The COVID-19 pandemic created an unprecedented state of emergency, during which medical doctors struggled to save lives, and scientists worked feverishly to study the virus, identify effective drugs or develop vaccines in record time. Several different approaches to vaccine production were used, some of which were traditional and others quite innovative. mRNA vaccines had never been licensed for humans before, but the remarkable results of the SARS-CoV-2 vaccines produced by Pfizer/BioNTech and Moderna rapidly dissolved all scepticism. The simplicity of its nature, the potent immunity it provides, and its safety, turned the mRNA vaccine into the most widely distributed type of vaccine during the pandemic.

The idea to use RNA for therapeutic applications was first introduced by John Wolfe in 1990. Katalin Karikó, a Hungarian biochemist who at the time was Assistant Professor at the University of Pennsylvania, saw this and became committed to mRNA therapeutics. However, the scientific community and funding agencies doubted the potential of this molecule due to its instability and its inefficient delivery. Drew Weissman, who moved to Penn in 1998 and worked in the same building as Karikó, shared her vision and passion for mRNA. Together, they started a fruitful collaboration, which would eventually lead to the development of the mRNA vaccine that has saved millions of lives during the ongoing pandemic.

Dr Weissman today is Roberts Family Professor in Vaccine Research at the Perelman School of Medicine, University of Pennsylvania, and Director of Vaccine Research in the Infectious Diseases Division. He leads cutting-edge research on RNA and innate immune response applied to the fields of vaccine research and gene therapy. Weissman and Karikó have received numerous awards this year for the development of the mRNA vaccine and the impact it had on humanity, and they are whispered by many to be potential candidates for the Nobel Prize.

We have interviewed Dr Weissman to learn more about how mRNA vaccines work and gain insight into what therapeutic applications RNA can have.

Initially, when you tried to use RNA for therapeutic applications, you found it was rapidly degraded and provoked an inflammatory response. How did you get the idea of using pseudouridines to avoid that?

Kati Karikó and I started studying RNA in 1998. My specialty was dendritic cells (DCs), immune cells that pick up foreign things and start immune responses with them. Kati gave me in vitro-transcribed RNA, and I added it to the DCs. The first thing I noticed was that they were highly activated, which was unexpected [1]. We spent seven years trying to figure out how RNA activates cells. We tried different kinds of RNA and found that not all RNAs were equally activating. The most striking thing was that tRNAs did not activate dendritic cells at all. Almost 25% of the nucleosides in tRNAs are modified. This observation gave us the idea to try and see whether using modified nucleosides would get rid of the inflammation. Sure enough, when we replaced uridines with pseudouridines, which are the most common RNA modification, the DCs were not activated [2].
How does pseudouridine bypass inflammation?

There are a total of 17 different receptors that can recognise RNA. We studied all the receptors that could recognise mRNA. We found that when the RNA had pseudouridine in it (Ψ-RNA), it did not activate those receptors [3]. Sometimes, the RNA did not bind to the receptor; sometimes, it bound but did not signal, and it could block other RNA from binding. There is a different mechanism for each receptor for how pseudouridine blocks activation, but for all of them, Ψ-RNA failed to induce the receptor to signal.

Another advantage of using Ψ-mRNA is that it is more stable due to not activating certain sensors, which means that it can be translated for a longer time and more efficiently.

Are all the uridines in the Pfizer and Moderna vaccines modified?

Yes, they are all 1-methyl-pseudouridines. Both Moderna and Pfizer/BioNTech licensed our technology, so they use identical modified nucleosides to make mRNA encoding the viral spike protein.

How is the Ψ-mRNA in the vaccine delivered to our cells?

Naked mRNA is not efficiently internalised by cells, and it gets degraded. To optimise uptake, the Moderna and Pfizer/BioNTech vaccines use lipid nanoparticles (LNPs), which contain ionisable cationic lipid, lipid-linked polyethylene glycol (PEG), cholesterol and phospholipids [4]. LNPs encapsulate the mRNA, protecting it from extracellular degradation, and facilitate endosomal release of the mRNA into the cytoplasm. When the LNP-Ψ-mRNA is injected into the muscle, every cell takes it up, but for muscle cells that is very inefficient, you can barely measure the protein that they make. The LNPs are 80nm in size, which is about the size of a virus. What happens is that the LNPs travel through the lymphatic drainage to lymph nodes, and in the lymph nodes, DCs take them up. There is also an infiltrate of lymphoid cells into the muscle that picks up the particles. Once the vaccine gets to a lymph node, the DC translates the mRNA and presents it to B and T cells to activate them, and that is how the immune response is started.

