

# BNT162b2 Vaccine: possible codons misreading, errors in protein synthesis and alternative splicing's anomalies

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## Abstract

BNT162b2 vaccine against Covid-19 is composed of an RNA having 4284 nucleotides, divided into 6 sections, which bring the information to create a factory of S Spike proteins, the ones used by Sars-CoV-2 (Covid-19) to infect the host. After that, these proteins are directed outside the cell, triggering the immune reaction and antibody production.

The problem is the heavy alteration of the mRNA: Uracil is replaced to fool the immune system with Ψ (Pseudouridine); the letters of all codon triplets are replaced by a C or a G, to extremely increase the speed of protein synthesis; replacement of some amino acids with Proline; addition of a sequence (3'-UTR) with unknown alteration.

These impairments could cause strong doubts about the presence of codon usage errors. An eventual mistranslation has consequences on the pathophysiology of a variety of diseases. In addition, mRNA injected is a pre-mRNA, which can lead to the multiple mature mRNAs; these are alternative splicing anomalies, direct source of serious long-term harm on the human health.

In essence, what will be created may not be identical with protein S Spike: just an error in translational decoding, codons misreading, production of different amino acids, then proteins, to cause serious long-term damage to human health, despite the DNA is not modified, being instead in the cell nucleus and not in the cytoplasm, where the modified mRNA arrives.

However, in this case, the correlation between speed of synthesis and protein expression with synthesis errors, as well as the mechanism that could affect the translation of the sequence remain obscure, many trials have not yet been performed.

**Keywords:** Covid-19, Coronavirus, BNT162b2, alternative splicing, misreading, codons

## 1. Introduction

### Hints on how vaccine works

*BioNTec/Pfizer's* Sars-CoV-2 (Covid-19) vaccine called **BNT162b2**, but also called *Tozinameran*, or *Comirnaty*, contains about **30 mcg of RNA**, which is injected into a **lipid** sphere inside the human body, specifically inside the **cytoplasm** of cells, but **outside the nucleus** (where the **DNA is located**); this RNA has **modified genetic information** (hence *modRNA*), i.e. an mRNA (messenger RNA) containing **instructions** to set in place a **factory of proteins**, clones of the protein **Spike**, i.e. the protein (and only the protein, not the whole virus) **used by Covid-19** to enter the host and **infect** it. Once they are serially produced by the **ribosomes**, they are transported **outside the cell**, beyond the lipid coating; in this way the **immune system** identifies these proteins as cell **invaders** and **attacks** them, through the **production of antibodies**. This is why it is **not conceivable** that the vaccine **induces Covid-19**, or that it **modifies human DNA**.

### Hints on protein synthesis

**Translation** is generally divided into **three phases**: *beginning*, *lengthening*, and *ending*.

1. The **ribosome** binds to the mRNA at the start codon;
2. The **polypeptide chain elongates** in one direction of ribosome movement, by successive **addition** of amino acids;
3. When a **Stop codon** is found, the polypeptide is released and the ribosome dissociates.

### Errors in sequence assembly and translation

Converting the sequence of **mRNA** into a **polypeptide** depends on **transfer RNA (tRNA)** to carry amino acids to the ribosome. At ribosomes, **tRNA pairs with mRNA** by **complementary base pairing** between mRNA **codon** nucleotides and tRNA **anticodon** nucleotides. Once the correct tRNA is bound by a codon, it **transfers its amino acid** to the end of a growing polypeptide chain.

**Deciphering mRNA codons** by transfer RNAs (tRNAs) in the **ribosome** involves **Watson-Crick** base pairing.

The **general error rates** of **genomic replication** (about  $10^{-8}$ ) are estimated to be approximately **10,000-fold lower** than those of **protein synthesis** (about  $10^{-4}$ ), and thus in most instances **mRNA translation** is the **key process** contributing to **inaccuracy** of the cellular proteome. The **discrepancy** between error rates in **DNA replication** and **mRNA translation** may partially relate to the fact that **DNA replication** occurs at the level of **individual nucleotides** (involving  $4^1 = 4$  possible permutations), whereas the **translation machinery** interprets **mRNA codons in triplets** (involving  $4^3 = 64$  possible permutations).<sup>1</sup>

