Bacteria: our ally in the fight against cancer

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Abstract

Cancer heterogeneity and development of resistance is the main limiting factor for the management and treatment of the disease. However, major technological innovations in precision medicine and immune-based therapies have renewed faith in having a cure for different types of cancers. Classical cancer treatment options include chemotherapy, radiotherapy, and immunotherapy. Emergence of novel tumor targeting bacteria could open up new therapeutic avenues. Bacteriotherapy alone or in conjunction with classical cancer therapies has given promising results on local tumor regression and distant metastasis. Moreover, bacteria exhibit direct anti-cancer effects that subsequently aid in the activation of innate and adaptive anti-tumor immune responses. Overall, genetically reprogrammed bacterial vectors holds great potential for the specific targeting of cancers as delivery vehicles. In this review article, we have reviewed the therapeutic potential of bacteriotherapy as monotherapy or combination therapy and discussed its benefits, challenges, and future directions.

Keywords: Cancer; bacteriotherapy; chemotherapy; radiotherapy; immunotherapy.

1. Introduction

Cancer is a major health concern and one of the leading causes of death. According to WHO estimates, 19.3 million new cases of cancer and nearly ten million cancer deaths will occur in 2020 [1]. Tobacco use, exposure to chemicals or other highly mutagenic agents, and infection with a microbe like HPV or HCV and HBV are all known to influence cancer development [2–5]. Most of the malignant neoplasms share similar etiopathogenecity but diverge on tissue of origin, histopathologic attributes, immunologic properties, intertumoral and intratumoral heterogeneity [6].

Cancer initiation and progression is a complicated multihit process involving a variety of functional and genetic abnormalities. Cancer disease is characterized by unchecked cell growth and proliferation that often spreads to secondary sites in the body termed as metastasis [7]. Cancer tumor microenvironment (TME) consists of a variety of stromal and immune cells. Immune cells commonly present in the TME include macrophages, mast cells, myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), NK cells, and T cells. Over the last century, significant advances in cancer treatment have been made. On the market, there are dozens of cytotoxic anticancer agents. For patient prognosis and treatment, traditional therapies such as surgery, chemotherapy, radiotherapy, immunotherapy, and combination therapy are used. Conventional cancer therapies often eradicate cancer cells at the expense of normal tissue damage and its use causes systemic toxicities [8]. These practices often have a number of adverse effects and limited penetrance. Therefore, efforts are underway to establish a treatment strategy, which is devoid of any negative outcomes.

Experiments and clinical studies were conducted over the last several years to assess microbes as a potential therapeutic option for cancer control

