

Review

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Bacteria in cancer therapy: beyond immunostimulation

Wheeler Torres¹, Víctor Lameda¹, Luis Carlos Olivar¹, Carla Navarro¹, Jorge Fuenmayor¹, Adrián Pérez¹, Andres Mindiola², Milagros Rojas¹, María Sofía Martínez¹, Manuel Velasco³, Joselyn Rojas^{1,4}, Valmore Bermudez^{1,5}

¹Endocrine and Metabolic Diseases Research Center, the University of Zulia, Maracaibo 4001, Venezuela.

²Miami Veterans Affairs Medical Center, Geriatric Research Education and Clinical Center (GRECC), Miami 33125, FL, USA.

³Department of Pharmacology, "JM Vargas" Medical School, Central University of Venezuela, Caracas 1050, Venezuela.

⁴Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston 02115, MA, USA.

⁵Advanced Frontier Studies Research Group (ALEF), Simón Bolívar University, Cúcuta 540006, Colombia.

Correspondence to: Dr. María Sofía Martínez, Endocrine and Metabolic Diseases Research Center, the University of Zulia, Maracaibo 4001, Venezuela. E-mail: mmartinez@fmed.luz.edu.ve

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Abstract

Currently, conventional therapies in cancer are improving; chemotherapy, radiotherapy and surgery have increased survival significantly. New therapies have arisen with the same goal; immunotherapy has appeared as a promising option in the fight against cancer stimulating the immune system by inducing innate and adaptive responses. These responses include release of pro-inflammatory cytokines, making the immune system capable to eliminate or protect against multiple tumors. Nowadays, many of these therapies are being used in clinical settings, such as checkpoint inhibitors, monoclonal anti cytotoxic T-Lymphocyte associated protein 4 (CTL-4) and programmed death protein 1 (PD1), with inspiring results; however, they may decrease immunotolerance, limiting their use. At the same time, chemotherapy works by passive transport across the cell membrane, limiting its capacity to penetrate in tumor cells. For these reasons, bacteria employment represents one of the best candidates for cancer treatment. They can surpass these barriers with their selective colonization which also has an oncolytic effect by increasing proliferation and immunostimulation in the tumor environment. Attenuated strains, such as *Mycobacterium bovis*, *Clostridium*, *Salmonella typhimurium* and *Listeria monocytogenes* have been studied showing promising results in experimental models. However, their application in clinical trials has shown the need to maximize their therapeutic effect. Genetic engineering and synthetic biology are necessary to prove the scope that this novel approach has against cancer due to implications of cancer therapy and public health.

Keywords: Bacterium, cancer, selective colonization, *salmonella*, *clostridium*, *listeria*



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INTRODUCTION

At present, cancer has one of the highest morbidity and mortality rates worldwide, nationwide and statewide^[1,2]. It comes from the growth of uncontrolled and invasive malignant cells with DNA mutations capable of producing multiple diseases^[3]. Most of these malignant neoplasms have the same etiopathogenesis. However, the diversity on the anatomic location, histologic origin, immunologic characteristics and intrinsic spreading capacity (intertumoral heterogeneity)^[4], and different genomic alterations inside the same tumor (intratumoral heterogeneity)^[5] have shown the need for specific biomarkers and individualized therapy to improve patient prognosis.

Currently, conventional therapy such as surgery, chemotherapy, radiotherapy, or mixed therapy have increased survival rates worldwide against cancer in different subtypes^[6,7]. However, these practices produce many adverse effects and have shown a limited tumor penetrance^[8]. The role of the immune system has been studied in order to find a therapeutic approach with equivalent therapeutic potency and controlled damage to healthy tissue; which gave rise to immunotherapy as a novel treatment^[9].

Nowadays, many of these therapies are being used in clinical settings, including the checkpoint inhibitors monoclonal antibodies anti cytotoxic T-Lymphocyte associated protein 4 (CTLA-4) and programmed death protein 1 (PD1). They have been shown to increase survival in patients with metastatic melanoma^[10] but their mechanism of action decreases immunotolerance with systemic administration. The latter may cause autoimmune adverse effects, limiting its use only for specific patients^[11]. In the last few decades, experimental studies and clinical trials have been aimed to assess bacteria therapeutic functions^[12-15]. Bacteria selective replication within the tumor microenvironment gives them antitumor effect and minimizes systemic adverse effects. On the other hand, expression of multiple ligands, immunostimulants, cytokines and tumor antigens can be achieved through gene manipulation to increase the therapeutic effect against specific tumors.

Cancer causes many physical and psychological effects to the patients and their families, but it also increases state expenditures. For these reasons, evaluation of these novel therapies in clinical settings has great importance. This review brings the basic science principles in genetics, immunology, and microbiology that gave rise to this therapeutic approach, in addition to its latest experimental and clinical advances.

THE BEGINNING: GENETIC AND IMMUNOLOGIC BASIS IN CARCINOGENESIS

Carcinogenesis begins as a result of multiple genomic alterations within a cell. They come from a prolonged exposure to different mutagens^[16], adverse epigenetic factors^[17], as well as chronic infections^[18]. These alterations increase proliferation and affect cell cycle through gene functioning^[19], in proto oncogenes^[20] and tumor suppressor genes^[21], causing different mutations^[22]. They ultimately modify the cell physiology making a mutated cell capable to generate its own mitogenic signals, resist against growth inhibitory signals, and acquires its own blood vessels. In advanced stages it can even invade and metastasize^[3].

The role of the immune system in tumor surveillance comes from the response to multiple oncogenic viruses and other infecting agents that can induce a chronic inflammatory environment leading to carcinogenesis^[23,24]. Identification of tumor-specific antigens (TSA) induces an immune response on carcinogenesis at an early stage^[24]. Tumor cells generate multiple modified surface proteins, decreasing immunologic tolerance as carcinogenesis progresses, and many TSA are expressed^[25]. The immune system can recognize and eliminate abnormal cells, in a continuous and bidirectional pathway between innate and adaptive immunity, which is called “*Immunosurveillance*”^[26]. Natural killer (NK) cells^[27] and cytotoxic

