Review

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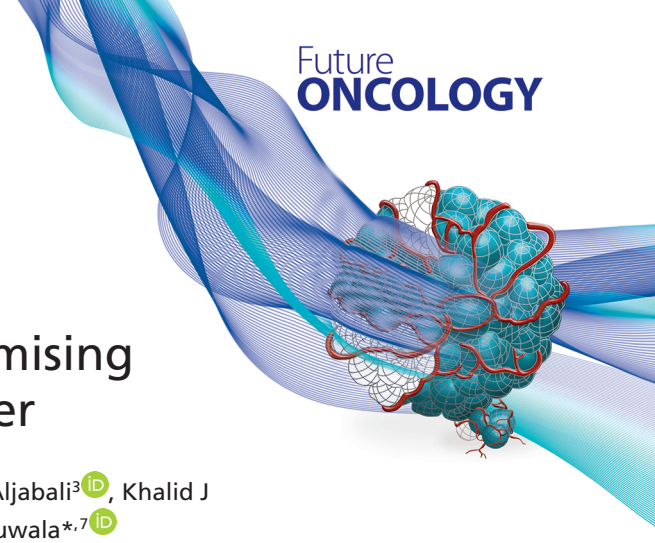

Bugs as drugs: neglected but a promising future therapeutic strategy in cancer

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Effective cancer treatment is an urgent need due to the rising incidence of cancer. One of the most promising future strategies in cancer treatment is using microorganisms as cancer indicators, prophylactic agents, immune activators, vaccines or vectors in antitumor therapy. The success of bacteria-mediated chemotherapy will be dependent on the balance of therapeutic benefit and the control of bacterial infection in the body. Additionally, protozoans and viruses have the potential to be used in cancer therapy. This review summarizes how these microorganisms interact with tumor microenvironments and the challenges of a 'bugs as drugs' approach in cancer therapy. Several standpoints are discussed, such as bacteria as vectors for gene therapy that shuttle therapeutic compounds into tumor tissues, their intrinsic antitumor activities and their combination with chemotherapy or radiotherapy. Bug-based cancer therapy is a two-edged sword and we need to find the opportunities by overcoming the challenges.

Plain language summary: Microbe-based cancer treatment strives to address urgent healthcare needs in patients experiencing difficult-to-treat cancers by using tumor-specific infectious microbes. Due to the ease of microbial culturing, microbes can be self-regenerating cancer therapeutics. Despite the fact that bacteria are usually believed to be the primary cause of cancer, the scientific literature has revealed exciting data indicating that bacteria might be efficient cancer prophylactic and therapeutic agents and ideal carriers for targeted cancer therapy. Advanced molecular engineering has recently been applied to bacterial therapy, resulting in increased efficacy with fewer adverse effects.

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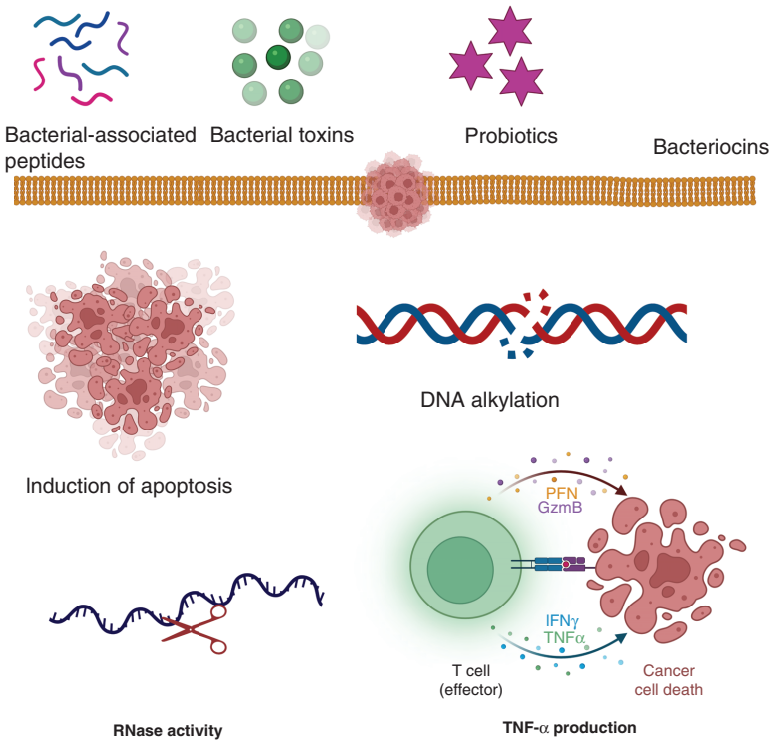
Graphical abstract:



Bacteriotherapy has been proposed to progress and raise the effectiveness of current conventional cancer therapy such as chemotherapy, surgery, radiotherapy and immunotherapy [1]. The challenges include high toxicity to normal cells, failure to treat deep tumor tissues and the challenge of persuading antibiotic resistance in tumor cells to develop other alternatives [2]. Microorganisms such as bacteria, viruses and protozoans have different effects on a developing tumor. The concept of using microorganisms in cancer treatment originated at the end of the 19th century, but a remarkable amount of research has been published during recent years with the progress of molecular biology. Several experimental studies have shown that bacteria can act alone as potent antitumor agents or attenuated/genetically modified bacteria or in combination with other conventional agents [3]. Due to the failure of conventional therapy, bacteria-mediated tumor therapy was successfully developed by exploiting probiotic *Escherichia coli* Nissle 1917 (EcN) for the selective delivery of a small cytotoxic protein (HlyE) against colorectal cancer. Also, studies were performed to increase the therapeutic efficacy [4]. Bacteria can be used as anticancer agents by enhancing human immunity through the activation of inflammasome pathways, either as vaccines that activate the immune system to fight against the disease or as vectors for transmitting antitumor therapeutics.

Probiotic bacteria and gut microbiota are likely to become essential components in cancer prevention and treatment. They can increase or decrease anti-inflammatory cytokines, which is a significant step in preventing carcinogenesis. The early-stage cancers also can be eliminated by activating phagocytes, and the risk of colon cancer can be reduced by consuming dairy products. Hence, probiotic bacteria in the future can be a promising tool in cancer diagnosis and prevention [5]. The pitch of using bacteria in cancer treatment is quite challenging.

Bacteria in cancer treatment

Some microorganisms have already been used in cancer therapy, and some are in the clinical trial stages [6]. The selection of a potential type of bacteria and their minimal pathogenicity to the host cell are essential in developing bacterial therapy. The common genera used in the studies, *Salmonella, Clostridium, Bifidobacterium, Lactobacillus, Escherichia, Pseudomonas, Caulobacter, Listeria, Proteus* and *Streptococcus*, have shown regional tumor growth with improved prognosis in animal model experiments [3,7,8]. Bacteria can be used as live, attenuated or genetically modified non-pathogenic bacteria as immunotherapeutic agents having direct tumoricidal effects [9]. There are also several attenuated bacteria used in cancer therapy. The ability of the attenuated bacteria to replicate in necrotic

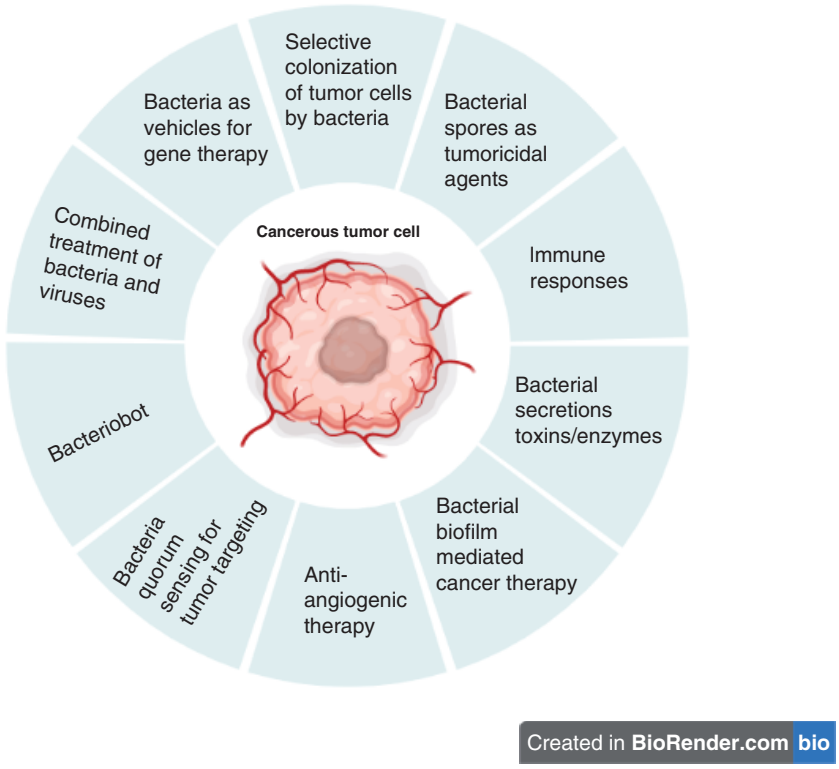


Figure 1. Role of bacteria in cancerous cells.
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tissues has several advantages in chemotherapy and radiotherapy. The attenuated bacterial strains commonly used in cancer therapy include *Salmonella typhimurium* and *Listeria monocytogenes* [10]. *S. typhimurium* attenuated strain VNP20009 exhibited strong inhibitory effects on tumor growth and metastasis in experiments using mice models [11]. Recombinant *S. typhimurium* induced tumor necrosis stx2 in acidic tumor microenvironment [3].

