Exploring light as a pan-variant therapeutic for COVID-19

Biosafety Considerations of the Biological Light Unit

Carolinas Biological Safety Association 8/10/2023

EmitBio™

Outline

- EmitBio – translating light into life (antiviral therapeutic device)
- Biosafety considerations for the biological light unit
- Development of the biological light unit to evaluate light as an antiviral
- Preclinical data supporting light as an antiviral

EmitBio, Inc.

- Operating subsidiary of KNOW Bio; established in January 2020

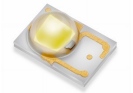
- Team of subject matter experts with decades long track-record of developing and deploying high volume, novel solutions to complex problems in the **life** and **light** science industries.

- Discovered how to use precise, monochromatic wavelengths of **visible** (not UV) light to **eliminate respiratory pathogens** and **stimulate host defense** in the body.

At EmitBio, we believe that everyone exposed to a respiratory pathogen deserves access to a treatment of their choice

Translating Light into Life

LEDs specifically identified for medical use



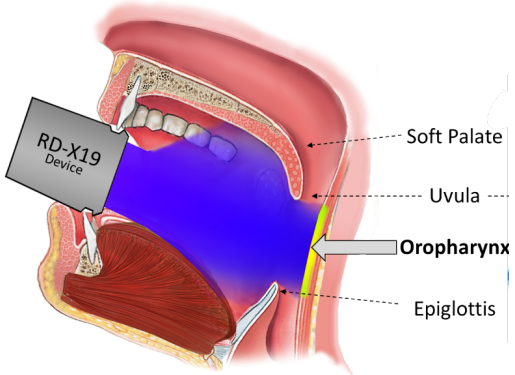
Advanced Development

Biological Efficacy and Safety Screening of UV, Visible & Infrared Light

Precise control of color and energy density

Engineering

Real World, Clinical Solutions



Light & Life Science expertise combined to leverage *in vitro* experiments into immediately deployable safe & effective treatment for COVID-19.

The use of light to inactivate viruses, including CoVs, is a common area of research

Rapid inactivation of SARS-CoV-2 with LED irradiation of visible spectrum wavelengths

Riccardo De Santis [a], Vincenzo Luca [a,b], Jonas Näslund [e], Rosina K. Ehmann [f], Marta De Angelis [d], Eva Lundmark [e], Lucia Nencioni [d], Giovanni Faggioni [a], Silvia Fillo [a], Donatella Amatore [a], Elisa Regalbuto [a], Filippo Molinari [a], Giancarlo Petralito [a], Roman Wölfel [f], Paola Stefanelli [c], Gianni Rezza [c], Anna Teresa Palamara [d], Markus Antwerpen [f], Mats Forsman [e], Florigio Lista [a,*]

[a] Scientific Department, Army Medical Center, Rome, Italy
[b] 7th CBRN Defence Regiment "Cremona", Civitavecchia, Italy
[c] Department of Infectious Disease, National Institute of Health, Rome, Italy
[d] Department of Public Health and Infectious Diseases, Laboratory Affiliated to Pasteur Italia-Fondazione Cenci Bolognetti, "Sapienza" University of Rome, Italy
[e] Department of CBRN Protection and Security, Swedish Defence Research Agency (FOI), Umeå, Sweden
[f] Section Viral and Intracellular Pathogens, Bundeswehr Institute of Microbiology, Munich, Germany

Efficacy and hazards of 425 nm oral cavity light dosing to inactivate SARS-CoV-2

Max A. Stockslager *, Jacob F. Kocher, Leslee Arwood, Nathan Stasko, Rebecca A. McDonald, Mark A. Tapsak, David Emerson

EmitBio Inc., 4222 Emperor Blvd, Suite 470, Durham, NC 27703, United States

Photodynamic Inactivation of Human Coronaviruses

Brett A. Duguay [1], Adrian Herod [1], Eric S. Pringle [1], Susan M. A. Monro [2], Marc Hetu [2], Colin G. Cameron [2,3], Sherri A. McFarland [2,3,*] and Craig McCormick [1,*]

Review of Virus Inactivation by Visible Light

Martin Hessling *, Bernhard Lau and Petra Vatter

A 265-Nanometer High-Power Deep-UV Light-Emitting Diode Rapidly Inactivates SARS-CoV-2 Aerosols

