Harnessing *Listeria monocytogenes*: A Promising Approach to Cancer Treatment

Abstract

Increasing mortality and morbidity rates have drawn global attention to cancer, prompting the exploration of new treatment options. The use of immunotherapy for recurrent or metastatic cancer has emerged as a promising option over the years despite its limitations as compared to traditional treatment options. Among the various immunotherapeutic approaches, bacterial-based vectors like *Listeria monocytogenes* (*Lm*) have garnered attention for their unique characteristics. Utilizing these vectors involves leveraging their ability to invade Antigen-Presenting Cells (APCs), grow intracellularly within immune cells, and spread intracellularly, enhancing their efficacy in tailoring immune responses. It is important to note that the use of bacterial vectors significantly minimizes the risks associated with off-target effects. The antitumor effects of *Lm* can be observed through the reduction of immunosuppressive cells in the tumor microenvironment as well as the stimulation of T cells. Various types of tumor cells can be targeted by modified Lm vaccines, according to research. However, it is recognized that *Lm* vaccines alone may not suffice for comprehensive cancer treatment. Therefore, using *Lm* vaccines in combination with other therapeutic modalities like radiotherapy, reactivated adoptive cell therapy, and immune checkpoint inhibitors could result in superior results. As a result of these developments, the current review aims to elaborate on recent developments in the understanding of how *Lm* vaccines perform their antitumor properties. This review aims to provide insights into optimizing the therapeutic potential of *Lm* vaccines by comprehensively examining their interplay with the immune system. In order to harness the full therapeutic potential of Lm vaccines for fighting cancer, researchers and clinicians need to gain a deeper understanding of these mechanisms.

Keywords: *Listeria monocytogenes*, Cancer, Cancer vaccine, Immunotherapy, Tumor

1. Context

Cancer is a broad category of diseases that can affect virtually any organ or tissue in the body, grow abnormally, invade other parts of the body, and eventually lead to death. World Health Organization (WHO) data indicates that cancer caused 9.6 million deaths in 2018 (1). There are several physical and psychological side effects associated with conventional cancer therapies, including chemotherapy, surgery, and radiation. Immunotherapies in cancer treatment have become more prevalent in recent years (2). Immunotherapies became a reality in 2010 with the approval of sipuleucel-T for prostate cancer therapy, even though immunologists and oncologists once viewed artificial stimulation of the immune system as a dream (3). Cancer immunotherapy has rapidly progressed in recent years (4). It is possible to categorize immunotherapies into passive and active types, so tumor immunotherapy has emerged to selectively destroy tumor cells by reactivating or activating host cellular immunity, mainly mediated by T cells (5). Additionally, immunomodulatory drugs can work in contrast to cancer cells by increasing the absorption of Dendritic Cells (DC), antibodies, macrophages, Natural Killer cells (NK), and cytokines that target tumors.

It has recently been shown that tumor immunotherapy can improve patients' prognoses and overcome the limitations of conventional treatments (6-9). Cancer vaccines developed from bacteria are a highly active research area, with applications expected soon. It was documented for the first time when William B. Coley, a New York Memorial Hospital surgeon, described using bacteria as anticancer agents in 1890. It has been reported that several live attenuated, dead but metabolically active, and genetically engineered microorganisms, including *Bacillus Clostridium*, *Listeria Monocytogenes* (*Lm*), and *Salmonella*, can target cancer cells and display anticancer properties (10). As a Gram-positive bacterium, *Lm* is mainly known as a food-borne pathogen that can cause sepsis, bacteremia, and encephalitis, among other serious infections.

2. Evidence Acquisition

There are several virulent factors produced by the organism, such as *Listeria* Lysin O (LLO), phospholipases specific to phosphatidylinositol, ActA proteins that recruit and polymerize host actin, and internalin that aid nonphagocytic cells in adhering to the pathogen and internalizing it. Internalin B (inlB) and Internalin A (inlA) are surface proteins on *Lm* that interact with C-Met and E-cadherin to allow *Lm* to enter nonphagocytic cells. In the host phagosome, *Lm* becomes enclosed after internalization. As a result of secreting lectin LLO and phospholipases (plcA and plcB), *Lm* perforates phagosomes, incoming the cytoplasm to avoid being killed by phagolysosomes. *Lm* secretes Tumor-Associated Antigens (TAAs),

which are degraded by proteasomes and stimulate specific CD8+ T cells through Major Histocompatibility Complex (MHC) class I molecules (11).