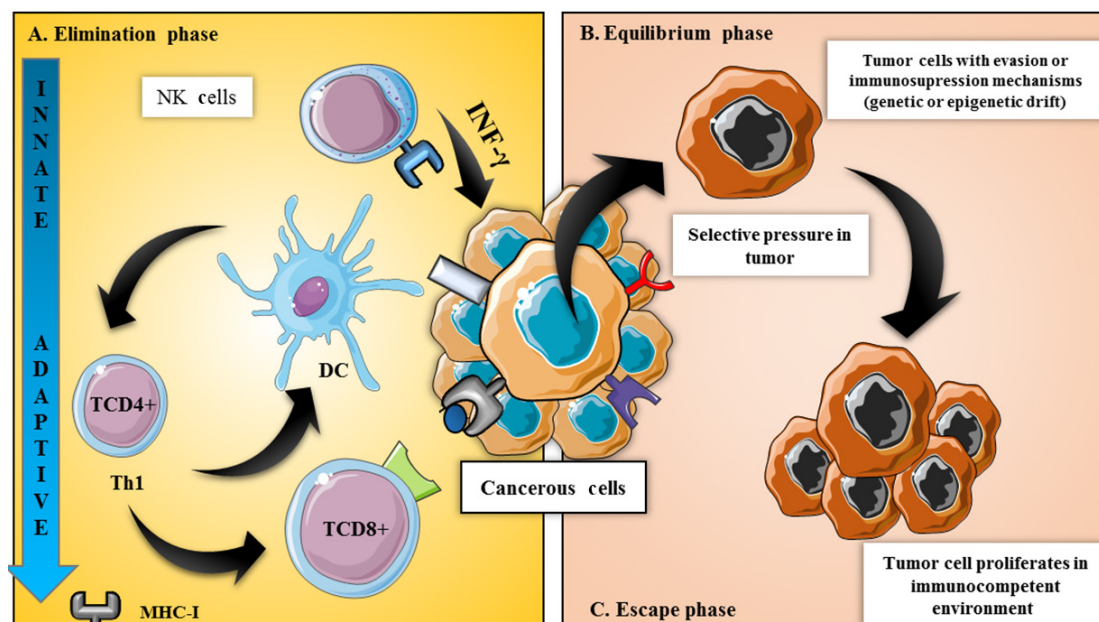


Figure 1. Immunosurveillance vs. immunoediting: key points in regulation of immune system in tumor progression/regression. Immunoediting comprises 3 phases: (A) elimination: when the tumor cells begin to proliferate, an inflammatory response is induced by the injured tissue. This causes the migration of cells from the immune system, orchestrating the innate immune response; (B) the equilibrium phase: in which this continuous process produces a selective pressure in these cells that can cause genetic or epigenetic rearrangement, causing certain cells to evade these immunological effector mechanisms; (C) the escape phase, when cells that have evaded these mechanisms also gained uncontrolled growth ability. DC: dendritic cells; MHC: major histocompatibility complex; NK: natural killers

T lymphocytes (T-CD8⁺) are the main mediators in this process^[28]; macrophages associated to tumors, dendritic cells (DC), naïve T cells, αβT-cell receptor (TCR)-expressing T cells, γδT-cells and regulatory T cells (T-reg) FOXP3⁺ also participate in the immune response towards the tumor. They interact with tumor cells, while some act inhibiting their growth and others stimulate it, composing the tumor microenvironment^[29].

NK cells are considered the main part of the innate immunity against tumors. They recognize and eliminate neoplastic cells effectively^[30,31]; but are not confined to the innate immune system. They also act with the adaptive immunity by working as T-lymphocyte response modulators^[32]. Damage associated molecule patterns (DAMPs) are released from tumor cell elimination mediated by NK cells^[33], increasing DC maturation^[34] and presentation to T-CD8⁺ lymphocytes on major histocompatibility complex (MHC)-1 molecules^[35]. Once activated, NK cells and T-CD8⁺ lymphocytes induce activation, proliferation and recruiting of other cells to the tumor site^[36]. This is achieved through the release of cytokines such as interferon gamma (IFN-γ), granulocyte and macrophage colonies stimulating factor (GM-CSF) and tumor necrosis factor (TNF)^[37]. IFN-γ carries important functions such as direct inhibition of tumor growth, macrophage activation, and increases Th1 expression among T-CD4⁺ lymphocytes. This represents their major role in modulating cellular response against tumors^[38]. T-CD8⁺ lymphocytes require the expression of tumor antigens on MHC-1 molecules and co-stimulatory signals in the tumor site in order to function appropriately^[39].

Cancer may become clinically detectable in advanced stages explained by the mechanisms in which tumor cells evade immune surveillance^[40]. This theory started with the “immunoediting” process^[41], where the immune system works inversely: making an immunosuppressed environment that favors tumor growth. This process is composed by three phases: elimination, equilibrium, and escape [Figure 1], being the elimination phase a homologous mechanism from those seen in immunosurveillance^[42].

Once the tumor cell has escaped the elimination phase, it enters the equilibrium phase^[43]. This phase consists in the destruction of cells expressing tumor antigens in their MHC-1 molecules by T-CD8⁺^[44]. Following this, less immunogenic cellular clones will be immunoselected and more aggressive tumor cells will grow, directing them to the escape phase^[43]. Although evasion of the immune system is not an isolated event; it also includes an immunological adaptation process. During this process an immunosuppressed microenvironment comes with recruitment T-reg FOXP3⁺^[45] and release of immunomodulatory molecules such as transforming growth factor β (TGF β), prostaglandin E2 (PGE2), indoleamin 2,3 dioxygenase (IDO), adenosine, and interleukin-10 (IL 10); also with decreased expression of co-stimulatory proteins and increased expression of inhibitor molecules such as CTL-4/CD28 and PD-1/PD-L1, called checkpoints^[46].

The discovery of these processes has led to research looking for novel immunologic therapies against cancer^[47]. Most of this therapy approaches have been dedicated to increase active or passive immune responses. Others have tried to modify tumor cells to increase recognition by the immune system^[48]. Despite of this, only few immunotherapies have achieved a response strong enough to be clinically effective^[49]. For these reasons, using bacteria to potentiate response has become a promising strategy.

INCOMING BACTERIOLOGY: ENEMIES OR ALLIES?

Chronic infection with biological agents represents a risk factor associated with cancer, with viral agents leading in this field^[50]. Bacteria have been associated with cancer because of their effect on cell cycle, and their capability to evade the immune system and cause immunosuppression through chronic infections^[51-53]. Bacterial infections stimulate phagocyte activity and increase oxidative stress on neighboring cells. The latter causes the release of reactive oxygen (ROS) and nitrogen (RNS) species such as peroxynitrite (ONOO⁻), reactive hydroxyl group (OH⁻) and other free radicals that damage cell membranes and DNA, affecting enzymatic activity and gene expression^[54]. Among DNA alterations mediated by oxidative stress, the most common includes the formation of 8-oxoguanine and/or 8-2'-desoxyguanosine. These modified nucleotides are caused by deregulated and repetitive metabolism, and lead to mutagenesis by inhibiting or enabling expression of altered genes^[55]. Chronic inflammation is considered carcinogenic^[56] by activation and preservation of nuclear factor κ B (NF- κ B) [Figure 2] which modulates gene expression related to cell cycle^[57,58], apoptosis^[59,60], proinflammatory cytokines, angiogenic processes^[58], invasion and metastasis^[61,62].

Infectious agents can act directly on the genome of their carrier and promote carcinogenesis by inactivation of tumor suppressor genes or mitotic stimulation. For example, chronic infections with *Helicobacter pylori* (*H. pylori*) carrying *CagA* positive virulence factor, causes mutations on p53 protein and *adenomatous polyposis coli* (APC) tumor suppressor genes; it can also induce loss of deleted in colorectal carcinoma (DCC) gene and microsatellite instability^[52]. Cases of infection by *Mycobacterium tuberculosis* affect tissue structure, generating a fibrotic scar that will probably increase the risk of carcinogenesis by blocking the lymphatic flow that decreases activated leucocyte depuration and increases risk for metastatic deposits. *Mycobacterium tuberculosis* can also modulate tumor immunity together with the frequent co-infection with the human immunodeficiency virus (HIV), promoting survival of the bacillus and inhibiting INF- γ secretion with increase in TNF- α secretion^[63].

Epidemiologic studies support a relationship between bacteria and cancer. *Salmonella typhi* chronic carrier state is related to gallbladder cancer^[64], *Streptococcus bovis*, found in bacteremia complications and infective endocarditis, is related to colorectal tumors^[53], *H. pylori*, known by its relationship with gastric adenocarcinoma, is also related to esophageal cancer^[52], and *Chlamydia pneumoniae* has been considered as an etiological factor in patients with lung cancer^[51].

