

Supplementary Appendix

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

Supplement to: Nair P, Pizzichini MMM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985-93.

Online supplement

Table of contents

Methods	2
Table 1 (individual patient data confirming asthma diagnosis	4
Table 2 (prednisone dose at beginning and end of study)	5
Table 3 (details of individual exacerbations)	6
Table 4A (sputum differential cell count before & after treatment with mepolizumab: subgroup analysis)	8
Table 4B (sputum differential cell count before & after treatment with placebo: subgroup analysis)	9
Table 4C (blood differential cell count before & after treatment with mepolizumab: subgroup analysis).....	10
Table 4D (blood differential cell count before & after treatment with placebo: subgroup analysis)	11
Figure 1 (time to exacerbation: subgroup analysis)	12
Figure 2 (correlation between blood and sputum eosinophils: subgroup analysis)	13
Figure 3a (change in FEV1 before and after treatment: subgroup analysis)	14
Figure 3b (change in FEV1 percent predicted before and after treatment: subgroup analysis)	15
Figure 3c (change in ACQ before and after treatment: subgroup analysis)	16

Methods

Patients

At the time of the study, seven of the patients (4 in the mepolizumab arm and 3 in the placebo arm) had normal methacholine airway responsiveness (PC20 >16 mg/ml) despite persistence of symptoms with prednisone withdrawal. Airway responsiveness or bronchodilator reversibility was not checked in nine subjects (4 in the active arm and 5 in the placebo arm) either because the FEV1 was too low or because of reluctance on our part to withhold long acting inhaled β 2-agonists to obtain a pre-bronchodilator FEV1 measurement.

Blinding

Drs FEH and PN did all the clinical assessments. MK did all the clinical measurements. AE did all the sputum measurements. Patients were recruited from our clinics. Sputum was induced by MK (clinical lab) and sent to AE (sputum lab). AE would call FEH or PN to let us know if the sputum eos was greater than 3% in which case the patients were randomized. Neither FEH or PN saw the results.

At each visit, MK would induce sputum and send it to AE. Melanie, FEH or PN were not told of the results. They continued with the steroid reduction according to schedule. If the patient developed clinical criteria of exacerbation, there were 4 possibilities for the sputum counts: 1) eosinophilia, 2) neutrophilia suggestive of bacterial infection (>15 million and 80% neutrophil), 3) neutrophilia not suggestive of bacterial infection (<15 million and 80% neutrophil) and 4) normal cell counts.

If the sputum lab detected a neutrophilia likely to be a bacterial infection (option 2), FEH or PN were informed about it and they would treat with antibiotic, and continue with the steroid reduction protocol. **If the exacerbation was associated with eosinophilia, or with a neutrophilia not**

likely to be due to bacterial infection or with normal cell counts, FEH or PN were not informed of the sputum cell counts. They followed the protocol, withdrew patients and treated them with prednisone. Blood eosinophil counts results were also kept away from both FEH and PN.

Thus the investigators were not able to guess the treatment allocations based on sputum or blood eosinophil counts.

