

Mepolizumab failed to affect bullous pemphigoid: A randomized, placebo-controlled, double-blind phase 2 pilot study

To the Editor,

Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease characteristically affecting the elderly.¹ The disease is due to an autoimmune response against hemidesmosomal antigens, involving autoreactive T and B cells. Interleukin (IL)-5 is abundantly expressed in lesional skin and is associated with blood eosinophilia and eosinophil infiltration in the skin of BP patients.^{2,3} There is evidence that eosinophils, following activation with IL-5 in the presence of BP antibodies, may directly contribute to blister formation.⁴

Superpotent topical and oral corticosteroids as well as doxycycline are currently used to treat BP patients.¹ Nevertheless, use of steroids is limited by their side effects, and after discontinuation of therapy, BP relapses in 85% of patients within 6 months.⁵ Since eosinophils are characteristically found in the skin at early stages of the disease before blisters occur, targeting eosinophils by reducing their number and activation promised an alternative therapeutic approach. Anti-IL-5 antibody (mepolizumab) therapy has been shown to be effective in eosinophilic bronchial asthma and hypereosinophilic syndrome.⁶⁻⁸

The objective of the presented trial was to investigate the efficacy and safety of mepolizumab vs placebo as an add-on therapy to oral corticosteroids (OCS) in patients with an acute flare-up of BP. We conducted a randomized, placebo-controlled, double-blind, parallel-group, phase 2 pilot study at a single academic center (NCT01705795). The trial was approved by the cantonal ethics committee Bern and the regulatory agency Swissmedic. All the participants provided written informed consent prior to enrollment in the study.

After screening, participants were randomly assigned in a 2:1 ratio to receive intravenous mepolizumab at a dose of 750 mg or matching placebo every 4 weeks over 12 weeks in addition to standard care with OCS and followed over a period of up to 6 months after treatment. The OCS dose was 0.5 mg prednisolone per kg body weight until no further blisters and/or BP lesions appeared and was then tapered by 20% every 2 weeks. Enrolled patients presented with either a newly diagnosed disease or a relapse of BP.¹ The primary endpoint was the cumulative rate of relapse-free patients after initiating therapy. Additional information on the study design (Figure

S1, Tables S1 and S2) and the statistical analyses is provided in this article's Appendix S1.

In total, 32 patients were enrolled, and 30 underwent randomization and received at least one dose of the trial regimen and thus were included in the intention-to-treat population. The patients' characteristics and diagnostic findings at baseline are given in Table S3 in this article's Appendix S1.

The proportion of patients free of BP relapse did not differ between mepolizumab and placebo groups as assessed 4 weeks after the end of treatment (week 16) and at the end of follow-up (week 36) (Table 1, Figure 1A). Moreover, there were no significant differences in median time until relapse between groups. Thereby, the trial failed to achieve the primary endpoint. In addition, the cumulative rates of patients achieving disease control and maintaining disease control did not show significant differences between mepolizumab and placebo groups (Figure 1B-C). Upon treatment, an overall improvement of BP clinical signs as indicated by a reduction of the severity score, and the intensity of pruritus was noted in both mepolizumab and placebo groups. Despite the fact that mepolizumab did not significantly affect the clinical outcome, we found significantly lower peripheral blood eosinophil levels in patients treated with mepolizumab compared to patients receiving placebo (Table 1).

Next, we were interested whether mepolizumab would have an effect on skin inflammation (Table S4, Figure S2 in this article's Appendix S1). In both groups, the mean number of skin-infiltrating eosinophils was reduced upon therapy. The mean difference in eosinophil numbers was higher in the mepolizumab compared with the placebo group (-62.8 vs -45.9 ; $P = 0.68$). The number of cells expressing IL-5 did not decrease in either mepolizumab or placebo groups. Furthermore, we did not observe any significant differences in the numbers of T cell, mast cells, and cells expressing IL-13 or IFN- γ .

The overall percentage of patients with adverse and serious adverse events was considerable, something which can be accounted for by the age of the patients and their various co-morbidities. Despite the relatively high number of serious adverse events, none of them was related to mepolizumab. The uncontrolled diabetes observed in one patient was most likely due to the OCS therapy. Five patients discontinued the study medication because of a lack of efficacy (Figure S1 and Table S5 in this article's Appendix S1).

