Clinical Microbiology and Infectious Diseases

Review Article ISSN: 2398-8096

Messenger RNA (mRNA) – A forgotten molecule; its past, present and future for therapeutics

Melin J. Khandekar[1] and Janardan D. Khandekar[2]*

[1]Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, USA

[2]Department of Medicine, Northshore University Health System, University of Chicago, USA

Abstract

The COVID-19 pandemic began in Wuhan, China in December of 2019. The disease rapidly disseminated, affecting at least 167 million people worldwide with a minimum of 3.4 million deaths. In the United States alone, close to six hundred thousand deaths can be attributed to Covid. (Johns Hopkins University Center for Systems Science and Engineering – My 24th 2020) The pandemic has caused worldwide economic recession and hardships to millions, if not billions, of people. Amongst this gloom, one molecule stands out as a shining light; messenger RNA-based (mRNA) vaccines. Rapid development and deployment of these vaccines in the community has resulted in decreasing infection rates, transmission of infections in the community, and reduced death rates in the countries that have been able to distribute them. Here, we briefly review the advances in molecular biology that led to the development of mRNA-based vaccines at lightning speed. We briefly discuss mechanisms by which these vaccines produce B and T cell immunity, as well as outstanding questions, including the duration of the protective effect of vaccines and the efficacy of vaccines in immuno-compromised people. We also briefly discuss other applications of mRNA-based technology in management of cancer and other diseases. The development of these vaccines show the amazing power of science to save lives and economic livelihoods, and how forgotten aspects of science can be redeveloped to great therapeutic effect.

Introduction

In 2020, the word mRNA leapt from biology textbooks to headlines across the world. As the world grappled with deadliest pandemic in a century, which has killed over 600,000 people in US alone, many began to put their faith in vaccines to prevent Covid-19 infection. The vaccine candidates that quickly rose to the forefront were based on mRNA technology – a platform that has never previously been used clinically for vaccines. There was much uncertainty and apprehension about success of this approach; the future of the physical and economic health of United States and the world rested on mRNA-based vaccines. The fact that 2 vaccines – one by Moderna in partnership with National Institute of Health and another by BioNTech/Pfizer- have shown a very high efficacy and minimal toxicity, have established the mRNA platform as a very promising tool for potential therapeutics for many diseases [1-3].

Here, we briefly review the discovery of mRNA, early attempts to use this molecule for therapy, and then chronicle the COVID-19 pandemic, and mRNA vaccine development against SARS-CoV-2. We will then briefly discuss possible applications to other diseases and conclude with remarks about the need for international cooperation in science.

Discovery of mRNA

Several well researched-articles and book chapters chronicle the discover of mRNA [4,5]. The story begins with the research of Avery, McLeod and McCarty of Rockefeller University in identifying DNA as the "Transforming Principle" in pneumococcal bacteria. In today's world, where discoveries are recorded and published in nanoseconds, Avery took many years to publish his results as he was unsure that scientific world was ready to accept his revolutionary findings. His thinking is probably validated by the fact that Avery, who survived 11 years after the publication of his landmark paper, did not receive the Nobel prize because the committee was not ready to accept that the heritable element is DNA and not protein [4,5].

The landmark Nobel prize winning publication in 1953 by James Watson, Francis Crick [6], Wilkins and Rosalind Franklin (unfortunately not recognized before her premature death in 1958) suggested that the sequence of four DNA bases contains specific genetic information and was copied during its replication [4,6]. However, how this genetic information is used to produce proteins – the workhorse of cells- remained elusive. As very elegantly described by M. Cobb, ribosomal RNA was identified and described in 1958, but its function remained elusive [4].

Scores of ingenious experiments by several researchers around the world suggested that there was a short-lived RNA intermediary produced by genes, but its structure and function remained unclear. It was left to Crick to postulate what then became the central dogma of molecular biology, i.e., DNA transcribes RNA which in turn gets translated into protein [4,6]. Further, it was postulated in the mid-1950's that the reverse process i.e., protein to RNA to DNA, does not occur [5]. As we know now, RNA can produce DNA by reverse transcription as shown by Baltimore and Temin [7,8].