Mechanisms of bacteria to target & suppress tumors

The capability of bacteria to target tumors precisely is a fundamental advantage in bacteria-based cancer therapy (Figure 1). The tumor suppression mechanism varies according to the type of bacteria used in therapy. After the systemic administration of specific bacteria, they localize to the tumor microenvironment. The specific targeting of the bacteria can be through active or passive mechanisms. Currently, it is thought that bacteria escape from the blood circulation and reach the tumor site. Initially, bacteria may enter a tumor via passive entrapment in the chaotic tumor vasculature and then flow into the tumor, owing to inflammation caused by a sudden increase in the amount of TNF-α tumor vessels [12]. Bacteria may use both pathways to target a tumor site. They can directly infect dendritic cells (DCs) or macrophages and other antigen-presenting cells, including myeloid-derived suppressor cells (MDSCs). *Listeria* cells that reside in the MDSCs are protected from immune clearance, but the cells residing in healthy tissues are soon get eliminated [13,14].

Active mechanisms may also involve chemotaxis, the attraction of bacteria to the chemical substances produced by dying tumor tissues. This mechanism can occur in hypoxic tumors with low oxygen concentration, which attracts obligate anaerobes such as *Clostridium* and *Bifidobacterium* [15,16]. A mathematical cylindroid model was developed to determine the chemotaxis mechanisms, which determine the relative contributions of chemotaxis and proliferation to the accumulation of *S. typhimurium* in a tumor. The particular mechanism behind selective homing is not understood, however, chemotaxis and hemodynamic seem to play a role in this process [3]. Kasinskas *et al*. demonstrated that intramural growth and chemotaxis were significantly higher in the model developed due to the chemoattractant secreted by the quiescent cells and necrotic cells, making them multiplicate in the tumor microenvironment [17].

The motility of the bacteria is a critical feature enabling them to penetrate deeply into tumor tissues. Bacteria can swim actively using self-propulsion away from the vasculature to disperse themselves throughout tumor tissues. It was demonstrated that *Salmonella* could penetrate tumor tissue and selectively target metastases in mice models. *Salmonella* started to form colonies in the tumor tissues and spread throughout the tumor microenvironment within 3 days after bacterial administration [18].

Bacteria as immunotherapeutic agents

The logical idea behind bacterial treatment is that bacteria stimulate an inflammatory response in patients. In 1867, a German physician, Willhem Busch, purposely infected a cancer patient with erysipelas – *Streptococcus pyogenes* – and rapid tumor regression was observed [19]. William B. Coley, a New York-based surgeon, observed that regression in malignant tumors in sarcomas in patients suffering from parallel bacterial infection. He hypothesized that the immune reaction against a toxin present in the bacteria cross-reacted and killed the tumor cells [20]. Gram-positive or Gram-negative bacteria will interact with the host as either standard or pathogenic flora. Pathogenic interaction of bacteria enhances the host's immune system in different ways [8]. Anaerobic bacteria such as *E. coli*, which can engulf solid tumors, are indirectly involved in the clearance of some tumor cells (e.g., CT26) through the infectious defense mechanism. Rapid tumor regression was observed when cancer patients with erysipelas were injected with *Streptococcus pyogenes* [2].

Cancer immunotherapy includes triggering a specific immune response in patients to allow various host immune cells, mainly tumor antigen-specific $CD8^+$ and $CD4^+$ T lymphocytes, to attack the cancer cells. Bacterial infection in cancer patients leads to heat shock proteins, such as Hsp70 (released from the necrotic cells), being released from the bacteria [21]. Hsp70 causes the maturation of dendritic cells, which are the professional antigen-presenting cells required to generate effective antigen-specific immune responses.

Bacterial derivatives as anticancer agents

Obligate or facultative anaerobic bacteria such as *Bifidobacterium, Clostridium, Salmonella* or *E. coli* specifically colonize and proliferate inside anaerobic tumor tissues. They kill the tumor cells and activate antitumor immunity. Anaerobic microorganisms have the unique ability to grow selectively in hypoxic anaerobic areas of solid tumors that often are not accessible to drugs. At the same time, molecular manipulation may create changes among *Salmonella* and *Clostridium novyi*, which can only infect hypoxic tumor tissues and are non-viable outside the hypoxic tumor microenvironment [19].

Experimental studies have demonstrated that the enzymes bacteriocin and phenazine secreted by the bacteria showed anticancer activities against cancer cell lines [8]. In particular, preparations for microbial products, the lipopolysaccharide (LPS)-based vaccines have also been shown to have an anticancer property [22].

Bacterial toxins

Experimental studies have proven that bacterial toxins can inhibit cancerous cells [9]. Bacterial toxins have dual activities against cancer cells, depending on the lethal and reduced levels. At lethal levels these toxins kill cancer cells, and at a reduced level they are able to change the cellular process by controlling cell proliferation, apoptosis and cell differentiation [1]. The toxins produced by bacteria can block or stimulate the eukaryotic cell cycle called 'cyclomodulins'. Cytolethal distending toxins (CDTs) and cell cycle inhibiting factors (Cifs) block the mitosis process and inhibit the clonal expansion of lymphocytes [23]. The antitumor activities of the toxins are categorized into toxins conjugated to ligands and toxins conjugated to tumor surface antigens. Enterotoxin such as *Clostridium perfringens* enterotoxin (CPE) will bind directly to the receptors such as CLDN3 and CLDN4 and inhibit tumor growth, because these receptors are regulated [9]. Diphtheria toxin (DT) binds to the tumor-specific antigens on the cell surface through receptors and is activated. Few recombinant toxins can fuse to a ligand that binds to receptors selectively on the target cell [24].

Highly potent *Corynebacterium diphtheriae*-produced DT binds to cell surface receptors and then it activates the antitumor pathways [9]. Later, the DT is modified to recombinant DT3895, which can selectively bind to the surface of various cancer cells and showes cytotoxic effect. DT385 possessed anti-angiogenic activity with cytotoxic effects against 18 human cancer cell lines and inhibited human and mouse tumors [24]. Other experimental studies have shown that DT can kill epithelial ovarian cancer in women over the age of 60 years [25].

CPE toxins produced from *C. perfringens* induce cytolysis very rapidly by binding directly to receptors CLDN3 and CLDN4, which are upregulated in tumor cells and can significantly inhibit tumor growth [26]. *In vitro* studies

revealed that the expression of CLDN3 and CLDN4 sensitizes primary breast carcinomas to CPE-mediated cytolysis and emphasizes the potential of CPE in breast cancer therapy when delivered locally. Also, *in vivo* experiments with CPE treatment resulted in cell necrosis, significantly reducing cell volume [27].

Like DT, the other toxin produced from *Pseudomonas aeruginosa*, an exotoxin T, induced cytotoxicity against a wide range of murine and human cancer cell lines [28]. Cytotoxicity of *Pseudomonas* exotoxin A in a mice model with a lethal dose of 0.3 μg was found to be effective in cancer therapy [1].

Immunotoxins are a class of targeted cancer therapeutics in which a toxin such as *Pseudomonas* exotoxin A (PE) is linked to an antibody or a cytokine to direct the toxin to a target on cancer cells [29]. 'Immunotoxin therapy' is a promising approach to cancer therapy. Research is ongoing to find suitable protein molecules to combine with immunotoxins having minimum immunogenicity and high potency for killing cancer cells [30].

Prodrug-converting enzymes

With the help of prodrug-converting enzymes, prodrugs can be converted into cytotoxic agents in the tumor region. This strategy can be achieved by using bacteria to improve cancer treatment efficacy and reduce the side effects associated with systemic administration [10]. The prodrug-converting enzyme cytosine deaminase (CD) converts nontoxic 5-fluorocytosine (5-FC) into the chemotherapeutic agent 5-fluorouracil (5-FU) and led to a marked reduction in tumor growth. This is highly toxic because it further interferes with RNA and DNA synthesis. It was shown to produce functional CD in the tumor region upon administration of the attenuated *S. typhimurium* (VNP20009) strain expressing *E. coli* CD and 5-FC into patients [31].

Peptides & other secondary metabolites

Bacteriocins

Bacteriocins are the proteinaceous and peptide toxins secreted by bacteria. They act as synergistic agents to conventional cancer drugs. Bacteriocins preferentially bind to the cancer cell membrane over normal cells. This selective binding is due to a negative charge on the cell membrane [8]. Colicins are the best-studied bacteriocins produced from *Enterobacteriaceae* and *E. coli* showed anticancer activity in *in vitro* studies [8].

Biosurfactants

Biosurfactants are surface-active molecules and structurally different organic compounds produced by both prokaryotic and eukaryotic microorganisms. In recent years the use of biosurfactants as anticancer agents has been highlighted [32]. Studies have shown that the surfactants produced by bacteria are superior to synthetic surfactants. Microbial biosurfactants have their application in various medical fields, as they are biodegradable and less toxic [33]. Biosurfactants are localized on the microbial surface consisting of hydrophobic and hydrophilic moieties [34]. They are classified into low and high molecular surfactants based on their chemical structure and their mode of action. Biosurfactants that are low in molecular weight possess higher surface-active activities due to their simple chemical structure. Rhamnolipids (glycolipid) and surfactin (lipopeptide) are the two extensively studied surfactants [32]. In recent years, studies have reported that biosurfactants act as anticancer agents by controlling various mammalian cell functions and by interfering with the cancer progression process, as presented in Table 1.

Bacterial biofilms in cancer therapy

A biofilm is a dense network of colonies that are embedded in an extracellular polymeric matrix adhered irreversibly to biological or non-biological surfaces. The biofilm formation process is regulated by quorum sensing that enables bacteria to persist in the host environment [43]. The bacterial biofilm can be used as an anticancer agent due to its ability to deliver therapeutics and restrict the spread of the tumor.

Anticancer drugs such as hydroxyurea and doxorubicin, used during cancer treatment, have induced and promoted biofilm formation in *Pseudomonas aeruginosa* [44]. The bacterial cells growing as a biofilm on cancer cells are triggered by the SOS response, an inducible DNA repair system, and bacteria attain phenotypes that attack or penetrate the cancer cells [45]. The bacterial macromolecules such as proteins and DNA necessary for biofilm formation coat the cancer cells to block metastasis [46]. The attachment of cancer cells to endothelial cells is inhibited by the polysaccharide released by the bacterium *Streptococcus agalactiae* [47]. The potential use of iron oxide nanowires as a carrier for cancer therapy from the biofilm waste of Zetaproteobacteria *Mariprofundus ferroxidase* was demonstrated by Kumeria *et al.* This biofilm-derived nanowireswere magnetic and can generate active and passive trigger responses with alternate magnetic fields, resulting in decreased cell viability. When these biofilm-derived

Table 1. Anticancer activity of biosurfactants against various human cancer cell lines.