Table 1 (online): Individual patient data confirming asthma diagnosis

PATIENTS	Year of onset of symptoms	Year of prednisone dependence	History prior to randomization				At randomization		Current Reversibility ml (%)	Outcome in the study
			Confirmed exacerbations in preceding 3 years (n)	Date of last exacerbation	Max fall in FEV ₁ , ml (%) with exacerbation	Max reversibility, ml (%) with salbutamol	Sputum Eosinophils (%)	FEV ₁ (% predicted)		
Mepolizumab										
01	2002	2004	9	Aug-05	1260 (50%)	260 (19%)	42.3	60%	No follow-up	Completed
02*	1995	2006	6	May-06	1050 (25%)	130 (4%)	25.5	95%	40 (1%)	Completed
03	1993	2002	4	Mar-05	1860 (69%)	260 (30%)	21.0	68%	130 (7%)	Completed
04	1970	1985	>10	Mar-04	300 (29%)	210 (25%)	10.7	34%	210 (24%)	Completed
05	2005	2005	13	Dec-05	2420 (65%)	530 (18%)	12.3	63%	520 (17%)	Completed
06**	1996	1996	19	Nov-04	1100 (37%)	70 (4%)	54.3	55%	130 (5%)	Completed
07	1979	1999	6	Apr-06	670 (29%)	480 (29%)	16.6	85%	70 (2%)	Completed
08	1968	1983	3	Jun-05	900 (47%)	210 (21%)	6.0	65%	70 (6%)	Withdrawn
09	1999	2004	7	Jun-05	500 (38%)	470 (37%)	1.7%	44%	460 (42%)	Not analyzed
Placebo										
01	1993	1999	13	Oct-04	1750 (60%)	710 (53%)	3.3	45%	200 (17%)	Completed
02	Unknown	2004	6	Jun-05	1650 (57%)	300 (22%)	4.0	87%	460 (28%)	Completed
03	1986	2005	19	Aug-05	890 (28%)	350 (18%)	3.6	71%	Deceased	Exacerbated
04	2001	2006	9	Mar-06	1600 (47%)	550 (29%)	4.6	73%	550 (29%)	Exacerbated
05	1995	1999	8	May-05	2610 (56%)	580 (18%)	4.7	91%	420 (14%)	Exacerbated
06	1997	2001	13	Jan-06	860 (45%)	280 (29%)	3.2	39%	350 (37%)	Exacerbated
07	1981	1995	9	Sep-05	1100 (48%)	420 (24%)	6.0	67%	200 (13%)	Exacerbated
08	2000	2004	5	Nov-04	950 (37%)	340 (21%)	4.5	83%	30 (1%)	Exacerbated
09	2004	2005	4	Jun-05	1100 (35%)	510 (24%)	35.3	60%	370 (15%)	Exacerbated
10	1973	2005	13	Jun-05	690 (38%)	240 (22%)	3.7	64%	140 (13%)	Exacerbated
11	1994	1999	13	Jan-06	2160 (51%)	470 (16%)	+++ granules	88%	310 (9%)	Not analyzed

* PC₂₀ methacholine 3.2 mg/ml, **PC₂₀ methacholine 2.0 mg/ml

Table 2 (online): Prednisone dose at the beginning and at the end of the study. The two patients who were not included in the sub-group analysis (protocol violators) are indicated in red.

Patients	Starting dose (mg)	Lowest dose without exacerbation (mg)	Minimum possible dose according to protocol (mg)	<i>% of maximum possible reduction</i>
Mepolizumab				
01	15	5	5	<i>100</i>
02	5	0	0	<i>100</i>
03	5	0	0	<i>100</i>
04	10	2.5	2.5	<i>100</i>
05	25	7.5	5	<i>87.5</i>
06	20	5	5	<i>100</i>
07	10	2.5	2.5	<i>100</i>
08	10	5	2.5	<i>66.7</i>
09	7.5	7.5	0	<i>0</i>
Placebo				
01	17.5	5	5	<i>100</i>
02	5	0	0	<i>100</i>
03	12.5	5	2.5	<i>75</i>
04	12.5	12.5	2.5	<i>0</i>
05	10	5	2.5	<i>66.7</i>
06	10	5	2.5	<i>66.7</i>
07	5	5	0	<i>0</i>
08	10	5	2.5	<i>66.7</i>
09	20	20	5	<i>0</i>
10	2.5	2.5	0	<i>0</i>
11	12.5	7.5	2.5	<i>50</i>

