COVID-19 Supplement No. 8

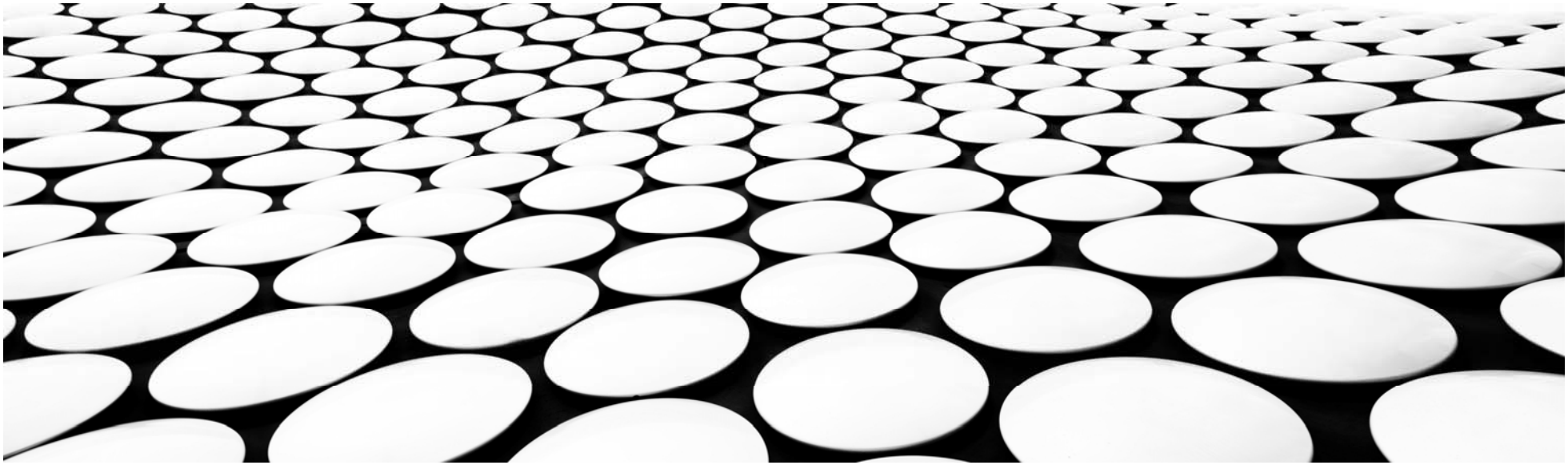
Fluvoxamine – an Antidepressant

Damping COVID-19 Inflammation Via a Novel Pathway

How does a SSRI Drug for Depression and Neurologic Diseases Work in COVID?

It has a high affinity for the Sigma-1 Receptor

BRMoyer & Associates, LLC, Amherst, NH. *Created: March 2021 Updated March 9, 2021*



Drugs Are Like a Game of Dominos – Where to Stop the Falling

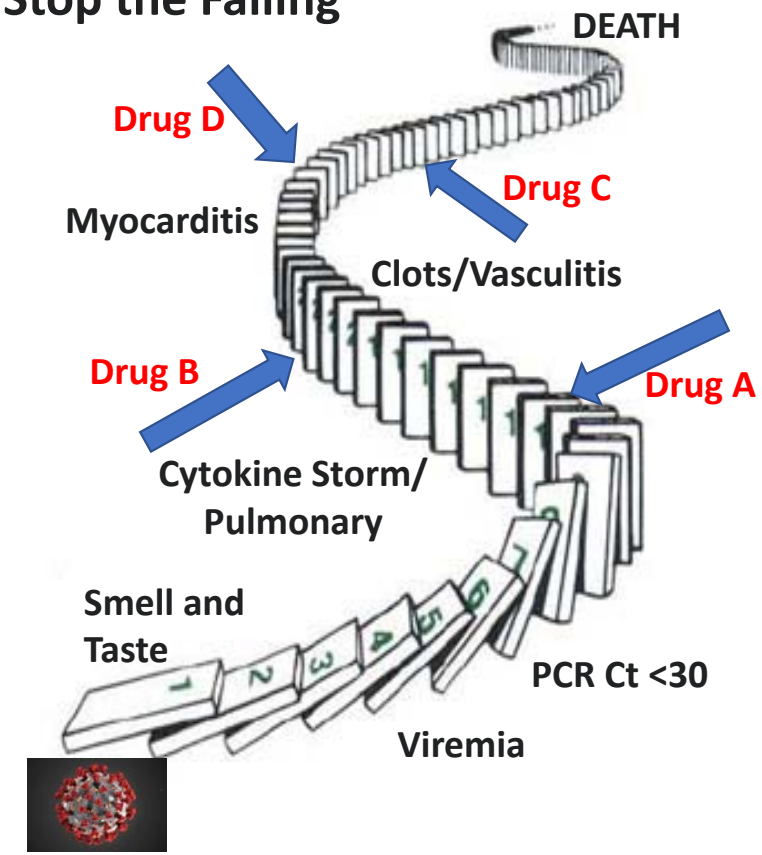
Drugs that can effectively damp the COVID-19 infection and the body's responses to the virus are a prize accomplishment. Investigations into the mechanisms of action of COVID-19 – **the step-by-step chain of events that act like a set of falling dominos** - have revealed a complex array of potential targets.

