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Complexes of oligoribonucleotides with D-mannitol inhibit the interaction of SARS-CoV-2 Spike pseudotyped lentiviral particles with host cells

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pharmaceuticals MDPI

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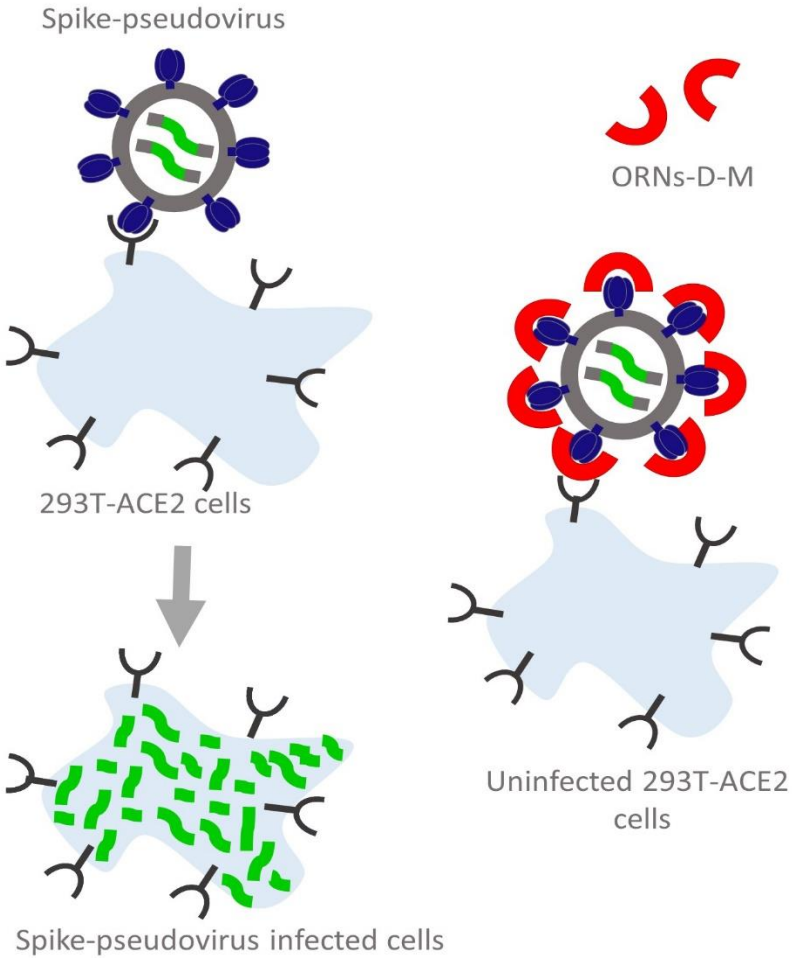
Title of the Presentation



