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REVIEW

Blades and barriers: Oral vaccines for conquering cancers and warding off infectious diseases

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Abstract Global public health faces substantial challenges from malignant tumors and infectious diseases. Vaccination provides an approach for treating and preventing these diseases. Oral vaccinations are particularly advantageous in disease treatment and prevention due to their non-invasive nature, high patient compliance, convenience, cost-effectiveness, and capacity to stimulate comprehensive and adaptive immune responses. However, the overwhelming majority of oral vaccines remain in experimental development, struggling with clinical and commercial translation due to their suboptimal efficacy. Thus, enhancing scientists' understanding of the interaction between vaccines and gastrointestinal immune system, creating antigen delivery systems suitable for the gut mucosal environment, developing more

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Gastrointestinal immune system

potent antigenic epitopes, and using personalized combination therapies are critical for advancing the next generation of oral vaccines. This article explores the fundamental principles and applications of current oral anti-tumor and anti-infective vaccines and discusses considerations necessary for designing future oral vaccines.

1. Introduction

The gastrointestinal tract (GIT) plays a pivotal role in the immune system, acting as the primary barrier between the human body and the external environment[1]. This crucial barrier is commonly referred to as the intestinal mucosal barrier. Within this region, a diverse array of tissues and cells collaboratively safeguard the host's health, vigilantly defending against the invasion of harmful pathogens and toxic substances. Given that the GIT serves as the body's largest immune organ, encompassing roughly 70% of its immune cells[2], there is a growing interest in harnessing its potential for developing oral vaccines[3].

Over the past few centuries, researchers have explored the possibility of delivering vaccines *via* the oral route. In the late 18th century to early 19th century, British physician Edward Jenner achieved a significant milestone in vaccine history by successfully developing the smallpox vaccine[4]. Although this vaccine was not administered orally, it served as the cornerstone for subsequent exploitation of oral vaccines. During the 20th century, scientists shifted their attention towards the application of oral vaccination to combat diseases. However, this journey was fraught with diverse obstacles, primarily due to technological constraints and a lack of immunological knowledge. A turning point was reached when Albert Sabin pioneered the development of an oral polio vaccine, which received approval in 1961. This breakthrough set the stage for the evolution of oral vaccine technologies[5-7]. As research advanced into the latter part of the 20th century and the dawn of the 21st century, scientists deepened their understanding of the intestinal immune system and the design principles of oral vaccines. The approval of oral rotavirus vaccine in 1998 marked a more mature technical platform for oral vaccine development[8]. In recent years, the rapid development of related disciplines has propelled oral vaccine research forward, leading to notable successes in vaccine applications[2,9].

Oral vaccines can be categorized into two primary types based on their intended purposes. The first type includes oral vaccines designed to prevent diseases caused by pathogens (*e.g.*, fungi, bacteria, and viruses). These vaccines are formulated to introduce pathogen antigens and adjuvants into the human body to activate innate and adaptive immune responses. Currently, the approved oral vaccines for preventive purpose primarily target pathogen of contagion, such as poliovirus, adenovirus, and *Bacillus anthracis*. Additionally, a plethora of oral vaccines, including those against pathogens like hepatitis B virus, influenza virus, respiratory syncytial virus, and SARS-CoV-2, are currently in clinical trials (Table 1). The second category is therapeutic oral vaccines, designed to treat malignant diseases, especially targeting tumors with poor prognoses. These vaccines aim to introduce the tumor-associated antigen (TAA) and adjuvant into the body to leverage the intestinal immune system, initiating a strong immune response that targets and eliminates tumor cells. Nowadays, the majority of oral tumor vaccines remain in the experimental stage rather than in clinical trials, primarily targeting malignancies, such as colorectal cancer (CRC)[10], breast cancer[11], melanoma[12], and fibrosarcoma[13]. Furthermore, oral vaccines can be comprehensively compared based on their vaccination objectives, dosing frequencies, immune response types, and target populations, summarized in Table 2.

Recently, oral vaccines have attracted burgeoning interest. The expanding knowledge of mucosal immunology, coupled with the swift advancements of genetic engineering, immunology, and pharmaceutics, has accelerated the development of oral vaccines. This review delineates the contemporary advancements in oral vaccines for oncological and infectious diseases from both therapeutic and prophylactic standpoints and looks forward to these research challenges and future directions.

2. Intestinal immune microenvironment

The GIT is vital in the mucosal immune system, as it is one of the body's major immunological organs. Hence, identifying the role and function of pertinent cells and lymphoid tissues in the gastrointestinal environment is crucial for exploiting oral vaccines (Fig. 1).

2.1. Intestinal mucosal immune system

2.1.1. Enterocytes

Enterocytes, known as intestinal absorptive cells, are simple columnar epithelial cells that line the inner surface of the small and large intestines. They have roughly 3000 microvilli on their apical surface[14]. These microvilli significantly increase the surface area of enterocytes, which are crucial for nutrient absorption from the intestinal lumen[15]. Tight junctions act as linking units, connecting enterocytes with other intestinal cells in a wall-like arrangement and controlling substance permeation. Their highly differentiated structures support nutrient absorption and mucus secretion. The intestinal epithelial layer is an effective barrier for the intestinal mucosa, which prevents harmful substances, pathogens, and toxins from entering the circulatory system[16]. At the same time, they also play a role in recognizing and responding to antigens within the gut[17,18].

2.1.2. Goblet cells

Goblet cells, characterized by their cup-shaped and oval appearance, are situated within the epithelial cell layer beneath the intestinal mucosa. They typically feature smooth cell surfaces and abundant intracellular mucin granules. One of their essential functions is the production and secretion of mucus, which lubricates the intestinal surface, reduces friction within the GIT, and prevents the invasion of harmful bacteria and other microorganisms[16,19]. In

Table 1 Oral vaccines available on the market or currently undergoing clinical trials.

Vaccine name	Number	Pathogen	Indication	Status
Reassortant rotavirus vaccine, live, oral, pentavalent (vero cell)	SJ20180002	Rotavirus	Used to prevent acute gastroenteritis	NMPA approved
Reassortant rotavirus vaccine, live, oral, trivalent (vero cell)	S20230022	Rotavirus	Used to prevent acute gastroenteritis	NMPA approved
Rotavirus vaccine, live, oral	S20010002	Rotavirus	Used to prevent acute gastroenteritis	NMPA approved
Human rotavirus, live attenuated	EMEA/H/C/ 000639	Rotavirus	Prevention of rotavirus infections	EMA approved
Rotavirus vaccine, live, oral, pentavalent	BL 125122	Rotavirus	For the prevention of rotavirus gastroenteritis caused by the types G1, G2, G3, G4, and G9 for use in infants 6 weeks−32 weeks of age	FDA approved
Poliomyelitis (live) vaccine type I type III (human diploid cell), oral	S20180013	Poliovirus	Used to prevent polio	NMPA approved
Poliomyelitis (live) vaccine type I type III (human diploid cell), oral	S20171001	Poliovirus	Used to prevent polio	NMPA approved
Poliomyelitis vaccine, live, human diploid cell, oral	S20130001	Poliovirus	Used to prevent polio	NMPA approved
Oral polio vaccine	NCT02580201	Poliovirus	Used to prevent polio	Phase 4
Freeze-dried oral bivalent live vaccine of flexneri 2a-sonnei	S20030058	*Salmonella, Shigella*	To prevent dysentery	NMPA approved
Cholera vaccine, live attenuated, oral	EMEA-001490-PIP01-13-M02	*Vibrio cholerae*	Prevention of cholera	EMA approved
Rabies vaccine for foxes and raccoon dogs, live, oral	EMEA/V/C/ 004387	Rabies virus	For the active immunization of foxes and raccoon dogs against rabies to prevent infection and mortality	EMA approved
Adenovirus type 4 and type 7 vaccine, live, oral	BL125296	Adenovirus Type 4 and type 7	Prevention of febrile acute respiratory disease	FDA approved
Typhoid vaccine live oral ty21a	103,123	*Salmonella typhi*	For immunization of adults and children older than 6 years of age against disease caused by *Salmonella typhi*	FDA approved
Oral shigellosis vaccine	E-2012-016	*Shigella*	For bacillary dysentery and intestinal fever	FDA approved
Adenovirus type 4 and type 7 vaccine, live, oral	BL 125296	Adenovirus	Indicated for active immunization for the prevention of febrile acute respiratory disease caused by adenovirus type 4 and type 7	FDA approved
Typhoid-plague bivalent vaccine	E-2011-007	*Yersinia pestis*, *Salmonella typhi*	Used to prevent plague and typhoid	FDA approved
A novel anthrax vaccine candidate	E-2003-032	*Bacillus anthracis*	For the prevention of anthrax	FDA approved
Oral cholera vaccine	NCT03373669	*Vibrio cholerae*	Used to prevent *Vibrio cholerae* infection	Phase 4
Live enterovirus vaccine and type 1 diabetes	NCT02961595	Enterovirus (poliovirus)	To explore the relationship between enterovirus and type I diabetes	Phase 1
Ad4-H5-VTN vaccination-tonsillar route	NCT01443936	H5N1 influenza	Used to prevent influenza infection	Phase 1
H. pylori vaccine	NCT02302170	*H. pylori*	Used to prevent *H. pylori* infection	Phase 3
VXA-G1.1-NN oral vaccine	NCT03125473	Norovirus	Prevent gastroenteritis caused by norovirus	Phase 1
VXA-BYW.10 oral vaccine	NCT02547792	Influenza B	Used to prevent influenza B	Phase 1

(*continued on next page*)

Table 1 (*continued*)

Vaccine name	Number	Pathogen	Indication	Status
			infection	
Salmonella typhi-vectored pneumonia vaccine	NCT01033409	*Streptococcus*	Used to prevent pneumonia caused by *Streptococcus*	Phase 1
VXA-RSV-f tablets	NCT02830932	Respiratory syncytial virus	An important pathogen for preventing bronchiolitis and pneumonia caused by respiratory syncytial virus	Phase 1
WRSs2 and WRSs3 vaccine	NCT01336699	*Shigella*	For bacillary dysentery and intestinal fever	Phase 1
ETEC/rCTB vaccine	NCT02556996	*E. coli*	Prevention and treatment of diarrhea	Phase 3
HIV-1 peptide vaccine, microparticulate monovalent	NCT00000798	Human immunodeficiency virus	Used to prevent HIV	Phase 1
Adenovirus anthrax vector candidate vaccines	NCT01979406	*Bacillus anthracis*	For the prevention of anthrax	Phase 1
COVID-19 oral vaccine consisting of *Bacillus Subtilis* spores	NCT05239923	SARS-CoV-2	Used to prevent COVID-19 Pneumonia	Not applicable
VXA-CoV2-1	NCT04563702	SARS-CoV-2	Used to prevent COVID-19 Pneumonia	Phase 1
BCG vaccine	NCT00396370	*Mycobacterium tuberculosis*	For the prevention of Tuberculosis	Phase 1

Data sourced from the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), National Medical Products Administration (NMPA), and ClinicalTrials.gov, obtained through a search for "oral vaccine". Only a minor number of vaccines with the same type are listed. For an expanded understanding of oral vaccine information, it is advisable to consult resources, such as the FDA, EMA, NMPA, and ClinicalTrials.gov databases.

addition, goblet cells play a pivotal role in the regulating the intestinal immune system. They contribute to immune responses *via* nonspecific endocytic activities and goblet cell-associated antigen passages. Consequently, they are instrumental in counteracting the invasion of exogenous bacteria and the intrinsic intestinal microbiota and modulating immune functionality[20].

2.1.3. *Microfold cells*

Microfold cells, or M cells, are specialized epithelial cells located primarily in the gut-associated lymphoid tissues[21]. They possess unique structural characteristics that enable them to fulfill their specialized functions. Compared to the surrounding intestinal epithelial cells, M cells are typically flatter and irregularly shaped, and they have a smoother cell surface. This shape allows them to form microfolds or cell invaginations in the mucosa, which provides a large surface area for increased contact with antigens and pathogens on the mucosal surface. From a functional perspective, M cells are the primary cells for monitoring, capturing, and delivering antigens within the intestinal lumen[22]. They can capture antigens and deliver them to antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, initiating immune responses. In the context of beneficial microorganisms residing in the intestines for an extended period, M cells play a role in recognizing and initiating immune tolerance, ensuring the normal function of the intestinal immune system[23].

2.1.4. *Peyer's patches (PPs)*

PPs are composed of multiple lymphoid follicles, typically located beneath M cells. Each lymphoid follicle consists of various lymphocytes, including DCs, macrophages, B cells, and T cells. The amount of B lymphocytes, especially immunoglobulin A^+ (IgA^+) B lymphocytes, is much higher than that of T lymphocytes. The lymphocytes within PPs can connect with other lymphoid tissues and lymph nodes *via* the lymphatic vessels, forming part of the lymphatic system[21]. PPs are indispensable for the initiation of intestinal mucosal immunity. When M cells recognize and capture pathogens, they are directly transmitted to the underlying PPs through endocytosis. Within the PPs, APCs process and present the pathogens and initiate an immune response in the intestinal mucosa[24].

2.1.5. *Isolated lymphoid follicles (ILFs)*

ILFs are lymphoid tissues similar to the PPs located within the intestinal mucosa[25], which also possess similar immune functions as PPs. Unlike PPs, they exist independently, disperse widely, and have a small quantity. Therefore, their impact on intestinal

Table 2 The distinction between preventive oral vaccines and therapeutic oral vaccines.

Index	Preventive oral vaccine	Therapeutic oral vaccine
Goal	Prevention of infectious diseases	Treating existing diseases
Vaccination time	Before the onset of the diseases	After the onset of the diseases
Type of immune response	Humoral, cellular, and mucosal immunity	Mainly for cellular immunity
Vaccination scale	Mass vaccination	Vaccination of specific populations

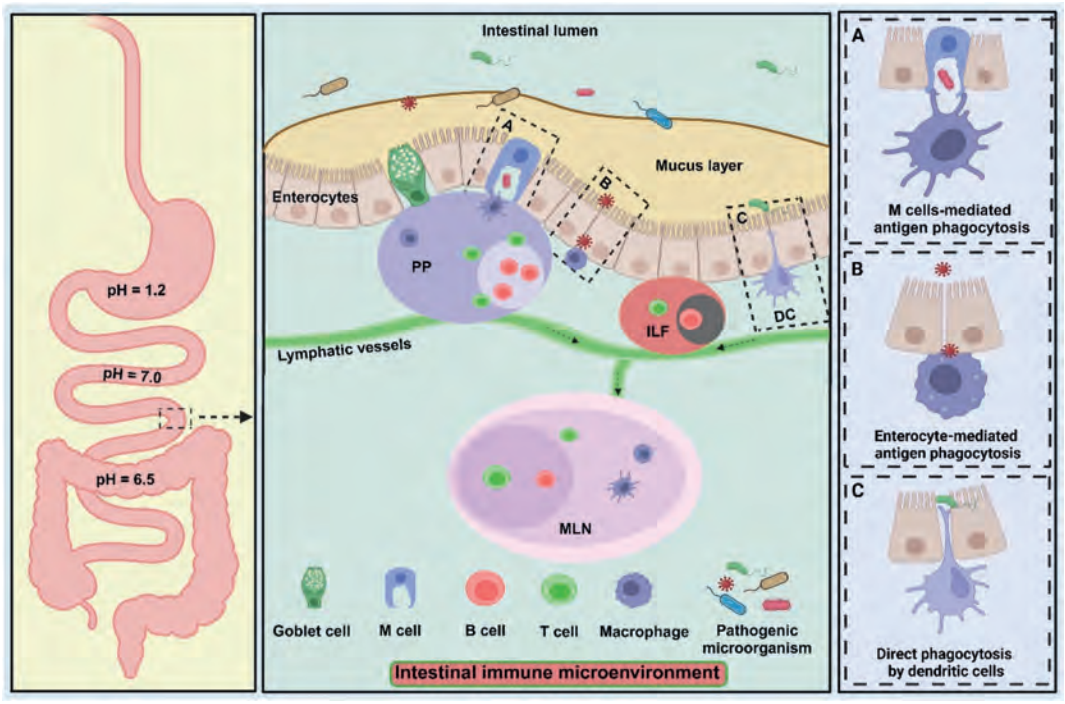

Figure 1 Schematic illustration of intestinal immune microenvironment. The intestinal immune microenvironment mainly comprises lymphoid tissues, such as MLNs, PPs, and ILFs, along with lymphocytes (*e.g.*, T cells, macrophages, and DCs). Pathogenic microorganisms in the intestine are apprehended and processed by APCs *via* multiple pathways and transported to the MLNs, initiating the host's adaptive immune responses. This figure was created with BioRender (https://biorender.com).

mucosal immunity is relatively minor[26,27]. Furthermore, mature ILFs encompass germinal centers (GCs), with these lymphoid formations being encapsulated by a follicle-associated epithelium that includes M cells. Studies have elucidated the distribution of ILFs within the intestines of various species, including mice, rats[28], guinea pigs[29], rabbits[30], and humans[31], highlighting their ubiquitous presence across different mammalian systems.

2.1.6. Mesenteric lymph nodes (MLNs)

MLNs are distributed throughout the abdominal mesentery of various gut regions, such as the duodenum, jejunum, ileum, and colon. Like other lymph nodes, MLNs consist of lymphatic follicles containing DCs, macrophages, B cells, and T cells. Through their lymphatic vessels, MLNs form a comprehensive lymphatic network along with PPs, ILFs, and other lymphoid tissues, helping the body to initiate a rapid immune response when pathogenic microorganisms invade[32]. Moreover, the mucosal immune system is perpetually confronted with immune challenges stemming from an array of gut microbiota and dietary antigens. Smith's studies suggest that MLNs are pivotal sites for developing intestinal immune tolerance. MLNs adeptly regulate the mucosal immune system's immune responses to dietary antigens and commensal bacteria in the GIT, thereby fostering immune tolerance towards dietary antigens and orchestrating the immune equilibrium of gut microbes[33].

3. Advantages and challenges of oral vaccines

Oral vaccines hold immense potential for the prevention and treatment of various diseases. Understanding the differences between oral route and other vaccination routes is crucial for the development of oral vaccines.

3.1. Advantages of oral vaccines

Orally administered vaccines offer a non-invasive strategy for the treatment and prevention of cancers and infectious diseases. This strategy holds pivotal potential for fighting tumors and curbing the spread of infectious diseases. Firstly, oral vaccines notably diminish the pain and distress linked to vaccination, eliminating the need for needles and syringes. This characteristic is notably favorable for children, who often exhibit a pronounced fear and aversion to vaccination procedures involving needles[34]. Secondly, these vaccines significantly lessen the risk of cross-contamination, since they circumvent the need for skin punctures or any blood contact, thus averting potential infections that could originate from unsanitary conditions and repeated use of medical instruments[35]. Thirdly, the logistics of storing and transporting these vaccines are generally more straightforward and more manageable, as they sidestep the rigorous cold chain requirements for injectable vaccines. This can potentially streamline the vaccine deployment process, reduce the production expense, and simplify the usage steps[36].

Furthermore, oral vaccines offer the distinct advantage of self-administration, rendering them ideal for use at home or in community, eliminating the necessity for professionally trained healthcare nurses. This advantage can broaden vaccine accessibility, especially in the regions constrained by limited resources and geographical remoteness. These vaccines significantly mitigate the production of medical waste, as they abstain from utilizing needles and syringes, alleviating the pressure on waste

management systems and curtailing environmental pollution risks. Lastly, they prompt cellular and humoral immune reactions through the intestinal immune system and adeptly initiate mucosal immune responses, a tissue region inefficiently stimulated by injectable vaccines[37-39].

