Evolving immune evasion during a SARS-CoV-2 chronic infection – a pathway to variants of concern

Suzanne Pickering[1(*)], Luke Snell[1,2(*)], Xiaohao Xu[1], Helena Winstone[1], Jeff Seow[1], Carl Graham[1], Rahul Batra[2], Michael Malim[1], Katie Doores[1], Jonathan Edgeworth[1,2], Gaia Nebbia[1,2], Stuart Neil[1], Rui Pedro Galão[1]

[1]Department of Infectious Diseases, King's College London, United Kingdom.
[2]Centre for Clinical Infection and Diagnostics Research, Dept. of Infectious Diseases, Guy's & St Thomas' NHS Foundation Trust, London, UK

Background

- Long-term persistent (LTP) infections with SARS-CoV-2 have been associated with immunocompromised individuals and can endure for over extended periods of time.
- Cases of LTP infections, and the viral persistence is likely due to impaired immune clearance.
- The rate of evolution of SARS-CoV-2 on LTP infections is noticeably higher when compared with transmission chains of acutely infected individuals.
- This is mostly evident in the virus' spike protein (S) often presenting several amino-acid changes mapping to the receptor-binding domain (RBD) and N-terminal domain (NTD).
- Many of these mutations in spike are associated with enhanced transmissibility and/or resistance to immune defences, particularly evasion from neutralizing antibodies
- Many of the same mutations are seen in the spikes of SARS-CoV-2 variants of concern (VOCs), and LTP infections are believed to greatly influence their emergence.
- Several other mutations can be found across the genomes of viruses from LTP patients, but their relevance to viral replication or the interplay with the host responses is unclear.

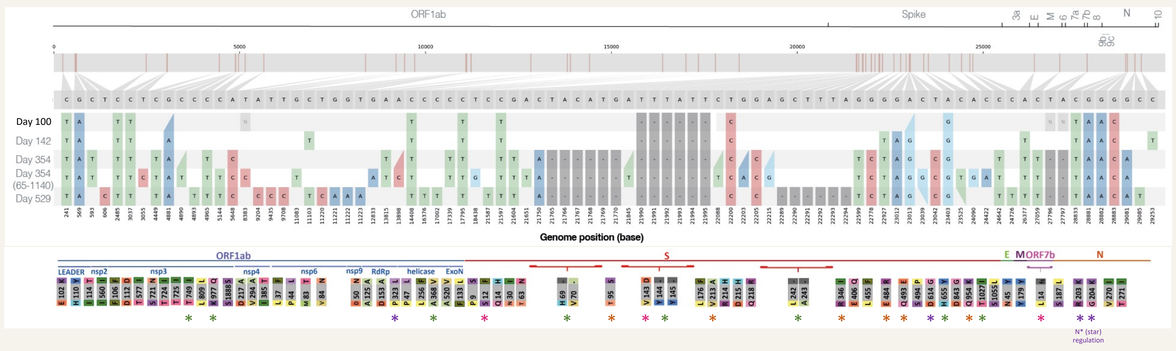
Aims

- Examine the viral genomic evolution during a very lengthy LTP-infection with SARS-CoV-2.
- Examine the capacity of viruses from LTP infected individuals to evade adaptive and innate immune responses.

Patient 2 – 20yo Male, with SARS-CoV-2 infection for >500 days

- Severe immunodeficiency of unknow genetic origin. Prolonged steroid treatment at young age.
- Previous history of CMV colitis and Pneumocystis pneumonia
- Became symptomatic and tested positive in April 2020. Small oxygen requirement which resolved.
- Admitted in June 2020 with ongoing infection (lineage B.1.1). Treated with remdesivir.
- No other therapies licensed and declined for compassionate nmAb use.
- **Outcome:** Multiple co-morbidities and overlapping acute illnesses, died in Sep 2021.

1. Whole genome evolution during LTP infection case 65

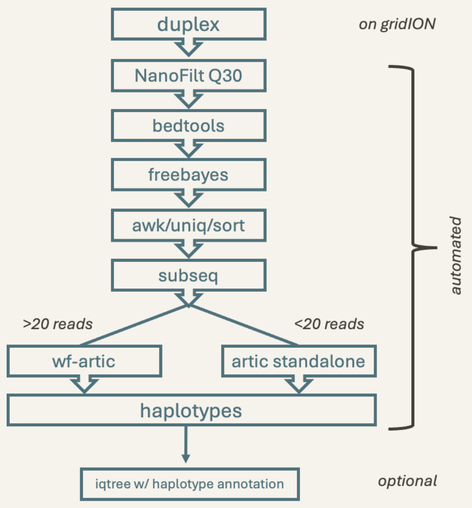


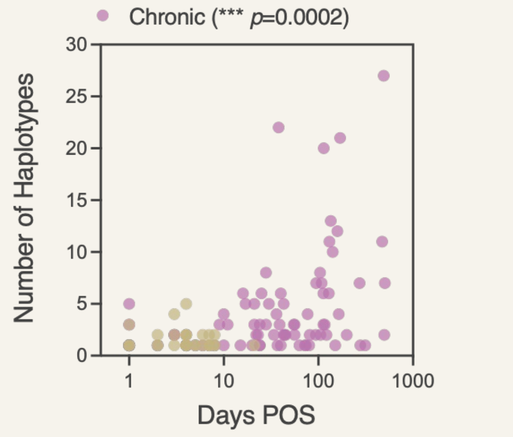
* B.1.1 defining * Common mutations found in VOCs * Other mutations found in VOCs * Recurrent mutations in immunodeficiency