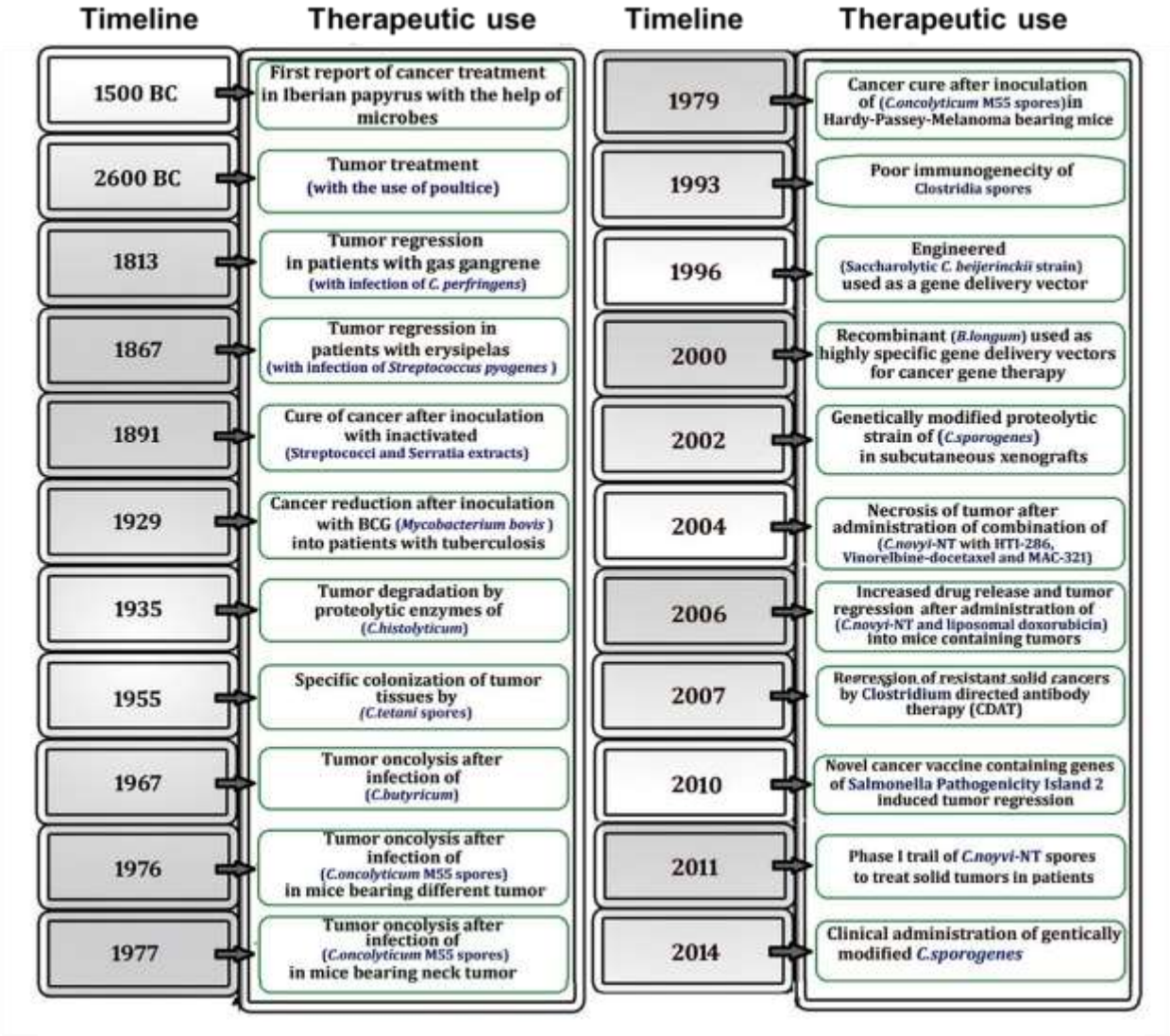

Figure 1. A timeline depicting the historical use of bacteria for cancer therapy. Intriguing research findings over the last few decades suggest that bacterial use could be an effective cancer treatment strategy.

[9–12]. For instance, one good example is the use of Bacillus Calmette–Guerin (BCG) for the treatment of bladder cancer [13]. The immunomodulatory activity of BCG is assumed to produce anti-cancer effects [9,14]. In addition, azurin, a bacterial redox protein, has been shown to cause apoptosis in melanoma cells. expressing functional p53 tumor suppressor protein [15]. Azurin interacts with p53 and forms a complex that stabilizes p53, which leads to the induction of p53-mediated apoptosis in melanoma cells [15,16]. p53 is a well-established tumor suppressor protein [17]. Like p53, its family members, p63 and p73 are also tumor suppressor genes and promote senescence and apoptosis [17,18].

Novel therapeutic strategies using live or genetically modified microbes to target the localized disease might be an effective way of immune system activation. Several microorganisms have been tested against cancers and some are going through phase II and phase III clinical trials (clinicaltrials.gov/ct2/show/NCT00004988, clinicaltrials.gov/ct2/show/NCT03358511,

Table 1: Summary of bacteria-derived oncolytic molecules targeting different human cancers.

Metabolite	Bacteria	Target cancer cells	References
Diphtheria toxin	Corynebacterium diphtheriae	Ovarian, pancreatic, and lung cancer	(104) (105) (106)
Streptolysin O	Streptococcus	Embryonic kidney fibroblast	(107)
Listeriolysin O	Listeria monocytogenes	Breast cancer (MDA-MB-231, MCF7)	(108)
Exotoxin A	Pseudomonas aeruginosa	Head and neck cancer cells (KCCT873)	(109)
Arginine Deaminase	Mycoplasma hominis Mycoplasma arginini	Glioblastoma (HROG02, HROG05, HROG10)	(110)
L- Asparginase	Escherichia coli	Breast (MCF7), Liver (HepG2), and lung (SK-LU-1) cancer	(111)
Nisin A	Lactococcus lactis	Colon cancer (SW480)	(112)
Colicin	Escherichia coli	Lung cancer (H460, H292)	(113)
Bovicin	Staphylococcus bovis HC5	Breast and liver cancer	(114)
Surfactin (cyclo lipopeptide)	Bacillus subtilis	Breast cancer (MDA-MB-231, MCF7)	(115)

clinicaltrials.gov/ct2/show/NCT01118819).

