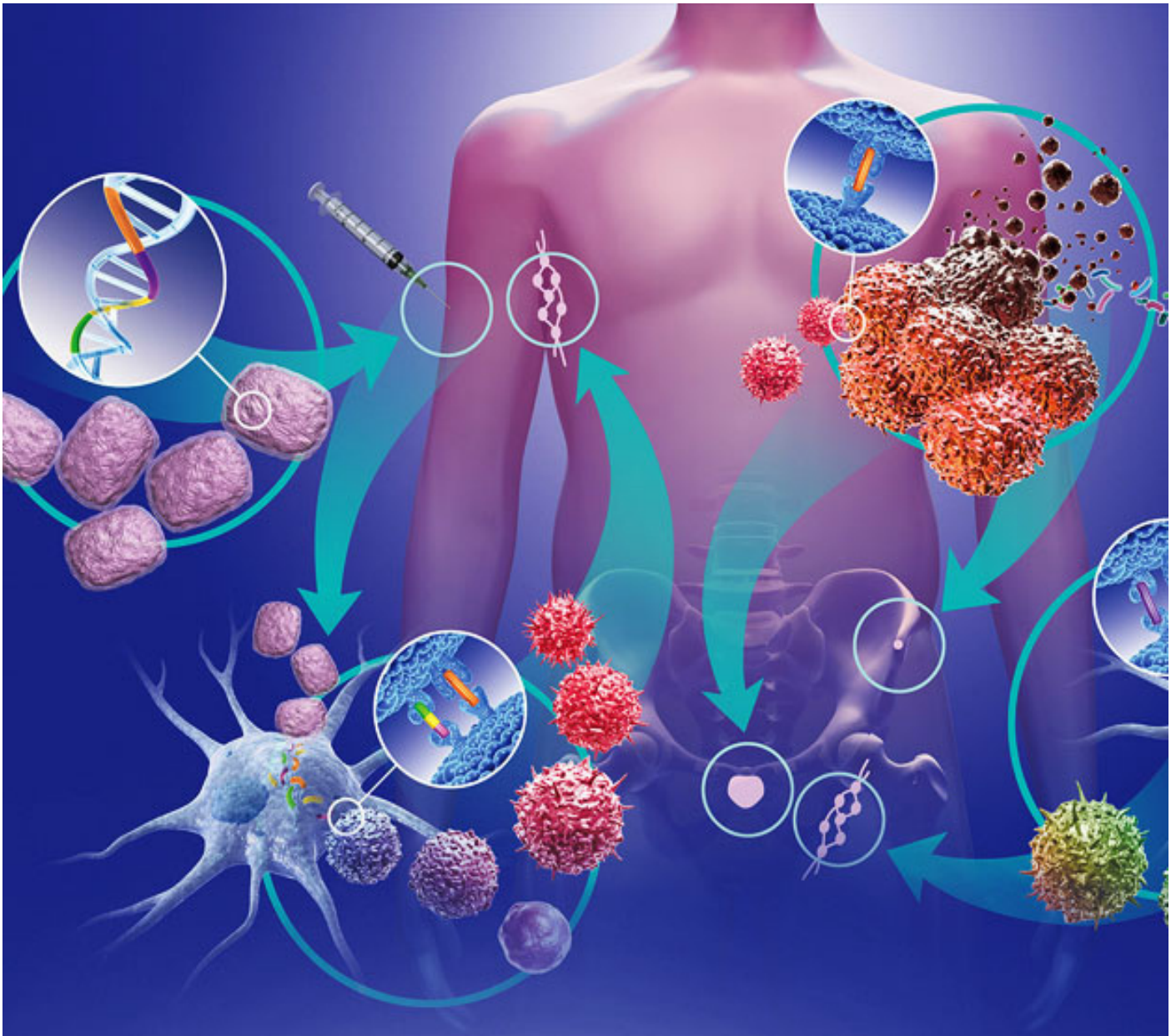

# CANCER THERAPY

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# Recent progress in bacterial-mediated cancer therapy

