Vaccine 33 (2015) 3322–3330

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and immunogenicity of a four-component meningococcal group B vaccine (4CMenB) and a quadrivalent meningococcal group ACWY conjugate vaccine administered concomitantly in healthy laboratory workers

Jamie Findlow[a,*], Xilian Bai[a], Helen Findlow[a], Emma Newton[a], Ed Kaczmarski[a], Elizabeth Miller[b], Ray Borrow[a,c]

[a] Public Health England, Public Health Laboratory, Manchester, Manchester Medical Microbiology Partnership, PO Box 209, Clinical Sciences Building II, Manchester Royal Infirmary, Manchester M13 9WZ, UK
[b] Immunisation Department, Health Protection Services, Public Health England, Colindale, London NW9 5EQ, UK
[c] University of Manchester, Inflammation Sciences Research Group, School of Translational Medicine, Stopford Building, Manchester M13 9PL, UK

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 12 May 2015
Accepted 13 May 2015
Available online 27 May 2015

Keywords:
Meningococcal
Vaccine
4CMenB
ACWY-CRM
Laboratory workers

ABSTRACT

Safety precautions for laboratory staff working with meningococci should primarily rely on laboratory procedures preventing exposure to aerosols containing viable meningococci. Despite this, vaccination is a key component of protection in the occupational setting. In the UK in 2009, there were no licensed vaccines for meningococcal capsular group B or conjugate vaccines for capsular groups A, C, W and Y. We therefore undertook a Phase II trial in laboratory workers to investigate the safety and immunogenicity of a four component group B vaccine (4CMenB) and a quadrivalent group A, C, W and Y conjugate vaccine (ACWY-CRM).

Enrolment was open to staff aged 18–65 years at the Public Health Laboratory, Manchester who may have had a potential occupational exposure risk to meningococci. 4CMenB was administered at 0, 2 and 6 months in the non-dominant arm and ACWY-CRM concomitantly at 0 months in the dominant arm. Pre- and post-vaccination blood samples were taken and analysed by the serum bactericidal antibody (SBA) assay against A, C, W and Y strains and a panel of seven diverse group B strains. Diary cards were used to record any local and systemic reactions following each vaccination.

In total, 38 staff were enrolled and received initial vaccinations with 31 completing the trial per protocol. Both vaccines were proven safe, with local reactogenicity being more commonly reported following 4CMenB than ACWY-CRM. High proportions of subjects had putative protective SBA titres pre-vaccination, with 61–84 and 61–87% protected against A, C, W and Y strains and diverse MenB strains, respectively. Post-vaccination, SBA titres increased with 95–100 and 90–100% of subjects with protective SBA titres against A, C, W and Y strains and diverse MenB strains, respectively.

These data suggest that 4CMenB and ACWY-CRM are safe when administered concomitantly and have the potential to enhance protection for laboratory workers.

www.clinicaltrials.gov identifier: NCT00962624.

1. Introduction

Invasive meningococcal disease (IMD) caused by the Gram-negative bacterium *Neisseria meningitidis* remains a significant cause of morbidity and mortality in many countries. In the general population, the greatest burden of disease is observed in infants, young children and adolescents. Asplenia and complement deficiency are specific risk factors irrespective of age [1]. As transmission of meningococci occurs via the aerosol/respiratory route, laboratory workers handling live meningococcal cultures in clinical, reference or research facilities have a potential occupational exposure risk. This is supported by a number of reports of probable/laboratory acquired cases [2] and a UK study which estimated laboratory workers to have a 184-fold increased risk of acquiring IMD compared to the general population [3].

* Corresponding author. Tel.: +44 0161 701 5303; fax: +44 0161 276 6792.
E-mail addresses: jamie.findlow@phe.gov.uk (J. Findlow), xilian.bai@phe.gov.uk (X. Bai), helen.findlow@phe.gov.uk (H. Findlow), emma.newton@phe.gov.uk (E. Newton), ed.kaczmarski@phe.gov.uk (E. Kaczmarski), elizabeth.miller@phe.gov.uk (E. Miller), ray.borrow@phe.gov.uk (R. Borrow).

http://dx.doi.org/10.1016/j.vaccine.2015.05.027
0264-410X/

Although protection of laboratory workers from IMD should principally rely on physical control measures to prevent exposure and acquisition [2], vaccination is an important form of defence. In Europe, the first quadrivalent (A, C, W and Y) glycoconjugate vaccine was licensed in 2010 [4] and a vaccine to provide protection against capsular group B strains was licensed in 2013 [5]. It is therefore now possible to implement an occupational vaccination programme designed to achieve protection against the five most prevalent capsular groups: A, B, C, W and Y which collectively account for almost all cases of disease [6]. The potential risk of disease in laboratory workers is longitudinal and therefore booster vaccinations are necessary to sustain protective antibody concentrations. The concern of immunological hyporesponsiveness induced by bivalent (A and C) and quadrivalent (A, C, W and Y) plain polysaccharide vaccines [7–10] has historically, complicated re-vaccination recommendations. The ability of glycoconjugate vaccines such as the monovalent MenC vaccines to overcome and not induce immunological hyporesponsiveness has resulted in these becoming the preferred option in groups requiring repeat vaccination [2].

In 2009, a quadrivalent (A, C, W and Y) glycoconjugate vaccine using a CRM carrier protein (ACWY-CRM) and the four component vaccine for capsular group B strains (4CMenB) were undergoing licensure review and in pre-licensure development, respectively. Both had acceptable safety profiles and proven immunogenicity [1,4]. We therefore undertook a single group phase II trial to evaluate the safety and immunogenicity of a single dose of ACWY-CRM and three 4CMenB doses in staff that may be at potential occupational exposure to meningococci. This trial incorporated the first evaluation of the concomitant administration of ACWY-CRM and 4CMenB and provided the potential of broader protection than that afforded by the vaccination programme in place at that time.

