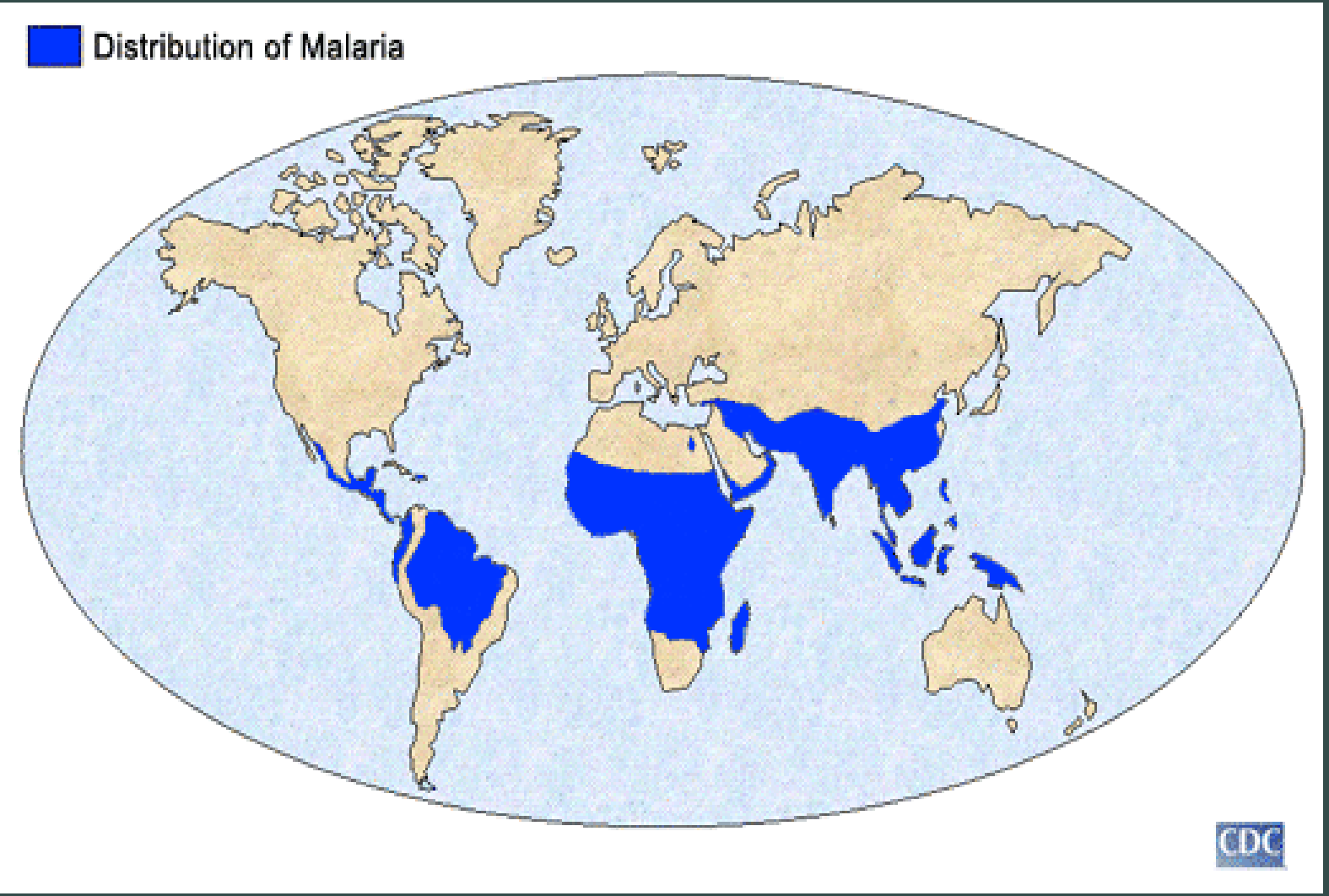
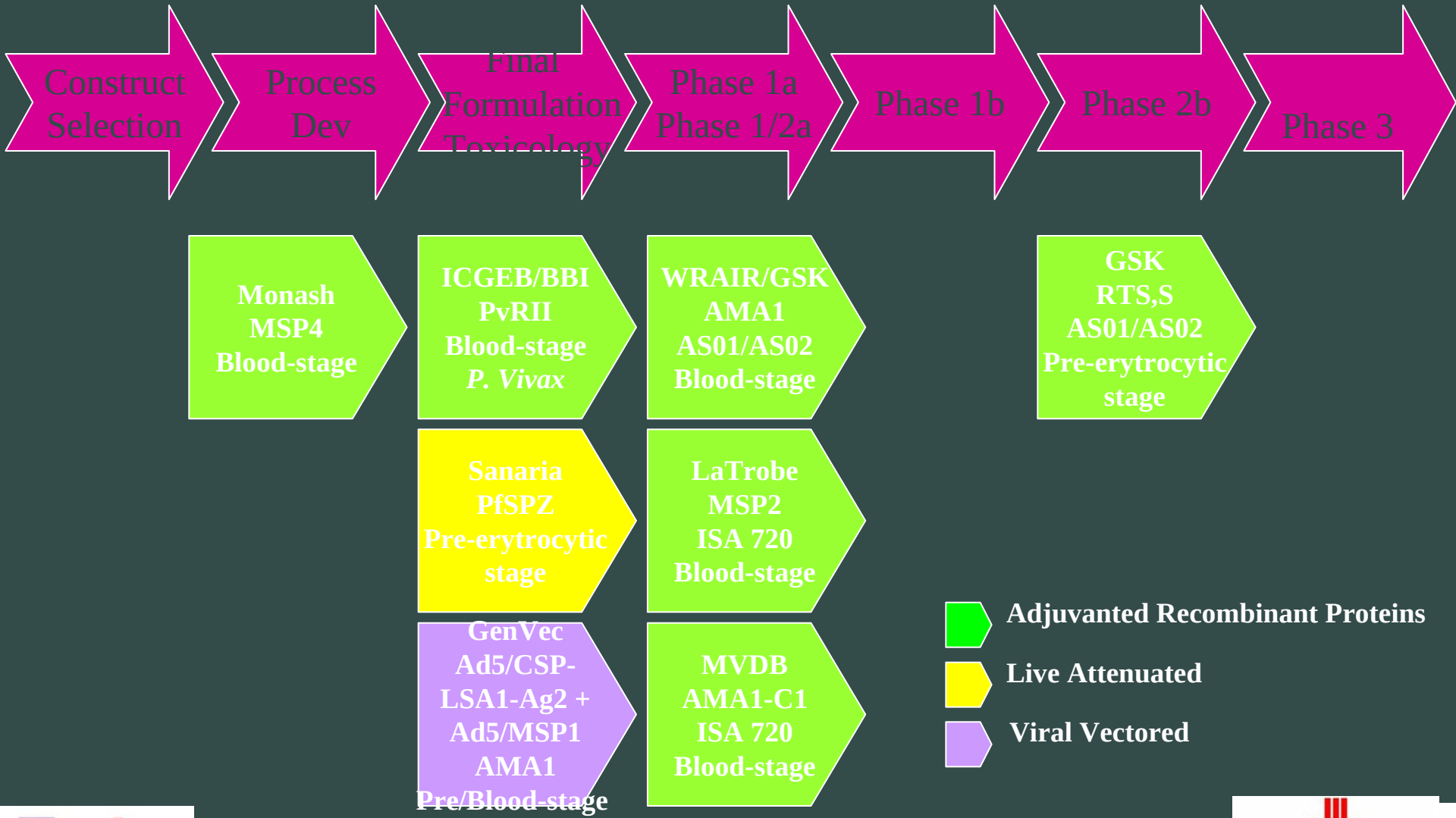
Recombinant Measles Malaria Vaccine

