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Review

Nirmatrelvir and COVID-19: development, pharmacokinetics, clinical efficacy, resistance, relapse, and pharmacoeconomics

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ABSTRACT

Nirmatrelvir/ritonavir (N/R) is one of the most effective antiviral drugs against SARS-CoV-2. The preclinical development, pharmacodynamics and pharmacokinetics of N/R are reviewed herein. Randomized clinical trials have been conducted exclusively with pre-Omicron variants of concern, but in vitro studies show that efficacy against all Omicron sublineages is preserved, as confirmed by post-marketing observational studies. Nevertheless, investigations of large viral genome repositories have shown that mutation in the main protease causing resistance to N/R are increasingly frequent. In addition, virological and clinical rebounds after N/R discontinuation have been reported in immunocompetent patients. This finding is of concern when translated to immunocompromised patients, in whom N/R efficacy has not been formally investigated in clinical trials. Economical sustainability and perspectives for this therapeutic arena are discussed.

Introduction

Oral small-molecule antivirals against SARS-CoV-2 have been approved worldwide. These drugs simplify the management of infection and reduce hospitalization rates in patients with COVID-19 who are at risk for disease progression.

One class of small-molecule antivirals targets the SARS-CoV-2 main polyprotein protease (M^{Pro}), which is often referred to as 3C-like protease ($3CL^{pro}$) or nonstructural protein 5 (Nsp5). M^{Pro} is a chymotrypsin-like cysteine protease, the catalytic site of which consists of H41 and C145 residues [1]. Homologous enzymes are found in most positive-sense, single-stranded RNA viruses [2]. Proteases have an indispensable role in the life cycle of a virus and this, combined with a high degree of conservation, renders M^{pro} an ideal target for drugs [3]. In this narrative review, the preclinical and clinical development, pharmacokinetics, and pharmacodynamics of N/R are discussed, with a focus on efficacy against the Omicron variant of concern (VOC). Also considered are two emerging phenomena, rebounds and resistance, as well as pharmacoeconomics and perspectives for M^{Pro} inhibitors.

Methods

On December 1, 2022, PubMed, medRxiv, bioRxiv, and ResearchSquare repositories were searched for English language manuscripts published after December 1, 2019 using the following queries: "nirmatrelvir AND resistance", "nirmatrelvir AND (rebound OR relapse)", "nirmatrelvir AND mutations". Case reports, case series, and clinical trials were included; secondary research was excluded. Search results were manually assessed for relevance, and references in each suitable manuscript were further screened for additional sources.

Preclinical and clinical development

During the SARS outbreak in 2002, Pfizer launched a research program that led to an intravenous ketone-based covalent M^{pro}

Abbreviations: N/R, nirmatrelvir plus ritonavir; VOC, variant of concern; RCT, randomized controlled trial.

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cysteine protease inhibitor, PF-00835231. As expected from the 96% overall homology and 100% catalytic domain homology between SARS-CoV-1 and SARS-CoV-2 M^{Pro}, PF-00835231 was effective against SARS-CoV-2 in vitro [3]. During the COVID-19 pandemic, Pfizer developed the phosphate prodrug, nirmatrelvir (PF-07321332), to be taken orally. A phase 1 clinical trial in healthy subjects (NCT04756531) investigated nirmatrelvir as either a single agent or in combination with the pharmacokinetic enhancer (CYP3A4 inhibitor), ritonavir. The combination (N/R) resulted in higher and prolonged nirmatrelvir serum levels. Consequently, Pfizer used N/R in the Evaluation of Protease Inhibition for COVID-19 (EPIC) series of clinical trials. In the phase 2/3 EPIC high-risk (EPIC-HR) randomized controlled trial (RCT), 2246 unvaccinated outpatients with COVID-19 at high risk of progression were treated within 3 days of symptom onset (NCT04960202). At the time, the predominant circulating lineage was the Delta VOC. Patients were randomized according to the protocol to receive N/R as 3 tablets (2 × 150 mg tablets of nirmatrelvir and 1 × 100 mg tablet of ritonavir), or placebo twice daily for 5 days. A total of 1120 patients were randomized to receive N/R and 1126 to receive placebo (twice daily for 5 days). On November 5, 2021, Pfizer announced the results of an interim analysis of the first 774 treated patients, showing that hospitalization was 0.77% (3 of 389) in the N/R arm and 7% in the placebo arm, which included 7 deaths by day 28 [4]. The final data were announced on December 14, 2021 [5] and published in NEJM on February 16, 2022: hospitalization rates through day 28 were 0.72% (5 of 697) in the N/R arm vs. 6.45% (44 of 682) with 9 deaths in the placebo arm by day 28. A similar ratio (0.77% vs 6.31%) was seen in those treated within 5 days (88.9% reduction in the relative risk of hospitalization: -5.81%) [6]. In what may be the fastest drug development project in modern pharmacology [7], Pfizer applied to the US Food and Drug Administration (FDA) for emergency use authorization (EUA) of nirmatrelvir tablets co-packaged with tablets of ritonavir (Paxlovid®/Bexovid®) on November 16, 2021, 11 days after the interim analysis results were made public. The treatment was indicated for use in COVID-19 outpatients aged over 12 years, and weighing more than 40 kg [8]. The EUA was granted on December 22, 2021 [9]. Regulatory agencies in other countries quickly followed suit, with N/R authorizations occurring after a further 4 days in Israel [10], 9 days in the UK [11], 35 days in Europe [12], and 50 days in China [13]. As of December 2022, N/R has been authorized in more than 50 countries [14].