In contrast to other bacteria, *Lm* can more easily escape immune surveillance because of hypoxia, suppressive Tumor Micro Environment (TME), and the ability to grow intracellularly. Increasing effector T cells in the presence of immunosuppressive cells can improve the effectiveness of immunotherapy by increasing innate immunity and restructuring TME. Many studies have examined *Lm* and irritated to use it as a vector for tumor immunotherapy. Despite this, *Lm*-based therapy alone still poses several problems associated with poor complications and therapeutic effects as a result of its potential pathogenic nature. Among the possible ways to overcome the limitations, *Lm*-based therapy may be combined with other treatments. *Listeria monocytogenes (LM)* cancer vaccines have great potential, and there is great hope for their role in developing innovative anticancer therapies for cancer patients (12). Specifically, the review will emphasize how *LM*-based immunotherapy modulates immune pathways to produce a promising antitumor response and the current advances in Lm-based immunotherapy.

3. Pathogenesis of *Listeria* infection

Typically, *Lm* enters the body via the gastrointestinal tract after contaminated food is consumed, crossing the intestinal epithelium and spreading in the blood. However, *Lm* is generally administered intravenously to treat immunologic conditions, bypassing the intestinal epithelium. *Listeria monocytogenes* can enter the bloodstream into various organs, including the placenta, brain, and liver. Although it can continue outside the cell, it prefers to replicate in the cytoplasm of the cell. Numerous immune mechanisms are activated in the intracellular compartments of *Lm* as it transitions into them. An internal family of proteins is primarily responsible for allowing *Lm* to enter mammalian cells. There is particular interest in InlA and InlB, which promote *Lm* invasion by binding to host receptors and initiating receptor-mediated endocytosis (13). Internalin A and InlB infect different types of cells due to their binding to other receptors. Internalin A binds E-cadherin on epithelial cells, whereas InlB relates with Met, the hepatocyte growth factor.

In addition to entering through cell surface receptors, APCs actively take up *Lm* through phagocytosis. Additionally, as *Lm* interacts with the outer surface of mammalian cells, the innate immune response to *Lm* is triggered. Pathogen-Associated Molecular Patterns (PAMPs) Expressions of *Lm* on the cell surface and inside phagosomes are recognized by mammalian toll-like receptors (TLRs), which trigger NF-B signaling and promote inflammation (14). *Lm* may undergo one of two fates once internalized into phagosomes. Phagosome–lysosome fusion is estimated to kill most bacteria, providing antigens to stimulate MHC class II-dependent exhibition and CD4+ T-cell response to Lms. As a result, *Lm* has developed mechanisms to

escape lysosome deprivation and enter infected cells' cytosols. In the host cell, *Lm* begins to express PrfA as it transitions from the extracellular environment.

Numerous virulence genes are activated by PrfA, including forms of the two types of phospholipases, PlcA and PlcB, and the pore-forming toxin LLO. Listerio lysin O molecules form a barrel-shaped cavity at the phagosome membrane for controlling *Lm* passage (15), and phospholipases regulate *Lm* exit by hydrolyzing membrane lipids directly (16). After leaving the phagosome, peptides secreted by *Lm* enter the host cell's cytosol, where proteasomes can degrade and present them to cytotoxic T cells via MHC class I molecules. As a result of the direct excretion of *Lm* antigens into the cytosol and their degradation in phagosomes, T cells respond to *Lm* antigens with potent CD4+ and CD8+ responses (17).

By expressing the virulence factor actin assembly-inducing protein (ActA), *Lm* escapes the phagosome and travels throughout the host cell cytosol. Acta proteins are attached to the surface of Lm, where they interact with the Arp2/3 complex to stimulate actin monomer nucleation and filament formation. *Lm* can be pushed throughout the cytosol and into the plasma membrane of an infected cell through actin polymerization, forming protrusions that neighboring cells can internalize and spread the infection (18).

4. Immunological mechanisms of *Listeria monocytogenes*

A biotechnology and medicine concept called path-biotechnology utilizes virulence factors and pathogenic stress. Delivery systems that use *Lm* have much potential. *Lm* is one of the capable delivery systems (19). Some reports have shown that the internal proteins of *Lm* (inlA and inlB) encourage phagocytosis in normal non-phage-cystic human cells. As a result, therapeutic drugs can be conjugated to inlA/inlB proteins to be delivered to cells. An actin rearrangement occurs when inlB binds to Met, a membrane protein present in nonphagocytic cells, leading to the invasion of the bacterial pathogen by the host cell. Additionally, E-catenin on host epithelial cells is activated by binding inlA to it, resulting in the cells' taking up bacteria. It has been reported that attenuated strains of the *Lm* strain have recently been used in clinical trials to deliver anticancer vaccines to humans (20). These studies have found that two essential genes, D-amino acid aminotransferase (dat) and alanine racemase (dal), play a key role in producing mucopeptides in the Lm cell wall.

