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# BACTERIA BASED CANCER THERAPY: A PROMISING FRONTIER IN ONCOLOGY

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**Abstract**

With a focus on its mechanisms, historical development, and potential advantages over more traditional treatments like radiation and chemotherapy, this article provides a comprehensive analysis of bacteria-based cancer treatment. It discusses the various pathogenic and non-pathogenic bacteria's components and engineered modifications that are employed in anticancer strategies. The study emphasizes the immunomodulatory and targeted delivery potential of bacterial therapies in addition to current concerns like toxicity and dosage control. With future prospects focused on genetic engineering and combination therapies to improve efficacy and safety, bacterial-based approaches are positioned as promising complementary or alternative options in the treatment of cancer.

**Keywords:** Cancer, bacteriotherapy, chemotherapy, radiotherapy, tumor targeting bacteria, cancer immunotherapy.

## 1. Introduction

Abnormal cells that proliferate uncontrollably and have the capacity to penetrate healthy body tissue are the hallmark of a disease called cancer. Humbly acknowledged that cancer ranks as the second leading cause of death worldwide, as reported by the WHO. According to estimates, cancer claims the lives of at least 9 million people annually. (1)

Malignancy is a major global general health concern and the 2nd leading reason for death in the United States (US). This complicated leading cause of death is associated with lifespan, gender, caste, and culture. According to a report published in January 2023 by the Cancer Society of America, there will be 1,958,310 new cases of malignancy and 609,820 deaths in the US. Every day, about 1670 people pass away from cancer; the most frequent reason of death for male are lung, prostate gland, & colon carcinoma while the most frequent reasons for female are lung, breast, & colon carcinoma. (2)

By 2030, malignancy is predicted to claim the lives of 20 million people worldwide. Cancer is a dangerous disease that has claimed many lives. It is exceedingly difficult to treat cancer in a way that causes more harm since chemotherapy kills healthy cells, and those cells eventually develop drug resistance. Drug resistance often occurs, which lowers chemotherapy's initial effectiveness and eventually leads to inadequate tumor control. (3)

## 2. Why To Use Bacterial Treatment Over Other Well Known Bacterial Therapies

Since Dr. William Coley's initial attempt to use bacterial products as immunotherapy nearly a century ago, using live, attenuated bacteria has emerged as a viable cancer treatment alternative. In recent years, research has mostly concentrated on biochemical and molecular approaches of controlling bacteria to fight cancer due to technical developments and our ability to reduce dangerous strains. Certain cancer-targeting treatments are enhanced, and side effects are decreased by drugs that bacteria deliver directly to the tumor site. As an alternative, bacteria can be employed to produce pharmaceuticals inside tumor cells.

Even though we now have a much better understanding of cancer-specific treatments, it is still possible to find good targets for cancer therapies, mainly by manipulating well-known microorganisms. Many bacterial species have shown promise in using antitumor immune response in preclinical and clinical studies by inducing innate and adaptive immunity , which increases the chance of neoplasm removal without any other side effects.

Human phase I and II clinical study have recently employed a variety of bacterial treatments. This article discusses the benefits and current challenges of using bacterial-mediated drugs and delivery systems as anti-cancer treatments, looking at recent advancements in this field. (4)

