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Plant-based technologies to enable rapid response to Ebola outbreak

Jerzy Karczewski
Fraunhofer CMB, Newark, Delaware, USA, jerzy.karczewski@fhcmb.org

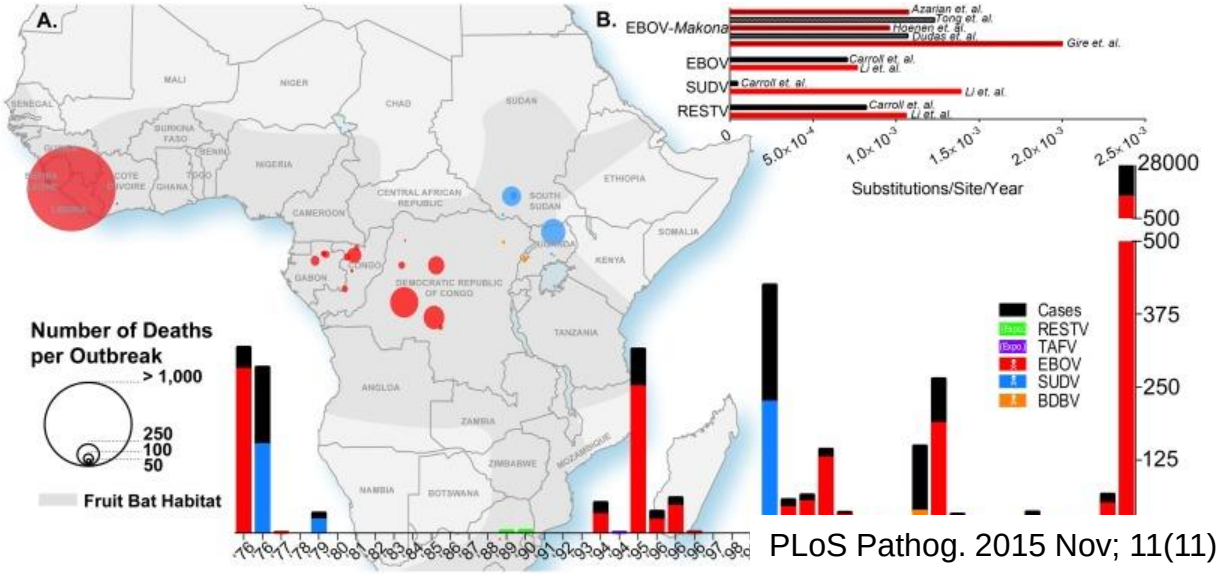
Vidadi Yusibov
Fraunhofer CMB, Newark, Delaware, USA

Plant-based technologies to enable rapid response to Ebola outbreak

Jerzy Karczewski, Ph.D.
on behalf of Fraunhofer USA Team
Fraunhofer USA Center for Molecular Biotechnology
Newark, Delaware

Vaccine Technology VI, June 12-17, 2016, Albufeira, Portugal.

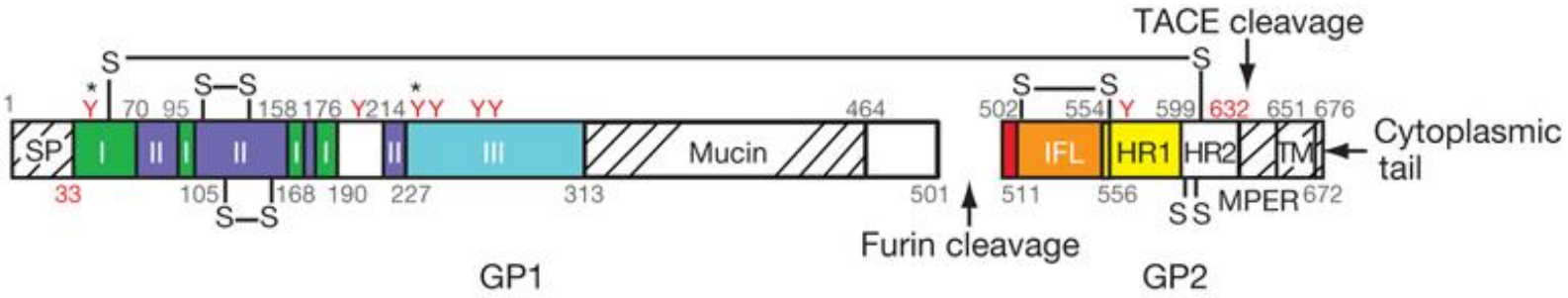
Ebolavirus outbreaks



PLoS Pathog. 2015 Nov; 11(11)

- **Ebola virus disease (EVD) is a severe, often fatal illness in humans (~50% fatality)**
- **The most recent (2014) outbreak was reported in Guinea (and 5 additional West African countries).**
- **Ebola virus (EBOV) is considered a biological warfare threat agent.**

Structure of the Ebola virus glycoprotein



Nature. 2008 Jul 10;454(7201):177-82

- **EBOV is an enveloped, negative-sense, single-stranded RNA virus. The genome of EBOV encodes seven proteins.**
- **Several vaccines and antibodies based on EBOV GP are under development, including viral vaccines and subunit GP vaccines, virus-like particles (VLP) and multiple recombinant monoclonal antibodies.**

Plant-expression system - upstream process

~28 days	Seeding and hydroponic growth
1 day	Vacuum Infiltration
2-8 days	Post-infiltration growth
1 day	Harvest & extraction

