## 16.5 Genotyping Report

This section contains the following document:

Preliminary Analysis of Viral Copy Number and Circulating Viral Variants in the mRNA-1273 Phase 3 COVE Trial dated 16 Jul 2021

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**Title:** **Preliminary Analysis of Viral Copy Number and Circulating Viral Variants in the mRNA-1273 Phase 3 COVE Trial**

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**Preliminary Analysis of Viral Copy Number and Circulating Viral Variants in the mRNA-1273 Phase 3 COVE Trial**

**Background:** Vaccine mRNA-1273 was highly effective in preventing Covid-19 illness in the recent coronavirus vaccine efficacy (COVE) phase 3 trial.(1) Following Emergency Use Authorization of mRNA-1273, the trial protocol was amended from the observer-blind part of the study to an open-label part that is ongoing. From July 2020 to the end of March 2020, a constant emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) mutants has re-shaped the epidemiological landscape. Viruses constantly evolve through mutation, and SARS-CoV-2 is no exception. Thus, a mutated SARS-CoV-2 virus, that diverges from the original strain is called a SARS-CoV-2 *variant*. A variant has one or more mutations that differentiate it from others in circulation. As expected, multiple variants of SARS-CoV-2 have been documented in the United States and globally throughout this pandemic.

Currently the Center for Disease Control (CDC) groups the variants in three groups (2):

> **Variants of Interest (VOI):** A variant with specific genetic markers that have been associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity

**Variants of Concern (VOC):** A variant for which there is evidence of an increase in transmissibility, more severe disease (increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures

**Variants of High Consequence:** A variant of high consequence has clear evidence that prevention measures or medical countermeasures (MCMs) have significantly reduced effectiveness relative to previously circulating variants

(For an up-to-date definition please visit: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Concern )

**Table 1: CDC Variants of Concern (VOC) definition table**

| Variant (PANGO)† | Spike protein substitutions | Name (Nextstrain)‡ | First Detected | WHO Label | VOC/VOI |
|---|---|---|---|---|---|
| B.1.1.7 | Δ69/70, Δ144, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H (K1191N*) | 20I/501Y.V1 | United Kingdom | Alpha | VOC |
| P.1 | L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I | 20J/501Y.V3 | Japan/ | Gamma | VOC |
| B.1.351 | D80A, D215G, Δ241/242/243, K417N, E484K, N501Y, D614G, A701V | 20H/501.V2 | South Africa | Beta | VOC |
| B.1.427 | L452R, D614G | 20C/S:452R | United States-(California) | Epsilon | Initially VOC, now VOI |
| B.1.429 | S13I, W152C, L452R, D614G | 20C/S:452R | United States-(California) | Epsilon | Initially VOC, now VOI |
| B.1.617.2 | T19R, (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N | 20A/S:478K | India | Delta | VOC |

†PANGO (Phylogenetic Assignment of Named Global Outbreak) nomenclature of lineages. PANGO is a software tool developed by members of the Rambaut Lab, and associated web application was developed by the Centre for Genomic Pathogen Surveillance in South Cambridgeshire, intended to implement the dynamic nomenclature of SARS-CoV-2 lineages. Nextstrain nomenclature. ‡Nextstrain collaboration between researchers in Seattle, USA and Basel, Switzerland, provides open-source tools for visualizing the genetics of outbreaks with a goal to support public health surveillance by facilitating understanding of the spread and evolution of pathogens. *detected in some sequences but not all. Source: CDC https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Concern

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)-detected Respiratory Pathogens during Symptomatic Study Visits in the mRNA-1273 P301 COVE Study (August 2020-June 2021)**

The presence of viral and bacterial respiratory pathogen sequences was evaluated in nasopharyngeal (NP) samples collected at "illness visits". Participants with symptoms of a respiratory illness were advised to contact the clinical site within 72 hours of onset. Such visit defined the "Day 1 Illness" date and triggered a SARS-CoV-2 molecular test, a test for additional respiratory pathogens (Biofire RP2) and a 28-day follow-up period with periodic sampling (SARS-CoV-2 RT-PCR) also at days 3, 5, 7, 9, 14, 21 and 28. Peak illness visits by participants occurred in December 2020 with 1002 visits. That month, 461 participants had positive results for SARS-CoV-2 or any other respiratory pathogen, with 78% attributed to SARS-CoV-2, and 22% resulting in human rhinovirus/enterovirus detection. On December 28$^{th,}$ following amendment of the COVE phase 3 study, the open-label phase of the trial was initiated and participants in the placebo arm started to receive the mRNA-1273 vaccine. By February 2021, the percentage of SARS-CoV-2 infection (regardless of symptoms) decreased to 39% of all positive results (n=309) and by June 2021 to 4% (n=286, Figure 1). As the rate of SARS-CoV-2 positivity decreased among participants, the detection (as a percent) of other respiratory pathogens increased. During August to December 2020, 54% of all positive illness visits were attributed to SARS-Cov-2 infections, and 46% to other respiratory pathogens, mainly rhinovirus/enterovirus infections. From January to June 2021, 70% of all positive illness results were attributed to other respiratory pathogens and not to SARS-COV-2 infections, the diversity of which increased through the spring months. There were 23 cases of co-infection with both SARS-CoV-2 and another respiratory pathogen during October 2020 to May 2021 in which participants tested positive for both. The majority (19/23) were co-infections with human rhinoviruses/enteroviruses, and there were 3 cases of co-infection with a seasonal Coronavirus strain and 1 with human parainfluenza virus type 3 (HPIV3).

**Figure 1. Respiratory pathogens detected in the COVE study per month.**
Respiratory pathogen sequences detected during August 2020 through June 2021 in samples from all participant illness visits: (A) Number of positive samples, (B) Percent of detected pathogen.

