SUPPLEMENTAL DIGITAL CONTENT 1 – SUPPLEMENTAL TEXT

Supplemental to:

Randomized trial to compare immunogenicity and safety of a CRM and TT conjugated quadrivalent meningococcal vaccine in teenagers who received a CRM or TT conjugated serogroup C vaccine at preschool age.

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SUPPLEMENTAL DIGITAL CONTENT – METHODS

Participants: From the original cohort of 832 pre-school age children who took part in a previous study between 1998 and 2000, a preliminary check on the health service demographics system suggested that 615 were still registered with GPs for health services in Hertfordshire and Gloucestershire, England (see Figure 1 in main article). Study vaccine research nurses and partners from the regional primary care research network (PCRN) contacted GPs to check their records. For 57 individuals, the records were inaccessible as GP configurations had either changed or their GPs were not able to participate in the checking process. Those who were confirmed to still be registered were invited to join this study, and eligible respondents were enrolled after providing fully informed written consent. Teenagers who received an MCC vaccine during the preschool study, but no further MCC vaccination thereafter, and no other vaccine within the 3 months preceding enrolment, were included. Exclusion criteria included immunosuppression, pregnancy, significant medical illness, antibiotic use within 14 days of enrolment, previous confirmed invasive meningococcal disease, and any other contraindication as per routine practice guidelines in the UK national immunisation guidance "Green Book"[1]. Study formal procedures commenced in June 2012 and recruitment took place between July and November 2012. For antibody persistence measurements, participants were randomised in order of inclusion

[1] Department of Health, England. Meningococcal meningitis and septicaemia (Updated 11 April 2014). In: Salisbury D, Ramsay M, eds. Immunisation against infectious disease: the Green Book (chapter 22). London: Crown copyright 2013; Open Government Licence v 2.0. URL: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/302904/Green_Book_Chapter_22_v2_5.pdf (accessed 14 August 2014).

using a computer-generated list, to either 6 or 9 months follow-up, with final study visits in August 2013. Participants completed a health diary to record oral temperature and any local or systemic reactions daily for the week following vaccination. Reactions and events were further monitored by vaccine research nurses during a telephone follow-up on the 8th day post-vaccination; and by directly enquiring from participants at each study visit.

Regulation: The trial was approved by the North West 3 NHS Research Ethics Committee (Reference 11/H1002/6) and conducted in accordance with the Helsinki Declaration (2008 amendment), the 1996 International Committee for Harmonisation Guidelines for Good Clinical Practice, and the 2004 EU Clinical Trial Directive. The supplementary section provides further information on participants, regulation, vaccines, serology, and analyses. After gaining all regulatory approvals, appropriate local research governance permissions in Hertfordshire and Gloucestershire were obtained. The trial EudraCT Number is 2010-022505-18. It was registered on the public website, www.ClinicalTrials.gov (identifier NCT01192997), and adopted and registered on the National Institute for Health Research (NIHR) Clinical Research Network (CRN) Portfolio database (ID 10242).

Vaccines: Novartis MenACWY (Menveo®) was licensed by the European Medicine Agency (EMeA) in 2009, and currently indicated for use from 2 years of age and above. It has 10 µg of MenA oligosaccharide; and 5 µg of each of MenC, W and Y, with a total *Corynebacterium diphtheriae* CRM197 protein content of 32.7-45.8 µg per dose. GSK MenACWY was an investigational product during preparation for this study. It became licensed [2] (as Nimenrix®) just before study commencement, but the already provided pre-

[2] European Medicines Agency (2012). EPAR-summary for the public: Nimenrex (EMA/CHMP/136315/2012). URL:

licensure batch was used. It had 5 µg oligosaccharide of each of the four serogroups, and a total tetanus toxoid content of ~44 µg per dose.

