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How Prepared are we to Control Severe Acute Respiratory Syndrome in Future

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ABSTRACT

No non-human reservoirs for smallpox- and polio-viruses has contributed to the success of worldwide eradication of smallpox and a significant control of poliomyelitis. Most emerging and re-emerging viruses including SARS Coronavirus (SARS-CoV), have animal reservoirs and therefore,they impose a constant threat of host jump leading tooutbreaksin humans. It is desirable to be ready for control of infections that are caused by zoonotic pathogens, even after an outbreak has ended. This literature review is a compilation of advances made so far for diagnosis and treatment of SARS.

Keywords: SARS Coronavirus (SARS-CoV), Antivirals, Vaccines, Human Monoclonal Antibody

1. INTRODUCTION

According to the World Health Organization, there were 8,098 reported cases and 774 deaths worldwide during the Severe Acute Respiratory Syndrome (SARS) outbreak in2002-2003. First case of SARS appeared in November 2002 in Guangdong Province, China. By April2003, it had spread to around 30 countries including, Vietnam, Hong Kong, Singapore, Taiwan, India, Canada and United States of America.

On 24th March 2003, Center for Disease Controland Prevention, Atlanta, USA announced that the possible etiologic agent of SARS is either a human metapneumovirus or a previously unrecognized coronavirus. Shortly, it was confirmed to be a novel coronavirus based on electron microscopy, immunostaining, seroconversion as well as RT-PCR and sequencing of polymerase gene fragment (Drosten *et al*., 2003; Ksiazek *et al*., 2003). Availability and affordability of DNA sequencing facilitated genotyping of several isolates of SARS-CoV. By 29th April 2003, complete genome sequences of SARS-CoVisolates, Tor2, Urbani, HKU-39849, CUHK-W1 and KYK were posted on the web. Facilities at the BCCA Genome Sciences Centre in Vancouver, Centers for Disease Control and Prevention in Atlanta, University of Hong Kong, Chinese University of Hong Kong, Genome Institute of Singapore and Beijing Genomics Institute were involved in thisseminal work. Many others are olates weresequencedand compared subsequently. The information obtained from such analyses was of epidemiologic significance. One, it revealed mutability of SARS-CoV, which, would have implications in vaccine development (Ruan *et al*., 2003; Tsui *et al*., 2003). Second, it led to the understanding that the organization of SARS-CoV genome is similar to the other coronaviruses although, at the primary sequence level, they were only distantly related (Rota *et al*., 2003). This ruled out the possibility of simple recombination event(s) among existing coronaviruses being responsible for the emergence of SARS-CoV. It was also indicative of the fact that this virus might have originated from animals. In fact, virus isolated from SARS patientswas able to cause a similar disease in cynomolgus macaques (Fouchier *et al*., 2003). Scientists began a search for the source of the SARS-CoV by scanning wild and domestic animals and indeed foundSARS virus-like coronaviruses from Himalayan palm civets and a raccoon dog found in a market in Guangdong, China. From sequence analysis, it was apparent that the viruses of human and civet origin shared more than 99% homology. However, phylogenetic analysis of S proteins, placed viruses of

human and civet origin in separate clusters. Also, the animalisolates contained a 29-nucleotide sequence in ORF 8 region that was absent in most human isolates (Guan *et al*., 2003).These analyses and the fact that palm civets did not show a widespread infection indicated that palm civetsmight not have been the natural reservoir host for SARS-CoV. Instead, they may have only served a medium to facilitate animal-to-human transition. This led to further searches inother animals including bats, rodents and monkeys for SARS-CoV host. SARS-CoV like viruses were found in bats. Sequence analysis showed that there was a significant homology between bat and human viruses. Interestingly, the 29-nucleotides in the ORF-8 was present in the virus of bat origin and the most variability between the two was found to be in the S1 region of spike protein, which is responsible for receptor binding (Li *et al*., 2005a). Findings from all such studies were put together to establish origin of SARS-CoV. The most accepted theory is that bats are the natural reservoirs of SARS-CoV. Civets and other wild animals came in contact with SARS-CoV infected bats in a market, where theyacquired the virus. It evolved in these animals before hoping to animals. Major species-specific determinants are traced to the viral S protein.

