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An Encouraging Approach to Combat SARS-CoV-2 Infections: Antisense Therapy

Anaïs M Quemener[1]*, Marie-Dominique Galibert[2]

[1]Univ Rennes, CNRS, IGDR (Institute of Genetics and Development of Rennes) - UMR 6290, Rennes, France
[2]Department of Molecular Genetics and Genomics, University Hospital of Rennes (CHU Rennes), Rennes, France

Corresponding author:
Anaïs M Quemener, Univ Rennes, CNRS, IGDR (Institute of Genetics and Development of Rennes) - UMR 6290, Rennes, France. E-mail: quemener_ana@gmail.com

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Abstract

As of July 25, 2020, over 643,412 individuals across 215 countries have fallen victim to the novel coronavirus, SARS-CoV-2. Consequently, significant efforts are underway to develop effective strategies to treat and prevent SARS-CoV-2 infections. These efforts primarily involve drug repurposing, the use of anti-SARS-CoV-2 antibodies from recovered individuals, and the development of vaccines. However, despite these endeavors, there remains a lack of specific treatment options for SARS-CoV-2-infected patients. As a result, ongoing research continues to explore new approaches and ideas for combating SARS-CoV-2 infections.

One promising approach under investigation is antisense therapy, which aims to target the genomic RNA of SARS-CoV-2 specifically and inhibit its activity by disrupting viral RNA processing. In this study, researchers have designed Antisense Oligonucleotide (ASO) candidates that target SARS-CoV-2 genomic RNA. Through this process, ASOs with high scores and significant potential to inhibit SARS-CoV-2 replication and transcription by inducing cleavage of the viral genome were identified among the candidate ASOs. Moving forward, these promising ASOs can be synthesized, undergo necessary modifications, and be tested on SARS-CoV-2-infected Vero cells to assess their efficacy for the treatment of individuals infected with SARS-CoV-2.

Keywords: Antisense oligonucleotides (ASOs), Antisense RNA therapy, Drug development, SARS-CoV-2

Abbreviations

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; ACE2: Angiotensin-Converting Enzyme 2; ASOs: Antisense Oligonucleotides; 5Me-Dc: 5-Methyl Deoxycytosine; 7-deaza-dG: deaza G; 2'-O-MOERNA: 2'-O-Methoxy-Ethyl 5me Uridine; PS: Phosphorothioate Linkage.

Introduction

SARS-CoV-2, a single-stranded RNA virus, infects mammals and birds by utilizing spike (S) proteins on its surface to bind to angiotensin-converting enzyme 2 (ACE2) receptors found predominantly on the surface of pulmonary alveolar epithelial cells. The receptor-binding domain (RBD) of the Spike (S) protein facilitates host attachment. Upon attachment, the virus enters host cells via endocytosis pathways or direct fusion, releasing its RNA genome into the cytoplasm. The positive-sense single-stranded viral genome is then translated directly by host ribosomes, followed by viral particle formation and assembly. COVID-19, caused by SARS-CoV-2 infection, has resulted in significant global morbidity and mortality [1-9].

As of July 25, 2020, the virus has affected millions worldwide, highlighting an urgent need for effective treatment strategies. Despite efforts, there is currently no specific treatment available for SARS-CoV-2 infection. Various repurposed antiviral drugs and antibody therapies have been explored, but their efficacy remains

uncertain. In contrast, antisense therapy presents a promising alternative for specifically targeting SARS-CoV-2.

Antisense Oligonucleotides (ASOs) can target specific RNA sequences, inhibiting translation through hybridization with target RNA strands. This mechanism suppresses translation either by preventing ribosomal assembly at the 5' cap or by cleavage by RNase H. ASOs have shown promise in treating viral infections, with examples such as miravirsen used to target human miRNA miR-122 in hepatitis C treatment.

In this study, the aim is to design ASOs targeting specific regions of the SARS-CoV-2 genomic RNA to inhibit viral replication and transcription. Drawing from references like fomivirsen, mipomersen, and miravirsen, regions of the SARS-CoV-2 genome devoid of secondary structures, such as the 5'UTR, 3'UTR, and start codon, were analyzed for ASO targeting. These ASO candidates were evaluated based on in vitro and in vivo parameters, with modifications determined for functionality. This approach holds promise for the development of effective treatments against SARS-CoV-2, leveraging the specificity, low toxicity, and cost-effectiveness of antisense therapy.

