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VACCINE INSIGHTS

SPOTLIGHT ON
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Analytical characterization in an era of precision vaccinology

Julia O'Neill

Designing vaccines to be safe and effective is challenging. In addition, global access to an affordable, reliable supply of key vaccines is essential. Vaccines need to work for vast, diverse populations. Safety events may be rare occurrences limited to certain groups. Analytical characterization provides predictors of safety and effectiveness but the links between product characteristics and outcomes during use have often been elusive. Incomplete knowledge of mechanisms, variability in traditional test methods, and indirect correlations between testing models and human immune responses can all obscure the view. Precision vaccinology enables vaccine developers to overcome these challenges, bringing clarity and focus and opening a development pathway bypassing traditional inherently variable assays. Developers have always had to predict safety based on product analytical characteristics. Empirical predictions are severely challenged by the low frequency of rare safety events. Ongoing monitoring of safety events in real-world use sets up the potential for deepening our understanding by employing multivariate analysis tools and artificial intelligence. The value of advanced analytics is in generating hypotheses for testing with precision vaccinology techniques to expand our understanding of mechanisms. Predicting effectiveness in use has its own challenges. New analytical characterization methods may provide greater insight for vaccine development than conventional bioassays, animal studies, and clinical trials. Overcoming quality by inspection mindsets may be difficult but is essential for delivering on all dimensions of quality needed by patients. Implementation of precision vaccinology opens new possibilities for adopting quality by design for vaccines, supporting patient relevant specifications, and expanding access, while ensuring continued safety and efficacy.

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INTRODUCTION

Developing vaccines has always been a complex and challenging task, even without the pressures of a pandemic. Vaccines have had tremendous positive impacts on global health, but some earlier vaccines depended on serendipity for discovery, and on slow experimental progress for development [1]. Incomplete understanding of mechanisms of action forced developers to rely on empirical approaches and end-product testing (quality by inspection [QbI]).

The fundamental weakness of QbI is that increased testing does not improve product quality. Quality must be built into the product [2]. Limitations of QbI include representativeness of samples, accuracy and precision of test methods, and absence of in-process feedback controls [3].

For vaccines and other biologics, the QbI strategy is summarized by the mantra 'the process is the product'. The hope was that a 'fixed process' would produce a 'fixed product'. In other words, once a process is finalized it should be locked with nothing allowed to change. The aim was to produce a product that, although not fully characterized, would at least be consistent with initial materials tested in clinical trials. This approach relies on extensive product testing to monitor consistency.

Although vaccines are painstakingly tested, full analytical characterization of traditional vaccines is daunting. In contrast to simpler small pharmaceutical molecules, proteins and viruses have primary, secondary, tertiary, and even quaternary structures that may be dynamic and biologically important.

Traditional bioassays are typically included in vaccine test panels but are fundamentally limited. Bioassays test function broadly by mimicking product use in animal or cell models, instead of quantifying specific product characteristics known to be important for function. Bioassays are plagued by variability from biological inputs, substrates, and test subjects. They are also limited by the degree to which the animal or cell model reflects mechanisms in humans.

Advances in immunology and biotechnology have ushered in an entirely new era of precision vaccinology, with the potential to overcome the limitations of QbI. Molecular biology and genetic engineering now enable developers to create vaccines more directly based on understanding of mechanism. These provide the foundation for integrating immunology and biotechnology to deliver precision vaccinology.

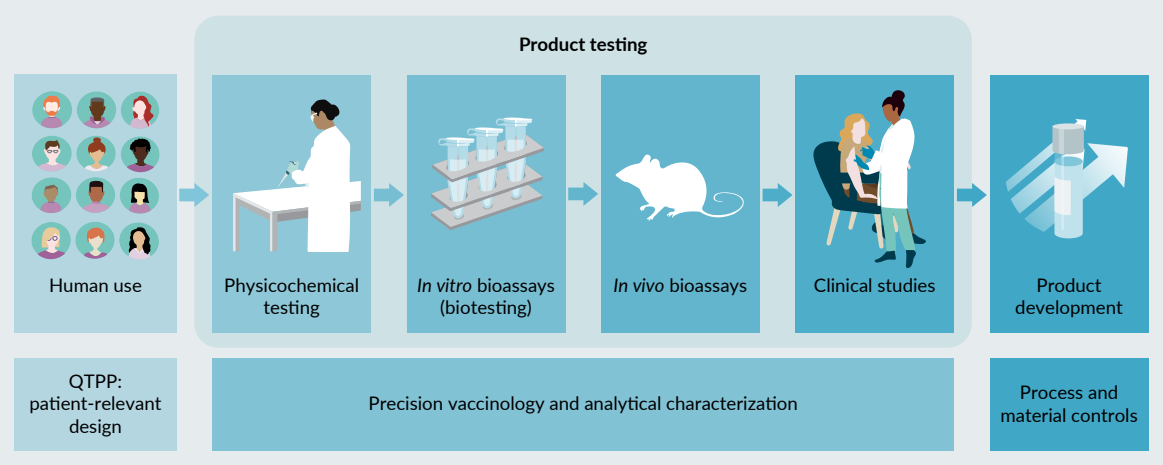
mRNA vaccines for COVID-19 provide instructive case studies of the application of precision vaccinology to vaccine design and development. The breadth of understanding of the immunological mechanisms and pharmacology of mRNA vaccines is summarized by Amirloo and Jimenez [4]. Vaccine optimization balancing immunogenicity and reactogenicity is described by Lee *et al.* [5].

Deeper and more precise understanding enables us to shift from traditional QbI to the modern quality by design (QbD) development paradigm. QbD based on a foundation of precision vaccinology can be executed much faster than empirical QbI. Other benefits include more consistent and predictable safety and efficacy, and expanded access. Developers and regulators have advanced this transition from QbI to QbD for biological products, but more work is needed to update regulatory frameworks and submissions [6].

QbD replaces the fixed-process design strategy with patient-relevant product design, as shown in Figure 1. The starting point is consideration of patient needs to define a quality target product profile [7]. The product is designed to fulfill those characteristics then tested in an array of studies and assays like those employed in QbI. Once a satisfactory product is demonstrated, process and material controls are defined to ensure the product remains within the design specifications [8]. A key difference between QbI and QbD is that the design process is reversed, to begin with what the patient requires instead of beginning with what the initial process is capable of producing.

FIGURE 1

QbD begins with patient needs, reversing the design process relative to the traditional QbI paradigm.



The fixed-process QbI strategy will ultimately deliver product test failures based on artificially narrow specifications derived from initial process performance, given inevitable future shifts and updates in input materials and other factors [9]. More importantly, it fails to address the need to continually improve processes, enable transfer to other manufacturing sites (for global access and supply expansion) and reduce the cost of supply.

Establishing specifications that are relevant to requirements for use allows more flexibility for technical transfer to sites beyond the initial manufacturing facility and provides room for continual process improvements [10]. These are key enablers for reducing costs and expanding reliable supply. In contrast to QbI, with its focus on safety and effectiveness alone, QbD delivers safety, effectiveness, and also access.

The transition from QbI to QbD is made more critical by faster clinical trials. According to Janet Woodcock, "In the past, efficient manufacturing scale-up was not that important because clinical development took so long." [11]. The rapid pace of clinical trials during the COVID-19 pandemic highlighted this issue, with some vaccine emergency use authorizations granted less than 1 year from virus sequencing. Faster clinical trials put development on the critical path for vaccines, which increases the need for a QbD approach based on precision vaccinology.