2. Materials and methods

2.1. Study population and schedule

Enrolment into this open-label phase II trial was open to adult (18–65 years of age) laboratory staff from the Public Health Laboratory, Manchester who were considered to be at potential occupational exposure to meningococci and provided written informed consent. Potential occupational exposure was defined as routine handling of live meningococcal cultures, which included both scientific staff and support staff. At enrolment, this incorporated a greater range of staff than were then scheduled to receive occupational meningococcal vaccination which was limited to scientific staff. During the course of this trial, ACWY-CRM gained licensure in Europe [4].

Exclusion criteria included known or suspected pregnancy, serious chronic disease (as evaluated by the trial clinician which would include progressive neurological disease or seizure disorder), bleeding diathesis or any other condition associated with prolonged bleeding time, history of severe allergic reactions after previous vaccinations, hypersensitivity to any vaccine component or receipt of another investigational agent within 90 days prior to enrolment or before completion of safety follow-up period.

MenACWY-CRM (Novartis Vaccines and Diagnostics, Siena, Italy) [4], from a single lot (Lot: X38D28N1Z) and 4CMenB (Novartis Vaccines and Diagnostics) [1], from a single lot (Lot: X79P45I1O) were administered to the deltoid muscle of the non-dominant and dominant arms, respectively at visit 1. Second and third doses of 4CMenB were administered at three and six months, respectively. Vaccination was deferred if the oral temperature measured was >38 °C or if there was acute illness on the day of vaccination. Subsequent immunisations were also delayed if any contradictions specified in the initial exclusion criteria were developed or any convulsions, neurological disturbances experienced following previous doses.

Blood samples were taken before and following each vaccination at months 0 (visit 1), 2 (visit 2), 3 (visit 3), 6 (visit 4) and 7 (visit 5).

2.2. Serology

Serum samples were assayed in the Vaccine Evaluation Unit at the Public Health England (PHE), Manchester, using a multiplexed fluorescent bead assay to quantify IgG antibody concentrations to A, C, W and Y polysaccharides [11]. Functional antibody activity against A, C, W and Y was determined in the serum bactericidal antibody (SBA) assay as previously described [12] utilising target strains presented in Table 1. Baby rabbit sera (Pel-Freeze Incorporated, Rodgerson, AZ, USA) was used as the exogenous complement source (rSBA) as recommended by the World Health Organization [13] in the standardised assay [12]. rSBA titres were expressed as the reciprocal of the final serum dilution giving 50% killing at 60 min. For computational purposes, rSBA titres lower than the serum starting dilution of 4 were assigned a value of 2. As baby rabbit complement is unsuitable for group B target strains, functional antibody activity against seven diverse strains (Table 1) was determined using human serum (Sera Laboratories International Ltd, UK) as the exogenous complement source (hSBA) as previously described [14]. hSBA titres were calculated as rSBA titres, with the exception that titres lower than the serum starting dilution of 2 were assigned a value of 1.

2.3. Correlates of protection

For group C, an rSBA titre of ≥8 is the putative protective titre which was shown to predict short-term clinical protection against disease in the UK [15,16]. The more discriminatory rSBA titre of ≥128 reliably predicts a hSBA titre of ≥4 [16], the accepted hSBA correlate of protection for group B [17,18]. Although an anti-capsular Ig concentration of ≥2 μg/mL has historically been used for group A [19], there are currently no established SBA correlates for groups A, W and Y; therefore, the group C rSBA correlate was applied in line with previous studies [20].

2.4. Safety

Following each vaccination, subjects were monitored for 30 min. Subjects were provided with a ruler and thermometer on enrolment to enable the completion of a health diary, for seven days recording and measuring oral temperature and any local reactions. Any visits to a doctor, systemic reactions (including headache and nausea) or any medication taken were also recorded in the diary. Severe reactions were classified as any local reaction >100 mm and any pain level which resulted in an inability to perform normal daily activities.

2.5. Analyses

All immunogenicity results gained were used in the analysis. For IgG antibody concentrations, geometric mean concentrations (GMCs) with 95% confidence intervals (95% CI) were calculated at each time point. For SBA results, geometric mean titres (GMTs) with 95% CI and proportions of subjects achieving rSBA titres ≥8 and ≥128 and hSBA titres ≥4 were calculated at each time point. Due to the small sample sizes, immunogenicity analysis did not include any formal statistical analysis.

Table 1
Meningococcal strains used in serum bactericidal antibody assays.

Strain	Capsular group	Subtype	Sequence type	Clonal complex	fHbp		NadA		NHBA	Comments
					Variant	Peptide[a]	Variant	Peptide[a]		
F8238	A	P1.20,9	5	5	1	5	2/3	8	27	Group A indicator strain
C11	C	P1.7-1,1	345	Unassigned	2	22	2/3	8	357	Group C indicator strain
M01 240070	W	P1.18,3	184	22	2	16	–	–	20	Group W indicator strain
M03 241125	Y	P1.5,2	11	11	2	22	2/3	3	29	Group Y indicator strain
44/76-SL	B	P1.7,16	32	32	1	1	–	–	3	Group B fHbp indicator strain
NZ 98/254	B	P1.7-2,4	42	41/44	1	14	–	–	2	Group B PorA indicator strain
5/99	B	P1.5,2	8	8	2	23	2/3	3	20	Group B NadA indicator strain
M00 242922	B	P1.7-2,4	41	41/44	1	4	–	–	2	Group B UK wild type strain (possessing same PorA as 4CMenB and NZ 98/254)
M01 240355	B	P1.22,14	213	213	3	31	4/5	12	18	Group B UK wild type strain (mismatched to 4CMenB antigens)
M01 240101	B	P1.19-1,15-11	1049	269	1	15	–	–	21	Group B UK wild type strain (mismatched to 4CMenB antigens)
M01 240364	B	P1.5,2	11	11	3	31	2/3	5	29	Group B UK wild type strain (mismatched to 4CMenB antigens)

[a]Classification scheme by www.neisseria.org which classifies peptide a sequential number on submission to the database.
– Does not harbour the NadA gene.

