At the Sharp End

TEXT **INGA OLFEN**

High tech is coming to the fore in the struggle against viruses and bacteria. Modern vaccines transport genetic information from pathogens into target human cells in order to trigger an immune reaction there. The transportation of these "blueprints" requires tiny lipid nanoparticles of the kind that Evonik is producing in Canada

"The Covid-19 pandemic is a catalyst for the development of gene-based vaccines"

STEFAN RANDL, HEAD OF RESEARCH, DEVELOPMENT, AND INNOVATION, EVONIK HEALTH CARE BUSINESS LINE

When the country doctor Edward Jenner took a knife to his gardener's son in the English county of Gloucestershire on May 14, 1796, he could not have suspected that he was starting a medical revolution. After making an incision in the healthy eight-year-old boy's skin, Jenner rubbed into it some pus taken from a milkmaid who was sick with cowpox. One week later, the child had fever and a headache, as well as small blisters at the places that Jenner had infected. A few days later, these symptoms disappeared.

Six weeks later, the doctor repeated this procedure, this time with secretions from a patient suffering from smallpox, an illness that was killing 400,000 people a year in Europe during the 18th century. The boy developed no symptoms of illness whatsoever: Vaccination had been invented. Jenner called his process vaccination, a word derived from *vacca*, the Latin word for cow. The scientific term "vaccine" is also derived from this Latin word.

Today, a good two centuries after the invention of vaccine, scientists are researching completely new types of vaccine technology. This time, they are not working behind the doors of a country doctor's small office; instead, they are doing their work in full view of the international public. They are driven by a pandemic caused by a virus that can be deadly: the coronavirus SARS-CoV-2. They are working under tremendous time pressure. "Until just a few years ago, people estimated that it would take around 15 to 20 years to go from the analysis of a virus to the approval of a vaccine," explains Stefan Randl. As the head of research, development, and innovation at Evonik's Health Care business line, he's familiar with the challenges along the way toward new or improved medicines and vaccine serums. "Empirical data and very new technologies, such as gene-based vaccines, can significantly accelerate the process," he adds.

Evonik, as a contract development and manufacturing organization for the pharmaceutical industry, is also part of this effort. As a specialty chemicals company, it has the expertise and the technology that are needed for the development and manufacture of complex and highly specialized injectable drug products. These products can require drug delivery technologies such as lipid nanoparticles (LNPs), which are a hundred times smaller than a human blood cell and play a very important role when it's necessary to encapsulate unstable active ingredients and release them at exactly the right place in the human body. "Today, when we're discussing topics such as gene-based vaccines that have much shorter development times yet are more effective than conventional serums, lipid nanoparticles play a central role," Randl says.

By producing lipid nanoparticles, the chemical industry is making an important contribution to the development of the vaccines of the future, says Professor →

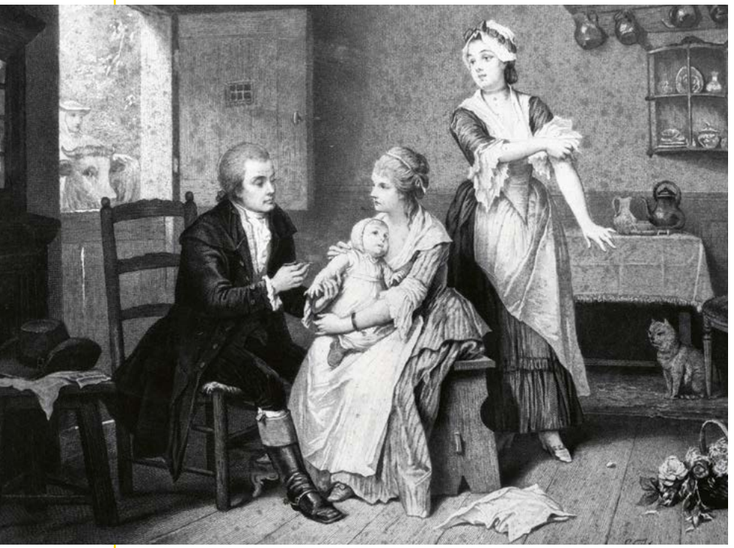
Hartmut Hengel. “The substances we call transfection reagents play a crucial role in the effectiveness of vaccines,” says Hengel, who is the medical director of the Institute of Virology at the Freiburg University Medical Center and the deputy chairperson of the Scientific Advisory Board of the Paul-Ehrlich-Institut (see the complete interview starting on page 15). “These reagents determine which cells the vaccine penetrates and how effective and stable it is.”

