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3 **Repurposing Functional inhibitors of Acid sphingomyelinase (FIASMA) drugs: an**
4 **opportunity against SARS-CoV-2 infection ?**
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Abstract

What is known and objective: SARS-CoV-2 outbreak is characterized by the absence of active compounds for the treatment. The purpose of this study was to present an hypothesis on the prophylactic and/or therapeutic use in SARS-CoV-2 infections of functional inhibitors of acid sphingomyelinase (FIASMAS) that are mostly molecules approved drugs for various diseases.

Comment: We present and discuss the potential interest of FIASMAS presenting a lysosomotropism that may explain in vitro activities against viral infections, and more specifically in infections mediated by coronaviruses the SARS-CoV-2. A successful in vitro to in vivo translation of FIASMA requires a particular attention to drug pharmacokinetics (dosing regimen, drug-drug interactions) and to viral kinetics.

What is new and conclusion: The drug repurposing approach with the hope to bring patients effective treatment faster has gathered much attention in the SARS-COV-2 pandemic era. The current knowledge suggests that FIASMAS, as small molecules having a lysosomotropism and clinically approved indications in various diseases, should be paid more attention for their repurposing as potential drugs against SARS-COV-2.

1 - What is known and objective

The current situation of the SARS-CoV-2 outbreak poses major therapeutic problems given the absence to date of a specific antiviral agent to treat this infection, decrease viral shedding and subsequent transmission. Numerous clinical trials are in progress with different disease severity at enrollment with a high majority of studies for treatment purpose compared to prevention purpose (1,628 trials versus 289 trials consulted on October 28, 2020, cf Living mapping and living systematic review of Covid-19 studies at <https://covid-nma.com/dataviz>). Efforts have to be maintained since it is likely that we will have to coexist in the future with pathogenic coronavirus type viruses (SARS-Cov-2 or other strains). Moreover, initial trials in the search for effective therapy have led somewhat disappointing results (1-2).

Given the difficulties and costs in rapidly bringing up treatments with a favorable benefit-risk balance, in particular with the aim of large-scale treatment either for treatment of patient population or for prevention in healthy subjects, it seems relevant to investigate whether - among the drugs marketed to date - some of them could have a therapeutic or a prophylactic potential. This so-called "drug repurposing" strategy is based on different methodological approaches, and to date the most successful examples of drug reuse have not involved a systematic approach but rather serendipity (3). In the repositioning strategy the first step which consists in identifying the candidate molecule (s) for a given indication is critical. The potential approaches are either experimental and / or computational (3). Among these, the use of new data sources - including clinical data repositories - offer new opportunities.

Some organic molecules, including currently marketed drugs, have the potential to functionally inhibit, from reversible and additive manner, the activity of acid sphingomyelinase (ASM) which is a lysosomal glycoprotein involved in a wide range of disorders (4) including virus infections. These molecules identified by the acronym FIASMA (i.e. functional inhibitors of acid sphingomyelinase) have the potential to inhibit this enzyme, and therefore disrupt the entry of viruses into cells. The purpose of this study was to present an hypothesis on the prophylactic and/or therapeutic use of FIASMAs (Functional Inhibitors of ASM) in SARS-CoV-2 infections through a literature search in PubMed, and medRxiv.org preprint server for Health Sciences.

2 – Comment

2.1 - FIASMA's (Table 1)

Acid sphingomyelinase (ASM) is a lysosomal glycoprotein anchored to the inner lysosomal membrane which is translocated to the external leaflet of the cell membrane under specific stimuli where it catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcholine. Sphingomyelin is the most abundant sphingolipid component of the mammalian plasma membrane where it is associated with cholesterol to form lipid rafts. ASM inhibitors work either by direct inhibition or by functional inhibition (FIASMA). Unlike FIASMA, there are few direct inhibitors, by these including biphosphonates, in particular