TABLE 1 Intention-to-treat analysis of efficacy

	Mepolizumab (N = 20)		Placebo (N = 10)		
		Cumulative survival rate (95% CI)		Cumulative survival rate (95% CI)	
	N events		N events		P ^a
Cumulative relapse-free survival rates					
End of treatment (16 wk)	6	66.7% (44.9-88.5)	4	55.6% (23.1-88.1)	0.74
End of follow-up (36 wk)	14	20.0% (0.6-39.4)	6	33.3% (2.5-64.1)	0.53
Median survival time (weeks)	20.0 (8.4-31.6)		24.6 (0.0-51.3)		
Cumulative rates of patients reaching disease control					
First 2 wk	15	86.1% (69.0-100)	8	80.0% (55.3-100)	0.27
End of treatment (16 wk)	17	100% (nc)	10	100% (nc)	0.24
Median time (weeks)	1.14 (1.03-1.26)		1.71 (1.42-2.00)		
Cumulative rates of patient maintaining disease control ^b					
End of treatment (16 wk)	5	68.6% (45.7-91.5)	4	50.0% (15.3-84.7)	0.48
End of follow-up (36 wk)	13	21.2% (0.8-41.6)	6	33.3% (2.5-64.1)	0.73
Median time (weeks)	19.0 (10.7-27.3)		16.0 (8.1-23.9)		
ABSI score reduction from baseline	Mean (95% CI) ^c		Mean (95% CI) ^c		
Average in the first 16 wk	25.6 (18.1-33.2)		21.6 (11.1-32.2)		0.52
Average in the overall study period (36 wk)	28.1 (20.3-35.9)		32.8 (14.8-50.8)		0.43
Pruritus NRS reduction from baseline	Mean (95% CI) ^c		Mean (95% CI) ^c		
Average in the first 16 wk	4.5 (3.4-5.5)		4.4 (2.9-5.8)		0.90
Average in the overall study period (36 wk)	4.3 (3.2-5.4)		4.8 (3.3-6.3)		0.62
Cumulative dose of systemic corticosteroids until clinical remission	Mean (95% CI) 257.5 (185.3-329.7)		Mean (95% CI) 445.0 (167.5-722.5)		0.16 ^d
Peripheral blood eosinophils	Mean (95% CI) ^e		Mean (95% CI) ^e		
Average in the first 16 wk	0.04 (0.00-0.09)		0.16 (0.09-0.22)		0.007
Average in the overall study period (36 wk)	0.05 (0.01-0.10)		0.26 (0.20-0.33)		<0.001

(Continues)

TABLE 1 (Continued)

	Mepolizumab (N = 20)		Placebo (N = 10)		
	N events	Cumulative survival rate (95% CI)	N events	Cumulative survival rate (95% CI)	P ^a
ELISA BP 180 AB (RU/mL)	Mean (95% CI) ^e		Mean (95% CI) ^e		
Average in the first 16 wk	10.0 (–12.9 to 33.1)		6.6 (–21.6 to 34.8)		0.66
Average in the overall study period (36 wk)	14.9 (–3.3 to 33.1)		7.5 (–20.5 to 35.5)		0.65
ELISA BP 230 AB (RU/mL)	Mean (95% CI) ^e		Mean (95% CI) ^e		
Average in the first 16 wk	3.9 (–1.6 to 9.4)		–0.3 (–7.2 to 6.7)		0.52
Average in the overall study period (36 wk)	4.5 (0.2–8.7)		–0.7 (–7.7 to 6.3)		0.52

Abbreviations: ABSI, autoimmune bullous skin disorder intensity; BP180 AB, BP230 AB, antibodies to bullous pemphigoid hemidesmosomal antigens BP180 and BP230; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; NRS, numerical rating scale; RU, relative units. Bold numbers indicate statistically significant differences.

^aLog-rank test.

^bComparisons were performed on patients who had achieved disease control (17 in the mepolizumab and 10 in the placebo groups).

^cEstimated marginal means from mixed linear regression models.

^dMann-Whitney *U* test.

^eEstimated marginal means from mixed linear regression models including baseline levels of each parameter as adjustment factors.