Table 3 (online): Details of all exacerbations, withdrawn due to protocol violation (1 each in each treatment arm) and withdrawn due to adverse event (1 in the active group). Exacerbations associated with sputum neutrophilia are indicated in bold and the corresponding visit with an *. The table has the following information: a) treatment group b) exacerbation visit c) prednisone dose at exacerbation d) FEV1 e) FEV1 % predicted f) FEV1/VC g) FEV1 post bd h) Likert composite score i) Likert cough score j) ACQ k) SABA: total number of puffs of salbutamol for the two days previous to the visit l) Sputum TCC m) Sputum Eos% n) Sputum N % o) Blood eos (absolute) p) criterion(a) used to define exacerbation

Any blank cells in SEOS or SNEU = missing/no DCC. Criteria to define exacerbation: Symptoms (nocturnal symptoms or albuterol use 4 or more puffs over baseline for two consecutive days or 2 unit change in cough score) or 15% change in FEV1 or both

	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Placebo Subject	EXAC	V#	PRED	FEV1	F%PD	FEVVC	FEV BD	LKRT	CGH	ACQ	SABA	TCC	SEOS	SNEU	BEOS	CRITERIA
1	V6	1	2.5	1.15	64	55	1.20	35	7	0.57	0	2.0	3.7	76.0	135.2	FEV1 Nocturnal wheeze
		2	2.5	1.05	58	59	1.23	34	6	0.71	0	15.6	9.3	69.3	223.1	
		5	0.0	0.99	55	56	1.21	34	7	0.71	0	14.7	8.0	52.3	558.0	
		6	0.0	0.94	52	59	1.10	33	6	0.71	4	15.0	18.7	39.3	589.3	
2	V7* V10* V11	1	12.5	2.78	71	73	2.87	33	7	0.86	0	4.4	3.6	74.4	558.0	FEV1 SABA Cough
		2	12.5	2.49	64	64	2.47	33	7	1.10	0	13.0	12.8	68.4	338.0	
		7	5.0	2.1	54	75	2.11	18	3	4.3	6	37.5	1.0	92.7	561.6	
		10	2.5	2.38	61	71	2.33	31	6	1.60	4	32.9	14.4	82.0	846.0	
3	V8	11	2.5	2.16	56	76	2.37	21	4	2.70	8	10.1	63.7	22.0	1040.3	
		1	10.0	3.96	91	81	3.96	35	6	0.71	0	6.2	4.7	33.4	121.8	FEV1 SABA
		2	10.0	3.70	85	81	3.41	35	7	0.30	0	1.2	2.0	26.3	403.2	
		7	5.0	3.61	83	80	3.71	35	7	0.30	2	14.2	32.4	26.5	1117.8	
8	5.0	3.35	77	78	3.58	31	6	0.43	8	15.9	62.3	17.0	499.2			
4	V9	1	10.0	1.78	84	69	1.90	30	6	1.10	0	2.6	4.5	13.5	333.0	Cough SABA
		2	10.0	1.75	83	66	1.75	31	6	1.60	0	2.7	3.1	11.3	246.0	
		8	5.0	1.67	79	67	1.80	31	6	1.00	0	1.8	45.7	21.3	408.1	
		8.1	2.5	1.69	80	67	1.66	25	4	2.10	4	3.2	13.3	72.4	474.5	
		9	2.5	1.76	83	67	1.84	25	4	2.10	18	2.6	25.3	46.3	624.8	
5	V3	1	20.0	1.94	60	62	2.08	29	7	2.70	32	7.6	35.3	41.4	238.0	FEV1 SABA
		2	20.0	1.67	51	60	1.94	27	6	2.60	32	4.5	47.0	17.0	506.0	
		3	20.0	1.69	51	62	2.27	21	6	3.30	38	15.9	47.0	17.0	143.0	