We have seen drugs like **hydroxychloroquine** (changes the pH of the cell environment to abate viral progress) that required high concentrations to achieve its purpose. Such levels gave a narrow **"therapeutic index"** – the ratio of the drug level for efficacy to that of toxicity (TI = dose efficacy/dose toxicity).
Then there are drugs that abate the cytokine storm like monoclonal antibodies to IL-6 (**Tocilizumab**) and to TNF-α (**Adalimumab**).

Among the most recent ideas is to stimulate the **Sigma-1 Receptor (**σ–1). Drugs known to be σ–1 receptor "agonists" (stimulators) include cocaine, morphine, PCP, methamphetamine, dextromethorphan and others which also stimulate the κ-opioid receptor the NMDA glutamate receptor. The σ–1 receptors can damp IL-6 and TNF-α synthesis reducing the cytokine storm via the **inositol-3-phosphate receptor**

Sigma-1 receptors are trans-membrane proteins placed in the endoplasmic reticulum (ER), which regulates the function of, and stabilizes, calcium signaling between ER and mitochondria at the MAM (a zone between the two). ,

One new drug being investigated for its Sigma-1 receptor activity is **Fluvoxamine (Luvox®)** which is used an antidepressant for the way it acts on SSRI (serotonin reuptake inhibitors). It is also a potent σ–1 receptor agonist.



Drug Discovery Involves Knowing "the Dominos"

https://www.sciencedirect.com/science/article/pii/S2405722316300214

Fluvoxamine and the Sigma-1 Receptor:

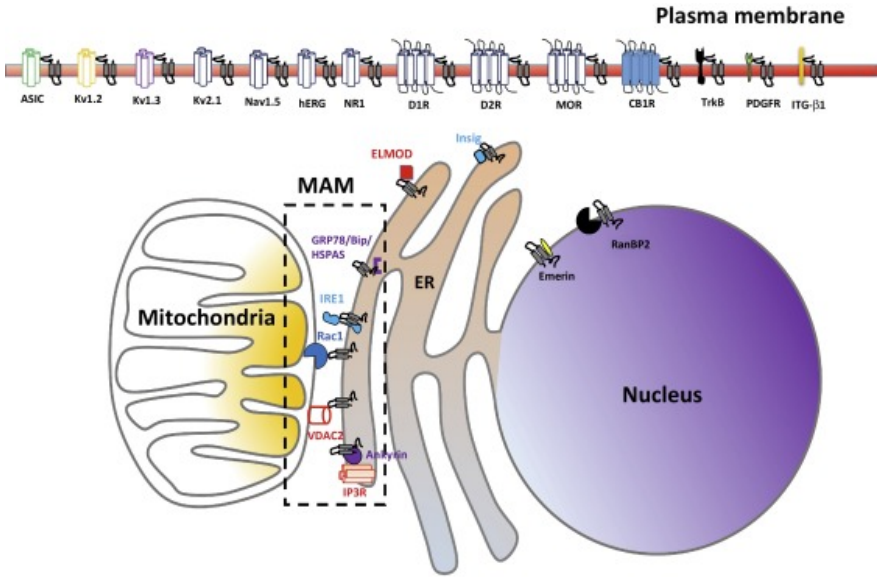
How does an SSRI Acting Antidepressant Modulate COVID Inflammation?

Fluvoxamine (Luvox®) is an SSRI (serotonin reuptake inhibitor) used as an **antidepressant,** but it also stimulates the **Sigma-1 receptor**. Upon stimulation by agonists or stressors, Sigma-1R can translocate to the Plasma Membrane to interact with ion channels, receptors, and kinases. Antidepressant dose: 50 mg/BID; Luvox CR: 100 mg.

CNS diseases reported to relate to Sig-1R include Alzheimer disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, stroke/ischemia, pain/neuropathic pain, and certain psychiatric disorders. fluvoxamine is a very potent agonist at sigma-1 receptors at 36 nM.