Historical scientific observations had revealed that bacterial infections enhance anti-tumor effects. In addition, bacteria can effectively invade hypoxic areas in the tumor tissue, which is reported to provide resistance to conventional cancer therapies. Furthermore, a few studies have shown that parasitic microbes can inhibit tumor progression [19–21]. One such parasite is *Plasmodium*, which causes the malarial disease in humans [22–25]. Several studies have found that malaria parasites can kill cancer cells by activating the host's innate and adaptive immune systems [19,26,27].

Due to the advancements in radiation therapies in the nineteenth century, the attention of the scientific community was diverted from microbe-based anti-cancer therapies [9]. However, in the 1990s the concept and understanding of the tumor microenvironment (TME) and DNA recombinant technology was broadly improved. More potent bacterial strains were developed and tested in animals for their anti-tumor potential. Bacteria can also be used as vectors to execute tumoricidal and immunomodulatory activities for the destruction of cancer cells. However, the major hurdle in developing bacteria as promising anti-tumor

agents is the toxicities associated with their infection. Although it is unlikely to have a cure for cancer anytime soon, innovative efforts will continue to leverage the power of bacteria for improving the cancer treatment. In this review article, we discuss the current state and future prospects for the using bacteria in anti-cancer therapy to advance cancer treatment.

2. Role of microbes in cancer regression: historical aspects

The origin of microbial therapy against cancer has been there for several centuries (**Fig. 1**). The first report describing the role of microorganisms in cancer treatment was documented by Iberian papyrus (1550 BC), followed by Egyptian pharaoh Imhotep (2600 BC) [28]. Various forms of cancer immunotherapy became popular in the 17th and 18th centuries. In 1813, Vautier observed tumor regression in patients with gas gangrene after infection of *C. perfringens* [11]. Similarly, in 1867, the German physician Wilhelm Busch described a case of cancer remission in a patient who had erysipelas, now known as *Streptococcus pyogenes* [29]. In the late 19th century, William B. Coley, who posited the hypothesis that Streptococcal bacterial infections triggered the immune system to restrict the tumor growth, leading to the discovery of an important bacteria-based therapeutic strategy against cancer. Coley developed Coley's toxin, a mixture of lysates derived from heat-killed *Streptococcus pyogenes* bacteria, which is recognized as the first attempt of cancer immunotherapy [30]. This fortuitous benefit of concurrent infection of bacterial pathogens in tumors prompted the investigation of microbial use for targeting cancer.

Bacteria produces a number of anti-cancer molecules including toxins, enzymes, bacteriocins, and biosurfactants (**Table 1**) Moreover, the most promising clinical application of an attenuated *Mycobacterium bovis,* Bacillus Calmette-Guerin (BCG), for the treatment of bladder cancer was established by Morales, Eidinger and Bruce in 1976 [31]. BCG, a tuberculosis vaccine, is the only bacterial agent approved by the FDA. However, a number of patients fail to respond to BCG therapy, it is recommended as the standard of care for high-risk urinary bladder tumors in conjunction with immunotherapy [32].

