UniSR	**Progetto proposto in applicazione del D.M. 1061 del 10/8/2021**

PROGETTO 3

DoS: NICOLA CLEMENTI

Titolo: **Molecular characterization of SARS-CoV-2 viral variant cell infection kinetic and evaluation of their infectivity from fomites**

Curriculum: Experimental and Clinical Medicine

Link alla propria pagina personale sul sito OSR/UniSR http://www.unisr.it/k-teacher/clementi-nicola/

Contenuto del progetto (*Numero di caratteri inclusi spazi*: max 600):

The project will investigate on possible mechanisms leading to indirect transmission of respiratory viruses. Molecular characterization of infection kinetic of SARS-CoV-2 viral variants will allow a better comprehension of the non-respiratory mediated virus infection.

Conformità del progetto proposto con le richieste per i progetti su tematiche indicate all'art. 2 del D.M. 1061 del 10/08/2021 e di seguito riportate (*Numero di caratteri inclusi spazi*: max 600):

"Pertinenza del progetto di percorso dottorale in relazione alla capacità di creare un alto valore aggiunto, attraverso la valorizzazione del capitale umano, in termini di ricadute scientifiche, sociali ed economiche sul territorio azionale, favorendo opportuni modelli di ricerca e di contaminazione di conoscenze e competenze in grado di favorire lo sviluppo di prodotti e servizi innovativi ad impatto ridotto sull'ambiente, focalizzati su temi orientati alla conservazione dell'ecosistema, alla biodiversità, nonché alla riduzione degli impatti del cambiamento climatico e alla promozione di uno sviluppo sostenibile, quale contributo per promuovere la ripresa verde e il superamento degli effetti della crisi nel contesto della pandemia di COVID-19.

The COVID-19 pandemics heavily impacted on economical and social aspects of our society. Respiratory transmitted infections will always be the main scourge under this perspective even in the future. The comprehension of molecular details involved in the viral transmission will certainly have a huge social impact in the case of this and next future pandemics.

Ipotesi Sperimentale (*Numero di caratteri inclusi spazi*: max 600):

Possible non-aerosol mediated transmission of SARS-CoV-2 virus variants

Obiettivi (*Numero di caratteri inclusi spazi*: max 600):

The main goals of the project are:
1) Molecular characterization of SARS-CoV-2 variants infectivity in terms of cell infection kinetic and phenotypic evaluation of spike protein mutants
2) To evaluate the susceptibility of SARS-CoV-2 variants to available sanitizing and disinfectant solutions and better addressing the choice of chemicals effective against SARS-CoV-2 clinical isolates for the decontamination of different materials and surfaces

Studio Design (*Numero di caratteri inclusi spazi*: 2000 - 3000)*:*

The different viral variants of interest (VOI) or the different variants of concern (VOC) will be further identified and cultured *in vitro* in the BSL-3 of the Microbiology and Virology Laboratory at University Vita-Salute San Raffaele. All the viral isolates will be titrated, sequenced through Sanger and NGS whole genome sequencing approaches and used for evaluating their cell infection kinetic through quantitative *RealTime PCR* based analyses based on infection assays as well as by using fluorescence microscopy techniques. Moreover, the resistance of the different variants on different materials and, their resistance to disinfectants and chemicals used for sanitation will be also assessed through cell infection assays. In this context, functional mutations on SARS-CoV-2 isolates possibly identified during the study of viral infection kinetic or in assays aimed at defining the susceptibility of SARS-CoV-2 to materials or environment will be also described. Influenza A viruses will be used as control.

Descrizione del Progetto (*Numero di caratteri inclusi spazi*: 2000 - 3000)*:*

The whole project will be organized as follows:
1) identification of SARS-CoV-2 from clinical samples of SARS-CoV-2 infected subjects or clinical samples of COVID-19 patients

2) Isolation in BSL-3 P3 laboratory of viral isolates
3) Sanger and/or NGS whole genome sequencing of isolates carrying mutations on the Spike protein
4) Titration of viral stocks and study of cell infection kinetic on different cell lines at different time points through PCR based analyses and indirect immunofluorescence high resolution microscopy
5) Evaluation of virus infectivity on different materials of selected viral variants by including those epidemiologically relevant and those endowed with fixed mutation the viral spike
6) Evaluation of inhibitory activity of chemicals and disinfectants against very high concentration of infectious viral particles exposed on different surfaces

Metodi e modelli (*Numero di caratteri inclusi spazi*: max 600):

All the procedure with non inactivated virus will be carried out in BSL-3 P3 Lab. Virus Isolation: transport medium of the nasopharyngeal swab (COPAN's kit UTM® universal viral transport medium—COPAN) of SARS-CoV-2 infected subjects will be mixed with DMEM and supplemented with double concentration of P/S and Amphotericin B. The mixture will be added to Vero E6 cells. After 1 h adsorption at 37°C, DMEM will be removed and replaced with fresh medium. Five days post-infection (dpi) cells and supernatant will be collected, aliquoted, and stored at −80°C.Virus titration: P3 virus stocks will be titrated using both Plaque Reduction Assay (PRA, PFU/mL) and Endpoint Dilutions Assay (EDA, TCID50/mL). TCID50/mL will be calculated according to the Reed–Muench method.

