The Joint Research on COVID-19

NO.	Current Research Progress and Latest Result on COVID-19	Research Plan
	Drug Research	
1	The world has encountered Coronavirus disease 2019 (COVID-19) since December 2019. One of the major pathological features of the severe COVID-19 patients is pulmonary fibrosis, a progressive disease that eventually leads to respiratory failure and death. Up to till now, there is no specific treatment for COVID-19. Clinical studies have found that mesenchymal stem cell (MSCs) therapy can significantly improve the prognosis of severe and critical patients, effectively avoid cytokine storm, with no obvious side effects. However, whether MSCs can alleviate the COVID-19 caused pulmonary fibrosis, and how MSCs improve the pulmonary fibrosis, still need more in vivo experiments to verify and elucidate. In the past several years, our group devoted to the treatment of pulmonary fibrosis by MSCs. We found that the MSCs migrated to the damaged lungs first after transplantation, and inhibited the collagen deposition in the lung of pulmonary fibrosis mice, thus alleviating the pulmonary fibrosis. Moreover, based on gold, gadolinium, and long persistent luminescence nanoparticles, we developed a series of multi-modality nanotracers for the labeling and in vivo tracking of the transplanted stem cells, realizing the real-time evaluation of the stem cell therapy. The MSCs could be labeled by the naontracers effectively, resulting in significantly increased cellular imaging contrast, without any obvious adverse effect on the functions, including proliferation and differentiation of the labeled stem cells. By the using of these nanotracers combined with computed tomography, magnetic resonance imaging, near-infrared fluorescence imaging, and bioluminescence imaging technologies, the migration, homing, and survival of the intravenously injected MSCs in pulmonary fibrosis mice had been in situ visualized. Moreover, the transplanted MSCs labeled with these nanotracers could be tracked for 30 d in vivo. Our researches may provide an insight into the role the transplanted MSCs play in pulmonary fibrosis therapy, and therefore promoting the stem cell-based COVID-19 treatment. These related studies have been published in international journals including Small, Nanoscale, Biomaterial Science, and ACS Applied Bio Materials.	It has been reported that mesenchymal stem cells (MSCs) can inhibit pulmonary fibrosis. However, the therapeutic effect of MSCs on COVID-19 caused pulmonary fibrosis is unclear, and the movement and function of the transplanted stem cells in vivo cannot be accurately and effectively observed. To address these issues, we plan to use MSCs to treat the COVID-19 caused pulmonary fibrosis, and clarify the therapeutic effect of MSCs. Meanwhile, we will construct a serious of nanotracers with imaging functions, combining with magnetic resonance imaging, computed tomography, and fluorescence imaging technologies to realize the effective, long-term, and safe in vivo tracking of the MSCs after transplantation, and reveal their mechanism of action during the treatment process. Based on the advantages and characteristics of our group and the group from Iran, relevant researchers from both groups will join the research. During the implementation of the project, the two groups will discuss the progress and existing problems of the project at any time by means of network communication, regular meetings and visits. We will share the data, and promote the team cooperation to the greatest extent. Moreover, we will co-cultivate young scientific and technological talents, and promote the academic and cultural exchanges between China and Iran.
2	COVID-19 pandemic remains as a global rampage without a cure. Current drug research focuses on 1)blockage of virus entry and 2)blockage of viral replication. Our project aims to develop a series of Nanobodies(12kDa) to specifically bind to the highly conserved membrane fusion domain of SAR-CoV-2 S protein, and thus block viral fusion to cells and subsequent replications. We first prepared the protein fragment of the non-glycosylated fusion domain, and then trimerized the fragment to restore its natural conformation, and then used it as a bait to select high affinity nanobodies using a yeast surface displayed nanobody library, and then to test nanobodies' capacity in blocking viral fusion in cells and in vivo. The advantage of using low molecular weight nanobody lies in its ability to bind the highly conserved, non-glycosylated yet cryptic epitopes of the membrane fusion domain.	To collaborate: 1)blockage of viral fusions in cell models; 2) blockage of viral entry in animal models; 3) blockage of viral load in human subjects; 4) pharmaceutical development of nanobody drugs. Forms of collaborations: 1) data exchange; 2) nonbiohazard material exchanges.
