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N° 11 — September 2021

Messenger RNA: The Outsiders Strike Back

by Fabrice Delaye

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Messenger RNA: The Outsiders Strike Back

by Fabrice Delaye

Explorations magazine
A publication of Heidi Media SA

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Content

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A word from the editor

An exceptional 11th edition

By Tibère Adler,
Editor and Director
of the publication

The magazine you're now holding is truly exceptional. Fabrice Delaye, one of the stalwarts of the Heidi.news team, tells the story of the emergence of messenger RNA (mRNA), the discovery that enabled anti-Covid vaccines to be created in record time. He has managed to get some of the key players from all over the world to tell their stories. The sheer richness of these personal testimonies and the narrative quality of this scientific adventure have already prompted admiration and wonder. Indeed, several French and English-speaking publishers are already queuing up to bring out a book based on this report.

Heidi.news is primarily a digital medium. From the outset, though, the media team wanted some of its best stories, reports and investigations to appear in print. The rationale behind our printed magazines has therefore been to enable the articles to leave a longer-lasting impression and to allow them to exist physically in space (there's nothing quite like holding a beautifully produced magazine). And a printed magazine also makes it more comfortable for our readers to enjoy a long and well-written story. Every three months, we select an Exploration piece (a long story broken down into episodes) that we feel is worthy of being printed. As time has gone on and the number of Explorations on the website *www.heidi.news* has grown, the competition has become ruthless!

But the choice for this eleventh edition was an easy one: it was impossible not to share the Exploration piece entitled "Messenger RNA: the Outsiders Strike Back" in print with our readers. The distribution of this magazine also marks a fundamental step in the development of *Heidi.news*. Two years after our launch (May 2019), most of the capital of our publishing company (Heidi Media SA) was bought on 18 May by the publishing company of *Le Temps*. The operation was financed by the Aventinus Foundation, which has owned *Le Temps* since January 2021. The Aventinus Foundation was created with the sole aim of supporting and financially encouraging the quality press in French-speaking Switzerland. The foundation is recognised as being of public utility and without profit-making objectives. It also has a long-term vocation: to enable the media it supports to find their own economic equilibrium in the medium term. It is therefore a great recognition of the work of the *Heidi.news* team for it to be able to join forces with the newspaper *Le Temps* in this exciting way. But it is the subscriptions and support of our readers that will continue to be the backbone of *Heidi.news*' future. So thank you to you, our readers and subscribers. Without you, none of this would be possible. And we hope you'll find this edition both interesting and stimulating.

Editorial

A scientific adventure

By Serge Michel,
Editor-in-Chief
of Heidi.news

What springs to mind when you hear the words 'scientific adventure'? Do you imagine Louis Pasteur poring over his microscope or Thomas Edison hunched over his telegraph? Maybe you picture Albert Einstein in his small flat in Bern working on his theory of special relativity or Marie Curie in Paris discovering radium in her makeshift laboratory at the *École municipale de physique et de chimie industrielles*?

These and others became icons, and were honoured with the first Nobel prizes. But are scientific adventures still possible in the 21st century, and if so just how do they look? You'll find the answers to these questions in the following pages because Fabrice Delaye's investigation describes a very contemporary scientific adventure. He wanted to understand how two laboratories managed to come up with an effective vaccine against Covid-19 in just ten months when such an exercise normally takes ten years.

It all began in 1961 with the discovery of mRNA by Jacques Monod and François Jacob at the Pasteur Institute. This groundwork would be continued in several places, not just in the major American universities but also, at the end of the 1970s, in the small Hungarian town of Szeged by Katalin Karikó, a butcher's daughter.

Fairly soon, hundreds of researchers who had studied mRNA became fascinated by how it worked. But how could it be used and translated from research into technology? What would be its first use as a treatment? Meanwhile another adventure was starting, one that would see reputations and fortunes made and lost.

Enter Robert Malone. This American was the main author of a 1989 publication on the possibility of transferring liposome-protected mRNA into cultured cells to provide the information needed for protein production. Malone's employer, the renowned Salk Institute, was unconvinced, though, and dropped his patent applications, thus depriving him of fame and fortune. Consumed with bitterness, Malone is now feeding conspiracy theories to the second-rate media.

But in the meantime, two pharmaceutical companies have done extremely well. Their earnings are commensurate with the achievement: Moderna is forecasting $30 billion in sales in 2022 and Pfizer $56 billion. But that's just the beginning: mRNA is a medical revolution that opens up endless possibilities for treating many diseases. And so the adventure goes on.

Foreword

Unlocking human capital

By Sébastien Eisinger,
Managing Partner,
Pictet Group

Never underestimate the power of human ingenuity. This has been Pictet's guiding philosophy since our founding in 1805. Over that time, we have placed our faith in the world-changing ambitions of countless companies and individuals. Doing so has served our clients well.

There have been several instances in our history, however, when we have found ourselves genuinely taken aback by humanity's capacity for innovation. Take the past year. The medical breakthroughs achieved during the coronavirus pandemic have been truly awe-inspiring. Developing a vaccine for Covid-19 within months of identifying the disease's genome was a scientific first. What would normally have taken virologists 10 years to complete was accomplished in less than one. Nobody within the medical establishment could have envisaged such rapid progress.

Yet the list of successes does not end there.

There are currently 15 different types of coronavirus vaccine being administered across the globe. And, as I write this, more than four billion people have received at least one inoculation. It is a remarkable medical and logistical feat. To say we are witnessing the modern-day equivalent of the moon landing is no exaggeration.

The events of the past 18 months also remind us that the world's innovators need constant nurturing. Too often, promising technologies are cast aside and visionaries deprived of vital capital because investors balk at the time required for returns to materialise. Such myopia is costly. It means society benefits from only a fraction of humanity's collective ingenuity. Unlocking intellectual capital almost always requires significant resources and patience.

All of which explains why, earlier this year, Pictet chose to take part in a project that sought to shed light on the exhaustive human effort that led to perhaps the most important pandemic-inspired vaccine breakthrough of all: the transformation of messenger ribonucleic acid (mRNA) into a therapeutic superweapon.

You will no doubt already be familiar with mRNA. It is the clever bio-molecular technology behind the Pfizer-Biontech and Moderna Covid vaccines. Its distinguishing feature is that it can instruct the human body to create specific missing – or new – proteins, the essential components of almost every one of our bodily processes. Put simply, mRNA can turn each of us into pharmaceutical factories.

Less familiar, perhaps, is the fascinating story behind the development of mRNA and the promise it holds for medical science.

As you will discover in this detailed account, supported by Pictet and written by health correspondents at Swiss media group Heidi News, mRNA was no overnight success; it was decades in the making. When the world's first mRNA Covid-19 vaccine shot was injected into the arm of the British grandmother and nonagenarian Margaret Keenan in December 2020, it represented the culmination of more than half a century of scientific research. Over those years, many reputations were forged and ruined and large fortunes lost. The history of mRNA is also one of fiercely contested lawsuits, complex patent disputes and numerous missed opportunities.

Its circuitous rise to prominence testifies to the 'human' aspect of scientific discovery – rational at times, defying all logic at others but continuously propelled forward by the willpower of visionaries who see a revolution within a deceptively simple idea.

It is a fair assumption that, by the time you begin to read this report, the scientists behind the mRNA success will have been added to the list of nominees for this year's Nobel Prize for Medicine. An award of this magnitude would seem a fitting tribute to all those who worked tirelessly towards realising the same world-changing ambition. We all look forward to the Nobel committee's decision in November.

Until then, I hope you enjoy reading the stories contained within these pages and that they leave you marvelling at the power and unpredictability of human ingenuity. For, as Albert Einstein famously observed, "Logic will get you from A to B. Imagination will take you everywhere."

$ 30 billion and $ 56 billion

Forecasts for vaccine sales by Moderna and Pfizer, respectively for 2022

10 months

Time between the publication of the sequencing of SARS-CoV-2 by the Chinese authorities and the approval of the first two RNA vaccines in the US

Number of Covid deaths worldwide up to 23 August 2021

4.4 million

Average lifetime of an mRNA molecule in the body if not protected by a small layer of fat

2 minutes

Number of doses of Moderna vaccine that the Lonza plant in Visp aims to produce

800 000

4.7 billion

Number of doses of coronavirus vaccine

1961

Year in which Jacques Monod and François Jacob, both former resistance fighters, **demonstrated the existence of mRNA,** for which they were awarded the Nobel Prize in 1965

11 billion dollars

US government grants and money distributed by the White House's Operation Warp Speed to accelerate the development, manufacture and distribution of Covid-19 vaccines.

Several recipients failed to come up with the goods by 2021, including Sanofi and GlaxoSmithKline

The price paid by both BioNTech and Moderna to

license the University of Pennsylvania patent

on the modified mRNA base by Kati Karikó and Drew Weissman

75 million dollars

Author

Fabrice Delaye, 56, was introduced to the internet on 18 July 1994 on the screens of the inventors of the World Wide Web at CERN. On that same day, NASA released near-live Hubble images of the collision of comet Shoemaker-Levy with the planet Jupiter... It was also the moment when Fabrice's passion for science and technology was born. The internet bubble, which subsequently ballooned, led him to progressively cover not only the digital economy, but also life sciences, renewable energies and, over time, all the sectors transformed by the knowledge and innovation economy. Fabrice worked for *Hebdo* and *Bilan* after being the correspondent for *Agefi* in the US in association with the *MIT Technology Review*. Fabrice is now a reporter at large for *Heidi.news*. A graduate of Sciences-Po Paris and holder of a Master's from EPFL in society, science and technology, he has also written a biography of Patrick Aebischer, EPFL president from 2000 to 2016.

Illustrator

Jean-Philippe Kalonji was born in Geneva and has lived in New York, London and Japan. A painter, illustrator and comic book writer now based in Carouge, Jean-Philippe penned the graphic novel *365 Samurais and a Few Bowls of Rice* in 2009 for Dark Horse Comics in the United States. In addition to his press assignments, he works with humanitarian organisations such as Amnesty International, UNESCO and the ICRC, as well as with other artists such as Wyclef Jean and brands like Thrasher and Burton snowboards, the McLaren Formula 1 team and the Langham Hotel in London.

Introduction

What happens when the jab goes into your arm?

If you haven't already been vaccinated against Covid-19, the chances are you will be soon. If you're living in Switzerland or the United States, it will be an mRNA vaccine – as it has been for millions around the world.

by Fabrice Delaye

Cultural resistance against the vaccine has largely resulted from the apparent speed at which the mRNA vaccine was developed – a mere ten months between the sequencing of the coronavirus in China and the approval of the first mRNA vaccines in the United States. Typically, it takes ten years to develop a vaccine. More than eighty new viruses have been identified since 1980, but far fewer vaccines have been developed in that time. Only a few smart investors saw the potential in this new avenue of mRNA vaccine research.

Unanswered questions

By February 2021 it had become clear that the knowledge and technology needed to bring mRNA vaccines to market could not possibly have been acquired in the short time since Covid-19's emergence. This investigation recounts the story of RNA vaccines as told by the individuals involved in this extraordinary thirty-year enterprise.

Dozens of interviews, mostly conducted via Zoom with scientists in their laboratories or their offices in American universities, revealed a great deal – not least that there was no one parent behind the development of mRNA as a therapeutic tool. It was a collective enterprise between people who were often as close as family. And as with any family, there were arguments, doubts, jealousy and mistakes, leaving a legacy of disputes, many surrounding patenting issues.

Future Nobel laureates

These researchers were mostly molecular biology outsiders – albeit a few pioneers with Nobel prizes among them and others probably destined to become future laureates. The majority, however, suffered the scepticism of their peers, seeing their articles rejected by prestigious journals and their studies snubbed by the pharmaceutical industry. Some have forfeited their careers, other millions of dollars.

The international triumph of mRNA vaccines against Covid-19 has clearly changed their fortunes. The medical revolution heralded by RNA-related technologies extends well beyond vaccines, to treatments for cancer, heart disorders, auto-immune diseases such as multiple sclerosis, and hereditary conditions like cystic fibrosis. It's a medical gold mine and one likely to prove a fierce battleground of intellectual property.

Before launching into the detail of mRNA's development

as a therapy, it's important to first consider the fundamental science behind it – starting with what happens when the vaccine is injected into the shoulder.

The spatial beauty of Calder and Tinguely

To my mind, the process has a spatial beauty reminiscent of the slightly ironic cascading logic of Jean Tinguely's machines and the fragile equilibrium of Alexander Calder's mobiles. Messenger RNA is a monument of Darwinian evolution. Messenger RNA converts the information contained in DNA – the molecule found in nearly every cell, and which holds the genetic blueprint for nearly everything that constitutes life – into myriad proteins that govern the functioning of our cells and, in turn, that of our tissue and organs.

Proteins are responsible for the majority of what goes on inside our bodies. They carry oxygen and nutrients. They produce movement and signals. They sound the alarm, detecting alien invaders and calling up the body's defences. This is the principle behind vaccination – vaccines hack the immune system by imitating a benign attack. A conventional vaccine is a virus that has been neutralised or killed; meeting it in benign form, the immune system learns to recognise and tackle the foreign agent without being put at risk.

"The principle behind vaccination: vaccines hack the immune system by imitating a benign attack. A conventional vaccine is a virus that has been neutralised or killed; meeting it in benign form, the immune system learns to recognise and tackle the foreign agent without being put at risk"

Computer games in the body

Dietmar Hopp, the founder of German tech giant SAP, and Bill Gates, are said to see mRNA as a type of biological software, a programme that can be coded. If that's the case, a good metaphor for how an mRNA vaccine works might be something like a video game.

The hero of the game is a postman (mRNA). His job is to deliver a blueprint to a workshop, for the creation of a molecule capable of detecting Covid-19. This blueprint is tattooed on his skin – a 4000-character sequence containing only four letters. He's also been given a cap for identification purposes, and a hard-wearing suit. The house (or cell) to which he needs to deliver the blueprint is in a dodgy neighborhood bristling with 300 watchdog species (the different ribonucleases) whose instinct is to kill him off. If the postman makes it to his destination – which isn't always the case, so these vaccines contain millions of mRNA postmen – then his suit changes colour (in reality, it changes electrical charge – a miracle of bioengineering as we shall see) to open the door.

But his job isn't finished there. Plenty of dangers lie in wait for our postman inside the house (the cell) too. More watchdogs (interferon) are lurking to tear him to pieces, but his clever tattoos help him give them the slip. The dogs bark, alerting the immune system's police, but they don't bite yet. When he reaches the workshop (the ribosomes), our postman's cap acts as his password.

Now, his assembled blueprint is used as a wanted notice and stuck up in the windows of the house. This alert is like an identikit image of the coronavirus for the police (the immune system). Should the coronavirus appear, the police can send out their Alsatians (antibodies) and snipers (T-lymphocytes) to deal with it. But nature is unforgiving. Once the postman has carried out his mission, he is thrown to the dogs. You, on the other hand, are protected.

This investigation tells the story of thirty years of research and technological development, mapping out the path to the creation of this vaccine. And many other therapies in the making. It's a picture showing scientific obstacles, epic patent battles and the scorn of researchers obsessed with a different molecule: DNA. And then there's the scepticism of big pharma, which, until it proved itself during the pandemic, remained largely impervious to mRNA's therapeutic potential.

Moroccan-born scientist Professor Moncef Slaoui watched what happened from a prime seat, as head of Donald Trump's Operation Warp Speed from May 2020. He will tell us what it was like working on the inside.

Stumbles in the final straight

Since the very first vaccine, the adoption of the technology into mainstream medicine has been slow. Edward Jenner, the 18th century inventor of vaccination, was forced to self-publish his experiments on smallpox because the Royal Society didn't dare, for fear of harming its reputation.

So Slaoui was unsurprised at how long it had taken for mRNA technology to be adopted. "Vaccines are injected into healthy people, so it takes an emergency like a pandemic to be able to introduce new vaccine technology."

The founders of mRNA:
François Jacob, Fred Sanger, Tom Cech
and Mary Edmonds

Prologue

1960-90: discovering messenger RNA

Fundamental research that took place between 1960 and 1990 solved the mystery of how RNA made the crucial step between DNA and the proteins it coded.

by Fabrice Delaye

The period created fertile conditions for the next phase, during which scientific understanding and newly developed technologies could be applied to medicine. As ever, it was pure research that led the way. Tom Cech, 1989 Nobel laureate in Chemistry and someone heavily involved in this research, says: "What motivated us at first was not the medical applications. It was to understand the mechanisms of life."

Biologist James Darnell gave a detailed insider's account in his 2011 book *RNA: Life's Indispensable Molecule*. As he explains, the whole enterprise was sparked by questions springing from British researchers at the University of Cambridge. Fred Sanger (winner of Nobel Prizes in 1958 and 1980) showed through his 1951 sequencing of insulin that proteins are arranged in molecular building blocks – in the form of twenty amino acids – uniquely ordered. Soon after, in 1953, James Watson and Francis Crick, the 1962 Nobel laureates, proved with their description of DNA's double-helix structure that the information on which all life is based is also organised in a specific way.

Proof that messenger RNA exists

The question arising from these discoveries was where these sequences interact, and how genetic information is transferred and transcribed into the proteins that shape cells and fulfil the thousands of essential functions of life.

In the 1950s, transfer RNA was discovered. This kind of RNA transports amino acids so that they can be incorporated as proteins by tiny synthesis factories: ribosomes. An article postulating the existence of mRNA in 1961, and the publication of proof by François Jacob, Jacques Monod and Sydney Brenner the following year, boosted research immensely. The missing link between DNA and proteins had been found. But this still didn't explain *why* a cell chooses a particular protein and *how* it produces it.

In the 1960s, US biochemists (including Marshall Nirenberg, Severo Ochoa and Gobind Khorana) explained for the first time how information is organised within RNA. They showed that the four bases of RNA – the nucleosides A, C, G and U, equivalent to DNA's four nucleotides A, C, G and T – combine to

"What motivated us at first was not the medical applications. It was to understand the mechanisms of life"

form sequences of triplets called codons. For instance, AUA, GCC, and so on, in sixty-four possible combinations. It is the arrangement of these codons – RNA's code words – that determines how the twenty amino acids are organised, and gives different proteins their specific properties. As scientists' knowledge of these structures deepened, they began to understand the mechanism by which DNA's code of life is transcribed into the proteins that shape existence. This is how we came to understand the role of mRNA.

A wave of seminal discoveries

These discoveries triggered a multitude of research projects. Bacteria was the subject of the first experiments in the late 1960s; an easy starting point, with only 2,000 to 3,000 different proteins, compared with tens or even hundreds of thousands in the human body. But during the 1970s, research increasingly focused on RNAs in more complex organisms, leading to a further wave of seminal discoveries.

Biologists Mary Edmonds (University of Pittsburgh) and Aaron Shaktin (Rutgers University in New Jersey) identified the structures that finish and cap mRNA. These are essential to protecting mRNA and connecting it to ribosomes, as well as differentiating it from other types of RNA. As the 1980s dawned, Tom Cech (University of Colorado) and Phillip Sharp (MIT) showed how RNA derived from DNA specialises to produce a plethora of proteins. Their work earned each of them a Nobel Prize in 1989 and 1993, respectively.

The magic of the molecule

"This is the magic of the molecule," Cech says from his office with its view of the Rockies. "It is both a vector of information and the matrix of functions, of chemical catalysis."

On a Zoom call from his office in MIT, Sharp explains that although these discoveries have not fed directly into the current vaccines, they did make them possible. "It is this knowledge that will, for example, help determine the optimal organisation of codons in the spike protein expressed by the vaccines."

Cech adds, "This quest for knowledge was also at the origin of the artificial RNA synthesis technologies that are the basis of those used today."

These research projects also identified the 300 or so enzymes (the watchtdogs) that work to break down RNA in our bodies, either because it has outlived its use or because it has been identified as an alien invader – a virus, for instance. These enzymes give the RNA molecule its "almost mythical reputation for instability," according to Juan Valcárcel Juárez, a Spanish biologist and former president of the RNA Society.

Scepticism and mockery from peers

RNA's reputation for instability had a considerable impact on future research. Its cousin, DNA, which was seen as stable, attracted significant interest from both researchers and funders, and the first recombinant molecules were produced by genetic engineering in 1975.

By contrast, in the late 1980s, not a single laboratory worldwide had yet discovered an RNA-based therapy.

For the next thirty years, the pioneers of RNA technology battled against this reputation. Many fellow biologists looked on with scepticism and mockery – until the advent of vaccines against Covid-19 conclusively proved the case for mRNA.

“This is the magic of the molecule,” Cech says from his office with its view of the Rockies. “It is both a vector of information and the matrix of functions, of chemical catalysis”

Robert Malone was not yet thirty, working at the Salk Institute, when he found a way to introduce lipid-coated messenger RNA into cultured cells. From there, the mRNA could induce the cell to produce proteins

Robert Malone was 29 when he made the major discovery that it was possible to bring mRNA protected by a fat ball into cultured cells to produce proteins.

Chapter 01

From science to technology at the Salk Institute

Robert Malone was not yet thirty, working at the Salk Institute, when he found a way to introduce lipid-coated messenger RNA into cultured cells. From there, the mRNA could induce the cell to produce proteins. His discovery has since proved to be a key towards RNA vaccines. He was able to confirm his results through trials conducted on frogs. But despite this, and the fact that the Salk Institute is a leading centre of genetic research, a surrendered patent and scepticism about mRNA's potential would hold back research for years. Today, Robert Malone is as bitter as only an unrecognised pioneer can be.

His Zoom background may be dressed in the bucolic shades of a Virginia fall, but Robert Malone is seething inside. "I'm very glad you contacted me," are his opening words, suggesting that I am the first to do so. Yet Robert Malone, endowed with a Hemingwayesque bushy white beard, is a true pioneer of the messenger RNA technology that produced the first vaccines against the ongoing Covid-19 pandemic.

The mRNA family tree has many branches. It has sprouted and grown on the fertile ground of decades of fundamental research and the findings of many collective projects. Nevertheless, the scientific literature is unequivocal. In August 1989 Robert Malone co-published the first article about research demonstrating that it was possible to introduce messenger RNA, coated with a small ball of fat, electrically charged (a cationic liposome), into cultured cells so that it could deliver the information required for protein production.

Research into mRNA has grown out of decades of fundamental research and the findings of many collective projects. But August 1989 was the starting point for much of this, when Malone's article, outlining his discovery, was published and opened the transfer of scientific knowledge to medical technology.

