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mRNA therapy at the convergence of genetics and nanomedicine

João Conde, Robert Langer & José Rueff

Since the early 1990s, the intersection of genetics and nanomedicine has found a home in the clinic as one of the game changers of the past decade, holding great promise in fighting diseases by rapidly developing much-needed therapeutic platforms, from cancer to infectious or genetic diseases. And this revolution was just triggered by the amazing evolving world of messenger RNA and its 'cues'.

A fascinating aspect of Mendel's theoretical biology was his conceptual novelty: genes are the 'particles' of heredity, and they are determined by quanta of genetic information. The discovery of chromosomal abnormalities and the birth of medical cytogenetics by Jérôme Lejeune in the second half of the twentieth century and, above all, the discovery of the double helix structure of DNA[1] in 1953 by James Watson and Francis Crick (and not forgetting Rosalind Franklin) paved the way to the 'molecularization' of genetics and medicine. Around the mid-1950s, André Boivin had already proposed that RNA might be involved, noting that "the macromolecular deoxyribonucleic acids govern the creation of macro-molecular ribonucleic acids, and, in turn, they govern the manufacturing of cytoplasmic enzymes[2]". However, at that time, few paid attention to these delicate molecules due to the lack of evidence to support this theory.

The discovery of messenger RNA began in 1961 (Fig. 1a) when Sydney Brenner and colleagues identified RNA as a fragile intermediary molecule that duplicates the DNA's informational code and controls protein synthesis[3]. Brenner was surrounded by a team that examined the gene expression in cells infected by viruses. They concluded that stable ribosomal RNA does not include protein-encoding information. Instead, the genetic code is translated by a transitory RNA molecule, mRNA. Ribosomes create proteins based on the instructions provided by mRNA. In 1969, Raymond Lockard and Jerry Lingrel collaborated to present the first proof of mRNA translation in vitro. Using a rabbit cell-free system, they showed that an mRNA transcript from a different species of mammal could be translated[4]. It was only a few years later that these molecules could be delivered into cells and tissues.

Indeed, in 1976, Robert Langer and Judah Folkman were the first to report the use of nano- and microparticles to package nucleic acids such as DNA and RNA[5], raising the possibility of using DNA or RNA as a drug. This discovery was expanded two years later with the use of liposomes to deliver mRNA to lymphocytes[6]. In 1989, Robert Malone and co-workers described a non-PEGylated cationic liposome mRNA delivery[7]. Later in 1994, PEG was added to the surface of nanoparticles to prevent aggregation and non-specific uptake by macrophages and liver cells[8].

At that time, mRNA was becoming more popular as a possible treatment modality. In 1990, Jon Wolff laid the groundwork for using mRNA as a therapeutic tool for expression in vivo[9]. To demonstrate the feasibility of direct gene transfer in vivo, the scientists administered naked mRNA to mouse muscles as a potential therapeutic molecule. In 1992, Gustav Jirikowski used mRNA to temporarily fix diabetes insipidus in rats that didn't have the hormone vasopressin[10].

Although the concept of mRNA vaccines as nanomedicines appears to be relatively new, it dates back to 1993, when Frédéric Martinon first developed mRNA-nanoparticle delivery systems encoding an influenza virus nucleoprotein[11]. In 1995, Robert Conry and colleagues developed a cancer antigen-encoding mRNA vaccine[12]. All this work in mRNA therapeutics laid the groundwork for establishing the first mRNA company, Merix Bioscience (1997), now called Argos Therapeutics, since 2004.

In 2005, Katalin Karikó and Drew Weissman discovered a way to prevent the immune response from being activated against the injected mRNA. It has been discovered that mRNA is responsible for activating toll-like receptors (TLR) on immune cells. Karikó and Weissman modified the RNA and overcame challenges such as the (unwanted) immune-stimulatory effects of the mRNA components by incorporating pseudo-uridine, a naturally occurring modified nucleoside[13]. This modified RNA transcript inhibits the TLR-mediated immune response and even improves translational capacity. We now know that, in addition to TLRs, type I interferons are the first line of defence against pathogens and have been extensively implicated in inhibiting mRNA translation (via the expression of interferon-stimulated genes), limiting the production of viral proteins and host factors essential for viral replication.

Several mRNA-based gene editing tools, such as zinc-finger nucleases, transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR–Cas9) were developed from 2008 to 2013. Over the course of the subsequent years, several different pre-clinical and clinical tests on mRNA-based vaccines against infectious diseases, hypersensitivity diseases and cancer were developed. Indeed, in 2008, Benjamin Weide and colleagues published a first-phase I/II clinical immunization experiment using direct injection of mRNA[14] in collaboration with CureVac. In 2009, the authors completed the first-ever experiment in cancer immunotherapy on patients with metastatic melanoma utilizing mRNA-based vaccinations[15]. According to the findings of this trial, the vaccination enhanced the number of T cells that fight cancer.