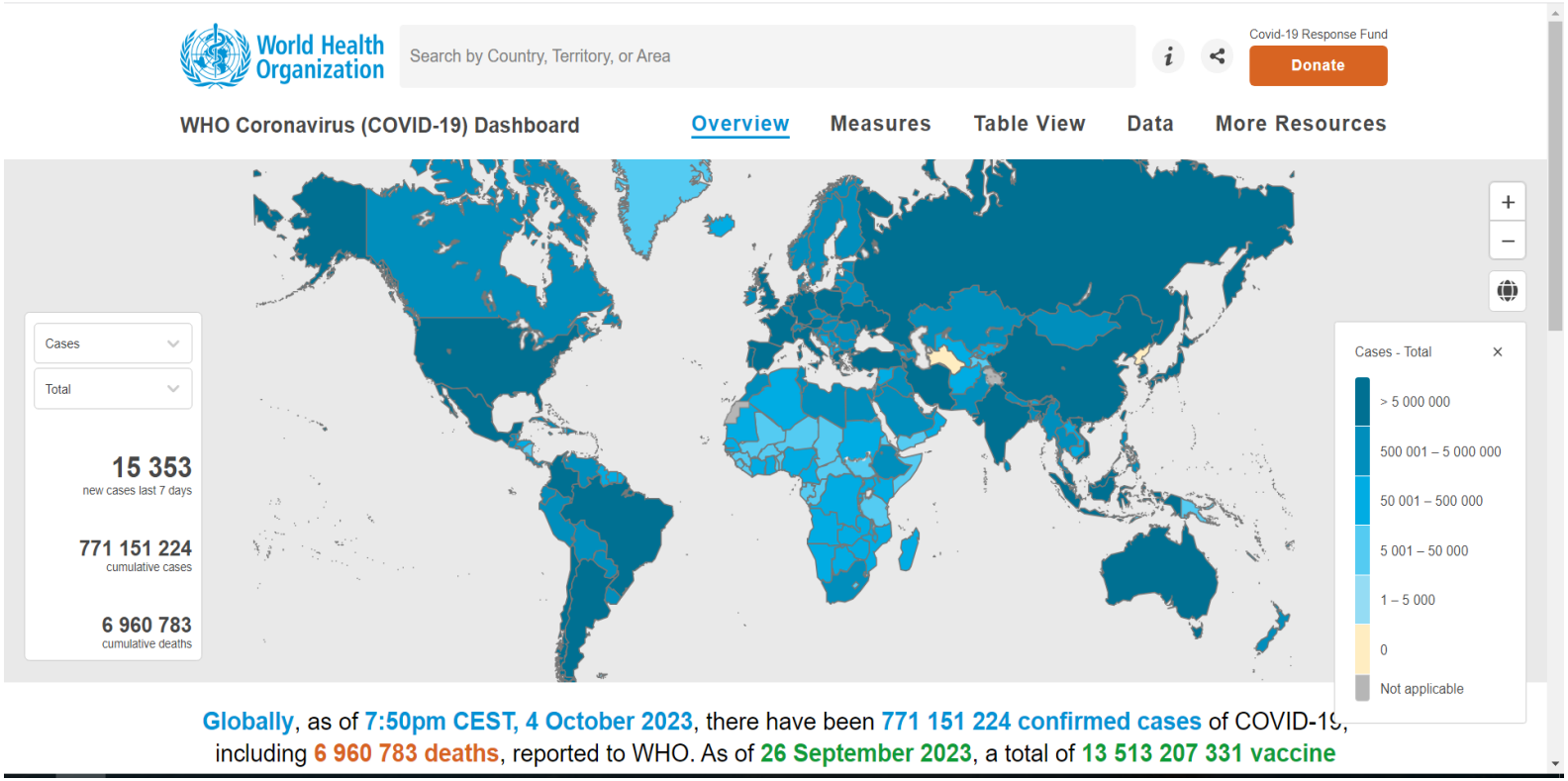
Abstract: Coronavirus disease 2019 (COVID19) is a respiratory illness that is caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV2) and leads to numerous deaths. SARS-CoV-2 enters cells using its Spike protein, which is also a prospective target for the development of new anti-COVID-19 drugs. Therefore, searching for novel therapeutic agents with Spike inhibitory activity is an urgent task. Oligoribonucleotides-D-mannitol (ORNs-D-M) complexes possess antiviral activity by inhibiting the activity of surface viral proteins. We hypothesized that the ORNs-D-M could inhibit SARS-CoV-2 Spike activity. Because SARS-CoV-2 is a biosafety-level-3 virus, one way to simplify such assays is to pseudotype biosafety-level-2 viral particles with Spike. In this study, we aimed to evaluate the ORNs-D-M efficiency against pseudotyped lentiviral particles with SARS-CoV-2 Spike.

We obtained SARS-CoV-2 Spike-pseudotyped lentaviral particles (Spike-pseudovirus) by transfecting the 293T cells with a plasmid complex (lentiviral backbone (Luciferase-IRES-ZsGreenbackbone), Spike SARS-CoV-2 and the other HIV proteins needed for virion formation). Microscope images showed the ZsGreen expression in the infected 293T-ACE2 cells with Spike-pseudovirus. Low ZsGreen fluorescence was observed in the infected 293T-ACE2 cells with Spike-pseudoviruses that were pre-incubated with the ORNs-D-M compared to the Spike-pseudovirus control. Luciferase expression was indicated in the infected 293T-ACE2 cells with Spike-pseudovirus. It was found that pre-incubation of the Spike-pseudovirus with the ORNs-D-M significantly reduced luciferase activity compared to the Spike-pseudovirus control. Decreased expression of ZsGreen and luciferase, marker proteins of cell infection with Spike-pseudovirus, potentially indicates that the ORNs-D-M interact with SARS-CoV-2 Spike and inhibit the interaction of Spike-pseudovirus with host cells. The obtained results show that the ORNs-D-M can have anti-COVID19 activity.

