



REVIEW

Bacterial-Based Methods for Cancer Treatment: What We Know and Where We Are

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ABSTRACT

A severe disease, cancer is caused by the exponential and uncontrolled growth of cells, leading to organ dysfunction as well as disorders. This disease has been recognized as one of the significant challenges to health and medicine. Various treatment procedures for cancer are associated with diverse side effects; the most conventional cancer treatments include chemotherapy, surgery, and radiotherapy, among others. Numerous adverse and side effects, low specificity and sensitivity, narrow therapeutic windows, and, recently, the emergence of tumor cells resistant to such treatments have been documented as the shortcomings of conventional treatment strategies. As a group of prokaryotic microorganisms, bacteria have great

potential for use in cancer therapy. Currently, utilizing bacteria for cancer treatment has attracted the attention of scientists. The high potential of bacteria to become non-pathogenic by genetic manipulation, their distinguished virulence factors (which can be used as weapons against tumors), their ability to proliferate in tissues, and the contingency to control their population by administering antibiotics, etc., have made bacteria viable candidates and live micro-medication for cancer therapies. However, the possible cytotoxicity impacts of bacteria, their inability to entirely lyse cancerous cells, as well as the probability of mutations in their genomes are among the significant challenges of bacteria-based methods for cancer treatment. In this article, various available data on bacterial therapeutics, along with their pros and cons, are discussed.

Keywords: Bacterial therapeutics; Bacteriotherapy; Cancer treatment; Immunotherapy; Microbial metabolites; Multimodal therapies

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Key Summary Points

The present review study was carried out to summarize the most recent data available on bacteria-based methods for cancer treatment to prepare a comprehensive basis for future research

Multiple side effects of available and conventional cancer treatment methods and emergence of resistant tumor cells have highlighted the importance of ingenious strategies for cancer therapy

Low specificity and sensitivity, a narrow therapeutic window, and high cellular toxicity of available cancer treatment methods have increased the importance of novel procedures like bacteriotherapy

The suitable properties of bacteria and their metabolites, such as their high potential for genomic or biochemical manipulation, etc., have made them a suitable candidate for cancer treatment

In conclusion, some microbial metabolites and bacteria have demonstrated remarkable antitumor impacts and can be used as novel approaches for cancer treatment solely or in combined therapies

INTRODUCTION

Having serious complications, cancer is a severe and critical disease that causes many deaths every year worldwide. Scientists are concerned about the growing rate of cancer. From a medical point of view, cancer can be defined as the unregulated and invasive proliferation of transformed cells. These neoplastic cells have the potential to disperse to various tissues and organs in the body via the metastatic process. According to a recent World Health Organization (WHO) report, cancer is the first, or at least second, cause of death in people under the age of 70 years in 112 out of 183 countries [1].

While lung, prostate, gastric, colon, and liver cancers are the prevailing cancers in males, breast, lung, colon, uterus, and thyroid cancers are the most prevalent in females. Inducing apoptosis and preventing the growth and proliferation of tumor cells have been the mainstays of cancer treatment so far [2]. Although the standard antitumor therapy methods, including surgery and tumor resection, radiotherapy, and chemotherapy, have been efficient and valuable in treating and improving most symptoms, they remain ineffective for nearly half of cancer cases. Therefore, alternative cancer treatments are being developed to target tumors and cancerous tissues more efficiently [3].

Currently, scientists are investigating and developing novel strategies for treating cancer [4]. Nevertheless, precise examination and modifications in these methods are required for selecting the best alternative therapies and using them in cancer treatment [5]. Thus, particular pioneer therapeutic approaches, like immunotherapy, stem cell-based treatment, hormone therapy, and dendritic cell-based immunotherapy, have recently been implemented. As a conventional and widely exploited treatment strategy against tumors, chemotherapy can be practical in concomitant-multimodal therapies. However, it may also result in the appearance of multidrug-resistant (MDR) malignant cells and metastasis in some patients [6]. Therefore, there is a tremendous demand for up-to-date cancer treatment methods and remedial substances with fewer adverse and side effects and higher efficiency. In the last 10 years, scientists and researchers have explored bacteria and their metabolites' potential in tumor cell destruction as a novel anti-neoplastic strategy. They have found that bacteria and their products not only have low toxicity, but also have limited side effects on normal and healthy cells. Some bacterial species in various forms and bacterial metabolites (including peptides, bacteriocins, etc.) have been utilized to treat cancer patients. The results have demonstrated that these agents can selectively multiply and influence tumors and restrain their growth [7]. In anticancer bacteria-based immunotherapy methods, the used

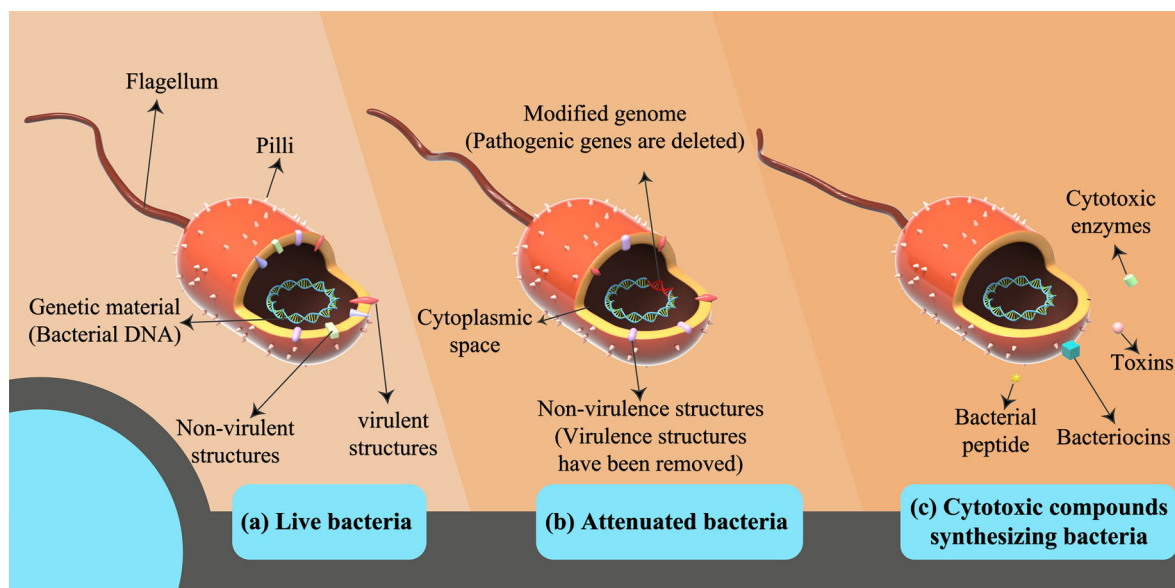


Fig. 1 Bacteria in the living form (a), attenuated (b), or their metabolites (c) can be used for cancer therapy. The pathogenic bacteria may turn into non-pathogenic and

appropriate weapons for cancer treatment by deleting a part of their genome or virulent structures

bacteria can be present in living or attenuated conditions, or even in genetically engineered forms, as illustrated in Fig. 1.

Adopting bacteria for cancer treatments has been associated with promising outcomes in diverse cases. Spores of anaerobic bacteria can be utilized to synthesize, develop and produce anticancer agents. They may also be used as carriers for gene and drug delivery to tumor tissues. These bacterial spores can obtain hypoxic-necrotic tissues, where they are able to germinate, multiply, and exert their antitumor function. The application of genetically modified bacteria to selectively destroy tumors and alter cellular enzymes expression has also been accompanied by successful outcomes [8].

Although other microorganisms such as *Neospora caninum* have also been observed to have anticancer properties, the current study has concentrated on the bacteria-based methods for cancer therapy [9]. Overall, this article has been carried out to gather and summarize the most recent available data and literature on

bacteria-based methods for cancer treatment because of the importance of reviewing and investigating these novel strategies. The study aims to create a reliable basis for researchers and their future research by rendering a comprehensive summary of clinical trials on bacteriotherapy and the scientific history of this procedure. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

DISCUSSION

Apart from briefly reviewing the history and literature of bacteriotherapy, the present study also discusses the bacterial species that can be effective in bacterial immunotherapy. These bacteria and their metabolites can eliminate the tumor tissues from the body and interrupt the growth of neoplastic cells. Moreover, certain bacteria such as *Clostridium* spp. and

Bifidobacterium longum strains—which can endure and progress in hypoxic conditions—can proliferate and surround the tumor, causing tumor destruction [7, 10].

The Scientific History of Bacteriotherapy: First Observations and Experiments

The first observations about the effect of bacteria on cancer date back to 150 years ago [11]. At that time, two physicians from Germany, namely W. Busch [12] and F. Fehleisen [13], individually observed the improved symptoms of cancer in patients who accidentally had been infected by erysipelas. Therefore, as the first physician who observed this phenomenon, Busch inoculated erysipelas-causing bacteria in a cancer patient and monitored him for a while [12]. A few years later, Fehleisen identified the cause of erysipelas infection after repeating the experiment and discovered that the *Streptococcus pyogenes* pathogen causes it [13].

The first official examination on utilizing bacteria for cancer treatment was performed during the past 2 centuries. William Coley, a medical doctor in New York City, developed a combination of two bacteria consisting of *S. pyogenes* and *Bacillus prodigiosus* (nowadays known as *Serratia marcescens*) in 1893. He combined the lytic compounds of these bacteria and named the product Coley toxin. He found that the injection of Coley toxin into the cancerous tissues in some patients ceased tumor growth and subsequently improved the patient's healing process [14]. After initial examinations by Coley, certain anaerobic bacterial species, such as those classified as *Clostridium* spp., were discovered by scientists. These bacteria could reduce oxygen pressure and cause oxygen deprivation in cancerous tissues as they grew, thereby making a hypoxic environment in which the tumor cells died.

