Is regulation preventing the development of therapeutics that may prevent future coronavirus pandemics?

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In the last century, hundreds of new emerging infectious diseases (EIDs) have arisen in human populations most of which originate from wild animals as zoonoses [1]. The recent surge of zoonotic EIDs in human populations is driven by a constellation of socioeconomic factors including human population growth, eroding public health infrastructures, changes in land use and agriculture and ease of global travel. HIV, Ebola virus, avian influenza (H5N1, H7N9, etc.), severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are but a few recent examples of highly virulent, zoonotic viral EIDs that have catastrophically affected global economies and public health [2]. Geopolitical flux since the 1990s and the potential for weaponizing EIDs provoked the creation of myriad policies aimed at protecting the USA from bioterrorist threats and the accidental release of potential pandemic pathogens from laboratories. Are these policies effective? Are they impacting countermeasure development for current and future EIDs? How are they shaping the direction of individual research programs, the recruitment of new investigators and the stability of impacted fields? Below, we discuss our experiences in developing therapeutics against SARS-, MERS- and zoonotic CoV in an ever changing regulatory environment.

Gain of function (GOF) research is critical for microbiological research in determining causal relationships between a mutation and its phenotype. In 2012, two studies describing GOF mutations facilitating the transmission of highly pathogenic avian influenza in ferrets set off a firestorm of debate ultimately resulting in a 'pause' of active GOF research and its future funding [3]. While influenza research was primarily targeted, GOF policies were also extended to studies that could enhance the pathogenicity and/or transmissibility of SARS- and MERS-CoV in mammals, requiring regulatory approval of each experiment by NIH or its equivalent. Since the types of mammals were not specified and the assessment of mutant virus in humans or all the other approximately 5400 mammals was obviously not possible, the GOF policies by nature of their vagueness could never be adequately satisfied. During the 'pause,' a risk and benefit analysis conducted by the National Science Advisory Board for Biosecurity (NSABB) helped guide the creation of 'Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight (P3CO)' released in January 2017 [4,5]. Almost a year later, US Department of Health and Human Services (HHS) lifted the pause and released policy intended to guide funding decisions of new grant applications involving potential pandemic pathogens that have been 'enhanced' for pathogenicity or transmissibility [6]. While the policies for new grant applications are now clear and in place, the triage process of amendments to currently funded grant applications remains unknown, as oftentimes a new recombinant must be made to address an evolving research question.

For CoV researchers, regulatory complexity increased in 2012 when SARS-CoV was designated as a 'select agent' by the HHS and the Centers for Disease Control and Prevention (CDC) [7]. The Federal Select Agent Program (FSAP) requires those working with pathogens or toxins that pose a severe threat to public health to register and meet certain safety and security standards. Many of the 66 pathogens and toxins accompanying SARS-CoV on the select agent list are infamous including Ebola virus, smallpox and anthrax. As such, the FSAP has many positive

consequences including the registration of pathogens/toxins with the government, increased oversight on biosafety and biosecurity, increased standardization of high containment facilities and procedures, and research funding prioritization. For SARS-CoV, both the virus and its RNA genome became select agents as the genome itself is 'infectious' and can produce live virus if injected into mammalian cells under highly defined conditions. But what is SARS-CoV? Is it the epidemic strain that spread across the globe? Is it a bat virus that is 99.9% similar to SARS-CoV at the amino acid level, yet has never circulated among humans? What about chimeric viruses containing pieces of SARS-CoV? These questions illuminate the complexities in defining the FSAP policy, as biology is not binary but an ever-evolving continuum. For FSAP compliance, many US institutions spent tens to hundreds of thousands of dollars renovating BSL3 facilities. In addition to BSL3 renovations at UNC, an FSAP compliant BSL2 laboratory was created to process and analyze (i.e., qRT-PCR, sequencing, microarray, RNA-seq, etc.) samples containing SARS-CoV RNA since they could no longer be processed by core facilities or contracted out. Upkeep of select agent paperwork and annual inventory requires periodic review by a team of UNC Environmental Health and Safety Officers and their work is supported by 5–10% effort of each BSL3 worker and 35% effort of a facility manager. Initially, the CDC estimated the cost for FSAP compliance would be less than US$20,000/lab, but this is a gross under-estimate especially when personnel time is included. New policies and paperwork also evolve like the FSAP's policy for "validated inactivation procedures." All told, select agent work is serious business and the University's commitment to retaining a SARS-CoV research program has not been without sacrifice, approaching US$500,000 in facility renovation/maintenance costs over the years. Thus, quantitative data detailing the financial impacts of these policies and their effect on the current and future research programs are desperately needed, as the true impact is unknown and likely under-estimated.

