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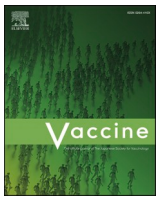

Short communication

Advancing regulatory dialogue: In silico models for improved vaccine biomanufacturing - an expert meeting report

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ABSTRACT

On March 30, 2022, Inno4Vac, a public-private partnership funded by the IMI2/EU/EFPIA Joint Undertaking (IMI2 JU), organised a hybrid workshop, titled "Regulatory Dialogue for Road Maps of Implementation of New Tools in Chemistry, Manufacturing, and Controls Dossiers." This event brought together modellers, regulatory experts, and academic and industry professionals specialising in vaccine process and product development. The sessions discussed key parameters and requirements for model development and verification relevant to vaccine biomanufacturing and shelf life.

The *stability model* was highlighted as having the most significant impact on the common technical document (CTD) due to its potential to streamline data requirements. Regulators are open to considering reliable reduced stability data packages (3–12 months) instead of the standard 36 months, potentially expediting product availability. Appropriate study design reduces uncertainty and therefore the risk of making poor decisions.

Upstream models are further from the final product, and their role in the control strategy of the product will define their level of risk and, therefore, requirements for validation and inclusion of information in the file.

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Regulators may consider *downstream models* high risk as these can be associated with the monitoring and/or control of critical quality attributes and/or be involved in the release of a product. However, requirements for validation and/or dossier content should always be linked to the intended use of the model and its overall role in the control strategy as per the new EMA Quality Innovation Group Considerations regarding Pharmaceutical Process Models.

The success of these models hinges on manufacturers providing enough quality data to prove their accuracy in representing real-world processes. Proactive engagement with regulators, supported by detailed evidence, can foster regulator understanding of new models and potentially lead to new guidelines and pathways for model acceptance.

1. Introduction

Inno4Vac, an Innovative Medicines Initiative 2 (IMI2)-funded consortium, was initiated in September 2021. One of its four objectives is to establish an open-source, in silico simulation platform to guide the design, scaling, operation, and transfer of vaccine manufacturing and stability testing [1]. The project focuses on developing models that will expedite vaccine process development for biomanufacturing and will be compliant with regulatory requirements for use.

Inno4Vac aims to facilitate the integration of these models into regulatory frameworks by initiating dialogue with relevant authorities. This dialogue is expected to pave the way for the future use of predictive stability modelling and process scale-up/scale-down modelling in chemistry, manufacturing, and control (CMC) dossiers (Module 3 of the Common Technical Document (CTD)) for new and existing vaccines.

Inno4Vac organised the first of two regulatory workshops on March 30, 2022, in Brussels, Belgium. The workshop brought together project modellers and international regulatory experts to discuss the criteria necessary for mathematical modelling platforms for stability prediction and process changes to be accepted by regulators as part of the CMC dossier for vaccines. The workshop served as a platform for modellers to understand the levels of reliability and safety their models need to ensure regulatory approval and for regulators to gain knowledge of new modelling and predictive technologies entering the industry. This report summarises the outcomes of this pivotal workshop.

2. Stability modelling according to the ICH/WHO Guidelines on Stability Evaluation of Vaccines

2.1. Introduction of Stability models

During the vaccine development process, one critical step is to ensure the product will be stable over time. Stability studies aim at assessing how a drug product or vaccine would degrade over time, which will help defining proper formulation and storage conditions, and ultimately provide a shelf-life under these conditions, i.e., the maximum storage time a manufacturer can claim their product is safe and efficacious.

Currently effective stability guidelines from the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and World Health Organization (WHO) (i.e., ICH Q1E, ICH Q5C and TRS_962, annex 3) define how stability studies must be conducted (i.e., long-term real-time stability studies, numbers of batches to be tested etc.). Although these guidelines provide a general guidance on statistical approaches and use of accelerated studies to support shelf-life setting, they lack to support the use of advance the use of advance computational models to predict long-term stability to support shelf-life setting from data obtained under accelerated conditions. To remediate this situation, two major shifts of paradigms are being developed within the Inno4Vac project. First, stability is evaluated during formulation characterisation and optimisation, through accelerated studies where various stresses (e.g., freeze-thaw, agitation) and high temperatures allows to provide faster degradations. The hypothesis is that a product remaining relatively stable under these conditions will stay optimal at the long-term storage condition, which can be defined in parallel. While ICH Q1E remains the reference for shelf-life determination, it is primarily designed for chemical entities. Current guidelines, such as ICH Q5C, are more applicable to biologicals, but even these may not fully address the complexity of vaccines. Regulatory requirements still adhere to the principle that at least 3 representative batches must undergo real-time, real-temperature stability studies to justify a shelf-life claim. This underscores the need for updated guidelines that better reflect the nature of biological products, including vaccines.

To better understand the differences between current regulatory guidelines and the innovative approaches developed within the Inno4Vac project, Table 1 provides a structured comparison of key aspects.