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**Abstract**

Worldwide incidence rate of cancer is increasing day by day and there is an urgent need of development of novel treatment strategies. In this regard bacterial therapy to target cancer is intensively investigated nowadays and may represent an important salutary option. Bacterial cancer therapy is ancestral concept but recognized in 19$^{th}$ century. In the past few decades tremendous efforts has been made to design a better cancer targeting bacterial host who can produce unique tumor targeting therapeutic proteins showing therapeutic efficacy alone or in combination with other therapeutic approaches. With the inimitable intrinsic anti-tumor property of anaerobic bacteria's, some distinctive bacterial strains are being used as therapeutic vectors by engineering them in such a way that they can facilitate shuttle therapeutic compounds in tumor proximity and studied are in clinical trials. Despite their safe efficacy from patients of view, these studies are limited and more apt exploitation and further refinement at larger scale is required to combat the limitations of conventional therapeutic approaches. In order to extend our clinical knowledge, we tried to summarize the achievements made in bacterial mediated cancer therapies till date.

## Concept of bacterial mediated therapy

Decade per decade major efforts have been made to deliberate the genetic basis of cancer and the knowledge gained is intensively being used worldwide to develop alternative treatment approaches such as targeting and demolishing tumor cells using gene therapy [1,2]. Successful use of gene therapy for tumor targeting require suitable vectors who can discriminate between cancerous and surrounding noncancerous tissue along with nullifying the factors comes across during therapy [3,4]. Though, for better delivery of therapeutic genes, various vectors have been designed but lack of tumor specificity is major challenge. Till today, tumor hypoxia remains one of the potent reasons in the limitation of cancer therapies. Tumor hypoxia is a condition which arises due to lack of adequate supply of oxygen, nutrients, and therapeutic agents as a result of muddled organization and irregular blood vessels in the tumor proximity [5]. This hypoxic tumor microenvironment ropes the favorable growth conditions for anaerobic bacteria's, creating the concepts of bacterial mediated cancer therapy [6,7]. The concept to eradicate the tumor using anaerobic bacteria is not new and has been came to light two centuries ago when tumor regression was seen in gas gangrene patients, a condition caused by anaerobic *Clostridium* strain [8]. Some species of anaerobic bacteria including *Clostridium*, *Bifidobacterium*, and attenuated *Salmonella* have a natural ability to target tumors, prosper, and consume oxygen poor cancerous tissues; hence, they can colonize only within the necrotic and hypoxic areas of tumors, and as a result, microbial growth within the tumor can result in a strong cytolytic and oncolytic effects [9-11]. Interestingly, bacterial cancer therapies have advantage over other therapies as their growth and expression of therapeutic compounds can be controlled using antibiotic markers, and these therapeutic strains are easy to store, making them accurate pick. One important consideration for bacterial therapies is the species and strain of bacteria used. Some of the different bacteria utilized so far include *Clostridia sp*., *Salmonella sp*., *Bifidobacteria sp*., *E. Coli sp*., lactic acid bacteria such as *Streptococcus sp*., *Lactobacillus sp*., and *Listeria sp*.. Each of these strains has its own unique properties affecting its potential use for cancer therapy including its tumor colonization potential, ability to invade tissue, interaction with the immune systems, and ease of genetic manipulation. Nowadays, bacterial-mediated cancer therapy has re-emerged with the development of non pathogenic strains, which can target solid tumors accurately and can be engineered as per required therapeutic gene expression.