3. Aiming for the perfect bacterium for cancer therapy

Tumor-bacterial biological interactions are important in anti-cancer properties that are either direct or immune-mediated. Bacteria-associated molecular factors that influence the magnitude of anti-tumor responses include tumor chemotaxis, cytotoxic potential, motility, invasive capacity, composition, and abundance of pathogen-associated molecular patterns (PAMPs) [12,33–37]. Current cancer therapies are unable to penetrate deeper in the hypoxic tumor microenvironment, however, certain bacteria can steadily migrate deeper in such areas. To improve the targeting of tumor cells, two approaches are commonly used. First is genetic engineering of the bacteria to display tumor-specific molecules on their surface. For instance, αvβ3 integrin, is highly expressed on tumors, *Salmonella* ppGpp-deficient strain SHJ2037 was modified to express the integrin binding peptide Arg-Gly-Asp (RGD) on the bacterial cell surface to facilitate the specific targeting of cancer cells [38]. Second method involves the genetic modification of the bacteria to express a prodrug converting enzyme herpes simplex virus thymidine kinase (HSV-TK) that can activate ganciclovir (GCV) to produce anti-tumor effects [39]. The standard that allows the microbe to turn out to be an appropriate tool for cancer therapy includes nontoxic to host, selective for tumor, and deeper penetration of TME. Additionally, it should not trigger an early immune response [40]. *Salmonella*, *Mycobacterium*, *Streptococcus*, *Clostridium*, *Listeria*, *Bifidobacterium*, and *Shigella* are among the bacteria commonly used in tumor-targeting therapies.

3.1 ***Mycobacterium:*** An obligate anaerobic, facultative intracellular, non-motile bacterium that infects cattle and has the potential to cause

similar disease in humans [41]. At the beginning of the twentieth century, there were a few evidences that highlighted a correlation between the occurrence of tuberculosis and tumor regression [42]. The attenuated *Mycobacterium bovis* (BCG) was widely used to treat superficial bladder cancer [43]. BCG therapy was originally designed as a tuberculosis vaccine and has since evolved into one of the most successful adjuvant therapy. Moreover, in several parts of the world, it became the standard of care for superficial bladder cancer. The mechanism of action of BCG is to stimulate the body's own innate and adaptive immune responses. The most commonly used intravesical immunotherapy for the treatment of early-stage bladder cancer is BCG [41]. It helps in decreasing tumor growth and its distant spread in the body, as well as prevents the relapse of cancer.

3.2 ***Streptococcus:*** *Streptococcus,* a gram-positive, facultative anaerobic bacterium, which was initially utilised in the treatment of bone sarcoma by Dr. William Coley [30]. Microbes also harness the host immune system for mediating their indirect anti-tumor effects. Activated immune cells disrupt the neoplasm and inhibit the progression of cancer. In addition, it reduces the lymphangioma [44]. After even first day of administration, the amount of immune cells including neutrophils, macrophages, as well as other lymphocytes, rapidly increase in the circulation [30,45]. The anti-cancer effects of bacterial administration are not immediately observed, but rather appear after a few months of treatment. Streptococcal strains are able to stimulate the immune system of the host to attack the tumor cells [46]. In particular, it activates the innate immune system that further promotes the production of TNF-, IFNγ-, IL-12, and other inflammatory mediators [46]. Overall, *Streptococcus spp*. mediates an important role in integrating complicated immune cell responses to enhance host's anti-tumor activity.

3.3 ***Clostridium*****:** *Clostridium*, an obligate or facultative anaerobe is another promising bacterium for cancer treatment. They were preferred for cancer therapy because they thrive in environments with little or no oxygen (hypoxia). Blood vessels transport oxygen to the cells, which only penetrate the surface of tumors. As a result, oxygen transfer into the tumor is impaired, resulting in hypoxia. The anaerobic milieu favours the growth and development of anaerobic microbes including *Clostridium spp., Salmonella spp., Bifidobacterium spp., or Listeria spp.* [47,48]. The primary advantage of using microbes over conventional cancer therapies is that they grow right inside the tumor areas and specifically kill cancer cells as opposed to chemotherapies, which affects the body systemically [48,49]. *Clostridium novyi* is one of the most extensively studied bacterium. Deletion of its α-toxin gene, enables *Clostridium novyi* to colonize the tumor with less to moderate negative effects [33]. It was used in experimental models for gliomas, colorectal cancer [50], and sarcomas [51] to observe its role in tumor cell death by analyzing its role in selective colonization, immune cell infiltration, and elevating their effector functions [51]. *Clostridium* spores are less immunogenic that allows them to colonize multiple organs when administered systemically in the body [52]. However, they stimulate an inflammatory immune response once they germinate in the body. This causes increased infiltration of immune cells that also produce oncolytic effects [53]. These strains have been genetically engineered as vectors and vehicles to enhance the production of cytokines including TNF-α, IL-12, and IL-2 [54–56]. Furthermore, *C. novyi*-NT and *C. sporogenes* have been shown to increase the antibody secretion against hypoxia inducing factor-1 (HIF-1) [57].