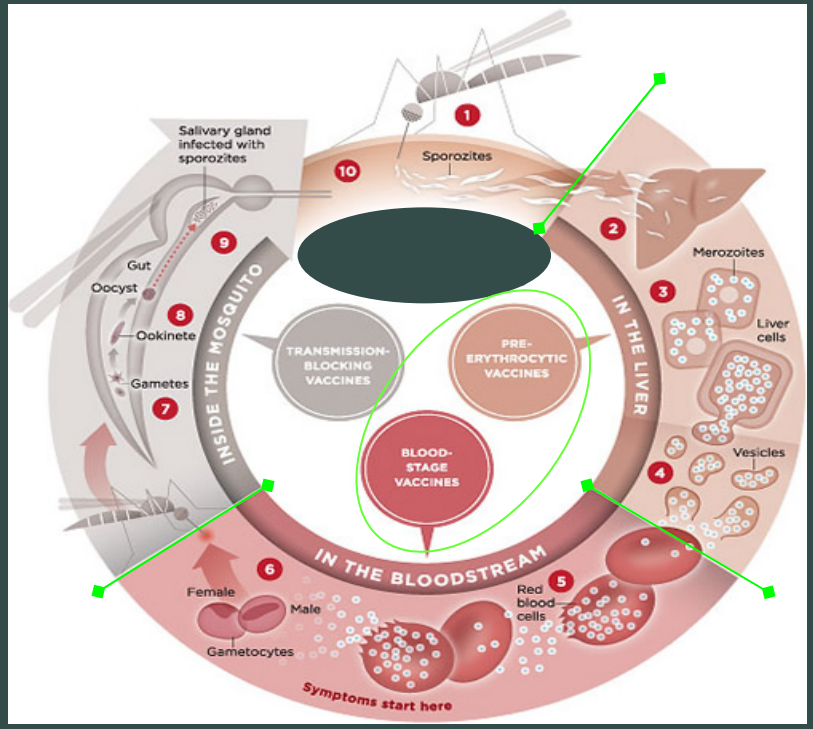
Malaria World Wide Distribution



Vaccine Candidates & Development Stage Target



Breaking *Plasmodium* Life Cycle With Multi-Antigen & Multi-Stage Vaccines



CSP

AMA-1

(DiCo1, DiCo2, DiCo3)

MSP-1 (3D7, FCB1)

Block2, p42, p83-30-38, GPI anchored and secreted

- **Combining pre-erythrocytic and blood-stage antigens would offer a higher yield approach to developing an efficacious vaccine**

Multivalent Recombinant Asexual Blood-Stage Vaccine Based On AMA1 & MSP1p19 Covering Allelic Diversity

- Collaboration with Alan Thomas, Biomedical Primate Res. Centre.
- This approach should help to overcome the problem of sequence polymorphism in MSP1 & AMA1, two leading malaria vaccine candidates.
- In previous studies these constructs elicited high invasion inhibitory antibody titers.

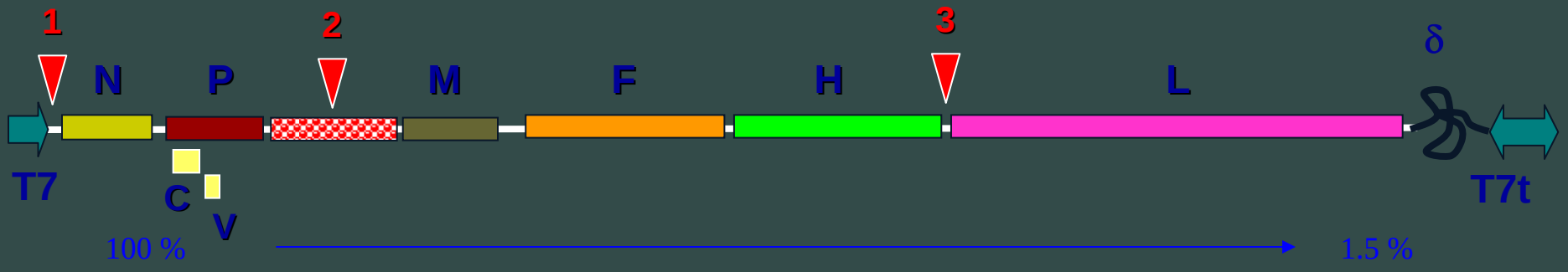
Multivalent Recombinant Measles Malaria Vaccine Containing Full Length MSP1 or Fragments of it

- Collaboration with Herman Bujard, Heidelberg.
- There is strong experimental evidence to suggest MSP1 is an essential protein for *P. falciparum*.
- Epidemiological evidence that MSP1 is important target of antibody response.
- Antigenic diversity is a problem for this antigen:
- Full Length protein could contain cross-reactive epitopes.

Multivalent Recombinant Measles Malaria Vaccine Containing MSP1 Block2+Block1 Hybrid

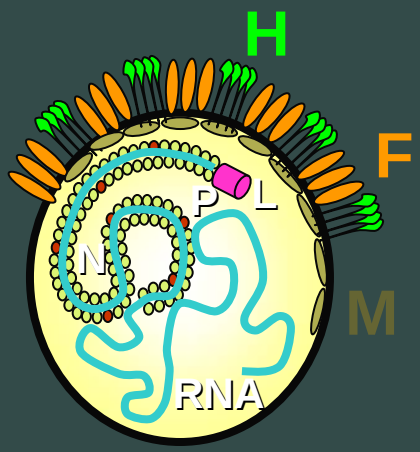
- Collaboration with David Cavanagh, Edinbergh
- Statistical survival analysis show that the block 2 region of MSP1 is a target of protective immunity against *P. falciparum*.
- Evidence of association between antibody responses to Block 2 & significantly reduce risk of subsequent clinical malaria.

