A Randomized, Controlled Study on the Safety and Efficacy of Maraviroc and/or Favipiravir vs Currently Used Therapy in Severe COVID-19 Adults. “COMVIVIR” Trial.

Adolfo Pérez-García  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0002-9103-5633

Alma Villalobos-Osnaya  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0002-0854-9407

Maria Luisa Hernández-Medel  
Hospital General de México "Dr. Eduardo Liceaga"

Lucia Monserrat Perez-Navarro  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0002-9281-9202

Elba O. Medina-Hernandez  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0002-6202-9939

Diana Sofia Cabrera-Orejuela  
Hospital General de México "Dr. Eduardo Liceaga"

Ana Maria Espinoza-Garcia  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0003-3255-4051

Helena Solleiro-Villavicencio  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0001-5137-3681

Mireya Leon-Hernandez  
Hospital General de México "Dr. Eduardo Liceaga"

Arianna Rodriguez-Cal Y Mayor  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0002-4619-2701

Manli M. Servin-Murillo  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0003-3283-8724

Jose Damian Carrillo-Ruiz  
Hospital General de México "Dr. Eduardo Liceaga"  
https://orcid.org/0000-0003-2271-0030

Raul Serrano-Loyola  
Hospital General de México "Dr. Eduardo Liceaga"

Guadalupe Mercedes Lucia Guerrero-Avendaño  
Hospital General de México "Dr. Eduardo Liceaga"

Gustavo Reyes-Teran
Method Article

Keywords: COVID-19, Maraviroc, CCR5, Favipiravir, RdRP

DOI: https://doi.org/10.21203/rs.3.rs-107427/v2

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Multiple studies have established that hyperinflammatory response induced by SARS CoV-2 is a main cause of complications and death in infected subjects. Such dysfunctional immune response has been described as a dysregulated and exacerbated production of cytokines and chemokines that attracts and activates inflammatory cells, which start and sustain pulmonary and systemic damage, thus causing complications that lead to multi organ failure and death. Therefore, we suggest that blocking key inflammation receptors could help to reduce migration and activation of T cells, monocytes/macrophages and neutrophils, thus mitigating the cytokine dysregulation and averting severe complications and death. Importantly, the optimum treatment for COVID-19 severe patients should combine a modulator of the immune response plus a direct antiviral drug against SARS-CoV-2, in order to address both the hyperinflammatory effects of the immune dysregulation and the viral load. Methods: Maraviroc (MVC), a CCR5 antagonist, and Favipiravir (FPV), an antiviral, will be evaluated single and combined, added to the treatment currently used at the Hospital General de México Dr. Eduardo Liceaga for severe COVID-19 patients. One hundred patients will be allocated in four arms [Current treatment only (CT), CT+MVC, CT+FPV, CT+MVC+FPV]. Percentage of patients free of mechanical ventilation or death at day 28, immunophenotyping and viral load will be compared between groups. Discussion: New immune focused therapies are targeting strong inflammation mediators such as IL-6 and IL1-β; nevertheless, to our best knowledge, only one study explores chemotaxis control. The use of a drug therapy that addresses both the regulation of the immune response and the inhibition of viral replication could at the same time, help to alleviate the hyperinflammatory condition and reduce the time of the viral clearance process, therefore improving treatment outcomes.

Introduction

The COVID-19 (Coronavirus Disease 2019) pandemic caused by infection of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) has caused a global mortality surrounding 3% (1). Multiple studies have found that the hyperinflammatory response induced by SARS-CoV-2 is one of the main causes of severity and death of infected subjects. In severe COVID-19 patients, an association was found between pneumonitis and/or ARDS (Acute Respiratory Distress Syndrome), high serum levels of proinflammatory cytokines, extensive lung damage and microthrombosis (2). The late stage of the disease is difficult to manage and many patients die (3,4). Based on the reports of the Chinese Disease Control Center, Cascella and cols. (5) classified the patients according to clinical severity in three groups (Table 1):
Table 1: Classification of COVID patients according to Cascella and cols. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/

| Feature | % Cases |
|---------|---------|
| **Mild Disease** | 81 |
| Mild or absent pneumonia | |
| **Severe Disease** | 14 |
| Dyspnea, Respiratory rate ≥ 30/min, Oxygen saturation (SpO₂) ≤ 93%, Kirby index (ratio between partial oxygen pressure, PaO₂ and oxygen fraction inspired, FiO₂) < 300, Pulmonary infiltrate in thorax imaging > 50%. | |
| **Critical Disease** | 5 |
| Respiratory failure | |
| Septic shock | |
| Multiple organ failure. | |

It has been proposed that the critical course of the disease that leads to complications and eventually death, is caused by an exacerbated and poorly understood immune response, linked to the phenomenon known as “cytokine storm” or cytokine release syndrome (6). Albeit it is not completely clear what initiates and propagates the cytokine storm, the severity of COVID-19, combined with rapid pandemic spread, has placed unprecedented pressure on the global healthcare system, and therapeutic strategies are urgently needed.

