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COVID-19 Variants & Disease Variability

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SARS-CoV-2 Variants

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus of the genus Betacoronavirus that uses a glycoprotein (spike protein) to bind to the angiotensin-converting enzyme 2 (ACE 2) receptor (1). After binding, a serine protease is activated which enables viral entry into the cell. The Neuropilin-1 receptor, which is expressed on neurons and cells within the respiratory tract and blood vessels, binds the S1 subunit of the SARS-CoV-2 spike protein, increasing infectivity (2). The SARS-CoV-2 genome consists of 14 open reading frames (ORFs) including 4 that encode structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) (3). The S protein is very flexible and hinges at three points permitting swaying and rotation (1). The S protein contains 2 subunits: S1 and S2. SARS-CoV-2 variants tend to have mutations in S1 where the receptor-binding domain is located, including Alpha and Delta (1,4). The Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and European Centre for Disease Prevention and Control list current and former SARS-CoV-2 Variants of Concern (VOC) (Table 1) and Variants of Interest (VOI) (Table 2) and their S protein mutations. A VOC has increased transmissibility, impact on immunity, and/or increased disease severity (5-7). A VOI has genetic changes and preliminary evidence that would suggest potential impact on transmissibility, immunity, and/or disease severity (5-7). A full list of all detected variants is maintained by the Pango Network (8-9).

Furin cleavage of the S protein occurs at the junction of S1 and S2 subunits at a site containing 5 amino acids: proline-arginine-arginine-alanine-arginine. Most mutations at this 5 amino acid furin cleavage site inhibit viral entry into human cells (1, 4, 10, 11). However, the Delta spike mutation, P681R, located at the furin cleavage site, enhances S1/S2 cleavage and increases infectivity (4). The most recently identified VOC, Omicron, has acquired multiple mutations, including three in the furin cleavage site (5; Table 1).

Table 1. WHO SARS-CoV-2 Variants of Concern

WHO Label	Pango lineage	S Protein mutations (Of parental variant)	Date of WHO VOC Designation
1. Alpha	B.1.1.7	N501Y, D614G, P681H	18-Dec-2020
2. Beta	B.1.351	K417N, E484K, N501Y, D614G, A701V	18-Dec-2020
3. Gamma	P.1	K417T, E484K, N501Y, D614G, H655Y	11-Jan-2021
4. Delta	B.1.617.2	L452R, T478K, D614G, P681R	11-May-2021 (Previous VOI designation 4-Apr-2021)
5. Omicron	B.1.1.529	67V, Δ69-70, T95I, G142D, Δ143-145, Δ211-212, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	26-Nov-2021

*Includes all descendent lineages such as AY.4.2, also known as "Delta plus", or variants of the Omicron lineage, BA.1, BA.2, and BA.3. For full lineage list see cov-lineages.org.

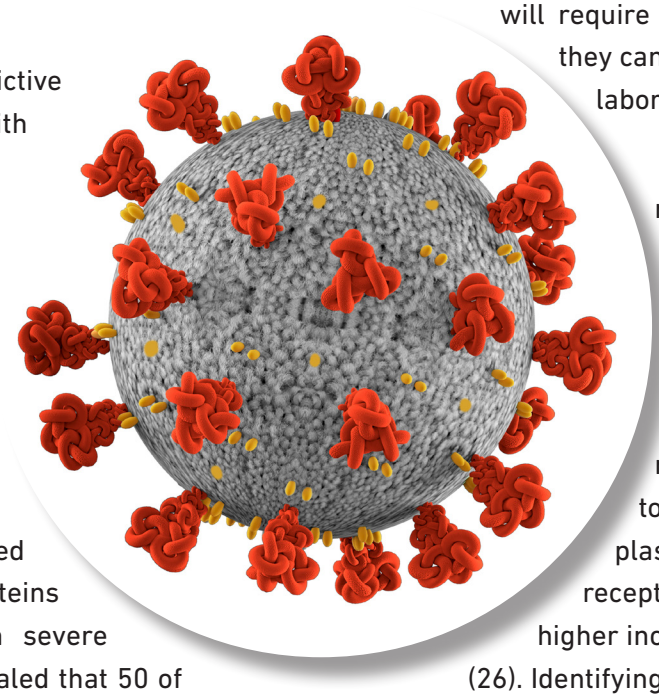
Table 2. WHO SARS-CoV-2 Variants of Interest

WHO Label	Pango lineage	S Protein mutations (Of parental variant)	Date of WHO VOC Designation
Current VOIs			
1. Lambda	C.37	L452Q, F490S, D614G	14-June-2021
2. Mu	B.1.621	R346K, E484K, N501Y, D614G, P681H	30-Aug-2021
De-escalated VOIs			
3. Zeta	P.2	E484K, D614G	17-Mar-2021 Reclassified 17-Aug-2021
4. Theta	P.3	E484K, N501Y, D614G, P681H	24-Mar-2021 Reclassified 17-Aug-2021
5. Epsilon	B.1.427 B.1.429	L452R, D614G	5-Mar-2021 Reclassified 9-Nov-2021
6. Eta	B.1.525	E484K, D614G, Q677H	17-Mar-2021 Reclassified 22-Dec-2021
7. Iota	B.1.526	E484K, D614G, A701V	24-Mar-2021 Reclassified 22-Dec-2021
8. Kappa	B.1.617.1	L452R, E484Q, D614G, P681R	4-Apr-2021 Reclassified 29-Dec-2021

*Includes all descendent lineages. For full lineage list see cov-lineages.org.

