Advantageous non-specific effects of live-attenuated vaccines in COVID-19 treatment

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Abstract

The currently ongoing COVID-19 pandemic has driven an urgent need to develop treatments and preventative measures against this phenomenon, particularly given the devastation that the ongoing situation has wrought on the global economics, medical, and social arenas. This dire situation has driven a monumental global effort has to urgently produce suitable vaccines to prevent and stem COVID-19. However, there remains a lack of consensus as to what constitutes a safe and effective COVID-19 vaccine strategy, with current trials not designed to detect a reduction in the likelihood of severe illness and stemming of disease transmission. Critically, however, most indicators suggest that millions of high-risk individuals will not gain access to vaccines any time soon (persons ≥ 65 years of age, persons with underlying conditions, the economically deprived, and various ethnic minorities). Considering such concerns, perhaps deployment of existing vaccinations with documented results could be deployed to assist in interim efforts to stem the spread of COVID-19. Some vaccines such as the Bacilli-Calmette-Guerin (BCG) vaccine may confer non-specific protection or effects (NSE) against disease other than its intended target. In this article, we discuss recent efforts to investigate how such approaches may be beneficial and present our hypothesis that such non-specific events of similar vaccines may assist in prevention of severe disease while specific COVID-19 vaccines are further developed and made available to the most high-risk individuals.

Keywords: COVID-19; SARS-CoV-2; Vaccines; Bacilli-Calmette-Guerin (BCG); Polio vaccine (OPV)

Introduction

The current COVID-19 outbreak in December 2019 caused a devastating ripple of events in the international community caused by SARS-CoV2, a zoonotic coronavirus that has increasingly become difficult to control. There is thus a pressing need to improve our understanding of the immunology of this disease to develop treatments and preventative measures against this phenomenon.[1-3]

Coronaviruses (CoV) are enveloped, single stranded positive sense RNA (ssRNA) belonging to the Coronavirinae family, of which seven are known to infect humans.[3] Human SARS-CoV-2 exhibits a classical flu-like clinical presentation in more than 80% of patients who have mild to moderate and self-limiting disease, with an estimated incubation period of anywhere between 2-14 days. Asymptomatic presentation has also been recorded in a significant number of individuals, underlying increases in unsuspected transmission.[4] SARS-CoV-2 enters target cells via a spike protein (S). An interesting feature of S is the mediation of cell fusion and formation of syncytia, used to elude immune cells.[3] The helical envelope of the SARS-CoV-2 also contains the matrix protein (M), nucleocapsid (N), and envelope protein (E). SARS-CoV-2 has been observed to enter the respiratory epithe-lium by means of the angiotensin converting enzyme 2 (ACE2) receptors, also present in the gastric endothelium, potentially explaining the diarrhea and nausea symptoms of COVID-19. A¬CE2 catalyzes the conversion of angiotensin I to angiotensin II, contributing towards maintenance of fluid and electrolyte homeostasis, and entry also interestingly seems to be aided by two host proteases ADAM17 and TMPRSS2.[5] Viral load and repeated expo- sure to the virus are important factors determining disease severity, with clinical deterioration usually observed at the end of the second week following development of a cytokine storm, causing disseminated intravascular coagulation (DIC). Many aspects of severe COVID-19 infection are unique, rarely occurring in other respiratory viral infections, including severe lymphopenia and eosinopenia, extensive pneumonia and lung tissue damage, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction (MOD). Marked elevation of the acute phase reactants levels like ESR, CRP, ferritin and lymphopenia are early indicators of high disease severity.[5-7]

