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Rapid Manufacture and Release of a GMP Batch of Zaire Ebolavirus Glycoprotein Vaccine Made Using Recombinant Baculovirus-Sf9 Insect Cell Culture Technology

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Abstract

For the ongoing 2014 Ebola virus outbreak, all viable options and technologies need to be evaluated as potential countermeasures to address this emerging biological threat. Novavax, Inc. has a rapid, practical vaccine development and manufacturing platform with the capability to deliver clinical trial material and, ultimately, commercial doses in response to novel infectious disease agents.[1-3] This report describes the application of our platform technology for the successful generation, manufacture, and release of a clinical batch of Zaire ebolavirus glycoprotein nanoparticle vaccine three months from project initiation. The key enabling factors were:

- An integrated project plan and frequent cross-functional response team meetings
- Forward processing of intermediates prior to completion of quality control testing
- Rapid development and confirmation of the platform process at laboratory-scale
- Advanced use of the baculovirus master virus seed to bypass production of the working virus seed
- Rapid analytical method development
- A manufacturing process that employs single-use manufacturing technology
- Collaboration and flexibility from key raw material suppliers and contracted service providers

Introduction

In 1976, a new virus (later designated genus *Ebolavirus*), which caused acute hemorrhagic fever in people of Zaire (now the Democratic Republic of Congo) was reported.[4] Since then, five ebolavirus species have been identified. The species designated *Zaire ebolavirus* (EBOV), *Sudan ebolavirus*, and *Bundibugyo ebolavirus* have caused multiple, major Ebola virus disease (EVD) outbreaks in Africa.[5] From 1976 to 2013, EVD is estimated to have claimed the lives of 1590 people.[6]

In March 2014, the United Nations World Health Organization (WHO) was notified of a new outbreak of EVD in Guinea that had expanded to Liberia. Further outbreaks were identified in Sierra Leone (May) and Nigeria (July).[6] In August, WHO Director-General Dr. Margaret Chan declared a public health emergency of international concern, characterizing the 2014 EVD outbreak as "the largest,

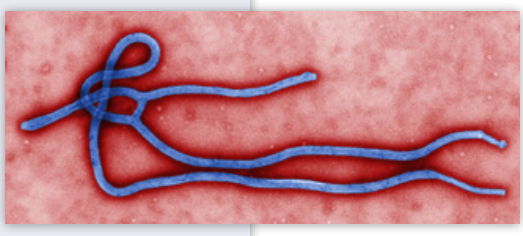

IMAGE: Created by microbiologist Cynthia Goldsmith, Centers for Disease Control and Prevention (CDC), this colorized transmission electron micrograph (TEM) revealed some of the ultrastructural morphology displayed by an Ebola virus virion. (http://phil.cdc.gov/phil/details.asp?pid=10816)

most severe and most complex outbreak in the nearly four-decade history of this disease."[7] Beyond the initial countries, a first-case-in-country was reported in both the Democratic Republic of Congo and Senegal in August, in the United States of America, Spain, and Mali in October, and in the United Kingdom in December.[8] Worldwide, reported statistics for the 2014 outbreak through February 1, 2015 summarize a total of 22,495 confirmed, probable, and suspected cases of EVD with 8981 associated deaths[9]—eclipsing five times the reported total from 1976 to 2013.

A landmark report by Gire *et al*., published in *Science* on September 12, 2014 (print date), showed that the Sierra Leone outbreak stemmed from the introduction of two genetically distinct Ebola virus strains from Guinea.[10] The investigators' genetic tracing revealed that these two 2014 Guinea virus lineages were present in 12 of the first EVD patients in Sierra Leone, all believed to have attended the funeral of an EVD case from Guinea, suggesting that the funeral attendees were most likely infected by the same isolate(s). Based on genetic sequence analysis, Gire *et al*. proposed that the current sustained human-to-human transmission (with no evidence of additional zoonotic sources) is due to a modified EBOV cluster 3 strain prominent in Sierra Leone, beginning in late May 2014, which was derived from the strain(s) that infected the funeral attendees. The glycoprotein (GP) gene sequence from this isolate, H. sapiens-wt/SLE/2014/Makona-G3798; cluster 3 (Genbank #AIG96283) (hereafter referred to as "EBOV/Mak"), was selected for our vaccine.

