Creating a vaccine for the tick-borne Powassan Virus

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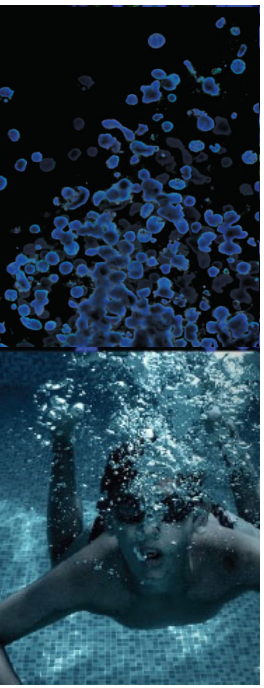
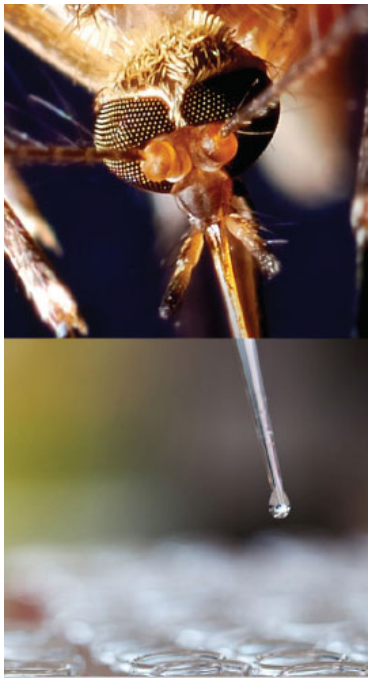
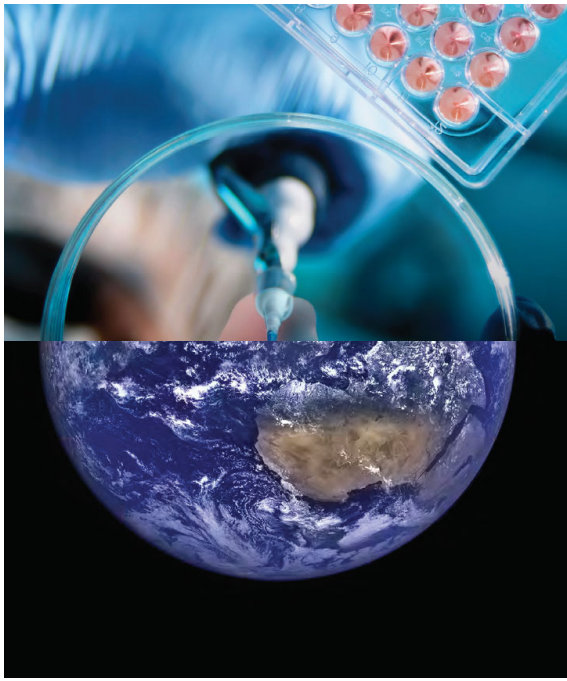
Scientist, ATCC

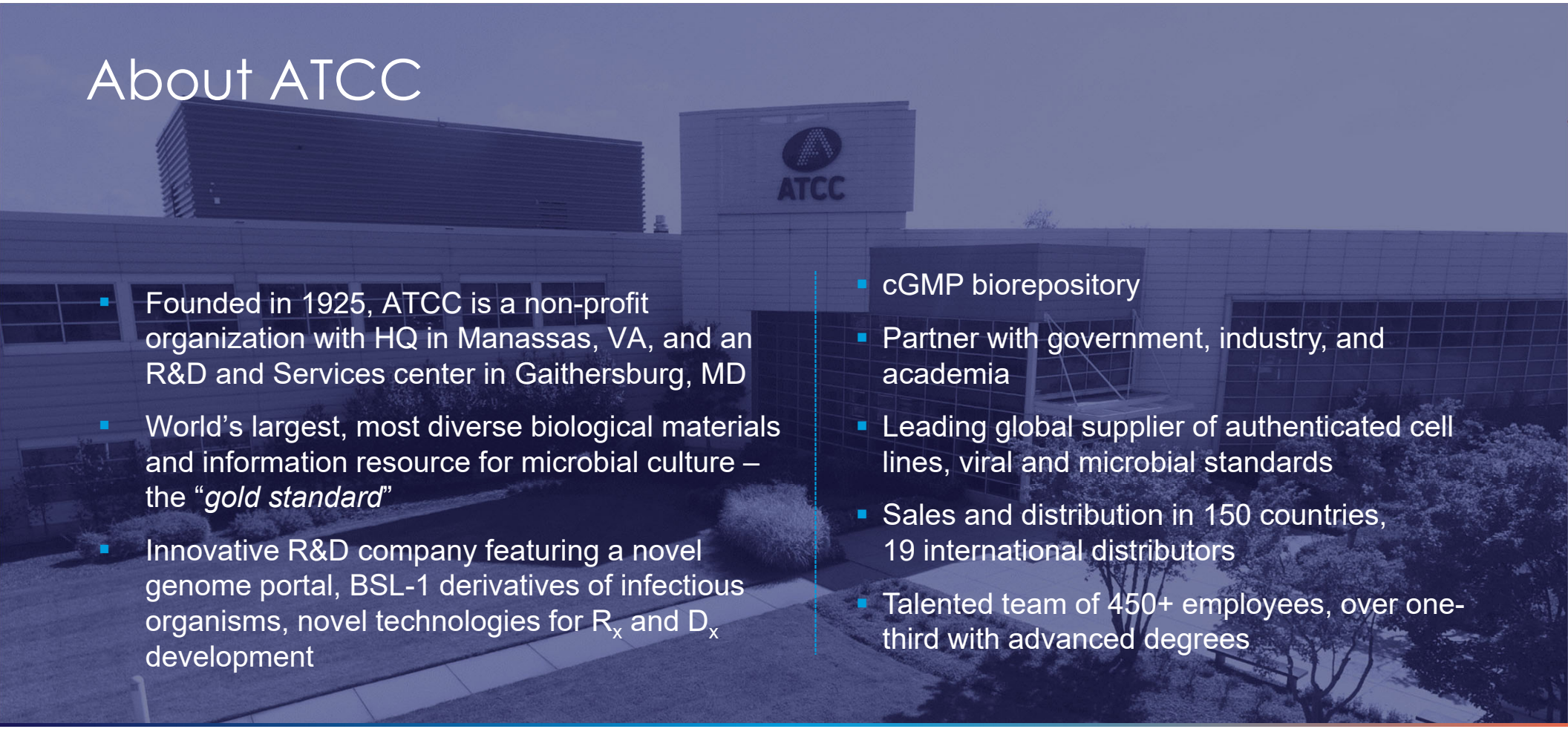
Credible Leads to Incredible™







About ATCC



- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbial culture – the "*gold standard*"
- Innovative R&D company featuring a novel genome portal, BSL-1 derivatives of infectious organisms, novel technologies for R_x and D_x development
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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Agenda

