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(54) **Title:** DNA APTAMER CONJUGATES RECOGNIZING AND DEGRADING CORONAVIRUS PROTEINS

(57) **Abstract:** The invention relates to single stranded DNA (ssDNA) aptamer conjugates comprising one or more ssDNA aptamers that bind to one or more proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and a cargo, which is a molecule that specifically binds or associates with a target of a protein degradation pathway. The invention also encompasses particular aptamers for S1 subunit of spike protein of SARS- CoV-2, aptamers for membrane (M) protein of SARS-CoV-2, and particular aptamers for nucleocapside (N) protein of SARS-CoV-2, all these aptamers highly specific and with high affinity for the indicated proteins. The invention also includes pharmaceutical composition with the conjugates and/or the isolated aptamers, as well as several applications of the same.

WO 2024/003300 A1

DNA aptamer conjugates recognizing and degrading coronavirus proteins

This application claims the benefit of European Patent Application 22382616.5 filed June 30th, 2022.

Technical Field

The present invention relates to the field of aptamers, in particular DNA aptamers that specifically bind to coronavirus proteins. The invention also relates to any of the fields of application of the aptamers, in particular in the field of therapeutics and in the field of diagnosis of infections caused by coronaviruses.

Background Art

Widely is known the critical scenario caused worldwide by the emergence of the highly pathogenic coronavirus (SARS-CoV-2). and its rapid international spread poses a serious global public health emergency. Covid-19, the pandemic disease caused by SARS-CoV-2 manifests through severe acute respiratory syndrome, as that caused by another coronavirus, such as (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012.

From a phylogenetic analysis on the coronavirus genomes SARS-CoV-2 is to be emplaces as a new member of the Betacoronavirus genus, including SARS-CoV, MERS-CoV, bat SARS- related coronaviruses (SARSr-CoV), as well as others identified in humans and diverse animal species. Each coronavirus contains four structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Among them, the Spike glycoprotein or S protein plays the most important role in viral attachment, fusion and entry, and it serves as the most promising target for development of antibodies, entry inhibitors and vaccines. The S protein mediates viral entry into host cells by first binding to a host receptor through the receptor-binding domain (RBD) in the S1 subunit and then fusing the viral and host membranes through the S2 subunit. Thus, the homotrimeric spike glycoprotein (S1 subunit and S2 subunit in each spike monomer) on the virus envelope is used to bind their cellular receptors. Such binding triggers a cascade of events leading to the fusion between cell and viral membranes for cell entry.

Many has been the research and development to identify agents which are directed against Coronavirus spike proteins and thus able to interfere with the infection process. Antibodies have been used as therapeutic approach against many virus diseases. However, the development of antibodies is usually expensive and for being big molecules

they are not free of, for example, immune reactivity causing severe secondary effects.

Thus, a new approach that has been proposed, and in particular to face SARS-CoV2 infections is the use of aptamers. Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. There are DNA aptamers (double-stranded or single-stranded), RNA aptamers and xenonucleic acid aptamers, the later comprising analogues of the natural nucleotides. In addition, there are also peptide aptamers, which consist of one (or more) short variable peptide domains, attached at both ends to a protein scaffold, and that specifically bind to target molecules.

Examples of DNA aptamers that have been proposed as therapeutical approach for treating SARS-CoV-2 infections are disclosed in the international patent application publication WO2021205012 (BERLIN CURES, GmbH).

Also, Sun et al., in “Aptamer Blocking Strategy Inhibits SARS-CoV-2 Virus Infection”, Angew. Chem. Int. - 2021, vol. no. 60, pp.:10266 –10272, disclose the aptamer called by the authors as CoV2-6, which was able to prevent, compete with, and substitute acetylcholinesterase 2 (ACE2) from binding to the RBD in the S1 protein of the virus. A circular more stable version of the aptamer (called cb-CoV2-6C3) was able to bind the RBD with a dissociation constant (Kd) of 0.13 nM and to block so SARS-CoV-2 with an IC50 of 0.42 nM.

Also, the document of Krüger et al., “Aptamer Applications in Emerging Viral Diseases”, Pharmaceuticals-2021, vol. no. 14, pp.: 622.https://doi.org/10.3390/ph1407062, discloses in a review the aptamers that are being developed or under research against coronaviruses. There, many of the aptamers are also proposed for diagnostic methods.

Notwithstanding all efforts, there is still a need of novel aptamers that can specifically recognize not only S protein of SARS-CoV-2. Also needed are aptamers with improved affinities and sensitivities to, respectively, block the virus and detect even very low amounts in an infected sample of a subject.

Summary of Invention

Inventors have surprisingly found that certain aptamers directed to three of the proteins in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) provided high affinity for these proteins with dissociation constants of the nanomolar or even a lower molar order, that make them useful in therapy. In addition, IC50 values of the aptamers were

also low, thus making them also useful for the carry-on of highly sensitive diagnostic methods.

Using aptamers for the recognition of proteins, in this case of SARS-CoV2 imply the advantages of supposing molecules that can be easily synthesized in an independent way of a cell or any biological system (i.e., (i.e., cells, hybridomas), which finally avoids the purification steps. Moreover, the process of synthesis is controlled with high reproducibility and giving stable molecules. Aptamers are, moreover, non-immunogenic. Thus, in relation to the use of antibodies to recognize SARS-CoV-2 proteins all these examples of advantages associated to the aptamers help to overcome, for example, the drawbacks of purification requirements associated to the production in cell systems of the antibodies, as well as the avoidance of the immunogenicity of the latter.

Thinking more specifically in lateral flow systems for the detection of proteins, there are several publications that highlight the advantages of using aptamers against antibodies for the development of said lateral flow systems. One of the biggest advantages is the difference in time and cost between the production of antibodies (months-years) and the selection of aptamers (weeks). Aptamers are stable to changes in temperature, which also reduces the cost of the supply chain because they do not require refrigeration and offer greater stability of the final product. The high sensitivity and specificity in detection are given by the system used for the selection of aptamers by SELEX. Furthermore, these two parameters are optimized when a sandwich system is used using a pair of aptamers that recognize two binding sites on the analyte of interest. Variability between batches of monoclonal antibody production due to changes in culture conditions or hybridoma mutations is solved with the use of aptamers obtained by chemical synthesis. The size of the aptamers is much smaller than that of the antibodies, facilitating the binding of a greater number of aptamer molecules to the gold nanoparticles during functionalization. All these advantages, together with the ease of use in places where complex laboratory equipment is not available, make LFA systems affordable, fast, robust and low-cost analytical methods (see for example in Table 1 of *Chen A, Yang S. Replacing antibodies with aptamers in lateral flow immunoassay. Biosens Bioelectron. 2015;71:230–242)*.

All these aptamers with high affinity for SARS-CoV-2 proteins are proposed by the inventors as moieties in a conjugate or multifunctional molecule that bind both to a SARS-CoV-2 protein and to lysosome.

Thus, a first aspect of the invention is a conjugate comprising:

- one or more single stranded DNA (ssDNA) aptamers that specifically bind to one or more target proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
- a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds to, or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

Generically, the conjugates of the invention are ones comprising:
- one or more single stranded DNA (ssDNA) aptamers that specifically bind to one or more target proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
- a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds to, or associates with a factor of a protein degradation pathway, or a molecule that recruits factors that trigger protein degradation, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

Thus, they are aptamer conjugates, comprising:

- An aptamer that specifically bind to a protein of the SARS-CoV-2, and
- A molecule associated or involved in a protein degradation cell pathway.

Or in other words, aptamer conjugates comprising
- one or more single stranded DNA (ssDNA) aptamers that specifically bind to one or more proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
- a cargo.

These aptamer conjugates are bifunctional (or multifunctional) conjugates or molecules due to the at least the one ssDNA aptamer and at least the compound that induces degradation of the target protein. Thus, the conjugates of the invention can also be termed bifunctional (or multifunctional) molecules.

Also disclosed is a conjugate comprising:
- one or more ssDNA aptamers that specifically bind to one or more target proteins of the SARS-CoV-2; and
- a cargo, which is a molecule that specifically binds or associates (or recruits) with a target of a protein degradation pathway, in particular that that specifically binds to, or

associates with lysosome, or with a molecule that specifically binds a lysosomal targeting molecule.

All the particular aptamers with a high affinity for the coronavirus proteins that have been identified are also aspects of this invention.

Thus, a second aspect of the invention is a single-stranded DNA (ssDNA) aptamer that specifically binds to a protein of the SARS-CoV-2, said aptamer selected from:

(a) One or more ssDNA aptamers that specifically bind to the subunit 1 (S1) of the spike protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(a1) atgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 1; [702]),

(a2) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccctaaatacgagcaac (SEQ ID NO: 2, [AB7]),

(a3) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccct (SEQ ID NO: 3, [703]),

(a4) agactggtgtgttggctctgggggtgtggaggggt (SEQ ID NO: 4, [704]),

(a5) tggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 5, [701])

(a6) tggggtcggtgtgtcgggtgggggttgggt (SEQ ID NO: 6, [901]),

(a7) gcggatgaagactggtgtgggtggggtcggtgtgtcgggtgggggttgggttgtgtgtgccctaaatacgagcaac (SEQ ID NO: 7, [900 or AB9]),

(a8) gcggatgaagactggtgtgggtgtggttagtgtttcccgttctgttgttttcttggttgccctaaatacgagcaac (SEQ ID NO: 8, [AB4]),

(a9) gttgctcgtatttagggcggaggggcgggggaggaggtggcgggaatgtccattgtctacaccagtcttc atccgc (SEQ ID NO: 9, [AB5])

(a10) ttggggcgggagttttgtttgggtgggggtt (SEQ ID NO: 10);

(b) One or more ssDNA aptamers that specifically bind to N protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(b1) acgcaagggaccacagggtttttcaatataaagatcggattctttattat (SEQ ID NO: 11, [N21]),

(b2) cacgacgcaagggaccacagggtttttcaatataaagatcggattctttattatctatagactacagcacgacaccgcagaggca (SEQ ID NO: 12, [N2]),

(b3) gtttttcaatataaagatcggattctttattatctatagacta (SEQ ID NO: 13, [N22]),

(b4) gcggatgaagactggtgtgtgtggcgctgtttctgttgggtggtgagtgggtctgcccgccctaaatacgagcaac (SEQ ID NO: 14, [N100]),

(b5) gcggatgaagactggtgtcgtgcagcgttaagcggtgaggtgtactgagggttacggggccctaaatacgagcaac (SEQ ID NO: 15, [N200]),

(b6) gcggatgaagactggtgtagagggccgtggaggggcttggcgtgttgaatgagtgagggccctaaatacgagcaac (SEQ ID NO: 16, [N300]),

(b7) gcggatgaagactggtgtgggcaagtgtgtttagtgatggacgcccatgtgtagtcgtgccctaaatacgagcaac (SEQ ID NO: 17, [N400])

(b8) gcggatgaagactggtgtaatatttgtgtgtgtttgttgttgtttttggtttgtttgtgccctaaatacgagcaac (SEQ ID NO: 18, [N600]),

(b9) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 19, [N700]),

(b10) gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 20, [N701]),

(b11) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggc (SEQ ID NO: 21, [N702])

(b12) tgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 22, [N703]),

(b13) gcggatgaagactggtgtcgggagggagtccgtgaggaggggggctgcggaatggtatgccctaaat (SEQ ID NO: 23, [N801]),

(c) One or more ssDNA aptamers specifically binding membrane (M) protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(c1) gttgctcgtatttagggcgggtgggcgggggatcgttgtgctgttgcttgttgtgtgtacaccagtcttcatccgc (SEQ ID NO: 24, [M1000]),

(c2) gttgctcgtatttagggcgggtgggtggggtgtatgtttcgtgccgtgtgctctgtgtacaccagtcttcatccgc (SEQ ID NO: 25, [M1100]),

(c3) gttgctcgtatttagggcggcaagggtgggagggtgggtgttgttgcggcgtgtctgtacaccagtcttcatccgc (SEQ ID NO: 26, [M1200]),

(c4) gttgctcgtatttagggccgaggggggacgctggtattgggtgggtgggagggcgttggacaccagtcttcatccgc (SEQ ID NO: 27, [M1300],

(c5) agggggacgctggtattgggtgggtgggagggcgttggacaccagtct (SEQ ID NO: 28, [M1301]),

(c6) agggggacgctggtattgggtgggtgggagggcgttgga (SEQ ID NO: 29, [M1302])

(c7) gttgctcgtatttagggctgagctgggtgcgttcctggggctgcattcctggggcccgacaccagtcttcatccgc (SEQ ID NO: 30, [M200]), and

(c8) gcggatgaagactggtgtaggggtgagggcgaggggtgggtggaggcaggtcgagggcgccctaaatacgagcaac (SEQ ID NO: 31, [M500])

The notation of numbers into brackets all along the description (e.g.., [M200]) corresponds to an internal notation, which finds equivalence to the indicated SEQ ID NO ID indicated into parenthesis.

As will be illustrated in the examples, SEQ ID NO: 1 and SEQ ID NO: 2 have Kd values for S1 proteins of 7.87e-5 nM and 1.5 nm, respectively. SEQ ID NO: 1 to 5 have as common, the sequence Tggctctgggggtgtggaggggt (SEQ ID NO: 32), thus they are structurally related.