COVID-19 Stages and Progression

Coronavirus disease 2019 (COVID-19), the disease caused by SARS-CoV-2 infection, is highly variable in patients. Clinically, four stages of COVID-19 have been identified: mild, moderate, severe and critical (12,13). The typical findings for each stage are listed in Table 3. A fifth stage, sometimes referred to as "long COVID," involves persistent symptoms that last weeks to months after the initial recovery (12, 13). The five most common persistent symptoms in these patients are fatigue (58%), headache (44%), attention disorders (27%), hair loss (25%) and dyspnea (24%) (14).



The search for predictive biomarkers associated with disease severity is ongoing (15-31). Shen *et.* al. (16) used proteomics to study serum protein differences among COVID-19 patients of varying disease severity compared to normal control individuals. In this study, 105 proteins were differentially expressed in COVID-19 sera compared to controls. Of these, 93 proteins showed specific modulation in severe patients. Pathway analyses revealed that 50 of these proteins belonged to 3 major pathways: activation of complement, macrophage function and platelet degranulation. Silvin *et.* al. (17) used high-dimensional flow cytometry and single-cell RNA sequencing of peripheral blood cells from severe COVID-19 patients and detected the disappearance of non-classical CD14low CD16high monocytes and the accumulation of HLA DRlow classical monocytes. They also reported the massive release of calprotectin in the blood of the severe COVID-19 patients.

Nasopharyngeal (NP) swabs from SARS-CoV-2 infected patients and controls have been evaluated by proteome profiling, which identified 577 proteins that were upregulated in SARS-Cov-2 positive specimens (18). Similar studies using transcriptome profiling of SARS-CoV-2 positive NP swabs and whole-blood samples demonstrated a 19-gene diagnostic classifier with >85% overall accuracy (80% sensitivity and 90% specificity) (19). These genomic and proteomic approaches to identifying potential clinically useful biomarkers will require further development before they can be utilized in routine clinical laboratory testing.

Multivariant analysis revealed that elevated lactate dehydrogenase (LDH) and low albumin were significantly associated with death. Lower LDH values were associated with lower risk of needing admission to intensive care (24). Higher plasma soluble urokinase receptor values associated with a higher incidence of acute kidney injury (26). Identifying a reliable set of biomarkers for stratifying patient responses to COVID-19 would provide novel therapeutic targets and inform selection of current treatment strategies, an effort that is an ongoing research initiative (29, 31). One confounding variable is that many of the biomarkers identified for COVID-19 are also biomarkers of comorbidities that impact COVID-19 disease progression (31). Table 4 summarizes some of the biomarkers that have been identified as increased or decreased in the four stages of COVID-19 disease.

Table 3. Stages of COVID-19 infection

Stage	Findings
1. Mild	Upper respiratory tract infection
2. Moderate	Epithelial cells express antiviral/interferon response genes, onset of dyspnea (31%), cough (82%), fever (83%)
3. Severe	Muted antiviral responses, cytokine storm with increase IL-6, IL-9 and INF-alpha, hyper inflammatory state, DIC, pulmonary embolism, high blood pressure, respiratory rate ≥30/min
4. Critical	Respiratory failure requiring mechanical ventilation, shock and end organ failures including thrombotic complications, myocardial dysfunction and arrhythmias, acute coronary syndrome, acute kidney injury, liver injury, GI symptoms, hyperglycemia and ketosis: death or recovery

DIC: disseminated intravascular coagulation
IL: interleukin

Table 4. COVID-19 Biomarkers

Stage	Findings
1. Mild	Increased • C-reactive protein, AST, ALT, GGT, LDH, transferrin, cortisol, ferritin, ESR, serum amyloid A Decreased • White blood cell count, neutrophils, lymphocytes, platelets
2. Moderate	Increased • CK and/or CKMD, BNP, myoglobin, troponin T and I Decreased • White blood cell count, neutrophils, lymphocytes, platelets
3. Severe	Increased • Prothombin time; d-dimer; cytokine storm: IL-6, IL-9, IFN gamma and TNF-alpha; circulating endothelial cell; IP-10; sVCAM-1, calprotectin, fibrinogen, procalcitonin, granulocyte stimulation factor Decreased • Lymphocytes; Type I interferon deficiency: IFN beta, IFN alpha; platelets, albumin
4. Critical	Increased • Bilirubin, BUN, creatinine, soluble urokinase receptor, LDH very high Decreased • Natural killer cells, CD3+ T cells including T cell subsets like CD8+ T cells, albumin – very low

IP-10: interferon gamma induced protein
sVCAM-1: soluble vascular cell adhesion molecule
IL: interleukin
IFN: interferon
TNF: tumor necrosis factor
LDH: lactate dehydrogenase

Therapeutic Strategies for COVID-19

There are now multiple treatment options available for COVID-19 patients that have received either full FDA-approval or FDA Emergency Use Authorization (EUA) which are summarized in Table 5. These treatments are indicated for patients at various disease stages and/or with specific medical histories. Available treatments include antivirals which work to block viral replication and transmission, immunosuppressive drugs that block the overactive immune response characteristic of COVID-19, monoclonal antibodies that target the SARS-CoV-2 S protein, and convalescent plasma collected from recovered patients (32). Each of these treatments are effective at different disease stages and have varying efficacy against SARS-CoV-2 variants.