BCG vaccine is possibly the most famous bacteria-based agent used for cancer treatment. Caused by *Mycobacterium tuberculosis*, tuberculosis (TB) is an infectious disease which generally infects the lungs, but it can also harmfully affect other parts of the body [15, 16]. *Bacillus Calmette-Guérin* (BCG) vaccine, named after its

inventors, is mainly injected into babies intramuscularly to prevent TB infection [17, 18]. A live attenuated strain of *M. bovis*—which belongs to the same genus as *M. tuberculosis*, but is a different species [18]—is used for BCG vaccine manufacture. It is primarily administered for TB prevention and can be 0 to 80% effective according to the geography and the laboratory where it is synthesized [19]. However, it has been demonstrated that BCG can be effective in preventing other infections such as Buruli ulcer [20] and treating non-muscle invasive bladder cancer (NMIBC) [21]. However, *M. bovis* was the first bacterium that underwent manipulation to become non-pathogenic. After applying modifications, BCG was administered intravesically to patients who suffered from NMIBC [22]. The intravesical injection of BCG in patients with NMIBC leads to a dramatic decrease in cancer relapse [23]. The primary action mechanism of the BCG vaccine for NMIBC treatment is thought to be due to innate and acquired immunity activation by this agent [24]. It has been further elaborated that BCG is able to activate the caspase-8 signaling pathway, which concerns Toll-like receptor 7 (TLR7), and results in tumor cell apoptosis [25]. It is also reported that BCG can directly cause cell necrosis and death [24]. The other action mechanism of BCG is related to its effects on immune cells. BCG can act as a pathogen-associated molecule pattern (PAMP) and activate pattern recognition receptors (PRR) present in the immune cells [26, 27], which ultimately results in the production of cytokines and the activation of the immune system against tumor cells [24, 28]. BCG is also capable of influencing natural killer cells (NK cells) and neutrophils, which can eliminate cancerous cells by phagocytosis or other mechanisms [24, 29]. BCG can inhibit the cellular proliferation of tumor cells as well [30, 31]. The summarized history of bacteriotherapy-related observations and studies is shown in Fig. 2.

Nonetheless, it is now well established that numerous bacteria are associated with tumor promoters and carcinogens. It has also been reported that some of them can play a beneficial role in cancer therapy. Some bacterial species, including *Clostridia*, *Shigella*, *Lactococcus*,

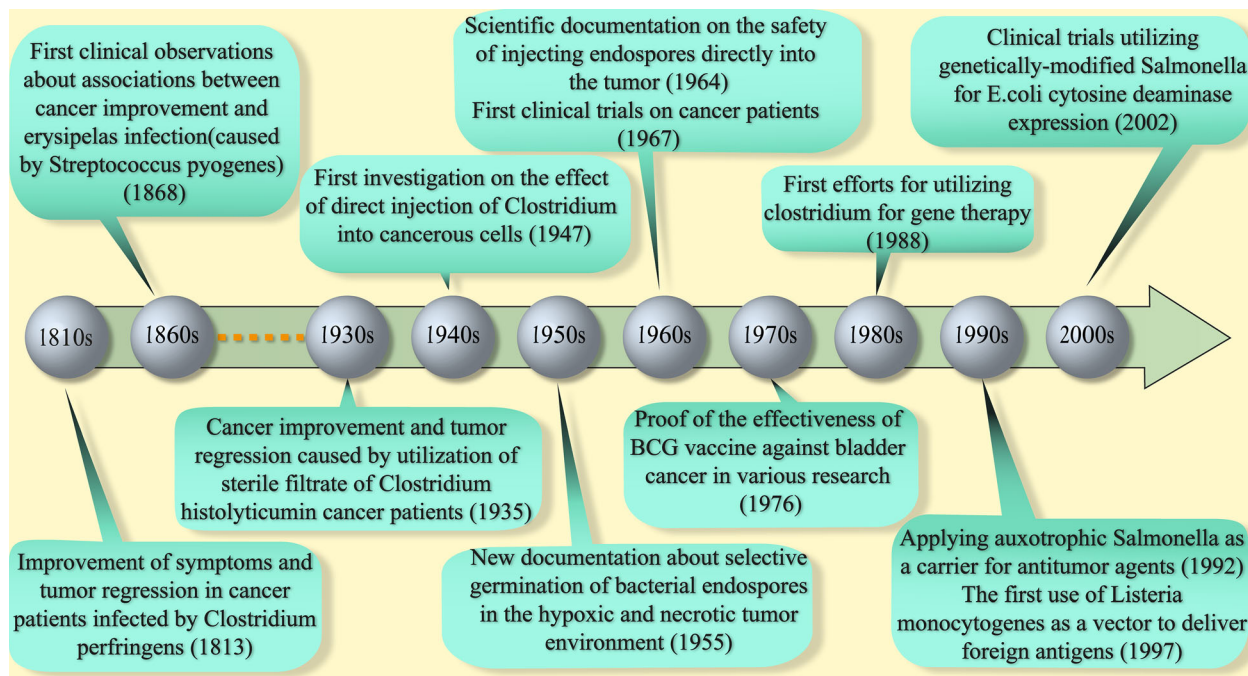


Fig. 2 The history and early observations on the bacteria-based methods for cancer treatment: the first evidence of relationships between bacteria and cancer was accidentally observed and recorded about 2 centuries ago. Since then,

many studies, examinations, and clinical trials have been performed to enhance bacteriotherapy’s effectiveness for cancer therapy

Bifidobacteria, Listeria, Salmonella, Vibrio, and Escherichia coli, have been indicated to have exceptional potential to be used for cancer treatment. They also appear to have a potent tumoricidal effect [32–35].

The Possible Effect of Bacteriotherapy on Immune Cells for Cancer Treatment

Although bacterial infections are mainly thought to be tumor-promoting—and some of them are even carcinogenic [36]—mounting scientific evidence suggests that some bacterial species can have antitumor effects, as mentioned previously. The interconnection of host cells with bacteria (commensal or pathogenic species) can improve the host’s immune response to malignant cells. Tumor cells may impair the immune system’s ability to fight diseases, but some bacteria may positively affect it. It is worth noting that immune cells’ ability is irrespective of bacterial infections [37, 38]; however, bacteria can positively enhance

immune cells. The most crucial members of the immune system that have a significant impact on suppressing malignant and abnormal cells are CD8 + T-lymphocytes, macrophages, NK cells, dendritic cells (DCs), and regulatory T cells (T-regs), which contain FOXP3 as a bio-marker. All of the mentioned cells have a remarkable effect on inhibiting the replication of cancerous cells and attacking as well as eliminating them [37]. CD8 + T-cells have been recognized as the most influential immune system components in inhibiting malignant cells growth [39].

Certain microbial infections and complications such as *E. coli*-induced infections can be used to suppress the tumor tissues. These induced infections improve and accelerate the differentiation of CD8 + killer T-cells, resulting in IFN- γ synthesis and an increase in the expression of major histocompatibility complex subtype I (MHC-I) on cancerous cells. This integrative mechanism can ultimately change CD8 + T-cell diapedesis into tumor tissue [39].

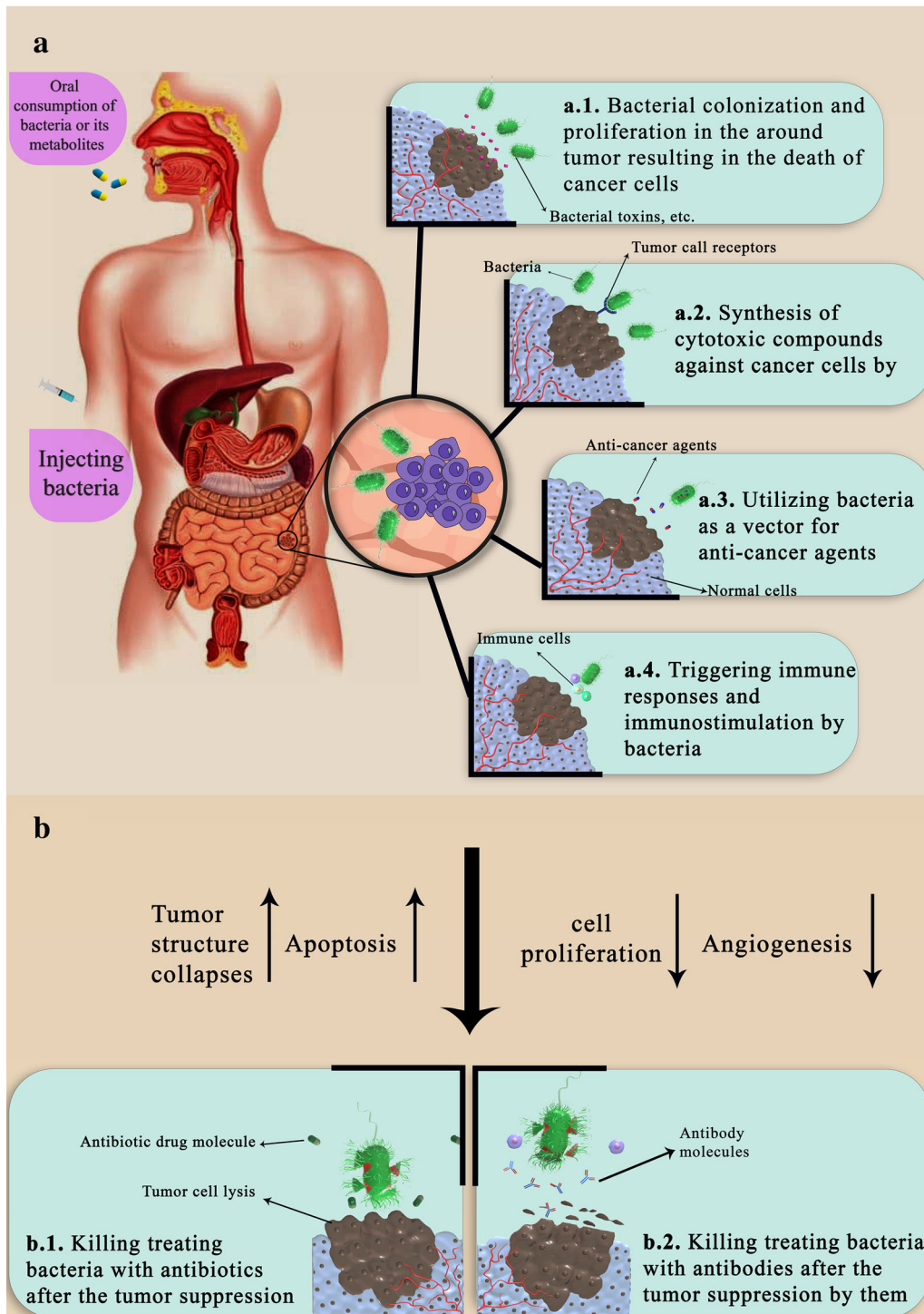
It should be added that the role of CD8 + T cells in attacking tumor cells is recognized independently from the bacterial activity, and these cells are able to attack cancerous tissues even in the absence of bacterial infections or activities [38]. However, bacterial activities can only enhance their effectiveness. Furthermore, other microbe-related compounds can also affect the CD8 + T-cells. For instance, a study conducted by Diwakar Davar et al. [40] indicated that the administration of responder-derived fecal microbiota transplant (R-FMT) with pembrolizumab can improve the CD8 + T cells' induction, reduce IL-8 synthesis, and thus strengthen the immune responses against tumor cells resistant to anti-PD-1 [40].

