THE USES OF BACTERIA IN CANCER THERAPY

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing the bachelor degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all the main sources of help.

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Abstract

Cancer is one of the leading cause of death worldwide. Cancer treatment success remains a challenge due to the unique pathophysiology of solid tumors and the predictable emergence of drug resistance. Traditional cancer treatments, including radiation therapy, chemotherapy, and immunotherapy, have limitations. A new approach, bacteriotherapy, used alone or in combination with traditional methods, has shown positive effects in regression of tumors and inhibition of metastases. Bacteriotherapy is the use of live, attenuated strains of bacteria, toxins, peptides, and bacteriocins in the treatment of cancer. They're also commonly employed as a vector for delivering genes, peptides, or medicines to tumor targets. Surprisingly, it was shown that combining them with traditional therapy techniques can improve outcomes. In the era of genome editing, it is possible to create a new generation of cancer-fighting bacteria with fewer side effects and higher efficacy. The goal of this paper was to summarize what is currently known about bacteria's involvement in cancer treatment. Here, we reviewed the recent improvements in the development of engineered bacteria for cancer therapy, as well as further engineering techniques to enhance the delivery of therapeutic payloads and recent advances in the field of *Salmonella typhimurium (S. typhimurium)* and *Escherichia coli* (*E. coli*) mediated cancer therapy. Finally, we discuss past and ongoing clinical studies involving tumor-targeting bacteria.

Keywords

Bacteriocin, bacterila toxin, bacterial peptides, bacterial vector, Enzymes, Bacterial ghost, Immunotherapy, Cytotoxin protein melanoma, Colon cancer, Gastrointestinal Cancer, Lung cancer, Gastrointestinal microbiota, Mycobacterium bovis, *Salmonella typhimurium, E.coli, Magnetococcus marinus, Clostridium, Lactic acid bacteria*

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List of acronyms

1. **NMIBC**: Non-muscle invasive bladder cancer
2. **DT**: Diptheria Toxin
3. **HB**-ECG)-Heparin binding epidermal growth factor
4. **MDR**- Multidrug resistant
5. **TNF** - tumor necrosis factor
6. **TIFP** = Tumor interstitial fluid pressure
7. **MTB** = *Magnetococcus Marinus* MC1
8. **rhIFNα** = Recombinant human interferon α 2B
9. **HSVTK** = Herpes simplex virus thymidine kinase
10. **RTK** = Receptor tyrosine kinase
11. **ePNR** = E. coli purine nucleoside phoshorylase
12. **wtp53** = Wild-type p53
13. **MePdR** = 6 methyl purine 2′ oxyriboside
14. **MoPdR** = 6 meprine 2′ oxyriboside
15. **MeP** = 6-methyl purine
16. **MoP** = 6-mephrin
17. **CPG2** = carboxy peptidase G2
18. **CD** = cytosine deaminase
19. **5-FC** = 5-fluorocytosine
20. **5-FU** = 5-fluorouracil
21. **siRNAs** = small interfering RNAs
22. **Stat3** = Signal converter and activator of transcription
23. **BCG - *Bacille Calmette Guerin***
24. **NMIBC** - non-muscle invasive bladder cancer
25. **BMTT** - Bacteria mediated tumor therapy
26. **MTB** - Magnetotactic bacteria

27. C.=*Clostridium*
28. ***B.=****Bifidobacterium*
29. ***L.=****Listeria*
30. ***S.=****Salmonella*
31. ***La.=****Lactobacillus*
32. ***E.*** *=Escherichia.*
33. **TLR4** - Toll-like receptor 4
34. **RenCa** - Renal adenocarcinoma
35. **GI-** Gastrointestinal
36. **CRC** - Colorectal cancer
37. **HDAC** - Histone deacetylases
38. **LPS**- Lipopolysaccharide
39. **SCLC** - Small-cell lung cancer
40. **NSCLC** - non-small-cell lung cancer
41. **CTLA-4** - Cytotoxic T-Lymphocyte Antigen 4
42. **LAB**- Lactic acid bacteria
43. **BGs** - Bacterial ghosts
44. **APCs** - Antigen presenting cells
45. **NK** – Natural killer cells
46. **DCs** - Dendritic cells

CHAPTER 1

Introduction

Cancer is a significant cause of mortality and morbidity across the world. According to the World Health Organization's (WHO) most recent study, the number of new cancer cases might increase by more than half to 15 million by 2020 (Siegel et al., 2020). For example Lung, prostate, stomach, colorectal, and liver cancers are the most common malignancies in males, whereas breast, lung, colorectal, cervix, and thyroid cancers are the most common cancers in women. Cancer is caused by the transformation of healthy cells into tumor cells due to the suppression of growth control systems and the proliferation of neoplasm-producing cell clones. As a result, the primary therapy for cancer is apoptosis induction and tumor cell growth suppression (Baba and Câtoi, 2007).

Surgery, radiation, and chemotherapy are the most common and traditional cancer therapies. There are also some new treatments available, including as stem-cell therapy, immunotherapy, hormone-based therapy, and CD-based immunotherapy (Arruebo, 2011; Mathis, 2019). All of these traditional and modern therapies, however, have certain drawbacks and side effects, such as a lack of particular toxicity against normal body cells. Because it must be followed up with chemotherapy and radiation, the surgical method for tumors is a restricted and inadequate strategy on its own (Arruebo et al., 2011).

Researchers have been considering the possibility of bacteria and their products in treating malignant cells as a novel cancer treatment with low toxicity and no side effects for normal cells in the last decade. Several bacterial species were utilized in live, attenuated, or genetically modified bacteria, as well as bacterial products (such as bacterial peptides, bacteriocins, and toxins, enzymes) that may preferentially multiply in tumors and limit their growth (Roberts., 2014; Fujimori., 2006). Bacteria generate toxins that disrupt cellular signals, causing cell development to be disrupted. In addition, they have the potential to stimulate tumor growth by causing inflammation (Lax, 2005). Similarly, tumor-detecting bacterium can be used to detect tumor recurrence, evaluate therapy effectiveness, and detect the development of metastatic illness in a sensitive and less invasive manner (Panteli et al., 2015). Furthermore, Bacteriotherapy has been used to treat cancer throughout history, with the first example history going back to 1891, when William Coley injected a heat-inactivated combination of *Serattia marcescence* and *Streptococcus pyogenes* into a patient suffering from incurable cancer. The bacterial combination used to treat cancer was known as Coley's toxin, and it caused tumor shrinkage in many patients, as well as total cure in certain cases (30 out of 1000 patients). For the first time in the 1970s, an attenuated strain of *Mycobacterium bovis, Bacillus Calmette–Guérin (BCG),* was utilized as an intravesical therapy for the treatment and prevention of non-muscle invasive bladder cancer (NMIBC). In NMIBC patients, intravesical BCG leads in a

substantial decrease in cancer recurrence (Gontero, 2010; Zlotta, 2019). On the other hand, many bacteria have been shown to be strongly linked to tumor promoters and carcinogens, and a number of them have been demonstrated to be beneficial in cancer treatment.Moreover, *Lactococcus*, *Clostridia, Shigella, Bifidobacteria, Listeria, Vibrio, Salmonella,* and *Escherichia* have all showed considerable potential for tumor invasion and colonization, resulting in tumor clearance. Furthermore, some bacteria, such as *Clostridia* strains and *Bifidobacterium longum*, can survive and proliferate in the hypoxic environment of the tumor, causing it to die (Roberts, 2014; Fujimori, 2006). Scientists, on the other hand, have employed genetic engineering in bacteriotherapy to create treatments with higher effectiveness and fewer adverse effects. In light of the above, the attenuated form of auxotrophic mutants of *Salmonella typhimurium* (*S. typhimurium)* may penetrate and conquer many types of cancer cells in vitro, and multiply in hypoxic and toxic tumor areas in vivo (Zhao et al., 2006). Such advances in genetic engineering have cleared the path for the development of potential bacteria-based cancer therapies (Danino et al., 2015). Another advantage of bacteria as an anticancer agent is their ease of genetic manipulation, which allows bacteria to be programmed to produce and release antitumor chemicals as well as change their metabolic pathways (Sabzehali et al., 2017) Bacterial delivery of medicinal and anticancer drugs can help to destroy tumor cells by delivering anti-tumor vectors (Jiang et al., 2013). The purpose of this review was to provide a comprehensive study that looked at cancer therapy's potential as cancer target delivery vehicles, immunotherapeutic agents, and bacterial substance-mediated anticancer therapy with an emphasis on the various mechanisms involved in tumor targeting and tumor suppression as well as advances in the field of cancer therapy via *Salmonella, Escherichia coli, lactic acid* and how specific bacterial strains are used to eradicate different kind of cancer such as lung cancer, gastrointestinal cancer and moreover their previous and ongoing trials have been discussed .

Chapter -02

Research Methodology

Databases such as Google Scholar, PubMed and ScienceDirect were used to find out scientific literature relevant to the topic. While searching, keywords such as Bacteriocin, bacterial toxin, bacterial peptides, bacterial vector, Enzymes, Bacterial ghost, Immunotherapy, Cytotoxin protein melanoma, Colon cancer, Gastrointestinal Cancer, Lung cancer, Gastrointestinal Microbiota, *Mycobacterium bovis, Salmonella Typhimurium, E.coli, Magnetococcus marinus, Clostridium, Lactic acid bacteria* had been used. The search result was kept specific by using Boolean operators "AND" "OR" and "NOT". Original research and review articles with good number of citations were chosen to retrieve relatable information with the subject.

2.1 Inclusion criteria

Original literature that described the bacterial substances used for cancer therapy, uses of genetically modified bacteria in cancer, bacterial vector, bacterial toxins, side effects of bacterial therapy. Moreover, literature that mentioned the challenges of bacterial therapy in cancer were also included in a brief manner.

2.2 Exclusion criteria

Literature that only stated the Bacterial Spore Mediated Cancer Therapy, Biofilm based Cancer Therapy, killed but metabolically active vaccines, Bacteria - based microrobot were excluded.

CHAPTER 3

3.1 Mechanisms by which bacteria target and suppress tumors

The capacity to target tumors selectively via distinct pathways is the primary benefit of bacteria-based cancer treatment. Bacteria are considered to escape from the blood circulation into tumor tissue via both passive and aggressive pathways at the moment. Moreover, Bacteria may enter the tumor by passive trapping in the chaotic tumor vasculature, then flow into the tumor due to inflammation produced by a rapid rise in tumor necrosis factor (TNF) levels in the tumor vessels (Leschner et al., 2009). The active mechanism in the TME is most likely chemotaxis toward molecules produced by dying tumor tissue and low oxygen concentrations in hypoxic tumors, the latter of which may be attractive to obligate anaerobes (Malmgren et al., 1955; Dang et al., 2001) and facultative anaerobes (Kasinskas et al., 2006; Kasinskas et al., 2007). Bacteria can theoretically employ their self-propulsion abilities to actively swim away from the vasculature after systemic administration to distribute themselves throughout tumor tissue. The host immune response appears to influence bacterial distribution in tumor tissue in addition to motility.

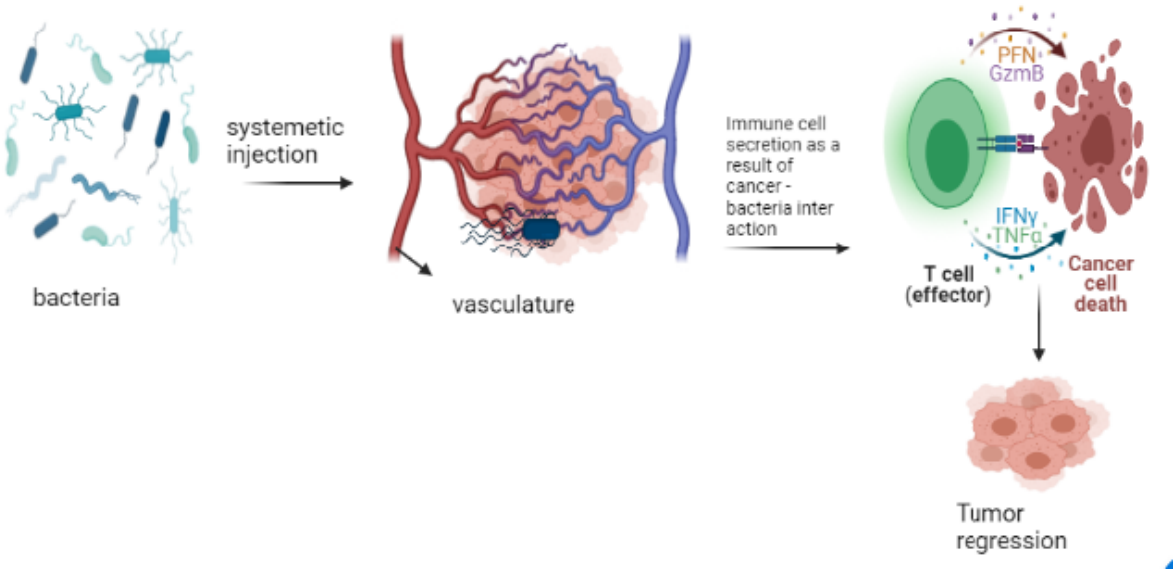

Figure 1: Mechanisms by which bacteria target tumors: Bacteria settle in the tumor microenvironment after systemic injection. Interactions between bacteria, cancer cells, and the surrounding microenvironment produce changes in tumor-infiltrating immune cells, cytokines, and chemokines, which aid tumor regression even further.

