Antiviral Research 240 (2025) 106208

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

# Unveiling SARS-CoV-2's heart: role, structure and inhibition of SARS-CoV-2 RNA-dependent RNA polymerase

Paolo Malune, Francesca Esposito, Enzo Tramontano *

*Department of Life and Environmental Sciences, Faculty of Biology and Pharmacy, University of Cagliari, Cittadella Universitaria di Monserrato, ss554 Km 4500, Monserrato, Cagliari, 09045, Italy*

A B S T R A C T

SARS-CoV-2 emergence in late 2019 represented an incredible challenge for virological research and the development of novel as well as repurposed antiviral agents. Among the targeted viral proteins, one is nsp12 that carries the RNA-dependent RNA polymerase (RdRp) activity, essential for the viral replication. Together with nsp7 and nsp8 cofactors, having an essential role in aiding processivity and associated with several other nonstructural proteins such as helicase, methyltransferase, endo- and exonuclease, nsp12 forms the large viral replication and transcription complex (RTC). Within such RTC, nsp12 catalyzes the synthesis of one of the longest RNA genomes in the viral world, requiring exceptional speed, processivity and fidelity compared to other viral RdRps. Moreover, the peculiar replication cycle of coronaviruses requires nsp12 to perform less conventional functions in backtracking on the viral genome for proof-reading activity and "jumping" during discontinuous synthesis of subgenomic mRNAs. The structure of the minimal RTC was resolved with a resolution <3 Å by cryo-electron microscopy in complex with RNA and with inhibitors, opening the doors to structural studies on its functions and drug development. Given its essential role in viral replication, extensive research was carried out over the last years to identify both nucleoside (NI) and non-nucleoside (NNI) inhibitors, resulting in two NIs reaching clinical use, although their efficacy *in vivo* is still under evaluation. This review aims at summarizing the currently known structural and functional aspects and the state-of-the art in drug discovery for SARS-CoV-2 RdRp.

## 1. Introduction

SARS-CoV-2 belongs to the β-coronavirus genus, subgenus Sarbecovirus, of the family *Coronaviridae*, which includes other 3 genera: α-coronavirus, γ-coronavirus, and δ-coronavirus, according to the International Committee on Taxonomy of Viruses. Among all known members of the *Coronaviridae* family, seven viruses are human pathogens: four of them (HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43) are low pathogenicity coronaviruses, etiological agents of seasonal and mild respiratory tract infections, while the remaining three (SARS-CoV, MERS-CoV and SARS-CoV-2) are responsible for important upper and lower respiratory diseases, with substantial pulmonary involvement and life-threatening clinical presentations (Brant et al., 2021; V'kovski et al., 2021).

SARS-CoV was identified in 2002 as the etiological agent of the SARS outbreak of ~8k cases (Z. Zhao, 2003), while MERS-CoV emerged in 2012 in Saudi Arabia and then spread to 27 countries, with total of ~2.5k cases (Bermingham et al., 2012; Zaki et al., 2012). This marked SARS-CoV-2 as the third highly pathogenic HCoV to have emerged only in the last 20 years. Both SARS-CoV and MERS-CoV showed high fatality rates but limited transmissibility, largely confined to localized outbreaks (Jiang et al., 2021; Pormohammad et al., 2020). In contrast, SARS-CoV-2 spread globally at an alarming rate. The high transmissibility of SARS-CoV-2 has made it responsible for over 777 million infections and 7 million deaths as of March 2025 (World Health Organization, 2025).

SARS-CoV-2 possesses a ~29.9 kb ssRNA(+) genome, making it one of the longest viral RNA genomes identified so far (C. Cao et al., 2021). Its genome includes 16 nonstructural proteins (nsps), 4 structural proteins (spike, membrane, envelope, and nucleocapsid), and several accessory proteins. These components coordinate to mediate viral replication, immune evasion, and host cell manipulation (Brant et al., 2021; Steiner et al., 2024; V'kovski et al., 2021). The spike protein allows viral entry into cell by binding to host receptor ACE2, while the envelope, membrane, and nucleocapsid proteins contribute to virion assembly. Once inside the cytoplasm, the viral RNA is translated into two polyproteins, pp1a and pp1ab, which are cleaved into monomeric nsps by viral proteases nsp3 and nsp5. The replication-transcription complex (RTC) then assembles at the 3′ end of the viral genome, synthesizing new genomic and subgenomic RNAs for further protein synthesis. Genomic RNAs are encapsidated by the nucleocapsid protein and assembled into virions at the ER-Golgi intermediate compartment (ERGIC). Virions are then transported to the cell surface via lysosomal

* Corresponding author.
*E-mail address:* tramon@unica.it (E. Tramontano).

https://doi.org/10.1016/j.antiviral.2025.106208
Received 27 March 2025; Received in revised form 11 May 2025; Accepted 1 June 2025
Available online 3 June 2025

exocytosis (Brant et al., 2021; Steiner et al., 2024; V'kovski et al., 2021).

Constituting the RTC catalytic core, nsp12 displays an RNA-dependent RNA polymerase (RdRp) activity, essential for viral genome replication and transcription, in complex with its cofactors nsp7 and nsp8, which are crucial for RNA binding and RdRp processivity (Brant et al., 2021; Steiner et al., 2024; V'kovski et al., 2021). Such RTC, synthesizing one of the largest genomes among RNA viruses, demands exceptional speed, processivity, and fidelity, which the virus carries on through different strategies. Furthermore, nsp12 functions extend beyond canonical RNA polymerization since this enzyme acts within a larger multiprotein assembly that includes nsps with helicase, methyltransferase, endonuclease and exonuclease functions, enabling the RTC to perform several and diverse tasks (Brant et al., 2021; Steiner et al., 2024; V'kovski et al., 2021). In the context of this multimeric complex, nsp12 performs specialized roles such as genome capping and template switching during the discontinuous synthesis of subgenomic mRNAs, and it is also involved in nsp13-aided genome backtracking for proofreading, underscoring its critical task in maintaining the integrity and efficiency of the replication process (Brant et al., 2021; Steiner et al., 2024; V'kovski et al., 2021). In confirmation of the importance of nsp12 in the viral replication cycle and as a drug target, as of March 2025, two of the three drugs currently used for COVID-19 treatment—remdesivir (Veklury) and molnupiravir (Lagevrio)—target the RdRp activity of SARS-CoV-2 (European Medicines Agency, 2023; U.S. Food and Drug Administration, 2021).

In this review, we aim to provide a comprehensive overview of SARS-CoV-2 nsp12, focusing on its structural and functional characteristics, its critical roles in the viral replication cycle, and its role as a target for antiviral therapies.

## 2. Structural and biophysical insights into SARS-CoV-2 RdRp

Comparison of a comprehensive set of available structures of viral RNA polymerases revealed the strong structural conservation among these proteins, with 6 common motifs (from A to F) forming the classical right-handed shape that is typical of the superfamily of DNA and RNA polymerases (Buonaguro et al., 2020; Jácome et al., 2015). The structural similarity is much less evident when positive-sense RdRps are compared to the ones of negative-sense viruses (Buonaguro et al., 2020). The first reported cryo-EM structure of SARS-CoV-2 nsp12 in complex with cofactors nsp7 and nsp8 dates to early 2020 with a reconstruction of 2.9 Å (Gao et al., 2020), subsequently integrated by other resolved cryo-EM reconstructions with and without the RNA substrate (Hillen et al., 2020; W. Yin et al., 2020) (Fig. 1A–B). The np12 + nsp7/8 complex, forming the minimal replication and transcription complex (RTC) of SARS-CoV-2, is globally similar to the one resolved for SARS-CoV, with a rmsd of 0.82 for 1078 Cα atoms (Gao et al., 2020). The structure was resolved as a complex of one monomer of nsp12, a dimer of nsp7 and nsp8 (referred to as nsp8-1) and one additional monomer of nsp8 (referred to as nsp8-2). The overall nsp12 structure confirmed the resemblance to the one of a "classical" viral polymerase, with a right-handed shape for the RdRp domain (spanning from residues S367 to F920), while the N-terminal region (from residues A4 to T28 and from Y69 to R249) forms the NiRAN domain (Nidovirus RdRp-associated nucleotidyl transferase), that is typical of coronaviruses. The two domains are connected by an interface region, constituted by residues A250-R365 (Gao et al., 2020), composed of three helices and five β-strands (W. Yin et al., 2020). The RdRp domain of nsp12 is composed of the conserved three subdomains: fingers (residues L366-A581 and K621-G679), palm (residues T582-P620 and T680-Q815) and thumb (residues H816-E920) (Gao et al., 2020) (Fig. 1C). The fingers

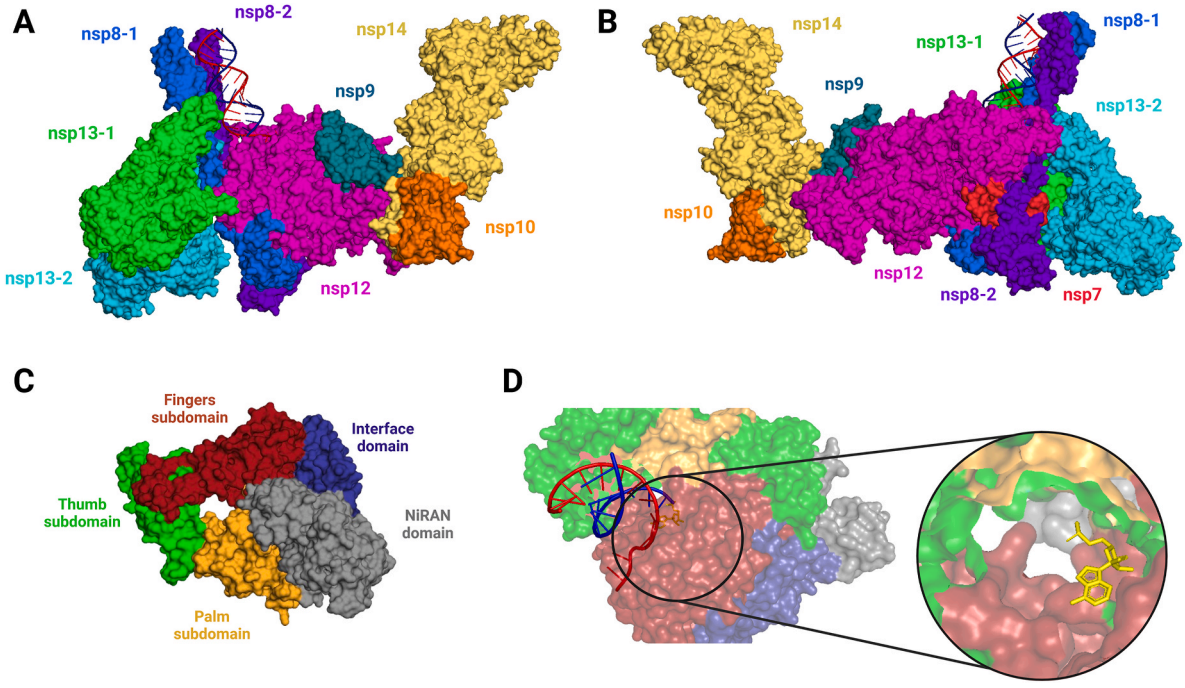


**Fig. 1.** Assembly and Structure of SARS-CoV-2 Replication and Transcription Complex
Fig. 1 (A-B) SARS-CoV-2 nsp12 (magenta) forms a multimeric complex with $nsp8_2$ (monomer 1 blue; monomer 2 purple), nsp7 (red), $nsp13_2$ (monomer 1 green; monomer 2 aquamarine), nsp9 (petrol), nsp14 (yellow) and nsp10 (orange). The complex takes contact with the double-strand RNA substrate (primer in blue, template in red). Cryo-EM reconstruction from PDB 7EIZ. Panel B shows an 180° oriented view of the structure in panel A. (C) Nsp12 is composed of two functional domains (NiRAN, grey) and RdRp, which is further divided into in three subdomains: fingers (dark red), palm (yellow) and thumb (green). The two NiRAN and RdRp domains are connected by an interface domain (dark blue). (D) Zoomed-in view of the catalytic site of nsp12 with primer (blue) and template (red) RNA duplex, and remdesivir triphosphate (yellow) poised for catalysis. Color-code is respected compared to panel C, showing that the RTC active site is made of a pocket formed by the palm, thumb and fingers subdomains of nsp12. Cryo-EM reconstruction from PDB 7BV2. Created in https://BioRender.com. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

subdomain forms a closed circle with the thumb subdomain. The NiRAN domain is composed of seven helices and three β-strands (W. Yin et al., 2020). Minimal differences are observed between the apo structure and the one determined in presence of RNA: in particular they are a minor shift of nsp7 towards the nsp12 core, a translocation of ~3 Å in the thumb subdomain and one in motif G to better allocate the RNA template, suggesting that only minimal amounts of energy are required for conformation changes by the polymerase to initiate replicase activity (W. Yin et al., 2020).

The RdRp active site is located in a central cavity of the palm subdomain, formed by motifs A-G, and opens up to the solvent through the NTP and RNA entry channels, which are positively charged (Gao et al., 2020). Motif A contains the well-conserved divalent cation-binding residue D618, while motif C contains the catalytic residues 759-SDD-761, also strongly conserved in most viral RdRps (Gao et al., 2020). Motif B binds the sugar/base moiety of the incoming NTPs (Neogi et al., 2020). The NTP entry channel, instead, is composed of several positively charged amino acids, such as K545, R553 and R555 in motif F (Gao et al., 2020), which are involved in binding the triphosphate moiety of the incoming NTPs (Neogi et al., 2020). F and G strands appear to direct RNA strands into the nsp12 active site. Motif F takes contact with the nascent primer strand, allowing its correct orientation for the incorporation of the incoming NTP. A total of 29 amino acids from nsp12 are observed to be involved in duplex RNA binding, but surprisingly no residue of nsp7 nor nsp8 appears to be involved in the direct interaction with the RNA, although their presence is essential for nsp12 catalytic activity (W. Yin et al., 2020). Most of the identified interactions involve the 2′-OH group in the ribose backbone of RNA, while no interaction is detected with the nitrogen bases, thus suggesting a sequence-independent mechanism of binding between nsp12 and RNA (W. Yin et al., 2020) (Fig. 1D).