## History of the bacterial therapy in cancer

Bacteria-mediated cancer therapy concept seems new but has its roots since 2600 BC when Egyptian physician Imhotep saw some correlation between infection and possibly cancerous tissue swellings. After that in 1893, similar findings were observed where regression of cancer tissue was reported in gas gangrene patients [12]. Later, W. Busch and colleagues performed first "clinical trial" In 1863 in Berlin when they intentionally infected a female cancer patient with *Streptococcus pyogenes* which causes Erysipelas. Interestingly the tumor size was retarded; however, patient died due to inability to control bacterial infections. Later, William Coley (1862-1936), an american physician came in to light with his pioneering contribution towards use of bacterial strains in cancer immunotherapy. During his treatment he observed high mortality rate using live bacteria and with his treatment strategies and clinical experience, he suggested that a balance is required between therapeutic approach and infection control. He developed a mixture of strains (heat-inactivated *Serratia marcescens* and *Streptococcus pyogenes*), later known as "Coley's toxin," and applied on thousands of cancer patients by injecting his toxin in cancerous tissue or in surrounding non cancerous tissue with increasing dose with the course of treatment [13,14]. During his treatment, although patients suffered with episodes of fever but balance between dose and fever leads to some success as tumor retardation as well as complete clearance of tumor tissue was observed in some patients. Coley's approach was not approved due to inability to explain the therapeutic mechanism and to control bacterial infections along with emergence of the other competitive conventional therapies such as Radiotherapy. Later in 1936, immediately after his death, American Medical Association approved his approach. Even after Coley's death, bacteria mediated tumor therapy was point of debate and remained dormant for several decades. Later in 1962, a controlled study held on 93 cancer patients out of which 20 patients showed therapeutic response [15]. Considering both Coley's and later attempts, it is important to choose accurate strains, duration and frequency of the treatment course, and prior or concurrent use of antipyretic as well as chemotherapeutic drugs along with bacterial therapy. Despite little knowledge about mechanism involved, these bacteria's are reported to boost immunity against tumor cells by activating natural killer cells. Advancement of genetic engineering as well as increased knowledge about bacterial behavior, are two important bases for the development of bacterial tumor therapy.

## Recent advances in bacterial cancer therapy

Some species of anaerobic bacteria have a natural ability to target tumors, prosper, and consume oxygen poor cancerous tissues; hence, they can colonize only within the necrotic and hypoxic areas of tumors, and as a result, microbial growth within the tumor can result in a strong cytolytic and oncolytic effects. Several Gram-negative and Gram positive bacterial strains have shown their potential in cancer therapy using various animal model systems. Table 1 summarizes former and current studies dealing with bacterial therapies.

### I. Gram positive bacteria

During late 20th century, the most commonly used gram positive anaerobic bacterial strains were Clostridia spores with the characteristic properties of safety point of view as well as to grow in the hypoxic regions of the cancerous tissue where they can germinate and grow to become active. With the advantage to grow in hypoxic regions of tumors, toxins produced by these bacteria have caused high mortality. *Clostridia* can express IL-2 and TNF-α with the property of stimulating antitumor immunity and direct antitumor features by genetic modifications. To minimize the side effects and to produce maximum cytotoxic drug in tumor proximity, Clostridial spore's antitumor potential has been increased by incorporating cytosine deaminase, a pro-drug converting enzymes which converts pro-drug 5-fluorocytosine (5-FC) to active 5-fluorouracil (5-FU) in a localized area of tumor [16]. To increase the safety, C. novyi strains were developed by deleting virulence factors like the α-toxin and shown to have an anticancer effect. In recent times, beside animal studies, attenuated *C. novyi*-NT strain has garnered renewed interest and is in preclinical and clinical trials using dogs as well as human patients (Table 2) where positively effects were seen in case of advanced leiomyosarcoma upon intra tumoral injection of *Clostridia* spores [17]. *C. novyi* spores shown success in targeting orthotopic glioblastomas upon intravenous infection in a rat model [18]. These studies indicate that the *C. novyi* spores are able to cross the blood brain barricade under certain circumstances. Despite poorly understood mechanism of Clostridia bacteria, they can effectively target neoplastic tissue as well as minimizes side effects to the host, making them potential candidate for bacterial-mediated cancer therapy. *Clostridia* strains are not able to target metastatic or small cancerous tissues and might also be a great disadvantage due to the restriction to anaerobic regions.

Other Gram-positive bacteria used as therapeutic delivery include lactic acid bacteria, *Bifidobacteria*, *Listeria*, and *B. subtilis*. *Bifidobacteria* and *lactobacillus* are probiotic strains naturally found in the human gut and are already in use as therapeutic agents through reinforcement stimulation of cancer-specific T cells and increases selective accumulation of antigen-specific CD8+ T cells in the tumor and thus demolition takes place [19]. Similarly, oral administered of natural non-pathogenic *L. lactis* produces IL-10 as a prominent therapeutic agent to treat inflammatory bowel disease and moderate ulcerative colitis [20]. As extensive research on *Listeria* was focused mostly as a vaccine, some groups have used it as a gene delivery vector for cancer therapy due to its intracellular life cycle [21-23]. *B. subtilis* is naturally competent and its full genome has been sequenced, although genetic circuitry has not been as developed and new tools are being emerged for genetic engineering in this species and others, though much work remains to be done.