Pathological studies of patients lethally infected by COVID-19 had reported acute pulmonary edema, abundant infiltration of inflammatory cells, multiple organ failure, thromboembolic complications and septicemia (7,8). A better understanding of the sequence and concatenation of these events could help to devise control strategies for the disease. One of the mechanisms that could precede functional and tissue damage is the infiltration of inflammatory cells, which is known to be triggered by the release of chemokines, which are leukocyte-attracting molecules (9,10).

Gene studies in lung samples identified overexpression of CCL2 and CCL3 chemokines (11). Furthermore, Huang and cols. (12) reported that besides leukopenia and lymphopenia,
hospitalized patients had higher plasma concentrations of CCL3 and CCL4 upon admission than healthy subjects. They also mention that SARS and MERS physiopathology is outlined by an increase of proinflammatory cytokines and chemokines in serum (IL-1β, IL-6, IL-12, IFN-γ and CCL2). Previously, serum CXCL10 and CCL7 were identified as predictors of progression (13). Hence, it is of notice that the cytokine storm is accompanied by chemokine-induced migration of white cells, particularly CCL2, CCL3, CCL7 and CXCL10.

CCR5 is a G-protein-coupled chemokine receptor expressed by dendritic cells, monocytes, macrophages, natural killer (NK) cells, Th1 cells, Th17 cells and Treg cells which has multiple ligands, namely CCL3, CCL4, CCL5, CCL7 and CCL8 (14).

In respiratory diseases, it has been shown that CCR5 is involved in neutrophil recruitment to the lungs (15). In that sense, it has been observed in human subjects with chronic pulmonary inflammatory diseases that infiltrated neutrophils overexpress CCR5, induced by activation of TLRs and NOD2 (16). Neutrophils’ infiltration in pulmonary capillaries, alveolar extravasation and neutrophilic mucositis have already been observed in COVID-19. (17). Despite the precise mechanism that drives such infiltration remains unknown, it is feasible that CCR5 may play a critical role in the immunopathology of COVID-19 (18).

Along with phagocytosis and oxidative burst, neutrophils have another resource to eliminate pathogens: NETosis, a distinct form of programmed, necrotic cell death characterized by the neutrophilic release of network organized protein and DNA structures known as “neutrophil extracellular traps” (NETs), which are able to capture and entangle such pathogens (19). Though beneficial against pathogens, NETs could stimulate certain disease processes, some of them viral (20). Excessive formation of NETs could trigger a chain of inflammatory reactions that destroys surrounding tissue and facilitates microthrombosis (21). Previous reports associate aberrant formation of NETs to pulmonary disorders, namely ARDS (22). The increase in D dimer described as a severity marker in COVID-19 severe patients, could be related to NETosis, since it has an essential role in the
start and progression of thrombosis in veins and arteries (23). Hence, all these neutrophil functions could be part of both the tissue damage and microthrombosis in COVID-19.

As previously mentioned, the chemokines increased in COVID-19 severe patients are CCL3, CCL5 and CCL7. All these are CCR5 ligands; thus, our group hypothesizes that a CCR5 blockade could prevent leukocyte migration to the lung and attenuate the cytokine storm, and can be considered a therapeutic target (24). Moreover, a monoclonal antibody targeted against CCR5 (Leronlimab, also known as PRO140) was able to restore lymphocyte levels and decrease IL-6 in 10 COVID-19 patients (18).

CCR5 targeted drugs have been tested in HIV, multiple sclerosis and hepatic fibrosis clinical trials (25). One of these drugs is Maraviroc (MVC), an oral CCR5 antagonist, mainly used as an anti-retro viral that impedes binding of the gp120 viral protein to CCR5, thus avoiding viral internalization by the cells. (26). MVC has not been widely studied in the context of reduction of hyperinflammatory conditions. However, some reports have found interesting effects in modulation or resolving of general inflammatory conditions, such as reduction of cytokine expression by *in vitro* human adipocytes (27), and as an alleviating agent of hemorrhage-induced hepatic injury in rats by a PPAR-γ depending pathway (28). Furthermore, it was used in a phase II study to minimize the graft vs. host disease in bone marrow stem cell transplant in pediatric patients (29). It has also been observed that MVC decreases mucosal inflammation (30), VCAM-1 (31), T cell infiltration, neuroinflammation (32) and endothelial dysfunction (33). Regarding the lung, a model of induced hemorrhagic shock in rats reported that MVC has a protective role against pulmonary damage (34). All the aforementioned, along with the broad safety range of MVC, good tolerance an low incidence of adverse effects (35) makes it an excellent candidate to be used as a modulator of the dysregulated immune response in COVID-19.