Subject	EXAC	V#	PRED	FEV1	F%PD	FEVVC	FEV BD	LKRT	CGH	ACQ	SABA	TCC	SEOS	SNEU	BEOS	CRITERIA
6	V6	1	5.0	1.74	67	74	1.88	32	7	2.30	2	2.1	6.0	70.5	491.4	SABA Nocturnal wheeze
		2	5.0	1.57	61	88	1.68	32	7	2.30	0	3.4	1.0	26.0	423.3	
		5	2.5	1.80	70	99	1.97	32	7	1.30	2	2.2	6.5	50.5	592.2	
		6	2.5	1.72	67	91	1.89	29	7	2.10	8	2.7	5.0	64.0	674.5	
7	V5	1	12.5	2.48	73	64	2.75	31	7	1.90	5	1.0	4.6	31.7	395.6	FEV1 SABA
		2	12.5	2.20	65	58	2.42	28	7	2.30	1	3.9	4.0	51.5	198.0	
		4	12.5	2.90	86	65	2.84	34	7	0.86	6	0.8	0.6	44.0	191.1	
		5	7.5	1.27	38	41	1.33	29	7	3.00	30	6.3	5.3	80.0	673.2	
8	V10	1	10.0	1.33	39	37	1.33	31	7	3.10	22	0.2	3.2	11.8	366.3	FEV1 SABA
		2	10.0	1.41	41	39	1.41	29	7	3.30	24				280.8	
		9	2.5	1.08	32	33	1.23	29	6	2.40	28	4.4	27.0	26.0	980.4	
		10	2.5	1.04	31	31	1.04	29	6	2.60	43	4.6	51.0	23.0	731.0	
9	V7	1	12.5	3.47	88	69	3.63	32	7	1.3	2	1.0			127.2	Protocol violation (exacerbated) Nocturnal wheeze, hypo- Adrenalism
		2	12.5	3.55	90	70	3.88	31	7	1.6	2	33	0	89	130.9	
		6	7.5	3.68	93	73	3.87	34	6	1.0	2	6.9			453.6	
		7	5.0	3.69	93	75	3.89	27	6	1.0	4	10.7	5	72.4	924.6	
10	V8*	1	5.0	2.44	87	70	2.66	30	7	2.7	4	2.4	4.0	54.3	962.5	Cough SABA
		2	5.0	2.35	84	73	2.35	32	7	2.6	4	3.5	3.4	49.0	608.4	
		8	0	2.22	80	77	2.49	30	4	1.9	12	26.2	5.7	79.0	1106.0	
Mepolizumab 11	V6	1	10.0	1.27	65	75	1.22	32	7	1.10	2	1.4	6.0	36.3	553.5	Withdrawn by PI (adverse event) No exacerbation
		2	10.0	1.14	58	75	1.23	29	7	1.60	2	0.8			335.8	
		5	7.5	1.30	67	79	1.34	31	6	1.40	4	5.8	2.0	13.0	24.0	
		6	7.5	1.34	69	76	1.32	34	7	1.70	2	6.9	0.3	36.7	10.4	
12	V5	1	7.5	1.11	44	60	1.57	24	6	4.1	8	1.2	1.6	25.2	510.6	Protocol violation (exacerbated) FEV1 SABA
		2	7.5	1.47	59	68	1.77	24	6	3.3	8	0.2	1.0	55.0	262.2	
		4	7.5	1.48	59	62	1.45	24	5	3.3	18				78.4	
		5	5.0	0.87	35	59	1.03	10	5	4.4	20	30.6	0.7	90.6	12.1	

Table 4A (online). Sputum differential cell counts before and after treatment with mepolizumab in the subgroup of patients with sputum eosinophilia at baseline.