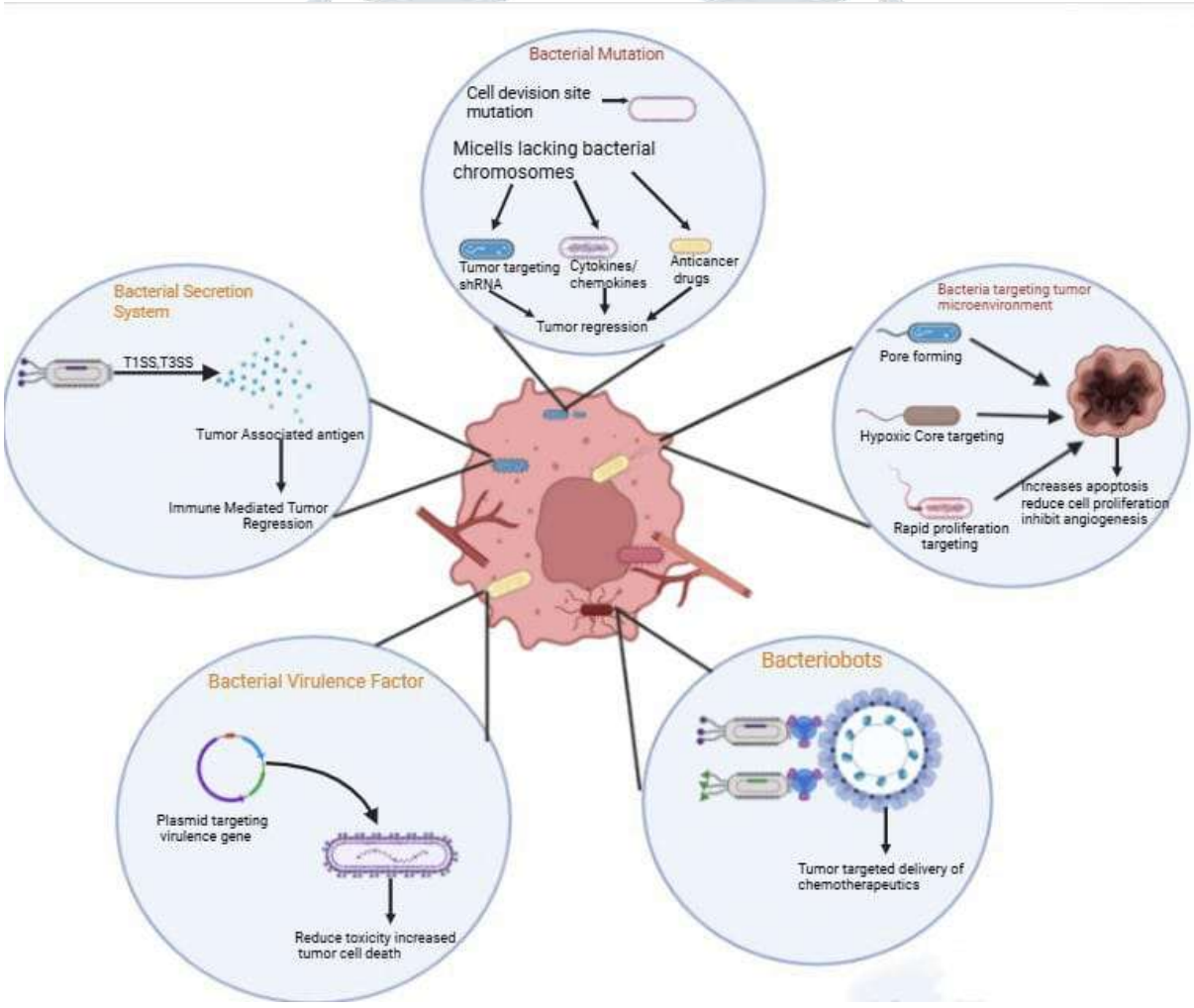


**Fig 1**. Schematic summary of the various bacterial mechanisms utilized in BBCT (4)

By giving therapeutic bacteria particular characteristics, a new class of cancer immunotherapies will be created. Surgery might not be able to completely remove tumors, which could lead to recurrence, and traditional treatments like radiation and chemotherapy can have systemic toxicities. There are still unmet needs because immunotherapies have a non-localized therapeutic effect and are not effective against immunosuppressive tumors.

The future generation of tailored bacterial therapeutics can elicit immune responses in malignancies that evade the immune system in addition to directly depositing medications in tumors. Through a variety of methods, such

as the administration of cytokines, short-hairpin ribonucleic acid (shRNA), and Tumor-associated antigens (TAAs), bacterial treatment have been created to boost the host defence mechanism. TAA administration by bacteria promotes the growth of cytotoxic T-lymphocytes, which specific myeloma cells precisely and produce memory T cells to stop recurrence. It has been feasible to create transgenic bacteria that both stimulate cytotoxic immune cells and repress immunosuppressive cells by producing cytokines and TAAs. Bacterial immunotherapies can treat cancers that are resistant to conventional treatments and prevent them from returning by altering these pathways.

The two species most commonly employed in the field of bacterial immunotherapies are Salmonella enterica serovar typhimurium (henceforth referred to as Salmonella) and Listeria monocytogenes (Listeria).

Salmonella has been shown to grow 10,000 times more in tumors than in healthy tissue, and Listeria has been shown to grow strongly in metastases over healthy tissue. The tumor microenvironment's reduced immune activity protects the bacteria after tumor invasion, making it difficult to eradicate them and allowing them to grow unchecked.

**3. Historical Development in This Field**

The first observations about the role of bacteria in cancer were made approximately 150 years ago. W. Busch and F. Fehleisen, two physicians from Germany at the time, independently observed that cancer symptoms were less severe in patients who had inadvertently contracted erysipelas. Being the first physician to observe this phenomenon, Busch inoculated a cancer patient with erysipelas-causing bacteria and monitored him for some time. Serratia marcescens is a bacterial mixture of S. pyogenes and B. prodigiosus that was developed in 1893 by a physician named William Coley in New York City. By the lytic substances produced by this bacterium, he produced a material he named Coley toxin.

The BCG vaccine is arguably the most well-known bacterial agent used to treat malignancy. The Mycobacterium tuberculosis (MTB) is the reason of the infectious disease tuberculosis (TB), which typically influence the lungs but can also damage other body organs. The Bacillus Calmette-Guerin (BCG) vaccine bears the names of its inventors [(5)].

Microbial treatments were among the initial biotherapy. In the late 1800s, Dr. William B. Coley of the Cancer Hospital of New York City (now Memorial Sloan Kettering Cancer Centre) made the discovery that cancer patients regressed due to bacterial infections. Streptococcus pyogenes injections, which he started using to treat cancer patients, caused inoperable tumors to shrink. In the end, he created what are known as Coley's Toxins by combining thermally deactivated S. pyogenes and Bacillus marcescens. Even though his trials were finally stopped, Helen Coley Nauts daughter of Coley gathered the data related to impact on peoples cured with Coley's Toxins and displayed that they were comparable to those of advanced drug therapy for malignancy. Coley is now recognized as the "father of immunotherapy of malignancy" because bacterial treatments were among the first successful immunotherapies ever documented.

One of the major accomplishments was the finding that bladder cancer may be treated with Bacillus Calmette-Guérin (BCG). It was first invented as a TB vaccination using a weakened strain of M. bovis. After promising animal studies. It was first studied as a therapy for bladder cancer in the 1970s. This patient produced anti-cancer immunoglobulins and lymphocytes that were toxic to carcinoma cells following a BCG injection, leading to long-term tumor immunity. The FDA authorized the intravesical administration of BCG in 1990 following additional clinical trials. Because of its effectiveness, BCG therapy remains the treatment protocols of care for non-muscle invensive bladder cancer. It was the first bacterial immunotherapy to be approved for use in clinical settings.

The No. of articles on bacterial treatments has enhanced dramatically since the 1990s, when the first ones addressed the use of Listeria and Salmonella as cancer treatments. The main reason for this increase has been evidence of using bioengineering to transform microorganisms into transport systems for cytotoxic and anticancer

medicines. The branch of bacterial bacterial therapy has advanced well beyond Coley's most hopeful projections because of developments in genetic modification and our knowledge of the immune response (6).

## 4. Types of bacteria

There are two types of bacteria one which causes malignancy (cancer) and other which are used to cure cancer by eliminating tumor.