Milestones of vaccination history

Vaccines are considered the greatest achievements in the struggle against viruses and bacteria. On the following pages we present the most important challenges and successes of medicine in the past two centuries

1796 SMALLPOX

PATHOGEN *Orthopoxvirus variolae* **FIRST VACCINATION** 1796 in England (image: Edward Jenner) **VACCINATION TYPE** Injection of cowpox lymph; it was followed later on in Germany by an attenuated vaccine containing the *Vaccinia* virus, which is closely related to *Variola.* **HISTORY** Between 15 and 30 percent of infected individuals died. After a smallpox epidemic that killed 125,000 people, the German Reich passed a law in 1874 to the effect that children must be vaccinated in their first and twelfth years. Since 1980, smallpox has been considered eradicated worldwide.



FROM SMALLPOX VIA MEASLES TO THE CORONAVIRUS

Ever since Edward Jenner’s pioneering work, some of the worst threats to human health have been mitigated or even eliminated through vaccination. That includes devastating diseases such as rabies, plague, diphtheria, and tuberculosis. For example, thanks to worldwide vaccination programs, smallpox has been considered eradicated since 1980. In 2002 the World Health Organization (WHO) declared that Europe was “polio-free,” and a few weeks ago it extended that declaration to include the continent of Africa.

The WHO had also sought to eradicate the measles virus by 2020. Measles can lead to serious complications, including blindness or fatal meningitis, especially in children younger than five. About 2.6 million people died of measles worldwide every year before the first vaccination became available in 1964. After that, the figures rapidly decreased—until 2016. Unfortunately, since then the illness has once again been gaining ground. In 2019 the WHO sounded the alarm: The number of registered cases of measles had increased by 700 percent in Africa and by about 300 percent in Europe in a single year. Many people who live in poor regions have no access to vaccines, but the spread of measles in rich countries is primarily due to growing skepticism about vaccination. In reaction to these figures, mandatory measles vaccination was introduced in Germany in March 2020.

In the struggle against the novel coronavirus, there are no plans for mandatory vaccination, even though fears about such a mandate are being voiced by vaccination skeptics and believers in conspiracy theories. At the moment the focus is on the search for a suitable vaccine and on the associated hopes for ending the pandemic as soon as possible. “There’s a lot of optimism,” said Professor Klaus Cichutek, the president of the Paul-Ehrlich-Institut (PEI—the Federal Institute for Vaccines and Biomedicines), which is responsible for approving vaccines in Germany, back in August. The initial results of ongoing studies have shown “that some vaccines can actually induce a specific immune reaction in human beings against SARS-CoV-2,” he said.

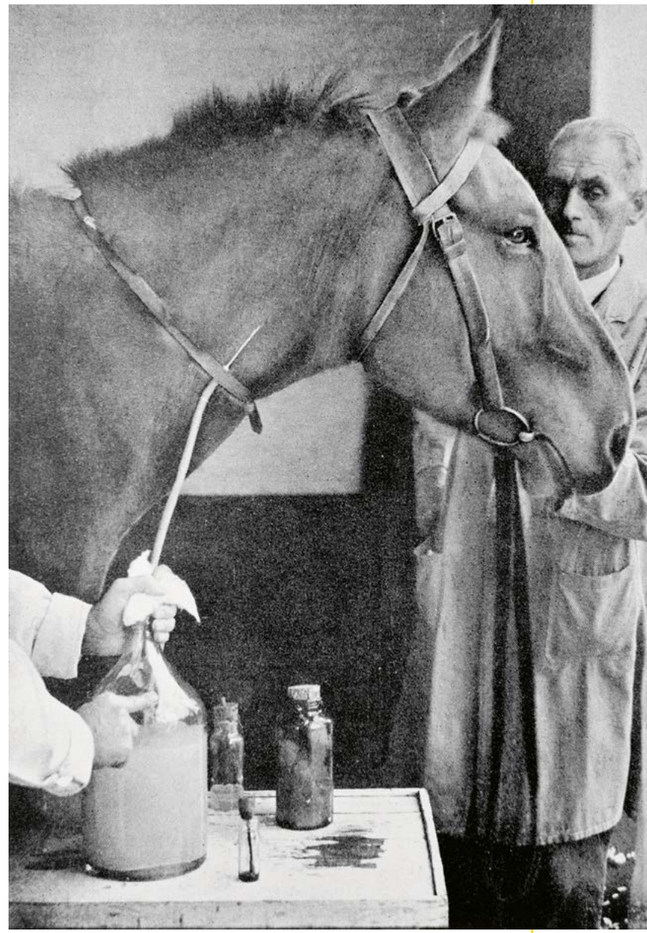
The new vaccine technologies are based on the classic processes that make use of the memory of our immune system. When it is invaded by a virus or a bacterium, the immune system reacts by forming antibodies. The information about these antibodies remains stored in special white blood cells. If there is a new infection by the same pathogen, these white blood cells can produce these antibodies very quickly and render the intruders harmless (see the diagram on pages 20/21).