Based on the observation that eosinophils are present in BP lesions and are capable of splitting the skin at the dermal-epidermal junction in an ex vivo model,⁴ we launched this investigator-initiated trial to study the effect of mepolizumab, an anti-IL-5 antibody, that might reduce the number and activity of eosinophils in patients with active BP. Unfortunately, we could not demonstrate any superiority of mepolizumab therapy over placebo in terms of the clinical and serological outcomes.

What reasons might explain the failure of mepolizumab to significantly improve BP? (a) The total number of patients enrolled in

this proof-of-concept study was modest. It can be assumed that small effects of mepolizumab might have been detectable in a larger cohort and by calculating a higher study power. (b) Since we could not estimate the effect of mepolizumab in these elderly patients in advance, for ethical reasons, we chose an add-on therapy, that is, mepolizumab or placebo were administered in addition to the standardized therapy with OCS. This design may have obscured possible effects of mepolizumab. (c) The treatment period with the study drug was limited to 12 weeks. This time period may have been rather short to achieve long-term control of BP.

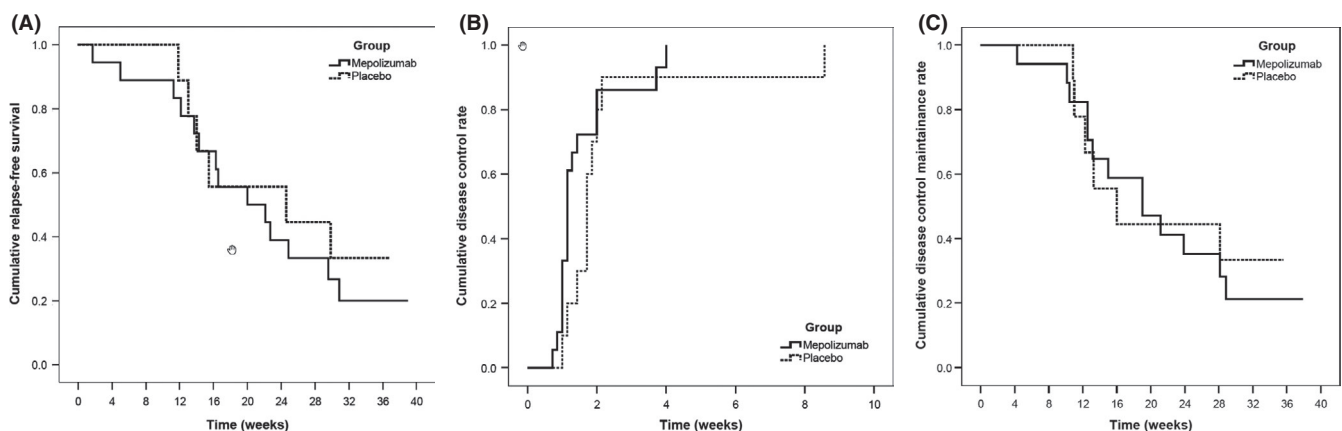


FIGURE 1 Rates of BP patients free of relapse, achieving disease control, and maintaining disease control. The Kaplan-Meier curves show (A) the cumulative relapse-free survival, (B) the cumulative disease-control rate, and (C) the disease-control maintenance rate in the mepolizumab (solid line) and placebo groups (dashed line)

As expected, mepolizumab significantly decreased blood eosinophil numbers. However, it did not significantly affect tissue eosinophil infiltration. Even after therapy, eosinophils were detectable in the skin both in patients with and without relapse of BP. The observation that mepolizumab insufficiently controls tissue eosinophil numbers has been reported in other trials with bronchial asthma and eosinophilic esophagitis.^{7,8}

Based on the experimental observations of a role for eosinophils in BP,⁴ we would still argue for continuing investigation of therapeutic strategies targeting eosinophil activity and/or numbers. In this context, novel therapeutic antibodies against the IL-5 receptor alpha subunit would seem to be promising since they mediate antibody-dependent cell-mediated cytotoxicity of both eosinophils and basophils.⁹ Moreover, bertilimumab, an anti-eotaxin antibody, has recently been designated as an orphan drug for the treatment of BP by the Food and Drug Administration.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Online Supporting Information