Correspondence to: Janardan D. Khandekar, Center for Molecular Medicine, Northshore University Healthsystem, 2650 Ridge Ave, Evanston, IL 60201, USA, E-mail: JKhandekar@northshore.org

Key words: *SARS-CoV2, RT-PCR, antigen test*

Received: May 14, 2021; **Accepted:** June 20, 2021; **Published:** June 30, 2021

It was during this time that transfer RNA was discovered, and several groups of scientists were converging on the idea of a "messenger" carrying the information stored on DNA to the ribosomes in the cytoplasm to direct protein synthesis. Two papers with authors including Gilbert, Brennan, Watson, and Jacob (all Nobel laureates for other work) were simultaneously published in same issue of Nature [9,10]. Jacob and Monod described mRNA as a tape that copied the information from DNA and carried it to ribosomes, which read the instructions and made the requisite proteins [11]. Cobb and others have described work of many groups on this subject, which exemplifies how science moves in tandem in many laboratories and demonstrates how collaboration is critical for scientific progress [4].

About 30 Nobel prizes have been given for discovery of various elements of cell and molecular biology, but not for identification of mRNA [4]. The reasons cited include; too many investigators involved as Nobel prize can be given to only 3 living people at a time- (6 if given for Medicine/physiology and chemistry); the discovery almost simultaneously of the genetic code by Marshall Nirenberg and his colleagues eclipsed the importance of mRNA [12]. However, as we discuss here, mRNA has made a big comeback in therapeutics and in fact has outdistanced other much-hyped species of RNA such micro RNAs, silencing RNAs and non-coding RNAs [13,14].

Summary of SARS Covid 2 Infection and Challenges

On December 26th, 2019, a 41-year-old man was admitted to Central Hospital in Wuhan, China with fever, chest tightness, cough, and fatigue, heralding the beginning of the COVID-19 pandemic [15]. It was soon established that the illness in China was caused by SARS–CoV-2 virus [15,16]. Of the seven known human coronaviruses, three are highly pathogenic (SARS- CoV, SARS-Cov-2 and middle east respiratory syndrome (MERS) while other 4 are less virulent, causing various "common colds" [16]. The proximal origins and timing of the pandemic are unclear, and under intense investigations by the WHO among others.

COVID-19 became a worldwide pandemic, spreading to Europe, Americas and to Asia. As of April 2021 -at least 136 million people have been infected with the virus (a lot more have not been diagnosed) and at least 2.9 million people have died from the illness. In the US, over 550,000 people have died from Covid. The symptoms and signs of disease resemble acute respiratory illness and have been well characterized. However, the mortality is 4-5 fold higher than influenza and is highly dependent of age of the patient. In addition, 10-20% of patients develop chronic symptoms such as fatigue, encephalopathy, shortness of breath, muscle weakness, myocarditis which may be caused by "rogue" autoimmune antibodies [17,18]. Thus, Covid -19 poses challenges with acute symptoms perhaps due to ineffective response of innate immunity, including low levels of interferon and or antibodies to interferon [19]. This is then followed with an exuberant immune response with damage to vital organs in 10-20% of patients [18].

The immunology of Covid-19 has been studied with record-breaking speed. Immune memory is generated after infection in all four major domains i.e., neutralizing antibodies against SARS CoV-2 spike and receptor binding domain, memory B cells and memory CD8 and Cd4 T cells [19,20]. The studies also indicate that in about 95 % of subjects infected with Covid, immune memory was retained at 12 months after infection [20-22]. Very recent studies indicate that antibodies to spike protein are present up to 6 months after Covid mRNA-based vaccine [22].