3	Yun Zhu has experiences in the study of important protein's structures and antiviral agents of multiple coronavirus. He has solved the crystal structure of fusion core of Middle East Respiratory Syndrome coronavirus for the first time globally, and designed effective viral entry inhibitors. After the outbreak of COVID-19, under the guidance of team leader Fei Sun, he quickly solved the crystal structure of fusion core 6-HB of SARS-CoV-2 for the first time in the world, and released the data through Protein Data Bank directly (PDB entry code: 6 LXT and LVN). This structure revealed important characteristics in the infection process of SARS-CoV-2 infection, which led to the design of high efficient and broad-spectrum viral inhibitor of EK1 and EK1C4. This work has been published as the cover story on the international famous academic journals "Cell Research". Most recently, he has solved the crystal structure of EK1 bound SARS-CoV-2 HR1 motif (PDB entry 7C53), which helps to further optimize this viral inhibitor and enhance its antiviral efficiency. They are trying to obtain a novel potent inhibitor against SARS-CoV-2 for clinical use as soon as possible. In addition, they are also working with other teams to solve the in situ structure of important SARS-CoV-2 proteins, to provide key foundation of research and development antiviral drugs.	He has expertise to rapid screen antiviral agents from natural source. Through previous communication, we plan to collaborate on rapid development and efficient screening antiviral agents of SARS-CoV-2 to give play to each other's strengths. Professor Majid have screened out several inhibitors against S protein and M protein of SARS-CoV-2, then we would quickly resolve the complex structure of viral proteins and inhibitors, which could further help to optimize the inhibor. Meanwhile, Professor Majid would use the unique plant expression system to improve the expression of EK1 peptide inhibitors efficiently to largely reduce costs of EK1 peptides.
4	The S1 antigen of SARS-cov-2 coronavirus was recombinantly expressed using the insect expression system and immunized laying hens. The egg yolk antibody （IgY） was extracted and prepared. The anti-S1-IgY was verified by pseudovirus neutralization assay	It is planned to cooperate in the research on passive immunity of aerosol on respiratory mucosa, mainly involving animal experiments and research on the immune mechanism of lower respiratory
5	The infection of SARS-CoV-2 Coronavirus outbreak in Wuhan causes acute respiratory syndrome and severe pneumonia. Studies have found that SARS-CoV-2 can spread widely in the population through droplets, contact and other ways. Symptoms vary among different populations, and asymptomatic infected individuals also spread the virus. As a result, the number of confirmed cases has risen rapidly, causing serious public health problems around the world. It is urgent and important to find clinical drugs against SARS-CoV-2 infection to prevent and cure COVID-2109. "Green bismuth" widely distributed in nature is a non-toxic and harmless ecological strategic cheap metal. Bismuth compounds have been widely used in environmental protection, photocatalysis, biological imaging and treatment. By using chemical synthesis of Bisthmuth nanoparticles, the antiviral effect of bismuth series compounds of common coronavirus, SARS-CoV-2 lenti-virus and SARS-CoV-2 at the cellular level, will be screened， and the protective efficacy evaluation of bisthmuth compounds in mice will be applied. In 2019, we synthesized three bismuth nanocompounds, conducted cytotoxicity experiment on human common coronavirus host cells. We need to find partners and financial support for further research.	The synthesis of bismuth nanoparticles compound needs to be done in China, other parts: detection of bismuth series compounds' antiviral effect on common coronavirus, SARS-CoV-2 lenti-virus and SARS-CoV-2 at cellular level, and the efficacy evaluation in mice to confirm the protective effects of coronavirus infection. Those parts could all be done in China, or one or two parts could be chosen and done in Iran by the Iranian partner. China and Iran may exchange visiting scholars or overseas students for collaboration.
