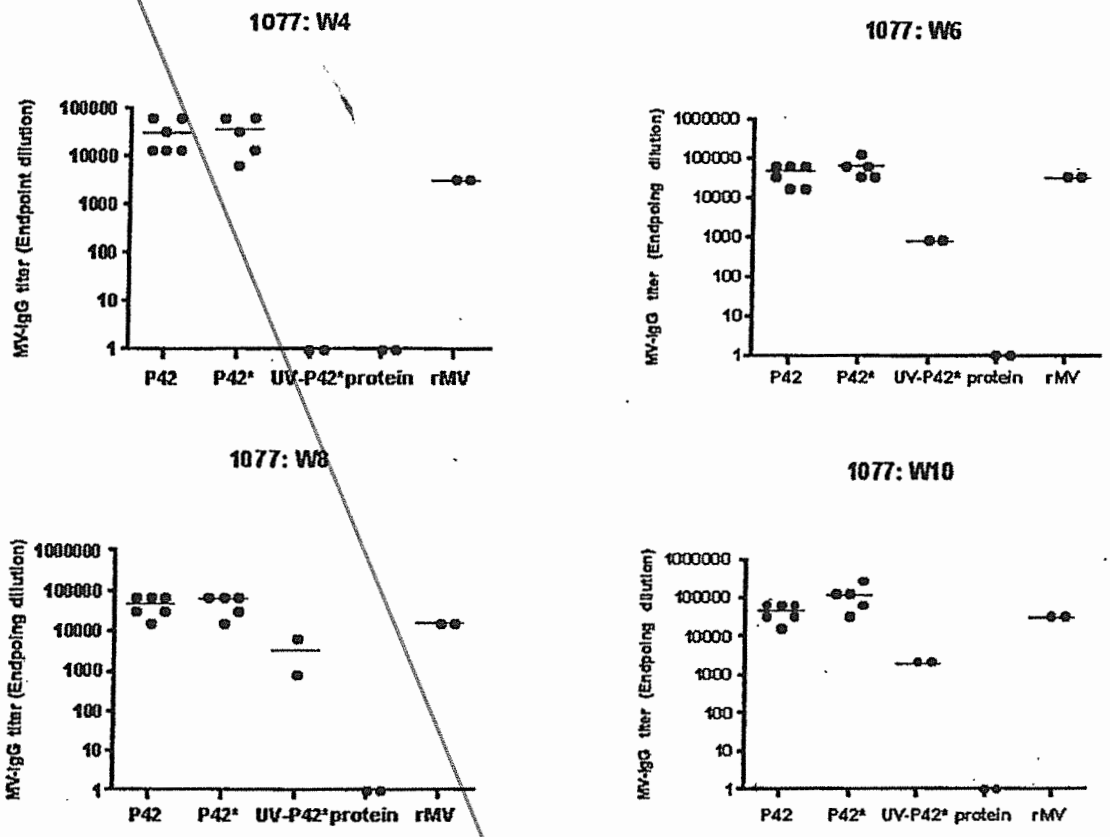
were immunized sub cutaneously with 20 µg of protein in Incomplete Freund's Adjuvant.

The presence of MV-specific antibodies in the sera from the immunised IFNAR/CD46 mice (6 per test group and 3 for control group) was determined by ELISA using 96-microwell plates, coated with Measles virus EIA bulk (ATCC VR-24), for IgG antibody detection. Protein was diluted 0.6 µg/ml with 0.05 M carbonate buffer (pH 9.4), and 100 µl per well was added to 96-well-microtiter plates. The plates were incubated overnight at 4°C, washed with PBS/0.05% Tween 20 (PT) (ph 7.4), incubated with PT (0.1 ml/well)-10% BSA for 60 min at 37°C, and washed again with PT. Serial 2-folds dilutions of the tested sera were added (100 µl/well), and the plates were incubated for 60 min at 37°C. The plates were washed with PT and were incubated with 100 µl of goat anti-mouse IgG HRP diluted 1:2000 in PT for 30 min at 37°C. The plates were washed with PT and incubated with 100 µl OPD (o-Phenylendiamin, Fluka 78411). The reaction was stopped after 3-4 min. Plates were read on a MicroElisa Reader at a wave length of 490 nm. Readings higher than three-folds negative controls were scored as positive reaction.

The presence of MV-Malaria-specific antibodies in the sera of immunised CD46 mice (at least 10 per test group) was determined by ELISA assay. Briefly, 96-microwell plates were coated 50 ng/ well MSP-1-d42 3D7 strains, diluted with carbonate buffer pH 9.4. The plates were incubated overnight at 4°C, washed with PBS/0.05% Tween 20 (PT). Subsequently, unspecific interaction were blocked with 10% defatted milk dissolved in PT for 1hour at 37°C and wells were washed again with PT. The plates were consecutively incubated with various dilutions of mouse sera (starting at 1:200, followed by serial two-fold dilutions), peroxidase-conjugate goat anti-mouse IgG and with OPD substrate. Optical density values were measured at 490 nm. Values above the cut-off background level (mean value of sera from MV immunised mice multiplied by a factor of 2.1) were considered positive. Titres were depicted as reciprocal end-dilutions.