Vaccine candidates against Ebola virus have been in development for more than a decade.[11-18] While some offer promise, they also have some accompanying challenges.[14-16] In particular, vaccine candidates listed on the ClinicalTrials.gov[19] website in 2014 using viral vector technology (which generally require high dose levels)[14, 16-18] are based on the glycoprotein of the 1976 Mayinga (Genbank #AAD14585) or 1995 Kikwit (Genbank #AGB56821) strains of EBOV[20, 21] (which differ by 20 and 17 amino acids, respectively, relative to the EBOV/Mak isolate), have limited thermal stability, requiring storage at temperatures below −60°C[17, 22] (necessitating a frozen, cold-chain distribution network), and induce an immune response to the vaccine vector[16, 17] (limiting their potential as a multi-dose vaccine).

Knowing that Novavax has the technology to develop 2–8°C stable vaccine candidates specific to emerging novel viral gene sequences rapidly[23-25], the company considered it imperative to initiate an accelerated development program. On September 16, 2014, following the report by Gire *et al*.[10], Novavax commenced a project to generate, manufacture, and release an EBOV/Mak GP nanoparticle vaccine candidate (hereafter referred to as "Ebola nanoparticle vaccine") in three months. Concurrently, Novavax's vaccine adjuvant Matrix-M™, which is a mixture of separately purified fractions of *Quillaja* saponins formulated with cholesterol and phospholipids into Matrix-A™ and Matrix-C™ particles[26, 27], was manufactured and released within six weeks. In 2013, we reported on Novavax's rapid response to the avian influenza A/Anhui/1/2013 (H7N9) virus by rapidly producing a vaccine candidate based on a flexible and agile manufacturing virus-like particle (VLP) platform.[1-3] This paper provides evidence that Novavax's protein-protein micelle nanoparticle vaccine manufacturing platform can be applied to emerging endemic disease threats caused by viral genome modifications.

The Ebola nanoparticle vaccine candidate, combined with Novavax's proprietary saponin-based adjuvant (Matrix-M), demonstrated immunogenicity and 100% cross-strain protection in mice challenged with a lethal dose of EBOV.[28] The vaccine drug product and the adjuvant drug product are planned for use in a Phase I clinical study.

Process Description

Novavax's protein micelle nanoparticle vaccine manufacturing technology is designed for flexibility as a platform process for the production of practically any viral surface protein and many other types of proteins intended to be used as a vaccine. Vaccine candidates under development by Novavax using this technology include the respiratory syncytial virus (RSV) F protein[29] and rabies G protein[30] vaccines, which are in clinical studies, and the Middle East respiratory syndrome (MERS) S protein vaccine, which is in preclinical studies.[24, 25] Because the manufacturing process is a platform, it can be rapidly implemented following completion of recombinant protein-specific process elements, including the generation of a specific baculovirus (BV) master virus seed (MVS), confirmation of protein-specific process parameters, and the generation of strain-specific assay reagents.

The core of Novavax's technology is the genetically engineered baculovirus, which is designed to express the vaccine-specific recombinant protein, in this case a protein corresponding to EBOV/Mak GP. When *Spodoptera frugiperda* (Sf9) insect cells are infected with the recombinant baculovirus, the EBOV GP is expressed on the surface of the cells. The cells are collected from the culture and lysed with a detergent-containing buffer solution to extract and solubilize proteins from the lipid membrane. After a series of purification steps, the purified EBOV GP

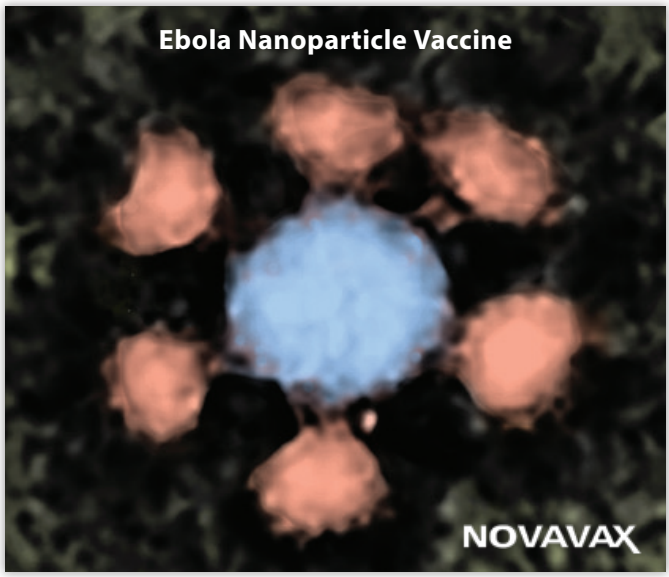

FIGURE 1. TEM negative stain image with 2D class average of one of Novavax's EBOV GP nanoparticles showing: (A) a dense core region which contains the GP2 fusion subunits (blue); with (B) six visible chalice-like GP1 trimer attachment subunits (pink) extending outward. (TEM with manual coloration by NanoImaging Services, Inc.)

