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Marginal effect of GC-376 as a SARS-CoV-2 antiviral in the K18 hACE2 transgenic mouse model

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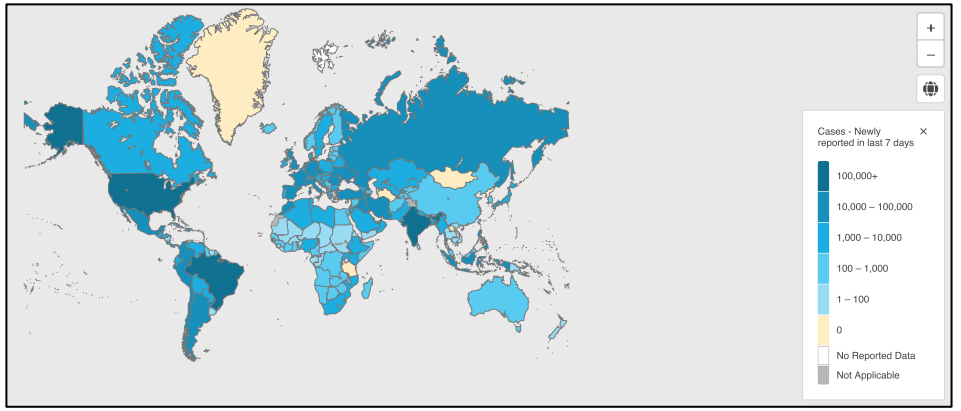
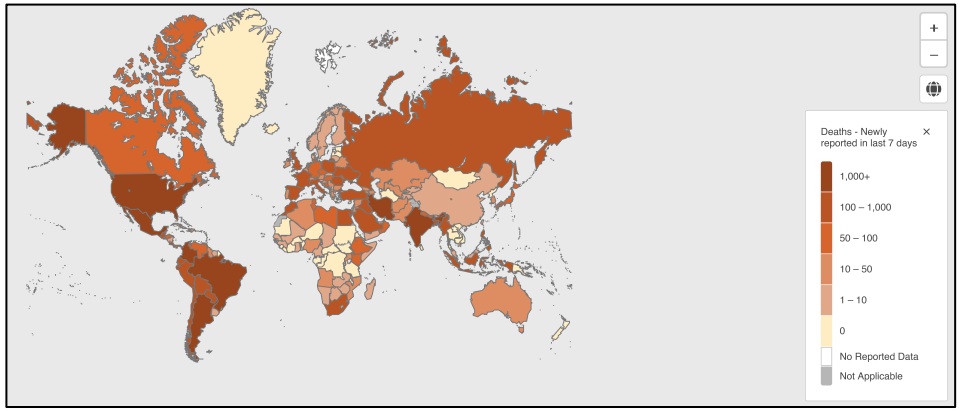
SARS-CoV-2 situation

- First cases of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) were reported on December 31st in Wuhan, China.
- SARS-CoV-2 is a beta coronavirus like SARS-CoV-1 and Middle Eastern respiratory syndrome coronavirus (MERS-CoV).
- SARS-CoV-2 was declared a pandemic that has spread to more than 203 countries.
- Currently, neither vaccines nor antivirals are approved against SARS-CoV-2.
- As of September 2020, more than 32 million cases and one million of deaths have been confirmed.

Number of cases (Last week of September 2020)