Keywords: Oligoribonucleotides with D-mannitol, SARS-CoV-2, Spike pseudotyped lentiviral particles

Introduction

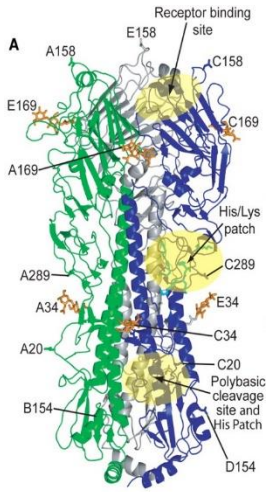
Coronavirus disease 2019 (**COVID-19**) caused by the Severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**) and lead to COVID-19 pandemic. SARS-CoV-2 enters cells using its Spike protein, which is also a prospective target for the development of new anti-COVID19 drugs.



https://covid19.who.int/

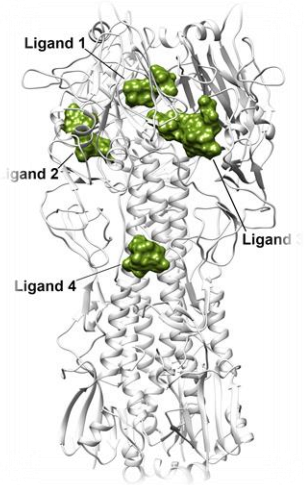
Complexes of natural oligoribonucleotides with D-mannitol (**ORNs-D-M**) based on total yeast RNA with the dominant fraction of 3–8 nucleotides modified with D-mannitol (D-M) have an antiviral effect against a wide range of DNA and RNA viruses.

The ORNs reduce the activity of the hemagglutinin of the influenza virus, which leads to the inhibition of the hemagglutinin-glycan interaction and a decrease in the infectivity of the virus. The low-affinity interaction of ORNs with the influenza virus glycoprotein reveals insights into the mechanism of antiviral action of ORNs against a wide range of viruses. We hypothesized that ORNs drugs can interact with the SARS-CoV-2 adhesion protein Spike, showing an anti-coronavirus effect.



Hemagglutinin structure

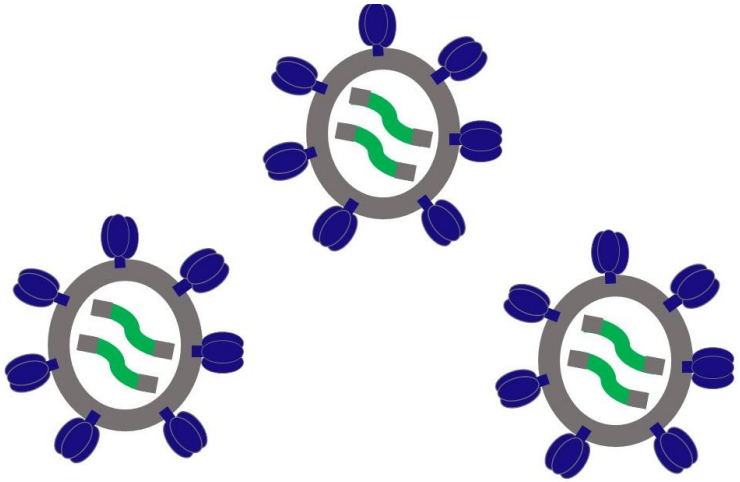
Stevens J. et al. Science. 2006. 312 (5772): 404-410



Visualization of docking results of the interaction of ORNs-D-M with hemagglutinin

Z. Tkachuk and al. Membranes 2021, 11, 757.

SARS-CoV-2 studies are limited by the fact that the virus is a biosafety-level-3 agent that must be worked with in specialized facilities. One way to simplify such assays is to pseudotype biosafety-level-2 viral particles with Spike. Therefore, we conducted our studies of the antiviral effect of ORNs-D-M against SARS-CoV-2 on a pseudotyping model of lentiviral particles with the SARS-CoV-2 adhesion protein.