The article that set the ball rolling

Malone's article was instrumental in developing the science behind RNA vaccines into fully fledged technology. The principle behind the Pfizer-BioNTech and Moderna mRNA Covid vaccines can be traced back to this article and its three authors. But it was a long three decades between its publication in the *Proceedings of the National Academy of Sciences* and the moment when vaccines based on their research began to help stem the pandemic. So what happened in those 30 years?

Before Covid-19, not one mRNA technology had achieved the pharmaceutical industry's holy grail – market authorisation. But in the wake of this revolutionary outcome – one that may well win Nobel Prizes – the claims for paternity are mounting.

RNA's long genesis is not entirely down to the challenges of molecular biology. The technology transfer process kickstarted by Malone has resulted in more than its fair share of rows, doubts, patents surrendered or never filed, scientific articles rejected by leading journals, and financial strategists getting the better of scientists. It forged some careers and derailed others.

Standing on the shoulders of the giants of gene therapy

Robert Malone tells the story of his scientific career, and it is clear that it has left many scars. One of the emails he sends me, complete with an abundance of attached files to back up his claims, even describes his founding experience as "intellectual rape". Whatever the wrongs he feels he suffered, his story illustrates the harsh realities of scientific research rather than the pretty pictures projected by elite universities and pharmaceutical PR teams. Then again, you don't inspire a medical revolution that goes far beyond vaccines without rocking the boat or even capsizing.

Back in 1988, Malone was twenty-nine and a molecular biology student at the University of California San Diego. He'd taken up medical studies late, after trying out several different jobs. It was the age of AIDS and he was fascinated by retroviruses.

In nearby Silicon Valley, the biotech firms that blossomed after Nixon's 1971 declaration of war on cancer were the new darlings of Wall Street. Genentech, now a subsidiary of Roche, was marketing the first so-called recombinant proteins, made by modifying bacteria to produce human insulin. Chiron, a company later absorbed into Novartis, had just cloned HIV. This was a step towards the first blood tests for the virus.

In this ecosystem, anything seemed possible, and an even more radical technology was just beginning to take shape: gene therapy. So far, genetic

engineering had been used to produce humanised proteins in bacteria or yeast that could be injected as medical drugs in patients. The challenge now was to go further and replace a defective gene in a human cell, or even prompt the cell to produce one of the proteins needed to fight any number of diseases.

The prevailing wisdom at the time was that to achieve this, it might be possible to capitalise on the natural ability of a virus to introduce its genetic material into cells. This ability could potentially be used as a means of transporting genetic information. Malone wasn't alone in thinking that retroviruses might make an ideal vector. The advantage of retroviruses is that they convert their single-strand RNA genomes into a double-strand DNA molecule, which is stably incorporated into the target cell's genome.

It was this question that persuaded Malone to continue studying at the University of San Diego for a master's degree and then a PhD. Then, in 1988, he joined the Salk Institute, a futuristic-looking temple of molecular biological research, overlooking the Pacific Ocean and built by Jonas Salk, the inventor of the polio vaccine.

"There, I literally stood on the shoulders of giants," says Malone. "At the time, the Salk Institute still housed the laboratory of Francis Crick [the co-discoverer of DNA] and six Nobel Prize winners."

Another side to the Salk Institute

The institute's teaching and research staff also included some of the leading pioneers of gene therapy. Theodore Friedman, who first conceived of it in 1972. Inder Verma, who created the world's first genetically modified virus with the potential to transport genes. David Baltimore, who had won the Nobel Prize in 1975 for his studies leading to the discovery of reverse transcriptase, a retrovirus enzyme with a genome made up of RNA that can insert genes into the DNA of its host cells.

Despite its beautiful location between the desert and the ocean, the polished concrete exterior of the Salk Institute is not quite as smooth as it looks. The giants of scientific research also have their flaws. David Baltimore spent 10 years fighting accusations of scientific fraud before finally clearing his name. Despite his trailblazing discoveries in the fields of cancer and immunology, Inder Verma vanished from public view following allegations of sexual harassment.

The young Robert Malone was oblivious to such matters. All he saw was a magical location and an amazing stream of information and new ideas in which he did not hesitate to immerse himself. "I was literally navigating in a sea of knowledge."

Having joined Verma's laboratory team, Malone embarked on a doctorate. His thesis focused on the issue of transforming a retrovirus into a viral vector capable of transporting genetic material. He set about studying how to synthesise RNA and introduce molecular information that would produce proteins in host cells. This information (known as 'codons' – the elements of RNA's code) is transported by RNA and will, in scientific terms, 'express' proteins in the host cells.

“We started by introducing RNA and DNA into culture cells, but nothing was happening. It was clear that something new was needed to transport the genes. That’s where the idea of building an artificial virus came from”

What is RNA?

Ribonucleic acid – RNA's full name – was discovered in 1961 by French biologists François Jacob and Jacques Monod who came to California the year after to prove its existence in an experiment with Sydney Brenner. It is a copy of a portion of DNA containing instructions for assembling a specific protein. Tiny molecular factories called ribosomes use this blueprint to produce relevant proteins.

Scientists soon realised that it would be useful to find a way of producing RNA specific to given proteins without having to start with the DNA masterplan. The strand of imported RNA could introduce the information the cell needs to produce proteins missing or corrupted by disease.

The snag was that, at the time, mRNA could only be synthesised in small, laboratory-produced, quantities, unlike DNA which was already being produced on an industrial scale.

"We knew only how to produce short RNAs in the laboratory and in small numbers," says Robert Malone in front of his autumnal Virginia background.

When at the Salk Institute, Malone became aware of Pablo Garcia, a doctoral student at the University of California in San Francisco, who had hijacked the main method of DNA production using plasmids (DNA molecules in bacteria, as distinct from DNA in chromosomes).

In the 1970s, scientists had manipulated plasmids' ability to replicate autonomously, to produce modified DNA. The result was the industrialisation of genetic engineering. Malone had set his sights on investigating whether the plasmid cassette developed by Garcia in San Francisco would be able to synthesise mRNA that could transport instructions to produce proteins on demand. It was an avenue of research that was of particular interest to Malone while at the time there were no commercial product to synthesise mRNA.

Enter the lipids

Malone used one of these mRNA production cassettes to purify and then produce, in various cell lines, mRNA encoding his control protein, luciferase.

To be sure that the proteins are being produced, biologists use several markers. Malone chose luciferase, the class of enzyme responsible for making fireflies glow. Its major advantage is that it is luminescent and therefore highly visible, meaning that it is easy to judge the quantity of protein being manufactured in petri dishes used for cultivating cells.

"I was particularly motivated to achieve this synthesis because it was the key to my PhD," he says.

A second challenge remained: how to transport the mRNA into cells to deliver instructions to the ribosomes – the protein factories. At the time, some successful experiments of mRNA delivery with liposomes had been conducted by Giorgos Dimitriadis at the National Institute for Medical Research in the United Kingdom and Marc Ostro at the University of Illinois, as early as 1978. But these initial experiments had no technological follow-up. That is because the most complicated aspect of this operation, known as transfection, is that cell membranes have a negative charge, as does mRNA (and DNA). Since negative

charges repel each other, the result is a force field around the cell preventing the DNA and RNA from entering.

Back then, there were two ways around this problem. Both are based on the use of chemicals: calcium phosphate and DEAE-Dextran. Several experiments had been conducted using these technologies for RNA transfection, for instance by the molecular biologist John Dunn at Brookhaven National Laboratory in 1985, using an RNA polio virus. Nevertheless, most of the technologies involved DNA. A new kind of liposomes was clearly much needed.

A battle between scientists and financiers

In November 1987, biochemist Philip Felgner had published an article in the *Proceedings of the National Academy of Sciences*. It put forward a new approach for introducing DNA into cells, using electrically charged lipid aggregates. These cationic liposomes bond to DNA, protecting it and easing its passage through the cell membrane. They were the ancestors of the lipid nanoparticles now used in the Moderna and Pfizer-BioNTech Covid vaccines.

Felgner, now a professor at the University of California Irvine, was at the time working at the Syntex Research Institute in Palo AltoAlt. "Syntex was then the model for the biotech company," he says via Zoom from his office.

Syntex was founded in 1944 in Mexico City by Russell Marker, the inventor of the octane rating system. The company found success through using Mexican yams to manufacture steroids such as cortisone and progesterone, which formed the basis for the first contraceptive pills.

But by the 1980s it was a company looking hard for its next big hit. It suffered from the typical pharmaceutical industry problem of reconciling the need to achieve quarterly results with research programmes that could span decades before bearing fruit. "[A colleague] told me at the time that it would take thirty years for my work to lead to a concrete application. That turned out to be true," Phil Felgner says.

Headquartered close to Stanford University, Syntex tilted in favour of research over profits in the early eighties. The company invested proportionally more in basic research than any of its competitors at the time.

"We started studying liposomes in 1983 because we thought it could be useful for drug development and in particular for transporting DNA or even RNA into cells," Felgner continues. "Genetic engineering had just begun, and we had the vision that we could introduce any gene into cells and get an effect from that gene. But people were still struggling to figure out how to do it. We started by introducing RNA and DNA into culture cells, but nothing was happening. It was clear that something new was needed to transport the genes. That's where the idea of building an artificial virus came from."

Mixing with liposomes

In natural respiratory viruses such as SARS and retroviruses, a lipid membrane coats the DNA or RNA to allow it to slip into cells so that the virus can reproduce.

In 1984 Richard Roman, a Syntex chemist, took inspiration from this mechanism and created a new artificial liposome called Lipofectin. Its chief property is that it carries a positive charge and can therefore attach itself easily to the negatively charged DNA or mRNA.

This was a huge step forwards, but one more problem was still without a solution.

"The inside of the cationic liposomes was too small to encapsulate the genetic material, and the encapsulation was not very effective, and impractical," Felgner explains. "So, we thought about simply mixing DNA with these liposomes – they stuck together like magnets. It worked beautifully on the first try. I was amazed that we were able to encapsulate all the nucleic acids in one liposome. Even though we didn't completely understand why, all of a sudden, there was a convenient gene delivery system that anyone could use."

By the time Felgner's article was published in late 1987, however, Syntex no longer viewed the development as a profitable pathway. Its president, Albert Bowers, underlined this in an interview with the *Los Angeles Times*: "To make money, you don't want to remain a research company."

Felgner had to admit defeat but did nonetheless score one major success. "Still, we agreed that we had to make this technology as accessible as possible. I started sending vials of cationic liposomes to dozens of researchers working on gene transport in cells."

From discovery to depression

One of those researchers was Robert Malone.

"He was producing RNA *in vitro*," says Felgner. "While we had used our artificial virus to transport DNA, he took the initiative to combine it with RNA and it also worked the first time."

"I had the genes coding for luciferase, a technology to synthesise them into messenger RNA, a vector capable of crossing the cell barrier and cell lines to express these RNAs," says Malone, whose research was about to accelerate. "I did tests on different cell types, and it worked very well *in vitro*. Automatically, the next question that came up was whether it would also work *in vivo*?"

At the time, Malone was funding his PhD by working as a research assistant on the embryology course at the University of San Diego.

"We had a large quantity of frog (Xenopus) embryos available, so I tried it. Again, it worked the first time."

Malone had just invented the first mRNA delivery system using a charged lipid coating (lipofectine) to facilitate the transfection of mRNA into cells and express proteins. This method was even more promising because the main alternative – vectors derived from naturally occurring viruses – still carried a risk of infection or the possibility of an unwanted immune response. But his luck proved too good to last.

The need for a patent proves fatal

At first, everyone was interested in the discovery presented by Robert Malone at meetings such as the one held on 2 May 1988 at the Salk Institute. Molecular biologist Tony Hunter (Wolf Prize in medicine 2005) sent us the notes he had taken at that meeting.

"Even the president of the Salk Institute, Frederic de Hofmann [an Austrian-born nuclear physicist who had been involved in the Manhattan Project] and senior scientists at the institution such as Dinko Valerio [who went on to found Crucell, now the jewel in the crown of Johnson & Johnson's vaccine department] were interested in my results," says Malone.

For the Salk Institute to give him their financial backing, Malone was required to file a patent application before publishing any scientific articles. To this end, he consulted the institution's specialist lawyers.

However, the scientific community had serious doubts that RNA could ever be used in treatment – not least because of the abundance of enzymes (ribonuclease) in an organism that existed with the purpose of destroying the molecule. Malone suggested in his correspondence with Salk's lawyers that it should be possible to produce mRNA vaccines, but gene therapy's predominant application was modifying defective genes in DNA. And if the aim was to produce proteins in the ribosomes, introducing genes into the cell nucleus made sense. Not least because they could be produced at industrial scale, while synthesising mRNA was still happening on benchtops in the laboratory.

Some of Malone's mentors at the Salk Institute argued that RNA would never feature in gene therapy because the molecule was too unstable.

"All I have done would be seen as a laboratory artefact," he says. And when he resists, he is told that his RNA synthesis cassette, his liposomes and his frog embryos were not obtained according to the rules... The Salk Institute will give up the patent.

Malone's article was published in 1989 in the *Proceedings of the National Academy of Sciences*. With his co-authors Phil Felgner and Inder Verma (also his mentor), he described the technology for cationic liposome transfection technology and luciferase production using mRNA in all kinds of different cells, including human ones. But it didn't mention his *in vivo* experiments on frog embryos.

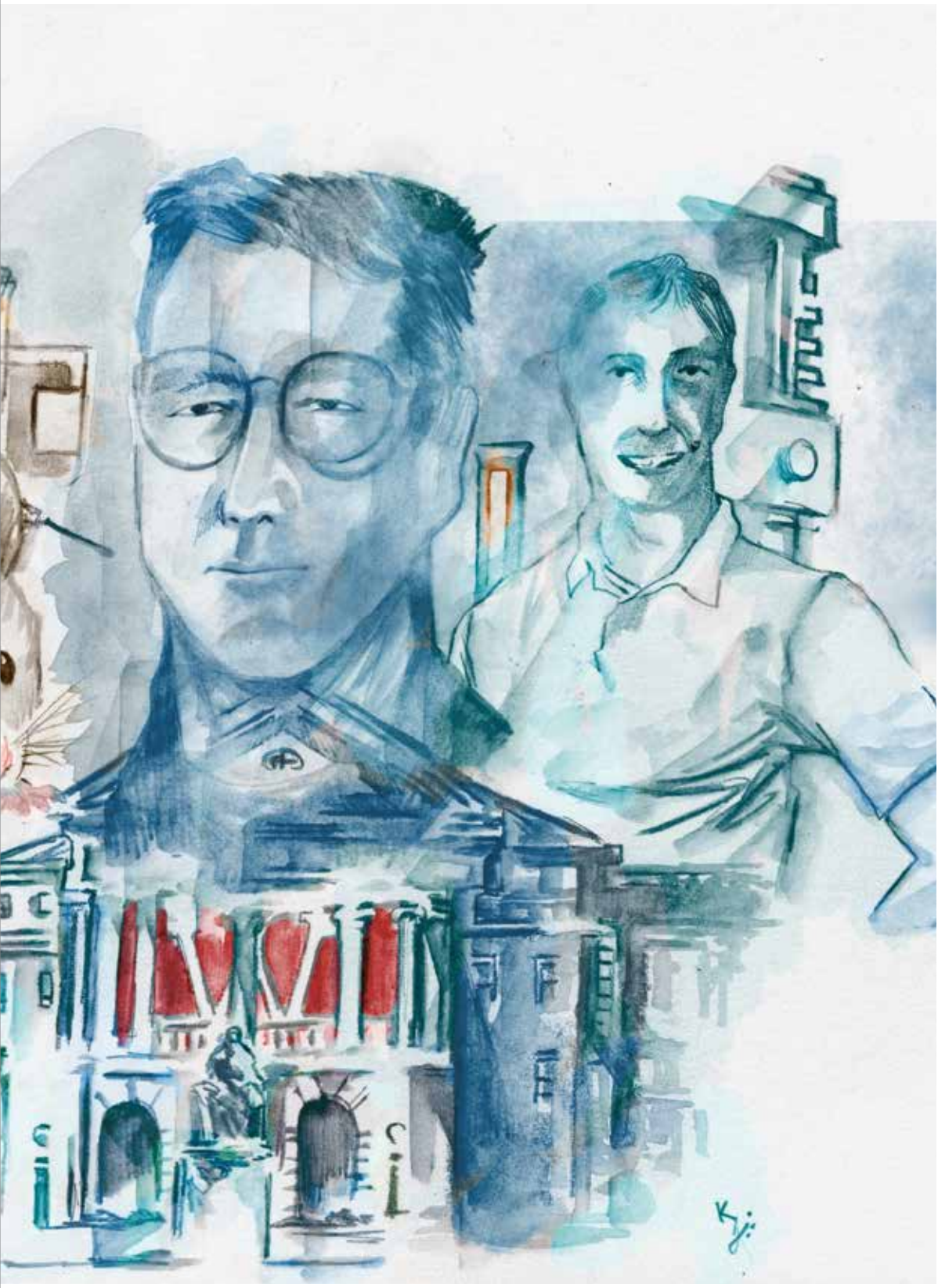
The surrender of his patent application was a severe blow to Malone. "I found myself completely depressed. To the point where a doctor diagnosed me with post-traumatic stress disorder." He was so down that he even abandoned his PhD.

Things didn't work out much better for Felgner at Syntex. Production of his cationic liposomes was licensed to another company, Bethesda Research Lab, which was then taken over by Thermo Fisher, a multinational supplying scientific instruments and other materials.

However, the business climate in San Diego was so febrile at the time that Phil Felgner had engineered a way out for himself. And Malone soon joined him.

Doubters underestimated scientific advances that would allow researchers to take full advantage of RNA. As a result, big pharmaceutical companies like Merck were missing a trick – and fell out of the race

Jon Wolff and Philip Felgner.



Chapter 02

RNA or DNA? How Merck missed the jackpot

RNA had a reputation for instability. It looked a poor second to DNA, in the eyes of molecular biologists and drugs companies. But these doubters underestimated scientific advances that would allow researchers to take full advantage of RNA. As a result, big pharmaceutical companies like Merck were missing a trick – and, as a result, fell out of the race to find a Covid-19 vaccine.

02

"Unstable!" The pioneers of messenger RNA – or mRNA – lost count of how many times they heard this verdict as they saw promising research abandoned, the wallets of funding bodies and drugs companies snap shut and, occasionally, their careers derailed.

DNA and RNA are closely related – both long chains of a handful of different molecules called nucleotides in the first case and nucleosides in the second and both crucial to biological processes. But where DNA's structure is robust, RNA's is anything but.

'Unstable', as biologists put it means fragile, in terms you and I would understand. Ineffective, pharmaceutical companies would say, which partially explains the near-constant scorn they later poured on mRNA.

Siberian mammoths

The moment RNA enters the body, it is met by certain molecules (the watchdogs of our introduction), both inside and outside cells, that have specifically evolved to destroy it. Faced with these pre-programmed pit bulls, mRNA's life expectancy inside the organism is a matter of minutes.

On the other hand, we find intact fragments of DNA in Siberian frosted mammoth fossils millions of years old. It's even proving itself as a long-term medium for storing digital data. By contrast, RNA – and its variants like messenger RNA (mRNA) are short-lived. RNA's life expectancy inside a living organism can be measured in minutes; there are legions of in-built biological mechanisms designed to destroy RNA.

DNA has historically behaved much more reliably in processes such as transfection, or deliberately introducing genetic material from a virus, to programme a cell to produce a given protein. Struggles to overcome RNA's relative fragility in this process led to its dismissal by drugs companies as ineffective, impractical, and unworthy of expensive research. mRNA research became a no-go area for pharmaceutical companies and scientists, as project after project failed, funding dried up and careers came to dead ends.

DNA's molecular stability encouraged scientists and managers at pharmaceutical companies like Merck to pursue it as the best avenue for transfection. But they were wrong. And it was this mistake that left big pharma out of the race to find this kind of Covid-19 vaccine in 2020.

The boom in gene therapy

Let's rewind a little. In 1987, when Syntex was shutting down its research into liposomes (see Chapter 1), Phil Felgner and other professors from the University of San Diego set up a company called Vical, with the aim of developing anti-viral drugs against AIDS.

At the time, all the entrepreneurs gravitating around San Diego's Salk Institute were keen to exploit the emerging possibilities of gene therapy. Among them was the molecular biologist Inder Verma (Robert Malone's mentor), founder of Viagene Biotech, a company working to develop viral vectors. Vical's Phil Felgner was working on the idea that liposomes could be useful for medical applications and invited Robert Malone to join him.

"Very few people are capable of seeing the future; he's one of them. We had the same vision back then," Phil Felgner explains. "He'd managed to get messenger RNA to express itself in cell culture, but now we had to prove that it was possible *in vivo*."

In fact, Robert Malone had already done this using frog embryos, but that wasn't enough (nor recognised – in molecular biology, the animal of choice for *in vivo* verification is the lab mouse.

Stunning *in vivo* results

On a visit, consulting pioneering gene therapist Theodore Friedman at the University of San Diego, Phil Felgner met post-doctoral student Jon Wolff.

"Jon told me that he was taking up a position at the University of Wisconsin-Madison and they had some lab rats available there."

Malone, Wolff and Felgner devised an experiment to show whether DNA and mRNA formulated in cationic liposomes could express proteins in the muscle cells of mice.

"At first, Wolff thought that his assistants must have got the mice and the data mixed up. However, the results were the same when he repeated the experiments. When injected into an animal's muscle, the naked RNA was expressed in the form of proteins"

The findings Wolff sent back from Madison were at first satisfying and then stunning. The data showed that the levels of protein expression achieved in the mice were comparable with the best results obtained by transfecting *in vitro* cells.

"It worked first time with both DNA and RNA," Felgner says. "It was the first successful transfection of genetic material in live mammals using lipid nanoparticles."

As is expected in best practice, Vical ran rigorous control studies. Injections of liposomes associated with RNA and DNA were used to express luciferase, a bioluminescent enzyme found in fireflies, and other markers. Another used liposome only as well as DNA, and mRNA separately. The experiments showed that 'naked' DNA or RNA (without liposomes) induced similar or greater protein expression than those encased in lipids.

"At first, Wolff thought that his assistants must have got the mice and the data mixed up. However, the results were the same when he repeated the experiments. When injected into an animal's muscle, the naked RNA was expressed in the form of proteins," says Robert Malone.