2.6. Governance

The trial was funded by the Meningitis Research Foundation, sponsored by PHE (formally the Health Protection Agency) and was conducted in accordance with the 1996 International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines, the 2000 Declaration of Helsinki and the 2004 EU Clinical Trial Directive. A favourable opinion was given by the UK Medicines and Healthcare products Regulatory Agency (MHRA) and the National Research Ethics Service, Wandsworth Research Ethics Committee. The EudraCT number was 2008-007182-23 and the trial was registered on the public website www.clinicaltrials.gov under the identifier: NCT00962624.

3. Results

The trial was undertaken between July 2010 and February 2011. A total of 42 staff responded to trial advertisements of whom 38 were available for the first visit and were enrolled and vaccinated (Fig. 1). Women accounted for 63% (24/38) of those enrolled reflecting the higher proportion of women within the eligible recruitment cohort. At enrolment, the mean age of subjects was 34 years 5 months (range 23–55 years). Seven subjects withdrew consent over the duration of the trial (2 not feeling well enough to continue, 1 local reaction to first 4CMenB dose, 1 needle phobia and 3 no reason given). Blood samples were available for all 38 subjects at visit 1 but due to missed time windows, failed venepuncture and needle phobia fewer samples were available at subsequent time points (Fig. 1). Safety data (from health diary/telephone follow up) were collected for all subjects for each vaccine received.

3.1. Immunogenicity

Prior to vaccination, high rates of baseline immunity were determined for groups A, C, W and Y with at least 60.5 and 57.9% of subjects having rSBA titres ≥8 and ≥128, respectively (Table 2). Two-month post-ACWY-CRM (and 1st 4CMenB), these proportions had increased to at least 94.6 and 89.2% for the two cut-offs, respectively. For groups A and C, a four-fold rise in rSBA GMT was determined two months following ACWY-CRM (and 1st 4CMenB), whereas for W and Y, a 23- and 14-fold rise was achieved, respectively (Table 3). The proportions of subjects with SBA titres at the cut-offs and rSBA GMTs remained relatively constant for the remaining three visits/five months encompassing the additional two 4CMenB doses. Increases of between 3.4- and 5.8-fold were observed in anti-capsular IgG GMC following ACWY-CRM which remained elevated for the remaining time points (Table 4).

High rates of baseline immunity were also determined for group B strains with at least 60.5% of subjects having hSBA titres ≥4 (Table 2). Increases in the proportions with hSBA titres ≥4 and hSBA GMT (Table 3) were observed against all seven strains following the first dose of 4CMenB. Subsequent increases in hSBA GMT were measured for all strains following the second 4CMenB dose, but this was not mirrored by increases in the proportions with hSBA titres ≥4 due to the high proportions already surpassing this level. The hSBA GMTs for all strains were shown to have decreased at the 6-month visit (pre-3rd 4CMenB dose) in comparison to month 3 although the proportions of subjects with hSBA titres ≥4 remained constant. The third dose of 4CMenB induced further responses, with the greatest number of subjects with hSBA titre ≥4 and highest hSBA GMT determined at the final seven-month visit for all group B strains.

3.2. Safety

Low rates of solicited injection site reactions were reported following ACWY-CRM, with two subjects (5%) reporting erythema and induration and eight subjects (21%) reporting pain (Fig. 2). In comparison, the proportions reporting erythema and induration following 4CMenB were at least three and six times greater, respectively. The most frequently reported reaction with 4CMenB was pain, reported by all subjects following the third dose and all but one subject following the first and second doses. Severe reactions were exclusively reported for 4CMenB vaccination and at least 16% of subjects reporting severe pain following each dose.

The greatest reports of systemic reactions of nausea and headache were following the first vaccination visit (ACWY-CRM

