E. coli: The Cure for Cancer?

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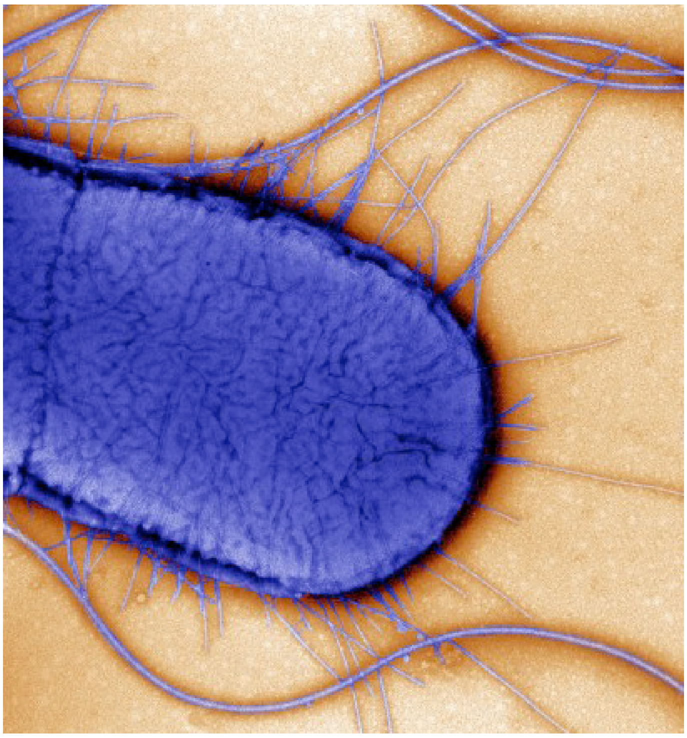
For years, researchers have been investigating the use of bacteria as programmed vehicles to deliver drugs. Few successes have been reported with these experiments due to the difficulty in finding a bacterium strong enough to kill pathogens without harming patients [1]. Dr. J. Christopher Anderson, an assistant professor of bioengineering at the University of California, Berkeley, is leading a project with high potential—to remodel *Escherichia coli (E. coli)* to fight cancerous tumors. *E. coli*, a species of bacteria sometimes associated with food poisoning, are being designed by researchers at Anderson Lab to travel through the bloodstream, identify and invade cancer cells, and destroy them [3]. Utilizing engineered bacteria in the manner Dr. Anderson is proposing to combat this deadly disorder could be a revolutionary step in the field of therapeutic bacteria [2]. Current cancer treatments including chemotherapy and radiotherapy, are inefficient and cause an array of side effects, giving Dr. Anderson's experiment much attention in the medical community [3].

At Anderson Lab, researchers are attempting to develop a drug that uses *E. coli* as vehicles, to deliver a cytotoxic chemical that kills tumor sites. The drug would be injected into a patient's bloodstream where it would to carry out three main tasks: avoid the immune system, find and invade the tumor site, and release the cytotoxin to kill the cancer cells [3]. The goal of the researchers is to engineer genetic devices, each comprised of multiple genes, that contain the instructions for the bacteria to carry out the assignment. Each genetic device specifies the what, when, where, and how of each step that the *E. coli* must execute to ultimately kill tumor sites.

The first step the *E. coli* would take once injected into the bloodstream of a cancer patient is to evade the various levels of the human immune system [3]. Usually considered a beneficial evolution of the human body, the immune system would be the enemy of this cancer-killing drug as the system's duty is to eliminate foreign substances that enter the body. To the immune system, the *E. coli* would be no different than any other foreign germ that enters the body. With that said, the *E. coli* are being engineered such that the immune system does not recognize the drug as a foreign substance. The immune system usually recognizes pathogens by features on their outer membrane; thus, to prevent the bacteria from being engulfed once inside the bloodstream, the *E. coli* are commanded to grow an alternate carbohydrate surface layer instead of chains that would induce an immunity response [4]. Without worry that the injected *E. coli* will cause a dangerous response from the immune system, they are free to carry out the rest of their tasks.

With the disguise to sneak past the immune system, the *E. coli* would then travel to the tumor site through the bloodstream. These facultative anaerobes are able to sense hypoxic regions, or regions of the body that are deprived of oxygen. This is perfect for a vector, or vehicle, that travels to cancerous sites as cancer cells usually consume high doses of oxygen to grow at their exponential rates, leaving a highly hypoxic region [5]. Tumor cells in sight, the *E. coli* would bind to surface proteins called β1-integrins on the cancer cells, causing the cells to uptake the bacteria by phagocytosis, a process by which the bacteria outside the cell is engulfed by the cell membrane and brought inside the cell [5,6]. The *E. coli* now inside the cancer cells' vacuoles, or organelles that act as compartments within the cell, would have completed their second set of instructions and would be ready to begin their third and final task.



Electron micrograph of *Escherichia coli*, close-up. Reproduced from [9].