As a rule, a vaccination actively introduces pathogens into the human body in order to stimulate the immune system to form antibodies. This is usually done by means of an injection into a muscle or subcutaneously. We distinguish between various classes of vaccine.

Live vaccines contain viruses or bacteria that have been attenuated to such an extent that they can still reproduce themselves but can no longer cause the disease. The protection afforded by a live vaccine lasts for many years. Examples include the live vaccines against measles, mumps, rubella, and chickenpox.

In inactivated or killed vaccines, the pathogen is first killed, so that it is unable to reproduce itself and cause the disease. In this case the protection gradually decreases and must therefore be regularly renewed with a booster shot. Vaccines of this type protect the recipients from polio, tick-borne encephalitis (TBE), and hepatitis B.

Other vaccines, such as those against tetanus, diphtheria, whooping cough, and the flu, only contain components of the pathogen, such as proteins or sugars that are recognized by our immune system. These vaccines also protect recipients for only a limited period of time. →



DIPHTHERIA 1925

PATHOGEN *Corynebacterium diphtheriae.* The symptoms are caused by a toxin made by the bacterium. **FIRST VACCINATION** In 1925 in Germany (approval: 1936), Emil von Behring (photo) and Erick Wernicke discovered a new method of immunization for treating infectious diseases. After the publication of the paper “On the Origin of Diphtheria Immunity and Tetanus Immunity in Animals,” it took only four years for the industrial production of diphtheria antitoxin serum to begin. **VACCINATION TYPE** Patients were injected with blood serum from infected animals that had already formed antibodies. Today the vaccine contains inactivated diphtheria toxin. **HISTORY** Between 1881 and 1886, an average of 25,000 infants and toddlers under the age of three died annually in Prussia as a result of the infection. Diphtheria was the most frequent cause of death among children between the ages of three and five. Today 97 percent of infants and preschool children in Germany are protected, thanks to a combined vaccination during their first year of life.

1955 POLIOMYELITIS

PATHOGEN Poliovirus **FIRST VACCINATION** 1955 with inactivated vaccine, 1961 with attenuated vaccine **VACCINATION TYPE** Inactivated polio vaccine as a killed vaccine, initially usually administered orally (photo: Oral vaccination in Stuttgart, 1962), but solely as an intramuscular injection since 1998. **HISTORY** After 1880, poliomyelitis occurred as an epidemic that sickened thousands of people annually. It primarily affected children, who either died or suffered from lifelong physical disabilities ("infantile paralysis"). Starting about 1910, regional epidemics were observed in Europe and the USA at intervals of approximately five or six years. As recently as 1961, a total of 4,670 new infections were registered in Germany; in 1965, only a few years after the start of the first vaccination campaigns, that figure was less than 50, representing a decrease of 99 percent.

GENE-BASED VACCINES OFFER HOPE

The time to develop traditional vaccines can typically take several years as a general rule. For one thing, large amounts of virus material are required. For another, the large-scale production of a vaccine requires a lot of time and effort. "For example, in the case of inactivated vaccines the pathogens have to be precisely specified under strict safety conditions," explains PEI President Cichutek. "Next, the strain is produced, cultured in large amounts, and only then inactivated."

This is why the researchers who are searching for effective protection against the coronavirus as well as diseases such as AIDS and certain types of cancer have been focusing in recent years on completely new candidates: gene-based vaccines that contain not the virus itself but only a blueprint that the human body can use in order to produce exactly that part of the virus that triggers the immune response. For example, in the case of the coronavirus this part could be the "spike protein" on the virus envelope. Serums of this kind can be produced in large amounts relatively quickly, and if the pathogens should mutate the serums can be adapted as necessary.