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3 zoledronic acid (5). Functional inhibitors of ASM (FIASMA's) are cationic amphiphilic
4 molecules relatively heterogeneous in terms of chemical structure but which have the
5 characteristic of being polycyclic molecules, with at least 1 basic nitrogen atom ($pK_a > 4$
6 which corresponds to a partially protonated functional group at acidic pH) and moderate to
7 high lipophilicity ($\log P > 3$) (6).
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10 The internal wall of the lysosomes is negatively charged due to the abundant presence of
11 phospholipids, and the positively charged proteins form electrostatic interactions with this
12 internal wall of the lysosome which protects them from lysosomal degradation; ASM is thus
13 attached to the lysosomal membrane. FIASMA's which are lipophilic molecules integrate into
14 the lysosomal membrane with their lipophilic part, and present their positively charged part
15 in the lysosomal lumen, which leads to detachment of the ASM from the lysosomal
16 membrane and its subsequent proteolytic degradation in the lysosomal lumen. Hence, ASM
17 activating stimuli cannot lead to the translocation of ASM to the external leaflet of the cell
18 membrane, leading to perturbation in the downstream of the signaling cascade and in lipid
19 raft formation (Figure 1).
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22 However, there remains residual ASM activity necessary for cell viability. There are
23 significant differences in intra-lysosomal capture speed depending on lipophilicity ($\log P$) and
24 degree of ionization (pK_a) (from minutes to hours in cell culture systems). Indeed,
25 desipramine, fluoxetine, maprotiline, paroxetine and protriptyline have rapid lysosomal
26 capture (<30 min) with moderate lysosomal accumulation (lysosome / extracellular
27 concentration ratio <100: 1) (6). Drugs known for their significant FIASMA's properties
28 (residual activity <50% at a concentration of 10 μM) have been described (7-10) and are
29 listed in Table 1.
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33 The interest in FIASMA drugs relies on their potential interest in treating pathologies in
34 which there is an increased ASM activity such as neuro-psychiatric pathologies, and in
35 particular in major depression (11) where the inhibition of ASM by modifying the
36 sphingomyelin-ceramide balance could contribute significantly to their mechanism of action
37 (12, 13). Other diseases include ischemic strokes (14), alzheimer's disease (15) and chronic
38 obstructive pulmonary disease (16) as well as intra-cellular infections (17). In addition, the
39 production of ceramide via ASM plays a key role in the production of pulmonary edema in
40 acute pulmonary insufficiency, and ASM inhibitors may be of therapeutic value in diseases
41 with chronic and acute pulmonary injury (6).
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47 **2.2 - FIASMA's and viral infections**

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49 Viruses usually take benefit of the endocytosis mechanisms to penetrate in the cytosol of
50 cells, and different mechanisms of internalization are involved. These are clathrin-mediated
51 endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, as well as several
52 less well characterized clathrin- and caveolin/lipid raft-independent mechanisms (18).
53 Viruses using lipid raft-dependent pathways depends on cholesterol, lipid rafts and a
54 complex signaling pathway and use glycosphingolipids as receptors.
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56 The role of ASM inhibition in intracellular infectious pathologies, and in particular by
57 enveloped viruses, is due to the fact that the life cycle of enveloped RNA viruses (e.g., Sars-
58 Cov-2) is critically dependent of the lipid biosynthesis of the host (19). Binding and entry of
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3 the virus involves interactions between the envelope's viral glycoproteins and cellular
4 receptors concentrated in micro-domains rich in sphingolipids present on the membranes of
5 host cells, and the sphingolipid itself is able to serve as an alternative receptor for certain
6 viruses (i.e., HIV). The subsequent infection leads to a reorganization of cell membranes and
7 lipid metabolism to support the production of new viral particles (19). Beyond the role in the
8 entry of the virus, sphingolipids also play an important role in modulating the replication of
9 the virus and in shaping the innate antiviral immune response (20).

10 More specifically, the role of FIASMA's in infection by intracellular bacteria has been shown
11 (17), and ASM activity has been found essential for optimal cycle progression of intracellular
12 bacterial pathogens. This study established the importance of ASM for infection by several
13 intracellular bacteria and suggested FIASMAs (namely desipramine, amitriptyline, and
14 nortriptyline) as potential therapies for diseases caused by intracellular pathogens. The role
15 of sphingomyelin and ASM in the intra-cellular penetration of an alpha-herpes virus has
16 been shown (21). Imipramine and amitriptyline (FIASMA's) have reduced cellular infection
17 with porcine alphaherpesvirus pseudorabies virus (PRV) by intracellular sphingomyelin
18 depletion. However, not all herpes viruses were dependent on sphingomyelin and ASM for
19 cell infection.

