

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: French N, Gordon SB, Mwalukomo T, et al. A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. *N Engl J Med* 2010;362:812-22.

Supplementary Material

Methods

Laboratory methods

We used standard microbiological techniques for processing clinical specimens. *Streptococcus pneumoniae* was identified on the basis of colony morphology, α haemolysis, sensitivity to Optochin and bile salt solubility. Serotyping of pneumococci was performed using latex agglutination and the Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). The Respiratory and Meningeal Pathogens Research Unit, Johannesburg, South Africa, undertook quality control of serotyping and molecular typing for serotype 6C by molecular characterisation of the *wciN* region of the capsular gene loci.

The HIV status of participants was confirmed following enrolment using a standardized twin EIA method (Vironostika HIV uni-Form II plus O, Biomerieux, Boxtel, Netherlands and Murex HIV-1.2.0, Dartford, UK).

CD4 T-cell counts were performed using a FACScount© system (Becton Dickinson, San Jose, California, USA). Whole blood parameters were measured using a HMX analyser (Beckman Coulter, Brea, California, USA). Viral loads were measured using the Roche Cobas amplicor HIV-1 monitor version 1.5 kits (Branchburg, USA).

The laboratories participated in the UK external quality assessment scheme, NEQAS.

Trial product handling

Study products were presented in single dose vials labelled in pairs with the study number. Labelling was done by two independent local clinicians who retained a single hard copy of the randomization list in Blantyre. Storage was in accordance with the manufacturer's recommendations in a temperature-monitored refrigerator. After inoculation, study product vials were signed and dated by the study nurse and retained for final audit.

Study product was injected into the deltoid muscle of the non-dominant arm.

Data management and analysis.

Study data were collected by manual recording of information in a standardized format. Double data entry and validation were performed in databases created in Microsoft Access© 2000 (Microsoft corp., Redmond, WA, U.S.A.) on a secure Microsoft SQL server. Analyses were carried out using Stata© version 10.0 (Stata corporation, College station, Texas, U.S.A.).

Table 1 web: Baseline demographic, clinical and laboratory parameters for all randomized participants, loss to follow up categorization and uptake of HIV¹ care (antiretroviral therapy [ART] and trimethoprim-sulphamethoxazole [CTX] prophylaxis).

Variable	Treatment group		P ²
	Vaccine	Placebo	
At baseline			
Number of patients	248	248	
Male sex	106 (42.7%)	111 (44.7%)	0.92
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Age (years)			
Median [Range]	31 [16 – 72]	33 [15 - 75]	0.74
15 – 24 years	37 (14.9%)	41 (16.5%)	
25 – 34 years	111 (44.8%)	105 (42.3%)	
35 – 44 years	58 (23.4%)	66 (26.6%)	
45 – 54 years	28 (11.3%)	21 (8.5%)	
55 – 75 years	14 (5.7%)	15 (6.1%)	
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Previous invasive pneumococcal disease – no (%)			
Bacteremic pneumonia	187 (75.4%)	196 (79.0%)	0.74
Meningitis	60 (24.2%)	51 (20.6%)	
Other invasive syndrome	1 (0.4%)	1 (0.4%)	
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Days since previous invasive pneumococcal disease			
Median [Range] ³	19 [7 – 1946]	20 [7 – 1715]	0.70
History of tuberculosis – no (%)	60 (24.2%)	83 (33.5%)	0.02
History of pneumonia before enrollment episode – no (%)	99 (39.9%)	116 (46.8%)	0.25
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Presence of HIV infection – no (%)			
Positive	219 (88.3%)	218 (87.9%)	0.70
WHO clinical stage 4	42 (16.9%)	45 (18.2%)	
Unknown HIV status	1 (0.4%)	1 (0.4%)	
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CD4+ count at baseline			
Median [Range]	212 [1 - 1342]	214 [1 - 1200]	0.60
≤100	46 (18.6%)	39 (15.7%)	
>100 - ≤200	62 (25.0%)	73 (29.4%)	

