

Daclizumab Improves Asthma Control in Patients with Moderate to Severe Persistent Asthma

A Randomized, Controlled Trial

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Rationale: Airway inflammation in asthma is associated with increased activated CD25⁺ T cells, IL-2, and soluble IL-2 receptors (IL-2Rs).

Objectives: A randomized, double-blinded, placebo-controlled study was used to evaluate the safety and efficacy of daclizumab, a humanized IgG1 monoclonal antibody against the IL-2R α chain (CD25) of activated lymphocytes, in adults with moderate to severe persistent asthma.

Methods: Patients with obstructive pulmonary functions, despite inhaled corticosteroids (ICS), were switched to equivalent dose inhaled triamcinolone acetate acetonide (TAA). Patients dependent on ICS were randomized (3:1) to daclizumab (intravenous loading dose, 2 mg/kg, then 1 mg/kg) or placebo every 2 weeks, added to stable-dose TAA through Week 12 (Treatment Period 1). Over Weeks 12–20 (Treatment Period 2), patients tapered TAA while on the study drug, and were followed for 16 weeks off the study drug.

Measurements and Main Results: Among 115 evaluable patients (88 daclizumab, 27 placebo), groups had similar age, disease duration, and length of ICS use. During Treatment Period 1, daclizumab improved FEV₁ (daclizumab, $4.4 \pm 1.80\%$ vs. placebo, $1.5 \pm 2.39\%$; $P = 0.05$), and reduced daytime asthma symptoms ($P = 0.018$) and short-acting inhaled β_2 -agonist use ($P = 0.009$). Daclizumab treatment prolonged time to exacerbation ($P = 0.024$). Adverse events were evenly distributed between groups, although there were more serious adverse events in the patients treated with daclizumab.

Conclusions: Daclizumab improved pulmonary function and asthma control in patients with moderate to severe chronic asthma inadequately controlled on ICS. The mechanism of action likely involves inhibition of proinflammatory cytokine generation by IL-2R blockade in activated T cells.

Clinical trial registered with www.clinicaltrials.gov (NCT00028288).

Keywords: pulmonary function; asthma; daclizumab; airway inflammation

Airway inflammation in asthma involves T-cell activation, with increased numbers of activated CD25⁺ T cells and increased levels of IL-2 and soluble IL-2 receptor (IL-2R) α chain (sCD25) found in airways of patients with severe asthma (1–9). After T-cell

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Daclizumab is a humanized monoclonal antibody that binds specifically to the α (Tac, CD25) subunit of the high-affinity IL-2R, and inhibits IL-2 binding and its biological activity. However, there is limited information concerning potential benefits of daclizumab in asthma.

What This Study Adds to the Field

The use of daclizumab, an anti-CD25 antibody, in patients with moderate to severe asthma was associated with a small improvement in lung function and asthma control along with a reduction in blood eosinophils.

activation, cytokine generation and secretion may contribute to the initiation and potentiation of inflammation, along with development of repair leading to airway remodeling in asthma (6).

Daclizumab (Zenapax; F. Hoffmann-La Roche [Roche]; Nutley, NJ) is a humanized monoclonal antibody that binds specifically to the α (Tac, CD25) subunit of the high-affinity IL-2R, and inhibits IL-2 binding and its biological activity (10). The recombinant genes encoding daclizumab are a composite of 90% human from the IgG1 constant domains and Eu myeloma framework regions, and 10% murine from murine anti-Tac complementarity-determining region sequences and, thus, the immunogenic potential of daclizumab may be minimized. Daclizumab is approved by the U.S. Food and Drug Administration as part of an induction regimen used for rejection prophylaxis in renal transplantation (11). Patients with renal transplantation, who were treated with daclizumab, did not demonstrate increased risk in the types or frequency of adverse events, including infections or malignancies, compared with placebo. Limited studies of daclizumab in the chronic treatment of patients with autoimmune disease suggest that the drug has been well tolerated (12–15).

The objectives of this study were to address the hypothesis that a novel T-cell-targeted therapy might improve asthma control in patients who still have reversible airflow obstruction despite inhaled corticosteroid (ICS) treatment. Current guidelines advise that clinicians classify asthma severity by using the domains of current impairment (e.g., assessment of asthma symptoms and lung function) and future risk of adverse events (e.g., exacerbations) (16). For clinical research studies, asthma severity can be inferred after optimal therapy is established by correlating levels of severity with the lowest level of treatment required to maintain control. Achieving asthma control by therapeutic intervention is aimed at reducing both impairment and risk.

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Specifically, the study was designed to determine if there was a signal of clinical activity that would warrant further development of daclizumab in asthma. Some of the results of this study have been previously reported in abstract form (17–20).