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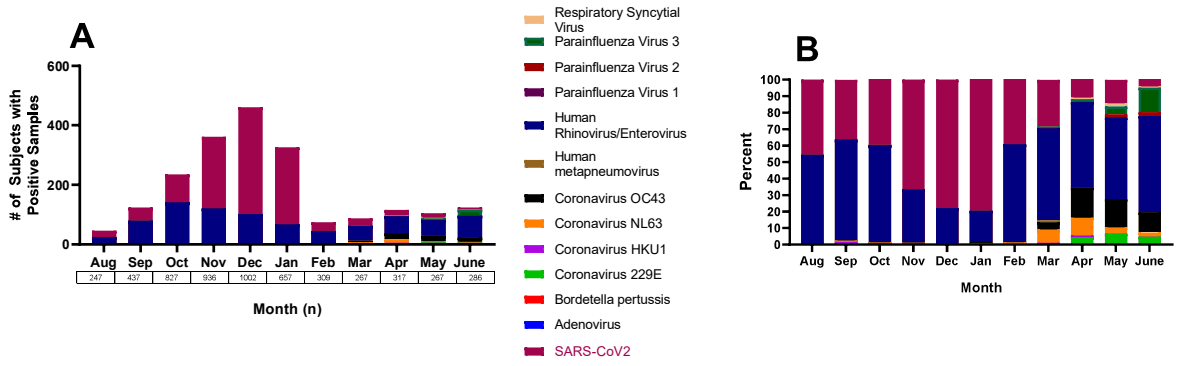


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**Effect of Vaccination with mRNA-1273 on SARS-CoV-2 Viral Load and Detectability of Infection**

Viral load was assessed in the adjudicated Covid-19 cases cohort of participants in the per-protocol (PP) population during the blinded phase of the COVE study (Figure 2).(1) The analysis population is comprised of participants in the PP Set who were SARS-CoV-2 negative by both binding antibody against nucleocapsid (bAb against NP, ROCHE Elecsys) and reverse transcriptase polymerase chain reaction (RT-PCR) at baseline and day 29, and negative bAb against NP at day 57. The baseline characteristics were generally balanced for the two treatment groups.

There were a total of 799 adjudicated cases starting 14 days after dose 2 in the PP Set in the study, (745 in placebo and 56 in the mRNA-1273 groups) and out of the 799 cases, 778 (723 in placebo and 55 in mRNA-1273) at day 29. Of these, 701 cases had negative RT-PCR and negative bAb against NP at day 29, and negative bAb against NP at day 57, and thus were used for the analysis of viral load data. Among these 701 adjudicated Covid-19 cases, 18 did not have a nasopharyngeal (NP) swab result, eight participants had an NP swab result of detected but no quantitative result available, and an additional 24 had a negative NP swab result but subsequent positive saliva results. These participants were excluded from the viral copy modeling, thus a total of 651 participants (610 placebo and 41 mRNA-1273 recipients) were included in this analyses (see Figure 2).

From illness day 1 through 9, the number of viral copies detected in the placebo arm were significantly higher than those in the mRNA-1273 arm (Figure 3, Panel A). Figure 3 panel B provides the difference and 95% confidence interval between the two arms, showing almost a 100-fold (2 log10) reduction in viral copies at day 1, and a 10-fold (1 log10) reduction at day 9, all of which were statistically significant.

**Figure 2. Analysis Population.**

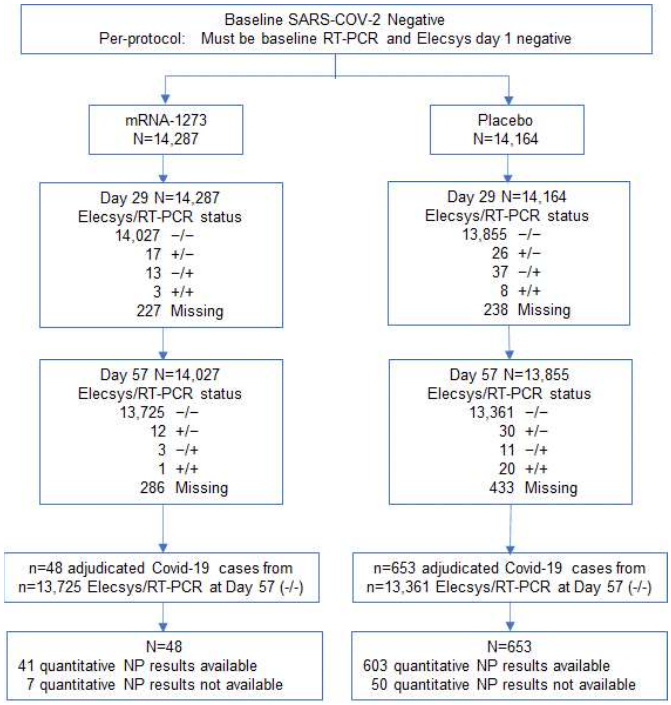


**Figure 2 legend. Analysis population for viral load analysis.** Included in the analysis were participants in the per-protocol population who were SARS-CoV-2 negative by both binding antibody against nucleocapsid (ROCHE Elecsys) and RT-PCR at baseline and day 29, and negative for binding antibody against nucleocapsid (ROCHE Elecsys) at day 57 (1). The analysis was limited to adjudicated Covid-19 cases in the blinded portion of the study, i.e. earlier of unblinding or data cutoff date of 26-Mar-2021 based on a database lock which occurred on 04-May-2021.(1)