SEQ ID NO: 6 and 7 have a Kd of 0.05 nM and 0.82 nM, respectively. Both have in common SEQ ID NO: 6. Thus, these aptamers with high affinity can be disclosed as ssDNA aptamers comprising SEQ ID NO: 6.

Although other ssDNA aptamers have been developed against the Spike protein, the proposed ones provide better affinity.

ssDNA aptamers consisting in SEQ ID NO: 11 and 12 are good aptamers also in terms of high affinity for the S1 protein. They have, respectively, Kd values of 5.8e-9 nM with an IC50 of 1.01e-8 nM, and 1.44 nM. SEQ ID NO: 11-13 have in common SEQ ID NO: 33 (Gtttttcaatataaagatcggattctttattat).

Other authors have disclosed ssDNA aptamers that specifically bind the nucleocapside protein in SARS CoV, with Kd values of 4.9 nM (See Cho et al., J. Biosci. Bioeng. 2011, 112, 535–540) or 0.49 nM (See Song et al., Anal. Chem 2020, 92, 3393-3402). Thus, present invention provides ssDNA aptamers comprising a nucleic acid sequence SEQ ID NO: 33, or any sequence with a % of identity of at least 85% that show high affinity and sensitivity values.

Aptamers consisting in SEQ ID NO: 14-18 have in common sequence Gcggatgaagactggtgt-(N)x-gccctaaatacgagcaac (SEQ ID NO: 34), wherein N is any nucleotide selected from a, g, c, and t, and x is an integer from 40 to 42. In other words, N is any nucleotide selected from a, g, c, and t, and N is repeated x times, being x an integer from 40 to 42 nucleotides, each nucleotide selected from a, g, c, and t.

ssDNA aptamers of SEQ ID NO: 19 to 21 have in common the sequence Gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 35). These aptamers showed Kd values for the nucleocapside protein (N) from 0.18 to 1.45 nM.

SEQ ID NO: 24-26 have in common SEQ ID NO: 36 (Gttgctcgtatttagggcgg-(N)x-gtcttcatccgc, wherein N is any nucleotide selected from a, g, c, and t, and x is an integer of 44 nucleotides. In other words, N is any nucleotide selected from a, g, c, and t, and N is repeated x times, being x 44 nucleotides, each nucleotide selected from a, g, c, and t.

ssDNA aptamers of SEQ ID NO: 27-29 have in common SEQ ID NO: 29. Thus they can also be defined as ssDNA aptamers that specifically recognize protein M of SARS-CoV2 comprising SEQ ID NO: 29 or any sequence with a percentage of identity from 85% to 100% with the said SEQ Id NO: 29. Moreover, they can be functionally defined as aptamers specifically binding peptide of M defined from AA 7 to AA 23, of 17 AA, LKKLLEQWNLVIGFLFL (SEQ ID NO: 37). In this description the protein M of SARS-CoV-2 is to be understood as the recombinant M protein obtained in E.coli, YP_009724393.1, version of sequence of 18-JUL-2020, in https://www.ncbi.nlm.nih.gov/protein/YP_009724393.1 (retrieved the 21st of February 2022)).

According to the inventor's knowledge, this is the first time ssDNA aptamers recognizing M protein have been provided.

Also disclosed is a multispecific targeting chimera comprising two or more of the aptamers as defined in the previous second aspect, covalently linked to a linker molecule (L). These multispecific aptamers (i.e., chimeras for containing several differentiated parts) are of

special interest, since they can specifically bind two or more of the proteins of the virus, increasing the probability of blocking the infection and/or the probability of its detection in an isolated sample.

The conjugates of the first aspect imply the advantage of degrading the virus once it has been quenched by the aptamer. Indeed, the molecule that specifically binds or associates (or recruits) with a target of a protein degradation pathway is generally a hydrophobic molecule capable to bind, or to associate or to recruit any target of a protein degradation cell pathway. Once the conjugate is bound to both the protein of SARS-CoV-2 in a viral particle and to the target (i.e., generally protein or enzyme) of the protein degradation pathway, the viral particle is degraded as will be illustrated in the examples below.

This behaviour of the conjugate makes the same useful not only in diagnostics for being able to recognize the proteins of the virus, but also in therapy due to the capability of degrading said particle.

Thus, also another aspect of the invention is an in vitro method for the diagnosis of SARS-CoV2 infections, comprising determining in an isolated sample of a subject, the presence and/or levels of SARS-CoV2 by contacting said sample with the aptamer conjugates and/or with one or more aptamers of the previous aspects, and/or with the multispecific aptamers as defined above; wherein if SARS-CoV2 is detected, the subject is diagnosed of SARS-CoV2 infection.

Thus, the aptamers and conjugates are conceived as reagent means in diagnostic methods of SARS-CoV2 infections. It is also another aspect of the invention the use of one or more aptamers and/or of the conjugates as defined in the previous aspects, and/or of a multispecific aptamer as defined above as means for the detection of SARS-CoV2 in an in vitro diagnosis method

For the easy distribution of these aptamers or aptamer conjugates conceived as tools for aiding the diagnostic, in another aspect of the invention are disclosed kits comprising one or more aptamers, or one or more aptamer conjugates as defined in the previous aspects, or a multispecific aptamer as defined above, and instructions for carrying out the method of the said diagnostic method.

Due to the high affinity shown by the aptamers of the invention, they are also useful as therapeutic agents when for example they bind the virus and do not allow to bind to the mammal cell receptors, or do not allow to complete the virus cycle (i.e., when the aptamers block or trap the membrane (M) protein or the protein N of SARS-CoV2). The

therapeutic effect can be increased if moreover said aptamers are linked to an additional cargo in the conjugate, being a drug or being as previously indicated an additional protein degradation system leading molecule (i.e., a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds or associates with a factor of a protein degradation pathway or a molecule that recruits factors that trigger protein degradation). This later approach allows the degradation of the virus particles one being specifically bound.

Thus, also another aspect of the invention is a pharmaceutical or veterinary composition comprising a therapeutically effective amount of an aptamer as defined in the previous second aspect, or a pharmaceutically acceptable salt thereof, or a multispecific aptamer as defined above or a pharmaceutically acceptable salt thereof, or an aptamer conjugate as defined in the previous first aspect or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable excipients or carriers.

Therefore, the aptamers or any derivative product (i.e., conjugate, multispecific aptamers), or any pharmaceutically acceptable salts thereof, or composition comprising them are proposed as medicaments. Thus, another aspect of the invention is an aptamer or a conjugate or a pharmaceutical composition as defined in any of the previous aspects, or a multispecific aptamer as defined above for use in therapy (i.e., for use as a medicament).

More specifically, these aptamers, conjugates and compositions are used in the prevention and/or treatment of an infection caused by a virus from the Coronaviridae family, precisely for having been designed for specifically binding proteins of the SARS-CoV2. Thus, in another aspect, the invention relates to an aptamer, or a conjugate, or a pharmaceutical composition as defined in any of the previous aspects, or a multispecific aptamer as defined above, for use in the prevention and/or treatment of an infection caused by a virus from the Coronaviridae family.

This later aspect may also be formulated as the use of an aptamer, or of a conjugate, or of a pharmaceutical composition as defined in any of the previous aspects, or of a multispecific aptamer as defined above, for the manufacture of a medicament for the prevention and/or treatment of an infection caused by a virus from the Coronaviridae family. The present invention also relates to a method for the treatment or prevention of an infection caused by a virus from the Coronaviridae family, comprising administering a pharmaceutically effective amount of an aptamer, or of a conjugate, or of a pharmaceutical composition as defined in any of the previous aspects, or of a multispecific aptamer as defined above, together with pharmaceutically acceptable excipients or carriers, in a subject in need thereof, including a human.

Brief Description of Drawings

FIG. 1 shows a Western-Blot of an assay with K562 cells in presence or absence of the nucleocapsid-targeted LYTAC, an example of conjugate of the invention comprising SEQ ID NO: 13 (also named in this description N22, and NP LYTAC in this figure) as the ssDNA aptamer that specifically binds to a SARS-CoV2 protein. 100 and 500 ng nucleocapsid relates to the two different amounts of the protein tested in the cell assay. The bars under the Western-Blot image illustrate the band intensity for quantitative purposes.

FIG. 2 shows also a Western-Blot image and an graphic with band intensity of an assay as in FIG. 1 but performed in HepG2 cells.

Detailed description of the invention

All terms as used herein in this application, unless otherwise stated, shall be understood in their ordinary meaning as known in the art. Other more specific definitions for certain terms as used in the present application are as set forth below and are intended to apply uniformly through-out the specification and claims unless an otherwise expressly set out definition provides a broader definition.

As used herein, the indefinite articles “a” and “an” are synonymous with “at least one” or “one or more.” Unless indicated otherwise, definite articles used herein, such as “the,” also include the plural of the noun.

For the purpose of this invention, the term "aptamer" refers to an oligonucleotide that binds specifically and with high affinity to a target molecule. Under defined conditions, aptamers may fold into a specific three-dimensional structure. In one preferred embodiment of the present invention, the claimed aptamers interact specifically and with high affinity with the target sequence.

If not indicated to the contrary all the sequences of nucleotides in this description are indicated in the 5’-3’ direction reading from left to right. In the same way, the sequences of peptides or proteins are indicated in the N-terminal to C-terminal direction, reading from left to right.

The term or expression “that binds specifically” or “specifically binding” refers to the capability of a molecule (e.g., an aptamer) to bind to a target molecule A by means of

either covalent or electrostatic (i.e., ionic) or coordination bonds, without excluding a cross-reactivity with other target B molecules but at a minor extent (lower affinity, Kd) in relation with target A molecule. Thus, it is to be understood that the aptamers of the invention or the conjugates comprising them bind to targets that are SARS-CoV-2 proteins. A "target protein" is, a protein that is desired to be in some way found or contacted. The expression "lysosomal targeting molecule" also referred in this description as "molecule that ultimately associates with lysosome" refers to the fact that the molecule ligand of the conjugate of the invention can specifically bind to a molecule or motif that it is not in the lysosome, but that leads the conjugate as a complex to the lysosome.

The invention encompasses as a first aspect a conjugate comprising:
- one or more single ssDNA aptamers that specifically bind to one or more target proteins of the SARS-CoV-2; and
- a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds to, or associates with lysosome, or with a molecule that specifically binds a lysosomal targeting molecule, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

In a particular embodiment of the conjugate, the one or more targeted proteins of the SARS-CoV-2 are selected from M protein; S protein, in particular S1 protein; and N protein.

In a particular embodiment of the conjugate, the ssDNA aptamer or multispecific aptamer are covalently linked to the compound that induces degradation of the target protein (i.e., like a cargo), said covalent bound optionally hydrolysable once the target (i.e., SARS-CoV2 protein) has been caught by the aptamer(s).

In another particular embodiment of the conjugate of the invention, the compound that induces degradation of the target protein is a ligand selected from the group consisting of an aptamer, a glycopeptide, an antibody, a nanobody, all with the capability to bind a cell-surface lysosome-shuttling receptor.

Thus, the expression "compound that induces degradation of the target protein" refers to a molecule (i.e., usually hydrophobic moieties) that associate with factors of a protein degradation pathway or a molecule that recruits factors that trigger protein degradation. The expression "factors of a protein degradation pathway" includes those molecules that are, for example able to bind or to associate with proteins or other compounds of a protein degradation pathway in the cell. In the same way, this compound may be a molecule or

moiety that does not directly associate with any molecule (i.e., protein or enzyme) of the protein degradation pathway, but that serve as recruiter molecule of other molecules that then lead all the complex to this protein degradation pathway.

The conjugate of the invention comprises, in another particular embodiment, the ligand molecule that specifically binds to, or associates with lysosome, or the molecule that specifically binds a lysosomal targeting molecule, and which is selected from the group consisting of:

- A molecule which specifically binds the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof, in particular being selected from an aptamer, an antibody, a nanobody, a peptide, mannose-6-phosphate, an analogue of mannose 6-phosphate (M6P), M6P-containing ligands, and non MP6 containing ligands; or alternatively
- an aptamer, an antibody, a nanobody, or a peptide that specifically binds an asialoglycoprotein receptor or a fragment thereof.

Using aptamers (or antibodies or fragments of antibodies) that specifically bind to these receptors allow the targeting of proteins that are membrane or extracellular proteins (i.e., to target and degrade the circulating viral particles of SARS-CoV-2).

In a more particular embodiment, the ligand that specifically binds a lysosomal targeting molecule is an aptamer, more in particular, it is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC), or an aptamer with a 90% of identity with this sequence.

In a more particular embodiment, when the ligand is an analogue of mannose 6-phosphate (M6P), it is an analogue of M6P functionalized at the anomeric position. There are many of these analogues of M6P. Indeed, any compound, such a glycoproteins with carbohydrate motifs that are ultimately recognized by the mannose 6-phosphate receptor (CI-M6PRc).

