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## Antisense oligonucleotides targeting an oncogenic mitochondrial long non-coding mtRNA to inhibit equine skin tumor cells proliferation

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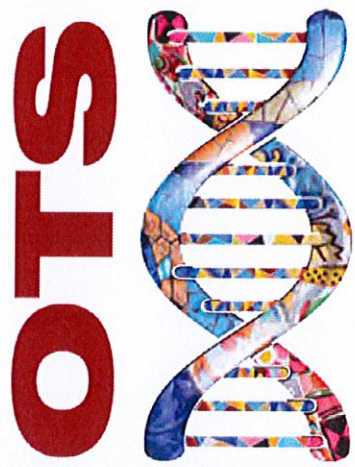
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The 2023

# OLIGO MEETING

BARCELONA | SPAIN

19<sup>th</sup> Annual Meeting of the  
Oligonucleotide Therapeutics Society  
Oct 22 – 25, 2023



**P020**

**Antisense oligonucleotides targeting an oncogenic mitochondrial long non-coding eca-ASncmtRNA to inhibit equine skin tumor cells proliferation**

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**Abstract**

**Introduction:** Cancer treatment is highly constrained by chemotherapy side-effects. ASncmtRNA family are mitochondrial long-non-coding RNAs, previously identified in mice and humans, containing both sense and antisense transcripts transcribed from 16S rRNA mDNA region (1), showing differential expression between tumor and normal cells. Knockdown of the ASncmtRNAs induces proliferative arrest and apoptosis in several tumor cell lines and mouse models without affecting normal cells (2-5). The aim of our project is to translate this technology into an equine skin tumor model by the design and screening of ASO candidates directed to the *Equus caballus* ASncmtRNAs ortholog (eca-ASncmtRNA) in two equine tumor cell culture models. Melanoma is a high prevalent in gray horses (6) and sarcoid tumors is a firosarcoma caused by BPV viral infection (7).

**Material and methods:** Equine eca-ASncmtRNAs were characterized using successive RT-PCR and amplicons Sanger sequencing. We designed in silico targeting the eca-ASncmtRNA. Melanoma cell lines (6) and sarcoid biopsy cultures were transfected with ASO candidates and controls and selected by differential anti-proliferative and cell death induction in tumor cells versus normal proliferative myoblasts. ATP luminescent cell viability assays and cell counts were used to perform screenings followed by flow cytometric cell death dye-based assays. Full phosphorotioate (PS) and locked nucleic acids (LNA) oligos were evaluated.

**Results:** Cell proliferation screenings showed three interesting ASO candidates inducing a significant reduction in cell viability and death in the melanoma cell line at 500nM at 72h post-transfection (65% reduction *versus* ASO-C, IC50 253nM). Flow cytometric cell death analyses showed ASO-08 induced cell death and increased mid-apoptotic cells significantly compared to ASO-C control (scrambled ASO). ASO-08 cell death induction was similar to doxorubicin treatment. ASO-08 repeated transfection increases the anti-proliferative effect (65 to 89%) at 500nM but not at 250nM compared to ASO-C. ASO-08 with full PS modifications induces higher anti-proliferative and cell death effects compared to the same sequence with LNA modifications. In sarcoid cell cultures, ASO-08 induced a higher anti-proliferative effect and cell death at 250nM for 72h (66% reduction *versus* ASO-C). Doxorubicin treatment did not significantly induce any anti-proliferative effect in these cells.

**Conclusion:** ASOs directed to the equine eca-ASncmtRNA induce selective antiproliferative effects in the two tumor cell models. Oligonucleotide antisense therapy will be evaluated in horse skin cancer cases by ASO local injections.

