SCIENTIFIC DISCUSSION

1. Introduction

ProQuad is a quadrivalent vaccine containing the components of measles, mumps, rubella vaccine (more attenuated vaccine strain of measles virus (derived from Enders' attenuated Edmonston strain), the Jeryl Lynn strain of mumps virus, the Wistar RA 27/3 strain of live attenuated rubella virus) and of VARIVAX (Oka/Merck strain of varicella virus). ProQuad is indicated for simultaneous vaccination against measles, mumps, rubella, and varicella in individuals from 12 months of age.

Measles (rubeola) is caused by a paramyxovirus of the genus Morbillivirus and is transmitted from person to person via aerosolized or large respiratory infectious droplets. The clinical presentation consists of prodromal fever, conjunctivitis, coryza, and cough. In some cases, Koplik spots (an erythema with white spots in the buccal mucosa) can be observed. Subsequently, a maculopapular rash usually appears, spreads from the head to the entire body, and fades within 4 to 7 days. Measles can result in otitis media, pneumonia, encephalitis and death.

Mumps is caused by a paramyxovirus of the genus Rubulavirus and is spread by direct contact via the respiratory route. The clinical presentation is characterized by swelling of one or more salivary glands (usually the parotid glands) and may be preceded by several days of non-specific symptoms, including fever, lymphadenopathy, headache, malaise, myalgias, and anorexia. Mumps can result in deafness, orchitis, pancreatitis, meningitis, encephalitis and death.

Rubella is caused by a togavirus of the genus Rubivirus and is spread via infectious droplets shed from the respiratory secretions of infected persons to susceptible individuals. The clinical presentation is characterized by nonspecific signs and symptoms including transient erythematous and sometimes pruritic rash, postauricular or suboccipital lymphadenopathy, and low-grade fever. The most important consequences of rubella are the miscarriages, stillbirths, fetal anomalies, and therapeutic abortions, associated with Congenital Rubella Syndrome (CRS) that result when rubella infection occurs during early pregnancy. Anomalies associated with CRS include sensorineural deafness, cataracts, glaucoma, and other ophthalmic disorders, cardiac defects, microcephaly, meningoencephalitis and mental retardation.

Varicella is caused by varicella-zoster virus (VZV), a herpes virus. The clinical presentation of varicella is characterized by fever, malaise, and a generalized rash. The rash is usually pruritic and consists of 300 to 500 maculopapular lesions that progress to vesicles, and crusts over the course of several days. The skin lesions are generally concentrated on the face, head, and trunk. Varicella may be associated with serious and life-threatening complications including bacterial superinfection of skin lesions with S*taphylococcus aureus or Streptococcus pyogenes,* viral or bacterial pneumonia, septic shock, secondary bacterial arthritis, fasciitis, cerebella ataxia and encephalitis.

2. Quality aspects

Introduction

The finished product is presented as a powder and solvent for suspension for subcutaneous injection in a single 0.5 ml dose. The lyophilised vaccine must be stored frozen at -15ºC or colder. The lyophilised powder is presented in a vial (Type 1 glass) with a butyl rubber stopper and flip-off aluminium seal. The finished product contains the following excipients: sucrose, hydrolysed gelatin (porcine), sodium chloride, sorbitol, monosodium glutamate, sodium phosphate, sodium bicarbonate, potassium phosphate, potassium chloride, Medium 199 with Hanks' Salts, Minimum Essential Medium Eagle (MEM), neomycin, phenol red, hydrochloric acid and sodium hydroxide (pH adjustment).
Before use, each vial is to be reconstituted with 0.7 ml water for injections supplied in either a vial (Type 1 glass) with a butyl rubber stopper or in a prefilled syringe (Type 1 glass) with plunger stopper and tip cap (chlorobutyl rubber).

The lyophilised vaccine must be stored frozen at -15ºC or colder, whereas the diluent should be stored refrigerated or at room temperature. Therefore, the product is shipped using a styrofoam box allowing packing the frozen component and the non-frozen component together. This polystyrene container is composed of two compartments. The frozen component is placed in the lower compartment where dry ice is used as the refrigerant. The non-frozen component is placed in the receptacle compartment in the lid, so it is not exposed to the freezing conditions of the dry ice.

After reconstitution, one dose (0.5 ml) contains:

Measles virus[1] Enders' Edmonston strain (live, attenuated) ..... not less than 3.00 $\log_{10}$ $TCID_{50}$*
Mumps virus[1] Jeryl Lynn™ (Level B) strain (live, attenuated) ..... not less than 4.30 $\log_{10}$ $TCID_{50}$
Rubella virus[2] Wistar RA 27/3 strain (live, attenuated) ..... not less than 3.00 $\log_{10}$ $TCID_{50}$
Varicella virus[3] Oka/Merck strain (live, attenuated) ..... not less than 3.99 $\log_{10}$ PFU**

* 50% tissue culture infectious dose
** plaque-forming units

([1]) Produced in chick embryo cells.
([2]) Produced in human diploid lung (WI-38) fibroblasts.
([3]) Produced in human diploid (MRC-5) cells.

The product also contains human serum albumin, used in the manufacturing process of the vaccine.

Active substance – measles

- Manufacture

Seed lot system

The Enders' Edmonston strain of measles virus was isolated in primary human kidney cell tissue culture from the blood of a child (Edmonston) in the early acute phase of measles. The virus (10 ml) was received by Merck from Dr. John Enders at the Children's Hospital of Harvard Medical School in 1960. Further passages were performed at Merck to develop the Moraten (more attenuated Enders) strain that served as a pre-master seed from which Master Seed was derived. The preparation of the Master Seed and the Stock Seed is appropriately described in the dossier.

Chicken embryo cells (CEC) as cell substrate

To prepare the cell substrate for virus propagation, eggs, sourced from a specific-pathogen-free (SPF) chicken flock, are incubated and prepared.

Manufacture of measles harvested virus fluids (HVFs)

A virus propagator, a stainless steel tank, is planted with CEC suspension. The cells are infected with an appropriate volume of thawed measles stock seed, added to the seeding medium, stirred and incubated. The cell sheets are rinsed and refed several times and virus propagators are harvested. HVF is sampled for virus potency and sterility.

Manufacture of redispensed bulk

Harvests from one or more batches of HVF may be used to produce a single batch of measles vaccine bulk. The final bulk is dispensed in cans (dispensed bulk) and stored frozen. The dispensed bulk cans comprise a batch of drug substance. The dispensed bulk is thawed and used for filling or redispensed into aliquots appropriate for filling (*redispensed bulk*). Samples for QC are drawn from the appropriate different bulk stages.

Control cell Cultures and Harvest Control Fluids (HCFs)

Uninfected harvested control fluids (HCF) are produced using the same cell substrate and culture media. Before the final collection of the HCF, control cell monolayers are examined microscopically throughout the harvest period.

Controls of materials and critical steps / process validation

The CEC substrate used in the manufacture of measles vaccine bulk is tested according to Ph. Eur. requirements.
Testing of the measles stock seed is consistent with the Ph. Eur., Section 2.6.16 and the monograph for Measles Vaccine (Live), with the exception of the virus identification test. Identity testing is instead performed post-clarification on the vaccine bulk, where antibody neutralization can be performed on a clarified bulk virus solution.

Critical process parameters (CPPs), critical quality attributes (CQAs), and their specifications/acceptance criteria are based on historical process capability, current manufacturing specifications, and the specifications defined in the company's monovalent measles vaccine license.

Process validation was both retrospective and prospective. Retrospective validation of measles vaccine was first used to determine acceptable ranges; a prospective validation of measles vaccine was then performed to demonstrate conformity of the processes to validation specifications. Within each manufacturing process step, goals, CPPs and CQAs were determined, along with appropriate specifications and acceptance criteria.

- Characterisation and specifications

The complete nucleotide sequences for the Stock Seeds and a monovalent measles filled container vaccine lot have been determined. Nucleotide sequence alignment showed complete agreement.

Process-related impurities arising from the measles vaccine bulk manufacturing processes are classified as cell substrate or cell culture derived.
Cell substrate derived impurities may include proteins derived from the host organism, such as CECs used as substrate for measles vaccine bulk production. Cell culture-derived impurities may include antibiotics (e.g., neomycin), serum, or other media components. Also low levels of particle-associated reverse transcriptase activity are found; however, no signal of infectious retrovirus could be detected.

Since the measles process uses cell growth medium containing fetal bovine serum (FBS), measures have been taken to minimize the concentration of bovine serum proteins in the vaccine bulk. The concentration of bovine serum albumin (BSA) is used as a surrogate marker for other bovine serum proteins. Each measles final bulk is tested for BSA. Measles vaccine bulk is an unpurified product whose potency was measured through a biological assay for the active substance rather than through evaluation of integrity of physical form. Degradation products are neither identified nor quantified.

Tests are performed at specified stages of vaccine bulk processing in order to confirm absence of extraneous agents, to verify potency and identity, and to provide a measure of quality and process consistency. Most assays performed on measles bulks are qualitative methods for which there are only two outcomes (growth or no growth, absence or presence, etc.). In many of these cases, the assay specifications are compendial.

The parameters that were evaluated as part of the method validation for the assays have been provided for each analytical procedure. When applicable, the assay parameters addressed were specificity, inter-assay precision, limit of detection, limit of quantification, linearity, range, ruggedness, and robustness.

Batch analysis results have been provided for HVF/HCF lots and dispensed bulk lots; all results met specifications.

The reference standard used in potency testing is a monovalent measles vaccine lot manufactured using currently approved processes. The applicant committed to characterize the performance of the measles potency assay with international reference standards.

- Stability

The stability studies were initiated using three lots of vaccine bulk. The current storage time of these lots was documented as the initial testing time point. Satisfactory stability results for these three lots of measles final bulk are available.

Stability results, combined with the production history of measles final bulk, were evaluated to determine the maximum hold time used for vaccine bulk prior to processing into the filled container. The resultant filled containers passed release specifications for potency. Formal stability studies are ongoing to justify the proposed hold time and results will be provided on an annual basis.

Active substance - mumps

- Manufacture

Seed lot system

The Jeryl Lynn strain of mumps virus was isolated from a throat washing specimen collected in 1963 from a clinical case of mumps (Jeryl Lynn) by Dr. M. R. Hilleman, Merck Research Laboratories, Merck & Co., Inc. Virus strain isolation was performed at the Merck West Point, Pennsylvania facility. The preparation of the master seed and the stock seed is described in detail in the dossier.

Manufacture of mumps harvested virus fluids (HVFs) and redispensed bulk

CEC are planted in analogy to the process described for measles. Post-infection, the virus propagators are refed and the spent medium is drained and discarded; the virus harvest is collected. The HVFs are sampled for virus potency and sterility and shell frozen.

The redispensed bulk is manufactured in analogy to the process described for measles.

Control cell Cultures and Harvest Control Fluids (HCFs)

The HCFs are manufactured in analogy to the process described for measles.

Controls of materials and critical steps / process validation

The seed testing is consistent with the Ph. Eur., Section 2.6.16 and monograph for Mumps Vaccine (Live), with the exception of the virus identification test. Identity testing is instead performed post-clarification on the vaccine bulk, where antibody neutralization can be performed on a clarified bulk virus solution.

Definition of CPPs and process validation were performed in a similar manner as for measles.

- Characterisation and specifications

To assess the population diversity of the stock seed and bulk product, the JL-strain specific nucleotide sequences were determined and results provided in the dossier.