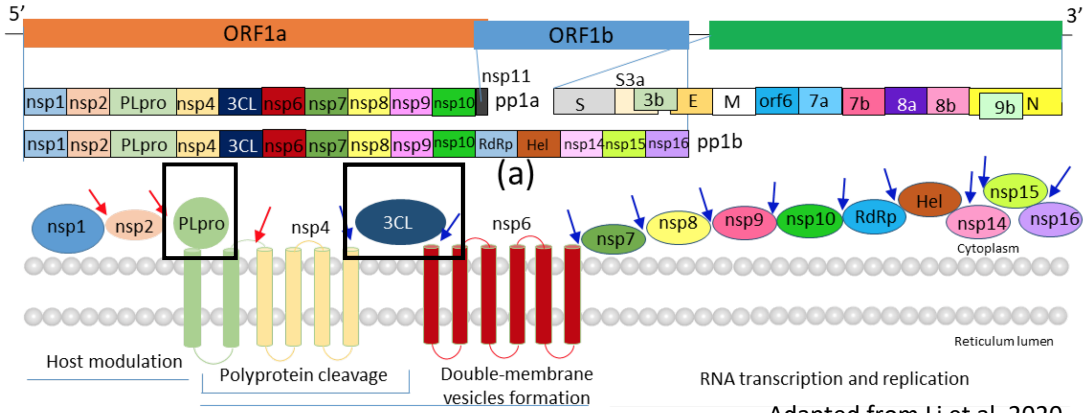
Number of deaths (Last week of September 2020)



Source: WHO

Antiviral development against SARS-CoV-2

- SARS-CoV-2 is a (+) strand RNA virus with a ~ 30kb genome.
- SARS-CoV-2 encodes two major polyproteins (ORF1a and ORF1b).
- Processing of ORF1a and ORF1b is mediated by viral proteases and required for virus replication
  - Papain-like protease (PL^{pro})
  - 3 chymotrypsin-like protease ($3CL^{pro}$)
  - Viral proteases are attractive antiviral targets.
  - Many efforts aimed at repurposing of current antivirals.

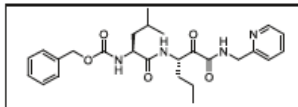
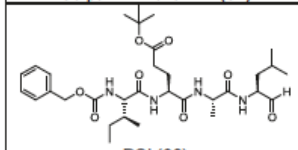
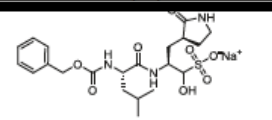
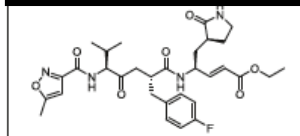


Adapted from Li et al. 2020

List of compounds proposed as potential SARS-CoV-2 antivirals

Number	Target	Drug name
1	HIV Protease	Saquinavir
2	HIV Protease	Ritonavir
3	HIV Protease	Indinavir
4	HIV Protease	Nelfinavir Mesylate
5	HIV Protease	Amprenavir
6	HIV Protease	Lopinavir
7	HIV Protease	Atazanavir sulfate
8	HIV Protease	Fosamprenavir
9	HIV Protease	Tipranavir
10	HIV Protease	Darunavir
11	HCV NS3 protease	Boceprevir
12	HCV NS3 protease	Telaprevir
13	HCV NS3 protease	Simeprevir
14	HCV NS3 protease	Asunaprevir
15	HCV NS3 protease	Grazoprevir
16	Proteasome	Carfilzomib
17	Proteasome	Bortezomib
18	3C-Like Protease	GC376 sodium

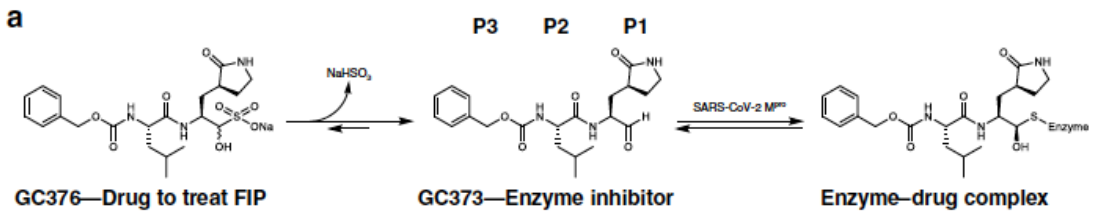
Adapted from Fu et al. 2020

 Calpain inhibitor XII (**62**)	0.45 ± 0.06	63.60 ± 0.01/7.86	>20	>20	Preclinical; not tested in animal model
 PSI (**63**)	10.38 ± 2.90[c]	N.T.	1.22	13.74 ± 3.86	Preclinical; tested in rats[63]
 GC-376 (**64**)	0.030 ± 0.008	74.04 ± 0.07/18.30	>20	0.136 ± 0.025	Preclinical; tested in felines[14, 15]
 Rupintrivir (**65**)	> 20	N.T.	>20	0.042 ± 0.014	Dropped out of clinical trial

