REVIEW ARTICLE

A Comprehensive Review of Bacteria-based Cancer Therapies

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Abstract

Target-based techniques are part of effective anti-cancer therapy tactics, and they have been shown to be quite effective when compared to more modern therapeutic approaches. Chemotherapy, radiation therapy, immunotherapy, and surgery are all part of traditional anticancer treatment. The ability of malignant cells to "survive even in adverse conditions such as low pH, hypoxia, and high pressure of interstitial fluid that make up the tumor microenvironment" (TME) is one of the main disadvantages of recent cancer treatment methods. We can easily overcome all the shortcomings of the most current treatment and contribute to boosting the effectiveness of target-based therapy by introducing bacteria as powerful innovative agents for anti-cancer therapy. Furthermore, since bacterial anti-cancer treatment is more effective in TME than other options, the sustainability and hyperplastic activity of these malignant cells may be significantly reduced. In addition, bacterial-based cancer treatment (BBCT) may be employed alone or in conjunction with conventional techniques to boost their effectiveness. This essay will discuss the latest developments, present difficulties, and possible future directions of BBCT.

Key words: Bacterial-based cancer therapy, bacterial vectors, cancer therapy, tumor microenvironment

INTRODUCTION

Definition of cancer

The pathologic buildup of clonally amplified cells coming from a common progenitor is what defines cancer. Mutations in DNA are the root cause of cancer, which may be either hereditary or acquired. Oncogenes, which promote cell growth, and tumor suppressor genes, which limit cell development, are often the culprits for unchecked cell proliferation. Additional genes that play a role in carcinogenesis include those that control DNA repair and programmed cell death (apoptosis). As stated in the most popular explanation of cancer genesis, the Knudson "2-hit" hypothesis, invasive cancer can only arise when a second mutation has taken place, and a mutation in one predisposing gene is required but not sufficient for malignancy.[1]

It is well-established that carcinogenic substances contribute to many human malignancies, even though the majority of *de novo* cancers lack a precisely identifiable etiology. Radioactive, chemical, infective, electromagnetic, and immunosuppressive agents include viruses, X-rays, gamma rays, and occupational, environmental, and medicinal chemicals.[2]

Cancer epidemiology

To design policies, conduct screenings, diagnose patients, and prevent health problems by gathering data on their causes and population patterns, a thorough and accurate understanding of the subject is necessary. We examine data supplied by the World Health Organization (WHO) and the American Cancer Society (ACS) (Montagnana and Lippi, 2017).[3] Since high-quality and relevant information is usually required in epidemiology (Global Health Estimates: Life Expectancy and Leading Causes of Death and Disability, n.d.).

According to the most recent data from the WHO (2016), cancer accounts for 244.6 million "Daly's," with 137.4 million men and 107.1 million women affected.

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Ischemic heart disease and stroke are the second and third leading causes of disability-adjusted life years, with 203.7 million and 137.9 million, respectively. On average, males have greater cancer prevalence than females (9.6% vs. 8.6%). Moreover, 124.2 million "Daly's" (or 50% of the total) are located in the 60+ age bracket in general. When looking at individuals aged 14 and under, the most common types of cancer are leukemias (37%), followed by cancers of the brain and nervous system (16%), and lymphomas (13%). The most prevalent malignancy encountered in persons aged 15–49 is breast cancer (13%), followed by liver cancer (12%) and lung cancer (9%). The most common malignancies in people aged 50–59 are lung cancer (18%), liver cancer (11%), and breast cancer (9%). In people aged 60 and up, the most common malignancies are lung cancer (21%), colorectal cancer (9%), stomach cancer (9%), and liver cancer (9%) (Life Expectancy and the Leading Causes of Death and Disability: Global Health Estimates, n.d.).

Overall, 18.08 million new cases were identified in 2018, with the most common ones being 1.37 million lung cases, 2.09 million breast cases, and 1.28 million prostate cases, according to the World Health Organization's Global Cancer Observatory 2018 (GLOBOCAN). Lungs (1.37 million), prostates (1.28 million), stomachs (0.68 million), and livers (0.60 million) are the most common organs in males. Among female cancer patients, the most common three types are breast (2.09 million cases), lung (0.72 million cases), cervix-uteri (0.57 million cases), and colon (0.58 million cases).

Breast cancer has the greatest incidence rate at 46.3/100,000 people, followed by prostate at 29.3/100,000, lung at 22.5/100,000, colon at 19.2/100,000, cervix-uteri at 13.1/100,000, and stomach at 11.1/100,000, according to GloboCan. (Cancer Today, n.d.).[5]

Mortality rate

While certain cancers have a greater mortality rate than others, GLOBOCAN reports that the top causes of cancer-related fatalities do not share a frequency rank. Most fatal malignancies are those of the lung (including the trachea and bronchi), liver (involving the intrahepatic bile ducts), and stomach. The fatality rates for male malignancies are as follows: Greatest for lung, liver, and stomach cancers; second highest for esophageal and prostate cancers; and seventh and eighth highest, respectively. Cancers of the breast, lungs, stomach, and colon organs account for the majority of cancer-related deaths in females. Among those aged 0–74, 10.6% die from cancer, with 12.7% of males and 8.7% of females succumbing to the disease. Females have a 1.41% breast cancer risk, a 1.32% lung cancer risk, and a 0.77% cervix-uteri cancer risk; males have a 3.19% lung cancer risk, 1.46% liver cancer risk, and 1.36% stomach cancer risk. Pancreatic cancer has the highest mortality rate at 94%, followed by liver and intrahepatic bile ducts at 93%, the esophagus at 89%, trachea-bronchi and lungs at 84%, and thyroid cancer at 7% (Life Expectancy and the Leading Causes of Death and Disability: Global Health Estimates, n.d.).[4]