Process-related impurities arising from the mumps bulk manufacturing processes may be classified as cell substrate-derived or cell culture-derived. Since the mumps process uses cell growth medium containing fetal bovine serum (FBS), mumps bulk lots were tested for BSA and the results for all of these lots were within the specification. Mumps vaccine is an unpurified product whose potency is

measured through a biological assay for the active substance rather than through evaluation of integrity of physical form. Degradation products are neither identified nor quantified.

The testing (and method validation) of the mumps bulk is essentially the same as for the measles bulk. Batch analysis results have been provided for HVF/HCF lots and dispensed bulk lots; all results met specifications.
The reference standard used in potency testing is a monovalent mumps vaccine lot manufactured using currently approved processes. The applicant committed to characterize the performance of the mumps potency assay with international reference standards.

- Stability

The stability studies with the mumps final bulk were initiated using three lots of vaccine bulk. The current storage time of these lots was documented as the initial testing timepoint. Stability results for these three lots of mumps final bulk are available.
Stability results, combined with the production history of mumps final bulk, were evaluated to determine the maximum hold time used for vaccine bulk prior to processing into the filled container. The resultant filled containers passed release specifications for potency formal stability studies are ongoing to justify the proposed hold time and results will be submitted on an annual basis.

Active substance - rubella

- Manufacture

Seed lot system

The Wistar RA 27/3 strain of rubella virus was isolated in 1964 by Dr. Stanley Plotkin, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, U.S., from a kidney explant obtained from a surgically aborted foetus. It was directly inoculated into WI-38 cells, and then attenuated. The preparation of the Master Seed and the Stock Seed is appropriately described in the dossier.

Release testing results were presented for Virus Stock Seed Lots.

Human diploid fibroblast cells (WI-38) as cell substrate

The source of the cell substrate used in the manufacture of rubella vaccine is female, embryonic, human, lung tissue (WI-38) obtained from the Karolinska Institut, Stockholm, Sweden. Primary cells were isolated and a cell suspension was prepared at a population doubling level (PDL) of 8. Frozen ampoules of cells at PDL of 8 were sent to the American Type Culture Collection (ATCC) for storage.

WI-38 working cell banks (WCBs) are prepared using appropriate cells from the ATCC. WCB lots have been used in clinical trials; in the meantime, the stock for these two WCBs has been depleted and a new WCB lot was manufactured by the method described in the dossier and has passed all release testing.

Manufacture of rubella harvested virus fluids (HVFs)

An appropriate number of WCB ampoules are expanded to create a sufficient amount of cell substrate. Post-plant, the spent medium is removed and discarded. A sufficient quantity of rubella stock seed is added. Following virus adsorption, the infected cells are refed and incubated.

Post-infection, the spent medium is removed and discarded; the cell sheets are rinsed, refed and incubated.

The HVF are collected, pooled and mixed with a stabilizer. The HVF is sampled for virus potency and sterility.

Manufacture of redispensed bulk

Harvests from one or more batches of HVF may be used to produce a single batch of rubella dispensed bulk which is redispensed into appropriate aliquots. The dispensed bulk cans comprise a batch of drug substance. The redispensed bulk is diluted to target fill potency during the formulation of ProQuad.

Control cell Cultures and Harvest Control Fluids (HCFs)

Control roller bottles and HCFs are prepared in analogy with the HVFs.

Controls of materials and critical steps / process validation

Historically, no direct qualification/certification of the WI-38 master cell bank was performed. Each WI-38 WCB is further tested to ensure freedom from extraneous agents and certify the bank for use in manufacturing. Cells from each WCB are passaged to the vaccine production PDL level or beyond to demonstrate safety and acceptable karyology at the PDL intended for use in harvested virus fluid (HVF) manufacturing. Release testing is described at appropriate process steps and will be performed in compliance with Ph. Eur 5.2.3.
Historically, no direct qualification/certification of the rubella master seed was performed. Release testing of stock seeds is performed at appropriate process steps.

Definition of CPPs and process validation were performed in a similar manner as for measles.

- Characterisation and specifications

Rubella virus Stock Seed Lots showed complete agreement in the nucleotide sequence alignment.

Process-related impurities arising from the rubella vaccine bulk manufacturing processes are classified as cell-substrate or cell-culture derived. Cell-substrate-derived impurities may include proteins derived from the host cell line; cell-culture-derived impurities may include antibiotics (e.g., neomycin), serum, or other media components.

Rubella vaccine bulk is an unpurified product whose potency is measured through a biological assay for the active substance rather than through evaluation of integrity of physical form. Degradation products have been neither identified nor quantified.

Drug substance release tests are performed at the specified stages of vaccine bulk processing in order to confirm absence of extraneous agents, to verify potency and identity, and to provide a measure of quality and process consistency. For qualitative assays, the specifications are based on historical data.
Assays involved in control of drug substance are performed according to approved control procedures that describe the main steps in a procedure.

The validation was performed using the assay procedure that was in place at the time the assays was validated. The parameters that were evaluated as part of the method validation for the assays were provided for each analytical procedure.

Batch analysis results have been provided for three HVFs, pooled bulk lots and ProQuad filled container lots. Consistency of production was demonstrated and all lots met the specifications.
The reference standard used in potency testing is a monovalent rubella vaccine lot manufactured using the currently approved process. The applicant committed to characterize the performance of the rubella potency assay with international reference standards.

- Stability

Formal stability studies with the rubella final bulk were initiated using three lots of vaccine bulk. The current storage time of these lots was documented as the initial testing timepoint. Stability results for

these three lots of rubella final bulk are available. The study is on-going and results will be submitted on an annual basis.

Active substance - varicella

Seed lot system

The Oka strain of the varicella –zoster virus (VZV) was isolated from fluid taken from the vesicles of a 3-year-old boy with a case of chicken pox. The virus was isolated in primary human, embryonic lung cells (HEL) and was passaged 11 times. The strain was further passaged 12 times in guinea pig embryo fibroblasts (GPE) to attenuate the strain and once in human diploid cells (WI-38) to passage 24. One vial of frozen infected cells of the passage 24 Oka VZV strain was received by Merck from Osaka University.
Although no clinical studies with ProQuad have been conducted using varicella virus from the passage levels intended for commercial production, varicella vaccine at the passage level for commercial production was developed and evaluated in the setting of the applicant's monovalent varicella vaccine, VARIVAX. The preparation of Master Seed and Stock Seed lots are appropriately described in the dossier.

Human diploid fibroblast cells (MRC-5) as cell substrate

MRC-5 cells, a human, embryonic, lung, fibroblast cell line (diploid, male) originally isolated by J.P. Jacobs at the National Institute for Medical Research (London, England) and deposited at approximately population doubling level (PDL) 7 at the National Institute for Biologicals Standards and Controls (NIBSC).

Manufacture of varicella harvested virus fluids (HVFs)

A vial from the MWCB is thawed and planted. Cells are trypsinized and finally planted for infection. A batch of HVF represents mechanically harvested, varicella-infected MRC-5 cells.

Based on appropriate criteria, the concentration of the working seed is adjusted. Each production roller bottle is planted with working seed cell suspension and incubated. The spent medium is removed and discarded, and each cell culture is rinsed. Stabilizer is added; the suspension is removed and stored with appropriate conditions.

Manufacture of dispensed bulk

Varicella dispensed bulk is a blend of HVF lots. The cells in the HVF suspension are disrupted and clarified. This volume is dispensed prior to freezing. The dispensed final bulk containers (dispensed bulk) comprise a batch of the active substance and are stored.

Control cell Cultures and Harvest Control Fluids (HCFs)

Final harvested control fluids are tested for sterility, mycoplasms, and tissue culture safety, while cells are tested for hemadsorption.

Controls of materials and critical steps / process validation

The MRC-5 MCB and WCB are tested to ensure freedom from extraneous agents and to ensure that the cells behave normally through production use PDL. Release testing is described at appropriate process steps and will be performed in compliance with Ph. Eur 5.2.3.
Release testing of the varicella master seed and stock seeds is performed in compliance with Ph. Eur. The applicant committed to consult the EMEA to discuss the need for monkey neurovirulence testing on any new varicella master seed if MNV is still required in the Ph. Eur.

Within each manufacturing process step, goals, CPPs, and CQAs were determined, along with appropriate specifications and acceptance criteria.

- Characterisation and specifications

The complete sequences of the Oka/Merck strain and the wild-type Oka parent have been determined. Process-related impurities arising from the rubella vaccine bulk manufacturing processes are classified as cell-substrate or cell-culture derived. Cell-substrate-derived impurities may include proteins derived from the host cell line; cell-culture-derived impurities may include antibiotics (e.g., neomycin), serum, or other media components. Varicella process uses cell growth medium containing fetal bovine serum. Serum protein clearance is provided by rinsing the cell layers to remove as much serum as possible prior to virus harvest. Each varicella final bulk is tested for BSA.

Assays are performed at several stages of processing of vaccine bulks in order to confirm absence of extraneous agents, to verify potency and identity, and to provide a measure of quality and process consistency. Assays involved in release testing of drug substance are performed according to approved control procedures that describe the main steps in a procedure. Most assays performed on varicella vaccine bulks and bulk intermediates are qualitative methods for which the experimental outcome is only: growth or no growth, absence or presence etc... In many of these cases, the assay specifications are compendial. For quantitative assays, the acceptance criterion is based on historical data.
The validation was performed using the assay procedure that was in place at the time the assays was validated. The parameters that were evaluated as part of the method validation for the assays were provided for each analytical procedure.
Batch analysis results have been provided for three HVFs, pooled bulk lots and ProQuad filled container lots. Consistency of production was demonstrated and all lots met the specifications.

The reference material used for the varicella potency and antigen content is described appropriately. To generate an antigen reference standard lot, material from multiple varicella final bulk lots is pooled, filled, and lyophilized according to procedures applied in VARIVAX vaccine manufacture. Varicella antigen content in a new reference standard is established by calibration against a previously qualified reference standard. The applicant satisfactorily demonstrated that varicella standards are stable, perform in concordance with test samples in a specific assay and that the assigned potencies of standards are linked to potencies of lots, shown in clinical studies to be efficacious. However, the difference between observed and assigned potencies of reference standards is not fully understood yet and therefore, sequential calibration of standards should be avoided. Therefore, the applicant committed to establish a ‘gold standard’ with a link to the clinic which in the future will be used to calibrate standards included in varicella potency testing.

- Stability

Formal stability studies with the varicella final bulk were initiated for three lots. Stability results for these three lots of varicella final bulk are available. Formal stability studies are ongoing to justify the proposed hold time.

Finished product

ProQuad is a sterile lyophilized vaccine preparation combining the four viruses used in the manufacture of currently licensed M-M-R II and VARIVAX vaccines from Merck. Sterile water for injections is provided for reconstitution. The product is intended for single-dose administration and contains no preservative.

- Pharmaceutical Development

The formulation composition of ProQuad is based on the formulation compositions of the currently licensed trivalent vaccine for measles, mumps and rubella, and for varicella vaccine. The individual viruses are known to be compatible with their own stabilisers. The compatibility of varicella virus with diluents used for measles, mumps and rubella and of the measles, mumps and rubella viruses with

the varicella stabiliser have been demonstrated through stability studies. The formulation of ProQuad vaccine is appropriately described in the dossier.

Three lots were tested in one Clinical Study aimed to determine the varicella dose necessary to elicit the minimum acceptable immune response. The filling potency of varicella in these lots was increased to compensate for the reduced immunogenicity observed in the presence of measles, mumps, and rubella viruses. Three lots of ProQuad vaccine were then manufactured to demonstrate clinical consistency and were considered the initial process validation series.
Each vial is reconstituted with 0.7 ml diluent (0.2 ml overage) to ensure that a 0.5 ml dose can be recovered.