METHODS

Study Design and Objectives

This randomized, double-blinded, placebo-controlled, parallel-group study in patients with suboptimally controlled moderate to severe asthma was conducted at 24 U.S. sites (*see* Figure 1). Patients met requirements for moderate to severe persistent asthma despite optimal treatment with medium- to high-dose ICSs (16), or partly controlled/uncontrolled asthma despite the need for step-3 therapy (21). Patients with atopy were defined (*post hoc*) as having at least one class-3 allergen-specific IgE response to a panel of six common aeroallergens (22). Patients with an FEV₁ of 50 to 80% predicted, despite 1,200 µg or more of inhaled triamcinolone acetate acetonide (TAA) (or equivalent ICS) for 3 months or longer before enrollment, were switched to equivalent-dose TAA (23). All patients were switched to TAA to eliminate confounding effects of the different ICSs and to facilitate graded steroid taper during run-in and treatment. During a 2- to 5-week run-in, patients were required to demonstrate a greater than 10% decrease in FEV₁ after reducing TAA by 25% per week, up to 50% of baseline TAA dose to be randomized. Patients were restabilized to an FEV₁ of at least 90% of baseline on the TAA dose before the demonstrated FEV₁ reduction.

For the first 12 weeks of study (Study Day 0–83, Treatment Period 1, ICS-stable phase), patients received daclizumab (2 mg/kg intravenously followed by 1 mg/kg intravenously) or placebo intravenous infusion (3:1 randomization) every 2 weeks added to stable TAA.

During Treatment Period 2 (ICS-taper phase, Study Day 84–140, Weeks 12–20), blinded study drug was continued, while ICS was tapered 25% every 2 weeks. Study drug was discontinued for the 16-week follow up. Occupancy of CD25 receptors on peripheral T cells was not assessed in this study, as prior data indicated that daclizumab fully saturates the CD25 receptor on peripheral T cells at the selected dose regimen. At the dose regimen approved in the renal transplantation indication (1 mg/kg every 2 wk), daclizumab saturates the CD25 subunit of the IL-2R for approximately 120 days after transplantation in adult patients (10).

Patients were monitored for adverse events, laboratory abnormalities, concomitant medication use, pharmacokinetics, pharmacodynamics, and immunogenicity during treatment and follow up.

Study visits occurred at screening, during the run-in period (up to four visits), every 2 weeks during Treatment Period 1 (six visits), every 2 weeks through Treatment Period 2 (four visits), and then every 2–4 weeks during the follow-up phase (five visits).

The study was conducted in accordance with good clinical practice, the latest revisions to the Declaration of Helsinki, and appropriate regulatory guidelines. An independent institutional review board approved the protocol at each participating site. Patients gave written informed consent to participate in the study.

Inclusion Criteria

The primary inclusion criteria were: nonsmoking adults with asthma; 18–70 years old; asthma history of 6 months or longer; FEV₁ of 50–80% of predicted; reversibility of at least 12% with inhaled short-acting β_2 -agonist; at least 1,200 µg daily inhaled TAA (or equivalent ICS) for 3 months or more before enrollment.

Exclusion Criteria

Major exclusion criteria were: 10 pack-years or more smoking history or within 12 months; oral or parenteral corticosteroids within 30 days; hospitalization for asthma within 60 days; and current allergen immunotherapy.

Efficacy and Safety Endpoints

The primary clinical endpoint was a change in pulmonary function (percent change in FEV₁) from randomization to Day 84. FEV₁ was selected as the primary clinical endpoint in this trial, as it represents an objective measure of asthma outcome. At the time this study was conducted, FEV₁ was the only endpoint approvable by the U.S. Food and Drug Administration for registration of new drugs in asthma. Secondary endpoints included asthma exacerbations, time to asthma exacerbation, morning/evening PEF, rescue medication use, daytime/nighttime asthma symptoms, and asthma-free days. Asthma symptoms, medication use, and PEF rates were assessed from each patient's diary record (performed twice daily by the interactive voice response system). This diary has previously demonstrated significant responsiveness to change in asthma due to therapy in clinical trials (24, 25).

An asthma exacerbation was defined as a loss of asthma control that was associated with one or more of the following (26):

- Inhaled albuterol $\geq$ 8 puffs/24 hours over baseline for 48 hours
- Inhaled albuterol $\geq$ 16 puffs/24 hours for 48 hours
- PEF < 65% reference level despite 60 minutes of rescue treatment
- Symptoms despite 60 minutes of rescue treatment.

See online supplement for additional details regarding study procedures.

