Phosphatidylserine Receptor Enhancement of SARS-CoV-2 Entry: AXL as a Therapeutic Target for COVID-19

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INTRODUCTION AND PURPOSE

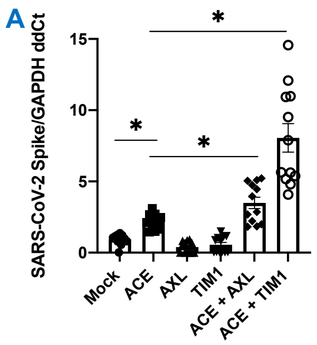
Therapeutics able to counter SARS-CoV-2 are currently limited. Dexamethasone finds utility with only the most critically ill, whereas convalescent sera and remdesivir have limited efficacy. Leveraging our understanding of RNA virus biology, we set out to identify host proteins that are exploited by SARS-CoV-2 for entry and identify efficacious inhibitors.
Phosphatidylserine (PS) receptors, TIM1 and AXL, bind to virion- or apoptotic body-associated PS, internalizing cargo into the endosomal compartment. These receptors enhance entry of a wide range of enveloped viruses, including filoviruses and flaviviruses. We show here that SARS-CoV-2 also utilizes PS receptors to enhance entry. Further, an inhibitor extensively tested and shown to be safe in humans, bemcentinib, inhibits SARS-CoV-2 infection in *in vitro* and *in vivo* models.

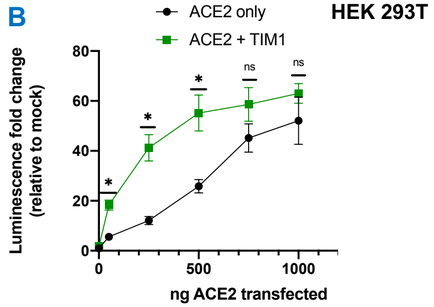
METHODS

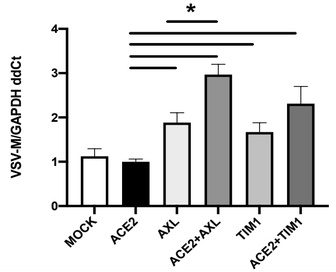
Cells were treated with inhibitors as noted 1 hour before infection. Bemcentinib (BGB324) concentrations were **0.1, 0.33, and 1 μM**. E-64 was used at 300μM, and BMS777607 at 0.1 and 1 μM. These are non-toxic doses, assessed by ATPlite. The SARS-CoV-2 WA1_2020 isolate was used in these studies and passaged in VeroE6 cells. Presence of the furin cleavage site in our stocks was verified by sequencing. MOI used was 0.5 unless otherwise noted, and all cells and supernatants were harvested 24 hours post infection (hpi). Transfections used 50 ng human ACE2 plasmid and 1 μg of all other plasmids with the addition of an empty vector to adjust plasmid concentrations so that equivalent plasmid quantities were transfected .

PS Receptors Enhance SARS-CoV-2 Binding and Entry

TIM1 or AXL expression in HEK 293T cells enhances hACE2-dependent entry of SARS-CoV-2 when transfected cells were challenged with BSL3 SARS-CoV-2. Viral loads were measured 24 hpi **(A)**. Exogenously TIM1 expression facilitated SARS-CoV-2-spike pseudovirion infection when hACE2 was low, but not at higher hACE2 concentrations **(B)**.



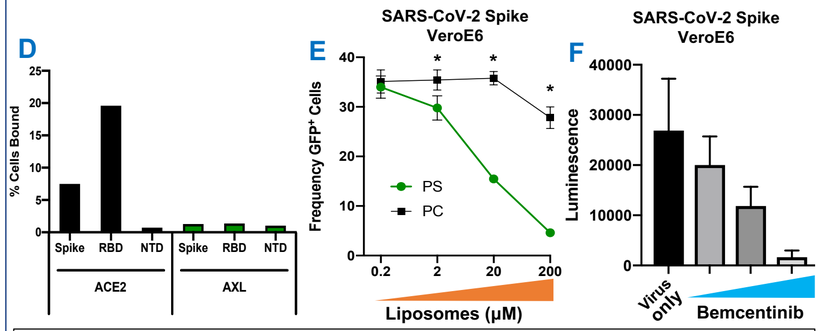




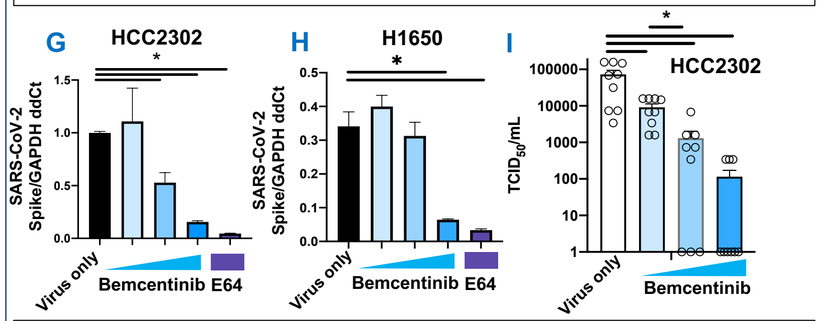
TIM1 and AXL enhance binding of SARS-CoV-2-Spike rVSV virions to transfected HEK 293T cells as assessed by bound viral load detected at 1 hour at room temperature **(C)**. Our data show that overexpression of sub-optimal ACE2 levels does not alter binding, whereas PS receptors significantly increase virion binding capacity.