Biosurfactants	Produced by	Cell lines	Description	Activity	Ref.
Surfactin or surfactin-like biosurfactants	*Bacillus subtilis* sp.	BEL7402	Hepatocellular carcinoma	Growth inhibition, apoptosis induction	[35]
		MCF7	Breast cancer	Growth inhibition, apoptosis induction	[36]
		K562	Myelogenous leukemia	Growth inhibition, cell-cycle arrest, apoptosis induction	[35]
		LoVo	Colon adenocarcinoma	Growth inhibition, apoptosis induction	[37]
Monoolein	*Exophiala dermatitidis*	HeLa	Cervical cancer	Growth inhibition	[38]
		U937	Leukemia cancer	Growth inhibition	[38]
Viscosin	*Pseudomonas libanesis*	PC3M	Metastatic prostate cancer	Migration inhibition	[39]
Serratamolide	*Serratia marcescens*	BCLL	B-chronic lymphocytic leukemia	Apoptosis induction	[40]
e-poly-L-lysine	*Bacillus subtilis* SDNS	HeLaS3	Cervix adenocarcinoma	Growth inhibition	[41]
		HepG2	Hepatocellular liver carcinoma	Growth inhibition	[41]
Mannosylerythritol lipids	*Candida antarctic*	K562	Myelogenous leukemia	Growth inhibition, differentiation	[42]
Glycoprotein	*Lactobacillus paracasei*	T47D/MDA-MB231	Breast cancer	Growth inhibition, cell-cycle arrest	[32]

nanowires were loaded with the drug doxorubicin and tested for carrier capabilities, they could cause cytotoxicity in human breast cancer cells [48]. In another study, colorectal cancer imaging was facilitated when the biofilm synthesized by *Lactobacillus reuteri* was coated with zinc gallogermanate [49].

Bacteria as vehicles for gene therapy for cancer

A gene delivery system can be a physical or chemical approach to introducing foreign DNA or RNA into host cells, and it must reach the host genome to induce gene expression. Bacteria and viruses can be used as vectors for gene delivery.

The innate biological properties of bacteria make them efficient at DNA delivery to cells or tissues. The use of bacteria to deliver therapeutics suggests many advantages over other gene delivery approaches. Malignant cell death via a gene therapy strategy can be used for direct or indirect killing. Introduction of a vector carrying killing gene into a malignant cell can directly kills the cancer cells. This can be achieved by delivering pro-apoptotic genes or suicide genes using a bacterial vector. Oncolytic vectors use replication-competent bacteria with the capacity to spread to tumor tissues and infect the neighboring cells, killing the cancer cells. Many bacteria are employed as gene delivery vehicles, such as *Salmonella, Escherichia, Listeria, Clostridium* and *Bifidobacterium,* for cancer gene therapy. Gene therapy may be a sucessful strategy in treating most of the cancers that involves delivery of a gene producing anticancer therapeutic protein directly to the cancer site. The use of bacteria as vectors has been divided into two broad categories: tumor-specific bacterial replication and bactofection.

Tumor-specific bacterial replication

In tumors, the hypoxic condition is created by an insufficient supply of blood, an established feature of tumors. This anaerobic nature of hypoxic regions promotes the growth of anaerobic or facultative bacteria. Another factor favoring the growth of bacteria is that in the areas of necrosis the availability of nutrients may also provide nutrients for the growth of bacteria [50]. Another factor is the chemotactic nature of bacteria toward the chemoattractant compounds that are produced in necrotic or cancer cells [17]. As per the hypoxia theory, the unique microenvironment in the solid tumors and aberrant neovascular and tumor suppression are also involved in bacterial colonization [51]. The tumor-specific colonization of bacteria now appears to be both bacterial species and tumor origin independent. During tumor development, neo-angiogenesis (the formation of new blood vessels) is occured, but the newly formed blood vessels are highly disorganized with an incomplete endothelial lining and blind ends, resulting in leaky blood vessels with sluggish blood flow. *E. coli*, *Vibrio cholerae*, *S. typhimurium* and *Listeria monocytogenes* are few bacteria having great importance those can use those leaky blood vessels to enter into the cancer cells [52].

Bactofection

The process of bactofection is defined as transgene expression through bacterial delivery system to produce therapeutic proteins at the target site or within the cell by genetic engineering. Invasive bacteria can deliver therapeutic proteins into the tumor cells, whereas the non-invasive cells can deliver the therapeutic proteins at the external site of the tumor microenvironment [53]. Steps such as bacterial attenuation, the enhancement of tumor targeting and strategies for delivering anticancer cargo are critical for carrying the therapeutic cargo by bioengineered bacteria.

Attenuation of bacterial virulence

When bacteria are employed as vectors, their pathogenicity is a significant concern. To use bacteria as safe vehicles, specific bacterial virulence genes must be attenuated, not affecting the antitumor activity. In Gram-negative bacteria, the LPS present on the outer membrane is responsible for sepsis, as it is a potent tumor necrosis factor stimulator. Various studies have shown how to address LPS-related complications. In Gram-negative bacteria such as *Salmonella, the* safety profile was attained by deleting the *mshB* gene, which leads to myristoylation of lipid A, and the toxicity was reduced drastically and by minimizing TNF-α expression [54]. The strain *S. typhimurium* VNP200009 was generated by deleting the *msbB* gene and purine auxotrophy gene (*purI*) [55]. However, in phase I clinical trials, these attenuated strains showed tumor specificity and inhibition activity in murine models but lacked tumor specificity in metastatic melanoma patients [56].

Tumor target enhancement

When facultative anaerobes such as *Salmonella* and *Listeria* target tumor cells, these bacteria can survive in the oxygen environment and lead to normal tissue toxicity. Tumor target enhancement can be achieved by genetically modifying bacteria to express tumor-specific ligands or by genetically engineering bacteria to target tumor-associated antigens [19]. On cancer cells, the $\alpha_v\beta_3$ integrin was overexpressed, and *Salmonella* strain SHJ2037, a deficient strain of ppGPP, was designed to display integrin-binding peptide Arg-Gly-Asp on the bacterial surface to target the cancer cells. This strain showed high tumor specificity and enhanced tumor activity in MDA-MB-231 breast cancer cells and MDA-MB-435 melanoma xenografts with overexpressed $\alpha_v\beta_3$ integrin [57].

Bacterial vectors for anticancer cargo

The therapeutics needed to deliver to tumor cells are DNA, RNA and proteins delivered by bacteria. Intracellular bacteria are genetically engineered to carry a plasmid-mediated gene that regulates therapeutic proteins inside tumor cells [53]. An exciting approach to therapeutics is the induction of apoptosis by using ligands such as TNF-α, FasL and TRAILs. However, factors such as toxicity and short circulation half-life after systemic administration limit this approach. Several studies have shown the anticancer potential of engineered bacteria that express these death inducers [54]. Engineered *S. typhimurium* to secrete murine FasL, a pro-apoptotic cytokine, has been shown to reduce the growth of tumors in murine breast carcinoma and CT-26 colon carcinoma cells [58]. In another study, the engineered *S. typhimurium* shown to induce TRAIL-mediated tumor growth suppression in mice bearing melanoma tumors [59].

Anticancer strategy using protozoans

Toxoplasma lysate antigen (TLA) contains antigens of the microorganisms that are used in cancer treatment. Many researchers have used uracil auxotrophic carbamoyl phosphate synthase (CPS) mutant *Toxoplasma gondii* to treat pancreatic cancer, melanoma, ovarian cancer and lung cancer [37,60,61]. An increase in the level of IL-12 was observed by using these strains; cytokine mediates the inflammation and activation of immune cells. Studies on the mouse model demonstrated that the use of the auxotrophic strain of *Toxoplasma gondii* (CPS) provides long-term protection against cancer [60]. *Plasmodium falciparum* protein VAR2CSA conjugated with Diphtheria toxin was tested against tumor cell lines and *in vivo* mice models. Both these *in vitro* and *in vivo* studies showed high expression of chondroitin sulfate on melanoma cells [6].

Combination therapy

Bacteriotherapy with chemotherapy or radiotherapy

Complete elimination of cancer cells from body with single cancer treatment is not an easy task because cancer is a multifactorial disease. Bacteriolytic therapy combined with a conventional treatment regime can be used successfully

with positive outcomes. Combining bacteriotherapy with chemotherapy is a new approach that may increase the effectiveness of cancer treatment and reduce the toxicity of chemotherapy. The efficiency of chemotherapy can be increased by sensitizing the tumors with bacteria and chemotherapy can be exploited as a vehicle for gene delivery or drug delivery [2].

Normal tissue damage can be reversed by using probiotics during or after radiotherapy and has been shown in many clinical and preclinical studies [62,63]. Probiotic formula VSL-3 has been shown to be effective in reducing the gastrointestinal effects of chemotherapy using rat model experiments [64]. Bacteriotherapy with radiotherapy also showed a remarkable potential for both therapeutic and diagnostic applications. Tumor shrinkage was observed using a combination of radiation therapy and the bacterial strain *Clostridium novyi-NT* against mice bearing HCT116 tumors. Suppression of tumor growth and prolonged survival of mice was observed upon administering x-rays 5 –15 Gy with injections of *Salmonella* 2×10^5 CFU into mice bearing B16F10 or Cloudman S91 melanomas [2].

