Covid-19 therapies, do we see substantial progress?

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Appearance of SARS-Cov-2 (severe acute respiratory syndrome coronavirus-2)[1] and its spread all over the World is connected with COVID-19 pandemia, which recently (end of February 2021) results in more than 112 million cases and almost 2.5 million deaths, not to mention unknown long-term or persistent side effects in convalescent individuals.

On Feb. 11th, 2020 International Committee on Taxonomy of Viruses (ICTV) has given a name SARS-Cov-2 to this virus which was classified to the genus *Betacoronavirus*, family *Coronaviridae*, order *Nidovirales*, Class *Pisoniviricetes*, Phylum *Pisuviricota*. Coronaviruses contain genetic material as a tight single helix of RNA o positive polarity, ssRNA(+). Their nucleocapsid is covered by lipid bilayer membrane containing proteins and glycoproteins. The electron microscope images showed crown-like shape of the virus envelope, which suggested the name of the entire group of animal and human viruses. In humans they induce mild infections of upper respiratory tract and also (rather seldom) of digestive tract. Namely 15-30% of mild, seasonal colds results from various coronavirus infections.

However, at the beginning of this millennium two more serious epidemic events took place: one, SARS-Cov (severe acute respiratory syndrome coronavirus) which appeared in China, province Guangdong in 2003 and spreaded in 26 countries and affected 8000 people and the other, MERS (Middle East respiratory syndrome) which started in Saudi Arabia in 2012 and also spreaded in 27 countries affecting 2500 individuals. Therefore both epidemic events did not show pandemic tendencies and did not prelude pandemic character and giant aggressiveness of SARS-Cov-2. It should be noted, that even now, more than a year from the epidemics start we do not have real therapy curing the disease.

The big hope brings vaccination, which recently is ongoing in most countries and hopefully, by prophylaxis will quench the Covid-19 pandemia. Still however, inspite great effort undertaken by scientist and medical professionals there are no substantial success to effectively treat ongoing COVID-19 disease.

Hitherto undertaken, rational attempts based on application of remdesivir, a broad spectrum antiviral drug, inhibiting viral RNA polymerase and proofreading nuclease [2] although apparently facilitate recovery from COVID-19 and reduce side effects require further studies [3], in particular when WHO Solidarity Trial Consortium found that therapy of Covid-19 hospitalized patients with remdesivir similarly as hydroxychloroquine, lopinavir, and interferon had no, or little effect as resulted in overall mortality, initiation of ventilation, and duration of hospital stay [4].

Another potent antiviral drug target was found SARS-CoV-2 PLpro (NSP3, viral papain-like cysteine protease) which is essential for SARS-CoV-2 replication and also due to its suggested important role in the innate immune response during viral infection analogous to SARS-CoV PLpro, known to be involved in inhibiting the production of cytokines and chemokines that are responsible for the activation of the host innate immune response against viral infection [5-7]. Proposed by Rut et al [6] inhibitors are potent anti-SARS-Cov-2 drug candidates, but still require further studies.

To this group of approaches belong studies on clinically approved protease inhibitors, first of all camostat mesylate [8, 9], which is the mesylate salt form of camostat, an orally bioavailable, synthetic serine protease inhibitor, with anti-inflammatory, antifibrotic, and potential antiviral activities suggested anti-Covid-19. This drug and its metabolite 4-(4-guanidinobenzoyloxyl)phenyl acetic acid (FOY 251) are potent inhibitors of a variety of serine proteases and also C1r- and C1 esterases. Along with blocking activation of trypsinogen in the pancreas which is known to play a crucial role in the development of pancreatitis, camostat is thought to suppress expression of numerous cytokines responsible for inflammation and fibrosis of the pancreas. Camostat and its active metabolite 4-(4-guanidinobenzoyloxy)phenylacetic acid (GBPA) are also known to inhibit the transmembrane protease, serine 2 (TMPRSS2) and other TMPRSS2-related host cell serine proteases (among them TMPRSS11D and TMPRSS13), which mediate viral cell entry for influenza virus and coronavirus,