Followings are the bacteria which causes cancer to human: -

**Table 1**. Experimental evidence for a link between bacteria and cancer

| Types of bacteria | Types of cancer | Reference |
|---|---|---|
| H. pylori | Non-cardia gastric carcinoma | (7) |
| S. typhi | Gallbladder Carcinoma | (7) |
| S. enteritids | Colon Carcinoma | (7) |
| Chlamydia Trachomatis | Carcinoma of cervix and ovaries | (7) |
| Bacteroid Fragilis | Colorectal Cancer | (8) |
| Fusobacterium Spp | Colorectal Cancer | (8) |
| Clostridia Spp | Colorectal Cancer | (8) |
| Streptococcus bovis | Colon Cancer | (9) |
| Chlamydia pneumoniae | Lung Carcinoma | (9) |
| Proteobacteria | Breast Cancer | (10) |
| Streptococcus Gallolyticus | Colorectal Cancer | (10) |
| Diaphorobacter | Lung Cancer | (10) |

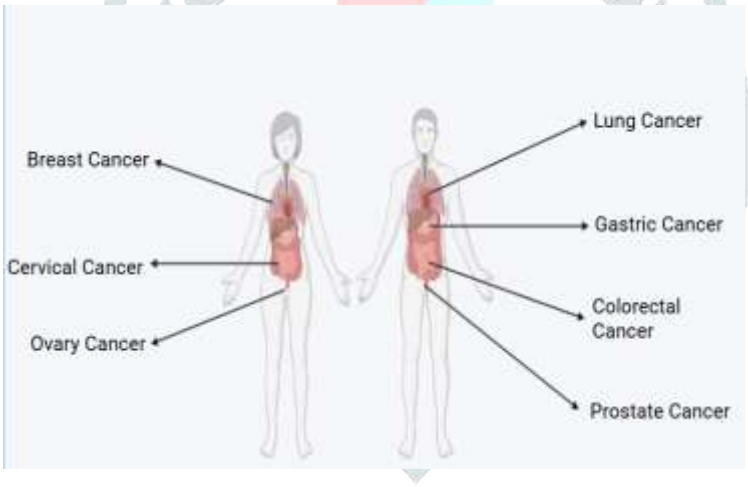


**Fig 2:** The role of bacterial infections in various forms of cancer (10)

## 5. Bacterial Elements of Antitumor Therapy

Bacterial toxins that are capable of effectively inhibiting cancer proliferation through mechanisms such as cell-cycle arrest, disruption of tumor-cell signalling pathways, and alternative modes of action include the Coley toxin, the measles toxin, Clostridium perfringens enterotoxin, bacterial enzymes such as L-asparaginase and arginine deaminase, as well as biosurfactants like surface-active and prodigiosin-like compounds. Furthermore, the immune system can be specifically activated to eradicate tumor cells by engaging with the outer surface, membrane, wall, and biofilm structures of bacteria.

### 5.1 Bacterial Toxins

The area of bacterial cancer treatment began in 1891 when Dr. Coley used a combination of living microbes and thermally deactivated S. pyogenes and Bacillus mirabilis bacteria, sometimes known as "Coley toxin," to successfully cure cancer patients. The 2 subclasses of cytotoxin (TcdB) and enterotoxin (TadA) found in

Clostridium difficile toxin can kill myeloma cells by inducing an immunological response by recruiting proinflammatory factors. In addition to its anticancer properties, enterotoxin from Clostridium perfringens attached to the upreregulating claudin-4 receptor on adenocarcinoma cells, causing dose-dependent severe intoxication.

### 5.2 Bacterial Enzymes

As well, Fiedler et al. showed that arginine deaminase produced by Streptococcus pyogenes, which can consume arginine in myeloma cells and inhibit the growth of arginine-deficient tumor glioblastoma multiform. The bacterial enzyme L-asparaginase, which is developed by Escherichia coli, is a potent cancer treatment that can stop the growth of cancerous cells by hydrolysing asparagine and lowering its blood levels.

### 5.3 Biosurfactant

Bacillus subtilis natto TK-1 produces cyclic lipopeptide, a biological surfactant with broad bactericidal and anticancer activities. By causing apoptosis and raising the ion calcium concentration in the protoplasm, cyclic lipopeptide was shown by Xiaohong Cao et al. to suppress the growth person's breast carcinoma MCF-7 cells. Epsilon-poly-L-lysine, an L-lysine biopolymer having antibacterial and anticancer characteristics, is also produced by the same genus, Marine Bacillus subtilis sp. It has been shown that epsilon-poly-L-lysine clearly cytotoxically targets the liver myeloma cell HepG2 and the cervical adenocarcinoma cell HeLaS3. Prodigiosin-like fragments had crucial cytotoxic action against the colorectal carcinoma cell line HCT-116, the liver carcinoma cell line HepG-2, and the breast carcinoma cell line MCF-7.

Table 2 shows antitumor agents biological surfactant that inhibit specific processes of cancer progression as well as their impact on the growth of myeloma cells. Biosurfactant applications in microemulsion-based drug formulations seem promising. Biosurfactants are thought to be safe carriers or components of medication delivery systems and are utilized in broad-spectrum anticancer therapies.