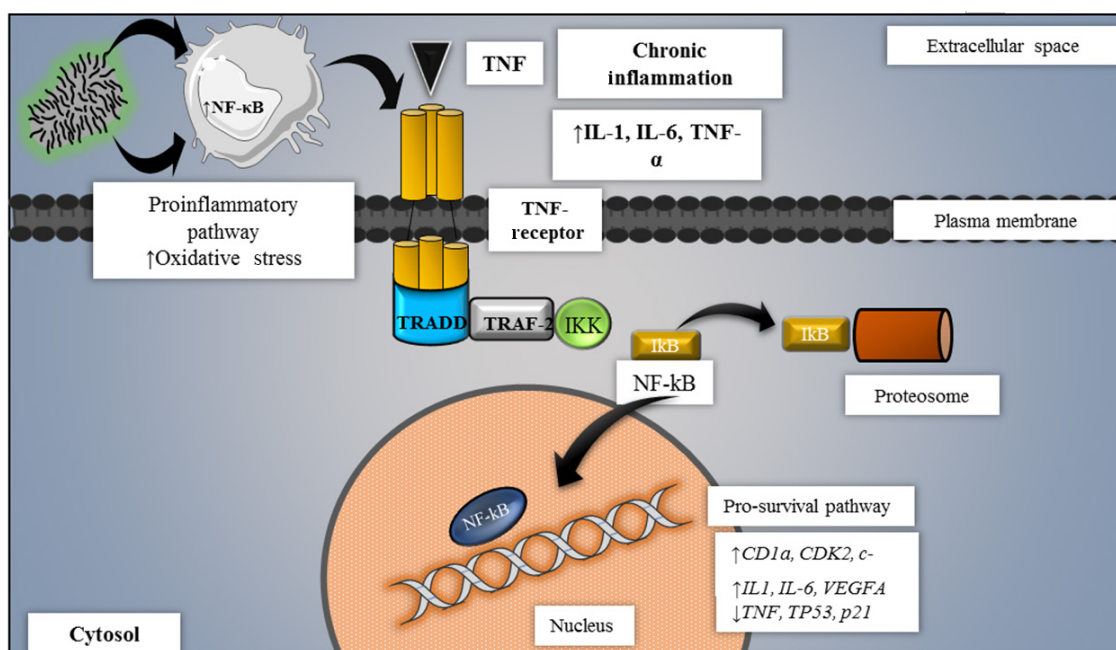


Figure 2. Carcinogenesis molecular mechanisms associated to chronic inflammation. Chronic inflammatory cascade is carcinogenic by the activation of the NF- κ B pathway. This leads to the degradation of such proteins, allowing that NF- κ B enter the nucleus to mediate the transcription of specific cell cycle-related genes while genes responsible for apoptosis are downregulated. IKK: I kappa B kinase; NF- κ B: nuclear factor kappa B; TNF: tumor necrosis factor; TRADD: tumor necrosis factor receptor type 1-associated DEATH domain protein; TRAF: TNF receptor-associated factor; I κ B: I-kappa-B

Learning from Coley's toxin

Human carcinogenesis is not related to all bacteria^[65]. Some bacterial properties work through mechanisms that stimulate the immune system and are capable to potentiate defenses against malignancy^[66]. Bacteria's role against cancer was recognized in the 19th century, when an American oncologist, Dr. William Coley observed tumor regression in patients with acute bacterial infections^[67]. After this observation, he decided to administer inactivated *Streptococcus pyogenes* and *Serratia marcescens*^[68] - in a mixture he called Coley's toxin - to a patient with an inoperable sarcoma, inducing tumor regression and curing the patient^[69-71]. Furthermore, it was used in cases with carcinomas, lymphomas, melanomas and myelomas, having significant results^[72].

The Coley's toxin mechanism of action became a key finding for immunotherapy^[73]. It is composed of *gram-negative bacterial endotoxin* (*Serratia marcescens*), a lipopolysaccharide released from the bacterial cell membrane that was considered a prototype for pathogen associated molecular patterns (PAMPs). This compound induces the secretion of TNF, IL-2, INF- α and IL-12^[74] from the immune system. Being IL-12 the most important in both innate and adaptive immunities since it stimulates T-CD4⁺ Th1 cells development, and increases NK/NKT and TCD8⁺ lymphocytes pathway mechanisms^[75].

These pathways require *Preexistent Immunization* in order to gain antineoplastic activity. This comes from expression of IL-12 receptors only on activated T cells^[76], explaining its major effectiveness in patients with previously sensitized T cells^[76]. On the other hand, bacterial intrinsic properties could also be used against tumors, such as *Streptokinase* from *Streptococcus pyogenes* cases, an enzyme considered as one of the active agents in Coley's toxin. This enzyme has anti-angiogenic effects, suppressing new vessel formation and decreasing tumor growth and invasion^[77]. Despite the fact that some clinical trials have shown effectiveness with this therapy, others have not shown any success, presenting multiple reasons for treatment failures^[78]. High doses of IL-12 used as support treatment with other cytokines have produced an immunologic response with high toxicity and its employment has been cancelled^[74].

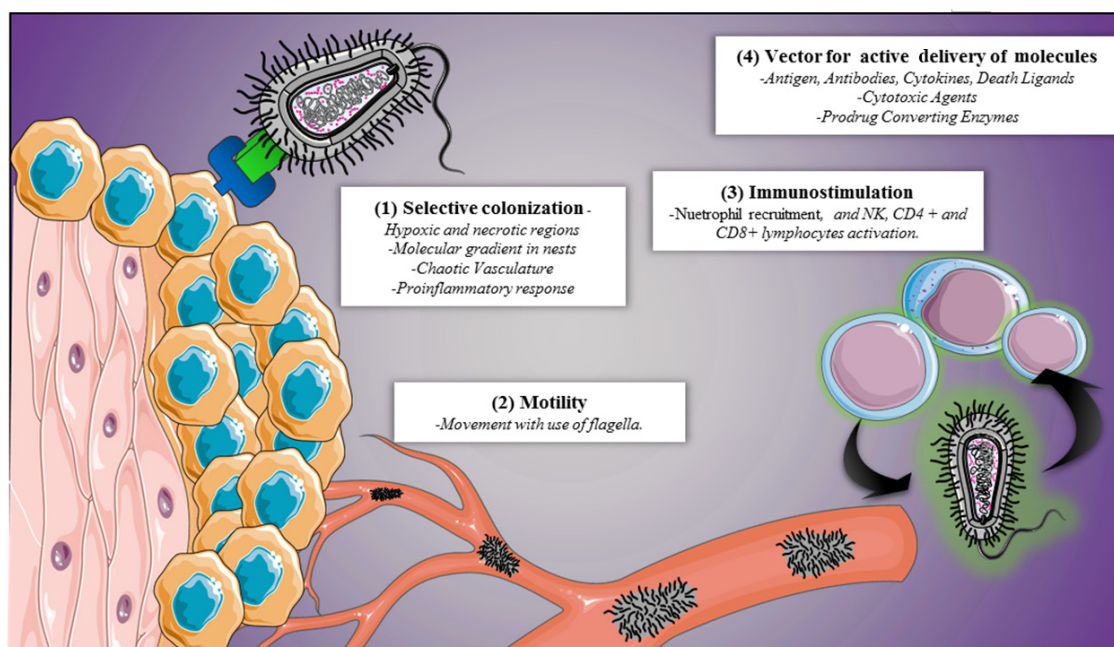


Figure 3. Bacterial therapeutic mechanisms. Multiple bacterial features that can be used to make novel therapies against cancer. NK: natural killer

BACTERIA SUPPORTING THE FIGHT AGAINST CANCER: A CROSSROAD FOR GENETICS, IMMUNOLOGY AND MICROBIOLOGY

Aiming for the perfect bacterium

Limited penetration in tumor tissue is considered a challenge for conventional therapy. This happens to chemotherapy and other specific biological therapies. They all depend on passive transport of the molecules into the tumor, limiting their efficacy and increasing their risk for toxicity^[8]. On the other hand, bacterial therapy works through mechanisms against cancer that cannot be achieved with standard conventional methods, becoming a great prospect^[79].

The main issue with therapeutic uses of microorganisms against cancer in the 19th century was the adverse effects associated to immunity, such as fever, septic shock, and death^[80,81]. Development in genetic engineering has led to use genetically modified bacteria- decreasing their pathogenicity- as cancer therapy^[82]. Their accessible genome manipulation make bacteria the best candidates among other microorganisms^[83]. Giving them the ability to enter cancerous tissue^[82,84], selecting tumor cells following specific chemical signals in their microenvironment^[85,86] and acting as vectors for molecule transportation^[87,88] assuming the fact they can be controlled from outside [Figure 3]^[89-92].