Even after the outbreakcame under control, scientists around the world continued to treat the situationas urgent and have made significant advances in understanding the SARS-CoV biology, developing diagnostics, identifying a number of drug targets, potential antivirals, tools for vaccines and immunotherapy for SARS.

1.1. Diagnostics

Early and sensitive detection of SARS-CoV is important not only for treatment but also for control of disease spread. Initially, clinical symptoms and epidemiologic linkage were diagnostics for SARS followed by serologic testing, viral culture and PCR-based methods (Wu *et al*., 2003; Yam *et al*., 2003). Now, reagents are also available for Nucleocapsid (N) protein and Spike (S) protein detection (Che *et al*., 2004; Sunwoo *et al*., 2012).

1.2. Antivirals

During the outbreak, spread of SARS-CoV was predominantly controlled by surveillance and quarantine. Agents that were usually adaptedfor treatment were ribavirin, corticosteroids, human interferons (IFN-β and IFN-γ) and convalescent plasma (Barnard *et al*., 2004; He *et al*., 2004; Keyaerts *et al*., 2004; Wu *et al*., 2004a; Cinatl *et al*., 2005; Groneberg *et al*., 2005; Lai, 2005; Morgenstern *et al*., 2005; Saijo *et al*., 2005; Lau *et al*., 2006; Stockman *et al*., 2006). However, a systematic review of clinical trials and *in vitro* studies revealed that although agents such as ribavirin, corticosteroids, lopinavir and type I interferon showed inhibition of SARS-CoV in tissue culture, their usefulness was inconclusive in most patient studies (Stockman *et al*., 2006). Some studies have in fact shown possible harm from some of them (Lau *et al*., 2006; Stockman *et al*., 2006). Since then, several other small moleculeshave been investigated for effect on SARS-CoV *in vitro* and are listed with their observed effects in **Table 1**. In addition, progress made in understanding cellular and biochemical processes of the virus has allowed the identification of several novel antiviral targets and molecules to inhibit them.

1.3. Entry Inhibitors

Three important steps for SARS viral entry into the host cell include its binding to the host cells through an interaction between viral spike protein (S protein) and its receptor, the angiotensin-converting enzyme 2 (ACE 2) followed by conformational changes in the S protein and its activation by proteolysis. Agents that target these steps have been identified and analyzed for their inhibitory effects on SARS-CoV entry. Classes of entry inhibitor include siRNA to spike protein gene (Qin *et al*., 2004), peptides or recombinant proteins derived from S protein (Ni *et al*., 2005; Sainz *et al*., 2006; Ujike *et al*., 2008; Struck *et al*., 2012) or ACE2 (Imai *et al*., 2005; Han *et al*., 2006), small molecules that bind S protein (Yi *et al*., 2004) and inhibitors of cellular protease (Simmons *et al*., 2005; Wang *et al*., 2007; Zhou *et al*., 2011). In addition, TNF-α Converting Enzyme (TACE) and lactoferrin bound to heparin sulfate proteoglycans have also been identified as targets for inhibition of viral entry (Haga *et al*., 2010; Lang *et al*., 2011).

1.4. Viral Protease Inhibitors

SARS viral replicase polyprotein is proteolytically processed by the viral proteases to generate functional enzymes. Owing to their essential role, 3CL protease, the main protease and the Papain-Like Protease (PLP2) of SARS-CoV are considered important drug targets. Based on homology modeling using crystal structures for human coronavirus and an inhibitor complex of porcine coronavirus, Anand *et al*. (2003) proposed that rhinovirus 3C protease inhibitors might be modified for inhibiting SARS protease (Anand *et al*., 2003; Regnier *et al*., 2009).

