An accidental discovery

Sometimes, the key to saving lives can be hidden in a protein that hasn't changed much over billions of years.

It was a warm summer day in 2011 and down at NTNU's medical research lab in Trondheim, researchers led by Marit Otterlei were just finishing another round of testing a promising new cancer drug.

The researchers put their cancer cells into little wells on a cell culture plate, as was the routine.

Then they added their drug, an anti-cancer peptide, in increasing doses to the different wells on the plates, so they could see the relationship between the strength of the drug and the cancer cell growth.

Some of the wells didn't get any of the drug, some wells got a little of the drug, and some wells got high doses of the drug.

But remember, it was a warm summer day, in a country where buildings aren't necessarily built for warm summer days. So the researchers had opened the windows.

What they didn't realize was that those warm summer breezes had brought an unwanted visitor – bacteria that decided the cell culture would be a good place to live and grow.

So when they came back the next day…

Marit:... what we saw then was that we were running an experiment which failed due to bacterial infections, but we noticed that in wells where we have added this anti-cancer peptide, at low doses, there were no bacteria. And the cancer cells looked fine.

So this indicated that this peptide not only targeted cancers, or human cells, but also was a pretty good antibacterial peptide, maybe better than cancer peptide, if you look directly at the dose.

Nancy: This is what you could call the Alexander Fleming moment for Marit. Alexander Fleming is the guy who discovered penicillin in 1928 when some mold infected an experiment he was conducting.

Marit: It happened by accident. So it was our Fleming discovery

Nancy: The comparison is even more applicable than you might think. It wasn't until 1939, when WWII was erupting, that researchers were able to take Fleming's discovery and eventually turn it into the drug we know today. And then it wasn't until 1942 that the first treatments were offered. Marit Otterlei's magic peptide is under

development now, but it's most likely to come to market first …. as a cancer treatment.

Nancy: I'm Nancy Bazilchuk, and you're listening to 63 Degrees North, an original podcast from NTNU, the Norwegian University of Science and Technology.

Today, I'm going to tell you about Marit Otterlei's accidental journey into drug development, not just for a new antibiotic but for a cancer drug too.

It's a peek into the challenging world of what it takes to bring a drug, especially an antibiotic, to market. But it's also an inside look into how some researchers, with their deep curiosity about the nuts and bolts of how life actually works, can come up with startling discoveries that may someday save our lives.

Sometimes, the key to saving lives can be hidden in a protein that hasn't changed much over billions of years.

Nancy: And this whole journey started with something called a beta clamp, made up of two identical proteins forming a ring.

I know this sounds like some kind of weird medical equipment, like maybe for keeping betas from leaking out of something somewhere, but it's actually a part of the biological machinery that every single organism contains.

Nancy: To understand why this is important, I need to review a little basic cell biology.

Remember that DNA contains the code that all organisms, from yeasts to humans, need to live and reproduce. Before a cell can divide, it has to copy that code exactly. And it doesn't happen by magic—it's a full-on molecular deconstruction – reconstruction site.

This deconstruction/reconstruction works because DNA is structured like a twisted ladder with rungs made of four different molecules, called nucleotides, abbreviated A,T, C and G.

And these nucleotides are picky – A will only pair up with T, and C will only pair up with G, and vice versa. When James Watson and Francis Crick figured out this structure back in 1953, they wrote (with dry scientific humor) "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

So when a cell is going to divide, it unzips its DNA ladder down the middle. It can then copy its DNA perfectly by using each side as a template for building its opposite side. You know this from high school biology, I bet.

But here's the twist: to actually *read* and *copy* that code, your cells need a little mechanism. In bacteria, it's called a **Beta clamp**. In humans, the equivalent mechanism is called **PCNA**. In both bacteria and humans, these mechanisms are

like sliding rings that hold the copying tool tight to the DNA, keeping it on track while the cell copies the recipe written in the rungs.

Marit: It is completely essential for all life, because you need it to copy your DNA. Without copying your DNA, there is no life.

Nancy: If you block that ring—jam it or pop it off—the copying process falls apart. The DNA can't replicate. And suddenly, that cell—whether it's a cancer cell or a bacterium—hits a dead end.

Nancy: What Marit and her colleagues discovered on that hot summer day was that the cancer drug that they were testing derailed the replication of the human cancer cells – *but not normal, healthy cells*. But it also appeared to work on *bacterial cells*.