CHAPTER 4

Bacterial substances as a cancer therapeutic agents

4.1 Bacterial toxins

Bacterial toxins are proteins that can perform a variety of activities. They work as self-contained molecular devices that target certain cells in an organism, punch holes in their membranes, and alter intracellular components. The toxins generated by bacteria are powerful chemicals that may target and kill host cells and tissues. They play a crucial role in deciding the infection's prognosis by altering cellular processes, triggering apoptosis, and altering cell proliferation and differentiation (Yaghoubi et al., 2020).

Some bacterial toxins have a lot of potential for cancer therapy described as follows (**Table 1**)

Table 1: Features of anticancer toxin of bacteria

Toxins	Origin	Molecular weight	GI Cancer Cell Line	Other Human Cancer Cells/Cell Lines	Ref
Diphtheria toxin	*Corynebacterium diphtheria*	60 kDa	Adrenocortical carcinoma H295R, coloncancer (SW480,SW620, HCT116,CaCo-2 and HT-29),	Glioblastomas (U118MG, U373MG, U87MG), cutaneous T cell lymphomas (CTCL), cervical adenocarcinoma (HeLa), breast cancer (MCF 7)	(Lewis et al., 2017; Lutz et al., 2014; RK et al., 2000)
TcdB	*Clostridium difficile*	270 Kda	Colorectal cancer (CT26)	Breast cancer (MDA-MB-231)	(Tuxiong et al., 2014; Yunli et al., 2018)
CPE enterotoxin	*Clostridium perfringens*	35-kDa, (319 aa)	Human colorectal cancer cell lines (SW480, SW620,HCT116, CaCo-2, and HT-29), Human gastric cancer cell lines (SGC7901, MKN45, AGS, MGC803, BGC823, and HGC27)	Human hepatocarcinoma (HepG2 cell, SK-HEP-1 cells), breast cancer (4T1), ovarian cancer, and prostate cancers	(Zheng et al., 2017; Saeki et al., 2009; Pahle et al., 2017; Black et al., 2015)
Exotoxin A		66 kDa	Pancreatic cancer	Melanomas (FEMX, Melmet-1, Melmet-5, Melmet44, MelRM, MM200), head and neck squamous carcinomas,	(Hemmati et al., 2017;

	Pseudomonas aeruginosa		(PaCa-2)	Burkitt's lymphoma (Daudi, CA46), leukemias (EHEB, MEC1), breast cancer (MCF-7, BT-20, CAMA-1, SKBR-3)	Bjorn et al., 1986)
Verotoxin 1	*E.coli*	70 kDa	Human colorectal cancer cell lines (HCT116)	Lung cancer (A-549, PC-14, and RERF-LC-AI), ovarian carcinoma, breast tumor cell lines (T47D, MCF-7)	(Bhattacharjee et al., 2005)
Arginine deiminase	*Mycoplasma hominis, M. arginine*	46.3 KDa	Hepatocellular carcinoma (HCC)	Prostate cancer (CWR22Rv1)glioblastoma HROG02,HROG05, HROG10, HROG17	(Kim et al., 2009; Fiedler et al., 2015)

Table 1 : Explained about the different kind of bacterial toxin with their origin and molecular weight and gastrointestinal cancer cell line, other Human cancer cells lines .Some of the toxins are discussed in detail below.(Soleimanpour et al., 2020)

4.1.1 Diptheria Toxin

Roux and Yersin discovered diphtheria toxin (DT) in Corynebacterium diphtheriae for the first time in 1888 (Holmes et al., 2000). The DT gene is found on a bacteriophage (Holmes et al., 2000), and is a single-chain protein with an enzymatic A domain (amino acids 1-193), a binding B domain (amino acids 482-535), and a translocation domain (amino acids 482-535) in the molecule's center (Rolf et al., 1990). DT attaches to the heparin-binding epidermal growth factor-like growth factor (HB-EGF) precursor on the surface of cells (Louie et al., 1997). The HB-EGF precursor forms a complex with CD9 and heparan sulfate proteoglycan (Frankel et al., 2002) on the plasma membrane. CRM197 is a mutant and harmless version of diphtheria toxin that has shown to have significant anticancer properties by reducing tumor proliferation, lowering angiogenesis, causing apoptosis, serving as an immunological adjuvant, and blocking heparin-binding epidermal growth factor. Furthermore, this mutant version is used with conventional treatment, such as doxorubicin, to reduce adverse effects while increasing cytotoxicitc (Martareli, 2009). DTAT, a DT-based immunotoxin is another modification form of this toxin that targets the tumor vascular endothelium and can promote tumor regression in mice (Vallera, Mortari, 2009). Diphtheria toxin has cytotoxic action against adrenocortical carcinoma cell lines H295R, colon cancer cell lines SW480, SW620, HCT116, CaCo-2, and HT-29, as well as gastrointestinal cancer cell lines (Lewis, 2017; Lutz, 2014; Rk, 2000).

4.1.2 *Clostridium difficile* toxin

Clostridium difficile generates cytotoxin (TcdB) and enterotoxin (TcdA) toxins, respectively (Eckert, 2015; Pothoulakis, 1996). TcdB inhibits cell proliferation and induces necrosis and apoptosis by generating pro-inflammatory chemokines and cytokines, reducing cell proliferation, and causing necrosis and apoptosis (Voth, 2015; Huang, 2014). Furthermore, data shows that TcdB is highly immunogenic, generating long-term anti-tumor immunity against a variety of cancer cell lines, including CT26 colorectal cancer cell lines, and that it might be utilized as an anti-tumor vaccine or immunotherapy agent in the treatment of cancer (Huang, 2014; Zhang, 2018).

4.1.3 *Clostridium perfringens* Enterotoxin

A single polypeptide with a molecular mass of 35 kDa and a length of 319 amino acids makes up this toxin (Gao et al., 2012). By interacting to the transmembrane tight junction proteins claudin-3 and -4, this pore-forming toxin can lyse epithelial cells (Ding, Cheng, 2013). These two junction proteins are found in large amounts in a variety of human cancers, including breast, ovarian, and colon cancers (Tsutsmi et al., 2017). CPE has anticancer properties through a variety of methods, including pore formation in the cell membrane and binding to claudins, which causes fast cell death. Furthermore, the optimized CPE expressing vector (optCPE), a recombinant form of CPE, is frequently employed in gene therapy and targeting gene of claudin-3 and/or -4 overexpression in human cancer (Saini, 2005). It also inhibits the proliferation of various human cancer cell lines, including HCT116, SW620, SW480, HT-29, and CaCo-2 cell lines of human colon cancer and HGC27, BGC823, MGC803, AGS, MKN45, and SGC7901 cell lines of human gastric cancer by disrupting the membrane, inducing necrosis in claudin overexpressing cells, and inhibiting the proliferation of HCT116, SW620, SW480 (Zheg et al., 2017).

4.1.4 Verotoxin 1

Enterobacteriaceae groups such as VT-producing E. coli (VTEC), enterohemorrhagic E. coli (EHEC), and hemolytic uremic syndrome release verotoxin 1 (VT1), also known as Shiga toxin-1 (Stx-1) (HUS) (Karmali et al., 1985). VT1 inhibits protein synthesis, inhibits cell growth, arrests the cell cycle in the S phase, and targets the membrane receptor Gb3, which is overexpressed in numerous multidrug resistant (MDR) human cancer cell lines (Obrig et al., 1987). The cell cycle of colon cancer cell lines HCT116 might be stopped by VT- (Bhattacharjee et al., 2005).

4.1.5 Exotoxin A

Pseudomonas aeruginosa produces this toxin, which has a molecular mass of 66 kDa. Exotoxin A suppresses protein synthesis and causes death in tumor cells via ADP-ribosylating elongation factor-2 (EF-2) (Karpioski et al., 2013). Exotoxin A deimmunized in conjunction with human epidermal growth factor (EGF) and interleukin-4 (IL-4) has potent anti-tumor action in PaCa-2 pancreatic cancer cell lines (Todhunteret al., 2012).

4.1.6 Immunotoxin

Immunotoxins are a novel approach to cancer treatment because they target cells with unique surface receptors or antigens. Immunotoxins have a ligand attached to a protein toxin, such as a growth factor (GF), monoclonal antibody (mAb), or antibody fragment. The molecule internalizes after the ligand subunit attaches to the target cell's surface, and the poison kills the cell. Pseudomonas exotoxin and diphtheria toxin are two bacterial toxins that have been used to target cancer cells and are ideally suited to generating recombinant single-chain or double-chain fusion poisons. Immunotoxins have been developed to target a range of growth factor receptors and antigens to target haematological malignancies and solid tumors (Pastan, 1998).

4.1.7 *Escherichia coli* toxins

The shiga toxin (Stx) family of *E. coli* toxins includes two types: Stx1 (VT1 or Shiga-like toxin: SLT1) and Stx2 (VT2, SLT2), both of which are encoded by bacteriophages. Because it attaches to particular receptors on the surface of some types of malignant tumor cells and kills them by blocking protein synthesis, VT1 has been investigated for its anticancer properties.

Select Therapeutics is working on a technique that uses human dendritic cells to express verotoxin receptors. The first malignancies to be targeted include ovarian, testicular, breast, and brain cancers. Verotoxin was used to treat human astrocytoma tumor xenografts in nude mice (Arab et al., 1999). In all treated animals, a single low-dose intratumoural injection of VT1 resulted in full tumor remission. Within the treated xenograft, apoptosis was seen in both tumor and vascular cells. In sections of primary glioblastoma multiforme, verotoxin binding to tumor cells and blood arteries was discovered.

4.1.8 Fusion toxins containing *Pseudomonas* exotoxin

Transforming growth factor- (TGF-) fused with PE40 was one of the first fusion toxins containing Pseudomonas exotoxin (PE). It was effective against epidermal growth factor receptor-positive tumor cells (EGFR). It was tested as an intravesical treatment for bladder cancer in clinical studies (Goldberg et al., 1995). This drug is being studied for cleansing the marrow of multiple myeloma patients prior to autologous bone marrow transplantation.

4.1.9 Transferin-CRM 107

This is a mixture of human transferin (TS) and a diptheria toxin (CRM 107) genetic mutant that lacks native toxin binding. Interstitial infusion has been utilized to cross the blood-brain barrier in the treatment of glioblastoma (Laske et al., 1997). In 9/15 of the patients, the tumor volume was reduced by at least 50%. There was no clinical systemic toxicity.

4.1.10 IL-4 fusion toxin

NBI-3001 (Neurocrine Biosciences, San Diego, CA, USA) is an experimental medication that combines IL-4 with a Pseudomonas exotoxin. It is injected directly into the tumor through a specific catheter and attaches to IL-4 receptors with a high affinity, which are found on malignant brain tumors but not on normal brain cells. As a result, it can eliminate a large portion

of the tumor while causing no damage to normal brain tissue. In patients with recurrent glioblastoma, a phase I/II dosage escalation, safety and effectiveness study is currently conducted (Puri, 1999).

4.2. Bacterial Peptides

Bacterial peptides may also have anticancer properties, according to some studies. Non-ribosomal peptides are secondary bioactive metabolites produced by non-ribosomal peptide synthetases, which are found in bacteria. Special chemical structures, including as N-terminally linked fatty acids, N-formulated residues, D-amino acids, heterocyclic elements, N- and C-methylated residues, glycosylated amino acids, and phosphorylated residues, have been identified in these peptides. In addition to antibacterial properties, these peptides with a bacterial origin have a lot of potential for cancer treatment (Sieber et al., 2003). Several studies show that bacterial peptides inhibit cancer through a variety of methods, including I activation of apoptosis or growth suppression; (ii) prevention of angiogenesis; and (iii) disruption of the tumor's signaling system, (iv) arresting the tumor cell cycle; (v) causing apoptosis by a caspase-dependent or independent mechanism (Karpiński et al., 2013; Goto et al., 2003; Yamada et al., 2002; Karpiński et al., 2012; Lee et al., 2005).

4.2.1 Arenamides

Arenamides A, B, and C are three cyclohexadepsipeptides that can be produced from Salinispora Arenicola. Arenamides A and B have been shown to decrease tumor cells by inhibiting (NO) and prostaglandin E2 (PGE2) production, as well as blocking tumor necrosis factor (TNF)-induced activation to effect NFkappaB activity. Arenamides A and B are cytotoxic to a variety of cancer cells, including HCT-116, which is a human colon carcinoma (Asolkar RNF et al., 2009; Asolkar RN et al., 2009).

4.2.2 Halolitoralins

Halolitoralins are cyclic peptides generated by Halobacillus litoralis YS3106, which is a marine-derived bacteria. Halolitoralin A (C27H48O6N6), a cyclic hexapeptide with a molecular mass of 575 Da, and halolitoralin B and C, both cyclic tetrapeptides with the chemical formula C23H42O4N4, are members of this family. These peptides have been exhibited to have anticancer properties in human gastric tumor cells (BGC) (Yang L et al., 2002).

4.2.3 Ieodoglucomides

This peptide is obtained from Bacillus licheniformis, which is a marine bacteria. In vitro, ieodoglucomides A and B exhibit little antibacterial action. Ieodoglucomide B has cytotoxic effect against human stomach cancer cells in addition to antibacterial activities (Tareq et al., 2012).

4.2.4 Lucentamycins

Lucentamycins are 3-methyl-4-ethylideneproline peptides isolated from Nocardiopsis lucentensis CNR-712, a marine actinomycete that is divided into four groups called Lucentamycins A-D. Lucentamycins A and B are members of this category that have in vitro cytotoxic action against the human colon cancer cell line HCT-116 (Cho et al., 2007).