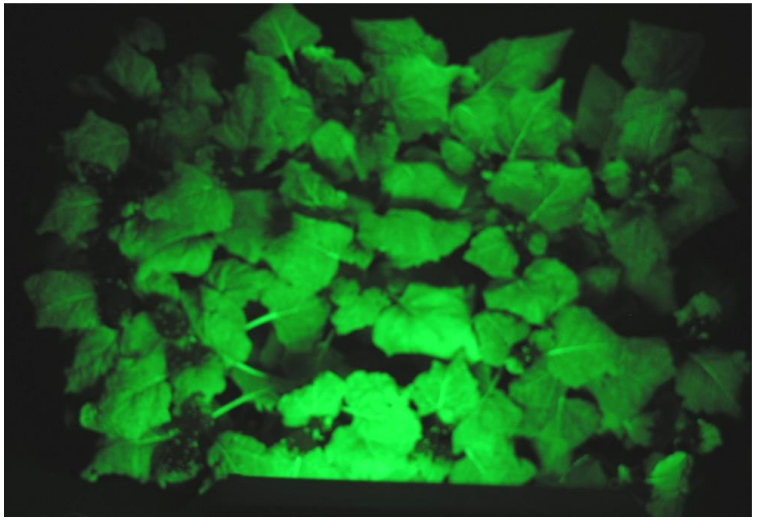
Agrobacteria

- ***Nicotiana benthamiana*** **plants are vacuum infiltrated with agrobacteria carrying GOI(s) in a non-viral vector, under control of strong plant-based promoters.**
- **Plants are allowed to grow for 2-8 days, then are harvested and accumulated protein is extracted.**

Plant-expression system - vacuum infiltration

Not Infiltrated

Infiltrated with GFP



- **Gene expression is observed mainly in leaves, as demonstrated in plants infiltrated with green fluorescent protein (above).**
- **Plant-based systems enable proper folding and disulfide bond formation**
- **Multiple proteins or subunits can be expressed simultaneously (such as heavy and light chain of IgGs).**

Plant-based expression of EBOV virus-like particle vaccines

Disclaimer :

This project has been funded in part with federal funds from JPM-Medical Countermeasure Systems.
Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army under subcontract to Battelle.

Design and production of non-enveloped VLPs

Purification

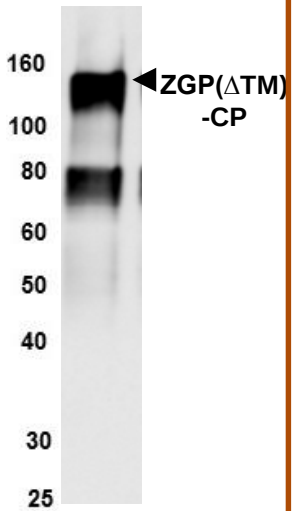
Particle formation

TEM

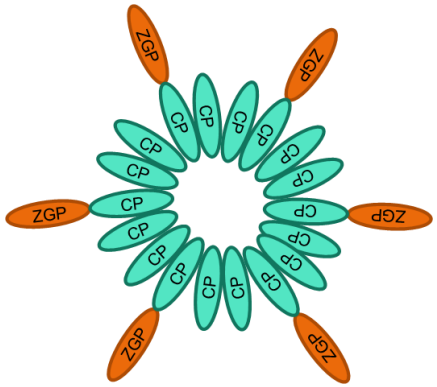
Extraction/
clarification

↓

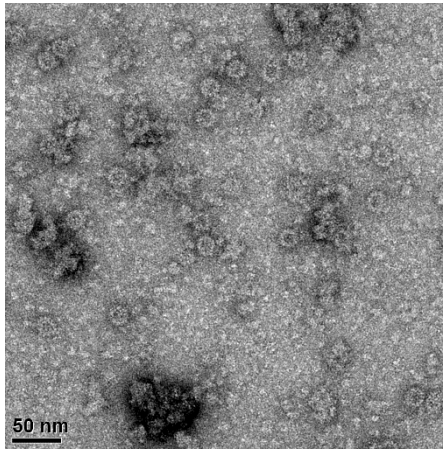
Affinity
purification



6D8 (anti-Zaire mAb)

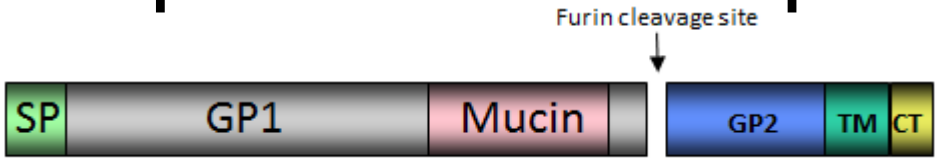


Dialysis/Concentration



- ZGP(ΔTM)-CP fusion protein is expressed in plants, extracted and affinity purified.
- The fusion protein assembles into VLPs during buffer exchange by dialysis (or ultrafiltration).

Design and production of enveloped VLPs



Purification

Extraction/
clarification

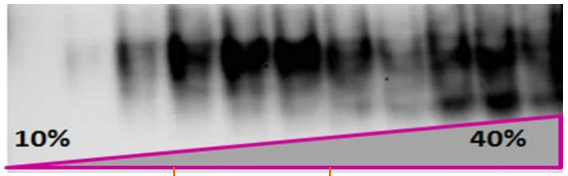
↓

High speed centrifugation
(100,000 xg)

↓

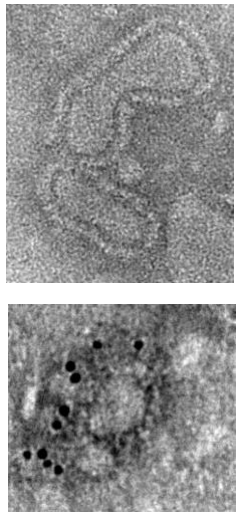
Sucrose gradient (10-40%)

Sucrose gradient



High speed centrifugation
(100,000 xg)