The nsp7/8 dimer takes contact with the thumb-finger interface of nsp12, with most of the interactions occurring via nsp7, while nsp8-1 establishes minimal direct contacts with nsp12. Instead, the additional nsp8-2 monomer sits on top of the finger subdomain (Peng et al., 2020; W. Yin et al., 2020). Both monomer and dimer of the cofactors have a role in stabilizing the closed conformation of the nsp12 subunit (Peng et al., 2020; W. Yin et al., 2020). In contrast with the role of primase proposed for the SARS-CoV nsp7/8 complex that came from the observation of *de novo* RNA synthesis in presence of this dimer (Imbert et al., 2006), the cryoEM structures of the complex showed that the nsp7/8 interaction site with nsp12 is too distant from the 3′-end of the RNA genome to act as primase, suggesting that different configurations of the cofactors in solution are physiologically relevant for the primase activity. Therefore, the several roles proposed for the nsp7/8 complex, such as processivity factor, primase or nsp12 activating factor, may depend on the conformation and stoichiometry of these proteins (Konkolova et al., 2020). In absence of cofactors, the fingers extension loops of nsp12 display significant flexibility, which would reduce the stability of the fingers subdomain and explain the functional inactivity of the nsp12 monomer alone (Peng et al., 2020). In fact, nsp12 alone was observed to retain no RNA binding capacity, and only the presence of the cofactors allows its binding to the RNA substrate (Chen et al., 2020; Jones et al., 2022; W. Yin et al., 2020). Additionally, biophysical methods such as SAXS and SANS confirmed that nsp12 alone is unstable and prone to aggregation, which suggests that this protein is unlikely to act alone in physiological conditions. The stability of nsp12 is greatly enhanced by the addition of the cofactors, especially nsp8, but the full RTC still does not form in a stable structure until RNA is present (Wilamowski et al., 2021). SARS-CoV-2 helicase (nsp13) was shown by single-particle cryo-EM to interact with two monomers for each RTC assembly (Chen et al., 2020). One nsp13 monomer was always found taking contact with nsp8-2 in all structures, in both $nsp13_1$-RTC and $nsp13_2$-RTC, while the second monomer, taking contact with nsp8-1, seems to be dissociable and never present without the first one. The unpaired 5′ extension of the RNA template is directed from nsp12 into the RNA-binding channel of nsp13-2 (Yan et al., 2020). An interesting feature of this assembly emerges from the apparent conflict between the orientation of nsp13 on RTC and the direction of translocation of nsp12. In fact, the interaction with nsp13 and nsp12 allows for the template RNA to enter the nsp13 active site from nsp12, but since nsp13 is a 5'→3′ translocase and nsp12 works on RNA synthesis in the 3'→5′ direction on the template, this leads to an apparent conflict of activity between the two enzymes and suggests a coordinated push/pull mechanism (Chen et al., 2020; Yan et al., 2020). This mechanism of backtracking of the complex on the RNA substrate is likely associated with two functions: 1) proof-reading activity, mediated by the ExoN complex and 2) template-switching during discontinuous synthesis of subgenomic mRNAs (Chen et al., 2020). The resolution of the SARS-CoV-2 nsp13-RTC backtracked complex by cryo-EM revealed that the template RNA was engaged by nsp13-2, with the extrusion of the 3′ ssRNA primer into the RdRp NTP entry tunnel underneath motif F (B. Malone et al., 2021).

Other cryo-EM structures of the extended SARS-CoV-2 complex showed how nsp9 appears to bind close to the catalytic center of nsp12 NiRAN. Interactions between nsp12 and nsp9 occur in two regions: region 1 is formed by nsp9 N terminus, palm domain and the NiRAN β-hairpin motif of nsp12, while region 2 consists of a flat region in nsp12 NiRAN that binds to the C-terminal α-helix of nsp9 (Yan et al., 2021a). Binding of nsp9 to nsp12 NiRAN results in the formation of a positively charged groove that extends from the catalytic center of nsp12 NiRAN to nsp9, which may act as an RNA-binding site to stabilize the conformation of the 5′ end of GpppA-RNA after the catalytic reaction (Yan et al., 2021a). Additionally, it was observed how the three N-terminal residues of nsp9 insert into the NiRAN catalytic site, forming a phospho-amide covalent bond with the α-phosphate of RNA in a reaction catalyzed by the NiRAN domain termed RNAylation (Yan et al., 2022). The resolution of the structure of the complex nsp7-$nsp8_2$-nsp9-nsp12-$nsp13_2$-RNA and nsp14/nsp10 showed that nsp9 and nsp12, through its NiRAN domain, bind to nsp14 ExoN to stabilize the assembly of nsp14/nsp10 to the RTC (Yan et al., 2021b). In this assembly, the nsp10/nsp14 complex integrates vertically into a "canyon" formed by nsp9 and nsp12 NiRAN, with nsp14 ExoN stabilizing the assembly through extensive interactions, while nsp14 N7-MTase remains distal from the interaction site (Yan et al., 2021b). The analysis of the catalytic residues in nsp14 ExoN and N7-MTase revealed conserved interactions with the other proteins of the complex, critical for the coupling of methylation and proofreading during RNA synthesis, suggesting a common mechanism for cap formation among coronaviruses (Yan et al., 2021b).

In addition to the $nsp12_1$-$nsp7_1$-$nsp8_2$ structure resolved by most structural studies, a dimeric form of the SARS-CoV-2 RTC was resolved at the resolution of 5.5 Å (Jochheim et al., 2021). In this reconstruction, the two complexes arranged in an antiparallel orientation, each containing a single copy of nsp8 and nsp7, lacking the second nsp8-1 which was shown to interact with nsp7 in the previously resolved monomeric complexes. Since the catalytic sites are not involved in RTC dimer formation, the authors speculated that the two RdRp enzymes remain functional and may be simultaneously involved in RNA elongation, but the challenges in purifying this dimeric RTC could not strengthen the physiological relevance of this configuration (Jochheim et al., 2021). A dimeric form was also observed for the extended nsp7-$nsp8_2$-nsp9-nsp12-$nsp13_2$-nsp14-nsp10 and RNA complex, where nsp12 NiRAN and the palm of two extended RTC protomers face each other and form the central core of the dimer, with the dimeric interactions stabilized through their nsp14 N7-MTase domains (Yan et al., 2021b).

## 3. Evolution, functional Complexity and unique features of the SARS-CoV-2 RdRp

### 3.1. Sequence analyses, mutations and Nsp12 evolution over time

After SARS-CoV-2 emergence in 2019, phylogenetic analysis of

nsp12 from a dataset of SARS-CoV, MERS-CoV, SARS-CoV-2 and Bat CoV-RaTG13 nsp12 sequences confirmed a strong conservation of the nsp12 RdRp domain, while the NiRAN domain -a feature of members of the *Nidovirales* order-showed a higher degree of variability (Mishra et al., 2021; Neogi et al., 2020; Shannon et al., 2020).

During the progression of SARS-CoV-2 pandemic, longitudinal analyses of SARS-CoV-2 global sequences observed the strong conservation of nsp12 among different viral strains, especially in the RNA-binding residues (Showers et al., 2022). However, the analysis of widely shared single-nucleotide polymorphisms on SARS-CoV-2 global sequences deposited in the GISAID database revealed the emergence in 2020 of a rapidly spreading nsp12 missense mutation, responsible for the P323L amino acid substitution (compared to the reference Wuhan sequence), which is located on the surface of the protein at the interface between the NiRAN and the RdRp domains (Morais et al., 2020). Given the rapid dominance of the nsp12-L323 substitution over P323, the role of this mutation was thoroughly investigated and different mechanisms explaining its prevailing over the wild type P323 variant were proposed. From the observation that the nsp12-323 amino acid residue is located close to one of the nsp8–nsp12 interfaces, the substitution of P323 with a leucine likely strengthens the interaction between nsp8 and nsp12 (Biswal et al., 2021; Ilmjärv et al., 2021). In addition, the P323L substitution frequently co-emerged with the D614G substitution in the spike protein, suggesting a synergistic effect, with P323L potentially contributing by enhancing replication efficiency or modulating polymerase activity (Ilmjärv et al., 2021). While *in vitro* studies on recombinant viruses carrying the nsp12-P323L substitution failed to demonstrate a clear advantage in viral fitness of the L323 over the P323 substitution (Goldswain et al., 2023), RdRp reporter assays conducted at 33 °C or 37 °C to mimic the upper and lower airways showed important differences in the enzymatic activity of P323L nsp12 mutants, with an additive effect of substitution G671S on P323L, likely due to the stabilization of the nsp12-nsp8 interaction (S.-M. Kim et al., 2023). In addition, a temperature-dependent replication kinetics *in vivo* study on ferrets confirmed the biochemical results, with nsp12-P323L, -G671S and -P323L/G671S mutant viruses showing higher viral replication rates and transmissibility over the WT virus only in the upper nasal tract, while the replication rate in the lungs remained unaffected, demonstrating a fitness advantage of the nsp12-L323 variant in the upper nasal tract (S.-M. Kim et al., 2023). The majority of the other detected missense mutations appeared to be poorly widespread, representing less than 4 % of total sequences (Showers et al., 2022), mostly located in two mutational hotspots, namely the polymerase RNA-binding cavity and the nsp12–nsp8 contact interface were identified (Ferrer-Orta et al., 2024).

### 3.2. SARS-CoV-2 Nsp12-Mediated discontinuous transcription

SARS-CoV-2 nsp12 is a pleiotropic protein, involved in several and diverse tasks (Fig. 2). Among these functions, discontinuous synthesis of sgRNAs is a unique feature of all coronaviruses and most viruses of the Nidovirales order, from which the name of the order derives ("nidus" is Latin for nest, from the nested set of subgenomic RNAs (Steiner et al., 2024). Before SARS-CoV-2 emergence, it has been demonstrated that discontinuous transcription takes place during the synthesis of negative-strand sgRNAs in coronaviruses (Sola et al., 2015). The transcription of canonical sgRNAs in coronaviruses is driven by a "jumping" mechanism of the RdRp, orchestrated by the transcription-regulatory sequences in the leader (TRS-L) and body (TRS-B) regions. These regions share conserved core sequences (CS), typically 6–7 nucleotides long, which play a pivotal role in facilitating the interaction between the TRS-L in the 5′-UTR and the TRS-B located upstream of each gene. This interaction enables base-pairing between the CS in the TRS-Leader (CS-L) and its complementary sequence (cCS-B) on the nascent negative strand, ensuring the alignment required for the polymerase to complete the full-length sgRNA (Fig. 2B) (Sola et al., 2015; Zúñiga et al., 2004).

SARS-CoV-2 transcriptome analyses revealed a heterogeneous

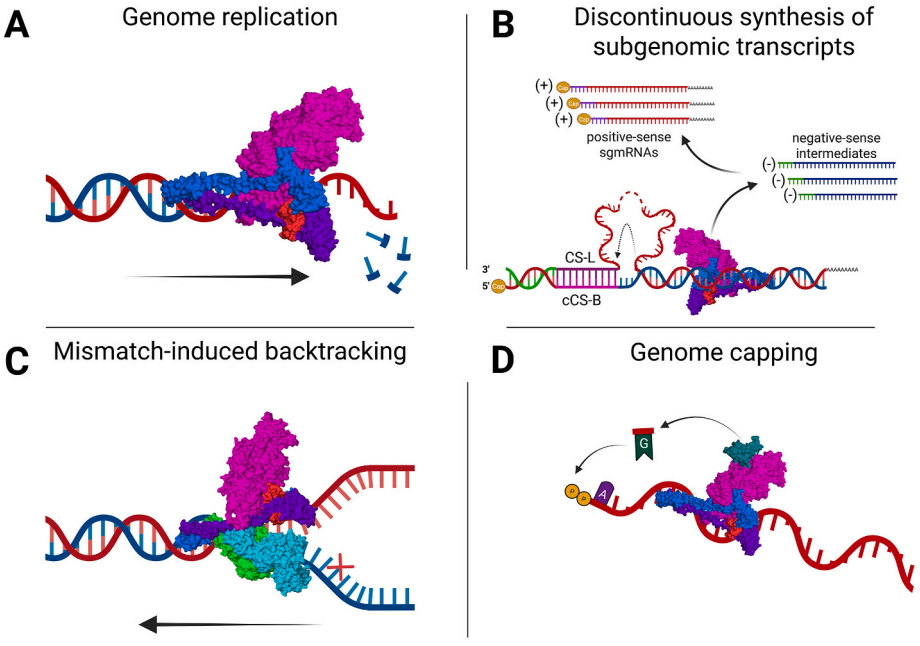


**Fig. 2.** Functions of SARS-CoV-2 nsp12

Fig. 2. (A) SARS-CoV-2 nsp12 catalyzes the replication of the (+)ssRNA genome by synthesis of a (−)ssRNA intermediate and (B) transcribes the 3′-located structural and accessory genes by discontinuous synthesis of subgenomic mRNAs (sgmRNAs) that incorporate the 5′-capped leader region of the genome. Nsp12 "jumps" on the template via the annealing of the CS-L (located in the TRS-L) and cCS-B (located in the TRS-B) sequences and synthesizes positive-polarity sgmRNAs via negative-polarity intermediates. (C) Moreover, nsp12 backtracks on the RNA template -aided by nsp13-in presence of mismatches, to allow ExoN-mediated proofreading and (D) is involved in the first step of genome capping, with the transfer of a GTP residue to the 5′ end of the genome. The protein color code is consistent with Fig. 1 (PDB 7EIZ). Created in https://BioRender.com. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

population of fusion transcripts, with TRS-L-dependent fusion of canonical ORFs, TRS-L-dependent in-frame and out frame non-canonical, and TRS-L-independent non-canonical template switch events (D. Kim et al., 2020). Over 433 unique sgRNA types were identified, including canonical leader-body sgRNAs and non-canonical sgRNAs with internal template switches, among which known positive-to-positive template switching were detected, in addition to novel negative-to-negative template switching during (+)-strand synthesis (D. Wang et al., 2021). Interestingly, the relative abundance of SARS-CoV-2 sgRNAs followed a descending order from the 3′–5′ direction of the viral genome, with the N sgRNA(+) being the most abundant, followed by ORF8, ORF7a/b, M, ORF6, E, ORF3a, and S sgRNA(+) being the least abundant transcript (D. Wang et al., 2021). During SARS-CoV-2 infection both in cell culture and *in vivo* models, non-canonical sgRNAs (nc-sgRNAs) make up a significant portion up to 33 % of the total viral sgRNAs in infected cells, which increased over the course of the infection and, surprisingly, are not associated with TRS-like homology (Nomburg et al., 2020). Many nc-sgRNAs are predicted to encode variants or truncated ORFs, which may lead to proteins with modified functions or non-functional proteins, with potential impacts on modulating viral pathogenesis, potentially contributing to immune evasion or altered host responses (Nomburg et al., 2020).