20 Several drugs, not considered as FIASMA drugs, may likely act via intracellular sequestration
21 and increase in endosomal and/or lysosomal pH to explain their antiviral activity.
22 Azithromycin, as well as chloroquine and hydroxychloroquine, may share this mechanism of
23 action, and act in a synergistic manner (22). A screen of 2,600 approved drugs with further
24 evaluation of antiviral activity using in vitro virus-like particle entry assay and a murine
25 model of EBOLA disease showed the cationic molecules had strong in vitro and in vivo
26 antiviral activities (23). Viral entry assays showed that these compounds blocked a late
27 stage of viral entry. The drugs with protective ability included sertraline and bepridil which
28 are known as FIASMA's. Hence, drug targeting the lysosomes (lysosomotropic drugs) may
29 have different mechanisms of action at the lysosomal level. The role of the Niemann-Pick C1
30 (NPC1) protein as an essential entry receptor was studied in a mouse model of EBOLA
31 disease and showed that imipramine inhibited the entry of Ebola virus (EBOV) in vitro and
32 protected against EBOV infection in a mouse model (24). Additionally, the increase in intra-
33 lysosomal pH may impair the activity of cathepsin-L that is a key protease for membrane
34 fusion as shown for SARS-CoV-2 (25).

35 Very recently, as part of a high throughput screening using a genomic approach to reposition
36 old molecules (DrugBank target database), amitriptyline has shown a reduction in cell death
37 in a model of A549 infected human lung carcinoma cells by the lethal avian influenza A H5N1
38 virus (26). In a mouse model infected with H5N1, amitriptyline showed a reduction in the
39 number of infiltrating cells, decreased the score of pulmonary lesions on the basis of
40 pulmonary histopathology, and reduced pulmonary edema and improved the survival of
41 mice infected with the H5N1 virus. Analysis of RNA-seq data from the lung tissues of mice
42 infected with H5N1 shows that the genes significantly influenced by amitriptyline were
43 mainly grouped in those corresponding to immune responses, neurophysiological processes
44 and processes related to apoptosis.

45 As a whole, drugs that target the lysosomes (lysosomotropic drugs) and especially FIASMA's
46 alter its functions and create an unfavorable host cell environment for viruses.

47 48 49 50 51 52 53 54 55 56 57 58 59 **2.3 - FIASMA's and coronaviruses (Table 2)** 60

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As mentioned above, different mechanisms of internalization are used by viruses to penetrate in the cytosol of cells, and lipid-raft mediated endocytosis (27) and clathrin-mediated pathway (28) have been identified in the Coronaviridae suggesting potential target for drugs acting at the internalization step (i.e., fusion and entry). However, targeting the late compartments of the endocytic pathway after delivery of the SARS-CoV genome in the cytoplasm should also be considered as potential target for disruption of the replication, as shown with amiodarone (29). Hence different targets are to be considered against SARS-CoV-2, and those targeting directly the virus may be considered as most effective (30) than targeting the host cells. However, targeting the biology of the host cells, instead of targeting the biology of the virus, has the advantage to be less influenced by genetic variations (much less frequent in host cells compared to the viruses (e.g., mutations)). As lysosomotropic drugs with specific activity, attention should be paid to FIASMA's interfering via ASM inhibition to lipid-raft mediated endocytosis, and for some by interfering with other steps in the endocytic pathways.

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In a docking study using two crystal structures from SARS-CoV main protease (M^{pro}), 4 drugs including the FIASMA drug promazine were identified used as templates for the rational design of anti-SARS proteinase drugs (31). However, negative results with promazine were obtained using *in vitro* and *in vivo* (mouse lung model) methods (32).

High-throughput screening through a library of current or previously FDA-approved drugs made it possible to identify on cell culture systems several molecules having antiviral activity against MERS-CoV and/or SARS-CoV viruses (33). Among the 27 compounds identified as active against both MERS-CoV and SARS-CoV, 9 of them were FIASMAs (i.e., emetine, tamoxifen, benztropine, fluphenazine, promethazine, astemizole, chlorpromazine, triflupromazine, clomipramine). A library screening of 348 FDA-approved drugs for anti-MERS-CoV activity in cell culture identified 4 compounds (chloroquine, chlorpromazine, loperamide and lopinavir) with activity in the low micromolar range (34). These drugs also inhibited the replication of SARS coronavirus and human coronavirus 229E. Among this set of compounds two of them (i.e., chlorpromazine and loperamide) are FIASMAs. In a mechanistic study on drugs. Taking into account previous results (33-34), as part of a mechanistic study on drug ability to block virus entry, the ability of a panel of drugs including 7 FIASMA's (i.e., chlorpromazine, promethazine, fluphenazine, astemizole, triflupromazine, clomipramine and tamoxifen) to inhibit MERS-CoV was confirmed (35).

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Very recently, several studies have been directed towards the search of drugs potentially inhibiting Sars-CoV-2. SARS-CoV-2 proteins have been cloned, tagged and expressed in human cells and the human proteins associated with each have been identified (36). SARS-CoV-2 human protein-protein interactions were identified ($n = 332$) and 66 of them were targeted by 69 compounds (including 29 FDA-approved drugs) whose antiviral activity was investigated using 2 viral assays. Among this panel of drugs clinically usable for targeting the human proteins, two are FIASMAs (i.e., clemastine and cloperastine).