Vector vaccines are a variant of these innovative vaccines. In vector vaccines, genetic material of the pathogen is inserted into harmless More on page 16 →

"This is a marathon, not a sprint"

The Freiburg-based virologist Professor Hartmut Hengel talks about the prospects for the rapid development of a vaccine against the coronavirus, the advantages of RNA technology, and the role of the chemical industry in new vaccination procedures

INTERVIEW **INGA OLFEN**

Prof. Hartmut Hengel is the medical director of the Institute of Virology at the Freiburg University Medical Center and the deputy chairman of the Scientific Advisory Board of the Paul-Ehrlich-Institut

Professor Hengel, in the discussion about a coronavirus vaccine, more and more skeptics are speaking up. They're saying that vaccinations have caused cancer and other diseases, overburdened the immune system, etc. Is there any truth to these assertions?
All of them can be scientifically refuted. Basically, even higher safety requirements apply to vaccines than to medications, because vaccines are administered to healthy people. Modern research is making huge efforts to offer vaccines that have a minimum of side effects. However, in the past some vaccines have in fact caused undesired effects. That's why comprehensive safety testing is so important.

That takes time, and during the battle against the coronavirus pandemic time is in especially short supply. What shortcuts could be taken so that we can administer a proven vaccine to end the pandemic as soon as possible?
Each society has to answer for itself the question of how to deal with this process at the ethical and political levels. In my opinion, we have to proceed carefully and conduct stringent testing. I think it would be not only risky but also unethical to administer a vaccine that has not been properly tested to the general population. You first have to investigate how long the protection will last and the safety aspects over time. Especially in cases where side effects occur only very rarely, these side effects might be seen only years later.

Many fears are related to gene-based vaccines, which are currently going through several approval processes. There are fears that they could alter the genetic material of the vaccinated individuals. Are these fears justified?
The vaccines that are currently being discussed in connection with SARS-CoV-2—the novel coronavirus—are primarily vaccines whose mechanism of action is based on ribonucleic acid, or RNA. The RNA converts genetic information into proteins, and—according to everything we know—it is not integrated into the genetic material of human beings. I have no fears of that.

One of the reasons why RNA-based vaccines look so attractive is that they can be produced in large quantities in a very short time. How soon will a vaccine be available?
In the case of a few vaccine candidates, we're hoping that one or more of them may still be approved in 2020. However, we can only arrive at a comprehensive safety assessment in the course of a vaccine's actual use. →

In the case of other viral diseases such as measles, it's known that 95 percent of the population must be immune in order for unvaccinated individuals to also be protected. Is that also true of the coronavirus?
We don't know. One difference between the measles virus and SARS-CoV-2 is that there are people who were infected with the coronavirus but did not produce any antibodies. It's even possible that herd immunity against SARS-CoV-2 will fail to materialize because coronaviruses are programmed for reinfection. If we succeed in developing vaccines that lack the functions of the virus that are responsible for "immune evasion," a vaccination could even protect us more effectively than a previous infection.

What role is the chemical industry playing in the development of potent vaccines?
A very important role. For example, take the lipid nanoparticles that are used in mRNA vaccines. Transfection reagents of this kind are a key determinant of the effectiveness of the vaccines, because they enable the mRNA to be transferred into the cells. They determine which cells the vaccine can penetrate, as well as how effective and stable it will be. If this method is successful against the SARS-CoV-2 virus, it would be an entry point into a new class of vaccines, and maybe even into completely new principles of vaccination.

Are you saying that this technology could solve problems for which there is no solution at present?
Absolutely! I can even imagine that in the future we will produce cocktails of messenger RNAs and thus combine multiple vaccinations. That would make it possible to generate much higher levels of immunity with far fewer individual vaccinations. It would be a big step forward.

Will there one day be vaccinations against all diseases, ranging from cancer to Parkinson's disease and all the way to diabetes?
It would be naive to believe that through vaccinations we can eliminate all diseases. However, I expect that in the future we will be able to block more infectious diseases by means of vaccinations. But we have to be patient. That's why I'm not happy at all about the current talk about a "race" to develop a vaccine against SARS-CoV-2. The winner is not necessarily the one who starts out fastest. This is a marathon, not a sprint.

"The lipid nanoparticles have to be put together out of a large number of different components"

JAY NATARAJAN, HEAD OF LIPID RESEARCH AT EVONIK IN VANCOUVER, CANADA

carrier viruses (such as the virus used for measles vaccine or attenuated adenoviruses). Initial vaccines against dengue fever and Ebola have already been approved. And Russia already dashed forward in August and produced a vector serum against Covid-19.