- Manufacture of the Product

All manufacturing operations are performed at Merck & Co., Inc, West Point, Pennsylvania, USA.

Due to the range of potency of the starting vaccine bulks, a dilution factor specific for each vaccine bulk component is calculated to target the desired potency in the filled container.

Predetermined amounts of measles, mumps, and rubella bulks are mixed into the intermediate stabilizer for a minimum time to ensure homogeneity, forming the measles, mumps, and rubella intermediate (MMR intermediate).

The required amounts of measles, mumps and rubella vaccine intermediate is transferred into the stabilizer. Varicella vaccine bulks are added and mixed for a minimum time to ensure homogeneity, forming the final formulated bulk (FFB). The FFB is then sampled for sterility and neomycin testing. The FFB is maintained at 2-8 °C with mixing throughout the pooling and subsequent vial filling process. CPPs are identified.

Vials are filled using an automatic filling machines. Filled vials are then lyophilised for an appropriate cycle time. The vials are removed from the lyophilization chamber and stored at appropriate temperature prior to sealing. The time that vaccine is held at room temperature during sealing, inspection, labelling, packaging and assembly operations is documented. Inspected sealed vials are stored at appropriate temperature until they are packaged. After packaging, the vaccine may be stored at < 15°C for a maximum of 18 months as stated in the Summary of Product Characteristics. CPPs and CQAs of the filling process are identified and include filling volume, time in solution of the active substances, and transfer time to the lyophilization cabinet.

Qualified insulated containers have been designed specifically for frozen vaccine shipments. During transport to Europe, a temperature below –20 ºC is maintained.

Process validation for ProQuad was successfully performed by comparing the results of three validation lots. The validation results demonstrate that the predefined specifications for CPPs and CQAs were met.

Both, the sterile diluent in a syringe with fixed-needle and the diluent in a syringe without needle syringe are manufactured by an outside vendor. The diluent in a vial is manufactured by Merck & Co., Inc, West Point, Pennsylvania, USA.

At the outside vendor, Water for Injection (WFI), manufactured by distillation of purified water, is filled into glass syringes and sterilized. Raw materials are tested according to standard operating procedures or according to specifications and methods described in pharmacopoeias. Raw materials are in accordance with specifications. The manufacturing process is described in detail and all relevant information regarding quality control, validation of the manufacturing process and stability of the diluent have been provided by the applicant.

At Merck, WFI, manufactured by distillation of purified water, is filled into glass vials and terminally sterilized. The manufacturing process is described in detail and all relevant information regarding

quality control, validation of the manufacturing process and stability of the diluent have been provided by the applicant

- Control of excipients

The excipients are derived from specific monovalent viral bulks and stabilisers. Culture media are also used as diluent to achieve a consistent chemical composition, since viral bulks of different potencies are diluted to a target potency (for each virus) at the time of formulation. Different proportions of viral bulk and diluent are necessary to ensure consistent release potency and chemical composition between lots. In addition, human serum albumin (HSA) is used as a component of the cell culture medium and consequently, is present in the drug product as a residual.

Except for hydrolyzed gelatine and phenol red, the stabilizers used are compliant with all existing compendial monographs. The excipients of human or animal origin, hydrolyzed porcine gelatine and human serum albumin (HSA), are derived from non-ruminant sources and therefore in compliance with Ph.Eur. Chapter 5.2.8. HSA is obtained from vendors that use validated ethanol precipitation and heat treatment. The plasma pools are tested in compliance with the CPMP Note for Guidance for Plasma Pool testing.

- Product Specification

The testing scheme of the finished product represents a combination of the regimens used for the testing of measles, mumps, rubella, and varicella virus-containing vaccines, all of which are in currently licensed products.

Tests are performed on the drug product to ensure safety, sterility, to confirm the identity and quantify the potency of the product, and to provide a measure of process consistency. Assays employed in control of the finished product lots are performed according to approved CPs that describe the main steps in a procedure.

Each dose of the vaccine contains at the end of its shelf-life a minimum of 3.00 log $TCID_{50}$ measles virus, 4.30 log $TCID_{50}$ mumps virus, 3.00 log $TCID_{50}$ rubella virus, and 3.99 log plaque forming unit (PFU) varicella virus. The release specifications have been selected to ensure that, at expiry, each dose will contain the aforementioned minimum potency for each virus when the vaccine is reconstituted and stored at room temperature for 30 minutes.

Several assays performed on the finished product are qualitative methods for which there are only two experimental outcomes (growth or no growth, absence or presence, etc.). In many cases, the assay specifications are compendial.

The potency specifications for filled container have been derived from several sources. Each virus release potency is described in the dossier.

The parameters that were evaluated as part of the method validation for the assays are listed for each analytical procedure. When applicable, the assay parameters addressed were specificity, inter-assay precision, limit of detection, limit of quantisation, linearity, range, ruggedness, and robustness.

Batch analysis was performed on three process validation lots within the range of a commercial lot size. All results met the pre-defined specifications.

Because ProQuad is a live virus vaccine composed of measles, mumps, rubella and varicella bulks prepared from cell culture fluids, it is not a highly purified product. To provide a marker for removal of fetal bovine serum used during the cell culture process, a quantitative test for residual BSA is conducted on the virus bulks. This BSA content is used to calculate the amount of BSA present in the filled container based on the dilution of each bulk during filling. A specification exists for BSA content in filled container (≤ 500 ng BSA per single human dose) as per the Ph. Eur. monograph 0648 even though filled container material is not directly tested.

The applicant committed to characterize the performance of his potency assay with international reference standards and to establish a 'gold standard' with a link to the clinic for calibration of future standards used in ProQuad potency testing.

- Viral safety and TSE

Adventitious Agents

The testing program for adventitious agents is described in detail in the chapters on the Measles, Mumps, Rubella, and Varicella active substances. All raw materials used in vaccine manufacturing are tested for adventitious agents prior to release and use in manufacturing. Validated processing steps that add additional levels of confidence for the absence of adventitious agents are filter sterilization and ultraviolet (UV)- or gamma -irradiation.

TSE

The manufacturing process for ProQuad™ was evaluated for the theoretical risk of transmission of infectivity associated with BSE prions, with the conclusion that the risk of BSE transmission in ProQuad is exceedingly remote. The rationale and the calculation for the theoretical risk of transmission of infectivity associated with BSE prions were provided.
Biological reagents used in the manufacture of the vaccine or intermediates include iron-enriched bovine calf serum (BCS), fetal bovine serum (FBS), porcine pancreatic trypsin, porcine-derived hydrolyzed gelatine, choline chloride, bovine or porcine tallow-derived polysorbate 80, fish or sheep wool-derived cholesterol, amino acids, and human serum albumin (HSA). Certificates of Suitability (CoS), which are granted by the European Directorate for the Quality of Medicines (EDQM), and the measures applied (e.g. regular audits of vendor facilities, testing to ensure that the appropriate quality standards are met, etc.) ensure that the ruminant-derived raw materials currently used in manufacturing are free of transmissible spongiform encephalopathy (TSE) or bovine spongiform encephalopathy (BSE) contamination.

- Stability of the Product

Stability tests have been designed to measure product performance under anticipated handling and storage conditions and under stressed conditions that might be encountered after distribution. The anticipated conditions following lyophilization were studied. Upon use, the vaccine is reconstituted and may be stored for up to 30 minutes at room temperature prior to injection.

Stability studies were conducted on different process validation lots at various temperatures described in the dossier suitable to support the storage conditions of the vaccine.
All viruses undergo a statistically significant loss of potency when stored at 2–8 °C or higher, which underscores the importance of frozen storage of the vaccine.

Available stability data indicate this vaccine to be satisfactorily stable for at least 18 months when stored at ≤ -15 °C (frost-free) and up to 30 minutes at room temperature following reconstitution immediately prior to use as stated in the SPC.

Stability studies are on-going In addition, post-launch vaccine lots will be placed on stability on an annual basis for the purpose of routine monitoring. Full testing will be performed at initial and expiry intervals; a subset of the tests will be performed at each time interval as appropriate.

Discussion on chemical, pharmaceutical and biological aspects

During the evaluation of ProQuad, two major objections were identified. Firstly, there was a question whether the varicella seed lot system used by the manufacturer conforms to Ph. Eur. Chapter 5.2.1; the terminology used in the dossier was clarified and it was shown to be conform to the Ph. Eur. requirements.

Secondly, the change of a higher passage level for the varicella component, as described in the dossier, raised concerns regarding safety and efficacy of the higher passage vaccine. However, a corresponding change had been approved for the monovalent Varivax vaccine, based on data from a clinical study. Since no clinical studies were performed for ProQuad to address this issue, data from Varivax are regarded as relevant and suitable to demonstrate, that the additional passage does not change the quality of the varicella component. Hence it can be concluded, that requesting a further clinical study, to investigate the safety and efficacy of ProQuad, comprising the higher passage varicella component, is not justified.

A number of other concerns, including setting potency specifications for HVFs and the dispensed bulk, setting a specification for BSA content in the measles, mumps and rubella bulks. The applicant was also asked to characterize the performance of the measles, mumps and rubella potency assay with international reference standards. Regarding the calibration of potency results for measles, mumps and rubella and calibration of varicella and ProQuad reference standards used for calibration of potency results for varicella, both issues are resolved, resulting in one follow-up measure. Data provided by the applicant satisfactorily address the concerns raised and support the view, that the calibration procedure is suitable and of significance to consistently manufacture vaccine of satisfactory quality.

Several commitments are made by the applicant, and several follow-up measures are defined to provide further information post-approval. In conclusion, all quality issues are resolved.

3. Non-clinical aspects

Introduction
ProQuad is a combination of known marketed antigens (measles, mumps, rubella vaccine and varicella vaccine). These authorised viral components have been administered safely to millions of children and adults. Furthermore the safety of these authorised components has been established pre-clinically by extensive safety testing of cell cultures, cell banks, seeds, viral bulks and final formulated vaccine and clinically by trials and post-marketing surveillance.

Pharmacology
Although the Note for guidance on preclinical pharmacological and toxcicological testing of vaccines, CPMP/SWP/465/95 mentions that "It is preferable to study new combined vaccines in comparison with the individual antigen in animals….", traditional pharmacodynamic studies have not been performed for either ProQuad (Measles, Mumps, Rubella and Varicella [Oka/Merck] Virus Vaccine Live) or its authorised component vaccines measles, mumps, rubella vaccine, and varicella vaccine.

Pharmacokinetics
Traditional pharmacokinetic studies have not been performed for either ProQuad (Measles, Mumps, Rubella and Varicella (Oka/Merck) Live Virus Vaccine) or its authorised component vaccines measles, mumps, rubella vaccine, and varicella vaccine. Pharmacokinetic studies are not normally needed such as in combined vaccines according to the Note for guidance on preclinical pharmacological and toxcicological testing of vaccines, CPMP/SWP/465/95.

Toxicology
Traditional toxicity studies have not been performed with ProQuad [Measles, Mumps Rubella and Varicella Vaccine Virus Live (Oka/Merck)]. However, exhaustive safety testing was performed for the absence of transmissible or infective viral agents according to requirements listed in the European Pharmacopoeia (Supplement 2002, Section 2.6.16).

Discussion on the non-clinical aspects
No non-clinical data have been submitted for ProQuad. However, with respect to the actual stage of the vaccine development and considering the extended clinical testing of the vaccine already performed, it appears not to be justified to request additional preclinical testing.

