

**BACTERIA IN CANCER THERAPY: AN EMERGING ROBUST STRATEGY**

Preenu P Sen, Gautham A, Murugan Manavalan, Mansoor Ani Najeeb\*

Department of Biomedical Engineering, Noorul Islam Centre for Higher Education, Kumarcovil, Kanyakumari Dist, Tamil Nadu, India

\*Corresponding Author Email: animansoor@gmail.com

Article Received on: 19/03/13 Revised on: 07/04/13 Approved for publication: 11/05/13

DOI: 10.7897/2230-8407.04501

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

© All rights reserved.

**ABSTRACT**

Cancer is one of the leading causes of death in the world. Solid tumors account for 90% of all cancers. Despite rapid developments in the conventional modalities of cancer treatment (chemotherapy and radiotherapy) there is still no definitive “cure” for cancer. The present observation aims to an eye on alternative cancer therapies. The search for alternative therapies dates back to early 1800’s when a patient was observed to recover spontaneously following an infection. This resulted in the birth of Coley’s toxin for the treatment of sarcomas and use of live Bacillus Calmette – Guerin (BCG) for bladder cancer. The same provoked for a series of researches in this field, but was heralded due to lack of specificity, lack of complete tumor lyses and ineffectiveness against small tumors. However with the advent of recombinant DNA technology and its developments, it has paved the way for a new era for a tumor- targeted bacterial vector system for gene therapy of solid tumors. In this review we will discuss the potential of genetically manipulated bacteria that will usher in the new era of bacterial therapy for solid tumors.

**Keywords:** Cancer; Coley’s toxin; BCG; Recombinant DNA; Bacterial therapy

**INTRODUCTION**

Cancer is distinguished by uncontrolled and invasive growth of cells. These groups of cells may spread to other parts of the body and is called as ‘Metastasis’. Cancer has been considered as one of the lethal diseases from antediluvian time itself. Even though various researches and studies about cancer therapeutics are going on, no proper cure for cancer has been invented so far. Although conventional therapeutic methods for cancer consisting of radiotherapy and chemotherapy exist, for about half of the cancer victims, these are ineffective<sup>1</sup>. As a result of the low successive rates in conventional cancer therapies, advanced researches and studies led to experimental cancer treatments. Experimental cancer treatment can be briefed as medical therapies used to treat cancer by remodeling, reinforcing or substituting prevailing methods. Experimental cancer treatments include Photodynamic therapy, HAMLET, Telomerase therapy, Gene therapy, Hyperthermia therapy, Dichloroacetate (DCA), Insulin potentiating therapy, Diet therapy, Complementary and Alternative therapy, Non-invasive RF cancer treatment and Bacterial treatment<sup>2</sup>. But many of these therapies are uncertain due to lack of evidence, availability, specificity, selectivity and other related factors. With the help of certain advanced research methodologies, it’s been found that certain microorganisms could be used for controlling the growth of cancerous cells over the affected areas. Many viruses like vaccinia virus, reovirus, Newcastle disease virus and adenovirus with an E1a deletion, which are engaged to achieve selective replica and destruction of tumour cells, were studied in detail<sup>3</sup>. Viruses are referred as the most commonly used micro-organisms to carry altered genes to cancer cells, and they latch on to the cancerous cells. Oncolytic viruses cause rupture of cancer cells but the effective use of such viruses is sometimes prohibited by the production of potentially overriding antibodies generated in contrast to them. It was then came the vital role of bacteria in cancer therapeutic methods<sup>4</sup>. Studies and researches have stated that certain bacterial species can be used as cancer therapeutic agents as active elements. Moreover they possess

certain advantageous features over viruses such as motility, capacity to simultaneously carry therapeutic proteins and nutrients and resistivity against antibiotics. These all features concluded bacterial treatment as a promising tactical method in cancer treatment.

**Background**

The role of bacteria as an anticancer agent was recognized almost hundred years back. Even though the invention was accidental, it led to many successful developments in cancer therapeutics. The German physicians W. Busch and F. Fehleisen separately noticed that certain types of cancers reverted following accidental erysipelas infections that occurred while the patients were hospitalized. Independently, an American physician William Coley observed that one of his patients suffering from neck cancer began to recover following an infection with erysipelas. Later, he started researching about the disease, erysipelas. As a result of his experimental strategies, he recognized the pathogen of erysipelas as *Streptococcus pyogenes*<sup>5</sup>. His inventions led the milestone of cancer therapy methods with the help of bacteria and began well documented use of bacteria and their toxins to treat solid tumours. He developed a vaccine in the late 1800’s with the help of two killed bacterial species *Streptococcus pyogenes* and *Serratia marcescens*<sup>6</sup> and this vaccine was widely used to treat patients who were suffering from sarcomas, carcinomas, lymphomas and related diseases. The treatment responded with prolonged regression of advanced malignant stages in most of the cases. ‘Coley’s toxin’ was also studied for potential anticancer therapy<sup>7</sup>. The prevent success of Coley’s Toxin accounted for current and further researches in this area.