Today, mRNA vaccination technology is used in a wide range of biomedical applications and nanotechnologies, from gene delivery using nanoparticles[16] to gene therapy using a variety of nanomedicines and nanomaterials, ushering in a new era of mRNA-nanomedicine.

A new mRNA-nanomedicine era

Many vaccines developed before mRNA vaccines weakened or inactivated the virus, triggering the body's immune system to fight disease.

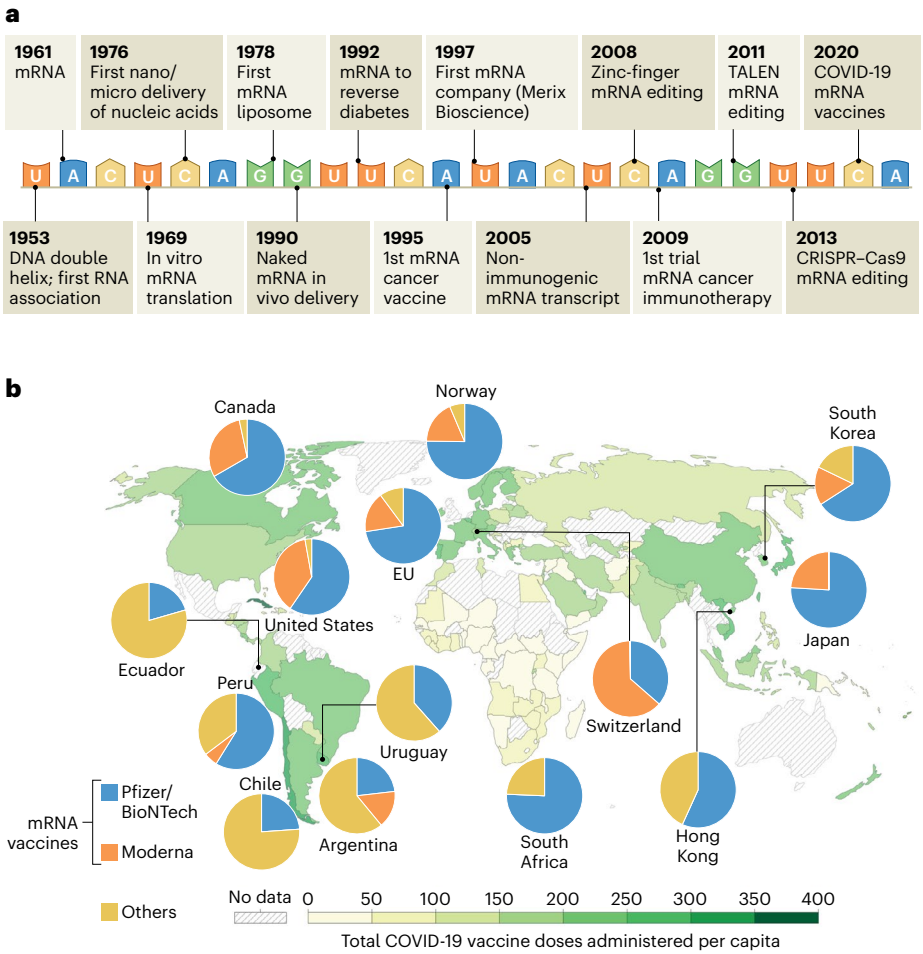

Fig. 1 | When genetics meets nanomedicine. a, Timeline depicting the evolution of the genetics landscape, from RNA discovery to the arrival of the new mRNA-nanomedicine. **b**, Total COVID-19 vaccine doses administered per capita (world map) and COVID-19 vaccine doses administered by manufacturer, worldwide (pie charts). All doses, including boosters, are counted individually. Data taken from Our World in Data.

This vaccine's development can be time-consuming and expensive. In contrast, mRNA vaccines guide the body's cells to make immune system-training proteins using genetic instructions. The final product will be plug-and-play mRNA vaccinations with expedited development timeframes and lower costs. If that's the case, why wasn't the first mRNA vaccine available to the public until the worldwide coronavirus disease 2019 (COVID-19) pandemic of 2020?