VACCINE EFFECTIVENESS AND SAFETY

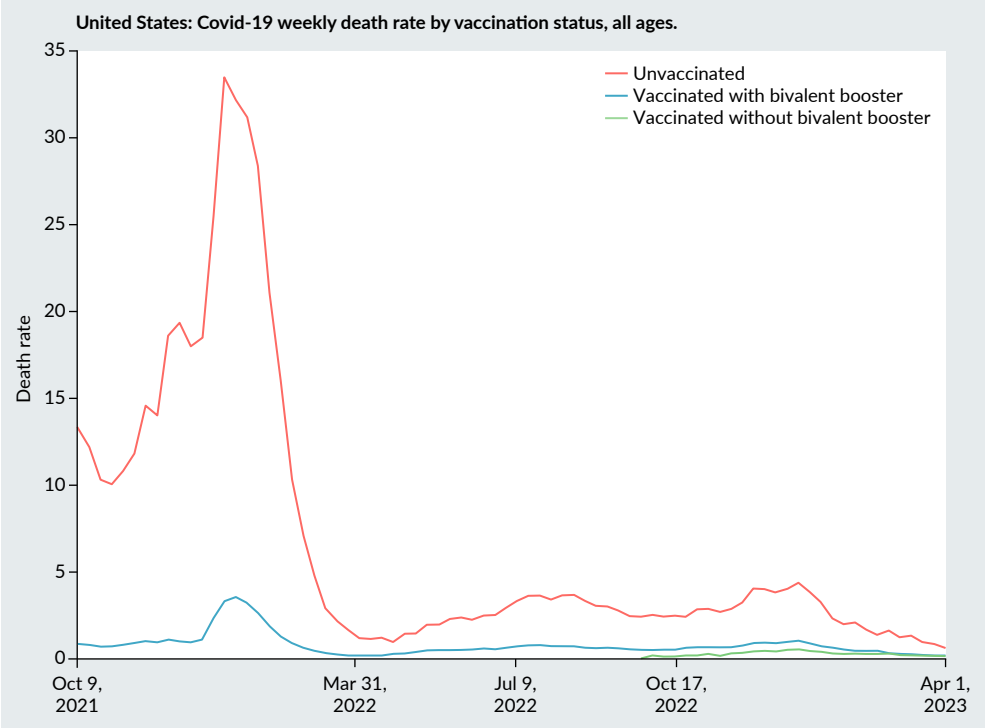
The ultimate measure of success for any vaccine is the safety and effectiveness delivered by use in real-world conditions. For example, Figure 2 shows the reduction in death rates due to COVID-19 for vaccinated compared to unvaccinated people in the USA during 2021 to 2023 [12]. The benefit of vaccination was most pronounced during the early weeks of virus circulation, when few people in the population had native immunity.

Effectiveness metrics, by definition, are not available until a vaccine has been in use for substantial time periods. They also rely on health monitoring systems that are inconsistent across regions and populations. These factors limit the use of real-world effectiveness as direct input to development of a first-generation vaccine, although it can be invaluable to developers working on vaccine updates.

Clinical efficacy provides a faster readout of vaccine effectiveness and is an essential measure of success for Phase 3 vaccine trials. Efficacy for both approved mRNA

FIGURE 2

Vaccine effectiveness demonstrated in real-world conditions (US) during 2021–2023.



Death rates are calculated as the number of deaths in each group, divided by the total; number of people in this group. This is given per 100,000 people. Data source: Centers for Disease Control and Prevention, Vaccine Breakthrough/Surveillance, and Analytics Team. The mortality rate for the 'all ages' group is age-standardized to account for the different vaccination rates of older and younger people. CC BY license held by OurWorldInData.org/coronavirus.

vaccines against COVID-19 (SpikeVax® and COMIRNATY®) was over 90% in their Phase 3 trials in 2020 [13,14]. However, efficacy represents effectiveness for a sampling of all possible vaccine recipients during a limited timeframe and cannot be a perfect predictor of real-world effectiveness over potentially many years of future use.

Responses to both infection and vaccination may vary depending on factors that are not fully known during development [15]. To address this uncertainty, vaccine developers may intentionally include a sampling of diverse groups to characterize effectiveness more comprehensively. For example, the Phase 3 trial for the Moderna COVID-19 vaccine recruited participants with locations or circumstances that put them at an appreciable risk of SARS CoV-2 infection, a high risk of severe COVID-19, or both.

Real-world effectiveness is a complex set of outcomes for each individual. Summarizing clinical efficacy requires aggregating the richness of individual responses from heterogeneous participants into a single number, typically with a binary outcome such as infection versus no infection. Efficacy is a snapshot in time and depends on external factors such as the disease attack rate and mutations in the virus of interest. These considerations interfere with the correlation between efficacy and effectiveness. And although clinical efficacy read-outs are available sooner than real-world effectiveness, efficacy is not available in real-time to inform optimization during early vaccine development.

TABLE 1

Estimated rates of myocarditis and expected events based on group size.

Stage	Example group size	Expected events		
		Unvaccinated	Vaccinated	Infected
Rate per million		6	22	103
Phase 1	100	0.00	0.00	0.01
Phase 2	500	0.00	0.01	0.05
Phase 3	3,000	0.02	0.07	0.31
Hypothetical population	1,000,000,000	6,391	22,369	103,424

Vaccines are designed to be both safe and effective, but rare safety risks may emerge only when vaccines are administered to populations much larger than those in clinical studies. Vaccines are developed and tested in logical stages. Once proof of concept has been demonstrated in laboratory studies, clinical studies may begin to test the vaccine in human participants. The Phase 1 clinical study tests safety in a small group of people (typically 100 or fewer) and may also collect immunological responses [16]. The Phase 2 trial includes up to 500 participants and provides expanded information on side effects and risks, as well as data on immune responses. The Phase 3 trial typically enrolls thousands of participants and generates information on efficacy and additional information on less common side effects. Typical clinical trial sizes are large enough to provide useful results on moderate, transient reactions to the vaccine, but not large enough to reliably detect rarer, potentially more serious adverse events. This was illustrated by our experience with COVID-19.

The intensive monitoring of COVID-19 vaccine administrations and reactions provides a rich source of information on reactogenicity to the vaccines. Data from the largest healthcare organization in Israel can be used to estimate rare safety events from a population that received the BNT162b2 mRNA COVID vaccine [17]. Table 1 illustrates the difficulty of detecting rare events during clinical studies. The estimated rates of myocarditis are based on observations reported in Israel. In 938,812 vaccinated subjects, 21 incidents of myocarditis were reported, compared to six incidents in an unvaccinated comparison group of 938,812. The corresponding rates are 22 expected events per million vaccinated, and 6 expected events per million unvaccinated. Another group of 183,710 people infected with Sars-CoV-2 experienced 19 events in the infected group, compared to 1 event in the comparison group of 183,710. The corresponding rates are 103 events per million for those infected, and 5 events per million for those uninfected.

Three main points are apparent from this analysis. First, the likelihood of detecting rare adverse events in clinical studies is exceedingly small. Second, the incidence of rare adverse events in background populations (uninfected, unvaccinated) is not zero, complicating the interpretation of events observed in vaccinated individuals. Third, the incidence of some rare adverse events is higher following infection than it is following vaccination, suggesting the possibility of reactions that may share similar underlying mechanisms.

PREDICTING SAFETY AND EFFECTIVENESS FROM ANALYTICAL TESTING

Typical test panels for vaccines include multiple critical quality attributes (CQAs), which are categorized as relating to safety, efficacy, or both. "Specifications are chosen to confirm the quality of the drug substance and drug product rather than to establish full characterization and should focus on those molecular and biological characteristics found to be useful in ensuring the safety and efficacy of the product." [18]. A few additional CQAs may be expected by regulators or compendia [19].