Second, the shelf-life can be defined through similar high temperature testing, where a panel of temperatures is applied during a shorter period of time (e.g., 6 months) to better uncover the degradation pathways, translate them into a mechanistic modelling (advanced kinetic models), and finally predict the shelf-life over an extended extrapolation of the time (e.g., 2 years) at the defined storage temperature (e.g., room temperature, −20 °C, or − 80 °C). In both cases, the Inno4Vac project shows that the amount of collected data to study these mechanisms is much larger than data obtained through classical stability studies, while being carried out in a shorter time frame. A more detailed comparison of specific aspects, including extrapolation approaches from real-time and elevated temperature data, is provided in Table 2.

Timothy Schofield (CMC Sciences LLC) delivered the keynote speech, which included an overview of vaccine stability modelling per ICH and WHO guidelines [2–4]. Vaccine potency was used to illustrate the application of stability modelling. Given that some vaccine components may degrade over time, understanding degradation kinetics is critical and is initially revealed through early accelerated stability studies. Many vaccine attributes follow first-order kinetics, but the order should be determined based on product characterisation.

Shelf-life determination based on ICH Q1E was presented, with the caveat that this method expresses the stability of only the specific lots being tested for long-term stability rather than future lots [2,5]. This increases the risk of releasing vaccine lots that could fall below the potency Lower Specification Limit (LSL) before the end of their shelf life. A model is proposed in the WHO guidelines to mitigate this issue and ensure with 95 % confidence that lots maintain adequate potency throughout their shelf life.

That model focuses on the batch lifecycle rather than the product lifecycle and accounts for the rates and uncertainties associated with different planned storage and handling conditions [3,4].

Additional considerations in vaccine stability evaluation were highlighted, such as statistical design. The ICH stability time points, originally designed for small-molecule chemical entities, are not optimal for modelling. Long-term and accelerated stability testing strategies should be tailored to address the uncertainties in the stability model.

Moreover, the presentation emphasised the importance of considering data structure in variability analysis. Stability measurements often cluster due to concurrent testing of batches in the same potency assay runs. When not accounted for properly in the modelling, this clustering can lead to inaccurate estimations of shelf life, release limits, and uncertainties. Studies could be designed to avoid this by using more

Table 1
Overall comparison of Stability Guidelines and Inno4Vac Approaches.

Aspect	ICH Q1E (Current Step 4 version dated 6 February 2003)	ICH Q5C (Current Step 4 version dated 30 November 1995)	WHO _TRS_962, annex 3	Inno4Vac Proposed Approach
Scope	Stability data evaluation for chemical entities.	Stability testing for biotechnological/biological products. Applies to well-characterised proteins and polypeptides. Vaccines consisting of well-characterised proteins or polypeptides are included. Applicable to conventional vaccines, after consultation with the appropriate regulatory authorities.	Global recommendations for stability testing, considering different climates. Applicable to all vaccines against infectious diseases.	Predictive in silico models for vaccine bio-manufacturing and stability.
Methodology	Statistical analysis to determine shelf life.	Testing tailored for biological products.	Stability testing protocols for diverse environments.	Universal kinetic predictive models to reduce reliance on empirical testing.
Data Requirements	Extensive empirical data. Few (3) batches advocated.	Detailed stability data for biologics on at least 3 batches for which manufacture and storage are representative of the manufacturing scale of production.	Robust data collection across various conditions. Data from at least three lots should be included from lots representative of the intended manufacturing scale production and formulation.	Uses computational models to minimize empirical data needs and accelerate timelines. It generally encompasses the evaluation of more batches over a shorter period of time, providing more understanding on degradation pathways.
Innovation	Standard statistical methods for stability evaluation.	Stability considerations for biological products.	Adaptable guidelines for multiple products and climates.	Open source in silico modelling involving multiple temperatures datasets for vaccine formulation stability and manufacturing optimization.
Regulatory Integration	Framework for regulatory submissions.	Specific guidance for biologics.	Reference for national regulatory authorities.	Developing strategies to integrate new models into existing regulatory frameworks. Justification of accelerated timelines through the collection of more data earlier, i.e. better understanding.

Sources: ICH Q1E - https://database.ich.org/sites/default/files/Q1E_Guideline.pdf;
ICH Q5C - https://database.ich.org/sites/default/files/Q5C%20Guideline.pdf;
WHO _TRS_962, annex 3 - https://www.who.int/publications/m/item/guidelines-on-stability-evaluation-of-vaccines;
Inno4Vac Proposed Approach - https://www.inno4vac.eu/st4vaxins

batches tested at different time points.

A key message was that the ICH Q1E stability guidelines advocate focusing on the mean kinetics of the tested lots or of the product, while the modelling of individual measurements can lead to overly conservative shelf life or release limits, thus restricting availability of quality vaccines. Modelling should be extended into post-licensure stability evaluation to warrant or validate the release limit or the shelf life over time. Mr. Schofield advocated employing Bayesian continual learning to integrate all elements of stability acquired throughout the vaccine's lifecycle, including supporting stability and post-licensure assessments.

A significant contradiction exists between the guidelines and the actual evaluation of vaccine stability post-licensure. The main issue lies in the statistical uncertainty, which differs when considering means versus individual measurements. According to ICH Q1E, shelf life is determined using a confidence limit, reflecting the average batch potency over time. However, individual stability measurements fall within a wider prediction limit. This discrepancy increases the probability of out-of-specification (OOS) occurrences over the product's shelf life [6], which can lead to difficulties when OOS results occur frequently in post-licensure programmes.