6	The severity of the ongoing COVID-19 pandemic has shown that it is critical to clarify how the SARS-CoV-2 virus enters host cells and, importantly, is there any clinic-approved drugs specifically targeting to host protein during viral entry, for more efficient therapies. SARS-CoV share the same ACE2 receptor and homogenous spike protein with SARS-CoV-2, which is worthwhile for the parallel study. Previous studies on SARS-CoV identifies that there is a direct interaction between host cytoskeletal protein vimentin and spike-ACE2 complex, promoting the viral entry. SARS-CoV virus-like particles (VLPs) and exogenously overexpress of spike protein dramatically recruit vimentin to the host cell membrane. Anti-vimentin antibodies specifically diminished the uptake of SARS-CoV VLPs, suggesting that vimentin is a putative anti-viral drug target for preventing/reducing the susceptibility to SARS-CoV infection. Furthermore, we found that cellular vimentin is super-sensitive upon stress (eg. osmotic shock, mechanical stimuli, Redox stress, bacterial infection) and being transported to the cell surface. Depletion of vimentin dramatically prevent the infection efficiency of a number of flaviviruses (eg. ZIKV, DENV, which are enveloped single strand positive sense RNA viruses as SRAS-CoV-2). Importantly, we identified eight clinical approved drugs from over 2000 compounds which regulate the distribution of vimentin, indicating its potential pharmacological application. Together, the current proposal aims to clarify the entry mechanism of SARS-CoV-2, analyze the temple- and spatial- regulation pattern of the entry process, study the hijack strategy of SARS-CoV-2 to host vimentin, establish screen and identify clinical molecules targeted to vimentin distribution which potentially can be used as anti-viral drugs. Successful implementation of this proposal will improve our understanding on the invasion mechanisms of SARS-CoV-2. With these cell biological and high-throughput compounds studies, it will also provide a novel insight into the therapeutic targets.	We would like to share the relevant SARS-CoV-2 research tools with Iran collaborators, for example, pseudotyped virus, virus-like particles (VLPs), expression plasmids of receptor protein, structural protein as well as nonstructural proteins. These tools could also be used as the readout for drug screen. Moreover, we have successfully generated the stable host cell lines with optimized expression of ACE2 (eg. Vero E6, Huh7, Caco-2, A 540, ect). We further generate the several gene knockout cell line tools for the entry mechanistic study, all of which we are pleased to share with Iran collaborators for common interest. It is worth to note that we take advantage of several cutting-edge bioimaging techniques aiming to 'visualize' the infection process on cellular and cub-cellular level together with Iran collaborators. Most importantly, we have the chance to access the BSL3 laboratory in Iran for the real virus and live animal experiments based on the novel findings on the entry targets and the potential drug we identified.

7	SARS-CoV-2, a novel coronavirus responsible for the ongoing COVID-19 pandemic, possesses an extraordinary ability to spread among human beings. Conventional chemical-based disinfection approaches can cause the secondary damages to the environment. Therefore, it is imperative to develop an efficient and environment-friendly approach to disinfect pathogens in a field setting to mitigate transmission. Photocatalysts are known to inactivate various microbes, but their application in disinfecting viral pathogens has not been systematically evaluated and the underlying antiviral mechanism remains elusive. In the past three years, we have developed a system to study the effects of nanosized TiO2 on viral pathogens under a low irradiation condition that mimics the field setting. We showed that photo-catalyzed TiO2 efficiently inactivates a broad range of human viral pathogens, including SARS-CoV-2. Mechanistic studies demonstrated that hydroxyl radicals produced by photo-activated TiO2 attack viral RNA genome, thus inactivating the virus. In conclusion, we showed that photo-catalyzed nanosized TiO2 inactivates pathogenic viruses, paving a way to its field application in control of viral infectious diseases. It has several advantages: effective, broad antiviral spectrum, easy to use, long-lasting effect and environment-friendly.	There is no approved therapeutic drug for COVID-19. This grant is aimed to (1) establish a subgenomic replicon model for SARS-CoV-2, an important tool for antiviral development in a Biosafety Level 2 (BSL-2) laboratory ; (2) use the replicon system to screen for lead compounds that inhibit viral replication and study the underlying antiviral mechanisms; (3) use the replicon system to identify novel host factors for SARS-CoV-2, and study the related molecular mechanisms and explore their potentials as a novel antiviral target. My research team has a track record in the areas of RNA virus replicon, host factors and antivirals. We are looking forward to a collaboration with Iranian scientists who are interested in these projects.