Variables	Visit 1 Baseline	Visit 4 4weeks post First dose	Visit 12 4weeks post Last dose	Visit 14 8 weeks post Last dose
N	8	8	7	7
Viability, %	50.4 (11.00)	51.8 (18.95)	56.2 (29.41)	63.7 (17.03)
TCC, x10 ⁶ cells/ml	6.6 (7.23)	5.0 (6.54)	2.3 (2.20)	5.0 (3.88)
Eosinophils, % *	18.8 (6.0-54.3)	0.0 (0.0-4.0)**	1.3 (0.0-11.3)**	0.3 (0.0-4.6)**
Neutrophils, %	45.0 (17.56)	57.7 (7.26)	58.9 (22.47)	54.0 (30.39)
Macrophages, %	28.4 (18.44)	37.5 (7.46)	36.6 (23.98)	42.1 (30.60)
Lymphocytes, %	2.0 (2.73)	1.4 (0.82)	0.2 (0.45)**	2.1 (2.38)
Epithelial cells, %	1.0 (1.39)	2.7 (3.48)	1.1 (1.26)	0.3 (0.39)

Data expressed as mean and SD, *median (min-max) **p<0.05 compared to baseline

Table 4B (online). Sputum differential cell counts before and after treatment with placebo in the subgroup of patients with sputum eosinophilia at baseline.

Variables	Visit 1 Baseline	Visit 4 4weeks post First dose	Visit 12 4weeks post Last dose	Visit 14 8 weeks post Last dose	Exacerbation	4 weeks post Exacerbation
N	10	9	2	2	8	8
Viability, %	65.6 (16.13)	51.1 (10.81)	62.9 (4.03)	48.1 (9.12)	47.4 (22.40)	72.6 (21.65)
TCC, x10 ⁶ cells/ml	3.4 (2.43)	5.0 (5.46)	2.4 (2.05)	2.1 (0.64)	9.1 (5.85)	4.0 (4.06)
Eosinophils, % *	4.3 (3.2-35.3)	5.3 (0.6-16.3)	5.3 (1.3-5.0)	5.0 (1.0-9.0)	25.3 (5.0-63.7)	3.8 (2.0-41.4)
Neutrophils, %	46.5 (23.98)	59.0 (17.59)	58.5 (9.97)	60.8 (27.15)	41.7 (23.59)	54.5 (31.09)
Macrophages, %	43.9 (24.90)	33.6 (17.12)	34.5 (9.19)	29.9 (22.42)	24.4 (9.54)	35.1 (26.01)
Lymphocytes, %	1.3 (1.45)	0.9 (0.97)	1.9 (1.98)	2.7 (1.41)	0.7 (0.81)	0.8 (0.77)
Epithelial cells, %	0.9 (1.27)	0.9 (1.20)	2.0 (1.41)	1.7 (2.33)	0.2 (0.16)	1.1 (2.02)

Data expressed as mean and SD, *median (min-max)

Table 4C (online). Blood differential cell counts before and after treatment with mepolizumab in the subgroup of patients with sputum eosinophilia at baseline.

Variables	Visit 1 Baseline	Visit 4 4weeks post First dose	Visit 12 4weeks post Last dose	Visit 14 8 weeks post Last dose
N	8	8	7	7
Lymphocytes (x10 ⁶ /L)	1.94 (1.05)	1.51 (0.63)	1.82 (0.67)	1.65 (0.64)
Monocytes (x10 ⁶ /L)	0.59 (0.24)	0.51 (0.25)	0.76 (0.24)	0.74 (0.21)
Neutrophils (x10 ⁶ /L)	7.58 (3.94)	8.30 (3.70)	5.94 (2.31)	6.37 (3.03)
Eosinophils (x10 ⁶ /L)	0.68 (0.52)	0.05 (0.04)**	0.07 (0.04)**	0.07 (0.04)**
Basophils (x10 ⁶ /L)	0.07 (0.04)	0.04 (0.03)	0.05 (0.02)	0.05 (0.03)

Data expressed as mean and SD, **p<0.05 compared to baseline

Table 4D (online). Blood differential cell counts before and after treatment with placebo in the subgroup of patients with sputum eosinophilia at baseline.

