nature microbiology

Review article

https://doi.org/10.1038/s41564-025-02070-z

Challenges and opportunities in mRNA vaccine development against bacteria

Received: 21 August 2023

Accepted: 18 June 2025

Published online: 29 July 2025

Check for updates

Ilke Aernout[1,13], Rein Verbeke[1,13], Fabien Thery[2,3], Patrick Willems[2,3,4,5], Uri Elia[6,7,8,9,10], Stefaan C. De Smedt[1], Rino Rappuoli[11], Dan Peer[6,7,8,9], Francis Impens[2,3,12,13] ✉ & Ine Lentacker[1,13] ✉

The global surge in antimicrobial resistance presents a critical threat to public health, emphasizing the urgent need for the development of new and more effective bacterial vaccines. Since the success of mRNA vaccines during the COVID-19 pandemic, this vaccine strategy has rapidly advanced, with most efforts focused on cancer immunotherapy and targeting viral pathogens. Recently, mRNA vaccines have entered the early phases of clinical development for bacterial diseases. However, bacteria present greater biological complexity compared with viruses, posing additional challenges for vaccine design, such as antigen selection, immune response and mRNA construct design. Here, we discuss critical aspects in the development of bacterial mRNA vaccines, from antigen selection to construct design. We also highlight the current preclinical landscape and discuss remaining translational challenges and future potential for mRNA vaccines against bacterial infections.

Antibiotics have been successfully used to treat bacterial infections for decades. However, recently, antimicrobial resistance (AMR) has been rising to dangerously high levels[1–4]. Bacterial vaccines represent an attractive tool to combat AMR as they can confer protection against infection and disease, while preventing the emergence and transmission of bacterial infections. This directly impacts on antibiotic prescription and overuse, and might thus limit the selection and dissemination of antibiotic-resistant strains[5,6].

Bacterial vaccines are typically based on whole-cell vaccines (inactivated or live attenuated pathogens), polysaccharides alone or conjugated to proteins, and protein subunit vaccines. Whole-cell bacterial vaccines have the advantage of inducing immunity against a wide repertoire of bacterial antigens. Within this category, live attenuated vaccines are often more effective compared to inactivated vaccines because they retain the ability to replicate, thereby inducing a broader immune response. This feature, however, limits their use in immunocompromised individuals. Several successful bacterial subunit vaccines have been licensed in recent years. Among them, glycoconjugate vaccines, created by covalently linking polysaccharides to an antigenic protein, have proven particularly effective and cost-efficient in preventing bacterial infections, such as *Haemophilus influenzae* type B, *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Salmonella* Typhi[7]. These vaccines also contribute to herd immunity by reducing transmission within the population[8]. Other successful bacterial vaccines have been based on protein subunits, including inactivated toxins (as for diphtheria and tetanus vaccines), virulence factors (as for the serogroup B meningococcus) or can be combined (as in the acellular pertussis vaccine). In addition, innovative approaches are currently in

[1]Ghent Research Group on Nanomedicines, Ghent University, Ghent, Belgium. [2]VIB-UGent Center for Medical Biotechnology, VIB, Ghent, Belgium. [3]Department of Biomolecular Medicine, Ghent University, Ghent, Belgium. [4]Center for Plant Systems Biology, VIB-UGent, Ghent, Belgium. [5]Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium. [6]Laboratory of Precision Nanomedicine, Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel. [7]Department of Materials Sciences and Engineering, Iby and Aladar Fleischman Faculty of Engineering, Tel Aviv University, Tel Aviv, Israel. [8]Center for Nanoscience and Nanotechnology, Tel Aviv University, Tel Aviv, Israel. [9]Cancer Biology Research Center, Tel Aviv University, Tel Aviv, Israel. [10]Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona, Israel. [11]Fondazione Biotecnopolo di Siena, Siena, Italy. [12]VIB Proteomics Core, VIB, Ghent, Belgium. [13]These authors contributed equally: Ilke Aernout, Rein Verbeke, Francis Impens, Ine Lentacker. ✉e-mail: Francis.Impens@vib-ugent.be; Ine.Lentacker@UGent.be

development, including engineered outer membrane vesicles (OMVs), derived from Gram-negative bacteria, and whole-cell vaccines developed using synthetic biology, as reviewed elsewhere[9]. Novel immune stimulants have also been investigated to enhance T cell-mediated immunity, boost immunogenicity and counteract immunosenescence in bacterial subunit vaccines. One example is the clinically approved AS01 adjuvant, a liposomal formulation of monophosphoryl lipid A with the purified saponin QS-21[10]. As a key final step in the development of the first new anti-tuberculosis vaccine in over a century, a phase III clinical trial is currently evaluating the efficacy of a vaccine candidate, composed of the M72 recombinant fusion protein—comprising two *Mycobacterium tuberculosis* (*Mtb*) antigens (Mtb32A and Mtb39A)—formulated with the AS01 adjuvant[11]. This vaccine is aimed at preventing pulmonary tuberculosis in adults and adolescents. Previous clinical data have already demonstrated that M72, formulated with AS01E, which enhances immune responses, provides over 50% protection against active pulmonary tuberculosis disease in *Mtb*-infected adults[12].

In addition to these approaches, the recent COVID-19 pandemic has demonstrated that mRNA vaccines can become next-generation approaches against infectious diseases due to their proven efficacy and safety. Their rapid manufacturing process also enables fast responses to emerging pandemics[13]. mRNA vaccines work by delivering genetic instructions to the body's cells for the temporary production of protein antigens. This is achieved by using lipid nanoparticles (LNPs) as a non-viral delivery system to transport the synthetic mRNA into cells. While mRNA vaccines continue to demonstrate success against viral diseases, the development of mRNA vaccines against bacterial disease has been more challenging. Unlike viruses, bacteria express thousands of proteins, complicating the selection of suitable antigens[14]. Although viral proteins tend to be more compatible and easier for host cells to translate—having evolved to exploit host cellular machinery—bacterial proteins encoded by mRNA may be poorly translated in mammalian cells. Additionally, the intracellular trafficking, processing and post-translational modifications of these foreign proteins may substantially affect their stability and immunogenicity. Both humoral and cellular immunity play a role in protection against bacteria, but their relative importance can vary depending on the bacterial species. Vaccine development against extracellular bacteria primarily focuses on inducing effective humoral responses; accordingly, bacterial proteins or polysaccharides that are surface-exposed or secreted are commonly regarded as potential vaccine candidates. However, this requires the secretion of stable bacterial proteins to enable recognition and binding to B cell receptors, leading to antibody production. Although antibodies may provide some protection against intracellular bacteria, especially before cellular entry, these bacteria become less accessible to antibodies once they reside within host cells. Therefore, cellular immunity becomes important by directly killing infected cells. Cellular $CD8^+$ and $CD4^+$ T cell responses are triggered by short antigenic peptides that are presented by major histocompatibility complex (MHC) class I or II molecules, respectively, on antigen-presenting cells (APCs). For loading onto MHC-I receptors, cytosolic peptides are degraded by the proteasome then translocated into the endoplasmic reticulum (ER), where they are loaded onto MHC-I receptors. MHC-II molecules bind peptides that are derived from proteins degraded in the endocytic pathway. Peptide-loaded MHC-I and MHC-II molecules are then transported to the cell membrane to present their cargo to $CD8^+$ and $CD4^+$ T cells.

Here, we discuss major challenges in bacterial mRNA vaccine design, including antigen identification and selection, as well as the impact of translational and post-translational processing of bacterial antigens in the eukaryotic cell. We also provide examples and strategies for tailoring mRNA construct design to promote either humoral or cellular immunity. Finally, we provide an overview of the current landscape of preclinical and clinical development of bacterial mRNA vaccines and discuss potential solutions to improve the translatability and immunogenicity of bacterial mRNA vaccines.

Challenges in bacterial antigen selection and post-translational processing

Cellular and subunit vaccines involve the direct engulfment of entire bacterial pathogens or purified bacterial proteins by APCs, but mRNA vaccines work by inducing the host's own cells to produce bacterial protein antigens encoded by the mRNA. As most protein-based bacterial vaccines are composed of several purified proteins, mRNA vaccines may have the advantage of easily encoding different protein antigens in multivalent formulations. This flexibility is particularly valuable given the antigenic complexity of bacterial pathogens, which will most probably require multivalent vaccine strategies to combat variant strains and to adeptly navigate variable antigen expression in bacteria during different stages of infection[15]. Reversible switches of gene expression (phase variation)[16–18] and expression of distinct (surface) antigens (antigenic variation)[19–21] are often associated with virulence and therefore important factors to consider. However, designing mRNA-based bacterial vaccines also comes with several challenges. Many bacterial antigens are complex multi-subunit proteins that fold in bacterial cells, often requiring specific chaperones. These proteins might not correctly fold inside mammalian host cells. Moreover, host cells may glycosylate proteins, a modification that is usually absent in bacteria. In addition to these challenges, mRNA vaccines cannot be used to encode non-protein antigens such as bacterial polysaccharides, excluding the possibility to make bacterial conjugate vaccines that can elicit protective antibody responses against the polysaccharide capsule of certain bacteria[22].

As with different vaccine platforms, the development of mRNA-based bacterial vaccines hinges on the careful selection of antigens that are efficiently expressed and correctly folded within host cells. Ideal antigens are typically low-molecular-weight protein subunits capable of self-assembly without requiring chaperones and designed to avoid unwanted glycosylation. An overview of current methodologies that can be applied for bacterial antigen discovery is provided in Box 1 and Fig. 1a,b. Computational immunology has emerged as a powerful tool for predicting vaccine candidates in a cost-effective and rapid manner[23,24]. Although these approaches might drastically shorten the list of potential vaccine candidates, they might still yield dozens of antigen candidates requiring experimental validation. Immunopeptidomic screens offer a complementary strategy that integrates data from diverse cellular and infection models[14]. Recently, we used immunopeptidomics to identify antigens presented on two human cell lines infected with *Listeria monocytogenes* and identified 13 bacterial antigens represented by two or more immunopeptides[25]. After excluding potential toxic antigens, seven candidates were retained and encoded in mRNA vaccine formulations to test their protective efficacy in a mouse model of *Listeria* infection. Interestingly, the antigen with the highest number of epitopes, an uncharacterized bacterial surface protein, LMON_0149, provided the highest level of protection. Moreover, we observed a positive correlation between the number of identified immunopeptides per antigen and the degree to which they reduced bacterial numbers following immunization[25]. This finding suggests that the number of immunopeptides identified through immunopeptidomics experiments could serve as a valuable criterion for prioritizing bacterial vaccine candidates, although further studies are required to confirm this. The flexibility of mRNA vaccine platforms could further accelerate antigen discovery approaches as their rapid and scalable production enables evaluation of the protective efficacy of many different protein antigens, a concept recently termed reverse vaccinology 3.0[26]. For example, inactive sequence variants of bacterial toxins such as the non-pore-forming E262K variant of listeriolysin O, used in the aforementioned *Listeria* study, can be quickly evaluated.