The **efficiency** of **mRNA decoding machinery** is also essentially **regulated** by **codon usage bias** that is distinguished by **over-** or **underrepresented synonymous** codons. Accordingly, optimizing of **tRNA wobble** and **codon usage** in mRNA can substantially enhance translation **efficiency** and **accuracy**.<sup>1</sup>

**Pre-** or **post-mRNA translation** may indirectly introduce **errors** of **protein synthesis** during **transcription** and **posttranslational** processing. However, the translation machinery can directly contribute to **mistranslation** by **tRNA misdecoding** (leading to **misincorporation** or stop-codon readthrough), **tRNA misacylation** (leading to wrong tRNA-amino acid coupling), **codon reassignment** or **ribosomal translocation**-provoked **frameshifts**.<sup>1</sup>

## 2. Investigation method

### Genetic sequence analysis

The vaccine is composed of **4284 nucleotides**, divided into **6 sections**: **cap** is the **beginning** of the sequence, which opens with the two **GA** nucleotides, **falsely** indicating that the **mRNA comes from the human cell** and thus be **accepted**; **5'** indicates the **direction** to be followed for translation, while **UTR** indicates the area where the **ribosome** must rest in order to manufacture proteins. In this section, the **U of Uracil** has been

replaced with one molecule of 1-methyl-3'-pseudouridine, indicated with the character  $\Psi$ , to bypass the immune system and prevent the degradation of the mRNA that has just entered; however, this is a factor that can lead to errors in protein production. Multiple  $\Psi$  synthases are involved in the modification of specific positions, and defects in several of them are linked to human disease<sup>2</sup>.

Then there is the sig section, called the *extended startup sequence of the S-glycoprotein signaling peptide*, whose information is needed to guide the newly formed protein out of the cell via the endoplasmic reticulum; here too are put in place changes to the triplets of nucleotides to make the RNA accepted by the immune system, changing some letters, that make up the information, with others (usually in 3rd position, "wobble"), apparently "harmless synonyms" (mainly by increasing the number of letters C and G, which encode the speed of protein synthesis). However, while they specify identical amino acids, the two synonyms are not precisely the same, at least when it comes to the act of translation. Mechanistic studies show that there are subtle but significant differences in how each interacts with its corresponding transfer RNA (tRNA), differences that affect both the speed and the accuracy of translation.<sup>3</sup> While it is true that 3 letters form a codon and more than one codon encodes for the same amino acid, it is also true that by disproportionately increasing the rate of protein production, could be risks of serious translational errors.

Also the characters that compose the sequence related to the construction of the real Spike protein S protein\_mut have been altered with more C and G that was possible to add, respecting the synonyms in the standard genetic code table, with substitution of the amino acids Lysine (AAA) and Valine (GUU) with Proline (CUU), to prevent the constructed protein to collapse on itself. At the end of this sequence there are 2 stop codons. It's not fully proven that the same elements will be formed with this substitution and won't be misreading errors.

**3'-UTR (Untranslated Region 3 First):** it should indicate the direction of translation of the sequence and improve protein synthesis, however many of its functions remain unknown; therefore it is impossible to ascertain its safety. What is known is stated by WHO and is the following sentence: the 3' UTR for the *BioNTech/Pfizer* vaccine was taken from "the amino-terminal enhancer of split (AES) mRNA and the mitochondrial encoded 12S ribosomal RNA".

**poly(A):** we then reach the end of the sequence and encounter 30 A's, then a 10-nucleotide GCAUAGACU linkage, followed by another 70 A's, since each mRNA can be reused by the organism multiple times.

When the A's run out, the mRNA is degraded.

All of these are proprietary modifications to increase protein expression, of which nothing is known about the actual translation implemented by the organism.

#### Alternative splicing and other errors

Another related problem is that the same pre-mRNA can give rise to different mature mRNAs, and therefore to slightly different proteins (anomalies of *alternative splicing*). An alteration in the process of protein synthesis has been found to be the cause of the development and growth of some cancers, and other diseases, without altering the DNA in any way.