Adapted from Ma et al. 2020

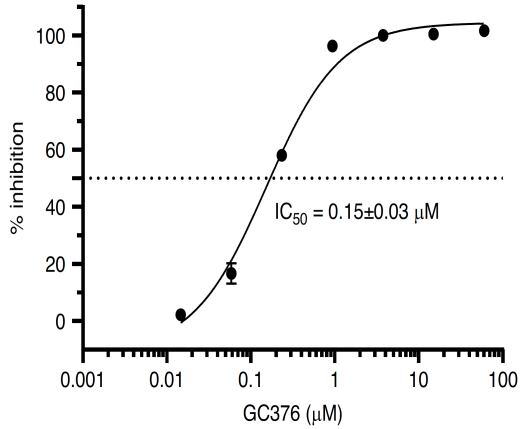
GC-376 shows antiviral activity against SARS-CoV-2 *in-vitro*

- **GC-376 shows antiviral activity against Picornaviruses and Coronaviruses *in-vitro*.**
- **GC-376 shows antiviral activity against Feline infectious peritonitis (FIP) *in-vitro* and *in-vivo* in cats.**
- **The antiviral activity of GC-376 against SARS-CoV-2 *in-vitro* has been reported recently.**



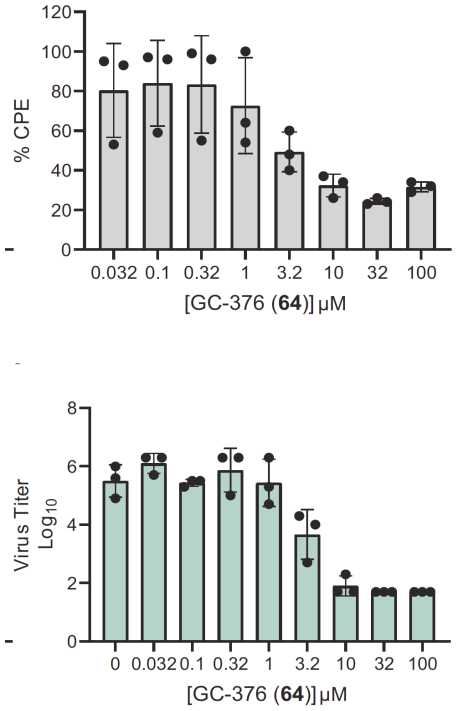
Adapted from Vuomg et al. 2020

GC-376 inhibition effect over SARS-CoV-2 3CL^{pro} activity