Hiroshi Ueki [a,b], Mutsumi Ito [a], Yuri Furusawa [a,c], Seiya Yamayoshi [a,b], Shin-ichiro Inoue [d], Yoshihiro Kawaoka [a,b,e,f]

The virucidal effects of 405 nm visible light on SARS-CoV-2 and influenza A virus

Raveen Rathnasinghe [1,2,3], Sonia Jangra [1,2], Lisa Miorin [1,2], Michael Schotsaert [1,2], Clifford Yahnke [6] & Adolfo García-Sastre [1,2,4,5]

Efficient Inactivation of SARS-CoV-2 and Other RNA or DNA Viruses with Blue LED Light

Chiara Terrosi [1], Gabriele Anichini [1], Jean Denis Docquier [1], Gianni Gori Savellini [1], Claudia Gandolfo [1], Francesco Saverio Pavone [2] and Maria Grazia Cusi [1,*]

Viral inactivation by light

Mohammad Sadraeian, Le Zhang, Farzaneh Aavani, Esmaeil Biazar & Dayong Jin

Direct inactivation of SARS-CoV-2 by low level blue photobiomodulation LED at 470, 454 and 450 nm

Luisa Zupin, Rossella Gratton, Margherita Milani, Libera Clemente, Francesco Fontana, Maurizio Ruscio, Sergio Crovella

Visible light to treat (respiratory) viral infections is a novel concept

Visible blue light inhibits infection and replication of SARS-CoV-2 at doses that are well-tolerated by human respiratory tissue

Nathan Stasko[1], Jacob F. Kocher[1], Abigail Annas[1], Ibrahim Henson[1], Theresa S. Seitz[2], Joy M. Miller[2], Leslee Arwood[1], Rachel C. Roberts[1], Thomas M. Womble[1], Emily G. Keller[1], Soren Emerson[1], Michael Bergmann[1], Ashley N. Y. Sheesley[3], Rebecca J. Strong[3], Brett L. Hurst[3], David Emerson[1], E. Bart Tarbet[3], Shelton S. Bradrick[2] & Adam S. Cockrell[1]

Visible blue light inactivates SARS-CoV-2 variants and inhibits Delta replication in differentiated human airway epithelia

Jacob Kocher, Leslee Arwood, Rachel C. Roberts, Ibrahim Henson, Abigail Annas, David Emerson, Nathan Stasko, M. Leslie Fulcher, Marisa Brotton, Scott H. Randell, Adam S. Cockrell

A randomized, controlled, feasibility study of RD-X19 in subjects with mild-to-moderate COVID-19 in the outpatient setting

Nathan Stasko[1] | Adam S. Cockrell[1] | Jacob F. Kocher[1] | Ibrahim Henson[1] | David Emerson[1] | Ye Wang[2] | Jonathan R. Smith[3] | Nathan H. Henderson[4] | Hillary Wood[4] | Shelton S. Bradrick[4] | Terry Jones[5] | Jorge Santander[6] | John G. McNeil[1]

Blue photobiomodulation LED therapy impacts SARS-CoV-2 by limiting its replication in Vero cells

Luisa Zupin[1*] | Rossella Gratton[1] | Francesco Fontana[2] | Libera Clemente[2] | Lorella Pascolo[3] | Maurizio Ruscio[2] | Sergio Crovella[4]

Biosafety considerations for the biological light unit

The Biological Light Unit is designed to…

- Deliver accurate, precise, and uniform doses of light
- Be capable of evaluating safety, efficacy, and biological mechanisms
- Be reproducible, transportable, and biosafety-compliant

- Biosafety considerations:
  - Needs to be transportable within BSL-3 facilities
  - Needs to be able to be decontaminated for removal from BSC and BSL-3
  - Needs to be able to prevent sample overheating while also eliminating aerosol generation
  - Needs to be able to minimize operator error during use

Consideration: transportable in BSL-2/3 labs

- The biological light unit is used in BSL-2/3 facilities with respiratory pathogens.
- Possible risks:
  - As a complete functioning unit, the biological light unit could be cumbersome to transport into and out of the BSC.
  - Slippery/slick materials could cause drops
- Solutions:
  - The biological light unit comes in 3 separate components that are assembled prior to use and disassembled following use.
  - The biological light unit remains in the BSC until completion. The BLU is not moved while test articles are within the BLU.
  - The biological light unit is compact enough to fit within the BSC.
  - The biological light unit includes a 3-D printed biobox with easy grab handles to limit potential drops.