What's more, the concentration of these proteins locally – in the area around the injection site – was fairly high. The surface of cells naturally repels both RNA and DNA, yet some cells had proved capable of absorbing the molecule. The reasons for this were unclear at first.

"We later realised that temporary membrane lesions in the muscle cells during the injection allow the DNA or RNA to penetrate the cell," Felgner explains.

This discovery would have major consequences. Vical immediately filed for a patent before publishing its studies in the journal *Science* in March 1990. The possibility of injecting naked DNA or RNA into patients' muscles to produce selected proteins opened a whole new field of therapy. Now it seemed there might be potential for inducing insulin production in diabetics, or coagulation factors in haemophiliacs – or even to stimulate red blood cell production in cancer patients undergoing chemotherapy.

However, with the possible exception of the latter, it soon became apparent that this targeted approach was not a realistic treatment for systemic diseases at the time. Although muscle cells had expressed proteins, the yield was still too low to be reliable when diluted in the six pints of plasma in the human bloodstream.

And there was a further complication, too, as Felgner explains. "We observed one annoying side effect during these experiments: occasionally there was an immune response against the expressed protein."

The vaccine option

Taking all these factors into account, the most promising option for naked DNA or mRNA appeared to be vaccines. That's because even small quantities of proteins known as antigens, commonly found in viruses and bacteria, can trigger a significant immune response. In short, the immune system has a multiplier effect. Vaccines exploit it to provoke such a response, either humoral (one resulting in the B-lymphocytes producing antibodies) or cell-mediated (attracting T-lymphocytes, which attack cells infected by the virus or bacteria).

Now focusing on gene therapy, Vical promptly raised USD130 million in development capital when it floated on the stock exchange. At the time, the major priority for the pharmaceutical industry was to find a vaccine against AIDS. Felgner partnered initially with scientists from the University of San Diego to develop a naked DNA coded for a specific protein in the HIV virus – the GP120 protein. GP120 is to AIDS what the spike protein is to coronavirus: the key that allows it to enter cells. This was shown to trigger antibodies in mice, and the National Institutes of Health (NIH) later funded a clinical trial.

However, the decision to privilege naked DNA saw Robert Malone depart for Northwestern University to complete his medical studies, pausing his research into mRNA. "The disappointment is still like a punch to the gut", Malone says today. Is this maybe the reason he is now making ambiguous comments about the approved Covid vaccines while vociferously asserting that he was their originator?

Still, in the early 1990s, research continued at Vical. But finding an HIV vaccine was one of the toughest goals imaginable. With this in mind, two of Felgner's assistants, Suzanne Parker and Gary Rhodes, began similar experiments targeting the flu virus – which promised more immediate results – presenting their initial work to scientists at Merck's West Point laboratories in Pennsylvania.

Merck prioritises DNA

Maurice Hilleman, the microbiologist who was then Merck's head of vaccine research, was enthusiastic about Parker and Rhodes' approach. Having already developed close to forty vaccines – including for measles, mumps, hepatitis A, hepatitis B, chickenpox, meningitis and pneumonia – Hilleman's support carried a lot of weight. Merck agreed to pay Vical an annual USD1 million to continue work on a universal flu vaccine, deciding to develop it using DNA rather than following the RNA route.

Margaret Liu, who now advises the WHO on DNA and mRNA vaccines from her base in San Francisco, was at the time leading a research group on vaccines for Merck. "The challenge was to produce not just antibodies but cytotoxic T-lymphocytes, meaning ones capable of killing infected cells," she says via video call, sat in front of a black lacquered screen that suggests a certain flair for staging. Liu, who was born to Chinese parents and grew up in Colorado, was later nicknamed "the Mother of DNA vaccines."

"We hoped that this form of immunological memory would get around the virus's mutations, which demand a new vaccine for each new strain, every year," she says. "There were several possible directions we could have taken. What we wanted was to target the stable proteins stored in the flu virus to obtain lasting immunity through T-lymphocytes, including against new strains."

She makes no bones about the fact that even within Merck there were doubts about the chances of success.

"It seemed about as elusive as cold fusion!" she says with a smile. "The research by Malone, Felgner and Wolff had demonstrated protein expression in muscle but not yet in cells expressing antigen proteins that provoke the cellular immune response."

John Donnelly, associate principal scientist at Merck for over twenty-five years, explains further. "We initially tried messenger RNA and the results were good, stronger even than for DNA," he wrote in an email. "But we [he and his colleague Donna Montgomery] had to use much lower doses than for DNA because we could only produce tiny volumes of mRNA using the standard method at the time. Technology based on mRNA clearly wasn't a marketable procedure. It also required incredibly expensive reagents."

The yield problem

Although at the time DNA was easier to use than mRNA, yield was a problem. Some viruses are so fiendishly effective at introducing their genome into cells that 1,000 viruses might infect 1,000 cells. On the other hand, naked DNA or liposome-coated DNA transfection is considerably less efficient, requiring somewhere nearer 10 million copies to guarantee transfecting 1,000 cells. Back then, producing enough mRNA was still challenging, whereas biotechnologies were already producing pharmaceutical-grade DNA on an industrial scale.

"We thought that DNA had a better chance because it was more stable," Liu says. Felgner explains further. "The priority for Merck was to establish if it could be industrialised, whereas making naked DNA without viral vectors was a risky business already."

But Merck never managed to develop its naked DNA flu vaccine. Liu's research group had managed to show the possibility of protecting mice against different flu strains *in vivo*, but rather than studying T-lymphocytes, the clinical trials then focused on producing antibodies. Despite laying the foundations for future DNA vaccines, these initial efforts were limited to veterinary therapies – some of which succeeded, such as ending the threat of the West Nile virus that was wiping out the Californian condor. "DNA transfection worked with small animals, but not big ones. They tried bigger and bigger doses of DNA, but it didn't work," explains Robert Malone.

"Merck overcame all the industrialisation hurdles to verify that there was no risk of this DNA interacting with the natural DNA of the infected subjects," Felgner says. "They were satisfied with safety. Now they had to move on to clinical trials on humans to demonstrate the efficacy of this naked DNA vaccine."

Air disasters

Still, Merck abandoned its pursuit of a universal flu vaccine without publishing the results – Margaret Liu had left for Chiron, along with some of her team. Fate seemed to conspire against further research. Mary-Lou Clements-Mann, the Johns Hopkins University immunologist overseeing the clinical trials, was killed when Swissair Flight 111 crashed on its way from New York to Geneva in 1998.

Coincidentally, only two years earlier, the TWA Flight 800 air disaster had claimed the life of Rodolphe Mérieux. The timing was uncanny; it was just as BioMérieux, the pharmaceutical company to which Mérieux was heir, was discontinuing research that might also have accelerated the arrival of mRNA vaccines in France.

1993. France was leading the race to develop the first messenger RNA vaccine

Pierre Meulien (left) and Frédéric Martinon in 1993 at the Cochin Institute.

Chapter 03

How French RNA pioneers took their eye off the target

1993. France was leading the race to develop the first messenger RNA vaccine. It had behind it a successful experiment, biotech ambitions, and was home to a pharmaceutical group founded by the far-sighted Mérieux family. There were risks, however, and a lot of work remaining. But in the end, the first project to create an mRNA vaccine was killed off – and it delayed it by 20 years.

03

Turn back the clock to 1979. Robert Lattès, a venture-capital specialist at Paribas, and his boss, Pierre Moussa, an inspector of public finances turned banker, were determined to put France at the forefront of the emerging international biotech field. Genentech, the star of the sector, had been founded three years earlier in California; in Geneva, the Swiss molecular biologist Charles Weissmann and other scientists had just set up Biogen. Venture capitalists in Silicon Valley and Boston were about to launch Amgen, Chiron and Genzyme.

Biotech 1.0

The first generation of biotech start-ups focused on developing recombinant proteins. Cultivated in genetically modified animal cells, these humanised proteins are used to replace the ones patients lack – for example, insulin in diabetics.

In 1981, Robert Lattès founded Transgène SA in Strasbourg, bringing together a group of stakeholders representing the cream of French capitalism (Elf-Aquitaine, Paribas, Moët-Hennessy, AGF and others), with the goal of developing its own proteins.

Through the 1980s, faced with financial realities, Transgène moved away from this aim, adopting a model prioritising short-term profit: research under contract for big pharma. But it maintained several of its own autonomous, cutting-edge projects under the aegis of leading scientists such as geneticist Pierre Chambon and immunologist Philippe Kourilsky.

Freedom to experiment

The late-eighties boom in recombinant proteins fuelled the genetic engineering sector. In 1990, the great project to sequence the human genome was launched, while DNA repair, the primary goal of gene therapy, seemed a realistic ambition.

At Transgène, molecular biologist Pierre Meulien was, like his American colleagues, seeking to repair faulty DNA as a treatment for genetic pathologies such as haemophilia. "For two years I did research on non-viral vectors, especially liposomes, to deliver genetic material into cells," he says.

Trained at the University of Edinburgh and the Institut Pasteur, Meulien is now head of the Innovative Medicines Initiative (IMI), a EUR5 billion public-private partnership between the European Union and the pharmaceutical industry.

"We spent a lot of time at Transgène thinking about our lack of contact with clinical research as practised with hospital patients."

Meulien was an enterprising man. He proposed to his mentor, Philippe Kourilsky, that they place a small research team in a hospital to work directly with patients. Kourilsky encouraged him to explore studying vectors capable of transporting DNA. The team set up shop at Paris's Cochin hospital. Little did Meulien know that it was here he would have a seminal encounter.

First steps in the fight against AIDS

The building next door to the Transgène headquarters on the Cochin campus housed a brand-new research centre, established by two stars of French medical research – geneticist Axel Kahn and virologist Jean-Paul Lévy. One of Lévy's students, Frédéric Martinon, had just taken up a research position at the new Cochin Institute for Molecular Genetics to investigate AIDS vaccines.

HIV attacks a very particular type of white blood cells called T4 lymphocytes, known as 'helper cells'. The healthy function of these cells is to memorise enemy pathogens and boost the defences of the immune system.

"I was working on these mechanisms," Frédéric Martinon explains, "and on another type of white corpuscle, T8 lymphocytes, which directly attack the cells infected by the virus to stop them from proliferating. AIDS research was booming at the time. We had identified how the virus attacked T4 lymphocytes

and prevented an antibody response. Now we had to find a way of activating a different line of defence, and that was T8 lymphocytes."

Like other scientists, Martinon had concluded that "a vaccine was the only way of generating this response."

The conventional approach at the time was to develop this kind of vaccine from peptides – short chains of amino acids. These are adapted to resemble the antigen proteins responsible for triggering an immune response. But the process resulted in the fragile peptides degrading before that response could be triggered. "Stimulating T8 lymphocytes with these peptides worked very well *in vitro* but not *in vivo* on mice," Martinon says.

The route to immunity

An article, published in the journal *Science* in 1990, alerted Martinon to Jon Wolff, Robert Malone and Phil Felgner's radically different approach, introducing DNA and mRNA to express proteins (see Chapter 2).

Their research conducted on mice had demonstrated that synthetic DNA or mRNA could cause protein production in muscle cells.

Up until this point, these proteins had been simply markers proving a concept. Another study, however, published in 1992 by Stephen Johnson's team at the University of Texas, outlined a different therapy. It involved using DNA, protected by gold particles, to produce a different protein – in this case, a growth hormone.

This gene therapy proved inconclusive on humans but inspired Frédéric Martinon. "I thought it would be different if this gene therapy was applied immunologically," he says. "If it managed to produce the protein we wanted – an antigen characteristic of an infectious disease, say – then the next step would necessarily be that the antigen would trigger the immune system."

Choosing the vaccine path

Pierre Meulien was all ears when he was told of this still-theoretical vaccine approach. A strategic move on the part of his employer heightened his interest further. Alain Mérieux, heir to the Institut Mérieux, took over Transgène in 1991, incorporating the company into the family holding, Pasteur-Mérieux. The Lyon-based company was then the world leader in vaccines.

Merck and Vical's ongoing trials of their DNA-based flu vaccine was making big news at the time. Meulien and Martinon discussed the work conducted by Wolff, Malone and Felgner and wondered whether it was better to use DNA or mRNA.

"RNA was obviously more difficult to produce," Frédéric Martinon says. "Also, it is harder for messenger RNA to travel through the body, where it comes up against a series of mechanisms that degrade it. Which is why the naked RNA they discovered in the States didn't work well. It produced extremely uneven levels of expression because they were destroyed at random. Last but not least, RNA needs to be stored at minus 80 degrees, whereas DNA can be kept at four degrees."

Advantage RNA

Nonetheless, Martinon and Meulien finally opted for RNA.

"We still had lots of questions at the time about the risk of inserting a DNA sequence into the genome and its potential consequences," says Martinon. As Meulien points out, "there was some cultural reticence among Pasteur-Mérieux's vaccine specialists. They considered DNA an impurity in the classical vaccines they produced." So, it was advantage RNA. Meulien initiated a study aiming to send mRNA into cells to produce antigen proteins and trigger an immune response in T8 lymphocytes.

Liposomes were also chosen to protect and transport the mRNA. The team's biochemist, Shiv Krishnan, successfully synthesised liposomes large enough to encapsulate the RNA molecular material, rather than mixing it with the lipid nanoparticles as Malone and Felgner had done. "That was one of the keys of our research," Meulien says. "It protected the messenger RNA properly until it entered the cells."

First immunity using RNA

Frédéric Martinon had been using one of the first *in vitro* kits, marketed by the US firm Stratagene, to produce messenger RNA from modified DNA. "It was quite a long process, but we succeeded in producing good-quality RNA."

By now the French team was making rapid progress. The next step was to use this mRNA to produce not just marker proteins as in the US experiments, but antigen proteins capable of activating the immune system.

"We selected proteins from the flu virus, which I knew well because I'd studied various molecular areas that trigger immune responses," Martinon says. "We could toy with a variety of immune reactions. We chose three and did three different experiments on mice."

They were successful. The mice displayed an immune response after being injected with liposome-coated mRNA. "It was the first time anyone had demonstrated that it was possible to induce cellular immunity [T8 lymphocytes] against an infectious disease like flu using antigens expressed by specially designed messenger RNA," Meulien says. In other words, it was the first demonstration of the effectiveness of an mRNA vaccine in a mammal.

Rejected by the major journals

Yet, as is so often the case in molecular biology, the path between a successful experiment on mice and a human application is steep and perilous – ever more so when it involves radical and innovative technologies.

Meulien and Martinon had difficulty persuading the scientific community to accept their discovery. Despite publishing a whole host of studies on DNA, leading journals such as *Nature* and *Science* refused the Cochin team's article on mRNA. Their research results were eventually published in 1993 in the *European Journal of Immunology*.

Martinon readily accepts that the discovery raised as many questions as it answered. "The immune response in mice varied greatly, not just between the three experimental models we selected but also within each model. It was strong in some of our mice, while nothing happened in others."

Moreover, some of the mice had been injected in an area naturally rich in the right kind of white blood cells – ones with the specific role of activating the immune system.

"Even with this advantage, nothing happened. Yet at the same time we managed to produce T-lymphocytes with a drip when the RNA was diluted in the bloodstream."

Funding and research working out of step

When Pasteur-Mérieux acquired Transgène, Pierre Meulien was also appointed Head of Research of the Mérieux Sérum & Vaccins division. He moved from Paris to Lyon.

"There, the engineers didn't really see how they could industrialise the technology we had developed at Cochin," Meulien says. A technology that worked on only half the mice, for reasons that remained unclear, was hard to defend. "We had actually filed a European patent for the technology in 1992, but I couldn't really say to my superiors, 'If you give me between five and ten years and the equivalent of EUR100 million, we'll get there.' It also looked suspiciously like the boss's pet project. So, in the end I myself decided to call time on my project and prioritise more traditional ones that stood a chance of reaching fruition more quickly."

In 1998, after a series of mergers which left the project floundering, the patent application for RNA technology was dropped. In much the same way as Merck, French pharmaceutical companies that had once led mRNA vaccine technology had left the game too early to capitalise on their head start.

Plenty of research was still required if these technologies were going to make the grade. The next phase was led by a small team of researchers at the University of Pennsylvania and the University of Tübingen in Germany. They too struggled to muster support, but the big difference was that they persevered. But with big pharma set against mRNA, they had to put their careers, their investments and their reputations on the line.

In much the same
way as Merck,
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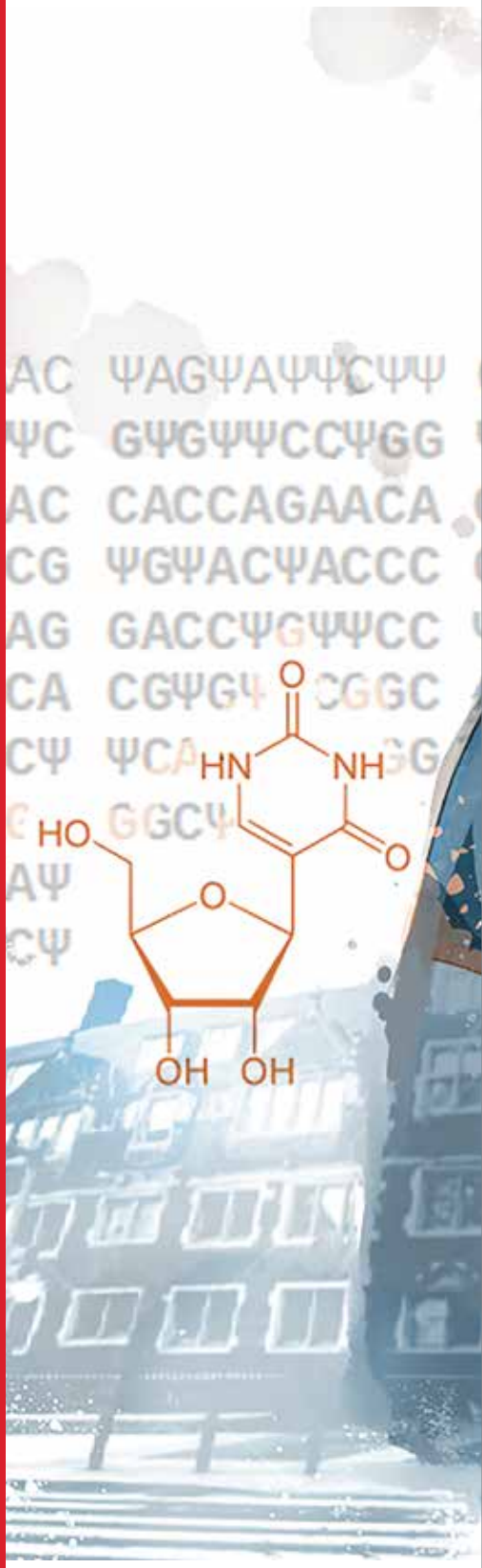
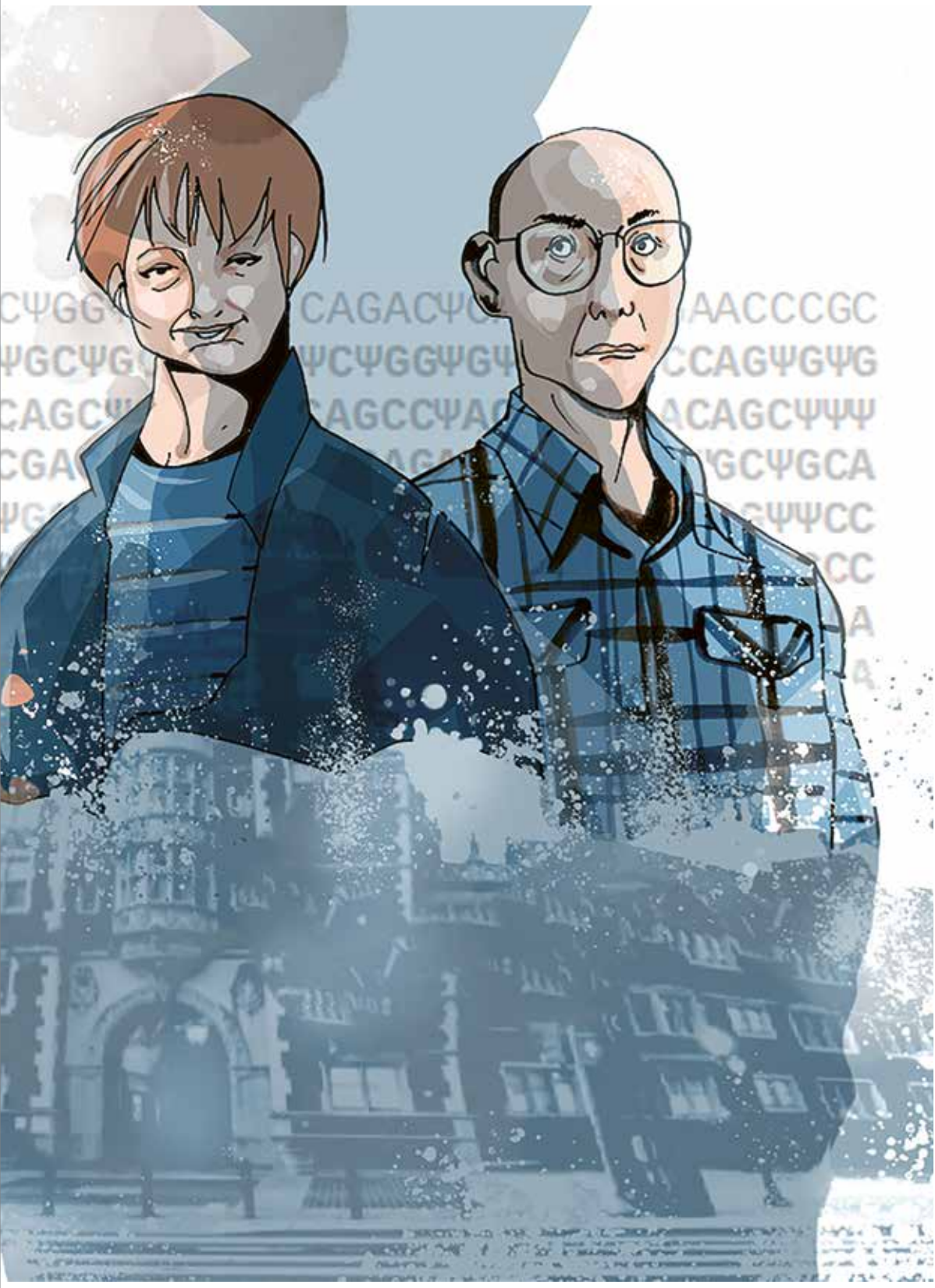
on their head start

Together, a pair of mRNA-obsessed scientists, the Hungarian Katalin Karikó and the American Drew Weissman, made a discovery that paved the way for therapeutic applications of mRNA



Kati Karikó and Drew Weissman met in 1998 at the University of Pennsylvania.