Why do some vaccines convey long-lasting immune protection and others don’t?

The hypothesis is that it is a function of the kinds of cells that are induced. Vaccines often have components that act as adjuvant – a substance that enhances the magnitude and durability of the immune response.

The LNPs of mRNA vaccines also act as adjuvant, because they activate certain helper T cells, called T follicular helper cells, that generate long-lived antibody responses. These cells lead to germinal centres (GCs) where B cells are activated. The mRNA vaccine makes very good GCs, so you have a very potent and long-lasting antibody response [4].

You can make antibodies without a GC, but the response is short-lived. For example, inactivated influenza does not make many GCs and the antibody response does not last very long. The durability of protein vaccines will vary, depending on what kind of adjuvant is used with them.

Are vaccines being developed for every variant that is considered more infectious?

Moderna and Pfizer/BioNTech are testing variants in immunised people to see whether they give protective responses against the variant. Apparently, a boost with the current vaccines raises the antibody levels enough to be protective against all current variants. The problem with the variants is that by the time you have made a vaccine against one variant, a new variant will come and take over, so you are constantly chasing the variants.

Last summer, we started working on a pan-coronavirus vaccine. This is a vaccine that targets conserved regions of all beta-coronaviruses. A conserved region means that the virus cannot mutate it because it cannot survive. By targeting conserved regions, it works against all beta-coronaviruses that we tested [5], and against all known variants. Our guess is that it will work against any new variant that arises because we are targeting the regions that cannot change.

mRNA is also used to make cancer vaccines: how do these work?

Cancer vaccines mainly have a therapeutic role rather than a prophylactic one. There is a couple of different techniques to make cancer vaccines. BioNTech and Moderna are doing personalised cancer vaccines. They have to get the tumour from a patient, sequence it, identify the mutations, produce mRNA encoding the protein with the mutations and make a vaccine, which will activate T cells that attack the tumour. The whole process is very expensive.

Other companies isolate tumour antigens and tumour-associated antigens. There are tumour antigens for prostate cancers, gastrointestinal cancers, melanoma and others. People have tried making vaccines against
those. Overall, the results have not been stellar, so far. A lot of scientific development is needed to get these vaccines to work better.

If the cancer vaccine simply encodes the protein with the somatic mutation in it and generates an immune response against the tumour, then why doesn’t the immune system recognise the somatic mutation directly without a vaccine?

Cancers have a very immunosuppressive environment. They are infiltrated with suppressive cells and make suppressive factors. The body cannot make good immune responses against cancers. In the early studies by Steve Rosenberg, he took cancers out of people, isolated the lymphocytes in the cancer and then turned them on to make them active [6]. When he gave them back to the patient, they could now attack the tumour. What he took out were turned off, suppressive anergic lymphocytes that could not attack the tumour.

We call tumours cold when they do not induce an immune response. There are ways of making them hot so that they do induce an immune response. It is a different approach that does not involve a vaccine, but just manipulating the tumour to make it immunogenic.

The current vaccines that BioNTech is using are made with unmodified RNA, which has inherent adjuvant activity that activates the immune system, so it can make responses against the tumour. The LNP also has adjuvant activity, so when you use a modified RNA that does not have adjuvant activity, the LNP supplies the adjuvant activity, and then you can make responses against those epitopes.

What other RNA-based therapeutics are being developed?

We are working with the Defense Advanced Research Projects Agency (DARPA) to make mRNA that encodes monoclonal antibodies against brand new unknown diseases. If a new epidemic breaks out somewhere, they want to be able to get blood from a survivor and within 60 days make a monoclonal antibody that neutralises whatever is causing that epidemic. The system is all set up: all you need to do is sequence an antibody from a survivor, and make the corresponding mRNA to produce the monoclonal antibody.