Variables	Visit 1 Baseline	Visit 4 4weeks post First dose	Visit 12 4weeks post Last dose	Visit 14 8 weeks post Last dose	Exacerbation
N	10	9	2	2	8
Lymphocytes (x10 ⁶ /L)	1.96 (0.96)	1.48 (0.30)	2.61 (0.50)	2.48 (0.34)	1.51 (0.26)
Monocytes (x10 ⁶ /L)	0.55 (0.23)	0.54 (0.18)	0.56 (0.14)	0.52 (0.08)	0.61 (0.18)
Neutrophils (x10 ⁶ /L)	7.29 (2.42)	8.58 (2.38)	4.13 (0.78)	4.67 (0.07)	5.77 (2.49)
Eosinophils (x10 ⁶ /L)	0.37 (0.26)	0.32 (0.20)	0.66 (0.41)	1.22 (1.38)	0.62 (0.25)
Basophils (x10 ⁶ /L)	0.06 (0.03)	0.05 (0.02)	0.04 (0.01)	0.05 (0.02)	0.08 (0.06)

Data expressed as mean and SD

Figure 1 (online). Sub-group analysis of patients with baseline eosinophilia: Time to exacerbation after randomization into study treatment (n=8) or placebo (n=10). None of the patients in the active arm exacerbated (one was withdrawn due to an adverse event). Eight out of 10 patients who received placebo had exacerbation associated with eosinophilia.