Chapter 04

Karikó and Weissman's invisibility cloak

Together, a pair of mRNA-obsessed scientists, the Hungarian Katalin Karikó and the American Drew Weissman, made a discovery that paved the way for therapeutic applications of mRNA. It's the story of a journey from Hungary to Germany via some prestigious US universities.

04

In scientific research, much of the work is done by scientists whose role is complete once the results are published; researchers move on to the next new project. Katalin 'Kati' Karikó and Drew Weissman are a different breed, paving their own ways throughout their careers.

In August 2005 Karikó and Weissman published their most significant article in the scientific journal *Immunity*. The article went almost unnoticed at the time. But it laid the groundwork for the first two mRNA Covid-19 vaccines, produced by Moderna and Pfizer-BioNTech and approved in autumn 2020.

The title may sound obscure – "Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA." But it answers the key questions of why transfections of mRNA trigger a parasitic immune response, causing strong inflammatory reactions, and why previous researchers had failed.

Karikó and Weissman first met in 1998, over their lab photocopier at the University of Pennsylvania, whereupon they developed a remarkable professional alchemy, despite having notably contrasting personalities. On Zoom from the living room of her home in Philadelphia, Katalin Karikó, usually known as Kati, is chatty, warm, and unfazed by being interviewed.

Drew Weissman, by contrast, conducts his calls from within the neoclassical buildings of the University of Pennsylvania. He rarely gives interviews. He enunciates his words with care. One can hear the point at the end of his sentences.

The journey begins near the Serbian border

For Kati Karikó, the journey that was to lead to her work with mRNA began in the late 1970s. The Hungarian biologist had just graduated from the University of Szeged, not far from the Serbian border. In 1978, Karikó joined chemist Professor Jenö Tomasz's laboratory at the Biological Research Centre of the Hungarian Academy of Sciences.

Tomasz was conducting essential research synthesising analogues of the mRNA cap for the American virologist Aaron Shatkin. Shatkin had discovered this structure three years earlier – the cap, located at one end of a messenger RNA molecule, enables its recognition by ribosomes – the protein 'factories' in cells.

As a young PhD student, Karikó found herself drawn to RNA research. The only biologist in a lab dominated by chemists, her role was to synthesise short segments of RNA for the purpose of testing their antiviral effects. In 1976, British biologist Ian Kerr had discovered that these segments of genetic material activated the immune response's first line of defence: the innate system. He initiated production of interferons – small proteins which, among other things, stop pathogens from self-replicating. The idea was to use these to combat viruses.

"I was discovering immunology, virology, the activities of RNA... I was amazed by the richness of what evolution had produced," Karikó says.

At the same time, she met two obstacles that were to have a lasting influence on her work. The first was the problem of how to introduce the synthesised sequences of nucleic acids – the four molecular building blocks, A, C, G and U, on which RNA code is based – into a cell.

"During my studies, I had been part of a research group on lipids and had worked on associations of phosphates and lipids to bring plasmid DNA [DNA from bacteria whose ability to self-replicate is used in gene cloning] into cells."

Her pursuit of this line of research led to her first published articles on phospholipids (protective fat molecules capable of delivering nucleic acids) and liposome-formulated mRNA delivered in mammalian cells. This was just the first of a series of studies, as the issue of how best to transport RNA was, and remains, a major challenge.

Her second problem was of a different kind: cash. Hungary remained firmly behind the Iron Curtain in the mid-eighties. European and American funding was out of reach, and her own country invested little in molecular biological research. "Since there was no money, I had to look for a job," she says.

Travels to Philadelphia

Karikó's only real prospect in Hungary was a job in the pharmaceutical sector. Fearing her curiosity would be stymied by industrial routine, she looked abroad. She wrote to Ian Kerr in Britain, Bernard Lebleu (a Montpellier-based specialist in interferons), and Luis Carresco (a pioneering expert in viral toxicity in cells at the University of Madrid). "I applied in England, France and Spain, and the answer was always the same: you can come, but get a grant first," she says.

This proved impossible from Hungary. But eventually, in 1985 Karikó found a job at Temple University, Philadelphia.

"We changed USD100; the maximum allowed. Fifty for my daughter Zsuzsika, fifty for my husband Béla. I was not allowed to do so because I had a contract to work abroad. We sold our Lada for the equivalent of USD900 hidden in Zsuzsika's teddy bear. Our tickets were one-way."

The seeds of obsession

Getting by on a salary of USD17,000 in a country where she had no friends was never going to be easy, but Kati Karikó didn't care. All that mattered was the pursuit of her work with RNA. She started by focusing on an enzyme, the production of which is induced when interferons degrade single-strand RNA during a viral infection.

By 1988, Karikó was working at the main Bethesda campus of the National Institutes of Health (NIH). She was one of the scientists who received the Lipofectin Phil Felgner sent out to disseminate the fruits of his research. She was first able to apply it at the University of Pennsylvania the following year, a time when fundamental research into mRNA was beginning to produce more effective synthesisers.

Karikó was convinced that RNA synthesisers and Felgner's Lipofectin would advance the development of mRNA therapies. In 1990 she suggested to her boss, the cardiologist Elliot Barnathan, that synthesised mRNA might be capable of increasing protein levels in blood vessels that had been removed and then reimplanted during coronary bypass operations.

Karikó was always looking to apply mRNA to a wide range of therapies, but it was to be eight years before she found someone who would listen. That person was Drew Weissman.

Having studied medicine and specialised in immunology, Drew Weissman spent seven years researching HIV in immunologist Anthony Fauci's research laboratory at the prestigious NIH in the 90s. Fauci, who was later appointed head of the Trump administration's Coronavirus Task Force, gave Drew Weissman his own research department at the NIH lab.

"I was particularly interested in dendritic cells," Weissman says. "It was a relatively new field for Tony [Fauci], but I convinced him of the value of these cells as the basis of acquired immune responses. Very quickly, I came up against the question of how to bring in the genetic information needed to trigger this immunity. We tried all sorts of avenues such as peptides or DNA, and even considered RNA although we had little experience in this field. What we wanted to do was to find the best way."

Heard at last

It was 1998 when Karikó first talked to Weissman about the potential of mRNA. He was immediately interested, not least because she had been part of the clinical trials for one of the first experimental RNA-based AIDS treatments at Temple University.

Karikó was elated to at last find someone who would listen. Since 1990, when her first application for funding had been turned down (to investigate an RNA-based therapy for cystic fibrosis), every one of her proposals had been rejected. She was nevertheless able to continue her research for a while thanks to her projects with Elliot Barnathan. She knew of the first successful *in vivo* transfection by Felgner, Malone and Wolff in 1990 and was encouraged by the publication of a first experimental therapy using mRNA in 1992. The neurobiologist Floyd Bloom's team at the Scripps Institute in La Jolla, California, had produced in the brains of mice a hormone – the deficiency of which was associated with a form of diabetes.

Nevertheless, Barnathan was having trouble raising further funding for RNA research.

"I was a nobody and it was hard to convince people to let me undertake molecular biology research in a cardiology department," Karikó says. But despite this, Barnathan asked her to clone receptors for an enzyme (urokinase) used to unclog venous catheters.

"I did it with messenger RNAs, and they were functional with ten times more binding between this enzyme and these receptors than normal. That familiarised me with the different processes of mRNA transfection."

Her findings were not published until 1998. By then, Karikó was facing all kinds of difficulties, the first being Barnathan's departure from the university to join a biotech company called Centokor. "I found myself with no salary," she says.

Karikó was diagnosed with cancer around this time and had to cope with the further disappointment of not being given tenure professorship. It would be another two years before she was given a post as a molecular biologist in the University of Pennsylvania Department of Neurology.

An invisibility cloak

Despite the difficult circumstances, Karikó continued to refine the process of synthesising RNA. With neurologist David Langer, who would much later star in the Netflix doc Lenox Hill, she developed a method to produce a molecule that dilates blood vessels to prevent a stroke.

Langer convinced the neurology department to give her a chance, and wanted to know if this promising molecule, which has such a short shelf life that it's impossible to inject, could be used in other ways. "A problem that could have been solved by producing this molecule directly in the cells from messenger RNA," she says today, true to her visionary habit of applying messenger RNA to all medical problems.

Although the following animal trials failed, her research showed an increase in protein production using mRNA. All the while, Karikó was also experimenting with new encapsulation techniques.

However, it was in 1998 that her research really took off. She began working with Drew Weissman, drawing on his experience of immunology and its clinical applications, leading towards some major discoveries.

The new research partnership was soon to meet difficulties. The first was that of parasitic inflammatory reactions. These broke out during the transfection of mRNA when they moved on from tests with cultured cells, to trials involving live animals.

"In the case of AIDS, which Drew was working on, you don't want to have that extra activation," she says.

This is the core problem of using mRNA for therapeutic applications. The aim is to induce the acquired immune response of B and T cells, while minimising the innate immune response triggered by interferons and inducing inflammation.

"The fundamental question was to know which receptor triggers this inflammatory response."

Karikó and Weiiessman began trying to make non-inflammatory mRNA. They included a control in their experiments using other types of RNA, in particular transfer RNA, the role of which is to carry the amino acids to the ribosome protein factories.

"We took different types of RNA from bacteria, animals, etc., and tested them. Some had this inflammatory effect, but others did not, and we wondered why," Weissman explains.

The answer was that mRNA and transfer RNA have different compositions. Transfer RNA has nucleosides slightly different from the four that make up mRNA (adenine, cytosine, guanine and uridine). Instead of uridine, transfer RNA contains a pseudo-uridine that acts as an invisibility cloak against the innate immune system.

The next step was to find a means of modifying mRNA using pseudo-uridine and other modified bases nucleosides. The team heard from a chemist at the University of Bonn that it was possible to buy synthetic pseudo-uridine indistinguishable from that found in nature. They bought several varieties for study, from different manufacturers. Five of these strains turned out to suppress the parasitic immune response during mRNA transfection, while maintaining the same capacity for protein production. Karikó and Weissman selected the one that performed best.

They looked for the causes of the different immune reactions, and an explanation for the invisibility. In 2001, researchers at Yale showed that a receptor (the "Toll-like receptor 3") triggered an innate immune response in the presence of a double-stranded type of RNA. This was a type that many viruses produce when they self-replicate. Karikó and Weissman searched for receptors belonging to the same family, which might also react to single-strain mRNA. They found that the receptors 7 and 8 triggered the innate response.

This was a major discovery. The Yale findings had begun a worldwide race to identify other similar receptors. But Karikó and Weissman were pipped to the post by a German research team from Munich, who published an article on the subject in *Science* in 2004. Still, Karikó and Weissman's 2005 article in the journal *Immunity*, on replacing uridine with pseudo-uridine in artificial mRNA, paved

“The aim is to induce the acquired immune response of B and T cells, while minimising the innate immune response triggered by interferons and inducing inflammation”

the way for developing therapeutic applications. The University of Pennsylvania applied for a patent.

A patent with a high price

Certain of the potential of their discovery, Karikó and Weissman approached pharmaceutical and biotech companies. With no success. Eventually they founded a start-up, RNARx, only to face further financial obstacles. The Bayh-Dole Act in US legislation meant that the University of Pennsylvania retained sole ownership of all intellectual property arising from research it had funded. Karikó and Weissman would have to buy a licence for their own invention, if they were to benefit from any linked opportunities. The price the university quoted – USD300,000 by some accounts – was beyond Karikó and Weissman's means. And potential investors wanted to see concrete prospects of clinical applications before they would commit. The proverbial chicken and egg problem.

RNARx initially tried to put together a project to produce erythropoietin (a hormone that increases the number of red blood cells) from mRNA, but it took three years to secure funding. In the meantime, UPenn had sold an exclusive licence lasting until 2026 to Gary Dahl and his company Cellscript. The university continues to pocket its share, which has become a river of cash under the impact of the new vaccines. Karikó and Weissman, on the other hand, received a fraction of the fraction of what UPenn gets.

"Few businesses are more profitable than an elite US university," Karikó says.

Karikó and Weissman published a second landmark study about the use of pseudo-uridine in 2008, reaffirming their belief in the potential therapeutic applications of their discovery. The modified nucleic acid reduced the inflammatory response and produced several hundred times more proteins in transfected mice.

Next, RNARx faced another problem. Karikó and Weissman had used RNA encapsulated in Lipofectin for their 2005 demonstration of dendritic cells. However efficient in animal experiments , Lipofectin is too toxic for use in humans, so they needed an alternative.

The discovery of other types of RNA called interfering RNA was a source of great hope at that time. The ability of these to block the expression of natural mRNA from defective genes in DNA seemed to offer a pathway to a new generation of gene therapy. Billions of dollars in investment poured into the development of new lipid nanoparticles to transport this interfering RNA.

"We tried forty formulations and it turned out that the best one was the one developed by a Canadian company called Protiva," Weissman says. But to obtain it was an intellectual property nightmare.

The advent of lipid nanoparticles

After merging with Tekmira, another Vancouver-area biotech company, in 2008, Protiva had initiated the development of its technique using lipid nanoparticles to transport RNA. Its inventors, biophysicist Pieter Cullis and biochemist

Ian MacLachlan, developed lipids whose electrical charge changed at different stages according to the acidity of their biological environment. This both greatly reduced their toxicity and increased the chances of the RNA passing through the cell membrane successfully. These lipid nanoparticles were to prove key to mRNA vaccines and to their future iterations.

"The problem was that they wouldn't supply us with their lipid nanoparticles. We had to wait seven years to get them from another source,' Drew Weissman says.

Protiva-Tekmira was keeping a tight hold on the technology because they had signed up with Alnylam Pharmaceuticals to develop therapies based on interfering RNA. The result was the first RNA-based technology, which was approved in 2018 (interfering RNA rather than messenger RNA). But before that, Alnylam and Tekmira were determined not to allow any parallel clinical tests with their lipid nanoparticles too soon. Any problems and their technology would be invalidated in the eyes of federal drug agencies.

Finally, in 2014, Weissman managed to source lipid nanoparticles from Acuitas, a company founded, coincidentally, by former Tekmira executives and Pieter Cullis. This was too late for RNARx and for Karikó, but not for Weissman's research.

A young Hungarian comes to the rescue

During previous work back in 2011, Weissman and Karikó had developed a purification process that further reduced the parasitic immune response during mRNA transfection. The problem was that some modified mRNA transcribed during in-vitro synthesis contained defects and caused inflammation. Weissman and Karikó's process removed the contaminants using high-performance liquid chromatography and increased the production of the desired proteins.

In 2011 Karikó had invited to Upenn a young researcher from Hungary called Norbert Pardi after she had become became his mentor. "My grandfather worked in the same butcher's shop as her father in our small hometown of Kisújszállás, and she gave me the taste for science when she came to visit her mother every summer," Pardi says via Zoom.

Promoted to a post-doc research position in Weissman's University of Pennsylvania lab, Pardi was involved in developing a platform of lipid nanoparticles and mRNA. This programme showed for the first time that mRNA really did work as a vaccine against infectious diseases.

Pardi now leads his own research team, working on RNA vaccines for malaria and other pathogens such as flu. He is planning a universal vaccine composed of the four messenger RNAs, each encoding a different flu virus protein, which he hopes will remain effective against annual mutations by targeting specific regions of the virus.

A trip to Switzerland

Weissman is preparing clinical trials of mRNA HIV vaccines in 2021. "It probably won't be the final vaccine yet, but bricks towards it," he says.

Meanwhile, Karikó's daughter, Zsuzsanna Francia – whose teddy bear hid the family's only money when they left Hungary – became a champion rower, winning gold with the US women's eight at the 2008 Beijing Olympics, going on to defend her title in London in 2012. It was after the London Olympics, when mother and daughter were travelling to Switzerland for another competition, that Karikó met Uğur Şahin, professor of oncology at Mainz University in Germany. Formerly a student in Zurich, where he was mentored by the Swiss Nobel laureate, Rolf Zinknagel, Şahin is also the founder of BioNTech, a company that was to become key in the mRNA story.

Karikó's track record had made her a scientist much in demand. Two of the other firms that also proved key in developing mRNA as a therapy – Moderna and CureVac – both wooed her, but she put her trust in Uğur Şahin. At the age of fifty-eight, she joined the staff of BioNTech as senior vice-president. By this time, the race had entered the home straight and BioNTech would go on to produce the Covid-19 vaccine that Pfizer began marketing in 2020.

This programme showed for the first time that mRNA really did work as a vaccine against infectious diseases

The 1998 World Cup: Croatia eliminates the German football team. It was an unexpected turn of events that would bring together in sporting solidarity the founders of CureVac, the world's first RNA vaccine company

At the University of Tübingen, Steve Pascolo (centre) and Ingmar Hoerr (top left) went on to create the first mRNA start-up with their professors.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Chapter 05

CureVac: the early leader falls behind

The 1998 World Cup: Croatia eliminates the German football team. It was an unexpected turn of events that would bring together in sporting solidarity the founders of CureVac, the world's first RNA vaccine company.

05

With the *Mannschaft* out of the running during the 1998 soccer World Cup, the German biologist Ingmar Hoerr switched his allegiance to the French national team, *Les Bleus*. His research group at the University of Tübingen at the time included a young French post-doctoral student, Steve Pascolo, and, having bonded over football, they decided to work together.

Trained in Paris at the École Normale Supérieure, Steve Pascolo had come to this university town in Baden-Württemberg to be supervised by the immunologist Hans-Georg Rammensee. The German professor was a leading authority in the field of T-lymphocytes – the white corpuscles that attack contaminated and cancerous cells.

From soccer to lymphocytes

The open-minded Rammensee was working to develop vaccines capable of activating T-lymphocytes to destroy cancerous cells. His idea was to boost the natural defence mechanisms that protect us from tumours throughout our lifetimes. These are the immune defences compromised when a cancer grows.

Rammensee had developed a large number of potential vaccines. Pascolo was eager to test them on the transgenic mice models he had produced during his PhD at the Institut Pasteur. There, under the supervision of François Lemmonier, he had developed 'humanised' animals. The genes and, in turn, immune systems of these mice are altered to include some of human origin, producing T-lymphocytes.

Natural technology

It was at a conference in Israel in early 1996 that Rammensee first heard about pioneering studies of mRNA offering a new future for vaccine projects. Eli Gilboa, a professor at Duke University, had extracted dendritic cells – cells that play an early role in the immune response – and transfected mRNA into them *in vitro*.

mRNA introduces into these extracted dendritic cells information about the proteins typical of cancer cells. Then, when reinjected into the body, these cells trigger the production of T-lymphocytes, in the same way as vaccines do, teaching the immune system to identify and attack cancerous cells. Drew Weissman reached the same conclusion about the same studies almost concurrently in the United States, inspired in part by his interest in Kati Karikó's research two years later (see Chapter 4).

A laborious method

"At that time, I was working on vaccines against cancers mainly based on peptides identified in cancer cells. But there were still a lot of challenges, and I was interested in all approaches," Hans-Georg Rammensee says. "The method presented by Eli Gilboa was quite laborious because you had to extract the cells, provide them with the necessary information with messenger RNA, and then reinject them. So, I asked myself, what would happen if we injected the messenger RNA directly into the body?"

When Rammensee returned to Germany, his PhD student Ingmar Hoerr tasked himself to find the answer to this question. Just as Malone, Felgner & Wolff had done, Hoerr began by injecting mRNA into mice ears – both naked RNA and RNA encapsulated in liposomes or peptides. In all three cases, proteins were expressed. Hoerr thought at first that he must have mixed up his findings when the naked RNA produced a better level of expression than the encapsulated RNA – just as it had in the experiments conducted at the University of Wisconsin back in 1990. His results were published in the *European Journal of Immunology* in 2000.

The superior efficacy of the naked RNA led to the development of a technology different from that used in today's Pfizer-BioNTech and Moderna vaccines, both of which rely on mRNA with one nucleoside (uridine – see Chapter 4) modified, as done by Kati Karikó and Drew Weissman.

"Unlike DNA material, messenger RNA is short-lived, and it doesn't go into the nucleus. This eliminates any possibility of integration into the recipient's genome"

A wall of scepticism

Turning the clock back to 1998, the year of the French victory over Brazil in the World Cup final and the start of Pascolo and Hoerr's partnership, vaccines from these technologies were a distant dream.

"We initially thought that this would be the way to make vaccines that were much safer than DNA vaccines," Pascolo explains. "Because, unlike DNA material, messenger RNA is short-lived, and it doesn't go into the nucleus. This eliminates any possibility of integration into the recipient's genome."

Their early experiments with RNA were conducted on modified mouse models of melanoma. This expanded into mouse models of cancer and infectious diseases such as HIV, hepatitis B and human papillomavirus.

"It worked even though the immune response was not very strong," Rammensee says. "It was promising, but we quickly ran into a wall of scepticism."

Pascolo continues: "We were indeed having success in inducing T cells with our messenger RNA, but nobody was interested. When I presented our results at conferences, there were no questions. So I would take a few slides out of my presentation to ask questions myself."

Ingmar Hoerr was planning to move into the pharmaceutical industry but he had encountered the same scepticism in his job interviews. It became clear that setting up their own company was the only way to pursue the research.

The first RNA start-up

CureVac was founded in 2000 after the University of Tübingen had filed a patent application for an mRNA vaccine. Although research focused then on naked RNA, this didn't signal the complete abandonment of encapsulation.

The earlier work by Frédéric Martinon and Pierre Meulien at the Institut Cochin (see Chapter 3) ruled out applying for a patent on liposome encapsulation. Instead, the patent was filed for encapsulation using peptides (protamine). This line of research is ongoing, though remains behind exploration of lipid nanoparticle options.

Biochemist Florian von der Mülbe turned down an opportunity at Roche to co-found CureVac with Hoerr and Pascolo. They raised an initial EUR100,000 from a Scottish investor, but when he backed out, the money had to be returned. They had already spent part of the sum but were saved by a last-minute EUR20,000 loan from a small local bank.