TABLE S1 Inclusion and exclusion criteria

Inclusion criteria	1) Men, women; > 18 years 2) Active BP, diagnosed with the typical clinical picture and based on skin biopsies and direct immunofluorescence and/or detection of anti-basement membrane antibodies and/or BP180, BP230 antibody in serum 3) Must have provided written informed consent
Exclusion criteria	1) Patients with other skin diseases, that might interfere with the evaluation of BP 2) Patients with severe diseases of other organ systems (e.g. cardiovascular, psychiatric, neurologic) that might put the patient at risk during the study or might interfere with the evaluation (in the opinion of the investigator) 3) Systemic treatment for BP (e. g. corticosteroids, azathioprine, mycophenolate mofetil, hydroxyurea, cyclophosphamide) or systemic treatment with immunosuppressive/immunomodulating substances including biologics for other indications within 14 days prior to baseline. 4) Topical therapy with corticosteroids and other anti-inflammatory substances 5) For female patients, unless postmenopausal or surgically sterile, unwillingness to practice effective contraception during the study (defined as methods with <1% failure rate: Abstinence from penile-vaginal intercourse, when this is the female's preferred and usual lifestyle; Oral contraceptive, either combined or progestogen alone; Injectable progestogen; Implants of levonorgestrel or etonogestrel; estrogenic vaginal ring; percutaneous contraceptive patches; intrauterine device (IUD) or intrauterine system (IUS) that has a <1% failure rate documented in the product label; male partner sterilization (vasectomy with documentation of azoospermia) prior to the female's subjects entry into the study, and this male is the sole partner for the subject. For this definition, "documented" refers to the outcome of the investigator's /designee's medical examination of the subject and/or semen analysis or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject's medical records; male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository); male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream or suppository).

	Since the effects of mepolizumab on the fetus have not been studied, double contraception is advised during the study (hormonal method in combination with mechanical method). The rhythm method is not to be used as the sole method of contraception. Female patients considering becoming pregnant while in the study are excluded. 6) Female patients who are currently pregnant or breast-feeding 7) Current abuse of alcohol and/or drugs 8) History of or a new diagnosis or treatment of an invasive malignancy within 5 years of enrollment. Patients with a history of treated squamous cell and/or basal cell carcinomas limited to the skin were not excluded. 9) History of recurrent clinically significant infection 10) Congenital or acquired immunodeficiency syndrome 11) Current enrollment in any other investigational drug study 12) Previous participation in this study or previous studies with mepolizumab 13) Hypersensitivity to mepolizumab or its constituents
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TABLE S2 Primary and secondary outcomes, and safety parameters

Endpoints	Parameter
<i>Primary endpoint</i>	Cumulative rate of relapse-free patients after initiating therapy *.
<i>Secondary endpoints</i>	
Clinical efficacy	Cumulative rate of patients attaining disease control
	Cumulative rate of patients maintaining disease control
	Absolute reduction of severity (ABSIS**)
	Absolute reduction of pruritus (NRS***)
	Absolute reduction of serum levels of BP180 and BP230 antibodies
	Peripheral blood eosinophil count
	Cumulative dose of systemic corticosteroids administered until clinical remission
Skin inflammation	H&E: presence of eosinophils, subepidermal blister formation
	Direct immunofluorescence: antibody or complement C3 deposition at the dermal-epidermal junction
	Immunofluorescence: number of eosinophils, mast cells, T cells, cytokine expression (IL-5, IL-13, IFN-gamma, TSLP)
Safety	physical examination

	monitoring white blood cell counts, liver and renal tests
	Concomitant therapies
	Adverse events (AE), serious adverse events (SAE)

*Relapse was defined as manifestation of new BP lesions and/or >3 blisters during or 4 weeks after the treatment period