- Introduction
- Scientific approach
- Results
- Summary

https://www.cdc.gov/ticks/gallery/index.html

Powassan Virus

An emerging global infection

- Flavivirus like Zika virus and dengue virus
- Transmitted by the tick species *Ixodes scapularis* and *Ixodes cookei*
- Circulating in North America and the Russian Far East
- Causes encephalitis, meningitis, or encephalomeningitis
- Medical treatment available is only supportive
- There is not an FDA-approved diagnostic test
- Increase in the number of infections in the U.S. in the last decade

https://www.cdc.gov/powassan/index.html

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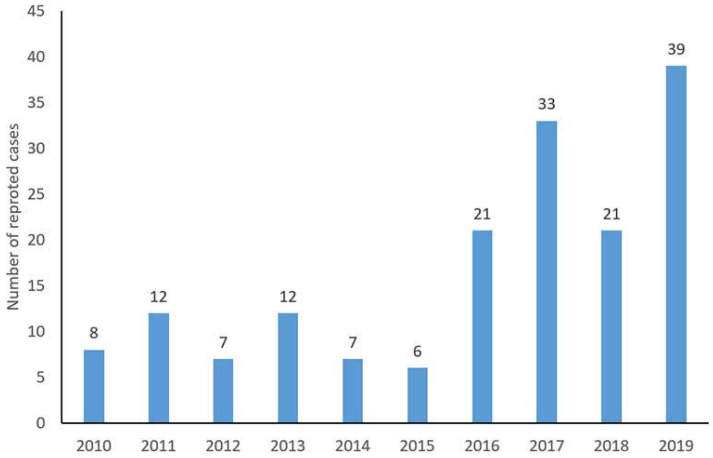
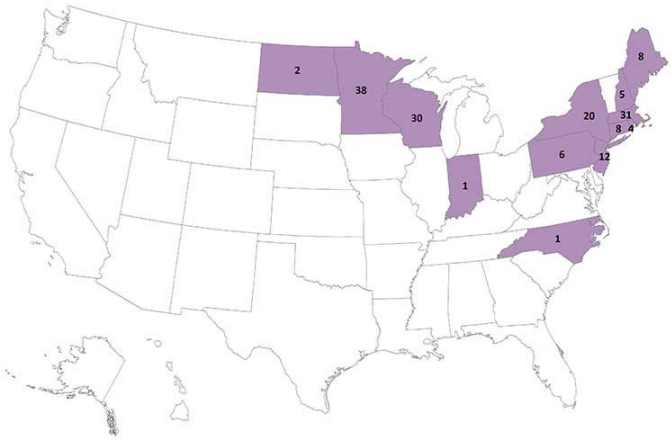
Powassan Virus Statistics (2010-2019)

Powassan and Emerging Infection in the US

Powassan virus neuroinvasive disease cases reported by year



Powassan virus neuroinvasive disease cases reported by state



https://www.cdc.gov/powassan/statistics.html

Factors important in the increase of Powassan infections:

- Increase in tick population due to global warming
- Reforestation, urban sprawl, and higher human density in rural area
- Possible adaptation of POWV in the tick vector

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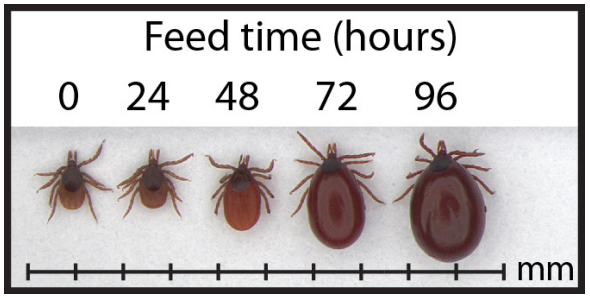
Ixodes scapularis main vector of Powassan Virus

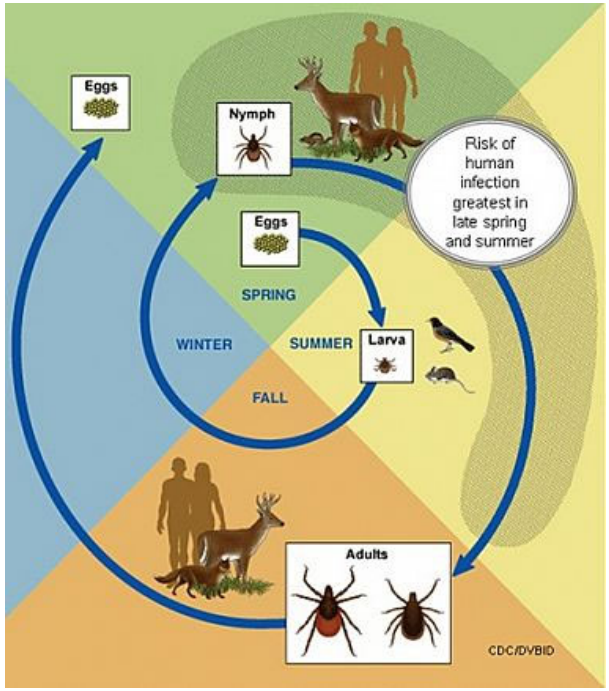
The tick vector population expansion in US

Ixodes scapularis, also called blacklegged tick, is present in the eastern part of U.S.

Stages of tick engorgement



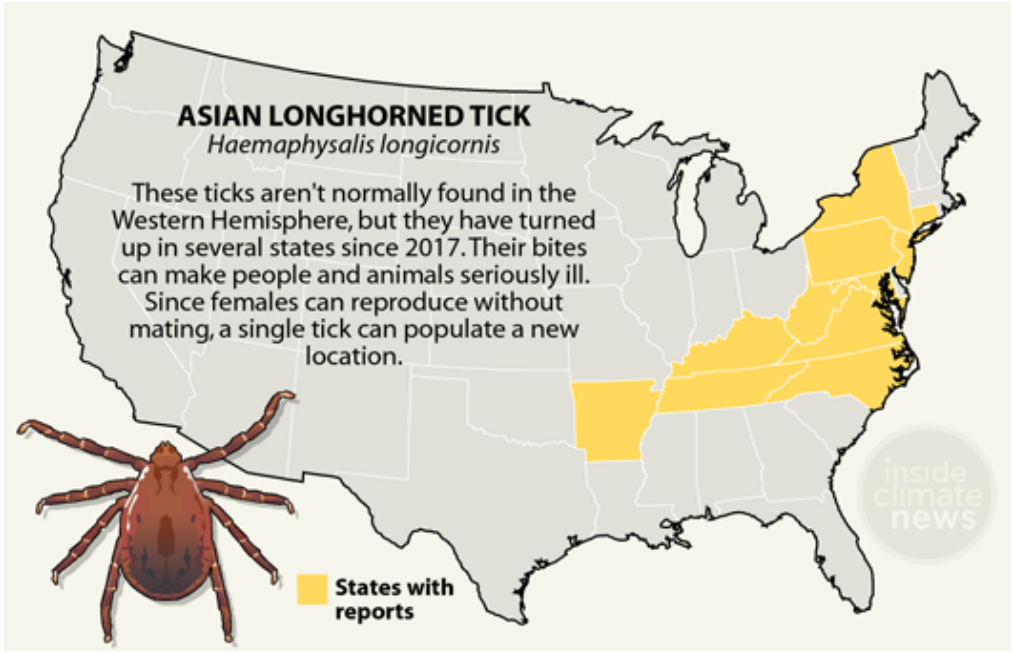
Ixodes scapularis has a life cycle divided in 4 stages



https://www.cdc.gov/ticks/surveillance/index.html

Asian Longhorned Ticks: A Potential New Vector

Asian longhorned ticks have spread to the U.S. and can potentially transmit Powassan virus



SOURCE: Centers for Disease Control and Prevention PAUL HORN / InsideClimate News

https://insideclimatenews.org/news/05072019/tick-disease-danger-species-longhorned-lonestar-climate-change/