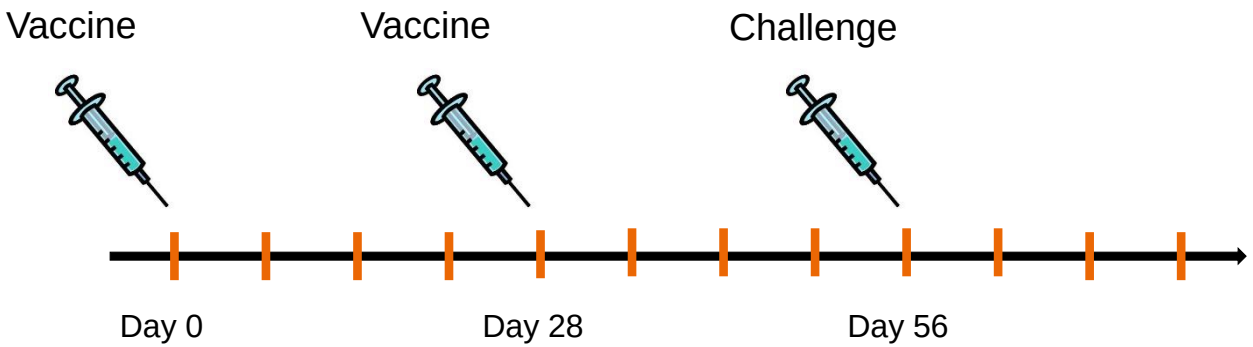
TEM analysis



Immunogold
(mAb 8-C10)

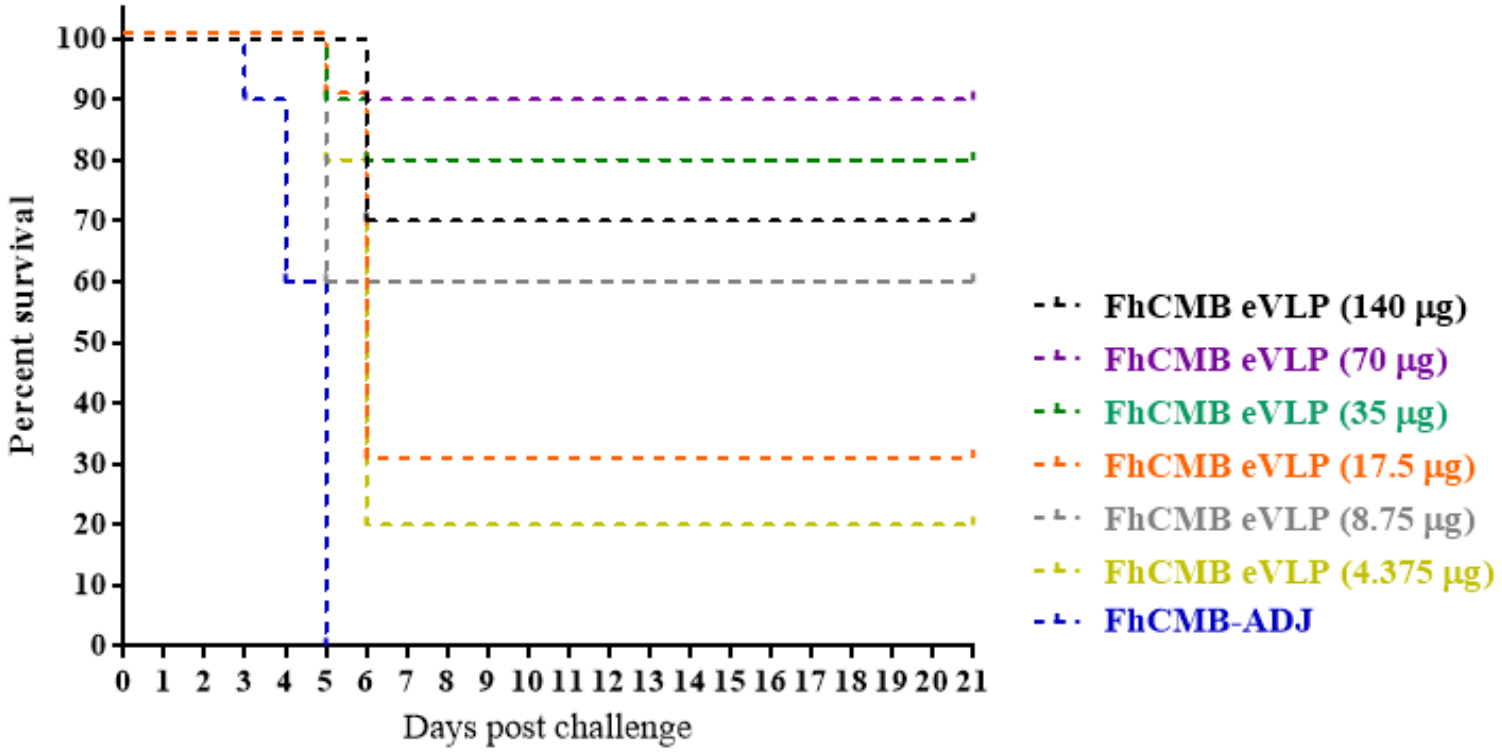
- The full-length ZGP is expressed in plants, eVLPs are extracted and fractionated using sucrose gradient, then concentrated by ultracentrifugation.