Consideration: decontamination

- The Biological Light Unit (except the power supply) is utilized within the BSC.

- Possible risks:
  - Components must withstand surface decontamination to be removed from the BSC.
  - Removal of the BLU from the BSL-3.

- Solutions:
  - The light used by the BLU itself is capable of inactivating virus.
  - All BLU components were designed to undergo 70% ethanol decontamination and VHP.
  - BLU components can be separated to ensure all surfaces are decontaminated.

Consideration: prevention of overheating without aerosols

- The Biological Light Unit requires a fan to prevent overheating of the test article

- Potential risks
  - Air from the fan if blown across the test article could lead to the aerosolization of test article
  - Standalone fan could fall over onto the test article

- Solutions
  - BLU is compact enough to fit in the BSC. No illuminations, and thus no use of the fan, occur outside the BSC.
  - BLU is calibrated to deliver desired light dose through tissue culture plate lid so air from the fan does not disturb test article.
  - Biobox contains a holder that retains the fan to the BLU.

Consideration: power supply connections to LED array

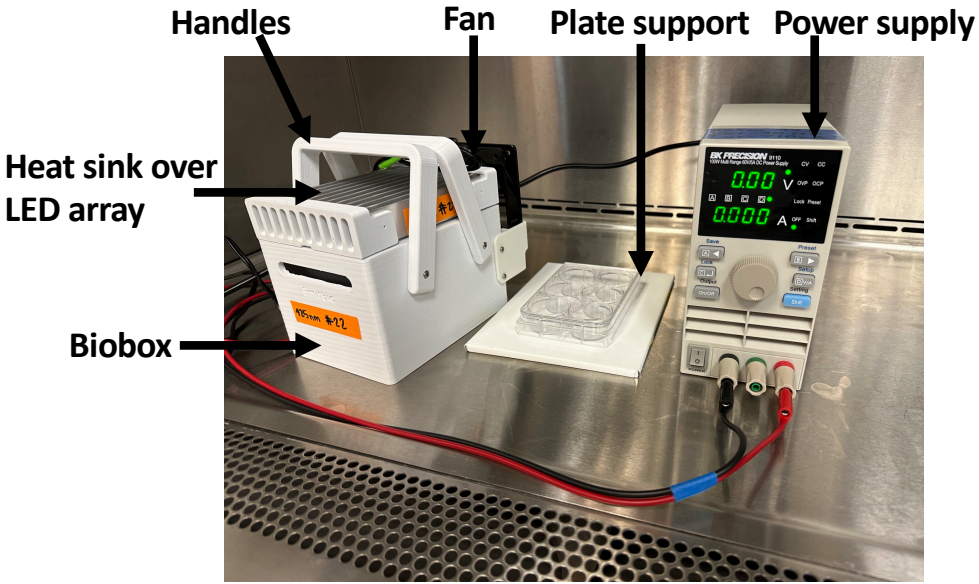
- The power supply sits outside the BSC and is connected to the LED array via leads

- Potential risks:
  - Leads for the power supply connection must withstand surface decontamination with 70% ethanol.
  - Leads could become tangled or snagged by the operator.

- Solutions:
  - No work is conducted within the BSC during illuminations. Work resumes only after the BLUs are removed from the BSC.
  - Designed and implemented longer leads that can run along the outer edge of the BSC to minimize potential snags during operator movements while changing test articles.

