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We previously published an article in *Science* discussing the role of ATP in cystic fibrosis (CF). (*1*) In this manuscript, we update the findings published in that article and add new evidence that the elevated levels of ATP in CF patients may have a protective benefit CF patients infected by SARS-CoV-2 and suffereing from COVID-19. By extension, we suggest a therapeutic regimen that may benefit COVID-19 in the general populaton.

Two recent publications – both available in late April 2020 – provide important findings concerning the effect of the COVID-19 pandemic on the cystic fibrosis (CF) population world-

wide. Colombo et al. present important information about the infection in the CF[1] population in the COVID-19 hotspot of Lombardia, Italy as well as elsewhere in Europe. (*2*) Cosgriff et al. presents a more detailed evaluation of the effects of COVID-19 in the cystic fibrosis population in eight countries on three continents. (*3*)

To more fully understand the ramifications of the two data points of Colombo et al., we constructed table 1. This table shows that the incidence of clinically detectable COVID-19 resulting from coronavirus 2 (SARS-CoV-2) infections in Italian CF individuals appears to be 1.43 times higher than in the non- CF population in Lombardia, while the incidence of COVID-19 specific mortality in the Italian CF population is zero. The presence of a reduced mortality rate despite an increased infection rate is truly unexpected and puzzling[2].

While the details of these findings may change with rapid improvements in detection and calculated exposure based on blood antibody titers and other assays, this data suggests that CF patients have an increased risk of contracting COVID-19 mitigated by a remarkably increased chance of survival following exposure. It is possible of course that some of the survival advantage shown in these CF patients may be due to the younger age of this group as compared to the general population; however, no age- matched population cohort could possibly have a better survival rate than the 100% survival seen in the CF patients.

Recent data published by the *Cystic Fibrosis Registry Global Harmonization Group* seems to corroborate this conclusion. (*3*) This group reported on the outcome of 40 CF patients with SARS-CoV-2 infection located in eight participating countries and concluded that the clinical course of these patients was "better than initially predicted". Remarkably, there were no deaths in these patients as of the time of publication. This is illustrated in table 2. This improved sur-

[1]Here and elsewhere in this communication, the acronym CF represents the homozygous presence of a mutated Cystic Fibrosis Transmembrane Conductance Regulator (CFTCR aka CFTR) gene leading to clinical cystic fibrosis.

[2]At present, there is no detailed information about SARS-CoV-2 infection rates and mortality rates in CFTR gene mutation heterozygotes.

vival is remarkable since CF patients begin the infection with a compromsed state of respiratory functon. (*1*) (*4*) (*5*)

In view of these unusual findings, we endeavored to understand the physiological factors in the CF state that offer protection against COVID-19 morbidity. While many potential factors could play a role (including the factors that provide benefit against diseases such as cholera, typhoid and tuberculosis), we eventually narrowed our investigation to focus on two promising candidates we thought might play a role: vitamin D and ATP. Recent evidence suggests that elevated vitamin- D levels offer some survival benefit against COVID-19. (*6*) However, CF patients have deficiencies in fat soluble vitamin (A, D, E, K) absorption and vitamin D levels are intrinisically lower in the CF population than in the general population. (*7*) Abraham et al. has even reported a patient with vitamin D deficiency presenting with rickets subsequently found to be suffering from CF. (*8*) As opposed to vitamin D levels, however, systemic levels of ATP are intrinscally elevated in the CF patient. We hypothesize that this is the crucial factor causing improved survival in view of ATP's central role in both intracellular metabolism and extracellular signalling, as discussed below. We explain the rationale supporting this hypothesis in what follows.

CFTR gene mutations have been isolated from the dental and skeletal remains of Stone Age Europeans using modern DNA analytic tools, demonstrating that the CF mutation extends far back into distant antiquity. Homozygous CFTR gene mutations result in clinical CF which universally resulted in death during childhood until the mid twentieth century. Heterozygous carriers of CFTR gene mutatons do not develop clinical CF. Any possible survival benefits of the CFTR gene mutations in the general population were thus conferred by their presence in the heterozygous CFTR gene mutation carriers. Postulated benefits of a mutated CFTR protein gene includes improved abilty to survive after infection with various microbes; as mentioned above, there is evidence for CF carrier state benefit against cholera, typhoid and tuberculo-