Other known ligands of the CI-M6PRc, herewith termed as natural ligands, are mainly proteins and include M6P-containing ligands, namely M6P-containing proteins, like lysosomal enzymes, and non M6P-containing ligands, namely non M6P-containing proteins like Insulin-like growth factor II (IGF-II), or compounds like retinoic acid with a non-proteic nature. Many others are listed in Table 1 of the document of Dahms, N. (2002). P-type lectins. Biochimica et Biophysica Acta (BBA) - General Subjects, 1572(2-3), 317–340. doi:10.1016/s0304-4165(02)00317-3.

The skilled person in the art will know which other kinds of compounds that induce degradation of the target protein can be in the conjugate of the invention, optionally covalently linked by means of a linker, or in the alternative, directly joined to the ssDNA aptamer that specifically binds to one or more target proteins of the SARS-CoV-2. Other examples are, thus, beside the mannose receptor analogues, glycopeptides or glycoproteins.

In another particular embodiment of the conjugate of the invention, the one or more ssDNA aptamers that specifically bind to one or more target proteins of SARS-CoV-2, and the compound that induces degradation of the target protein are covalently linked by a linker molecule. This covalent bonding can be represented by the following schematic formula:

Aptamer to SARSCov2-Linker-compound to degradation,
or abbreviated A-L-Cd,
wherein
- A is an ssDNA aptamer that that specifically binds to one or more target proteins of SARS-CoV-2, and can be joined to the linker (L) by means of any of its 5'- or 3'-terminal (or 5'-end, or 3'-end), and more in particular it is joined to the linker by means of its 3'-terminal (or 3'-end);
- L is a linker; and
- Cd is the compound that induces degradation of the target protein.

In the more particular embodiment when the ligand molecule that specifically binds to, or associates with lysosome, or the molecule that specifically binds a lysosomal targeting molecule is an aptamer, the following schematic formula applies:

5'-Aptamer to SARSCov2-3'-Linker-5'-Aptamer to degradation, or abbreviated 5'-AptSARS-3'-L-5'-AptLys-3'.

In a particular embodiment of the conjugate when a linker is present, said linker (L) is a polymeric compound, more in particular selected from a polynucleotide, such as dsDNA, ssDNA, RNA and combinations thereof. In another also particular embodiment, the linker is a peptide nucleic acid (PNA). In another particular embodiment, the linker is selected from the group consisting of a C_1-C_{10}-alkyl, a C_1-C_{10}-alkyl linked to a polyethylene glycol (PEG) of formula H−(O−CH_2−CH_2)n−OH, wherein *n* is an integer from 5 to 100, and a polyethylene glycol (PEG) of formula H−(O−CH_2−CH_2)n−OH, wherein *n* is an integer from 5 to 100. In particular, it is a polyethylene glycol selected from polyethylene glycol of n=6-

80, more in particular with an *n* around 70 or an hexaethylene glycol (i.e., $H(OCH_2CH_2)_6OH$), in which n=6.

Following the commonly known techniques by the skilled person in the art, the linker(s) and the two or more aptamers are joined. The skilled man will also understand that if the linker is a ramified polymeric compound, such as a ramified PEG, more than one ssDNA aptamers can be covalently linked to more than one of the compounds that induces degradation of the target protein.

Besides the previously indicated linkers, additional linkers are, in another particular embodiment, and optionally in combination with any embodiments above or below, selected from the group consisting of 1,2-ethylene glycol, 1,3-propanediol, 1,4-butanediol, 1,5-pentanediol, 1,6-hexanediol, an other diols of C_1-C_{10}-alkanes (i.e., C_1-C_{10}-alkanediols); C_1-C_{10}-cycloalkanediols, such as 1,2-cis-cyclobutanediol, 1,2-trans-cyclobutanediol, cis and trans-1,2-cyclopentanediols, cis and trans-1,3-cyclopentanediols, and aromatic spacers such as orto- meta-, para-xylenediol.

Similarly, in another particular embodiment optionally in combination with any embodiments above or below, the linker is selected from an amino-C_1-C_{10}-alkanol, in particular 2-aminoethanol, 3-aminopropanol, 4-aminobutanol, 5-aminopentanol, 6-aminohexanol.

In also another particular embodiment, optionally in combination with any embodiments above or below, the linker is an amino-C_3-C_{10}-cycloalkanol, in particular cyclic aminodiol, in particular selected from 2-cis-aminocyclobutanol, 2-trans- aminocyclobutanol, cis and trans-2- aminocyclopentanol, cis and trans-1,3- aminocyclopentanol.

In also another particular embodiment, the linker is an aminoethylenglycol derivative, such as orto- meta-, para-aminoxylanol.

In a more particular embodiment of the conjugate of formula 5'-Aptamer to SARSCov2-3'-Linker-5'-Aptamer to degradation, the Aptamer to degradation is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC), or an aptamer with a 90% of identity with this sequence;
and the linker is selected from the group consisting of any of the combined lists of linkers disclosed above; more in particular the linker is selected from a C_1-C_{10}-alkyl, a C_1-C_{10}-alkyl linked to a polyethylene glycol (PEG) of formula $H-(O-CH_2-CH_2)n-OH$, wherein *n* is an integer from 5 to 100, and a polyethylene glycol (PEG) of formula $H-(O-CH_2-CH_2)n-OH$, wherein *n* is an integer from 5 to 100, even more in particular the linker is a polyethylene

glycol selected from polyethylene glycol of n=6-80, more in particular with an *n* around 70 or an hexaethylene glycol (i.e., $H(OCH_2CH_2)_6OH$), in which n=6.

In even a more particular embodiment, the conjugate has the formula:

5'-Aptamer to SARSCov2-3'-Linker-5'-Aptamer to degradation, wherein the Aptamer to degradation is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC); and the linker is an hexaethylene glycol (i.e., biradical $-(OCH_2CH_2)_6O-$).

Thus, the cargo or synonymously the compound that induces degradation of the target protein is, in a particular embodiment of the conjugate of the invention, a molecule that specifically binds or associates with a target of a protein degradation pathway in the cell and it is selected from an aptamer that leads the whole aptamer conjugate to the lysosome, or a glycopeptide that leads the whole conjugate to the lysosome.

Therefore, generically disclosed are also conjugates, namely aptamer conjugates, comprising:

- an (i.e., one or more) aptamer that specifically binds to a protein of the SARS-CoV-2, and
- a molecule associated or involved in a protein degradation cell pathway (i.e., a molecule that specifically binds or associates with a target of a protein degradation pathway in the cell).

These conjugates imply the advantage of being able to degrade the virus once it has been quenched by the aptamer. Thus, they are conceived as therapeutic approaches for use in the prevention and/or treatment of an infection caused by coronavirus.

In a particular embodiment of the aptamer conjugate of one of the aspects of the invention, the said conjugate comprises:

- one or more aptamers that specifically bind M protein of SARS-CoV2, in particular selected from an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 24 to 31; and
- a compound that induces degradation of the M protein, and which is a ligand molecule that specifically binds or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, and which is selected from the group consisting of:

(i) a molecule which specifically binds the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof, in particular being selected from an aptamer, an antibody, a nanobody, a peptide, mannose-6-phosphate, an analogue of mannose 6-phosphate (M6P), M6P-containing ligands, and non MP6 containing ligands; or alternatively

(ii) an aptamer, an antibody, a nanobody, or a peptide that specifically binds an asialoglycoprotein receptor or a fragment thereof.

wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

In another more particular embodiment, the aptamer conjugate comprises one or more aptamers that specifically bind M protein of SARS-CoV2; and an aptamer which specifically bind the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof.

Even in a more particular embodiment, the aptamer conjugate has the schematic formula below:

5'-Aptamer that specifically binds M protein of SARS-CoV2-3'-Linker-5'-Aptamer to degradation, wherein the Aptamer to degradation is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC); and the linker is an hexaethylene glycol (i.e., biradical $-(OCH_2CH_2)_6O-$).

In another particular embodiment of the aptamer conjugate of one of the aspects of the invention, the said conjugate comprises:

- one or more aptamers that specifically bind S protein of SARS-CoV2, in particular S1 protein, and more in particular selected from an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 10; and
- a compound that induces degradation of the S protein and which is a ligand molecule that specifically binds or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, and which is selected from the group consisting of:

(i) a molecule which specifically binds the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof, in particular being selected from an aptamer, an antibody, a nanobody, a peptide, mannose-6-phosphate, an analogue of mannose 6-phosphate (M6P), M6P-containing ligands, and non MP6 containing ligands; or alternatively

(ii) an aptamer, an antibody, a nanobody or a peptide that specifically binds an asialoglycoprotein receptor or a fragment thereof.

wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

In another more particular embodiment, the aptamer conjugate comprises one or more aptamers that specifically bind S protein of SARS-CoV2, in particular S1 protein; and an aptamer which specifically bind the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof.

Even in a more particular embodiment, the aptamer conjugate has the schematic formula below:

5'-Aptamer that specifically binds S protein of SARS-CoV2-3'-Linker-5'-Aptamer to degradation, wherein the Aptamer to degradation is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC); and the linker is an hexaethylene glycol (i.e., biradical -$(OCH_2CH_2)_6O$-). In a more particular embodiment, the 5'-Aptamer that specifically binds S protein of SARS-CoV2, specifically binds S1 protein, and even more in particular is selected from an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 10.

In another particular embodiment of the aptamer conjugate of one of the aspects of the invention, the said conjugate comprises:

- one or more aptamers that specifically bind N protein of SARS-CoV2, in particular selected from an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 11 to 23; and
- a compound that induces degradation of the N protein and which is a ligand molecule that specifically binds or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, and which is selected from the group consisting of:

(i) A molecule which specifically binds the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof, in particular being selected from an aptamer, an antibody, a nanobody, a peptide, mannose-6-phosphate, an analogue of mannose 6-phosphate (M6P), M6P-containing ligands, and non MP6 containing ligands; or alternatively

(ii) an aptamer, an antibody, a nanobody, or a peptide that specifically binds an asialoglycoprotein receptor or a fragment thereof.

wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

In another more particular embodiment, the aptamer conjugate comprises one or more aptamers that specifically bind N protein of SARS-CoV2; and an aptamer which specifically bind the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof.

Even in a more particular embodiment, the aptamer conjugate has the schematic formula below:

5'-Aptamer that specifically binds N protein of SARS-CoV2-3'-Linker-5'-Aptamer to degradation, wherein the Aptamer to degradation is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC); and the linker is an hexaethyleneglycol (i.e., biradical -$(OCH_2CH_2)_6O$-).

In a more particular embodiment, the aptamer conjugate comprises the aptamer of SEQ ID NO: 13 (gtttttcaatataaagatcggattctttattatctatagacta, or N22 in this description), which specifically binds N protein of SARS-CoV2, covalently linked by means of an hexaethylenglycol (HEG) molecule (i.e., biradical -$(OCH_2CH_2)_6O$-)) to the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC) that specifically binds CI-M6PR.

In another particular embodiment of the aptamer conjugate of one aspect of the invention, optionally in combination with any of the embodiments of the conjugate disclosed above or below, the one or more ssDNA aptamer that specifically bind to the spike protein of SARS-CoV-2, is an aptamer that specifically binds to the subunit 1 (S1) of the spike protein of SARS-CoV-2, said aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(a1) atgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 1; [702]),

(a2) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccctaaatacgagcaac (SEQ ID NO: 2, [AB7]),

(a3) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccct (SEQ ID NO: 3, [703]),

(a4) agactggtgtgttggctctgggggtgtggaggggt (SEQ ID NO: 4, [704]),

(a5) tggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO; 5, [701])

(a6) tggggtcggtgtgtcgggtgggggttgggt (SEQ ID NO: 6, [901]),

(a7) gcggatgaagactggtgtgggtggggtcggtgtgtcgggtgggggttgggttgtgtgtgccctaaatacgagcaac (SEQ ID NO: 7, [900 or AB9]),

(a8) gcggatgaagactggtgtgggtgtggttagtgtttcccgttctgttgttttcttggttgccctaaatacgagcaac (SEQ ID NO: 8, [AB4]),

(a9) gttgctcgtatttagggcggaggggcgggggaggaggtggcgggaatgtccattgtctacaccagtcttc atccgc (SEQ ID NO: 9, [AB5])

(a10) ttggggcgggagttttgtttgggtgggggtt (SEQ ID NO: 10) [301];

In another particular embodiment of the aptamer conjugate of one aspect of the invention, optionally in combination with any of the embodiments of the conjugate disclosed above or below, the one or more ssDNA aptamer that specifically binds to N protein of SARS-CoV-2, is an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(b1) acgcaagggaccacagggtttttcaatataaagatcggattctttattat (SEQ ID NO: 11, [N21]),

(b2) cacgacgcaagggaccacagggtttttcaatataaagatcggattctttattatctatagactacagcacgacaccgcagagg ca (SEQ ID NO: 12, [N2]),

(b3) gtttttcaatataaagatcggattctttattatctatagacta (SEQ ID NO: 13, [N22]),

(b4) gcggatgaagactggtgtgtggcgctgtttctgttgggtggtgagtgggtctgcccgccctaaatacgagcaac (SEQ ID NO: 14, [N100]),

(b5) gcggatgaagactggtgtcgtgcagcgttaagcggtgaggtgtactgagggttacggggccctaaatacgagcaac (SEQ ID NO: 15, [N200]),

(b6) gcggatgaagactggtgtagagggccgtggaggggcttggcgtgttgaatgagtgagggccctaaatacgagcaac (SEQ ID NO: 16, [N300]),

(b7) gcggatgaagactggtgtgggcaagtgtgtttagtgatggacgcccatgtgtagtcgtgccctaaatacgagcaac (SEQ ID NO: 17, [N400])

(b8) gcggatgaagactggtgtaatatttgtgtgtgtttgttgttgtttttggtttgtttgtgccctaaatacgagcaac (SEQ ID NO: 18, [N600]),

(b9) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 19, [N700]),

(b10) gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 20, [N701]),

(b11) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggc (SEQ ID NO: 21, [N702])

(b12) tgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 22, [N703]),

(b13) gcggatgaagactggtgtcgggagggagtccgtgaggaggggggctgcggaatggtatgccctaaat (SEQ ID NO: 23, [N801]).