4. Clinical aspects

Introduction
ProQuad is a sterile, lyophilized preparation of the components of measles, mumps, rubella vaccine (live) and varicella vaccine (live [Oka/Merck]). The vaccine is comprised of the:

- more attenuated vaccine strain of **measles virus** (derived from Enders' attenuated Edmonston strain),
- Jeryl Lynn strain of **mumps virus**
- Wistar RA 27/3 strain of live attenuated **rubella virus**
- Oka/Merck strain of **varicella virus**

ProQuad is administered subcutaneously in a single 0.5-mL dose. The vaccine must be stored frozen at -15ºC or colder and shelf life of the product is 18 months.

A formal efficacy trial was not conducted with ProQuad. The efficacy of the product was determined through the use of serologic correlates of protection previously established in the evaluation of the efficacy of the monovalent measles, mumps, rubella and varicella vaccines.

In all of the clinical trials performed with ProQuad, the specifications for the measles, mumps, and rubella components of the product remained the same as those used for the production of measles, mumps, rubella vaccine. Studies performed with an early formulation of a combination measles, mumps, rubella vaccine and varicella vaccine (referred to as MMRV) using doses of each of the components similar to the doses in measles, mumps, rubella vaccine and varicella vaccine indicated that the measles, mumps, and rubella immune responses were not affected by the presence of varicella virus. Using the same specifications for the measles, mumps, and rubella components of ProQuad allowed the extensive safety, immunogenicity, and efficacy database for measles, mumps, rubella vaccine to be used in support of this Application for ProQuad. The studies performed with MMRV also showed that the varicella immune response was diminished in the presence of measles, mumps, and rubella viruses. Therefore, dose ranging of the varicella component was performed in order to determine the appropriate specifications for the varicella component of ProQuad.
The safety and immunogenicity of ProQuad were demonstrated in clinical trials involving over 5400 subjects 12 to 23 month of age and 399 subjects 4 to 6 years of age. The safety profile of ProQuad also is supported by the extensive data generated with measles, mumps, rubella vaccine and varicella vaccine in prelicensure clinical trials and post licensure experience

The clinical program to support licensure of ProQuad consisted of 5 randomized, controlled studies s in which over 5800 subjects received ProQuad with a varicella virus release potency ≥3.97 log10 plaque-forming units (PFU), the lowest dose of varicella virus in the product determined to be clinically acceptable.

Four (4) of the studies 009, 011, 012, and 013) evaluated the immunogenicity and safety of ProQuad compared with measles, mumps, rubella vaccine and varicella vaccine in children 12 to 23 months of age. Study 014 evaluated the immunogenicity and safety of ProQuad in place of measles, mumps, rubella vaccine in children 4 to 6 years of age. The studies included in this Application are summarized in the table below:

Table 1: Summary of pivotal studies

Study Number	Study Title	Primary Study Objectives
009	A Pilot Study to Compare the Safety, Tolerability, and Immunogenicity of Measles, Mumps, Rubella and Varicella (MMRV) Vaccine and the Concomitant Administration of the Currently licensed varicella vaccine and measles, mumps, rubella vaccine in Healthy Children.	(1) To determine if 1 or 2 doses of ProQuad can elicit a similar immune response to varicella as the concomitant administration of 1 dose of the currently licensed varicella vaccine and measles, mumps, rubella vaccine (2) To assess the safety and tolerability of ProQuad after 1 and 2 doses

011	A Dose Selection Study in Healthy Children Comparing Measles, Mumps, Rubella, and Varicella (ProQuad) Vaccine to measles, mumps, rubella vaccine Given Concomitantly With Process Upgrade Varicella Vaccine (PUVV) in Separate Injections	To select at least 1 dose level and regimen of ProQuad that has a similar immune response to varicella as the control group of mumps, rubella vaccine and PUVV given concomitantly but in separate injections (2) To demonstrate that there is similar immunogenicity for measles, mumps, and rubella between at least 1 dose level and regimen of ProQuad and the control group of and PUVV given concomitantly but in separate injections (3) To demonstrate that ProQuad is generally safe and well tolerated
012	Comparison of the Safety, Tolerability, and Immunogenicity of 3 Consistency Lots of Frozen , and Varicella Vaccine (ProQuad) in Healthy Children	(1) To demonstrate that the 3 consistency lots of ProQuad will elicit similar immune responses to , and varicella (2) To determine whether the 3 consistency lots of ProQuad combined will elicit an immune response similar to MMR II and varicella vaccine given concomitantly, but at separate injection sites (3) To demonstrate that each of the 3 consistency lots of ProQuad provides an acceptable immune response to measles, mumps, and rubella (4) To demonstrate that the 3 consistency lots of ProQuad are well tolerated (5) To evaluate the persistence of antibodies to all 4 vaccine antigens 1 year postvaccination
013	An Open, Randomized, Multicenter Study of the Safety, Tolerability, and Immunogenicity of ProQuad (Frozen) Given Concomitantly Versus Nonconcomitantly With Other Pediatric Vaccines in Healthy Children 12 to 15 Months of Age	(1) To demonstrate that ProQuad can be administered concomitantly with DTPa and Hib-Hep B without impairing the immune response to , varicella, diphtheria, tetanus, pertussis toxin (PT), pertussis filamentous haemagglutinin (FHA), hepatitis B, or *Haemophilus influenzae* type b (Hib) (2) To demonstrate that the concomitant administration of ProQuad, DTPa, and Hib-Hep B provides an acceptable immune response to , and varicella (3) To show that ProQuad is generally well tolerated when administered concomitantly with DTPa and Hib-Hep B at the same visit or separated by an interval of 6 weeks (4) To show that ProQuad, whether administered concomitantly with DTPa and Hib-Hep B at the same visit or separately by an interval of 6 weeks, is generally well tolerated compared with the concomitant administration of MMR II and varicella vaccine
014	Administration of Frozen , and Varicella (ProQuad) Vaccine to Healthy Children at 4 to 6 Years of Age	(1) To show that the antibody responses to measles, mumps, and rubella following a dose of ProQuad at 4 to 6 years of age will be similar to the antibody responses after the recommended second dose of MMR II (2) To show that the antibody responses to , and varicella following a dose of ProQuad at 4 to 6 years will be similar to the antibody responses after a second dose of and varicella vaccine administered concomitantly at separate injection sites (3) To show that a dose of ProQuad at 4 to 6 years will be generally well tolerated (4) To summarize the following immunogenicity parameters by treatment group: seroconversion rates to measles, mumps, and rubella in subjects initially seronegative to the respective antigen; seropositivity rates to measles, mumps, and rubella in all subjects; the percent of subjects with postvaccination varicella antibody titer ≥5 gpELISA units/mL in subjects initially seronegative to varicella, in subjects with predose varicella titer ≤1.25 gpELISA units/mL, and in all subjects; for each of , and varicella, the percent of subjects achieving ≥4-foldrise in antibody titer

Abbreviations:
ProQuad = and varicella (Oka/Merck) virus vaccine live.
varicella vaccine = Varicella virus vaccine live (Oka/Merck).
MMR II = Measles, mumps, and rubella virus vaccine live.
DTPa = Diphtheria and tetanus toxoids and acellular pertussis vaccine absorbed.
Hib-Hep B = Haemophilus b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccine.
PUVV = Process upgrade varicella vaccine.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.

The applicant claims that the studies were conducted following appropriate Good Clinical Practice (GCP) guidelines.

Pharmacokinetics
Pharmacokinetic studies are not applicable for this vaccine (Note for guidance on clinical development of new vaccines (CPMP/EWP/463/97))

Pharmacodynamics
This information is provided in the Clinical efficacy section.

Clinical efficacy
Studies performed in the early 1990s with an earlier formulation of a combined measles, mumps, rubella and varicella vaccine (referred to as MMRV) had demonstrated that the measles, mumps, and rubella responses were adequate, but the varicella response was suboptimal compared with measles, mumps, rubella vaccine and varicella vaccine. Modifications to the manufacturing process for varicella vaccine were made to improve the varicella vaccine yield; these changes were incorporated into ProQuad.

- Main studies

METHODS

Study participants and treatments
Study 009: A multicenter (2 centres in the US), partially-blind (subjects were blinded with regard to the treatment group until the second visit), controlled, small-scale trial in which healthy children 12 to 23 months of age were assigned to one of two treatments: concomitant injections of either ProQuad and placebo or measles, mumps, rubella vaccine and varicella vaccine on Day 0.

Subjects who received ProQuad and placebo on Day 0 received a second dose of ProQuad at approximately Day 90 in the first group. The second group received measles, mumps and rubella vaccine + varicella vaccine on Day 0.

Subjects were randomised 2:1 (ProQuad: measles, mumps, rubella vaccine + varicella vaccine) according to a computer generated allocation scheme provided by the applicant.
Study 011: A multicenter (18 centers in the U.S.), partially double-blind (blinded with regard to specific formulations of ProQuad, but not to treatment group), controlled trial, in which healthy children 12 to 23 months of age**,** were assigned to 1 of 4 treatment groups: the first 3 groups received 1 dose of ProQuad containing 1 of 3 different potencies for the varicella component (3.48, 3.97, or 4.25 log10 PFU) or measles, mumps, rubella vaccine and varicella vaccine.

Subjects randomized to receive ProQuad on Day 0 received a second dose of ProQuad containing the same potency as the first dose on Day 90. The fourth group received measles, mumps and rubella vaccine + varicella vaccine (PUVV) on Day 0.

Subjects were randomised to one of 3 different doses of ProQuad or measles, mumps and rubella vaccine + varicella vaccine PUVV

Study 012: A multicenter (35 U.S. centers, 5 Canadian centers), partially-double blind (blinded with regard to specific formulation of ProQuad, but not to treatment group), controlled trial randomized study in which healthy children were assigned to 1 of 4 treatment groups: the first 3 groups received 1 of 3 consistency lot formulations of ProQuad (frozen) in which the dose level of the varicella component contained 4.40, 4.61, and 4.73 log10 PFU/dose, henceforth referred to as Lot 1, Lot 2, and Lot 3, respectively. The fourth group received marketed measles, mumps and rubella vaccine and varicella vaccine concomitantly at separate injection sites. All subjects were randomized on Day 0 to 1 of the 4 groups.

Subjects were randomised to one of 3 ProQuad lots or measles, mumps and rubella vaccine + varicella vaccine.

Study 013: Open, multicenter (48 U.S. centers), randomized study, in which healthy children, 12 to 15 months of age, were assigned to 1 of 3 treatment groups:
Group 1 (concomitant group) received ProQuad, DTPa (diphtheria and tetanus toxoids and acellular pertussis vaccine), and Hib-Hep B (haemophilus b conjugate and hepatitis B vaccine) concomitantly at separate injection sites on Day 0.
Group 2 (non-concomitant group) received ProQuad on Day 0 (Visit 1), and on Day 42 (Visit 2) received DTPa and Hib-Hep B concomitantly at separate injection sites.
Group 3 (control) received measles, mumps and rubella vaccine and varicella vaccine on Day 0 (Visit 1) concomitantly at separate injection sites and on Day 42 (Visit 2) received DTPa and Hib-Hep B concomitantly at separate injection sites.

Subjects were randomised to one of 3 treatment groups (ProQuad + DTPa + Hib-Hep B, ProQuad followed 6 weeks later by DTPa + Hib-Hep B, measles, mumps and rubella vaccine + varicella vaccine followed 6 weeks later by DTPa + Hib-Hep B).