Populaton	Population size	# Infected	Incidence	# Deaths	Death rate
Italy $\pm$ CF	60 360 000	190 000	314	25 549	42.3
Lombardia, - CF	10 058 994	70 155	697	12 940	128.6
Lombardia, + CF	1 006	10	994	0	0

Table 1: A characterization of the COVID-19 infection in Lombardia from its beginning – taken as 21 FEB 2020 – until 31 MAR 2020. Incidence refers to those first manifesting COVID-19 during this time period. "Deaths" ("death rate") are COVID-19 - specific deaths (death rates). The incidence and death rate are given per 100 000 people of the indicated population group. The negative vs. positive signs indicate the absence vs. presence of clinical CF, respectively; the $\pm$ sign indicates the total population (i.e., including those with and without CF). Data calculated from Colombo et al. (*2*) and other publications. The total number of CF patients in Lombardia was calculated based on the CF prevalence in Lombardia of 10.0 per 100 000 people as published in (*9*).

sis[3]. Survival benefits have presumably led to the current high prevalence of CFTR mutations present in the Caucasian population; approximately one out of every 20 Caucasian individuals is at least heterozygous for a CFTR protein gene mutation. It now appears that homozygous CFTR gene mutation is demonstrating a survival benefit against a new class of microbial agent (i.e., SARS-CoV-2) with a potentially new and unknown mechanism of action. Whether heterozygous carriers of the CFTR gene mutation are also protected (as with the previously mentioned bacterial infections) is an open question. (*1*) (*4*) (*5*)

CF is associated with increased viscosity of mucous of the airways and other organs. From table 1 above, it is evident that the thick CF mucous does not impede SARS-CoV-2 viral entry

[3]The heterozygous presence of a CFTR mutation may have produced better survival in cholera and typhoid by reducing the lethality of the diarrhea characterstic of the infection. The homozygous presence of a CFTR gene mutation may have also produced protection against cholera and typhoid, but such protection was offset by the universal lethality of the homozygous mutation.

Country	Population size (millions)	# Deaths	Death rate CF ±	Death rate CF +
Australia	24.99	97	0.4	0
Belgium	11.46	8 415	73.4	0
France	66.99	25 897	38.7	0
Germany	83.02	7 392	8.90	0
Ireland (Republic)	4.904	1 403	28.6	0
Netherlands	17.28	5 288	30.6	0
United Kingdom (UK)	66.65	30 615	46.0	0
United States (US)	328.20	76 513	23.3	0

Table 2: A characterization of the worldwide COVID-19 pandemic. Deaths (death rates) are COVID-19 - specific deaths (death rates). Death rates are per 100 000 people. CF + means patients positive for cystic fibrosis. CF ± indicates general population (i.e., those with CF and those without CF). Data from Cosgriff et al. (*3*)

into the airway cells and the establishment of COVID-19. CF is also associated with abnormal chloride and electrolyte abnormalities as demonstrated by the chloride sweat test. The laboratory of Abraham et al. was the first to investigate the association between CF and abnormal ATP and purine transport. (*1*) (*4*) (*5*) (*10*) They demonstrated that the CFTR protein is associated with ATP transport to the extracellular surface of epithelial cells. In normal and CF physiology, the level of extracellular ATP is also controlled by CD39 and CD73, which dephosphorylate ATP and produce extracellular adenosine. Extracellular ATP and adenosine control a variety of epithelial functions through interaction with the P1 and P2 purinergic receptors. Extracellu-

lar adenosine returns to the intracellular space via adenosine transporters resulting in renewed intracellular ATP and adenylates.

The laboratory of Abraham et al. demonstrated that ATP blood levels are increased in CFTR knockout animals as well as in CF patients. In fact, ATP levels in the blood of patients with CF are approximately twice the levels measured in normal individuals. (*5*) (*10*) (*11*) The mechanism of this increased ATP level is still under active investigation.

Based on the data just presented, we hypothesize that the mitigation of respiratory failure in COVID-19 infected CF patients is mediated by this increased serum ATP level. Furthermore, blood ATP levels decrease monotonically with the normal ageing process and in a variety of disease states. This could help to explain why COVID-19 infection is significantly more lethal in older individuals and individuals with these diseases.