The only FDA-approved therapy is Veklury, also known as remdesivir, which is approved for treatment of patients hospitalized with COVID-19 that are over the age of 12 (33). A previous EUA had allowed the use of remdesivir for a broader group of patients. Remdesivir is an antiviral drug that blocks the RNA-dependent RNA polymerase used by coronaviruses for replication, including SARS-CoV-2 (34).

Olumiant (baricitinib), originally approved for the treatment of rheumatoid arthritis (RA), received EUA in 2020 to be used alongside remdisivir, showing better clinical results compared to remdesivir alone (35). The EUA was amended in 2021 to also allow for Olumiant treatment without remdesivir. Olumiant is an immunosuppressive drug with anti-cytokine activity (36), with the goal of blocking the "cytokine storm" characteristic of severe COVID-19 (37). Actemra (tocilizumab) is another immunosuppressive drug originally used to treat RA that has received EUA for the treatment of COVID-19. Olumiant and Actemra are only approved for use for adult and pediatric patents, aged 2-years or older, who are receiving systemic corticosteroids and require supplemental oxygen, mechanical ventilation, or ECMO. In clinical trials, Actemra reduced mortality and likelihood of needing a ventilator in hospitalized patients (38).

Therapeutic Strategies for COVID-19. *Cont.*

There are now several monoclonal antibodies that target the SARS-CoV-2 S protein that have received EUA. This treatment is most effective when administered early in the disease course to reduce viral load. REGEN-COV is a cocktail of two monoclonal antibodies (casirivimab and imdevimab) that target different regions of the SARS-CoV-2 spike protein and resulted in reduced viral load and reduced risk of hospitalization in a Phase 3 clinical trial (39). REGEN-COV received EUA for treatment of mild-to-moderate COVID-19 in adult and pediatric patients 12 years of age or older who are at high risk of developing severe COVID-19. It is also used as a post-exposure prophylaxis to prevent COVID-19 in high-risk individuals (40). The combination of bamlanivimab and etesevimab monoclonal antibodies is also available for adults and some children with mild-to-moderate COVID-19. The FDA also granted EUA for use of the monoclonal antibody sotrovimab for adults and children over the age of 12 with mild-to-moderate COVID-19 at risk for developing severe disease (41).

Convalescent plasma from patients who have recovered from COVID-19 contain antibodies against SARS-CoV-2; it was used as an early form of treatment, and has received EUA from the FDA in August 2020. However, there are growing concerns given the elevated autoantibodies that neutralize Type I IFNs present in the plasma of recovered patients who experienced severe COVID-19 that account for approximately 20% of COVID-19 deaths and may pose a risk to recipients (42). Additionally, more recent findings have indicated it isn't an effective treatment option (43).

In addition to treatment, there are vaccines available against COVID-19. While there are currently 24 COVID-19 vaccines in use globally, eight of those have been approved for use by the World Health Organization (44). In the United States there is one FDA-approved (Comirnaty, aka Pfizer-BioNTech) vaccine and two (Moderna and Janssen) vaccines granted FDA EUA (45). Pfizer-BioNTech received EUA for the use in children over 5 years of age in the United States in October 2021 (46). The European Union has so far provided four vaccines with conditional approval, which include the three available in the United States as well as AstraZeneca (47).

Table 5. COVID-19 Therapeutics

Drug	Approval
Velkury (remdesivir)	Full FDA-approval for hospitalized patients 12 years of age and older
Olumiant (baricitinib)	EUA approval for hospitalized patients 2 years of age or older requiring supplemental oxygen, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO)
Actemra (tocilizumab)	IEUA for adult and pediatric patents, aged 2-years or older who are receiving systemic corticosteroids and require supplemental oxygen, mechanical ventilation, or ECMO.
REGEN-COV (casirivimab and imdevimab)	EUA approval for treatment of mild to moderate COVID-19 in adult and pediatric patients 12 years of age or older who are at high risk of developing Severe COVID-19. This monoclonal antibody cocktail is also approved as prophylaxis to prevent COVID-19 following exposure in this same population of individuals.
Bamlanivimab and etesevimab	The FDA revoked the EUA for bamlanivimab alone on April 16, 2021. The combination of bamlanivimab and etesevimab antibodies have received EUA for treatment of mild to moderate COVID-19 in adult and some pediatric patients.
Sotrovimab	EUA for treatment of mild-to-moderate COVID19 in adults and children 12 years of age or older who are at high risk of progressing to severe COVI-19 disease
Convalescent plasma	EUA for the treatment of hospitalized COVID-19 patients

Considerations for Clinical Trial Design and Regulatory Submission

The mechanisms of action of the available and potential therapeutic strategies are specific to disease stage and patient characteristics. For this reason, clinical trials and regulatory submissions require careful and deliberate consideration of demographics, disease presentation, and patient history when determining the inclusion and exclusion criteria for trial participation or when making recommendations for patient populations for regulatory submissions. Further, COVID-19 has disproportionately affected some demographics with racial and ethnic minorities being infected at higher rates and experiencing worse disease outcomes (48). The reasons for this are complex and can be attributed to location, occupation, healthcare access, socioeconomic status, prevalence of comorbidities, and several other factors (48).