Post-licensure stability evaluation typically involves placing one lot on long-term stability (per formulation) and requires companies to report OOS results. Suggested alternatives for evaluation include monitoring all lots continuously in post-licensure stability and employing more complex modelling techniques, such as accelerated stability studies and coupling stability after process changes [3,5,7–10].

All in all, advanced statistical methods, such as random coefficients or mixed effects modelling, and other types of statistical approaches, including Bayesian analysis to better model the release of the vaccine product, were recommended to enhance stability evaluation.

Elements of a scientifically sound vaccine stability approach that should meet regulatory acceptance were summarised. These include thorough product characterisation to establish degradation pathways and, thus, the appropriate kinetics model; study designs that focus on parameter and measurement uncertainties versus regulatory expectations; analysis tools that acknowledge data structure, such as short-term variability, analytical variability (e.g., run effect) and batch testing; advanced Bayesian analysis for continuous integration of available platforms data throughout the vaccine lifecycle including supporting stability and post-licensure activities; establishing meaningful product specifications (end of shelf life limit); and an agreement on individual measurements vs batch mean vs product mean.

Table 2
Comparison of specific aspects: ICH Q1E, ICH Q5C, WHO Guidelines vs. Inno4Vac.

Aspect	ICH Q1E	ICH Q5C	WHO Guidelines	Inno4Vac Proposed Approach	Novelty Assessment
1. Extrapolation from Limited Real-Time Data	Allows extrapolation to propose a retest period or shelf life based on statistical analysis of available data. Any retest period or shelf life granted on the basis of extrapolation should always be verified by additional long-term stability data as soon as these data become available.	Emphasises real-time data; limited guidance on extrapolation. Dating is based upon the real-time/real-temperature data. Continuing updates of initial stability data should occur during the review and evaluation process.	Recommends real-time, real temperature storage stability studies; extrapolation is generally conservative. Regression analysis may be used to analyse real-time vaccine stability data. Increased testing of larger numbers of lots at increased numbers of time-points reduces the statistical uncertainty.	Proposes advanced computational models to predict long-term stability from consolidated data obtained in shorter timeframe.	Novel approach applying Bayesian statistics by utilising predictive modelling to enhance the evaluation of the extrapolation uncertainty, particularly when modelling multiple batches together.
2. Extrapolation from Elevated Temperature Data	Provides guidance on using accelerated data to support shelf-life estimation.	Suggests accelerated studies but with caution due to the complex nature of biological products. Studies under accelerated conditions may provide useful support data for establishing the expiration date. Studies under stress conditions may be useful in determining whether accidental exposures to conditions other than those proposed.	Advises on the use of accelerated studies, emphasising the need for real-time, real temperature data correlation.	Develops models incorporating data from elevated temperatures to predict degradation kinetics at long-term storage conditions.	Innovative in applying computational models and Bayesian statistic to predict stability across temperatures.
3. Modelling Inter-Assay and Inter-Batch Variability	Recommends statistical analysis to evaluate variability but lacks detailed modelling approaches. It is recommended that that a statistical test for batch probability is performed using a level of significance of 0.25.	Acknowledges variability; focuses on empirical data analysis.	Recognises variability; suggests comprehensive data collection.	Utilises Bayesian statistics and degradation kinetics to model and understand variability, enhancing predictive accuracy.	Introduces advanced modelling techniques to address variability comprehensively.
4. Incorporation of Additional Data Sources	Limited discussion on integrating mechanistic degradation data or prior knowledge.	Focuses on empirical stability data; minimal emphasis on mechanistic insights.	Encourages thorough characterisation but lacks specific guidance on integrating prior knowledge.	Integrates mechanistic degradation data and leverages prior knowledge on kinetic degradation general shape (not the specific rates), including platform technologies, to inform models.	Novel in systematically incorporating diverse data sources to inform stability predictions.

Source: IHI Europe - https://www.ihi.europa.eu/news-events/newsroom/vaccine-stability-predicted-inno4vacs-advanced-computer-models