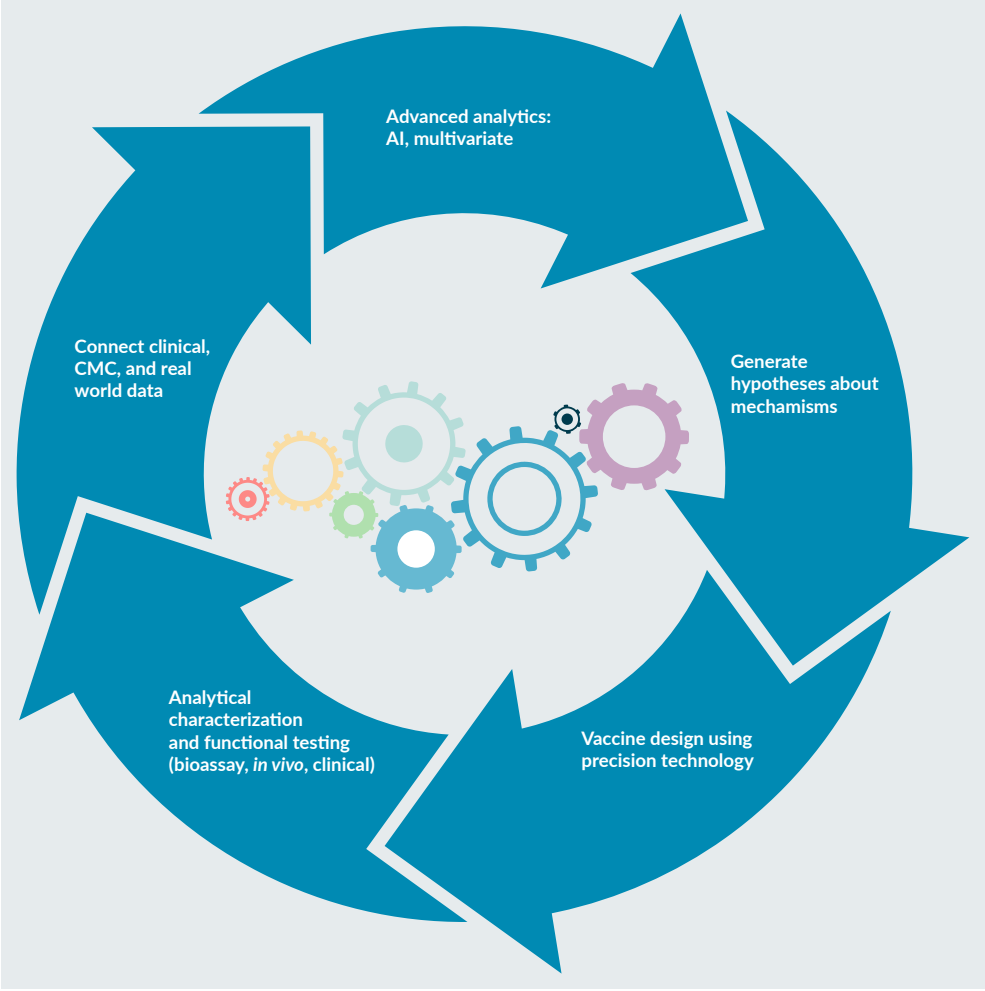
In essence, these CQAs predict safety and effectiveness for vaccines in real-world use. The difficulty of detecting rare safety events empirically in clinical trials makes predicting safety based on mechanistic understanding particularly important. However, the links between product attributes and safety events were obscured for earlier vaccines by our partial understanding of immunology and limited array of characterization techniques.

Precision vaccinology provides the opportunity to bring these mechanisms into focus. Figure 3 outlines a workflow incorporating both precision vaccinology and advanced analytics (machine learning, multivariate statistical analysis, artificial intelligence, etc., to continuously improve vaccine design based on enhanced understanding of mechanisms. Advanced analytics are powerful tools for generating hypotheses for consideration and testing by vaccinologists. Adoption of this strategy requires establishing connected data sets with highly granular information on both product characteristics (specific to each dose delivered, when possible) and clinical outcomes (specific to individual participants, when possible). Although challenging to execute, this collaboration between CMC and clinical teams can greatly improve vaccine design.

The ultimate purpose of analytical characterization is to assure that vaccine doses are

FIGURE 3

Precision vaccinology and advanced analytics bring mechanisms into focus.



safe and effective during ongoing use by their intended recipients. Safety and effectiveness cannot be demonstrated in individual recipients until after administration, when corrections to the vaccine design are no longer possible. Instead, safety and effectiveness in use are essentially predicted by measuring levels of the CQAs, and these predictions inform vaccine design and optimization.

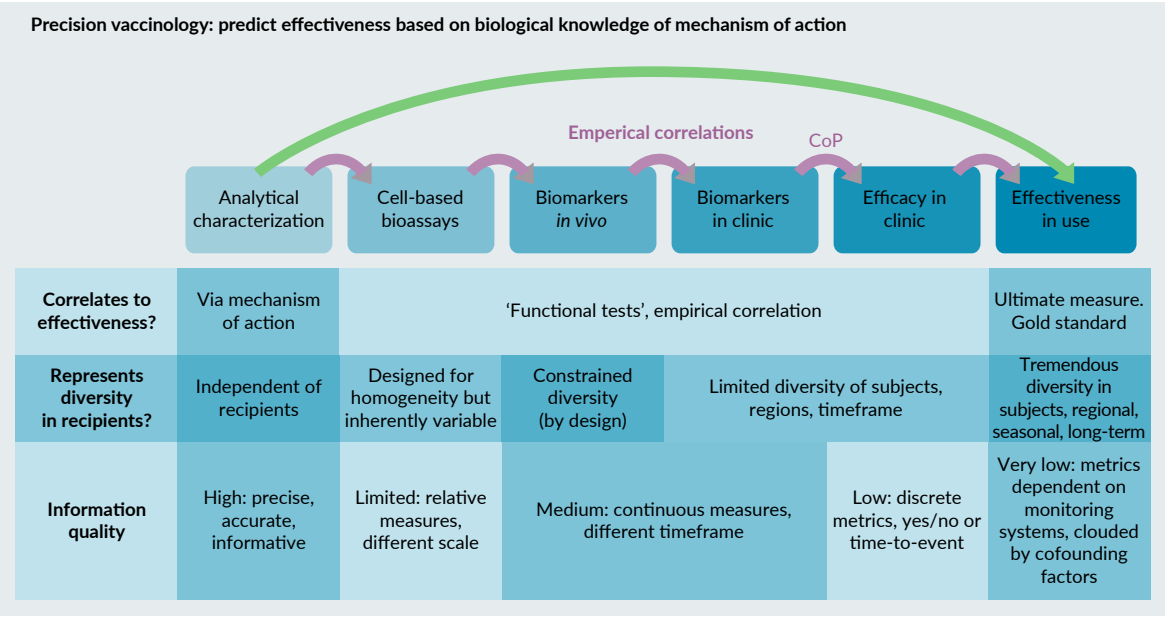
Predicting effectiveness based on product attributes has challenges different from those for predicting safety. Figure 4 shows some of the testing that may be performed to characterize product attributes relevant to effectiveness. Analytical characterization assays should provide precise, accurate and informative measures of a vaccine's characteristics (e.g., purity, impurities, infectivity, expression, structure, etc.). Although these characteristics may indeed correlate with mechanism of action, the relationship is seldom a simple univariate correlation based on just one attribute in isolation. More commonly, a composite of multiple attributes working in combination correlates with effectiveness. For example, a vaccine may be most effective when the levels of strength, purity, infectivity, and expression are all jointly within their optimum ranges. If any single attribute is out of range, correlation between the others and effectiveness is broken. Multivariate modeling techniques provide powerful means of predicting effectiveness directly based on these composite attributes.

Other assays and studies are 'functional' tests, in the sense that they attempt to mimic in a controlled laboratory or clinical trial setting the function of the vaccine in use. From left to right in Figure 4, these tests move closer to replicating real-world use of the vaccine. Cell-based bioassays and *in vivo* studies can be run under more controllable laboratory conditions, but their ability to mimic vaccine use depends on finding an appropriate animal or cell-based model similar to human immune function. Although these tests may more closely replicate ultimate vaccine use compared to analytical characterization, their results may not easily translate to human outcomes. These tests also tend to be subject to high levels of inherent variability from biological inputs (media, reagents, cell banks, animals, etc.).

Clinical trials most closely mimic real-world use but are limited in their ability

FIGURE 4

Predicting vaccine effectiveness from product attributes.



to represent the full range of heterogeneity in human populations in spite of best efforts to include diverse representation in clinical trial study groups. They also represent only a snapshot in time, capturing results within a limited timeframe relative to disease prevalence and virus evolution.

Even though the ensemble of results from these assays and trials is useful for informing vaccine design and optimization, none of these perfectly predict vaccine effectiveness in use. The selection of assays for the product quality test panel should be informed by the fullest possible understanding of mechanisms of action, emerging through application of precision vaccinology.