Human cells AND bacterial cells??? The tree of life branched away from bacteria 3 BILLION years ago. Yet those little sliding rings that were key to cell replication in bacteria– the beta clamp– and in humans – PCNA – were still similar enough that this one substance looked like it could stop them both!

Marit: So this was very scientifically interesting because these very conserved proteins, what kind of functions are conserved? …It's in all, all kingdoms of life, actually. So that made us explore this further.

Nancy: A little translation here: Biologists say that a feature, like a gene or a protein, is conserved when it is found in all life forms, from the simplest cells to human beings. That's because as life evolved, the mechanisms that worked well were passed up the evolutionary tree.

Nancy: So let's summarize a bit. Remember Marit and her team were testing their substance, called a peptide, in wells that had cancer cells in them. The cell cultures got infected with bacteria from an open window in the lab.

Marit: Where we hadn't added the peptide, there was overgrowth (of bacteria) …So in wells where we had the peptide, no bacteria in wells, without peptides overgrown with bacteria.

Nancy: Marit already knew that the peptide drug worked to kill cancer cells but not normal cells. That's because cancer cells are stressed in a way that healthy cells aren't. The experiment they were running was actually to see if they could make the peptide drug stronger and more effective in different doses and different combinations.

Marit: And then we were testing a lot of conditions, combining them with different drugs to see if we can increase their sensitivity. Because that was the focus of the cancer therapy project.

Nancy: They knew enough about the effectiveness of their peptide drug that Marit and some colleagues had already formed a company, APIM Therapeutics, in 2009, as a spinoff from NTNU. I'll put a link in the show notes to the company's website.

Nancy: But this antibacterial ability of the peptide drug, that was something altogether different. Because if you've listened this far, you probably know that antibiotic resistant bacteria are a huge and growing problem. Society needs more drugs that can kill bacteria that are resistant to existing antibiotics. In 2022, a giant international collaboration called the GBD Antimicrobial Resistance Collaborators came up with an estimate that was a real wake up call.

They found that by 2050, unless we find better ways to combat antibiotic resistance, an estimated 10 million people will either die directly, as a result of bacterial infections that can't be treated, or because antibiotic resistant bacteria or fungi were a contributing factor.

This is at a time when the need for antibiotics in medicine is getting bigger and broader. These drugs are not just for treating a bad cut or strep throat. No, no, no…

One key group of patients that gets antibiotics as a preventative measure are cancer patients. Chemotherapy or bone marrow transplants can wipe out their immune systems, so doctors regularly treat these patients to make sure they don't get sick. Patients with chronic illnesses like Cystic Fibrosis may need repeated or long-term treatment of lung infections. And think about organ transplants and even simple surgeries.

Nancy: So remember, what made the peptide really interesting is that Marit and her colleagues had structured it to work specifically on the human DNA replication tool, **PCNA**, in cancer cells. But now what they saw suggested it also worked on bacteria. That would be completely different from the way most antibiotics worked.

Marit: So, if we could show that the mammalian motif was actually interacting with the bacterial protein, that would be interesting. It's scientifically interesting also, not only to explore it as a new drug.

Nancy: Still, they needed to prove that the bacterial apocalypse they were seeing on their little plates was actually due to their peptide drug targeting the replication machinery in bacteria. That turned out not to be that easy.

Marit: We started to look into this antibacterial activity, but it was very technically difficult to show that it actually interacted with the beta clamp. So we spent quite a lot of time doing that.

Nancy: Not just months, but years.

Marit: I think the first four, five years, it was a lack of experience both with microbiology and with these antibacterial peptides.

Marit: It was very hard to find out the specific mode of action of these peptides. In bacteria that can stop growing, it doesn't necessarily mean that you have something that is antibacterial.

Nancy: Details matter. Still, they had enough ammunition by 2015 to get a patent.

Marit: Yeah. We patented the ability of these different peptides to inhibit bacterial growth in 2015.

Nancy: And by this time their little peptide had a name: Betatide.

Then it was time to talk to some pharmaceutical companies. And they were interested.. Sort of….

By this point, NTNU's Technology Transfer Office had gotten involved. This is a limited liability company owned by NTNU and the Central Norway Regional Health Authority that helps researchers commercialize promising ideas. They helped Marit with creating APIM Therapeutics, the spin-off company for the cancer drug version of this peptide, back in 2009-2010. So when it came time to talk to the pharma companies, Siril Bakke, the project manager from NTNU's Technology Transfer Office who is responsible for Betatide, was also at the table.

