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Role of some microorganisms in the treatment of cancer: A review

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Abstract
Cancer is a disorder that results from genetic or epigenetic alterations in the somatic cells and has abnormal cell growth which may be spread to other body parts. There are various types of cancer treatments, which depend upon the cancer type and how to advance it is. Several decades after ago, interest re-emerged in the use of bacteria to treat cancer. Experiments showed that pathogenic species of the anaerobic bacteria such as clostridia were able to proliferate preferentially within the necrotic (anaerobic) regions of tumours in animals compared to normal tissues. Research in this field is growing and new strains of bacteria are being investigated as anticancer agents: *Mycobacterium bovis*, *Streptococcus pyogenes*, *Salmonella choleraesuis*, *Vibrio cholerae*, *Listeria monocytogenes* and even *Escherichia coli* have all been shown to replicate within tumours. This paper was aimed to review the use of microorganisms in anticancer therapy. It presents microorganisms that have already been commonly used and those going through phase II and phase III clinical trials.

Keywords: bacteria, cancer, microorganisms, treatment

Introduction
Cancer incidence and mortality are rapidly growing worldwide [1]. The reasons are complex but reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development [1]. It should be stressed that the statistics may be underestimated as many cancer lesions develop over the years and are only diagnosed at a high stage of the disease. There are many factors that influence the development of cancer. One of the best recognized risk factors is tobacco smoking, which can cause cancers in lungs, head, and neck [2]. Other examples are chemicals, including those being in use in the research laboratories, such as ethidium bromide, which is a highly mutagenic agent [3]. Mutations in the genetic material may also be the result of irradiation, such as UV or X rays [4], or the effect of infection with a pathogen such as HPV (cervical cancer) [5] or HCV and HBV (liver cancer) [6, 7]. Neoplasms can also be inherited as a polygenic disorder. This is due to the overlap of hereditary changes in the carriers of the defective gene and the DNA damage at sites that are important for the process of cancerogenesis that occurred during human development [8].
It was predicted by Global demographic characteristics that about 420 million new cases of cancer by 2025 annually, which means increasing cancer incidence in years. Cases of cancer about 18 million in 2018 were recorded worldwide; in men, about 9.5 million and women about 8.5 million. Globally about 9.6 million deaths were estimated in cancer [1, 9]. The commonest cancers are prostate cancer (1.28 million), female breast cancer (2.09 million), colorectal cancer (1.1 million), stomach cancer (1.03 million) and non-melanoma skin malignancies (1.04 million) [10]. Cancer-related deaths, from most to least frequent, are due to lung cancer (1.76 million), colorectal cancer (862,000) and stomach cancer (783,000), liver cancer (782,000). Over 100 types of cancers affect humans [11]. There are various types of cancer treatments, which depend upon the cancer type and how to advance it is [12]. Among the known and practiced anticancer therapies, the use of microbes appears to be one of the most original strategies.

Epidemiology of Cancer
Globally about 9.6 million in 2018 deaths were estimated in cancer which represents the cancer as the second leading cause of deaths and about 1 in 6 deaths are due to cancer [1]. About 70% of deaths in middle- income and Low Countries are due to cancer [9]. The main and the most important cause of cancer is tobacco use, approximately 22% [13, 14]. There are also some infections that cause cancer, like Human papilloma Virus (HPV) are causes 25% of cancer in middle and low-income countries [14]. In 2017, solely twenty-sixths of low-income countries reported having pathology services usually obtainable within the public sector. More than ninetieth of high-income countries reported treatment services square measure obtainable compared to but a half-hour of low-income countries [15].
There are many causes which may cause cancer in different body parts like mainly 22% deaths are due to tobacco consumption,

10% of deaths are due to poor diet, obesity, lack of physical activity, excessive drinking of alcohol or other facts include certain exposure to ionizing radiation, environmental pollutants, and infection [16]. About 15% of cancer in the world is due to some infections like hepatitis b, hepatitis c, human papillomavirus infection, helicobacter pylori, and immunodeficiency virus (HIV), Epstein - Barr virus. These factors are at least partly responsible for changing the genes. Inherited genetic defects from patient's parents are also responsible for 5-10% of cancer [16, 17].

Immunotherapy

Immunotherapy deals with the treatment of disease (cancer) by adjusting the body's immune response. In this therapy, the immune system is boost by medication or other treatments. The immune system is made up of WBC and tissues of lymph nodes help to provide the strength to the body to fight against the disease and infection. It is also called biological therapy, which means the substances used in the treatment made from living organisms to treat cancer. It is not yet widely used, but many immunotherapies are studied in clinical trials [12].

