Heliyon 9 (2023) e17148

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Multidimensional futuristic approaches to address the pandemics beyond COVID-19

Shifa Bushra Kotwal [a,1], Nidhi Orekondey [a,1], Gargi Prasad Saradadevi [a,1], Neha Priyadarshini [a,1], Navinchandra V. Puppala [a,1], Mahak Bhushan [b], Snehasri Motamarry [a], Rahul Kumar [a], Gireesha Mohannath [a,**], Ruchi Jain Dey [a,*]

[a] Department of Biological Sciences, BITS Pilani, Hyderabad Campus, Telangana 500078, India
[b] Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Kolkata, West Bengal 741246, India

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Diagnostics
Therapeutics
RNA mimics Of GFP
RPA
Lab-on-Chip
Ribozyme
Vaccines
BCG

ABSTRACT

Globally, the impact of the coronavirus disease 2019 (COVID-19) pandemic has been enormous and unrelenting with ~6.9 million deaths and ~765 million infections. This review mainly focuses on the recent advances and potentially novel molecular tools for viral diagnostics and therapeutics with far-reaching implications in managing the future pandemics. In addition to briefly highlighting the existing and recent methods of viral diagnostics, we propose a couple of potentially novel non-PCR-based methods for rapid, cost-effective, and single-step detection of nucleic acids of viruses using RNA mimics of green fluorescent protein (GFP) and nuclease-based approaches. We also highlight key innovations in miniaturized Lab-on-Chip (LoC) devices, which in combination with cyber-physical systems, could serve as ideal futuristic platforms for viral diagnosis and disease management. We also discuss underexplored and underutilized antiviral strategies, including ribozyme-mediated RNA-cleaving tools for targeting viral RNA, and recent advances in plant-based platforms for rapid, low-cost, and large-scale production and oral delivery of antiviral agents/vaccines. Lastly, we propose repurposing of the existing vaccines for newer applications with a major emphasis on Bacillus Calmette–Guérin (BCG)-based vaccine engineering.

1. Introduction

The world has been fighting a fast-spreading global pandemic caused by the novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). So far, more than ~765 million people across the world have been infected with ~6.9 million deaths (as of 2nd May 2023) (https://covid19.who.int/). However, according to the WHO, the number of infections and deaths is estimated to be much more than what has been reported. The prolonged raging of the pandemic has caused loss of livelihoods and negatively impacted the global economy, mental well-being, education, and other essential services and human activities.

The coronaviruses (CoVs) are single-stranded ribonucleic acid (RNA) viruses that carry a genome of ~30 kb and are of size ranging

* Corresponding author.
** Corresponding author.
E-mail addresses: gireesha@hyderabad.bits-pilani.ac.in (G. Mohannath), ruchij80@hyderabad.bits-pilani.ac.in (R.J. Dey).
[1] Equal contributing authors.

https://doi.org/10.1016/j.heliyon.2023.e17148
Received 27 October 2022; Received in revised form 1 June 2023; Accepted 8 June 2023
Available online 11 June 2023

between 65 and 125 nm in diameter [1]. CoVs belong to the order Nidovirales, family *Coronaviridae*, and subfamily *Orthocoronavirinae*, which is further divided into four genera: Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV) [2,3]. The SARS-CoV-2 shares genomic similarities with other β-CoVs associated with the past epidemics [4, 5], and it consists of 16 non-structural proteins (nsps) and four structural proteins [1].

Mammals are known to be reservoirs of α-CoVs and β-CoVs, while birds mainly harbour γ-CoV and δ-CoV, with some reports indicating infections in mammals [6]. In general, bats, rodents, and avian species are known to be the natural reservoirs of diverse CoVs [7–10]. SARS-CoV-2 has been predicted to be transmitted to humans from bats through an unknown intermediate, which remains to be proved with supporting data [11]. It is also speculated that the virus might have undergone virulence- and transmission-enhancing mutations in humans, further resulting in human-to-human transmission [12–14]. For details regarding SARS-CoV-2 variant-specific virulence and transmission rates, refer to Carabelli et al. [14].

The genome of SARS-CoV can mutate at a rate of 0.80–2.38 $\times$ 10^{-3} nucleotide substitutions per site per year. Such high mutation rates are ascribed to a lack of or poor proof-reading activities of the viral RNA polymerase [15]. Consequently, these viruses evolve rapidly, aiding them in evading the host immune system accompanied by enhanced viral infectivity, replication, and mortality [16]. Faster mutation rates help viruses develop resistance to drugs, escape antibody response [14], switch hosts, and could reduce the efficiency of nucleic acid-based viral diagnosis [12,17].

Over the last two years, several mutant variants differing in their genomic and antigenic properties have been characterized. Among several variants of SARS-CoV-2 identified, some have been declared as variants of concern by the WHO based on their global impact concerning infectivity and mortality. A recent epidemiological survey by the WHO (as of November 2021) (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/), suggests five variants of concern, namely, Alpha (B.1.1.7; first reported in United Kingdom (UK) in late December 2020); Beta (B.1.351, first reported in South Africa in December 2020); Gamma (P.1, first reported in Brazil in early January 2021), Delta (B.1.617.2, first reported in India in December 2020) [18] and Omicron (B.1.1.529, first reported from multiple countries). All the variants of concern were found to have mutations in the receptor-binding domain (RBD) and the N-terminal domain of the spike (S) protein which may have implications for the viral pathogenesis. Some of these mutations are predicted to cause increased affinity of the S protein to human angiotensin-converting enzyme 2 (hACE 2) receptors enhancing viral attachment and entry into the host cells. These domains are also targets of the neutralizing antibodies produced in response to vaccines.

The research findings over the past two years have enhanced our understanding of SARS-CoV-2 aetiology and pathophysiology of the disease. The SARS-CoV-2 infection causes multiple disease phenotypes such as pulmonary dysfunction, haematological alterations, inflammation, electrolyte imbalance, coagulation dysfunction with an increased risk of venous and arterial thromboembolism, liver and kidney dysfunctions and cardiac muscle injuries [19]. Due to the diverse symptoms, it has been challenging to diagnose and manage the disease in a timely manner. Moreover, a lack of clarity about the impact of comorbidities (e.g., cardiomyopathies, hypertension, diabetes, etc.) on SARS-CoV-2 infection and disease progression made disease management even more challenging [20].

Clinical studies have played a significant role in identifying pathological markers to differentiate disease progression and resolution. These studies have also played a key role in determining the antigenic/protective nature of specific viral proteins, leading to the development of viral diagnostics, therapeutic, and protective strategies. Despite the faster pace of vaccine development and mass vaccination, the continuous emergence of variants of concern poses a significant hurdle in the global efforts to end the pandemic [18].

Upon the dawn of any new viral pandemic, until the arrival of vaccines or drugs to effectively prevent or treat the viral infections, reliable and rapid early diagnosis of the virus and quarantining the infected patients is critical to control the spread of the outbreaks. The symptomatic diagnosis of COVID-19 was challenging, as most of its symptoms are shared by infections caused by other common respiratory viruses. Therefore, viral genome detection-based diagnostics were developed and became the mainstay of pandemic management.

2. Viral diagnostics

2.1. Methods employed to detect viral genome

Several methods are currently being used to detect SARS-CoV-2. One of the most used approaches is based on the molecular detection of viral RNA by nucleic acid amplification test (NAAT). With a detection capability of as low as 1 to 10 copies of the viral genome/μL, NAAT has been considered one of the most sensitive assays for viral diagnostics [21]. Labs across the world have primarily been using reverse transcription followed by polymerase chain reaction (RT-PCR)-based methods for detecting the virus using RNA extracted from the nasopharyngeal or oral swab samples of the suspects. It takes approximately 5–6 h for the RT-PCR method to generate results, excluding the time taken for sample collection and transportation. It is considered as the gold standard method for diagnosis. It has some advantages, such as it can be automated, multiplexed, and used for high-throughput testing and analysis. A major disadvantage of this method is that it requires expensive equipment (thermocycler) and expertise for testing and analysis. Therefore, this method is not suitable for point-of-care (POC) or resource-poor settings.

Another method of significance that has been used to detect viral genomes including SARS-CoV-2 is loop-mediated isothermal amplification (LAMP) method [22–25]. To detect RNA, LAMP coupled to reverse transcription (RT-LAMP) is used. LAMP involves auto-cycling strand displacement DNA synthesis by utilizing a DNA polymerase possessing high strand displacement activity and two pairs of primers [26]. LAMP is performed at a constant temperature of 60–65 °C in a single tube and it does not require a thermocycler [26]. It is cost effective and one of the rapid assays requiring only ~30 min to complete a test, making it suitable for POC or resource-poor settings.

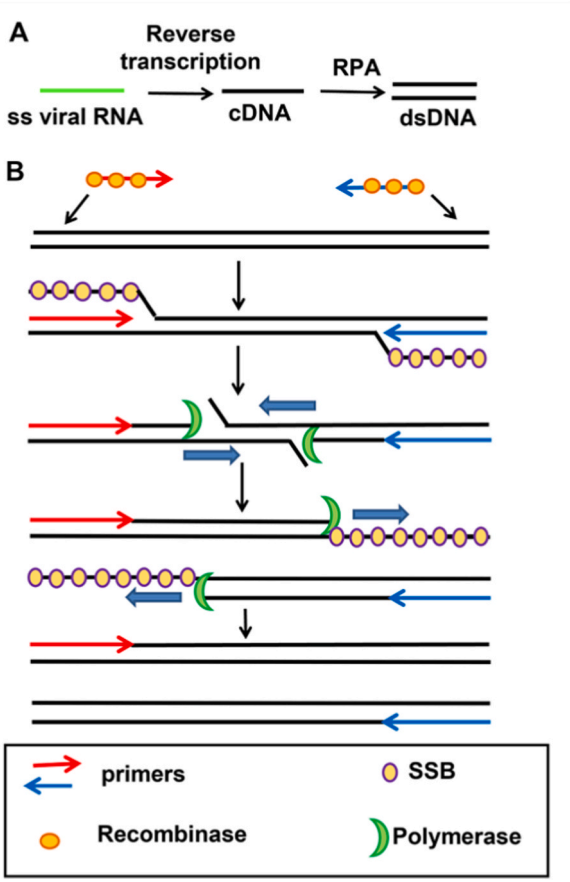

Fig. 1. A flow chart for reverse transcription and Recombinase Polymerase Amplification (RT-RPA)-based method for detection of viral RNA. (A) Single-stranded RNA (ssRNA) isolated from virus-infected samples is reverse transcribed. (B) cDNA subjected to isothermal amplification using Recombinase polymerase amplification (RPA) reaction. ds-double-stranded; SSB–single-stranded DNA binding proteins.

Reverse transcription followed by isothermal recombinase polymerase amplification (RT-RPA) is one of the recently developed methods to detect viral RNA (Fig. 1). Unlike conventional PCR, RPA involves DNA amplification at constant temperature (usually 23–42 °C), eliminating the need for thermocyclers, and the time taken for amplification is a lot less (15–20 min) [27–29]. In RPA, recombinase inserts primers onto complementary DNA strands via strand exchange. Single-strand binding proteins (SSB) are used to displace the non-complementary strand and to stabilize the bound primers. Then, DNA polymerase (e.g., Bsu) initiates amplification, and the whole process continues iteratively (Fig. 1). Moreover, the RPA enzyme mixture can be lyophilized and stored at room temperature for several months. The amplified DNA can be detected by different platforms, including lateral flow devices and portable incubation and detection instruments [30]. RPA has the potential to become an ideal POC test in clinical settings due to its, simplicity, speed, and requirement of minimal low-cost equipment [31]. Its effectiveness as a POC test has already been demonstrated for detecting human immunodeficiency virus (HIV) type 1 [32,33], foot-and-mouth disease virus [34], avian influenza A H7 virus [35], rabies [36], ebola virus [37], and bacterial infections [38]. It has also been employed for developing rapid corona diagnosis kit [39,40] either as a stand-alone or in combination with CRISPR Cas13a [30,41]. Compared to RT-PCR, POC methods are cost-effective, less time-consuming, and can be performed by minimally trained technicians in POC settings (Fig. 2). However, RT-RPA suffers from lower sensitivity compared to RT-PCR and RT-LAMP. Also, it requires expensive reagents, and primer and probe designing is more challenging [42].

Some key innovations in diagnostic platforms, such as the development of lab-on-chip (LoC) devices, could significantly accelerate the pace of diagnosis. An LoC device involves encasing the miniaturized functional equivalents of the laboratory settings (e.g., heating, centrifugation, mixing, and other treatments) onto a chip. Although the LoC was envisioned way back in the early 1990s [43], it is only due to the recent advancements in the field of microelectronics and micro-electromechanical systems (MEMS), internet of things (IoT) and artificial intelligence (AI), the feasibility of using LoC in a point-of-care (POC) setting has increased [44]. The LoC devices are typically fabricated to work under low resource and remote settings [45]. Relatively, smaller input samples are processed on LoC to amplify the target nucleic acid sequences either by a simple loop-mediated amplification (LAMP) or RPA followed by on-chip detection. The test results are detected either as a visual output or fluorescence readout. Fig. 3 illustrates the components of a typical LoC.

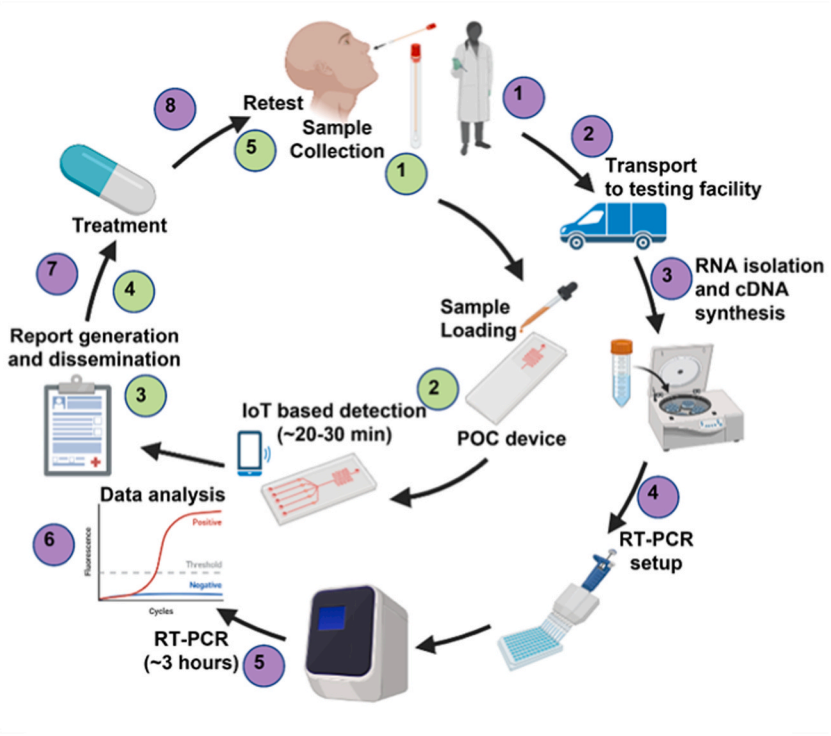

Fig. 2. Comparative flow charts of RT-PCR and Point-of-care (POC) detection methods. POC devices (workflow depicted by numbers in green circles) are cost-effective, less time-consuming, simpler and can be performed by minimally trained technicians in point-of-care settings compared to RT-PCR-based diagnostics (workflow depicted by numbers in purple circles). RT-PCR: Reverse transcriptase-polymerase chain reaction; IoT: Internet of things. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

RT-RPA and other nucleic acid detection methods can be performed using LoC [46] and can be integrated with automation and cyber-physical systems, enabling smart diagnostics and patient monitoring. The diagnosis time can be further reduced by employing rapid RNA isolation protocols, which take less than a minute for RNA extraction [47]. Very recently, RNA-extraction-free methods have also been developed to detect SARS-CoV-2 [48].

2.2. *Potentially novel non-PCR-based methods for RNA detection*

Most of the current viral diagnostic methods rely on RT-PCR-based detection. Based on recent advances in the field of nucleic acids research, we propose two new non-PCR methods for detecting the SARS-CoV-2 genome (and other RNA) sans amplification steps. Unlike most of the existing methods, our proposed methods involve single-step detection.

2.2.1. *RNA mimics of GFP for SARS-CoV-2 detection*

RNA mimics of green fluorescent protein (GFP) are RNA aptamers that bind fluorophores such as 4-hydroxybenzylidene imidazolinone (HBI), resulting in activation of their fluorescence [49,50]. HBI resembles the fluorophore naturally found in GFP. The first version of an RNA aptamer that mimicked GFP was called 'Spinach' [51]. Subsequently, several improved versions of RNA aptamers called 'Spinach 1.2', 'Broccoli' and 'Corn' have been developed, which exhibit enhanced binding to the fluorophore, increased fluorescence, and thermostability [49–52]. Similarly, multiple fluorescent dyes containing HBI fluorophores such as 3,5-dimethoxy-4-hydroxybenzylidene imidazolinone (DMHBI), 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI), DFHBI-1T and BI were developed, which exhibit higher extinction coefficient, lower background fluorescence in cells, and a red-shifted excitation and emission spectra that match the commonly used filter cubes [53–55]. The latest versions of the RNA aptamer and the fluorophore can detect a single molecule of mRNA inside live mammalian cells [53]. The proposed *in vitro* RNA aptamer binding assays can be carried out between 20 and 60 °C [52]. These RNA aptamers can be fused to any RNA molecules through cloning followed by *in vitro* transcription, which can then be used as probes to detect any target RNA molecules. A brief illustration of the proposed method is given in Fig. 4(a–d). This could potentially be one of the simplest and most rapid assays because it does not require reverse transcription or amplification of nucleic acids. Nor does it require any enzymes or proteins and thus can significantly bring down the cost of diagnosis without necessarily compromising sensitivity, specificity, and reliability. Moreover, the method can be tweaked by altering assay temperature to detect the target RNA even if it carries a few mismatches. Such a feature can be highly advantageous in detecting rapidly-evolving viral variants. RNA aptamers, which mimic other coloured fluorescent proteins such as red fluorescent protein (RFP),

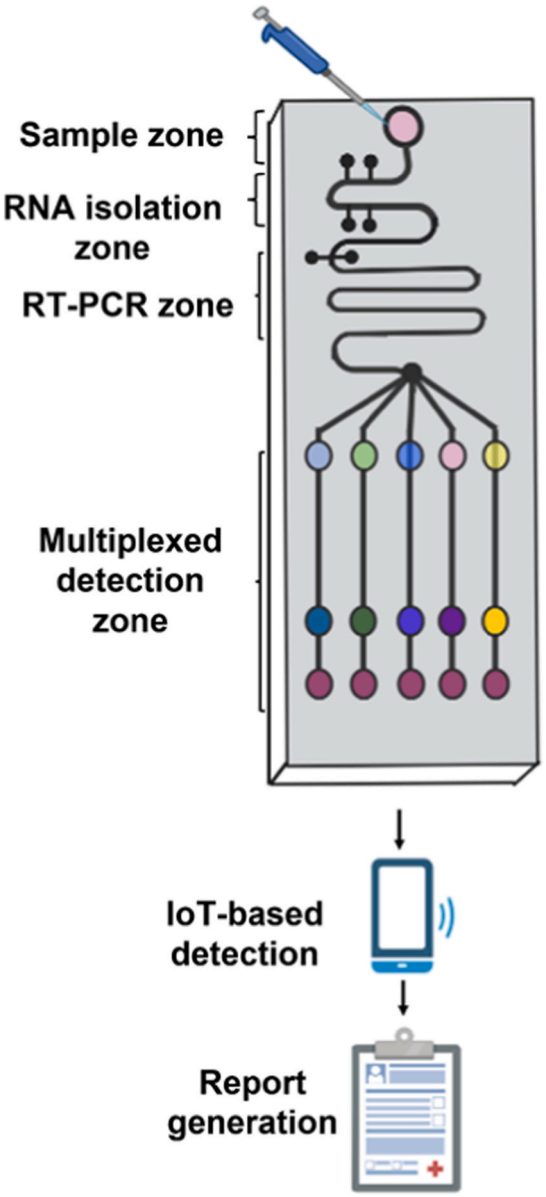

Fig. 3. Diagrammatic representation of miniaturized Lab-on-chip (LoC) module. Microfluidic Lab-on-chip (LoC) consists of specialized microfluidic patterning to ensure proper flow, release, and mixing of reagents required for RNA isolation and RT-PCR. Thermal loops present in the RT-PCR zone provide suitable temperatures to carry out the reaction. The Serpentine micro channels ensure a controlled flow rate and minimal sample loss. The multiplexed detection zone facilitates the detection of multiple pathogens in parallel chambers along with a suitable control either via colorimetric (visual) or fluorescence-based methodologies. Internet of Things (IoT)-based methods can be employed for automating and sharing the data.

blue fluorescent protein (BFP), and cyan fluorescent protein (CFP) are also available which can be multiplexed for simultaneous detection of more than one target RNA [52]. This method is yet to be tested for molecular diagnosis. However, a recent publication reports development and use of DNA aptamer mimic of GFP for SARS-CoV-2 detection [56]. Currently, most of the estimated detection cost of this method is attributed to the cost of fluorophores, which are synthesized commercially. In-house synthesis of these fluorophores can potentially bring down the cost further. Other potential disadvantages include time and cost involved in synthesizing and storing (cold-storage) labile RNA probes and the need for an expensive fluorescence-detecting equipment. A recent discovery of DNA aptamer mimic of GFP could do away with the problems associated with synthesis and handling of RNA [56]. Similarly, recent development of cost-effective smartphone-based devices coupled with machine learning-driven software could bring down the cost of fluorescence detection associated with this method [57].