**Table 2**. Antitumor biosurfactants that combat malignant cells

| **Biosurfactant** | **Anti-tumor activity** | **Carcinoma Type** |
|---|---|---|
| lipopeptidase | B. subtilis natto TK-1 | Breast carcinoma |
| Surfactin | B. subtilis natto T-2 | Breast carcinoma |
| L-lysine biopolymer Epsilon-poly-L-lysine | Marine B. subtilis sp. | Liver cancer<br>Cervix glandular cancer |
| Viscosin | Pseudomonas libanensis m9-3 | Breast carcinoma |
| AT514 | Serratia marcescens | Chronic B-cell lymphocytic blood cancer |
| BE18591 | Streptomyces sp. | Gastric carcinoma |
| Roseophilin | Streptomyces sp | Blood carcinoma<br>Colorectal cancer cancer |

### 5.4 Extracellular Cell Membrane

Lactobacillus bacteria release carbohydrate molecules called gram-positive exopolysaccharides (EPS), which typically find their way into the culture medium during growth and metabolism. Capsular polysaccharides are polysaccharides that stick to the peptidoglycan to create a capsule. They have a dose-dependent and time-dependent anticancer impact of growth inhibition and increase cell death as part of their anticancer activity. Gram-positive bacteria have an outermost cell surface called the S-layer, which is made of protein and glycoprotein and possesses anticancer properties. According to research, Lactobacillus acidophilus CICC 6074's S-layer protein may be used as a possible anticancer medication.

### 5.5 Bacterial plasma Membrane

Membrane fragments, negative bacteria's extracellular membrane vesicles, and Gram-positive bacteria's cytoplasmic membrane vesicles are examples of bacterial membrane components employed in anticancer therapy. Antigen-presenting cells (APCs) can recognize the bacterial membrane and trigger T cell immune response because it is rich in pathogen-associated molecular patterns (PAMPs). Additionally, it has the ability to bind to and activate the toll-like receptor (TLR), that controls the synthesis of CD40 and other component molecules as well as proinflammatory cytokines like IL-12. Following the Th1-dependent immunity, which is mostly driven by CD8+ effector cells, these mediators produce interferon (IFN), which in turn sets off a potent immunity against myeloma cells in the cancer stroma. Min Li et al. found that neutrophils were able to identify and internalize the PAMP on E. coli OMVs during revascularization. Once OMVs had travelled through the blood arteries, neutrophils directed them to target inflammatory tumors [11].

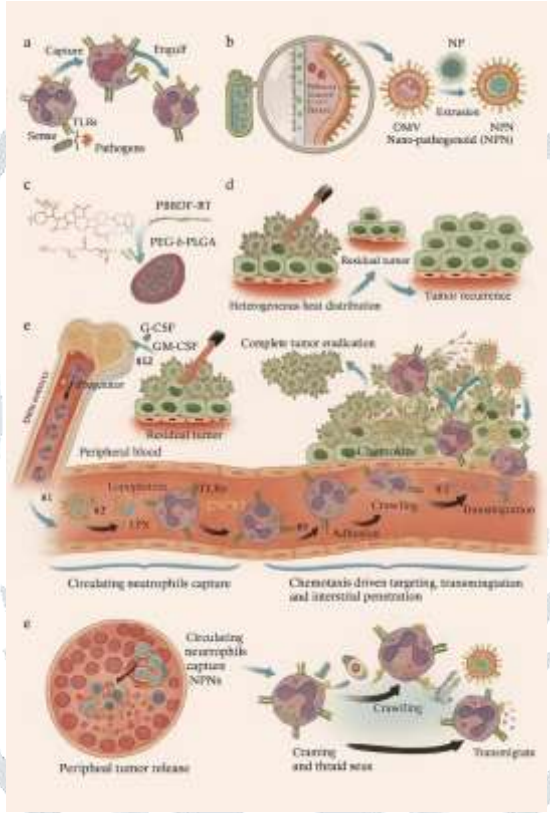


**Fig**. **3** Diagrammatic representation of the chemotaxis-based delivery of NPNs to totally destroy tumors following phototherapy. (a) Neutrophils use toll-like receptors (TLRs) to recognize PAMPs in order to find, capture, and swallow pathogens. (b) OMVs are coated on NPs, which then receive PAMPs from the OMVs to create NPNs. (c) PBIBDF-BT (PBT) encapsulation in PEG-b-PLGA NPs as a photothermal transducer. (d) Laser cancers' restricted penetration, which causes tumor recurrence. (e) Granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), and the chemokines CXCL1 and MIP-2 are produced when treatment-induced cell death occurs in the residual tumor.

### 5.6 Bacterial Ghosts

Bacterial envelopes, or BGs, are produced by gram-negative bacteria and contain PAMPs and other surface elements but don't have the entire cytoplasm and parts of the microorganism. Because the bacterial plasma membrane is totally intact and hasn't been denaturated, BGs can trigger immune responses just like live bacteria. Because of this, BGs are one of the most researched bacterial element-dependent delivery systems for tumor-targeting anticancer vaccines. Nowadays, chemical and genetic engineering are the two most popular methods for producing BGs, as Figure 4 illustrates. Xie et al. suggested using electroporation to transfect phage-derived lysis gene E (EcN). Antibiotics were applied to deactivate the unlysed bacteria after the expression of the EcN-induced lysis gene E was discovered.

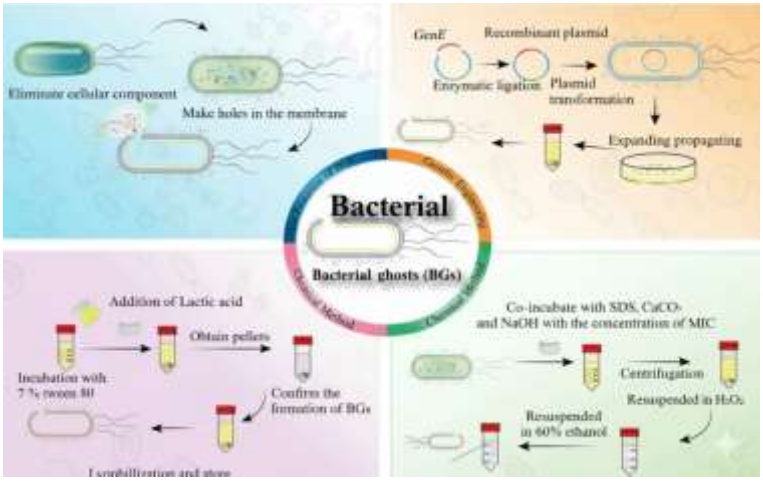


**Fig 4.** Bacterial ghosts: structure, composition, isolation, and purification

### 5.7 Bacterial Spores (BSPs)

Recently, BSPs have been used as a therapeutic transport system system and have demonstrated encouraging outcomes in increasing the effectiveness of anti-tumor vaccinations. It has been demonstrated that commonly used Clostridium Novyi-NT spores are free of deadly toxins and can flourish in the anaerobic, low pH environment found in solid tumors. According to clinical research, Clostridium Novyi-NT spores have strong antitumor properties. However, further research is still needed to address its limited therapeutic potential and strong immunological response.

