COVID-19 is an infectious disease that targets primarily the respiratory tract, caused by the positive-strand RNA coronavirus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). Infection is mostly by respiratory droplet transmission, underscoring the importance of social distancing and wearing face masks in public health advice. Viral spike proteins drive entry into cells through angiotensin-converting enzyme 2 (ACE2), the essential receptor for SARS-CoV2 that is found on multiple cell types.\(^1\) Viral replication triggers cell damage and pyroptosis, which is followed by immune cell infiltration, expression of pro-inflammatory cytokines, and dysregulated activation of the adaptive immune system causing acute respiratory distress syndrome, multiple organ failure and death.
Early studies on 41 COVID-19-confirmed cases in China revealed a significant increase in numerous cytokines compared with healthy controls, and several studies reported elevation of interleukin-6 (IL-6) in severe cases. It was soon proposed that a “cytokine storm” might underlie severe COVID-19, and that anti-cytokine therapies, and especially IL-6 antagonists, might present an effective therapeutic strategy.

Tocilizumab is a humanised monoclonal antibody against the IL-6 receptor used to treat rheumatoid arthritis. Preliminary observational studies suggested the antibody reduced mortality in COVID-19 patients. However, the much-anticipated Phase 3 COVACTA trial with tocilizumab reported disappointing results. More recent data suggest the cytokine dysregulation seen in severe COVID-19 might not be a conventional cytokine storm and tissue damage cannot simply be explained by elevated IL-6. Because other cytokines are also elevated, a broader strategy would be to target a common step in the synthesis of multiple cytokines and chemokines. One such step involves the CRAC channel-calcineurin-NFAT triumvirate.

CRAC channel-calcineurin-NFAT axis

Nuclear factor of activated T cells (NFAT) transcription factors are extensively phosphorylated at rest and confined to the cytoplasm. On dephosphorylation by the Ca\(^{2+}\)-activated protein phosphatase calcineurin, a nuclear localization sequence is exposed, enabling NFAT proteins to translocate into the nucleus and regulate the expression of numerous...
chemokines and cytokines. In immune and other non-excitable cells, store-operated Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels have a privileged communication with NFAT: following stimulation, the scaffolding protein AKAP79 interacts with Orai1, the pore-forming subunit of the CRAC channel. AKAP79 binds calcineurin and NFAT, placing both close to the open Ca\(^{2+}\) channel.\(^5\) Local activation of calcineurin results in dephosphorylation of tethered NFAT, which then dissociates from AKAP79 and migrates to the nucleus.

**The quest for CRAC channel blockers suitable for use in the clinic**

One hypothesis therefore is that a CRAC channel blocker might be beneficial to COVID-19 patients by modulating a pathological cytokine response. However, despite pharmaceutical interest in this target and a growing number of small molecule tool compounds that block the channels in an *in vitro* setting, no CRAC channel blockers are in current clinical use.\(^6\) Calcimedica recently completed a phase 2 clinical trial assessing efficacy in acute pancreatitis ([https://clinicaltrials.gov/ct2/show/NCT03709342](https://clinicaltrials.gov/ct2/show/NCT03709342)) and are examining the impact of their lead compound on respiratory function in COVID-19 patients ([https://clinicaltrials.gov/ct2/show/NCT04345614](https://clinicaltrials.gov/ct2/show/NCT04345614)), but many other potential drug candidate molecules have not progressed into clinical development.\(^6\) Why is this? Because of their wide expression, targeting CRAC channels might affect multiple cell types and organ systems with deleterious consequences. Preliminary safety observations from two completed phase I clinical trials, however, suggest this might not be the case. Given their central functional role in various immune cell types in particular, CRAC channel inhibition might also be predicted to result in immunosuppression and risk from opportunistic infection. However, it is
unlikely that therapeutic effects require full block of CRAC channels. Indeed, partial CRAC channel inhibition impairs Th17 activity but has little effect on killing of virally infected cells by cytotoxic T cells. A therapeutic window should therefore manifest for CRAC channel blockers in treating certain immune disorders.

One major confounding issue impacting drug discovery campaigns is the ability of candidate molecules to suppress Ca$^{2+}$ entry without affecting the CRAC channel directly, by altering membrane potential. Drugs that block K$^+$ channels, for example, depolarize the membrane potential and reduce the driving force for Ca$^{2+}$ entry through CRAC channels, which in fluorescence-based flux measurements might be incorrectly interpreted as CRAC channel blockade. Cromoglicic acid, the first non-corticosteroid anti-asthma drug, is described as a mast cell stabilizer and was long considered an inhibitor of Ca$^{2+}$ influx channels. However, it actually partially inhibits Cl$^-$ channels, depolarizing the membrane potential and indirectly reducing CRAC channel activity. K$^+$ channel blockers are also effective inhibitors of CRAC channel-driven cytokine release from T cells. Therefore, targeting ion channels that indirectly inhibit CRAC channel activity through effects on the membrane potential could be a helpful strategy to regulate store-operated Ca$^{2+}$ entry in the absence of a clinically useful CRAC channel blocker.