The “artificial medical bacteria” also have a role in the diagnostic process (detecting molecules or tumor markers related to certain diseases), therapeutic decision making (detection of chemical stimuli and production of therapeutic agents) and most importantly, can be controlled^[93]. Synthetic biology has been used to design and build biologic machineries based on vehicles. Bacteria compounds integrated on genes, proteins and molecules coming from multiple origins can affect their security and therapeutic effect^[94]. Systemic administration of these compounds would be better. Less concentration would be needed and multiple agents could be made without requiring neither formulation nor purification processes to amplify their effect^[93].

Selective colonization in cancerous tissue

Blood supply in cancerous tissue is insufficient, which results in acidity, deprivation of nutrients and presence of hypoxic areas^[95]. Hypoxia is more associated with expression of malignant phenotypes

characterized by genomic instability, angiogenesis and metastatic qualities^[96]; leading to new approaches against this feature^[97-100]. Strict anaerobic bacteria with spore germination qualities, such as *Clostridium*, cannot proliferate in highly oxygenated environments, restricting their colonization to hypoxic and necrotic regions of the tumor^[101]. This is affected by tumor morphology, with central necrotic areas and well perfused cells in the periphery, allowing anaerobic bacteria to proliferate in the center. Following this, the immune system gets activated and makes a peripheral ring of immune cells prepared to eradicate the tumor completely^[102].

Facultative anaerobic bacteria such as *Salmonella* act differently. They are capable to identify and penetrate into tumors by detecting chemotactic factors including molecular gradients of serine, aspartate, and ribose^[86,103]. Necrotic cancer cells release these compounds after being exposed to hypoxia for prolonged periods of time^[103]. A strain of obligate anaerobic *Salmonella* has been associated with antitumor features by replacing the *asd* gene with recombinant technology. Making the gene expressed only with hypoxia-inducible promoters^[104] to maximize selective colonization. Selective colonization consists of the bacteria's ability to be confined to the tumor chaotic vasculature^[105]. An increase in cytokines production such as TNF- α is observed in response to primary colonization of bacteria that leads to a secondary colonization^[106]. In addition to this, auxotrophic microorganisms-capable to grow in environments with nutrients produced only in tumor nests- have been synthesized with mutations generated from null alleles lacking biological capability^[107,108].

Recently, bacterial motility has shown to be critical in tumor colonization. Many bacteria have flagella that work with consumption of energy^[109]. Bacteria use this feature to migrate and stay for longer periods of time on places distal to tumor vasculature, in contrast to passive transport with chemotherapy^[83]. In addition to this, differences between diffusion and pressure gradients limit movement of molecules by passive transport and most of this happens on poorly perfused tumor areas^[105].

Immunostimulation in tumor microenvironment

There is no bacterium capable of completely inhibiting tumor growth just through colonization^[110]. However, it represents an important prospect for cancer treatment as an immunostimulator or as a vector for therapeutic components that can be released inside a tumor^[111,112] [Table 1].

The main theory for this approach comes from the bacterial intrinsic ability to immunostimulate after colonizing tumor tissue. They can proliferate inside the tumour where an increased activity of the immune system has been observed. Neutrophils, T CD8⁺ and CD4⁺ cells recruitment, cytokine and chemokine release, potentiate immune response with no effect on the surrounding healthy tissue^[130]. This approach has shown better results than conventional therapy since it can affect healthy and cancerous tissue altogether.

Bacteria have one of the largest genomes that exist. They can express multiple therapeutic transgenes and increase immune activity with cytokines and tumor antigens presentation^[131]. They can transfer those genes to eukaryotic cells and get expressed or repressed^[132,133]. Systemic administration of cytokines such as IL-2, IL-8, and CCL21A1 may show certain limitations related to their short half-life and adverse effects^[134]. Their manufacture is highly expensive and they lack tumor orientation, which may cause severe systemic inflammatory reactions restricting their clinical use^[134]. In contrast, gene modified bacteria are manufactured with low expenses, can be directed to specific tumor tissue, and may be easily eradicated with antibiotics^[114,135]. Bacteria *in situ* cytokine production may benefit those with difficult DNA recombination methods and/or protein instability in production and purification. To achieve oncolytic activity genes are introduced to increase cytokine expression and promote tumor regression^[113].

Antitumor activity can be achieved without significant toxicity and related to inflammatory cell infiltration such as granulocytes, T lymphocytes and NK cells. Induction of intratumor production of

Table 1. Pre-clinical studies for evaluation of molecular antitumor effects made by genetic engineering bacteria

Bacterium	Molecule	Most relevant results	Reference
<i>Salmonella typhimurium</i>			
VNP20009	CCL21	Increased intratumoral production INF γ , CXCL9 and CXCL1	Loeffler <i>et al.</i> ^[87]
VNP20009	LIGHT (TNFSF14)	Prominent reduction in tumor growth was observed. Evidenced with an inflammatory infiltrate (B lymphocytes, CD4 ⁺ ,CD8 ⁺ in models treated with this bacterium	Loeffler <i>et al.</i> ^[113]
VNP20009	IL-18	Inhibition of tumor growth was observed. Evidenced with a leukocytic infiltrate (especially NK cells) and increased secretion of INF- γ , TNF- α , IL-1 β and GM-CSF	Loeffler <i>et al.</i> ^[114]
VNP20009	FASL	Significant reduction of tumor size was observed in primary tumors and lung metastases, increasing neutrophil recruitment	Loeffler <i>et al.</i> ^[115]
VNP20009	TRAIL	TRAIL expression increased tumor cells apoptosis dependent on caspase 3 and 8	Ganai <i>et al.</i> ^[116]
<i>S. choleraesuis</i>	Endostatine	Inhibition of tumor growth was observed in 40%-70%. Evidenced with a decrease in intratumoral microvasculature, VEGF expression and increase in T CD8 ⁺ lymphocyte recruitment	Lee <i>et al.</i> ^[117]
<i>S. choleraesuis</i>	Thrombospondin	Selective colonization was observed in a 1000:1 to 10000:1 ratio with respect to liver and spleen. Evidenced with inhibition of tumor growth and increase in survival by angiogenic effects	Lee <i>et al.</i> ^[118]
<i>Nula phoP/ phoQ LH430</i>	RNAi-STAT3	RNAi inhibited significantly tumor growth, the number of metastatic lesions decreased, increased survival rate in animal models	Zhang <i>et al.</i> ^[119]
<i>S. typhiTy21</i>	VEGFR-2	Vaccination for this molecule showed inhibition of tumor growth, decreased metastasis growth and prevented new spontaneous metastasis, increasing survival rate in models	Niethammer <i>et al.</i> ^[120]
<i>aroA SL7207</i>	PSA-CtxB*	This vaccine administration conjugated with <i>Salmonella</i> showed protective effects by reducing tumor size in 8-14 days since its inoculation. This mechanism depends on T CD8 ⁺ lymphocyte activity and a prototype of the <i>E. coli</i> Hemolysin secretion system	Fensterle <i>et al.</i> ^[121]
<i>Clostridium</i>			
<i>C. beijerinckii</i>	NR	Nitroreductase activity increased <i>in vitro</i> antitumor activity of CB in 1954, by a factor of 22	Lemmon <i>et al.</i> ^[122]
<i>C. beijerinckii</i>	Cytosine deaminase	Tumor cells sensitivity to 5-fluorocytosine increased by 500 times	Fox <i>et al.</i> ^[123]
<i>C. sporogenes</i>	IL-12	Increased selective secretion of INF- γ with effects on tumor growth, without signs of toxicity	Zhang <i>et al.</i> ^[124]
<i>C. novyi-NT</i>	AC anti-HIF α	A heterologous gene transfer was satisfactory in this bacterium. Showing increased antibody secretion (with adhesion capacity and specificity)	Groot <i>et al.</i> ^[125]
<i>Listeria monocytogenes</i>			
<i>Lm-LLO-E7</i>	HPV16-E7*	This therapy induced regression in 75% of tumors expressing E7 antigen. This response depends on TCD4 ⁺ and TCD8 ⁺ lymphocytes and INF γ secretion	Gunn <i>et al.</i> ^[126]
<i>ADX531-164</i>	HER-2/neu (Human)*	An increase in TCD8/Tregs ratio was observed with this therapy. It also prevented more breast tumor formation and delayed more metastasis growth than other vaccines based on this bacterium	Shahabi <i>et al.</i> ^[127]
<i>LM-LLO-Mage-b/2nd</i>	MAGE-b*	The most effective vaccine for breast tumors, decreasing number of metastasis by 96%, correlating to a strong CD8 ⁺ lymphocytic response in spleen after restimulation with antigen use	Kim <i>et al.</i> ^[128]
<i>Lm-LLO-HMW-MAA-C</i>	HMW-MAA	This therapy immunization prevented tumor growth not only in models that expressed the antigen, but in melanoma, renal carcinoma and breast carcinoma. TCD4 ⁺ and TCD8 ⁺ lymphocytes were needed to achieve this	Maciag <i>et al.</i> ^[129]

