Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Could imiquimod (Aldara 5% cream) or other TLR7 agonists be used in the treatment of COVID-19?

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ABSTRACT

Toll-like receptor 7 is critical in recognition of single strand RNA viruses, including SARS CoV-2, and generation of anti-viral immunity. Coronaviruses evolved strategies to dampen the host immunity. Herein, we discuss the potential use of TLR7 agonists in the early stages of COVID-19 treatment.

Coronavirus Disease 2019 (COVID-19) was first described in 2019 in Wuhan city of Hubei state, China, as a pneumonia caused by a previously unknown pathogen. The International Coronavirus Study Group named the responsible virus initially as 2019-nCoV and later as SARS-CoV-2. World Health Organization (WHO), named the disease caused by SARS-CoV-2 as COVID-19 [1,2]. SARS-CoV-2 belongs to beta-coronavirus family, which also includes SARS-CoV and MERS-CoV, and is an enveloped, linear, positive strand RNA virus. Coronaviruses are zoonotic in origin and spread from human to human, causing flu-like or more severe diseases such as middle east respiratory syndrome (MERS) or severe acute respiratory syndrome (SARS). Studies showed SARS-CoV might have originated from civic cats whereas MERS-CoV might have crossed from camels to humans. Bats and pangolins have been suggested as the likely origin of SARS-CoV-2 [3,4]. There are several other coronaviruses that have not made their way to humans. Fatality rate for SARS-CoV was ~11%, for MERS ~35–50%; currently, for SARS-CoV2 is ~2% [2].

There is no specific antiviral drug for treatment of COVID-19. Currently, there is also no vaccine. The patients are treated with combined anti-viral, anti-malarial drugs and corticosteroid and interferon (IFN) β. Critical patients in the intensive care unit receive combined antivirals (oseltamivir, ganciclovir, lopinavir, ritonavir, Remdesivir), anti-malarials (chloroquine, hydroxy-chloroquine) and oxygen support and mechanical ventilation [5].

Early Type I IFN response is critical in antiviral immunity [6–10]. Production of Type I IFNs are induced by viral nucleic acids upon interaction with their respective membrane bound or cytoplasmic sensors TLR3, TLR7, TLR9, RIG-I, MDA5 and cGAS etc. Type I IFN signaling induce expression of various interferon stimulated genes (ISG) whose products allow direct inhibition of viral replication/production, presentation of viral antigens by MHC I molecules, recruitment of associated myeloid and lymphoid lineages to the site of infection and initiation of local inflammatory response [6]. Viruses in general, evolved strategies to overcome host immune responses [10]. SARS-CoV and MERS CoV escape IFN-mediated growth inhibition by preventing the induction of IFN-β [11]. This is possibly partly due to transient rather than long lasting IRF3 nuclear localization [11]. Additionally, SARS-CoV and MERS-CoV, compared with SARS-CoV-2, produce IFN antagonists, open reading frame (ORF) 3b and ORF6 which hijack the host's anti-viral response [9]. Thus, early administration of Type I IFN into mice in MERS infection models had protective effects and blunted the viral replication [7]. SARS-CoV-2, however, lacks ORF3b and have alterations in ORF6, possibly due this, SARS-CoV-2 displays dramatic sensitivity to IFNα in vitro [12]. Accordingly, IFNα2b sprays may reduce the infection rate of SARS-CoV-2 [9,13] These findings suggest that IFN-I or therapeutic approaches which will augment Type I IFNs may be used as prophylaxis against SARS-CoV-2. This notion has also been supported by the in vitro efficacy of interferon pretreatment against the virus [12], while the replication of MERS-CoV and SARS-CoV, was reported to be less sensitive to IFN-I prophylaxis owing to presence of inhibitory ORF3b, ORF6 and others [9,14–16]. Among Type I IFNs, IFN-β (IFNβ1b or IFNβ1a) appears to be more potent inhibitor of coronaviruses (SARS-CoV) compared with IFN-α [9,17,18].

