A CRISPR-based approach as a novel therapy against SARS-CoV-2

Research project proposal | Final thesis | Bachelor's degree in Microbiology | Mateo Piantelli Ortigosa

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BACKGROUND

- **CRISPR/Cas** technology is a powerful tool for nucleic acid editing and targeting.
- The system recognizes the pathogen genome by base pairing of CRISPR-RNA (**crRNA**).
- **RNA-targeting Cas13d** nuclease was recently discovered and has shown a robust interference activity with no off-targets in mammalian cells.
- Severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**) is a **ssRNA** virus.
- Cell entry is mediated by the interaction between **viral S protein** and **ACE2 receptor**.
- The lack of numerous antiviral strategies against COVID-19 represents an **urgent need** for an **effective solution** besides prophylaxis.

HYPOTHESIS AND OBJECTIVES

The following **hypothesis** has been set to lead the research project:

CRISPR/Cas technology is able to efficiently target and neutralize SARS-CoV-2 as a potential treatment for COVID-19.

To corroborate the hypothesis proposed, the overall **objectives** of the project are:

- To design four CRISPR-based approaches able to combat SARS-CoV-2.
- To successfully produce the pertinent constructs.
- To compare the strategies developed in terms of efficacy.

ABACAS (Antibody and Cas)

An antibody fragment (**scFv**) against viral S protein will be fused to Cas13d in form of ribonucleoprotein complex (Cas-crRNA). The recombinant protein will be expressed from plasmid pET28b-scFv-Cas13d (**Fig. 1**) in an *E. coli* BL21 expression system.

ABACAS is expected to conduct a highly specific delivery of the CRISPR system into infected cells together with the virus (**Fig. 2**).

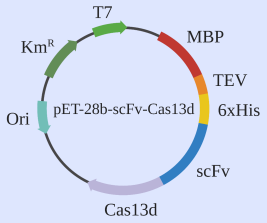


Fig. 1: Map of plasmid pET-28b-scFv-Cas13d.

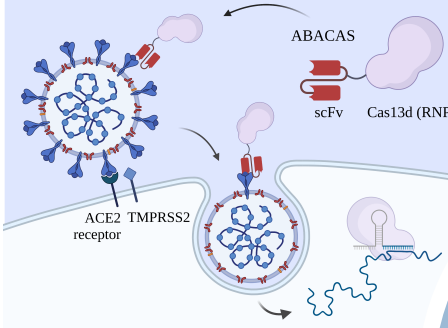


Fig. 2: Schematic representation of ABACAS delivery strategy.

CasACE2

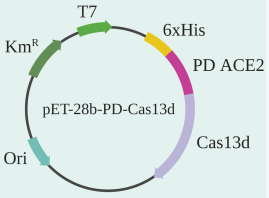


Fig. 3: Map of plasmid pET-28b-PD-Cas13d.

Delivery of Cas13d will be conducted by the interaction between the **peptidase domain (PD)** of ACE2 and the spike. An *E. coli* BL21 expression system will be employed as well, transfecting plasmid pET-28b-PD-Cas13d (**Fig. 3**).

In addition, a **neutralization** of the virus is expected for viral particles with no free spike protein due to the binding of ACE2 domain (**Fig. 4**).

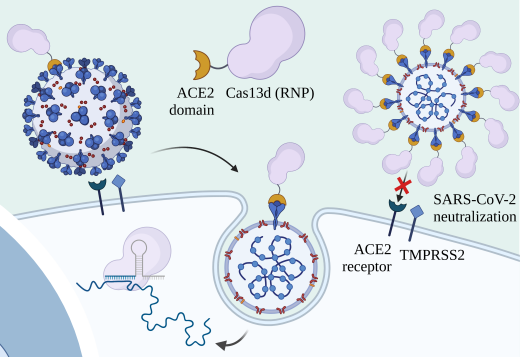
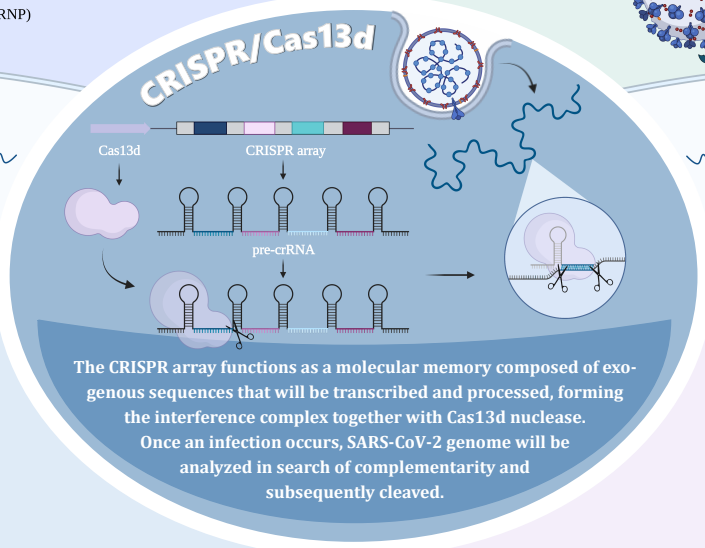


Fig. 4: Schematic representation of CasACE2 delivery strategy.