In another particular embodiment of the aptamer conjugate of one aspect of the invention, optionally in combination with any of the embodiments of the conjugate disclosed above or below, the one or more ssDNA aptamer that specifically binds to membrane (M) protein of SARS-CoV-2, is an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(c1) gttgctcgtatttagggcgggtgggcgggggatcgttgtgctgttgcttgttgtgtgtacaccagtcttcatccgc (SEQ ID NO: 24, [M1000]),

(c2) gttgctcgtatttagggcgggtgggtggggtgtatgtttcgtgccgtgtgctctgtgtacaccagtcttcatccgc (SEQ ID NO: 25, [M1100]),

(c3) gttgctcgtatttagggcggcaagggtgggagggtgggtgttgttgcggcgtgtctgtacaccagtcttcatccgc (SEQ ID NO: 26, [M1200]),

(c4) gttgctcgtatttagggccgaggggacgctggtattgggtgggtgggagggcgttggacaccagtcttcatccgc (SEQ ID NO: 27, [M1300],

(c5) aggggacgctggtattgggtgggtgggagggcgttggacaccagtct (SEQ ID NO: 28, [M1301]),

(c6) aggggacgctggtattgggtgggtgggagggcgttgga (SEQ ID NO: 29, [M1302])

(c7) gttgctcgtatttagggctgagctgggtgcgttcctggggctgcattcctggggcccgacaccagtcttcatccgc (SEQ ID NO: 30, [M200]), and

(c8)
gcggatgaagactggtgtaggggtgagggcgaggggtgggtggaggcaggtcgagggcgccctaaatacgagcaac (SEQ ID NO: 31, [M500])

In yet another particular embodiment of the aptamer conjugate of one aspect of the invention, optionally in combination with any of the embodiments of the conjugate disclosed above or below, the one or more ssDNA aptamers comprise a multispecific targeting chimera comprising two or more of the aptamers with a sequence selected from any of (a1) to (c8) or with a percentage of identity from 85% to 100% with these sequences, said two or more aptamers covalently linked to a linker molecule (L). More in particular L is selected from a polymeric compound, more in particular selected from a polynucleotides, such as dsDNA, ssDNA, RNA and combination thereof. In another also more particular embodiment the linker is a peptide nucleic acid (PNA). When a multispecific targeting chimera is comprised in the one or more ssDNA aptamers of the conjugate, it is in particular, a bispecific targeting chimera of formula (I)

(A)-L-(A') (I), wherein A and A' are equal or different aptamers as defined in the previous aspect or in any of the particular embodiments, and L is a linker covalently linked to A and A', said linker being in more particular embodiments selected from the previously disclosed. In another particular embodiment, the linker is a polyethylene glycol of formula $H-(O-CH_2-CH_2)n-OH$, wherein n is an integer from 1 to 100. In particular, it is a polyethylene glycol selected from polyethylene glycol of n=6-80, more in particular with an *n* around 70 or an hexaethylene glycol (i.e., $H(OCH_2CH_2)_6OH$), in which n=6.

In another particular embodiment of the conjugate, it further comprises a nanovehicle, which is loaded with one or more therapeutic agents, said agents selected from the group consisting of small interfering RNA (siRNA), micro RNA (miRNA), short hairpin RNA (shRNA), a DNA plasmid, an oligopeptide, a protein, a small molecule, and combinations thereof.

Further, the aptamers or conjugates according to any of the aspects of the invention, or of any of their embodiments, they are encapsulated in suitable vehicles to protect their structural integrity as well as to promote their delivery inside cells. Preferred vehicles include liposomes, lipid vesicles, microparticles, and the like.

In another particular embodiment of any of the aspects of the invention, the aptamers are conjugated to a carrier molecule and/or to a reporter molecule. "Carrier molecules" comprise such molecules that, when conjugated to the aptamer, prolong the plasma half-

life of the conjugated aptamer in human plasma, e.g., by enhancing the stability and/or by affecting the excretion rate. One example of a suitable carrier molecule is PEG.

"Reporter molecules" comprise molecules that allow for the detection of the conjugated aptamer. Examples of such reporter molecules are GFP, biotin, cholesterol, dyes like e.g. fluorescence dyes, electrochemically active reporter molecules and/or compounds comprising radioactive residues, in particular radionuclides suitable for PET (positron emission tomography) detection like e.g.,18 F,11 C,13 N,15 0,82 Rb or68 Ga. The skilled person is well aware of suitable carrier and reporter molecules and of ways of how to conjugate them to the aptamers of the invention.

As previously indicated, another aspect of the invention is an ssDNA aptamer that specifically binds to a protein of the SARS-CoV-2, the aptamer selected from:

(a) One or more ssDNA aptamers that specifically bind to the spike protein of SARS-CoV-2, in particular to the subunit 1 (S1) of the spike protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(a1) atgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 1; [702]),

(a2) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccctaaatacgagcaac (SEQ ID NO: 2, [AB7]),

(a3) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccct (SEQ ID NO: 3, [703]),

(a4) agactggtgtgttggctctgggggtgtggaggggt (SEQ ID NO: 4, [704]),

(a5) tggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO; 5, [701])

(a6) tggggtcggtgtgtcgggtgggggttgggt (SEQ ID NO: 6, [901]),

(a7) gcggatgaagactggtgtgggtggggtcggtgtgtcgggtgggggttgggttgtgtgtgccctaaatacgagcaac (SEQ ID NO: 7, [900 or AB9]),

(a8) gcggatgaagactggtgtgggtgtggttagtgtttcccgttctgttgttttcttggttgccctaaatacgagcaac (SEQ ID NO: 8, [AB4]),

(a9) gttgctcgtatttagggcggaggggcgggggaggaggtggcgggaatgtccattgtctacaccagtcttc atccgc (SEQ ID NO: 9, [AB5])

(a10) ttggggcgggagttttgtttgggtgggggtt (SEQ ID NO: 10);

(b) One or more ssDNA aptamers that specifically bind to N protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(b1) acgcaagggaccacagggtttttcaatataaagatcggattctttattat (SEQ ID NO: 11, [N21]),

(b2) cacgacgcaagggaccacagggtttttcaatataaagatcggattctttattatctatagactacagcacgacaccgcagaggca (SEQ ID NO: 12, [N2]),

(b3) gtttttcaatataaagatcggattctttattatctatagacta (SEQ ID NO: 13, [N22]),

(b4) gcggatgaagactggtgtgtgtggcgctgtttctgttgggtggtgagtgggtctgcccgccctaaatacgagcaac (SEQ ID NO: 14, [N100]),

(b5) gcggatgaagactggtgtcgtgcagcgttaagcggtgaggtgtactgagggttacggggccctaaatacgagcaac (SEQ ID NO: 15, [N200]),

(b6) gcggatgaagactggtgtagagggccgtggaggggcttggcgtgttgaatgagtgagggccctaaatacgagcaac (SEQ ID NO: 16, [N300]),

(b7) gcggatgaagactggtgtgggcaagtgtgtttagtgatggacgcccatgtgtagtcgtgccctaaatacgagcaac (SEQ ID NO: 17, [N400])

(b8) gcggatgaagactggtgtaatatttgtgtgtgtttgttgttgtttttggtttgtttgtgccctaaatacgagcaac (SEQ ID NO: 18, [N600]),

(b9) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 19, [N700]),

(b10) gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 20, [N701]),

(b11) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggc (SEQ ID NO: 21, [N702])

(b12) tgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 22, [N703]),

(b13) gcggatgaagactggtgtcgggagggagtccgtgaggaggggggctgcggaatggtatgccctaaat (SEQ ID NO: 23, [N801]),

(c) One or more ssDNA aptamers specifically binding membrane (M) protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(c1) gttgctcgtatttagggcgggtgggcgggggatcgttgtgctgttgcttgttgtgtgtacaccagtcttcatccgc (SEQ ID NO: 24, [M1000]),

(c2) gttgctcgtatttagggcgggtgggtggggtgtatgtttcgtgccgtgtgctctgtgtacaccagtcttcatccgc (SEQ ID NO: 25, [M1100]),

(c3) gttgctcgtatttagggcggcaagggtgggagggtgggtgttgttgcggcgtgtctgtacaccagtcttcatccgc (SEQ ID NO: 26, [M1200]),

(c4) gttgctcgtatttagggccgaggggacgctggtattgggtgggtgggagggcgttggacaccagtcttcatccgc (SEQ ID NO: 27, [M1300],

(c5) aggggacgctggtattgggtgggtgggagggcgttggacaccagtct (SEQ ID NO: 28, [M1301]),

(c6) aggggacgctggtattgggtgggtgggagggcgttgga (SEQ ID NO: 29, [M1302])

(c7) gttgctcgtatttagggctgagctgggtgcgttcctggggctgcattcctggggcccgacaccagtcttcatccgc (SEQ ID NO: 30, [M200]), and

(c8) gcggatgaagactggtgtaggggtgagggcgaggggtgggtggaggcaggtcgagggcgccctaaatacgagcaac (SEQ ID NO: 31, [M500])

In a particular embodiment of this second aspect, the ssDNA aptamer is an aptamers that specifically binds to the S1, said aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, and SEQ ID NO: 7. More in particular the ssDNA aptamers consist in any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, and SEQ ID NO: 7, thus they have 100% of identity with these sequences.

As indicated, these sequences gave good values in terms of affinity and as will be depicted in the examples, also of specificity for the target protein. In addition, low IC50 values make them useful as sensitive diagnostic tools.

In another particular embodiment of the second aspect, the ssDNA aptamer is an aptamer that specifically binds to N protein of SARS-CoV-2, said aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 11, and SEQ ID NO: 12. More in particular the ssDNA aptamers consist in any of SEQ ID NO: 11, and SEQ ID NO: 12, thus they have 100% of identity with these sequences.

In another particular embodiment of the second aspect, the ssDNA aptamer is an aptamer that specifically binds to M protein of SARS-CoV-2, said aptamer specifically binding a peptide defined by sequence LKKLLEQWNLVIGFLFL (SEQ ID NO: 37), also termed peptide of M, the aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29. More in particular the ssDNA aptamers consist in any of SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, thus they have 100% of identity with these sequences.

Dissociation constant values (Kd) for the aptamers of the invention are listed in examples below. These Kd values were preferably determined by using a dilution series (see below and in examples). The Kd is by definition a specific type of equilibrium constant that measures the propensity of a larger object to separate (dissociate) reversibly into smaller components, as when a complex falls apart into its component molecules, or when a salt splits up into its component ions. In the particular case of the invention, referring to the binding of aptamers to a target, Kd is defined according to the following formula:

Kd = [free aptamer][free target] / [aptamer-target]

The lower the Kd the high the affinity of the aptamer and the target.

The IC50 or dissociation constant (KD) is for example determined by direct ELONA assay. High protein-binding capacity plates (44-2404-ThermoFisher) are coated with 3-5 ug/mL of the target protein (S1, M or N) overnight at 4ºC. Different concentrations of the biotinylated tested aptamer are added in buffer (e.g., 1X-PBS, 1 mM Mg Cl2 for 1 h at 25ºC). 50 ng/mL streptavidin-Poly-HRP (SP80C SDT-Reagents for live) are then added in 1X-PBS, 0.05 % Tween, pH 7.4 for 30 min at 25ºC. Between each step, three times washing step is performed with 1X-PBS, 0.05 % Tween, pH 7.4. BioFX TMB Super sensitive (TMBS-1000-01 Surmodics) is then added for 5 min and the reaction is stopped with 1M H2SO4. The optical density at 450 nm is read, for example with with SynergyHTX spectrophotometer. GraphPad Prism 9 is then for example used to perform nonlinear regression, one site-specific binding with Hill slope model.

In the present invention, the term "identity" refers to the percentage of residues that are identical in the two sequences when the sequences (either of amino acid or of nucleic acid) are optimally aligned. If, in the optimal alignment, a position in a first sequence is occupied by the same amino acid or nucleotide residue as the corresponding position in the second sequence, the sequences exhibit identity with respect to that position. The

percentage of identity determines the number of identical residues over a defined length in a given alignment. Thus, the level of identity between two sequences or ("percent sequence identity") is measured as a ratio of the number of identical positions shared by the sequences with respect to the number of positions compared (i.e., percent sequence identity = (number of identical positions/total number of positions compared) x 100). A gap, i.e., a position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues and is counted as a compared position.

