Bugging cancer

Microbes have a natural affinity for tumours, opening up incredible new ways to fight cancer, discovers **Alice Klein**

PETER STRAIN

SALMONELLA bacteria are normally associated with violent food poisoning. But in 2019, Irit Balboul, a 71-year-old Canadian, volunteered to drink a liquid containing 1 billion live *Salmonella typhimurium* bacteria as a last resort against her pancreatic cancer, which had spread to other organs and given her just months to live.

The bacteria had been genetically engineered to trigger an immune attack on her cancer cells, while being less toxic than regular *Salmonella* to the rest of her body. Balboul was the first person in the world to trial them along with chemotherapy and her tumours shrank to just 10 per cent of their former size.

It may sound strange to use bacteria to treat cancer, but the approach harks back to the late 1800s, when William Coley, a New York surgeon, began injecting *Streptococcus* bacteria into the inoperable tumours of some of his patients, resulting in regressions he described as "nothing short of marvellous". Despite this promise, the approach fell out of favour. More than a century later, however, we are witnessing Coley's comeback.

It is now clear that many bacteria are naturally attracted to tumours, which are home to a rich microbial ecosystem. This tumour microbiome can influence how cancer progresses and responds to treatment. A clearer appreciation of this system is leading to the development of new microbial medicines targeting cancer, some of which are now in clinical trials – including the engineered *Salmonella*. These microbes can burrow deep into places that are hard to reach with existing treatments such as chemotherapy and can be equipped with extra, cancer-fighting weapons through genetic engineering, offering alternative ways to attack tumours.

Most people are now familiar with the gut microbiome – the complex community of bacteria, fungi, viruses and other microbes that inhabit our guts and influence our health in myriad ways. But the idea that tumours are also teeming with microbes only came to the fore in 2020, when Ilana Livyatan at the Weizmann Institute of Science in Israel and her colleagues analysed bacteria in more than 1000 human tumour samples.

Using genetic sequencing, they identified bacteria in all eight tumour types they studied: breast, brain, lung, skin, bone, ovary, pancreas and colon. "It made us raise our eyebrows and say, 'Hey, what are they doing there?'," says Livyatan. On average, they found roughly one bacterial cell for every 150 tumour cells, but some tumour types, such as those of the colon and breast, typically harboured more bacteria than others. Even more surprising was the discovery that each tumour type had its own distinct microbial signature.

Last year, Livyatan and her colleagues dropped another bombshell: they had found fungi in tumours too, in all eight types that had previously tested positive for bacteria. At the same time, a team led by Iliyan Iliev at Cornell University in New York revealed that it had also sequenced fungal DNA in a range of tumours. These included *Candida* fungi – which are more commonly associated with vaginal and oral thrush – in cancers of the stomach, colon, head and neck (though the amounts were tiny: about one fungal cell per 10,000 tumour cells). "It was kind of mind-blowing," says Iliev. "Something seems to be attracting the fungi to some tumours because when we look at tumour tissue and the adjacent tissue from the same patient, we see that the fungal cells are preferentially abundant in the tumour," he says.

There are many reasons why microbes might be attracted to tumours (see "Ideal home for bacteria", page 42), but what is becoming clear is that their presence can influence the course of cancer and its treatment.

Bacteria called *Fusobacterium nucleatum*, for example, can promote colon cancer, accompany its spread to other parts of the body and dampen its response to chemotherapy. These bacteria mostly live in the mouth, but can invade the bloodstream and then circulate to the colon. There, they can colonise polyps – clumps of cells that form on the lining of the colon – and turn them cancerous. Similarly, the presence of *Malassezia globosa* fungi in breast tumours, which are thought to travel there via ducts connecting the skin and breast, has been associated with poorer survival rates.

