

Oral Methioninase for Covid-19 Methionine-restriction Therapy

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Abstract. *The Covid-19 pandemic is a world-wide crisis without an effective therapy. While most approaches to therapy are using repurposed drugs that were developed for other diseases, it is thought that targeting the biology of the SARS-CoV-2 virus, which causes Covid-19, can result in an effective therapeutic treatment. The coronavirus RNA cap structure is methylated by two viral methyltransferases that transfer methyl groups from S-adenosylmethionine (SAM). The proper methylation of the virus depends on the level of methionine in the host to form SAM. Herein, we propose to restrict methionine availability by treating the patient with oral recombinant methioninase, aiming to treat Covid-19. By restricting methionine we not only interdict viral replication, which depends on the viral RNA cap methylation, but also inhibit the proliferation of the infected cells, which have an increased requirement for methionine. Most importantly, the virally-induced T-cell- and macrophage-mediated cytokine storm, which seems to be a significant cause for Covid-19 deaths, can also be inhibited by restricting methionine, since T-cell and macrophage activation greatly increases the methionine requirement for these cells. The evidence reviewed here suggests that oral recombinant methioninase could be a promising treatment for coronavirus patients.*

Coronavirus SARS-CoV-2 is responsible for the world-wide pandemic of Covid-19. At the present time there is no effective documented therapy. “Repurposed” drugs such as Kaletra, a mixture of lopinavir and ritonavir, used as protease inhibitors for HIV and SARS, failed in a clinical trial for Covid-19 in China (1). Hydroxychloroquine, a malaria drug, also failed in a recent clinical trial in China (2). Remdesivir an adenosine analog tested for Ebola on monkeys is being tested for Covid-19 (3). The *in*

vitro activity of remdesivir and hydroxychloroquine against SARS-CoV-2 is at micro-molar concentrations, which are quite high, indicating possible toxicity *in vivo* (4). An influenza drug called Arbidol (umifenovir) was not able to improve the clinical outcome of Covid-19 patients in China (5). Arbidol is an inhibitor that blocks the fusion of the influenza virus with the cell and is active against coronavirus *in vitro* (5). Tocilizumab (6), which targets the IL-6 receptor, is being tested for Covid-19-induced cytokine storm. A mesenchymal stem-cell therapy is also being tested for Covid-19 (7).

SARS-CoV-2 is a positive single-stranded RNA virus which uses its genomic RNA both for translation and replication (8-11). For proper RNA replication and translation, the cap of the viral RNA must be methylated (8-14). It appears that two methylation sites are present in the viral RNA of coronaviruses; one site is necessary for replication and translation, and the other site possibly for the viral RNA to evade the host intracellular immunity system which would degrade the RNA if not for the cap methylation (8-14). For example, Wang *et al.* showed that a designed peptide named TP29 targeted the 2'-O-methyltransferase of coronaviruses and inhibited both the transmethylation reactions and viral replication (8). Non-structural proteins (nsp) of the coronavirus, especially nsp10, nsp14 and nsp16 appear to be RNA methyltransferases or associate proteins of the methyltransferases (10-14). Methyltransferases use S-adenosylmethionine (SAM) which is the universal methyl donor, in order to transfer a methyl group to the viral genome (8-14). Synthesis of SAM depends on the availability of methionine. If methionine is in low concentration, the viral methyltransferases cannot complete their reaction due to the lack of SAM (15). Each time a methyl group is transferred to SAM, the resulting product is the methylated molecule, as well as S-adenosylhomocysteine (SAH) (8-15). The ratio of SAM to SAH is critical for whether methyltransferase reactions can proceed; if the SAM to SAH ratio is low, methyltransferase reactions will not occur, and the coronavirus RNA will not be methylated (8-15). As a result, the virus will not be able to replicate and the viral genomes present in the cell will be susceptible to degradation (8-15). The SAM to SAH ratio depends on the availability of methionine, which is converted to SAM by the enzyme methionine adenosyl transferase (MAT) (14, 15). In addition, virally-infected cells have an increased requirement for methionine, and they cannot survive at low

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methionine levels (16). Therefore, restricting methionine availability may inhibit or eliminate the replication and translation of the virus and make it susceptible to be degraded in the host cells, as well as prevent proliferation of cells infected with SARS-CoV-2.