8	We examine the molecular basis of SARS-CoV-2 RNA replication by determining the cryo-EM structures of the stalled pre-/post- translocated polymerase catalytic complexes. The structures show notable structural rearrangements occurring to nsp12 and its cofactors nsp7/nsp8 to accommodate the nucleic acid compared to the apo complex, while there are highly conserved residues in nsp12 positioning the template and primer for an in-line attack on the incoming nucleotide. Furthermore, we investigate the inhibition mechanisms of the triphosphate metabolite of remdesivir through structural and kinetic analyses. A transition model from the nsp7-nsp8 hexadecameric primase complex to the nsp12-nsp7-nsp8 polymerase complex is also proposed to provide clues for the understanding of the coronavirus transcription/replication machinery. The related work has been published in Cell (Wang et al., Structural Basis for RNA Replication by the SARS-CoV-2 Polymerase, Cell (2020), https:// doi.org/10.1016/j.cell.2020.05.034).	By taking advantage of the already established SARS-CoV-2 polymerase assays, we seek to test the anti-SARS-CoV-2 activity of various nucleotide analogs, to understand their intervention mechanisms, and to develop novel nucleotide analogs as anti-CoV drugs.
9	Evaluated the effects of multiple chemical products on pregaration of 2019-nCov on cell level, those products include natural products, small molecules, and bacterial secondary metabolites.	Cooperation content: screening and verification of antiviral drugs. Cooperation mannor: external parties provide potential compounds, CAS side conduct antiviral activity evaluation;
10	S protein (Spike protein) is the most important pathogenic protein of the new coronavirus. It binds to the receptor on the surface of human cells and enters the cell through endocytosis for viral replication. Severe acute respiratory syndrome coronavirus (SARS-CoV) and new coronavirus (SARS-CoV-2) infect human cells via a key receptor, i.e., angiotensin converting enzyme II (ACE2). Therefore, blocking the binding of viral S protein to the ACE2 receptor of human cells is an important strategy for the research of anti-SARS-CoV-2 therapeutic drugs. In our pilot study, we constructed a 293 engineered cell line with stable expression of human ACE2, and the ACE2+ engineered cells were capable of neutralizing the SARS-CoV-2 S pseudovirus, which indicates that the ACE2 receptor on the surface of the engineered cell can effectively interact with the viral S protein and thus antagonize viral infection of human cells. This result suggests that the expression of ACE2 on engineered cells can be used as a potential therapeutic drug.	S protein (Spike protein) is the most important pathogenic protein of New Coronavirus (SARS-CoV-2). Its binding to ACE2 receptor on the surface of human cells is the key mechanism of virus invasion. This proposed project is to study a biomimetic nanovesicle with ACE2 receptor on its surface as a new biotechnology drug for neutralizing SARS-CoV-2. The core technology is to fuse and reconstruct the ACE2 high-expression engineering cell membrane and liposome to prepare the biomimetic nanovesicles characterized by the ACE2-receptor anchored membrane. The biomimetic nanovesicles will yield a long circulation effect in the body. When cruising in the body, it is conducive to binding the SARS-CoV-2 S protein through its ACE2 receptor on the surface of the membrane, thereby blocking the virus infection on the human body.
11	Currently, no specific vaccines or drugs for COVID-19 have been approved. Antibody therapeutics are important options in antiviral drug discovery due to their longer half-lives, better specificities and safety profiles compared to small molecule compounds. However, there are 66 prone-to-mutation glycosylation sites on the surface of COVID-19, twice and 6-12 times more than those of HIV and influenza viruses, which makes it a challenge to develop antibodies to directly target COVID-19. ACE2 is the shared receptor for COVID-19 and other coronavirus' Spike protein and the entry point for viral infection. Thus, to target ACE2 with antibody may block multiple coronavirus infection and also avoid drug resistance caused by viral mutation. Also, enzymatic function domain of ACE2 may not overlap with the binding site of Spike protein, which makes ACE2 a safe target. We have successfully immunized mice with ACE2 protein and constructed a phage display library of antibodies. Several hit molecules blocking the interaction between ACE2 and Spike protein were discovered by ELISA. After epitope binning and affinity measuring, a highly active antibody molecule has been obtained. Pseudo-type and wild-type COVID-19 and ACE2 catalytic function assay and ACE2 knock-in mouse model have been successfully established with collaborators. Our work has laid the necessary foundation for development of coronavirus therapeutic antibodies targeting ACE2.	We propose to collaborate with Iranian scientists in the following aspects: 1. Increasing the diversity of lead antibody molecules: antibody library of our group is mainly obtained through the mouse hybridoma technology. Increasing the phage display library of rabbit antibody or nanobody will increase the diversity of lead molecules for more and better antibodies. 2. Sharing evaluation resources with viruses: we successfully established the pseudotyped virus system, but BSL-3 laboratory is necessary for testing in wild COVID-19. We hope to optimize the resources of both sides and accelerate the development speed through collaboration. 3. Clinical trials: Antibodies with high drugability will be advanced to clinical development. At present, COVID-19 patient resources are diminishing in China, but there are more than 30,000 newly-diagnosed patients in Iran, where clinical trials can be possibly conducted.

