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Treatment and vaccines for severe acute respiratory syndrome

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The causative agent of severe acute respiratory syndrome (SARS), which affected over 8000 individuals worldwide and was responsible for over 700 deaths in the 2002–2003 outbreak, is a coronavirus that was unknown before the outbreak. Although many different treatments were used during the outbreak, none were implemented in a controlled fashion. Thus, the optimal treatment for SARS is unknown. Since the outbreak, much work has been done testing new agents against SARS using in-vitro methods and animal models. In addition, global research efforts have focused on the development of vaccines against SARS. Efforts should be made to evaluate the most promising treatments and vaccines in controlled clinical trials, should another SARS outbreak occur.

Severe acute respiratory syndrome (SARS) is an infectious disease characterised by substantial morbidity and mortality, first recognised after an outbreak in 2002–2003. The WHO issued a global alert on SARS on March 12, 2003 after receiving reports from China’s Guangdong province, Hong Kong, and Vietnam regarding clusters of respiratory illness of unknown aetiology. One of the first reports was made by WHO scientist Carlo Urbani, who was called to investigate cases of pneumonia of unclear aetiology in a hospital in Hanoi; he later died of SARS. Following the WHO alert, probable SARS cases were also reported from other regions in China, and other Asian countries including Singapore, Taiwan, Indonesia, Thailand, and the Philippines. Other countries, including Canada, the USA, and Germany also identified cases. In retrospect, SARS originated in Guangdong at the end of 2002. It first spread to other regions in Asia and then international travel facilitated its spread to other continents. A cumulative total of 8096 probable cases of SARS were recorded during the period from November 1, 2002 to July 31, 2003, with 774 deaths in 29 countries.

Soon after SARS was identified as a new disease, the WHO initiated a collaborative global network striving to work together to identify the aetiological agent of SARS. In unprecedented time, a novel coronavirus—SARS coronavirus—was identified as the probable causative agent of SARS (figure 1) and Koch’s principles were demonstrated to be fulfilled by this agent. This virus belongs to the coronavirus family—enveloped, positive-sense RNA viruses associated with respiratory disease in human beings and animals. Evidence suggests that SARS coronavirus originated from SARS-like viruses in animals in the southern Chinese province of Guangdong; the most frequently implicated animal is the Himalayan palm civet, an animal found in food markets and eaten as a delicacy. 

SARS coronavirus is organised into 13–15 open reading frames (ORFs) containing approximately 30 000 nucleotides. In total, 61 SARS-coronavirus sequences derived from different SARS epidemic phases have been analysed and genotypes characteristic of each phase have been identified. The different SARS-coronavirus ORFs represent typical viral genes such as protease and replicase, spike, envelope, membrane, and nucleocapsid, all of which may represent potential therapeutic targets (figure 2). In common with all infections caused by coronaviruses, after infection SARS coronavirus induces the synthesis of 3’ coterminal sets of subgenomic mRNAs in target markets and eaten as a delicacy.15,16

Figure 1: Morphology of SARS coronavirus

Electron microscopy reveals virus particles on the surface of infected cytopathic Vero E6 cells (A). The diameter of the virus particle ranges between 60 nm and 120 nm with a round to oval shape. Virions are also localised intracellularly within vesicles (B). Scale bars represent 100 nm. Reproduced with permission from Chin J Biochem Biophys 2003; 35: 587–91.3
In laboratory settings, SARS coronavirus is able to infect macaque monkeys, mice, ferrets, and domestic cats.

Clinically, SARS is characterised by systemic symptoms such as fever and myalgia, followed by respiratory symptoms including a non-productive cough and dyspnea. Laboratory findings include lymphopenia, and chest radiographs commonly exhibit unilateral or bilateral infiltrates. Approximately 15% of cases deteriorate, requiring intubation and mechanical ventilation. The overall mortality rate has been reported to be about 10%. However, SARS mortality rates in those over 60 years old have been reported to be as high as 50%. Affected children seemed to have milder symptoms with no reports of death.

