High infection rates achieved with the world's first RSV B challenge model:

a key component of our RSV-hMPV combination vaccine efficacy testing platform

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INTRODUCTION & OBJECTIVES

Controlled human infection studies have been critical in advancing RSV vaccine and therapeutic development, most notably through the Vero cell-grown RSV A Memphis 37 challenge model. However, no model has existed for RSV B, despite its substantial contribution to global disease burden. To address this gap, we have developed the world's first RSV B challenge model utilising our contemporary strain cGMP manufactured RSV B challenge virus, assessing its safety profile and ability to reliably establish infection in healthy adults. This will add to our existing RSV A and hMPV challenge models ready for combination vaccine efficacy testing.

Since the first RSV challenge models were developed over 20 years ago utilising Vero cell grown viruses, it was subsequently determined that Vero cells cause a post-translational truncation in the RSV G protein (Kwilas *et al.* 2009). Vero cell RSV with truncated G proteins were shown to be attenuated with lower disease severity in the lamb model (Derscheid *et al.* 2013). Consequently, we have switched production for our next generation RSV challenge viruses, described here, from Vero cells to Wi-38 cells to produce fully wild-type RSV A and RSV B challenge viruses.

METHODS

Virus Selection and Manufacturing process

Multiple RSV B clinical samples were collected from patients with community acquired symptomatic RSV infection and aliquoted before use. The isolates were triaged for suitability to produce a challenge agent based on the following criteria: ability for the virus to grow in GMP Wi-38 human cell line, absence of adventitious agents, suitable genome sequence. Once the growth conditions for the isolate had been optimised in the GMP Wi-38 cell line, an unused aliquot of the clinical sample was used to re-isolate the virus and produce the seed virus (p3) for GMP manufacture (p4). The chosen isolate was subsequently names RSV B London, after the city of the primary infection. The manufacturing process for RSV B London is described below in Fig 1.

To update our previous cGMP RSV A Memphis stock, we used the Vero-cell grown batch seed virus bank to inoculate GMP Wi-38 cells and follow the same process described for RSV B manufacture.

Manufacturing Process

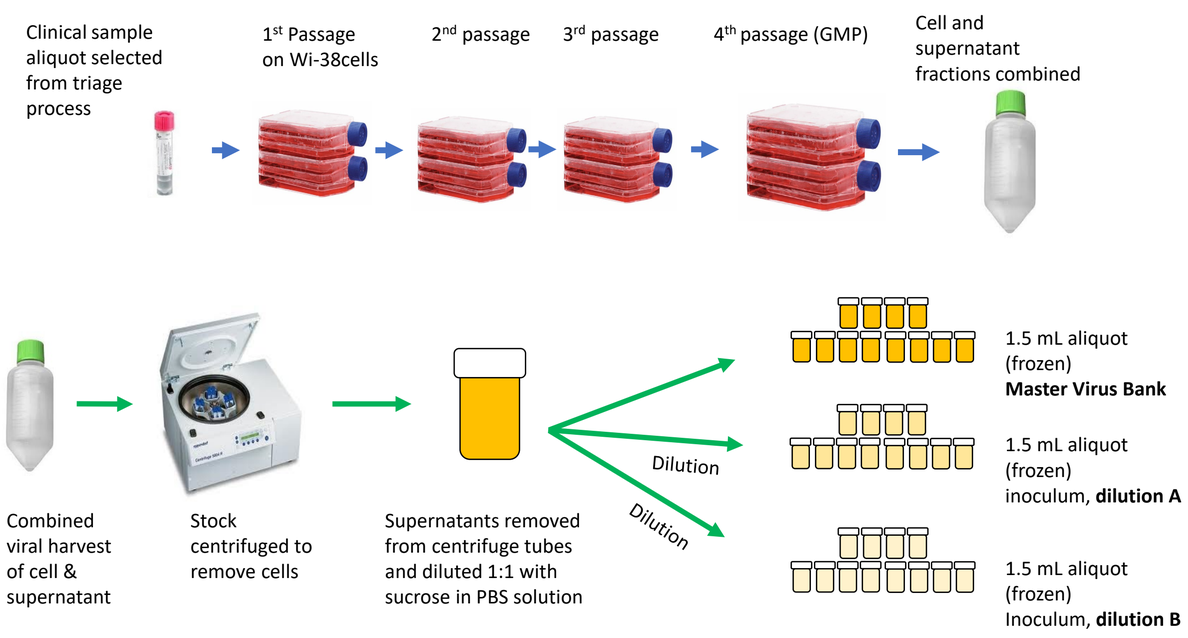


Figure 1: RSV B London challenge virus manufacturing process

RSV B London hVIVO Human Challenge Model

Volunteers were screened for suitability for participation based on their health status, excluding all volunteers with known risk factors for severe disease from participation. In addition, volunteers with high serological antibody levels against the challenge strain were excluded from participation. Fig. 2 illustrates the challenge model process, showing the screening, inpatient and outpatient phases.