CURRENT TREATMENT STRATEGIES

Antibiotics

The primary objective of cytotoxic chemotherapeutic drugs is to shorten the time it takes for cancer cells to multiply and then die off after each cycle of therapy.[6] Some antibiotics used in chemotherapy damage DNA while others function as powerful intercalating agents. Among the many antibiotics employed, actinomycin, and doxorubicin stand out. However, it has been shown that other medicines also work by stimulating and enhancing the host's pre-excitation defence system. Many chemotherapy medicines target DNA as their principal molecular target. There have been recent advances in the development of DNA-specific drugs, and these newer ones are expected to be even more targeted and successful in their targeting of DNA. Although DNA-targeted treatment has several drawbacks, one of them is that it primarily targets neoplastic cells – which are characterized by a high proliferation rate and genomic instability – while also damaging benign cells. As a result of DNA-targeted therapies, normal cells can withstand low levels of DNA damage, but they have a hard time dealing with the repair process.[7] Anthracycline, liomycin, actinomycin-d, and mitomycin are a few of the other antibiotics utilized in anti-cancer treatment.[8]

Anthracycline antibiotic doxorubicin, a hydroxyl derivative of daunorubicin, alters membrane function and generates free radicals to serve as an intercalating agent.[9] Solid tumors (e.g., ovarian, small lung, bladder, stomach, liver, and thyroid) are best treated with this drug, which is known to induce resistance in cancer cells and has serious side effects (e.g., dose-dependent cardiotoxicity). This agent needs to be changed so that a structural equivalent may be made that is less toxic and has a broader spectrum of action. Only two analogues, epirubicin and idarubicin, have received clinical approval.[10] Gastric, ovarian, breast, and lung cancers are all treatable with epirubicin. When compared to other medications in the same family, epirubicin has a lower risk of cardiotoxicity. Acute myelogenous leukemia is treated with idarubicin,[11] whereas early bladder cancer is treated with valrubicin,[12] which has a faster cell penetration rate than doxorubicin.

Reactive oxygen species are produced by DNA topoisomerase-2, which is inhibited in its activity by anthracyclines, which in turn impede DNA transcription and replication.[13] The cytotoxic side effects of anthracycline are the same as those of any other chemotherapeutic drug. There is also vomiting, nausea, and baldness. Myelosuppression and irreversible cardiotoxicity are two of the main toxicities.[14] By intercalating DNA, the complex compound actinomycin inhibits RNA

production.[15] Mitoxantrone, a quinone antibiotic, is less hazardous than anthracycline antibiotics.[16] Isolated from Streptococcus verticullis, bleomycinic acid, blma2, and b2 are members of the glycol peptide-derived antibiotic family; they are structurally related to tallysomycin and phleomycin; and they are utilized in the treatment of squamous cell carcinoma and malignant lymphoma.[17,18] In the absence of oxygen, the broad-spectrum antibiotic mitomycin acts as a bioreductive alkylating agent by forming covalent bonds to DNA.[19] It comes from *Streptomyces caespitosus* and has been isolated. The medication attaches to a single DNA strand for alkylation, and the process involves cross-linking two complementary DNA strands. Reactive oxygen species, or ROA, may also be formed. It often exhibits unexpected and dose-dependent adverse effects. Almost all patients will have some ulcer, anorexia, necrosis, etc. Along with pulmonary responses, this therapy is associated with renal failure and anemia.[20]

Anti-metabolites

Apoptosis in neoplastic cells is caused by DNA-damaging agents, which include anti-metabolites.[21]

The treatment result is determined by events generated by signalling contacts, which in turn affect the gene regulatory factor in the cell death machinery. The susceptibility of cells leading to cell death is proportionate to this factor.[22,23]

- 5-flurouracil(fura), analog of 5-flurouracil(fura), and folate-based inhibitors of thymidylate synthase

On the studies conducted on anti-metabolites such as 5-flurouracil (fura), 5-flurodeoxyuridine (FdUrd), capecitabine (prodrug of fura), fura and FdUrd are commonly used in colorectal cancer treatment and have metabolisms within the cell.[24]

Mechanism of action

In the presence of 5,10-methylene tetrahydrofolate, the inhibiting effect of 5-fluorodeoxyuridine's metabolite 5-fluorodeoxyuridylate on thymidylate synthase leads to a decrease in thymidine 5'triphosphate and an increase in 2'deoxyadenosine-5 triphosphate, the latter of which induces DNA damage and S-phase arrest.[25,26] Misincorporation of 2' deoxyuridine 5' triphosphate (dutp) or 5-fluorodUTP (fdutp) into DNA is another possible complication.[27]