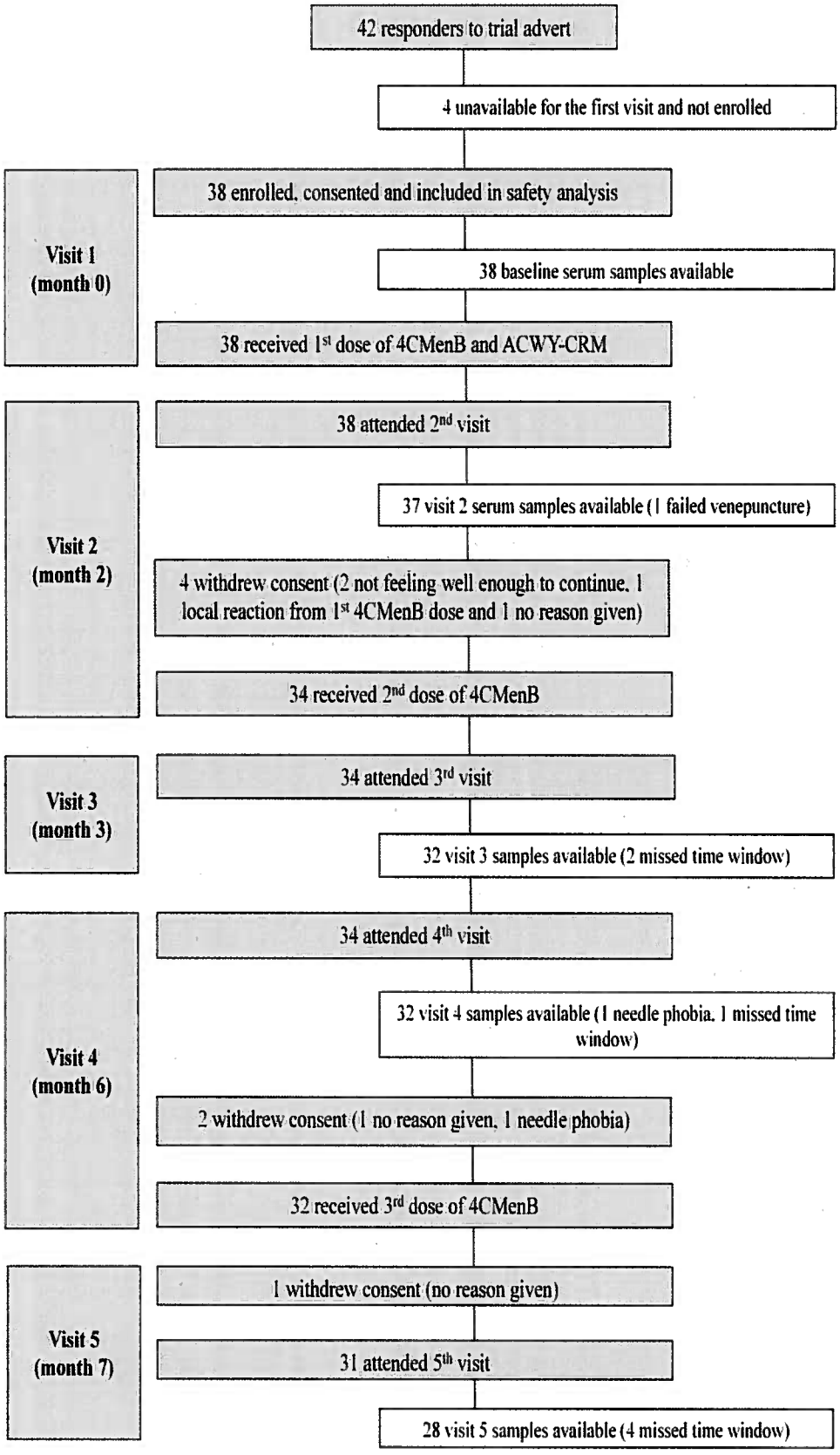

Fig. 1. Participant flow chart.

and 4CMenB) and consecutively reduced following the second and third doses of 4CMenB (Fig. 3). Over 40% of participants reported using pain relief following ACWY-CRM/4CMenB with fewer following the second and third doses of 4CMenB (Fig. 3).

Overall, 13 adverse events were reported of which 9 were considered by the investigators to be either related or possibly related to 4CMenB. These consisted of eight injection site reactions (all associated with 4CMenB) and a general episode of myalgia and

ody titres above cut-offs, pre- and post-vaccination.

)ff	Month 0 (pre-vaccination)	Month 2 (2 months post-ACWY-CRM, 2 months post-1st 4CMenB)	Month 3 (3 months post-ACWY-CRM, 1 month post-2nd 4CMenB)	Month 6 (6 months post-ACWY-CRM, 4 months post-2nd 4CMenB)	Month 7 (7 months post-ACWY-CRM, 1 month post-3rd 4CMenB)
≥ 8	32/38 (84.2%)	35/37 (94.6%)	31/32 (96.9%)	30/32 (93.75%)	28/30 (93.3%)
≥ 128	31/38 (81.6%)	35/37 (94.6%)	31/32 (96.9%)	30/32 (93.8%)	28/30 (93.3%)
≥ 8	26/38 (64.4%)	35/37 (94.6%)	32/32 (100.0%)	30/32 (93.8%)	29/30 (96.7%)
≥ 128	24/38 (63.2%)	33/37 (89.2%)	27/30 (90.0%)	28/32 (87.5%)	27/30 (90.0%)
≥ 8	23/38 (60.5%)	37/37 (100.0%)	32/32 (100.0%)	31/32 (96.9%)	30/30 (100.0%)
≥ 128	22/38 (57.9%)	36/37 (97.3%)	31/32 (96.9%)	28/32 (87.5%)	29/30 (96.7%)
≥ 8	24/38 (63.2%)	37/37 (100.0%)	32/32 (100.0%)	31/32 (96.9%)	30/30 (100.0%)
≥ 128	22/38 (57.9%)	35/37 (94.6%)	32/32 (100.0%)	30/32 (93.8%)	30/30 (100.0%)
\ ≥ 4	33/38 (86.8%)	35/37 (94.6%)	32/32 (100.0%)	32/32 (100.0%)	28/28 (100.0%)
\ ≥ 4	27/38 (71.1%)	33/37 (89.2%)	32/32 (100.0%)	31/32 (96.9%)	30/30 (100.0%)
\ ≥ 4	27/38 (71.1%)	37/37 (100.0%)	32/32 (100.0%)	32/32 (100.0%)	30/30 (100.0%)
\ ≥ 4	25/38 (65.8%)	31/37 (83.8%)	31/32 (96.8%)	31/32 (96.9%)	30/30 (100.0%)
\ ≥ 4	23/38 (60.5%)	31/37 (83.8%)	29/32 (90.6%)	29/32 (90.6%)	27/30 (90.0%)
\ ≥ 4	33/38 (86.8%)	37/37 (100.0%)	32/32 (100.0%)	32/32 (100.0%)	30/30 (100.0%)
\ ≥ 4	29/38 (76.3%)	33/37 (89.2%)	30/32 (93.8%)	29/31 (93.6%)	29/30 (96.4%)

)5% confidence intervals) pre- and post-vaccination.