forms a nanoparticle similar to Novavax's RSV F protein[23], rabies G protein[30], MERS S protein, and SARS S protein[24-25] nanoparticle vaccines. Based on structural analysis previously reported for EBOV GP[31], the dense core region of the vaccine nanoparticle (**Figure 1**) is believed to contain the glycoprotein (GP2) fusion subunits with the visible, chalice-like glycoprotein 1 (GP1) trimer attachment subunits extending concentrically outward. The EBOV GP nanoparticles are about 36 ± 4 nm, as measured using dynamic light scattering.

A specific sequence of events was required for the generation of a baculovirus MVS. First, the GP gene sequence derived from the EBOV/Mak strain was codon-optimized for expression in insect cells and then biochemically synthesized (Genscript). These genes were cloned into a pFastBac™1 baculovirus transfer vector (Life Technologies™) and transposed into a baculovirus genome using the Bac-to-Bac® baculovirus expression system (Life Technologies) using *Escherichia coli* host cells. Recombinant bacmid DNA was purified from the bacteria and transfected into Sf9 insect cells from which a recombinant baculovirus expressing EBOV GP was identified, plaque-purified, and amplified in cell culture as passage 1 (P1) pre-master virus.

Manufacturing of the MVS was initiated with the thaw of a frozen vial of Sf9 cells (**Figure 2A; following page**). The culture was continuously passaged in flasks (Corning) of increasing culture volume to achieve the number of cells needed for inoculation of a 50 L WAVE Bioreactor™ (GE Healthcare Life Sciences). The 50 L bioreactor was infected with the P1 virus to produce passage 2 (P2) MVS. Most of the MVS harvest was frozen for use in future EBOV nanoparticle vaccine campaigns. A portion was held at 2–8°C for inoculation of the Xcellerex™ 200 L bioreactor (GE Healthcare Life Sciences) for the first GMP clinical batch.

A similar cell expansion process (as used for the MVS) was initiated for the clinical batch (**Figure 2B;** following page). This time, the 50 L bioreactor was used to inoculate the 200 L bioreactor. Following a few days of cell growth in the 200 L bioreactor, the culture was infected with the MVS stored at 2–8°C (**Figure 2A;** see orange arrow). Typically, a passage 3 (P3) working seed is prepared to inoculate the production bioreactor (**Figure 2B**).

For the GMP clinical batch, the recombinant baculovirus infection of the bioreactor Sf9 cells at harvest was evaluated by fluorescence-activated cell sorting (FACS). The increase in fluorescence associated with anti-EBOV GP mAb (4F3; IBT Bioservices) binding to the glycoprotein on the infected cell surfaces, compared to an uninfected control culture, confirmed effective progression of baculovirus infection and recombinant protein production (**Figure 3**).

For the upstream portion of the platform manufacturing process, general operating ranges are known for the multiplicity of infection (MOI) and harvest time (post-BV infection) for the production bioreactor process. The MVS for EBOV GP was evaluated at laboratory-scale to establish the specific MOI and harvest time within the known operating ranges.

For the harvest portion of the manufacturing process, the bioreactor cells were collected using a semi-continuous, single-use centrifuge (UniFuge®, PneumaticScaleAngelus) (**Figure 2B**). The pelleted cells were suspended in a wash

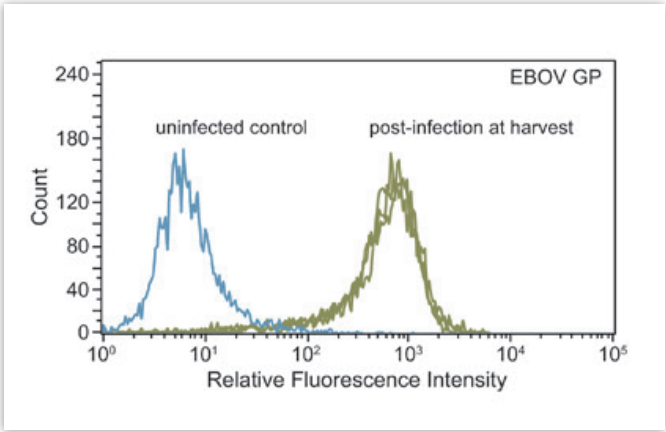

FIGURE 3. FACS analysis of recombinant baculovirus-infected Sf9 cells at the final harvest timepoint for the GMP batch. The increase in fluorescence associated with anti-EBOV GP binding to the glycoprotein on the surface of infected cells (green), compared to an uninfected control culture (blue), confirms effective progression of baculovirus infection and recombinant protein production.