Table 1. Effect of antiviral agents on SARS-CoV *in vitro*

Agent	Major effects reported
Calpain	A class of cellular cysteine proteinases that inhibited SARS virus yield with an effective concentration in micro molar range (Barnard *et al*., 2004)
Niclosamide	An existing antihelminthetic drug that abolished viral antigen synthesis at concentration of 1.56 uM (Wu *et al*., 2004b)
Aurintricarboxylic Acid (ATA)	ATA is known to inhibit protein and nucleic acid interaction. It was reported to be 10 or 100 times more potent inhibitor of SARS-CoV than IFN-∝ and IFN-β, respectively (He *et al*., 2004)
Chloroquine	A clinically approved drug for malaria was effective with an IC50 in lower μM range (Keyaerts *et al*., 2004). In addition to its effect through elevation of endosomal pH, chloroquine seems to interfere with terminal glycosylation of ACE2, the the receptor for SARS-CoV (Vincent *et al*., 2005)
Nitric oxide	Nitric oxide donor S-nitroso-N-acetylpenicillamine inhibited SARS-CoV by 2 logs at 100 μM (Akerstrom *et al*., 2005)
Hydrocortisone	Only at very high concentrations, hydrocortisone showed a moderate effect on chemokine production by SARS-CoV (Cinatl *et al*., 2005)
Procyanidins and butanol extracts of cinnamomi cortex	A moderate inhibitory activity in wild-type SARS-CoV and HIV/SARS-CoV pseudovirus assay is reported (Zhuang and Jiang, 2009)
Synthetic peptides outside of spike protein heptad	S protein fragments spanning sequence variation hotspots reduced SARS-CoV infectivity significantly (Guan *et al*., 2003)
Dipeptide glutaminyl fluoromethyl ketone	An inhibition with EC50 value in low μmolar range is observed (Zhang *et al*., 2008)
Cyclopentenyl carbocyclic nucleosides	1,2,3-trizole analogs which, exhibited an antiviral activity with an EC50 of21 μM or 47 μM (Cho and Bernard, 2006)
Indomethacin	Inhibits viral RNA synthesis with > 1,000 fold reduction in CCo-V infected dogs (Amici *et al*., 2006)
Phenanthroindolizines	Tylophorine compounds inhibited SARS-CoV with EC50 in nM range (Yang *et al*., 2010)
Emodin	Emodin is shown to inhibit SARS-CoV via its ion channel protein, 3a (Schwarz and Wang, 2011)
Glycyrrhizin	Glycyrrhizin inhibits SARS but some of its derivatives showed reduced specificity (Hoever and Baltina, 2005)
Antisense Peptide Nucleic Acids (PNAs)	PNAs that were targeted to interfere with programmed -1 ribosomal shifting and fused to cell penetrating peptides resulted in inhibition of SARS-CoV replication with IC50 of 4.4 μM (Ahn *et al*., 2011)
Antisense morpholino oligomers	Oligomers targeted to Transcription-Regulatory Sequence (TRS) are reported to show a low inhibitory activity against SARS-CoV (Neuman *et al*., 2005)
SiRNA	siRNAs for various targets including, interferons, leader sequence or N protein have been tested (Li *et al*., 2005a; 2005b; Wu and Huang, 2005; Zhao and Qin, 2005; Tang and Li, 2008)

Homology modeling also formed a basis for designing mechanism-based irreversible inhibitors of 3CLpro with an activity of wide spectrum across coronaviruses (Yang *et al*., 2005a). Besides, several groups have identified a number of inhibitors of 3CLpro using a variety of approaches. Virtual screening (Plewczynski *et al*., 2007; Mukherjee *et al*., 2008; 2011; Nguyen *et al*., 2011) or a high-throughput screening of small molecule libraries have identified inhibitorsincluding an anti-HIV agent and serotonin antagonist, cinanserin (Blanchard *et al*., 2004; Kao *et al*., 2004; Wu *et al*., 2004a; Chen *et al*., 2005). Other 3CL protease inhibitors identified so far belongto categories such as plant derived phenolic or flavonoid compounds (Lin *et al*., 2005; Nguyen *et al*., 2012), active site, non-active site or competitive inhibitors (Kaeppler *et al*., 2005; Lee *et al*., 2005; Du *et al*., 2007; Ryu *et al*., 2010), ketones or ester based inhibitors (Goetz *et al*., 2007; Zhang *et al*., 2007; Ghosh *et al*., 2008; Shao *et al*., 2008; Verschueren *et al*., 2008; Zhang *et al*., 2008), modified peptidomimetic inhibitors (Ghosh *et al*., 2007), metal conjugated inhibitors (Lee *et al*., 2007; 2009), common inhibitors of Corona and Picornaviruses

(Kuo *et al*., 2009) and pyrimidines (Ramajayam *et al*., 2010). Protease inhibitors have also been reviewed elsewhere (Liang, 2006; Ramajayam *et al*., 2011).