>200 - ≤ 500	83 (33.5%)	67 (27.0%)	
> 500 / HIV –ve	42 (16.9%)	53 (21.4%)	
Missing, not HIV -ve	15 (6.1%)	16 (6.5%)	
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Viral load at baseline - Log ₁₀			
Median [Range]	4.9 [2.5 - 5.9] ⁴	5.0 [2.5 - 6.8]	0.16
≥100,000 counts/ml - no (%)	94 (42.7%) ⁵	101 (46.1%)	
Missing, not HIV –ve - no	13	10	
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ARTuse ^{4,5}			
ART at enrolment – no (%)	32 (14.6%) ⁴	26 (11.9%)	0.48
Median duration (days) [Range]	91 [3 - 337]	84 [2 - 652]	
ART commenced during follow-up – no (%)	63 (28.6%)	73 (33.3%)	0.35
Median time from recruitment to start (days)	146	190	
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CTX use ⁴			
CTX at enrolment – no (%)	20 (9.1%)	20 (9.2%)	1.00
Median duration (days)	41	39	
CTX commenced during follow-up – no (%)	111 (50.4%)	125 (57.1%)	0.15
Median time from recruitment to start (days)	179	182	
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At follow-up			
Combined ART & CTX use at any time during study – no (%)	89 (40.6%)	95 (43.6%)	0.56
Patients who received 2 nd dose of vaccine between 28 and 56 days – no (%)	221 (89.1%)	224 (90.3%)	0.62
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Lost to follow-up			
Declined participation	11 (4.4%)	13 (5.2%)	0.28
Left the study area	17 (6.9%)	24 (9.7%)	
Not found at given address	9 (3.6%)	7 (2.8%)	

¹ HIV denotes human immunodeficiency virus

² Between group comparisons are only for patients with HIV infection. Comparison were calculated with the use of Fisher's exact, Pearson's chi-square or the Wilcoxon rank sum test..

³ 13 recruits had more than twelve months from IPD event to recruit

⁴ Data on viral load, antiretroviral and trimethoptim-sulphamethoxazole use are provided only for patients with HIV infection.

⁵ All patients received Triomune® (Cipla pharmaceuticals, Mumbai, India) consisting of Stavudine, Lamivudine and Nevirapine. Two patients substituted Efavirenz for Nevirapine with and two participants substituted Stavudine with Zidovudine, because of intolerance.

Table 3 web: Primary and secondary end-point numbers, adverse events and loss to follow-up categorized by enrolment CD4 count in the 437 HIV-infected patients¹.

End-point	<u>Number of patients (events)</u>		<u>Hazard ratios for First event (95%CI)²</u>		<u>Incidence rate ratios for Recurrent events (95%CI)</u>	
	Vaccine	Placebo	Unadjusted	Adjusted	Unadjusted	Adjusted
<u>Primary end-point</u>						
Vaccine serotype or 6A (Intention-to-treat analysis)	5 (5)	19 (19)	0.26 (0.10-0.70)	0.31 (0.11-0.84) ³	-	-
CD4≤200	2(2)	16 (16)				
200<CD4≤500	3 (3)	1 (1)				
>500	0 (0)	1 (1)				
CD4 missing	0 (0)	1 (1)				
<i>Vaccine serotype or 6A (Per protocol analysis)</i>	<i>4 (4)</i>	<i>18 (18)</i>	<i>0.22 (0.08-0.66)</i>	<i>0.26 (0.08-0.78)</i>	-	-
<u>Secondary end-point</u>						
Vaccine serogroup	7 (7)	19 (20)	0.37 (0.15-0.87)	0.41 (0.17-1.02)	0.19 (0.06-0.66)	0.30 (0.09-1.02)
Any invasive pneumococcal disease ⁴	22 (29)	30 (38)	0.72 (0.42-1.25)	0.80 (0.45-1.44)	0.35 (0.12-1.01)	0.34 (0.11-1.02)
CD4≤200	11 (11)	21 (26)				
200<CD4≤500	10 (17)	7 (9)				
>500	0 (0)	1 (1)				
CD4 missing	1(1)	1(2)				
All cause pneumonia	32 (44)	41 (58)	0.75 (0.47-1.19)	0.71 (0.43-1.17)	0.54 (0.26-1.14)	0.49 (0.20-1.21)