We have also known for decades that

certain viruses can trigger cancer formation: for instance, human papillomavirus (HPV) and hepatitis B virus (HBV), which are associated with cancers of the cervix and liver respectively. HPV inserts its DNA into the genomes of healthy cervical cells and causes them to grow out of control, becoming cancerous, and we are now getting a clearer picture of the mechanisms by which other microbes can influence the progression of cancer (see "Microbial manipulations", page 43).

Vaccine hope

Fortunately, we now have HPV and HBV vaccines to help prevent their associated cancers. In the same way, we may be able to develop vaccines against tumour-promoting bacteria, says Robert Holt at the British Columbia Cancer Research Institute in Canada. These have the potential to inhibit the growth of bacteria in existing tumours, slowing their progression and making them more responsive to chemotherapy. They could even stop tumours from forming in the first place.

Holt and his team are specifically working on a vaccine against *F. nucleatum*. The vaccine contains messenger RNA (mRNA) that is designed to instruct the body to make certain protein fragments found in this bacterium. The idea is to train the immune system to recognise and kill the microbe. At this stage, the vaccine is still being tested in mice, but Holt and his colleagues hope to one day trial it in people with *F. nucleatum*-positive colon cancer that is resistant to conventional treatments.

Taking "bad" microbes out of tumours is one strategy to tackle cancer, but another may be to send in "good" microbes. This is the approach that Coley took, inspired by reading about a man whose large neck tumour disappeared after he developed a *Streptococcus pyogenes* infection at the site where his surgeon had unsuccessfully tried to cut the tumour out.

When Coley injected live *S. pyogenes* into 10 of his patients' tumours, half had partial or complete tumour regressions. However, he found it difficult to get the dose right and some of his later patients died from the treatment. The turn of the 20th century saw the arrival of radiation therapy, which was seen as safer, and Coley's approach fell by the wayside.

We still aren't sure how Coley's treatment worked. It is most likely that the *Streptococcus* bacteria he injected into his patients' tumours made them more visible to their immune systems, says Livyatan. Although small ›

"The tumour shrinkage was striking. None of us had seen anything quite like it before"

Ideal home for bacteria

The reason certain bacteria are attracted to tumours is still an open question, says Ilana Livyatan at the Weizmann Institute of Science in Israel. "If you grow a tumour in a mouse and then inject bacteria into its bloodstream, the bacteria will home to the tumour, but we don't really know why yet."

One suggestion is that blood vessels in tumours grow in a fast, chaotic way, forming twists and dead ends that may trap bacteria. Another is that the rapid metabolism of tumours provides abundant nutrients for bacteria to feed on. Low-oxygen pockets that typically form inside tumours may also attract oxygen-hating bacteria called anaerobes. And tumours often develop ways to evade detection from the immune system, making them perfect hiding spots for microbes.

Certain tumours provide the right kind of food for particular microbes. Lung tumours of smokers, for instance, seem to favour the growth of bacteria that can degrade components of cigarette smoke such as nicotine.

amounts of bacteria can hide in tumours because of cancer's immune-evading mechanisms, injecting large doses of them into tumours may be enough to trigger alarm bells so that the immune system launches an assault on the cancer cells that the bacteria are living in, she says.

Research by another group at the Weizmann Institute of Science led by Yardena Samuels has provided some clues as to how Coley's bacteria may have made tumours more visible to the immune system. The team discovered that protein fragments from bacteria that colonise melanoma skin cancers are expressed on the surface of the cancer cells. These signpost the tumour as foreign to the immune system, so that it realises "Hey, something's wrong here" and attacks the cancer cells, says Livyatan.

The *Salmonella* that were used to treat Balboul had been genetically engineered to rev this mechanism up even further. They were made to express a protein called interleukin-2 that activates the immune system. "The *Salmonella* goes directly to the tumour and it brings along the interleukin-2 so that the whole body's not exposed to the interleukin-2, just the tumour," says Gerald Batist, the oncologist who oversaw Balboul's treatment at the Jewish General Hospital in Montreal. "It creates an immune activity against the tumour right at the site of the tumour."