Combined treatment with bacteria & viruses

Bacteria and oncolytic viruses (OVs) share the property of tumor-selective replication following systemic administration. Attenuated viruses and attenuated bacteria have been successfully used to generate immunity against pathogens, but their general use for tumor treatment is still lagging. The approach of using viruses in cancer treatment is more effective and less toxic than other treatment regimes. The viral-mediated oncolysis cancer treatment approach is possibly more effective and less toxic than current treatment regimes. Though bacteria have better action on a tumor, viruses possess unique abilities to kill cancer cells [65]. In a study by Cronin *et al.*, two diverse microorganisms (vesicular stomatitis virus and non-pathogenic *E. coli*) were rationally combined and demonstrated improved therapeutic outcomes in the mice model [66].

Gut microbiome & probiotics in cancer therapy

Several studies have investigated probiotic bacteria's ability to modulate cancer cell proliferation and apoptosis *in vitro* and *in vivo*. Goldin and Gorbach were the first to demonstrate that a diet enriched with *Lactobacillus* reduced the incidence of colon cancer. However, the exact mechanism associated with antitumor properties remains unclear [67].

Bacteria such as *E. coli* and *C. perfringens* are natural residents of the gut and are putrefactive. Several studies have proven that they produce carcinogenic compounds using enzymes such as nitroreductase, β-glucuronidase and azoreductase. In late 1970, the study conducted by Goldin and Gorbach showed that the consumption of fermented milk products resulted in an increase in the number of *Lactobacillus acidophilus* probiotic in rat's gut and a subsequent reduction of putrefactive bacteria [67]. The mutagenic compounds that are generally associated with foods and fried meat increase the risk of colon cancer. Ingestion of the *Lactobacillus* strain reduces the mutagenic effect of a diet rich in cooked meat and decreases the excretion rate of heterocyclic amines (HCAs) through urine or fecal extraction [68,69]. Several other *in vitro* studies have shown the ability of probiotic bacteria to bind or metabolize mutagenic compounds such as HCAs, aflatoxins, mycotoxins, nitrosamines, phthalic acid esters (PAEs) and polycyclic aromatic hydrocarbons (PAHs) [5]. In maintaining gut homeostasis and suppressing carcinogenesis, many beneficial compounds are produced by the gut microbiota. The gut microbiota produces several short-chain fatty acids (SFCAs), such as butyrate, acetate and propionate, due to the fermentation of fiber-rich probiotic bacteria. Short-chain fatty acids are not only known for their energy source. They also act as a signaling molecule that affects the immune system, cell death and cell proliferation [70]. They are also involved in hormone production and lipogenesis, which explains their essential role in epithelial integrity maintenance [71]. Certain probiotic bacteria such as *Bifidobacteria* and *Lactobacilli* affect the production of SCFAs by modulating the gut microbiome [72]. Colorectal cancer is highly related due to decreased SCFAs and SCFAs producing bacteria dysbiosis [73]. *Butyrivibrio fibrisolvens* MDT-1 is known for its production of a high amount of butyrate. When these bacteria are administered in a mouse model of colon cancer, the progression of tumor development is inhibited and immune response is increased [74]. The other species belonging to *Firmicutes* families (*Ruminococcaceae*, *Clostridiaceae* and *Lachnospiraceae*) have been proven to promote apoptosis and inhibit proliferation in cancer cells cultured *in vitro* [71].

Probiotic bacteria in cancer treatment & prophylaxis

The characteristic features of probiotic bacteria, such as the ability to tolerate the gastrointestinal tract environment, the ability to colonize the mucosal surface and prolonged residence in the gut by maintaining protective properties, make these bacteria to be used as vehicles to deliver various therapeutics such as drugs, cytokines, enzymes or DNA in recent years and such approach has been successfully applied in colorectal cancer treatment [5]. The recombinant

Table 2. A summary of the clinical trials on bacteria-based cancer drugs with their clinical trial phases and the cancer types used in these studies.

Bacterial strain	Trial phase	Cancer type	Ref.
Clostridium novyi-NT	I	Colorectal cancer	[81]
	I	Leiomyosarcoma	[82]
	I	Solid tumor malignancies	[83]
	Ib (ongoing)	Refractory advanced solid tumors	[84]
Listeria monocytogenes	III	Cervical cancer	[85]
	II	Metastatic pancreatic tumors	[86]
	II	Cervical cancer	[87]
Salmonella typhimurium VNP20009	I	Metastatic renal cell carcinoma and metastatic melanoma	[56]
	I	Melanoma	[88]
	I	Advanced or metastatic solid tumors	[89]
S. typhimurium VNP20009 expressing TAPET-cytosine deaminase	I	Head, neck or esophageal adenocarcinoma	[31]
S. typhimurium expressing human IL-2	I	Liver cancer	[90]
S. typhimurium Ty21a VXM01	I	Pancreatic cancer	[91]
S. typhimurium VNP20009	I	Neoplasm metastatic tumors	[66]

strains of *Lactobacillus lactis* in intragastric applications were able to express anti-inflammatory compounds such as cytokine, IL-10, antioxidants or human interferon-β has been able to improve intestinal inflammation and demonstrated cytoprotective effect [75]. The catalase expressing *L. lactis* has been proven to reduce the production of reactive oxygen species (ROS) such as H_2O_2, reducing colonic damage and inflammation [76]. In recent years, studies on targeting the inhibition of inflammatory-related carcinogenesis with different combinations of probiotic vectors expressing different therapeutic molecules, drugs or cytokines have need demonstrated. However, the limitations of probiotic bacteria with recombinant vectors are that the antibiotic resistance genes generally used as selective markers in cloning could be transferred to resident intestinal microbiota [77].

OMVs as cancer vaccines

Outer membrane vesicles (OMVs) are lipid-based vesicular nanostructures containing various porins; they can carry heterologous substances to accomplish adjuvant and delivery functions [78]. OMVs have great potential in vaccine development due to their ability to transport and delivery of antigens to endothelial cells or antigen-presenting cells (APCs). The mode of OMV transport to the site of the desired immune response has an important influence on potency. Both humoral and cell-mediated immunity were stimulated by OMVs similar to bacteria; they are also superior to bacteria in the safety contour and ease of production. In addition to Gram-negative bacteria, Gram-positive bacteria such as *Lactobacillus acidophilus* and *Staphylococcus aureus* can also be used as targets for designing vesicle-based cancer vaccines [79].

Bacteria-mediated cancer drugs: status of clinical trials

Several preclinical and clinical studies involving cancer vaccines with varying stages of success have been described over the last two decades. *In vitro* and *in vivo* studies of tumor-bearing rats and mice under phase I clinical trials are performed to investigate the potential therapeutic use of bacterial strains in cancer treatment. In phase III randomized trials, clinical efficacy was achieved for sipleucel-T (*ex vivo* DC vaccine for prostate cancer); T-VEC modified herpes virus for melanoma [79]. There are varying clinical trial results because of the types of patients recruited for the studies, trial designs and other host-related factors. Regrettably, the results of the clinical trials so far have been disappointing. In a pooled analysis in bacterial vaccine trials against cancer, only 3.6% of the patients benefited from the vaccination provided [80]. So, it is essential to address all these issues to improve vaccines against cancer. Results of clinical trials allow determinations of whether a particular product can be intended for general use. Table 2 provides some examples of bacteria-based cancer drugs that are in clinical trials.

Challenges & their solutions in bacteria-based anticancer therapy

There are several advantages and disadvantages of bacterial toxins and their spores used in cancer therapy [9]. An essential step in the development of bacterial therapeutics is the identification of potential species and strains of bacteria possessing minimal pathogenicity to the host [3].

Bacteria-mediated cancer therapy was still an investigatory topic after William Coley's death (1936). It had been marginalized for several decades. His work was met with firm success but failed to explain the therapeutic mechanisms in controlling tumors and the therapy associated side effects. Meanwhile, radiotherapy came into existence and provided a competitive alternative to bacterial therapy [92]. In the late 1960s, clinical trials were performed with different formulations similar to Coley's toxin. Oncologists developed vaccine variants from the bacteria to treat cancer [93]. Research carried out in the past forms a foundation for future investigations. Biomedical research led to experimental models for studying bacterial tumor therapy using the prominent bacterial genera *Listeria, Salmonella* and *Clostridia*. In another experiment, experimental mice have developed progression in cancer and infection after bacteria-mediated tumor therapy (BMTT) using *Salmonella.* However, inadequately attenuated virulent strain after heat treatment could successfully regress tumor development [92]. The development of adequately balanced strains in treating cancer remains a challenge. Hence, the bacterial virulence factors are more important in inducing immune responses for therapeutic effects. The technology was not well developed 100 years ago during Coley's time, but heat inactivation experiments with *Salmonella* generated a proper balance between the intrinsic therapeutic benefit and the safety of bacteria-mediated tumor therapy [92].

Certain drawbacks were observed in earlier studies; however, further experimental studies were performed to overcome the existing problem and to gain supreme microbe-associated molecular patterns (MAMPs) essential structures for the microbes. Examples for MAMPs include LPS of Gram-negative bacteria; lipoteichoic acids (LTAs) of Gram-positive bacteria; peptidoglycan; lipoproteins generated by palmitylation of the N-terminal cysteines of many bacterial cell wall proteins; and lipoarabinomannan of mycobacteria. The studies showed that the intrinsic antitumor responses of bacteria are likely connected to MAMPs. Although the antitumor activity of *Salmonella* is partially understood, designing them suitably for therapy remains challenging [94]. There are few therapeutically active compounds or toxins secreted from the bacteria during tumor colonization – for example, α-Hemolysin or azurin [95]. The delivery of these toxin molecules to the extracellular environment signifies an essential task.

The other obstacle in bacteria-mediated cancer therapy is incomplete tumor lysis. Bacterial toxins or spores failed to colonize and multiply in the small tumors, leaving the viable rim, small tumors and metastases unaffected in later studies. After administering clostridial spores, rat models were used by injecting vascular targeting compound combretastatin A-4 phosphate (combreAp). The studies indicated that long-term production of therapeutic proteins from recombinant *Clostridium* is possible in tumors [9].