In contrast to DNA-directed cytotoxicity, which is crucial in the chemotherapeutic response, 5-fluorouridine triphosphate incorporation into RNA inhibits RNA processing, leading to fura toxicity in normal gastrointestinal tissues.[28] Thymidylate synthase (ts) inhibitors have been the subject of substantial research into cell death, which is impacted by the p53 tumor suppressor gene.[29] As a response toward thymidylate synthase inhibition, S-phase arrest occurs.[30]

Anti-metabolite side effects

Hyperbilirubinemia, hand-foot syndrome, and diarrhea are the most prevalent adverse reactions to capecitabine monotherapy that occur at dosage limits. Some people have had symptoms such as myelosuppression, weakness, nausea, and stomach discomfort. Medical care of stomatitis, alopecia, and neutropenia was necessary when capecitabine was compared to bolus fu/lv delivery; however, hand foot syndrome was more common with the former. In addition, it has been seen to increase the blood phenytoin level and the international normalized ratio in individuals taking warfarin and phenytoin at the same time.

Dose

During the first 2 weeks of each 3-week cycle, the US Food and Drug administration-approved dosage of capecitabine for breast cancer and metastatic colorectal cancer is 1,250 mg/m^2 taken orally twice daily, separated by 12 h.[31]

Natural products

Taxol has shown efficiency in the treatment of refractory ovarian, breast, and other cancers. Synthetic modification of podophyllotoxin has led to the discovery of etoposide, which is found to be effective for lung cancer and testicular cancer. Isolated product from *Camptotheca*-acuminate camptothecin has also been studied.

Other molecules - vincristine, colchicine, ellipticine, lapachol, flavopiridol.

Ellipticine, which is a pyridol indole alkaloid and is isolated from the leaves of *Ochrosia* species. The most important chemotherapeutic target includes DNA topoisomerase; different groups of neoplastic agents identify their biochemical target as topoisomerase-I and topoisomerase-II.

Etoposide analogues

Etoposide is a potent topoisomerase inhibitor and a derivative of podophyllotoxin. Etoposide phosphate, or etopophos, is a brand name for anti-cancer medications. Molecular analyses informed the synthesis of several semi-synthetic etoposide analogue derivatives. A 4-amino-epipodophyllotoxin derivative, Gl-333, has a p-nitroaniline group at the 4 b position. The way they work is by blocking topoisomerase-Â½, which leads to DNA double-strand breaks and the G2 phase arrest.

The specific side effects of platinum drugs can be grouped into 7 categories:

1. Nephrotoxicity
2. Ototoxicity
3. Neurotoxicity
4. Cardio toxicity

5. Hematological toxicity
6. Hepatotoxicity
7. Gastrointestinal toxicity.

Nephrotoxicity

Acute kidney damage or hypomagnesemia is one of the frequent nephrotoxic side effects of cisplatin, seen in 90% of patients. Damaged kidneys are unable to reabsorb magnesium, leading to hypomagnesemia.

Ototoxicity

A loss of hearing due to injury to the inner ear is known as ototoxicity. Tinnitus, vestibular disturbance, and Otalgia (ear pain) are among the possible adverse effects. There is a direct correlation between the frequency of hearing loss and the dosage or cumulative dose of cisplatin.

Neurotoxicity

Pain, weakness, or numbness in the limbs might be symptoms of neurotoxicity, which is damage to the neurological system. Oxaliplatin and cisplatin both have a high incidence of neurotoxicity.

Cardiotoxicity

Cardiotoxicity refers to toxic effects on the heart, whether they harm the heart muscle directly or alter the sinus rhythm electrophysiologically. Among these symptoms are pericarditis, chronic heart failure, myocardial infarction, angina, cardiac ischemia, diastolic abnormalities, thromboembolic events, and silent and symptomatic arrhythmia.

Hematological toxicity

Among the hematological side effects are anemia, leukopenia, neutropenia, thrombocytopenia, all of which impact the bone marrow's ability to produce blood cells.

Hepatotoxicity

Chemotherapeutic medicines may produce a variety of liver-damaging effects, collectively known as hepatotoxicity. Sinusoids are blood veins that provide oxygen to the liver; natural products such as cisplatin and oxaliplatin harm them. Swelling and discomfort in the abdomen, as well as a narrowing of the sinusoids and a decrease in liver function, are symptoms of this injury.

Gastrointestinal toxicity

The use of platinum medications may lead to gastrointestinal side effects such as nausea, vomiting, and dyspepsia, which are symptoms of chemotherapy. Despite the frequency of side effects associated with cisplatin medicines, individuals treated with the radiation seldom have diarrhea and stomach discomfort.