The COVID-19 vaccine landscape

As of writing this article, SARS-CoV-2 has resulted in well over 58 million global infections, a global death toll exceeding 1.3 million, and an almost unquantifiable destruction of the global economy far exceeding trillions of dollars.[8] The current pandemic has, continues to, and will further, devastate the most vulnerable in our societies; persons ≥65 years of age, persons with underlying conditions, the economically deprived, and perhaps by extension, various ethnic minorities.[9] Thus, a monumental and herculean effort has been mounted worldwide to urgently produce a suitable vaccine to prevent and stem COVID-19.[10] Indeed, it seems to have become widely accepted that global normalcy will not return until safe and effective vaccines are available and deployed via an effective global vaccination programme.[11,12] However, considering that COVID-19 represents a new phenomenon to afflict humanity, the nature of protective immune responses is poorly understood, compounded further by a lack of clarity or consensus regarding mechanisms and pathogenesis of infection. To this degree, despite at least 166 vaccine candidates are currently in preclinical and clinical development (Table 1), it is unclear which strategies will be most successful.[11] However, despite such monumental efforts, there remains a lack of

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Table 1: *Details of candidate vaccines currently under clinical evaluation at Phase 3 trials. Table modified from (WHO Draft landscape of COVID-19 candidate vaccines–12 November 2020. Available from https://www.who.int/publications/m/item/draft- landscape-of-covid-19-candidate-vaccines last Accessed 23rd November 2020)*

COVID-19 Vaccine developer/ manufacturer	**Vaccine platform**	**Type of candidate vaccine**	**Number of doses**	**Timing of Doses**
Sinovac	Inactivated	Inactivated	2	0, 14 days
Wuhan Institute of Biological Products/ Sinopharm	Inactivated	Inactivated	2	0, 21 days
Beijing Institute of Biological Products/ Sinopharm	Inactivated	Inactivated	2	0,21 days
Bharat Biotech	Inactivated	Whole-Virion Inactivated	2	0,28 days
University of Oxford/ AstraZeneca	Non - Replicating Viral Vector	ChAdOx1-S	2	0,28 days
CanSino Biological Inc./Beijing Institute of Biotechnology	Non - Replicating Viral Vector	Adenovirus Type 5 Vector	1	-
Gamaleya Research Institute	Non - Replicating Viral Vector	Adeno-based (rAd26-S+rAd5-S)	2	0,21 days
Janssen Pharmaceutical Companies	Non - Replicating Viral Vector	Adenovirus Type 26 vector	1	0
			2	0,56 days
Novavax	Protein Subunit	Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	2	0,21 days
Moderna/NIAID	RNA	LNP-encapsulated mRNA	2	0,28 days
BioNTech/Fosun Pharma/Pfizer	RNA	3 LNP-mRNAs	2	0,28 days

consensus as to COVID-19 vaccine strate- gy.[12] The rapid pace of COVID-19 vaccine development has been enabled by several factors including existing knowledge of spike protein pathogenesis in coronaviral infection; advances allowing creation and prompt manufacture of thousands of vaccine doses; and refinement of vaccine development for stages to be conduct- ed in parallel, rather than sequentially, without increasing risks for study participants.[10-15]

Potential setbacks to a newly designed COVID-19 vaccine

A number of uncomfortable truths require confrontation to view the recent advances made in the context required. Traditionally, the vaccine industry has long been underfunded, espousing low production capacity, difficulty in predicting infectious outbreaks, and assessing the risk to benefit ratio. Moreover, vaccines are generally manufactured for healthy adult populations between the ages of 18 to 65 years, and do not include the pediatric, geriatric or immunocompromised populations – perhaps the largest population vulnerable to COVID-19. There has also been a long history of scarcity of interest in vaccine production by pharmaceutical companies.[16]

COVID-19 vaccine development has employed both traditional methods (live attenuated and inactivated vaccines), and next generation vaccines, with the latter including vaccines that are already known in the market such as DNA vaccines and novel vaccine techniques such as mRNA vaccines and recombinant spike protein (S) vaccine.[17] Considering that the usual timeline for vaccine development is three to nine years in normal vaccine trials, most COVID-19 vaccine trials are occurring at breakneck speed (Figure 1).[10,18] While such monumental efforts have led to a number of vaccine candidates that have elicited much hope regarding effectiveness, including those from Pfizer/BioNTech, University of Oxford/Astrazeneca, Moderna/NIAID, and Sinovac/Sinopharm/CanSino candidates, there still are concerns regarding whether these vaccines will enable an effective response to the virus itself.[11,19]