On the other hand, severe COVID-19 patients have elevated levels of pro-inflammatory cytokines IL-6, IL-1β, IL-2, IL-8, IL-17, G-CSF, GM-CSF, M-CSF, IP10, MCP1, MIP1α (CCL3) and TNF-α in their sera [19,20], this burst of cytokines is defined as cytokine release syndrome (CRS) and is also common during CAR T cell therapies, macrophage activation syndrome (MAS) or hemophagocytic lymphohistiocytosis (HLH) [21–23], COVID-19 pathology mainly consists of pulmonary lesions, and thus, presents similar characteristics with interferonopathies which root from intrinsic hyperactive IFN response. Additionally, IL-6, IL-1β and GM-CSF were considered to be the major cytokines contributing to CRS. Indeed, neutralizing IL-6 (via tocilizumab) showed promise in severe COVID-19 patients [24,25], IL-1β or TNF-α blockers are considered or planned for clinical trials [19,22,23], It's yet unclear how SARS CoV-2 overcomes host immune responses collectively, and if and how it may suppress initial IFN mediated anti-viral immune response of the host which is critical for limiting destructive capacity of virus [7–10,18].

Imiquimod (IMQ) is a heterocyclic molecule that belongs to imidazoquinoline family and is a Toll-like receptor (TLR) 7 agonist [26,27]. Anti-viral and anti-tumor properties of IMQ has been defined. As topical ointment, Aldara 5% cream (of IMQ) has been approved by US Food and Drug Administration (FDA) in the treatment of genital and perianal warts, molluscum contagiosum, actinic keratosis and superficial basal cell carcinoma [26]. IMQ activates receptor bearing-antigen presenting cells, DCs (mostly plasmacytoid DCs in skin) and macrophages, induces their maturation and migration to draining lymph nodes (dLNs) [28,29]. IMQ-driven TLR signaling results in expression of

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IFN-α/β and downstream ISGs, including IFIT family members in DCs [26,27]. When applied to skin Aldara activates and mobilizes Langerhans' cells to dLNs. Additionally, IL-12, IL-23, IL-6, IL-1β or TNF-α cytokines' production is induced by its topical application. Importantly, though skin tissue has mostly IFN-α expression, both IFN-α and β are elevated in dLNs [30]. Imiquimod also activates Th1-mediated immunity and promotes cross-presentation by DCs of viral or tumor antigens [26].

IMQ has been considered as an adjuvant and antiviral molecule in the recent years. Adjuvant power of IMQ has been demonstrated in several studies against *Influenza* virus [31–33]. *Influenza* viruses are negative strand RNA viruses with potentially similar epidemic, pandemic, morbidity and mortality capacity to coronaviruses. When used as adjuvant, IMQ was effective in inducing virus specific IgG and IgM production, against inactive Influenza virus [33]. Aldara or TLR7 agonists were also very potent as an adjuvant. More importantly however, in a study by To *et al.* intranasal application of TLR7 agonist IMQ reduced peak viral replication, weight loss, pulmonary inflammation and neutrophil infiltration to lungs [34]. Prophylactic intramuscular injections of poly I:C another TLR7 ligand, or TLR4, TLR9 agonists LPS and CpG, respectively, also conferred protection of chickens against avian influenza virus (AIV) [35]. In their very recent exciting paper, Bryden *et al.* showed in a mouse model and human skin explants that topical application of Aldara at virus inoculation site (mimicking mosquito bites) protected against systemic infection of arboviruses from the Alphavirus, Flavivirus, and Orthobunyavirus genera [30].

In summary, based on presented literature above, we believe and hypothesize that Aldara 5% cream, probably other TLR7 agonists when applied early during infection as nasal, spray/cream, or topically over the chest or armpits, may prove useful in providing the initial innate immunity-mediated antiviral responses. Additionally, already FDA-approved Aldara 5% cream can be combined with more specific biologicals, IL-6 or IL-1β blockers to evoke anti-viral immunity while keeping CRS cytokines in check. We believe further clinical trials and animal studies are warranted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110202.

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