Using the Biological Light Unit to develop a novel therapeutic medical device

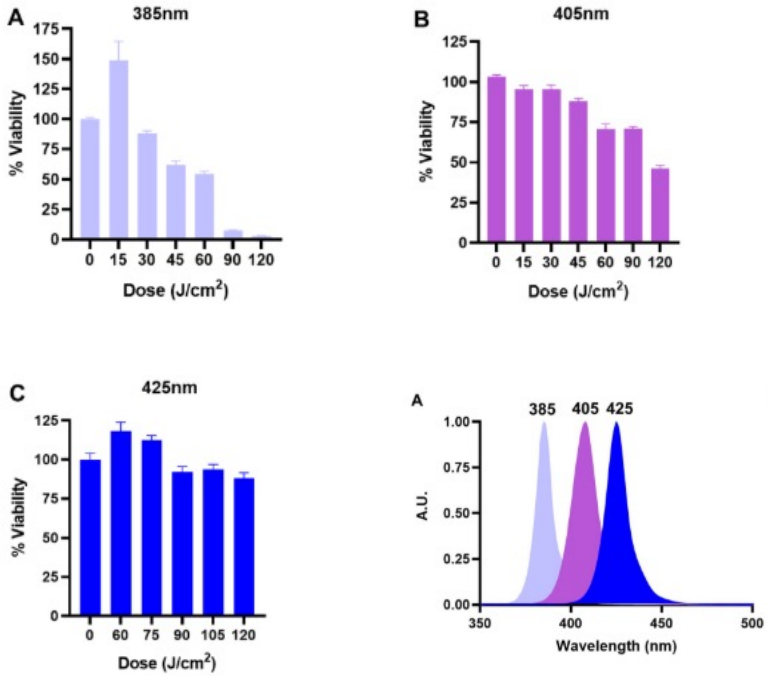
The Biological Light Unit (BLU) is reproducible, transportable, and biosafety-compliant



*For illustration purposes (power supply sits outside BSC)

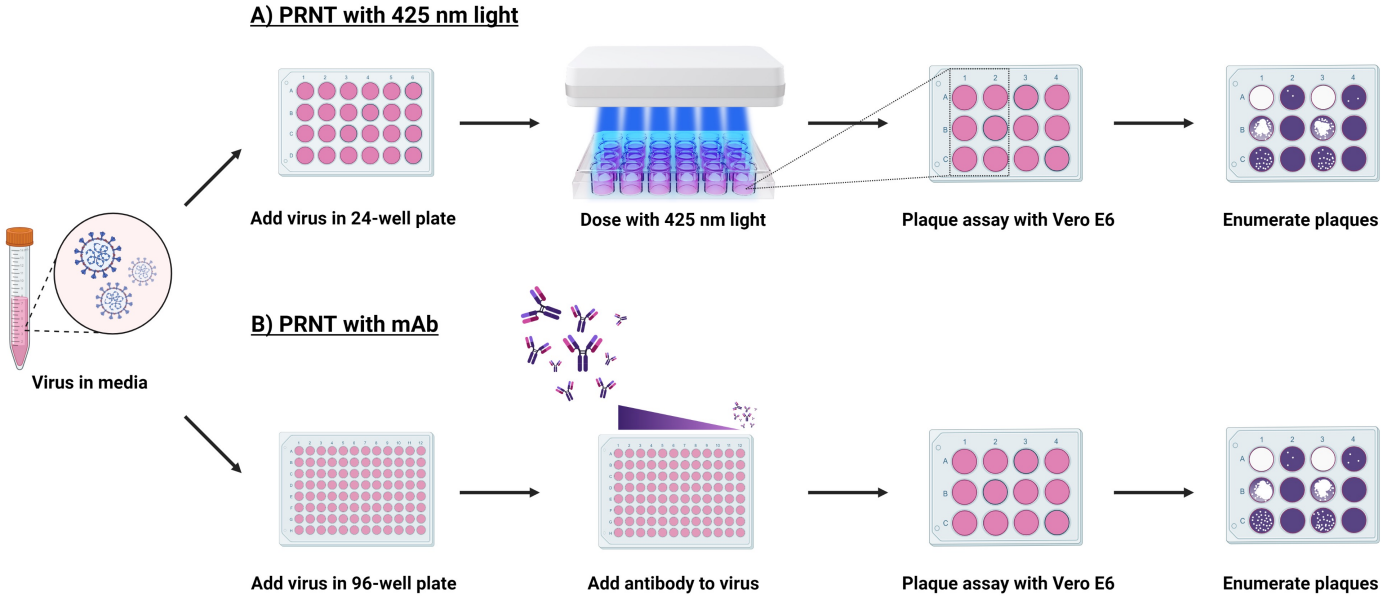
- Easy to use
  - Place plate support on BSC surface
  - Place test article on plate support
  - Center biobox over test article
  - Turn on the power supply
- Fan attached to prevent overheating of sample
  - Plate lid remains on throughout dose
- Designed to accurately deliver dose through tissue culture lid