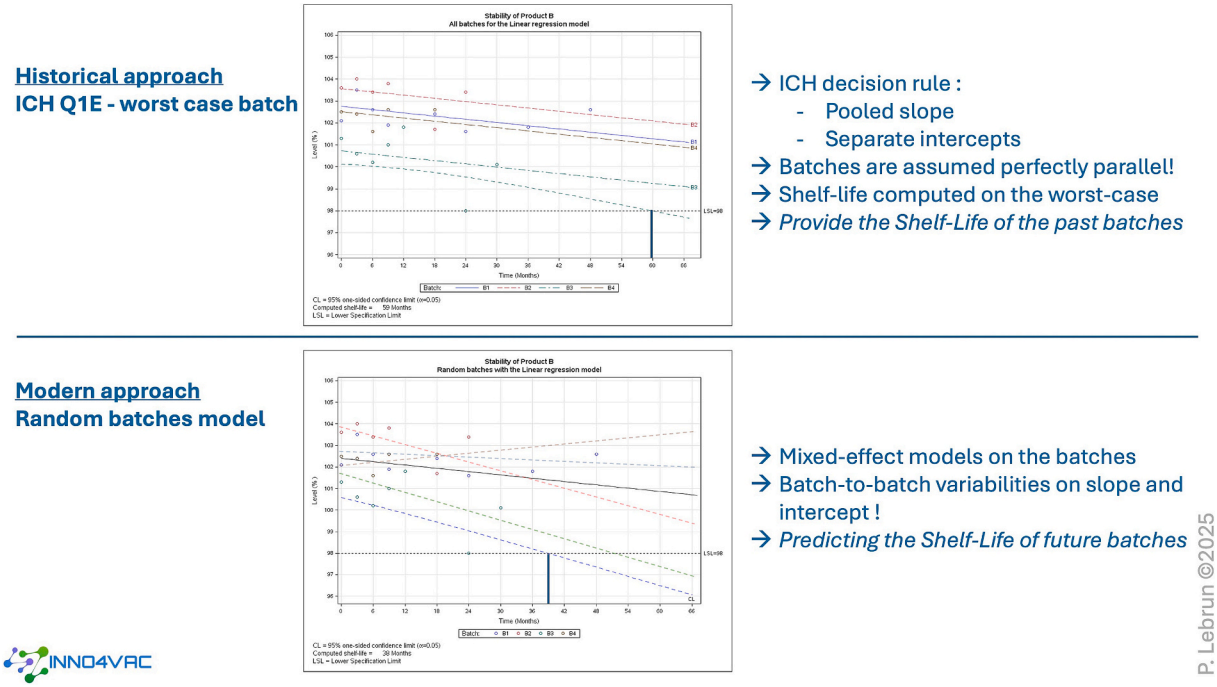

Fig. 1. Stability studies - Shelf-Life determination process following ICH Q1E (top) and using a modern mixed-effect modelling approach (bottom) to ensure uncertainty is managed properly. ICH Q1E is often referred to as a worst-case approach, as it is making the decision made on the worst-case batch. However, this simple illustration shows that embracing properly the variability (of the batches with respect to the release and slopes) can lead to shorter shelf-life when variability is too large. Model simplification as governed by Q1E thus prevented a proper use of the batch variability and provided an optimistic and unlikely large shelf-life.

Pierre Lebrun (Pharmalex Belgium) presented a summary of the stability prediction models proposed by Pharmalex for protein-based vaccines, which began by comparing the *classical long-term stability approach* per ICH guidelines to state-of-the-art *accelerated predictive stability (APS)*. In the classical ICH Q1 stability model, typically, three product batches are tested, and poolability is assessed to determine whether the data from different batches can be combined for an overall estimate of shelf life [2]. This is a major pitfall of ICH Q1; one cannot really claim a product shelf life because only three batches are analysed, and the results often depend on the worst-case batch, leading to the loss of a considerable amount of data. To improve upon this, Pharmalex developed a predictive mixed-effect stability model encompassing the batch-to-batch variability in its parameters (e.g., intercepts and slopes.) *Long-term stability models* based on the mixed model approach are currently available (e.g., linear and mono-exponential models), and they are used to predict shelf life and release limits for small molecules or vaccines. Bayesian statistics offers a more relevant approach to enhancing stability studies and supporting decision-making in industrial processes. This methodology supports complex models while simplifying the prediction process by integrating past data and accounting for uncertainty. By leveraging Bayesian statistics, the probability of a product being out of specification can be more accurately estimated, as it can provide the optimal release window for ensuring a high probability of success. A comparison of the historical versus the modern approach is shown in Fig. 1.

An overview of *APS* was given, detailing how classical linear degradation models have been incorporated by industry partners into more sophisticated kinetic models, referred to as `Advanced Kinetic Modelling' (AKM), to describe reaction progress independently of the complexity of degradation pathways for vaccines, biotherapeutics, adjuvants, etc., [11,12]. The ICH guidelines revision process explicitly recognise modelling using multiple temperature conditions as a valid approach for stability claims, signalling a shift away from traditional testing alone. AKM uses Arrhenius-based equations to fit accelerated stability data obtained at +5 °C (i.e., recommended storage condition) and under accelerated conditions (e.g., +25 °C, +37 °C) and predict long-term realisation (e.g., 24 or 36 months). Considering unlimited types of models, from simple zero-order linear reactions up to competitive two-step reactions, such empirical models are informed by prior knowledge of kinetics and consider multiple mechanisms of degradation simultaneously. If properly formulated, they can accurately predict long-term stability. These models have already been discussed with health authorities and have had various successes [11–13].

The next steps are to apply Bayesian methodologies to these advanced kinetic models on stability-indicating attributes, which will be used to determine critical process parameters (CPP) and critical material attributes, introduce run-to-run analytical variabilities, and test random batches through mixed-effect modelling. Predictive stability approaches, as potentially obtained through Bayesian statistics and AKM, are already included in the draft revision of ICH guidelines. These methods allow for shelf-life claims (e.g., 36 months) based on limited experimental data (e.g., 12 months) when supported by modelling. While accelerated stability models allow for shorter long-term stability studies (conducted over 3–6 months versus the regular 2–3-year assessment), it is also the opportunity to obtain more data and perform more relevant testing (e.g., more batches together with more extensive analytical testing) at various temperature conditions, which strengthen the understanding of the degradation patterns compared to classical approaches. This ultimately leads to a better understanding and predictability of the product's stability within a significantly shorter period. Furthermore, using accelerated stability data would allow models to rapidly detect batches that may present an unexpected trend after a process change, to compute internal release limits (IRLs) to ensure batches remain within specifications until the end of the claimed shelf life.