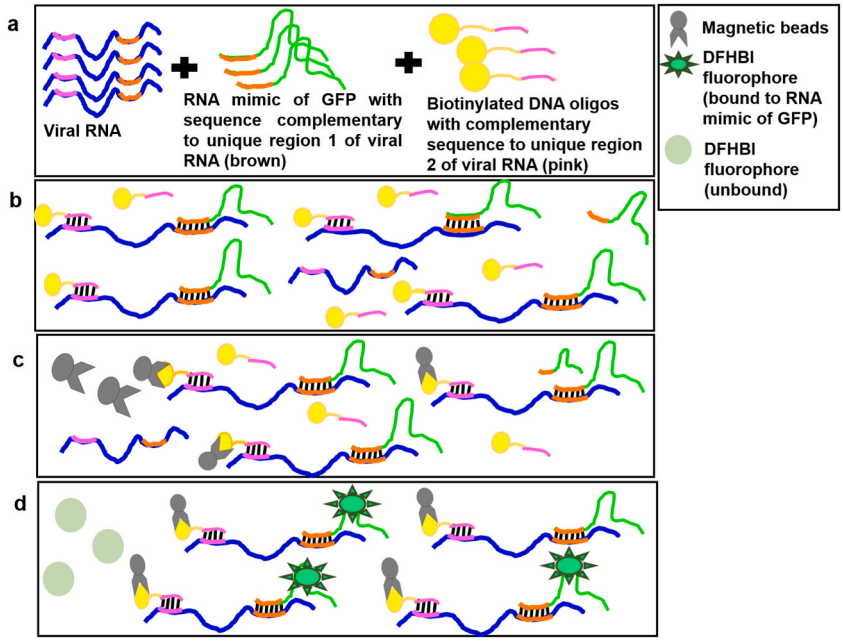

Fig. 4. A flowchart illustrating how RNA mimics of GFP-based viral RNA detection works. (a) Step 1: Mix viral RNA, customized RNA mimic of GFP and custom synthesized DNA oligos. (b) Step 2: Hybridization of complementary sequences (RNA: RNA and DNA: RNA). (c) Step 3: Add streptavidin-coated magnetic beads and pull-down complexes through biotinylated DNA oligos and wash the unbound molecules. (d) Step 4: Add DFHBI fluorophore (light green molecules), which upon binding to RNA aptamer results in green fluorescence. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

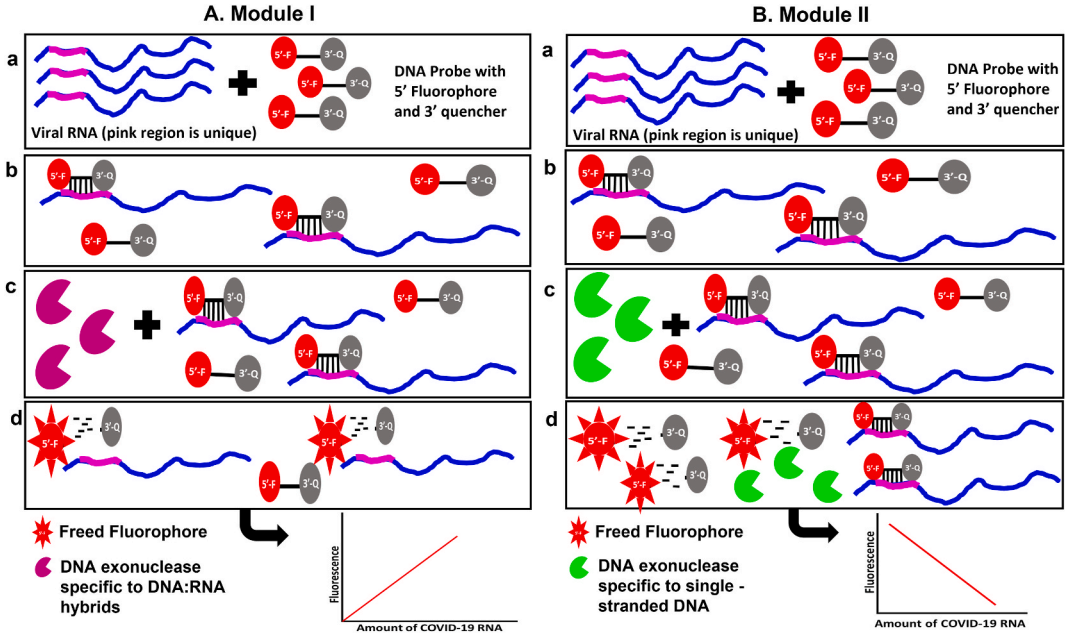

Fig. 5. A flowchart illustrating how specific nuclease and TaqMan probes-based detection of viral RNA works. A, Module I and B, Module II. (A) Module I involves the following steps: (a) Step 1: Mix viral RNA with the customized TaqMan probes. (b) Step 2: Hybridization of complementary sequences (DNA: RNA). (c) Step 3: Add DNA-specific T7 5′-3′ exonuclease, which preferentially cleaves DNA: RNA hybrid (least activity on ssDNA). (d) Step 4: Fluorescence detection and measurement. (B) Module II involves the following steps: (a) Step 1: Mix viral RNA with the customized TaqMan probes. (b) Step 2: Hybridization of complementary sequences (DNA: RNA). (c) Step 3: Add Nuclease P1 (endonuclease) which preferentially cleaves ssDNA or ssRNA and not DNA:RNA hybrid. (d) Step 4: Fluorescence detection and measurement.

2.2.2. Fluorescent probes and nucleases for SARS-CoV-2 detection

Another potentially novel non-PCR approach we propose uses hybridization of viral RNA and specifically designed fluorescent DNA Probes (labelled with 5′ fluorophore and 3′ quencher) that are complementary to the unique regions of viral RNA, followed by a nuclease-mediated cleavage. Nucleases that are commercially available can be exploited to either preferentially cleave (e.g., T7 exonuclease from New England Biolabs) the fluorescent probe DNA from DNA:RNA hybrids leaving the unbound fluorescent ssDNA probe uncleaved (Fig. 5A) or preferentially cleave (e.g., Nuclease P1 from New England Biolabs) the fluorescent ssDNA probe leaving the fluorescent probe DNA:RNA hybrids uncleaved (Fig. 5B). In both cases, nuclease-mediated cleavage of the fluorescent probe results in emission of the fluorescence due to the release of the fluorophore quenching by the quencher. However, in one case, the fluorescence intensity will be positively correlated with the viral genome copy number (Fig. 5A) while in the other, the fluorescence intensity will be negatively correlated with the viral genome copy number (Fig. 5B). For this method, it is advisable to employ multiple probes that are complementary to unique regions of SARS-CoV-2 RNA because relying on one probe could lead to false negatives originating from higher mutagenic rates of viral nucleic acids. Such false negatives constitute an important and frequently reported issue in diagnostic virology [58–60]. Employing several fluorescent probes also increases the sensitivity of detection. In Fig. 5, we have depicted the working principle of this method for only one probe targeting one of the unique regions of viral genome. The method can be modified to include multiple probes targeting multiple unique viral RNA regions. Potential disadvantages of this method include cost of the enzymes along with cold-storage requirement, and fluorescence-detecting equipment. The disadvantage associated with fluorescence detection can be addressed as discussed above, but technologies to enable mass production of enzymes that can be stored at room temperature are yet to be developed.

Our proposed new methods can be used to detect RNA from any other pathogens, and these methods can be integrated into LoC-based diagnostics. Upon implementation, these novel methods are expected to provide new avenues for research and development of alternative, low-cost, and more efficient fluorescent riboprobes and deoxy riboprobes, nucleases with increased specificity towards DNA in DNA:RNA hybrids, fluorophores with enhanced extinction coefficient, and new RNA/DNA aptamers which serve as mimics of different coloured fluorescent proteins such as GFP, RFP, and BFP.

2.3. Serological diagnosis of SARS-CoV-2

Serological tests are currently used as an alternative or to complement nucleic acid-based detection of SARS-CoV-2 due to the ease and low cost of diagnosis. The majority of the antigen and antibody-based tests are currently available as POC test strips, which are advantageous over nucleic acid detection that requires expensive reagents, equipment, and a laboratory set up for diagnosis [61]. These tests use serum samples or other biological fluids such as respiratory lavage, nasopharyngeal swabs, or faecal samples for detecting viral antigens or antibodies specific to the viral antigens. The specificity of SARS-CoV-2 diagnosis based on detection of IgM and IgG antibodies has been ~95–100%, with sensitivity of 84% and 94% for IgM and IgG, respectively [62]. In addition, it is recommended to use an antibody test in conjunction with a RT-PCR test to increase the diagnostic accuracy.

Antibodies are typically detected using enzyme-linked immunosorbent assay (ELISA) or lateral flow assays (LFA). The most commonly detected antigens of SARS-CoV-2 are S, N, E, and M proteins. Clinical studies suggest that the detection of N protein has shown the highest sensitivity in SARS-CoV-2 diagnosis as N protein is the most abundant and highly immunogenic viral protein produced in the host cells. On the other hand, antibodies targeting the receptor-binding domain (RBD) of the S protein are found to be the most specific and neutralizing in nature [63]. Therefore, detection of more than one viral antigen is recommended to improve both the sensitivity and the specificity of viral diagnosis. A major drawback of serological detection of viral antigens is the cross-reactivity of the antibodies with the epitopes not unique to SARS-CoV-2 [64]. This problem can be addressed by designing more specific antibodies based on bioinformatic identification of potentially unique epitopes [65,66].

3. Therapeutics for COVID-19 and beyond

3.1. Host-directed therapies

SARS-CoV-2 infections cause hyper inflammation and excessive cytokine responses, resulting in end-organ dysfunction/mortality [67,68]. The COVID-19 infections also dampen the natural killer (NK) cell response, as evident from the reduced circulating NK cell number and exhausted phenotype [69]. Several host-directed therapies are currently being evaluated to ameliorate aberrant host immune and inflammatory responses as part of a holistic and long-term treatment of COVID-19 patients [70,71]. NK cell-based immunotherapies are being clinically evaluated (ClinicalTrials.gov Identifier: NCT04344548, NCT04365101, NCT04280224) to boost antiviral immune responses against COVID-19 [72,73].

Mesenchymal stem cells (MSCs) from dental pulp and umbilical cord with regenerative and differentiation properties act as immuno-modulators and prevent inflammatory response by reducing the cytokine levels [74,75]. Phase 1 and 2 clinical trials involving COVID-19 patients with moderate to severe lung damage showed that MSC isolated from the umbilical cord are safe, well-tolerated, and they markedly reduced lung lesion size in the patients [76–78]. Moreover, MSC-derived extracellular vesicles have been explored to express decoy ACE-2 protein to capture SARS-CoV-2 S protein to limit viral infections [79]. Similarly, the wild-type and affinity-matured soluble decoy ACE-2 have been proposed as potential antiviral therapeutic strategy [80,81].

Next, based on findings from *in vitro* studies, several promising monoclonal antibodies (mAbs) exhibiting neutralizing activity against SARS-CoV-2 have been selected and subjected to preclinical [82,83] and clinical trials (ClinicalTrials.gov Identifier: NCT04545060) to treat COVID-19. These mAbs are mostly specific to epitopes originating from the RBD and non-RBD regions of the S

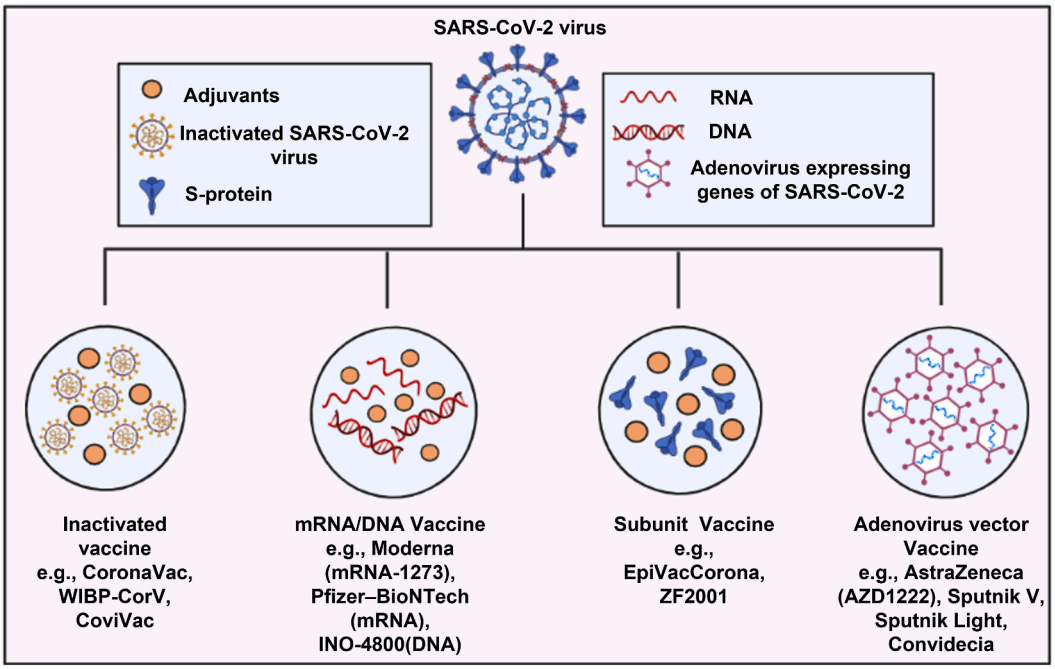

Fig. 6. Methods being employed to develop vaccines against SARS-CoV-2. The figure summarizes various methods currently used to develop vaccines against SARS CoV-2. Candidate vaccines belonging to these categories are either in various phases of clinical trials or approved for human use (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines).

protein. Results from some of these trials show reduced viral load in the patients treated with these mAbs [84]. Similarly, nanobodies (nAbs) are being explored to target SARS-CoV-2. nAbs are single-domain antibody fragments comprising a single monomeric variable domain of the antibody. A variety of nAbs that displayed effective viral neutralization in *in vitro* assays are currently in preclinical development. Some of these are designed to target the RBD of SARS-CoV-2 S protein in order to disrupt its interaction with human angiotensin-converting enzyme 2 (hACE2) [85]. These nAbs are yet to be tested in animal models and human trials. Interestingly, when a cocktail of non-competing, unique, neutralizing antibodies targeting the S protein was tested *in vitro*, antibody escape mutants were not generated compared to treatments with individual mAbs or a cocktail of competing mAbs (mAbs targeting the overlapping epitopes).

Another therapy involves targeting complement protein 5a (C5a). Experimental evidence showed that SARS-CoV-2 nucleocapsid protein binds to mannan-binding lectin serine protease 2, activating the downstream complementation pathway and C5a generation [86,87]. Also, higher concentrations of C5a and other complement proteins were found in patients with severe COVID-19 [87,88]. C5a is a well-known anaphylatoxin that causes widespread tissue damage in addition to attracting neutrophils and monocytes to the site of infection [89]. It also activates the blood coagulation system [90]. Therefore, monoclonal antibodies targeting C5a has been explored as a host-directed therapy to reduce acute respiratory distress syndrome and thrombotic microangiopathy associated with CoV infections [89,91].

Type I interferons (IFN–I) with a broad antiviral activity have been clinically evaluated to treat COVID-19 either independently or in combination with other drugs [92–94]. IFN-I are a group of cytokines comprising seven subtypes; IFNα, -β, - δ, -τ, -ε, -ω, and -κ [95,96]. Due to their non-specific antiviral effects, IFN-I interferons are often evaluated in the context of emerging viral infections, including those caused by Influenza viruses and the CoVs closely related to SARS-CoV-2, such as Middle East respiratory syndrome (MERS), and SARS [92,97–103]. A review of several studies has revealed that IFN-I may account for safe, efficient, and easy to upscale COVID-19 treatment during the early stages of infection [92]. A recent retrospective study of ~450 COVID-19 patients revealed that the early use of IFN-α decreased mortality, but the late use of IFN-α resulted in increased mortality and delayed recovery [104]. Furthermore, some reports indicate that IFN-I interferons exacerbate the inflammation in the patients with severe COVID-19 but not in the cases of mild COVID-19 [105,106]. Therefore, more precise information is required to establish the therapeutic effects of IFN-I to treat COVID-19. Further studies along these lines should consider dose, route, and the timing of administration of IFN-I and the target population for effectively utilizing the IFN-based therapies [106].

3.2. Vaccines

3.2.1. Current status of approved vaccines against COVID-19

In the era of modern medicine, vaccination has proved to be one of the most effective, least expensive, and simplest intervention strategies, dramatically altering the way infectious diseases, epidemics and pandemics have been managed over the past century.

Table 1
Non-specific or heterologous immunity of BCG against different diseases.

Vaccine	Name of the disease against which BCG provides cross protections	Clinical Trial ID	Study model (Human/Animal)	Reference
BCG	Yellow fever	–	Healthy adults	[110,131]
	Influenza	NCT02114255	Healthy adults (male)	[113,131]
	Hepatitis	NCT02444611	Children	[131,132]
	HIV	NCT00331474	Infants	[131,133]
		NCT02062580	Infants	[131,134]
		NCT02606526	Infants	[131,135]
	Malaria	NCT00126217	Children	[131,136]
		NCT00131794	Children	[131,137]
		NCT02692963	Healthy adults	[131,138, 139]
	Pertussis	NCT02771782	Healthy adults (female)	[131,140]
	Diphtheria	NCT02771782	Healthy adults (female)	[131,140]
	Sepsis, reduced childhood mortality	–	Neonates and children	[131,141]
	Pneumonia and sepsis	–	Neonates	[131,142]
	Respiratory tract infections	–	Tuberculosis negative adolescent	[131,143]
	Pneumonia in geriatric population	–	Tuberculin negative adults (≥65 years old)	[131,144]
	Melanoma	–	Retrospective study in adult melanoma patients vaccinated with BCG in childhood	[131,145]
	Bladder cancer	–	Adult bladder cancer patients and healthy adults	[131,146]
Ongoing clinical trials involving BCG	COVID-19	NCT04328441	Healthy adult (Health care workers)	[147–149]
		NCT04327206	Healthy adult (Health care workers)	[148–150]
		NCT04348370	Healthy adult (Health care workers)	[148]
		NCT04417335	Healthy adult (≥60 years)	[149,151]
		NCT04350931	Healthy adult (Health care workers)	[148,151]
		NCT04373291	Healthy adult (Health care workers)	[131,151, 152]
		NCT04414267	Healthy adult (≥50 years)	[131,151, 152]
		NCT04379336	Healthy adult (Health care workers)	
		NCT04384549	Healthy adult (Health care workers)	
		NCT04475302	Healthy dult (≥60 years)	
		NCT04461379	Healthy dult (Health care workers)	
		NCT04537663	Healthy dult (≥60 years)	
		NCT04369794	Healthy dult (≥18 years)	
		NCT04534803	Healthy dult (≥60 years)	
		NCT04542330	Healthy dult (≥65 years)	

Vaccination across various age groups also protected the human population against several other deadly infectious diseases over the last century. Vaccines break the chain of disease transmission in a short span of time and bring down disease-associated morbidity and mortality. Although infection and vaccination can both presumably induce B and T cell responses, they could significantly vary qualitatively and quantitatively owing to the immune evasion strategies employed by the active virus.

Natural exposure to SARS-CoV-2 can achieve herd immunity, but mass vaccination is still considered the most promising means to induce rapid and more uniform herd immunity across the human population. As a result, approvals for pre-clinical and clinical safety and protective efficacy of the various vaccine candidates against SARS-CoV-2 have been fast-tracked to bring the most promising candidates to market. The WHO website describes the most updated landscape of the vaccines in various stages of clinical trials [107].

Currently, multiple vaccines have been approved for administration to the public to protect against COVID-19 (Fig. 6). Among SARS-CoV-2 proteins, the S protein has been a major target for vaccine development as it plays a critical role in viral pathogenicity. Among the recently approved vaccines, the mRNA-based vaccine Moderna mRNA-1273 (developed by Moderna, Inc, USA) encoding for the S protein of SARS-CoV-2 is a new type of vaccine approved for the first time for human use. Lately, one more S protein-encoding mRNA vaccine called Comirnaty (produced by Pfizer and BioNTech) has been approved both for adults and children above 12 years of age. Unlike the other vaccines, mRNA-based vaccines do not involve the usage of virus particles but have a shorter shelf life and require cold storage (−20° Celsius or colder). Among other approved vaccines, Covishield (developed by Oxford-AstraZeneca), Janssen COVID-19 (developed by Johnson and Johnson, USA), Sputnik V (developed by Gamaleya Research Institute of Epidemiology and Microbiology, Russia), Covaxin (co-developed by Bharat Biotech, India, and Indian Institute of Medical Research-National Institute of Virology, India), and CoronaVac (developed by Sinovac, China) are prominent vaccines being mass-produced and currently being administered. Among these, Covishield, Janssen COVID-19, and Sputnik V use adenoviral vectors expressing the S protein of SARS-CoV-2, while Covaxin and CoronaVac make use of inactivated SARS-CoV-2 virus particles. All these vaccines are being monitored to test their efficacy against the multiple new variants of SARS-CoV-2, which underlie the recent resurgence in COVID-19 infections in several countries. For the list of other vaccines, which are currently under various stages of clinical trials, refer to (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines).

Thanks to the overwhelming number of global COVID-19 infections, there has been a heightened demand for vaccines that has not

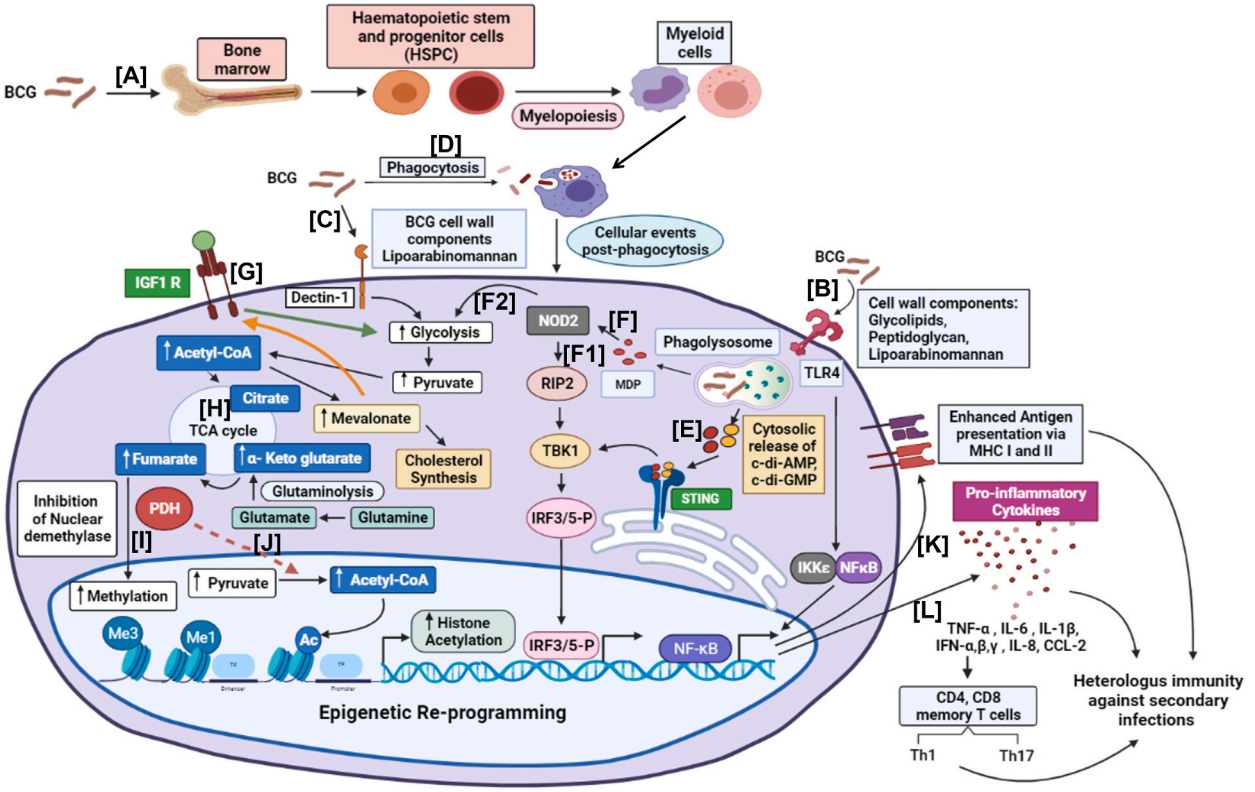

Fig. 7. BCG-induced trained immunity and heterologous protection against secondary infections. The figure describes various mechanisms underlying induction of trained immunity by BCG involving a complex network of circulating peripheral myeloid cells, metabolic rewiring, epigenetic reprogramming, and hematopoietic stem and progenitor cells' activation leading to myelopoiesis. (A) BCG induced activation of hematopoietic stem and progenitor cells, leading to myelopoiesis. (B) Activation of TLR4 receptors by BCG cell wall lipids triggering downstream signalling events leading to activation of NF-kB pathway. (C) Activation of Dectin-1 receptor causing increased glycolysis. (D) Initiation of post-phagocytic events in phagocytic cells. (E) Release of mycobacterial cyclic dinucleotides such as c-di-AMP and c-di-GMP, which bind to the endoplasmic reticulum (ER)-associated sensor called *stimulator of interferon genes* (STING) with subsequent activation of IRF3-dependent transcription and cytokine production. (F) Muramyl dipeptides (MDP) released by BCG binds to the cytosolic nucleotide-binding oligomerization domain 2 (NOD2) receptor. (F1) Upon stimulation with MDP, the NOD2 receptor binds to RIP2, activating the IRF pathway. (F2) NOD2 also causes increased glycolysis. Enhanced production of acetyl-CoA induces epigenetic reprogramming via histone modifications causing widespread expression of inflammation-related genes. (G) Insulin-like Growth Factor 1 receptor (IGF1 R) activation by mevalonate enhances glycolysis. (H) Increase in glycolytic intermediates further increases the flux of acetyl-CoA into the TCA cycle. Glutamine lysis further augments α-ketoglutarate and fumarate levels. (I) Increased fumarate levels inhibit histone demethylases, enhancing histone methylation of the promoters of the genes governing inflammation. (J) Pyruvate dehydrogenase complex (PDH) also translocates into the nucleus and causes the production of acetyl-CoA and increases histone acetylation. (K) The metabolic and epigenetic reprogramming from various pathways leads to enhanced antigen processing and presentation via MHC I and II. (L) Enhanced production of several pro-inflammatory cytokines in combination with antigen presentation, amplifies the T cell response (CD4 and CD8 T and Th17 response) against secondary infections.

been matched by global manufacture and supply. To address this shortage, there is a renewed interest in heterologous prime-boost vaccination strategies wherein individuals primed with one type of vaccine are advised to take an alternative vaccine as their second dose. For example, a recent trial was conducted in the UK to test the reactogenicity and safety of two vaccines, Covishield and Comirnaty, in two alternative combinations [108]. Both the combinations of heterologous vaccine schedules induced higher immune responses compared to their homologous counterparts. Similar trials are going on involving other vaccine combinations.