### II. Gram negative bacteria

Currently, the two most extensively investigated gram negative bacteria for bacterial mediated cancer therapy are *S. typhimurium* and *E. coli*. Both have been shown to colonize in tumors in mice at a higher rate compared to normal tissues [24]. As Gram-negative bacteria naturally contain high lipopolysaccharide content, they can stimulate the immune system [25]. *E. coli*, a model organism extensively studied in recombinant biology, is the next most extensively used bacteria in the field of cancer therapeutics. In contrast to *S. typhimurium*, *E. coli* strains are non pathogenic in nature and found in the human gut and some of them have a positive effect on health after administration. The capability to use without attenuation and their status as clinically approved probiotics makes them striking candidates for use in therapies. The most widespread probiotic strain in use is *E. coli* Nissle 1917, but other strains have been explored also. While *E. coli* has been used more than *S. typhimurium* in the overall field of synthetic biology, *Salmonella*

strains are most widely used strain in bacterial mediated tumor biology and extensive research upon *E. coli* and *S. typhimurium* states that these two species have similar ease of engineering as they have fully sequenced genomes, knockout collections, and easily used tools for genetic manipulation.

## Advantage of Using Salmonella as Bacterial Cancer Therapy

*S. typhimurium* is the most widely studied cancer therapeutic approach as it has potential to grow in the hypoxic core of tumors as well as the non-hypoxic regions and has been used for a variety of applications, reaching as far as clinical trials. Several strains of *Salmonella* such as VNP20009, A1-R, SL7207 and CRC2631 have been used as antitumor therapeutic agents and have been tailored time to time for their safer use and targeted delivery.

### I. VNP20009 Strain

VNP20009 is most intensively studied strain due its safety applications and was developedfrom *S. Typhimurium* ATCC14028 at Yale University. This strain was developed by deleting purI gene responsible for virulence, msbB gene responsible for septic shock potential, and changes in antibiotic resistance potential, using genetic attenuation method in such a way that even after multiple passages both in In vivo and In vitro conditions, it can remain stable genetically and phenotypically. In tumor-model of mice and human tumor xenografts, VNP20009 strain has shown its accumulation thousand times higher, preferentially in tumor part over normal organs [26]. Various models suggested that clearance of VNP20009 strain takes place within 2 hours from blood while takes several days from all organs. Dose of 1×106 CFU/m2 to 1×109 CFU/m2 of VNP20009 strain has been evaluated in clinical trials for human renal cell carcinoma and nonresponsive metastatic melanoma and results were promising with significant elevation of IL-1β, IL-6, and IL-12 and TNF- α in the serum of subjects. Despite local colonization of VNP20009 shown localized necrosis of tumors but failed to show any evidence of tumor regression [27]. By introducing some modifications, the safety and anti-tumor efficiency of this strain has been significantly improved. With the accomplishment of VNP20009 complete genome sequencing, some new mutations like deletion of 108 kb purM and 50 non synonymous SNPs has been detected whose biological importance is under investigation.

### II. A1/A1-R Strain

A1 is another tumor-targeting modified strain developed from *S. typhimurium* ATCC14028 at University of California by giving exposure to mutagenic Nitrosoguanidine (NTG) and selected strains was identified as a leucine and arginine auxotroph that had the property to grow in tumor xenografts by using these amino acids and unable to grow in normal tissue environment [28]. In vitro experiments show, that A1 is capable to invade and replicate intra-cellular in human PC-3 cell line developed from prostate cancer and very low quantity of bacteria even 10-50 CFU found to be effective with a tumor vs normal tissue invading ratio of as high as 10,000:1 and strains clears approximately within 4th day to 2 weeks [28]. To further improve tumor targeting potential and toxicity, *S. typhimurium* A1 strains were genetically modified as A1-R strains with a potential of more than six fold potential when compared with earlier [29]. Enhanced virulence and tumor targeting potential showed that A1-R had more than 100 times CFU in PC-3 cancer tissue in vivo when compared to A1. Forty percent of all orthotopic metastatic human prostate tumor-bearing mice were completely cured within a week using A1-R strain [30]. The potential of A1-R has been tested with promising results in many primary cancers such as pancreatic, prostate, breast, and spinal-cord tumor models, as well as some metastatic tumors [31-34]. Very recently, A1-R shown its therapeutic potential capability of decoying quiescent cancer cells to S/G2/M phase and sensitize them to cytotoxic chemotherapy [35].

