors.	N/A
Tissue accessibility	N/A	CAR-Ts proliferate, distribute and persist in different tissues. Both flow cytometry and qPCR assays are limited to only liquid samples in clinical studies. Hence, they provide only a fraction of tissue distribution data except where tissue biopsies are possible. Currently, noninvasive PET based imaging techniques are used to enumerate CAR-Ts in animal models.
Long-term analysis	Useful in monitoring the persistence of CAR-Ts for a very long time. CAR-Ts can persist up to 10 years after therapy.	The numbers measured do not reflect the functional, transgene expressing cells. RNA-seq might provide some information to predict.
Flow cytometry - phenotypic measurement		
Enumeration of viable cells	Detection of functional T cells expressing the CAR protein. No chance of counting non-living cells.	Difficulty distinguishing low-level CAR expression from background fluorescence. For BCMA CAR-Ts, flow cytometry sensitivity is 0.4%, whereas for qPCR it went as low as 0.02% to 0.01%. Difference of 20 to 40-fold.
Multiple cell type identification on a per cell basis	Potential to simultaneously assess expression of other proteins on CAR-Ts on a per-cell basis. This allows, for example, the separate enumeration of CD4+ and CD8+ subsets of CAR-T cells, and the determination of memory phenotype of circulating CAR-Ts.	Need for intact cells to perform the assay and, in some instances, a paucity of available reagents for CAR detection.
Assay standardization	Method transfer guidelines and best practices are published. Where possible, same reagents and same SOPs. centralized gating are recommended.	Lack of assay standardization between laboratories.
Monitoring of cells persisting for a long term	N/A	Detection of persisting CAR Ts up to 10 years after therapy was reported by qPCR and flow cytometry.

cfDNA, cell free DNA; CK, cellular kinetic; PCR, polymerase chain reaction; dPCR, digital PCR; N/A, not applicable; PET, positron emission tomography; qPCR, quantitative PCR; SOP, Standard Operating Procedures.

Immunogenicity

As for therapeutic proteins, product-, process-, treatment-, and patient-related immunogenicity risk factors should be assessed in the overall immunogenicity risk of CAR-T and TCR-T therapies (Table 2). It is important to note that comparison between incidence of immunogenicity should be interpreted with caution due to differences in assay format and assay sensitivity. Current assay formats for anti-drug antibodies (ADAs) detection include immunoassays and flow cytometry assays, and interestingly, there has been no clear evidence that ADAs impact CKs, efficacy, or safety of approved CAR therapies. An important question is whether a cellular immune assay can provide timely, robust, and sensitive enough information to drive decision-making. Overcoming the technical challenge to obtain the required sensitivity may not be warranted in cases where only altered CKs are the main consequences of cellular responses, as these can readily be observed through CK profiles. However, understanding cellular immunogenicity will be important if the same patient is going to receive future cell therapy treatment given the risk of identical elements of the CAR in reactivating the immune response.

One promising approach to mitigate the immunogenicity risk of CAR-T therapies involves the use of *in silico* MHC class I and binding algorithms to identify potential T cell epitopes within the CAR constructs. Several studies have demonstrated the feasibility of this approach, showing that *in silico*-designed CAR-Ts can have reduced immunogenicity and increased persistence in preclinical models. Other critical tools are using *in vitro* T cell proliferation assays or replacing CAR constructs with humanized/human ScFv.

Conclusion

The best practices and recommendations by the IQ consortium working group provided a comprehensive analysis of the bioanalytical strategies to support CAR-T modalities. Digital PCR-based assays were considered optimal for monitoring CKs due to ease of use, sensitivity, and robustness. This is particularly beneficial when quantifying low copy numbers during the persistence phase of CAR-T cells. However, qPCR provides a wider dynamic range than dPCR and is therefore better suited for assessments during the CAR-T cell expansion phase. The highly referenced product array of Applied Biosystems qPCR solutions has been specifically designed to allow fast and easy experimentation and to reduce the time spent on development and compliance. Thanks to Applied Biosystems optimized assays, reagents, and workflows, researchers can easily improve biopharmaceutical drug development. All products are produced under GMP manufacturing principles and the pipeline is complimented with **automated instruments**, premium service and support, as well as a vertically integrated supply chain.

