Self-amplifying RNA vaccine ZIP1642 induces potent adaptive immune response and protects Syrian hamsters from wild-type WA1/2020 and B.1.351 SARS-CoV-2 infection

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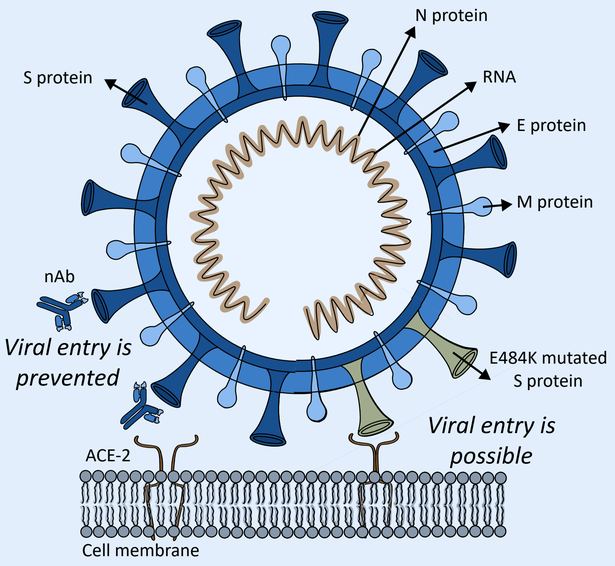
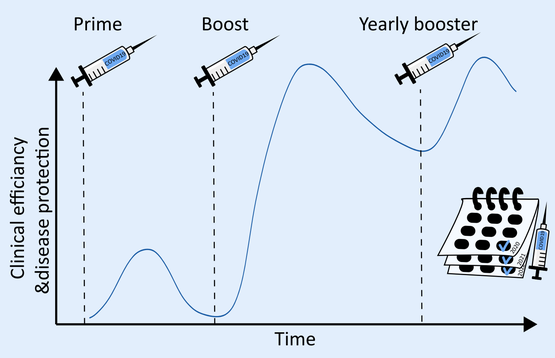
ZIPHIUS VACCINES
GAME CHANGER IN VACCINATION -

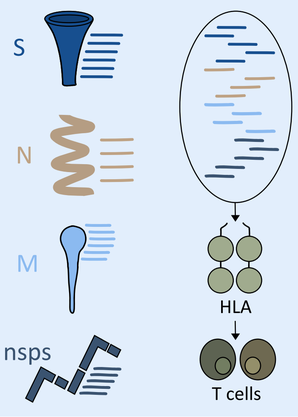
Introduction

Current authorized vaccines protect against COVID-19 disease by inducing humoral immunity towards the spike (S) glycoprotein of SARS-CoV-2. However, evidence on evasion of neutralizing immunity by variants of concern (e.g. B.1.351 lineage) is emerging.





Periodical updates of the current vaccines might be needed to compensate for viral evolution.



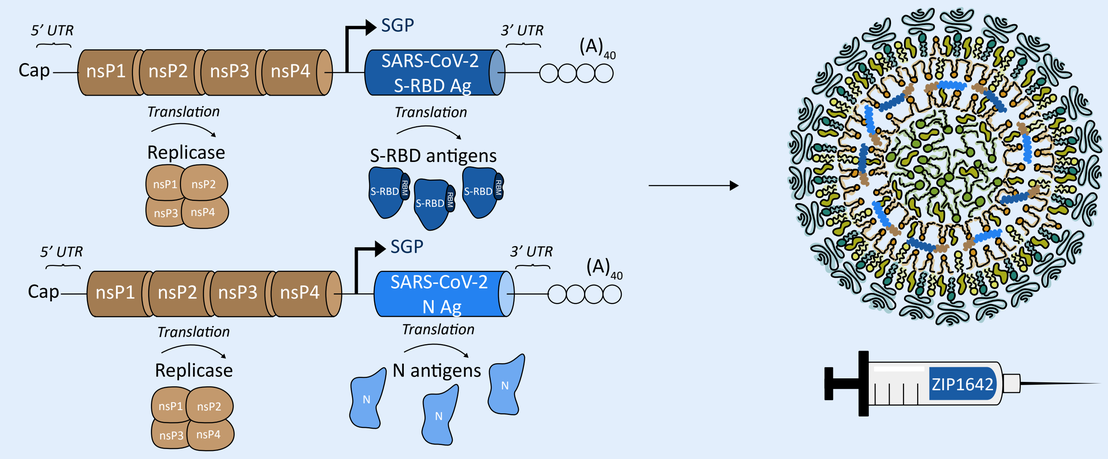
Concomitantly, T-cell-mediated immunity (CMI) plays a pivotal role in COVID-19 disease resolution and waning antibody responses

Distinct patterns of immunodominance are observed for CD4+ T helper (Th) cells, CD8+ cytotoxic T cells, and antibodies.