3.2. Challenges to oral vaccines

The GIT is a complex system encompassing diverse cells, microbial communities, and biochemical factors that significantly challenge the development of oral vaccines[40,41]. Firstly, the harsh gastrointestinal environment, characterized by gastric acid and digestive enzymes, might compromise the vaccines, leading to their structural damage and inactivation[42], which prevents the vaccines from effectively awakening the immune responses. Secondly, the mucosal barrier might impede the effective absorption of vaccines, preventing their internalization by APCs and reducing their bioavailability[9,43]. Moreover, the intestinal microbiota can potentially engage in adverse interactions with vaccines. This includes scenarios where intestinal microbes compete for receptor-binding sites with vaccine components and degrade vaccine ingredients, consequently diminishing vaccine efficacy[44,45].

Concurrently, the development of oral vaccines also confronts a distinct challenge of potential gut immune tolerance. The gut, the primary interface for the body's interaction with external substances, is routinely exposed to various food and microbial antigens. Under normal circumstances, the gut's immune system is inclined towards a state known as "oral immune tolerance" to maintain immune equilibrium and avert hyperactive responses to these typical benign antigens. This systemic non-responsiveness is primarily facilitated by the interplay between regulatory T cells (Tregs) and DCs, mitigating deleterious inflammatory reactions to food antigens and symbiotic bacteria[46]. Nonetheless, this antigen-specific hypo-responsiveness could undermine the efficacy of oral vaccines, as they require the induction of a sufficient immune response for protection[47]. Hence, the design of oral vaccines necessitates a meticulous approach to circumventing the gut's inherent immune tolerance mechanism, ensuring the vaccine's capacity to stimulate the gut immune system.

In conclusion, a comprehensive understanding of the advantages and disadvantages of different vaccination routes (Table 3), along with addressing the current challenges faced by oral vaccines, is crucial for their applications in cancer treatment and infectious disease prevention.

4. Oral vaccine adjuvants

The primary purpose of oral vaccine adjuvants is to enhance the immune responses to antigens within the intestinal mucosal immune system. Adjuvants, such as double-mutant heat-labile toxin (DMLT) and multi-mutant cholera toxin (MMCT), can significantly boost the production of specific IgA antibodies by targeting specific receptors in the GIT, providing strong immune protection. Adjuvants like liposomes can protect vaccine antigens from gastrointestinal degradation and prolong antigen release in the GIT, thus improving antigen utilization and immune efficacy. However, some adjuvants (*e.g.*, cholera toxin and the heat-labile enterotoxin of *E. coli*), effective as oral vaccine adjuvants, have toxicity issues that limit their clinical application. Traditional injectable adjuvants, including aluminum hydroxide, complete Freund's adjuvant, and incomplete Freund's adjuvant, cause limited immune effects when used in oral vaccines[48,49]. Therefore, there is a need to develop adjuvants with the ability to stably pass through the GIT and boost mucosal immunity. Several promising oral mucosal adjuvants have been identified, such as modified bacterial enterotoxins, small molecule immune modulators, biodegradable polymers, and synthetic toll-like receptor (TLR) agonists and their derivatives.

Various oral adjuvant candidates have been categorized into biologically derived adjuvants, non-biologically derived adjuvants, and their composites, based on their physicochemical properties. For instance, oral liposomes or water/oil/water emulsions loaded with the TLR2 adjuvant (Pam2Cys) and tumor antigen peptides significantly activated T cells and reduced tumor volume in tumor-bearing mice[50]. mesoporous silica nanoparticles (MSNs) modified with yeast β-glucan, which possess immune-stimulating activity and M cell-targeting capability, increased the survival time of tumor-bearing mice when administered orally[51]. Furthermore, the combination of whole-cell *Helicobacter pylori* (*H. pylori*) vaccines with the MMCT elevated the levels of *H. pylori*-specific IgG and intestinal mucosal IgA[52]. The co-administration of Thymosin α1 with classical swine fever virus antigens orally effectively protected pigs from virus challenge *via*

Table 3 Comparison of the advantages and drawbacks of various vaccines *via* different administration routes.

Vaccination route	Advantage	Disadvantage	Primary immune response
Intramuscular injection	Rapid absorption; easy to control dosage; widely used	Can cause pain; requires professional administration; high cost	Humoral immunity and cellular immunity
Subcutaneous injection	Long-lasting immunity; suitable for various vaccines; fewer side effects	May cause local reactions; requires professional administration; slow absorption	Humoral immunity and cellular immunity
Inhalation	Painless; needle-free; rapid local immune responses; easy for mass vaccination	Dosage control is difficulty; may cause respiratory discomfort; high technical requirements	Humoral immunity, cellular immunity, and mucosal immunity
Oral administration	Painless; convenient to take; suitable for mass vaccination	May be degraded by gastrointestinal contents; lower bioavailability; not suitable for all vaccines	Humoral immunity, cellular immunity, and mucosal immunity

the oral route[53]. Overall, the development of composite adjuvants that integrate both biologically and non-biologically derived materials represents a potent strategy to improve the immune efficacy of oral vaccines. In addition, several excellent reviews have elaborated in detail on the development and selection of oral vaccine adjuvants[54-57].

5. Mechanism of oral vaccine-mediated immune responses

Intramuscular injection, as the most common vaccination route, primarily involves directly injecting vaccines (adjuvants and antigens) into the muscle. APCs in the muscle capture the vaccines and migrate to the lymph nodes, where they present the antigens to lymphocytes. This process activates strong humoral and cellular immune responses, enabling rapid immune responses when the body encounters pathogens carrying the same antigenic signals[58]. Intramuscular vaccines can bypass the digestive barriers, requiring relatively low antigen stability, but they are less effective in inducing mucosal immune responses. In contrast, oral vaccines are able to activate intestinal immune responses *via* the intricate interactions between vaccines and the intestinal immune system[35,59,60]. The mechanisms underlying the immune responses elicited by anti-tumor and anti-infective vaccines within the organism might vary significantly. Elucidating the distinct mechanisms of these immune responses holds substantial implications for the design and construction of oral vaccines.

5.1. Anti-tumor mechanism of oral vaccines

The working mechanism of oral tumor vaccines revolves around effectively initiating the body's immune responses. The activation of the immune system by oral tumor vaccines primarily encompasses efficient delivery of vaccines within the GIT, antigen presentation, T-cell activation, and tumor cell killing. Firstly, oral tumor vaccines must traverse the gastrointestinal barrier and be internalized and presented by APCs[2]. This poses significant challenges for the selection of vaccine delivery vectors, and several excellent reviews have detailed the selection of vaccine delivery platforms[54,55,61]. Secondly, delivering tumor vaccines to gut-associated lymphoid organs, such as MLNs and PPs, and subsequent stimulating T cells are critical steps[51]. The specific binding of T-cell receptors (TCRs) to major histocompatibility complex (MHC) molecules on the surface of APCs, particularly DCs, in these lymphoid tissues triggers T-cell activation. This binding sets off a chain of signals from the TCR−MHC complexes that alter the gene expression profiles and induce T cells from the rest state to the activated and proliferative states. However, the signaling cascade from the TCR−MHC complex alone cannot fully activate T lymphocytes. They also require concurrent co-stimulation from molecules (*e.g.*, CD80, CD86, OX40L, and CD40) on the surface of APCs. Additionally, cytokines like Type I interferons, interleukin-2 (IL-2), and interleukin-12 (IL-12) play a significant role in T-cell activation[62].

In summary, the activation of T lymphocytes, especially cytotoxic lymphocytes (CTLs), is effectively achieved through the combined action of TCR-MHC binding, co-stimulatory molecules, and various cytokines. Indeed, the specific and efficient recognition of tumor cells by CTLs is pivotal for their effective destruction. Once activated by the neoantigens presented by APCs, CTLs precisely accumulate in the tumor tissues. Meanwhile, the interactions between TCR on the CTL surface and MHC molecules on the tumor cell surface, in conjunction with co-stimulatory molecules (*e.g.*, CD80, CD86, and CTLA4), and the combined action of granzymes and perforins, initiate the specific killing of tumor cells[63]. Moreover, oral immunization can induce a robust mucosal immune response. IgA, as a unique product of mucosal immunity, may induce a more potent antibody-dependent cell-mediated cytotoxicity (ADCC) compared to immunoglobulin G (IgG) through its specific binding with tumor cells[64], potentially enhancing the cytotoxic action of neutrophils against tumor cells[65] (Fig. 2).

5.2. Anti-infective mechanism of oral vaccines

In contrast to oral tumor vaccines, oral anti-infective vaccines are primarily designed to safeguard the body against the onslaught of pathogens. The principle of oral anti-infective vaccines is to train the body to produce adaptive immunity against infectious pathogens. Acting as a "drill instructor", oral anti-infective vaccines orchestrate the immune system training. This "immune training" process predominantly encompasses the following components: efficient vaccine delivery[66], antigen capture and presentation by APCs, activation of T lymphocytes, and the establishment of immune memory[67].

Like oral tumor vaccines, oral anti-infective vaccines require effective antigen delivery to the gut mucosa and internalization by intestinal cells, particularly M cells. Subsequently, these antigens, conveyed *via* M cells, reach lymphatic structures, such as PPs, where APCs within the lymphoid follicles process and present them to T and B lymphocytes in the GCs[68]. B lymphocytes undergo rapid proliferation and differentiation into the plasma and memory cells, producing antibodies (*e.g.*, IgG, IgA, and IgM), countering potential extracellular infections. Concurrently, T lymphocytes facilitate the activation and proliferation of CTLs and memory cells, countering potential intracellular infections. During this phase, immature APCs evolve into mature forms, as indicated by the increased expression of surface molecules like CD40, CD80, CD86, and MHC[69]. Eventually, these immune and co-stimulatory signals are conveyed to the MLNs *via* the intestinal lymphatic vessels, dramatically amplifying the immune responses and effectively mobilizing the body's defense against specific pathogens through the association between blood vessels and lymphatics throughout the body and mucosal tissue (lung mucosa, intestinal mucosa, and vaginal mucosa, Fig. 3).

6. Application of oral vaccines

Nowadays, oral vaccines have been constrained in clinical practice and clinical trials. Besides a handful of oral preventive vaccines, either marketed or having concluded clinical evaluations, the realm of oral therapeutic vaccines targeting tumors remains notably underserved (Table 1). Here, we explore the application of oral vaccines with a dual focus on tumor treatment and infectious disease prevention.

6.1. Tumor therapy

The malignancy of tumors poses an increasingly severe threat to human health, burdening not only individual patients and their families but also creating significant societal pressure[70]. Oral tumor vaccines hold substantial importance for treating malignant

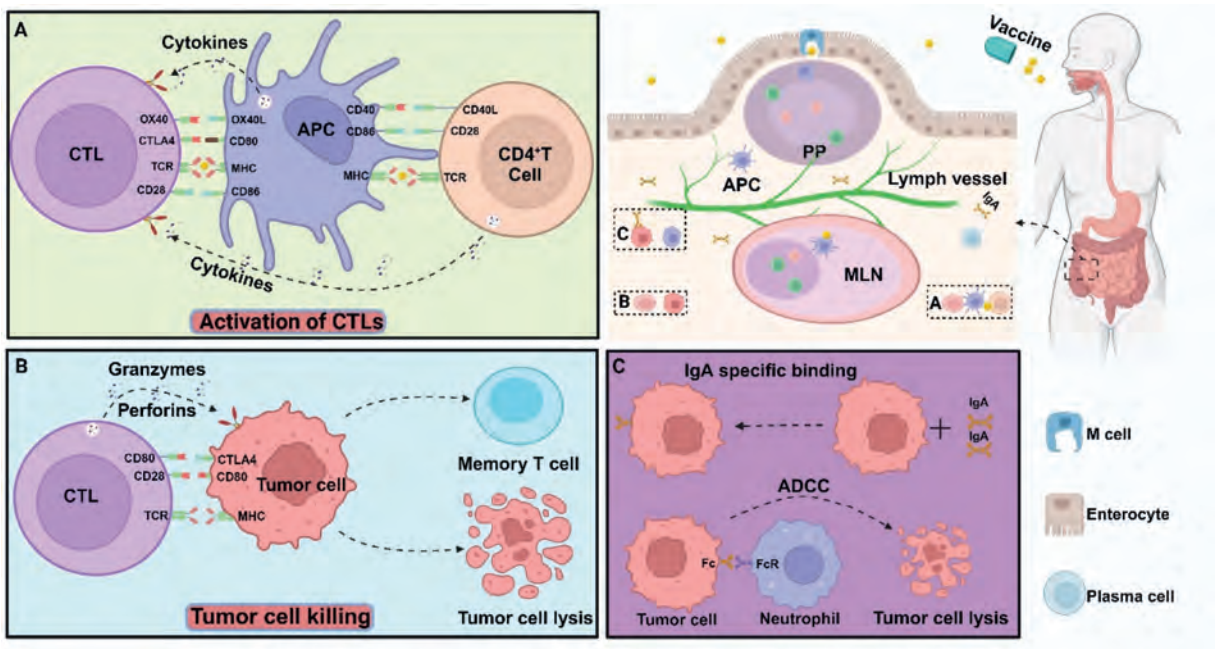

Figure 2 Anti-tumor mechanism of oral vaccination. After oral administration, vaccines traverse the gastrointestinal barriers, where they are internalized by M cells, facilitating access to the PPs. Within the PPs, the vaccines elicit a humoral immune response, promoting plasma cells to secrete anti-tumor IgA. This IgA navigates through lymphatic pathways to the tumor site, where it specifically binds to tumor antigens, initiating ADCC. Concurrently, APCs at the PPs uptake and process the vaccine, presenting its "immune signal" to the MLNs *via* lymphatic routes. In the MLNs, the "immune signal" undergoes further amplification, presented to immune cells, including $CD4^+$ T cells and CTLs, facilitating CTL-mediated specific recognition and destruction of tumor cells. This figure was created with BioRender (https://biorender.com).

tumors. The representative oral tumor vaccines are summarized in Table 4[71-73,51,74-76,12,77,43,50,78-79,10,80-82,38,83,11,84-86,38,87-91,13].

The clinically available treatment modalities against cancer mainly include surgery, chemotherapy, radiation therapy, and new approaches (*e.g.*, gene therapy, targeted biological agents, and chimeric antigen receptor T-cell therapy (CRA-T))[92-96]. Each of these strategies offers distinct advantages, yet it also comes with its own set of drawbacks. For instance, surgery may not eradicate all tumor cells and induce potential surgical risks[97]. Treatments like chemotherapy and radiation can inadvertently damage healthy cells, triggering unwanted adverse effects on the hosts[98]. While hormone therapy is primarily used for hormone receptor-positive cancers, it can lead to adverse impacts related to hormonal changes[99]. Groundbreaking techniques like gene therapy and CAR-T cell therapy offer a beacon of hope for treating certain cancers but may potentially harbor unknown long-term risks and significant costs[93,100]. Compared to the treatment modalities mentioned above, oral tumor vaccines represent a non-invasive therapeutic approach with enormous potential for anti-tumor applications. (i) These vaccines effectively circumvent the acute trauma and subsequent inflammation induced by invasive surgical procedures. (ii) They present a notable advantage as they do not involve chemotherapy and radiation therapy, thereby avoiding relevant toxic effects, such as typical cell damage, bone marrow suppression, and immune system impairment, which potentially increase the risk of secondary cancers. (iii) They reduce the psychological stress and anxiety that patients might otherwise experience during cancer treatment. (iv) They distinguish themselves from targeted therapies by not relying on complex biomarkers and genetic testing. This not only streamlines the treatment process but also reduces the overall treatment cost. Additionally, the vaccine components can inhibit the expression levels of PD-L1 and TGF-β1 in the tumor tissues, enhancing the overall immunotherapeutic efficacy[101]. (v) They offer numerous benefits, including ease of massive production, low technological barriers, and high biosafety. These benefits are instrumental in circumventing the challenges associated with the previously mentioned therapies, characterized by their high expenses, intricate technicalities, and risk of adverse effects[2,38,80,102].

6.1.1. Oral vaccination strategies against CRC

CRC ranks third in global cancer diagnoses and stands as the second leading cause of cancer-related deaths, according to GLOBOCAN 2023[103]. Although various CRC treatments are available, oral vaccines are gaining traction due to their high patient acceptability and the potential to stimulate local mucosal immune responses[2,43,50,78,79].

Liposomes and oil emulsions with superior manufacturing and therapeutic benefits have been extensively employed to the delivery of various therapeutics. These delivery systems enhance the therapeutic efficacy and stability of the encapsulated agents, offering a versatile platform for drug and gene delivery[104-106]. Naciute and his team[50] formulated a single long peptide antigen by merging AH1 (an antigen peptide of CT-26 colorectal adenocarcinoma) with a chimeric tetanus−diphtheria toxoid $CD4^+$ T cell helper peptide. Subsequently, they encapsulated this combined antigen in liposomes or a water/oil/water emulsion, and the formulations were administered with the immune adjuvant (Pam2Cys) *via* the oral route. The results demonstrated that this emulsion-based oral vaccine showcased promising results. There were significant increases in the amounts of $CD4^+$ T, $CD8^+$ T, and B cells within the MLNs of tumor-bearing mice. This vaccine

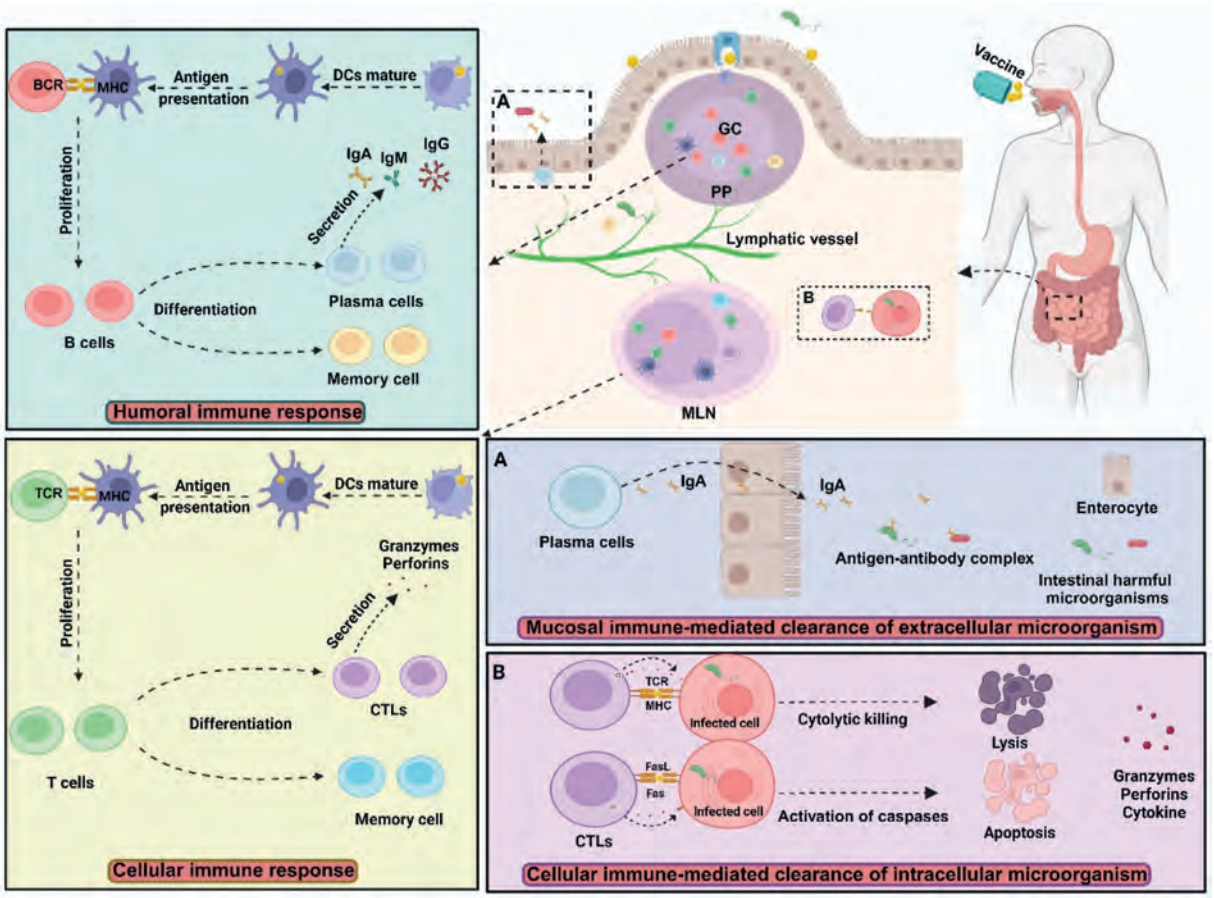

Figure 3 Anti-infection mechanism of oral vaccination. After oral ingestion, vaccines are transported to the gut, where M cells uptake and deliver them to the PPs. Within the GCs of PPs, DCs and other APCs process and present the vaccines to B cells. Upon receiving the vaccine's "immune signal", B cells proliferate and differentiate into plasma cells and memory B cells. Plasma cells produce secretory antibodies (*e.g.*, sIgA), which specifically bind to mucosal pathogens, exposing and eliminating these pathogens. After oral vaccination and the subsequent intracellular pathogen infection, the host's immune system is swiftly activated by memory T cells. T cells proliferate, differentiating into numerous CTLs, which circulate throughout the body, rapidly identifying and inducing lysis or apoptosis of infected cells through TCR−MHC pathways and Fas/FasL interactions. This figure was created with BioRender (https://biorender.com).

stimulated the production of $CD44^+CD122^+$ memory $CD8^+$ T cells and $CD62L^-CD44^+$ cells in the spleen, ultimately effectively suppressing tumor growth.