CD4≤200	21 (26)	31 (41)				
200<CD4≤500	9 (14)	6 (8)				
>500	0 (0)	2 (3)				
CD4 missing	2(4)	2(6)				
Minor adverse events ⁵	27 (41)	9 (13)		-	-	-
Serious adverse events ⁶	3 (3)	17 (17)		-	-	-
	Number of patients (Number on ART)					
Death ⁷	73	63	1.18 (0.84-1.66)	1.24 (0.88-1.75)	-	-
CD4<200	51 (6)	47 (11)				
200<CD4≤500	20 (4)	7 (3)				
CD4>500	0 (0)	7 (0)				
HIV +ve, CD4 unknown	2 (1)	2 (0)				
Definite, probable and possible pneumococcal related death ⁸	35	35	1.02 (0.64-1.63)	1.14 (0.71-1.85)	-	-
CD4<200	22 (4)	26 (5)				
200<CD4≤500	11 (1)	5 (1)				
CD4>500	0 (0)	3 (0)				
HIV +ve, CD4 unknown	2 (1)	1 (1)				

Loss to follow-up

Number of patients

CD4<200	13	20
200<CD4≤500	9	12
CD4>500	1	4
HIV +ve, CD4 unknown	3	2

¹No episodes of pneumococcal disease or pneumonia were recorded in the 57 patients who were not infected with the human immunodeficiency virus (HIV). Data regarding the status of HIV infection were missing for one patient in each study group.

²Hazards are for the vaccine group as compared with the placebo group . Values were adjusted for age, sex, viral load, clinical stage and CD4+ cell count at baseline

³ When the proportional-hazards assumption was violated, the Cox model was stratified according to the year of recruitment and baseline CD4+ count.

⁴ In vaccine group, two patients each had two recurrent events, and one patient had six recurrent events. In the placebo group, six patients had two recurrent events and one had three recurrent events.

⁵ P=0.003 by Fisher's exact test

⁶ P=0.002. The serious adverse events consisted of 2 deaths and 1 hospitalization in the vaccine group and 7 deaths and 10 hospitalizations (5 of which were invasive pneumococcal disease, 1 of which was caused by vaccine serotype) in the placebo group.

⁷ Of the patients who died, 11 in the vaccine group and 14 in the placebo group were receiving antiretroviral therapy

⁸ Of the 70 deaths in this category, the diagnosis of pneumococcal disease was definite for 9, probable for 4 and possible for 57; among these patients, 6 in the vaccine group and 7 in the placebo group were receiving antiretroviral therapy.

Supplementary table 5 List of minor adverse events by vaccine group. Thirty six individuals reported 54 minor adverse events, 19 with first vaccine, 17 with second and five patients reporting events with both vaccines.

Description	Vaccine	Placebo
Number of patients reporting any minor adverse event	27	9
Primary symptoms reported (number reporting for both vaccinations, when repeated)		
Pain at injection site	15 (2)	2
Local reaction	3	2
Fever ¹	11 (1)	3
Myalgia	5	1
Abdominal pain	1	1
Diarrhoea	1	1
Headache	1	0
Nasal discharge	0	1
Sneezing	0	1
Weakness/fatigue	1	0
Anorexia	0	1

¹ Reported by patient. An axillary temperature greater than 37.5°C was not documented in any episode.

Supplementary table 6: Description of all serious adverse events by vaccine group, HIV status and relationship to vaccine dose.

