Inhibition of Fatty Acid Synthase *in vitro* and *in vivo* Reduces RSV Replication

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Introduction

- 3-V Biosciences has developed a series of antiviral drugs which reduce the replication of multiple respiratory viruses by inhibiting cellular fatty acid synthase (FASN).
- FASN is a multifunctional enzyme that catalyzes the *de novo* synthesis of palmitate, a fatty acid utilized for both synthesis of more complex fatty acids and for the post-translational palmitoylation of specific proteins, including many viral proteins.
- 3-V Bioscience's FASN inhibitors are highly selective, reversible, orally available and have potent antiviral activity at nanomolar concentrations.

Results

FASN inhibitors TVB-2722 and TVB-3166 reduce RSV A and B replication in A549 cells

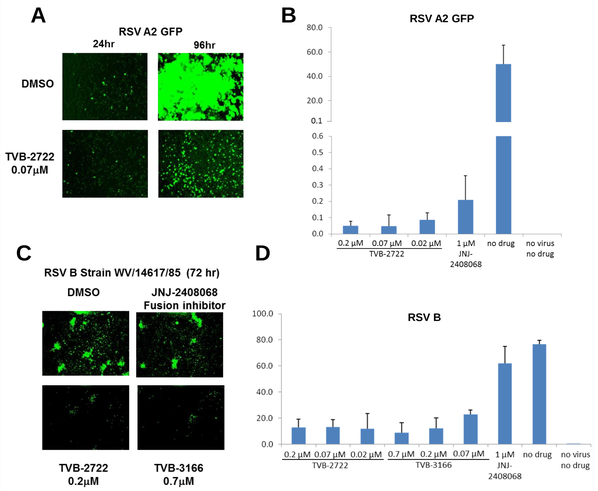

Figure 1
FASN inhibition reduces RSV spread and production of infectious RSV progeny
A549 cells were infected at MOI of 0.03 (**A,B**) or 0.05 (**C,D**) (**B**,**D**) Progeny virus harvested from A549 cells infected for 72 hours in the presence or absence of the indicated drugs was used to infect Vero cells. The percent of infected Vero cells was quantified by automated microscopy. RSV B was visualized with an anti-F antibody.

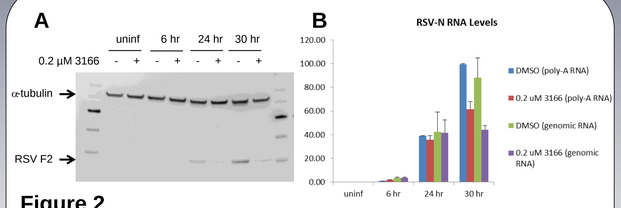

Figure 2
FASN inhibition reduces synthesis of RSV protein and RNA
A549 cells were infected with RSV A2 GFP (MOI 2) and subsequently treated with TVB-3166 or DMSO. At the indicated times post-infection, cells were harvested for protein or RNA. (**A**) Detection of RSV-F protein levels by Western blot (**B**) RSV N RNA levels determined by qRT-PCR. First strand cDNA synthesis by oligo dT or genomic primers

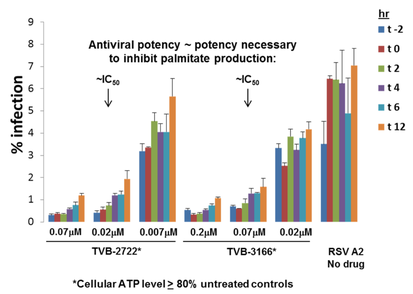

Figure 3
FASN inhibitor added up to 12 hours after RSV infection exhibits antiviral effect
A549 cells were infected with RSV A2 GFP (MOI 0.03) and treated with TVB-2722, TVB-3166 or DMSO at the indicated times. Infection levels were quantified at 72 hours post-infection by automated microscopy.

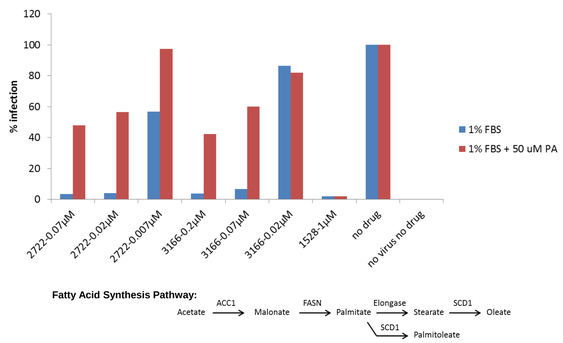

Figure 4
Anti-RSV activity is mediated by palmitate reduction
A549 cells were infected with RSV A2 GFP (MOI 0.03) followed by treatment with TVB-2722, TVB-3166, DMSO or the fusion inhibitor JNJ-2408068 in the presence or absence of 50 μM palmitate-BSA (PA). Infection levels were quantified at 72 hours post-infection by automated microscopy.