Just a few hundred euros a month

Eager to compete with Bavaria in the biotech field, the Baden-Württemberg government supported the tiny start-up through its Young Innovators scheme. This meant the firm could use a small, converted laboratory in the chemistry department at Tübingen. Hoerr and von der Mülbe drew minimal salaries of EUR800 a month, with Pascolo paid only slightly more, thanks to various university research grants.

Salaries aside, money to progress the project soon became a pressing daily concern for the three novice entrepreneurs. Investors were in a state of shock after the dot-com bubble had burst, so CureVac's priority was to conduct clinical trials fast.

In 2001 the CureVac team were awarded an EU grant to carry out a trial on swine influenza, but veterinary work proved to be financially unrewarding. Winning over doubting financiers would take a human clinical trial. For this, they needed lab facilities compliant with the pharma industry's Good Manufacturing Practice (GMP).

Meeting these standards would be a titanic leap for a start-up. Yet, by 2003 the small team had built the world's first industrial plant producing mRNA. In the same year, a Mannheim-based venture capitalist invested EUR2.7 million. This gave CureVac some breathing space, aided by a move to new premises at the Tübingen Technology Park. The town had invested EUR5 million in brand-new GMP-compliant laboratories that CureVac leased. Now the serious business could commence.

German medical legislation on human testing is thorough. However, there is a clause that enabled doctors to test any substance on a consenting patient – if the doctor produced the substance personally. Pascolo and Hoerr saw an opportunity to begin a first clinical trial on melanomas, in conjunction with clinicians from the university's dermatology department, who would produce the mRNA.

The first human vaccinated with RNA

Hoerr suggested trying it out on a first subject. Pascolo volunteered and Benjamin Weide, one of the dermatologists, was instructed to inject mRNA coding for luminescent control proteins (luciferase) into Pascolo's leg, to prove the technology safe. It was 2003 and Pascolo was the first human ever to receive mRNA produced *in vitro*.

Clinical trials began on patients with melanomas or cancer of the kidney skin, who had exhausted all other possible treatments. Despite encouraging results for a promising technology, every leading journal turned down the team's articles. Their first study was finally published five years later, in 2008, in the *Journal of Immunotherapy*.

Nevertheless, the trials had an impact. First and foremost, they attracted the attention of Friedrich von Bohlen und Halbach, an influential German biotech entrepreneur. A member of the Krupp family and alumnus of the Swiss Federal Institute of Technology in Zurich, in 1997 he had co-founded Lion Biosciences, a bioinformatics firm that flourished after floating on the stock market in 2000.

A welcome supporter

Von Bohlen introduced the CureVac executives to the billionaire Dietmar Hopp, founder of the German multinational software corporation SAP. As a computer scientist, he was immediately interested in CureVac's approach of

transmitting molecular information without using DNA. In late 2005 Hopp's venture capital fund invested EUR35 million in CureVac.

For the company this was a new beginning. Steve Pascolo had agreed to stay on at CureVac for another year but his attention was now on the university hospital in Zurich, and a pioneering researcher, Thomas M. Kündig. With the team there, Pascolo set up an RNA vaccine trial for lung cancer.

"He [Kündig] is the father of intralymphatic vaccination," Pascolo says. "Since the lymph nodes contain the largest number of cells involved in the immune response, I thought it would be interesting to try out something other than our intradermal RNA injections to produce antigenic proteins that would have to go to the lymph nodes anyway to stimulate T cells."

Cancer: the problem everyone wants to solve

In the mid-2000s, there was little investment in vaccines against infectious diseases. Cancer was where the real money was to be made; mention of immune-oncology was a sure path to piquing the interest of potential funders.

The pharmaceutical industry was then lucratively developing hundreds of monoclonal antibodies, produced *ex vivo* by genetically modified cells. There was no indication, however, that they couldn't be produced *in vivo* by the patient's own cells. In theory, blueprints of these proteins carried by mRNA could enable cells to produce them as required.

Pascolo had come up with the idea of 'therapeutic' mRNA when he observed a scientist in Rammensee's research team having difficulty producing an elaborate form of antibody known as "bi-specific." He had developed this technology at CureVac, but the move to Zurich made his continued involvement difficult; wrangling over patents continued until as recently as 2016.

Baby steps

CureVac had initially worked with naked RNA. The challenge was to improve the proportion absorbed by cells and transcribed as proteins, and the simplest way of doing this was to increase the dose and inject more mRNA. Through the 2000s, each injection contained up to 800 micrograms of mRNA. CureVac's Covid vaccine now contains seventy times less than this, in its final phase trials.

CureVac had no licence for the technology developed by Karikó and Weissman for modifying one of the bases of RNA (see Chapter 4), so the company developed other ways of refining its technology. The fruits of this are now used in its Covid vaccine development.

mRNA has coding regions and non-coding regions important to its stability and the way it binds to ribosomes. CureVac began by optimising these. Its mRNA is also enriched with two of the four nucleic bases of RNA (guanine and cytosine), to reduce the concentration of those (particularly uridine) that trigger the innate immune response.

Improving the RNA cap

Named 'GC rich' and patented by CureVac in 2002, the method improved the efficiency of mRNA's translation into proteins. It didn't eliminate the inflammatory reaction, however. For protein expression to occur, uridine is required by ten out of the twenty amino acids. CureVac also optimised the codons – molecular information that prompts protein production in host cells. This enabled enrichment of the proteins with amino acids, in turn prompting a stronger response from the immune system.

Other research looked at improving the mRNA caps (see Chapter 4). These were inconsistent in the produced RNA, so the aim was to retrospectively 'cap' all of it, ensuring the artificial caps were optimised to bind reliably to ribosomes. But it wasn't until 2017 that the California-based company Trilink developed a technique to ensure capping of RNA during production, making it 100 per cent recognisable by the ribosomes. "That was a giant leap, when for everything else we've been taking baby steps for 20 years," says Pascolo.

The improvements resulted in more stable mRNA that produced more proteins. For CureVac, however, the final validation depended on the trial results of its Covid vaccine. Published in June 2021, they have been disappointing with an efficiency of only 48% and no market authorisation.

DARPA revives viral vaccines

Still, CureVac's primary focus for some time had been cancer vaccines. Success was varied and its most advanced programme to find a vaccine for prostate cancer was drawn to a close in 2017.

But interest in funding viral vaccines, which had dwindled through the 2000s, was seeing a resurgence. The Defense Advanced Research Projects Agency (DARPA) had inaugurated a large-scale scheme against infectious diseases in 2011, stating the risks of bacteriological warfare and bioterrorism.

CureVac announced a venture with Johnson & Johnson to develop an RNA influenza vaccine and in 2015 had received a grant from the Bill & Melinda Gates Foundation for several vaccines. Funded by this were CureVac's first clinical trials of a rabies vaccine in 2017. The results, which were incomplete, sparked controversy. Balancing the high risk of toxicity, which demanded the vaccine be administered in very small doses, against the need for a reasonable level of RNA to ensure efficacy, was challenging.

CureVac and Sanofi Pasteur also joined forces in a programme to develop vaccines against infectious diseases for which they received funding worth EUR33.1 million.

Ahead in the game again

The experience CureVac gained working on these programmes has been crucial for its work towards a Covid vaccine. It enabled the company to build industrial facilities equal to those leading the field, and to make progress in another critical area of the field – lipid nanoparticles. As part of its joint venture

with Sanofi, CureVac is now working with In-Cell-Art, a small French start-up specialising in lipid nanoparticles.

Still, for its Covid vaccine CureVac has, like BioNTech, chosen to work with the Canadian firm Acuitas. But with less success. Its results showed an efficiency of less that 50% in third-phase clinical trials while Moderna and BioNTech were above 90%. CureVac's choice of a very low dose of 12 micrograms (only a third of BioNTech's and a tenth of Moderna) for its Covid vaccine may explain these disappointing results. But it is not the end of the story.

mRNA printers

CureVac is working on two huge projects in parallel. Its Covid vaccine technology is cheaper than the Pfizer-BioNTech and Moderna jabs and can be stored at higher temperatures. These features would make this technology an ideal contender for many vaccines used in developing countries, a need still to be met.

Also, in conjunction with Tesla, CureVac's other major project is to develop mRNA printers – small production units that can be used locally to produce personalised medicines on demand. This could play a decisive role in applying mRNA technologies to many more diseases beyond those caused by viruses.

Moderna had ambitions to start a medical revolution with mRNA. The story behind the realisation of that ambition is an American tale led by Europeans

Stéphane Bancel draws on the work of Derrick Rossi, the expertise of Robert Langer and the funds of Noubar Afeyan (from left to right) to launch Moderna.


E484K
K417N
N501Y
R246I
L18F
Δ242-24
D80A
D215G
D614G
A701V

Chapter 06

Moderna's moonshot

Moderna had ambitions to start a medical revolution with mRNA. The story behind the realisation of that ambition is an American tale led by Europeans.

Stéphane Bancel, CEO of Moderna, is not the average mRNA outsider. His career is one of a brilliant global go-getter – he was educated at the Lycée Sainte-Geneviève in Versailles, reputedly the best high school in France; École Centrale Paris (a leading science and engineering university); University of Minnesota; and Harvard Business School, as well as being named a Young Global Leader at Davos.

And yet Bancel also plays Dungeons and Dragons, which in my humble opinion is the world's best training ground for creativity. He is a geek of the Apple generation too, and I suspect that he is driven by the American dream.

After a meteoric rise that took him by the age of 34 to the summit of the BioMérieux Group, a company with 6,000 employees and turnover of EUR2 billion in 2007, he packed it all in five years later to join Moderna, a tiny start-up in Cambridge, Massachusetts, in 2011. Now, 10 years on, the whole world is familiar with the biotech company that developed one of the first Covid vaccines. When Bancel switched jobs, though, the response was more like: Modern-what?

King Noubar

"On my first day on the job, there was only one employee. We had no offices or labs and just one experiment that had produced results in ten rats," Stéphane Bancel explains, via Zoom from his Boston office. He is wearing a fine rollneck sweater. Not black like Steve Jobs' uniform, but beige.

He is not exaggerating still; they really did have next to nothing at the start. What they did have, though, was Flagship Ventures, the venture capital firm that had persuaded him to leave the wheel of the BioMérieux super tanker to take the helm of a flimsy skiff named by combining the words "modified" – a reference to the modified RNA nucleic base invented by Kati Karikó and Drew Weissman (see chapter 4) – and RNA.

First of all, a word about Flagship. The venture-capital industry is a patchwork of territories and networks, a technological Game of Thrones that has its own dynasties and scientific fiefdoms. Flagship is one of the lords of the biotech kingdom that sprouted in the late 1980s between MIT and the Harvard Medical School in the Boston areal.

No major deal was done without the involvement of Flagship's founder, Noubar Afeyan. Partly because this Canadian-educated Armenian-Lebanese biochemical engineer had a nose for innovation, partly because his address book was bursting with R&D contacts in industry and academia. It was indeed one of his university contacts, Robert Langer, who first talked to him about the small experiment on 10 rats that Stéphane Bancel mentioned earlier.

Biotech rock star

A professor of bio-engineering at MIT, Robert Langer is a biotech "rock star", according to the many scientists who mention his name during our conversations. A few years ago, the journal *Nature* described his typical day. It features a three-page printout of meetings and a constant stream of emails. Most of the people in his timetable get 15 minutes, half an hour maximum. His assistant errs on the generous side with me.

It is hard to hold Robert Langer's attention as he sits in his dimly lit office at MIT. He fires off emails as we speak. He only looks at the camera to answer very specific questions and is not inclined to flatter you by saying they're interesting.

Langer confirms somewhat distractedly that a Harvard scientist called Derrick Rossi sought him out in May 2010 for advice about a discovery he had just made and that their conversation was the spark for Moderna's creation. It was reportedly the Harvard immunologist Tim Springer who introduced his colleague Derrick Rossi, then associate professor at the Stem Cell and Regenerative Biology Department at Harvard Medical School, to Langer to get his opinion on a start-up project. With something like 25 start-ups to his name, Robert Langer was a prince not only among scientists but also among entrepreneurs. There was an additional factor, though.

From stem cells to myriad therapies

The discovery Rossi had made involved stem cells – unspecialised cells. He had hopes of modifying them to specialise as replacement cells – red blood cells, for example, or cardiac muscle cells, or neurons. But Rossi had the same problem as all his US colleagues at the time: the main source of stem cells was unused human embryos from fertility clinics. Using these embryos for research was at

the centre of a fierce bioethics battle. In 2001 President George W. Bush had banned federal investment in research on any newly created embryonic cells lines, reducing research to those already existing. President Obama overturned this decision in 2009.

In 2006, the Japanese researcher Shinya Yamanaka succeeded in reprogramming normal adult cells so that they reverted to stem cells. He was awarded the 2012 Nobel Prize for Medicine for this discovery. Rossi had been at Stanford when the discovery was made and saw it as a solution to his problem. But there was a new obstacle. Yamanaka had used retroviruses to transport the sequences of genetic material that were intended to reprogramme the cells. The risk was that this would produce mutations in the genome, and mutations can cause cancer.

Rossi was among the few who had read Karikó and Weissman's 2005 article in *Immunity* (see Chapter 4). He wondered if it might be possible to use mRNA to reprogramme the cells, eliminating the risk of provoking mutations.

When he was hired by Harvard in 2007, Rossi appointed one of his post-doc students, Luigi Warren, to work on this idea. Like Karikó and Weissman, Warren synthesised mRNA using modified nucleic bases nucleosides such as pseudouridine, to avoid parasitic inflammation. In November 2009 he too managed to transfect mRNA into mice cells. They were reprogrammed as stem cells and the results were published in the specialist journal *Stem Cell* in November 2010.

The discovery still had to overcome a number of challenges to qualify as a technology. One was the question of how to protect the RNA during transport. TransIT, the commercial technology Warren and Rossi used, worked well for research, but its efficacy and toxicity problems limited its clinical use. Human trials required a different means of transporting RNA. Langer, who had been honing his expertise in this since 1971, would provide the path towards the solution.

"Derrick Rossi and I discussed several subjects during our meeting," Robert Langer says. "We talked about setting up a company and about the technologies that still needed to be developed to transport messenger RNA into the cells, which remained a challenge."

Just like Hoerr, and Karikó, Langer saw a vast range of therapeutic applications for this technology, beyond stem cells. Three days on from the meeting, Rossi was invited to make a second presentation to Flagship Ventures, with whom Langer had set up Selecta Biosciences the previous year.

Where Flagship Ventures rules

Later, in a 2015 article in *Nature*, Noubar Afeyan said that he had found Rossi's innovation "immediately intriguing." He'd brought in another Harvard scientist, Ken Chien, known for his work on cardiac stem cells. Chien conducted several experiments and observed that cardiac cells also absorbed Rossi's modified mRNA and expressed proteins. Then, in late 2010 Rossi, Langer, Afeyan and Chien founded Moderna. They assembled a prestigious scientific advisory board featuring the Nobel laureate Jack Szostak and Doug Melton, the head of

the Harvard Stem Cell Institute. What they needed now was an entrepreneur capable of transforming their research into saleable applications.

Many pharmaceutical firms had done as Novartis did, establishing their research and development departments close to MIT, the Broad Institute and the Whitehead Institute. The outpost for BioMérieux was nearby Kendall Square, and Bancel had followed from Lyons.

Like all venture capitalists, Noubar Afeyan needed people with business sense to build up companies and market scientific studies. He had been courting Bancel for some time before presenting Moderna's plans to him at the Flagship offices.

"That was the first time I had heard about therapeutic uses of messenger RNA," says Stéphane Bancel, unaware at the time that his own company, BioMérieux, had come close to the same path in 1993 (see Chapter 3). "Noubar had Rossi's data, the company had just been established and he wanted my opinion as a biochemical engineer."

Bancel had worked for the pharmaceutical group Eli Lilly and Company, the manufacturer of Prozac, and gained solid experience in the production of recombinant proteins. These proteins were produced by genetic engineering in bioreactors and then injected into patients.

"He wanted me to get involved, but I was pretty sceptical. He told me I could be whatever I wanted – investor, board member or CEO."

Applying Cartesian logic as any good Frenchman would, Stéphane Bancel thought things through.

"My first question was if it was possible to have a product before we burned through the investors' money. Most biotech companies crash, so do most start-ups, and this was both at once. Also, since no one had ever produced an RNA-based therapy, it was impossible to know whether it would take two years to have a solid candidate or 50 years or forever."

The project had piqued his curiosity nonetheless, and his experience with recombinant proteins had opened his eyes to RNA's potential.

"Two-thirds of the proteins produced from our DNA are intracellular or express in the cell membrane," Bancel explains. "That means you cannot produce them in bioreactors and inject them like insulin. The only solution is to produce them *in vivo* using messenger RNA. The technology had never been proven, of course. And yet once you have one approved product, there is a clear pathway to hundreds of other products."

A leap of faith

At their home in the historic Beacon Hill area of Boston, Bancel talked through the plans with his wife. "I told her, it's extremely risky but if it works, it will change medicine. It may be an even bigger opportunity than the recombinant proteins that made biotech firms a fortune."

His American wife encouraged him to take a leap of faith, a bit like Indiana Jones in the Last Crusade.

With the USD2 million invested by Flagship, Tim Springer and Stéphane Bancel during the first round of financing, Bancel rented a small laboratory, and

moved Moderna to premises at 200 Technology Square, a hundred metres from BioMérieux's offices.

Jason Schrum, originally the company's sole employee, set about finding out if there were alternatives to Karikó and Weissman's pseudo-uridine invisibility cloak, so that Moderna could avoid buying a licence of the patent. Bancel also realised that transporting the RNA would be a huge challenge. The option Rossi had developed was unsuitable for human applications, and the kind Alnylam was using at the time had been developed for interfering RNA 100 times smaller than mRNA.

"My first hire was a chemist," Bancel says, stressing that RNA transport was "the number one problem." The rest of the team were biologists, and their first job was to test if it could really work. "I asked them three questions," Bancel continues. "What are the limitations, particularly in terms of toxicity? What are the constraints, for example modes of injection? And what is the potential?"

The team conducted three separate experiments between summer 2011 and summer 2012 to answer these questions. They tested huge doses of mRNA on cultured cells and animals to find the upper toxicity limit. They tried different modes of injection (subcutaneous, intradermal, intramuscular, and intravenous) to find the most effective route of administration.

Bancel also asked them to carry out trials on mice, for the production of a hundred different proteins (insulin, EPO, growth factors, etc.) to get an idea of the potential scope.

"There we clearly saw the advantage of having a technology platform," Bancel says. "You need very little cell culture. Once you have produced the messenger RNA you want [from a DNA matrix from bacteria DNA called plasmids], you use enzymes which make lots of copies of this matrix to make lots of messenger RNA. The production process is always the same, and you can switch from one type of RNA to another in a month. For recombinant proteins, on the other hand, it's more like a year. That's because you can't risk contaminating your new product with cells from the old one."

Swiss investment

Bancel's industrial logic proved crucial. In 2012, however, Moderna was still a long way from having anything to industrialise. The results with mice may have been encouraging but it is notoriously difficult to make the move from rodents to humans. As a venture capitalist once remarked, "Today, we know how to cure any cancer – that is in mice!"

Keen to refine their results, Moderna's scientists went to Montreal to carry out two experiments on primates. The similarities between monkey's immune systems and ours would give a much clearer picture of the possibilities and limitations.

"It's very expensive," Stéphane Bancel says, "but we decided to do two experiments because if you do only one, people can always say that you got lucky."

Now that he had a clearer overall picture of mRNA's limits and potential, Bancel had to convince possible funders. In June 2012 he attended a meeting at

the Hôtel Métropole in Geneva, where a financier had assembled a small group of Swiss investors. Ernest Loumaye, founder of several biotech companies including ObsEva in Switzerland, remembers Bancel's presentation clearly.

"He told us that the company would have the first results of its technology from testing growth hormone factors on primates in two months' time. And that we could wait for these results before firming up our financial commitment."

Two months later, the primate experiments showed that their cells had indeed produced proteins. These were erythropoietin, which boosts red blood cell production, and GCSF, a hormone that stimulates production of white blood cells. These first results encouraged investors in Switzerland and elsewhere to release USD20 million as part of a second round of funding. They were joined the following year by a number of new investors including Banque Pictet. The serious business was about to start.

Until now Moderna had kept its plans under its hat. The company had evolved in stealth mode and its ultra-low-key website contained only a vague description of its activities.

On 6 December 2012 Moderna sent out its first press release, in which Noubar Afeyan announced, "Moderna's promise rivals that of the earliest biotechnology companies." An online technology media even went as far as to compare the firm to a future Genentech, the biotech star of the Roche Group. Not so fast.

Moderna's laboratories had some way to go. The first question was over which therapeutic area to concentrate the application of mRNA. Growth factors for red and white blood cells, chosen for the primate trials, were of particular interest to Moderna's cardiologist co-founder Ken Chien. In March 2013, these experiments formed the basis for the firm's first joint project with a big pharmaceutical company. AstraZeneca signed a USD240 million contract for forty drug options, including an innovative application for the cardiac muscle (see Chapter 9).

A eureka moment with lipids

Another interesting area was rare diseases: there were no available treatments and clinical trials were easier to organise than for other therapeutics. The Orphan Drug Act of 4 January 1983 provides generous support in the US for the development of new treatments for diseases with a small market, extending from tax credits through to 50 per cent of the cost of clinical trials, and accelerated authorisation by the Food and Drug Administration (FDA). It is one of the factors behind the success of US biotech firms. In Moderna's case, it led to a USD100 million deal with biotech company Alexion in early 2014.

During 2013, an event beyond Moderna's control was to have a huge impact on the company's fortunes; Stephen Hoge, a doctor, visionary scientist, and now the company's president, called it "a turning point" during the company's Vaccines Day on 14 April 2021, when Moderna presented its developments.

Remember all the trouble Drew Weissman had getting hold of lipid nanoparticles from Canadian company Tekmira and Boston based Alnylam? Those companies were involved in a lawsuit that had been running since 2011.

“I asked them three questions,” Bancel continues. “What are the limitations, particularly in terms of toxicity? What are the constraints, for example modes of injection? And what is the potential?”

Moderna has obtained some lipid nanoparticles from Tekmira, which had since been renamed Arbutus, but for legal reasons Moderna could use these so-called MC3 liposomes for research only and not for clinical trials.