3.4 ***Listeria*****:** *Listeria*, a gram positive, facultative intracellular bacterium, which can be used to inhibit the tumor progression or metastasis in an immune-privileged microenvironment. As it selectively colonizes the tumor and helps in their elimination through ROS production [58]. Additionally, this bacterium tends to show immunomodulatory activities as it decreases the recruitment of T-reg cells in tumor

microenvironment [59]. Because it only infects APCs, this bacterium is thought to be a valuable immunostimulant or immunotherapeutic agent. *Listeria* infection strongly activates innate immunity and promotes the production of several pro-inflammatory cytokines [60]. After phagocytic internalization, *Listeria monocytogenes* (Lm) escapes its clearance in the phagolysosomes by producing a virulence protein known as listeriolysin O (LLO) [61]. LLO acts like a hemolysin, perforates the phagosomes, and comes out in the cytosol. They can multiply and releases antigens once they enter the cytosol [61]. This process allows antigen processing and presentation through class I and II MHC molecules to stimulate effector responses from both CD4+ and CD8+ T cells. These features of *Listeria* can be leveraged for the production of tumor antigens by means of genetic engineering to potentiate the anti-tumor responses.

3.5 *Bifidobacterium*: *Bifidobacterium* is an anaerobic gram-positive bacterium that does not produce bacterial spores. *Bifidobacteria* are reported to have favorable effects on human health, which includes vitamin synthesis, pathogen defense, and the secretion of antimicrobial agents. In addition, it reduces the secretion of pro-inflammatory cytokines, enhances immune protective functions including activation of cytotoxic T cells and influences the interactions between natural killer (NK) cell and dendritic cells [62,63]. A number of reports have confirmed anti-tumor roles of *Bifidobacteria* [64–67]. However, as opposed to other microbes like *Clostridium spp*., *Bifidobacterium* monotherapy did not show potent anti-tumor effects in preclinical studies in spite of successful tumor colonization [68]. In contrast, these findings suggest that *Bifidobacterium* can be used as a delivery vehicle that can be easily genetically engineered to synthesize proteins of interest [69]. *Bifidobacteria* plays an important role in cancer prevention both in-vivo and in-vitro. Furthermore, some preclinical reports have shown its role in protecting DNA damage from carcinogens to inhibit the associated genotoxicities [70]. Few other reports have also described a protective role of *Bifidobacteria* through immune surveillance and host's immune system activation [71]. Preclinical melanoma treatment study in mice have revealed that oral administration of *Bifidobacterium* provides an additive effect on tumor regression [72]. Furthermore, tumor growth was significantly abolished when *Bifidobacterium* was used in combination with immune checkpoint inhibitors [72,73]. These anti-tumor effects were primarily manifested in the TME by increasing dendritic cell activation and CD8+ T-cell priming.

4. Classical cancer treatment regimen in conjunction with microbial therapy

Conventional cancer treatments include chemotherapy, radiotherapy, and immunotherapy. However, development of resistance to these therapies poses a major problem for improving overall survival of cancer patients. Both host and tumor secreted factors contributes in the development of resistance. As a result, there is an urgent need for the development of innovative therapies to combat the disease more effectively.

4.1 Radiotherapy

Radiation therapy has long been considered as one of the most effective cancer treatment. Radiotherapy can cause damage to both healthy and malignant cells but the primary goal of radiotherapy is to kill cancer cells while minimizing damage to normal cells. Radiotherapies are not very successful against solid tumors, as these tumors tend to have hypoxic regions that are resilient to radiation effects[74,75]. Use of anaerobic (obligate or facultative) bacteria can turn this limitation into an advantage against malignant cells. Bacterial therapy against solid tumors is an emerging area of investigation where lower and safer doses of ionizing radiations can be utilized to effectively kill cancer cells causing less to no harm to normal healthy cells. Importantly, bacteria-based anti-tumor therapies also alleviates the risks associated with gene therapies involving genetic