Ideally, study participants should reflect the demographics of those most affected by the disease, or at a minimum reflect the diversity of the entire population. However, this is often not the case either because trials cannot recruit a representative cohort or because certain conditions are expressly excluded from the trial. Researchers are unable to recruit a representative cohort for a variety of reasons including financial barriers, investigator bias, medical mistrust, limited health and research literacy, and lack of transportation (49, 50).

Disease stage and patient factors such as age and health history are critical in trial design. Investigators cannot combine all COVID-19 cases into a single group as severity and disease course are highly variable, often based on the health history of individual patients. For example, throughout the course of this pandemic, it has been evident that age is a significant factor in disease outcomes (51, 52), though that may be due to the incidence of comorbidities and likelihood of living in long-term care facilities amongst this population (52). Similar demographic considerations must be made regarding regulatory submissions when making recommendations for treatment recipients.

Considerations for Clinical Trial Design and Regulatory Submission. *Cont.*

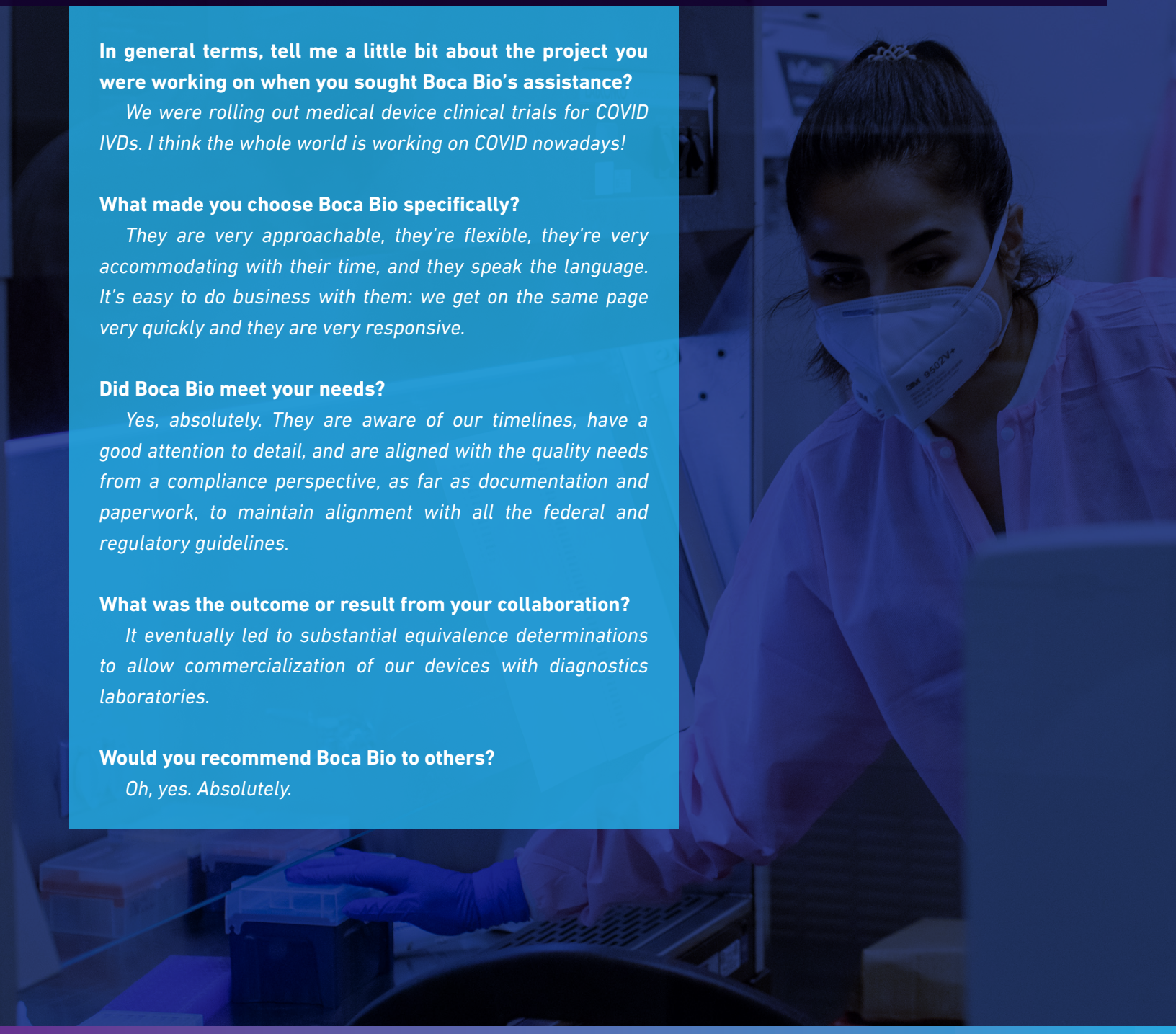
In addition to demographic considerations, the current dominant variant in the geographical area where the trial takes place has a significant impact. COVID-19 vaccine trials that occurred in 2020 encountered a different dominant SARS-CoV-2 strain than those trials that are currently ongoing. Clinical trials were also conducted in various geographical areas where the dominant SARS-CoV-2 variants differed. For this reason, vaccine efficacy was much lower in some parts of the world compared to others. Thus, efficacies cannot be directly compared (53). Investigators cannot control for the shifting variant landscape, but they can acknowledge differences among data collected at varying times.