Unfortunately, therapies to date have been limited. Remdesvir – a nucleotide prodrug of an adenosine analogue – and a broad-spectrum antiviral, reduces hospitalization days but has no effect on mortality. Tocilizumab, a competitive inhibitor of IL-6 receptor binding, improves outcomes in some studies but not others. The use of convalescent plasma has produced mixed results. Monoclonal antibodies against the spike protein of virus are promising but definitive studies are still on going. The only therapy proven to improve mortality from COVID-19 is the steroid dexamethasone, which was effective in patients with severe disease [23]. Thus, best therapeutic measures continue to be old rules of preventative hygiene i.e., wearing a mask, hand washing and social distancing. Thus the stakes were high for an effective vaccine for Covid infections in order to contain and reverse this devastating pandemic [24].

Design of Vaccines against SARS-CoV-2

Vaccines for SARS-CoV-2 are based on structure and function of the virus itself. This has been well studied with other related viruses such as Zika and Hepatitis C [20]. The viruses causing these diseases are positive strand RNA (+-RNA) [20]. These viruses package their genomes in infectious virions as sense mRNA. The SARS-CoV-2 virus is the 3rd human virus that enters cells by co-opting the peptidase angiotensin converting enzyme 2(ACE2) [25]. Entry of virus is dependent on its 180 k Da spike(S) protein which mediates binding of the virus to ACE2 and fusion of viral and cellular membranes [25,26]. Infection of organs requires host proteolytic activation of spike at a furin cleavage site. Membrane fusion also requires cleavage of other proteolytic proteases, particularly Transmembrane protease serine 2 (TMPRSS2), a host cell surface protease that cleaves spike protein shortly after it binds to ACE2 [25].

Once virus enters the cell through its trimeric spike protein, it presents about 2 dozen genes to cellular ribosomes, which then produce proteins. The virus then traverses through endoplasmic reticulum and using its own polymerases to produce duplicate RNA, 70% of which is used for replication [16]. When infected, cells mount a vigorous defense response with innate immunity (interferon, monocytes), and adaptive immunity with humoral antibodies and cytotoxic T-cells, helper T-cells and possibly memory cells [18,25]. The prominent scientist and author Siddhartha Mukherjee has succinctly and eloquently summarized the challenges of immune reactions [19].

mRNA Vaccines

mRNA has been advocated as vaccine platform as it offers strong safety advantages [27]. As its genetic construct contains elements that are required for expression of encoded proteins it can be tailored to specific targets [28,29]. Perhaps even more importantly, as this is a single stranded molecule, recombination between strands or with DNA rarely occurs. This lack of genomic integration, in combination with mRNA being non- replicative and metabolically unstable (with a half-life of a few days,) renders this molecule safer than other vectors as it is transient carrier of information [29]. Despite considerable progress, optimal delivery of vaccines remains a challenge.

Naked mRNA is rapidly degraded by RNases and can be immunogenic [27,29]. It cannot penetrate the cell membrane and thus delivery to cells cannot occur [29]. Many techniques have been developed and tested to incorporate mRNA in various vehicles. Cao and Gao have briefly summarized history of development of mRNA vaccines [29]. In 1990, Wisconsin researchers showed the practicality of mRNA as vaccine in mice in a paper in Science [30]. Subsequent work

by others further demonstrated possible applications of this approach. However, a major concern was that the constructs generate an innate immune response and the technology was not pursued. In 2005, researchers found an effective way to evade innate immune response by modifying mRNA's nucleosides [31]. This was a key discovery which prevented innate immune rejection of the construct and allowed delivery of mRNA into cells. This advance also sparked further innovation in mRNA purification, and in modifications to improve mRNA stability. As we discuss, very few researchers paid attention to this finding.

The most efficient preparations for vaccines seem to be cationic lipid nanoparticles. (LNPs). One of the substances used is polyethylene glycol (PEG,) which reduces particle size, prevents particle aggregation and increases circulation time. However, PEG may also be a culprit in causing allergic reactions [32].