In the new era of precision vaccinology, traditional potency assays may no longer be the best predictors of vaccine effectiveness. Instead, potency can be assured by a suite of precise analytical characterization assays and confirmed by a cell- or animal-based potency assay if needed. This precedent was established for mRNA vaccines. The EMA assessment report for the Moderna COVID-19 vaccine lists active substance specifications for identity, content, purity, and several additional attributes characterizing the mRNA–LNP structure. A traditional cell-based potency assay is not among the tests required for product release [20].

The FDA draft guidance for potency assurance of cell and gene therapy products offers alternatives to traditional cell-based potency assays, although no corresponding guidance is yet available for vaccines [21]. "Clinical data may be used to establish a correlation(s) between biological activity and a more practical potency measurement(s) that can be used for lot release, stability, and/or comparability studies." Correlation is defined as a statistical and biological relationship between two or more variables such that systematic changes in one stimulate systematic changes in the other.

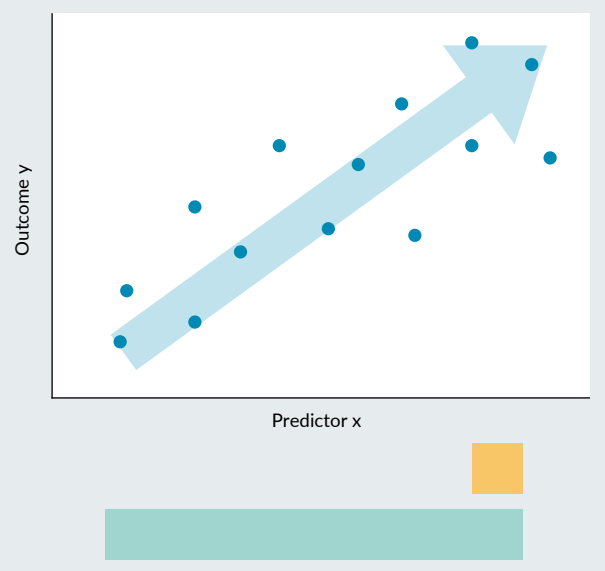
Note that the draft guidance indicates a correlation relationship should be both 'statistical and biological'. This is an important nuance, implying that there should be a plausible scientific basis for expecting a statistical correlation. Reliance on statistical (empirical) correlation in the absence of a scientific basis does not provide a credible basis for potency assurance.

Statistical correlation outside the context of scientific understanding is fraught with risk. Correlation is essentially a signal-to-noise ratio between a predictor x and outcome y, with significance relative to the noise (or variability) in y. The numerical value of the correlation coefficient between x and y is heavily dependent on the range of x. This concept is illustrated in Figure 5. When x is constrained to a very narrow range (yellow box), the estimated correlation between x and y will be obscured by the variability in y. Conversely, when x is pushed to its limits to cover a very broad range (teal box), numerical correlation with y, if present, is increased.

Spurious correlation between unrelated attributes can occur by chance, or because both attributes change across time, or via a third attribute (a 'lurking variable') related to both of the first two. Correlation does not demonstrate causation [22]. In order to demonstrate causation, the level of at least

FIGURE 5

Illustration of statistical correlation between predictor x and outcome y.



one attribute must be changed intentionally in a controlled study.

The study of correlations between clinical outcomes and product attributes creates a fundamental tension between the goals of the CMC and Clinical teams. The clinical experience can be described in terms of a clinical design space with each CQA serving as a dimension [23]. The clinical team aims to demonstrate efficacy and safety, and as a result may be motivated to allow clinical experience only with the expected optimum product design within narrow attribute ranges. The CMC team aims to support adoption of patient relevant specifications by demonstrating with clinical results that a wider range of attributes is equally effective and safe. Collaboration between CMC and clinical teams is essential to overcome this tension.

SUMMARY

Precision vaccinology can deliver revolutionary gains in accelerating vaccine design and development for safety and effectiveness. Access to affordable vaccines with reliable supply is equally important. To deliver on all three essential dimensions of quality (safety, effectiveness, and access) precision vaccinology must be applied within a QbD framework [24].

The QbI paradigm, which was conventionally accepted for pharmaceutical development, delivers safe and effective product but falls short of enabling affordability and availability. This shortcoming is aggravated for biological products. QbI begins with development and manufacture of a candidate, tests that candidate to demonstrate it has a reasonable benefit/risk profile, and sets specifications to check for consistency. QbD begins with understanding patient requirements and mechanisms of action, sets specifications relevant to patient needs, and directs vaccine development to fulfill those requirements.

Precision vaccinology overcomes traditional challenges in vaccine development and enables patient-relevant design. Predicting effectiveness based on analytical characterization has the potential to deliver greater insight than cell-based potency assays. QbD employing precision vaccinology delivers safety, efficacy, and access.

TRANSLATIONAL INSIGHT

Precision vaccinology has enabled tremendous advances in vaccine design. Deeper understanding of immunological mechanisms, and enhanced analytical characterization of vaccine candidates, open the door to adoption of QbD for expanded access to safe and effective vaccines. The mRNA COVID-19 vaccines illustrate the potential for modernizing development and regulatory paradigms.

To further advance this progress, vaccine developers should make the case for the strongest possible assay (or suite of assays) to predict real-world safety and effectiveness. Health authorities can help by clarifying guidance for assurance of vaccine potency to elaborate on the potential for acceptance of less-traditional assays (i.e., not just the classic bioassay).

Ongoing optimization of vaccines will benefit from mining the depths of connected clinical, CMC, and real-world data using advanced analytics including AI, machine learning, and multivariate statistical modeling. Hypotheses generated from real-world use and tested with the full battery of precision vaccinology tools, may result in breakthrough discoveries in understanding mechanisms and optimizing vaccine design for greater safety and effectiveness.

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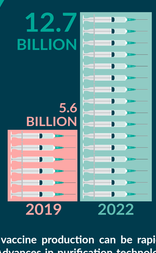
Vaccine
MANUFACTURING & ANALYTICS



The manufacturing of COVID-19 vaccines demonstrated how vaccine production can be rapidly scaled up with faster regulatory approval and time to market. Advances in purification technology and analytical tools in recent years has led to an increased market value and manufacturing capacity for vaccines; however, challenges remain. In particular, rapid, scalable and cost-effective downstream processing is required to meet increasingly rigorous regulatory standards for product identity, characterization, and impurities. This infographic offers a step-by-step overview of manufacturing and analytical methods for four key vaccine types: VLP (virus-like-particle), subunit, mRNA, and viral vector vaccines.

VLP & SUBUNIT PROTEIN VACCINES
Human Papillomavirus (HPV), hepatits B and malaria

mRNA VACCINES
COVID-19 and Candidate Zika Virus

VIRAL VECTOR VACCINES
COVID-19 and Ebola

UPSTREAM PROCESSING

	VLP & SUBUNIT PROTEIN VACCINES	mRNA VACCINES	VIRAL VECTOR VACCINES
Generation of materials	Cloning of genes of interest.	Target gene discovery through techniques such as next-generation sequencing. Integration of target sequence into a plasmid.	Cells, plasmids, and/or seed virus.
Expansion	Viral structural proteins expressed in the expression system.	Plasmid DNA (pDNA) is amplified in host bacteria, typically *E. coli*, which grows in a single-use fermenter. Linearization of supercoiled plasmid and purification and recovery of linearized plasmid.	Cell expansion using adherent or suspension systems to increase cell concentration.
Generation	Cell lysis to aid in extracting VLPs from the expression system (bacteria, yeast, insect cells, mammalian cells and plant cells).	mRNA is synthesized using *in vitro* transcription (IVT) with linearized pDNA template.	Infection and production of replication-competent viral vector; transient transfection of plasmids that contain viral vector genes and therapeutic gene of interest.
Harvest	Clarification to remove remaining cell debris and aggregates.	DNase I digestion of DNA template and recovery of mRNA.	Obtain viral vectors from cell culture supernatant or through cell lysis.

PURIFICATION SOLUTIONS

It is important to select a purification process that is robust, reliable, scalable, cost-effective, and GMP-compliant. Here we highlight the methods that can be used in scalable vaccine manufacturing, as well as mentioning some alternative methods that are not typically suitable for manufacturing.

Manufacturing-friendly methods

Affinity chromatography

C-tag (VLP and subunit vaccines)

Binding of a C-terminal tag (composed of four amino acids) to protein of interest.

