Review Article

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

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ABSTRACT

The escalating mortality and morbidity rates have prompted global attention to focus on cancer, with the exploration of new treatment options being a key priority. The utilisation of immunotherapy for recurrent or metastatic cancer has emerged as a promising option over the years, despite its limitations in comparison to traditional treatment options. Among the various immunotherapeutic approaches, bacterial-based vectors such as Listeria monocytogenes (Lm) have attracted considerable attention on account of their distinctive characteristics. The utilisation of these vectors entails the exploitation of their capacity to invade antigen-presenting cells (APCs), proliferate intracellularly within immune cells, and disseminate within these cells, thereby augmenting their efficacy in modulating immune responses. It is important to note that the use of bacterial vectors significantly minimises 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. Research has indicated that a range of tumour cell types can be targeted by modified Lm vaccines. However, it is acknowledged that Lm vaccines alone may not be sufficient for a comprehensive cancer treatment. Consequently, the employment of Lm vaccines in conjunction with other therapeutic modalities, such as radiotherapy, reactivated adoptive cell therapy, and immune checkpoint inhibitors, has the potential to yield superior outcomes. Consequently, the present review aims to elaborate on recent advancements in the understanding of the antitumor properties of Lm vaccines. The objective of this review is to provide insights into optimising 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 in the fight against cancer, researchers and clinicians must gain a deeper understanding of the mechanisms involved.

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. Cancer is a broad category of diseases that can affect virtually any organ or tissue in the body. It is defined by uncontrolled growth and subsequent invasion of other parts of the body, which can ultimately lead to death. According to data from the World Health Organization (WHO), cancer was responsible for 9.6 million deaths in 2018 (1). Conventional cancer therapies, including chemotherapy, surgery and radiation, are associated with a number of physical and psychological side effects. Immunotherapies have become increasingly prevalent in the treatment of cancer in recent years (2). The advent of immunotherapies in 2010, marked by the approval of sipuleucel-T for the treatment of prostate cancer, signified a pivotal shift in the landscape of cancer therapy. This development stood in stark contrast to the prevailing views of immunologists and oncologists, who previously regarded the artificial stimulation of the immune system as a mere aspiration. Cancer immunotherapy has undergone rapid development in recent years (4). Immunotherapies can be categorised into two main types: passive and active. The latter has emerged as a means of selectively destroying tumour cells by reactivating or activating host cellular immunity, primarily mediated by T cells (5). Furthermore, immunomodulatory drugs have been shown to exert their therapeutic effects by increasing the absorption of dendritic cells (DC), antibodies, macrophages, natural killer cells (NK), and cytokines that target tumours. Recent findings have demonstrated the efficacy of tumor immunotherapy in enhancing patient prognoses and circumventing the constraints imposed by conventional therapeutic modalities (6-9). The development of cancer vaccines derived from bacteria represents a highly active research domain, with imminent applications anticipated. The first documented instance of this approach occurred in 1890, when William B. Coley, a surgeon at New York Memorial Hospital, described the use of bacteria as anticancer agents. It has been documented that a variety of live attenuated, dead but metabolically active, and genetically engineered microorganisms, including Bacillus, Clostridium, Listeria monocytogenes (Lm), and Salmonella, have the capacity to target cancer cells and exhibit anticancer properties (10). Lm is a Gram-positive bacterium that is primarily recognized as a food-borne pathogen, capable of causing sepsis, bacteremia, and encephalitis, among other serious infections.