** ABSIS; Autoimmune bullous skin disorder intensity score

*** NRS; numerical rating scale (1-10)

TABLE S3 Characteristics of patients

		Mepolizumab (N=20)		Placebo (N=10)		P**
		N*	%	N*	%	
Patient characteristics						
Age (yrs)	<i>mean, SD</i>	74.5	10.6	78.4	8.0	0.33
Sex	M	7	35.0%	5	50.0%	0.46
	F	13	65.0%	5	50.0%	
BP manifestation	First	15	75.0%	9	90.0%	0.63
	Recurrent	5	25.0%	1	10.0%	
PGA	Clear	0	0.0%	0	0.0%	0.29
	Mild	0	0.0%	1	10.0%	
	Moderate	6	30.0%	4	40.0%	
	Severe	14	70.0%	5	50.0%	
Blisters/dd (last 3 dd)	<i>mean, SD</i>	3.7	0.5	3.4	0.7	0.24
ABSIS	<i>mean, SD</i>	41.6	27.4	53.9	40.8	0.58
Pruritus (VAS)	<i>mean, SD</i>	7.3	2.9	7.0	3.5	0.93
Vital signs, laboratory parameters						
Systolic blood p. (mmHg)	<i>mean, SD</i>	137.0	19.2	128.8	14.0	0.46
Diastolic blood p. (mmHg)	<i>mean, SD</i>	79.1	12.5	71.1	15.2	0.41
Pulse No.	<i>mean, SD</i>	72.7	10.7	74.1	7.9	0.73
ECG result	Normal	18	100.0%	9	90.0%	0.36
	Abnormal	0	0.0%	1	10.0%	
WBC count (total, 10 ⁶ /l)	<i>mean, SD</i>	9255.0	3148.0	8970.0	1568.5	0.61
Eosinophils (Gpt/l)	<i>mean, SD</i>	0.68	0.93	0.57	0.70	0.77
Creatinine	Normal	13	68.4%	8	80.0%	0.67
	Abnormal	6	31.6%	2	20.0%	

		Mepolizumab (N=20)		Placebo (N=10)		p**
		N*	%	N*	%	
ASAT	Normal	18	100.0%	10	100.0%	nc
	Abnormal	0	0.0%	0	0.0%	
ALAT	Normal	18	100.0%	10	100.0%	nc
	Abnormal	0	0.0%	0	0.0%	
GGT	Normal	13	86.7%	7	77.8%	0.61
	Abnormal	2	13.3%	2	22.2%	
BP diagnostic parameters						
Subepidermal blister	No	5	27.8%	3	30.0%	1
	Yes	13	72.2%	7	70.0%	
Dermal infiltrate	No	0	0.0%	0	0.0%	nc
	Yes	18	100.0%	10	100.0%	
DIF	Linear IgG deposits	4	21.1%	1	10.0%	0.63
	Linear complement deposits	15	78.9%	9	90.0%	
ELISA BP 180 AB (RU/ml)	mean, SD	55.8	48.7	75.8	57.6	0.48
ELISA BP 230 AB (RU/ml)	mean, SD	26.1	35.8	43.1	42.9	0.24

AB: antibodies, ABSIS: autoimmune bullous skin disorder intensity score, ALAT: alanine amino transferase, ASAT: aspartate amino transferase, BP: bullous pemphigoid, dep: deposition, DIF: direct Immunofluorescence, ECG: electrocardiogram, ELISA: enzyme-linked immunosorbent assay, GGT: gamma-glutamyl transferase, Gpt: gigaparticles, IgG: Immunoglobulin G, nc: not computable, PGA: physician global assessment, RU: relative units, SD: standard deviation, VAS: visual analogue scale, WBC: white blood cells

* Numbers may not sum up to the total due to missing data

** Mann-Whitney U test and X² test (or Fisher's exact test where required) were used for continuous and categorical variables respectively

TABLE S4 Antibodies used for immunofluorescence staining

	Target	Source
Cell surface markers	Eosinophil cationic protein (ECP)	Pharmacia & Upjohn Diagnostic AB, Uppsala, Sweden
	Mast cell tryptase (MCT)	Dako, Glostrup, Denmark
	CD3	Dako, Glostrup, Denmark
Cytokines	Thymic stromal lymphopoietin (TSLP)	Santa Cruz Biotechnology, Dallas, USA

	Interleukin (IL)-5	Santa Cruz Biotechnology, Dallas, USA
	Interleukin (IL)-13	Santa Cruz Biotechnology, Dallas, USA
	Interferon (IFN)-gamma	R&D Systems, Minneapolis, USA
Secondary antibodies	Alexa Fluor 488	Life Technologies, Thermo Fisher Scientific, Carlsbad, USA
	Cy3	Jackson ImmunoResearch Ltd, Suffolk, UK

