Title
Efficacy of SIGMAR1-based therapy in the early treatment of confirmed mild symptomatic COVID-19 patients

Running Title
Efficacy of E-52862 in the early treatment of COVID-19

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Dear Editor,

We read with interest the recent Letter to Editor by Vladimir Trkulja[1] arguing against the reported efficacy of fluvoxamine for the treatment of COVID-19 patients in two published meta-analyses. Fluvoxamine is a serotonin reuptake inhibitor that binds to other molecular targets, including sigma-1 receptors (SIGMAR1). The activity on SIGMAR1, a resident chaperone in the endoplasmic reticulum (ER), is believed to account for its antiviral activity and that of other non-selective drugs such as amitryptiline, haloperidol or amiodarone.[2] However, their low affinity, functional profile and/or lack of selectivity against SIGMAR1 make them suboptimal for proof-of-concept studies of SIGMAR1-based therapies. To this end, we undertook an investigators-initiated, non-industry-sponsored clinical study to assess the efficacy of E-52862 (4-[2-[[5-methyl-1-(2-naphthalenyl)-1H-pyrazol-3-yl]oxy]ethyl] morpholine; S1RA), a selective SIGMAR1 antagonist[3] with demonstrated human safety in Phase I studies,[4] in the early treatment of mild symptomatic COVID-19 patients.

Our study (EudraCT 2020-003603-33) was a phase II, placebo-controlled, randomized, double-blinded, parallel, multicentre study including nonhospitalized adult patients receiving E-52862 (400 mg/day; once daily by oral route) or placebo for 14 days, recruited within five days of having initiated signs or symptoms of COVID-19, across the pandemic (from 3th February 2021 to 20th July 2022) in Catalonia (Barcelona city) and Galicia (Santiago de Compostela, A Estrada and Barbanza Area), Spain. The evolution of symptoms and adverse effects included face-to-face visits on days 0 (recruitment), 4, 7, and 14 of treatment (±1), at which specific symptoms and SARS-COV-2 viral load were determined, plus a follow-up visit on day 21 (±1).

Patient disposition and demographics are shown in Fig. 1 and Supplementary Table 1 (Table S1), respectively. The per-protocol analysis included 108 patients that received treatment (E-52862, n=57) or placebo (n=51) (Fig. 1). No notable differences were observed in the demographic variables between groups of treatment. The mean age was 47.7 years, with nine patients aged ≥ 65 years. Men accounted for 40.7%. Most patients were infected with Omicron (63%) or Delta (28%) variants (genome sequencing was performed) and were vaccinated (79.6% received one or more doses of mRNA SARS-CoV-2 vaccine) (Table S1).

The mean SARS-CoV-2 $\log_{10}$ viral load (Fig. 2A-D) from nasopharyngeal swabs decreased with time up to day 14, similarly in active and placebo groups using RT-qPCR nucleocapsid (N)1 and N2 probes, with no significant differences in either the time and treatment group interaction or the area under the curve (AUC) (Table S2). However, the fold-change of SARS-CoV-2 viral expression relative to the human RP gene (Fig. 2E-H) was significantly decreased in N1 and N2 assays on day 14 in the active group compared to day 0 (statistical analyses in Table S2), suggesting a subtle beneficial effect of E-52862 on this outcome at the end of treatment. No significant differences were found in the effect of treatment between groups clustered according to their viral variant, vaccination status, or geographical area (Catalonia and Galicia).

Clinical signs across the study are shown in Table S3, and represented in Fig. 2I-N (most relevant ones) and Fig. S1 as the percentage of individuals with the absence of clinical signs at the different time points (statistic analyses in Table S4 and Table S5). Headache, a core clinical sign of COVID-19, was significantly reduced in patients treated with E-52862 respect to placebo (7% *vs* 19.6%) (Table S3), with a significantly higher percentage of individuals without headache on days 4 and 14 (Fig. 2I). Another core symptom of SARS-CoV-2 infection is cough, which was also significantly ameliorated by treatment with E-52862 (15.8% *vs* 27.5%) (Table S3), with absence of this clinical sign in an increased percentage of patients at day 14 (Fig. 2J and Table S4). Finally, sore throat was also significantly inhibited by the active treatment (0%) respect to placebo (7.8%) (Table S3), with significant amelioration at day 14 (Fig. S1H and Table S5). Fever, asthenia, anosmia, ageusia, and rhinorrhea (Tables S3-S5, Fig. 2K-N and S1L), and other symptoms unfrequent in mild symptomatic COVID-19 patients (Table S3, S5 and Fig. S1), were not modified by treatment. Notably, the percentage of individuals with dizziness was significantly superior in the active respect to the placebo group (Table S3 and Fig. S1A), suggesting that E-52862 boots this clinical sign and common adverse effect. Overall, 17 (28.8%) and 35 (59.3%) patients in the placebo and active groups, respectively, reported TEAE, most of which were mild in severity (Table S6).