https://www.cdc.gov/ticks/longhorned-tick/index.html

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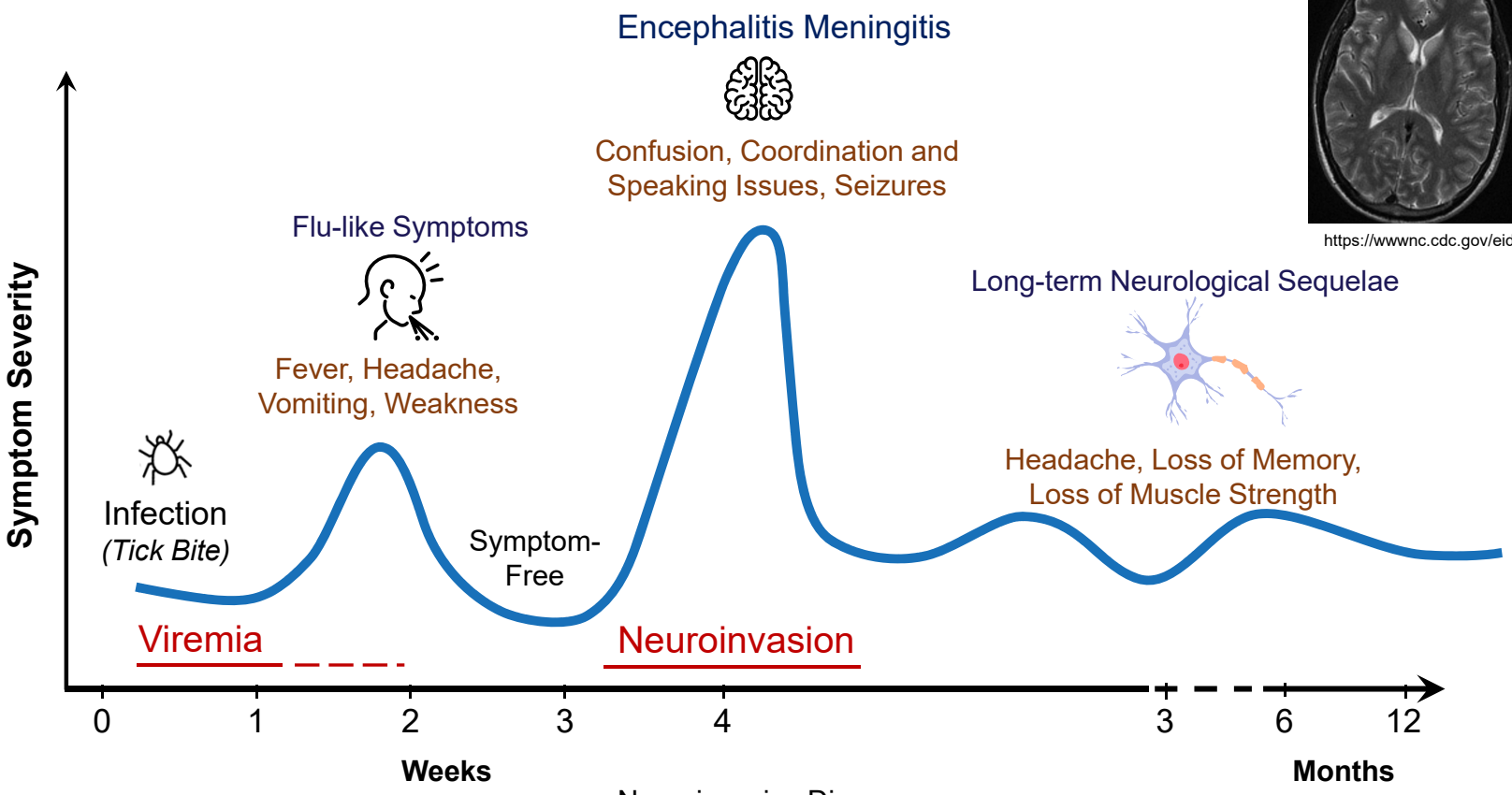
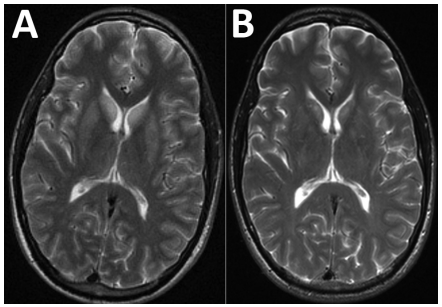
Powassan Virus Disease

Powassan virus-induced encephalitis and meningoencephalitis





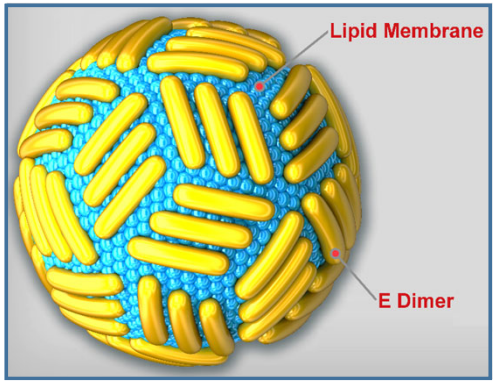
https://wwwnc.cdc.gov/eid/article/25/10/18-1262-f1

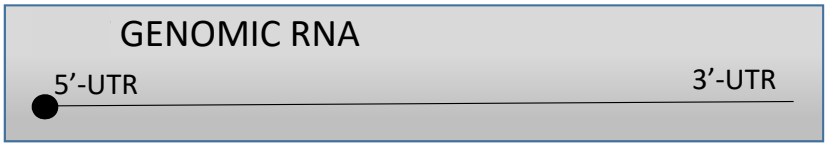
Neuroinvasive Disease:
~12% Mortality Rate

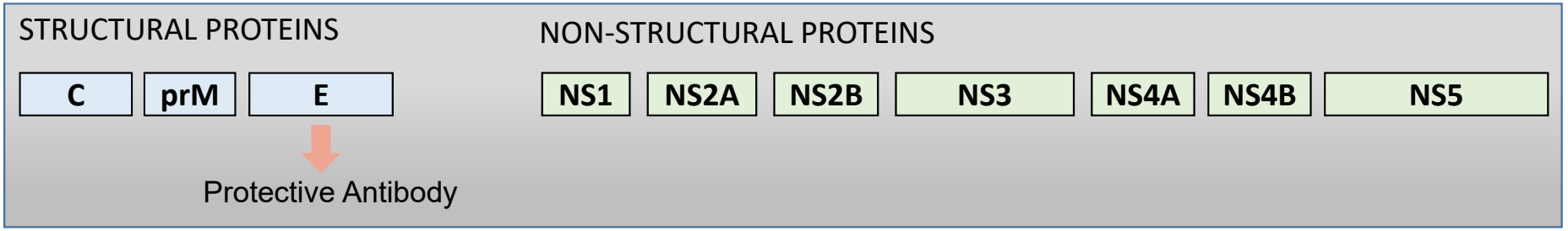
Powassan Virus Organization

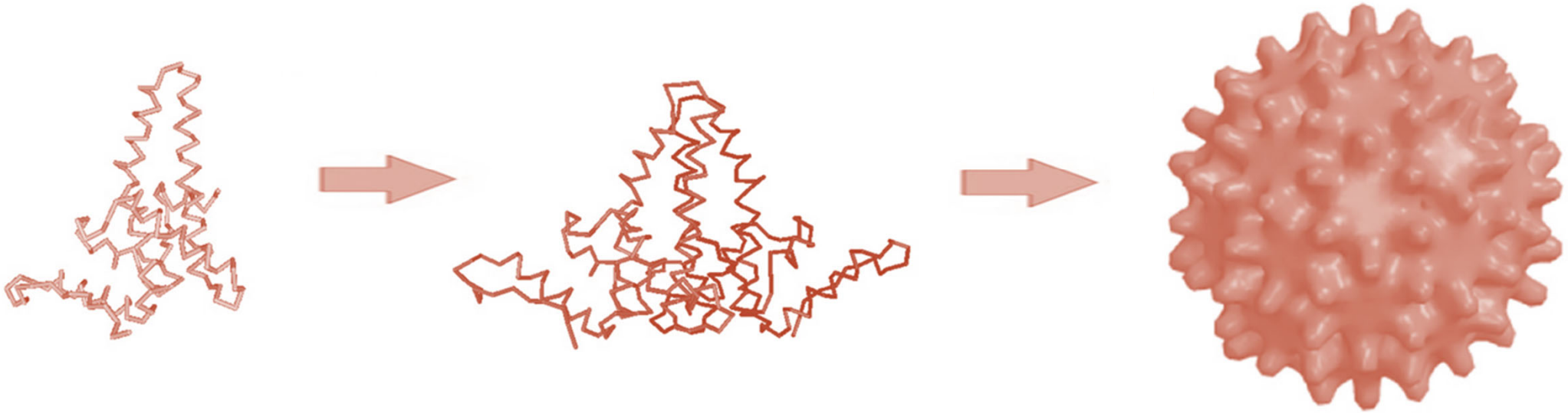
Powassan virus genome

- Powassan virus genome is a single-stranded positive RNA (10.7 kb)
- Powassan genome codes a polyprotein that is cleaved in structural and non-structural proteins
- Envelope (E) protein is the main Powassan immunogen