Tissue-specific AAV

Adeno-associated virus (**AAV**) are the leading platform for gene therapy because of their numerous advantages. Therefore, genes coding for Cas13d and the CRISPR array (region flanked by ITRs in pAAV-MCS, **Fig. 5**) will be delivered by AAV serotype 6, with **tissue tropism** to the airway tract (**Fig. 6**).

Moreover, CAG promoter presents better expression rates in the airway tract, **reducing off-tissue effects**.

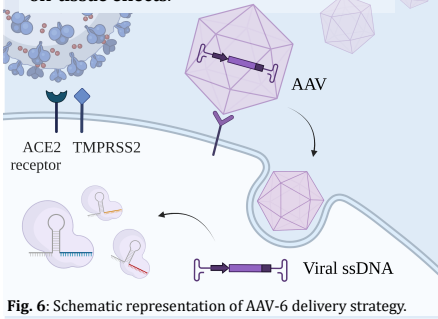


Fig. 6: Schematic representation of AAV-6 delivery strategy.

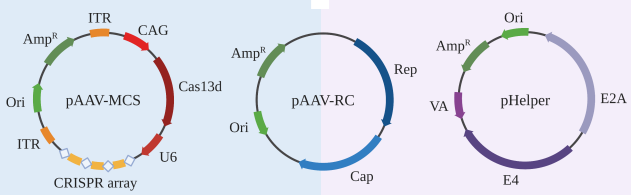


Fig. 5: Map of plasmids involved in AAV vector production. pAAV-MCS includes the genes of interest, flanked by ITRs. pAAV-RC and pHelper provide *trans*-acting genes required for viral replication. The three plasmids will be co-transfected in HEK293T cells for vector production.

AAV displaying anti-ACE2 antibody

Aiming to display an **antibody against ACE2** on the viral capsid, an IgG binding domain sequence will be inserted within amino acid 587 of *cap* gene (**Fig. 5**). IgG binding molecule will bind to the antibody via its **Fc region**.

Consequently, engineered AAV will enable the delivery of the CRISPR system into cells susceptible to be infected by SARS-CoV-2 (**Fig. 7**).

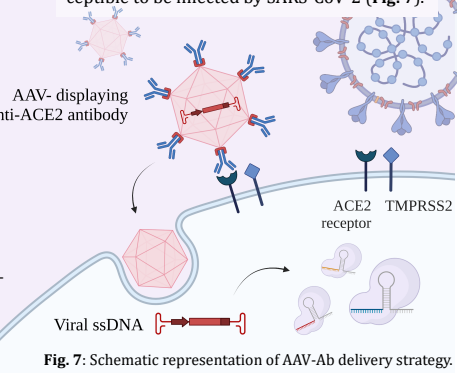


Fig. 7: Schematic representation of AAV-Ab delivery strategy.

IN VITRO ASSAYS

- Infection assays will be conducted using **human alveolar epithelial cell line A549**, stably expressing human **ACE2 receptor**.
- A reporter **nanoluciferase SARS-CoV-2 strain** (SARS-CoV-2-Nluc) will be co-plated at a MOI 0.025 with different concentrations of each CRISPR-based strategy.
- Measurment of relative luciferase signals will give an insight into the **efficacy** of the strategies proposed.

EXPECTED RESULTS

- Absent or minimal cytotoxicity effects.
- Reduction of Nluc signal in a **dose-dependent** manner.

Initial discussion of the optimal strategy from a comprehensive view.

FUTURE PERSPECTIVES

- **Dissemination** of the results.
- **Validation** via *in vivo* and subsequent clinical assays.
- Define **therapeutic indications.**
- **Further studies** about both CRISPR/Cas13d and SARS-CoV-2 are required.

REFERENCES

Carmignotto GP, Azzoni AR. On the expression of recombinant Cas9 protein in E. coli BL21(DE3) and BL21(DE3) Rosetta strains. J Biotechnol. 2019;306:62-70; Halbert CL, Lam SL, Miller AD. High-efficiency promoter-dependent transduction by adeno-associated virus type 6 vectors in mouse lung. Hum Gene Ther. 2007;18(4):344-354; Kimura T, Ferran B, Tsukahara Y, et al. Production of adeno-associated virus vectors for in vitro and in vivo applications. Sci Rep. 2019;9(1); Konermann S, Lotfy P, Brideau NJ, Oki J, Shokhirev MN, Hsu PD. Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors. Cell. 2018;173(3):665-676.e14; O'Connell MR. Molecular Mechanisms of RNA Targeting by Cas13-containing Type VI CRISPR–Cas Systems. J Mol Biol. 2019;431(1):66-87; Reche-Perez FJ, Plesselova S, De Los Reyes-Berbel E, et al. Single chain variable fragment fused to maltose binding protein: A modular nanocarrier platform for the targeted delivery of anti-tumorals. Biomater Sci. 2021;9(5):1728-1738; Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA - J Am Med Assoc. 2020;324(8):782-793; Xie X, Muruato AE, Zhang X, et al. A nanoluciferase SARS-CoV-2 for rapid neutralization testing and screening of anti-infective drugs for COVID-19. Nat Commun. 2020;11(1):1-11. Figures created with BioRender.com.