TABLE S5 Summary on adverse events in mepolizumab and placebo groups

	Mepolizumab group N=20	Placebo group N=10	P value
Adverse events (AEs)			
All	100%	100%	
Moderate to severe	49.3%	52%	0.51
Serious adverse events	27.5%	16.7%	0.70
AEs associated with investigational product	13 possible, 1 likely, 1 certain		
	fatigue (n=3), vertigo (n=3), diarrhea (n=3), malaise/nausea (n=3), flushing (n=1), high blood pressure (n=1) and vesicular dermatitis (n=1)		

Statistical analyses

For this investigator-initiated, proof-of-concept study, a 2-stage adaptive trial design was chosen. For the first stage, we calculated 30 patients would be required in order to reach an acceptable study power while keeping the numbers of patients as low as possible to ease recruitment. Aiming to detect a relapse-free survival rate of 60% in the experimental and 30% in the control arm with a 2:1 ratio for random allocation, one can calculate that at least 30 patients (20 in the experimental and 10 in the control arm) should be recruited in the first stage in order to detect a statistically significant difference ($\alpha=0.05$, $\beta=0.6$, two sided log-rank test). Based on the results of the interim analysis, we refrained from undertaking a second stage, for which another 30 patients would have to have been recruited in order to reach an overall power of 70%.

For descriptive purposes, continuous variables are presented as means with standard deviations (SD), while categorical variables are shown as numbers with percentages. Cumulative rates were computed by using Kaplan-Meier estimator along with their 95% confidence intervals (CI). Median (survival) times together with their 95% CI were computed as well.

Efficacy end points were assessed in the intention-to-treat population, which included all patients who underwent randomization and received at least one dose of mepolizumab or placebo, and were analyzed according to the randomization group (mepolizumab; n=20; placebo; n=10). The Mann-Whitney U test and X² test (or Fisher's exact test where required) were used for the comparison of continuous and categorical variables between study arms respectively. Survival rate differences between study groups were assessed by means of log-rank test. Comparison of continuous outcomes that were assessed at multiple time points, was performed using mixed linear regression models including their baseline values as adjustment factors. Analyses were carried out with the SPSS software v.20.0 (IBM Corp., Armonk, NY, US) or Graph Pad software Prism 6 (La Jolla, CA, US). All statistical tests were considered significant at p-values of <0.05.