*Antigen expressed on tumor

cytokines^[114,136,137], including IL-18, is important to enhance cytokine production in T lymphocytes and NK cells, to increase MHC-1 expression, and to favor differentiation of Th1 CD4⁺ cells; leading to an immune response mediated by NK cells, macrophages, and T CD8⁺ cells^[114,138].

Bacteria induce expression of ligands in cancerous cells with antitumor activity. For example, the FAS ligand (FASL), member of TNF family, enhances chemotaxis and IL-23 production from dendritic cells with T cell proliferation^[115]. TNF related to apoptosis inducing ligand (TRAIL) protein expression has been achieved in models with breast cancer^[116], gastric cancer^[139] and melanoma by employment of controlled bacteria^[140].

An interesting fact about cancer prognosis is the advanced stage by the time it is diagnosed, decreasing patient survival. Therefore, bacteria have been employed to work as vaccine vectors. These vaccines would increase tumor antigen expression on cancerous cells. Among these: prostatic specific antigen (PSA) in prostate cancer^[121], C-rapidly accelerated fibrosarcoma in pulmonary adenoma^[141], and alpha-fetoprotein (α -FP) for hepatocellular carcinoma^[142]. They can work by inducing an adaptive immune response to protect against these tumors. APCs recognition of these antigens is followed by a specific T-CD8⁺ cell proliferation with immunologic memory, in contrast to systemic administration of antibodies or adoptive T cell leading to loss of immunotolerance and healthy tissues affected^[143].

Other use as therapeutic vectors

Expression of hemolytic toxins could be achieved in tumors resistant to conventional therapy. Cytolysin A (*ClyA*) with *E. coli* K-12 use^[144] is an example of these. Transcription factors could be induced in cases with *S. Typhimurium* JRG5356 where genes for *HlyE* activation are expressed so pore-forming cytolysins are made by activating the FF+20 promoter^[85]. On the other hand, inhibition of angiogenetic processes with TSP-1 or endostatin genes could be used to decrease capillary density and reduce expression of vascular endothelial growth factors (VEGF)^[117,118].

Lastly, protein repression could also be induced using RNA interference (RNAi). Bacteria carrying plasmids such as *pSi-Stat3* are capable of changing specific portions of DNA and increase expression of small interference RNA (siRNA)^[145] or short hairpin RNA (shRNA). All of these cause degradation of specific mRNA sequences leading to a dysfunctional tumor gene expression^[146,147].

POTENTIAL OF BACTERIA UTILIZATION IN ONCOLOGY

Research on bacteria employment against malignant tumors in human subjects is expanding in diagnostics (for their selective colonization and external control) and therapeutics (for their antitumor effect). The next sections will discuss experimental and clinical evidence supporting bacteria utilization against cancer.

Bacteria utilization as cancer diagnostic method and to monitor therapeutic efficacy

Bacteria utilization is not limited to the therapeutic scope but also to diagnostic methods. Developments in genetic engineering have shown expression of bacterial genes that can be detected and monitored externally by fluoroscopy^[148], magnetic resonance imaging (MRI)^[90] and positron emission tomography (PET)^[149] scan. These genes can code for light-emitting proteins, such as luciferase and green fluorescent protein (GFP), making them observable in real time under low light image processing; and also under micro-to-micro fluorescent microscopes^[150]. *E. coli* bacterium remains as a prototype carrying PLITE201 plasmid that codes for luxCDABE protein^[151] giving luminescent features. This bacterium also carries the pMW211 plasmid that codes for dsred protein^[152] making cancerous cells recognizable in their exact localization by turning them luminescent without any invasive approach. *Salmonella typhimurium* and *Vibrio cholerae* remain under study for their utilization in colon and breast cancer diagnosis, respectively^[153].

MRI is routinely used for tumor diagnosis and treatment evaluation. *Magneto spirillum* is a bacterium employed in this radiologic study. It consists of a microaerophilic microorganism with magnetic properties on its magnetosome which contains magnetic crystals formed mainly of magnetite (Fe₃O₄) covered by a lipid bilayer membrane^[154]. Experiments with AMB-1 strains of this bacterium have shown positive contrast features in T1-enhanced imaging when they were cultured under iron deprived (FeCl₃) conditions^[90]. Contrast was intensified with expression of *MagA* gene. This gene codes for an iron transporter that gets positively regulated in presence of low iron concentrations^[155]. *In vitro* experiments with colon carcinoma models in HT-29 human subjects did not show any evidence of toxicity and tumor necrosis was observed

on both histologic slides and MRI^[156]. Employment of these magnetic features implies new advances with clinical use potential.

Regarding PET scans, other modified strains of *E. coli*: *E. coli Nissle (EcN)* 1917^[152] along with pyrimidine nucleotide analogs have been considered for diagnosis of breast tumors. Increased local accumulation of radio-isotopes has shown a positive correlation with the number of bacteria containing radioactive drugs. These bacteria selectively colonized tumors, making them detectable via PET. Other bacterium employed for these studies was Salmonella VNP20009-TK. The latter has had similar results with a positive correlation between intratumor bacteria and fialuridine sequestration (FIAU), a radio-marked nucleoside analog used for tumor identification^[157].

Other bacteria diagnostic features can be used in oncology. This includes their employment as probiotics in cancer screening^[158]. Use of *EcN* with modified genetic circuits enhances detection of focal metastasis in urine samples. Two principles were applied: first, bacteria produced a luminous signal that can be detected through imaging techniques; and second, LacZ enzymatic activity on a substrate composed of luciferine and galactose (LuGal) results in luciferine traveling into the circulatory system and serving as a colorimetric indicator with fluorescent or luminescent features; these features could be detected in urine samples^[158].

Current perspective in bacteria based therapy in medicine

Recently, bacterial strains with therapeutic characteristics against cancer have been discovered. *Mycobacterium bovis* (BCG) is considered a prototype. It is an obligate anaerobic, acid-alcohol-resistant, facultative intracellular and non-motile bacterium that has been employed in the past for tuberculosis vaccine manufacturing^[159]. For more than 30 years it has been utilized in bladder cancer patients as immunotherapy. A decrease in tumor recurrence has been observed with this therapy along with its well tolerated adverse effects^[160]. Even though the first-choice treatment for non-muscle invasive bladder cancer (NMIBC) is still transurethral resection for bladder tumor (TURBT), a high recurrence (50%-70%) and progression rates (10%-20%) after two years have been observed with this procedure^[161]. In these cases, intravesical instillation with BCG is one of the main therapeutic options, decreasing long term appearance of distant metastasis^[162]; also an increase in global survival in 5 years with long term maintenance of this therapy has been observed^[163]. However, its long term use may have adverse effects such as drug induced cystitis, hematuria, and systemic toxicity^[164]. Despite of this, BCG is still considered^[164] the standard treatment for NMIBC after transurethral resection for bladder tumor has been performed in patients with intermediate and high risk of progression or recurrence^[12].

