Chapter

SARS-CoV-2 Variant Surveillance in Genomic Medicine Era

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Abstract

In the genomic medicine era, the emergence of SARS-CoV-2 was immediately followed by viral genome sequencing and world-wide sequences sharing. Almost in real-time, based on these sequences, resources were developed and applied around the world, such as molecular diagnostic tests, informed public health decisions, and vaccines. Molecular SARS-CoV-2 variant surveillance was a normal approach in this context yet, considering that the viral genome modification occurs commonly in viral replication process, the challenge is to identify the modifications that significantly affect virulence, transmissibility, reduced effectiveness of vaccines and therapeutics or failure of diagnostic tests. However, assessing the importance of the emergence of new mutations and linking them to epidemiological trend, is still a laborious process and faster phenotypic evaluation approaches, in conjunction with genomic data, are required in order to release timely and efficient control measures.

Keywords: SARS-CoV-2 viral genome, variant surveillance, genomic medicine, molecular diagnostic tests, public health decisions

1. Introduction

In the current COVID-19 pandemic context, with a count of 544 million cases including 6.33 million deaths [1], a worldwide collective effort for observing the SARS-CoV-2 genomic landscape, monitoring the virus mutational rate and the emergence of new variants is noted. As a result of SARS-CoV-2 genome modification, the virus may gain selective advantage regarding transmission and virulence, increase disease severity and eventually, significantly impact public health either by affecting the current vaccine performance or interfering with the current diagnostic and therapeutic strategies [2].

A series of measures have been taken globally for optimal management of the emergence of SARS-CoV-2 variants. World Health Organization (WHO),

international health expert networks, and researchers have continuously been monitoring virus evolution by examining the mutations occurring in the SARS-CoV-2 genome.

During an emergency situation in a health care system, the most important actions are the rapid and effective identification of pathogen and epidemiological surveillance to allow disease control reaction. The development of next-generation sequencing (NGS) techniques has led to an enormous amount of genomic sequence data [3]. In COVID-19 genomic era, the accumulation of this considerable amount of genomic data shared in international repositories, such as GISAID EpiCOV, COG-UK, or NCBI, allowed the evaluation of the transmission pattern of viral strains, the impact of each variant, and also the comparison between the available vaccines and the circulating viral variants [4] and it also oriented global public health measures.

At present, two and a half years after the beginning of the pandemic, we can say that given the mutations and recombination of the viral genes, we are facing a different type of SARS-CoV-2 than the one that emerged in China in December 2019. On November 26, 2021, Omicron, B.1.1.529 Pango lineage has emerged, and currently, it represents the circulating variant of concern, still threatening several countries with its sub-lineages.

In this chapter, we summarize the genomic medicine impact on the identification of the new infectious agent that causes COVID-19, on development of molecular tests for diagnostic and surveillance of the emergent variants, describe their importance in managing transmission, preventing failure of diagnostic tests, on sustaining effectiveness of vaccines and therapeutics and eventually, to inform the public health policies.

2. SARS-CoV-2 viral genome – development of molecular tests for diagnostic and surveillance of the emergent variants

One of the first events that led to the diagnosis of Coronavirus disease (COVID-19) following *SARS-CoV-2* infection was the identification of the infectious agent that causes a new disease of unknown origin by characterizing the nucleic acid signature.

The first patient was hospitalized on 12th of December 2019 and on 10th of January a viral genome sequence was already released. The first metagenomic RNA sequencing report of a sample of bronchoalveolar lavage fluid from a patient who was admitted to the Central Hospital of Wuhan on 26th of December 2019 while experiencing a severe respiratory syndrome, identified a new RNA virus strain from the family *Coronaviridae*, which was later named *SARS-CoV-2* (Wuhan-Hu-1, GenBank accession number MN908947) [5]. Confirmation of the results obtained by deep meta-transcriptomic sequencing, regarding the genome sequence of this virus and also its termini, was done by real-time reverse-transcription PCR (rRT – PCR), and this was the beginning of a new era, the era of "COVID-19" because at that time rRT-PCR was routinely used to detect causative viruses from respiratory secretions, but it was not considered a gold standard diagnostic technique. What followed turned this technique into the gold standard in terms of diagnosing COVID-19 disease and SARS-CoV-2 infection [6–9].

The first three determined genomes of the novel coronavirus (SARS-CoV-2), namely: Wuhan/IVDC-HB-01/2019 (GISAID accession ID: EPI_ISL_402119) (HB01), Wuhan/IVDC-HB-04/2019 (EPI_ISL_402120) (HB04), and Wuhan/IVDC-HB-05/2019 (EPI_ISL_402121) (HB05) were compared [10]. The three genomes were almost identical and the findings showed that the SARS-CoV-2

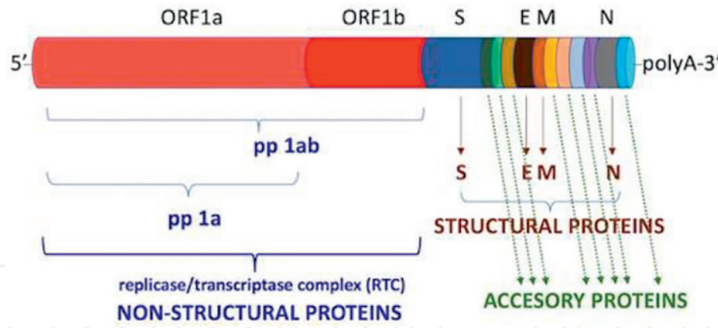

Figure 1.
Schematic diagram of SARS-COV-2 virus genome and most important encoded proteins.

genome, which is approximately 30 kb in size, was a positive sense, single-stranded RNA with a 5′-cap and a 3′-poly-A tail that contained 14 open reading frames (ORFs) encoding 27 proteins (**Figure 1**). The 5′-terminus contains orf1ab and orf1a genes, which encode the polyproteins pp1ab and pp1a. These two polyproteins are further processed by viral proteinases Nsp3 and Nsp5 resulting in 16 nonstructural proteins (Nsps), Nsp1 to Nsp10 and Nsp12 to Nsp16, responsible for viral replication. The 16 nonstructural proteins form a replicase/transcriptase complex (RTC) together. The activity of this complex is dependent on the involvement of viral enzymes Nsp7-Nsp8 primase, the Nsp12 RNA-dependent RNA polymerase (RdRp), the Nsp13 helicase/triphosphatase, the Nsp14 exoribonuclease (the first identified proofreading enzyme encoded by an RNA virus), Nsp15 endonuclease, and Nsp10-Nsp16 N7- and 2′O-methyltransferases. The 3′-terminus encode the four structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) and eight accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14) [8, 10–13].