Test Kits Development

12	Nucleic acid detection is the standard for the diagnosis of COVID-19, but antibody detection is convenient and fast, with large detection flux and short time. It can better screen the patients with mild disease and evaluate the vaccination titer, so it has irreplaceable clinical value. At present, the detection of SARS-CoV-2 antibody is mainly based on chemiluminescence and colloidal gold technology. Chemiluminescence detection method has high sensitivity and specificity, but the detection process is relatively complex, which needs professional institutions, professional equipment and professionals to complete. However, the detection method of colloidal gold is simple, convenient and rapid, and does not need special instruments, but its sensitivity is not high. In this project, a fluorescent antibody test strip for SARS-CoV-2 is designed, which can not only improve the sensitivity and accuracy of detection, but also make test quickly and conveniently. To build this approach, firstly we will modify the specific SARS-CoV-2 biological ligand molecules on the nanoparticles which can emit strong fluorescence, then disperse the modified nanoparticles in the sample cushion of the test strip, and fix the secondary antibody of the specific SARS-CoV-2 antibody in the middle of the test strip. When the serum sample to be tested is added to the sample pad at the end of the test strip, the SARS-CoV-2 antibody in the serum will combine with the biological ligand molecules on the nanoparticles. At the same time, the combined nanoparticles SARS-CoV-2 antibody complex moves to the upper part of the test strip through capillary action. When it moves to the fixed area of the secondary antibody, the complex is trapped by the secondary antibody and gathered on the detection band. At this time, the SARS-CoV-2 antibody can be determined by the intensity of fluorescence of test zone by naked eye observation on simple UV light irradiation. Current work progress: (1) we constructed EB virus antibody fluorescence test strip. This work provides a good research foundation for the development of SARS-CoV-2 antibody detection and test note. At present, we have completed the biological modification of nanoparticles, the preliminary preparation of the test strip, and the fixation of secondary antibodies. (2) In terms of the SARS-CoV-2 antibody test strip, we have completed the preparation, physical and chemical properties characterization, surface functionalization and other work of new fluorescent nanomaterials, and are carrying out the modification of biological ligands.	The cooperation includes: (1) raw material preparation: mainly including the synthesis of fluorescent nanomaterials (including various fluorescent nanoparticles and quantum dots), the selection of SARS-CoV-2 specific antigen spike glycoprotein (s protein) and nucleocapsid protein (N protein), the source determination of S protein and N protein, the source determination of SARS-CoV-2 specific antibody IgG and IgM, the source determination of secondary antibody OK, etc. (2) Test strip reaction system construction: it mainly includes the construction of test strip nitrocellulose membrane main body, the preparation of fluorescent nano material placement pad, the preparation of sample pad, the control area, the fixation of biomaterials in the detection area, etc. (3) Verify the test effect of test strip: mainly including the exploration of various test conditions, the detection of standard samples, the measurement of detection concentration, the calculation of detection limit, the detection of simulated samples and real samples, the probability calculation of false positive and false negative, etc. (4) Product forming: optimization of products, matching reagents and materials, etc. Cooperation mode: the two sides work together to complete the project. Chinese task division: be responsible for all aspects of the whole project, the application of Chinese project, the development of the overall research content, the management of the project research process, the sorting of the final materials, and the writing of the thesis. Provide AIE fluorescent nanoparticles, blank materials of test strips, etc. synthesized by the Iraqi side, and also provide finished products to the Iraqi side for testing. Iran: complete the submission and application of Iran's project, prepare fluorescent silicon shell nano materials, complete the construction of test strip reaction system, and verify the test effect of the test strip. To assist the Chinese side in sorting out and publishing scientific research results and in completing the project conclusion.