A protein-protein interaction network containing the genes co-expressed with the angiotensin converting enzyme 2, a main cell receptor of SARS-CoV and SARS-CoV-2, has been set up (37). These authors found that 36 drugs were associated with the gene network, including one FIASMA (i.e., amiodarone). In a screen of 3,000 FDA-approved drugs against SARS-CoV, tested *in vitro* on Vero cells, 24 drugs were retained including two FIASMAs (i.e.,

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3 loperamide and cepharanthine) (38). Using a systems pharmacology-based network
4 medicine platform, the quantification of interplay between the human coronaviruses
5 interactome and drugs targets found 16 potential drugs including four FIASMAs (i.e.,
6 paroxetine, melatonin, quinacrine, carvedilol) (39). Four studies using docking, in vitro or in
7 vivo studies reported that emetine could be a potential inhibitor of SARS-CoV-2 (40-43).
8 Using artificial intelligence technology to identify marketed drugs with potential activity
9 against SARS-CoV-2, the FIASMA drug clofazimine was identified, in a subset of 8 drugs, as a
10 potential candidate based on in vitro cell activities against a feline coronavirus (44).
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14 Studies currently in a review process, have evaluated either in silico (45) or in vitro (46) the
15 potential of approved drugs. A docking was carried out with the papain-like protease
16 (PLpro) of SARS-CoV-2 using a panel (n = 2,525) of molecules approved by the FDA (45) in
17 order to find inhibitors. This work made it possible to select those who had the best affinity
18 including chloroquine, formoterol and a FIASMA (i.e., amitriptyline). In an in vitro study, a
19 subset of 17 drugs with activity as inhibitors of SARS-CoV-2 were identified (46). This subset
20 of drugs included 5 FIASMAS (i.e., fluphenazine, chlorpromazine, clomipramine,
21 promethazine, and emetine), and also highlighted chloroquine derivatives (e.g.,
22 hydroxychloroquine) as potential SaS-CoV-2 antiviral drugs. About 30 FDA approved drugs
23 have been selected by computational analysis to inhibit the SARS-CoV-2 main protease
24 (M^{pro}) (47). Among 7 drugs, the 3 more potent were ebastine and 2 FIASMA's (i.e., pimozone
25 and bepridil). Using quantitative high-throughput screen coupled with artificial intelligence
26 based machine learning from a library of 1,425 FDA-approved drugs, a subset of 16
27 compounds with in vitro antiviral effects were identified including amiodarone as a FIASMA
28 drug (48). In a cell culture model 3 FIASMAS (amiodarone, fluoxetine, and imipramine)
29 inhibited the entry and propagation of SARS-CoV-2 without cytotoxic adverse effects and
30 had also antiviral activity against two currently circulating influenza A virus subtypes (49). In
31 the study, fluoxetine was associated with increased cholesterol storage as well as
32 dysregulated acidification. A very recent paper, showed that amitriptyline, desipramine,
33 escitalopram, fluoxetine, imipramine, maprotiline, and sertraline displayed an almost
34 complete *ex vivo* inhibition of the infection of human epithelial cells and different human
35 cell lines with SARS-CoV-2 and pp-VSV-SARS-CoV-2 spike particles (50).
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42 Based on a potential prophylactic activity of the anti-psychotic chlorpromazine against Sars-
43 CoV-2, and as a result of a lower prevalence of COVID-19 infections in psychiatric patients
44 (4%) comparatively to health care professionals (14%), suggestions of a prophylactic effect of
45 with psychoactive compounds have emerged (51-53), and a clinical trial (i.e., The reCoVery
46 study) is ongoing. The antiviral activity of chlorpromazine involves the inhibition of clathrin-
47 mediated endocytosis and, once again, the status of FIASMA was not mentioned.
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51 As a whole, numerous in vitro or in vivo study have been identified 28 FIASMA's as potential
52 antiviral drug candidates against SARS-COV, MERS-COV or SARS-COV-2. In this subset of
53 drugs, 6 can be identified as active against both the 3 coronaviruses (chlorpromazine,
54 clomipramine, emetine, fluphenazine, loperamide, promethazine, see Table 2). It should be
55 noticed that there are many unknowns in SARS-CoV-2 cell penetration. Apart from
56 caveolar/lipid raft-mediated endocytosis, clathrin-mediated endocytosis and
57 micropinocytosis potentially involved in cell entry may be modulated by some drugs with
58 FIASMA properties such as chlorpromazine, sertraline and promethazine as inhibitors of
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3 clathrin-mediated endocytosis, and imipramine as inhibitors of micropinocytosis (54).
4 Furthermore, the endocytic mechanisms for coronaviruses can vary along the level in the
5 respiratory tract. Nasal epithelial cells express a wide variety of endocytic markers while
6 pneumocytes have a more restricted pattern with micropinocytosis as a predominant
7 endocytic pathway (54). Type II alveolar pneumocytes as preferential targets of SARS-CoV-2
8 should explain the late alveolar damage observed in some patients.
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17 **2.4 - In vitro-to-in vivo translation**

