



# Selective Pressure-Free Treatments for COVID-19

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**Abstract:** The new severe acute respiratory syndrome coronavirus (SARS-CoV-2) has caused more than 40 million human infections since December 2019, when a cluster of unexplained pneumonia cases was first reported in Wuhan, China. Just a few days after the coronavirus was officially recognized, it was identified as the causative agent of this mysterious pneumonia. This paper discusses the pros and cons of antiviral drugs from the selective pressure and possible drug resistance point of view. We also address the key advantages of potential selective pressure-free treatment methods such as the use of sparsely and densely ionizing low-dose radiation (LDR). It is known that LDR has the capacity to modulate excessive inflammatory responses, regulate lymphocyte counts and control bacterial co-infections in patients with COVID-19 and different modalities. Substantial evidence shows that viruses are constantly mutating and evolving. When an antiviral immune response is unable to eliminate a virus, viral evolution is promoted. Therefore, it is of crucial importance to limit the use of antivirals/vaccines against SARS-CoV-2 when their effects on viral fitness are not fully understood. Furthermore, to limit the spread of the virus, it is essential to develop a vaccine that is available for as many people as possible. However, with the advent of vaccines or new therapies, the new situation may force the virus to evolve. Given this consideration, selective pressure-free treatments for COVID-19 are of great importance.

**Keywords:** SARS-CoV-2; COVID-19; low-dose radiation; radiotherapy; selective pressure

## 1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19 [1], like SARS-CoV and MERS-CoV, is a single-stranded RNA beta-coronavirus. To produce strands containing multiple copies of their RNA, these viruses enter host cells and replicate. The patients infected with SARS-CoV-2 show a broad spectrum of clinical symptoms. While about 40% to 45% of the infected people remain asymptomatic, some patients show mild and moderate to severe illness of the upper respiratory tract, and even fatal events such as sepsis, severe pneumonia, acute respiratory distress syndrome (ARDS) and respiratory failure. The large proportion of the people who remain asymptomatic indicates that the potential of SARS-CoV-2 for spreading silently and widely through human populations may be much greater than what was estimated previously. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a global pandemic. As of 17 November 2020, the number of COVID-19 cases exceeded 55 million worldwide, with more than

1.3 million verified deaths. Despite controversies about the origin of SARS-CoV-2, it is likely that the virus originated from bats. However, the question whether mutations allowed this jump from animals to humans, namely bats and pangolins which are believed to be the missing link for SARS-CoV-2 transmission to humans, cannot be answered, at least for now [2]. Coronaviruses, the spherical viruses coated with protein spikes, are a large family of viruses that usually cause upper respiratory tract infections. Although the virus spike (S) protein helps the virus to bind to human cells through binding to the angiotensin-converting enzyme 2 (ACE2) receptor, it can also stimulate the immune system to produce neutralizing anti-S antibodies at the same time. ACE2 is expressed in type II alveolar cells of the lungs and can severely affect the function of the lungs. To develop the most effective vaccines against rapidly spreading SARS-CoV-2, there are different methods: innovative RNA vaccines, and viral vector- or protein-based vaccines [3]. Many attempts have been focused on the SARS-CoV-2 S protein to design a possible effective vaccine to control COVID-19 [4]. In addition to the lungs, ACE2 is expressed in many organs such as the intestine, kidney, bladder, liver, heart and brain; thus, SARS-CoV-2 can damage multiple organs and cause organ failure, in particular ARDS [5–7].

The large expansion of SARS-CoV-2, high rate of cell death (through necrosis, apoptosis and pyroptosis), damage to lung epithelial and endothelial cells, vascular leakage, failure of the renin-angiotensin system, thrombosis, hyper-inflammatory responses and cytokine storm are among the major pathological events occurring in COVID-19 [8,9]. In addition to ARDS, other abnormalities such as thrombosis, coagulopathy, lung fibrosis and lymphopenia may occur in severe COVID-19 patients.

Although scientists around the globe are trying to develop novel vaccines and effective therapies to mitigate the spread of the novel coronavirus (SARS-CoV-2), reducing the pandemic impact on human life including the immediate effect on unemployment, food supplies and the local and global economy and even challenging issues such as preventing potential virus recurrence are still unsolved problems. It is well known that if a treatment is ineffective and some genomes find the opportunity to replicate, selective pressures may lead to rapid adaptation causing viral resistance. Therefore, when the treatment is not robust, it may cause an even worse pandemic than the current one. In this paper, we review and discuss literature presenting information about the selective pressure and possible drug resistance of viruses, especially SARS-CoV-2. Different published treatment methods of COVID-19 are also reviewed, and results from randomized trials with dexamethasone, remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon- $\beta$ 1a (initially with Lopinavir, later not) are presented. In summary, the results from the larger trials have unfortunately been negative and none of the tested drugs showed any major positive effect on hospitalized COVID-19 patients up to now.

