ViroStatics' Testing Capabilities to Determine Efficacy of Drugs, Disinfectants, or Devices against SARS-CoV-2 Variants on Surfaces and in Aerosol

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Graphical Abstract

Keywords:

Testing, SARS-CoV-2, drug, compound, natural product, aerosol, variants, efficacy, cytotoxicity, device

Summary:

ViroStatics is a privately held small pharmaceutical company dedicated to discovering and developing novel compounds for a multifaceted treatment of cancers and viral diseases and focuses on novel, selective, host cell kinase targeted inhibitors in its state-of-the-art Biological Safety Level 3 Laboratory. The company has strong expertise in testing drugs, natural substances, disinfectants, or any material for activity against SARS-CoV-2.

Purpose and Rationale

Thanks to its thorough expertise in infectious diseases,^{1,2,3} ViroStatics can test any drug against various strains of SARS-CoV-2 both on surfaces and in aerosol. The purpose of this technical note is to introduce the competencies of the company to potentially interested researchers.

Introduction

ViroStatics is dedicated to discovering and developing novel compounds for a multifaceted treatment of cancers and viral diseases. The team and its advisors combine scientific, business, legal, and technology expertise, spanning the entire process of drug

[†] Technical and commercialization notes convey useful information regarding new technologies, capabilities, business and regulatory issues, intellectual property, nanotechnology law, etc.

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development from translational science through preclinical and clinical drug development.

Thanks to its thorough expertise in infectious diseases,^{1,2,3} ViroStatics can test any drug against various strains of SARS-CoV-2, measuring in cell lines and primary cells at the same time cytotoxicity (through MTS viability assay) and antiviral activity (through cytoprotection assay upon observation of the viral cytopathic effect on the cells or quantification of viral replication with antigen ELISA assay), both on surfaces and in aerosol.

Cytotoxicity determination.

Exponentially growing cells (e.g., Vero E6 cells) are seeded into a 96-well plate at their optimal density in a complete medium. 24 hours later, cells are exposed to different concentrations of drugs in a complete medium (2% FBS) for 72 hours. The cytotoxic effect is then evaluated through MTS colorimetric assay. Cell viability is expressed as the percentage of untreated control. A cytotoxic concentration 50% (CC₅₀) value is calculated through interpolation of the dose-response curves generated by Magellan™ software.

Antiviral activity studies.

Like cytotoxicity experiments, exponentially growing cells are seeded into a 96-well plate at their optimal density in a complete medium, 24 hours later, cells are exposed to different concentrations of drugs, then infected with the virus and cultured for 72 hours. At the end of the incubation period, antiviral activity is examined through both ELISA assay (Sino Biological, quantifying SARS-CoV-2 nucleoprotein) as well as through cytopathic effect observation at the microscope. Antiviral activity is expressed as a percentage of untreated control. An inhibitory concentration 50% (IC₅₀) value is calculated. A selectivity index (SI₅₀=CC₅₀/IC₅₀) can be extrapolated to define the therapeutic window.

We have validated the system testing several drugs already in a clinical setting that demonstrated efficacy against SARS-CoV-2. Figure 1 reports cytotoxicity and antiviral activity dose-response curves for three representative drugs of different classes: remdesivir (nucleoside analogs), darunavir (protease inhibitors), hydroxychloroquine (indirect inhibitors).

Fig 1. Cytotoxicity and antiviral activity curves for representative drugs. Calculated indexes for these drugs are shown in Table 1 below.

Table 1. CC₅₀, IC₅₀, and SI₅₀ values for test drugs.

	CC ₅₀ (μM)	IC ₅₀ (μM)	SI ₅₀
Remdesivir	>100	6	>17
Darunavir	>100	50	>2
Hydroxychloroquine	66	10	6.6

ViroStatics has access to different SARS-CoV-2 strains, including the 2019-nCoV strain 2019-nCoV/Italy-INMI1, SARS-CoV-2-UNIBS-AP66: ERR4145453, and the Alpha, Beta, and Delta variants.

ViroStatics can also test disinfectants – sanitizers – antimicrobial – medical devices – personal care products against SARS-CoV-2. Efficacy of test substance is determined with ASTM (American Society for Testing and Materials, one of the internationally recognized standards organizations) E1052 assay or according to EN14476 guidelines (European standards for chemical disinfectants and antiseptics – quantitative suspension test) assessing the activity of microbicides against the virus in suspension for varying amounts of contact times. Devices with virucidal activity (e.g., UV lamps or filters) can also be tested, either in liquid suspension or bioaerosol. Test in bio-aerosol is of particular relevance as several pieces of evidence support the hypothesis that SARS-CoV-2 is transmitted primarily by the airborne route.⁴

Stock virus with elevated titer ($>10^6$ TCID₅₀/mL) is employed, the viral suspension is exposed to a test agent for varying exposure time. Viral titer reduction is then determined. 10-fold serial dilutions (usually 10^{-1} to 10^{-9}) of the viral suspension is used to infect Vero E6 cell monolayers prepared to suitable confluency in 96-well plates, in 6 replicates for each dilution. Cells are then cultured for 72 hours, and then infection is determined by cytopathic effect quantification at the microscope. Images can be acquired by means of a Leica microscope. The viral titer is then determined according to Reed and Muench method.⁵

Figure 2 shows viral titer reduction according to exposure time variation when employing virucidal agents.

Fig 2. Inhibition of viral titer obtained testing a microbicide or a UV-C device

Other viruses (e.g., HIV) can also be considered for testing, and other experimental systems to study efficacy on different viruses can be implemented upon request.

Discussion

ViroStatics performs studies by employing qualified personnel with documented training and qualification records. All equipment is qualified, maintained, and calibrated as per manufacturer specifications and ViroStatics Standard Operating Procedures, and all records are retained in equipment logs.

Reports for the experimental work are supplied in the form of a final report.

ViroStatics conducts the work under the leadership of a Project Manager. Project Managers have outstanding expertise in a wide range of therapeutic areas and different classes of compounds and offer a comprehensive and

tailored management service to assist the Sponsor with their compound(s) progression through the various stages of development.

To ensure an effective flow of information and rapid resolution of any issues, Project Managers also serve as the primary point of contact for communication between the Sponsor and ViroStatics. They establish and sustain a cohesive and highly effective project team throughout the project lifecycle and oversee the various steps integrated across ViroStatics from initiation through planning, execution, and monitoring until to close out. Project issues that may arise are acknowledged and addressed as early as possible. Lastly, the Project Manager ensures that all aspects of the project are agreed upon and made visible to the Client.

Conclusions

ViroStatics has the background, instrumentation, and the necessary expertise to test drugs, materials, or devices against various strains of SARS-CoV-2 using cell lines or primary cells, both on surfaces and in aerosol, having the capability to work in a BSL3 area according to CDC biosafety guidelines (<https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html>). Further information is available at <http://www.virostatics.com/services/>.

Conflict of Interests

The authors declare that they are employees at ViroStatics. For a signed statement, please contact the journal office: editor@precisionnanomedicine.com

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