"Stephen Hoge came into my office sometime during summer 2013 and began to sketch an explanation linking messenger RNA, lipid nanoparticles and industrial processes," Stéphane Bancel says. "It was pretty complicated, and I made him repeat it twice."

This was Moderna's eureka moment. Stephen Hoge's explanation led to a logical conclusion: mRNA was not just therapeutic, it was also a vaccine technology. By using mRNA to induce an immune response, it was possible to build immune defences against an infectious disease and even against cancer cells – options CureVac and BioNTech were already exploring at the same time.

You may think Moderna had several irons in the fire now. And, now that it had made its media debut, it was the target of a number of attacks. The *Boston Globe* and the influential website StatNews questioned the company's seemingly overblown ambitions, and the impetuousness of its French boss.

Choosing from a cornucopia

Moderna had discovered a cornucopia in mRNA but had to make a choice. "For a long time we were wondering if it was important to use modified RNA [like Karikó's] or not. We concluded that it was," says Bancel.

Attempts to develop its own modified RNA bases had proved unsuccessful, and Moderna bought a wide-ranging licence to Karikó and Weissman's discovery from the University of Pennsylvania and Crucell, who own the patent. "It cost us USD75 million," Bancel comments.

Their research into lipid nanoparticles continued. Moderna's scientists first used the MC3 vector Tekmira had developed for Alnylam's interfering RNA. Though effective in the liver, it degraded in other parts of the body, limiting its usefulness. This led the company to develop its own lipid formulations. "The one in our Covid vaccine disappears in two hours," Bancel states.

A fleet of applications

The strategy Bancel and his board adopted was to consider the wide range of possibilities for using mRNA. They justified building a whole fleet of applications. So, between 2013 and 2016 Moderna set up four subsidiaries, each specialising in a different field: Valera in infectious diseases, Elpidera in rare diseases, Onkaido in oncology, and Caperna in customised anti-cancer vaccines – a double commitment to cancer that wasn't to last. Moderna dropped this plan in 2017 but did not lower its ambitions.

In 2016 the company had been running no fewer than fifty programmes of preclinical trials. Stéphane Bancel had managed to raise a total of USD1.6 billion prior to Moderna's record-breaking IPO in December 2018. This stock-market flotation valued the company at USD7.5 billion – a value that was to increase twentyfold by August 2021.

Clinical trials, however, are the ultimate arbiter in the biotech industry: Moderna's first was to have a decisive impact on what came later.

On Moderna's *Vaccines Day* in 2021, Stephen Hoge reminded his audience that "we gave a dose to our first subject in a clinical trial in December 2015. For a vaccine."

Bancel explains more. "It was our first product, a vaccine against H10N8 flu, which is endemic in Germany. We'd organised animal challenge trials first," he says. The animal trials involved injecting the flu virus into vaccinated and unvaccinated mice to test whether the vaccine worked. Everyone was stunned when the results came in.

"The increase in the acquired immune response was incredible," recalls biologist Melissa Moore, advisor to the company at the time, now its chief scientific officer. Like Jon Wolff (see Chapter 2) or Ingmar Hoerr (see Chapter 5), Bancel thought there must have been an error and asked for the experiment to be repeated.

"But there was no doubt that it worked and that gave us the confidence to take the next industrial gamble on the Norwood plant," he explains.

Platform capitalism

Although they did not lead to a commercial product, the results of the H10N8 flu vaccine and its early clinical trials had a profound impact on the company. In September 2016, Moderna invested USD110 million in a two-year scheme to transform a former Polaroid factory in Norwood, south-west of Boston, into an industrial platform for the future. It was virtually unheard of for a biotech firm with no approved products (not even any advanced clinical trials), to invest in a production facility.

"Moderna took the same approach as Amazon or Tesla. Spend a lot of money before making any to corner the market," says the Swiss entrepreneur Andrin Oswald, head of Centogene after 10 years at Novartis.

But the facility also enabled Moderna to capitalise on and investigate mRNA's many uses. "You always make messenger RNA from the same four chemical bases [the four nucleosides or nucleic acids in RNA – A, C, G and U]," Bancel says. "Which means that you don't have to change much to produce different RNA expressing different proteins. It's very similar for lipid nanoparticles. If it works for one, you can produce others with small tweaks. You don't need to rethink the whole industrial platform."

Norwood, whose production capacity has doubled during the pandemic, produces Moderna's range of mRNA. The company has taken this platform logic further: its mRNA Design Studio produces mRNA on demand. This facility allows both in-house and external scientists to design bespoke mRNA on their computers, which is then made by an automated, modular production line. Researchers submit their desired digital blueprint of the sequence to obtain a sample – similar to any company buying computing power from Amazon Web Services or Microsoft Azure.

The quest for immunity

The H10N8 results altered the company's priorities. Alongside its own research, Moderna naturally continued its cancer vaccine collaboration with Merck, and its work with Vertex, to find a treatment for cystic fibrosis. It also expanded its programme with AstraZeneca, covering everything from cardiology to cancer. It maintained ambitions to find mRNA capable of producing proteins for rare diseases. With the flu vaccine, the company would focus on boosting immunity with antibodies produced from mRNA within the body's own cells.

Moderna began honing its pandemic response skills in September 2016, with an mRNA vaccine against the Zika virus. The virus subsided, but the programme established a lasting relationship with the Biomedical Research and Development Authority (BARDA), which would contribute approximately USD1 billion to the company's Covid vaccine development costs in 2020.

Like CureVac (see Chapter 5), Moderna also began working with the Defense Advanced Research Projects Agency (DARPA) through their ADEPT programme. This eight-year, USD291 million scheme was intended to generate therapies and vaccines capable of countering an epidemic caused by an emerging pathogen or a bioterror attack. The man behind it, Dan Wattendorf, a former US Air Force physician, was an advocate of mRNA technology. He first worked with Moderna on clinical trials of antibodies against Chikungunya, a virus contracted from infected mosquitoes.

When Wattendorf joined the Bill & Melinda Gates Foundation in 2016, he came to continue to collaborate with Moderna to launch a different project focusing on AIDS antibodies. Moderna organised clinical trials for a vaccine with the International AIDS Vaccine Initiative founded by Seth Berkley, now CEO of Geneva-based Gavi, the Vaccine Alliance. As of early summer 2021, Moderna's twenty-four clinical programmes included ten prophylactic vaccines. The company has just launched its first clinical trial for an AIDS vaccine.

There were other signs of how infectious diseases and vaccines were making their way up Moderna's list of priorities. One was the addition to the board of immunologist Moncef Slaoui (see Chapter 7), with his thirty years of experience in vaccine development at GlaxoSmithKline. He stayed at Moderna for only three years before getting a call from the White House to go and lead Operation Warp Speed. Furthermore, although vaccines accounted for only USD35 billions of a pharmaceutical market worth USD1 trillion in total, there is logic to developing a more preventive than curative form of medicine.

It was a significant moment when Moderna developed a vaccine containing six different mRNA varieties against cytomegalovirus (CMV) – a form of herpes. Although most people tend not to notice this form of the virus – it usually takes a benign course – it can drain the immune system and distract it from its constant battle to destroy cancer cells and prevent them from taking hold.

"Like Epstein-Barr virus, multiple studies have shown that CMV is linked to reduced life expectancy," Stéphane Bancel says.

Moderna obtained good results during phase two trials of its CMV vaccine in 2019, which led to an unexpected side effect: on 2 March 2020 Bancel found

himself called to the White House by Donald Trump, along with the CEOs of all the biggest pharmaceutical companies.

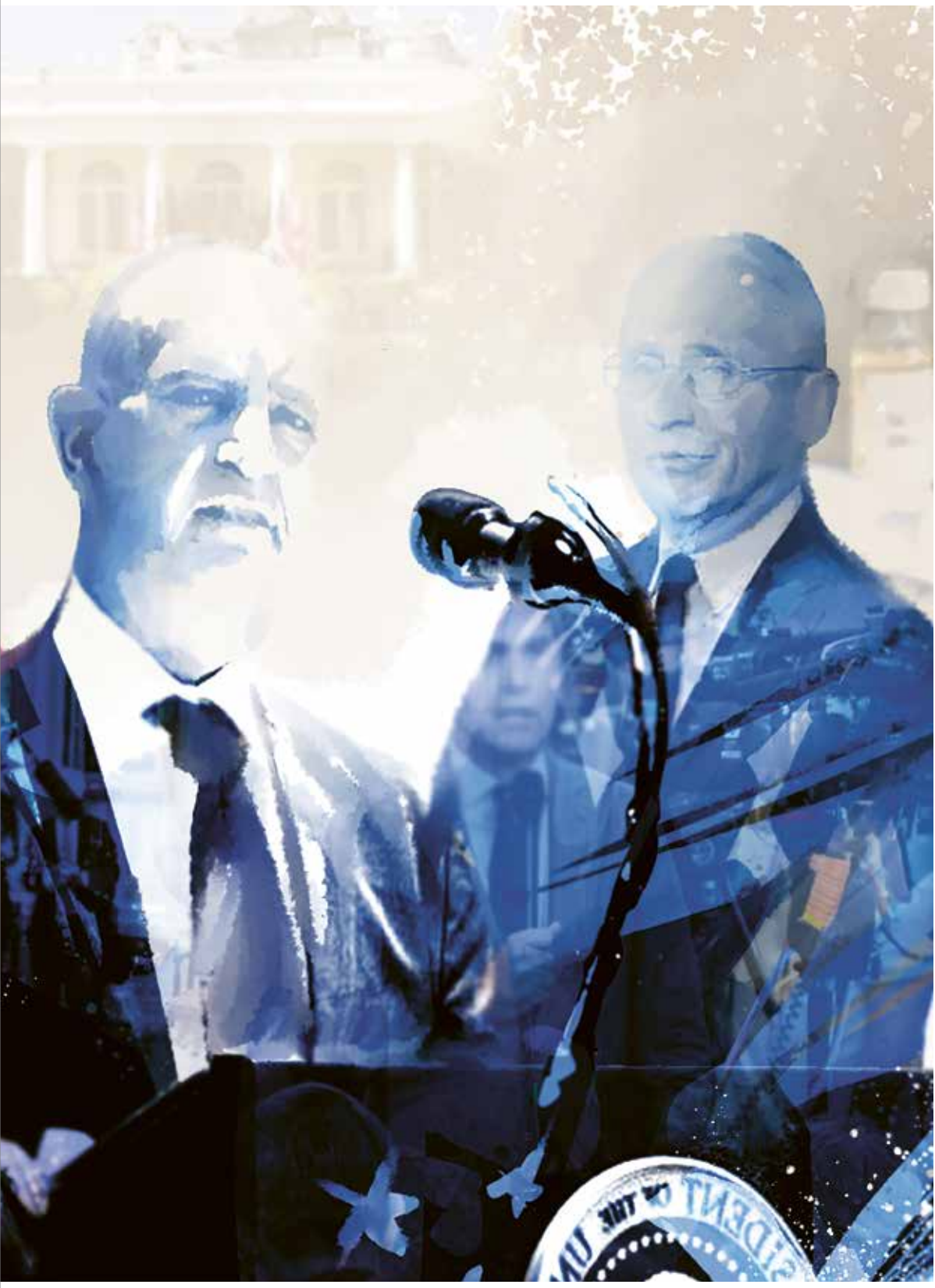
The president wanted to know how long it would take to develop a Covid-19 vaccine. Bancel's rivals around the table suggested a few years but the Moderna boss was able to give a clear answer, with a confidence built on 600 patents, fifty scientific articles and the promising CMV clinical trials. What's more, his teams had come up with an RNA candidate vaccine within weeks of China publishing the genomic sequence of coronavirus.

"In our case, we're talking about months," he told Donald Trump. And the countdown began.

On 2 March 2020 Bancel found himself called to the White House by Donald Trump, along with the CEOs of all the biggest pharmaceutical companies

The White House's Operation Warp Speed was a big driver of the race to find an RNA vaccine for coronavirus. There are now approved British, Chinese, Russian, Cuban and Indian vaccines, but the first approved and widely available ones across the world were mRMA vaccines

Warp Speed boss Moncef Slaoui (right) organised the biggest vaccine race in history.



Chapter 07

The race for mRNA vaccines as told by its referee

The White House's Operation Warp Speed was a big driver of the race to find an RNA vaccine for coronavirus. There are now approved British, Chinese, Russian, Cuban and Indian vaccines, but the first approved and widely available ones across the world were mRMA vaccines. Moncef Slaoui organised and refereed the scientific and clinical competition for Trump, in which start-ups beat big pharma hands down.

07

It was March 2013 and Andrew Geall, the then head of RNA vaccine research for Novartis, took an urgent phone call. His boss, Rino Rappuoli, told him that three people in China had been infected by a new strain of bird flu. He was asked if his group was capable of developing a vaccine fast.

Geall didn't need the urgency spelled out to him; the WHO regarded mutations of the bird flu virus capable of jumping the species barrier as the main likely source of a future pandemic.

His group had already developed an RNA vaccine (self-replicating, as Chapter 9 will reveal) against a respiratory alphavirus. It had been tested successfully on mice. Having received the genome sequence of the new bird flu strain, the sixty-strong team at Novartis set to work.

"Three days later we were synthesising the first RNA. By the end of the week, we had tested the first vaccine candidates on cells and a week later on mice." In short, Novartis had managed to develop a vaccine candidate within a month.

The troubling surrender of big pharma

It is the speed of development that is the most stunning characteristic of the RNA vaccines now keeping Covid in check even if boosters may be needed because of variants. But in this race, the big global vaccine makers – Novartis, Sanofi, Merck and GlaxoSmithKline – were laggards. Why did they fall behind?

Novartis's experience with bird flu back in 2013 suggests why the big pharmaceutical companies have struggled to keep up in the field of mRNA vaccines: for one thing, Andrew Geall's RNA vaccine was never tested on humans after that epidemic quickly died down.

Novartis, who had strengthened their position on the vaccine market in 2006 by acquiring Chiron, decided around the same time to sell off the division, focusing instead on more profitable treatments.

The sale proved a complex transaction. US anti-trust laws prevented the Swiss group from selling its flu vaccine operation to rival GlaxoSmithKline (GSK), and it was bought instead by the Australian firm CSL in 2015, consolidating activities at its North Carolina laboratories. This led to the disbanding of the RNA vaccine research team, whose members moved to RNA leaders like Moderna and Pfizer. Phil Dormitzer was among them. His move to Pfizer might well have contributed to the company's success, in partnership with BioNTech, in being first across the line with a useable vaccine.

Moncef Slaoui, who had been GSK's long-time head of vaccine research before moving to Moderna and eventually leading Operation Warp Speed, gives a little more detail on the problems there.

"The large vaccine corporations all suffered during previous pandemics. When the H1N1 influenza strain appeared in 2008-9, we had to defend ourselves at GSK against accusations that we'd created the epidemic in order to profit from it. Then, with Ebola in 2014, we worked around the clock for seven months, and the virus simply stopped. Same with Zika in 2016. Those were investments that never paid off, along with the opportunity costs. When your teams are mobilised on a vaccine emergency, they fall behind on other health projects that are just as important."

A Warp Speed breakaway

Warp Speed fuelled the breakaway from the peloton in the vaccine race. By mid-April 2021 there were no fewer than 184 Covid vaccines at the animal-trial stage and eighty-four undergoing human trials, while thirteen have been approved. But it was the RNA candidates selected by Warp Speed that won the race. It's these that have shown the highest efficacy so far, and that have been most widely distributed.

"It all began with a call from Jim Greenwood," Slaoui says. At the time, the former Republican congressman was president and CEO of BIO, the American biotech lobbying group. He was phoning to ask Slaoui to lead Operation Warp Speed. "I told him that I could only accept if I had a free rein and no political interference." Greenwood answered, "the administration will call you."

Slaoui had two reasons for wanting carte blanche to do as he saw fit. It was widely known that the Trump administration was a treacherous place for anyone whose politics didn't align with those of the president. More importantly, Slaoui had spent his whole professional life fighting pandemics.

"There's no time in a pandemic to get distracted by back-room bargains and killer tweets," he says.

When he took up his position on 15 May 2020, there were ninety-four vaccine projects taking place across the globe, five or six of which were in the first phase of clinical trials.

"It was impossible to examine them all. We decided to select eight in four technology tracks based on criteria of their probable efficacy, safety, speed and suitability for large-scale production."

That selection process was condensed into just four days.

Four competing technologies

The conventional vaccine pathway was excluded from the outset, considered by the operation as "slower and also more dangerous."

"You have to cultivate living viruses for these traditional vaccines," Slaoui says, "and so you have to make sure there is no contamination risk and no possibility of leaks into the environment. For attenuated viruses, which is the other conventional vaccine pathway, the difficulty is striking a balance between sufficient virus replication to obtain a solid immune response and overdoing it, in which case it becomes a pathogen. That takes a long time."

The four technological platforms that made the cut included the Oxford/AstraZeneca and the Johnson & Johnson DNA vaccines.

"The day I came into post we announced a first order for 300 million doses from AstraZeneca," he says.

In the second category were the recombinant protein vaccines already used in the hepatitis B and papillomavirus vaccines. Here, the Novavax and Sanofi-GSK candidates were selected. Slaoui also inherited two attenuated vaccine projects, from Merck in partnership with the International AIDS Vaccine Initiative (IAVI). These had been developed not from the Covid virus but from Indiana vesiculovirus (a rabies-related virus). These had already been shown to work against Ebola and a measles virus.

Also in the mix were two mRNA vaccines from Moderna and Pfizer-BioNTech.

USD 10 billion for Warp Speed

Originally called Manhattan Project 2.0, Operation Warp Speed was launched in late March 2020 as a public-private partnership to accelerate vaccine and therapeutic developments. Its initial budget was USD10 billion.

Slaoui's personal interest in Moderna (though he sold his shares when he took up the US government post) raised questions over possible conflicts of interest. But as this was a new technology, Slaoui saw his personal connections as an advantage. "Moderna had received the phase II results of an RNA vaccine against

cytomegalovirus in 2019. They showed that vaccinated individuals developed more antibodies against the disease than people infected naturally. That inspired a lot of confidence in RNA technology's potential."

This forecast was confirmed by Moderna's Chief Scientific Officer, Melissa Moore. "Using the genome sequence of the virus, we chose a single messenger RNA coding the virus's spike protein. That was how confident we were."

Big money

At BioNTech, Uğur Şahin had been testing four different versions of RNA vaccines since mid-April – two modified with pseudo-uridine at different dosages, one without and one with self-replicating RNA. At the same time, his German rival CureVac, using Acuitas's lipid nanoparticles, was slowed by the search for the optimal dose. A prior trial for rabies had shown a risk of toxicity connected to dosage. Moderna was therefore out in front, with phase I trials (during which small groups of humans are given the vaccine) underway by 16 March, a mere sixty-three days after the publication of the genome sequence. By mid-May AstraZeneca was also in phase I, while Johnson & Johnson, Merck, Sanofi and Novavax were still at the animal trials stage.

The phase I trials had already been financed by the companies themselves and by public agencies. The role of Operation Warp Speed was to fund the most expensive and most difficult part of the trials – phase III on groups of roughly 30,000 people in hundreds of clinical centres. "They cost about USD800–900 million per trial," Slaoui says.

The rules of the game

Slaoui and General Perna agreed on a sleek organisational structure for Warp Speed. Slaoui would oversee research and development, Perna logistics. A task force was created, to include the Secretary of Health: the Defence Secretary (a role whose occupant changed three times in the period); Anthony Fauci; NIH director Francis Collins; Robert Redfield, head of the CDC; Dr Deborah Birx; and, finally, President Trump's son-in-law, Jared Kushner, to monitor the process.

"Flexible supervision," says Slaoui. "The task force met only once a fortnight."

For daily monitoring and rapid decision-making, Slaoui set up a team composed essentially of BARDA scientists and, at first, the head of the US Food and Drug Administration. However, when the board became too influential in the process, Slaoui replaced it with Matt Hepburn, an epidemiological specialist from DARPA, the military research body.

"To be able to act fast, we needed to work without any bureaucracy and without a committee of independent experts who would weigh up five different solutions," Slaoui explains.

But you still need some rules to referee a race. Establishing these mainly involved drawing up a clinical protocol for measuring comparisons.

"We agreed on the definition of a Covid case for the sake of homogeneous results," Slaoui says. "Then we defined six essential criteria which, if fulfilled, would give access to funding."

Merck received USD38 million, Johnson & Johnson USD1 billion, AstraZeneca USD1.2 billion, Moderna USD1.53 billion, Novavax USD1.6 billion, and Sanofi-GSK USD2.1 billion. As for Pfizer and BioNTech, they funded their clinical trials themselves, only requesting assistance in selecting clinical centres.

"Since it was impossible to predict where the epidemic would hit hardest, and therefore where the results would be fastest, we had to recruit the huge number of 100 to 200 centres for each trial, covering the whole territory," Slaoui says.

The field narrows

Spring 2020 began with the first shedding of companies still stuck in the pre-clinical phase.

"The main risk with DNA vaccines is viral vectors," Slaoui explains. "Johnson & Johnson's and Oxford/AstraZeneca's vectors had already been tested for other vaccines, so they were able to start fairly quickly."

Johnson & Johnson were able to bring forward their planned phase I trials from September to July, and AstraZeneca started their phase III on 31 July, its advance on Johnson & Johnson now cut to three weeks.

Things weren't looking quite so good for the other technologies. On the recombinant protein track, Novavax had launched its phase I trial on 25 May but only started phase III testing at the end of September.

"The method was well known, but since it supposed producing copied proteins from sub-particles of the coronavirus's S protein, purifying and stabilising them is a serious challenge," Slaoui says.

Sanofi and GSK would pay the price. Based on cell lines from flies, the technology initially produced not only particles of S protein but also another similar one, which hampered purification, resulting in some batches containing between 50 per cent and 70 per cent contaminants.

The result was that the phase II trials only began in September. Further delays were announced in December 2020, this time due to a dosage error. Phase II testing resumed in February 2021. By then Merck had thrown in the towel after a disappointing first phase. Novavax and Johnson & Johnson, on the other hand, announced efficacy of 89 per cent and 70 per cent respectively, and applied to the drug administration for authorisation.

Three months after the RNA vaccines

The phase III trials launched in July 2020 by Moderna and Pfizer-BioNTech, and in August by AstraZeneca, were no easy ride.