Chronology of mRNA-based vaccines against Covid infections

Damian Garde of STAT news and Jonathan Saltzman of Boston Globe published the story of mRNA vaccine developed by Moderna [33]. Other publications more or less corroborate this story. This has been a truly remarkable story with twists of successes, failures and some fortuitous occurrences. The Pfizer vaccine was developed by BioNTech (abbreviated form Biopharmaceutical New Technologies), a company based in Mainz, Germany [33-35]. It was founded by the husband-and-wife team Ugur Sahin and Ozlem Tureci of Turkey. Both are physician/scientists with a background in immunology. BioNTech was developing mRNA-based vaccines for treatment of cancers including melanoma [34]. However, when the pandemic started, it had no FDA approved products.

On January 10th, 2020, Chinese scientists posted viral sequence of Covid 2 online. Sahin rushed to laboratory and himself constructed few genetic vectors that could be deployed in cells to induce an immune response to SARS-CoV-2 [35]. The company hired Katalin Kariko who, along with Drew Weissman, developed a method of incorporating modified nucleosides into mRNA to avoid immune recognition, which has been key to success for an mRNA vaccine [31]. The company already had communicated with Pfizer, and Sahin called Pfizer's top vaccine expert Kathrin Jansen. This started the collaboration for vaccine development. Pfizer did not accept government funding for vaccine development but received funding for vaccine deployment [35,36].

Moderna (Mode RNA), a Cambridge, Mass based company, originated from the concept of Derrick Rossi of Harvard who used the techniques developed by Kariko and Weissman to modify mRNA to reprogram adult cells into an embryonic state [33]. According to Garde and Saltzman, Rossi took his findings to Professors Timothy Springer of Harvard Medical School and Robert Langer of MIT. They immediately saw the tremendous potential of introducing mRNA into cells to produce proteins and formed Moderna. While Moderna had focused on using the mRNA platform to develop cancer vaccines and other therapeutics, when the pandemic started they switched their focus to COVID-19 [33]. The company, which had yet to produce an FDA-approved drug, was able to manufacture the Covid vaccine in 42 days! The vaccine basically encodes a stabilized version of SARS-Cov-2 full length spike trimeric S2 glycoprotein, which has been modified to include 2 Proline substitutions at the top of the central helix in S2 subunit. S Glycoprotein is stabilized in its prefusion conformation and mediates host attachment for viral entry.

Both the Moderna and BioNTech/Pfizer vaccines moved rapidly and supported by Operation Warp Speed, were tested using novel trial designs, allowing rapid generation of data for approval [1,2].

Efficacy of these vaccines have exceeded even optimistic expectations, demonstrating 95% efficacy in preventing Covid infection and entirely preventing severe disease and death [1-3]. Furthermore, the results of the clinical trials have been validated in mass vaccination of 596,618 persons in Israel [37]. Furthermore, patients of all ages and with coexisting conditions benefit from the BNT162b2 mRNA vaccine. This is very encouraging as there has been concern as to whether vaccines will be equally effective in elderly as their immune system not vigorous in responding to infection [38].

Recently, there has been concern that emerging mutant variants may lead to vaccine resistance, although the data thus far are reassuring [39,40]. However, the mRNA vaccine platform is ideally suited to rapidly adapt and deploy new vaccines to prevent infections with these variants. However, it has been difficult to scale up in production and that has resulted in some delays in delivery of vaccines. A very detailed discussion of this topic is beyond the scope of this essay.

Other Applications of the mRNA Based Vaccines

As stated before, investigators have attempted to use this technology for other therapeutic indications. Recently Buris and colleagues reported a phase 1 dose escalation personalized RNA- based vaccine (known as mRNA 4157) in combination with Pembrolizumab in cancer [41]. Based on safety profile and encouraging results researchers are moving into phase 2 trial [42]. Anecdotally, we have seen responses and prolonged disease free survival in patients treated in clinical trials with these types of vaccines.

Success of mRNA vaccines in Covid has led to activation of several clinical trials with this platform against other viruses, such as cytomegalovirus, and cancer and even cell-based therapies such as Chimeric-Antigen Receptor T cells. Other trials are looking at using this platform replace missing or defective proteins [42,43].