In addition to its role in proteolytic processing of the viral polymerase, PLP2 is also involved in host evasion. Some of the first identified small molecule lead compounds for inhibition of PLP2 were thiopurine analogs (Chou *et al*., 2008; Chen *et al*., 2009). Besides, Ratia *et al*. (2008) have synthetically evolved a noncovalent inhibitor demonstrating an IC_{50} of around 15 μM in a cell based SARS-CoV replication assay (Ratia *et al*., 2008). Dooley *et al*. (2006) and Ghosh *et al*. (2009) have identified small molecules with EC_{50}valuesin lower micromolar range (Dooley *et al*., 2006; Ghosh *et al*., 2009). Recently, a yeast-based assay for measurement of papain-like protease activity that is suitable for screening of inhibitors was established (Frieman *et al*., 2011).

1.5. Helicase Inhibitors

Bismuth complexes and RNA aptamers have been shown to inhibit activity of SARS-CoV helicase (Yang *et al*., 2007; Jang *et al*., 2008; Adedeji *et al*., 2012; Keum and Jeong, 2012).

1.6. Host Pathway Inhibitors

Although, inhibitors of viral proteins have been used for treating some other viral infections, asignificant issue with targeting the viral proteins has been the development of drug resistant virus. This is likely due to the selection of mutant virus under drug pressure. Inhibitors of host systems, including immune and housekeeping, that may be critical for virus survival are alternatives that are worth an investigation. Cyclosporine and FK506 have emerged as examples of such inhibitors (De Wilde *et al*., 2011; Pfefferle *et al*., 2011; Carbajo-Lozoya *et al*., 2012). Other host pathway proteinsthat are potential antiviral drug targets have been identified (Ma *et al*., 2010; Bhardwaj *et al*., 2012; Millet *et al*., 2012; Smith *et al*., 2012; Zhao *et al*., 2012).

1.7. Vaccines and Immunotherapy for SARS-CoV

Coronaviruses cause significant infections in humans and animals. Although, no vaccines against coronaviruses are available at this time for use in human, they are produced for use in animals (Olsen *et al*., 1993; Anton *et al*., 1996). A need for prophylactic treatment ora vaccine is underscored by what happened during the 2002-2003 SARS outbreak. In the Vietnamese outbreak of SARS, all patients who died apart from the index patient were healthcare professionals including a WHO scientist, Dr. Carlo Urbani. It was Dr. Urbani's initiatives that led to the successful containment of the disease in Hanoi. He died of SARS on March 29th 2003.

Roberts *et al*. (2008) and Roper and Rehm (2009) have reviewed the SARS animal models and the initial vaccine studies in great detail (Roberts *et al*., 2008; Roper and Rehm, 2009). Several animal models that have been developed for SARS vaccine studies include mice, African green monkey, ferrets, macaques, hamsters and Chinese masked palm civet. Multiple labshave demonstrated the feasibility of various types of vaccines (**Table 2**). However, vaccine efficacy and safety issues are still being investigated (**Table 2**). Studies related to SARS vaccinehave taught us several lessons about pathogenesis and host responses to SARS-CoV, in addition to unraveling the need for caution. With certain experimental vaccines, such as the viral vector based ones, immunopathology and redirection of the viral vector to brain was reported (Czub *et al*., 2005; Deming *et al*., 2006; Kam *et al*., 2007; Jaume *et al*., 2011; Tseng *et al*., 2012). Subsequent studies demonstrated that a sub lingual immunization can prevent the viral vector entry into the brain (Shim *et al*., 2012). Also, an intranasal route of vaccination was shown to protect mice from SARS-CoV challenge better than an intramuscular delivery of the same vaccine (See *et al*., 2006; Hu *et al*., 2007). Expression of full length S protein is shown to result in enhanced hepatitis or infection whereas, expression of just the ectodomain of S protein eliminated infection enhancement (Weingartl *et al*., 2004; Yang *et al*., 2005b). All these reports point to that it will be important to establishan appropriatecombination of vaccination route, vaccine vector andchoice of epitopes for each vaccine type.SARS vaccines thatgeneratea predominantly cellular or a predominantly humoral response, as well as therapeutic monoclonal antibodies, have been shown protectiveeffects in animal models. Therefore, what kind of responses are important for protection has not been clear (Subbarao *et al*., 2004; Zakhartchouk *et al*., 2005; Lin *et al*., 2007; See *et al*., 2008; Zhao and Perlman, 2010). Cameron *et al*. (2012) have recently reported an analysis of transcripts expressed during SARS-CoV infection in vaccination and reinfectiontrials in ferrets (Cameron *et al*., 2012). Such studies can potentially reveal new therapeutic options in addition to providing the basic understanding of host responses during infection, vaccination and re-infection.