	Month 0 (pre-vaccination)	Month 2 (2 months post-ACWY-CRM, 2 months post-1st 4CMenB)	Month 3 (3 months post-ACWY-CRM, 1 month post-2nd 4CMenB)	Month 6 (6 months post-ACWY-CRM, 4 months post-2nd 4CMenB)	Month 7 (7 months post-ACWY-CRM, 1 month post-3rd 4CMenB)
	251.37 (116.56–542.09)	1083.20 (600.35–1945.39)	1545.38 (889.06–2686.06)	1002.06 (509.59–1970.45)	615.95 (316.53–1198.60)
	112.66 (44.48–285.36)	449.07 (239.69–841.35)	512.00 (259.20–1011.34)	331.99 (175.00–629.80)	561.57 (291.84–1080.62)
70	75.41 (26.91–211.35)	1762.96 (1027.75–3024.10)	1448.16 (854.72–2453.62)	918.89 (449.22–1879.60)	1203.76 (677.04–2140.27)
25	45.25 (20.03–102.24)	653.19 (426.52–1000.30)	939.01 (608.39–1449.31)	546.38 (317.23–941.04)	523.97 (360.02–762.58)
	15.84 (9.73–25.79)	66.14 (34.05–128.45)	192.58 (112.34–330.16)	121.67 (64.18–230.68)	303.44 (198.53–463.78)
	8.84 (4.76–16.44)	58.25 (29.16–116.37)	271.61 (144.13–511.83)	127.76 (62.21–262.36)	272.49 (147.39–503.79)
	6.98 (4.26–11.43)	113.38 (61.80–208.00)	650.35 (355.89–1188.46)	180.34 (96.69–336.36)	866.43 (501.20–1497.82)
22	8.01 (4.51–14.26)	33.75 (17.81–63.96)	75.57 (39.33–145.21)	70.29 (35.30–139.97)	134.42 (68.93–262.14)
55	8.50 (4.44–16.30)	18.41 (10.72–31.63)	52.26 (27.99–97.59)	36.64 (19.59–68.52)	65.48 (31.33–163.83)
01	44.04 (23.10–83.95)	200.86 (129.06–312.69)	305.50 (166.23–561.45)	261.74 (140.62–487.17)	457.60 (270.00–775.54)
54	6.50 (4.43–9.53)	17.47 (9.60–31.77)	81.69 (40.47–164.89)	34.90 (18.24–66.76)	82.94 (48.17–142.82)

ch time point/strain are the same as those presented in Table 2.

Table 4
Anti-capsular IgG geometric mean concentrations (μg/mL) (95% confidence intervals) pre- and post-vaccination.

Capsular group	Month 0 (pre-vaccination)	Month 2 (2 months post-ACWY-CRM, 2 months post-1st 4CMenB)	Month 3 (3 months post-ACWY-CRM, 1 month post-2nd 4CMenB)	Month 6 (6 months post-ACWY-CRM, 4 months post-2nd 4CMenB)	Month 7 (7 months post-ACWY-CRM, 1 month post-3rd 4CMenB)
A	3.70 (2.17–6.29)	12.65 (7.72–20.70)	14.30 (8.55–23.91)	9.19 (5.38–15.72)	11.91 (6.94–20.44)
C	2.61 (1.16–5.86)	10.99 (5.93–20.35)	11.05 (6.42–19.00)	8.41 (4.91–14.40)	8.70 (4.99–15.19)
W	0.59 (0.24–1.46)	3.41 (1.55–7.52)	4.30 (1.99–9.26)	2.88 (1.30–6.17)	3.78 (1.85–7.73)
Y	0.95 (0.45–2.00)	5.21 (2.67–10.16)	5.38 (2.70–10.70)	5.42 (2.59–11.34)	7.62 (3.95–14.72)

The number of subjects used to calculate GMCs for each time point/strain are the same as those presented in Table 2.

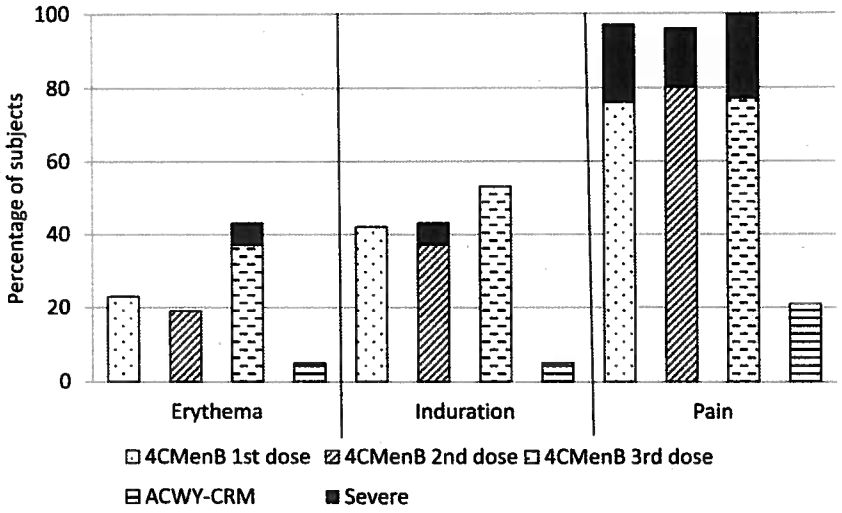

Fig. 2. Solicited injection site reactions following each vaccination visit.

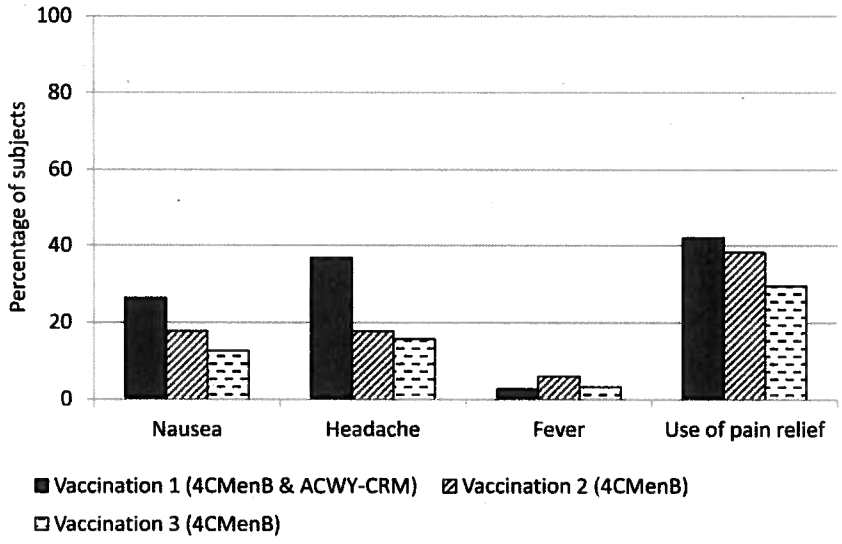

Fig. 3. Solicited systemic reactions and use of pain relief following each vaccination visit.

nausea three-day post-2nd dose of 4CMenB resulting in a General Practitioner visit.