Sig-1R occurs specifically at the MAM, the interface between the endoplasmic reticulum (ER) and mitochondria (M), where it promotes cellular survival. Sig-1R also occurs at the nuclear envelope, where it recruits chromatin-remodeling factors to affect the transcription of genes.

Experimental and bioinformatics studies have identified interactions between Sig-1R as an inhibitor of endoplasmic reticulum-driven **Inositol-Requiring Enzyme 1α (IRE1**) which drives nuclear signaling synthesis of TNF-α and IL-6 that mediates inflammation.



Bottom Line:

Fluvoxamine (Luvox®) ➔ Stimulation of S1R in the MAM (mitochondria/endoplasmic reticulum):

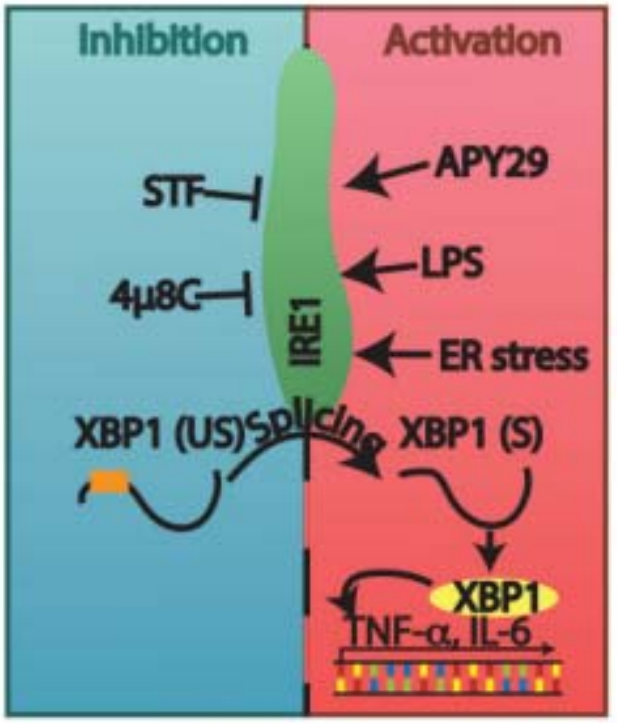
Damps an ER stress sensor (enzyme IRE1) which blocks TNF-α and IL-6 synthesis ➔ reduces the inflammatory triggers.

Su, et al., 2016, Sigma-1 Pluripotency; https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147(16)00004-3

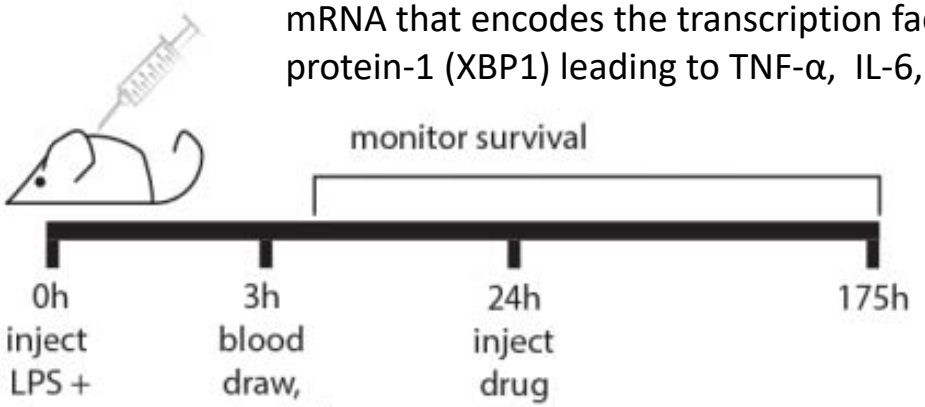
Preclinical LPS Sepsis Model - Fluvoxamine 20 mg/kg; C57/Bl6 mice

Major depression and anxiety are two of the major psychiatric disorders that have some overlapping pathophysiologies, the most significant being the dysfunction in the monoaminergic, GABAergic and glutamatergic systems. The Sigma-1 receptor is a new alternative therapeutic targets https://pubmed.ncbi.nlm.nih.gov/19589051/

The **Sigma-1 receptor** is also a critical inhibitor of ER-driven **Inositol-Requiring Enzyme 1α**, or **IER1** (or inositol 1,4,5-triphosphate, IP3, receptor-mediated Ca+2 signaling) inflammation and a potential therapeutic target in septic shock. IRE1 is activated by the TLR4 ligand lipopolysaccharide (LPS). The endoplasmic reticulum (ER) is increasingly recognized as a powerful controller of inflammatory signaling and the response of immune cells. IRE1 activity is required for cytokine production.