Statistical Methods

The study was powered to detect potential differences between the active treatment and the placebo groups. A total of 90 patients in the active treatment and 30 patients in the placebo arms would yield 81%

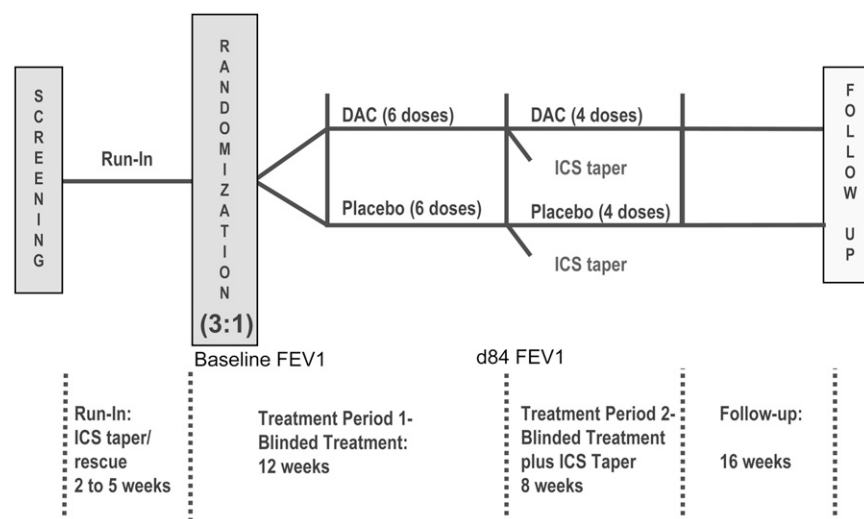


Figure 1. Study design included screening, run-in (2–5 wk), treatment (20 wk), and follow-up (16 wk). Patients who met screening criteria entered run-in to demonstrate dependence on medium- to high-dose inhaled corticosteroids (ICS). Patients were then randomized to daclizumab or placebo, initially as add-on to stable dose ICS (12 wk), followed by taper of ICS (8 wk). After the treatment period, patients discontinued study drug for a 16-week washout. The prerandomization FEV₁ was assessed before the first administration of study drug, and the Day 84 FEV₁ was assessed before administration of study drug.

power to detect an 8% point difference in the respective changes in FEV₁ (two-sided $\alpha = 0.05$). Moreover, the same sample sizes would yield 92% power to detect (at the same significance level) a difference between an 80% exacerbation rate in the placebo group from a 45% rate in the active group. The 3:1 randomization allocation was selected to increase power and to obtain additional safety data on active treatment. The major drawback to this allocation is the small number of patients receiving placebo, which can result in increased statistical variability.

Results are included for all valid paired measurements. Data were analyzed by biostatisticians at PDL BioPharma (more information available on online supplement).

RESULTS

Patient Characteristics and Disposition

A total of 208 patients enrolled in the study; 92 patients were not randomized. Of the 92 patients who did not meet eligibility for randomization, 61 patients (66.3%) failed to demonstrate dependence on medium- to high-dose ICS ($\geq 1,200$ μg inhaled TAA or equivalent) during run-in. Of the remaining patients not randomized, 13 were withdrawn due to patient choice, three due to an asthma exacerbation, three due to noncompliance, three to investigator judgment, two to protocol violation, one to an adverse event that led to discontinuation, and six to withdrawal for no specified reason. Thus, 116 patients were randomized, and 115 patients met all requirements for intention-to-treat (ITT) analysis (one patient was randomized, but not dosed with study drug due to investigator judgment, and therefore was not included in the analysis).

In total, 88 (77%) patients were randomized to daclizumab and 27 (23%) to placebo. White females comprised the majority of patients in both daclizumab treatment and placebo groups. There were no significant differences between these groups at baseline (Table 1). The mean prerandomization dose of TAA was $2,089 \pm 769$ $\mu\text{g}/\text{day}$ (mean $\pm$ SD) for patients receiving daclizumab and $2,178 \pm 622$ $\mu\text{g}/\text{day}$ for patients receiving placebo. Mean FEV₁ values were 2.34 ± 0.07 L for patients treated with daclizumab and 2.25 ± 0.1 L for patients receiving placebo. Approximately two-thirds of patients were atopic based on assessment of serum-specific IgE to an allergen panel.