3.2.2. Repurposing old vaccines against COVID-19: harnessing the benefits of heterologous (trained) immunity

Among various vaccines that were considered for repurposing against COVID-19, Bacillus Calmette-Guerin (BCG) vaccine appeared to be a promising prophylactic approach. BCG is a live attenuated vaccine derived from *Mycobacterium bovis.* Due to the high antigenic similarity between *M. bovis* and *M. tuberculosis*, BCG was initially developed for its utility as a childhood vaccine given at birth to protect against tuberculosis [109]. Clinical evidence collected over the last century revealed that BCG also provides non-specific protection called "trained or heterologous immunity" against several diseases, including those caused by viruses such as, influenza A virus (H1N1), herpes simplex virus 2 (HSV-2), respiratory syncytial virus (RSV), yellow fever virus, human papilloma virus (HPV), vaccinia virus, hepatitis B, herpes simplex virus (HSV), Japanese encephalitis, and ectromelia virus. Earlier, BCG was also used as a

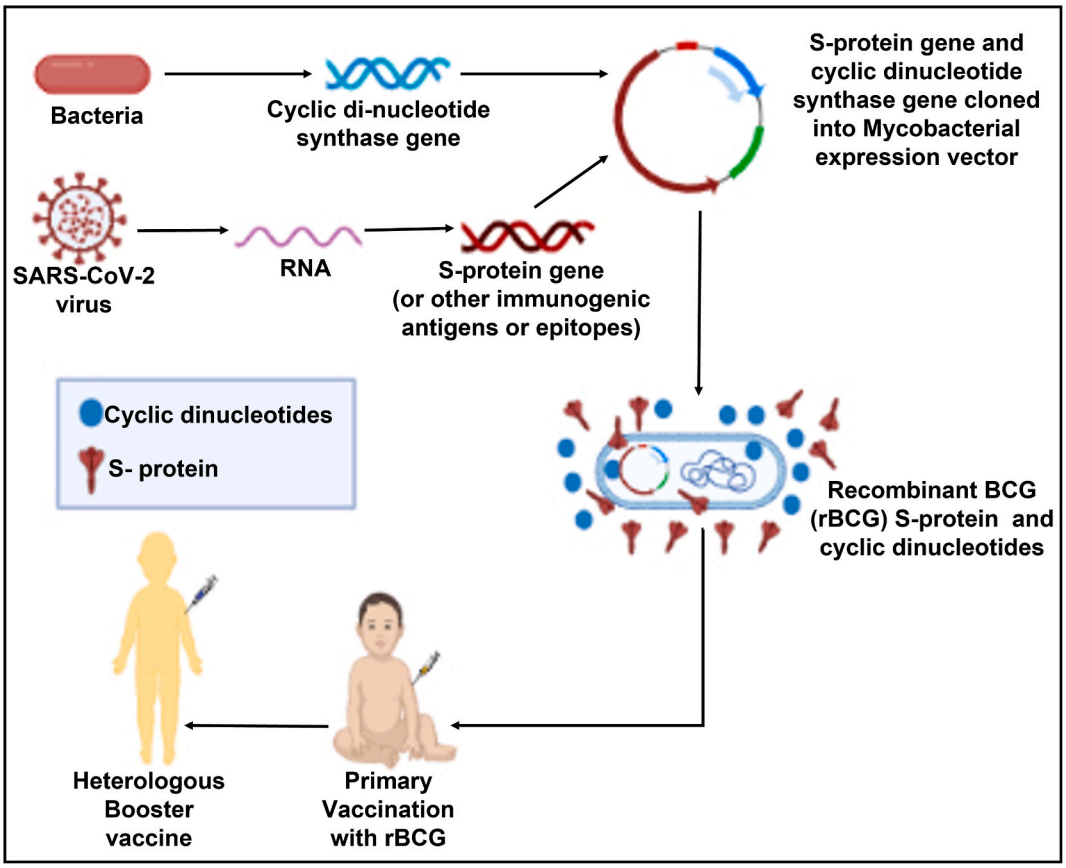

Fig. 8. Engineering BCG to protect children against TB and COVID-19.
The figure describes how the anti-viral benefits of BCG and cyclic dinucleotide-based STING agonists are harnessed by employing the recombinant BCG (rBCG) approach. BCG vaccine can be genetically engineered to simultaneously express STING agonists (cyclic dinucleotides) and SARS-CoV-2 antigen(s) (e.g., S protein). The rBCG approach can be utilized as a primary vaccination for children aged 0–5 years, conferring protection against both childhood TB and COVID-19. Based on the duration of protection conferred against prevailing strains of SARS-CoV-2, primary immunization can be followed by a heterologous booster vaccine (other than BCG) at later time points to augment SARS-CoV-2 or pan-corona virus-specific immune responses further.

therapeutic vaccine against bladder cancer, Buruli ulcer, diabetes, and leprosy in adults [110–120]. Therefore, BCG was subjected to clinical testing on frontline nursing staff and the adult populations in several countries to investigate its protective ability against COVID-19 (Table 1). In countries where childhood BCG vaccination is followed, the relative incidence and mortality rate due to COVID-19 appeared to be lower compared to the other countries with no childhood BCG vaccination. Several nations, including the United States, Australia, Germany, India, and the Netherlands conducted clinical trials to evaluate the ability of the BCG vaccine to protect against COVID-19 among high-risk individuals. These studies are in different stages of clinical trials, and preliminary studies indicate that BCG is safe and well-tolerated among the immunized individuals [121]. Children (age 0–5 years) in countries with a very high coverage rate of BCG vaccination were found to be far less susceptible to COVID-19 compared to nations with no BCG policy [122–127]. Similar conclusions were drawn from observational studies in large paediatric cohorts, showing reduced hospitalization rate, morbidity, and mortality among BCG-vaccinated children [128].

Further clinical evidence suggests that BCG vaccination stimulates the innate and adaptive immunity both in a specific as well as a non-specific manner towards secondary infections [129]. For example, BCG therapy was found to be highly effective in HPV patients suffering from respiratory papillomatosis by restoring antiviral T-cell response and balancing Th1/Th2/Th17 cytokines [130].

Analysis of underlying mechanisms suggests that innate immune cells, such as monocytes and NK-cells, undergo activation of cellular signalling events, metabolic rewiring, and epigenetic reprogramming upon exposure to the BCG vaccine (Fig. 7). These mechanisms collectively constitute trained immunity, imparting memory-like characteristics to innate immune cells. Activating various pro-inflammatory cytokines such as Interleukin-1 (IL-1) β, Interferon (IFN)-γ, Tumour Necrosis Factor (TNF)-α, and IL-6 results in enhanced antigen-presenting capability of the trained innate immune cells, thereby stimulating adaptive and innate immunity towards other pathogens [128,153,154].

As illustrated in Fig. 7, induction of trained immunity is initiated by the recognition of BCG cell wall components (glycolipids, peptidoglycan, lipoarabinomannan, and muramyl dipeptide) by cell surface and intracellular pattern recognition receptors (PRRs),

such as Toll-like receptor (TLR) 4 and nucleotide-binding and oligomerization domain (NOD)-2. Activation of TLR4 triggers signalling events leading to activation of the NF-kB pathway and transcriptional activation of genes involved in inflammatory processes. Some studies suggest that resting innate immune cells prefer oxidative phosphorylation over glycolysis. However, BCG-activated cells switch to glycolysis, which plays a crucial role in epigenetic modulations of the genes encoding the inflammatory cytokines (e.g., TNFα and IL6) [155]. Further, exposure to BCG causes modulations in glutamine and cholesterol metabolism resulting in similar epigenetic effects [155,156]. All these events cooperatively augment responsiveness to other pathogens. BCG-induced trained immunity has been observed in circulating cells, self-renewing hematopoietic progenitor cells and tissue-resident macrophages [157–160], and confers long-term memory (up to several decades) to the innate immune cells.

3.2.3. Engineered BCG as a vaccine against diverse infectious agents

Next, we focus on how BCG has been engineered to serve as a vaccine against diverse infections by introducing genes encoding specific antigens. As highlighted in Fig. 8, we propose a dual approach involving genetically engineered BCG vaccine that expresses *Stimulator of Interferon Gene* (STING) agonist and a single or multiple highly conserved epitopes (e.g., epitopes originating from N and S proteins) belonging to a wide variety of coronaviruses, including SARS-CoV-2 [161]. Note that STING is a well-known inducer of IFN-I-mediated antiviral immunity. In a recent study, a synthetic STING agonist confers protection against severe SARS-CoV-2 infection, especially if administered early during the disease process [162]. We have shown earlier that such a STING-based dual approach confers significant protection against *M. tuberculosis* [163] and bladder cancer [164]. This approach could possibly help in developing pan-coronavirus BCG-based vaccines for all age groups. Moreover, BCG, being a highly attenuated slow-growing microorganism, it is less likely to cause severe side effects often associated with protein and RNA-based vaccines caused by heightened immune responses. Indeed, a recent study demonstrated the immunogenicity of a recombinant *Mycobacterium paragordonae* expressing SARS-CoV-2 RBD [165], further underscoring the utility of live vaccines in developing COVID-19 vaccines. Owing to its several advantages, such as safety, mass vaccination coverage (~75% of children worldwide), low cost, amenability to genetic engineering, the convenience of production and vaccination, and thermal stability, the BCG vaccine can serve as a promising tool for mitigating SARS-CoV-2 and other viruses.

Further, a genome-wide analysis comparing the protein sequences of *M. bovis* BCG and various antigenic proteins of other childhood vaccines with SARS-CoV-2 revealed a list of shared epitopes [166]. For example, the hsp65 protein of BCG shares an epitope with the S protein of SARS-CoV-2 [167]. It is speculated that having shared epitopes may potentially activate cross-reacting memory B and T cells. These data assert the need for future epidemiological studies in paediatric population to determine the protective effect of various childhood vaccines (those sharing epitopes with SARS-CoV-2) against COVID-19 [168–170]. A recent study demonstrating some protection by measles, mumps, and rubella (MMR) booster vaccine against COVID-19 infection and disease severity in adults appears to support the above-mentioned hypothesis of cross-protection against COVID-19 by other vaccines [169].

3.2.4. Reverse vaccinology: A desirable strategic plan to design new vaccines

The term "reverse vaccinology", coined by Rappuoli, is a genome-based approach for vaccine design and development [171,172]. Traditional vaccine development approaches involve laborious and time-consuming testing of immunogenic and protective potential of several pathogen-derived antigens to identify and develop the best candidate vaccines. On the other hand, reverse vaccinology employs *in silico* analysis of genome and protein sequences of pathogens, facilitating systematic identification of all the potential antigens for downstream testing and development of vaccine candidates [172]. This approach can significantly reduce the time and the cost needed to identify vaccine candidates and is also suitable for the difficult-to-culture pathogens [173]. Reverse vaccinology approaches are currently being used for designing vaccines against diverse viruses, bacteria, and other parasitic infections [171,174,175]. The bioinformatic approaches used in reverse vaccinology consider the following features of a pathogen to identify the potential useful antigens/epitopes: (a) unique protein sequence, (b) high-scoring B cell and T cell epitopes, (c) highly conserved sequences, (d) peptide sequences that can bind to pattern recognition receptors of the innate immune system, and (e) ability to be presented by the major histocompatibility complex (MHC) molecules irrespective of their diversity among human populations [176].

In reverse vaccinology for viruses, the viral genomes are sequenced and aligned to identify the regions unique to the given strain of the virus. So far, hundreds of thousands of SARS-CoV-2 genomes have been sequenced and their sequences are available in public databases (https://www.ncbi.nlm.nih.gov/sars-cov-2/). Further, an open-source genome browser was developed for interactive sequence visualization with downstream applications, including vaccine design [177]. For example, a recent study has designed potentially effective vaccine targets using reverse vaccinology and machine learning (ML) [178] In this study, the authors compared genomes of several CoVs using Vaxign-ML and predicted the S, nsp3, and nsp8 proteins to be highly antigenic. As an additional strategy, a comparison of the three-dimensional structures of the viral proteins has been used to gain further understanding of the motifs involved in viral adhesion, entry, host recognition, presentation by MHC molecules, binding affinities to antibodies, and pattern recognition receptors of the innate immune system [179]. Often, transcriptomic and proteomic data sets are integrated during the antigen selection procedure. Peptides predicted to exhibit the most favourable interactions with immune receptors will be selected for downstream testing [180,181].

There are certain disadvantages of reverse vaccinology approaches. First, it assumes that all the genes are expressed by the pathogen all the time. Second, it assumes that all the antigens are expressed during infection and are abundantly available to elicit immune responses. Third, this approach relies exclusively on protein antigens and omits other important immunogens such as polysaccharides and glycolipids. Fourth, the lack of a high-throughput system to evaluate the protective immunity of the shortlisted candidate antigens is still a limitation [182,183]. Nonetheless, reverse vaccinology has been successfully used to develop vaccines against various pathogens, including viruses. The pace of vaccine discovery can be significantly enhanced by employing AI. For

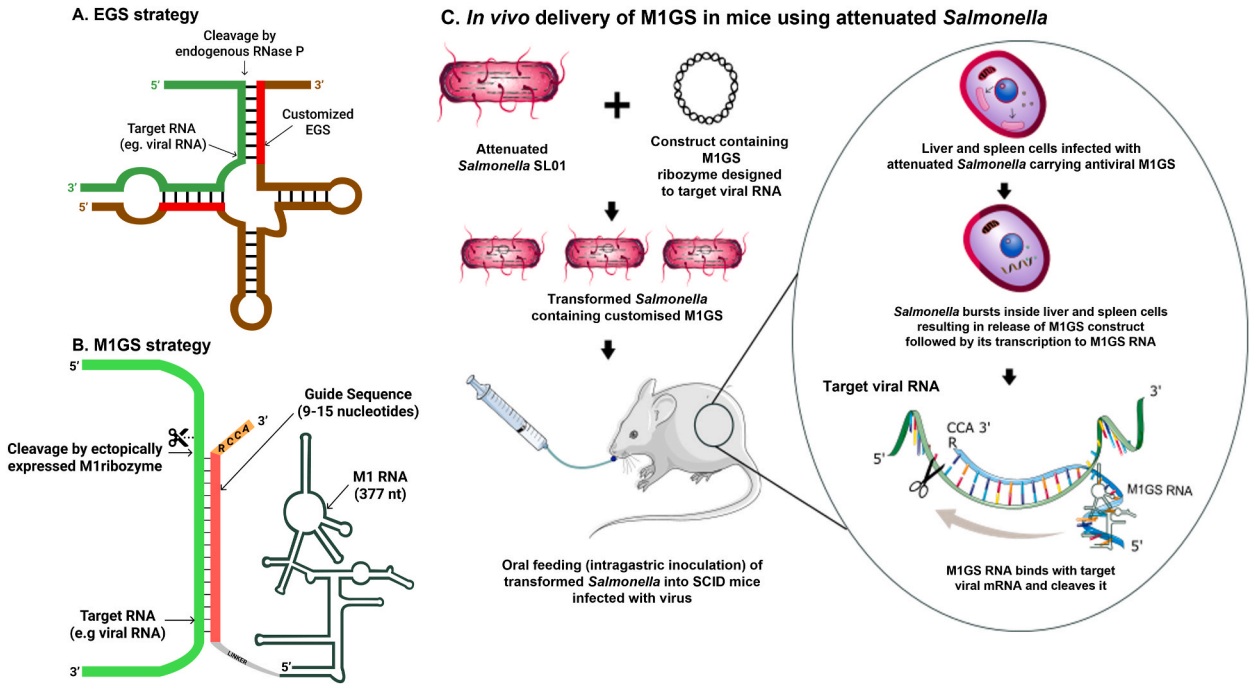

Fig. 9. Engineered bacterial M1GS ribozyme-based strategy to cleave the target viral RNA. (A) Cartoon depicting the mode of target RNA cleavage by engineered RNase P ribozyme using EGS strategy. (B) Cartoon depicting the mode of target RNA cleavage by engineered RNase P ribozyme using M1GS strategy. (C) The cartoon illustrates a scheme for oral delivery of M1GS ribozymes in mouse models using engineered *Salmonella* bacteria to target viral mRNA in virus-infected mice [205]. EGS-External guide sequence; M1GS-M1 RNA-Guide sequence; SCID-Severe combined immunodeficiency.

example, Ong et al. [178] employed the *Vaxign* reverse vaccinology-machine learning platform to predict possible vaccine candidates for COVID-19.

3.2.5. Challenges and bottlenecks in vaccine development

Vaccines are considered to be the best antidote in our fight against several pathogens, including viruses. However, several challenges and bottlenecks underlie the successful development of a safe and effective vaccine. The foremost is the identification of the antigens/epitopes of the pathogen that can induce the most protective and sustainable immune responses. Although predictions about the immunogenicity of the potential antigens can be made based on *in silico* analysis of nucleic acid and protein sequences, it still remains at large as a hit-and-miss strategy in finding a successful vaccine candidate. For instance, despite several years of research endeavours, vaccines for several deadly diseases, including acquired immunodeficiency syndrome (AIDS), tuberculosis (TB), and malaria, are still unavailable [184,185]. Therefore, it entails parallel testing of multiple potential candidates in preclinical animal models, followed by clinical testing in human populations. Unfortunately, no reliable markers exist to predict the success of a vaccine against a given infection. One of the formidable challenges stems from the life cycle of the pathogens and their subcellular locations [186,187]. For example, viruses like HIV, Hepatitis B, and Hepatitis C, can stay in the lysogenic or latent stage for several years before reactivation [188,189]. Similarly, localization of the pathogens to several subcellular locations, such as phagosome, mitochondria, nucleolus, and endoplasmic reticulum, poses additional challenges in detecting and/or targeting the pathogen by the immune system [190]. *In silico* identification of mitochondrial localization signals in SARS-CoV-2 genomes by a recent study indicates that the virus can hijack host mitochondria for replication and infection [187,191]. Should the virus reside in mitochondria, it may evade its detection by the host cytosolic surveillance system.

The next challenge comes from the lack of ideal animal models for preclinical evaluation of the vaccine candidates for their immunogenicity and protective efficacy [192]. Significant variation in their biology, genetics, and expression levels of viral entry receptors (e.g., ACE2 for SARS-CoV-2) amongst several animal models and humans results in differential sensitivity to viral infection and varied pathogen-specific immune responses [193,194]. Therefore, detailed viral infection studies using animal models need to be carried out to decide the best model for vaccine testing [192]. Recently, humanized animal models, reminiscent of human tissues, are being developed and explored to understand viral pathogenesis and to evaluate the efficacy of vaccine candidates [194,195].

Another bottleneck is the duration of protection imparted by the vaccine candidates. Akin to natural viral infections, antibodies generated due to vaccination may dwindle within a few months, entailing a need for booster doses at regular intervals, for sustained protection [196]. Lastly, rapid production and immunization of the entire human population remain a greater challenge as some vaccines have shorter shelf lives and require cold storage [197].

4. Targeted RNA-cleaving tools as potential antiviral therapeutics

Over the last two decades, tools to cleave viral RNA have been explored to develop alternative antiviral strategies. We briefly describe below some of the RNA-cleaving tools with the potential to be developed as novel antiviral therapies for future use. RNase H, a ubiquitous ribonuclease that specifically cleaves RNA in a RNA:DNA was explored to cleave viral RNA by exogenously providing DNA oligos that are complementary to viral RNA [198,199]. DNA oligos were proposed to protect against viruses via at least two different mechanisms. One, by blocking viral RNA translation and/or by blocking interactions of the viral RNA with the host or other viral components necessary for the viral pathogenesis. The other mechanism is through RNase H-mediated cleavage of viral RNA [200].

Another method used to cleave viral mRNA in human cell lines and animal models involves the use of engineered bacterial ribozymes [201–204]. Several studies have reported usage of engineered RNase P and hammerhead ribozymes to cleave viral RNA. In this review, we focus on RNase P-based ribozymes. RNase P, which is primarily involved in the 5′ maturation of tRNAs in all three domains of life, has been engineered to cleave any target RNA. This strategy uses two alternative approaches. The first approach employs an external guide sequence (EGS) designed to base-pair with the target RNA to be recognized by the endogenous RNase P, resulting in the target RNA cleavage [201] (Fig. 9A). The second approach employs the catalytic M1 RNA of bacterial (*Escherichia coli*) RNase P fused to a guide sequence (GS), designed to base-pair with the target RNA, which is then cleaved by M1 RNA. This approach is called M1GS [201] (Fig. 9B). Customized EGSs and M1GSs have been successfully used to block viral replication in bacterial and mammalian cultured cells and mouse models [205–210]. Further, *in vitro* evolution procedure has been used to create ribozyme variants with improved target RNA cleavage efficiency [206,211–215]. In spite of these developments, progress has not been made to enable testing ribozyme-based antiviral strategies in clinical settings. This lack of progress is perhaps due to some challenges the ribozyme-based antiviral strategies currently face.