13	We have carried out a series of research work around the molecular diagnosis of COVID-19. First of all, a series of core enzymes for molecular diagnosis have been successfully developed, such as the core enzyme BST DNA polymerase and reverse transcriptase for loop mediated amplification (lamp) system, the hot start Taq DNA polymerase and anti pollution UDG enzyme for routine fluorescent quantitative PCR detection, etc. the performance of these core enzymes is close to the same products of international famous brands. On this basis, two sets of detection systems for molecular diagnosis ofCOVID-19 are established, which are based on the loop mediated isothermal amplification system and RT-PCR detection system. The sensitivity and stability of these two systems have reached a high level. In addition, we have independently developed a constant temperature amplification instrument with lamp isothermal detection system. At present, the evaluation of clinical samples and the development of detection kits are being carried out for these two systems.	We are deeply concerned about the COVID-19 situation in Iran, and we hope that our COVID-19 detection system can serve the epidemic prevention and control in Iran. Therefore, we suggest that we use our molecular diagnostic core enzyme and detection system to carry out the development and promotion of diagnostic kits. We are responsible for the R & D and evaluation of LAMP and RT-PCR detection systems, and the developed kits are clinically evaluated for registration in Iran. Once the kits are mature, we are willing to transfer these two kits formulas to Iran in the form of technology transformation, and we'd like to provide sufficient core raw materials enzymes. We wish this kind of coorperation would help Iran defeat the epidemic as soon as possible.
14	High-throughput and fast chip-based real-time digital nucleic acid detection system: The technology is based on digital loop-mediated isothermal amplification and the detection chip and reagent have been developed. 500 reagents/ chips have been produced, and 100 reference materials of COVID-19 have been tested. The limit of detection reaches 800 copies per milliliter, and the CV of quantitative result is smaller than 10% compared with reference material. The prototype of instrument has been developed with the function of sample loading, encapsulation, amplification, detection and data analysis. In addition, the industrial design of the prototype has also been completed. Digital real-time fluorescence quantitative detection module and tube real-time fluorescence quantitative module are currently being adjusted in order to extend the trial range of the instrument. At present, the detection time is less than 50 min.	China provides instruments, chips and reagents. Iran carries on sample testing and data analysis. It can also carry out R & D according to the actual situation of Iran, We can also cooperate with enterprises in Iran, as well as in R & D and achievement transfer.
15	Rapid detection box of epidemic virus: We have developed a portable box which is in compliance with the biosafety regulations for the rapid point-of-care detection of COVID-19. This portable box is totally-enclosed, and can detect COVID-19 on the spot with high precision and less time. Currently, the preparations for medical device registration (CFDA, CE etc.) are in progress. This instrument is also expected to be used for rapid point-of-care detection of HIV, dengue fever, avian influenza, African swine fever and other viroses in the future.	China provides instruments, chips and reagents. Iran carries on sample testing and data analysis. It can also carry out R & D according to the actual situation of Iran, We can also cooperate with enterprises in Iran, as well as in R & D and achievement transfer.

16	For the rapid detection of 2019 novel coronavirus (SARS-CoV-2), we have developed a series of detection kits and corresponding devices. Previously, we have developed lateral flow device and 2019-nCoV IgG/IgM Test Kit (colloidal gold method). It have been proved by clinical experiments with a detection rate of 80%, and meanwhile it can identify antibody types. On the other hand, we have developed antigen Test Kit for the detection of N protein based on fluorescence microsphere immunochromatography assay, resulting in a higher sensitivity in contrast to colloidal gold test strip. In addition, we have developed a portable filed detection system, which was composed of sample collection module, automatic inactivation module, rapid DNA amplification detection kit for COVID-19 and portable detection device. The results shown by 200 clinical samples are well proven by qPCR (100%).	To further improve and establish a more efficient and accurate diagnostic standard for COVID-19, we are able to develop a antigen detection method for COVID-19, from the perspective of sing-cell level and clinical verification to evaluate antigen expression levels, detection method and clinical application values. The collaborative research includes: (1) using single-cell sequencing technology, quantitative analysis of N and S protein levels in epithelial cells from throat swabs of COVID-19 patiens; (2) establishing highly sensitive detection methods targetting to N and S proteins of COVID-19; (3) clinical sample verification and statistical analysis were performed to evaluate the clinical value of antigen detection mehtod, and finally establish an improved diagnostic criteria for COVID-19.