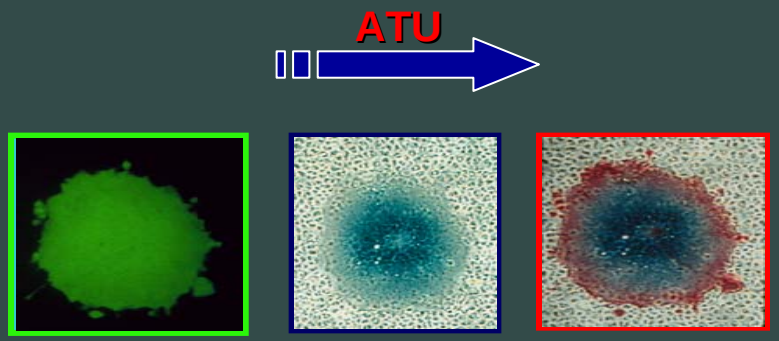
The Patented Technology: Virus From DNA



mRNA transcription gradient

Measles virus

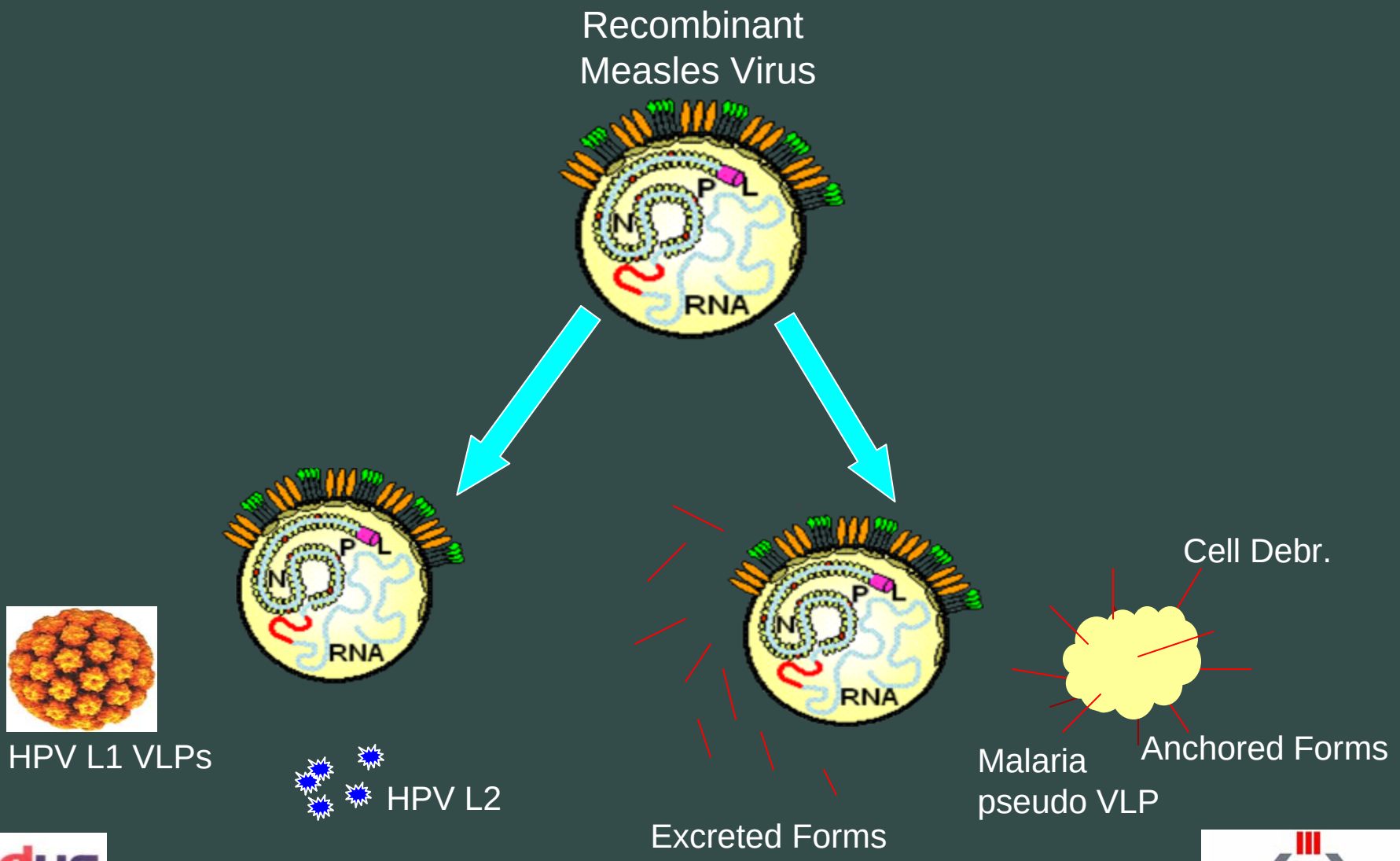




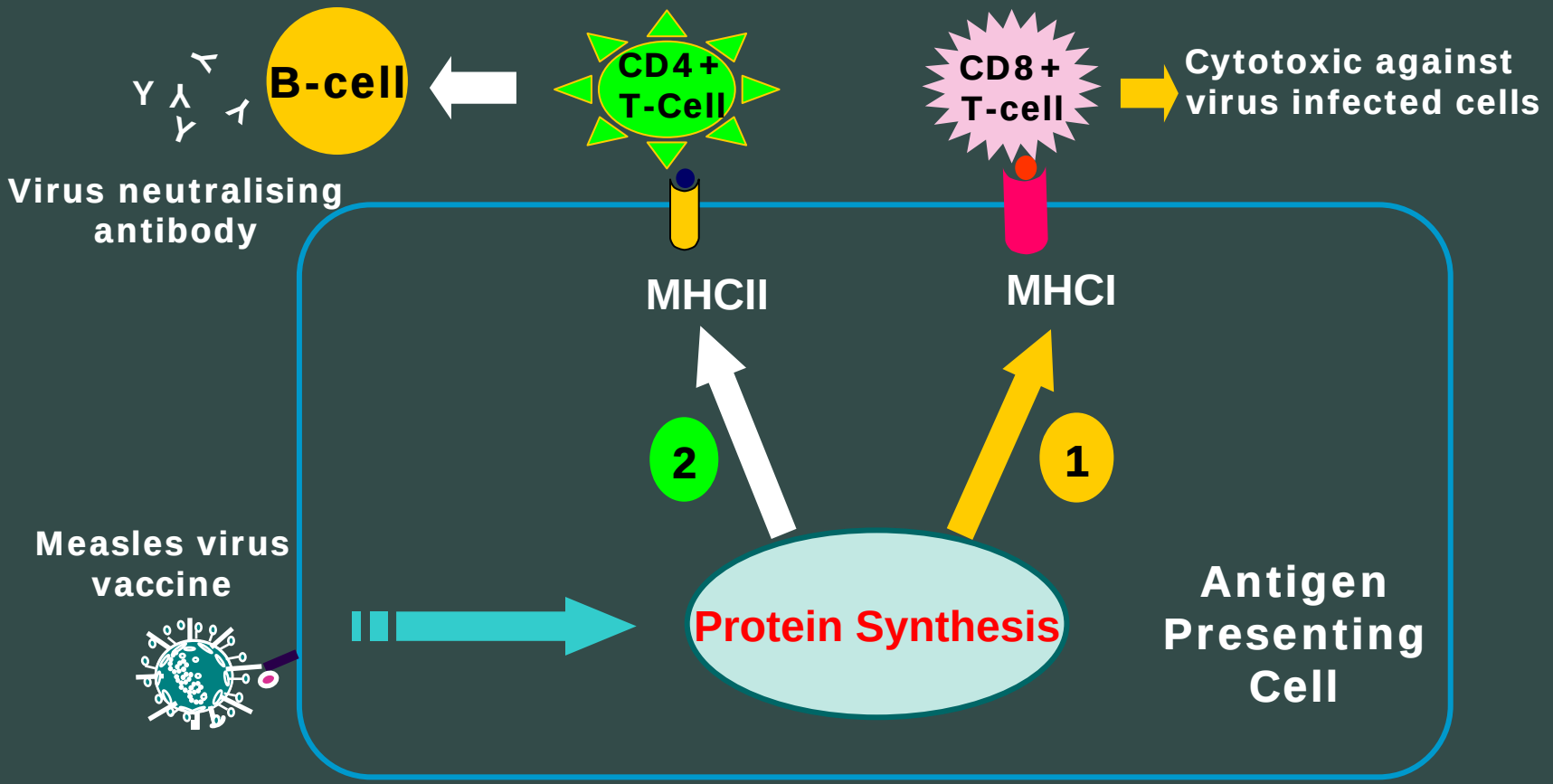
eGFP LacZ CAT

Recombinant Measles virus

Expression Forms From MV



Measles Vaccine-Broad Protective Immunity



Efficacy, Safety & Stability Of MeV Vaccines

- Traditional MeV vaccines confer life-long protection against measles infection in humans.

- In animal models, after inoculation of recombinant MeV vaccines a complete protection against the pathogen of the transgene expressed is well documented (Brandler *et al*., 2007).

- Thus, could be expected that in humans vaccination with recombinant MeV will elicit a comparable strong and durable protection, also against additional pathogens.

Efficacy, Safety & Stability Of MeV Vaccines Contd...