### III. CRC2631 Strain

CRC2631 is a candidate therapeutic tumor-targeting *Salmonella* strain, developed from LT2 strain of *S. typhimurium* at the Cancer Research Center, Columbia [36]. These strains were developed automatically by storing in agar stabs for more than four decades at normal temperature and generated remarkable genetic diversity by various deletion, duplication, frameshift and inversion mutations [37,38]. The Demerec collection strains were screened by co incubating them with MCF-7 human breast cancer cells and it was found that one strain, CRC1674, which targeted and destroyed breast cancer cells more effectively. Genetic alterations of CRC1674 include his-2550 (plus suppressor mutation, DIIR49B), mutated rpoS start signal (UUG), and diminished HPI and HPII. Foe better performance, CRC1674 was further manipulated by disrupting aroA, thyA, and rfaH genes and a LPS deficient auxotrophic strain was developed. These strains shown high potential to colonize in tumor environment and high destruction rate as destroyed tumor cells mitochondria's within one hour; whereas, decreased their toxicity drastically with very high ratio from >200 : 1 to >1000 : 1. Strain CRC2631 recovered from TRAMP mouse prostate tumors showed significant loss of wild-type motility and flagella, indicating phenotypic evolution of this therapeutic strain within the tumor environment and further optimization of CRC2631 are under way [39].

## Current Tumor-Targeting approaches using engineered Salmonella as therapeutic vectors

Although *Salmonella* strains possess native bacterial cytotoxicity against tumors, they have the advantage of being engineered easily and has long track record as a bacterial genetic model and engineered strains show enhanced antitumor effect. Currently, the most popular strategies are (a) expressing enzymes to activate anticancer "prodrugs" at tumor sites; (b) expressing anticancer agents directly; (c) expressing tumor-specific antigens and antibodies; (d) transferring eukaryotic expression vectors into tumor cells; and (e) expressing oncogene silencing RNA. Some other strategies, such as delivery of tumor-killing nanoparticle therapies, are still being developed.

### I. Pro-drug converting enzymes approach

To avoid the side effects and toxicity of non targeted cytotoxic chemotherapy, many suicide gene therapies have been introduced to exclusively express pro-drug converting enzymes inside or close to tumor cells [40]. Pro-drugs are biologically inactive compounds which produces active drug following metabolism in the body. Salmonella has been engineered to introduce Herpes Simplex Virus Thymidine Kinase (HSV TK) with a beta-lactamase secretion signal to phosphorylate the prodrug ganciclovir (a nucleoside analogue) and results were promising in terms of better tumor retardation and prolonged survival [41]. S. typhimurium has been engineered by introducing the Purine Nucleoside Phosphorylase (ePNP) gene of E. coli, which converts MoPdR into methoxy purine. Engineered VNP20009 expresses Carboxypeptidase G2 (CPG2), a pro-drug activating

enzyme, to significantly reduce the growth of xenografts [42]. Engineered Salmonella vectors introduced with pro-drug converting enzymes, cytosine deaminase have capability to convert 5-Fluorocytosine (5-FC) to 5-Fluorouracil (5-FU) showing high therapeutic potential [43].

**II. Anticancer agents approach**

Anticancer agents are being used since long time when William Coley used his "Coley's toxin". Till date, several categories of bacterial toxins have been introduced to tumor targeting *Salmonella* strains which include Colicin E3 (ColE3), HlyE, TNF-Related Apoptosis-Inducing ligand (TRAIL), TNF-alpha and FAS ligand (FASL) [44-48]. These agents have shown increased tumor-killing ability when expressed in *S. typhimurium*. Limitations of these cytotoxic agents such as hepatotoxicity and short circulatory half-life can be trounce by *Salmonella*-mediated targeting to the tumor site to maximize the localized concentrations of anticancer agents. Immunomodulatory molecules such as IL-2, IL18, LIGHT, and CCL21 are able to stimulate the host immune system to clear tumors. These molecules can be delivered by *Salmonella* vector and have been proven to inhibit tumors both primary and metastatic tumors when expressed by *Salmonella* vectors [48-50].