Another anticancer function of different bacteria and their products is improving the body's immunity against malignant cells. They can counteract cancer cells in multiple ways; the most noticeable of these mechanisms are as follows: (1) consuming and reducing a large amount of needed nutrients for cancer cell metabolism in the tumor environment; (2) being capable of generating and proliferating in necrotic regions creating hypoxic conditions for neoplastic cells; (3) having the potential to be used as a means for delivering anticancer drugs; (4) killing malignant cells by forming biofilms and surrounding them [41–46]. Furthermore, bacteria can be effective in cellular apoptosis through other mechanisms such as hosting and consuming free foreign DNA as well as improving their ability in cancer treatment and tumor elimination [47]. The most important strategies of utilizing bacteria to overcome the restrictions and side effects of conventional cancer treatment methods (e.g., chemotherapy, surgery, radiotherapy, etc.) are illustrated in Fig. 3.

Bacteria also have numerous influences on other immune cells, which are involved in tumor suppression. As cancer cells form and begin to grow, a large number of leukocytes, tumor-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tyrosine kinase receptor (TIEs)-2-expressing monocytes, and tolerogenic dendritic cells (TDCs) migrate to the cancerous region, improving the density of such

cells in that area [48]. Diapedesis of monocytes in response to chemokines and adsorbent chemicals released by tumor cells occurs in the tumor microenvironment. Compounds like colony-stimulating factor 1 (CSF1) and (C–C motif) ligand 2 (CCL2) chemokine are among the substances that absorb monocytes and cause their differentiation into macrophages [49]. As a group of activated immune cells contributing to tissue regeneration, these monocytes are named M2-type macrophages. After being converted to M2 macrophages, these monocytes express and secrete immune inhibitory molecules such as arginase-1 (Arg1) and interleukin 10 (IL-10) cytokine. M2 macrophages suppress host anti-tumor immune responses, resulting in the formation of malignant cells and tumors. Unlike M2 macrophages, M1 macrophages, also recognized as normal active macrophages or classical killers, express an enzyme called nitric oxide synthase 2 (NOS2), leading to tumor death. They exert their antitumor effect by expressing tumor necrosis factor-alpha (TNF- α) or other cytokines [50–52]. The effects of bacteriotherapy on immune cells involved in anticancer activities, such as MDSCs, tumor-associated lymphoid cells (TALCs), TAMs, and T-reg cells, are demonstrated in Fig. 4.

Induction of immune responses by bacteria and bacterial immunotherapy has been regarded as an emerging cancer treatment method. This therapeutic technique may have a high chance of breaching the immune barriers caused by conventional immunotherapy, such as immune-related adverse events (irAEs), which are associated with the immune checkpoint inhibitors (ICIs) [53, 54]. ICIs are a drug family that block a particular type of proteins called immune checkpoints, which are expressed by some immune cells like T-cells [55]. These immune checkpoints such as PD-1/PD-L1 and CTLA-4 prevent the overactivation of immune cells and restrain strong responses which are harmful [56, 57]. ICIs block these checkpoints and result in robust immune responses to the cancerous tissues. However, they may also cause adverse side effects such as rash, pruritus, diarrhea, and colitis, which are usually counted as irAEs [53]. Immunotherapy is also capable of eliciting an immune system



◀ **Fig. 3** The most important usages of bacteria for cancer treatment. Bacteria can bind to cancer cells through their surface ligands and colonize and proliferate there, thereby reducing the available oxygen pressure, which consequently leads to cancer cell destruction (**a.1**). Synthesis of cytotoxic compounds by bacteria against the cancer cells in the tumor microenvironment after colonization (**a.2**). Using bacteria as carriers of anticancer agents to increase their specificity (**a.3**). Stimulation of immune cells to eliminate tumors by bacteria colonized in the tumor microenvironment (**a.4**). One of the benefits of utilizing bacteria for cancer therapy is the ability to limit them when needed. Bacteria can be eliminated indirectly by triggering and strengthening the immune system (actively or passively) (**b.1**) or directly with antibiotics (**b.2**) after cancer treatment

response to tumor cells with limited side effects [58]. The basic idea of immunotherapy is to enhance and strengthen patients' immune systems and make them able to destroy cancer cells. In fact, although scientists recognized the link between the immune system and various diseases a long time ago, they did not explicitly know how the immune system suppresses and affects tumors [59]. Nevertheless, to ward off several infectious diseases and cancers [60], many vaccines have been developed using weakened (such as BCG vaccine, which comprises live attenuated *M. bovis* [61]) or killed bacteria (e.g., the first type of pertussis vaccine, which contains whole-cell killed *Bordetella pertussis* [62, 63]) species. Bacteria have also been used in multiple forms as vaccine carriers to transmit distinct types of antigenic signals to activate the immune system against tumors [64].

Bacteria-Based Microrobots (Bacteriobots): New Gadgets for Cancer Therapy

For a long time, scientists have focused on researching and designing different types of biomedical microrobots as a practical means for drug delivery, microscopic surgery, and cancer treatment. Recently, bacteria-based microrobots have been posited as a viable alternative for cancer treatment. The concept of bacteriobots is an innovative and novel bacteria-based method

for triggering antitumor actions [65]. Bacteriobots are bacteria-based devices with a similar structure that can be applied for targeting specific cells (i.e., for drug delivery or attacking them), and can be exploited in free or granulated form.

As described to before, to increase bacteriobots' efficiency, bacteria can be encapsulated/granulated in biodegradable and biocompatible microbeads, which are safe for human cells. The microbeads protect bacteria against opsonization and other physiological modifications which may occur in the body. These microbeads are in close contact with normal organs, and that is why it is vital to utilize appropriate microbeads to synthesize efficient bacterial robots for tumor targeting. The rate and mechanism of entrapped bacteria release from microbeads should be considered in the following steps of bacteriobots preparation. Ultimately, the bacterial flagella should be added to the microbial granules to facilitate the movement of bacterial robots and simplify the agents reaching the target tissue [66].

In one study, *Salmonella typhimurium* was adopted as part of a bacteria-based microbot. In this research, biocompatible alginate granules were used to encapsulate bacteria, and then *S. typhimurium* flagella were attached to the microbeads as a motility section. The experiments demonstrated that these robotic bacteria were able to target the tumor effectively [66].