ATP can be safely administered to patients both orally and intravenously. (*4*) (*12*) (*13*) In patients with various solid tumors with depleted ATP levels, Abraham et al. conducted a phase II clinical trial demonstratng that ATP can be safely administered as a prolonged infusion. Such infusions replenish depleted ATP stores in chronically debilitated cancer patients with treatment refractory metastatic disease. This elevated blood and extracellular ATP level may explain why some solid tumors intrinsically expressing purine receptors are largely absent from the CF population; malignant melanomas are one such example. In advanced hormone refractory prostate cancer, purinergic receptors are over-expressed and as a result, the addition of oral ATP to palliative agents such as bisphosphonate- chelated samarium-153 dramatically reduces the prostate cancer biomarker PSA.

Since deterioration of pulmonary, renal and gastrointestinal epithelia are characteristics of COVID-19, we now suggest considering a trial of ATP designed to prevent organ failure in the host. We have previously conducted studies administering high dose oral ATP to athletes and advanced cancer patients resulting in improved athletic performance and cancer patient

survival. (*12*) (*14*) (*15*) (*13*) This approach is uncomplicated and easily applied in the setting of SARS-CoV-2 infected individuals who are not yet in intensive care and has the potential to reduce rates of hopsitalization and intubation. For COVID-19 individuals who are critically ill and require intensive care, intravenous ATP infusion as described in Phase II study publications could be given. ATP therapy could potentially avoid the need for intubation and ventilation of SARS-CoV-2 infected individuals, thus providng time for other agents and the immune system to engage and clear the viral infection.

There is substantial evidence that infection with SARS-CoV-2 is associated with blood clotting in lungs, brain and elsewhere. It seems likely that microclots in the lungs prevent oxygenation of hemoglobin and cause a decrease in $Pa\,O_2$ resulting in decreased tissue metabolic ATP production. ATP is necessary for survival of pulmonary tissues and other organs. However, low $Pa\,O_2$ levels are also a trigger for ATP loaded red blood cells to release ATP. Therefore, treatment with intravenous ATP would deliver ATP to these areas of critical need via the red blood cell CFTR mediated delivery complex; this may be able to save organs. IV ATP has also been shown to reverse pulmonary artery hypertension. Pulmonary hypertension is observed in COVID-19 patients who are showing rapid deterioration in their clinical status.

The conventional view of cystic fibrosis focuses on the presence of CFTR on the apical surfaces of epithelial cells lining the respiratory tract, the hepatobiliary tract and pancreas, the gastrointestinal tract and sweat ducts. CFTR is important in the regulation of chloride / electrolyte transport across these epithelial surfaces and mutation of the CFTR gene affects the secretions of these epithelial cells.

A newer view of CF stimulated by the recently described clinical course of COVID-19 in CF patients focuses on CFTR as a modulator of ATP transport across the cell membrane, either directly or indirectly through modulation of separate membrane- bound ATP transporters. We were the first to demonstrate that CFTR protein is detectable in the red blood cell membrane.

We also found that when the CFTR gene is mutated or absent, there is an associated elevation of blood and plasma ATP levels. These elevated blood ATP levels reflect increased liver and systemic ATP pools. Another manifestation of increased liver and systemic ATP pools is increased ATP levels in the airways. We hypothesize that it is this increased ATP level (either in the blood, liver, total body or some combination of all three) that aids cystic fibrosis patients in their battle for survival with the SARS-CoV-2 virus. This new view is illustrated in figure 1.

This hypothesis suggests that mimicking the systemic CF state with oral and/ or intravenous ATP may help spare SARS-CoV-2 infected non-CF individuals the need for placement on ventilators and avoid complications of radical treatment and/ or death. We do not recomment ATP supplementation in SARS-CoV-2 infected CF patients.