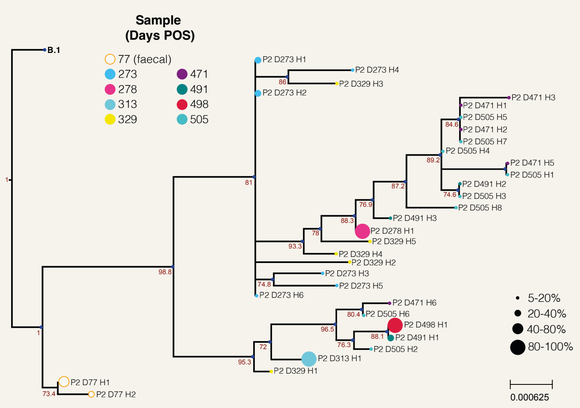
Whole genome sequencing of longitudinal timepoints, with SNPs compared to the SARS-CoV-2 reference genome horizontally. Timepoints are represented chronologically from top to bottom and location of single-nucleotide polymorphisms and amino-acid deletions are indicated.

2. Determining spike haplotypes in LTP infections (see also poster #310 by Snell L)

Using nanopore to resolve low-frequency variants





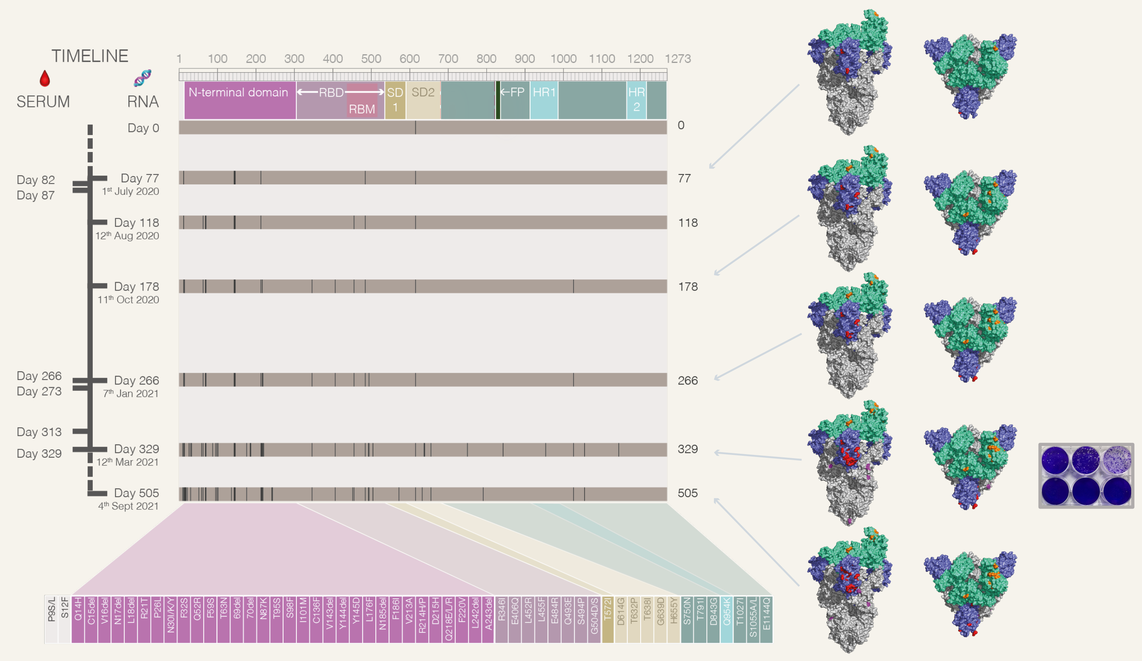


A pipeline was developed to resolve full-length spike haplotypes using high-fidelity nanopore-based sequencing.

The number of haplotypes resolved in a sample positively correlated with the duration of infection (but not with the Ct value of the sample or the number of Q30 reads obtained).

Spike haplotypes representing at least 5% of total at a given time point demonstrate diversity during the LTP infection of patient 2.

3. Longitudinal accumulation of mutations within spike



Longitudinal accumulation of spike mutations during Patient 2 LPT infection occurs mostly in the NTD and RBD antigenic domains.

Full-length virus was isolated from day 329 POS.