Another aspect of the pathology of SARS-CoV-2 is that it induces overactivation of T-cells and macrophages, resulting in a cytokine storm (17-19). A cytokine storm is one of the factors thought to be lethal in patients with a severe SARS-Cov-2 infection, especially in the lower part of the lungs (17-20). Since, upon activation, T-cells have an increased requirement for methionine (17-18), restriction of methionine may prevent or alleviate the cytokine storm.

The studies described above indicate that three important aspects of SARS-CoV-2 infection and pathogenicity require methionine. The question is how to sufficiently restrict methionine availability in the body. A low-methionine diet, such as a vegan diet can significantly lower the circulating levels of methionine (17, 18, 21). However low-methionine diets have to be extremely low in the amount of protein which can have adverse effects on patients. Previous studies in which patients were placed on a low-methionine diet, especially for cancer patients, have shown that it is difficult for them to maintain it (21). Therefore, alternative ways to lower methionine in the body are necessary.

A very promising and proven way to lower methionine in the body is by methioninase, which is an enzyme that degrades methionine (22). Our laboratory has developed methioninase over the past 30 years, which is coded by a gene originally present in the soil bacterium *Pseudomonas putida*. The gene has been cloned into *E. coli* enabling high levels of production of the enzyme. Methioninase is very robust, and highly stable even at 50°C. In a previous study, we purified and administered this enzyme to cancer patients by intravenous infusion, and found that methionine levels in the blood were lowered by approximately 300-fold (23, 24). On the other hand, long-term studies of intravenously administered methioninase in macaque monkeys have shown potential anaphylactic reactions, unless methioninase is modified by PEGylation; however, this is a difficult and expensive procedure (25).

A breakthrough occurred approximately 3 years ago when our laboratory found that methioninase could be successfully administered orally (26). Numerous studies have shown that oral methioninase is highly effective as an antitumor drug in mouse models of cancer (26, 27). Oral administration of methioninase resulted in 80% depletion of methionine within 3 h (28). Oral methioninase restricts methionine in the gut and does not enter the blood stream, thereby eliminating immunological problems (28). In a previous study, we showed that oral administration of methioninase (5 mg twice a day), as a supplement, to both healthy people and cancer patients does not cause any side effects, even after months of administration (29).

It is our intention to make methioninase available now as a supplement and in the future as a drug after clinical trials, to patients with severe Covid-19 to alleviate their acute symptoms of viral pneumonia especially, in the lower respiratory system, which make it difficult or impossible to breathe without the assistance of a ventilator. Present data suggest that up to 80% of patients with Covid-19 who have to be ventilated do not survive (1) since there is no efficient therapy.

Recently there has been much publicity about the therapeutic efficacy of hydroxychloroquine against Covid-19. However, the results of the clinical trial that took place in China suggested that hydroxychloroquine had no benefit to the patients (2) while another two very recent trials also showed no benefit (30, 31). The drug remdesivir (Gilead Sciences), which causes RNA chain termination and can be toxic, was provided on a compassionate use and may have some benefit for Covid-19 patients with assisted breathing (32). In Japan, the drug Avigan (favipiravir), which has been previously used for influenza, is now being promoted for Covid-19. Favipiravir is a purine analog that inhibits RNA polymerase, and is probably toxic (33). A randomized clinical trial of this drug for Covid-19 in China showed no improvement over Arbidol (34), which had failed in another clinical trial for Covid-19. Recently, a triple combination of interferon beta-1b, lopinavir-ritonavir and ribavirin showed moderate benefits to Covid-19 patients (35). A recent meta-study of 96,032 patients worldwide showed hydroxychloroquine and chloroquine are harmful when used for Covid-19 (36). Recently a double-blind, randomized, placebo-controlled trial of intravenous remdesivir for Covid-19 patients showed a moderate benefit of a decrease in recovery time from 15 to 11 days (37). A combination of oral methioninase and remdesivir may provide more benefit.