### 5.8 Other Bacterial Component

Microbial biopolymer, which include polysaccharides, polyamides, poly (γ-glutamic acid), polyphenols, polyesters, and hyaluronic acid, are important components of microbes that present promising chances for the growth of agents developed from bacteria in the synthesis of nanomaterials. In the field of nanomedicine, these bacterial polymers have enormous potential for improving osmotic effectiveness and controlled medication release. Clinical experiments have demonstrated the efficacy of bacterial extracellular membrane proteins from Neisseria meningitides and Klebsiella pneumonia as immunostimulant to strengthen the defence mechanism for treatment of malignancy.

## 6. Bacteria/Bacterial Element-Based Delivery Vector Benefits and Difficulties

Because of their intrinsic qualities, bacteria and their constituent parts have become attractive medication delivery methods. Such features allow the delivery of therapeutic drugs to disease area, increase the stability of encapsulated drugs, and protect physiologically active molecules from degradation. Effective tumor-targeting, intrinsic immunostimulatory properties, high drug delivery efficiency, and ease of modification are just a few advantages of bacterial therapy over traditional treatments (Fig 5). However, due to harmful effect and antigenicity, unpredictable drug dosage and concentration, uncertain mechanism, and effectiveness, the clinical use of bacteria and bacterial elements as drug delivery vehicles is currently restricted.

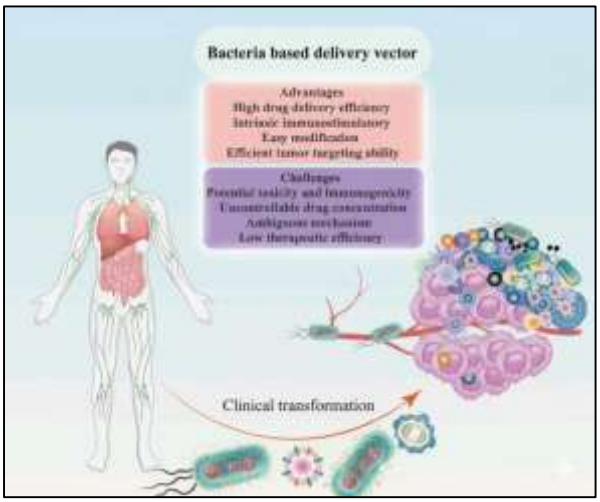


**Fig 5.** Bacteria/Bacterial Elements-Based Delivery Vector Benefits and Difficulties in clinical transformation

**Table 3**. Summary of recent research on bacteria and bacterial component-based drug delivery vectors for tumor therapy [12]

| Microbes/Bacterial Element | Microorganism | Approaches | Result |
|---|---|---|---|
| Microbes/Bacteria | Salmonella typhimurium VNP20009 Escherichia coli Nissle 1917 | Chemical binding | Immune responses that are triggered Specific intra-tumor localization. |
| Microbes/Bacteria | Escherichia coli BL21 (DE3) | Chemical binding | Tumor immune activation's dual ability |
| Microbes/Bacteria | Escherichia coli MG1655 | Genetic engineering | Tumor cell autophagy was triggered by active targeting of solid tumor regions. |
| Microbes/Bacteria | Escherichia coli Nissle 1917 | The use of genetic engineering Surface coating that mimics biology | Microbial translocation in distal tumors was enhanced by improved antitumor efficacy in vivo. |
| Microbes/Bacteria | Attenuated S. typhimurium VNP20009 | Biomimetic surface coating | Systematic and cooperative anticancer immunity prevented the growth and metastasis of tumors |
| Outer Membrane vesicles | Escherichia coli BL21 (DE3) | The use of genetic engineering Surface alteration | Long-term adaptive immune response with TME remodelling |
| Outer Membrane vesicles | Escherichia coli DH5α | Genetic engineering | Promoted apoptosis of tumor cells and inhibited tumor angiogenesis |
| Outer Membrane vesicles | S. Typhimurium ATCC14028 | Simple incubation | Increased tumor cell autophagy and apoptosis |
| Outer Membrane vesicles | Attenuated K. pneumonia ACCC60095 | Simple incubation | Macrophages inducted in TME accelerated apoptosis of tumor cells |

| | | | |
|---|---|---|---|
| Bacterial Ghosts | Escherichia coli Nissle 1917 | Simple incubation | Promoted DC maturation enhanced CD4+ and CD8+ T-cell growth |
| Bacterial Ghosts | Escherichia coli Nissle 1917 | Simple incubation | Accelerated maturation of DC Increased proliferation of CD4+ and CD8+ T cells |
| Bacterial Spores | C.novyi-NT | Simple incubation | triggered a cytokine response in the systemic immune system Increased T-cell activation specific to tumor cells |
| Bacterial Spores | Clostridium butyricum ATCC19398 | Simple incubation | enriched and targeted in tumor sites |

## 7. Bacterial species used in cancer therapy

### 7.1 Pathogenic Bacteria

Anaerobic bacteria are able to thrive even in oxygen-rich conditions because of their amazing capacity to create extremely robust spores. Bacterial spores have shown promise in targeted drug delivery as a novel way to deliver anti-cancer agents, remain inactive until they find suitable conditions, like low-oxygen areas in malignant tissues. These adaptable carriers can transport a range of curative payloads, including genetic elements, cytotoxic peptides, and therapeutic proteins, opening the door to new and effective anti-cancer treatments.