After SARS-CoV-2 genome virus sequences were obtained, the similarities and differences between SARS-CoV-2 and other SARS viruses offered the possibility to establish key sequences in the genome for use in diagnosis and surveillance. The release of the first SARS-CoV-2 sequence allowed rapid evaluation of the rRT – PCR techniques for the detection of specific sequences of the SARS-CoV-2 genome and immediately a diagnostic workflow was established [6]. Sequences that offered sensitivity and specificity to the diagnosis were selected, so the detection of a sequence in the E gene provided sensitivity to the test, but not specificity is given the high percentage of similarity with other coronaviruses. The specificity of the test was given by the use of specific primers for certain sequences in genes with less homology to other coronaviruses, as N, S, Orf1ab, and RdRp (located in ORF1ab gene), and in order to increase the sensitivity of the test, the simultaneous detection of several targets have been employed [6, 8].

In addition to rRT-PCR as a standard method for diagnosing SARS-CoV-2 infection, other methods involving the amplification of nucleic acids (NAATs) have been used to detect viral RNA, including digital PCR (dPCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR)-based assays. All of that could be a useful tool for surveillance and timely identification of emerging strains. Moreover, NGS has been used since the beginning of the COVID-19 pandemic for the characterization and analysis of viral genetic material and mutation surveillance [8].

There are many studies that evaluated different NAAT strategies for the detection of SARS-CoV-2 and compared their sensitivity and specificity and their conclusion

was that rRT-PCRs were significantly more sensitive than other methods [14]. However, for population surveillance, there are need for detection methods that have an increased specificity, are less expensive, and are faster than NGS and rRT-PCR.

dPCR has many advantages over rRT-PCR including higher precision with absolute nucleic acid quantification, it has higher sensitivity and it is not as sensitive to PCR inhibitors or mismatch primer/template. However, this technique has a complicated workflow and depends on expensive instruments and consumables, which results in a higher cost per test [15, 16]. As an important advantage, there are studies that propose using dPCR for SARS-CoV-2 viral load measurement directly from crude lysate without nucleic acid purification [17].

RT-LAMP was previously used for the detection of the Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) global outbreaks. RT-LAMP is a reliable and rapid screening test, which can also be used under non-laboratory conditions, but the sensitivity of RT-LAMP is poor, with an important percentage of positive patients remaining undetected [18].

CRISPR-based assays represent a system based on CRISPR-associated endonucleases (Cas), CRISPR-Cas12a, and CRISPR-Cas13a, that recognizes and cleaves nucleic acids in a sequence-specific way. Recently, a CRISPR-based diagnostic platform that combines nucleic acid pre-amplification with CRISPR-Cas enzymology was established for the detection of SARS-CoV-2 RNA. The great advantage is that the detection via fluorescent and colorimetric readouts provides results in less than 1 hour, but even if it is highly sensitive and specific, the multistep nucleic acid amplification process may affect precise target quantification. Additionally, the preparation and testing of reaction components need optimization [19, 20].

Although NAAT techniques have high sensitivity and specificity for the detection of SARS-CoV-2, in the management of the COVID-19 pandemic, a faster detection method was required, which would involve lower costs and also non-laboratory conditions and expertise. These needs have led to the development of rapid tests that detect SARS-CoV-2 viral proteins, intensively used in the detection of other viral and bacterial infections, but which have as a limitation the lower specificity and sensitivity than NAAT-type tests. During the infection with SARS-CoV-2 in the nasopharynx and oropharynx of infected people, high concentrations of S and N protein were detected and because of that, they became ideal candidates for diagnostic targets for the **detection of viral protein** by antigen–antibody (Ag-Ab) reaction. Thus, monoclonal antibodies against viral N and S proteins react with the viral proteins N and/or S present in patients' specimens and this interaction can be easily visualized [21–23]. The major limitations of this technique are that it could generate false negative results for patients with low viral loads, and has lower sensitivity for cycle of quantification >30. The negative results need to be confirmed using molecular tests, particularly when the clinical context is suggestive of SARS-CoV-2 infection.

SARS-CoV-2 has proofreading mechanisms, which make the mutation rate lower compared to other RNA viruses such as HIV and influenza, however, the selection pressure and immune evasion mechanisms have led to mutations that can affect the properties of the virus, thus surveillance of viral evolution is utterly necessary.

Genomic surveillance involves the analysis of similarities and differences between sequences obtained by **viral genome sequencing**.

The development of **NGS** techniques has led to a huge amount of genomic sequence data [3]. As it was shown for emerging infectious diseases, such as SARS, MERS, Zika, and Ebola, **whole-genome sequencing (WGS) metagenomics**

technique offers the possibility to rapidly obtain the full sequence of pathogen genomes, tracing origins, spread and transmission chains of outbreaks, and monitoring the pathogen evolution [24–28].

Metagenomics applications were used for rapid identification and characterization of SARS-CoV-2 and brought critical novel information [5, 29]. The application is simple, cost-effective, and does not require reference sequence for analysis.

In order to obtain complete or nearly complete assemblies of the genome of SARS-CoV-2 clinical samples, shotgun metatranscriptomics – saturation RNA sequencing – has been successfully used. The principle of the method was based on host gene expression monitoring and consists of either enrichment of the poly(A) + RNA fraction, or depletion of host rRNA [30]. Depending on the manufacturer and NGS technology the workflow consists of RNA fragmentation, first- and second-strand cDNA synthesis, and library preparation. Most of the studies were developed on Illumina platforms and the Oxford Nanopore Technology (ONT) [31].

Amplicon-based sequencing approach was developed later, after the enrichment of the knowledge regarding the SARS-CoV-2 genome, as the method is highly specific. The typical workflow consists of first-strand cDNA synthesis followed by genome amplification with multiplex PCRs. The primers used in multiplex PCRs produce a pool of amplicons that cover almost the entire viral genome. Amplicon sequencing is highly specific and robust, but it presents some limitations regarding differences in primer efficiency, amplification across the genome can be biased, with decreased coverage in specific genomic regions and/or 3′ and 5’ UTRs regions are not targeted leading to an incomplete assembly [30]. For library preparation, several commercial and noncommercial protocols are available, and libraries can be sequenced on benchtop platforms (i.e., Illumina NextSeq and Miseq; Ion torrent platforms, etc.) [30].

Hybrid capture-enrichment sequencing is similar to amplicon-based sequencing that allows to target regions of a genome and enrich through hybridization to specific biotinylated probes. This approach was initially developed for exome sequencing [32]. Libraries obtained can be sequenced on benchtop platforms (Illumina NextSeq and MiSeq, Ion torrent, etc.). Hybrid capture-enrichment method uses a larger number of fragments/probes, providing more complete profiling of the target sequences and more robust to genomic variability [33].

Direct RNA sequencing is relatively recent approach in sequencing technologies that do not require RNA revers-transcription and allow the direct determination of the sequence of single nucleic acid molecules, without amplification [34]. This technology provides longer reads than regular NGS methods, but with higher error rates [35]. However, this method can provide the sequence of a single mature and precursor transcripts, and information about complex transcriptional patterns, which accompany coronavirus infection (recombination, alternative transcript maturation, rare transcriptional isoforms, etc.) [12].

The global effort of NGS for SARS-CoV-2 in COVID-19 pandemics generated a massive number of reads that had to be analyzed organized and stored in international databases with global access. Basically, the NGS data analysis involves several essential steps: quality control of the NGS data, removal of host/rRNA data, reads assembly, taxonomic classification, and virus genome verification [36].