Scientific Approach

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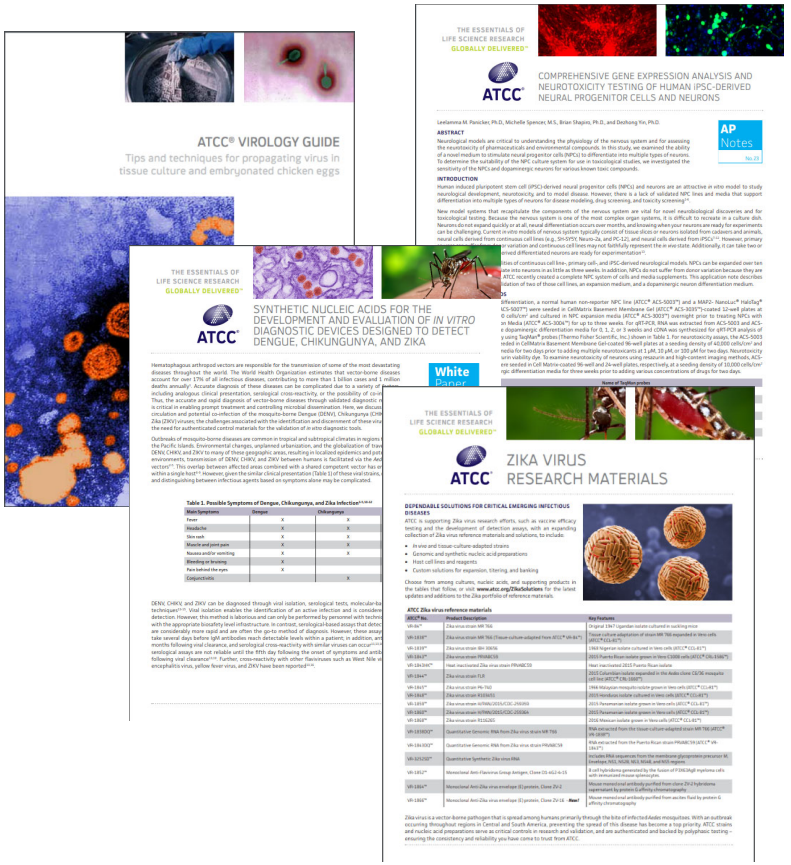
ATCC Powassan Resources

ATCC® No.	Product Description
VR-1262™	Powassan virus LB strain
VR-3273SD™	Quantitative Synthetic Powassan virus lineage I RNA
VR-3275SD™	Quantitative Synthetic Powassan virus lineage II RNA
VR-1262AF™	Powassan virus immune ascitic fluid [V-518-711-562]
VR-1262CAF™	Powassan virus control ascitic fluids
CRL-11973™	Tick cell line, IDE8
CRL-11974™	Tick cell line, ISE6

www.atcc.org/vectorborne



Principles for Vaccine Design

Strategies for developing a novel, efficacious, and safe Powassan vaccine

Virus-like particle (VLP) technology for delivering highly immunogenic Powassan antigens

Non-invasive Powassan-specific:

- Protection from vector-borne transmission
- Sterilizing immunity
- High level of safety and tolerability
- Immunogenicity in immunocompromised
- Stable for standard storage and distribution
- Easy to manufacture

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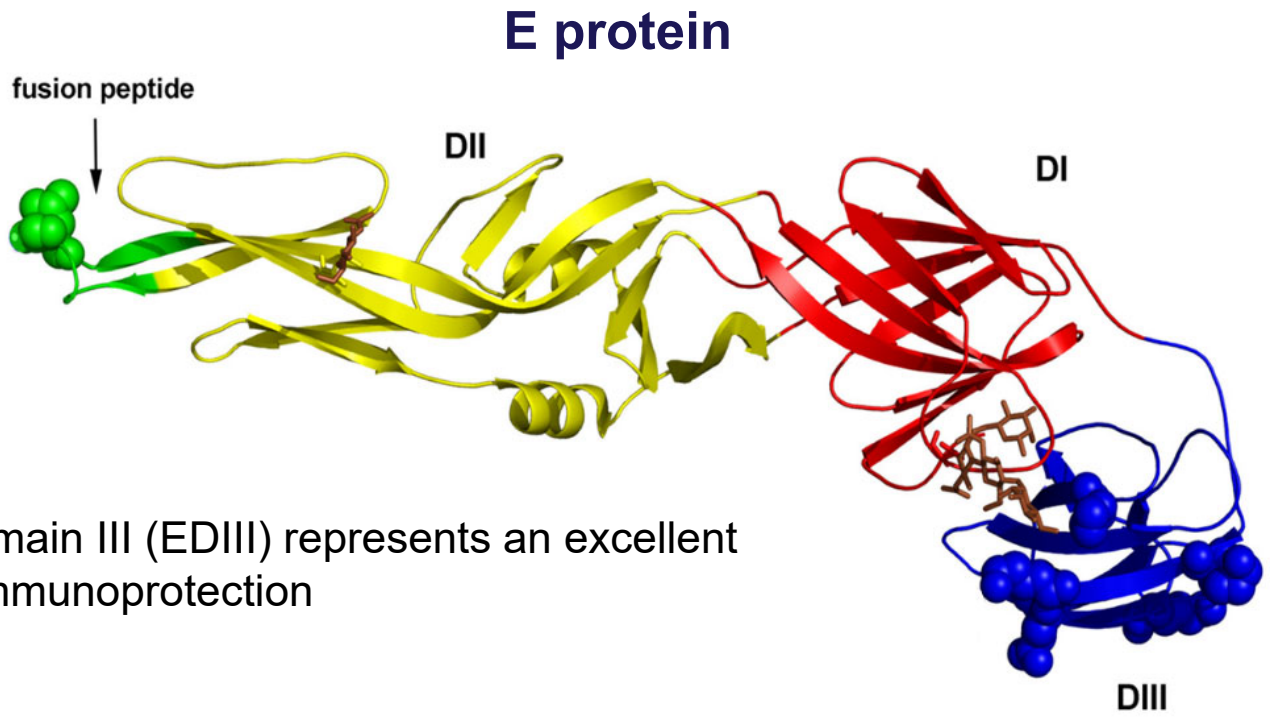
Rational Design

Aims to improve vaccine immunogenicity, specificity, and inter-strain cross-protection



E protein Domain III (EDIII) represents an excellent antigen for immunoprotection

Crlll WD, et al. PLoS One. 4(4):e4991, 2009. doi: 10.1371/journal.pone.0004991

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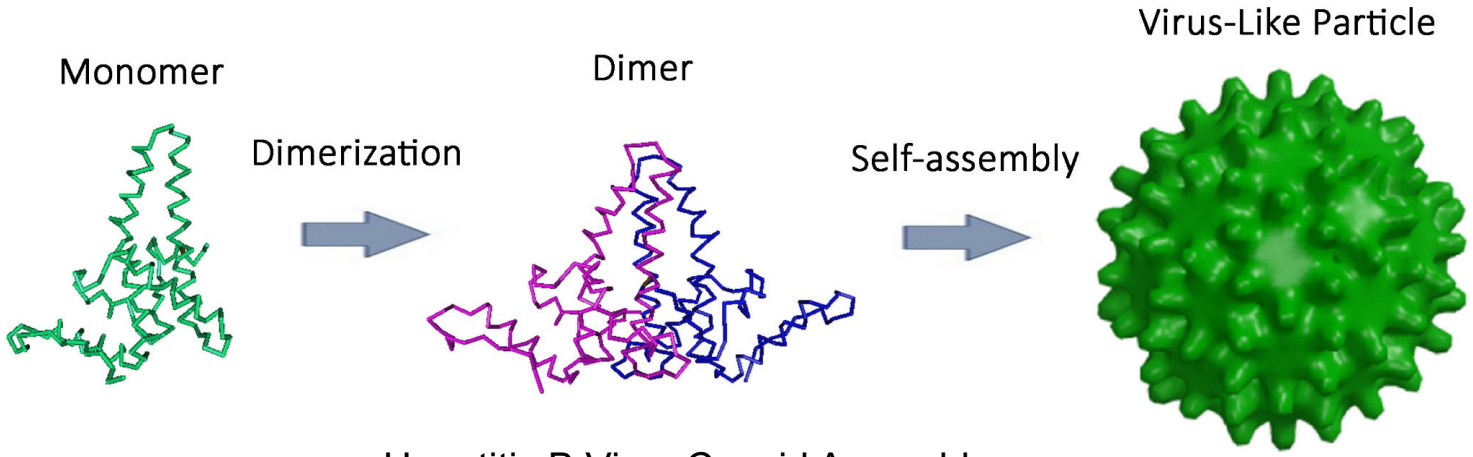
Virus-like Particle (VLP) Technology