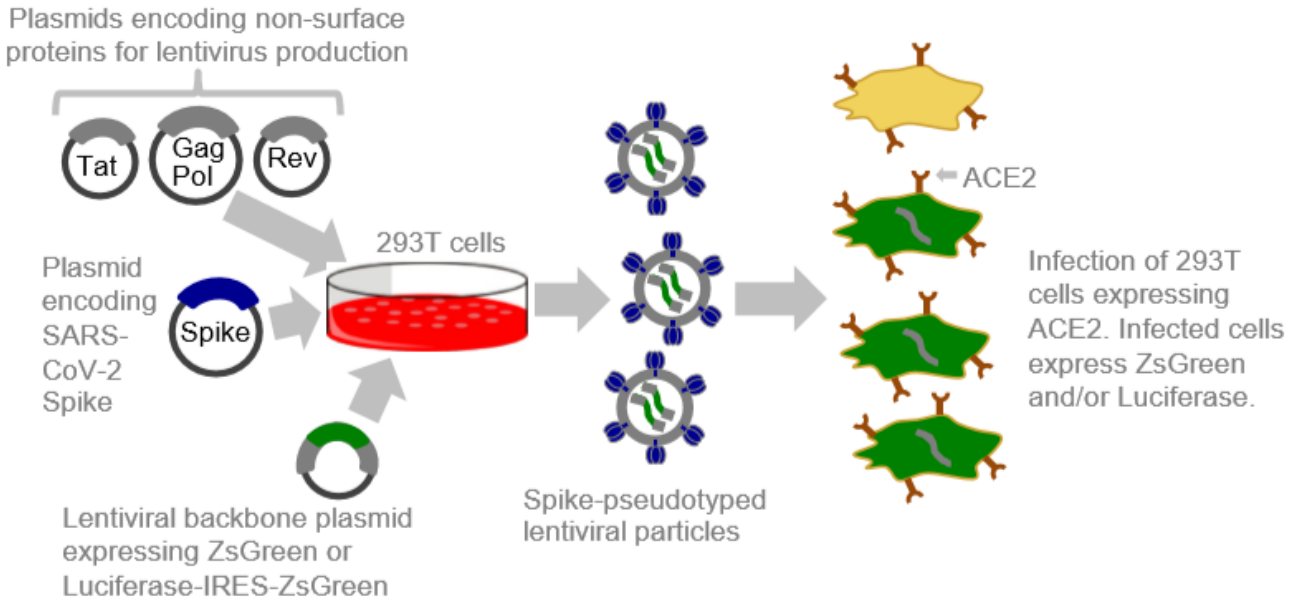


Spike-pseudotyped lentiviral particles

Results and discussion

Generation of Pseudotyped Lentiviral Particles with SARS-Spike

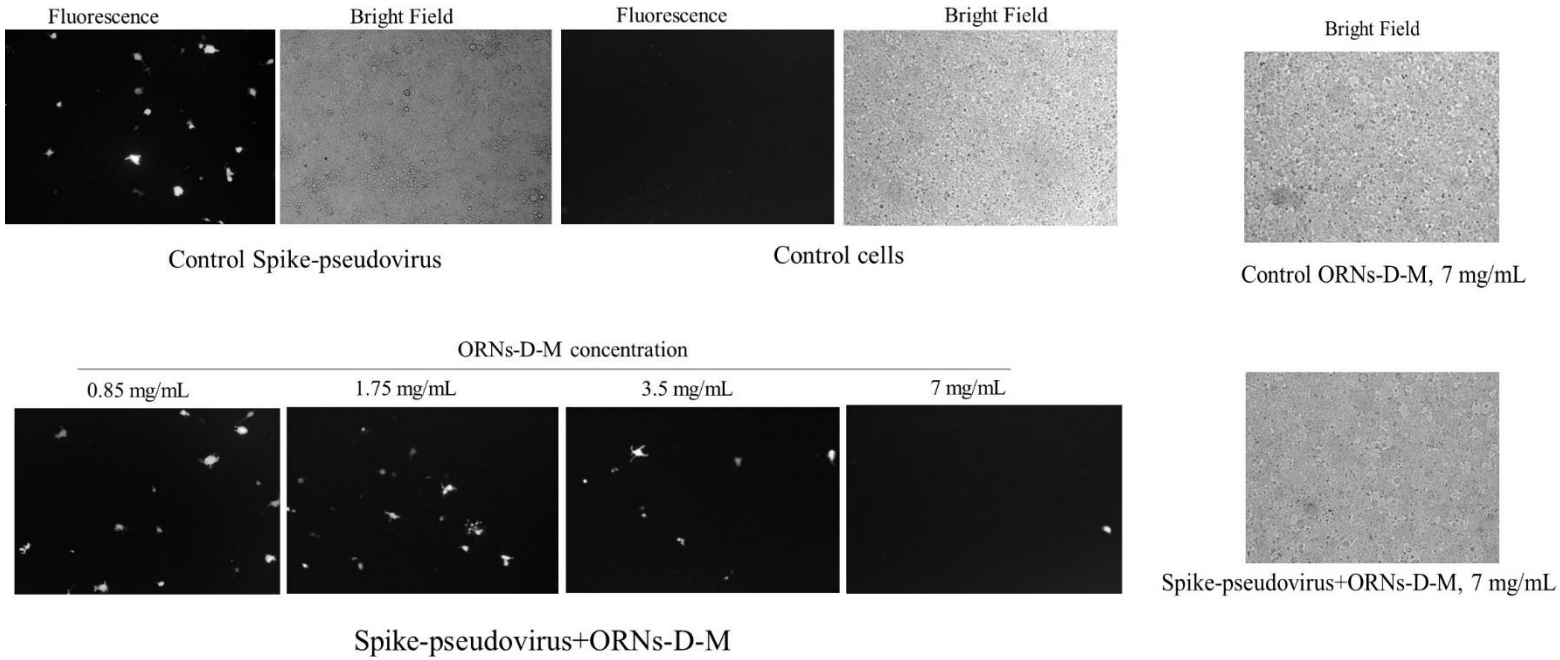
We generated the SARS-CoV-2 Spike-pseudotyped lentiviral particles (Spike-pseudovirus) by transfecting the 293T cells with a plasmid complex (lentiviral backbone (Luciferase-IRES-ZsGreenbackbone), Spike SARS-CoV-2 and plasmids expressing the other HIV proteins needed for virion formation (Tat, Gag-Pol, and Rev)).



Crawford et al. Viruses 2020, 12, 513; doi:10.3390/v12050513