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Table 2

Overview of product-, process-, patient- and treatment-related immunogenicity risk factors and associated mitigation approaches

Immunogenicity risk factors identification

Risk category	Risk factor	Example of higher risk	Impact	Recommended mitigation
Product-related risk factors	Cell origin: autologous vs. allogeneic	Allogeneic due to MHC mismatch Autologous is lower risk	GVHD Diminished <i>in vivo</i> expansion and persistence	Partial MHC matching; MHC I/II knock-out with insertion of MHC-E to prevent killing by NK cells of the host and/or co-expression of accessory proteins to improve overall cell expansion and persistence
	Nonhuman or partially human nature of various components of the CAR construct	Nonhuman sequence or protein sequence with known polymorphism used for - CAR (ScFv or receptor)/ linker/domain junctions/ intracellular domain - accessory proteins (armored cells)	ADA: potential for neutralization of drug, changed expansion and persistence references For CAR based on receptor domains and accessory protein(s), potential cross-reactivity of ADA with endogenous counterpart Complement activation and cytotoxic elimination Cellular cytotoxic response: CTL-mediated loss of efficacy, persistence, and cytotoxicity Safety, efficacy, initial expansion, and persistence not impacted.	Use of fully human, highly conserved (germline) sequences with low polymorphism to avoid potential cross-reactivity of ADA/NAB with endogenous counterpart For antibody fragment-based CARs run <i>in silico</i> T cell epitope analysis and sequence optimization to remove T cell epitopes Immunosuppressive rituximab therapy prior to infusion
	Post-translational changes and chemical modifications	Deamidation, oxidation, etc.	Increase protein uptake by APC enhance ADA development risk	Replace potential CMC-relevant risky residues in the genetic sequence of CAR/accessory proteins
Process-related risk factors	Residual viral proteins: Plasmid vector used to transfer into the T cells	Presence of elevated impurity levels (product and/or process related) including: viral proteins from transduction; mAbs and streptavidin from expansion process; gene editing components	Innate an adaptive immune response develop between 24 hours and 7 days after infusion Loss of efficacy, persistence, and cytotoxicity	Optimize production process; <i>in vitro</i> assessment (toxicological assessment); consider levels of residual proteins/ gene editing components after cell expansion prior to administration Immunosuppressive rituximab therapy prior to infusion Risk-based approach for residual proteins: use of innate activation assays to set CQA concentration cutoffs
Patient-related risk factors	Immune and disease status	Activated e.g., autoimmune disease Oncology/hematological malignancies is lower risk	Altered immune reactivity depending on immune status of patients	Consider concomitant medication, and close monitoring
	Genetic disposition	MHC mismatch in allogeneic therapy	Increased cellular and humoral unwanted immunogenicity development	See allogeneic vs. autologous therapy (product-related risk factors)
	Treatment regimen: dosing frequency	Multiple dose; chronic treatment; previously treated with (different) CAR-T (serial dosing)	Increased cellular and humoral unwanted immunogenicity development	Enhanced and appropriate monitoring of cellular and humoral immune responses vs. cell exposure and persistence
	Co-medication	Lymphodepletion in allogeneic setting lower risk for GvHD; higher risk for GvHD caused by alloreactive T cells No lymphodepletion in autologous setting	Decreased or delayed onset of immunogenicity development due to lymphodepletion	Consider inclusion of lymphodepletion; include risk-benefit-evaluation for allogeneic therapies
	Pre-existing ADAs to CAR and/or accessory protein/gene editing components (e.g., Cas9, AAV)	High ADAs titers	Loss of efficacy, persistence, and cytotoxicity Safety risk if accessory protein has an endogenous counterpart	Consider appropriate exclusion criteria and include pre-screening of patients, real-time monitoring for high-risk projects; consider short-term immunosuppressive treatment in extreme cases

AAV, adeno-associated virus; ADAs, anti-drug antibodies; Cas9, CRISPR associated protein 9; CMC, Chemistry, Manufacturing and Controls; CQA, critical quality attributes; GvHD, graft vs. host disease; mAbs, monoclonal antibodies; MHC, major histocompatibility complex; NAB, neutralizing antibodies; ScFv, Single Chain Variable Fragment.