### 3.3. A novel function of Nsp12 is mediated via its NiRAN domain

The RdRp-associated NiRAN domain of nsp12 is one of the characteristic genetic markers of *Nidovirales*, although it is quite variable among viruses belonging to this order (Shannon et al., 2024). Nsp12 NiRAN domain has structural similarity to pseudo-kinase selenoprotein-O (SelO), which has AMPylation activity that entails transfer of AMP to Ser/Thr/Tyr residues on protein substrates, and it was demonstrated to possess nucleotidylation activity with UTP/GTP preference, as well (Lehmann et al., 2015; Yan et al., 2021a). It was shown that coronavirus NiRAN domains transfer NMP residues to its own RdRp domain (Lehmann et al., 2015). Site-directed mutagenesis that affected this nucleotidyl transferase activity also strongly negatively impacted viral replication, as shown for SARS-CoV, EAV and HCoV-229E, demonstrating its fundamental role in viral replication (Lehmann et al., 2015; Slanina et al., 2021).

These findings suggested that the NiRAN domain acts as the second step in cap synthesis of SARS-CoV-2 genome. Preliminary studies on *in vitro* GTase-assays revealed that SARS-CoV-2 nsp12 facilitates the transfer of GMP to ppA-RNA, resulting in the formation of GpppA-RNA. This process requires the presence of nsp13, which acts as a CoV RTPase to convert pppA-RNA into ppA-RNA (Yan et al., 2021a) (Fig. 3A). The association of nsp12 with nsp9 resulted in attenuated nucleotidyl transferase activity by nsp12, which was confirmed by site-directed mutagenesis of a series of residues involved in the nsp12/nsp9 interface (Walker et al., 2021; Yan et al., 2021a).

In contrast, parallel studies observed how coronavirus NiRAN domains transfer a single NMP to the N-terminal primary amine of nsp9 instead of RNA, forming a covalent phosphoramidate bond, in addition to self-attaching an NMP residue, with the formation of covalent nsp12-NMP adducts (Park et al., 2022; Slanina et al., 2021). This nucleotidyl transferase reaction to nsp9 appeared to be $Mn^{2+}$-dependent and showed a slight preference for UMP, although AMP, GMP, and CMP can also be substrates. The N-terminal dipeptide sequence of nsp9 (N3825-N3826) was found to be critical for NMPylation and substitution or deletion of these residues significantly reduced or abolished NMPylation activity and impaired viral replication (Slanina et al., 2021). In addition, NMPylation appears to be dynamically regulated, since the process is fully reversible in the presence of pyrophosphate (PPi), which promotes de-NMPylation by pyrophosphorolysis (B. Wang et al., 2021). In contrast, RNAylation of nsp9 was found to be irreversible in the presence of PPi, indicating that RNAylation is likely the physiologically relevant modification of nsp9 during viral RNA capping (Park et al., 2022).

Different models have been proposed to explain the role of the NiRAN domain in the SARS-CoV-2 replication cycle. Given the distance of the NMPylated Asn1 residue in nsp9 from the putative RNA-binding

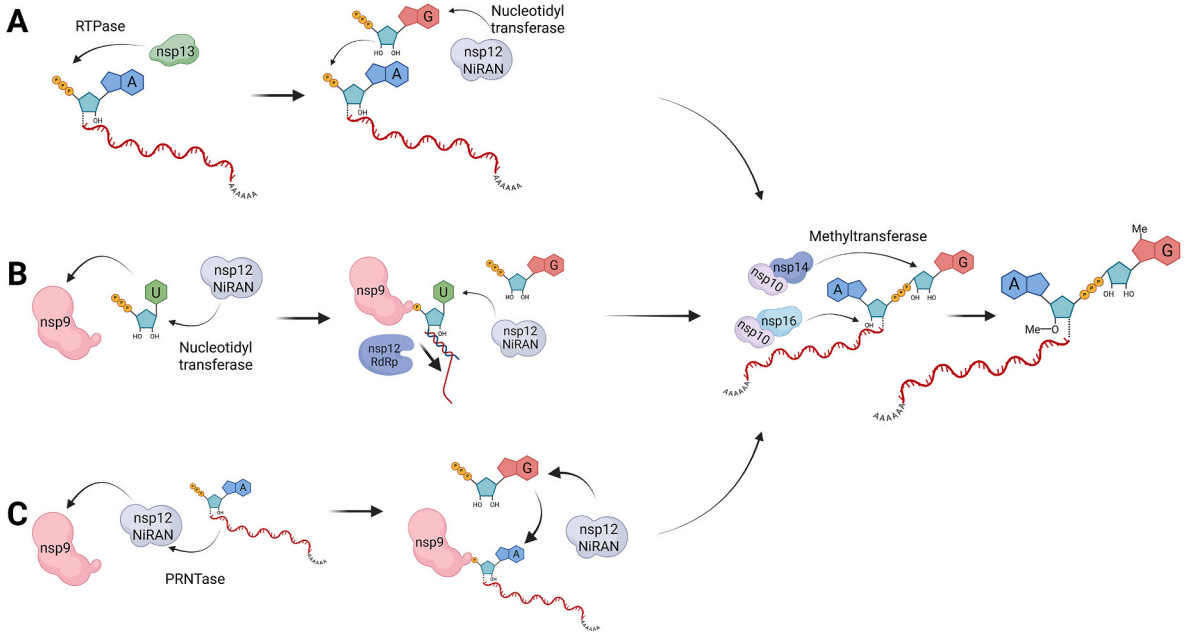


**Fig. 3.** Different models proposed for NiRAN-mediated capping activity
Fig. 3. (A) SARS-CoV-2 nsp13 removes the γ-phosphate from the ATP at the 5′-end of the RNA genome. Subsequently nsp12-NiRAN transfers a GTP moiety to the ppA end, forming a GpppA pre-cap structure. (B) Nsp12 mediates the transfer of NMPs to nsp9, which then acts as primer for RdRp-mediated genome replication. The NMP-nsp9 complex then binds again to nsp12 NiRAN, which catalyzes the transfer of the capping nucleotides. (C) Nsp12 works as PRNTase, mediating the transfer of RNA to nsp9, which then acts as intermediate for the formation of GpppA. The final steps mediated by the nsp10-aided nsp14 and nsp16 methyltransferase activities are well-established and common to all proposed mechanisms. Created in https://BioRender.com.

site, and the lack of any significant theoretical thermodynamic contribution of this modification in nsp12-nsp9 binding, which would make nsp9 an ideal candidate deliver NMP to secondary acceptor, nsp9 was suggested to act both in priming and capping mechanisms. In this model: 1) nsp9 would bind and be NMPylated by the NiRAN domain; 2) NMP-nsp9 could bind to the nsp12 RdRp site and serve as a primer for RNA synthesis; 3) the newly formed primer RNA-nsp9 rebinds to the NiRAN domain and a second nucleotidyl transfer reaction removes the NMP residue to cap the primer RNA (B. Wang et al., 2021) (Fig. 3B). Similarly, the NiRAN domain was proposed not to function as a conventional GTase, but rather as a GDP–PRNTase catalyzing the transfer of 5′-monophosphate RNA (pRNA) to a GTP or GDP via a covalent nsp9–pRNA intermediate (Park et al., 2022; Yan et al., 2021b) (Fig. 3C).

### 3.4. Biochemical characterization of the RdRp activity of SARS-CoV-2 Nsp12

SARS-CoV-2 nsp12 is the fastest viral RdRp known, with maximal catalytic rates ($k_{cat}$) between 90 and 150 $s^{-1}$ at 22 °C, depending on the template used in pre-steady state rapid-quench experiments. This is significantly higher as compared to other known viral RdRps, such as HCV and picornavirus, and is expected to increase by 3–5 folds at 37 °C (Shannon et al., 2020). Using a high-throughput magnetic tweezers approach on a 1 kb-long template, RNA synthesis by the SARS-CoV-2 RTC was observed to occur in bursts of rapid nucleotide addition, interspersed with pauses of varying durations, likely driven by distinct structural or conformational states within the complex, or by specific events such as nucleotide misincorporation (Bera et al., 2021). Over the course of 1000 monitored nucleotide addition cycles, no evidence was found for the exchange of polymerase components or dissociation of the complex, highlighting its stability and high processivity (Bera et al., 2021). In fact, the dissociation rate of nsp12 and cofactors nsp7 and nsp8 with RNA is slow, as assessed by quench flow double-mixing experiment with heparin, where the best fit obtained by simulation gives an RNA dissociation rate constant ($k_{off}$) of $0.013 \pm 0.001$ $s^{-1}$ (Dangerfield et al., 2020).

As the coronavirus genome is one of the longest known among RNA viruses, it was shown that, in order to exert the high processivity and fast rates required to replicate it, SARS-CoV-2 nsp12 displays poor fidelity of incorporation. This phenomenon was particularly marked at high and close-to-physiological concentrations of nucleotides, with a mis-incorporation rate of ~0.2 $s^{-1}$, which is significantly less accurate than the generally admitted $10^{-4}$–$10^{-6}$ error rate of viral RdRps (Shannon et al., 2020). The presence of ATP seems to play a pivotal role in modulating the fidelity of the SARS-CoV-2 RdRp, significantly enhancing error-prone ribonucleotide incorporation when added in any combination of other nucleotides, while the lack of ATP was poorly substituted by mismatches of the other NTPs (Pourfarjam et al., 2022; X. Yin et al., 2023). Such low fidelity could be explained by a structural point of view since viral RdRps catalyze NTP positioning by exploiting an arginine residue to interact with triphosphate charges. In most viral RdRps, this arginine residue is stabilized by a salt bridge with a glutamate residue, which is notably absent and substituted with an alanine residue in CoV RdRps (Shannon et al., 2020). This “relaxed” NTP-binding site could explain the low fidelity and speed of this polymerase, mitigated by the presence of an exonuclease proof-reading complex (Shannon et al., 2020).

The evaluation of the activity of SARS-CoV-2 RdRp on different substrates confirmed the structural importance of 2′-OH in the ribose in the RTC-RNA binding and demonstrated that no binding with DNA (which lacks the 2′-OH) could be detected (Jones et al., 2022). Enhanced RNA binding was observed when the 2′-OH was substituted with 2′-O-Me or the ribose group was replaced with a phosphorothioate nucleic acid variant, modifications that both increase RNA rigidity (Jones et al., 2022). Biochemical elongation assays with 2′-O-Me RNA substrate yielded no elongation by RdRp, while elongation was observed using the phosphorothioate RNA substrate, which suggested that a 2′-O atom in the ribose is essential and sufficient for RNA binding, but a free 2′-OH group is necessary for elongation (Jones et al., 2022). The evaluation of the activity of SARS-CoV-2 RdRp on epigenomic modifications, by generating RNA templates containing various types of ribonucleotide modifications, showed a modulation of the enzymatic activity, with 3-meU, 1-meG and 2′-O-meG strongly blocking the polymerization activity, while others (N6meA, 5-meC and Ψ, which do not change base pairing) did not affect extension of the primer (Petushkov et al., 2023). In addition to these modifications, N1-methyladenosine (m1A), which disrupts hydrogen bonding of canonic base pair, was shown to significantly inhibit RdRp activity by halting RNA extension at the modified nucleotide, resulting in truncated products. Conversely, N3-methylcytosine (m3C), despite disrupting canonical base pairing, is efficiently read through by RdRp, similarly to Ψ, m5C, and m6A (Apostle et al., 2023). Interestingly, the observation that m3C severely inhibits HIV-1-RT and MMLV-RT suggests a different adaptation of the SARS-CoV-2 RdRp, possibly to evade host-mediated epigenetic modifications (Apostle et al., 2023).

## 4. Development of antiviral assays and discovery of RdRp inhibitors

### 4.1. Different cell-based and biochemical assays to evaluate SARS-CoV-2 RdRp inhibition

Since viral polymerases are among the most important targets for the development of antiviral drugs (De Clercq, 2004; De Clercq and Li, 2016; Tian et al., 2021), also for SARS-CoV-2 the effort focused on this essential enzyme, among other nonstructural proteins.

To identify and validate novel antiviral compounds that are capable of inhibiting SARS-CoV-2 RdRp activity, different assays have been set up since the emergence of the virus in late 2019. One of the first assays developed is a classical primer-elongation assay, which utilized as substrate a previously annealed fluorescently labeled substrate and an unlabeled template, then separated by denaturing urea-PAGE (Lu et al., 2020). Another approach exploited the properties of double stranded nucleic acid intercalating agents to detect the RdRp activity by the SARS-CoV-2 RTC complex, by emitting a fluorescence signal only when intercalated to the dsRNA produced during the enzymatic reaction (Eydoux et al., 2021; W. Yin et al., 2021). Additionally, a high-throughput screening (HTS) assay exploiting FRET (Förster resonance energy transfer) entailed coupling of a single stranded RNA with a fluorophore and its partially complementary RNA strand with its quencher. This substrate was then used to detect RdRp activity by strand displacement, which occurred when the SARS-CoV-2 RTC elongated a primer annealed to the free 3’ end of the fluorescently-labeled RNA strand and displaced the quencher-labeled strand (Bertolin et al., 2021). A Surface Plasmon Resonance (SPR)-based assay was developed to assess the interference in the RdRp-RNA binding. By ligating a biotinylated RNA to a sensor chip and by injecting the SARS-CoV-2 RTC in the flow cells, it was possible to assess the binding affinity of the complex to the RNA and observe the reduction of this binding in dose-dependent manner when suramin was added to the sample (Mravinec et al., 2021). Bai et al. (2022) developed a fluorometric approach based on dsRNA quantification by a fluorescent nucleic acid-binding dye of a self-priming substrate, to assess the catalytic activity of the SARS-CoV-2 RdRp complex.

In parallel to biochemical assays, cell-based assays were designed to study the RdRp activity of SARS-CoV-2 in a cellular context. A cell-based CoV-RdRp-Gluc reporter assay was set up using the Gaussia luciferase (Gluc) as a reporter, whose transcription is initiated by the CMV promoter. When the CoV-Gluc reporter-expressing cells were transfected with nsp12-expressing vector together with nsp7 and nsp8 in a 1:3:3 ratio, the Gluc RNA was further amplified by viral RdRp, resulting in the increase of Gluc protein and luminescent signal (Zhao et al., 2021a).