During experimental studies, animal models are dying because of infection caused by pathogens, so it is essential to use the most effective strain that destroys cancer. Ensuring the safety of patients with the use of an attenuated strain of microorganisms is of great importance. Additionally, the costs associated with clinical trials and the introduction of new products to the market are high and legal protocols are also very complex . It is also essential to ensure that the microbes are administered in different ways: intramuscularly, intravenously or directly into the tumor and the microorganisms that have already been commonly used to be tested through phase II and phase III clinical trials [6].

Bacteriobot

To overcome the challenge of delivering adequate quantities of a cancer drug to target cells, research groups have investigated a new drug-delivery system (DDS) called microbot, which uses biodegradable and biocompatible materials. This innovative approach uses bacteria-based fabrication for cancer treatment, in which bacteria act as microactuators and microsensors to deliver microstructures to tumors. A study developed Bacteriobot using strong therapeutic bacteria (*Salmonella typhimurium*) to Cy5.5-coated polystyrene microbeads. Both *in vitro* and *in vivo* tests of the bacteriology in a mouse model showed higher migration velocity toward tumor cell lysates or spheroids than toward normal cells [96]. Hence, bacteria can be protected from the immune system, which is also a safer method than the direct inoculation of bacteria into tumor cell [97].

To develop microbot therapy, Park *et al.* [97] encapsulated therapeutic bacteria (*S. typhimurium*) with biocompatible/biodegradable alginate microbeads to fabricate a bacteria-based microrobot in a targeted tumor region. The bacteria-encapsulated delivery system protects the bacteria from being attacked by the immune system, which is safer than the direct inoculation of bacteria. Targeted prodrug therapy for cancer has diverse target selection, tumor specificity, systemic safety and efficacy. Recombinant CD95L-based prodrug appears to be a multifunctional drug very much like a Swiss army knife in cancer treatment with reduced side effects [98].

Bacteria are a double-edged sword in cancer therapy. Using bacteria for cancer therapy is feasible, and their potential to treat solid tumors has been known for decades. However, clinical application of this therapy never

became routine because of the uncontrollable adverse side effects. Some attenuated species of bacteria capable of treating cancer have recently been identified and studied to overcome these side effects. These species of bacteria are considered safe for cancer therapeutic application with few or no side effects. While bacteria alone may not entirely demonstrate therapeutic potential, their modifications as antitumor agents, anti-oncogenes or immunogenic antigens and their combination with other therapeutic processes will improve their potential for cancer therapy. The arena of using bacteria as anticancer agents is still new; further studies are imperative to scrutinize the clinical significance of bacteria-based cancer therapy. The findings presented in this review suggest that this promising cancer therapy needs to be optimized and developed further.

Microbiomes as cancer indicators

It is well documented in the literature that there is a direct link between microbiota and cancer. Studies of germination-free (gnotobiological) mice with faulty immunological pathways have provided considerable insight into the function of microbiota and cancer. An IL-10 knockout mouse study revealed that the mutated mouse had spontaneous colitis. However, the illness was worse when the mice were contained in a specific pathogen-free (SPF) habitat [99]. In a subsequent investigation, the same team found that an unregulated Th1 reaction, most likely in response to microbiota, exacerbates colitis in mice lacking IL-10 and culminates in development of adenocarcinoma in older adults of these mice [100]. Likewise, STAT3-conditional animals suffer chronic colitis from macrophages and neutrophils, perhaps by interrupted IL-10 and active Th1 response.

Further investigations of toll-like receptor (TLR) signals in mice have helped our understanding of microorganisms and cancers. Mice deficit in TLR-4 and significantly less vulnerable to colitis-related malignancies are the primary receptors for LPS [101]. These findings show that local microbiota may induce overwhelming immunological reactions (colitis) if critical immune tolerance components are disrupted. Colitis does not develop in germ-free IL-10 knockout mice, and there is no indication of any unnatural stimulation of the immune system, but if colonized by the NC101 *E. coli* strain, tumors form much more rapidly, probably because DNA damage is increasing [102]. Pathogenic bacteria play a significant role in several illnesses, including colorectal cancer, in addition to their unbalance in the commensal bacterial makeup. Many harmful microorganisms, including *E. coli, Streptococcus bovis*, *Helicobacter pylori*, *Bacteroides fragilis*, *Enterococcus* spp. and some *Enterobacteriaceae* family members, promote colorectal cancer (CRC) [103]. These microorganisms can attach epithelial layers of the target tissue, such as the colon, to epithelial cells that lead to hyperplasia. They can cause direct proliferation. They may also create toxins that compromise the epithelium barrier's functionality, harm endothelial cells and induce inflammation. This was stressed in numerous recent reviews [104,105].

Escherichia coli can stimulate tumor growth, caused by hyperproliferation and inflammation, through attachment to colonic epithelial cells. Moreover, essential components of virulence exhibit pro-tumorigenic activities by degrading the DNA or the mucous layer/epithelia. The polyketide synthase (PKS) complex is a significant source of cytotoxicity in *E. coli* that promotes the development of colon cancer in IL-$10^{-/-}$ murine models and thus PKS are significantly concentrated in individuals with colon cancer with poor expression of IL-10 [102].

Anticancer secondary bacterial metabolites & their mode of actions

The secondary microbial metabolites are low-molecular-weight compounds that are not required but are significantly vital to human health for producing cultures. These include antibiotics, anticancer agents, medicinal products that reduce cholesterol and more. They are generated in the late development of the manufacturing microorganisms, with impressive structures. Microorganisms produce many varieties of secondary metabolites with distinct chemical structures and bioactivities. Typically, such secondary metabolites act as bioregulators, quorum sensors, signaling moieties and antimicrobial drugs [106]. However, many secondary metabolites have been reported focusing on molecules of clinical relevance mainly derived from Gram-positive bacteria highlighting the *Streptomyces* genus [107]. The compounds reported most often have been from four general types of biosynthetic systems, including the polyketides-I, nonribosomal peptides, isoprenoids and derivatives of shikimate [107]. An efficient protein treatment swiftly kills low-dose cancer cells. Most rapidly killing compounds are too toxic for systemic treatment; however, a precise release from the tumor by bacteria would reduce this toxicity. The current range of therapeutic proteins can be classified under three categories based on their mechanism of actions: cytotoxic components such as bacterial toxins; proteins that specifically target cancer pathways and promote cell death; and immunoregulatory activator proteins [108].

Expression of cancer drugs through bacterial systems

Bacterial cancer therapy is not new and has been widely used for a considerable time. As mentioned earlier, William B. Coley, who established that significant bacterial infections have therapeutic effects for tumor patients in 1890, was one of the pioneers of bacterial therapy for cancer. He treated advanced cancers and produced complete disease regression with a vaccination developed from *Streptococcus pyogenes* and *Serratia marcescens*, termed Coley's toxin [109]. Bacteria must also be engineered to reduce their pathogenicity to the human immune system. It is important to note that some bacterial components may be accountable for their inherent anticancer action. A reduction without removal of its antitumor action must thus be achieved. For instance, essential virulence genes have been deleted in human diseases, and the deadly strains have been transformed into essentially safe variants [110]. Most payloads supplied by tumor-targeting bacteria are harmful to both tumor cells and normal cells; hence, precise production control over constitutional expression is desirable.

There have been several bacterial species accumulating in tumor tissue. Because of their oxygen metabolism, they may be broken up into two groups: anaerobes (*Bifidobacterium* and *Clostridium*) and facultative anaerobes (*Salmonella*, *Escherichia* and *Listeria*) [111]. Anaerobic strains only thrive in the necrotic areas of tumors due to anaerobic tissues in the human body. *Clostridium* and bifidobacteria are discovered exclusively in tumors as compared to the healthy tissue in tumor-bearing mice [112].

Bacteria that release therapeutic proteins can be produced using their intrinsic metabolic pathways. Therapeutic proteins need to be produced readily and need to be toxic exclusively to cancer cells to be used as cancer therapeutics. Long-term release of the selected therapeutic chemical will sustain a deadly level of tumor concentration. Several aspects need to be considered for optimum synthesis of a therapeutic protein; for example the issue of lower synthesis rates of smaller proteins to be overcome [112]. Rare codons are replaced by more frequent codons, which enhances the pace of translation. However, this may lead to detrimental effects on bacterial metabolism or development; bacteria have high protein levels. Non-native proteins may be bacterial-toxic via inhibiting protein synthesis or directly inducing bacterial deaths [113]. Proteins must be released into the extracellular space after transcription and translation. This can be accomplished by adding sequences to the sequence of protein-coding or lysis. Multiple methods for the release of recombinant proteins are present in bacteria. For instance, *Salmonella* employs the type 3 secretion system to directly provide protein into the host cell cytoplasm [114]. Controlled and targeted expression of the gene containing cytotoxic compounds through bacterial system is critical to avoid the delivery of the drug to healthy tissue. Upon injection, microbes are cleared out of the blood and healthy organs. During this early colonization, constitutive gene expression would systemically disseminate medicines and have harmful side effects. Either externally inducible developers or microenvironmental promotors can manage gene expression [115]. Bacterial vectors have also been utilized for targeting cancer cell signalling pathways. Several pro-apoptotic protein strains of bacteria, including Noxa (Noxa is a p53-based pro-apoptotic protein downstream of p53 that causes apoptosis through induced mitochondrial dysfunction), TRAIL (a cytokine that causes cellular death by binding to the apoptosis receptor) and FasL (induces apoptosis) [18]. The monoclonal antibodies that inhibit ligand binding are another therapeutic method, mainly designed to target cell receivers. The discovery and purification of single-domain antibodies allow the synthesis of functional antibodies by bacteria. By providing high levels, especially to the tumors, bacteria may enhance antibody-based cancer treatments. *C. novyi* has demonstrated the expression and release of monoclonal antibodies [116]. Theoretically, a controlled gene expression system may be created by placing a particular promoter sequence upstream of a drug-producing gene, thereby providing transcription control via external cues. This method enables the time and location of the synthesis of drugs to be managed *in vivo*. Three techniques are primarily used to control or triggering this gene: internal trigger; auto triggering and external triggering [117]. For example, fumarate and nitrate reduction in the hypoxic environment in tumor tissue is triggered by hypoxia-inducible promoters such as HIP-1 and pepT [118]. Bacteria alone are frequently not adequate for complete tumor suppression, despite studies on bacteria's antitumor properties.