In parallel, Bayesian statistics are also used to optimise the vaccine formulations (critical formulation parameters and material attributes) that maximise long-term stability, and to define the design space of CPP and formulation components that will guarantee robust manufacturing by computing the predicted probability that the specifications will be

met at release and at shelf-life.

3. Upstream models (bioreactor and metabolic modelling)

3.1. Introduction to Upstream models

Vaccine production involves a sequence of unit operations, where the specific sequence varies depending on the specific vaccine. The focus was on vaccine manufacturing using an *Escherichia coli*-expressed antigen process. The first part of the process involves upstream cell cultivation (fermentation), a process that can be conducted at different scales, i.e., at lab and pilot scale for process development and full-scale for production purposes. One of the key elements to efficiently transfer a process across scales is to possess software tools that can use data collected at lab scale to predict process performance at larger scales.

Krist V. Gernaey (Denmark Technical University) presented an overview of an upstream model created by integrating a compartment model derived from detailed computational fluid dynamic (CFD) simulations with a statistical-mechanistic kinetic cell model based on the *E. coli* expression system. The resulting model is used to simulate the kinetics of upstream biological processes in each of the reactor compartments in the model. An illustration of this can be found below in Fig. 2.

When moving from laboratory-scale bioreactors to pharmaceutical production scale, a systematic approach is the best strategy to ensure a successful scale-up. This means using a model-based approach [14], and a CFD model is one such example. CFD is a discipline that uses computational numerical analysis to investigate systems involving fluid flows. ANSYS (CFX and FLUENT), STAR CCM+, COMSOL Multiphysics ®, and OpenFoam are examples of widely used software tools. The CFD model provides a detailed description of bioreactor mixing phenomena, including the potential occurrence of gradients, thus allowing for extensive system characterisation. However, CFD requires lengthy computational times to characterise a system in such detail. To avoid this, a simplified representation called a compartment model was extracted from the detailed CFD simulation of a large-scale reactor. These compartment models allow for the design of scaled-down experiments that mimic the processes of a large-scale reactor, allow for rapid simulation of non-ideal reactor behaviour, and allow for in silico study of bioreactor configurations, ultimately leading to improved mixing efficiency and oxygen mass transfer. A CFD model can also support process transfer, such as moving a process from one industrial plant to another, by simulating different reactor configurations (e.g., bench scale, pilot, industrial scale).

Kinetic cell models are the second element in the upstream model. In one implementation of the *E. coli* model developed as part of Inno4Vac, the metabolic processes of the organism are connected in a metabolic network model and coupled to a neural network system. The latter is then used to identify parameters in the metabolic network model. An alternative kinetic model implementation consists of differential equations, where model parameters are identified based on data.

Data supplied by two industry partners (GSK and Sanofi) were used to train the bioreactor compartment and kinetic models. The resulting hybrid model (CM + Kinetic [mechanistic/data-driven model]) is able to predict the behaviour within the fermenter (linked to growth and antigen expression) to ensure a consistent process control within the design space, i.e., a digital twin of the laboratory-scale system, which can be converted to a digital twin of a large-scale process. This hybrid model is a simulator and application software that can virtually represent a bioreactor and reactions or events taking place in that bioreactor for mammalian/microbial cell cultivation, regardless of scale. The model allows for exploring different physical phenomena, process conditions, machines, equipment, and other factors, to provide hints on optimal process or manufacturing conditions.

