

## Lipid-based nano delivery of Tat-peptide conjugated drug or vaccine—promising therapeutic strategy for SARS-CoV-2 treatment

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### 1. Introduction

Ever since the outbreak of SARS-CoV-2 in December 2019 at Wuhan, China, it has already become a global epidemic, leaving millions infected (5,652,091) in over 213 countries and killing over 350,398 people [1]. SARS-CoV-2 belongs to the family *Coronaviridae* and subgenus beta-CoV [2]. Other known coronaviruses that cause severe respiratory diseases in human are the Middle East respiratory syndrome (MERS) [3] and severe acute respiratory syndrome (SARS) [4]. The novel SARS-CoV-2 is the seventh known human coronavirus (HCoV) from the same family after 229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV [5]. SARS-CoV-2 is a single-stranded positive-sense RNA (+ssRNA) viruses with ~30kb of the genome, which is the largest among all RNA viruses [6,7]. The crystal structures of two bat SARS-like CoVs (ZC45 and ZXC21), share ~89% sequence identity with novel SARS-CoV-2. Recently, it has been reported that the sequence of bat CoV, RaTG3, share 92% sequence identity with SARS-CoV-2, which strengthens the fact, as mentioned earlier about the origin of SARS-CoV-2 [7].

The world will count the social and economic costs of this pandemic for decades to come. It is therefore absolutely critical to developing an effective drug that can treat and prevent COVID-19 before a vaccine can be developed. To date, there is no specific anti-COVID-19 medicine or vaccine has been developed for the treatment of this highly contagious novel virus. The search for new medicines for immediate treatment of COVID-19 infection is the most complex and challenging task endured by the scientific community around the globe. The effort to develop a drug followed by extensive clinical trials may take several years. Further, the screening of new drugs or vaccines using live SARS-CoV-2 includes a high-level biosafety facility that is a challenge for those scientists and

researchers who do not have access to such facilities. However, some of FDA approved repurposed antiretroviral drugs (lopinavir & ritonavir), broad-spectrum antiviral (remdesivir & favipiravir), antimalarial (hydroxychloroquine) and monoclonal antibodies alone or/and in combination have emerged as magic bullets for the treatment of COVID-19 infection.

### 2. Nanoformulations-based drug delivery of repurposed drugs

The limited intracellular intake/uptake of repurposed antiviral drugs or molecules is one of the major challenges to successful treatment of COVID-19. There are other challenges, too, such as decreased biological potentials, therapeutic efficacy, bioavailability, limited aqueous solubility, short half-life, and enhanced toxicity [8]. These limitations open up new windows for the development of alternative strategies that can enhance the efficacy of repurposed drugs and promote their intracellular penetration. To overcome these limitations, various nanoformulation-based nano-drug delivery platforms have been designed that can enhance the therapeutic potential of repurposed antiviral drugs for the treatment of COVID-19 such as liposomes, solid lipid nanoparticles, polymeric nanoparticles, polymeric micelles, dendrimers, cyclodextrin derivatives, nanoemulsions, nanosuspensions or nanocrystals, self-assembled nanostructures [8,9]. Nanoformulations-based drug delivery systems may increase the effectiveness of the drugs by facilitating local and specific sustained-release, modified pharmacokinetics and pharmacodynamics, enhanced bioavailability, reduced side effects and target specific sites of action [8]. The surface modification of nanocarriers by appropriate linkers may enhance their capability to interact with virus structures, overcome biological barriers and

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accomplish effective concentrations of drug in viral reservoirs. Further, these engineered nanocarriers can easily reach specific extracellular or intracellular targets site and thus compete for attachment to the cell surface receptors with viruses [8]. Therefore, nanoformulation-based nano-drug delivery strategies can be used to develop the broad-spectrum antiviral drugs that may either target the viral membrane or cell surface receptor or both to compete with the virus adherence, attachment and their entry into the target cell that finally prevents the virus internalization [8].

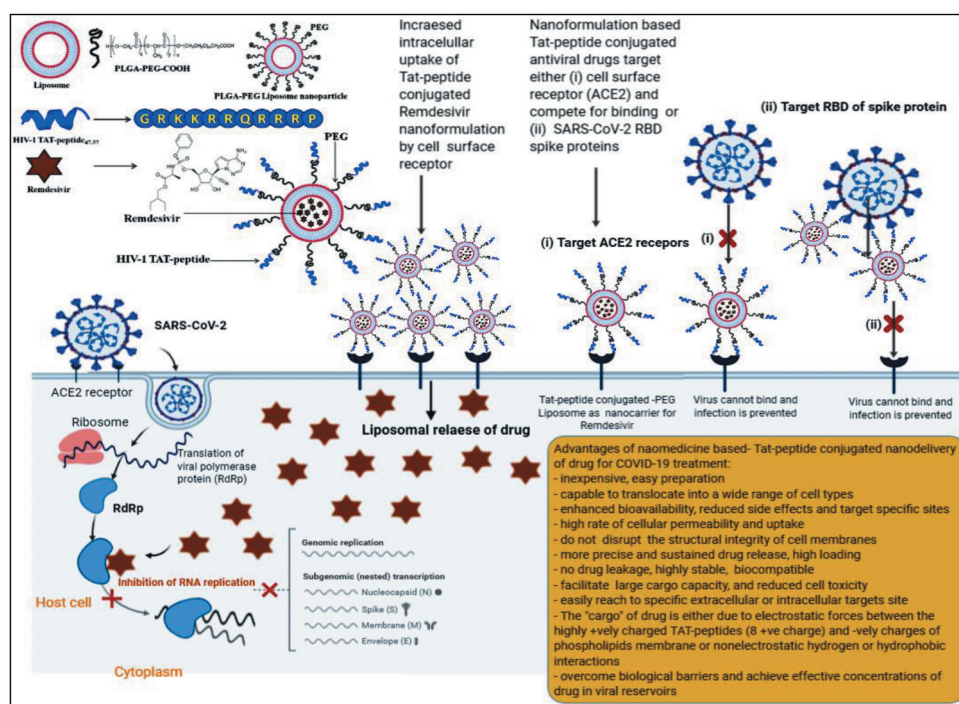
### 3. Cell-penetrating peptides (CPPs) based-nano delivery of repurposed drugs