The benefit of eukaryotic and prokaryotic expression vectors, including cytotoxic substances, drug conversion enzymes, immune regulation systems, tumor stream-targeting molecules and syndromes, has been documented to improve the good results of bacterial cancer therapy [119]. Prodrug conversion enzyme expression can transform medicines into cytotoxic substances, particularly in the tumor area. The method's utility has been studied in enhancing cancer therapy efficiency and reducing the adverse effects of systemic management. Bacteria have been given many drug-converting enzymes [120]. For example, Nontoxic 5-FCs are converted into chemotherapy drug 5-FU by cytosine deaminase. 5-FU is very hazardous, as it is converted into a substance that affects the production of

DNA and RNA. The conversion of 5-FC to 5-FU shows the bacterial development of available CD in the tumor after co-administration of the attenuated *S. typhimurium* (VNP20009) strain expressing *E. coli* CD and 5-FC in patients [31].

Conclusion

Tumor-targeting bacteria exhibit unique features that make them optimal carriers able to provide cancer-specific therapeutic payloads, including tumor selectivity and free gene packaging capacity. This limitless capacity of gene packing not only enables significant or numerous target genes to be expressed but also facilitates the building of signaling networks that allow bacteria in cancer treatment to carry out complex activities. Although engineered tumor-targeting bacterial therapeutics have enormous promise, effective cancer therapy may require combinations, as it is challenging to obtain remission with single anticancer drugs with cancer heterogeneity on both the molecular and histological levels. However, the clinical applications of these techniques are not routine because of the adverse side effects.

Future perspective

As an improvement to existing immunotherapy techniques, an intratumorally bacterial infection could be an interesting addition to chemotherapy and radiation; its anticancer effects can be synergistic with those of bacteria. Thus, there is a need for further research in this area if we aim to use bugs as drugs. The advanced knowledge of bacterial pathogenesis, immunology and cancer biology provides a better understanding of bacteria-based cancer therapy. Genetic manipulation and new molecular technologies have made it possible to revisit bacteria-based cancer therapy from a future perspective. Bacteria-based cancer therapy has great potential to address the gaps in past failures, such as the complexity of tumor bacteria interactions, so the field remains challenging to researchers. The past obstacles can be overcome by the use of genetically modified microorganisms and sophisticated genetic engineering technology, which may have great applications in cancer therapy. In the future, bacterial combinations can be used to enhance the efficacy of immunotherapy, chemotherapy and radiotherapy.

Executive summary

Bacteria in cancer treatment
- This review highlights the importance of bacteria, viruses and protozoans in cancer therapy.
- The microbiome can be an indicator of various cancers and probiotics may help in cancer prophylaxis.

Bacteria as immunotherapeutic agents
- Bacteria and their metabolites can target and suppress tumors as immunotherapeutic agents.

Bacterial derivatives as anticancer agents
- Bacteria and bacterial biofilms may deliver therapeutic drugs in the target region of the tumor.

Bacteria as vehicles for gene therapy for cancer
- Bacteria can be used in a gene delivery system in cancer treatment.

Combination therapy
- 'Bugs' can be used in combination with chemotherapy or radiotherapy.

Bacteria-mediated cancer drugs: status of clinical trials
- This review also highlights the status of bacteria-based drugs in clinical trials.

Challenges & their solutions in bacteria-based anticancer therapy
- The challenges and strategies for overcoming them in bacteria-based cancer treatment are discussed.

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References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Patyar S, Joshi R, Byrav DSP, Prakash A, Medhi B, Das BK. Bacteria in cancer therapy: a novel experimental strategy. *J. Biomed. Sci.* 17(1), 1–9 (2010).
2. Sedighi M, Zahedi Bialvaei A, Hamblin MR *et al.* Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. *Cancer Med.* 8(6), 3167–3181 (2019).
3. Nallar SC, Xu DQ, Kalvakolanu DV. Bacteria and genetically modified bacteria as cancer therapeutics: current advances and challenges. *Cytokine* 89, 160–172 (2017).
4. Chiang CJ, Huang PH. Metabolic engineering of probiotic *Escherichia coli* for cytolytic therapy of tumors. *Sci. Rep.* 11(1), 1–18 (2021).
5. Górska A, Przystupski D, Niemczura MJ, Kulbacka J. Probiotic bacteria: a promising tool in cancer prevention and therapy. *Curr. Microbiol.* 76(8), 939–949 (2019).
6. Łukasiewicz K, Fol M. Microorganisms in the treatment of cancer: advantages and limitations. *J. Immunol. Res.* 2018, 1–8 (2018).
7. Thamm DH, Kurzman ID, King I *et al.* Systemic administration of an attenuated, tumor-targeting *Salmonella typhimurium* to dogs with spontaneous neoplasia: phase I evaluation. *Clin. Cancer Res.* 11(13), 4827–4834 (2005).
8. Song S, Vuai MS, Zhong M. Role of bacteria in cancer. *Infect. Agent. Cancer* 13(9),1–7 (2018).
9. Weerakkody LR, Witharana C. The role of bacterial toxins and spores in cancer therapy. *Life Sci.* 235(August), 116839 (2019).
10. Duong MTQ, Qin Y, You SH, Min JJ. Bacteria–cancer interactions: bacteria-based cancer therapy. *Exp. Mol. Med.* 51(12), 1–15 (2019).
11. Min JJ, Kim HJ, Park JH *et al.* Noninvasive real-time imaging of tumors and metastases using tumor-targeting light-emitting *Escherichia coli*. *Mol. Imaging Biol.* 10(1), 54–61 (2008).
12. Leschner S, Westphal K, Dietrich N *et al.* Tumor invasion of *Salmonella enterica* serovar *Typhimurium* is accompanied by strong hemorrhage promoted by TNF-α. *PLoS ONE* 4(8), e6692 (2009).

• **General introductory article that summarizes some specific aspects of the use of microbes for cancer.**

13. Quispe-Tintaya W, Chandra D, Jahangir A *et al.* Nontoxic radioactive Listeriaat is a highly effective therapy against metastatic pancreatic cancer. *Proc. Natl Acad. Sci. USA* 110(21), 8668–8673 (2013).
14. Chandra D, Jahangir A, Quispe-Tintaya W, Einstein MH, Gravekamp C. Myeloid-derived suppressor cells have a central role in attenuated *Listeria monocytogenes*-based immunotherapy against metastatic breast cancer in young and old mice. *Br. J. Cancer* 108(11), 2281–2290 (2013).
15. Malmgren RA, Flanigan CC. Localization of the vegetative form of *Clostridium tetani* in mouse tumors following intravenous spore administration. *Cancer Res.* 15(7), 473–478 (1955).
16. Dang LH, Bettegowda C, Huso DL, Kinzler KW, Vogelstein B. Combination bacteriolytic therapy for the treatment of experimental tumors. *Proc. Natl Acad. Sci. USA* 98(26), 15155–15160 (2001).

•• **A key review in the area with a focus on microbe-based drug-delivery systems.**

17. Kasinskas RW, Forbes NS. *Salmonella typhimurium* lacking ribose chemoreceptors localize in tumor quiescence and induce apoptosis. *Cancer Res.* 67(7), 3201–3209 (2007).
18. Ganai S, Arenas RB, Forbes NS. Tumour-targeted delivery of TRAIL using *Salmonella typhimurium* enhances breast cancer survival in mice. *Br. J. Cancer* 101(10), 1683–1691 (2009).
19. Sawant SS, Patil SM, Gupta V *et al.* Microbes as medicines: harnessing the power of bacteria in advancing cancer treatment. *Int. J. Mol. Sci.* 21(20), 1–24 (2020).
20. Linnebacher M, Maletzki C, Klier U, Klar E. Bacterial immunotherapy of gastrointestinal tumors. *Langenbeck's Arch. Surg.* 397(4), 557–568 (2012).
21. Gelman AE, Turka LA. Autoimmunity heats up. *Nat. Med.* 9(12), 1465–1466 (2003).
22. Chakrabarty AM. Microorganisms and cancer: quest for a therapy. *J. Bacteriol.* 185(9), 2683–2686 (2003).
23. Nougayrède JP, Taieb F, De Rycke J, Oswald E. Cyclomodulins: bacterial effectors that modulate the eukaryotic cell cycle. *Trends Microbiol.* 13(3), 103–110 (2005).
24. Zhang Y, Schulte W, Pink D *et al.* Sensitivity of cancer cells to truncated diphtheria toxin. *PLoS ONE* 5(5), e10498 (2010).
25. Mizrahi A, Czerniak A, Levy T *et al.* Development of targeted therapy for ovarian cancer mediated by a plasmid expressing diphtheria toxin under the control of H19 regulatory sequences. *J. Transl. Med.* 7, 1–11 (2009).
26. Michl P, Buchholz M, Rolke M *et al.* Claudin-4: a new target for pancreatic cancer treatment using *Clostridium perfringens* enterotoxin. *Gastroenterology* 121(3), 678–684 (2001).
27. Kominsky SL, Vali M, Korz D *et al. Clostridium perfringens* enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins Claudin 3 and 4. *Am. J. Pathol.* 164(5), 1627–1633 (2004).
28. Goldufsky J, Wood S, Hajihossainlou B *et al. Pseudomonas aeruginosa* exotoxin t induces potent cytotoxicity against a variety of murine and human cancer cell lines. *J. Med. Microbiol.* 64(2), 164–173 (2015).

29. Dieffenbach M, Pastan I. Mechanisms of resistance to immunotoxins containing pseudomonas exotoxin a in cancer therapy. *Biomolecules* 10(7), 1–13 (2020).