Human CD24 protein, a biomarker of hepatocellular carcinoma, was expressed and secreted in human cells based on a new approach based on genetic alteration of the replication-deficient *Lm* strain dal dat (Lmdd). T-regulatory cells (Treg) and tumor growth were significantly reduced in mice treated with Lmdd-CD24 (21). In cancer immunotherapy, Cholesterol-Dependent Cytolysin (CDC) LLO, a pore-forming toxin

derived from *Lm*, is an effective adjuvant in increasing immune responses against TAAs. In the future, a detoxified, nonhemolytic form of LLO (dtLLO) may act as a molecular pattern associated with a pathogen and interact with Pathogen-Recognition Receptors (PRRs), such as Toll-like receptor 4, to elicit innate and cellular immunity. As an example, dtLLO fusions with or mixed with Human Papillomavirus (HPV)16 recombinant E7 proteins (dtLLO-E7 fusion or dtLLO + E7) may promote DC maturation, enhance and eradicate tumors, indicating its adjuvant effect and antitumor immune responses (induction of Interleukin (IL-12) and Tumor Necrosis Factor(TNFα)) (22).

In addition, seeligeriolysin O, one of the CDCs associated with *Listeria seeligeri*, activates both TLR2 and TLR4 in macrophages, triggering IL-12 production (22). Meanwhile, mast cells showed microbicidal activity, including DNA release and granular proteins embedded in DNA called Mast Cell Extracellular Traps (MCETs). The cells cause a wide variety of immunological diseases. It has been reported that *Lm* induces mast cells to produce microbicidal MCETs and essential levels of ROS, inhibits Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, and reduces DNA release from mast cells (23). It was reported that some differences were observed when generating DCs for cancer treatment. The *Lm* bacteria activate DCs via PRRs. To find the most efficient DC activation components, researchers examined *Lm* stimulation. Protein components are more effective at inducing DC maturation than DNA components. There was a considerable improvement in DC maturation and CD8+ T cell proliferation stimulation via a lysate fraction containing 109 proteins when compared with lipopolysaccharide maturation of DCs. An analysis of bioinformatics data on 109 proteins revealed that elongation factor Tu acted as a ligand for PRR during DC maturation (24).

Because *Lm* could spread between cells without entering the extracellular matrix, an adequate antibody response was prevented from being produced. The hemolytic activity of its surface protein can induce a CD8+ CTL phenotype and CD4+ Th1 phenotype *in vivo*, similar to other intracellular pathogens (25). The low immunogenicity of TAAs is a severe problem because of their homology to self-proteins. As a result, *Lm*'s ability to stimulate the immune system efficiently allowed the presentation pathways of MHC class I and class II to be loaded with poorly immunogenic TAAs in professional APCs. In APCs, *Lm* can activate several signaling pathways, including the TLR/MyD88 pathway, which promotes the expression of suppressive/regulatory or inflammatory cytokines, autophagy, and ROS production. Another pathway is governed by STING/IRF3, which results in the expression of co-regulated genes and Interferons (IFN-β). Furthermore, another pathway is inflammasomes, which are activated by AIM-2/Caspase-1-mediated signaling pathways that result in proteolytic activation and secretion of IL-1β and IL-18 (26).

Lm activates pathways in monocytes and macrophages to elicit an innate immune response. Furthermore, *Lm* stimulates the excretion of TNF-V8 cytokines, IL-2, IL-6, and IL-12 by DCs, as well as the upregulation

of other proteins like CD40 and Programmed Death-Ligand 1 (PD-L1) in DCs. The adaptive immune response against *Lm* infection provides two primary functions, including the specific lysis of infected cells and the rapid secretion of IFN-γ in response to the innate production of IL-12 and IL-18. There are interesting results from a mouse model showing that neonatal innate cells produce higher quantities of IL-10 and lower levels of Th1-eliciting cytokines when stimulated with Lm, resulting in suboptimal stimulation of CD8+ T cells and CD4+ Th1 (26). However, when appropriate stimuli are administered before birth, newborns can protect Th1-type immune responses and induce robust despite established differences between them and adult adaptive immune responses (26).

5. Application of Lm-based vaccine in solid tumors

There have been numerous clinical trials and preclinical involving Lm-based vaccines for cancers, including malignant pleural mesothelioma, Breast Cancer (BC), cervical cancer, prostate cancer, and melanoma.