Cytotoxicity testing of AIR-100 tissues with BLU to pick the proper wavelength

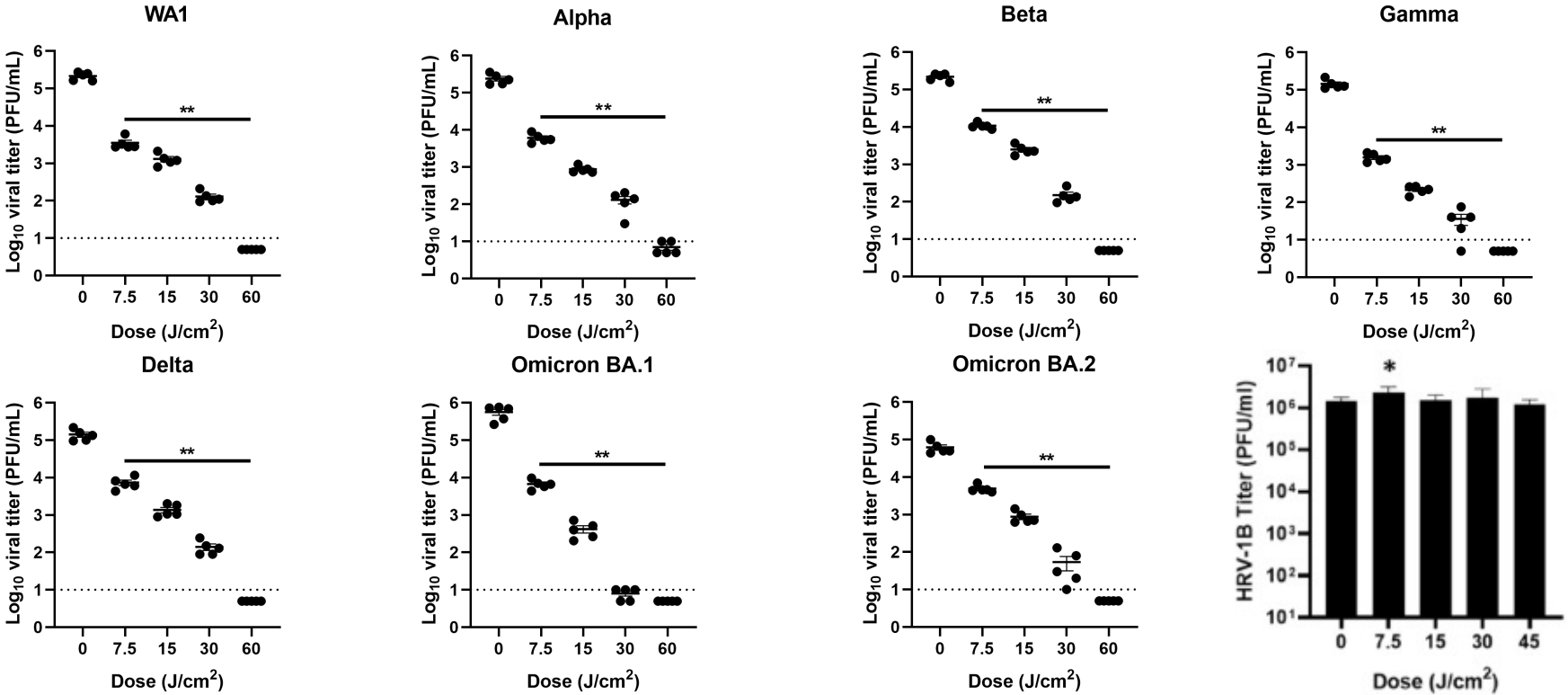


- MatTek AIR-100 tissues dosed with varying wavelengths and doses of light
- Cytotoxicity evaluated 3 hours post-illumination
- Visible light cytotoxicity is wavelength-dependent and dose-dependent
  - Moving forward discussing 425 nm