qPCR Cell and Gene Therapies

Interview with Prof. Dr. Mikael Kubista



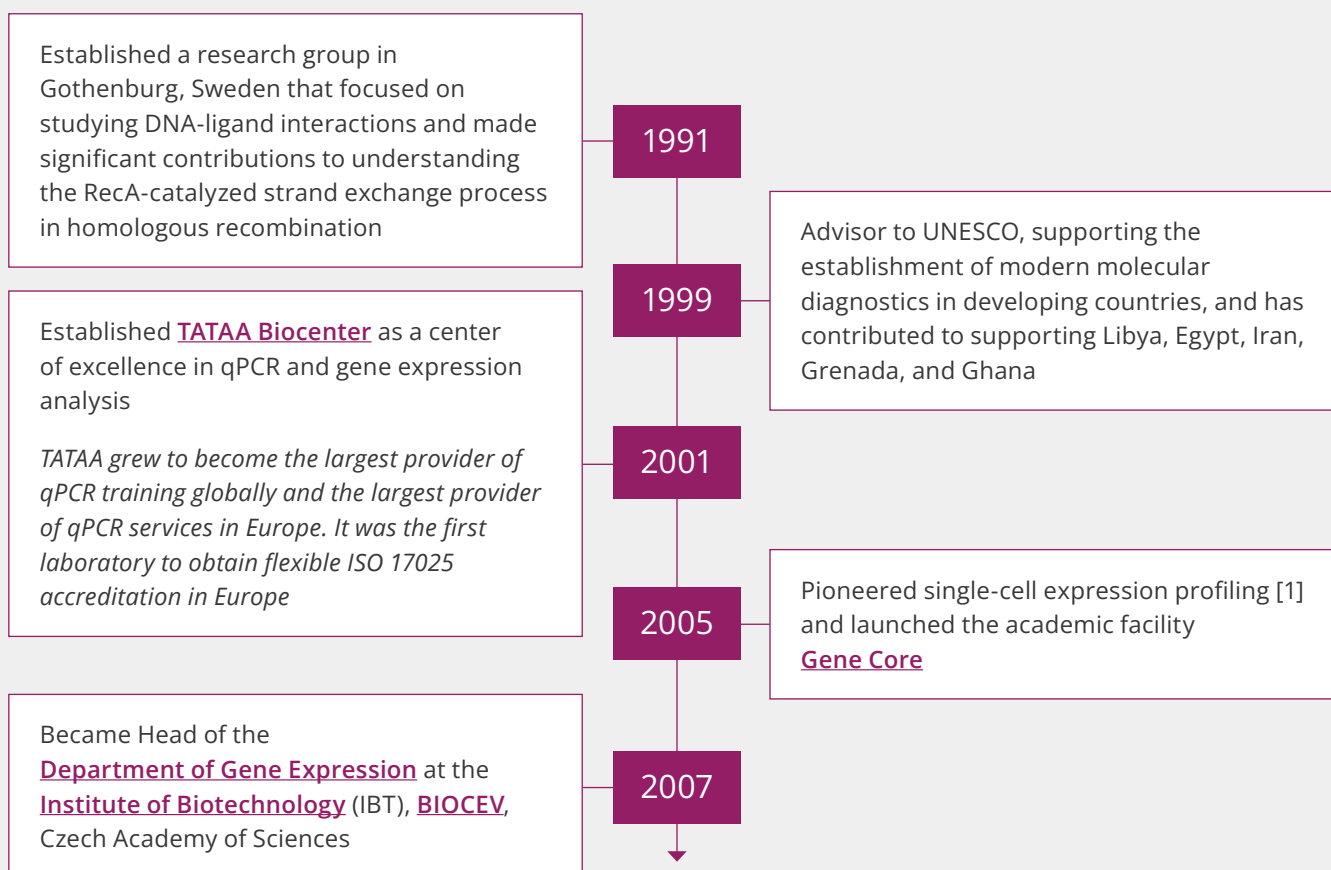
Róisín Murtagh from Wiley Analytical Science interviewed Prof. Dr. Mikael Kubista, a renowned pioneer in real-time quantitative polymerase chain reaction (qPCR) who discovered a novel mechanism of transcriptional activation of oncogenes, leading to the development of a new class of anticancer drugs that target quadruplex DNA structures.