2.1 SARS-CoV-2 genome data analysis

The assembly of the SARS-CoV-2 genome is a quite straightforward process, as the viral genome is small and does not contain any large repetitive sequence. The

main method for the assembly of NGS data that provides a complete and accurate representation of the genome (highly contiguous and accurate assemblies) is based on Overlap Layout Consensus, de Bruijn graphs, or, in general, reference-based assembly [37]. SARS-CoV-2 sequencing with a 30x coverage is generally considered sufficient to generate high-quality assembly [30]. The coverage is dependent on the sequencing platform and on the sequencing strategy, however, data obtained from targeted-enrichment-based library preparation methods (hybrid capture and amplicon sequencing) provide a sufficient viral genomic read.

The first step of bioinformatics workflow is to establish the quality of the reads. Fastq files are processed for subsequent analyses as follows: removing the adapter sequences and filtering low-quality/complexity reads, error correction, etc. [36].

Metagenomics sequencing protocols provide uniform coverage, but the number of viral reads depends on the viral load of the sample and may contain reads, derived from viral sub-genomic RNAs and replication intermediates [38]. For metagenomics reads assembly-efficient software tools are currently available [39].

In order to obtain an accurate representation of the genomic sequence of a SARS-CoV-2 strain, de-novo assembly method could be used, but there were also available, less sensitive methods, such as reference-guided assembly algorithms [40, 41].

2.2 SARS-CoV-2 genome verification and classification

Taxonomic classification is the following step after the reads are assembled into contigs. The quality of contigs can be evaluated by read mapping. The reliable contigs with unassembled overlaps are fused to form longer viral contigs using contig assembly tools (e.g., SEQMAN and Geneious).

2.3 SARS-CoV-2 phylogenetic analyses

The best-known portals for the real-time monitoring of the evolution SARS-CoV-2 strains are Nextstrain [42] and the HyPhy COVID-19 [43]. These systems provide real-time information of on worldwide distribution of different clades and lineages of SARS-CoV-2 (Nexstrain), and detailed phylogenetic analyses of SARS-CoV-2 protein-coding genes (Hyphy).

2.4 SARS-CoV-2 genomic data deposition and exploratory access

At present, the GISAID [44] with EpiCov portal represents the most widely used repository of SARS-CoV-2 genomic data. Along with sequencing data, metadata are provided including the type of sample, the sequencing technology and protocol, patient status (e.g. hospitalized or released), vaccination, etc.

Exploratory access is available from the three most popular portals for SARS-CoV-2 genome data: COG-UK [45], GISAID EpiCoV [44], and the NCBI [46].

Technical advances in NGS and bioinformatics have permitted a fast identification of causative agent of COVID-19, tracking its global spread and confirming the genomic modifications when they occurred. Current bioinformatics resources are multiple, but big datasets pose challenges for data storage and analysis and a solution must be found not only for the control of the current COVID-19 pandemic but for future outbreaks response.

Although NGS is a very precise tool, allowing the detection of each mutation in a sample (thus being considered the gold standard in tracking the viral variants), it has a few drawbacks regarding the price, the duration, and the accessibility [47–49].

To overcome these limitations, other genotyping strategies have been developed [50]: Multiplex PCR tests that use either TaqMan probes or molecular beacon probes, identify and monitor specific SARS-CoV-2 variants, and, even if they target preselected known mutations, they are more rapid, cheaper options, and could easily be deployed in settings with limited resources as an alternative to genome sequencing methods [47, 51].

New appeared mutations had an important effect on the detection sensitivity of RT-PCR that could be reduced if the mutations were located where probes and primers bind [52]. Because of this, commercial variants of kits that detected several genes that included the RdRp and Orf1ab genes in addition to the S and N genes were used and commercial multiplexing tests for tracking mutations in the population, for the surveillance and sequencing prioritization were rapidly developed.

The occurrence of the mutations in the S gene led to S gene target failure or so-called S gene dropout, which generated false negative RT-PCR results. This test failure, however, turned later in new pre-screening rRT-PCR assays that analyzed simultaneous detection of del-HV69/70 and N501Y in order to distinguish between B.1.1.7 and B1.351 lineages or have been used as a marker of B.1.1.529 variant [51, 53].

A TaqMan SNP genotyping test, recently developed by a Taiwanese team [50], targets nine mutations in receptor-binding domain of the spike protein of SARS-CoV-2 (delH69/V70, K417T, K417N, L452R, E484K, E484Q, N501Y, P681H, and P681R), and it is designed to simultaneously detect five important variants (Alpha, Beta, Gamma, Delta, and Omicron).

Molecular diagnostic companies are closely tracking data collected from laboratories all over the world in order to develop commercial multiplex genotyping kits that identify and screen variants as new significant functional mutations emerge.

3. Emergence of SARS-CoV-2 variants – genotype to phenotype analysis and global public health effects

A series of measures have been taken globally for optimal management of the emergence of SARS-CoV-2 variants. A global system has been established to detect SARS-CoV-2 lineages and to assess the potential risk for the circulating viral variants. For effective surveillance and viral characterization worldwide, it was essential to better describe the recently emerging variants and to establish a joint nomenclature.

Considering the large amount of collected data generated by sequencing, WHO experts after consultation with the Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE) establish the current nomenclature in use when referring to SARS-CoV-2 variants [54]. Thus, for each identified lineage, it was assigned a Greek alphabet letter (e.g., Alpha, Beta, Delta, or Omicron) and when choosing the working terminology for SARS-CoV-2 variants, it was considered the current terminology adopted by well-known open-source databases that performed phylogenetic analysis. Such examples are **PANGOLIN** – The Phylogenetic Assignment of Named Global Outbreak Lineages [55]; **Nextstrain** [56] or **GISAID** – The Global Initiative on Sharing All Influenza Data [57]. At the same time, the term "index virus" was established when referring to the SARS-CoV-2 genome characterized at the beginning of the pandemic (December 2019) in the situation of the first cases reported [58]. Considering the rapid evolution of the virus from a mutational point of view, the present nomenclature may undergo changes.

Specific viral variants that pose a risk to public health have been named, are considered a priority in monitoring, and are categorized using the following working terms:

- Variants of Interest (VOIs)
- Variants of Concern (VOCs)
- VOC lineages under monitoring (VOC-LUM).
- Variants under monitoring (VUM)

Variant of Interest (VOI) - a SARS-CoV-2 variant presenting a certain genetic constellation that is predicted to cause changes in virus properties impacting its transmissibility, virulence, the diagnostic and treatment methods, the severity of the disease, and immune system escape. It is also acknowledged to be responsible for high community spread, prevalence in multiple clusters or many countries, or hint of an emerging risk to global public health. At present, there are no circulating VOIs [58].