Table 2. Experimental vaccines for SARS

Vaccine type	Safety	Efficacy
Inactivated virus	Safe and immunogenic in humans (Lin *et al*., 2007). Hypersensitive reaction upon post-immunization viral challenge in mice (Kam *et al*., 2007; Tseng *et al*., 2012)	Efficacy needs to be established in appropriate animal model (See *et al*., 2008)
Recombinant vector Vaccines (Adenovirus, Poxvirus or recombinant)	Disease exacerbation upon SARS-CoV challenge in some cases (Czub *et al*., 2005)	Protective in mice, ferrets, monkeys, hamsters (Bukreyev *et al*., 2004; See *et al*., 2006; Napoli *et al*., 2007; See *et al*., 2008)
Subunit or virus like particle vaccines	Immunopathology is observed in some cases (Kam *et al*., 2007; Jaume *et al*., 2011; Tseng *et al*., 2012). Hypersensitive reaction upon post-immunization viral challenge in mice (Kam *et al*., 2007; Tseng *et al*., 2012)	Protective in hamsters and ferrets (Kam *et al*., 2007; Tseng *et al*., 2012)
DNA vaccines	Safe and immunogenic in healthy humans (Martin *et al*., 2008). Hypersensitive reaction upon post-immunization viral challenge in mice (Kam *et al*., 2007; Tseng *et al*., 2012)	Protective in mice (Yang *et al*., 2004)
Attenuated vaccines	Safety needs to be established	E protein lacking-or ExoN mutant vaccine is immunogenic and efficacious (Lamirande *et al*., 2008; Graham *et al*., 2012)

2. CONCLUSION

Although, a myriad of compounds have been identified to show inhibitory effects on SARS-CoV*in vitro*, only a few of those are reported for their safety and efficacy in animal models. Of the tested compounds, a hybrid interferon alpha (IFN-α) and an IFN- inducer, a mismatch double stranded RNA, have shown potent inhibition of SARS-CoV replication in the lungs of infected mice (Barnard *et al*., 2006). It would be useful and desirable to evaluate the other compounds in animal models for their safety and efficacy. With identification of epitopes that will not generate antibodies cross-reactive to self-antigens andcare taken to eliminate antibody dependent enhancement of disease, therapeutic use of human monoclonal antibodies seems a promising option for SARS. Coughlin and Prabhakar (2012) have reviewed the human monoclonal antibodiesgenerated for anti-SARS therapy (Coughlin and Prabhakar 2012). For active immunization, efficacy of the developed vaccines needs to be established in a most relevant disease model. Efforts have gone into improving animal models for SARS; however, they still have limitation.

A novel SARS-like human coronavirus, HCoV-EMC/2012 was identified earlier this year (Lu and Liu, 2012; Boheemen *et al*., 2012). Although, HCoV-EMC/2012 is only distantly related to SARS-CoV, the knowledge and reagents acquired from SARS-CoV research may prove useful in understanding and controlling this novel and other coronavirus (Elshabrawy *et al*., 2012; Graham *et al*., 2012).

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