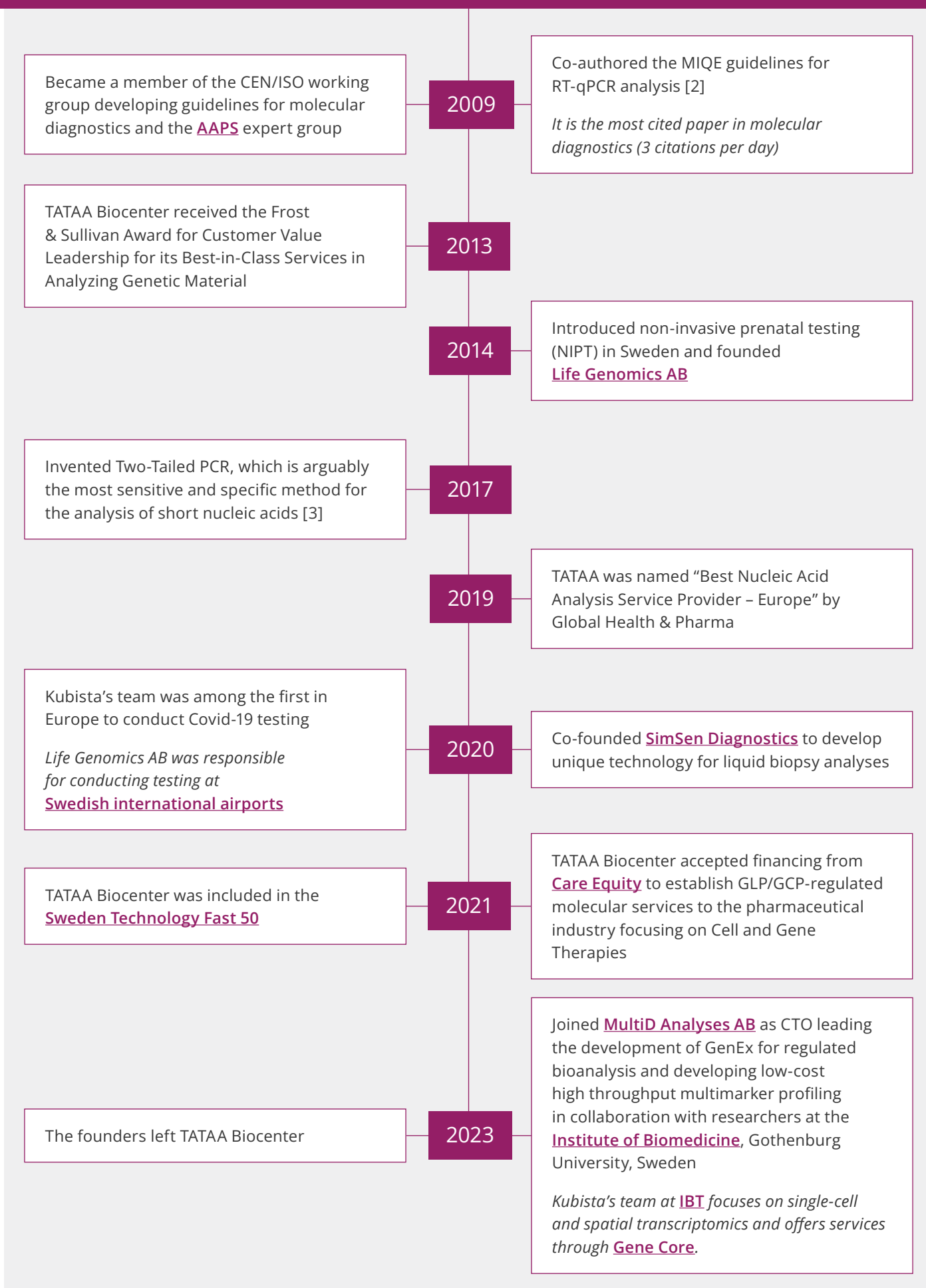
Dr. Kubista, recognized for his significant contributions to science, is a successful entrepreneur and also the founder of multiple biotech companies. He developed methods for multidimensional data analysis, forming the basis for the establishment of MultiD Analyses AB. He also invented light-up probes for nucleic acid detection

in homogeneous solutions, leading to the creation of LightUp Technologies AB, Europe's first company focusing on qPCR-based diagnostics. His groundbreaking work in qPCR, particularly with Two-Tailed PCR and single-cell profiling, has been transformative.

His expertise has played a crucial role in translating scientific advancements into practical healthcare solutions, and he is acknowledged as one of the top 2% **most cited** researchers globally. He co-authored the MIQE guidelines and has contributed to the development of over 20 industry standards. Currently, Mikael is actively supporting Cell and Gene Therapy (CGT) through MultiD Analysis at **GoCo Health Innovation City**. He leads the Department of Gene Expression at the Institute of Biotechnology of the Czech Academy of Sciences in Prague and serves as a visiting professor at Gothenburg University.

Career Timeline





Can you describe your professional journey leading up to your current role?

I have been interested in life sciences my whole life. After my bachelor's degree in Chemistry in 1984 at Gothenburg University, I joined the team at Astra Hässle (today part of AstraZeneca) that developed the K⁺/H⁺-ATPase inhibitor omeprazole. This drug, marketed under the trade names Losec (Prilosec in the US) and Nexium, became the top-selling pharmaceutical product at that time, used for treating ulcers. I obtained a Ph.D. in chemistry in 1988 at Chalmers University of Technology in Gothenburg and did postdocs at La Trobe University, Melbourne, Australia in 1990, and in 1991 at Yale University, New Haven, USA.

Can you provide an overview of your Gene Therapy and Cell Therapy research focus and the main areas of study in your laboratory?

At the Laboratory of Gene Expression, Czech Academy of Sciences, we use antisense technologies to study regeneration and wound healing, while our core facility offers assay design and validation service, as well as single-cell profiling and spatial transcriptomics. At the Institute of Biomedicine, Gothenburg University, we develop low-cost, high-throughput, multimarker profiling. At MultiD Analyses AB we develop statistics and software for the analysis of the nucleic acid targets that consider the entire workflows accounting properly for the pre-analytics and is ready for GXP-regulated workflows and integration with LIMS such as [Watson](#) from Thermo Fisher Scientific. [CCRM Nordic](#) at GoCo Health Innovation City in Gothenburg is a non-profit organization modeled on the highly successful [CCRM in Canada](#) that will set up cell line production to support the CGT industry. Learning the unmet needs, we will develop new molecular methods to assess the quality of the various constructs. qPCR is base technology, but also digital PCR, [Hyperplex PCR](#), and [SimSen Sequencing](#) will be used.