**References:** 1-Villegas et al (2007) PMID: PMC2175360 /2-Burzio et al (2009) PMID: PMC2695051 /3-Vidaurre et al (2014) PMID: PMC4175353 /4-Fitzpatrick et al (2019) PMID: PMC6541642 /5-Lobos-Gonzalez et al (2016) PMID: PMC5295434 /6-Rosengren et al (2008) PMID: 18641652 /7-Ogluszka et al (2012) PMID: 33461443

# Antisense oligonucleotides targeting an oncogenic mitochondrial long non-coding mtRNA to inhibit equine skin tumor cells proliferation



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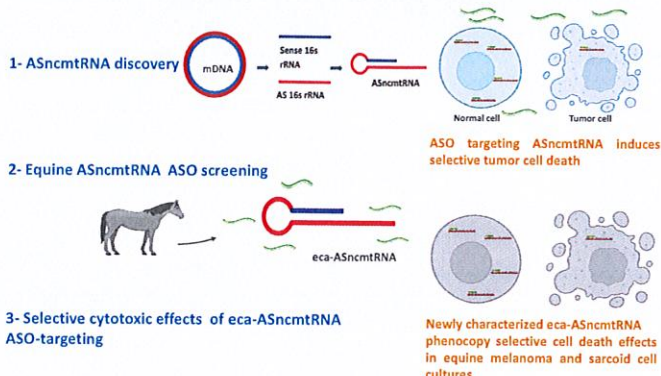
## INTRODUCTION

- Cancer treatment is highly constrained by chemotherapy side effects.
- The mitochondrial long non-coding RNA ASncmRNA is differentially expressed in normal vs. tumor cells in humans and mice (1)
- The ASncmRNA has been tested as a target for human and mouse cancer treatment *in vitro* and *in vivo* by Antisense oligonucleotides (ASO) inducing proliferative arrest and cell death in human and mouse cancer models (2)
- We characterized eca-ASncmRNA and screened for antitumor properties of ASO targeting.
- Our aim is to translate this technology into equine melanoma and sarcoid tumors as an experimental treatment approach.

### References:

(1) Vidaurre S, Fitzpatrick C, Burzio VA, et al. Down-regulation of the antisense mitochondrial non-coding RNAs (ncRNAs) is a unique vulnerability of cancer cells and a potential target for cancer therapy. *J Biol Chem.* 2014;289(33):27182-27198. doi:10.1074/jbc.M114.558841  
 (2) Fitzpatrick C, Bendek MF, Briones M, et al. Mitochondrial ncRNA targeting induces cell cycle arrest and tumor growth inhibition of MDA-MB-231 breast cancer cells through reduction of key cell cycle progression factors. *Cell Death Dis.* 2019;10(6):423. Published 2019 May 29. doi:10.1038/s41419-019-1649-3

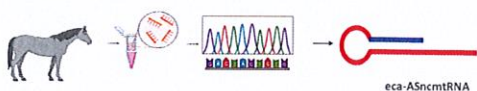
## GRAPHICAL ABSTRACT



## MATERIAL & METHODS

### Eca-ASncmRNA characterization

Equus caballus ASncmRNA is characterized by reverse transcription and specific amplicon sequencing from horse biopsies in normal and tumor tissue



Tumor and muscle biopsies were used as starting material. eca-ASncmRNA containing similar characteristics to the human ASncmRNAs (Loop region, long inverted repeat, 5' single strand).

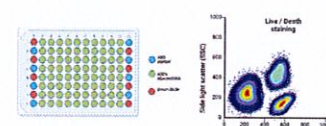
### In silico design ASO targeting eca-ASncmRNA



ASOs are designed to target accessible regions in the eca-ASncmRNA with desirable ASO properties.

### In vitro screenings and validation

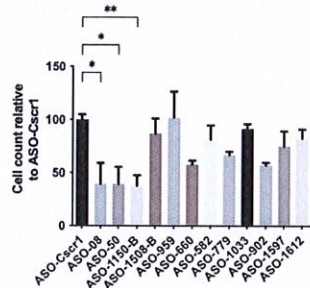
Anti-proliferative screening effects in equine melanoma and sarcoid tumor cells



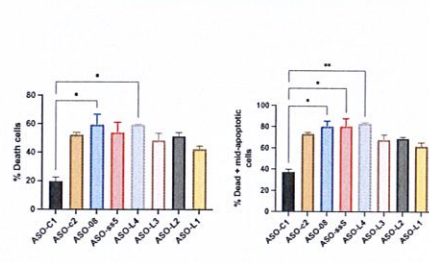
ASOs targeting eca-ASncmRNA were screened for anti-proliferative and cell death effects in tumor cells and challenged against normal cells using lipofectamine 2000 as transfection agent.