The humoral immune responses against Measles are shown in Figure ~~A~~ 42. The humoral immune responses against Malaria p42 are shown in Figure ~~B~~ 43.

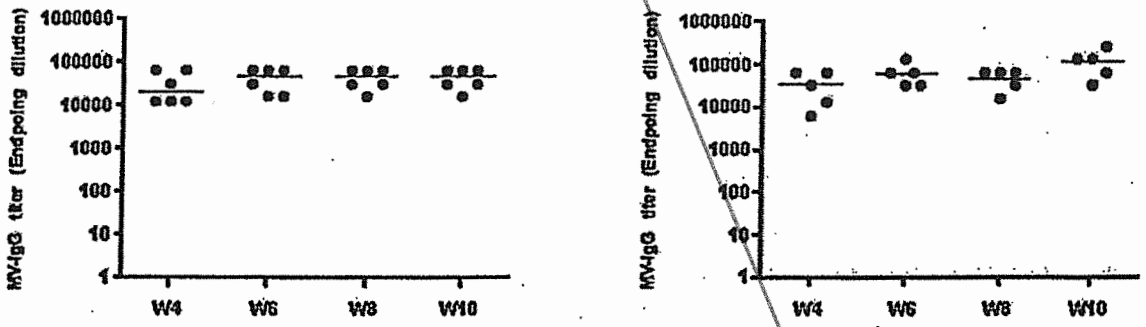
Figure A: Humoral response against Measles



rMeV2EZ-d-p42-SgrAI

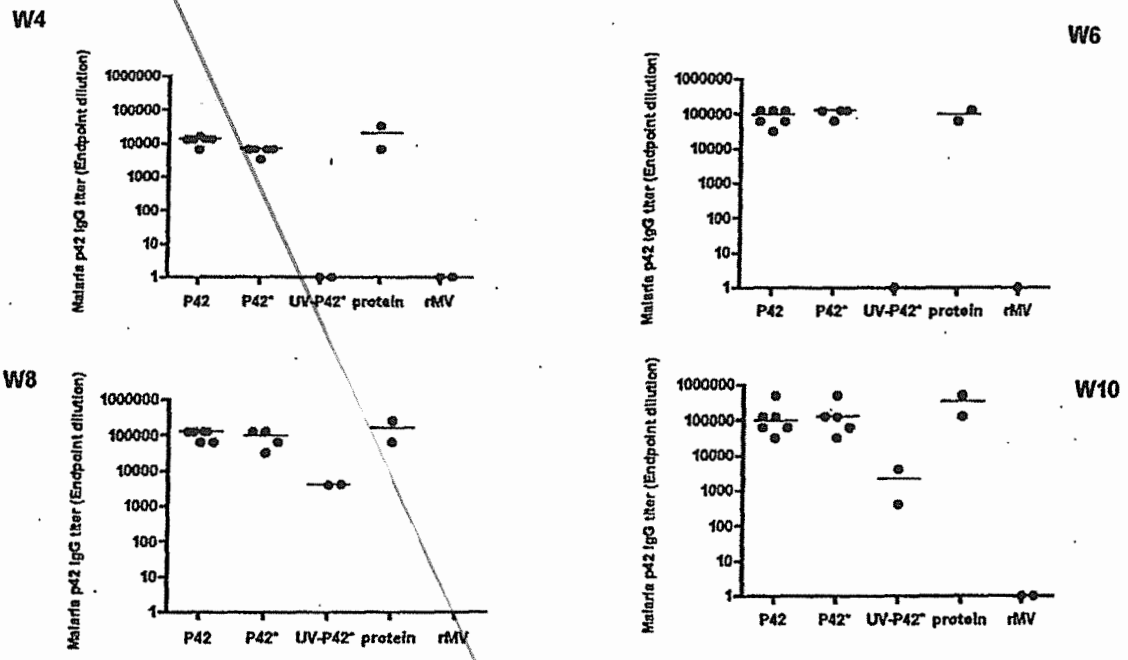
rMeV2EZ-d-p42*-

5 SgrAI



Blood was taken regularly and tested for Measles IgG Titers.