Stimulated/dimerized IRE1 leads to endonuclease splicing of mRNA that encodes the transcription factor X-box binding protein-1 (XBP1) leading to TNF-α, IL-6, and other cytokines.



IRE1 Inhibitors:
STF 083010 (in-vivo)
Alt: 4μ8C 5μM (in-vitro)

IRE1 Activators:
APY29 10μM (in-vitro)
LPS 6 mg/kg (in-vivo)

LPS:
@ 100 ng/mL in-vitro and 6mg/kg in-vivo

Fluvoxamine, administered at 20 mg/kg IP (with LPS) leads to higher survival

Rosen, et al., 2019, Fluvoxamine anti-sepsis model; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6936250/

Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy?

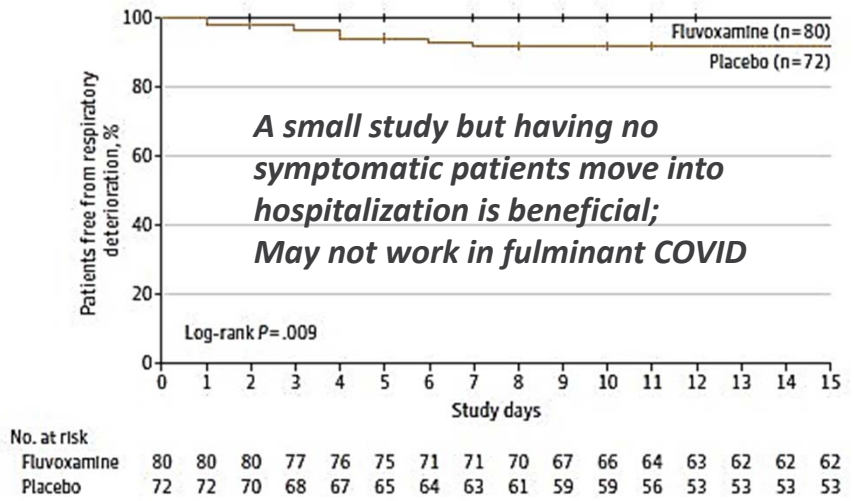
Approximately 40% of SARS-CoV-2 interacting proteins are associated with endomembrane compartments or vesicle trafficking pathways. In particular, the viral NS protein Nsp6 has been seen to specifically interact with Sig,a-1R.

The SARS-CoV-2 genome encodes as many as 14 open reading frames (Orfs) into proteins The Orf1a/Orf1ab at the 5' two-thirds of the genome encodes precursor polyproteins, which are auto-proteolytically processed into 16 NS proteins (Nsp1-16) that form the replicase/transcriptase complex.

At the 3' end of the viral genome, as many as 13 additional Orfs are expressed from sub-genomic mRNAs encoding Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) structural proteins and putative accessory proteins. The viral replication machinery is thought to localize in ER membranes thanks to Nsp3, Nsp4 and Nsp6. Nsp6 forms complexes with Nsp3 and Nsp4 to anchor the viral replicase/transcriptase complex to ER membranes

Vela, 2020; Repurposing S1R agonists for COVID; https://www.frontiersin.org/articles/10.3389/fphar.2020.582310/full

Lenza, et al, Dec 2020 (JAMA) describes a small clinical trial using fluvoxamine in **community-living, non-hospitalized adults (few symptoms)** with confirmed PCR COVID-19; FLUVOX: 80 pts; Placebo: 72 Pts. Dose: 100 mg/TID x 15 d.
Result: FLUVOX group had Zero pts moved into hospitalization vs Six in the Placebo group.



Lenze_2020; clinical trial using Fluvoxamine, https://jamanetwork.com/journals/jama/fullarticle/2773108

Fluvoxamine: How can a Sigma-1-R Agonist Work in COVID?

Preclinical Studies and a Small Clinical Trial Show Promising Results

Fluvoxamine is a potent **Sigma-1 Receptor (S1R) agonist (36 nM)** useful in treating depression but also reduces inflammation

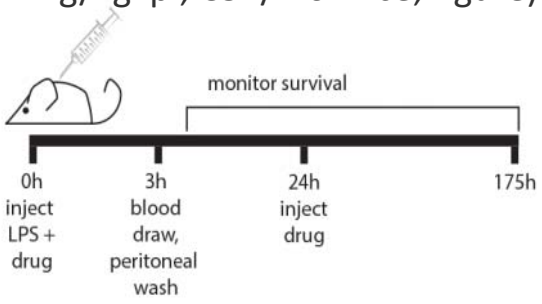
Trial Design: A randomized trial design that included 152 adult outpatients with confirmed COVID-19 and symptom onset within 7 days, 100 mg of fluvoxamine (n = 80) or placebo (n = 72) 3 times daily for 15 days