Listeria monocytogenes, a Gram-positive facultative intracellular bacterium, can reside within the cytoplasm of cells while having the extraordinary potential to trigger anti-tumor immunity against tumor-specific antigens[107,108]. Pan et al.[78] established mouse tumor models using three tumor cell lines: CT-26 cells (CRC), Renca cells (kidney cancer), and B16-F10 cells (melanoma), all of which express the influenza nucleoprotein. They constructed a *Listeria* strain (Lm-nucleoprotein) to express and secrete the nucleoprotein antigen. After orally administering the Lm-nucleoprotein vaccine to the tumor-bearing mice, tumor regression was observed. The results indicated that oral administration of Lm-nucleoprotein elicited a potent anti-nucleoprotein immune response in mice. This was directly correlated with a survival rate of 60% in Renca-nucleoprotein tumor-bearing mice and 50% in CT-26-nucleoprotein tumor-bearing mice. Additionally, there was a significant reduction in the tumor growth trend in B16-F10-nucleoprotein tumor-bearing mice, coupled with an approximately 70% increase in survival rate. These findings suggest that this oral Lm-nucleoprotein vaccine holds promise as an effective strategy for cancer immunotherapy.

Constructing a synchronized lysis circuit (SLC) in clinically relevant bacteria, which controls the lysis and release of intracellular cargos when the bacterial population reaches a threshold density, is a genetic circuit strategy in synthetic biology. This strategy has attracted attention for combating infectious diseases and tumors[109-111]. Zhang and colleagues[43] used SLC technology to engineer a bacterium capable of expressing a unique protein complex (BMC) in conjunction with a TAA (AH1-A5). This genetically modified bacterial vaccine, BMCH-AH1-A5, was encapsulated in a hydrogel for oral administration, followed by close monitoring of the anti-tumor outcomes. Experimental results revealed a significant activation of $CD3^+$ T lymphocytes in the MLNs, accounting for 69.2%, with $CD3^+CD4^+$ T cells at 33.5% and $CD3^+CD8^+$ T cells at 18.7%. Significantly, the percentage of $CD3^+CD8^+$ T cells was 3.9-fold higher than that of the control group, underscoring the CTL immune response to the AH1-A5 antigen induced by oral BMCH-AH1-A5 vaccine. To further substantiate the immune activation efficacy of the oral BMCH-AH1-A5 vaccine, the levels of TNF-α and IFN-γ were measured

Table 4 Examples of oral vaccines against various cancers.

Vaccine name	Tumor model	Oral delivery system	Ref.
rLM-E7	Cervical cancer	*Listeria monocytogenes*	71
OVA/RGD/PEOP	T Lymphocytic tumor	Mimicking-bacteria-membrane (MBM) nano-vesicle (RGD-PEOP)	72
Longum 420	Bladder cancer	*Bifidobacterium*	73
Len/OVA/yMSN	Liver cancer	*Saccharomyces*-inspired mesoporous silicon nanoparticle (NP) (yMSN)	51
WGP-OVA	Melanoma	Whole glucan particle, WGP	74
pUb-M, pUb-H	Melanoma	Attenuated strain of *Salmonella typhimurium*	75
TL-NE (MHS)	Melanoma	Tomato lectin-modified nanoemulsion	76
Tat-gp100	Melanoma	Multi-faceted blending-by-blending nanogels	12
pNSspH-HBx	Melanoma	Live attenuated *Salmonella*	77
BMCH-AH1-A5	CRC	Chitosan-sodium alginate microcapsules	43
E-Vac/L-Vac	CRC	Liposomes or water/oil/water double emulsions	50
Lm-NP	CRC/Renal carcinomas	Recombinant *Listeria monocytogenes*	78
Bac L-Vac/Bac E-Vac	CRC	Bacteria biohybrid-based vaccine delivery systems	79
NZ-SPK1-L-68V-DT/NZ-SPKM19-L-68V-DT/NZ-SPK1-L-WT/ NZSPKM19-L-WT	CRC	*Lactococcus lactis*	10
Longum 420	Oral cancer	Recombinant *Bifidobacterium*	80
Vaccine + Cy + GM-CSF	Prostate cancer	Microparticles	81
WCL prostate cancer vaccine	Prostate cancer	HPMCAS and Eudragit FS 30D in a ratio of 1:1:1 as delivery system	82
–	Ovarian cancer	HPMCAS and Eudragit® FS 30D were dissolved in an alkaline solution as delivery system	38
Oral vaccine microparticles	Breast cancer	Delivery systems were prepared by spray drying an aqueous suspension containing EC, trehalose, HPMCAS, and AAL.	83
mEndoglin	Breast cancer	Double attenuated *Salmonella typhimurium*	11
A.C.NPs-legumain	Breast cancer	Alginic acid-coated chitosan NPs (A.C.NPs)	84
pUb-Fra-1 pIL-18	Breast cancer	Attenuated *Salmonella typhimurium*	85
AAV6-neu	Breast cancer	Adeno-associated virus vectors	86
T07 Oral vaccine	Breast cancer	Delivery system was prepared by β-cyclodextrin, ethyl cellulose, trehalose, HPMCAS, and targeting agent AAL	38
Longum 420	Renal cell carcinoma	*Bifidobacterium*	87
–	Gastric cancer	Attenuated *Salmonella typhimurium*	88
Δpml2pGK20	Fibrosarcoma	Attenuated *Listeria monocytogenes* Δmpl2 mutant	89
SL7207 pCMVβ	Fibrosarcoma	*Salmonella typhimurium*	90
–	Fibrosarcoma	*Salmonella* type III secretion system	91
SB824 (pHR241)	Fibrosarcoma	*Salmonella* type III secretion system	13

–, not applicable.

during the treatment phase. On Day 14, the serum TNF-α concentrations for the AH1-A5 and BMCH-AH1-A5 groups were 273.7 and 253.0 pg/mL, respectively. By Day 21, both TNF-α and IFN-γ maintained elevated levels, suggesting that the oral BMCH-AH1-A5 vaccine successfully stimulated the production of anti-tumor-associated cytokines. The above results showed that tumor growth was significantly retarded, and the survival time was prolonged in mice vaccinated with the BMCH-AH1-A5 vaccine *via* the oral route.

6.1.2. Oral vaccination strategies against breast cancer

While there have been significant advancements in breast cancer treatment over recent decades, this disease claims the lives of over 40,000 women each year in the US. Globally, approximately one in three patients with breast cancer succumbs to the illness[112,113].

Cancer patients often possess a heightened presence of Tregs and a distinct subset of $CD4^+$ T cells. These cells play a pivotal role in suppressing the ability of CTLs to recognize and eradicate tumor cells[114,115]. Mulla and his team[83] developed oral vaccine microparticles (OVs) encapsulating breast tumor antigens. To further enhance the anti-breast tumor efficacy of OVs, they combined with a Treg inhibitor, cyclophosphamide, devising a strategy for breast cancer treatment termed OCy. This therapeutic strategy aims to deplete Tregs and activate CTLs. The results demonstrated that OCy effectively reduced the amount of Tregs to about one-fifth of that in the negative control group. It also initiated a robust CTL response, with the number of activated $CD8^+$ T cells being four times higher than that of the negative control group. Furthermore, OCy induced a potent immune memory for tumor cells, with the generated memory T cells

approximately twice more than that in the negative control group. Notably, OCy significantly inhibited tumor growth in tumor-bearing mice, with a survival rate of 60%−70% for these mice, markedly higher than other treatment groups. This innovative approach underscores the potential of OCy in treating breast cancer. It highlights the profound impact of strategically combining immune modulators with vaccine platforms to combat cancer more effectively.

In normal tissues, the formation of new blood vessels, or angiogenesis, is tightly regulated. However, this process can become dysregulated within tumors, leading to continuous angiogenesis that supplies tumors with oxygen and nutrients[116,117]. Lee and colleagues[11] devised a DNA vaccine targeting the endoglin gene to combat breast cancer. Endoglin is highly expressed in tumor vasculature, a critical regulator of tumor angiogenesis. Consequently, they employed attenuated *Salmonella* to deliver DNA plasmids encoding the endoglin gene, evaluating the vaccine's efficacy (mEndoglin) through oral immunization. The study revealed that the mEndoglin vaccine significantly activated DCs and $CD8^+$ T cells, effectively initiating the cellular immune response. Compared to other control groups with a survival time of 27 days, the mEndoglin vaccine effectively extended the survival of mice with pulmonary metastases to 50 days. The lung weight of mice in the mEndoglin vaccine group was about 200 mg, half of that detected in the control group. They further found that the tumor vascular growth of mice receiving oral administration of the mEndoglin vaccine was effectively inhibited, approximately 2/5 that of the empty vector group.

The adeno-associated virus (AAV), a member of the parvoviridae family, can infect a diverse range of human and nonhuman cells without being linked to any known diseases. This favorable safety profile positions AAV as a compelling vector for therapeutic gene transfer studies[118,119]. The neuroepithelial cell transforming gene 1 (NEU) encodes a receptor for neurotrophic growth factors and plays a crucial role in cell signaling and proliferation. Mutations or abnormal expression of the NEU gene are associated with the onset and progression of various tumors, particularly breast cancer. Functional abnormalities in the NEU gene can lead to excessive cell proliferation and transformation into malignant tumor cells. Hence, the NEU gene is a significant target in cancer research and a potential target for developing anti-tumor therapeutic methods[120,121]. Steel and his co-workers[86] developed an AAV vector carrying the NEU gene for breast cancer treatment. They evaluated the anti-tumor effect of the AAV-neu vaccine through both oral administration and intramuscular injection. The findings revealed that mice orally immunized with AAV 5-neu or AAV 6-neu achieved a significant survival advantage compared to those treated with phosphate-buffered saline (PBS). Moreover, the mouse survival rate was correlated with oral vaccine dosages. In tumor cell challenge experiments, all mice immunized with 10^{12} viral genome (VG) of AAV 6-neu, and 80% of those immunized with 10^{12} VG of AAV 5-neu survived beyond 100 days. In contrast, the survival rates for mice vaccinated with 10^{11} VG of AAV 6-neu and AAV 5-neu were only 50% and 10%, respectively.

Subsequently, they reached the cytotoxic capabilities of splenocytes from mice intramuscularly and orally immunized with AAV 5-neu and AAV 6-neu against tumor cells. The results indicated that mice orally immunized with AAV 6-neu generated a more potent CTL immune response than the intramuscularly injected AAV 5-neu immunized groups. Similar outcomes were observed in the detection of the splenic secretion of IFN-γ. To assess the long-term protective effects of the AAV-neu vaccine, the researchers re-challenged the surviving mice with tumor cells 120 days post-immunization. It was found that 60% of the mice orally vaccinated with AAV 5-neu survived up to 300 days post-rechallenge, while all mice in the AAV 6-neu group survived up to this time point. When the remaining surviving mice in the AAV 6-neu group were re-challenged with tumor cells after 320 days, 80% survived up to 420 days. The oral vaccine using AAV as a vector demonstrated exceptional performance in combating breast cancer, providing valuable insights for subsequent tumor vaccine research[86].

6.1.3. Oral vaccination strategies against melanoma

Metastatic melanoma stands as a critical global public health issue, characterized by a dismal survival rate[122,123]. The oral tumor vaccine is garnering attention for its promise to enhance treatment outcomes against metastatic melanoma, offering a beacon of hope in the battle against this aggressive disease[12,75-77,124].

Bacterial outer membrane vesicles (OMVs) are inherently nanoscale spherical entities (50−400 nm) originating from the outer membrane of Gram-negative bacteria. Modifiable to express designated antigens, OMVs are employed in vaccine development as adjuvants, antigen carriers, and delivery platforms. The utility of OMVs in vaccine platforms is attributed to their natural immunostimulatory characteristics through the activation of both innate and adaptive immune responses[125,126]. Additionally, their nanometric dimensions facilitate their internalization and processing by immune cells[127]. Yue et al.[2] constructed an engineered bacterial oral vaccine (ClyA-OVA-mFc-OMVs) for melanoma treatment. This innovative vaccine seamlessly incorporates the expression of bacterial toxin (ClyA), a specific antigen, and the Fc fragment of mouse IgG within the C terminus of the surface protein (mFc). The genetically modified bacteria secrete ClyA-OVA-mFc into the intestine *via* the OMVs (Fig. 4A). The *in vivo* study found that ClyA-OVA-mFc-OMVs markedly augmented the population of antigen-specific T cells within the spleens (Fig. 4B) compared with other control groups, and enhanced the capacity of T lymphocytes to kill tumor cells (Fig. 4C). In lung metastasis assays, ClyA-OVA-mFc-OMVs significantly fostered the populations of IFN-γ^+CD8^+ T cells and facilitated the elimination of tumor in the pulmonary tissues. ClyA-OVA-OMVs led to a substantial reduction in tumor metastasis to the lungs and achieved an inhibition rate of approximately 60% compared to the control. More importantly, ClyA-OVA-mFc-OMVs virtually eliminated lung metastatic tumors (Fig. 4D and E). Furthermore, pre-immunization with ClyA-OVA-mFc-OMVs elicited a heightened response of memory T cells against tumor cells (Fig. 4F). This investigation unequivocally establishes ClyA-Ag-mFc-OMVs as a groundbreaking oral tumor vaccine, propelling this field with an innovative paradigm.

Glycoprotein 100 (gp100) is an antigen present on the surface of melanoma cells, playing an essential role in mediating intracellular transport and regulating melanosome biogenesis. It has been commonly used in developing vaccines targeting melanoma, harnessing its potential to trigger a robust immune response against melanoma cells[128]. In a study by Qiu and co-workers[12], a DNA plasmid expressing the gp100 gene, known as Tat-gp100, was developed for oral immunotherapy against melanoma. They employed a novel "mix−mix" approach to create a multidimensional alginate nanogel for oral delivery of Tat-gp100, termed Alg-Tat-gp100. *In vitro* results demonstrated that compared with the negative control group, the Alg-Tat-gp100 vaccine significantly

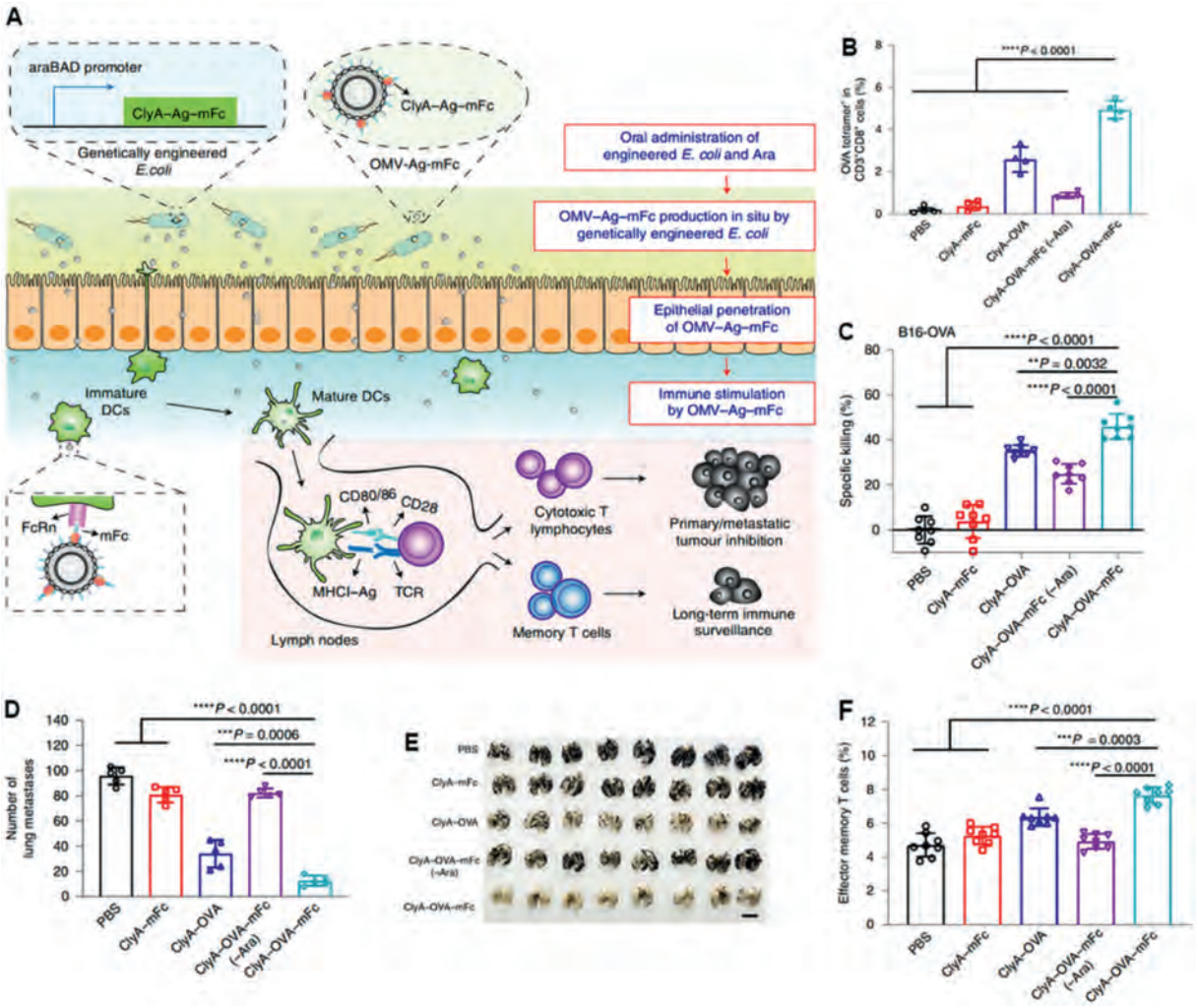

Figure 4 (A) Engineered *E. coli* was produced by transformation with a plasmid encoding ClyA (a surface protein on OMVs) fused with a tumor antigen and Fc fragment of mouse IgG (ClyA-Ag-mFc). An Ara-inducible promoter (araBAD) was introduced to control the expression of fusion protein. After oral administration of engineered *E. coli* and expression inducer (Ara), OMVs containing tumor antigens (OMV-Ag-mFcs) were produced in the intestine. Due to the inherent characteristics of OMVs, OMV-Ag-mFcs efficiently penetrated through the intestinal epithelial barrier, where they were internalized by DCs in the lamina propria through the interaction between mFc and FcRn. The presence of pathogen-associated molecular patterns (PAMPs) in the OMVs induced the rapid maturation of DCs, their migration to the lymph nodes, and the subsequent completion of tumor antigen presentation. This process resulted in the expansion of antigen-specific CTLs and memory T cells. TCR, T-cell receptor. (B) On Day 12, following three vaccinations, splenocytes were harvested and subjected to re-challenge with OVA peptides. Flow cytometry was employed to analyze the proportions of OVA tetramer-positive cells within the $CD3^+CD8^+$ population ($n = 4$). (C) The cytotoxic effect of splenocytes against B16-OVA cells (with OVA antigen). (D) Quantitative analysis of lung metastasis ($n = 5$). (E) The lungs were collected on Day 65. (F) The proportions of central memory T cells ($CD3^+CD8^+CD44^+CD62L^+$). Reprinted with the permission from Ref. 2. Copyright © 2022 Springer Nature.