Mouse challenge - study outline



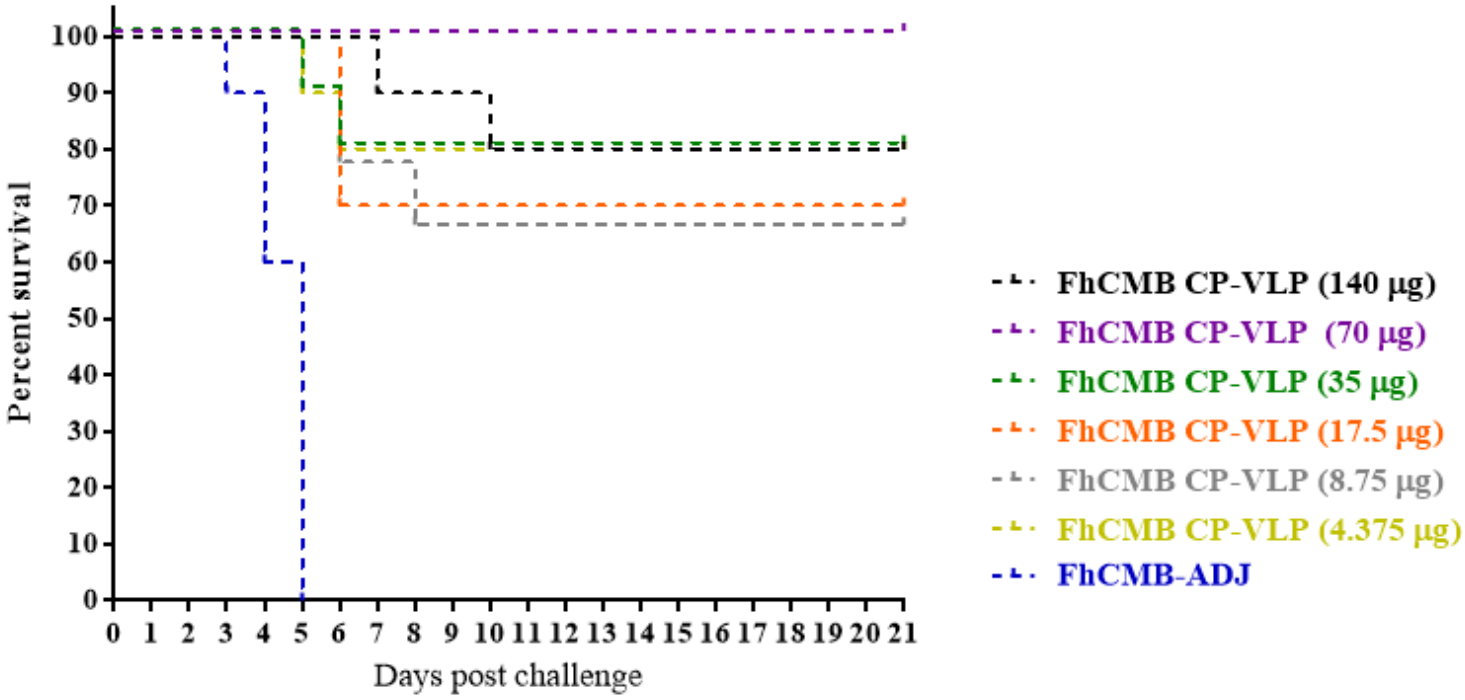
- Groups of 10 mice were vaccinated (i.m.) with :
  - FhCMB CP-VLP vaccine (FhCMB adjuvant, saponin-based)
  - FhCMB eVLP vaccine (FhCMB adjuvant, saponin-based)
  - FhCMB adjuvant only
- Two immunizations, 4 weeks apart
- Mice were challenged (i.p) with 1,000 PFU/mL in 0.25 mL of mouse-adapted EBOV (in ABSL-4)

Survival rates for FhCMB eVLP vaccinated groups



- EBOV-infected control animals died within 3-5 days, significant proportion of vaccinated animals survived the challenge up to 21 days post infection.
- The extent of protection was dependent on the dose of vaccine.

Survival rates for FhCMB CP-VLP vaccinated groups



- EBOV-infected control animals died within 3-5 days, significant proportion of vaccinated animals survived the challenge up to 21 days post infection.
- At least 70% survival was observed in groups vaccinated with CP-VLP vaccine.

Plant-based expression of monoclonal antibodies

Disclaimer :

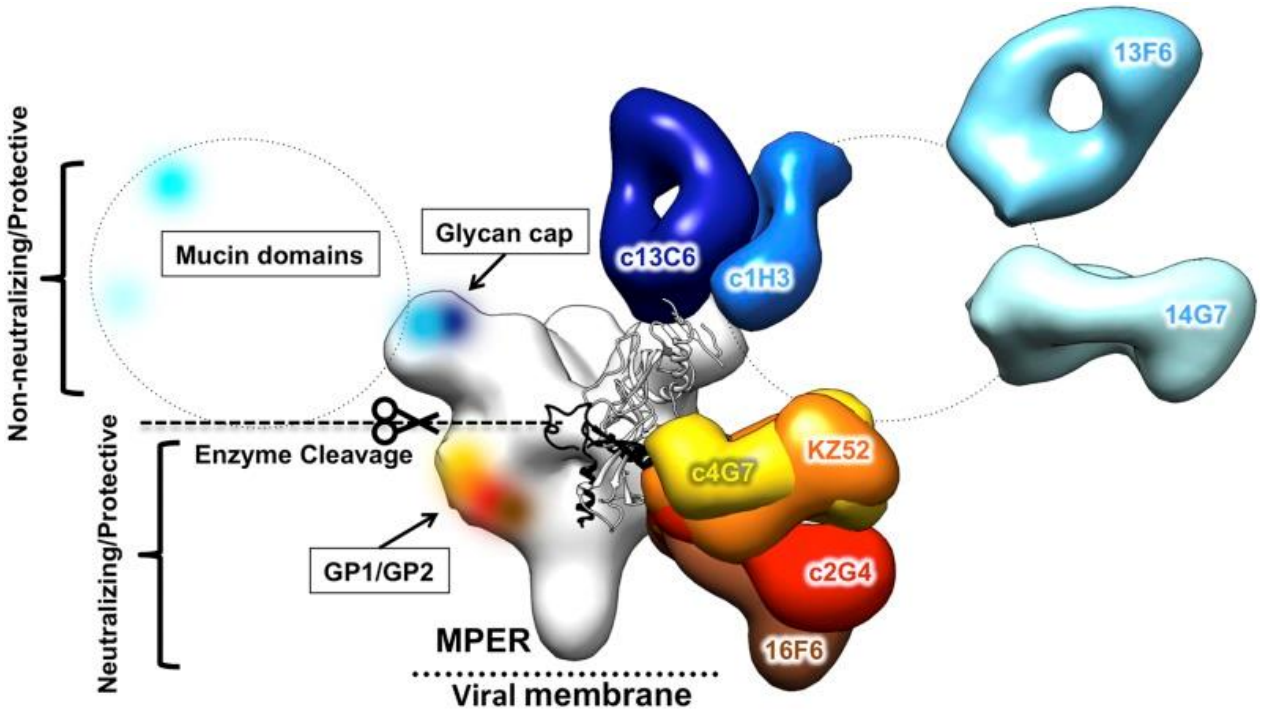
This project has been funded with federal funds from BARDA. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by BARDA/ASPR/HSS. The goal was to validate Nicotiana benthamiana expression systems for anti-Ebola mAbs.