One week after starting its trial in the US, the emergence of some neuronal side effects meant that AstraZeneca had to put testing on hold. The FDA requested more detailed data, including on the Oxford University scientists' ten years of experimentation with the viral vector. It took six weeks to produce this, delaying progress further. The American trials did not resume until late October. AstraZeneca's DNA vaccine was approved in the UK in late December and in

“There’s no time
in a pandemic
to get distracted by
back-room bargains
and killer tweets”

Europe at the end of January, on the basis of parallel trials conducted in Great Britain, South Africa and Brazil.

In September, Pfizer announced that despite already having 44,000 patients for its trials, it would be recruiting another 44,000. Officially, this was for the purpose of including teenagers aged 16–18, and immune-depressed patients, but it looked as if Pfizer needed to rebalance the ethnic demographics of its test patients to represent the diversity of the US population, which meant roughly a third African-American, Hispanic or Asian-American. The company abandoned its first preliminary evaluation, concluding that the placebo group had 32 more infections than the vaccinated group, although the full results were never published.

Pfizer-BioNTech announced its results on 9 November – a difference of ninety-four cases, meeting the bar set for the second preliminary assessment. Too late, though, for Donald Trump to declare success before the presidential elections. Moderna followed a week later (151 cases).

The RNA vaccines demonstrated over 90 per cent efficacy. The FDA would have been satisfied with 50 per cent. In the following weeks, the two victorious vaccine candidates were awarded emergency use authorisation in the United States and Europe. Now it came down to logistics. Here too, the new technology would triumph, despite being more expensive and requiring a more complex cold chain.

A sprint to industrialise

The companies hadn't waited for the clinical trial results before stepping up their production capacity. Since mRNA synthesis is essentially a chemical operation, it doesn't require cell cultures of the kind needed for viral vectors, which must be more strictly confined. But the challenge of encapsulating mRNA in nanoparticles remained.

"It is not the same thing to produce 10 litres in a laboratory or 500 litres in a factory," Slaoui observes. "Fundamentally, though, the infrastructure required for RNA is smaller."

Moderna had experience from its factory in Norwood near Boston (see Chapter 6). It passed on this knowledge to its Swiss production partner, Lonza. BioNTech, which had never managed production on anything like this scale, was helped by its partner giant Pfizer. In both cases, involvement in Warp Speed ramped up their operations.

Warp Speed pushed various ministries to invoke the Defense Production Act no fewer than eighteen times, to obtain priority access to equipment and material – especially lipids. The US Army was also called in to help with logistics, closing highways and ringfencing airport runways.

The US administration was much criticised for its pandemic response. More than 600,000 deaths were recorded in America as of late July 2021. But it proved its technological and logistical ability during the vaccine roll-out, delivering approximately 350 million doses, mainly of the two mRNA vaccines, by the same date.

Slaoui sees another triumph. "Warp Speed's pre-order contracts covered all development costs with the proviso that the first 100 million doses were reserved for the United States as well as options for future orders. The European contracts, on the other hand, cover only supplies of doses."

Slaoui has now resigned from Operation Warp Speed and moved on to pastures new. He's certain that demonstrating RNA's efficacy in vaccines was just the start. "RNA promises a medical revolution that is going to keep us busy for at least the next ten years."

The technology can be applied to almost any disease. Cancer, for starters – the priority of the winner of the vaccine race, BioNTech.

The *raison d'être* of BioNTech, founded thirteen years ago by two Turkish-German oncologists, Uğur Şahin and Özlem Türeci, was to develop cancer vaccines

Kati Karikó (centre) joined BioNTech founders Ugur Sahin and Özlem Türeci.

Chapter 08

BioNTech: where is it headed?

Founded by a couple of oncologists, BioNTech's primary goal is to use messenger RNA to teach our immune system to overcome cancer. All cancers. To do this, it is developing anti-cancer vaccines that are likely to be the first applications of RNA beyond Covid-19. The third medical revolution, that of messenger RNA nucleic acids after those of chemical and then biological drugs, has already begun in Mainz, Germany.

08

In the Paddock Club at a Formula 1 race, each team has an open bar for VIPs and journalists. Each team except two: Ferrari and Mercedes, both guarded like Fort Knox. In the race for pole position in the medical revolution promised by mRNA-based treatments, Moderna is Ferrari and BioNTech is Mercedes. What everyone is wondering is what BioNTech is going to do after winning the race to find a Covid vaccine.

The *raison d'être* of BioNTech, founded thirteen years ago by two Turkish-German oncologists, Uğur Şahin and Özlem Türeci, was to develop cancer vaccines. Developing the Covid vaccine wasn't the ultimate challenge for the German company; its overarching goal is to cure cancer. For every form of cancer, to be effective treatments need to be personalised for every patient. mRNA makes this possible. What's more, it does so without financially flooring our health systems because here, as for vaccines, the method is to teach the body to heal itself.

The windfall from the Covid vaccine will allow BioNTech to invest up to USD850 million (the equivalent in a single year of its entire investment over the past twelve years) in this research, starting this year. The company is running eleven clinical trials involving 440 patients and seventeen different forms of cancer.

An all-time record

Flashback to Saturday 25 January 2020. Uğur Şahin was sitting at his computer. The epidemic that first appeared in China didn't yet have a name, and it wouldn't be until 30 January that the WHO would declare a state of emergency. Four days earlier, however, the first cases had been detected in the United States, and in France just the previous day. Şahin sensed a pandemic was on its way.

Like many others, he had the complete genome sequence of the coronavirus, published by the Chinese authorities on 12 January. On his computer he produced ten models of mRNA coding for the spike protein that allows the coronavirus to enter cells and multiply. The teams at BioNTech later developed ten other versions of this molecule that, following vaccination, acts as the wanted notice to antibodies and other white blood cells for recognising the coronavirus. One of those versions led to the first Covid vaccine approved by the British Medicines and Healthcare Products Regulatory Agency (MHRA) on 2 December 2020.

The results of the trials of this BNT162b2 vaccine were published eight days later, in the *New England Journal of Medicine*, which hailed it a "triumph." During the trials, the vaccine created by Şahin and his staff was the most effective, a whisker ahead of Moderna's.

The complexity of cancerous tumours

When he sat at his computer in January 2020, Şahin was already aware of mRNA's potential to produce an antigen protein in the body, capable of triggering an immune response. He and his wife Özlem Türeci had been working with BioNTech's research team for over ten years to develop synthetic RNA as a means of activating the immune system. Their target, rather than viruses, was cancerous tumours. Among other things, the mutations caused by cancer can make them invisible to our natural defences.

A mountain of previous research had also taught Şahin that the mRNA needed to code the coronavirus spike protein for antibodies to recognise it must not be a *copy* of the natural virus – rather, it had to be a slight variation.

The maths

The sequence of BioNTech's mRNA, published by the WHO, is made up of precisely 4284 characters composed of the four constituent bases of RNA – A, C, G, and U, or more accurately, pseudo-U. Like Moderna, BioNTech used Kati Karikó and Drew Weissman's pseudo-uridine (see Chapter 4). And like Moderna,

BioNTech paid the University of Pennsylvania and Cellscript USD75 million to obtain a licence to use this nucleoside.

There are sixty-four possible combinations of these four nucleosides – the 64 codons – but there is a total of only twenty amino acids. This means that several codons produce the same amino acid. It is therefore possible to replace one with another. By increasing the codons containing more G and more C, Şahin managed to achieve an mRNA that produces more proteins and, logically, stronger immunity. A technique also used by CureVac (see chapter 5).

He made other innovations too. One was a customised cap, allowing the RNA to be clearly identified by ribosomes, the protein factories, in the cells. BioNTech also slightly modified the tail of its mRNA to slow down the enzymes that degrade it, giving it a longer life and a better chance of generating maximum proteins. Last but not least, this mRNA was also designed to concentrate a specific amino acid (proline). The result is that the spike proteins do not lose shape, keeping them as close in form as possible to the natural virus, for when they finally meet the immune system.

A lower dose than Moderna

Şahin demonstrated his perfectionism at every stage of development of the mRNA vaccine. The efficacy of BioNTech's Covid vaccine resulted from these improvements, as well as its efficiency: this vaccine contains 30 micrograms per dose whereas Moderna's has 100. Moderna used its own formula, whereas BioNTech joined forces with Canadian biotech company Acuitas.

Şahin also brought his perfectionism to the final-phase clinical trials of the Covid vaccine in April 2020. They tested four versions to compare different doses, and even a different technology (using self-replicating RNA).

25 February 2020: outline on the computer. 23 April: first injection on a German volunteer!

Speaking of speed, Şahin named the project Lightspeed. But it wasn't just the pace of the mRNA vaccine project that was remarkable. The commitment shown by the company's staff was admirable: from the end of February, BioNTech's 1,000 employees reorganised their schedules to work seven days a week. All leave was cancelled. Everyone's efforts were focused on pushing the project forward.

Steamroller Pfizer

Nevertheless, BioNTech had neither pandemic-scale production capacity nor the necessary clinical trial arrangements. According to the *Wall Street Journal*, these issues were settled over the phone on 1 March 2020, the night before Donald Trump's meeting with pharmaceutical company bosses.

Şahin talked to Kathrin Jansen, head of vaccine research and development at Pfizer. Sixty-two-year-old Jansen, who started out at the Glaxo Institute for Molecular Biology in Geneva in the late 1980s, had scored two huge vaccine successes in the past: Gardosil against papillomavirus, developed with Merck; and Prevnar 13, a pneumococcal vaccine on which she worked at Wyeth.

Following Pfizer's USD68 billion acquisition of Wyeth in 2009, Prevnar 13 became the cash cow of a group that had invented Viagra but was absent from the vaccine market. Prevnar 13 is Pfizer's best-selling product and the highest-grossing vaccine worldwide in 2020. No need to say that Kathrin Jansen had the ear of Pfizer's big boss, Albert Bourla. So when she took Şahin's call and expressed her enthusiasm, it was as good as a done deal.

Jansen had also studied in West Germany after her parents fled from the East just before the Berlin Wall went up, so there were geographical links between her and Şahin. And the professional relationship had a precedent too – Pfizer and BioNTech had been collaborating since 2018 on an RNA flu vaccine, BioNTech's only vaccine for an infectious disease.

The call put an American steamroller at the service of the German company. The Greek-born Albert Bourla, a trained veterinary surgeon, began signing cheques. He turned down Warp Speed funding for clinical trials of the BioNTech vaccine, investing USD2 billion of the multinational's own funds instead. Even before the first results were in, he ordered the reorganisation of a factory in Massachusetts to produce vaccines for clinical trials and bought seven machines costing USD200 million apiece to gear up for mass production.

In March 2020, scientists from Pfizer and BioNTech began to whittle down the number of RNA candidates, through animal testing. By 12 April, four remained in the running. Şahin took the decision to test them all and two frontrunners emerged – one produced a partial spike protein, the other a complete version. The latter was selected on 23 July, the day before Pfizer was required to submit its dossier to the US Food and Drug Administration for the large-scale clinical trials.

After the honeymoon

Developing the Covid vaccine has earned BioNTech and Pfizer a fortune – an estimated USD26 billion in 2021, divided 50/50 between the two partners. BioNTech's profit in just the first quarter of 2021 was EUR1.13 billion on a turnover of EUR2.1 billion; until the start of this year, the company had only ever made a loss.

The pandemic has delivered something else BioNTech had never before had factories. In Marburg, north of Frankfurt, it acquired from Novartis the Behringwerke, built in 1904 by Emil von Behring with the money from his Nobel Prize in Medicine, the very first awarded, for his research into diphtheria and tetanus. The price of BioNTech's transaction with the Swiss group was not made public, but the announcement came a few days after BioNTech received a EUR375 million grant. This site, which came on stream on 10 February 2021, employs 3000 staff and can produce 750 million vaccine doses per year. The company has also declared its plans to build an mRNA factory in Singapore by 2023 and another in China with its local partner, Fosun.

It's good news for BioNTech, because the honeymoon period with Pfizer appears to be over. In an interview for a talk reported by the *Wall Street Journal* on 23 March 2021, Albert Bourla said, "We like working with BioNTech, but we don't need to work with BioNTech. We have our own expertise developed."

"BioNTech's profit in just the first quarter of 2021 was EUR1.13 billion on a turnover of EUR2.1 billion; until the start of this year, the company had only ever made a loss"

A revealing comment

By the strict standards of pharmaceutical industry etiquette, Bourla's statement represented more than a simple split. It reveals much about how the future might look; Pfizer's expansion into other mRNA vaccines will reduce the group's dependence on its cancer drugs, which currently account for a quarter of its sales and one-third of all the medicines it has in development.

Pfizer's anti-cancer medications, however, are a legacy of the second medical revolution, using genetic engineering to produce recombinant proteins in cell culture. RNA marks the dawn of a third revolution, of which cancer will be the primary focus. Instead of cultivating proteins in genetically modified cells, protein expression will be induced within the patient's cells by mRNA, teaching the immune system to attack cancerous cells.

BioNTech put its cancer research on hold to develop the coronavirus vaccine. Şahin had to throw his whole weight behind the proposal to persuade his board, which, like those of CureVac and Moderna, had its reservations about this change of strategy.

However, BioNTech never took its eye off the ultimate goal of curing cancer, and Şahin has remained clear on this whenever interviewed. There's a firm grounding for the company's interest in this area. For cancer, BioNTech has a good ten-year head start on Pfizer, and on everyone else in the field.

Doctors above all else

Uğur Şahin was born in 1965 in Iskenderun on Turkey's Mediterranean coast. He moved to Germany four years later when his father was hired by a Ford factory near Cologne. His oncologist partner, Özlem Türeci, was born in 1967, after her father, a surgeon, came to Germany to work in a hospital in a small town near Bremen.

After studying medicine at the University of Cologne, Şahin followed his PhD supervisor to Saarland University Hospital in Homburg, where Özlem Türeci was completing her own medical studies. Both had chosen to specialise in oncology and were greatly affected by the helplessness and suffering of patients who had run out of treatment options. Uğur Şahin and Özlem Türeci are doctors above all else.

"They are incredibly genuine people," says Sonja Kastilan, editor of the Science section of the *Frankfurter Allgemeine Zeitung*, who has interviewed them several times.

White coat wedding

The Swiss Nobel laureate Rolf Zinknagel, who hosted them in his laboratory at the University Hospital Zurich in 2000 and 2001, told the *Neue Zürcher Zeitung*: "He is an innovative scientist, and she is an exceptional clinician with a great flair for business management."

Şahin was specialising in identifying cancer antigens at the time, and Türeci in immunotherapy. In 2002, when they were invited by the head of haematology

and oncology to join the Johannes Gutenberg University Mainz, their two closely linked areas of research were about to forge in more ways than one. The two scientists threw themselves headlong into their work and were married in their lab coats during their lunchbreak.

Şahin and Özlem Türeci were soon promoted to professorships and their specialist fields were key to all that has happened since. Cancer is a disease of the genome; it originates in DNA mutations due to copying errors that happen during the never-ending process of cell renewal. These genetic mutations produce natural mRNA that transport false information, creating antigen proteins (known as neo-antigens) on the surface of cancerous cells.

These mutations are produced continuously throughout our lives. Most of the time, their neo-antigens, which are not found on the surface of normal cells, are identified by the immune system in the same way as viruses or foreign microbes. White corpuscles detect and destroy them, preventing them from growing into tumours. But the cells are malign too and, through accumulated mutations, they create a small habitat within which they are invisible to the immune system. This micro environment is how cancer takes hold.

The age of antibodies

The central principle of the immunotherapy that emerged in the early 2000s was to replace or supplement cancer-killing chemotherapy and radiotherapy, by either standing in for the immune system or sparking it into action to attack neo-antigens.

The first immunotherapies of the mid-nineties were based on monoclonal antibodies. Produced through cell culture, they imitated the antibodies created naturally by the immune system, to provoke a targeted attack. It soon became apparent that these antibodies were able to detect and block the growth of tumour cells. That was the case, for example, with Roche's drug Herceptin, which went on the market in 1998. Since then, over seventy monoclonal antibodies have been launched, with cumulative annual sales of USD125 billion.

In 2001 Şahin and Türeci followed the same path by founding Ganymed Pharmaceuticals with funding from twin brothers Andreas and Thomas Strüngmann, who had sold their drug company to Novartis for USD7 billion. They have a proven instinct for a good investment. Among other treatments, Ganymed Pharmaceuticals developed a monocolonal antibody against pancreatic cancer, and was bought by the Japanese pharmaceutical company Astellas for USD1.4 billion in 2014.

Cancer vaccines

Monoclonal antibodies target an antigen, or sometimes two, in which case they are termed bi-specific. There are dozens or even hundreds of these neo-antigens in cancerous cells. Furthermore, 95 per cent of mutations in one patient are not present in a different patient with the same form of cancer. The ideal solution would be to personalise the treatments for each individual patient.

It was this insight that led to the creation of BioNTech in 2008, once more with the backing of the Strüngmann brothers.

In an interview with the *Frankfurter Allgemeine Zeitung*, Uğur Şahin says, "I tried to find out which structures the immune system can really identify in cancerous cells. Since the mid-nineties it has been possible to break down these tumour antigens more and more effectively."

BioNTech devoted time early on to developing a high-throughput screening system, a way of testing thousands of biological samples. It uses bioinformatics – computer analysis of biological data – to map the genetic mutations in each patient's cancers. What Şahin calls the 'mutanome' is a map of targets, which the immune system will learn to identify using mRNA vaccines.

Şahin started out by evaluating every available vaccine technology (DNA, peptide, etc.) before concluding that mRNA combinations were most efficient at producing not one but several types of neo-antigens. These, just like spike proteins, act as a wanted notice for the immune system.

Fixvac, BioNTech's main technology platform, develops mRNAs to induce the production of tumour neo-antigens from each patient's mutanome. These vaccines are then delivered directly into what are called dendritic cells, which play a preliminary role in establishing immunity.

New immunotherapeutic pathways

Şahin and Türeci had to wait until 2017 to obtain the first clinical proof of an mRNA vaccine's efficacy against cancer. They delivered a cocktail of bespoke mRNA into the lymph nodes of 13 patients with advanced melanomas. This technology was based on selecting the neo-antigens most suitable for triggering the desired immune response, after an analysis of the patients' respective mutanomes. In most cases the clinical trials succeeded. Since then, BioNTech has launched eleven clinical trials involving 440 patients and tackling seventeen different types of cancer.

Şahin and Türeci's ambitions stretch even further. Recent progress in immunotherapy has hinted at other possible mRNA anti-cancer uses. For example, Japanese biologist Tasuku Honjo and American immunologist James Allison have shown that the action of white blood cells (T-lymphocytes) on cancerous cells is reduced by a kind of molecular brake, known as a 'checkpoint'. They were awarded the 2018 Nobel Prize in Medicine. Before that, though, in the early 2000s, their discovery made it possible to develop a new type of antibody, to prevent the activation of these brakes, or 'checkpoint inhibitors'.

The next race

BioNTech is not the only biotech company exploring this approach, of course. In fact, it was their German rival CureVac that conducted the first clinical trials of an mRNA cancer vaccine, though without any success as yet (see Chapter 5). There are no fewer than forty-six ongoing clinical trials of RNA cancer vaccines, and Mainz's "Mercedes" races onwards.

For now, the BioNTech team can draw on remarkable revenues from its Covid vaccine. In a recent interview with the research magazine *Horizon Europe*, Türeci mentioned a timescale of two years for developing the first market-ready mRNA cancer vaccine. It's possible, but many questions remain over whether mRNA can produce sufficient concentrations of antibodies, and if RNA vaccines can be combined with other therapies, as seen in BioNTech's trial with Roche.

Auto-immune diseases

Even if cancer remains its primary focus, BioNTech could also choose to diversify. The company has launched a programme with the University of Pennsylvania to develop vaccines against ten infectious diseases. Early in 2021, BioNTech's scientists also outlined a range of new applications for mRNA in the field of auto-immune diseases such as multiple sclerosis. There are many other possibilities including heart disease and hereditary diseases.

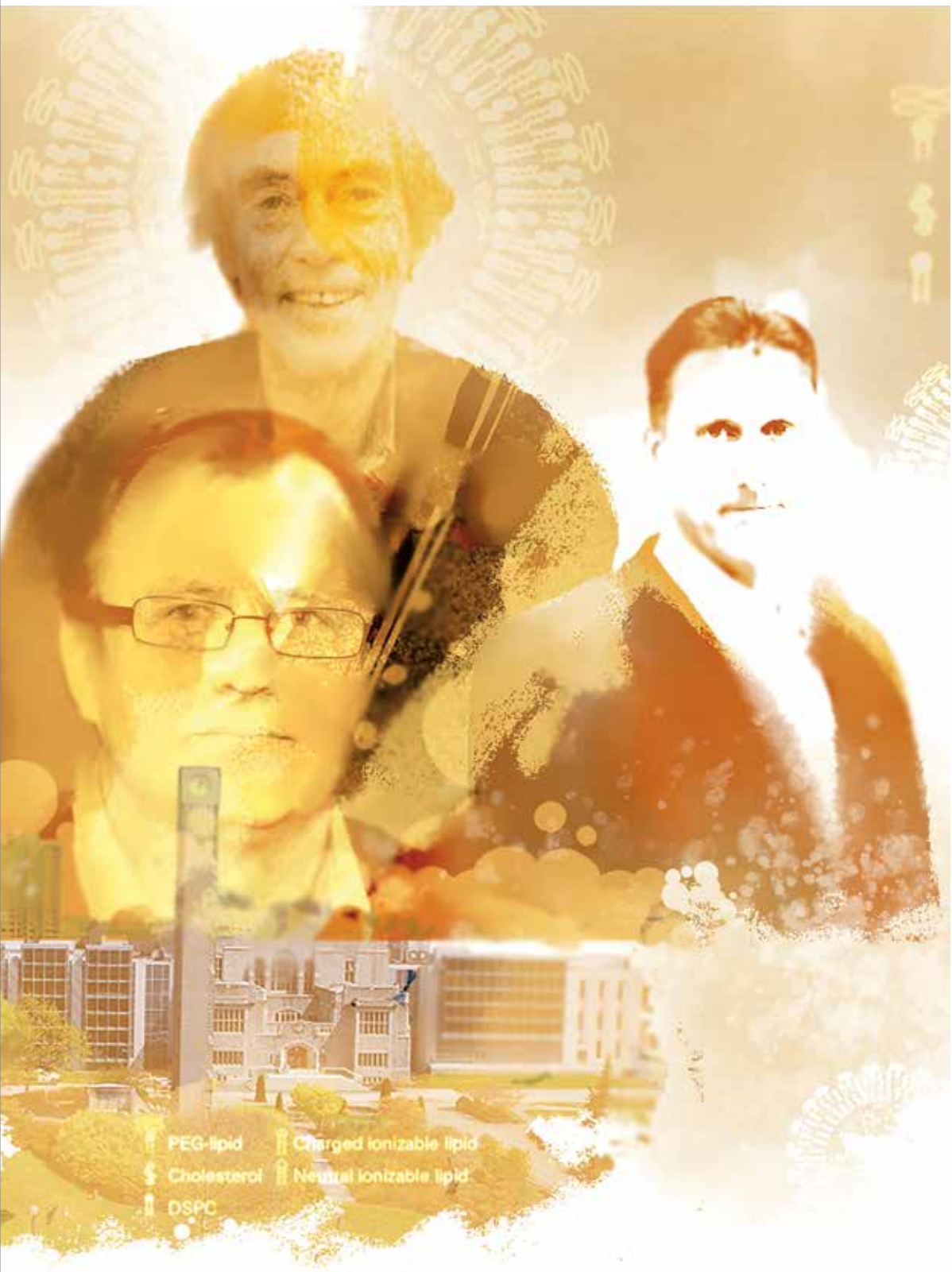
The big problem is that therapeutic applications beyond vaccines cannot rely on the immune system's multiplier effect and are likely to require far higher doses. Even with vaccines, rare cases have been seen where the RNA expresses in unexpected places such as in heart cells, causing inflammation.