Another cell-based reporter assay was developed using a bicistronic p (+)FLuc-(−)UTR-NLuc reporter plasmid, where FLuc expression was used as internal control of transfection, while NLuc activity was present only in presence of SARS-CoV-2 nsp12 expression (Min et al., 2021). In this assay, in contrast to the majority of the previously published works, the authors did not find a significant difference in RdRp activity when nsp12 was expressed alone or in complex with cofactors nsp7 and nsp8 (Min et al., 2021). A similar assay was set up with a bicistronic construct including the Renilla luciferase (RLuc) under the CMV promoter, which serves as an internal control, and the Firefly luciferase (FLuc), flanked by the 5′- and 3′-UTRs of SARS-CoV-2, which is released from the construct by an internal ribozyme ensuring that only RdRp transcription leads to correct expression of FLuc. Co-expression of a polyprotein encoding SARS-CoV-2 nsp5-nsp7-nsp8-nsp12, where nsp5 serves as protease to allow release of the other monomeric proteins, allows the transcription of the FLuc gene, which -normalized to the basal RLuc signal-permits to monitor the RdRp activity (Uppal et al., 2022).

### 4.2. RdRp as a target for antiviral drug development: Nucleos(t)ide analogues

The design of nucleos(t)ide analogues (NAs) requires a careful balance between structural and functional impact. Modifications must be sufficiently minor to ensure compatibility with host enzymatic systems, such as nucleos(t)ide kinases and hydrolytic enzymes, which are responsible for their activation. Simultaneously, these alterations must be substantial to effectively disrupt viral replication through mechanisms like chain termination, template-dependent inhibition, polymerase stalling, or induction of lethal mutagenesis, following their incorporation into the viral RNA (Chien et al., 2020; Ju et al., 2020).

#### 4.2.1. Remdesivir

Remdesivir (Fig. 4A) (RDV or GS-5734) is the monophosphoramidate prodrug of a 1′-cyano-substituted adenine C-nucleoside ribose analogue, first developed as a treatment against acute Ebola virus infections (Warren et al., 2016) and evaluated in phase 1 and 2 randomized controlled clinical trials (Mulangu et al., 2019). Soon after the emergence of SARS-CoV-2, a massive effort was directed to find effective antiviral therapy and, among the first positive results, RDV showed promising antiviral activity against SARS-CoV-2 replication in Vero E6 cells, with an $EC_{50}$ value of 0.77 μM (Wang et al., 2020). RDV was shown to act in a post-entry stage of the SARS-CoV-2 replication cycle, in agreement with its putative mechanism as the prodrug of a nucleotide analogue to be incorporated into the nascent viral RNA genome (M. Wang et al., 2020). In a cell-based SARS-CoV-2 RdRp reporter assay, RDV displayed an $EC_{50}$ of 1.1 μM confirming its activity as

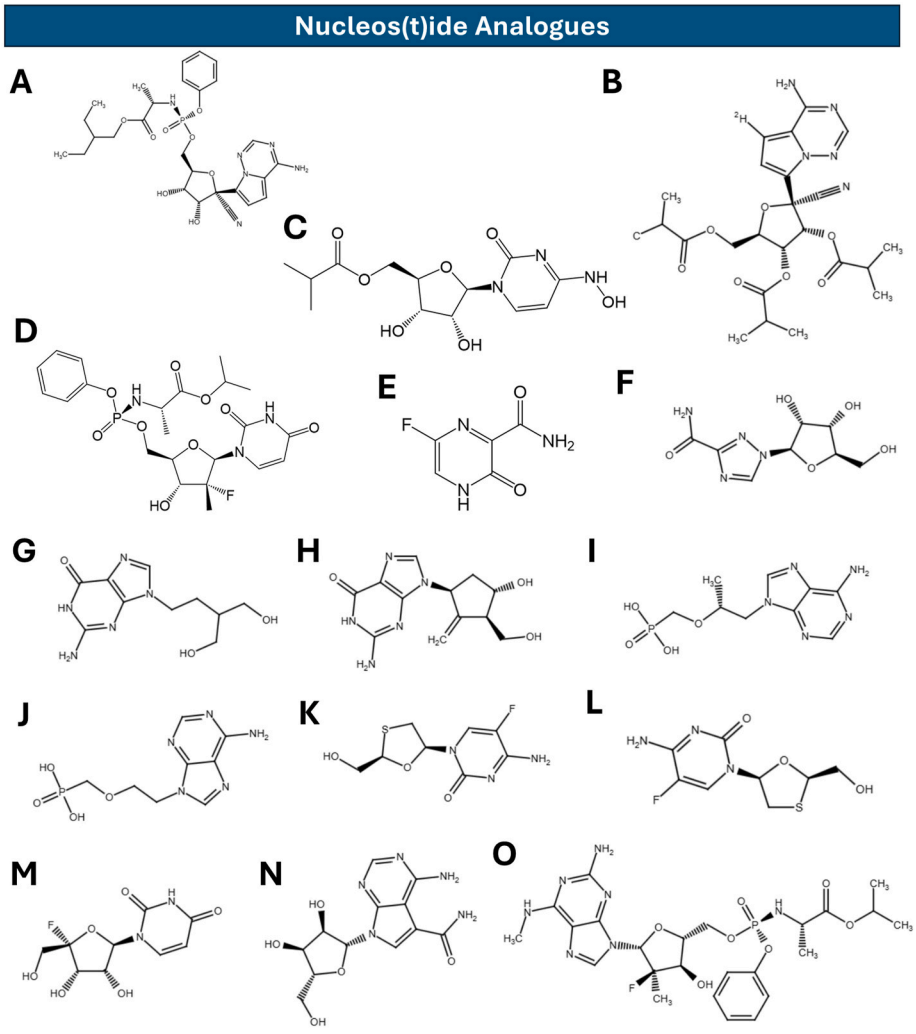


**Fig. 4.** Structure of Identified SARS-CoV-2 RdRp Nucleos(t)ide Inhibitors
Fig. 4. Chemical structures of some of the SARS-CoV-2 nsp12 identified nucleos(t)ide inhibitors: (A) remdesivir, (B) VV116, (C) molnupiravir, (D) sofosbuvir, (E) favipiravir, (F) ribavirin, (G) penciclovir, (H) entecavir, (I) tenofovir, (J) adefovir, (K) emtricitabine, (L) telbivudine, (M) 4′-fluorouridine, (N) sangivamycin and (O) bemnifosbuvir.

a viral RdRp inhibitor, although its potency decreased 2-fold when the nsp14/nsp10 ExoN complex was co-expressed with the RTC (Zhao et al., 2021a).

The active triphosphate form of remdesivir (RDV-DP or GS-443902) was shown to be incorporated by the MERS-CoV polymerase into the RNA nascent strand without causing immediate chain terminator, instead allowing elongation at position i + 3 (Gordon et al., 2020). The same mechanism was identified for the SARS-CoV and SARS-CoV-2 RdRp (Gordon et al., 2020). *In-silico* model predicted a steric clash at position i + 4 of the 1′-CN group of RDV-DP with residue S861 of nsp12, which was found to be conserved among all α, β and γ coronavirus sequences (Gordon et al., 2020b). This was confirmed by mutagenesis studies with the generation of a SARS-CoV-2 nsp12 S861A mutant, which resulted in less potent chain termination in presence of RDV in position i + 3 (Q. Wang et al., 2020). It was also suggested that this delayed termination, and the additional natural nucleotides incorporated after the monophosphate form of RDV (RMP), may have a protective effect on nsp14-mediated excision during proofreading (Gordon et al., 2020).

A selectivity value < 1 was measured for RDV-DP versus ATP in a steady-steady kinetic approach for all coronaviruses nsp12, indicating a preference of the enzyme for the nucleotide analogue in place of the natural nucleotide (Gordon et al., 2022; Gordon et al., 2020). However, with the limitations of biochemical studies, RDV-induced delayed chain termination could be overcome with increasing concentrations of NTPs, which can reach low millimolar values in cells, while remdesivir usually reaches high micromolar intracellular concentrations (Gordon et al., 2020). The understanding of RDV mechanism of action was expanded by the demonstration that, in addition to delayed polymerase stalling at position i + 3, RDV incorporation into the template strand acts with a template-dependent mechanism, suggesting a potential a dual mode of inhibition. In fact, incorporated RDV inhibits UTP incorporation in the complementary strand in multiple viral RdRps, including those of SARS-CoV-2 (Gordon et al., 2022). In addition to inhibiting the RdRp activity of SARS-CoV-2 nsp12, RDV-DP was demonstrated to inhibit the GTase function of the NiRAN domain *in vitro*, but with a much lower potency compared to its RdRp-directed inhibition (Walker et al., 2021).

The cryo-EM structures of SARS-CoV-2 RTC in complex with remdesivir showed that RMP was incorporated in position +1 in the elongated primer, with only two other natural nucleotides incorporated in position +2 and + 3, confirming the non-obligate chain terminator mechanism (W. Yin et al., 2020). Cryo-EM structures with different RNA templates containing RMP either in position −3 or −4 revealed how the addition of one more nucleotide was still feasible in the −3 structure, while the −4 structure led the polymerase to undergo into a pre-translocation state and cannot bind incoming nucleotides, given how the 1′-CN group causes a steric clash with the S861 residue (Kokic et al., 2021; Q. Wang et al., 2020). Moreover, in this structure, RMP is buried inside nsp12 active site, which would explain why remdesivir is at least partially resistant to excision from the ExoN complex (Kokic et al., 2021). In a pre-incorporation complex of SARS-CoV-2 RTC with RDV-DP, the 1′-cyano moiety on the ribose of RMP inserts into a hydrophilic pocket formed by residues T687, N691 (motif B) and S759 (motif C), and is stabilized by a network of polar interactions. This pocket was reconstructed with a bound water molecule in the ATP-bound structure, and the displacement of this water molecule and formation of novel hydrogen bonds with the 1′-cyano moiety can explain the higher selectivity and lower $K_M$ of RDV-DP compared to ATP (B. F. Malone et al., 2023).

A preliminary report on RDV administration to a small cohort of patients hospitalized for severe forms of COVID-19 showed an improvement in oxygen-support status for 68 % of total patients and a mortality of 18 % over 18 days of post-treatment median follow-up for patients requiring invasive ventilation, compared to a mortality of 66 % of the closest comparison group to this study (Grein et al., 2020). The clinical relevance of RDV treatment for COVID-19 is still under debate, since other clinical trials, in contrast, found no or small significant clinical improvement in COVID-19 patients treated with RDV, especially those in late-stages of disease (Ader et al., 2022; Pan et al., 2022; Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results', 2021; Wang et al., 2020).

Given the low oral bioavailability of RDV, which requires intravenous administrations in hospital settings, and the low efficacy in late-stage COVID-19, novel oral RDV derivatives with better *in vitro* antiviral activity, selectivity and pharmacokinetic properties, such as VV116 (Fig. 4B), have been tested in clinical trials (Qian et al., 2022; Shen et al., 2022) or in pre-clinical models (L. Cao et al., 2022; Cox et al., 2021; Y. Zhang et al., 2023).

#### 4.2.2. Molnupiravir

Molnupiravir (Fig. 4C), an oral prodrug of β-D-N4-hydroxycytidine (NHC, or EIDD-1931), was identified as a highly potent broad-spectrum inhibitor of SARS-CoV-2, SARS-CoV and MERS-CoV replication and pathogenesis in cell culture ($EC_{50}$ = 0.08 μM in Calu-3 cells) and *in vivo* mice models, reducing lung viral load and improving pulmonary function, although with a limited early window of intervention (Sheahan et al., 2020). *In vitro* analysis suggested that lethal mutagenesis is the molecular mechanism of action of molnupiravir, since NGS sequencing of MERS-CoV genome after treatment of cells with molnupiravir was found to have increased nucleotide transitions, which led to error catastrophe by inducing an error rate beyond the threshold allowed for viral replication (Sheahan et al., 2020). In fact, NHC-monophosphate incorporated into the RNA template showed ambiguous base pairing, allowing both GTP and ATP incorporation in the complementary strand with no preference, due to its ability to adopt two tautomeric forms, which provided the molecular basis for the mutagenic effect induced by molnupiravir (Gordon et al., 2021).

Phase 1 randomized, double-blind, placebo-controlled trial demonstrated the overall safety and tolerability of molnupiravir in healthy individuals, and the achieved plasma exposures of the active metabolite NHC in humans were consistent with efficacious levels in animal models for SARS-CoV-2 (Painter et al., 2021). On December 23rd, 2021, the U.S. Food and Drug Administration agency issued an emergency use authorization (EUA) for molnupiravir for "mild-to-moderate coronavirus disease (COVID-19) in adults with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19, including hospitalization or death, and for whom alternative COVID-19 treatment options authorized by the FDA are not accessible or clinically appropriate" (U.S. Food and Drug Administration, 2021). On November 11th, 2024, EMA issued an advice to assist national authorities in making decisions about the potential early use of the molnupiravir, such as in emergency scenarios, before marketing authorization is granted (European Medicines Agency, 2021).

However, molnupiravir has been linked to the emergence of novel SARS-CoV-2 variants by elevating the viral mutation rate. This is supported by the identification of molnupiravir-associated mutational signatures in circulating viruses, particularly in regions and age groups where the drug has been widely used (Sanderson et al., 2023). Molnupiravir has been discontinued in Europe after Merck Sharp and Dohme withdrew its application for marketing authorization to the European Medicines Agency (EMA). This decision followed the EMA Committee for Medicinal Products for Human Use's (CHMP) unfavorable assessment of the drug's risk-benefit balance (European Medicines Agency, 2021).

#### 4.2.3. Sofosbuvir

Sofosbuvir (Fig. 4D), the prodrug of a uridine analogue developed against the RdRp NS5B of HCV (Sofia et al., 2010), was found to inhibit SARS-CoV-2 in different cell lines, such as Huh7 and Calu-3, with $EC_{50}$ values between 5 and 7 μM (Sacramento et al., 2021). Incorporation of sofosbuvir into the nascent RNA strand by SARS-CoV-2 RdRp in biochemical assays demonstrated to induce chain termination and to be

more resistant than RDV to nsp14-mediated exonuclease excision (Jockusch et al., 2020; Ju et al., 2020). Sofosbuvir-DP was found to have less steric hindrance compared to RDV in the 2′ position (2′-F and 2′-Me substituents) and it does not have the 1' CN group, that is likely responsible for RDV steric hindrance on nsp12 elongation (Jockusch et al., 2020b; Ju et al., 2020). Given its previously established safety profile and efficacy in inhibiting SARS-CoV-2 RdRp, coupled with its relatively high resistance to ExoN-mediated excision, sofosbuvir (in combination with velpatasvir or daclatasvir) has been tested in preliminary small-cohort clinical trials (Sadeghi et al., 2020; Sayad et al., 2020).