2. Evidence Acquisition

The organism produces several virulent factors, including Listeria Lysin O (LLO), phospholipases specific to phosphatidylinositol, ActA proteins that recruit and polymerize host actin, and internalin, which aids nonphagocytic cells in adhering to and internalizing the pathogen. The internalins B (inlB) and A (inlA) are surface proteins on Lm that interact with C-Met and E-cadherin to facilitate the entry of Lm into nonphagocytic cells. In the host phagosome, Lm becomes enclosed after internalization. The secretion of lectin LLO and phospholipases (plcA and plcB) by Lm results in the perforation of phagosomes. This process enables the intrusion of the cytoplasm, thereby facilitating the evasion of phagolysosomes and the subsequent avoidance of cell death. Lm has been found to secrete Tumor-Associated Antigens (TAAs), which are subsequently degraded by proteasomes, thereby stimulating specific CD8+ T cells through Major Histocompatibility Complex (MHC) class I molecules (11). In contrast to other bacteria, Lm can more easily evade immune surveillance due to a combination of factors, including hypoxia, suppressive Tumor Micro Environment (TME), and its capacity for intracellular growth. The augmentation of effector T cells in the presence of immunosuppressive cells has been demonstrated to enhance the efficacy of immunotherapy through the potentiation of innate immunity and the restructuring of the tumor microenvironment (TME). A substantial body of research has examined the potential of Lm as a vector for tumor immunotherapy. Notwithstanding, Lm-based therapy in isolation continues to engender a multitude of complications and therapeutic shortcomings, attributable in part to its purported pathogenic characteristics. Among the potential strategies for overcoming these limitations, the combination of Lm-based therapy with other treatments is a promising avenue for further investigation. Listeria monocytogenes (LM) cancer vaccines have demonstrated significant potential, and there is considerable optimism regarding their potential to contribute to the development of novel anticancer therapies for cancer patients (12). The review will specifically address the manner in which Lm-based immunotherapy modulates immune pathways to produce a promising antitumor response, as well as 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, the intravenous administration of Lm is typically employed in the treatment of immunological conditions, thereby circumventing the intestinal epithelium. Listeria monocytogenes, a bacterium that can cause infections in humans, can enter the bloodstream and infect various organs, including the placenta, brain, and liver. While it is capable of persistence outside the cell, its replication preference is for the cytoplasm of the cell. A multitude of immune mechanisms are initiated within the intracellular compartments of Lm as it undergoes this transition. The primary function of the internal family of proteins is to facilitate the entry of Lm into mammalian cells. Of particular interest are InlA and InlB, which have been demonstrated to promote Lm invasion by binding to host receptors and initiating receptor-mediated endocytosis (13). It has been demonstrated that the internalin A and internalin B toxins infect different types of cells due to their binding to other receptors. The internalin A protein has been observed to bind to E-cadherin on epithelial cells, while the internalin B protein has been shown to interact with Met, which is also known as hepatocyte growth factor. In addition to entering through cell surface receptors, APCs actively take up Lm through phagocytosis. Furthermore, as Lm engages with the outer surface of mammalian cells, the innate immune response to Lm is initiated. The expression of pathogen-associated molecular patterns (PAMPs) on the cell surface and within phagosomes of Lm is recognized by mammalian toll-like receptors (TLRs), which in turn trigger nuclear factor-κB (NF-κB) signaling and promote inflammation (14). Upon internalization into phagosomes, Lm may undergo one of two fates. It is estimated that phagosome–lysosome fusion results in the killing of most bacteria, thereby providing antigens that stimulate MHC class II-dependent exhibition and CD4+ T-cell response to Lms. Consequently, Lm has evolved mechanisms to evade lysosome deprivation and gain entry into the cytosols of infected cells. In the host cell, Lm initiates the expression of PrfA as it transitions from the extracellular environment. PrfA activates numerous virulence genes, including those encoding the two types of phospholipases, PlcA and PlcB, and the pore-forming toxin LLO. Listeria monocytogenes (Lm) lysin O molecules form a barrel-shaped cavity at the phagosome membrane, thereby regulating the passage of Lm (15). In addition, phospholipases regulate Lm exit by directly hydrolyzing membrane lipids (16). Subsequent to exiting 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. Consequently, the direct excretion of Lm antigens into the cytosol, coupled with their subsequent degradation within phagosomes, results in the potent CD4+ and CD8+ responses exhibited by T cells in response to Lm antigens (17). The expression of the virulence factor actin assembly-inducing protein (ActA) enables Lm to evade the phagosome and disseminate within the host cell cytosol. Actin proteins are attached to the surface of Lm, where they interact with the Arp2/3 complex to stimulate actin monomer nucleation and filament formation. It has been established that Lm can be propagated throughout the cytosol and into the plasma membrane of an infected cell through the process of actin polymerisation. This process gives rise to protrusions that are able to be internalised by neighbouring cells, thereby facilitating the dissemination of the infection (18).