Natural product

Refractory, ovarian, breast, and other malignancies have all responded well to Taxol, one of the most remarkable agents. Another prominent molecule includes podophyllotoxin. Etoposide, a treatment for lung and testicular small cell tumors, was created by synthetically altering this chemical. Separately, camptothecin from *Camptotheca acuminata* has also been the subject of much research. An extensive list of other significant compounds is provided, including vincristine, vinblastine, colchicine, ellipticine, lapachol, flavopiridol, an Indian semi-synthetic counterpart of the chromone alkaloid rohitukine, a pyridoindole alkaloid derived from *Ochrosia* species' leaves, and many more. The review also covers the less well-known sub-Himalayan plant species. A pyridoindole alkaloid known as ellipticine was found in the leaves of several *Ochrosia* species. Some tried to enhance ellipticine's pharmacological profile of ellipticine by reducing its hazardous side effects. One of the most important and prominent goals of cancer chemotherapy is to inhibit DNA topoisomerases. A wide variety of anticancer drugs have topoisomerase I and II as their biological target. New topoisomerase-directed agents, such as 9-aminocamptothecin, are being developed, and our understanding of their molecular and cellular mechanisms of action, clinical utility, and the structure-activity relationship is helping us to understand better how to test for topoisomerase involvement.[32]

Current challenges in cancer treatment

Two revolutions that altered cancer therapy were immune oncology and targeting actionable changes in oncogene-driven tumors. However, still there are many important challenges in both the fields. The use of next-generation sequencing is on the rise in clinical research, particularly for molecular pre-screening. However, there are a number of challenges associated with interpreting genomic data, and these concerns are rather extensive, making their clinical use challenging. Two major obstacles to the effectiveness of precision oncology are acquired resistance and handling tumor heterogeneity. Thanks to immunological checkpoint inhibitors such as monoclonal antibodies against cytotoxic T lymphocyte antigen-4 and anti-programmed death cell protein-1/programmed death cell ligand-1 [pd-1/l1]. Validation of predictive markers identifies the following subgroups and aids in optimising therapy delivery and selection; nevertheless, they are not used for the whole population. A future precision approach for cancer therapy, combining medications that target molecular change and cancer hallmarks to produce long-term survival gains, will be both the most promising and the most difficult.[33]

BACTERIA USED IN CANCER THERAPY

History

Nearly a century ago, scientists discovered that bacteria may be used in cancer treatments. Accidental erysipelas infections

(*Streptococcus pyogenes*) in hospitalized patients were associated with regression in certain malignancies, according to distinct observations made by German doctors Nauts Helen Coley and Busch.[34] William Coley, a doctor from the United States, saw a patient whose neck cancer had improved after contracting erysipelas. Toxins produced by bacteria were first documented by him as a potential treatment for advanced cancer. Lymphoma, sarcoma, carcinoma, melanoma, and myeloma were all effectively treated with a vaccination that stimulated an infection with fever in the late 1800s. The vaccine consisted of two dead bacterial species, *Serratia marcescens* and *S. pyogenes*. Many patients with advanced cancer were found to have prolonged regression.[35,36]

Bacterial therapy

Scientists have shown that the Clostridium genus of anaerobic bacteria feeds on malignant cells, which are low in oxygen. However, these bacteria die when they come into touch with the oxygenated side of the tumor, thus they have no effect on healthy tissues.[37] These results suggest that bacteria might be useful as agents that target cancer. However, chemotherapeutic treatment must be administered in conjunction with this therapy since bacteria do not devor all components of cancerous tissues. The use of endotoxins and other bacterial products in cancer therapy has previously been investigated. Bacterial toxins may function as tumor distractors, and immunological toxins derived from bacteria can form the basis of cancer vaccinations. They have further potential use as gene therapy vectors and anti-cancer medication delivery agents. The aforementioned methods may be used using anaerobic bacteria's spores because, as previously stated, only spores can penetrate the tumor's oxygen-deficient region, where they can then germinate, grow, and eventually become active. Bacterial gene-guided enzyme pro-drug treatment and genetically engineered bacteria for tumor selective destruction show promise.[38]

Bacteria as tumoricidal agents

Using live, attenuated, or genetically modified non-pathogenic bacteria as potential anticancer medicines has begun to gain traction. These bacteria may either directly cassssuse tumors or carry substances that cause tumor's. Animal studies have shown that pathogenic anaerobic *Clostridia* can thrive in the tumor's necrotic (anaerobic) areas rather than normal tissues, leading to tumor regression. However, this process is accompanied by acute toxicity, and most of the animals get sick or die as a result.[39] In light of this, researchers began to focus on a Clostridium strain that is not harmful, known as "m55," which showed that it could colonise anaerobic cancer areas after intravenous administration but had no effect on tumor size reduction.[40] Recent studies have investigated the ability of several anaerobic bacterial species, such as pathogenic *Clostridia*, lactobacilli, and bifidobacteria, to colonise animal malignancies in a controlled environment. *Clostridium novyi* showed significant anti-tumor benefits; however, these trials were also deadly. The attenuated strain *C. novyi-nt* was produced by deleting a gene that encoded a lethal toxin. While it did exhibit some encouraging benefits, it was also hazardous. Consequently, typical chemotherapy medicines such as dolastatin-10, mitomycinc, vinorelbine, and docetaxel were administered with *C. novyi-nt* spores.