The CoV family has a proclivity for emergence. This paradigm was established with the emergence of SARS-CoV in 2002 and was solidified with the identification of MERS-CoV in 2012 [8]. While epidemic SARS-CoV is no longer a threat, similar 'prepandemic' viruses (i.e., Bat CoV WIV1 and SHC014) have been found in bats in China that can readily infect human cells without adaptation and are thus poised for human emergence [9,10]. To maximize the benefit of therapeutics targeting virus families prone to emerge, they should be broadly active against both human and zoonotic viruses, which will likely seed future EID. To this end, we have constructed recombinant virus from infectious cDNA clones for multiple human and zoonotic CoV [9,11–15]. Mutation and manipulation of the cDNA clone is essential to identify determinants of pathogenesis and to gauge zoonotic virus pandemic potential. Unfortunately, the study of zoonotic and human CoV outside of their natural host often times requires genetic manipulation and GOF to be useful. For example, the epidemic strain of SARS-CoV, SARS Urbani, replicates to low levels in laboratory mice and does not cause clinical disease. Since mouse models mimicking human disease more powerfully test therapeutic benefit than those with virus replication only, SARS Urbani was passaged in mice producing a mouse-adapted strain (SARS-MA) with pathogenesis paralleling human disease [16]. SARS-MA, used across the globe, has been an invaluable tool for the rigorous assessment of therapeutics and also helped critically identify inactivated vaccine associated immune pathology leading to a refocusing of vaccine efforts into safer technologies [17]. Similarly and prior to the 'pause', we reconstructed SARS-like (i.e., Bat CoV HKU3) and MERS-like (Bat CoV HKU5) bat CoV that could replicate in mammalian cell lines but could not propagate suggesting receptor and virus spike glycoprotein incompatibilities [15,18]. To facilitate sustained replication in cell culture and mice, portions of the Bat CoV HKU3 and HKU5 spike glycoproteins were exchanged with those of SARS-CoV. As defined by the 2014 GOF policy, the creation of SARS MA15, HKU3 and HKU5 adapted viruses would have been prohibited, seriously eroding the development of key systems for therapeutic evaluation. In fact, the 'pause' halted all US efforts to adapt MERS-CoV in small animals. This moratorium was lifted months later for a few laboratories leading to the development of several animal models that replicate many aspects of human disease. Given that there are no US FDA approved therapies for any CoV, these mouse models have been essential for the preclinical development of multiple MERS-CoV therapeutics.

We recently reported a nucleoside analog (GS-5734) capable of preventing SARS-CoV disease in mice and could inhibit replication of multiple CoV in cell culture [19]. Interestingly, GS-5734 is also efficacious against Ebola virus and respiratory syncytial virus [20]. Phase I clinical trial for GS-5734 has been completed and a feasibility assessment for conducting a Phase II trial for MERS-CoV is underway. Importantly, we determined GS-5734 had broad-spectrum efficacy against the CoV family through challenge with our diverse panel of human and zoonotic bat CoV discussed above. This analysis would not have been possible without passage of CoV to increase pathogenicity, the shifting of zoonotic CoV host range to infect human cells, and the reconstruction of 'prepandemic' SARS-like bat CoV poised for emergence (i.e., WIV1, SCH014). Since several branches of the CoV family tree have not

yet been evaluated with GS-5734, additional zoonotic CoV must be constructed, which will likely require genetic manipulation, adaptation or a host range shift to be tractable for use in the laboratory. The generation of genetically diverse panels of emerging virus is absolutely essential to rigorously evaluate therapeutics and proactively gauge their efficacy against pre-epidemic zoonotic viruses. Given the massive amount of morbidity and mortality associated with EIDs over the past 30 years, the balance between public health risk and inaction is clear. For EIDs, the myopic 'one bug, one drug' approach will forever be regressive as the therapy specifically designed for the past epidemic strain (e.g., SARS-CoV vaccine) will likely fail against future emergence (i.e., MERS-CoV). Therefore, we must change the paradigm to 'one drug, many bugs' and prospectively develop broadly active countermeasures within and across virus families. Regulation must adequately protect public safety but should also be fluid enough to evolve in step with EIDs. Despite the best intentions, one of the greatest risks of biosafety policy is unintended over-reach that limits our understanding of EID biology and prevents countermeasure development that could limit or prevent future pandemics.

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