All splicing events identified in the three PHT series genes involve the loss of the messenger sequence reading frame, and the introduction of a Premature Termination Codon (PTC) always located more than 50-55 nucleotides upstream of the last exon-exon junction, which makes the alternative transcripts targets of the NMD (*Nonsense-mediated mRNA Decay*) surveillance system. For human and rat slc15a4/PHT1, this was demonstrated by NMD inhibition experiments in different cell lines, in which the expression of alternative variants to canonical transcripts was always stabilized following inhibition.<sup>4</sup>

### 3. Conclusions

#### Possible long-term risks on human health

We can say that besides **not being optimized**, the **sequence** presents strong **doubts** about the presence of **codon usage errors**. It's possible to **hypothesize** that an **excessive alteration** aimed at an **extreme** increase in **protein expression** may be the **source of errors** in the **assembly** of the **mRNA** gene sequence.

Altering **tRNA availability** can lead to **neurodegenerative diseases** ([Ishimura et al., 2014](#)) and **upregulation** of specific **tRNAs** drives **metastasis** by enhancing **stability** of transcripts enriched in their cognate codons.<sup>5</sup>

**Mistranslation** has very serious **consequences** on the pathophysiology of a **variety of diseases**, including **multiple sclerosis, neurodegeneration, mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes, Parkinson's disease, and cancer** (genesis, growth acceleration and metastasis).<sup>6</sup>

The correlation between the **speed of protein synthesis**, increased by **100%**, with the **translation errors of the sequence**, as well as the **mechanism** that affects the **production of amino acids** remain in this case for now **obscure**, being many **trials** have **not yet** been **performed**.

Basically it can be said that the code of the **total sequence is intrinsically altered in an unbalanced way**, too much **compared** to the **natural** viral counterpart, and **too much** to be able to say that the human organism **reproduces exactly the S Spike proteins**, equal to each other, thus **risking** to bring serious **damage to human health** in the **long term**, in addition to **failure** to be efficient in **immunization**.

**What will be produced** from that sequence is far from well defined, but it is **written in the genes** of each individual, by **ribosomal profile**, how it will be **translated** and what will be **produced**, thus the **benefits or damages** that will be caused.

### References

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# Frequently Asked Questions

1. For the immune system also the Spike protein is not ok, in fact it eliminates it, so the same thing will happen for any eventual wrong products?

Not true. The fruits of translational errors may not be targeted by any antibody, or incorrect antibodies that are not designated to eliminate the S Spike protein, thus ineffective for the immune purpose against Covid-19.

2. Does mRNA degrade after a few days?

The mRNA degrades in a time frame based on the structure of the poly(A) gene sequence tail and other minor factors, but what is produced remains in the body and, as mentioned before, may not be eliminated and might create long-term harm.

3. We are submerged in RNAs, the possibility of alternative splicing abnormalities and translational errors can happen all the time and do they?

It is true, but the speed of protein production excessively increased, the modifications made by incorporating Pseudouridine and other proprietary alterations induce the organism to commit errors with much more probability and severity. After all we are talking about introducing, for the first time in the whole population, including healthy subjects, some genetic material.



#### 4. Studies on mRNA began at least 10 years ago, is there a valid testing?

There are **studies** and **clinical trials** in patients with **other diseases**. Indeed, if it were possible, the **mRNA** would have been **harnessed to treat** disorders other than and **pre-existing** to **Covid-19**, but the **risk outweighed the benefit**. It is **unclear**, therefore, how in this case it **could be suitable** to immunize the **world's population** without leading to **serious short- and long-term effects**.

#### 5. However, the Pfizer vaccine has passed EMA and AIFA safety standards, why?

The **BNT162b2** vaccine has only been **put on the market** with **conditioned authorization**, due to the ongoing **pandemic emergency**, after **heavy pressure** from the **Commission of the Member States**. In reality, this vaccine has **no indication** in the preventive treatment of **any pathology**, i.e. it's **not authorized** with **specific clinical indications**.