3.3. Discussion

To our knowledge, this trial incorporated the first investigation of the concomitant administration of ACWY-CRM and 4CMenB in healthy adults. In the trial population, both vaccines were proven safe and immunogenic when concomitantly administered. These results provide support of the recommendation [21] of the use of these vaccines following their licensure in various at-risk groups including laboratory staff which may have a potential occupational exposure to meningococci.

Determination of the true immunogenicity of the vaccines in this trial is hindered by the lack of a naive control group and being undertaken in laboratory workers, of which 33 out of 38 had previously received meningococcal vaccination. Two subjects had received monovalent group C conjugate vaccination during the catch-up campaign in 1999/2000 [22], and 30 had been vaccinated with multivalent polysaccharide vaccines within the previous decade. Furthermore, 11 subjects had previously received three doses of the 'Norwegian' outer membrane vesicle vaccine

six years previously [23] and 12 subjects had received a combined group C and *Haemophilus influenzae* type b conjugate vaccine three years previously [24]. This was the likely explanation for the high baseline immunity which was at least double than previously reported for age-matched UK general population for both group C (C11) and B strains (NZ 98/254) [25,26].

Of the 38 subjects enrolled, 30 had received prior meningococcal polysaccharide vaccination which has been associated with immunological hyporesponsiveness [7–10]. Due to the lack of a naive control group it was not possible to determine any hyporesponsiveness or the ability of ACWY-CRM to overcome it as demonstrated in previous laboratory staff trials [27,28].

Two months following ACWY-CRM (and 4CMenB), high increases in rSBA GMT were observed and the proportions with rSBA titres >8 had increased to >94% for group A, C, W and Y strains. As the SBA assay determines all functional anti-meningococcal antibody, it is possible that responses to 4CMenB may have augmented the predominantly anti-capsular killing against A, C, W and Y strains. This is likely as the A strain contained an fHbp variant/peptide that has shown to be recognised on group B strains by post-4CMenB sera [29]. For NadA, the group Y strain had an identical peptide to that on the group B strain 5/99 used as an indicator for the NadA component in 4CMenB. Although both the MenA and MenC strains possessed a different NadA peptide to the Y and 5/99 strains, they were the same variant group, suggesting that they would be recognised by 4CMenB-induced antibody [30]. Each of the A, C, W and Y strains possessed NHBA, but the level of killing attributable to antibodies raised against this is unknown. The other key consideration in trying to elucidate any contribution of 4CMenB-induced antibody to rSBA titre against A, C, W and Y strains is the level of protein expression. The Meningococcal Antigen Typing System (MATS) has been developed to collectively evaluate protein expression in conjunction with recognition by 4CMenB-induced antibody and therefore considering cross-reactivity [31]. It has however, only been developed and validated for group B strains in reference to the hSBA assay, meaning that it cannot currently be applied to non-group B strains.

The highest rSBA GMTs for A, C and Y were determined at the three-month visit which was three months following ACWY-CRM and 1-month post-2nd dose of 4CMenB. This could lead to the postulation that this was in part due to the sub-capsular responses induced by the second dose of 4CMenB. It must however, be considered that the highest anti-capsular IgG GMCs were also determined at this time point. At the 7-month visit for A, C, W and Y, rSBA GMTs and anti-capsular IgG GMCs had declined but remained elevated in comparison to baseline levels. Comparison of the A, C, W and Y responses to other reported trials is difficult as the only other trial undertaken in laboratory staff reported results one-month post-vaccination [32] and in accordance with all other adult trials utilised the hSBA assay [33,34].

Responses to 4CMenB were investigated using diverse strains as performed in the previous UK studies [35–37]. This consisted of three group B indicator strains 44/76-SL, NZ 98/254 and 5/99 to determine responses predominately against fHbp, PorA and NadA, respectively and four wild-type strains from the UK with known protein expression levels [35]. Broad responses were induced following 4CMenB as evidenced by the increases in hSBA GMT against all seven strains following first and second doses. hSBA data against the UK strains support previous predictions that cross-reactivity of 4CMenB-induced antibody to non-identical variants increases with age [29]. In this study, adults responded to both M01 240101 and M01 240364, as was demonstrated in infants when 4CMenB was administered at 6 and 8 months [36] but not at 2, 4 and 6 months of age [35]. Robust responses were also demonstrated in this study by adults against M01 240355. This was predictable as responses to this strain had been previously observed when 4CMenB was administered at 60 but not 40 months of age [37] or in infancy [35,36]. Due to the breadth of responses in adults, it is difficult to determine the immunogenicity and cross-reactive response directed against each individual component of 4CMenB against any of the SBA assay target strains. For example, responses against M01 240355 are unlikely to be as a result of fHbp, PorA or NadA due to previous studies suggesting that the variants expressed are too divergent to be recognised by 4CMenB-raised antibody [1,29,30]. Responses are therefore likely to be as a result of either NHBA or the minor components of the OMV.

Group B hSBA GMTs declined over the three months following the second 4CMenB dose as demonstrated in previous adolescent and adult studies [32,38]. These hSBA GMTs, however, were at least seven- and four-fold greater than baseline levels for indicator and UK strains, respectively. The highest hSBA GMTs were observed for all strains following the third dose of 4CMenB and the pattern similar to that reported by previous three-dose adolescent and adult studies [32,38]. During later stage pre-licensure studies, the number of 4CMenB doses was reduced to two [38,39] and the vaccine is now licensed in Europe for ages two years and above as a two-dose schedule [5]. This is supported by trial data generated against the three indicator strains. This study is the first to report data from non-indicator strains following three doses in adults which demonstrate that the benefit of a third dose is strain specific. For M01 240355 and M01 240364, similar hSBA GMTs were determined following the third as those following the second dose. For M01 242922 and M01 240101, however, the third dose was responsible for the induction of higher GMTs.