## 2. The Challenging Issue of Mutations

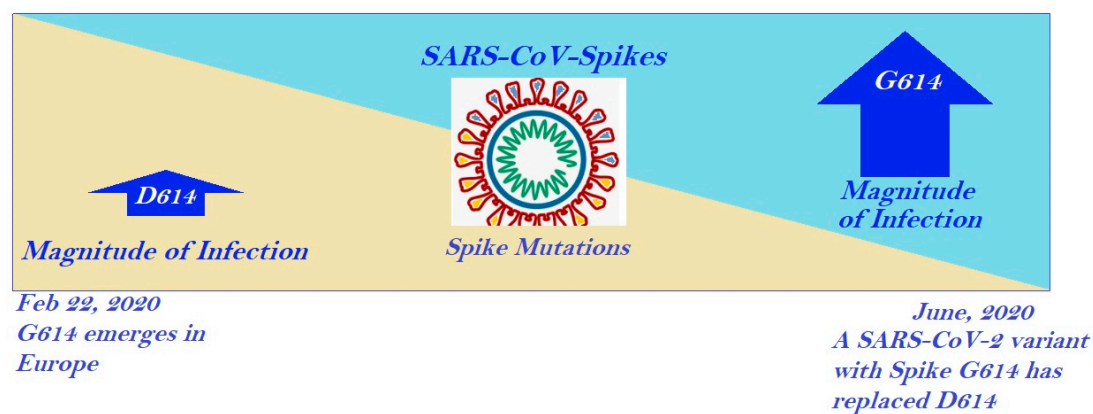
Viruses mutate constantly, and no doubt SARS-CoV-2 is not an exception. Although some mutations assist in the replication of the virus, some may prevent the process of reproduction and other mutations are neutral. The mutations that are capable of making the virus more or less virulent or transmissible can be used to trace the spread of the virus around the world. Given this consideration, only some mutations give the virus some advantage. Some mutations also have the potential to decrease the efficacy of vaccines through altering the ability of antibodies and T cells to detect pathogens. In the absence of selective pressure, viruses can remain stable in their host. However, when something exerts selective pressure on the viral populations, they can evolve rapidly. Thus, as long as the virus can spread easily within a species, it does not need to change. The widespread transmission of a certain mutation can be due to the so-called “founder effects”, in which lineages that appear early in the centers of transmission (e.g., Wuhan or Northern Italy) happen to have a mutation that is passed on when they spread to other areas [10].

## 3. Antiviral Drugs, Selective Pressure and Drug Resistance

Catanzaro et al. discussed that antiviral drugs which inhibit the replication of the virus may select for mutational escape that makes the therapy ineffective [11]. Although currently the efficacy of

different antiviral drugs, such as remdesivir, lopinavir and ritonavir, either alone or in combination, are being tested for SARS-CoV-2 in several clinical trials around the world, these drugs might render this therapeutic approach ineffective through mutational escape [12,13]. Given this consideration, by avoiding the use of antiviral drugs and focusing on the modulation of the activity of the immune responses of the host, we would be able to take the potential advantages of putting less selective pressure on viral populations [14]. It has been reported that different people infected with SARS-CoV-2 around the globe have variations in their genome and expression of the ACE2 receptor coding sequence, a factor that affects the binding ability of SARS-CoV-2. Das predicted that the variations in the ACE2 receptor exerted strong selective pressure on the circulating SARS-CoV-2 genome [15]. Therefore, the virus can be adapted to infect people with its different genetic variants [16]. The host body environment and the external environment are the two environments that can be considered as the main sources of genetic evolution of viral infections and their control [15]. A key question regarding potential vaccines against SARS-CoV-2 is how long a particular antiviral, antibody or vaccine would be effective. Some evidence shows that it strongly depends on the speed and magnitude of the evolution of the target gene or protein [17].

While in the early stages of the COVID-19 outbreak in Wuhan, China, the L type that is more aggressive and has a higher transmission rate than the S type was more prevalent (96.3% L type vs. 3.7% S type), the spread of the aggressive L type dropped after early January 2020. It is hypothesized that human intervention that caused severe selective pressure on the L type is the main reason as to why the milder type of the virus has become common due to selective pressure [18–20]. Now, some evidence shows that the strain circulating in the Western region has possibly evolved during the pandemic [21]. Moreover, in mid-June, a variant of the European type was found in Tokyo that seems to have emerged after three months of mutations. As shown in Figure 1, the rapid rise of D614G in Europe has strongly drawn scientists' attention.



**Figure 1.** The magnitude of infection of a SARS-CoV-2 variant with spike G614 that has replaced D614 in the US and Europe, which is much greater than the original strain [22,23].