Inhibition of the Spike-pseudovirus-induced ZsGreen expression by the ORNs-D-M

Fluorescent microscope images showed the ZsGreen expression in the infected 293T-ACE2 cells with the Spike-pseudovirus. Low ZsGreen fluorescence was observed in the infected 293T-ACE2 cells with the Spike-pseudoviruses that were pre-incubated with the ORNs-D-M compared to the Spike-pseudovirus control.

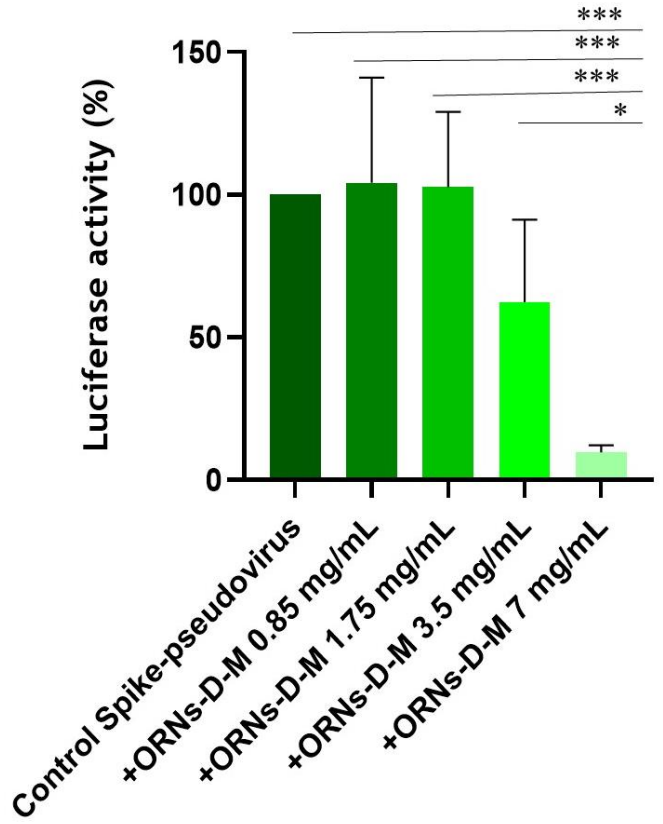


The expression of ZsGreen in the 293T-ACE2 cells after 48 h infection by the Spike-pseudovirus with or without the ORNs-D-M pre-incubation was detected using a fluorescent microscope

