# Multivalent sialic acid-binding proteins as a novel preventative and treatment of RSV infection

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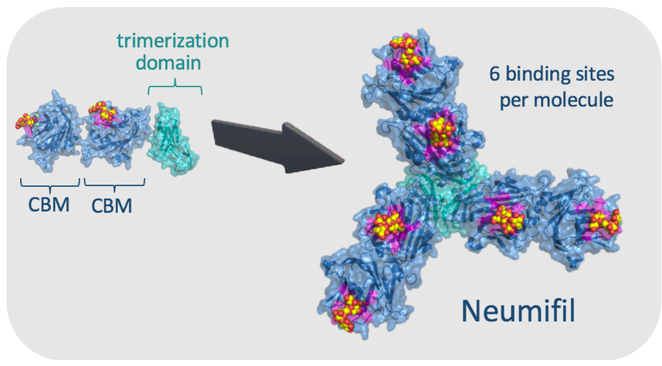
## Introduction

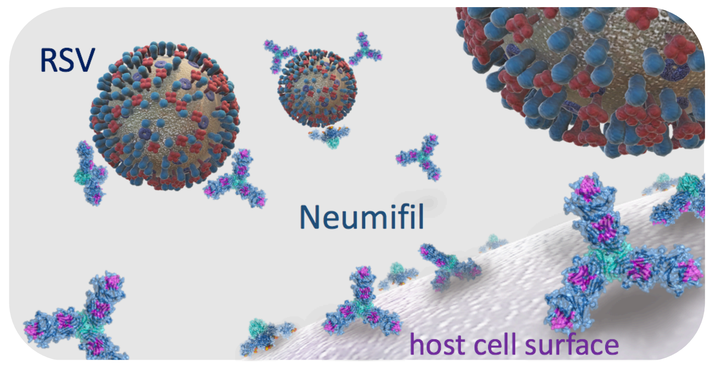
Sialic acids (SA) decorate the surfaces of most animal cells and are the receptor for the binding of many respiratory pathogens, such as the influenza virus (IFV). A host-targeted approach to the prevention or treatment of disease caused by SA-targeting pathogens is to mask the receptor to prevent cell binding, entry and replication. We have developed a multivalent SA-binding protein named Neumifil™, using carbohydrate binding modules (CBMs), which we have shown to protect mice from lethal doses of a range of IFVs[1,2]. To further explore the potential application of Neumifil™ to other respiratory pathogens, we recently investigated the effect of Neumifil™ in *in vitro* and *in vivo* models of Respiratory Syncytial Virus (RSV) infection. Remarkably, despite the fact that RSV does not, to our knowledge, utilize SA as a receptor, Neumifil™ reduces RSV cell attachment *in vitro*, through both host-targeting and viral-targeting mechanisms. Furthermore, these *in vitro* effects translate to significant reductions in viral titre *in vivo*.

### Neumifil™

- Multivalent Carbohydrate Binding Module (mCBM)
- Sub-nanomolar affinity to SA
- Exquisite specificity to cognate glycan
- Broad-acting against multiple respiratory pathogens
- Dual mode of action in RSV