enhanced the expression of co-stimulatory molecules (*e.g.*, CD80, CD86, and MHC-II) on the surface of bone marrow-derived DCs (BMDCs), indicating the vaccine's efficacy in promoting DC maturation. *In vivo* studies further revealed that this Alg-Tat-gp100 vaccine effectively stimulated the proliferation and differentiation of spleen-associated T lymphocytes. The proportion of $CD3^+CD4^+$ T cells in the Alg-Tat-gp100 group was 31.0 ± 3.4%, higher than 27.7 ± 1.4% observed in the Tat-gp100 group. Moreover, the activation efficiency of $CD3^+CD8^+$ T cells was notably elevated. In a murine tumor challenge experiment, the Alg-Tat-gp100 vaccine significantly delayed tumor growth in tumor-bearing mice. The findings from these experiments indicate that employing gp100 as a tumor antigen holds considerable potential and serves as a valuable reference in the therapeutic approach to melanoma.

Melanoma antigen (MAGE) is a typical tumor-specific antigen (TSA) expressed in most tumors[129]. Heat shock protein (HSP) is a molecular chaperone, which plays a crucial role in the processing and expression of tumor antigens[130]. Staphylococcal enterotoxin A (SEA), a traditional superantigen model, can stimulate a strong $CD4^+$ and $CD8^+$ T cell pool[131]. Long and colleagues[76] developed an oral anti-melanoma vaccine by encapsulating the MAGE1-HSP70/SEA (MHS) antigen within a nanoemulsion (NE). To address the challenges of stability and bioactivity associated with oral tumor vaccines, they coated the NE surface with tomato lectin (TL), resulting in TL-NE (MHS). Mice orally immunized with

TL-NE (MHS) exhibited a significant induction of IFN-γ production, approximately five times higher than the NE-immunized mice. The typical antibodies produced by mice immunized with TL-NE (MHS) were about four times higher than those of the NE group. Thereafter, the researchers evaluated the impact of TL-NE (MHS) vaccination on the survival of tumor-bearing mice *via* the intramuscular injection and oral route. It was noted that intramuscular injection of TL-NE (MHS) extended the average survival duration of tumor-bearing mice to 34 days, while oral vaccination with TL-NE (MHS) extended it to 62.6 days. The preventive effect of the TL-NE (MHS) vaccination against B16-MAGE-1 tumor metastasis was also evaluated. The findings indicated that oral immunization with TL-NE (MHS) effectively inhibited the metastasis of 16-MAGE-1 tumors, with the number of transfer tumors being 5.3 ± 1.6, significantly lower than 12.7 ± 2.4 in the oral NE group and 8.7 ± 3.1 in the intramuscular TL-NE (MHS) group. Undoubtedly, the approach of oral immunization utilizing a variety of tumor antigens has proven to be effective in treating melanoma, offering valuable insights for advancing future melanoma therapeutic platforms.

The Type III secretion system (T3SS) is a protein delivery system found in certain Gram-negative bacteria capable of transporting bacterial effector proteins directly into host cells. This mechanism is typically used in the pathogenic process of bacteria, aiding them in establishing infections within the host body. Several studies have employed T3SS-based vectors for anti-tumor immunotherapy. By delivering antigens to immune cells through T3SS, a strong immune response against tumors is activated[132,133]. Wang et al.[77] explored strategies to enhance the immunogenicity of tumor antigens and develop more effective tumor vaccines. They chose the hepatitis B virus x gene (HBx), expressed on melanoma cells, as the target antigen, which was introduced into *Salmonella* using two approaches based on the T3SS system. The first approach involved cloning HBx into a eukaryotic plasmid (PCDB), and the second approach entailed the fusion of HBx with *Salmonella*-secreted protein H2 (SspH2). Mice were orally vaccinated with these two vaccine strains, and their immune responses against melanoma were evaluated.

Initially, the secreted levels of lactate dehydrogenase (LDH) from spleen cells of mice orally immunized with the PCDB-HBX vaccine and pNSspH-HBx vaccine were measured to evaluate the vaccine's capability to induce a CTL immune response. The obtained results revealed that mice with oral vaccination of the PCDB-HBX vaccine produced a more considerable amount of LDH than that of the pNSspH-HBx vaccine at the same dilution ratio, indicating a robust induced CTL immune response from the PCDB-HBX vaccine. The LDH results were verified by the levels of IFN-γ secreted by spleen lymphocytes, confirming the superior CTL immune response induced by the PCDB-HBX vaccine. Regarding the tangible outcomes of tumor suppression, the performance of the two vaccine strategies exhibited no notable disparity. This suggests that while the PCDB-HBx approach appears more potent from a cellular immunological standpoint, both methodologies equally manifest their efficacy in curbing tumor growth.

6.1.4. Oral vaccination strategies against fibrosarcoma

Fibrosarcoma is a non-rhabdomyosarcoma soft tissue sarcoma, with an incidence of about 5 per million in children under one year of age[134]. Characterized by aggressive local invasion, it generally presents a favorable overall prognosis. A significant 80% of fibrosarcoma occurs within the first two years of life, leading many studies to refer to fibrosarcoma in children aged 2 to 3 as infantile fibrosarcoma[135,136]. The potential of orally administered tumor vaccines for preventing and treating fibrosarcoma is substantial, offering a promising avenue for enhancing patient outcomes[13,90,91,137].

β-Galactosidase (β-gal) has a relatively large molecular weight and multiple epitopes, which is recognized by the body as an exogenous protein, effectively stimulating an immune response. Thus, it has been frequently used in developing vaccine antigens[138]. Medina and colleagues[137] employed recombinant DNA technology to construct a β-gal plasmid and utilized *Salmonella* as a delivery carrier to prepare oral vaccines, SL7207 [pAH97] and SL7207 [pLS1000]. Subsequently, they assessed the anti-fibrosarcoma immunological effects of these oral vaccines. It was detected that mice orally immunized with SL7207 [pAH97] and SL7207 [pLS1000] produced anti-β-gal specific IgG antibodies approximately 6 and 8 times higher than the control group, and these immunized mice had a level of anti-β-gal specific IgM antibodies 2-fold higher than the control mice. Specific lysis assays revealed that both oral vaccines could induce splenic lymphocytes to specifically lyse tumor cells, with SL7207 [pLS1000] showing a slightly superior effect to SL7207 [pAH97]. Further tumor cell challenge results indicated that 54% and 85% of mice immunized with SL7207 [pAH97] and SL7207 [pLS1000] remained tumor-free post−challenge, compared to only 20% in the control group. Importantly, tumor formation in the vaccinated mice was significantly inhibited compared to the control group. The outcomes of this trial suggest that β-gal represents a TAA with significant therapeutic potential in oncology.

Effector $CD8^+$ T cells are significant in anti-tumor immunity[139]. Roider et al.[13] explored the application of *Salmonella*-induced effector $CD8^+$ T cells as an intervention for treating invasive fibrosarcoma. To this end, they engineered *Salmonella* to express p60 and employed it as a TAA for therapeutic immunization. Experimental findings showed that this approach induced a substantial population of p60-specific $CD8^+$ T cells, representing approximately 26.4%. To elucidate the relationship between p60-specific $CD8^+$ T cells and tumor regression, they analyzed the distribution of these cells in the blood and tumor tissues using co-staining with CD62L and CD127. It was observed that the frequency of p60-specific effector and memory $CD8^+$ T cells in both blood and fibrosarcoma tissues correlated positively with the dynamics of tumor regression. The therapeutic efficacy of this approach was evident, with 71%−80% of intravenously immunized mice and 50%−52% of orally immunized mice exhibiting complete tumor regression by Day 14, underscoring the effective treatment of invasive fibrosarcoma.

Researchers, including Paglia and colleagues[89], evaluated the anti-tumor effect of an oral vaccine utilizing an attenuated *Listeria monocytogenes* (ΔmpM mutant) as a carrier against murine fibrosarcoma. They transfected plasmids encoding the TAA (β-gal) or its immunodominant CTL epitopes into *Listeria monocytogenes* strains for anti-fibrosarcoma therapy. The findings revealed that mice exhibited significant immune protection against tumor challenges after oral vaccination. Specifically, 55%−64% of the vaccinated mice showed no tumor growth post−challenge. When the tumor antigen was switched from β-gal to CTL dominant epitope, the immune protection rate against tumor challenge was 43%. Compared to the non-vaccinated control group, the tumor volume in vaccinated mice was notably reduced.

Furthermore, the researchers identified that post-vaccination, mice generated specific antibodies capable of recognizing β-gal epitope and produced CTLs that killed tumor cells expressing β-gal. Additionally, the vaccinated mice manifested a long-term memory immune response, offering adequate protection against the injected tumor cells and achieving desirable treatment outcomes against invasive fibrosarcoma.

6.1.5. Oral vaccination strategies against other cancers

Liver cancer is a malignant tumor that severely impacts global public health, and it is characterized by an exceptionally high mortality rate[140]. Due to its subtle early symptoms, many patients are already in the advanced stages by the time of diagnosis, leading to increased treatment complexity and a poor prognosis[141]. *Saccharomyces*-glucan, a type of β-1,3-D-glucan derived from the fungal cell wall, exhibits stability against strong gastric acid and digestive enzymes. This compound explicitly targets C-type lectin domain family 7 member A (dectin-1) receptors, abundantly present on the surface of intestinal M cells, macrophages, and DCs. This interaction initiates a cascade of immune responses, which are mediated by TLRs on immunocytes, stimulating innate and adaptive immune defenses[142,143].

Mao and colleagues[51] designed a yeast-glucan-based nanovaccine (Len/OVA/yMSN) for liver cancer treatment *via* the oral route and targeting the lymph and tumor sites (Fig. 5A). The results indicated that Len/OVA/yMSNs could be specifically recognized and internalized by M cells (Fig. 5B), subsequently being presented by macrophages and DCs. Moreover, Len/OVA/yMSNs promoted macrophage migration and induced the polarization of M1-type macrophages in the tumor microenvironment (Fig. 5C and D), thereby enhancing the anti-tumor immune effect. Compared to the control group, Len/OVA/yMSNs significantly inhibited tumor growth with an inhibition rate of 89.5% and suppressed tumor re-challenge by inducing specific anti-tumor immune memory. Further studies suggested that Len/OVA/yMSNs increased the infiltration of $CD8^+$ T cells by 39.1%, which was 4.9 times more than those in the negative control group (Fig. 5E). Overall, this study provides a new strategy for oral tumor vaccines, showcasing the potential anti-tumor effects of this vaccine based on yeast-glucan and its ability to induce long-term immune responses.

Gastric cancer stands as a predominant malignancy that severely undermines global public health, which is marked by an alarmingly high mortality rate. The insidious onset, characterized by inconspicuous early symptoms, often leads to a late-stage diagnosis[144,145]. The MG7 antigen (MG7-Ag) is highly overexpressed on the surface of gastric tumor cells, and it has been recognized as a crucial biomarker for the early detection and treatment of gastric cancer. This antigen, associated with gastric cancer, is identified as an exogenous protein by the body, capable of effectively stimulating specific immune responses[146]. Recently, researchers developed an oral MG7-Ag-encoding DNA vaccine to combat gastric cancer. Initially, they designed PCR primers containing MG7-Ag mimic epitopes and universal auxiliary T cell epitopes. Through PCR amplification, a fusion gene of these two epitopes was obtained. This fusion gene was cloned into the pcDNA3 plasmid and transfected into the attenuated *Salmonella* strain SL3261, and mice were orally immunized with this transfected strain. The experimental results revealed a significant increase, about 2.8 times compared to the control group, in the MG7-Ag antibody titer in the serum of the vaccinated mice. However, no statistical difference was observed among the three groups in the *in vitro* splenocyte proliferation experiment. Within 14 days after the tumor cell challenge, approximately 28% of mice in the MG7-Ag-vaccinated group did not develop tumors, while all mice in the control group did[88]. These results demonstrate that applying MG7-Ag as an antigen in oral immunotherapy for gastric cancer yields specific therapeutic efficacies, yet it necessitates ongoing enhancements for optimal effectiveness.

Lymphoma, a malignant tumor in the lymphatic system, including lymph nodes, spleens, and other immune tissues, is categorized mainly into Hodgkin's and non-Hodgkin's lymphoma[147,148]. This malignancy not only poses a severe threat to the physiological health of patients but also impacts their psychological well-being and quality of life, bringing substantial economic and emotional burdens to the patients and their families. The pervasive and detrimental effects of lymphoma underscore the urgency for advanced research and innovative therapeutic strategies to alleviate the multifaceted challenges faced by lymphoma patients[149].

Cell membrane-derived biomimetic platforms have ushered in a new era in the design of cancer vaccines, offering targeted delivery, antigen presentation, precise recognition, and immune stimulation[150]. A diverse array of membrane vesicles, including those from immune cells, blood cells, bacteria, and cancer cells, have been harnessed to develop innovative vaccine designs[151]. Through oral administration, Liu and his team[72] sought to elicit a robust anti-lymphoma immune response. In their innovative approach, they engineered nanovesicles, designated RGD-modified polymersomes (RGD-PEOPs), that paralleled bacterial membrane structural and functional attributes. These nanovesicles encapsulated the model antigen OVA, forming a vaccine formulation (OVA/RGD-PEOP) to evaluate its anti-tumor efficacy. The experimental findings demonstrated that OVA/RGD-PEOPs induced a robust antibody-mediated immune response. On Day 28 following oral administration, the IgG1 titers against OVA/RGD-PEOPs were 5.8 and 1.8 times higher compared to the groups treated with OVAs and OVA/PEOPs, respectively; similarly, the IgG2a titers were 6.0 and 2.2 times higher in comparison. Moreover, OVA/RGD-PEOPs effectively increased the amounts of $CD3^+CD8^+$ T cells within the spleens and tumor sites. The infiltration percentage of $CD3^+CD8^+$ T cells in the tumor tissues reached $20 \pm 7\%$, which was double that of the control group ($10 \pm 1\%$). Additionally, the IFN-γ and IL-4 levels of the OVA/RGD-PEOP group were 2.0 and 2.3 times higher than those in the control group, respectively. Tumor growth in mice treated with OVA/RGD-PEOPs was significantly inhibited, corroborating the above results.

Drawing on their understanding of gut microenvironment and tumor immunology, scientists have successfully developed oral vaccines for treating various cancers in preclinical studies through the application of gene engineering and nanomedicine technologies. These studies have laid a solid research foundation for subsequent researchers to overcome the clinical challenges associated with oral tumor vaccines.