4. Downstream models (Centrifugation and Chromatography)

4.1. Introduction to downstream models

Following the production of crude vaccine intermediates from their

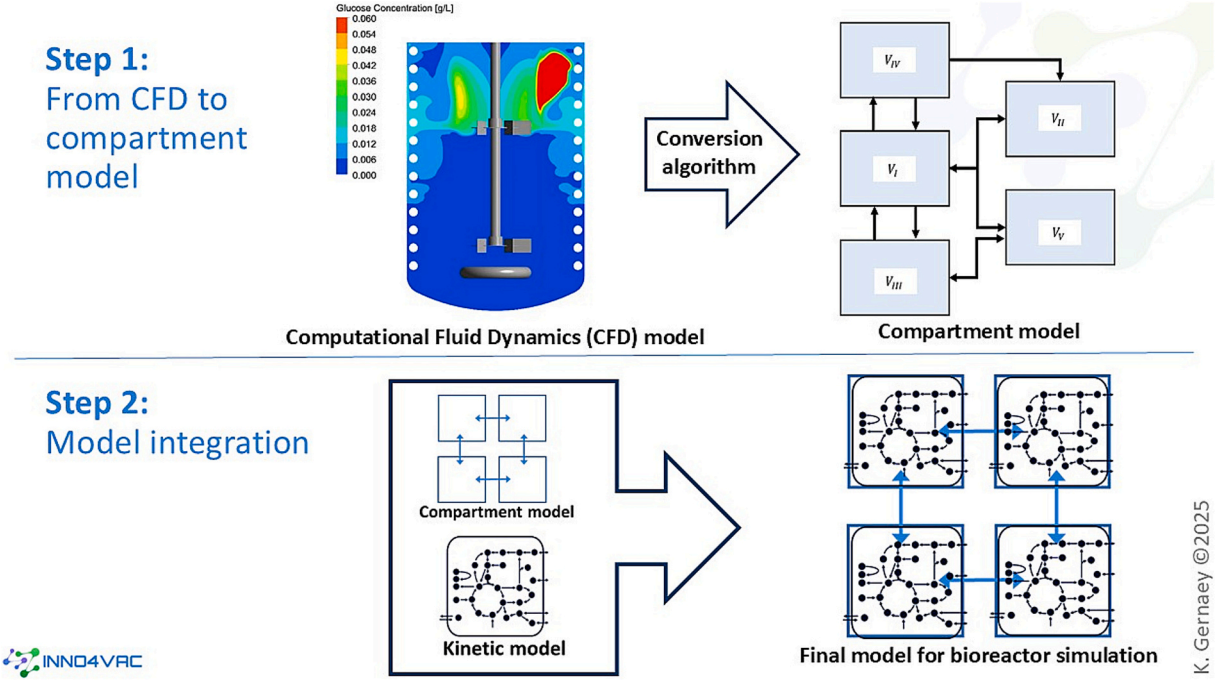

Fig. 2. Illustration of a step-wise model-building strategy. Detailed information about potential formation of bioreactor gradients, represented in the CFD model, is summarised in a much simpler compartment model by a conversion algorithm. In a second step, a kinetic model is integrated in each compartment of the compartment model, and the resulting final model is used for rapid simulation of biological processes in a bioreactor while taking into account potential bioreactor concentration gradients.

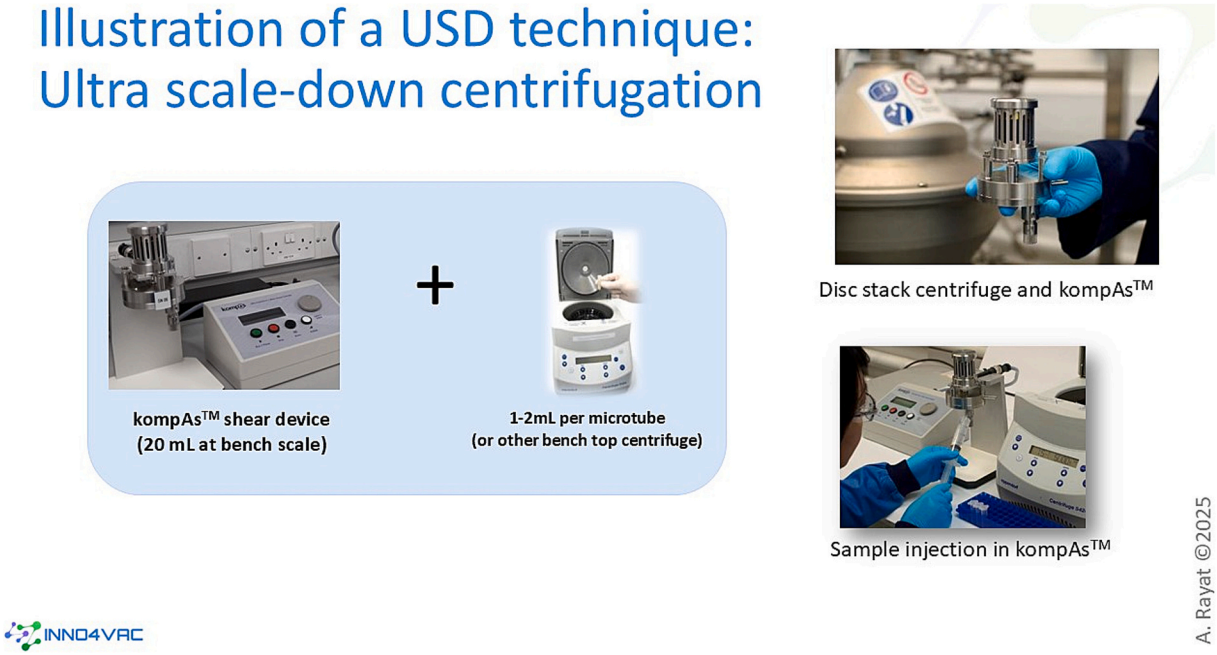

Fig. 3. A sample slide from the meeting depicting the ultra scale-down methodology for centrifugation using the kompAs™ device.

expression systems by fermentation, this production step is followed by several process steps to isolate, recover and purify the active substance. These steps are collectively called the downstream process. The project focused on two of these steps: the early recovery of the product by disc stack centrifugation and purification by column chromatography. The former aims to recover the crude vaccine active substance from the production cells and cell debris, while the latter removes process- and product-related impurities. Removing debris and other particles prior to chromatography also helps protect the chromatography column, thereby improving operation performance.