FASN inhibitor TVB-3166 reduces RSV A (Long) replication in Balb/c mouse model

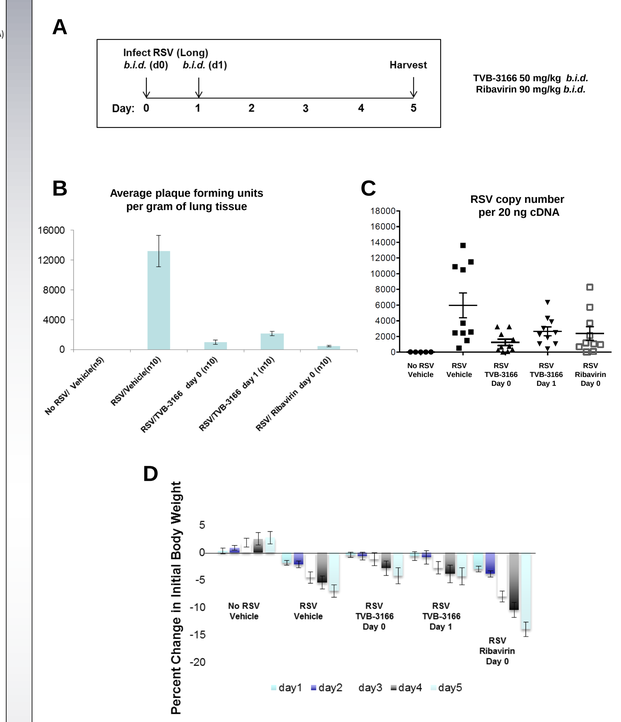

Figure 5
TVB-3166 exhibits both prophylactic and therapeutic anti-RSV effects in Balb/c mice
(**A**) Twice daily treatment with TVB-3166 (p.o.), Ribavirin (i.p.) or drug vehicle (p.o.) was begun 2 hours after intranasal inoculation of mice with 5.5 x 10^5 pfu. One group received vehicle on day 0 and TVB-3166 on subsequent days. (**B**) Lung titers were determined by plaque assay on HEp2 cells (**C**) RSV N RNA level was determined by qRT-PCR (**D**) Percent change in initial body weight was determined daily

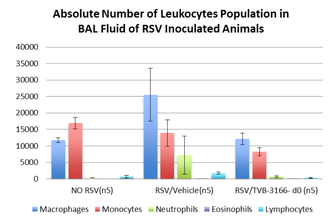

Figure 6
TVB-3166 reduces macrophage and neutrophil levels in RSV infected mice
On day five post-infection, lungs were harvested from 5 animals of the indicated groups and BAL collected. Leukocyte populations in the BAL were determined by differential count.

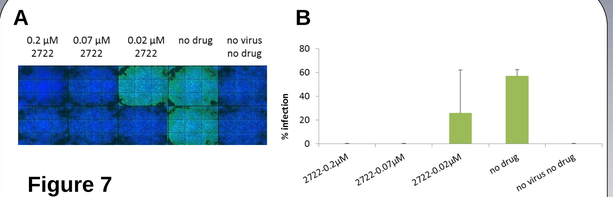

Figure 7
FASN inhibition reduces yield of PIV3 from HEp2 cells
HEp2 cells were infected with PIV3 (MOI 0.0001) and subsequently treated with TVB-2722 or DMSO for 72 hours. Supernatants were used to infect Vero cells for 72 hours. Then cells were fixed and stained with anti-PIV3 polyclonal antibody (**A**) Microscopic image (**B**) Percent infection as determined by automated imaging

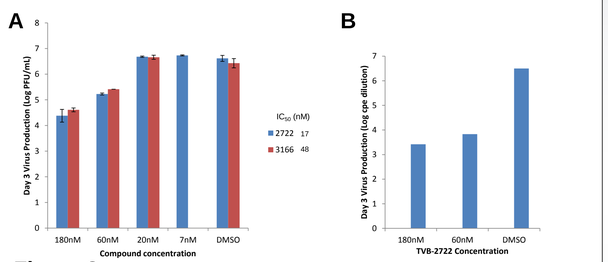

Figure 8
FASN inhibition reduces yield of HRV16 from Hela cells
Hela cell monolayers in 12 well dishes were infected with HRV16 (50 pfu/well). After incubation at 37^0 C for 3 days, supernatants were collected for virus titration. (**A**) Treatment with $FASN_I$ initiated 16 hours pre-infection (**B**) Treatment with $FASN_I$ initiated immediately post-infection

Conclusions

- **3-V Biosciences has developed potent inhibitors of FASN which reduce the *in vitro* production of RSV A, RSV B, PIV3 and HRV16 progeny from infected human cell lines.**
- **Antiviral activity is mediated by inhibition of palmitate production.**
- **In BALB/c mice, oral administration of the FASN inhibitor TVB-3166 on the day of RSV challenge or one day post-challenge resulted in reduced levels of RSV in the lung.**
- **TVB-3166 treated mice had lung titers comparable to Ribavirin treated animals but exhibited much less weight loss.**

Acknowledgements

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