4.2.5 Mixirins

Mixirins are cyclic acyl-peptides generated from the marine bacteria Bacillus species. Mixirins A (C48H75N12O140), B (C45H69N12O14), and C (C47H73N12O14) are the three main components, each with a molecular mass of 1 kDa. These three cyclic acyl-peptides exhibit anticancer properties and can stop the proliferation of the HCT-116 human colon carcinoma cell line (Zhang et al., 2004).

Antibacterial and anticancer properties have been reported in a variety of bacterial peptides, as described below.

Table: 2 Features of anticancer peptides

Proteins/Peptide	Origin	Molecular weight	GI Cancer Cell Line	Other Human Cancer Cells/Cell Lines	Reference
Arenamides A, B	*Salinispora arenicola*		Colon cell line (HCT116)		(Asolkar RNF et al., 2009; Asolkar RN et al., 2009).
Halolitoralins A–C	*Halobacillus litoralis*		Human gastric tumour (BGC)		(Yang L et al., 2002).
Ieodoglucomide B	*Bacillus licheniformis*		Human stomach cancer	Human lung cancer	(Tareq FS et al., 2012).
Lucentamycins A, B	*Nocardiopsis lucentensis CNR-712*		human colon carcinoma cells (HCT-116)		(Cho et al., 2007).

Mixirins A–C	*Bacillus sp.*		Human colon tumour (HCT-116)		(Zhang et al., 2004).
Proximicins A–C	*Verrucosispora sp. MG-37 and AB-18-032*		Human gastric adenocarcinoma (AGS), human hepatocellular carcinoma (HepG2)	Human breast carcinoma (MCF 7)	(Fiedler et al., 2008).
Urukthapelstatin A	*Mechercharimyces asporophorigenens YM11-542*	733 Da	Colon cancer (HCT-116)	Human lung cancers (A549, DMS114, NCIH460), ovarian cancers (OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV3), breast cancer (MCF-7),	(Matsuo et al., 2007).
Azurin	*Pseudomonas aeruginosa*	16 kDa	Liver cell line (HEPG2), colon cell line (HCT116)	Normal melanocytes (HFB4), progressive pediatric CNS tumors, breast cancer (MCF7, ZR-75-1, T47D, MDA-MB-157, MDD2, MDA-MB- 231)	(Goto et al., 2003; Gao et al., 2017)
p28	*Pseudomonas aeruginosa*	2.8 kDa	Colon cancer (HCT116, HT29), pancreatic cancer (MIA-Paca2)	Breast cancer (MCF7,ZR-75-1, T47D,MDA-MB-157,MDD2, MDA-MB-231), melanoma (UISO-Mel-6, UISO-Mel-23, UISO-Mel-29, UISO-Mel-2), lioblastoma (U87,	(Yamada et al., 2009;Mehta et al., 2011;Yamada et al., 2016)

				LN229), prostate cancer (DU145, LNCaP, PC-3), ovarian cancer (SK-OV3, ES-2), fibrosarcoma (HT1080),eiomyosarcoma (HTB-88), osteosarcoma (TE85), , Burkitt's lymphoma (Raji, HEK-293), neuroblastoma (IMR-32, SK-N-BE2), rhabdomyosarcoma (RD)	
Pep27anal2	*Streptococcus pneumoniae*	3.3kDa	Gastric cancer cells (SNU-601)	Leukemia cells (AML-2, HL-60, Jurkat), breast, cancer (MCF-7)	(Lee et al., 2005;T K et al., 2012)
Entap	*Enterococcus sp. strains*	6.2kDa	Gastric adenocarcinoma cells (AGS), colorectal adenocarcinoma (HT-29)	Uterine cervix adenocarcinoma cells (HeLa), prostate carcinoma (22Rv1), , breast cancer (MDA-MB-231)	(Karpioski et al., 2013; Karpioski et al., 2012)
Proximicins	*Verrucosispora sp. MG-37 and AB-18-032*	3.19 kDa	Human gastric adenocarcinoma (AGS), human hepatocellular carcinoma (HepG2)	Breast cancer (MCF-7)	(Fiedler et al., 2008)
HPRP-A1	*Helicobacter pylori*	15aa	Human liver cancer cell line (HepG2), human	HeLa, lung cancer (NSCLC) A549 cell line	(Hu et al., 2018; Hao et al., 2019)

			gastric cancer cells (BGC-823 & SGC-7901)		

Table 2 : Explained about the different kind of bacterial peptides with their origin and molecular weight and gastrointestinal cancer cell line, other Human cancer cells lines .Some of the peptides are discussed in detail below. (Soleimanpour et al., 2020)

4.2.6 *Halolitoralins*

Halolitoralins are cyclic peptides generated by *Halobacillus litoralis* YS3106, which is a marine-derived bacteria. *Halolitoralin A* (C27H48O6N6), a cyclic hexapeptide with a molecular mass of 575 Da, and *halolitoralin B* and *C*, both cyclic tetrapeptides with the chemical formula C23H42O4N4, are members of this family. These peptides have been exhibited to have anticancer properties in human gastric tumor cells (BGC) (Yang L et al., 2002).

4.2.7 *Ieodoglucomides*

This peptide is obtained from *Bacillus licheniformis*, which is a marine bacteria. In vitro, *ieodoglucomides A* and *B* exhibit little antibacterial action. *Ieodoglucomide B* has cytotoxic effect against human stomach cancer cells in addition to antibacterial activities (Tareq et al., 2012).

4.2.8 *Lucentamycins*

Lucentamycins are 3-methyl-4-ethylideneproline peptides isolated from *Nocardiopsis lucentensis CNR-712*, a marine actinomycete that is divided into four groups called *Lucentamycins A-D. Lucentamycins A* and *B* are members of this category that have in vitro cytotoxic action against the human colon cancer cell line HCT-116 (Cho et al., 2007).

4.2.9 *Urukthapelstatin A*

Urukthapelstatin is a cyclic thiopeptide antibiotic that was discovered in the marine bacteria *Mechercharimyces asporophorigenens* YM11-542 with a molecular mass of 733kDa. This peptide, C34H30N8O6S2, has anticancer action against HCT-116, a human colon carcinoma cell line (Matsuo et al., 2007).

4.2.10 *Proximicins*

Proximicins, particularly *proximicins B* (413 kDa) and C (436 kDa), inhibit gram-positive bacteria, while gram-negative bacteria, such as *Escherichia coli K12* and *Pseudomonas fluorescens*, are resistant. Proximicins have unique chemical structures that include 4-amino-furan-2-carboxylic acid, a previously unidentified amino acid. Among the variety of proximins, proximicin C demonstrates considerable anticancer efficacy by stopping the cell cycle during the G0/G1 phase, triggering apoptosis, upregulating the intracellular levels of p53, and acting as a cyclin kinase inhibitor p21 in different cancer cell lines such as AGS and

HepG2, which are a human gastric adenocarcinoma and a human hepatocellular carcinoma, respectively (Fiedler et al., 2008).

4.2.11 *Azurin*

Pseudomonas aeruginosa produces this peptide, which is a copper-containing metalloprotein with redox activity. With a length of 128 amino acids and a molecular mass of 14 kDa, this peptide has a strong anticancer effect (Yamada MG VP et al., 2002; Jia GSG LUC et al., 2011). In addition to *Azurin*, other smaller variants of this peptide, dubbed p28 having a molecular weight of 2.8 kDa and containing 50 to 70 amino acids of *azurin*, have shown to have significant anticancer action. This peptide and its derivatives exhibit anticancer effect by boosting p53 intracellular levels, which causes the cell cycle to be arrested at the G2/M stages. Furthermore, this peptide inhibits tumor cell proliferation by interfering with the receptor tyrosine kinase EphB2-mediated signaling process, which can prevent angiogenesis by decreasing the activity of VEGFR-2 tyrosine kinase, increasing the level of E-cadherin and interfering with the expression of P-cadherin protein (Yamada T et al., 2009; Mehta RR et al., 2011;Yamada T et al., 2016). Several investigations have confirmed azurin and p28 as tumor suppressors with potent anticancer action in gastrointestinal cancer cell lines including liver cell line (HEPG2), colon cell line (HCT116, HT29), and pancreatic cancer (MIA- Paca2) (Joo KR et al., 2012; Lulla SG TY et al., 2016)

4.2.12 *Pep27anal2*

This peptide, which has a length of 27 amino acids and a molecular mass of 3.3–3.6 kDa, is an analog of the *Streptococcus pneumoniae* signal peptide Pep27. In this bacteria, *Pep27anal2* is responsible for initiating the cell death mechanism, as well as permeating the cell membrane and triggering caspase-independent and cytochrome-independent apoptosis. This peptide not only has antibacterial properties, but it also has anticancer properties. In gastric cancer cell lines like SNU-601, *Pep27anal2* has been shown to inhibit cell growth (Sung WS et al., 2007; Lee DG et al., 2005).

4.2.13 Entap

Enterococcus strains synthesize this peptide, which has 58-62 amino acids and has a molecular mass of 6.2 kDa. Entap inhibits cell growth, arrests the cell cycle at the G1 phase, and induces autophagic apoptosis, all of which have anticancer properties. This peptide has been shown to have cytotoxic effect against a variety of human cancer cell lines, along with gastrointestinal cancer cell lines such the human colon adenocarcinoma cell line (HT-29) and gastric adenocarcinoma cell line (AGS) (T K et al., 2012;T SA et al., 2013).

4.2.14 *Helicobacter pylori* Ribosomal Protein (HPRP)

HPRP-A1 and its enantiomer HPRP-A2 are made up of 15 all-L or all-D amino acids and are obtained from the N-terminus of *Helicobacter pylori* ribosomal protein L1 (RpL1) (Pütsep K. et al., 1999) HPRP-A1 and its enantiomer have anticancer activity through a variety of mechanisms, including activation of caspase-3, -8, and -9-dependent pathways leading to apoptosis, cell cycle arrest at G0/G1 and G2/M, inhibiting cell growth, interfering with mitochondrial function, and increasing reactive oxygen species production (ROS). These peptides are cytotoxic to a variety of human carcinoma cell lines, as well as human

gastrointestinal cancers, including liver cancer cell line (HepG2) and human gastric cancer cells (BGC-823 & SGC-7901) (Cho E et al., 2018; Hao W et al., 2019).

However, employing tumor-targeting peptides (TTP) could boost the bacteria's ability to deliver drugs to tumor cells. Furthermore, they increase the bacterial agents' tumor-inhibitory action (Deutscher SL et al., 2010). Moreover, they increase the bacterial agents' tumor-inhibitory efficacy. Different types of TTP have been studied recently, and they have been categorized into three groups. TCP-1 belongs to the first group of TTP peptides that can target the vasculature of orthotopic colorectal cancer (Li ZJ et al., 2010). The next group is contained peptides that are able to target the broad-spectrum antigenic tumor markers include RGD- 4C, NGR, Lyp-1, TMTP1, etc. (Sugahara KN et al., 2009). iRGD and TAT are members of the third category, which, in addition to particular target ability, have a high cell-penetrating capacity and can deliver therapeutic compounds deep into tumor tissue. To improve specific targeting and toxicity, all of these tumor-targeting peptides are frequently conjugated with the N-terminal or C-terminal of bacterial peptides or proteins (Sugahara KN et al., 2009).. However, there are a number of advantages to employing bacteria to treat cancer that scientists are interested in, such as selective toxicity against tumor cells with minimal or no adverse effects on normal cells (Giuliani A et al., 2007).

4.3 Bacteriocins

Bacteriocins were first employed in the food sector as a preservative to extend the shelf life of goods. However, new research suggests that these non-immunogenic chemicals, in addition to their antibacterial action, have significant anticancer potential as shows below in (Table 3). Bacteriocins are peptide or protein-based toxins generated by bacteria to inhibit or even kill other bacteria that are closely related. Microcins (less than 20 kDa in size), colicins (20 to 90 kDa in size), and tailocins (more than 90 kDa in size) are the three types of bacteriocins (high molecular weight bacteriocins). Bacteriocins are divided into four categories (Belkum et al., 2011).