It is worth noting that amelioration of headache, cough, and sore throat was found despite the low number of patients, the different prevalence of clinical symptoms across SARS-CoV-2 variants, varying levels of immunity depending on vaccination status, exposure history and age. The benefit on clinical signs by treatment with E-52862 was not accompanied by a decrease in nasopharyngeal viral load, with the nuance of a decreased viral (N1 and N2) relative to human RP expression on day 14 compared to day 0 in the active, but not in the placebo group. Indeed, drugs inhibiting the viral replicase, such as remdesivir failed to change nasopharyngeal viral load despite its beneficial clinical effects,[5] supporting that SARS-CoV-2 nasopharyngeal viral loads do not reliably predict treatment outcomes in COVID-19 as virus clearance from the nasopharynge occurs early and quickly, being not representative of viral spreading to lower respiratory tract and beyond.[6] In addition, treatment with a SIGMAR1 drug is expected to slow down the spreading and prevent disease aggravation but not to reduce established viral replication (SIGMAR1 is rate limiting for launching RNA replication, but dispensable once viral replication is established).[7] Mechanistically, SIGMAR1 is required for the virus to assemble its replicative machinery to host ER membranes.[2] Direct interaction is known to occur between SARS-CoV-2 nsp6 and host SIGMAR1.[8] The presumable effect exerted by SIGMAR1 ligands is to prevent this virus-host protein interaction (Fig. S2), precluding nsp6 to fold properly, anchor to the ER and/or homodimerize to build a membrane-bound functional replication organelle.[9] Accordingly, the activity of E-52862 against SARS-CoV-2 *in vitro* is accompanied by cellular changes suggestive of inhibition of host cellular membrane remodeling.[10]

All together, E-52852 provides a safe therapeutic intervention with moderate beneficial effect on COVID-19 in patients with mild symptoms. These findings have the intrinsic limitations of an exploratory study. Further studies with larger/homogeneous patient cohorts should be done to assess the potential of a SIGMAR1 drug for treating infections by coronaviruses and other mRNA viruses engaging nsp6-SIGMAR1 interaction.

Funding

This work was supported by the Spanish "Minsterio de Ciencia e Innovación (MICIN), Agencia Estatal de Investigación (AEI)" (PID2020-120029GB-I00/MICIN/AEI/10.13039/501100011033) to RM; the "Generalitat de Catalunya, AGAUR" (2017 SGR-669) to RM; the "ICREA-Acadèmia" (2020) to RM; and the Spanish "Instituto de Salud Carlos III, RETICS-RTA" (RD16/0017/0020) to RM. We gratefully acknowledge grant support from Xunta de Galicia through the call for proposals RESCATA-COVID (IN845D 2020/19) and European Regional Development Fund (ERDF). Finally, this work received partial financial support by ESTEVE PHARMACEUTICALS S.A.

Data availability

All data used in this work are presented in the manuscript (Figs. 1 and 2) and supplementary figures (Figs. S1 and S2) and tables (Tables S1-S6). Supplementary material can be found, in the online version, at doi: XXX.

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 letter.

Acknowledgments

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PHARMACEUTICALS S.A. for supplying the study medication and for their partial financial support.

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Figure legends

Fig. 1. Patient selection.

Scheme of the process of selection of patients. Some patients were excluded from the analysis populations for more than one reason. Abreviations: ITT, intention-to-treat.

Fig. 2. Efficacy of treatment measured by the viral load and clinical signs.