Microorganism as a component of immunotherapy

Intrusion of microorganisms into the body leads to the activation of immune mechanisms, which manifests itself in increasing the number and recruitment of congenital immune cells (such NK cell, monocytes/macrophages and neutrophils), activation of acquired immunity cells, that is, T and B lymphocytes, and intensification of proinflammatory cytokine production. It is assumed that the "mobilized" immune system, by intentionally introducing microorganisms into the oncological patient, is able to at least limit the development of cancer. This is a method in which microbes indirectly lead to cancer regression—especially in those in whom other commonly used treatments have failed [18]. The safety of the used microorganisms is extremely important, because the aim of the therapy is to fight cancer, not to harm the patient's organism by infecting it with a pathogen. Various methods are used to ensure the safety of the formulations [19]. First and foremost, microbes are deprived of their pathogenicity (attenuation), for example, by culturing under appropriate environmental conditions or by the treatment of certain substances, resulting in mutation and weakening/loss of pathogenic properties [20].

Bacteria can be applied in various forms for therapeutic purposes. Apart from whole, living attenuated cells, we can use genetically engineered bacteria expressing particularly desirable factors [21]. Microorganisms are also applied as vectors, which are carriers of specific antineoplastic agents (e.g., chemotherapeutics) or enzymes useful in cancer cell destruction. The use of bacteria as a vector to transfer a chemotherapeutic agent directly into the tumor allows a significant reduction of the side effects of treatment that usually accompany traditional chemotherapy [21, 22]. In addition, there is a therapeutic potential in using bacterial secretion products, for example, toxins. Their presence in the tumor environment could have destructive effect on cancer cells [23, 25]. The use of sporangial bacteria, which can survive under unfavorable environmental conditions, represents another approach, which has been applied in the experiments with *Clostridium novyi*. This microorganism prefers anaerobic conditions, which are found in the tumor. Instead of spreading over the entire organism, the bacteria are directed to the tumor site only, where they have the optimal conditions for growth. This bacterial property allows the patient to be protected against the development of serious infections [19].

Bacteria Used as Anticancer Agents

The antitumor efficacy of microorganisms is extremely diverse. Results of clinical trials allow determining whether a particular product can be intended for general use. Currently used anticancer bacterial microbial preparations have the status of a therapy complementary to standard treatment, increasing the patient's chances of complete recovery [8].

Mycobacterium bovis

Bacillus Calmette-Guérin (BCG) is a strain of Mycobacterium bovis developed by Albert Calmett and Camille Guérin as a tuberculosis vaccine and has been used since 1921. In many countries, this vaccine has been induced in the mandatory vaccination schedule and is administered to children within 24 hours after birth, in a single dose, intra-dermally. Mycobacterium bovis is an etiological agent of bovine tuberculosis. However, in certain circumstances (e.g., after ingestion of untreated milk from an infected animal), it can cause tuberculosis symptoms in humans as well. That is why it was necessary to attenuate this microorganism. Calmett and Guérin have passaged *M. bovis* (231 passages in total) for 13 years on a medium consisting mainly of cooked potato slices soaked in ox bile and glycerin. Only then did it become safe for human use, as an avirulent but immunogenic strain [26].

At the beginning of the twentieth century there were some links between the occurrence of tuberculosis and cancer regression [26]. However, only after Morales and his colleagues demonstrated in 1976 that the use of BCG was accompanied with the cancer regression, the vaccine was approved as the complementary treatment of bladder cancer [27]. Treatment of this type of cancer with the M. bovis BCG strain requires the intravesical infusion of the microbial suspension using urethral catheters. This therapy is most often used after resection to eliminate accurately the cancer cells and to prevent recurrence [27]. The dose and duration of treatment are strictly dependent on the stage of cancer. Clinical observations show that recurrence is much less likely to occur after tumor resection or resection and chemotherapy when BCG is administered intravesically [28]. BCG's mechanism of action is based on stimulating the patient's immune system. It appears that IFN-γ and effector cells, that is, CD4+ and CD8+ lymphocytes, play an extremely important role in the recognition of tumor antigens. In addition, the pool of proinflammatory cytokines is increasing, which enhances the immune response of the body by activating the phagocytosis of cancer cells. Providing the selected vitamins during therapy may increase the survival of M. bovis BCG cells, which improves the quality of therapy [19, 26, 29, 30].

Streptococcus pyogenes

Streptococcus pyogenes was originally used in the treatment of bone sarcoma by Dr. William Coley [8]. However, the emergence and development of other treatments for cancer, especially chemotherapy and radiotherapy, caused that for many years, the concept of using this microorganism was forgotten [8].