Siril: It took some time to generate a lot of the data that the pharma industry wanted to see before they might be interested in the license agreement. And then the years went by, and then also the patent time, ending up where we were sitting at a table with a pharma actor that was really interested, but said like, oh, it's so interesting. You have so many interesting data, but the patent time has already run so many years and we need to do clinical trials that might take another seven, 10 years.

And then they have nothing left with the competitive advantage afterwards because the total time would be 20 years.

Nancy: That's right, drug patents last only 20 years.

Siril: So what we did was to make a second generation of Betatide. We have not patented that yet.

Nancy: Siril told me they will apply for the new patent soon. Still, just having a new patent isn't enough. The pharma companies need a lot of basic questions to be answered.

Siril: They would like to see how it works, that you have proof for how it works. They would like to see animal experiments showing if it's toxic or not, where it's distributed in organs, how it's eliminated, if it's through urine, for instance. So it could be an easy task and it could also be a difficult task depending on what kind of models and what kind of experience and whatever you have in the lab as well.

Nancy: There's also another major stumbling block in the way of developing new antibiotics: It's called the broken market. I talked to Christine Årdal, who's a senior scientist at the Norwegian Institute for Public Health.

Christine: My specialty area of research is access to antibiotics. I also look at innovation for new antibiotics but also how to ensure access to them, both old and new.

Nancy: She says that bringing *any* new medicine to the market is really hard.

Christine: If you look at the development pipeline, those that start phase one of clinical trials, 95% will fail for scientific reasons.

Nancy: But that's not the reason why drug companies tend to shy away from developing new antibiotics. It's because they can't make money from selling new kinds of antibiotics. Christine explains.

Christine: When you think about antibiotics, what do we wanna do with a new antibiotic? We want to put it on a shelf and put some glass in front of it, and a sign that says "Break only in the case of emergency". (04:34) because what we know about antibiotic resistance is the more that an antibiotic is used, the more likely that the bacteria will develop resistance.

Nancy: So, you're a pharmaceutical company, you're going to invest tons of money in developing a whole new kind of antibiotic, just to have it sit on the shelf? Not happening. Pharma companies make money by selling drugs, not creating them so they won't hardly be used.

Christine also told me that the antibiotics that pharmaceutical companies have in the pipeline now aren't actually new… they're just variations on existing antibiotics.

Christine: Yep, it's a whole lot easier and less expensive. It's still expensive, but it's less expensive to develop a new antibiotic in an existing class. And that's mostly what happens today. What that means is, it could be good because maybe you get better results for the patient. Maybe it doesn't have so many side effects or something, so it can be absolutely something that's worthwhile to do, but it can also potentially cause cross resistance to the existing antibiotics already in that class. And so you have to be careful.

Nancy: Enter the universities.

Christine:There's not a lot of innovation. And that's what's so interesting about the work that they're doing at NTNU, because they're trying to look at innovative approaches here.

Nancy: Countries across the globe are trying to find ways to make new antibiotics available. The UK has now begun what has popularly been called the Netflix subscription model, where companies get paid a fixed price for selected antibiotics. The idea is to provide companies revenue that's independent of sales – since the whole idea with new antibiotics is to save them as a last resort. Sweden has done a pilot of the same approach but hasn't adopted it yet. EU countries have supported a third model: a revenue guarantee scheme that would see developers' coffers topped up from an EU fund if sales of a new antibiotic don't hit a threshold.

Nancy: Above and beyond that though, Marit's little accidental discovery has one powerful advantage. Remember, when bacteria are exposed to antibiotics, the toughest survive and multiply. They can mutate. They can even share resistance genes with other bacteria, or scoop resistance up from the environment.

BUT *Betatide dismantles the ability of the bacteria to copy its DNA*. So Betatide also inhibits bacteria from evolving resistance.

Marit: That is important in bacteria because we have shown that these peptides, they are able to inhibit mutations. That means that they reduce the mutation frequency, and if we use them in combination with other antibiotics, we lower the mutation frequency. And, you know, mutations are what cause antibiotic resistance. So we lower the bacteria's ability to develop resistance against other antibiotics.