Among the circulating VOIs reported in the past [58, 59] (presented chronologically with spike mutation of interest and using the WHO and Pango lineage terminology) are:

- **Eta (B.1.525/VOI: 17-Mar-2021)**- E484K, D614G, Q677H;
- **Theta (P.3/VOI: 24-Mar-2021)**- E484K, N501Y, D614G, P681H;
- **Iota (B.1.526/VOI: 24-Mar-2021)**- E484K, D614G, A701V;
- **Kappa (B.1.617.1/VOI: 4-April-2021)**- L452R, E484Q, D614G, P681R;
- **Lambda (C.37/VOI: 14-Jun-2021)**- L452Q, F490S, D614G**;**
- **Mu (B.1.621/VOI: 30-Aug-2021)**- R346K, E484K, N501Y, D614G, P681H.

According, to WHO a **Variant of Concern (VOC)** is defined as a viral variant that meets the criteria for a VOI and in addition correlates with a higher degree of virulence and transmission or a change in COVID-19 epidemiology or clinical presentation, or a decrease in the effectiveness of currently available public health measures or of diagnostics, therapeutics, or vaccines [58].

Current VOC is **Omicron lineage (B.1.1.529/VOC: 26-Nov-2021)** including sublineages **BA.1, BA.2, BA.3, BA.4, BA.5,** and **XE** (**BA.1/BA.2** circulating recombinant) that WHO recommend to be monitored by public health authorities as distinct lineages.

The mutational profiles in spike sequence in Omicron sub-lineages are listed below:

- **BA.1** - A67V, Δ69–70, T95I, G142D, Δ143–145, N211I, Δ212, ins215EPE, 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;

- **BA.2** - G142D, N211I, Δ212, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K;

- **BA.3** - A67V, Δ69–70, Δ143–145, N211I, Δ212, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, D796Y, Q954H, N969K;

- **BA.4** -L452R, F486V, R493Q;

- **BA.5** - L452R, F486V, R493Q.

From the collected data, a higher transmission is observed also accompanied by a lower severity for the Omicron BA.1 and BA.2 variants with BA.2 displaying a dominant transmission in the EU/EEA countries. On the other hand, for the BA.4 and BA.5 there is not enough evidence regarding the impact of these variants on transmission rate or severity [59].

VOCs circulating in the past include:

- **Alpha (B.1.1.7/VOC: 18-Dec-2020)** - N501Y, D614G, P681H;

- **Beta (B.1.351/VOC: 18-Dec-2020)** - K417N, E484K, N501Y, D614G, A701V;

- **Gamma (P.1/VOC: 11-Jan-2021)** - K417T, E484K, N501Y, D614G, H655Y;

- **Delta (B.1.617.2/VOC: 11-May-2021)** - L452R, T478K, D614G, P681R.

VOC lineages under monitoring (VOC-LUM) are characterized as variants that, from a phylogenetic point of view, belong to VOCs, which are currently circulating, nevertheless compared to the circulating VOCs exhibit some genetic alterations that show better transmission and also present amino acids changes that might explain the epidemiologic modifications compared to circulating variants [58].

Currently, all **VOC-LUMs** are Omicron sublineages being under surveillance. Thus, the **BA.4 and BA.5** sublineages from a phylogenetic point of view belong to the same circulating VOC, presenting the same mutations profile for S (**BA.2-like** + del69/70, L452R, F486V, Q493) and also displaying two Nsp4 reversions: L438 and ORF6:D61. On the other hand, these two VOC-LUMs vary from each other through mutations outside spike, thus BA.4 shows the following additional mutations: ORF7b:L11F, N: P151S while BA.5 exhibits D3N mutation in M gene. **BA.2.12.1** is another current VOC-LUM belonging to BA.2 sublineage and which shares the same S mutational pattern plus L452Q and S704F. **BA.2.9.1** and **BA.2.13** come from BA.2 sublineage with which they share S mutational profile and also L452M additional mutation, while **BA.2.9.1** displays other genomic mutations outside of S, respectively, in N gene (P67S, S412I) and in ORF3a:H78Y [58].

It is very important to keep these lineages and their modifications under close observation in key sites or hotspots, in order to detect in real-time a divergence from the generating VOC and a possible new risk to global public health.

Variants under monitoring (VUM) are defined as variants that are suspected to pose future risk but with unclear evidence of phenotypic or epidemiological impact which requires enhanced monitoring. Currently, there is no variant under monitoring.

In the generation of SARS-CoV-2 variants, two processes, replication and recombination, have major implications. The SARS-CoV-2 replication process requires an RNA-dependent RNA polymerase (RdRp) that is prone to an error leading to the replication-associated changes. Due to the proofreading process assured by the virus-encoded 3′ exonuclease, nsp14, the mutation rate remains in the low range if we consider the magnitude of the SARS-CoV-2 extension [60].

The factors that may impact SARS-CoV-2 replication, error rate accumulation, and the variants selection include wildlife reservoirs (permissive host species and species-specific adaptation in different hosts) [61], biochemical characteristics of different infected cell types generating a heterogeneous mix of viral proteins [62], and population-level immunity driving selection of these variants [63]. Recombination can occur in a cell coinfected with more than one virus variant through discontinuous transcription of SARS-CoV-2 genomes via 'strand switching' by the viral RdRp or through breakage and rejoining of genomes based on homology regions to form chimeric genomes [63, 64].

Careful monitoring of new SARS-CoV-2 variants must reduce transmission rate, pathogenicity, and resistance to immune responses. This refers especially to molecules involved in cell entry and those that provide antigenicity.

The characteristics of SARS-CoV-2 variants are determined by the structural spike (S) protein cleaved in infected cells by a cellular protease, furin, yielding two subunits, S1 and S2. The S1 subunit binds the receptor ACE2 and the S2 subunit anchors the S protein to the membrane and mediates membrane fusion [65, 66].

In order to identify SARS-CoV-2 genome hotspots, a phylogenetic analysis of the virus is required. This analysis enables detection of the occurrence of variants that may present concern [67]. Until now numerous SARS-CoV-2 genome hotspots have been recognized. Being considered an important SARS-CoV-2 hotspot, spike genomic sequence is frequently altered (various substitutions/deletions) causing modifications in the protein sequence that ultimately may have a significant impact on virus evolution and can cause major difficulties in pandemic management. Consequently, certain mutations in spike protein can determine an increase in infectivity and also a more severe disease, while on the other hand can affect the therapeutic effectiveness [68].

During the early evolution of SARS-CoV-2, the **D614G** substitution in the receptor-binding domain (RBD) on Spike is considered to be one of the earliest S hotspots being detected in almost all previously circulating VOCs (Alpha, Beta, Delta, Gamma) as well in the current circulated VOC Omicron [69]. The mutation, originated from genetic drift and obtained a selective advantage [70, 71], being detected first in Europe in January 2020 and accounting for 98% of the SARS-CoV-2 spread in September 2021 [72]. In these variants, replacing the aspartic acid with glycine at position 614 of the spike protein induced structural change that increased binding affinity to ACE2 and virus entry [73], associated with increases in transmissibility (*in vivo* infectivity) but without affecting the severity of disease [71, 74].

Another recognized S hotspot is considered to be N501Y substitution also in RBD region, shared by Alpha, Beta, and Gamma lineages from previous VOCs. N501Y is known to promote viral replication through increasing affinity between the receptor-binding domain (RBD) and ACE2 and to facilitate the antibody escape [75]. P681H augments viral infectivity being located in the furin cleavage site [75], and delH69/V70 detected in Alpha and Omicron VOCs, was associated with disease severity and long-term infection [76].