4. Immunological Mechanisms of *Listeria Monocytogenes*

A concept in biotechnology and medicine known as path-biotechnology employs virulence factors and pathogenic stress. Delivery systems that utilise Lm demonstrate considerable potential. Lm is one of the capable delivery systems (19). It has been demonstrated in several reports that the internal proteins of Lm (inlA and inlB) promote phagocytosis in normal non-phage-cystic human cells. Consequently, therapeutic drugs can be conjugated to inlA/inlB proteins for targeted delivery 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. Furthermore, 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). The present studies have determined that two essential genes, D-amino acid aminotransferase (DAT) and alanine racemase (ALR), play a pivotal role in the production of mucopeptides within the Lm cell wall. The present study proposes a novel approach for the expression and secretion of human CD24 protein, a biomarker of hepatocellular carcinoma, in human cells. This approach is based on the genetic alteration of the replication-deficient Lm strain dal dat (Lmdd). The administration of Lmdd-CD24 to mice resulted in a significant reduction in both T-regulatory cells (Treg) and tumour growth (21). In the domain of cancer immunotherapy, Cholesterol-Dependent Cytolysin (CDC) LLO, a pore-forming toxin derived from Lm, has been shown to be an effective adjuvant in enhancing 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. For instance, the combination of dtLLO fusions with Human Papillomavirus (HPV)16 recombinant E7 proteins (dtLLO-E7 fusion or dtLLO + E7) has been observed to promote dendritic cell maturation, enhance and eradicate tumours, thereby indicating its adjuvant effect and antitumour immune responses (induction of Interleukin (IL-12) and Tumor Necrosis Factor (TNFα)) (22). Furthermore,

seeligeriolysin O, a CDCs associated with Listeria seeligeri, has been observed to activate both TLR2 and TLR4 in macrophages, thereby triggering IL-12 production (22). Concurrently, mast cells exhibited microbicidal activity, characterised by the release of DNA and the presence of granular proteins embedded within DNA, known as Mast Cell Extracellular Traps (MCETs). The cells in question have been demonstrated to be the causative agent of 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 has been documented that discrepancies were identified during the process of generating DCs for the purpose of cancer treatment. The activation of dendritic cells (DCs) by Lm bacteria is initiated through the engagement of pattern-recognition receptors (PRRs). In order to ascertain the most efficient DC activation components, researchers examined Lm stimulation. It has been demonstrated that protein components are more effective at inducing dendritic cell (DC) maturation than DNA components. A substantial enhancement in the maturation of dendritic cells (DCs) and the stimulation of CD8+ T cell proliferation was observed when a lysate fraction containing 109 proteins was employed, in comparison with the maturation of DCs by lipopolysaccharide (LPS). An analysis of bioinformatics data on 109 proteins revealed that elongation factor Tu acted as a ligand for PRR during DC maturation (24). It was hypothesised that Lm could spread between cells without entering the extracellular matrix, thereby preventing an adequate antibody response. The hemolytic activity of its surface protein has been observed to induce a CD8+ CTL phenotype and CD4+ Th1 phenotype in vivo, a phenomenon analogous to that observed in other intracellular pathogens (25). The low immunogenicity of TAAs is a significant problem due to their high degree of similarity to self-proteins. Consequently, Lm's capacity to effectively stimulate the immune system enabled the presentation pathways of MHC class I and class II to be loaded with poorly immunogenic TAAs in professional APCs. In APCs, Lm has been observed to activate several signalling pathways, including the TLR/MyD88 pathway, which has been demonstrated to promote the expression of suppressive/regulatory or inflammatory cytokines, autophagy, and ROS production. An alternative pathway is governed by STING/IRF3, which results in the expression of co-regulated genes and Interferons (IFN-β). Furthermore, an alternative pathway is inflammasomes, which are activated by AIM-2/Caspase-1-mediated signalling pathways that result in proteolytic activation and secretion of IL-1β and IL-18 (26). It has been established that Lm activates pathways in monocytes and macrophages with a view to eliciting an innate immune response. Furthermore, Lm has been observed to stimulate 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. A study utilising a mouse model yielded intriguing findings. The investigation revealed that neonatal innate cells exhibited elevated levels of IL-10 and diminished levels of Th1-eliciting cytokines in response to stimulation with Lm. This outcome resulted in suboptimal stimulation of CD8+ T cells and CD4+ Th1 (26). However, when appropriate stimuli are administered before birth, it has been demonstrated that newborns can protect Th1-type immune responses and induce robust immune responses in the absence of established differences between them and adult adaptive immune responses (26).