Conclusion

Based on the evidence reviewed here, a new therapeutic approach against Covid-19 that targets the biology of SARS-CoV-2 would be more beneficial than the use of repurposed drugs, developed for other diseases. Previous studies of oral administration of recombinant methioninase in healthy people and cancer patients have shown that it may be safely administered orally to patients without causing side effects (29). Therefore, targeting methionine with oral recombinant methioninase seems to be a promising strategy to treat Covid-19 patients. We are looking forward to promising results of methioninase treatment of Covid-19 patients.

Conflicts of Interest

None.

Authors' Contributions

RMH and QH wrote the paper.

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References

- Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Li H, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Wang J, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D and Wang C: A trial of lopinavir-ritonavir in adults hospitalized with severe COVID-19. *N Engl J Med*, 2020. PMID: 32187464. DOI: 10.1056/NEJMoa2001282
- Chen Z, Liu D, Lui Li, Liu P, Xu Q, Xia L, Ling Y, Huang D, Song S, Zhang D, Qia Z, Li T, Shen Y and Lu H: A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). *J Zhejiang Univ (Med Sci)* 49(2): 215-219, 2020. DOI: 10.3785/j.issn.1008-9292.2020.03.03
- Warren TK, Jordan R, Lo MK, Ray AS, Mackman RL, Soloveva V, Siegel D, Perron M, Bannister R, Hui HC, Larson N, Strickley R, Wells J, Stuthman KS, Van Tongeren SA, Garza NL, Donnelly G, Shurtleff AC, Retterer CJ, Gharaibeh D, Zamani R, Kenny T, Eaton BP, Grimes E, Welch LS, Gomba L, Wilhelmsen CL, Nichols DK, Nuss JE, Nagle ER, Kugelmann JR, Palacios G, Doerffler E, Neville S, Carra E, Clarke MO, Zhang L, Lew W, Ross B, Wang Q, Chun K, Wolfe L, Babusis D, Park Y, Stray KM, Trancheva I, Feng JY, Barauskas O, Xu Y, Wong P, Braun MR, Flint M, McMullan LK, Chen SS, Fearnis R, Swaminathan S, Mayers DL, Spiropoulou CF, Lee WA, Nichol ST, Cihlar T and Bavari S: Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* 531(7594): 381-385, 2016. PMID: 26934220. DOI: 10.1038/nature17180
- Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W and Xiao G: Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. *Cell Res* 30(3): 269-271, 2020. PMID: 32020029. DOI: 10.1038/s41422-020-0282-0
- Li Y, Xie Z, Lin W, Cai W, Wen C, Guan Y, Mo X, Wang J, Wang Y, Peng P, Chen X, Hong W, Deng X and Li L: An exploratory randomized, controlled study on the efficacy and safety of lopinavir/ritonavir, or arbidol treating adult patients hospitalized with mild/moderate COVID-19 (ELACOI). medRxiv, 2020. DOI: 10.1101/2020.03.19.20038984
- Schoels MM, van der Heijde D, Breedveld FC, Burmester GR, Dougados M, Emery P, Ferraccioli G, Gabay C, Gibofsky A, Gomez-Reino JJ, Jones G, Kvien TK, Murakami M, Nishimoto N and Smolen JS: Blocking the effects of interleukin-6 in rheumatoid arthritis and inflammatory rheumatic diseases: systematic literature review and meta-analysis informing a consensus statement. *Ann Rheum Dis* 72(4): 583-589, 2013. PMID: 23144446. DOI: 10.1136/annrheumdis-2012-202470
- Dooley LM, Abdalmula A, Washington EA, Kaufman C, Tudor EM, Ghosh P2, Itescu S, Kimpton WG and Bailey SR: Effect of mesenchymal precursor cells on the systemic inflammatory response and endothelial dysfunction in an ovine model of collagen-induced arthritis. *PLoS One* 10(5): e0124144, 2015. PMID: 25950840. DOI: 10.1371/journal.pone.0124144