• **General introductory article that summarizes some specific aspects of the use of microbes for cancer.**

30. Allahyari H, Heidari S, Ghamgosha M, Saffarian P, Amani J. Immunotoxin: a new tool for cancer therapy. *Tumor Biol.* 39(2), 1–11 (2017).

31. Nemunaitis J, Cunningham C, Senzer N *et al.* Pilot trial of genetically modified, attenuated *Salmonella* expressing the *E. coli* cytosine deaminase gene in refractory cancer patients. *Cancer Gene Ther.* 10(10), 737–744 (2003).

32. Gudiña EJ, Rangarajan V, Sen R, Rodrigues LR. Potential therapeutic applications of biosurfactants. *Trends Pharmacol. Sci.* 34(12), 667–675 (2013).

33. Marchant R, Banat IM. Microbial biosurfactants: challenges and opportunities for future exploitation. *Trends Biotechnol.* 30(11), 558–565 (2012).

34. Banat IM, Franzetti A, Gandolfi I *et al.* Microbial biosurfactants production, applications and future potential. *Appl. Microbiol. Biotechnol.* 87(2), 427–444 (2010).

35. Cao X, Wang AH, Jiao RZ *et al.* Surfactin induces apoptosis and G2/M arrest in human breast cancer MCF-7 cells through cell cycle factor regulation. *Cell Biochem. Biophys.* 55(3), 163–171 (2009).

36. Cao X-H, Wang A-H, Wang C-L *et al.* Surfactin induces apoptosis in human breast cancer MCF-7 cells through a ROS/JNK-mediated mitochondrial/caspase pathway. *Chem. Biol. Interact.* 183(3), 357–362 (2010).

37. Kim JO, Jung SS, Kim SY *et al.* Inhibition of Lewis lung carcinoma growth by *Toxoplasma gondii* through induction of Th1 immune responses and inhibition of angiogenesis. *J. Korean Med. Sci.* 22(Suppl.), 38–46 (2007).

38. Chiewpattanakul P, Phonnok S, Durand A, Marie E, Thanomsub BW. Bioproduction and anticancer activity of biosurfactant produced by the dematiaceous fungus *Exophiala dermatitidis* SK80. *J. Microbiol. Biotechnol.* 20(12), 1664–1671 (2010).

39. Saini HS, Barraga BE, Lebro A *et al.* Efficient purification of the biosurfactant viscosin from *Pseudomonas libanensis* strain M9-3 and its physicochemical and biological properties. *J. Nat. Prod.* 71(6), 1011–1015 (2008).

40. Escobar-Díaz E, López-Martín EM, Hernández del Cerro M *et al.* AT514, a cyclic depsipeptide from *Serratia marcescens*, induces apoptosis of B-chronic lymphocytic leukemia cells: interference with the Akt/NF-κB survival pathway. *Leukemia* 19(4), 572–579 (2005).

41. El-Sersy NA, Abdelwahab AE, Abouelkhiir SS, Abou-Zeid DM, Sabry SA. Antibacterial and anticancer activity of ε-poly-L-lysine (ε-PL) produced by a marine *Bacillus subtilis* sp. *J. Basic Microbiol.* 52(5), 513–522 (2012).

42. Isoda H, Nakahara T. Mannosylerythritol lipid induces granulocytic differentiation and inhibits the tyrosine phosphorylation of human myelogenous leukemia cell line K562. *Cytotechnology* 25(1–3), 191–195 (1997).

43. Rizzato C, Torres J, Kasamatsu E *et al.* Potential role of biofilm formation in the development of digestive tract cancer with special reference to *Helicobacter pylori* infection. *Front. Microbiol.* 10(April), 1–21 (2019).

44. Groizeleau J, Rybtke M, Andersen JB *et al.* The anti-cancerous drug doxorubicin decreases the c-di-GMP content in *Pseudomonas aeruginosa* but promotes biofilm formation. *Microbiol. (United Kingdom)* 162(10), 1797–1807 (2016).

45. Podlesek Z, Žgur Bertok D. The DNA damage inducible SOS response is a key player in the generation of bacterial persister cells and population wide tolerance. *Front. Microbiol.* 11(August), 1–8 (2020).

46. Adnan M, Khan S, Al-Shammari E, Patel M, Saeed M, Hadi S. In pursuit of cancer metastasis therapy by bacteria and its biofilms: history or future. *Med. Hypotheses* 100, 78–81 (2017).

47. Miyake K, Yamamoto S, Iijima S. Blocking adhesion of cancer cells to endothelial cell types by *S. agalactiae* type-specific polysaccharides. *Cytotechnology* 22(1–3), 205–209 (1996).

48. Kumeria T, Maher S, Wang Y *et al.* Naturally derived iron oxide nanowires from bacteria for magnetically triggered drug release and cancer hyperthermia in 2D and 3D culture environments: bacteria biofilm to potent cancer therapeutic. *Biomacromolecules* 17(8), 2726–2736 (2016).

49. Wang ZH, Liu JM, Li CY *et al.* Bacterial biofilm bioinspired persistent luminescence nanoparticles with gut-oriented drug delivery for colorectal cancer imaging and chemotherapy. *ACS Appl. Mater. Interfaces* 11(40), 36409–36419 (2019).

50. Al-Mariri A, Tibor A, Lestrate P, Mertens P, De Bolle X, Letesson JJ. *Yersinia enterocolitica* as a vehicle for a naked DNA vaccine encoding *Brucella abortus* bacterioferritin or P39 antigen. *Infect Immun.* 70(4), 1915–1923 (2002).

51. Samoszuk MK, Walter J, Mechetner E. Improved immunohistochemical method for detecting hypoxia gradients in mouse tissues and tumors. *J. Histochem. Cytochem.* 52(6), 837–839 (2004).

52. Yu YA, Shabahang S, Timiryasova TM *et al.* Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. *Nat. Biotechnol.* 22(3), 313–320 (2004).

53. Baban CK, Cronin M, O'Hanlon D, O'Sullivan GC, Tangney M. Bacteria as vectors for gene therapy of cancer. *Bioeng. Bugs* 1(6), 385–394 (2010).

54. Zhou S, Gravekamp C, Bermudes D, Liu K. Tumour-targeting bacteria engineered to fight cancer. *Nat. Rev. Cancer* 18(12), 727–743 (2018).

- **General introductory article that summarizes some specific aspects of the use of microbes for cancer.**

55. Jia LJ, Wei DP, Sun QM, Huang Y, Wu Q, Hua ZC. Oral delivery of tumor-targeting *Salmonella* for cancer therapy in murine tumor models. *Cancer Sci.* 98(7), 1107–1112 (2007).
56. Toso JF, Gill VJ, Hwu P *et al.* Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *J. Clin. Oncol.* 20(1), 142–152 (2002).
57. Massa PE, Paniccia A, Monegal A, De Marco A, Rescigno M. *Salmonella* engineered to express CD20-targeting antibodies and a drug-converting enzyme can eradicate human lymphomas. *Blood* 122(5), 705–714 (2013).
58. Loeffler M, Le'Negrate G, Krajewska M, Reed JC. Inhibition of tumor growth using *Salmonella* expressing fas ligand. *J. Natl Cancer Inst.* 100(15), 1113–1116 (2008).
59. Chen J, Yang B, Cheng X *et al. Salmonella*-mediated tumor-targeting TRAIL gene therapy significantly suppresses melanoma growth in mouse model. *Cancer Sci.* 103(2), 325–333 (2012).
60. Sanders KL, Fox BA, Bzik DJ. Attenuated *Toxoplasma gondii* therapy of disseminated pancreatic cancer generates long-lasting immunity to pancreatic cancer. *Oncoimmunology* 5(4), 1–5 (2016).
61. Baird JR, Fox BA, Sanders KL *et al.* Avirulent *Toxoplasma gondii* generates therapeutic antitumor immunity by reversing immunosuppression in the ovarian cancer microenvironment. *Cancer Res.* 73(13), 3842–3851 (2013).
62. Giralt J, Regadera JP, Verges R *et al.* Effects of probiotic *Lactobacillus Casei* DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. *Int. J. Radiat. Oncol. Biol. Phys.* 71(4), 1213–1219 (2008).
63. Blanarova C, Galovicova A, Petrasova D. Use of probiotics for prevention of radiation-induced diarrhea. *Bratislava Med. J.* 110(2), 98–104 (2009).
64. Bowen JM, Stringer AM, Gibson RJ, Yeoh ASJ, Hannam S, Keefe DMK. VSL#3 probiotic treatment reduces chemotherapy-induced diarrhea and weight loss. *Cancer Biol. Ther.* 6(9), 1449–1454 (2007).
65. Cronin M, Le Boeuf F, Murphy C *et al.* Bacterial-mediated knockdown of tumor resistance to an oncolytic virus enhances therapy. *Mol. Ther.* 22(6), 1188–1197 (2014).
66. ClinicalTrials.gov. Treatment of patients with cancer with genetically modified *Salmonella Typhimurium* bacteria (2008). www.clinicaltrials.gov/ct2/show/NCT00004988
67. Goldin BR, Gorbach SL. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *J. Natl Cancer Inst.* 64(2), 263–265 (1980).
68. Lidbeck A, Övervik E, Rafter J, Nord CE, Gustafsson JA. Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. *Microb. Ecol. Health Dis.* 5(1), 59–67 (1992).
69. Hayatsu H, Hayatsu T. Cancer urinary mutagenicity arising from ingestion of fried ground beef. *Mutation* 73, 173–179 (1993).
70. Garrett WS. Cancer and the microbiota. *Science* 348(6230), 80–86 (2015).
71. Requena T, Martínez-Cuesta MC, Peláez C. Diet and microbiota linked in health and disease. *Food Funct.* 9(2), 688–704 (2018).
72. LeBlanc JG, Chain F, Martín R, Bermúdez-Humarán LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Fact.* 16(1), 1–10 (2017).
73. dos Reis SA, da Conceição LL, Siqueira NP, Rosa DD, da Silva LL, Peluzio M do CG. Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutr. Res.* 37, 1–19 (2017).
74. Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T. Erratum: oral administration of *Butyrivibrio fibrisolvens*, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. *J. Nutr.* 139(1), 194 (2009).
75. Kaye J, Porcelli S, Jones B *et al.* Treatment of murine colitis by *Lactococcus lactis* secreting IL-10. *Proc. Natl Acad. Sci. USA* 76(August), 104 (1994).
76. De LeBlanc AM, LeBlanc JG, Perdigón G *et al.* Oral administration of a catalase-producing *Lactococcus lactis* can prevent a chemically induced colon cancer in mice. *J. Med. Microbiol.* 57(1), 100–105 (2008).
77. Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E. Lactic acid bacteria: reviewing the potential of a promising delivery live vector for biomedical purposes. *Microb. Cell Fact.* 14(1), 1–12 (2015).
78. Kaparakis-Liaskos M, Ferrero RL. Immune modulation by bacterial outer membrane vesicles. *Nat. Rev. Immunol.* 15(6), 375–387 (2015).
- **General introductory article that summarizes some specific aspects of the use of microbes for cancer.**
79. Zhang Y, Fang Z, Li R, Huang X, Liu Q. Design of outer membrane vesicles as cancer vaccines: a new toolkit for cancer therapy. *Cancers (Basel)* 11(9), 1–24 (2019).
80. Klebanoff CA, Acquavella N, Yu Z, Restifo NP. Therapeutic cancer vaccines: are we there yet? *Immunol. Rev.* 239(1), 27–44 (2011).
81. Staedke V, Roberts NJ, Bai R. *Clostridium novyi-NT* in cancer therapy. *Genes Dis.* 3(2), 144–152 (2016).
82. Roberts NJ, Zhang L, Janku F *et al.* Intratumoral injection of *Clostridium novyi-NT* spores induces antitumor responses. *Sci. Transl. Med.* 6(249), 111 (2014).