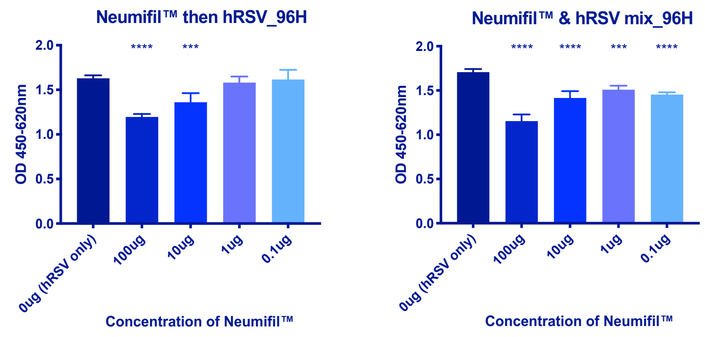

**Neumifil™: high-affinity sialic acid binding**




**Neumifil™ prevents virus attachment to the host**

## *In vitro* analysis of Neumifil™ against hRSV

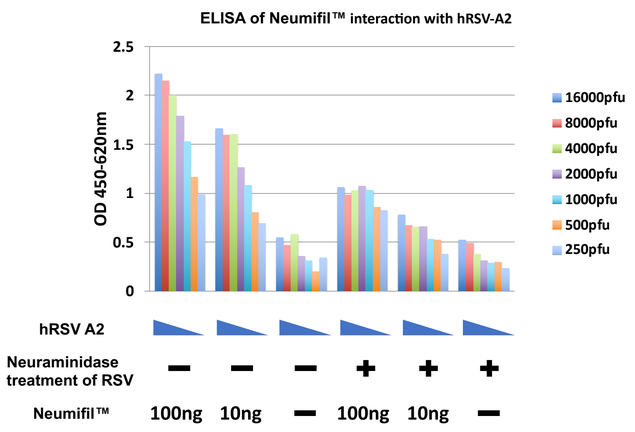
### 1 Neumifil™ inhibits hRSV host cell attachment



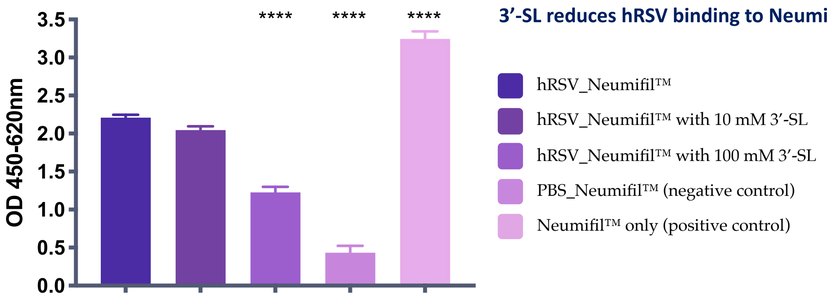

**Neumifil™ inhibits hRSV binding to the cell in a dose-dependent manner.** Neumifil™ and hRSV-A2 were added to human HEp2 cells either sequentially (left panel) or pre-mixed (right panel). After 96 h, hRSV infection was evaluated by immunodetection.

### 2 Neumifil™ targets the virus ...

**Neumifil™ binds RSV via SA.** ELISA: Plates were coated with varying amounts of hRSV, with or without neuraminidase treatment. Neumifil™ or PBS was added for 1h followed by immunodetection of bound Neumifil™. Direct binding to hRSV-A2 occurs in a dose-dependent manner and the binding level is decreased after the virus was treated with neuraminidase.

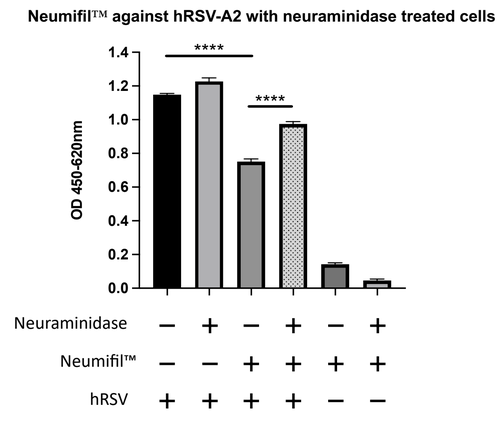



The interaction between Neumifil™ and hRSV-A2 was reduced after the binding site of Neumifil™ was blocked using 3'-sialyllactose (3'-SL). The effect is dose-dependent.




### 3 ... and the host

The protective effect of Neumifil™ is reduced when cell surface SA is removed. HEp2 cells were treated with neuraminidase to remove SA, or left untreated. Neumifil™ was added for 1 h, followed by hRSV for 1 h. hRSV infection was evaluated by immunodetection after 72 h.

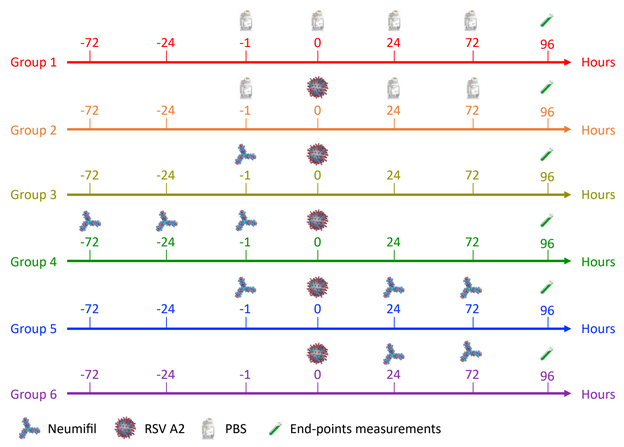



Similar *in vitro* results were observed using a different RSV strain (RSV B 18537) (not shown).