Adapted from Fu et al. 2020

GC-376 inhibition effect over SARS-CoV-2 replication *in-vitro*

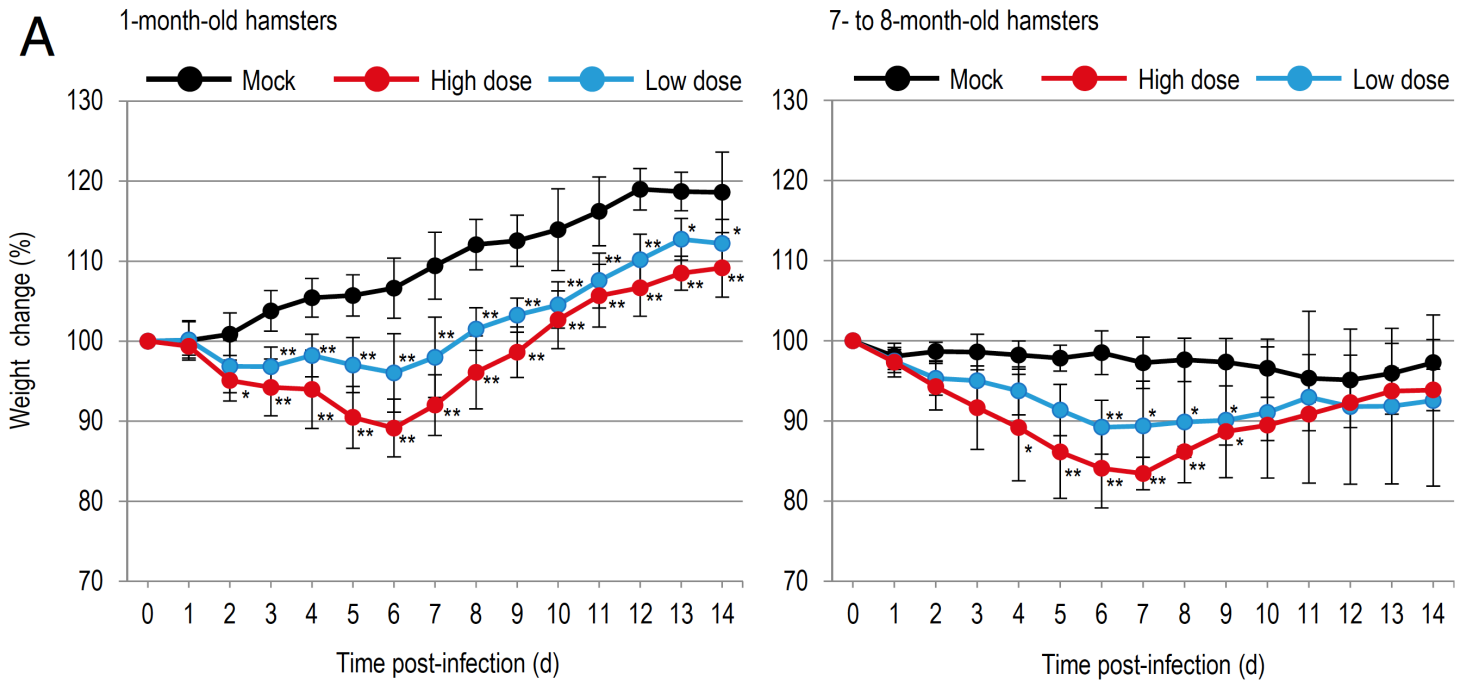


Adapted from Ma et al. 2020

Animal models for the study of SARS-CoV-2

- **Mice are not naturally susceptible to SARS-CoV-2.**
- **Hamsters, ferrets, and macaques are susceptible to SARS-CoV-2.**
- **However, these animal models does not show the full spectrum of clinical signs and mortality observed in COVID-19 cases in humans.**

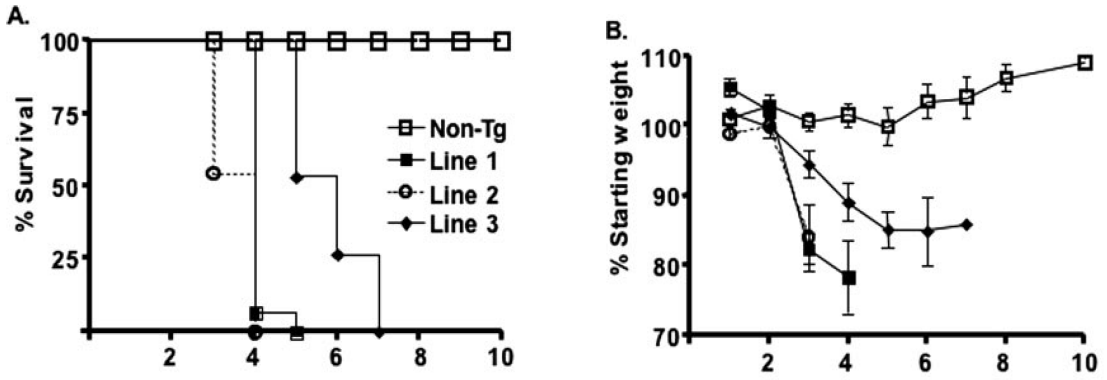
Infection of hamsters with SARS-CoV-2 results in mild weight loss.