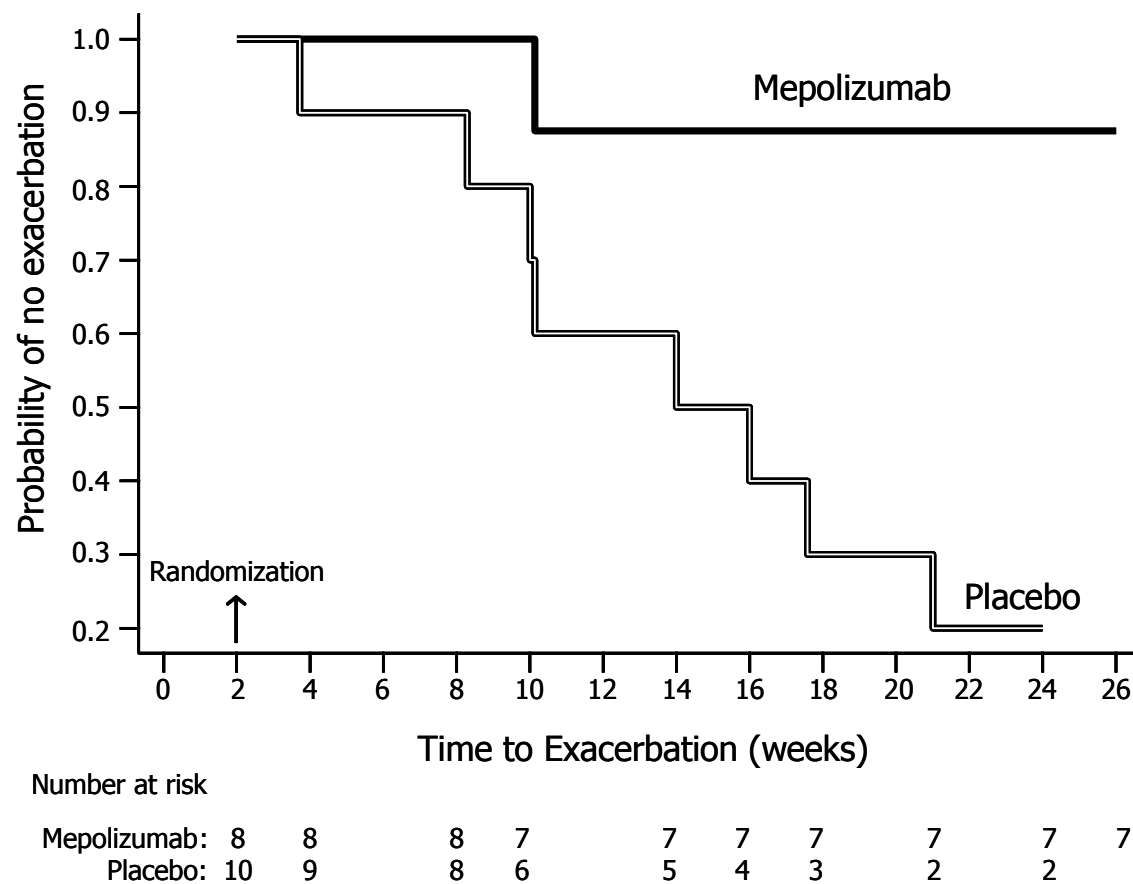


Figure 2 (online): correlation between blood eosinophils and sputum eosinophils at the beginning and at the end of the study in the subgroup of patients with sputum eosinophilia at baseline. Closed circles represent treatment with mepolizumab and the open circles represent placebo.

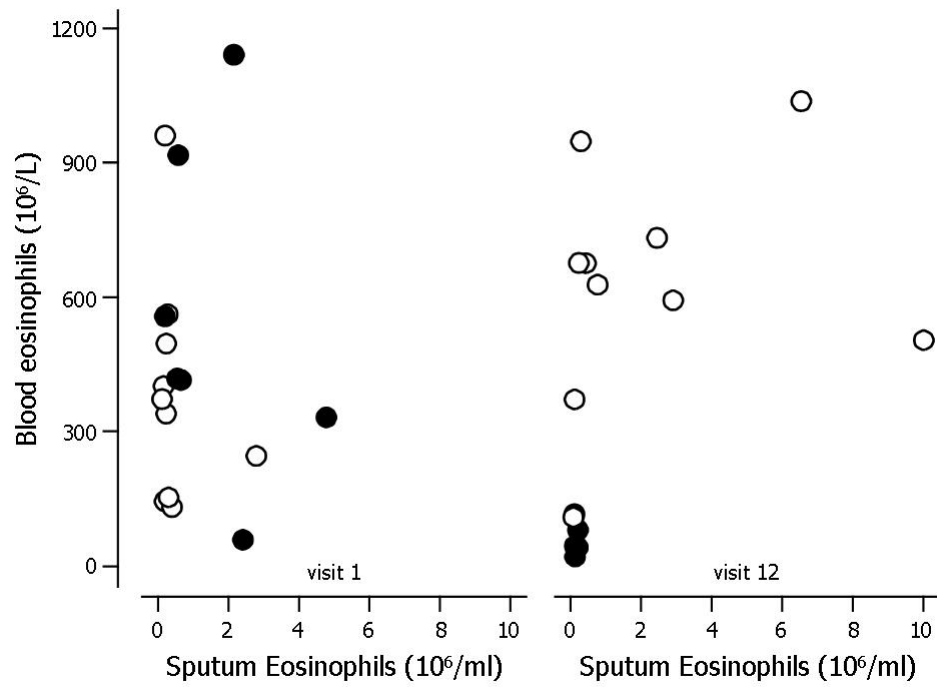


Figure 3a (online): Changes in FEV₁ (L) before and after treatment with mepolizumab or placebo in the subgroup with sputum eosinophilia at baseline