TABLE 1. BASELINE DEMOGRAPHICS AND CLINICAL CHARACTERISTICS

Characteristics	Daclizumab (n = 88)	Placebo (n = 27)	P Value
Female, n (%)	56 (63.6)	19 (70.4%)	0.68
White, n (%)	75 (85.2)	20 (74.1%)	0.30
Age, yr	43.2 ± 12.5	41.0 ± 12.4	0.42
Disease duration, yr	22.6 ± 13.6	24.9 ± 14.0	0.47
ICS use, yr	5.4 ± 6.1	4.6 ± 5.3	0.53
Prerandomization dose of TAA, $\mu\text{g}/\text{d}$	$2,089 \pm 769$	$2,178 \pm 622$	0.54
FEV ₁ , L	2.3 ± 0.7	2.2 ± 0.5	0.46
FEV ₁ % predicted	68.8 ± 11.3	68.0 ± 11.1	0.75
Daily symptom score	8.0 ± 4.1	9.7 ± 4.2	0.09
Nocturnal symptom score	0.4 ± 0.89	0.6 ± 0.48	0.37
Daily β_2 -agonist rescue, puffs/d	4.1 ± 3.3	4.5 ± 3.0	0.54
Daily morning PEF, L/min	367.6 ± 96.0	344.6 ± 80.6	0.26
Daily evening PEF, L/min	359.4 ± 94	329.6 ± 67	1.0
Whole blood eosinophils, k/m^3	0.2 ± 0.02	0.2 ± 0.02	0.55
Serum ECP, ng/ml	20.1 ± 23.4	17.5 ± 16.2	0.51
Total serum IgE, IU/ml	307.5 ± 574.8	301.9 ± 388.2	0.96
Reversibility, % change	21.9 ± 9.8	21.0 ± 9.4	0.68

Definition of abbreviations: ECP = eosinophil cationic protein; ICS = inhaled corticosteroids; TAA = triamcinolone acetate acetonide.

All values are for evaluable patients, presented as means $\pm$ SD, unless noted otherwise.

Enrollment of patients with atopy was similar between the two groups (patients treated with daclizumab, 65%; patients receiving placebo, 61.5%).

Efficacy

Markers of current impairment. LUNG FUNCTIONS. Daclizumab significantly improved pulmonary function (FEV₁) from baseline to Day 84 of treatment compared with placebo ($P = 0.05$). The percent increase in FEV₁ from baseline to Day 84 was $4.4 \pm 1.80\%$ ($P = 0.02$ within group) for daclizumab versus a decrease of $1.5 \pm 2.39\%$ ($P = 0.53$ within group) for placebo (represented as % of baseline in Figure 2). This difference corresponds to a placebo-corrected change of 5.9% for daclizumab. The maximum effects of daclizumab and placebo were seen by Day 28. Patients treated with daclizumab had an absolute increase in FEV₁ (L) from 2.34 ± 0.07 (baseline) to 2.4 ± 0.08 (Day 84), whereas the patients receiving placebo had a decrease in FEV₁ from 2.25 ± 0.1 to 2.2 ± 0.1 L. The relative change in FEV₁ % predicted from baseline to Day 84 was $2.45 \pm 1.06\%$ ($P = 0.02$ within group) for daclizumab versus $-0.97 \pm 1.42\%$ ($P = 0.50$ within group) for placebo (between group, $P = 0.06$) (Figure 3A). The change in the ratio of FEV₁/FVC from baseline was 0.02 ± 0.01 ($P = 0.001$) for daclizumab versus -0.02 ± 0.01 ($P = 0.12$) for placebo (between group, $P = 0.01$) (Figure 3B). Change in mean forced expiratory flow during the middle half of the FVC (forced expiratory flow_{25-75%}) from baseline to Day 84 was 0.16 ± 0.05 ($P = 0.002$) versus -0.10 ± 0.07 ($P = 0.19$) (between group, $P = 0.005$). Compared with placebo, daclizumab treatment significantly increased evening peak flow values ($P = 0.029$). Changes in morning peak flow rates did not differ significantly in patients treated with daclizumab compared with the placebo group ($P = 0.217$).

During the ICS taper in Treatment Period 2 (Days 84–140), FEV₁ values decreased for patients in both treatment groups. The difference of percent change in FEV₁ from Day 84 between daclizumab and placebo (daclizumab: -5.37% ; placebo: -4.3%) was not significant ($P = 0.61$). No significant differences were

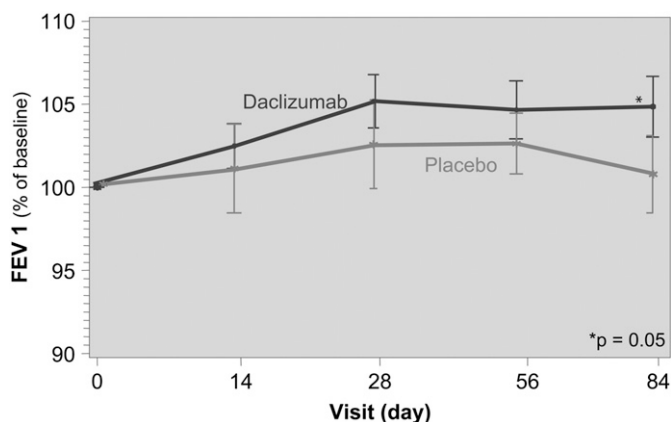


Figure 2. Change in FEV₁ from baseline through Day 84 for patients treated with daclizumab or placebo as add-on to stable-dose ICS. Change is expressed as a percent of baseline. Error bars indicate SEM. Within-group changes were evaluated by paired *t* tests, and between-group significance by *t* tests. Last-observation-carried-forward methods were used for patients who prematurely withdrew due to efficacy-related reasons. Patients who required treatment with systemic corticosteroids had their last FEV₁ before initiation of treatment with systemic corticosteroids carried forward. Available data were analyzed for patients who withdrew prematurely due to non-efficacy-related terminations.