Materials and Methods

ASO Design

The design of antisense oligonucleotide (ASO) candidates targeting the SARS-CoV-2 genomic RNA (GenBank: MT385474) was initiated by focusing on key regions, including the 5UTR, 3UTR, and start codon (Figures 1 and 2). ASO candidates were selected to target regions devoid of secondary structures, as predicted by m-fold analysis for each target site. Parameters outlined by Aartsma-Rus et al. [10-13] were employed for ASO scoring, which involved assessing Tm values, ASO length, molecular weight, number and percentage of GC nucleotides, secondary structure formation, and dimer formation using the Oligonucleotide Properties Calculator tool [14]. Only ASO candidates with high scores based on these parameters were retained for further analysis.

ASO Analysis

To evaluate off-target risks, high-scored ASOs were subjected to BLAST analysis against the GenBank database. ASO candidates showing significant similarity (E-value<10) to the human genome were excluded. Binding energy calculations were performed to assess the stability of ASO-target complexes. Free energy values for ASO-target complexes and SARS-CoV-2 genomic RNA were determined using the RNA structure server (version 4.5). The binding energy of ASO-target complexes was determined using the following equation.

ASO candidates with binding energies of ASO-target complexes lower than -20 kcal/mol were considered inefficient and eliminated, as per the criteria established by Aartsma-Rus et al.

ASO Modifications

Promising ASOs underwent modifications to enhance their cellular efficiency, guided by insights from relevant literature (Figure 3). These steps ensured the selection of ASOs with optimal targeting potential and improved efficacy for further investigation in SARS-CoV-2-infected cells.

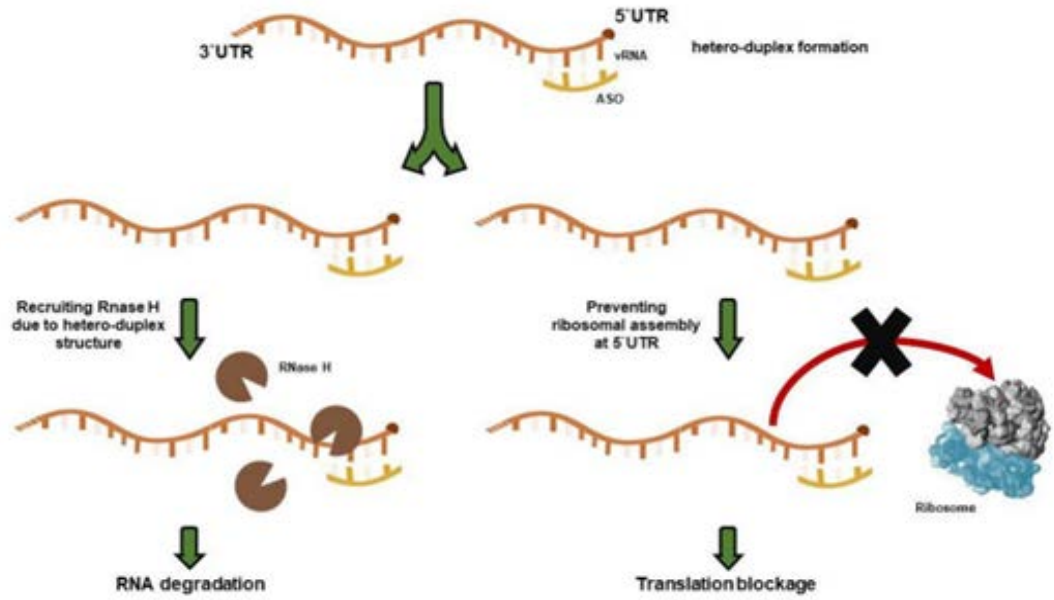

Figure 1: The principle of antisense RNA therapy. ASOs targeting specific sequences on viral genomic RNA enter the cell through an unclear mechanism. They recognize and hybridize specifically to targets based on Watson-Crick base pairing which results in the hetero-duplex formation (ASO/target RNA). At this point, hetero-duplex structures lead to suppression of translation through either by preventing ribosomal assembly at the 5' cap directly or cleavage by RNase H.

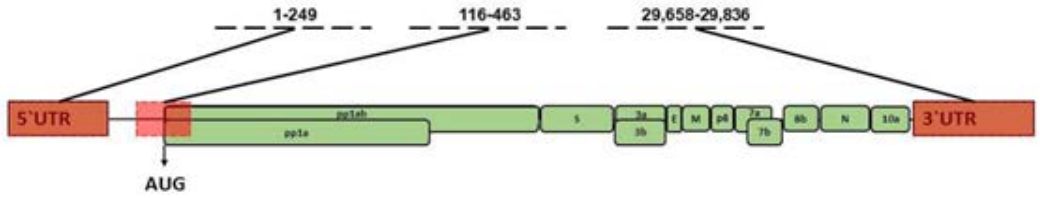

Figure 2: Locations targeted by ASOs on SARS-CoV-2 genomic RNA; 5`UTR (1-249 bases), 3`UTR (116-463 bases), and start codon (29,658- 29,836 bases).