VLP technology demonstrated a strong impact in vaccinology

- Multimeric assembly of viral protein in 20 to 200 nm particle diameter
- Highly immunogenic for mimicking viral morphology
- Very safe because of the lack of virus genetic material
- FDA-approved vaccines against Hepatitis B virus and Human papillomavirus



Hepatitis B Virus Capsid Assembly

Adapted from Peyret H, et al. PLoS One 10(4), 2015. doi:10.1371/journal.pone.0120751

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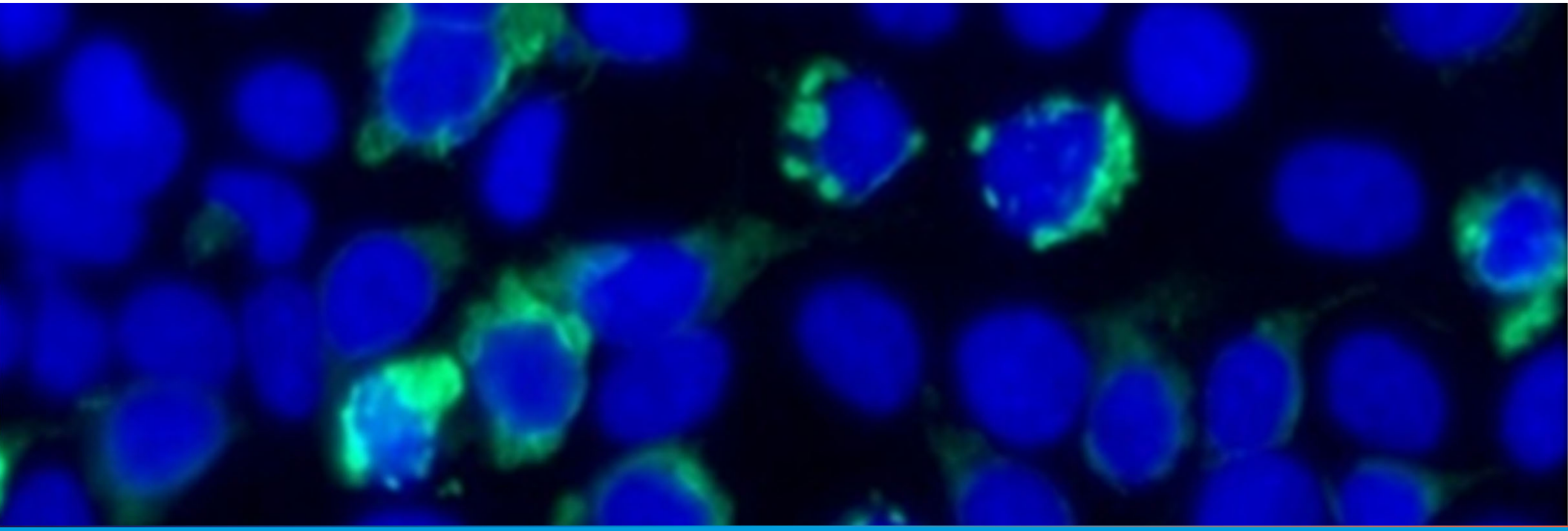
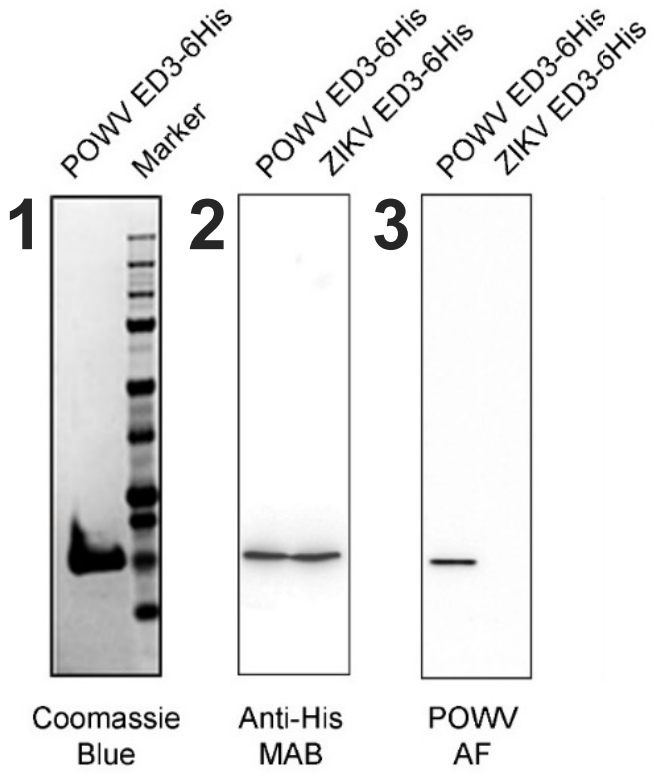


Results

POW-EDIII Recombinant Protein Standard

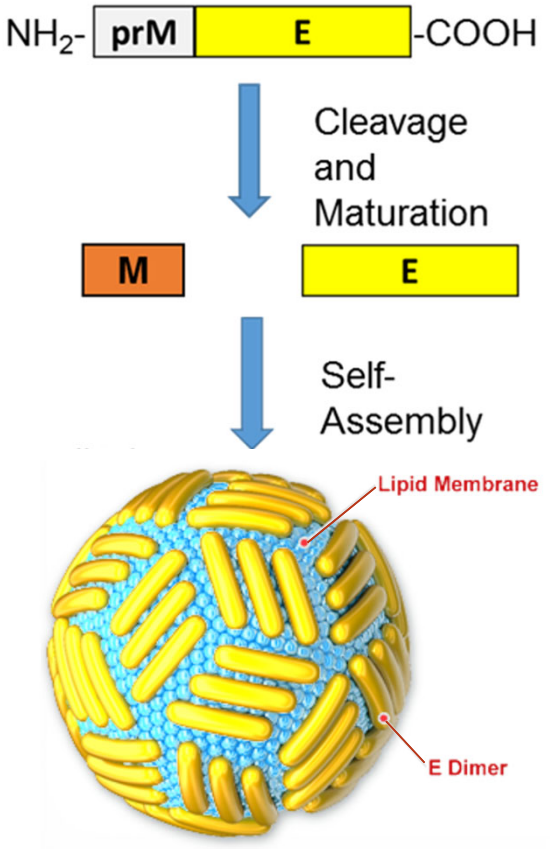
POW-EDIII testing by biological and immunological assays



- POW-EDIII was produced in yeast and purified using chromatography
- SDS-PAGE Coomassie staining demonstrate high purity of POW-EDIII protein (Panel 1)
- POW-EDIII reacted with anti-histidine tag antibody and migrated with similar size of Zika-EDIII recombinant protein standard (Panel 2)
- POW-EDIII reacted specifically with Powassan monoclonal antibodies (Panel 3)