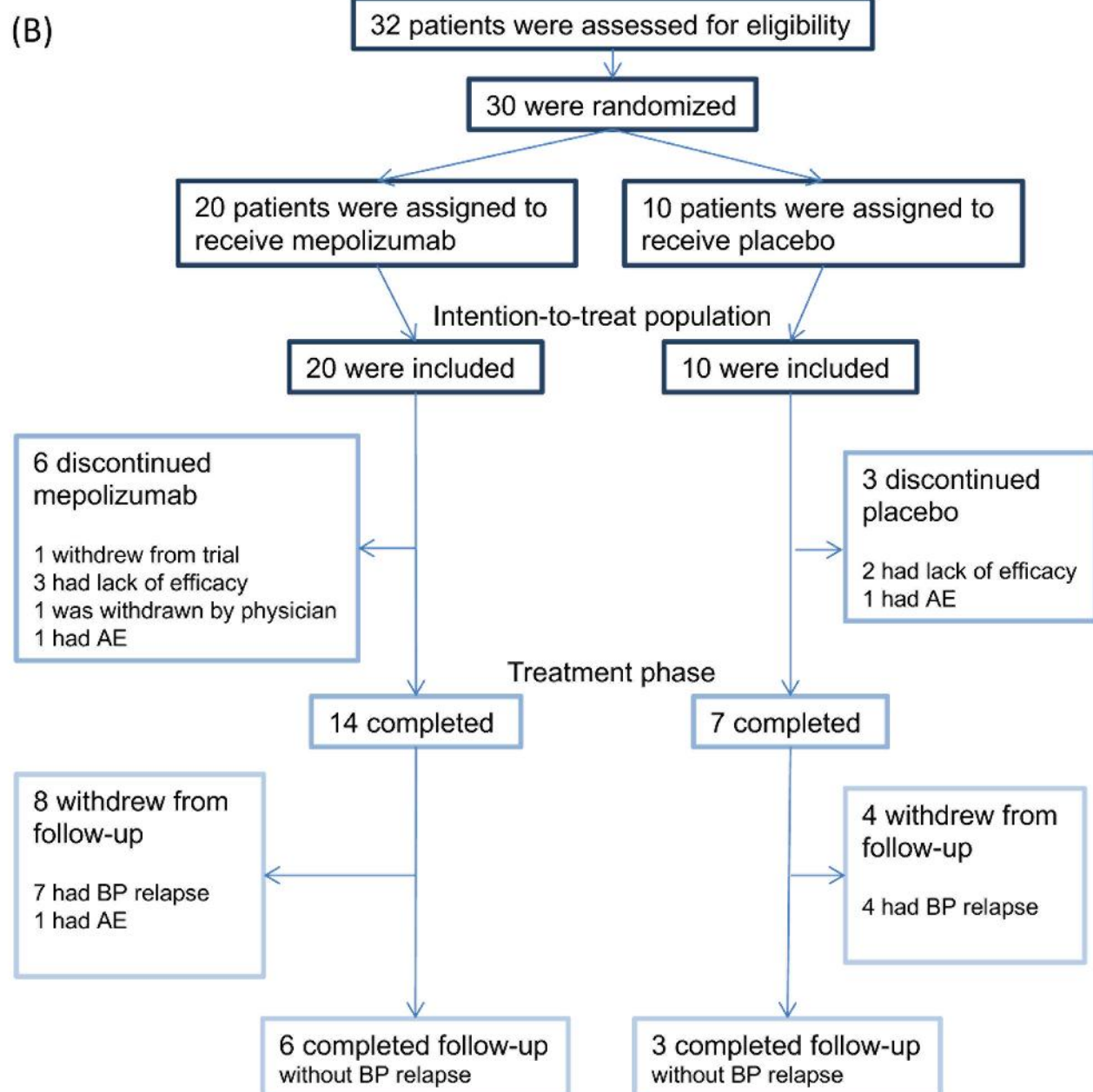
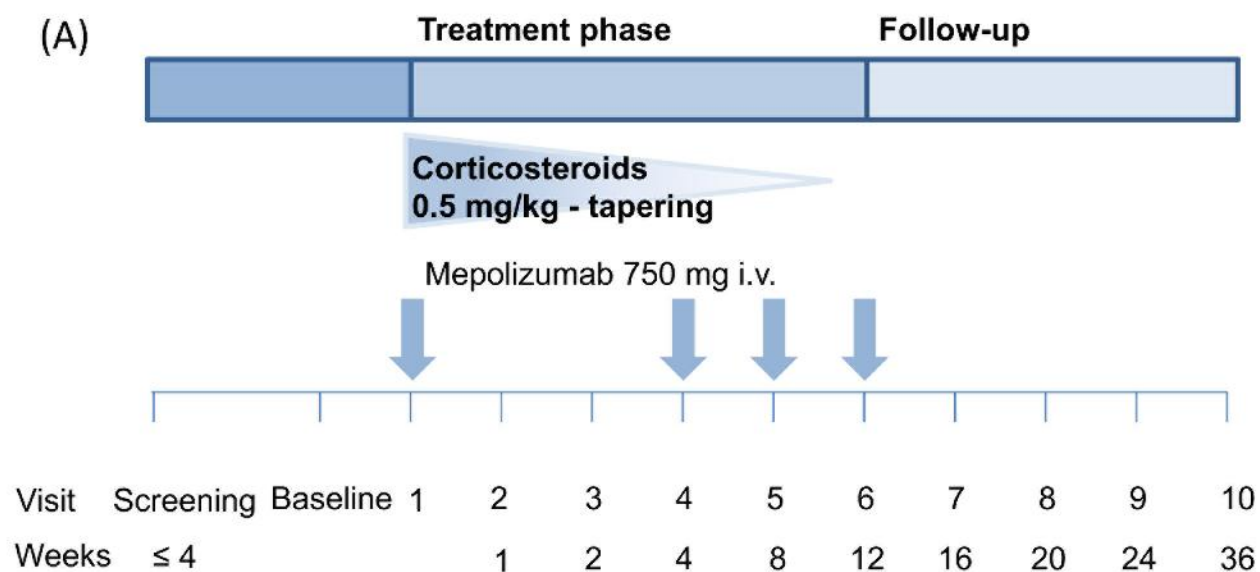
Randomisation

Randomisation (sequence generation, random allocation as well as preparation of mepolizumab and placebo infusions, identical in appearance) were done at the Institute of Hospital Pharmacy, Inselspital, by staff otherwise not involved in the trial.