On the other hand, an *in silico* study by Shams and cols.(36), aimed to find possible candidates for the treatment of SARS-CoV-2, and found that MVC could have a direct antiviral activity by binding to the main protease of the virus (Mpro). Altogether, this body
of evidence suggests that MVC could not only block the CCR5-dependent migration to the lung, but also reduce the viral load. This drug has been available in the market for 10 years, commercialized as Selzentry® (GSK®), and used as anti-retroviral therapy for HIV patients.

We hypothesize that an effective treatment for COVID-19 severe patients could combine a modulator of the immune response with a direct antiviral drug against SARS-CoV-2, in order to address both the hyperinflammatory effects of the immune dysregulation and the viral load, thus yielding best results. One of these antivirals is Favipiravir (FPV), that directly inhibits viral replication and transcription by selective inhibition of the viral enzyme RNA-dependent RNA polymerase (RdRP) \(^{(37,38)}\). FPV is a ribonucleotide analogue (fluorinated base analogue with a pyrazine carboxamide) \(^{(39)}\). Studies of the nucleotide addition to the elongation complex of the viral RdRP showed that FPV is capable to make RdRP pause during the reading process, which leads to backtracking (an attempt to recover reading errors), that when repetitive, causes the enzyme to interpret them as a prematurely terminated product, thus stopping the elongation and therefore interrupting the viral replication process \(^{(40)}\).

FPV has been used successfully against A H1N1 influenza \(^{(41,42)}\). Regarding COVID-19, an open randomized study in 80 mild patients found that FPV reduced the time of viral clearance by 50% compared to Lopinavir/Ritonavir with less adverse effects \(^{(43)}\); however, the study population had no risk comorbidities and subjects with SpO\(_2\) <93% were excluded. Another open randomized study in moderate patients reported FPV to be more effective in clinical recovery compared to Arbidol \(^{(44)}\). An \textit{in vitro} study found that FPV is capable to suppress the SARS-CoV-2 infection at high concentrations \(^{(45)}\). Finally, to date there are 37 studies registered in the U.S. National Library of Medicine (ClinicalTrials.gov) that evaluate FPV and 3 studies with MVC in COVID-19 patients, one of which is the present study, which noteworthy, is the only that combines both drugs.

**Design And Methods**
This open label, randomized, controlled study will be carried out in hospitalized patients at the Hospital General de México “Dr. Eduardo Liceaga” (HGMEL), located near Downtown Mexico City, Mexico. The protocol has been duly registered and approved by the Institutional Committees of Research, Ethics and Biosafety.

**Design**

Four arms will be conducted: (Figure 1)

1. **Current therapy (CT) only:** The current treatment for hospitalized COVID-19 patients at the HGMEL, consisting in enoxaparin, dexamethasone, and antibiotics if associated bacteremia is present, as per currently used at HGMEL.

2. **CT+MVC:** [CT] + [300 mg. MVC p.o. *bid* for 10 days]

3. **CT+FPV:** [CT] + [FPV p.o. for a 7-day period: 1600 mg *bid* on day 1 and 600 mg *bid* days 2-7]

4. **CT+MVC+FPV:** [CT] + [600 mg. MVC *bid* for 10 days] + [200 mg. FPV for a 7-day period. 1600 mg *bid* on day 1 and 600 mg *bid* days 2-7]

**Eligibility criteria**

**Screening:** Upon admission, screening criteria will be applied in the Emergency service to select the candidates

- Adult patients (18-70)
- Within 12 days of the appearance of symptoms
• Severe non-critical clinical stage at admission
• At least one of the following risk factors: DM, obesity (BMI>30), hypertension history or age >65
• Respiratory rate 25-34/min AND absence of other clinical signs of respiratory distress (nasal flaring, intercostal pulling, thoracoabdominal dissociation, hypoxic encephalopathy
• Respiratory rate 25-34/min
• Thorax USG with LUS >23
• O₂ saturation 90-81% (Unassisted)

**Inclusion criteria:** These will confirm the selected subjects for enrollment.

• Positive for SARS-CoV-2 confirmed by PCR
• PaFi 250-100
• LDH (Lactate dehydrogenase) >350
• >50% pulmonary infiltration as determined by thoracic imaging
• FiO₂ requirement >60% to maintain oxygenation goals
• Normal hepatic function, defined as a maximum of a fivefold increase of transaminases.
• Signed informed consent.
  ◦ Women in fertile capability must accept the use of a contraceptive method for 90 days after treatment completion.