Study Identifier	HIV status	CD4+ count	Vaccine dose	Time after vaccine (days)	Investigator assessment ¹	Outcome	Diagnosis ²
<u>Conjugate vaccine</u>							
1036	+	420	1	2	R	Recovered	Lobar pneumonia
1210	+	5	1	7	R	Died	Undiagnosed sudden collapse
1456	+	19	1	3	N	Died	Undiagnosed acute febrile illness
<u>Placebo</u>							
1017	+	182	2	14	R	Recovered	Undiagnosed acute febrile illness
1035	+	152	1	14	R	Died	TB pericardial effusion
1062	+	538	1	8	R	Died	Acute febrile respiratory illness
1083	+	180	2	1	N	Recovered	Pneumococcal pneumonia (IPD serotype 10B)
1098	+	386	2	7	R	Recovered	<i>Klebsiella oxytoca</i> septicaemia
1099	+	145	2	6	R	Recovered	Pneumococcal pneumonia (IPD serotype 4)
1166	+ ³	Missing	1	1	N	Died	Renal failure
1170	+	143	2	6	R	Recovered	Lobar Pneumonia
1219	+	179	1	6	R	Recovered	Pneumococcal pneumonia (IPD serotype not typed)
1221	+	539	2	9	R	Recovered	Undiagnosed collapse
1236	+	3	2	11	R	Died	Undiagnosed acute febrile illness with reduced consciousness
1239	+	94	2	9	R	Recovered	Pneumococcal pneumonia (IPD serotype 25)
1299	+	753	2	13	R	Died	Undiagnosed acute febrile illness
1312	+	227	1	10	R	Recovered	Pneumococcal meningitis, (IPD serotype 12F)
1314	+	345	1	6	R	Recovered	Lobar pneumonia

1442	+	320	2	1	B	Died	Undiagnosed cardio-respiratory arrest
1487	+	98	2	6	R	Died	Pulmonary embolus

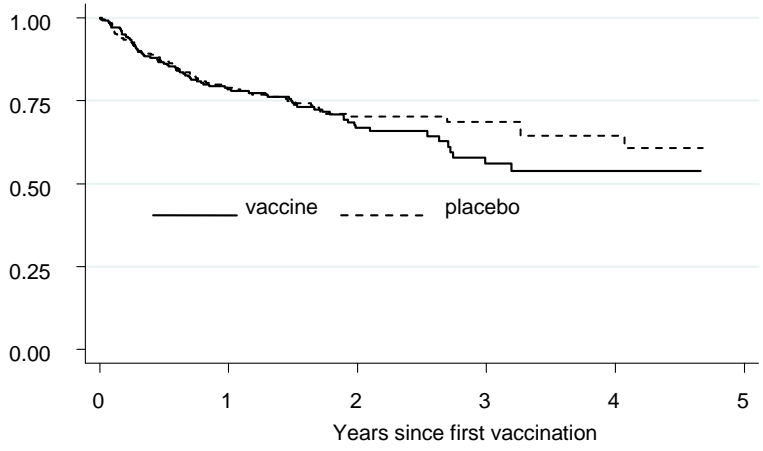
¹ Relationship of event to vaccine as assessed by principal investigator at the time of the incident. Categories are Definitely, Probably, Possibly (B) Probably not (R) Not related (N).

² Diagnosis based on clinical assessment or on verbal autopsy when death outside of hospital

³ Unconfirmed by study protocol, patient reported HIV-infected.

Figure 2:

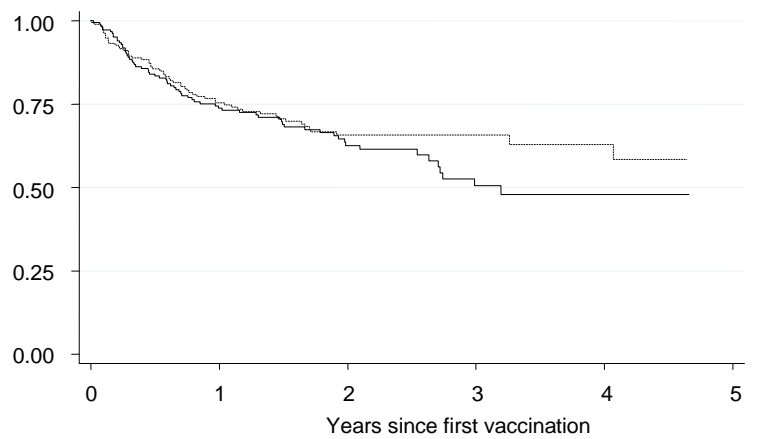
(A)