Design of POW-VLP

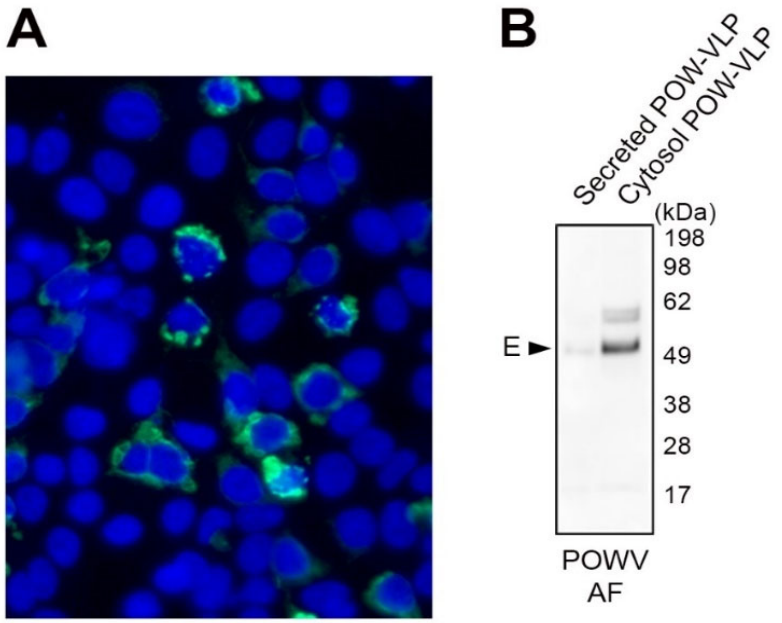
Powassan VLPs were generated using expression of structural proteins prM-E



prM-E polyprotein is cleaved by host proteases and self-assemble in VLPs

Production of POW-VLP

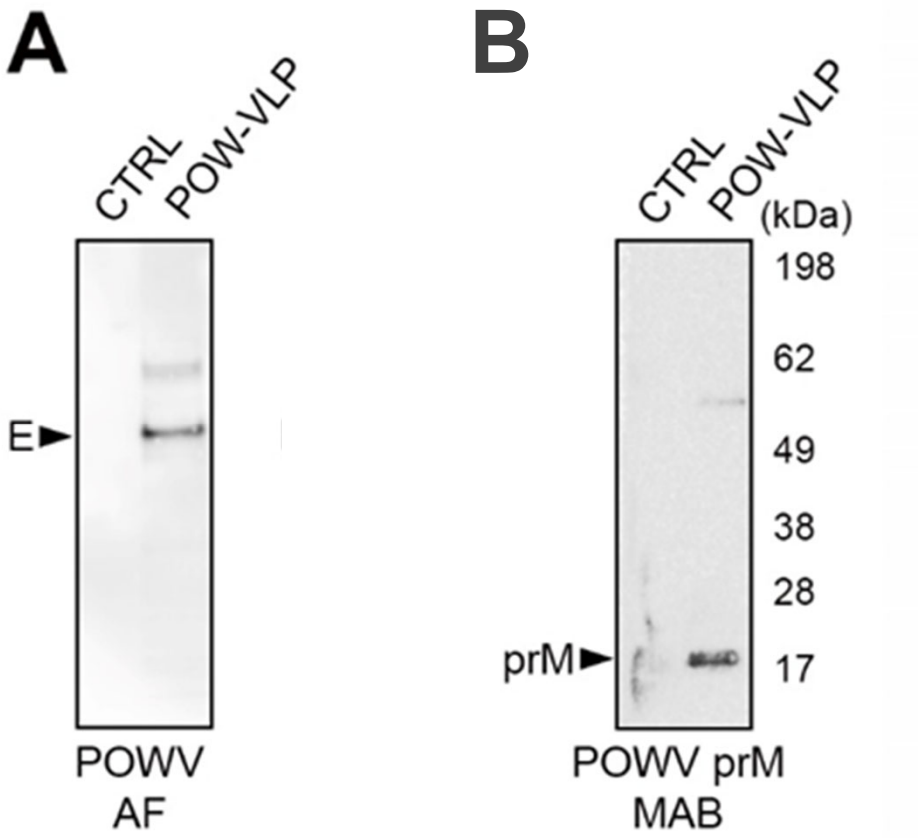
POW-VLP produced in mammalian cells 293T (ATCC® CRL-3216™)



- Transfected cells show production of POW-VLP (Panel A: DAPI nuclear staining in blue; FITC green POWV E)
- POW-VLP were expressed and accumulated in the cytosol (Panel B)

POW-VLP antigenicity

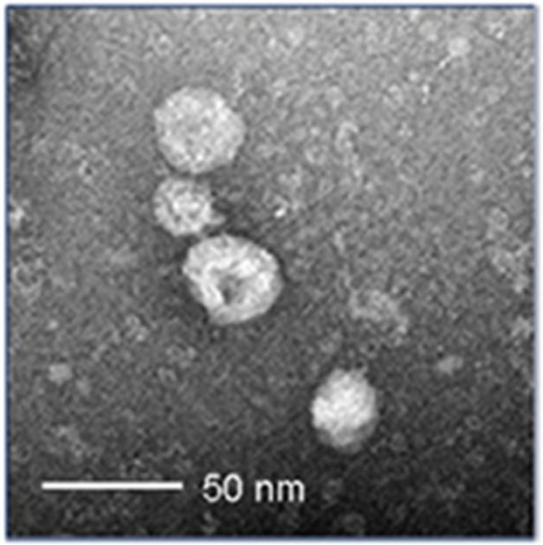
Immunological assays for testing POW-VLP antigenicity

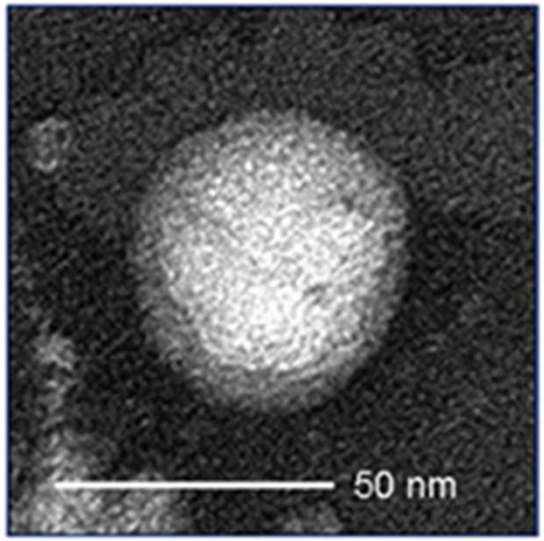


POW-VLP showed a high level of antigenicity for Powassan antibodies against E (Panel A) and prM (Panel B) proteins

POW-VLP morphology

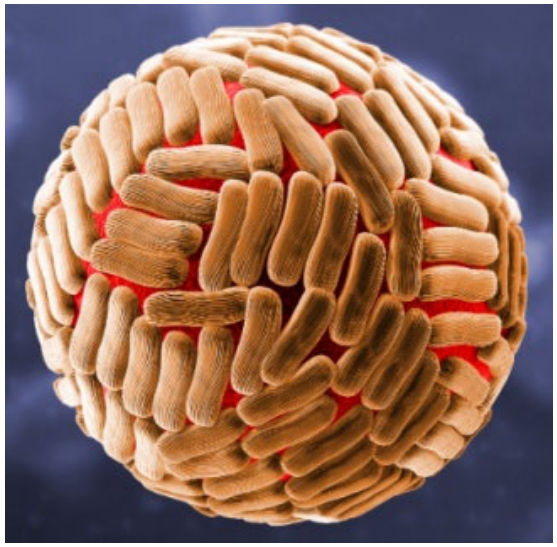
Transmission electron microscopy (TEM) is used to study VLP morphology