#### 4.2.4. Favipiravir

Broad-spectrum antiviral favipiravir (Fig. 4E) in its ribofuranosyl triphosphate form showed less efficient incorporation rate and selectivity over natural nucleotides, compared to RDV-DP or molnupiravir (Gordon et al., 2020), which is consistent with the finding of high micromolar $EC_{50}$ values ($EC_{50}$ = 109.5 μM, SI > 3.65) in cell culture against SARS-CoV-2 replication (M. Wang et al., 2020). Cryo-EM structure of SARS-CoV-2 RTC in complex with favipiravir-RTP shows that the analogue is inefficiently incorporated into the growing RNA strand due to a nonproductive binding mode at the catalytic site, where favipiravir forms a noncanonical base pair with cytosine, and this inefficient alignment prevents optimal nucleophilic attack on the β-phosphate (Naydenova et al., 2021). Further characterization of favipiravir mechanism of action revealed that this NA, although inefficiently, is incorporated as a purine analogue and shows an increase in total mutation frequency, especially transitions, therefore acting on viral replication by a lethal mutagenesis mechanism (Shannon et al., 2020). A randomized clinical trial involving 150 patients with mild-to-moderate COVID-19 evaluated the effects of oral favipiravir. Although the study did not find a statistically significant reduction in time to RT-PCR negativity as the primary endpoint, early administration of favipiravir was associated with a significantly shorter duration of clinical signs and symptoms, suggesting a potential benefit in achieving faster clinical recovery with favipiravir oral treatment of COVID-19 (Udwadia et al., 2021).

#### 4.2.5. Other Nucleot(s)ide analogues

Other nucleoside analogues were reported as active against SARS-CoV-2 *in vitro*. Alovudine (3′-fluoro-3′-deoxythymidine) and AZT (3′-azido-3′-deoxythymidine), originally developed for treating HIV infections, have been studied for their potential against SARS-CoV RdRp. The active triphosphate form of both compounds is incorporated by the enzyme and acts as obligate chain terminator, preventing further strand elongation (Ju et al., 2020). Nucleotide analogues ganciclovir (an acyclic guanosine analogue), carbovir (carbocyclic guanosine analogue lacking 2′- and 3′-OH groups), and entecavir (carbocyclic guanosine analogue missing the 2′-OH group) effectively inhibit RNA synthesis catalyzed by the SARS-CoV-2 RdRp by causing immediate termination upon incorporation, while cidofovir diphosphate (an acyclic cytidine nucleotide) demonstrates delayed termination similar to RDV, allowing further RNA extension before termination, potentially making it resistant to exonuclease activity. Stavudine triphosphate, a thymidine analogue, was demonstrated to cause complete termination of SARS-CoV-2 RdRp activity, due to the absence of both 2′- and 3′-OH groups, which potentially renders it a poor substrate for the viral exonuclease (Jockusch et al., 2020).

In cell-based SARS-CoV-2 RdRp reporter assays, ribavirin (Fig. 4F), penciclovir (Fig. 4G), entecavir (Fig. 4H) and tenofovir (Fig. 4I) showed relatively high $EC_{50}$ values between 87 and 120 μM (Zhao et al., 2021a). Other nucleos(t)ide analogues, such as adefovir (Fig. 4J), emtricitabine (Fig. 4K) and telbivudine (Fig. 4L), were identified by Min et al. (2021) to target the RdRp function of SARS-CoV-2, with $IC_{50}$ values in the range of 3.8 and 46 μM. An orally bioavailable nucleoside analog, 4′-fluorouridine (4′-FlU) (Fig. 4M), displayed potent broad-spectrum inhibition of different strains of SARS-CoV-2 with $EC_{50}$ values ranging from 0.2 to 0.6 μM, by causing sequence-dependent delayed stalling of the RdRp (Sourimant et al., 2022). Preclinical studies demonstrated its efficacy in human airway epithelial organoids and animal models, including significant viral load reductions against SARS-CoV-2 variants and RSV (Sourimant et al., 2022). Sangivamycin (Fig. 4N), an adenosine nucleoside analog, has shown potent antiviral activity against multiple SARS-CoV-2 variants with $EC_{50}$ values between 15 and 80 nM, depending on the viral strain and the cell line, outperforming RDV that was used as positive control (Bennett et al., 2022). Interestingly, sangivamycin incorporation into the nascent RNA by the SARS-CoV-2 RdRp does not induce chain termination nor significantly alter the viral mutation rate, suggesting its antiviral mechanism operates downstream of RdRp activity, potentially involving effects on viral RNA structure or subsequent interaction of the elongated RNA with the replication machinery (Bennett et al., 2023).

AT-9010, the active triphosphate form of AT-527 (bemnifosbuvir, Fig. 4O), is a guanosine analogue that carries a 2′-fluoro-2′-C-methyl modified ribose, structurally similarly to prodrug sofosbuvir, which demonstrated a double mechanism of action, by inducing immediate chain termination upon incorporation - resistant to the addition of high NTP concentrations and showing reduced ExoN-mediated excision compared to sofosbuvir-DP - and additionally by inhibiting nsp9 and nsp8 UMPylation by binding to the N-terminal NiRAN domain of SARS-CoV-2 nsp12 (Shannon et al., 2022). Another approach, widely used to improve NA stability and resistance to exo/ribonuclease degradation, consisting in the substitution of a non-bridging oxygen of the phosphodiester bond by a sulfur atom, has been investigated with the synthesis of a novel 5′-α-thiotriphosphate derivative of AT-9010, AT-9052 (Shannon et al., 2024). This analogue showed stereoselective incorporation into the nascent strand as rGTP-analogue, opposite to CTP, inducing immediate chain termination. More interestingly, the resulting α-thiophosphodiester linkage is completely resistant to cleavage by the viral ExoN complex, as compared to the low -but not absent-excision rate of incorporated AT-9010 (Shannon et al., 2024). However, AT-9052 loses its ability to additionally target the NiRAN activity, which was observed in the case of AT-9010 (Shannon et al., 2024).

### 4.3. RdRp as a target for antiviral drug development: Non-Nucleos(t)ide analogues

Following the massive scientific effort to develop novel tools to prevent and fight SARS-CoV-2 infections, several non-nucleos(t)ide analogues were identified to inhibit its RdRp activity. However, it should be noted how the development of non-nucleos(t)ide inhibitors can be hampered by the accidental identification of PAINS (Pan-Assay Interference compounds), which can give positive results by interfering with assay readout in an unspecific manner. Among these compounds, isothiazolones, curcumin, phenol-sulfonamides are only few examples (J. B. Baell, 2016; J. Baell and Walters, 2014). (te Velthuis et al., 2021; Elli et al., 2021; Walker et al., 2020; Holubovska et al., 2024; Steverding et al., 2024; Salgado-Benvindo et al., 2020)(W. Yin et al., 2021; Salgado-Benvindo et al., 2020)(W. Yin et al., 2021).

Among compounds that were not classified as PAINS, daclatasvir (Fig. 5A), an NNI approved for the treatment of HCV targeting the non-structural NS5A protein, showed potent antiviral activity, with $EC_{50}$ values ranging from 0.6 to 1.1 μM, across several cell types, including human lung epithelial cells (Calu-3) (Sacramento et al., 2021). Daclatasvir was shown to inhibit SARS-CoV-2 RNA synthesis by targeting the folding of viral RNA secondary structures and favoring RNA denaturation even at lower temperatures (Sacramento et al., 2021).

Novel 2-((1H-indol3-yl)thio)-N-phenyl-acetamide derivatives (Fig. 5B) showed potent activity against SARS-CoV-2 RdRp in a SARS-CoV-2-RdRp-Gluc reporter and biochemical RdRp assay, with $IC_{50}$ values as low as 1 μM, with broad-spectrum activity against other HCoVs (G.-N. Zhang et al., 2021).

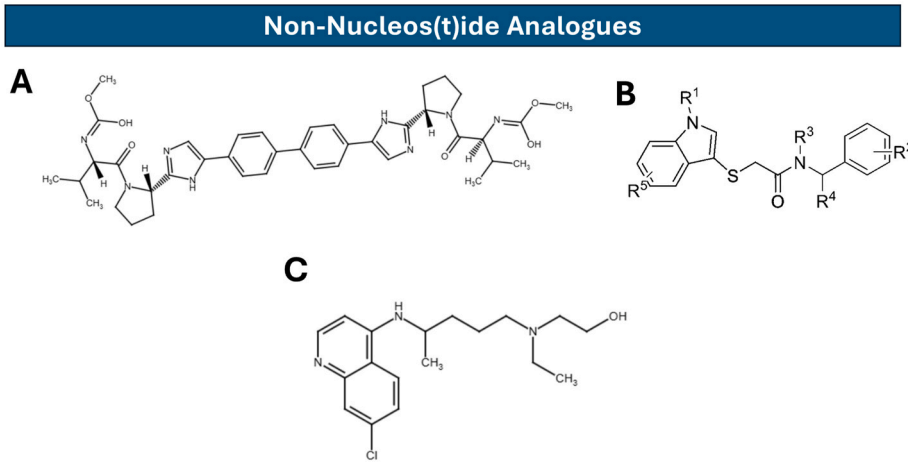


**Fig. 5.** Structure of Identified SARS-CoV-2 RdRp Non-Nucleos(t)ide Inhibitors
Fig. 5. Chemical structures of some of the SARS-CoV-2 nsp12 identified nucleos(t)ide inhibitors: (A) daclatasvir, (B) 2-((1H-indol3-yl)thio)-N-phenyl-acetamide scaffold and (C) hydroxychloroquine.

A series of quinoline derivatives, similar to hydroxychloroquine (Fig. 5C), showed promising activity in their inhibition of SARS-CoV-2 RdRp activity in a cell-based CoV-RdRp-Gluc reporter assay, with broad-spectrum activity against low pathogenicity HCoVs, with $EC_{50}$ values as low as 1 μM (Zhao et al., 2021b).

## 5. Conclusions and perspectives

The SARS-CoV-2 RdRp nsp12 has demonstrated to be a critical enzyme in the viral replication and transcription processes and in the maintenance of genome integrity, making it a key component of the viral RTC and an attractive target for antiviral intervention. Recent structural and functional studies of nsp12, in complex with its cofactors nsp7 and nsp8, have provided significant insights into its architecture and catalytic mechanisms. The resolution of different cryo-EM assemblies has revealed the conformational dynamics of nsp12 in various states, including its interactions with RNA, other nonstructural proteins of the RTC machinery and inhibitors. The multiple and diverse tasks that nsp12 carry out emphasize the versatility of this enzyme to meet the demands of the coronavirus replication cycle. Despite these achievements, significant knowledge gaps remain. The precise mechanisms by which nsp12 coordinates with other components of the RTC in non-canonical processes, such as discontinuous synthesis of sgRNAs, genome capping and backtracking during proofreading, are not yet fully elucidated.

From a therapeutic perspective, the successful development of nucleoside analogues such as remdesivir and molnupiravir against SARS-CoV-2 has validated nsp12 as a key antiviral target. However, these drugs have limitations, including debatable *in vivo* efficacy and potential for resistance. The recent discontinuation of molnupiravir in Europe due to concerns over its risk-benefit profile further highlights the need for safer and more effective alternatives. Understanding the molecular aspects of the nsp12 functions is crucial for developing therapies that target multiple steps of the replication process, reducing the likelihood of resistance. Other nucleos(t)ide or non-nucleos(t)ide inhibitors targeting RdRp and/or auxiliary functions of nsp12, such as its NiRAN domain, may offer novel prospects for the expansion of the antiviral arsenal. Additionally, high-throughput screening of small-molecule libraries, coupled with advanced *in silico* modeling, could accelerate the discovery of next-generation inhibitors.

Therefore, the ongoing evolution of SARS-CoV-2, supported by the evidence of emerging new variants with altered replication kinetics and antiviral resistance profiles, highlights the need to focus on the understanding of nsp12 structure and function to fight not only SARS-CoV-2 but also future coronavirus threats. In fact, given the high structural conservation of nsp12 amongst different members of the *Coronaviridae* family, the development of RdRp inhibitors has the potential to provide tools to prepare against current and future coronavirus-related threats.

## CRediT authorship contribution statement

**Paolo Malune:** Writing – original draft, Conceptualization. **Francesca Esposito:** Writing – review & editing. **Enzo Tramontano:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) spoke 5 and by RAS LR 7/07 project antivirale-unica F73C22001570002.

## Data availability

The article is a review.

## References

Ader, F., Bouscambert-Duchamp, M., Hites, M., Peiffer-Smadja, N., Poissy, J., Belhadi, D., Diallo, A., Lê, M.-P., Peytavin, G., Staub, T., Greil, R., Guedj, J., Paiva, J.-A., Costagliola, D., Yazdanpanah, Y., Burdet, C., Mentré, F., Egle, A., Greil, R., et al., 2022. Remdesivir plus standard of care versus standard of care alone for the treatment of patients admitted to hospital with COVID-19 (DisCoVeRy): a phase 3, randomised, controlled, open-label trial. Lancet Infect. Dis. 22 (2), 209–221. https://doi.org/10.1016/S1473-3099(21)00485-0.

Apostle, A., Yin, Y., Chillar, K., Eriyagama, A.M.D.N., Arneson, R., Burke, E., Fang, S., Yuan, Y., 2023. Effects of epitranscriptomic RNA modifications on the catalytic activity of the SARS-CoV-2 replication complex. Chembiochem 24 (8). https://doi.org/10.1002/cbic.202300095.

Baell, J.B., 2016. Feeling nature's PAINS: natural products, natural product drugs, and Pan assay interference compounds (PAINS). J. Nat. Prod. 79 (3), 616–628. https://doi.org/10.1021/acs.jnatprod.5b00947.

Baell, J., Walters, M.A., 2014. Chemistry: Chemical con artists foil drug discovery. Nature 513 (7519), 481–483. https://doi.org/10.1038/513481a.