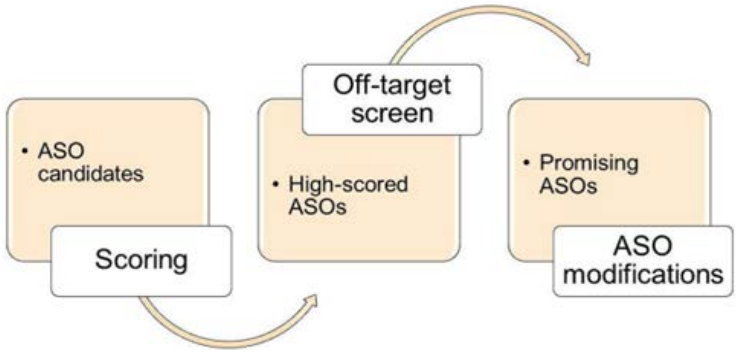

Figure 3: Design algorithm. ASO candidates primarily based on targeting 5`UTR, 3`UTR, and start codon were scored. High-scored ASOs were screened for possible off-targets. Promising ASOs after off-target screening were modified for the ASOs` purposes.

Results and Discussion

Antisense Oligonucleotide (ASO) Design for SARS-CoV-2 Genomic RNA

To develop ASOs targeting the SARS-CoV-2 genomic RNA (GenBank: MT385474), the reference sequence was obtained and analyzed. ASOs, chemically modified oligonucleotides with base-complementary capabilities to specific RNA targets, were designed to target regions near the 5UTR, start codon, and 3UTR. These ASOs aimed to regulate translation by inhibiting the genetic material's translation into functional proteins. Through m-fold analysis, theoretical open regions in the secondary structure of the SARS-CoV-2 genomic RNA were identified for ASO complementarity. Twenty-four ASO candidates were designed based on these predictions.

Selection of Promising ASO Candidates

Among the ASO candidates, seven high-potential ASOs were identified for their ability to inhibit SARS-CoV-2 replication and transcription by inducing cleavage of the viral genomic RNA. These ASOs exhibited optimal characteristics, including lengths ranging from 20 to 24 nucleotides (nt), Tm values higher than 50°C, and GC content exceeding 30%. Additionally, secondary structure formation and dimerization potential were considered detrimental to ASO efficacy and were thus screened out (Figure 3) [15-23].

Off-Target Analysis and Modifications

BLAST analysis was performed to assess off-target risks on the human transcriptome. ASOs with significant similarity to human genes were excluded to mitigate potential side effects. Subsequently, modifications were applied to enhance ASO stability and resistance to cellular nucleases. Various modifications, such as 5-methyl deoxycytosine (5Me-dC), 7-deaza-dG, 2'-O-methoxy-ethyl 5me Uridine (2'-O-MOE-RNA), and Phosphorothioate linkage (PS), were employed to improve ASO efficiency and resistance [12,24].

Efficiency Evaluation

The binding energy of ASO-target complexes was assessed to determine efficacy. ASOs with binding energies lower than -20 kcal/mol were considered inefficient and eliminated. Our results indicated that all ASOs demonstrated binding free energies less than -20 kcal/mol, suggesting their potential effectiveness[25-28].

Conclusion

the designed ASOs exhibit promising characteristics for targeting SARS-CoV-2 genomic RNA. Through careful design and modifications, these ASOs hold potential as effective therapeutic agents against SARS-CoV-2 infection, warranting further investigation in vitro and in vivo.

In summary, this study underscores the potential of antisense therapy as a novel strategy in combating the ongoing COVID-19 pandemic by directly targeting SARS-CoV-2 genomic RNA. ASOs designed to target specific regions such as the 5UTR, start codon, and 3UTR of the viral genome underwent rigorous screening, resulting in the identification of seven high-scored ASOs for further investigation. These promising ASOs hold potential for future studies and could be synthesized and subjected to necessary modifications to assess their efficacy in inhibiting viral replication and transcription in SARS-CoV-2-infected Vero cells. This research paves the way for the development of innovative therapeutic approaches against COVID-19.

Conflict of Interest

No conflicts of interest have been declared.

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