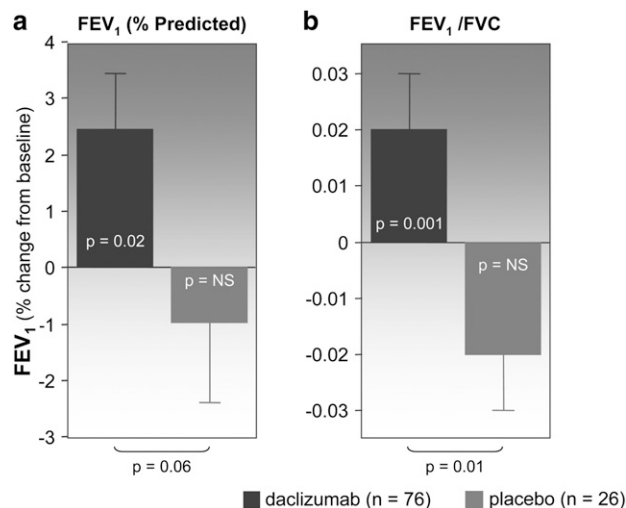


Figure 3. Change in FEV₁ % predicted and FEV₁/FVC from baseline at Day 84 for patients treated with daclizumab or placebo as add-on to stable-dose ICS. Change is expressed as a percent change from baseline. Error bars indicate SEM. Within-group changes were evaluated by paired *t* tests, and between-group significance by *t* tests. Last-observation-carried-forward methods were used for patients who prematurely withdrew due to efficacy-related reasons. Patients who required treatment with systemic corticosteroids had their last FEV₁ before initiation of treatment with systemic corticosteroids carried forward. Available data were analyzed for patients who were premature non-efficacy-related terminations.

observed between the two groups for changes in morning and evening PEF rates during Treatment Period 2.

ASTHMA SYMPTOMS. Compared with placebo, daclizumab treatment significantly reduced daytime asthma symptom scores ($P = 0.018$) and use of rescue short-acting inhaled β_2 -agonist ($P = 0.009$). No significant differences were observed in patients treated with daclizumab compared with the placebo group for nocturnal asthma symptoms ($P = 0.136$) and asthma-free days ($P = 0.35$), although all outcomes favored daclizumab (Table 2). No significant differences were observed between the two groups for changes in daytime and nocturnal asthma symptoms, short-acting inhaled β_2 -agonist use, or asthma-free days during Treatment Period 2.

Markers of future risk. ASTHMA EXACERBATIONS. Patients treated with daclizumab experienced a prolonged time to a severe exacerbation requiring systemic corticosteroid rescue compared with patients receiving placebo during both the treatment and follow-up periods (Days 0–252) ($P = 0.024$) (see Figure 4). There was no significant difference between the two groups in the time

TABLE 2. CHANGE FROM BASELINE TO DAY 84 IN MEASURES OF ASTHMA CONTROL

	Daclizumab	Placebo	<i>P</i> Value
Daytime asthma symptoms	-1.2 ± 0.4	0.1 ± 0.4	0.018
Nocturnal asthma symptoms	-0.2 ± 0.1	0.0 ± 0.1	0.136
Rescue short-acting β_2 -agonist use, puffs/day	-0.9 ± 0.4	0.5 ± 0.3	0.009
Morning peak flow rates, L/min	12.9 ± 3.8	-0.1 ± 9.5	0.217
Evening peak flow rates, L/min	15.1 ± 4.1	-4.1 ± 7.3	0.029
Asthma-free days, %	12.3 ± 2.9	7.5 ± 4.2	0.35
Asthma exacerbations requiring systemic corticosteroids	5/75 (6.7%)	3/25 (12%)	0.41

All values are means $\pm$ SE, unless noted otherwise.

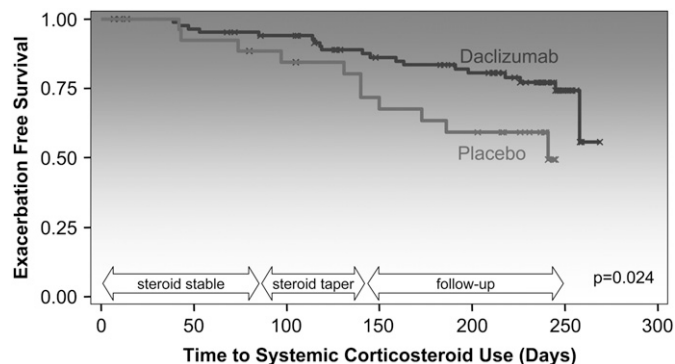


Figure 4. Kaplan-Meier plot showing time to first exacerbation requiring systemic corticosteroid rescue for patients through treatment and follow-up. During study Days 0–140, patients were treated with daclizumab or placebo, initially as add-on to stable-dose ICS (Days 0–83) followed by taper of ICS (Days 84–140). After the treatment period, patients discontinued study drug (Days 141–250). Kaplan-Meier and log-rank methods were used to assess time-to-event variables.