Monoclonal antibody projects at FhCMB

Therapeutic target	Sponsor	Scale	Confirmed activity
Ebola (Zaire) Three mAbs	DoD	10-30 grams	In vivo
Ebola (Sudan) Multiple mAbs	DoD	0.1-0.5 grams	In vitro
Anthrax	DoD	Bench scale	In vivo
VEEV	DoD	12-15 grams	In vivo
Clostridium difficile	Internal	Bench scale	In vitro
Influenza	Internal	Bench scale	In vivo

- Recent examples of plant-based biologics are Ebola Zaire and Sudan mAbs (under contract from DoD)

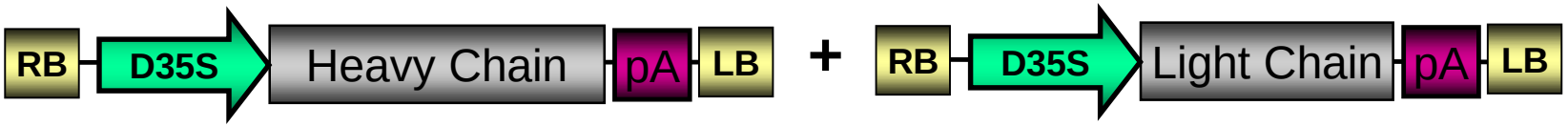
Sites of interaction between EBOV GP and neutralizing antibodies



- Neutralizing mAbs (such as c2G4, c4G7) bind at the GP1–GP2 interface and can prevent structural changes in GP2 required for membrane fusion.
- Non-neutralizing antibodies (such as c13C6, 13F6) bind outside of the core GP.

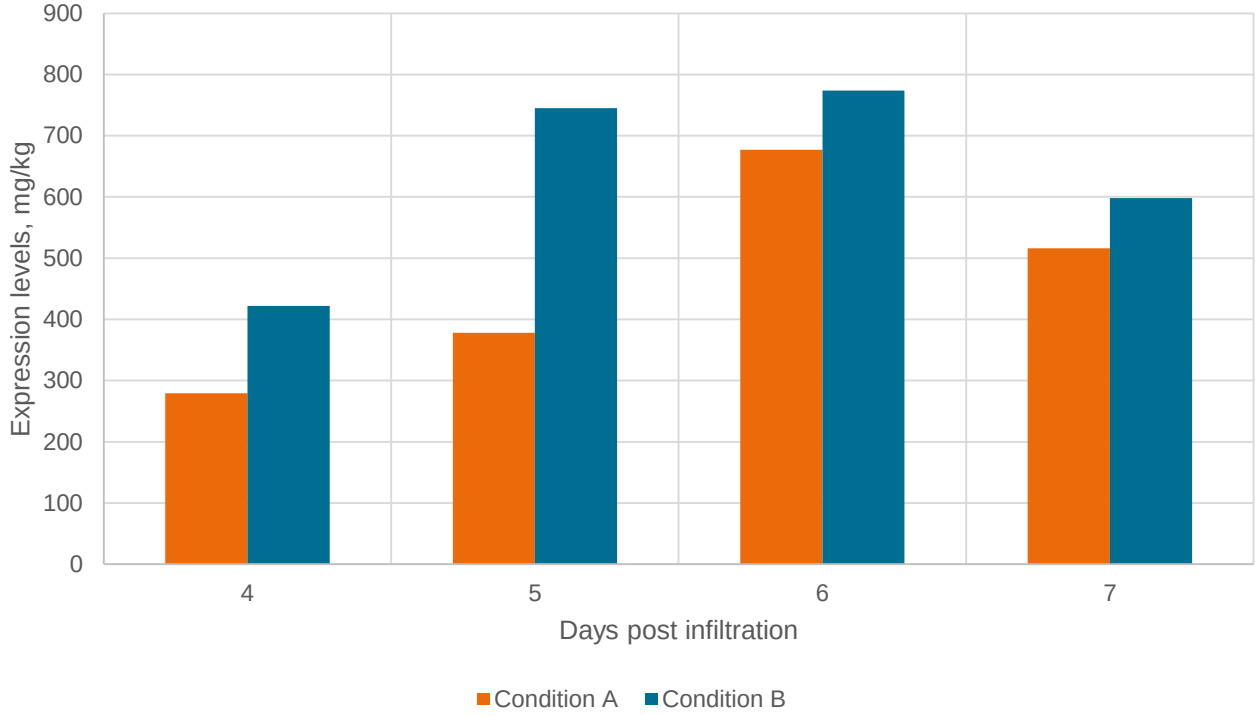
Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17182

Schematic representation of plant expression vectors



- Variable regions of heavy or light chain are cloned into a *Nicotiana benthamiana* expression vector containing human antibody constant regions

Optimization of Ebola mAb (c2G4) expression in *N. benthamiana*



- Plasmids carrying light and heavy chains are agroinfiltrated into *N. benthamiana*, and plants are harvested at 4, 5, 6, and 7 days post infiltration.
- The extracts are prepared and clarified, and antibodies are affinity purified on Protein A.
- Growth conditions and harvest time are optimized to maximize yields.