Fortunately, the concept of anticancer therapy with the use of *S. pyogenes* has endured and the bacteria are currently applied in the treatment of lymphangiomas in children. Presently, the *S. pyogenes* OK-432 strain has been used in that way in many countries around the world [31, 32]. Lymphangiomas are tumors formed by excessive division of lymphatic vessels' endothelial cells. They are most often found in the head and neck area of children under the age of two. The pathological development of lymphatic vessels is primarily associated with impaired lymph flow, which in turn manifests itself in the formation of cysts. Changes in children resemble goiter, similar to that one, which is associated with an enlarged thyroid gland. Treatment primarily involves surgical removal of the cyst, but this is not an easy task, and is often burdened with numerous adverse effects, including death [33, 34]. An alternative and safer method of treatment is sclerotherapy. *Streptococcus pyogenes* OK-432 is injected into pathologically changed lymphatic vessels. In Japan, this microorganism has been successfully used in the treatment of lymphangiomas in children since 1987. Studies show that the strain is safe and results in at least 50% reduction of cyst volume [32, 34].

The mechanism of action of the microorganism is also based on the sensitization of the immune system. Activated cells destroy the neoplasm, further growth is inhibited, and the lymphangioma is reduced. Studies using flow cytometry have shown that the first day after suspension administration, the numbers of neutrophils and macrophages, as well as lymphocytes, rapidly increase. NK CD56+ cells, TNFα, IL-6, IL-8, IFNγ, and VEGF (vascular endothelial growth factor) levels also increase. Due to the appearance of inflammation immediately after the procedure, the lesion may be swollen, but therapeutic effects are noticeable after a few months [32, 34, 35]. Moreover, studies conducted in the years 2005–2015 showed the great effectiveness of this strain also in the treatment of intraoral ranula. Complete regression occurred in 78.2% of patients [36].

Clostridium spp

Obligate anaerobes and facultative anaerobes have potential to be used in anticancer therapies because they grow best under conditions of significant oxygen unavailability (hypoxia). Oxygen is delivered to the cells through blood vessels which penetrate mainly the tumor surface area. That results in impaired diffusion of oxygen into the tumor and hypoxia. The anaerobic environment creates favourable conditions for the development of anaerobic bacteria, for example, *Clostridium* spp., *Bifidobacterium* spp., or *Listeria* spp. [19, 37]. The greatest advantage of using these microorganisms is that they locate directly inside the tumor, in contrast to chemotherapeutics, which spread throughout the body with blood, also destroying normal, healthy cells [37, 39].

In the context of hypoxia and the antineoplastic therapy, the most common type of bacteria being in use is *Clostridium*, due to the anaerobic nature of the rods. Bacteria develop in the tumor's necrotic areas and can directly damage tumor cells [37-39]. The history of the use of *Clostridium* in the fight against cancer dates back to 1935, when Connell published an article describing the regression of advanced cancer under the influence of enzymes produced by *Clostridium histolyticum* [40]. Since then, more research has been done on the use of *Clostridium*. The attenuated strain of *Clostridium novyi*-NT has positively undergone phase I and phase II clinical trials, giving extremely promising results for the treatment of leiomyoma [37, 39]. The mechanism of the anticancer activity of *Clostridium* spp. is unknown yet, but it is common knowledge that bacterium is capable of producing specific enzymes and toxins that destroy cancer cells. In addition, it produces specific proteins that can be conjugated to specific chemotherapeutics. This allows the drug to enter the tumor. In traditional chemotherapy, drugs are not able to penetrate into the tumor precisely due to its external vascularization and internal hypoxia [37, 39].

Salmonella Typhimurium

Salmonella enterica serovar Typhimurium, an etiological agent of typhoid fever, shows similar features as *Clostridium*. It is a relatively anaerobic rod that can also be located in the necrotic tumor regions. In the treatment of cancer, the attenuated strain *Salmonella typhimurium* VNP20009 is used for safety reasons [41]. Clinical trials on the use of this microorganism for melanoma treatment began in 2002 [19]. In addition, the VXM01 antitumor vaccine, which is based on the attenuated strain of Salmonella typhi, has successfully passed phase I clinical trials. This bacterium has a plasmid encoding expression of VEGFR2 (vascular endothelial growth factor receptor-2). The vaccine blocks the angiogenesis process. The formulation was tested in individuals with pancreatic cancer [42].

Conclusion

Following the personal and historical observations of tumor regression associated with acute bacterial infections, a combination of *Streptococcus pyogenes* and gram-negative *Bacillus prodigiosus* (*Serratia marcescens*), providing evidence that a severe localized infection may induce a systemic antitumor immune response. Subsequently, several bacteria or bacterial preparations, such as *Corynebacterium parvum* and the streptococcal preparation OK-432, were tested in cancer therapy; local treatment with Bacille Calmette Gu´erin (BCG), an attenuated strain of *Mycobacterium bovis*, is still a first-line therapy for superficial bladder carcinoma. Many genera of bacteria, including *Salmonella*, *Escherichia*, and *Clostridium*, preferentially accumulate in tumors when delivered systemically, and they have been tested as anticancer agents.

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