The introduction of bacterial spores into tumor cells causes significant changes, such as mitochondrial dysfunction, increased metabolism, and improved cellular signaling, which lead to an increase in reactive oxygen species (ROS). A sequence of events that culminate in the tumor cell's demise can occur when ROS levels in cancer cells surpass a specific threshold. However, avoiding possible tissue injury is one of the main difficulties in cancer bacteriotherapy employing facultative aerobic-anaerobic bacteria. For these bacteria to be used safely and effectively in medicine, their virulence must be sufficiently reduced. Using a variety of mechanistic techniques, the utilization of several pathogenic bacteria as curative agents in myeloma treatment has been thoroughly investigated.

#### 7.1.1 Salmonella typhimurium

The Gram-negative Salmonella enterica serovar Typhimurium (S. Typhimurium) is the cause of gastroenteritis in humans. Recent studies have shown that a evolved strain of S. Typhimurium can transport medications to specific organs. The genes that create tumor-suppressive proteins can also have their genomic sequences altered using gene therapy. Researchers created the genetically modified bacterium used to treat cancer using S. Typhimurium.

Remarkably, this bacterial carrier effectively eliminated carcinoma cells which are spread to other areas of the body, having a major impact on numerous developed tumors. Several myeloma cell lines, including human pancreatic carcinoma (ASPC-1) and colorectal carcinoma cell lines (such as C38, WiDr, and CT26), have shown an amazing ability to eradicate tumor forms when exposed to genetically modified S. Typhimurium strains. Daniel Saltzman also conducted an important study on the possible use of Saltikva, a recombinant attenuated strain of S. Typhimurium, as a cancer treatment.

#### 7.1.2 Escherichia coli

Anaerobic bacteria like Escherichia coli (E. coli) have been widely used in many scientific and healthcare sector, including treatment of malignancy. Such bacteria have been shown to be able to proliferate and eat the solid tumor microenvironment, which either inhibits the tumors from growing or even kills them, causing the body to eliminate them. E. Coli Nissle 1917, which can be given orally as a probiotic or as a diagnostic compound, is one

prominent example. This bacterium helps physicians correctly identify liver metastases by discharging unique chemical markers that are readily discover in urine. Such report demonstrate the great potential of bacteriotherapy for the detection and treatment of cancer.

### 7.1.3 Listeria monocytogenes

The Gram-positive facultative anaerobic bacteria Listeria monocytogenes is one of the primary vehicles for cancer immunotherapy. Although Listeria is associated with foodborne illnesses, its potentially harmful characteristics are now being exploited to deliver cancer treatments. With the help of Listeria's special properties, therapeutic agents can now be administered precisely, opening up new possibilities for targeted cancer therapy treatments. LLO is crucial to the bacterium's survival because it stops phagolysosomes from developing inside host cells. This mode of action allows Listeria monocytogenes to survive in the intracellular environment while evading the host's immune response. Additionally, Listeria monocytogenes cancer is frequently treated with the human colorectal cancer cell line Colo205.

### 7.1.4 Clostridium

Bacteria in the Clostridium genus showed remarkable tolerance to harsh environmental conditions, including dehydration and high temperatures, by producing endospores. Many Clostridium subtypes, including as Clostridium butyricum, Clostridium tetani, Clostridium histolyticum, and Clostridium acetobutylicum, have been thoroughly studied as possible anti-cancer therapies. This study has examined how different strains of Clostridium may help fight malignancy. Some Clostridium strains can target and partially break down cancer cells and tumors. Traditional remedies like chemotherapy are losing their effectiveness in treating malignancy. Therefore, some Clostridium strains and their spores are suggested as promising options for combination tissue therapies. [13]

### 7.2 Non-Pathogenic Bacteria

### 7.2.1 Bifidus spp

Bifidobacterium (Bifidus) species are anaerobic, immotile bacteria bacteria that live in human digestive tracts. They exhibit oncolytic qualities by concentrating on tumor cells and releasing anticancer agents. Breve is the most significant strain of Bifidobacterium. It has undergone genetic modification to produce interleukin-12, which promote the death of cancerous cells. At the moment, head and neck tumors are treated with it.

Enterolactone, which may transform fatty acids into pectin oligosaccharides (POS), is produced by certain bioengineered bifidobacterium species. Because of its anti-proliferation characteristics, POS prevents cancers from growing. Breast cancer may be treated with Bifidobacterium longum. It produces the enzyme cytosine deaminase when combined with 5-fluorocytosine (5-FC). The enzyme converts 5-fluorocytosine to 5-fluorouracil (5-FU), a cytotoxic compound that destroys myeloma cells. In mice, it has displayed that a significant decrease in xenografted human HER2-positive neoplasm. In vitro studies on Heps liver carcinoma animal models have shown a marked reduction in the growth of the cancer.

### 7.2.2 Lactobacillus spp

One of the primary probiotics in the digestive tract is Lactobacillus species. gram-positive bacterium with a rod-like form. By promoting the production of mucus and barrier-related proteins, they fortify the intestinal barrier. To combat infections, they also generate antibacterial substances including bacteriocins, hydrogen peroxide (H2O2), and short-chain fatty acids (SCFAs). Lactobacillus Plantarum is the most significant species being studied for cancer treatment. Research has shown that the L-14 form of L. plantarum extract inhibits the expression of genes associated with cancer cell motility. It also reduces the viability and migration of A375 cells.