Genome Sequencing

17	The 2019 Novel Coronavirus Resource (2019nCoVR) developled by Beijing Institute of Genomics (BIG), Chinese Academy of Sciences / China National Center for Bioinformation (CNCB) was issued on January 22, which is an open-accessed information resource on the novel coronavirus. As of June 15, 2019ncCoVR has intergrated 47,360 novel coronavirus genomes, 10,719 genome variants, 208 clinical cases, and 29,635 related literatures. It has served nearly 150,000 users from 173 countries/regions. The sequences have been downloaded for more than 40 million times. 2019ncCoVR is one of the major resources for the novel coronavirus, and has been linked with NCBI, ViralZone, Elsevier, UCSC Genome Browser, etc. 2019nCoVR offers virus data submission and sharing services for raw sequence reads and assembled sequences. Through a data exchange mechanism established with NCBI, data submitted to 2019nCoVR are shared with international databases. The first complete genome sequence of SARS-CoV-2 from Pakistan was released in CNCB and NCBI on March 25. A total of 150 SARS-CoV-2 RNA samples from the NIH of Pakistan was also delivered to CNCB on June 10 for genome sequencing and analysis.	Content of collaboration: To conduct joint research and analysis of SARS-CoV-2 genomes with Iran counterparts, carry out dynamic monitoring on the international spread and variation of COVID-19, study the influence of genome variants to the pathogenicity, spread, vaccines, drugs and test kits of the novel coronavirus, and try to establish a uniform standard and mechnism for data access and sharing of COVID-19. Way of collaboration: We hope that Iran counterparts could provide SARS-CoV-2 samples to us for genome sequencing, or submit local genome sequences of SARS-CoV-2 to 2019nCoVR for comparative analysis, and share the research results together.
18	**Genomic mutation spectrum of novel coronavirus populations in individual patients** Previous studies assumed the 2019 novel coronavirus genome was highly homogenous in an individual patient. Nearly ten thousand 2019-nCoV virus genome sequences have been revealed and collected in database 2019nCoVR and GISAID. In fact, the coronavirus in an individual patient is in the form of population so that its genome sequences are highly heterogeneous. Based on our in-house detection algorithm for low-frequency mutations, we investigated the mutational spectrum of 2019-nCoV from deep-sequencing data of patients. Hundreds of low-frequency (lower than 0.05) positive point mutations among virus population were detected in four patients. Further, we evaluated the effect of these mutations on the coding protein structure and analyzed the distribution and enrichment of these low-frequency mutations in the virus sequences of infected patients collected in databases such as 2019nCoVR and GISAID. These results lay a foundation for the study of mutation hotspots and possible population evolution patterns among viral genome sequences, and provide a possibility for early warning of possible mutation direction for 2019-nCoV.	**Collaborating content:** This research is based on the hypothesis that "the new coronavirus in an individual patient exists in the form of a population, and its genome pattern is highly heterogeneous". The samples of new coronavirus in multiple patients at multiple time points during disease development process will be deeply sequenced. We aim to investigate the low-frequency mutation spectrum and population heterogeneity of new coronavirus population in an individual patient. The mutation characteristics in subclones and the evolution pattern of clonal population will be revealed. This research plan would provide the possibility for early warning of the possible evolutionary direction of 2019-nCoV mutations. **Collaborating mode**: Comparative sampling is carried out from China and Iran respectively. We two parts will collaborate to study the evolution pattern and the differences of new coronavirus populations between China and Iran.
19	We have recently developed a RIC-seq (RNA in situ conformation sequencing) technology for global profiling of the in situ higher-order structures and interactions of both mRNA and noncoding RNA. RIC-seq not only accurately captures the tertiary structure of RNAs but also enables the transcriptome-wide screening of transcripts interacted with various RNAs (Cai et al. Nature, 2020). Ar present, we successfully collected virus particles from in vitro cultured Vero cells and performed high-throughput RIC-seq. RIC-seq data were highly reproducible between two biological replicates. According to the intramolecular interactions detected by RIC-seq, we proposed a heuristic algorithm to predict the secondary structure of the entire COVID-19 virus RNA genome. Taking advantage of genome conservation and variation across different coronaviruses strains, we plan to design high-efficiency single guide RNAs targeting virus genome and employ CasRx module to induce the degradation of virus RNA and repress the infectivity of COVID-19. Besides, we also collected cells infected by COVID-19 virus at different time-points and constructed RIC-seq libraries for each sample with at least two biological replicates. Next, we plan to identify the potential target transcripts interacting with the virus RNA using the intermolecular interactions in RIC-seq data. We hope these efforts could help to understand the pathological mechanisms of COVID-19 from RNA-RNA interaction and eventually provide potential therapeutic targets.	1. Collect genome sequences for different coronaviruses strains and identify conserved or frequently mutated nucleotides in the genome. 2. Based on the predicted secondary structure of COVID-19 virus RNA genome by RIC-seq, design and synthesize highly-efficient single guide RNA targeting virus genome 3. Emply CasRx module to induce the degradation of virus RNA and repress the infection. 4. Delineate the dynamics for the target profiles of COVID-19 virus during the infection process, decipher the mechanism for the pathogenicity of COVID-19 virus, and provided potential targets for clinical therapy.