How has the use of qPCR revolutionized research in Gene Therapy and Cell Therapy, and can you provide examples of its impact?

qPCR has enabled cell and gene therapies. PCR is used in every aspect of the development and production including quality control, clinical validation, and sometimes also treatment and relapse monitoring.

Regarding the role of qPCR in biologics quality control and bioanalysis – Is there any specific brand you prefer or use in your labs?

We are very picky about the assays, reagents, instruments, and software we use. We typically design assays ourselves and validate them using an established pipeline, estimating PCR efficiency including confidence intervals, limit of detection, limit of quantification, and linear dynamic range. The specifics vary based on the application context. We have recently published [general recommendations](#) for cell and gene therapy as part of an AAPS expert group. Unlike the MIQE guidelines, which outline reporting requirements, the AAPS recommendations focus on validation and quality assessment. We typically conduct measurements using one of the QuantStudio platforms (5, 6, 7 Pro, or 12K Flex) with an appropriate mastermix from Thermo Fisher Scientific. Data are analyzed using [GenEx](#) from Thermo Fisher Scientific or with the new [GenEx GXP](#) software.

Can you elaborate more on your experience using QuantStudio platforms?

I believe we have utilized most QuantStudio instruments manufactured, including the QuantStudio 12K, which we used also for dPCR. We loved it, but today it is replaced by the Absolute Q, which is more cost-efficient and user-friendly. The QuantStudios are excellent. They have a small footprint, which saves valuable laboratory bench space, excellent and user-friendly software, and, of course, great performance. Thermo Fisher's application support and service, which is critical for labs like ours, has been excellent in the countries we have been active.

What recent advancements or breakthroughs have you and your team achieved using qPCR about which you are particularly excited?

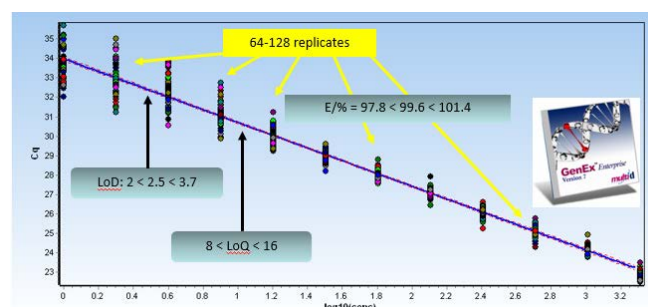
We published an article on how to determine the limit of detection (LoD) and limit of quantification (LoQ) including confidence intervals [4]. This was not trivial, because of the logarithmic response in qPCR, which prohibits calculating the standard deviation of the measurand for low concentrated samples, which is the normal procedure when a response is in linear scale. Instead, LOD calculations in qPCR are based on replicating positive signals with confidence (Video 1).

Video 1



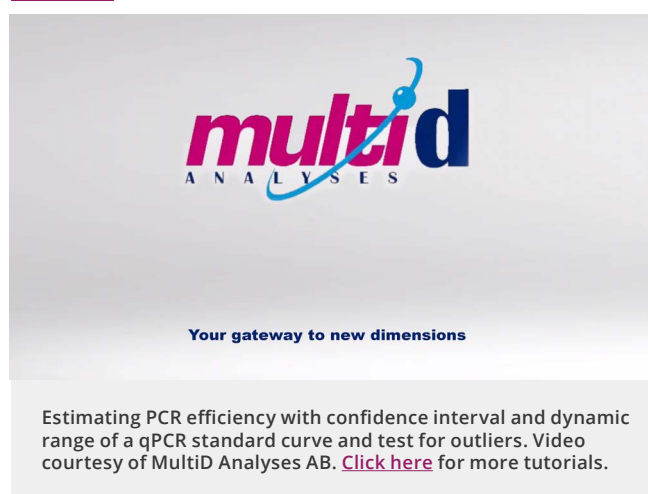
We also published an article on how to estimate PCR efficiency including confidence intervals (Fig. 1) and how to estimate concentrations of unknowns based on a standard curve including confidence intervals [5]. Many researchers are surprised when they hear confidence intervals of estimated concentrations in linear scale are asymmetric (Video 2).

Figure 1



qPCR standard curve with a standard assay targeting conserved loci present in one copy per haploid human genome. 64-128 replicates were performed at each concentration. The spread of replicates is seen to increase at lower concentrations, which is due to sampling ambiguity (Poisson noise). LoD is around 3 molecules, which is the theoretical limit when 95 % of replicates are positive. LoQ here defined as 35 % relative error in linear scale is around 10 molecules, which also is theoretically expected. PCR efficiency is around 100 %. Figure courtesy of MultiD Analyses AB.