Intervention timing is another important factor. For example, monoclonal antibodies are best administered early on or even prior to the onset of symptoms, while immunosuppressive treatments can be used for patients already experiencing severe COVID-19 to blunt the overreactive immune response. Administering treatment at the wrong time could yield the false conclusion that a treatment is ineffective. Dose and dose spacing are also important considerations. Many trials build in dose escalation or de-escalation arms into the study design of a Phase I or II trial to determine the most effective dose or the minimum effective dose. An example of this is the dose escalation incorporated into the current Phase II clinical trial (NCT04796896) investigating the Moderna mRNA-1273 COVID-19 Vaccine in children aged 6 months to 12 years of age. Similarly, dose spacing arms were included in trials for Pfizer, Moderna, and Oxford-AstraZenaca COVID-19 vaccines.

Considerations when Sourcing SARS-CoV-2 / COVID-19 Biospecimens

The search for the most effective diagnostic and therapeutic tools to end the global pandemic will require meticulously designed studies. With the significant variability in disease course among infected individuals and an ever-growing list of variants, however, research efforts on SARS-CoV-2 and COVID-19 are increasingly complicated. Additionally, vaccination status and the vaccines used vary based on location and time. With all these variables it can be difficult to find standardized specimens with which to conduct studies. Obtaining and using fully characterized specimens should be considered necessary whenever human biospecimens are used. As we have already seen that variants can affect the accuracy of diagnostics (54) and the efficacy of treatments (55), well-conducted studies will appropriately consider such effects. Optimal study design will additionally consider vaccination status and clinical presentation to obtain a full perspective on the efficacy of any investigational intervention.

Boca Biolistics is proud that its large repository of COVID-19 biospecimens has helped to advance therapeutics and diagnostics which are being put to use to help end the global pandemic. Below, a Director of Clinical Affairs for a major manufacturer of COVID-19 diagnostics shares his experience. The interview has been lightly edited for clarity and abridged for length.



In general terms, tell me a little bit about the project you were working on when you sought Boca Bio's assistance?

We were rolling out medical device clinical trials for COVID IVDs. I think the whole world is working on COVID nowadays!

What made you choose Boca Bio specifically?

They are very approachable, they're flexible, they're very accommodating with their time, and they speak the language. It's easy to do business with them: we get on the same page very quickly and they are very responsive.

Did Boca Bio meet your needs?

Yes, absolutely. They are aware of our timelines, have a good attention to detail, and are aligned with the quality needs from a compliance perspective, as far as documentation and paperwork, to maintain alignment with all the federal and regulatory guidelines.

What was the outcome or result from your collaboration?

It eventually led to substantial equivalence determinations to allow commercialization of our devices with diagnostics laboratories.

Would you recommend Boca Bio to others?

Oh, yes. Absolutely.

Boca Biolistics Biobanx COVID-19 Variant Collections

Country	Pangolin Lineage	NextStrain Clade	# Donors
Dom. Rep.	B.1	20C	3
Dom. Rep.	P.1	20J (Gamma; V3)	5
Dom. Rep.	B.1.621.1	21H (Mu)	13
Dom. Rep.	B.1.621	21H (Mu)	1
Dom. Rep.	B.1.630	20C	5
Dom. Rep.	B.1.1.7	20I (Alpha; V1)	1
Dom. Rep.	B.1.351.3	20C	2
Dom. Rep.	B.1.526	21F (Iota)	2
Honduras	B.1.1	20B	1
Honduras	AY.113	21J (Delta)	5
Honduras	B.1.1.432	20B	2
Honduras	B.1.153	20A	1
Honduras	B.1.2	20G	2
Honduras	AY.100	21J (Delta)	2
Honduras	B.1	20A	10
Honduras	B.1.627	20A	2
India	B	21A (Delta)	9
India	A	21A (Delta)	2
India	B.1	21A (Delta)	1
India	B	19A	3
India	B.1.604	21A (Delta)	2
India	B	20A	1
Peru	B.1.1.1	20D	1
Peru	P.1	20J (Gamma; V3)	8
Peru	B.1.1.251	20B	1
Peru	BA.1.1	21K (Omicron)	15
Peru	B.1.525	20A	1
Peru	P.1.12.1	20J (Gamma; V3)	3
Peru	AY.122.4	21J (Delta)	2
Peru	C.14	20D	2
Peru	B.1.1.28	20B	1
Peru	C.32	20D	1
Peru	C.11	20D	3
Peru	AY.119.1	21J (Delta)	2
Peru	B.1.1.67	20B	1
Peru	B.1.1	20I (Alpha; V1)	1
Peru	B.1.1.348	20B	10
Peru	B.1.1.279	20B	1
Peru	C.37	20D	22
Peru	C.4	20D	3
Peru	AY.39.2	21J (Delta)	12
Peru	AY.122	21J (Delta)	1
Peru	P.1.12	20J (Gamma; V3)	1
Peru	B.1.1.1	20B	2
Peru	B.1.1.29	20B	2
Peru	B.1.205	20A	1
Peru	B.1.429	20C	1
Peru	AY.102	21J (Delta)	1

