# Synthetic Biology: Using Microbes and Nanotechnology to Fight Cancer

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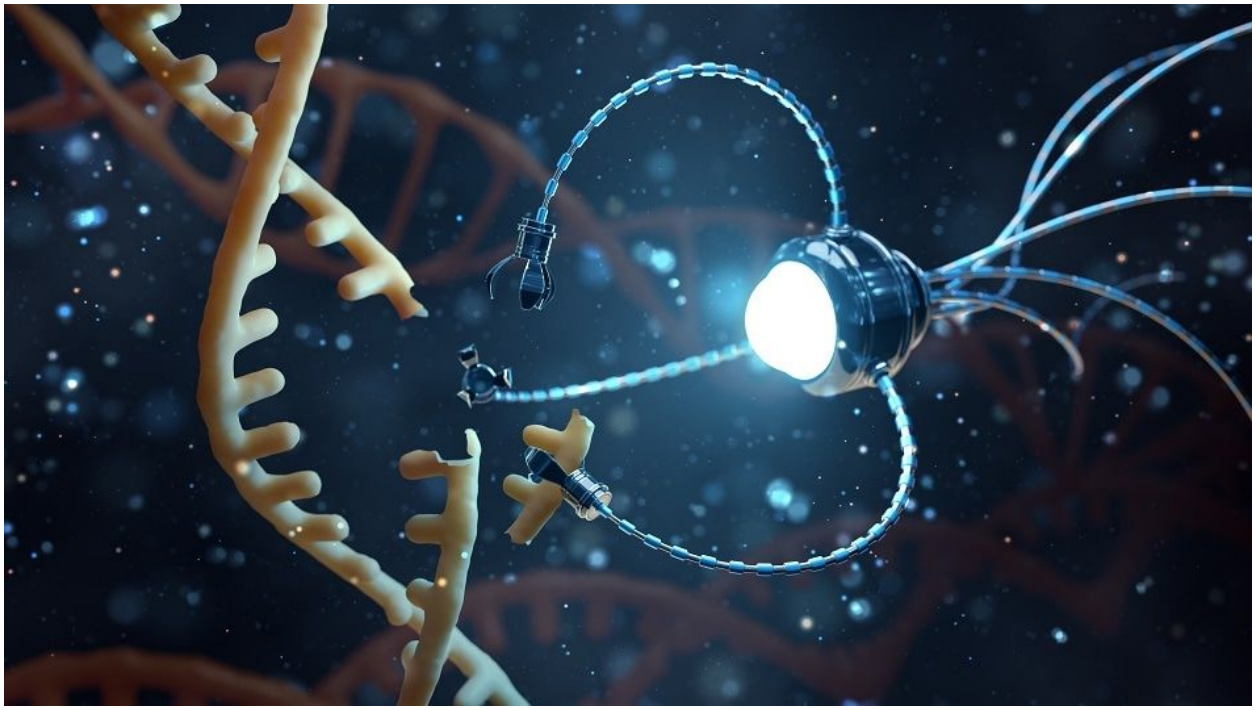


Abstract

Cancer is the third leading cause of death in the world, following only cardiovascular and infectious diseases. The general form of cancer is when cells divide uncontrollably and harm body tissues. Over time, the buildup of cells will form tumors in the body. Successful treatment for cancer remains a challenge, due to the unique way that cancer proliferates and its ability to adapt to almost all situations. Furthermore, most cancer treatments, such as chemotherapy, damage and destroy both healthy and cancerous cells, and may actually do more harm than good.

This treatment will be conducted in a lab and carried out by injecting nanobots with thrombin attached to them into the body to cut off the blood supply of tumor cells. Then viruses and bacteria would be injected into the tumor to destroy it. Materials needed would include needles and syringes for injections, and devices to monitor the tumor such as MRIs, CT scans, and X-rays machines. Our entire experiment would be at least 1 week, and depending on how patients react to treatment and how effective it is early on, we could possibly repeat our treatment as weekly cycles.

If we use nanobots and viral/bacterial therapy together, then the cancer patients will have a much higher rate of survival. The hypothesis for this treatment is that if nanobots and virus/bacterial therapy are used as a combination treatment, the tumor will go through cell death and the patients' survival rates will increase. Instead of only one form of attack on the tumor, we will use two different methods of attack to increase chances of success.