Interfering also with antibody escape and thus being associated with vaccination failure were another two hotspots found in RBD region namely E484K and K417N/T

substitutions. E484K is found in Beta, Gamma, Delta, and Omicron VOCs while K417N/T is only in Beta and Gamma [77]. Hoter and Naim analyzed the biosynthetic forms and glycosylation of intracellular and secreted forms of double mutants L452R and E484Q (Indian B.1.617 variant) in comparison with E484K and N501Y (B.1.351 and P.1 variant) and observed that the double mutants L452R and E484Q were comparatively highly secreted, associated with a strong interaction with ACE2 in the human lung Calu3 cells [78]. L452R and T478K hotspots, identified in Delta variant, were associated with an increased ACE2 binding and also with antibody escape, which led to an increase in virulence [79, 80].

Compared with wild-type Wuhan-1 bearing D614G, the **Delta (B.1.617.2)** was six-fold less sensitive to serum neutralizing antibodies from previously infected individuals, and eight-fold less sensitive to vaccine-elicited antibodies, lower in ChAdOx1 vaccinees than in BNT162b2 vaccinees [81]. The B.1.617.2 variant proved to be highly fusogenic and notably more pathogenic than its parental virus due to the highly conserved P681R mutation in the spike protein facilitating cleavage of the spike protein and enhancing viral fusogenicity [82].

It is crucially important to determine spike mutations that affect antigenic profiles and the level of cross-protection provided by prior infection with other viruses. The immunogenic regions of the spike refer especially to the spike receptor-binding domain (RBD) because ~90% of the serum neutralizing antibodies from SARS-CoV-2 infected individuals target this region [83–85]. But, also, the N-terminal domain (NTD) of the S protein is targeted [86–89].

4. SARS-CoV-2 vaccine development in COVID-19 genomic era

A major advantage in reducing the COVID-19 pandemic was the development of vaccines against SARS-CoV-2 since all the approved COVID-19 vaccines, although based on the initial SARS CoV-2 strain, continue to preserve efficacy against hospitalization and death, especially after administration of a booster dose [90]. Until the appearance of Omicron, all variants had a convergent evolution pattern [91], selecting similar mutations in particularly vulnerable genomic sites and clustering in similar serotypes. Omicron is highly antigenically divergent from the other VOCs [92] and is characterized by a continuous antigenic drift, giving rise to several sublineages, with limited cross-antigenicity. The most recently selected sub-lineages, designated as variants of concern (BA4, BA5) have higher neutralization escape capacity [93], and vaccination efficacy seems to decrease, although not significantly, even in terms of protection against severe forms of the disease.

Currently, according to the WHO, more than 150 vaccines are in clinical development and almost 200 are in preclinical development [94]. Reported studies demonstrated that the benefits of COVID-19 vaccination compensate for the risks that involve rare but serious adverse effects [95]. For example, a study focused on the administration of almost two million first doses of the vaccine Pfizer-BioNTech vaccine in the US reported only 21 cases of anaphylaxis after administration, with no fatalities reported [96].

COVID-19 vaccines developed so far and tested or approved for clinical trials can be classified into inactivated vaccine, live attenuated, viral vector-based vaccine, RNA, DNA, protein subunit, and virus-like particle (VLP) vaccines [97].

Inactivated vaccines are obtained from a virus multiplied on cell cultures and then chemically inactivated. This system can sustain stably expressed,

conformationally native antigenic epitopes [98]. The advantages of inactivated vaccines include the capacity of the vaccine to induce an immune response that results in production of antibodies against many epitopes of the SARS-CoV-2, including S protein, N protein, and E protein [99]. On the other hand, the vaccine is well tolerated, the adverse reactions reported are rare, without reported deaths, and the study and development of this type of vaccine are relatively complete [100]. The disadvantages arise mainly from the fact that the living virus must be manipulated in a biosafety level-3 laboratory at least and there is a limitation in vaccine production that depends on viral productivity [97].

Viral vector-based vaccine is also considered a classic vaccine since this medical technology was introduced in 1972 by Jackson et al., and uses a secondary virus as a transient gene expression vector. Nonreplicating viral vector-based vaccine, the most commonly utilized, uses viral vectors deficient in replication, to deliver a specific antigen to the host cell in order to induce immunity against the desired antigen. The vector used in the viral vector-based vaccine developed against SARS-CoV-2 infection is adenovirus [101]. Administration of this type of vaccine against SARS-CoV-2 infection seems to induce rapid and complex antibody responses as well as cellular immune responses by activation of Th1 cell responses [102]. Comparing to inactivated vaccines the production of viral vector vaccines is safer as there is no need to manipulate live SARS-CoV-2. However, in the case of adenovirus-based viral vector vaccines, rare but severe reactions have been reported, especially thrombocytopenia, sustaining the need of monitoring platelet levels. The mechanism that triggers these effects is mainly the development of pathological anti-platelet factor 4 (PF4) antibodies, as result of vaccine administration that activates platelets and the coagulation system. Also, the immunogenicity of these vaccines can be reduced in some people that present neutralizing antibodies against several adenoviruses [103].

Live attenuated vaccines are developed by a recoding of the virus genome, being a well-known method of immunization against pathogens. Thus, the virus is attenuated by *in vitro* or *in vivo* passage or reverse-genetic mutagenesis, resulting in a weakly pathogenic that is also able to mimic the live virus infection. Usually, this type of vaccine can produce a durable immune response, but the apparition of secondary mutations that can cause reversion into virus wild-type strains, especially in the case of RNA viruses is considered a disadvantage for this type of vaccine [99, 104, 105].

DNA vaccines use a sequential transcription-to-translation process that sustain the production in host cells of a viral antigen that is encoded by a recombinant DNA plasmid, inducing neutralizing antibodies [105]. DNA vaccines present a higher stability compared with mRNA vaccines**,** the production risk of DNA vaccines is relatively low and does not require the presence of an infectious agent. On the other hand, the immunogenicity of the DNA vaccine is low and the efficacy depends on the injection method [97].

mRNA-based vaccines comprise mRNA molecules that encode viral protein antigens and the main problem of this type of vaccine is removed by utilization of nanoparticle delivery carriers that overcome mRNA instability. Moreover, these nanoparticles are adjuvants to sustain the activation of the immune response. The method to obtain these vaccines is based on the *in vitro* transcription process for obtaining mRNA and the technology is quite developed today to allow obtaining large doses in a short time for any pathogen [106]. Due to the high vaccination rate with this type of vaccine, it can be clearly demonstrated that SARS-CoV-2 mRNA-based vaccination induces a persistent germinal center B cell response and Th1 cell responses, which allows the development of strong humoral immunity [107]. The main

disadvantages are the reported adverse reactions, especially myocarditis and the necessity to preserve the vaccines at low temperatures to avoid mRNA degradation [108].