**Bacterial Therapy**

After Coley had developed the toxin (1800’s), advanced researches in bacterial therapy for cancer was introduced by a group of scientists. They identified certain species of anaerobic bacteria; those belonging to *Clostridium* genus

were the ones responsible for the actual cause of the positive response shown by Coley's toxin<sup>8</sup>. These findings made bacteria as one of the primary Oncolytic agents. Since, bacteria alone were not able to cure all parts of the malignant tissues, by combining radiotherapy and chemotherapy with bacterial therapy, a new methodology was invented for cancer therapy. Thus bacteria can be significant supporting element for current anticancer therapies which include chemotherapy and radiotherapy<sup>9</sup>.

Bacterial products like endotoxins have already shown better results in anticancer therapy. These cancer vaccines are mainly based on immunotoxins of bacterial origin. Bacteria can be used as delivery agents for anticancer drugs and as vectors for gene therapy.

Our body is comprised of numerous cells which undergoes cell division. All these activities are controlled by molecules in our body. Normal cells regenerate in a proper manner and they will be shattered after the proposed cell life. But, in a cancerous site, the cells regenerate again and again inconsistently and pass through uncontrolled multiple cell divisions. This abnormal activity leads to the formation of cancer lumps and masses at different part/s of the body, which in turn leads to solid tumors. Cancerous cells multiply continuously contrary to normal cell growth.

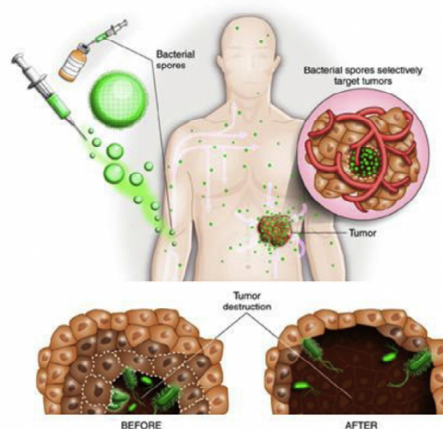
Nutrients are one of the essential elements required for the body. Our body has its own mechanism in procurement of nutrients. Normally, blood vessels act as carriers of nutrients all over the body. In a cancerous state, nutrients won't be reaching the cancer affected areas properly. In this state, alternative blood vessels will be formed by the cells for meeting the adequate nutrient requirements. Even though alternative blood vessels exist, they won't be able to meet the normal functionality of a blood vessel. Moreover enormous cancer masses will be present here. These areas will be oxygen deficient and are commonly termed as Oxygen deprived areas<sup>10</sup>.

Since the cancerous area consist anaerobic portions too, the ideology of using anaerobic bacteria for the treatment of solid tumors paved a new lead against solid tumors. Several scientists and genetically expertise people discussed about this and concluded anaerobic bacteria as a promising assisting agent for solid tumors or cancers. The process what they explained was about inflowing anaerobic bacteria to the anaerobic portions of the cancerous area. Since anaerobic bacteria becomes active in oxygen deprived environment, the anaerobic sites of cancerous area was considered as perfect sites for breeding of these species. These bacteria multiply and destroy the cancerous cells present in the anaerobic portions present in the solid tumors. This was considered as an efficient treatment procedure against cancer until the problem of infections caused by bacteria aroused<sup>11</sup>. Bacteria cannot be directly inoculated inside the body. Several risk factors like occurrence of bacterial infections, disturbing the immune system of the body etc. aroused. As a result of all these problems, bacterial treatment procedures in cancer therapy were immobilized, since these problems could not be stabilized at ancient times. Later, the knowledge about Polymerized Chain Reaction, Recombinant DNA Technology and Genetic Engineering paved the way for further developments in the treatment of cancer with the help of anaerobic bacteria<sup>12</sup>.