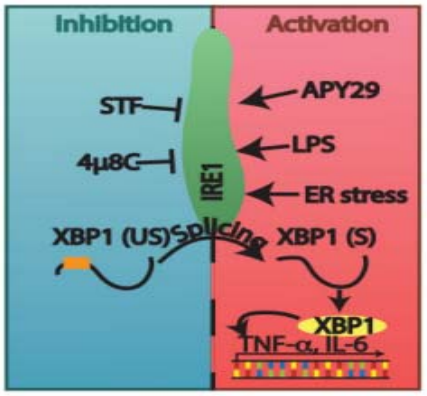
Outcome: clinical deterioration occurred in 0 patients treated with fluvoxamine vs 6 (8.3%) patients treated with placebo over 15 days

S1R is an inhibitor of IRE1 during inflammation. Patients treated with fluvoxamine, compared with placebo, had a lower likelihood of clinical deterioration over 15 days. However, the study is limited by a small sample size and short follow-up duration

Fluvoxamine is a strong Sigma-1 receptor (S1R agonist that is highly lipophilic, has rapid intracellular uptake, and has been shown in preclinical models to reduce inflammation and septic events. The receptor is an ER-resident protein acting as an essential inhibitor of cytokine production in a preclinical model of septic shock (LPS). Mechanistically, we find that S1R restricts the endonuclease activity of the ER stress sensor IRE1 and hinders cytokine expression**. It does not inhibit the classical inflammatory signaling pathways.**

(Preclinical model; Rosen; 20 mg/kg ip ; C57/Bl6 mice; figure)





Fluvoxamine ➔ Stimulation of S1R restricts the endonuclease activity of the ER stress sensor IRE1 thus blocking a path for TNF-a and IL-6 synthesis

Vela, 2020; Repurposing S1R agonists for COVID; https://www.frontiersin.org/articles/10.3389/fphar.2020.582310/full

Lenze, et al., JAMA, Fluvoxamine vs Placebo in symptomatic outpatients; https://jamanetwork.com/journals/jama/fullarticle/2773108

Rosen DA, Seki SM, Fernández-Castañeda A, et al. Modulation of the sigma-1 receptor-IRE1 pathway is beneficial in preclinical models of inflammation and sepsis. Sci Transl Med. 2019;11 (478):eaau5266. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6936250/

More Data on Repurposing Sigma-1 Receptor Ligands for COVID-19

- The order of potency for certain SSRIs at sigma-1 receptor is as follows:
- fluvoxamine (Ki =17.0 nM) > sertraline (Ki = 31.6 nM) > fluoxetine (Ki = 191.2 nM) > escitalopram (Ki=288.3 nM) > citalopram (Ki = 403.8 nM)
  Fluvoxamine Kd values for: SERT (serotonin) Kd= 2.2 nM ; NET (norepinephrine) Kd = 1300 ; DAT (dopamine): 9200 nM https://www.frontiersin.org/articles/10.3389/fphar.2013.00045/full

- In its dormant state, the sigma-1receptor forms a complex with another chaperone binding **immunoglobulin protein (BiP**), also known as 78 kDa glucose-regulated protein (GRP78), in the lumen of the ER.
  - It mediates the Inositol 1,4,5-triphosphate (IP3) receptor- affecting Ca2 signaling.

- **Fluvoxamine** demonstrates itself as a very potent inhibitor of the high-affinity O-deethylation of phenacetin, which is catalyzed by cytochrome P4501A2 (CYP1A2) in the liver, Thus, the apparent inhibitor constant of fluvoxamine, Ki, ranged from 0.12 to 0.24 µM
  https://www.researchgate.net/publication/222784402_Fluvoxamine_is_a_potent_inhibitor_of_cytochrome_CYP1A2

(PDF) ***Activation of sigma-1 receptor chaperone in the treatment of neuropsychiatric diseases and its clinical implication*****.** Available from: https://www.researchgate.net/publication/272750545_Activation_of_sigma-1_receptor_chaperone_in_the_treatment_of_neuropsychiatric_diseases_and_its_clinical_implication [accessed Mar 09 2021].