**Figure 3. Reduction in SARS-CoV-2 viral load with mRNA-1273 compared with placebo.**

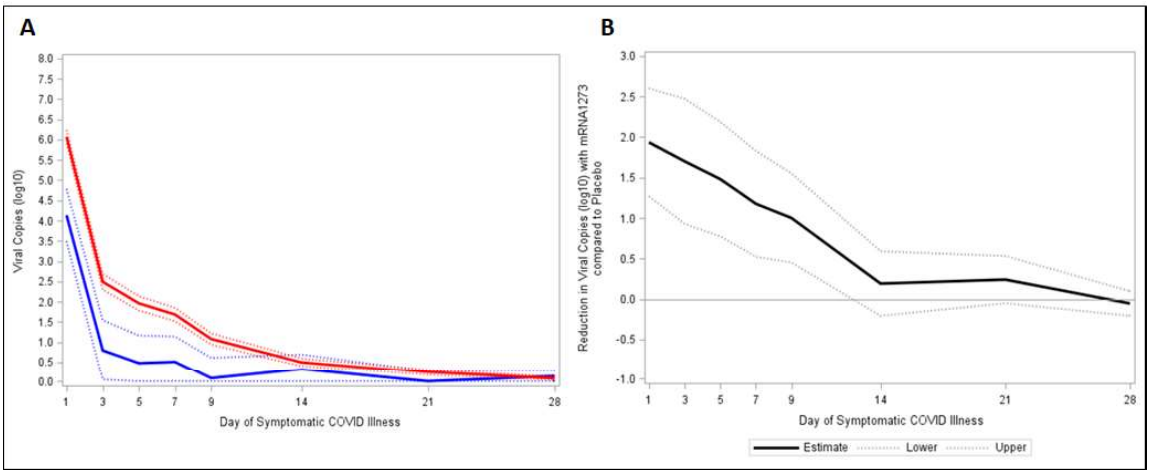


**Figure 3 legend: SARS-CoV-2 Viral load (log10).** Mixed model repeated measures (MMRM) analysis was performed comparing absolute and change from baseline log10 viral load between vaccinated and placebo participants from the NP swabs from day 1 of illness through saliva sample days 3, 5, 7, 9, 14, 21, and 28 of illness. **A:** Solid lines represent placebo (Red) and mRNA-1273 (Blue), while dotted lines correspondingly denote 95% confidence intervals. **B:** Difference between the mRNA-1273 and placebo subjects in viral copies (log10) black solid line, and 95% CI in gray.

**Spike-associated SARS-CoV-2 Variants Detected in COVE mRNA-1273 Trial**

We attempted to obtain sequence information from all available SARS-CoV-2 RT-PCR positive NP samples collected from July 2020 through May 2021 from participants in the blinded portion of the COVE trial. Sequence data of the spike gene were generated from 832 different samples, corresponding to 791 trial participants (720 from placebo recipients, 71 from mRNA-1273 recipients; Figure 4 panel A). For 41 participants we sequenced more than one sample within the illness period. The analysis of these cases is still ongoing and will be a subject of additional analyses/reporting at a later date. To assess the relative prevalence of key variant lineages detected in the clinical dataset, Pango lineages (3) were inferred for each isolate based on amino acid mutations detected in the spike gene. The prevalence of selected lineages were then compared to those from a United States time-matched subset of the Global Initiative on Sharing All Influenza Data (GISAID) database (4) (supplementary Table S1). This comparison revealed that the sequences detected in clinical case samples were essentially a

representation of the circulating strains in the US during the trial, with similar frequencies between the clinical and GISAID subset (Figure 4B and 4C).

**Figure 4. Spike sequence-associated lineages found among COVE trial participants**

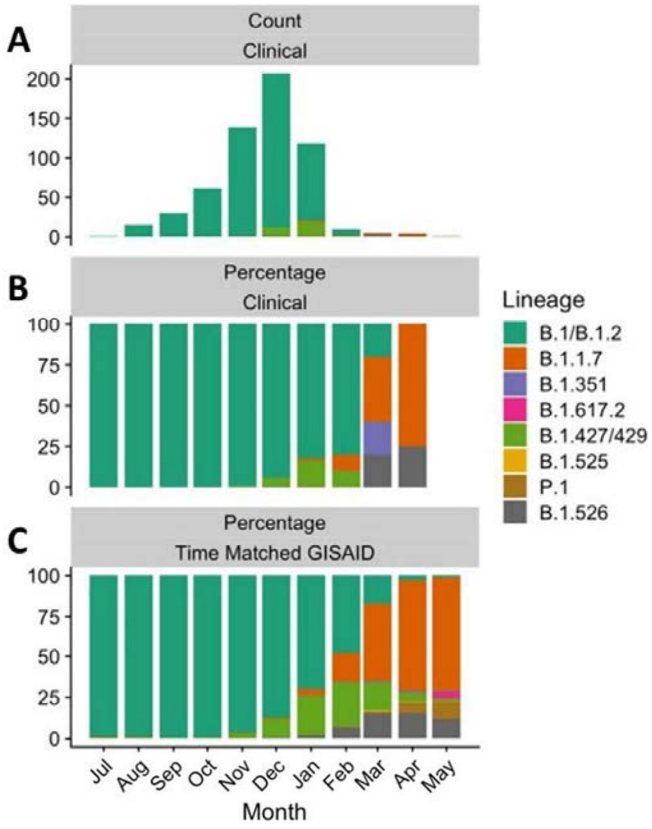


**Figure 4 legend: Spike sequence-associated lineages found among COVE trial participants.** Summary of spike sequence-associated lineages found among COVE trial participants (regardless of symptoms) from July 2020 to May 2021. Y axis: Number of sequences, X axis: Month. A: Number of sequences in the clinical dataset, B: Percent of assigned lineages in the clinical data set, C: Percent of lineages circulating in the US in the same period (time-matched sequence set) obtained from GISAID (4, 5).

