Welcome, and thank you for watching. Every month, the LUMC organises a so-called pep talk for patients and staff: a story about our research. This month was scheduled to feature the new coronavirus. Unfortunately, as you may be aware, some companies have got less work on at the moment due to the coronavirus. This is definitely not the case for the LUMC. The virus is keeping us very busy and we are also worried it might spread to the audience coming to listen to a~~the~~ pep talk. For this reason, we have decided that this month's pep talk should be online only.

Coronaviruses. Over the next twenty minutes, I would like to tell you about coronaviruses, and how unique they actually are. How does the coronavirus work once it infects a cell? And what are the possible solutions for the outbreak we are dealing with right now? You can see the crown. What are coronaviruses? The name is derived from their appearance, as you can see from the electron microscopic image here. You can see a virus particle, but this is actually not a very good image of such a virus. The image is used a lot because it is freely available on the Internet, but a virus particle should be considered more like a juicy tomato. And what we just saw is more like the flattened, sun-dried version of this tomato. What it does show is the reason why they are called coronaviruses. The crown of spikes on the surface of the virus, and also the approximate size of the particle. A diameter of one hundred nanometres, which is small - too small to be visible to the naked eye. If we put the virus particle next to a space hopper, you can see the space hopper is ten million times bigger. And if we consider the space hopper in relation to planet earth, we have to multiply by ten million again. So, it is quite fascinating that this miniscule globule is holding the entire world in its grip at the moment. A very ingenious little globule indeed. I have this globule here, a model of the virus particle, enlarged about a million times. You can see the familiar spikes on the surface. This globule is filled with something; I will try to explain the contents to you. Inside, there is mainly genetic material. Your hereditary characteristics are captured in a genome. This is also true for a virus: these are the data of the virus. They determine the structure of the virus and how it behaves. The genome is enclosed in a Capsid, the so-called N-protein. The Nucleocapsid Protein is able to bind to the genome and encapsulate it. This leads to this little worm here in the virus particle, and around it is the so-called viral envelope. This grey layer is a biological membrane, like a fatty layer, stolen from the host. This envelope contains two viral proteins too; the M-protein and the membrane protein. As well as the S-protein. The Spike protein is the protein that causes the well-known protrusions on the viral surface. You could say the membrane is robbed from the host itself when it puts together new virus particles. I will get back to this at the end of my story. It may also be useful to take a little detour into virus inactivation, because this envelope, the biological membrane there, is very vulnerable. It is one of the vulnerable aspects of the virus. This membrane plays a very important role when we talk about virus inactivation using soap or alcohol, because soap or alcohol can be used to dissolve the membrane. When this happens, the virus particle disintegrates by itself and the spikes and the virus disappear suddenly. So, the explanation for the disinfecting effect of these substances is very closely linked to the structure of a virus particle. Back to the virus. Viruses are dead, which may surprise you, but it is true when talking about virus particles outside of the body. It is a biological complex of the genome and those proteins, but it is not actually doing anything. The virus only comes to life when it infects a cell. You probably know your body is made up of cells, billions of them. All these cells have an internal structure with a number of cell organelles. The virus knows its way around these cells, it knows how to enter and how to use what's in the cells to copy itself and then leave the cell. Your body is made up of billions of cells. So, if these cells become ill with a virus infection, the entire body will become ill eventually too. The virus knows the way, knows your cell's PIN codes and the passwords. For example, there is a so-called route for endocytosis which means the cell is effectively taking small bites of the environment outside the cell. It is how the cell eats and drinks, and this route is used by the virus to enter. There is an exit route that works