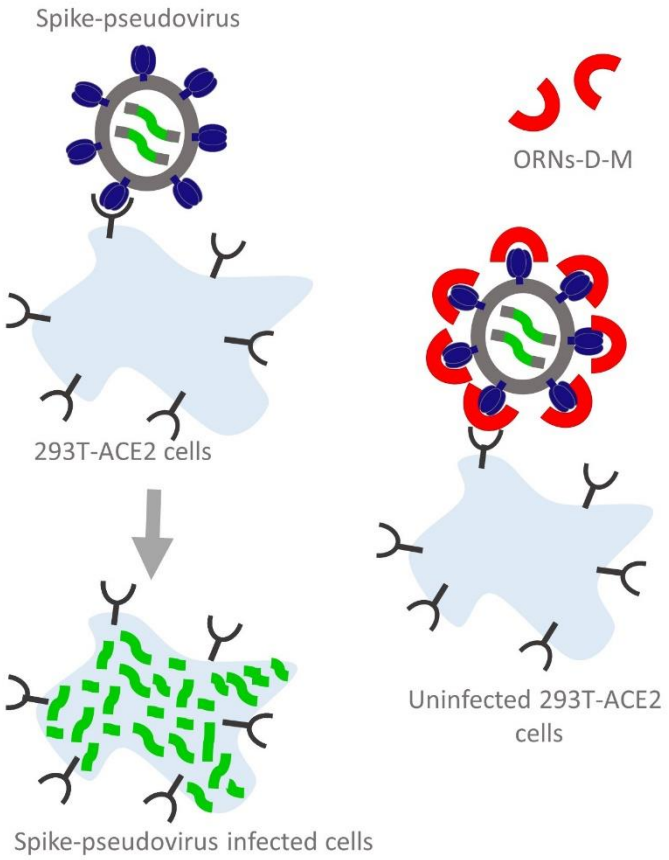
Inhibition of the Spike-pseudovirus-induced luciferase activity by the ORNs-D-M

The luciferase expression was indicated in the infected 293T-ACE2 cells with Spike-pseudovirus and also was a marker of cell infection. Using luciferase luminescence assay was found that pre-incubation of the Spike-pseudovirus with the ORNs-D-M reduced significantly luciferase activity compared to the Spike-pseudovirus control.

Statistical significance was evaluated using the one-way ANOVA test (Tukey's multiple comparisons test). * P<0.05, ***P<0.001, vs. Spike-pseudovirus control.



Luciferase activity in the infected 293T-ACE2 cells by the Spike-pseudovirus with or without the ORNs-D-M



Decreased expression of ZsGreen and luciferase, marker proteins of cell infection with Spike-pseudovirus, potentially indicates that the ORNs-D-M interact with SARS-CoV-2 Spike and inhibit the interaction of Spike-pseudovirus with host cells.

The obtained results show that the ORNs-D-M can have an anti-COVID-19 activity.

Conclusions

- The ORNs-D-M suppress the Spike-pseudovirus-induced ZsGreen expression in the infected 293T-ACE2 cells;
- Luciferase activity was inhibited significantly in the Spike-pseudovirus-infected 293T-ACE2 cells by the ORNs-D-M;
- Decreased expression of ZsGreen and luciferase, marker proteins of cell infection with Spike-pseudovirus, potentially indicates that the ORNs-D-M interact with SARS-CoV-2 Spike and inhibit the interaction of Spike-pseudovirus with host cells;
- By inhibiting the interaction of SARS-CoV-2 Spike pseudotyped lentiviral particles with host cells, the ORNs-D-M can have an anti-COVID-19 activity.