## RESULTS

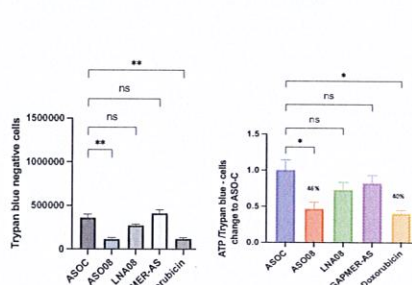
ASOs targeting eca-ASncmRNA induce anti-proliferative effects.



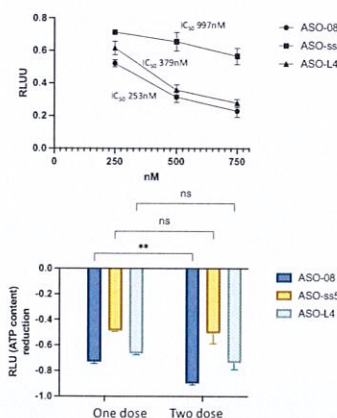
Cell death staining flow cytometric analyses show Eca-ASncmRNA targeting induces cell death at 72h post-transfection (Guava ViaCount assay system)



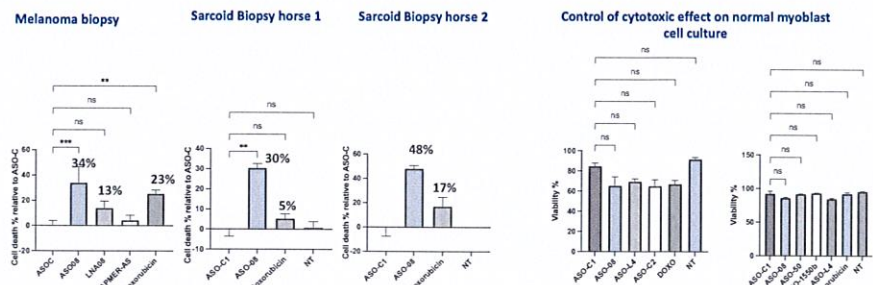
ASO-08 phosphorptioate induced higher anti-proliferative and ATP inhibition effects than LNA modification.



ASO-08 induced a higher anti-proliferative effect and repeated transfection improved the overall effects.



ASO-08 induced cell death in Tumor cells, but does not significantly affects normal myoblasts viability



### Conclusion:

- ASOs directed to the mitochondrial equine long non-coding RNA eca-ASncmRNA targeting inhibits proliferation and induces selective tumor cell death.
- This result phenocopies human and mouse ASncmRNAs targeting in cells, supporting the development of specific cancer treatments with reduced side effects for potential human and veterinary cancer treatments



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**P061**

## Antisense oligonucleotides targeting the SARS-CoV-2 genome to inhibit viral replication

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### Abstract

#### Introduction

SARS-CoV-2 is a positive, single-strand RNA coronavirus responsible for the COVID-19 pandemic that has yielded nearly 700 million cases and 7 million deaths worldwide. Additionally, 45% of patients suffer from long-covid with at least one symptom 3 months after infection (1). Despite the availability of vaccines, once a person is infected, their clinical fate will depend almost uniquely on their age, concomitant chronic diseases, and genetic background. Thus, antiviral treatments against SARS-CoV-2 are still required. Antiviral oligonucleotide targeting has been widely explored and successfully approved for cytomegalovirus treatment in immunocompromised patients (2). Our group has proposed and tested antisense oligonucleotides to inhibit SARS-CoV-2 replication by targeting regions under low selective pressure. Selected ASOs were validated in human and simian cellular models (3).