One of the challenges associated with the M1GS strategy is the accessibility of the target RNA inside the cells. To address this challenge, specific sequences can be added to the ribozymes to facilitate their cellular colocalization with the target RNA to enhance their cleavage efficiency. One such strategy involves the fusion of ribozymes with the constitutive transport element (CTE), an RNA motif that can interact with intracellular RNA helicases [216]. The idea behind this strategy is to produce helicase-bound hybrid ribozymes in cells. Such a modification enhanced ribozyme activity *in vivo*, permitting cleavage of sites previously found to be inaccessible [216]. Another such strategy involves tethering ribozymes to viral packaging signal, facilitating colocalization of the ectopically expressed ribozyme with the target viral RNA [217]. Similarly, different promoters are used to drive the expression of customized ribozymes to control their subcellular localization and expression levels [218]. For example, Bertrand and colleagues (1997) [218] designed ribozymes against HIV-1 and Simian immunodeficiency virus (SIV) using various expression cassettes containing promoter sequences from the human U1 snRNA, U6 snRNA, $tRNA^{Meti}$ genes, or Pol II fused to different processing/stabilizing sequences. The ribozymes driven by $tRNA^{Meti}$, U1, and U6 promoters were nuclear-localized and expressed at high levels, while the ribozymes produced by the standard Pol II promoter were cytoplasmic and expressed at lower levels. Further, they demonstrated that ribozymes against SIV and HIV were effective only when they were cytoplasmically co-localized with its target RNA. In a recent study in human cell lines, the CRISPR-Cas9 system was used to cleave RNA of SARS-CoV-2 and influenza viruses [219], further emphasizing the need to pursue RNA-cleaving tools as antiviral approaches.

Another challenge in developing nucleic acid-based therapeutics originates from the lack of a safe, effective, and targeted *in vivo* delivery of the nucleic acids. However, due to an increased interest in developing nucleic acid-based therapeutic agents to cure various diseases, several attempts are being made to effectively deliver RNA and DNA to cells using different technologies, including nanotechnology, in *in vitro* and *in vivo* models [220–223]. In this context, it is vital to note the development of attenuated strains of *Salmonella* as a vehicle to deliver nucleic acid-based vaccines. *Salmonella*, a group of invasive bacteria, were engineered to serve as gene delivery agents because of their ability to enter human cells and transfer genetic material to the host cells [224–227]. These attenuated strains can target specific cells, such as dendritic cells, macrophages, and epithelial cells [225,228]. Most notably, Bai et al. [205] demonstrated that customized M1GS could be delivered not only to cell lines but also to mice through oral delivery using an attenuated strain of *Salmonella* bacteria (SL101), which carries deletions of virulence loci ssrA/B [205,229] (Fig. 9C). This strain exhibited efficient gene transfer activity in the inoculated mice with little cytotoxicity and pathogenicity. In the strategy used by Bai et al. (2010) [205], the *Salmonella* strain was transformed with a construct containing M1GS, customized to target and cleave the overlapping mRNA region of M80.5 and protease, two murine cytomegalovirus (MCMV) proteins essential for the viral replication. The transformed *Salmonella* bacteria were orally fed to MCMV-infected mice, resulting in *Salmonella* infection of the liver and spleen cells. In these cells, lysis of *Salmonella* bacteria released plasmid DNA containing M1GS followed by its expression to produce M1GS ribozyme, which then pairs with target viral mRNA and cleaves it. As a result, the treated mice displayed reduced viral gene expression, decreased viral titres, and improved survival compared to the untreated mice or mice treated with *Salmonella* containing control ribozyme (inactive) sequences.

The next challenge is to develop a safe, effective and targeted delivery system for humans. Interestingly, some viral vector systems have been utilized to deliver and express ribozymes to cleave HIV RNA in humans. Some of these ribozyme-based antiviral tools are currently in various stages of human clinical trials [230]. Moreover, a recent innovation that could possibly provide additional solutions includes the generation of bacterial cells controlled by chemically synthesized genomes [231]. Such a strategy can be used to create harmless bacteria that can serve as carriers of ribozymes or other viral RNA-cleaving tools to target and inhibit viral multiplication in humans. Further, it might be worth exploring engineering suitable bacterial strains to recognize the hACE2 receptor (as in the case of SARS-CoV-2) to gain systemic entry in humans, ensuring the entry of the engineered, antiviral ribozyme-carrying bacteria into the cells invaded by the virus. Lastly, efforts should be made to test the therapeutic efficacy of the above-described approaches individually and in combination with other known approaches to control viral multiplication synergistically. Given the lives and

Table 2
List of viral antigens, antibodies, and antiviral agents produced using plant-based systems.

S. No.	Product	Plant species used for production	Application	Status of Clinical trials	Reference
Antigen/Vaccine/Antiviral therapeutics					
1	Covifenz	*Nicotiana benthamiana*	A vaccine against COVID-19	Phase 3 completed, approved for usage in Canada (2022)	[258]
2	HAI-05	*Nicotiana benthamiana*	Recombinant hemagglutinin (rHA) Influenza vaccine	Phase 1 completed (2011)	[263, 264]
3	Quadrivalent virus-like particles (VLP) Vaccine	*Nicotiana benthamiana*	Vaccine against Influenza	Phase 3 completed (2019)	[265, 266]
4	Locteron	*Lemna minor*	Recombinant Interferon (IFN-α2b); Induces antiviral activity in Hepatitis C Virus (HCV) patients	Phase 1 and 2 completed (2009)	[267, 268]
5	Poliovirus capsid viral protein 1 (VP1)	Chloroplasts of *Nicotiana tabacum cv. Petit Havana* and *Lactuca sativa*	Viral protein 1 (VP1) subunit oral booster vaccine against polio	NA	[269]
6	Human Immunodeficiency Virus type I (HIV-1) and rabies virus antigenic peptide	*Nicotiana benthamiana*	Viral protein used for immunization against HIV-1 and rabies	NA	[270]
7	HIV-1 p24 capsid protein	*Nicotiana tobacum*	For production of p24 capsid peptide as antigen to develop HIV vaccines	NA	[271]
8	HIV gp120 multi-epitopic envelope protein	*Lactuca sativa*	Vaccine against multiple HIV strains	NA	[272]
9	CPDrg24 peptide of rabies virus	*Nicotiana benthamiana and Spinacia oleracea*	Viral protein used for immunization against rabies	NA	[273]
10	Hepatitis B viral envelope protein	*Lupinus luteus and Lactuca sativa*	Edible vaccine for Hepatitis B	NA	[274]
11	Hepatitis B core antigen (HBcAg)	*Nicotiana benthamiana*	Production of Hepatitis B core antigen (HBcAg)	NA	[275]
12	Human respiratory syncytial virus (RSV) A2 strain G-protein	*Nicotiana tabacum*	Expression of RSV G-proteins in plant viruses and used for immunizing mice to test their immunogenicity and protective efficacy against RSV	NA	[276]
13	Human Papilloma Virus (HPV) 16 L1-based chimeric virus-like particle (cVLP)	*Solanum lycopersicum*	Prophylactic and therapeutic vaccines against HPV-induced tumors.	NA	[277]
14	Dengue-Ebola recombinant immune complexes (DERIC) complexes	*Nicotiana benthamiana*	Vaccine against dengue and Ebola	NA	[278]
15	Ebola glycoprotein (GP) in fusion with 6D8 anti-Ebola IgG	*Nicotiana benthamiana*	Antigen-antibody fusion vaccine against Ebola	NA	[279]
Antiviral antibodies					
1	Zmapp	*Nicotiana benthamiana*	Chimeric antibody used for monoclonal antibody (mAb) therapy for Ebola	Phase 3 completed (2020)	[260]
2	P2G12	*Nicotiana tabacum*	mAb therapy for HIV-1	Phase 1 completed (2011)	[280]
3	Humanized 6D8 mAb	*Nicotiana benthamiana*	IgG1 targeting the Ebola virus glycoprotein GP1	NA	[281]
4	Antibody 2A10G6	*Nicotiana benthamiana*	Neutralizing mAb against flavivirus group of viruses (dengue, yellow fever, West Nile, Japanese encephalitis virus, and tick-borne encephalitis virus	NA	[282]
5	mAb HSV8	*Nicotiana benthamiana*	Human-derived antibody which neutralizes Herpes Simplex Virus (HSV)	NA	
6	Anti-HIV IgG	*Nicotiana benthamiana*	Anti-HIV IgG antibodies	NA	[275]

livelihood of millions at stake, intensifying global efforts to develop effective alternative antiviral therapeutic strategies could be a vital step in fighting the current and future pandemics.

5. Plant-based platforms for the production of antiviral agents

5.1. Large-scale production of viral antigens/antibodies in plants

Large-scale synthesis of proteins plays a major role in dealing with viral infections, from detection to disease management. The proteins produced constitute viral antigens, antibodies to detect viral antigens, and antiviral agents, including protein-based vaccines. Currently, large-scale production of therapeutic proteins (TPs) involves the usage of mammalian, yeast, or bacterial cell culture systems. However, these systems suffer from several disadvantages such as the involvement of complex processes of production and purification, high cost, a requirement of cold storage and transportation, short shelf-lives of purified TPs, the need for health care

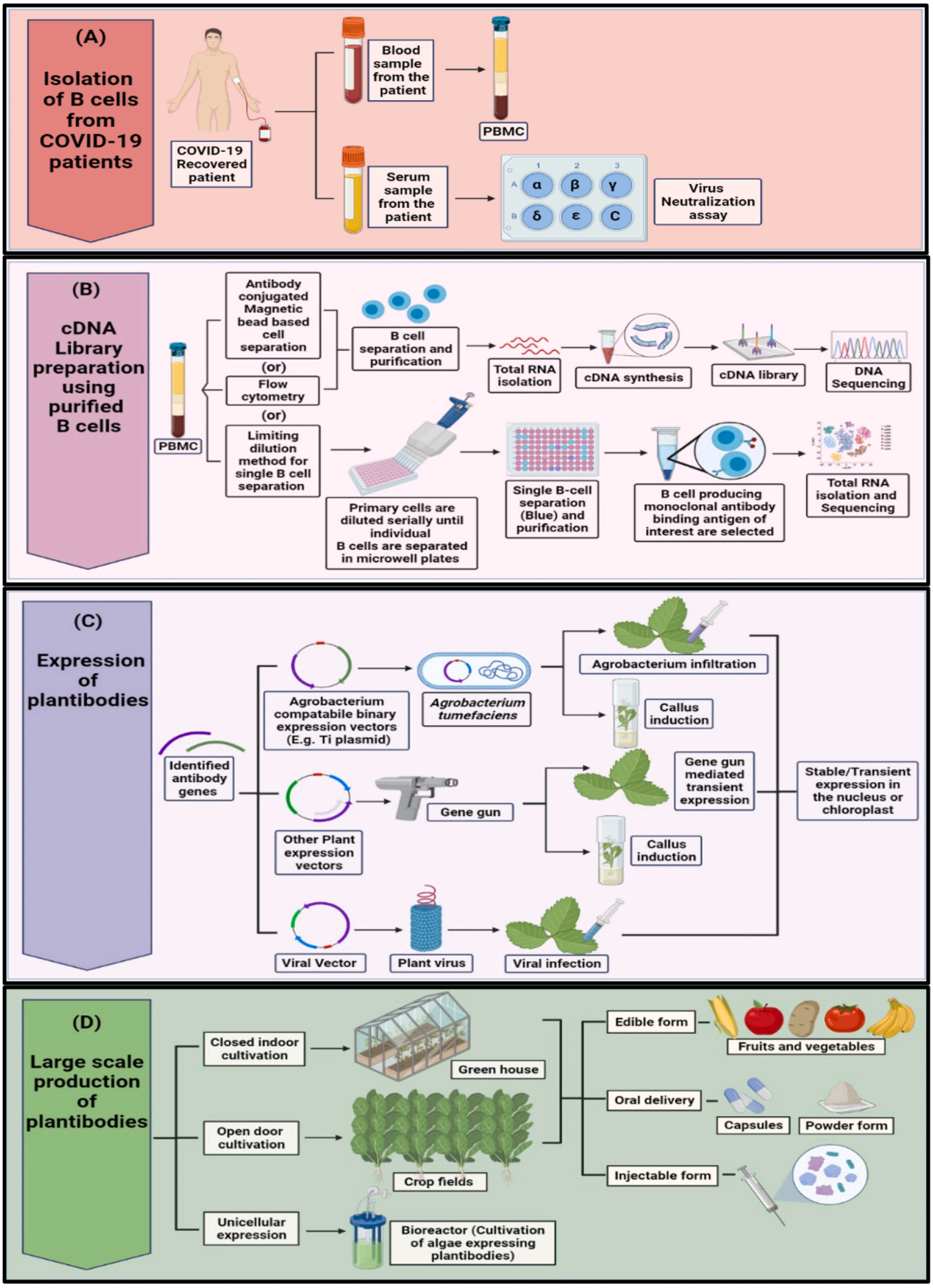

Fig. 10. Plant-based platforms for cloning and expression of antibodies derived from pathogen-specific B cells. The figure depicts various steps involved in antibody production. (A) Isolation of B cells from COVID-19 patients. (B) Purification of B cells and cDNA library preparation. (C) Isolation of the antibody-producing gene followed by cloning and expression using various expression systems such as *Agrobacterium tumefaciens*, plant or viral expression vectors. Various methods are used for the transformation of these expression systems (e.g., leaf infiltration, callus induction, gene gun). These methods allow for transient as well as stable expression of the transgenes. (D) The plants transiently or stably expressing antibodies can be grown on a large scale using either green houses, fields, bioreactors, or hydroponic systems. The antibodies thus produced can be used either directly by consuming fruits and vegetables or unpurified powder. In addition, antibodies can be purified to develop injectable or capsule forms. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

personnel for administration, and the risk of human pathogenic contamination during production/storage/transportation [232–234].

An additional challenge associated with TPs is that they are not feasible for oral delivery because they will be degraded by stomach acids and proteases in the digestive system. Moreover, systemic delivery of TPs is also not possible due to their inability to cross intestinal membrane barriers [235]. Collectively, these shortcomings have become limiting factors in the large-scale production of TPs. Although several attempts have been made to modify TPs to facilitate their oral delivery, no clinically approved oral peptide drugs are available. Therefore, TPs are primarily delivered through injection, but their targeted delivery poses several challenges. Production of recombinant TPs in plants offers an ideal alternative platform to traditional manufacturing processes with several advantages as described below (for details, see the review by Kwon and Daniel) [233]. In a significant development, US Food and Drug Administration (FDA) has recently approved plant cells for cost-effective large-scale production of protein drugs using current good manufacturing practice (cGMP) hydroponic growth facilities [233].

By and large, plants are safe hosts and are free of adventitious agents that can infect humans and animals. Large-scale production of TPs, also called biopharming, can be achieved either using whole plants or plant cell cultures. Between the two, plant cell cultures are preferred to minimize the regulatory concerns and to expedite approval by the regulatory bodies [236]. Akin to mammalian cells, plant cells carry out post-translational modifications of foreign proteins, including the formation of disulphide bonds, glycosylation, folding, and assembly [237,238]. Moreover, tests are available to determine if recombinant TPs expressed in plants are bioactive or not. For example, different human interferons having antiviral properties were expressed in plants and shown to be bioactive using human cell lines [239–242].

Plant cells are totipotent, wherein a single cell can develop into a complete plant. This feature has been exploited to generate transgenic plants expressing proteins of interest through tissue culture methods. Commonly, TP-coding genes are inserted into the nuclear or chloroplast genomes (transplastomic plants) mainly by using *Agrobacterium* (a group of gram-negative bacteria) or a biolistic gun (gene gun) [233]. Codon-optimization of the foreign genes for expression in chloroplasts can increase protein expression levels by 5 to 50-fold [243]. Ectopic expression of proteins in chloroplasts offers several advantages. First, the inserted gene copy number can reach >10,000 per cell because a plant cell contains several chloroplasts and each chloroplast carry several copies of the circular chloroplast genome. Due to this high copy number of the inserted gene, maximum expression levels of transgenes can reach up to 70% of the total leaf protein [244]. Second, it eliminates the risk of foreign gene dispersal through pollen because chloroplasts follow maternal inheritance in most plant species [245]. Third, site-specific integration of genes of interest eliminates positional effects, and multiple genes can be inserted in a single transformation event using homologous recombination [246,247]. Fourth, gene silencing has not been observed in chloroplasts, and the effects of toxic proteins are minimized by sequestering them within these organelles [248]. Most importantly, TPs expressed in chloroplasts can indefinitely maintain their stability in lyophilized plant cells by preserving their folding and functional efficacy even when stored at ambient temperature, eliminating the need for cold storage [249].

Several transient expression systems are available to express TPs in whole plants that do not require generation of stable transgenic plants. One such expression vector system involves using a high-efficiency tobacco mosaic virus RNA-based overexpression vector (TRBO) [250] for transient expression of proteins of interest. This system is rapid (~6 weeks from cloning to obtain purified proteins) and low-cost because it neither requires the generation of stable transgenic plants nor expensive infrastructure. More importantly, foreign proteins can be expressed at as high as 5.5 mg/g of fresh weight of plant tissue, the highest among the existing plant-based expression systems [250,251]. For the TRBO vector, infection efficiency with *Agrobacterium tumefaciens* is very high, thus minimizing hyper response-mediated cell death in infiltrated leaves. Although this is a virus-based vector, infectious virus particles will not be formed since it lacks the viral coat protein. Therefore, the biocontainment of these viral vectors is not a problem, as they will be confined to the infiltrated leaves. In the recent past, plant-made TPs had been orally delivered to treat Alzheimer's disease, diabetes, hypertension, and Gaucher's or ocular diseases in mouse/rat models [252–256]. In Table 2, we have listed some examples of antigens, antibodies, and other proteins produced in plants that are used against viral infections, including their clinical trial status. For more examples, refer to Altindis et al. [257]. The most notable example from the list is Covifenz, the recent-most vaccine developed by Medicago Inc. and approved by Canada for use against COVID-19 [258]. Covifenz consists of plant-based virus-like particles (VLP) of SARS-CoV-2 spike protein belonging to the original strain [258].

Another example is ZMapp, a combination of three humanized monoclonal antibodies generated in tobacco plants against the Ebola virus [259]. These antibodies not only resulted in the reversion of advanced Ebola virus disease in nonhuman primates [260] but also showed promising results in clinical trials in humans [261]. Manufacturing economics of plant-made therapeutic and industrial enzymes revealed that plants offer substantial cost advantages over alternative platforms [262]. Delivery of TPs without the need for expensive purification procedures can dramatically reduce the cost further. In Fig. 10, we provide a schematic pipeline outlining how plant-based platforms can be employed to mass-produce pathogen-specific antibodies (e.g., antibodies against SARS-CoV-2). This scheme involves isolation of the B cells from the patients, followed by total RNA isolation, cDNA library construction, and sequencing to identify genes that produce the pathogen-specific antibodies. Following this, the genes expressing the antibodies of interest can be cloned into any of the desirable plant expression vectors. Upon production, the antibodies shall be tested for their efficacy, and the promising antibodies can undergo large-scale production, as outlined in Fig. 10. Once established, this kind of pipeline can be easily extended to any new pathogens, including viruses and their variants.

5.2. Oral delivery of plant-made therapeutic proteins

Plants have cell walls filled with tightly packed lignin and cellulose, which provide natural protection to ectopically expressed TPs when orally ingested because human enzymes cannot degrade these carbohydrates. However, bioencapsulated TPs are released into the gut lumen upon digestion by gut bacteria [283,284]. Various tags, such as receptor-binding proteins and cell-penetrating peptides,

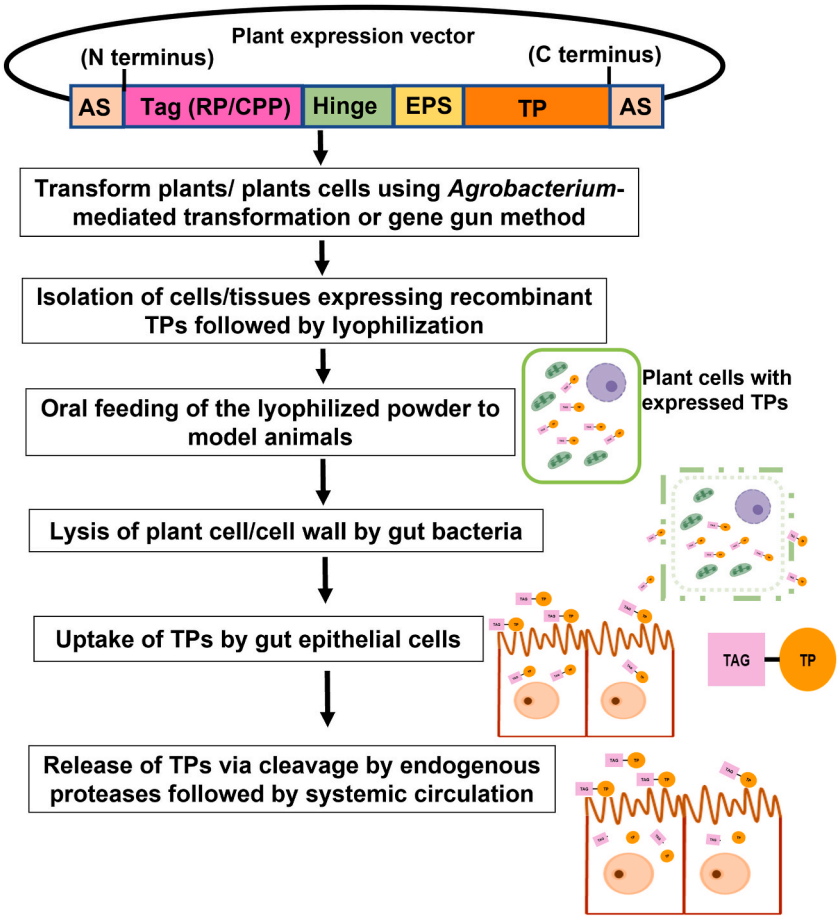

Fig. 11. A flowchart illustrating the expression of therapeutic proteins (TPs) in plants and their oral delivery. AS-Accessory sequence; CPP-Cell-penetrating peptide; EPS-Endogenous protease cleavage site; RP-Receptor protein.

are fused to TPs to facilitate their uptake by gut epithelial cells via the membrane barriers and deliver them to the circulatory or the immune system [285]. These tags use different cellular routes and mechanisms for delivering the fused TPs [286–290]. The introduction of endogenous protease cleavage sites (e.g., Furin) as part of the tags fused to TPs facilitates the release of TPs from the rest of the tag upon their uptake [291].

Plant-based protein expression systems have recently been used for oral delivery of TPs using leaf powder in mouse models, eliminating the time-consuming and cost-enhancing protein purification and cold storage [233,252,255,256,285,292–296]. Fig. 11 provides a brief illustration of the steps and mechanisms involved in expressing TPs in plants and their oral delivery in mammalian systems. In these experiments, the cholera toxin B subunit (CTB) proved to be a highly desirable tag for efficient systemic delivery of TPs. Note that recombinant CTB (rCTB) is a component of the clinical human cholera vaccine being used internationally for over a decade [297]. If the TPs must be delivered to the intestine as their site of action, then fusing tags is not required. ACE-2 expression has been found to be the highest in the brush border of intestinal enterocytes [298]. Moreover, gastrointestinal symptoms are found in a subset of patients [299,300] and viral RNA can be detected in rectal swabs [301,302]. Therefore, oral delivery of non-purified plant-expressed antiviral TPs to the GI tract could be worth exploring as a treatment option.