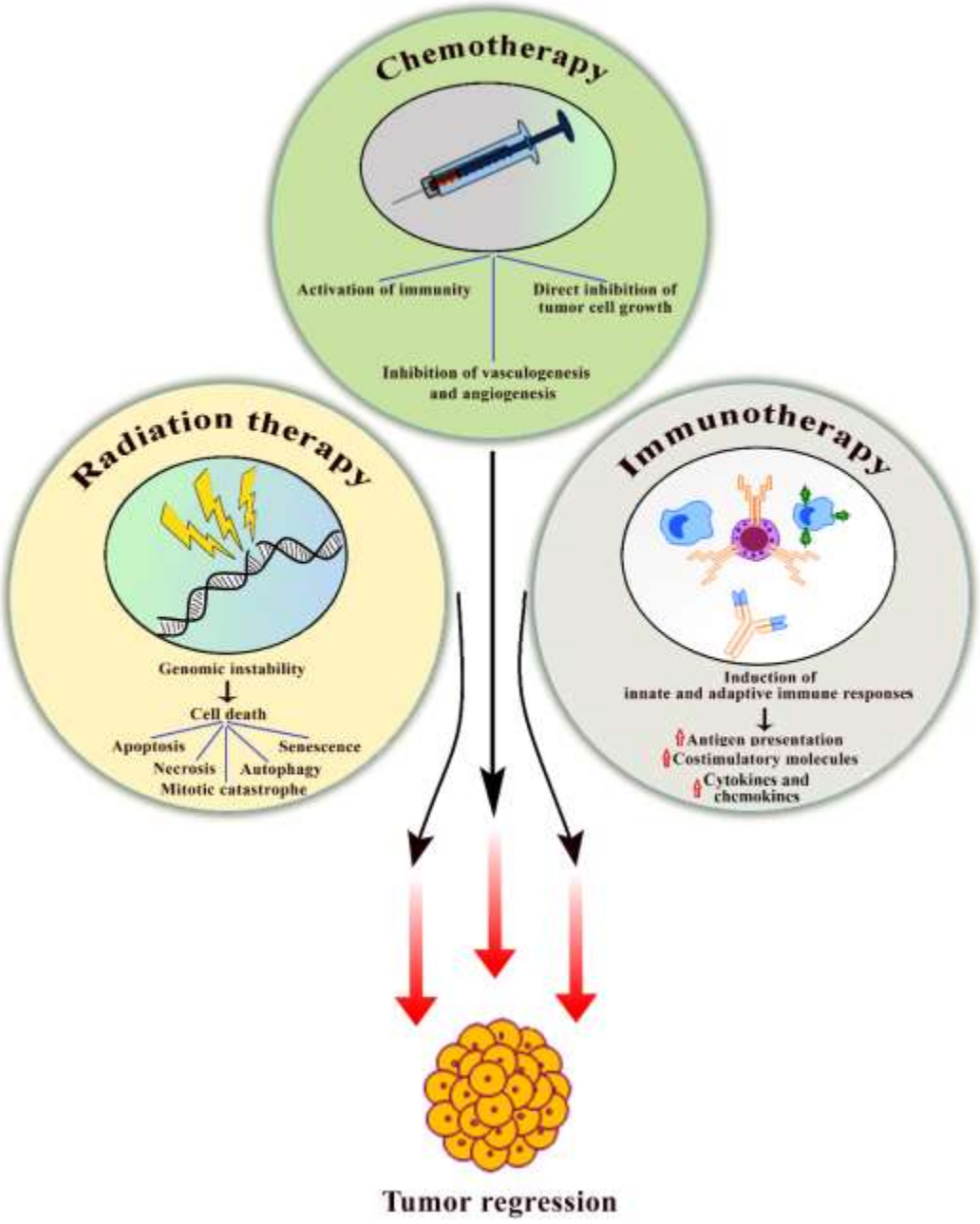

Figure 2. Schematic diagram highlights the use of bacteriotherapy in conjunction with classical cancer therapies and associated distinct mechanisms for targeting cancer cells. Over the last several years, bacteriotherapy in combination with traditional cancer therapies such as chemotherapy, radiotherapy, and immunotherapy has shown promising results. Bacteria inhibits tumor cell growth and induces apoptosis or senescence. Bacteria also helps in the activation of innate and adaptive immune system for the clearance of malignant cells.