In contrast to viruses, which rely on host translational machinery upon infection, bacteria have their own cellular machinery for protein production. The first issue that arises from this distinction is that bacterial antigen sequences delivered through mRNA vaccines may be poorly

https://doi.org/10.1038/s41564-025-02070-z

BOX 1

Methods for untargeted bacterial antigen discovery

Recent technological advances enable direct experimental detection of bacterial antigens. Contemporary mass spectrometry (MS)-based proteomics workflows allow the sensitive identification of even low-abundance bacterial peptides presented by MHC-I or MHC-II molecules on infected host cells[14,116]. This method, also referred to as immunopeptidomics, has recently proven effective in identifying bacterial immunopeptides derived from the intracellular bacterial pathogens *Mycobacterium tuberculosis*[84,117,118] (*Mtb*) and *Listeria monocytogenes*[25,119] presented on the surface of infected cells. Using a diverse range of host cell types with differing HLA alleles along with sampling at various stages of infection can help increase the diversity of bacterial epitopes detected. In addition to MHC-I-presented peptides, surface-exposed bacterial proteins[120,121] or those bound to circulating antibody–antigen complexes[122] can also be detected by MS, further informing vaccine antigen candidate selection. An alternative approach is proteome-wide T cell immunoreactivity screening, for instance by recording ex vivo cytokine production when exposing patient's peripheral blood mononuclear cells to synthetic peptide libraries spanning 4,000 *Mtb* open reading frames[85]. Various screening strategies are also used to identify antigen targets of specific T cell receptors (reviewed in ref. 123), such as T cell receptors associated with *Mtb* infection[124,125]. In addition to experimental methods, computational immunology can steer the rational selection or prioritization of bacterial antigens starting from gene or protein sequences, an approach generally referred to as reverse vaccinology[23,24,126]. Protein features such as surface exposure, adhesin probability and others can be used to identify antigen candidates[127,128]. Additional considerations include possible variable expression[16] and conservation of antigens across strains of the pathogen species[126]. Alternatively, machine learning algorithms trained on curated sets of protective bacterial antigens aim to score and rank the antigenicity of bacterial proteins[129,130]. In addition to antigen prediction, B and T cell epitopes can be predicted by a multitude of bioinformatic tools (reviewed in refs. 131,132) and can assist in antigen selection[127,133], but also immunopeptide annotation[25,84], the rational design of peptide pools[134,135] and synthetic multi-epitope mRNA vaccines[136,137]. Although immunoinformatics is being increasingly adopted, caution is warranted, as the available models or imposed selection criteria may not generalize well for certain pathogens or populations[138–140]. Following their prediction or identification, prioritization of bacterial antigens as vaccine candidates is a critical step[14]. Toxic antigens should be avoided or rendered inactive, for example, as demonstrated for the pore-forming agent listeriolysin O[25]. Additionally, as HLA alleles occur at different frequencies in different human populations, antigens targeted by HLA alleles carried at high frequency in the targeted population should also be prioritized[141] Conversely, sharing of cross-reactive epitopes between pathogens and gut bacteria may influence the composition of intestinal microbiota as well as vaccine efficacy, as reported for the BCG vaccine[142].

translated in the eukaryotic host, although this can be partially resolved by codon optimization[27]. Vaccine development against extracellular bacteria mainly focuses on inducing effective humoral responses, by targeting surface-exposed or secreted bacterial proteins as potential antigen candidates. This strategy requires stable bacterial protein secretion for recognition and binding to B cell receptors. To support this in mRNA vaccine constructs, the native bacterial secretion signal is often replaced with a mammalian secretion signal to ensure proper protein processing in the mammalian host cells. This approach was used in a study on the development of a self-amplifying mRNA vaccine against *Streptococcus pyogenes* and *Streptococcus agalactiae*. When the truncated BP-2a (pilus 2a backbone protein) antigen was compared with the same antigen preceded by the signal sequence of the murine immunoglobulin (Ig) κ chain, higher antibody titres and enhanced protection were observed when the signal sequence was encoded[28]. A study on *Pseudomonas aeruginosa* also found higher antibody responses by fusing the signal peptide (SP) sequence of human tissue plasminogen activator (h-tPA) to PcrV, a *P. aeruginosa* antigen[29]. Similarly, in a study by Messou and colleagues[30] on *Salmonella*, enhanced protein expression was obtained for most mRNA-encoded antigens when adding the SP sequence of human Ig K. However, this strategy did not prove to be effective for all bacterial proteins. Replacing the native SP sequence was also tested in a recent study by the Peer laboratory, where an mRNA vaccine was developed for the extracellular bacterium *Yersinia pestis*, encoding its capsule antigen, the F1 protein[31]. In contrast to the recombinant F1 protein vaccine, which was immunogenic, the corresponding mRNA-encoded antigen fused to a mammalian SP sequence originating from the IgG κ light chain yielded cellular but no humoral responses. In this case it was hypothesized that post-translational modifications may have suppressed or masked critical immunogenic epitopes, thereby reducing B cell recognition of the bacterial epitopes. This, in turn, may have resulted in weaker humoral responses and only partial protection against the bacteria compared to the recombinant protein vaccine.

In addition, it is well known that post-translational processes also vary substantially between prokaryotic and eukaryotic cells. Bacterial proteins expressed by host cells may thus differ structurally from their native bacterial counterparts due to post-translational and co-translational modifications, with glycosylation being the most notable example. Glycans added by the host may cause structural differences or sterically hinder the recognition of B cell epitopes in the bacterial antigen. Glycans can also generate new target sites for receptors that may be involved in stimulatory and inhibitory immune pathways (reviewed in ref. 32). By utilizing bioinformatic tools such as NetNglyc and NetOglyc, one can predict potential glycosylation sites within bacterial vaccine targets and design mutated antigens accordingly[33]. The substantial impact host glycosylation can have on the immune response generated by nucleic acid vaccines was demonstrated for the *Mtb* surface protein, Ag85A[34]. Upon immunization in mice, glycosylation impaired humoral and even cellular immunity compared to the native bacterial protein. However, immunogenicity could be restored through mutagenesis of the N-glycosylation site. Most importantly, the authors hypothesized that this might, at least partially, explain the clinical failure of two viral vector-based vaccine candidates encoding the *Mtb* antigen, MVA85A[35,36] and AERAS-402[37]. A study by Muir and colleagues[38] further highlights the complexity associated with expressing bacterial proteins in mammalian cells. In that study, the authors aimed to enhance the production and secretion of a bacterial enzyme, chondroitinase ABC, which is not naturally secreted by mammalian cells in its native gene form. They demonstrated that removal of some glycosylation sites resulted in increased protein secretion. However, two other glycosylation sites were identified as essential for the production and secretion of the active enzyme. This indicates that some degree of glycosylation might even be necessary for the secretion of the bacterial protein. Interestingly, in the study by Kon and colleagues[31], vaccination with an mRNA vaccine encoding *Y. pestis* F1 protein devoid of a secretion signal resulted in robust IgG titres, providing full protection in

https://doi.org/10.1038/s41564-025-02070-z

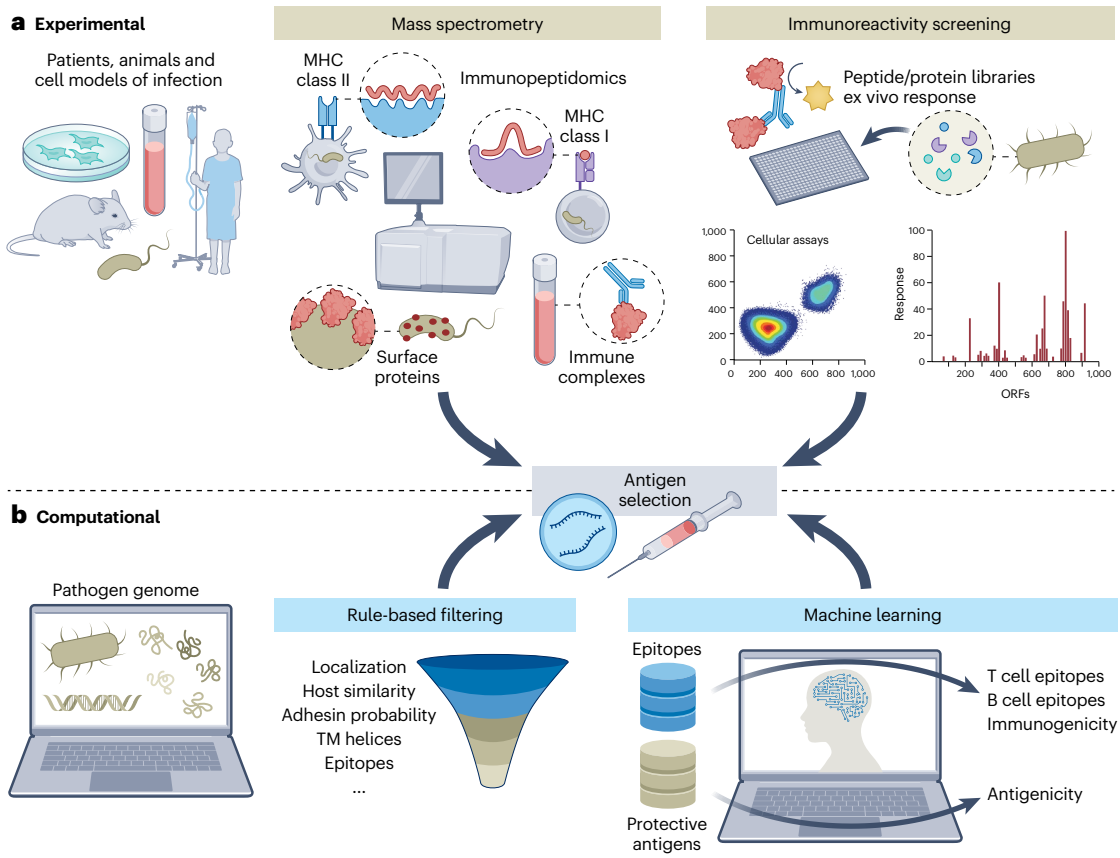

Fig. 1 | Antigen discovery methods in bacterial vaccine development. **a**, Antigens can be identified experimentally using samples from infected patients, animals or cell models of infection. Experimental methods include MS-based detection of antigens via purification and identification of T cell epitopes presented on MHC molecules (immunopeptidomics), circulating immune complexes or bacterial surface proteins. Alternatively, various immunoreactivity-based screening methods can reveal immunodominant bacterial antigens. ORF, open reading frame. **b**, Computational immunology methods can facilitate antigen discovery by applying rule-based filtering to bacterial proteins based on known features of existing antigens to prioritize potential antigen vaccine candidates. Additionally, machine learning models trained on experimental immunology data can be used to score and rank possible protective antigens, and presented epitopes, among other relevant features such as antigenicity.

a lethal *Y. pestis* Kimberley 53 challenge mouse model. This suggests that the F1 protein was secreted into the extracellular environment via alternative routes, collectively known as unconventional protein secretion. These pathways, which may include stress-induced membrane pore formation and secretion of membrane-bound organelles, do not involve transition of the protein through the Golgi apparatus and ER, and therefore avoid post-translational modifications (PTMs) and other protein alterations that occur in the traditional secretory pathway[39]. Collectively, these studies suggest that the use of mammalian SP sequences and manipulation of host glycosylation pathways are key factors that warrant further investigation as they influence the fate of translated bacterial proteins, the resulting immune effects and ultimately the degree of protective efficacy achieved.