A-B log10 viral load of SARS-CoV-2 in patients that received placebo or active compound (E-52862) during days 0, 4, 7, and 14 of the study. Data are expressed as individual values with median and interquartile ranges in N1 and N2. **C-D** Area under the curve (AUC) of viral load of SARS-CoV-2 in patients that received placebo or active compound during the study period. Data are expressed as mean ± SEM in N1 and N2. **E-F** Fold change of SARS-CoV-2 viral expression with respect to RP gene in patients that received placebo or active compound during days 0, 4, 7, and 14 of the study. Data are expressed as mean ± SEM in N1 and N2 (***P*<0.01, ****P*<0.001 day 14 *vs* day 7 in the active group). **G-H** AUC of fold-change of SARS-CoV-2 expression in patients that received placebo or active compound during the study period. Data are expressed as individual values with median and interquartile ranges in N1 and N2 or with meas ± SEM in the case of the AUC. **I-N** Percentage of absence of the clinical signs of headache (**I**), cough (**J**), fever (**K**), asthenia (**L**), anosmia (**M**), and ageusia (**N**) during days 0, 4, 7, and 14. **P*<0.05, ***P*<0.01 active *vs* placebo group. The sample sizes are N=51 for the placebo group and N=57 for the active group. For statistical details of data analyses of A-H and I-N see supplementary table 2 (Table S2) and table 4 (Table S4), respectively.

Fig. S1. Clinical Efficacy/adverse effects measured by the clinical signs.

A-L Percentage of absence of the clinical signs of dizziness (**A**), artralgias (**B**), broken speech (**C**), diarrhoea (**D**), vomiting (**E**), pleuritic pain (**F**), congestion (**G**), sore throat (**H**), shivers (**I**), dysgeusia (**J**), choking sensation (**K**), and rhinorrhea (**L**) in patients treated with placebo or active compound during days 0, 4, 7, and 14. ****P*<0.001 active *vs* placebo group. The sample sizes are N=53 for the placebo group and N=57 for the active group. See supplementary table 5 (Table S5) for statistical details of data analyses.

Fig. S2. Proposed mechanism of host SIGMAR1-mediated modulation of SARS-CoV-2 infection.

Early after primary translation of the viral genome, SARS-CoV-2 nsp6 interacts with host sigma-1 receptor (SIGMAR1) at the endoplasmic reticulum (ER) to initiate the formation of the reticulovesicular network of modified ER membrane (replication organelle) and assembly of the functional viral replicase complex. In the presence of the SIGMAR1 antagonist E-52862 the interaction nsp6-SIGMAR1 is disrupted and subsequent host cell ER membrane reprogramming and functional replicase complex assembly are precluded. Note that SIGMAR1 is involved early after primary translation of viral RNA genome and is required for the biogenesis of the replication organelle and launching RNA replication, but it is dispensable once the replication organelle has been formed and viral replication machinery has been established, as observed in persistently infected cells. Note also that SIGMAR1 regulates calcium homeostasis and ER stress, unfolded protein response (UPR) and autophagy, which are hijacked (reprogrammed) by the virus and this may account for potential antiviral effects secondary to SIGMAR1 antagonism.[2] The 3D structure of nsp6 has not been experimentally determined yet and we used the by Alphafold generated de novo model to represent it. The 3D of SIGMAR1 was generated from the crystal structure.