The downstream process (mAbs)

Harvest, homogenization, clarification

↓

Concentration (UF) of clarified extract

↓

Capture: Protein A

↓

Neutralization

↓

Dilution prior to AEX

↓

Polish: AEX

↓

UFDF into final formulation

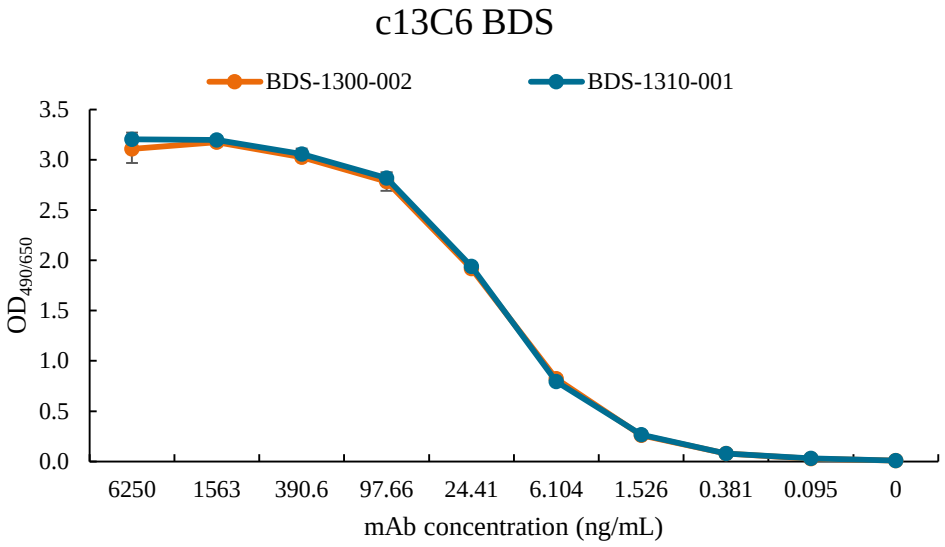
↓

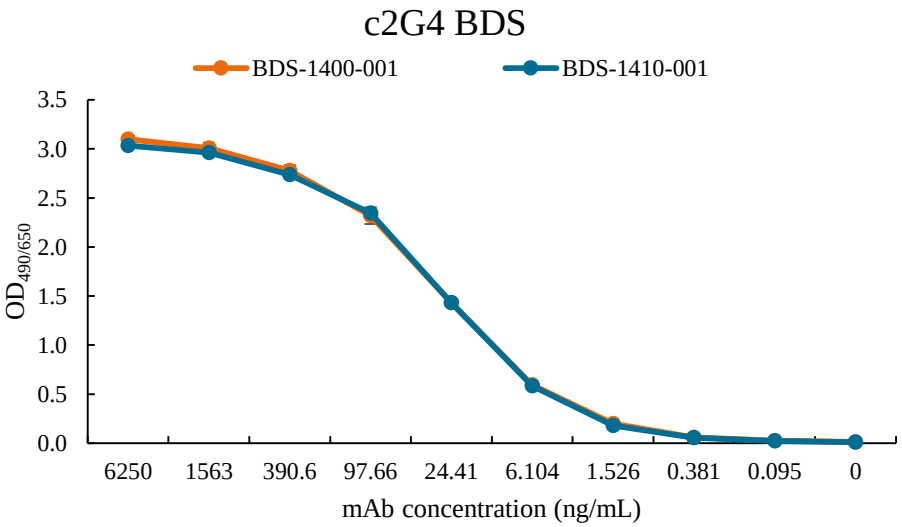
Filtration 0.22 µm/bottling for BDS

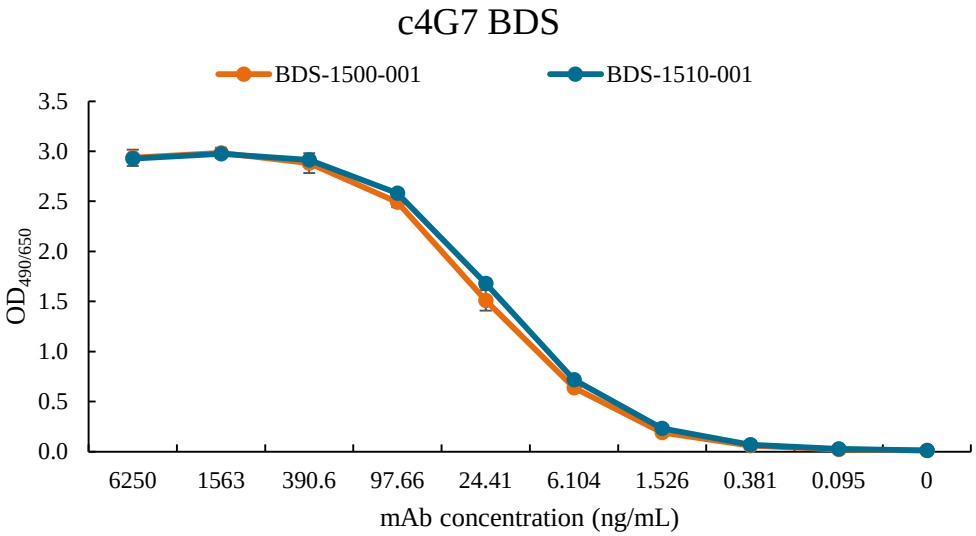
Quality Control Release Data for Antibody c13C6