> **"The *E. coli* bursts inside the vacuole, releasing the cytotoxin"**

Once inside the tumor cell, the bacteria would deliver a cytotoxin to the cytoplasm of the tumor cell [2]. The *E. coli* would undergo lysis, or cell suicide, and burst inside the vacuole, releasing the cytotoxin and a protein that degrades the membrane of the vacuole, allowing the cytotoxin to spread through the cytoplasm and kill the cancer cell.

Dr. Anderson has also been researching alternative methods to deliver the toxin. The second option would be to first instruct the *E. coli* to escape from the vacuole and then to secrete the cytotoxin into the cytoplasm. The advantage of this method is that each bacterium's potency is optimized within each tumor cell. The third and last set of instructions is similar to the second in that the *E. coli* would escape from the vacuole and secrete the cytotoxin, with the added instructions for the bacteria to continuously release the toxin while spreading from cell to cell, maximizing the number

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of tumor cells that would receive the drug [7]. Each set of instructions, with its distinct advantages and disadvantages, is suited for different types of tumors. In the end, all three sets result in the destruction of cancer cells, the ultimate goal of the bacteria.

This project is still early in the development phase; however, advancements are being made. Dr. Anderson states, "the drug can deliver protein payloads to cancer cells." With further experimentation, researchers at Anderson Lab hope to be able to regress a tumor in a live mouse [3].

"Drugs would no longer be limited to a single function"

The success of this project could mean an alternative form of cancer treatment to the present-day chemotherapy and radiotherapy [2]. The tumor-killing bacteria could prove to be safer, more efficient, and less expensive due to its biospecificity, or ability to localize and target a specific site [8]. Dr. Anderson notes that "there are very few drugs out there that work on the basis of recognizing the cancer cell surface; most cancer drugs work as inhibitors of some process vital to the cancer cell" [3]. Moreover, unlike other cancer treatments that rely on the movement of blood to travel throughout the body, *E. coli* are able to be engineered to grow flagella, tail-like structures that provide movement, allowing them to travel to tumor sites [6]. The ability of the bacteria to release the drug only once they are inside the tumor cell, as well as their self-destruction after they perform their task, prevents damage to other healthy cells, a feat that most other treatments cannot accomplish [7,8]. The multitudes of side effects that are commonly associated with cancer treatments today are usually attributed to damage to healthy cells in the body, thus this drug may be able to boast fewer side effects [8]. Additionally, the use of bacteria as a vector to deliver a cytotoxic chemical allows higher carrying capability than conventional methods. These characteristics make this developing drug a promising treatment that boasts specificity and efficiency over current cancer treatments.

Like most synthetic biology experiments, researchers must take an additional precaution with this experiment. The risk of a mutation occurring within the DNA of the *E. coli* could compromise the safety aspect of this drug [8]. Dr. Anderson discusses potential instabilities: "For example, if a growth control device were inserted into a cell that actively killed the cell after growing for six generations, this would be the type of device that might go awry. You have to consider what happens when you mutate the things you add into the cell. In this case, one loss-of-function point mutation would destroy the safety mechanism" [3]. While mutation only has a one per million chance of occurring per DNA base pair, scientists take the risk into serious consideration. Devices are being upgraded to include multiple safeguards that cause the bacteria to lyse if certain objectives are not met. The improvement of these safeguards could promote the use of therapeutic bacterial drugs in the future [8].

With the increasing potential of the use of therapeutic bacteria, Dr. Anderson worries that his drug could pose a societal problem—it is difficult for people to cope with the idea of injecting live bacteria into their bloodstream as a cure for a deadly disease [3]. With hope for the future of this project, Dr. Anderson believes that this obstacle is something that people will be able to overcome in time. "One reason we pick cancer above all others is because it is a disease for which people are willing to try something that they aren't willing to try for any other disease," [3]. Other therapeutic bacteria are already on the market in the form of dietary supplements, otherwise known as probiotics [3]. Many dairy products, breads, meats, and other ordinary food items eaten daily are supplemented with probiotics for an assortment of health benefits; however, it is rare to see people avoiding the yogurt aisle because live bacteria are added. This may be due to the common belief that there are already germs in food anyway, so people are accustomed to ingesting bacteria, unlike a cancer-killing drug, which would need to be injected [3].

Dr. Anderson's project pioneers unchartered territory in the use of therapeutic bacteria. Although it may require some adaptations in terms of societal attitudes, the space for potential design is tremendous. Only limited by the lack of methodology, which can be developed in time, this cutting-edge technology has a key advantage of infinite modification. "Nothing is off limits in terms of what we can change about these organisms" says Dr. Anderson [3]. The use of bacteria as a delivery method for drugs allows for a complex mechanism of action. Drugs would no longer be limited to a single function, as bacteria can have multiple queues of behaviors engineered into their genetic code [3]. Dr. Anderson's project would not only result in one usage of these bacteria, but could also provide the tools for further development. It is one of the first of its kind, a great advancement in therapeutic bacteria and a potential to be a new cure for cancer. ■

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