AXL Inhibition Reduces SARS-CoV-2 Entry, Infection

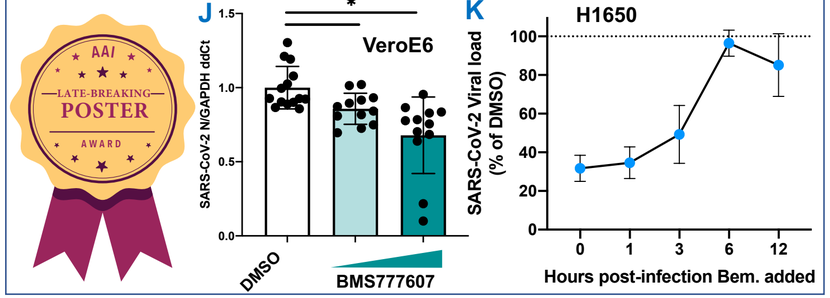
PS receptors were assessed for direct binding to SARS-CoV-2 Spike **(D)** or to virion-associated PS **(E)**. As expected, spike binds to ACE2, but fails to bind AXL. Instead, PS liposomes competed for PS receptor mediated entry. Further, the AXL inhibitor bemcentinib reduces SARS-CoV-2 pseudovirion infection **(F)**.



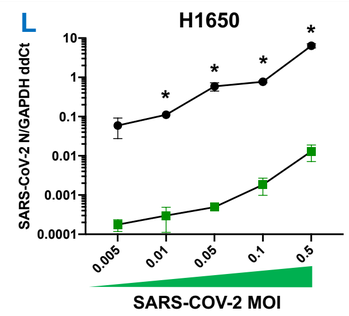
To investigate the effect of AXL inhibition in permissive, disease-relevant lung epithelial cell lines (HCC2302 and H1650), we treated cells with bemcentinib and challenged with SARS-CoV-2. Bemcentinib dramatically reduced viral loads in all both cell lines **(G, H)** and dramatically reduced infectious titers 48 hpi in HCC2302 cells **(I)**. Cathepsin inhibitor E64 also inhibited virus infection.

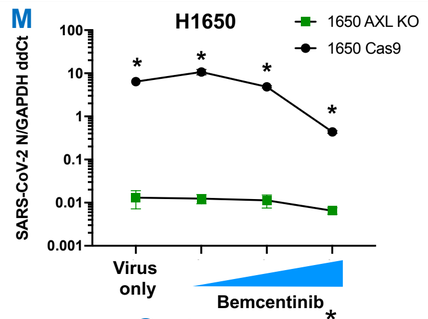


An unrelated TAM inhibitor, BMS777607, also inhibited SARS-CoV-2 infection in VeroE6 cells **(J)**. To determine the timing of efficacy of bemcentinib on SARS-CoV-2 infection, we added drug at various times during infection **(K)**. In H1650 cells bemcentinib is most effective when present at the earliest stages of infection, supporting our hypothesis that AXL enhances viral entry.

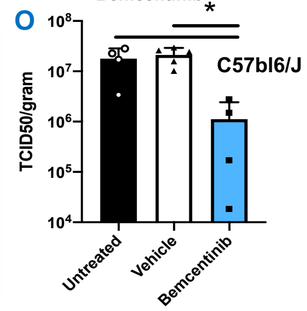


AXL Knockout Impairs Infection + *in-vivo* Model



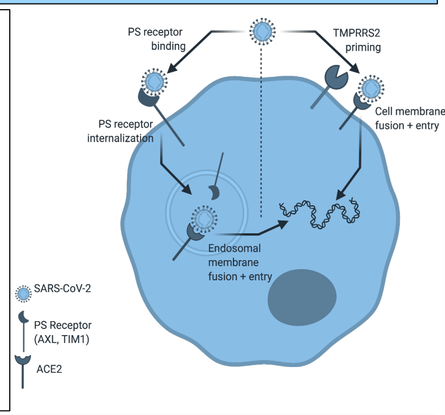


As further assessment of AXL in SARS-COV-2 infection, we generated CRISPR/Cas9 AXL knockout H1650 cells. These cells were infected at multiple MOIs **(L)** or in the presence of bemcentinib **(M)**. In the absence of AXL, SARS-CoV-2 viral load at 24 hours was suppressed at every MOI tested and bemcentinib had no antiviral effect on AXL KO cells. These data demonstrates the critical role of AXL during infection. Translating these observations to a mouse model of MHV (a related β CoV), treatment with bemcentinib by oral gavage significantly reduced infectious titers in liver at 5 dpi **(O)**. Viral loads were similarly reduced in these experiments (not shown).



CONCLUSIONS

We identify that PS receptors (TIM1 and AXL) play major roles in the entry of SARS-CoV-2 virions. Our studies suggest that virus utilization of PS receptors internalizes virus through the endosomal compartment rather than at the plasma membrane. Inhibition of AXL signaling and cargo internalization by bemcentinib reduces infection as measured by viral loads and infectious titers. This inhibitory activity is specific to the entry stage of infection, and AXL knockout lung cells are far less permissive than wild type cells. This inhibitory activity was also observed in a coronavirus mouse model.
In summary, PS receptors enhance SARS-CoV-2 infection and inhibition of AXL is a promising therapeutic target for COVID19.



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