Adapted from Imai et al. 2020

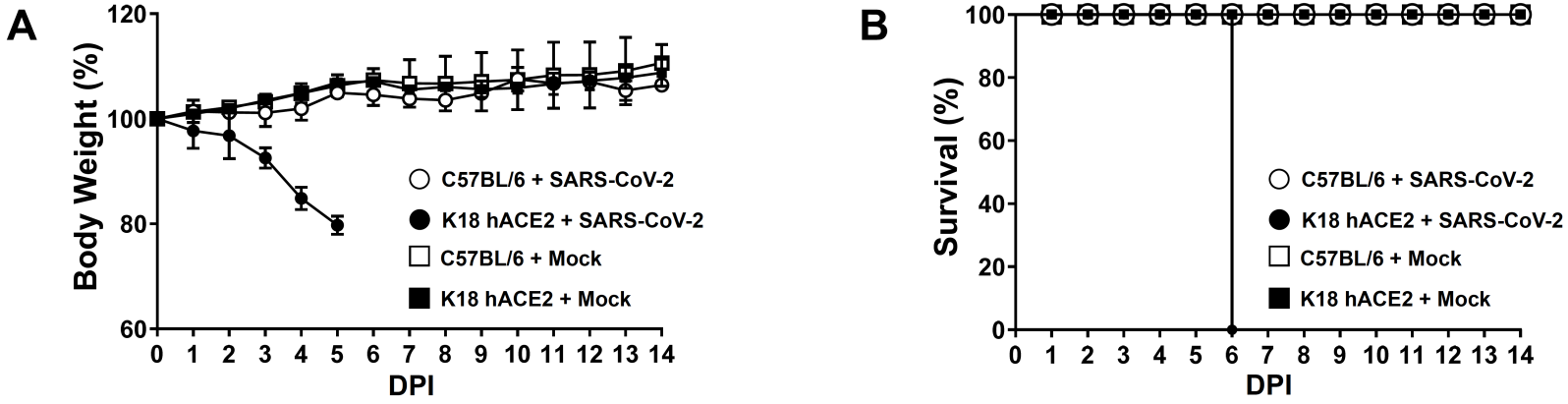
K18-hACE2 mice

- **Transgenic mice, K18-hACE2, were generated by introducing the coding sequence of the human angiotensin-converting enzyme 2 (hACE2).**
- **K18-hACE2 mice are highly susceptible to SARS-CoV-1.**



Adapted from McKay et al. 2007

- **Since SARS-CoV-2 utilizes the same hACE2 receptor, K18-hACE2 mice are potentially a suitable animal model for SARS-CoV-2 research. Preliminary reports showed high susceptibility of the K18-hACE2 mice against SARS-CoV-2.**



