**Dr. Balevic, *et al*, reply**

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**To the Editor:**

We read with great interest the very thoughtful commentary by Drs. Joob and Wiwanitkit. We agree with the authors that, despite conflicting clinical data to date, it is possible that HCQ may have a protective effect in the setting of COVID-19. More importantly, we agree that optimal HCQ dosage and timing is a critical underpinning for clinical trials. However, we highlight several considerations regarding treatment with high dose HCQ.

In order to optimize dosing, the relationship between drug concentration in the target tissue and response must be well characterized. Because the precise mechanism of action of HCQ for SARS-CoV-2 is unknown, current dosing strategies are based on extrapolating the in-vitro antiviral activity targets (e.g., EC50, EC100). Herein lies one of the first challenges; the reported EC50 target for inhibiting viral replication varies in different reports (0.72-17.31 uM).[1,2] Based on in-vitro EC50 targets, dosages of HCQ 400 mg orally every 12 hours for 2 doses followed by 200 mg every 12 hours to achieve target unbound lung concentrations has been proposed.[1] However, the authors relied on animal-derived lung partition coefficients for HCQ, and it was unclear how they accounted for differences in the fraction of unbound drug in tissue compared to plasma.[1] Differences in these assumptions significantly alter whether the proposed dosing would achieve target concentrations.[3] Similarly, we modeled total serum concentrations and observed that only the lowest EC50 target (0.72 uM) is achievable in serum using proposed loading doses, and all concentrations were significantly less than those needed for complete viral inhibition.[4]

Although our analysis suggests that most in-vitro target concentrations cannot be achieved in plasma and pulmonary interstitial fluid even with higher dosing, this does not preclude a potential benefit for HCQ in the setting of COVID-19. In addition to possible anti-inflammatory benefits, intracellular lung concentrations may be critical for the drug's potential antiviral effect. HCQ appears to block intracellular transport of SARS-CoV-2, with only 0.03% of virions in HCQ treated cells localizing to endolysosomes, compared to 34.3% in untreated cells.[2] Because animal studies suggest substantially higher HCQ concentrations in lung tissue compared to plasma, it is possible that antiviral target concentrations can be achieved in the lungs cells compared to interstitial fluid with conventional dosing.[2,5] However, because HCQ accumulates in lung tissue over several months,[5] the duration of therapy (as opposed to the dose) may be the key to understanding the drug's potential in COVID-19. In addition, we are aware of early reports that suggest patients with lupus and SARS-CoV-2 infection

develop severe COVID-19 at a similar frequency regardless of prior HCQ use, underscoring the importance of further study.[6]

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