- Scalable, highly selective

Oligo-DT (mRNA vaccines)

Separation of mRNA from the byproducts of the IVT manufacturing process through binding of mRNA to the polyA tail.

AAV affinity (viral vector vaccines)

Binding of a specific protein on the AAV capsid to an immobilized ligand on the chromatography resin.

- Fast, highly selective, scalable, binding capacity
- Developed for non-mAb modalities as the standard capture step for high purity and yield

Ion-exchange chromatography

Separation based on charge.

Cation-exchange chromatography (CEX)

Negatively charged medium binds positively charged molecules.

Anion-exchange chromatography (AEX)

Positively charged surface binds negatively charged molecules.

- High sensitivity, can detect a wide range of ions
- Ineffective for the separation of closely related charged species

Hydrophobic interaction chromatography (HIC)

Separates proteins according to differences in their surface hydrophobicity.

- Large volume, binding capacity
- May require significant process development

Tangential flow filtration (TFF)

Removal of smaller impurities which are held inside the beads using core bead chromatography.

- Can achieve quick separation of species based on size
- Impurity clearance can vary and be minimal for certain biomolecules

Alternative methods

Reversed-phase chromatography (RPC)

Reversed-phase chromatography separates molecules on basis of hydrophobicity. Surface of RPC medium is very hydrophobic that typically requires non-polar organic solvents for elution.

- dsRNA impurities removed
- Challenging to scale—costly and uses toxic reagents

Size exclusion chromatography (SEC)

Separates molecules based on their size by filtration through a gel.

- Not scalable—requires a large footprint to implement

Cellulose-based chromatography

dsRNA binds to cellulose in ethanol for removal.

- Low capacity and not scalable

Hydroxyapatite chromatography (HAC)

Mixed-mode resins attract and repel protein through hydrophobic, ion-exchange and hydrogen bonding interactions.

- Improved resolution
- Not scalable or manufacturing friendly

DOWNSTREAM PROCESSING

Purification of the final product from process-related impurities, formulation, fill and finish

	VLP & SUBUNIT PROTEIN VACCINES	mRNA VACCINES	VIRAL VECTOR VACCINES
Clarification	Removal of cells and cellular debris from the cell culture medium. Cell sedimentation, depth filtration, TFF, ultrafiltration/diafiltration (UF/DF).	Remove small process-related impurities. UF, TFF.	Remove process impurities such as cells and cell debris. Centrifugation and/or microfiltration.
Capture & concentration	Protein capture and removal of process impurities such as proteins, DNA and lipids. AC (C-Tag or CaptureSelect options), AEX, CEX, HI.	Remove process-related impurities such as rNTPs, DNA fragments, nucleotides, enzymes and buffer components. mRNA-AC (oligo dT), AEX, HIC, RPC.	Concentration: to reduce bulk volume. TFF, UF. Capture: to remove process and product-related impurities. AEX, TFF, AAV affinity.
Polishing	pH adjustment, removal of lipopolysaccharides, endotoxins, etc. AEX, CEX, SEC, and HIC.	Reduce dsRNA and uncapped RNA products from the final product. HIC, AEX, cellulose-based chromotography.	Removal of empty capsids. AEX.
Formulation	Formulation into (typically) sucrose and detergent.	Buffer exchange, concentration adjustments, sterile filtration. Purified mRNA encapsulated in drug delivery vehicle such as a lipid nanoparticle (LNP) or another lipid or carbohydrate.	Concentration and buffer exchange into formulation buffer.
Fill and finish	Filtration to sterilize and the addition of buffers and preservatives for stabilization.	Terminal filter sterilization and aseptic vial fill.	Terminal filter sterilization and aseptic vial fill.
	PURIFICATION CHALLENGE: Filtration of disintegrated protein fragments from particle suspensions.	**PURIFICATION CHALLENGE:** Denaturation of enzymes, NTPs and DNA template molecules in nucleic acid solutions.	**PURIFICATION CHALLENGE:** Removal of pathogen contaminants and disintegrated genetic materials from mammalian culture systems while maintain the integrity and infectivity of viral particles.

ANALYTICS

	VLP & SUBUNIT PROTEIN VACCINES	mRNA VACCINES	VIRAL VECTOR VACCINES
Identity	Western blot, peptide mapping.	Sequence confirmation: Sanger or NGS, RT-PCR. RNA content: RT-qPCR, RT-dPCR, UV absorbance, fluorescence-based RNA-specific assays.	Genomic DNA: PCR and sequencing. Viral protein: western blot and mass spectrometry, Transgene ID, capsid/serotype ID, envelope protein ID (VSV-G). Nucleic acid techniques.
Purity	SEC-HPLC, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), RP-HPLC.	Process and product-related impurities: qPCR, RT-qPCR. Residual DNA template: qPCR. Protein and dsRNA: immunoblot.	Viral structure: electron microscope, genome integrity and protein purity. Residual HCPs: ELISA and mass spectrometry. Residual HC-DNA: PCR, empty/full capsid ratio (AAV only), process impurities.
Integrity	Molecular weight, SDS-PAGE, RP-HPLC, CEX- and AEX-HPLC.	% intact & fragment mRNA: capillary gel electrophoresis. % 5' capped: UPLC, RP-HPLC and LC/MS. % 3' polyA: RP-HPLC. mRNA integrity: gel electrophoresis.	Total particle counts: TFF, flow virometry.
Safety	Binding of functional epitopes: immunoassays such as surface plasmon resonance (SRP). Antigenicity: ELISA, endotoxin assay.	Endotoxin assay, bioburden, sterility, appearance.	Exdotoxin assay, bioburden, sterility, appearance, mycoplasma testing.
Characterization	MALDI-TOF MS. Peptide mapping: LC-MS, size. Visualization: Cryo-EM, AFM, HPSEC, DLS, AUC. Host cell protein: ELISA.	Lipid content: LC/MS, HPLC. Particle size: Dynamic light scattering, electron microscopy. % RNA encapsulation: RiboGreen RNA assay, fluoresence-based mRNA assay. Lipid identity & impurities: LC-MS. Fatty acid analysis: HPLC.	Aggregate analysis. Capsid protein: size exclusion chromatography, HPLC, mass spectrometry.
Quantity	ES-DMA, AF4-MALS, HPSEC-MALS. Total particles: dynamic light scattering (DLS), Electrospray Differential Mobility (ES-DMA) flow cytometry, HA, or NA. Antigens: RP-HPLC.	RT-qPCR, RT-dPCR, UV absorbance, fluorescence-based RNA-specific assays.	Total vectors: ELISA, NTA, TRPS, FFF-MALS, flow virometry. Infectious particles: plaque assay, TCID50. Vector genome particles: PCR.
Potency	Morphology, size, polydispersity.	Lipid identity: RT-qPCR, RT-dPCR, UV absorbance, fluorescence-based RNA-specific assays. Impurities: LC-MS. Fatty acid analysis: HPLC.	Infectious titer (LV only). Functional analysis: *in vivo* and cell-based assay.

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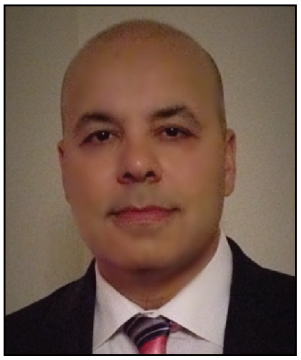
VACCINE INSIGHTS

CMC AND ANALYTICS

SPOTLIGHT

INTERVIEW

Clinical assays for vaccine development: the role of CEPI centralized laboratory network



Established to offer free sample testing for vaccine developers during the COVID-19 pandemic, CEPI's centralized laboratory network has continued to expand, and is now addressing a range of pandemic threats. **Charlotte Barker**, Commissioning Editor, *Vaccine Insights*, speaks with **Ali Azizi**, Project Lead, CEPI, to find out more.

Vaccine Insights 2024; 3(4), 113–117

DOI: 10.18609/vac.2024.021

Q How did you get involved in vaccine development?