to severe asthma exacerbation from baseline to the end of the treatment period (Days 0–140) ($P = 0.25$). Six of 76 (7.9%) patients receiving daclizumab and 4 of 26 (15.4%) patients receiving placebo experienced exacerbations by Day 84 ($P = 0.27$). Exacerbations requiring oral corticosteroid rescue therapy occurred in 5 of 75 (6.7%) patients receiving daclizumab and in 3 of 25 (12.0%) patients receiving placebo ($P = 0.41$). Exacerbations requiring treatment with systemic corticosteroids occurred in 8 of 69 (11.6%) patients treated with daclizumab and 6 of 21 (28.6%) patients receiving placebo from baseline through Day 140 ($P = 0.08$). Exacerbations requiring treatment with systematic corticosteroids occurred in 17 of 68 (25%) patients treated with daclizumab and 10 of 21 (47.6%) patients receiving placebo through Day 252 ($P = 0.06$). Approximately equal numbers of patients required treatment with ICS during the follow-up period (62.5% of patients receiving daclizumab compared with 59.3% of patients receiving placebo).

Biomarkers. EOSINOPHILS AND SERUM EOSINOPHIL CATIONIC PROTEIN. Peripheral blood eosinophil values and serum eosinophil cationic protein (ECP) were measured as surrogate markers of airway inflammation. Daclizumab therapy was associated with a significant reduction in the mean peripheral blood eosinophil count from baseline to Day 84 (26% decrease) compared with placebo (9% increase) ($P = 0.04$), with a peak reduction by Day 28. During the first 56 days, patients treated with daclizumab with elevated baseline serum ECP of 20 ng/ml or greater had a significant reduction in serum ECP from baseline compared with patients receiving placebo ($P < 0.01$). Patients treated with daclizumab demonstrated reductions in peripheral eosinophils compared with placebo through Day 140 ($P = 0.004$).

Safety. ADVERSE EVENTS. No overall increase in frequency or severity of adverse events was observed in patients treated with daclizumab compared with patients receiving placebo. The frequency of patients reporting adverse events was similar for both groups (87.4%, daclizumab, vs. 88.9%, placebo). Although most of the adverse events reported were mild or moderate in severity, six patients reported seven serious adverse events (see subsequent text). The most frequently reported adverse events among patients treated with daclizumab included upper respiratory tract infection (18.4%), nasopharyngitis (16.1%), nasal congestion (9.2%), rash (9.2%), and nausea (9.2%). Of these events, only nasopharyngitis, rash, and nausea were observed

more frequently in the daclizumab group than in the placebo group.

SERIOUS ADVERSE EVENTS. Six patients experienced serious adverse events in the study: five patients receiving daclizumab and one patient receiving placebo. Three patients (daclizumab) had events considered related to the study drug. These events included one patient who had an anaphylactoid reaction after the infusion of the first dose, with no prior history of exposure to daclizumab. Approximately 30 minutes after initiation of infusion, the patient developed flushing, chest tightness, dyspnea, and cyanosis that progressed rapidly to respiratory arrest. The patient required epinephrine and intubation, requiring hospitalization. The patient was discharged in stable condition. A second patient developed varicella zoster viral meningitis and myelitis after infusion of the first dose, events which were assessed as possibly related to daclizumab. This patient was diagnosed with an aseptic meningitis, radiculopathy, and myelopathy, with rapidly progressing weakness of his lower extremities and cauda equina syndrome, as well as cranial neuropathies. Lumbar puncture was positive on polymerase chain reaction testing for varicella zoster, and a small vesicular rash on the coccyx was positive for varicella zoster. The patient had also noted sacral and lower extremity paresthesias before the administration of daclizumab. One additional patient developed breast cancer four months after the last infusion of daclizumab, which was assessed as possibly related to the study drug.

The following serious adverse events were considered unrelated to the study drug: exacerbation of ulcerative colitis (daclizumab); diabetic ketoacidosis and coronary artery disease (daclizumab); Stevens-Johnson syndrome due to metronidazole (daclizumab); and uterine prolapse (placebo).

Pharmacokinetic Analysis

A total of 32 patients were evaluated for the pharmacokinetic analysis. Daclizumab had a long elimination half-life of 20 days (473 ± 157 h), with no accumulation of drug observed after the initial loading dose.