Country	Pangolin Lineage	NextStrain Clade	# Donors
Peru	C.37	21G (Lambda)	15
Peru	C.37.1	21G (Lambda)	1
Peru	BA.1	21K (Omicron)	5
South Africa	AY.91	21J (Delta)	3
South Africa	AY.45	21J (Delta)	15
South Africa	AY.99	21J (Delta)	1
South Africa	BA.1.1	21K (Omicron)	5
South Africa	AY.107	21J (Delta)	3
South Africa	AY.32	21J (Delta)	28
South Africa	BA.3	21M (Omicron)	1
South Africa	AY.38	21I (Delta)	3
South Africa	BA.1	21K (Omicron)	80
South Africa	AY.33	21J (Delta)	1
South Africa	AY.116	21J (Delta)	2
South Africa	AY.6	21J (Delta)	5
Ukraine	B.1.1.7	20I (Alpha; V1)	8
Ukraine	B.1	20A	2
Ukraine	B.1.1	20B	1
United States	AY.44	21J (Delta)	2
United States	AY.43	21J (Delta)	1
United States	AY.25	21J (Delta)	2
United States	AY.13	21A (Delta)	2
United States	B.1.2	20G	7
United States	B.1.369	20C	2
United States	B.1.324	20C	1
United States	B.1.429	21C (Epsilon)	1
United States	B.1.617.2	21J (Delta)	5
United States	AY.103	21J (Delta)	2
United States	B.1.429	20C	2
United States	B	20A	2
United States	AY.39	21J (Delta)	1
United States	B.1.577	20C	3
United States	AY.74	21I (Delta)	1
United States	B.1	20C	3
United States	B.1.234	20A	3
United States	AY.117	21J (Delta)	1
United States	BA.1	21K (Omicron)	12
United States	B.1.596	20G	3
United States	BA.1.1	21K (Omicron)	10
United States	B.1.1.316	20B	1
United States	B.1	20A	3
United States	B.1.617.2	21I (Delta)	1
United States	B.1.232	20A	2
United States	B.1.1.7	20I (Alpha; V1)	2
United States	AY.25.1	21A (Delta)	1
United States	B.1.545	20A	1
United States	B.1.243	20A	1
United States	P.1.17	20J (Gamma; V3)	1

Boca Biolistics Biobanx COVID-19 Sample Subtype Collections

Sample Subtype	Total Donors	Matrix Type	Total Specimens
COVID-19 Positive Test	4857	Swab	157
		Whole Blood	1574
		Serum	33240
		Oropharyngeal swab	38
		Plasma	21757
		Nasopharyngeal swabs	3461
		Saliva	2783
		Nasal Swab	297
		Dried Blood Spot	122
		Urine	50
COVID-19 Symptomatic	891	Serum	3769
		Plasma	550
		Nasopharyngeal swabs	1484
		Saliva	698
		Nasal Swab	734
		Swab	100
COVID-19 Vaccinated	233	Serum	5446
		Plasma	2268
SARS-CoV-2 Remnant Sample	39158	Nasopharyngeal swabs	23176
		Serum	4442
		Swab	15377
		Plasma	82
TOTAL	**45139**		**121605**