Moving forward, plants offer promising options for similar oral drug delivery of TPs in humans as a rapid, safe, and low-cost alternative. It appears that such promising plant-based technologies are currently underutilized and underexplored. We reckon that management of current and future pandemics would greatly benefit by utilizing these tools to their fullest potential, not just as stand-alone tools but as a significant component of integrated approaches in fighting viral infections.

6. Concluding remarks

Due to the severity and widespread nature of the COVID-19, the pandemic has pushed the scientific community to carry out studies at an unprecedented pace to find solutions to control the viral infection and its spread. Global efforts have resulted in developing several diagnostic tools and clinically approved vaccines for use across age groups. Although the pandemic currently appears to be waning worldwide, having diverted most of the resources to deal with this pandemic has enormously impacted the research and

management of other diseases. Moreover, the prolonged duration of the COVID-19 pandemic has resulted in an unprecedented negative impact on all aspects of human life, including the global economy, public health, mental well-being, education, and allied sectors. Therefore, the need for the global scientific community to collectively address the current and the future pandemics has never been higher before. Finally, we conclude that coordinated efforts to explore, integrate, and streamline the current and newer technologies are required to strengthen the viral diagnostics and therapeutics, aimed at rapid control of morbidity and mortality rates. Hopefully, such combined efforts would leave us better prepared for future pandemics.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

RJD and GM are thankful to Birla Institute of Science and Technology (BITS) Pilani, Hyderabad campus for their support and for providing an Institute Fellowship to SBK, GPS and NP. RJD is also thankful to the Department of Biotechnology (DBT), Government of India, for Ramalingaswami fellowship (BT/RLF/Re-entry/18/2016) and DBT grant (BT/PR34478/MED/15/206/2019) and Life Science Research Board, Defence Research & Development Organisation (DRDO) grant (LSRB/81/48222/LSRB-367/BTB/2020) for supporting junior research fellowship to SBK and senior research fellowship to NO for their doctoral research, respectively. GM is thankful to Science and Engineering Research Board (SERB), Government of India, for Ramanujan Fellowship (SB/S2/RJN-062/2017) and CRG grant (CRG/2020/002855), Indian Council of Medical Research (ICMR) for senior research fellowship to NP. Authors also profusely thank Prof. Venkat Gopalan, the Ohio state university, USA, for his critique of this manuscript. Figs. 2 and 3 and 6–8 and 10 were created using BioRender.com.

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
BCG	Bacillus Calmette–Guérin
BFP	Blue fluorescent protein
CAS	CRISPR associated protein
CFP	Cyan fluorescent protein
cGMP	Current good manufacturing practice
COVID-19	Coronavirus disease 2019
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CTB	Cholera toxin B subunit
CTE	Constitutive transport element
DFHBI	3,5-difluoro-4-hydroxybenzylidene imidazolinone
DMHBI	5-dimethoxy-4-hydroxybenzylidene imidazolinone
DNA	Deoxyribonucleic acid
EGS	External guide sequence
ELISA	Enzyme-linked immunoassay
FDA	Food and Drug Administration
GFP	Green fluorescent protein
GI	Gastrointestinal
hACE 2	Human angiotensin-converting enzyme 2
HBI	4-Hydroxybenzylidene imidazolinone
HPV	Human papilloma virus
IFN	Interferon
IL	Interleukin
IoT	Internet of things
LFA	Lateral flow assays
LoC	Lab-on-chip
M1GS	M1 guide sequence

mAbs	Monoclonal antibodies
MCMV	Murine cytomegalovirus
MERS	Middle East respiratory syndrome
NAAT	Nucleic acid amplification test
nAbs	Nanobodies
NOD	Nucleotide-binding and oligomerization domain
PCR	Polymerase chain reaction
POC	Point-of-care
RBD	Receptor-binding domain
rCTB	Recombinant cholera toxin B subunit
RFP	Red fluorescent protein
RNA	Ribonucleic acid
RPA	Recombinase polymerase amplification
RSV	Respiratory syncytial virus
RT	Reverse transcription
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIV	Simian immunodeficiency virus
STING	Stimulator of Interferon Gene
TB	Tuberculosis
TLR	Toll-like receptor
TNF	Tumour Necrosis Factor
TP	Therapeutic protein
TRBO	Tobacco mosaic virus RNA-based overexpression vector
MMR	Measles, mumps, and rubella
NSP	Non-structural protein

References

[1] M.A. Shereen, S. Khan, A. Kazmi, N. Bashir, R. Siddique, COVID-19 infection: origin, transmission, and characteristics of human coronaviruses, J. Adv. Res. 24 (2020) 91–98, https://doi.org/10.1016/j.jare.2020.03.005.

[2] M. Ciotti, M. Ciccozzi, A. Terrinoni, W.-C. Jiang, C.-B. Wang, S. Bernardini, The COVID-19 pandemic, Crit. Rev. Clin. Lab Sci. 57 (2020) 365–388, https://doi.org/10.1080/10408363.2020.1783198.

[3] Y.A. Helmy, M. Fawzy, A. Elaswad, A. Sobieh, S.P. Kenney, A.A. Shehata, The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control, J. Clin. Med. 9 (2020) 1225.

[4] P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270–273.

[5] F. Wu, S. Zhao, B. Yu, Y.-M. Chen, W. Wang, Z.-G. Song, Y. Hu, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265–269.

[6] J. Cui, F. Li, Z.-L. Shi, Origin and evolution of pathogenic coronaviruses, Nat. Rev. Microbiol. 17 (2019) 181–192.

[7] F.A. Rabi, M.S. Al Zoubi, G.A. Kasasbeh, D.M. Salameh, A.D. Al-Nasser, SARS-CoV-2 and coronavirus disease 2019: what we know so far, Pathogens 9 (2020) 231.

[8] D. Vijaykrishna, G.J. Smith, J.X. Zhang, J. Peiris, H. Chen, Y. Guan, Evolutionary insights into the ecology of coronaviruses, J. Virol. 81 (2007) 4012–4020.

[9] D.K. Chu, C.Y. Leung, M. Gilbert, P.H. Joyner, E.M. Ng, T.M. Tse, Y. Guan, J.S. Peiris, L.L. Poon, Avian coronavirus in wild aquatic birds, J. Virol. 85 (2011) 12815–12820.

[10] P.C. Woo, S.K. Lau, C.S. Lam, C.C. Lau, A.K. Tsang, J.H. Lau, R. Bai, J.L. Teng, C.C. Tsang, M. Wang, Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus, J. Virol. 86 (2012) 3995–4008.

[11] M.A. Turcios-Casco, R.C. Gatti, Do not blame bats and pangolins! Global consequences for wildlife conservation after the SARS-CoV-2 pandemic, Biodivers. Conserv. 29 (2020) 3829–3833.

[12] W.T. Harvey, A.M. Carabelli, B. Jackson, R.K. Gupta, E.C. Thomson, E.M. Harrison, C. Ludden, R. Reeve, A. Rambaut, S.J. Peacock, SARS-CoV-2 variants, spike mutations and immune escape, Nat. Rev. Microbiol. 19 (2021) 409–424.

[13] A.S. Lauring, E.B. Hodcroft, Genetic variants of SARS-CoV-2—what do they mean? JAMA 325 (2021) 529–531.

[14] A.M. Carabelli, T.P. Peacock, L.G. Thorne, W.T. Harvey, J. Hughes, S.J. Peacock, W.S. Barclay, T.I. de Silva, G.J. Towers, D.L. Robertson, SARS-CoV-2 variant biology: immune escape, transmission and fitness, Nat. Rev. Microbiol. 21 (2023) 162–177, https://doi.org/10.1038/s41579-022-00841-7.

[15] Z. Zhao, H. Li, X. Wu, Y. Zhong, K. Zhang, Y.-P. Zhang, E. Boerwinkle, Y.-X. Fu, Moderate mutation rate in the SARS coronavirus genome and its implications, BMC Evol. Biol. 4 (2004) 1–9.

[16] M. Pachetti, B. Marini, F. Benedetti, F. Giudici, E. Mauro, P. Storici, C. Masciovecchio, S. Angeletti, M. Ciccozzi, R.C. Gallo, Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant, J. Transl. Med. 18 (2020) 1–9.

[17] FDA. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests#mutations, 2021.

[18] M. Cascella, M. Rajnik, A. Aleem, S. Dulebohn, R. Di Napoli, Features, Evaluation, and Treatment of Coronavirus (COVID-19), StatPearls, 2021.

[19] M. Ciaccio, L. Agnello, Biochemical biomarkers alterations in coronavirus disease 2019 (COVID-19), Diagnosis 7 (2020) 365–372, https://doi.org/10.1515/dx-2020-0057.

[20] B. Bordallo, M. Bellas, A.F. Cortez, M. Vieira, M. Pinheiro, Severe COVID-19: what have we learned with the immunopathogenesis? Adv. Rheumatol. 60 (2020) 50, https://doi.org/10.1186/s42358-020-00151-7.

[21] J.F.-W. Chan, C.C.-Y. Yip, K.K.-W. To, T.H.-C. Tang, S.C.-Y. Wong, K.-H. Leung, A.Y.-F. Fung, A.C.-K. Ng, Z. Zou, H.-W. Tsoi, et al., Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/hel real-time reverse transcription-PCR assay validated In vitro and with clinical specimens, J. Clin. Microbiol. 58 (2020), e00310-00320, https://doi.org/10.1128/jcm.00310-20.

[22] S.J. Ahn, Y.H. Baek, K.K.S. Lloren, W.S. Choi, J.H. Jeong, K.J.C. Antigua, H.I. Kwon, S.J. Park, E.H. Kim, Y.I. Kim, et al., Rapid and simple colorimetric detection of multiple influenza viruses infecting humans using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform, BMC Infect. Dis. 19 (2019) 676, https://doi.org/10.1186/s12879-019-4277-8.

[23] C. Amaral, W. Antunes, E. Moe, A.G. Duarte, L.M.P. Lima, C. Santos, I.L. Gomes, G.S. Afonso, R. Vieira, H.S.S. Teles, et al., A molecular test based on RT-LAMP for rapid, sensitive and inexpensive colorimetric detection of SARS-CoV-2 in clinical samples, Sci. Rep. 11 (2021), 16430, https://doi.org/10.1038/s41598-021-95799-6.

[24] S. Bhadra, Y.S. Jiang, M.R. Kumar, R.F. Johnson, L.E. Hensley, A.D. Ellington, Real-time sequence-validated loop-mediated isothermal amplification assays for detection of Middle East respiratory syndrome coronavirus (MERS-CoV), PLoS One 10 (2015), e0123126, https://doi.org/10.1371/journal.pone.0123126.

[25] T.C. Hong, Q.L. Mai, D.V. Cuong, M. Parida, H. Minekawa, T. Notomi, F. Hasebe, K. Morita, Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus, J. Clin. Microbiol. 42 (2004) 1956–1961, https://doi.org/10.1128/jcm.42.5.1956-1961.2004.

[26] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Loop-mediated isothermal amplification of DNA, Nucleic Acids Res. 28 (2000) E63, https://doi.org/10.1093/nar/28.12.e63.

[27] O. Piepenburg, C.H. Williams, D.L. Stemple, N.A. Armes, DNA detection using recombination proteins, PLoS Biol. 4 (2006), e204, https://doi.org/10.1371/journal.pbio.0040204.

[28] S. Xia, X. Chen, Single-copy sensitive, field-deployable, and simultaneous dual-gene detection of SARS-CoV-2 RNA via modified RT–RPA, Cell Discovery 6 (2020) 1–4.

[29] Y. Sun, L. Yu, C. Liu, S. Ye, W. Chen, D. Li, W. Huang, One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a, J. Transl. Med. 19 (2021) 1–10.

[30] P. Maffert, S. Reverchon, W. Nasser, C. Rozand, H. Abaibou, New nucleic acid testing devices to diagnose infectious diseases in resource-limited settings, Eur. J. Clin. Microbiol. Infect. Dis. 36 (2017) 1717–1731.

[31] J. Li, J. Macdonald, F. von Stetten, A comprehensive summary of a decade development of the recombinase polymerase amplification, Analyst 144 (2018) 31–67.

[32] M. Kong, Z. Li, J. Wu, J. Hu, Y. Sheng, D. Wu, Y. Lin, M. Li, X. Wang, S. Wang, A wearable microfluidic device for rapid detection of HIV-1 DNA using recombinase polymerase amplification, Talanta 205 (2019), 120155.

[33] L. Lillis, D.A. Lehman, J.B. Siverson, J. Weis, J. Cantera, M. Parker, O. Piepenburg, J. Overbaugh, D.S. Boyle, Cross-subtype detection of HIV-1 using reverse transcription and recombinase polymerase amplification, J. Virol Methods 230 (2016) 28–35.

[34] L. Liu, J. Wang, R. Zhang, M. Lin, R. Shi, Q. Han, J. Wang, W. Yuan, Visual and equipment-free reverse transcription recombinase polymerase amplification method for rapid detection of foot-and-mouth disease virus, BMC Vet. Res. 14 (2018) 1–8.

[35] S. Wang, B. Huang, X. Ma, P. Liu, Y. Wang, X. Zhang, L. Zhu, Q. Fan, Y. Sun, K. Wang, Reverse-transcription recombinase-aided amplification assay for H7 subtype avian influenza virus, Transbound. Emerg. Dis. 67 (2020) 877–883.

[36] J. Coertse, J. Weyer, L.H. Nel, W. Markotter, Reverse transcription recombinase polymerase amplification assay for rapid detection of canine associated rabies virus in Africa, PLoS One 14 (2019), e0219292.

[37] M. Yang, Y. Ke, X. Wang, H. Ren, W. Liu, H. Lu, W. Zhang, S. Liu, G. Chang, S. Tian, Development and evaluation of a rapid and sensitive EBOV-RPA test for rapid diagnosis of Ebola virus disease, Sci. Rep. 6 (2016) 1–7.

[38] L.D. Renner, J. Zan, L.I. Hu, M. Martinez, P.J. Resto, A.C. Siegel, C. Torres, S.B. Hall, T.R. Slezak, T.H. Nguyen, Detection of ESKAPE bacterial pathogens at the point of care using isothermal DNA-based assays in a portable degas-actuated microfluidic diagnostic assay platform, Appl. Environ. Microbiol. 83 (2017).

[39] H.M. Amer, A. Abd El Wahed, M.A. Shalaby, F.N. Almajhdi, F.T. Hufert, M. Weidmann, A new approach for diagnosis of bovine coronavirus using a reverse transcription recombinase polymerase amplification assay, J. Virol. Methods 193 (2013) 337–340, https://doi.org/10.1016/j.jviromet.2013.06.027.

[40] T. Yang, Y.-C. Wang, C.-F. Shen, C.-M. Cheng, Point-of-care RNA-Based Diagnostic Device for COVID-19, Multidisciplinary Digital Publishing Institute, 2020.

[41] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (2017) 438–442.

[42] M. Tan, C. Liao, L. Liang, X. Yi, Z. Zhou, G. Wei, Recent advances in recombinase polymerase amplification: principle, advantages, disadvantages and applications, Front. Cell. Infect. Microbiol. 12 (2022), 1019071, https://doi.org/10.3389/fcimb.2022.1019071.

[43] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip, Science 261 (1993) 895–897.

[44] T. Leichle, L. Nicu, T. Alava, MEMS biosensors and COVID-19: missed opportunity, ACS Sens. 5 (2020) 3297–3305.

[45] L. Syedmoradi, M. Daneshpour, M. lvandipour, et al., Point of care testing: the impact of nanotechnology, Biosens. Bioelectron. 87 (2017) 373–387.

[46] S. Lutz, P. Weber, M. Focke, B. Faltin, J. Hoffmann, C. Müller, D. Mark, G. Roth, P. Munday, N. Armes, et al., Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA), Lab Chip 10 (2010) 887–893, https://doi.org/10.1039/b921140c.

[47] Y. Zou, M.G. Mason, Y. Wang, E. Wee, C. Turni, P.J. Blackall, M. Trau, J.R. Botella, Nucleic acid purification from plants, animals and microbes in under 30 seconds, PLoS Biol. 15 (2017), e2003916, https://doi.org/10.1371/journal.pbio.2003916.

[48] U. Kiran, C. Gokulan, S.K. Kuncha, D. Vedagiri, B.T. Chander, A.V. Sekhar, S. Dontamala, A.L. Reddy, K.B. Tallapaka, R.K. Mishra, Easing diagnosis and pushing the detection limits of SARS-CoV-2, Biol. Methods Protocols 5 (2020) bpaa017.

[49] G.S. Filonov, J.D. Moon, N. Svensen, S.R. Jaffrey, Broccoli: rapid selection of an RNA mimic of green fluorescent protein by fluorescence-based selection and directed evolution, J. Am. Chem. Soc. 136 (2014) 16299–16308, https://doi.org/10.1021/ja508478x.

[50] W. Song, R.L. Strack, N. Svensen, S.R. Jaffrey, Plug-and-play fluorophores extend the spectral properties of Spinach, J. Am. Chem. Soc. 136 (2014) 1198–1201, https://doi.org/10.1021/ja410819x.

[51] J.S. Paige, K.Y. Wu, S.R. Jaffrey, RNA mimics of green fluorescent protein, Science 333 (2011) 642–646, https://doi.org/10.1126/science.1207339.

[52] R.L. Strack, M.D. Disney, S.R. Jaffrey, A superfolding Spinach2 reveals the dynamic nature of trinucleotide repeat-containing RNA, Nat. Methods 10 (2013) 1219–1224, https://doi.org/10.1038/nmeth.2701.

[53] X. Li, H. Kim, J.L. Litke, J. Wu, S.R. Jaffrey, Fluorophore-promoted RNA folding and photostability enables imaging of single broccoli-tagged mRNAs in live mammalian cells, Angew. Chem. Int. Ed. 59 (2020) 4511–4518.

[54] K.D. Warner, L. Sjekloca, W. Song, G.S. Filonov, S.R. Jaffrey, A.R. Ferre-D'Amare, A homodimer interface without base pairs in an RNA mimic of red fluorescent protein, Nat. Chem. Biol. 13 (2017) 1195–1201, https://doi.org/10.1038/nchembio.2475.

[55] M. You, S.R. Jaffrey, Structure and mechanism of RNA mimics of green fluorescent protein, Annu. Rev. Biophys. 44 (2015) 187–206, https://doi.org/10.1146/annurev-biophys-060414-033954.

[56] B.S. VarnBuhler, J. Moon, S.K. Dey, J. Wu, S.R. Jaffrey, Detection of SARS-CoV-2 RNA using a DNA aptamer mimic of green fluorescent protein, ACS Chem. Biol. 17 (2022) 840–853, https://doi.org/10.1021/acschembio.1c00893.

[57] A. Samacoits, P. Nimsamer, O. Mayuramart, N. Chantaravisoot, P. Sitthi-Amorn, C. Nakhakes, L. Luangkamchorn, P. Tongcham, U. Zahm, S. Suphanpayak, et al., Machine learning-driven and smartphone-based fluorescence detection for CRISPR diagnostic of SARS-CoV-2, ACS Omega 6 (2021) 2727–2733, https://doi.org/10.1021/acsomega.0c04929.

[58] A. Nagy, L. Cernikova, E. Vitaskova, V. Krivda, A. Dan, Z. Dirbakova, H. Jirincova, B. Prochazka, K. Sedlak, M. Havlickova, Correction: MeltMan: optimization, evaluation, and universal application of a qPCR system integrating the TaqMan qPCR and melting analysis into a single assay, PLoS One 13 (2018), e0196444, https://doi.org/10.1371/journal.pone.0196444.

[59] A. Nagy, E. Vitaskova, L. Cernikova, V. Krivda, H. Jirincova, K. Sedlak, J. Hornickova, M. Havlickova, Evaluation of TaqMan qPCR system integrating two identically labelled hydrolysis probes in single assay, Sci. Rep. 7 (2017), 41392, https://doi.org/10.1038/srep41392.

[60] D. Steensels, A. Vankeerberghen, H. De Beenhouwer, Towards multitarget testing in molecular microbiology, Int. J. Microbiol. 2013 (2013), 121057, https://doi.org/10.1155/2013/121057.

[61] M. Lisboa Bastos, G. Tavaziva, S.K. Abidi, J.R. Campbell, L.P. Haraoui, J.C. Johnston, Z. Lan, S. Law, E. MacLean, A. Trajman, et al., Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis, BMJ 370 (2020) m2516, https://doi.org/10.1136/bmj.m2516.

[62] M. Nuccetelli, M. Pieri, S. Grelli, M. Ciotti, R. Miano, M. Andreoni, S. Bernardini, SARS-CoV-2 infection serology: a useful tool to overcome lockdown? Cell Death Dis. 6 (2020) 38, https://doi.org/10.1038/s41420-020-0275-2, 275[pii].

[63] R. Kubina, A. Dziedzic, Molecular and serological tests for COVID-19 a comparative review of SARS-CoV-2 coronavirus laboratory and point-of-care diagnostics, Diagnostics 10 (2020), https://doi.org/10.3390/diagnostics10060434 diagnostics10060434 [pii].

[64] R.W. Peeling, C.J. Wedderburn, P.J. Garcia, D. Boeras, N. Fongwen, J. Nkengasong, A. Sall, A. Tanuri, D.L. Heymann, Serology testing in the COVID-19 pandemic response, Lancet Infect. Dis. 20 (2020) e245–e249, https://doi.org/10.1016/S1473-3099(20)30517-X. S1473-3099(20)30517-X [pii].

[65] B.E. Pickett, D.S. Greer, Y. Zhang, L. Stewart, L. Zhou, G. Sun, Z. Gu, S. Kumar, S. Zaremba, C.N. Larsen, et al., Virus pathogen database and analysis resource (ViPR): a comprehensive bioinformatics database and analysis resource for the coronavirus research community, Viruses 4 (2012) 3209–3226, https://doi.org/10.3390/v4113209, v4113209 [pii].

[66] B.E. Pickett, E.L. Sadat, Y. Zhang, J.M. Noronha, R.B. Squires, V. Hunt, M. Liu, S. Kumar, S. Zaremba, Z. Gu, et al., ViPR: an open bioinformatics database and analysis resource for virology research, Nucleic Acids Res. 40 (2012) D593–D598, https://doi.org/10.1093/nar/gkr859, gkr859 [pii].

[67] R. Heiss, D.M. Grodzki, W. Horger, M. Uder, A.M. Nagel, S. Bickelhaupt, High-performance low field MRI enables visualization of persistent pulmonary damage after COVID-19, Magn. Reson. Imaging 76 (2021) 49–51, https://doi.org/10.1016/j.mri.2020.11.004.

[68] J.W. Song, C. Zhang, X. Fan, F.P. Meng, Z. Xu, P. Xia, W.J. Cao, T. Yang, X.P. Dai, S.Y. Wang, et al., Immunological and inflammatory profiles in mild and severe cases of COVID-19, Nat. Commun. 11 (2020) 3410, https://doi.org/10.1038/s41467-020-17240-2.