Table 3: Features of anticancer bacteriocins

Bacteriocins	Origin	Molecular weight	GI Cancer Cell Line	Other Human Cancer Cells/Cell Lines Bovicin	Ref.
Bovicin HC5	*Streptococcus bovis HC5*	2.4 kDa	Liver hepatocellular carcinoma (HepG2)	Breast cancer (MCF-7)	(Kaur et al., 2015)
Colicin E1 and A	*Escherichia coli*	40 to 80 kDa	Colon cancer (HCT116)	Osteosarcoma (HOS), fibrosarcoma (MRC5, HS913T), leiomyosarcoma (SKUT-1 cells), lung cancer (A-549, PC-14, RERF-LC-AI), ovarian carcinoma,	(Chumchalova et al., 2003)

				Breast cancer (MCF7, ZR75, BT549, BT474, MDA-MB-231, SKBR3 and T47D)	
Nisin A	*Lactococcus lactis*	3.5 kDa	Colon cancer (LS180, SW48, HT29, Caco2), liver hepatocellular carcinoma (HepG2)	Head and neck squamous cell carcinoma (UM-SCC-17B, UM-SCC-14A, HSC-3, acute T cell leukaemia (Jurkat), Breast cancer(MCF-7)	(Begde et al., 2011)
Pediocin K2a2-3	*Pediococcus acidilactici K2a2-3*	4.6 kDa	Colon adenocarcinoma (HT29)	Hepatocellular carcinoma (HepG2)	(Villarante et al., 2011)
Pyocin S2	*Pseudomonas aeruginosa*	73.8 kDa	Hepatocellular carcinoma	Multiple myeloma (Im9), cervical adenocarcinoma(HeLa), embryonal ovary carcinoma (AS-II	(Watanabe et al., 1980)
Fermentic in HV6b	*Lactobacillus fermentum*	6.6 kDa	Spleen lymphoblast cell line (Sp2/0- Ag14 ATCC-CRL-1581), hepatocarcinoma cell line (HepG2)	Cervical cell lines (Hela ATCC CCL2), breast carcinoma cell line (MCF7 ATCC-HTB-22), kidney embryonal cell line (HEK293 CRL-1573)	(Kaur et al., 2013)

Table 3: Explained about the different kind of bacteriocin with their origin and molecular weight and gastrointestinal cancer cell line, other Human cancer cells lines some of the bacteriocin are discussed in detail below. (Soleimanpour et al., 2020)

4.3.1 Bovicin HC5

Bovicin HC5 has a molecular weight of 2.4 kDa and is structurally similar to nisin (Kaur et al., 2015). It belongs to the class I bacteriocins generated by *Streptococcus bovis*. Bovicin HC5 is a broad-spectrum antimicrobial peptide that can cause potassium efflux in target cells by forming a hole in the cell membrane. In addition to antibacterial action, this peptide has been shown to have high cytotoxicity against human cancer cells, including HepG2, a human liver hepatocellular carcinoma cell line (Paiva et al., 2012).

4.3.2 Nisin A

This peptide is a polycyclic antibacterial peptide with a 34-amino-acid length that belongs to the lantibiotics family. By interfering with phospholipid rearrangement, Nisin A, a phospholipid rearrangement inhibitor generated by Lactococcus lactis subsp, inhibits tumor cell growth, alters cell membrane integrity, creates holes, and enhances ion penetration (Nam et al., 2017). In a range of human cancer cells, including liver hepatocellular carcinoma (HepG2) cells, this lantibiotic has been demonstrated to inhibit tumor cell invasion and metastasis (Norouzi et al., 2018).

4.3.3 Pediocins

Pediocins belong to the bacteriocin lass IIa. The 44 amino acids in Pediocin CP are obtained from *Pediococcus acidilactici* MTCC 5101 (Balgir et al., 2010). In addition to antibacterial action, this member of class IIa has potent anticancer activity against a variety of cancer cell lines, including the mouse spleen lymphoblast cell line (Sp2/O-Ag14) and hepatocarcinoma (HepG2) (Kumar et al., 2012). Pediocin K2a2-3, another pediocin generated by *P. acidilactici* K2a2-3, can suppress the growth of tumor cells such as human colon adenocarcinoma cells (HT29) (Villarante, 2011, Kumar, 2012).

4.3.4 Fermenticin HV6b

Fermenticin HV6b has a molecular mass of 6.6 kDa and belongs to the class IIa of bacteriocins. Lactobacillus fermentum HV6b MTCC 10770, isolated from the human vaginal environment, produces this antimicrobial peptide (Kaur, 2013, Kameron, 2019). Fermenticin HV6b has anticancer properties through causing apoptosis in vascular endothelial cells, cell shrinkage, and DNA breakage, among other methods. Fermenticin HV6b appears to have a cytotoxic impact on a variety of malignant cell lines, as well as Sp2/0-Ag14, a spleen lymphoblast cell line (Kaur et al., 2019).

4.3.5 Colicins

Colicins are bacteriocins with a high molecular mass (40 to 80 kDa) generated by *Escherichia coli* (Cameron et al., 2019). Colicins A, B, E1, Ia, Ib, K, L, N, U, 5, and 10 are members of this family that disrupt target cells by altering the electric charge distribution, resulting in plasma membrane depolarization. Colicins E2, E7, E8, and E9, for example, have non-specific DNase activity, but colicins E3, E4, E6, E5, and D have RNase activity, which is a highly specific RNase activity. Murein synthesis can also be inhibited by colicin M and pesticin. Colicins have

been shown to decrease tumor cell growth and exhibit cancer cell-specific toxicity. Colicin E1, for example, shows cytotoxic action against the human colon cancer cell line HT29 (Kaur, 2015, Chumchalova, 2003).

4.3.6 Pyocin S2

Pyocin S2, which has a molecular mass of 73.8 kDa and has cytotoxic action on tumor cells, is generated by *Pseudomonas aeruginosa* 42A and can suppress the development of cancer cells such as HepG2, a hepatocellular carcinoma cell line (Chumchalova et al.,2003).

4.4 Bacterial enzymes in cancer therapy

Essential amino acids are necessary for healthy cell development and metabolism. These amino acids can function as a limiting factor, preventing tumor cells from growing uncontrollably and rapidly. Alternative cancer treatment involves deprivation of these vital amino acids. To do so, bacteria create a variety of enzymes that act on these important amino acids and inhibit a variety of cellular processes that are necessary for tumor growth. The most widely studied bacterial enzymes are L-asparaginase, arginine deiminase, and arginine decarboxylase (Laliani et al., 2020). The enzyme L-asparaginase (L-ASNase) catalyzes the hydrolysis of asparagine, while arginine catabolism is catalyzed by enzymes such arginine deiminase and arginine decarboxylase. These enzymes deprive tumor cells of the amino acids needed for protein synthesis, resulting in tumor cell death (Zam, 2017).L-ASNase comes from Escherichia coli, Erwinia species, Streptomyces, and Bacillus subtilis. According to Nguyen et al., the L-ASNase enzyme reduced blood asparagine levels, limiting protein synthesis and inducing cell cycle arrest in leukemia cells in the G1 phase (Nguyen et al., 2018). Acute lymphoblastic leukemia, lymphosarcoma, neoplasia, and other cancers have all been shown to respond to L-ASNase (Nguyen, 2018, Ghasemian, 2019). L-glutaminase activity, antigenicity, and hypersensitivity responses are all characteristics of commercial asparaginase. Side effects such as hepatotoxicity and immunosuppression are caused by the L-glutaminase coactivity. Nguyen et al. found that Erwinia chrysanthemi-derived L-ASNase is extremely successful in the treatment of acute lymphoblastic leukemia, with fewer side effects and lower toxicity than other FDA-approved asparaginases because it lacks L-glutaminase coactivity (Nguyen et al., 2018).

CHAPTER - 5

5.1 Bacteria as a target delivery vector for cancer therapeutic agents

The considered bacterium has gained interest as anticancer agents for more than a decade, and is now frequently employed as anticancer target delivery vectors. As mentioned below (Table 2), bacteria are utilized as live, attenuated, or genetically modified vectors for targeted delivery

or expression of anticancer genes, conventional drugs, antiangiogenic genes, and tumoricidal chemicals (Soleimanpour et al., 2020).

Table 4: Application of genetically engineered bacteria as vectors for anti-cancer treatment

Treatment Strategy	Bacterial Strain	Gene/Drug	Mechanism of Action	Application	References
Prodrug Therapy	*Salmonella*	Thymidine kinase polypeptide Prodrug: Ganciclovir	Inhibits deoxyguanosine triphosphate, dGTP, incorporation into DNA	Melanoma	Rooseboom et al., 2004
Prodrug Therapy Anti-Angiogenic Therapy	*Escherichia Coli*	Uridine phosphorylase Prodrug: Capecitabine	Impede thymidylate synthase enzyme	Colon, rectum, head and neck cancers	Guise et al., 2012
	S. choleraesuis	Endostatin	Increases infiltration of CD8(+) T cells	Melanoma Bladder tumor, Hepatoma	Lee et al., 2004
Anti-Angiogenic Therapy Anti-Angiogenic Therapy Immunotherapy	*S. typhimurium SL7207*	VEGFR-2	Upregulates vascular-endothelial growth factor receptor 2 (FLK-1) of proliferating endothelial cells in the tumor vasculature	Melanoma Colon carcinoma Lung carcinoma	Niethammer et al., 2002
	S. typhimurium RE88	IL-18	Activation of T, natural killer and dendritic cells	Breast carcinoma	Luo et al., 2003
Anti-Angiogenic Therapy Immunotherapy	*Serratia marcescens*	Endotoxin	Releases pro inflammatory cytokines, making the immune system eliminate or protect against multiple tumors	Melanoma Leukemia, Lymphoma	Rosenberg et al., 2014
			Releases proinflammatory		

Quorum Sensing peptides for anti-tumor action	*Listeria monocytogenes* *Pseudomonas. aeruginosa*	Listeriolysin O N-3 oxo dodecanoyl homoserine lactone (3OC12-HSL)	cytokines and increases expression of costimulant molecules in antigen presenting cells surfaces leading to maturation and activation of high affinity T cells Inhibition by protein kinase	Prostate cancer Cystic fibrosis	Shahabi et al., 2014 Roussel et al., 2017
Biofilms as Anti-Cancer Agents	*Streptococcus agalactiae*	Polysaccharids	Inhibit adhesion of cancer cells to endothelial cells	Colon cancer	Miyake et al., 1996

Table 4: Explained about the treatment strategy of some genetically engineered bacteria with their mechanism and applications in different types of cancer. Some of the genetically engineered bacterial vector are discussed in detail below. (Soleimanpour et al., 2020)

5.1.1 *Bifidobacterial* vectors

Cancer gene treatment using Bifidobacteria may be classified into four categories: I tumor suppressor gene delivery; (ii) suicide gene therapy; (iii) anti-angiogenesis; and (iv) chemosensitization.

Bifidobacterium longum is the best studied of the many *Bifidobacteria* strains. The human phosphatase and tensin homologue (PTEN) gene was effectively inserted into B. longum for tumor suppressor gene therapy, and the recombinant bacteria inhibited the development of solid tumors in mice (Hou et al., 2006) The CD gene was inserted into *Bifidobacterium longum* and *Bifidobacterium* breve to test the efficacy of suicide gene therapy (Hidaka et al., 2007). In addition, methods for interfering with tumor angiogenesis have been investigated. Both *B. longum* and *B. adolescentis* were genetically engineered to express endostatin, a tumor angiogenesis inhibitor. Both recombinant bifidobacterial strains reduced tumor angiogenesis and proliferation, according to the findings. (Xuyf, 2007, Li, 2003).The major benefit of *Bifidobacteria* as a vector system is that it is a common flora of the human gut, offering a minimal risk of bacterial illness transmission. Bifidobacteria can also be administered orally as well as intravenously (Wei et al., 2008).

5.1.2 *Salmonella* vectors

Several recent investigations have shown that an attenuated strain of *S. typhimurium* might be utilized as a selective target delivery vector for the therapeutic gene encoding anticancer proteins. To increase efficacy, two major genes were deleted in attenuated strains: the purI gene, which was deleted to increase the need for an external source of adenine, and the msbB gene, which was deleted to reduce toxicity by reducing the production of proinflammatory cytokines (TNF) and nitric oxide (Clairmont and Jesenberger., 2000).These strains are genetically stable and do not have antibiotic resistance indicators, which slow tumor growth by enhancing bacterial survival and multiplication in the tumor microenvironment. Attenuated *S. typhimurium* has completed a phase I clinical trial and is now being utilized as a delivery vehicle for the *E. coli* cytosine deaminase gene. *S. typhimurium* attenuated strains have potent anticancer activity against a variety of cancer cell lines, including human colon carcinoma cell lines (C38, WiDr, and CT26) and pancreatic cancer cell lines (ASPC-1) (Chonnam and Wang., 2016). One study showed that *S. typhimurium* was successfully engineered to secrete murine FasL, a pro-apoptotic cytokine, and demonstrated reduced tumor growth in murine breast carcinoma and CT-26 colon carcinoma cells. (Loeffler et al., 2008) In another study, *S. typhimurium* was designed to secrete TRAIL which induced tumor growth suppression in mice bearing melanoma tumor. (Chen et al., 2012) In both the cases, tumor growth was suppressed by induction of caspase-3 mediated apoptosis. (Niethammer et al., 2002).

5.1.3 *Listeria monocytogenes*

Listeria monocytogenes produces listeriolysin O (LLO), which can permit phagolysosome bacteria to survive by forming hemolytic activity and puncturing the phagosomal membrane, allowing the bacteria to escape and enter the intracellular space. This bacterium species is commonly employed as a vaccine delivery vector that produces anti-tumor action by modifying host immune responses. Different variants of this anti-tumor vector are currently in phase I and II of clinical trials (Gedde et al., 2000). Lm-LLO-E7 is a recombinant strain of L. monocytogenes (rLm) that expresses the E7 protein of the human papillomavirus-16 (HPV-16). The Lm-LLO-E7 recombinant form of *L. monocytogenes* (rLm) produces the *papillomavirus*-16 (HPV-16) E7 protein, which is fused to the non-hemolytic listeriolysin O (LLO) (Gunn et al., 2001). ADXS31-142 is a recombinant version of this vector that conjugates attenuated *Listeria* vector LmddA and has the capacity to express HER2/neu. This bacterium vector is commonly used to treat cancer and the Colo205 human colon cancer cell line (Shahabi, 2011, Singh, 2005).