Experiences in the treatment of human immunodeficiency virus (HIV) infection, that currently is primarily based on the inhibition of the viral enzymes reverse transcriptase (RT) and protease (PRO), has taught us that these compounds are not capable of fully suppressing the replication of the virus. In many patients, in the presence of the selective pressure of antiviral drugs, residual replication leads to the emergence of drug-resistant strains that finally causes therapeutic failure [24]. Over the past two decades, the advent of highly effective antiviral drugs has revolutionized the treatment of chronic viral diseases, including human immunodeficiency virus (HIV) and hepatitis B and C viruses (HBV and HCV). These drugs have been successful in substantially reducing morbidity, mortality and transmission, but unfortunately they also impose a strong selection pressure, which has led to observations of drug resistance and associated treatment failure [25].

However, in the presence of selective pressure of antiviral drugs, secondary compensatory mutations may occur that can restore the replication fitness [26]. Kupferschmidt [27] stated that scientists believe that as the novel coronavirus raced through millions of immunologically naive people, the selective pressure on the virus was not remarkable. However, this situation might change with the advent of new therapies and vaccines that force the virus to evolve [28]. The Japan Times quotes a professor at Oxford University that will be involved in the “COVID-19 Genomics U.K. Consortium”, which is a GBP 20 million (USD 23 million) project, to highlight that although viruses accumulate mutations at different rates, this process has only just started for SARS-CoV-2. “All viruses accumulate mutations over time, some faster than others, . . . For Covid-19, this has only just begun—But this emerging variation can be tracked in detail.” [28].

In mid-June, 2020, “an apparent variant of the European type was found in Tokyo that is believed to have emerged after more than three months of mutations,” according to the Japan Times. “And a virus derived from that variant was later found in many other parts of Japan” [29]. Furthermore, Reuters quoted Kurokawa, a physician that served as a science adviser to the Japanese cabinet from 2006 to 2008 and currently advises the Japanese government on the coronavirus pandemic, “I think the virus is mutating all the time... it may be a much stronger virus that triggers a second wave” [30]. The finding that within a narrow section of the genome of SARS-CoV-2 there are six fragments of the HIV genome has led to growing concerns about the source of this virus. Given this consideration, there are distinguished scientists who believe that the presence of HIV genome elements in such a small fraction (<1% of the SARS-CoV-2 genome) could not have been inserted naturally. Thus, they hypothesize that SARS-CoV-2 must have been artificially engineered. Although world-famous French newspapers and magazines such as *Le Monde* and *Le Figaro* published reports on this issue, scientists from the Pasteur Institute of France and the France National Center for Scientific Research have declined the theory that SARS-CoV-2 is man-made, as they argue that information exchange occurs frequently among viruses in nature. Scientists from other countries remained skeptical about the challenge claim that SARS-CoV-2 is man-made [31]. Andersen et al. in their paper “The proximal origin of SARS-CoV-2” that was published in *Nature Medicine* in mid-March, 2020, outlined evidence that SARS-CoV-2 is not the product of purposeful manipulation. Regarding the spike proteins on the surface of the virus, Andersen et al. believe that they are not optimally configured to bind to receptors on human cells and suggest that “natural selection” only, rather than “artificial engineering”, was involved in the spike’s binding to the human receptor ACE2. The spike protein of SARS-CoV-2 is a key mediator of viral infectivity that is needed for attachment and entry into target cells by binding the ACE2 receptor. Antiviral antibodies can be a therapeutic approach to the management of COVID-19 through targeting of these spike proteins. In a recent paper, Baum et al. [32] published a note in *Science* that stated that although antiviral antibodies can be a therapeutic approach to the management of COVID-19, antiviral therapeutics can increase the risk of acquiring drug resistance due to the rapid mutations of viruses. Baum et al. also state that when selective pressure is exerted in the setting of drug treatment, such resistance would be more remarkable [31]. Korber et al., in their paper published in *Cell*, showed that G614 (the amino acid at position 614 of the SARS-CoV-2 spike that has been changed from an aspartic acid (D) to a glycine (G) due to mutation) has become more common everywhere (Figure 1). Another paper published in *Science* states that the risk of drug resistance due to rapid mutation of viruses is a significant concern when using antiviral therapeutic agents. The authors believe that this type of resistance becomes more remarkable when there is a significant selective pressure in the setting of drug treatment [31]. The rate of mutation of a virus while it spreads through a population is a real concern. Some authors have confirmed the need to study the viral genome when new vaccines become available because these databases currently are collected from a population naive to the virus.

#### 4. Selective Pressure-Free Treatment Methods

A number of treatment methods for COVID-19 have been suggested and evaluated, in different non-randomized and randomized trials, around the world since COVID-19 was first identified 2019. In February, 2020, a WHO COVID-19 research forum recommended evaluation of treatments in large organized randomized trials [33]. In March, 2020, the most promising treatments were dexamethasone (a steroid), remdesivir (an antiviral agent), hydroxychloroquine, lopinavir (fixed-dose combination with ritonavir) and interferon- $\beta$ 1a (mainly subcutaneous; initially with lopinavir, later not), and it was decided that the effects of these treatments should be evaluated [33]. In April, National Institute of Allergy and Infectious Diseases Director Anthony S. Fauci, MD, declared that remdesivir should be the standard of care for treating COVID-19 patients [34] and in October, 2020, some media outlets in the USA were reporting that remdesivir is a miracle drug [35]. Another method, which has been discussed for the treatment of COVID-19 patients, is low-dose radiation therapy (LDRT). Below follows a short description of each method, followed by a discussion.