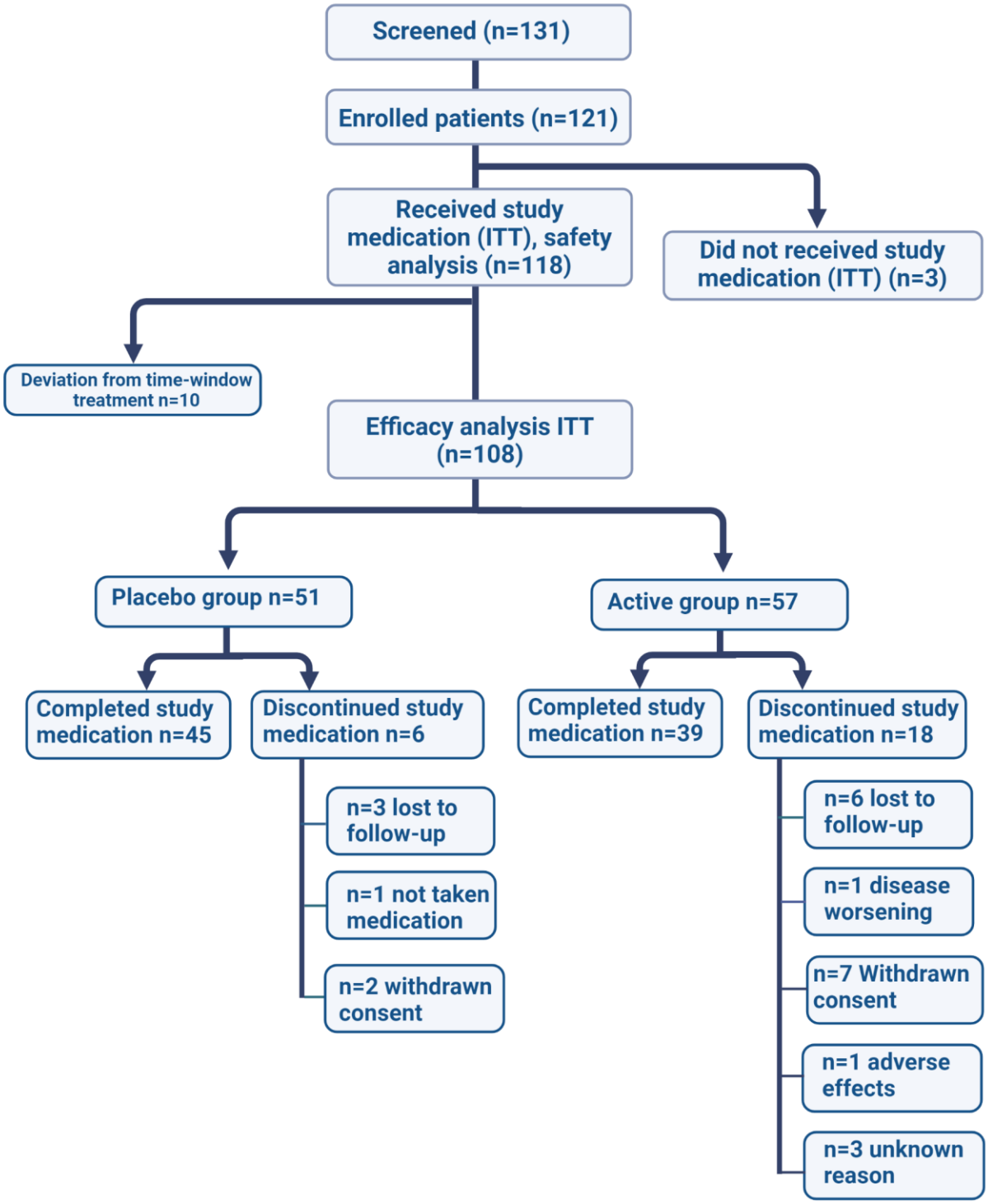

Fig. 1

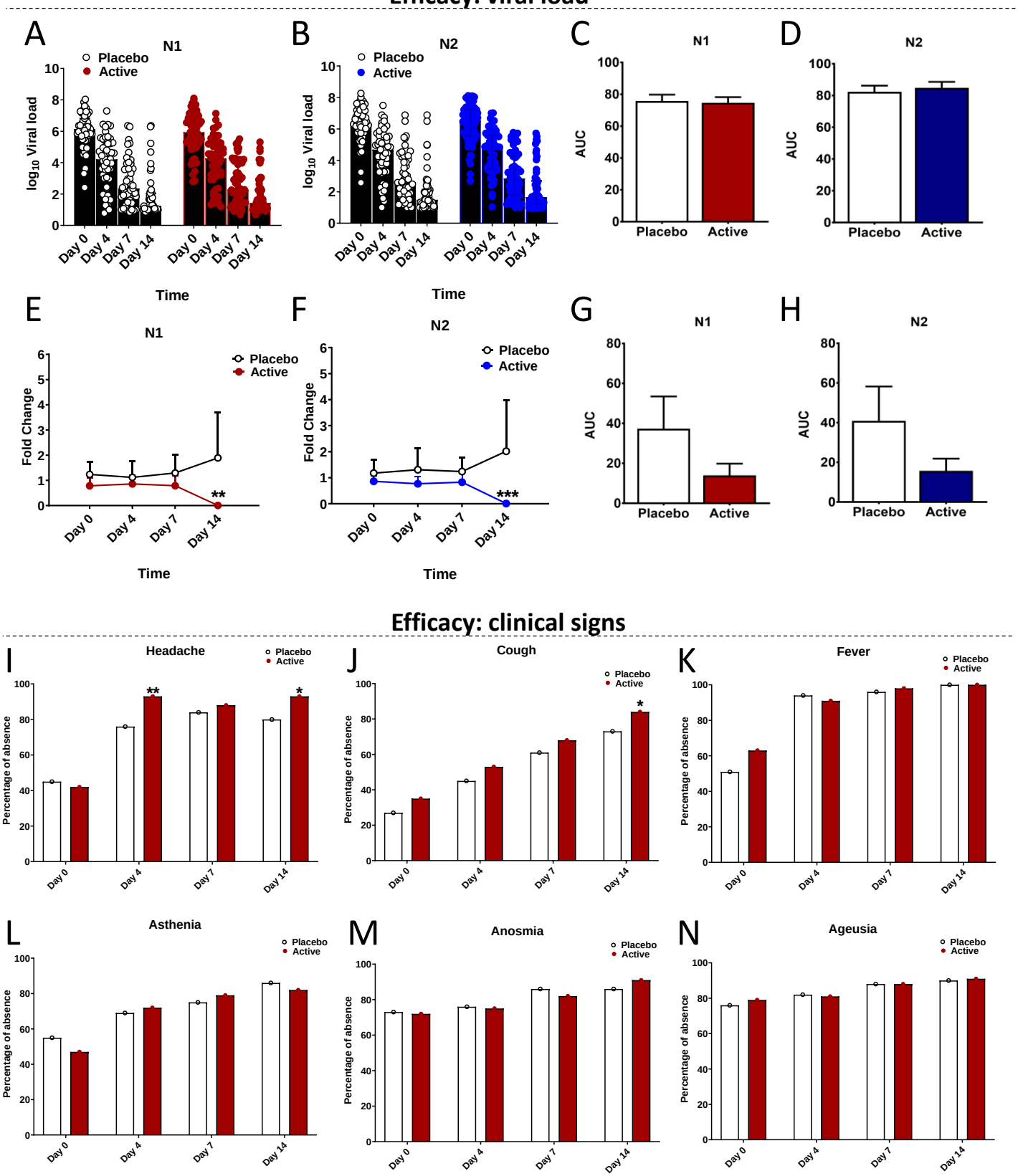
Efficacy: viral load
A
N1
Placebo
Active
log10 Viral load
Day 0
Day 4
Day 7
Day 14
Time
B
N2
C
AUC
D
E
Fold Change
**
F
***
G
H
Efficacy: clinical signs
I
Headache
Percentage of absence
J
Cough
K
Fever
L
Asthenia
M
Anosmia
N
Ageusia