Serology: After blood sample collection, sera were separated and testing for tetanus and diphtheria antitoxin performed at Public Health England (PHE) Microbiology Services laboratories at Porton Down; aliquots were transported on dry ice to the PHE Vaccine Evaluation Unit, Manchester, for meningococcal antibody measurements. Laboratory staff remained unaware of participants' study groups. Sera were tested for serogroup-specific IgG antibodies using a standardised enzyme-linked immunosorbent assay (ELISA) protocol. They were tested for serogroup-specific SBA using a standardized assay incorporating serum from 3-4 week old rabbits as the exogenous complement source. The target meningococcal strains used were MenC C11, MenW M01.240070, and MenY M00.241125 (S1975), MenA M99.243594. SBA titers were expressed as the reciprocal serum dilutions yielding ≥50% killing after 60 min. Diphtheria and tetanus-specific antibodies (IgG) were quantified using standardized ELISAs with the National Institute for Biological Standards and Control (NIBSC) National Diphtheria reference serum 00/496 and the first International Tetanus reference serum 26/488.

Statistics: Outcomes were measured at three time points; pre-booster, 28 days post-boost, and either 6 or 9 months post-boost. The proportion of participants with serogroup A, C, W and Y-specific SBA titers ≥8 and ≥128 (not shown); as well as SBA geometric mean titers (GMTs) were calculated with 95% confidence intervals (95% CIs) at each time point.

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http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/002226/WC500127665.pdf (accessed 14 August 2014)

Proportions with ≥4-fold rises in SBA titer from baseline (results not shown) and geometric mean (*n*-fold) rises in SBA titer from baseline were also calculated with 95% CIs. Similarly, serogroup-specific IgG geometric mean concentrations (GMCs) were calculated with 95% CIs at each time point, along with geometric mean rises (*n*-fold) in concentration from the baseline. For antibody responses to the vaccine carrier proteins, GMCs of antibody to TT and diphtheria toxin, as well as proportions with specific IgG concentration ≥0.1 IU/mL, were calculated with 95% CIs at each time point (CIs not shown). SBA titers below the lower detection limit of 4 were assigned a value of 2 for computational purposes, and antibody titers were log-transformed for geometric mean calculations. The 95% CIs were calculated for each of the groups as well as overall by the three primary vaccines and overall by the two trial vaccines. Comparisons between boosters and by primary vaccination overall were done at a 5% significance level. Post-boost measurements were compared by primary vaccine and by booster vaccine, with multivariable normal errors regression on logged antibody levels, adjusting for pre-vaccination titers and time from vaccination to blood sample, and testing for interactions. Previously-reported post-primary antibody responses from the original study were compared with pre-booster antibody responses from the current trial, by regression of logged post-primary (original study) titers on logged teenage (current study) pre-boost titers, taking account of between-group differences in primary vaccine and booster vaccine. Comparisons across the 6 arms were by non-overlapping 95% CIs, being a conservative approach (equivalent to a $P < 0.01$ approximately) to allow for the high numbers of possible comparisons.

SUPPLEMENTAL DIGITAL CONTENT – RESULTS

The male/female distribution was 16 /30 (MenACWY-TT group) and 20/27 (MenACWY-CRM group). The median 1, 6 and 9-month post-booster intervals to blood sampling were

27 (range 17-42), 273 (251-306), and 189 (161-231) days, respectively. All were included in analysis as they were within pre-set time point limits. Per-protocol analysis only was carried out since there was no difference to modified intention-to-treat data. For all antigen group comparisons, age and gender were not associated with responses and not included in the models.

LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure S1. Meningococcal group C serum bactericidal antibody (SBA) (***upper panel***, n=40) and IgG (***lower panel***, n=81). Antibody titers after the original primary vaccination with a meningococcal C conjugate vaccine at pre-school age (post-primary, x-axis), were compared to titers one month after teenage booster vaccination (1-month post-boost, y-axis). Trend lines were fitted. Regression of logged post-boost on post-primary titers, allowing for primary and booster vaccines, showed weakly positive association for both SBA and IgG, but neither reached statistical significance (SBA p=0.26; IgG p=0.09).

Supplemental Figure S2. Decline in meningococcal serogroup-specific IgG titers over time, after teenage booster vaccination, with fitted trend lines. Fold titer changes per doubling time since day 28 (and 95% CI) estimated from a fixed effects model were as follows: ***Upper left panel:*** MenC IgG 0.61 (0.58-0.64); ***Upper right panel:*** MenW 0.67 (0.64-0.71); ***Lower left panel:*** MenA 0.66 (0.62-0.7); and ***lower right panel:*** MenY 0.78 (0.74-0.82).