- Wang Y, Sun Y, Wu A, Xu S, Pan R, Zeng C, Jin X, Ge X, Shi Z, Ahola T, Chen Y and Guo D: Coronavirus nsp10/nsp16 methyltransferase can be targeted by nsp10-derived peptide *in vitro* and *in vivo* to reduce replication and pathogenesis. *J Virol* 89(16): 8416-8427, 2015. PMID: 26041293. DOI: 10.1128/JVI.00948-15
- Ginalski K, Godzik A and Rychlewski L: Novel SARS unique adoMet-dependent methyltransferase. *Cell Cycle* 5(20): 2414-2416, 2006. PMID: 17102613. DOI: 10.4161/cc.5.20.3361
- Decroly E, Imbert I, Coutard B, Bouvet M, Selisko B, Alvarez K, Gorbalenya AE, Snijder EJ and Canard B: Coronavirus nonstructural protein 16 is a Cap-0 binding enzyme possessing (Nucleoside-2'-O)-methyltransferase activity. *J Virol* 82(16): 8071-8084, 2008. PMID: 18417574. DOI: 10.1128/JVI.00407-08
- Bouvet M, Debarnot C, Imbert I, Selisko B, Snijder EJ, Canard B and Decroly E: *In vitro* reconstitution of SARS-coronavirus mRNA Cap methylation. *PLoS Pathog* 6(4): e1000863, 2010. PMID: 20421945. DOI: 10.1371/journal.ppat.1000863
- Menachery VD, Yount BL Jr, Josset L, Gralinski LE, Scobey T, Agnihothram S, Katze MG and Baric RS: Attenuation and restoration of severe acute respiratory syndrome coronavirus mutant lacking 2'-O-methyltransferase activity. *J Virol* 88(8): 4251-4264, 2014. PMID: 24478444. DOI: 10.1128/JVI.03571-13
- Chen Y, Su C, Ke M, Jin X, Xu L, Zhang Z, Wu A, Sun Y, Yang Z, Tien P, Ahola T, Liang Y, Liu X and Guo D: Biochemical and structural insight into the mechanisms of SARS coronavirus RNA ribose 2'-O-methylation by nsp16/nsp 10 protein complex. *PLoS Pathog* 7(10): e1002294, 2011. PMID: 22022266. DOI: 10.1371/journal.ppat.1002294
- Aouadi W, Blanjoie A, Vasseur JJ, Debart F, Canard B and Decroly E: Binding of the methyl donor S-adenosyl-L-methionine to Middle East respiratory syndrome coronavirus 2'-O-methyltransferase nsp 16 promotes recruitment of the allosteric activator nsp10. *J Virol* 91(5): e02217-16, 2017. PMID: 28031370. DOI: 10.1128/JVI.02217-16
- Stollar V, Mensah V, Adams S and Li ML: Evolution of sindbis virus with a low-methionine-resistant phenotype is dependent both on a pre-existing mutation and on the methionine concentration in the medium. *PLoS One* 8(3): e60504, 2013. PMID: 23544150. DOI: 10.1371/journal.pone.0060504
- Koziorowska J, Mazurowa N and Tautt J: Methionine dependence of virus-infected cells. *Exp Cell Res* 190(2): 290-293, 1990. PMID: 2170157. DOI: 10.1016/0014-4827(90)90199-k
- Tang S, Li X and Locasale JW: Dietary methionine in T-cell biology and autoimmune disease. *Cell Metab* 31(2): 211-212, 2020. PMID: 32023442. DOI: 10.1016/j.cmet.2020.01.007
- Roy DG, Chen J, Mamane V, Ma EH, Muhire BM, Sheldon RD, Shorstova T, Koning R, Johnson RM, Esaulova E, Williams KS, Hayes S, Steadman M, Samborska B, Swain A, Daigneault A, Chubukov V, Roddy TP, Foulkes W, Pospisilik JA, Bourgeois-Daigneault MC, Artyomov MN, Witcher M, Krawczyk CM, Larochelle C and Jones RG: Methionine metabolism shapes T helper cell responses through regulation of epigenetic reprogramming. *Cell Metab* 31(2): 250-266, 2020. PMID: 32023446. DOI: 10.1016/j.cmet.2020.01.006
- Merad M and Martin JC: Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol* 1-8, 2020. PMID: 32376901. DOI: 10.1038/s41577-020-0331-4