##### 4.1. Dexamethasone

Hydrocortisone and dexamethasone, as corticosteroids, have been known for their anti-inflammatory, antifibrotic and vasoconstrictive effects and they have been used for decades to treat patients with ARDS. Dexamethasone, which is similar to a natural hormone produced by the adrenal gland, has been tested on patients infected with SARS-CoV-2. In a clinical trial [36], performed in the United Kingdom (RECOVERY), researchers showed that administration of dexamethasone (6 mg/day orally or intravenously, for 10 days) decreased the numbers of deaths associated with COVID-19 by 35% in patients who needed intensive care and required mechanical ventilation [37]. For non-ventilated patients who were on oxygen therapy, the mortality rate was decreased by 20%. Furthermore, using dexamethasone resulted in a shorter period of hospitalization and a larger chance of discharge from hospital within the 28 days of the trial [36]. However, this advantage was only observed in seriously ill COVID-19 patients and was not seen in patients with milder levels of the disease. WHO has welcomed the findings of this trial [38]. However, clinicians should be highly advised to carefully monitor the COVID-19 patients who receive dexamethasone for any adverse effects (mostly, but not limited to, hyperglycemia, secondary infections, psychiatric effects, avascular necrosis). The COVID-19 Treatment Guidelines of the US National Institutes of Health (NIH) [39] suggest consideration of the following key points:

- Prolonged use of systemic corticosteroids may increase the risk of reactivation of latent infections (e.g., hepatitis B virus (HBV), herpesvirus infections, strongyloidiasis, tuberculosis).
- The risk of reactivation of latent infections for a 10-day course of dexamethasone (6 mg once daily) is not well defined. When initiating dexamethasone, appropriate screening and treatment to reduce the risk of *Strongyloides* hyperinfection in patients at high risk of strongyloidiasis (e.g., patients from tropical, subtropical or warm, temperate regions or those engaged in agricultural activities) or fulminant reactivations of HBV should be considered.
- Dexamethasone is a moderate cytochrome P450 (CYP) 3A4 inducer. As such, it may reduce the concentration and potential efficacy of concomitant medications that are CYP3A4 substrates. Clinicians should review a patient's medication regimen to assess potential interactions.
- Coadministration of remdesivir and dexamethasone has not been formally studied, but a clinically significant pharmacokinetic interaction is not predicted.
- Dexamethasone treatment should continue for up to 10 days or until hospital discharge, whichever comes first.

##### 4.2. Remdesivir

Based on the preliminary phase 3 trial results by Beigel et al. [40], on May 1, 2020, the US Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for emergency use of



remdesivir, which is sold under the brand name Veklury, for treatment of hospitalized patients with severe SARS-CoV-2 coronavirus disease (COVID-19) [41]. This is pursuant to Section 564 of the Act.5. Since 7 May 2020, remdesivir has also been approved in Japan [42] for patients with severe SARS-CoV-2 infection. Remdesivir was also the first drug against SARS-CoV-2 recommended for approval in the EU and was evaluated in an exceptionally short time. Remdesivir is a known broad-spectrum inhibitor of the viral RNA-dependent RNA polymerase developed by the biopharmaceutical company Gilead Sciences [43] and is administered for intravenous (IV) use [44,45]. This antiviral agent was originally developed to treat hepatitis C and was later tested against Ebola virus disease and Marburg virus disease. However, remdesivir was not found to be fully effective in these viral infections. Scientists believe that even if remdesivir is approved for treatment of severe cases of COVID-19, it is not without controversy. Shortly before remdesivir was authorized in the USA and Japan, the findings of a Chinese clinical trial by Wang et al. were published in the Lancet [46]. This trial showed that the effect of remdesivir on clinical improvement was stronger in patients with severe SARS-CoV-2 infection who started treatment within 10 days of symptom onset compared to those who started later, although it did not show significant benefits for the patients. According to the large-scale study funded by the WHO [33], remdesivir had “little or no effect” on hospitalized COVID-19 patients, from overall mortality, initiation of ventilation and duration of hospital stay points of view.