Adapted from Oladunni et al. 2020

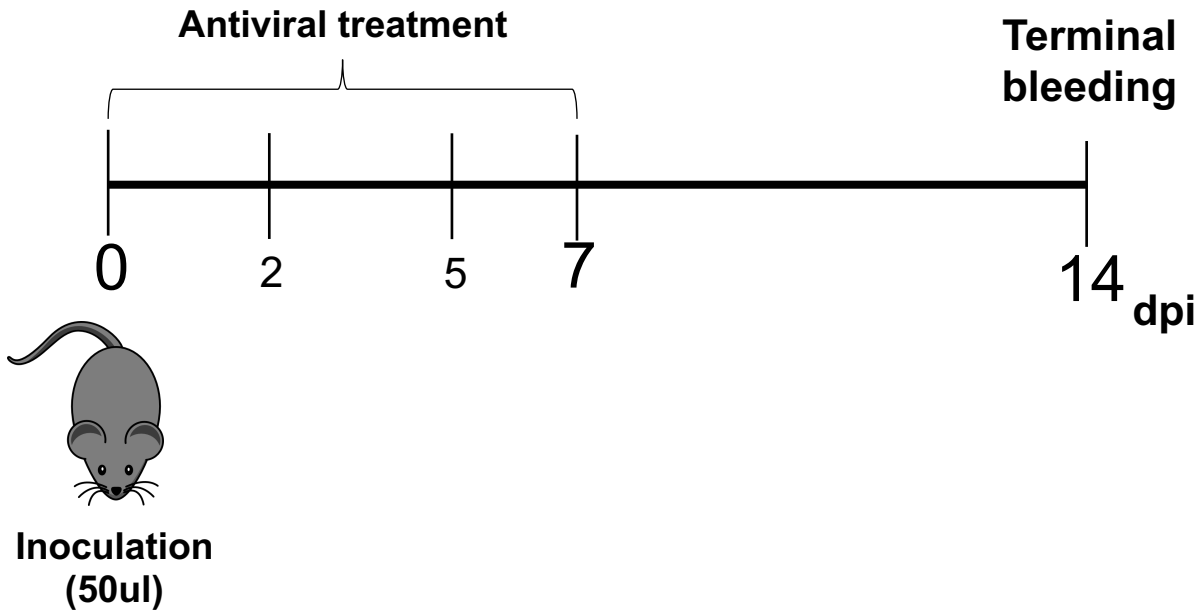
Objectives

- **Characterize the pathogenesis of SARS-CoV-2 in the K18 hACE2 transgenic mice model.**

- **Evaluate the efficacy of GC-376 as an antiviral *in-vivo* in K18 hACE2 mice.**

Experimental design

- **Mice were randomly distributed into six different groups.**
- **Two different doses of SARS-CoV-2 were used: 1x10ˆ5 TCID50/mouse (high dose) and 1x10ˆ3 TCID50/mouse (low dose).**
- **Antiviral treatment was performed 2 times per day for 7 days after SARS-CoV-2 inoculation.**
- **A standard dose of GC-376 was used (20mg/kg).**

