CLOSTRIDIUM NOVYI: A NEW EPOCH IN CANCER TREATMENT

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Abstract: The present paper reviews and summarises the role of Clostridium novyi NT as a newer approach in the treatment of Cancer. Cancer is one of the leading causes of deaths worldwide but there is still no definite cure of cancer despite the rapid developments in the conventional modalities of treatment. Organisms such as Clostridium novyi NT, Streptococcus pyogenes, Salmonella typhimurium etc were used in the treatment. C. Novyi NT is a clone derived from C. Novyi after elimination of the major systemic toxin gene from the parenteral strains. It is used mainly due to its ability to propagate in tumor generated hypoxia. The bacteria are an oncolytic agent that’s why it is used with the combination of chemotherapy & radiotherapy. C Novyi NT has shown promising results in many researches without or lesser side effects. Further research works are needed to study effects in various forms of cancer, toxic effects and to standardize the treatment regimen so as to make it effective in all types of cancers which may eventually reduce the cost of treatment and make it available for common sufferers.

Keywords: Clostridium novyi NT, Tumor, Anticancer
INTRODUCTION

Cancer is one of the leading causes of death worldwide\textsuperscript{[1,2]}. It is generated by a mass of tissues that grows faster than normal in an uncontrolled manner, and continues to grow even after the initial stimulus has ceased. Metastasis is the process in which the cancer spread from one organ to another organ \textsuperscript{[3-5]}. Cancer results from a series of molecular events which fundamentally alter the normal properties of cells. In cancer cells the normal control systems preventing cell overgrowth and invasion of other tissues do not function properly. These altered cells divide and grow in the presence of signals that normally inhibit cell growth. Depending on it tumours are classified as benign or malignant. Benign grows & confined in one place whereas malignant totally grows throughout the body \textsuperscript{[4,6,7]}.

Solid tumours accounts 90% of all cancer \textsuperscript{[2-4]}. The oncology is the branch of medicine that deals with the study, diagnosis, treatment, and prevention of cancer \textsuperscript{[4,8]}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cancer_cellDevelopment.png}
\caption{cancer cell development}
\end{figure}

CAUSES

The environmental agents which are responsible for causing cancer are called as carcinogens. Cancer is caused by both external factors such as tobacco, chemicals, radiation, and infectious organisms and internal factors like inherited mutations, hormones, immune conditions, and mutation that occurs from metabolism \textsuperscript{[4,9,7]}. Alcohol & Tobacco causes mainly oral cancer, laryngeal cancer, esophageal, liver cancer \textsuperscript{[9,10]}. Common environmental factors leading to cancer death include: tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation, stress, lack of physical activity, and environmental pollutants. These environmental factors cause abnormalities in the genetic material of cells \textsuperscript{[4,8,11]}. Life style causes mainly oesophageal, chlorectal cancer \textsuperscript{[12]}. 
TREATMENT

Science is still unable to show a definite way of cure of cancer despite the rapid developments in the conventional treatment like chemotherapy & radiotherapy \[3,5\]. Cancer recurrence is an added complexity in treatment approach. \[13\]. Experimental cancer treatments are medical therapies intended or claimed to treat cancer by improving, supplementing or replacing conventional methods. These includes photodynamic therapy, HAMLET (human alphalactalbumin made lethal to tumor cells), gene therapy, telomerase therapy, hyperthermia therapy, dichloroacetate (DCA), non-invasive RF cancer treatment, complementary and alternative therapy, diet therapy, insulin potentiating therapy and bacterial treatment \[2,5,7,14\]. For example, use of antibiotics reduces the capacity of intestinal microflora to metabolize phytochemicals into compounds that may protect against cancer \[15,16\].

ORGANISMS HELP IN CANCER TREATMENT

Parker and colleagues were first evaluate an anaerobic bacteria in mouse tumor. The spores of Clostridium Butyricium firstly evaluated in clinical studies which were injected into variety of cancer . But difficulty in management of toxicity and lack of a complete clinical responses delayed further studies of such therapy \[17\]. Streptococcus pyogenes also used as anticancer agent \[18\] The Strain of Salmonella typhimurium recently attenuated \[3\] has been used in a clinical trial to treat metastatic melanoma and carcinoma in renal cells. But due to insufficient bacterial colonization in the tumors studies failed to give a proof of significant clinical efficacy \[17\].According to Coley's view there are a variety of natural and genetically modified non-pathogenic bacterial species which either provide direct tumoricidal effects or deliver tumoricidal molecules \[19\]. The sporeforming anaerobic bacterium Clostridium novyi NT has capability to propagate in tumor generated hypoxia,which leading oncolysis Bifidobacterium ,Listeria and Escherichia also used for this purpose \[2,19,20\]. Except these above organisms , Escherichia coli Nissle which decreased the number of lung metastases and restrained growth of subcutaneous melanoma in mice.Other wise Listeria monocytogenese, Mycobacterium bovis (BCG) which is approved for cancer management as in BCG therapy, and more effective than intravesical chemotherapy. Also Shigella flexneri , used as anti cancer agent\[21\]