At the time of the 2002–2003 outbreak, physicians shared their personal experiences supporting or rejecting various treatments for SARS. Because of the rapid progression of the outbreak, multicentre, randomised, controlled interventional trials were not possible, and the success of various treatments remains largely anecdotal. Thus, a consensus on therapeutic strategies has not yet been reached. Since the outbreak, global research efforts have focused on testing new agents against SARS with in-vitro methods and animal models. In addition, much effort has been placed on developing effective vaccines against SARS. This review summarises the clinical experience of the use of various treatments during the outbreak and provides an overview of the data, both in vitro and in vivo, supporting, or otherwise, the effectiveness of these treatments and those that have been proposed since the outbreak. In addition, we summarise the progress made to date regarding SARS vaccines.

### Treatment of SARS

A summary of the pharmacological agents that have been used or proposed for the treatment of SARS is shown in figure 3.

### Antibiotics

During the 2002–2003 outbreak, suspected SARS cases were usually treated initially with broad-spectrum antibacterial drugs effective against typical bacterial causes of acute community-acquired pneumonia. The administration of broad-spectrum antibiotics—e.g., respiratory fluoroquinolones, second-generation cephalosporins, or third-generation cephalosporins—plus a macrolide is recommended at the first signs of the SARS, because the initial features of the disease are non-specific. However, after SARS coronavirus is identified as the causative agent, antibiotics may be withdrawn, because there is no evidence that antibiotics are clinically beneficial in the treatment of SARS.

### Antiviral drugs

#### Ribavirin

Even before the causative agent of SARS was discovered, treatment with ribavirin was used empirically to treat patients with SARS. Ribavirin is a synthetic nucleoside with broad-spectrum antiviral activity. Clinical studies that have assessed the effectiveness of ribavirin in SARS range from anecdotal case reports and retrospective case series to one randomised clinical trial with multiple treatment arms. However, none of these studies definitively determine whether or not ribavirin is effective against SARS.

Case reports and case series suggest that combined treatments including ribavirin may be beneficial to some extent; however, in all of these studies, the effect of ribavirin is confounded by the concomitant use of other agents. For example, studies describe clinical and radiological improvements in patients treated with ribavirin and steroids, but, without a control group, it is difficult to determine whether the improvements result from ribavirin, steroids, the combination of both, or the natural course of the illness. One study showed that the delayed initiation of combined therapy with ribavirin and steroids was among the risk factors associated with severe complicated disease, suggesting that ribavirin might be beneficial, but it is difficult to delineate the role of delaying the use of ribavirin from the delay in the use of steroids. The results of a randomised clinical study in Guangdong, involving multiple different treatment arms, suggest that ribavirin given at a low dose (400–600 mg/day) was less effective compared with an early and aggressive use of steroids with interferon alfa. However, the lack of a control arm in this study does not allow for one to make definitive conclusions about whether or not ribavirin has any positive effect on SARS compared with no treatment. In-vitro testing showed that ribavirin was not able to inhibit
SARS-coronavirus replication at clinically achievable concentrations.\textsuperscript{33,34} This finding, combined with post-mortem findings demonstrating high viral loads in most patients despite treatment with ribavirin,\textsuperscript{35} suggests that if ribavirin has any effect against SARS coronavirus, it is likely to have only a small beneficial effect at best. This is important when the side-effects that have been associated with ribavirin use are considered. Knowles and co-workers reported common adverse events in 110 people with suspected or probable SARS who were treated with ribavirin.\textsuperscript{36} 61% of these people had evidence of haemolytic anaemia; hypocalcaemia and hypomagnesiaemia were reported in 58% and 46% of the people, respectively.

**SARS-coronavirus protease inhibitors**

The combination of the protease inhibitors lopinavir and ritonavir was used less frequently during the SARS outbreak compared with ribavirin. The lopinavir/ritonavir combination was first considered a potentially useful treatment after in-vitro studies showed it had antiviral activity against SARS coronavirus.\textsuperscript{37,38} Chan and colleagues\textsuperscript{39} compared outcomes in people who received lopinavir/ritonavir as initial treatment, and as rescue therapy, with matched controls; all patients were given ribavirin and steroids according to a standardised protocol. The addition of lopinavir/ritonavir as initial treatment was associated with a statistically significant reduction in the overall death rate and intubation rate compared with matched controls (p<0·05). However, the subgroup that received lopinavir/ritonavir as rescue therapy did not show a significant difference in these endpoints. Chu and co-workers\textsuperscript{40} also assessed treatment with lopinavir/ritonavir compared with historic controls; all patients were also treated with ribavirin and steroids in a similar protocol to that of Chan and colleagues. Adverse events (development of acute respiratory distress syndrome [ARDS] or death within 21 days) were significantly lower in the lopinavir/ritonavir group than in the historic controls (p<0·001). In addition, a significant reduction in the need for rescue pulsed steroids for severe respiratory deterioration (p<0·001) and significantly lower nosocomial infections were also noted in those treated with lopinavir/ritonavir, compared with controls (p<0·043). By multivariate analysis, it was demonstrated that the lack of treatment with lopinavir/ritonavir, age 60 years old or greater, and positive hepatitis B carrier status were independent predictors of an adverse outcome including death or the development of ARDS requiring intensive care within 21 days of onset of illness.\textsuperscript{37} Based on these studies, lopinavir/ritonavir appears to be a promising anti-SARS-coronavirus agent.