Average steady-state serum daclizumab concentrations were maintained at approximately 24 $\mu\text{g/ml}$. Serum levels were maintained at greater than 1 $\mu\text{g/ml}$ for at least 2 months after the last infusion, the lowest level thought capable of maintaining saturation of IL-2R α on peripheral T cells (11, 12).

Anti-Daclizumab Antibody Analysis

Of the 113 patients tested for anti-daclizumab antibodies, 86 were from the daclizumab group and 27 from the placebo group; 3 of 84 (3.6%) patients receiving daclizumab with available postdosing samples tested positive for neutralizing antibodies. No patient appeared to have safety events related to the development of anti-daclizumab antibodies. The patient who had the anaphylactoid reaction did not have detectable anti-daclizumab antibody.

DISCUSSION

This was the first trial of a novel humanized antibody targeting the α chain of the high-affinity IL-2R on activated T cells in patients with asthma. Daclizumab improved both markers of asthma impairment and future risk in patients with moderate to severe persistent asthma who were not optimally controlled on high-dose ICS at enrollment. Compared with patients receiving placebo, patients treated with daclizumab had significant improvements in measures of pulmonary function, as well as significant reductions in asthma symptoms and rescue β_2 -inhaler therapy when daclizumab was added to high-dose ICS. In addition, patients treated with daclizumab demonstrated

increased time to asthma exacerbation after ICS taper compared with patients receiving placebo.

No treatment differences in spirometry, asthma symptoms, rescue medication use, or PEF were observed during the ICS-taper phase of the study, most likely due to the dependence these patients had on high-dose ICS to maintain asthma control. However, differences in the frequency of exacerbations favoring patients treated with daclizumab did become apparent during the ICS-taper phase and continuing into the follow-up period (Figure 4), suggesting that daclizumab may offer protection against exacerbations while ICSs are being withdrawn. Asthma exacerbations represent an important clinical outcome, as these events may lead to hospitalization, increased cost, and poor quality of life.

The mechanism of action of daclizumab has been recently reviewed (27). *In vitro* studies conducted with daclizumab and with a parent murine anti-Tac antibody demonstrate that this antibody inhibits various lymphocyte functions, including IL-2-dependent mitogen- and antigen-induced T-cell proliferation (28, 29), mixed lymphocyte reaction (28, 29), decreased secretion of Th1 and Th2 cytokines by activated lymphocytes (30, 31), blockade of CD28-dependent CD40L expression on activated T cells (31), and immunoglobulin production by activated B cells (32). Thus, it is hypothesized that daclizumab may act as an antiinflammatory agent in patients with immune-mediated diseases, such as asthma. Although daclizumab can also bind to CD25 expressed on the naturally occurring human regulatory CD4⁺CD25⁺ T cells, it does not alter the function of these cells in renal transplant recipients (33).

The reduction of peripheral eosinophils and serum ECP observed in patients treated with daclizumab suggests a further antiinflammatory effect of this compound. Airway mucosal inflammation involving lymphocytes, mast cells, eosinophils, and neutrophils is a feature of patients with chronic, persistent asthma. The synthesis and release of cytokines, including IL-13, -9, -4, and -5 (6), predominantly from Th2 cells, is thought to initiate and sustain the inflammatory process (34). Based on previously well characterized pharmacodynamics of daclizumab (11), exposure to daclizumab in this study was sufficient to fully saturate peripheral CD25 receptors during the treatment and for at least 2 months after the last infusion. In addition, based on bronchoalveolar lavage fluid (BALF), bio-distribution data obtained with humanized monoclonal antibodies, including anti-IL-4 (35) and anti-IgE (36), it is possible that, at steady-state levels, daclizumab can reach biologically active levels in BALF.

Targeting CD25⁺ T cells with daclizumab may inhibit Th2 cytokine release in a subset of Th cells, and thus disrupt the cascade of immunological events causing airway inflammation in asthma. Elevated plasma concentrations of sCD25 have been observed in oral glucocorticoid-dependent patients with asthma compared with patients controlled without oral glucocorticosteroids (37). A significant association was observed between CD25⁺ T cells in BALF and asthma symptom scores, airway methacholine responsiveness, baseline FEV₁, and eosinophils in BALF (9). In patients with chronic, severe asthma, treatment with cyclosporine resulted in clinical improvement associated with a decrease in serum concentrations of sCD25 (2). These findings further support CD25 on activated T cells as a potential therapeutic target (2, 3, 7–9). In fact, *in vitro*, daclizumab strongly inhibits production of Th2-associated cytokines by activated T cells (30, 31). In addition, *in vitro* investigations demonstrate a synergistic effect of daclizumab and dexamethasone in blocking T cell activation (11, 30), and suggests that low doses of daclizumab may enhance the immunosuppressive effects of dexamethasone and reduce the need for corticosteroid therapy in populations with immune-mediated inflammatory

disease, such as asthma. Daclizumab may also have a direct effect on eosinophils that have been shown to express functional IL-2R (38, 39). Because this study was conducted as an early proof of clinical concept in patients with moderate to severe asthma, no specific assessments were conducted to define the role of the antibody in the airway compartment.