The great challenges of the next few years will be the dosage and targeted transport of RNA. These are the keys to the third medical revolution.

There are no less than forty-six ongoing clinical trials of RNA cancer vaccines

The Covid vaccine is only the first big therapeutic step for messenger RNA. The technology can be used to treat a host of other diseases from cancer to cystic fibrosis

RNA pioneers such as Frank De Rosa, Peter Liljeström and Andrew Geall (left to right) were able to make progress by using nanoparticles developed by Pieter Cullis (top centre).


PEG-lipid
Cholesterol
DSPC
Charged ionizable lipid
Neutral ionizable lipid

Chapter 09

Lipids: the key to the third medical revolution

The Covid vaccine is only the first big therapeutic step for messenger RNA. The technology can be used to treat a host of other diseases from cancer to cystic fibrosis. But it's subject to the not insignificant hurdle of getting RNA into cells. The lipid coating that's used for mRNA vaccines won't work in all cases. Current research is looking at other fats or alternative to find the key to unlock specific cell membranes and allow for new therapies.

One of the possible future paths in RNA-based therapies can be traced back to an unexpected source. 5,000 metres up East Africa's Ruwenzori Mountains, the Semliki River, which streams down to feed the White Nile, passes through forests teeming with mosquitoes.

It was here, in 1944, that scientists from the Uganda Virus Research Institute isolated a virus, known as the Semliki virus, that would become a model for virological research. It also resulted in the discovery of self-replicating RNA. This is a technology now used by both Imperial College London and a Californian biotech firm called Arcturus, to create vaccines to compete with those developed by Moderna, BioNTech and CureVac. The technology is very similar to mRNA but its history gives a glimpse of the huge challenges of the third medical revolution that has been sparked by successful mRNA vaccines.

From the Nile to Stockholm

In the late 1980s, Peter Liljeström, was researching the Semliki virus. He was at the time associate professor at the Centre for Life Sciences at Stockholm's Karolinska Institute, the organisation that awards the Nobel Prize in Physiology and Medicine. Research into viral vectors was a major focus at the time, and Peter Liljeström was investigating how the envelope of the Semliki virus might serve to deliver nucleic acids (the four bases of RNA and DNA).

The Semliki virus was of particular interest because its envelope has a positive charge, enabling it to overcome the repulsion between the negatively charged DNA or RNA and the cell membrane. More importantly, this RNA virus has a very peculiar mechanism that allows it to self-replicate in great volumes inside the cell, making it a particularly potent mRNA. It copies its message thousands of times and, as a result, induces large quantities of protein production.

"I realised in 1990 that if I could use this mechanism to introduce biological material into the cells and produce antigen proteins, I would have a vaccine," Liljeström says.

The subtleties of life

He began by testing this concept with a self-replicating RNA encoding typical influenza proteins in cell cultures. He made some important discoveries.

"The effect of the replication mechanism is that for just one RNA that gets into the cell, you quickly have at least 50,000 copies," he says. "That significantly and lastingly increases your chances of producing the proteins needed for a vaccine. And you will also need to inject a much lower dose at the start, with a lower risk of side effects. What's more, after a few days the self-replicating RNA causes the cell to die [by apoptosis, a natural phenomenon balancing out the constant regeneration of our tissue with new cells]. So much so that their debris is full of the antigens that will also stimulate the immune response."

Self-replicating RNA vaccines consistently produce an even stronger immune response than those provoked by their mRNA or DNA counterparts. But Peter Liljeström says they have a further advantage. He estimates that a self-replicating RNA vaccine will need only one-tenth of the dose of existing mRNA vaccines.

"That means we will have to produce 10 times less, and it will therefore be 10 times cheaper."

But producing these is not without difficulties. First, self-replicating RNA contains an additional structure (replicase) that makes it far larger than mRNA, though this also makes it more resilient to being broken down by the mechanisms that destroy mRNA. Its biggest disadvantage is that it is hard to encapsulate.

In the early nineties, Peter Liljeström got around this problem by using the envelope of his alphavirus to transport self-replicating RNA coding the antigen proteins of the influenza virus. It worked in the laboratory, and in 1994 he published the first proof of self-replicating RNA transfection in cultured cells. He then tested the technology on all sorts of animals – mice, pigs, monkeys, and so on. Next, he tried it out against viruses such as hepatitis C, HIV and Ebola.

"It worked well with animal models, but no one believed me when I presented the results to pharmaceutical and biotech companies," he says.

Familiar hurdles

He had unveiled his first results in 1993 at the prestigious Cold Spring Harbour Laboratory on Long Island – the mecca of molecular biology. At the time, however, the whole focus of vaccine development had been not on RNA but on the DNA projects of Vical and Merck (see Chapter 2). As a result, his research fell below the radar.

"For many years I was often the only person presenting results about experimental self-replicating RNA vaccines. People always brought up the problem of industrial production," he says.

The second difficulty inherent to Liljeström's first approach proved too big a hurdle. The viral vector he used to transport the self-replicating RNA was almost identical to natural RNA. *In vivo* it triggered a strong immune response which destroyed both the vector and its load of RNA. Liljeström tried a different method in the 1990s using electroporation – the application of a small electrical field to facilitate the introduction of RNA injected subcutaneously into cells.

"It worked well in the laboratory, but it was inconceivable to use this technology for vaccination campaigns," he says.

The lipid solution

Like Drew Weissman and Kati Karikó (see Chapter 4), like Moderna, CureVac and BioNTech, and like all scientists studying RNA-based therapies, Peter Liljeström had to wait until the 2000s and the development of effective non-viral vectors before he could pursue RNA-based therapies. These vectors were lipid nanoparticles (known as LNPs or liposomes), the tiny balls of fat that protect the mRNA in Covid vaccines.

"The main challenge is no longer the biology. It's transporting the RNA and particularly the capacity to address a specific type of cell," Dan Anderson says during a Zoom call. A specialist in the technologies for transporting the active pharmaceutical ingredient, he is professor of bio-engineering at the Massachusetts Institute of Technology.

Smart fat

Lipid nanoparticles worked well for vaccines, but different lipids are needed for other medical applications. For investors, the stakes are high.

The search "will keep us busy for the next 10 years," says Anderson.

Pieter Cullis is a biochemist at the University of British Columbia and the man who supplied Drew Weissman with his first lipid nanoparticles after a seven-year wait (see Chapter 4). He was also the founder of Acuitas Therapeutics, the Canadian company that supplies the liposomes for the BioNTech and CureVac vaccines. He accomplished the bioengineering feat that made these lipids capable

of delivering the maximum amount of RNA into the cells, while minimising the risks of toxicity.

A Canadian serial entrepreneur

"It all began in the 1980s because I set out to study the several thousand different lipids you find in cell membranes," he explains from his Vancouver home.

His work led him to select some of these lipids as vectors for small chemical molecules in the 1980s and 90s, mainly against cancer. At the same time, he invented the extrusion technology for making liposomes.

Cullis set up a series of companies to market his discoveries. In 1987 he founded the first of these, the Canadian Liposome Company, which he ran until 1991. But it was Inex Pharmaceuticals, established in the mid-1990s, that would develop the first lipid nanoparticles used for RNA.

"We came from cancer research, but if you were trying to raise money back then, investors were only interested in gene therapy," Cullis explains.

An electrifying brainwave

"Positively charged liposomes like the ones Phil Felgner developed at Syntex (see Chapter 1) have a toxicity problem," the Canadian scientist says. "It's fairly easy to understand...There are no positive lipids in our bodies. They're all either neutral or negative. And so even if Felgner's positive lipids attached themselves like magnets to the RNA and then the negative cell membrane, they also have a harmful effect. They cause the cells to lose their impermeability."

His training as a physicist allowed Cullis to solve this problem of electrical charge using ionisable lipids. The electrical charge of these lipids varies according to the acidity of their environment. When acidity is low – in this case above a pH of about 7, as happens in the serum and plasma between cells after an injection – the lipids are neutral and in no danger of becoming toxic. But if they attach to the cells' entrance gates (endosomes) the increasing acidity of this environment turns them negative. This allows them to embed themselves and the RNA code in cells.

"You find about 50 per cent of these ionisable lipids in RNA vaccines like BioNTech's. The rest is made up of three other lipids [36 per cent cholesterol, 10 per cent DSPC and 4 per cent PEG] which are there mainly to give the liposomes some structure," he says.

A non-messenger RNA

As with mRNA technology, this pioneering innovation comes with a difficult back story.

Cullis recalls the chaotic environment of the early 2000s. Inex Pharmaceuticals started a partnership with Alnylam. The Boston biotech firm was looking at the time for a way to transport a different type of RNA – in many ways the opposite of mRNA – called interfering RNA (iRNA).

Discovered in 1998 by Andrew Fire and Craig Mello, who won the 2006 Nobel Prize for their work, interfering RNA controls the expression of mRNA. Whereas messenger and self-replicating RNA are responsible for protein production, iRNA stops it. Specifically, it prevents mRNA that is generated by defective genes from producing deformed or unwanted proteins, which in turn cause diseases.

The gold rush

In the 2000s, the ability to inhibit the production of toxic proteins seemed to offer the prospect of a new type of gene therapy. In September 1999, eighteen-year-old Jesse Gelsinger died while taking part in clinical trials of a DNA-based therapy conducted by the University of Pennsylvania. This made researchers wary of pursuing projects involving the genome itself. But now there was the possibility of using interfering RNA to block the production of toxic proteins manufactured by damaged mRNA, rather than trying to repair flawed genes in DNA.

The pharmaceutical industry was enraptured by the potential and versatility of iRNA. Soon there were many collaborations with specialist start-ups. But, with a single exception, the clinical trials were disappointing and, as had happened a decade earlier, pharmaceutical companies pulled out of this line of research in the early 2010s. But the invested billions weren't entirely wasted. For one thing, the funds allowed Pieter Cullis to build on his invention of lipid nanoparticles through a spinoff from Inex: Tekmira.

High stakes

In 2008 Tekmira merged with its competitor, Protiva. For a while it kept its name (it is now called Arbutus), but it was a difficult relationship from the start. By 2011 the partnership had fallen apart, leading to legal disputes over intellectual property rights relating to lipid nanoparticles. There was a further court battle, this time with Moderna, after Tekmira had been renamed Arbutus.

The collaboration between Tekmira and Alnylam ultimately resulted in the first approved therapy based on interfering RNA. In 2018 the U.S. Food and Drug Administration authorised Alnylam's Onpattro drug. This treatment used iRNA to combat a hereditary mutation in liver cells, which engendered a protein malformation affecting the cerebrospinal fluid, causing neuropathy. This disease (ATTR) is rare but usually fatal.

"That was the first clinical demonstration of lipid nanoparticles," says Cullis. It was also central to the emergency approval of mRNA vaccines two years later. "The lipid nanoparticles used in the Pfizer-BioNTech Covid vaccine [and CureVac's] were very similar to Onpattro's," he says.

Those nanoparticles were developed by another company, Acuitas, set up with colleagues formerly at Tekmira. Founded in 2009, also in Vancouver, Acuitas swiftly moved from encapsulating interfering RNA to doing the same with messenger RNA. Drew Weissman put them in touch with BioNTech and Pfizer when they began their influenza research in 2018.

“Lipid nanoparticles worked well for vaccines, but different lipids are needed for other medical applications. For investors, the stakes are high”

"After small chemical molecules and recombinant proteins, bio-medicines and therapies based on nucleic acids herald the third medical revolution. In theory, you can use messenger RNA to treat any disease," Cullis says.

In practice, future applications will be determined by how the RNA is delivered into the cells. For instance, lipid nanoparticles that have proven effective for vaccines or, in Onpattro's case, for reaching the liver, are not necessarily effective when it comes to other therapies.

"We're at the beginning," argues Robert Langer (see chapter 6). Few are more aware of the hurdles still faced – he has tested thousands of lipids. "The challenges that remain are targeting specific cells but also stability, toxicity, degradability, industrial production and storage life," he says.

Efforts at developing a therapy against cystic fibrosis offer insights into the issues. These vaccine therapies must be given every week for the rest of the patient's life, rather than simply being a matter of two doses of 60 30 or 200 100 micrograms, as with the Pfizer or the Moderna Covid vaccines. One leading researcher into these therapies is Frank DeRosa, Chief Technology Officer of Translate Bio, a Boston-based biotech firm that grew out of a spinoff of Shire in 2017. The company is currently developing an mRNA Covid vaccine with Sanofi and a therapy against cystic fibrosis.

"The challenge of messenger RNA for therapies is on a totally different scale from vaccines," DeRosa says. "With a vaccine you benefit from the multiplier effect of the immune system. But with messenger RNA that is meant to stand in for a missing or deformed protein, you have to be able to renew the doses regularly."

A huge challenge

Cystic fibrosis is a genetic disorder which most seriously affects the lung. With this, the challenge is enormous. And that's not just because of the lungs' large surface area – the surface area of a pair of lungs laid flat could cover a tennis court.

"There are something like 2,000 different mutations of the [CFTR] gene that causes this disease, leading to a reduction in the amount of water excreted by the mucous membranes and a thickening of the mucous which can be fatal," DeRosa explains. "And since the mucous membrane cells are constantly renewing themselves, the defective gene keeps returning."

DNA-based corrective gene therapy has been running into these same two problems for as long as telethons have been funding research into them.

Ten years ago, at Shire, DeRosa began investigating the possibility of introducing mRNA to code into the lungs the protein lacking in CF patients – the production of which prevents the thickening of secretions.

"One of the main challenges was to be able to produce messenger RNA on the scale of the lungs, something like half a kilo per batch, while removing the impurities that cause inflammations," he says. "Next, we developed a lipid formula that was not only tolerated but could be sprayed and introduced into the lung by aerosol."

Translate Bio is currently running phase I and II trials of this therapy. Its advantage is that it can be applied to any patient suffering from cystic fibrosis, regardless of which mutations of the CFTR gene are the cause of their illness.

This same logic could be applied to other genetic diseases. The enormous advantage of industrial RNA production – as with Moderna and BioNTech – is that the same facility can produce different types for different symptoms within a few weeks by adapting its production lines.

But transporting the RNA, whether messenger, interfering or self-replicating, remains the key hurdle.

With prophylactic vaccines it's all a lot simpler. Although delivered in small doses, they have a large impact thanks to the immune system's multiplier effect. Micro-doses (less than one microgram) with self-replicating RNA, could yet be possible – lowering the risk that lipid nanoparticles build up in the body and cause harmful side effects.

In Stockholm, Peter Liljeström has developed a vaccine platform to manufacture self-replicating RNA to increase the production of antigens against viruses like HIV, Ebola, Chikungunya and, more recently, coronavirus.

Homing missiles

Andrew Geall, the scientist who developed a self-replicating RNA vaccine against bird flu for Novartis in 2013 (see Chapter 7), is now chief scientific officer at Precision Nanosystems in Vancouver, another company founded by Pieter Cullis. With scientists from Yale University, he's working to develop a vaccine based on self-replicating RNA capable of installing immune memory in cells to avoid frequent reinfection with malaria.

Geall has also recently founded Replicate Biosciences in San Diego, to further his work on self-replicating RNA and its medical applications – particularly cancer. "The idea is to destroy cancerous cells that have grown resistant to conventional drugs," he says on video from his kitchen. "It is estimated that 90 per cent of cancer-related deaths are due to resistance to drugs caused by changes in the tumours. We are creating new treatments to prevent and reverse this resistance."

But as ever, the challenge is to get the RNA into cells. Alnylam's solution of getting its iRNA to the liver via a drip into the bloodstream and Translate Bio's aerosol technique of sending mRNA into the lungs are sufficiently targeted to be effective. It becomes more difficult when it comes to clusters of cells deep inside other organs.

Missing the target

With cancer, the immune system pathway is efficient because it is powered by antibodies and white blood cells, which have learned to recognise the cells that need eliminating. It is a systemic approach. Complications arise when it's a question of delivering RNA into specific cancerous cells without affecting their neighbours.

"The present targeting methods aren't very effective," Pieter Cullis says. "It is still very difficult to add molecules that could guide them, like monoclonal antibodies or ligands [molecules that bind naturally to certain receptors]. Their lifespan is so short that they miss their targets."

The challenges are greatest when it comes to organs like the kidneys, the heart, bone marrow or the brain. "Especially if you want to target a very particular type of cells within these organs," says DeRosa.

This doesn't put off bioengineers like Robert Langer. "Making a specific nanoparticle for a type of cell means first of all identifying the cell's receptors so that you can add the right homing device to guide the RNAs and their vectors towards their targets. There are several ways of doing that. We, for instance, developed specific ligands for endothelial cells [the barriers between vessels and tissue]."

Moderna is exploring a path in a phase II trial with AstraZeneca, to correct a heart disorder by producing a protein that increases the supply of oxygen to the cardiac muscle.

"We added a microRNA to the sequence of this messenger RNA," says Melissa Moore, Moderna's chief scientific officer. "It acts as a switch to stop the messenger RNA expressing if it isn't the right type of cell."

Proteins 'R' Us!

This is the kind of inventiveness that will separate the winners from the losers over the coming ten years. Proteins give structure to our cells, send and receive messages, control our organ functions, ensure that we grow, defend us, and oxygenate us. The possibility of using RNA to produce them on demand or correct flawed proteins opens a vast field of potential therapies.

It carries the promise of personalised cancer treatment specific to each individual patient, regenerating failed organs or extending the preventive principle of vaccines to protect us from many things other than just viruses and microbes.

There are many more clinical trials to come and new transport vehicles to discover before the full potential of these therapies can be realised.

One indisputable fact, however, is that by skipping the DNA stage, the RNA outsiders are now at the centre of molecular biology. Covid has triggered a revolution.

La marche du monde, sur LeTemps.ch

Certificat covid: attention aux zones d'ombre

Article publié le vendredi 4 juin 2021

LE TEMPS

Avec l'avènement du certificat covid et à mesure que se précise son utilisation, des questions éthiques profondes émergent.
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Impressum

Exploration Magazine
No. 11 — September 2021

A publication of Heidi Media SA, publisher of heidi.news

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Remerciements
L'équipe de Heidi.news remercie l'imprimerie Courvoisier-Attinger Arts graphiques SA de son soutien pour la réalisation de cette publication.

Les abonnements sont la principale source de financement de Heidi.news.
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ISSN 2673-1584
ISBN 9782940660124

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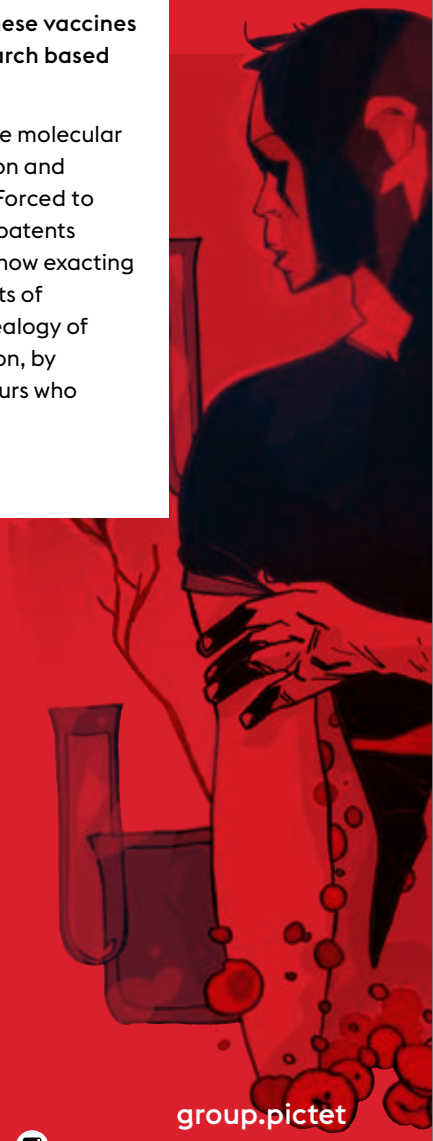
Investigation

mRNA vaccine: The revenge of the outsiders

The development of effective vaccines against the coronavirus in the space of just 10 months has elevated mRNA to the status of a virtual saviour of the world. But the creation of these vaccines is rooted in more than 30 years of technological research based on as many decades of fundamental discoveries.

It's also the story of scientists considered outsiders in the molecular biology community, the story of broken careers, litigation and missed opportunities by pharmaceutical companies. Forced to publish their work in secondary journals, or to see their patents squandered on multiple occasions, these scientists are now exacting resounding revenge. Fabrice Delaye, one of the stalwarts of *Heidi.news*, has delved into the untold story of the genealogy of this technology, which now promises a medical revolution, by interviewing more than 30 researchers and entrepreneurs who were involved in it.



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