Figure 1: *Schematic representations indicating a) the traditional length of time required for vaccine development in relation to the exhaustive steps requiring completion before a vaccine can be approved for use, compared to b) vaccine development against SARS- CoV-2 in comparison to vaccine development for more recent viral examples. Figure 1a was inspired by Heaton (2020), while 1b was modified and re-used from Funk et al., [54] under the Creative Commons Attribution License (CC BY).*

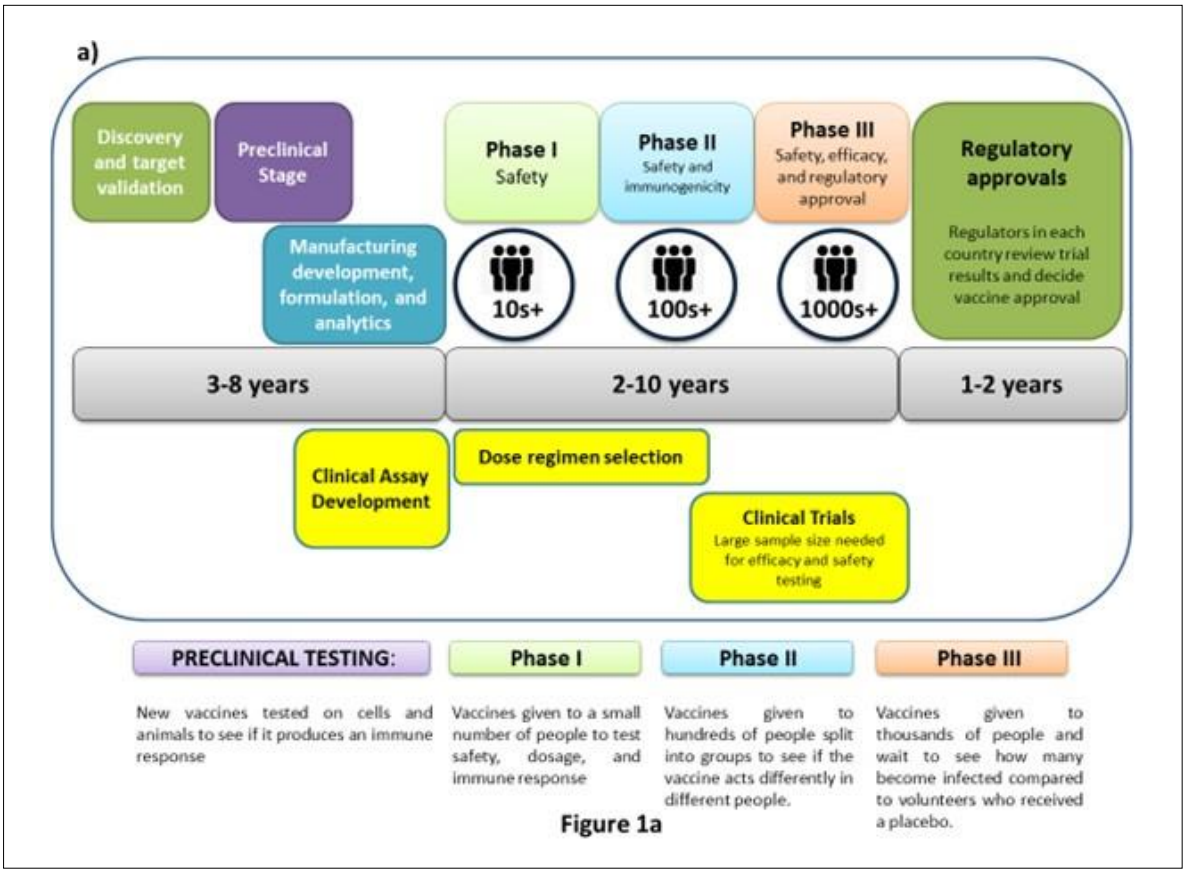

Figure 1a

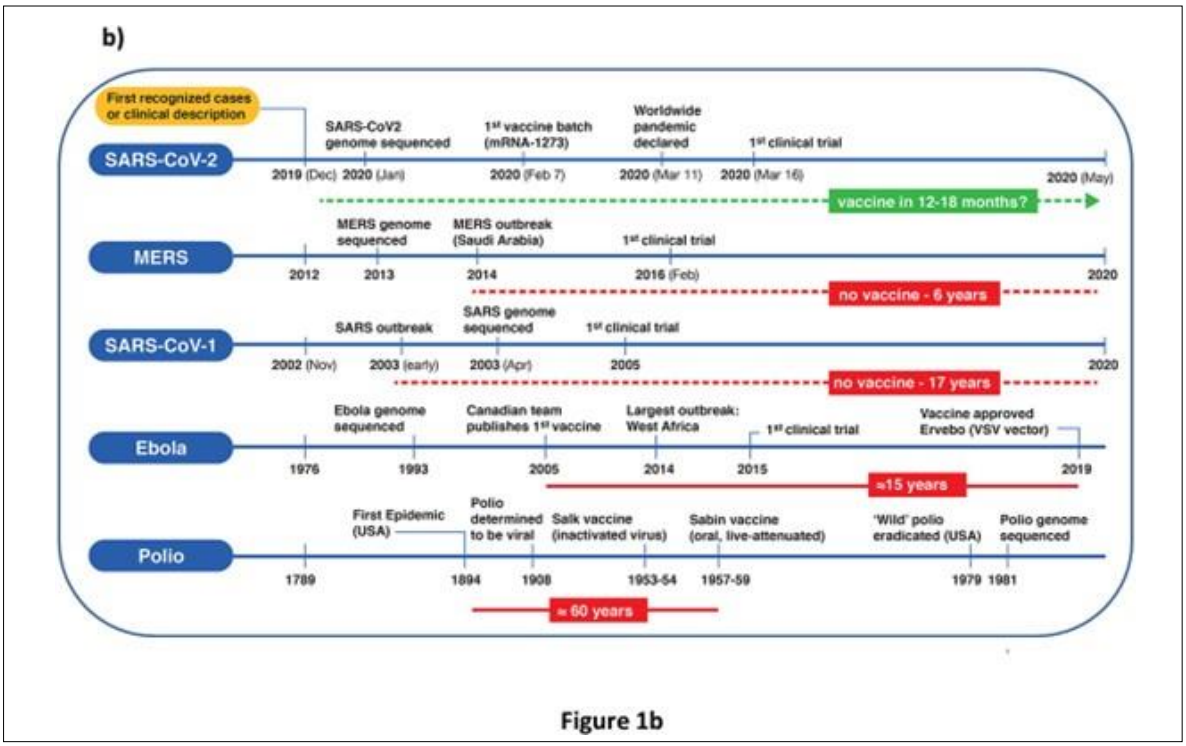

Figure 1b

It is well accepted that drugs or biological agents will cause specific side effects or adverse reactions, and utility of such agents is determined by balancing the advantageous against disadvantageous affects.[19,20] Current criteria, at least in the USA, limit the window to identify adverse reactions to 2 months. Adenoviral vaccine adverse effects include fever, pneumonia, diarrhoea, transient neutropenia and lymphopenia, fatigue, laboured breathing, headaches, liver damage, and fasting hyperglycaemia. Further more significant, yet rarer, side effects include neuropathies, Guillain-Barré syndrome, gait disturbance, specific inflammatory conditions.[20] Perhaps these side effects are caused by pre-existing acquired anti-adenoviral immunity from previous infections, promoting long-lived B and T cell responses and increased production of antibodies.[20]