Protein subunit vaccines use key viral proteins or peptides that can be obtain *in vitro* using bacteria, yeast, or mammalian cells [99]. COVID-19 protein subunit vaccine can induce Th1 cell responses and a high titer of neutralizing antibodies but due to the large molecular weight, the *in vitro* synthesis rate of the viral S protein is relatively low [97].

Virus-like particle vaccines use viral capsid proteins or replication-defective virus particles without the viral genomes but the technology for obtaining this type of vaccine is more complex [109].

Although studies to date show that the administration of COVID-19 vaccines may reduce the risk of symptomatic infection and decrease mortality, a decline in virus-neutralizing activity with the emergence of new variants has been reported. Therefore, the strong contagious activity of Alpha and Delta variants and the powerful immune escape ability of Beta and Gamma variants were outclassed by the capacity of the Omicron variant to evade the immunity induced by the COVID-19 vaccines [97]. For example, the efficacy of BNT162b2 COVID-19 vaccine (Pfizer BioNTech) against SARS-CoV-2 VOCs starts at almost 90% in the case of B.1.1.7 (Alpha) [110] and decreases (in some reports) to about 35% in the case of B.1.1.529 (Omicron) [111].

Several studies have shown that breakthrough infections including Omicron VOC increase the breadth of the immune response in vaccinated persons [112]. As such, Omicron-specific vaccine candidates have been developed by several pharmaceutical companies and might be administered as booster doses for the recipients of a primary vaccination scheme or for persons already infected with previously circulating variants. In recent press releases, both Pfizer and Moderna [113, 114] announced that a second booster with Omicron-adapted vaccine candidates (either in a monovalent or bivalent formulation with the classic vaccine) increased significantly the magnitude of the immune response against the Omicron sublineages. It is hoped that such broad responses will be preserved for a longer period of time, as shown by data from trials with a previous version of a Moderna vaccine candidate developed against Beta-another highly immune evasive variant. Presently, administration of an Omicron-specific vaccine in unvaccinated persons is not recommended, due to insufficient data on the level of cross-protection against unrelated variants [115].

Future vaccination strategies are envisioned, aimed at finding better administration regimens (using extended intervals between doses, increased antigen concentrations, heterologous prime-boost schemes), or better vaccine formulations (multivalent vaccines, encoding the Spike protein of multiple VOCs, pan-coronavirus vaccines, and mucosal vaccines, administered intra-nasally).

5. Antiviral treatment development and emergent SARS-CoV-2 variants

The development of direct antiviral drugs was rather slow, direct medication being replaced by vaccines in the treatment of COVID-19. However direct antiviral treatment proved to be effective, indifferent to the mutations that have accumulated while SARS-CoV-2 variants have emerged. Currently, the main therapeutic strategies are directed toward (a) direct inhibition of the viral entry, (b) inhibition of viral replication, and (c) immunomodulatory treatment to block the cytokine release storm that underlies COVID-19 severe evolution [116].

5.1 Direct inhibition of the viral entry

In the first category, there are several anti-spike protein monoclonal antibodies (MAB) such as bebtelovimab, sotrovimab, casirivimab and imdevimab, bamlanivimab and etesevimab used for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (12 years of age and older weighing at least 40 kg). Additionally, tixagevimab and cilgavimab were authorized for emergency use as pre-exposure prophylaxis for prevention of COVID-19 in adults and pediatric individuals. However, in the context of high frequency of the Omicron BA.2 sub-variant, authorizations for all of these monoclonal antibodies were revoked, except for Bebtelovimab that has a broad neutralizing activity, unaffected by the most common mutations present in all of the known variants of concern of SARS-CoV-2, including the Omicron subvariants BA1/BA2 [117, 118].

The administration of MABs has several significant drawbacks, including the need for intravenous administration in healthcare units by qualified healthcare personnel who have access to emergency medications to treat severe reactions, including anaphylaxis. Common side effects include hypersensitivity, with anaphylaxis and infusion-related reactions, nausea, vomiting, pruritus, and rash. Among advantages, we can count no drug-drug interactions.

This strategy can be used against the receptor binding domain only as long as no mutations in the spike glycoprotein occur. One possible way around this problem would be administration of several MAB antibodies that could simultaneously bind to different parts of the receptor binding domain of the SARS-CoV-2 spike protein.

5.2 Inhibition of viral replication

To date, several antivirals received conditional authorizations for usage in the interest of public health because they address an unmet medical need and the benefit of immediate availability outweighs the risk from less comprehensive data than normally required (**Table 1**) [119]. The oldest is remdesivir, a drug that has to be intravenously administrated, with plenty of adverse effects, which drastically reduce its utility, conditioning its administration by hospitalization. The newest molnupiravir and nirmatrelvir – ritonavir are both oral antivirals, less expensive, with huge advantage that they can be administered in patients isolated at home. However, the treatment has to be started early, and Paxlovid came with several drug–drug interactions that can complicate its use in patients taking other medications. Moreover, there is a concern about molnupiravir regarding a potential impairment of bone and cartilage growth, being restricted for usage in children [120].

Molnupiravir is a slightly modified small-molecule drug developed from a ribonucleoside known as NHC (β-d-N^4-hydroxycytidine) by a research team at Emory University in Atlanta, Georgia. Intended initially to enter clinical trials against influenza, tested it as a treatment for COVID-19 by Ridgeback Biotherapeutics Company, which partnered later with Merck in May 2020, for large-scale clinical trials [121].

After oral administration, molnupiravir breaks down to form NHC that is further phosphorylated to NHC triphosphate. Under this form, NHC is linked by SARS-CoV-2 RNA-dependent RNA polymerase (RdRP) and used for RNA chain elongation during viral replication instead of guanosine or adenosine. This leads to an accumulation of errors in the viral genome that ultimately render the virus noninfectious and unable to replicate [122–124].

Compound	Molnupiravir	Nirmatrelvir - Ritonavir	Remdesivir
Brand name:	Lagevrio	Paxlovid	Veklury
Market authorization:	Merck Sharp and Dohme (UK) Limited	Pfizer Limited	Gilead Sciences, Inc
Alternative names:	MK MK-4482, EIDD-2801	PF-07321332	GS-5734
Pharmacological classification	inhibitor of the viral RNA-dependent RNA polymerase (RdRp)	Mpro viral protease inhibitor	inhibitor of the viral RNA-dependent RNA polymerase (RdRp)
Indications:	Treatment of mild-to-moderate COVID-19 in adults with symptom onset within 5 days from diagnosis with risk for developing severe illness.	Treatment of mild-to-moderate COVID-19 patients aged 12 years and older, with risk for progression to severe COVID-19, without need for supplemental oxygen.	Treatment of mild-to-moderate COVID-19 patients with symptom onset within the previous 7 days, with at least one risk factor for disease progression (age ≥ 60 years, obesity, or certain coexisting medical conditions)
Dose:	800 mg every 12 hours for 5 days	300 mg nirmatrelvir with 100 mg ritonavir, every 12 hours for 5 days	200 mg on day one, followed by 100 mg daily for up to 9 additional days

Table 1.
Anti-viral drugs currently used for COVID-19 therapy.