**Exclusion criteria**

• Pregnant or lactating women
• Participating in another clinical trial
• Clinical evidence of an infectious disease different from COVID-19 at the time of admission
• Glasgow coma score <13

• Clinical signs of respiratory distress (nasal flaring, intercostal pulling, thoracoabdominal dissociation, hypoxic encephalopathy AND persisting Glasgow score ≤ 13

• Glomerular filtration rate <60ml/min/1.73m² and known history of pre-existing chronic kidney disease (Chronic kidney disease stage 3,4,5)

• Coronary disease (acute or chronic)

• Previous history of allergies to MVC or FPV

• Autoimmune disorders

• History of any previous transplant

• Under treatment with psychotropic drugs of any kind.

• Cancer of any kind

Elimination criteria

• Withdrawal of the informed consent

Endpoints

• Primary: Percentage of patients free of mechanically assisted ventilation [time frame: day 28]

• Secondary:
  ○ Percentage of patients free of mechanically assisted ventilation [Time frame: day 5]
  ○ Time of improvement in at least 2 categories in the WHO 7-category ordinal scale (46) [Time frame: day 15]
Evaluation of the response to treatment of the hyperinflammatory condition by analysis of the change rate in percentage of lymphocytes, monocytes, and neutrophils, as well as proinflammatory chemokine and cytokine levels [Time frame: day 10-1]

Sample size
The calculation was performed to compare survival curves using EPIDAT 4.2, based upon the current casuistry observed at the Infectology Department of the HGMEL, with a mechanical ventilation free survival rate in severe cases of 65% and an expected value of 80%, with a confidence level of 80%. For a 4-arm study, the following is obtained:

Sample size and power for survival curves comparison:

Groups: 4

Estimated losses: 15%
Confidence level: 80.0%

Survival probability (%)
Group 1: 65
Group 2: 75
Group 3: 75
Group 4: 80

| Power (%) | Total |
|-----------|-------|
| 80.0      | 98    |

Therefore, 25 patients will be allocated by group: 100 totals.
**Randomization**
Subjects will be randomized using EPIDAT v. 4.2.

**Statistical analysis**
Quantitative data such as demographics and basic clinical characteristics will be analyzed by comparison of means and/or medians with standard deviations and interquartile ranges (unless otherwise stated). Variables will be compared between groups, to verify the randomization process.

For the calculation of the efficacy, all those patients that completed the study with no major deviations from the foreseen results (improvement, progression to critical or death) will be considered as the Population per Protocol (PP). In order to avoid bias by the effect of termination of treatment due to causes different from the foreseen ones, the modified intention to treat (mITT) will be calculated, based on the criterion of at least one dose taken and at least one measurement after basal of the endpoints.

The primary endpoint (Percentage of patients free of mechanically assisted ventilation [time frame: day 28]) will be compared between the 4 arms using a Cochran-Mantel-Haenszel test stratified in time.

The secondary endpoints will be compared between arms as follows:

- Percentage of patients free of mechanically assisted ventilation [Time frame: day 5]: Cochran-Mantel-Haenszel test stratified in time.
- Time of improvement in at least 2 categories in the WHO 7-category ordinal scale (46) [Time frame: day 15]: Kruskal-Wallis test.
- Change rate in expression of cytokines and chemokines in serum [Time frame: day 10-1]: One-way ANOVA will be used to compare change rate of each cytokine/chemokine. Additionally, a Principal Component Analysis (PCA) will be performed for clusters identification and their relationship with treatment response.
• Change rate in the patterns of activation, trafficking and exhaustion in peripheral blood lymphocytes, monocytes and neutrophils [Time frame: day 10-1]: Data will be analyzed for subpopulations, frequencies (%) and mean fluorescence intensities (MFI) for each molecule and their interactions.
  ○ By subsets: Gating using Flowing Software
  ○ By clusters: PCA using RStudio

Data will be controlled by confounding factors (BMI, sex, hypertension, and diabetes). A Cox regression survival analysis adjusted by age, BMI, sex and comorbidities will be performed to estimate the relative risk of each of the following variables: Discharge by improvement or death, days of hospital stay and days free of ventilatory support.

IBM SPSS version 21 and RStudio will be used for the statistical analysis. A confidence interval of 95% and a significance level of $p \leq 0.05$ will be considered.

**Procedure**

Patients eligible by screening criteria will be invited to participate in the protocol. Upon acceptance, a detailed explanation will be given, and signature of an informed consent form will be requested.

If the confirmatory tests for inclusion criteria show that not all are met, or any exclusion criterion if found, the subject will be informed and will not be able to participate.