- 20 Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S and Zhang Z: Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med*, 2020. PMID: 32398875. DOI: 10.1038/s41591-020-0901-9
- 21 Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, Richie JP Jr., Ciccarella A, Calcagnotto A, Mikhael PG, Mentch SJ, Liu J, Ables G, Kirsch DG, Hsu DS, Nichenametla SN and Locasale JW: Dietary methionine influences therapy in mouse cancer models and alters human metabolism. *Nature* 572(7769): 397-401, 2019. PMID: 31367041. DOI: 10.1038/s41586-019-1437-3
- 22 Hoffman RM: Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey. *Expert Opin Biol Ther* 15(1): 21-31, 2015. PMID: 2543928. DOI: 10.1517/14712598.2015.963050
- 23 Tan Y, Zavala J Sr, Xu M, Zavala J Jr and Hoffman RM: Serum methionine depletion without side effects by methioninase in metastatic breast cancer patients. *Anticancer Res* 16(6C): 3937-3942, 1996. PMID: 9042316.
- 24 Tan Y, Zavala J Sr, Han Q, Xu M, Sun X, Tan X, Tan X, Magana R, Geller J and Hoffman RM: Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. *Anticancer Res* 17(5B): 3857-3860, 1997. PMID: 9427792.
- 25 Yang Z, Wang J, Lu Q, Xu J, Kobayashi Y, Takakura T, Takimoto A, Yoshioka T, Lian C, Chen C, Zhang D, Zhang Y, Li S, Sun X, Tan Y, Yagi S, Frenkel EP and Hoffman RM: PEGylation confers greatly extended half-life and attenuated immunogenicity to recombinant methioninase in primates. *Cancer Res* 64(18): 6673-6678, 2004. PMID: 15374983. DOI: 10.1158/0008-5472.CAN-04-1822
- 26 Kawaguchi K, Miyake K, Han Q, Li S, Tan Y, Igarashi K, Kiyuna T, Miyake M, Higuchi T, Oshiro H, Zhang Z, Razmjooei S, Wangsiricharoen S, Bouvet M, Singh SR, Unno M and Hoffman RM: Oral recombinant methioninase (o-rMETase) is superior to injectable rMETase and overcomes acquired gemcitabine resistance in pancreatic cancer. *Cancer Lett* 432: 251-259, 2018. PMID: 29928962. DOI: 10.1016/j.canlet.2018.06.016
- 27 Kawaguchi K, Han Q, Li S, Tan Y, Igarashi K, Murakami K, Unno M and Hoffman RM: Efficacy of recombinant methioninase (rMETase) on recalcitrant cancer patient-derived orthotopic xenograft (PDOX) mouse models: A review. *Cells* 8(5): 410-423, 2019. PMID: 31052611. DOI: 10.3390/cells8050410
- 28 Tashiro Y, Han Q, Tan Y, Sugisawa N, Yamamoto J, Nishino H, Inubushi S, Higuchi T, Aoki T, Murakami M and Hoffman RM: Oral recombinant methioninase prevents obesity in mice on a high-fat diet. *In Vivo* 34(2): 489-494, 2020. PMID: 32111745. DOI: 10.21873/invivo.11799
- 29 Han Q, Tan Y and Hoffman RM: Oral dosing recombinant methioninase is associated with a 70% drop in PSA in a patient with bone-metastatic prostate cancer and 50% reduction in circulating methionine in a high-stage ovarian cancer patient. *Anticancer Res* 40(5): 2813-2819, 2020. PMID: 32366428. DOI: 10.21873/anticancer.14254