Although it was not without its share of animal fatalities, combination bacteriolytic therapy–more often known as Cobalt–had significant anti-tumor benefits.[41] Radiation, radioimmunotherapy, and further chemotherapy have all been investigated in conjunction with *C. Novyi* in experimental tumor models.[42] Future cancer therapies that combine multi-modality approaches offer potential based on the results. To enhance the release of medications contained in liposomes inside malignancies, *C. novyi-nt* has been used due to its evident ability to rupture cell membranes. It has been discovered that the enhanced medication release is caused by liposomes, an enzyme produced by bacteria. A single dose of liposomal doxorubicin mixed with *C. novyi-nt* resulted in the astonishing elimination of tumors in mice with large, established tumors, which has inspired further study in the field.[43] To access the tumor's undervascularized regions, *C. novyi-nt* was administered in conjunction with anti-microtubule medications. The findings demonstrated that microtubule destabilising medications, such as hti-286 and vinorelbine, significantly decreased blood flow to tumors, hence increasing the hypoxic zone conducive for spore germination, in contrast to microtubule stabilising therapies, such as taxanes, docetaxel, and mac-321.[44] So far, Bacillus Calmette–Guerin (bcg) is the most successful bacterial therapy for superficial bladder cancer. A new strain of *Salmonella* Typhimurium, vnp20009, has been developed for cancer therapy purposes. The loss of two of its genes – msbb and puri – led to its complete attenuation and reliance on external purine supplies for life, since it prevented toxic shock in animal hosts. This need means that the bacterium cannot replicate in normal tissues such as the liver or spleen, but it may flourish in tumors that contain purine. Several experimental malignancies showed long-lasting action against this vector, and it might target metastatic lesions as well.[45] In comparison to Costridium and Bifidobacterium, *Salmonella* has the benefit of being able to grow in both aerobic and anaerobic conditions, which demonstrates its effectiveness against small tumors. Patients with cancer have shown encouraging responses to vnp20009 in phase 1 clinical trials. *Clostridia* and Bifidobacterium are two examples of live, attenuated bacteria that will likely be studied in future human therapeutic trials. Some novel bacterial strains are now under investigation for their potential as anticancer agents, including *Salmonella* choleraesuis, *Vibrio cholerae*, *Listeria monocytogenes*, and *Escherichia coli*.[46]

Bacteria as a vector for gene therapy

One major drawback of using bacteria as anti-cancer medications is that, even at therapeutic doses, they are toxic, and reducing the amount makes them less effective. The

primary obstacle in cancer gene therapy is the exact targeting of treatment to a solid tumor.

One way to get past, these limits are to utilize microorganisms that have been genetically engineered to express a certain therapeutic gene. In the tumor microenvironment, these bacterial vectors may preferentially produce the target protein, making them a powerful adjuvant therapy to many cancer therapies. Bacteria perform the role of vectors or carriers to transport cytotoxic peptides, therapeutic proteins, pro-drug converting enzymes, anticancer medicines, and other medicinal substances to solid tumors.[47]

Bacteria as carriers of tumoricidal agents

A mutant strain of *Salmonella* Typhimurium 4550 has been engineered to express interleukin-2 for the treatment of liver cancer in preclinical models. This strain contains mutated *cya/crp* genes, which encode proteins necessary for the control of cyclic amp levels.[48] It is believed that cytokines might be administered locally to the liver with a decrease in hepatic metastases, utilizing *Salmonella* Typhimurium's attenuated form, because this strain generally colonizes the liver. Among the therapeutic proteins cloned and manufactured in vnp20009 are Tnf- and platelet factor 4 fragment.[49] The genes for hil-12, hgm-csf, mil-12, and mgm-csf were cloned into the auxotrophic *Salmonella* Typhimurium sl3261 using a cytomegalovirus (cmv) promoter. Oral injection of *Salmonella* expressing mgm-csf or mgm-csf + mil12 led to tumor regression in mice with Lewis lung carcinomas.[50] Functional tnf has been cloned and expressed in *Clostridium acetobutylicum*. Recently, *Bifidobacterium adolescentis* supplied the antiangiogenic protein endostatin. Systemic administration of its spores through the tail vein of tumor-bearing mice resulted in potent angiogenesis inhibition and reduced tumor development.[51]