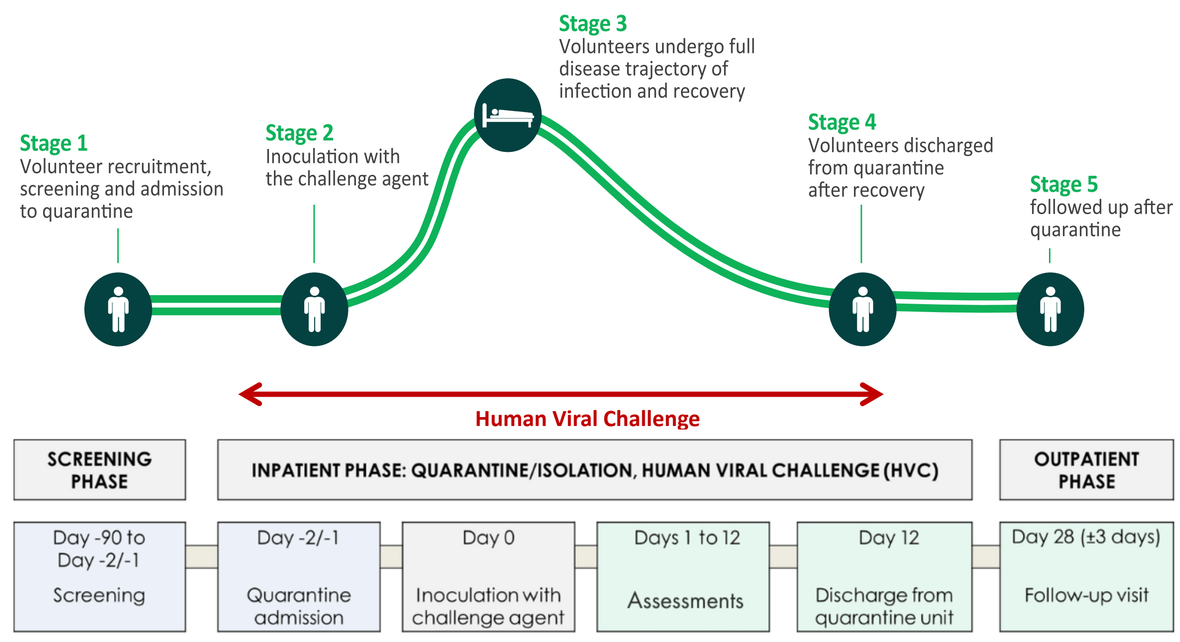
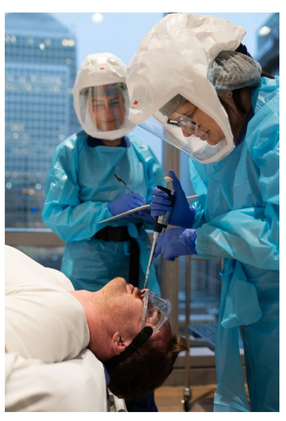


Figure 2: RSV human challenge model process



During the quarantine phase, post intranasal inoculation with the GMP RSV challenge virus, volunteers were monitored closely via safety assessments and examinations. Their clinical disease was monitored by completion of a symptom diary card (3x daily), vital signs (daily), nasal discharge / mucus weight (daily) and nasopharyngeal swabs (NPS) for viral load determination (2x daily). Sera was collected pre-inoculation and at follow-up to assess the relationship between infection and antibody levels.

RESULTS

Clinical Outcomes

In total, 20 participants were challenge with the newly developed GMP RSV B London virus and 11 with the next-generation RSV-A Memphis 37 Wi-38 cell grown virus. All volunteers were inoculated with ~4.5 Log_{10}PFU/ml.

- Excellent safety profiles were obtained for both viruses was obtained:
  - No SAEs, all AEs mild & resolved
- High symptomatic infection rates observed, substantially higher than the original RSV A Memphis 37 Vero cell virus.
- Robust AUC (Area Under the Curve) virology determined by qRT-PCR