On March 9, 2022, Pfizer initiated EPIC-PEDS, a phase 2/3 trial in 140 children aged 6-18 years that compared 300 mg vs. 150 mg nirmatrelvir within the N/R formulation. Pfizer is also working to develop a body weight-adjusted formulation in 3 additional cohorts below the age of 6 years [14].

In September 2021, Pfizer began the phase 2/3 study, EPIC-PEP (Post-Exposure Prophylaxis; NCT05047601) to evaluate the efficacy and safety of N/R in adult household contacts of COVID-19 patients within 3 days of exposure. On April 29, 2022, the company reported that the 5- or 10-day courses led to statistically nonsignificant reductions in infection of 32% and 37%, respectively [15].

In August 2021, Pfizer initiated the phase 2/3 trial, EPIC in Standard-Risk Patients (EPIC-SR; NCT05011513). On December 14, 2021, the company disclosed that the primary endpoint of symptom amelioration for 4 consecutive days was not met in an interim analysis of 954 patients. A 70% relative risk reduction in hospitalization or death (treatment: 3/428; placebo: 10/426) was reported, but this was not statistically significant [5]. Results from an updated analysis of 1153 patients, reported on June 14, 2022, showed a 51% relative risk reduction of hospitalization (treatment: 5/576; placebo: 10/569), but again this was not statistically significant. The trial was halted by the company [16]. On June 30, 2022, Pfizer announced the submission of a New Drug Application (NDA) to the FDA for high-risk patients aged over 12 years and weighing more than 40 kg [17].

Another emerging off-label usage for N/R is the treatment of post-acute sequelae of SARS-CoV-2 (PASC), a multifaceted entity colloquially called 'long COVID' [18,19] and sometimes related to persistent infection [20,21].

Pharmacokinetic interactions and dose adjustments

Pharmacokinetic interactions with N/R were reviewed recently [22,23]. Ritonavir substantially elevates blood levels of co-administered CYP3A-dependent drugs, with increases in area-under-the-curve (AUC) blood concentrations ranging from 1.8- to 20-fold. Consequently, N/R is contraindicated in patients receiving drugs that are highly dependent on CYP3A for clearance and for which elevated concentrations are associated with serious reactions, including amiodarone, flecainide, propafenone, quinidine, colchicine, clozapine, lovastatin, simvastatin, sildenafil, triazolam, and midazolam. N/R is also contraindicated in patients treated with drugs that are potent CYP3A inducers, such as carbamazepine, phenobarbital, phenytoin, and rifampin, which lead to reduced N/R plasma concentrations. In a large US study, the cumulative prevalence of these contraindications among hospitalized patients was estimated to be 14.6%, with higher rates in men vs. women (18% vs. 11.3%), in older patients vs. younger patients (26.9% vs. 8.8%), and in those with comorbidities vs. those without comorbidities (>37% vs. 3.9%); notably, cumulative prevalence was 50.7% among those who died [24].

N/R should also be avoided in organ transplant patients receiving medications to prevent rejection, such as tacrolimus, cyclosporin, sirolimus and everolimus, unless blood levels of those drugs can be followed closely. This is particularly challenging for solid organ transplant recipients with COVID-19 who are on such drugs. These patients are at increased risk for complications of COVID-19 but frequent blood testing to measure drug levels during isolation is logistically difficult. An N/R dose reduction strategy to minimize drug-drug interactions has been studied. The manufacturer recommends dose adjustment to 150 mg nirmatrelvir and 100 mg ritonavir twice daily for 5 days for patients with moderate renal impairment (estimated glomerular filtration rate [eGFR] ≥30 to <60 mL/min). Lingscheld et al. administered N/R 150/100 mg twice daily to 4 patients with end-stage renal disease under hemodialysis and showed high nirmatrelvir blood concentrations that were still within the range known from patients without renal failure; no accumulation took place and levels declined to zero within a few days after the end of treatment [25]. Although not formally recommended, a dose of 300 mg nirmatrelvir (with 100 mg ritonavir) daily and after hemodialysis on dialysis days, is anticipated to provide effective blood concentrations for enzyme inhibition [26]. Brown et al. administered such a modified 5-day N/R regimen to 15 patients with COVID-19 and the treatment was well tolerated and effective, with only 1 patient manifesting rebound symptoms, which resolved in 2 days [27].

Efficacy against Omicron in vitro

All the commercially available anti-spike monoclonal antibodies have lost activity against recent Omicron sublineages [28–30]. In contrast, all the authorized small-molecule antivirals have thus far retained efficacy against the various Omicron sublineages. The common M^{pro} mutations in Omicron (P132H) do not affect the catalytic site of nirmatrelvir [31], and this drug has retained in vitro efficacy against the following VOC Omicron sublineages: BA.1 [31–38], BA.1.1 [32], BA.2 [32,36,39], BA.2.12.1 [32], BA.4 [32], BA.5 [32], BA.2.75 [40], BQ.1.1 and XBB [29] (<2-fold increases in IC_{50}).