#### Material and methods

We designed *in silico* antisense oligonucleotides (ASOs) targeting the SARS-CoV-2 viral genome mainly at the 5', nucleocapsid (N) coding region and the 3'UTR genomic regions. We screened for anti-SARS-CoV-2 putative targeting regions by a luciferase reporter system. Validation of selected ASOs with full phosphorothioate chemical modifications was performed with infectious SARS-CoV-2/ZsGreen reverse-engineered virus in Vero-E6 cells and tested with the wild-type SARS-CoV-2 strain in HEK-293/ACE2 cells.

#### Results

Antisense oligonucleotides directed to the N coding sequence and the 3' UTR of the SARS-CoV-2 genome induced a stronger reduction in luminescence at nanomolar concentrations, with an IC<sub>50</sub> of 3.5 nM. These ASOs significantly inhibited viral gene expression in SARS-CoV-2/ZsGreen-infected Vero-E6 cells. Wild-type SARS-CoV-2 challenge in HEK-293/ACE2 cells showed a 96% viral RNA reduction by ASOs directed to the N gene, as evidenced by RT-qPCR, consistent with the reporter assays.

#### Conclusion

The abovementioned results present a simple procedure for the screening of ASOs as antiviral candidates. ASOs targeting the SARS-CoV-2 N gene are currently under evaluation in the Syrian hamster infection model (4).

#### References

- (1) O'Mahoney et al. (2022) PMID: 36474804
- (2) Roehr (1998) PMID: 11365956
- (3) Dhorne-Pollet et al. (2022) PMID: 36386681
- (4) Antisense molecules and their uses for the treatment of coronavirus infection, in particular the treatment of covid-19 related to SARS-CoV-2. European Patent # 23 305055.8

# Antisense oligonucleotide targeting of the SARS-CoV-2 genome to inhibit viral replication

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## INTRODUCTION

shortcut

## CONCLUSION

- SARS-CoV-2 is a positive, single-strand 30k nucleotides long RNA coronavirus responsible for the COVID-19.
- Despite the availability of vaccines, patients still getting infected, so the need for efficient and specific antivirals is still urgent.
- Our group has designed and tested antisense oligonucleotides to inhibit SARS-CoV-2 replication by targeting regions under low selective pressure
- ASOs were validated in human and simian cellular models

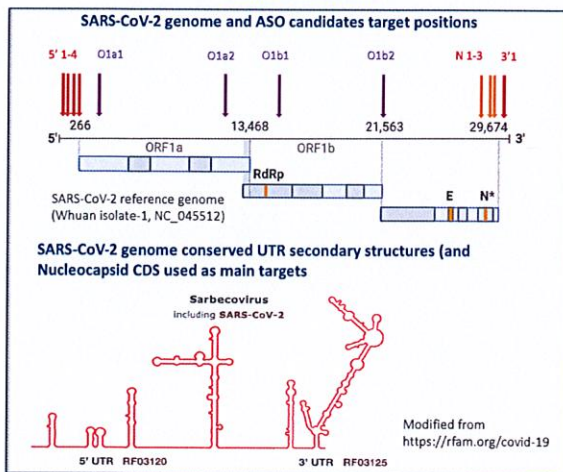
- Antisense oligonucleotides directed to the nucleocapsid coding sequence (N) of the SARS-CoV-2 genome induced a stronger reduction in luminescence at nanomolar concentrations, with an IC<sub>50</sub> of 3.5 and 15nM in the best ASO candidates.
- ASOs directed to the (N) coding sequence decrease the fluorescence of the SARS-CoV-2 infectious reporter and effectively inhibit SARS-CoV-2 viral RNA.

