Are Kinases Double-Agents?: Host Kinase Overexpression as a Guide for Antiviral Drug Development

Tesia Bobrowski[1]; Eric Lenarcic[2]; Eugene Muratov[1]; Nathaniel Moorman[2]; Alexander Tropsha[1]
UNC Eshelman School of Pharmacy[1] and Department of Microbiology & Immunology[2], The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

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Background

There are only a handful of small-molecule antiviral medications currently on the market. Many deadly viruses such as Ebola, Nipah, and SARS-CoV-2 lack acute antiviral treatments.

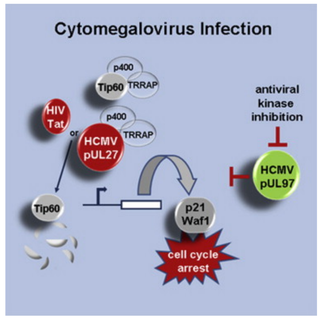

Fig. 1. Downstream effects of inhibiting viral kinase pUL97 in HCMV.

How can we find new antivirals?
We can search for new protein targets for which we can develop small-molecule inhibitors. One of these targets are *kinases*, host proteins involved in cell proliferation, replication, and death.

What is so special about kinases?
Viruses regulate specific kinases when they infect cells to enhance virus replication. By targeting these abnormally expressed kinases, we could modify viral replication in cells.

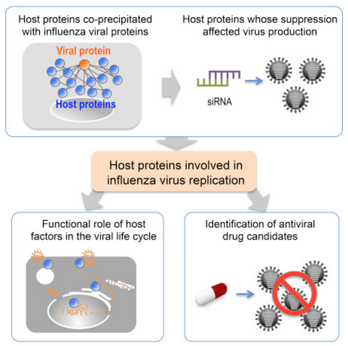

Fig. 2: Workflow for the identification of antiviral drug candidates.

How do we find compounds that target these kinases?
Open-source chemical databases such as ChEMBL and PubChem provide assay data for specific protein targets such as kinases.

We hypothesize that we can curate and streamline this publicly available assay data to identify kinase inhibitors that could function as novel antiviral drug candidates.

Methods

We used published kinome expression profile data on human cytomegalovirus (HCMV)[1] to identify kinases enhanced by virus infection.

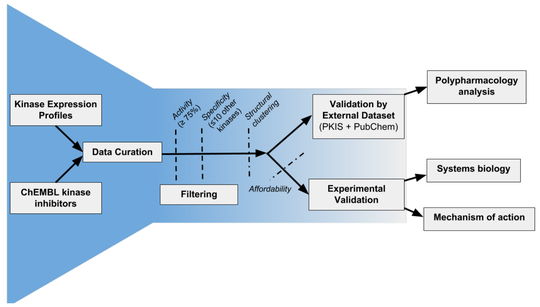

Fig. 3: Workflow of data curation and validation.

1. Identified 75 kinases with statistically significant upregulation
2. All publicly available data for these targets was collected and curated

Hit criteria:
- ≥ 75% inhibition
- active against ≤ 10 other kinases
- Clustered by structure to get wider range of chemotypes for top hits
- Tanimoto similarity to current antivirals, comparison to inhibitors in PKIS1/2

Results

- Experimental validation was performed via a novel flow cytometry-based assay and a fluorescent reporter virus[1]
  - Selection of compounds based on largest % inhibition value, structural diversity, and affordability - chose 11 compounds

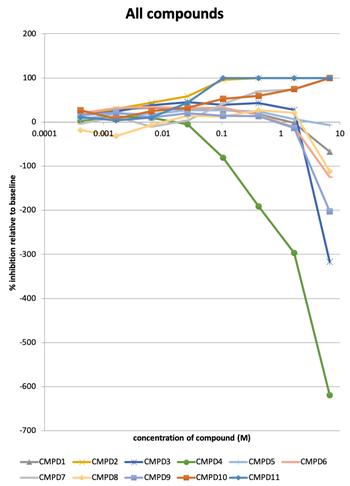

Fig. 4: percent inhibition values obtained over logarithmic range of concentrations for all 11 compounds.

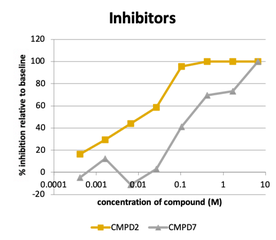

Fig. 5: percent inhibition values obtained over logarithmic range of concentrations for the inhibitory compounds 2 and 7.

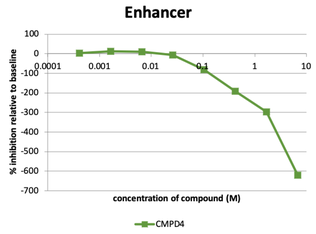

Fig. 6: percent inhibition values obtained over logarithmic range of concentrations for the enhancer identified, compound 4.

Conclusions

The method we developed to identify possible antiviral drug candidates is isotropic: **effective** but **non-directional**, resulting in a collection of inducers and inhibitors of virus replication.

We identified:
- Two inhibitors of HCMV replication (compounds 2 and 7)
- One potent inducer following a dose-response curve (compound 4)

We successfully created a method to identify compounds that influence virus replication.

Future Directions

- Include inhibition metrics other than % inhibition data, such as K_i & IC50, and compare accuracy of prediction
- Pharmacology analysis to identify non-promiscuous compounds that hit multiple targets
- Perform systems biology and mechanism of action studies on the successful enhancer and inhibitors already tested against HCMV
- Automate to make approach easily accessible to experimental scientists

Acknowledgements

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References

[1] Arend, K. C., et. al (2017). *Molecular & Cellular Proteomics*. https://doi.org/10.1074/mcp.m116.065375
Fig. 1. Reitsma, J., Savaryn, J., Faust, K., Sato, H., Halligan, B., & Terhune, S. (2011). Antiviral Inhibition Targeting the HCMV Kinase pUL97 Requires pUL27-Dependent Degradation of Tip60 Acetyltransferase and Cell-Cycle Arrest [Online Image].
Fig. 2. Watanabe, T., et al. (2014). Influenza Virus-Host Interactome Screen as a Platform for Antiviral Drug Development. [Online Image]