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Pioneering work in the field of mRNA-based innovations

A case study

Dr. Katalin Kariko and Dr. Drew Weissman have been named the winners of the 2023 Nobel Prize in Physiology and Medicine for their innovations concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19.

Katalin Kariko's and Drew Weissman's invention based on modified mRNA technology is revolutionary in the field of RNA based therapeutics. Their main focus has been on RNA mediated mechanisms and their work has been instrumental in widening the therapeutic potential of mRNA. Kariko's work has accorded her various accolades including the prestigious Japan Prize, the Paul Ehrlich award, the Gairdner award, the Kovalenko medal, the Breakthrough prize and the Lasker prize.

Figure 1: Katalin Karikó (Right) and Drew Weissman in 2022 (Picture Courtesy *creative commons*)

The 2023 Nobel Prize in Physiology or Medicine was awarded jointly to Katalin Karikó and Drew Weissman "for their discoveries concerning base modifications that enabled the development of effective mRNA vaccines against COVID-19"

- By "The Nobel Prize

Born in Hungary, Kariko's interest in RNA and modified nucleosides has been constant since her early years of research at the Biological Research Centre (BRC), Szeged. In 1985, she moved to the US, where she went on to work at the University of Pennsylvania (Penn) and hasn't looked back ever since. It was at Penn, with Dr. Elliot Barnathan, a cardiologist, where she demonstrated that successful introduction of mRNA into cells could be used to direct the expression of specific proteins of interest. With this initial break through, Kariko was determined to extend her work to gene therapy applications.

While her determination and persistence in exploiting RNA mechanisms have always been untiring and unwavering, her struggle in making a successful mark in the field has not been easy, especially at a time when RNA based therapeutics were considered unconventional. As is the case with many remarkable innovators, finding resources and support was a challenge. Even Kariko, who is now considered a pioneer and forerunner of advanced mRNA vaccine, was then moving against the tides with her revolutionary approaches. Securing funds and getting grants for continuing her research in an area that was considered new and far-fetched was unsurprisingly even more difficult. Her love for work and out-of-the-box thinking are what kept her moving forward in her pursuit of successful integration of RNA mechanisms in gene therapy based applications.

A huge breakthrough came in 2005, when Kariko, along with her colleague Drew Weissman, successfully developed modified mRNA molecules having reduced immunogenicity. This technology and various other modifications of RNA arising therefrom have been meticulously protected through a series of patents in multiple jurisdictions. One such PCT Application *(WO2007024708)* titled *"RNA containing modified nucleosides and methods of use thereof"* which was filed by the Trustees of the University of Pennsylvania (Penn), matured to granted patent in several jurisdictions, including the US and Europe. The main claim, in these patents, is directed towards an RNA molecule containing a pseudouridine residue. Kariko successfully showed that mRNA containing pseudouridine did not activate double stranded RNA (dsRNA)-dependent protein kinase (PKR). One of the reasons for the increase in translation efficiency was later demonstrated by evaluation of the translation efficiency of pseudouridine modified mRNA and unmodified mRNA in PKR knockout cells[1] Kariko also found that RNA containing modified nucleosides such as s^2U,

[1]*Anderson BR, Muramatsu H, Nallagatla SR, Bevilacqua PC, Sansing LH, Weissman D, Karikó K. Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res. 2010 Sep;38(17):5884-92. doi: 10.1093/nar/gkq347. Epub 2010 May 10. PMID: 20457754; PMCID: PMC2943593.)*

5-methylcytidine (m^5C) or 6-methyladenosine (m^6A) showed a similar enhancement in translation of efficiency which could be further increased by 10 folds on adding a Poly-A tail. Further, the application also includes the transcribed RNA molecule, gene therapy vector, invitro transcription kits, method of synthesis and double stranded RNA molecules containing the pseudouridine residue or a modified nucleoside.

While the *PCT* application was filed in 2006 (claiming priority from EP19168984.3A) and published in 2007, it was only later, through a series of experimental studies, that Kariko and her colleagues found that the increase in translation efficiency is attributable to the poor binding of modified mRNA to PKR, which in turn led to the inhibition in the activation of PKR. It was observed that unmodified mRNA strongly binds and activates PKR which led to a supersession in translation.

The inventor's modified mRNA was successfully applied to various gene therapies for treating various conditions such as cystic fibrosis, x-linked agammaglobulinemia, vasospasm, niemann-pick disease, prevention of organ rejection, restoration of hair growth, etc. The patent includes claims directed towards methods of treating anemia, vasospasm, decreasing an incidence of a restenosis of a blood vessel, increasing a hair growth from a hair follicle in a scalp, inducing expression of an enzyme in a cell, treating cystic fibrosis, X-linked agammaglobulinemia, adenosine deaminase severe combined immunodeficiency (ADA SCID), etc. using the modified RNA molecules.