Video 2



We then developed a strategy to optimize (RT)-qPCR workflows [6]. I wish more people would use it, so they can discover that very rarely, if ever, is the qPCR analytics the most confounding step of a workflow. Improving the qPCR step in most cases has a negligible impact on the experimental precision of the analysis. Instead, the most confounding step should be optimized. The confounding steps can be identified in a simple pilot study (Video 3) as described in our paper.

Video 3



Your gateway to new dimensions

Analysis of a pilot experiment based on nested design with workflow split into four levels: animals, sampling, RT, and qPCR. In between the levels, the samples were divided into triplicates ending with $3 \times 3 \times 3 \times 3 = 81$ measurements. Data are analyzed using GenEx nested Anova to estimate the confounding variation of each level. Very different results are obtained when analyzing liver and blood samples. Video courtesy of MultiD Analyses AB. [Click here](#) for more tutorials.

Also, the Two-Tailed PCR technology we developed enables the quantification of very short nucleic acids with exceedingly high specificity and sensitivity [3]. Two-tailed PCR is arguably the best option for quantitative siRNA and microRNA analysis, and it can also be used to quantify certain antisense oligonucleotides (ASOs).

What are some of the current challenges or obstacles in the field of biotechnology, and how are you addressing them in your work?

It is challenging to account properly for the pre-analytics in regulated bioanalysis. In biodistribution studies, for example, every tissue has a unique matrix, and its effects should be accounted for. We need standardized procedures to prepare these matrices and spike representative standard materials into them, such that they reflect the drug target when preprocessed, i.e., showing similar yield and imprecision. We are addressing this using various spikes, protections, and extensive protocol optimization to maximize reproducibility and similarity to the target.

Can you discuss any ongoing or upcoming research projects about which you are particularly excited?

We are developing statistics and software to analyze workflows ending with qPCR or dPCR quantification that accounts also for the pre-analytics. This is a challenging task as we expect heteroscedastic variance contributions, which require extensive validation. We welcome laboratories in the field to contact us for collaborations. The more data we get access to, the better modeling of the imprecision we can do.

Furthermore, we are developing intelligent assay designs for the very special targets that may be encountered in cell and gene therapy. For example, highly modified nucleotides, circular vs. linear targets, cleaved vs. uncleaved targets, single vs. double-stranded, integrity testing, etc. We reach substantially higher sensitivities and specificities than with conventional approaches.

Based on the knowhow generated in the European Union-funded [SPIDIA/4P](#) projects on pre-analytics, which has already contributed to over 20 industry standards, we optimize bioanalytical workflows for cell and gene therapy applications, often reaching sensitivities 10-100 fold, occasionally even 1000-fold higher than with conventional approaches and with dramatically improved reproducibility. This work is so important, that we are considering establishing a pre-analytical center of excellence.

How do you stay updated with the latest developments and advancements in biotechnology?

I am fortunate to have a broad network with which I interact. Many are with innovative start-ups with exciting new technologies, who I support as an advisor or board member, and these guys must keep up with the latest developments. I learn from them.

We also offer courses in various aspects of molecular analyses, often in collaboration with solution providers like Thermo Fisher Scientific. The nice thing about courses is they attract the best researchers from the field, from whom we learn the needs.

I am also a member of several scientific advisory boards, which allow interaction with peers who have been recognized for their excellence in the field.

I keep a foot in academia by having an appointment at the Institute of Biotechnology at the Czech Academy of Sciences, where my department, and associated Gene Core, are among the best in Europe in single-cell profiling and spatial transcriptomics. Currently, I also have a mobility grant to collaborate with researchers at the Institute of Biomedicine at Gothenburg University to develop low-cost, high-throughput multimarker profiling.

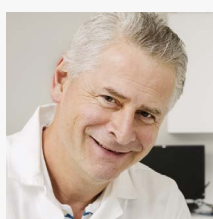
How do you envision the future of biotechnology and its impact on various industries and society as a whole?

We will see much more personalized treatments and personalized therapies not only in sickness but also in health. This development is catalyzed by dramatic improvements in diagnostics including important cost reductions. For example, with the SimSen technology from [SimSen Diagnostics](#), we design personalized targeted panels for ultrasensitive digital sequencing, which allows monitoring of cancer patients using liquid biopsies at affordable cost. With Hyperplex PCR developed at [Aplex Bio](#), we perform a 50-plex biomarker analysis with a streamlined workflow that requires only approximately four hours. This approach allows for the simultaneous analysis of RNA, DNA, and protein biomarkers within the same run. Single-cell profiling is also coming down in price. Powerful software, even offered for free to academics, like the Scarfweb from [Nygen](#), who also offer access to single-cell databases,

is dramatically increasing our knowledge about cell types, subtypes, and even transient variants like the Regeneration Initiating Cells (RICs) Radek Sindelka's group at IBT recently discovered. The RICs are vital for regeneration and scar-free wound healing. Combined with spatial transcriptomics and even multiomics these platforms are dramatically increasing our knowledge of how tissues are made up, which identifies new drug targets and opens for novel treatments. In collaboration with Milos Pekny and Marcela Pekna, our team conducted studies using single-cell and spatial transcriptomics of brain trauma led by Lukas Valihrach's group at the IBT. Through this research, a peptide was identified that can be administered as a nasal spray one week after a stroke, effectively reducing its consequences.