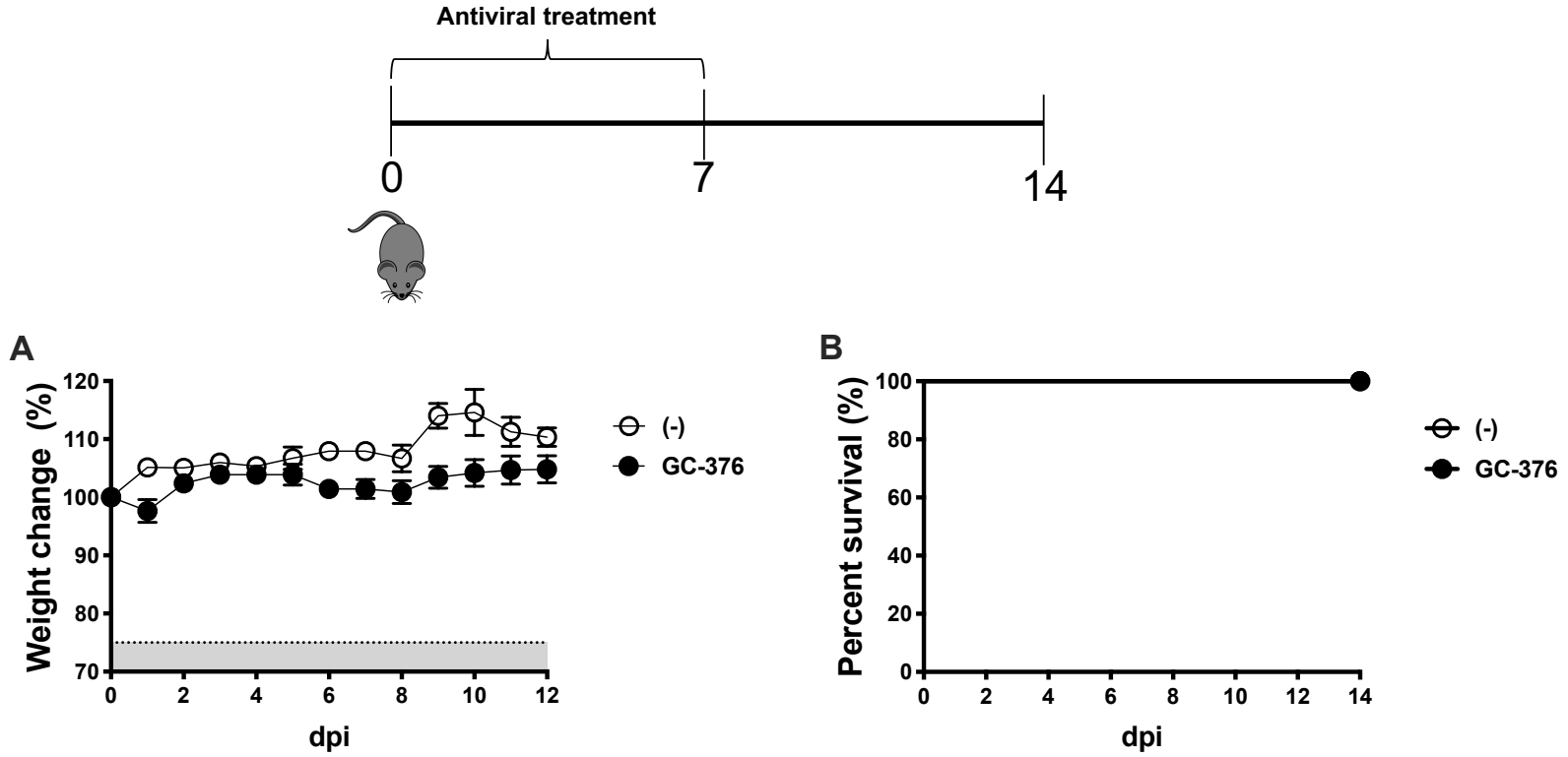


1. **PBS**
2. **Mock infection/GC-376**
3. **SARS-CoV2 low dose/Drug vehicle**
4. **SARS-CoV2 high dose/Drug vehicle**
5. **SARS-CoV2 low dose/GC-376**
6. **SARS-CoV2 high dose/GC-376**

GC-376 is not toxic in the K18-hACE2 model

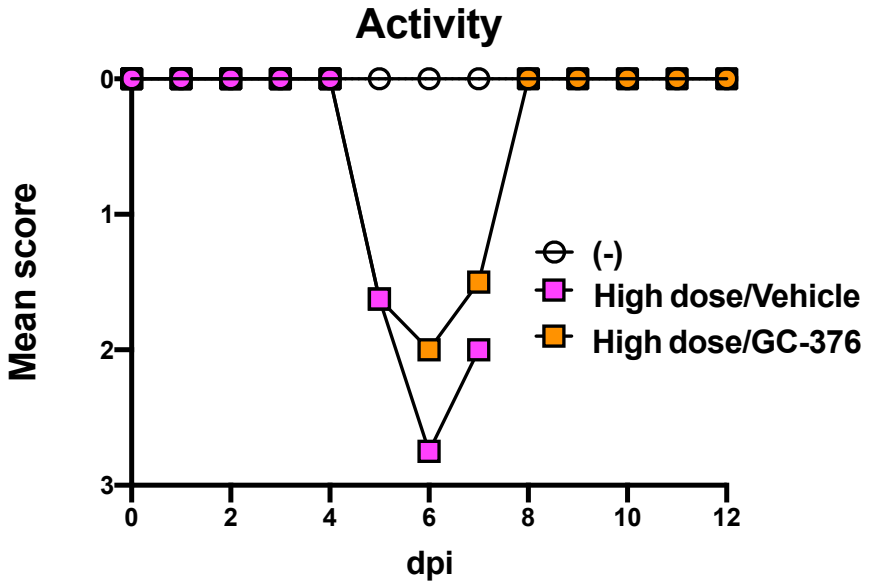
- **Clinical signs (Weight change, physical appearance, activity and survival) was monitored and compared against the mice without antiviral treatment.**