ATTRIBUTE	METHOD	ACCEPTANCE CRITERIA	RESULTS
Identity	Non-Reducing & Reducing SDS-PAGE	Comparable to reference standard or described MWt of bands	Comparable to reference standard or described MWt of bands
Protein Concentration	UV (A_{280})	15 - 30 mg/mL	27.3 mg/mL
Purity	Bioanalyzer	≥ 90% of main target peak	98.5%
Purity	SEC	≤ 10% aggregates	< 10% aggregates (no aggregates detected)
Appearance	Visual	Clear to slight amber solution	Clear slight amber solution
Osmolality	Freezing point depression	Report value	304 mOsm/kg
pH	Potentiometric	5.5 - 6.5	6.2
Bacterial Endotoxin	LAL	Report value	0.05 EU/mg
Bioburden	Aerobic growth	≤ 10 CFU/mL	< 10 CFU/mL (no growth observed)

Reactivity of plant-based Ebola antibodies with a recombinant EBOV GP fragment



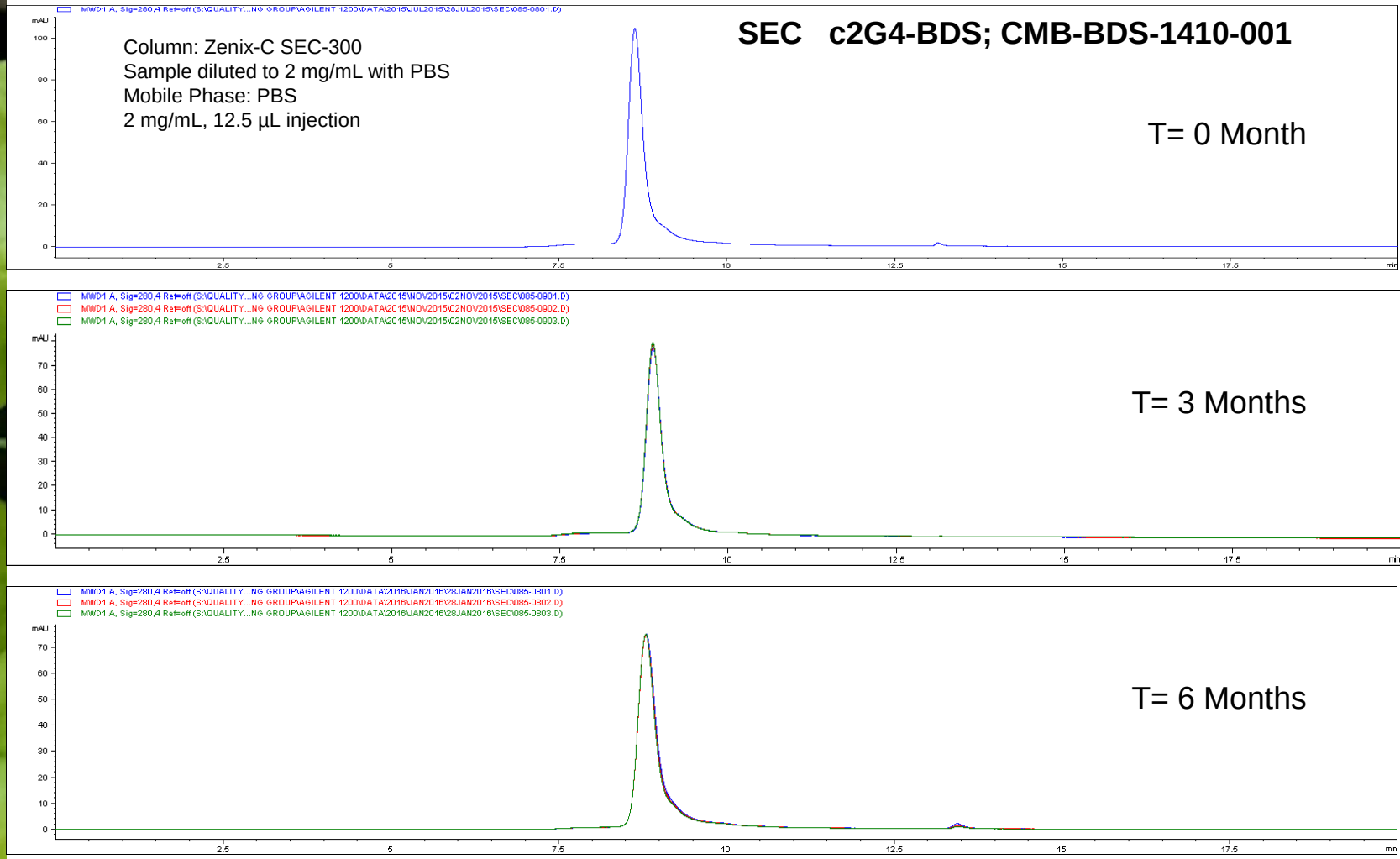




mAb	EC_{50} ng/mL (Ave (STDV))	
	BDS-001	BDS-002
c13C6_FhCMB	14.5 (1.29)	15.0 (2.45)
c2G4_FhCMB	26.0 (2.16)	27.6 (1.71)
c4G7_FhCMB	20.0 (1.41)	17.5 (1.73)
c13C6_IBT	12.0 (1.13)	

ELISA against rZEBOV GPdTM (IBT Bioservices)
Two batches of mAbs (001 and 002) were tested