Optimizing mRNA construct design for improved cellular immunity

The generation of pathogen-specific antibodies is generally considered essential for disease protection; however, cellular immunity also plays a crucial role, especially in the case of intracellular bacteria such as *Mtb*[40,41]. Both $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes (CTLs) can contribute to host defence through direct killing of infected cells upon antigen recognition[42,43]. Activated $CD4^+$ and $CD8^+$ T cells also produce cytokines, including interferon-γ (IFNγ), which can stimulate (infected) macrophages, inducing autophagy and intracellular production of nitric oxide, leading to the apoptosis of bacteria-infected macrophages[44]. When mammalian cells are transfected by mRNA vaccines, without SP, the encoded protein is expressed in the cytosol where proteins are typically degraded by the ubiquitin-proteasome system[45]. In the case of bacterial virulence factors that are normally secreted or injected in the host cell during pathogen infection, adaptation to the eukaryotic host cell environment might result in relatively stable expression of such encoded antigens[46]. Other bacterial proteins, especially cell wall or membrane proteins, may quickly degrade in mammalian cells owing to improper folding or other factors. Such unstable expression and fast proteasomal degradation might be beneficial for MHC-I presentation and the activation of $CD8^+$ T cell responses, because defective ribosomal products, short-lived and non-canonical proteins, constitute a substantial portion of the immunopeptidome[47–49]. In a study conducted by us, although the mechanism was not fully elucidated, vaccination with the bacterial surface protein LMON_0149, encoded without an SP in an mRNA construct, conferred protection against *L. monocytogenes* in a mice challenge study. This protection could well be the result of such unstable protein expression[25], especially as protective immunity against this intracellular pathogen strongly depends on $CD8^+$ T cell responses[50]. This has further been exploited in the context of a mycobacterial DNA vaccine where ubiquitin fusion constructs with the mycobacterial antigen MPT64 were encoded to target ubiquitin-proteasomal degradation and favour cytotoxic T cell stimulation, while no humoral responses were detected[51].

Because $CD4^+$ and $CD8^+$ T cells synergize to generate immunity against intracellular bacteria, antigens and vaccine design should be selected to optimally target and elicit both T cell subsets. As

https://doi.org/10.1038/s41564-025-02070-z

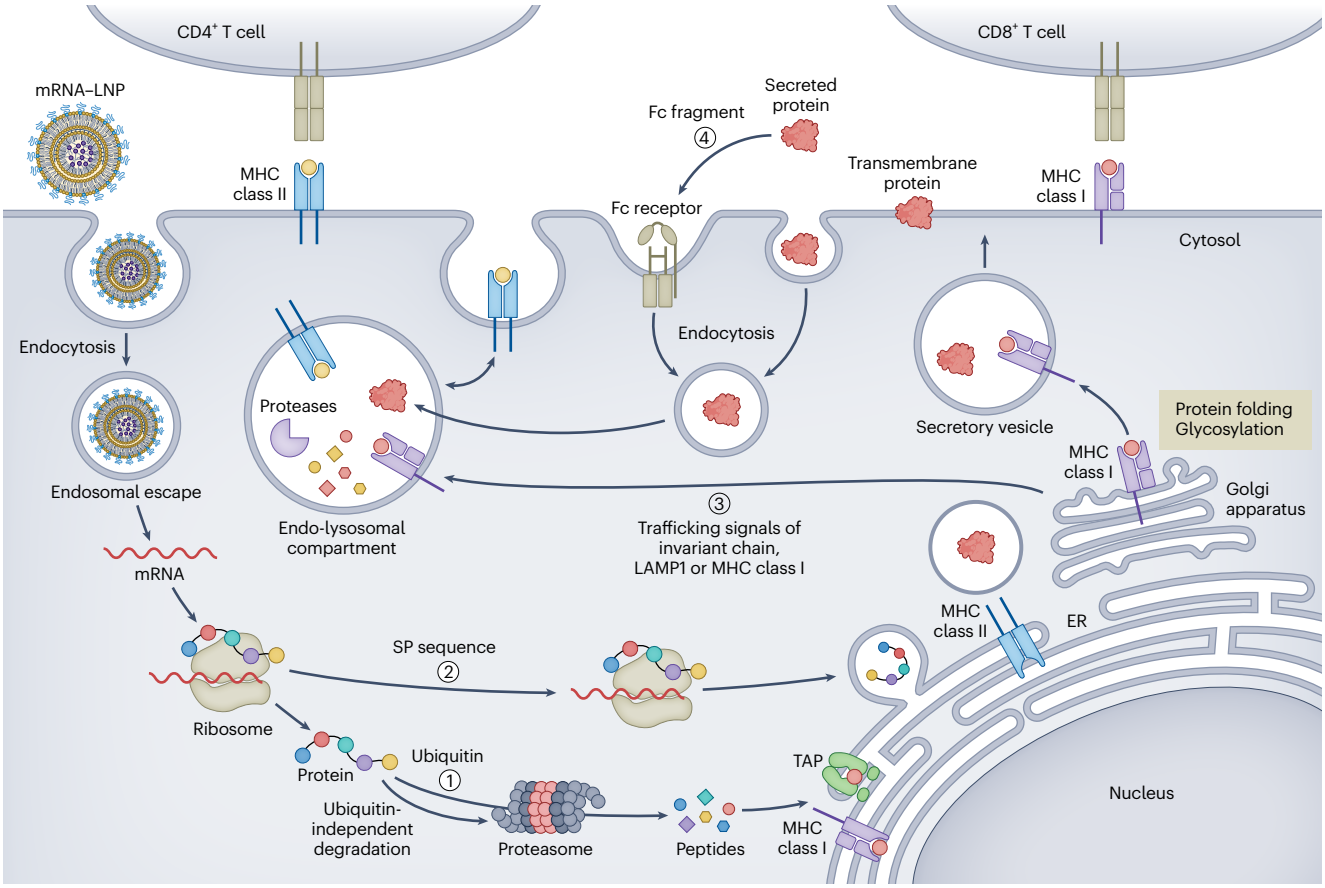

Fig. 2 | Inclusion of different signalling peptides and trafficking motifs directs intracellular processing and localization of the mRNA-encoded antigen after ribosomal translation. Cytosolic proteins are targeted to the ubiquitin-proteasome pathway or alternative ubiquitin-dependent pathways for degradation (1). Subsequently, protein fragments can be loaded on MHC-I complexes within the ER after transport through the transporter associated with antigen-processing (TAP), then trafficked to the cell surface where they can activate CD8$^+$ T cells. Proteins with a signalling peptide are imported in the ER and guided into the secretory pathway (2). In this process, proteins undergo conformational changes and can be subject to post-translational modifications, including glycosylation. Proteins linked to trafficking motifs derived from invariant chain, LAMP1, MHC-I or others are guided into the endo-lysosomal compartments (3). After degradation, protein fragments can be loaded in MHC-I and MHC-II complexes and delivered to the cell surface, stimulating CD8$^+$ and CD4$^+$ T cells, respectively. Proteins linked to an Fc fragment can become re-internalized through Fc-mediated endocytosis, entering endo-lysosome compartments, where they are degraded and loaded onto MHC-II molecules, then trafficked to the cell surface where they mainly activate CD4$^+$ T cells (4).

mRNA-encoded antigens are expressed in the cellular cytoplasm, it is expected that they will mainly be presented in the context of MHC-I complexes[52]. However, it has been shown that MHC-II molecules can also acquire access to endogenously synthesized antigens within specialized endo-lysosomal compartments[53]. Therefore, fusion constructs of antigens with human leukocyte antigen (HLA) class II sorting signals have been evaluated for their ability to guide cytoplasmic antigens to these compartments, mainly in the context of viral vaccine design. More specifically, those HLA class II sorting signals contain endocytic-sorting motifs present at the cytoplasmic domain of endosomal or lysosomal transmembrane proteins, which are recognized by trafficking components and guide these proteins into endo-lysosomal compartments[53]. Some examples are the sorting signals derived from the transmembrane protein invariant chain (Ii) stabilizing and controlling the intracellular transport of MHC-II molecules[54], or the lysosomal associated membrane protein (LAMP1), present in the endo-lysosomal compartment[55,56]. Importantly, the LAMP1-mediated transport of cytoplasmatic proteins to the endo-lysosomal compartment is dependent on their translocation to the ER, which is guaranteed by including the LAMP1 SP in the construct (Fig. 2)[56]. Kreiter and colleagues[57] explored an alternative strategy that entailed inserting the genetically encoded antigen between the SP sequence and the cytoplasmic domain of MHC-I (MITD), which was pivotal for endo-lysosomal targeting and cross-presentation of exogenously derived antigens in dendritic cells[58]. This resulted in enhanced MHC-I and MHC-II presentation of the antigen, and CD8$^+$ and CD4$^+$ T cell activation, respectively[57]. However, it remains unclear whether the SP sequence also routes the protein to the extracellular space, after which re-internalization and MHC-II loading can take place[59]. Notably, these constructs were used to design BioNTech's clinical cancer vaccines[60,61]. Finally, it has been shown that coupling the Fc fragment of IgG to the mRNA-encoded antigen can indirectly target antigens to the MHC-II compartment, as secreted proteins will be re-internalized by Fcγ receptor-mediated endocytosis (Fig. 2)[62]. In addition, it has been reported that this can extend the plasma half-life of proteins[63], but it is not clear to what extent this contributed to the enhanced specific immune responses observed after Fc-conjugation in the design of a SARS-CoV-2 vaccine and a *Y. pestis* vaccine[31,64]. Taken together, these studies illustrate that, depending on the targeted bacteria and desired immune response, several approaches involving different mRNA construct designs can be explored to promote specific intracellular pathways and thereby steer adaptive immune responses, with varying degrees of success (Fig. 3).