5.1.4 *Lactobacillus*

Lactobacillus is generally recognized as a key component of the human microbiota and as a probiotic agent (Makarova, 2006, Zhu, 2011). Several species of this genus have showed significant promise in cancer therapy, such as *Lactobacillus acidophilus*, which suppresses tumors by boosting the host immune response by raising IFN-, IL-10, CD4+, and CD8+ T cells, and lowering blood levels of tumor markers such as CEA and CA19-9 (Agah et al., 2016). *Lactobacillus brevis* SBL8803 is another species having anticancer properties. This bacteria

produces polyphosphate (polyP), an anticancer chemical that causes death in tumor target cells by activating the ERK pathway (Sakatani et al., 2016). *Lactobacillus casei* BL23 also inhibits tumor cell proliferation by upregulating caspase-7, caspase-9, and Bik, and adenoma formation by activating the host immune response, reducing the amount of IL-22, and inhibiting tumor cell proliferation by upregulating caspase-7, caspase-9, and Bik (Jacouton et al., 2016).

5.1.5 Live attenuated bacteria as a cancer vaccine vector

Strategies employing live-attenuated bacterial vectors have matured in terms of academic and industry research in the developing field of active and targeted cancer immunotherapy. Because of their microbial origin, different bacterial species may be genetically modified to transport antigen to APCs with significant adjuvant effects. Antigen delivery methods based on proteic or DNA-encoding antigens, as well as natural bacterial tropisms, may differ between species, allowing for various uses. Some companies have lately begun clinical studies utilizing live *Listeria* or *Salmonella* spp. after numerous academic attempts to overcome safety and effectiveness problems (Toussaint et al., 2013).

5.1.6 Bacteria as protein vectors for cancer immunotherapy

Bacteria have long been employed to deliver proteins as cancer vaccine carriers. The antigenic proteins are generated in this scenario by the bacteria in situ rather than by the host cells. Adjuvants are also produced by the bacterium. *L. monocytogenes* delivered heterologous protein antigens to the immune system and induced CTL responses for the first time in 1992 (Schafer et al., 1997). Since then, *L. monocytogenes* has been successfully investigated as a vaccine vector for the delivery of antigens in a variety of models (Gentschev, 2002, Paterson, 2004) and Aduro Biotech Inc. has examined it as an experimental novel medication in the United States for the first time. Antitumor effectiveness was established in virtually all preclinical investigations using *L. monocytogenes* as a carrier to deliver neoplastic antigens when the antigen was produced as a fusion protein with the shortened listerial virulence factors LLO or ActA.

5.1.7 *Magnetococcus marinus*:

In the field of controlled release systems, targeted and efficient delivery of therapeutic drugs is still an unprecedented goal. The non-specific distribution in the systemic circulation can cause considerable toxicity and side effects. One strategy to reduce unnecessary side effects and increase the therapeutic index of hypoxic areas is to use targeted propulsion drugs, which can offset the impact of TIFP after tumor metastasis and increase the ability of drugs to target hypoxic areas in early treatment. (Taherkhani et al., 2014) is investigating the magnetotactic bacterium Magnetococcus Marinus MC1 (MTB) as a potential therapeutic vector. (Taherkhani et al., 2014). Magnetococcus marinus is a type of αproteobacteria, which has the special ability to form magnetosomes, which are a kind of mineral crystals formed by bio mineralization. The membrane seals single magnetic domains, allowing cells to move along the earth's magnetic field. (Bazylinski et al., 2013). By combining these targeted self-driven magnetotactic micro aerobic control, more therapeutic agents can be administered to cross the diffusion limit of large drug molecules and avoid the complex treatment of solid tumors of the hypoxic area.
The potential advantages of these vectors indicate the need to find a suitable method to combine therapeutic delivery, such as studying the mobility and magnetic response of MTBLP. The

results confirmed that a large number of nanoliposomes (about 70) actually bind to MTB. Without affecting function and fluidity, cytotoxicity tests on three different cell types (J774, NIH/3T3 and Colo205) showed that the combination of liposomes and MTB drugs improved the biocompatibility of MTB, and the combination does not affect the absorption of liposomes. (Taherkhani et al., 2014) Another study showed that the migration behavior of the MC1 strain of magnetotactic bacteria can be used to transport drug-loaded nanoliposomes to the hypoxic area of tumors. Based on the two-stage aerodynamic detection system, nanoparticles containing iron oxide nanocrystalline magnetic chains tend to float along local magnetic field lines at low oxygen concentrations. The results show that detecting the accumulation of microbes with magnetotactic behavior can significantly improve the therapeutic index of various nanocarriers in hypoxic tumors. (Felfoul et al., 2016)

CHAPTER - 6

6.1 Genetically Altered Bacteria for Cancer Therapy

Bacterial genetic engineering is a well-established biotechnology process. This technique was used to produce recombinant proteins. Bacterial genomic knowledge allows the modification of bacterial characteristics by deleting or introducing genes.

6.1.1 Bioengineered *Escherichia coli* Nissle 1917 for tumour-targeting therapy

The *Escherichia coli* strain nissle 1917 (EcN) strain was discovered by Alfred Nissle in 1917 during the First World War from the faeces of a German soldier (Nissle, 1918; Nissle, 1925). Escherichia coli strain Nissle 1917 (EcN) is undoubtedly the most intensively researched gram-negative bacterium with probiotic qualities today. The EcN strain has been utilized as the active pharmaceutical ingredient in a licensed medicinal product supplied in Germany and several other countries for nearly a century. Furthermore, microorganisms can be genetically modified to detect and respond to the tumor microenvironment, resulting in both innate and adaptive anti-tumor immune responses. (Zhou et al., 2018). However, bacteria's anti-tumor activity is often weak, and many bacteria and treatment methods have been created to improve their anti-tumor effect. (Pinero-Lambea et al., 2015b). Additionally, some bacteria like *Escherichia coli*, is currently being bioengineered to make biologically active compounds utilizing a range of molecular tools. EcN-mediated tumor therapies have been proven in a number of studies to successfully regress tumors and enhance survival in mice. (Yu et al., 2019). These tiny living factories may lower production costs, lessen side effects, need fewer doses of biological material, and generate more molecules as a next-generation therapy. (Pedrolli et al., 2019). This means EcN is a versatile probiotic that can be used in a variety of clinical applications as a live therapy. Engineering these microorganisms to express prodrug converting enzymes is another prevalent method. The main benefit of these enzymes is that the cytotoxic chemicals they produce can penetrate the cell membrane and spread further inside the solid tumor. (Lehouritis et al., 2016). EcN can be genetically modified to act as a live therapy in the treatment of solid tumors. (Singh et al., 2017; Chua et al., 2017). These data support EcN's potential as a cancer-fighting probiotic. By inserting synthetic genes into the EcN's genome and reducing homologous recombination, for example, genetic stability could be increased. Moreover, EcN may be rationally engineered for clinical investigations due to its genetic flexibility, resulting in a strong cancer weapon. (Yu et al., 2019).

6.1.2 The mechanisms of EcN as an antitumour agent

Different bacterial species have different intrinsic cancer-killing capabilities. EcN, as a facultative anaerobe, has the potential to target tumors through multiple pathways. Within the pathogenic *E. coli* serotype O6 lineage, the EcN serotype O6:K5:H1 is a perfect example of bacterial genome evolution. (Behnsen et al., 2013).Furthermore, the EcN membrane's serum-sensitive LPS ensures that the strain is quickly eliminated from normal organs, and it has no immunological toxic side effects in patients (Grozdanov et al., 2002). Bacteria have been shown to preferentially multiply within solid tumors and this property likely facilitates EcN's targeting to the tumor, resulting in preferential proliferation within the tumor microenvironment. (Pawelek et al., 2003).

6.1.3 Exploration of EcN for tumour-targeting therapy

EcN is a potential *E. coli* strain that can be genetically modified to cause tumor colonization only in living mice (Stritzker et al., 2007). The tumor-targeting activity of EcN has been monitored using positron emission tomography PET and optical imaging (Brader et al., 2008). Bioengineered EcN has several unique properties, including I interaction with the host immune system (Sturm et al., 2005), (ii) antimicrobial activity via secretion of microcins and bacteriocins and (iii) biofilm formation leading to the production of defensins (Sassone-Corsi et al., 2016). EcN has opened up new possibilities for next-generation medicinal and probiotic therapies because to its unique function and high adaptability. (Lasaro et al., 2009). Researchers have seen reduced tumor volume, enhanced longevity, and elimination of metastatic illness in animal models using these modalities, all while avoiding injury to healthy cells (Yu et al., 2019).

6.1.4 Expression of prodrug-converting enzymes in EcN

Another option is to develop prodrug-converting enzymes that can metabolize and convert prodrug substrates into cytotoxic products, resulting in a powerful by stander effect (a therapeutic impact on cells that is unaffected by bacteria) (Ho et al., 2018). It chose the alanine-deficient EcN to use constitutive promoters to co-express INP-HlpA (Protein HlpA from *Streptococcus gallolyticus* with an INP tag) and YebF-I1 (Myrosinase from *Armoracia rusticana* with a YebF-secretion tag). EcN that had been engineered was given orally and bound to the heparan sulfate proteoglycan on colorectal cancer cells. As a result, dietary glucosinolate was transformed to sulforaphane, an organic compound having anticancer potential, via secreted myrosinase. In vitro, this strategy resulted in an almost total reduction of growth in murine and human colorectal cancer cell lines. (Lehouritis et al., 2016).

6.1.5 Engineering of EcN-derived minicells

Minicells and bacterial ghosts (BGs) generated from EcN can be manipulated and filled with tumor-targeting medicines (MacDiarmid et al., 2007). The minicells absence of a genome prevents them from proliferating, but they retain other traits inherited from their parent's bacteria. Minicells have been utilized to deliver of siRNA or chemotherapeutic medicines to tumors with extreme accuracy. These drug-loaded minicells can target tumors and release anticancer drugs after being modified with antibodies to cancer cell receptors. (MacDiarmid et al., 2009; MacDiarmid et al., 2016). Additionally, Zhang et al. (2018) also discovered that

pHLIP-mosaic minicell treatment resulted in considerable orthotopic breast tumor shrinkage in a BALB/c mouse model, as well as high biocompatibility and low toxicity.

6.1.6 Engineering of EcN BGs

Bacterial ghosts are non-living bacterial cell envelopes that are empty and complete and can be employed as a chemical delivery method (Langemann et al., 2010; Krasko et al., 2017). EcN BGs were subsequently loaded with Epothilone B, an anticancer drug that promotes apoptosis in HeLa cells via the mitochondrial route (Zhu et al., 2018).BGs have excellent carrier capacity and immunogenicity (Ganeshpurkar et al., 2014). EcN BGs were used as candidate adjuvants due to the exterior immunologic characteristics of living bacteria. This was accomplished using a syngeneic murine lung carcinoma model and cell lysate-based anticancer immunization (Krasko et al., 2017). These findings suggest that EcN BGs could be a potential medication delivery vehicle for cancer therapy drug candidates. Overall, studies of EcN-mediated tumor therapies have shown that probiotic EcN can be manipulated to transport therapeutic payloads to the tumor microenvironment safely and selectively, and that they can be exploited as an ideal chassis for living cancer therapeutics (Yu et al., 2019).

6.2 Anticancer activity of *Lactic Acid bacteria*

According to several studies, distinct species of LAB (Lactic acid bacteria) isolated from various sources have anticancer potential and properties (Kim et al., 2011). LAB contain anticancer qualities that inactivate or block carcinogenic substances in the gastrointestinal tract, increase the immune response, and inhibit the enzymes glucuronidase, azo reductase, and nitro reductase, which are known to transform pre carcinogens to carcinogens (Vamanu et al., 2006).In addition to its anticancer qualities, new research has shown that LAB inhibits colon cancer cell proliferation through synergistic interactions between cancer cell adhesion (Azcárate- et al. 2011). Several mechanisms have been proposed to prevent cancer cells, including an increase in the host's immune response, binding and degradation of potential carcinogens, qualitative changes in the intestinal microflora that produce putative carcinogens and promoters (e.g., bile–acid degrading bacteria), production of antitumorigenic or antimutagenic compounds in the colon, and alteration of the metabolic activities of intestinal microflora (Hirayama and Rafter, 2000; Kim et al., 2008).

6.2.1 Anticancer properties of LAB

The majority of studies, have focused on the effects of lactobacilli on cancer cell viability or tumor size reduction (Kim et al., 2002 and Lee et al., 2004). Oral treatment of *Lactobacillus rhamnosus* was reported to lower the faecal concentration of glucuronidase in people, signifying a decrease in the conversion of procarcinogens to cancinogens, according to research finding of (Salminen et al., 1993). Chiu et al. (2010) found that *Lactobacillus casei* and *Lactobacillus rhamnosus* released soluble substances that caused apoptosis in a human monocytic leukemia cell line. Antimutagenic activity of milk fermented with mixed cultures of various lactic acid bacteria and yeast was researched and it was discovered that fermented milks produced with mixed cultures of lactic acid bacteria had a wider range of antimutagenic activity than those produced with a single strain of lactic acid bacteria, (Tamai; et al., 1995). Nandhini and Palaniswamy (2013) observed similar results when they investigated the anticancer activity of goat milk hydrolysate fermented by *Lactobacillus plantarum and Lactobacillus paracasei*. *Lactobacillus (L. acidophilus 606 and L. casei A TCC 393, L.*

rhamnosus GG and L. brevis A TCC 8287) inhibited the growth of several human cancer cell lines, according to Choi et al (2006).