5.1. Cervical cancer

There are several risk factors associated with chronic HPV infection, especially type 16, that contribute to cervical cancer, the fourth most common cancer among women (27). A substantial amount of evidence must be gathered before current therapeutic interventions can be considered productive. The second-line alternatives remain a source of disagreement due to the poor prognosis (28). The new vaccine Axalimogen filolisbac (ADXS11-001) is based on a weakened *Lm* containing the HPV-16 E7 and LLO virus antigen (29). ADXS11-001 can stimulate specific immune responses against malignant cells expressing the E7 molecule (29). As a result, Tumor-Infiltrating Lymphocytes (TIL) are increased, and the immunosuppression status of TME is also alleviated (30).

Clinical trials such as phase I, II, and III have generated encouraging results for cervical cancer patients in the future. A growing body of evidence suggests that recombinant *Lm* strains are more effective when combined than when used alone. A significant improvement in disease regression was observed when HPV-infected mice were treated with LIΔactAplcB-E6E7 (LIΔ-E6E7) and LMDactAplcB-E6E7 (LMΔ-E6E7) before these vaccines were administered. Furthermore, some studies show that optimizing codon usage improves host immunity against TAAs (31). By comparing the codon-enhanced *LM*4Δhly:: E7-1 to *LM*4Δhly::E7, stronger Th1-biased immunity was observed and increased specific CTL activity and lymphocyte proliferation —furthermore, *LM*4Δhly::E7 significantly improved tumor establishment efficacy (32).

5.2. Melanoma

Melanoma can be classified as the most destructive form of skin cancer arising from melanocytes. Inhibition of melanoma growth by attenuated DactA/DinlB *Lm* that express Melanoma Inhibitory Activity (MIA) is possible by reducing the density of blood vessels (33). It can induce cell-mediated immune responses against HMW-MAA by expressing *Lm* with a high molecular weight melanoma-associated antigen (HMW-MAA-C) targeting pericytes within the tumor vascular system and cells (34). It still appears that non-targeting *Lm* can cause cell death in melanoma without a specific antigen target. Genetically engineered Braf/Pten mice with melanoma showed significant reductions in their volume, size, and metastatic burden due to Lmat-LLO, which produces ROS and induces apoptosis (35). After transplanting B16F10 cells into a mouse model with OVA-expressing *Lm* with deletions of phospholipase C and actA, the mouse model showed protection against melanoma due to robust CD8+ T cell responses. As a result of combined ICI and RT, the *Lm* vaccine shows superior effects in reducing tumor size and increasing the infiltration of antigen-specific CD8 + T cells and NK cells (36).

5.3. Breast cancer

One of the most common cancers in women is Breast Cancer (BC). There was a mortality rate of more than 20% among patients with resistance to intervention and metastatic lesions. Currently, surgery is the first line of treatment for metastatic cancer, followed by chemotherapy or radiation. Although BC therapy has advanced recently, primary or metastatic tumor cells are rarely eliminated after primary treatment. Several aggressive strategies are needed, but few options exist, so other practical measures are urgently required. The immunotherapy treatment is a promising option and can be an essential alternative for patients with BC. A cDNA-expressing Mage-b *Lm* (*LM*-LLO- Mage-b/2nd) administered before establishing tumors eliminates metastases more effectively than *Lm*-LLO in 4T1 BC models (37).

A TAA Mage-b-expressing *Lm* (*Lm*-Mage-b) combines with immunological adjuvants to stimulate immunity. As a result of a synergistic interaction with a-galactosylceramide, *LM*age-b promotes the growth of NK T cells in the spleen and eliminates metastatic colonies without harming the cells (38). Three variations of Triple-Negative Breast Cancer (TNBC) include subtypes that lack the estrogen receptor, the progesterone receptor, and the Human Epidermal growth factor receptor 2 (HER-2 receptor). The effects of curcumin are enhanced by robust CD8+ T cell responses and the inhibition of Myeloid-Derived Suppressor Cells *(*MDSC*)*-derived IL-6, resulting in an advanced level of efficacy against metastasis (39). A high level of *Listeria* does not increase the number of MDSCs in primary tumors or blood. As a signal to stimulate CD8+ T cell clonal expansion, IL-12 plays a role (40).

5.4. Prostate cancer

According to estimates, the number of new cases of prostate cancer will increase to 288,300 in 2023, and the number of deaths will rise to 34,700 (41). It has been reported that nearly 20% of American men diagnosed with prostate cancer have localized or metastatic disease at the time of their diagnosis. Approximately 80% of patients with metastatic castration-resistant prostate cancer who have been treated with androgen deprivation therapy for 1-3 years will ultimately develop metastatic disease. In most prostate cancer cases, Prostate Specific Antigen (PSA) can be detected and is known to be the target antigen. A new live attenuated *Lm*-based immunotherapy, ADXS31-142, mimics the activity of *Lm* Lysozyme toxin (tLLO) by producing truncated fragments of the toxin and a fusion protein called tLLO-PSA (42).

