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

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Abstract

The *BioNTech/Pfizer* **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 subject. 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**: the **Uracil** is replaced to fool the immune system, the **letters of all codon triplets** are replaced by a **C** or a **G**, to **extremely increase the speed of protein production**, replacement of some amino acids with **Proline**, the addition of a **sequence (3'-UTR),** combined with anomalies of ***alternative splicing***, which is the possibility of **errors in translation of the sequence and synthesis of proteins**; they are not produced equal, but slightly different. All this can be the cause of many **hereditary diseases** and various types of **tumors**, from appearance to their growth, up to the metastasis formation.

In essence, what will be created is **anything but well defined as protein S Spike**: just a **transcription error**, misreading codons (codon bias) wrong production of 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.

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**[1], 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**.

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**[1]; **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 a molecule of **1-methyl-3'-pseudouridine**, indicated with the character **Ψ**, to **bypass the immune system** and prevent the **degradation of the RNA** 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**[1] 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 the position of 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 production of proteins**). 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**.[2] 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 and making at least one change to all triplets, one risks serious translation 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 is **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.

There is a strictly **correlation** with **codons misreading** (***codon bias***).

2. Investigation method

Hints on proteins synthesis

Translation is generally divided into **three phases:** *beginning*, *lengthening*, and *ending*.[2]

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.

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** (***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. Specifically, the **genesis of 200 hereditary diseases** and **33 types of cancer** are implicated, in addition to **neurodegenerative deseases**. 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.[3]

3. Conclusions

Possible long-term risks on human health

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 owned by *BioNTech/Pfizer***.

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** 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**.

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.

References

[1]*Biomolecules* **2020**, *10*(5),729; https://doi.org/10.3390/biom10050729

[2]Robinson R (2014) Which Codon Synonym Is Best? It May Depend on What's on the Menu. PLoS Biol 12(12): e1002014. doi:10.1371/ journal.pbio.1002014

[3]Ghent University Faculty of Veterinary Medicine, "*mRNA modification and delivery strategies towards the establishment of a platform for safe and effective gene therapy*"