Fig. 2

Efficacy: clinical signs

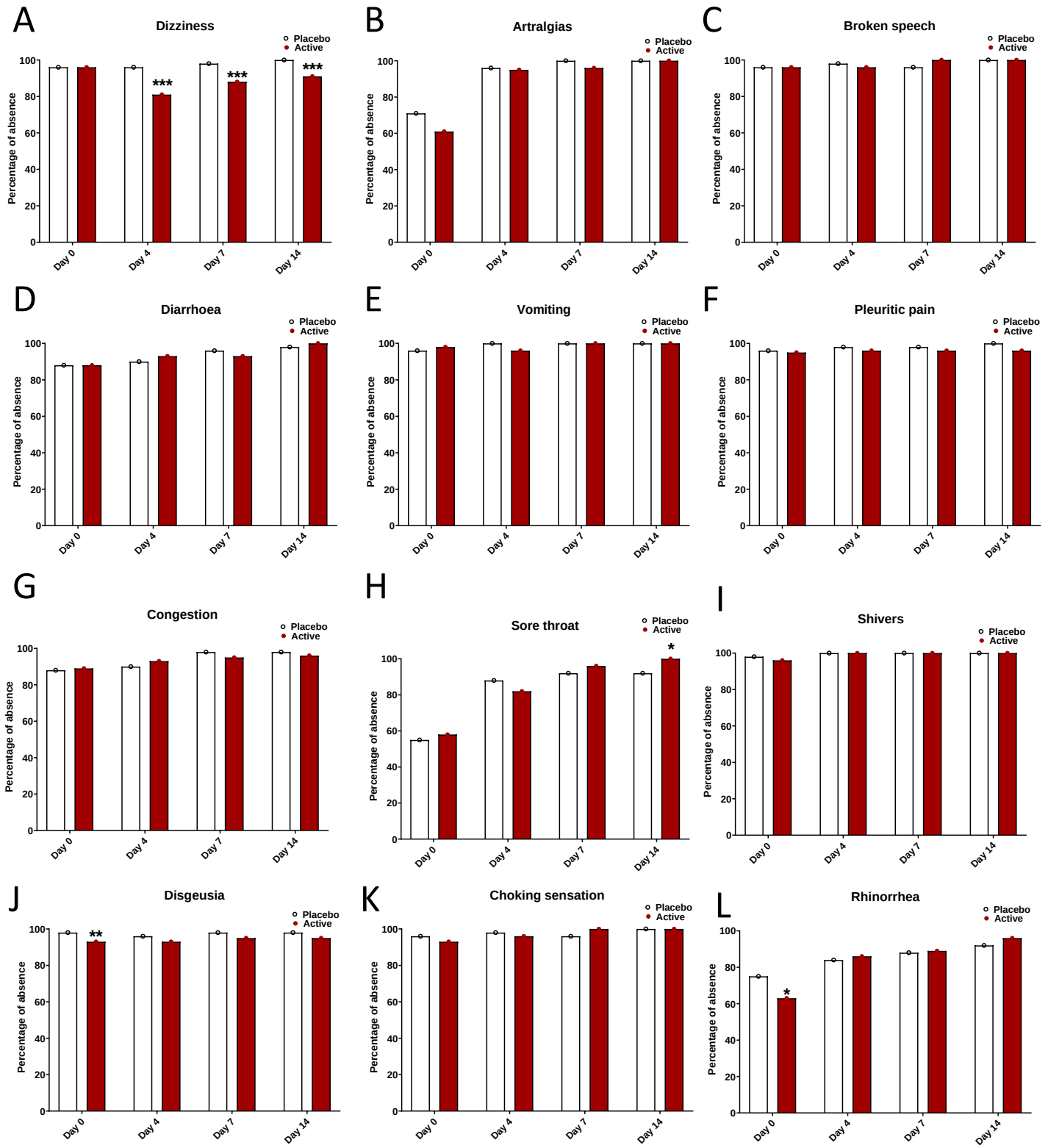

Fig. Supp. 1

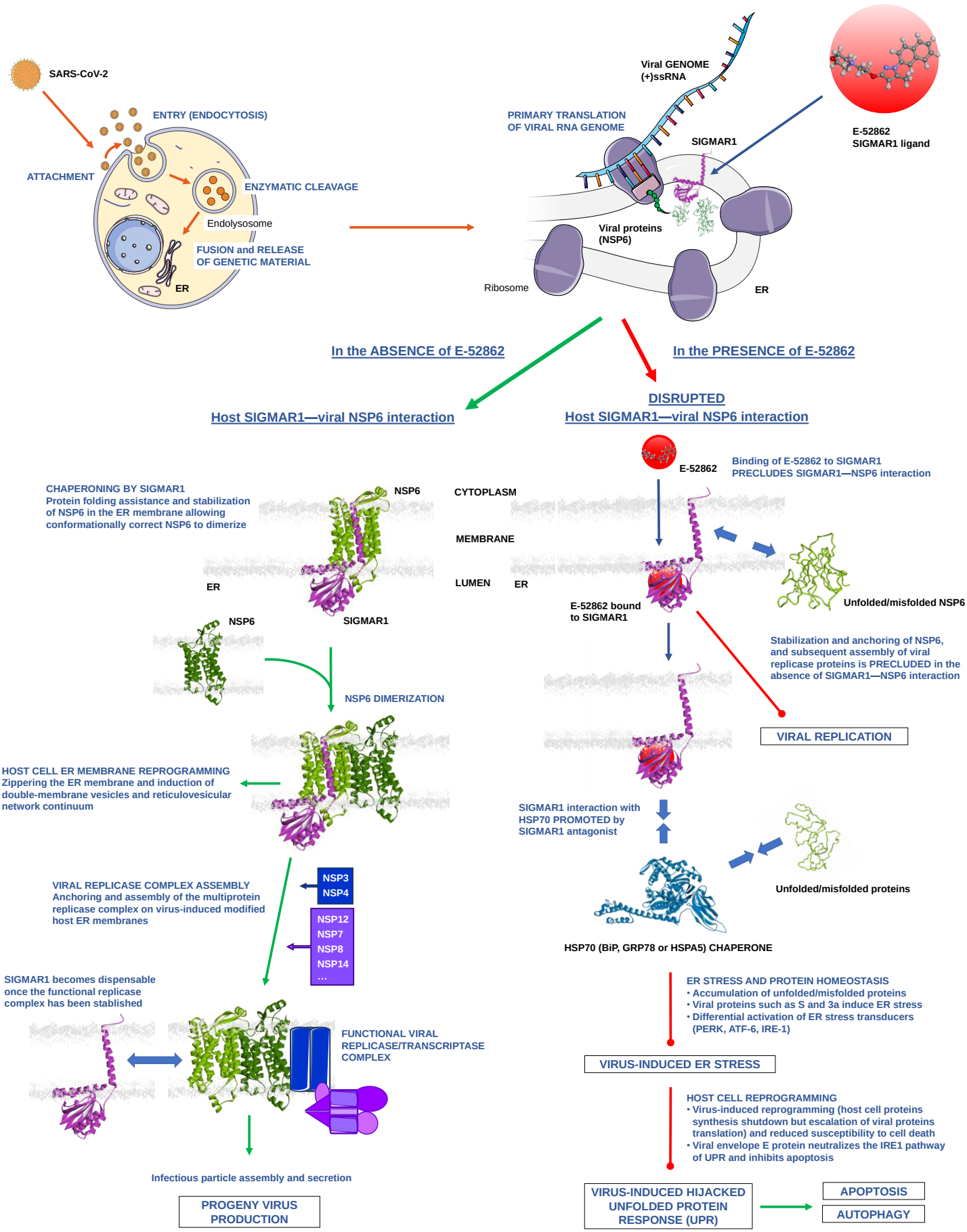
SARS-CoV-2
ENTRY (ENDOCYTOSIS)
ATTACHMENT
ENZYMATIC CLEAVAGE
Endolysosome
FUSION and RELEASE OF GENETIC MATERIAL
ER
Viral GENOME (+)ssRNA
PRIMARY TRANSLATION OF VIRAL RNA GENOME
SIGMAR1
E-52862
SIGMAR1 ligand
Viral proteins (NSP6)
Ribosome
ER
In the ABSENCE of E-52862
In the PRESENCE of E-52862
Host SIGMAR1—viral NSP6 interaction
DISRUPTED
Host SIGMAR1—viral NSP6 interaction
CHAPERONING BY SIGMAR1
Protein folding assistance and stabilization of NSP6 in the ER membrane allowing conformationally correct NSP6 to dimerize
NSP6
ER
SIGMAR1
NSP6
NSP6 DIMERIZATION
HOST CELL ER MEMBRANE REPROGRAMMING
Zippering the ER membrane and induction of double-membrane vesicles and reticulovesicular network continuum
VIRAL REPLICASE COMPLEX ASSEMBLY
Anchoring and assembly of the multiprotein replicase complex on virus-induced modified host ER membranes
NSP3
NSP4
NSP12
NSP7
NSP8
NSP14
...
SIGMAR1 becomes dispensable once the functional replicase complex has been stablished