Bai, X., Sun, H., Wu, S., Li, Y., Wang, L., Hong, B., 2022. Identifying small-molecule inhibitors of SARS-CoV-2 RNA-dependent RNA polymerase by establishing a fluorometric assay. Front. Immunol. 13. https://doi.org/10.3389/fimmu.2022.844749.

Bennett, R.P., Postnikova, E.N., Eaton, B.P., Cai, Y., Yu, S., Smith, C.O., Liang, J., Zhou, H., Kocher, G.A., Murphy, M.J., Smith, H.C., Kuhn, J.H., 2022. Sangivamycin is highly effective against SARS-CoV-2 in vitro and has favorable drug properties. JCI Insight 7 (1). https://doi.org/10.1172/jci.insight.153165.

Bennett, R.P., Yoluç, Y., Salter, J.D., Ripp, A., Jessen, H.J., Kaiser, S.M., Smith, H.C., 2023. Sangivamycin is preferentially incorporated into viral RNA by the SARS-CoV-2 polymerase. Antivir. Res. 218, 105716. https://doi.org/10.1016/j.antiviral.2023.105716.

Bera, S.C., Seifert, M., Kirchdoerfer, R.N., van Nies, P., Wubulikasimu, Y., Quack, S., Papini, F.S., Arnold, J.J., Canard, B., Cameron, C.E., Depken, M., Dulin, D., 2021. The nucleotide addition cycle of the SARS-CoV-2 polymerase. Cell Rep. 36 (9), 109650. https://doi.org/10.1016/j.celrep.2021.109650.

Bermingham, A., Chand, M.A., Brown, C.S., Aarons, E., Tong, C., Langrish, C., Hoschler, K., Brown, K., Galiano, M., Myers, R., Pebody, R.G., Green, H.K., Boddington, N.L., Gopal, R., Price, N., Newsholme, W., Drosten, C., Fouchier, R.A., Zambon, M., 2012. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. : Bulletin Europeen Sur Les Maladies Transmis. = European Commun. Dis. Bulle. 17 (40), 20290.

Bertolin, A.P., Weissmann, F., Zeng, Jingkun, Posse, V., Milligan, J.C., Canal, B., Ulferts, R., Wu, M., Drury, L.S., Howell, M., Beale, R., Diffley, J.F.X., 2021. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp12/7/8 RNA-dependent RNA polymerase. Biochem. J. 478 (13), 2425–2443.

Biswal, M., Diggs, S., Xu, D., Khudaverdyan, N., Lu, J., Fang, J., Blaha, G., Hai, R., Song, J., 2021. Two conserved oligomer interfaces of NSP7 and NSP8 underpin the dynamic assembly of SARS-CoV-2 RdRP. Nucleic Acids Res. 49 (10), 5956–5966. https://doi.org/10.1093/nar/gkab370.

Brant, A.C., Tian, W., Majerciak, V., Yang, W., Zheng, Z.-M., 2021. SARS-CoV-2: from its discovery to genome structure, transcription, and replication. Cell Biosci. 11 (1), 136. https://doi.org/10.1186/s13578-021-00643-z.

Buonaguro, L., Tagliamonte, M., Tornesello, M.L., Buonaguro, F.M., 2020. SARS-CoV-2 RNA polymerase as target for antiviral therapy. J. Transl. Med. 18 (1), 185. https://doi.org/10.1186/s12967-020-02355-3.

Cao, C., Cai, Z., Xiao, X., Rao, J., Chen, J., Hu, N., Yang, M., Xing, X., Wang, Y., Li, M., Zhou, B., Wang, X., Wang, J., Xue, Y., 2021. The architecture of the SARS-CoV-2 RNA genome inside virion. Nat. Commun. 12 (1), 3917. https://doi.org/10.1038/s41467-021-22785-x.

Cao, L., Li, Y., Yang, S., Li, G., Zhou, Q., Sun, J., Xu, T., Yang, Y., Liao, R., Shi, Y., Yang, Y., Zhu, T., Huang, S., Ji, Y., Cong, F., Luo, Y., Zhu, Y., Luan, H., Zhang, H., et al., 2022. The adenosine analog prodrug ATV006 is orally bioavailable and has preclinical efficacy against parental SARS-CoV-2 and variants. Sci. Transl. Med. 14 (661). https://doi.org/10.1126/scitranslmed.abm7621.

Chen, J., Malone, B., Llewellyn, E., Grasso, M., Shelton, P.M.M., Olinares, P.D.B., Maruthi, K., Eng, E.T., Vatandaslar, H., Chait, B.T., Kapoor, T.M., Darst, S.A., Campbell, E.A., 2020. Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex. Cell 182 (6), 1560–1573.e13. https://doi.org/10.1016/j.cell.2020.07.033.

Chien, M., Anderson, T.K., Jockusch, S., Tao, C., Li, X., Kumar, S., Russo, J.J., Kirchdoerfer, R.N., Ju, J., 2020. Nucleotide analogues as inhibitors of SARS-CoV-2 polymerase, a key drug target for COVID-19. J. Proteome Res. 19 (11), 4690–4697. https://doi.org/10.1021/acs.jproteome.0c00392.

Cox, R.M., Wolf, J.D., Lieber, C.M., Sourimant, J., Lin, M.J., Babusis, D., DuPont, V., Chan, J., Barrett, K.T., Lye, D., Kalla, R., Chun, K., Mackman, R.L., Ye, C., Cihlar, T., Martinez-Sobrido, L., Greninger, A.L., Bilello, J.P., Plemper, R.K., 2021. Oral prodrug of remdesivir parent GS-441524 is efficacious against SARS-CoV-2 in ferrets. Nat. Commun. 12 (1), 6415. https://doi.org/10.1038/s41467-021-26760-4.

Dangerfield, T.L., Huang, N.Z., Johnson, K.A., 2020. Remdesivir is effective in combating COVID-19 because it is a better substrate than ATP for the viral RNA-dependent RNA polymerase. iScience 23 (12), 101849.

De Clercq, E., 2004. Antiviral drugs in current clinical use. J. Clin. Virol. 30 (2), 115–133. https://doi.org/10.1016/j.jcv.2004.02.009.

De Clercq, E., Li, G., 2016. Approved antiviral drugs over the past 50 years. Clin. Microbiol. Rev. 29 (3), 695–747. https://doi.org/10.1128/CMR.00102-15.

Elli, S., Bojkova, D., Bechtel, M., Vial, T., Boltz, D., Muzzio, M., Peng, X., Sala, F., Cosentino, C., Goy, A., Guerrini, M., Müller, L., Cinatl, J., Margitich, V., te Velthuis, A.J.W., 2021. Enisamium inhibits SARS-CoV-2 RNA synthesis. Biomedicines 9 (9), 1254. https://doi.org/10.3390/biomedicines9091254.

European Medicines Agency, 2021. In: EMA issues advice on use of Lagevrio (molnupiravir) for the treatment of COVID-19. Ema.Europa.Eu/En/News/Ema-Issues-Advice-Use-Lagevrio-Molnupiravir-Treatment-Covid-19.

European Medicines Agency, 2023, March 13. Lagevrio (Molnupiravir). Ema.Europa.Eu/En/Medicines/Human/EPAR/Lagevrio.

Eydoux, C., Fattorini, V., Shannon, A., Le, T.-T.-N., Didier, B., Canard, B., Guillemot, J.-C., 2021. A fluorescence-based high throughput-screening assay for the SARS-CoV RNA synthesis complex. J. Virol. Methods 288, 114013. https://doi.org/10.1016/j.jviromet.2020.114013.

Ferrer-Orta, C., Vázquez-Monteagudo, S., Ferrero, D.S., Martínez-González, B., Perales, C., Domingo, E., Verdaguer, N., 2024. Point mutations at specific sites of the nsp12–nsp8 interface dramatically affect the RNA polymerization activity of SARS-CoV-2. Proc. Natl. Acad. Sci. 121 (29). https://doi.org/10.1073/pnas.2317977121.

Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., et al., 2020. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Sci. Technol. Humanit. 368 (6492), 779–782. https://doi.org/10.1126/science.abb7498.

Goldswain, H., Dong, X., Penrice-Randal, R., Alruwaili, M., Shawli, G.T., Prince, T., Williamson, M.K., Raghwani, J., Randle, N., Jones, B., Donovan-Banfield, I., Salguero, F.J., Tree, J.A., Hall, Y., Hartley, C., Erdmann, M., Bazire, J., Jearanaiwitayakul, T., Semple, M.G., et al., 2023. The P323L substitution in the SARS-CoV-2 polymerase (NSP12) confers a selective advantage during infection. Genome Biol. 24 (1), 47. https://doi.org/10.1186/s13059-023-02881-5.

Gordon, C.J., Lee, H.W., Tchesnokov, E.P., Perry, J.K., Feng, J.Y., Bilello, J.P., Porter, D.P., Götte, M., 2022. Efficient incorporation and template-dependent polymerase inhibition are major determinants for the broad-spectrum antiviral activity of remdesivir. J. Biol. Chem. 298 (2), 101529. https://doi.org/10.1016/j.jbc.2021.101529.

Gordon, C.J., Tchesnokov, E.P., Feng, J.Y., Porter, D.P., Götte, M., 2020a. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. J. Biol. Chem. 295 (15), 4773–4779. https://doi.org/10.1074/jbc.AC120.013056.

Gordon, C.J., Tchesnokov, E.P., Schinazi, R.F., Götte, M., 2021. Molnupiravir promotes SARS-CoV-2 mutagenesis via the RNA template. J. Biol. Chem. 297 (1), 100770. https://doi.org/10.1016/j.jbc.2021.100770.

Gordon, C.J., Tchesnokov, E.P., Woolner, E., Perry, J.K., Feng, J.Y., Porter, D.P., Götte, M., 2020b. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. J. Biol. Chem. 295 (20), 6785–6797. https://doi.org/10.1074/jbc.RA120.013679.

Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., Feldt, T., Green, G., Green, M.L., Lescure, F.-X., Nicastri, E., Oda, R., Yo, K., Quiros-Roldan, E., Studemeister, A., Redinski, J., Ahmed, S., Bernett, J., Chelliah, D., et al., 2020. Compassionate use of remdesivir for patients with severe covid-19. N. Engl. J. Med. 382 (24), 2327–2336. https://doi.org/10.1056/NEJMoa2007016.

Hillen, H.S., Kokic, G., Farnung, L., Dienemann, C., Tegunov, D., Cramer, P., 2020. Structure of replicating SARS-CoV-2 polymerase. Nature 584 (7819), 154–156.

Holubovska, O., Babich, P., Mironenko, A., Milde, J., Lebed, Y., Stammer, H., Mueller, L., te Velthuis, A.J.W., Margitich, V., Goy, A., 2024. RNA polymerase inhibitor enisamium for treatment of moderate COVID-19 patients: a randomized, placebo-controlled, multicenter, double-blind phase 3 clinical trial. Adv. Respiratory Med. 92 (3), 202–217. https://doi.org/10.3390/arm92030021.

Ilmjärv, S., Abdul, F., Acosta-Gutiérrez, S., Estarellas, C., Galdadas, I., Casimir, M., Alessandrini, M., Gervasio, F.L., Krause, K.-H., 2021. Concurrent mutations in RNA-dependent RNA polymerase and spike protein emerged as the epidemiologically most successful SARS-CoV-2 variant. Sci. Rep. 11 (1), 13705. https://doi.org/10.1038/s41598-021-91662-w.

Imbert, I., Guillemot, J.-C., Bourhis, J.-M., Bussetta, C., Coutard, B., Egloff, M.-P., Ferron, F., Gorbalenya, A.E., Canard, B., 2006. A second, non-canonical RNA-dependent RNA polymerase in SARS Coronavirus. EMBO J. 25 (20), 4933–4942. https://doi.org/10.1038/sj.emboj.7601368.

Jácome, R., Becerra, A., Ponce de León, S., Lazcano, A., 2015. Structural analysis of monomeric RNA-dependent polymerases: evolutionary and therapeutic implications. PLoS One 10 (9), e0139001. https://doi.org/10.1371/journal.pone.0139001.

Jiang, Y., Yin, W., Xu, H.E., 2021. RNA-dependent RNA polymerase: structure, mechanism, and drug discovery for COVID-19. Biochem. Biophys. Res. Commun. 538, 47–53. https://doi.org/10.1016/j.bbrc.2020.08.116.

Jochheim, F.A., Tegunov, D., Hillen, H.S., Schmitzová, J., Kokic, G., Dienemann, C., Cramer, P., 2021. The structure of a dimeric form of SARS-CoV-2 polymerase. Commun. Biol. 4 (1), 999. https://doi.org/10.1038/s42003-021-02529-9.

Jockusch, S., Tao, C., Li, X., Anderson, T.K., Chien, M., Kumar, S., Russo, J.J., Kirchdoerfer, R.N., Ju, J., 2020a. A library of nucleotide analogues terminate RNA synthesis catalyzed by polymerases of coronaviruses that cause SARS and COVID-19. Antivir. Res. 180, 104857. https://doi.org/10.1016/j.antiviral.2020.104857.

Jockusch, S., Tao, C., Li, X., Chien, M., Kumar, S., Morozova, I., Kalachikov, S., Russo, J. J., Ju, J., 2020b. Sofosbuvir terminated RNA is more resistant to SARS-CoV-2 proofreader than RNA terminated by Remdesivir. Sci. Rep. 10 (1), 16577.

Jones, A.N., Mourão, A., Czarna, A., Matsuda, A., Fino, R., Pyrc, K., Sattler, M., Popowicz, G.M., 2022. Characterization of SARS-CoV-2 replication complex elongation and proofreading activity. Sci. Rep. 12 (1), 9593. https://doi.org/10.1038/s41598-022-13380-1.

Ju, J., Li, X., Kumar, S., Jockusch, S., Chien, M., Tao, C., Morozova, I., Kalachikov, S., Kirchdoerfer, R.N., Russo, J.J., 2020. Nucleotide analogues as inhibitors of SARS-CoV polymerase. Pharmacol. Res. Perspec. 8 (6). https://doi.org/10.1002/prp2.674.

Kim, D., Lee, J.-Y., Yang, J.-S., Kim, J.W., Kim, V.N., Chang, H., 2020. The architecture of SARS-CoV-2 transcriptome. Cell 181 (4), 914–921.e10. https://doi.org/10.1016/j.cell.2020.04.011.