**III. Therapeutic Vectors Based Tumor-Specific Antigens and Antibodies Approach**

In today's world of innovation, bacterias are engineered in such a way that numerous tumor-specific antigens can be expressed that sensitizes the host immune system to prevent tumor formation or inhibit tumor growth. *Salmonella* based type III Secretion System (TTSS) is one of the approaches, extensively used to deliver tumor antigens [51-53]. TTSS approach is controlled via a needle-like structure which contains a sensory probe to detect the presence of eukaryotic cells and directly inject proteins into host cells, thus making it an effective antigen translocation candidate [54]. Other secretion system such as, the antigen C-Raf (a serine-threonine kinase) was expressed by an attenuated *S. typhimuriumaro* A strain using the *E. coli* hemolysin secretion system, who, drastically knocked down cancer growth in Raf oncogene induced lung adenomas of transgenic mouse models. Combination of *S. typhimuriumaro* A strain type I secretion system and *Cholera* toxin subunit B, induced cytotoxic CD8+ T-cell responses during delivery of PSA (prostate-specific antigen) in vivo [55]. Similarly *Salmonella fimbrial* display system has been shown to express NY-ESO-1 p157-165 or p157-167 (T-cell epitopes) to induce a human cancer antigen NY-ESO-1 p157–165-specific CD8 (+) T cells in in vivo experiments [56].

**IV. Silencing RNA approach**

*Salmonella* as a vector have potential to transfer therapeutic expression plasmids to mammalian host cells in both type of In vivo and In vitro systems, and on the basis of this approach cytotoxic agents, cytokines, and tumor antigens have been designed to be expressed in tumor cells using transfection. However, these strategies have limitations due to its uncontrollability and low efficiency. Recently, oncogene STAT3-specific silencing RNA, introduced into tumor-targeting Salmonella, significantly inhibited cancerous growth and metastasis and extended the life of C57BL/6 mice bearing a prostate tumor when compared to bacterial treatment alone [57].

**V. Engineered salmonella strains as tumor-detection tools**

The feature to target both anaerobic and aerobic conditions, better tumor-targeting, and accumulation phenotype united with genetic tools for strain reengineering made Salmonella a high-quality tumor-detection tool. Use of Green Fluorescent Proteins as markers to visualize Salmonella as well as other tumor-targeting bacterial strains has been used and seems successful for whole-mouse imaging but may be limited for use in the human body due to thick tissues [58]. Recently magnetic resonance and positron emission have been used to detect the presence of bacteria in tumors such as fluorine-19 magnetic resonance spectroscopy has been tested to monitor the conversion of 5-fluorocytosine (5-FC) in to 5-fluorouracil (5-FU), using recombinant *Salmonella* strain [43]. Similarly, engineered VNP20009 for Herpes Simplex Thymidine Kinase (HSV1-tk) reporter gene selectively phosphorylate radio labeled 2'-deoxy-2-Fluoro-Beta-D-Arabinofuranosyl-5-Iodouracil (FIAU) and PET images could easily recognized multiple tumor sites [59]. With the improvement of technology a Zs Green fluorescent protein was introduced in engineered attenuated *Salmonella* strain and then detection was achieved using a antibody against bacterially produced Zs Green with significantly increased sensitivity and could detect tumors more than 2500 times smaller than the current limit of tomographic techniques [60]. These results indicate that the noninvasive *Salmonella* vectors have the potential to be used in clinical applications to either diagnose or cure tumors, still more extensive research is required.