Efficacy against Omicron in clinical trials

In addition to in vitro data, nirmatrelvir can restrict viral infection in the respiratory organs of hamsters infected with BA.2 [41]. However, a pharmacokinetic human-equivalent dose of N/R did not significantly reduce shed SARS-CoV-2 titers in ferrets and failed to block virus transmission to untreated direct-contact ferrets, whereas transmission was fully suppressed in a group of animals treated with a human-equivalent dose of molnupiravir. Prophylactic administration of molnupiravir to uninfected ferrets in direct contact with infected animals blocked productive SARS-CoV-2 transmission, whereas all contacts treated with prophylactic N/R became infected [42].

Hence, clinical data are needed. In a study of eligible Clalit Health Services patients, among the 42 819 aged over 65 years, the 2504 who were treated with N/R during the Omicron wave had 72% fewer hospitalizations and 81% less mortality, but no benefit was seen in the 40-65 years age group [43].

Considering the EPIC-SR RCT was halted, it is unlikely that any further RCT evidence will be forthcoming during the Omicron wave; therefore, any new information will have to come from cohort studies, which have intrinsic biases.

Among 1 072 004 non-hospitalized COVID-19 patients in Hong Kong during the BA.2.2 wave (March-April 2022), the 5663 who received N/R had a lower risk of mortality (hazard ratio [HR] 0.25) and hospitalization (-31%, HR 0.69) than those who did not receive N/R, regardless of vaccination status and age (dichotomized at 65 years) [44].

Similarly, among 6036 patients (87% vaccinated) prescribed N/R in Massachusetts and New Hampshire during the Omicron wave (Jan-May 2022), the overall risk of hospitalization within 14 days (<1% following an outpatient diagnosis) was 45% lower compared with in the 24 286 patients who did not take the protease inhibitor [45].

In a retrospective cohort of 5287 patients in the Kaiser Permanente Southern California (KPSC) healthcare network who received prescriptions for N/R from December 31, 2021 to May 26, 2022, 6 (0.11%) patients were hospitalized for symptoms consistent with COVID-19 during the 5–15 days after treatment was dispensed. All hospitalized patients were in groups at high risk for severe COVID-19 and 2 died [46].

In a retrospective cohort of 111, mostly vaccinated, patients in Italy treated with N/R between February and June 2022 (BA.1 and BA.2 waves), Gentile et al. reported only 1 (0.9%) hospitalization [47].

A propensity score-matched (PSM) study from the US-Optum dataset during the period December 22, 2021 to June 8, 2022 showed that the incidence of hospitalization within 30 days was 1.21% for 2808 patients in the N/R group and 6.94% for 10 849 patients in the non-N/R group, with an HR of 0.16 (84% relative risk reduction) [48].

In another PSM study from the USA at the time of BA.2/BA.2.12.1, 3614 N/R patients vs. 4835 untreated patients had lower all-cause hospitalization (0.9% vs. 1.3%), COVID-19-related hospitalization (adjusted odds ratio [aOR]: 0.42), 28-day all-cause mortality (aOR: 0.05), and 28-day emergency room (ER) visits (3.9% vs 4.2%) [49].

Schwartz et al. showed that among 8876 outpatients treated with N/R in Ontario, hospitalization or death within 30 days was lower compared with that in unexposed individuals (2.1% vs 3.7%). In the secondary analysis, the relative odds of death were significantly reduced (1.6% vs 3.3%). The number of patients that needed to be treated to prevent one case of severe COVID-19 was 62. Findings were similar across strata of age, vaccination status, and comorbidities [50].

Compared with untreated matched controls, 1587 N/R-treated patients in the Veterans Health Administration (VHA) had a lower 30-day risk of hospitalization (27.10/1000 vs. 41.06/1000, risk difference [RD] -13.97) and death (3.15/1000 vs. 14.86/1000, RD -11.71). Among individuals who were alive at day 31, there were no further significant reductions in 31-180-day incidence of hospitalization (sub-HR 1.07) or death (HR 0.61). A statistically significant difference in 30-day or 31-180-day risk of hospitalization or death was not observed between matched N/R- or molnupiravir-treated participants. Incidence of most post-COVID conditions was similar across groups [51].

Hospitalization is not the only efficacy endpoint. Among 9217 outpatients in the healthcare databases of the US Department of Veterans Affairs, treatment with N/R in March-June 2022 was associated with reduced risk of PASC (HR 0.74, absolute risk reduction [ARR] 2.32) compared with control, including reduced risk of 10 of 12 post-acute sequelae in the cardiovascular system (dysrhythmia and ischemic heart disease), coagulation and hematologic disorders (deep vein thrombosis, and pulmonary embolism), fatigue, liver disease, acute kidney disease, muscle pain, neurocognitive impairment, and shortness of breath. N/R was also associated with reduced risk of post-acute death (HR 0.52, ARR 0.28), and post-acute hospitalization (HR 0.70, ARR 1.09). N/R was associated with reduced risk of PASC in people who were unvaccinated, vaccinated, and boosted, and in people with primary SARS-CoV-2 infection and reinfection [50].