The mechanism behind these benefits has not been clarified, but the antitumoral effects of BCG are considered to come from the immune response^[165,166]. Once urothelial cells or macrophages internalize the bacillus, they induce an immune response with secretion of TNF- α , IL-6, IL-10, INF- γ , FEC-GM, CC and expression of CXC chemokine receptors^[167-169]; this stimulates recruitment of neutrophils, macrophages, T-CD4⁺ cells and increases expression of MHC-I, MHC-II and IL-2 receptors^[170-172]. TRAIL is one of the main mediators in bacteria based therapy. This ligand appears to be upregulated in response to INF- γ , causing urothelial cell death^[173,174]. BCG remains as reference for novel cancer therapies in development such as vaccines, and also for nonbacterial therapies, having similar efficacy and reliability (NCT02010203). Next sections discuss the most important bacteria used for these goals, going from their experimental research to current clinical evidence [Table 2].

Clostridium: heading to tumor specificity

Necrosis and hypoxia in tumor tissues make them resistant to conventional therapies^[106], therefore, research on *Clostridium* began because of its natural anaerobic features^[101]. In regards to utilization of this

Table 2. Clinical evidence evaluating the safety, tolerance, adverse and therapeutic effects of bacteria against cancer

Reference	Bacterium/compound	Methodology	Clinical phase	Results
Nemunaitis et al. ^[175]	Samonella TAPET-CD	Open clinical trial that included 3 patients with solid and/or metastatic tumors, 5-FU sensitive, without any response to conventional therapies. With intratumor administration of bacteria	Phase I	A favorable response was observed in 2 patients at their injection site. Bacterial CD dependent conversion of 5-FC to 5-FU. Presented adverse effects not related to therapy
Toso et al. ^[110]	Salmonella VNP20009.	Open clinical trial that included 24 patients with metastatic melanoma and one patient with renal cell carcinoma to assess safety, tolerability and clinical response	Phase I	From the 25 patients treated with VNP20009, none experimented an objective tumor regression. Dose-limiting toxicity was associated to TNF- α and IL1- β secretion, despite the majority of adverse effects showed reversibility
Schmitz-Winnenthal et al. ^[176]	S. typhiTy21/ Anti-VEGFR-2 (VXM01)	Randomized, double-blind clinical trial to assess safety, tolerability, and clinical and immunologic responses in 45 patients with locally advanced stage IV pancreatic cancer	Phase I	Treatment was well tolerated in all applied doses. No dose-limiting toxicity was found. There was an effector T lymphocyte dependent response and a decrease in tumor perfusion in patients with preexisting immunologic memory
Roberts et al. ^[113]	Clostridium novyi-NT	Clinical trial including 1 patient with retroperitoneal leiomyosarcoma and received intratumoral administration of spores in a metastatic lesion on shoulder	Phase I	Extensive tumor destruction was observed, compatible with necrosis. By day 4 after administration, biopsy showed absence of viable tumor cells. By day 55, presented with a pathologic fracture. Therapy improved his quality of life
Maciag et al. ^[185]	Lm-LLO-E7	Non randomized clinical trial to assess safety of the therapy in 15 patients with advanced stage cervical cancer, refractory or recurrent	Phase I	LI patients presented adverse effects, including severe (grade 3) in 6 patients (40%). At the end of the study, 2 patients died, 5 developed disease progression, 7 showed stable disease and partial tumor response was observed in one patient
Le et al. ^[177]	ANZ-100/CRS-207	Open multicentric clinical trial to assess safety and induction of immune system in two groups: 1) ANZ-100 = 9 patients with colorectal cancer (6), pancreatic cancer (2), and melanoma (1). 2) CRS-207 = 17 patients with pancreatic cancer (7), mesothelioma (5), lung cancer (3) and ovarian cancer (2)	Phase I	In both groups, therapy was well tolerated with self-limited adverse effects. In group 1, no dose-limiting toxicity was found with ANZ-100 administration, and was related to NK cell (CD38) activation and increase in MCP-1, MIP-1 β and INF γ secretion. In group 2, CRS-207 was well tolerated. The majority of observed adverse effects were grade 2. Like IN group 1, an increase in proinflammatory cytokines was observed. CRS-207 induced a specific response dependent on T cells towards mesothelin and listeriolysin-O
Le et al. ^[178]	CRS-207	Randomized multicentric clinical trial to assess safety and clinical response in 90 patients with stage IV pancreatic adenocarcinoma and administration of Cy/GVAX+CRS-207 (A) in contrast to Cy/GVAX only (B)	Phase II	The mean follow-up was 3.4 months. The global survival rate was higher in patients treated with Cy/GVAX+CRS-207 ($n = 61$) than those treated with GVAX/Cy only ($n = 29$) (HR: 0.59; IC 95%: 0.36-0.97, $P = 0.02$). Nevertheless, increase in T CD8+ lymphocytic specific response to mesothelin was associated to a higher global survival rate, independent on treatment group

gram-positive, obligate anaerobe, spore forming bacteria to develop a therapy against cancer in tumors with necrosis associated to bad prognosis^[179-181], *Clostridium novyi* is one of the most studied. After a deletion of its α -toxine gene, *Clostridium novyi-NT* becomes capable to colonize selectively; in addition to diminished adverse effects because of its decreased exotoxin production^[82]. It was used in experimental models with colorectal cancer, renal carcinoma^[99], gliomas^[182], and sarcomas^[13] to observe its selective colonization, immune cell infiltration, and cytokine release leading to tumor tissue necrosis^[125]. Phase I clinical trials were initiated on one patient with retroperitoneal leiomyosarcoma presenting multiple metastasis and refractoriness to conventional therapy. After intratumoral application-preferred over systemic administration to decrease adverse effects - the tumor located in this patient right shoulder regressed with an extensive necrotic area; and medically managed adverse effects^[13,183]. Further clinical trials are currently in patient recruitment (NCT01924689).

Genetic therapy was employed to increase the oncolytic effects of this strain and presented promising results^[122,184,185]. *Clostridium sporogenes*^[13] was utilized because of its tumor directed features. Genes derived from *E. coli* serve for nitroreductase (NR) and cytosine deaminase (CD) codification^[185,186]. These enzymes metabolize cytotoxic drugs inside tumors, having *in vivo* antitumoral effects.

Clostridium spores have low immunogenicity and can colonize multiple organs after systemic administration^[187]. However, once they germinate, they induce an inflammatory response with infiltration of immune cells with oncolytic effects^[99]. These strains have been employed in genetic engineering as vectors for cytokines secretion such as TNF- α ^[188], IL-12^[124] and IL-2^[189], achieving high concentrations inside tumor tissue without systemic toxicity. Also *C. novyi-NT* and *C. sporogenes* increase secretion of specific antibodies against hypoxia inducing factor-1 (HIF-1), main component observed in hypoxia response regulation inside tumors^[125].

Salmonella: multi-use bacterium

Salmonella enterica serivar typhimurium (*S. typhimurium*) is one of the most studied bacterium for its adaptative qualities leading to new strains with bacterial engineering showing antitumor activity^[119]. In the beginnings of the 21st century, phase I clinical trials were conducted to show their efficacy with gene modification via deletion in *msbB* and *purI* genes. The *msbB* gene is required for lipid A synthesis and its deletion was made to reduce TNF- α related toxicity, preventing septic shock^[190]. On the other hand, by deletion of *purI* gene, the bacterium became able to colonize tumors more selectively. All of this made the strains depend on purine external sources for survival restricting their growth to areas with substantial cell renewal^[191]. Tumor tissues with their purine rich activities would be perfect regions for their selective colonization^[191]. *Salmonella typhimurium* VNP20009 is one of the main strains in experimental studies originated from this theory.