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19 Solid evidence of antiviral activity based on in vitro and on pre-clinical studies is a
20 prerequisite to further studies in humans. However, in vivo translation of antiviral activity
21 requires that the drugs have suitable profiles of drug exposure (concentration and duration)
22 at the site(s) of action. Hence, drug pharmacokinetics and drug regimens are essential
23 parameters for a successful translation.
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26 FIASMA's are lipophilic basic amine drugs and as such have a very high volume of
27 distribution. Indeed, steady-state volume of distribution (V_{dss}) of amitriptyline and of
28 chlorpromazine is 14.4 L per kg (55) and 8.9 L per kg (56). As lipophilic drugs, they are highly
29 metabolized with significant first-pass effect. Indeed, the systemic clearance of amitriptyline
30 and chlorpromazine is quite high (0.9 L/min and 1.3 L/min), and they have a low and
31 variable oral bioavailability: ranging from 4 to 38 % for chlorpromazine (56) and from 33 to
32 62 % for amitriptyline (55). Even though they have a high volume of distribution, as a result
33 of a high clearance, their apparent elimination half-life of is not too long (approx. 11 h and
34 18.5 h for amitriptyline and chlorpromazine, respectively). However, reaching steady-state
35 to obtain a maximal effect would require a delay (approximately 7-times the half-life) that
36 may prove unsuitable in an epidemic context either for prevention or for a curative use.
37 Alternatively, a loading dose may be used to reach more rapidly the steady state provided
38 that the tolerance profile is not a limiting factor. Furthermore, as illustrated for these two
39 prototypic drugs, the variable oral bioavailability is a factor of inter-individual that should be
40 considered.
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42 Other variability factors that may impact dosing such renal and hepatic impairment as well
43 as drug-drug interactions with concomitant medications should also be considered with
44 these drug candidates as lipophilic drugs usually have significant first-pass effect.
45 Furthermore, a particular attention should be paid to FIASMA interacting with P-gp either as
46 substrate and/or as inhibitor (Table 2). P-gp is present in the lysosomal membrane (57) and
47 P-gp substrates may have a higher lysosomal distribution compared to those driven only by
48 passive diffusion (58). Hence, co-administration of strong P-gp inhibitor (e.g., clarithromycin,
49 itraconazole, verapamil and some HIV protease inhibitor) with FIASMAs may decrease their
50 lysosomal accumulation. Such feature should be considered as part of drug combination
51 therapy in the management of SARS-CoV-2 infection.
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53 As part of prophylaxis use in individual contacts, knowledge of the pharmacokinetics in
54 different sub-populations (including pediatrics and elderly people) should be estimated.
55 Besides drug pharmacokinetics, viral kinetics has to be considered to optimize dosing
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3 regimens given the limited window to initiate an antiviral therapy in a curative setting. The
4 viral profile kinetics (i.e., time to peak viral load and duration of viral shedding) varies among
5 viruses and is not yet well defined for SARS-CoV-2. These elements are mandatory for the
6 definition of the initiation and of the duration of an antiviral treatment (59).

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8 These elements highlight the fact that drug pharmacokinetics and viral kinetics has to be
9 known with precision in order not to be lost in translation.
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11 **2.5 – Framework of future investigations**