Kim, S.-M., Kim, E.-H., Casel, M.A.B., Kim, Y.-I., Sun, R., Kwak, M.-J., Yoo, J.-S., Yu, M., Yu, K.-M., Jang, S.-G., Rollon, R., Choi, J.H., Gil, J., Eun, K., Kim, H., Ensser, A., Hwang, J., Song, M.-S., Kim, M.H., et al., 2023. SARS-CoV-2 variants with NSP12 P323L/G671S mutations display enhanced virus replication in ferret upper airways and higher transmissibility. Cell Rep. 42 (9), 113077. https://doi.org/10.1016/j.celrep.2023.113077.

Kokic, G., Hillen, H.S., Tegunov, D., Dienemann, C., Seitz, F., Schmitzova, J., Farnung, L., Siewert, A., Höbartner, C., Cramer, P., 2021. Mechanism of SARS-CoV-2 polymerase stalling by remdesivir. Nat. Commun. 12 (1), 279.

Konkolova, E., Klima, M., Nencka, R., Boura, E., 2020. Structural analysis of the putative SARS-CoV-2 primase complex. J. Struct. Biol. 211 (2), 107548. https://doi.org/10.1016/j.jsb.2020.107548.

Lehmann, K.C., Gulyaeva, A., Zevenhoven-Dobbe, J.C., Janssen, G.M.C., Ruben, M., Overkleeft, H.S., van Veelen, P.A., Samborskiy, D.V., Kravchenko, A.A., Leontovich, A.M., Sidorov, I.A., Snijder, E.J., Posthuma, C.C., Gorbalenya, A.E., 2015. Discovery of an essential nucleotidylating activity associated with a newly delineated conserved domain in the RNA polymerase-containing protein of all nidoviruses. Nucleic Acids Res. 43 (17), 8416–8434. https://doi.org/10.1093/nar/gkv838.

Lu, G., Zhang, X., Zheng, W., Sun, J., Hua, L., Xu, L., Chu, X., Ding, S., Xiong, W., 2020. Development of a simple *in vitro* assay to identify and evaluate nucleotide analogs against SARS-CoV-2 RNA-dependent RNA polymerase. Antimicrob. Agents Chemother. 65 (1). https://doi.org/10.1128/AAC.01508-20.

Malone, B., Chen, J., Wang, Q., Llewellyn, E., Choi, Y.J., Olinares, P.D.B., Cao, X., Hernandez, C., Eng, E.T., Chait, B.T., Shaw, D.E., Landick, R., Darst, S.A., Campbell, E.A., 2021. Structural basis for backtracking by the SARS-CoV-2 replication–transcription complex. Proc. Natl. Acad. Sci. 118 (19). https://doi.org/10.1073/pnas.2102516118.

Malone, B.F., Perry, J.K., Olinares, P.D.B., Lee, H.W., Chen, J., Appleby, T.C., Feng, J.Y., Bilello, J.P., Ng, H., Sotiris, J., Ebrahim, M., Chua, E.Y.D., Mendez, J.H., Eng, E.T., Landick, R., Götte, M., Chait, B.T., Campbell, E.A., Darst, S.A., 2023. Structural basis for substrate selection by the SARS-CoV-2 replicase. Nature 614 (7949), 781–787. https://doi.org/10.1038/s41586-022-05664-3.

Min, J.S., Kwon, S., Jin, Y.-H., 2021. SARS-CoV-2 RdRp inhibitors selected from a cell-based SARS-CoV-2 RdRp activity assay system. Biomedicines 9 (8), 996. https://doi.org/10.3390/biomedicines9080996.

Mishra, D., Suri, G.S., Kaur, G., Tiwari, M., 2021. Comparative insight into the genomic landscape of SARS-CoV-2 and identification of mutations associated with the origin of infection and diversity. J. Med. Virol. 93 (4), 2406–2419. https://doi.org/10.1002/jmv.26744.

Morais, I.J., Polveiro, R.C., Souza, G.M., Bortolin, D.I., Sassaki, F.T., Lima, A.T.M., 2020. The global population of SARS-CoV-2 is composed of six major subtypes. Sci. Rep. 10 (1), 18289.

Mravinec, M., Bajc, G., Butala, M., 2021. Surface plasmon resonance approach to study drug interactions with SARS-CoV-2 RNA-dependent RNA polymerase highlights treatment potential of suramin. J. Virol. Methods 298, 114283. https://doi.org/10.1016/j.jviromet.2021.114283.

Mulangu, S., Dodd, L.E., Davey, R.T., Tshiani Mbaya, O., Proschan, M., Mukadi, D., Lusakibanza Manzo, M., Nzolo, D., Tshomba Oloma, A., Ibanda, A., Ali, R., Coulibaly, S., Levine, A.C., Grais, R., Diaz, J., Lane, H.C., Muyembe-Tamfum, J.-J., 2019. A randomized, controlled trial of Ebola virus disease therapeutics. N. Engl. J. Med. 381 (24), 2293–2303. https://doi.org/10.1056/NEJMoa1910993.

Naydenova, K., Muir, K.W., Wu, L.-F., Zhang, Z., Coscia, F., Peet, M.J., Castro-Hartmann, P., Qian, P., Sader, K., Dent, K., Kimanius, D., Sutherland, J.D., Löwe, J., Barford, D., Russo, C.J., 2021. Structure of the SARS-CoV-2 RNA-dependent RNA polymerase in the presence of favipiravir-RTP. Proc. Natl. Acad. Sci. 118 (7). https://doi.org/10.1073/pnas.2021946118.

Neogi, U., Hill, K.J., Ambikan, A.T., Heng, X., Quinn, T.P., Byrareddy, S.N., Sönnerborg, A., Sarafianos, S.G., Singh, K., 2020. Feasibility of known RNA polymerase inhibitors as anti-SARS-CoV-2 drugs. Pathogens 9 (5), 320. https://doi.org/10.3390/pathogens9050320.

Nomburg, J., Meyerson, M., DeCaprio, J.A., 2020. Pervasive generation of non-canonical subgenomic RNAs by SARS-CoV-2. Genome Med. 12 (1), 108. https://doi.org/10.1186/s13073-020-00802-w.

Painter, W.P., Holman, W., Bush, J.A., Almazedi, F., Malik, H., Eraut, N.C.J.E., Morin, M. J., Szewczyk, L.J., Painter, G.R., 2021. Human safety, tolerability, and pharmacokinetics of molnupiravir, a novel broad-spectrum oral antiviral agent with activity against SARS-CoV-2. Antimicrob. Agents Chemother. 65 (5). https://doi.org/10.1128/AAC.02428-20.

Pan, H., Peto, R., Henao Restrepo, A.M., Preziosi, M.-P., Sathiyamoorthy, V., Karim, Q.A., Alejandria, M., Hernàndez García, C., Kieny, M.-P., Malekzadeh, R., Murthy, S., Reddy, K.S., Periago, M.R., Hanna, P.A., Abutidze, A., Ader, F., Al-Bader, A., Alhasawi, A., Allum, E., et al., 2022. Remdesivir and three other drugs for hospitalised patients with COVID-19: final results of the WHO Solidarity randomised trial and updated meta-analyses. Lancet (N. Am. Ed.) 399 (10339), 1941–1953. https://doi.org/10.1016/S0140-6736(22)00519-0.

Park, G.J., Osinski, A., Hernandez, G., Eitson, J.L., Majumdar, A., Tonelli, M., Henzler-Wildman, K., Pawłowski, K., Chen, Z., Li, Y., Schoggins, J.W., Tagliabracci, V.S., 2022. The mechanism of RNA capping by SARS-CoV-2. Nature. https://doi.org/10.1038/s41586-022-05185-z.

Peng, Q., Peng, R., Yuan, B., Zhao, J., Wang, M., Wang, X., Wang, Q., Sun, Y., Fan, Z., Qi, J., Gao, G.F., Shi, Y., 2020. Structural and biochemical characterization of the nsp12-nsp7-nsp8 core polymerase complex from SARS-CoV-2. Cell Rep. 31 (11), 107774.

Petushkov, I., Esyunina, D., Kulbachinskiy, A., 2023. Effects of natural <scp>RNA</scp> modifications on the activity of <scp>SARS-CoV</scp> -2 <scp>RNA</scp> -dependent <scp>RNA</scp> polymerase. FEBS J. 290 (1), 80–92. https://doi.org/10.1111/febs.16587.

Pormohammad, A., Ghorbani, S., Khatami, A., Farzi, R., Baradaran, B., Turner, D.L., Turner, R.J., Bahr, N.C., Idrovo, J.-P., 2020. Comparison of confirmed COVID-19 with SARS and MERS cases - clinical characteristics, laboratory findings, radiographic signs and outcomes: a systematic review and meta-analysis. Rev. Med. Virol. 30 (4), e2112. https://doi.org/10.1002/rmv.2112.

Pourfarjam, Y., Ma, Z., Kim, I.-K., 2022. ATP enhances the error-prone ribonucleotide incorporation by the SARS-CoV-2 RNA polymerase. Biochem. Biophys. Res. Commun. 625, 53–59. https://doi.org/10.1016/j.bbrc.2022.07.087.

Qian, H., Wang, Y., Zhang, M., Xie, Y., Wu, Q., Liang, L., Cao, Y., Duan, H., Tian, G., Ma, J., Zhang, Z., Li, N., Jia, J., Zhang, J., Aisa, H.A., Shen, J., Yu, C., Jiang, H., Zhang, W., et al., 2022. Safety, tolerability, and pharmacokinetics of VV116, an oral nucleoside analog against SARS-CoV-2, in Chinese healthy subjects. Acta Pharmacol. Sin. 43 (12), 3130–3138. https://doi.org/10.1038/s41401-022-00895-6.

Repurposed antiviral drugs for covid-19 Interim WHO solidarity trial results, 2021. N. Engl. J. Med. 384 (6), 497–511. https://doi.org/10.1056/NEJMoa2023184.

Sacramento, C.Q., Fintelman-Rodrigues, N., Temerozo, J.R., Da Silva, A., de, P.D., Dias, S. da S.G., da Silva, C., dos, S., Ferreira, A.C., Mattos, M., Pão, C.R.R., de Freitas, C.S., Soares, V.C., Hoelz, L.V.B., Fernandes, T.V.A., Branco, F.S.C., Bastos, M. M., Boechat, N., Saraiva, F.B., Ferreira, M.A., Jockusch, S., et al., 2021. *In vitro* antiviral activity of the anti-HCV drugs daclatasvir and sofosbuvir against SARS-CoV-2, the aetiological agent of COVID-19. J. Antimicrob. Chemother. 76 (7), 1874–1885. https://doi.org/10.1093/jac/dkab072.

Sadeghi, A., Ali Asgari, A., Norouzi, A., Kheiri, Z., Anushirvani, A., Montazeri, M., Hosamirudsai, H., Afhami, S., Akbarpour, E., Aliannejad, R., Radmard, A.R., Davarpanah, A.H., Levi, J., Wentzel, H., Qavi, A., Garratt, A., Simmons, B., Hill, A., Merat, S., 2020. Sofosbuvir and daclatasvir compared with standard of care in the treatment of patients admitted to hospital with moderate or severe coronavirus infection (COVID-19): a randomized controlled trial. J. Antimicrob. Chemother. 75 (11), 3379–3385. https://doi.org/10.1093/jac/dkaa334.

Salgado-Benvindo, C., Thaler, M., Tas, A., Ogando, N.S., Bredenbeek, P.J., Ninaber, D.K., Wang, Y., Hiemstra, P.S., Snijder, E.J., van Hemert, M.J., 2020. Suramin inhibits SARS-CoV-2 infection in cell culture by interfering with early steps of the replication cycle. Antimicrob. Agents Chemother. 64 (8). https://doi.org/10.1128/AAC.00900-20.

Sanderson, T., Hisner, R., Donovan-Banfield, I., Hartman, H., Løchen, A., Peacock, T.P., Ruis, C., 2023. A molnupiravir-associated mutational signature in global SARS-CoV-2 genomes. Nature 623 (7987), 594–600. https://doi.org/10.1038/s41586-023-06649-6.

Sayad, B., Sobhani, M., Khodarahmi, R., 2020. Sofosbuvir as repurposed antiviral drug against COVID-19: why were we convinced to evaluate the drug in a registered/approved clinical trial? Arch. Med. Res. 51 (6), 577–581. https://doi.org/10.1016/j.arcmed.2020.04.018.

Shannon, A., Chazot, A., Feracci, M., Falcou, C., Fattorini, V., Selisko, B., Good, S., Moussa, A., Sommadossi, J.-P., Ferron, F., Alvarez, K., Canard, B., 2024. An exonuclease-resistant chain-terminating nucleotide analogue targeting the SARS-CoV-2 replicase complex. Nucleic Acids Res. 52 (3), 1325–1340. https://doi.org/10.1093/nar/gkad1194.

Shannon, A., Fattorini, V., Sama, B., Selisko, B., Feracci, M., Falcou, C., Gauffre, P., El Kazzi, P., Delpal, A., Decroly, E., Alvarez, K., Eydoux, C., Guillemot, J.-C., Moussa, A., Good, S.S., La Colla, P., Lin, K., Sommadossi, J.-P., Zhu, Y., et al., 2022. A dual mechanism of action of AT-527 against SARS-CoV-2 polymerase. Nat. Commun. 13 (1), 621. https://doi.org/10.1038/s41467-022-28113-1.

Shannon, A., Le, N.T.-T., Selisko, Barbara, Eydoux, C., Alvarez, K., Guillemot, J.-C., Decroly, E., Peersen, O., Ferron, F., Canard, B., 2020a. Remdesivir and SARS-CoV-2: structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. Antivir. Res. 178 (104793), 104793.

Shannon, A., Selisko, B., Le Nhung-Thi-Tuyet, Huchting, J., Touret, F., Piorkowski, G., Fattorini, V., Ferron, F. cois, Decroly, E., Meier, C., Bruno, Coutard, Peersen, O., Canard, B., 2020b. Rapid incorporation of Favipiravir by the fast and permissive viral RNA polymerase complex results in SARS-CoV-2 lethal mutagenesis. Nat. Commun. 11 (1), 4682.

Sheahan, T.P., Sims, A.C., Zhou, S., Graham, R.L., Pruijssers, A.J., Agostini, M.L., Leist, S. R., Schäfer, A., Dinnon, K.H., Stevens, L.J., Chappell, J.D., Lu, X., Hughes, T.M., George, A.S., Hill, C.S., Montgomery, S.A., Brown, A.J., Bluemling, G.R., Natchus, M. G., et al., 2020. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci. Transl. Med. 12 (541). https://doi.org/10.1126/scitranslmed.abb5883.