Bacterially directed enzyme pro-drug therapy

This technique avoids the unacceptable side effects of bacterial treatment using anaerobic bacteria engineered with an enzyme that can convert a non-toxic prodrug into a toxic drug. Bacterial proliferation in the tumor's necrotic and hypoxic regions is the only reason the enzyme is expressed there. As a result, the transformation of a systemically delivered prodrug into the lethal drug occurs only in the tumor.[52] There are numerous enzyme/prodrug combinations. The enzyme nitro reductase (nr) and the enzyme cytosine deaminize (cd), which convert prodrug cb1954 into a DNA cross-linking agent and 5-fluorocytosine (5fc) into 5-fluorouracil (5fu), have both been tested with clostridium sporogenes. Despite these combinations' ability to kill tumor cells *in vitro* and deliver considerable amounts of enzymes to model tumors, the effects *in vivo* have been disappointing so far. Evidence that cancers may selectively absorb the active exogenous enzyme has also been shown in cd generated in *C. acetobutylicum*.[53] The cloning and production of cd in the same clostridium strain have been shown in recent studies, and the vascular targeting medication combretastatin a-4 phosphate was found to significantly enhance cd expression. The amplification might be due to the fact that tumors have an expanded necrotic area.[54] In addition, the *Salmonella* vector has been used to successfully pair nr with cd, leading to positive results *in vivo*. Both are now undergoing phase I clinical trials with cancer patients. By producing an *E. coli* cd utilising vnp20009, an attenuated strain of *Salmonella* Typhimurium, as a bacterial vector, tumor amplified protein expression treatment (TAPET) facilitates the selective delivery of anticancer drugs to solid tumors. An increase in anticancer activity was seen in a purine auxotroph when the prodrug-converting enzyme hsv-thymidine kinase (tk) was enhanced in the presence of ganciclovir, the corresponding prodrug.[55] A preferential buildup of hsv-tk was seen in subcutaneously implanted murine colon 38 tumors when expressed in vnp20009.[56] Coupling *Salmonella* with the enzyme carboxypeptidase g2 (cpg2) makes it possible to convert many mustard prodrugs into DNA cross-linking agents. Results showing significant activity in tumor after *in vivo* administration have prompted further research. Both the prodrug and the activated drug must be able to cross biological membranes for the treatment to be highly effective, since the prodrug must be activated within bacterial cells before penetrating the cancer cells. Research has shown that pbles100-s-ecd-transfected b. Longum produces cytosine deaminase in hypoxic tumors, which is an effective prodrug-enzyme treatment.[57]

Bacterial toxins for cancer treatment

Cancer treatments using bacterial toxins have been studied to a certain degree. Toxins produced by bacteria may either kill cells directly or, at low doses, alter cellular processes that control proliferation, apoptosis, and differentiation. These alterations are associated with carcinogenesis and may cause cellular abnormalities or disrupt normal cell control.

Cytolethal distending toxins (cdts) and cycle inhibiting factor (cif) are examples of cell-cycle inhibitors that are believed to reduce immunity by blocking the formation of lymphocyte clones. In contrast, cell-cycle promoters such as cytotoxic necrotizing factor (cnf) hinder cell differentiation while simultaneously encouraging cell proliferation.[58] The host eukaryotic cell cycle is disrupted by a class of bacterial toxins called cyclomodulins. One example is the cell-cycle stimulant cnf, which is secreted by certain bacteria such as *E. coli*. Cnf initiates DNA replication and the g1-s transition. Still, there is no change to the total number of cells. Rather, the cells undergo multi-nucleus division, which may be caused by the toxin's ability to inhibit cell death and differentiation.[59] Among the gram-negative bacteria that carry cdts are *Salmonella* Typhimurium and Campylobacter jejuni, and among the enteropathogenic and enterohemorrhagic strains of *E. coli*, cif is present. The anti-tumor effects of toxins are probably less severe than those of

more traditional tumor therapies. Thus, bacterial toxins may enhance the efficacy of cancer treatment, either alone or in integration with chemo drugs or radiation.[60]

Bacterial toxins binding to tumor surface antigens

The surface of cells that are making the precursor of heparin-binding epidermal growth factor (hb-egf) becomes infected with diphtheria toxin (dt). The dt-hb-egf complex is absorbed by clathrin vesicles during endocytosis. To form the catalytically active toxin dt fragment a, dt undergoes a series of posttranslational modifications. This leads to the catalytic ribosylation of elongation factor-2 (ef-2), which in turn suppresses protein synthesis, cell lysis, and/or the start of cell death.[61] Protein synthesis is inhibited by pseudomonas exotoxin a, which acts similarly to dt by catalytically ribosylating ef-2. This toxin shows great promise as a targeted cancer treatment due to its very high cytotoxicity; 0.3 g is the fatal dosage when administered intravenously to mice.[62] Clostridium perfringens, a kind of bacteria that causes gastroenteritis, produces clostridium perfringens enterotoxin (cpe). It is believed that cytotoxicity requires the n-terminal of cpe, but high affinity binding to the cpe receptor (cpe-r) requires the c-terminal.[63] Studies have shown that cpe in its purified form has a rapid cytotoxic effect on pancreatic cancer cells, leading to tumor necrosis and inhibiting tumor growth in living organisms. Scientists are studying its potential effects on malignancies of the stomach, breast, and colon. Before its potential use in systemic cancer treatment, it is essential to establish that cpe is effective in the long run and does not cause any harm to living organisms. Botulinum neurotoxin (bont) momentarily dilates tumor arteries, allowing radiation and chemotherapy to more efficiently destroy cancer cells, according to a new research. Bonts may influence the tumor's microenvironment rather than specifically targeting cancer cells for cytotoxicity.[64] Several bacterial toxins, such as cholera toxin, shiga-like toxins, ac-toxin from Bordetella pertussis, and alfa-toxin from staphylococcus aurous (u-1690), are now being studied using two cell lines: Mesothelioma cells (p31) and small lung cancer cells. The first results with ac-toxin showed that the toxin considerably increased apoptosis and that the cytotoxicity increased with increasing dosage in both cell lines. However, apoptosis was not caused by cholera toxin.[65]