6.2.2 LAB in preventing colon cancer

Some LAB strains, such as *Lactobacillus delbrueckii* subsp. *Bulgaricus*, may exhibit anti-mutagenic properties due to their capacity to bind to carcinogenic heterocyclic amines (Wollowski et al., 2001). LAB has been shown to protect rodents from colon cancer in animal tests. Some human trials have also suggested that certain varieties of LAB may be anti-carcinogenic due to their capacity to reduce the activity of an enzyme called β glucuronidase. (Brady *et al*., 2000) (Which can generate cancer producing substances in the digestive system). Lactic acid bacteria are recognized for their antibacterial, antioxidant, anti-inflammatory, and anticancer properties, in addition to balancing intestinal flora (Dethlefsen et al., 2008). The cell-free filtrate and cell-free lyophilized filtrate of LAB (*Pediococcus pentosaceus, Lactobacillus plantarum,* and *Weissella confusa)* were reported to have antiproliferative effects on human colorectal adenocarcinoma cells in a recent study (Knecht et al., 2014). *Lactococcus lactis* KC24 isolated from kimchi has anticancer activity against gastric carcinoma (AGS), colon carcinoma (HT-29 and LoV o), breast carcinoma (MCF-7), and lung carcinoma (SK-MES-1) cells (>50 percent cytotoxicity), according to Lee NKet al (2008). Sevda ER et al. the effect of cell-free filtrate and cell-free lyophilized filtrate of *Pediococcus pentosaceus*, *Lactobacillus plantarum* and *Weissellaconfusa* suppress the growth of colon cancer cell in a dose-dependent manner, by using MTT assay and *L.* plantarum showed the strongest inhibitory effect. Similarly, Ewaschuk et al. found that *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *Bifidobacterium breve*, *B newbornis*, *B longum*, and *Streotococcus thermophilus* reduced the viability and induced apoptosis in human colon cancer cells. Moreover, It is feasible to deduce from the aforementioned data that lactic acid bacteria strains have anticancer activity and are not hazardous to normal cells.Another *Lactococcus lactis* has anti colonic cancer action, according to Kim et al. (2003), because of its ability to enhance the amount of antiproliferative protein and diminish the effects of mutagenic protein. From the evidence presented thus far, it is obvious that LAB has an important role in colon cancer antiproliferation, metastasis, growth and development, and spread.

6.2.3 Immune responses induced by lactic acid bacteria

Several elements of the immune system, such as antigen presenting cells (APCs), a variety subsets of T cells, B cells, natural killer (NK) cells, and dendritic cells (DCs), is usually activated by damage, invasion or mutation (Jounai et al., 2012). Recent research has linked LAB to immune responses that are important for colorectal cancer prevention and treatment (Gabrilovich and Pisarev, 2003). *Lactobacillus* species, in particular, have the potential to play a significant role in antimicrobial activity as a contributor to the host's immune system. In mouse peritoneal macrophages, *L. plantarum* strain YU, which was isolated from food products, demonstrated strong interleukin 12-inducing activity (Kawashima et al., 2012).

6.2.4 Cytotoxic effects of lactic acid bacteria

According to several studies, certain species of LAB isolated from animals, plants, and fermented foods have cytotoxic effects on cancer and tumor cells. The most common lactic acid bacteria are *Lactobacilli* and *Bifidobacteria* (Wang et al., 2014). The different fractions of LAB such as whole cells, heat-killed cells, the cell wall, peptidoglycan, and cytoplasmic fractions of LAB all have anti-cancer properties against human cancer cell lines (Kim et al.,

2003). Furthermore, polysaccharide fractions derived from *Lactobacillus* cultures and glycoproteins identified in *Lactobacillus* culture supernatants have been shown to have the similar impact. The anti-proliferative effects of the cell-free filtrate and cell-free lyophilized filtrate of 3LAB (*P. pentosaceus, L.plantarum, and W. confusa*) on the human colorectal cancer cell line were studied by Sevda et al (2015). Paolillo et al. (2009) investigated the cytotoxicity of live *L. plantarum* cells on Caco cells. Some LAB strains' anticarcinogenic and/or antimutagenic qualities have been shown to have a dose-dependent response (Salminen et al., 1998). In general, *P. pentosaceus*, *L. plantarum*, and *W. confusa* have the ability to stop cancer cells from multiplying. Moreover, Villarante et al. (2011) have discovered that bacteriocin isolated from *Pediococcus acidilactici* has a cytotoxic effect on HT29 (human colon cancer) and HeLa cells, as measured by the MTT assay. In the modern era, functional foods, pharma foods, and nutraceuticals are in high demand in the for illness prevention (Khan, 2014). Globally, rising interest has sparked innovation and new product creation in the food business (Vinderola, 2008).

6.2.5 LAB Induction Apoptosis

Several studies have shown that LAB can regulate cell apoptosis via intrinsic and extrinsic routes, which are potentially important strategies in cancer cell prevention. Chen et al. (2013) .According to findings, the effects of *Lactobacillus acidophilus* (*L. acidophilus)* oral supplementation investigated on colorectal cancer in mice. In treated mice, *L. acidophilus* reduced the severity of colorectal cancer cytogenesis and increased apoptosis. *Lactobacillus reuteri (L. reuteri*) has been demonstrated to protect against colorectal cancer by inhibiting nuclear factor-kappaB (NF-B)-dependent gene products that control cell proliferation (Cox-2, cyclin D1) and survival (Bcl-2, Bcl-xL) (Iyer et al., 20 08).The application and usage of LAB and its elements is a promising approach in the search for novel, unconventional therapies and prevention techniques to prevent cancer and tumor cells.

6.3 Genetically Engineered *Salmonella Typhimurium* for Anti-Cancer Therapy:

The ability of bacteria to colonize tumors has been used to create genetically modified bacteria that restrict their activity to the tumor area. (Shruti et al., 2020). Transgenic bacteria invade target cells and express transgenes, resulting in the expression of therapeutic proteins. This process is called bacterial infection. Invasive bacteria can deliver genes to tumor cells within the cell, thereby promoting bacterial infection in tumor cells, while non-invasive strains are designed to release therapeutic proteins outside the tumor microenvironment. (Baban et al., 2010)

6.3.1 Tumor Targeting Enhancement:

Facultative anaerobes such as *Salmonella* cause toxicity to normal tissues that survive in an oxygen-rich environment. Therefore, it is very important to improve the tumor direction of facultative anaerobes. In order to improve tumor targeting, two methods can be implemented. One of them is to identify certain tumors through bacterial genetic engineering. For example, integrin αvβ3 is overexpressed in cancer cells, and *Salmonella* ppGpp deletion strain SHJ2037 was developed to attack cancer cells by presenting ArgGlyAsp integrin binding peptide (RGD) on the surface of the bacteria. Antitumor activity of MDAMB231 breast cancer cells and MDAMB435 melanoma xenografts that overexpress integrin αvβ3 (Park et al., 2016). The

second method is bacterial genetic engineering of tumor-associated antigens. To this end, a surface strain of Salmonella lymphoma-associated antigen CD20 was developed to transport a prodrug enzyme, the *herpes simplex* virus thymidine kinase (HSVTK). Simultaneous administration of HSVTK effectively treated chitin xenograft mice. (Mas et al., 2013)

6.3.2 *S. Typhimurium* mediated Cancer Treatment Strategies:

Attenuated *Salmonella typhimurium* can inhibit various cancers in mouse models. Various strategies are being developed, including combined therapy with radiation or chemicals and genetically modified bacteria to express therapeutic agents such as cytotoxic proteins, cytokines, prodrug enzymes, regulators, DNA vaccines, or genetic materials to suppress genes (Jin et al., 2016).

Table 5: *S. Typhimurium* mediated cancer treatment strategies

Strategies	Released Products	Results	Reference
1. Native cytotoxicity and combinational therapy	Bacterial components (such as LPS, flagellin, and CpG), signals/molecules and IL-18,TNF-α released from damaged cancer cells	The production of the inflammatory cytokine IL1 mediates the anti-tumor immune response by activating the TLR and NLR signaling pathways. Bacteria that grow rapidly also deprive tumors of nutrients, leading to starvation and death of cancer cells.	kim et al., 2015; Zhang et al., 2015; wang et al., 2013; Zhao et al., 2005; Momiyama et al., 2012; Yam et al., 2010; Yano et al., 2014; Sznol et al., 2000; Hiroshima et al., 2014
2. Cytotoxic proteins	used as a vector	Administration and expression of tumor-specific cytotoxic agents to slow tumor growth; however, the expression of toxic genes must be strictly regulated by inducible or tumor-specific promoters to avoid inadvertent damage to normal tissues.	Nguyen et al., 2010; Jiang et al., 2013; Shi et al., 2016; Ryan et al., 2009
	Engineered to transfer immune-eligible cytokines such as IL2, IL18,	IL2: induced tumor suppression correlates with decreased angiogenesis and increased necrosis in tumor tissue.	

3. Cytokines	CCL21 and LIGHT for targeted cancer immunotherapy.	IL18 (known as IFNɣ inducer) produces cytokines and increases the cytolytic activity of T cells and NK cells. IL18 also up-regulates the expression of MHC class I antigens and promotes the differentiation of CD4+ helper T cells. Th1 cells inhibit angiogenesis by inhibiting the proliferation of endothelial cells, amplifying anti-tumor effects through NK cells, macrophages, and CD8+ T cells.	Loeffler et al., 2009; al-Ramadi et al., 2009; Ha et al., 2012; Loeffler et al., 2008; Xiang et al., 2005; Loeffler et al.,2007
		CCL21: regulates the migration of lymphocytes, dendritic cells, and NK cells.	
		LIGHT (also known as TNFSF14 or HVEML) is a TNF family kinase that is homologous to lymphocytes and binds to both the lymphoid toxin α receptor (LTβR) and herpes virus invasion mediator (HVEM) expressed in cell carcinoma. It is expressed by T lymphocytes.	
4.Regulatos	SPRY1/2 proteins, E6 viral oncoprotein, p53 and mdm2 siRNA	SPRY1 / 2 Protein is an endogenous regulator of the receptor tyrosine kinase (RTK) signaling pathway. Activation of the RTK signaling pathway often correlates with cancer cell proliferation, angiogenesis and progression. Passing SPYR to tumor tissue through engineered VNP20009 significantly suppressed melanoma growth in vivo. Tumor suppression was mediated primarily through suppression of ERK1 / 2 phosphorylation.	Liu et al., 2015; Jiang et al., 2015; Jeong et al., 2015
		The E6 viral oncoprotein binds to wild-type p53 (wtp53) in the host cell and disrupts its function. Thus, in HPV-positive cervical cancer, E6 gene silencing restores cell cycle arrest and apoptosis-related p53 function and provides cancer suppression both in vitro and in vivo.	

		Engineered *Salmonella* coexpressing p53 and mdm2 siRNA increased the therapeutic effect of cisplatin on prostate cancer. *Noxa's* MTD-expressing ΔppGpp strain increased cytosolic calcium concentration and mitochondrial permeability, inducing cell death.	
5. Enzymes	Prodrug activation enzyme, carboxypeptidase G2 (CPG2), Attenuated VNP20009 expressing *E.coli* cytosine deaminase (CD)	Administration of an attenuated *S. typhimurium* expressing *E. coli* purine nucleoside phoshorylase (ePNR) resulted in the release of two prodrugs, 6 methyl purine 2′ oxyriboside (MePdR) and 6 meprine 2′ oxyriboside (MoPdR). It converts to toxic substances called 6-methyl purine (MeP) and 6-mephrin (MoP) which causes tumor-specific cell death. The prodrug activating enzyme, carboxy peptidase G2 (CPG2), activates a variety of prodrugs and induces cytotoxicity in human tumor cells, but not in the host bacteria.	Fu et al.,2008; Chan et al.,2013; Friedlos et al.,2008; Tjuvajev et al.,2001; Soghomonyan et al.,2005
		Attenuated VNP20009 expressing *E.coli* cytosine deaminase (CD) have been injected directly into the tumors which converts 5-fluorocytosine (5-FC) into a cytotoxic anticancer drug 5-fluorouracil (5-FU) to treat different types of cancer.	
6. RNA interference	Production of small interfering RNAs (siRNAs), mdm2 silencing	Production of small interfering RNAs (siRNAs): *S. typhimurium* is treated with an enzyme cutter to produce small interfering RNA (siRNA) that triggers the breakdown of target RNA. Signal converter and activator of transcription (Stat3), a factor that inhibits apoptosis and promotes cell growth, is overexpressed in many cancers. It is an attractive target for shRNA-mediated gene suppression and has been extensively researched to prevent metastasis and inhibit tumor growth.	Zhang et al., 2007; Manuel et al., 2011; Zhang et al., 2007; Li et al., 2013; Tian et al., 2012; Jiang et al., 2013; Manuel et al., 2015; Blache et al., 2012; Deng et al., 2015; Liu et al., 2012; Li et al., 2013
		The silencing of mdm2 restores the activity of p53 to regulate cell cycle and function, thereby inhibiting tumor growth.	

Table 5: Different types of strategies to inhibit the cancer cells are briefly discussed.

6.4 *Mycobacterium bovis*:

Melanoma is a benign disease caused by the malignant transformation of normal melanocytes. It is the most aggressive type of skin cancer (Bandarchi et al., 2010), accounting for 4% of all skin cancers (Siegel et al., 2019). The etiology is multifactorial, including environmental and genetic factors. BRAFV600E is the most common mutation which promotes tumor growth, angiogenesis, and metastasis progression in approximately 50% of melanomas (Garnett et al., 2004; Marais et al., 2004; Haass et al., 2004; Haass et al., 2004). Many recent studies have identified new treatment strategies and goals to reduce the possibility of melanoma metastasis (Orgaz et al., 2013; Sanz et al., 2013; Mattia et al., 2018). One such treatment is *Mycobacterium bovis* BCG (BCG). BCG is an attenuating agent that has been used as an immunotherapeutic agent for melanoma and superficial urothelial carcinoma (Begnini et al., 2015; Maruf et al., 2016). Currently, wild-type BCG and recombinant strains are used together with chemotherapy and immunotherapeutic to enhance immune response and tumor regression (Stewart et al., 2011; Levine et al., 2011) for immunotherapy and vaccination. (Yuan et al., 2010; Zheng et al., 2015). BCG is divided into other strains (Leung et al., 2008; Hayashi et al., 2009; Liu et al., 2009). BCG shows anti-proliferative activity and produces cytokines including IL6 and IL8. (Secanella et al., 2013).