Hence, vaccine design should not only ensure inclusion of regions targeted by neutralizing antibodies, such as the S-RBD region, but also include regions that are suitable targets of T cells, such as membrane (M), envelope (E) and nucleocapsid (N) structural and non-structural proteins (nsps).

Methods

ZIP1642 is a self-amplifying mRNA (saRNA) vaccine, which contains two RNA molecules encoding either the WA1/2020 S-RBD or N protein that are encapsulated into one lipid nanoparticle (LNP).



ZIP1642 and its controls were administred to mice and hamsters to assess expression duration of the saRNA, potential immune responses,and effect on disease severity upon infection.

Vaccination study in female SWISS mice (intramuscular I.M., *n*=6, 1 µg doses)

Prime — 3 weeks — Boost — 2 weeks — Immune response & expression

- *Unvaccinated control (SHAM)*
- *Luciferase saRNA (negative control)*
- *S-RBD saRNA*
- *N saRNA*
- *ZIP1642*

Challenge studies in a Syrian golden hamster infection model (I.M., *n*=6, 1, 2 or 5 µg doses)

Prime — 3 or 4 weeks — Boost — 2 weeks — Challenge with WA1/2020 or B.1.351 — 4 days — Immune response & disease

*Data is presented as mean ± SD, except histological (presented as median), with ns (non significant), *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.*

Results

Vaccination study in SWISS mice

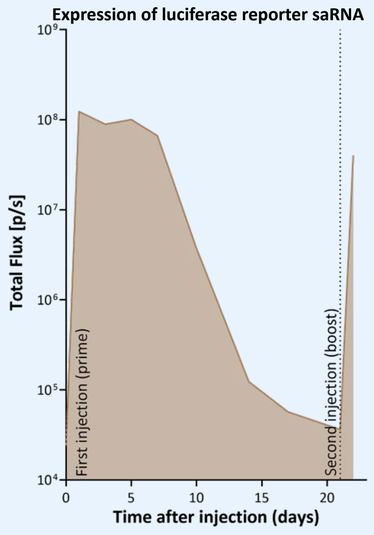


Figure 1 Injection with luciferase saRNA leads to high and sustained levels of protein expression up to 10 days after first injection (*in vivo* imaging).

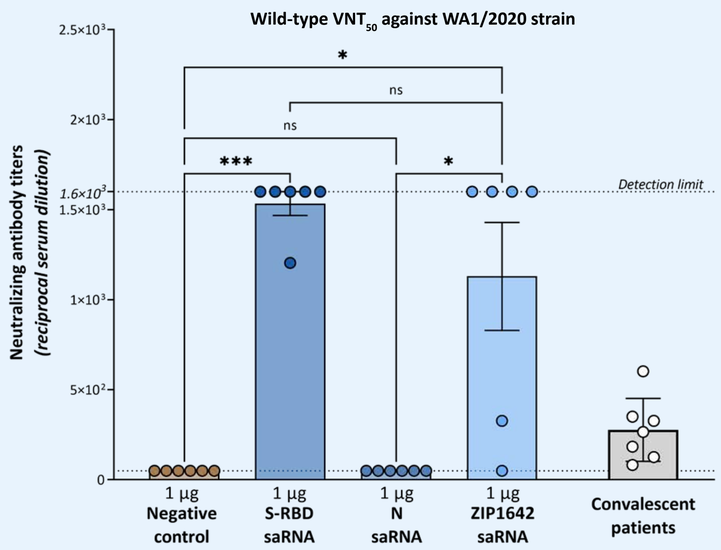


Figure 2 After boost vaccination, S-specific neutralizing antibody (nAb) responses in mouse sera were analyzed using a cell-based viral neutralization test (VNT). Significantly high nAb titers against wild-type WA1/2020 strain (2019-nCoV-Italy-INMI1) were induced after S-RBD and ZIP1642 vaccination, and were found to be much higher compared to convalescent patient serum samples.