Scientists believe that additional opportunities are offered by vaccines based on nucleic acids, which are the carriers of genetic information inside cells in the form of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). Because the production of DNA or RNA vaccines requires not the entire virus but only its genetic material, it is much easier and faster than the production of other types of vaccine. Dozens of studies are now being conducted in this area. However, by mid-October no vaccines against Covid-19 had been approved for use on human beings.

In the case of DNA vaccines, the sequence of the desired antigen is inserted into the genetic information of a bacterium. After the bacterium has entered the target cell, this information is read in the cell's nucleus and the antigen is produced directly inside the cell. Researchers have already been working on these vaccines for many years. Pharmaceutical companies are currently working on DNA vaccines against about 20 diseases including rabies, leukemia, and AIDS. So far, the possibility of foreign DNA being inserted into human genetic information, which in the worst case could lead to increased tumor formation, has not been documented in any studies. "We have spent many long decades investigating a theoretical risk that might be harbored by DNA vaccines, but in fact this risk has never materialized in animal testing or in clinical trials," said PEI President Cichutek at a press briefing in April 2020 in order to calm any fears.

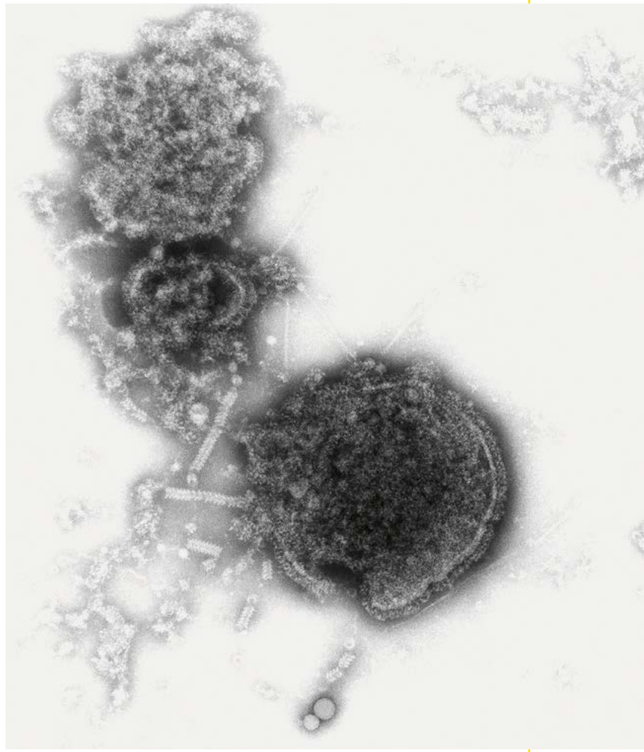
In order to completely exclude this risk, there is the option to use not the entire DNA of a protein but only its mRNA, or messenger RNA. A protein's mRNA is basically a copy of its blueprint, which is read out from the DNA. The mRNA transports this blueprint directly to those places in the cell where the desired protein is produced. In other words, it is not incorporated into the cell's nucleus, and thus it cannot be inserted into the DNA there.

THE EPICENTER FOR LIPID FORMULATIONS

However, in order to unfold its effect the mRNA must first reach the right place inside the human body. "For a long time this was a big problem for scientists, because it is a very unstable construct," explains Stefan Randl. This is where the lipid nanoparticles from Evonik—ultrafine particles of fats and waxes—once again come into play. "If I were to inject mRNA without having previously formulated it—in other words, without having packaged it inside a protective layer—it would disintegrate in the bloodstream within seconds."

Evonik produces lipid nanoparticles and complete mRNA serums at a facility in the town of Burnaby near Vancouver, Canada. In 2016 Evonik expanded its portfolio for advanced drug delivery to include the development and production of liposomal formulation technologies by acquiring the local company Transferra Nanosciences. "Vancouver is an epicenter for the development and the production of LNPs," says Randl. Research on lipid nanoparticles has been conducted there for almost 30 years. "The researchers have already developed hundreds of LNP formulations for gene-based and cell-based therapies, and they are connected with pharmaceutical and biotech companies all over the world." A whole series of the LNP-based drug products that have already been approved or are currently in development have been supported either by Evonik or, before that, by Transferra Nanosciences.