POW-VLP vaccine demonstrates flavivirus morphology

Flavivirus morphology



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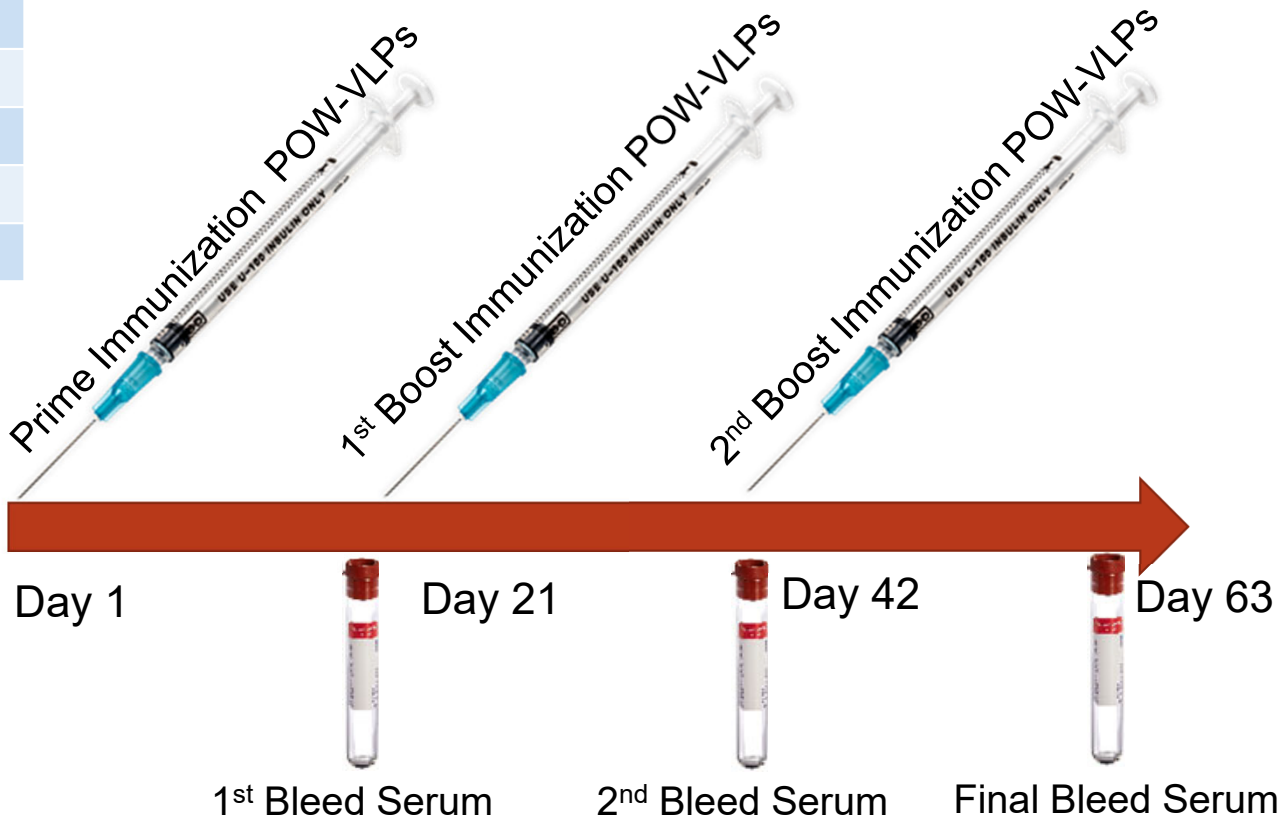
Murine model for POW-VLP testing

Safety and immunogenicity is tested in a mouse model

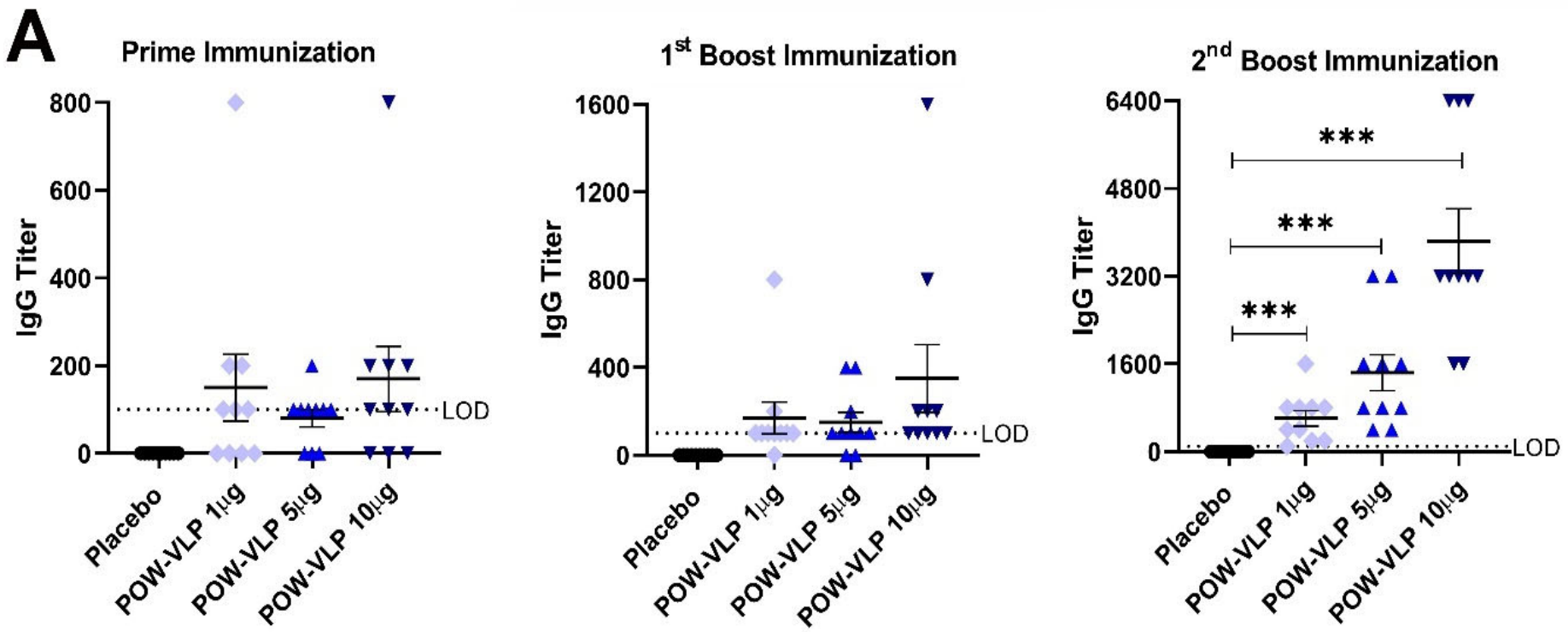
Group	Female	Male
Placebo	5	5
POW VLP 1 µg	5	5
POW VLP 5 µg	5	5
POW VLP 10 µg	5	5
Total	20	20

BALB/c



POW-VLP immunogenicity

ELISA method is used to assess mouse POW-VLP antibody response



POW-VLP afforded 100% seroconversion (*** = p value < 0.0005)

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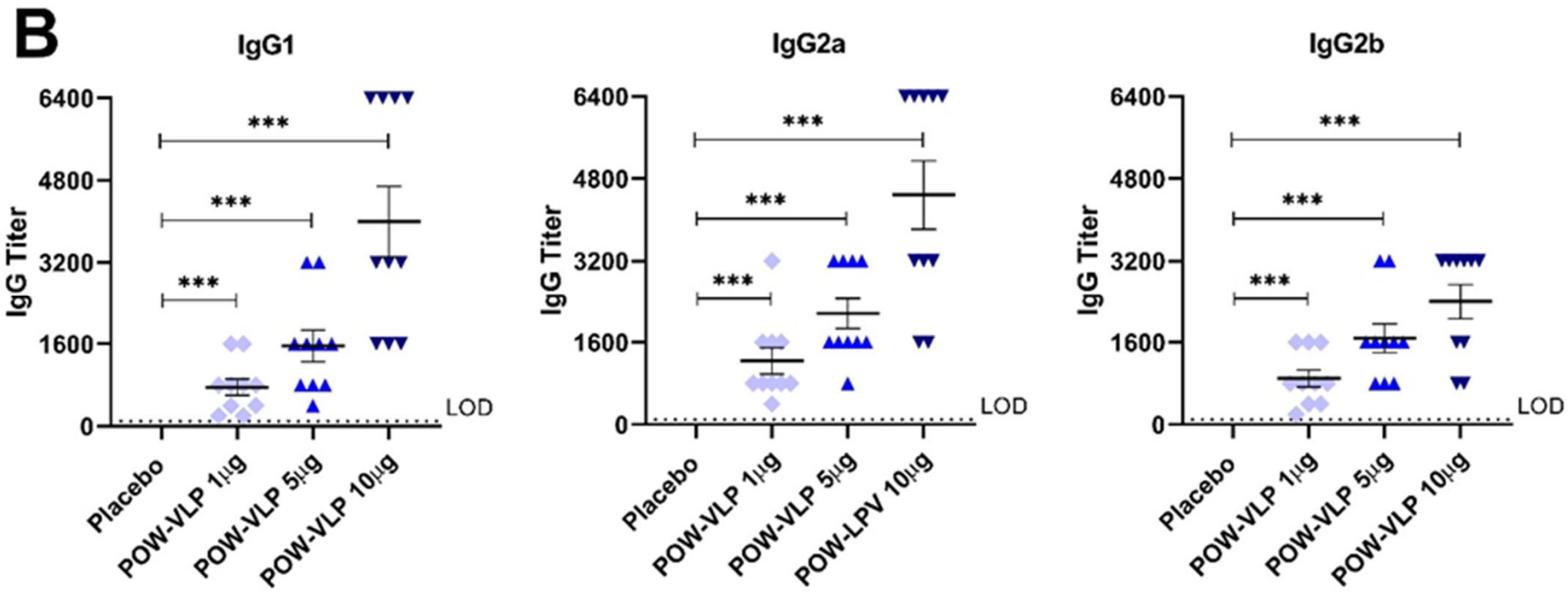
POW-VLP immunogenicity