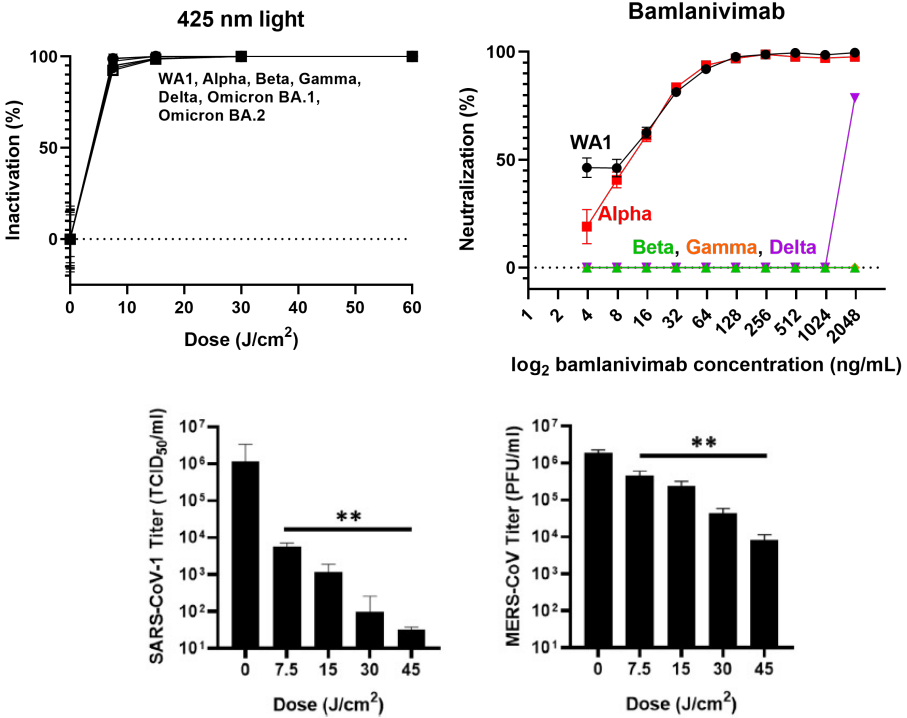
BLU allows for the development of comparable assays to those of monoclonal antibodies and



425 nm light inactivates all SARS-CoV-2 variants of concern, but not human rhinovirus

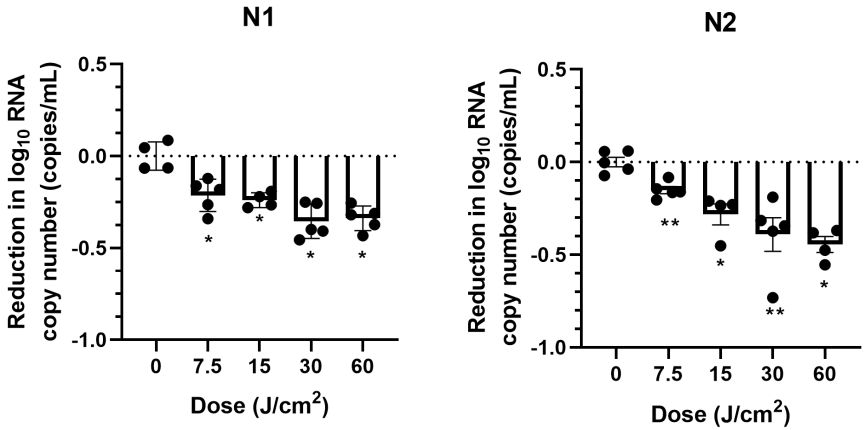


Side-by-side comparison reveals that 425 nm light inactivates variants that monoclonal antibodies cannot