6.4.1 rBCG strains as therapeutic agents for melanoma:

Enhance the immune response to rBCG: Ag85BIFNγ, for C57BL/6 mice and TNFα and IFNγ induced tumor necrosis factor (Liu et al., 2017). It has also been found that transgenic BCG: Rv2645 can enhance antigen presentation on dendritic cells (DC) and enhance the Th1/Th17 immune response in tuberculosis (Luo et al., 2018). An auxotrophic pantothenic acid strain of *Mycobacterium bovis* BCG expressing HIV1 Gag, Gp120 and RT (BCGDpanCD) induces T cells to produce IL2 and TNFα (Chapman et al., 2013) have shown that another recombinant BCG-Line (ΔureC :: hly) expressing heterologous protein from Listeria monocytogenes to induce the production of caspase, IL18 and IL1β (Saiga et al., 2015) Study on ureChly + BCG (Desel et al., 2011) are tested to prove their clinical efficacy in terms of immunogenicity and safety (Nieuwenhuizen et al., 2017).

6.4.2 BCG in melanoma immunotherapy:

The development of vaccines containing BCG adjuvants can promote anti-tumor immunity by recruiting NK, CD4 + and CD8 + T cells, thereby triggering an immune response (Murphy et al., 1993; Kim et al., 2014; Cantor et al., 2014; Kaufmann et al., 2016). This results in the release of cytokines into the tumor microenvironment. (Lardone et al., 2017). The use of BCG as an immunotherapeutic agent for melanoma has been shown to induce tumor regression (Morton et al., 1976), and local or intratumoral injection of BCG can cause infiltration and increase long term effects. The expression of chemokines and cytokines, including CXCL9, CXCL10, CXCL11, IL 15, TNFα and IFNγ. (Yang et al., 2017) Due to the clinically observed high tumor heterogeneity and relatively low immunogenicity of melanoma-associated antigens, the immunotherapy strategy for melanoma with rBCG strains capable of secreting functional cytokines is the ability to stimulate anti-tumor immune responses has broad prospects. (Sanlorenzo et al., 2014). The development of recombinant BCG strains has led to the development of new vaccines. Compared with traditional systems, these vaccines can

provide multiple antigens and induce long-memory CD8 T cell immune responses. (Costa et al., 2014; Liang et al., 2015; Oliveira et al., 2017). In addition, these rBCG strains have been genetically modified to display different phenotypes, such as using Hsp60, Hsp7, pAN and 18 kDa promoters. (Newton et al., 2013; Gey et al., 2013 Van et al., 2013; Oliveira et al., 2017), which will increase the expression of cytokines and improve the immune response. (Himmelrich et al., 2000; Slobbe et al., 1999). The use of different rBCG strains that secrete different cytokines (such as IL4, IL6, IL2, IFNγ, and GMCSF) can change and possibly enhance the anti-tumor response. (Murray et al., 1996; Zhou et al., 2015). In addition, this method can also develop auxotrophic strains, including strains that exclude genes involved in metabolite synthesis, strains without antibiotic resistance markers, and strains that exhibit stable expression in vitro and in vivo. (Borsuk et al., 2007; Seiksas et al., 2010; Rizzi et al., 2017). Several studies have reported the use of rBCG strains to treat melanoma. One of the most commonly used rBCG strains for cancer treatment is the rBCG strain that expresses interleukin 2 and GMCSF. (Fujimoto et al., 1996). One of the most important strains used is the Pasteur strain, which contains a promoter for the heat shock protein 60 (hsp60) that controls the expression of IL2. When administered by intratumoral injection, this rBCG strain showed immunomodulatory properties and resulted in a 45% reduction in tumor size in the B16 melanoma mouse model (C57BL/6 mice) (Duda et al., 1995). In addition, rBCG can express recombinant human interferon α 2B (rhIFNα) under the control of the hsp60 promoter, thereby increasing the production and immunostimulatory properties of IFNγ (Luo et al., 2001).

CHAPTER: 7

Use of Bacteria in Cancer

Although a variety of bacteria have been used for cancer treatment however in some types of cancer it has shown potential improvement such as Gastrointestinal cancer ,Lung cancer, Colon cancer ,Breast cancer, stomach cancer so some of them have been discussed in detail in the following sections.

7.1 Lung and Gut Microbiota as Potential Hidden Driver of Immunotherapy Efficacy in Lung Cancer

Lung cancer is one of the world's deadliest and most frequent cancers, representing one of the most difficult challenges in cancer treatment (Carbone et al., 2019). Some of the major findings depicting bacteria as a crucial gatekeeper for the immune response against tumors, as well as their role as a driver of immunotherapy efficacy in lung cancer, have been discussed, with a special focus on the distinct role of gut and lung microbiota in the efficacy of immunotherapy treatment. Small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) (also known as lung cancer "LC") are one of the most deadly cancers in the world. According to the American Cancer Society, there will be 116,440 new LC cases and 111,710 new LC deaths in 2019, with 24 % and 23 % of new fatalities for men and women, respectively. (Siegel et al., 2019). Many studies have shown that the direct contact between the microbiota and the human epithelial barrier is needed for immune system maturation and function, which affects the host's health and also immunotherapy's ability to promote anticancer response. The release of proinflammatory cytokines, metabolites, or nucleic acids by the microbiota could potentially modify and eventually amplify an immune response, allowing for a microbiome-based selection of individuals who could benefit from specialized immunotherapy treatment. (Carbone et al., 2019). Because this field of study is still in its infancy, additional efforts are

needed to define the role of the microbiota in the response to immunotherapeutic drugs, as well as to depict the gut-lung axis and its consequences in detail.

7.2 Role of Commensal Bacteria in Cancer Response to Immunotherapy

The increasing evidence supports the idea of a dynamic connection between immune cells, microbiota, and the tumour microenvironment. Disruption of the microbiota reduces the efficiency of CpG-oligonucleotide immunotherapy by changing the activities of myeloid-derived cells in the tumor microenvironment (Iida et al., 2013). Furthermore, oral administration of *Bifidobacterium* increases anti-PD-L1 antibody response in mouse models of cancer by enhancing CD8+ T cell accumulation in the tumor microenvironment and activating dendritic cell activity (Sivan et al., 2015). The immune stimulatory effects of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) blockage are also influenced by the nature of the microbiota composition.In a recent study *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were found in abundance in responding patients that evaluated baseline stool samples from 42 metastatic melanoma patients before immunotherapy treatment. Anti-PD-L1 therapy was improved by fecal transplantation of germ-free mice with stool from responding patients, which increased immune-mediated tumor suppression by inducing T cell response (Matson et al., 2018). All of these studies demonstrated the value of manipulating the gut microbiota in cancer treatment, particularly in tumours where immunotherapy is currently used in clinical practice, such as LC and melanoma. In 30-40% of melanoma patients, PD-1 inhibitors provide long-term responses. Recent research demonstrated that the gut microbiome could influence immunotherapy responsiveness. Moreover, in a mouse melanoma model, gut commensals such as *B. thetaiotaomicron* or *B. fragilis* are predictive factors for anti-CTLA-4 treatment (Vetizou et al., 2015). These findings imply that in LC patients, the gut microbiota influences the anti-cancer immune response (Viaud et al., 2013).

7.3 Bacteriotherapy in Gastrointestinal Cancer

The gastrointestinal (GI) malignancies, which include stomach cancer, colorectal cancer, liver cancer, pancreatic cancer, gastric cancer, and spleen cancer, are among the most often diagnosed cancers in both men and women (Dizdar O et al.,2019). According to WHO reports, colorectal cancer is the most prevalent diagnosed GI cancer, with over 1.80 million cases, stomach cancer with 1.03 million cases, and liver cancer with 782 000 fatalities per year (Bray F et al.,2018:Dizdar O et al.,2019). One of the most difficult aspects of conventional cancer treatment is nonspecific toxicity to normal cells. In recent decades, scientists have been searching for new effective anticancer medicines, and bacteriotherapy has been identified as a promising new strategy with fewer side effects in the field of cancer treatment. Bacteriotherapy refers to the use of bacteria strains, peptides, and proteins, as well as bacteriocins and toxins produced by bacteria, in cancer treatment. (Soleimanpour et al., 2020).

7.4 GI Microbiota as a Tumor-Suppressor

Evidence suggests that the GI microbiota's tumor-suppressing action is related to the release of chemicals that may have anticancer properties. Butyrate and propionate are produced by gut bacteria and have an anticancer impact by inhibiting tumor cell histone deacetylases (HDAC). HDAC suppression led to alterations in JAK2/STAT3, VEGF, and other key carcinogenic signaling pathways (Gao S-m et al., 2013: Sawa H et al., 2002). Butyrate suppresses cancer cell motility by deactivating Akt/ERK signaling in a histone deacetylase dependent way, which has anticancer effects in vitro and in vivo against colorectal cancer (CRC) and lymphoma

cancer cell lines (Li Q et al., 2017). Butyrate has the ability to obstruct both mitochondrial and extrinsic apoptotic pathways. Butyrate can also lower gut inflammation by encouraging T-regulatory cell differentiation through reduced NF-B and STAT3 pathway activity. Furthermore, microRNAs and methylation modulate oncogenic signaling molecules by butyrate (Schwab JM et al., 2007: Chen J et al, 2018). Additionally, microbiota has an anticancer effect via regulating the immune system of the host. According to many studies, gram-negative bacteria's lipopolysaccharide (LPS), which is a significant component of the outer membrane, can activate the host's cell surface receptor toll-like receptor 4 (TLR4), which belongs to the pattern recognition receptors ((PRRs) family, resulting in the activation of T cell-mediated response toward the tumor cells (Paulos et al., 2007).

CHAPTER: 8

Side effects of bacterial therapy in cancer:

Bacterioterapy has different advantages and disadvantages, like all the other therapeutic approaches. The pathogenic character or toxicity of the bacteria that may cause illnesses or even death is one of the most prominent concerns in the field of bacteria-mediated cancer therapy (Soleimanpour et al., 2020). To overcome this constraint, numerous investigations have used attenuated and genetically engineered strains. Other limitations of bacteriotherapy include short half-life of bacterial peptides or proteins, as well as unstable DNA. (Hu et al., 2011, Chen et al., 2005). In recent studies, genetic engineering approaches have been applied to improve the efficiency of bacteriotherapy drugs. Chemical modifications such as D- amino acid substitution, cyclization, and replacement of labile amino acids, for example, were used in several research to enhance the half-life and stability of therapeutic bacterial agents (Riedl et al., 2011; Torfoss et al., 2012). Despite the promising results of bacteria-mediated cancer therapy, this technique is still in its early stages, and further research is needed to overcome the limits of bacteriotherapy and develop more effective bacteriotherapy agents in the field of cancer therapy. Unfortunately, despite the fact that bacterial agents have potent anticancer properties, most studies have ended in the in vitro stage, with only a few making it to the clinical trial stage (Soleimanpour et al., 2020). Another major difficulty is incomplete tumor lysis, which occurs when bacteria do not consume all of the malignant tissue, necessitating the use of therapy in conjunction with chemotherapeutic therapies. Moreover, Small non-necrotic metastases of large primary tumors are more challenging problem to treat, as metastasis is the leading cause of cancer-related death. Bacterial targeting of these metastases is problematic because of their tiny hypoxic areas. The main challenge with bacteria-based vector therapy is accessibility, as most of the time an intra-tumor injection is necessary (Hatefi and canine, 2009). Although recombinant DNA technology has alleviated some of the safety concerns, it still requires additional development (Patyar et al., 2010).
As a result, more future in vivo studies or even more clinical trials are required to establish the anticancer potential of this unique therapeutic method in the field of cancer treatment before the compounds may be licensed as an anticancer drug. (Soleimanpour et al., 2020).

CHAPTER: 9

Pre-clinical and clinical trials:

Several bacteria and bacterial products have already been clinically trialed and some are in different clinical trial phases. **Table 6** have demonstrated about recent pre-clinical trials of bacteria mediated tumor therapy on some specific bacterial strains with their particular results and models such as dog, rat, mouse in some specific organ like brain, breast, bladder, ovary, pancreases, glioblastoma. Clinical trials have been conducted with prominent representatives of the *Listeria*, *Clostridia*, and *Salmonella* genera. While *Listeria monocytogenes* (ANZ-100) was primarily investigated as a recombinant attenuated therapeutic live vaccination for advanced cancer patients, (Maciag et al., 2009; Shahabi et al., 2010; Le et al., 2012) *Clostridia* and *Salmonella* trials mostly focused on the bacteria's intrinsic anticancer impact **(Table 7).** The first bacterial filtrates of *C.histolyticum* spores were tested in therapeutic trials in 1947 (Barbe et al., 2012).