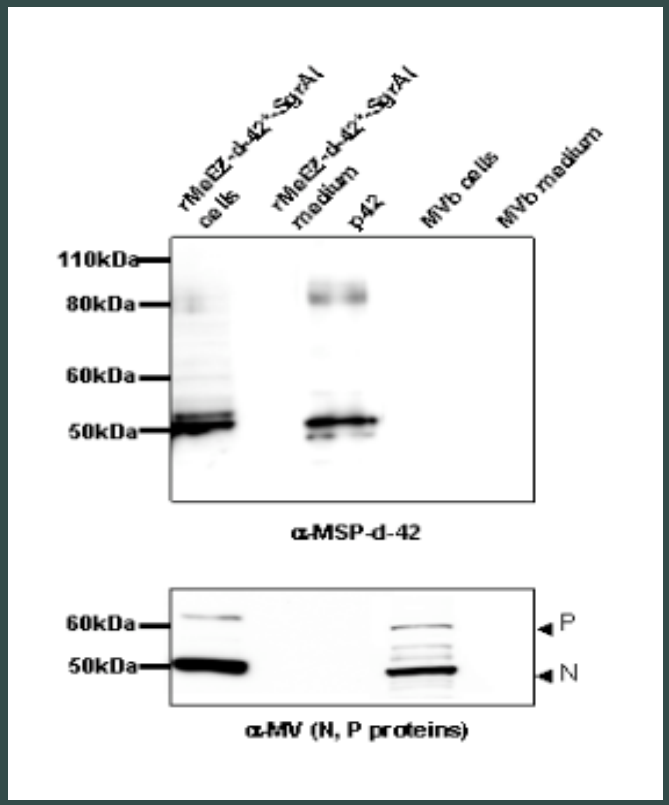
- In terms of safety compared to other technologies using different vectors for the delivery of genetic materials, MeV-based platform is favourable cause never have been observed reversion to wild-type strain and integration of the recombinant viral genetic material into the host's genome.

- High genetic stability in comparison with other RNA virus vectors: the virtual absence of recombination is due to the very tight RNP structure, as illustrated by the "rule of six".

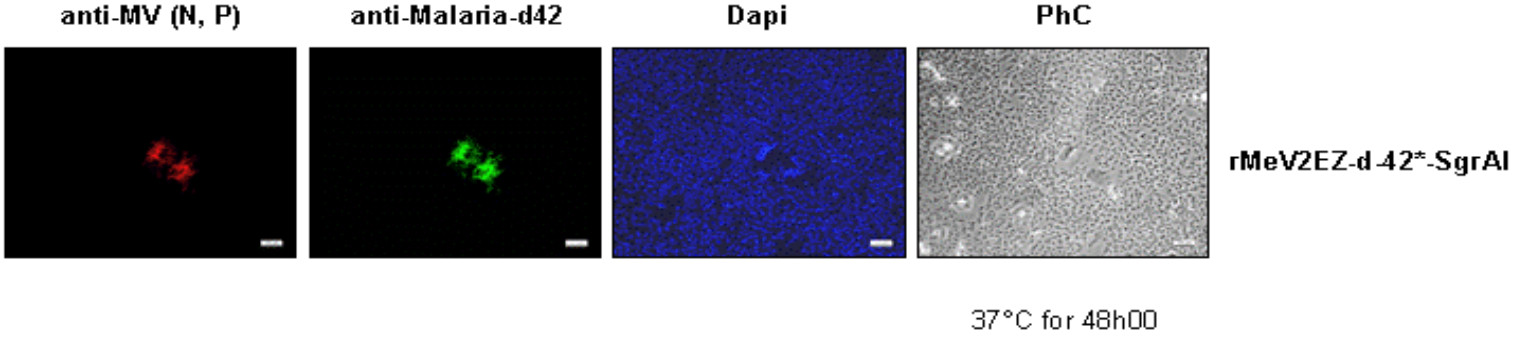
Western Blot

Western blot analysis of rMeV2-d-42*-SgrAI infected Vero cells. Malaria antigens were detected using antisera directed against the respective proteins. Additionally, the MV nucleocapsid (N) and phosphoprotein (P) were detected with a specific antiserum (bottom pannel).