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thereby inhibiting viral infection and replication. Possible application of this drug is under a clinical trial [10], but it is unknown whether the drug concentrations are sufficient to suppress viral entry to cells of respiratory epithelium. Also we have to remember that Protein S of SARS-Cov-2 could be cleaved by other proteases such as cathepsins, CatB/L [8] to facilitate fusion. Several clinical trials on this drug as Covid-19 treatment (NCT04321096, NCT04353284, NCT04355052, NCT04374019) are on the way. Recently, Pfizer announced initiation of Phase I clinical trial (ClinicalTrials.gov Identifier: NCT04756531) on a protease inhibitor PF-07321332 for oral administration in infected individuals. They also investigate protease inhibitor PF-07304814 for intravenous delivery of hospitalized patients (Balfour H, Eur. Pharm. Rev. 24.03.21). The Company does not provide readers with the formulas of investigated compounds.

One of the most considered, although recently most disputable and known for a long time approaches is the therapy by intravenous delivery of blood plasma of convalescent individuals. This approach seems disputable first of all because it includes limited pool of donors i.e. individuals age of 18-60 and in good health condition. Also reasonable volume (~200 ml) of plasma is required for each patient. There were numerous initial reports on the effectiveness of this therapy from several hospitals e.g. [11, 12]. However, we have to mention that recent data on clinical studies of this therapy do not confirm its effectiveness, in terms of clinical status of Covid-19 patients treated with plasma of convalescent individuals versus those who received placebo [13, 14].

Another, intensively studied rational approach is construction of human antibodies or their variable domains responsible for binding of spike protein (protein S) in its receptor, ACE2 recognition region [15, 16], or humanized mouse monoclonal antibodies [17] for therapeutic use. The authors of both approaches point to the necessity of using antibody mixtures due to potentially large mutagenic variability of the virus [18].

Further approach for Covid-19 treatment and/or prophylaxis is proposed by a group of authors from Netherlands, USA, and Italy. Namely they developed a PEG-lipid conjugate of the peptide derived from the C-terminal heptad repeat (HRC/HR2) domain of SARS-CoV-2 protein S which was shown to inhibit SARS-Cov-2 fusion with target cell and therefore the viral infection in vitro and in vivo in experimental animals [19, 20]. The mechanism of inhibitory action of this peptide is based on the fact that that fusion between viral and host cell membrane relays on: 1. cleavage of viral protein S at cleavage site S1/S2, S2 by TMPRSS2 primes SARS-2-S for entry the fusion process via facilitating penetration of host cell membrane by so called fusion domain (peptide). 2. formation of stable hexameric structure via antiparallel interaction of three HRC/HR2 domains with trimeric HRN/HR1 bundle is responsible for bringing viral membrane close to the host cell membrane and facilitate their fusion[21]. Invented by the mentioned above group of authors intranasal spray administered daily was shown to prevent SARS-Cov-2 infection of experimental animals when co-housed with infected animals in conditions in which 100% control animals got infected[19].

Our idea for invention liposome-based nano-therapeutic is based on known fact that the receptor for SARS-Cov and deadly SARS-Cov-2 appeared ACE2 (angiotensin converting enzyme 2) [8, 22]. Moreover, it was determined earlier that two relatively distant in primary structure sections but close in conformation (amino acid residues 22-44 and 351-357) were responsible for high affinity binding of the S-glycoprotein of SARS-Cov viruses [23-26]. Structural studies provided presumable explanation why SARS-Cov-2 spike glycoprotein S binds ACE2 receptor sequence with ~five times higher affinity than one of SARS-Cov (44 vs 185 nM) [27].

In our study project we propose a unique approach to the virus-host cell inhibition which is liposome-based nanotherapeutic formulation which hosts on the liposome surface polyethylene glycol derivative conjugated to one of the designed peptides mimicking the SARS-Cov-2 binding sequence of ACE2 e.g. residues 22-44 or 351-357 or both peptides or peptide of sequence of both peptides conjugated via an appropriate linkage. It is known that such peptides competitively inhibit binding of SARS-Cov-2 glycoprotein S with cellular receptor of target host. We expect that such a nano-formulation of peptide inhibitor would not only inhibit SARS-Cov-2 binding to the cell surface of the host's

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respiratory tract epithelia but also immobilize virus on the liposome surface, therefore impede the progress of infection (See Fig. 1).