In another study conducted by Al-Fandi et al., non-pathogenic *E. coli* were applied as live nanobots for cancer treatment. These live microrobots were equipped with naturally synthesized nano-biosensor systems bound to vascular endothelial growth factor (VEGF). The microbial nanobots were designed to detect and bind to tumor cells and the VEGF receptor was overexpressed in their membrane [67]. It is reported that robotic bacteria have greater chemotactic motility, more efficient cancerous tissue targeting, and higher migration rates to tumors compared to healthy cells. Moreover, this new generation of bacterial robots also acts as micro-stimuli and micro-sensors. This means that they can be applied as carriers for delivering therapeutic nanoparticles (NPs) and drugs to tumors. Therefore, bacteriobots may be a new

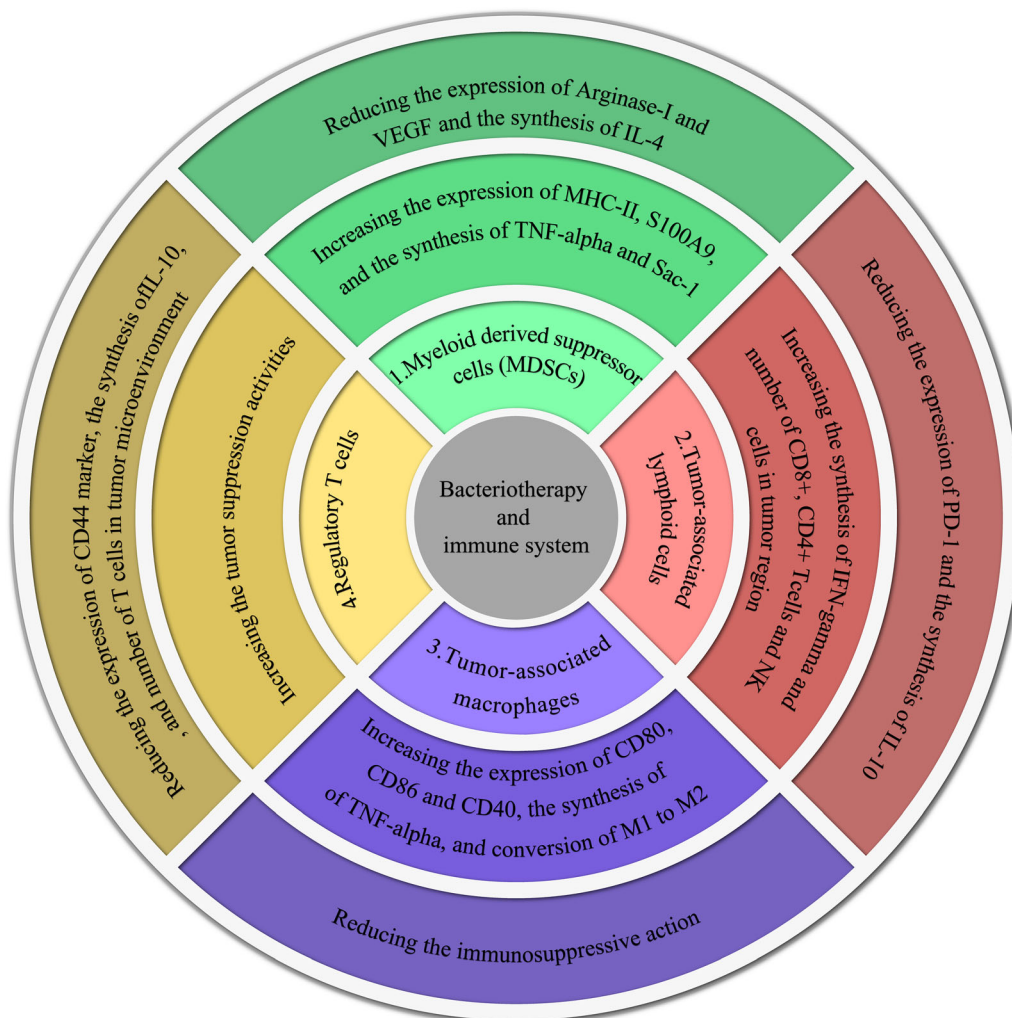


Fig. 4 The effect of bacteria on different antitumor immune cells: bacteria can be effective in cancer immunotherapy by accurately regulating the increasing-reducing responses against the tumor cells

therapeutic tool for detecting and fighting solid tumors [68]. In Fig. 5, the merits and benefits of utilizing bacteribots, how they move to target tissues, and their effect on immune cells are illustrated.

Most-Utilized Bacterial Species Applied for Cancer Treatment

Many anaerobic bacteria studied so far can make spores under brutal conditions. These spores are highly resistant to unfavorable circumstances. They also permit bacteria to survive even in oxygen-rich environments, which

are not desirable for anaerobic bacteria. These spores can get active and germinate to the living form of bacteria as soon as they find favorable conditions, such as hypoxic areas in the necrotic tissue within tumors. These features make spore-producing anaerobic bacteria an ideal choice for targeting cancer cells. Genetically modified strains of *C. novyi-NT* are free of lethal toxins and do not cause any systemic side effects on the injected host. Cell lysis in the tumor tissues was observed in mice with uterine cancer that had received *C. histolyticum* spores via intrauterine injection. The same result was recorded in mice that received *C. sporogenes* spores by intravenous (I.V.) injection [69].

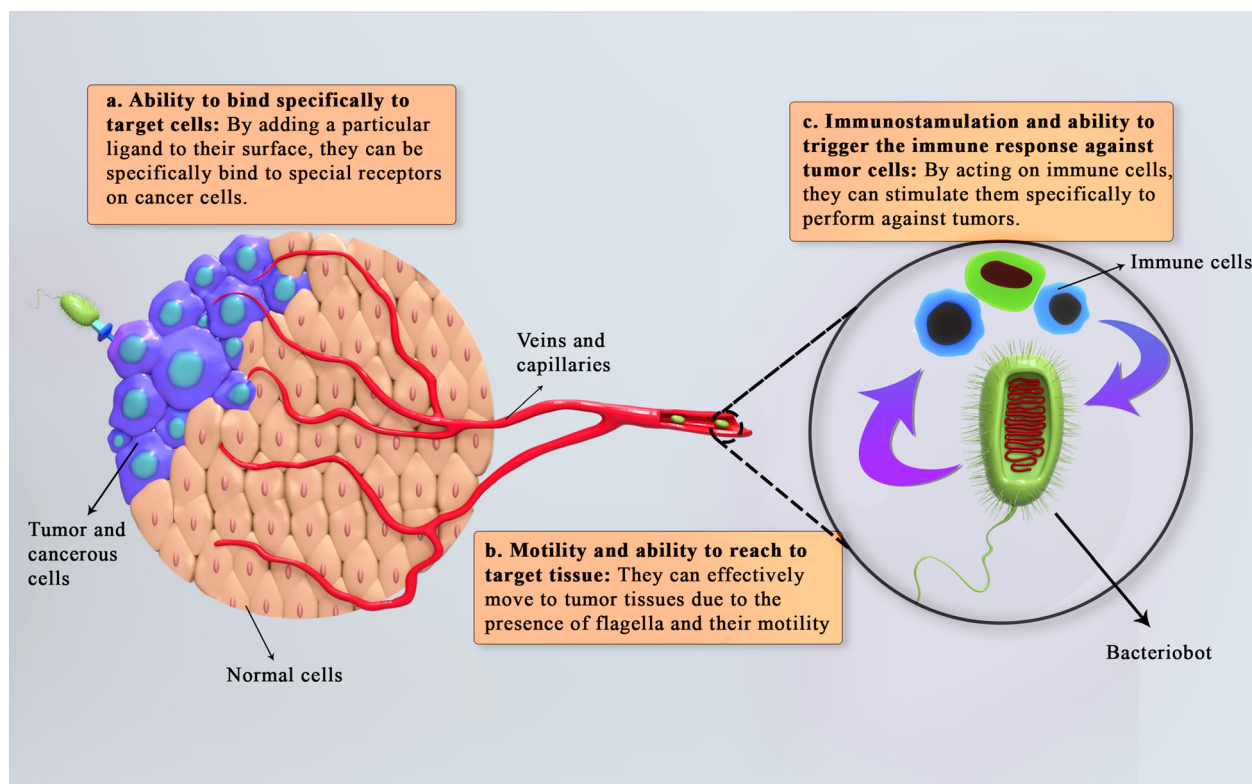


Fig. 5 Schematic in vivo image of bacteria applied for bacteriotherapy: specific binding of bacteria to tumor cells and non-binding to normal cells (a). How bacteria move in capillaries and veins (b) and their effect on immune cells (c)

Pharmacological and toxicological evaluation of *C. novyi-NT* spores indicated that the reticuloendothelial system (RES) rapidly eliminates spores from the circulatory system. No clinically harmful toxins were found in the healthy mice or rabbits that received these spores, even at high doses [70]. Spores produced by bacteria have also been utilized as carriers for delivering anticancer compounds. Bacterial spores can also deliver various agents like cytotoxic peptides, therapeutic proteins, and genetic materials.

Some modifications in tumor cells following spore injection, such as mitochondrial failure, increased metabolism, cellular signaling, etc., increase the reactive oxygen species (ROS) concentration. An excessive increase in ROS in cancer cells can lead to tumor cell death [71]. Bacteria can be used to elevate the level of ROS in cancer cells and kill them. The application of bacteria as therapeutic agents against solid

tumors also has been studied and tested. Facultative anaerobic bacteria seem to be able to invade and multiply in the hypoxic regions of solid tumors. As a result, they can slow down the growth rate of tumors or cause them to regress. However, a significant challenge for cancer bacteriotherapy using facultative aerobic-anaerobic bacteria is to prevent typical tissue damage. Consequently, the virulence of the bacteria has to be sufficiently reduced for medical utilizations [72].

As mentioned, the live, attenuated, killed, and genetically modified bacteria can be exploited as vectors to deliver conventional drugs, anti-angiogenic genes, and tumor-killing molecules. In the following section, the most important bacterial species used for therapeutic aims are discussed.

Salmonella typhimurium

Extensive current research has shown that an altered strain of *S. typhimurium* can be applied to target tissues as a carrier for drug delivery. It can also be used as a gadget to make genomic alterations in genes that encode tumor-suppressing proteins through gene therapy. Scientists have designed genetically modified bacteria using *S. typhimurium* that were used for cancer therapy. Two genes (i.e., *msbB* and *purI*) were deleted entirely from the wild strain in the modified bacteria. This deletion was aimed at ultimately weakening these bacteria to prevent the occurrence of disease or toxic shock in the host or experimental animals. The *purI* gene deletion increases the bacteria's demand for external sources of adenine. The *msbB* gene was the second gene to be removed, aiming to decrease *S. typhimurium* toxicity by reducing nitric oxide and proinflammatory cytokine (such as TNF- α) synthesis [73]. Deleting these two genes has effectively attenuated this bacterium by making it dependent on the exterior depots of purine nucleotides for endurance. On the one hand, this dependency prevents the microorganism from germinating and multiplying in the healthy tissues of the host body, such as the liver or spleen, but on the other hand, it enables it to grow in tumors in which purine nucleotides are abundant for bacteria. This bacterial vector displayed a long-term impact against many induced tumors and could kill metastatic cancer cells [74].