FUNCTIONAL VIRAL REPLICASE/TRANSCRIPTASE COMPLEX
Infectious particle assembly and secretion
PROGENY VIRUS PRODUCTION
CYTOPLASM
MEMBRANE
LUMEN
ER
E-52862
Binding of E-52862 to SIGMAR1 PRECLUDES SIGMAR1—NSP6 interaction
Unfolded/misfolded NSP6
E-52862 bound to SIGMAR1
Stabilization and anchoring of NSP6, and subsequent assembly of viral replicase proteins is PRECLUDED in the absence of SIGMAR1—NSP6 interaction
VIRAL REPLICATION
SIGMAR1 interaction with HSP70 PROMOTED by SIGMAR1 antagonist
Unfolded/misfolded proteins
HSP70 (BiP, GRP78 or HSPA5) CHAPERONE
ER STRESS AND PROTEIN HOMEOSTASIS
• Accumulation of unfolded/misfolded proteins
• Viral proteins such as S and 3a induce ER stress
• Differential activation of ER stress transducers (PERK, ATF-6, IRE-1)
VIRUS-INDUCED ER STRESS
HOST CELL REPROGRAMMING
• Virus-induced reprogramming (host cell proteins synthesis shutdown but escalation of viral proteins translation) and reduced susceptibility to cell death
• Viral envelope E protein neutralizes the IRE1 pathway of UPR and inhibits apoptosis
VIRUS-INDUCED HIJACKED UNFOLDED PROTEIN RESPONSE (UPR)
APOPTOSIS
AUTOPHAGY

Fig. Supp. 2