the same way, the exocytosis route, whereby small volumes of material are ejected from the cell. This is the route used by the virus to leave the cell. The best way to imagine a virus is as a burglar, someone who knows the way - which might be through the front door, but if not, they'll use a window. The virus will find a way in and knows how to use the stuff in the house to multiply itself, preferably before the police arrives. In this example, your immune system is the police. The immune system really starts to respond properly about a week after the viral infection, and the virus will eventually be eliminated. But in the meantime, the virus has free reign and will use as many things from your house to try and copy itself. Over to the coronavirus family. Here we have a coronavirus particle and various hosts: bats, birds, humans and all ~~manner~~ kinds of other mammals. We can divide the coronavirus family into alpha, beta, gamma and delta coronaviruses. This is not very relevant per se, except that it signifies the variation of these viruses. There are hundreds. These viruses could be described as guests at the same mammal hotel, all in rooms at the same hotel. It is probably not that difficult to move from one room to the next. This is the idea that makes us believe, or even be quite certain, that the new virus originated in the animal world. A virus doesn't care whether it has to multiply in a human cell, or a cell in the body of a dog, a cow or a bat. They are all quite alike. This step only requires minor ~~adjustments~~ adaptations by the virus. To use our example: sometimes a room switch takes place. There are four coronaviruses in humans that have done this before - take a look below. HCoV is used to denote a human coronavirus, plus a code to signify the type. We are quite certain that these four viruses made the jump from the animal world, some probably many centuries ago. Related viruses are present in cows, for example, and in dromedaries, and also in abundance in all those bat species. And there are many species! What do these viruses cause? Common colds. Fifteen percent of human colds can be traced back to these four human coronaviruses, and recur every winter. In addition, there are those dangerous coronaviruses such as the first SARS virus. This is the SARS coronavirus that appeared in Asia in 2003. It was probably transmitted to humans by civets, this animal here. But there are reliable indications that the original source was a species of bat. Nine years later, in 2012, the MERS coronavirus appeared. A virus that was transmitted by dromedaries in the Middle East, and is actually still doing the rounds eight years after it was first discovered. There are new introductions all the time, whereby dromedaries infect people. This virus is not very strong and doesn't spread very efficiently, but because it keeps entering the human population it is still an ongoing issue. Finally, there is the new virus we have officially started calling SARS coronavirus 2. Precisely because it is so ~~incredibly~~ similar to the first SARS virus. Eighty percent of the genetic information of these two viruses is identical, a pretty close match. There is no agreement on the source of this virus yet. This animal, a so-called pangolin, was among those mentioned. A virus was found in this animal which is 96 percent identical to the new human SARS virus. Very similar, but it could also be that this is an intermediate host; that this animal picked up the virus from bats for example, and has only transmitted it to humans. These viruses are much less harmless and, in the worst case, could cause pneumonia. Although it should be stressed that 80 percent of people who contract the SARS coronavirus 2 will only suffer from a mild infection that passes without too many complications. Let's take a look at a comparison of the three outbreaks. This is the first twelve months after discovery of the three viruses I mentioned earlier. This is the MERS coronavirus, which only amounted to 108 infections over a year. But this virus is still ongoing. The SARS virus 1 in 2003: eight thousand infections in six months, eight hundred deaths and contained using the same measures we are trying now - isolation and quarantine. Now, the outbreak of the new virus. This image is a little out of date; by now, there are almost 120,000 infected people in about two months. It is this strongly rising curve in particular that is different from the first SARS outbreak and poses the biggest worry. As virologists, we look into the genetic information of viruses. The coronaviruses can be structured into a family tree; here it is, with the three isolates of this new Wuhan coronavirus in red. Wuhan is the location where the outbreak