**SARS-CoV-2 VOC Among Adjudicated Covid-19 cases in the COVE Trial and Vaccine Efficacy**

Of the total adjudicated Covid-19 cases started 14 days after dose 2 in the per-protocol group of the COVE trial during the blinded phase with sequence data, 20 (2.8%) were attributed to VOC or VOI in the placebo group and 3 (4.2%) in the mRNA-1273 group (Table S1). Of these, 15 (2.1%) were CA variants in the placebo group and 3 (4.2%) in the mRNA-1273 group, including 9 (1.3%) B.1.429 and 6 (0.8%) B.1.427 variants in the placebo and 3 (4.2%) B.1.429 in the mRNA-1273 groups. There were also 2 (0.3%) B1.117 variants in the placebo group and none in the mRNA-1273 group.

An exploratory analysis of variant-specific vaccine efficacy (VE) was performed for variants first detected in California as the total number of such Covid-19 cases was ≥10 using the competing risk method to estimate the VE of mRNA-1273. Specifically, Fine and Gray's (FG) sub-distribution hazard model was used and Covid-19 cases with variants other than the specific variant were considered as competing risk. Exploratory analyses of VE against VOC and VOI were also performed given the interest in these variants.

The VEs (95% CI) of mRNA-1273 to prevent Covid-19 were 82.4% (40.4-94.8) for VOC, 100.0% (100.0-100.0) for VOI, and 81.2% (36.1-94.5) for variants detected in California and 68.9% (-12.8-91.4) for the B.1.429 variant (Table 2).

**Table 2. Exploratory Analysis of Vaccine Efficacy Against Variants in the COVE Trial - Per-Protocol Set**

| Covid-19 Variant Cases in COVE | Placebo N=14164 | mRNA-1273 N=14287 |
|---|---|---|
| Covid-19 adjudicated cases n (%) | 744 | 55 |
| Vaccine Efficacy Based on Hazard Ratio (95% CI)[†] | | 93.2 (91.0-94.8) |
| Incidence Rate per 1,000 Person-Years (95% CI)[‡§] | 136.6 (127.0-146.8) | 9.6 (7.2-12.5) |
| Covid-19* cases with VOC, n (%) | 16 (0.1) | 3 (0) |
| Number with competing events, n (%) | 728 (5.1) | 52 (0.4) |
| Vaccine Efficacy Based on Hazard Ratio (95% CI)[†] | | 82.4 (40.4-94.8) |
| Incidence Rate per 1,000 Person-Years (95% CI)[‡§] | 2.9 (1.7-4.8) | 0.52 (0.11-1.5) |
| Covid-19* with VOI, n (%) | 2 (0) | 0 |
| Number with competing Events, n (%) | 742 (5.2) | 55 (0.4) |
| Vaccine Efficacy Based on Hazard Ratio (95% CI)[†] | | 100.0 (100.0-100.0) |
| Incidence Rate per 1,000 Person-Years (95% CI)[‡§] | 0.38 (0.04-1.4) | - |

| | | |
|---|---|---|
| Covid-19* first detected in California¶ n (%) | 15 (0.1) | 3 (0) |
| Number with competing events, n (%) | 729 (5.1) | 52 (0.4) |
| Vaccine Efficacy Based on Hazard Ratio (95% CI)† | | 81.2 (36.1-94.5) |
| Incidence Rate per 1,000 Person-Years (95% CI)‡§ | 2.8 (1.5-4.5) | 0.52 (0.11-1.5) |
| Covid-19* first detected with B.1.429, n (%) | 9 (0.1) | 3 (0) |
| Number with competing events, n (%) | 735 (5.2) | 52 (0.4) |
| Vaccine Efficacy Based on Hazard Ratio (95% CI)† | | 68.9 (-12.8-91.4) |
| Incidence rate per 1,000 Person-Years (95% CI)‡§ | 1.7 (0.8-3.2) | 0.52 (0.11-1.6) |

VOC=variant of concern (includes B.1.427, B.1.429, and P.1); VOI=variant of interest (includes P.2). *Covid-19 cases with variant lineages other than the specified variant(s) assessed are considered as competing events. Based on participants with adjudicated assessment starting 14 days after second injection in the per-protocol set. †Vaccine efficacy, defined as 1 - hazard ratio (mRNA-1273 vs. placebo), and 95% CI are estimated using Fine and Gray's sub-distribution hazard model with disease cases as competing events and with the treatment group as a covariate, adjusting for stratification factor. ‡Person-years defined as the total years from randomization date to the earliest of the date of symptomatic SARS-CoV-2 infection, the date of asymptomatic SARS-CoV-2 infection, last date of study participation, or efficacy data cutoff date, whichever was earlier. Incidence rate is defined as the number of participants with an event divided by the number of participants at risk and adjusted by person-years (total time at risk) in each treatment group. The 95% CI was calculated using the exact method (Poisson distribution) and adjusted by person-years. ¶Both B.1.427 and B.1.429 variants. Note that variants B.1.427 and B.1.429 were originally categorized as VOC but are reconsidered as VOI by the CDC and in this analysis were included in the VOC group.

## Discussion

The preliminary analysis of respiratory pathogens, SARS-CoV-2 viral copy number, and circulating viral variants in the mRNA-1273 Phase 3 COVE trial are reported.(1)

During the blinded, randomized, portion of the trial, the majority of symptomatic respiratory cases were caused by SARS-CoV-2 infection and mostly among placebo recipients. This finding further confirms the high efficacy of mRNA-1273 vaccine in the prevention of symptomatic, molecularly confirmed, Covid-19 disease. Correspondingly, by February 2021 and through June 2021, the percentage of SARS-CoV-2 infection among trial participants continued to drop, while the presence (percent detected) of non-SARS-CoV-2 respiratory pathogens increased. This observation was interpreted as another signal of the high efficacy of the vaccine now reflecting the end of the blinded phase of the trial and the completion of the cross-over of placebo recipients to mRNA-1273.