[69] M. Zheng, Y. Gao, G. Wang, G. Song, S. Liu, D. Sun, Y. Xu, Z. Tian, Functional exhaustion of antiviral lymphocytes in COVID-19 patients, Cell. Mol. Immunol. 17 (2020) 533–535, https://doi.org/10.1038/s41423-020-0402-2.

[70] M. Maeurer, R. Ramalho, F.-S. Wang, A. Zumla, Host-directed therapies for COVID-19, Curr. Opin. Pulm. Med. 27 (2021) 205–209, https://doi.org/10.1097/mcp.0000000000000769.

[71] A. Zumla, D.S. Hui, E.I. Azhar, Z.A. Memish, M. Maeurer, Reducing mortality from 2019-nCoV: host-directed therapies should be an option, Lancet 395 (2020) e35–e36, https://doi.org/10.1016/s0140-6736(20)30305-6.

[72] M. Market, L. Angka, A.B. Martel, D. Bastin, O. Olanubi, G. Tennakoon, D.M. Boucher, J. Ng, M. Ardolino, R.C. Auer, Flattening the COVID-19 curve with natural killer cell based immunotherapies, Front. Immunol. 11 (2020).

[73] F. Ahmed, D.H. Jo, S.H. Lee, Can natural killer cells Be a principal player in anti-SARS-CoV-2 immunity? Front. Immunol. 11 (2020), 586765 https://doi.org/10.3389/fimmu.2020.586765.

[74] X. Cui, L. Chen, T. Xue, J. Yu, J. Liu, Y. Ji, L. Cheng, Human umbilical cord and dental pulp-derived mesenchymal stem cells: biological characteristics and potential roles in vitro and in vivo, Mol. Med. Rep. 11 (2015) 3269–3278, https://doi.org/10.3892/mmr.2015.3198.

[75] S. Croci, M. Bonacini, G. Dolci, M. Massari, N. Facciolongo, E. Pignatti, A. Pisciotta, G. Carnevale, A. Negro, G. Cassone, et al., Human dental pulp stem cells modulate cytokine production in vitro by peripheral blood mononuclear cells from coronavirus disease 2019 patients, Front. Cell Dev. Biol. 8 (2020), 609204, https://doi.org/10.3389/fcell.2020.609204.

[76] F. Meng, R. Xu, S. Wang, Z. Xu, C. Zhang, Y. Li, T. Yang, L. Shi, J. Fu, T. Jiang, et al., Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial, Signal Transduct. Targeted Ther. 5 (2020) 172, https://doi.org/10.1038/s41392-020-00286-5.

[77] L. Shi, H. Huang, X. Lu, X. Yan, X. Jiang, R. Xu, S. Wang, C. Zhang, X. Yuan, Z. Xu, et al., Effect of human umbilical cord-derived mesenchymal stem cells on lung damage in severe COVID-19 patients: a randomized, double-blind, placebo-controlled phase 2 trial, Signal Transduct. Targeted Ther. 6 (2021) 58, https://doi.org/10.1038/s41392-021-00488-5.

[78] L. Tang, Y. Jiang, M. Zhu, L. Chen, X. Zhou, C. Zhou, P. Ye, X. Chen, B. Wang, Z. Xu, et al., Clinical study using mesenchymal stem cells for the treatment of patients with severe COVID-19, Front. Med. 14 (2020) 664–673, https://doi.org/10.1007/s11684-020-0810-9.

[79] J.M. Inal, Decoy ACE2-expressing extracellular vesicles that competitively bind SARS-CoV-2 as a possible COVID-19 therapy, Clin. Sci. 134 (2020) 1301–1304, https://doi.org/10.1042/cs20200623.

[80] D. Batlle, J. Wysocki, K. Satchell, Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? Clin. Sci. 134 (2020) 543–545, https://doi.org/10.1042/cs20200163.

[81] J.J. Sims, S. Lian, R.L. Meggersee, A. Kasimsetty, J.M. Wilson, High activity of an affinity-matured ACE2 decoy against Omicron SARS-CoV-2 and pre-emergent coronaviruses, PLoS One 17 (2022), e0271359, https://doi.org/10.1371/journal.pone.0271359.

[82] X. Chi, R. Yan, J. Zhang, G. Zhang, Y. Zhang, M. Hao, Z. Zhang, P. Fan, Y. Dong, Y. Yang, et al., A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2, Science 369 (2020) 650–655, https://doi.org/10.1126/science.abc6952.

[83] L. Liu, P. Wang, M.S. Nair, J. Yu, M. Rapp, Q. Wang, Y. Luo, J.F. Chan, V. Sahi, A. Figueroa, et al., Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike, Nature 584 (2020) 450–456, https://doi.org/10.1038/s41586-020-2571-7.

[84] P. Chen, A. Nirula, B. Heller, R.L. Gottlieb, J. Boscia, J. Morris, G. Huhn, J. Cardona, B. Mocherla, V. Stosor, et al., SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with covid-19, N. Engl. J. Med. 384 (2021) 229–237, https://doi.org/10.1056/NEJMoa2029849.

[85] S. Jiang, X. Zhang, Y. Yang, P.J. Hotez, L. Du, Neutralizing antibodies for the treatment of COVID-19, Nat. Biomed. Eng. 4 (2020) 1134–1139, https://doi.org/10.1038/s41551-020-00660-2.

[86] T. Gao, M. Hu, X. Zhang, H. Li, L. Zhu, H. Liu, Q. Dong, Z. Zhang, Z. Wang, Y. Hu, et al., Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement over-activation, medRxiv (2020), https://doi.org/10.1101/2020.03.29.20041962, 2020.2003.2029.20041962.

[87] J. Carvelli, O. Demaria, F. Vély, L. Batista, N. Chouaki Benmansour, J. Fares, S. Carpentier, M.L. Thibult, A. Morel, R. Remark, et al., Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis, Nature 588 (2020) 146–150, https://doi.org/10.1038/s41586-020-2600-6.

[88] M. Cugno, P.L. Meroni, R. Gualtierotti, S. Griffini, E. Grovetti, A. Torri, M. Panigada, S. Aliberti, F. Blasi, F. Tedesco, F. Peyvandi, Complement activation in patients with COVID-19: a novel therapeutic target, J. Allergy Clin. Immunol. 146 (2020) 215–217, https://doi.org/10.1016/j.jaci.2020.05.006.

[89] A.P.J. Vlaar, S. de Bruin, M. Busch, S. Timmermans, I.E. van Zeggeren, R. Koning, L. Ter Horst, E.B. Bulle, F. van Baarle, M.C.G. van de Poll, et al., Anti-C5a antibody IFX-1 (vilobelimab) treatment versus best supportive care for patients with severe COVID-19 (PANAMO): an exploratory, open-label, phase 2 randomised controlled trial, Lancet Rheumatol. 2 (2020) e764–e773, https://doi.org/10.1016/s2665-9913(20)30341-6.

[90] K. Kambas, M.M. Markiewski, I.A. Pneumatikos, S.S. Rafail, V. Theodorou, D. Konstantonis, I. Kourtzelis, M.N. Doumas, P. Magotti, R.A. Deangelis, et al., C5a and TNF-alpha up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome, J. Immunol. 180 (2008) 7368–7375, https://doi.org/10.4049/jimmunol.180.11.7368.

[91] A.M. Risitano, D.C. Mastellos, M. Huber-Lang, D. Yancopoulou, C. Garlanda, F. Ciceri, J.D. Lambris, Complement as a target in COVID-19? Nat. Rev. Immunol. 20 (2020) 343–344, https://doi.org/10.1038/s41577-020-0320-7.

[92] E. Sallard, F.X. Lescure, Y. Yazdanpanah, F. Mentre, N. Peiffer-Smadja, Type 1 interferons as a potential treatment against COVID-19, Antivir. Res. 178 (2020), 104791, https://doi.org/10.1016/j.antiviral.2020.104791.

[93] M.A. Martinez, Compounds with therapeutic potential against novel respiratory 2019 coronavirus, Antimicrob. Agents Chemother. 64 (2020), https://doi.org/10.1128/aac.00399-20.

[94] D. Belhadi, N. Peiffer-Smadja, F. Lescure, Y. Yazdanpanah, F. Mentré, C. Laouénan, A brief review of antiviral drugs evaluated in registered clinical trials for COVID-19, preprint, medRxiv (2020), https://doi.org/10.1101/2020.03.18.20038190.

[95] C.E. Samuel, Antiviral actions of interferons, Clin. Microbiol. Rev. 14 (2001) 778–809, https://doi.org/10.1128/cmr.14.4.778-809.2001, table of contents.

[96] A. Murira, A. Lamarre, Type-I interferon responses: from friend to foe in the battle against chronic viral infection, Front. Immunol. 7 (2016), https://doi.org/10.3389/fimmu.2016.00609.

[97] L. Gao, S. Yu, Q. Chen, Z. Duan, J. Zhou, C. Mao, D. Yu, W. Zhu, J. Nie, Y. Hou, A randomized controlled trial of low-dose recombinant human interferons alpha-2b nasal spray to prevent acute viral respiratory infections in military recruits, Vaccine 28 (2010) 4445–4451, https://doi.org/10.1016/j.vaccine.2010.03.062.

[98] M.R. Loutfy, L.M. Blatt, K.A. Siminovitch, S. Ward, B. Wolff, H. Lho, D.H. Pham, H. Deif, E.A. LaMere, M. Chang, et al., Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study, JAMA 290 (2003) 3222–3228, https://doi.org/10.1001/jama.290.24.3222.

[99] A.S. Omrani, M.M. Saad, K. Baig, A. Bahloul, M. Abdul-Matin, A.Y. Alaidaroos, G.A. Almakhlafi, M.M. Albarrak, Z.A. Memish, A.M. Albarrak, Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study, Lancet Infect. Dis. 14 (2014) 1090–1095, https://doi.org/10.1016/s1473-3099(14)70920-x.

[100] J.F. Chan, Y. Yao, M.L. Yeung, W. Deng, L. Bao, L. Jia, F. Li, C. Xiao, H. Gao, P. Yu, et al., Treatment with lopinavir/ritonavir or interferon-beta1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset, J. Infect. Dis. 212 (2015) 1904–1913, https://doi.org/10.1093/infdis/jiv392.

[101] F. Chen, K.H. Chan, Y. Jiang, R.Y. Kao, H.T. Lu, K.W. Fan, V.C. Cheng, W.H. Tsui, I.F. Hung, T.S. Lee, et al., In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds, J. Clin. Virol. 31 (2004) 69–75, https://doi.org/10.1016/j.jcv.2004.03.003.

[102] T.P. Sheahan, A.C. Sims, S.R. Leist, A. Schafer, J. Won, A.J. Brown, S.A. Montgomery, A. Hogg, D. Babusis, M.O. Clarke, et al., Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV, Nat. Commun. 11 (2020) 222, https://doi.org/10.1038/s41467-019-13940-6.

[103] K.L. Shen, Y.H. Yang, Diagnosis and treatment of 2019 novel coronavirus infection in children: a pressing issue, World J. Pediatr.: WJP (2020), https://doi.org/10.1007/s12519-020-00344-6.

[104] N. Wang, Y. Zhan, L. Zhu, Z. Hou, F. Liu, P. Song, F. Qiu, X. Wang, X. Zou, D. Wan, et al., Retrospective multicenter cohort study shows early interferon therapy is associated with favorable clinical responses in COVID-19 patients, Cell Host Microbe 28 (2020) 455–464.e452, https://doi.org/10.1016/j.chom.2020.07.005.

[105] J.S. Lee, S. Park, H.W. Jeong, J.Y. Ahn, S.J. Choi, H. Lee, B. Choi, S.K. Nam, M. Sa, J.S. Kwon, et al., Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19, Sci. Immunol. 5 (2020), https://doi.org/10.1126/sciimmunol.abd1554.

[106] J.S. Lee, E.C. Shin, The type I interferon response in COVID-19: implications for treatment, Nat. Rev. Immunol. 20 (2020) 585–586, https://doi.org/10.1038/s41577-020-00429-3.

[107] WHO, Draft landscape of COVID-19 candidate vaccines. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines, 2021.

[108] R.H. Shaw, A. Stuart, M. Greenland, X. Liu, J.S.N. Van-Tam, M.D. Snape, Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data, Lancet (2021), https://doi.org/10.1016/s0140-6736(21)01115-6.

[109] I. Abubakar, L. Pimpin, C. Ariti, R. Beynon, P. Mangtani, J.A. Sterne, P.E. Fine, P.G. Smith, M. Lipman, D. Elliman, et al., Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis, Health Technol. Assess. 17 (2013) 1–372, https://doi.org/10.3310/hta17370, v–vi.

[110] R.J.W. Arts, S. Moorlag, B. Novakovic, Y. Li, S.Y. Wang, M. Oosting, V. Kumar, R.J. Xavier, C. Wijmenga, L.A.B. Joosten, et al., BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity, Cell Host Microbe 23 (2018), https://doi.org/10.1016/j.chom.2017.12.010, 89.e105–100.e105. S1931-3128(17)30546-2 [pii].

[111] Y.C. Chang, C.J. Lin, Y.H. Hsiao, Y.H. Chang, S.J. Liu, H.Y. Hsu, Therapeutic effects of BCG vaccination on type 1 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials, J. Diabetes Res. 2020 (2020), 8954125, https://doi.org/10.1155/2020/8954125.

[112] N. Curtis, A. Sparrow, T.A. Ghebreyesus, M.G. Netea, Considering BCG vaccination to reduce the impact of COVID-19, Lancet 395 (2020) 1545–1546, https://doi.org/10.1016/S0140-6736(20)31025-4. S0140-6736(20)31025-4 [pii].

[113] J. Leentjens, M. Kox, R. Stokman, J. Gerretsen, D.A. Diavatopoulos, R. van Crevel, G.F. Rimmelzwaan, P. Pickkers, M.G. Netea, BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study, J. Infect. Dis. 212 (2015) 1930–1938, https://doi.org/10.1093/infdis/jiv332, jiv332 [pii].

[114] S. Moorlag, R.J.W. Arts, R. van Crevel, M.G. Netea, Non-specific effects of BCG vaccine on viral infections, Clin. Microbiol. Infect. 25 (2019) 1473–1478, https://doi.org/10.1016/j.cmi.2019.04.020. S1198-743X(19)30197-1 [pii].

[115] A. Morales, D. Eidinger, A.W. Bruce, Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors, J. Urol. 116 (1976) 180–183, https://doi.org/10.1016/s0022-5347(17)58737-6. S0022-5347(17)58737-6 [pii].

[116] F. Nackers, M. Dramaix, R.C. Johnson, C. Zinsou, A. Robert, D.E.B.B. E, J.R. Glynn, F. Portaels, R. Tonglet, BCG vaccine effectiveness against Buruli ulcer: a case-control study in Benin, Am. J. Trop. Med. Hyg. 75 (2006) 768–774, 75/4/768 [pii].

[117] L.J. Old, D.A. Clarke, B. Benacerraf, Effect of Bacillus Calmette-Guerin infection on transplanted tumours in the mouse, Nature 184 (Suppl 5) (1959) 291–292, https://doi.org/10.1038/184291a0.

[118] L.F. Pittet, N. Curtis, Does bacillus Calmette-Guerin vaccine prevent herpes simplex virus recurrences? A systematic review, Rev. Med. Virol. 31 (2021) 1–9, https://doi.org/10.1002/rmv.2151.

[119] E. Rey-Jurado, J. Soto, N. Galvez, A.M. Kalergis, A safe and efficient BCG vectored vaccine to prevent the disease caused by the human Respiratory Syncytial Virus, Hum. Vaccines Immunother. 13 (2017) 2092–2097, https://doi.org/10.1080/21645515.2017.1334026.

[120] M.S. Setia, C. Steinmaus, C.S. Ho, G.W. Rutherford, The role of BCG in prevention of leprosy: a meta-analysis, Lancet Infect. Dis. 6 (2006) 162–170, https://doi.org/10.1016/S1473-3099(06)70412-1. S1473-3099(06)70412-1 [pii].

[121] A.R. Sharma, G. Batra, M. Kumar, A. Mishra, R. Singla, A. Singh, R.S. Singh, B. Medhi, BCG as a game-changer to prevent the infection and severity of COVID-19 pandemic? Allergol. Immunopathol. 48 (2020) 507–517, https://doi.org/10.1016/j.aller.2020.05.002. S0301-0546(20)30106-3 [pii].

[122] I. Kristensen, P. Fine, P. Aaby, H. Jensen, Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa Commentary: an unexpected finding that needs confirmation or rejection, BMJ 321 (2000) 1435.

[123] J. Vaugelade, S. Pinchinat, G. Guiella, E. Elguero, F. Simondon, Non-specific effects of vaccination on child survival: prospective cohort study in Burkina Faso, BMJ 329 (2004) 1309.

[124] E. Elguero, K.B. Simondon, J. Vaugelade, A. Marra, F. Simondon, Non-specific effects of vaccination on child survival? A prospective study in Senegal, Trop. Med. Int. Health 10 (2005) 956–960.

[125] P. Aaby, A. Roth, H. Ravn, B.M. Napirna, A. Rodrigues, I.M. Lisse, L. Stensballe, B.R. Diness, K.R. Lausch, N. Lund, Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J. Infect. Dis. 204 (2011) 245–252.

[126] L.H. Moulton, L. Rahmathullah, N.A. Halsey, R. Thulasiraj, J. Katz, J.M. Tielsch, Evaluation of non-specific effects of infant immunizations on early infant mortality in a southern Indian population, Trop. Med. Int. Health 10 (2005) 947–955.

[127] J.P. Higgins, K. Soares-Weiser, J.A. López-López, A. Kakourou, K. Chaplin, H. Christensen, N.K. Martin, J.A. Sterne, A.L. Reingold, Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review, BMJ 355 (2016).

[128] L.A. O'Neill, M.G. Netea, BCG-induced trained immunity: can it offer protection against COVID-19? Nat. Rev. Immunol. 20 (2020) 335–337.

[129] J. Kleinnijenhuis, J. Quintin, F. Preijers, C.S. Benn, L.A. Joosten, C. Jacobs, J. Van Loenhout, R.J. Xavier, P. Aaby, J.W. Van Der Meer, Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity, J. Innate Immun. 6 (2014) 152–158.

[130] E.K. Vetskova, M.N. Muhtarova, T.I. Avramov, T.R. Stefanova, I.J. Chalakov, M.H. Nikolova, Immunomodulatory effects of BCG in patients with recurrent respiratory papillomatosis, Folia Med. 55 (2013) 49–54.

[131] J. Li, L. Zhan, C. Qin, The double-sided effects of Mycobacterium Bovis bacillus Calmette-Guérin vaccine, NPJ Vaccines 6 (2021) 14, https://doi.org/10.1038/s41541-020-00278-0.

[132] ClinicalTrials.gov, A Trial Investigating the Influence of BCG and Hepatitis B Immunisation at Birth on Neonatal Immune Responses: the Early Life Vaccines and Immunity Study, US National Library of Medicine, 2017.

[133] ClinicalTrials.gov, The Effect of Bacille Calmette Guerin (BCG) Vaccination on Immune Responses in HIV-Exposed and Unexposed Infants, US National Library of Medicine, 2009.

[134] C.T. Tchakoute, A.C. Hesseling, E.B. Kidzeru, H. Gamieldien, J.A. Passmore, C.E. Jones, C.M. Gray, D.L. Sodora, H.B. Jaspan, Delaying BCG vaccination until 8 weeks of age results in robust BCG-specific T-cell responses in HIV-exposed infants, J. Infect. Dis. 211 (2015) 338–346, https://doi.org/10.1093/infdis/jiu434.

[135] V. Nankabirwa, J.K. Tumwine, O. Namugga, T. Tylleskär, G. Ndeezi, B. Robberstad, M.G. Netea, H. Sommerfelt, Early versus late BCG vaccination in HIV-1-exposed infants in Uganda: study protocol for a randomized controlled trial, Trials 18 (2017) 152, https://doi.org/10.1186/s13063-017-1881-z.

[136] ClinicalTrials.gov, Revaccination of Young Children with Bacille Calmette Guerin (BCG) Vaccine, US National Library of Medicine, 2008.

[137] ClinicalTrials.gov, Bacillus Calmette-Guerin (BCG) Vaccine and Morbidity from Malaria, US National Library of Medicine, 2016.

[138] J. Walk, L.C.J. de Bree, W. Graumans, R. Stoter, G.J. van Gemert, M. van de Vegte-Bolmer, K. Teelen, C.C. Hermsen, R.J.W. Arts, M.C. Behet, et al., Outcomes of controlled human malaria infection after BCG vaccination, Nat. Commun. 10 (2019) 874, https://doi.org/10.1038/s41467-019-08659-3.

[139] ClinicalTrials.gov, Safety and Protective Efficacy of BCG Vaccination against Controlled Human Malaria Infection, US National Library of Medicine, 2017.

[140] B.A. Blok, L.C.J. de Bree, D.A. Diavatopoulos, J.D. Langereis, L.A.B. Joosten, P. Aaby, R. van Crevel, C.S. Benn, M.G. Netea, Interacting, nonspecific, immunological effects of bacille calmette-Guérin and tetanus-diphtheria-pertussis inactivated polio vaccinations: an explorative, randomized trial, Clin. Infect. Dis. 70 (2020) 455–463, https://doi.org/10.1093/cid/ciz246.

[141] A. Rieckmann, M. Villumsen, S. Sørup, L.K. Haugaard, H. Ravn, A. Roth, J.L. Baker, C.S. Benn, P. Aaby, Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010, Int. J. Epidemiol. 46 (2017) 695–705, https://doi.org/10.1093/ije/dyw120.

[142] S. Biering-Sørensen, P. Aaby, N. Lund, I. Monteiro, K.J. Jensen, H.B. Eriksen, F. Schaltz-Buchholzer, A.S.P. Jørgensen, A. Rodrigues, A.B. Fisker, C.S. Benn, Early BCG-Denmark and neonatal mortality among infants weighing <2500 g: A randomized controlled trial, Clin. Infect. Dis. 65 (2017) 1183–1190, https://doi.org/10.1093/cid/cix525.

[143] E. Nemes, H. Geldenhuys, V. Rozot, K.T. Rutkowski, F. Ratangee, N. Bilek, S. Mabwe, L. Makhethe, M. Erasmus, A. Toefy, et al., Prevention of M. tuberculosis infection with H4:IC31 vaccine or BCG revaccination, N. Engl. J. Med. 379 (2018) 138–149, https://doi.org/10.1056/NEJMoa1714021.

[144] T. Ohrui, K. Nakayama, T. Fukushima, H. Chiba, H. Sasaki, [Prevention of elderly pneumonia by pneumococcal, influenza and BCG vaccinations], Nihon Ronen Igakkai Zasshi 42 (2005) 34–36, https://doi.org/10.3143/geriatrics.42.34.

[145] K.F. Kölmel, J.M. Grange, B. Krone, G. Mastrangelo, C.R. Rossi, B.M. Henz, C. Seebacher, I.N. Botev, M. Niin, D. Lambert, et al., Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients, Eur. J. Cancer 41 (2005) 118–125, https://doi.org/10.1016/j.ejca.2004.09.023.