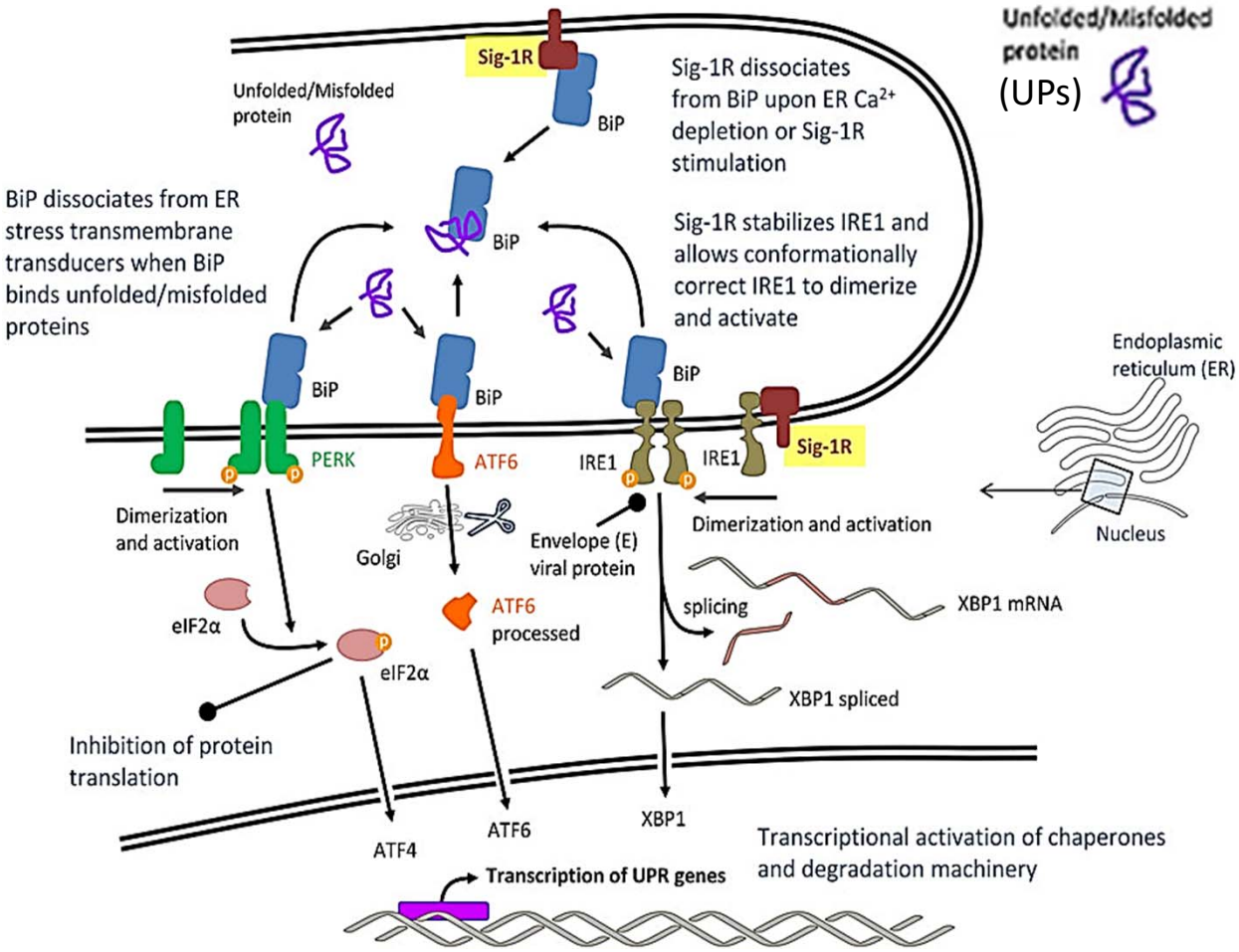
S1Rs stabilize IRE1 ➔ 2 IRE1 are activated (phos + dimerized)➔ IRE1 then binds BiP

S1R agonists control disrupted Ca+2 homeostasis by releasing ER-bound and S1-R-bound BiP (immuno-heavy chain binding globulin protein) from the ER.

BiP (also called glucose regulating protein 78, GRP78; or heat shock 70 kDa protein 5GRP78, HSPA5) is a "clean-up" protein which preferentially binds to unfolded or misfolded proteins (UPs; ie damaged or incorrect).

S1-R agonists also help S1-R stabilize IRE1 **(inositol required enzyme α)** which gets activated via phos /dimerization allowing BiP to trigger nuclear repair, apoptosis, etc.

Bound BiP with UPs acts with the IRE1 dimer to allow mRNA splicing for transcription within the factor X-box binding protein-1 (XBP1) that: splices mRNA ➔ stimulates nucleus UPR (UPR = unfolded protein response genes via 3 pathways: PERK➔ ef2a or ATF4 ➔ ATF6 or IRE1➔ XBP1) ➔ then with UPR transcription, cytokine silencing, or apoptosis.