Bacteriocins, which are produced by some lactobacillus species, prevent cancer cells from proliferating by stopping the G2 phase of the cell cycle. Nanoparticles that transport ginsenoside compound K (CK) to tumor locations have been produced using Lactobacillus kimchicus DCY51. It mostly stops breast cancer that is hormone-independent.

### 7.2.3 Lactococcus spp

Lactococcus bacteria show promise as gene delivery systems and anticancer drugs for several cancer types. Lactococcus lactis NZ9000-401 is the most promising strain. It has undergone genetic modification to express the kisspeptin-coding KiSS-1 gene. These peptides prevent cancers from growing and spreading. In (HT-29) colorectal carcinoma cells, this strain has shown apoptosis and morphological changes. Additionally, it is believed to be a potent anticancer drug for the treatment of breast cancer in humans. To promote tumor resistance, their anti-angiogenic qualities are crucial. (14)

## 8. Combining Bacteriotherapy with Various Methods for Cancer Treatment

Bacterial therapy has demonstrated exceptional promise for both diagnostic and therapeutic uses when combined with other forms of cancer treatment. Chemotherapy, radiotherapy, and immunotherapy are recognized as the three primary standard cancer treatment modalities. However, in addition to typically failing to kill all of the cancer cells, conventional techniques can cause a number of difficulties for patients, such as immune suppression, systemic toxicity, and the induction of cancer cell resistance. Chemotherapy and radiation therapy are known to be difficult to deliver to the acidic and hypoxic areas of tumors.

Improving local tumor control could prolong the lives of a third of cancer patients, as it accounts for about 30% of cancer-related deaths. Numerous strategies to enhance local tumor management are presently being researched. It seems like a promising approach to combine cutting-edge methods with well-established therapeutic modalities, such as gene therapy and ionizing radiation (15).

### 8.1 Combination Of Bacteriotherapy With Radiotherapy

Radiation therapy has a strong therapeutic effect on a range of cancers as a first-line clinical cancer treatment. Radion therapy may damage DNA and kill cancer cells by ionizing radiation from high-energy rays. Between 50% and 70% of cancer patients receive radiotherapy as part of their treatment, and it can cure about 40% of cancers. A rising number of research in recent years have concentrated on the combining of radiation therapy with other treatments including immunotherapy and chemotherapy in an effort to circumvent these limitations. (16)

A expanding number of research refers that by reducing the quantity of immune-suppressing chemicals, microbial metabolites like SCFAs may increase the effectiveness of immunotherapy, chemotherapy, and radiation therapy. Tumor-killing CD4 + and CD8 + T cells, tregs, and SCFAs, especially butyrate, have immunomodulatory qualities that can affect anti-tumor responses. The enzymes known as histone deacetylases (HDACs), which are important in cell cycle regulation and proliferation, are specifically inhibited by butyrate. A few HDAC inhibitors have been investigated as possible anti-cancer medications. According to recent research, SCFAs like butyrate can boost anti-tumor immunity, which can increase the effectiveness of radiation therapy. (17)

### 8.2 Combination Of Bacteriotherapy with Chemotherapy

One crucial cancer treatment is chemotherapy. Among the issues limiting its effectiveness are systemic toxicity, insufficient drug concentrations in tumors, the formation of drug-resistant tumor cells, and a absence of selectivity for neoplastic cells over normal cells (18). It should be mentioned that tumor cells that survive chemotherapy often grow more aggressive and have the ability to penetrate lymphatic and blood vessels, which increases the chance that they will spread. (19,20)

Therefore, new approaches must be developed to increase the effectiveness of chemotherapy while reducing its toxicity. Combination of chemotherapy and bacteriotherapy could be one of these innovative methods (21). Bacterial gene-directed enzyme prodrug treatment and selective tumor targeting are two applications for genetically modified bacteria. Bacterial endotoxins can also be utilized to combat cancer, especially when combined with chemotherapy (22).

Bowen et al. suggested that probiotics be investigated for chemotherapy side effects despite the overall paucity of hard data. [23] In a different trial, patients with colorectal cancer receiving continuous 5FU infusion as a postoperative adjuvant therapy in addition to 5FU and leucovorin bolus injections were randomized to receive fiber (11 g guar gum) and L rhamnosus GG (1–2 × 10(10) CFU) daily, while the other patients did not. [24]

The COBALT technique (combined bacteriolytic therapy; concurrent use of C novyi-NT spores with conventional chemotherapeutic drugs) is another way to lessen the negative effects of chemotherapy. COBALT was unable to completely prevent animal deaths even though it showed strong antitumor effects. [25] The primary obstacles in this process was inadequate tumor lysis. Because bacteria do not completely consume all of the components of malignant tissue, bacteriotherapy should be used in conjunction with chemotherapy. [26] Kasinskas et al, [27] examined the connection between solid tumor microenvironments and S typhimurium. They proposed that the quantity and location of bacterial accumulation were controlled by the interaction between S typhimurium and the surroundings. [28]

### 8.3 Bactria In Theranostic Approaches

The utilization of multioperational techniques to contemporarily image, monitor, and treat tumors is known as "theranostics," and it has received a lot of attention recently (29–31). Bacteria can be used in theranostics because they can replicate in tumor cells and specifically target tumors. [32]

Sheng-Nan Jiang et al. used an E. coli strain K-12 (MG1655) that produces the toxic protein and pore-forming hemolytic cytolysin A to kill colorectal malignant cells. They also used the bacterial luciferase (Lux) operon as an in vivo imaging marker.). They discovered that mice's survival rates rose and tumor metastasis decreased when bacteriotherapy and radiation therapy were combined. [33] For some bacteria (Haloarchaea) that develope gas vesicles that are 45–250 nm wide and 100–600 nm long, theranostic medications have been studied. [34–35] According to a recent study by Shapiro et al., gas vesicles—which are made from Anabaena flosaquae and Halobacterium NRC-1—are employed as ultrasonic contrast agents for molecular imaging in mice. They also suggested that these vesicles might be employed as therapeutic agents and drug or gene delivery vehicles. [36].