[146] K. Buffen, M. Oosting, J. Quintin, A. Ng, J. Kleinnijenhuis, V. Kumar, E. van de Vosse, C. Wijmenga, R. van Crevel, E. Oosterwijk, et al., Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer, PLoS Pathog. 10 (2014), e1004485, https://doi.org/10.1371/journal.ppat.1004485.

[147] T. Ten Doesschate, S. Moorlag, T.W. van der Vaart, E. Taks, P. Debisarun, J. Ten Oever, C.P. Bleeker-Rovers, P.B. Verhagen, A. Lalmohamed, R. Ter Heine, et al., Two Randomized Controlled Trials of Bacillus Calmette-Guérin Vaccination to reduce absenteeism among health care workers and hospital admission by elderly persons during the COVID-19 pandemic: a structured summary of the study protocols for two randomised controlled trials, Trials 21 (2020) 481, https://doi.org/10.1186/s13063-020-04389-w.

[148] N.A. Brooks, V. Narayan, P.K. Hegarty, H. Zafirakis, X.Y. Han, A.M. Kamat, The role of the urologist, BCG vaccine administration, and SARS-CoV-2: an overview, BJUI Compass 1 (2020) 87–92, https://doi.org/10.1002/bco2.21.

[149] S. Moorlag, R.C. van Deuren, C.H. van Werkhoven, M. Jaeger, P. Debisarun, E. Taks, V.P. Mourits, V. Koeken, L.C.J. de Bree, T. Ten Doesschate, et al., Safety and COVID-19 symptoms in individuals recently vaccinated with BCG: a retrospective cohort study, Cell Rep. Med. 1 (2020), 100073, https://doi.org/10.1016/j.xcrm.2020.100073.

[150] ClinicalTrials gov, BCG Vaccination to Protect Healthcare Workers against COVID-19, US National Library of Medicine, 2022.

[151] H. Motamedi, M.M. Ari, S. Dashtbin, M. Fathollahi, H. Hossainpour, A. Alvandi, J. Moradi, R. Abiri, An update review of globally reported SARS-CoV-2 vaccines in preclinical and clinical stages, Int. Immunopharm. 96 (2021), 107763, https://doi.org/10.1016/j.intimp.2021.107763.

[152] J. Hensel, K.M. McAndrews, D.J. McGrail, D.P. Dowlatshahi, V.S. LeBleu, R. Kalluri, Protection against SARS-CoV-2 by BCG vaccination is not supported by epidemiological analyses, Sci. Rep. 10 (2020), 18377, https://doi.org/10.1038/s41598-020-75491-x.

[153] R.J.W. Arts, A. Carvalho, C. La Rocca, C. Palma, F. Rodrigues, R. Silvestre, J. Kleinnijenhuis, E. Lachmandas, L.G. Goncalves, A. Belinha, et al., Immunometabolic pathways in BCG-induced trained immunity, Cell Rep. 17 (2016) 2562–2571, https://doi.org/10.1016/j.celrep.2016.11.011. S2211-1247(16)31552-2 [pii].

[154] S. Kamat, M. Kumari, BCG against SARS-CoV-2: second youth of an old age vaccine? Front. Pharmacol. 11 (2020) 1050, https://doi.org/10.3389/fphar.2020.01050.

[155] R.J. Arts, L.A. Joosten, M.G. Netea, Immunometabolic Circuits in Trained Immunity, In 5, Elsevier, 2016, pp. 425–430.

[156] S. Bekkering, R.J. Arts, B. Novakovic, I. Kourtzelis, C.D. van der Heijden, Y. Li, C.D. Popa, R. Ter Horst, J. van Tuijl, R.T. Netea-Maier, Metabolic induction of trained immunity through the mevalonate pathway, Cell 172 (2018), 135.e139 146.e139.

[157] E. Kaufmann, J. Sanz, J.L. Dunn, N. Khan, L.E. Mendonça, A. Pacis, F. Tzelepis, E. Pernet, A. Dumaine, J.-C. Grenier, BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis, Cell 172 (2018), 176.e119 190.e119.

[158] I. Mitroulis, K. Ruppova, B. Wang, L.-S. Chen, M. Grzybek, T. Grinenko, A. Eugster, M. Troullinaki, A. Palladini, I. Kourtzelis, Modulation of myelopoiesis progenitors is an integral component of trained immunity, Cell 172 (2018), 147.e112 161.e112.

[159] M.G. Netea, J. Domínguez-Andrés, L.B. Barreiro, T. Chavakis, M. Divangahi, E. Fuchs, L.A. Joosten, J.W. van der Meer, M.M. Mhlanga, W.J. Mulder, Defining trained immunity and its role in health and disease, Nat. Rev. Immunol. 20 (2020) 375–388.

[160] T. Chavakis, I. Mitroulis, G. Hajishengallis, Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation, Nat. Immunol. 20 (2019) 802–811.

[161] S. Forcelloni, A. Benedetti, M. Dilucca, A. Giansanti, Identification of conserved epitopes in SARS-CoV-2 spike and nucleocapsid protein, Curr. Genom. 22 (2021) 541–549, https://doi.org/10.2174/1389202923666211216162605.

[162] F. Humphries, L. Shmuel-Galia, Z. Jiang, R. Wilson, P. Landis, S.-L. Ng, K.M. Parsi, R. Maehr, J. Cruz, A. Morales, A diamidobenzimidazole STING agonist protects against SARS-CoV-2 infection, Sci. Immunol. 6 (2021), eabi9002.

[163] R.J. Dey, B. Dey, A.K. Singh, M. Praharaj, W. Bishai, Bacillus Calmette-Guérin overexpressing an endogenous stimulator of interferon genes agonist provides enhanced protection against pulmonary tuberculosis, J. Infect. Dis. 221 (2020) 1048–1056.

[164] K.A. Lombardo, A. Obradovic, A.K. Singh, J.L. Liu, G. Joice, M. Kates, W. Bishai, D. McConkey, A. Chaux, M.-L. Eich, et al., BCG invokes superior STING-mediated innate immune response over radiotherapy in a carcinogen murine model of urothelial cancer, J. Pathol. 256 (2022) 223–234, https://doi.org/10.1002/path.5830.

[165] B.-J. Kim, H. Jeong, H. Seo, M.-H. Lee, H.M. Shin, B.-J. Kim, Recombinant Mycobacterium paragordonae expressing SARS-CoV-2 receptor-binding domain as a vaccine candidate against SARS-CoV-2 infections, Front. Immunol. 12 (2021).

[166] S. Haddad-Boubaker, H. Othman, R. Touati, K. Ayouni, M. Lakhal, I.B. Mustapha, K. Ghedira, M. Kharrat, H. Triki, In silico comparative study of SARS-CoV-2 proteins and antigenic proteins in BCG, OPV, MMR and other vaccines: evidence of a possible putative protective effect, BMC Bioinf. 22 (2021) 1–14.

[167] P. Finotti, Sequence similarity of HSP65 of Mycobacterium bovis BCG with SARS-CoV-2 spike and nuclear proteins: may it predict an antigen-dependent immune protection of BCG against COVID-19? Cell Stress Chaperones (2021) 1–7.

[168] E. Yengil, Y. Onlen, C. Ozer, M. Hambolat, M. Ozdogan, Effectiveness of booster measles-mumps-rubella vaccination in lower COVID-19 infection rates: a retrospective cohort study in Turkish adults, Int. J. Gen. Med. 14 (2021) 1757.

[169] J.E. Gold, W.H. Baumgartl, R.A. Okyay, W.E. Licht, P.L. Fidel Jr., M.C. Noverr, L.P. Tilley, D.J. Hurley, B. Rada, J.W. Ashford, Analysis of measles-mumps-rubella (MMR) titers of recovered COVID-19 patients, mBio 11 (2020), e02628-02620.

[170] E. Marakasova, A. Baranova, MMR vaccine and COVID-19: measles protein homology may contribute to cross-reactivity or to complement activation protection, mBio 12 (2021), e03447-03420.

[171] R. Rappuoli, Reverse vaccinology, a genome-based approach to vaccine development, Vaccine 19 (2001) 2688–2691, https://doi.org/10.1016/s0264-410x(00)00554-5. S0264410X00005545 [pii].

[172] K.L. Seib, X. Zhao, R. Rappuoli, Developing vaccines in the era of genomics: a decade of reverse vaccinology, Clin. Microbiol. Infect. 18 (Suppl 5) (2012) 109–116, https://doi.org/10.1111/j.1469-0691.2012.03939.x. S1198-743X(14)61369-0 [pii].

[173] H.S.A.T.S. Raman, M.F. Sjaugi, N.E. Mohamed, A.M. Khan, Bioinformatics for Vaccine Target Discovery, Asia Pacific Biotech News, Singapore, 2014. https://www.asiabiotech.com/18/1809/18090043x.html.

[174] A.K. Gupta, K. Kaur, A. Rajput, S.K. Dhanda, M. Sehgal, M.S. Khan, I. Monga, S.A. Dar, S. Singh, G. Nagpal, et al., ZikaVR: an integrated zika virus resource for genomics, proteomics, phylogenetic and therapeutic analysis, Sci. Rep. 6 (2016), 32713, https://doi.org/10.1038/srep32713 srep32713 [pii].

[175] H. Zahroh, A. Ma`rup, U.S. Tambunan, A.A. Parikesit, Immunoinformatics approach in designing epitope-based vaccine against meningitis-inducing bacteria (Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae type b), Drug Target Insights 10 (2016) 19–29, https://doi.org/10.4137/DTI.S38458, dti-10-2016-019 [pii].

[176] R.E. Soria-Guerra, R. Nieto-Gomez, D.O. Govea-Alonso, S. Rosales-Mendoza, An overview of bioinformatics tools for epitope prediction: implications on vaccine development, J. Biomed. Inf. 53 (2015) 405–414, https://doi.org/10.1016/j.jbi.2014.11.003. S1532-0464(14)00233-0 [pii].

[177] J.D. Fernandes, A.S. Hinrichs, H. Clawson, J.N. Gonzalez, B.T. Lee, L.R. Nassar, B.J. Raney, K.R. Rosenbloom, S. Nerli, A.A. Rao, et al., The UCSC SARS-CoV-2 genome browser, Nat. Genet. 52 (2020) 991–998, https://doi.org/10.1038/s41588-020-0700-8.

[178] E. Ong, M.U. Wong, A. Huffman, Y. He, COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning, Front. Immunol. 11 (2020) 1581, https://doi.org/10.3389/fimmu.2020.01581.

[179] J.W. Saville, A.M. Berezuk, S.S. Srivastava, S. Subramaniam, Three-dimensional visualization of viral structure, entry, and replication underlying the spread of SARS-CoV-2, Chem. Rev. 122 (2022) 14066–14084, https://doi.org/10.1021/acs.chemrev.1c01062.

[180] S.N. Crooke, I.G. Ovsyannikova, R.B. Kennedy, G.A. Poland, Immunoinformatic identification of B cell and T cell epitopes in the SARS-CoV-2 proteome, Sci. Rep. 10 (2020), 14179, https://doi.org/10.1038/s41598-020-70864-8.

[181] M. Enayatkhani, M. Hasaniazad, S. Faezi, H. Guklani, P. Davoodian, N. Ahmadi, M.A. Einakian, A. Karmostaji, K. Ahmadi, Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: an in silico study, J. Biomol. Struct. Dyn. (2020) 1–16, https://doi.org/10.1080/07391102.2020.1756411.

[182] L. Bruno, M. Cortese, R. Rappuoli, M. Merola, Lessons from Reverse Vaccinology for viral vaccine design, Curr. Opin. Virol. 11 (2015) 89–97, https://doi.org/10.1016/j.coviro.2015.03.001. S1879-6257(15)00032-2 [pii].

[183] A.S. Kanampalliwar, Rajkumar, Amandeep Girdhar, Archana Tiwari, Reverse vaccinology: basics and applications, J. Vaccine Vaccin. 4 (2013) 6.

[184] J.G. Beeson, L. Kurtovic, C. Dobano, D.H. Opi, J.A. Chan, G. Feng, M.F. Good, L. Reiling, M.J. Boyle, Challenges and strategies for developing efficacious and long-lasting malaria vaccines, Sci. Transl. Med. 11 (2019), https://doi.org/10.1126/scitranslmed.aau1458, 11/474/eaau1458 [pii].

[185] P. Oyston, K. Robinson, The current challenges for vaccine development, J. Med. Microbiol. 61 (2012) 889–894, https://doi.org/10.1099/jmm.0.039180-0.

[186] G. Voss, D. Casimiro, O. Neyrolles, A. Williams, S.H.E. Kaufmann, H. McShane, M. Hatherill, H.A. Fletcher, Progress and challenges in TB vaccine development, F1000Res 7 (2018) 199, https://doi.org/10.12688/f1000research.13588.1.

[187] K.E. Wu, F.M. Fazal, K.R. Parker, J. Zou, H.Y. Chang, RNA-GPS predicts SARS-CoV-2 RNA residency to host mitochondria and nucleolus, Cell Syst. 11 (2020), 102.e103 108.e103.

[188] D.H. Barouch, Challenges in the development of an HIV-1 vaccine, Nature 455 (2008) 613–619, https://doi.org/10.1038/nature07352, nature07352 [pii].

[189] Y. Zhou, Y. Zhang, J.P. Moorman, Z.Q. Yao, Z.S. Jia, Viral (hepatitis C virus, hepatitis B virus, HIV) persistence and immune homeostasis, Immunology 143 (2014) 319–330, https://doi.org/10.1111/imm.12349.

[190] P. Escoll, S. Mondino, M. Rolando, C. Buchrieser, Targeting of host organelles by pathogenic bacteria: a sophisticated subversion strategy, Nat. Rev. Microbiol. 14 (2016) 5–19, https://doi.org/10.1038/nrmicro.2015.1, nrmicro.2015.1 [pii].

[191] K.K. Singh, G. Chaubey, J.Y. Chen, P. Suravajhala, Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis, Am. J. Physiol. Cell Physiol. 319 (2020) C258–C267, https://doi.org/10.1152/ajpcell.00224.2020.

[192] R.E.J.W. Herati, What is the predictive value of animal models for vaccine efficacy in humans? Consideration of strategies to improve the value of animal models, Cold Spring Harbor Perspect. Biol. 10 (2018) a031583, https://doi.org/10.1101/cshperspect.a031583. PMID: 28348037; PMCID: PMC5880169.

[193] S. Kumar, P.K. Yadav, R. Srinivasan, N. Perumal, Selection of animal models for COVID-19 research, Virusdisease (2020) 1–6, https://doi.org/10.1007/s13337-020-00637-4, 637 [pii].

[194] P. Lee, D.J. Kim, Newly emerging human coronaviruses: animal models and vaccine research for SARS, MERS, and COVID-19, Immune Netw 20 (2020) e28, https://doi.org/10.4110/in.2020.20.e28, 2020200401 [pii].

[195] M.D. Johansen, A. Irving, X. Montagutelli, M.D. Tate, I. Rudloff, M.F. Nold, N.G. Hansbro, R.Y. Kim, C. Donovan, G. Liu, et al., Animal and translational models of SARS-CoV-2 infection and COVID-19, Mucosal Immunol. 13 (2020) 877–891, https://doi.org/10.1038/s41385-020-00340-z.

[196] CDC, Facts about COVID-19 vaccines. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/facts.html#:~:text=It%20typically%20takes%20a%20few,vaccination%20and%20still%20get%20sick, 2021.

[197] M. Fischetti, The COVID cold chain: how a vaccine will Get to you, in: Scientific American, Scientific American, 2020.

[198] M.L. Stephenson, P.C. Zamecnik, Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 285–288, https://doi.org/10.1073/pnas.75.1.285.

[199] P.C. Zamecnik, M.L. Stephenson, Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide, Proc. Natl. Acad. Sci. U. S. A. 75 (1978) 280–284, https://doi.org/10.1073/pnas.75.1.280.

[200] S. Agrawal, S.H. Mayrand, P.C. Zamecnik, T. Pederson, Site-specific excision from RNA by RNase H and mixed-phosphate-backbone oligodeoxynucleotides, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1401–1405, https://doi.org/10.1073/pnas.87.4.1401.

[201] V. Gopalan, A. Vioque, S. Altman, RNase P: variations and uses, J. Biol. Chem. 277 (2002) 6759–6762, https://doi.org/10.1074/jbc.R100067200.

[202] J.J. Rossi, N. Sarver, Catalytic antisense RNA (ribozymes): their potential and use as anti-HIV-1 therapeutic agents, Adv. Exp. Med. Biol. 312 (1992) 95–109, https://doi.org/10.1007/978-1-4615-3462-4_9.

[203] N. Sarver, E.M. Cantin, P.S. Chang, J.A. Zaia, P.A. Ladne, D.A. Stephens, J.J. Rossi, Ribozymes as potential anti-HIV-1 therapeutic agents, Science 247 (1990) 1222–1225, https://doi.org/10.1126/science.2107573.

[204] R.J. Scarborough, A. Gatignol, HIV and ribozymes, Adv. Exp. Med. Biol. 848 (2015) 97–116, https://doi.org/10.1007/978-1-4939-2432-5_5.

[205] Y. Bai, H. Li, G.P. Vu, H. Gong, S. Umamoto, T. Zhou, S. Lu, F. Liu, Salmonella-mediated delivery of RNase P-based ribozymes for inhibition of viral gene expression and replication in human cells, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 7269–7274, https://doi.org/10.1073/pnas.0912813107.

[206] A.F. Kilani, P. Trang, S. Jo, A. Hsu, J. Kim, E. Nepomuceno, K. Liou, F. Liu, RNase P ribozymes selected in vitro to cleave a viral mRNA effectively inhibit its expression in cell culture, J. Biol. Chem. 275 (2000) 10611–10622, https://doi.org/10.1074/jbc.275.14.10611.

[207] K. Kim, S. Umamoto, P. Trang, R. Hai, F. Liu, Intracellular expression of engineered RNase P ribozymes effectively blocks gene expression and replication of human cytomegalovirus, RNA 10 (2004) 438–447, https://doi.org/10.1261/rna.5178404.

[208] G. Kraus, R. Geffin, G. Spruill, A.K. Young, R. Seivright, D. Cardona, J. Burzawa, H.J. Hnatyszyn, Cross-clade inhibition of HIV-1 replication and cytopathology by using RNase P-associated external guide sequences, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 3406–3411, https://doi.org/10.1073/pnas.052651199.

[209] S.M. Raj, F. Liu, Engineering of RNase P ribozyme for gene-targeting applications, Gene 313 (2003) 59–69, https://doi.org/10.1016/s0378-1119(03)00677-2.

[210] P. Trang, A. Kilani, J. Kim, F. Liu, A ribozyme derived from the catalytic subunit of RNase P from Escherichia coli is highly effective in inhibiting replication of herpes simplex virus 1, J. Mol. Biol. 301 (2000) 817–826, https://doi.org/10.1006/jmbi.2000.4022.

[211] A. Berzal-Herranz, S. Joseph, J.M. Burke, In vitro selection of active hairpin ribozymes by sequential RNA-catalyzed cleavage and ligation reactions, Genes Dev. 6 (1992) 129–134, https://doi.org/10.1101/gad.6.1.129.

[212] S. Joseph, A. Berzal-Herranz, B.M. Chowrira, S.E. Butcher, J.M. Burke, Substrate selection rules for the hairpin ribozyme determined by in vitro selection, mutation, and analysis of mismatched substrates, Genes Dev. 7 (1993) 130–138, https://doi.org/10.1101/gad.7.1.130.

[213] J.R. Lorsch, J.W. Szostak, In vitro evolution of new ribozymes with polynucleotide kinase activity, Nature 371 (1994) 31–36, https://doi.org/10.1038/371031a0.

[214] Y. Yuan, S. Altman, Selection of guide sequences that direct efficient cleavage of mRNA by human ribonuclease P, Science 263 (1994) 1269–1273, https://doi.org/10.1126/science.8122108.

[215] T. Zhou, J. Kim, A.F. Kilani, K. Kim, W. Dunn, S. Jo, E. Nepomuceno, F. Liu, In vitro selection of external guide sequences for directing RNase P-mediated inhibition of viral gene expression, J. Biol. Chem. 277 (2002) 30112–30120, https://doi.org/10.1074/jbc.M200183200.

[216] M. Warashina, T. Kuwabara, Y. Kato, M. Sano, K. Taira, RNA-protein hybrid ribozymes that efficiently cleave any mRNA independently of the structure of the target RNA, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5572–5577, https://doi.org/10.1073/pnas.091411398.

[217] B.A. Sullenger, T.R. Cech, Tethering ribozymes to a retroviral packaging signal for destruction of viral RNA, Science 262 (1993) 1566–1569, https://doi.org/10.1126/science.8248806.

[218] E. Bertrand, D. Castanotto, C. Zhou, C. Carbonnelle, N.S. Lee, P. Good, S. Chatterjee, T. Grange, R. Pictet, D. Kohn, et al., The expression cassette determines the functional activity of ribozymes in mammalian cells by controlling their intracellular localization, RNA 3 (1997) 75–88.

[219] T.R. Abbott, G. Dhamdhere, Y. Liu, X. Lin, L. Goudy, L. Zeng, A. Chemparathy, S. Chmura, N.S. Heaton, R. Debs, et al., Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza, Cell 181 (2020) 865–876.e812, https://doi.org/10.1016/j.cell.2020.04.020.

[220] H. Shen, X. Huang, J. Min, S. Le, Q. Wang, X. Wang, A.A. Dogan, X. Liu, P. Zhang, M.S. Draz, J. Xiao, Nanoparticle delivery systems for DNA/RNA and their potential applications in nanomedicine, Curr. Top. Med. Chem. 19 (2019) 2507–2523, https://doi.org/10.2174/1568026619666191024170212.

[221] H. Shin, S.-J. Park, Y. Yim, J. Kim, C. Choi, C. Won, D.-H. Min, Recent advances in RNA therapeutics and RNA delivery systems based on nanoparticles, Adv. Therapeut. 1 (2018), 1800065, https://doi.org/10.1002/adtp.201800065.

[222] X. Xia, N. Pollock, J. Zhou, J. Rossi, Tissue-specific delivery of oligonucleotides, Methods Mol. Biol. 2036 (2019) 17–50, https://doi.org/10.1007/978-1-4939-9670-4_2.

[223] F. Liu, Ribonuclease P as a tool, in: F. Liu, S. Altman (Eds.), Protein Reviews, Springer, 2010, pp. 257–275, https://doi.org/10.1007/978-1-4419-1142-1_14.

[224] A. Darji, C.A. Guzmán, B. Gerstel, P. Wachholz, K.N. Timmis, J. Wehland, T. Chakraborty, S. Weiss, Oral somatic transgene vaccination using attenuated S. typhimurium, Cell 91 (1997) 765–775, https://doi.org/10.1016/s0092-8674(00)80465-1.

[225] C. Grillot-Courvalin, S. Goussard, P. Courvalin, Bacteria as gene delivery vectors for mammalian cells, Curr. Opin. Biotechnol. 10 (1999) 477–481, https://doi.org/10.1016/s0958-1669(99)00013-0.

[226] G. Vassaux, J. Nitcheu, S. Jezzard, N.R. Lemoine, Bacterial gene therapy strategies, J. Pathol. 208 (2006) 290–298, https://doi.org/10.1002/path.1865.