AA: I have always had a strong interest in infectious disease and the development of vaccines, starting from when I was a kid in the 1980s and first heard about HIV. I remember wishing I could help by finding a cure. Led by that desire, I completed a BSc in microbiology and chose a PhD in immunology focused on HIV vaccine development. While my PhD project never reached the clinical trial, my enthusiasm for the field remained, and I have spent

the past 25 years in academia, small biotech, and big pharma, developing vaccines for several emerging pathogens.

Q What led you to join CEPI's CLN and what do you hope to achieve?

AA: I am currently a Project Lead at CEPI's centralized laboratory network (CLN). I jumped at the opportunity to join CEPI because the organization's mission to accelerate the development of vaccines against epidemic and pandemic threats is so well aligned with my own personal and career goals. I hope to leverage my expertise and experience to support the development of vaccines and assay that can make a difference in people's lives. By contributing to vaccine development, particularly against rare diseases with epidemic or pandemic potential, I can make a meaningful impact on global scale. To maximize that impact, all of CEPI's assays, protocols and standard operating procedures are freely available to all—that principle is very important to me.

Q Tell us about the origins of the CLN—why was setting up the network a priority for streamlining vaccine development? How were the initial partners chosen?

AA: In response to COVID, CEPI established a CLN in March 2020, in order to standardize immunological assay for SARS-CoV-2 and support vaccine developers. Additionally, the CLN assists in characterization of immune correlates of protection and helps to approve and distribute effective vaccine candidates.

The initial partners for CEPI CLN were selected through two calls for proposals, through which ten high-quality laboratories were invited to join the network. They were chosen based on the quality of the applicant, their willingness to enter an open partnership, previous experience with working on assays for different diseases, and the quality and budget that they proposed for each individual assay. Geographical region was another consideration; we didn't want all the labs to be based in North America or Europe.

Q The network has now expanded to include more labs around the world—why is that important?

AA: Having labs around the world will increase and enhance our ability to detect, respond, and control future pandemics—ultimately saving lives and reducing the impact of infectious diseases on global health. For instance, having labs in different regions can help to

"...having labs in different geographic regions allows us to share data, research funding, and best practices..."

detect new local outbreaks early, allowing for a rapid response to contain the spread of infectious diseases.

In addition, having labs in different geographic regions allows us to share data, research funding, and best practices, leading to a more coordinated and effective response to pandemics.

Q How do you ensure standardization between labs in the network? What are the key barriers/risks and how were they overcome?

AA: To achieve standardization, we partner with the UK National Institute for Biological Standards and Control (NIBSC), now part of the UK Medicines and Healthcare Products Regulatory Agency (MHRA), to generate well-characterized, traceable controls and reference standards. We also make sure that our standards and controls are suitable to be authorized by WHO. Plus, we ensure that all laboratories within our network are using the same reagents and protocol, and comparable instruments.

As you would expect, standardizing processes in labs around the world carries risks and challenges; however, we have been able to resolve most of them by proper planning and communications, and collaboration with experts in the field. One of our biggest challenges remains the logistics of coordinating transfer of materials and protocols to the receiving labs, especially the labs in low- and middle-income countries (LMICs). We are working on resolving these issues with advanced planning, local partnerships, and using alternative shipping methods.

Q What will be the most important projects for the CLN going forward?

AA: Our goal is to provide crucial laboratory support for development and evaluation of vaccines against emerging infectious disease, including any Disease X that might arise. We aim to be well prepared for any emerging pathogens that may become endemic or pandemic. We have a list of priority pathogens, which is largely aligned with the WHO Blueprint List of Priority Diseases.

For instance, we are currently working on development of assays for Lassa fever, Nipah virus, monkeypox, Marburg virus, MERS, and various human coronaviruses. Many of these pathogens are rare or neglected—even with decades of experience in infectious diseases, I knew little

"...in the very near future, artificial intelligence and other new technologies will change the field dramatically."

about some of these diseases before joining CEPI! They are all diseases that have a potential to be epidemic or pandemic, and we are currently developing assay for them.

Q Having viewed vaccine development and analytics from different perspectives (academic, pharma, nonprofit), what do you see as the biggest roadblocks and most exciting advances facing the field?

AA: One major roadblock is the sheer complexity of pathogen-host interactions. There is a lighthearted saying that vaccines have done more for immunologists than immunologists have done for vaccines. The mechanisms of action of many vaccines or diseases are still not completely clear, and immunologists have learned a lot by studying how vaccines work. The complexity of diseases such as HIV, hepatitis C, tuberculosis, or malaria makes them very difficult to develop vaccines for, but our understanding is growing, and with it the potential for effective vaccines.

Regulatory hurdles can also pose a significant challenge. Meeting regulatory requirements for vaccine approval can be sometimes expensive and lengthy, and delay the availability of new vaccines.

Another important roadblock to implementation of vaccines is public perception. Despite what vaccines have done for society, we still see hesitance or even anti-vaccine sentiment. Combatting public concern and misinformation about vaccines is crucial.

While there are challenges, there have also been some exciting advances in the field in recent years, with the success of the mRNA vaccines for COVID-19. Within a year, we were able to produce a vaccine and save millions of lives.

Artificial intelligence and machine learning will play a larger role in the vaccine field in future. At a recent conference, I heard several people discuss using artificial intelligence for design of vaccines or clinical trials, and this approach is attracting support from regulators too. I believe in the very near future, artificial intelligence and other new technologies will change the field dramatically.

BIOGRAPHY

ALI AZIZI is a Project Lead at the Coalition for Epidemic Preparedness Innovations (CEPI), Oslo, Norway. With an extensive career spanning over 25 years, he has demonstrated exceptional leadership and technical skills in numerous international vaccine projects, guiding them from inception to commercialization. Ali has a notable publication record and has

received several research awards including the Exceptional Leadership Award 2021 from University of Ottawa, presented for professional excellence, leadership, and dedication to the community. Currently, Ali is spearheading efforts to establish the largest global network of laboratories dedicated to the development and validation of standardized assays targeting emerging pathogens.

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VACCINE INSIGHTS

LATEST ARTICLES

FASTFACTS

Accelerate vaccine development with novel affinity purification solutions

Manuel Matos, Field Application Scientist, Purification Bioproduction group (BPG), Thermo Fisher Scientific

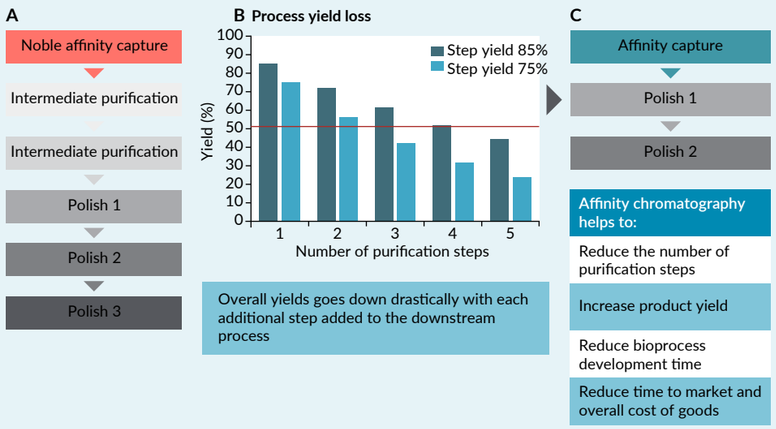
As the landscape of vaccine production continues to expand, it becomes crucial to develop highly efficient purification solutions that can address the growing diversification of vaccine modalities. This poster explores the advantages of affinity chromatography in streamlining the vaccine purification bioprocesses.

ADVANTAGES OF AFFINITY CAPTURE IN BIOMOLECULE PURIFICATION

One of the main challenges in downstream processing of vaccine development is the high number of purification steps, which leads to yield loss. As seen in Figure 1A, non-affinity capture methods require multiple steps to reach the desired purity, depending on the molecule and the number of impurities in the feedstock. Each purification step results in a yield loss—even high step yields such as 85% or 75% may decline to 50% and 30%, respectively (Figure 1B).

This issue can be addressed by using affinity chromatography, which reduces the number of purification steps and increases total product yield (Figure 1C). Additionally, affinity chromatography shortens bioprocess development time, speeding up market entry and reducing the overall cost of goods.

Figure 1. Comparison of required steps and step yields in non-affinity capture and affinity capture for biomolecule purification.