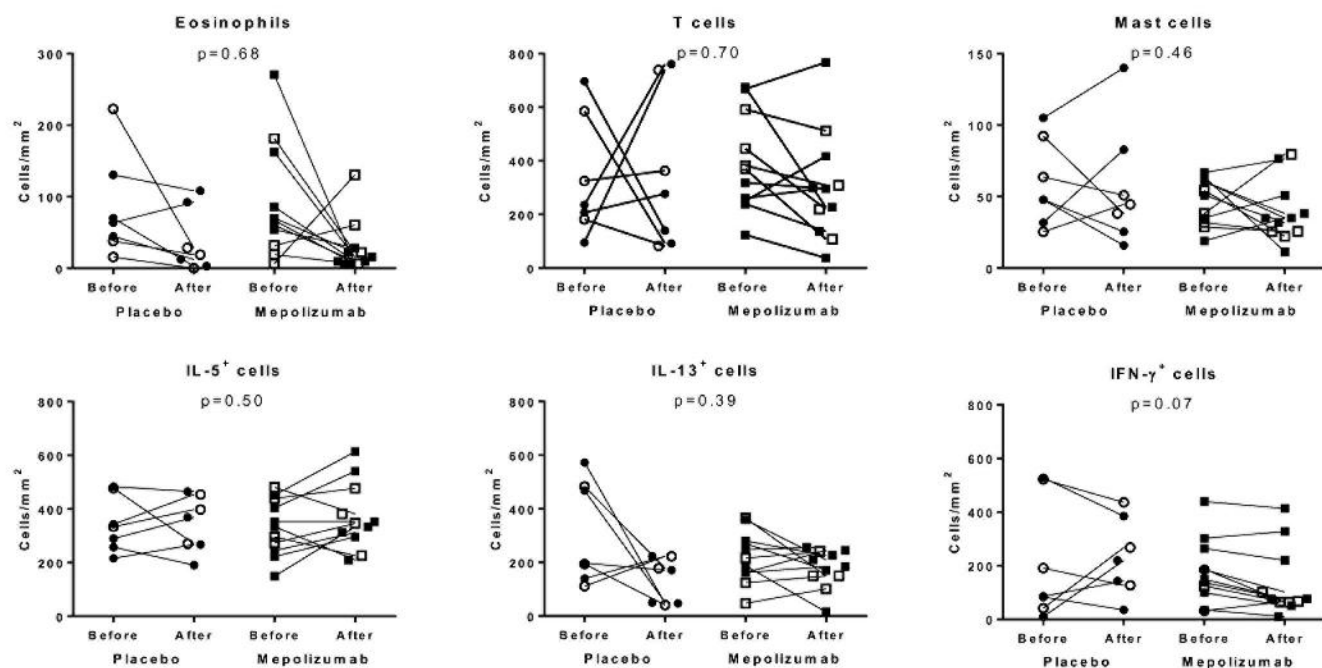
Online Supporting Information Figures

FIGURE S1 Trial design. (A) Patients with active BP received 4 intravenous injections of mepolizumab 750 mg or placebo, 4 weeks apart over 12 weeks in addition to corticosteroids (initial dose 0.5 mg/kg body weight) and were followed up over 36 weeks. (B) Randomization and follow-up of patients.

FIGURE S2 Skin inflammatory cells present and cytokine expression. Biopsies taken before and after (week 16) therapy, were analyzed by immunofluorescence techniques. Graphs show the number of eosinophils, T cells and mast cells, as well as cells expressing interleukin (IL)-5, IL-13 and interferon (IFN)- γ in patients receiving mepolizumab (squares) or placebo (circles). Open symbols represent patients with relapse. The p value indicates the comparison of mean differences (before and after therapy numbers) between the mepolizumab and placebo groups.



Online Supporting Information Figure S1



Online Supporting Information Fig S2