•• **A key review in the area with a focus on microbe-based drug delivery systems.**

83. ClinicalTrials.gov. Safety study of intratumoral injection of *Clostridium novyi-NT* spores to treat patients with solid tumors that have not responded to standard therapies (2019). https://clinicaltrials.gov/ct2/show/NCT01924689
84. ClinicalTrials.gov. Pembrolizumab with intratumoral injection of *Clostridium novyi-NT* (2021). https://clinicaltrials.gov/ct2/show/NCT03435952
85. ClinicalTrials.gov Study of ADXS11-001 in subjects with high risk locally advanced cervical cancer (AIM2CERV) (2020). https://clinicaltrials.gov/ct2/show/record/NCT02853604
86. Le DT, Wang-Gillam A, Picozzi V *et al.* Safety and survival with GVAX pancreas prime and *Listeria monocytogenes*-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J. Clin. Oncol.* 33(12), 1325–1333 (2015).
87. Basu P, Mehta A, Jain M *et al.* A randomized phase 2 study of ADXS11-001 *Listeria monocytogenes*-listeriolysin O immunotherapy with or without cisplatin in treatment of advanced cervical cancer. *Int. J. Gynecol. Cancer* 28(4), 764–772 (2018).
88. Jia LJ, Wei DP, Sun QM *et al.* Tumor-targeting Salmonella typhimurium improves cyclophosphamide chemotherapy at maximum tolerated dose and low-dose metronomic regimens in a murine melanoma model. *Int. J. Cancer* 121(3), 666–74 (2007).
89. ClinicalTrials.gov VNP20009 in treating patients with advanced or metastatic solid tumors that have not responded to previous therapy (2013). www.clinicaltrials.gov/ct2/show/NCT00004216
90. ClinicalTrials.gov. IL-2 expressing, attenuated *Salmonella Typhimurium* in unresectable hepatic spread (2020). www.clinicaltrials.gov/ct2/show/NCT01099631
91. Schmitz-Winnenthal FH, Hohmann N, Schmidt T *et al.* A phase 1 trial extension to assess immunologic efficacy and safety of prime-boost vaccination with VXM01, an oral T cell vaccine against VEGFR2, in patients with advanced pancreatic cancer. *Oncoimmunology* 7(4), e1303584 (2018).
92. Felgner S, Kocijancic D, Frahm M, Curtiss R, Erhardt M, Weiss S. Optimizing *Salmonella enterica* serovar *Typhimurium* for bacteria-mediated tumor therapy. *Gut Microbes* 7(2), 171–177 (2016).
93. Morales A, Eidinger D, Bruce AW. Intracavitary bacillus Calmette-Guerin in the treatment of superficial bladder tumors 1976. *J. Urol.* 167(2 Pt 2), 891–893 (2002).
94. Leschner S, Weiss S. *Salmonella*-allies in the fight against cancer. *J. Mol. Med.* 88(8), 763–773 (2010).
95. St Jean AT, Swofford CA, Panteli JT, Brentzel ZJ, Forbes NS. Bacterial delivery of *Staphylococcus aureus* α-hemolysin causes regression and necrosis in murine tumors. *Mol. Ther.* 22(7), 1266–1274 (2014).
96. Park SJ, Park SH, Cho S *et al.* New paradigm for tumor theranostic methodology using bacteria-based microrobot. *Sci. Rep.* 3, 1–8 (2013).
97. Park SJ, Lee YK, Cho S *et al.* Effect of chitosan coating on a bacteria-based alginate microrobot. *Biotechnol. Bioeng.* 112(4), 769–776 (2015).
98. Kassahn D, Nachbur U, Brunner T. CD95L pro-drug: a novel Swiss army knife in cancer therapy? *Cell Death Differ.* 14(3), 393–394 (2007).
99. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75(2), 263–274 (1993).
100. Berg DJ, Davidson N, Kühn R *et al.* Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ Th1-like responses. *J. Clin. Invest.* 98(4), 1010–1020 (1996).
101. Santaolalla R, Sussman DA, Ruiz JR *et al.* TLR4 activates the β-catenin pathway to cause intestinal neoplasia. *PLoS ONE* 8(5), e63298 (2013).
102. Arthur JC, Perez-Chanona E, Mühlbauer M *et al.* Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science (80-.)* 338(6103), 120–123 (2012).
103. Collins D, Hogan AM, Winter DC. Microbial and viral pathogens in colorectal cancer. *Lancet Oncol.* 12(5), 504–512 (2011).
104. Goldszmid RS, Dzutsev A, Trinchieri G. Host immune response to infection and cancer: unexpected commonalities. *Cell Host Microbe* 15(3), 295–305 (2014).
105. Schwabe RF, Jobin C. The microbiome and cancer. *Nat. Rev. Cancer* 13(11), 800–812 (2013).

•• **A key review in the area with a focus on microbe-based drug-delivery systems.**

106. Challinor VL, Bode HB. Bioactive natural products from novel microbial sources. *Ann. NY Acad. Sci.* 1354(1), 82–97 (2015).
107. Schwarzer D, Finking R, Marahiel MA. Nonribosomal peptides: from genes to products. *Nat. Prod. Rep.* 20(3), 275–287 (2003).
108. Van Dessel N, Swofford CA, Forbes NS. Potent and tumor specific: arming bacteria with therapeutic proteins. *Ther. Deliv.* 6(3), 385–399 (2015).

• **General introductory article that summarizes some specific aspects of the use of microbes for cancer.**

109. Coley Nauts H, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. *Cancer Res.* 6(4), 205–216 (1946).

110. Kim JE, Phan TX, Nguyen VH *et al. Salmonella typhimurium* suppresses tumor growth via the pro-inflammatory cytokine interleukin-1β. *Theranostics* 5(12), 1328–1342 (2015).
111. Sun HK, Castro F, Paterson Y, Gravekamp C. High efficacy of a *Listeria*-based vaccine against metastatic breast cancer reveals a dual mode of action. *Cancer Res.* 69(14), 5860–5866 (2009).
112. Kimura T, Aoki K, Baba T. Selective localization and growth of *Bifidobacterium bifidum* in mouse tumors following intravenous administration. *Cancer Res.* 40(6), 2061–2068 (1980).
113. Rocha EPC, Danchin A. An analysis of determinants of amino acids substitution rates in bacterial proteins. *Mol. Biol. Evol.* 21(1), 108–116 (2004).
114. Gal JE. *Salmonella* interactions with host cells: type III secretion at work. *Annu. Rev. Cell Div. Biol.* 17, 53–86 (2001).
115. Stritzker J, Weibel S, Hill PJ, Oelschlaeger TA, Goebel W, Szalay AA. Tumor-specific colonization, tissue distribution, and gene induction by probiotic *Escherichia coli* Nissle 1917 in live mice. *Int. J. Med. Microbiol.* 297(3), 151–162 (2007).
116. Groot AJ, Mengesha A, van der Wall E, va Diest PJ, Theys J, Vooijs M. Functional antibodies produced by oncolytic *Clostridia*. *Biochem. Biophys. Res. Commun.* 364(4), 985–989 (2007).
117. Liang K, Liu Q, Li P, Luo H, Wang H, Kong Q. Genetically engineered *Salmonella typhimurium*: recent advances in cancer therapy. *Cancer Lett.* 448(2), 168–181 (2019).
118. Javan B, Shahbazi M. Hypoxia-inducible tumour-specific promoters as a dual-targeting transcriptional regulation system for cancer gene therapy. *Ecancermedicalscience* 11, 1–10 (2017).
119. Berger E, Soldati R, Huebener N *et al. Salmonella* SL7207 application is the most effective DNA vaccine delivery method for successful tumor eradication in a murine model for neuroblastoma. *Cancer Lett.* 331(2), 167–173 (2013).
120. Tang W, He Y, Zhou S, Ma Y, Liu G. A novel *Bifidobacterium infantis*-mediated TK/GCV suicide gene therapy system exhibits antitumor activity in a rat model of bladder cancer. *J. Exp. Clin. Cancer Res.* 28(1), 1–7 (2009).