Bacteria as immunotherapeutic agents

There is much hope for cancer immunotherapy as a novel and effective therapeutic option. Tumors are immunogenic; hence, the goal of the immunotherapeutic strategy is to eliminate cancer cells by stimulating the immune system. However, the biggest problem is that tumors may learn to evade the immune system via tolerance development, even if they are very weakly immunogenic and the body sometimes confuses them with self-antigens. Thus, microbes are used by one of the most recent immunotherapeutic approaches to increase the immunogenicity of cancer cells.[66] *In vitro* and *in vivo* studies have shown that *Salmonella* Typhimurium may infect cancer cells, triggering an immunological response. Although reduced, it is quite intrusive. Attenuated *Salmonella* Typhimurium has been shown to successfully infiltrate melanoma cells, which may then express antigenic signals originating from bacteria and function as targets for anti-*Salmonella*-specific T lymphocytes. Nonetheless, animals with tumors fared better after being vaccinated with *Salmonella* Typhimurium before receiving intratumoral *Salmonella* injections.[67] The capacity to modulate infection immunity and decrease the growth of experimental melanomas has been shown by attenuated strains of *Salmonella* Typhimurium that have been genetically engineered to express murine cytokines. Studies have shown that cytokine-expressing *Salmonella* organisms are more able to prevent tumor development compared to their non-expressing parental strain.[68] Animal models now exhibit immunity to *Salmonella* and *Listeria* thanks to the addition of tumor antigen DNA sequences. Evidence suggests that a cd8 cytotoxic t-cell response specific to tem8 may be generated after oral administration of a xenogenic DNA vaccine expressing human tumor endothelial marker 8 (tem8) given by attenuated *Salmonella* Typhimurium. Results showing less tumor growth, protection of mice against potentially fatal insults to cancer cells, and reduction of tumor angiogenesis all lend credence to the promise of antiangiogenesis immunotherapy.[69] Severe inflammation and leukocytosis are symptoms that have been linked to *C. novyi*. Furthermore, inflammation is known to have anti-cancer effects. Systemically given *C. novyi-nt* spores destroy cancer cells in the area and trigger an inflammatory response by secreting cytokines such as il-6, mip-2, g-csf, timp1, and kc, which attract inflammatory cells such as monocytes, lymphocytes, and neutrophils. The inflammatory response helps destroy cancer cells and limits bacterial infection through producing enzymatic degraders such as proteases and reactive oxygen species. Ultimately, it eliminates any lingering cancer cells by triggering a robust cellular immune response. Phase 1 clinical study with *C. novyi-nt* spores and an antimicrotubuli medication has commenced. Due to its ability to robustly elicit innate and cell-mediated immunity, the cancer vaccine has used the facultative intracellular bacteria *L. monocytogenes* as its vector. Recombinant *L. monocytogenes* vaccine strain lm-np expressing nucleoprotein (np) from influenza strain a/pr8/34 showed promising therapeutic promise in preclinical tests by decreasing the development of macroscopic tumors of all sorts. Another recombinant listerial strain, lm-llo-e7, had remarkable curative effects in the majority of tumor-bearing mice. Furthermore, lm-llo-e7 is now being tested in clinical settings for its potential as an immunotherapeutic medication for cervical cancer.[70] The synergenic, aggressive 4t1 mouse breast tumor model has shown that a vaccination expressing truncated listeriolysin o (llo) based on attenuated *L. monocytogenes* (lm) may remove all metastases and almost the whole primary tumor.[71] Several mouse tumor models using murine carcinoma cell lines in immunocompetent mice have shown that a recombinant strain of attenuated

Salmonella Typhimurium, which expresses a gene encoding light, a cytokine known to promote tumor rejection, inhibits the growth of primary tumorand the spread of pulmonary metastases. This suggests that a *Listeria*-based approach may be useful in this context. Achieving anti-cancer activity was not associated with any significant toxicity. Mycobacterium bovis bacillus Calmette Guerin (BCG-CWS) cell wall skeleton has been an effective adjuvant for immunotherapy in several cancer patients.[72]

DISCUSSION

Cancer is the leading cause of disability-adjusted life years (244.6 million), and the most common malignancies in the 15–49 years of age group are breast, liver, and lung cancers, according to data provided by the ACS and the WHO. The disease is more common in men. According to the WHO Global Cancer Observatory 2018 (GLOBOCAN), there were 18.08 million new cases of cancer in 2018. The most common types of cancer were lung, breast, and stomach cancers. Interestingly, the top causes of cancer deaths did not share a frequency rank; men and women died from lung, liver, and stomach cancers, respectively. Although the precise origins of cancer are still unclear, Hill propose that it is ultimately linked to a basic disruption of normal life processes or the variables that produce this disruption. However, there is reason to be optimistic about the future of our knowledge thanks to recent developments in cytogenesis and molecular biology. Propose that ionising irradiation, chemicals, heavy smokers, hormones, arsenic exposure, sexual activity, and hereditary factors are all variables linked to cancer. While mechanical variables like trauma are also not entirely disregarded, the link between the two is a persistent problem in medical and legal circles, making it all the more important to understand the origins of cancer in order to improve public health.