Living longer

Balboul trialled the treatment alongside standard chemotherapy. The dramatic shrinkage of her tumours was a far stronger response than would be expected with chemotherapy alone. "It was very striking – none of us had seen anything quite like it before," says Batist. What's more, the bacteria didn't make her sick, he says. "The idea that we would have someone drinking bacteria really freaked out the infectious disease people on our ethics committee, but she had no side effects whatsoever."

After Balboul's promising response to these bacteria, Batist and his colleagues launched a phase II clinical trial in 2020 involving 20 people with stage 4 metastatic pancreatic cancer – considered one of the worst of the worst cancers. The trial participants were all treated with standard chemotherapy plus the engineered *Salmonella*. The results, which were released in January, showed that the participants lived for 24 months on average, which is more than double the typical survival length of 11 months for standard chemotherapy alone. The *Salmonella* didn't make these volunteers sick either.

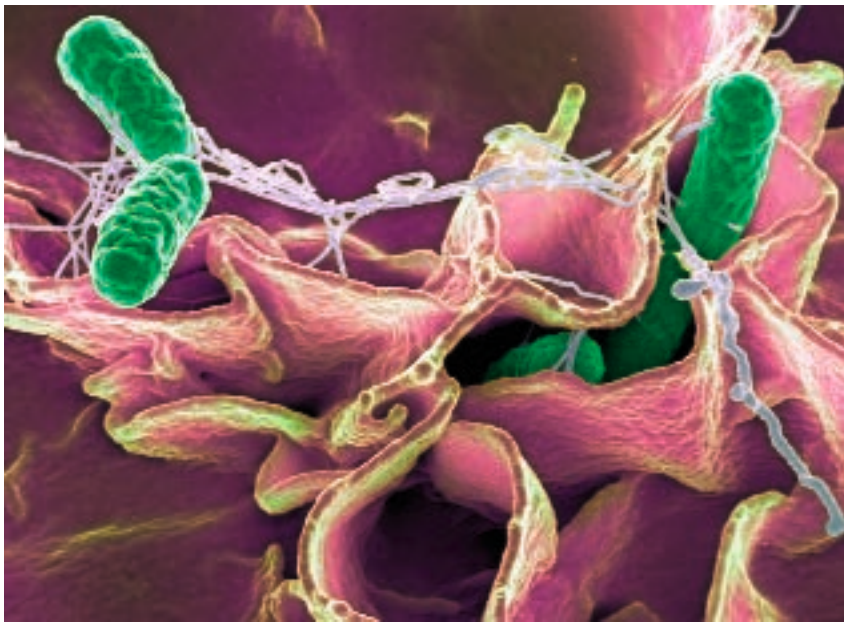
"It's not a miracle cure, but a doubling of survival is quite significant if we can confirm it in larger trials," says Batist. A major advantage of this approach is that bacteria are far cheaper to manufacture than other cancer immunotherapies like checkpoint inhibitors, CAR T-cell therapies and personalised vaccines, he says. The US Food and Drug Administration (FDA) recently gave the *Salmonella* therapy fast-track status to make it available as soon as possible if it passes future trials. And last year, a study in mice showed that tumour-homing bacteria could be controlled by ultrasound to release cancer-fighting substances, offering a potential new route to precise targeting.

A company in Switzerland called T3 Pharma is also using bacteria to try to supercharge the immune system against cancer. It is mainly working with a bacterium called *Yersinia enterocolitica*, which is a contaminant of pork that can cause food poisoning, but has been genetically engineered to "down-tune its virulence", says Christoph Kasper, the firm's chief scientific officer.

An extra bonus of *Y. enterocolitica* is that it has special "nano-syringes" on its surface that it uses to inject proteins into cells. T3 Pharma has engineered a strain of *Y. enterocolitica* that can inject proteins into tumour cells to make them release signalling molecules that "act like warning signals to bring the immune system into play to fight the tumour", says Kasper.