A number of mathematical algorithms for rapidly obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. For purposes of the present invention, the sequence identity between two amino acid sequences or two nucleotide sequences is preferably determined using algorithms based on global alignment, such as the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453), preferably implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277); or the BLAST Global Alignment tool (Altschul et al., “Basic local alignment search tool”, 1990, J. Mol. Biol, v. 215, pages 403-410), using default settings. Local alignment also can be used when the sequences being compared are substantially the same length.

In a particular embodiment of the aptamers of the aspect of the invention, optionally in combination with any one of the embodiments provided below, the percentages of identity with any of the therein identified sequences (i.e., aptamers) are selected from at least 85%, at least 86%, at least 87%, at least 88%, at least 88.5%, at least 89%, at least 89.5%, at least 90%, at least 90.5%, at least 91%, at least 91.5%, at least 92%, at least 92.5%, at least 93%, at least 93.5%, at least 94%, at least 94.5%, at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%, at least 97.5%, at least 98%, at least 98.5%, at least 99%, or at least 99.5% identical to the indicated sequences, or even 100% identity with the indicated sequences.

As indicated, also disclosed herewith is a multispecific targeting chimera comprising two or more of the aptamers as defined in the first aspect, covalently linked to a linker molecule (L).

In a particular embodiment of this multispecific targeting chimera, L is selected from a polymeric compound, more in particular selected from a polynucleotides, such as dsDNA, ssDNA, RNA and combination thereof. In another also more particular embodiment the

linker is a peptide nucleic acid (PNA). In another particular embodiment, the linker is a polyethylene glycol of formula H−(O−CH_2−CH_2)n−OH, wherein n is an integer from 1 to 100. In particular, it is a polyethylene glycol selected from polyethylene glycol of n=6-80, more in particular with an *n* around 70 or an hexaethylene glycol (i.e., $H(OCH_2CH_2)_6OH$), in which n=6.

Following the commonly known techniques by the skilled person in the art, the linker(s) and the two or more aptamers are joined.

In another particular embodiment of the multispecific targeting chimera, it is a bispecific targeting chimera of formula (I)

(A)-L-(A') (I), wherein A and A' are equal or different aptamers as defined in the previous (first) aspect or in any of the particular embodiments, and L is a linker covalently linked to A and A', said linker being in more particular embodiments selected from the previously disclosed.

Another aspect of the invention is the vitro method for the diagnosis of SARS-CoV2 infections, comprising determining in an isolated sample of a subject, the presence and/or levels of SARS-CoV2 by contacting said sample with one or more aptamers, or aptamer conjugates as defined in the previous aspects and respective embodiments, and/or with a multispecific aptamer as defined above; and wherein if SARS-CoV2 is detected, the subject is diagnosed of SARS-CoV2 infection.

In a particular embodiment of the in vitro method the subject is an animal, more in particular a mammal, and even more in particular a human.

In also another particular embodiment of the in vitro method, the isolated sample is a biofluid, more in particular selected from blood (including whole blood, plasma and serum), saliva, mucosa. Particular samples are those obtained from the use of swabs to collect respiratory material found in the animal nose (i.e., nasal swans or nasopharyngeal swabs).

In also another particular embodiment of the method for the diagnosis of the invention, the aptamers, conjugates and/or multispecific aptamers are labelled with a reporter molecule being, in particular selected from the ones listed above.

A person skilled in the art will understand the conditions required for binding to occur between the aptamers or aptamers in the conjugates described herein and the target coronavirus protein as defined herein.

Thus, in order the method can be easily implemented, herewith provided are also kits comprising one or more aptamers or conjugates as defined in the previous aspects, or a multispecific aptamer as defined also herewith, and instructions for carrying out the method for the diagnosis of SARS-CoV2 infections.

In a particular embodiment, the aptamers and/or the conjugates as defined in the previous aspects, or a multispecific aptamer as defined above, are the only reagents in the kit for detecting the presence and/or levels of the coronavirus in the sample.

In another particular embodiment, the kit further comprises other reagent means for the carrying out of the method. Example of other means include solvents (e.g., isotonic buffers), and optionally a compartment where to put the sample in contact with the aptamers, and/or colorimetric or fluorescent reagents for the visualization of the binding.

In another particular embodiment, the kit is for the carrying out of a sandwich method for the detection of the SARS-CoV2 protein.

In another particular embodiment of the kits, they comprise a solid support with the aptamers, conjugates and/or multispecific aptamers immobilized on the said solid support.

Due to the high affinity of the aptamers of the invention for the SARS-CoV2 proteins, they are useful as therapeutic agents as indicated, and they can be used as actives in pharmaceutical compositions.

The term “pharmaceutical” encompasses also the concept of “veterinary composition”. Thus, they relate to compositions that are therapeutically effective when administered by any desired or applicable route to any animal, including humans.

In a particular embodiment, the aptamer or conjugates are in form of their pharmaceutically acceptable salts. Phosphate groups of the polynucleotide strand form salts in particular with alkaline or alkaline-earth cations. More in particular, the salts are alkaline salts of the aptamers or conjugates, more in particular selected from sodium and potassium salts.

The expression "therapeutically effective amount" as used herein, refers to the amount of

a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disease which is addressed. The particular dose of compound administered according to this invention will of course be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and the similar considerations.

The expression "pharmaceutically acceptable excipients or carriers" refers to pharmaceutically acceptable materials, compositions or vehicles. Each component must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the pharmaceutical composition. It must also be suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity or other problems or complications commensurate with a reasonable benefit/risk ratio. Examples of suitable acceptable excipients are solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

Examples of suitable pharmaceutically acceptable excipients are solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

The relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents,

preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as coloring agents, coating agents, sweetening, and flavoring agents can be present in the composition, according to the judgment of the formulator.

The pharmaceutical compositions containing the polynucleotide construct according to the invention, the vector according to the invention, or the cells of the invention, can be presented in any dosage form, for example, solid or liquid, and can be administered by any suitable route, for example, oral, parenteral, rectal, topical, intranasal, intraocular, intraperitoneal or sublingual route, for which they will include the pharmaceutically acceptable excipients necessary for the formulation of the desired dosage form, for example, topical formulations (ointment, creams, lipogel, hydrogel, etc.), eye drops, aerosol sprays, injectable hydrogels, injectable solutions, osmotic pumps, etc.

Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, corn-starch, powdered sugar, and combinations thereof.

Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked polyvinylpyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and combinations thereof.

Exemplary binding agents include, but are not limited to, starch (e.g., corn-starch and starch paste); gelatin; sugars (e.g., sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol); natural and synthetic gums (e.g., acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, polyvinylpyrrolidone), magnesium aluminium silicate (Veegum), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; and combinations thereof.

Exemplary preservatives may include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, ascorbyl palmitate, ascorbyl stearate, ascorbyl oleate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and trisodium edetate.

Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and combinations thereof.

Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

The preparation of all these pharmaceutical compositions is according to the common practices known by the skilled person in the art.

As indicated, another aspect of the invention is a conjugate or a pharmaceutically acceptable salt thereof, or an aptamer or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition, all as defined in previous aspects, for use in the prevention and/or treatment of an infection caused by a virus from the Coronaviridae family, in particular by a virus selected from the group comprising MERS-CoV, SARS-CoV and SARS- CoV-2. In a more particular embodiment, the infection is caused by SARS-CoV-2.

Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps. Furthermore, the word “comprise” encompasses the case of “consisting of”. Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

Examples

The aptamers selected as effective for the diagnosis and treatment of SARS-CoV2 infections was performed using the combinatorial chemistry technique known as Systematic evolution of ligands by exponential enrichment (SELEX).

Enrichment of the subsequent rounds of selection, varying from 6 to 25, were checked by Polymerase Chain Reaction (PCR) or Enzyme-Linked OligoNucleotide Assay (ELONA). The populations were then sequenced and analysed for the structure characterization. 10 aptamers for each of the selected proteins (S1, M and N) could be chosen.

Example 1. Obtention of the aptamers specific for S1 protein.

Two independent SELEX were performed for the Spike protein, in one of them it was used as target the S1 subunit and in the other the RBD region. Proteins were used in both recombinants with a C-terminal histidine tag.

The sequence of S1 protein used for the selection of the aptamers was the one in NCBI database with sequence entry identifier YP_009724390.1, produced in Baculovirus-Insect Cells, version of sequence of 18-JUL-2020, in https://www.ncbi.nlm.nih.gov/protein/1796318598 (retrieved the 21st of February 2022).

After 6 rounds of selection, the enrichment of the aptamer populations obtained in each of them in relation to the initial population was analyzed by Enzyme-Linked OligoNucleotide Assay (ELONA). The results showed that the populations obtained in round 6 showed significant enrichment, so they were cloned and sequenced by Sanger and also by NGS (Next-Generation Sequencing).

NGS sequencing allowed knowing all the selected aptamers. The sequences that had the highest percentage of representation were identified and, after a bioinformatic analysis, 10 aptamers were chosen that were synthesized and used in the affinity studies.

The recognition of the RBD domain and the S1 subunit of SARS-CoV-2 was evaluated by ELONA. In general, a preferential recognition of the 10 aptamers by the S1 subunit was observed. However, the AB9 aptamer (SEQ ID NO: 7) recognizes the RBD domain similarly to the entire S1 subunit.

Using direct ELONA, the dissociation constant (Kd) was determined for the 10 aptamers for the SARS-CoV-2 spike protein. Aptamers AB4 (SEQ ID NO: 8), AB5 (SEQ ID NO: 9), AB7 (SEQ ID NO: 2) and AB9 (SEQ ID NO: 7) showed dissociation constants less than 6 nM.

For the ELONA assays the following procedure was followed. The IC50 o dissociation constant (KD) was determined by direct ELONA assay. High protein-binding capacity plates (44-2404-ThermoFisher) were coated with 3-5 ug/mL of recombinant target protein overnight at 4ºC. Different concentrations of the biotinylated aptamer were added in 1X-PBS, 1 mM Mg Cl2 for 1 h at 25ºC. 50 ng/mL streptavidin-Poly-HRP (SP80C SDT-Reagents for live) were added in 1X-PBS, 0.05 % Tween, pH 7.4 for 30 min at 25ºC. Between each step, three times washing step was performed with 1X-PBS, 0.05 % Tween, pH 7.4. BioFX TMB Super sensitive (TMBS-1000-01 Surmodics) was added for 5 min and the reaction was stopped with 1M H2SO4. The optical density at 450 nm was read with SynergyHTX spectrophotometer. GraphPad Prism 9 was used to perform nonlinear regression, one site-specific binding with Hill slope model.

Table 1

Aptamer	Kd (nM)	R^2
AB4	4.615	0.977
AB5	5.81	0.969
AB7	1.51	0.993
AB9	2.24	0.980

Data obtained from direct ELONA for the S1 subunit of SARS-CoV-2. Each of the aptamers were conjugated with biotin and incubated with the S1 subunit adhered to the plate. Kd values were calculated. The number of replicates (n) was of 3 for each condition (n=1).

In the same type of direct ELONA assay, the IC50 was determined for each aptamer by

incubating different concentrations of the aptamers conjugated with biotin with the S1 protein attached to the plaque, as indicated above. Of the 10 aptamers, 4 showed the best values of affinity in the nanomolar range (2 - 7 nM).

Table 2

Aptamer	IC50 (nM)	R^2
AB4	4.72	0.994
AB5	6.97	0.994
AB7	1.983	0.993
AB9	2.19	0.973

Data obtained from ELONA directo para la subunidad S1 de SARS-CoV-2. Each of the aptamers were conjugated with biotin were incubated with the S1 subunit adhered to the plate. IC50 values from n=3 of each condition (n=1).

Tests were done to form aptamer pairs that were able to recognize S1 in a sandwhich assay.

For the sandwich ELONA assay the following procedure was followed:

The maleimide plate (15150-Fisher scientific) was washed three times with 1X-PBS, 0.05 % Tween, pH 7.4. As capture aptamer, 500 nM thiolated aptamers were used to coat the well overnight at 4ºC. To prevent unspecific binding, 5% skim milk (70166-SIGMA) was added for 1 hour at 25ºC. Target protein at different concentrations was added in 1X-PBS, 1 mM Mg Cl2 for 1 hour at 25ºC. As detection aptamer, 100 – 200 nM biotin aptamer was added in 1X-PBS, 1 mM Mg Cl2 for 30 min at 25ºC. 50 ng/mL streptavidin-Poly-HRP (SP80C SDT-Reagents for live) were added in 1X-PBS, 0.05 % Tween, pH 7.4 for 30 min at 25ºC. Between each step, three times washing step was performed. BioFX TMB Super sensitive (TMBS-1000-01 Surmodics) was added for 5 min and the reaction was stopped with 1M H2SO4. The optical density at 450 nm was read with SynergyHTX spectrophotometer.

As a result, a pair of aptamers was obtained as useful for the sandwich assay to detect S1. The detection limit was determined using different amounts of S1 protein. The pair of aptamers was AP4; SEQ ID NO: 2 and SEQ ID NO: 7, which allowed a detection limit of (84 ng/mL).

Truncated versions of some aptamers were designed, consisting of sequences with some

nucleotides omitted.