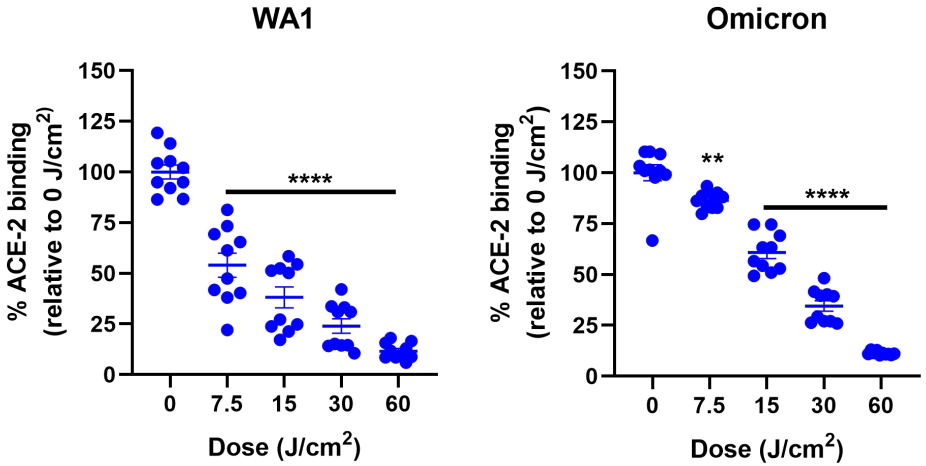
- 425 nm light demonstrates consistent inactivation of SARS-CoV-2 variants regardless of mutations acquired
- Bamlanivimab does not neutralize Beta, Gamma, or Delta variants
  - Also limited efficacy against Omicron variants
- 425 nm light also inactivates SARS-CoV-1 and MERS-CoV

425 nm inactivation of SARS-CoV-2 does not significantly reduce genomic RNA



- Conducted real-time PCR on SARS-CoV-2 suspensions illuminated with 425 nm light
- Saw statistically significant reductions in genomic RNA, but not to sufficient levels to inactivate whole virus suspensions
- 425 nm light does impact viral RNA, but not enough to inactivate virus

425 nm light reduces SARS-CoV-2 spike binding to ACE-2 *in vitro*

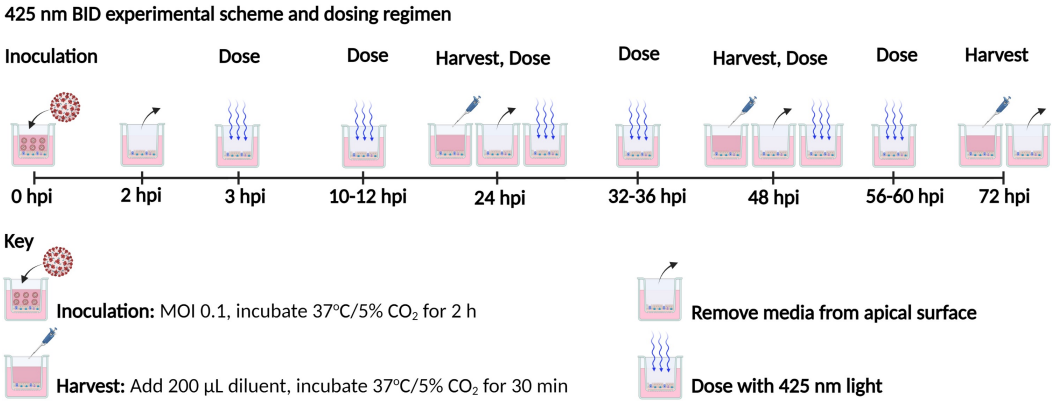


- Illuminated spike trimers and then assessed binding to ACE-2
- Dose-dependent reduction in spike binding to ACE-2 *in vitro*
- Reductions consistent across multiple variants, including those heavily mutated in RBD
- 425 nm light inhibits spike binding to ACE-2

Summary

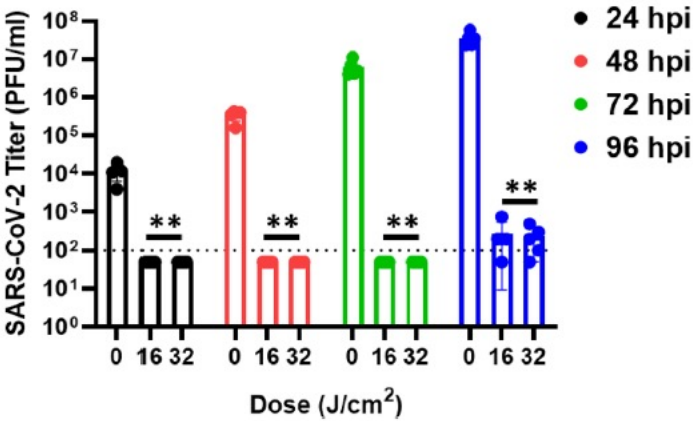
- EmitBio has implemented biosafety guidelines in developing a preclinical platform to evaluate the antiviral capabilities of light.
- These studies informed the development of the EmitBio RD-X19, an investigational medical device to treat COVID-19.
- Light inactivates SARS-CoV-2 by inhibiting spike binding to ACE-2.
- These studies did not evaluate potential host effects (e.g. ROS) and their role in inhibiting SARS-CoV-2 replication.