References

1. Scudellari M. How the coronavirus infects cells - and why Delta is so dangerous. Nature. 2021 Jul;595(7869):640-644. doi: 10.1038/d41586-021-02039-y.
2. Kyrou I, Randeva HS, Spandidos DA, Karteris E. Not only ACE2-the quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19. Signal Transduct Target Ther. 2021 Jan 18;6(1):21. doi: 10.1038/s41392-020-00460-9.
3. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends Immunol. 2020 Dec;41(12):1100-1115. doi: 10.1016/j.it.2020.10.004. Epub 2020 Oct 14.
4. Johnson BA, Xie X, Kalveram B, *et al.* Furin Cleavage Site Is Key to SARS-CoV-2 Pathogenesis. bioRxiv [Preprint]. 2020 Aug 26:2020.08.26.268854. doi: 10.1101/2020.08.26.268854.
5. European Centre for Disease Prevention and Control. SARS-CoV-2 variants of concern as of 26 November 2021. Accessed January 10th, 2022. https://www.ecdc.europa.eu/en/covid-19/variants-concern
6. Centers for Disease Prevention and Control. SARS-CoV-2 Variant Classificiations and Definitions. Accessed January 10th, 2022. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html
7. World Health Organization. Tracking SARS-CoV-2 Variants. Accessed January 10th, 2022. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
8. O'Toole Á, Hill V, Pybus OG et al. Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2. 2021 *Wellcome Open Res* DOI:10.12688/wellcomeopenres.16661.1
9. Lineage List. Accessed January 10th 2022. https://cov-lineages.org/lineage_list.html
10. Liu Y, Liu J, Johnson BA, *et al.* Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. bioRxiv [Preprint]. 2021 Sep 5:2021.08.12.456173. doi: 10.1101/2021.08.12.456173.
11. Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell. 2020 May 21;78(4):779-784.e5. doi: 10.1016/j.molcel.2020.04.022. Epub 2020 May 1.
12. Stasi C, Fallani S, Voller F, Silvestri C. Treatment for COVID-19: An overview. *Eur J Pharmacol.* 2020;889:173644. doi:10.1016/j.ejphar.2020.173644
13. Clinical Spectrum of SARS-CoV-2 Infection. National Institutes of Health. 2021. Accessed November 25, 2021. https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/
14. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, *et. al.* More than 50 Long-term effects of COVID-19: a systematic review and meta-analysis. medRxiv [Preprint]. 2021 Jan 30:2021.01.27.21250617. doi: 10.1101/2021.01.27.21250617. Update in: Sci Rep. 2021 Aug 9;11(1):16144.
15. Ziegler CGK, Miao VN, Owings AH, *et. al.* Impaired local intrinsic immunity to SARS-CoV-2 infection in severe COVID-19. Cell. 2021 Sep 2;184(18):4713-4733.e22. doi: 10.1016/j.cell.2021.07.023. Epub 2021 Jul 23.
16. Shen B, Yi X, Sun Y, *et. al.* Proteomic and Metabolomic Characterization of COVID-19 Patient Sera. Cell. 2020 Jul 9;182(1):59-72.e15. doi: 10.1016/j.cell.2020.05.032. Epub 2020 May 28.
17. Silvin A, Chapuis N, Dunsmore G, *et. al.* Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19. Cell. 2020 Sep 17;182(6):1401-1418.e18. doi: 10.1016/j.cell.2020.08.002. Epub 2020 Aug 5.
18. Mun DG, Vanderboom PM, Madugundu AK, *et. al.* DIA-Based Proteome Profiling of Nasopharyngeal Swabs from COVID-19 Patients. J Proteome Res. 2021 Aug 6;20(8):4165-4175. doi: 10.1021/acs.jproteome.1c00506. Epub 2021 Jul 22.
19. Ng DL, Granados AC, Santos YA, *et. al.* A diagnostic host response biosignature for COVID-19 from RNA profiling of nasal swabs and blood. Sci Adv. 2021 Feb 3;7(6):eabe5984. doi: 10.1126/sciadv.abe5984.
20. Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis. 2020 Jun;95:304-307. doi: 10.1016/j.ijid.2020.04.061. Epub 2020 Apr 25.
21. Weidmann MD, Ofori K, Rai AJ. Laboratory Biomarkers in the Management of Patients With COVID-19. Am J Clin Pathol. 2021 Feb 11;155(3):333-342. doi: 10.1093/ajcp/aqaa205.
22. Fei Y, Tang N, Liu H, Cao W. Coagulation Dysfunction. Arch Pathol Lab Med. 2020 Oct 1;144(10):1223-1229. doi: 10.5858/arpa.2020-0324-SA.
23. Alnor A, Sandberg MB, Gils C, Vinholt PJ. Laboratory Tests and Outcome for Patients with Coronavirus Disease 2019: A Systematic Review and Meta-Analysis. J Appl Lab Med. 2020 Sep 1;5(5):1038-1049. doi: 10.1093/jalm/jfaa098.
24. Aloisio E, Chibireva M, Serafini L, *et. al.* Comprehensive Appraisal of Laboratory Biochemistry Tests as Major Predictors of COVID-19 Severity. Arch Pathol Lab Med. 2020 Dec 1;144(12):1457-1464. doi: 10.5858/arpa.2020-0389-SA.
25. Tjendra Y, Al Mana AF, Espejo AP, *et. al.* Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers. Arch Pathol Lab Med. 2020 Dec 1;144(12):1465-1474. doi: 10.5858/arpa.2020-0471-SA.
26. Azam TU, Shadid HR, Blakely P, *et. al.* International Study of Inflammation in COVID-19. Soluble Urokinase Receptor (SuPAR) in COVID-19-Related AKI. J Am Soc Nephrol. 2020 Nov;31(11):2725-2735. doi: 10.1681/ASN.2020060829. Epub 2020 Sep 22.
27. De Michieli L, Ola O, Knott JD, Akula A, *et. al.* High-Sensitivity Cardiac Troponin T for the Detection of Myocardial Injury and Risk Stratification in COVID-19. Clin Chem. 2021 Aug 5;67(8):1080-1089. doi: 10.1093/clinchem/hvab062.
28. Zinellu A, Sotgia S, Fois AG, Mangoni AA. Serum CK-MB, COVID-19 severity and mortality: An updated systematic review and meta-analysis with meta-regression. Adv Med Sci. 2021 Sep;66(2):304-314. doi: 10.1016/j.advms.2021.07.001. Epub 2021 Jul 7.