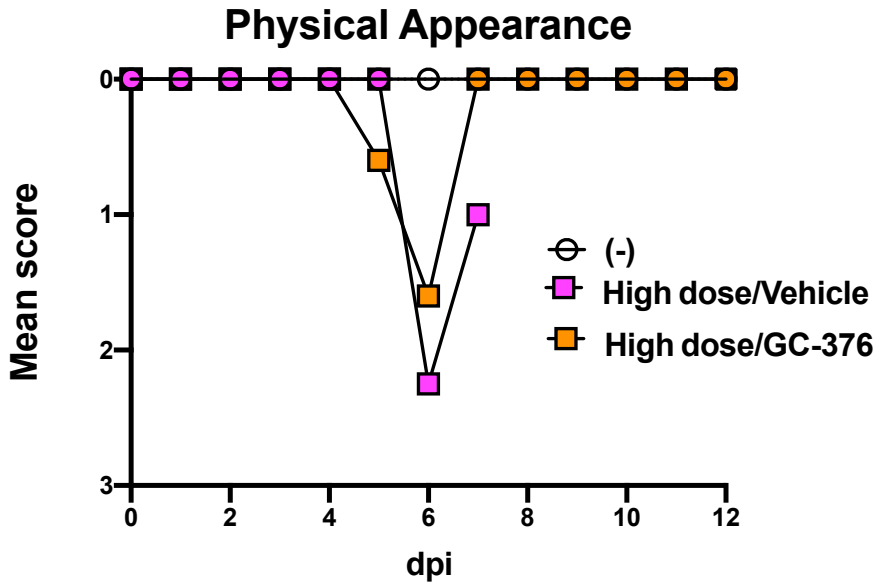
- **No significant differences were observed in the presence of GC-376.**



Marginal effect in clinical signs were observed with GC-376 treatment

- Activity and physical appearance were recorded daily.
- Lethargy and rough coat were observed after SARS-CoV-2 inoculation.
- Slight differences were observed between the mice treated with GC-376 and mice treated with vehicle.
- The results suggest a marginal effect of GC-376 as a SARS-CoV-2 antiviral.





Future directions

- **A subset of mice were euthanized, and tissues were collected at different time points. We are currently evaluating the viral load in those tissues.**

- **We are analyzing the distribution of viral antigens and cellular immune markers by immunohistochemistry.**

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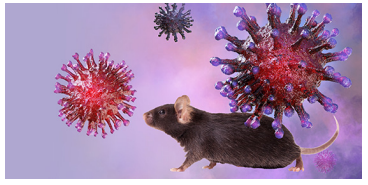
Conclusions

- **K18 hACE2 mice are highly susceptible to SARS-CoV-2 infection. Morbidity and mortality were observed at two different virus challenge doses.**

- **GC-376 did not show signs of toxicity in the K18 hACE2 mice.**

- **Marginal effects of GC-376 in terms of morbidity and survival was observed in K18-hACE2. The results were observed independently of the SARS-CoV-2 doses used.**



Acknowledgments

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