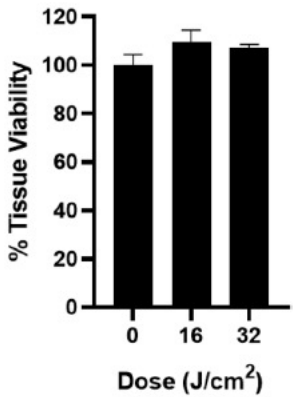
Using 425 nm light to reduce viral titers in a well-differentiated model of the human airway



- Primary tracheobronchial cells cultured at the air-liquid interface differentiate and mimic airway *in vivo*
  - Cilia beating
  - Mucus production
- Model used for preclinical testing of:
  - Remdesivir (Veklury)
  - Nirmatrelvir/ritonavir (Paxlovid)
  - Molnupiravir (Lagevrio)

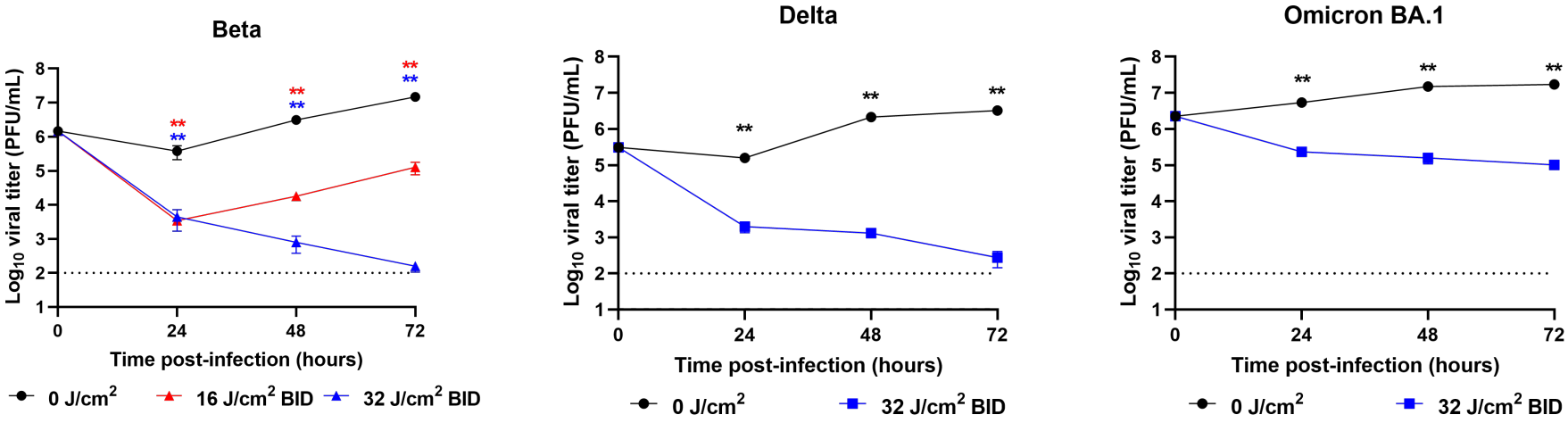
Non-cytotoxic doses of 425 nm light reduces SARS-CoV-2 WA1 viral titers in models of the human airway





- Low and undetectable viral titers at each timepoint tested following twice daily dosing with 425 nm light
- No cytotoxicity observed in time-matched, uninfected inserts
- Thus, 425 nm light has antiviral capability at non-cytotoxic doses

Same dosing regimen retains antiviral capability against SARS-CoV-2 VOCs



- Similar dose responses observed with Alpha and Gamma VoCs