Current status of bacterial mRNA vaccines

Numerous studies have explored the use of mRNA vaccines against viral targets, but only a limited number of publications have shown protective efficiency of mRNA vaccines against bacterial infections. Table 1 summarizes bacterial mRNA vaccines that have been

https://doi.org/10.1038/s41564-025-02070-z

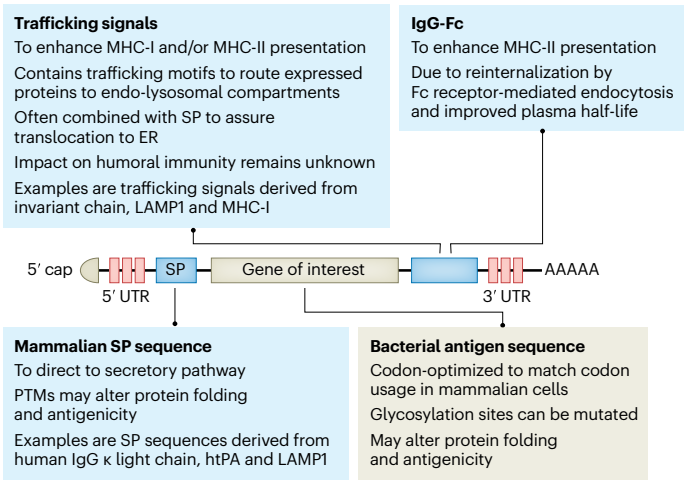

Fig. 3 | Optimization of mRNA construct design to enhance protein expression or to direct expressed antigens towards specific antigen presentation pathways. These approaches can aid in tailoring adaptive immune responses to mRNA vaccines, depending on the desired correlates of protection of the bacterial disease, but come with certain disadvantages. IgG-Fc, crystallizable fragment of immunoglobulin G antibody. UTR, untranslated region.

pre-clinically evaluated (Table 1, top) or that have entered clinical evaluation (Table 1, bottom). As discussed above, mRNA vaccines have shown promise in preclinical models of various bacterial diseases caused by *Y. pestis*[31,65], *L. monocytogenes*[25,66], *S. pyogenes* and *S. agalactiae*[28], *P. aeruginosa*[29,67] and *S. typhimurium*[30]. Other bacterial diseases being evaluated at the preclinical stage are also listed in Table 1 (top).

By contrast, only a few vaccines have entered the phase of clinical evaluation. This encompasses Moderna's Phase I/II clinical trial (NCT05975099), which aims to evaluate two mRNA vaccines against Lyme disease caused by *Borrelia burgdorferi*. This bacterial pathogen is transmitted to humans through the bite of infected ticks. Lyme disease is now recognized as the most common vector-borne disease in both Europe and North America and there is no vaccine available[68]. Moderna's first candidate, mRNA-1982, encodes the outer surface protein A serotype 1 (OspA SR1) of *B. burgdorferi*, whereas mRNA-1975 is a heptavalent vaccine encoding OspA serotypes 1–7, designed to induce protection against four major *Borrelia* species causing Lyme disease in the United States and Europe[69]. Interestingly, vaccine-induced antibodies directed against the OspA antigen can neutralize bacteria within the midgut of the tick while feeding and, as such, inhibit transmission of the bacteria from the tick to the host. In a preclinical mouse study, an OspA-encoding mRNA–LNP vaccine was found to be superior to an alum-adjuvanted OspA protein vaccine in inducing humoral and cellular immunity, thereby showing successful protection against *B. burgdorferi* infection after a single immunization[70]. Notably, an effective alum-adjuvanted recombinant OspA protein vaccine (LYMErix) developed by GlaxoSmithKline was already on the market in 1998 but was discontinued after only three years due to declining sales. Indeed, LYMErix suffered from lack of acceptance as it was suggested that OspA was cross-reactive with human lymphocyte function-associated antigen 1 (hLFA1), potentially causing arthritic symptoms in vaccinated individuals[71]. This was later disproven by several independent studies[72].

In collaboration with West Virgina University, Moderna also recently reported on a multivalent mRNA vaccine targeting both *Bordetella pertussis* antigens, and diphtheria and tetanus toxoids. mRNA constructs for the pertussis and tetanus antigen were optimized with SPs of either Ig K or bovine prolactin, whereas the native signal sequence was maintained in the diphtheria antigen construct. Some of the antigens were additionally modified with mutations to avoid N-linked glycosylation sites, and/or to eliminate toxin-related activities. Prime-boost vaccination with a 6- to 10-valent mRNA vaccine resulted in similar levels of protection in a murine *B. pertussis* challenge model compared to 1/20th of the human dose of either acellular multivalent pertussis vaccine (DTaP) or whole-cell pertussis vaccine. Additionally, they found that the mRNA vaccine elicited more balanced Th1 and Th2 immunity than DTaP, which induced a Th2 polarized response, which was evident from higher IgG2a and IgG2b antibody levels, and an IFNγ-dominant $CD4^+$ T helper response[73]. This is important, as Th1- and Th17-mediated immune responses are known to be essential for bacterial clearance and long-lasting protection against *B. pertussis*[22].

In 2023, tuberculosis ranked as the world's leading cause of death from a single infectious agent disease[74]. The live attenuated vaccine, bacillus Calmette-Guérin (BCG), has been used since 1921, and remains the only licensed vaccine against *Mtb*. Although effective in young children, BCG is less effective in adults, especially in low-and middle-income countries (LMICs)[75]. Larsen and colleagues[76] evaluated the efficacy of the fusion protein ID91 encoded in self-amplifying RNA complexed through a nano structured lipid carrier (NLC) against *Mtb* in a preclinical setting. Of interest in these studies is that by heterologous vaccination of RNA and protein subunit vaccines, promising protective results were obtained, which could be explained by the stronger induction of multifunctional T cells. BioNTech also announced that they are working on an mRNA vaccine against *Mtb*. In the patent filed by BioNTech (WO 2024/028445 A1), the following antigens are listed: Ag85A, ESAT6, VapB47, Hrp1, RpfA, RpfD, Mtb32a, Mtb39a and HbhA, covering antigens present at various stages of infection. Different combinations of these antigens were evaluated, encoded as fusion proteins or co-formulated as single mRNA strands. In addition, the use of alternative SPs and/or transmembrane and cytoplasmic domains of MHC-Is were screened, resulting in variable cellular and humoral responses against the different antigens. This indicates the complexity of predicting the impact of vaccine construct design on intracellular processing and immunogenicity. Based on the patent information, it is likely that BioNTech combined four different mRNA strands in their final vaccine, each encoding a fusion protein of two antigens proceeded by the SP of MHC-I[77].

Using two different clinical studies, BioNTech aims to compare vaccine candidates against *Mtb* containing either unmodified mRNA (BNT164a1) or N1-methylpseudouridine (m1Ψ)-modified mRNA (BNT614b1). These vaccines will be administered to either BCG-naïve, non-tuberculosis-exposed subjects (NCT05537038, Phase Ia trial in Germany) or BCG vaccinated healthy volunteers, who will be stratified by interferon gamma release assay (IGRA) (NCT05547464, Phase Ib trial in countries in Africa and Asia, including Republic of South Africa, Mozambique and Republic of the Philippines). The fact that BioNTech is retesting the role of mRNA modifications in their *Mtb* candidates is also intriguing, given that m1Ψ-modified mRNA was chosen over unmodified mRNA and self-amplifying mRNA in the development of their COVID-19 mRNA vaccine[78]. The main distinction in their formulations is in their delivery systems. It should be noted that the unmodified mRNA is complexed in spleen-targeting lipoplexes for intravenous administration, similar to BioNTech's ongoing cancer clinical trials (NCT04526899, NCT04534205, NCT05557591, NCT03815058, NCT05968326). By contrast, the m1Ψ-modified mRNA is formulated in LNPs containing ionizable lipids and is delivered intramuscularly. Separately, the replacement of uridines is critical in immune recognition of the mRNA. The replacement of normal uridines in in vitro transcribed (IVT) mRNA with naturally occurring modified uridines, such as m1Ψ, enables mRNA to evade intracellular RNA sensors. This prevents type I IFN-mediated antiviral immune responses, resulting in enhanced translation capabilities and improved tolerability compared to its unmodified counterparts, as extensively summarized elsewhere[79,80]. Notably, Mulroney and colleagues[81] recently reported that the incorporation of m1Ψ in IVT mRNA affects ribosome stalling and thereby can lead to 1+ frameshifting. Consequently, a certain amount of the

https://doi.org/10.1038/s41564-025-02070-z

Table 1 | Overview of bacterial mRNA vaccines that underwent a preclinical evaluation or that are currently under clinical evaluation