The affinity calculation for the truncated aptamers was determined by ELONA, as indicated above. 5 ug/mL of S1 protein and different concentrations of the biotinylated aptamer were evaluated. The affinities for aptamers 700 (SEQ ID NO: 2), 702 (SEQ ID NO: 1), 900 (SEQ ID NO: 7) and 901 (SEQ ID NO: 6) are shown in the Table 3 below. No change in affinity was observed for the truncated aptamer 901 (SEQ ID NO: 6), relative to the original aptamer 900 (SEQ ID NO: 7). While with aptamer 702 (SEQ ID NO: 1), which corresponds to the truncated version of aptamer 700 (SEQ ID NO: 2), a large increase in affinity was obtained.

Table 3

Aptamer	Kd (nM)
700 (SEQ ID NO: 2)	0.004
702 (SEQ ID NO: 1)	7.87e-5
900 (SEQ ID NO: 7)	0.82
901 (SEQ ID NO: 6)	0.05

Example 2. Obtention of the aptamers specific for the nucleocapsid (N) protein.

Independent SELEX were performed in different libraries to increase success to get the aptamers. ELONA direct assays were performed as indicated in Example 1.

Library A:

Nucleocapside of SARS-CoV-2 expressed in baculovirus with a histidine tag at C-terminal was used to allow binding to the Ni-NTA Agarose resin. The sequence of the protein was the one disclose in NCBI protein database with entry YP_009724397.2, produced in Baculovirus-Insect Cells, version of sequence of 18-JUL-2020, in https://www.ncbi.nlm.nih.gov/protein/1798174255 (retrieved the 21st of February 2022). Aptamers were added for the selection rounds in PBS 1mM MgCl2 pH 7.4. ssDNA bound to the resin was amplified after two washing steps. After the second round, a counter-selection in front of agarose-NTA resin was carried out. In the third round an ELONA assay was performed to value the enrichment of the populations, by immobilizing N protein. The sequences were labelled with digoxigenin and an HRP-labelled anti-digoxigenin antibody was used, further seen with ABTS.

Further, a counter-selection was performed in front of other N protein of other human coronavirus (MERS-CoV, SARS-CoV and HCoV-HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E). To this aim an equimolar amount of the proteins was prepared and bound to the resin, and finally incubated with population of the third round.

From this library and procedure the aptamers of Table 4 were selected. Kd is indicated

Table 4

Aptamer	Kd (nM)
N-100 (SEQ ID NO: 14)	0.05
N-200 (SEQ ID NO: 15)	0.02
N-300 (SEQ ID NO: 16)	2.49
N-700 (SEQ ID NO: 19)	0.18
N-701 (SEQ ID NO: 20)	1.45
N-801 (SEQ ID NO: 32)	0.03
N-703 (SEQ ID NO: 22)	2.04

Libraries B and C:

Using nucleocapside protein of SARS-CoV-2 and of other coronaviruses (HCoV-HKU1, HCoV-NL63, HCoV-OC43) bound to superparamagnetic Dynabeads® MyOne™ Carboxylic Acid.

Selected aptamers showed a preferential binding for N protein in SARS-CoV-2.

From this libraries B and C and procedure the aptamers of Table 5 were selected. Kd and IC50 is indicated

Table 5

Aptamer	Kd (nM) /IC50 (nM)
N2 (SEQ ID NO: 12)	1.44 / 3.08
N21 (SEQ ID NO: 11)	5.8e-9 / 1.01e-8

Kd and IC50 obtained from different aptamer concentrations and using the GraphPad Prism 9 software.

Example 3. Obtention of the aptamers specific for the membrane (M) protein.

Two approaches were applied for selectin specific aptamers for the M protein in SARS-CoV-2. In the first approach a peptide of 17 amino acids in the M protein was used. The peptide resulted from the N-terminal region of the protein and had the sequence Biotin-LKKLLEQWNLVIGFLFL, (SEQ ID NO: 37) (also termed in this description peptide of M). In the second approach the whole M protein was used. The sequence of M was the recombinant obtained in E.coli, YP_009724393.1, version of sequence of 18-JUL-2020, in https://www.ncbi.nlm.nih.gov/protein/ YP_009724393.1 (retrieved the 21st of February 2022).

The most abundant structural protein of coronaviruses is the M glycoprotein; it spans the membrane bilayer, leaving a short NH2-terminal domain outside the virus and a long COOH terminus (cytoplasmic domain) inside the virion. The M protein can bind to all other structural proteins. Binding with M protein helps to stabilize N proteins and promotes completion of viral assembly by stabilizing the N protein-RNA complex, inside the internal virion. *Thomas S. Pathog Immun. 2020;5(1):342-363. Published 2020 Oct 19. doi:10.20411/pai.v5i1.377.*

In next Table 6, selected aptamers and Kd values are shown

Aptamer	Kd (nM)
Recognizing peptide 7-23 of M protein (SEQ ID NO: 36)	
M-1000 (SEQ ID NO: 24)	1.58
M-1200 (SEQ ID NO: 26)	2.19
M-1300 (SEQ ID NO: 27)	0.79
M-1301 (SEQ ID NO: 28)	2.351
M-1302 (SEQ ID NO: 29)	0.5578
Recognizing a recombinant M protein in E. coli	
M-200 (SEQ ID NO: 30)	2.01
M-500 (SEQ ID NO: 31)	3.07

As previously indicated for Example 1, all Kd for aptamers were determined from data in an ELONA assay using different biotin-labelled aptamers (50, 100, 150, 200, 250 nM), which were incubated with 5 µg/ ml of the proteinat 25 ºC. Kd was calculated using the GraphPad Prism 9 software.

Example 4. Protein degradation assay

To assess the feasibility of the aptamers of the invention, a conjugate was tested to bind both the N protein of SARS-CoV2 and the cation independent mannose receptor (CI-M6PR). This experimental was named aptamer- based LYTACs for being CI-M6PR a cell-surface lysosome-shuttling receptor. LYTAC stands for Lysosome-targeting chimaeras, and some of these kinds of compounds are disclosed, for example by Banik et al., "Lysosome-targeting chimaeras for degradation of extracellular proteins", Nature-2020, vol. no. 584, pp.: 291–297 (2020). https://doi.org/10.1038/s41586-020-2545-9.

The hypothesis behind this experimental test was that LYTAC-mediated degradation of the N protein of SARS-CoV2 by the cells would decrease the amount of nucleocapsid protein in the cell media at an increased rate compared to any nucleocapsid protein degradation mediated only by the cells themselves.

Briefly, SARS-CoV-2 nucleocapsid protein (N) was added to the culture media of cells along with a N-targeted LYTAC and then incubated for 22 hours. The nucleocapsid LYTAC (i.e., conjugate) was comprised of two aptamers, acting as directed recognition elements, one aptamer against the nucleocapsid protein with sequence gtttttcaatataaagatcggattctttattatctatagacta (N22 or SEQ ID NO: 13) and other against the cation independent mannose receptor (CI-M6PR), GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC, (SEQ ID NO: 38) linked together by a HEG molecule (hexaethylene glycol, or also $H(OCH_2CH_2)_6OH$), in which n=6). The schematic formula was 5´-SEQ ID NO: 13-HEG-SEQ ID NO:38-3'. After the incubation time, the cell media was recovered and its protein content isolated by precipitation. The presence of nucleocapsid protein was finally quantified by Western-Blot. Two different cell lines, HepG2 and K562 (both commercially available from different suppliers) with different cell biology, and levels of expression of the CI-M6PR were used for the experiment to evaluate the response in different cell types. HepG2 are epithelial-like cells derived from a hepatocellular carcinoma and are widely used as hepatocyte models. K562 are non-adherent cells, lymphoblasts derived from leukemia. In both cell lines a reduction of N protein was observed when the N-targeted LYTAC was present. Suggesting a LYTAC-mediated degradation where N protein is captured by the LYTAC, internalized by the cells via CI-M6PR and finally protein degradation likely following the lysosomal pathway.

Methods

K562 cells ($5x10^5$) were incubated in RPMI growth medium without serum and supplemented with two different amounts of nucleocapsid protein, 100 ng or 500 ng (40588-V08B Sinobiologicals), and 300 nM nucleocapsid LYTAC (i.e., aptamer-based

LYTAC) for 22 hours at 37ºC in 5% CO_2. Cells were also incubated with the aforementioned protein amounts and without nucleocapsid LYTAC to assess any degradation by N protein mediated by the cells themselves.

HepG2 cells ($2x10^5$) were seeded in complete DMEM low glucose medium with 10% FBS for 24 hours. Previous to the incubation with the HepG2 cells, two different amounts of N protein, 100 ng or 500 ng, were firstly incubated with 300 nM of nucleocapsid LYTAC (i.e., the conjugate also termed aptamer-based LYTAC) for 50 min at 25ºC in static conditions. Cells were then washed twice using DMEM low glucose medium without FBS. The preincubated N protein-LYTAC complexes were added to serum deprived cells, according to experimental conditions, and incubated for 22 hours at 37ºC in 5% CO_2. As in the previous experiment, controls with cells and with both amounts of N protein were also incubated to evaluate any protein degradation mediated by the cells themselves.

In both sets of experiments controls of N protein degradation, where both amounts of N-protein were incubated either with or without aptamer conjugate in absence of cells, were performed.

After the incubation times, cell media was recovered and cleared of any cell or cellular debris by centrifugation. Then a trichloroacetic acid /acetone precipitation of proteins was performed. Precipitated proteins were loaded into a 12% SDS–PAGE gel and separated by electrophoresis.

Separated proteins were transferred to a PVDF membrane. After transfer, the PVDF membranes were blocked in blocking buffer (5% Skim milk in PBS-0,05% Tween), first 1 h at room temperature and then overnight at 4ºC. Membranes were incubated with the primary antibody against nucleocapsid protein (40143-MM05 Sino Biological 1:1000) in blocking buffer at 25 °C for 1 hour. Membranes were washed three times with PBS-0,05% Tween for five minutes each to remove any excess antibody. Then, membranes were incubated with a goat anti-mouse IgG-HRP secondary antibody (P0447 Dako 1:1000) in blocking buffer for 1 h at room temperature. Finally, membranes were washed three times with PBS-0,05% Tween.

Protein bands in the membrane were revealed with SuperSignal™ West Pico PLUS Chemiluminescent Substrate. Gel images were taken in a ChemiDoc gel reader at different exposition times. Bands intensity were analyzed with image Studio Lite Ver 5.2 and ImageJ.

For the experiment with K562 cells, data are depicted in FIG. 1.

K562 cells further decrease the quantity of nucleocapsid in cell media in presence of the nucleocapsid-targeted LYTAC. The amount of nucleocapsid protein present after its incubation with K562 cells in presence or absence of the nucleocapsid-targeted LYTAC was determined by Western-Blot as indicated. Columns 1& 2 show the amount of nucleocapsid protein detected after 22 hours of incubation with K562 cells without nucleocapsid LYTAC (i.e., the conjugate of the invention). Column 3 represents the background signal obtained when incubated with K562 cells for 22 hours without nucleocapsid protein. Finally, columns 4&5 show the nucleocapsid levels after 22 hours of incubation with K562 in presence of the nucleocapsid LYTAC. For the 100 ng and the 500 ng protein experiments a reduction of 57% and 25% was observed, respectively. These results suggest a LYTAC-mediated degradation of the nucleocapsid protein.

For the experiment with HepG2 cells, data are depicted in FIG. 2.

HepG2 cells further decrease the quantity of nucleocapsid in cell media in presence of the Nucleocapsid-targeted LYTAC (i.e., the conjugate of the invention). Nucleocapsid protein levels present after its incubation with HepG2 cells in presence or absence of the nucleocapsid-targeted LYTAC were determined by Western-Blot. Columns 1& 2 shown the amount of nucleocapsid protein detected after 22 hours of incubation with the HepG2 in presence of the nucleocapsid LYTAC, a 50-minute protein-LYTAC preincubation step was taken before adding the nucleocapsid protein and the LYTAC to the cell medium. Column 3 represents the background signal obtained when incubated with HepG2 cells for 22 hours in absence of nucleocapsid protein. Finally, Columns 4&5 show the nucleocapsid levels after 22 hours of incubation with HepG2 without the LYTAC. For the 100 ng and the 500 ng protein experiments a diminution in the nucleocapsid protein quantity of 17% and 38% was observed, respectively. These results suggest a LYTAC-mediated degradation of the nucleocapsid protein.

Both cell assays illustrate the therapeutic activity of the conjugate of the invention.

Citation List

- WO2021205012 (BERLIN CURES, GmbH)
- Sun et al., in “Aptamer Blocking Strategy Inhibits SARS-CoV-2 Virus Infection”, Angew. Chem. Int. - 2021, vol. no. 60, pp.:10266 –10272
- Krüger et al., “Aptamer Applications in Emerging Viral Diseases”, Pharmaceuticals-2021, vol. no. 14, pp.: 622.https://doi.org/10.3390/ph1407062.
- Cho et al., J. Biosci. Bioeng. 2011, 112, 535–540.