6.2. Prevention of infectious diseases

In today's globalized world, infectious diseases can rapidly cross national borders. An epidemic in one country can quickly escalate into a global crisis, as evidenced by the 2009 H1N1 influenza

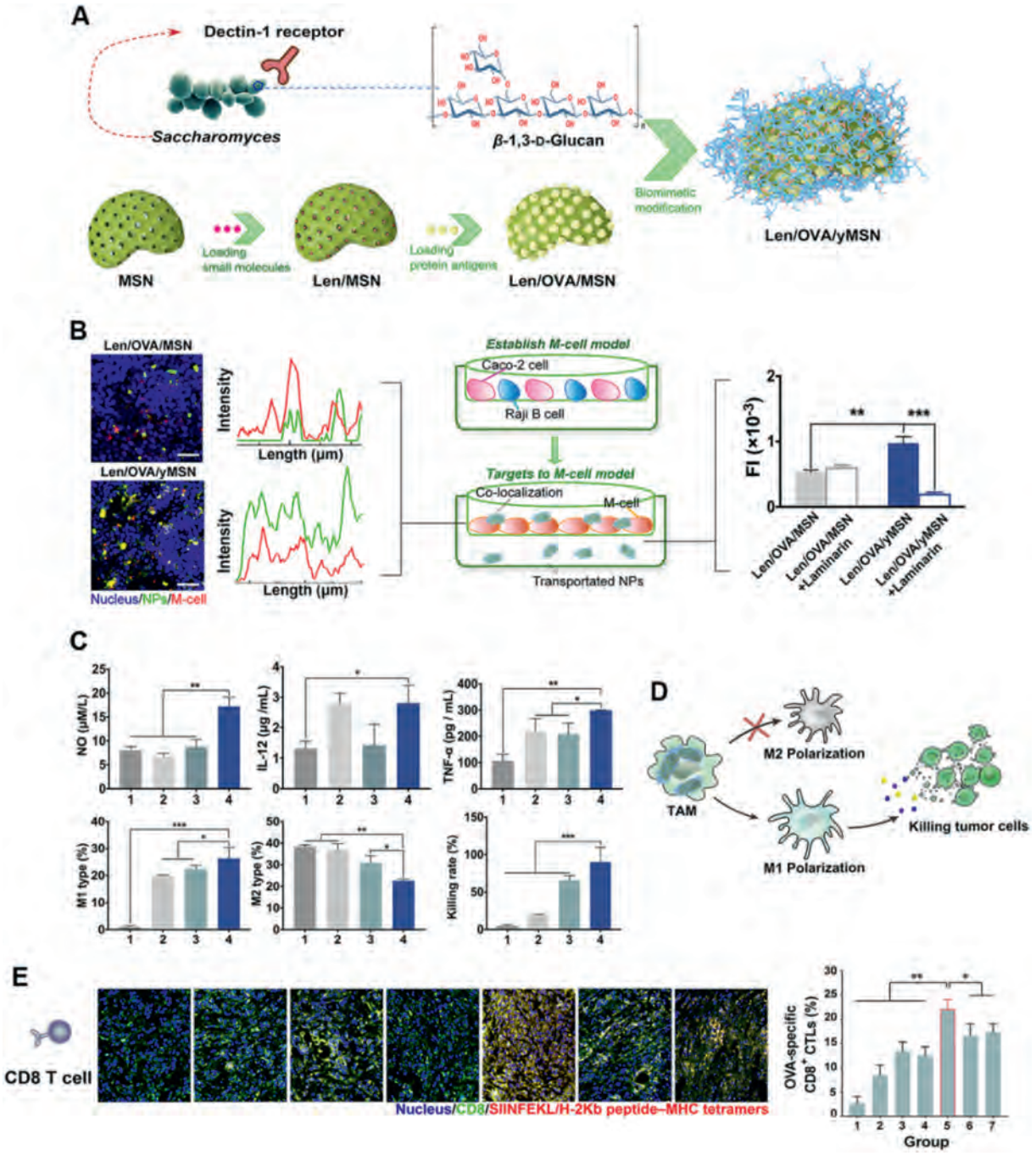

Figure 5 (A) Schematic illustration of the construction and therapeutic outcomes of Len/OVA/yMSN. (B) Diagrammatic representation depicting the establishment of the M-cell model and co-localization. (C) Relative quantification of the ratio of M1-type and M2-type macrophages induced by yMSN, secretion levels of cytokines (NO, IL-12, and TNF-α), and the cell-killing rate of macrophages carrying Len/OVA/yMSN to Hepa1-6 tumor cells ($n = 3$) (1. Saline; 2. MSN; 3. Len/OVA/MSN; 4. Len/OVA/yMSN). (D) Schematic depiction illustrating the innate immune responses triggered in macrophages by yMSN. (E) Immunofluorescence images of the tumors showing the polarization of the infiltration of OVA-specific $CD8^+$ T cells (1. saline; 2. Len; 3. Len/yMSN; 4. Len/OVA/MSN; 5. Len/OVA/yMSN; 6. OVA/yMSN; 7. Len/yMSN ± OVA/yMSN). Reprinted with the permission from Ref. 51. Copyright © 2022 Elsevier Ltd.

pandemic and the 2020 COVID-19 pandemic[152,153]. Understanding the research advancements in oral vaccines for preventing infectious diseases is essential in combating the infringement of contagious diseases in human society. The current preclinical, clinical, and market results of oral anti-infective vaccinations are summarized in Tables 1 and 5[154−176].

6.2.1. Oral vaccination strategies against infectious viruses

Viral infection has wreaked havoc globally, leading to significant mortality and morbidity and imposing a heavy burden on public health systems and the global economy[177,178]. From historical smallpox to the modern COVID-19 pandemic, viral infectious diseases continuously threaten human well-being, causing

Table 5 Examples of oral ant-infectious vaccines.

Vaccine name	Infectious disease model	Oral delivery system	Ref.
CaP-Chi-Alg@OVA	−	Chitosan- and alginate-coated CaP NPs	154
OVA@Al-MOFs/YCs	−	A biomimetically mineralized aluminum-based metal−organic framework (Al-MOF) system	155
FS30D/PLGA/ProtAg + TLRL	−	PLGA NPs were coated with methacrylate-based polymer Eudragit FS30D21	156
GP-OVA	−	β-glucan particles (GP)	157
OVA + MPLA + PLGA NPs	−	Monophosphoryl lipid A (MPLA) were incorporated in polymeric NPs based on PLGA	158
NTD + RBD + S2	SARS-CoV-2	*Salmonella* type III secretion system	159
Pjwnl432 in VLP	HIV	Virus-like particles	160
SHO4/SHO8	Edwardsiellosis	Starch hydrogel	161
−	Haemorrhagic septicemia virus/ *Streptococcus parauberis*	Chitosan-PLGA	162
pPG612-T7g10-VP2/LP	Infectious bursal disease virus	*Lactobacillus plantarum* strains	163
rLpPG-2-FaeG	ETEC	rLpPG-2-LTAK63-co-LTB or rLpPG-2-LTAK63-fu-LTB	164
EDIII-1-4	Dengue virus	Lettuce chloroplasts	165
CS/p146 NPs	Hepatitis E virus	Chitosan NPs	166
Lettuce-E1E2	Hepatitis C virus	Lettuce	167
GRA4	Toxoplasma	Tobacco plants	168
SV/TGLMSN	Influenza virus	MSNs	169
Eno1P	*Candidiasis*	*Saccharomyces cerevisiae* cells	170
Lp-pPG-Malt	*Aeromonas hydrophila*	Recombinant *Lactobacillus plantarum*	171
EBY100/pYD1	*H. pylori*	*Saccharomyces cerevisiae*	172
Mix-Ag + adjuvants	*H. pylori*	HP55/PLGA	173
GP5	Porcine reproductive and respiratory syndrome virus	Transgenic banana	174
BmpB-CKS9-WSC-PLGA MPs	*Brachyspira hyodysenteriae*	Porous PLGA microparticles coated with M cell homing peptide-coupled chitosan	175
OV	*Mycoplasma hyopneumoniae*	Nanostructured mesoporous silica (SBA-15)	176

−, not applicable.

extensive social and economic issues, including strained healthcare resources, economic activity disruption, and global supply chain breakdowns[179]. This highlights the urgent need for more robust public health measures and accelerated vaccine research and development[180]. Indeed, oral vaccines against infectious diseases have garnered notable interest in the scientific community owing to their distinct benefits.

Influenza viruses pose a global threat to human health, leading to unpredictable yet recurrent pandemics. This virus has been responsible for four significant pandemics over the past century[181,182]. Inspired by the lipid-specific absorption pathway, Lu and his team[169] fabricated a biomimetic intestinal lymphatic delivery system based on large-pore mesoporous silica NPs (LMSN), whose surface was further functionalized with dioleoyl phosphatidylethanolamine (PEG-DP) (Fig. 6A). The resultant TGLMSN@PEG-DPs possessed strong mucosal permeability and lymphatic tissue-targeting capabilities. Their transport levels in the duodenum, jejunum, and ileum were 4.7, 3.9, and 4.5 times higher than those of TGLMSNs, respectively. Four hours post-administration, the lymphatic tissue-targeting ability of TGLMSN@PEG-DPs was evidenced by the apparent fluorescence signals in the MLNs (Fig. 6B). Moreover, compared to other control groups, TGLMSN@PEG-DPs significantly promoted the expression levels of CD40, CD80, CD86, MHC, and other molecules in BMDCs, suggesting their potential as a vaccine adjuvant to activate immune cells (Fig. 6C). Oral administration of TGLMSN@PEG-DPs carrying influenza antigen elicited strong specific mucosal IgA responses in the intestine, nasal cavity, lung, and vagina, and high hemagglutination inhibition (HI) antibody titers were also detected in the blood (Fig. 6D). This biomimetic delivery system undoubtedly provides a new insight into oral anti-influenza vaccines.

Inspired by the challenges of oral antigens being easily degraded in the GIT and not efficiently taken up by M cells, Shastri and colleagues[183] developed an oral influenza vaccine based on enteric-coated microparticles (MP F1 and MP F2). They detected that the levels of serum IgG antibody produced by a low dose of MP F1 (5 μg antigen) were comparable to those of MP F2 (5 μg antigen). However, the immunological response was significantly amplified with a higher dose of MP F2 (20 μg antigen), producing IgG antibody levels approximately twice of that of MP F1 (20 μg antigen). Moreover, influenza virus challenge tests revealed that both MP F formulations offered protection against homologous and heterologous influenza viruses, with protection rates of 50% for MP F1 and 80% for MP F2. These results indicate that a reasonable antigen dose and adjuvant design will benefit the development of oral influenza vaccines.

Hepatitis viruses represent a significant contributor to the global health dilemma, with hepatitis B virus (HBV) and hepatitis C virus (HCV) being particularly noteworthy culprits. An estimated 296 million individuals globally are afflicted with chronic HBV, while around 170 million are chronically infected with

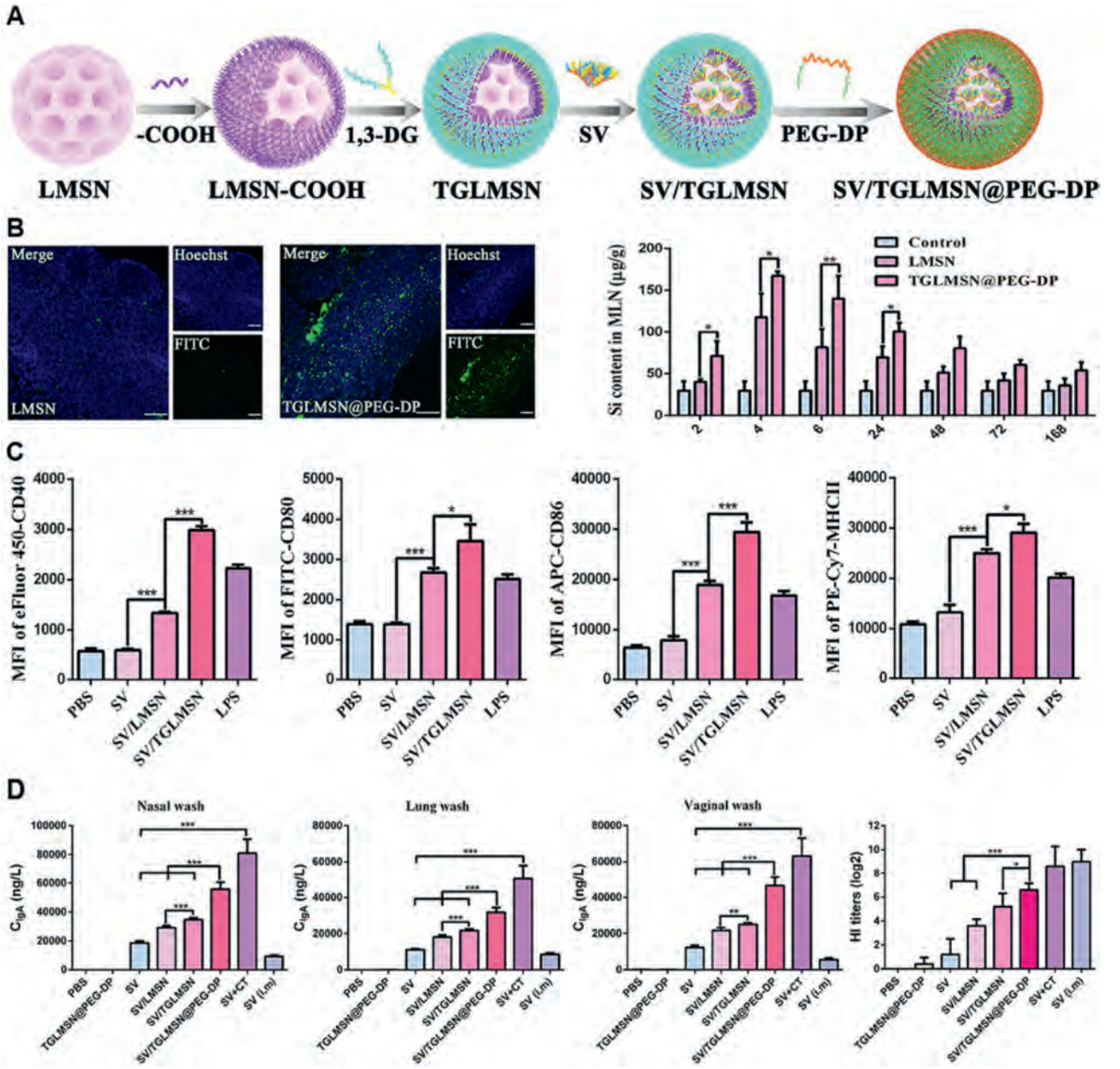

Figure 6 Schematic illustration of the construction procedure of the influenza split vaccine bionic delivery platform (SV/TGLMSN@PEG-DP). (B) Fluorescent microscopy images of NPs in the MLN tissue sections after oral administration for 4 h. Scale bars, 100 μm. Si element contents in MLNs after oral administration of LMSNs and TGLMSN@PEG-DPs at different time points ($n = 3$). (C) Flow cytometric examination for the expression levels of BMDC maturation markers (CD40, CD80, CD86, and MHC II). (D) Specific mucosal IgA levels in nasal wash, lung wash, and vaginal wash. Serum HI titers were detected by HI assay using 4 hemagglutinating units of the HINI influenza virus strain. Reprinted with the permission from Ref. 169. Copyright © 2023 Elsevier Ltd.

HCV[184-186]. Kong and colleagues[187] explored the feasibility of oral immunization using transgenic plants expressing hepatitis B surface antigen (HBsAg). Initially, they prepared recombinant HBsAg using a yeast expression system, and the obtained antigen was orally administered to mice in combination with mucosal adjuvant (cholera toxin, CT), delivered once a week for two consecutive weeks. A control mouse group received a combination of PBS and CT. Six weeks post-administration, mice were given a vaccination of yeast-derived HBsAg as a booster immunization. The results revealed that mice receiving oral HBsAg presented an immediate secondary antibody response, with a response peak of 175 mIU/mL, while no HBsAg-specific antibody was detected in the control group. This study demonstrates that oral administration of HBsAg expressed in transgenic potatoes causes a lasting immune memory response in mice. Upon receiving the yeast-derived HBsAg vaccine, only the mice with oral administration of HBsAg transgenic potatoes exhibited a strong secondary antibody response. This suggested that the immune response elicited by the oral vaccine is an authentic immunological memory response rather than just a primary response caused by the injected HBsAg.

Thus, the study underscored that oral plant-derived HBsAg could induce an immune response in mice and generate a sustained immune memory response.

Clarke and colleagues[167] developed another innovative approach using *Agrobacterium*-mediated transient expression technology to produce hepatitis C virus dimeric antigens (HCV E1E2 and HCV E1E2ΔN6) in an edible form of lettuce. The immunogenicity of HCV E1E2 and HCV E1E2ΔN6 was subsequently assessed. Mice orally vaccinated with HCV E1E2 and HCV E1E2ΔN6 heterodimers generated low HCV IgM antibodies, with no detectable HCV IgG antibodies. Interestingly, HCV IgA antibodies were identified in the feces of mice orally vaccinated with HCV E1E2ΔN6 heterodimers. On the contrary, mice inoculated with HCV E1E2 and HCV E1E2ΔN6 heterodimers by intramuscular injection produced a strong antibody immune response. Drawing upon the elucidated data, the dimer exhibited pronounced immunogenicity, evoking a robust immune response *via* the intramuscular injection. Nevertheless, the lettuce antigen demonstrated susceptibility to the rigorous conditions of the gastrointestinal milieu, culminating in its degradation and, consequently, the thwarting of the anticipated oral vaccine-mediated immune responses[61].

Since the declaration of COVID-19 as a global pandemic by the World Health Organization on March 11, 2020, the virus has infected over 300 million individuals worldwide and has been responsible for millions of deaths. This situation has posed a significant threat to global public health infrastructure. Immunization remains the most effective strategy for curtailing the transmission of COVID-19[188]. Langel et al.[189] developed an oral adenovirus type 5 vector-loaded vaccine expressing the SARS-CoV-2 spike protein (Oral r-Ad-S). They demonstrated that administrating this vaccine *via* oral or intranasal routes (IN r-Ad-S) in hamsters induced high titers of IgG and IgA. Upon challenging these vaccinated hamsters with SARS-CoV-2, a significant reduction in viral RNA and viral load was observed in the nasal and lung tissues of the Oral r-Ad-S and IN r-Ad-S groups, in compared with the oral PBS group. Histopathological examination of the lungs revealed a marked decrease in pathology in the Oral r-Ad-S and IN r-Ad-S groups, with lung pathology scores of less than or equal to 1, in contrast to a score of 4 in the Mock group and 2 in the IM r-Ad-S group. Subsequently, they established a model of SARS-CoV-2 infection post-mucosal vaccination using a relatively high dose of virus and exposed these animals to naive animals within a containment chamber. It was found that mucosal vaccination significantly reduced the transmission of SARS-CoV-2 from the vaccinated hamsters to the unvaccinated hamsters. The data presented herein suggest that mucosal immunization represents a viable strategy for mitigating SARS-CoV-2 disease and its airborne transmission.

6.2.2. Oral vaccination strategies against infectious bacteria

Bacterial infections have contributed significantly to the global health burden, and their negative impact on human health has become increasingly pronounced in recent years. Annually, infections result in the death of 13.7 million individuals worldwide, with 7.7 million of these fatalities associated with 33 specific bacterial pathogens and 11 infectious syndromes. These deaths account for 13.6% of the total global mortality and 56.2% of sepsis-related deaths[190]. Considering this data and the prevailing circumstances, bacterial infections pose a grave threat to global health. This highlights the urgent need for more robust public health measures and accelerated the development of vaccines to prevent bacterial infections[191-193]. Indeed, oral vaccines to prevent bacterial infection have garnered notable interest in the scientific community owing to its distinct set of benefits.

H. pylori is a spiral-shaped bacterium that infects over half of the world's population. In most hosts, *H. pylori* infection leads to asymptomatic chronic gastritis, which is closely associated with the progression of peptic ulcers and gastric cancer[194,195]. Currently, vaccination is considered an economically viable alternative to control the prevalence of *H. pylori*[196]. Cen et al.[172] successfully cloned *H. pylori*'s UreB and VacA genes into plasmid pYD1 and electroporated them into the brewing yeast EBY100. They utilized EBY100/pYD1-UreB, EBY100/pYD1-VacA, and EBY100/pYD1-UreB + EBY100/pYD1-VacA as oral vaccines. It was found that these vaccines effectively activated the body's immune responses, generating specific IgG and IgA antibodies, with antibody levels increasing over time gradually. In contrast, no significant antibody levels were observed in the control group. Thereafter, they conducted a *H. pylori* challenge test, where mice receiving oral vaccine showed a substantial reduction in *H. pylori* colonization after infection, with the bacterial colonization efficiency being about 1/10 of that in the control group.

Zhang and colleagues[197] developed an oral nanovaccine composed of poly-L-arginine (R12), recombinant Urease B subunit (rUreB), and a hydrophilic PEG derivative (PEGSuc), designated as R12/rUreB/PEGSuc, and aimed at preventing *H. pylori* infection. Animal studies indicated that the R12/rUreB/PEGSuc nanovaccines significantly elevated the levels of IgG, IgG1, and IgG2a in mice and enhanced the quantities of splenic $CD8^+$ T cells producing IFN-γ and $CD4^+$ T cells secreting IL-4, in compared with the groups receiving the treatment of rUreB alone or R12/rUreB combination. After that, challenge trials with *H. pylori* revealed that the R12/rUreB/PEGSuc vaccine resulted in a 1.6 $\log_{10}$ reduction in the bacterial load, in contrast to a colonization count of 5.9 $\log_{10}$ in the control group, 5.6 $\log_{10}$ with rUreB, and 5.1 $\log_{10}$ with R12/rUreB. Furthermore, the inflammatory scores of gastric tissues indicated that mice vaccinated with R12/rUreB/PEGSuc exhibited an average inflammatory score of 0.2, approximately 1/12 of the control group (average score of 2.5). These observations demonstrate that oral vaccines can protect mice from *H. pylori* infection.

