Review

# Review: N1-methyl-pseudouridine (m1Ψ): Friend or foe of cancer?

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## Abstract

Due to the health emergency created by SARS-CoV-2, the virus that causes the COVID-19 disease, the rapid implementation of a new vaccine technology was necessary. mRNA vaccines, being one of the cutting-edge new technologies, attracted significant interest and offered a lot of hope. The potential of these vaccines in preventing admission to hospitals and serious illness in people with comorbidities has recently been called into question due to the vaccines' rapidly waning immunity. Mounting evidence indicates that these vaccines, like many others, do not generate sterilizing immunity, leaving people vulnerable to recurrent infections. Additionally, it has been discovered that the mRNA vaccines inhibit essential immunological pathways, thus impairing early interferon signaling. Within the framework of COVID-19 vaccination, this inhibition ensures an appropriate spike protein synthesis and a reduced immune activation. Evidence is provided that adding 100 % of N1-methyl-pseudouridine (m1Ψ) to the mRNA vaccine in a melanoma model stimulated cancer growth and metastasis, while non-modified mRNA vaccines induced opposite results, thus suggesting that COVID-19 mRNA vaccines could aid cancer development. Based on this compelling evidence, we suggest that future clinical trials for cancers or infectious diseases should not use mRNA vaccines with a 100 % m1Ψ modification, but rather ones with the lower percentage of m1Ψ modification to avoid immune suppression.

## Introduction

When the COVID-19 pandemic broke out in early 2020, there was an immediate need for COVID-19 vaccines. Creating new vaccine technologies was necessary to increase vaccine effectiveness and decrease production time [1]. mRNA vaccines, one of the cutting-edge new technologies, attracted a lot of interest and offered a lot of hope [2,3]. Fast development and manufacturing speeds were made possible by this technique, which were crucial capabilities that could be successfully employed in biotechnological and therapeutic scenarios [4]. The manufacturing of mRNA vaccines can be completed in a matter of days or weeks as opposed to months or years required for the manufacture of, for example, attenuated or inactivated viruses [5]. It is possible to achieve this using in vitro transcription of mRNA, in which nearly any mRNA sequence may be generated from a DNA template [6,7]. Additionally, an mRNA vaccine would give the cell-specific instructions for using cytoplasmic translation to create a desired immunogenic protein [8]. The development of mRNA therapies, like other nucleic acid-based treatment methods, has been hampered by several delivery challenges. Before arriving at the ribosomes, an RNA molecule, for example, may be destroyed by ribonucleases or captured by endosomes [9]. A further obstacle in the mRNA delivery is related to the RNA crossing biological membranes due to its negatively charged phosphodiester backbone [10].

This problem was resolved by encasing the RNA in a wrap made of lipid nanoparticles (LNPs) and guiding it to the ribosomes. These lipids were explored as delivery systems for RNA to mammalian cells decades ago [[11], [12], [13]]. In addition to the aforementioned delivery difficulties, therapeutic mRNA faced at least two other significant obstacles: When administered to animals, in vitro transcribed (IVT) mRNA would: 1) be susceptible to nuclease breakdown; and 2) induce innate immunogenicity comparable to that experienced when infected by a pathogen [14]. Pseudouridine (Ψ), a widely recognized RNA alteration that can be utilized to substitute uridine in the IVT mRNA, provided a solution to these problems. It has been shown that Ψ inclusion increases RNA stability while concurrently dampening the anti-RNA immune response [15,16]. Since it was shown that the Ψ-modification could help mRNA to avoid innate immune responses [16], a search for Ψ-derivatives with the enhanced characteristics was conducted. As a result, it was discovered that N1-methyl-Ψ (m1Ψ) decreased the functionality of innate immune sensors, and performed properly (and even better than Ψ) when tested in several basic human cells. In mice, m1Ψ enhanced the translational efficiency and lowered the cytotoxicity of modified mRNA delivered intramuscularly and through the skin [17].