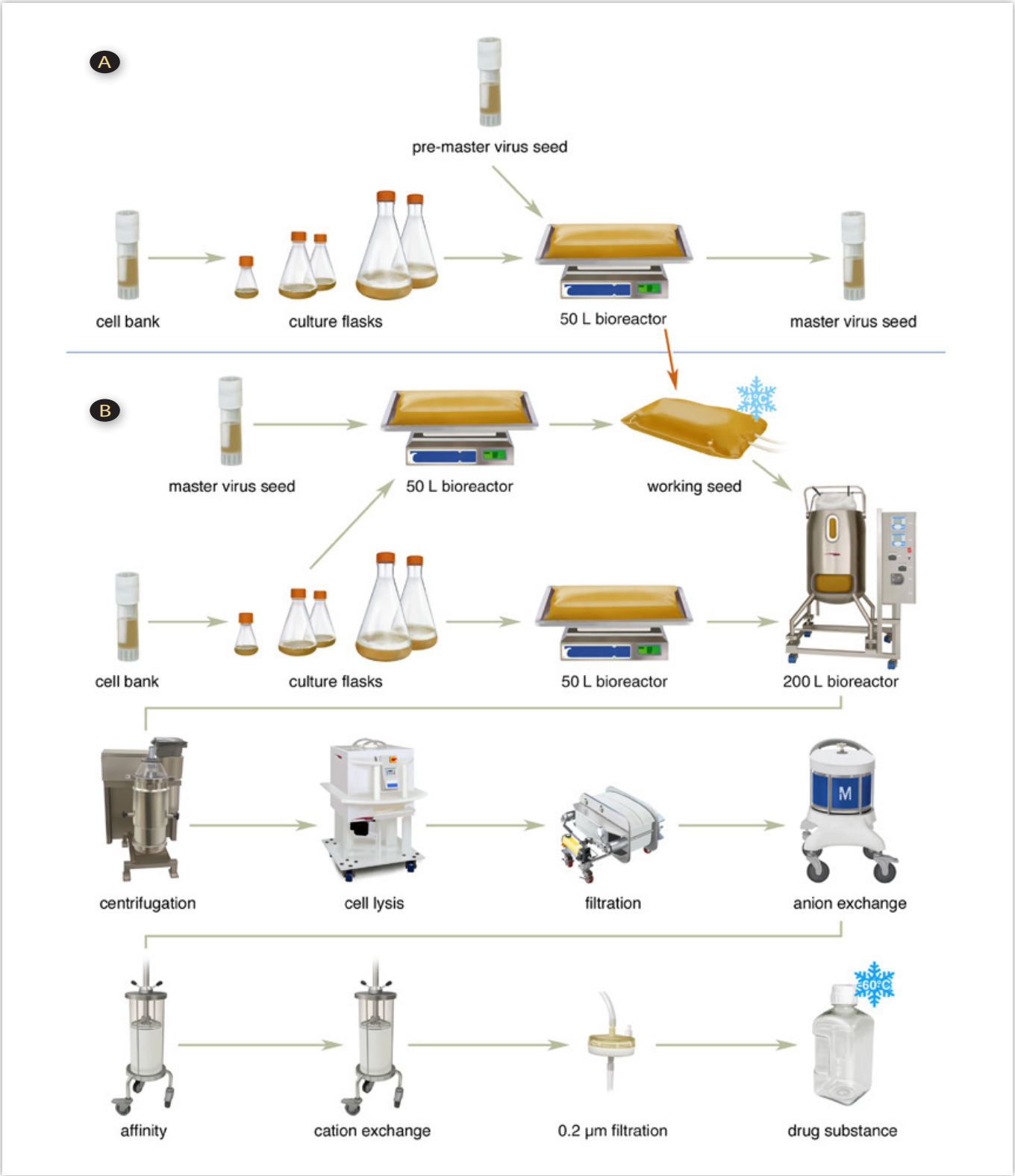

FIGURE 2. Process flow diagram: **(A)** master virus seed is produced by Sf9 cell culture (expanded in culture flasks from a cell bank vial) in a 50 L bioreactor infected with pre-master virus seed; **(B)** the Novavax clinical-scale nanoparticle vaccine production process begins with the thaw of a cell bank vial and continues with the culture of cells in flasks, followed by a 50 L bioreactor, and then a 200 L bioreactor. Cells are infected with seed in the 200 L bioreactor and then harvested, washed, and lysed to prepare a solubilized solution of recombinant protein. After filtration, the protein is purified by anion exchange, affinity, and cation exchange chromatography. The 0.2 µm-filtered drug substance is stored at ≤−60°C. The orange arrow shows the use of the master virus seed in place of the working seed for the first bioreactor infection.

solution and re-pelleted. Afterward, the cells were lysed in a detergent-containing buffer solution and clarified through a depth filter (EMD Millipore Millistak+® Pod).

The purification portion of the platform process typically uses the same three types of chromatography resins, in this order: anion exchange (AEC), affinity, and cation exchange. Depending on the isoelectric point (pI) of the recombinant protein, the pH and salt concentrations of buffers may be modified based on laboratory-scale studies, and AEC may be performed in either a flow-through or capture mode.

For the Ebola nanoparticle vaccine, the standard resins were used, some buffers were modified, and the EBOV GP was eluted following binding to the AEC resin. The AEC product was further purified by an affinity chromatography step. Final processing to the formulation buffer was achieved by cation exchange chromatography in bind and elute mode.