Resistance

As with any other antiviral, resistance to nirmatrelvir can be either basal or treatment-emergent.

Mutations in M^{pro} causing resistance to nirmatrelvir are already found in circulating SARS-CoV-2 viruses (Table 1 and Fig. 1). For example, in May 2022, the M49I mutation was found in 1883 genomes, with a slight uptick in late 2021 [52], while 6 different types of mutations at position 191 (nt 10625-10627) were found in 9262 sequences (https://coronavirus3d.org/#/drug). Hu et al. identified in GISAID sequences 66 prevalent M^{pro} mutations located at the nirmatrelvir binding site, 11 of which (including S144M/F/A/G/Y, M165T, E166Q, H172Q/F, and Q192T/S/V) showed <10-fold change in enzymatic activity and resistance to nirmatrelvir (K_i > 10-fold increase) [53]. Sasi et al. identified 5 mutations (N142L, E166M, Q189E, Q189I, and Q192T) that reduce the potency of nirmatrelvir: in particular, the IC_{50} of nirmatrelvir was reduced by 24-fold against E166M [54]. Dias Noske et al. reported that N/R retained most of its in vitro activity against most of the 14 naturally occurring polymorphisms close to the binding site, with only G143S and Q189K linked to higher resistance. Of interest, ensitrelvir had a different resistance profile, driven by M49I, G143S and R188S, but not for Q189K [55]. Phylogenetic analyses indicate that nirmatrelvir-resistant variants pre-existed the introduction of nirmatrelvir into the human population and are transmissible [56].

The widespread use of N/R could make even low probability events more likely. N/R-resistant variants selected in vitro harbored different sets of M^{pro} mutations, with the L50F and E166V combination driving an 80-fold increase in EC_{50} [57]. Of interest, the N/R-resistant variants have high fitness but, as expected, remain sensitive to remdesivir [58]. The L50F + E166A + L167F combination is instead associated with a >20-fold increase in EC_{50} values for nirmatrelvir [59]. In vitro passaging of SARS-CoV-2 in increasing concentrations of nirmatrelvir generated mutants harboring T21I, P252L, or T304I mutations in M^{pro}: E166V mutation lead to ~300-fold reduction in nirmatrelvir activity, but resulted in a loss of viral replicative fitness compensated by L50F and T21I [60].

Table 1
Summary of main M^{Pro} variants associated with N/R resistance reported to date, with prevalence estimate in the GISAID databank.

Variant [lineage]	Variant grouping	Ref	Domain location	Subsite location (as defined by [94])	Total mutational counts (specific mutation; total at that position) [via GISAID CoVsurver: https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/]	Effect of variant on proteolytic activity	Nirmatrelvir inhibition
High frequency potential resistance mutations predicted by Coronavirus3D. Residues of interest are identified based on atomic distance from nirmatrelvir inhibitor and computational predictions of ligand-protein interactions. The emergence and dynamics of mutations at these positions observed in circulating virus are tracked via data from GISAID. [52] (https://coronavirus3d.org/)							
M49I	T	[52]	D1	S2	1898; 2062	n.a.	n.a.
V186F	P	[52]	SBL	none	1966; 3211	n.a.	n.a.
R188K	P	[52]	SBL	S2	243; 408	n.a.	n.a.
T190I	P	[52]	SBL	close to S4	1868; 1945	n.a.	n.a.
A191V	P	[52]	SBL	close to S4	8492; 9320	n.a.	n.a.
Experimentally characterized M^{pro} variants							
G15S [C.37 Lambda]	T	[53]	D1	outside of binding site	27289; 29511	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[95]				k_{cat}/K_m = 16,500 S^{-1} M^{-1} (1.9-fold decrease); Crystal structure shows binding mode and M^{pro} conformation is not altered compared to WT complex	K_i = 4.07 nM* (4.3-fold increase, $P = 0.0002^*$)
		[96]				k_{cat}/K_m = 15,000 S^{-1} M^{-1} (1.08-fold decrease)	K_i =10.3 nM (1.05-fold decrease)
T21I [B.1.1.318]	T	[53]	D1	outside of binding site	15618;15890	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[96]				k_{cat}/K_m = 10,000 S^{-1} M^{-1} (1.58-fold decrease)	IC_{50} =10.1 nM (1.11-fold increase)
		[59]					EC_{50} 1.4-fold increase
H41M/T/Y	IA	[53]	D1	S1 pocket/ catalytic residue	84/26/19; 378	Enzymatically inactive (H41 forms catalytic dyad with C145)	Inactive M^{pro} variant, unlikely to be tolerated in circulating variants
M49I/T/L/V	T	[53]	D1	S2 pocket	2059/78/71/54; 2346	k_{cat}/K_m of M49I and M49L showed 1.69 and 1.74-fold increase	All remained sensitive to nirmatrelvir (< 3-fold change in IC_{50} value)
L50F	T	[59]	D1	Close to S2	4532; 4821	Interferes with dimerization (K_d = 2287 nM); 95.5% reduction in protease activity	1.4-fold EC_{50} increase
L89F [B.1.2]	T	[53]	D1	Outside of binding site	153727; 153995	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[96]				k_{cat}/K_m = 12,000 S^{-1} M^{-1} (1.33-fold decrease)	IC_{50} =10.5 nM (1.06-fold decrease)
K90R [B.1.351 Beta]	T	[53]	D1	Outside of binding site	175764; 176349	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[96]				k_{cat}/K_m = 9,000 S^{-1} M^{-1} (1.79-fold decrease)	IC_{50} = 12.7 nM (1.13-fold increase)
		[95]				k_{cat}/K_m = 28,300 S−1 M−1; (1.1-fold decrease); Crystal structure shows binding mode and Mpro conformation is not altered compared to WT complex	K_i = 1.05 nM (1.1-fold increase, n.s.)
P108S [B.1.1.284]	T	[53,97]	D2	Outside of binding site	25774; 30707	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
P132H [B.1.1.529 Omicron]	T	[53]	D2	Outside of binding site	4255443; 4274244	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[95]				k_{cat}/K_m = 20,800 S^{-1} M^{-1}1; (1.5-fold decrease); Crystal structure shows binding mode and Mpro conformation is not altered compared to WT complex	K_i =0.635 nM (1.4-fold decrease, n.s.)