Whether mRNA-1273 vaccination had a discernible effect on the reported RT-PCR cycle threshold (Ct) values and associated SARS-CoV-2 viral loads was also explored. We found a highly significant reduction in both viral load at day 1 of reported disease onset, and detectability of viral shedding up to day 9. This observation is highly relevant due to its potential implications on the transmissibility of the SARS-CoV-2 virus.

Although the transmission dynamics of Covid-19 are still under study, the observed estimated 100-fold reduction on day 1 illness viral load, with statistically significant reduction in copies through day 9, may provide a significant reduction in Covid-19 disease spread, even in vaccine breakthrough cases.

With the analyses of the spike gene sequences obtained from Covid-19 cases in the trial we were able to explore a key aspect: albeit the high efficacy of the vaccine demonstrated during a period of relative lower diversity of circulating strains (July 2020 through December 2020), the data also suggest maintained high efficacy throughout the open-label phase of the trial (approximately February through May 2021) which encompasses not only the peak of the US epidemic (January 2021), but also the emergence of B.1.1.7, B.1.351, P.1, and B.1.427/429 variants.

In the trial as a whole, or when looking at each phase (blinded or open-label), the sequences found among trial participants match the respective epidemiological patterns prevalent in the US during the same timeframe. While more sophisticated sequence analyses are underway, we have not yet observed evidence of a vaccine-resistant strain driven by the vaccination campaign.

Supported by the sequence data, an exploratory analysis of the VE of mRNA-1273 against circulating variants was performed. Against the small number of VOIs circulating through April 2021, the mRNA-1273 vaccine maintained 100% efficacy. Of all VOC, only a sufficient number of cases was accrued to allow for a formal analysis of the variant first detected in California (B.1.427 and B.1.429 variants) with a VE (95% CI) of 81.2% (36.1-94.5) against both California variants B.1.427 and B.1.429. These data support that Moderna's mRNA-1273 vaccine is a highly efficacious vaccine, and indicate the potential for variants to generate vaccine breakthroughs as more aggressive and divergent variants emerge amidst waning immune response following months after vaccination.

**Limitations:** This study has several limitations. The analyses presented here are exploratory in nature, based on data that continue to accrue over time. The data will be subject to future updates. The sequence data comes also with known caveats, some demonstrated through this work. For example, the fact that the vaccine has a marked

effect on lowering SARS-CoV-2 viral loads hampered our efforts to generate an unbiased sequence data set. Our sequencing efforts were performed in a "blinded pipeline" with the performing team not being aware of the treatment received by the participant. Yet, the success rate in obtaining good quality sequences from samples obtained from vaccine breakthrough cases was 50% or less, while it was over 80% for placebo-originated samples. While this is a clear, unavoidable bias in the available sequence data, it is one we anticipated as it is widespread among all the teams working in this field. Conversely, we have not seen a highly-aggressive variant that can cause both a higher number of cases among vaccine recipients and higher viral loads in the same population. Such a variant would be anticipated to yield more readily sequenceable samples and hence there would be a detection bias in its favor.

Finally, we must also recognize that the sample size of variants detected was small and thus makes it difficult to accurately assess the proportions of viral variants and the associated VE. Nonetheless, the variants detected in the study were representative of circulating variants over the time course of the study.

In summary, the preliminary analysis of the viral load and the circulating viral variants in the mRNA-1273 Phase 3 COVE trial suggests that vaccination with Moderna's mRNA-1273 vaccine leads to a significant reduction of the SARS-CoV-2 viral load and shedding period. mRNA-1273 vaccine showed remarkable efficacy through the observation period of the blinded phase and continues to have a marked effect in reducing symptomatic Covid-19 cases among vaccine recipients. The shifting landscape in terms of new emerging variants and the potential waning of the immune response suggest that a continuation of these studies is needed.

**Annex**

**Estimates of SARS-CoV-2 Variants circulating in the US Prevalence at the time of analysis**

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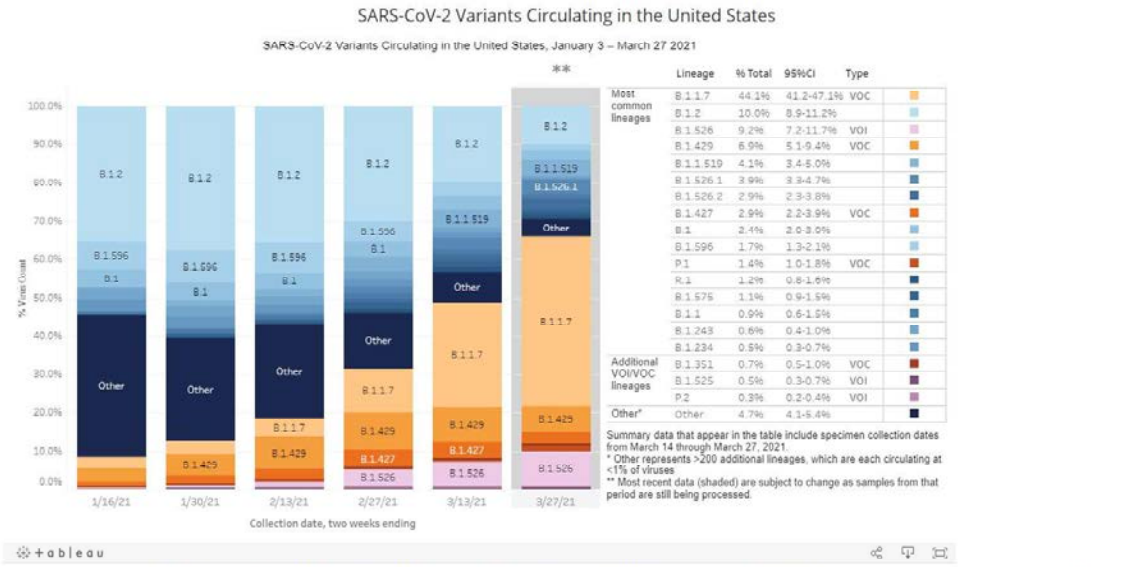
The data below shows the estimated biweekly prevalence of the most common SARS-CoV-2 lineages circulating in the United States, based on >40,000 sequences collected through CDC's national genomic surveillance since Dec 20, 2020 and grouped in 2-week intervals. Variant proportions in Figure 1 are adjusted using statistical weighting† to correct for the non-random sampling of sequencing data over time and across states and to provide more representative national estimates.