- All MV genes and rMV transgenes are expressed.



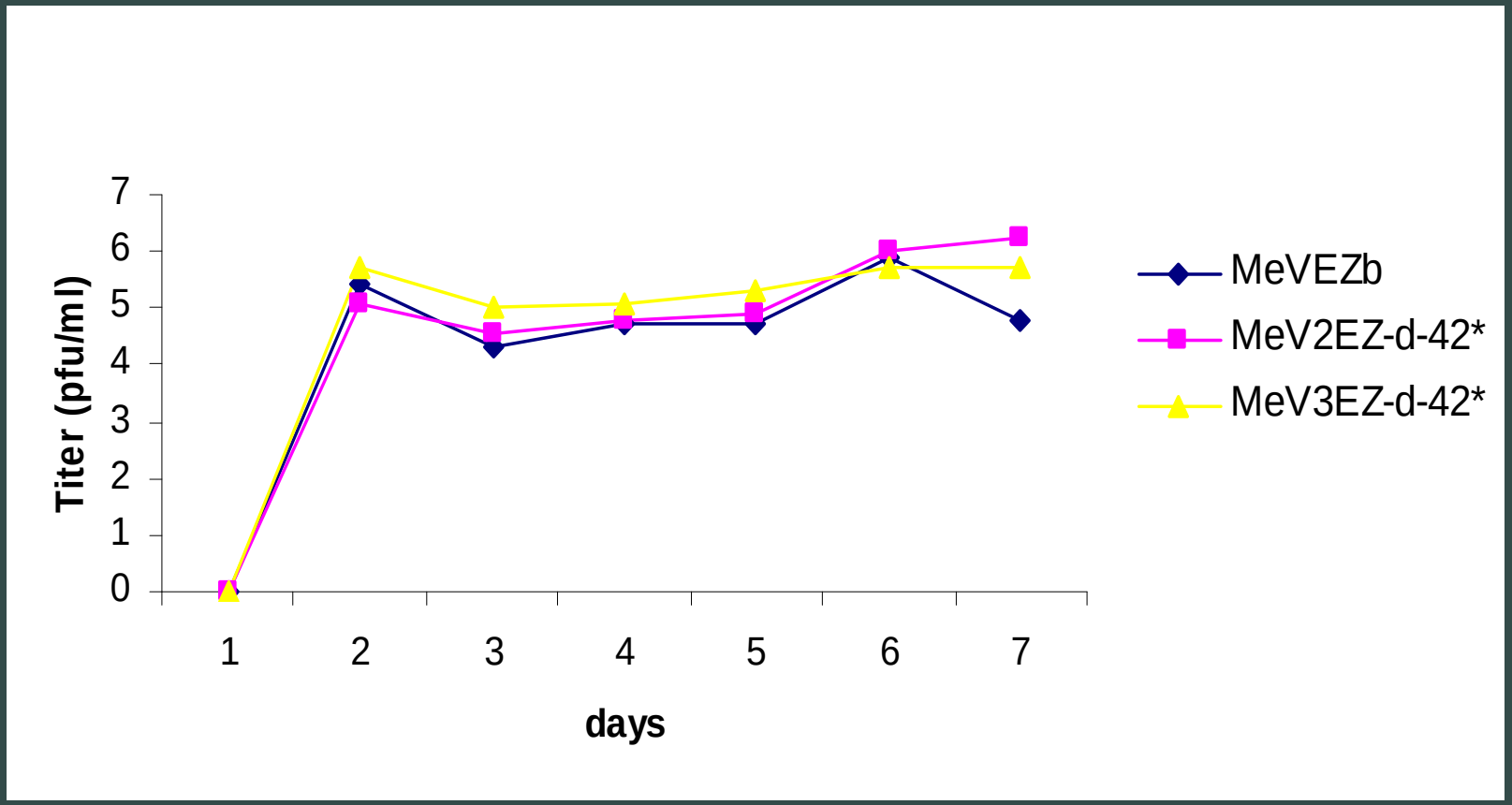
Immuno-fluorescence



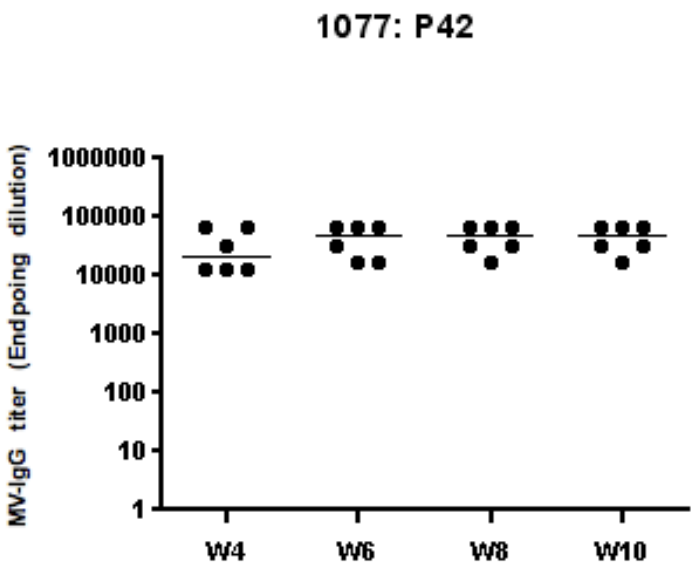
Virus	Avrage of Syncytia/well	Number of wells	Syncytia expressing MV-N and P proteins (%)	Syncytia expressing the transgene (%)
rMeV2EZ-d-42*-SgrAI	52	4	100	100