Acute infectious diarrhea stands as the second most prevalent cause of death for children in developing countries, surpassed only by acute respiratory diseases, which accounts for approximately 20% of all mortalities within this demographic[198]. Enterotoxigenic *E. coli* (ETEC) emerges as one of the primary pathogens responsible for these acute diarrheal conditions, underscoring the pressing need to develop a more efficacious vaccine to combat ETEC infections[199]. To protect mice from ETEC infection through oral immunization, Yu and colleagues[164] tackled this challenge by constructing a recombinant *Lactobacillus casei* vaccine expressing multivalent enterotoxins. Initially, they divided the mice into four groups (rLpPG-2, rLpPG-2-FaeG, rLpPG-2-FaeG + rLpPG-2-LTAK63-fu-LTB, and rLpPG-2-FaeG + rLpPG-2-LTAK63-co-LTB), and each group receiving these recombinant *lactobacillus* vaccines with the same dosage of antigens. Following immunization, they collected serum, fecal, and mucosal samples, assessed the levels of FaeG-specific antibodies, and evaluated the extent of lymphocyte proliferation before subjecting them to an ETEC challenge test. The findings revealed that compared to the monovalent vaccine (rLpPG-2-FaeG), multivalent vaccines (rLpPG-2-FaeG + rLpPG-2-LTAK63-fu-LTB and rLpPG-2-FaeG + rLpPG-2-LTAK63-co-LTB) were able to induce higher

levels of FaeG-specific antibodies (IgG and IgA), more effectively stimulate spleen T lymphocyte proliferation, and provide 100% protection against ETEC challenge. On the contrary, under the same condition, the monovalent vaccine (rLpPG-2-FaeG) only offered an 80% protection rate. This study lays significant theoretical and experimental groundwork for developing oral vaccines to prevent intestinal infections.

In addition to oral vaccines developed to combat the above mentioned human bacterial threats, academic research has also delved into oral vaccines targeting other bacterial strains. For instance, oral vaccines have been explored to counteract bacteria severely impeding the economic growth of the fisheries and aquaculture industries, such as *Edwardsiella*[161], *Vibrio anguillarum*[200], *Mycoplasma hyopneumoniae*[176], *Aeromonas hydrophila*[171,201], and *Aeromonas salmonicida*[202].

6.2.3. Oral vaccination strategies against other infectious microorganisms

The parasitic protozoan *Toxoplasma gondii* causes toxoplasmosis, which can infect most mammals and birds. In the realm of human medicine, *Toxoplasma gondii* leads to complications in pregnant women and individuals with compromised immune systems. Similarly, this parasite results in complications in veterinary medicine in animals with weakened immunity. Given these challenges, developing an effective vaccine against *Toxoplasma gondii* holds significant importance[203,204]. Del and colleagues[168] embarked on a quest to develop an oral vaccine to prevent toxoplasmosis. Chloroplast engineering technology was employed to introduce the *Toxoplasma gondii* GRA4 antigen gene into tobacco chloroplasts. Subsequently, the obtained GRA4 vaccine was used to immunize mice *via* the oral route, and its immunogenicity and protective efficacies were assessed. The results suggested that the oral GRA4 vaccine effectively induced the production of GRA4-specific antibodies (IgG and IgA), with the highest IgG titer reaching 1000 and the maximal IgA concentration in the intestinal lavage fluid being 32.6 ± 2.5 ng/mL. Furthermore, this vaccine significantly promoted the expression levels of relevant cytokines in the mice, including IFN-γ, IL-4, and IL-10. Notably, the number of brain cysts in the vaccinated mice was substantially lower than in the control and PBS groups, indicating the vaccine's effective defense against the *Toxoplasma gondii* challenge. These experiments demonstrate that an oral vaccine can elicit *toxoplasma*-specific antibodies and cellular immune responses, protecting against *toxoplasma* infection.

Candida is a common fungal, manifesting in various forms depending on the affected body area. It can cause infections like oral thrush, vaginal candidiasis, and invasive candidiasis, which affects the bloodstream or internal organs like the kidney, heart, and brain[205,206]. Shibasaki and colleagues[170] opted for *Candida albicans*' Eno1p protein as a model antigen, employing cell surface display technology to express Eno1p on the exterior of *Saccharomyces cerevisiae* (*S. cerevisiae*). This technique used a-agglutinin protein on the surface of *S. cerevisiae* to anchor the target antigenic protein, exposing heterologous proteins externally. The cell surface display method of *S. cerevisiae* has been widely applied to express red sea bream iridovirus antigens. Subsequently, they investigated the variations of anti-*Candida* antibody levels and the survival of mice subjected to lethal doses of *Candida* infection by oral administration, intranasal administration, and subcutaneous injection of the Eno1p yeast vaccine. It was found that oral and intranasal administration significantly elevated anti-Eno1p antibody titers (maximum titer of 5.2×10^4) and prolonged the survival of *Candida*-infected mice (60% survival). These results suggest that oral vaccines generated using yeast molecular display systems are convenient for preventing infectious diseases. This system offers rapid, convenient advantages, enabling quicker provision of antigenic proteins without requiring an antigen purification process like that required for *E. coli*-produced recombinant proteins.

7. Future directions

Aside from the classical oral vaccines presented in Table 1, most oral vaccine research remains in the preclinical stage rather than in clinical trials. The successful development of oral vaccines for diseases, such as poliovirus and rotavirus, is attributed to their primary mode of invasion and infection through the GIT. Oral administration of these vaccines can mimic the natural infection process of these viruses, more effectively stimulating the intestinal mucosal immune response and the body's innate immune response, resulting in enhanced protection[207]. Viruses like influenza and COVID-19 primarily infect the host through the respiratory tract, where mucosal immunity plays a crucial role. Given that the GIT houses the largest number of immune cells in the body, oral vaccination holds the potential to elicit a strong mucosal immune response[208]. Therefore, among the various oral tumor vaccines, those targeting cancers originating in the gut are more likely to achieve clinical breakthroughs[209].

Developing more effective oral vaccines remains a formidable challenge, yet their immense potential in combating cancers and infectious diseases continues to captivate scientists[2,9,210,211]. A multifaceted understanding of this approach is imperative to pave the way for more efficacious oral vaccines. First and foremost, a profound knowledge of the interaction mechanism between the intestinal mucosal immune system and vaccines is crucial[212]. This interaction encompasses a series of intricate biological processes. Vaccines aim to induce immune memory and protective immune responses, with the intestinal mucosal immune system playing a pivotal role in this endeavor[213]. Researchers have to delve deeper into the interplay between the intestinal mucosal immune system and oral vaccines to design and construct more effective vaccines[214].

Developing antigens with multiple epitopes for oral vaccines is essential. An epitope represents a specific region on an antigen molecule that can be recognized by the immune system and bind to antibodies or T-cell receptors. Designing antigens with multiple epitopes can enhance the potency and breadth of oral vaccines, which is critical for preventing the immune escape of tumor cells and frequently mutating viruses, such as influenza virus and SARS-CoV-2[215]. It alleviates the immunological resistance that can manifest with oral vaccines[216]. Cutting-edge advancements in antigen prediction platforms and genetic engineering technology have propelled the development of multi-epitope antigens[217,218]. Notably, the strategic development of an antigen delivery platform tailored for oral vaccines is crucial. An optimal delivery system should exhibit enhanced mucosal penetration, targeted delivery to intestinal cells, notably M cells, and a specific affinity towards APCs. For antigens characterized by suboptimal immunogenicity, the delivery platform should bolster their immunogenicity, ameliorate the challenges associated with antigen internalization and cross-presentation, and promote APC maturation[55,219]. Achieving this necessitates a synergistic endeavor between immunology and pharmaceutics experts.

Currently, the approved adjuvants are only used for injectable vaccines (*e.g.*, CpG, Freund's adjuvant, Alum, AS01, AS03,

AS04, and LNP)[220]. There is no adjuvant specifically approved for oral vaccines[221]. Only DMLT has undergone phase I/II clinical trials (NCT02531802)[222,223], while most other oral adjuvants are still in the experimental stages. This significantly limits the widespread application of oral vaccines in cancer treatment and infectious disease prevention. Therefore, the development of oral vaccine adjuvants is urgently needed. An ideal oral vaccine adjuvant should have part or all of the following characteristics. (i) It can enhance the immunogenicity of antigens and effectively promote the body's adaptive immune responses[224]. (ii) It possesses carrier properties that facilitate its safe passage through the GIT. (iii) It is able to effectively target and activate intestinal APCs, which are typically tolerant and less reactive, making it challenging to elicit strong mucosal immune responses[225]. Last but not least, the development of composite adjuvants combining biological and non-biological materials is an effective strategy to enhance the immune efficacy of oral vaccines.

For oral tumor vaccines, the design of personalized vaccines predicated on an individual's tumor genomic profile and immunological response patterns is pivotal. Vaccines tailored to incorporate patient-specific tumor antigens can significantly augment the immune system's capacity to identify and assail tumor cells. Meanwhile, integrating oral tumor vaccines with bespoke therapeutic strategies, including surgical excision, immune checkpoint inhibitors, and adoptive cellular immunotherapy, may potentiate the overall therapeutic impact of oral tumor vaccines[226,227]. In the end, upon the successful resolution of the predominant challenges inherent in oral vaccines, the future of vaccine exploitation endeavors is anticipated to prioritize oral vaccination strategies, thereby broadening their application in the prevention of global pandemics (*e.g.*, influenza, COVID-19, HIV, and hepatitis B) and the therapeutic management of malignancies (*e.g.*, CRC, gastric cancer, and breast cancer). Vaccine research and clinical application necessitate a collaborative effort involving corporations, governments, research institutions, and regulatory bodies. Although the development of oral vaccines presents significant challenges, it represents a revolutionary field with broad application prospects.

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Author contributions

Kun Yang: Writing − review & editing, Writing − original draft, Investigation, Conceptualization. Jinhua Liu: Writing − original draft, Conceptualization. Yi Zhao: Writing − original draft. Haiting Xu: Writing − original draft. Menghang Zu: Writing − original draft. Baoyi Li: Writing − original draft. Xiaoxiao Shi: Writing − review & editing. Rui L. Reis: Writing − review & editing. Subhas C. Kundu: Writing − review & editing. Bo Xiao: Writing − review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Allaire JM, Crowley SM, Law HT, Chang SY, Ko HJ, Vallance BA. The intestinal epithelium: central coordinator of mucosal immunity. *Trends Immunol* 2018;**39**:677−96.
2. Yue Y, Xu J, Li Y, Cheng K, Feng Q, Ma X, et al. Antigen-bearing outer membrane vesicles as tumour vaccines produced *in situ* by ingested genetically engineered bacteria. *Nat Biomed Eng* 2022;**6**: 898−909.
3. Ogra PL, Faden H, Welliver RC. Vaccination strategies for mucosal immune responses. *Clin Microbiol Rev* 2001;**14**:430−45.
4. Riedel S. Edward Jenner and the history of smallpox and vaccination. *SAVE Proc* 2005;**18**:21−5.
5. Sabin AB. Oral poliovirus vaccine: history of its development and use and current challenge to eliminate poliomyelitis from the world. *J Infect Dis* 1985;**151**:420−36.
6. Sabin AB. Properties and behavior of orally administered attenuated poliovirus vaccine. *JAMA, J Am Med Assoc* 1957;**164**:1216−23.
7. Sabin AB, Hennessen WA, Winsser J. Studies on variants of poliomyelitis virus. I. Experimental segregation and properties of avirulent variants of three immunologic types. *J Exp Med* 1954;**99**:551−76.
8. Vesikari T, Giaquinto C, Huppertz HI. Clinical trials of rotavirus vaccines in Europe. *Pediatr Infect Dis J* 2006;**25**:S42−7.
9. Serradell MC, Rupil LL, Martino RA, Prucca CG, Carranza PG, Saura A, et al. Efficient oral vaccination by bioengineering virus-like particles with protozoan surface proteins. *Nat Commun* 2019;**10**:361.
10. Alias NAR, Hoo WPY, Siak PY, Othman SS, Mohammed Alitheen NB. Effect of secretion efficiency of mutant KRAS neoantigen by *Lactococcus* lactis on the immune response of a mucosal vaccine delivery vehicle targeting colorectal cancer. *Int J Mol Sci* 2023;**24**:8928.
11. Lee SH, Mizutani N, Mizutani M, Luo Y, Zhou H, Kaplan C, et al. Endoglin (CD105) is a target for an oral DNA vaccine against breast cancer. *Cancer Immunol Immunother* 2006;**55**:1565−74.
12. Shen Y, Qiu L. Effective oral delivery of gp100 plasmid vaccine against metastatic melanoma through multi-faceted blending-by-blending nanogels. *Nanomedicine* 2019;**22**:102114.
13. Roider E, Jellbauer S, Kohn B, Berchtold C, Partilla M, Busch DH, et al. Invasion and destruction of a murine fibrosarcoma by *Salmonella*-induced effector CD8 T cells as a therapeutic intervention against cancer. *Cancer Immunol Immunother* 2011;**60**:371−80.
14. Chevalier C, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, et al. Gut microbiota orchestrates energy homeostasis during cold. *Cell* 2015;**163**:1360−74.
15. Moor AE, Harnik Y, Ben-Moshe S, Massasa EE, Rozenberg M, Eilam R, et al. Spatial reconstruction of single enterocytes uncovers broad zonation along the intestinal villus Axis. *Cell* 2018;**175**:1156.
16. Pelaseyed T, Bergström JH, Gustafsson JK, Ermund A, Birchenough GMH, Schütte A, et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev* 2014;**260**:8−20.
17. Snoeck V, Goddeeris B, Cox E. The role of enterocytes in the intestinal barrier function and antigen uptake. *Microb Infect* 2005;**7**: 997−1004.
18. Hershberg RM, Mayer LF. Antigen processing and presentation by intestinal epithelial cells - polarity and complexity. *Immunol Today* 2000;**21**:123−8.
19. Dolan B, Ermund A, Martinez-Abad B, Johansson MEV, Hansson GC. Clearance of small intestinal crypts involves goblet cell

mucus secretion by intracellular granule rupture and enterocyte ion transport. *Sci Signal* 2022;**15**:eabl5848.
20. Gustafsson JK, Johansson MEV. The role of goblet cells and mucus in intestinal homeostasis. *Nat Rev Gastroenterol Hepatol* 2022;**19**: 785−803.
21. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. *Nat Rev Immunol* 2014;**14**:667−85.
22. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology (New York, N Y, 1943)* 1977;**72**:440−51.
23. Beumer J, Clevers H. Cell fate specification and differentiation in the adult mammalian intestine. *Nat Rev Mol Cell Biol* 2020;**22**:39−53.
24. Jung C, Hugot J-P, Barreau F. Peyer's patches: the immune sensors of the intestine. *Int J Inflamm* 2010;**2010**:1−12.
25. Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, et al. Natural Aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Sci Technol Humanit* 2011; **334**:1561−5.
26. Knoop KA, Newberry RD. Isolated lymphoid follicles are dynamic reservoirs for the induction of intestinal IgA. *Front Immunol* 2012;**3**: 84.
27. Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, et al. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity (Camb, Mass)* 2008;**29**:261−71.
28. Hitotsumatsu O, Hamada H, Naganuma M, Inoue N, Ishii H, Hibi T, et al. Identification and characterization of novel gut-associated lymphoid tissues in rat small intestine. *J Gastroenterol* 2005;**40**: 956−63.
29. Rosner AJ, Keren DF. Demonstration of M cells in the specialized follicle-associated epithelium overlying isolated lymphoid follicles in the gut. *J Leukoc Biol* 1984;**35**:397−404.
30. Keren DF, Holt PS, Collins HH, Gemski P, Formal SB. The role of Peyer's patches in the local immune response of rabbit ileum to live bacteria. *J Immunol* 1978;**120**:1892−6.
31. Moghaddami M, Cummins A, Mayrhofer G. Lymphocyte-filled villi: comparison with other lymphoid aggregations in the mucosa of the human small intestine. *Gastroenterology (New York, N Y, 1943)* 1998; **115**:1414−25.
32. Sabine A, Davis MJ, Bovay E, Petrova TV. Characterization of mouse mesenteric lymphatic valve structure and function. *Methods Mol Biol* 2018;**1846**:97−129.
33. Macpherson AJ, Smith K. Mesenteric lymph nodes at the center of immune anatomy. *J Exp Med* 2006;**203**:497−500.
34. Giudice EL, Campbell JD. Needle-free vaccine delivery. *Adv Drug Deliv Rev* 2006;**58**:68−89.
35. Bhattacharjee A, Hand TW. Role of nutrition, infection, and the microbiota in the efficacy of oral vaccines. *Clin Sci* 2018;**132**: 1169−77.
36. Ramirez JEV, Sharpe LA, Peppas NA. Current state and challenges in developing oral vaccines. *Adv Drug Deliv Rev* 2017;**114**:116−31.
37. Holmgren J, Svennerholm AM. Vaccines against mucosal infections. *Curr Opin Immunol* 2012;**24**:343−53.
38. Tawde SA, Chablani L, Akalkotkar A, D'Souza C, Chiriva-Internati M, Selvaraj P, et al. Formulation and evaluation of oral microparticulate ovarian cancer vaccines. *Vaccine* 2012;**30**:5675−81.
39. Bartlett S, Eichenberger RM, Nevagi RJ, Ghaffar KA, Marasini N, Dai Y, et al. Lipopeptide-based oral vaccine against hookworm infection. *J Infect Dis* 2020;**221**:934−42.
40. Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, et al. Enterotypes in the landscape of gut microbial community composition. *Nat Microbiol* 2018;**3**:8−16.
41. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 1995;**16**:351−80.
42. Coria LM, Risso GS, Guaimas FF, Ferrero MC, Bruno L, Pasquevich KA, et al. Oral co-administration of a bacterial protease inhibitor in the vaccine formulation increases antigen delivery at the intestinal epithelial barrier. *J Control Release* 2019;**293**:158−71.
43. Zhang Y, Kang R, Zhang X, Pang G, Li L, Han C, et al. A programmable oral bacterial hydrogel for controllable production and release of nanovaccine for tumor immunotherapy. *Biomaterials* 2023; **299**:122147.
44. Gilmartin AA, Petri Jr WA. Exploring the role of environmental enteropathy in malnutrition, infant development and oral vaccine response. *Philos Trans R Soc Lond B Biol Sci* 2015;**370**:20140143.
45. Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms. *Nat Rev Immunol* 2022;**22**:33−46.
46. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, et al. DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*−specific immune tolerance, and asthma protection. *J Clin Investig* 2012;**122**:1082−96.
47. Ruff WE, Greiling TM, Kriegel MA. Host−microbiota interactions in immune-mediated diseases. *Nat Rev Microbiol* 2020;**18**:521−38.
48. Lavelle EC, Ward RW. Mucosal vaccines - fortifying the frontiers. *Nat Rev Immunol* 2022;**22**:236−50.
49. Holmgren J, Czerkinsky C, Eriksson K, Mharandi A. Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges. *Vaccine* 2003;**21**:S89−95.
50. Naciute M, Niemi V, Kemp RA, Hook S. Lipid-encapsulated oral therapeutic peptide vaccines reduce tumour growth in an orthotopic mouse model of colorectal cancer. *Eur J Pharm Biopharm* 2020;**152**: 183−92.
51. Mao Y, Wang X, Chen C, Zhao Q, Liu Y, Zhang J, et al. Immune-awakening Saccharomyces-inspired nanocarrier for oral target delivery to lymph and tumors. *Acta Pharm Sin B* 2022;**12**:4501−18.
52. Holmgren J, Nordqvist S, Blomquist M, Jeverstam F, Lebens M, Raghavan S. Preclinical immunogenicity and protective efficacy of an oral *Helicobacter pylori* inactivated whole cell vaccine and multiple mutant cholera toxin: a novel and non-toxic mucosal adjuvant. *Vaccine* 2018;**36**:6223−30.
53. Xu YG, Guan XT, Liu ZM, Tian CY, Cui LC. Immunogenicity in swine of orally administered recombinant *lactobacillus* plantarum expressing classical swine fever virus E2 protein in conjunction with Thymosin alpha-1 as an adjuvant. *Appl Environ Microbiol* 2015;**81**: 3745−52.
54. Frizzell H, Woodrow KA. Biomaterial approaches for understanding and overcoming immunological barriers to effective oral vaccinations. *Adv Funct Mater* 2020;**30**:1907170.
55. Lee Y, Kamada N, Moon JJ. Oral nanomedicine for modulating immunity, intestinal barrier functions, and gut microbiome. *Adv Drug Deliv Rev* 2021;**179**:114021.
56. Zhang Y, Li M, Du G, Chen X, Sun X. Advancedoral vaccine delivery strategies for improving the immunity. *Adv Drug Deliv Rev* 2021;**177**:113928.
57. Ou B, Yang Y, Lv H, Lin X, Zhang M. Current progress and challenges in the study of adjuvants for oral vaccines. *BioDrugs* 2023;**37**: 143−80.
58. Jiang H, Wang Q, Sun X. Lymph node targeting strategies to improve vaccination efficacy. *J Control Release* 2017;**267**:47−56.
59. Duerkop BA, Vaishnava S, Hooper LV. Immune responses to the microbiota at the intestinal mucosal surface. *Immunity (Camb, Mass)* 2009;**31**:368−76.
60. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. *Nat Med* 2005;**11**:S45−53.
61. Xu Y, Shrestha N, Preat V, Beloqui A. Overcoming the intestinal barrier: a look into targeting approaches for improved oral drug delivery systems. *J Control Release* 2020;**322**:486−508.
62. Nandi D, Pathak S, Verma T, Singh M, Chattopadhyay A, Thakur S, et al. T cell costimulation, checkpoint inhibitors and anti-tumor therapy. *J Biosci (Tueb)* 2020;**45**:1−36.
63. Irvine DJ. Function-specific variations in the immunological synapses formed by cytotoxic T cells. *Proc Natl Acad Sci U S A* 2003;**100**: 13739−40.