Our preliminary experiments may indicate that 125 nm in diameter liposomes containing on their surface joined by glycine residue peptide 1 (residues 22-44) with peptide 2 (residues 351-357)(Sikorski et al, Polish Patent Application, No. P.435921] exerted an inhibitory effect on ACE2-SARS-Cov-2 interaction in "syncytia-forming" test of two populations of HEK293T cells, one transformed with DNA-vector carrying virus-binding sequence of ACE2 (cell surface receptor of the virus) and the other transformed with a vector carrying SARS-Cov-2 Protein S receptor binding domain (RBD). It was also shown that this nano-drug formulation was also nontoxic to human cells in culture. Further studies are on the way towards specific and effective therapeutic formulation fighting early stages of infection with SARS-Cov-2 virus. This should prove useful for patients who had not been vaccinated yet or to those who cannot be vaccinated due to the health condition preventing vaccination or to those for whom there is no approved vaccination yet.

We think that our approach allows overcoming at least one aspect of the disease, namely virus mutations. As we are using an artificial human ACE-2 receptor sequence, so its mutation would not matter and in the case of mutation in virus gene encoding RBD of Protein S (probability of which is rather high) the affinity of Protein S towards ACE2 may increase, therefore the affinity of our formulation towards virus also increases, when it decreases, or disappears the virus becomes neutral.

In summary, mentioned above (by no means complete), multidirectional approach to the anti-Covid-19 therapy undertaken by many research and industrial centers all over the world, in our opinion, will hopefully help to control the disease.

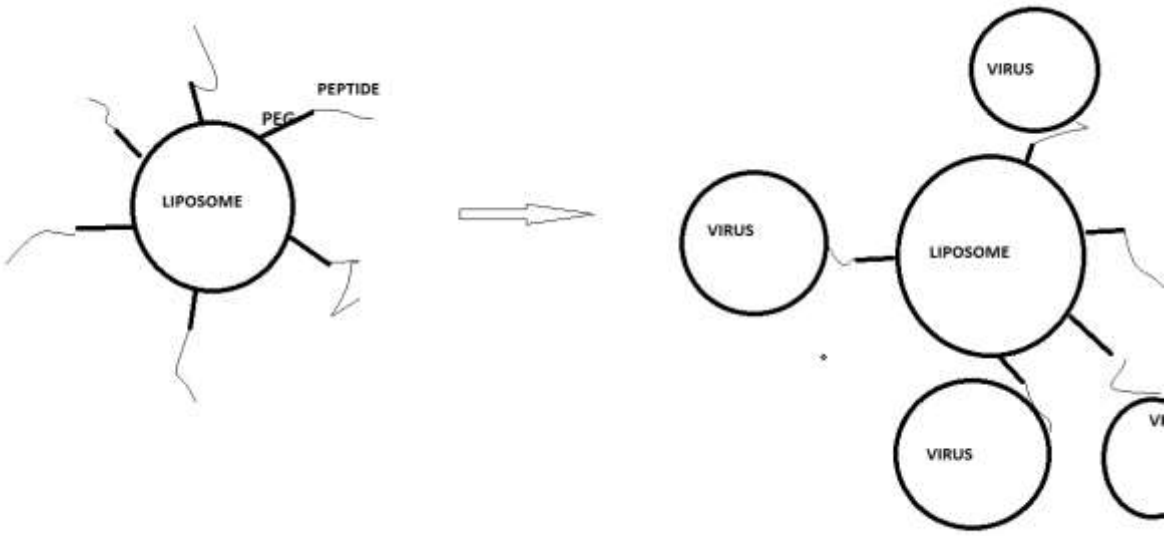

Fig.1 Schematic representation of action of the proposed nano-drug formulation. Peptide, means one of the peptides mimicking virus-binding site in ACE2 protein. For other details see the text.

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