Recently, it has been suggested that the genetic backgrounds of patients might be among the reasons for controversial findings reported in the Chinese and American remdesivir clinical trials [47]. Recently, in a study [48], a gene cluster on chromosome 3 was identified that can be a risk locus for respiratory failure in people infected with SARS-CoV-2. Moreover, a different study [49] conducted on 3199 hospitalized COVID-19 patients and controls confirmed that this issue can be considered as a major genetic risk factor for severe SARS-CoV-2 infection and hospitalization. In addition, the authors of [50] report that a genomic segment of about 50 kb that is inherited from our Neanderthal ancestors and is carried by about half of the people living in South Asia and ~16% of people in Europe is a major genetic risk factor for severe SARS-CoV-2 infection. However, further studies must be performed to verify this.

#### 4.3. Hydroxychloroquine

Hydroxychloroquine was previously used as a medication to prevent and treat malaria. It was also used for treatment of rheumatoid arthritis (RA), lupus and porphyria cutanea tarda (PCT). Recent studies of hydroxychloroquine to prevent and treat COVID-19 patients have concluded that this drug is ineffective and may cause dangerous side effects [33,51–54].

#### 4.4. Lopinavir (Fixed-Dose Combination with Ritonavir)

Lopinavir is an antiretroviral drug of the protease inhibitor class that is widely used for the treatment of AIDS. Lopinavir is given together with another protease inhibitor, ritonavir (lopinavir/ritonavir available in the market under the brand names Kaletra or Aluvia). By inhibiting the metabolizing enzyme cytochrome P450 3A, ritonavir increases the half-life of lopinavir. Preliminary evidence indicates that lopinavir/ritonavir might be effective against other coronaviruses. Given this consideration, it was also considered a potential candidate for the fight against COVID-19. However, the recent findings obtained from the large-scale study funded by the WHO [33] showed that lopinavir has no significant effect on hospitalized COVID-19 patients, from the overall mortality, initiation of ventilation and duration of hospital stay points of view.

#### 4.5. Interferon- $\beta$ 1a (Mainly Subcutaneous; Initially with Lopinavir, Later Not)

Interferon- $\beta$ 1a (IFN- $\beta$ 1a) is a cytokine in the interferon family, produced by mammalian cells, used to treat multiple sclerosis (MS). It has been suggested that IFN- $\beta$ 1a may be effective as a treatment for SARS-CoV infections, but the large WHO-funded COVID-19 organized randomized trial [33] showed that IFN- $\beta$ 1a had little or no positive effect on the tested COVID-19 patients.

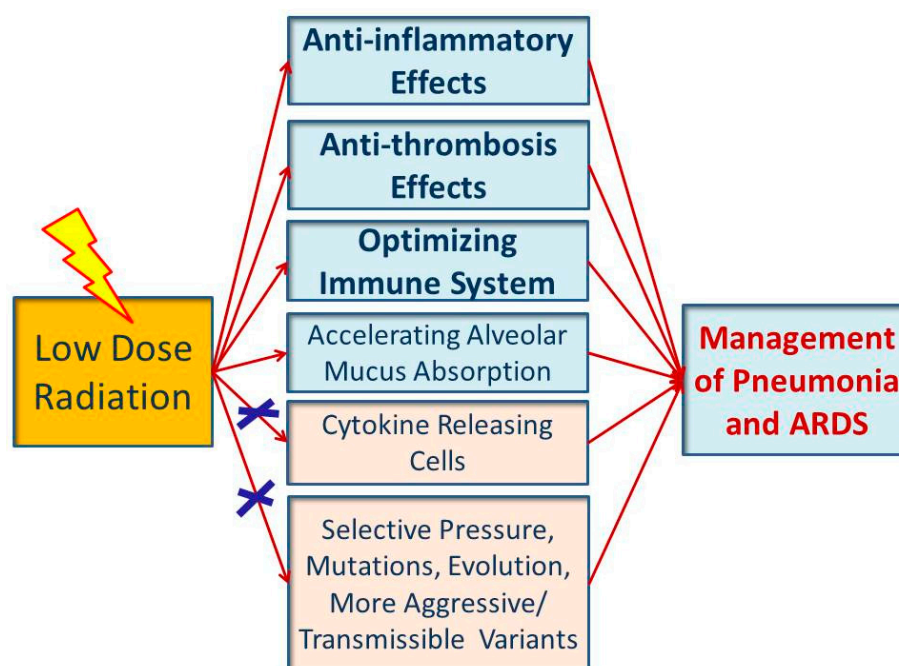
#### 4.6. Low-Dose Radiation Therapy (LDRT)

It is well known that low-dose radiation can induce anti-inflammatory responses due to its potential for inducing polarization of both M1 and M2 macrophage phenotypes [55]. The use of ionizing radiation to inactivate viruses has been studied since around 1940 [56] and there are several reports on studies of direct effects of X-rays on different types of viruses already performed during the 1950s (see, e.g., [57,58]). The history of radiation therapy for viral pneumonia dates back to 1946 [59] and since then, using low doses of ionizing radiation has been tested with promising findings for the treatment of infectious diseases like pneumonia [60,61]. In early 2020, several groups around the globe started discussing the concept of using external beam LDRT for COVID-19 [62]. These researchers mostly focused on some key bioeffects of LDRT such as anti-inflammatory and immunomodulatory effects and proposed that local irradiation of lungs with radiation doses ranging from 0.1 to 1 Gy could be beneficial for treatment of severe pneumonia in patients infected with SARS-CoV-2. The first paper by Ghadimi-Moghadam et al. [62] not only introduced LDRT as an effective treatment for pneumonia in COVID-19 patients but also discussed, in detail, the disadvantages of alternative treatment methods that were mainly based on using antiviral drugs.