Once participation is confirmed, patients will be registered in the data collection sheet and will undergo a general clinical and medical record assessment. Samples for blood count, blood chemistry and blood gases will be collected, as well as saliva for viral load and blood for evaluation of immune status. A general approach to the study is presented in table 2

**Table 2.** Overview of the study, including time frames, eligibility, allocation of subjects and sampling
## Study Period

| Enrollment | Allocation | Post-allocation |
|------------|------------|-----------------|
| Screening period | Treatment period | Follow-up period | Close out |
| EVENT | D2 | D0 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D15 | D28 | D180 |
| Informed consent | X | | | | | | | | | | | | | |
| Screening criteria | X | | | | | | | | | | | | | |
| Inclusion criteria | X | | | | | | | | | | | | | |
| Allocation | X | | | | | | | | | | | | | |

## Intervention

| Arm A: Current Therapy (CT) | Arm B: MVC + CT | Arm C: FVP + CT | Arm D: MVC + FVP + CT |
|----------------------------|-----------------|-----------------|-----------------------|
| X | X | X | X |

## Assessments

| Variables                   | X | X | X | X | X | X | X | X |
|-----------------------------|---|---|---|---|---|---|---|---|
| Hematic biometry            |   |   |   |   |   |   |   |   |
| Electrocardiogram           |   |   |   |   |   |   |   |   |
| Viral Load                  |   |   |   |   |   |   |   |   |
| Chemokines                  |   |   |   |   |   |   |   |   |
| Cytokines                   |   |   |   |   |   |   |   |   |
| Chest x-ray                 |   |   |   |   |   |   |   |   |
| Disease progress            |   |   |   |   |   |   |   |   |
| Days free of mechanical ventilation |   |   |   |   |   |   |   |   |

NPE will be used to measure SARS-CoV-2. Blood samples will consist of 25ml: for cytokines and chemokines measurement on serum and activation and trafficking markers on leukocytes. Saliva, plasma, and leukocytes samples will be transported to the BSL3 laboratory of CIENI-INER (Research Center for Infectious Diseases at the National Respiratory Diseases Institute) for these analysis.

Participation of the subjects will end upon discharge by improvement or death. If patients progress to critical condition, tablets will be crushed and administered using a nasogastric tube.

Even in the occurrence of anticipated discharge or death, the patient’s information will be kept and processed for data analysis, as long as the patient or their relatives do not retire their consent. Likewise, participation of the subjects will be terminated before term if a
complication or adverse effect occurs that can be related to the drug under study and is ruled so by the responsible physician. If that is the case, the necessary medical support will be given to correct the adverse effect, and the patient will be kept in observation.

**Follow up**

Discharged patients will be contacted by telephone on a weekly basis until day 28. Questions will be addressed for symptoms and possible adverse effects.

The study will be considered finished when all the enrolled subjects calculated in the sample size complete their participation. The resulting data will be collected and processed by the researchers.

**Discussion**

In this study, we propose the use of MVC and/or FPV as a therapeutic resource to prevent or ameliorate the immune dysregulation, decrease the viral load and reduce complications and death in severe patients in risk of progressing to critical. The cells accountable for such dysregulation express receptors like CCR5, which mediate their trafficking to the lungs. Therefore, it is considered a therapeutic target. MVC is a CCR5 antagonist that could also have an antiviral effect, albeit it has not been evaluated in the context of COVID-19. Alongside, the results could be potentiated by the direct effect of an antiviral such as FPV.

Currently, there is not a vaccine able to effectively prevent the disease, and the pharmacologic strategies used for treatment have not proven completely effective. Thence, more studies of different therapeutic targets are needed to search for resources that help to improve the patients’ prognosis. The use of a drug therapy that addresses both the regulation of the immune response and the inhibition of viral replication could at the same time, help to alleviate the hyperinflammatory condition and reduce the time of the viral clearance process. This study is intended to compare the treatment currently used at HGMEL for COVID-19 for severe non-critical patients, which does not comprise either an inflammation modulator or an antiviral, to a strategic therapy focused on the aforementioned mechanisms of pathogenicity.

**Limitations**

1) Viral strains were not considered. 2) MVC is an expensive drug. 3) Albeit the sample size calculation was focused on reliable parameters and therefore is solid, the sample size is limited for a better
understanding of the interaction of other variables such as clinical improvement and days of hospital stay. Thence, a greater number of subjects is desirable for further studies. 4) Open studies can be potentially affected by the effect of multiple biases (e.g. placebo effect)

**Trial Status**

At the time of submission of this paper (September 2020), recruiting has not yet started. The Institutional Committees of Research, Ethics and Biosafety of the HGMEL approved the protocol on June 24, 2020 (registration number DI/20/407/04/38). Registered in NIH's Clinical Trials (https://clinicaltrials.gov) on July 17 2020, COMVIVIR NCT 04475991. Recruitment is estimated to be completed by January 2021