Despite this success story, many challenges remain about the delivery, immunogenicity, and potential toxicities of these vaccines. Further work is needed to better understand mechanisms of efficacy of this class of therapeutics [44,45].

While the pandemic has caused incredible harm, with millions of deaths and trillions of economic losses, it has also indirectly opened up new vistas for science. mRNA, a somewhat forgotten molecule, has come to rescue in the form of vaccines against Covid infections [46]. This molecule, identified over 60 years ago and which constitutes only 2% of the genome, has become an object of great curiosity and many new possible applications. One has to conclude that mRNA is the comeback molecule of the year, particularly as vaccines based on Adenoviruses have been established to cause rare unusual complications like cerebral venous sinus thrombosis with low platelets like heparin induced thrombocytopenia [47]. Vascular complications have not been seen with mRNA-based vaccines. Recent evidence also is reassuring that immunity from these vaccines probably lasts up to a year. Further, so far, vaccine immunity protection is against all known virus variants including the most dangerous – delta variant [48]. In science, one never knows the turns and twists that lay ahead, and which discoveries that are left on the back burner will prove to be essential to meet the new challenges ahead.

Acknowledgement

We express our thanks to Ms. Shahida Qazi for her editorial assistance in preparation of this.

References

1. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, et al. (2021) Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* 384: 403-416. [Crossref]
2. Desmond A, Offit PA (2021) On the Shoulders of Giants - From Jenner's Cowpox to mRNA Covid Vaccines. *N Engl J Med* 384: 1081-1083. [Crossref]
3. Mullard A (2020) COVID-19 vaccine development pipeline gears up. *Lancet* 395: P1751-P1752. [Crossref]
4. Judson, H.F. The Eighth Day of Creation: Makers of the Revolution in Biology. Plainview: Cold Spring Harbor Laboratory Press, 1996.
5. Cobb M (2015) Who discovered messenger RNA? *Curr Biolo* 25: R526-R532. [Crossref]
6. Watson JD, Crick FHC (1953) Genetical implications of the structure of deoxyribose nucleic acid. *Nature* 171: 964-967. [Crossref]
7. Baltimore D (1970) RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 226: 1209-1211. [Crossref]
8. Temin HM, Mizutani S (1970) RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* 226: 1211-1213. [Crossref]
9. Gros F, Hiatt H, Gilbert W, Kurland CG, Risebrough RW, et al. (1961) Unstable ribonucleic acid revealed by pulse labelling of Escherichia coli. *Nature* 190: 581-585. [Crossref]
10. Brenner S, Jacob F, Meselson M (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* 190: 576-581. [Crossref]
11. Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* 3: 318-356. [Crossref]
12. Nirenberg M (2004) Historical review: Deciphering the genetic code – a personal account. *Trends Biochem Sci* 24: 46-54. [Crossref]
13. Bartel DP (2009) MircoRNAs: Target regulation and regulatory functions. *Cell* 136: 215-233. [Crossref]
14. Slack FJ, Chinnaiyan AM (2019) The Role of Non-coding RNAs in Oncology. *Cell* 179: 1033-1055. [Crossref]
15. Wu F, Zhao S, Yu B, Chen YM, Wang W, et al. (2020) A new coronavirus associated with human respiratory disease in China. *Nature* 579: 265-269. [Crossref]
16. Fischetti M (2020) Inside the coronavirus: What scientists know about the inner workings of the pathogen that has infected the world. *Scientific American*: 32-37.
17. Wadman M, Couzin-Frankel J, Kaiser J, Matacic C (2020) A rampage through the body. *Science* 368: 356-360. [Crossref]
18. Couzin-Frankel J (2020) The long haul. *Science* 369: 614-617. [Crossref]
19. Mukherjee S (2020) Before virus, after virus: A reckoning. *Cell* 183: 308-314. [Crossref]
20. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, et al. (2021) Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 371: eabf4063. [Crossref]
21. Unchwaniwala N, Ahlquist P (2020) Coronavirus dons a new crown. *Science* 369: 1306-1307. [Crossref]
22. Cyranoski D (2020) Profile of a killer: The complex biology powering the coronavirus pandemic. *Nature* 581: 22-26. [Crossref]
23. Sette A, Crotty S (2021) Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 184: 861-880. [Crossref]
24. Waldholz M (2020) Three ways to make coronavirus drugs in a hurry. *Scientific American* 322: 32-35.
25. Matheson NJ, Lehner PJ (2020) How does SARS-CoV-2 cause COVID-19? *Science* 369: 510–511. [Crossref]
26. Wong JP, Damania B (2021) SARS-CoV-2 dependence on host pathways. *Science* 371: 884-885. [Crossref]
27. Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ (2012) Developing mRNA-vaccine technologies. *RNA Biol* 9: 1319-1330. [Crossref]
28. Haynes BF (2021) A New Vaccine to Battle Covid-19. *N Engl J Med* 384: 470-471. [Crossref]
29. Cao Y, Gao GF (2021) mRNA vaccines: A matter of delivery. *EClinicalMedicine* 32: 100746. [Crossref]
30. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, et al. (1990) Direct gene transfer into mouse muscle in vivo. *Science* 247: 1465-1468. [Crossref]
31. Karikó K (2019) In vitro-transcribed mRNA therapeutics: Out of the shadows and into the spotlight. *Mol Ther* 27: 691-692. [Crossref]
32. Zhang H, You X, Wang X, Cui L, Wang Z, et al. (2021) Delivery of mRNA vaccine with a lipid-like material potentiates antitumor efficacy through Toll-like receptor 4 signaling. *Proc Natl Acad Sci U S A* 118: e2005191118. [Crossref]
33. Garde D, Saltzman J (2020) The story of mRNA: How a once-dismissed idea became a leading technology in the Covid vaccine race. *STAT*.
34. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, et al. (2020) An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* 585: 107-112. [Crossref]
35. Leaf C (2021) The conversation with Albert Bourla. *Fortune* 9-12.
36. Haider M (2021) How Pfizer's head of vaccine development made Covid-19 vaccine in record time and led a large team. *Forbes*.
37. Roest S, Hoek RAS, Manintveld OC (2021) BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 384: 1968-1970. [Crossref]
38. Willyard C (2020) How anti-ageing drugs could boost COVID vaccines in older people. *Nature* 586: 352-354. [Crossref]
39. Mascola JR, Graham BS, Fauci AS (2021) SARS-CoV-2 Viral Variants-Tackling a Moving Target. *JAMA* 325: 1261-1262. [Crossref]
40. Hopkins J, Eastwood J, Moriarty D (2021) mRNA Covid-19 vaccines are fast to make, but hard to scale. *Wall Street J*.
41. Burris H (2019) Novel mRNA-based vaccine shows promise as monotherapy and in combination with checkpoint inhibition. *Am Society Clin Oncol*. [Crossref]
42. Servick K (2020) mRNA's next challenge: Will it work as a drug? *Science* 370: 1388-1389. [Crossref]
43. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F (2020) The promise of mRNA vaccines: a biotech and industrial perspective. *NPJ Vaccines* 5: 11. [Crossref]
44. Gebre MS, Brito LA, Tostanoski LH, Edwards DK, Carfi A, et al. (2021) Novel approaches for vaccine development. *Cell* 184: 1589-1603. [Crossref]
45. Servick K, Couzin-Frankel J, Matacic C (2021) Medicine's longest year. *Science* 371: 1195-1199. [Crossref]
46. Rubin EJ, Baden LR, Morrissey S (2021) Audio Interview: Antibody Responses to Natural Infection and Vaccination. *N Engl J Med* 384: e65. [Crossref]
47. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, et al. (2021) Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* 384: 2092-2101. [Crossref]
48. Ledford H (2021) Six months of COVID vaccines: What 1.7 billion doses have taught scientists. *Nature* 594: 164-167. [Crossref]