(*continued on next page*)

Table 1 (*continued*)

Variant [lineage]	Variant grouping	Ref	Domain location	Subsite location (as defined by [94])	Total mutational counts (specific mutation; total at that position) [via GISAID CoVsurver: https://www.gisaid.org/epiflu-applications/covsurver-mutations-app/]	Effect of variant on proteolytic activity	Nirmatrelvir inhibition
High frequency potential resistance mutations predicted by Coronavirus3D. Residues of interest are identified based on atomic distance from nirmatrelvir inhibitor and computational predictions of ligand-protein interactions. The emergence and dynamics of mutations at these positions observed in circulating virus are tracked via data from GISAID. [52] (https://coronavirus3d.org/)							
		[96]				k_{cat}/K_m = 23,000 S^{-1} M^{-1}1 (1.44-fold increase)	IC_{50} =12.2 nM (1.09-fold increase)
		[98]				k_{cat}/K_m =10,000 S^{-1} M^{-1} (1.1-fold increase); Tm=53.6 C, 2.6 C lower than WT	IC_{50}= 32nM (1.23-fold increase)
T135I	T	[53]	D2	Outside of binding site	1325; 1481	Similar k_{cat}/K_m value	Remained sensitive inhibition (<2.9-fold change in Ki values
N142X	T	[53]	D2	Close to S1	431	All have similar enzymatic activity as the WT (<4.1-fold change in k_{cat}/K_m value)	All remained sensitive to nirmatrelvir (<3.5-fold change in IC_{50} values)
S144M/F/AG/Y	R	[53]	D2	S1 pocket (forms oxyanion hole)	15/14/9/2/2; 235	k_{cat}/K_m values are comparable to WT (from 2.8 to 8.0-fold)	K_i increase 19.2-38.0-fold
H163W	IA	[53]	D2	S1 pocket	4656; 5032	Enzymatically inactive, hydrogen bond with P1 is critical for substrate binding	Inactive M^{pro} variant, unlikely to be tolerated in circulating variants
H164N	T	[53]	D2	S1 pocket (via hydrogen bond from main chain carboxyl)	4664; 5031	4.2-fold lower k_{cat}/K_m value; is comparable to WT	Remained sensitive (<4.1-fold change in K_i values)
M165T	R	[53]	D2	S2	7; 5180	8.3-fold decrease in k_{cat}/K_m value	K_i = 56.3 nM (29.9-fold increase)
E166Q/A/V	R	[53]	D2	S1	4665; 5084	E166Q: Same enzymatic activity as WT	E166Q: K_i= 22.0 nM (11.7-fold increase)
		[59]				Interferes with dimerization (K_d = 235 nM); 86.4% reduction in protease activity	E166A: K_i= 230 nM (10-fold increase)
		[58]				n.a.	E166V: 267-fold increase in EC_{50}
		[57]				n.a.	E166V: EC_{50} = 14.08 uM (265-fold increase)
L50F/E166V	R	[58]	n.a.	n.a.	n.a.	n.a.	80-fold increase in EC_{50}
L167F	T	[59]	D2	Close to S4	18; 442	Interferes with dimerization (K_d = 127 nM); 83.6% reduction in protease activity	IC_{50} = 100 nM (4.4-fold change)
L50F/E166A/L167F	R	[59]	D1/D2/D2	n.a.	n.a.	Interferes with dimerization (K_d = 966 nM); 94.7% reduction in protease activity	IC_{50} = 1600 nM (72-fold change)
P168S		[57]	D2	None	456; 778		P168R: Scored as inactive
H172Q/F	R	[53]	D2	Close to S1	12/5; 260	H172Q: k_{cat}/K_m = 3.2-fold lower; H172F: k_{cat}/K_m = 9.9-fold lower H172Y: k_{cat}/K_m = 790 M-1S-1 (13.9-fold decrease)	K_i increase by more than 10-fold H172Y: K_i =275 nM (146.3-fold increase)
Q189X	T	[53]	SBL	Close to S2	1436	All retained similar k_{cat}/K_m values (between 1.9- and 9.2-fold) Q189E: 20669 S^{-1} M^{-1} (1.88-fold increase)	No significant resistance for any variants (<3.1-fold change in IC_{50})
H172Y/Q189E	R	[53,99]	D2/SBL	n.a.	n.a	k_{cat}/K_m = 1009 S^{-1} M^{-1} (10.9-fold decrease)	K_i = 528 nM (281.1-fold increase)
Q192T/S/V	R	[53]	SBL	S4	181/27/6; 1462	Q192T: k_{cat}/K_m = 9.2-fold lower; Q192S: k_{cat}/K_m = 8.9-fold lower; Q192V: k_{cat}/K_m = 9.0-fold lower	All showed inhibition resistance (K_i increase >22.2-fold)
L205V [P.2 Zeta]	T	[53]	D3	Outside of binding site	6013; 6256	Activity similar to WT	no significant IC_{50} or K_i value shifts observed (< 2-fold)
		[96]				k_{cat}/K_m =15 000 S^{-1} M^{-1} (1.08-fold decrease)	IC_{50} = 10.7 nM (1.05-fold decrease)