In a study of patients with metastatic castration-resistant prostate cancer, ADXS31-142 combined with pembrolizumab demonstrated safety and well-tolerance. As a newly developed personalized immunotherapy, JNJ-64041809 (JNJ-809) is based on DactA/DinlB *Lm* and targets four antigens found in prostate cancer, most importantly, prostatic acid phosphatase (42). Synovial sarcoma X breakpoint 2, Prostate-specific membrane antigen, and NKX3.1 are the homeobox proteins involved in prostate carcinoma (43). Despite the risks associated with JNJ-809, the safety of the drug is manageable, and early interventions may result in a more robust response. The amount of antigen-specific immune response observed is restricted and has not converted into an objective clinical response despite the limited observable immune response.

5.5. Malignant pleural mesothelioma

Among the rare forms of Malignant Pleural Mesothelioma (MPM), asbestos exposure or exposure to other tiny carcinogenic fibers is most likely to cause the disease. In patients with unresectable MPM, pemetrexed and cisplatin are commonly used as standard first-line treatments. A high mortality rate has prompted the search for alternative therapies. As more evidence accumulates, immunotherapeutic approaches may be promising treatment options for MPM. Most epithelial MPMs overexpress mesothelin, and CRS-207 increases NK cell and T cell infiltration and converts macrophages from immunosuppressive M2 to proinflammatory M1. Combining CRS-207 with pemetrexed/cisplatin increased the CD8+ T cell ratio and DC penetration in mice with MPM in a phase Ib study (44). Therapy can significantly reduce tumor size without causing severe side effects. Cytoreduction surgery may also reduce immunosuppression, making mesothelin-expressing *Lm* vaccines more effective (Figure 1) (Table 1).