**Figure S1: SARS-CoV-2 Circulating in the US January-March and March-June 2021**

**A: January-March 2021**




† Estimated weights come from laboratory data providing the number of reverse transcription polymerase chain reaction (RT-PCR) tests and number of positive RT-PCR test results stratified by state, specimen collection date, and by genomic surveillance data source, using a survey-design-based approach. COVID-19 laboratory data sources include commercial and reference laboratories, public health laboratories, hospital laboratories, and other testing locations

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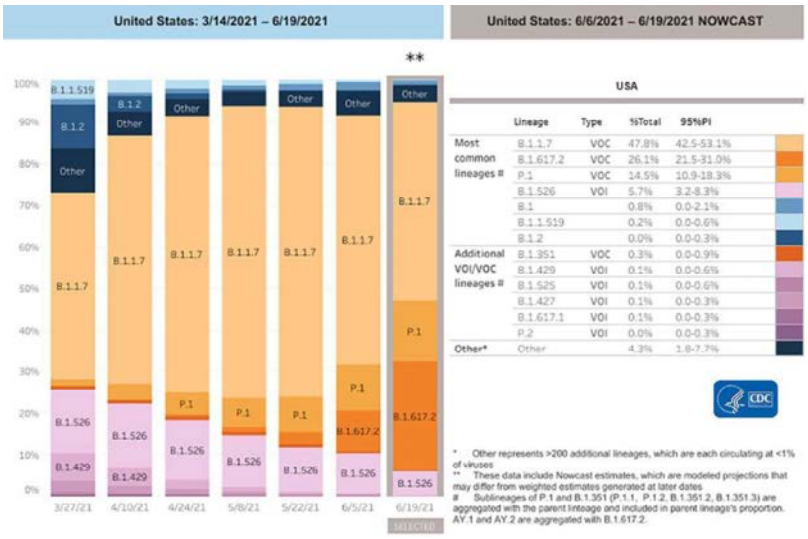
**B: March-June 2021**




(source: https://covid.cdc.gov/covid-data-tracker/?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fcases-updates%2Fvariant-proportions.html#variant-proportions)

**Table S1: Summary of COVID-19* based on Adjudication Committee Assessments Starting After Randomization by Variant Groups, Per-Protocol Set**

| Covid-19 n (%) | Placebo N=14164 | mRNA-1273 N=14287 |
|---|---|---|
| COVID-19* cases, n (%) | 769 (5.4) | 56 (0.4) |
| Number of events by lineage, n (%) | | |
| B.1 | 5 (0) | - |
| B.1.1 | 1 (0) | - |
| B.1.1.128 | 1 (0) | - |
| B.1.1.186 | 2 (0) | - |
| B.1.1.207 | 1 (0) | - |
| B.1.1.222 | 8 (0.1) | - |
| B.1.1.316 | 1 (0 | - |
| B.1.1.337 | 1 (0) | - |
| B.1.1.432 | 1 (0) | - |
| B.1.1.434 | 1 (0) | - |
| B.1.1.519 | 2 (0) | - |
| B.1.2 | 394 (2.8) | 13 (0.1) |
| B.1.232 | 1 (0) | - |
| B.1.234 | 6 (0) | - |
| B.1.240 | 1 (0) | - |
| B.1.243 | 23 (0.2) | 1 (0) |
| B.1.311 | 6 (0) | - |
| B.1.349 | 1 (0) | - |
| B.1.369 | 2 (0) | - |

| | | |
|---|---|---|
| B.1.375 | 1 (0) | - |
| B.1.382 | 1 (0) | - |
| B.1.396 | 1 (0) | - |
| B.1.404 | 2 (0) | - |
| B.1.427 | 6 (0) | - |
| B.1.429 | 9 (0.1) | 3 (0) |
| B.1.517 | 2 (0) | - |
| B.1.526.3 | 1 (0) | - |
| B.1.544 | 2 (0) | - |
| B.1.551 | 1 (0) | - |
| B.1.561 | 5 (0) | - |
| B.1.564 | 3 (0) | - |
| B.1.587 | 8 (0.1) | - |
| B.1.595 | 2 (0) | - |
| B.1.596 | 13 (0.1) | - |
| B.1.599 | 1 (0) | - |
| B.1.605 | 1 (0) | - |
| B.1.609 | 1 (0) | - |
| NONE | 20 (0.1) | - |
| P.1 | 1 (0) | - |
| P.2 | 2 (0) | - |
| R.1 | 3 (0) | - |
| WILD TYPE | 1 (0) | - |
| By First Detected, n (%) | | |
| Brazil | 1 (0) | - |
| P.1 | 1 (0) | - |
| California | 15 (0.1) | 3 (0) |
| B.1.427 | 6 (0) | - |
| B.1.429 | 9 (0.1) | 3 (0) |
| Variant of Concern | | |
| B.1.427† | 6 (0) | - |
| B.1.429† | 9 (0.1) | 3 (0) |
| P.1 | 1 (0) | - |
| Variant of Interest | | |
| P.2 | 2 (0) | - |