The authors Brockmann *et al*. talk about antibiotics are chemotherapeutic agents with a variety of modes of action, including acting as strong intercalating agents or damaging cell DNA; cytotoxic chemotherapeutic agents were developed and are now used to shorten the time it takes for neoplastic cells to multiply and die during treatment cycles. Among them, DNA-targeted treatments have shown the most promise, but they are not without their limits; this is particularly true when dealing with neoplastic cells, which exhibit both rapid cell proliferation and genetic instability. The regimen also includes alternative prescriptions for antibiotics with lower toxicity, such as epirubicin, idarubicin, and mitomycin, which have applications in treating squamous cell carcinoma and malignant lymphoma, in addition to antibiotics with solid tumor treatment applications, such as doxorubicin, which can cause severe side effects and toxicity. Anti-metabolites, including 5-flurouracil (fura) and 5-flurodeoxyuridine (FdUrd), which induce cell death in neoplastic cells, are detailed in Arcamone *et al*. Side effects such as hyperbilirubinemia, hand foot syndrome, diarrhea, myelosuppression, weakness, nausea, stomach discomfort, and exhaustion are prevalent with regularly used and very successful medications for colorectal cancer therapy, such as capecitabine.

Refractory ovarian and breast tumors may be effectively treated with natural products like Taxol, as mentioned by Moore *et al*. and Minotti *et al*. Endotoxin-free treatment for cancers of the testicles and lungs, The most popular natural products are ellipticine, camptotheca, vincristine, colchicine, lapachol, and flavopiridol; these are pyridoindole alkaloids derived from *Ochrosia* leaves that target DNA topoisomerase and other components. There are additional dangers associated with these treatments that might lower the quality of the therapy's final result. For example, platinum drugs can cause nephrotoxicity, ototoxicity, neurotoxicity, cardiotoxicity, hematological toxicity, liver toxicity, and gastrointestinal toxicity. Precision oncology is impeded by newly discovered concepts that promise to transform cancer therapy, such as oncogene-driven cancer treatment and immunological oncology, genomic interpretation, acquired resistance, and tumor heterogeneity. However, there are still challenges with combining medications that target molecular change and cancer hallmarks, even if this provides promise precision techniques.

It is necessary to emphasize and refine the potential of bacterial-based cancer treatment in this context, which includes bacterial products, spores, and genetically engineered bacteria. Research on this topic dates back over a century, to findings made by Nauts, who first brought the idea to light through their work documenting cancers and observing regression. More recent findings, such as anaerobic bacteria eating cancerous tissues with low oxygen levels and potentially acting as oncolytic agents, highlight the need to integrate it with current chemotherapeutic treatment strategies.

In recent years, there has been a surge in the use of non-pathogenic bacteria as prospective anticancer medicines. Strains such as "m55" and vnp20009, a *Salmonella* Typhimurium derivative, have shown promise in triggering tumor regression and dramatically lowering tumor size. Also showing promise as cancer therapies include combinations of bacteriolytic therapy, radiation, radioimmunotherapy, and chemotherapy. Although there are several challenges associated with using bacteria as anti-cancer medications, such as toxicity and precision targeting, genetically engineered microorganisms have the potential to transmit tailored therapies to solid tumors (Li *et al*.). Additional therapeutic proteins that they help produce and clone include tumor necrosis factor- and platelet factor 4-fragment. To avoid the side effects of bacterial treatment, anaerobic bacteria that have been engineered with enzymes may transform non-toxic prodrugs into harmful drugs. Consequently, techniques such as TAPET, which primarily target solid tumors with anti-cancer drugs, have been shown to exhibit substantial levels

of tumor activity upon *in vivo* administration, as reported in publications by Brüggemann and Gottschalk.

Toxins produced by bacteria may 1 day be used as cancer treatments if they can damage cells or modify cellular activities in a way that prevents carcinogenesis. Cytotoxic necrotising factor and cytolethal distending toxins are examples of cell-cycle promoters and inhibitors that may reduce the efficacy of the immune system and enhance the efficacy of cancer treatments. The cytotoxic and apoptotic effects of other bacterial toxins are being studied by Ansiaux and Gallez, Nougayrède *et al*., among which are Diphtheria toxin (dt), Pseudomonas exotoxin-A, cpe, which has an immediate effect on pancreatic cancer cells, and Botulinum neurotoxin, which momentarily widens tumor arteries, allowing radiotherapy and chemotherapy to have an unrealized potential for targeted cancer therapies. Research conducted by Ruan *et al*. and Kim *et al*. suggests that immunotherapy, which involves stimulating the immune system to eliminate cancer cells, could be a viable treatment option." The researchers demonstrated that *Salmonella* Typhimurium, a weakly immunogenic bacterium, infects cancer cells both in laboratory settings and in living organisms, leading to an invasive immune response. Cancer vaccine vectors including *Salmonella*, *L. monocytogenes*, and Candida novyi-NT spores have also shown promising therapeutic outcomes.

CONCLUSION

The development of new strategies is the need of the hour, and bacteria-based cancer therapy has the potential to offer a credible answer, given that cancer is a chronic disease whose management has been the longest-standing question to modern medicine. Its shown use across a range of fields, including tumoricidal agents, gene therapy vectors, tumoricidal medication carriers, immunotherapeutic agents, bacterial toxins, and more, lends credence to the idea that it should be embraced as a novel strategy to combat cancer. Cancer therapy will benefit from more research and advancements in these areas.

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