Some other more recent vaccine candidates against SARS-CoV-2 are also utilising more cutting-edge technologies incorporating DNA/RNA-based vaccine approaches. Such approaches utilise fragments of recombinant genetic material coding viral components (such as S), injection of which induces the body to produce this antigen, inducing an immune response and memory.[21] However, while such vaccines can be rapidly designed and cheaply manufactured, there are currently no approved similar vaccines types for medical use in humans. Furthermore, such vaccines would only allow immunity against specific fragments of the virus, potentially prompting a relatively poor protective immune response requiring multiple 'top-ups'. Then there is also the relatively low, yet still theoretically valid, possibility that such DNA/RNA fragments could be incorporated into the human genome.[21] Indeed, these and many other vaccine types are currently being considered and investigated, each specific strategy containing its own specific pros and cons which need considering (Table 2).[19,22]

Table 2: *Details of advantages, disdvantages, and currently used examples of vaccine types currently in use. Table modified from Ng et al., (2020).*

Vaccine type	Advantages	Disadvantages	Currently approved examples
DNA	Low Cost Safe	Unstable immunogenicity	
		Multiple doses required	
RNA	Low Cost Safe Stable No anti-vector immunity	Unstable/low immunogenicity	
		Potential genome integration	
Viral vectors	High efficiency and specificity increased and robust immune responses	Low titer Anti-vector immunity Potential tumorigenesis	JYNNEOS (Smallpox/Monkeypox) • ACAM2000 (Smallpox) • Adenovirus type 4 and type 7 vaccine, live, oral (febrile acute respiratory)
Inactivated viruses	Safe Wide patient use	Low titer Multiple doses required	Polivax (polio) Flucelvax Quadrivalent (Influenza) Ixario (Japanese Encephalitis) Imovax (Rabies)
Live attenuated viruses	High and long lasting potency Low cost	Possible regression to virulence strain Limited population applicability	ERVEBO (Ebola virus) MMR II (Measles, Mumps, and Rubella) BCG vaccine (Tuberculosis)
Protein subunits	Safe Wide patient use	Low immunogenicity Potential batch-wise variation	PedvaxHIB (Haemophilusinfluenzae type b) Engerix-B (Hepatitis B) Recombivax HB (Hepatitis B)

Hypothesis: Non-specific effects of vaccines meant for other targets may prove beneficial towards preventing severe COVID-19.

Considering the various concerns surrounding the production of a COVID-19 vaccine, perhaps it would be advisable to consider existing strategies, utilised for a number of years with documented results and potential side effects, to aid in the battle against COVID-19 and perhaps assist in interim efforts to stem the spread of this disease. Some vaccines such as the Bacilli-Calmette-Guerin (BCG) vaccine are able to broadly stimulate the innate immune system to confer non-specific protection or effects (NSE) against disease other than tuberculosis, such as bladder carcinoma, asthma or influenza.[17] BCG confers immunity by "training" components of the innate immune system through NOD2 receptor signaling pathways in macrophages, leading to macrophage stimulation of cytokine release such as TNF-α and IL-6 against non-specific pathogens, and upregulation of toll-like receptor (TLR) ligands. Natural killer (NK) cells, another innate cell component, are also "trained" to develop an immunological memory.[23]