*With the censoring rules for efficacy analyses. COVID-19 case is based on eligible symptoms and positive RT-PCR within 14 days. Percentages based on adjudicated cases starting 14 days post-randomization in the per-protocol set. If a participant had a positive RT-PCR test at pre-dose 2 visit (day 29) without eligible symptoms with 14 days, or positive Elecsys at scheduled visits prior to becoming a COVID-19 case, the participant was censored at the date with positive RT-PCR or Elecsys. †Now considered variants of interest by the CDC.
Table 14.2.1.1.2.1.4.1

**Detection of respiratory pathogens**

***SARS-CoV-2 RT-PCR Test (Eurofins Viracor (Kansas City, MO))***

SARS-CoV-2 specific real time reverse transcription polymerase chain reaction (RT-qPCR) assay was used to detect SARS-CoV-2 RNA in upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage (BAL) samples. This assay, performed by Eurofins Viracor Laboratories, (Kansas City, MO) provides qualitative detection of RNA from SARS-CoV-2 virus in specimens collected from individuals meeting SARS-CoV-2 virus clinical criteria (https://www.fda.gov/media/136740/download). Briefly, the SARS-CoV-2 RT-qPCR assay was performed as a multiplex reaction with the MS2 internal control assay. Oligonucleotide primers and Taqman probes are used for the detection of two regions of

the viral nucleocapsid (N) protein gene region of SARS-CoV-2 and an internal extraction and amplification control target (the RNA bacteriophage MS2) was used. The limit of detection (LOD) for this assay is determined to be 73 copies/mL for BAL, nasal wash and NP swab with a cycle threshold (Ct) of 38 being the cutoff for positive result.

***BioFire Respiratory Panel (RP2) (Eurofins Viracor (Kansas City, MO))***

Respiratory pathogens were detected using the BioFire Respiratory Panel RP2, a diagnostic multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from 20 viral and bacterial respiratory organisms. The disposable closed system pouch was run on the Filmarray® 2.0 system which lyses samples, extracts, and purifies all nucleic acids, and performs nested multiplex PCR. Endpoint melting curve data was used to detect target-specific amplicons and analyze data to generate a result for each analyte. Nasopharyngeal swabs were used as qualitative diagnostic assays for the detection of 20 different viruses and bacteria associated with respiratory tract infection. Assay results are provided as negative or positive for each pathogen in the BioFire Respiratory Panel RP2.

**Sequencing Methodology and Sequence Data Analyses Narrative**

Sequencing data was generated using three different approaches in two different laboratories.

***Spike-Gene Sequencing Assay from Eurofins Viracor (Kansas City. MO):***

Viral RNA from nasal swabs was extracted using the NucliSENS® easyMag® extraction kit. Extracted RNA was used as template in a Qiagen QIAquick One-Step Reverse Transcription-PCR (RT-PCR) reaction for cDNA synthesis using SARS-CoV-2 S gene Conventional RT-PCR primer mixes. The Agilent 2200 or 4200 TapeStation in conjunction with D5000 ScreenTapes, D5000 reagents, and the TapeStation Analysis software was used to assess post-amplified and purified PCR reactions for the presence, size, and concentration of any products generated. Library preparation was

performed using Illumina Nextera XT Library Prep Kit. The Agilent 2200 or 4200 TapeStation in conjunction with D5000 ScreenTapes, D5000 reagents and the TapeStation Analysis software was used to assess purified libraries for presence, average fragment size, and concentration of the fragment distributions generated. Analysis of next generation sequencing data for the SARS-CoV-2 S gene NGS assay was done using Qiagen CLC Genomics Workbench v20.0.1 using NC_045512.2 as the reference strain. The custom workflow in CLC Genomics Workbench processed the sequencing data as follows: paired fastq files were imported, primer sequences were trimmed from 5'-ends of reads, reads were mapped to the full SARS-CoV-2 reference genome (NC_045512.2), single nucleotide and insertion/deletion variants relative to reference were called and annotated, and a consensus sequence of the spike gene (bases 21615 to 25436) was generated. The analysis workflow reported annotated variant tables, spikegene coverage tables, and spike-gene consensus sequences. Upon TapeStation D5000 assessment and subsequent analysis of data using the TapeStation Analysis software, if the viral load was insufficient to obtain a correct band for the SARS-CoV-2 S gene targets (S1 (1026bp), S2 (893bp), S3 (1178), and S4 (1264bp), these results were considered negative. Positive results for the RT-PCR reactions were identified by 1) the presence of a band at the appropriate size for the SARS-CoV-2 S gene PCR products (S1 (1026bp), S2 (893bp), S3 (1178), and S4 (1264bp) relative to the D5000 ladder and 2) a peak table reporting a concentration for the specific bands for the sample.

SARS-CoV-2 S Gene NGS runs using MiSeq v2 chemistry reagents running paired-end 2 x 151 reads must exhibit the following criteria: Cluster densities approximating 600 - 1,200K/mm2; >80% of bases called exhibit Q-scores ≥30. Individual library sequence quality metrics were assessed by referencing the sequencing quality reports generated by analysis through the Qiagen CLC workbench program. For SARS-CoV-2 S gene NGS runs, up to 24 libraries can be sequenced on a single flow cell and the number of reads displayed in the trim summary section of the trim report should be ≥50,000 reads for each amplicon (prior to the reads being trimmed). Nucleotide positions between 21615-25436 should have a coverage of ≥100.

SARS-CoV-2 whole virus was used as a positive control. The LOD with all replicates for all 4 (S1-S4) amplicons was 6,667 copies/mL.