References. *Cont.*

29. Zhang L, Guo H. Biomarkers of COVID-19 and technologies to combat SARS-CoV-2. Adv Biomark Sci Technol. 2020;2:1-23. doi: 10.1016/j.abst.2020.08.001. Epub 2020 Aug 19.
30. Bonaventura A, Vecchié A, Dagna L, *et. al.* Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. Nat Rev Immunol. 2021 May;21(5):319-329. doi: 10.1038/s41577-021-00536-9. Epub 2021 Apr 6.
31. Gogate N, Lyman D, Bell A, *et. al.* COVID-19 biomarkers and their overlap with comorbidities in a disease biomarker data model. Brief Bioinform. 2021 Nov 5;22(6):bbab191. doi: 10.1093/bib/bbab191.
32. Coronavirus Disease 2019 (COVID-190 EUA information. Accessed November 29, 2021. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs
33. FDA Approves First Treatment for COVID-19. 2020 Oct 22. Accessed November 29, 2021. https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19
34. Kokic, G., Hillen, H.S., Tegunov, D. *et al.* Mechanism of SARS-CoV-2 polymerase stalling by remdesivir. *Nat Commun* **12,** 279 (2021). https://doi.org/10.1038/s41467-020-20542-0
35. Kalil AC, Patterson TF, Mehta AK, *et. al.* Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. N Engl J Med. 2021 Mar 4;384(9):795-807. doi: 10.1056/NEJMoa2031994. Epub 2020 Dec 11.
36. Stebbing J, Krishnan V, de Bono S, *et. al.* Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients. EMBO Mol Med. 2020 Aug 7;12(8):e12697. doi: 10.15252/emmm.202012697.
37. Fajgenbaum DC, June CH. Cytokine Storm. N Engl J Med. 2020 Dec 3;383(23):2255-2273. doi: 10.1056/NEJMra2026131.
38. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021 May 1;397(10285):1637-1645. doi: 10.1016/S0140-6736(21)00676-0.
39. Weinreich DM, Sivapalasingam S, Norton T, *et. al.* REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. N Engl J Med. 2021 Sep 29:NEJMoa2108163. doi: 10.1056/NEJMoa2108163. Epub ahead of print.
40. FDA Authorizes REGEN-COV monoclonal antibody therapy for post-exposure prophylaxis (prevention) for COVID-19. Accessed November 30, 2021. https://www.fda.gov/drugs/drug-safety-and-availability/fda-authorizes-regen-cov-monoclonal-antibody-therapy-post-exposure-prophylaxis-prevention-covid-19
41. Coronavirus (COVID-19) Update: FDA Authorizes Additional Monoclonal Antibody for Treatment of COVID-19. Accessed November 30, 2021. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-monoclonal-antibody-treatment-covid-19
42. Bastard P, Gervais A, Le Voyer T, *et. al.* Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. Sci Immunol. 2021 Aug 19;6(62):eabl4340. doi: 10.1126/sciimmunol.abl4340.
43. COVID19 Vaccine Tracker. Accessed November 30, 2021. https://covid19.trackvaccines.org/agency/who/
44. Early treatment with convalescent plasma for COVID-19 doesn't show benefit. 31 August 2021. Accessed November 30, 2021. https://www.nih.gov/news-events/nih-research-matters/early-treatment-convalescent-plasma-covid-19-doesnt-show-benefit
45. COVID-19 Vaccines. Accessed November 29, 2021. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines
46. FDA Authorizes Pfizer-BionTech COVID-19 Vaccine for Emergency Use in Children 5 through 11 Years of Age. Accessed November 29, 2021. https://www.fda.gov/news-events/press-announcements/fda-authorizes-pfizer-biontech-covid-19-vaccine-emergency-use-children-5-through-11-years-age
47. Safe COVID-19 vaccines for Europeans. Accessed November 30, 2021. https://ec.europa.eu/info/live-work-travel-eu/coronavirus-response/safe-covid-19-vaccines-europeans_en
48. Azar KMJ, Shen Z, Romanelli RJ, Lockhart SH, Smits K, Robinson S, Brown S, Pressman AR. Disparities In Outcomes Among COVID-19 Patients In A Large Health Care System In California. Health Aff (Millwood). 2020 Jul;39(7):1253-1262. doi: 10.1377/hlthaff.2020.00598. Epub 2020 May 21.
49. Representation in Clinical Trials: A Review on Reaching Underrepresented Populations in Research. 10 August 2020. Accessed November 30, 2021. https://acrpnet.org/2020/08/10/representation-in-clinical-trials-a-review-on-reaching-underrepresented-populations-in-research/
50. Bogart LM, Ojikutu BO, Tyagi K, *et. al.* COVID-19 Related Medical Mistrust, Health Impacts, and Potential Vaccine Hesitancy Among Black Americans Living With HIV. J Acquir Immune Defic Syndr. 2021 Feb 1;86(2):200-207. doi: 10.1097/QAI.0000000000002570.
51. Mahase E. Covid-19: Why are age and obesity risk factors for serious disease? BMJ. 2020 Oct 26;371:m4130. doi: 10.1136/bmj.m4130.
52. D'ascanio M, Innammorato M, Pasquariello L, Pizzirusso D, Guerrieri G, Castelli S, Pezzuto A, De Vitis C, Anibaldi P, Marcolongo A, Mancini R, Ricci A, Sciacchitano S. Age is not the only risk factor in COVID-19: the role of comorbidities and of long staying in residential care homes. BMC Geriatr. 2021 Jan 15;21(1):63. doi: 10.1186/s12877-021-02013-3.
53. Ledford H. Why COVID vaccines are so difficult to compare. Nature. 2021 Mar;591(7848):16-17. doi: 10.1038/d41586-021-00409-0.
54. SARS-CoV-2 Viral Mutations: Impact on COVID-19 Tests. December 7, 2021. Accessed December 7, 2021. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests
55. Bernal JL, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, Stowe J, Tessier E, Groves N, Dabrera G, Myers R, Campbell CNJ, Amirthalingam G, Edmunds M, Zambon M, Brown KE, Hopkins S, Chand M, Ramsay M.

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