In the early stages of mRNA research, there was a clear degree of excitement around the technology. Still, several challenging technical obstacles required a significant amount of creativity to solve. The fact that mRNA would be absorbed by the body and rapidly broken down before it could 'deliver' its message — the RNA transcript — and be translated into proteins by the cells was one of the most difficult obstacles to overcome. Naked mRNA delivery is still challenging because it cannot cross cell membranes easily. Encasing mRNA in nanoparticles to avoid degradation has helped scientists protect and transport it. Lipid nanoparticles[17], which bubbled the mRNA in a protective vesicle and enabled it to enter cells, were a step towards a safe, effective and stable delivery method.

All the necessary components, such as the proper chemistry of mRNA and the invention of nanoparticles to preserve and deliver mRNA, were in place. As a result, the scientific community refocused its efforts on getting a COVID-19 mRNA vaccine to patients as soon as possible without jeopardizing public safety. Based on these findings, Pfizer/BioNTech[18] and Moderna[19] developed two new vaccines based on mRNA nanoparticles, demonstrating more than 90% efficacy in preventing COVID-19 illness, including severe disease. This may be explained by their one-of-a-kind nanocarrier, the lipid nanoparticles. Once the mRNA was delivered into the cell using these nanoparticles, it could be translated into proteins, such as the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The immune system would then be prepared to recognize the foreign protein. Due to the instability of the mRNA molecule, these companies used chemical modifications (that is, uridines) to stabilize the mRNA and packaged it into an injectable intramuscular form using lipid nanoparticles. In 2020, for the first time in history, Pfizer/BioNTech and Moderna were granted emergency use authorization for the two mRNA COVID-19 vaccines, which were produced and distributed in less than one year (developed within only three months of sequencing the viral genome of SARS-CoV-2), reaching more than 80% of vaccinated people in Europe (Fig. 1b) and more than 95% in the United States, according to Our

Table 1 | Current mRNA therapeutics development examples by disease, strategy, and company

mRNA therapeutic area	Strategy	Indication	Company
Oncology	Vaccine	Melanoma	BioNTech, eTheRNA, Curevac
	Chimeric antigen receptor T-cell	Pancreatic cancer	BioNTech
	Antibody	Pancreatic cancer	BioNTech
Genetic diseases	Protein replacement	Cystic fibrosis	Moderna, Arcturus, Translate Bio
	Protein replacement	Haemophilia	Sirnaomics
Infectious diseases	Vaccine	COVID-19	Moderna, BioNTech, Curevac, Sirnaomics
	Antibody	COVID-19	BioNTech, Sirnaomics
Cardiology	Protein replacement	Hypercholesterolemia	Sirnaomics
	Protein replacement	Myocardial ischaemia	Moderna

World in Data. A similar pattern was observed worldwide, especially in countries with the highest vaccine doses administered per capita.

Now, the next step could be the realization of an oral or nasal multimodal nanovaccine for targeted delivery of a synthetic mRNA of the virus to the respiratory tract, with the purpose of enhancing the immunostimulatory activity of the vaccine. A pill or nasal spray can reduce patient hesitancy and increase compliance. The benefits of an intranasal spray include the ease of combining vaccinations, simple vaccine editing to accommodate new variants, a combined mucosal and systemic immune response, protection at distant mucosal regions, and a faster onset of a robust immune response. Another advantage could be gained by relying on quadrivalent mRNA vaccines[20], which contain up to four antigens that protect against multiple strains and can also serve as a unique tool in the fight against future pandemics.

Therefore, developing mRNA vaccines for different diseases will undoubtedly be a priority in healthcare. Several mRNA vaccines are now in development to lower the health risks of latent viruses such as Epstein–Barr virus and cytomegalovirus and to address additional unmet needs, such as those to treat seasonal influenza, respiratory syncytial virus, herpes simplex virus, hepatitis B virus, cancer or HIV. Although these therapies continue to face challenges, such as regulatory issues, large-scale production and accessibility to the public, as well as the fact that, for specific pathogens, the mRNA platform may not be sufficient to induce protective immune responses (for example, biological/immunological problems in HIV), they also offer several benefits over traditional vaccines, including safety, effectiveness, quick preparation and adaptability. The main future goal of the scientific community as well as companies should be to focus on developing novel and specialized delivery systems that can survive several biological obstacles to reach the target site and provide long-term protection. Based on these technological advances, researchers can now develop mRNA vaccines against emerging, rare and neglected diseases worldwide.