Efficacy and safety were evaluated in phase 3 double-blind, randomized, placebo-controlled trial MOVe-OUT clinical trials [NCT04575597] on unvaccinated and seronegative subjects, with final results published on February 10, 2022, reporting a relative risk reduction of 30% for hospitalization or death at 29 days [122]. Moreover, new results in evaluating virological outcomes presented at the 2022 European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) (Abstract #4514) showed that molnupiravir was associated with more rapid elimination of infectious viruses than placebo. Thus, at 3 and 5 days of treatment, no infectious virus was detected in patients who received LAGEVRIO compared with 21.8%, and 2.2% respective, of patients who received placebo [125]. The risk for adverse effects was 30% with molnupiravir vs. 33% with placebo. The most common adverse effects reported were diarrhea (3%) and nausea (2%) classified as mild or moderate. Also, no drug–drug interactions have been identified [122].

Molnupiravir received an Emergency Use Authorization (EUA) issued by the Food and Drug Administration (FDA) on December 23, 2021 for LAGEVRIO as treatment of mild-to-moderate COVID-19 in adults with positive results of direct SARS-CoV-2 viral testing, who are at high-risk for progression to severe COVID-19, including hospitalization or death [126]. Also, received authorization in the United Kingdom (U.K.) for molnupiravir (MK-4482, EIDD-2801), and currently is under review for authorization by European Medicines Agency (EMA).

Nirmatrelvir – Ritonavir (Paxlovid) is a combination of two drugs, ritonavir (a pharmacokinetic enhancer) and newly developed protease inhibitor nirmatrelvir

(PF-07321332), a structure-based potent inhibitor of SARS-CoV-2 3-chymotrypsin–like cysteine protease enzyme (Mpro) [127]. Mpro protease is involved in processing viral polyproteins into functional units, and since essential to viral replication. Within the absence of recognized human analog, Mpro is an attractive antiviral target across a wide spectrum of coronaviruses. Nirmatrelvir is administrated with ritonavir, an inhibitor of cytochrome P450 (CYP) 3A4, that blocks the metabolism of nirmatrelvir increasing and maintaining its plasma concentration approximately five to six times higher than the in vitro 90% effective concentration [127, 128].

Evidence for efficacy and safety comes from a phase 2–3 double-blind, randomized, and controlled trial, which enrolled unvaccinated, non-hospitalized adults with high risk for progression to severe COVID-19. Subjects that were treated within 3 days after symptom onset with 300 mg of nirmatrelvir plus 100 mg of ritonavir had an 89% lower risk of progression to severe COVID-19 than placebo group [NCT04960202]. The risk for adverse effects was 22.6% with nirmatrelvir plus ritonavir vs 23.9% with placebo. The most common adverse effects were dysgeusia (5.6%) and diarrhea (3.1%) [129].

Paxlovid is associated with several drug–drug interactions that could complicate its use in the community due to ritonavir association that inhibits CTP3A and therefore may increase plasma concentration of drugs that may be associated with serious, life-threatening events (e.g., colchicine, clozapine, diazepam, simvastatin, etc.). Conversely, products that may increase the metabolism of nirmatrelvir/ritonavir and reduce their concentrations may be associated with a loss of antiviral effect (e.g., rifampicin, carbamazepine) [130]. Thus, a careful assessment of patients' medication is needed before administrating Paxlovid. A web tool developed by the University of Liverpool, which monitors drug interactions with current anti-COVID-19 therapies, may be helpful [131].

Nirmatrelvir – Ritonavir received a EUA issued by the FDA on December 22, 2021, for PAXLOVID for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (12 years of age and older weighing at least 40 kg) with high risk for progression to severe COVID-19 [132]. It is also authorized for use in European Union [133] since January 2022, under a conditional marketing authorization.

Both molnupiravir and nirmatrelvir, effectively inhibited viral replication of the Delta variant and the Omicron variant however, slight differences in antiviral response among wild-type, Delta, and Omicron variants were observed [134].

Remdesivir is a broad-spectrum antiviral medication administered via intravenous injection. The compound is a prodrug whose metabolizing leads to the release of a nucleoside analog GS-441524 monophosphate with subsequent biotransformation into GS-441524 triphosphate. Under this form, it acts as an inhibitor of the viral RNA-dependent RNA polymerase (RdRp) with potent activity against an array of RNA virus families including Filoviridae, Paramyxoviridae, Pneumoviridae, and Orthocoronavirinae [135]. Various studies have documented its inhibitory activity against SARS-CoV-1, Middle East respiratory syndrome (MERS-CoV), and SARS-CoV-2 in vitro [136, 137].

Data on its efficacy on COVID-19 infected patients came from ACTT-1 clinical trial [NCT04280705], which showed that remdesivir treatment shorten recovery times in hospitalized patients with COVID-19 (median recovery time of 10 days (95% confidence interval [CI], 9 to 11), in remdesivir treated patients as compared with 15 days (95% CI, 13 to 18) among those who received placebo) [138] and reduces chances of hospitalization or death with 87% for patients at high risk of severe disease [139].

The most common adverse effects that occurred in 5% of patients treated with remdesivir were respiratory failure, decreased glomerular filtration rate with increased creatinine in the blood, decreased lymphocyte and hemoglobin counts, anemia, and increased blood sugar levels [138]. Also, 5% of patients experienced nausea, headache, and cough.

In October 2020, remdesivir received the first FDA EUA as a treatment for COVID-19, which was extended in April 2022, for the first time for pediatric patients under 12 years of age including those who are older than 28 days, weighing at least 3 kg [140]. Also, in July 2020 received a conditional marketing authorization from EMA.

Remdesivir showed similar antiviral activity against the wild-type virus and the VOCs Alpha, Beta, Gamma, Delta, and Omicron variants. These findings are justified by the fact that the target proteins of these antivirals, viral RNA dependent RNA polymerase, and the viral main protease Mpro, respectively, are highly conserved. These results indicate that is a high probability that VOC that might emerge in the future will remain susceptible to antivirals that do not target the spike protein [141].

5.3 Immunomodulatory treatment

Many of the complications associated with COVID-19 are due to an augmented host immune response, which contributes to the increased severity of COVID-19 and death. Several immunomodulatory drugs can be administered in-hospital to severely ill patients to reduce inflammation and prevent a cytokine storm. These include corticosteroids, monoclonal antibodies that block the IL-6 receptor (tocilizumab, sarilomab, and siltuximab), monoclonal antibodies that block the IL-1 beta receptor (anakinra), and selective Janus kinase 1 and 2 inhibitors (JAK1 and 2) (baricitinib and ruxolitinib).

Immunomodulatory treatment leads to an improvement in clinical outcome. Among positive results, a decrease in hospitalization lengths, duration of mechanical ventilation, and mortality with 8.7% in the critically ill, and 6.7% in patients with severe COVID-19, are the most notable ones. Coadministration with anti-viral such as remdesivir, improved the clinical outcome, reducing the number of patients who experience progression to severe respiratory failure or death [142].