Other protease inhibitors have been studied in vitro for potential antiviral effects in SARS. For example, Yamamoto and colleagues\textsuperscript{41} screened a set of compounds that included antiviral drugs already widely used, and found that nelfinavir strongly inhibited SARS-coronavirus replication. Nelfinavir inhibited the cytopathic effect induced by SARS-coronavirus infection, and the expression of viral antigens was much lower in infected cells treated with nelfinavir than in untreated, infected cells. In addition, Barnard and colleagues\textsuperscript{42} found that two protease inhibitors—calpain inhibitor VI (Val-Leu-CHO) and calpain inhibitor III (Z-Val-Phe-Ala-CHO)—inhibited SARS coronavirus, interacting with the S1 domain of the spike protein.\textsuperscript{43} Thus, peptides and small compounds that bind to ACE2\textsuperscript{44,45} are possible agents for the treatment and prevention of SARS. In addition, a soluble form of the receptor, antibodies to it, or the receptor-binding domain of the spike protein, may be candidate treatments. Indeed, Sui and co-workers\textsuperscript{46} searched a non-immune human antibody library and successfully identified an anti-S1 human monoclonal antibody, 80R, that potently neutralises SARS-coronavirus infection.
and efficiently inhibits syncytium formation by blocking binding to ACE2. 80R was shown to compete with soluble ACE2 for association with the S1 domain of the spike protein and bound to it with high affinity.

**Fusion inhibitors**

Theoretical reasoning and in-vitro evidence suggest that fusion inhibitors are promising treatment candidates for SARS.45,46 Peptides derived from the heptad repeat regions 1 and 2 of HIV-1 gp41—a transmembrane protein involved in the fusion of HIV and target cells—are the basis for anti-HIV fusion inhibitors. Based on similarities between the heptad repeat regions of gp41 in HIV-1 and the heptad regions in the spike protein of SARS coronavirus, a common mechanism mediating fusion between each virus and target-cell membranes was postulated.45,46 Liu and colleagues45 tested two sets of peptides corresponding to the heptad regions in the spike protein for inhibitory activity against SARS coronavirus, and found that one peptide—CP1— inhibited SARS-coronavirus infection in vitro. It has been postulated that CP1 binds to heptad region 1 of the spike protein and interferes with the conformational changes needed to allow fusion with target cells.

**RNA interference**

RNA interference (RNAi) treatment is a process by which small interfering RNAs (siRNA) are administered, leading to degradation of mRNA with identical sequence specificity.47 This technology has been used to silence genes in cultured cells and in animals, and to target HIV, hepatitis B, and hepatitis C viral infections.48–50 To explore the possibility of interrupting SARS-coronavirus replication with siRNAs, specific siRNAs targeting the spike gene in SARS coronaviruses were synthesised. These siRNAs effectively and specifically inhibited gene expression of the spike protein in SARS-coronavirus-infected cells.51 Another study assessed the in-vitro efficacy of six siRNA molecules targeting different sites of the replicase 1A region of the SARS-coronavirus genome.52 Judged by morphological changes, three of the molecules markedly inhibited the cytopathic effects caused by viral infection and replication. The three siRNAs also inhibited the infection and replication of different strains of SARS coronavirus, indicating that siRNAs targeting the replicase 1A region may be an option for future clinical use.52

**Glycyrrhizin**

In-vitro studies have shown that glycyrrhizin, a component of liquorice roots, is able to inhibit SARS-coronavirus replication.53 Glycyrrhizin inhibits HIV replication in vitro54 and has been used clinically in the treatment of hepatitis C55 and hepatitis B56 with some success. The mechanism of glycyrrhizin-induced inhibition of viral replication—and specifically SARS-coronavirus replication—is unclear, but possibly involves inhibition of replication through an antiviral effect of nitric oxide (NO). Glycyrrhizin upregulates expression of inducible NO synthase and production of NO in mouse macrophages.57 In addition, preliminary results by Cinatl and colleagues34 show that glycyrrhizin induces NO synthase in Vero cells used to cultivate SARS coronavirus.