Study 014: This was a double-blind (operating under in-house blinding procedures), multicenter, randomized study in which healthy children, 4 to 6 years of age, were enrolled. Subjects were stratified based on whether their primary doses of measles, mumps and rubella vaccine and varicella vaccine were received concomitantly or non-concomitantly. Subjects within each stratum were enrolled into 1 of the 3 treatment groups.
Group 1 received 1 dose each of ProQuad and placebo administered concomitantly at separate injection sites.
Group 2 received 1 dose each of measles, mumps and rubella vaccine and placebo administered concomitantly at separate injection sites. Subjects enrolled into
Group 3 received 1 dose each of measles, mumps and rubella vaccine and varicella vaccine administered concomitantly at separate injection sites.

Objectives
See Table 1; Summary of Pivotal studies

Outcomes/endpoints
With regard to the design and conduct of the clinical studies the following items were evaluated:
(1) Evaluation of immunogenicity using validated assays could be used as a surrogate measure for efficacy;
(2) Seroconversion and GMTs were evaluated for all studies supporting Marketing Authorisations;
(3) Non-inferiority or equivalence margins were implemented to establish the similarity of ProQuad with measles, mumps, rubella vaccine and varicella vaccine administered concomitantly at separate injection sites; no more than a 5-10 percentage points difference for measles, mumps, rubella vaccine and no more than a 10-15 percentage points difference for varicella vaccine.
(4) Definition of the minimum clinically acceptable dose of varicella virus in ProQuad

The choice of a wider non-inferiority threshold for varicella has been justified by the Applicant by a wider variability in response rate for varicella, compared to the other ProQuad components and by the lower acceptable response rate which has been set at 76%.
Comparisons of antibody response rates and/or GMTs ~6 weeks following vaccination were used as the primary serologic endpoints in each study. All serologic assays were developed and performed by Merck Research Laboratories, West Point, Pennsylvania, with the exception of serologic assays for diphtheria antitoxin, tetanus antitoxin, and antibodies to pertussis toxin (PT) and pertussis FHA, which were accomplished by Commonwealth Serum Laboratories, Sydney, Australia. Levels of antibody for each assay were evaluated by an appropriately sensitive and reliable method and each assay was rigorously validated.

The response rate for **varicella** was defined as the percent of subjects with a postvaccination VZV antibody titer ≥5 gpELISA units/mL.

The response rate for **measles** was originally defined as the percent of subjects with a postvaccination measles antibody titer ≥207.5 milli International units (mIU/mL) for Studies 009 and 011 and ≥120 mIU/mL for Studies 012, 013, and 014.

For Studies 009 and 011, a **mumps** enzyme-linked immunosorbent assay (ELISA) based on the mumps vaccine strain was used. The response rate for mumps in these 2 studies was the percent of subjects with a postvaccination mumps antibody titer above the optical density (OD) cutoff. For Studies 012, 013, and 014, a mumps ELISA based on the wild-type mumps virus was used. The response rate for mumps in these 3 studies was the percent of subjects with a postvaccination mumps antibody titer ≥10 ELISA units.

The response rate for **rubella** in each study was defined as the percent of subjects who had a postvaccination rubella antibody titer ≥10 IU/mL.

The following Table 2 summarizes the serological criteria that must be fulfilled in order meet the clinical endpoint requirements for MMRV vaccines, i.e. protection from infection with measles, mumps, rubella and varicella virus were identified in each study.

Table 2: Assays, baseline inclusion criteria, and definition of vaccine response used to evaluate the immune response in clinical trials of PROQUAD

Antigen	Study	Assay	Baseline inclusion criterion for primary analysis	Definition of post-vaccination response
Measles	009, 011	EIA	<OD cutoff	≥21.3 Ab units (=207.5 mIU/ml)
	012, 013, 014	Modified EIA	<120mIU/ml	≥120 mIU/ml
Mumps	009, 011	EIA	≤OD cutoff (generally <2Ab units)	>OD cutoff
	012, 013, 014	Wild-type EIA	<10 ELISA Ab units	≥10 Ab units
Rubella[1]	009, 011	EIA	≤OD cutoff	≥12.8 Ab units (=10 IU/ml)
	parts of 012, 012 persistence, and 013	EIA	<12.8 ELISA Ab units (=10 IU/ml)	≥12.8 ELISA Ab units (=10 IU/ml)
	parts of 012 persistence and 013, 014	Modified EIA	<10 IU/ml	≥10IU/ml
Varicella	009, 011, 012, 013, 014	gpELISA	<1.25 gp ELISA units/ml	≥ 5gp ELISA units/ml

The modified Rubella ELISA was introduced in the middle of the testing of samples from Studies 012 and 013.
OD = Optical density.
Ab units = Antibody units.
IU = International units.
EIA = Enzyme immunoassay.
gpELISA = Glycoprotein-based enzyme-linked immunosorbent assay.
ELISA = Enzyme-linked immunosorbent assay.

Publications and epidemiological surveillance provide sufficient evidence that cut off ELISA values indicating protection from measles, mumps, rubella and varicella were correctly chosen.

Similarly serological correlates account for efficacy analysis of other vaccines used concomitantly in Study 013, DTPa and Hib-Hep B. These parameters are summarised in Table 3:

Table 3: Response Criteria for *Haemophilus influenzae* Type b, Hepatitis B, Diphtheria, Tetanus, and Pertussis

Antigen	Study	Assay	Definition of post-vaccination response
Diphtheria	013	EIA	≥0.1 IU/ml
Tetanus	013	EIA	≥0.1 IU/ml
Pertussis Toxin (PT)	013	EIA	≥4-fold rise from pre- t0 post-vaccination
Pertussis FHA	013	EIA	≥4-fold rise from pre- t0 post-vaccination
Hepatitis B	013	RIA	≥10mIU/ml
Hib	013	RIA	≥1.0µg/ml
The definitions of post-vaccination response for diphtheria, tetanus, pertussis PT, and pertussis FHA are based on data in the package circular for DTPa which (diphtheria, and tetanus toxoids and acellular pertussis vaccine, Aventis Pasteur) was used with ACTHib (tetanus, toxoid conjugate, Aventis Pasteur MSD) as well as the Clinical Development Plan for ARBI (ACTHib, RECOMBIVAX HB, DTPBiken, Inactivated Polio). The definitions of post-vaccination response for hepatitis B and *Haemophilus influenzae* type B (Hib) are based on the Hib-Hep B (Haemophilus b conjugate [meningococcal protein conjugate] and hepatitis B [recombinant] vaccine) package circular. EIA = Enzyme immunoassay. RIA = Radioimmunoassay. *FHA = Filamentous haemagglutinin.* Hib = *Haemophilus influenzae* type b.			

Sample size
The immunogenicity data described are presented from each clinical study separately and also pooled across all studies within the same subject population.

The 4 clinical trials in healthy children 12 to 23 months of age (studies 009, 011, 012 and 013) included 5833 subjects who received ProQuad (4884 who received ProQuad alone and 949 who received ProQuad concomitantly with other paediatric vaccines [DTPa and Hib-Hep B]). Study 012 investigating the consistency of the manufacturing process for ProQuad evaluated the largest number (2915) of recipients of ProQuad. In the additional Study 014, 399 children received ProQuad at 4 to 6 years of age.

Statistical methods With respect to immunogenicity all studies were planned and analysed as non-inferiority or equivalence (in case of lot-to-lot consistency) studies. In general the analyses were performed on per-protocol populations of patients initially seronegative to the component analysed.
With regard to the different studies the following statistical methods were applied.

- Similarity based on rates (e.g. seroconversion rates):
  - Similarity hypotheses were tested by expanding the method of Farrington and Manning to multicenter studies
  - Confidence intervals on the average difference in proportions across study centers were calculated using the method proposed by Miettinen and Nurminen
- Acceptability of rates:
  - Two-sided (exact) binomial tests for rates were applied
- Similarity based on GMTs:
  - While in studies 011, 012 and 013 ANOVA models with the natural log of individual titers and fixed effects for study center, treatment group and center-by-treatment interaction were applied, no interaction term was used in study 009. In study 014 the

same model as in studies 09 adding primary vaccination status and log predose titer level as covariate was applied.

To account for the multiplicity issues arising from the multiple comparisons in each study, different methods were applied. The experiment-wise Type I error in studies 009 and 011 was set to 0.025 (one-sided) while in studies 012, 013 and 014 an experiment-wise Type I error of 0.05 (one-sided) was used.

RESULTS

Participant flow and recruitment
Five pivotal (009, 011, 012, 013, 014) clinical trials were conducted from 1998 to 2002 using ProQuad.
The studies were conducted to address the safety and immunogenicity of the vaccine. Around 95% of subjects enrolled completed the studies. Drop-outs were mainly due to technical reasons and a few number of subjects discontinuing due to severe adverse drug reactions.

Conduct of the study and Baseline data
A number of study amendments were made during the conduct of all pivotal studies. These changes were mainly of technical nature or refinements to the original study plans. None of these changes modified the initially defined objectives and hypotheses. No asymmetries in treatment arms were observed in any of the pivotal studies in terms of age, sex, gender and ethnicity.

Numbers analysed
Table 4: Overview on subject accounting in pivotal clinical studies 009, 011, 012, 013 and 014:

Study 009				
	PROQUAD + Placebo followed by PROQUAD	**vaccine + Varicella vaccine**		
entered	323	157		
completed	303	153		
Study 011				
	PROQUAD low dose	**PROQUAD middle dose**	**PROQUAD high dose**	**vaccine +PUVV**
entered	387	393	381	390
completed	336	343	346	370
Study 012				
	PROQUAD lot 1	**PROQUAD lot 2**	**PROQUAD lot 3**	**vaccine + Varicella vaccine**
entered	985	968	962	1012
completed	950	924	918	965
Study 013				
	Concomitant group	**Non-concomitant group**	**Control group**	
entered	949	485	479	
completed	884	453	442	
Study 014				
	PROQUAD + placebo	**vaccine + placebo**	**vaccine + Varicella vaccine**	
entered	401	205	195	
completed	392	201		

Outcomes and estimation

Proof-of-Concept Study
Study 009 evaluated the primary immunogenicity hypothesis comparing varicella responses of 1 and 2 doses of ProQuad to measles, mumps and rubella vaccine + varicella vaccine.

The percent of subjects (initially seronegative) achieving a post-vaccination varicella antibody titer ≥5 gpELISA units is estimated to be 91.2% after receiving 1 dose of ProQuad, 99.2% after receiving 2 doses of ProQuad, and 92.2% after receiving measles, mumps and rubella vaccine + varicella vaccine.

Table 5: Study 009 - Statistical Analysis of the Percent of Subjects With Varicella Antibody Titer ≥5 gpELISA Units 6 Weeks Postvaccination (Per-Study Analysis)

Popu-lation	Comparison of 1 and 2 injections of PROQUAD to control						*Estimated difference (Group A-GroupB) (95%CI)	*One-sided p-value	*Conc-clusion
	Group A (323 subjects enrolled)			Group B (157 subjects enrolled)					
	PROQUAD + placebo followed by PROQUAD			vaccine + varicella vaccine					
	Clinical material	N	Estimated response°	Clinical material	N	Estimated response°			
Initially sero-negative subjects^	PROQUAD (1 injection)	250	91.2%	vaccine + varicella vaccine	128	92.2%	-0.9 (-6.5,5.7)	<0.001	similar
	PROQUAD (2 injections)	239	99.2%	(1 injection)			7.0 (3.2, 13.1)	<0.001	similar
Subjects with baseline varicella titer < 1.25 gpELISA units	PROQUAD (1 injection)	290	91.1%	vaccine + varicella vaccine	128	92.4%	-1.3 (-6.5,4.8)	<0.001	similar
	PROQUAD (2 injections	278	98.8%	(1 injection)			6.5 (2.9,12.1)	<0.001	similar

*Below optical density (OD) cutoff at baseline.
N = Number of subjects with serology evaluable.