Beneficial or stimulatory biological effects, after exposure to low-dose radiation, possibly through mechanisms such as stimulating the immune system, have been reported by different researchers. For example, while high doses certainly weaken the immune system, exposure to low-dose radiation can boost the immune system [63,64]. Moreover, some studies show that LDRT boosts the fundamental anti-viral immune parameters that alter the natural killer cell activity [65]. Recently, Rödel et al. [66] published an article aimed at reviewing the data regarding LDRT for treatment of COVID-19-related pneumopathy and concluded that this method is worth investigating in the current clinical situation. However, they highlighted the need for strict monitoring and disease phase-adapted treatment using lung function tests as well as clinical markers including interleukin 6 (IL-6) and serum D-dimer detection. There seems to be a large body of evidence in support of the concept of low-dose radiation-induced enhancement of the immune system [66]. Besides anti-inflammatory effects, *in vitro*, animal and human evidence also indicates that low-dose radiation is capable of controlling bacterial pneumonia. Given this consideration, LDR might also reduce bacterial co-infections in patients infected with SARS-CoV-2. This hypothesis needs to be carefully investigated in well-designed clinical trials. Different mechanisms suggested for the efficiency of LDRT for COVID-19-associated pneumonia and ARDS including the anti-inflammatory and anti-thrombosis effects of low-dose radiation, as well as optimization of the activity of the immune system, are shown in Figure 2.

Development of an effective vaccine and developing effective treatment methods for COVID-19 patients are of crucial importance. SARS-CoV-2 uses its spike glycoprotein (S) to bind its receptor and mediate membrane fusion and entry into humans. It also causes activation of inflammatory cells, particularly CD4 lymphocytes that subsequently transform to T helper 1 (Th1) cells. These cells are involved in enhancing production of different pro-inflammatory cytokines and chemokines. Different methods have been proposed to develop an effective vaccine against SARS-CoV-2, including innovative RNA vaccines, and viral vector- or protein-based vaccines. As of October, 2020, there were more than 320 vaccines in development. However, to date, none of these vaccines have passed clinical trials to confirm their safety and efficacy [68]. However, on 9 November 2020, Pfizer and BioNTech presented their preliminary data obtained from their phase III trial reporting that their mRNA-based vaccine, BNT162b2, has a vaccine efficacy rate above 90%, at seven days after the second dose [69]. Meanwhile, results from other advanced trials are expected soon. There are different pathways to form SARS-CoV-2 proteins to prompt an immune response so a human can form antibodies against the virus, but in all considered protein-based pathways, sparing of membrane epitopes is essential to elicit the immune response toward vaccine generation. One method that is used for vaccine development, and that was already in use at the end of the 1950s, is ionizing radiation [70]. Recently, Feng et al. presented Monte Carlo simulations of a physical SARS-CoV-2 virus [71], built based on the detected gene sequence of a Wuhan patient from the National Center for Biotechnology Information

(NCBI). They defined the damage (“inactivation”) efficiency to be the ratio of the total loss energy in the virus to the total incident energy, and they found that the irradiation electron energy corresponding to the greatest damage can reach 55% around 2 keV. There has also been a suggestion of using high-energy protons or higher densely ionizing radiation to inactivate SARS-CoV-2, with minimal damage to the envelope proteins, to create the needed viral proteins. However, the production of high-energy ions requires expensive accelerators and the radiation fields are inhomogeneous, so there are several economical and practical challenges to this pathway.

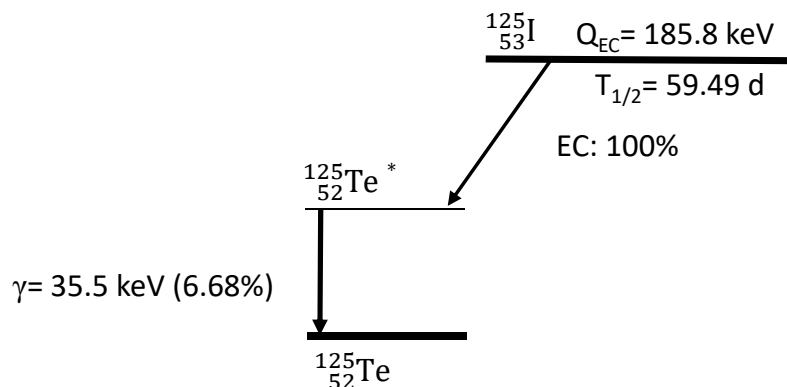


**Figure 2.** Different mechanisms suggested for the efficiency of low-dose radiation therapy (LDRT) for COVID-19-associated pneumonia and ARDS [67].