The EBOV GP drug substance was formulated to the appropriate dilution at Novavax and then transferred to our fill/finish contract manufacturer Advanced BioScience Laboratories (Rockville, Maryland) for filling, inspection, labeling, and packaging of the final drug product. Drug substance and drug product release testing was performed by Novavax and testing service providers, BioReliance (Rockville, Maryland) and GENEWIZ (Frederick, Maryland). Product was released by the Quality Assurance department at Novavax.

Results and Discussion

The relationship of the GP gene sequence associated with the EBOV/Mak to the current outbreak in West Africa (Guinea, Liberia, and Sierra Leone) was expressly established.[10] Based on the significance of this finding and an expectation that a vaccine to EBOV/Mak would be most effective, Novavax initiated a plan on September 16, 2014 to manufacture and release a GMP batch of an Ebola nanoparticle vaccine for clinical study. The team's key activities for this project are presented in **Figure 4**. The five main areas of focus were: (1) EBOV GP gene processing to produce the P1 virus; (2) production of MVS, drug substance, and drug product; (3) process and assay development; (4) quality control (QC) testing; and (5) quality assurance review.

Construction of the plasmid was completed on September 23 and then the gene was integrated, cloned, amplified, and prepared as a bacmid for Sf9 cell transfection. Baculovirus was produced and plaque purified. The cloned P1 virus was harvested, and potency was determined on October 10. MVS production was initiated on October 12 and completed on October 30.

On October 17, in advance of MVS completion, Novavax began the expansion of the cell culture steps through to the production bioreactor in order to coordinate inoculation of the production bioreactor with the availability of the MVS. MVS was manufactured in sufficient quantity to infect the production bioreactor and to freeze a suitably sized MVS bank. A portion of the seed was held at 2–8°C to inoculate the production bioreactor.