### References

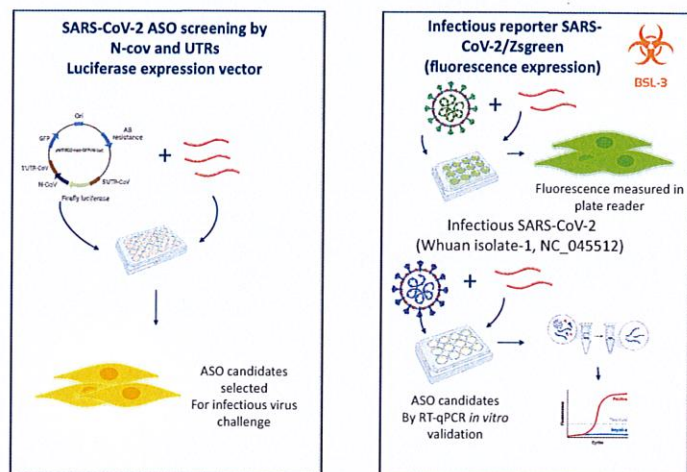
- O'Mahoney et al. (2022) PMID: 36474804
- Roehr (1998) PMID: 11365956
- Dhorne-Pollet et al. (2022) PMID: 36386681
- Antisense molecules and their uses for the treatment of coronavirus infection, in particular, the treatment of COVID-19 related to SARS-CoV-2. European Patent # 23 305055.8

## MATERIAL & METHODS

### In silico predictions

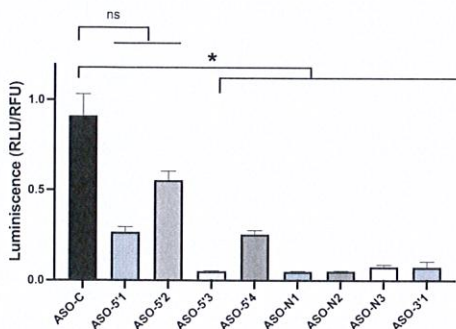


### In vitro screenings and validation

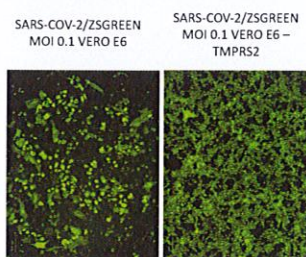


## RESULTS

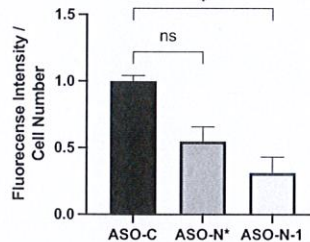
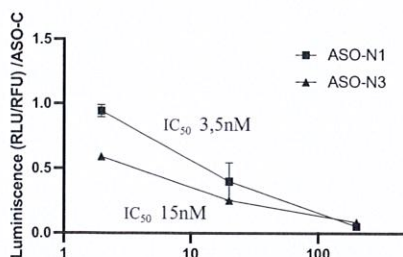
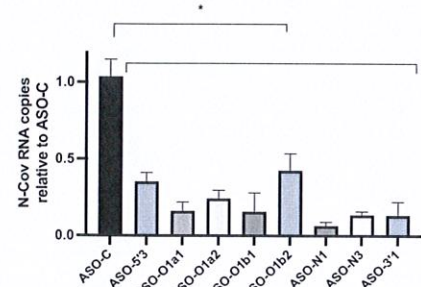
### SARS-CoV-2 luciferase reporter screening show ASO candidates



### ASOs directed to the Nucleocapsid gene (N) inhibit SARS-CoV-2 infectious reporter fluorescence



### ASOs directed to the Nucleocapsid gene (N) effectively inhibit SARS-CoV-2 RNA expression



### Results in a nutshell

- ASOs directed to the nucleocapsid CDS and 3'UTR of the SARS-CoV-2 genome induce higher luciferase inhibition
- The IC<sub>50</sub> of the most efficient ASOs in the luciferase screening is 15 and 3,5 nM
- ASOs directed to (N) effectively decreased fluorescence in the Engineered SARS-CoV-2/ZsGreen reporter virus assay
- ASOs directed to nucleocapsid CDS inhibit SARS-CoV-2 viral RNA