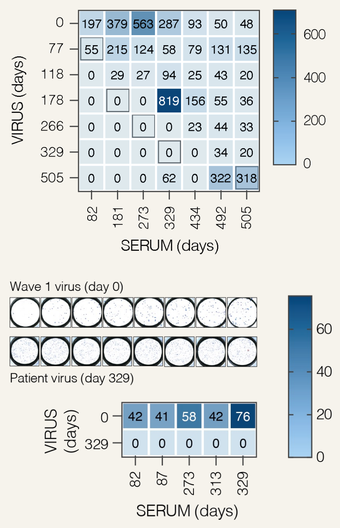
4. Ongoing evolution of neutralising antibody escape

Autologous

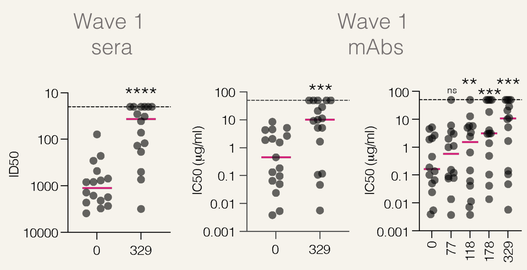
TOP: Neutralisation assays performed with lentiviral particles pseudotyped with representative longitudinal spike samples from case 65 patient and autologous longitudinal sera.

BOTTOM: Neutralisation assays performed with full-length virus isolated from day 329, compared with parental D614G virus.

Heatmaps show IC50 values (sera dilution) for each virus-serum pair.



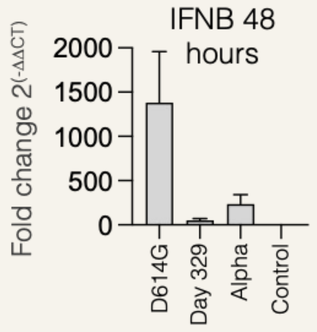
Heterologous



Spike from late time point (day 329) demonstrates significant evasion of neutralisation by wave 1 heterologous sera (left) and monoclonal antibodies (center), compared to original infecting virus (D614G). Evasion of neutralization evolves during the course of LTP infection (right).

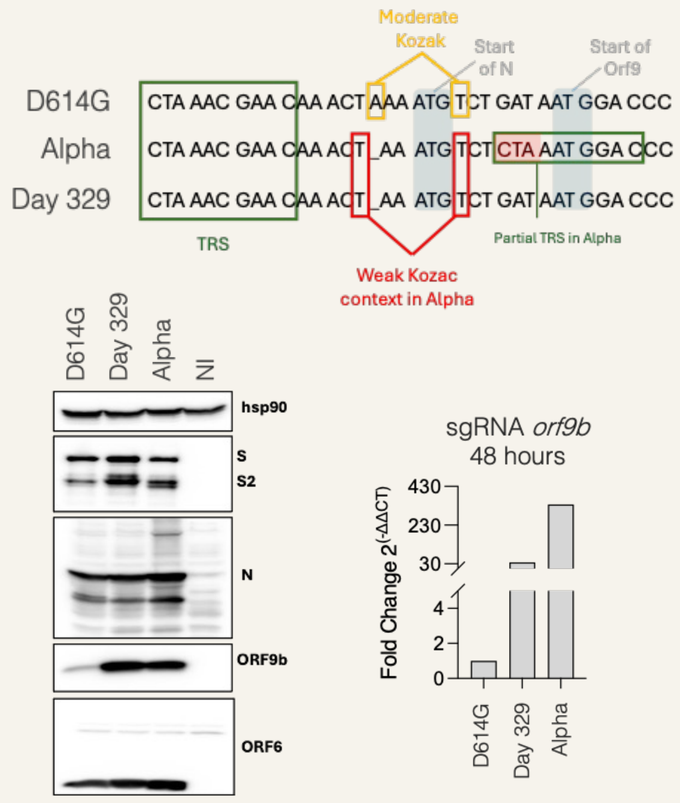
5. Virus isolated from day 329 evades effects of IFN-β

Virus isolated from day 329 has evolved to evade innate immunity, as shown by reduced IFNβ expression in lung epithelial cells (similar to that seen with alpha virus) compared with parental D614G virus.



This can be attributed to a weakened Kozac sequence before the *n*-gene start codon, leading to enhanced expression of the innate immune antagonist Orf9b.

This same mutation has arisen independently in VOCs such as alpha, delta and omicron BA.2.



Conclusions

- To our knowledge, this clinical case is the longest SARS-CoV-2 infection on record.
- Intra-host evolution of an ancestral SARS-CoV-2 virus led to the appearance of mutations typically present in several VOCs, including omicron.
- We have developed a sequencing approach to evaluate the presence of spike haplotypes in clinical samples.
- We present clear evidence that LTP infections can lead to the evolution of evasion mechanisms of both adaptive and innate immune responses.