Most importantly, the nano delivery of repurposed antiviral drugs can be further enhanced by conjugating to cell-penetrating peptides (CPPs). The discovery of CPPs, a short cationic peptide with a high content of basic amino acid residues [10], readily facilitate intracellular intake and delivery of a variety of nanosize, small and large molecules into cytoplasm or nucleus without causing toxicity and damaging the cell membranes integrity [11,12]. The 'cargo' of these molecules is either due to electrostatic forces between the positively charged TAT-peptide and negatively charges of phospholipids membrane or nonelectrostatic hydrogen or hydrophobic interactions [12,13]. CPPs deliver the cargo into the cell either through macropinocytosis, caveolae-mediated endocytosis or clathrin-independent endocytosis mechanism. It has also been studied that HIV1 TAT-peptides directly penetrate the membranes by generating nanoscale pores [13]. CPPs has several advantages over other delivery and translocation approaches as it is inexpensive, easy to manufacture and usually nontoxic [14]. They have a higher capability to translocate into a wide range of cell types, higher rate of cellular permeability and uptake, more comfortable to pass other biological barriers. Additionally, they have a large cargo capacity and reduced cell toxicity with no immunological response [14,15]. Apart from the several advantages of CPPs, these peptides have also limitations, such as low cell specificity, uptake into intracellular endosomes and inactivation of CPPs by proteases could be the most significant drawbacks of the first generation of CPPs [16]. Hence, to the therapeutic application, these CPPs should be stabilized via the incorporation of multifunctional organic polymeric or lipid-based nanocarriers to improve selectivity, efficiency, and capacity of cargo transport to avoid inactivation by proteases [17]. It has been reported that CPP-based drug delivery systems have reached clinical trials for cancer diagnosis and therapy, where they showed enhanced efficacy [18]. It is, therefore, reasonable to expect similar progress in the development of nanoformulation-based CPP-conjugated nano delivery of repurposed antiviral drugs for the treatment of COVID-19. The HIV-1 TAT-peptide<sub>47-57</sub> (GRKKRRQRRRP), a short

cationic (8 positive charges) and high content of essential amino acid (2 lysine and 6 arginine), rapidly became a popular and powerful research tool to enhance the transport and delivery of proteins, DNA/RNA, viruses, drugs and nanoparticles inside the cells [12,15]. CPPs might be promising immune enhancers when incorporated into appropriate nanoformulation-based nanocarriers systems. According to Milken Institute, ~123 vaccines, ~21 repurposed antiviral drugs, ~58 antibodies, ~15 cell-based, 6 RNA-based and more than 80 such as immune enhancers, immune-modulating, antimalarial, antiparasitic and anti-inflammatory are at different stages of clinical trials for the treatment of COVID-19 infections [19]. Unluckily, none of the ongoing clinical trials and treatments is based on nanocarriers and Tat-peptide conjugated-nanoformulation strategies despite the many advantages in nanocarriers and Tat-peptide drug delivery systems. Therefore, we suggest that the efficacy of antiviral activity of repurposed drug or vaccine against COVID-19 can be improved and enhanced by conjugating it to the Tat-peptides by utilizing nanoformulation-based nanocarriers delivery systems. We do not have high-level biosafety facilities for testing or analyzing the antiviral efficacy of nanoformulations-based nanocarriers for the delivery of Tat-peptide conjugated repurposed drug or vaccine using live SARS-CoV-19 either *in vitro* or *in vivo*. The promising therapeutic strategy of lipid-based nano delivery of Tat-peptide conjugated drug or vaccine for SARS-CoV-2 treatment was presented in Figure 1.

### 4. Conclusion and future direction

The present hypothesis might be an excellent platform for the development of nanoformulations-based nano delivery of Tat-peptide conjugated repurposed drug or vaccine which may be a silver bullet for the doctors around the globe who are fighting SARS-CoV-2 battle on the front line for the treatment of deadly COVID-19 infections. Therefore, I would kindly urge the researcher, scientists, pharmaceutical companies and funding agencies around the globe to come forward immediately to conduct *in vitro*, *in vivo*, preclinical or/and clinical trials on the efficacy of nanoformulations-based nano delivery of Tat-peptide conjugated repurposed drug or vaccine. The advantages offered by lipid-based nano delivery of drug or vaccine make it a versatile therapeutic alternative for the treatment of infectious disease outbreaks such as COVID-19, MERS, SARS, Ebola etc. because the lipid-based nano delivery of Tat-peptide conjugated drug or vaccine systems is inexpensive, easy to prepare, enhance the bioavailability, cellular permeability, uptake and stability of drug and vaccine, and most importantly it is more precise and sustained drug release. Therefore, the demand for lipid-based delivery of drug or vaccine is high, and positively, it might be fulfilled in the future.



**Figure 1.** Tat-peptide in COVID-19 treatment: lipid-based nanocarriers for the delivery of Tat-peptide conjugated repurposed drug – promising therapeutic strategy for SARS-CoV-2 treatment.

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## Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Papers of special note have been highlighted as either of interest (\*) or of considerable interest (\*\*) to readers.

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  - **This link provides the current drug and vaccine development for the treatment of Covid-19 at the preclinical level and Phase I/II/III/IV clinical trials.**