Andrea Rayat (University College London) shared an overview of centrifuge modelling with ultra-scale-down (USD) technology developed by University College of London. USD seeks to mimic large-scale manufacturing processes using millilitre quantities of material (<50 mL) that is representative of the full manufacturing scale. These devices and technology provide insights into how the production environment, in this case, a centrifuge, can affect the process material, including the product. Using such small quantities of materials allows for process understanding far earlier in the course of product development than traditionally possible, thus de-risking process development and ensuring robust technology transfer across scales [15]. An illustration of the technique can be found below in Fig. 3.

Traditional centrifuge scale-up is based on the flow rate to sigma value (Q/Σ) ratio, where each centrifuge has a specific equivalent settling area, Σ, dependent on its design. To predict the flow rate when transitioning from centrifuge A to centrifuge B, the known Σ of centrifuge B can be used alongside the new flow rate and Σ of centrifuge A, assuming constant particle settling velocity and, therefore, constant particle size. However, a UCL study found that large-scale centrifuges can damage biological materials due to the shear into the feed zone, causing variability in particle sizes [16]. To address this, UCL developed a rotating shear device to replicate the entry feed zone shear and linked it to a bench-top centrifuge to effectively mimic the larger scale centrifugation in terms of the separation performance with just 20-30 mL of material [16,17]. This device has been further developed over the years, with the most recent version used to study floc centrifugation [18].

This USD methodology enables insights into how particular process materials and bioreactor conditions are processed across various continuous centrifuge types. The specific methodology presented was for disc stack centrifuges to remove solids from the feed (i.e., for clarification). Data derived from the USD experiments can then be used to develop a model that can be run to understand process robustness, for example, using the Monte Carlo simulation based on typical input variations. These input variations can be informed by the preceding upstream models and, in turn, provide inputs for subsequent models, such as those for filtration and/or chromatography (filtration often follows centrifugation before the process stream is fed to the chromatography step). The models offer significant benefits, including the ability to predict scale-up behaviour from millilitre quantities of feed material and to anticipate performance when using different industrial centrifuges. They also allow for quantifying the sensitivity of an operation to upstream variability, providing a basis for connecting to filtration and bioreactor modelling to predict the impact of upstream changes on downstream processing. The model could replace some process development data at pilot-scale centrifugation and some post-approval validation batches and reduce the number of pilot/comparability runs required when working on multiple sites with different centrifugation equipment.

A representative of MCT Bioseparation presented the second downstream model detailing further product purification through an ion-exchange chromatography model. This is a mechanistic model that consists of two models: a fluid dynamic model and an adsorption model. The fluid dynamic model describes the transport of each component being modelled in the system from the chromatography column inlet to the outlet column. It considers mass transport in the mobile, pore, and stationary phases. The adsorption model describes how each component adsorbs/desorbs to ligands attached to resin material within the column. This mechanistic model is augmented with artificial intelligence (neural network) to produce a digital twin calibrated using bioprocessing data from small-scale equipment. The models are implemented and numerically solved using CADET (https://cadet.github.io). The fluid dynamic model can be calibrated from simple pulse experiments to determine the different volumes in the system. In contrast, the adsorption model requires a time-dependent UV signal, fractionation analysis data at the column outlet, and data from batch experiments.

One client case study previously undertaken involved a fine-polishing step in the purification of an antibody from pre-monomer

and size variants using ion exchange. The performance parameters received gave a yield of 60 % and productivity just below 1 g per litre solvent. The goal was then to improve this step by using modelling. Experimental data were used to calibrate the digital twin and predict the optimal conditions. The predictions were in good agreement with the actual experiment and resulted in a yield increase of 15 %.

Combining experimental data with predictions from calibrated small-scale chromatography twins enables robust process development, validation, and scale-up/scale-down to de-risk clinical development. This model minimises experimental efforts and sample material usage, accelerates R&D project timelines, and lowers manufacturing and capital costs through optimised process designs. Key benefits include predicting optimal design variables with reduced physical experimentation, determining optimal salt gradient parameters and load times for polishing steps, and optimal flow trajectories for capture steps. It also performs robustness analysis under varying process inputs and environmental conditions, predicts scale-up and scale-down effects, and links upstream manufacturing with downstream processing to enhance overall plant performance. Furthermore, it supports monitoring and optimal control of purification processes. Also, it provides a model-based fractionation control system for real-time release testing, increasing quality assurance and reducing retrospective end-product testing.

5. Roundtable discussion

Following the presentations, Mónica Perea-Vélez (GlaxoSmithKline) led a roundtable discussion, during which industry partners could pose general questions to the regulators. The main themes of the discussion are summarised below, and the panel of experts included Timothy Schofield (CMC Sciences), Dean Smith (Health Canada), Mats Welin (Medical Products Agency), Koen Brusselmans (Sciensano), Marcel Hoefnagel (Medicines Evaluation Board), Julius Carl Pollinger (Paul Ehrlich Institute), Volker Öppling (Paul Ehrlich Institute), and Greger Abrahamsen (Norwegian Medicines Agency).

Q1: Taking into account the limited amount of detailed guidance currently existing for these models, are there any recommendations to industry partners when bringing these models before regulatory agencies? Will existing guidance and standards for modelling in medical devices, particularly artificial intelligence (AI), also apply to the models in the Inno4Vac project?