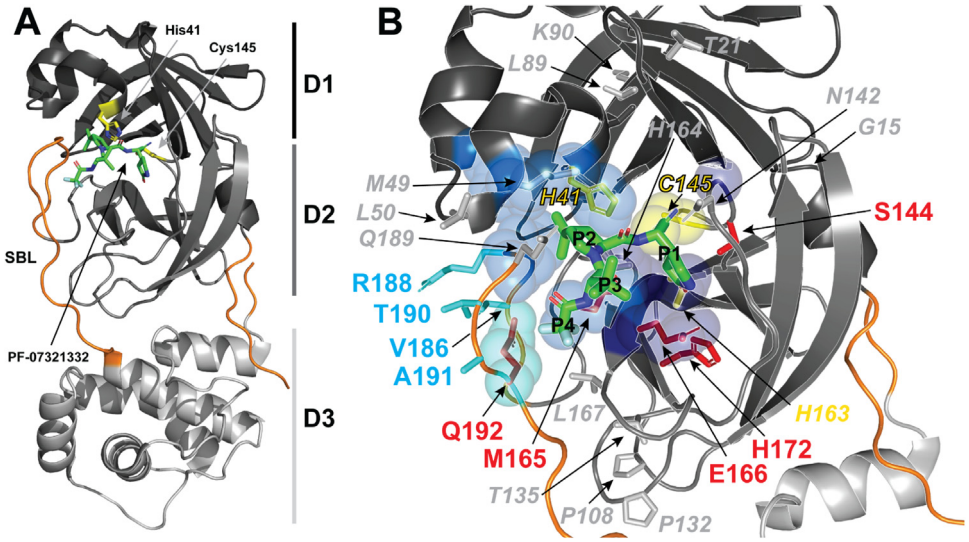

Fig. 1. The structure of SARS-CoV-2 M^{pro} in complex with the PF-07321332 inhibitor (PBD 7VH8). **A)** The M^{pro} monomer is displayed in cartoon representation to illustrate the domain organization of the protease. Domain I-III (residues 10-99, 100-184, and 201-303, respectively) are colored black, dark grey and light grey, respectively. The substrate binding loop (SBL), amino- and carboxy-termini are colored orange, and PF-07321332 is displayed as green sticks. The catalytic triad (Cys145 and His41) is displayed as yellow sticks. **B)** Positions of frequent amino acid substitution in M^{pro} variants are mapped to a detailed view of the contacts between PF-07321332 and M^{pro} at the interface between Domain I and II. Substrate residue numbers are indicated in black and viral protease amino acids involved in each subsite are displayed as spheres, with S1, S2 and S4 subsites colored dark blue, marine blue and cyan, respectively. Amino acid positions with quantified resistance to nirmatrelvir inhibition, positions that can tolerate multiple mutations without affecting activity or inhibitor binding, positions that yield inactive M^{pro}, and positions with common variations that are predicted to impart nirmatrelvir resistance are displayed as sticks and colored red, grey, yellow, and cyan, respectively.

Although the short (5-days) treatment course reduces selective pressure for N/R resistance, widespread deployment as a monotherapy, including to immunosuppressed patients who could fail to clear the virus after the 5-day schedule, increases the probability of resistance emergence.

Relapses

Since April 2022, there have been multiple reports of Omicron-infected patients experiencing virological and clinical relapse within days after completion of the 5-day N/R regimen. A search of PubMed, ResearchSquare preprint server and Google News performed on August 3, 2022 retrieved 32 cases [61–67], the features of which are summarized in Supplementary Table 1. Most patients were vaccinated, and no patient died, but 6/31 (5%) required hospitalization. A few authors reported the isolation of infectious SARS-CoV-2 [63,64,66] and transmission during the rebounds [61]. Accordingly, the CDC recommended wearing a mask for 10 days in case of rebound [68]. All relapses resolved without additional antiviral treatment (with a single exception in the Chief Medical Advisor to the President of the United States [69]) within a couple of weeks. Sequencing in many patients indicated that relapse was not due to a treatment-emergent mutation or infection with a different viral strain [64].