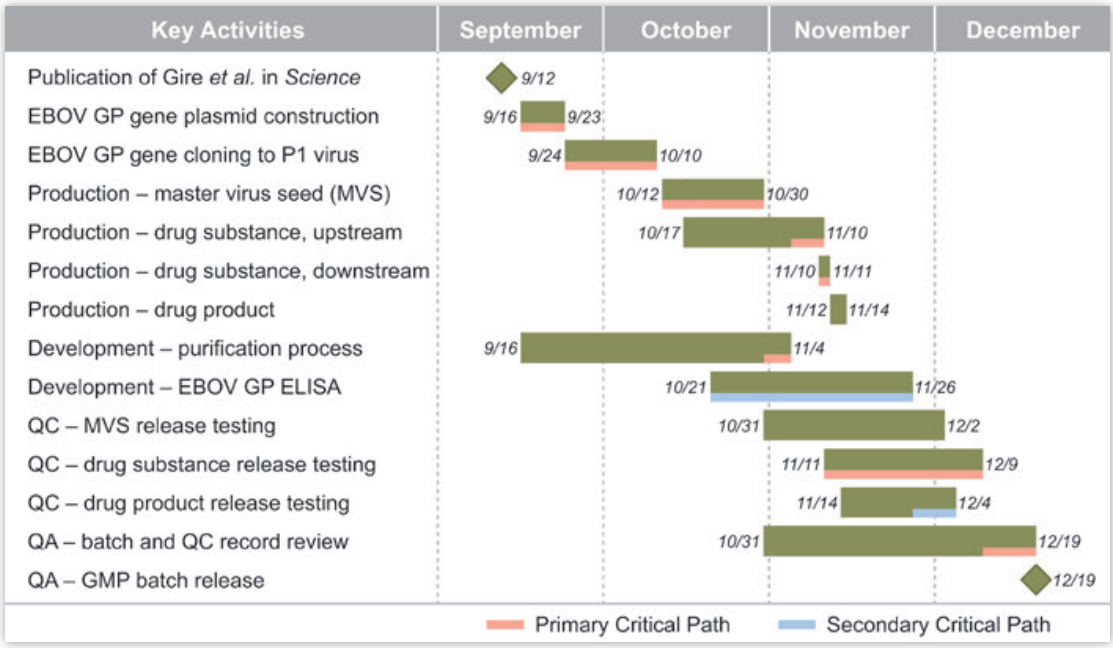

FIGURE 4. Timeline with primary and secondary critical paths for the production and release of a GMP batch of EBOV GP vaccine drug product three months from project initiation.

After the MVS was manufactured, the critical path went through downstream process development. The final process conditions were defined and approved through the quality system change control procedure on November 4. The bioreactor culture was then harvested on November 10, and purification was completed on November 11. The drug substance was formulated to the proper dilution (based on protein content) on November 12, and filled on November 13–14. The final gating activities for the primary critical path were drug substance release testing and batch record review. EBOV GP nanoparticle vaccine drug product was released on December 19.

Manufacture and release of our proprietary saponin-based adjuvant, Matrix-M, was not on the critical path and was performed at our Novavax AB manufacturing site in Uppsala, Sweden (**Figure 5**). Using our inventory of raw material components, including the fractionated saponin raw material, one drug substance batch of Matrix-C was produced (October 20–24) and then one drug substance batch of Matrix-A was produced (November 3–7). Matrix-M (an 85:15 ratio of Matrix-A to Matrix-C) was formulated on November 14. The Matrix-M was filled, labeled, and packaged at our contract service provider Apotek Produktion & Laboratorier AB (Umeå, Sweden). The adjuvant drug product was released on December 2, in advance of the release date for the Ebola GP nanoparticle vaccine drug product.

Key enabling factors in the vaccine product's successful timeline included:

- An integrated project plan and cross-functional response team meetings
- Forward processing of intermediates prior to completion of quality control testing

FIGURE 5. Timeline for the production and release of a GMP batch of Matrix-M adjuvant drug product (DP) prepared from Matrix-A and Matrix-C drug substances (DS).

- Rapid development and confirmation of the platform process at laboratory-scale
- Advanced use of the baculovirus master virus seed to bypass production of the working virus seed
- Rapid analytical method development
- A manufacturing process that employs single-use manufacturing technology
- Collaboration and flexibility from key raw material suppliers and contracted service providers

An integrated project plan and cross-functional response team meetings. Best practices were applied right from the project's initiation to ensure appropriate planning, monitoring, and control for real-time assessment of risks and status. A detailed project plan with dependencies within and across departments was developed and updated throughout the project to handle the overlapping and integrated tasks. Several important intermediate milestone completion dates were set *a priori* and then activities began under the assumption that all required prerequisites would be completed as scheduled. The primary critical path and the secondary critical path (which could have become a gating event for release had it extended beyond the primary critical path) were monitored with mitigation actions taken as needed. Any manufacturing or testing deviation was rapidly identified, investigated, and resolved. Upon completion of batch record review, the GMP batch was released.

Forward processing of intermediates prior to completion of quality control testing. Forward processing was an essential part of completing the production and release of the GMP batch of an Ebola nanoparticle vaccine in three months. The MVS was forward processed to the drug substance manufacturing process prior to completion of the MVS QC testing. Likewise, drug substance was forward processed to drug product prior to completion of QC testing. This strategy enabled QC testing of MVS, drug substance, and drug product to proceed simultaneously. The last QC test completed on December 9 was the 28-day *in vitro* assay on the bioreactor harvest sample. All other QC testing was completed within the timeframe defined by this assay. Forward processing followed approved quality system procedures.

