Rationale of a loading dose initiation for hydroxychloroquine treatment in COVID-19 infection in the DisCoVeRy trial

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Around the world, several dose regimens of hydroxychloroquine have been used for COVID-19 infection treatment, with the objective of identifying a short-term course. Hydroxychloroquine was found to decrease the viral replication in a concentration-dependent manner in vitro and to be more active when added prior to the viral challenge. A loading dose is used to rapidly attain a target drug concentration, which is usually considered as approximately the steady-state concentration. With a loading dose, the minimum effective concentration is reached much more rapidly than when using only the maintenance dose from the start. Thus, we propose a hydroxychloroquine sulphate dose regimen of 400 mg twice daily at Day 1 then 400 mg once daily from Day 2 to Day 10. We aim to evaluate this in the C-20-15 DisCoVeRy trial.

In France and all around the world, many different dose regimens (200 to 1200 mg daily, one to three times daily, with or without a loading dose) of hydroxychloroquine have been used and are recommended for COVID-19 infection treatment (Table 1). In many sites and countries, clinicians use dose regimens recommended for chronic autoimmune disease treatment, such as rheumatoid arthritis or systemic lupus erythematosus. The objective and exigencies of a short-term course of hydroxychloroquine treatment against COVID-19 infection are, however, quite different from the usual chronic autoimmune disease treatment. A large number of clinical trials are being initiated all around the world to evaluate the efficacy and toxicity of this drug in this infection. It is important for these trials to use hydroxychloroquine doses based on a specific scientific rationale.

Based on the severe acute respiratory distress syndrome (ARDS) reported, marked by an uncontrolled cytokine release, eradication of the novel coronavirus (SARS-CoV-2) that causes COVID-19 requires rapid drug penetration into pulmonary tissues, intracellular drug uptake and anti-inflammatory and immunomodulatory effects.

Among the different candidates for COVID-19 infection treatment, hydroxychloroquine is a racemic 4-aminoquinoline derivative, chemically related to chloroquine but less toxic in animals, used as the sulphate salt for oral administration (200 mg hydroxychloroquine sulphate is equivalent to 155 mg hydroxychloroquine base) and demonstrating favourable in vitro antiviral activity against SARS-CoV-2.

Hydroxychloroquine was found to decrease viral replication in a concentration-dependent manner in vitro [EC50 of 0.72 μM (242 ng/mL) at 48 h] and to be more active when added prior to the viral challenge. Hydroxychloroquine effectively inhibited the entry step, as well as the post-entry stages of SARS-CoV-2 infection, by changing the glycosylation of the ACE2 receptor and the spike protein.

Besides its antiviral activity, hydroxychloroquine is also known for its modulation of the immune response. In particular, hydroxychloroquine can increase the intracellular pH and inhibit lysosomal activity in antigen-presenting cells, reducing T cell activation, differentiation and expression of co-stimulatory proteins (e.g. CD154 on CD4+ T cells) and cytokines produced by T cells and B cells (e.g. IL-1, IL-6 and TNF). Hydroxychloroquine can also interrupt binding between toll-like receptors (TLR7 and TLR9) and their RNA/DNA ligands and interfere with the interaction between cytosolic DNA and the nucleic acid sensor cyclic GMP-AMP synthase, attenuating the subsequent pro-inflammatory signalling activation and production of cytokines, such as IL-1 and TNF.

Following oral dosing, hydroxychloroquine is rapidly and extensively absorbed, with a time to maximum blood concentration
Table 1. Clinical trials evaluating hydroxychloroquine in COVID-19 prevention or treatment using a loading dose