Short term stability of Ebola mAb

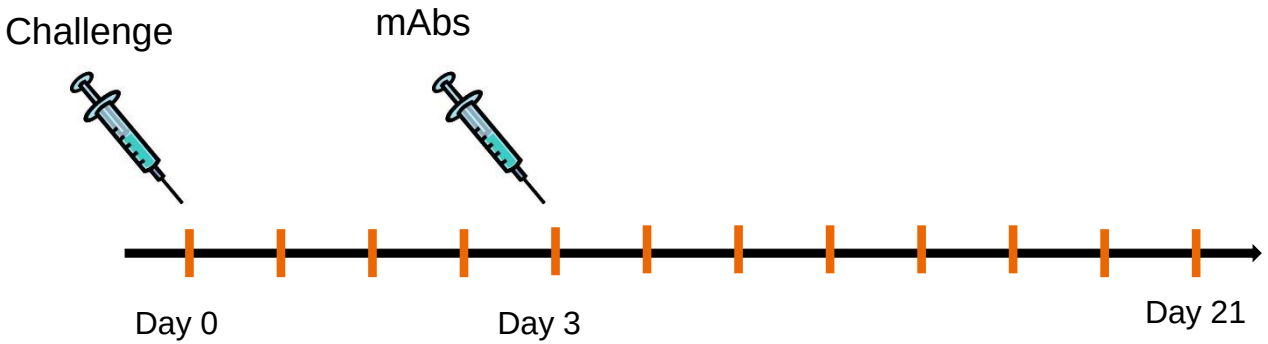


- **Product is stable up to 6 months when stored at -60ºC or below and no aggregation was observed**

Rapid production timeline example

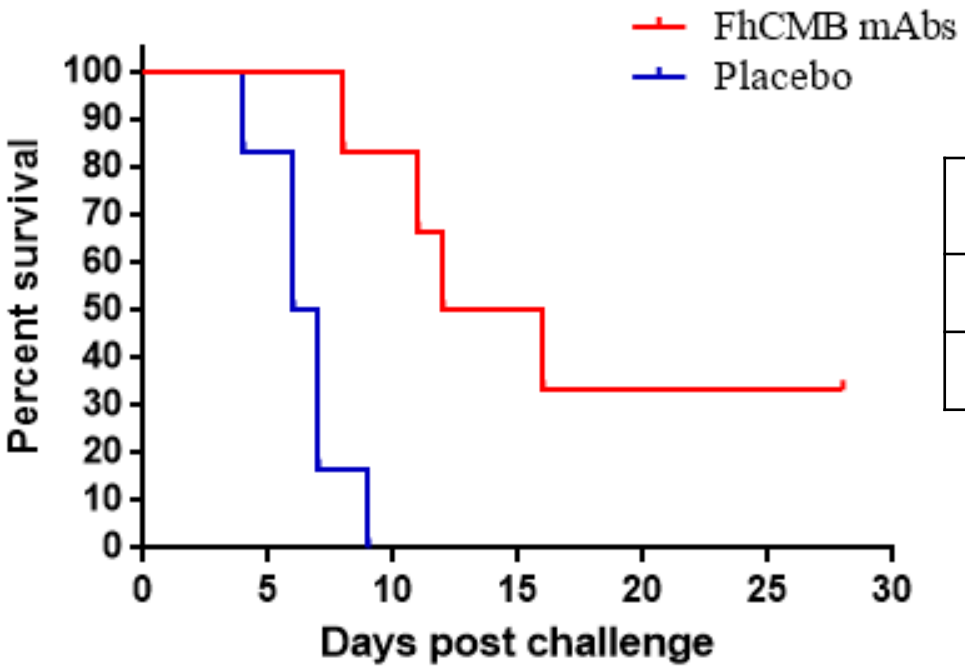
DNA synthesis and cloning	4 weeks
Development of upstream process	2 weeks
Development of downstream process	2 weeks
Establishment of assays	2 weeks
Large scale manufacturing of mAbs:	
Plant growth	~5 weeks
Target expression	1 week
Bulk purification	2 days
Total	~ 3 months

Guinea pig study outline



- **On Day 0, the animals received 1000 pfu of EBOLA Virus Guinea Pig-adapted Mayinga by the intraperitoneal (i.p.) route.**
- **Antibodies were administered i.p. 72 +/- 2 hours after infection.**
- **Animals were monitored and blood samples collected for virology, hematology and clinical chemistry.**

Efficacy of FhCMB Ebola mAbs in Guinea Pigs



Group	Median Survival Day	*p* value*
FhCMB mAbs	14	0.0031
Placebo	6.5	N/A

*Gehan-Breslow-Wilconxon test

- **Six Hartley Guinea pigs (250 to 350 grams) received 1000 pfu of EBOLA Virus Guinea Pig-adapted Mayinga by the i.p. route.**
- **Antibodies (combined dose of 5 mg/animal) were administered 3 days post infection.**
- **Treatment with anti-Ebola mAbs extended survival to 14 days, as compared to placebo (PBS) group, for which median survival was 6.5 days.**

Summary

- Two recombinant vaccine candidates based on Ebola surface GP were expressed in *N.benthamiana* and purified as enveloped and non-enveloped VLPs and their in vivo efficacy demonstrated.
- Rapid production of three Ebola monoclonal antibodies in *N. benthamiana* was demonstrated *and in vivo* protective activity was confirmed.
- Major advantages of plant-based expression systems include:
  - Ability to produce large quantities of target proteins at low cost
  - The presence of an eukaryotic post-translational modification machinery
  - Low risk to introduce human pathogens
  - Plant tissues can be inexpensively processed for oral delivery
- The results warrant further development of a novel plant-based vaccines and biologics.

Acknowledgements

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Battelle

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