5. Application of Lm-based Vaccine in Solid Tumors

A plethora of clinical trials and preclinical studies have been conducted employing Lm-based vaccines for various 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 body of evidence must be amassed before contemporary therapeutic interventions can be regarded as efficacious. The second-line alternatives remain a source of disagreement due to the poor prognosis (28). The novel vaccine, designated Axalimogen filolisbac (ADXS11-001), is predicated on a genetically modified Lm strain that harbours the HPV-16 E7 and LLO virus antigen (29). It has been demonstrated that ADXS11-001 has the capacity to stimulate specific immune responses against malignant cells that express the E7 molecule (29). Consequently, an increase in Tumor-Infiltrating Lymphocytes (TIL) has been observed, accompanied by a reduction in the immunosuppression status of the TME (30). The results of clinical trials, categorised as phase I, II, and III, have yielded encouraging outcomes for patients diagnosed with cervical cancer. A mounting body of evidence indicates that recombinant Lm strains exhibit enhanced efficacy when employed in combination rather than as standalone treatments. 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 the administration of these vaccines. Moreover, research has indicated that optimising codon usage can enhance the host immune response to TAAs (31). In the experiment, E7-1 to LM4Δhly::E7 was observed to demonstrate a stronger Th1-biased immunity, with increased specific CTL activity and lymphocyte proliferation. Furthermore, LM4Δhly::E7 significantly improved tumour establishment efficacy (32).

5.2. Melanoma

Melanoma is classified as the most destructive form of skin cancer arising from melanocytes. The 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 has been demonstrated that this 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 tumour vascular system and cells (34). It is evident that non-targeting Lm can induce cell death in melanoma, independent of a specific antigen target. Genetically engineered Braf/Pten mice with melanoma demonstrated significant reductions in volume, size, and metastatic burden following treatment with Lmat-LLO, which produces ROS and induces apoptosis (35). Following the transplantation of B16F10 cells into a mouse model that expressed OVA and had undergone deletions of phospholipase C and actA, the mouse model demonstrated a robust CD8+ T cell response, which resulted in protection against melanoma. In consequence of the combination of ICI and RT, the Lm vaccine has been demonstrated to engender superior effects in terms of the reduction of tumour size and the augmentation of the infiltration of antigen-specific CD8+ T cells and NK cells (36).

5.3. Breast Cancer

Breast cancer (BC) is one of the most prevalent forms of cancer among the female population. The mortality rate among patients exhibiting resistance to intervention and metastatic lesions exceeded 20%. At present, surgery is the primary treatment for metastatic cancer, with chemotherapy or radiation therapy being used as secondary treatment. Despite recent advancements in BC therapy, primary or metastatic tumour cells are seldom eradicated following primary treatment. A range of assertive strategies is required, yet there is a paucity of available options. Consequently, the implementation of additional pragmatic measures is imperative. 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 the establishment of tumours has been shown to be more effective in the elimination of metastases than Lm-LLO in 4T1 BC models (37). The process of TAA Mage-b-expressing Lm (Lm-Mage-b) combines with immunological adjuvants to stimulate immunity. In consequence of a synergistic interaction with a-galactosylceramide, LMage-b has been demonstrated to promote the growth of natural killer T cells in the spleen and to eliminate metastatic colonies without causing harm to the cells (38). There are three distinct variations of triple-negative breast cancer (TNBC). These include subtypes that are characterised by the absence of the oestrogen receptor, the progesterone receptor and the human epidermal growth factor receptor 2 (HER2 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). Furthermore, an elevated level of Listeria does not appear to result in an increase in the number of MDSCs present within primary tumours or blood. IL-12 has been demonstrated to play a role in stimulating CD8+ T cell clonal expansion (40).