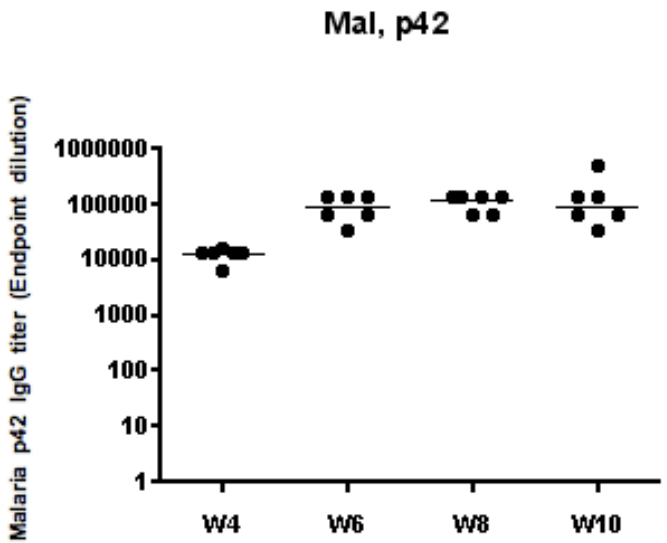
Vero cells were cultured and inoculated with 200 pfu rMV or rMeV2EZ-d-42*-SgrAI. Expression of measles and the transgene was detected after 48h by staining with specific antibodies. The malaria proteins are expressed and present in all infected cells.

rMeV$_{2\text{-}3}$EZ-d-42*-SgrAI Growth Kinetics



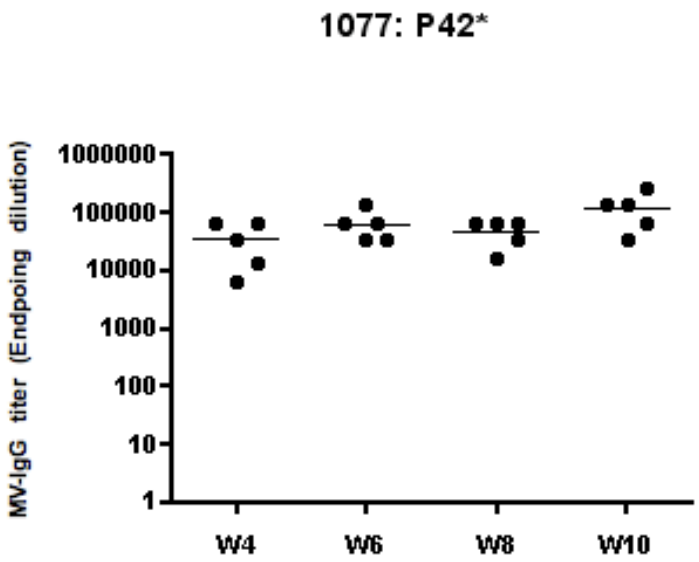
Humoral Response Against Measles & Malaria

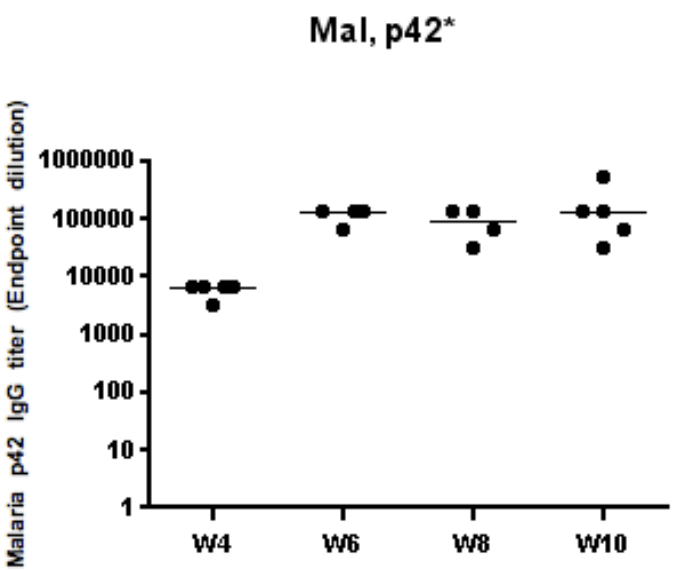




ETNA BIOTECH
Zydus Cadila Research Center

Humoral response against Measles & Malaria





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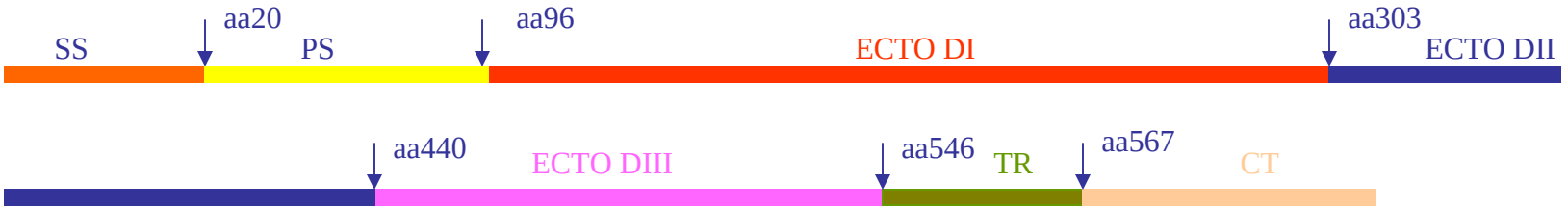
Zydus Cadila Research Center

Recombinant Measles-Malaria AMA-1 DiCos vector construction

- **Apical Membrane Antigen-1 (AMA-1) from *Plasmodium falciparum* (Pf) is a promising candidate for a blood-stage malaria vaccine.**

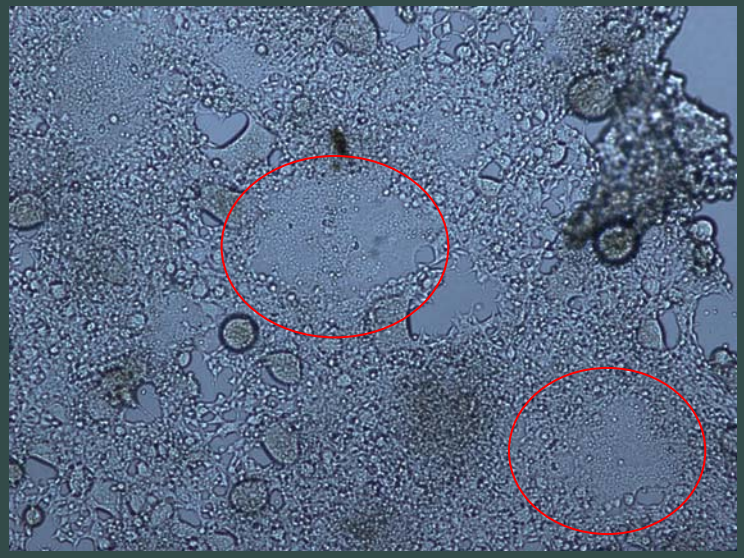
- **The AMA-1 protein is 622 aa long: it comprises a pro sequence (aa 1-96), an ecto domain (I, aa 97-303; II, 304-440; and III, 441-546), a trans membrane region (aa 547-567), and a cytoplasmic tail (aa 568-622).**