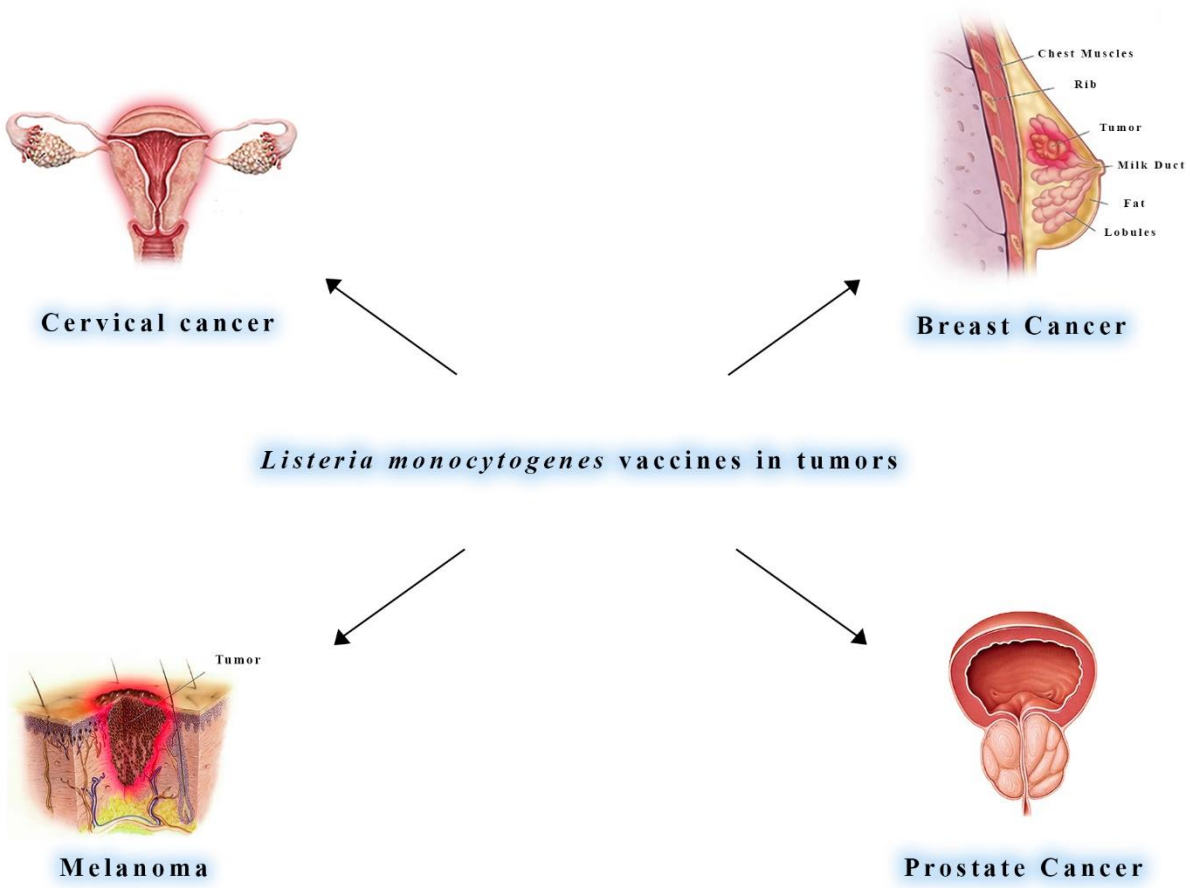

Figure 1: *Listeria monocytogenes* (*Lm*) vaccines in various tumor types

Listeria monocytogenes (Lm) vaccines across multiple tumor types, including cervical, prostate, breast, and melanoma. *Lm*-based vaccines have shown promising results in enhancing antitumor immune responses and overcoming the immunosuppressive microenvironment in these cancers.

Table 1. Important recent studies regarding using *LM* to fight cancers.

Author	Year	Method	Results
Yi-Dan Ding	2023	Selectively deleting virulence genes of wild-type *LM*, including inlB, actA, alanine racemase (dal), and D-amino acid aminotransferase (dat), have	the result indicates that the competition with immunodominant *LM*-derived CD8+ T-cell epitope may be involved.

		been widely used to develop an ideal vector	
Azam Bolhassani et al	2017	activate different signaling pathways in APCs containing: (a) a TLR/MyD88-dependent pathway promoting the expression of inflammatory or suppressive/regulatory cytokines (e.g. TNF-α, IL-12, and IL-10), autophagy and ROS production; (b) a STING/IRF3-dependent pathway resulting in expression of IFN-β and co-regulated genes; and (c) an AIM-2/ Caspase-1-dependent inflammasome pathway leading to pro- teolytic activation and secretion of IL-1β and IL-18	These vectors stimulate MHCI and MHCII pathways and the proliferation of antigen-specific T lymphocytes.
John C. Flickinger Jr	2018	Vaccines using ΔactA/ΔinlB strains also exhibit rapid clearance of infection from the liver and spleen compared to single ΔactA or ΔinlB mutants.	Lm vaccines targeting tumor-associated angiogenic proteins, including CD105 and VEGFR2, have demonstrated inhibited tumor growth

Mark Tangney	2010	The cytoplasmic location of L. monocytogenes is significant as this potentiates entry of antigens into the MHC Class I antigen processing pathway, leading to priming of specific CD8+ T cell responses	studies have demonstrated the ability of *L. monocytogenes* for intracellular gene or protein delivery in vitro and in vivo, and this vector has also displayed safety and efficacy in clinical trial
Jorge H. Leitão	2020	Deleting virulence genes, constructing strains that ectopically express virulence or metabolic genes, and killing but metabolically active strains. virulence factors ActA and LLO by fusing them with tumor-associated antigens	successful *Listeria monocytogenes* cancer vaccines are immense, and there is great hope that such vaccines will contribute to innovative anti-cancer therapies that benefit cancer patients

6. Safety of *Lm* strains and future perspectives

Infections caused by *Lm* are most common in immunocompromised or immunosuppressed patients, such as the elderly, pregnant women, and the very young. The wild-type strain was highly attenuated compared to the live vectors used for clinical trials. Clinical trials can be highly effective with their attenuation. Antibiotic-free vectors enable them to be treated more readily for side effects after vaccination because of their use. *Lm* can also be grown on a media free from animal products, and its DNA cannot integrate into an organism's genome as viral vectors can (45). Clinical trials evaluating Lm's safety found that its administration caused flu-like symptoms less severe than those caused by chemotherapy or radiation treatment. To minimize potential adverse events, patients may be screened for immune deficiencies before treatment, and a suitable antibiotic should be included in clinical protocols after dosing for infection prevention.

It is also possible for *Lm* infections to spread to healthcare workers or a patient's family from the patient. In clinical studies, the strains used are highly attenuated and not infectious, even in the wild type. Finally, there is a concern regarding the possibility that the vector could spread in the environment, especially if an antibiotic-resistant gene is included in the vector design. *Lm* has recently been demonstrated to be effective as a vector for delivering intracellular genes or proteins *in vitro* and *in vivo*. Clinical trials have shown that

this *Listeria* is safe and effective and that an innate and adaptive immune response is required to remove the organism from the host. Cy/GVAX and CRS-207 heterologous prime/boost treatment with pancreatic cancer extended survival with minimal side effects (46).