**Bacterial mediated therapy and clinical trials**

Several anaerobic bacterial strains of the genera *Clostridia*, *Listeria*, and *Salmonella* have been shown their potential and clocked the clinical trials. However, recombinant *Listeria monocytogenes* (ANZ-100) mainly used as a therapeutic live vaccine for complex cancer patients, the clinical trials with Salmonella and *Clostridia* relied largely on the intrinsic antitumor effects o these strains (Table 2). With the emerging engineering technologies new strains of bacteria's are being developed and are in preclinical studies such as six client-owned dogs were treated with *C. novyi*-NT strains and promising results were obtained with some side effects such as fever, diarrhea and nausea [61]. Despite some side effects, clinical trials were carried out in human patients with advanced leiomyo-sarcoma and tumor regression was observed after several intra-tumoral injections of spores. Attenuated *Salmonella* strain VNP20009 was specifically designed for bacterial cancer therapy and its potential was investigated both in humans as well as dogs in 2002 and 2005, respectively. Findings observed with this strain were promising in murine but results in canine and human hosts were not as prominent as in dogs, as they were able to colonize only in 42% subjects with a response rate of 25% only, while in humans, therapeutic response completely failed [61]. This assessment may be due to translational challenges as a result of turning from one host (mice) to the other (humans). Nowadays, it is well known that bacteria expresses Microbial Associated Molecular Patterns (MAMPs), recognizable by pattern recognition receptors and are heat stable and are being evaluated in preclinical and clinical trials. MAMPs stimulate immune system cells which generates therapeutic response but partly. It has been observed that Toll like receptor 4 (TLR4) or MyD88 deficient mice, lacks any antitumor response when injected with *Salmonella* strain [62,63]. Thus, potential of a MAMP-based therapy does not depend only on invading capacity but also depends on the immunogenitic nature of the tumors as well as efficacy of its escape

mechanisms. On the basis of these considerations new strains can be engineered with the characteristic properties of high immunogenicity and preserved attenuated character.

## Use of bacterial-mediated therapy with other conventional approaches

Although, various wild-type and genetically modified strains have shown success when used alone, simultaneous treatment plans have been explored to enhance these strains therapeutic efficacy. Combining bacterial-mediated therapy with radiotherapy and vascular targeting agents are the two extensively investigated approaches with promising future prospects.

### I. Bacterial mediated tumor-therapy with radiotherapy

Combining radiation therapy with bacterial mediated therapy may be very promising as use of radiation is very efficient to kill well oxygenated cells, while anaerobic bacteria's are very effective to target hypoxic cells. Since bacteria's distinctively targets these radiation sensitized hypoxic cells, combined effects may enhance the therapeutic potential. The combining effect of *C. novyi*-NT and VNP20009 strains with radiotherapy has been seen in athymic nu/nu mice bearing HCT116 xenographs where significant tumor retardation was observed when compared with either treatment in alone [64]. It is predicted that radiation targets the microvascular endothelial cells, creates the hypoxic regions in cancerous tissue which provides the increased niche for *C. novyi*-NT growth. Fascinatingly, the effectiveness of combined therapy shown to be independent of the cancer size signifying that a all types of tumor volumes may be targeted with this approach. Furthermore, the addition of radio sensitizing drugs such as 5-FU is already in investigations using Pro drug therapy approach. All together, various bacterial strains including Salmonella and *C. novyi*-NT have been shown to be very efficient when united with radiotherapy. However, further combinational standardization of therapeutic dose is required.

### II. Vascular targeting agents with bacterial mediated cancer therapy

Vascular targeting mediators are an attractive approach that expended the use of bacterial sp. like *Clostridia* to very small tumors, which are not yet of necrotic nature. These agents exclusively targets dividing endothelial cells, and rapidly inhibit vascular activity in tumors, with promotion of hypoxia and necrosis [65]. Systemic administration of Combreta statin A4-phosphate (CombreAp) is very effective and causes severe vascular blockage very rapidly, specifically in the cancerous tissue and starts necrosis. These findings show that CombreAp helps in high levels of clostridial colonization. Similarly in animal models, use of CombreAp with CD-recombinant *C. acetobutylicum* spores increased the CD-positive tumors occurrence significantly [66]. However, this combined treatment strategy also shown noteworthy toxicity as a result of vascular targeting and chemotherapeutic agents. However, these existing studies definitely indicate the potential benefit of this combination therapeutic approach, yet further investigations are required.