## Section snippets

### The role of pattern recognition receptors in cancer

Pattern recognition receptors (PRRs) were discovered in 1990 [18], and their roles in stimulating cells of the innate and adaptive immune systems have been at the center of attention of many researchers since that time [19]. For this work, Jules A. Hoffman and Bruce A. Beutler were awarded The Nobel Prize in Physiology or Medicine 2011, along with the acknowledgment of the contributions of Ruslan Medzhitov and Charles A. Janeway Jr. Germline-encoded receptors, or PRRs, are essential for both

## mRNA vaccination impairs the RIG-I signaling pathway: implications for cancer development

Cytoplasmic PRRs known as RLRs are capable of identifying both internal and viral double-stranded RNAs. The DEXH box RNA helicases RIG-I, melanoma differentiation-associated gene 5 (MDA5), and RIG-I-like receptor LGP2 (also known as ATP-dependent RNA helicase DHX58) are the three members of the RLRs family that have been discovered at this point [20]. Through their Caspase Activation and Recruitment Domains (CARD), they initiate a signaling process. Type-I interferons (IFN) and pro-inflammatory

## m1Ψ use in COVID-19 mRNA vaccines

m1Ψ was added in 2020 to Pfizer-BioNTech's COVID-19 mRNA candidate vaccine (Comirnaty® or BNT162b2), which codes for the entire transmembrane spike (S) protein of SARS-CoV-2 [36]. A significant amount of m1Ψ-modified SARS-CoV-2 (COVID-19) spike mRNA was generated by extensive IVT. After demonstrating a favorable safety record and 95 % protection from the disease after a two-inoculation protocol (intramuscular injection), the Pfizer vaccine became the first mRNA vaccine to be fully licensed

## Is m1Ψ a friend or foe of cancer?

The creators of the mRNA vaccines against SARS-CoV-2 have emphasized only the positive aspects related to the addition of m1Ψ: it was critical to diminish the disintegration of this synthesized mRNA as well as its immunogenicity to avoid an overly aggressive immune response. However, important investigations performed during this pandemic have demonstrated that mRNA-based and inactivated vaccines temporarily disrupt IFN signaling [[46], [47], [48], [49]]. It is important to reveal here that in

## Imperfect translation of m1Ψ mRNA leading to the synthesis of different proteins as opposed to uniform production of the spike protein

Surprisingly little is known about how ribonucleotide alteration influences protein synthesis, especially for translation of therapeutic IVT mRNAs, considering their widespread use. A new investigation found that during mRNA translation, m1Ψ dramatically increases +1 ribosomal frame-shifting [90]. The process of mRNA translation is a strictly regulated and strongly conserved method of protein synthesis. Even with sophisticated protein quality control mechanisms, amino acid deficiency in

## Discussion

The COVID-19 pandemic's impact caused an unprecedented level of biomedical research community participation, which made it possible for the fastest vaccine production process in history [36]. Using mRNA vaccines has a number of benefits over other platforms. This platform combines the well-defined composition and safety of killed or subunit vaccines with the immunological properties of live attenuated vaccines, including endogenous antigen expression and T cell induction [126]. At provisional

## Glossary

AEBP1
: Adipocyte Enhancer-Binding Protein

ApoE
: Apolipoprotein E

CARD
: Caspase Activation and Recruitment Domains

CHIC1
: Cysteine Rich Hydrophobic Domain 1

CLRs
: C-type lectin receptors

CTLs
: cytotoxic T lymphocytes

CXCL
chemokine ligand

DAMPs
damage-associated molecular patterns

DCs
dendritic cells

FDG
fluorodeoxyglucose

HMGB1
high-mobility group box 1

HSPs
heat shock proteins

IFNβ
interferon beta

IFN-γ
interferon gamma

IL-10
interleukin 10

IRF
interferon regulatory factor

ISGs
interferon-stimulated genes

LDLR
low-density lipoprotein

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## CRediT authorship contribution statement

**Alberto Rubio-Casillas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **David Cowley:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Mikolaj Raszek:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Vladimir N. Uversky:** Writing – review & editing, Writing – original

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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