At the time we started writing this article (07 MAY 2020), we began offering oral ATP on a voluntary basis as a nutritional supplement to patients in a COVID-19 ward at a local assisted living center. So far, ten COVID-19 patients requested the supplement. The ATP has been in the form of 400 mg capsules or ATP powder at 450 mg per scoop. The powder can be mixed with pudding, applesauce or beverages according to the patient's preference. Patients are currently tolerating a gradual dose escalation well. They are starting with two doses on the first day (800 to 900 mg total) and increasing the oral ATP to a maximum of 800 x 4 = 3200 mg or 900 x 4 = 3600 mg a day in divided doses as tolerated for the duration of the COVID-19 hospitalization and convalescence. We have observed objective and subjective signs of clinical improvement. None of the patients receiving ATP have required hospitalization, intensive care admission or intubation to date. [4] In the near future, we will retrospectively compare the clinical course of the patients receiving ATP with those patients not receiving ATP. In particular, we will compare length of convalescence and occurrence of hospitalization, intensive care admission, intubation and COVID-19 - specific death.

[4]The source of the ATP 400 mg pills and ATP powder is Technical Sourcing International Group (TSI Group), Missoula, Montana.

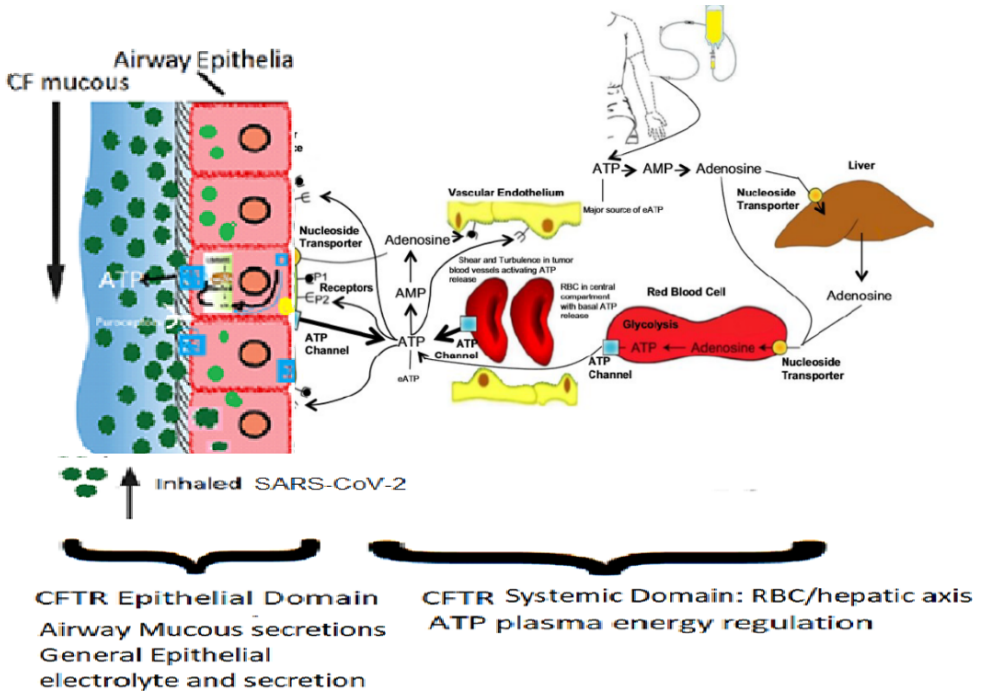

Figure 1: CFTR distribution can now be divided between CFTR in epithelial apical and basolateral membranes and CFTR with systemic distribution, most notably in the erythrocyte membrane. The former distribution modulates epithelial secretions including airway mucous and sweat duct sweat. The latter helps regulate systemic ATP and energy distribution. CF individuals have approximately twice the RBC and plasma as the non- CF general population. ATP distribution can be modified via the detailed pathways illustrated for ATP and adenosine before, during, and after continuous intravenous infusions of ATP in humans. The major source of extracellular ATP (eATP) is the infused ATP, which is delivering ATP directly into the blood plasma compartment. After a short infusion time, the elevated eATP pools induce higher activities of catabolic ecto-enzymes. At the termination of a continuous intravenous infusion of ATP, the majority of the exogenously administered ATP is sequestered in the erythrocytes and all the eATP has been degraded. In Phase II clinical trials we have been able to safely bring the ATP levels of elderly patients with a variety of Stage IV solid tumors up to the CF plasma and CF RBC ATP levels with significant clinical and laboratory improvements. This is similar to the demographics of the populations faring worst with COVID-19.

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16. Non- author contributions

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16. Data and materials availability

There are no restrictions on the data in this paper. All data is available in the manuscript; there are no supplementary materials.