Lm vaccines have exposed promising performance in primary and metastatic cancers by enhancing targeted antitumor immune responses and overcoming the immunosuppressive microenvironment (47). In contrast, *Lm* vaccines can cause systemic immune reactions, hypertension, and fatigue, similar to other classical immunotherapies. *Lm* is a pathogenic organism that has raised safety concerns, even risking bacteremia. Several educations have maintained a delicate balance between Lm-based vaccines' potential effectiveness and safety. In addition, a lack of technical facilities limits the ability to test if enough vaccines can reach one's tumor site. Additionally, lacking technical facilities limits testing whether enough vaccines can reach a tumor site due to a lack of technical infrastructure. The primary method of achieving this goal is to delete virulence factors or develop Lm-RIID and KBMA strains.

As developed strains become more sophisticated, more effective evaluation ways will be required to select the best applicants. Research has also examined the combination of Lm-based vaccines and ReACT cells, radiotherapy, Immune Checkpoint Inhibitors (ICI), and other treatments with remarkable results (48). Vaccines based on *Lm* can improve the immune response of CD8+ T cells and reform TME, allowing them to use synergistic antitumor effects (48). Future clinical research may also focus on how Lm-based vaccines contribute to other approaches that regulate TME immune status. The immune system differences between humans and experimental animals continue to hinder Lm-based vaccine development. It is possible to elicit robust CD8+ T cell immunity by using Lm-based vaccines to target mouse tumor antigens. It has not yet been confirmed in humans. Several studies have suggested that the differences between human and mouse Gamma Delta (gd) T cells might explain this phenomenon. A T cell can be classified into two types based on gd receptor expression: gd-positive cells (gd+) and ab-negative cells (gd-). Gamma Delta T cells in humans are mostly Vg9Vd2+, whereas GD T cells in murine are mostly Vg5Vd1+.

It has been shown that human DC infected with *Lm* induces Vg9Vd2 T cells by upregulating cholesterol metabolism (49). In contrast to mice, *Lm* infection in humans increases Vg9Vd2 T-cell proliferation. Lm-based vaccines expressing Lm-GUCY2C can induce strong Lm-specific immunity rather than anti-GUCY2C immunity rather than anti-GUCY2C immunity against colorectal cancer antigens (Lm-GUCY2C) (50). As a result of the finding, the immunodominant CD8+ T-cell epitope derived from the *Lm* may be competing with it. Some evidence suggests that weak antigens, such as GUCY2C, are susceptible to competition from peptides derived from Lm. Even though the principal mechanisms of Lm-derived peptides may explain recent advances and the unresolved problems of different species, future research must explore the synergies between treatments and vaccines (Figure 2).