| Study identifier | Sponsor | Phase | Primary purpose | Day 1 | Maintenance daily dose | Duration of treatment |
|------------------|---------|-------|-----------------|-------|------------------------|-----------------------|
| NCT04323631      | Rambam Health Care Campus | 1     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 10 days |
| NCT04318444      | Columbia University | 2     | prevention      | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04303507      | University of Oxford | NA    | prevention      | 10 mg base/kg     | 200 mg once daily | 200 mg | 90 days |
| NCT04321616      | Oslo University Hospital | 2     | treatment       | 800 mg twice daily | 400 mg twice daily | 800 mg | 10 days |
| NCT04315948      | Institut National de la Santé Et de la Recherche Medicale, France | 3     | treatment       | 400 mg twice daily | 400 mg once daily | 400 mg | 10 days |
| NCT04304053      | Fundacio Lluita Contra la SIDA | 1     | prevention      | 800 mg once daily | 400 mg once daily | 400 mg | 7 days |
| NCT04308668      | University of Minnesota | 3     | treatment       | 1400 mg once daily | 600 mg once daily | 600 mg | 5 days |
| NCT04325893      | University Hospital, Angers | 3     | prevention      | 400 mg twice daily | 200 mg once daily | 400 mg | 8 days |
| NCT043113-21     | University of Oxford | 2/3   | treatment       | 800 mg twice daily | 400 mg twice daily | 800 mg | 10 days |
| NCT04342221      | University Hospital Tubingen | 3     | treatment       | 800 mg once daily | 600 mg once daily | 600 mg | 7 days |
| NCT04342156      | Tan Tock Seng Hospital | 3     | prevention      | 800 mg once daily | 400 mg once daily | 400 mg | 5 days |
| NCT04342169      | University of Utah | 2     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04341441      | Henry Ford Health System | 3     | prevention      | 400 mg once daily | 200 mg once daily | 200 mg | 8 weeks |
| NCT04339816      | Charles University, Czech Republic | 3     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04338906      | Heinrich-Heine University, Duesseldorf | 4     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 7 days |
| NCT04335552      | Duke University | 2     | treatment       | 800 mg once daily | 600 mg once daily | 600 mg | 5 days |
| NCT04334967      | Providence Health & Services | 4     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04334382      | Intermountain Health Care, Inc. | 3     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04332991      | Massachusetts General Hospital | 3     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04332094      | Fundación Instituto de Recerca de l’Hospital de la Santa Creu i Sant Pau | 2     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 7 days |
| NCT04330144      | Gangnam Severance Hospital | 3     | prevention      | 800 mg once daily | 400 mg once daily | 400 mg | 5 days |
| NCT04329832      | Intermountain Health Care, Inc. | 2     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04329611      | University of Calgary | 3     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| NCT04329572      | Azidus Brasil | 1     | treatment       | 400 mg twice daily | 400 mg twice daily | 400 mg | 5 days |
| ChiCTR2000029898  | Peking University Third Hospital | 4     | treatment       | 600 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| IRCT201002280    | Tehran University of Medical Sciences | 2/3   | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5 days |
| 03449N30         | Bassett Healthcare | 3     | treatment       | 400 mg twice daily | 200 mg twice daily | 400 mg | 5–14 days |
| NCT04328012      | Ayub Medical College, Abbottabad | 3     | treatment       | 600 mg twice daily | 400 mg twice daily | 800 mg | 7 days |

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achieved in approximately 4 h. Oral absorption of hydroxychloroquine sulphate tablets is not stereoselective and comparable fractions of each enantiomer are absorbed (74% of the dose for the R enantiomer compared with 77% for the S enantiomer). According to the Plaquenil label information, under normal circumstances and because they are film-coated, tablets should not be crushed or cut in half. In the exceptional case of a nasogastric tube for patients in the ICU, tablets might be crushed after removal of the film coating and dispersed slowly in water before administration. Absorption of any altered hydroxychloroquine formulation should be verified with therapeutic drug monitoring when available.

To date, the specific metabolic pathways of hydroxychloroquine are unknown and considered likely to be similar to those of chloroquine. By extrapolation, human P450 enzymes such as CYP2D6, -2C8, -3A4 and -3A5 are believed to be the major enzymes responsible for the N-desethylation of hydroxychloroquine. Whether genotypic polymorphism in these oxidative enzymes affects the antiviral efficacy or toxicity of hydroxychloroquine is still unknown.

The very long mean ± SD terminal elimination half-life of hydroxychloroquine (50±16 days) appears to be mainly due to its extensive volume of distribution (5522 L, estimated from blood data), which is consistent with the lipophilicity of the hydroxychloroquine molecule (logP = 3.84). Based on pharmacokinetic predictions in healthy volunteers, a period of 6 months on oral hydroxychloroquine sulphate 200 mg tablets once daily is required to achieve 96% steady-state concentrations of hydroxychloroquine. Elimination is predominantly via the faeces, with approximately 20%–25% of the dose excreted as unchanged drug in the urine.