It was soon realized that in the protocol of the RCT that led to drug approval, nasopharyngeal or nasal swabs were collected on day 1 (baseline) and days 3, 5, 10, and 14, but the final publication only reported outcomes on day 5 [6]. A look back at the Center for Drug Evaluation and Research (CDER) review identified that *"Several subjects appeared to have a rebound in SARS-CoV-2 RNA levels around Day 10 or Day 14* [i.e. days 13 and 17 since onset of first symptoms], *although this occurred among subjects with or without potential resistance-associated substitutions detected at Day 1 or Day 5"*, but clinical symptoms were not reported, and the subset with available samples was very small [70]. In response to FDA inquiries, the EPIC-HR investigators conducted real-time polymerase chain reaction (PCR) and next-generation sequencing at days 10 and 14. The proportion of present/persistent rebounds was 1.73% (17/980) vs. 2.32% (23/990) and that of transient rebounds was 2.35% (23/980) vs. 4.65% (46/990) in placebo vs. N/R participants, respectively. The authors concluded that this was not associated with low nirmatrelvir exposure, hospitalization or death, severe symptom relapse, serological status, or M^{pro} gene treatment-emergent mutations [71]. Accordingly, in the placebo arm of the ACTIV-2/A5401 trial, 12% of participants had viral rebound (viral rebounders being older than non-rebounders), symptom rebound occurred in 27% of participants after initial symptom improvement and in 10% of participants after initial symptom resolution, and the combination of high-level viral rebound to ≥5.0 log10 RNA copies/mL and symptom rebound after initial improvement was observed in 1-2% of participants [72].

Explanations proposed so far for the rebound phenomenon include the shortness of the schedule, insufficient dosing in obese patients, pharmacokinetic interactions with concurrent medications lowering plasma levels of nirmatrelvir, and/or failure of the drug to eradicate the virus from as yet unidentified drug-inaccessible sanctuary tissues [73]. Fumagalli et al. used a mouse model of SARS-CoV-2 infection and showed that nirmatrelvir administration soon after infection blunts the development of SARS-CoV-2-specific antibody and T cell responses. Accordingly, upon secondary challenge, nirmatrelvir-treated mice recruited significantly fewer memory T and B cells to the infected lungs and to mediastinal lymph nodes, respectively [74].

Although the efficacy of N/R at preventing hospitalization [6] is clear, it is essential to establish the exact frequency of relapses (regardless of mechanism) with the currently circulating Omicron sublineages in fully vaccinated and boosted subjects. In a cohort of 483 high-risk COVID-19 patients treated with N/R, 2 patients (0.4%) required hospitalization by day 30; 4 (0.8%) experienced mild clinical relapses at a median of 9 days after treatment, and all resolved without additional COVID-19-directed therapy, but virological monitoring was not performed [75]. In a study of 11 270 patients treated with N/R and 2374 patients treated with molnupiravir in the USA, the 7-day and 30-day COVID-19 rebound rates

after N/R treatment were 3.53% and 5.40% for COVID-19 infection, 2.31% and 5.87% for COVID-19 symptoms, and 0.44% and 0.77% for hospitalizations, respectively. The 7-day and 30-day COVID-19 rebound rates after molnupiravir treatment were 5.86% and 8.59% for COVID-19 infection, 3.75% and 8.21% for COVID-19 symptoms, and 0.84% and 1.39% for hospitalizations, respectively. This clearly shows the phenomenon is shared across antivirals. Patients with COVID-19 rebound had a significantly higher prevalence of underlying medical conditions than those without [76]. Hence, prospective observational studies reporting higher incidence of viral rebounds in 3-dose mRNA-vaccinated N/R-treated compared with N/R-untreated cohorts that are not PSM (e.g., in BA.2 [77]) have limited meaning.

In the largest dataset of untreated mRNA-vaccinated individuals infected with the Omicron variant, viral rebound (defined with PCR monitoring) occurred in 6% of 494 infections [78]. In a large, retrospective cohort study, the risks of both COVID-19 rebound infections and symptoms 2-8 days after N/R treatment were higher in the BA.5 cohort than in the PSM BA.2.12.1 cohort (HR 1.32) [79]. In the absence of biological rationales, a notoriety bias may explain this finding.

In a prospective cohort study based on 12 sequential quick antigen assays over 16 days conducted between August 4, 2022 and November 1, 2022 in California, viral rebound incidence was 14.2% in the N/R group (18/127) and 9.3% in the control group (4/43). The incidence of COVID-19 symptom rebound was higher in the N/R group (18.9%) than in the control group (7.0%) [80].

Considering the aforementioned findings, there is an urgent need to establish whether prolonging antiviral therapy can prevent the rebound phenomenon. Of interest, on June 30, 2022, Pfizer launched a triple-blind, phase 2 study (NCT05438602) in which immunocompromised patients were to be randomized to treatment with N/R for 5, 10 or 15 days and followed-up for 24 weeks.