- Song et al., Anal. Chem 2020, 92, 3393-3402.
- Rice et al., 2000, Trends Genet. 16: 276-277
- Altschul et al., "Basic local alignment search tool", 1990, J. Mol. Biol, v. 215, pages 403-410.
- Banik et al., "Lysosome-targeting chimaeras for degradation of extracellular proteins", Nature-2020, vol. no. 584, pp.: 291–297 (2020). https://doi.org/10.1038/s41586-020-2545-9.
- Thomas S. The Structure of the Membrane Protein of SARS-CoV-2 Resembles the Sugar Transporter SemiSWEET. Pathog Immun. 2020;5(1):342-363. Published 2020 Oct 19. doi:10.20411/pai.v5i1.377.
- Chen A, Yang S. Replacing antibodies with aptamers in lateral flow immunoassay. Biosens Bioelectron. 2015;71:230–242.
- Dahms, N. (2002). P-type lectins. Biochimica et Biophysica Acta (BBA) - General Subjects, 1572(2-3), 317–340. doi:10.1016/s0304-4165(02)00317-3.

Claims

1.- A conjugate comprising:

- one or more single stranded DNA (ssDNA) aptamers that specifically bind to one or more target proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and
- a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds to, or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

2.- The conjugate according to claim 1, wherein the one or more proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are selected from M protein; S protein, in particular S1 protein; and N protein.

3.- The conjugate according to any one of claims 1-2, wherein the compound that induces degradation of the target protein is a ligand selected from the group consisting of an aptamer, a glycopeptide, an antibody, a nanobody, all with the capability to bind a cell-surface lysosome-shuttling receptor.

4- The conjugate according to any one of claims 1-3, wherein the ligand molecule that specifically binds to, or associates with lysosome, or the molecule that specifically binds a lysosomal targeting molecule is selected from the group consisting of:

(i) A molecule which specifically binds the cation independent mannose 6-phosphate receptor (CI-M6PRc or IGFR2) or a fragment thereof, in particular being selected from an aptamer, an antibody, a nanobody, a peptide, mannose-6-phosphate, an analogue of mannose 6-phosphate (M6P), M6P-containing ligands, and non MP6 containing ligands; or alternatively

(ii) an aptamer, an antibody or a peptide that specifically binds an asialoglycoprotein receptor or a fragment thereof.

5.- The conjugate according to any one of claims 1-4, wherein the ligand that specifically binds a lysosomal targeting molecule is an aptamer, more in particular, it is the aptamer of SEQ ID NO: 38 (GGGCGCGTAGATGACGAGCAGTCCTAACATCGTTTAGGAC), or an aptamer with a 90% of identity with this sequence.

6.- The conjugate according to any to any one of claims 1-5, which comprises one or more aptamers that specifically bind M protein of SARS-CoV2.

7.- The conjugate according to any to any one of claims 1-6, which comprises: one or more aptamers that specifically bind S protein of SARS-CoV2, in particular S1 protein.

8.- The conjugate according to any to any one of claims 1-7, which comprises one or more aptamers that specifically bind N protein of SARS-CoV2.

9.- The conjugate according to any to any one of claims 1-8, wherein the one or more ssDNA aptamer that specifically binds to the spike protein of SARS-CoV-2, is an aptamer that specifically binds to the subunit 1 (S1) of the spike protein of SARS-CoV-2, said aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(a1) atgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 1),

(a2) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccctaaatacgagcaac (SEQ ID NO: 2),

(a3) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccct (SEQ ID NO: 3),

(a4) agactggtgtgttggctctgggggtgtggaggggt (SEQ ID NO: 4),

(a5) tggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 5)

(a6) tggggtcggtgtgtcgggtgggggttgggt (SEQ ID NO: 6),

(a7) gcggatgaagactggtgtgggtggggtcggtgtgtcgggtgggggttgggttgtgtgtgccctaaatacgagcaac (SEQ ID NO: 7),

(a8) gcggatgaagactggtgtgggtgtggttagtgtttcccgttctgttgttttcttggttgccctaaatacgagcaac (SEQ ID NO: 8),

(a9) gttgctcgtatttagggcggaggggcgggggaggaggtggcgggaatgtccattgtctacaccagtcttc atccgc (SEQ ID NO: 9)

(a10) ttggggcgggagttttgtttgggtgggggtt (SEQ ID NO: 10);

10.- The conjugate according to any to any one of claims 1-9, wherein the one or more ssDNA aptamer that specifically binds to N protein of SARS-CoV-2, is an aptamer

comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(b1) acgcaagggaccacagggtttttcaatataaagatcggattctttattat (SEQ ID NO: 11),

(b2) cacgacgcaagggaccacagggtttttcaatataaagatcggattctttattatctatagactacagcacgacaccgcagaggca (SEQ ID NO: 12),

(b3) gtttttcaatataaagatcggattctttattatctatagacta (SEQ ID NO: 13),

(b4) gcggatgaagactggtgtgtgtggcgctgtttctgttgggtggtgagtgggtctgcccgccctaaatacgagcaac (SEQ ID NO: 14),

(b5) gcggatgaagactggtgtcgtgcagcgttaagcggtgaggtgtactgagggttacggggccctaaatacgagcaac (SEQ ID NO: 15),

(b6) gcggatgaagactggtgtagagggccgtggaggggcttggcgtgttgaatgagtgagggccctaaatacgagcaac (SEQ ID NO: 16),

(b7) gcggatgaagactggtgtgggcaagtgtgtttagtgatggacgcccatgtgtagtcgtgccctaaatacgagcaac (SEQ ID NO: 17)

(b8) gcggatgaagactggtgtaatatttgtgtgtgtttgttgttgtttttggtttgtttgtgccctaaatacgagcaac (SEQ ID NO: 18),

(b9) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 19),

(b10) gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 20),

(b11) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggc (SEQ ID NO: 21)

(b12) tgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 22),

(b13) gcggatgaagactggtgtcgggagggagtccgtgaggaggggggctgcggaatggtatgccctaaat (SEQ ID NO: 23).

11.- The conjugate according to any to any one of claims 1-10, wherein the one or more ssDNA aptamer that specifically binds to membrane (M) protein of SARS-CoV-2, is an aptamer comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(c1) gttgctcgtatttagggcgggtgggcgggggatcgttgtgctgttgcttgttgtgtgtacaccagtcttcatccgc (SEQ ID NO: 24),

(c2) gttgctcgtatttagggcgggtgggtggggtgtatgtttcgtgccgtgtgctctgtgtacaccagtcttcatccgc (SEQ ID NO: 25),

(c3) gttgctcgtatttagggcggcaagggtgggagggtgggtgttgttgcggcgtgtctgtacaccagtcttcatccgc (SEQ ID NO: 26),

(c4) gttgctcgtatttagggccgaggggacgctggtattgggtgggtgggagggcgttggacaccagtcttcatccgc (SEQ ID NO: 27),

(c5) aggggacgctggtattgggtgggtgggagggcgttggacaccagtct (SEQ ID NO: 28),

(c6) aggggacgctggtattgggtgggtgggagggcgttgga (SEQ ID NO: 29)

(c7) gttgctcgtatttagggctgagctgggtgcgttcctggggctgcattcctggggcccgacaccagtcttcatccgc (SEQ ID NO: 30), and

(c8) gcggatgaagactggtgtaggggtgagggcgaggggtgggtggaggcaggtcgagggcgccctaaatacgagcaac (SEQ ID NO: 31)

12.- The conjugate according to any one of claims 1-11, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are covalently linked by a linker molecule.

13.- The conjugate according to claim 11, wherein the linker is a molecule selected from the group consisting of: a polynucleotide; a peptide nucleic acid (PNA), a C_1-C_{10}-alkyl; a C_1-C_{10}-alkyl linked to a polyethylene glycol (PEG) of formula H−(O−CH_2−CH_2)n−OH, wherein *n* is an integer from 5 to 100; a polyethylene glycol (PEG) of formula H−(O−CH_2−CH_2)n−OH, wherein *n* is an integer from 5 to 100; 1,2-ethylene glycol; a C_1-C_{10}-alkanediols; a C_1-C_{10}-cycloalkanediol; an orto- meta-, para-xylenediol; an amino-C_1-C_{10}-alkanol; an amino-C_3-C_{10}-cycloalkanol; and an orto- meta-, para-aminoxylanol.

14.- A single-stranded DNA (ssDNA) aptamer that specifically binds to a protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) selected from:

(a) One or more ssDNA aptamers that specifically bind to the subunit 1 (S1) of the spike protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(a1) atgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO: 1),

(a2) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccctaaatacgagcaac (SEQ ID NO: 2),

(a3) gcggatgaagactggtgtgttggctctgggggtgtggaggggtgtgtggtgggttggccct (SEQ ID NO: 3),

(a4) agactggtgtgttggctctgggggtgtggaggggt (SEQ ID NO: 4),

(a5) tggctctgggggtgtggaggggtgtgtggtgggtt (SEQ ID NO; 5)

(a6) tggggtcggtgtgtcgggtgggggttgggt (SEQ ID NO: 6),

(a7) gcggatgaagactggtgtgggtggggtcggtgtgtcgggtgggggttgggttgtgtgtgccctaaatacgagcaac (SEQ ID NO: 7),

(a8) gcggatgaagactggtgtgggtgtggttagtgtttcccgttctgttgttttcttggttgccctaaatacgagcaac (SEQ ID NO: 8),

(a9) gttgctcgtatttagggcggaggggcgggggaggaggtggcgggaatgtccattgtctacaccagtcttc atccgc (SEQ ID NO: 9)

(a10) ttggggcgggagttttgtttgggtgggggtt (SEQ ID NO: 10);

(b) One or more ssDNA aptamers that specifically bind to N protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(b1) acgcaagggaccacagggtttttcaatataaagatcggattctttattat (SEQ ID NO: 11),

(b2) cacgacgcaagggaccacagggtttttcaatataaagatcggattctttattatctatagactacagcacgacaccgcagagg ca (SEQ ID NO: 12),

(b3) gtttttcaatataaagatcggattctttattatctatagacta (SEQ ID NO: 13),

(b4) gcggatgaagactggtgtgtggcgctgtttctgttgggtggtgagtgggtctgcccgccctaaatacgagcaac (SEQ ID NO: 14),

(b5) gcggatgaagactggtgtcgtgcagcgttaagcggtgaggtgtactgagggttacggggccctaaatacgagcaac (SEQ ID NO: 15),

(b6) gcggatgaagactggtgtagagggccgtggaggggcttggcgtgttgaatgagtgagggccctaaatacgagcaac (SEQ ID NO: 16),

(b7) gcggatgaagactggtgtgggcaagtgtgtttagtgatggacgcccatgtgtagtcgtgccctaaatacgagcaac (SEQ ID NO: 17),

(b8) gcggatgaagactggtgtaatatttgtgtgtgtttgttgttgtttttggtttgtttgtgccctaaatacgagcaac (SEQ ID NO: 18),

(b9) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 19),

(b10) gttgctcgtatttagggctgcgtgggcgggggt (SEQ ID NO: 20),

(b11) gttgctcgtatttagggctgcgtgggcgggggttttttgtgtgtggcggc (SEQ ID NO: 21)

(b12) tgtgtgtggcggcgagtttgtacaccagtcttcatccgc (SEQ ID NO: 22),

(b13) gcggatgaagactggtgtcgggagggagtccgtgaggaggggggctgcggaatggtatgccctaaat (SEQ ID NO: 23),

(c) One or more ssDNA aptamers specifically binding membrane (M) protein of SARS-CoV-2, said aptamers comprising a sequence with a percentage of identity from 85% to 100% with sequences selected from the group consisting of:

(c1) gttgctcgtatttagggcgggtgggcgggggatcgttgtgctgttgcttgttgtgtgtacaccagtcttcatccgc (SEQ ID NO: 24),

(c2) gttgctcgtatttagggcgggtgggtggggtgtatgtttcgtgccgtgtgctctgtgtacaccagtcttcatccgc (SEQ ID NO: 25),

(c3) gttgctcgtatttagggcggcaagggtgggagggtgggtgttgttgcggcgtgtctgtacaccagtcttcatccgc (SEQ ID NO: 26),

(c4) gttgctcgtatttagggccgaggggacgctggtattgggtgggtgggagggcgttggacaccagtcttcatccgc (SEQ ID NO: 27),

(c5) aggggacgctggtattgggtgggtgggagggcgttggacaccagtct (SEQ ID NO: 28),

(c6) aggggacgctggtattgggtgggtgggagggcgttgga (SEQ ID NO: 29)

(c7) gttgctcgtatttagggctgagctgggtgcgttcctggggctgcattcctggggcccgacaccagtcttcatccgc (SEQ ID NO: 30), and

(c8)
gcggatgaagactggtgtaggggtgagggcgaggggtgggtggaggcaggtcgagggcgccctaaatacgagcaac (SEQ ID NO: 31)

15.- An in vitro method for the diagnosis of SARS-CoV2 infections, comprising determining in an isolated sample of a subject the presence and/or levels of SARS-CoV2 by contacting said sample with the conjugate as defined in any of claims 1-13, and/or with one or more aptamers as defined in claim 14; and wherein if SARS-CoV2 is detected, the subject is diagnosed of SARS-CoV2 infection.

16.- A pharmaceutical or veterinary composition comprising a therapeutically effective amount of a conjugate as defined in any of claims 1-13 or a pharmaceutically acceptable salt thereof, or an aptamer as defined in claim 14 or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically acceptable excipients or carriers.