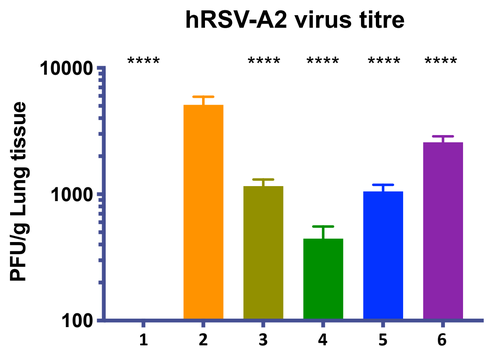
*p* value <0.05 was considered statistically significant (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

## *In vivo* analysis of Neumifil™ against hRSV

### 4 Neumifil™ reduces RSV infection *in vivo*




- Female BALB/c mice (n=8/group).
- Single 100 µg/dose of Neumifil™ was administered I.N. on specified days.
- Non-lethal dose of RSV-A2 at 5 x $10^6$ pfu was administered I.N. on Day 0



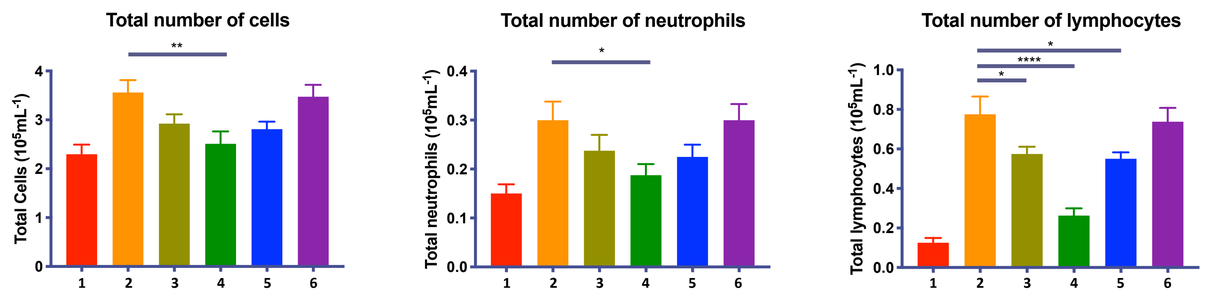

**Neumifil™ improves the clearance of virus from lung.** Reductions in virus titres reached statistical significance in all treated groups.

*p* value <0.05 was considered statistically significant (****p<0.0001).

(Reduction in viral titre)

3 4 fold | 4 >10 fold | 5 4 fold | 6 2 fold

Total & differential cell counts of the BAL fluid samples were analyzed using flow cytometry. Neutrophil and lymphocyte cell counts were significantly lower in treated groups.




## Conclusion

- Neumifil™'s dual mechanism of action prevents hRSV replication at the level of virus entry.
- Neumifil™ treatment either as a prophylactic or given post-exposure significantly reduces hRSV replication in an animal model.
- Immune components (known to be correlated with immune-mediated hRSV disease in humans) from Neumifil™-treated mice were significantly reduced compared to untreated mice.
- Neumifil™ is well-tolerated in all dosing regimens.
- Neumifil™ is a potential universal drug for respiratory tract infections.

## Acknowledgement

- We would like to thank Martin Schutten and SGS Life Sciences for advice in the development of the RSV *in vivo* protocol.
- The *in vivo* work was performed at Pharmidex Ltd, UK

## References

1. Connaris *et al.*, 2014 PNAS 111, 6401-6406;
2. Govorkova *et al.*, 2015 AAC 59, 1495-1504
3. Pictures resource from https://www.pneumagen.com. https://www.st-andrews.ac.uk. http://www.resvinet.org.

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