Knowledge of antibody persistence is important to inform re-vaccination recommendations, particularly in populations where direct protection is essential. The only available persistence data for 4CMenB are for up to two years following two doses in subjects aged 11–17 years against indicator strains [38]. Although we did not investigate persistence, we did observe that the UK stains generally had lower SBA GMTs than indicator strains. Assuming that antibody decay is the similar for all strains, antibody levels will drop below protective levels sooner for UK strains than indicator strains which had the higher post-vaccination GMTs. The lower GMTs following the licensed two-dose schedule, in comparison to those following three doses will also impact on persistence. These have implications for future re-vaccination timing decisions particularly when protection is required against a broad spectrum of group B meningococci.

Local injection site reactions of some type were reported by all subjects receiving 4CMenB while only 23% of subjects reported a reaction associated with ACWY-CRM. Due to concomitant administration, we were unable to determine the cause of the systemic reactions although based on previous reports it is likely that the fever was associated with 4CMenB [35,36,38,40]. These results are consistent with previously published studies where these vaccines were administered separately. To reduce systemic reactions, prophylactic paracetamol has been recommended in infants when receiving 4CMenB [21] and has been shown not to adversely impact immunogenicity [40]. Although not studied in this trial, it is likely that paracetamol could also be used to reduce the reactogenicity profile of 4CMenB when administered to adults.

The immunogenicity results reported incorporate all serology results gained, including those at initial time points for those whom subsequently withdrew. This was shown not to introduce any bias and also enabled data for subjects whom missed an intermediate blood draw to be included in the final analyses. One potential limitation of this trial is the lack of a naive control group which limits the applicability of the results to vaccine naive individuals. It must be considered however, that the aim of this trial was to investigate these vaccines in a population previously and currently recommended to receive meningococcal vaccination. Results are

therefore likely a combination of primary and secondary responses, representative of responses in such at-risk groups.

4. Conclusions

This first investigation of the concomitant administration of ACWY-CRM and 4CMenB has demonstrated that they are safe and immunogenic in healthy adults. These vaccines are suitable for providing protection in laboratory workers against the five major pathogenic meningococcal capsular groups and supporting the recommendation for their use in this setting [21]. These data also support the ongoing development of a combined vaccine comprising of both ACWY-CRM and 4CMenB components [41].

Conflict of interest statement

J.F., X.B., H.F., E.N. and R.B. perform contract research on behalf of PHE for Novartis, GSK, Baxter Bioscience, Pfizer, Sanofi Pasteur and Sanofi Pasteur MSD. J.F. has acted as a consultant on behalf of PHE for Novartis, GSK and Baxter Bioscience. None declared for other authors.

Acknowledgements

We would like to thank all the staff who participated in the trial and Lynne Joslin, our dedicated Vaccine Research Nurse. We are grateful to the Meningitis Research Foundation for the provision of funding, PHE (formerly Health Protection Agency) for acting as the Sponsor and Novartis Vaccines and Diagnostics for the provision of vaccines.

We are indebted to Dr Jo Southern for expert advice in facilitating regulatory and ethical approval processes. Finally, we would like to acknowledge the contribution and professionalism of the staff in the Vaccine Evaluation Unit who undertook the laboratory analysis of the samples.