**Abbreviations**
| Acronym | Description |
|---------|-------------|
| ARDS   | Acute Respiratory Distress Syndrome |
| bid    | Twice a day |
| BSL-3  | Biosafety Level 3 |
| BMI    | Body Mass Index |
| CCL2   | Chemokine C-C motif 2 |
| CCL3   | Chemokine C-C motif 3 |
| CCL4   | Chemokine C-C motif 4 |
| CCL5   | Chemokine C-C motif 5 |
| CCL7   | Chemokine C-C motif 7 |
| CCL8   | Chemokine C-C motif 8 |
| CCR5   | Chemokine C-C motif 5 Receptor |
| COVID-19 | Coronavirus Disease 2019 |
| CT     | Current Treatment |
| CXCL-10 | Chemokine C-X-C motif 10 |
| DM     | Diabetes mellitus |
| DNA    | Deoxyribo nucleic Acid |
| FiO$_2$ | Fraction of Inspired Oxygen |
| FPV    | Favi piravir |
| HGMEL  | Hospital General de México “Dr. Eduardo Liceaga” |
| H1N1   | Hemagglutinin Neuraminidase Influenzavirus |
| HIV    | Human Immunodeficiency Virus |
| IFN-γ  | Interferon-γ |
| IL-6   | Interleukin 6 |
| IL-12  | Interleukin 12 |
| IL-1β  | Interleukin 1β |
| LDH    | Lactic Dehydrogenase |
| LUS    | Lung Ultrasonographic Score |
| Mpro   | Main protease |
| MVC    | Maraviroc |
| NET    | Neutrophil Extracellular Traps |
| NOD2   | NOD like receptor 2 |
| NPE    | Nasopharyngeal Exudate |
| PaFi   | Quotient between PCO$_2$ and FiO$_2$ (Kirby Index) |
| PaO$_2$ | Partial Oxygen Pressure |
| PCA    | Principal Component Analysis |
| p.o.   | Oral administration |
| PPAR-γ | Peroxisome proliferator-activated receptor gamma |
| RdRP   | RNA depending RNA Polymerase |
| RNA    | Ribonucleic Acid |
| SARS   | Severe Acute Respiratory Syndrome |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus-2 |
| SpO$_2$ | Blood oxygen saturation and pulse |
| Th1    | T Helper 1 Cells |
| Th17   | T Helper 17 Cells |
| TRL    | Toll-Like Receptors |
| USG    | Ultra sonographic imaging |
| VCAM-1 | Vascular cell adhesion protein 1 |
Declarations

Ethical approval

The protocol was originally approved by the Institutional Research Ethics Committee of the Hospital General de México “Dr. Eduardo Liceaga” on the 23rd of June 2020 (Original in Spanish and English translation attached), and assigned the registration number DI/20/407/04/38. The Ethics Committee has a valid registration in the National Commission of Bioethics (registration number CONBIOETICA-09-CEI-005-20160531). Written, informed consent to participate will be obtained from all participants.

Consent for publication

Not applicable

Availability of data and materials

All data generated from this protocol will be kept in official Hospital computers. Only the members of the staff who work at the Hospital will have access to them (APG, MLHM, LMPN, EOMH, AMEG, AVO, MLH, GMLGA, RSL, ARCM, MMSM, JHR). Data in government computers are protected by law under penalty of criminal liability.

Competing interests

All the authors declare that they have no competing interests.

Funding

The protocol will be funded by Federal Government funds as a part of the budget that the Hospital receives. Those documents are out of access to the authors. The funding party will have no involvement in the design of the study, analysis and interpretation of data, nor in writing the manuscript.

Authors’ contributions

APG is the Chief Investigator. He co-conceived the study, searched the literature, assembled the protocol, participated in the methodologic design, submitted the protocol for approval of the Committees, coordinates inter institutional cooperation, led the proposal and protocol development. MLHM is the
Medical Officer of the protocol. She co-first authored the protocol. She participated in the design and contributed to outline the outcomes and the eligibility criteria. She will also monitor patient care, keep record of adverse effects and evaluates if treatment should be continued in their occurrence. AVO participated in the design of cytometry panels and viral load tests. She co-first authored the protocol. She also contributed in the sampling scheduling and logistics. LMPN participated in the methodological and statistical design, as well as in the outlining of the outcomes. She conducted the design of a data collection tool (Project EXCELEN, currently being developed at the Hospital). EOMH and DSCO will monitor patient care, keep record of adverse effects and evaluates if treatment should be continued in their occurrence. MMSM participated in defining quality issues and internal monitoring. AMEG will coordinate the laboratory procedures for sample processing. HSV participated in the design of cytometry panels and viral load tests. MLH participated in the design of the schedule for sample collection. She designed the nursery plan to accomplish the tasks within the study. GMLGA participated in the design of the study, assignation of resources and managed national and international exchanges. RSL participated in the logistic design and assignation of resources. ARCM participated in the design of the schedule for sample collection. She will also take part in laboratory procedures for sample preparation. GRT participated in the design of the study, by proposing treatment schemes for different scenarios based upon drug interactions and pharmacokinetics. JSM participated in the design of the study, collaborated in the outlining of primary and secondary outcomes. GSMO participated in the design of the cytometry panels. He will also conduct the cytometry tasks. SAR participated in the design of the cytometry panels. JCLA outlined the statistical methodology. JHR co-conceived the study, participated in the design of cytometry panels and the logistic design. He will act as Corresponding Author.