Targeted bacterial pathogen	Intra- or extracellular	Type of RNA	Target antigen and RNA construct	Formulation	Administration route	Publication date	Ref.
Under development							
M. tuberculosis	Facultative intracellular	Unmodified	**MPT83**	Naked	IM	2004	108
M. tuberculosis	Facultative intracellular	Unmodified	**Hsp65**	Naked	IN	2010	109
S. pyogenes and *S. agalactiae*	Extracellular	saRNA	**LOdm** or murine Ig κ ss+**BP-2a**	Cationic nano-emulsion	IM	2017	28
S. typhimurium	Facultative intracellular	Nucleoside-modified	Human Ig κ ss+antigen (**Mig14**, **OmpC/F/L**, **SlyB**, **SseB**, **CpoB** or **T1855**)	LNP	IM	2018	30
Chlamydia trachomatis	Obligate intracellular	saRNAs	**MOMP**	CAFs with R848, 3M-052 or Poly I:C adjuvants	IM	2019	110
Staphylococcus aureus	Extracellular	Nucleoside-modified	h-tPA ss+**AdsA**+MITD	mRNA-InstantFECT nanocomplex	IM versus SC	2020	111
B. burgdorferi	Extracellular	Nucleoside-modified	**19ISP** (derived from *Ixodes scapularis*)	LNP	ID	2021	112
L. monocytogenes	Facultative intracellular	Nucleoside-modified	**LMON_0149**, **_276**, **_0442**, **_1501**, **_2272**, **_1065** or **LLO_E262K**	α-Galactosylceramide adjuvanted cationic lipoplexes	IV	2022	25
M. tuberculosis/ *Mycobacterium avium*	Facultative intracellular	saRNA	Fusion protein of **exV**, **RpfD**, **PPE60** and **Ag85B**	LION	IM	2023	76,113
Y. pestis	Facultative intracellular	Nucleoside-modified	ss-devoid or human Ig κ chain ss Cp-**caf1** and/or human IgG-Fc domain	LNP	IM	2023	31
P. aeruginosa	Extracellular	Nucleoside-modified	**OprF-I** or h-tPA ss **PcrV**	LNP	IM	2023	67
P. aeruginosa	Extracellular	Nucleoside-modified	h-tPA ss **PcrV**	LNP	IM	2023	29
B. burgdorferi	Extracellular	Nucleoside-modified	**OspA**	LNP	IM	2023	70
Y. pestis	Facultative intracellular	saRNA	**F1** and **V antigen**	LNP	IM	2023	65
Rhodococcus equi	Facultative intracellular	Nucleoside-modified	Equine-specific ss **VapA**	LNP	IM versus IN (nebulized)	2023	114
L. monocytogenes	Facultative intracellular	Nucleoside-modified	**LMON_0149**	LNP	IM	2024	66
B. pertussis	Extracellular	Nucleoside-modified	Mammalian Ig κ ss or bovine prolactin ss or native ss+antigen (**PTX-S1**, **FHA3**, **FIMD2/3**, **PRN**, **DT**, **TT**, **RTX**, **TCFA**, **SPHB1** and/or **BRKA**)	LNP	IM	2024	73
Clostridioides difficile	Obligate anaerobe and spore-forming	Nucleoside-modified	IL-2 ss **TcdA**, **TcdB**, **PPEP1** and **CdeM**	LNP	IM	2024	115
Under clinical evaluation							
B. burgdorferi	Extracellular	Nucleoside-modified	**OspA SR1** or **OspA SR1-7**	LNP	IM	2023	NCT05975099
M. tuberculosis	Facultative intracellular	Unmodified versus nucleoside-modified	Multivalent undisclosed	LNP	IM	2023	NCT05547464 NCT05537038

Target antigens are highlighted in bold. 19ISP, 19 *Ixodes scapularis* salivary proteins; AdsA, adenosine synthase A; Ag85B, antigen85B; BP-2a, pilus 2a backbone protein; BRKA, BrkA autotransporter; CAF, cationic adjuvant formulation; CdeM, exosporium morphogenetic protein CdeM; Cp-caf1, circular permutated F1 capsule antigen; CpoB, cell division coordinator protein; DT, diptheria toxin; exV, exonuclease V protein; FHA3, forkhead-associated domain protein 3; FIMD2/3, outer membrane usher protein FimD2/3; Hsp65, heat shock protein 65; h-tPA, human TPA; ID, intradermal; IgG-Fc, crystallizable fragment of immunoglobulin G; IL-2, interleukin 2; IM, intramuscular; IN, intranasal; IV, intravenous; LION, lipid inorganic nanoparticle; LLO, listeriolysin O; LMON, *L. monocytogenes*; LOdm, double-mutated streptolysin-O; MITD, MHC class I trafficking domain; Mig14, migration inhibitor gene 14; MOMP, major outer membrane protein; MPT83, *Mycobacterium tuberculosis* protein 83; Omp, outer membrane protein; OprF-I, outer membrane porin F; PcrV, *P. aeruginosa* V antigen; PPE60, proline–proline–glutamic acid protein 60; PPEP1, Pro-Pro endopeptidase 1; PRN, pertactin autotransporter; PTS-S1, pertussis toxin S1 subunit; RpfD, resuscitation-promoting factor; RTX, repeats-in-toxin exoprotein; saRNA, self-amplifying RNA; SC, subcutaneous; SPHB1, autotransporter subtilisin-like protease; SlyB, outer membrane lipoprotein SlyB; SR, serotype; ss, secretion signal; SseB, *Salmonella* secreted effector B; TcdA, *N*-acetylglucosaminyltransferase TcdA; TcdB, glucosyltransferase TcdB; TCFA, autotransporter TcfA; VapA, virulence associates protein A; TT, tetanus toxoid.

translation proteins can be altered, which in turn could potentially result in off-target T cell and antibody responses. This highlights an additional complexity in the design of mRNA sequences when m1Ψ is incorporated, not indicating yet what the clinical outcome will be. For this reason, BioNTech's choice of putting forward both RNA platforms for clinical testing probably originates from their observation that they achieved similar protective effects in a *Mtb* challenge model in mice. However, neither vaccine showed superiority to BCG vaccination.

Taken together, comparative clinical studies, like those currently conducted by BioNTech, may provide new directions for the design of mRNA vaccines against intracellular bacteria, which will probably require a multifaceted immune response to provide adequate protection.

Concluding remarks and outlook

Recent efforts by industry and academic groups indicate that bacterial diseases have become a desired target for mRNA vaccine development, potentially offering an alternative to traditional bacterial vaccines and antibiotics. In addition to their proven efficacy for COVID-19, mRNA vaccines are attractive in terms of the flexibility of their design, speed of production and scalability, but we also anticipate some specific challenges that will need to be overcome in the development of mRNA vaccines against bacteria to compete with other vaccine platforms.

First, selecting the right antigens is a challenging task for a bacterial vaccine. Although we envision that recent technological advances in both experimental and computational methods will boost bacterial antigen discovery in the years to come, antigen prioritization for further preclinical validation will remain a critical step. An interesting question is whether the speed and flexibility of mRNA vaccine production could allow more functional screenings of different antigen combinations or fusion constructs in comparison with subunit platforms. It is clear that the antigenicity and immunogenicity of bacterial mRNA vaccines is substantially influenced by the expression, processing and transportation of the encoded antigen in the transfected host. Consequently, several approaches to optimize antigen expression and/or presentation of bacterial proteins in mammalian cells, such as the use of trafficking and secretion signals and bioinformatic tools to predict and overcome host glycosylation, are under evaluation. However, the results obtained so far suggest that their impact on protein stability, intracellular trafficking and antigen presentation is rather unpredictable and may require proper validation for each specific antigen, thereby further hindering antigen screening. Moreover, predicting an optimal mRNA construct design is further complicated by the fact that for many bacterial diseases, the immune correlates of protection are largely unknown. In their recent review, Rappuoli and colleagues[82] emphasize that advances in systems biology, which integrate data from multiple 'omics technologies, offer unique prospects for the in-depth characterization of protective immune responses. Combined with the ability to rapidly generate and produce various mRNA constructs, this approach can be utilized to establish a protective 'target immune profile' for specific bacteria. In turn, this could help shape future mRNA vaccine designs against bacterial pathogens.

A key aspect to consider might be the obligate or facultative intracellular lifestyle of certain bacteria, including important human pathogens such as *Mycobacteria*, *Shigella* or *Salmonella* species with rising AMR[9]. Once intracellular, these bacteria can adopt a vacuolar or cytosolic lifestyle[83]; however, how this affects the downstream induction of effective immune responses and the implications of this for mRNA vaccine design are not yet known. In the case of virulence effector proteins, which are often secreted or injected by these bacteria into the host cell cytosol, further research should elucidate how such virulence factors can be encoded in mRNA vaccines to elicit the most protective response. For *Mtb*, recent immunopeptidomics and proteome-wide peptide screens revealed substrates of the type VII secretion systems as major antigens (for example, ESAT6)[84,85]. However, it remains unclear whether encoding such effector antigens in mRNA vaccines should mimic cytosolic delivery (without SP) or rather result in endocytic targeting (with SP and/or sorting motif).

A key advantage of mRNA vaccines compared to subunit vaccine platforms is that mRNA vaccine design enables different bacterial antigens to be encoded concurrently. Both mRNA vaccines that are currently in clinical trials against *Mtb* and *B. burgdorferi* comprise multivalent formulations to include multiple antigens or serotypes (Table 1). Current data do not always clarify whether fusion constructs are preferred and how immunodominance, the phenomenon where certain epitopes elicit a stronger immune response than others, might impact these multivalent vaccination strategies. One additional challenge is that this might require the use of higher mRNA vaccine doses to accomplish sufficient immunogenicity, compared to the current COVID-19 mRNA vaccines encoding a single antigen. Wolf et al.[73] acknowledged that dosing also complicates proper comparisons between mRNA vaccines and other vaccine platforms as it is often difficult to define exactly the amount of protein that is produced after mRNA vaccination. To this end, it is uncertain whether mRNA vaccines will be beneficial compared to vaccines currently considered gold standards. Based on BioNTech's patent information, no superiority to BCG was seen with their two mRNA formulations in preclinical studies. Additionally, any protection that may have been conferred by application of the mRNA booster following BCG prime vaccination was not reported[77].

As mRNA vaccines against bacteria are in their infancy, it remains debatable whether the current generation of mRNA–LNP vaccines will be sufficiently capable of inducing effective and durable immune protection against bacteria. The COVID-19 mRNA vaccines were characterized by high titres of neutralizing antibodies and superior vaccine efficacy in the early period of evaluation, but several studies indicated that humoral immunity and protection waned rapidly over six months. However, it was suggested that mRNA vaccine-induced T cell responses are more long-lived and may potentially last for years[86,87]. High-risk populations, such as elderly and immunocompromised individuals, typically exhibited weaker responses to primary COVID-19 mRNA vaccination and needed booster shots to achieve adequate protection[88,89]. More recently, a single dose of Moderna's mRNA vaccine candidate (mRNA-1345) against respiratory syncytial virus (RSV) was found to be safe and effective against RSV-associated lower respiratory tract disease and RSV-associated acute respiratory disease among a study population of those who were 60 years and older, including frail adults, albeit persons with certain immunocompromising conditions were not included in this trial[90]. Because these vaccines are developed for seasonal viral outbreaks, durability may be a more critical consideration when developing vaccines against bacterial pathogens.