## 9. Mechanism Of Bacteria In Treatment of Cancer

Live strains of Streptococci and Clostridium were the first to be employed in cancer treatment trials. Numerous techniques can be used to treat bacteria in order to achieve tumor therapy. [37] Numerous bacteria have been shown to be able to destroy tumors, including those belonging to the genera Pseudo monas, Caulobacter, Listeria, Proteus, Bifidobacteria, and Salmonellae, among many others. Among the mechanisms are their bacterial toxicity, the synthesis of immunotherapeutic components, enzymes, biofilms, bacteriocins, RNA interference capabilities, and prodrug cleavage. [38] The potential therapeutic benefits of these bacterial species have also been tested in animal models of cancer. [39-40]

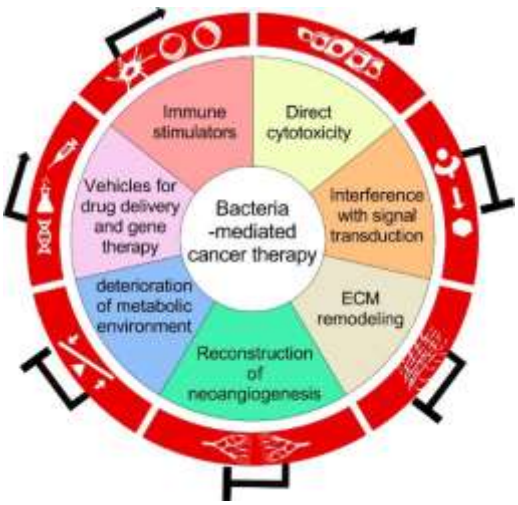


**Fig 6.** Bacteria Mediated Cancer Therapy

## 10. Current uses and constraints (limitations) of bacterially mediated cancer treatment

Numerous cell types and pathogenic pathways contribute to the complexity of cancer. Current treatment guidelines are challenged by cancer cells' capacity to evade host immunity. Bacteria have the exciting strength to affect immunity of malignancy and improve curative success because of their immunomodulatory actions, which are essential to their capacity to infect a host and were covered in the preceding section. Since Coley's time, technological developments and a deeper comprehension of the molecular structure of bacteria have led to advances in bacterial-mediated cancer therapy. Nevertheless, despite these advancements, bacterially mediated cancer treatment still has drawbacks. (41)

### 10.1 Limitations: -

The risk of widespread infection in patients with compromised immune systems is a significant disadvantage of bacterially mediated cancer treatment. Live-attenuated vaccinations, such as BCG or Listeria, have strong anti-tumor effects and can strengthen the innate and acquired immunity. (42-43)

Determining the appropriate agent dosage presents another challenge. Tolerable, effective, and toxic are all in a delicate balance. Many of the current and upcoming bacterial treatments are short-term and require maintenance. Overuse of combination therapies can result in negative side effects and increased toxicity, which can be dangerous for those with weakened immune systems. (44) Therefore, in order to obtain the desired results without side effects, methods for delivering safe and effective doses without the requirement for combination bacteriolytic therapy must be developed.

### 10.2 Current uses

As discussed in the previous section, the limitations of bacterial based cancer treatment can be overcome by manipulating bacteria and bacterial components thanks to recent advancements in recombinant technology and bioengineering. Clostridium strains can be altered to express genes that trigger cell death, such as eukaryotic host molecules like TNF-α or the bacterial enzyme cytosine deaminase. (45)

The ability of AR-1 to promote tumor cell progression from the G0/G1 phase of the cell cycle to the S/G2/M phase is one of the primary explanations for its efficacy. This can improve treatment sensitivity in resistant malignancies because the majority of cell toxic drug-based therapies only identify and destroy tumors in the S/G2/M phase. (46)

The live-attenuated double-deleted (LADD) strain of Listeria is presently the main biomarkers for vaccines used in in-vitro & in-vivo studies due to its decreased toxic hepatitis and quick clearance from the liver and spleen, which is produced by Act A and inlB deletion. (47)

Nevertheless, there is currently little proof that it works for patients who are resistant to treatment.

## 11. Future Perspective

Recent advances in the field of cancer medication discovery are frequently associated with reasonably designed medicines that target a particular chemical or signaling system. Small natural or synthetic compounds, drug and prodrug combinations, and monoclonal antibodies are among the many therapeutic modalities that are currently commercially available and used in clinical practice.

In addition to using live bacteria, there has been recent interest in using bacterial proteins that can selectively enter cancer cells and either kill or disrupt their growth through a variety of mechanisms.

Over the past ten years, the single-target, single-compound paradigm has served as the cornerstone for the development and discovery of cancer drugs. This idea stands in contrast to a more modern approach to drug development that makes it possible for a single medication to effectively target several stages of cancer growth.

The pathogenic bacterium P. aeruginosa produces a protein called azourin, which is particularly important in this situation. Azourin inhibits the development of cancer cells by either inhibiting receptor tyrosine kinase-mediated cell signalling or angiogenesis. [48]

## 12. Conclusion

A potential new area in oncology is bacteria-based cancer therapy, which offers targeted delivery and anti-tumor immune boosting. By delivering therapeutic molecules, activating cytotoxic immune cells, and inhibiting immunosuppressive cells, bacteria can overcome the drawbacks of traditional medicines including toxicity and drug resistance thanks to advancements in genetic engineering. Although bacterial immunotherapy, particularly with species like Salmonella and Listeria, has demonstrated therapeutic potential, problems with dose control and safety still exist, particularly in individuals with impaired immune systems. To fully grasp the benefits and overcome barriers for successful incorporation into cancer therapy regimens, more research and clinical trials are necessary.

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