alterations of cancer cells [74,75]. Human cancers have unique TME where tumor and stromal interactions play an important role in tumor growth and progression [76]. TME is immune-dormant with altered metabolic state that allows the bacteria to colonize and thrive successfully

within these tumors. Genetically engineered *Salmonella* holds the ability to selectively thrive and proliferate in the tumor microenvironment [33,77]. *Salmonella* can also express effector genes including the herpes simplex thymidine kinase (HSV-TK), which converts inactive GCV (ganciclovir) into its activated form leading to induction of anti-tumor activity [78]. The combination of radio and bacteriotherapy has yielded much more than just additive anti-tumor effects. Attenuated *S typhimurium* strain ΔppGpp (guanosine 5'-diphosphate-3'-diphosphate) was genetically modified to encode a cytotoxic protein named as cytolysis A (ClyA) [79]. ClyA is a pore-forming hemolytic protein that is normally absent in *S typhimurium*. The ClyA is cytotoxic to mammalian cells and induces caspase-mediated apoptosis. In addition, *Clostridium novyi* (*C novyi)*-NT spores have been used in conjunction with radiotherapy to treat tumors[77]. *C novyi*-NT spores alone had limited anti-tumor efficacy whereas in combination with radiotherapy yielded significant tumor regression. Combination therapies involving radiation and bacteria are significantly safer and can be successfully utilized to target selectively cancer cells without harming the adjacent normal healthy cells (**Fig. 2**). Certain probiotic bacteria, such as *Lactobacilli* and *Bifidobacteria*, have been shown to reduce the negative effects of radiotherapy[80]. These probiotic bacteria have also been shown to aid in the recovery of damaged normal cells.

4.2 Chemotherapy

Chemotherapy still represents one of the mainstays of cancer treatment, in spite of causing systemic toxicity [81]. Nowadays, chemotherapy-based treatments are gradually being set aside with the emergence of newer and effective treatment options like immunotherapy. The major limitation of chemotherapy is the development of drug-resistance, which occurs after the completion of the treatment course, when some of the residual cells acquire a higher invasive and metastatic potential, worsening the disease outcome. Henceforth, there is a greater need for the development of newer therapeutic targets and treatment strategies to combat the disease. Combination of chemo and bacteria-based therapy seems to be an attractive therapeutic strategy. Development of hypoxic regions is a hallmark feature of many human solid tumors, which are often resistant to anti-tumor therapies [74,75]. Importantly, bacteria not only holds the ability to thrive in such environment but also aid in specific killing of malignant cells.

Most commonly, these bacteria help in sensitizing the cancer cells, thereby increasing the therapeutic efficacy of chemotherapy drugs [74]. In addition, these bacteria can also be used either directly or as vectors. Bacteria produces a number of toxins that show anti-tumor activity and mediate killing of cancer cells. *Botulinum* neurotoxin (BoNT) has been reported to target and lyse tumor cells directly; however, complete destruction of tumor cells has not been achieved [82]. Interestingly, bacteria like *S choleraesuis* alone has demonstrated higher anti-cancer abilities and addition of chemotherapy drug cisplatin prolonged the development of tumor and improved overall survival [83]. Therefore, addition of chemotherapy can compensate this insufficient lysis of target tumor cells and thereby, improve overall anti-tumor efficacy. These bacteria can also be used for developing bacterial cancer vaccines. With the advancement of genetic engineering, bacteria can be easily genetically engineered for specific targeting of cancer cells. Such genetically improved bacteria can specifically target cancer cells and can be used alone or in combination with canonical cancer treatment regimens (**Fig. 2**). Common side effects of chemotherapy are often associated with gastrointestinal tracts [84]. Probiotic bacteria like *lactobacillus* plays an important role in culminating gastrointestinal side effects of chemotherapy [85]. Use of probiotic bacteria during chemotherapy has resulted in fewer intestinal side effects in cancer patients.

4.3 Immunotherapy

Cancer cells avoid the immune mediated clearance through a variety of mechanisms including recruitment of immunosuppressive cells, secretion of cytokines/chemokines, expression of immune checkpoints, and downregulating major histocompatibility 1 (MHC1) surface presentation [86]. Tumor-infiltrating immune cells in solid tumors are mainly derived from the innate immune system including macrophages. Tumor associated macrophages (TAMs) are the most common type of immune cell found in the tumor microenvironment [87]. TAMs are known to promote tumor growth and metastasis [87]. Macrophages are classified as M1 or M2, and TAMs are shown to have an M2-like phenotype. [87]. Therefore, M2-like TAMs are considered as promising targets for cancer immunotherapy. Macrophages are highly plastic in nature and can rapidly change their phenotypes in response to their local signals in order to facilitate local tumor growth and distant metastasis [88]. Cancer immunotherapy is based on boosting the host immune response that can specifically recognize and kill malignant cells. Cytotoxic T cells including CD8+ and CD4+ cells play predominant role in clearance of tumor cells. Moreover, combination of immune- and bacteriotherapy seems to be an attractive strategy for inhibiting tumor growth and progression (**Fig**. **2**).

