ORF1 Region of SARS-CoV-2 Genomic RNA as a Promising Target for siRNA-based Therapy

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Shubhankar S Ambike[1], Domizia Baldassi[2], Cho-Chin Cheng[1], Martin Feuerherd[1], Gerald Burgstaller[2], Chunkyu Ko[1], Olivia Merkel[2] and Thomas Michler[1]
[1]Technical University of Munich, Munich, Germany, [2]Ludwig Maximilian University of Munich, Munich, Germany

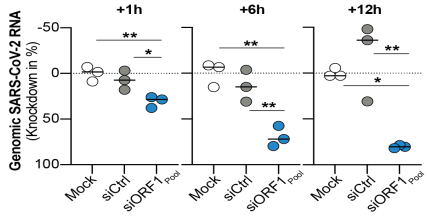
BACKGROUND

Local delivery of highly-active antiviral siRNAs against SARS-CoV-2 could be a promising approach to treat COVID-19. Coronaviruses replicate in semi-discontinuous manner via negative sense RNA intermediates. It results in presence of 3 common regions shared by genomic and sub-genomic RNA transcripts - Leader sequence(L), Nucleocapsid gene (N) and 3'UTR region (See graphic "Rational of Study"). Targeting these regions was thought to be efficacious previously. Until now, however it is unclear which RNA species are accessible for a siRNA-based therapy. Here we prove that an early therapeutic or pyrophytic application of siRNAs contrastingly against ORF1 could be key in achieving cure.

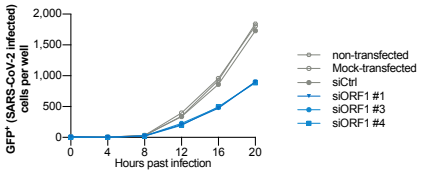
METHODS

siRNAs designed against four genomic regions of SARS-CoV-2 were preselected using luciferase reporters. Antiviral activity of siRNAs was evaluated by transfecting before or after infection of VeroE6 cells with wildtype (*wt*) or GFP expressing SARS-CoV-2[1]. Luciferase competition experiments were done in HEK293T cells by co-transfecting siRNAs with respective psiCHECK™2 reporters followed by *wt*SARS-CoV-2 infection. Highly efficacious siRNAs were further delivered by VIPER polymer in human lung explants, confirming their therapeutic potential. Viral replication *in vitro* and *ex vivo* was assed via reverse-transcriptase PCR (RT-qPCR) or time-lapse fluorescence microscopy.

1 siRNAs degrade SARS-CoV-2 upon entry, at early time points

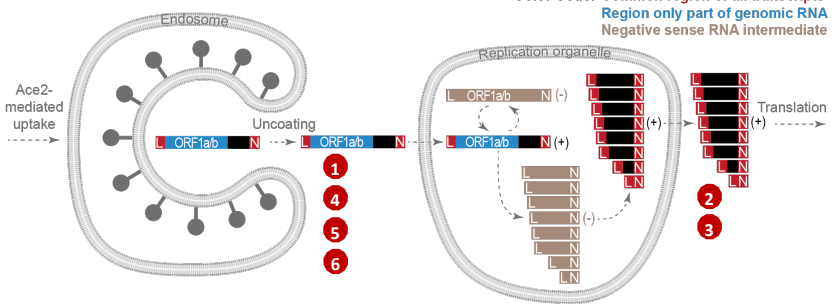


Knockdown of *wt*SARS-CoV-2 genomic RNA was quantified by qPCR after siORF1 treatment in VeroE6 cells



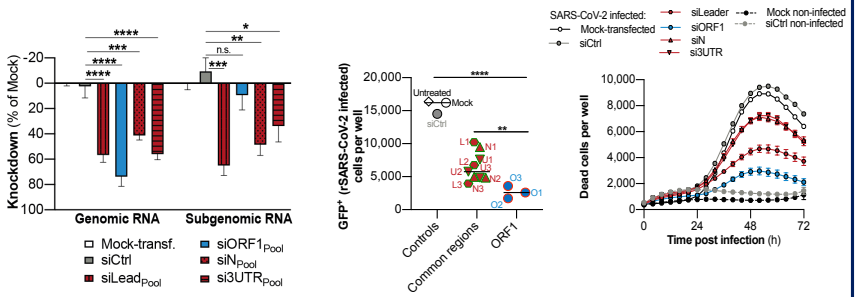
Reduction in viral spread confirmed by siORF1 upon rSARS-CoV-2-GFP infection in VeroE6 cells

Rational of Study



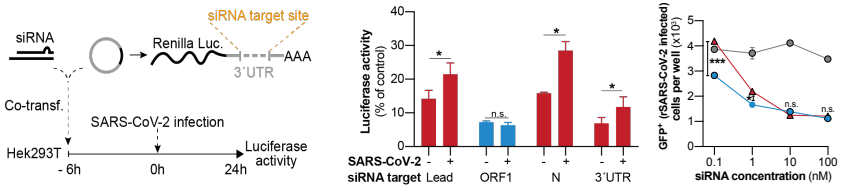
We investigated which RNA species of SARS-CoV-2 are available for siRNA therapy

2 Higher efficacy of siRNAs targeting ORF1 comes from silencing genomic RNA more efficiently than common region targeting siRNAs



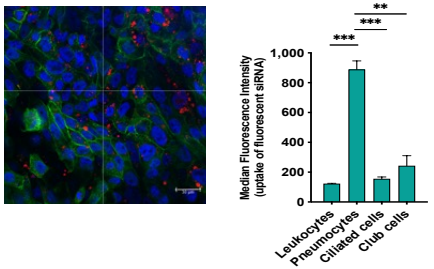
siORF1 do not suppress sub-genomic RNA of *wt*SARS-CoV-2 (Left). siORF1 exhibit higher antiviral activity against rSARS-CoV-2 (Mid) and prevent viral induced cell death than other siRNAs in VeroE6 cells (Right).

3 Highly abundant sub-genomic RNAs (sgRNAs) out-compete siRNAs rendering them less effective



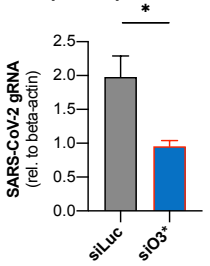
Setup of luciferase competition experiments performed in HEK293T (left). Reduction in luciferase activity explains out-competition of siRNAs by highly abundant sgRNAs (mid). Higher amounts of common region-targeting siRNA are essential to achieve antiviral efficiency against rSARS-CoV-2 in VeroE6 cells

4 VIPER delivers siRNA efficiently *in vitro* and *in vivo*



VIPER delivered fluorescently labelled siRNA was delivered efficiently to Calu-3 cells in vitro (red; left) and to Pneumocytes *in vivo*

5 Chemically modified siRNA delivered with VIPER demonstrate therapeutic potential



Suppression of viral RNA in human lung explants confirm the therapeutic potential of siRNAs

CONCLUSIONS

- siRNAs terminate SARS-CoV-2 replication before initiation of transcription
- Single siRNA targeting common regions of SARS-CoV-2 allow simultaneous suppression of genomic and sgRNA but leads to a loss of efficacy
- Highly abundant siRNAs out-compete siRNAs targeting common regions unlike siRNAs targeting ORF1
- VIPER delivers siRNAs to murine pneumocytes and to human lung slices *ex vivo*
- Chemically modified siRNAs succeeded as therapy in human lung explants

References

1. Thi Nhu Thao, T. *et al.* Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature* 2020

Contact: shubhankar.ambike@tum.de