Pharmacoeconomics

In the USA, a 5-day N/R course costs USD 529 (£410; €490) according to the independent US non-profit Institute for Clinical and Economic Review (ICER) [81]. This is estimated to correspond to an expenditure of USD 21 000 per hospital admission averted. By contrast, the per-patient hospitalization cost in the USA for COVID-19 is estimated at USD 24 826, without taking into consideration personal and societal costs [82]. In the post-vaccine Omicron era, the cost-benefit further worsened: the ARR dropped from 5.8% (in the original RCT that led to authorization) to 1.8% [43], causing an increase in the number needed to treat to prevent a single hospitalization from 19 to 56 patients. In other words, about 35 000 USD have to be spent to prevent a single hospital admission.

On March 15, 2022, the Drugs for Neglected Diseases initiative (DNDi) expressed concern, on behalf of a consortium of 26 African and global research bodies, that Pfizer would not provide access to N/R for testing in combination with other drugs at later disease stages as the company wanted to run those trials internally [83]. On March 17, the United Nations-backed Medicines Patent Pool (MPP) signed agreements with 35 manufacturers of generic drugs in Europe, Asia, and Central and South America to make N/R and supply it to 95 poorer countries.

Then, on March 22, 2022 Pfizer agreed with UNICEF to supply 4 million courses of treatment to 95 low- and middle-income countries, beginning in April 2022, pending authorization or approval [84]. Two days later, the Africa Centers for Disease Control and Prevention agreed to a memorandum of understanding with Pfizer to provide N/R for African countries [85]. However, availability in India of a locally made generic version of N/R (made by Hetero Labs and Optimus) has been delayed by a requirement of the country's Central Drugs Standard Control Organization for additional local clinical trials [86]. That situation remained unchanged as of June 14, 2022 [87]. An additional problem is the distribution of substandard and falsified medical products on the black market, of which inequality is a driving force [88].

Perspectives

The experience with monotherapy for HIV-1 and HCV shows that viral resistance may develop rapidly when using a single drug. Viral resistance that develops while on therapy has also been described for influenza virus infection, particularly in immunocompromised patients. The ability of SARS-CoV-2 to escape antiviral therapies has been amply demonstrated by the emergence of monoclonal-antibody-resistant variants in treated individuals [28], particularly those who are immunocompromised and cannot clear the infection. Given the historical precedents for resistance to other antiviral therapies, the fact that mutations associated with resistance to N/R are already found in circulating SARS-CoV-2 genomes, the enormous number of patients being treated, and the rebound phenomenon showing that not all SARS-CoV-2 virions are eradicated with 5 days of treatment, N/R resistance is likely to occur rapidly. One potentially effective strategy to reduce the likelihood of N/R resistance is to combine it with other small molecule antivirals or antibody-based therapies. However, considering that antibody-resistant SARS-CoV-2 variants emerged in immunocompromised individuals, who have reduced capacity to clear infection, perhaps this population should be targeted for combination therapy to reduce the probability that N/R-resistant variants emerge. Such individuals could be treated with a combination of other small-molecule antivirals (e.g., additive and synergic effects are expected when M^{pro} inhibitors are combined with RNA-dependent RNA polymerase [RdRp] inhibitors, such as remdesivir or molnupiravir [91,92]) or antibody-based therapies, which may increase the therapeutic benefit and preserve the efficacy of nirmatrelvir.

Novel oral M^{pro} inhibitors that do not require ritonavir boosting and are administered once daily, such as ensitrelvir/S-217622 (Xocova®, Shionogi) [93], are in advanced stages of development. These inhibitors would largely be free of pharmacokinetic interactions. The clinical pipeline also includes EDP-235 (Enanta) and PBI-0451 (Pardes Bio), whereas the preclinical pipeline includes boceprevir, STI-1558 (Sorrento), SH-879 (Sosei Heptares), EDDC-2214 (Everest Medicine), ASC-11 (Ascletis), GC376 (Anivive Lifesciences), and NLC-V-01 (Tollovir®, Todos Medical). Rupintrivir is selective for rhinoviruses but its derivative, M^{pro}-1, is also effective against SARS-CoV-2.

Conclusions

N/R preclinical development has been fast and furious and has contributed to alleviating the COVID-19 healthcare burden in 2022. This drug has proven to be remarkably effective, with preserved in vitro and clinical efficacy against Omicron. N/R has become the most prescribed antiviral in the world, generating $1.5 billion in sales in the first quarter of 2022. For example, in the USA more than 160 000 patients were prescribed this drug per week as of May 2022 [89], and in Italy, as of June 21, 2022, N/R had been prescribed to more than 17 000 unique patients [90]. Despite this, pharmacokinetic interactions are preventing deployment in frail and comorbid patients, which represent the residual burden of the pandemic. Furthermore, treatment-emergent resistance, early relapse, and economical sustainability represent clouds on the horizon that could undermine the long-term future of N/R. For such reasons, research on alternative M^{Pro} inhibitors should not be discontinued because of the success of N/R.

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Declaration of Competing Interests

A.C. is on the scientific advisory board of SAB Therapeutics and owns stock options. The other authors declare no conflict of interest related to this manuscript.

Ethical Approval

Not require.

Sequence Information

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2022.106708.

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