However, reported side effects are major. For example, anakinra treatment induces a decrease in hematological parameters, headache, diarrhea, an increase of liver function tests, and hyperglycemia [143]. Treatment with tocilizumab also caused neutropenia with severe infections, thrombocytopenia, and increased the liver enzyme levels. Moreover, several cases of bowel perforation were also reported [144, 145].

Another strategy that has been used in COVID-19 treatment included the use of COVID-19 convalescent plasma or ultrapotent antibodies isolated from SARS-CoV-2 elite neutralizers. These are individuals that displayed a highly potent neutralizing response with IgG 50% inhibitory concentration (IC50) values of <20 μg/mL. The ultrapotent antibodies are directed against conserved viral epitopes with broad spectrum activity against ancestral variant and the variant that emerged lately: B.1.1.7, B.1.351, B.1.429, B.1.617, and B.1.617.2 variants [146].

Convalescent plasma administration is nevertheless limited to high-titer products. It was associated with allergic and anaphylactic reactions febrile nonhemolytic reactions, hemolytic reactions, metabolic complications, transfusion-transmitted infections, and thrombotic events. Moreover, there is a theoretical risk of antibody-mediated enhancement of infection and suppressed long-term immunity [147].

Currently, due to the intense studies carried out during the SARS-CoV-2 pandemic, several treatment modalities are available for COVID-19. There are both

molecules that block the virus from entering the cell, and molecules that interfere with and block viral replication. In addition, there are immunological modulators that can prevent severe development and even death. These therapeutic strategies are supported by prophylactic ones (e. g. vaccines), all in conjunction with aim of avoiding the disruption of social and economic calm.

6. The impact of genomics on public health decisions

The emergence of SARS-CoV-2 in December 2019 triggered an unprecedented cascade of public health measures aiming at delaying the virus introduction in specific countries; prevention or limitation of viral transmission in the community; rapid tracing, identification, and isolation of contacts; and sheltering of the most vulnerable populations. These measures benefited from almost real-time surveillance of viral spread using genomic characterization.

At the beginning of the pandemic, the rapid development of sensitive real-time PCR tests was facilitated by the immediate sharing of genome sequence data. This allowed the implementation of national NAAT-based testing programs and supported the rapid diagnosis of infection, followed by preventive measures of contact tracing and isolation and quarantine – a policy known as TETRIS or TTI or TTIQ - test, track, isolate (and quarantine).

Whole genome sequencing has identified independent introduction of SARS-COV-2 from international travels, followed by local transmission clusters in individuals with no previous travel history, triggering interdictions of mass gathering and stay-at-home orders in many European countries [148, 149]. Further on, large nationwide programs of routine genetic sequencing implemented by several countries across all continents allowed for the rapid identification of new viral variants, further labeled as variants of interest (VOIs) and variants of concern (VOCs). The COG-UK consortium has identified the emergence of B.1.1.7/Alpha VOC at the end of 2020, a finding that triggered a reinstatement of lock-down in the UK [150].

Mathematical modeling of many epidemiological and social parameters were important pieces in the complicated scenarios of policymaking, as they sometimes furnished reliable predictions on the shape, amplitude, and severity of the pandemic. These parameters were adapted each time a new variant of concern was identified. In addition, genomic sequencing revealed specific mutations in the circulating viral strains that allowed rapid testing for variant monitoring – such as S target failure in a specific PCR test in the case of the Alpha VOC (B.1.1.7), due to deletion at positions 69 and 70 of the spike protein (delH69/V70). These data were conducted for a fast implementation of this biomarker in the SARS-CoV-2 community PCR testing program of Public Health England in the early autumn months of 2021 [151]. Whole genome sequencing allowed a rapid warning of the global community when variants of concern emerged and further monitoring of their dissemination and displacement of previously circulating variants. The availability of free giant repositories for whole viral genomes such as GISAID, sometimes with associated epidemiological and clinical metadata, enabled a fast follow-up of the viral spread, and thorough characterization of the variants' transmissibility and pathogenicity. For example, the genetic surveillance network in South Africa has rapidly spotted the Beta variant (B.1.351. identified in October 2020) harboring mutations associated with immune evasiveness) and the highly-mutated Omicron variant (B.1.1.529; first identified in November, 2021) [152, 153]. This information was used to back up reinforcements or

relaxations of some of the most drastic public health measures, such as lockdowns, border control, closing working places and schools, social distancing, mobility restrictions, and obligatory green passes. For example, accumulating genomic information on the spread of the highly transmissible, yet low pathogenicity Omicron variant triggered a progressive abandonment of the "zero COVID" policy with compulsory curfews, testing and strict mobility control initially adopted by a series of Eastern-Asian countries [154].

The importance of genomics for public health is underlined by the unexpected emergence of the Omicron variant, attributed either to (a) a continuous, baseline circulation of a slowly changing ancillary strain in a region with low genomic surveillance; (b) persistent infections with prolonged viral shedding and high variability in immunosuppressed persons; and (c) spill out from an unknown animal reservoir [155]. To prevent a similar episode, genomic informed public health measures must be upscaled, including:

- The establishment of an extended global network of pathogen surveillance pursuing a "one health" policy at the human-animal interface
- The use of portable nanopore DNA sequencers for rapid outbreak monitoring in low resource settings
- Early viral detection and characterization using wastewater surveillance systems. Cryptic SARS-CoV-2 lineages, previously unreported by sequencing of symptomatic human cases of SARS CoV-2 infections, harboring common mutations with the Omicron variant and partially resistant to neutralizing antibodies from vaccinated and especially from previously infected patients have been detected in New York City wastewater in 2021 [156].

Genomic data can inform public health policies by:

- Identifying the source of initial clusters of cases in particular settings (health care settings, long-term care facilities, travel vehicles-airplanes, and cruises ships) or in the community, by linkage of the genomic data to demographic, epidemiological, and clinical data sets [157]
- Analyzing super-spreading events to identify human behaviors that are more prone to viral transmission [158]
- Monitoring the viral variability and assessing their potential impact on the community by associating new genotypic features with changes in the antigenicity, infectivity, pathogenicity, and susceptibility to available antivirals and vaccines.
- Adaptation of the vaccine composition to ensure the production of the most efficient vaccines in a timely manner [159].

7. Conclusions

The rapid development of diagnostic tools and COVID-19 vaccines was possible due to the characterization of viral genome and of the structure of the main viral immunogen-the spike glycoprotein.

Surveillance of the emergent variants and assessing their potential impact on the community by genotype to phenotype analysis may control reduction of the effectiveness of available antivirals and vaccines. COVID-19 vaccines developed to date proved to be highly active against hospitalizations and death across all ages, and their large-scale deployment in the middle and high-income countries, has decreased the pressure on the medical system and helped in reopening the economy.

Currently available antiviral therapies prove to be reasonably effective regardless of viral variants and their development should be an important strategy together with vaccination strategy improvement.

With the emergence of viral variants more antigenically distant from the vaccine strains, the utility of adaptation of the vaccine composition, either by adding a variant specific version or by developing a pan-coronavirus vaccine become an important point on the public health agenda.

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Conflict of interest

The authors declare no conflict of interest.

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