17.- A conjugate as defined in any of claims 1-13 or a pharmaceutically acceptable salt thereof, or an aptamer as defined in claim 14 or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition as defined in claim 16 for use in therapy.

18.- A conjugate as defined in any of claims 1-13 or a pharmaceutically acceptable salt thereof, or an aptamer as defined in claim 14 or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition as defined in claim 16, for use in the prevention and/or treatment of an infection caused by a virus from the Coronaviridae family, in particular by a virus selected from the group comprising MERS-CoV, SARS-CoV and SARS- CoV-2.

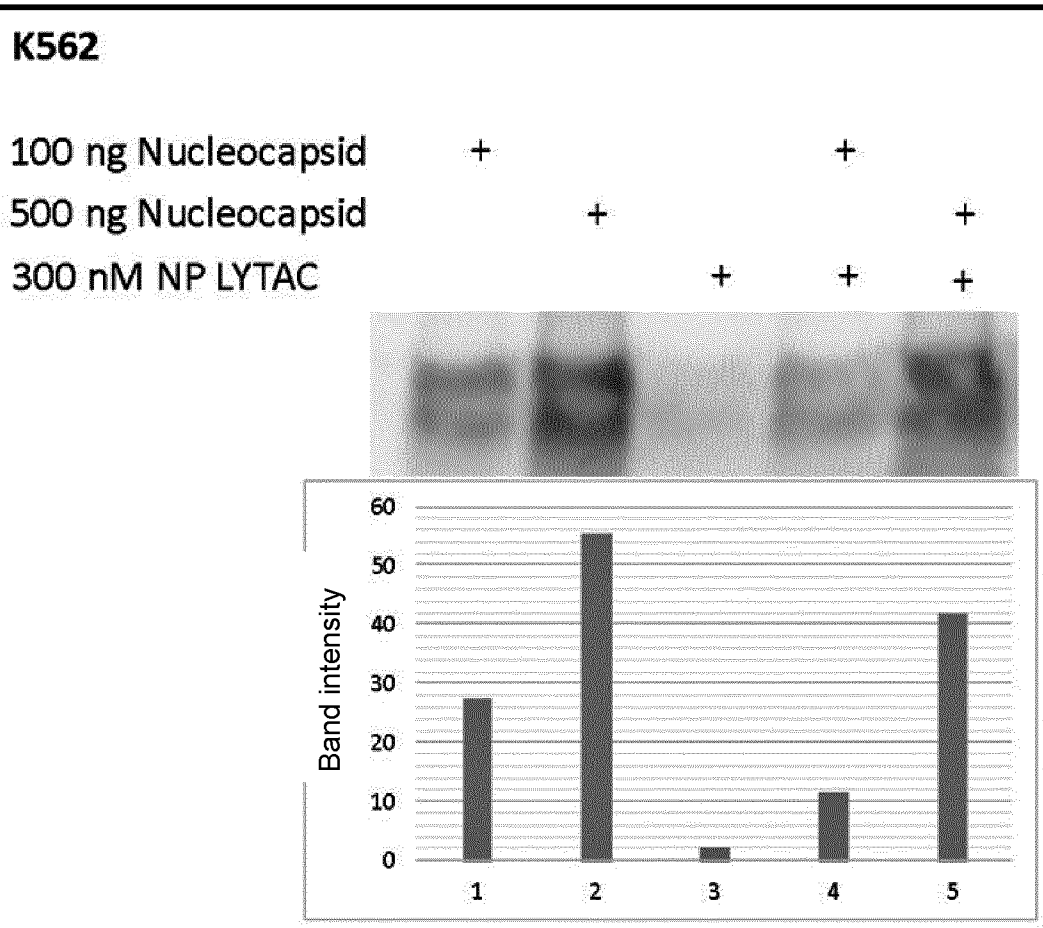
K562
100 ng Nucleocapsid + +
500 ng Nucleocapsid + +
300 nM NP LYTAC + + +
Band intensity
60
50
40
30
20
10
0
1
2
3
4
5

FIG. 1

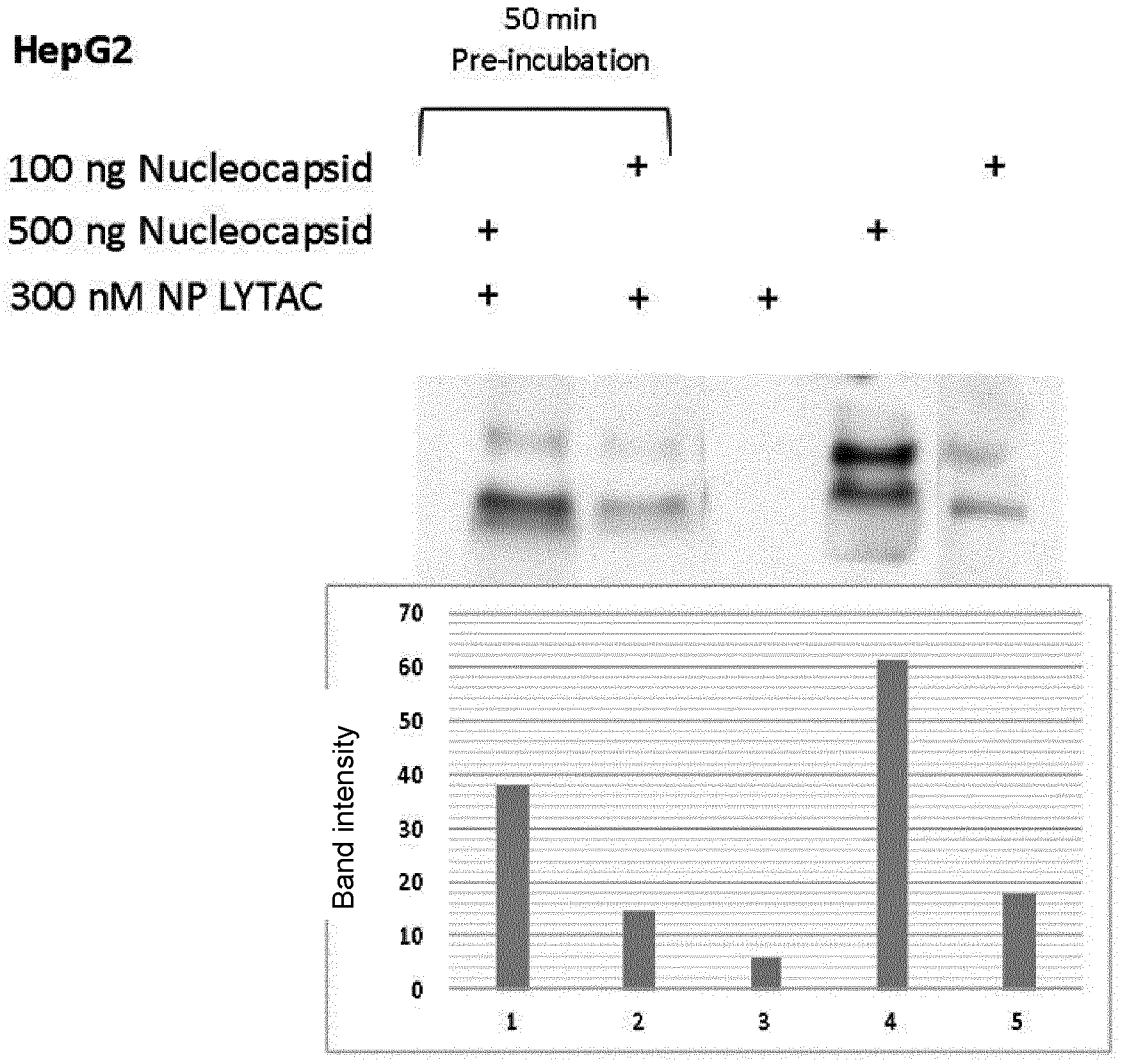
HepG2
50 min
Pre-incubation
100 ng Nucleocapsid
500 ng Nucleocapsid
300 nM NP LYTAC
Band intensity
70
60
50
40
30
20
10
0
1
2
3
4
5

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/067905

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/115
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2022/081827 A1 (DANA FARBER CANCER INST INC [US]) 21 April 2022 (2022-04-21)	1-4, 6-13, 15-18
A	the whole document	5
Y	DING YU ET AL: "Emerging New Concepts of Degrader Technologies", TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER, HAYWARTH, GB, vol. 41, no. 7, 23 April 2020 (2020-04-23) , pages 464-474, XP086181137, ISSN: 0165-6147, DOI: 10.1016/J.TIPS.2020.04.005	1-4, 6-13, 15-18
A	pages 467,468; table 1	5

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 September 2023	08/11/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KRÜGER ARNE ET AL: "Aptamer Applications in Emerging Viral Diseases", PHARMACEUTICALS, vol. 14, no. 7, 28 June 2021 (2021-06-28), page 622, XP055980060, DOI: 10.3390/ph14070622 cited in the application table 1	1-13, 15-18
A	WO 2022/095853 A1 (INTELLINOSIS BIOTECHNOLOGY SHANGHAI CO LTD [CN]) 12 May 2022 (2022-05-12) the whole document	1-13
A	WO 2020/132100 A1 (UNIV LELAND STANFORD JUNIOR [US]) 25 June 2020 (2020-06-25) the whole document	1-13
A	HANIFF HAFEEZ S. ET AL: "Targeting the SARS-CoV-2 RNA Genome with Small Molecule Binders and Ribonuclease Targeting Chimera (RIBOTAC) Degraders", ACS CENTRAL SCIENCE, vol. 6, no. 10, 30 September 2020 (2020-09-30), pages 1713-1721, XP055886604, ISSN: 2374-7943, DOI: 10.1021/acscentsci.0c00984 the whole document	1-13
A	HOMOLAK J ET AL: "Widely available lysosome targeting agents should be considered as potential therapy for COVID-19", INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, ELSEVIER, AMSTERDAM, NL, vol. 56, no. 2, 6 June 2020 (2020-06-06), XP086234692, ISSN: 0924-8579, DOI: 10.1016/J.IJANTIMICAG.2020.106044 the whole document	1-13
A	XU SHUJING ET AL: "Newly Emerging Strategies in Antiviral Drug Discovery: Dedicated to Prof. Dr. Erik De Clercq on Occasion of His 80th Anniversary", MOLECULES, vol. 27, no. 3, 27 January 2022 (2022-01-27), page 850, XP093001884, DOI: 10.3390/molecules27030850 the whole document	1-13

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/067905

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BÉKÉS MIKLÓS ET AL: "PROTAC targeted protein degraders: the past is prologue", NATURE REVIEWS DRUG DISCOVERY, NATURE PUBLISHING GROUP, GB, vol. 21, no. 3, 18 January 2022 (2022-01-18), pages 181-200, XP037710236, ISSN: 1474-1776, DOI: 10.1038/S41573-021-00371-6 the whole document	1-13
A	DI GIORGIO AUDREY ET AL: "New Chemical Modalities Enabling Specific RNA Targeting and Degradation: Application to SARS-CoV-2 RNA", ACS CENTRAL SCIENCE, vol. 6, no. 10, 2 October 2020 (2020-10-02), pages 1647-1650, XP093001893, ISSN: 2374-7943, DOI: 10.1021/acscentsci.0c01187 the whole document	1-13

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2023/067905

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2023/067905

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

1-13(completely); 15-18(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13(completely); 15-18(partially)

A conjugate comprising:- one or more single stranded DNA (ssDNA) aptamers that specifically bind to one or more target proteins of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and- a compound that induces degradation of the target protein and which is a ligand molecule that specifically binds to, or associates with lysosome, or a molecule that specifically binds a lysosomal targeting molecule, wherein the one or more single stranded DNA aptamers and the compound that induces degradation of the target protein are optionally covalently linked by a linker molecule.

2-6. claims: 14-18(partially)

groups of ssDNA aptamer targeting S protein of SARS-CoV-2 with a common motif grouped as SEQ ID NOs:1-5, 6-7, 8, 9, 10

7-11. claims: 14-18(partially)

groups of ssDNA aptamer targeting N protein of SARS-CoV-2 with a common motif grouped as SEQ ID NOs:11-13, 14-18, 19-21, 22, 23

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12. claims: 14-18(partially)

ssDNA aptamers targeting M protein of SARS-CoV-2 as defined by SEQ ID NOs:24-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/067905

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022081827 A1	21-04-2022	AU 2021360898 A1	23-03-2023
		CA 3193261 A1	21-04-2022
		CN 116801908 A	22-09-2023
		EP 4228699 A1	23-08-2023
		US 2023330238 A1	19-10-2023
		WO 2022081827 A1	21-04-2022
WO 2022095853 A1	12-05-2022	CN 114438088 A	06-05-2022
		WO 2022095853 A1	12-05-2022
WO 2020132100 A1	25-06-2020	AU 2019404061 A1	22-07-2021
		BR 112021012113 A2	08-09-2021
		CA 3123086 A1	25-06-2020
		CN 113301925 A	24-08-2021
		EP 3897747 A1	27-10-2021
		IL 283958 A	29-07-2021
		JP 2022513299 A	07-02-2022
		KR 20210104828 A	25-08-2021
		SG 11202106209R A	29-07-2021
		US 2022023434 A1	27-01-2022
		US 2022025057 A1	27-01-2022
		WO 2020132100 A1	25-06-2020