C novyi infection results in the production of various heat shock proteins including (Hsp70) [89]. Hsp70 is mainly released from necrotic cells while bacteria release PAMPs [90]. Hsp70 predominantly aids in the maturation of professional antigen presenting cells (APCs) such as dendritic cells [74,91]. These professional APCs are very important for the development of adaptive immunity, particularly cell-mediated immunity. PAMPs are unique molecules shared by various microbes that are required for bacterial survival but are absent in mammalian hosts. Bacterial lipopolysaccharide (LPS) and endotoxins are classical bacterial PAMPs. PAMPs elicit innate responses, which protect the host from infection. Inflammasomes are also activated by PAMPs, particularly NLRC4 inflammasomes [92]. Inflammasomes are oligomer protein complexes that activates inflammatory signaling [93]. Moreover, PAMPs are known to interact with toll-like receptors (TLRs) for inducing the secretion of pro-inflammatory cytokines including IL-12 [94]. In addition, it enhances the expression of costimulatory molecules like CD40. Furthermore, these changes lead to the development of Th1 type immune response and increases the production of interferon gamma (IFN-γ) [95]. Th1-type immune response includes the activation of tumor reactive cytolytic CD8+ effector T cells for tumor clearance [96]. Bacteria like *S typhimurium* utilizes the type-3 secretion system (T3SS) for infecting and penetrating melanoma cells while T3SS mutant strains lack the ability to penetrate host cells [97]. *S typhimurium* does not completely kill infected target cells but their antigens are processed and presented over tumor cells that ultimately leads to their immune detection and clearance [97]. Furthermore, bacterial compounds including CpG oligonucleotides can also activate APCs especially dendritic cells in melanoma, which further helps in tumor cell killing [98]. *E. coli* strains are long been used for the production of recombinant proteins. They are also used for the delivery of tumor specific antigens in dendritic cells [99].

Cytokines and chemokines play important roles in tumor regression and immune activation. Several cytokines including IL-2, IL-15, IL-4, IFN-γ, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been delivered using genetically modified bacteria [9]. Delivery of IL-2 through attenuated *S. typhimurium* has been progressed to human clinical trials (https://clinicaltrials.gov/ct2/show/NCT01099631). Furthermore, attenuated *S. typhimurium* injected in a mouse model promotes the secretion of flagellin B from another bacterium, *Vibrio vulnificu*s in the tumor microenvironment that leads to macrophage polarization from M2 to M1 type [100]. M2-type macrophages are pro-tumor and anti-inflammatory in nature as opposed to M1-type macrophages that are anti-

tumor and pro-inflammatory. In addition, it promotes the production of anti-tumor cytokines including TNF-α and IL-1β. Another anti-cancer therapeutic approach to counter the immune suppressive mechanisms include the use of genetically engineered bacteria that make neutralizing nanobodies (nb) in the tumor microenvironment. These nb will help in boosting the overall anti-tumor immune response [101]. For instance, *E. coli* Pir1+ was genetically modified to secrete CD47 nb that stimulates tumor-resident T cells and ultimately caused tumor regression [102].

5. Conclusion

Canonical anti-cancer therapies face significant challenges because of the uncanny behavior of cancer cells. There are both benefits and drawbacks in using bacteria therapeutically for cancer therapy. Although conventional cancer therapies remain the mainstay treatment, microbial therapy has produced impressive results due to high specificity, manageable post administration, and anti-cancer effects. However, many challenges still prevail in using microbes as anti-tumor agents, which primarily includes toxicities associated with bacterial administration, DNA instability, modest efficiency, and selection of safer and effective bacterial strain. In the near future, genetically modified bacteria may overcome these limitations, making its therapeutic use for targeted anti-tumor therapies more feasible.

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