All authors read and approved the final manuscript.

Aknowledgements

The authors wish to thank the medical staff of the Emergency Service of the Hospital General de México “Dr. Eduardo Liceaga”: Adolfo Alejandro Velasco-Medina, Benjamín Ortega-Flores, Leonor Zapata-Altamirano, Eduardo Daniel Anica-Malagón. Also to Neyla Baltazar-López from the Central Laboratory of the Hospital General de México “Dr. Eduardo Liceaga”.

Maraviroc will be donated by Glaxo Smith Kline México.

Favipiravir will be supplied by CCINSHAE

References

1. World Health Organization. WHO coronavirus disease (COVID-19) dashboard. [Internet]. 2020. Available from: https://covid19.who.int/
2. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Vol. 20, Nature Reviews Immunology. Springer US; 2020. p. 355–62.

3. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 2020 Mar 17;323(11):1061.

4. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020 May 19;17(5):533–5.

5. Cascella M, Ranjik M, Cuomo A, Dublebohn SC, Di Napoli R. Features, Evaluation and Treatment Coronavirus (COVID-19) [Internet]. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/

6. Mehta P, Mcauley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033–4.

7. Kong SL, Chui P, Lim B, Salto-Tellez M. Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. Virus Res. 2009 Nov;145(2):260–9.

8. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol Res. 2014 Aug 21;59(1–3):118–28.

9. Qi B, Fang Q, Liu S, Hou W, Li J, Huang Y, et al. Advances of CCR5 antagonists: From small molecules to macromolecules. Eur J Med Chem. 2020;208:112819.

10. Vangelista L, Vento S. The expanding therapeutic perspective of CCR5 blockade. Front Immunol. 2018;8(JAN):1–7.

11. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol. 2020 Jun;215:108427.

12. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb;395(10223):497–506.

13. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med. 2020 May 12;

14. Rautenbach A, Williams AA. Metabolomics as an Approach to Characterise the Contrasting Roles of CCR5 in the Presence and Absence of Disease. Int J Mol Sci. 2020 Feb 21;21(4):1472.

15. Grommes J, Drehслer M, Soehnlein O. CCR5 and FPR1 mediate neutrophil recruitment in endotoxin-induced lung injury. J Innate Immun. 2014;6(1):111–6.

16. Hartl D, Krauss-Etschmann S, Koller B, Hordijk PL, Kuijpers TW, Hoffmann F, et al. Infiltrated Neutrophils Acquire Novel Chemokine Receptor Expression and Chemokine Responsiveness in Chronic Inflammatory Lung Diseases. J Immunol. 2008;181(11):8053–67.

17. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med. 2020;217(6):1–7.
18. Patterson BK, Seethamraju H, Dhody K, Corley MJ, Kazempour K, Lalezari JP, et al. Disruption of the CCL5/RANTES-CCR5 Pathway Restores Immune Homeostasis and Reduces Plasma Viral Load in Critical COVID-19. medRxiv. 2020;2020.05.02.20084673.

19. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlmann Y, Weiss DS, et al. Neutrophil Extracellular Traps Kill Bacteria. Science (80- ). 2004 Mar 5;303(5663):1532 LP – 1535.

20. Schönrich G, Raftery MJ. Neutrophil Extracellular Traps Go Viral. Front Immunol. 2016;7:366.

21. Jorch SK, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease. Nat Med. 2017;23(3):279–87.

22. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. J Clin Invest. 2012 Jul 2;122(7):2661–71.

23. Fuchs TA, Brill A, Wagner DD. Neutrophil Extracellular Trap (NET) Impact on Deep Vein Thrombosis. Arterioscler Thromb Vasc Biol. 2012 Aug;32(8):1777–83.

24. Sorbera L, Graul A, Dulsat C. Taking aim at a fast-moving target: targets to watch for SARS-CoV-2 and COVID-19. Drugs Future. 2020;45(4):1–6.

25. Fantuzzi L, Tagliamonte M, Cristina M, Lucia G. Dual CCR5 / CCR2 targeting: opportunities for the cure of complex disorders. Cell Mol Life Sci. 2019;76(24):4869–86.

26. Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, et al. Maraviroc (UK-427, 857), a Potent, Orally Bioavailable, and Selective Small-Molecule Inhibitor of Chemokine Receptor CCR5 with Broad-Spectrum Anti-Human Immunodeficiency Virus Type 1 Activity. Antimicrob Agents Chemother. 2005;49(11):4721–32.