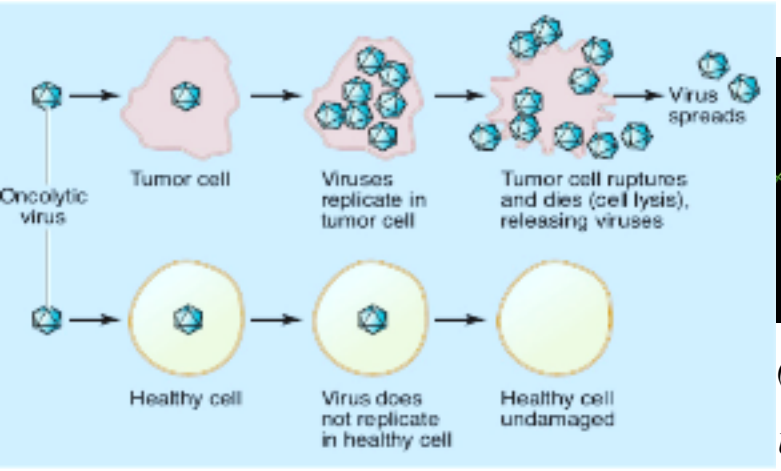
# Introduction

In 1884, a patient with a tumor in his neck developed an unrelated bacterial skin infection. As the infection was fought off, the tumor also began to recede. This response marked the beginning of bacteriotherapy, or intentionally injecting bacteria into patients with cancer, successfully curing 50% of all sarcoma patients. This is part of synthetic biology, the field of science that involves redesigning organisms for useful purposes by engineering them to have new abilities. Many synthetic biologists have designed and reprogrammed microbes to battle diseases. Doing this is important because researching them can provide treatments and cures to untreatable conditions, such as cancer. Cancer is the second leading cause of death in the United States and the third in the world. Even though many attempts have been made to cure this disease, there is still no specific cure for cancer. Our project is important since cancer is such a major leading cause of death and finding an effective cure is crucial to global health and can save many lives. The part of the experiment that is new is the use of both nanobots and bacteriotherapy. This is a new combination that uses two therapies that have been tested and proved efficient as a form of treatment for cancer.

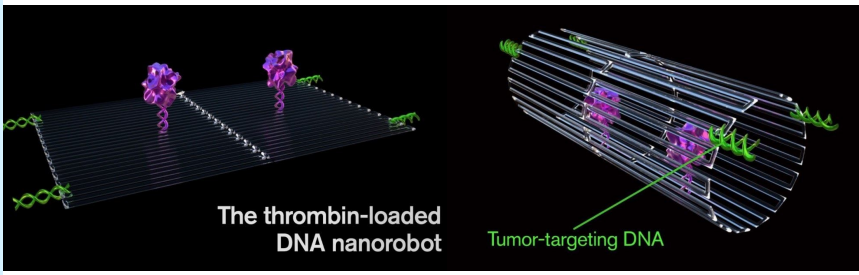
Cancer is a disease where there is uncontrollable cell growth. The cancerous cells keep on multiplying, and begin to crowd out normal cells, which causes a tumor. However, tumor cells need more nutrients and oxygen than the average cell. With recent research related to nanobots, scientists believe they could be the way to cure cancer. These microscopic robots can cut off the blood supply of tumors, which depletes cell growth. This is done using a blood-clotting enzyme called thrombin. Another method that scientists think could cure cancer is by hacking bacteria and using virotherapy to attack the tumor. The methods of bacteria and virotherapy were first developed in 1904 when a 42-year-old woman with leukemia went into

remission after contracting influenza. Therapies use bacteria such as *Escherichia coli* and oncolytic, or cancer killing, viruses to battle the tumor and destroy it. The bacteria and viruses are specifically programmed to kill cancerous cells and spare nearby healthy ones. Some bacteria can be programmed to deliver cancer-killing drugs to the tumor. The center of the tumor is also an ideal environment for bacteria growth, where it is protected from immune cells.