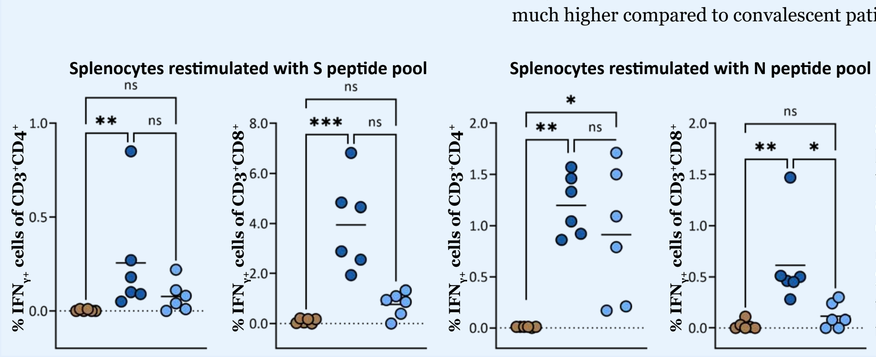


Figure 3 Upon restimulation of splenocytes with S and N peptide pools, strong cell-mediated immunity was observed, consisting of both S- and N-antigen specific IFNγ positive CD4+ Th1 and CD8+ T lymphocyte response in S-RBD (dark blue ●) & ZIP1642 (light blue ●) saRNA vaccinated mice, compared to negative controls (luciferase saRNA, brown,●) .

Challenge studies in the SARS-CoV-2 Syrian Golden hamster model

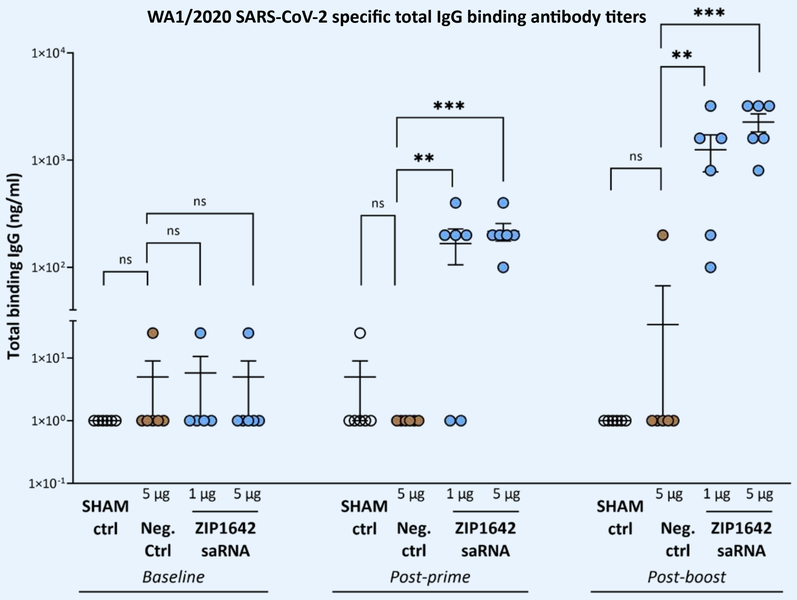


Figure 4 Prime/boost vaccination with both 1 and 5 µg of ZIP1642 induces robust SARS-CoV-2 binding antibody responses, compared to unvaccinated (SHAM) and luciferase saRNA (negative control) groups. Total IgG responses were quantified via ELISA.

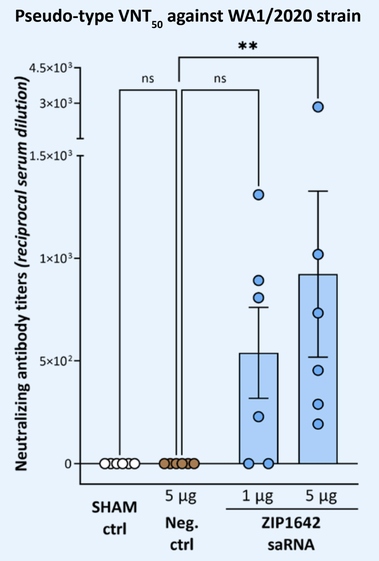


Figure 5 Boost vaccination with 5 µg of ZIP1642 was able to significantly induce S-specific neutralizing antibodies against pseudo-type WA1/2020 (via VNT).