This study results showed the maximum tolerated dose of this bacteria, its toxicity limit dose, and adverse effects by increasing production of proinflammatory cytokines. The observed adverse effects included thrombocytopenia, anemia, persistent bacteremia, hyperbilirubinemia, nausea, vomit, elevated alkaline phosphatase and hypophosphatemia. However, tumor colonization was detected only in 3 patients, and no tumor regression was observed^[110]. Despite the fact that the study did not show promising results, it was the start line for prospect studies to find doses that could be adjusted for efficiency and tumor localization and for other therapeutic features.

In order to increase this bacterium therapeutic effect, a study was initiated to use them as vectors in tumor gene therapy^[192]. A pilot study was performed with an attenuated and gene modified *Salmonella* strain with expression of *E. coli* CD, called suicide prodrug-activating enzyme^[193]. These genes were integrated in VNP20009 chromosome through Donnenberg and Karper method resulting in TAPET-CD strain^[175]. The mechanism of action of this enzyme consists in conversion of 5-fluorocytosine (5-FC, antifungal agent with limited cytotoxicity) to 5-fluorouracil (5-FU, cytotoxic antimetabolite capable of producing cellular apoptosis)^[193]. No promising results were obtained 2 out of 3 treated patients did not present any tumor regression but an improvement of their disease was observed^[175].

Another example from *S. typhimurium* is strains would be A1-R, which currently is on preclinical studies against different cancerous tissues such as prostate^[14], pancreatic^[194,195], glioma^[196], colorectal^[197], and ovary^[198]. *S. typhimurium* A1-R colonization seems to be more selective and effective than other strains and less toxic than VNP20009 strain. It also has safer systemic administration than *C. novyiNT*^[199]. Therefore, clinical trials for this strain are coming. A1-R is a gene modified strain, auxotrophic for leucine and arginine by nitroguanidine mutagenesis (NTG)-preventing healthy tissue invasion. It was utilized in animal models with prostatic cancerous cells PC3 and also in humans showing tumor regression, inhibition and prevention of cancer^[14].

Bacteria therapeutic use has been confirmed in cancer models with stem cell characteristics. This represents the only method capable to reduce *in vivo* tumor sizes in relation to chemotherapy (5-FU in monotherapy, cisplatin and gemcitabine). The efficacy increased when combined with 5-FU^[195]. *S. typhimurium* A1-R

could induce cell entrance from G0/G1 to S/G2/M and reduced significant portion of cells in quiescent state, making them sensible to chemotherapy^[200,201].

Other approach in gene modification of *Salmonella* was the study of strains to decrease or inactivate gene expression. This inactivation could be achieved with utilization of iRNA^[119,202]. *S. typhimurium* LH340 strain was made with deletion on popP/phoQ operon required for its virulence resulting in its attenuation^[203,204]. The signal transducer and activator of transcription protein-3 (STAT3) is the goal with these therapies. Confirmation of its role in immune system depression^[205] and expression of target genes such as *VEGF*, *Cyclin D1*, *Cyclin D2*, *c-Myc*, *p53*, *Bcl-XL*, *Bcl-2*, *Mcl-1* and *Survivin* have been observed^[206]. A relationship between inhibition of these genes expression and suppression of tumor growth was found^[207].

Strains with expression of iRNA for Stat3 suppressed tumor growth significantly, reduced metastasis and increased survival in experimental models with prostate^[119] and hepatocellular carcinoma^[202]. These tumors are usually highly vascularized and angiogenesis inhibition through plasmids required for endostatin codification (SL/pEndostatin) may increase efficacy to the novel therapy^[133]. By introducing Stat3, (SL/pEndo-Si-Stat3) more antitumor effects were observed. These effects were related to angiogenesis inhibition and increase in TCD8⁺ lymphocyte proliferation, NK cytotoxicity and T-regs proliferation. The later came from inhibition probably by stimulation of INF- γ and TNF- α secretion with significant decrease in TGF- β concentrations^[202].

In clinical settings *Salmonella typhi Ty21a* is one of the new therapy prospects. It was studied to find a vaccine to prevent typhoid fever^[173]. The bacterium was introduced to cancer therapy strategies with the VXM01 vaccine. This is an oral vaccine made of live attenuated strains of *S. typhi ty21a* capable to induce a T cell response; it also contains a plasmid that codes for VEGFR2 and plays an important role in tumor angiogenesis^[208]. It can also induce both humoral and cellular responses^[176] observed in experimental models with melanoma, colorectal cancer and lung cancer. Suppression of primary tumor growth and metastatic lesions mediated by T-CD8⁺ cells activity was observed in these models^[117]. In clinical settings, it was recently evaluated on 45 patients with stage IV pancreatic cancer and it showed the importance of preexisting immunologic memory for effector T cells to achieve an antiangiogenic effect^[176].

Clinical trials have shown that *Salmonella* still lacks therapeutic efficacy and selective tumor colonization but could be considered as a multi-use bacterium for its diverse features. It can work as a vector, and a better inducer of antitumoral response because of its efficient type III secretion system^[209]. Prospecting studies should be focused on this objective with specific molecules for each cancer type, getting major effectiveness.

Listeria monocytogenes: the perfect antigenic vector

Listeria monocytogenes (Lm) is a gram positive, facultative intracellular bacterium^[131]. Over the last few decades multiple studies have shown that it can work cancer therapeutic agent with multiple effect or mechanisms^[210]. It can be used against primary and metastatic tumors in an immune-privileged microenvironment. The latter helps its selective colonization and favors their elimination with ROS production^[211]. In addition to this, Lm decreases T-regs cells and immunomodulation molecules such as TGF β and IL10 in tumor microenvironment^[212]. However, main feature of Lm consists on selectively infecting APCs favoring self-antigen and heterolog antigens processing and presentation^[213]. These characteristics make Lm to be considered as a valuable immunostimulant agent.

Intracellular life cycle of Lm favors its use as an immunotherapeutic agent. Once infection has occurred, Lm strongly activates innate immunity with the release of proinflammatory cytokines such as IL-2, IL-6, IL-

12, and TNF- α ; and increases expression of co-stimulant molecules in APCs surfaces leading to maturation and activation of high affinity T cells^[214]. After internalization by phagocytes, Lm is capable to escape from phagolysosomes using its virulence factor called listeriolysin O (LLO)^[215]. It works as a hemolysin that perforates the phagosomal membranes of the bacterium could escape into the cytosol. Once in the cytosol, they can replicate and secrete its antigens^[216]. This mechanism makes antigen processing and presentation to be via both class I and II MHC molecules^[217] inducing potent specific responses from both T-CD4⁺ and T-CD8⁺ cells^[218].

These features of Lm have been studied with genetic engineering looking for recombinant strains capable to secrete tumor antigens^[219]. They could be employed as live vectors through vaccines to potentiate cellular response and overcome immunotolerance towards certain types of cancers^[131]. This could be achieved with insertion of plasmids encoding the tumor antigen^[126], or by their integration in the bacterial chromosome^[220]. These antigens would be expressed as chimeric proteins along with Lm virulence factors^[221] such as LLO or actin assembly inducing-protein (ActA)^[222]. Lm uses ActA for motility and intercellular propagation and its immunogenic features increase the immune response towards tumor antigens with poor immunogenicity^[223]. These experimental studies were oriented to measure efficacy in recently developed vaccines. Among these vaccines, Lm-LLO-E7 was studied for cervical cancer models^[224], Lm-her2-neu for metastatic breast cancer^[211], Lm-LLO-PSA for prostate cancer^[225], Lm-MPFG for hepatocellular carcinoma^[226] and LM-Kras for pancreatic ductal adenocarcinoma^[227] and others; all of them reporting suppression in growth and even regression^[228].