Ruolo del Dottorando (*Numero di caratteri inclusi spazi*: max 600):

The student will evaluate the kinetic of cell infection of the SARS-CoV-2 variants of concern and the variant of interest (VOI) identified in the Virology laboratory in clinical samples from SARS-CoV-2 infected subjects. Secondly, the student will study the resistance of viruses on surfaces of different materials. For the molecular characterization the student will be responsible for all phenotypic assays, sequence assays and *in silico* modelling.

Skill da acquisire dal dottorando (*Numero di caratteri inclusi spazi*: max 600):

Starting from the earlier phases of the project, the student will be trained on microbiology and virology cultural and molecular techniques. In particular, the student will be introduced to BSL-2 and BSL-3 working procedures indispensable for working with microbial pathogens. Moreover, the student will be also familiar with *in vitro* laboratory techniques such as cell culture, enzyme immunoassays, FACS analysis, confocal microscopy, molecular biology techniques and virus envelope protein tailored mutagenesis. Importantly, the student will be trained to the virus isolation procedures, viral culture, viral titration and evaluation of virus infectivity *in vitro*, study of sequence and *in silico* evaluation of amino acid mutation of SARS-CoV-2 S protein. Finally, the student will use the most recent microscopy techniques available at ALEMBIC (San Raffaele) and in collaboration with Ranieri Bizzarri from Scuola Normale Superiore, Pisa

Impatto dei risultati attesi nel campo della ricerca (*Numero di caratteri inclusi spazi*: max 600):

The study of cell entry of the different SARS-CoV-2 viral variants and other respiratory viruses is of pivotal importance for defining the infectivity of the different VOC and VOI so far described and of those possibly circulating in the next future. Person to person transmission represents the better described route of transmission for SARS-CoV-2. Since the very beginning of the COVID-19 pandemic, a countless number of sanitizing and disinfectant solutions are being produced by companies worldwide. However, little is known on the role of fomites in the transmission of SARS-CoV-2. Given the high number of clinical isolates of the most relevant SARS-CoV-2 variants available in the Virology Lab at UniSR, testing the virus susceptibility to different environmental conditions, sterilizers, chemicals and gasses possibly useful for sanitization of public areas and surfaces, would be of paramount importance for optimizing resources for fighting COVID-19 pandemics and avoiding wasting money, public resources and chemicals. The use of other respiratory viruses could allow a better definition of fomite mediated infection.

Referenze (max. 15)

A spatial multi-scale fluorescence microscopy toolbox discloses entry checkpoints of SARS-CoV-2 variants in VeroE6 cells
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The efficacy of UV light-emitting technology against coronaviruses: a systematic review
Federica Chiappa, Beatrice Frascella, Giacomo Pietro Vigezzi, Matteo Moro, Luca Diamanti, Leandro Gentile, Paolo Lago, Nicola Clementi, Carlo Signorelli, Nicasio Mancini, Anna Odone Journal of Hospital Infection; https://doi.org/10.1016/j.jhin.2021.05.005

Fast inactivation of SARS-CoV-2 by UV-C and ozone exposure on different materials
Elena Criscuolo, Roberta A. Diotti, Roberto Ferrarese, Cesare Alippi, Gabriele Viscardi, Carlo Signorelli, Nicasio Mancini, Massimo Clementi, and Nicola Clementi
Emerging microbes & infections 10 (1), 206-210;
https://doi.org/10.1080/22221751.2021.1872354

Characterization of a Lineage C. 36 SARS-CoV-2 Isolate with Reduced Susceptibility to Neutralization Circulating in Lombardy, Italy
Matteo Castelli, Andreina Baj, Elena Criscuolo, Roberto Ferrarese, Roberta A Diotti, Michela Sampaolo, Federica Novazzi, Daniela Dalla Gasperina, Daniele Focosi, Davide Ferrari, Massimo Locatelli, Massimo Clementi, Nicola Clementi, Fabrizio Maggi, Nicasio Mancini. doi: https://doi.org/10.3390/v13081514

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