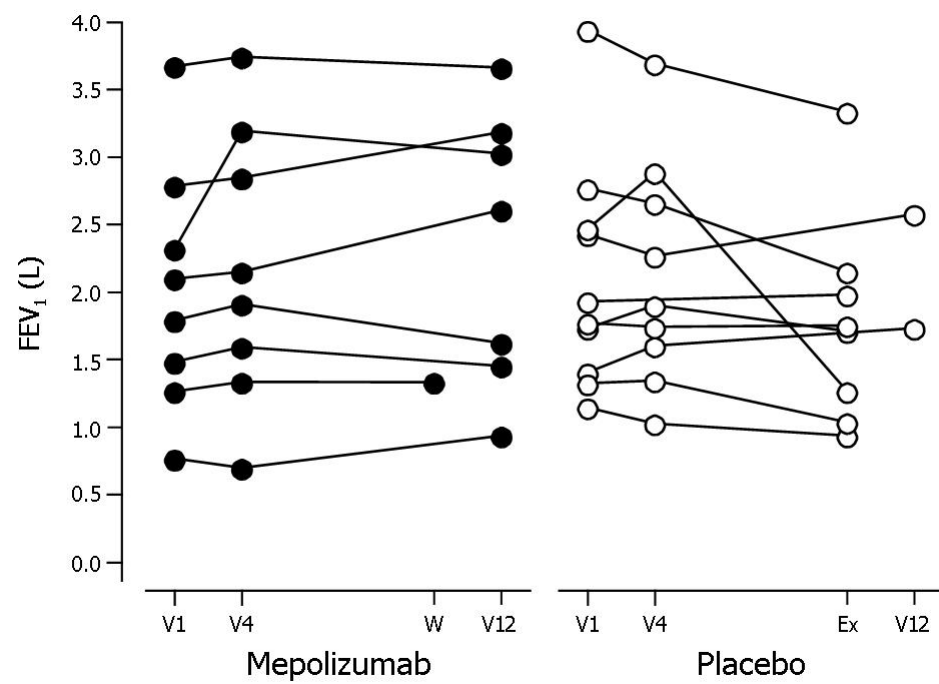


Figure 3b (online): Changes in FEV₁ (% predicted) before and after treatment with mepolizumab or placebo in the subgroup of patients with sputum eosinophilia.

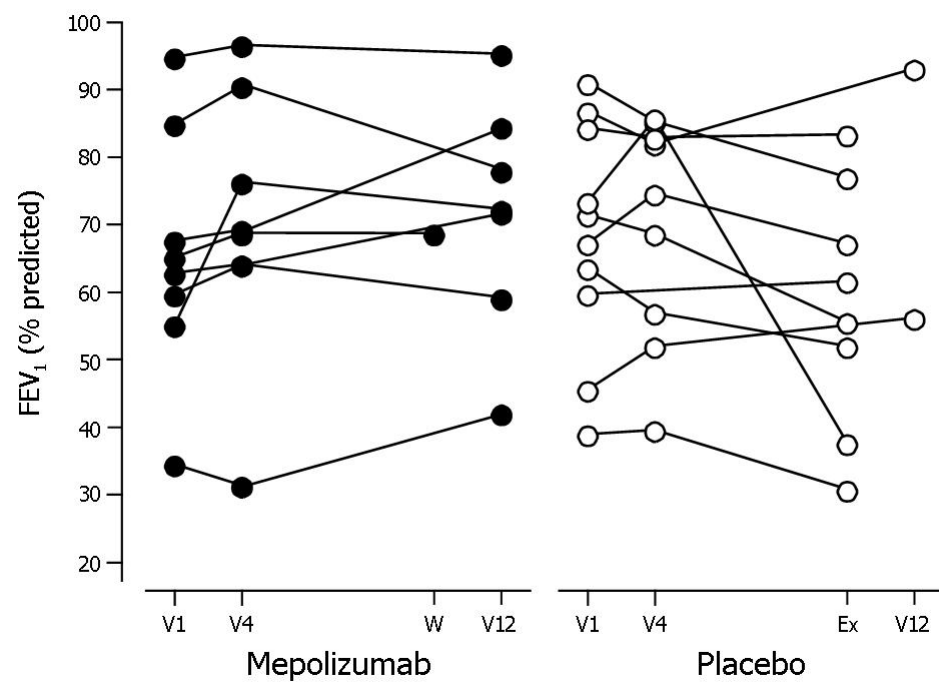


Figure 3c (online): Changes in ACQ before and after treatment with mepolizumab or placebo in the subgroup with sputum eosinophilia at baseline.