Today LNP technology is regarded as the "gold standard" for the development of complex parenteral medications—in other words, those that are admin- →



MEASLES 1963

PATHOGEN *Measles morbillivirus* **FIRST VACCINATION** 1963 in the USA **VACCINATION TYPE** Initially an inactivated vaccine, since 1968 an attenuated vaccine **HISTORY** Before a vaccine was developed, about 2.6 million people died annually worldwide; vaccination campaigns helped to reduce the number of deaths due to measles by 84 percent worldwide—from over 500,000 to around 90,000—between 2000 and 2016. Since 2018 a massive increase in cases of measles has been seen again in Europe, with more than 100 deaths between January 2018 and June 2019. Measles can be regarded as "eliminated" only after at least 95 percent of a population is immune. Vaccination against measles has been mandatory in Germany since March 2020.

istered via injection—against diseases such as cancer or amyloidosis, which is triggered by protein deposits in the body and can lead to organ dysfunction. This was the first application of an RNA-based therapy. "Certain combinations of active ingredients, as well as personalized medications, would also be unthinkable without LNPs," Randl adds. In the future, serums based on lipid nanoparticles could play an important role in the market for vaccines and many therapeutic drug products.

By means of its highly specialized and complex production processes for LNP-based medicines, Evonik develops formulations for pharmaceutical companies in Vancouver from start to finish. "For example, the customer sends us the mRNA, and we then conduct research in order to find out the proportions in which lipids must be mixed with other ingredients," says Jay Natarajan, the head of research in Burnaby. The tiny lipid particles have to protect the nucleic acids from destructive enzymes and thus enable them to pass through the cell membrane.

LAYERED LIKE AN ONION

"To make sure the mRNA safely reaches its target, the LNPs themselves have to consist of many different lipid and buffer components, so there's a long list of ingredients," explains Natarajan. The lipid ingredients are first dissolved using ethanol and combined with the mRNA, which has been dissolved in a buffer solution. This is done using a very rapid micromixing process that creates lipid nanoparticles encapsulating the mRNA just like the layers of an onion. These lipid nanoparticles are then subjected to a downstream purification process to form a final drug product that is ready for clinical trials on humans.

When the particles reach the target cells, they fuse with the cell membrane and release the mRNA into the cell exactly where it is needed. There the information that is required to manufacture the desired protein is read out, and the production of the antigens begins.

As soon as the right formulation for the customer's mRNA has been determined in Burnaby, serums can be produced in amounts sufficient for reaching Phase I /II of clinical testing. Looking ahead, Evonik is planning future operations that go beyond this stage. The laboratories at the company's location in Birmingham, Alabama have the capability to produce larger batches. The company has already developed and produced drugs based on bioresorbable polymer microparticles.

Incidentally, the story of the little boy in England had a good ending. Not only did the vaccination make him immune to smallpox—to show his gratitude, the country doctor Edward Jenner later on gave him a cottage to live in with his family. Eventually, this house became the first Jenner Museum.

2015 EBOLA

PATHOGEN Ebola virus from the family *Filoviridae* **FIRST VACCINATION** 2015, approved by the EMA at the end of 2019 **VACCINATION TYPE** The vector vaccination VSV-EBOV is a combination of different variants of a viral vector that is based on the *Vesicular stomatitis virus* (VSV) (photography: Vaccination in Conakry/Guinea 2015). In order to create the vaccine, a gene from the Ebola virus is inserted into the genome of the VSV virus; this gene encodes the viral glycoprotein (GP) of the Ebola virus. **HISTORY** A large percentage of people infected with this virus die from it. Precise numbers are hard to determine. It is assumed that between 30 and 90 percent of infected individuals die, depending on the severity of the outbreak. The average mortality is estimated to be 50 percent.

COVID-19 2020

PATHOGEN SARS-CoV-2 **FIRST VACCINATION** The first vaccinations reportedly took place in China and Russia in the fall of 2020. A comprehensively tested vaccine is not expected to be available before the spring of 2021. **VACCINATION TYPE** Vector vaccination, inoculation with DNA or mRNA **HISTORY** The initial cases of patients infected with this "novel coronavirus" occurred at the end of 2019. Originating in China, the virus has spread throughout the world since the spring of 2020. By mid-October 2020, around 37 million people were infected and more than one million patients had died. According to the World Health Organization (WHO), more than 100 vaccines against Covid-19 are currently in development.

Inga Olfen is a science journalist based in Hamburg. She has a degree in biology and previously worked for eight years as an editor in the science department of *Stern* magazine. She founded the communications agency Kontenta in 2017.