C-TAG FOR SCREENING VACCINE CANDIDATES

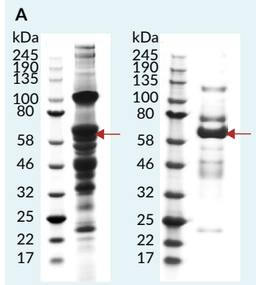
Epitope tagging is a technique that employs genetic engineering to fuse a known epitope, called an affinity tag, to the C or N terminus of a recombinant protein, facilitating its purification and detection.

C-tag is the smallest affinity tag in the market, consisting of only four amino acids: glutamic acid-proline-glutamic acid-alanine (E-P-E-A). The C-tag can be easily fused onto the C-terminal end of a protein through genetic engineering, and allows for easy detection and purification. This technology can be applied to rapid screening of vaccine candidates.

AFFINITY SOLUTIONS FOR PROTEIN-BASED VACCINES AND VLPS: CAPTURESELECT™ C-TAGXL RESIN

CaptureSelect™ C-tagXL is a novel affinity tag system offering unique selectivity for E-P-E-A peptide tag, enabling high-quality and single-step purification of C-tagged proteins. Being the smallest affinity tag in the market, the C-tag minimally affects protein functionality while achieving high yield and purity compared to larger tags such as GST and His6.

Figure 2. Purity and yield comparisons of downstream processing using His6-tag and C-tag for PfRH5 protein-based malaria vaccine candidate.



A: Purity comparison on gel, **B**: Size exclusion comparison, **C**: Yields comparison of both processes

C

Process yield (after)	His6-tagged construct	C-tagged construct
Culture supernatant	100%	100%
TFF	82.1%	91.0%
Affinity chromatography	52.5%	77.4%
SEC	25.5%	43.3%

TFF: tangenital flow filtration, SEC: size exclusion chromatography.

As seen in Figure 2A, the purity of PfRH5 protein reached 72% when using the C-tag affinity system, compared to just 20% with the His-tag system. In addition, the C-tag affinity system produced two well-resolved populations, compared to multiple overlapping protein populations with His-tag (Figure 2B). Finally, the C-tag system resulted in higher yields at every purification step, with a final recovery of 43.3% compared to the His-tag's 25.5% (Figure 2C). Furthermore, the incorporation of the tag did not significantly alter the binding affinity constant to the target [1,2].

TOWARD THE CLINIC: AFFINITY-TAGGED PURIFICATION FOR A VLP-BASED MALARIA VACCINE

The Phase I and II clinical trials of the R21/Matrix-M malaria vaccine, purified using the C-tag affinity system, were carried out in 450 children in Somalia, and the vaccine was demonstrated to be safe, effective and well-tolerated. Utilizing the C-tag in the manufacturing process is also being evaluated for other malaria vaccine candidates such as VLP-based RH 5.1.

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VACCINE INSIGHTS

Vaccine Insights 2024; 3(4), 279; DOI: 10.18609/vac.2024.025

In partnership with: ThermoFisher SCIENTIFIC

INTERVIEW

HIV vaccine research at Gates Foundation: mRNA and beyond

In the next few years, scientists around the globe are making plans to achieve effective immune responses considered to be relevant for developing an efficacious prophylactic HIV vaccine. What role will mRNA technology play in this mission? **Charlotte Barker**, Commissioning Editor of *Vaccine Insights*, speaks with **Pervin Anklesaria**, Deputy Director, HIV Vaccines & Biologics at the Gates Foundation, to find out. Additionally, Anklesaria provides an update on the Gates Foundation's research priorities in HIV and shares insights on successful collaboration.

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Q How did you first get involved in the infectious disease field, and how have your interests evolved since then?

PA: My early career focused on the cancer field, particularly hematological cancers and immunology. This initial work sparked my interest in gene therapy during its initial stages in the early 1990s. Subsequently, I transitioned to a position at Targeted Genetics, a small, Seattle-based biotech company that was one of the first small biotech companies to form a private-public partnership, notably with the International AIDS Vaccine Initiative (IAVI).

The collaboration with IAVI led me into the area of HIV vaccine R&D and I found myself drawn to the challenge of developing a prophylactic HIV vaccine.

Following the transfer of Targeted Genetics assets to Sanofi (formerly Genzyme), I supported IAVI with the development of various viral vectors to either deliver HIV antigens for vaccine discovery or to deliver antibodies for immuno-prophylaxis. This further solidified my commitment to HIV vaccine development.

I then joined the Gates Foundation, where I now lead our efforts for HIV vaccine development and biologics.

Q What are the priorities for HIV vaccine development at the Gates Foundation?

PA: Our overarching goal is 'impact first.' The foundation works collaboratively with other funding institutions. Specifically, in the global health R&D space, our primary role revolves around developing products or supporting their development with the objective of ensuring equitable access. Our main implementation/funding partners include IAVI, the NIH, and the US Agency for International Development (USAID).

A critical aspect of our overall HIV strategy is to accelerate the reduction of HIV incidence. To effectively combat the HIV epidemic, we are leveraging existing prophylactic tools alongside optimizing testing and treatment strategies. However, while existing tools and emerging interventions, such as long-acting antiretrovirals for prophylaxis, may further decrease HIV incidence, a highly efficacious and durable prophylactic vaccine may be needed to sustain low incidence rates in order to maintain control of the HIV epidemic. The Gates Foundation is also exploring innovative approaches towards a sustained viral remission, albeit with a long-term horizon.

The strategic focus of the HIV vaccine team is to obtain proof-of-concept for a HIV vaccine that is safe, efficacious, and cost-effective, to ensure equitable access to all communities that are impacted by this pandemic.

Given the unprecedented viral diversity observed in HIV, any HIV vaccine must address this diversity. Variant-specific vaccines are not viable options. We aim to elicit a comprehensive, potent, broad immune response—specifically, a highly potent, durable, broadly neutralizing antibody response targeting specific regions of vulnerability on the HIV envelope (preferably two to three regions).

Since HIV integrates into host cells, we cannot rely solely on neutralizing antibodies to prevent infection. In the event of infection, we must ensure robust immune responses, particularly CD8 T cells, which kill infected cells at the site of primary infection and prevent viral spread. We are therefore also investing in the development of vaccine components capable of eliciting CD8 T cell responses.

One of our key goals is to generate proof of concept within the next 5 years, demonstrating an HIV vaccine's ability to elicit potent and durable broadly neutralizing cross-reactive activity in serum. It must also be capable of eliciting a robust CD8 T cell response against HIV epitopes that results in a reduction in viral fitness.

Further, we are actively working to simplify two key aspects of vaccine administration. Firstly, we aim to simplify the vaccine regimen through innovations such as pulsatile release, reducing the necessity for multiple immunizations. Secondly, we prioritize ensuring the durability of vaccine response and efficacy. Long-term simplicity and practical ways of delivering the HIV vaccine is essential, and we are dedicated to addressing this aspect in our ongoing R&D.

"Long-term simplicity and practical ways of delivering the HIV vaccine is essential, and we are dedicated to addressing this aspect in our ongoing R&D."

Q How is the Gates Foundation investing in mRNA technology for vaccine R&D?

PA: The foundation's journey with mRNA technology predates the COVID-19 pandemic. In collaboration with Moderna, multiple investigators are exploring its potential applications to develop a vaccine that can elicit broadly neutralizing antibodies. We believe that the mRNA platform holds significant value, particularly in facilitating rapid immunogen identification and testing.

As a platform, we are excited about the opportunities presented by mRNA technology and remain committed to advancing its potential in vaccine development. Considering the importance of durability in eliciting a sustained immune response, we are remaining flexible so that we can potentially transition to other modalities, such as protein-based vaccines, to improve durability.

In partnership with USAID, IAVI, and Scripps, we recently concluded two Phase 1 discovery medicine studies to assess the feasibility of the mRNA platform to test specific HIV immunogens that may be components of an HIV vaccine and demonstrated comparable immunogenicity to traditional protein-based vaccines.

Recently a *Science* article from staff reporter Jon Cohen noted skin-related adverse events associated with mRNA vaccines [1]. Moderna, along with other key stakeholders Gates Foundation, NIH, USAID, Scripps and IAVI, are diligently working to understand these reactions and explore mitigation strategies. Moving forward, NIH's Division of AIDS (NIAID) and Moderna are planning a study to investigate safety and immunogenicity at lower doses.