Drug discovery in industry is often disease-led and new candidates are compared with current best-in-class treatments. CRAC channel blockers are potentially considered less useful when assessing efficacy in a disease confined largely to one cell type, but should be a more attractive strategy in more complex disorders involving multiple cell types at the
same time. One clear example of this is in acute pancreatitis. Although a major trigger of this disease is the premature activation of trypsin within acinar cells and autodigestion,\textsuperscript{9} immune cells contribute to the local inflammation and necrosis.\textsuperscript{10} CRAC channel blockers like the Calcimedica compound are effective in murine \textit{in vivo} models of acute pancreatitis by targeting multiple cell types simultaneously.\textsuperscript{10} Asthma is another complex disease involving multiple cell types and where CRAC channel blockers seem effective, at least in animal models.

\textit{Asthma- channels galore but a paucity of drugs}

Current treatments for asthma include a combination of corticosteroids and $\beta_2$ agonists garnished occasionally with cysteinyll leukotriene type I receptor antagonists (montelukast), theophylline and the biologic omalizumab (Xolair), although these are seemingly less effective in a growing cohort of severe asthmatics.

CRAC channels are robustly expressed in several cell types in the lung that contribute to asthma pathogenesis. CRAC channel activation drives degranulation of mast cells, the release of pro-inflammatory leukotrienes, and the secretion of cytokines and chemokines, which all contribute to a sustained inflammatory response.\textsuperscript{6} In fact, as shown in Figure 1, there are many ion channels expressed throughout the lung. Some of these are thought to contribute directly to bronchoconstriction and the lung remodelling that is characteristic of chronic asthma. Others impact on Ca$^{2+}$ influx through CRAC channels.
Ion channels particularly in the heart and peripheral neurons have been targeted successfully in the clinic. But remarkably, lung ion channels have been targeted therapeutically only for a rare form of cystic fibrosis. In ~5% of cases, mutations in cystic fibrosis transmembrane conductance regulator (CFTR) that impair channel gating can be rescued by the channel potentiator ivacaftor. This general lack of success in targeting lung ion channels is all the more remarkable when one considers the advantages of inhalation for drug delivery to the lung.

An urgent unmet clinical need

The lack of success in targeting lung ion channels is not due to lack of knowledge. A crystal structure and cryo-EM reconstruction of Drosophila Orai have been reported and detailed structure-function studies on genes encoding many lung channels have delineated key amino acids in gating, selectivity, permeation and inactivation.

The recent euphoria greeting the approval of vaccines against the COVID19 coronavirus is understandable. Nevertheless, antigenic drift as the virus replicates, the expectation that SARS-Cov2 will not be the last “new” infectious agent we encounter, the alarming number of individuals in the West who may refuse vaccination, and the potential for other diseases that evoke a cytokine storm in the lungs all point toward the importance of alternative therapies including those targeting pulmonary ion channels. The development of improved therapies targeting ion channels coupled with a better basic understanding of lung mechanisms to combat inflammatory processes have become ever more pressing needs.
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Conflict of Interest Statement:
The authors declare no conflicts of interest.

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Figure legend:

Figure 1. Cartoon depicts ion channels reported in various cell types found in the lung. Ion channel acronyms are defined as follows:

| Acronym                                      | Description                                                                 |
|----------------------------------------------|------------------------------------------------------------------------------|
| Epithelial sodium channel (ENaC)             |                                                                              |
| Cyclic nucleotide-gated channels (CNGs)      |                                                                              |
| Cystic fibrosis transmembrane conductance regulator (CFTR) |                                                |
| Ca\(^{2+}\)-activated chloride channels (CaCCs), e.g. TMEM16 |                                      |
| Voltage-gated K\(^{+}\) channels (K\(_{\mathrm{v}}\)1.5, K\(_{\mathrm{v}}\)2.1, K\(_{\mathrm{v}}\)7.1-5) |                                                  |
| Large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (BK; K\(_{\mathrm{Ca}}\)1) |                                                  |
| Intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (IK; K\(_{\mathrm{Ca}}\)3.1) |                                                  |
| Small-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (SK; K\(_{\mathrm{Ca}}\)2) |                                                  |
| Two-pore K\(^{+}\) channels (K2P, e.g. TASK-1/KCNK3 and TREK-1/KCNK2) |                                                  |
| ATP-sensitive potassium channel (K\(_{\mathrm{ATP}}\)) |                                                  |
| Basolateral outwardly rectifying Cl\(^{-}\) channel (BORC) |                                                  |
| Basolateral inwardly rectifying Cl\(^{-}\) channel (BIRC) |                                                  |
| TRPA1, TRPC4, TRPC6, TRPM2, TRPM8, TRPV1, TRPV4 |                                                  |
| Voltage-gated Ca\(^{2+}\) channels (Ca\(_{\mathrm{v}}\)) |                                                  |
| Store-operated Ca\(^{2+}\) channel (Orai) |                                                  |
Figure 1.