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14 Firstly, retrospective investigations on COVID-19 confirmed studied could be conducted to
15 explore the prevalence and the potential effects of FIASMAs. Indeed, it seems worthwhile to
16 investigate if patients infected with Sars-Cov-2 virus and exposed to FIASMA's and have a
17 more favorable evolution of the disease. Indeed, a recent retrospective study (59) on 65
18 inpatients tested positive for SARS-CoV-2 and treated or not by calcium channel blockers
19 (CCB, i.e., nifedipine or amlodipine as a FIASMA) for hypertension found that patients
20 treated by CCB were significantly more likely to survive than those not treated by CCB (50 %
21 survived in the CCB group versus 14.6 % survived in the No-CCB group). As a retrospective
22 study involving few patients, these data have to be considered with caution. Moreover, a
23 submitted study (61) has explored the association between antidepressant use and the risk
24 of intubation or death in inpatients with COVID-19. Significant association was reported
25 between the use of any antidepressant and reduced risk of intubation or death. When
26 individual antidepressant was taken into account, significant associations were found for
27 escitalopram, fluoxetine and venlafaxine. Among the 3 antidepressants one was a FIASMA
28 (fluoxetine). Data available in the clinical hospital data warehouses may help us testing this
29 assumption and to identify marketed drugs to be repositioned. For example, in the Erasme
30 hospital from Brussels, 370 (27 %) of the 1373 hospital admission for COVID-19 (March to
31 October 2020) took at least one FIASMA. Alternatively, in the Rennes University Hospital, the
32 prevalence of FIASMA in patients admitted for COVID-19 was 8.5 % (55 on 642 until May 5,
33 2020).
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36 Secondly, pharmaco-epidemiological studies could be carried out exploring in general
37 population if the chronic prescription of particular FIASMAs would be associated with a low
38 prevalence of severe symptoms of the COVID-19 infection.

39 Thirdly, all the compound identified as FIASMAs could be tested in vitro to explore their
40 activity against SARS-COV-2.

41 Fourthly, the FIASMAs having either a protective effect in general population or a significant
42 activity in vitro could be tested in vivo using notably randomized double blinded study
43 against placebo.
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51 **3 - What is new and conclusion**

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53 The drug repurposing approach with the hope to bring patients effective treatment faster
54 has gathered much attention in the SARS-COV-2 pandemic era. The current knowledge
55 suggests that FIASMAs, as small molecules having a lysosomotropism and clinically approved
56 indications in various diseases, should be paid more attention for their repurposing as
57 potential drugs against SARS-COV-2. We strongly suggest to explore the potential protective
58 effect of the FIASMAs in subjects treated chronically for various diseases, and in case of
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3 positive results to test the corresponding FIASMA in off-label therapy using notably large-
4 sized, randomized and double-blinded controlled clinical trials.
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9 Author Contributions : All authors performed the literature search and wrote the
10 manuscript.
11
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14 Conflict of interest :

15
16 All the authors (PLC & GL) disclose any financial and personal relationships with other people
17 or organizations that could inappropriately influence their work.
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3 Figure 1. Mechanisms of action of functional inhibitors of acid sphingomyelinase (ASM).
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6 Left : ASM is anchored to the inner leaflet of the lysosomal membrane by electrostatic forces
7 so that the enzyme is protected from proteolytic degradation. Specific stimuli allow the
8 translocation of ASM from the inner lysosome to the external leaflet of the cell where ASM
9 catalyzes the hydrolysis of sphingomyelin into ceramide and phosphorylcholine.
10 Shingomyelin is the most abundant sphingolipid component of the mammalian plasma
11 membrane where it is associated with cholesterol to form lipid rafts.
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14 Right : FIASMA are cationic drugs with lipophilic properties that diffuse in the lysosome by
15 passive diffusion and potentially via an additional mechanism using ABCB1 transporter
16 located on the lysosomal membrane. These drugs become protonated in the intra-lysosomal
17 acidic environment and increase the intra-lysosomal pH so that ASM is detached from the
18 inner leaflet of the lysosomal membrane, and is further degraded by proteolysis. ASM
19 translocation is no longer effective and the formation of lipid rafts is altered. Hence,
20 different mechanisms of internalization used by viruses (i.e., fusion and entry) to penetrate
21 in the cytosol of cells are altered.
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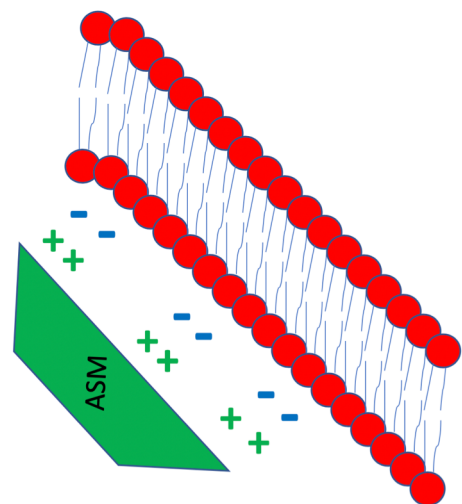
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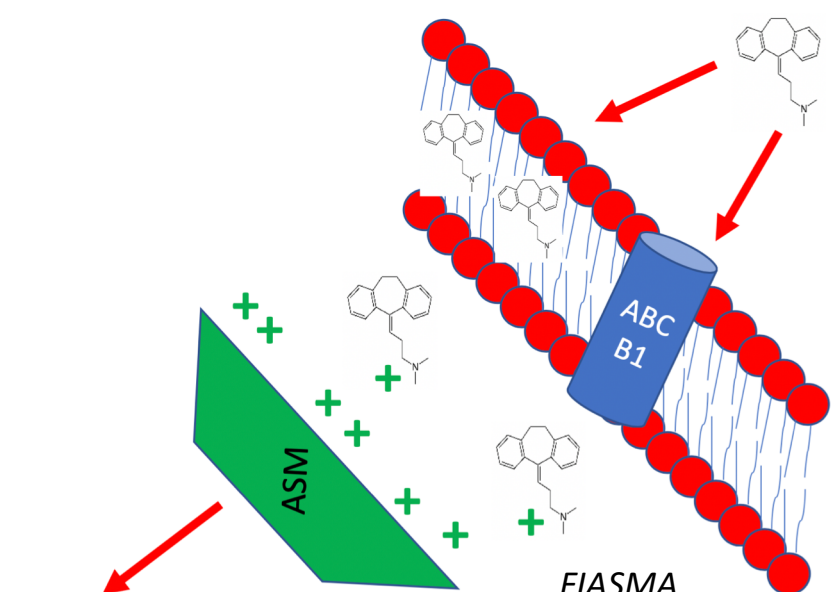
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Functional acid sphingomyelinase (ASM) anchored to the inner lysosomal membrane via electrostatic forces.