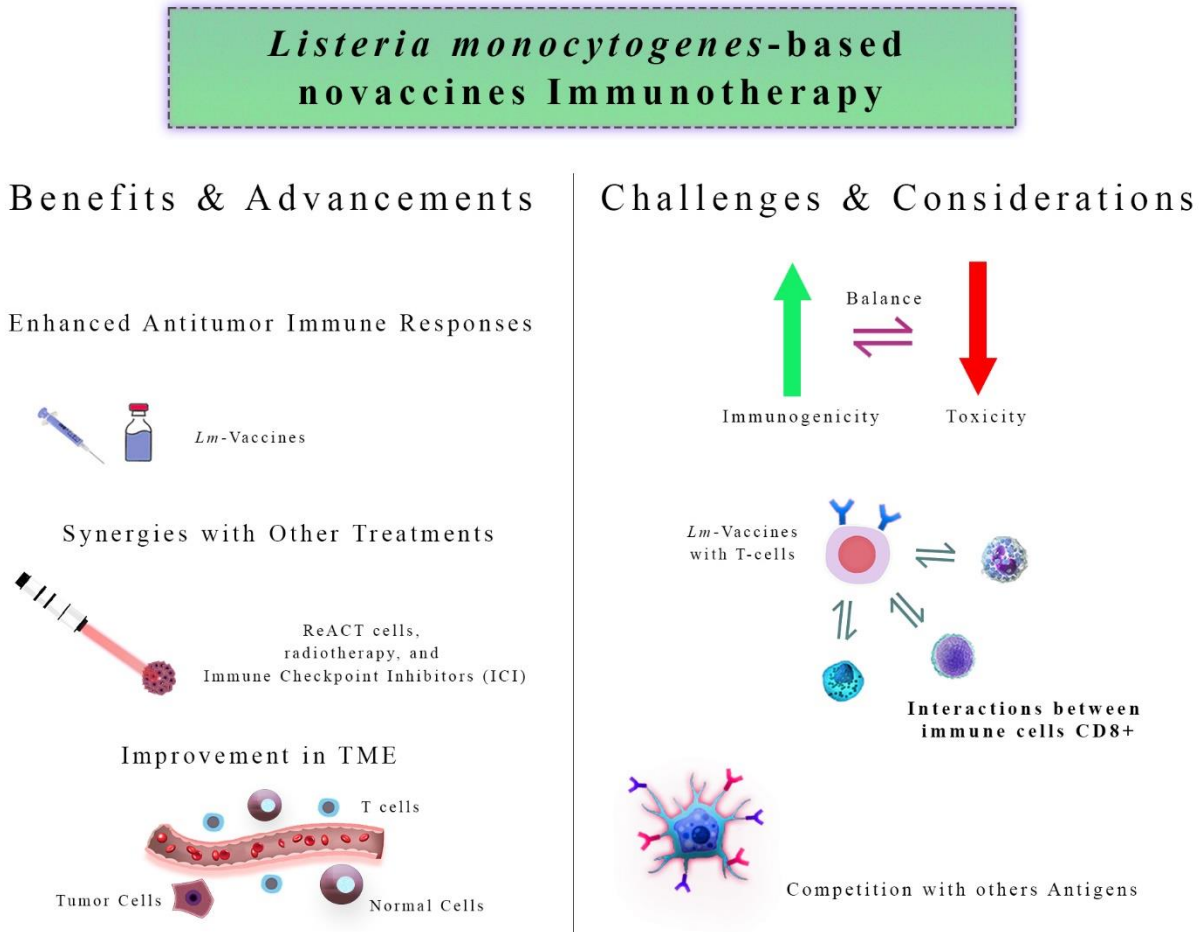

Figure 2: Overview of Challenges, Considerations, Benefits, and Advancements in *Listeria monocytogenes* (*Lm*)-Based Cancer Vaccines.

Challenges and considerations include safety concerns, technical limitations in evaluating vaccine efficacy, species differences affecting translational research, competition with weak antigens, and the need for effective evaluation methods for sophisticated *Lm* strains. On the other hand, benefits and advancements highlight enhanced antitumor immune responses, synergies with other treatments, improvements in the tumor microenvironment, and the potential of *Lm* vaccines in inducing strong *Lm*-specific immunity against colorectal cancer antigens.

Conclusion

Therefore, *Lm*-based immunotherapies may be a promising new avenue in the fight against cancer. Research has shown that bacteria can enhance the effectiveness of cancer treatments while minimizing side effects by exploiting their unique characteristics, such as their ability to invade host cells and induce robust

immune responses. As a therapeutic agent, modified *Lm* are versatile, reshaping the tumor microenvironment, stimulating T cells, and reducing immunosuppressive cells. Despite the vaccine's considerable promise, it will likely not be an all-inclusive solution for treating *Lm*. A more effective approach would be to combine vaccines with other complementary treatments, such as adoptive cell therapy, radiotherapy, and ICI. To further advance the understanding of the molecular mechanisms involved in its contribution to antitumor immunity, more research needs to be undertaken into the mechanisms involved in its effect and providing optimal combination therapies tailored to the needs of individual patients.

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Conflict of interests

The authors declare no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval

On behalf of all co-authors, I hereby confirm that I have reviewed and complied with the relevant Instructions to Authors, the Ethics in Publishing policy, and Conflicts of Interest disclosure.

Author contribution

Conceptualization: [Alireza Omeanzadeh, Mahdi Soroushianfar], …; Methodology: [Samin Safarian, Danial Soltani, Mahdi Soroushianfar], …; Formal analysis and investigation: [Fatemeh Arzani, Zahra Mostofi Fakhrani, Alireza Omeanzadeh], …; Writing - original draft preparation: [Fatemeh Arzani, Zahra Mostofi Fakhrani, Alireza Omeanzadeh, Samin Safarian, Danial Soltani, Mahdi Soroushianfar]; Writing - review and editing: [Fatemeh Arzani, Zahra Mostofi Fakhrani, Alireza Omeanzadeh], …; Funding acquisition: [Self-funding], …; Supervision: [Alireza Omeanzadeh]. All authors checked and approved the final version of the manuscript for publication in the present journal.

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