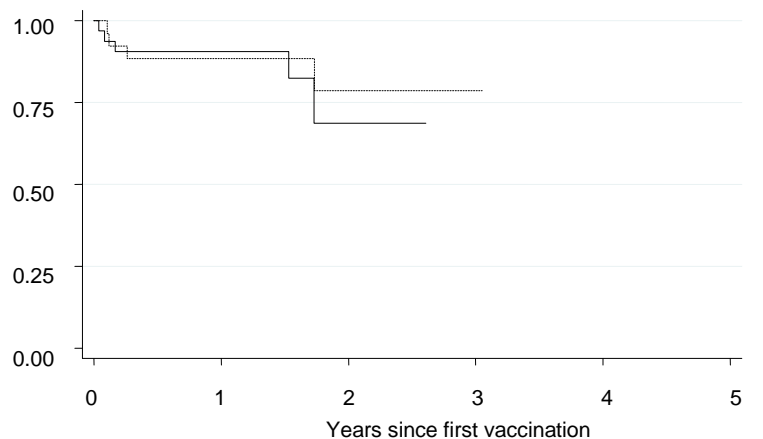
Number at risk

vaccine	248	154	78	31	15	0
placebo	248	156	86	40	19	0

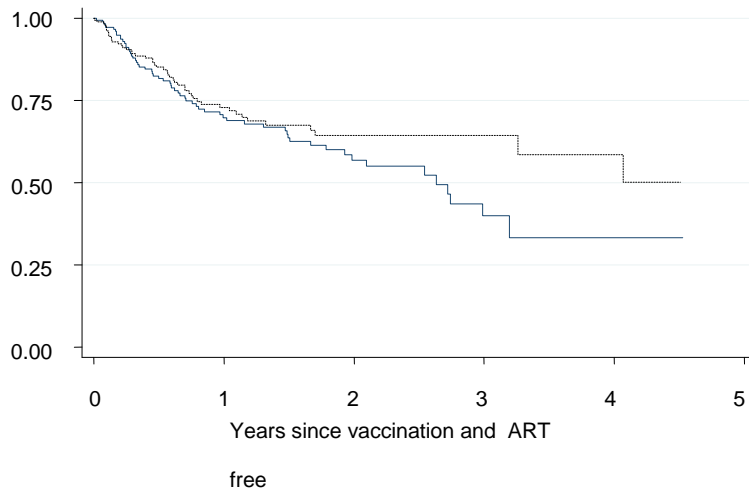
(B)



(C)



(D)



(E)

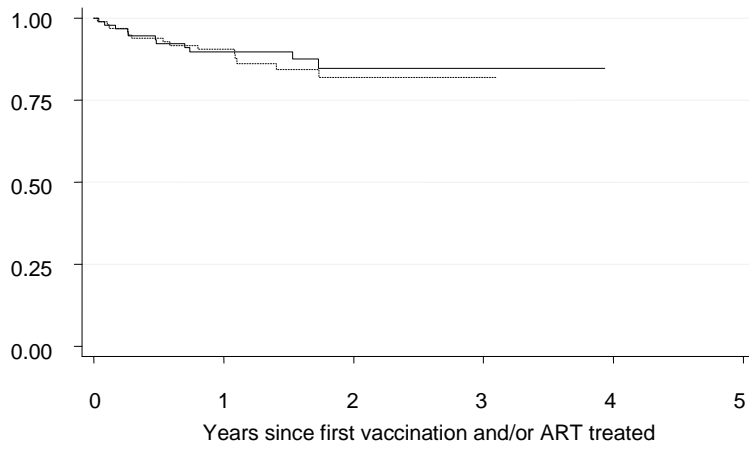


Figure 2 legend: (A) Kaplan-Meier plot of time to death for the whole cohort by vaccine group. Plots (B-E) show survival in the HIV-infected participants by vaccine group. Plots (B & C) show survival in relation to antiretroviral (ART) use at enrolment. Plot (B) shows those not receiving ART, plot (C) those receiving ART at enrolment. Plots (D & E) show survival by ART treatment but allowing for movement from the ART naïve cohort on commencement of ART after enrolment. Plot (D) shows the cohort remaining ART naïve throughout their participation in the study, plot (E) shows the survival of those who were on ART at enrolment or who commenced ART during follow-up.