64. Brandsma AM, Bondza S, Evers M, Koutstaal R, Nederend M, Jansen JHM, et al. Potent Fc receptor signaling by IgA leads to superior killing of cancer cells by neutrophils compared to IgG. *Front Immunol* 2019;**10**:704.
65. Hedrick CC, Malanchi I. Neutrophils in cancer: heterogeneous and multifaceted. *Nat Rev Immunol* 2022;**22**:173−87.
66. Booth JS, Goldberg E, Barnes RS, Greenwald BD, Sztein MB. Oral typhoid vaccine Ty21a elicits antigen-specific resident memory CD4 (+) T cells in the human terminal ileum lamina propria and epithelial compartments. *J Transl Med* 2020;**18**:102.
67. Schjetne KW, Thompson KM, Nilsen N, Flo TH, Fleckenstein B, Iversen JG, et al. Cutting edge: link between innate and adaptive immunity: toll-like receptor 2 internalizes antigen for presentation to $CD4^+$ T cells and could be an efficient vaccine target. *J Immunol* 2003;**171**:32−6.
68. Antel JP, Becher B, Ludwin SK, Prat A, Quintana FJ. Glial cells as regulators of neuroimmune interactions in the central nervous system. *J Immunol* 2020;**204**:251−5.
69. Aziz MA, Midha S, Waheed SM, Bhatnagar R. Oral vaccines: new needs, new possibilities. *Bioessays* 2007;**29**:591−604.
70. Liu J, Fu M, Wang M, Wan D, Wei Y, Wei X. Cancer vaccines as promising immuno-therapeutics: platforms and current progress. *J Hematol Oncol* 2022;**15**:28.
71. Lin CW, Lee JY, Tsao YP, Shen CP, Lai HC, Chen SL. Oral vaccination with recombinant *Listeria* monocytogenes expressing human papillomavirus type 16 E7 can cause tumor growth in mice to regress. *Int J Cancer* 2002;**102**:629−37.
72. Shen Y, Hu Y, Qiu L. Nano-vesicles based on phospholipid-like amphiphilic polyphosphazenes to orally deliver ovalbumin antigen for evoking anti-tumor immune response. *Acta Biomater* 2020;**106**: 267−77.
73. Kitagawa K, Tatsumi M, Kato M, Komai S, Doi H, Hashii Y, et al. An oral cancer vaccine using a *Bifidobacterium* vector suppresses tumor growth in a syngeneic mouse bladder cancer model. *Mol Ther Oncolytics* 2021;**22**:592−603.
74. He L, Bai Y, Xia L, Pan J, Sun X, Zhu Z, et al. Oral administration of a whole glucan particle (WGP)-based therapeutic cancer vaccine targeting macrophages inhibits tumor growth. *Cancer Immunol Immunother* 2022;**71**:2007−28.
75. Xiang R, Lode HN, Chao TH, Ruehlmann JM, Dolman CS, Rodriguez F, et al. An autologous oral DNA vaccine protects against murine melanoma. *Proc Natl Acad Sci U S A* 2000;**97**:5492−7.
76. Long P, Zhang Q, Xue M, Cao G, Li C, Chen W, et al. Tomato lectin-modified nanoemulsion-encapsulated MAGE1-HSP70/SEA complex protein vaccine: targeting intestinal M cells following peroral administration. *Biomed Pharmacother* 2019;**115**:108886.
77. Wang YJ, Hou Y, Huang H, Liu GR, White AP, Liu SL. Two oral HBx vaccines delivered by live attenuated *Salmonella*: both eliciting effective anti-tumor immunity. *Cancer Lett* 2008;**263**:67−76.
78. Pan Z-K, Ikonomidis G, Pardoll D, Paterson Y. Regression of established tumors in mice mediated by the oral administration of a recombinant *Listeria* monocytogenes vaccine. *Cancer Res* 1995;**55**: 4776−9.
79. Naciute M, Kiwitt T, Kemp RA, Hook S. Bacteria biohybrid oral vaccines for colorectal cancer treatment reduce tumor growth and increase immune infiltration. *Vaccine* 2021;**39**:5589−99.
80. Kitagawa K, Oda T, Saito H, Araki A, Gonoi R, Shigemura K, et al. Development of oral cancer vaccine using recombinant *Bifidobacterium* displaying Wilms' tumor 1 protein. *Cancer Immunol Immunother* 2017;**66**:787−98.
81. Parenky AC, Akalkotkar A, Mulla NS, D'Souza MJ. Harnessing T-cell activity against prostate cancer: a therapeutic microparticulate oral cancer vaccine. *Vaccine* 2019;**37**:6085−92.
82. Akalkotkar A, Tawde SA, Chablani L, D'Souza MJ. Oral delivery of particulate prostate cancer vaccine: *in vitro* and *in vivo* evaluation. *J Drug Target* 2012;**20**:338−46.
83. Mulla N, Chablani L, Parenky AC, D'Souza MJ. Boosting *in-vivo* anti-tumor immunity with an oral microparticulate breast cancer vaccine and low-dose cyclophosphamide. *Vaccines (Basel)* 2023;**11**: 543.
84. Liu Z, Lv D, Liu S, Gong J, Wang D, Xiong M, et al. Alginic acid-coated chitosan nanoparticles loaded with legumain DNA vaccine: effect against breast cancer in mice. *PLoS One* 2013;**8**:e60190.
85. Luo Y, Zhou H, Mizutani M, Mizutani N, Reisfeld RA, Xiang R. Transcription factor Fos-related antigen 1 is an effective target for a breast cancer vaccine. *Proc Natl Acad Sci U S A* 2003;**100**:8850−5.
86. Steel JC, Di Pasquale G, Ramlogan CA, Patel V, Chiorini JA, Morris JC. Oral vaccination with adeno-associated virus vectors expressing the Neu oncogene inhibits the growth of murine breast cancer. *Mol Ther* 2013;**21**:680−7.
87. Ueki H, Kitagawa K, Kato M, Yanase S, Okamura Y, Bando Y, et al. An oral cancer vaccine using *Bifidobacterium* vector augments combination of anti-PD-1 and anti-CTLA-4 antibodies in mouse renal cell carcinoma model. *Sci Rep* 2023;**13**:9994.
88. Guo CC, Ding J, Pan BR, Yu ZC, Han QL, Meng FP, et al. Development of an oral DNA vaccine against MG7-Ag of gastric cancer using attenuated *salmonella* typhimurium as carrier. *World J Gastroenterol* 2003;**9**:1191−5.
89. Paglia P, Arioli I, Frahm N, Chakraborty T, Colombo MP, Guzman CA. The defined attenuated *Listeria* monocytogenes delta mp12 mutant is an effective oral vaccine carrier to trigger a long-lasting immune response against a mouse fibrosarcoma. *Eur J Immunol* 1997;**27**:1570−5.
90. Paglia P, Medina E, Arioli I, Guzman CA, Colombo MP. Gene transfer in dendritic cells, induced by oral DNA vaccination with salmonellatyphimurium, results in protective immunity against a murine fibrosarcoma. *Blood* 1998;**92**:3172−6.
91. Panthel K, Meinel KM, Sevil Domenech VE, Trulzsch K, Russmann H. *Salmonella* type III-mediated heterologous antigen delivery: a versatile oral vaccination strategy to induce cellular immunity against infectious agents and tumors. *Int J Med Microbiol* 2008;**298**:99−103.
92. Pomeroy AE, Schmidt EV, Sorger PK, Palmer AC. Drug independence and the curability of cancer by combination chemotherapy. *Trends Cancer* 2022;**8**:915−29.
93. Printezi MI, Kilgallen AB, Bond MJG, Štibler U, Putker M, Teske AJ, et al. Toxicity and efficacy of chronomodulated chemotherapy: a systematic review. *Lancet Oncol* 2022;**23**:e129−43.
94. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer* 2021;**21**:145−61.
95. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. *Sci Technol Humanit* 2018;**359**:6372.
96. Wang J, Hu K, Cai X, Yang B, He Q, Wang J, et al. Targeting PI3K/AKT signaling for treatment of idiopathic pulmonary fibrosis. *Acta Pharm Sin B* 2022;**12**:18−32.
97. Li Z, Ding Y, Liu J, Wang J, Mo F, Wang Y, et al. Depletion of tumor associated macrophages enhances local and systemic platelet-mediated anti-PD-1 delivery for post-surgery tumor recurrence treatment. *Nat Commun* 2022;**13**:1845.
98. Ribeiro TP, Moreira JA, Monteiro FJ, Laranjeira MS. Nanomaterials in cancer: reviewing the combination of hyperthermia and triggered chemotherapy. *J Control Release* 2022;**347**:89−103.
99. Farzat M, Weib P, Sukhanov I, Rosenbauer J, Tanislav C, Wagenlehner FM. Effect of neoadjuvant hormonal therapy on the postoperative course for patients undergoing robot-assisted radical prostatectomy. *J Clin Med* 2023;**12**:3053.
100. Zheng J, Xiao Y, Wu XQ, Xiao QZ, Feng C, Gao KB. Double systemic cytokine release syndrome following sequential infusion of anti-CD22 and anti-CD19 chimeric antigen receptor T cells after autologous hematopoietic stem cell transplantation for a central diffuse large B-cell lymphoma patient: a case report and literature review. *Front Immunol* 2023;**14**:1098815.
101. Yi L, Jiang X, Zhou Z, Xiong W, Xue F, Liu Y, et al. A hybrid Nanoadjuvant simultaneously depresses PD-L1/TGF-β1 and activates cGAS−STING pathway to overcome radio-immunotherapy resistance. *Adv Mater* 2024;**36**:e2304328.

102. Shirakawa T, Kitagawa K. Antitumor effect of oral cancer vaccine with *Bifidobacterium* delivering WT1 protein to gut immune system is superior to WT1 peptide vaccine. *Hum Vaccines Immunother* 2018; **14**:159−62.
103. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023;**73**:17−48.
104. Neumann S, Young K, Compton B, Anderson R, Painter G, Hook S. Synthetic TRP2 long-peptide and alpha-galactosylceramide formulated into cationic liposomes elicit $CD8^+$ T-cell responses and prevent tumour progression. *Vaccine* 2015;**33**:5838−44.
105. Iwanaga K, Ono S, Narioka K, Kakemi M, Morimoto K, Yamashita S, et al. Application of surface-coated liposomes for oral delivery of peptide: effects of coating the liposome's surface on the GI transit of insulin. *J Pharm Sci* 1999;**88**:248−52.
106. Liau JJ, Hook S, Prestidge CA, Barnes TJ. A lipid based multi-compartmental system: liposomes-in-double emulsion for oral vaccine delivery. *Eur J Pharm Biopharm* 2015;**97**:15−21.
107. Wood LM, Paterson Y. Attenuated *Listeria* monocytogenes: a powerful and versatile vector for the future of tumor immunotherapy. *Front Cell Infect Microbiol* 2014;**4**:51.
108. Disson O, Moura A, Lecuit M. Making sense of the biodiversity and virulence of *Listeria* monocytogenes. *Trends Microbiol* 2021;**29**: 811−22.
109. Brune KD, Buldun CM, Li Y, Taylor IJ, Brod F, Biswas S, et al. Dual plug-and-display synthetic assembly using orthogonal reactive proteins for twin antigen immunization. *Bioconjug Chem* 2017;**28**: 1544−51.
110. Qi M, Zhang XE, Sun X, Zhang X, Yao Y, Liu S, et al. Intranasal nanovaccine confers Homo- and Hetero-Subtypic influenza protection. *Small (Weinh)* 2018;**14**:e1703207.
111. Zhou S, Gravekamp C, Bermudes D, Liu K. Tumour-targeting bacteria engineered to fight cancer. *Nat Rev Cancer* 2018;**18**:727−43.
112. Casanovas O. Limitations of therapies exposed. *Nature* 2012;**484**: 44−6.
113. Wolf DM, Yau C, Wulfkuhle J, Brown-Swigart L, Gallagher RI, Lee PRE, et al. Redefining breast cancer subtypes to guide treatment prioritization and maximize response: predictive biomarkers across 10 cancer therapies. *Cancer Cell* 2022;**40**:609−23.e6.
114. Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* 2017;**27**:109−18.
115. Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 2003;**9**:606−12.
116. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci* 2020;**77**:1745−70.
117. Papetti M, Herman IM. Mechanisms of normal and tumor-derived angiogenesis. *Am J Physiol-Cell Ph* 2002;**282**:C947−70.
118. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov* 2019;**18**:358−78.
119. Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 2007;**369**:2097−105.
120. Cato ACB, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE* 2002;**2002**:re9.
121. Descamps S, Toillon RA, Adriaenssens E, Pawlowski V, Cool SM, Nurcombe V, et al. Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. *J Biol Chem* 2001;**276**:17864−70.
122. Bhatia S, Tykodi SS, Thompson JA. Treatment of metastatic melanoma: an overview. *Oncology (Williston Park)* 2009;**23**:488−96.
123. Lu J, Song J, Zhang P, Huang Y, Lu X, Dai H, et al. Biomineralized polydopamine nanoparticle-based sodium alginate hydrogels for delivery of anti-serine/threonine protein kinase B-rapidly accelerated fibrosarcoma siRNA for metastatic melanoma therapy. *ACS Nano* 2023;**17**:18318−31.
124. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 2011;**480**:480−9.
125. Weyant KB, Oloyede A, Pal S, Liao J, Jesus MR, Jaroentomeechai T, et al. A modular vaccine platform enabled by decoration of bacterial outer membrane vesicles with biotinylated antigens. *Nat Commun* 2023;**14**:464.
126. Zhu Z, Antenucci F, Villumsen KR, Bojesen AM. Bacterial outer membrane vesicles as a versatile tool in vaccine research and the fight against antimicrobial resistance. *mBio* 2021;**12**:e0170721.
127. Krishnan N, Kubiatowicz LJ, Holay M, Zhou J, Fang RH, Zhang L. Bacterial membrane vesicles for vaccine applications. *Adv Drug Deliv Rev* 2022;**185**:114294.
128. Howard O, Buzas K, Dong H, Oppenheim JJ. Gp100 is chemotactic for mononuclear cells and utilizes chemokine receptors. *J Immunol* 2007;**178**:S183.
129. Sudo T, Kuramoto T, Komiya S, Inoue A, Itoh K. Expression of MAGE genes in osteosarcoma. *J Orthop Res* 1997;**15**:128−32.
130. Ma J-H, Sui Y-F, Ye J, Huang Y-Y, Li Z-S, Chen G-S, et al. Heat shock protein 70/MAGE-3 fusion protein vaccine can enhance cellular and humoral immune responses to MAGE-3 in *vivo*. *Cancer Immunol Immunother* 2005;**54**:907−14.
131. Balaban N, Rasooly A. Staphylococcal enterotoxins. *Int J Food Microbiol* 2000;**61**:1−10.
132. Nishikawa H, Sato E, Briones G, Chen LM, Matsuo M, Nagata Y, et al. In *vivo* antigen delivery by a *Salmonella* typhimurium type III secretion system for therapeutic cancer vaccines. *J Clin Investig* 2006;**116**:1946−54.
133. Jiang GM, Tan Y, Wang H, Peng L, Chen HT, Meng XJ, et al. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. *Mol Cancer* 2019;**18**:17.
134. Atalay IB, Togral G. Unusual localization and aggressive progression of large infantile fibrosarcoma. *Acta Orthop Traumato* 2019;**53**: 507−11.
135. Parida L, Fernandez-Pineda I, Uffman JK, Davidoff AM, Krasin MJ, Pappo A, et al. Clinical management of infantile fibrosarcoma: a retrospective single-institution review. *Pediatr Surg Int* 2013;**29**: 703−8.
136. Ainsworth KE, Chavhan GB, Gupta AA, Hopyan S, Taylor G. Congenital infantile fibrosarcoma: review of imaging features. *Pediatr Radiol* 2014;**44**:1124−9.
137. Medina E, Guzmán CA, Staendner LH, Colombo MP, Paglia P. *Salmonella* vaccine carrier strains: effective delivery system to trigger anti-tumor immunity by oral route. *Eur J Immunol* 1999;**29**: 693−9.
138. Brown A, Hormaeche C, Hormaeche RDd, Winther M, Dougan G, Maskell D, et al. An attenuated aroA *Salmonella* typhimurium vaccine elicits humoral and cellular immunity to cloned β-galactosidase in mice. *J Infect Dis* 1987;**155**:86−92.
139. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, et al. CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. *Clin Cancer Res* 2012;**18**:3281−92.
140. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology (New York, N Y, 1943)* 2004;**127**:S5−16.
141. Anwanwan D, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches. *Bba-Rev Cancer* 2020;**1873**:188314.
142. Brown TD, Whitehead KA, Mitragotri S. Materials for oral delivery of proteins and peptides. *Nat Rev Mater* 2020;**5**:127−48.
143. Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, Ostroff GR, et al. Mechanism by which orally administered β-1, 3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *J Immunol* 2004;**173**:797−806.
144. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet* 2020;**396**:635−48.