When the engineered *Y. enterocolitica* were injected into the blood of mice with melanoma or other tumours, they selectively homed in on the tumours and caused them to disappear in up to two-thirds of the animals, with no serious observable side effects. The company has just launched a clinical trial of the engineered *Y. enterocolitica* in people with solid tumours. "We're very excited," says Kasper.

BioMed Valley Discoveries, a company based in Missouri, is pursuing a different approach to target the low-oxygen pockets inside tumours that are often resistant to existing cancer treatments. It is injecting spores of anaerobic *Clostridium novyi* bacteria, which have been genetically modified to make them less toxic, directly into solid tumours. The spores only germinate in the low-oxygen regions of tumours and, as they proliferate, they alert the



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Above: Bacteria such as *Salmonella* (green) can be weaponised to fight cancer. Left: Vaccines against tumour-promoting bacteria are in development

Microbial manipulations

We know that microbes can cause cancer or influence tumour growth, but how exactly do they do this?

Some bacteria, like *Escherichia coli* (*E. coli*) and *Campylobacter jejuni*, release toxins that directly damage the DNA of human cells, turning them cancerous. And microbes that make their homes in already-established tumours can also help them to expand. For example, certain tumour-dwelling bacteria release chemicals that suppress the activity of nearby immune cells, making it easier for the cancer to grow unchecked.

When bacteria are inside tumour cells, they can also reshape the cells' cytoskeletons – the structures that maintain their shapes – to make them stronger. This allows the cells to resist damage while travelling through the body and seeding new tumours. In contrast, some bacteria seem to dampen tumour progression. For instance, *Helicobacter hepaticus* has been shown to shrink colorectal tumours in mice, most likely by attracting nearby immune cells.

immune system to start attacking these areas.

In a first-in-humans trial published in 2021, the bacteria were injected directly into the tumours of 24 people. There, they were found to essentially liquefy the tumour's low-oxygen innards. "You still had a ring of tumour cells on the outside, where the tumours were well-oxygenated and the bacteria couldn't grow, but in the middle, it was just fluid and gas where the cells had been destroyed," says Brent Kreider, president of BioMed Valley Discoveries.

The *Clostridium* bacteria are now being evaluated in a second clinical trial, this time in combination with the checkpoint inhibitor pembrolizumab – more commonly known as Keytruda – that can target the outer tumour cells. "The idea is for the bacteria to fight the tumour from the inside out and the pembrolizumab [to fight it] from the outside in," says Kreider. The results will be made available later this year, he says.

Bacteria aren't the only microbes getting attention, however. Several tumour-fighting viruses are also under investigation. One of these, called T-VEC, was approved by the FDA in 2015 for treating melanoma that had spread to other parts of the body. T-VEC uses a strain of herpes simplex virus 1, which normally causes cold sores, but has been genetically engineered to make it less harmful to healthy tissue and to invite an immune attack when it is inside tumours.

These are some of the most promising stories, but there have also been disappointments in the field. A trial of genetically engineered *Listeria* bacteria in people with lung cancer, for instance, was discontinued in 2018 after it was found to be ineffective, and another of genetically engineered *Bifidobacterium* in people with a range of tumour types was terminated in 2021 for the same reason.

"It's still a young field and it's not a classical way to approach cancer intervention – you're injecting something live, it's not like a pill – so there is still a lot to learn and it can be a bit of a struggle," says Kreider. "But I think there is great hope."

As for Balboul, after being told she only had months to live, she ended up living over three more years until recently passing away at the age of 74. "Her cancer came back and she decided she didn't want further treatment," says Batist. "But when I told her that we were able to get a phase II trial started [of the *Salmonella* bacteria], she was extremely excited," he says. Thanks to her courage in trialling the unusual treatment, Coley's once-shelved approach may finally find a place in modern medicine. ▮

Alice Klein is a reporter for *New Scientist*