- 30 Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, Labella A, Manson D, Kubin C, Barr RG, Sobieszczyk ME and Schluger NW: Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med* 7: NEJMoa2012410, 2020. PMID: 32379955. DOI: 10.1056/NEJMoa2012410
- 31 Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, Weinberg P, Kirkwood J, Muse A, DeHovitz J, Blog DS, Hutton B, Holtgrave DR and Zucker HA: Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York state. *JAMA* e208630, 2020. PMID: 32392282. DOI: 10.1001/jama.2020.8630.
- 32 Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Feldt T, Green G, Green ML, Lescure FX, Nicastri E, Oda R, Yo K, Quiros-Roldan E, Studemeister A, Redinski J, Ahmed S, Bennett J, Chelliah D, Chen D, Chihara S, Cohen SH, Cunningham J, D'Arminio Monforte A, Ismail S, Kato H, Lapadula G, L'Her E, Maeno T, Majumder S, Massari M, Mora-Rillo M, Mutoh Y, Nguyen D, Verweij E, Zoufaly A, Osinusi AO, DeZure A, Zhao Y, Zhong L, Chokkalingam A, Elboudwarej E, Telep L, Timbs L, Henne I, Sellers S, Cao H, Tan SK, Winterbourne L, Desai P, Mera R, Gaggari A, Myers RP, Brainard DM, Childs R and Flanigan T: Compassionate use of remdesivir for patients with severe covid-19. *N Engl J Med*, 2020. PMID: 32275812. DOI: 10.1056/NEJMoa2007016
- 33 Abdelnabi R, Morais ATS, Leyssen P, Imbert I, Beaucourt S, Blanc H, Froeyen M, Vignuzzi M, Canard B, Neyts J and Delang L: Understanding the mechanism of the broad-spectrum antiviral activity of favipiravir (T-705): Key role of the F1 motif of the viral polymerase. *J Virol* 91(12): e00487-17, 2017. PMID: 28381577. DOI: 10.1128/JVI.00487-17
- 34 Chen C, Huang J, Yin P, Zhang Y, Cheng Z, Wu J, Chen S, Zhang Y, Chen B, Lu M, Luo Y, Zhang J and Wang X: Favipiravir versus arbidol for COVID-19: A randomized clinical trial. medRxiv, 2020. DOI: 10.1101/2020.03.17.20037432
- 35 Hung IF, Lung KC, Tso EY, Liu R, Chung TW, Chu MY, Ng YY, Lo J, Chan J, Tam AR, Shum HP, Chan V, Wu AK, Sin KM, Leung WS, Law WL, Lung DC, Sin S, Yeung P, Yip CC, Zhang RR, Fung AY, Yan EY, Leung KH, Ip JD, Chu AW, Chan WM, Ng AC, Lee R, Fung K, Yeung A, Wu TC, Chan JW, Yan WW, Chan WM, Chan JF, Lie AK, Tsang OT, Cheng VC, Que TL, Lau CS, Chan KH, To KK and Yuen KY: Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet* S0140-6736(20): 31042-31044, 2020. PMID: 32401715. DOI: 10.1016/S0140-6736(20)31042-4
- 36 Mehra M, Desai S, Ruschitzka F and Patel A: Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. *Lancet* May 22, 2020 (online first). DOI: 10.1016/S0140-6736(20)31180-6
- 37 Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh MD, Ruiz-Palacios GM, Benfield T, Fätkenheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC; ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Preliminary Report. *N Engl J Med* May 22, 2020 (online first). PMID: 32445440. DOI: 10.1056/NEJMoa200776410.1056/NEJMoa2007764

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