Such strains, which are biologically stable, demonstrated no resistance to antibiotics. Thus, they can be used to prevent cancer cell proliferation. A weakened form of *S. typhimurium* has favorably passed phase I clinical trials [75] and has been tested in other experiments [76]. The bacteria have also been utilized to deliver the genes that encode cytosine deaminase to *E. coli*. It has been observed that strains of *S. typhimurium* are significantly effective in collapsing tumor structures. Diverse cancer cell lines such as human pancreatic cancer (ASPC-1) and colon cancer cell lines (including C38, WiDr, and CT26) have been observed to be damaged by altered *S. typhimurium* [77]. In another study conducted by Daniel Saltzman [47], a recombinant attenuated *S. typhimurium* called Saltikva

was used for cancer treatment. It is reported that Saltikva contains the human IL-2 gene, which causes robust antitumor responses by expressing this gene in the tumor microenvironment [47].

Listeria monocytogenes

Listeria monocytogenes synthesizes listeriolysin O (LLO). LLO is one of the virulence factors of this bacterium that can cause bacterial survival by preventing phagolysosome formation in the host cells [78, 79]. LLO can also perforate the phagosome membrane to help bacteria escape from phagosomes and enter the intracellular space [79]. This type of bacteria has a broad domain of usages, such as serving as a carrier for vaccine delivery that induces antitumor responses by triggering the host immune system's cells [80, 81]. Various kinds of this antitumor vector are in phases I and II of clinical trials [82, 83]. ADXS31-142 is a recombinant Lm-LLO, invested in and developed by Advaxis Inc. against prostate cancer [83]. *Listeria monocytogenes* is also broadly used for cancer treatment against the Colo205 cell lines (human CRC cell line) [84].

Bifidobacteria

Bifidobacteria can target tumor cells by influencing the host immune responses and exert their anticancer effects by modifying the intestinal microbiota and synthesizing anticancer molecules [85]. *Bifidobacterium adolescentis* SPM0212 has demonstrated antitumor actions against Caco-2 and HT-29 cell lines by increasing TNF- α and nitric oxide synthesis. In contrast, *B. longum* SPM1205 is known for its TNF- α inhibition performance and its ability to interfere and disrupt toxic fecal enzymes such as α -glucuronidase, tryptophanase, α -glucosidase, and urease, which leads to an increment in the chance of gastrointestinal cancer incidence [86]. An in vivo study was conducted by Hyeyoon Kim et al. [87] about the effect of *B. longum* RAPO, as a member species, on the cancer treatment with anti-PD-1 (anti-death protein 1) antibody as an immune checkpoint inhibitor (ICI). This study's results indicated that the combination of immunotherapy with anti-PD-1

agents and bacteriotherapy with *B. longum* RAPO, respectively, can modulate the antitumor immune responses and enhance the effect of anti-PD-1 in treating triple-negative breast cancer (TNBC) [87].

Clostridium

Spores of some *Clostridium* species, as anaerobic bacteria, are naturally absorbed into the hypoxic nuclei of tumors. They are able to target and partially lyse cancerous cells and tumors. As a consequence, the cancerous tissue is no longer able to resist conventional therapies, including chemotherapy. That is why these strains of *Clostridium* bacteria and their spores are indicated to be an appropriate option for combination treatments [88]. The antitumor effect of these bacteria can be increased by making modifications in genetically engineered strains, which cause the expression of prodrug-converting enzymes (PCE). It can also convert an inactive form of prodrugs to active and cytotoxic drugs at the tumor site [89, 90].

Lactic Acid Bacteria

Lactic acid bacteria (briefly identified as LABs) are beneficial microorganisms (also known as probiotics) that help improve the nutrition of foods, balance the microbial population of the gut microbiota, strengthen the immune and intestinal tract, and lower cholesterol ranges in the blood [91]. Although these bacteria are present in many foods, most experiments have been performed to study them in the laboratory and on dairy products [92].

Weissella cibaria

Weissella is a member of LABs isolated from the Lactobacillus family recently identified by DNA analysis techniques. This gram-positive microorganism, which lacks the catalase enzyme, is generally not pathogenic to the human body. Studies show that probiotic microorganisms, including LABs, can exert antitumor, anti-inflammatory, immune-modulating, and cholesterol-lowering functions by exopolysaccharides (EPS) [93].

***Escherichia coli* Nissle**

Escherichia coli and other anaerobic bacteria can multiply in solid tumors and surround their microenvironment, often stopping tumor growth or even destroying and clearing them from the body. *Escherichia coli* Nissle 1917 may be used as a probiotic medicine or diagnostic agent and should be prescribed orally. This bacterium can help physicians distinguish liver metastasis by producing chemical signals that are easily detectable in the urine. These outcomes reveal that bacteriotherapy can be used for the treatment and diagnosis of cancer [92]. Another example of using *Escherichia coli* to affect tumor regression is a study conducted by Sreyan Chowdhury et al. [94]. In the mentioned study, Chowdhury et al. manipulated a non-pathogenic *E. coli* to be programmable lysed in the tumor microenvironment while releasing an encoded nanobody antagonist of CD47. Their experiment outcomes show a remarkable tumor regression, T cell activation, and metastasis prevention [95].

Bacterial Virulence Factor as Anticancer Particles

Virulence is defined as the ability of a microorganism, such as a bacterium, to cause disease and infection in the host. Virulence factors are cellular structures, chemical compounds, or biological molecules synthesized by that organism that help it bind to a niche, survive, replicate, eliminate competitors, or infect the host [96]. Pathogenic bacteria have various factors, including bacterial peptides, bacteriocin compounds, and enzymes or toxins. These compounds occasionally have antitumor properties and can be utilized for medical applications. Other cytotoxic compounds can be consumed to target tumor cells. These substances include phytochemical molecules [97], marine compounds (occasionally produced by marine bacteria), etc. Some marine and bacterial compounds can kill cancer cell lines by triggering specific signaling pathways such as mitogen-activated protein kinase (MAPK) [98].

Table 1 The most important bacterial peptides with antitumor effects and their diverse properties

Peptide name	Biochemical family	Diverse subtypes	Isolated from:	Chemical formula or composition	Effective against:	Molecular weight	Significant notes and details	Ref(s)
Arenamides	Cyclohexadep peptide	A, B, and C	<i>Salinispora arenicola</i>	C ₃₆ H ₅₇ N ₅ O ₇ (A)/ C ₃₄ H ₅₃ N ₅ O ₇ (B)	Various cancer cells: HCT-116(human CRC cell line)	671.9 Da	Suppressing tumor cells by inhibiting synthesis (NO) and prostaglandin E2 (PGE2) (A and B)	[99–101]
Halitoralins	Cyclic peptides	A, B, and C	<i>Halobacillus litoralis</i> YS3106	C ₂₇ H ₄₈ O ₆ N ₆ (A)/ C ₂₃ H ₄₂ O ₄ N ₄ (B and C)	Human gastric tumor cells	575 Da	Blocking activation of tumor necrosis factor (TNF) cleavage pathways Affecting NF-κappaB activity A is a cyclic hexapeptide B and C are cyclic tetrapeptides	[102, 103]
Ioglobomides A and B	Glycolipopptides	A and B	<i>Bacillus licheniformis</i>	C ₃₀ H ₅₃ NO ₁₂ (A)	Human gastric cancer cells, lung cancer cells, and stomach cancer cell lines	619.67 Da	A particular peptide with a unique biochemical mixture, composed of one amino acid, one fatty acid, one carbohydrate, and one succinic	[103, 104]
Lucentamycins	Non-ribosomal peptide	A, B, C, and D	<i>Nocardopsis lucentensis</i> CNR712	C ₂₈ H ₄₂ N ₆ O ₅ (A)/ C ₂₆ H ₄₆ N ₆ O ₅ (C)	Human colon carcinoma cells	542.7 Da	Officially known as 3-methyl-4-ethylideneproline-containing peptides	[105–107]
Mixirins	Cyclic acetate lipopeptides	A, B, and C	<i>Bacterium bacillus</i>	C ₄₈ H ₇₅ N ₁₂ O ₁₄ (A), C ₄₃ H ₆₉ N ₁₂ O ₁₄ (B), C ₄₇ H ₇₃ N ₁₂ O ₁₄ (C)	Human tumor cells (such as HCT-116)	975.56 Da	Investigation of its biological properties shows its anticancer and antitumor effects, but not the antibacterial ones	[109–112]
Urukthapostatins A	Cyclic thiopeptide	One subtype	<i>Bacterium Mechercharimyces asporoharigenens</i> YM11-542	C ₃₄ H ₃₀ N ₈ O ₆ S ₂	Various cell lines such as the MCF-7, HCT-116, A549, DMS114, and NCIH460	710.8 Da	Studies proved its antitumor effect by inhibiting differential cell growth, but did not indicate any telomerase inhibitory or histone deacetylase activity for it	[113, 114]