RNA preparations comprising purified modified RNA for reprogramming cells are another pathbreaking innovation patented across multiple jurisdictions, including the US, Europe and Japan. With the discovery of the Yamanaka factors in 2006[2], the research fraternity was stirred with the innovative concept of reprogramming differentiated somatic cells to obtain induced pluripotent stem cells (iPSC). In 2007, successful reprogramming of human adult somatic cells using the Yamanaka factors was also reported. It paved the way for development of allogenic and personalized cell-based therapies. Sir John B. Gurdon and Shinya Yamanaka were awarded the 2012 Nobel Prize in Physiology or Medicine for the discovery that mature cells can be converted to stem cells. The delivery of the genes encoding the Yamanaka factors was popularly through lentiviral and retroviral delivery systems. It was crucial, at the time, to find alternative non-viral ways of delivery, as viral delivery was associated with risks of unpredictability and tumorigenicity[3]. With these advances in technology, Kariko and her colleagues were motivated to explore the possibilities of mRNA mediated cellular reprogramming.

[2]*Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76. doi: 10.1016/j.cell.2006.07.024. Epub 2006 Aug 10. PMID: 16904174.*

[3]*Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol. 2008 Jan;26(1):101-6. doi: 10.1038/nbt1374. Epub 2007 Nov 30. PMID: 18059259.*

Kariko's deep understanding of RNA mediated mechanisms and mRNA mediated immuno-stimulation culminated into the patented technology described in *WO2017036889*. Kariko's modified mRNA molecules, having reduced immunogenicity, demonstrated greater than 40-fold efficiency in delivering the reprogramming factors as compared to, then popular, lentiviral delivery systems. Key advantages achieved by the modified mRNA mediated delivery are that the RNA does not incorporate into the genome and its translation is instantaneous. Additionally, the lack of immunogenicity of the modified mRNA enables repeated delivery without the generation of inflammatory cytokines.

While the concept of *in vitro* transcribed mRNAs (IVT mRNA) as a new class of therapeutics was first developed in 1992, Kariko and her colleague's work solved the long existing problem of immunogenicity associated with IVT mRNAs which blocked the way for protein based therapeutic approaches. With the discovery of Pseudouridine modified IVT mRNA which paved way for many other future works, Kariko and her colleague were fuelled to explore alternative IVT mRNA technologies to be translated into vaccines. They further went on to find that IVT mRNA does not necessarily require the use of modified nucleosides for exhibiting low immunogenicity and increased translatability, and that the same could be achieved using mRNA constructs with low uridine and increased adenosine content. A PCT Application (WO2017036889A1), filed in 2016 by BioNTech, is a PCT application directed towards a method for reducing immunogenicity of RNA using mRNA having such modified nucleotide sequences.

Kariko's work with BioNTech started in 2013, where she was the vice president at RNA protein replacement therapies and went on to developing various technologies that were protected by BioNTech.

With the outbreak of Coronavirus in 2019, Kariko and her colleagues were determined to extend their findings on modified mRNA mediated efficient translation systems to develop a vaccine against SARS-CoV-2, famously called as mRNA vaccine. The mRNA based vaccine, claimed in the patent application *WO2021213924* filed in 2021 by BioNTech, was undoubtedly one of the significant achievements in protein-based approaches for vaccines against Coronavirus. The claims of this PCT Application are directed towards composition and methods for inducing an immune response against Coronavirus. The vaccine comprises a modified RNA encoding an immunogenic fragment of SARS-CoV-2 spike protein (S protein) for eliciting an immune response against the Coronavirus. Kariko's and BioNTech's innovations have also been protected using patent applications including *WO2019175356A1* directed towards 5'-cap-trinucleotide- or higher oligonucleotide compounds and their uses in stabilizing RNA molecules, expressing proteins and in therapy; *WO2021214204A1* describing RNA polynucleotides with a 5' Cap, a 5' UTR comprising a cap proximal sequence; and *WO2017182524A1* directed towards a method to remove double-stranded RNA (dsRNA) contaminants from

single-stranded RNA (ssRNA) suitable for therapy, in addressing certain challenges that are associated with RNA based therapeutics.

Kariko's objectives aligned with BioNTech's mission to improve public health at large. BioNTech's operational excellence and Kariko's expertise amalgamated into achieving various innovative immunotherapeutic platform technologies. Their approach to exploit the full potential of the immune system to effectively recognise and combat external and internal threats has been successful in crystallizing on novel therapeutics for cancer and vaccines to combat COVID-19. With Kariko's illustrious achievements, and her decade long association with BioNTech, she still continues to share her extensive experience and knowledge by taking on the role of an external consultant with BioNTech.