### Bacteria as Anticancer Agents

Since after the progressions in Genetical Engineering, several techniques were proposed for hosting anaerobic bacteria

inside the body for treatment of solid tumors and cancers. Among them the most prevailed was the use of attenuated bacteria for cancer therapy. The use of live, attenuated / weakened or genetically modified bacteria begun to emerge as potential anticancer agents. Spores of anaerobic bacteria are used in cancer therapeutic methods. It's mainly because only spores that reach the oxygen deprived area could germinate, multiply and become lively and thereby energizing the activity<sup>13</sup>. The primary bacteria experimented for the cancer therapy was bacteria belonging to clostridium class. Even though it showed better results the animals which were experimented on died due to acute toxicity. This resulted to the focus of a non-pathogenic strand of Clostridium such as M55, which showed promising results. The species were able to colonize the anaerobic areas of the cancerous tissues and succeeded in preventing the activity of proposed cancerous site but were not able to produce substantial tumor regression. Recently, a lot of bacterial species have been checked for their ability against cancers, but most of them concluded in death. *C. novyi* – NT was one among them obtained after removing a gene arrangement for a lethal toxin showed promising results but produced toxicity which made them inappropriate for cancer therapy<sup>14</sup>.



**Figure 1: hypoxia targeting therapy with bacterial spores**

(Source: <http://www.chordomafoundation.org/latest-updates/clinical-trial-using-novel-cancer-killing-bacteria-opens-to-chordoma-patients/> [Date of Browsing: 14-05-2013])

Since *C. Novyi*- NT spores showed promising results with toxin generation as a major defect, a series of researches were carried out about using the same vector for cancer therapy along with conventional methods like chemotherapy and radiotherapy. This strategy was known as Combination Bacteriolytic Therapy (COBALT)<sup>15</sup>. It showed significant anti- a cancer therapy but still wasn't able to provide completely successive results. Later, lipomase was identified as the factor responsible for enhanced drug release in bacteria. In- vivo experiments were carried out in many species of animals for screening the efficiency of the bacterium. *C. novyi*- NT along with a single dose of liposomal doxorubicin led to further studies in this area. Later, Recombinant DNA Technology was introduced and *C. novyi*- NT undergone this method by combining with anti-microtubule destabilizers such as vinorelbine and HTI- 286 helped in reducing the blood flow to cancerous sites and thereby helped in forming a hypoxic area for spore germination for anaerobic bacteria. The most successfully tested bacteria so far are

Bacillus Calmette- Geurin (BCG). It is generally used for the treatment of superficial bladder cancer<sup>16</sup>.

Salmonella typhimurium is another species which has been developed for cancer treatment. Deletion of its two genes from the normal DNA strand resulted in complete attenuated form of the species making it suitable for cancer therapy. This vector showed prolonged efficiency against wide range of tumors and cancers, and was able to destroy metastatic lesions. The major advantage of using Salmonella typhimurium instead of clostridium species is its ability to germinate in both aerobic and anaerobic environment, indicating its activity against normal tumors too. VNP20009 is another vector which has been successfully tested in clinical trials in cancer patients. It is sure that other alive, attenuated forms of bacteria like Bifidobacterium will be assessed in human medical trials in future. Some of the new strains of bacteria species which are being investigated as anti- cancer agents are: Vibrio cholera, Listeria monocytogenes, Salmonella choleraesuis, E. coli and certain bacteria from Clostridium species<sup>17</sup>.

### Bacteria and Gene Therapy

Bacterial approaches in the treatment of cancer showed promising results with minor faults. The major problem with using bacterial species as anti- cancer agents is their amount of toxicity generated. Researchers were not sure whether the toxicity produced by the vectors were much enough for the therapeutic efficiency. Another obstacle faced was the targeting of specific area to a solid tumor. As a solution for this, gene therapy was encompassed with the existing anaerobic bacterial species. Genetically engineered bacteria were used as a result of this technology to prompt a precise therapeutic gene. Proteins of desired interests were developed in the cancerous environment; these bacteria acted as drug enhancing agents to various cancer therapies. Thus genetically modified bacteria were developed and they serve

as active vectors for delivering anticancer cancer agents, therapeutic proteins and cytotoxic peptides<sup>18</sup>.

### Enzyme Prodrug Therapy

This strategy is used to overcome the side effects caused by the bacterial therapy and uses the hypoxic bacterial species that have been genetically modified with a desired enzyme which converts a non- toxic prodrug into a toxic drug for bacterial cancer therapy. Thus a scientifically derived drug was used in cancer therapy. Nowadays, several enzyme producing prodrug are available. The salmonella species were also tested successfully. High levels of activity are detected in tumors after in vivo administration motivating further research. Both the prodrug and activated drug must be able to cross biological membranes for significant, because the prodrug will be stimulated within bacterial cells and the active drug will then need to enter the cancerous cells. Reformed B. longum by pBLES100-S-eCD yields cytosine deaminase in the anaerobic tumor, and studies have concluded this as an effective prodrug-enzyme therapy<sup>19</sup>.