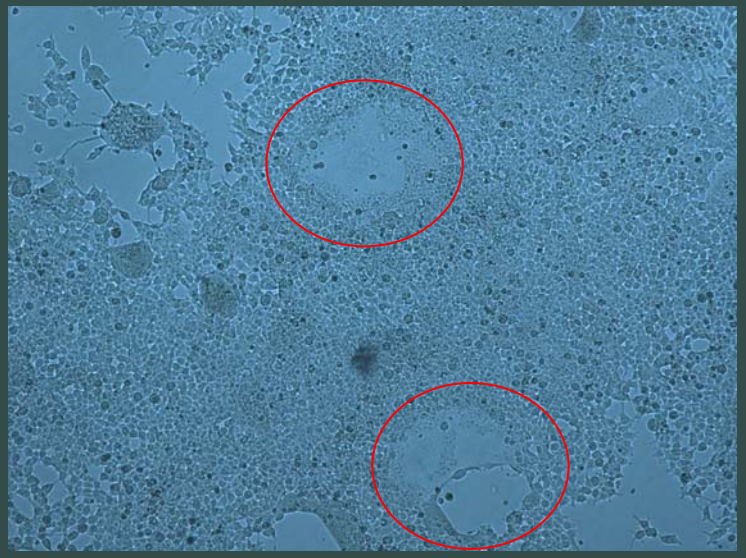
- **Diversity Covering (DiCo1, 2, 3) PfAMA-1 are synthetic sequences that together cover about 97% of polymorphisms in the 355 AMA-1 sequences present in data base.**

- **We received the DiCo 1 , 2 and 3 aminoacid sequences from Alan Thomas BPRC, the Netherland.**