CI = Confidence interval.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.

Although similarity criteria have been met for post-dose 1 and post-dose 2 it is very clear from study 009 that a single immunisation is not sufficient to provide optimal immune protection against VZV infections. This impression is corroborated by the fact that GMTs increase significantly post-dose 2 as summarized in the brief Table shown below:

Table 6; Summary of the Percent of Subjects With Varicella Antibody Titer ≥–5 gpELISA Units, Varicella Geometric Mean Titers (GMTs) 6 Weeks Postvaccination and Associated 95% Confidence Intervals (Per-Study Analysis)

	PROQUAD + placebo followed by PROQUAD (GMTs post-dose 1) (95%CI)	**PROQUAD + placebo followed by PROQUAD (GMTs post dose 2) (95%CI)**
Population		
Initially sero-negative subjects	13 (11.8,14.4)	588.1 (494.1,699.9)
Subjects with baseline varicella titer < 1.25 gpELISA units	12.7 (11.5,13.9)	610.4 (514.5,724.2)
Subject numbers N as outlined in Table 5		

In contrast to the VZV component of ProQuad, a second dose only marginally impacts response (seroconversion) rates mediated by the measles, mumps and rubella components. Likewise, although GMTs increase post-dose 2, this effect is far less pronounced compared to VZV confirming the present view that a second dose of measles, mumps and rubella vaccine constitutes a catch-up immunisation rather than a true booster.

Dose-Ranging Study
Study 011 represents a formal dose finding study for the VZV component contained in ProQuad. Dose ranges of the other components remained unchanged compared to authorised vaccine and no new dose finding studies were performed. PUVV (process upgrade varicella vaccine) monovalent varicella vaccine corresponds to varicella vaccine used in the other pivotal studies and to the varicella component present in ProQuad.

Thus, it could be concluded that all three **2-injection** regimens of ProQuad - Low Dose, Middle Dose, and High Dose (4.25, 3.97 and 3.48, log10 PFU) and the **1-injection** regimen of ProQuad (High Dose; 4.25 log10 PFU) were statistically non-inferior (<10 percentage points decrease) to the control group. However, the data did not support the hypotheses that the 1-injection regimens of Low Dose and Middle Dose ProQuad (3.48, 3.97 log10 PFU) were non-inferior to the control group.

There is no interference with the other vaccine components of any of the VZV doses present in ProQuad formulations under investigation in Study 011. It is also evident that unlike for VZV a single dose vaccination schedule is sufficient to achieve acceptable response rates and titers against the vaccine components. A second dose of ProQuad only marginally impacts seroconversion rates. Anti measles, mumps and rubella-antibody titers are generally increased following dose 2, however, since GMTs were high already following administration of the first dose the further increase might have no clinical significance. For any of the VZV doses included in ProQuad investigational vaccine, there is no statistical difference in the performance of the vaccine components.

Consistency Lots Study And Persistence Of Antibody
Study 012 provided clinical confirmation of the consistency of the manufacturing process for ProQuad. This study evaluated the largest number (2915) of recipients of ProQuad.

Equivalent antibody responses to measles, mumps, rubella and varicella were achieved by the 3 consistency lots of ProQuad. Similar immune responses to all four antigens could be demonstrated by comparison of the combined lots of ProQuad with the control group receiving measles, mumps and rubella vaccine + varicella vaccine concomitantly. The immune responses specific to measles mumps, rubella and varicella met the predefined acceptability criteria at 6-weeks post-vaccination. Thus the study objectives are met.
The antibody persistence rates for all four antigens among the 3 consistency lots of ProQuad were comparable to each other, and the combined antibody persistence rate from the 3 consistency lots of

ProQuad was comparable to that of measles, mumps and rubella vaccine and varicella vaccine administered concomitantly at separate injection sites. Persistence of antibodies to all four antigens was demonstrated at 1 year postvaccination. The increase between 6 weeks and 1 year in varicella titers can indicate a continuous exposure to circulating varicella wild type virus.

A higher number of varicella breakthrough cases were observed in the ProQuad arm.

Overall Immunogenicity Results Following the Primary Dose of ProQuad
Evaluation of the response in subjects 12- to 23-months old, 6 weeks following administration of a primary dose, was used to determine the equivalence of the immune response to all 4 antigens between recipients of ProQuad (containing a varicella virus dose ≥3.97 log10 PFU) and recipients of measles, mumps, rubella vaccine and varicella vaccine.

The results suggest that 1 dose of ProQuad containing a varicella virus dose ≥3.97 log10 PFU in 12- to 23-month-old initially seronegative subjects is highly immunogenic with response rates 6 weeks postvaccination of 91.2% for varicella, 97.4% for measles, 98.8% for mumps (vaccine strain ELISA), 95.8% for mumps (wildtype ELISA), and 98.5% for rubella. Studies **009, 011, and 012** demonstrated the similarity of ProQuad to and varicella vaccine using preplanned objectives, hypotheses, and statistical methods.

Concomitant Use With Other Routine Pediatric Vaccines
Study 013 was designed to evaluate whether ProQuad could be administered with other pediatric vaccines without impairing the immune response to any of the vaccine antigens. The immunogenicity of the concomitant administration of ProQuad, Haemophilus b conjugate and hepatitis B vaccine (Hib-Hep B), and diphtheria, tetanus, and acellular pertussis vaccine (DTPa) at separate injection sites was evaluated among 1915 healthy children, 12 to 16 months of age.

Table 7: Statistical Analysis of the similarity/noninferiority of the concomitant group compared with the nonconcomitant group— Stage 2: Antibody response to DTPa and Hib-Hep B at 6 weeks postvaccination (Per-Protocol Analysis)

Vaccine Com-ponent (Assay)	Parameter	Concomitant Group (N=949)	Noncon-comitant Group (N=485)	Estimated Difference† (90% CI)	Criterion	One-Sided p-Value	Conclusion
		Estimated Response†	Estimated Response†				
Diphtheria	% ≥ 0.1 IU/mL	98.7%	98.4%	0.3 (-1.2, 2.4)	LB>-10.0	<0.001*	Unable to simultaneously show similarity between Concomitant and Nonconcomitant Groups for all components of DTPa and Hib-Hep B
Tetanus	% ≥ 0.1 IU/mL	100.0%	100.0%	0.0 (-0.6, 1.1)	LB>-10.0	<0.001*	
Pertussis PT	% ≥ 4-fold rise in titer	80.5%	90.0%	-9.5 (-13.8, -5.1)	LB>-15.0	0.017*	
Pertussis FHA	% ≥ 4-fold rise in titer	69.6%.	87.4%.	-17.7 (-22.6, -12.7)	LB>-15.0	0.815	
Hep B	% ≥ 10 mIU/mL	95.9%	98.8%	-2.8 (-4.5, -1.2)	LB>-10.0	<0.001*	
Hib	% ≥ 1µg/mL	94.6%	96.5%	-1.9 (-3.8, 0.3)	LB>-10.0	<0.001*	

N = Number of subjects vaccinated in each treatment group.
PT = Pertussis toxin.
FHA = Filamentous hemagglutinin.
LB = Lower Bound (of 2-sided 90% confidence interval).

As for the Pertussis FHA component, the p-value for non-inferiority (0.815) does not show non-inferiority; a conclusion of a similar immune response for the concomitant group compared with the non-concomitant group, with respect to immune responses to all antigens in *both* Hib-Hep B and DTPa could not be made based on this analysis.

Concomitant use of these vaccines led to a statistically significant reduction in antibody responses to Pertussis FHA. Furthermore, the expected antibody response rates to Pertussis PT of 85% and Hep B of 98%, respectively, which are based on former clinical experience and are outlined in the respective package circulars, were not met. Taking these findings as a signal, ProQuad should not be given concomitantly with these two vaccines.

Noninferiority was demonstrated for the concomitant use of ProQuad and Hib-Hep B. However, the antibody response rates and the GMTs for the HepB component of Hib-Hep B are numerically lower for the concomitant group than for the nonconcomitant group. In the light of the current discussion on the low immunogenicity of Hep B component in several of the applicant's vaccine formulations and due to the highly diverse national childhood immunization programs throughout the EU, using far more complex DTPa vaccines compared to DTPa, concomitant administration of ProQuad with other childhood vaccines must be avoided for the time being.

Immune Response to ProQuad When Used to Administer a Second Dose of measles, mumps and rubella vaccine and Varicella Vaccines
In 3 studies, ProQuad was used to administer a second dose of measles, mumps and rubella vaccine and varicella vaccines to children.

In **Studies 009 and 011**, a total of 1097 recipients of ProQuad received a second dose of ProQuad **3 months** after the first dose (at ~15 months of age). For measles, mumps, and rubella, the response rates remained above 98% and the GMTs increased 1.7-fold to 2.4- fold in subjects who received a second dose of ProQuad ~3 months following the primary dose. The varicella responses increased significantly from **86.6%** after 1 dose to 99.4% after 2 doses, with up to an approximate 41-fold increase in GMTs after 2 doses.

In **Study 014**, 399 children were administered ProQuad instead of the routinely recommended dose of measles, mumps, rubella vaccine at 4 to 6 years of age.

Table 8: Summary of observed vaccine response rates and 95% CI to , and varicella at both prevaccination and postvaccination in subjects who had previously received vaccine and varicella vaccine (Per-Protocol Population)

Antigen	**Time Point**	**ProQuad + Placebo** (N=399/n=367)	**vaccine + Placebo** (N=205/n= 205)	**vaccine + varicella vaccines** (N=195/n= 195)
Measles (% ≥120 mIU/mL)	Prevaccination	97.8 (95.8, 99.1)	98.9 (96.1, 99.9)	98.2 (95.0, 99.6)
	6 weeks Postvaccination	100 (99.0, 100)	100 (98.0, 100)	99.4 (96.8, 100)
Mumps (% ≥10 ELISA Ab U/mL)	Prevaccination	96.2 (93.7, 97.9)	94.1 (89.6, 97.0)	98.2 (95.0, 99.6)
	6 weeks Postvaccination	99.5 (98.0, 99.9)	100 (98.0, 100)	100 (97.9, 100)
Rubella[§] (%≥10 IU/mL)	Prevaccination	98.1 (96.1, 99.2)	95.7 (91.7, 98.1)	98.2 (95.0, 99.6)
	6 weeks Postvaccination	100 (99.0, 100)	100 (98.0, 100)	99.4 (96.8, 100)
Varicella (% ≥5 gpELISA U/mL)	Prevaccination	88.0 (84.2, 91.2)	N/A	88.9 (83.2, 93.2)
	6 weeks Postvaccination	98.9 (97.2, 99.7)		99.4 (96.8, 100)
§: Rubella antibody titers obtained by the legacy format were converted to their corresponding titers in the modified format. Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.				

The study does not show significant differences in observed response rates between ProQuad and measles, mumps and rubella vaccine + varicella vaccine given concomitantly at separate injection sites, in children 4 to 6 years of age. For varicella and rubella, the GMTs were numerically higher in the recipients of ProQuad compared to the measles, mumps and rubella vaccine + varicella vaccine group, whereas for mumps the GMTs were lower in the recipients of ProQuad. The percent of subjects achieving a ≥4- fold rise in titers from prevaccination to postvaccination are low for measles (~4.5%), moderate for mumps (27%-41%) and rubella (27%-33%), but are substantially high for varicella (80.7% - 72%).

- Clinical studies in special populations

None

- Analysis performed across trials (pooled analyses and meta-analysis)

None

- Supportive studies

The following studies were submitted as supportive clinical information:

- Study 007 is a vaccine / varicella vaccine concomitant use study with the primary objective to define an acceptable end of expiry titer of the mumps component in vaccine. Immunogenicity of the varicella component is not specifically investigated in this study.