5.4. Prostate Cancer

Projections indicate that 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 documented that approximately 20% of American men diagnosed with prostate cancer have either localized or metastatic disease at the time of their diagnosis. It is estimated that approximately 80% of patients diagnosed with metastatic castration-resistant prostate cancer who have undergone androgen deprivation therapy for a period of 1-3 years will eventually progress to metastatic disease. In the majority of prostate cancer cases, Prostate Specific Antigen (PSA) can be detected and is recognised as the target antigen. A novel live attenuated Lm-based immunotherapy, designated ADXS31-142, has been developed to mimic the activity of Lm Lysozyme toxin (tLLO) by producing truncated fragments of the toxin and a fusion protein termed tLLO-PSA (42). A study of patients with metastatic castration-resistant prostate cancer revealed that the combination of ADXS31-142 and pembrolizumab was both safe and well-tolerated. As a newly developed personalised 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). The homeobox proteins implicated in prostate carcinoma are synovial sarcoma X breakpoint 2, prostate-specific membrane antigen and NKX3.1. Notwithstanding the risks associated with JNJ-809, the safety of the drug is manageable, and early interventions may result in a more robust response. Despite the observation of an antigen-specific immune response, no objective clinical response has been demonstrated.

5.5. Malignant Pleural Mesothelioma

Among the rare forms of Malignant Pleural Mesothelioma (MPM), exposure to asbestos or other tiny carcinogenic fibres is the most probable cause of the disease. In patients with unresectable MPM, pemetrexed and cisplatin are commonly used as standard first-line treatments. The high mortality rate has prompted the search for alternative therapies. As further evidence accumulates, immunotherapeutic approaches may emerge as a promising treatment option for MPM. The majority of epithelial MPMs overexpress mesothelin, and CRS-207 has been observed to increase NK cell and T cell infiltration, as well as converting macrophages from the immunosuppressive M2 phenotype to the proinflammatory M1 phenotype. In a phase Ib study, the combination of CRS-207 with pemetrexed/cisplatin resulted in an increase in the CD8+ T cell ratio and DC penetration in mice with MPM (44). It has been demonstrated that therapeutic intervention can

result in a substantial reduction in tumour size without the occurrence of severe adverse effects. It has been hypothesised that cytoreduction surgery may also result in a reduction of immunosuppression, thus rendering mesothelin-expressing Lm vaccines more effective (Figure 1 and Table 1).

6. Safety of *Lm* Strains and Future Perspectives

Infections caused by Lm are most prevalent among those with compromised or suppressed immune systems, including the elderly, pregnant women, and infants. The wild-type strain exhibited a significantly higher degree of attenuation in comparison to the live vectors that were utilised in the clinical trials. The efficacy of clinical trials is contingent upon the attenuation of their inherent risks. The employment of antibiotic-free vectors facilitates more straightforward management of any adverse reactions that may ensue post-vaccination, owing to their utilisation. Furthermore, Lm can be cultivated in a medium devoid of animal products, and its DNA is incapable of integrating into an organism's genome, in contrast to viral vectors (45). A series of clinical trials were conducted in order to evaluate the safety of Lm. The results of these trials indicated that the administration of Lm caused symptoms that were less severe than those caused by chemotherapy or radiation treatment, and which were comparable to the symptoms of the flu. In order to minimise potential adverse events, patients may be screened for immune deficiencies prior to treatment, and a suitable antibiotic should be included in clinical protocols following dosing for the prevention of infection. Furthermore, it is possible for Lm infections to be transmitted to healthcare workers or a patient's family from the patient. In clinical studies, the strains utilised are characterised by their high degree of attenuation, rendering them non-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. Recent studies have demonstrated the efficacy of Lm as a vector for the delivery of intracellular genes or proteins, both in vitro and in vivo. It has been demonstrated by clinical trials that this Listeria is both safe and effective. Furthermore, it is imperative to remove the organism from the host, for which an innate and adaptive immune response is required. The administration of a combination of Cy/GVAX and CRS-207, in a heterologous prime/boost configuration, has been demonstrated to prolong survival in cases of pancreatic cancer, with minimal occurrence of adverse effects (46). The administration of Lm vaccines has demonstrated the potential for enhanced antitumour immune responses and the ability to overcome an immunosuppressive microenvironment, offering a promising solution for the treatment of primary and metastatic cancers (47). In contrast, Lm vaccines have been observed to elicit systemic immune reactions, hypertension, and fatigue, which are analogous to the adverse effects associated with other classical immunotherapies. Lm is a pathogenic organism that has raised safety concerns, even risking bacteremia. It is evident that a number of educational institutions have succeeded in preserving a tenuous equilibrium between the potential efficacy and safety of Lm-based vaccines. Furthermore, a paucity of technical facilities engenders limitations in the capacity to ascertain whether an adequate supply of vaccines can reach the site of the tumour. Furthermore, the absence of adequate technical facilities hinders the capacity to assess the sufficiency of vaccines in reaching the tumour site, owing to a paucity of technical infrastructure. The primary method of achieving this objective is to delete virulence factors or develop Lm-RIID and KBMA strains. As developed strains become more sophisticated, more effective evaluation methods will be required to select the most suitable 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 have been shown to enhance the immune response of CD8+ T cells and modify the TME, thereby facilitating synergistic antitumour 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 tumour antigens. However, the validity of these findings remains to be substantiated in human subjects. A plethora of studies have posited that the disparities between human and mouse Gamma Delta (gd) T cells may provide a rationale for this phenomenon. In human subjects, the majority of Gamma Delta T cells are of the Vg9Vd2+ type. In contrast, in murine subjects, the majority of Gamma Delta T cells are of the Vg5Vd1+ type. It has been demonstrated that human DC infected with Lm induce Vg9Vd2 T cells by upregulating cholesterol metabolism (49). In contrast to the murine model, Lm infection in humans has been shown to induce Vg9Vd2 T-cell proliferation. It has been demonstrated that Lm-based vaccines expressing Lm-GUCY2C can induce robust Lm-specific immunity, as opposed to anti-GUCY2C immunity, when administered against colorectal cancer antigens (Lm-GUCY2C). Consequently, the finding suggests that the immunodominant CD8+ T-cell epitope derived from the Lm may be competing with it. It has been hypothesised that weak antigens, such as GUCY2C, may be susceptible to competition from peptides derived from Lm. Despite the fact that the principal mechanisms of Lm-derived peptides may provide explanations for recent advances and unresolved problems in different species, it is imperative that future research explores the synergies between treatments and vaccines (Figure 2).