References

[1] Bai X, Findlow J, Borrow R. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. Expert Opin Biol Ther 2011;11:969–85.
[2] Borrow R, Findlow J, Gray S, Taylor S, Kaczmarski E. Safe laboratory handling of *Neisseria meningitidis*. J Infect 2014;65:305–12.
[3] Boutet R, Stuart JM, Kaczmarski EB, Gray SJ, Jones DM, Andrews N. Risk of laboratory-acquired meningococcal disease. J Hosp Infect 2001;49:282–4.
[4] Pace D. Novel quadrivalent meningococcal A, C, W-135 and Y glycoconjugate vaccine for the broader protection of adolescents and adults. Future Microbiol 2010;5:1629–40.
[5] Martin NG, Snape MD. A multicomponent serogroup B meningococcal vaccine is licensed for use in Europe: what do we know, and what are we yet to learn. Expert Rev Vaccines 2013;12:837–58.
[6] Abio A, Neal KR, Beck CR. An epidemiological review of changes in meningococcal biology during the last 100 years. Pathog Glob Health 2013;107:373–80.
[7] Richmond P, Kaczmarski E, Borrow R, Findlow J, Clark S, McCann R, et al. Meningococcal C polysaccharide vaccine induces immunologic hyporesponsiveness in adults that is overcome by meningococcal C conjugate vaccine. J Infect Dis 2000;181:761–4.
[8] Granoff DM, Gupta RK, Belshe RB, Anderson EL. Induction of immunological refractoriness in adults by meningococcal C polysaccharide vaccination. J Infect Dis 1998;178:870–4.
[9] Findlow H, Sow S, Borrow R, Tapia M, Haidara FC, Akinsola AK, et al. Meningococcal group C and W135 immunological hyporesponsiveness in African toddlers. Clin Vaccine Immunol 2011;18:1492–6.
[10] Jokhdar H, Borrow R, Sultan A, Adi M, Riley C, Fuller E, et al. Immunological hyporesponsiveness to group C but not group A following repeated meningococcal A/C polysaccharide vaccination in Saudi Arabia. Clin Diagn Lab Immunol 2004;11:83–8.
[11] Lal G, Balmer P, Joseph H, Dawson M, Borrow R. Development and evaluation of a tetraplex flow cytometric assay for quantitation of serum antibodies to *Neisseria meningitidis* serogroups A, C, Y, and W-135. Clin Diagn Lab Immunol 2004;11:272–9.
[12] Maslanka SE, Gheesling LL, Libutti DE, Donaldson KBJ, Harakeh HS, Dykes JK, et al., The Multilaboratory Study Group. Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. J Clin Microbiol 1997;4:156–67.
[13] World Health Organization. Requirements for meningococcal polysaccharide vaccine. World Health Organisation Technical Series No. 594. Geneva: WHO; 1976.
[14] Borrow R, Aaberge IS, Santos GF, Eudey TL, Oster P, Glennie A, et al. Interlaboratory standardization of the measurement of serum bactericidal activity by using human complement against meningococcal serogroup B, strain 44/76-SL, before and after vaccination with the Norwegian MenBvac outer membrane vesicle vaccine. Clin Diag Lab Immunol 2005;12:970–6.
[15] Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine using efficacy estimates from post-licensure surveillance in England. Clin Diag Lab Immunol 2003;10:780–6.
[16] Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: a re-evaluation of correlates of protection. Infect Immun 2001;69:1568–73.
[17] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus: I. The role of humoral antibodies. J Exp Med 1969;129:1307–26.
[18] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus: II. Development of natural immunity. J Exp Med 1969;129:1327–48.
[19] Makela PH, Kayhty H, Weckstrom P, Sivonen A, Renkonen OV. Effect of group-A meningococcal vaccine in Army recruits in Finland. Lancet 1975;2:883–6.
[20] Findlow H, Borrow R. Immunogenicity and safety of a meningococcal serogroup A, C, Y and W glycoconjugate vaccine, ACWY-TT. Adv Ther 2013;30:431–58.
[21] Department of Health. Immunisation against infectious disease—'The Green Book'. http://immunisation.dh.gov.uk/category/the-green-book/ (2006).
[22] Miller E, Salisbury D, Ramsay M. Planning registration and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 2001;20(Supplement 1):S58–67.
[23] Findlow J, Taylor S, Aase A, Horton R, Heyderman R, Southern J, et al. Comparison and correlation of *Neisseria meningitidis* serogroup B immunologic assays and human antibody responses following three doses of the 'Norwegian' meningococcal outer membrane vesicle vaccine, MenBvac. Infect Immun 2006;74:4557–65.
[24] Findlow J, Findlow H, Frankland S, Holland A, Holme D, Newton E, et al. Evaluation of the safety and immunogenicity in United Kingdom laboratory workers of a combined *Haemophilus influenzae* type b and meningococcal capsular group C conjugate vaccine. JOMT 2014;9:26.
[25] Ishola DA, Borrow R, Findlow H, Findlow J, Trotter C, Ramsay ME. Prevalence of serum bactericidal antibody to serogroup C *Neisseria meningitidis* in England a decade after vaccine introduction. Clin Vaccine Immunol 2012;19:1126–30.
[26] Trotter C, Findlow J, Balmer P, Holland A, Barchha R, Hamer N, et al. Seroprevalance of bactericidal and anti-outer membrane vesicle antibodies to *Neisseria meningitidis* group B in England. Clin Vaccine Immunol 2007;14:863–8.
[27] Borrow R, Southern J, Andrews N, Peake N, Rahim R, Acuna M, et al. Comparison of antibody kinetics following meningococcal serogroup C conjugate vaccine between healthy adults previously vaccinated with meningococcal A/C polysaccharide vaccine and vaccine-naïve controls. Vaccine 2001;19:3043–50.
[28] Southern J, Deane S, Ashton L, Borrow R, Goldblatt D, Andrews N, et al. Effects of prior polysaccharide vaccination on magnitude, duration, and quality of immune responses to and safety profile of a meningococcal serogroup C tetanus toxoid conjugate vaccination in adults. Clin Diagn Lab Immunol 2004;11:1100–4.
[29] Brunelli B, Del Tordello E, Palumbo E, Biolchi A, Bambini S, Comanducci M, et al. Influence of sequence variability on bactericidal activity sera induced by Factor H binding protein variant 1.1. Vaccine 2011;29:1072–81.
[30] Commanducci M, Bambini S, Brunelli B, Adu-Bobie J, Arico B, Capecchi B, et al. NadA, a novel vaccine candidate of *Neisseria meningitidis*. J Exp Med 2002;195:1445–54.
[31] Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, et al. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci USA 2010;107:19490–5.
[32] Kimura A, Toneatto D, Kleinschmidt A, Wang H, Dull P. Immunogenicity and safety of a multicomponent meningococcal serogroup B vaccine and a quadrivalent meningococcal CRM197 conjugate vaccine against serogroups A, C, W-135, and Y in adults who are at increased risk for occupational exposure to meningococcal isolates. Clin Vaccine Immunol 2011;18:483–6.
[33] Alberer M, Burchard G, Jelinek T, Reisinger EC, Meyer S, Forleo-Neto E, et al. Immunogenicity and safety of concomitant administration of a combined hepatitis A/B vaccine and a quadrivalent meningococcal conjugate vaccine in healthy adults. J Travel Med 2014;22:105–14.
[34] Alberer M, Burchard G, Jelinek T, Reisinger EC, Beran J, Hlavata LC, et al. Safety and immunogenicity of typhoid fever and yellow fever vaccines when administered concomitantly with quadrivalent meningococcal ACWY glycoconjugate vaccine in healthy adults. J Travel Med 2014;22:48–56.
[35] Findlow J, Borrow R, Snape MD, Dawson T, Holland A, John TM, et al. Multicentre, open-label, randomized phase II controlled trial of an investigational recombinant meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. Clin Infect Dis 2010;51:1127–37.
[36] Snape MD, Dawson T, Oster P, Evans A, John TM, Ohene-Kena B, et al. Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life- a randomized comparative trial. Pediatr Infect Dis J 2010;29:71–9.

[37] McQuaid F, Snape MD, John TM, Kelly S, Robinson H, Houlden J, et al. Persistence of bactericidal antibodies to 5 years of age after immunization with serogroup B meningococcal vaccines at 6, 8, 12 and 40 months of age. PIDJ 2014;33:760–6.

[38] Santolayla ME, O'Ryan ML, Valenzuela MT, Prado V, Vergara R, Munoz A, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in Healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. Lancet 2012;379:614–24.

[39] Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. Lancet 2014;384:2123–31.

[40] Prymula R, Esposito S, Zuccotti GV, Xia F, Toneatto D, Kohl I, et al. A phase II randomized controlled trial of a multicomponent meningococcal serogroup B vaccine (I). Hum Vaccin Immunother 2014;10:1993–2004.

[41] **ClinicalTrials.gov identifier: NCT01272180. www.clinicaltrials.gov (accessed 09.01.2015).**