- Studies 003, 004, 005, 006 were conducted in the early '90s of the past century using an MMRV formulation unrelated to the current PUVV manufacturing process for ProQuad, thus being unrelated to the present application
- Study 745 is an early study on Merck's varicella vaccine, conducted 1982 –1989 focussing on the immunogenicity and safety as well as long-term efficacy of the monovalent varicella vaccine.

- Discussion on clinical efficacy

Although no immunogenicity data have been provided for children aged 24 to 47 months and 7 to 12 years it can be assumed based on previous experience that the immunogenicity profile after administration of ProQuad will be comparable in children from 12 months of age.

Manufacturing consistency was confirmed in clinical studies through the evaluation of 3 consistency lots of ProQuad, all of which were shown to be similar to each other, as well as to the concomitant administration (at separate injection sites) of measles, mumps, rubella vaccine and varicella vaccine in terms of the immune responses to each of the 4 antigens in ProQuad.
The minimum clinically acceptable doses for the measles, mumps, and rubella components of ProQuad are the same as those for measles, mumps, rubella vaccine because use of the same dose of these 3 components in ProQuad resulted in similar measles, mumps, and rubella immune responses between recipients of ProQuad and recipients of measles, mumps, rubella vaccine. The minimum clinically acceptable dose of varicella virus in ProQuad is 3.97 log10 PFU. The immune response in terms of percent of subjects with a VZV antibody titer ≥5 gpELISA units/mL with this dose is comparable to the response obtained with varicella vaccine (at end-expiry).

The need for a 2nd dose to provide optimal protection from varicella
There is no major concern on the efficacy of the measles, mumps and rubella component of ProQuad.

However, for the varicella component the following major objection was raised and discussed:
From Studies 009 and 011 where ProQuad was given in a two dose schedule, the second dose received 90 days after the first dose, it is concluded that a single dose of ProQuad is not sufficient to provide optimal protection from varicella. This is evident from the increase of seroconversion rates from below 90% to virtually 100%, and from the 10 to 40 fold increase in anti varicella GMTs. A prime-boost concept is highly unusual for live attenuated vaccines (in contrast to inactivated vaccines) but can be explained by the high attenuation level of the OKA/Merck varicella vaccine strain and/or interference phenomena between vaccine components.

A one-dose schedule would leave the vaccinee with a considerable risk to acquire varicella despite vaccination due to an insufficient quality of the immune response following a single dose of ProQuad. The higher number of varicella breakthrough cases observed in the ProQuad arm might be indicative for improper protection after only one ProQuad dose. This supports the *CHMP* conclusion on Studies 009 and 011 that the first dose of ProQuad constitutes a priming of the anti varicella immune response whereas the second dose has a booster function.

Although a second dose of varicella vaccine for this age group is not recommended in any Member State, administration of a second dose of ProQuad could increase the efficacy of the varicella component of the vaccine by further reducing the rate of breakthrough varicella. From the perspective of the significant increase in GMTs observed post-dose 2, it was concluded that for protection from VZV infection, the first dose of ProQuad should be considered as a primary vaccination, while the second dose of ProQuad is mandatory to booster the primary immune response mediating long term persistence of immune response and protection from disease. The need for a second dose of a varicella-virus containing vaccine to increase immunity to varicella to prevent further varicella outbreaks was discussed extensively, and agreed by the CHMP.

Thus the first dose considered as the primary immunisation, and a second booster dose, is clearly stated in the SPC.

Non-inferiority margin for varicella
The choice of a wider non-inferiority threshold for varicella has been justified by the Applicant by a wider variability in response rate for varicella, compared to the other ProQuad components and by the lower acceptable response rate which has been set at 76%.

However it should be noted that varicella is a highly infectious disease and extended use of vaccination should provide the same protection level ensured for the other vaccine components. The fact that the varicella vaccine is less stable than measles, mumps and rubella vaccine components does not justify the acceptance of lower sero-response rates and wider non-inferiority margins. These arguments on the non-inferiority margins should be taken into account also in view of the future type II variation already planned to be requested for the refrigerated product suitable for the EU market.

Concomitant use
In Study 013 – concomitant use of ProQuad and DTPa and ProQuad and Hib-HepB – non-inferiority of concomitant versus non-concomitant use could not be shown for ProQuad and DTPa given concomitantly. Response against the Pa components (PT and FHA) was insufficient. Concomitant use of ProQuad and Hib-Hep B vaccine showed a reduced response rate against the Hep B component of the Hib-Hep B vaccine in the concomitant group.

ProQuad should not be used concomitantly with other vaccines as stated in the SPC.

Long term persistence
Long term persistence studies conducted with Varivax will provide helpful information on the persistence of antibodies in a changing environment as well as the rate of breakthrough cases and the risk of HZ. The applicant has committed to conduct long-term follow-up studies.

Clinical safety

- Patient exposure

Over 93% of the 12- to 23-month-old subjects and over 97% of the 4- to 6-year-old subjects completed their respective studies. A subject was considered to have completed the study if he/she received all scheduled vaccinations, completed all safety follow-up, and provided blood samples as defined in the study.

Approximately 53% of the 12- to 23-month-old subjects and ~53% of the 4- to 6-year-old subjects who received ProQuad were male. The median age of the 2 groups was 12.0 months (range: 11 to 23 months) and 4.0 years (range: 4 to 6 years), respectively. The largest racial/ethnic groups in both age groups were Caucasian (66.0% and 78.4%, respectively), followed by African American (13.0% and 12.3%, respectively), and Hispanic (9.8% and 3.8%, respectively). These demographic characteristics appeared to be consistent with those observed in recipients of the respective control groups.

The safety data for the 4,497 children (12-23 month) from the ProQuad groups in studies 009, 011, 012 and 013 (non-concomitant) who were administered lots of ProQuad with varicella virus potencies greater than the minimum clinical acceptable dose of 3.97 log10 PFU were compared with the data obtained from the 2038 children administered who were measles, mumps and rubella vaccine + varicella vaccine.

- Adverse events

In general, increase of the varicella dose was paralleled by an increase of vaccine related adverse experiences.

When comparing the overall rate of subjects experiencing one or more injection-site adverse experiences following a single dose of ProQuad versus subjects who received both measles, mumps and rubella vaccine and varicella vaccine, the rate was significantly lower among recipients of

ProQuad (31.3% versus 34.6%, respectively). However, in parallel to the increase of the varicella dose an increase of the injection site adverse experiences (except for Study 011) was observed.

The rate of pain at the injection site was significantly lower among recipients of ProQuad compared with the rate at either site for recipients of measles, mumps and rubella vaccine and varicella vaccine (22.0% versus 26.8%, respectively). However, increasing doses of Varicella prompted higher rates of pain. A significantly higher rate of pain was observed when ProQuad was given concomitantly with other pediatric vaccines compared to the administration of ProQuad only. In addition, in Study 014 (4-6 year old children, second vaccination) the rate of pain was higher in the ProQuad than in the control group.

Erythema at the injection site for ProQuad occurred more frequently than erythema at the injection site for measles, mumps and rubella vaccine and varicella vaccine (24.4% for ProQuad, 15.6% and 14.5% for each of the injection sites for measles, mumps, rubella vaccine, and 15.5% for varicella vaccine). Rash at the injection site of ProQuad occurred at a higher rate than rash at the injection site of measles, mumps and rubella vaccine or varicella vaccine (2.4% versus 0.5% versus 1.4%, respectively).

In was also observed that in Study 014 older children showed a higher rate of swelling compared to the corresponding control-group (measles, mumps and rubella vaccine and varicella vaccine).

There was a higher rate of subjects with one or more clinical adverse experiences in the ProQuad group than in the measles, mumps and rubella vaccine + varicella vaccine group (81.5% versus 79.6%, respectively). The number of subjects who reported one or more systemic clinical adverse experiences was higher in recipients of a single dose of ProQuad compared with recipients of measles, mumps and rubella vaccine + varicella vaccine (76.1% versus 72.3%, respectively).

Systemic clinical adverse experiences that were reported at a higher rate among subjects who were administered a single dose of ProQuad were fever (37.2% versus 31.5%, respectively), upper respiratory infection (23.5% versus 20.7, respectively), and measles-like rash (3.2% versus 2.2%, respectively). When comparing only systemic clinical adverse experiences that were assessed to be related to the study vaccine, upper respiratory infection was numerically but not significantly different between the two groups.

Fever (≥40.0°C) was a systemic adverse experience that was statistically higher in recipients of ProQuad than in those who received measles, mumps and rubella vaccine + varicella vaccine (37.3% versus 31.6%, respectively). The rate of temperature - 40.0°C oral equivalent was also significantly higher in recipients of ProQuad when compared with the control group (5.8% versus 4.7%, respectively). There were more subjects with fever rated as severe in the ProQuad as in the control groups. In Study 013 the intensity of fever was described to be moderate for 39.2% (55.4% mild) of subjects in the concomitant group but for only 27.9% in the nonconcomitant (69.9% mild) and only for 19.6% in the control group (76.1% mild***).*** It is also noted that when comparing all children (12-23-months of age) vaccinated with ProQuad (first dose) higher rates of fever were observed in older children.

The rate of measles-like rash was significantly higher (all children, 12-23 months, together) in recipients of ProQuad than in recipients of measles, mumps and rubella vaccine + varicella vaccine (3.0% versus 2.1%, respectively).

The distribution of the day of onset of the upper respiratory infections, the average duration, the intensity and concurrent adverse experiences were similar between both groups. Although it is difficult to distinguish, nearly 95 % of the infections in both groups were considered not to be vaccine related.

However, 35,2% of the upper respiratory infections reported in recipients of ProQuad were not rated as mild in intensity. Even if there is no plausible biological explanation a significant difference was observed related to the rate of upper respiratory infections between both groups. Therefore, a follow-up observation is recommended.

- Serious adverse event/deaths/other significant events

Sixty-four (64) subjects reported one or more serious adverse experience during the 42-day safety follow-up period.

Thirteen (13 from 5731) of febrile seizures were reported among recipients of ProQuad and eight (8 from 1997) were reported among recipients of measles, mumps, rubella vaccine and varicella vaccine (i.e 0.23% ProQuad versus 0.4% measles, mumps, rubella vaccine and varicella vaccine. Thus, ProQuad appears to be less reactive with respect to febrile seizures compared to measles, mumps and rubella vaccine + varicella vaccine.

In the ProQuad –group, five of the thirteen febrile seizures were classified as vaccine related, one is unknown. By day 12 postvaccination eight of the thirteen febrile seizures appeared. In the measles, mumps, rubella vaccine and varicella vaccine group, two of the eight febrile seizures were classified as vaccine related. By day 12 postvaccination six of the eight febrile seizures appeared.

Incidence rates of SAEs between treatment groups appear to be comparable; however, it is noted that the clinical studies were not powered to detect significant differences.

- Laboratory findings

The objective of the sub-study of Studies 012 and 013 (Reference P012C1) was to determine if subjects who developed measles-like rashes were more likely to have increased levels of circulating measles virus postvaccination with either a single dose of ProQuad or the concomitant administration of a single dose of measles, mumps and rubella vaccine and a single dose of varicella vaccine than subjects who did not develop a measles-like rash.

A total of 193 samples were tested for measles RT-PCR. Samples were collected between 5 and 44 days postvaccination, with the majority of both RT-PCR control and measles-like rash samples collected between 10 and 17 days postvaccination. Fifty-eight (58) samples were from subjects who developed measles-like rash (45 from subjects who received ProQuad and 13 from subjects who received measles, mumps and rubella vaccine + varicella vaccine) and 135 samples were from RT-PCR control subjects (69 from subjects who received ProQuad and 66 from subjects who received measles, mumps and rubella vaccine + varicella vaccine).