**Nitric oxide**

Cinatl and colleagues34 showed that SARS-coronavirus replication is inhibited when DETA NONOate—a NO donor compound—is added to the culture medium. This finding has been further corroborated by Keyaerts and co-workers58 using a different NO donor compound, S-nitroso-N-acetyl-penicillamine. Keyaerts and colleagues also report their findings on the use of inhaled NO gas to treat a number of people with SARS. Their results suggest an associated immediate improvement in oxygenation and a lasting effect after termination of inhalation of NO, which is known to be a potent mediator of airway inflammation.59,60

**Niclosamide**

Wu and colleagues60 screened a set of marketed drugs that were not registered for antiviral use to determine if any had in-vitro activity against SARS coronavirus. They found that niclosamide, an existing antihelmintic drug, was able to inhibit replication of SARS coronavirus. The underlying mechanism by which the drug exerts this effect is unclear, but the study shows that niclosamide does not interfere with the virion’s attachment to, or entry into, cells, nor does it appear to inhibit the protease activity.

**Others**

New compounds continue to be tested, with the goal of finding more potential candidate treatments for SARS. For example, from over 10 000 agents tested, Wu and colleagues61 found 15 compounds with potent antiviral activity against SARS. The rationale for their use was based on the paradoxical finding that, despite a fall in SARS-coronavirus viral load became a mainstay of SARS therapy in many centres.62 The rationale for their use was based on the paradoxical finding that, despite a fall in SARS-coronavirus viral load and a rise in SARS-specific IgG typically seen during the 3rd week of illness, a clinical deterioration was observed in some people.63 In addition, pathological findings consistent with bronchiolitis obliterans organising pneumonia and ARDS led to the hypothesis that immune hyperactivity resulting from cytokine dysregulation may be a component of SARS that could be reduced by steroid treatment.64

In most cases, steroids were administered as adjunctive therapy to ribavirin treatment. If the patient’s...
respiratory condition worsened clinically, pulsed, high-dose steroids were added. However, most studies were confounded by the concomitant use of other agents, and none of the studies contained a control group. Thus, whether or not steroids have a beneficial effect in the treatment of SARS cannot be readily determined.

In some studies, treatment regimens containing steroids seemed to be associated with chest radiographic improvements, fever defervescence, and improvement in oxygenation rates earlier than patients not treated with steroids. However, in a study by Hsu and colleagues, adding steroids was not associated with clinical improvement, although the dose of steroids in this study was lower than in those where benefit was seen. Ho and co-workers retrospectively compared the clinical and radiographic outcomes of people with probable SARS who received ribavirin, 17 of whom initially received pulsed, high-dose steroids and 55 of whom initially received low-dose steroids. Pulsed, high-dose steroids were also given to any patient as rescue therapy in the presence of deteriorating respiratory status. The cumulative steroid dose, intensive care unit admission rate, need for mechanical ventilation, and mortality rates were similar in both groups after 21 days. However, those people initially given pulsed steroids required less oxygen and had earlier radiographic improvement. In addition, they required substantially less rescue pulsed steroids. This study suggests that early initiation of pulsed steroids may have a role in the treatment of SARS. However, definitive studies are needed and the potential benefits of steroids must be compared with the associated risks, such as the development of avascular necrosis, secondary sepsis, and fatal aspergillosis, some of which have been described in people with SARS. In Beijing, Hong and Du evaluated 67 people with SARS who had received steroids and ribavirin, and who presented with large joint pain, potentially caused by avascular necrosis, between March and May 2003. Both plain radiographs and magnetic resonance imaging examination were completed on the same day. 28 people were identified with avascular necrosis. The mean time to diagnosis of avascular necrosis was 119 days after the onset of SARS, or 116 days after steroid use.