Table 1 continued

Peptide name	Biochemical family	Diverse subtypes	Isolated from:	Chemical formula or composition	Effective against:	Molecular weight	Significant notes and details	Ref(s)
Entap	Simple peptide	One subtype	<i>Enterococcus sp.</i>	–	Human gastric adenocarcinoma (AGS) cell lines, colorectal adenocarcinoma (HT-29), mammary gland adenocarcinoma (MDA-MB-231), uterine cervix adenocarcinoma (HeLa) and also prostatic carcinoma (22Rv1)	6000 Da	Entap, which has excellent stability in high temperatures, is composed of hydrophobic amino acids; and shows its anticancer impact by interrupting cell proliferation, blocking cell division in G2, and inducing apoptosis	[107, 115, 116]
Pep27	Bacterial peptide	Six subtypes	<i>Streptococcus pneumonia</i>	Composed of 27 amino acids	AML-2, HL-60, Jurkat SNU-60, MCF-7 cancer cell lines	3.3–3.6 kDa	Evidence indicated that Pan-caspase inhibitor (Z-VAD-fmk) or cytochrome c release from mitochondria could not degrade the anticancer effect of Pep27	[117, 118]
Helicobacter pylori Ribosomal protein	Bacterial proteinaceous	Two subtypes (HPRP-A1, HPRP-A2)	<i>Helicobacter pylori</i>	Composed of 15 amino acids (all L- or D- enantiomer monomers)	Human cancer cell lines like HepG2, BGC-823, and SGC-7901	–	Activating of the caspase-3, -8, and -9- an associated mechanism that leads to apoptosis Inhibiting cell growth by stopping the cell cycle at G0/G1 and G2/M and by interfering with mitochondrial metabolisms Increasing the synthesis and concentration of ROS	[118–120]

Bacterial Peptides

Bacterial peptides are substances released from bacteria and can have anticancer properties. Anti-bacterial and anticancer properties of diverse types of bacterial peptides are being studied and identified currently. The most well-known and critical bacterial peptides with antitumor features include Arenamides, Halitoralins, Idoglobomides A and B, Lucen-tamycins, Mixirins, Urukthapelstatin, Entap, Pep27anal2, and *Helicobacter pylori* ribosomal protein. The most important data about these bacterial peptides and their anticancer properties are provided in Table 1.

Bacteriocins

Bacteriocins are known mainly as protein compounds synthesized by different bacteria to prevent other bacterial species' growth or even kill them. These bacterial products include microcins (which have < 20 kDa weight), colicins (which weigh 20 to 90 kDa), and taticians (which are heavier than others). Bacteriocins are divided into four groups. The first type of bacteriocin is called class I, also named lantibiotics. They have a molecular weight < 5 kDa and contain unusual/abnormal amino acids in their structure. Specific instances of this group are nisin, lectin, and miracidian [121]. Class II bacteriocins are the second type of bacteriocins, including heat-resistant peptides with a weight < 10 kDa, divided into three subclasses IIa, IIb, and IIc [122]. Heat-sensitive peptides with a molecular weight > 30 kDa are the third type of bacteriocins. It can disrupt cell membranes' function and structure and is partitioned into subclasses IIIa and IIIb. The fourth group includes proteins that are very high in lipids and carbohydrates [123]. Bovicin HC5, nisin A, pediocins, fermenticin HV6b, colicins, and S2 pyocin are crucial bacteriocins with antitumor properties that can be exploited in cancer therapy. The various characteristics of these bacteriocins have been studied from different perspectives and are summarized in Table 2.

Bacterial Toxins

Bacteria can synthesize toxins that can cause infections and disease by directly damaging host tissues and organs, weakening or disabling the immune system, or reducing a person's resistance to pathogens. By apoptosis induction and making modifications in the human cell division cycle and differentiation, these bacterial substances can disrupt the growth and expansion of host cells so that they can interpret cancerous cell mechanisms. Furthermore, various toxins (which are synthesized by bacteria) are able to act against various cancer cells in the human body, some of which are described below.

Diphtheria Toxin

Corynebacterium diphtheria produces a specific two-component toxin with a 60-kDa molecular weight consisting of 538 amino acids known as diphtheria toxin (DT). DT-coding gene can be transmitted to *C. diphtheria* via *Bacteriophage B*. DT contains two subunits, namely the (1) beta subunit, which can attach to the receptors of host cells and transmit the alpha subunit, and (2) alpha subunit, which interferes with the cellular function. Alpha subunits are responsible for ADP-ribosylation of cytoplasmic elongation factor 2 (EF-2), which ultimately causes the cessation of protein assembly and cell death [141]. Cross-reactive material 197 (CRM197) is an alerted and non-toxic type of DT that acts through various pathways such as inhibiting tumor metastasis and proliferation, reducing angiogenesis in the tumor site, inducing apoptosis, and acting as an immunological additive. Inhibition of heparin-binding epidermal growth factor has anticancer effects and exhibits antitumor properties. In addition, when CRM197 is used concomitantly with conventional chemotherapy drugs such as doxorubicin, this alerted form of DT reduces this drug's side effects and enhances its cytotoxic effect [142]. DTAT is another example of the other forms and molecules of DT toxin that have been chemically modified. This DT-based immunotoxin can target the tumor's vascular endothelium, causing cancerous tissue to regress and

not grow in mice. DTAT is one of the modified forms of this DT toxin. Diphtheria toxin has shown toxicity against various human cancer cell lines as well as gastrointestinal cancer cells, including cortical adrenal carcinoma cell lines, colorectal cancer cell lines SW480, SW620, HCT116, CaCo-2, and HT-29 [143].

Toxins of *Clostridium difficile*

Clostridium difficile synthesizes distinct toxins, and two prominent ones are called cytotoxins (TcdB) and enterotoxins. (TcdA) [144]. TcdB demonstrates its anticancer activity by inducing the synthesis of proinflammatory chemokines and cytokines, inhibiting the reproduction of tumor cells, and inducing necrosis as well as apoptosis [144]. Furthermore, evidence from laboratory studies indicates that TcdB has remarkable immunogenicity features; it also promotes long-term antitumor immunity to various cancer cell lines and CT26 colon cancer cell lines and has the potential to be utilized as a vaccine to prevent or as antitumor drugs or immunotherapeutic agents to treat cancers and tumor cells [145].

Verotoxin 1

Verotoxin 1 (VT-1), mostly recognized as Shiga toxin-1 (Stx-1), is produced by members of the *Enterobacteriaceae* family, such as *E. coli*—which has VT and causes hemolytic uremic syndrome (HUS)—*E. coli* enterohemorrhagic (EHEC), and *Shigella* [146]. VT-1 inhibits protein synthesis and cell growth and interferes with the S phase of the cell cycle. It has a membrane receptor called Globotriaosylceramide (Gb3), expressed in a broad range of MDR human cancer cell lines. VT-1 has shown antitumor activity and can stop the cell cycle in the HCT116 cell line of colon cancer [147].

Exotoxin A

This toxin is synthesized by *Pseudomonas aeruginosa*, having 66 kDa molecular mass. Exotoxin A is a toxin that has an ADB-ribosylation function and inhibits protein elongation factor-2 (EF-2) activity. This toxin inhibits protein synthesis, causing apoptosis in the cancerous cells [148]. Exotoxin A, in its

immunomodulatory phase, in combination with human epidermal growth factor (EGF) and interleukin-4 (IL-4), has demonstrated potent antitumor activity in PaCa-2 cell lines of pancreatic cancer [149].

Antitumor Enzymes with Bacterial Origin

Arginine deiminase-I (ADI) is a bacterial enzyme with about 46 kDa molecular weight synthesized by *Mycoplasma hominis* or *M. arginine*. Cutting and converting arginine to citrulline, which terminally results in ammonium release, is metabolized by this enzyme [149]. ADI-PEG20 is the name of arginine deaminase, which is conjugated to polyethylene glycol (PEG) polymers. It exerts its anticancer effect by inducing caspase-independent apoptosis, reducing tumor cells' physiological metabolism, inhibiting tumor cell growth and reproduction, and inducing autophagy [149]. It has led to the utilization of arginine deiminase as an anticancer agent, and its related clinical trials are in clinical phase II. This enzyme demonstrates excellent cancer treatment potential in different cancerous cell lines and liver carcinoma cell lines [150].

Utilizing Bacteria for Cancer Therapy by Gene Delivery

One of the significant challenges in utilizing bacteria as anticancer devices has been balancing the toxicity with the dose required for their effectiveness. These bacteria may be highly toxic to the host at high doses. On the other hand, a major barrier to gene therapy cancer treatment is specifically targeting solid tumors. One way to obviate these restrictions is to apply bacteria in a genetically modified manner to express specific proteins when used as the vectors for gene therapy [151]. As previously stated, the bacteria can contribute to the gene therapy process by carrying a specific gene and inducing a target protein synthesis in the cancer cells' environment. These bacterial carriers can be an effective and remedial adjuvant for a variety of cancer therapy methods. Chemotherapeutic compounds, cytotoxic peptides, remedial

proteins, prodrug-to-drug-converting enzymes in solid tumors, and even coding and non-coding gene delivery can all be delivered using bacteria [152]. Various ways by which bacteria can directly or indirectly have an impact on and kill tumor tissues and cancerous cells are illustrated in Fig. 6.