Shen, Y., Ai, J., Lin, N., Zhang, H., Li, Y., Wang, H., Wang, S., Wang, Z., Li, T., Sun, F., Fan, Z., Li, L., Lu, Y., Meng, X., Xiao, H., Hu, H., Ling, Y., Li, F., Li, H., et al., 2022. An open, prospective cohort study of VV116 in Chinese participants infected with SARS-CoV-2 omicron variants. Emerg. Microb. Infect. 11 (1), 1518–1523. https://doi.org/10.1080/22221751.2022.2078230.

Showers, W.M., Leach, S.M., Kechris, K., Strong, M., 2022. Longitudinal analysis of SARS-CoV-2 spike and RNA-dependent RNA polymerase protein sequences reveals the emergence and geographic distribution of diverse mutations. Infect. Genet. Evol. 97, 105153. https://doi.org/10.1016/j.meegid.2021.105153.

Slanina, H., Madhugiri, R., Bylapudi, G., Schultheiß, K., Karl, N., Gulyaeva, A., Gorbalenya, A.E., Linne, U., Ziebuhr, J., 2021. Coronavirus replication–transcription complex: vital and selective NMPylation of a conserved site in nsp9 by the NiRAN-RdRp subunit. Proc. Natl. Acad. Sci. 118 (6). https://doi.org/10.1073/pnas.2022310118.

Sofia, M.J., Bao, D., Chang, W., Du, J., Nagarathnam, D., Rachakonda, S., Reddy, P.G., Ross, B.S., Wang, P., Zhang, H.-R., Bansal, S., Espiritu, C., Keilman, M., Lam, A.M., Steuer, H.M.M., Niu, C., Otto, M.J., Furman, P.A., 2010. Discovery of a β- <scp>d</scp> -2′-Deoxy-2′-α-fluoro-2′-β- *C* -methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. J. Med. Chem. 53 (19), 7202–7218. https://doi.org/10.1021/jm100863x.

Sola, I., Almazán, F., Zúñiga, S., Enjuanes, L., 2015. Continuous and discontinuous RNA synthesis in coronaviruses. Annu. Rev. Virol. 2 (1), 265–288. https://doi.org/10.1146/annurev-virology-100114-055218.

Sourimant, J., Lieber, C.M., Aggarwal, M., Cox, R.M., Wolf, J.D., Yoon, J.-J., Toots, M., Ye, C., Sticher, Z., Kolykhalov, A.A., Martinez-Sobrido, L., Bluemling, G.R., Natchus, M.G., Painter, G.R., Plemper, R.K., 2022. 4′-Fluorouridine is an oral antiviral that blocks respiratory syncytial virus and SARS-CoV-2 replication. Sci. Technol. Humanit. 375 (6577), 161–167. https://doi.org/10.1126/science.abj5508.

Steiner, S., Kratzel, A., Barut, G.T., Lang, R.M., Aguiar Moreira, E., Thomann, L., Kelly, J. N., Thiel, V., 2024. SARS-CoV-2 biology and host interactions. Nat. Rev. Microbiol. 22 (4), 206–225. https://doi.org/10.1038/s41579-023-01003-z.

Steverding, D., Troeberg, L., 2024. 100 years since the publication of the suramin formula. Parasitol. Res. 123 (1), 11, 10.1007/s00436-023-08027-7.

te Velthuis, A.J.W., Zubkova, T.G., Shaw, M., Mehle, A., Boltz, D., Gmeinwieser, N., Stammer, H., Milde, J., Müller, L., Margitich, V., 2021. Enisamium reduces influenza virus shedding and improves patient recovery by inhibiting viral RNA polymerase activity. Antimicrobial Agents and Chemotherapy 65 (4). https://doi.org/10.1128/AAC.02605-20.

Tian, L., Qiang, T., Liang, C., Ren, X., Jia, M., Zhang, J., Li, J., Wan, M., YuWen, X., Li, H., Cao, W., Liu, H., 2021. RNA-dependent RNA polymerase (RdRp) inhibitors: the current landscape and repurposing for the COVID-19 pandemic. Eur. J. Med. Chem. 213 (113201), 113201.

Udwadia, Z.F., Singh, P., Barkate, H., Patil, S., Rangwala, S., Pendse, A., Kadam, J., Wu, W., Caracta, C.F., Tandon, M., 2021. Efficacy and safety of favipiravir, an oral RNA-dependent RNA polymerase inhibitor, in mild-to-moderate COVID-19: a randomized, comparative, open-label, multicenter, phase 3 clinical trial. Int. J. Infect. Dis. 103, 62–71. https://doi.org/10.1016/j.ijid.2020.11.142.

Uppal, T., Tuffo, K., Khaiboullina, S., Reganti, S., Pandori, M., Verma, S.C., 2022. Screening of SARS-CoV-2 antivirals through a cell-based RNA-dependent RNA polymerase (RdRp) reporter assay. Cell Insight 1 (4), 100046. https://doi.org/10.1016/j.cellin.2022.100046.

U.S. Food and Drug Administration, 2021. In: Coronavirus (COVID-19) update: FDA authorizes additional oral antiviral for treatment of COVID-19 in certain adults. Fda.Gov/News-Events/Press-Announcements/Coronavirus-Covid-19-Update-Fda-Authorizes-Additional-Oral-Antiviral-Treatment-Covid-19-Certain.

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., Thiel, V., 2021. Coronavirus biology and replication: implications for SARS-CoV-2. Nat. Rev. Microbiol. 19 (3), 155–170. https://doi.org/10.1038/s41579-020-00468-6.

Walker, A.P., Fan, H., Keown, J.R., Knight, M.L., Grimes, J.M., Fodor, E., 2021. The SARS-CoV-2 RNA polymerase is a viral RNA capping enzyme. Nucleic Acids Res. 49 (22), 13019–13030. https://doi.org/10.1093/nar/gkab1160.

Walker, A.P., Fan, H., Keown, J.R., Margitich, V., Grimes, J.M., Fodor, E., te Velthuis, A. J.W., 2020. Enisamium is a small molecule inhibitor of the influenza A virus and SARS-CoV-2 RNA polymerases. https://doi.org/10.1101/2020.04.21.053017.

Wang, B., Svetlov, D., Artsimovitch, I., 2021. NMPylation and de-NMPylation of SARS-CoV-2 nsp9 by the NiRAN domain. Nucleic Acids Res. 49 (15), 8822–8835. https://doi.org/10.1093/nar/gkab677.

Wang, D., Jiang, A., Feng, J., Li, G., Guo, D., Sajid, M., Wu, K., Zhang, Q., Ponty, Y., Will, S., Liu, F., Yu, X., Li, S., Liu, Q., Yang, X.-L., Guo, M., Li, X., Chen, M., Shi, Z.-L., et al., 2021. The SARS-CoV-2 subgenome landscape and its novel regulatory features. Mol. Cell 81 (10), 2135–2147.e5. https://doi.org/10.1016/j.molcel.2021.02.036.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., Xiao, G., 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 30 (3), 269–271. https://doi.org/10.1038/s41422-020-0282-0.

Wang, Q., Wu, J., Wang, H., Gao, Y., Liu, Q., Mu, A., Ji, W., Yan, L., Yan, Zhu, Zhu, C., Fang, X., Yang, X., Huang, Yucen, Gao, H., Liu, F., Ge, J., Sun, Qianqian, Yang, X., Xu, W., Liu, Z., Yang, Haitao, Lou, Z., Jiang, B., et al., 2020. Structural basis for RNA replication by the SARS-CoV-2 polymerase. Cell 182 (2), 417–428.e13.

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., Fu, S., Gao, L., Cheng, Z., Lu, Q., Hu, Y., Luo, G., Wang, K., Lu, Y., Li, H., Wang, S., Ruan, S., Yang, C., Mei, C., et al., 2020. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet (N. Am. Ed.) 395 (10236), 1569–1578. https://doi.org/10.1016/S0140-6736(20)31022-9.

Warren, T.K., Jordan, R., Lo, M.K., Ray, A.S., Mackman, R.L., Soloveva, V., Siegel, D., Perron, M., Bannister, R., Hui, H.C., Larson, N., Strickley, R., Wells, J., Stuthman, K. S., Van Tongeren, S.A., Garza, N.L., Donnelly, G., Shurtleff, A.C., Retterer, C.J., et al., 2016. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. Nature 531 (7594), 381–385. https://doi.org/10.1038/nature17180.

Wilamowski, M., Hammel, M., Leite, W., Zhang, Q., Kim, Y., Weiss, K.L., Jedrzejczak, R., Rosenberg, D.J., Fan, Y., Wower, J., Bierma, J.C., Sarker, A.H., Tsutakawa, S.E., Pingali, S.V., O'Neill, H.M., Joachimiak, A., Hura, G.L., 2021. Transient and stabilized complexes of Nsp7, Nsp8, and Nsp12 in SARS-CoV-2 replication. Biophys. J. 120 (15), 3152–3165. https://doi.org/10.1016/j.bpj.2021.06.006.

World Health Organization, 2025. In: WHO COVID-19 dashboard. Data.Who.Int/Dashboards/Covid19.

Yan, L., Ge, J., Zheng, L., Zhang, Y., Gao, Y., Wang, T., Huang, Y., Yang, Y., Gao, S., Li, M., Liu, Z., Wang, H., Li, Y., Chen, Y., Guddat, L.W., Wang, Q., Rao, Z., Lou, Z., 2021a. Cryo-EM structure of an extended SARS-CoV-2 replication and transcription complex reveals an intermediate state in cap synthesis. Cell 184 (1), 184–193.e10.

Yan, L., Huang, Y., Ge, J., Liu, Z., Lu, P., Huang, B., Gao, S., Wang, J., Tan, L., Ye, S., Yu, F., Lan, W., Xu, S., Zhou, F., Shi, L., Guddat, L.W., Gao, Y., Rao, Z., Lou, Z., 2022. A mechanism for SARS-CoV-2 RNA capping and its inhibition by nucleotide analog inhibitors. Cell 185 (23), 4347–4360.e17. https://doi.org/10.1016/j.cell.2022.09.037.

Yan, L., Yang, Y., Li, M., Zhang, Y., Zheng, L., Ge, J., Huang, Y.C., Liu, Z., Wang, T., Gao, S., Zhang, R., Huang, Y.Y., Guddat, L.W., Gao, Y., Rao, Z., Lou, Z., 2021b. Coupling of N7-methyltransferase and 3′-5′ exoribonuclease with SARS-CoV-2 polymerase reveals mechanisms for capping and proofreading. Cell 184 (13), 3474–3485.e11. https://doi.org/10.1016/j.cell.2021.05.033.

Yan, L., Zhang, Y., Ge, J., Zheng, L., Gao, Y., Wang, T., Jia, Z., Wang, H., Huang, Y., Li, M., Wang, Q., Rao, Z., Lou, Z., 2020. Architecture of a SARS-CoV-2 mini replication and transcription complex. Nat. Commun. 11 (1), 5874.

Yin, W., Luan, X., Li, Z., Zhou, Ziwei, Wang, Q., Gao, M., Wang, X., Zhou, F., Shi, J., You, E., Liu, M., Wang, Q., Jiang, Y., Jiang, H., Xiao, Gengfu, Zhang, L., Yu, X., Zhang, S., Eric Xu, H., 2021. Structural basis for inhibition of the SARS-CoV-2 RNA polymerase by suramin. Nat. Struct. Mol. Biol. 28 (3), 319–325.

Yin, W., Mao, C., Luan, X., Shen, D.-D., Shen, Q., Su, H., Wang, X., Zhou, F., Zhao, W., Gao, M., Chang, S., Xie, Y.-C., Tian, G., Jiang, H.-W., Tao, S.-C., Shen, J., Jiang, Y., Jiang, H., Xu, Y., et al., 2020. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Sci. Technol. Humanit. 368 (6498), 1499–1504. https://doi.org/10.1126/science.abc1560.

Yin, X., Popa, H., Stapon, A., Bouda, E., Garcia-Diaz, M., 2023. Fidelity of ribonucleotide incorporation by the SARS-CoV-2 replication complex. J. Mol. Biol. 435 (5), 167973.

Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. 367 (19), 1814–1820. https://doi.org/10.1056/NEJMoa1211721.

Zhang, G.-N., Zhao, J., Li, Q., Wang, M., Zhu, M., Wang, J., Cen, S., Wang, Y., 2021. Discovery and optimization of 2-((1H-indol-3-yl)thio)-N-benzyl-acetamides as novel SARS-CoV-2 RdRp inhibitors. Eur. J. Med. Chem. 223, 113622. https://doi.org/10.1016/j.ejmech.2021.113622.

Zhang, Y., Sun, Y., Xie, Y., Shang, W., Wang, Z., Jiang, H., Shen, J., Xiao, G., Zhang, L., 2023. A viral RNA-dependent RNA polymerase inhibitor VV116 broadly inhibits human coronaviruses and has synergistic potency with 3CLpro inhibitor nirmatrelvir. Signal Transduct. Targeted Ther. 8 (1), 360. https://doi.org/10.1038/s41392-023-01587-1.

Zhao, J., Guo, S., Yi, D., Li, Q., Ma, L., Zhang, Y., Wang, J., Li, X., Guo, F., Lin, R., Liang, C., Liu, Z., Cen, S., 2021a. A cell-based assay to discover inhibitors of SARS-CoV-2 RNA dependent RNA polymerase. Antivir. Res. 190, 105078. https://doi.org/10.1016/j.antiviral.2021.105078.

Zhao, J., Zhang, Y., Wang, M., Liu, Q., Lei, X., Wu, M., Guo, S., Yi, D., Li, Q., Ma, L., Liu, Z., Guo, F., Wang, J., Li, X., Wang, Y., Cen, S., 2021b. Quinoline and quinazoline derivatives inhibit viral RNA synthesis by SARS-CoV-2 RdRp. ACS Infect. Dis. 7 (6), 1535–1544. https://doi.org/10.1021/acsinfecdis.1c00083.

Zhao, Z., 2003. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. J. Med. Microbiol. 52 (8), 715–720. https://doi.org/10.1099/jmm.0.05320-0.

Zúñiga, S., Sola, I., Alonso, S., Enjuanes, L., 2004. Sequence motifs involved in the regulation of discontinuous coronavirus subgenomic RNA synthesis. J. Virol. 78 (2), 980–994. https://doi.org/10.1128/JVI.78.2.980-994.2004.