We are also developing gene therapy approaches. The way gene therapy is usually done is that you take cells out of a patient and you treat them. There is a gene therapy that is approved for sickle cell anaemia in the United States. They take out a lot of bone marrow, they infect it with a lentivirus that corrects the disease, and they give it back to the patient. The problem is that in Africa, 200 000 people a year are born with the disease, and you cannot do 200 000 bone marrow biopsies in Africa considering it costs about half a million dollars per treatment and requires specialised treatment centres.

So, we are looking at in vivo gene therapy. We figured out how to target LNPs to specific cells. We can now target T cells [7], lung [8], brain [9], heart or bone marrow stem cells by binding an antibody or a piece of an antibody to the surface of the LNP. The antibody binds to the cell of interest and tows in the LNP. We are able to target the LNPs to bone marrow stem cells, carrying RNA that encodes proteins such as Cas9 that through CRISPR in a cell-specific manner can fix the β-globin gene [10]. Basically, with a single intravenous injection, you can cure sickle cell anaemia.

The current pandemic provided a melting pot for vaccine development. What advances were made as a consequence?

We have been working on RNA for over 20 years and on nucleoside-modified mRNA-LNP vaccines for over 8 years. The pandemic happened at a time when RNA vaccines were ready to go, and it was easy to plug the spike sequence into an RNA vaccine and quickly make it. Initially, when the vaccine was first approved, the problem was raw materials. Pfizer and Moderna set up GMP facilities (production plants for manufacturing pharmaceutical products), but the enzymes, the nucleotides and other components needed to make the vaccine were not available in large quantities. So the companies that made them had to scale up production. We learned how to make large amounts of Ψ-mRNA-LNPs under GMP conditions, and Pfizer and Moderna figured out how to make the vaccines stable at ~20 °C and at 4 °C, by changing some salts and sugars in the excipients.

For the adenoviral vaccines, it is the same thing: they had been used in clinical trials, so when COVID hit, scientists were able to take the spike sequence and put it in the adenovirus and made the vaccine very quickly. The technology was not invented during COVID.

Do you think that patenting pseudouridine has delayed the development of potentially better vaccines?

The University of Pennsylvania patented the pseudouridine 15 years ago, and both Moderna and
BioNTech licensed that technology. Other companies are using it and considering licensing it, but the patent would not delay anybody’s research. In fact, you do not have to have the licence to start the research, you have to have it to sell the product. A pharmaceutical company will develop a drug. If it looks like it is going to work, then they will go and buy a licence. The LNPs also have to be licensed.

**What can be done to improve the availability of vaccine to low-income countries?**

I started to work with the Thai government last spring. I had been working with the University of Chulalongkorn for five years to develop different vaccines. In spring 2020, they came to me and said that they were worried that any vaccine made in the West would not be available to South-East Asia for many years and they were not willing to be shut down for years. The government agreed to make their own vaccine and to set up their own production facility. So, I made an RNA vaccine for them and helped them set up a GMP facility. They have now completed phase 1 clinical trials and they will start producing vaccine and distributing it to seven South-East Asian countries by the end of this year.

I am also working with two countries in Africa to help them set up GMP facilities to make mRNA vaccines, to have local production that can be distributed locally. The big pharma companies (Pfizer, Moderna, Johnson&Johnson) are selling or giving vaccine to low-income countries, but there are 7.4 billion people in the world: that is a lot of doses. I think we need to have local production of vaccine.

**Are the African and South-East Asian vaccines different from the Pfizer and Moderna ones?**

They are not significantly different. With the Thai vaccine, we used a slightly different immunogen and a LNP from a different company. But our results are as good if not better than Pfizer’s.

**Considering the development of the pandemic, is there anything you would do differently?**

The world made a lot of mistakes. It would take hours to list all of the things I would do differently. However, the pharmaceutical companies stepped up, and they started producing vaccine before it was approved at a cost of billions of dollars. Moderna got money from the government, but Pfizer did not. They took the risk: if their vaccines did not work, they would have lost billions of dollars. I think the US government funding vaccines early on was a great thing. On the other hand, a lot of problems with the US government made the pandemic much worse. There is a lot of good and a lot of bad.

**But from your side, it’s all good.**

Yes, the vaccine worked great and we are happy about that. We just wish we could get it to the world quickly.

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