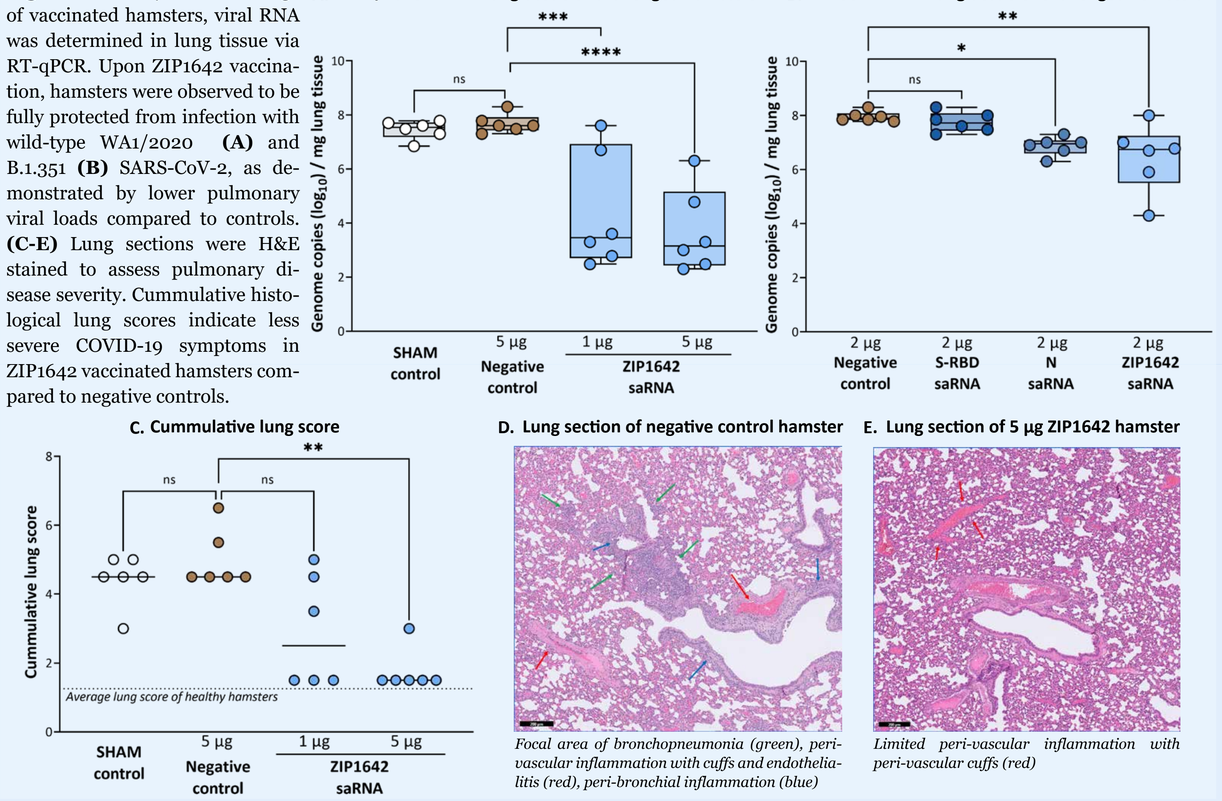


Figure 6 Four days after challenge of vaccinated hamsters, viral RNA was determined in lung tissue via RT-qPCR. Upon ZIP1642 vaccination, hamsters were observed to be fully protected from infection with wild-type WA1/2020 **(A)** and B.1.351 **(B)** SARS-CoV-2, as demonstrated by lower pulmonary viral loads compared to controls. **(C-E)** Lung sections were H&E stained to assess pulmonary disease severity. Cummulative histological lung scores indicate less severe COVID-19 symptoms in ZIP1642 vaccinated hamsters compared to negative controls.

Focal area of bronchopneumonia (green), peri-vascular inflammation with cuffs and endotheliatis (red), peri-bronchial inflammation (blue)

Limited peri-vascular inflammation with peri-vascular cuffs (red)

Conclusion

Our findings have important implications for next-generation vaccine development to go beyond the goal of inducing neutralizing antibodies, and additionally aim for coordinated and lasting multi-antigenic specific T cell responses. In the context of recent regulatory advice in support of booster vaccinations, heterologous vaccination regimes comprising a potent multi-antigenic COVID-19 candidate such as ZIP1642, are promising in providing superior protection against emerging SARS-CoV-2 variants of concern.

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