Regulators' response - Due to the novel nature of these models and artificial intelligence, the regulators felt that not much guidance could be given. These topics will need to be discussed further between the EMA, specialists, academia, and potentially, industry as well to find a balance that ensures product quality and safety without unintentionally inhibiting the implementation of valuable innovations by writing overly restrictive guidelines based on limited knowledge of how these models work and the practical data associated with them. Until more is known, the option to seek scientific advice from regulatory bodies remains available for modellers. Currently, internal discussions regarding models are ongoing. A general rule of thumb is that the more information available that demonstrates the applicability, validity, and reliability of the models, the more accommodating regulatory authorities will be. The same reasoning can be applied to artificial intelligence.

Q2: How do you envision periodic review of machine learning (ML) models, which are continually adapted, and what are the major concerns? Do ML models that are updated periodically (instead of continuously), validated before deployment, and traced with a versioning system represent a reasonable risk-benefit balance?

Regulators' response - Regarding software updates, regulators advised modellers that, as long as the model remains the same, it should not be a regulatory issue but rather more in line with the responsibilities of the inspectorate.

If the model is continually updated and refined with newly collected data, regular demonstrations that it is staying on track may be necessary. A significant factor is the intended use of the model. Regulatory agencies have long understood that knowledge and technology will continue to grow, necessitating adaptation. However, official guidelines governing the implementation of ML models and AI have since been established [19].

It was suggested that ML and AI both have the potential to result in continuous improvement and reduced reporting and that manufacturers could consider good manufacturing practice (GMP) oversight of continual improvement as opposed to citing changes as they come along. It was suggested as a possible way forward for manufacturers to present regulators with a clear path to demonstrate progress by continuously monitoring improvements in manufacture – tracking the ongoing manufacture and improved outcomes and providing means to monitor ML- and AI-related processes over time to confirm to regulators that outcomes continue to improve – and establishing with the regulatory agencies which criteria can be used to demonstrate this.

Q3: How do the regulatory agencies view the fact that the CADET codebase with which these models are incorporated and simulated will be open source?

Regulators' response - The regulators believed that, although many hold positive opinions regarding open-source software, it is not a specific issue for regulators. Open-source software does, however, provide a great deal of transparency, thus allowing for verification and validation of computations, which is not possible with closed platforms.

6. Conclusions and Recommendations

Wim Van Molle (Sciensano) concluded the workshop by providing an overview of the general regulatory views of the models presented and some general conclusions.

Three categories of models were proposed: stability, upstream, and downstream. The regulators present were of the view that, of these three, the stability model will probably have the most impact on the common technical document (CTD). Companies should consider what needs to be included in the file. For example, if indeed a reduced stability data package (3–12 months instead of 36 months) could be submitted at the time of authorisation, this could significantly impact the CTD structure. At the same time, the product would be available earlier. Regulators acknowledged the fact that the shorter period of time considered could be compensated through enhanced testing of time-points, temperatures, and potentially batches while remaining unsure of the data package design to support shelf-life and release limits claims.

When considering upstream and downstream models, regulators require assurance from companies that models are delivering as per their intended use, and that model validation/verification and data in the files is managed in accordance with the role the model has in the control strategy. Manufacturers know their products best and could educate regulators about these new models. This need for education or help in understanding the models will be crucial in the coming years. However, the most essential regulatory consideration is that the manufacturer demonstrates that the model accurately represents real-life processes.

During the workshop, a discussion was initiated on the inspectors' vs assessors' role with regards to the review of model information as part of the dossier or in the company's Pharmaceutical Quality System (PQS), e.g., equations, software characteristics, updates in software versions, etc. Industry recommended regulators engage with their inspector colleagues to provide future guidance on the model details expected to be included in the file vs. the details that can be included and managed via the PQS.

Demonstrating that the models are effective with substantial data will, in the first instance, make regulators more open to learning and understanding how they work and, in the second stage, start thinking about developing guidance, which will eventually lead to regulatory acceptance.

To conclude, the members or the Inno4Vac consortium would like to acknowledge the efforts that regulators bring to further understand and

eventually accept the models. In addition to the participation to this workshop, the Quality Innovation Group (QIG) of the EMA is, on a regular basis, organising Listen and Learn Focus Group meetings (LLFG) to interact with external stakeholders. These initiatives are held to gather information on the latest innovations, bringing together representatives from academia, industry, the regulatory network and QIG to share knowledge and experience. The role of the QIG is to support the EU regulatory network in its role to keep pace with innovation, identify and addresses gaps in the EU regulatory framework and create a reliable and predictable pathway for developers of innovative technologies. Members of the Inno4Vac consortium (Upstream models) participated at the second LLFG meeting (12 and 13 October 2023, [20]) were the focus was on Digital Novel Technologies applied to manufacturing and/or quality control testing (e.g., Artificial intelligence, machine learning, digital twins, robotics, internet of Things (IoT), virtual reality, etc.). The meeting allowed for a close interaction between developers and regulators.

Any future dialogue with regulators through additional regulatory workshops and/or participation in LLFG meetings of the QIG is highly welcomed and appreciated.

CRediT authorship contribution statement

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Disclaimer

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