Another approach to treating COVID-19 patients with ionizing radiation is with internal therapy, which often is performed for local solid tumors. As suggested in [72], a selective, molecularly targeted vector could be used. One example of such a targeted vector is the monoclonal antibody (mAb) CR3022, which could serve as a delivery agent for  $^{125}\text{I}$ , a commonly used radionuclide in brachytherapy to treat diseases such as prostate cancer, uveal melanomas and brain tumors.  $^{125}\text{I}$  decays by electron capture (EC) to an excited state of  $^{125}\text{Te}$ , which decays to the ground state of Te-125 by gamma decay, as shown in Figure 3.

This excited state decays immediately by  $\gamma$ -decay with a maximum energy of 35.5 keV. The SARS-CoV-neutralizing antibody CR3022 binds to the SARS-CoV-2 receptor binding domain (RBD) with a KD of 6.3 nM [73]. Moreover,  $^{131}\text{I}$  is another potential radionuclide for use in targeted SARS-CoV-2 treatment. This radionuclide is widely used as a standard treatment for some types of thyroid cancers as well as imaging procedures (e.g., scintigraphy and whole-body SPECT imaging) [74]. Since treatment of SARS-CoV-2 by Auger emitters possibly is not capable of eliminating all virions, brachytherapy in combination with other treatments can be used to improve the treatment outcomes. Currently tested treatments such as anti-IL-6 antibodies, remdesivir and dexamethasone can be among these treatment combinations. Moreover, by radiolabeling CR3022 for imaging, a non-invasive readout of viral load that can indicate the success of the treatment would be available. In summary, as stated by Bevelacqua and Mortazavi, treatment approaches other than antivirals, like LDRT, not only offer significant advantages, but also prevent the accelerated evolution of the virus. Table 1 summarizes the key therapeutic characteristics of LDRT, dexamethasone and remdesivir.





**Figure 3.** The decay of  $^{125}\text{I}$  to an excited state of  $^{125}\text{Te}$  by electron capture (EC), and then immediately to the ground state of Te-125 by gamma decay. “\*” shows the excited state of tellurium-125.

**Table 1.** Comparison of the potential effects of LDRT, dexamethasone and remdesivir concerning COVID-19 pathogenesis and treatment.

	Low-Dose Radiation Therapy (LDRT)	Dexamethasone (Corticosteroid)	Remdesivir (Antiviral)
Main Advantages	<ul style="list-style-type: none"> <li>Single dose</li> <li>Almost available everywhere</li> </ul>	<ul style="list-style-type: none"> <li>Once daily for 7–10 days</li> <li>Almost available everywhere</li> </ul>	Once daily for up to 9 days
Disadvantages	Cancer risk increase for doses > 0.5 Gy	Hyperglycaemia that can worsen diabetes	May induce selective pressure on SARS-CoV-2 possibly leading to viral evolution
Current Limitations	<ul style="list-style-type: none"> <li>Is particularly suggested for old patients</li> </ul>	<ul style="list-style-type: none"> <li>Is only suggested for severe and critical cases by WHO</li> </ul>	
Cost	Moderate	Low	High
Direct anti-inflammatory effects	✓	✓	-
Inhibiting cytokine storm	✓	✓	-
Direct anti-virus effects	✓	-	✓
Direct anti-bacterial effects	✓	May promote bacterial growth (causes superinfection)	-
Anti-thrombotic Effects	✓	-	-
Tissue repair	✓	-	-
Bone marrow stimulation	✓	-	-

In Table 1, a comparison of the potential effects of LDRT, dexamethasone and remdesivir concerning COVID-19 pathogenesis and treatment is presented.

## 5. Discussion

The severe acute respiratory syndrome coronavirus SARS-CoV-2 has caused a large pandemic since December 2019, when a cluster of unexplained pneumonia cases was first reported in Wuhan, China. Since viruses usually have different sensitivities to physical and chemical damaging agents such as antiviral drugs, heat, UV and ionizing radiation, ranging from extremely sensitive (ES) to extremely resistant (ER), it is of crucial importance to choose a robust treatment strategy. It is well documented that where the treatment course is effective (i.e., viral fitness is impaired sufficiently), no viral genomes will be successfully replicated, but where the treatment is ineffective and some genomes find the opportunity to replicate, selective pressures may lead to rapid adaptation causing viral resistance [75]. Therefore, when the treatment is not robust, it may cause an even larger disaster