Species of Clostridium spores

Clostridium spores belonging into 3 types, those are

C. sporogenes

C. novyi

C. beijerinckii\[21\]
C. butyricum[17]

*Clostridium Novyi –NT*

*Clostridium novyi* is alive, mobile spore forming [2,18] sensitive to oxygen, Gram variable, (gram positive in young cultures, often gram negative in older cultures) anaerobic [2,18,22], attenuated or genetically modified [19] nonpathogenic [3] bacteria, belonging into the family *Clostridiaceae*. *C. novyi-NT* is a clone derived from *C. novyi* after major systemic toxin genes are eliminated from the parental strain. *C. novyi* is found in the soil [2,3,24]. These are capable of multiplying selectively in tumors and inhibiting their growth [19].

![Clostridium Nvyi](image)

**Fig2: Clostridium Nvyi**

*Streptococcus pyrogenes*

It is a one type of anaerobic bacteria which have been used for this purpose. Solid tumors are treated with this bacteria and its toxins.[8] But it is failed to produce a viable anticancer agent, due to poor reproducibility and unacceptable toxicity [18]. The vaccine of two species, *Streptococcus pyrogenes* and *Serratia marcescens* were developed by Coley which widely used to treat Sarcomas, Carcinomas and related disease [2,3].

*Salmonella typhimurium*

Instead of Clostridium or Bifidobacterium, it has ability to grow in both aerobic and anaerobic condition [2].

*Clostridium butyricum*

Variety of cancer including glioblastoma, lung carcinoma, osteosarcoma, and malignant teratomas are treated with the Spores of *Clostridium butyricum*. But due to lack of a complete clinical responses and difficulty in managing toxicity delayed further studies of such therapy [17].
**Clostridia**

It is able to proliferate preferentially within the necrotic regions of tumors, show result in tumour regression. But most of the animals became ill or died due to acute toxicity \[^{[19]}\]. So the focus shifted on Clostridium but it can’t produce significant tumour regression. A number of anaerobic bacterial species (bifidobacteria, lactobacilli and pathogenic clostridia) have been used recently \[^{[19,23]}\]. Because of toxicity of C. novyi –NT and experiments too culminated in death\[^{[2]}\], this strain is administered in combination with conventional chemotherapeutic agents like dolastatin-10, mitomycin -c, docetaxel etc\[^{[19]}\].

Clostridium is responsible for actual cause of positive response. These bacteria are one oncolytic agent that’s why bacteria alone were not capable to cure all malignant cells. Hence combination of chemotherapy, radiotherapy and bacterio therapy used for cancer treatment \[^{[3]}\]. Remarkable tumor shrinkage and complete lysis of all tumor cells are produced by this combination therapy \[^{[25]}\].

*C. botulinum* or *tetani* also used for this purpose. Although clostridia are strictly anaerobic, not growth in aerobic conditions \[^{[26]}\].

**Types of clostridium Novyi**

Clostridium Novyi are 3 types.

1) Type A

2) Type B

3) Type C

Type A of clostridium Novyi contains alpha, gamma, delta, epsilon toxins.

Alpha, beta, zeta toxins present in Type B.

And type C contain Gamma toxin.

According to Some authors *Clostridium novyi* type D is considered by *Clostridium heamolyticicum* \[^{[27]}\]. C. Novyi-NT is an derivative of the wild type strain by removal of Alpha toxin gene \[^{[26]}\].

**Spore coat architecture**

An amorphous layer is surrounded the spores which intertwined with honeycomb parasporal layers. The sporecoat layers had apparently self assembled, which was likely to be governed by crystal growth. At the time of germination and outgrowth, the honeycomb layers, as well as the
underlying spore coat and undercoat layers, sequentially dissolved until the vegetative cell was released.[28]

**TREATMENT WITH C. NOVI**

Clostridium is the largest amongst prokaryotic genera, that comprises a diverse assemblage of obligatory anaerobic, Gram-positive, rod-shaped sporulating bacteria. It represents a several pathogens producing potent toxins such as *Clostridium tetani, C. botulinum, C. difficile* and *C. perfringens*.[26] The microbe can thrive only in poor oxygen environments, so it becomes a targeted means of destroying oxygen starved cells in tumors that meets difficulties treating with chemotherapy and radiation.[23]. Promising results is shown by C. novyi without any side effects. Pharmacological and toxicological evaluation of these species also found that spores were rapidly wiped from the circulation by the normal retiendothelial tissues.[3]

These strains makes bacteria of targeting specific tumor regions, that also enable bacteria to target tumors over normal tissue. This is the main reason for the exceptional targeting ability of bacteria of which tumors contain unique microenvironments, which are not present in normal tissue and are attractive to bacteria.[29]. Scientist was particularly interested in immune response to the bacteria that might diminish the effects of combination bacteriolytic therapy (COBALT). Surprisingly, they observed that a significant fraction of immunocompetent animals treated with C. novyi-NT spores exhibited complete tumor regression in the absence of any additional chemotherapy or radiation, which results in long-term cures.[30]

**HISTEROLOGICAL BACKGROUND OF CLOSTRIDIUM NOVYI IN CANCER TREATMENT**[31]

1813- Clostridium associated with cancer.