[227] N. Yang, X. Zhu, L. Chen, S. Li, D. Ren, Oral administration of attenuated S. typhimurium carrying shRNA-expressing vectors as a cancer therapeutic, Cancer Biol. Ther. 7 (2008) 145–151, https://doi.org/10.4161/cbt.7.1.5195.

[228] H. Loessner, A. Endmann, M. Rohde, R. Curtiss 3rd, S. Weiss, Differential effect of auxotrophies on the release of macromolecules by Salmonella enterica vaccine strains, FEMS Microbiol. Lett. 265 (2006) 81–88, https://doi.org/10.1111/j.1574-6968.2006.00470.x.

[229] Y. Bai, H. Gong, H. Li, G.P. Vu, S. Lu, F. Liu, Oral delivery of RNase P ribozymes by Salmonella inhibits viral infection in mice, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 3222–3227, https://doi.org/10.1073/pnas.1014975108.

[230] W. Bao Chi, S. Umama, T. Hock Siew, Ribozymes as therapeutic agents against infectious diseases, in: L. Irina Vlasova-St (Ed.), RNA Therapeutics - History, Design, Manufacturing, and Applications, IntechOpen), 2022, https://doi.org/10.5772/intechopen.107141. Ch. 6.

[231] D.G. Gibson, J.I. Glass, C. Lartigue, V.N. Noskov, R.Y. Chuang, M.A. Algire, G.A. Benders, M.G. Montague, L. Ma, M.M. Moodie, et al., Creation of a bacterial cell controlled by a chemically synthesized genome, Science 329 (2010) 52–56, https://doi.org/10.1126/science.1190719.

[232] H. Daniell, N.D. Singh, H. Mason, S.J. Streatfield, Plant-made vaccine antigens and biopharmaceuticals, Trends Plant Sci. 14 (2009) 669–679, https://doi.org/10.1016/j.tplants.2009.09.009.

[233] K.C. Kwon, H. Daniell, Oral delivery of protein drugs bioencapsulated in plant cells, Mol. Ther. 24 (2016) 1342–1350, https://doi.org/10.1038/mt.2016.115.

[234] K.C. Kwon, D. Verma, N.D. Singh, R. Herzog, H. Daniell, Oral delivery of human biopharmaceuticals, autoantigens and vaccine antigens bioencapsulated in plant cells, Adv. Drug Deliv. Rev. 65 (2013) 782–799, https://doi.org/10.1016/j.addr.2012.10.005.

[235] J. Renukuntla, A.D. Vadlapudi, A. Patel, S.H. Boddu, A.K. Mitra, Approaches for enhancing oral bioavailability of peptides and proteins, Int. J. Pharm. 447 (2013) 75–93, https://doi.org/10.1016/j.ijpharm.2013.02.030.

[236] J. Xu, M.C. Dolan, G. Medrano, C.L. Cramer, P.J. Weathers, Green factory: plants as bioproduction platforms for recombinant proteins, Biotechnol. Adv. 30 (2012) 1171–1184, https://doi.org/10.1016/j.biotechadv.2011.08.020.

[237] R. Sanz-Barrio, A. Fernández-San Millán, J. Carballeda, P. Corral-Martínez, J.M. Seguí-Simarro, I. Farran, Chaperone-like properties of tobacco plastid thioredoxins f and m, J. Exp. Bot. 63 (2012) 365–379, https://doi.org/10.1093/jxb/err282.

[238] E. Stoger, R. Fischer, M. Moloney, J.K. Ma, Plant molecular pharming for the treatment of chronic and infectious diseases, Annu. Rev. Plant Biol. 65 (2014) 743–768, https://doi.org/10.1146/annurev-arplant-050213-035850.

[239] S. Ahangarzadeh, M.H. Daneshvar, H. Rajabi-Memari, H. Galehdari, K. Alamisaied, Cloning, transformation and expression of human interferon α2b gene in tobacco plant (nicotiana tabacum cv. xanthi), Jundishapur J. Nat. Pharm. Prod. 7 (2012) 111–116.

[240] G.A. De Zoeten, J.R. Penswick, M.A. Horisberger, P. Ahl, M. Schultze, T. Hohn, The expression, localization, and effect of a human interferon in plants, Virology 172 (1989) 213–222, https://doi.org/10.1016/0042-6822(89)90123-2.

[241] O. Edelbaum, D. Stein, N. Holland, Y. Gafni, O. Livneh, D. Novick, M. Rubinstein, I. Sela, Expression of active human interferon-beta in transgenic plants, J. Interferon Res. 12 (1992) 449–453, https://doi.org/10.1089/jir.1992.12.449.

[242] Y.R. Sindarovska, I.M. Gerasymenko, Y.V. Sheludko, Z.M. Olevinskaya, N.Y. Spivak, N.V. Kuchuk, Production of human interferon alfa 2b in plants of Nicotiana excelsior by Agrobacterium-mediated transient expression, Tsitol. Genet. 44 (2010) 60–64.

[243] K.C. Kwon, H.T. Chan, I.R. Leon, R. Williams-Carrier, A. Barkan, H. Daniell, Codon optimization to enhance expression yields insights into chloroplast translation, Plant Physiol. 172 (2016) 62–77, https://doi.org/10.1104/pp.16.00981.

[244] T. Ruhlman, D. Verma, N. Samson, H. Daniell, The role of heterologous chloroplast sequence elements in transgene integration and expression, Plant Physiol. 152 (2010) 2088–2104, https://doi.org/10.1104/pp.109.152017.

[245] H. Daniell, Transgene containment by maternal inheritance: effective or elusive? Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 6879–6880, https://doi.org/10.1073/pnas.0702219104.

[246] B. De Cosa, W. Moar, S.B. Lee, M. Miller, H. Daniell, Overexpression of the Bt cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals, Nat. Biotechnol. 19 (2001) 71–74, https://doi.org/10.1038/83559.

[247] T. Quesada-Vargas, O.N. Ruiz, H. Daniell, Characterization of heterologous multigene operons in transgenic chloroplasts: transcription, processing, and translation, Plant Physiol. 138 (2005) 1746–1762, https://doi.org/10.1104/pp.105.063040.

[248] H. Daniell, S.B. Lee, T. Panchal, P.O. Wiebe, Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts, J. Mol. Biol. 311 (2001) 1001–1009, https://doi.org/10.1006/jmbi.2001.4921.

[249] J. Su, L. Zhu, A. Sherman, X. Wang, S. Lin, A. Kamesh, J.H. Norikane, S.J. Streatfield, R.W. Herzog, H. Daniell, Low cost industrial production of coagulation factor IX bioencapsulated in lettuce cells for oral tolerance induction in hemophilia B, Biomaterials 70 (2015) 84–93, https://doi.org/10.1016/j.biomaterials.2015.08.004.

[250] J.A. Lindbo, TRBO: a high-efficiency tobacco mosaic virus RNA-based overexpression vector, Plant Physiol. 145 (2007) 1232–1240, https://doi.org/10.1104/pp.107.106377.

[251] G. Mohannath, J.N. Jackel, Y.H. Lee, R.C. Buchmann, H. Wang, V. Patil, A.K. Adams, D.M. Bisaro, A complex containing SNF1-related kinase (SnRK1) and adenosine kinase in Arabidopsis, PLoS One 9 (2014), e87592, https://doi.org/10.1371/journal.pone.0087592.

[252] D. Boyhan, H. Daniell, Low-cost production of proinsulin in tobacco and lettuce chloroplasts for injectable or oral delivery of functional insulin and C-peptide, Plant Biotechnol. J. 9 (2011) 585–598, https://doi.org/10.1111/j.1467-7652.2010.00582.x.

[253] N. Kohli, D.R. Westerveld, A.C. Ayache, A. Verma, P. Shil, T. Prasad, P. Zhu, S.L. Chan, Q. Li, H. Daniell, Oral delivery of bioencapsulated proteins across blood-brain and blood-retinal barriers, Mol. Ther. 22 (2014) 535–546, https://doi.org/10.1038/mt.2013.273.

[254] Y. Shaaltiel, S. Gingis-Velitski, S. Tzaban, N. Fiks, Y. Tekoah, D. Aviezer, Plant-based oral delivery of β-glucocerebrosidase as an enzyme replacement therapy for Gaucher's disease, Plant Biotechnol. J. 13 (2015) 1033–1040, https://doi.org/10.1111/pbi.12366.

[255] V. Shenoy, K.C. Kwon, A. Rathinasabapathy, S. Lin, G. Jin, C. Song, P. Shil, A. Nair, Y. Qi, Q. Li, et al., Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension, Hypertension 64 (2014) 1248–1259, https://doi.org/10.1161/hypertensionaha.114.03871.

[256] P.K. Shil, K.C. Kwon, P. Zhu, A. Verma, H. Daniell, Q. Li, Oral delivery of ACE2/Ang-(1-7) bioencapsulated in plant cells protects against experimental uveitis and autoimmune uveoretinitis, Mol. Ther. 22 (2014) 2069–2082, https://doi.org/10.1038/mt.2014.179.

[257] E. Altindis, S. Gulce Iz, M. Ozgun Ozen, P. Nartop, I. Deliloglu Gurhan, A. Gurel, Plant derived edible vaccines and therapeutics, in: Frontiers in Clinical Drug Research: Anti-infectives, 2014, pp. 200–236, https://doi.org/10.2174/9781608058549114010007.

[258] An advisory committee statement (ACS) national advisory committee on immunization (NACI). https://www.canada.ca/content/dam/phac-aspc/documents/services/immunization/national-advisory-committee-on-immunization-naci/recommendations-use-medicago-covid-19-vaccine/recommendations-use-medicago-covid-19-vaccine.pdf, 2022.

[259] J. Gao, L. Yin, Drug development for controlling Ebola epidemic - a race against time, Drug Discover. Therapeut. 8 (2014) 229–231, https://doi.org/10.5582/ddt.2014.01040.

[260] X. Qiu, G. Wong, J. Audet, A. Bello, L. Fernando, J.B. Alimonti, H. Fausther-Bovendo, H. Wei, J. Aviles, E. Hiatt, et al., Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp, Nature 514 (2014) 47–53, https://doi.org/10.1038/nature13777.

[261] R.T. Davey Jr., L. Dodd, M.A. Proschan, J. Neaton, J. Neuhaus Nordwall, J.S. Koopmeiners, J. Beigel, J. Tierney, H.C. Lane, A.S. Fauci, et al., A randomized, controlled trial of ZMapp for ebola virus infection, N. Engl. J. Med. 375 (2016) 1448–1456, https://doi.org/10.1056/NEJMoa1604330.

[262] D. Tusé, T. Tu, K.A. McDonald, Manufacturing economics of plant-made biologics: case studies in therapeutic and industrial enzymes, BioMed Res. Int. 2014 (2014), 256135, https://doi.org/10.1155/2014/256135.

[263] J.A. Chichester, R.M. Jones, B.J. Green, M. Stow, F. Miao, G. Moonsammy, S.J. Streatfield, V. Yusibov, Safety and immunogenicity of a plant-produced recombinant hemagglutinin-based influenza vaccine (HAI-05) derived from A/Indonesia/05/2005 (H5N1) influenza virus: a phase 1 randomized, double-blind, placebo-controlled, dose-escalation study in healthy adults, Viruses 4 (2012) 3227–3244.

[264] Y. Shoji, J.A. Chichester, M. Jones, S.D. Manceva, E. Damon, V. Mett, K. Musiychuk, H. Bi, C. Farrance, M. Shamloul, Plant-based rapid production of recombinant subunit hemagglutinin vaccines targeting H1N1 and H5N1 influenza, Hum. Vaccine 7 (2011) 41–50.

[265] S. Pillet, É. Aubin, S. Trépanier, D. Bussière, M. Dargis, J.-F. Poulin, B. Yassine-Diab, B.J. Ward, N. Landry, A plant-derived quadrivalent virus like particle influenza vaccine induces cross-reactive antibody and T cell response in healthy adults, Clin. Immunol. 168 (2016) 72–87.

[266] S. Pillet, T. Racine, C. Nfon, T. Di Lenardo, S. Babiuk, B. Ward, G. Kobinger, N. Landry, Plant-derived H7 VLP vaccine elicits protective immune response against H7N9 influenza virus in mice and ferrets, Vaccine 33 (2015) 6282–6289.

[267] L.G. De Leede, J.E. Humphries, A.C. Bechet, E.J. Van Hoogdalem, R. Verrijk, D.G. Spencer, Novel controlled-release lemna-derived IFN-α 2b (locteron): pharmacokinetics, pharmacodynamics, and tolerability in a phase I clinical trial, J. Interferon Cytokine Res. 28 (2008) 113–122.

[268] K.M. Cox, J.D. Sterling, J.T. Regan, J.R. Gasdaska, K.K. Frantz, C.G. Peele, A. Black, D. Passmore, C. Moldovan-Loomis, M. Srinivasan, Glycan optimization of a human monoclonal antibody in the aquatic plant Lemna minor, Nat. Biotechnol. 24 (2006) 1591–1597.

[269] H.T. Chan, Y. Xiao, W.C. Weldon, S.M. Oberste, K. Chumakov, H. Daniell, Cold chain and virus-free chloroplast-made booster vaccine to confer immunity against different poliovirus serotypes, Plant Biotechnol. J. 14 (2016) 2190–2200.

[270] V. Yusibov, A. Modelska, K. Steplewski, M. Agadjanyan, D. Weiner, D.C. Hooper, H. Koprowski, Antigens produced in plants by infection with chimeric plant viruses immunize against rabies virus and HIV-1, Proc. Natl. Acad. Sci. USA 94 (1997) 5784–5788.

[271] G.G. Zhang, L. Rodrigues, B. Rovinski, K.A. White, Production of HIV-1 p24 protein in transgenic tobacco plants, Mol. Biotechnol. 20 (2002) 131–136.

[272] D.O. Govea-Alonso, N. Rubio-Infante, A.L. García-Hernández, J.T. Varona-Santos, S.S. Korban, L. Moreno-Fierros, S. Rosales-Mendoza, Immunogenic properties of a lettuce-derived C4 (V3) 6 multiepitopic HIV protein, Planta 238 (2013) 785–792.

[273] A. Modelska, B. Dietzschold, N. Sleysh, Z.F. Fu, K. Steplewski, D.C. Hooper, H. Koprowski, V. Yusibov, Immunization against rabies with plant-derived antigen, Proc. Natl. Acad. Sci. USA 95 (1998) 2481–2485.

[274] J. Kapusta, A. Modelska, M. Figlerowicz, T. Pniewski, M. Letellier, O. Lisowa, V. Yusibov, H. Koprowski, A. Plucienniczak, A. Legocki, A plant-derived edible vaccine against hepatitis B virus, FASEB J. 13 (1999) 1796–1799.

[275] F. Sainsbury, P.O. Lavoie, M.A. D'Aoust, L.P. Vézina, G.P. Lomonossoff, Expression of multiple proteins using full-length and deleted versions of cowpea mosaic virus RNA-2, Plant Biotechnol. J. 6 (2008) 82–92.

[276] H. Belanger, N. Fleysh, S. Cox, G. Hartman, D. Deka, M. Trudel, H. Koprowski, V. Yusibov, Human respiratory syncytial virus vaccine antigen produced in plants, FASEB J. 14 (2000) 2323–2328.

[277] A. Monroy-García, M.A. Gómez-Lim, B. Weiss-Steider, J. Hernández-Montes, S. Huerta-Yepez, J.F. Rangel-Santiago, E. Santiago-Osorio, M.d.L.M. García, Immunization with an HPV-16 L1-based chimeric virus-like particle containing HPV-16 E6 and E7 epitopes elicits long-lasting prophylactic and therapeutic efficacy in an HPV-16 tumor mice model, Arch. Virol. 159 (2014) 291–305.

[278] M.-Y. Kim, R. Reljic, J. Kilbourne, I. Ceballos-Olvera, M.-S. Yang, J. Reyes-del Valle, H.S. Mason, Novel vaccination approach for dengue infection based on recombinant immune complex universal platform, Vaccine 33 (2015) 1830–1838.

[279] W. Phoolcharoen, S.H. Bhoo, H. Lai, J. Ma, C.J. Arntzen, Q. Chen, H.S. Mason, Expression of an immunogenic Ebola immune complex in Nicotiana benthamiana, Plant Biotechnol. J. 9 (2011) 807–816.

[280] J.K.C. Ma, J. Drossard, D. Lewis, F. Altmann, J. Boyle, P. Christou, T. Cole, P. Dale, C.J. van Dolleweerd, V. Isitt, Regulatory approval and a first-in-human phase I clinical trial of a monoclonal antibody produced in transgenic tobacco plants, Plant Biotechnol. J. 13 (2015) 1106–1120.

[281] Z. Huang, W. Phoolcharoen, H. Lai, K. Piensook, G. Cardineau, L. Zeitlin, K.J. Whaley, C.J. Arntzen, H.S. Mason, Q. Chen, High-level rapid production of full-size monoclonal antibodies in plants by a single-vector DNA replicon system, Biotechnol. Bioeng. 106 (2010) 9–17.

[282] A.G. Diamos, J.G. Hunter, M.D. Pardhe, S.H. Rosenthal, H. Sun, B.C. Foster, M.P. DiPalma, Q. Chen, H.S. Mason, High level production of monoclonal antibodies using an optimized plant expression system, Front. Bioeng. Biotechnol. 7 (2020) 472.

[283] H.J. Flint, K.P. Scott, S.H. Duncan, P. Louis, E. Forano, Microbial degradation of complex carbohydrates in the gut, Gut Microb. 3 (2012) 289–306, https://doi.org/10.4161/gmic.19897.

[284] E.C. Martens, E.C. Lowe, H. Chiang, N.A. Pudlo, M. Wu, N.P. McNulty, D.W. Abbott, B. Henrissat, H.J. Gilbert, D.N. Bolam, J.I. Gordon, Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts, PLoS Biol. 9 (2011), e1001221, https://doi.org/10.1371/journal.pbio.1001221.

[285] Y. Xiao, K.C. Kwon, B.E. Hoffman, A. Kamesh, N.T. Jones, R.W. Herzog, H. Daniell, Low cost delivery of proteins bioencapsulated in plant cells to human non-immune or immune modulatory cells, Biomaterials 80 (2016) 68–79, https://doi.org/10.1016/j.biomaterials.2015.11.051.

[286] J. Durzyńska, Ł. Przysiecka, R. Nawrot, J. Barylski, G. Nowicki, A. Warowicka, O. Musidlak, A. Goździcka-Józefiak, Viral and other cell-penetrating peptides as vectors of therapeutic agents in medicine, J. Pharmacol. Exp. Therapeut. 354 (2015) 32–42, https://doi.org/10.1124/jpet.115.223305.

[287] H. Noguchi, M. Matsushita, S. Matsumoto, Y.F. Lu, H. Matsui, S. Bonner-Weir, Mechanism of PDX-1 protein transduction, Biochem. Biophys. Res. Commun. 332 (2005) 68–74, https://doi.org/10.1016/j.bbrc.2005.04.092.

[288] D.E. Saslowsky, Y.M. te Welscher, D.J. Chinnapen, J.S. Wagner, J. Wan, E. Kern, W.I. Lencer, Ganglioside GM1-mediated transcytosis of cholera toxin bypasses the retrograde pathway and depends on the structure of the ceramide domain, J. Biol. Chem. 288 (2013) 25804–25809, https://doi.org/10.1074/jbc.M113.474957.

[289] X. Wang, A. Sherman, G. Liao, K.W. Leong, H. Daniell, C. Terhorst, R.W. Herzog, Mechanism of oral tolerance induction to therapeutic proteins, Adv. Drug Deliv. Rev. 65 (2013) 759–773, https://doi.org/10.1016/j.addr.2012.10.013.

[290] N.L. Wernick, D.J. Chinnapen, J.A. Cho, W.I. Lencer, Cholera toxin: an intracellular journey into the cytosol by way of the endoplasmic reticulum, Toxins 2 (2010) 310–325, https://doi.org/10.3390/toxins2030310.

[291] G. Thomas, Furin at the cutting edge: from protein traffic to embryogenesis and disease, Nat. Rev. Mol. Cell Biol. 3 (2002) 753–766, https://doi.org/10.1038/nrm934.
[292] T. Arakawa, J. Yu, D.K. Chong, J. Hough, P.C. Engen, W.H. Langridge, A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes, Nat. Biotechnol. 16 (1998) 934–938, https://doi.org/10.1038/nbt1098-934.
[293] A. Muheem, F. Shakeel, M.A. Jahangir, M. Anwar, N. Mallick, G.K. Jain, M.H. Warsi, F.J. Ahmad, A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives, Saudi Pharmaceut. J. 24 (2016) 413–428, https://doi.org/10.1016/j.jsps.2014.06.004.
[294] T. Ruhlman, R. Ahangari, A. Devine, M. Samsam, H. Daniell, Expression of cholera toxin B-proinsulin fusion protein in lettuce and tobacco chloroplasts–oral administration protects against development of insulitis in non-obese diabetic mice, Plant Biotechnol. J. 5 (2007) 495–510, https://doi.org/10.1111/j.1467-7652.2007.00259.x.
[295] D. Verma, B. Moghimi, P.A. LoDuca, H.D. Singh, B.E. Hoffman, R.W. Herzog, H. Daniell, Oral delivery of bioencapsulated coagulation factor IX prevents inhibitor formation and fatal anaphylaxis in hemophilia B mice, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 7101–7106, https://doi.org/10.1073/pnas.0912181107.
[296] J. Yao, Y. Weng, A. Dickey, K.Y. Wang, Plants as factories for human pharmaceuticals: applications and challenges, Int. J. Mol. Sci. 16 (2015) 28549–28565, https://doi.org/10.3390/ijms161226122.
[297] D.R. Hill, L. Ford, D.G. Lalloo, Oral cholera vaccines: use in clinical practice, Lancet Infect. Dis. 6 (2006) 361–373, https://doi.org/10.1016/s1473-3099(06)70494-7.
[298] F. Qi, S. Qian, S. Zhang, Z. Zhang, Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses, Biochem. Biophys. Res. Commun. 526 (2020) 135–140, https://doi.org/10.1016/j.bbrc.2020.03.044.
[299] G. Cholankeril, A. Podboy, V.I. Aivaliotis, B. Tarlow, E.A. Pham, S.P. Spencer, D. Kim, A. Hsing, A. Ahmed, High prevalence of concurrent gastrointestinal manifestations in patients with severe acute respiratory syndrome coronavirus 2: early experience from California, Gastroenterology 159 (2020) 775–777, https://doi.org/10.1053/j.gastro.2020.04.008.
[300] J. Gu, B. Han, J. Wang, COVID-19: gastrointestinal manifestations and potential fecal-oral transmission, Gastroenterology 158 (2020) 1518–1519, https://doi.org/10.1053/j.gastro.2020.02.054.
[301] W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, W. Tan, Detection of SARS-CoV-2 in different types of clinical specimens, JAMA 323 (2020) 1843–1844, https://doi.org/10.1001/jama.2020.3786.
[302] F. Xiao, M. Tang, X. Zheng, Y. Liu, X. Li, H. Shan, Evidence for gastrointestinal infection of SARS-CoV-2, Gastroenterology 158 (2020), https://doi.org/10.1053/j.gastro.2020.02.055, 1831.e1833 1833.e1833.