In 2018, researchers from Arizona State University and the National Center for Nanoscience and Technology (NCNST) in China programmed nanobots to cut off the blood supply of tumors in mice, and as a result, shrinking them. The nanobots were made from a DNA sheet and had thrombin attached to its surface. Next, DNA aptamers were attached to the surface of the nanobots. "The DNA aptamer could specifically target a protein, called nucleolin, that is made in high amounts only on the surface of tumor endothelial cells — and not found on the surface of healthy cells."(Caspermeyer 19). Once it was bound to the tumor, the nanobot released the thrombin onto the tumor cells. In the experiment, human tumor cells were injected into a mouse and allowed to grow for a period of time. Then nanobots were injected into the mouse with an IV. The nanobots proved to be both safe and effective for shrinking tumors. After 24 hours, almost all of the nanobots had been expelled from the mice. No side effects were shown.




*(Viral Oncolysis, The Microbial Challenge, 2013)*




*(The Cancer-Fighting Nanobot, Arizona State University, 2018)*

On January 10th 2018, a two stage trial testing 20 patients with melanoma was conducted. Before this study occurred, patients took screening tests including CNS imaging if clinically suspected, tumor biopsy, and serological/stool studies to confirm whether or not they are appropriate for FMT administration. Then, patients that were eligible received an FMT endoscopically and an intestinal biopsy. Next, this will be followed by the first cycle of pembrolizumab and eventually, followed by cycles 2-4. Then they will perform a rescreening to test for the melanoma. Lastly, patients with consistent signs of melanoma after the 8th cycle of pembrolizumab will continue to receive therapy even after the study. Since this study began a few years ago, test results have not yet been concluded and will most likely be released in 2021. This study is relevant because it shows how bacterial injections can be used to help fight cancer cells. One phase 2 clinical trial aimed to test the effectiveness of paclitaxel albumin-stabilized nanoparticle formulation in patients of all different ages with metastatic breast cancer. They wanted to test the toxicity and how well the drug spread throughout the body, along with dosage size and response time of the drug. They recruited 40 patients across the US of all different ages and began. Patients received paclitaxel albumin-stabilized nanoparticle formulation IV over 30 minutes once daily on days 1, 8, and 15 as planned. They repeated treatment every 28 days in the absence of disease progression or toxicity. Conclusions to the study stated that 26% of patients experienced grade 3 toxicity, however it was not age related. The scientists suggest that "There is an association between toxicity risk score and grade $\geq$ 3 chemotherapy toxicity and pharmacokinetic variables,"(Hurria 14). However, they also claim that the treatment was well tolerated across all age groups. This study could expand research being done in the nanotechnology field. Lastly, a study by Durham University in the United Kingdom in 2017 showed that nanobots could drill through cellular lipid bilayers to cause programmed cell death. The molecular "motors" are attached onto the lipid bilayers. These motors then are activated

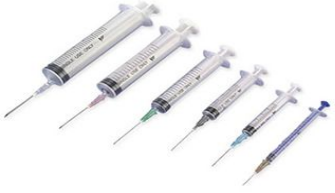
using ultraviolet light causing them to spin at rates up to 2 to 3 million rotations per second, rotations of such high rates can open cell membranes. Researchers have proven that these nanobots could become a way of injecting and removing chemical substances from live cells or cause cell death. These nanobots are made in different sizes and could inject peptides which can be designed to target and kill particular cancer cells. This study has significance to our problem because it is another way nanobots can be used to kill tumors. Our hypothesis is, if we could use nanobots, and bacteria and virotherapy concurrently, the tumor would shrink and be destroyed. The nanobots will then make their way to the tumor mass. Once there, the thrombin enzyme will suppress the blood flow, effectively shrinking the tumor. Because of the speed and frequency of blood flow in the body, the nanobots will most likely be swept away and expelled from the body after a certain period, which is where the bacteria and virotherapy come in. The bacteria or virus will be injected into the body, and will infect and destroy the cancer cells from inside, preventing it from being swept out of the body while the tumor exists. After one or more cycles of both the nanobots and bacteriotherapy, the tumor will go through cell death and the patients' survival chances would increase. Then, scientists will observe the response of the tumor to our therapies. If the tumors go through cell death, then the nanobots and bacteriotherapy worked. If the tumor did not completely go through cell death or showed partial response, that means we could repeat the cycle of treatments again to see if giving more therapy will increase the death rate of the cancer cells.