ASM membrane detachment and further inactivation

FIASMA interference with ASM membrane binding

FIASMA :
Functional inhibitors of acid sphingomyelinase (ASM)

FIASMA diffusion in the lysosome via passive diffusion or transporter-mediated diffusion

Table 1 : Functional inhibitors of acid sphingomyelinase with their ATC classification (functional inhibition is defined by a reduction to ASM activity below 50 % at a 10 μ M concentration, Kornhuber et al. 2013 and Hoehn et al. 2016).

Denomination	ATC-5	ATC-4
Alverine	A03AX08	Drugs for functional gastrointestinal disorders
Amiodarone	C01BD01	Class 1 and 3 antiarrhythmic drugs
Amitriptyline	N06AA09	Antidepressant drugs
Amlodipine	C08CA01	Selective calcium channel blockers
Astemizole	R06AX11	Antihistamines for systemic use
Benzotropine	N04AC01	Antiparkinson drugs
Bepidil	C08EA02	Non-selective calcium channel blockers
Camyllofine	A03AA03	Drugs for functional gastrointestinal disorders
Carvedilol	C07AG02	Beta blocking agents
Cepharanthine	(1)	
Chlorpromazine	N05AA01	Antipsychotic drugs
Chlorprothixene	N05AF03	Antipsychotic drugs
Cinnarizine	N07CA02	Antivertigo preparations
Clemastin	R06AA04	Antihistamines for systemic use
Clofazimine	J04BA01	Drugs for treatment of lepra
Clomiphene	G03GB02	Gonadotropins and other ovulation stimulants
Clomipramine	N06AA04	Antidepressant drugs
Cloperastine	R05DB21	Cough suppressants
Cyclobenzaprine	M03BX08	Muscle relaxants, centrally acting agents
Cyproheptadine	A15AA01	Appetite stimulants
Desipramine	N06AA01	Antidepressant drugs
Desloratadine	R06AX27	Antihistamines for systemic use
Dicyclomine	A03AA07	Drugs for functional gastrointestinal disorders