Lm utilization as live vector could induce systemic disease in immunocompromised individuals limiting its use for human vaccines^[221]. Different strains have been cultured with specific gene deletions to guarantee their safety^[229-232]. Among these new strains, only XFL-7 and Lm Δ actA/ Δ plcB have been used in clinical trials. The XFL-7 strain was created with chromosomal deletion in its *Prfa* gene. This gene codes for an activating transcription factor needed for bacterial virulence factor expression. In order to increase its expression, a complementation of a multicopy plasmid with a heterolog gene was introduced^[231]. The Lm Δ actA/ Δ plcB strain was made with a deletion of its virulence genes *ActA* and *inlB*-used for surface proteins codification that favors cell invasion-to prevent capture from non-phagocytic cells and reduce hepatic damage^[232].

The first clinical trial to assess safety with Lm administration in cancer patients utilized attenuated strains as vaccines, specially Lm-LLO-E7^[126]. The latter was made from XFL-7 strains to express E7 oncoantigen from human papilloma virus serotype 16 (HPV16). This vaccine was also designed to treat cervical cancer^[15], and other tumors induced by HPV16 such as oropharyngeal cancer^[224]. In this open, nonrandomized, uncontrolled study, Maciag *et al.*^[185] assessed safety and viability of Lm-LLO-E7 via intravenous administration with intervals of 21 days. Doses of 1×10^9 , 3.3×10^9 or 1×10^{10} Colony-Forming Units (CFU) were administered to 15 patients with invasive cervical carcinoma in advanced stages and refractory to conventional therapy. Despite the fact that all the patients presented systemic adverse effects in the study (fever, vomit, headache, muscle aches, tachycardia, hypotension, anemia) most of them were alleviated during the first 12-h post dose, responding to symptomatic treatment whenever necessary^[15].

Safety of Lm-LLO-E7 administration in humans is still under study with insertion of plasmid encoded resistance to chloramphenicol required for bacterial survival *in vivo*^[233]. Phase II clinical trials to assess efficacy and safety in patients with oropharyngeal cancer (NCT01598792) were suspended after a patient developed systemic listeriosis following vaccination^[234]. This shows the need for a new attenuation, especially for their use on immunocompromised patients.

The *Listeria* strain Lm Δ actA/ Δ plcB with application of two vaccines called ANX-100 and CRS-207 has been studied^[177]. ANX-100 consisted of a vector without antigen that was administered to 9 patients with colon cancer and hepatic metastasis from colon cancer and demonstrated its safety and tolerability to a dose of 1×10^8 CFU. It induced an antitumor inflammatory response. CRS-207 consisted of a modified strain to express mesothelin, which is an overexpressed antigen that is frequently found in multiple solid tumors, including mesothelioma, pancreatic adenocarcinoma, non-small cell lung carcinomas and ovarian cancer^[235-237]. Phase I clinical trials in patients with these characteristics showed their efficacy and tolerability to a dose of 1×10^9 CFU^[177]. Seven patients were treated during these trials. Six patients had increased survival in 15 months, showing treatment efficacy. But 3 patients with high survival rates had been treated with GVAX previously. This vaccine was designed to increase GM-CSF expression for its ability to induce cellular immunity against tumor antigens. Phase II clinical trials were performed posteriorly^[178]. They evaluated the safety and efficacy of the combined treatment with GVAX and cyclophosphamide (GVAX/Cy) with CRS-207 in contrast to exclusive administration of GVAX/Cy in patients with pancreatic cancer. Reports showed a global survival rate of 6.1 months in patients treated with GVAX/Cy+CRS-207, more than patients treated with GVAX/Cy exclusively (HR: 0.59; 95% CI: 0.36-0.97, $P = 0.02$).

Based on these results, current research is focusing on efficacy evaluation of vaccines based on Lm attenuated strains along with other immunological or conventional therapies. Among these, combining LM-LLO-E7 with anti-PD1 antibodies^[238], or using the strain as adjuvant therapy after chemotherapy against cervical cancer (NCT02853604). There was also found that combination of CRS-207 strain with an IDO1 inhibitor increases immunotherapeutic effects in ovarian and peritoneal cancer treatment (NCT02575807); which could be used as adjuvant therapy after chemotherapy for malignant pleural mesothelioma (NCT01675765).

Other bacteria under study

Research for bacteria use in cancer treatment is not limited to the cited genres. *Lactococcus lactis* NK34, generally used as a probiotic, showed significant antitumor activity against lung, colorectal, gastric and breast cancers on *in vitro* models^[239]. These effects appear to be mediated by an increase in tumor expression of p21 and p53 leading to apoptosis^[240,241]. Intratumor *Streptococcus pyogenes* was employed in pancreatic cancer models and complete tumor regression was observed and associated to cytokine release and immune cell infiltration^[242]. Recently, *Bacillus subtilis* and *Bifidobacterium infantis* are being included in preclinical studies to find more evidence supporting bacteria as life-saving prospects^[243-245].

CONCLUSION

The main advantage of bacterial therapy is its selective colonization in tumor tissue decreasing its toxicity. This direct oncolytic effect resides on proliferation and immunostimulation that take place in cancerous tissues. Despite lacking significant effects in initial models and multiple adverse effects, it has overcome these barriers. Development in genetic engineering has led to better therapeutic effects and the reinforcement of therapies with molecules such as cytokines, tumor antigens, drug metabolizing enzymes, death receptors, and even RNA interference. Promising results have been observed with these therapies during clinical trials. Research is beginning to determine their use as main, or supportive therapy in contrast to conventional therapy against cancer. Their toxicity, antitumor effect, and their long half-life represent critical variables to consider in future research protocols and clinical trials. However, microorganisms versatility remains a feature that may show encouraging results in the future [Table 3] with significant improvements in cancer diagnosis and treatment.

Table 3. Current clinical trials to evaluate bacteria use in cancer treatment

Bacterium	Indication	Clinical phase	NCT identification
<i>Clostridium novyi-NT</i>	Patients with malignant solid tumors refractory to treatment	Phase I	NCT01924689
<i>Lm-LLO-E7</i>	Patients with grade 2 cervical intraepithelial neoplasia	Phase II	NCT01116245
	Patients with non-small cell lung carcinoma, HPV positive	Phase II	NCT02531854
	Patients with anorectal carcinoma	Phase II	NCT02399813
	Patients with HPV positive oropharyngeal cancer	Phase II	NCT02002182
	Patients with high risk of locally advanced cervical cancer	Phase III	NCT02853604
<i>CRS-207</i>	Adults with previously treated pancreatic adenocarcinoma	Phase II	NCT02004262
	Patients with malignant pleural mesothelioma	Phase I	NCT01675765
	Patients with ovarian or peritoneal cancer	Phase I/II	NCT02575807
<i>ADU-623</i>	Patients with astrocytic tumors	Phase I	NCT01967758
<i>ADX531-142</i>	Patients with prostate cancer	Phase I/II	NCT02325557

HPV: human papilloma virus

DECLARATIONS

Authors' contributions

Article conception and design: Olivar LC, Lameda V, Torres W

Acquisition and analysis of bibliographic information: Torres W, Martinez MS, Lameda V, Olivar LC, Navarro C, Fuenmayor J, Pérez A, Rojas M

Drafting of the manuscript: Torres W, Martinez MS, Lameda V, Olivar LC, Navarro C, Fuenmayor J, Pérez A, Rojas M, Rojas J

Critical revision and final approval: Bermúdez V, Velasco M, Mindiola A, Rojas J

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