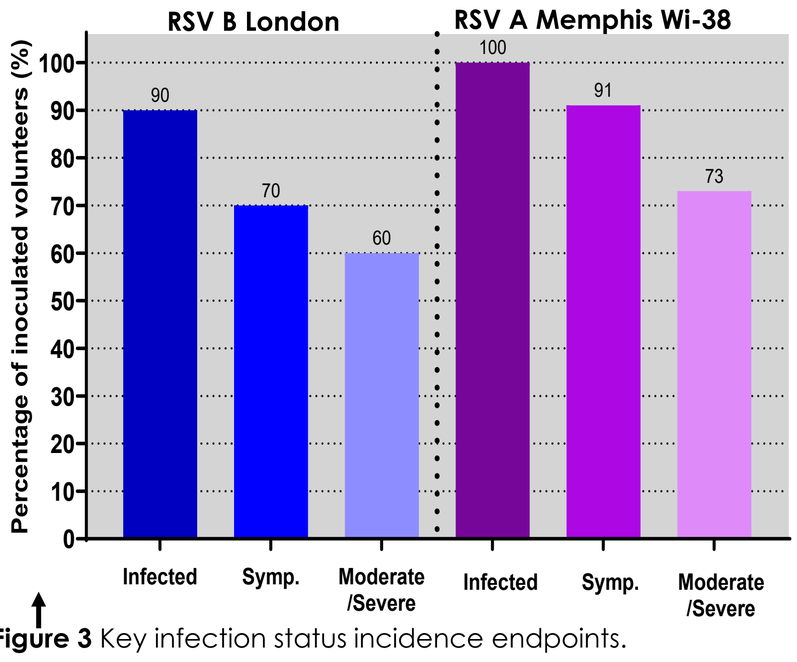


Figure 3 Key infection status incidence endpoints.

"infected": Lab-confirmed infection (LCI): >=2 quantifiable PCR over 48 hours

"Symp." Lab-confirmed symptomatic infection: LCI plus total symptom score ≥ 2

"Moderate / Severe" : LCI plus ≥ grade 2 symptoms

(diary card scored 0, 1, 2 or 3 for each symptom)

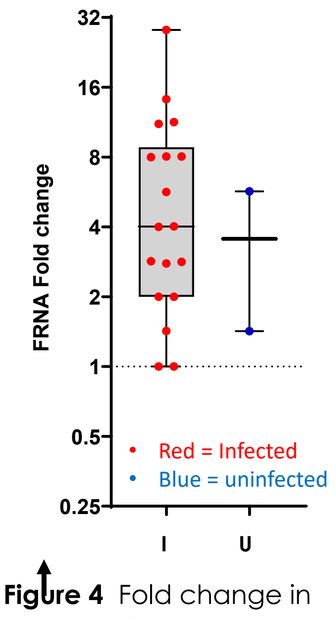
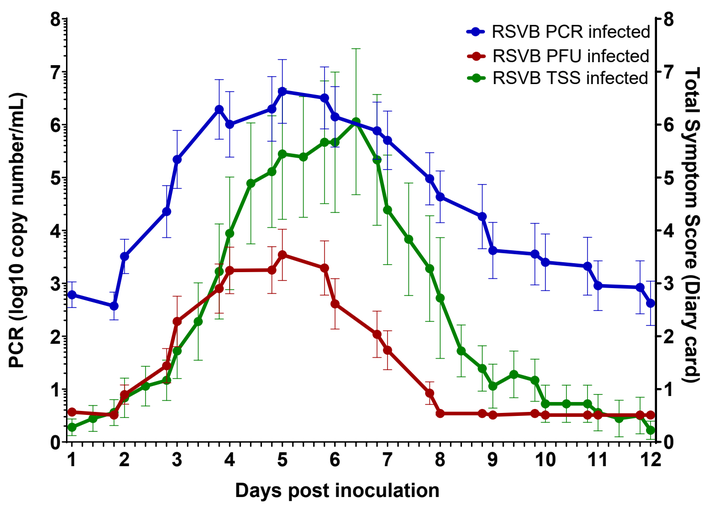


Figure 4 Fold change in serum antibody titres.

Day-1 (pre) to day 28 post viral inoculation.

I, Infected ; U, uninfected



← Figure 5: RSV B London

Disease profile time course of infected study participants (n=18).

Group mean

- viral load by qPCR (blue)
- Viral load by PFU (red)
- total symptom score (TSS)(green

← Figure 6: RSV A Memphis Wi-38

Disease profile time course of infected study participants (n=11).

Group mean

- viral load by qPCR (blue)
- Viral load by PFU (red)
- total symptom score (TSS)(green

Infection rates for the two next generation RSV challenge viruses RSV B London and RSV A Memphis 37 Wi-38 were 90 and 100%, respectively (Fig 3), which is 20-30% higher than historical Vero grown RSVA Memphis 37 (data not shown). Viral load profiles for were extremely similar to each other for the two Wi-38 cell grown viruses (Fig. 5 and 6). Both show peak qPCR a day 4-6, 2-3 days earlier than historical Vero grown RSV A Memphis (data not shown).

ACKNOWLEDGEMENTS

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Use QR code for further information

References

Kwilas S *et al.* 2009 J of Virology, 83,(20) 10710-10718

Derscheid RJ *et al.* Viruses 2013, 5(11) 2881-2897

CONCLUSIONS

We have successfully developed **the world's first RSV B challenge model**. The RSV B London model exhibits **very high infection rates** with robust moderate to severe symptomatic disease, which is an endpoint that translates well to traditional field-trial endpoints. Similarly, using the same Wi-38 cell platform for the next generation RSV A Memphis 37 virus, we have demonstrated that this infection rates are markedly increased in comparison to known Vero cell grown virus infection rates of the same strain. We propose that this is likely due to Vero cell grown RSV viruses having a truncated G protein from post-translational cleavage. Both of these new viruses have also shown an excellent safety profile making them **ideal for product efficacy testing**.