Available Clinical Data and Trials on the Efficacy of Bacteria-Based Methods for Cancer Therapy

Although promising outcomes in various situations have accompanied the utilization of bacteria-based methods, unfortunately, these innovative therapies have not been used widely in the clinical oncology [42]. As Shiyu Song et al. [42] stated, while the high potential of bacteria-based methods for solid tumors has been known in the last decades, the principal possible reason for not using bacteria, these “double-edged swords,” may be their adverse and unmanageable side effects. As mentioned before, the BCG vaccine—which is the attenuated form of *M. bovis*—is the only bacteria-based agent authorized by the US Food and Drug Administration (US-FDA) and is widely employed as the standard of care for the treatment of patients suffering from the NMIBC [153, 154]. However, the available clinical data have demonstrated that BCG vaccine treatment in 30–50% of the NMIBC patients fails to result in discernable and positive outcomes [155]. Moreover, 5% of the NMIBC patients who received BCG encountered adverse effects such as tissue sepsis and infections [155].

While available clinical data on bacteriotherapy seem to be limited, recently, there has been great enthusiasm in the scientific community resulting in various pre-clinical and clinical trials on bacteria-based methods. For example, intratumoral administration of *C. novyi* spores has been utilized to treat one patient suffering from advanced leiomyosarcoma, and the results were remarkably efficient [7]. Furthermore, the usage of attenuated *L. monocytogenes* species for treating patients with advanced cancer has been reported to be safe and effective [156, 157]. A phase I clinical trial

reported by Wood et al. [158] on the expression of HPV16 E7 (as an oncoprotein) by attenuated *L. monocytogenes* has shown a 30% tumor reduction in overall survival. There were also some common side effects, such as flu-like symptoms, in the patients [158]. As described above, there were also some pre-clinical studies on bacteria-based methods. A pre-clinical study [159, 160] on commensal Bifidobacterium was conducted to evaluate the efficacy of selectively expressed CD in hypoxic mammary cancerous tissue in rats and converting the rate of 5-FC into 5-FU [161, 162]. In the following section, recent clinical trials on bacteria-based methods and their outcomes in the last 10 years are briefly discussed in Table 3.

Bacteriotherapy: Risks and Challenges

While radiotherapy and chemotherapy are well known as the foundations of cancer treatment, their outcomes are associated with some ups and downs in cancer patients [163]. As a result of previous inadequate and restricted anticancer therapies, mediated cancer treatment has drawn scientists' attention. Bacteriotherapy for cancer treatment can be considered a novel treatment strategy with fewer side effects if applied correctly and can be utilized alone or as a booster with typical therapeutic methods. Bacteriotherapy, like other therapeutic approaches, has several advantages and disadvantages. One important point related to cancer therapy utilizing bacteria is that bacteria's pathogenicity leads to infection or even death in patients. Many studies have exploited weakened, alerted, or genetically manipulated species to eliminate these restrictions. Another downside of bacteriotherapy is the short half-life of bacterial peptides and proteins and unstable-mutable DNA [164]. Various investigations have proved that using genetic engineering techniques to improve bactericidal agents' effectiveness by research can enhance antitumor effects of the bacteriotherapy method. For example, in some research, scientists have consumed biochemical changes and reactions such as D-amino acid replacement,

Table 2 Some of the reputable bacteriocins in anticancer treatments and their remarkable properties

Name	Bacteriocin family	Biochemical classification	Origin	Composed of:	Molecular weight	Cancer cell lines	Significant details	Ref(s)
Bovicin HC5	Class I bacteriocins	Lantibiotic	<i>Streptococcus bovis</i>	22 amino acids	2.4 kDa	Vero, MCF-7, and HepG2 cancer cell lines	Killing target cells by interrupting the function of cell membrane via forming pores and holes which affect potassium flow and its entering/exiting	[124, 125]
Nisin A	Class I bacteriocins	Anti-bacterial polycyclic peptide toxin	<i>Lactococcus lactis</i>	34 amino acids	3.3 kDa	Astrocytoma cell line (SW1088), (AGS and KYSE-30), hepatic (HepG2), and blood (K562) cancer cell lines	It exerts its antitumor and anti-metastasis influence by destabilizing cell membranes, suppressing tumor cell growth, altering cell membrane, pore deformation, and elevating ion penetration which interferes with phospholipid arrangement	[107, 126–129]

Table 2 continued

Name	Bacteriocin family	Biochemical classification	Origin	Composed of:	Molecular weight	Cancer cell lines	Significant details	Ref(s)
Pediocins	Class IIa bacteriocins	Proteinaceous antimicrobials	<i>Bacterium pediococcus acidilactici</i> <i>MTCC 5101</i>	44 amino acids	4.6 kDa	Various cancer cells and gastrointestinal tumors, including rat splenic cancer lymphoblast cells (Sp2/O-Ag14) and HepG2	Preventing the cell division of tumor cells by disrupting the cell division cycle	[130–132]
Fermenticin HV6b	Class IIa bacteriocin	Antimicrobial peptide	<i>Bacterium Lactobacillus fermentum HV6b</i> <i>MTCC 10,770</i>	–	6.7 kDa	Spleen lymphoblast cell line (Sp2/0-Ag14 ATCC-CRL-1581), hepatocarcinoma cell line (HepG2)	It demonstrates its antitumor activity by induction of apoptosis in vascular endothelial cells, cell contraction, and DNA breakdown	[107, 133, 134]
Colicins	Class III bacteriocins	Pore-forming proteins (PFPs)	<i>Escherichia coli</i>	Colicin Z (151 aminoacids)	40 to 80 kDa	Human uterine carcinoma cell line, MCF7 line, cancer cell line HST913T, HT29 (a human colon cancer cell line)	Changing the distribution of electric charge in the target cell (which leads to its death) Non-specific DNase activity RNase activity	[107, 135–137]

Table 2 continued

Name	Bacteriocin family	Biochemical classification	Origin	Composed of:	Molecular weight	Cancer cell lines	Significant details	Ref(s)
S2 Pyocin	Class III bacteriocins	S-type bacteriocins	<i>Pseudomonas aeruginosa</i> 42A	777 amino acids	73.9 kDa	Hepatocellular carcinoma (HepG2), multiple myeloma (Im9), cervical adenocarcinoma (HeLa), embryonal ovary carcinoma (AS-II)	It shows its anticancer effect by blocking cell lipid synthesis and disrupting the DNA replication	[138–140]

unstable amino acid replacement, etc., to increase the bactericidal agent's shelf-life and stability [165].

As mentioned, a tremendous obstacle to applying bacteria-derived medications as anti-cancer compounds is their low cytotoxicity at the dose required for therapeutic efficacy. Furthermore, systemic bacterial infections can be a significant risk factor for living organisms. Besides, even deletion of genes encoding the toxin and virulence factors could result in the death of about 15–45% in test mice [166]. Another significant challenge to applying bacteria for cancer treatment is incomplete tumor lysis. The bacteria do not lyse any part of the cancerous tissue and may not effectively eradicate it. Therefore, the administration of concomitant therapies such as chemotherapy plus bacteriotherapy is essential to achieve the desired outcomes. A more difficult obstacle with bacteriotherapy is that it is challenging to treat small non-necrotic tumors from the metastasis of large tumors (the leading cause of cancer death) with this method. Due to the physiological problems and communication knots with hypoxia in these cancerous tissues, it is difficult for bacteria to target these issues accurately. In bacterial therapy based on live bacteria, the main problem is the lack of accurate access of these bacteria to tumors since, in most cases, an intra-tumor infusion is required [167]. Another primary risk of bacterial immunotherapy is the chance for modifications in nucleic acids and DNA mutations. Suppose the host body's bacterial DNA has a mutation or its genetic material changes through nucleic acid modification. This can lead to the bacterium losing its original anticancer function because of the conversion. It can prelude various problems such as failure to treat tumors or severe bacterial infections in the host organs. Although some concerns about treating cancer with bacterial therapy have been addressed using recombinant DNA technology, more research, studies, and experiments are warranted [168]. A summary of the pros and cons of applying bacteriotherapy for treating cancer is given in Fig. 7.

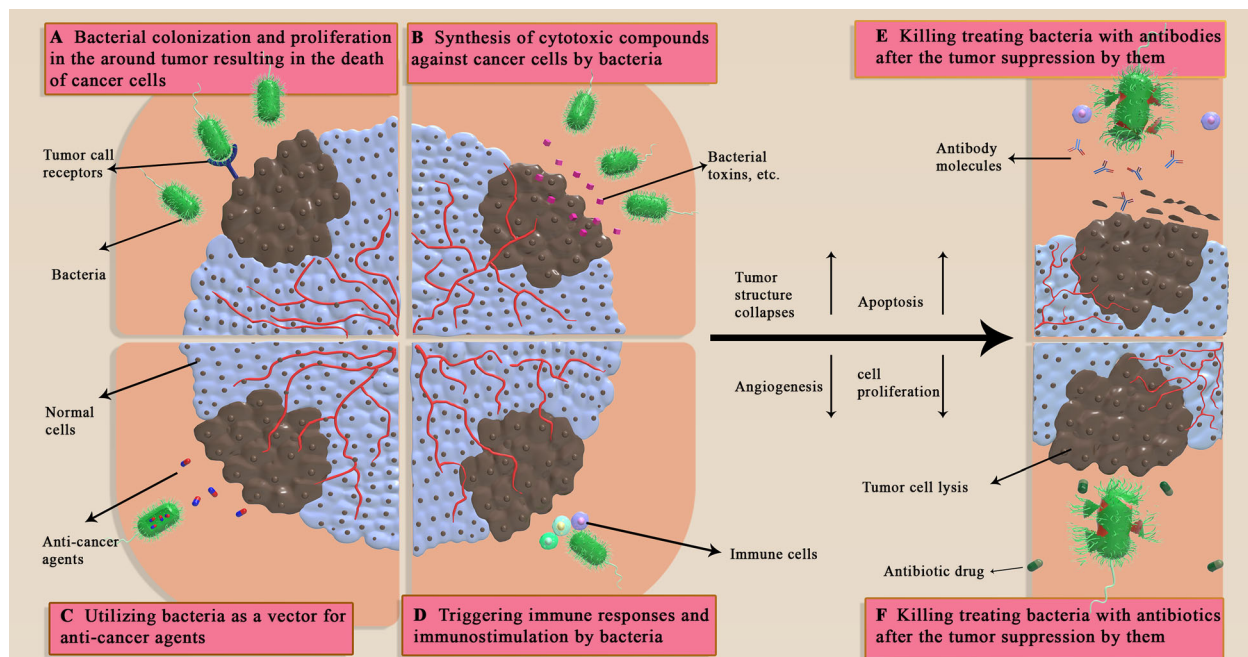


Fig. 6 Bacteria or their chemical derivations can have anticancer effects. Colonized bacteria consume available oxygen and nutrients and may result in the cancerous cells’ death (A). Bacteria can also synthesize various chemical compounds that may have antitumor effects (B); they have the potential to be used as a carrier for anticancer agents