The future of mRNA vaccine (nano)technology

Researchers worldwide are rapidly developing novel, ground-breaking applications for mRNA technology in the diagnosis and treatment of diseases, owing to their rapid development and production times without the need for a massive manufacturing facility. Besides, mRNA

vaccines are produced via biochemical rather than biological processes, in contrast to traditional vaccine technologies that rely on cell culture or other means (for example, chicken eggs). This simplifies the manufacturing process, making it more reliable and portable than previous vaccine producers. The time it takes to make the active pharmaceutical ingredient for an mRNA vaccine is also drastically reduced due to its ease of production, taking only three to seven days compared with one month for a non-replicating viral vector and a DNA-based vaccine. In fact, according to the World Health Organization, due to the unique properties of mRNA technology and the absence of cell-based biological components, mRNA vaccines may be manufactured in large quantities by already operating pharmaceutical manufacturers, even if these firms have no prior expertise with vaccines.

Billions of dollars have been invested in mRNA therapy, and a growing number of biotechnology companies, including Moderna, CureVac, BioNTech, Argos Therapeutics, Translate Bio, Ethris and Arcturus have emerged with applications in oncology, and genetic, cardiovascular or infectious diseases (Table 1). In fact, according to ClinicalTrials.gov, there are more than 200 mRNA-based vaccine clinical trials for other diseases than COVID-19 (almost 100 just for cancer) that have either been completed or are actively recruiting participants. Based on the results of these studies, we know that a vaccine's risk–benefit profile must strike the right balance between immunological and inflammatory activation. In addition, early cancer clinical trial findings with mRNA vaccines as monotherapy and in combination with checkpoint inhibitors have shown positive results. This indicates that these vaccines exhibit promising benefits even for complex diseases such as cancer or HIV.

In fact, from a clinical perspective, mRNA vaccines have the potential to provide broad-spectrum immunity, along with the implementation of quick-response manufacturing. Since mRNA vaccines are constrained only by the efficacy of the recipient's immune system against the disease, locating the corresponding mRNA is a straightforward task if a promising protein candidate is discovered. With a speedy manufacturing pipeline in place, these novel vaccine technologies might enable production and distribution within 1–3 months of the emergence of a new variant.

In the future, the next generation of lipid nanoparticles will face new challenges, such as improved stability and multifunctionality, which should be considered in their design to increase tolerance and safety[18]. Forthcoming developments also include single-dose second-generation vaccines and 'multi-variant' vaccines that might offer defence against newly developing viruses. The development of mRNA vaccines, which can prevent a variety of diseases with a single injection, has the potential to drastically streamline the current immunization schedules. In the search for a 'multi-variant' vaccine, researchers at the National Institutes of Health have identified a new target — the N (nucleocapsid) protein — which rarely mutates and targets multiple chemokines, weakening the body's immune response. Personalized vaccines are another future application of mRNA vaccines, which are manufactured using a generic approach that may be used to produce mRNA vaccinations targeting patient-specific antigens quickly. In addition to directly immunizing patients, mRNAs can be used in cellular therapies to transfect patient-derived cells ex vivo to change cell phenotype or function. These cells are then expanded and delivered into the patient.

Moreover, artificial intelligence and machine learning will certainly be useful to design highly structured 'superfolder' mRNA strands[21] and make mRNA vaccines safer and more durable (with fewer refrigeration requirements). A multi-pronged approach to reducing the world's substantial disease burden by making mRNA vaccines more widely available, affordable, efficient and safe is of utmost importance. Another potential development would be self-boosting vaccines protected and delivered by stable nanoparticles or local scaffold patches (for example, microneedles) that can be administered in a single injection and do not require the patient to return for boosters. These self-boosting platforms can be loaded with multiple doses into a single shot, which is especially relevant for populations that don't have easy access to medical services.

This is where genetics meets nanomedicine. This discussion demonstrates how genetics has evolved in the past 20 years since the Human Genome Project, allowing hundreds of millions of people to be vaccinated with mRNA vaccines and hindering the pandemic. The ability of mRNA therapeutics to better link the biology of human physiological systems with new mRNA payloads and in vivo nanodelivery systems, by providing options for continuous dosage with acceptable safety profiles and greater precision, length and duration, may be critical to their success. It is a new age for the technology and manufacture of mRNA vaccines, which stands as a monument to the advances that science has made over decades of research at the intersection of genetics and nanomedicine. This intersection will go down in history as one of science and medical research's greatest achievements.

João Conde [1,2] ✉, **Robert Langer** [3,4] ✉ **& José Rueff**[1,2] ✉

[1]NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade NOVA de Lisboa, Lisbon, Portugal. [2]ToxOmics, NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade NOVA de Lisboa, Lisbon, Portugal. [3]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA. [4]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.
✉e-mail: joao.conde@nms.unl.pt; rlanger@mit.edu; jose.rueff@nms.unl.pt

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