Only 3 samples (2/135 RT-PCR control and 1/58 measles-like rash) collected at ~5 to 20 days postvaccination were above the limit of quantitation for the RT-PCR assay. No conclusions can be drawn from this study. In wild-type measles infection, the peak of measles viremia occurs just prior to the rash onset and then falls rapidly during the next 2 to 3 days. This suggests that samples in this clinical trial may have been collected at a suboptimal time postvaccination because the samples were collected either after the onset of a measles-like rash, or after the time the peak in viremia might have been seen for subjects who donated a control sample.

- Discontinuation due to adverse events

Eleven subjects discontinued the studies due to adverse events.

There were no discontinuations due to serious AES in pivotal studies 009, 011, 012, 013 or 014.

The proportion of children who completed participation was >95% in all studies except Study 011 (89%) and 013 (93%). Both these studies were conducted in 12-23 month old children and included the administration of a second dose, at a 3-month interval from the first one. The drop-out from the first to second dose was approximately 7% in Study 011, and 4% in Study 013. The Applicant has described the reasons for discontinuation reported for children who did not receive the second dose, of which 6 /76 subjects in Study 011 were discontinued due to a clinical adverse experiences; 3 of which reported clinical adverse experience(s) related to the study vaccine. One subject reported respiratory infection, a second subject reported fever, viral infection, and injection-site pain, and a third subject

reported measles/rubella-like rash. In Study 013 a total of 57 subjects (4.0%) discontinued the study, but not due to adverse events.

- Post marketing experience

Since the marketing of M-M-R™II vaccine in 1978, more than 446 million doses of vaccine have been distributed worldwide. Postlicensure experience with measles, mumps and rubella vaccine collected through passive reporting of spontaneous adverse experiences to Merck & Co., Inc. has confirmed the excellent safety profile of the vaccine, with a very low frequency of reported serious adverse reactions. This is demonstrated by the overall AE reporting rate of 2.87 reports per 100,000 doses distributed, and the serious AE reporting rate of 0.61 serious reports per 100,000 doses of measles, mumps and rubella vaccine distributed worldwide. The most frequently reported serious adverse recations are pyrexia, febrile convolution, convolutions NOS (new onset seizures), autism (published literature reports have found no scientific basis for a casual relationship between autism and vaccination with a combination measles, mumps and rubella vaccine) and rashes.

Since the marketing of varicella vaccine in 1995, over 45 million doses of vaccine have been distributed. Post-licensure experience with varicella vaccine collected through passive reporting of spontaneous adverse experiences to Merck & Co., Inc. and to the Vaccine Adverse Event Reporting System (VAERS) has confirmed that varicella vaccine has an excellent safety profile and is generally well tolerated with a very low frequency of serious adverse experiences in all age. This is demonstrated by the overall rate of 31.5 reports per100.00 doses distributed, and the serious AE reporting rate of 1.4 serious reports per 100.00 doses of varicella vaccine distributed worldwide. The most frequently reported serious adverse events are varicella, pyrexia, abortion spontateous and induced, convolutions and herpes zoster.

Selected adverse reactions reported to VAERS for Varicella vaccines during 1995-1998 were: rash, injection site reaction, HZ, pharyngitis, cellulites, hepatic pathology, pneumonia, erythema multiforme, arthropathy, thrombocytopenia, anaphylaxis, vasculitis, aplastic anemia, neuropathy, convulsion, ataxia, encephalopathy and meningitis. A recent description of a 4-year old girl with an ischemic stroke two weeks after receiving varicella vaccine has also been reported.

- Discussion on clinical safety

The incidence of adverse reactions with ProQuad did not differ dramatically from the simultaneous use of measles, mumps and rubella vaccine + varicella vaccine. The number of subjects with erythema and rashes at the injection-site, with measles like rashes, with upper respiratory infections and with fever increased. Statistically significant difference in the number of vaccine-related SAEs could not be found, although an increase in the rate of elevated temperature following the administration of ProQuad may lead to an increase in the incidence of postvaccination febrile seizures.

Although no other safety issue is expected after the use of ProQuad, it should be mentioned that no investigations were made with children at the age of 24 - 47 month and 7 to12 years.

Postmarketing experience with varicella vaccine suggests that transmission of the varicella vaccine virus may occur, rarely, between healthy vaccine recipients who develop a varicella-like rash and healthy susceptible contacts. Therefore, recipients of ProQuad must try to avoid, whenever, possible, contact with susceptible high-risk individuals (e.g., newborns, pregnant women, immunocompromised persons) for up to 6 weeks following vaccination. This has been has been adequately reflected in the SPC (4.4 Special warnings and precautions)

The Applicant has agreed to further evaluate the occurrence of rare adverse events following administration of ProQuad in a post-marketing follow-up study. The primary objective of this intended study will be to evaluate rates of medically-attended (i.e., resulting in a medical encounter) febrile convulsions associated with the administration of ProQuad. In addition to the main objective of assessing the incidence of febrile convulsions, this study is also designed assess the general short-term

(30 day) safety of ProQuad including the rate of respiratory infections in children 12 months to 12 years of age.

The Applicant has also agreed to evaluate the occurrence of rare and very rare events in older seronegative children (older than 23 month) that are vaccinated for first time in a post-marketing follow-up study.

In previous studies with children who received either one or two doses of varicella vaccine, subjects were observed for around 10 years. In these studies no evidence of an increasing risk of zoster has been found so far. On the other hand the short intervals between vaccination and HZ occurrence in several patients seem consistent with the intriguing hypothesis that varicella vaccine might, in rare cases, provoke reactivation of latent wild-type VZV. Whether or not a second dose of varicella vaccine can trigger similar effects cannot be answered for the time being but requires long-term observation of varicella-vaccinated individuals. The Applicant has also agreed to carry out a long-term follow-up study to assess the risk of HZ in vaccinated individuals after the first and second varicella vaccination as well as in non-vaccinated individuals having experienced natural disease.

5. Overall conclusions, benefit/risk assessment and recommendation

The applicant has performed satisfactory readability testing of the product information.

Quality
During the evaluation of ProQuad, two major objections were identified. These concerned the nomenclature used for the seed lot system and also the passage number for the varicella component. Satisfactory justification has been provided to resolve these concerns.

Other minor concerns have been adequately addressed, however, several commitments are made by the applicant, and several follow-up measures are defined to provide further information post-approval. In conclusion, all quality issues are resolved.

Non-clinical pharmacology and toxicology
No non-clinical data have been submitted for ProQuad. However, with respect to the actual stage of the vaccine development - after extended clinical testing of the vaccine was already performed, requesting additional preclinical testing appears not to be justified.

Efficacy
The two issues of major concern were identified in relation to clinical efficacy were:

1. Insufficient seroconversion rates and GMTs in relation to the varicella component after only a single dose of ProQuad. This is in contrast to the other ProQuad components (measles, mumps and rubella) for which efficacy is not different from well established trivalent measles, mumps and rubella vaccines.

2. Insufficient efficacy of the acellular pertussis component FHA of the DTPa vaccine when administered concomitantly with ProQuad

With respect to the first major concern, clinical evaluation of available scientific information on the applicant's varicella vaccine component (OKA/Merck varicella virus vaccine strain) revealed that regardless of the potency (plaque forming units/ml) a single dose of ProQuad is to be considered as a primary immunisation dose requiring a second dose of ProQuad (or monovalent varicella vaccine), 4 – 12 weeks after the first dose to complete the varicella vaccination course. A second dose of varicella vaccine shifts seroconversion rates in terms of gpELISA units ≥ 5 to almost 100% and increases GMTs by a factor of 40 to 50. Most notably gpELISA units ≥ 5 according to the current knowledge is a good and widely accepted correlate for protection from disease over a period of at least 10 years. Section 4.2 of the SPC has now been appropriately worded highlighting that a second dose of varicella

vaccine should be administered to complete immunization against varicella disease, and this concern has now been satisfactorily addressed.

With respect to the second major concern the applicant's proposal to recommend concomitant use of ProQuad with other childhood vaccines was not substantiated by appropriate clinical data. Firstly, the applicant failed to demonstrate non-inferiority of the Pa component (FHA) of a DTPa vaccine when administered concomitantly with ProQuad. Secondly, the type of DTPa vaccine investigated does not adequately reflect the more complex combination vaccines commonly used in the EU such as the penta – and hexavalent DTPa containing vaccines. This concern has now been satisfactorily addressed by stating in section 4.5 of the SPC that: "there are insufficient data to support use of ProQuad with other vaccines."

Safety

No major safety concerns were identified during the evaluation phase. The safety profile of ProQuad does not deviate significantly from those known from the applicant's measles, mumps and rubella vaccine and monovalent varicella vaccine administered separately or concomitantly. However, a number of issues have been identified requiring further investigation in the post-marketing phase that the applicant has committed to put in place. These include:

- Safety of ProQuad in older, seronegative children
- Rate of respiratory infection following ProQuad vaccination
- Rate of febrile seizures following ProQuad vaccination
- Long-term follow-up studies concerning long term persistence of protective post vaccination antibodies to varicella and with special regard to an epidemiologically changing environment and the rate of break-through cases
- Long -term follow-up studies with special regard to the risk of herpes zoster (HZ) in vaccinated individuals

Different storage conditions of the lyophilized powder and the diluent

During the evaluation of ProQuad, the appropriateness of the applicant's approach on handling the different storage conditions for the lyophilized powder and the diluent has been extensively discussed at the Biologics Working Party, the Vaccine Working Party and CHMP.

The diluent is supplied together with the lyophilized powder to avoid that the vaccine is reconstituted with the wrong diluent (e.g. 0.9% NaCl solution for injection) or with the wrong volume of diluent. It is believed that this risk outweighs the potential risk of incorrect use or error caused by the different storage conditions of the vaccine components.

As the components of ProQuad cannot be stored together, a link between the lyophilized powder and the diluent is made through the text on the SPC, labeling and package leaflet, including a clear reference to each other and specific recommendation of use/method of administration. This is not a self-administered product and it is expected that reconstitution and administration by a health care professional according to the instructions ensures correct storage and use of the vaccine. In order to secure traceability of all the batches that have been shipped together and to maintain the link between active substance and diluent, the applicant will use a validated computerized system, which will assign a specific code for each combination that is shipped.

The applicant will also ensure that the expiry date of the diluent shipped with the active substance is at least equivalent to the expiry date of the active substance. If either component of the product has to be replaced (for ex. if a component has expired or if a component is missing) the applicant will provide a replacement of the complete product (active substance and diluent).

The applicant also pointed out that they currently market a frozen varicella vaccine presented in the same way as ProQuad on the Italian and US markets; in the US alone, over 40 million doses have been distributed over the past 10 years where the different storage conditions have been implemented in distribution centers, pharmacies, physician's practices and public health clinics. The CHMP considers that the distribution channels within the EU (e.g Italian) are comparable to the US ones.

Taking into account the above, the CHMP concludes that the company has provided a satisfactory justification for this approach and has implemented appropriate measures to ensure the safe and correct use of the vaccine.

Benefit/risk assessment

All major immunogenicity and safety concerns have been satisfactorily addressed and the Applicant has agreed on appropriate follow-up measures. The benefit/risk assessment is therefore considered to be favourable.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of ProQuad for simultaneous vaccination against measles, mumps, rubella and varicella in individuals from 12 months of age was favourable and therefore recommended the granting of the marketing authorisation.