1935- use of Clostridium filtrate for cancer treatment.

1947- first deliberate infection of tumor with Clostridium.

1955- Demonstration of selective spore germination in hypoxic/necrotic tumor region.


1967- Human clinical trial.

1988- First attempt to introduce therapuric gene into Clostridium.

1996- development of recombinant Clostridium for use in cancer therapy.

2001- Large scale screening for better colonizing strain and COBALT.
*C. novyi* Strains are modified to deliver pro-drug converting enzymes, cytokines, antibodies against tumor antigens. Spores are administered systematically germinate and multiply only in hypoxic/necrotic areas of tumors which causing significant oncolysis. Ongoing phase 1 clinical trial of *C. novyi*-NT spores is done in patients with solid tumor malignancies \(^{(32)}\)

![Tumor targeting mechanisms of obligate and facultative anaerobic bacteria that includes specific chemotaxis, preferential growth and hypoxic germination.](image-url)
MECHANISM

*C. novyi*-NT effectively reduces tumors and mounts an immune response that will clear any remaining tumor cells left by the bacteria. In the immune response, innate immune cells that aggregates at the site of the tumor and destroy any remaining tumor cells which is from outside the tumor. The result is destruction of tumor from within by bacteria and outside by immune cells. In about 30% of animal cases, the tumors were completely removed [17]. However, *C. novyi*-NT works best on larger tumors (>150 mm³) because of the extensive anoxic (anaerobic) environment within them [30]. This increased size may often leads to increase in toxicity [17].

![Schematic representation of the mechanism of tumor targeting.](image)

APPLICATIONS

One of the major goal of cancer therapy today is to achieve specificity. Current efforts to meet this challenge are focused on developing targeted therapeutics which are specific to the cancer cell. An alternative approach is to deliver cytotoxic agents to the tumour site selectively [33]. Oncolytic strains such as *Clostridium* show their ability to kill cancer cells due to their own replication within the tumor cores. The strictly anaerobic *Clostridium novyi* was engineered by the deletion of α-toxin (so-called *C. novyi*-NT)α gene coding for the alpha toxin [34,35].

When spores of the anaerobic bacterium *Clostridium novyi*-NT are systemically injected into the animals, within the hypoxic regions of cancers they germinate exclusively. The germinated bacteria destroy adjacent tumor cells but spare a rim of well oxygenated tumor cells that subsequently expand [30]. Various studies are performed to better understand the factors...
influencing the efficacy and toxicity of this form of therapy. It is found that spores were rapidly cleared from the circulation by the reticuloendothelial system. Even after large doses were administered, no clinical toxicity was observed. However, the drug doses required for such efficacy often generated substantial toxicities. Fortunately, C. novyi-NT possessed a unique device, allowing targeted release of SSL-encapsulated drugs in tumors, thereby increasing efficacy while diminishing the toxicity of chemotherapy delivered in conjunction with C. novyi-NT. The therapeutic effects were increased further when a combination bacteriolytic therapy (COBALT) was set up in order to increase the therapeutic effect (see below). Although these results are certainly very promising, it should also be noted that the observations made were tumor-type dependent and that some combinations led to severe toxicity called ‘tumor lysis’ syndrome.

![Diagram of therapeutic effects shown by C. novyi bacteria after injecting into the tumor region](31)

**CONCLUSION**

Cancer is characterized by uncontrolled growth of cells. These cells may spread to other parts of the body and causes metastasis. There are some carcinogenic factors in the environment and
lifestyle also a major factor for causing cancer. Current treatment approach for most solid tumors is surgery. Other options like chemotherapy and radiotherapy were broadly used in this purpose. But they cause damage both healthy and cancerous cells. The revolutionary role of bacteria in cancer therapy are focused as an alternative for this purpose. There are some bacteria which acts as an anticancer agent. Among them Clostridium novyi NT are able to germinate in and destroy hypoxic regions of tumors. This approach shows some alternative and effective way for the treatment of cancer which is still a matter of great concern. Further investigations and developments in these studies will add a new dimension to cancer treatment which will may establish itself as a new epoch in this arena.

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