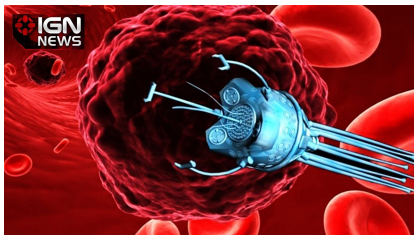
## Materials and Methods

This procedure would be carried out by injecting DNA origami-style nanobots with thrombin attached to the surface to cut off the blood supply of the tumor. Because tumor cells use a different metabolism that requires more nutrients and oxygen to be used, "starving" the cells of these tumors could cause them to regress rapidly. Injecting bacteria such as *Bifidobacterium longum* and *Clostridium novyi*, which are capable of colonizing the hypoxic areas of the tumor, could further damage and destroy tumor cells. And because bacteria such as *Escherichia coli* survive particularly well inside of tumors, where they are protected from the immune system and have space to multiply, they could be used to kill the tumor from inside out. If nanobots, bacteria, and virotherapy could be used together, then it may increase the chances of destroying the tumor. First, nanobots are programmed to recognize the unique antigens on cancer cells by making special claws that match the cancer antigens using a sample of tumor cells. Next, the nanobots would be introduced into the bloodstream to cut off the tumor's blood supply. This would weaken the tumor and prevent it from being able to multiply and spread. Then specific strains of viruses and bacteria would be injected to target the cancerous cells from inside and destroy them. Once the tumor is destroyed, the viruses and bacteria would be exposed to the person's immune system, which would wipe them out. Eventually, they will all die. This way, both treatments can be used in conjunction with one another to increase the chances of remission. Therefore, using nanobots and bacterial/viral therapy together will increase cancer patients' survival rate. The experiment would be done in a research facility that has access to nanobots and other biomedical supplies. This is because it would be the proper environment for conducting the experiment as it has all the necessary equipment. It would be easy to monitor the nanobots and grant the ability to modify anything quickly. The materials we would need for this experiment include MRIs, CT scans, X-rays machines, nanobots, syringes, and PET scans. In

this experiment, a syringe and needle would be used to insert the nanobots into the bloodstream, and from there, a magnetic resonance imaging (MRI) device would be used to track the position of the nanobots and maneuver it. Then, scientists could insert the virus/bacteria into the human body by also using a syringe. While the treatments are doing its job, the size of the tumor could be monitored by using CT scans, bones scans, PET scans, or x rays, depending on the type of cancer. The experiment would be over the course of a couple weeks. A cycle of the experiment would include an injection of nanobots into the body. The nanobots would take up to 24 hours to cut off the blood supply. After these 24 hours, we would perform tests such as a CT scan, magnetic resonance imaging (MRI), positron emission tomography (PET) scan, ultrasound, X-ray, and a biopsy. Depending on whether or not patients show complete response, a bacteria such as *Escherichia coli* or *Clostridium novyi* will be injected into the center of the tumor to attack it from within. Depending on the patients' response to both treatments, the doctor will suggest whether to continue with more cycles of both treatments or to stop if patients are experiencing side effects. We came up with this timeline based off of other studies which injected nanobots into mice. When injecting into mice, it took them several hours for the nanobots to cut off the blood flow so, we decided it would be best to wait for upt 24 hours for the nanobots to stop blood flow and then test to see how effective the nanobots were in degrading the tumor and if it was not enough to cause complete response, we would inject bacteria into the center of the tumor to complete cell death. If both treatments did not cause CR, based off of whether or not they had side effects from the treatments, we would continue with more cycles of both the nanobot injection and bacteriotherapy.

(A photo of different sized syringes,  photos of nanobots )



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# Reflection

Our five group members each played significant roles in this research paper. Jacob helped to do research, write, and helped organize parts of the project. The parts he helped write are, the second nanobot study, the FMT study, parts of the abstract, part of the materials and methods, and part of the first introduction paragraphs. Lena added to the project with a solid front to many parts in the essay and provided important information to the studies. She wrote parts of the abstract, the majority of the first page of the introduction, the ASU study, part of the hypothesis, and part of the materials and methods. Belinda helped by providing the first parts of the information and reconstructing parts of the essay to make it sound better. She wrote the majority of the first introduction paragraph, part of the materials and methods, part of the hypothesis, and part of the third paragraph of the introduction. Eddy promoted the project by writing much of the introduction and researching important information. He created the first nanobot study, parts in the hypothesis, materials and methods, and the abstract. Bryce also helped with writing and researching for the project. The parts Bryce contributed to include parts of the second introduction paragraph, part of the materials and methods, and part of the hypothesis.