27. Díaz-Delfín J, Domingo P, Giralt M, Villarroya F. Maraviroc reduces cytokine expression and secretion in human adipose cells without altering adipogenic differentiation. Cytokine. 2013;61(3):808–15.

28. Liu F-C, Tsai Y-F, Yu H-P. Maraviroc Attenuates Trauma-Hemorrhage-Induced Hepatic Injury through PPAR Gamma-Dependent Pathway in Rats. Mohanraj R, editor. PLoS One. 2013 Oct 18;8(10):e78861.

29. Khandelwal P, Fukuda T, Teusink-Cross A, Kashuba ADM, Lane A, Mehta PA, et al. CCR5 inhibitor as novel acute graft versus host disease prophylaxis in children and young adults undergoing allogeneic stem cell transplant: results of the phase II study. Bone Marrow Transplant. 2020 Apr 9;

30. Mencarelli A, Cipriani S, Francisci D, Santucci L, Baldelli F, Distrutti E, et al. Highly specific blockade of CCR5 inhibits leukocyte trafficking and reduces mucosal inflammation in murine colitis. Sci Rep. 2016 Nov 5;6(1):30802.

31. Francisci D, Falcinelli E, Baroncelli S, Petito E, Cecchini E, Weimer LE, et al. Potential anti-inflammatory effects of maraviroc in HIV-positive patients: A pilot study of inflammation, endothelial dysfunction, and coagulation markers. Scand J Infect Dis. 2014;46(6):466–70.

32. Mondal S, Rangasamy SB, Roy A, Dasarathy S, Kordower JH, Pahan K. Low-Dose Maraviroc, an Antiretroviral Drug, Attenuates the Infiltration of T Cells into the Central Nervous System and Protects the Nigrostriatum in Hemiparkinsonian Monkeys. J Immunol. 2019;202(12):3412–22.
33. Francisci D, Pirro M, Schiaroli E, Mannarino MR, Cipriani S, Bianconi V, et al. Maraviroc Intensification Modulates Atherosclerotic Progression in HIV-Suppressed Patients at High Cardiovascular Risk. A Randomized, Crossover Pilot Study. Open Forum Infect Dis. 2019 Apr 1;6(4):1–7.
34. Liu F-C, Zheng C-W, Yu H-P. Maraviroc-Mediated Lung Protection following Trauma-Hemorrhagic Shock. Biomed Res Int. 2016;2016:1–8.
35. Abel S, Van Der Ryst E, Rosario MC, Ridgway CE, Medhurst CG, Taylor-Worth RJ, et al. Assessment of the pharmacokinetics, safety and tolerability of maraviroc, a novel CCR5 antagonist, in healthy volunteers. Br J Clin Pharmacol. 2008;65(SUPPL. 1):5–18.
36. Shamsi A, Mohammad T, Anwar S, Alajmi M, Hussain A, Rehman MT, et al. Glecaprevir and Maraviroc are high-affinity inhibitors of SARS-CoV-2 main protease: Possible therapeutic implication in COVID-19. Biosci Rep. 2020 May 22;
37. Venkatasubbaiah M, Dwarakanadha Reddy P, Satyanarayana S V. Literature-based review of the drugs used for the treatment of COVID-19. Curr Med Res Pract. 2020 May;10(3):100–9.
38. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res. 2013;100(2):446–54.
39. Janissen R, Woodman A, Lee K, Moustafa I, Huang P, Kuijpers L, et al. Induced copy-back RNA synthesis as a novel therapeutic mechanism against RNA viruses. bioRxiv. 2020;1–24.
40. Shaevitz JW, Abbondanzieri EA, Landick R, Block SM. Backtracking by single RNA polymerase molecules observed at near-base-pair resolution. Nature. 2003 Dec 23;426(6967):684–7.
41. Bank C, Renzette N, Liu P, Matuszewski S, Shim H, Foll M, et al. An experimental evaluation of drug-induced mutational meltdown as an antiviral treatment strategy. Evolution (N Y). 2016;70(11):2470–84.
42. Jensen JD, Lynch M. Considering mutational meltdown as a potential SARS-CoV-2 treatment strategy. Heredity (Edinb). 2020;124(5):619–20.
43. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering. 2020 Mar;xxxx(5):5–11.
44. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, et al. Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. medRxiv. 2020;2020.03.17.20037432.
45. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269–71.
46. World Health Organization. Novel Coronavirus COVID-19 Therapeutic Trial Symptoms. WHO R&D Blueprint. 2020.

Figures
Figure 1

General design of the study and description of the arms. CT: Currently used treatment. MVC: Maraviroc. FPV: Favipiravir