### Bacterial Spores

The majority of the anaerobic bacteria can form exceedingly resistant spores which allow them to survive even in aerobic environment, even though they cannot germinate and multiple there. But once they meet the anaerobic conditions, the species become active and germination process will flourish making them ideal to target cancers. C. novyi- NT has shown promising results without any side effects. Pharmacologic and toxicological evaluation of these species found that the spores were rapidly wiped from the circulation by the normal reticuloendothelial system. Bacterial spores are considered as one of the best delivery agents for cancer therapeutics. A summary of significant clinical trials using bacteria are shown in the table<sup>20</sup>.

Table 1: List of genetically modified bacteria and their research phase

Intervention	Clinical trial phase	Present status	Disease conditions	Reference
VNP20009 (Attenuated Salmonella)	Phase I	Completed	Metastatic solid tumors	Bacteria in Cancer Therapy: A Novel Experimental Strategy (S Patyar et al.,2009)
TAPET-CD (Attenuated strain of Salmonella typhimurium)	Phase I	Completed	Neck, head and esophagus cancer	Bacteria in Cancer Therapy: A Novel Experimental Strategy (S Patyar et al.,2009)
Tf-107 CRM (Diphtheria toxin)	Phase I	Completed	Brain and Nervous system tumors	Bacteria in Cancer Therapy: A Novel Experimental Strategy (S Patyar et al.,2009)
IL4-PE- Interleukin-4- Pseudomonas exotoxin	Phase I	In progress	Brain and Nervous system tumors	Bacteria in Cancer Therapy: A Novel Experimental Strategy (S Patyar et al.,2009)
IL13-PE- Interleukin-13- Pseudomonas exotoxin	Phase I	In progress	Malignant Glioma, GlioblastomaMultiforme, Anaplastic Astrocytoma, Anaplastic Oligodendroglioma and Mixed Oligoastrocytoma	Bacteria in Cancer Therapy: A Novel Experimental Strategy (S Patyar et al.,2009)
Listeria Bacteria (Genetically modified Radioactive Bacteria)	Phase I	Completed	Malignant Pancreatic Cancer	www.einstein.yu.edu (DOA: 14-05-2013)

### Problems Regarding Bacterial Therapy

One of the major problems with using bacteria as an anticancer agent was the release of toxic substances within the body which may rupture the immune system of the body. Systemic infections caused by bacteria were another concern. Another main problem was incomplete tumor lysis i.e., bacteria won't consume all areas of the solid tumor, mainly anaerobic sites are considered as the active sites for spore

germination and further procedures. In case of bacterial gene therapy, the major obstacle is the inaccessibility because most of the times an intratumoural injection is essential. Another major concern was about the potential for DNA mutations. Any loss in functionality due to mutations can result into various problems. Although some of the above concerns have been solved with the recombinant DNA technology further developments are yet to be made<sup>21</sup>.

**CONCLUSION**

The journal focuses on the revolutionary role of bacteria in cancer therapy. Various trials conducted in mouse tumor models have proved beyond doubt the potency of bacteria in eradicating this deadly disease. However, the successful translation of these preclinical strategies into clinical practice will depend on the outcome of ongoing clinical trials. The availability of recombinant DNA technology and other modern modalities which includes the use of radioactive bacterial species along with conventional cancer therapy methods of chemotherapy and radiotherapy has provided a cutting edge for ongoing clinical trials.