Q How has the success of the mRNA COVID-19 vaccines advanced the field?

PA: The extensive safety databases associated with the mRNA vaccine have been significant in advancing confidence in its capabilities. Additionally, collaborating with Moderna to leverage their mRNA platform has further advanced our research.

While it is still early in our R&D efforts, the promise of mRNA technology in terms of its ease of use, cost-effectiveness, and rapid production capabilities is indisputable. The mRNA platform plays a pivotal role in realizing the objective of proof of concept.

The pharmaceutical and biotech sectors are witnessing substantial investment aimed at refining the mRNA platform, which bodes well for future advancements. The Gates Foundation has been collaborating with Moderna since 2016, and this has proved invaluable as they continually enhance the platform. The Gates Foundation also has other investments to ensure the affordability, thermostability, and accessibility of vaccines based on mRNA technology.

We remain open to providing strategic funding to drive down the cost of vaccine production. Embracing a collaborative approach allows for the exploration of diverse vaccine approaches and fosters innovation within the field.

Q Whether working in academia, biotech, or nonprofit sectors, collaboration has been a core component of your work. What insights have you gained into the factors behind successful R&D partnerships?

PA: From my personal experience, I believe that trust and transparency are crucial for long-standing and productive collaborations. Trust entails having confidence in our partner's ability to fulfill their responsibilities and being open to their input on important issues. Transparency, both from our partners and ourselves, is equally essential.

As one of the funding agencies working to develop an effective HIV vaccine, we must clearly communicate our expectations and listen to the insights provided by scientists and communities across the globe, especially those most impacted by the HIV pandemic. It is imperative that we do not operate in isolation but rather engage in open dialogue with all stakeholders, especially community members. To enable such an environment, the Gates Foundation has established the Collaboration for AIDS Vaccine Discovery, which emphasizes the standardization of laboratory techniques and data analysis and the sharing of scientific information with the broader scientific community and other stakeholders to facilitate collective progress and prevent redundant efforts.

By fostering a collaborative environment for HIV vaccine R&D and implementation and prioritizing open communication, we can achieve more significant scientific breakthroughs and equitable health outcomes for communities most impacted by HIV.

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BIOGRAPHY

PERVIN ANKLESARIA leads the HIV vaccines and biologics prevention efforts for the Gates Foundation's HIV team, Seattle, WA, USA. She joined the foundation in 2012 as a senior program officer working on the foundation's HIV vaccine and biologics initiatives.

Anklesaria has held leadership roles in complex programs within the foundation's HIV vaccine and biologics initiatives, including the CMV-HIV and tuberculosis vaccine program with Vir Biotechnology, the IAVI Neutralizing Antibody Consortium, mRNA-based vaccines with Moderna, and passive administration of broadly neutralizing antibodies and bi-specifics with Rockefeller University and Dr David Ho.

Before joining the foundation, Pervin worked at Targeted Genetics Corporation from 1993–2009, leading the clinical development of AAV vectors for inherited diseases and evaluation of that platform for HIV vaccines, in collaboration with IAVI and the National

Institutes of Health. In 2009, after the company's assets were sold to Genzyme, now Sanofi, she spent 2 years at IAVI as a project director and senior advisor, working on some of IAVI's HIV vaccine candidates. Pervin earned a PhD in India at the University of Mumbai, Mumbai, Maharashtra, India, followed by a productive research career at UMass Memorial Medical Center in Worcester, MA, USA.

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INTERVIEW

Developing new nanocage-based systems for multivalent vaccine delivery

A new approach to engineering ferritin nanocages could provide a convenient delivery system for multi-strain or multi-pathogen vaccines. **Charlotte Barker**, Editor, *Vaccine Insights*, speaks with King's College London Lecturer **Kourosh Ebrahimi** and PhD student **Yujie Sheng** about the technology.

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DOI: 10.18609/vac.2024.024

Q What led you to your current research?

KE: My background is biochemical engineering, bioinorganic chemistry, and virology. During my PhD, I studied how naturally forming ferritin nanocages store iron, which is fundamental to protecting the body from iron toxicity. After postdoctoral studies in virology and immunology, I started my research group at King's College London, and we began to explore using ferritin nanocages to deliver therapeutics.

"...you can have multiple antigens on the surface and the whole nanocage will act as a virus-like particle that can mimic different viruses entering the body." — Kourosh Ebrahimi

YS: After majoring in pharmaceutical engineering, I embarked on a Master's degree in biology, drug development, and discovery. Now, I am focusing my PhD studies on the control of ferritin assembly for biomedical applications such as vaccines and antiviral candidates.

Q Tell us about your recent study demonstrating a new approach to engineering ferritin nanocages [1].

KE: Ferritin is made up of 24 interlocking connected subunits that self-assemble to form a hollow sphere. To add therapeutic drugs inside the sphere or multiple proteins on the surface requires breaking it open—for drug delivery this has typically been done using acids, but this can be damaging to the proteins and unsuitable for water-insoluble drugs.

With our approach, we start with the disassembled subunits—which we call PREcursors of nanoCage (PREC)—and induce spontaneous self-assembly using protease cleavage to form protease-induced nanocages (PINCs). This was inspired by HIV-1 capsid formation from protease cleavage of Gag polypeptide precursors.

PRECs can be modified genetically to attach various proteins or peptides to them and during their protease-mediated assembly, they can encapsulate hydrophilic or hydrophobic drugs to form PINCs as a drug delivery vehicle or vaccine candidate. The precursors can also be mixed and matched to form a mosaic nanocages decorated with different surface molecules. It is a 'plug and play' platform to which a variety of molecules can be added inside and outside the PINCs.

Q Why is this delivery system particularly relevant for vaccines?

KE: We hope our platform ferritin nanocage technology will be able to create a multivalent vaccine that is effective against multiple strains of a virus, or even against different types of viruses.

Essentially, you can have multiple antigens on the surface and the whole nanocage will act as a virus-like particle that can mimic different viruses entering the body. The immune response will therefore be much broader than a single antigen.

"...we hope to be able to combine a vaccine and an antiviral together to attack viruses from multiple angles." — Yujie Sheng

Another point that is important for the pharmaceutical industry, and vaccines particularly, is that ferritin—and our nanocage system—is very stable. You can easily produce it in a large amount in a host like *E. coli*, and the nanocage can be lyophilized.

Q How well-established is ferritin nanocage technology in vaccine development, and do you have a sense of whether the ferritin itself is likely to have immune impacts?

KE: Ferritin has recently received a lot of attention in vaccine development, with ferritin-based technology being used for influenza virus, HIV, and SARS-CoV-2 vaccines under development, with some showing promise in clinical trials. One reason is that ferritin is a 3D spherical structure similar to a virus, and therefore more easily mimics the way that a virus induces immune responses.

Clinical trial data so far suggests that there is no significant toxicity or immunogenicity associated with the ferritin nanocages themselves. Ferritin is a protein that every organism produces so it is not expected to cause the immunogenicity seen with some synthetic nanocages.

Q What's next for this work?

KE: We are currently working with experts at King's College London and the UK Health Security Agency (UKHSA) who can produce antigens or antivirals to be delivered using PINCs, and help us to develop novel formulations, such as sprays for inhalation. We hope to develop, within the next few years, a therapeutic candidate that we can take further against influenza or other types of respiratory viruses.

YS: Using this platform technology, we hope to be able to combine a vaccine and an antiviral together to attack viruses from multiple angles.

REFERENCE

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BIOGRAPHIES

KOUROSH EBRAHIMI finished his MSc in Biochemical Engineering and subsequently his PhD in Enzymology at TU Delft, Delft, Netherlands. After doing postdocs in Virology (Scripps Research Institute), Microfluidics (TU Delft), and Bioinorganic Immunology (Oxford), he started his research group at King's College London, London, UK, in October 2021. His group combines fundamental science and biological engineering to shed insight into the immune system's function and develop multifunctional therapeutics like mosaic vaccines.

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AUTHORSHIP & CONFLICT OF INTEREST

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