Journal **XX** (XXXX) XXXXXX https://doi.org/XXXX/XXXX

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

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Received xxxxxx
Accepted for publication xxxxxx
Published xxxxxx

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

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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 **S 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).[1]

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**.[1]

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**.[1]

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 **Ψ**, 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 **Ψ** synthases are involved in the **modification of specific positions**, and **defects** in several of them are linked to **human disease**[2].

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**.[3] 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** GCAUAUGACU 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.[4]

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.[5]

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).[6]

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 **ribosomial profile**, how it will be **translated** and what will be **produced,** thus the **benefits or damages** that will be caused.

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