A loading dose is used to rapidly attain a target drug concentration, which is usually considered as approximately the steady-state concentration. With a loading dose, the minimum effective concentration is reached much more rapidly than when using only the maintenance dose from the start. With some drugs, especially those with a large volume of distribution such as hydroxychloroquine, it may be necessary to give a large dose initially to get above the minimum effective concentration and get the beneficial effect quickly. However, the loading dose will not maintain the target

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**Table 1. Continued**

| Study identifier       | Sponsor                                                                 | Phase | Primary purpose | Day 1                  | Maintenance dose | Maintenance daily dose | Duration of treatment |
|------------------------|-------------------------------------------------------------------------|-------|----------------|------------------------|-----------------|------------------------|-----------------------|
| NCT04345861 (COVIDOC)  | University Hospital, Montpellier                                         | 2/3   | treatment      | 800 mg once daily     | 600 mg once daily | 600 mg                 | 10 days               |
| NCT04328285 (COVIDAXIS)| Centre Hospitalier Universitaire de Saint Etienne                      | 3     | prevention     | 400 mg twice daily    | 200 mg once daily | 200 mg                 | 60 days               |
| NCT04347512 (TEACHCOVID) | University Hospital, Strasbourg, France                               | 3     | treatment      | 400 mg once daily     | 200 mg once daily | 200 mg                 | 5 days                |
| NCT04345692 (OAUH-COVID19) | Queen’s Medical Centre                                                | 3     | treatment      | 400 mg twice daily    | 200 mg twice daily | 400 mg                 | 5 days                |
| NCT04346667 (PEACE)    | Government of Punjab, Specialized Healthcare and Medical Education Department | 4     | treatment      | 400 mg twice daily    | 200 mg twice daily | 400 mg                 | 5 days                |
| NCT04344444            | LCMC Health                                                             | 3     | treatment      | 400 mg twice daily    | 200 mg twice daily | 400 mg                 | 5 days                |
| NCT04341727            | Washington University School of Medicine                               | 3     | treatment      | 400 mg twice daily    | 200 mg twice daily | 400 mg                 | 5 days                |
| NCT04334148 (HERO-HCQ) | Duke University                                                          | 3     | prevention     | 600 mg twice daily    | 400 mg once daily | 400 mg                 | 30 days               |
| NCT04333654            | Sanofi                                                                   | 1     | treatment      | 800 mg + 400 mg       | 200 mg thrice daily | 600 mg                 | 10 days               |
| 2020-001270-29         | Sanofi                                                                   | 2/3   | treatment      | 800 mg + 400 mg       | 200 mg thrice daily | 600 mg                 | 10 days               |

NA, not applicable.
concentration unless an appropriate maintenance dose is also used. Theoretically, this loading dose is calculated by multiplying the desired target drug concentration by the volume of distribution of the drug.

In the case of hydroxychloroquine, the desired target concentration might be the EC50 against SARS-CoV-2 (242 ng/ml) and we used the volume of distribution (552 L) for a calculated loading dose of approximately 1336 mg at Day 1. According to the within-subject variability, this volume of distribution is very close to the apparent volume of distribution (Vss/F) estimated by Ducharme et al. for hydroxychloroquine sulphate (200 mg film-coated tablets), the loading dose might be 600 mg twice daily to approach the calculated loading dose.

Based on results obtained by simulation using a physiologically based pharmacokinetic (PBPK) model that can predict hydroxychloroquine concentrations in human lung tissues in silico, several dosing regimens for hydroxychloroquine sulphate including a loading dose were evaluated. They showed that plasma concentrations of hydroxychloroquine rapidly increased after each of the three different regimens of loading dose (800 + 400 mg versus 600 + 600 mg versus 400 + 400 mg at Day 1) followed by different maintenance doses. Moreover, the free lung tissue inhibitory quotients (IQs) for hydroxychloroquine (ratio of free lung tissue trough concentration to EC50) were calculated for the different regimens. The regimen consisting of 400 mg twice daily at Day 1 (loading dose) then 200 mg twice daily from Day 2 to Day 5 (maintenance dose) was expected to achieve a free lung IQ of 21 on the first day, 39 on the third day and 85 on the tenth day. Owing to the uncertainty surrounding the in vitro susceptibility of SARS-CoV-2, particularly in pulmonary tissue, we decided to choose the longer option of treatment, given the best in vitro and in vivo susceptibility of SARS-CoV-2, particularly in pulmonary tissue, and 154 for 10 day treatment durations.

The same maintenance dose was previously tested by Furst et al. as the daily dose indicated in active rheumatoid arthritis, where mostly gastrointestinal adverse events occurred during the first 6 weeks of treatment. According to the discontinuations for adverse effects being proportionally related to the cumulative dose (16 800 mg) in that study, the lower loading dose of 400 mg q12h and a maintenance dose of 400 mg q24h for a 10 day treatment duration (cumulative dose of 4400 mg) were chosen in the C-20-15 DisCoVeRy study (NCT04315948).

In the light of these pharmacological, virological and safety data from the literature, we believe that our hydroxychloroquine dosing regimen should be evaluated in a multicentre, adaptive, randomized clinical trial versus lopinavir/ritonavir, with or without β-1b IFN, versus remdesivir versus standard of care for the treatment of adults hospitalized with COVID-19.

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