More research is also needed to fully understand the effects of mRNA vaccines on innate immune cells. Attenuated vaccines such as BCG or *Salmonella* Typhi strain TY21a are well known for inducing an innate form of immunological memory, termed trained innate immunity[91,92]. This has been attributed to the reprogramming of epigenetic and transcriptional processes of myeloid progenitors in the bone marrow, resulting in an elevated innate immune response towards homologous or even heterologous pathogens[93]. Studies so far have shown that although mRNA vaccination resulted in a transcriptional upregulation of innate and antiviral gene signatures in circulating monocytes[94], there is no evidence of long-lasting trained immunity, or beneficial non-specific effects induced by the COVID-19 mRNA vaccines[95–98]. Notably, Hellgren and colleagues[99] recently suggested that pre-existing adaptive immunity formed by primary vaccination or infection may better explain the augmented innate immune response observed after mRNA vaccination, rather than there being a trained innate immune effect. Although mRNA vaccines possess an inherent adjuvant activity generating robust adaptive immune activation[79], we believe that the incorporation of immune adjuvants in mRNA vaccines could be considered to strengthen or broaden the innate immune

activation[25,100]. One example is the use of LNPs to formulate mRNA vaccines, which provides opportunities to incorporate bacterial glycolipid antigens, as we previously demonstrated[66,101,102].

Another major challenge for the future of antibacterial mRNA vaccines is vaccine inequity. AMR has the highest burden in countries in a low-resource setting. These countries have suffered disproportionately from vaccine unavailability due to socioeconomic disparities[4]. For example, modelling estimated that during the COVID-19 pandemic, vaccine inequity was estimated to account for more than 50% of deaths in LMICs[103]. mRNA vaccines can be produced quickly at large scale, but they were not distributed equally globally during the COVID-19 pandemic. Among the many reasons for this, mRNA vaccines had the limitations of (ultra-)cold chain requirements and were relatively expensive compared to other vaccine platforms. However, many efforts have been made to produce thermostable formulations to avoid cold chain needs, such as by optimization of mRNA structure[104] and the development of lyophilized products[105,106]. In addition, several initiatives have been launched for local mRNA vaccine production hubs and training of local personnel in LMICs, which may improve access to mRNA vaccine technology in the future[107]. With rising levels of AMR, the importance of bacterial vaccines will also increase in high-income countries. Depending on the remaining treatment options, mRNA vaccines against multi-resistant hospital-acquired infections might become in scope, protecting immunocompromised or surgery patients for the duration of their hospitalization, for instance against ESKAPE pathogens (a group of six bacterial species known for high virulence and antibiotic resistance). mRNA vaccine technology offers the option to customize (for example, to specific strains circulating in the hospital) or even personalize such vaccines, similar to neo-antigen encoding cancer vaccines.

Taken together, the flexibility and rapid development of mRNA vaccines, along with ongoing efforts towards more equitable and affordable production and distribution, suggest bright prospects for mRNA vaccines, especially in the context of preparedness to tackle emerging infectious diseases, including those caused by bacterial pathogens. Although the first mRNA vaccines against bacterial pathogens are under clinical evaluation, many more are in early or late-stage preclinical development. These studies will help to fill remaining gaps in our knowledge on mRNA vaccine design, educating on the optimal ways to enhance the expression and presentation of the encoded bacterial antigens. Moreover, we expect that continuing research on the adaptive and innate immune response induced by mRNA vaccines, preferably in targeted populations, will teach us more about the differences between mRNA platforms, and how they could potentially compete with other advancing vaccine technologies.

References

1. Patel, J. et al. Measuring the global response to antimicrobial resistance, 2020–21: a systematic governance analysis of 114 countries. *Lancet Infect. Dis.* **23**, 706–718 (2023).
2. Urgent call for better use of existing vaccines and development of new vaccines to tackle AMR. *World Health Organization* https://www.who.int/news/item/12-07-2022-urgent-call-for-better-use-of-existing-vaccines-and-development-of-new-vaccines-to-tackle-amr (2022).
3. CDC. *Antibiotic Resistance Threats in the United States 2019* (US Department of Health and Human Services, 2019).
4. Murray, C. J. et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629–655 (2022).
5. *Bacterial Vaccines in Clinical and Preclinical Development 2021* (World Health Organization, 2021).
6. Bloom, D. E., Black, S., Salisbury, D. & Rappuoli, R. Antimicrobial resistance and the role of vaccines. *Proc. Natl Acad. Sci. USA* **115**, 12868–12871 (2018).
7. Frost, I. et al. The role of bacterial vaccines in the fight against antimicrobial resistance: an analysis of the preclinical and clinical development pipeline. *Lancet Microbe* **4**, e113–e125 (2023).
8. Moore, M. R. et al. Effectiveness of 13-valent pneumococcal conjugate vaccine for prevention of invasive pneumococcal disease in children in the USA: a matched case-control study. *Lancet Respir. Med.* **4**, 399–406 (2016).
9. Micoli, F., Bagnoli, F., Rappuoli, R. & Serruto, D. The role of vaccines in combatting antimicrobial resistance. *Nat. Rev. Microbiol.* **19**, 287–302 (2021).
10. Baker, S. J., Payne, D. J., Rappuoli, R. & De Gregorio, E. Technologies to address antimicrobial resistance. *Proc. Natl Acad. Sci. USA* **115**, 12887–12895 (2018).
11. Adepoju, P. New TB vaccine on trial. *Nat. Africa* 10.1038/d44148-024-00101-1 (2024).
12. Olivier, V. D. M. et al. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. *N. Engl. J. Med.* **379**, 1621–1634 (2018).
13. Chen, W. Will the mRNA vaccine platform be the panacea for the development of vaccines against antimicrobial resistant (AMR) pathogens? *Expert Rev. Vaccines* **21**, 155–157 (2022).
14. Mayer, R. L. & Impens, F. Immunopeptidomics for next-generation bacterial vaccine development. *Trends Microbiol.* **29**, 1034–1045 (2021).
15. van der Woude, M. W. & Bäumler, A. J. Phase and antigenic variation in bacteria. *Clin. Microbiol. Rev.* **17**, 581–611 (2004).
16. Tan, A., Atack, J. M., Jennings, M. P. & Seib, K. L. The capricious nature of bacterial pathogens: phasevarions and vaccine development. *Front. Immunol.* **7**, 586 (2016).
17. Seib, K. L., Srikhanta, Y. N., Atack, J. M. & Jennings, M. P. Epigenetic regulation of virulence and immunoevasion by phase-variable restriction-modification systems in bacterial pathogens. *Annu. Rev. Microbiol.* **74**, 655–671 (2020).
18. Trzilova, D., Anjuwon-Foster, B. R., Torres Rivera, D. & Tamayo, R. Rho factor mediates flagellum and toxin phase variation and impacts virulence in *Clostridioides difficile*. *PLoS Pathog.* **16**, e1008708 (2020).
19. Georgieva, M., Kagedan, L., Ying-Jie, L., Thompson, C. M. & Lipsitch, M. Antigenic variation in *Streptococcus pneumoniae* PspC promotes immune escape in the presence of variant-specific immunity. *mBio* **9**, e00264-18 (2018).
20. Vink, C., Rudenko, G. & Seifert, H. S. Microbial antigenic variation mediated by homologous DNA recombination. *FEMS Microbiol. Rev.* **36**, 917–948 (2012).
21. Ernst, J. D. in *Strain Variation in the Mycobacterium Tuberculosis Complex: Its Role in Biology, Epidemiology and Control* (ed. Gagneux, S.) 171–190 (Springer, 2017).
22. Rappuoli, R. Glycoconjugate vaccines: principles and mechanisms. *Sci. Transl. Med.* **10**, eaat4615 (2018).
23. Rappuoli, R. Reverse vaccinology. *Curr. Opin. Microbiol.* **3**, 445–450 (2000).
24. Sette, A. & Rappuoli, R. Reverse vaccinology: developing vaccines in the era of genomics. *Immunity* **33**, 530–541 (2010).
25. Mayer, R. L. et al. Immunopeptidomics-based design of mRNA vaccine formulations against *Listeria monocytogenes*. *Nat. Commun.* **13**, 6075 (2022).
26. Pardi, N. & Krammer, F. mRNA vaccines for infectious diseases—advances, challenges and opportunities. *Nat. Rev. Drug Discov.* **23**, 838–861 (2024).
27. Nagata, T., Uchijima, M., Yoshida, A., Kawashima, M. & Koide, Y. Codon optimization effect on translational efficiency of DNA vaccine in mammalian cells: analysis of plasmid DNA encoding a CTL epitope derived from microorganisms. *Biochem. Biophys. Res. Commun.* **261**, 445–451 (1999).