## Summary

Bacterial-mediated cancer therapy holds bright future and till date, several anaerobic bacterial strains of *Clostridia sp*., *Salmonella sp.*, *Bifidobacteria sp*., *Listeria sp*., *E.Coli sp*., lactic acid bacteria such as *Streptococcus sp*., and *Lactobacillus sp*., have shown their anti-tumor potential through germination in hypoxic regions of cancers. A among all gram negative and gram positive tumor-targeting bacteria's, *Salmonella sp*. are the most extensively, genetically modified, tumor-targeting strains with their properties to grow both in hypoxic as well as in normal tissue and ability to express therapeutic compounds. With advancement of technology and genetic engineering, new recombinant *Salmonella* strains such as VNP20009, A1-R, SL7207 and CRC263 have been designed to improve their applications, such as ability to target tumor regions very specifically, removal of virulence for safety aspects, ability to express therapeutic compounds to consume cancerous cells. Currently, the most popular tumor-targeting strategies using engineered *Salmonella* as vector are, expression of cancer specific pro-drug converting enzymes, expression of anticancer agents directly in tumor premises, expression of tumor-specific antigens and antibodies, expression of oncogene silencing RNA and transfer of eukaryotic expression vectors into tumor cells. Along with these bacterial-mediated tumor targeting strategies, use of other conventional therapies such as radiotherapy and vascular targeting agents have shown improved outcome however a balance of dose and time is required in combination to enhance the tumor targeting potential.

## Tables

**Table 1:** Current preclinical bacterial-mediated cancer therapy studies

| Species | Year | Model | Result |
|---|---|---|---|
| *C. novyi* | 2014 | Spontaneous, dog | Colonization and enhanced survival |
| *C. novyi* | 2015 | Glioblastoma, rat | Colonization and enhanced survival |
| *B. infantis* | 2013 | Bladder, rat | Enhanced tumor specificity using engineered strain |
| *L. monocytogenes* | 2014 | Ovary, mouse | M2-M reprogramming, iNOS-mediated tumor destruction |
| *L. monocytogenes* (ANZ-100) | 2014 | Pancreas, mouse | Enhanced survival |
| *S. Typhimurium* (A1-R) | 2012 | Breast, nude mouse | Effective on intravenous administration |
| *S. Typhimurium* (A1-R) | 2012 | Brain, nude mouse | Enhanced survival with tumor retardation |
| *S. Typhimurium* (A1-R) | 2014 | Bone metastasis, nude mouse | Breast cancer bone metastasis Inhibition |

| | | | |
|---|---|---|---|
| *S. Typhimurium* (A1-R) | 2014 | Pancreas, nude mouse | Tumor growth retardation |
| *S. Typhimurium* (A1-R) | 2015 | Ovary, nude mouse | Enhanced survival |
| *S. Typhimurium* | 2015 | Carcinoma (CT26), mouse | Total tumor clearence in all cases using recombinant strain |
| *E. coli* | 2015 | Breast (4T1), mouse | Retarded tumor volume |
| *La. acidophilus* | 2014 | Carcinoma (CT26), mouse | Enhanced apoptosis, tumor growth inhibition |

C: Clostridium; L: Listeria; S: Salmonella; E: Escherichia; B: Bifidobacterium; La: Lactobacillus.

**Table 2:** Current clinical trials with bacterial- mediated cancer therapy

| Species | Year | Group | Result |
|---|---|---|---|
| *S. Typhimurium* (VNP20009) | 2002 | Metastatic melanoma patients-24; Metastatic renal cell carcinoma patient-1 | Enhanced immune response, tumor colonization in 3 cases, no anti-tumor response |
| *S. Typhimurium* (TAPET-CD) | 2003 | Metastatic solid tumors-3 | 66% tumor colonization, measurable activity of cytosine deaminase in tumor |
| *L. monocytogenes* (ANZ-100 and CRS-207) | 2011 | 26 patients with solid tumors (liver, pancreas, lung, or ovary) | Strain used as vaccines, enhanced immune system |
| *C. novyi*-NT | Active | Solid tumors patients; Non responsive to conventional therapy | Recruitment (NCT01924689) |
| *C. novyi*-NT | 2014 | Advanced leiomyosarcoma patient-1 | Tumor volume decrease within and adjoining |
| *L. monocytogenes* (CRS-207) | Active | Pancreatic cancer patients-90 | Enhanced survival, reduced toxicity |

L: Listeria; C: Clostridium; S: Salmonella.

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