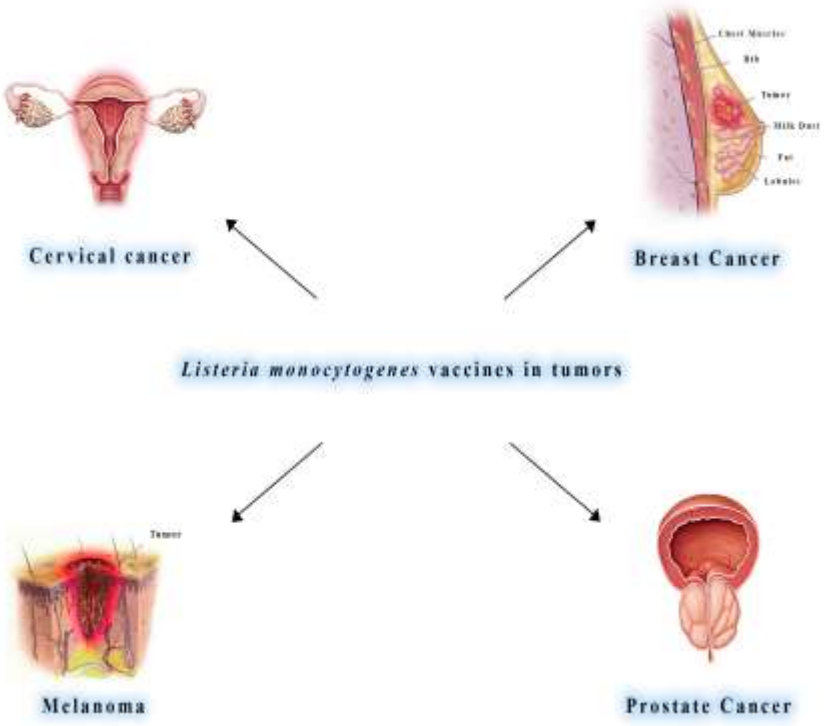

Figure 1: *Listeria monocytogenes* (*Lm*) vaccines in various tumor types
Listeria monocytogenes (*Lm*) vaccines in various tumor types, including cervical, prostate, breast, and melanoma. *Lm*-based vaccines have shown promising results in enhancing antitumor immune responses and overcoming the immunosuppressive 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 been widely used to develop an ideal vector	the result indicates that the competition with immunodominant *LM*-derived CD8+ T-cell epitope may be involved.
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