(C). Stimulation of the immune system by bacteria can result in immunotherapeutic effects (D). After killing tumor cells, used bacteria can be eliminated to prevent unintended bacterial infections. Bacteria are mainly cleared by antibiotics (E) and immune cells (F)

CONCLUSION

Bacteriotherapy has been posited and considered to be one of the most advanced methods that can be used for cancer therapy. The ability to replicate in the body and having no requirement for repetitive injections as well as specificity in exerting cytotoxic impacts on cancer cells, and the possibility of genetic modification to eliminate virulence factors, etc., are among the crucial attributes of bacteria that make them an appropriate candidate for cancer treatment. Like any other method, bacteriotherapy faces some challenges and risks. The possibility of pathogenicity of bacteria against healthy cells, their potential to have excessive and uncontrollable growth, their inability to lyse necrotic cells completely, and the lack of symmetrical growth in all tumor tissues are some of these challenges. Despite in vitro and

in vivo studies, this method is still not widely utilized clinically, and its application against tumors has been associated with some concerns.

Thus, further investigations are essential to increase our knowledge in this area and to determine the advantages and disadvantages of bacteriotherapy. Moreover, reviewing all the research achievements can help scientists reach a consensus and make this method more efficient in future applications. Further investigations are essential to increase knowledge in this area and determine the advantages and disadvantages of bacteria-based strategies for cancer treatment.

Table 3 Information of the recent clinical trials on bacteria-based methods and their outcomes in the last 10 years

Study first post ^a	Bacteria name	Cancer type	Enrolled participants	Primary outcome measures	Clinical trial phase	Current status	ClinicalTrials.gov identifier (references ^b)
March 26, 2012	<i>Bifidobacterium longum</i> (recombinant APS001F)	Solid tumors	75 participants (estimated enrollment)	Adverse events as a measure of safety and tolerability	I	Suspended	NCT01562626
August 30, 2012	<i>Listeria monocytogenes</i> (recombinant CRS-207)	Malignant pleural mesothelioma	60 participants (actual enrollment)	Number of subjects reporting adverse events	I	Completed	NCT01675765
December 25, 2014	<i>Listeria monocytogenes</i> (recombinant ADXS31-142)	Metastatic castration-resistant prostate cancer	51 participants (actual enrollment)	Adverse events in each dose level	I and II	Active, not recruiting	NCT02325557
August 3, 2016	<i>Listeria monocytogenes</i> (recombinant ADXS31-142)	Advanced cervical cancer with high risk	450 participants (estimated enrollment)	Disease free survival (DFS)	III	Active, not recruiting	NCT02853604
December 30, 2016	<i>Listeria monocytogenes</i> (recombinant CRS-207)	Metastatic pancreatic cancer	44 participants (estimated enrollment)	Recommended dose of epacadostat and 6-month survival	II	Recruiting	NCT03006302
June 16, 2017	<i>Listeria monocytogenes</i> (recombinant CRS-207)	Pancreatic cancer	63 participants (estimated enrollment)	Objective response rate (ORR)	II	Recruiting	NCT03190265
November 21, 2018	Attenuated <i>Salmonella typhi</i> Ty21a (recombinant VXM01)	Progressive glioblastoma	30 participants (estimated enrollment)	Treatment-emerging adverse events	I	Recruiting	NCT03750071
December 3, 2018	<i>Salmonella</i> CYD908:ssb (recombinant TXSVN)	Multiple myeloma	24 participants (estimated enrollment)	Number of patients with dose-limiting toxicity (DLT)	I	Recruiting	NCT03762291

Table 3 continued

Study first post ^a	Bacteria name	Cancer type	Enrolled participants	Primary outcome measures	Clinical trial phase	Current status	ClinicalTrials.gov identifier (references ^b)
February 20, 2019	Live attenuated <i>L. monocytogenes</i> (recombinant ADXS 503)	Non-small cell lung cancer (NSCLC)	74 participants (estimated enrollment)	Safety/tolerability of ADXS-503 monotherapy	I	Recruiting	NCT03847519
July 18, 2019	Live, genetically modified <i>Bifidobacterium longum</i> (bacTRL-IL-12)	Solid tumors	5 participants (actual enrollment)	Incidence and severity of adverse events	I	Terminated (sponsor decision)	NCT04025307
August 8, 2019	Attenuated <i>S. enterica</i>	Relapsed neuroblastoma	12 participants (estimated enrollment)	Adverse events, immune response; minimal residual disease (MRD)	Early phase I	Recruiting	NCT04049864
November 18, 2019	<i>Escherichia coli</i> Nissle 1917 (recombinant SYNBI891)	Metastatic solid neoplasm and lymphoma	70 participants (estimated enrollment)	Incidence of dose-limiting toxicities (DLTs)	I	Recruiting	NCT04167137

^aFirst availability date of study record at <https://www.clinicaltrials.gov/>

^bRegistration number of the clinical trial. For reference, see the number at <https://www.clinicaltrials.gov/>

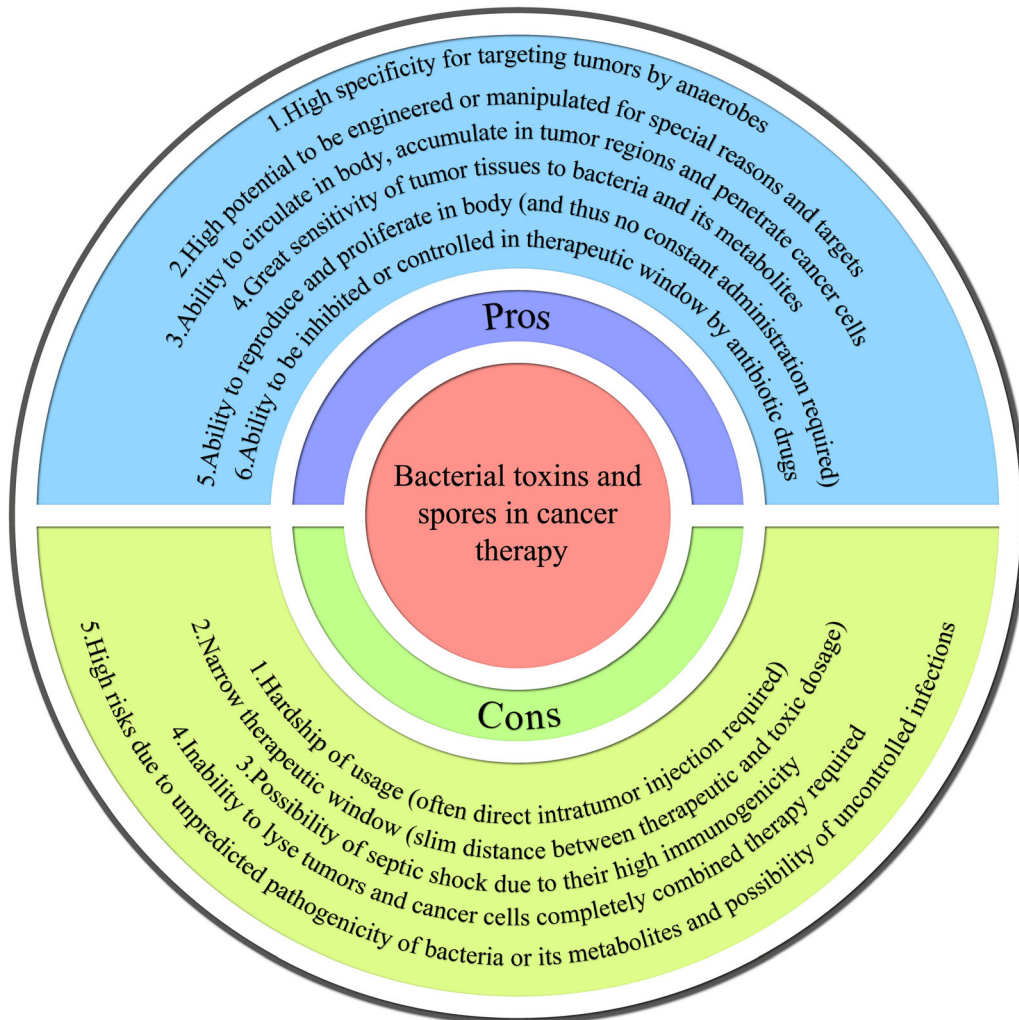


Fig. 7 Opportunities and challenges of bacteriotherapy for cancer treatment: the utilization of bacteria for cancer therapy should be comprehensively viewed, and its advantages and disadvantages must be considered

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