than the current one. In this paper, pros and cons of antiviral drugs are discussed from the selective pressure and possible drug resistance point of view. To date, a number of treatment methods of COVID-19 have been suggested and evaluated in different non-randomized and randomized trials around the world since March, 2020, when the WHO declared COVID-19 a pandemic. Especially, dexamethasone, remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon- $\beta$ 1a (initially with lopinavir, later not) were evaluated in a large WHO-funded randomized trial [33]. The results from this trial were unfortunately negative and none of the drugs were found to have any major positive effect on hospitalized COVID-19 patients, as indicated by overall mortality, initiation of ventilation and duration of hospital stay. Substantial evidence shows that viruses are constantly mutating and evolving, and when an antiviral immune response is unable to eliminate viruses, viral evolution is promoted. Based on this fact and the results from the WHO-funded trial, we should consider the risk of using ineffective antivirals on large populations. The lack of efficiency is one thing, but even worse is that using ineffective antivirals might drive the virus to adaptive mutations for increasing its viral fitness. This could lead to an even more contagious and lethal variant of the virus, so it is important to limit a large-scale use of antivirals/vaccines against the SARS-CoV-2 virus when their effects on viral fitness are not yet fully understood. We address the key advantages of using potential selective pressure-free treatment methods instead, such as the use of sparsely and densely ionizing low-dose radiation (LDR). It is known that LDR has the capacity to modulate excessive inflammatory responses, regulate lymphocyte counts and control bacterial co-infections in patients infected with COVID-19 and different modalities. A recently published article presented a simple planning technique for low-dose whole-lung treatment in real patient anatomy, including both non-COVID and COVID-19 patients, showing that the dose delivered to the lungs had reasonable uniformity even with this simple and quick setup [76]. This could be further evaluated for the treatments of COVID-19 patients with ARDS. Moreover, to stop the spread of the SARS-CoV-2 virus, not only is an effective antiviral needed, but also a vaccine. We therefore discussed how ionizing radiation could be used to inactivate the SARS-CoV-2 virus, with minimal damage to the envelope proteins, to create the needed viral proteins. The positive phase 3 trial results about the mRNA-based vaccine candidate, BNT162b2, released by Pfizer and BioNTech in early November, 2020, induced hope concerning an end to the pandemic [69]. There are also indications that Astra Zeneca's vector-based vaccine candidate AZD1222 might be a cheaper but still very efficient vaccine. However, more trials need to be performed before any conclusion can be made that it can be a real vaccine. Pfizer and BioNTech's lead means that they might be able to deliver vaccines earlier than other manufacturers, but there are a number of complications that are related to the nature of the vaccine. The largest problem is that the vaccine must be cooled down to at least minus 70 °C and then stored for a maximum of five days in a refrigerator. This makes the distribution of the vaccine to the public very difficult. On 16 November 2020, the biotech company Moderna Inc. said that its experimental vaccine was 94.5% effective in preventing the disease, according to an analysis of its clinical trial [77]. Moderna uses the same technology as Pfizer and BioNTech, which means creating an mRNA with the code for making the coronavirus spike protein.

Can we learn anything about selective pressure on viruses from human immunodeficiency virus (HIV), which is a lentivirus (a type of retrovirus, which uses RNA as its genetic material) that may have jumped from chimpanzees in Central Africa to humans as far back as the late 1800s or early 1900s but was not identified until in the 1980s? Two main types of HIV (HIV-1 and HIV-2) affect humans, but neither of them can reproduce on its own. Instead, the virus infects CD4 cells of the human immune system. Then, it takes control of the cell's DNA, makes copies of itself inside the cell and finally releases more HIV into the blood. HIV will continue to multiply and spread throughout the body. Retro viruses occur as extremely diverse populations and they can undergo spontaneous mutations along the genome appearing within a person every day [78–81]. Over time, the CD4 cells are killed by HIV and the body's ability to recognize and fight some types of infections begins to decline. If HIV is not controlled by treatment, the loss of CD4 cells makes it hard for the body to fight off infections which leads to the development of serious illnesses, severe infections and certain

HIV-related cancers. The existing antiretroviral therapies (ART) of HIV prevent HIV from multiplying (making copies of itself) but do not cure the illness and if the treatment is not followed as prescribed, the viral load increases again and the HIV virus may become drug-resistant. This might also be a risk with COVID-19 if the treatment is not efficient enough.

## 6. Conclusions

Up to now, the results from published larger clinical trials for treatment of COVID-19 patients have unfortunately been negative and none of the tested drugs were found to have any major positive effect on hospitalized COVID-19 patients. It is well known that if an antiviral treatment course is not effective (i.e., viral fitness is not altered sufficiently), viral genomes can be replicated and selective pressures may lead to rapid adaptation causing viral resistance. It is therefore important to certify that the developed drugs for treatment of COVID-19 are effective enough to not cause an even worse situation than the current one with a mutated virus. Early results of LDR are promising in this aspect since it is known to be less subject to virus mutation [76]. To limit the spread of the virus, it is also essential to develop a vaccine that is available for as many people as possible.

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