Rapid development and confirmation of the platform process at laboratory-scale. After a laboratory-grade EBOV GP baculovirus seed was prepared, studies were initiated to confirm upstream and downstream process parameters. The bioreactor infection parameters were readily set, based on small-scale studies. Establishing the downstream process parameters was more challenging as the pI of the EBOV GP required the AEC step to operate in capture mode. The pH and salt concentrations of buffers

needed to be modified, so several small-scale studies were executed to establish operating parameters while incorporating some practical constraints (including the available formulations of released buffers in GMP inventory and the volume of media needed for each step). The final process used for the GMP batch was confirmed at laboratory-scale prior to implementation.

Advanced use of the baculovirus master virus seed to bypass production of the working virus seed. The standard production process includes the generation of a baculovirus working virus seed (P3) from the MVS (P2), which is used to infect the production bioreactor. For the first few lots of a production campaign (when the MVS is first manufactured), in place of the working virus seed, the process allows the use of the MVS culture to infect the production bioreactor, with the remainder of the MVS culture being frozen for use in future production (**Figure 2**; see orange arrow). The Ebola nanoparticle vaccine batch was manufactured using this approach, thereby reducing the primary critical path timeline by approximately one week.

Rapid analytical method development. Quality control release assays for general properties, quantity, purity, process-related impurities, and contaminants are based on platform analytical technologies across all of the vaccine candidates produced by the nanoparticle vaccine manufacturing process and, therefore, require limited development to demonstrate that methods are suitable for their intended use. Product characterization assays, including surface plasmon resonance and mass spectrometry, are also generally applicable across many different vaccine candidates. The challenge for the Ebola nanoparticle vaccine project was the rapid development of a robust potency assay.

The development of a sandwich-type ELISA method for potency determination of the Ebola nanoparticle vaccine was initiated on October 21 with screening of several commercial sources of capture, detection, and horseradish peroxidase-conjugated antibodies. Work continued with evaluation of assay conditions including antibody concentrations, type of microplate, blocking buffer, assay diluent, and sample diluent. The capture antibody selected, mouse anti-EBOV GP mAb (4F3; IBT Bioservices), was raised against purified recombinant Zaire Ebola virus protein and demonstrated specific binding to purified Zaire virions, recombinant Ebola protein, and Ebola-infected cells.[32] The detection antibody selected, a murine/human chimeric anti-EBOV GP mAb (h13C6 FR1; IBT Bioservices), recognizes an N-terminal conformational epitope of GP1 and exhibits neutralizing activity against the Ebola virus.[33] Given the binding specificity of the capture and detection mAbs, the potency (as measured by conformational integrity) was assured. On November 26, the assay was qualified to ensure specificity, accuracy, linearity, precision, and reproducibility, in advance of the deadline for testing and release.

The ELISA binding curves of the Ebola nanoparticle vaccine show a well-defined sigmoid shape with saturated binding at high concentrations across the linear range of 4.0–16.0 µg/mL (**Figure 6 A–B**). Relative to a control stored frozen at ≤−60°C, a comparable profile was obtained for a sample stored at 2–8°C for 48 hours (**Figure 6C**). When a sample was subjected to stressed treatment (pH 3.7 for 48 hours), decreased binding activity and loss of parallelism were