**REFERENCES**

- Jain RK, New approaches for the treatment of cancer. *Adv Drug Delivery, Rev* 2001, Chapter 46. Page No.149-168.
- Parato KA, Senger D, Forsyth PA, Bell JC, Recent progress in the battle between Oncolytic viruses and tumours. *Nat Rev Cancer*, 2005, Chapter 5, Page No.965-976.
- Nauts H, Fowler G, Bogatko F, A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man. *Acta Medica Scandinavica*, 1953, Chapter 276, Page No.1-103.
- Nauts HC, McLaren, Coley's toxins-the first century. *Adv Exp Med Bio*, 1990, Chapter 267, Page No.483-500.
- Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B, An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci*, 1975, Chapter 72, Page No.3666-3670.
- Fujimori M, Amano J, Taniguchi S, The genus *Bifidobacterium* for cancer gene therapy. *Curr Opin Drug Discov Devel*, 2003, Chapter 5, Page No.200-203.
- Cheong I, Huang X, Bettgowda C, Diaz LA Jr, Kinzler KW, Zhou S, Vogelstein B, A bacterial protein enhances the release and efficacy of liposomal cancer drugs. *Science*, 2006, Chapter 314(5803), Page No.1308-1311.
- Richardson MA, Ramirez T, Russell NC, Moye LA, Coley toxins immunotherapy: a retrospective review. *Altern Ther Health Med*, 1999, Chapter 5, Page No.42-47.
- Wei MQ, Ellem KAO, Dunn P, West MJ, Bai CX, Vogelstein B, Facultative or obligate anaerobic bacteria have the potential for multimodality therapy of solid tumours. *Eur J Cancer*, 2007, Chapter 43, Page No.490-496.
- Pastan I, Targeted therapy of cancer with recombinant immunotoxins. *Biochim Biophys Acta*, 1997, Chapter 1333, Page No.C1-C6.

- Minton NP, Clostridia in cancer therapy. *Nat Rev Microbiol*, 2003, Chapter 1, Page No.237-242.
- Dang LH, Bettgowda C, Huso DL, Kinzler KW, Vogelstein B, Combination bacteriolytic therapy for the treatment of experimental tumors. *Proc Natl Acad Sci*, 2001, Chapter 98(26), Page No.15155-15160.
- Bettgowda C, Dang LH, Abrams R, Huso DL, Dillehay L, Cheong I, Agrawal N, Borzillary S, McCaffery JM, Watson EL, Lin KS, Bunz F, Baidoo K, Pomper MG, Kinzler KW, Vogelstein B, Zhou S, Overcoming the hypoxic barrier to radiation therapy with anaerobic bacteria. *Proc Natl Acad Sci*, 2003, Chapter 100(25), Page No.15083-15088.
- Dang LH, Bettgowda C, Agrawal N, Cheong I, Huso D, Frost P, Loganzo F, Greenberger L, Barkoczy J, Pettit GR, Smith AB, Gurulingappa H, Khan S, Parmigiani G, Kinzler KW, Zhou S, Vogelstein B, Targeting vascular and avascular compartments of tumors with C. novyi-NT and antimicrotubule agents. *Cancer Biol Ther*, 2004, Chapter 3(3), Page No.326-337.
- Low KB, Ittensohn M, Lin S, Clairmont C, Luo X, Zheng L-M, King I, Pawelek JM, VNP20009, a genetically modified *Salmonella typhimurium* for treatment of solid tumors. *Proc Am Assoc Cancer Res*, 1999, Chapter 40, Page 851.
- Al-Ramadi BK, Fernandez-Cabezudo MJ, El-Hasasna H, Al-Salam S, Attoub S, Xu D, Chouaib S, Attenuated Bacteria as Effectors in Cancer Immunotherapy. *Ann N Y Acad Sci*, 2008, Chapter 1138(1), Page No.351-357.
- Hatefi A, Canine BF, Perspectives in vector development for systemic cancer gene therapy. *Gene Ther Mol Biol*, 2009, Chapter 13(A), Page No.15-19.
- King I, Ittersson M, Bermudes D, Tumor-targeted *Salmonella typhimurium* overexpressing cytosine deaminase: a novel, tumor-selective therapy. *Methods Mol Biol*, 2009, Chapter 542, Page No.649-659.
- Loeffler M, LeNegrata G, Krajewska M, Reed JC, Attenuated *Salmonella* engineered to produce human cytokine LIGHT inhibit tumor growth. *Proc Natl Acad Sci*, 2007, Chapter 104(31), Page No.12879-12883.
- Avogadri F, Martinoli C, Petrovska L, Chiodoni C, Transidico P, Bronte V, Longhi R, Colombo MP, Dougan G, Rescigno M, Cancer Immunotherapy Based on Killing of *Salmonella*-Infected Tumor Cells. *Cancer Res*, 2005, Chapter 65(9), Page No.3920-3927.
- Ansiaux R, Gallez B, Use of *Clostridium* toxins in cancer therapy. *Expert Opin Invest Drugs*, 2007, Chapter 16(2), Page No.209-218.

**Cite this article as:**

Preenu P Sen, Gautham A, Murugan Manavalan, Mansoor Ani Najeeb. Bacteria in cancer therapy: An emerging robust strategy. *Int. Res. J. Pharm.* 2013; 4(5):1-4

Source of support: Nil, Conflict of interest: None Declared