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Email: mikael.kubista@ibt.cas.cz

Affiliation: [Institute of Biotechnology of the Czech Academy of Sciences](#)

ORCID: 0000-0002-2940-352X

Website: [Laboratory of Gene Expression](#)

Social media: [LinkedIn](#)

Precision and Efficiency:

How qPCR and dPCR Revolutionize Cell & Gene Therapy

Quantitative PCR (qPCR) and digital PCR (dPCR) are powerful tools in the field of cell and gene therapy.

They aid in the discovery of target genes, lead new molecular entities (NMEs), and biomarkers, which are essential for the development of effective therapies. These techniques enable accurate genetic analysis during the discovery research phase, helping to identify and characterize drug targets and biomarkers.

Benefits of qPCR



High sensitivity

Detects very low levels of gene expression or DNA/RNA targets



Speed

Results are provided within a few hours, making it a rapid technique for gene expression analysis



Quantification

Provides a precise measurement of the amount of target present



Versatility

Used for a wide range of applications, including gene expression analysis, copy number variation analysis, microbial detection, quantitative genotyping, and environmental monitoring with a wide dynamic range



Accuracy

High accuracy and reproducibility, allowing for reliable and consistent results



Cost-effective

Relatively cost-effective technique, user-friendly, and acceptable by regulatory authorities especially when compared to other methods like droplet dPCR (ddPCR), dPCR microarray analysis, or sequencing



Multiplexing

Can detect and quantify multiple targets simultaneously, enabling the analysis of different genes



Automation

Easily automated, saving time and reducing the risk of human error



Small sample requirement

Only a small amount of starting material is required, making it suitable for precious or limited samples



Standardization

qPCR assays can be standardized and validated, ensuring consistency and reliable results across different laboratories and studies

Benefits of dPCR



Absolute quantification

Enables absolute quantification of DNA or RNA targets without the need for a standard curve, making it highly accurate and reliable



High precision

Higher precision and lower variability compared to qPCR, particularly when quantifying low target concentrations



Enhanced sensitivity

Detects and quantifies small amounts of target nucleic acids, even in complex samples with high background noise or low target abundance



Improved robustness

Less prone to inhibition from PCR inhibitors present in complex sample matrices, ensuring more reliable and consistent results



Copy number variation analysis

Particularly suitable for detecting and quantifying copy number variations (CNVs) or rare genetic mutations, providing valuable insights into genomic alterations



Detecting rare alleles and small-fold changes

Identifies and quantifies rare alleles making it useful for detecting low-frequency mutations or monitoring minimal residual disease



Increased resolution

Can offer improved resolution by partitioning samples into thousands of individual reactions, allowing for absolute quantification of targets with high precision



Accurate detection of target DNA/RNA

High tolerance for PCR inhibitors and is less affected by sample quality or degradation, making it a robust method for detecting and quantifying target DNA or RNA



No need for perfect amplification efficiency

Unlike qPCR, dPCR does not require amplification efficiency to be perfect for accurate quantification, making it less sensitive to variations in PCR efficiency



Flexibility in sample types

dPCR can be performed on a wide range of sample types, including challenging samples such as degraded DNA, fragmented RNA, or samples with low target abundance