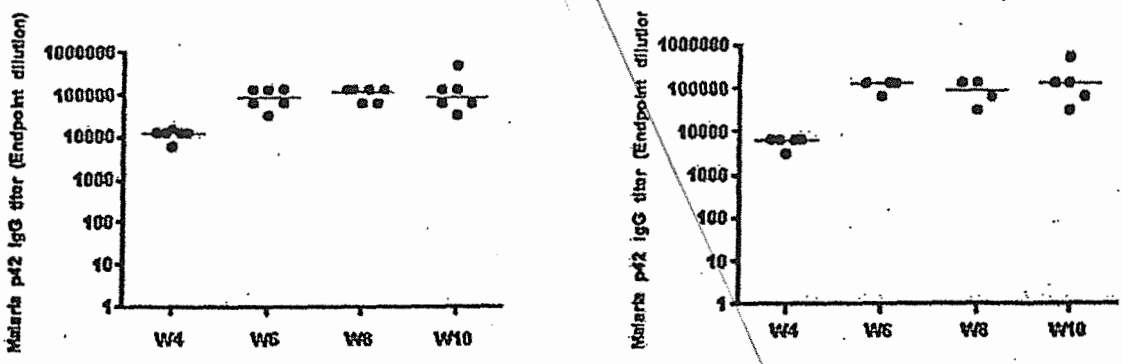
Figure B: Humoral response against Malaria



5

rMeV2EZ-d-p42-SgrAI

rMeV2EZ-d-p42*-SgrAI



10

Blood was taken regularly and tested for Malaria IgG Titers

Example 12: Purification of recombinant measles virus expressing malaria antigens from Defecting Interfering Particles (DIs) by plaque purification.

It is known from literature that after a certain number of passages with *Paramyxoviruses*, and in particular with measles virus, an accumulation of defective interfering particles (DIs) will occur (23, 24). It has been described that these DIs develop various defects: negative impact on vaccine safety, negative influence on virus yields in production, genome instability and suppression of immune reaction after vaccination. In order to avoid such DIs with our new recombinant viruses, we have applied the method of plaque purification as described in example 6 with the exception that we use MRC5 cell instead of 293T cells. After the formation of clear, well defined syncytia we aspirated under the microscope with a micropipette such material for further passaging in a fresh MRC5 tissue culture.

Example 13: Purification of recombinant measles virus expressing malaria antigens from Defecting Interfering Particles (DIs) by end point dilution.

The end point dilution technique was applied in microplates: in all wells a fresh monolayer of MRC5 cells had just developed. The virus suspension containing recombinant measles-malaria viruses was prepared in two fold dilutions. From the well of the latest monolayer where a syncytia was detected the supernatant was aspirated with a pipette. The supernatant was mixed with a suspension containing MRC5 cells. This mixture was incubated at 4°C for 1 hour. Finally, it was transferred in a small Costar flask and incubated at 35°C/5%CO_2 and harvested for purify recombinant measles-malaria virus after ten days.

Example 14: Production of a combined measles-malaria vaccine

The working seed of the described recombinant measles-malaria virus has been incubated on MRC5 cell monolayer in 1750cm² roller bottles at 35 °C for ten days. The cells have been monitored every day for status of health and confluence. On day ten at highest level of syncytia formation, the supernatant was pumped in a steel cylinder for storage in liquid nitrogen. The same procedure was repeated two days later. After performing of all the tests (virus titer, genome stability, virus safety, cell safety, chemical analysis, sterility and others), the harvests have been thawed up and mixed with stabilizer containing gelatine, sorbitol, amminoacids and other sugars to final dilution of 10^5. With a automated filling machine small lyo bottles (F3) have been inoculated with 0.5ml each. A specially calculated lyophilisation program was used to guarantee maximal survival of the product during the freeze-drying process.

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We claim:

1. A combined measles-malaria vaccine comprising a recombinant measles vaccine virus which express malaria antigens capable of eliciting immune response and protection both against measles and malaria.
2. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses single or different malaria antigens.
3. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses MSP1 malaria antigen.
4. The combined measles-malaria vaccine as claimed in claim1 wherein MSP1 malaria antigen is 190 to 200KDa (d190).
5. The combined measles-malaria vaccine as claimed in claim1 wherein MSP1 malaria antigen is d83-30-38.
6. The combined measles-malaria vaccine as claimed in claim1 wherein MSP1 malaria antigen is d42.
7. The combined measles-malaria vaccine as claimed in any preceding claims wherein the recombinant measles vaccine virus expresses MSP1 malaria antigen in both anchored and secreted forms.
8. The combined measles-malaria vaccine as claimed in any preceding claims wherein the recombinant measles vaccine virus expresses MSP1 malaria antigen in both anchored and secreted forms 3D7 strain and MAD 20 preferably the FCB1 strain.
9. The combined measles-malaria vaccine as claimed in any preceding claims wherein the recombinant measles vaccine virus expresses MSP1 malaria antigen in both anchored and secreted forms FCB1 strain.
10. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses Diversity Covering (DiCo) AMA1 malaria antigen.
11. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses DiCo-1 of AMA1 malaria antigen
12. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses DiCo-2 of AMA1 malaria antigen
13. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses DiCo-3 of AMA1 malaria antigen
14. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses DiCo-1, DiCo-2 and DiCo-3 of AMA1 malaria antigen

15. The combined measles-malaria vaccine as claimed in claim10 to claim 14 wherein the recombinant measles vaccine virus expresses Diversity Covering (DiCo) of AMA1 malaria antigen in trans membrane and secreted forms.