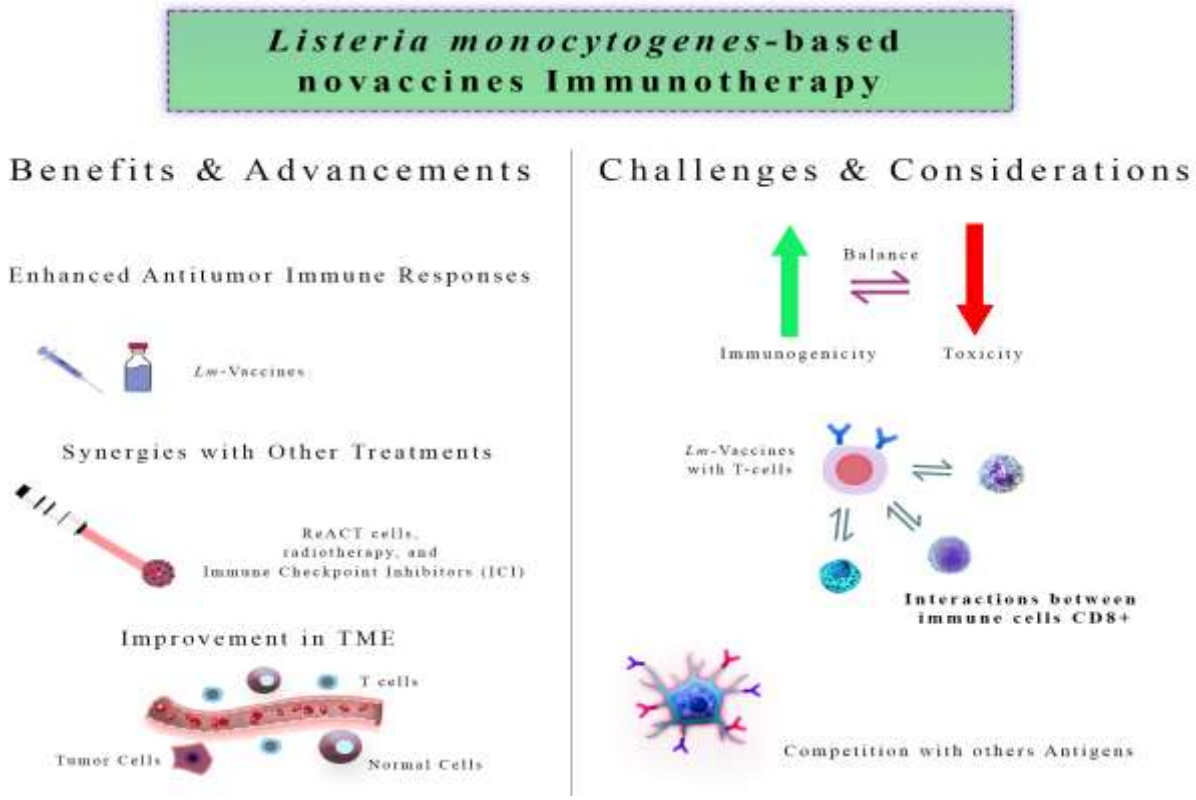

Figure 2: Overview of Challenges, Considerations, Benefits, and Advancements in *Listeria monocytogenes* (*Lm*)-Based Cancer Vaccines.
Challenges and considerations include safety concerns, technical limitations in evaluating vaccine efficacy, species differences affecting translational research, competition 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.

7. Conclusion

Consequently, Lm-based immunotherapies may represent a novel and promising approach in the fight against cancer. Research has demonstrated that bacteria have the capacity to enhance the effectiveness of cancer treatments while minimising 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 has been demonstrated to be versatile, with the capacity to reshape the tumour microenvironment, stimulate T cells, and reduce immunosuppressive cells. Notwithstanding the considerable promise of the vaccine, it is unlikely to constitute a panacea for the treatment of Lm. A more efficacious approach would be to combine vaccines with other complementary treatments, such as adoptive cell therapy, radiotherapy, and ICI. In order to further advance the understanding of the molecular mechanisms involved in its contribution to antitumor immunity, further research is required into the mechanisms involved in its effect and the provision of optimal combination therapies tailored to the needs of individual patients.

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Authors' Contribution

Conceptualization: A. O, M. S.
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Writing - original draft preparation: F. A, Z. MF, A. O, S. S, D. S, M. S.
Writing - review and editing: F. A, Z. MF, A. O.
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Ethics

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 on behalf of all co-authors.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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