Better control over PCR bias

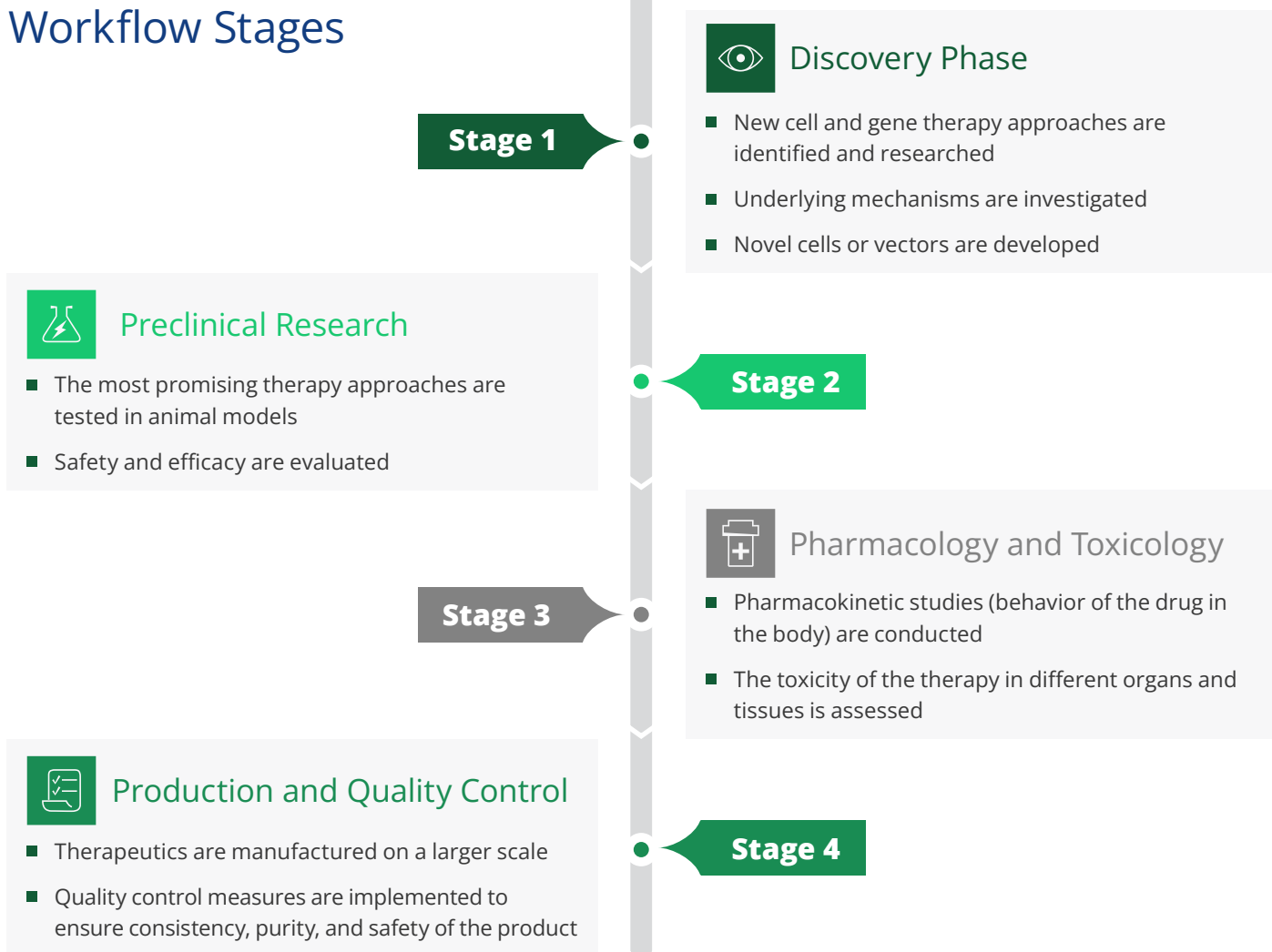
dPCR provides better control over PCR bias, as it works on the basis of absolute quantification rather than relying on relative amplification efficiencies, reducing the impact of bias on results



Digital readout

dPCR provides a clear digital readout of positive or negative results, making it easier to interpret and analyze data compared to qPCR, which relies on fluorescence signals and amplification curves

Workflow Stages



The Main Advantages of qPCR & dPCR in Cell and Gene Therapies in the Discovery Phase



Identify genes of interest (GOI) or targets



Biomarker discovery



Lead identification

Application in Cell and Gene Therapy Development

- Optimizing plasmid construction
- Assessing gene copy number
- Testing and characterizing therapy components
- Ensuring purity and stability of the final product and batches under cGMP production
- Used in *in vivo* analysis or bioanalysis (preclinical and clinical studies):
 - Biodistribution
 - Pharmacokinetics/ pharmacodynamics (PK/PD)
 - Safety and Efficacy



Conclusion



qPCR and dPCR are essential techniques in the field of cell and gene therapy. They allow for the discovery of causal variants, target genes, and biomarkers, vital components in the development of effective therapies.



By providing accurate genetic analysis during the early stages of research, these techniques help identify and characterize drug targets and biomarkers, thus avoiding costly lead elimination in later stages.



qPCR and dPCR also enable the confirmation and screening of biomarkers, optimization of plasmid construction, assessment of gene copy number, and testing and characterization of various components in cell and gene therapy development. These techniques also play a significant role in ensuring the purity, safety, stability, and identity of therapy products.



With their precision and reproducibility, qPCR and dPCR are indispensable tools for advancing the field of cell and gene therapy.

Further reading and resources

[Gene Expression Analysis using Real-Time PCR](#)

[qPCR, dPCR & CE tools for BioPharma](#)

[Assay design hub](#)

[TaqPath qPCR and RT-qPCR master mixes for post-delivery biopharma applications](#)

App note: [Uniformity of gene expression measurements on the QuantStudio 7 Pro Real-Time PCR System](#)

App note: [qPCR and dPCR probe and primer design](#)

Video: [qPCR Biopharma video](#)

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Email: info@wiley-vch.de

Editor: Róisín Murtagh

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