Interferons

Type 1 interferons have been shown to inhibit SARS-coronavirus replication in in-vitro studies. Because of initial reports describing these in-vitro results, interferons were used clinically during the latter part of the outbreak. Loufy and colleagues described their clinical experience with interferon alfacon 1—a recombinant, non-naturally occurring type 1 interferon containing common aminoacids from several natural interferon alfa subtypes—in 22 people with probable SARS treated in an open-label study in Toronto. 13 people with SARS who received treatment with steroids alone were compared with nine people who received steroids plus interferon alfacon 1. The group treated with interferon alfacon 1 had significantly improved oxygen saturation levels (p=0.02) and a more rapid resolution of radiographic lesions. In addition, this group exhibited substantially less elevation in creatine kinase levels and a trend towards a more rapid normalisation of lactate dehydrogenase levels. However, this group also received higher doses of steroids, so it is difficult to determine whether or not the beneficial effects were due to the interferon alfacon 1.

Haagmans and co-workers investigated the prophylactic use of interferons in a macaque model. 3 days before inoculation with SARS coronavirus, macaques were given pegylated interferon alfa. Substantially reduced viral replication, viral excretion, viral antigen expression by type 1 pneumocytes, and pulmonary damage were noted in the treated macaques compared with untreated macaques. Post-exposure treatment with pegylated interferon alfa yielded intermediate results. These results suggest that interferons have a role in the treatment of SARS.

Immunisation

Because most patients develop antibodies against SARS coronavirus and survive the disease, passive and active immunisation are viewed as possible effective means to prevent and/or treat SARS. Indeed, the development of various vaccines is one of the most important goals of ongoing SARS research.

Passive immunisation

One of the initial proposals to treat SARS was to use sera from people convalescing from SARS as passive immunotherapy. This passive immunisation was attempted with anecdotal success. Since then, prior infection and passive transfer of murine neutralising antibodies have been shown to prevent replication of SARS coronavirus in the respiratory tract in mice. Technological advances enabling the development and purification of human monoclonal antibodies can be exploited to create specific monoclonal antibodies in large-scale production. Indeed, monoclonal antibodies obtained from immortalised B lymphocytes isolated during convalescence from people with SARS have been shown to neutralise virus infection in vitro and to prevent virus replication in a mouse model of SARS-coronavirus infection. In addition, ter Meulen and colleagues showed that prophylactic administration of a human IgG monoclonal antibody reactive with whole inactivated SARS coronavirus was able to reduce replication of SARS coronavirus in the lungs of infected ferrets, completely prevent the development of SARS-coronavirus-induced macroscopic lung pathology, and stop the shedding of virus in pharyngeal secretions.
**Active immunisation**

Although passive immunisation strategies appear promising, the ideal approach to ensure rapid control of future outbreaks of SARS is to generate an effective and safe vaccine. There are numerous teams worldwide working on the creation of vaccines using inactivated SARS coronavirus, recombinant subunits, recombinant DNA, and viral vectors. Given the potential for antibody-directed viral enhancement and disease exacerbation, as reported for vaccines directed against another coronavirus (feline infectious peritonitis coronavirus), it is important that all vaccines created be carefully evaluated before being used clinically. Of all of the vaccines in development, most work relates to viral vectored vaccines and DNA vaccines.

**Viral vectored vaccines**

To date, three different viral vectored vaccines have been described with successful results reported in animal models. Gao and colleagues reported using three adenoviral-based vectors expressing codon-optimised SARS coronavirus spike, membrane, and nucleocapsid proteins. Intramuscular vaccination with all three vaccines at day 0 and day 28 was shown to induce broad, virus-specific immunity in rhesus macaques. All six vaccinated macaques had antibody responses against the spike protein and T-cell responses against the nucleocapsid protein. In addition, all vaccinated animals showed strong neutralising-antibody responses to SARS coronavirus infection in vitro. Challenge tests to determine whether or not this immune response was able to prevent, or reduce the severity of, infection with SARS coronavirus were not completed.

Bisht and co-workers constructed recombinant forms of the highly attenuated modified vaccinia virus Ankara (MVA) containing the gene encoding the full-length SARS coronavirus spike protein and assessed whether expression of the spike protein alone in MVA could raise neutralising antibodies and protectively immunise mice. Both intranasal and intramuscular administration of the vaccine to BALB/c mice at 0 and 4 weeks led to the production of serum antibodies against the spike protein that neutralised SARS coronavirus in vitro. 4 weeks after the second immunisation, vaccinated animals and control animals were challenged with SARS coronavirus. Those given the vaccine had reduced titres of SARS coronavirus in the respiratory tract. Likewise, the passive transfer of serum from mice immunised with the vaccine to naive mice led to a reduction in SARS coronavirus replication. These findings suggest that this MVA-based vaccine is a promising SARS coronavirus vaccine candidate.

