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

PROJECT 1

DoS: Prof. Marco E. Bianchi

Title: LNA-ASO based therapies for the treatment of SARS-CoV2 infections in pre-clinicals models

Curriculum: Basic and Applied Immunology and Oncology

Link to OSR/UniSR personal page: https://www.unisr.it/docenti/b/bianchi-marco-emilio

Context of the project (*Number of characters*, *including spaces*: max 600):

The aim of this project is to study the therapeutic effects of antiviral antisense oligonucleotides targeting SARS-CoV-2, previously predicted computationally, that will be tested *in vivo* using an infection-sensitive mouse model.

Experimental hypothesis (*Number of characters*, *including spaces*: max 600):

By designing antisense oligonucleotides (ASOs) targeting the SARS-CoV-2 genome, we should be able to alter and/or block viral replication and spread.

Objectives (*Number of characters*, *including spaces*: max 600):

Objective 1: Design ASOs that modulate the expression of SARS-CoV-2 messenger RNA and/or are capable of inducing the degradation of specific genomic elements important for the replication phase of the virus
Objective 2: Test the effectiveness of ASOs in in vitro models
Objective 3: Test the efficacy of ASOs in the hyACE2 mouse model we have previously developed

Study Design (Number of characters, *including spaces: 2.000 - 3.000):*

FOR CLINICAL PROJECT ONLY, specify:

1. *Observational (prospective, cross-sectional, or retrospective) OR- Interventional (and, if a drug will be used, indicate the Phase (I, II, III, or IV)*
2. *If a drug will be used, specify whether it has the marketing authorization (Autorizzazione In Commercio (AIC)), whether it will be used according to the AIC, or if it does not have the AIC.*
3. *If the study is not of a drug, please specify what will be studied (eg. Medical device, surgical procedure, diagnostic procedure, dietary supplement, etc.). If the study will use a medical device, specify: if it has the CE marking (marcatura CE) or it does not have the CE marking. If it has the CE marking, indicate whether it will be used according to the approved use or for a new use.*

Task 1:
We will design a maximum of 5 antisense oligonucleotides that effectively hybridize with the target sequences and activate degradation mediated by the host's RNAse H enzyme.
Task 2:
The selected ASO sequences will be tested in vitro in the HEK-293T cell line expressing hyACE2 and a fluorescent protein (GFP). GFP-encoding DNA will be engineered to express non-coding domains of SARS-CoV-2 containing the unstructured regions recognized by ASOs. The effectiveness of the latter will be evaluated by flow cytometry and real-time PCR.

Task 3:

To determine if ASOs successfully and efficiently modulate the expression of target regions we will utilize our hyACE2 mouse model of post infection SARS-CoV-2 (one strain isolated from San Raffaele and one in China). The hyACE2 mouse allows viral replication and is a model for COVID-19 disease in paucisymptomatic patients. We will measure by Rt-PCR the number of viral genomes in the lung and other tissues at the peak of the disease (day 3).
For this purpose, 2 groups of mice (20 mice per group) will be selected for the experiment and divided into treated and control groups according to the following experimental scheme:
1) hyACE2 mice treated with ASO control (scrambled)
2) ASO-treated hyACE2 mice selected in (2)

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

The COVID-19 pandemic has posed a significant burden to social, societal, as well as economic structures, globally [1]. Even today, many countries are facing new waves of contagion and the number of infections is increasing again. The ability of coronaviruses to evolve rapidly and adapt has caused the spread of more contagious variants capable in some cases of partially evading immunity, mediated by vaccines and antibodies [2]. The development of new effective and non-invasive therapeutic approaches could complement current vaccine campaign efforts, both in the short and long term.
Based on in silico models of the interaction between specific residues of the SARS-CoV-2 Spike protein and the human ACE2 receptor, encoded on exon II, III and IX of the gene, our team generated a knock-in mouse susceptible to viral infection (hyACE2 mice). In particular, murine exons II and III were replaced with equivalent human exons, leaving 99.5% of the original gene sequence intact, including regulatory elements.
The expression of the hybrid gene is measurable in the lungs, therefore hyACE2 mice are permissive to infection after intra-nasal administration of SARS-CoV-2, recapitulating the dynamics of infection in humans. In addition, the virus manages to replicate and remains in the mouse for about a week. This makes the model suitable for studying possible therapeutic approaches to counteract infection and its replication.
Based on a recently published study on the structural conformation of the SARS-CoV-2 genome [3], we have identified a subset of regulatory sequences potentially important for the replication cycle of the virus. These sequences show a high degree of “co-variation” between different coronavirus strains and display a single-stranded structure with little or no structural domains. This makes them ideal targets for antisense oligonucleotides (ASOs) that can induce efficient degradation of target RNAs. This project involves the therapeutic treatment of hyACE2 mice with LNA-ASOs, after infection with SARS-CoV-2. Both the effectiveness and the possible side effects of the treatment will be evaluated.
LNA-ASOs are particularly stable and can be easily modified to recognize different target regions of the virus. They are also relatively low-cost, so they would be of particular relevance in cases where the availability of vaccines is limited.