Nancy: Betatide can also be used in combination with existing antibiotics, Marit told me, to overcome resistance.

Marit: You can use them in combination, and then you will lower the dose needed. So you will lower the resistance to the bacteria because you target the bacteria two different ways, two different pathways.

Nancy: The road remains long before Betatide finds its way onto the market, though. Before any pharmaceutical company will take on a new drug, a developer needs two things, Siril and Marit told me.

Siril: Within pharma, they want to see that you know how the drug is working. They want to see that you have tested in animal experiments, they want to know if it's toxic or not. So they have a big list of things they would like to see before they're interested. And the problem here is within the university, this will take a lot of time, and you also need a lot of time, a lot of funding to manage to have this kind of package that pharma requires. So this is a big challenge.

Nancy: The good news is the idea has gotten support both from the university and from the Norwegian government, and has been accepted into the portfolio of a European incubator called INCATE. This group helps antibiotic startups get off the ground, offering funding, advice, and connections to investors and pharma partners. It’s where promising ideas get their first serious shot.

Siril: We have been fortunate to get national and local funding from NTNU Discovery, and also from the Research Council of Norway, the commercialization funds there. So right now we are using these funds for doing animal experiments to provide a proof of concept for Betatide. So it's an infection model of animal experiments.

Nancy: Siril told me they are testing Betatide to see how it treats lung infections.

Siril: So the animals will be infected by bacteria in the lungs. And then we treat them with Betatide afterwards. And then we also see how much bacteria are left in the lungs afterwards. So we are using a contract research organization for doing this because we don't have that infrastructure here in Trondheim.

Nancy: Then there’s a second test called GLP toxicity. The GLP stands for Good Laboratory Practices, and the toxicity study is more or less standardized.

Marit: That is the study toxicity that you have to do in two animal species, and you have to do it in a certified laboratory. So we cannot do it ourselves, and it's kind of expensive. What they do then is they test the treatment regime that you are going to use, and then they expand it. They test different doses to find the maximum tolerated dose and then evaluate the toxicity of the peptide. And this is what determines the starting dose in the phase one study in man.

Nancy: Marit doesn't strike me as someone who runs down the halls, yelling with joy when she has discovered something fundamental like this little peptide she has called Betatide. But it's an amazingly powerful discovery. And even as she's working to commercialize this peptide, she's continuing to explore what other secrets the bacterial beta clamp and its human counterpart, PCNA, contain.

Marit: My group is still working with these peptides targeting the beta clamp. But during these years we have seen that PCNA is involved in additional roles in the cell, not only in replication and repair. It is very important. We and another group have shown that PCNA is involved in cellular signaling, it's involved in regulation of apoptosis.

Nancy: A little translation here: Apoptosis is where a cell actively triggers its own death when it's no longer needed or is damaged. In cancer, this self-destruct system breaks down — the cells lose the ability to die and keep multiplying instead.

Marit: Last year we published that PCNA was involved in regulation of metabolism, via direct interaction with metabolic enzymes. So PCNA has a lot of additional, what we can maybe call non-canonical roles. And what we have seen is that some of these roles might also be conserved in bacteria. So that is interesting for us to further explore these new roles of these DNA sliding clamps that are conserved all the way from bacteria up to mammalian cells. If you look at the basic science, that is what my group is working with.

Nancy: Here I have to applaud researchers like Marit, who are essentially driven to learn more about how life works, simply out of the pure joy of curiosity. As the American nature filmmaker Louie Schwartzberg observed: "What is the intersection between technology, art and science? Curiosity and wonder, because it drives us to explore, because we're surrounded by things we can't see."

Marit: It's very inspiring to actually, to find new things. And if we are lucky, maybe we can develop something that can be of use that is also very inspiring, I think.

Nancy: I'm Nancy Bazilchuk, and you've been listening to 63 Degrees North, an original podcast from NTNU, the Norwegian University of Science and Technology. My guests on today's show are Professor Marit Otterlei, Siril Bakke, business development manager at NTNU Technology Transfer AS, and Christine Årdal, senior scientist at the Norwegian Institute of Public Heath.

If you want to learn more, you can see some of the publications from the research we've talked about today in the show notes. And if you've enjoyed today's show, leave me a review, and even better, tell your friends! We're on all major podcast

platforms, so we're easy to find. Writing, editing, sound design and production by me, Nancy Bazilchuk. Thanks for listening