Vaccine Development

20	The purpose for this research is to develop a new subunit vaccine against SARS-CoV-2 (2019nCoV) the new coronavirus, mainly for nasal immunization. The vaccine is safe, effective, easy to use and adapt to a wide range of people. Before the body generates immune response, it could attach to the nasal mucosa, effectively blocking the virus spread through the respiratory tract; once the immune response is generated in the body, a preventive effect is produced through systemic immunity response to cope with early infection and pathogen spread. We fused the receptor binding domain (RBD) of the SARS-CoV-2 S protein with the antibody IgG1 Fc fragment and obtained the SARS2-RBD-Fc fusion protein as a candidate vaccine. The vaccine can induce highly efficient neutralizing antibodies in mice by nasal immunization and intramuscular immunization. On this basis, we have applied for a national invention patent (application number: 202010316204.1), and we also conducted a preliminary evaluation of the vaccine's developability. The results showed that the vaccine protein has high yield in mammalian cell expression system, and it stable and uniform. Related technologies have been applied in the development of vaccine against Zika virus (patent application: 201811613477.1; published paper: Yang C, et al., J Biol Chem., 2019, 294(27): 10638-10648), and the immunization effect was very good. The successful case shows that the vaccine development route in this project is feasible. We hope that through in-depth research, the optimized candidate vaccine has can improved immunogenicity and could be finally applied to the clinical use.	Wuhan Institute of Virology will conduct the optimization of the vaccine, and the animal nasal immunization to finally determine the efficiency of the candidate on prevention of SARS-CoV-2 infection. Both China and Iran research team complete the preclinical research, including the pharmaceutical research and non-clinical research required for clinical trial application (including general pharmacology, efficacy, pharmacokinetics and safety evaluation); we aim to spend 1-2 years to get the Clinical Trial Permission or begin the application for Clinical Trial Permission.
21	In the development of coronavirus vaccines, recombinant protein based vaccines are especially preferred due to their specific antigen targeting and less safety concern. However, a critical weakness is the poor immunogenicity especially when a short epitope is used. Recently, we have established a ferritin nanoparticle based click vaccine technique. With this technique, we have generated a lot of vaccines. These vaccines demonstrated impressively high level, high affinity and long-lasting antibody responses against many weak immunogenic antigens. Some of the related results have been published on Nature Nanotechnology, etc. Employing this technique, we have currently constructed SARS-CoV-2 Spike RBD vaccine and coronavirus universal vaccines. In the mouse model, all of them generated impressive antibody responses. Further investigation on their in vitro neutralizing efficacy and in vivo protection against viral challenge are being planned. Further optimization of the immunogenic epitopes is required to improve the vaccine efficacy.	Collaborative partners are expected to have expertise on protein structure analysis, bioinformatics and epitope design. Or, they are expected to have recourses and expertise on coronavirus related in vitro and in vivo models.
22	Targeting the nsp12 polymerase and nsp13 helicase of SARS-CoV-2, we have established the enzymatic assay platform. By computer-based virtual screen and enzymatic assay, we have obtained several clinically-approved and under-clinic chemical compounds that can inhibit the live virus at cell level. We are still working on the action mechanism of these chemcial durgs. Hope to establish coopertation of cilnical trials of these chemical drugs, and also we can screen the new potent drugs together. We also developed a probe-based genome sequencing platform, and with this technology, we can sequence the clinical samples with low virus content, to trace the virus mutation and evolution.	Drug screen and clinical trial cooperation, and genome sequencing of the SARS-CoV-2. Development of diagonosis kit based on nuclei acid and antibody/antigen.