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3	Dilazep	C01DX10	Vasodilators used in cardiac diseases
4			
5	Doxepin	N06AA12	Antidepressant drugs
6			
7	Emetine	P01AX02	Antiprotozoal
8			
9	Ethopropazine	N04AA05	Anticholinergic agents for Parkinson
10			
11	Fendiline	C08EA01	Calcium channel blockers
12			
13	Flunarizine	N07CA03	Antivertigo preparations
14			
15	Fluoxetine	N06AB03	Antidepressant drugs
16			
17	Flupenthixol	N05AF01	Antipsychotic drugs
18			
19	Fluphenazine	N05AB02	Antipsychotic drugs
20			
21	Fluvoxamine	N06AB08	Antidepressant drugs
22			
23	Hydroxyzine	N05BB01	Anxiolytic drugs
24			
25	Imipramine	N06AA02	Antidepressant drugs
26			
27	Lofepamine	N06AA07	Antidepressant drugs
28			
29	Loperamid	A07DA03	Antipropulsives
30			
31	Loratadine	R06AX13	Antihistamines for systemic use
32			
33	Maprotiline	N06AA21	Antidepressant drugs
34			
35	Mebeverine	A03AA04	Drugs for functional bowel disorders
36			
37	Mebhydroline	R06AX15	Antihistamines for systemic use
38			
39	Melatonin	N05CH01	Hypnotics and sedatives drugs
40			
41	Mibefradil	C08CX01	Selective calcium channel blockers
42			
43	Nortriptyline	N06AA10	Antidepressant drugs
44			
45	Paroxetine	N06AB05	Antidepressant drugs
46			
47	Penfluridol	N05AG03	Antipsychotic drugs
48			
49	Perhexiline	C08EX02	Non-selective calcium channel blockers
50			
51	Perphenazine	N05AB03	Antipsychotic drugs
52			
53	Pimethixene	R06AX23	Antihistamines for systemic use
54			
55	Pimozide	N05AG02	Antipsychotic drugs
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3	Promazin	N05AA03	Antipsychotic drugs
4			
5	Promethazin	D04AA10	Antipruritics, including antihistamines, anesthetics...
6			
7	Protriptyline	N06AA11	Antidepressant drugs
8			
9	Quinacrine	P01AX05	Antiprotozoal drugs
10			
11	Sertindole	N05AE03	Antipsychotic drugs
12			
13	Sertraline	N06AB06	Antidepressant drugs
14			
15	Suloctidil	C04AX19	Peripheral vasodilators
16			
17	Tamoxifen	L02BA01	Hormone antagonists and related agents
18			
19	Terfenadine	R06AX12	Antihistamines for systemic use
20			
21	Thioridazin	N05AC02	Antipsychotic drugs
22			
23	Trifluoperazine	N05AB06	Antipsychotic drugs
24			
25	Triflupromazine	N05AA05	Antipsychotic drugs
26			
27	Trimipramine	N06AA06	Antidepressant drugs
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33	Footnote : (1) Cepharanthine is only approved by Japan health authority in some indications		
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Table 2 : Functional inhibitors of acid sphingomyelinase with activity against SARS-CoV, MERS-CoV and SARS-CoV-2 on *in silico*, or *in vitro* and in *ex vivo* models. Interaction with P-gp (substrate and/or inhibition) was retrieved from Metrabase a public cheminformatics and bioinformatics database for transporter data analysis (<http://www-metabase.ch.cam.ac.uk>).

FIASMA	SARS-CoV		MERS-CoV		SARS-CoV-2		P-gp substrate	P-gp inhibitor
Amiodarone	□■	(29,37)			□□■	(37,48, 49)	o	+
Amitriptyline					□❖	(45,50)	+	+
Astemizole	■	(33)	■ ■	(33,35)			+	+
Bepidil					■	(47)	+	+
Benztropine	■	(33)	■	(33)			o	o
Carvedilol					□	(39)	o	+
Cepharanthine					■	(38)	+	+
Chlorpromazine	■ ■ ■	(28, 33, 34)	■ ■	(33,35)	■	(46)	+	+
Clemastine					□	(36)	-	o
Clofazimine					■	(44)	o	+
Clomipramine	■	(33)	■ ■	(33,35)	■	(46)	+	+
Cloperastine					□	(36)	o	o
Desipramine					❖	(50)		
Emetine	■	(33)	■	(33)	□■■■■■	(40-43, 46)	+	+
Fluoxetine					■❖	(49, 50)	+	+
Fluphenazine	■	(33)	■ ■	(33,35)	■	46	o	+
Imipramine					■❖	(49,50)	+	+
Loperamide	■	(34)	■	(34)	■	(38)	+	+
Melatonin					□	(39)	o	o
Maprotiline					❖	(50)	o	+
Paroxetine					□	(39)	+	+
Pimozide					■	(47)	+	+
Promazine	□■†	(31,32)					-	+
Promethazine	■	(33)	■ ■	(33,35)	■	(46)	-	+
Quinacrine					□	(39)	o	o
Sertraline					❖	(50)	+	+
Tamoxifene	■	(33)	■ ■	(33,35)			-	+
Triflupromazine	■	(33)	■ ■	(33,35)			+	+

Foot note :

In bold : drugs active against the 3 coronaviruses

in silico (□), in vitro (■), *ex vivo* (❖) and negative result (†).

P-gp interaction : no information (o), positive information (+), negative information (-)