started. You can see these are very close together, indicating this was a single transmission. A jump from an animal host to humans and then a spread within the human population. This family tree also shows that the first SARS coronavirus, at the top in blue, is placed relatively ~~near~~ close to the new virus. Much nearer than the MERS virus, in any case. This is also new; it was not around in 2003. These websites show you almost in real time where the virus is and how it spreads. This image from 19 February is around three weeks old, and we can see that the issue was mainly centred in Asia, around Wuhan, and there were several red dots in Europe and North America. This is last night's image: 118,252 infections. And you'll see that Europe, North America and the Middle East are quite full of red dots, unfortunately. You will most likely have heard the story through the media. The media are playing an important role in this outbreak. Reporting is in real time. The first outbreak, the continuation and the conclusion that we could've learned more from the first SARS outbreak in 2003. Even conspiracy theories that us virologists ~~are not expected~~do not have to take too seriously, luckily. Of course, it is important to realise that if a virus like this is spreading, an invisible virus, a virus that could potentially kill you, a virus that flies through the air, that this could create all kinds of anxiety. This might be a little over the top, but it is very normal that people are worried. And that's why it might be useful if the second part of our story looks at that invisible threat and makes it visible for a change. It's something we can do in the laboratory. Techniques have been developed that can visualise this miniscule globule and what it does to a cell. It all starts with a cell layer. We do not use patients or animals for our work, we use cultivated cells. Cells that have been grown in a bottle, on some kind of tasty medium. This creates a nice cell layer on the bottom of the bottle. These cells can be infected with virus particles and we can then take a look at what happens in the cells, using microscopy for instance. Two types of microscopy are important in this story. We have the so-called fluorescence~~t~~ microscopy, where we work with fluorescent labels. It allows us to make parts of the virus visible using a fluorescence microscope. This is really just a light microscope. Then we also have the electron microscope. This is a much bigger piece of equipment that does not work with a light beam~~s~~, but uses an electron beam. The difference is that we can achieve much higher magnification so that virus particles become clearly visible. We have been using this method since the first SARS outbreak in 2003, together with our colleagues from the LUMC electron microscopy section at the department of Cell and Chemical Biology. Let's take a look at the steps in the lifecycle of the virus. It starts with the entrance of the virus particle, in the corner here, and if we zoom in to this corner of the cell, we can see that the spikes are not just on the virus surface, but also on the cell itself. The cell membrane around the cell contains various proteins that protrude and fulfil many functions. One of those proteins is used by the virus to gain entrance. The virus binds to the protein, and abuses~~along~~ the endocytosis route I showed you earlier - the route to get in. Little sacks called vesicles are formed and pinched off, they enter the cell and the virus particle enters inside such a vesicle. Then, the Spike protein from the virus works together with these receptors to organise a fusion. A fusion whereby the membrane of the virus ~~combines~~ merges with the membrane ~~is~~ of this transportation vesicle. Once this happens, the viral genome is released into the cell: the first important step by the virus. Take a look at this electron microscopic image where this process is visible. These are coronavirus particles stuck to the ~~open~~ cell surface as shown just now. Next step. The virus comes to life. The virus starts to use its genetic information to reprogram the cell. This means the viral genome has to produce proteins. These black structures are ribosomes: the cell's protein factories. There are attracted to the viral genome and start to read the information inside, leading to a major restructuring of the cell. The first thing that's made is a type of viral copying machine. 16 viral proteins working together to start copying the viral~~us's~~ genome. They are not just swimming around in the cell; they produce special structures in the cell that are needed to make this process efficient. The electron microscope is great for nice images of these structures. This is a healthy cell. It might look quite messy to you already! But it's not as bad as it seems. In fact, it looks

great: this cell is doing everything it should be doing. But if we infect this cell with the SARS coronavirus and check again seven hours later, we see this: Many new membrane structures: a lot has clearly changed and this cell is already falling ill, as you can imagine. This is the viral copier that's going to turn out many new copies of the genome. These are needed because we want to start making new virus particles, and ~~each~~ a new genome copy will have to be packaged inside each new virus particle. So, we will also need new packaging material. You'll recall that the virus particle had those N, M and S proteins, so they need to be made. This happens in the next phase. The N protein is made, swims through the cell and finds the new genome to form a new capsid. What's more, the M and S proteins are being pre-sorted to these membrane structures in the cell where the virus particles are going to be made. Take a look at the fluorescence microscopy.