Cytopathic Effect Of The rMeVEZ-SgrAI-DiCos In 293T Cell

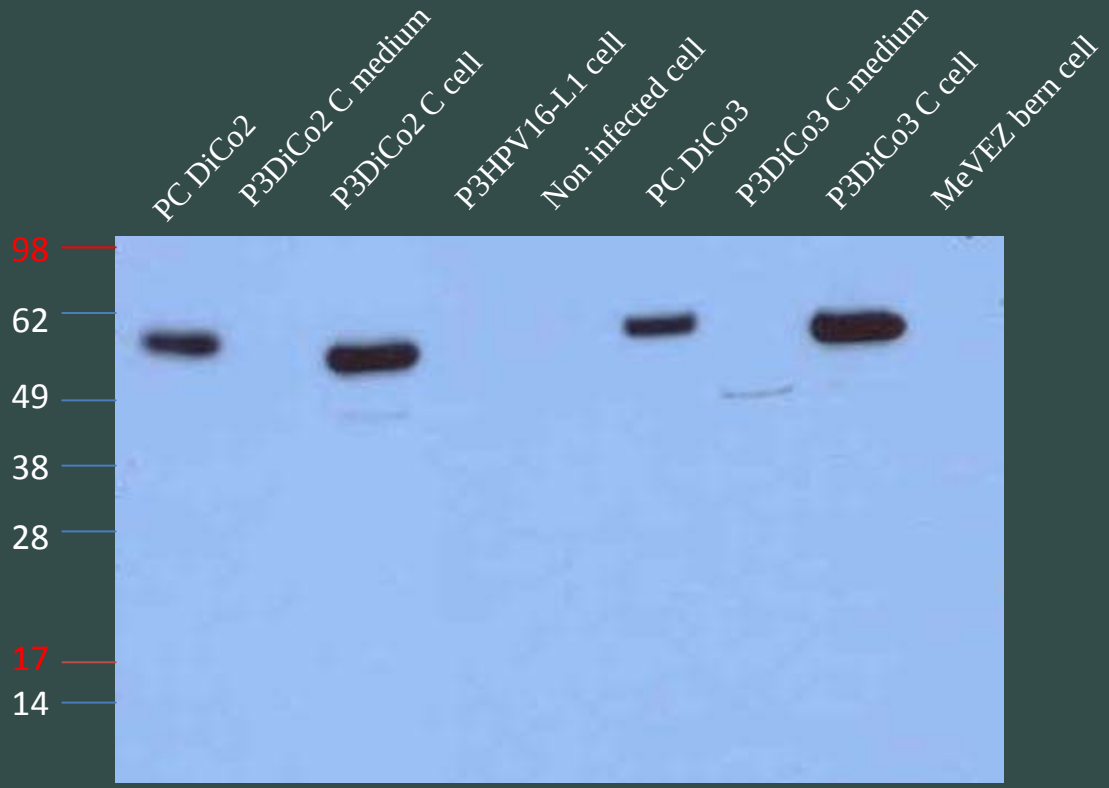


MeV_3EZ-SgrAI-DiCo3 ecto



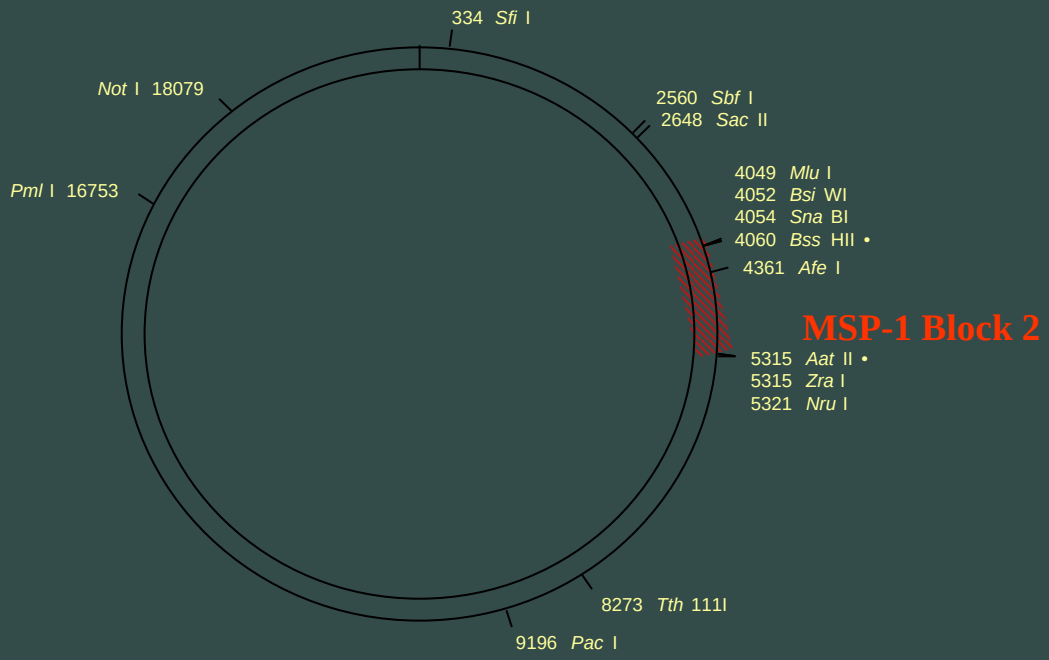
MeV_3EZ-SgrAI-DiCo1 ecto

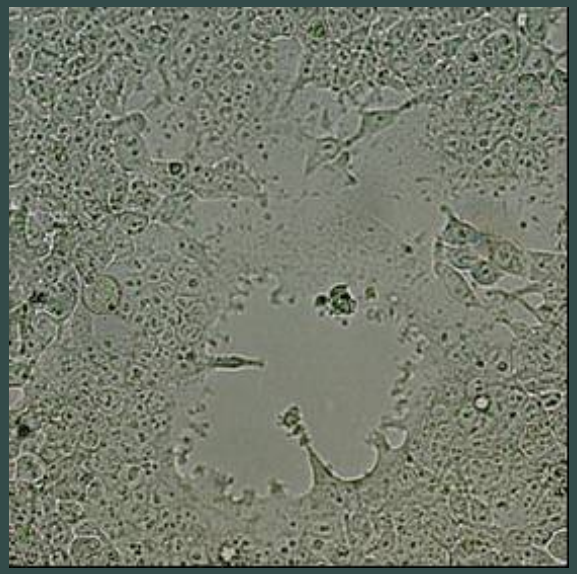
Western Blot Anaysis useing AMA-1 Specific Mouse Monoclonal Ab 4G2



Rescue Of The rMeV$_2$EZ-MSP-1 Block 2 Virus In 293T Cell

The Block2 region is composed of three major allelic types, found throughout the world: the K1, the MAD 20 and the R033 type to elicit immuneresponse to parasites of all three serotypes





p(+)MeV$_2$EZ-MSP-1 Block 2

20291 base pairs

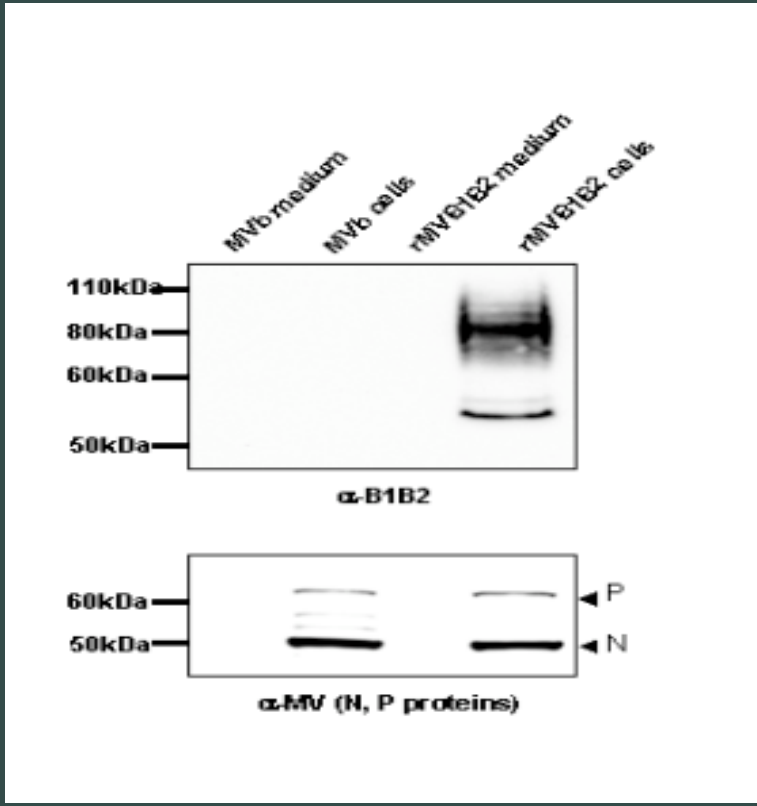
Unique Sites

R. Spilotri, D. Cavanagh, Edinburgh

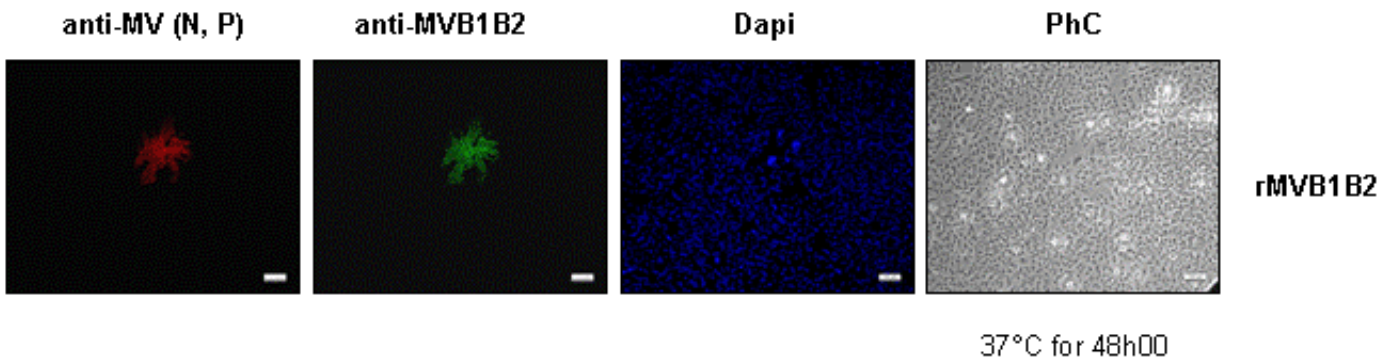
Western Blot

Western blot analysis of rMV-B1B2 infected Vero cells. Malaria antigens were detected using antisera directed against the respective proteins. Additionally, the MV nucleocapsid (N) and phosphoprotein (P) were detected with a specific antiserum (bottom pannel).

All MV genes and rMV transgenes are expressed



Immunofluorescence



Virus	Avrage of Syncytia/well	Number of wells	Syncytia expressing MV-N and P proteins (%)	Syncytia expressing the transgene (%)
rMVB1B2	46	4	100	100

Vero cells were cultured and inoculated with 200 pfu rMV or rMV-B1B2. Expression of measles and the transgene was detected after 48h by staining with specific antibodies. The malaria proteins are expressed and present in all infected cells.

Humoral Response Against Measles & Malaria