There is considerable evidence that BCG has non-tuberculosis

protective effects.[24] BCG may also provide non-specific protection against S. aureus and C. Albicans, leading to a heightened secretion of pro-inflammatory cytokines such as IL-6, IL-1 by natural killer cells (NK cells).[25] Collectively, evidence suggests immune-boosting effects of BCG on COVID-19 patients, alerting the immune system to prepare against SARS-CoV2. Indeed, an increased mortality rate was observed in countries with no mandatory BCG vaccination, namely the USA.[26] However, while epidemiological studies have shown a lack of association between a nation-wide BCG vaccination policy and decreased COVID-19 mortality, this is not entirely representative due to a of lack of adjustment for huge differences between countries, rural or urban areas, time of pandemic start, the criteria for testing, or number of tests. Indeed, when a BCG index (the degree of national BCG vaccination) was employed, a 10.4% decrease in COVID-19 mortality for every 10% increase in the BCG index was observed.[24]

Several other live attenuated vaccines including those against Measles, Mumps, and Rubella (MMR) and the Polio vaccine (OPV) also show non-specific immunity, reducing mortality, hospital stay and development of herd immunity. The measles component of the MMR vaccine specifically led to NSE, in contrast to the deleterious effects of inactivated vaccines, such as diphtheria-tetanus-pertussis (DTP), that lead to increased mortality.[27] The beneficial effects of OPV have also been studied since the 1960's, reducing morbidity and mortality. Such benefits have also been shown in measles, smallpox and BCG vaccine. An OPV vaccination study in 1998, Guinea-Bissau showed decreased mortality in children under the age of five, for diseases other than polio.[28] A randomized clinical trial is currently being undergone in USA on "OPV as Potential Protection against COVID-19", while milder COVID-19 symptoms in US Navy recruits may have been a consequence of the MMR vaccine.[29,30]

Antigen sharing:

To explore potential antigen sharing, Shelly et al., [31] compared SARS-CoV-2 epitopes with OPV and BCG epitopes using protein basic local alignment search tool (BLAST).[31-33] Finding a 80% similarity between SARS-CoV-2 open reading frame 7a (Orf7a) protein epitope with the human poliovirus type 3 Sabin strain epitope. Similarly, the SARS-CoV-2 nucleocapsid epitope shared an 87% similarity with part of the human poliovirus type 1 Mahoney epitope.[31] While no such direct epitope sequence matching was observed found between BCG and SARS-CoV-2. However, such analyses only examined antigen similarity not taking into account 3D structure and subcellular localization, which are required to fully antibodies cross-reactivity.[31]

For most of these vaccines, the exact measurement of protection provided is unknown. However, it would seem indubitable that at least some level of immunity is provided. Measles-containing vaccines reduce mortality by 30-86%; this reduction offered is more than the mortality caused by measles itself. A possible mechanism of action of the NSE is cross reactivity between various vaccines and antigens. An alternative hypothesis states that BCG activates T helper 1 and 17 cells polarization, T-helper cell generation, NK memory and cytokine induction.[28,34] BCG had become the main vaccine to protect children against tuberculosis since 1924, substantiated by a clinical study showing a 40% reduction in neonatal mortality in infants that were immunized with BCG at birth.[28]

BCG vaccine applications for COVID-19

Two mechanisms have been proposed to explain BCG's non-specific effects, namely heterologous or trained immunity. The heterologous immunity theory proposed that BCG vaccine antigens elicit cross-reactive antibody production against other pathogens, while the trained immunity hypothesis suggests that innate immune cells are 'trained' to develop a pro-inflammatory response following BCG stimulation.[35] Indeed, derivatives of fungi and bacteria, such as BCG, Lipopolysaccharide (LPS), and Glucan, can indeed train innate immune cells, which in the case of BCG is characterized by metabolic alterations, and upregulation of innate immune receptors with BCG-induced trained-immune responses in humans noted for upto 3-12 months. [28,28,36,37-41] Studies utilising animal models indicate an enhanced protection from viruses including herpes simplex, influenza A, vaccinia, and Japanese encephalitis infection, following BCG vaccination.[34]