16. The combined measles-malaria vaccine as claimed in claim1 wherein the recombinant measles vaccine virus expresses CS malaria antigen

17. The combined measles-malaria vaccine as claimed in any preceding claims wherein the malaria antigen is cloned between P and M or H and L protein of recombinant measles vaccine virus.

18. A measles vaccine virus vector comprising the nucleotide sequence of antigen of malaria.

19. The vector as claimed in claim 18 wherein the nucleotide sequence is selected from figure 26 to figure 35.

20. The vector as claimed in claim 18 and claim 19 wherein the measles vaccine virus vector further comprises nucleotide sequence selected from figure 24 to figure 25.

21. The vector as claimed in claim 18 wherein the nucleotide sequence encodes malaria antigens selected from d83-30-38 and d42 and d190 fragments of MSP1 or Diversity Covering (DiCo) AMA1 or CS protein.

22. A host comprising the vector of claim 18.

23. The host as claimed in claim 22 is selected from E.coli or mammalian cell line.

24. The combined measles-malaria vaccine as claimed in any preceding claims wherein the recombinant measles virus originating from a vaccine strain derived from Edmoston Zagreb.

25. The combined measles-malaria vaccine as claimed in any preceding claims wherein recombinant measles vaccine virus which expresses at least one malaria antigens selected from d83-30-38 and d42 and d190 fragments of MSP1 or Diversity Covering (DiCo) AMA1 or CS protein.

26. The combined measles-malaria vaccine as claimed in any preceding claims wherein recombinant measles vaccine virus which expresses two or more malaria antigens selected from d83-30-38 and d42 and d190 fragments of MSP1 or Diversity Covering (DiCo) AMA1 or CS protein or combination thereof.

27. The combined measles-malaria vaccine as claimed in any preceding claims wherein recombinant measles vaccine virus comprises the following sequences.

MSP-1 d-190-3D7	AN101TE
MSP-1 d-190*-3D7	AN102TE
MSP-1 d-83-30-38-3D7	AN103TE

MSP-1 d-83-30-38*-3D7	AN104TE
MSP-1 d-42-3D7	AN105TE
MSP-1 d-42*-3D7	AN106TE
MSP-1 d-190-FCB1	AN107TE
CS	AN108TE
DiCo1-complete	AN109TE
DiCo1-ecto	AN110TE

28. The combined measles-malaria vaccine as claimed in any preceding claims wherein recombinant measles vaccine virus encods in addition to the malaria antigens protein with adjuvantic properties.

29. The vaccine as claimed in any preceding claims comprising recombinant measles viruses encoding in addition to the malaria antigens an interleukin, preferably interleukin 2.

30. The vaccine as claimed in any preceding claims comprises one of the described recombinant measles malaria viruses or a mixture of two to several such viruses.

31. The vaccine as claimed in any preceding claims wherein the described recombinant measles malaria viruses or a mixture of two to several such viruses devoid of defective interfering particles (DIs)

32. The vaccine as claimed in any preceding claims wherein the adventitiously arisen DI particles have been eliminated by plaque purification.

33. The vaccine as claimed in any preceding claims wherein the adventitiously arisen DI particles have been eliminated by end point dilution.

34. The vaccine as claimed in any preceding claims wherein the adventitiously arisen DI particles have been eliminated by physical methods such as differential centrifugation.

35. The vaccine as claimed in any preceding claims being a component of a combined vaccine where the other components are rubella, mumps, varicella or another life attenuated vaccine virus, naturally attenuated or recombinant, alone or in combination.

36. The vaccine as claimed in any preceding claims comprises suitable stabilizer, such as gelatin and/or human serum albumin and sorbitol as main components, ideal for parenteral application.

37. The vaccine as claimed in any preceding claims comprises with a suitable stabilizer and/or adjuvant ideal for intranasal application.

38. The vaccine as claimed in any preceding claims comprises suitable stabilizer and/or adjuvant ideal for inhalation application.

39. The vaccine as claimed in any preceding claims comprises suitable stabilizer and/or adjuvant ideal for oral application.

40. The vaccine as claimed in any preceding claims comprises suitable stabilizer and/or adjuvant ideal for trans dermal application.

41. The vaccine as claimed in any preceding claims comprises suitable stabilizer and/or adjuvant ideal for any suppository formulation.

42. A composition of combined measles-malaria vaccine comprising stabilizer and/or adjuvant.