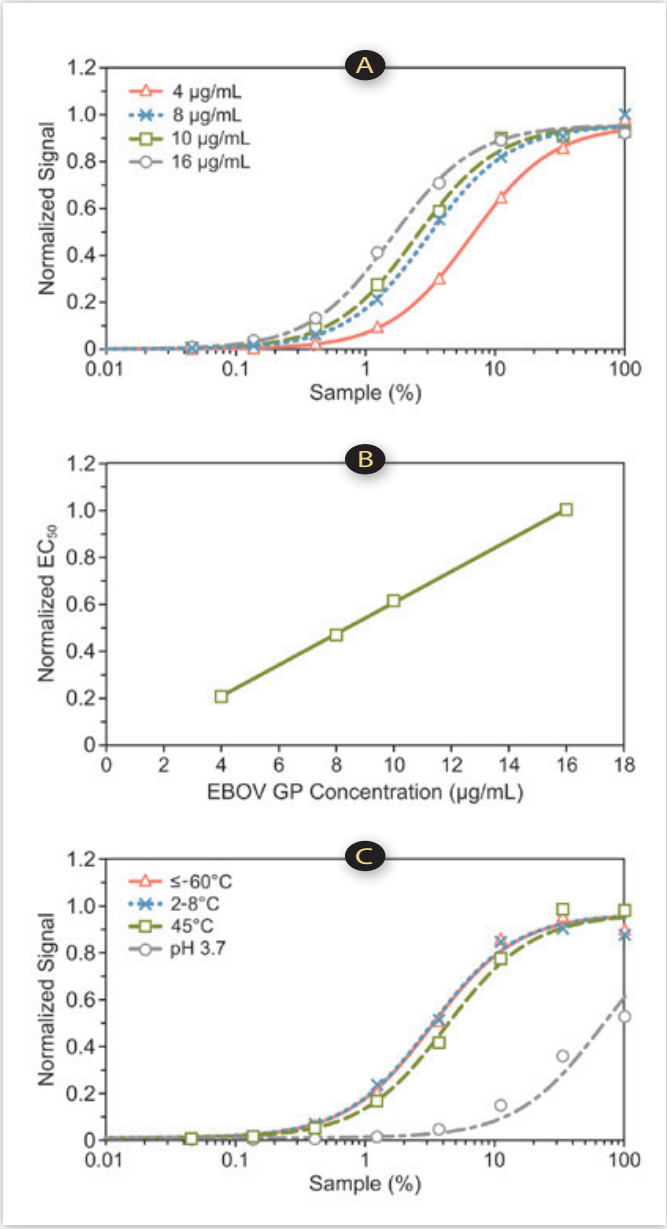

FIGURE 6. ELISA binding curve of Ebola nanoparticle vaccine. (A) dilution curves of 4, 8, 10, and 16 µg/mL samples; **(B)** normalized standard curve derived using EC_{50} points; and **(C)** dilution profiles for reference standard (10 µg/mL) stored at ≤−60°C (pink), stored at 2–8°C (blue) for 48 hours, stored at 45°C (green) for 48 hours, or treated at pH 3.7 for 48 hours (gray).

observed as compared to the frozen control (**Figure 6C**). Vaccine held at an elevated temperature (45 °C for 48 hours) had decreased binding, but parallelism was maintained (**Figure 6C**). The results demonstrate that the binding ELISA is stability-indicating and is suitable for monitoring biological activity of the Ebola nanoparticle vaccine during lot release and thermal stability testing.

A manufacturing process that employs single-use manufacturing technology. Novavax is a clinical stage vaccine company with a robust pipeline that often demands full utilization of the manufacturing facility. The opportunity to schedule an unplanned GMP batch of a novel EBOV GP vaccine would not likely have been possible with conventional stainless steel process equipment due to the cleaning qualification and changeover procedures that are typically necessary between product campaigns. By utilizing single-use components, from culture flasks through the lysate clarification step, including single-use bioreactors and a single-use centrifuge, our facility turnover procedures allowed us to manufacture the GMP batch of EBOV GP vaccine and maintain the production schedule for our other clinical products.

Collaboration and flexibility from key raw material suppliers and contracted service providers. As an independent company, Novavax readily adjusted priorities to align internal resources on this critical project. That said, the project success was not achieved by Novavax alone. It was necessary for key service providers (especially Advanced BioScience Laboratories, BioReliance, and Apotek Produktion & Laboratorier AB) to also adjust their priorities to coordinate efforts and minimize the time to product release. They provided exceptional collaboration by synchronizing their schedules around our project plan (and necessary modifications made to the plan).

Conclusion

Novavax, Inc. has a rapid, practical vaccine development and manufacturing platform with the capability to deliver clinical trial material and, ultimately, commercial doses in response to rapidly emerging disease agents. Novavax previously reported that it generated, manufactured, and released an avian influenza A/Anhui/1/2013 (H7N9) VLP vaccine candidate clinical batch three months from project initiation using its platform recombinant DNA and

baculovirus (Sf9) cell culture-based technology.[1-3] This new report describes the successful generation, manufacture, and release of a clinical batch of Ebola virus GP nanoparticle vaccine three months from project initiation using the protein-protein micelle nanoparticle vaccine production platform. With these two examples, Novavax continues to build a documented body of work, confirming its capability to respond quickly to emerging infectious disease threats.

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All authors are employees of Novavax, Inc.

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