28. Maruggi, G. et al. Immunogenicity and protective efficacy induced by self-amplifying mRNA vaccines encoding bacterial antigens. *Vaccine* **35**, 361–368 (2017).
29. Kawaguchi, K. et al. mRNA vaccine induces protective immunity against the type III secretory virulence of *Pseudomonas aeruginosa*. Preprint at https://www.biorxiv.org/content/10.1101/2023.06.09.544431v1.full.pdf (2023).
30. Messou, M.-A. A. *Harnessing a mRNA Platform Against Salmonellae* (Harvard Univ. Press, 2018).
31. Kon, E. et al. A single-dose F1-based mRNA-LNP vaccine provides protection against the lethal plague bacterium. *Sci. Adv.* **9**, eadg1036 (2023).
32. Ozdilek, A. & Avci, F. Y. Glycosylation as a key parameter in the design of nucleic acid vaccines. *Curr. Opin. Struct. Biol.* **73**, 102348 (2022).
33. Gupta, R. & Brunak, S. Prediction of glycosylation across the human proteome and the correlation to protein function. In *Proc. Pacific Symposium on Biocomputing 2002* (eds Altman, R. B. et al.) 310–322 (World Scientific, 2002).
34. Ozdilek, A., Paschall, A. V., Dookwah, M., Tiemeyer, M. & Avci, F. Y. Host protein glycosylation in nucleic acid vaccines as a potential hurdle in vaccine design for nonviral pathogens. *Proc. Natl Acad. Sci. USA* **117**, 1280–1282 (2020).
35. Tameris, M. D. et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* **381**, 1021–1028 (2013).
36. Ndiaye, B. P. et al. Safety, immunogenicity, and efficacy of the candidate tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, placebo-controlled, phase 2 trial. *Lancet Respir. Med.* **3**, 190–200 (2015).
37. Tameris, M. et al. A double-blind, randomised, placebo-controlled, dose-finding trial of the novel tuberculosis vaccine AERAS-402, an adenovirus-vectored fusion protein, in healthy, BCG-vaccinated infants. *Vaccine* **33**, 2944–2954 (2015).
38. Muir, E. et al. Trafficking and processing of bacterial proteins by mammalian cells: insights from chondroitinase ABC. *PLoS ONE* **12**, e0186759 (2017).
39. Rabouille, C. Pathways of unconventional protein secretion. *Trends Cell Biol.* **27**, 230–240 (2017).
40. Lin, P. L. & Flynn, J. A. L. CD8 T cells and *Mycobacterium tuberculosis* infection. *Semin. Immunopathol.* **37**, 239–249 (2015).
41. Grant, N. L. et al. *Mycobacterium tuberculosis*-specific CD4 T cells expressing transcription factors T-Bet or RORγT associate with bacterial control in granulomas. *mBio* **14**, e00477-23 (2023).
42. Tian, L. et al. CTLs: killers of intracellular bacteria. *Front. Cell. Infect. Microbiol.* **12**, 967679 (2022).
43. Osterloh, A. Vaccination against bacterial infections: challenges, progress and new approaches with a focus on intracellular bacteria. *Vaccines* **10**, 751 (2022).
44. Shanmuganathan, G. et al. Role of interferons in *Mycobacterium tuberculosis* infection. *Clin. Pract.* **12**, 788–796 (2022).
45. Baker, H. A. & Bernardini, J. P. It's not just a phase; ubiquitination in cytosolic protein quality control. *Biochem. Soc. Trans.* **49**, 365–377 (2021).
46. Weigele, B. A., Orchard, R. C., Jimenez, A., Cox, G. W. & Alto, N. M. A systematic exploration of the interactions between bacterial effector proteins and host cell membranes. *Nat. Commun.* **8**, 532 (2017).
47. Dersh, D., Hollý, J. & Yewdell, J. W. A few good peptides: MHC class I-based cancer immunosurveillance and immunoevasion. *Nat. Rev. Immunol.* **21**, 116–128 (2021).
48. Ruiz Cuevas, M. V. et al. Most non-canonical proteins uniquely populate the proteome or immunopeptidome. *Cell Rep.* **34**, 108815 (2021).
49. Yewdell, J. W. Hide and seek in the peptidome. *Science* **301**, 1334–1335 (2003).
50. Pamer, E. G. Immune responses to *Listeria monocytogenes*. *Nat. Rev. Immunol.* **4**, 812–823 (2004).
51. Delogu, G., Howard, A., Collins, F. M. & Morris, S. L. DNA vaccination against tuberculosis: expression of a ubiquitin-conjugated tuberculosis protein enhances antimycobacterial immunity. *Infect. Immun.* **68**, 3097–3102 (2000).
52. Pishesha, N., Harmand, T. J. & Ploegh, H. L. A guide to antigen processing and presentation. *Nat. Rev. Immunol.* **22**, 751–764 (2022).
53. Lizée, G., Basha, G. & Jefferies, W. A. Tails of wonder: endocytic-sorting motifs key for exogenous antigen presentation. *Trends Immunol.* **26**, 141–149 (2005).
54. Lotteau, V. et al. Intracellular transport of class II MHC molecules directed by invariant chain. *Nature* **348**, 600–605 (1990).
55. Rowell, J. F. et al. Lysosome-associated membrane protein-1-mediated targeting of the HIV-1 envelope protein to an endosomal/lysosomal compartment enhances its presentation to MHC class II-restricted T cells. *J. Immunol.* **155**, 1818–1828 (1995).
56. Wu, T. C. et al. Engineering an intracellular pathway for major histocompatibility complex class II presentation of antigens. *Proc. Natl Acad. Sci. USA* **92**, 11671–11675 (1995).
57. Kreiter, S. et al. Increased antigen presentation efficiency by coupling antigens to MHC class I trafficking signals. *J. Immunol.* **180**, 309–318 (2008).
58. Lizée, G. et al. Control of dendritic cell cross-presentation by the major histocompatibility complex class I cytoplasmic domain. *Nat. Immunol.* **4**, 1065–1073 (2003).
59. Diken, M., Kranz, L. M., Kreiter, S. & Sahin, U. mRNA: a versatile molecule for cancer vaccines. *Curr. Issues Mol. Biol.* **22**, 113–128 (2017).
60. Sahin, U. et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* **547**, 222–226 (2017).
61. Rojas, L. A. et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature* **618**, 144–150 (2023).
62. You, Z. et al. Induction of vigorous helper and cytotoxic T cell as well as B cell responses by dendritic cells expressing a modified antigen targeting receptor-mediated internalization pathway1. *J. Immunol.* **165**, 4581–4591 (2000).
63. Noy-Porat, T. et al. Acetylcholinesterase-Fc fusion protein (AChE-Fc): a novel potential organophosphate bioscavenger with extended plasma half-life. *Bioconjug. Chem.* **26**, 1753–1758 (2015).
64. Elia, U. et al. Design of SARS-CoV-2 hFc-conjugated receptor-binding domain mRNA vaccine delivered via lipid nanoparticles. *ACS Nano* **15**, 9627–9637 (2021).
65. Shattock, R. J. et al. A self-amplifying RNA vaccine provides protection in a murine model of bubonic plague. *Front. Microbiol.* **14**, 1247041 (2023).
66. Meulewaeter, S. et al. Alpha-galactosylceramide improves the potency of mRNA LNP vaccines against cancer and intracellular bacteria. *J. Controlled Release* **370**, 379–391 (2024).
67. Wang, X. et al. Strong immune responses and protection of PcrV and OprF-I mRNA vaccine candidates against *Pseudomonas aeruginosa*. *NPJ Vaccines* **8**, 76 (2023).
68. Mead, P. Epidemiology of Lyme disease. *Infect. Dis. Clin. North Am.* **36**, 495–521 (2022).
69. Moderna announces clinical and program updates at 4th vaccines day. *Moderna* https://www.accesswire.com/748485/Moderna-Announces-Clinical-and-Program-Updates-at-4th-Vaccines-Day (2023).
70. Pine, M. et al. Development of an mRNA-lipid nanoparticle vaccine against Lyme disease. *Mol. Ther.* **31**, 2702–2714 (2023).

71. Gross, D. M. et al. Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. *Science* **281**, 703–706 (1998).
72. Bobe, J. R. et al. Recent progress in Lyme disease and remaining challenges. *Front. Med.* **8**, 666554 (2021).
73. Wolf, M. A. et al. Multivalent mRNA-DTP vaccines are immunogenic and provide protection from *Bordetella pertussis* challenge in mice. *NPJ Vaccines* **9**, 103 (2024).
74. *Global Tuberculosis Report 2023* (World Health Organization, 2023).
75. Mangtani, P. et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin. Infect. Dis.* **58**, 470–480 (2014).
76. Larsen, S. E. et al. An RNA-based vaccine platform for use against *Mycobacterium tuberculosis*. *Vaccines* **11**, 130 (2023).
77. Neha, A., Albrecht, S. S., Mustafa, D., Annette, V. & Ugur, S (2024). RNA for preventing or treating tuberculosis. WIPO patent WO2024028445A1 (2023).
78. Polack, F. P. et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
79. Verbeke, R., Hogan, M. J., Loré, K. & Pardi, N. Innate immune mechanisms of mRNA vaccines. *Immunity* **55**, 1993–2005 (2022).
80. Morais, P., Adachi, H. & Yu, Y.-T. The critical contribution of pseudouridine to mRNA COVID-19 vaccines. *Front. Cell. Dev. Biol.* **9**, 789427 (2021).
81. Mulroney, T. E. et al. N^1-methylpseudouridylation of mRNA causes +1 ribosomal frameshifting. *Nature* **625**, 189–194 (2024).
82. Rappuoli, R., Alter, G. & Pulendran, B. Transforming vaccinology. *Cell* **187**, 5171–5194 (2024).
83. Petit, T. J. P. & Lebreton, A. Adaptations of intracellular bacteria to vacuolar or cytosolic niches. *Trends Microbiol.* **30**, 736–748 (2022).
84. Leddy, O., White, F. M. & Bryson, B. D. Immunopeptidomics reveals determinants of *Mycobacterium tuberculosis* antigen presentation on MHC class I. *eLife* **12**, e84070 (2023).
85. Panda, S. et al. Identification of differentially recognized T cell epitopes in the spectrum of tuberculosis infection. *Nat. Commun.* **15**, 765 (2024).
86. Sette, A. & Crotty, S. Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines. *Immunol. Rev.* **310**, 27–46 (2022).
87. Arunachalam, P. S. et al. Durability of immune responses to mRNA booster vaccination against COVID-19. *J. Clin. Invest.* **133**, e167955 (2023).
88. Fedele, G., Palmieri, A. & Onder, G. The immune response to SARS-CoV-2 vaccination in older people. *Lancet Healthy Longev.* **4**, e177–e178 (2023).
89. Kamar, N. et al. Three doses of an mRNA COVID-19 vaccine in solid-organ transplant recipients. *N. Engl. J. Med.* **385**, 661–662 (2021).
90. Walsh, E. E. et al. Efficacy and safety of a bivalent RSV prefusion F vaccine in older adults. *N. Engl. J. Med.* **388**, 1465–1477 (2023).
91. Pennington, S. H. et al. Nonspecific effects of oral vaccination with live-attenuated *Salmonella* Typhi strain Ty21a. *Sci. Adv.* **5**, eaau6849 (2024).
92. Kleinnijenhuis, J. et al. Bacille Calmette-Guérin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc. Natl Acad. Sci. USA* **109**, 17537–17542 (2012).
93. Ziogas, A., Bruno, M., van der Meel, R., Mulder, W. J. M. & Netea, M. G. Trained immunity: target for prophylaxis and therapy. *Cell Host Microbe* **31**, 1776–1791 (2023).
94. Arunachalam, P. S. et al. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature* **596**, 410–416 (2021).
95. Yamaguchi, Y. et al. Consecutive BNT162b2 mRNA vaccination induces short-term epigenetic memory in innate immune cells. *JCI Insight* **7**, e163347 (2022).
96. Benn, C. S., Schaltz-Buchholzer, F., Nielsen, S., Netea, M. G. & Aaby, P. Randomized clinical trials of COVID-19 vaccines: do adenovirus-vector vaccines have beneficial non-specific effects? *iScience* **26**, 106733 (2023).
97. Föhse, K. et al. The impact of BNT162b2 mRNA vaccine on adaptive and innate immune responses. *Clin. Immunol.* **255**, 109762 (2023).
98. Stevens, N. E. et al. No evidence of durable trained immunity after two doses of adenovirus-vectored or mRNA COVID-19 vaccines. *J. Clin. Invest.* **133**, e171742 (2023).
99. Hellgren, F. et al. Modulation of innate immune response to mRNA vaccination after SARS-CoV-2 infection or sequential vaccination in humans. *JCI Insight* **9**, e175401 (2024).
100. Han, X. et al. Adjuvant lipidoid-substituted lipid nanoparticles augment the immunogenicity of SARS-CoV-2 mRNA vaccines. *Nat. Nanotechnol.* **18**, 1105–1114 (2023).
101. Hayashizaki, K., Kamii, Y. & Kinjo, Y. Glycolipid antigen recognition by invariant natural killer T cells and its role in homeostasis and antimicrobial responses. *Front. Immunol.* **15**, 1402412 (2024).
102. Pifferi, C., Fuentes, R. & Fernández-Tejada, A. Natural and synthetic carbohydrate-based vaccine adjuvants and their mechanisms of action. *Nat. Rev. Chem.* **5**, 197–216 (2021).
103. Gozzi, N. et al. Estimating the impact of COVID-19 vaccine inequities: a modeling study. *Nat. Commun.* **14**, 3272 (2023).
104. Leppek, K. et al. Combinatorial optimization of mRNA structure, stability, and translation for RNA-based therapeutics. *Nat. Commun.* **13**, 1536 (2022).
105. Meulewaeter, S. et al. Continuous freeze-drying of messenger RNA lipid nanoparticles enables storage at higher temperatures. *J. Control. Release* **357**, 149–160 (2023).
106. Muramatsu, H. et al. Lyophilization provides long-term stability for a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine. *Mol. Ther.* **30**, 1941–1951 (2022).
107. Saied, A. A. Building Africa's first mRNA vaccine facility. *Lancet* **402**, 287–288 (2023).
108. Xue, T. et al. RNA encoding the MPT83 antigen induces protective immune responses against *Mycobacterium tuberculosis* infection. *Infect. Immun.* **72**, 6324–6329 (2004).
109. Lorenzi, J. C. C. et al. Intranasal vaccination with messenger RNA as a new approach in gene therapy: use against tuberculosis. *BMC Biotechnol.* **10**, 77 (2010).
110. Blakney, A. K. et al. Effects of cationic adjuvant formulation particle type, fluidity and immunomodulators on delivery and immunogenicity of saRNA. *J. Control. Release* **304**, 65–74 (2019).
111. Arya, S., Lin, Q., Zhou, N., Gao, X. & Huang, J. D. Strong immune responses induced by direct local injections of modified mRNA-lipid nanocomplexes. *Mol. Ther. Nucleic Acids* **19**, 1098–1109 (2020).
112. Sajid, A. et al. mRNA vaccination induces tick resistance and prevents transmission of the Lyme disease agent. *Sci. Transl. Med.* **13**, 9827 (2021).
113. Rais, M. et al. Immunogenicity and protection against *Mycobacterium avium* with a heterologous RNA prime and protein boost vaccine regimen. *Tuberculosis* **138**, 102302 (2023).
114. Legere, R. M. et al. Intramuscular but not nebulized administration of a mRNA vaccine against Rhodococcus equi stimulated humoral immune responses in neonatal foals. *Am. J. Vet. Res.* **85**, ajvr.23.09.0208 (2023).
115. Alameh, M.-G. et al. A multivalent mRNA-LNP vaccine protects against *Clostridioides difficile* infection. *Science* **386**, 69–75 (2024).