Vela, 2020; Repurposing S1R agonists for COVID; https://www.frontiersin.org/articles/10.3389/fphar.2020.582310/full

Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy?

Sigma-1R (S1R) regulates key mechanisms of the adaptive host cell stress response and early steps of viral replication.

It is enriched in lipid rafts and detergent-resistant ER membranes, where it colocalizes with viral replicase proteins, such as the protein Nsp6 which does interact with S1R.

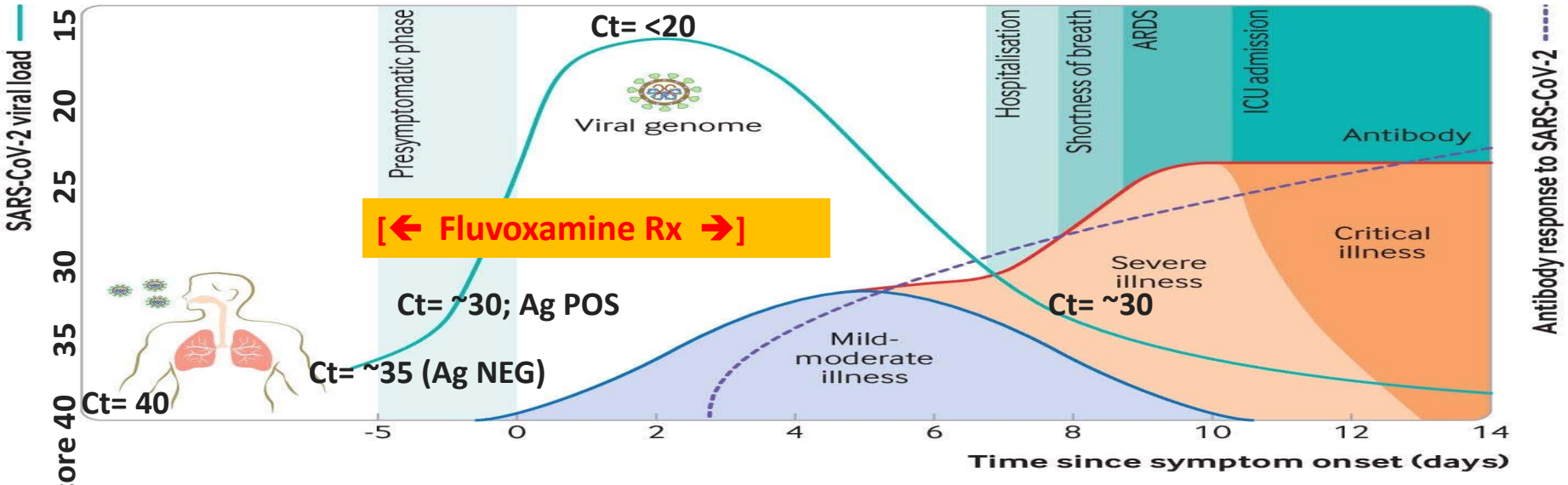
CoV replication is structurally and functionally associated with the endoplasmic reticulum (ER). Targeting S1R will not reduce already established viral replication, but it might interfere with early steps of virus-induced host cell reprogramming and potentially aid in slowing down the course of infection.

The receptor normally reside at the ER-mitochondrion contact called the (mitochondrion-associated ER membrane (MAM), where it regulates ER-mitochondrion signaling and ER-nucleus crosstalk. Mitochondrial function regulation by S1R includes bioenergetics and free radical generation/oxidative stress.

When cells undergo stress, S1R translocates from the MAM to the ER reticular network and plasma membrane. S1R regulates protein folding/degradation, calcium (Ca^{2+}) homeostasis, ER stress responses, autophagy, and ultimately cell survival through regulating BiP. S1R has been shown to colocalize with viral replicase proteins in membranous compartments and with the non-structural (NS) SARS-CoV-2 protein Nsp6.

Chlorpromazine, clomipramine, desipramine, perphenazine, imipramine, raloxifene, tamoxifen, clomiphene, hydroxyzine, benztropine and fluoxetine) bind to S1R with significant affinity. Interestingly, **chloroquine and hydroxychloroquine** also bind to S1R but they are non-selective S1R ligands and their affinities for this molecular target are suboptimal.