A study examining adults aged 35–41 years suggested that BCG vaccination during childhood was associated with a similar rate of positive SARS-CoV-2 test rates compared with no vaccination indicating that the BCG vaccine may not reduce the likelihood of acquiring the virus.[42] However, a retrospective cohort study examining individuals 18 or older in the USA found that COVID-19 patients with BCG vaccination were less likely to require hospital admission, perhaps suggesting a potential effect in preventing severe symptoms rather than preventing infection.[23,43,44]

OPV applications for COVID-19

The OPV consists of live attenuated polioviruses, administration of which not only seemed to result in protection from poliomyelitis, but also seemed to reduce the number of other viruses isolated from immunized children, with the most plausible explanation proposed being viral interference mediated by innate immunity.[45,46] Further studies indicated that OPV administration was effective against influenza infection in reducing morbidity, and a therapeutic effect on genital herpes simplex virus infections. OPV also demosntrated oncolytic properties, both by directly destroying tumor cells and by activating cellular immunity toward tumors.[47,48,49]

Indeed, mass immunization with OPV helped to control an outbreak of unrelated acute poliomyelitis-like disease caused by Enterovirus 71 in Bulgaria.[47] Furthermore, OPV reduced the burden of bacterial diarrheal disease in infants, and was associated with decreased middle ear infections which can be caused by both viruses and bacteria.[50,51] Furthermore, OPV usage also seemed associated with reduced hospital admissions for respiratory infections in children.[46] Perhaps OPV ameliorates/prevents COVID-19 as both the poliovirus and coronavirus are positive-strand RNA viruses and may be governed by common innate immunity mechanisms. Such data, coupled with a recorded safety, more than one serotype to sequentially prolong protection, low cost and ease of administration, and availability, could indicate the supreme usefulness of OPV in the fight against SARS-CoV-2 and COVID-19.[46,48,49] Indeed, OPV seems safer compared to BCG, with ~1% of BCG recipients exhibiting adverse reactions requiring medical attention, while such risks are relatively rarely associated with OPV.[46]

Non-specific effects of live-attenuated vaccines may prove useful in COVID-19 treatment and spread prevention

Considering the collective evidence, there is substantial ground to test NSE of vaccines such as BCG, OPV and measles-containing vaccines in combating the current challenges of the pandemic. Ideally, an antiviral vaccine should reduce the likelihood of severe illness and hospitalisation, and interrupt further disease transmission. However, the current trials underlying the most promising candidates do not seem designed to detect a reduction in any of these criteria.[52] Furthermore, Pfizer and Moderna

collectively estimate availability of enough doses for ~35 million individuals in 2020, reaching ~1 billion by 2021, indicating that millions of high risk individuals will not gain access to vaccines any time soon.[53]

Even if one does not consider the considerable logistical challenges of manufacturing and distributing successful vaccine candidates, equitable access is still an unaddressed issue. While COVAX, a financing mechanism intended to provide COVID vaccines to low and middle-income countries has raised US$ 2 billion, it still requires another $ 5 billion to meet its targets in 2021. Furthermore, Pfizer and Moderna have not yet reached agreements with COVAX to supply vaccines, while a handful of high-income countries have already bought hundreds of millions of doses. Indeed, profit limitation does not seem a priority area for most vaccine producers.[53] To this degree, utilisation of existing, and perhaps more reliable resources would benefit a maximal number of people compared to only a 'new vaccine'-centric approach. However, perhaps a more pertinent point to consider is that it remains likely that vaccines will perhaps be just one determinant affecting our responses to the ongoing pandemic. The sheer number of unknowns regarding the current crisis is astounding; the nature and length of immune responses, possibility/severity of reinfection following vaccination, immunity variation in gender ethnicity and age, the possibility of seasonal outbreaks and viral re-emergence.[54]

Acknowledgements

The manuscript was conceived by AY and JK, and all authors contributed towards writing the manuscript, which was submitted following approval of all authors. This work was supported by a COVID-19 project grant (#951) awarded by the Saudi Arabian Ministry of Health awarded to AY, JK and KK.

Conflict of interest statement

The authors report no conflict of interest.

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