ELISA method is used to assess mouse POW-VLP antibody response



POW-VLP afforded high level of Th1- and Th2- mediated immune (*** = p value < 0.0005)

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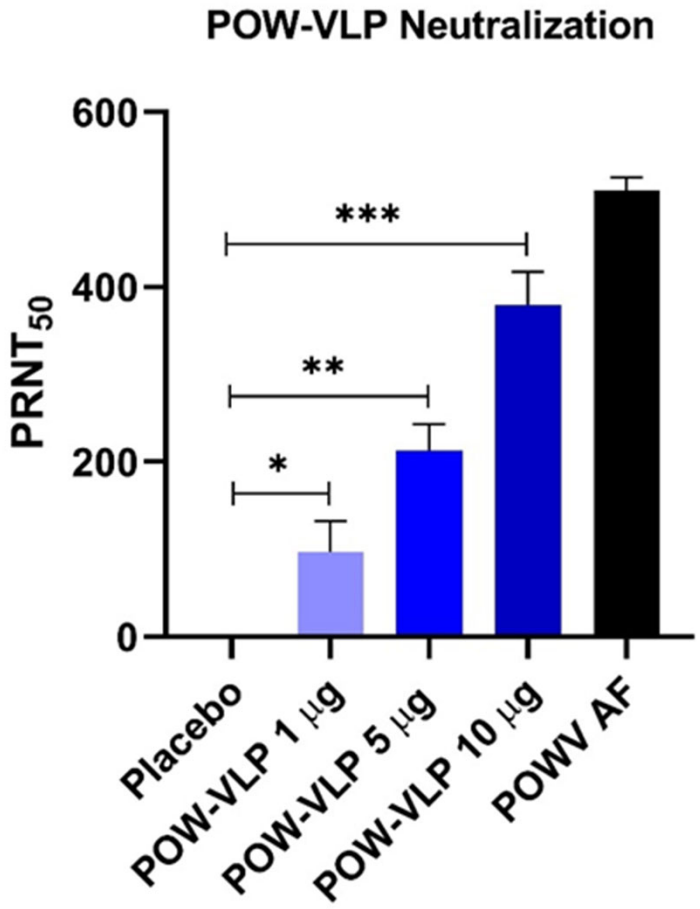
Powassan virus immune response

Neutralization analysis of serum antibody of mice immunized with POW-VLP



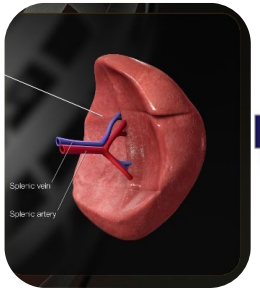
- POW-VLP induces a high level of neutralizing antibodies (*** = p value <0.0005)
- Neutralizing activity is dose dependent

Antibody Generated by POW-VLP Immunization

Hybridoma were generated by POW-VLP vaccine candidate

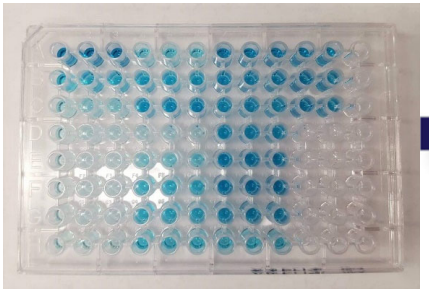
Mouse immunized POW-VLP

Splenectomy



335 Hybridoma Clones

ELISA Screening



30 Hybridoma Candidates

https://commons.wikimedia.org/wiki/File:3D_Medical_Animation_Spleen_Anatomy.jpg
https://commons.wikimedia.org/wiki/user:Ajpolino

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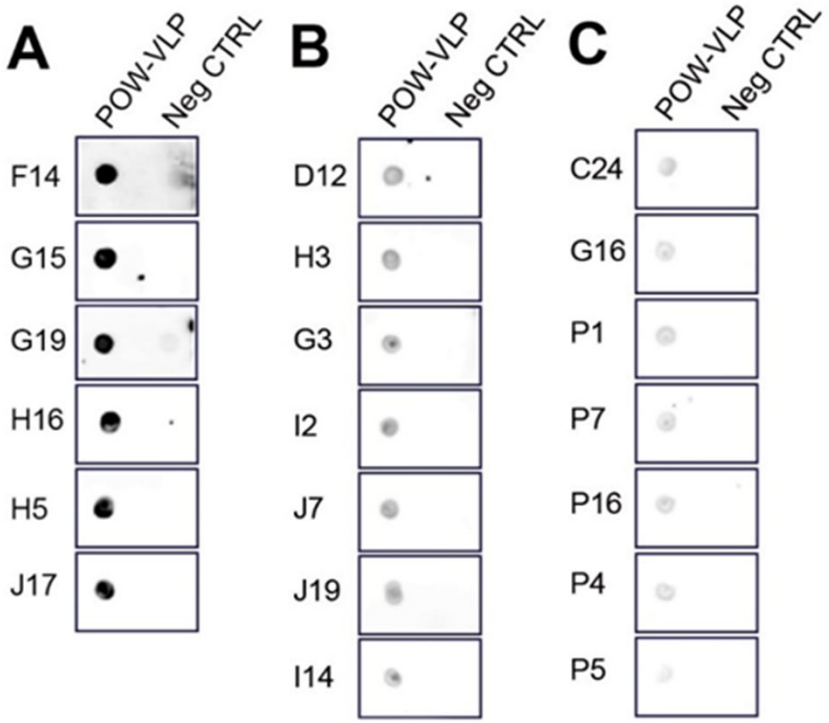
POW-VLP Monoclonal Development

Immunoblotting analysis of monoclonal antibody against POWV



Monoclonal with different level of reactivity have been generated using POW-VLP

Summary

Summary

Novel ATCC POW-VLP vaccine

- Main study goal: Apply effective and safe immunization strategies for the generation of improved vaccines

- Complementary fields provided the foundation for a rational approach to creating novel vaccines
  - Structural biology
  - Virology
  - Adjuvant formulation
  - Immunology

- ATCC's novel Powassan vaccine candidate has demonstrated in murine models:
  - Safety
  - Immune response
  - Protection activity

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Future Perspectives

Countermeasures against Powassan virus

- POW-VLP will be tested in mouse models for protection against tick-mediated Powassan transmission
- The Powassan vaccine candidate will be optimized for achieving complete sterilizing activity
- Development of neutralizing antibodies for a treatment against Powassan infection
- Testing broad spectrum anti-viral in vitro and in vivo

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Acknowledgements

The ATCC Team

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