145. Chia NY, Tan P. Molecular classification of gastric cancer. *Ann Oncol* 2016;**27**:763−9.
146. Wu S, Qu X, Wang N, Zhang L, Zhao X, Wu Q, et al. MG7-Ag, hTERT, and TFF2 identified high-risk intestinal metaplasia and constituted a prediction model for gastric cancer. *Chin Med J (Engl)* 2023;**136**:610−2.
147. Küppers R. The biology of Hodgkin's lymphoma. *Nat Rev Cancer* 2009;**9**:15−27.
148. Shankland KR, Armitage JO, Hancock BW. Non-hodgkin lymphoma. *Lancet* 2012;**380**:848−57.
149. Kojima N, Biao L, Nakayama T, Ishii M, Ikehara Y, Tsujimura K. Oligomannose-coated liposomes as a therapeutic antigen-delivery and an adjuvant vehicle for induction of in *vivo* tumor immunity. *J Control Release* 2008;**129**:26−32.
150. Ye X, Liang X, Chen Q, Miao Q, Chen X, Zhang X, et al. Surgical tumor-derived personalized photothermal vaccine formulation for cancer immunotherapy. *ACS Nano* 2019;**13**:2956−68.
151. Chen Q, Huang G, Wu W, Wang J, Hu J, Mao J, et al. A hybrid eukaryotic-prokaryotic nanoplatform with photothermal modality for enhanced antitumor vaccination. *Adv Mater* 2020;**32**:e1908185.
152. Osztovits J, Balazs C, Feher J. H1N1 influenza - pandemic, 2009. *Orv Hetil* 2009;**150**:2265−73.
153. Pfefferbaum B, North CS. Mental health and the Covid-19 pandemic. *N Engl J Med* 2020;**383**:510−2.
154. Cao P, Han FY, Grøndahl L, Xu ZP, Li L. Enhanced oral vaccine efficacy of polysaccharide-coated calcium phosphate nanoparticles. *ACS Omega* 2020;**5**:18185−97.
155. Miao YB, Pan WY, Chen KH, Wei HJ, Mi FL, Lu MY, et al. Engineering a nanoscale Al-MOF-Armored antigen carried by a "Trojan Horse"-like platform for oral vaccination to induce potent and long-lasting immunity. *Adv Funct Mater* 2019;**29**:1904828.
156. Zhu Q, Talton J, Zhang G, Cunningham T, Wang Z, Waters RC, et al. Large intestine−targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. *Nat Med* 2012;**18**:1291−6.
157. De Smet R, Demoor T, Verschuere S, Dullaers M, Ostroff GR, Leclercq G, et al. β-Glucan microparticles are good candidates for mucosal antigen delivery in oral vaccination. *J Control Release* 2013; **172**:671−8.
158. Sarti F, Perera G, Hintzen F, Kotti K, Karageorgiou V, Kammona O, et al. In *vivo* evidence of oral vaccination with PLGA nanoparticles containing the immunostimulant monophosphoryl lipid A. *Biomaterials* 2011;**32**:4052−7.
159. Wu L, Li L, Yin X, Li C, Xin W, Liu L, et al. A SARS-CoV-2 oral vaccine development strategy based on the attenuated *Salmonella* type III secretion system. *J Appl Microbiol* 2022;**133**:2484−500.
160. Takamura S, Niikura M, Li TC, Takeda N, Kusagawa S, Takebe Y, et al. DNA vaccine-encapsulated virus-like particles derived from an orally transmissible virus stimulate mucosal and systemic immune responses by oral administration. *Gene Ther* 2004;**11**:628−35.
161. Jun JW, Kang JW, Giri SS, Yun S, Kim HJ, Guen Kim S, et al. Immunostimulation by starch hydrogel-based oral vaccine using formalin-killed cells against edwardsiellosis in Japanese eel, Anguilla japonica. *Vaccine* 2020;**38**:3847−53.
162. Kole S, Shin SM, Kwak IS, Cho SH, Jung SJ. Efficacy of Chitosan-PLGA encapsulated trivalent oral vaccine against viral haemorrhagic septicemia virus, Streptococcus parauberis, and Miamiensis avidus in olive flounder (Paralichthys olivaceus). *Fish Shellfish Immunol* 2022; **127**:843−54.
163. Maqsood I, Shi W, Wang L, Wang X, Han B, Zhao H, et al. Immunogenicity and protective efficacy of orally administered recombinant *Lactobacillus* plantarum expressing VP2 protein against IBDV in chicken. *J Appl Microbiol* 2018;**125**:1670−81.
164. Yu M, Qi R, Chen C, Yin J, Ma S, Shi W, et al. Immunogenicity of recombinant *Lactobacillus* casei-expressing F4 (K88) fimbrial adhesin FaeG in conjunction with a heat-labile enterotoxin A (LTAK63) and heat-labile enterotoxin B (LTB) of enterotoxigenic Escherichia coli as an oral adjuvant in mice. *J Appl Microbiol* 2017; **122**:506−15.
165. Van Eerde A, Gottschamel J, Bock R, Hansen KEA, Munang'andu HM, Daniell H, et al. Production of tetravalent dengue virus envelope protein domain III based antigens in lettuce chloroplasts and immunologic analysis for future oral vaccine development. *Plant Biotechnol J* 2019;**17**:1408−17.
166. Wei W, Behloul N, Wang W, Baha S, Liu Z, Shi R, et al. Chitosan nanoparticles loaded with truncated ORF2 protein as an oral vaccine candidate against hepatitis E. *Macromol Biosci* 2021;**21**:e2000375.
167. Clarke JL, Paruch L, Dobrica MO, Caras I, Tucureanu C, Onu A, et al. Lettuce-produced hepatitis C virus E1E2 heterodimer triggers immune responses in mice and antibody production after oral vaccination. *Plant Biotechnol J* 2017;**15**:1611−21.
168. Del LYM, Farran I, Becher ML, Sander V, Sanchez VR, Martin V, et al. A chloroplast-derived Toxoplasma gondii GRA4 antigen used as an oral vaccine protects against toxoplasmosis in mice. *Plant Biotechnol J* 2012;**10**:1136−44.
169. Lu H, Gao Y, Chen J, Chai J, Li A, Sun T, et al. A novel oral biomimetic delivery system enhancing both the mucosal and systemic immunity of influenza virus vaccine. *Chem Eng J* 2023;**470**:144371.
170. Shibasaki S, Aoki W, Nomura T, Miyoshi A, Tafuku S, Sewaki T, et al. An oral vaccine against candidiasis generated by a yeast molecular display system. *Pathog Dis* 2013;**69**:262−8.
171. Yang Q, Yang BT, Kang YH, Cong W. Efficacy of a recombinant *Lactobacillus* plantarum Lp-pPG-Malt as an oral vaccine candidate against Aeromonas hydrophila infection in crucian carp. *Fish Shellfish Immunol* 2023;**136**:108737.
172. Cen Q, Gao T, Ren Y, Lu X, Lei H. Immune evaluation of a Saccharomyces cerevisiae-based oral vaccine against *Helicobacter pylori* in mice. *Helicobacter (Camb, Mass)* 2021;**26**:e12772.
173. Peng X, Zhang R, Wang C, Yu F, Yu M, Chen S, et al. E. coli enterotoxin LtB enhances vaccine-induced anti-H. *pylori* protection by promoting Leukocyte migration into gastric mucus via inflammatory lesions. *Cells* 2019;**8**:982.
174. Chan HT, Chia MY, Pang VF, Jeng CR, Do YY, Huang PL. Oral immunogenicity of porcine reproductive and respiratory syndrome virus antigen expressed in transgenic banana. *Plant Biotechnol J* 2013;**11**:315−24.
175. Jiang T, Singh B, Li HS, Kim YK, Kang SK, Nah JW, et al. Targeted oral delivery of BmpB vaccine using porous PLGA microparticles coated with M cell homing peptide-coupled chitosan. *Biomaterials* 2014;**35**:2365−73.
176. Ferreira GC, Sanches TVC, Mechler-Dreibi ML, Almeida HMS, Storino GY, Sonalio K, et al. Efficacy evaluation of a novel oral silica-based vaccine in inducing mucosal immunity against Mycoplasma hyopneumoniae. *Res Vet Sci* 2023;**158**:141−50.
177. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. *Nature* 2004;**430**:242−9.
178. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature* 2007;**447**:279−83.
179. Pybus OG, Rambaut A. Evolutionary analysis of the dynamics of viral infectious disease. *Nat Rev Genet* 2009;**10**:540−50.
180. Excler JL, Saville M, Berkley S, Kim JH. Vaccine development for emerging infectious diseases. *Nat Med* 2021;**27**:591−600.
181. Petrova VN, Russell CA. The evolution of seasonal influenza viruses. *Nat Rev Microbiol* 2018;**16**:47−60.
182. Harrington WN, Kackos CM, Webby RJ. The evolution and future of influenza pandemic preparedness. *Exp Mol Med* 2021;**53**:737−49.
183. Shastri PN, Kim MC, Quan FS, D'Souza MJ, Kang SM. Immunogenicity and protection of oral influenza vaccines formulated into microparticles. *J Pharm Sci* 2012;**101**:3623−35.
184. Revill PA, Tu T, Netter HJ, Yuen LK, Locarnini SA, Littlejohn M. The evolution and clinical impact of hepatitis B virus genome diversity. *Nat Rev Gastro Hepat* 2020;**17**:618−34.
185. Krajden M. Hepatitis. *Can J Infect Dis* 2001;**12**:329−31.

186. Jeng WJ, Papatheodoridis GV, Lok ASF. Hepatitis B. *Lancet* 2023; **401**:1039−52.
187. Kong Q, Richter L, Yang YF, Arntzen CJ, Mason HS, Thanavala Y. Oral immunization with hepatitis B surface antigen expressed in transgenic plants. *Proc Natl Acad Sci U S A* 2001;**98**:11539−44.
188. Bartsch SM, O'Shea KJ, Ferguson MC, Bottazzi ME, Wedlock PT, Strych U, et al. Vaccine efficacy needed for a COVID-19 coronavirus vaccine to prevent or stop an epidemic as the sole intervention. *Am J Prev Med* 2020;**59**:493−503.
189. Langel SN, Johnson S, Martinez CI, Tedjakusuma SN, Peinovich N, Dora EG, et al. Adenovirus type 5 SARS-CoV-2 vaccines delivered orally or intranasally reduced disease severity and transmission in a hamster model. *Sci Transl Med* 2022;**14**:eabn6868.
190. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;**399**:629−55.
191. Frost I, Sati H, Garcia-Vello P, Hasso-Agopsowicz M, Lienhardt C, Gigante V, et al. The role of bacterial vaccines in the fight against antimicrobial resistance: an analysis of the preclinical and clinical development pipeline. *Lancet Microbe* 2022;**4**:e113−25.
192. Jansen KU, Knirsch C, Anderson AS. The role of vaccines in preventing bacterial antimicrobial resistance. *Nat Med* 2018;**24**:10−9.
193. Poolman JT. Expanding the role of bacterial vaccines into life-course vaccination strategies and prevention of antimicrobial-resistant infections. *NPJ Vaccines* 2020;**5**:84.
194. Wang Y-C. Medicinal plant activity on *Helicobacter pylori* related diseases. *World J Gastroenterol* 2014;**20**:10368.
195. Papagiannakis P, Michalopoulos C, Papalexi F, Dalampoura D, Diamantidis MD. The role of *Helicobacter pylori* infection in hematological disorders. *Eur J Intern Med* 2013;**24**:685−90.
196. Wilson KT, Crabtree JE. Immunology of *Helicobacter pylori*: insights into the failure of the immune response and perspectives on vaccine studies. *Gastroenterology (New York, N Y, 1943)* 2007;**133**: 288−308.
197. Zhang Y, Li H, Wang Q, Hao X, Li H, Sun H, et al. Rationally designed self-assembling nanoparticles to overcome mucus and epithelium transport barriers for oral vaccines against *Helicobacter pylori*. *Adv Funct Mater* 2018;**28**:1802675.
198. Qadri F, Svennerholm AM, Faruque AS, Sack RB. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clin Microbiol Rev* 2005;**18**:465−83.
199. Khalil I, Walker R, Porter CK, Muhib F, Chilengi R, Cravioto A, et al. Enterotoxigenic *Escherichia coli* (ETEC) vaccines: priority activities to enable product development, licensure, and global access. *Vaccine* 2021;**39**:4266−77.
200. Joosten P, Tiemersma E, Threels A, Caumartin-Dhieux C, Rombout J. Oral vaccination of fish againstVibrio anguillarumusing alginate microparticles. *Fish Shellfish Immun* 1997;**7**:471−85.
201. Thirumalaikumar E, Lelin C, Sathishkumar R, Vimal S, Anand SB, Babu MM, et al. Oral delivery of pVAX-OMP and pVAX-hly DNA vaccine using chitosan-tripolyphosphate (Cs-TPP) nanoparticles in Rohu, (Labeo rohita) for protection against Aeromonas hydrophila infection. *Fish Shellfish Immun* 2021;**115**:189−97.
202. Jun JW, Kang JW, Giri SS, Kim SW, Kim SG, Kwon J, et al. Preventive effect of starch hydrogel-based oral vaccine against Aeromonas *salmonicida* infection in rainbow trout (Oncorhynchus mykiss). *Aquaculture* 2022;**555**:738202.
203. Chu K-B, Quan F-S. Advances in Toxoplasma gondii vaccines: current strategies and challenges for vaccine development. *Vaccines* 2021;**9**:413.
204. Wang J-L, Zhang N-Z, Li T-T, He J-J, Elsheikha HM, Zhu X-Q. Advances in the development of anti-Toxoplasma gondii vaccines: challenges, opportunities, and perspectives. *Trends Parasitol* 2019; **35**:239−53.
205. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. *Nat Rev Dis Primers* 2018;**4**: 18026.
206. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012;**36**:288−305.
207. Lundgren A, Leach S, Tobias J, Carlin N, Gustafsson B, Jertborn M, et al. Clinical trial to evaluate safety and immunogenicity of an oral inactivated enterotoxigenic Escherichia coli prototype vaccine containing CFA/I overexpressing bacteria and recombinantly produced LTB/CTB hybrid protein. *Vaccine* 2013;**31**:1163−70.
208. Kim L, Liebowitz D, Lin K, Kasparek K, Pasetti MF, Garg SJ, et al. Safety and immunogenicity of an oral tablet norovirus vaccine, a phase I randomized, placebo-controlled trial. *JCI Insight* 2018;**3**: e121077.
209. Jain S, Singh P, Mishra V, Vyas SP. Mannosylated niosomes as adjuvant−carrier system for oral genetic immunization against Hepatitis B. *Immunol Lett* 2005;**101**:41−9.
210. Bandyopadhyay AS, Zipursky S. A novel tool to eradicate an ancient scourge: the novel oral polio vaccine type 2 story. *Lancet Infect Dis* 2023;**23**:e67−71.
211. Singh R, Lin S, Nair SK, Shi Y, Daniell H. Oral booster vaccine antigen—expression of full-length native SARS-CoV-2 spike protein in lettuce chloroplasts. *Plant Biotechnol J* 2023;**21**:887−9.
212. Brandtzaeg P. Induction of secretory immunity and memory at mucosal surfaces. *Vaccine* 2007;**25**:5467−84.
213. Sheridan Brian S, Romagnoli Pablo A, Pham Q-M, Fu H-H, Alonzo F, Schubert W-D, et al. *Γδ* T cells exhibit multifunctional and protective memory in intestinal tissues. *Immunity (Camb, Mass)* 2013;**39**:184−95.
214. Perez-Lopez A, Behnsen J, Nuccio SP, Raffatellu M. Mucosal immunity to pathogenic intestinal bacteria. *Nat Rev Immunol* 2016;**16**: 135−48.
215. Kumar P, Lata S, Shankar UN, Akif M. Immunoinformatics-based designing of a multi-epitope chimeric vaccine from multi-domain outer surface antigens of Leptospira. *Front Immunol* 2021;**12**: 735373.
216. Cai Q, Peng G, Bu L, Lin Y, Zhang L, Lustigmen S, et al. Immunogenicity and in vitro protective efficacy of a polyepitope Plasmodium falciparum candidate vaccine constructed by epitope shuffling. *Vaccine* 2007;**25**:5155−65.
217. Saadi M, Karkhah A, Nouri HR. Development of a multi-epitope peptide vaccine inducing robust T cell responses against brucellosis using immunoinformatics based approaches. *Infect Genet Evol* 2017; **51**:227−34.
218. Meza B, Ascencio F, Sierra-Beltrán AP, Torres J, Angulo C. A novel design of a multi-antigenic, multistage and multi-epitope vaccine against *Helicobacter pylori*: an in silico approach. *Infect Genet Evol* 2017;**49**:309−17.
219. Vela Ramirez JE, Sharpe LA, Peppas NA. Current state and challenges in developing oral vaccines. *Adv Drug Deliv Rev* 2017;**114**: 116−31.
220. Zhao T, Cai Y, Jiang Y, He X, Wei Y, Yu Y, et al. Vaccine adjuvants: mechanisms and platforms. *Signal Transduct Targeted Ther* 2023;**8**: 283.
221. Davitt CJH, Longet S, Albutti A, Aversa V, Nordqvist S, Hackett B, et al. Alpha-galactosylceramide enhances mucosal immunity to oral whole-cell cholera vaccines. *Mucosal Immunol* 2019;**12**:1055−64.
222. Qadri F, Akhtar M, Bhuiyan TR, Chowdhury MI, Ahmed T, Rafique TA, et al. Safety and immunogenicity of the oral, inactivated, enterotoxigenic *Escherichia coli* vaccine ETVAX in Bangladeshi children and infants: a double-blind, randomised, placebo-controlled phase 1/2 trial. *Lancet Infect Dis* 2020;**20**:208−19.
223. Akhtar M, Chowdhury MI, Bhuiyan TR, Kaim J, Ahmed T, Rafique TA, et al. Evaluation of the safety and immunogenicity of the oral inactivated multivalent enterotoxigenic *Escherichia coli* vaccine ETVAX in Bangladeshi adults in a double-blind, randomized, placebo-controlled phase I trial using electrochemiluminescence and ELISA assays for immunogenicity analyses. *Vaccine* 2019;**37**: 5645−56.

224. Savelkoul HF, Ferro VA, Strioga MM, Schijns VE. Choice and design of adjuvants for parenteral and mucosal vaccines. *Vaccines (Basel)* 2015;**3**:148−71.
225. van Aalst S, Ludwig IS, van Kooten PJS, van der Zee R, van Eden W, Broere F. Dynamics of APC recruitment at the site of injection following injection of vaccine adjuvants. *Vaccine* 2017; **35**:1622−9.
226. Lorentzen CL, Haanen JB, Met Ö, Svane IM. Clinical advances and ongoing trials on mRNA vaccines for cancer treatment. *Lancet Oncol* 2022;**23**:e450−8.
227. Mackensen A, Haanen JB, Koenecke C, Alsdorf W, Wagner-Drouet E, Borchmann P, et al. CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. *Nat Med* 2023;**29**:2844−53.