***Spike-Gene Sequencing Assay from Monogram Biosciences (LabCorp, South San Francisco, CA):***

Viral RNA from nasal swabs stored in UTM is extracted using the Kingfisher Flex platform. Following extraction, nested RT-PCR reactions are performed to amplify the entire SARS-COV-2 Spike (S) coding region. The resulting amplicon is purified and normalized before undergoing NGS library preparation (Kapa). Resulting barcoded libraries are normalized, pooled, and undergo 2x150 bp paired end sequencing on the Illumina MiSeq platform. FASTQ files are analyzed using a semi-automated bioinformatics pipeline. Briefly, reads are quality trimmed and overlapping paired reads are joined. Reads are aligned in a codon aware manner that maintains reading frame through the S gene. Codon and amino acid variants are determined and reported along with consensus sequences. Results are reviewed to ensure appropriate coverage levels and quality metrics were obtained for each run and each individual sample.

A sensitivity to amplify of ~1,000 copies/mL and a minor variant detection threshold of 3% is validated.

***Whole Genome Sequencing Assay (WGS) from Eurofins Viracor (Kansas City, MO):***

Viral RNA from nasal swabs is extracted using the Kingfisher Flex platform and GSD NovaPrime® RNA extraction kit. Extracted RNA is used as template in a one-step RT-PCR for cDNA synthesis. Each cDNA is subjected to amplification using ARTIC SARS-CoV-2 Primer Pools. These primer pools were designed to amplify approximately 90 amplicons each with each amplicon averaging ~400bp. Mapping these amplicons to a reference sequence illustrates the 'tiled' approach used for primer design resulting in coverage of the entire SARS-CoV-2 genome. Purification of the ARTIC PCR reactions was performed manually with Beckman-Coulter SPRIselect magnetic beads. The concentration of amplified amplicons in each sample was quantified using the Qubit FLEX fluorometer. Preparation of libraries was performed using the NEBNext Ultra II

FS library prep kit in conjunction with the BRAVO liquid handling platform. Automated purification of the library reactions was performed using the Agilent BRAVO liquid handler and Beckman-Coulter SPRIselect magnetic beads. The fragment size distribution of the final pooled library was confirmed using the Agilent TapeStation 4200 or ThermoFisher BioAnalyzer 2100 DNA fragment analyzer prior to preparation for sequencing. Pooled libraries were denatured and sequenced on the NextSEQ 500 or 550 instrument using a NextSEQ Mid Output 500/550 flow cell and reagents running a 2x150 cycle paired-end sequencing protocol.

A Twist SARS-CoV2 RNA positive control was processed in parallel with each verification run for positive control of the reverse transcription, ARTIC PCR amplification, and library preparation. The LoD for SARS-CoV2 WGS was determined to be 100 copies/ARTIC PCR assay reaction.

**Variant Data Analyses**

SARS-CoV2 variants in the study population were assessed based on amino acid mutations in the spike protein relative to the reference strain (spike mutations). For each of the three sequencing datasets, (spike-gene sequencing from Eurofins Viracor; spike-gene sequencing from Monogram Biosciences (LabCorp, South San Francisco, CA), and whole genome sequencing from Eurofins Viracor), spike protein amino acid mutations were acquired directly from the sequencing service provider. For each specimen, a single spike haplotype was designated as the ordered set of spike mutations. The same analysis was performed for the global SARS-CoV-2 genomic database available from the Global Initiative on Sharing All Influenza Data (GISAID).(4, 5) Pango Lineages (3) were inferred for the clinical specimens in two ways. First, they were inferred from the lineage annotations of matching spike haplotypes included in the GISAID database. In cases when a single spike haplotype was associated with more than one Pango Lineage in GISAID, the dominant lineage was used. In addition, Pango Lineages were inferred based on the presence of core backbone mutations from CDC VOIs and VOCs (2) in a specimen's spike haplotype. To assess the relative prevalence of select lineages, the full clinical dataset and full GISAID dataset were subset to include only records annotated with the selected lineages. Prevalence for each selected

lineages was then computed as the percentage of total records for a given month within each data subset.

**Variant-specific vaccine efficacy of mRNA-1273 - Statistical Analysis Method**

For specific variants with sufficient number of variant cases during the blinded phase of the study, competing risk method was used to estimate the vaccine efficacy of mRNA-1273, specifically, Fine and Gray's (FG) sub-distribution hazard model was used. COVID-19 cases of specific variant were considered as cases, and COVID-19 cases of variant other than that of interest were considered as competing events in this analysis.

**Viral load assessment**

***Study design and population***

The study population for analysis of viral load were those participants in the per-protocol population who had binding antibody (Elecsys; nucleocapsid protein [NP]) negative baseline values (baseline SARS-CoV-2 negative), and also had follow-up Elecsys-negative values at days 29 and 57 and RT-PCR-negative results at baseline, day 29, and at day 57 (only Elecsys). The analysis period was limited to the blinded portion of the study and the data cut-off date was 26-March-2021 (or earlier unblinding).

***Statistical Analysis***:

For the cohort of Covid-19 adjudicated subjects, the day 1 illness NP swab and the days 3, 5, 6, 9, 14, 21, and 28 of illness saliva specimens were matched to assess the qualitative and quantitative result for each. Conversion from cycle time (CT) to viral copies for the quantitative RT-PCR were Log10ViralCopies=CT -40.9578)/-3.3385 for swabs (day1) and Log10ViralCopies = CT -41.0349)/-3.3346) for saliva (days 3, 5, 7, 9, 14, 21 and 28). If the qualitative result was negative, the log10viral copies was assumed to be 0. A mixed model repeated measures (MMRM) analysis was performed comparing absolute and change from baseline log10 viral load between vaccinated and placebo participants from the NP swab day 1 of illness through saliva sample days 3, 5, 7, 9, 14, 21, and 28 of illness. Only subjects with a quantitative result in the day 1 illness NP swab were included in the MMRM modeling. Any MMRM result estimate under 0 copies, was truncated at 0. There was no imputation for missing data.

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