Vela, 2020; Repurposing S1R agonists for COVID;
https://www.frontiersin.org/articles/10.3389/fphar.2020.582310/full



TIMELINE OF A COVID-19 INFECTION (The NATURAL HISTORY):

INFECTION → Asymptomatic period → Symptomatic period → Resolution → "Long Hauler"

Innate Immunity ……..Adaptive Immunity ……..Memory T and B cells

interferon blockade ……viremia ……. T and B-cell …. Hypoxia/lung/heart damage…… fibrosis

Inhale >20,000 virus Day 1-14 Day 14-28 Day 28-48 6 months – longer

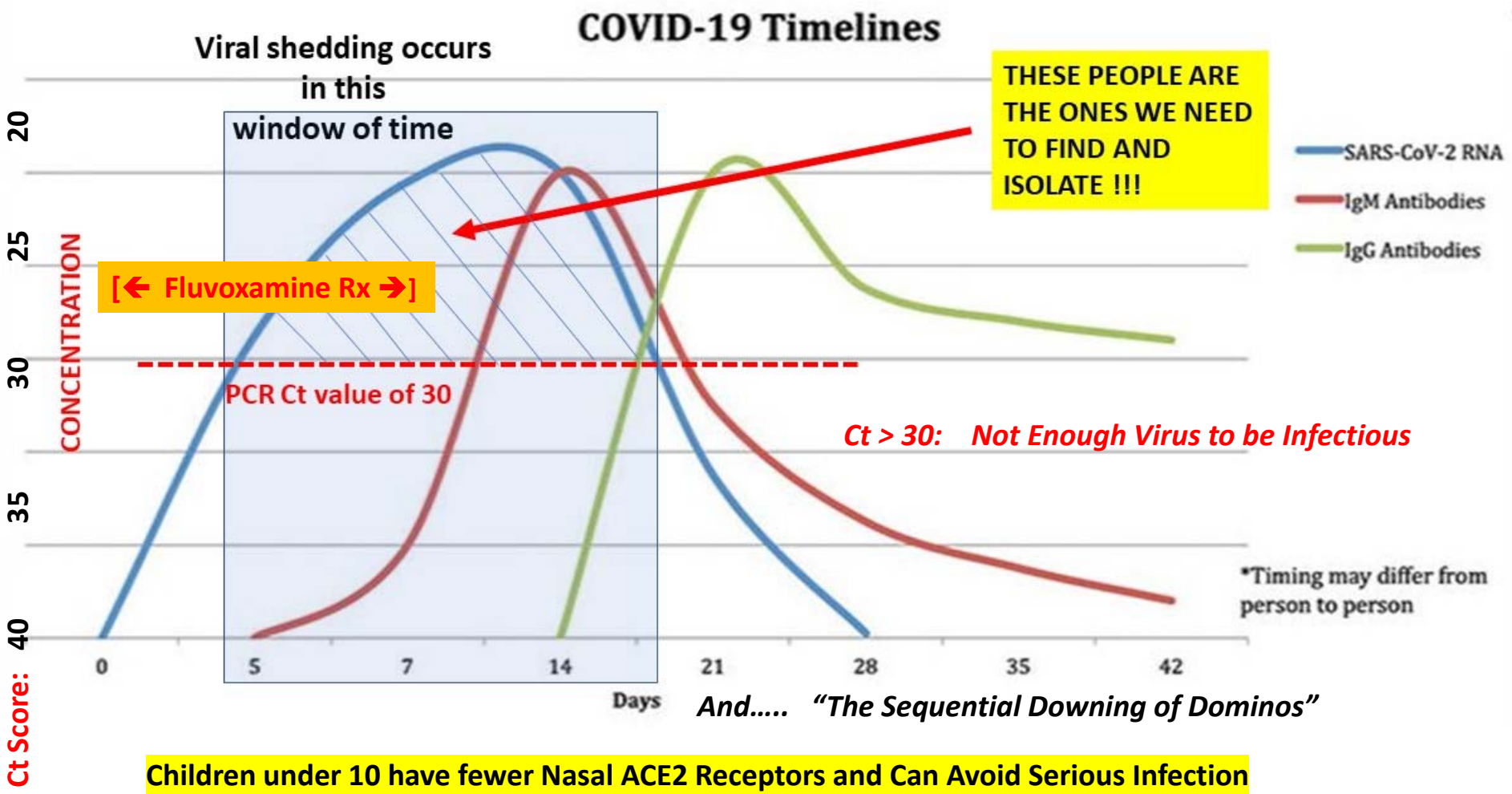
PCR NEG …Day 5 Ct = 30 Ct = 25 Ct= 20 Ct = 25 Ct = 30 (Ag test NEG) Ct >35

PCR POS period (infected) PCR POS (Ct score <40)

INFECTIOUS = Ct <30 Ag Test POS

The Natural History of COVID-19: It is the Same for all Ages

The Difference in Children is the Peak of the Blue Line is LOWER



Children under 10 have fewer Nasal ACE2 Receptors and Can Avoid Serious Infection