Bukreyev and colleagues reported their successful experience with the mucosal immunisation of African green monkeys with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein. The complete SARS coronavirus spike protein gene was incorporated into a recombinant attenuated parainfluenza virus that is being developed as a live attenuated, intranasal paediatric vaccine against human parainfluenza virus type 3. Four African green monkeys were vaccinated with a single dose of the vaccine, administered via the respiratory tract, and four other monkeys were vaccinated with a control. All monkeys were challenged with SARS coronavirus 28 days after immunisation. Neutralising serum antibodies were noted in all of the vaccinated animals. After SARS coronavirus challenge, viral shedding was documented in all of the control animals but not in any of the vaccinated animals. The authors concluded that a vectored mucosal vaccine expressing the SARS coronavirus spike protein alone may be highly effective for the prevention of SARS in a single-dose format.

**DNA vaccines**

DNA vaccines are also an attractive option for SARS vaccines. Thus far, three experimental studies have been published addressing DNA vaccination in SARS. Yang and colleagues showed that giving mice a SARS coronavirus DNA vaccine encoding the spike glycoprotein induced T-cell responses, neutralising antibody responses, and protective immunity. Alternative forms of the spike protein were assessed and all were found to induce substantial neutralising-antibody titres and strong immune responses mediated by CD8 and CD4 cells. In addition, a reduction in viral replication in the lungs by more than six orders of magnitude was noted after SARS coronavirus challenge; the protection was shown to be mediated by a humoral, but not a T-cell-dependent, immune mechanism. These findings show that DNA vaccines based on the spike glycoprotein may lead to effective immune responses with protective immunity in animal models.

Kim and co-workers reported the generation and characterisation of DNA vaccines targeting the nucleocapsid protein of SARS coronavirus by antigen linkage to calreticulin, which has been shown to enhance MHC class I presentation to CD8(+) T cells. With a murine model, it was shown that the vaccination with this DNA vaccine leads to the generation of a more potent nucleocapsid-specific humoral and T-cell-mediated immune responses, compared with nucleocapsid DNA alone. In addition, mice vaccinated with the DNA vaccine were capable of substantially reducing the titre of challenging vaccinia virus expressing SARS coronavirus nucleocapsid protein. In a similar study by Zhu and colleagues, immunisation of mice with a nucleocapsid-based DNA vaccine led to nucleocapsid-specific antibodies and specific cytotoxic T-cell activity. Challenge tests were not completed.

Together, the data presented on potential vaccines reflect enormous international efforts. Because a vaccine usually takes 6–8 years of clinical development after...
entering phase I clinical trials before being licensed, it is not expected that any of these vaccines will be available for clinical use in the near future. However, given the pace and amount of progress to date, the period of time before clinical production of a SARS vaccine may be substantially shortened compared with other vaccines.

**Strategies in the event of a recurrence of SARS**

Whether or not SARS will re-emerge is a matter of debate.5,10 However, in the event that SARS does recur, the most promising—and immediately available—agents for the treatment of the syndrome seem to be type 1 interferons, steroids, and lopinavir/ritonavir, based on the available data at that time. Ideally, the most promising—and immediately available—agents eg, SARS-coronavirus-specific receptor-binding inhibitors, fusion inhibitors, and siRNAs—may have been approved for clinical use. The choice of agents will need to be determined based on the available data at that time. Ideally, the most promising agents would be given in a controlled clinical trial. The difficulties in designing and implementing controlled clinical trials—which limited the ability of researchers to do such trials during the past SARS outbreak, and which will continue to pose problems in the event of future outbreaks of SARS or other novel pathogens—have been summarised.11–20 The best solution to facilitate the implementation of clinical trials in future outbreaks would be the establishment of an international collaborative clinical-trials group with access to appropriate contingency funds, and an internationally accepted ethics review board. Until then, research based on in-vitro studies and in-vivo animal models should be continued to determine the best agent, or combination of agents, worthy of further clinical consideration.

**Conflicts of interest**
We declare that we have no conflicts of interest.

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