N protein in green. Throughout the entire cell, except for the nucleus. The purple structure. This is where you DNA is stored. This virus will not invade the cell's nucleus, it does not need the nucleus. Multiplication takes place in what we call the cell's cytoplasm. This cytoplasm contains these membrane structures where the M and S proteins will start to gather. They amass there and wait until the capsid~~ule~~ with the new genome passes by so they can form a virus particle. This is the last phase of the infection, as I showed you earlier. This is how the virus entered and in fact, the creation of new virus particles is actually a reversal of this route. We reverse the arrows and the new viral capsid is able to detect a build-up of these M and S proteins in the membranes and bind, then part of this transport~~ation~~ vesicle is folded around the virus particle. In other words: the virus steals its membrane from the host cell. The virus particle ends up in this transport~~ation~~ vesicle and then leaves the cell along the flow. The last image shows how exuberant this process can be. Thousands of new virus particles can be released from a single infected cell. This only takes about twelve hours. It is no surprise that the cell meets a tragic end. This is the entire cycle once more, from entering and copying to creating new virus particles and finally the release of those particles. Everything in the cell is used for this purpose, the cell is exhausted. And if we look at the same cell twelve hours later, we can see that not much is left of the beautiful, original cell layer. This explains why these cells become ill, why your body becomes ill from this virus infection. Finally, the most important thing is to consider what you can do to stop this outbreak and this infection. I would suggest four steps; delay, prevent, treat and prepare. Just a quick rundown.

Unfortunately, this doesn't work. There are no antivirus programmes available that can easily eliminate this virus, but it is possible to delay it. A delay is very important, because we want to prevent this virus from making all of us ill at the same time. It would disrupt our society even more than is the case at the moment. This involves quarantine, finding infected patients, isolating patients and ensuring that the virus can only spread slowly. And maybe skip cruise ships altogether. It goes without saying that the hygiene I mentioned before - the use of soap and alcohol - is an important part of these tactics for limiting the spread of a virus. We no longer shake hands at the LUMC and we make sure that we avoid infecting one another accidentally by temporarily cancelling meetings, especially with external people, and to move more towards these online presentations. Prevention is about vaccination. We can ~~generate~~ induce an~~the~~ immune response in advance. It can offer protection against these types of viral infections, but it only works if a good vaccine is available, which is currently not the case. Those spikes that protrude from the virus particle's surface are important in the strategy to create a vaccine. They not only bind to the host itself, but they are also the main target of the immune response. If these spikes can be ~~duplicated~~administered upfront, the result will probably be antibodies that offer protection when the real virus arrives. An important strategy that is being developed from many different angles to try to produce a vaccine as soon as possible. Treatment is also important. For example, if people are already infected, it is very useful to block a virus ~~replica~~ using small molecules - medication - that are able to block a specific step in virus

replication. For instance, the fusion: the moment the virus particle enters the cell. If you could block this step and use a specific medicinal product, you have made significant progress. By the same token, the copying machine - those sixteen proteins that together copy the viral genome - are also a target for which very specific inhibitors could be developed that are able to block multiplication, probably without too many side effects. Finally, preparednessation is always important. After all, there are hundreds of coronaviruses and it is extremely unlikely this is the last one to move onjump to humans. This virus could come back once it is under control. There could be other coronaviruses that are trying to get lucky. We are better off being prepared by having strategies in place to combat these types of viruses. At this point I have to make a reference to Darwin and his theory of evolution: survival of the fittest. I'm sure you've heard this phrase, and it is very apt for these types of viruses. They are constantly making coincidental random mutations. The positive mutations will be selected and stored retained within the virus population. Viruses change, so if a vaccine is found, the virus will start to adapt. It will make small changes to the spike proteins, for example, so that the vaccine becomes less effective and has to be adjusted. If we develop virus inhibitors, chancges are that resistance develops against the particular inhibitors. This can only be tackled by using a combination of several virus scannersinhibitors, minimising the chances of resistance against all substances. Viruses change, and coronaviruses are no exception. I think we have to be strong right now to combat this outbreak in many ways. I hope this explanation was helpful, and I wish you all the best. Thank you for your time.