Method and models (*Number of characters*, *including spaces*: max 600):

FOR CLINICAL PROJECT ONLY, specify:

1. *whether or not subjects (patients and/or healthy volunteers) will be recruited*
2. *whether or not human samples will be collected from subjects (patients and/or healthy volunteers)*
3. *whether or not human samples will be stored in a Biobank (and specify which Biobank)*
4. *whether or not the human samples are already stored and available in a Biobank (specify which Biobank)*
5. *whether or not any **human samples** or data will be collected in addition to those already included in the routine standard of care? (specify the kind of samples/data and the quantity and timeline)*

*6.whether or not any **procedures** will be required in addition to those already included in the routine standard of care? (eg. Visits, laboratory exams, clinical/instrumental exams). Specify the additional procedures and the quantity and timeline).*

HyACE2 mice were generated by genetically modifying ES cells via the CRISPR/ AS9 system and subsequent homologous recombination of a donor fragment carrying the mouse-human hybrid sequence.
ASOs for the treatment of infected hyACE2 mice will be designed on poorly structured regions of the viral genome and chemically modified to increase stability (LNA). Sequences poor in CpG motifs will be selected when possible, to reduce their immunogenicity; alternatively, the cytosines present will be replaced with 5-methyl cytosine, which has been show to greatly reduce immunostimulation.

Role of the PhD student (*Number of characters*, *including spaces*: max 600):

From the experimental point of view, the PhD student will mainly deal with testing the therapeutic effect of ASOs in modulating the replication of SARS-Cov-2 both in vitro and in vivo (Objective I and II).

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

The PhD student will acquire skills in managing and manipulating the mouse model, alongside basic cellular and molecular biology skills, such as; cell culture, transfection, RNA extraction, real time PCR and basic flow cytometry.
The student will also acquire basic skills on the biomolecular and bioinformatics principles underlying the design and selection of the ASOs to be tested.

Impact of the expected results in the field of research (*Number of characters*, *including spaces*: max 600):

By combining the use of a mouse model sensitive to SARS-CoV-2 with ASOs-based technology, this study could pave the way for new therapeutic approaches to counteract SARS-CoV-2 infections.
Furthermore, the data gathered has the potential to generate a map of viral replication-related sequences.

In case of clinical research, insert time line for the "Comitato Etico" approval process.

References (max. 15)

[1] Zhou P., Yang X.-L., Wang X.-G., Hu B., Zhang L., Zhang W., Si H.-R., Zhu Y., Li B., Huang C.-L. et al. . A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579:270-273.
[2] Kuzmina A, Khalaila Y, Voloshin O, Keren-Naus A, Boehm-Cohen L, Raviv Y, Shemer-Avni Y, Rosenberg E, Taube R. SARS-CoV-2 spike variants exhibit differential infectivity and neutralization resistance to convalescent or post-vaccination sera. Cell Host Microbe. 2021 Apr 14;29(4):522-528.e2. doi: 10.1016/j.chom.2021.03.008. Epub 2021 Mar 20.
[3] Ilaria Manfredonia, Chandran Nithin, Almudena Ponce-Salvatierra, Pritha Ghosh, Tomasz K Wirecki, Tycho Marinus, Natacha S Ogando, Eric J Snijder, Martijn J van Hemert, Janusz M Bujnicki, Danny Incarnato, Genome-wide mapping of SARS-CoV-2 RNA structures identifies therapeutically-relevant elements, Nucleic Acids Research, Volume 48, Issue 22, 16 December 2020, Pages 12436-12452, https://doi.org/10.1093/nar/gkaa1053.