Table: 6 (Recent representative pre-clinical examples of bacteria mediated tumor therapy (BMTT) in vivo from 2012 to 2015)

Species	Year	Model	Result	Reference
C. novyi	2014	Spontaneous, dog	Colonization and prolonged survival	(Roberts et al.,2014)
C. novyi	2015	Glioblastoma, rat	Colonization and prolonged survival	(Staedtke et al.,2015)
B. infantis	2013	Bladder, rat	High tumor specificity by engineered strain	(Yazawa et al.,2001)
L. monocytogenes	2014	Ovary, mouse	Reprogramming of M2-Mϕ, iNOS-mediated tumor cell lysis	(Lizotte et al.,2014)
L. monocytogenes (ANZ-100)	2014	Pancreas, mouse	Prolonged survival	(Keenan et al.,2014)
S. Typhimurium (A1-R)	2012	Breast, nude mouse	Intravenous administration most effective for tumor targeting	(Zhang et al.,2012)
S. Typhimurium (A1-R)	2012	Brain, nude mouse	Tumor growth inhibition and prolonged survival	(Momiyama et al.,2012)

S. Typhimurium (A1-R)	2014	Bone metastasis, nude mouse	Inhibition of breast cancer bone metastasis	(Miwa et al.,2014)
S. Typhimurium (A1-R)	2014	Pancreas, nude mouse	Tumor growth retardation, A-VEGF supports therapy	(Hiroshima et al.,2014)
S. Typhimurium (A1-R)	2015	Ovary, nude mouse	Prolonged survival, less metastasis formation	(Matsumoto et al., 2015)
S. Typhimurium	2015	Carcinoma (CT26), mouse	Complete tumor rejection in all cases by recombinant strain	(Frahm et al.,2015)
E. coli	2015	Breast (4T1), mouse	Secretion of toxic compound, reduced tumor volume	(Jean et al.,2014)
La. acidophilus	2014	Carcinoma (CT26), mouse	Increased apoptosis and tumor growth suppression	(Chen et al.,2012)

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

Table: **7 (Previous and ongoing clinical trials of Bacterial with bacteria mediated tumor therapy (BMTT))**

Bacterial Strain	Phase	Cancer type	n	Year	Result	Reference
S.Typhimurium VNP 20009	I	Metastatic melanoma, metastatic renal cell carcinoma	25	2002	Increased level of several proinflammatory cytokines (IL-Iβ, TNF- α, IL-6 and IL-12), tumour colonization were found in some patients,	(Toso et al., 2002)

					no objective tumour regression was observed even in patients with colonized tumour.	
S.Typhimurium VNP 20009	I	Melanoma	4	2003	There was no objective tumour response and only minor and transient side effects were observed.	(Heimann & Rosenberg,2003)
S.Typhimurium VNP 20009 expressing TAPET-CD(Cytosine deaminase)	I	Head and neck esophageal adenocarcinoma	3	2003	Two patients showed tumour colonization.	(Nemunaitis et al., 2003)
C.novyi -NT	NG	Advanced leiomyosarcoma	1	2014	Tumour reduction within and surrounding the bone	(Robert et al.,2014)
C.novyi -NT	NG	Solid tumour	NG	Active	Recruitment(NCT01924689)	_
L.monocytogenes(ANZ-100 and CRS-207)	NG	Solid tumour(liver, pancreas, lung,ovar)	26	2011	Safe vaccines that resulted in immune activation	(Le et al.,2012)
L. momocytogenes (CRS-207)	NG	Pancreatic Cancer	90	active	Extended survival with minimal toxicity	(Le et al.,2015)
S. Typhimurium VNP20009	I	soft tissue sarcoma (AUS, FSA, RMS, HPC or MXS), melanoma, carcinomas, osteosarcoma, haemangiosarcoma, lymphoma or mast	41		tumour colonization observed in 42% cases, with 4 CRs and 2 PRs	(Thamm et al., 2005)

		cell tumour(in canine)				
S. Typhimurium SalpIL2 (S. Typhimurium χ4550 expressing IL-2)	I	appendicular osteosarcoma (in canine)	19		No dose-limiting toxicity observed; tumour colonization not evident in tumours from 5 patients assayed; and disease-free interval of patients treated with amputation, SalpIL2 and doxorubicin significantly longer than historical comparison group treated with amputation and doxorubicin	(Fritz et al.,2016)
C. novyi-NT	NG	haemangiosarcoma, lingual SCC, osteosarcoma, nasal adenocarcinoma or fibrosarcoma (in canine)	6		tumour abscess observed in 3 patients	(Krick et al.,2012)
S. Typhimurium VNP20009	I	superficial solid tumours (Human)	NG		injected lesions are stable or responding to treatment and non- injected lesions are not progressing	NCT00004216
S. Typhimurium SalpIL2 (S.	I	liver metastases of solid	22		PO with dose escalation, with	NCT01099631

Typhimurium χ4550 expressing IL-2)		tumours (Human)			1×10^5–1×10^{10} CFU/dos	
S. Typhimurium VNP20009	I	patients with metastatic cancer(Human)	45		fusion with dose escalation and up to 12 total doses every 35 days for patients with SD, PRs or CRs	NCT00004988

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

Furthermore, *Clostridia* spore inefficiency in normoxic environments most likely promoted tumor progression (Mellaert et al., 2006). As a result, new clinical trials utilizing nonpathogenic *Clostridia sp.,* such as *Clostridia butyricumM-55 or Clostridia novyi-NT*, have been conducted (Table 7). Six client-owned dogs were treated with *C. novyi-NT* in 2012 after failing to respond to usual therapy. The spores germinated preferentially in the tumor once again, although they were still able to cause some acute toxic symptoms such as fever, nausea, and diarrhea (Krick et al., 2012). Historical studies with the oncolytic M-55 strain of *Clostridium butyricum* (later reclassified as C. sporogenes ATCC 13732) has been shown to colonize and lyse tumors in a variety of cancer types (Heppner & Mose, 1978; Carey et al., 1967; Schmidt et al., 2006). Clinical signs of tumor colonization (for example, pain, erythema, swelling, and spontaneous drainage at the target tumor, systemic signs of infection such as fever, and laboratory findings) have been observed in a large fraction of patients treated with *C. novyi-NT* spores administered intravenously or intra tumourally in more recent phase I trials (NCT01118819, NCT01924689). These trials also provided objective evidence of tumor response. In a patient who underwent direct injection of *C. novyi-NT* spores into a metastatic shoulder lesion of retroperitoneal leiomyosarcoma a computed tomography scan revealed substantial tumor destruction with gas pockets, a characteristic of infection with the gas-forming *Clostridium spp* (Roberts et al., 2014, NCT01924689)The lesion had significant tumor necrosis and no viable tumor cells, according to biopsies. Furthermore, *C. novyi-NT* was found in anaerobic culture of the biopsied material, implying that it was involved in tumor destruction (Roberts et al., 2014). However, these oncolytic bacteria therapies alone were unable to destroy all cancer cells, which inevitably led to progression or relapse (Roberts et al., 2014, Heppner & Mose, 1978; Carey et al., 1967; Schmidt et al., 2006).In addition, after numerous intra tumoral administrations of spore, Roberts and colleagues found continuous tumor reduction in a human patient with advanced leiomyosarcoma. While it is now well acknowledged that *Salmonella's* antitumor impact is due to an activated anticancer immune response (Leschner et al., 2009; Stern, 2012), In the case of *Salmonella*, in 2002 and 2005, the highly attenuated strain VNP20009 was evaluated in humans and dogs (Toso et al., 2002 ; Thamm et al., 2005). This strain was created specifically for the treatment of bacterial cancers. It has the purIxyl genotype and has been proven to be hyper invasive in M2 melanoma cells (Pawelek et al., 1997). Furthermore, the msbB gene was removed to make the Lipid A molecule less immunogenic (Low et al., 1999; Needham et al., 2013). While this strain has been demonstrated to colonize murine tumors rapidly and have potent anticancer effects, the results in canine and human hosts were not significant (Toso et al., 2002; Thamm et al., 2005). In dogs, colonization was only accompanied by a 25% therapeutic response, whereas colonization and therapeutic response in the human environment were essentially non-existent.The human

research showed that the majority of germs were removed from the circulation within 60 minutes. This might indicate a distinguishing role for complement lysis and phagocytic clearance in human patients when given systemically. These are the most crucial considerations for a successful bacteria-based cancer therapy (Frahm et al., 2015). In Another clinical trial, four additional metastatic melanoma patients were included in a clinical trial with *S. Typhimurium* VNP20009, but there was no objective tumor regression and only minimal and temporary adverse effects were observed (Heimann, D. M. & Rosenberg, 2003). Furthermore, mice were often kept in pathogen-free environments and had not been exposed to *Salmonella Typhimurium* prior to treatment. Some humans and dogs, on the other hand, may have been pre-exposed to the bacteria and hence have some immunity to it (Mandell & Bennett, 2010).Mice lacking the Toll-like receptor 4 (TLR4) or deficient in MyD88 signaling did not demonstrate any antitumor response when given *Salmonella* (Lee et al., 2008; Kaimala et al., 2014). Nonetheless, preclinical and clinical trials are currently being conducted on a variety of pure or synthesized MAMPs (Goutagny et al., 2012). The VNP20009 *Salmonella* strain was given to cancer patients with metastatic melanoma in a clinical trial in 2001. VNP20009 failed to colonize tumors in the great majority of patients, with a few outliers. As a result, any anticancer response must have been triggered by MAMPs delivered from outside the tumor. In this trial, however, no significant anticancer effects were detected within the patient cohort. Studies in mice have shown that , While some highly immunogenic tumors, such as the colon carcinoma CT26, can be easily impacted by systemically injected pure LPS or dead bacteria, more resistant tumors, such as RenCa (a renal adenocarcinoma), may not be treated in the same way (Frahm et al., 2015). As a result, the efficiency of a MAMP-based therapy is determined not only by the bacterial infection's potency, but also by the tumor's immunogenicity and the effectiveness of its escape mechanisms. There could be a variety of reasons for VNP20009's failure. VNP20009 was presumably eliminated long before it could elicit a proper immune response or colonize a tumor. The aforementioned substantial gene deletion and the loss of flagellar production, in particular, could have had a significant impact on the bacterial strain's fitness and immunogenicity (e.g., by its inability to trigger TLR5 via flagellar PAMPs) (Hayashi et al., 2001). Based on these concerns, a novel strain design concept must be created to suitably boost a mutant strain's immunogenicity while maintaining its attenuated character.

CHAPTER: 10

Challenges in Bacterial Cancer Therapy

Cancer is a multifactorial disease, and using bacteria as an immune stimulant or a vector for transporting therapeutic cargo is a potential treatment technique (Sawant et al., 2020). However, toxicity is a major issue with bacteria-mediated cancer therapy. Lower doses can alter therapy efficacy, whilst higher amounts can be toxic and have adverse effects (Patyar et al., 2010). So, the balance between the trial subject's benefit and safety must be maintained (Curran et al., 2018). Preclinical animal models and human individuals have different tumor structures, which can affect bacterial penetration and growth in the tumor (Nallar et al., 2017). As a result, the dose and route of administration must be optimized. Furthermore, bacterial clearance by the immune system before to reaching the tumor site may lead to therapeutic failure (Kramer et al., 2018). Moreover, any bacterial alterations may result in therapeutic loss

and exaggerated infections (Patyar et al., 2010). For example, there are some challenges with magnetotactic bacteria. One of them is increasing MTB production and looking at synthesis tools to tune MTB's magnetic behavior by controlling the magnetosome biomineralization process. Alternative options include importing and expressing the genes required in magnetosome formation into other bacterial species that are simple to grow and culture on a large scale to avoid scaling-up difficulties related with MTB production (Kolinko et al., 2014). Controlling the magnetosome composition, size, and morphology during the biomineralization process is another technique to modify MTB's magnetic response.However, we are still far from having a firm handle on the situation (Fdez et al., 2020).

Chapter 11

Conclusion:

Currently, in pre-clinical animal tumor models, bacteria have showed promising and significant effectiveness in clearing existing tumors. Nevertheless the successful translation of these preclinical techniques in clinical practice, will be determined by the results of clinical studies. Among these, anaerobic bacterium vector mediated cancer therapy and immunotherapy (Patyar et al., 2010) are extremely promising. Furthermore, tumor-targeting bacteria have unique characteristics, such as tumor selectivity and infinite gene packaging capability, that make them ideal vehicles for delivering cancer-specific therapeutic payloads. This unlimited gene packaging potential not only allows for the expression of large or many target genes, but it also allows for the creation of engineered signaling networks that allow bacteria to conduct complex cancer-fighting functions. Despite the tremendous therapeutic promise of modified tumor-targeting bacteria, successful cancer therapy will almost certainly require combinatorial techniques, as cancer heterogeneity (at both the molecular and histologic levels) renders a cure with single anticancer drugs extremely difficult. In addition to chemotherapy and radiotherapy, which can have anticancer effects that are synergistic with bacteria. Despite this, there are still a number of issues with employing bacteria as anticancer agents in clinical practice, including bacterial toxicity, DNA instability, low targeting efficiency, the selection of practical and safe bacterial strains, and evaluating combinations with other medicines (Forbes, 2010;Van et al., 2006;Wei et al., 2007) . Another big risk with bacterial therapy is the potential of DNA mutations, or the loss of functioning caused by mutations, which can result in a number of issues such as therapy failure or increased infection. Hopefully, more sophisticated genetic engineering of customized strains will be able to overcome these limitations (Sedighi et al., 2019).In the near future, clinical research with such "smart" bacteria will hopefully establish this method as another powerful weapon in the arsenal in our fight against cancer (Duong et al., 2019).

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