116. Leddy, O., White, F. & Bryson, B. Leveraging immunopeptidomics to study and combat infectious disease. *mSystems* **6**, 10–21 (2021).
117. Leddy, O. et al. Immunopeptidomics informs discovery and delivery of *Mycobacterium tuberculosis* MHC-II antigens for vaccine design. Preprint at https://www.biorxiv.org/content/10.1101/2024.10.02.616386v1.full (2024).
118. Leddy, O., Yuki, Y., Carrington, M., Bryson, B. D. & White, F. M. PathMHC: a workflow to selectively target pathogen-derived MHC peptides in discovery immunopeptidomics experiments for vaccine target identification. Preprint at https://www.biorxiv.org/content/10.1101/2024.09.11.612454v1.full.pdf (2024).
119. Gul, A. et al. Immunopeptidomics mapping of *Listeria monocytogenes* T cell epitopes in mice. *Mol. Cell. Proteom.* **23**, 100829 (2024).
120. Rodríguez-Ortega, M. J. et al. Characterization and identification of vaccine candidate proteins through analysis of the group A *Streptococcus* surface proteome. *Nat. Biotechnol.* **24**, 191–197 (2006).
121. Montemari, A. L. et al. A shaving proteomic approach to unveil surface proteins modulation of multi-drug resistant *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients. *Front. Med.* **9**, 818669 (2022).
122. Menikou, S. et al. A proteomics-based method for identifying antigens within immune complexes. *PLoS ONE* **15**, e0244157 (2021).
123. Joglekar, A. V. & Li, G. T cell antigen discovery. *Nat. Methods* **18**, 873–880 (2021).
124. Musvosvi, M. et al. T cell receptor repertoires associated with control and disease progression following *Mycobacterium tuberculosis* infection. *Nat. Med.* **29**, 258–269 (2023).
125. Huang, H., Wang, C., Rubelt, F., Scriba, T. J. & Davis, M. M. Analyzing the *Mycobacterium tuberculosis* immune response by T-cell receptor clustering with GLIPH2 and genome-wide antigen screening. *Nat. Biotechnol.* **38**, 1194–1202 (2020).
126. Mora, M., Donati, C., Medini, D., Covacci, A. & Rappuoli, R. Microbial genomes and vaccine design: refinements to the classical reverse vaccinology approach. *Curr. Opin. Microbiol.* **9**, 532–536 (2006).
127. He, Y., Xiang, Z. & Mobley, H. L. T. Vaxign: The first web-based vaccine design program for reverse vaccinology and applications for vaccine development. *J. Biomed. Biotechnol.* **2010**, 297505 (2010).
128. Vivona, S., Bernante, F. & Filippini, F. NERVE: New Enhanced Reverse Vaccinology Environment. *BMC Biotechnol.* **6**, 35 (2006).
129. Rawal, K. et al. Vaxi-DL: a web-based deep learning server to identify potential vaccine candidates. *Comput. Biol. Med.* **145**, 105401 (2022).
130. Ong, E. et al. Vaxign-ML: supervised machine learning reverse vaccinology model for improved prediction of bacterial protective antigens. *Bioinformatics* **36**, 3185–3191 (2020).
131. Peters, B., Nielsen, M. & Sette, A. T cell epitope predictions. *Annu. Rev. Immunol.* **38**, 123–145 (2020).
132. Sanchez-Trincado, J. L., Gomez-Perosanz, M. & Reche, P. A. Fundamentals and methods for T- and B-cell epitope prediction. *J. Immunol. Res.* **2017**, 2680160 (2017).
133. De Groot, A. S. et al. Better epitope discovery, precision immune engineering, and accelerated vaccine design using Immunoinformatics tools. *Front. Immunol.* **11**, 442 (2020).
134. Lindestam Arlehamn, C. S. et al. Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and largely contained in a CXCR3+CCR6+Th1 subset. *PLoS Pathog.* **9**, e1003130 (2013).
135. da Silva Antunes, R. et al. T cell reactivity to *Bordetella pertussis* is highly diverse regardless of childhood vaccination. *Cell Host Microbe* **31**, 1404–1416.e4 (2023).
136. Mubarak, A. S., Ameen, Z. S., Hassan, A. S. & Ozsahin, D. U. Enhancing tuberculosis vaccine development: a deconvolution neural network approach for multi-epitope prediction. *Sci. Rep.* **14**, 10375 (2024).
137. Shi, J. et al. In silico designed novel multi-epitope mRNA vaccines against *Brucella* by targeting extracellular protein BtuB and LptD. *Sci. Rep.* **14**, 7278 (2024).
138. Prachar, M. et al. Identification and validation of 174 COVID-19 vaccine candidate epitopes reveals low performance of common epitope prediction tools. *Sci. Rep.* **10**, 20465 (2020).
139. Buckley, P. R. et al. Evaluating performance of existing computational models in predicting $CD8^+$ T cell pathogenic epitopes and cancer neoantigens. *Brief. Bioinform.* **23**, bbac141 (2022).
140. Conev, A., Fasoulis, R., Hall-Swan, S., Ferreira, R. & Kavraki, L. E. HLAEquity: examining biases in pan-allele peptide-HLA binding predictors. *iScience* **27**, 108613 (2024).
141. Gonzalez-Galarza, F. F., Christmas, S., Middleton, D. & Jones, A. R. Allele Frequency Net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Res.* **39**, D913–D919 (2011).
142. Zimmermann, P. The immunological interplay between vaccination and the intestinal microbiota. *NPJ Vaccines* **8**, 24 (2023).

Acknowledgements

This work was supported by a Research Foundation-Flanders (FWO-Vlaanderen) PhD fellowship for strategic basic research to I.A. (1S40923N) and postdoctoral fellowships to R.V. (1275023N), F.T. (12AN524N) and P.W. (12T1722N). D.P. acknowledges support from the European Research Council (ERC adv. grant no. 101055029), The EXPERT project (European Union's Horizon 2020 Research and Innovation Programme under grant no. 825828), ISF grant 2012/20 and the Shmunis Family Foundation. F.I. and I.L. acknowledge support from Ghent University Concerted Research Action grant BOF21/GOA/033 and from European Union's Horizon Europe research and from the Horizon Europe Project BAXERNA 2.0 (101080544). F.I. is further supported by starting grant BOF/STA/202209/011 from Ghent University.

Author Contributions

I.A., R.V., P.W., F.T., F.I. and I.L.: conceptualization, visualization, drafting and review of the manuscript. U.E., S.C.D.S., R.R. and D.P.: review and editing.

Competing interests

D.P. receives licensing fees (to patents on which he was an inventor), has invested in, consults for (or is on scientific advisory boards or boards of directors) or is a founder and hold shares or conducts sponsored research at Tel Aviv University for the following entities: ART Biosciences, BioNTech SE, Earli Inc., Geneditor Biologics Inc. Kernal Biologics, Merck, Newphase Ltd, NeoVac Ltd, RiboX Therapeutics, Roche, SirTLabs Corporation and Teva Pharmaceuticals Inc. R.R. holds shares in the GSK and Novartis group of companies. The remaining authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Francis Impens or Ine Lentacker.

Peer review information *Nature Microbiology* thanks Timothy Keys and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.