In this study, the frequency and severity of nonserious adverse events were similar for daclizumab and placebo. Serious adverse events were observed with increased frequency in the patients treated with daclizumab. The acute allergic reaction after daclizumab administration was unlikely to have been IgE-mediated, as the event appeared on first-dose exposure. Similar reactions occurring in patients receiving intravenous immune globulin (IVIG) have been attributed to the rapid infusion of large-molecular weight protein. Anaphylactic-like reactions have occurred rarely with daclizumab (11). The patient with severe varicella zoster infection likely had an early varicella zoster syndrome existing at the time of study drug administration, which possibly was worsened by administration of the daclizumab. Prior studies of daclizumab in large numbers of transplantation patients and limited patients with autoimmune disease have not shown a tendency toward increased risk of severe viral infections. Further clinical trials are needed to better define the safety of long-term daclizumab administration in patients with asthma.

This proof-of-concept study demonstrates that daclizumab, a humanized monoclonal antibody that targets the α chain of the high-affinity IL-2R, improved asthma control in patients with moderate to severe, persistent asthma who were suboptimally controlled on high-dose ICS. Daclizumab treatment was generally well tolerated, with no apparent differences in the overall frequency and severity of adverse events in patients receiving daclizumab compared with placebo. Further studies in asthma are needed to more fully define daclizumab's potential efficacy as an add-on treatment in patients with asthma who are inadequately controlled despite the use of ICS.

Conflict of Interest Statement: W.W.B. has provided consultancy/advisory board services for Isis (2006–2008), Altana (2006–2007), Hoffman-LaRoche (2006), Cepion (2006), Amgen (2006–2008), Centocor (2006–2008), Alza (2006), GlaxoSmithKline (2006–2008), Johnson & Johnson (2006–2007), Wyeth (2006–2008), Takeda (2006), CV Therapeutics (2006–2008), Genentech/Novartis (2006–2008), Dynavax (2007), Abbott Laboratories (2007–2008), Mellenium (2007), MAP Pharmaceuticals (2007), Merck (2006–2008), Asthmatic (2007), AstraZeneca (2007–2008), Pfizer (2006–2008), MedImmune (2007), Memory Pharmaceuticals (2007), Altair (2007–2008), PDL BioPharma (2007–2008), Schering Corporation (2008), and TEVA (2008); he has received lecture fees from Novartis (2007–2008), Merck, AstraZeneca (2006–2008), and GlaxoSmithKline (2006–2008); and has received industry-sponsored grants from Novartis (2006–2008), Centocor (2006–2008), GlaxoSmithKline (2006–2008), Medicinova (2006), Dynavax (2006), Wyeth (2006), Pfizer (2006), Dey (2006), Astellas (2006), Inflazyme (2006), Biowa (2006), and Cepion Therapeutics (2008). E.I. has received less than \$10,000 as a consultant for PDL BioPharma. H.S.N. has consultant arrangements with Genentech/Novartis, GlaxoSmithKline, Schering-Plough, Johnson & Johnson, Dyson, AstraZeneca, Dynavax Technologies, Abbott Laboratories, Medicinova, Boehringer-Ingelheim, and TEVA from 2007–2008; he has active grant/research support from Schering-Plough, Novartis, Wyeth, Altana, Cepion, Boehringer-Ingelheim, AstraZeneca, Sepracor, and Genentech; and is part of a speaker's bureau for GlaxoSmithKline and AstraZeneca. J.W.B. participated in an advisory group meeting for PDL in 2001 and was paid \$2,000 for the meeting. B.L.C. has participated in a PDL scientific advisory board meeting in 2003 and 2008, and received research grants in 2002–2003 for participation in this Daclizumab trial. D.Y.Y. was a full-time employee of PDL BioPharma during the design, conduct, and analysis of this study; his relationship with the company and its stock terminated in March 2006. V.V. is an employee of PDL BioPharma. R.S.S. is an employee of PDL BioPharma, Inc.

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Daclizumab improves asthma control in patients with moderate to severe persistent asthma: A randomized, controlled trial

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Online Data Supplement

Methods

Study Procedures

Eligible patients were enrolled from October 2001 to December 2003. As each patient qualified for the randomized phase of the study, a member of the study site called the Interactive Voice Recording System (IVRS) center (Synteract, Inc.) to receive a randomized number. The randomization schedule was generated by PDL and sent to Synteract, Inc. for uploading to IVRS. The IVRS center communicated the patient's randomization number to the study center. All persons directly involved in the study were blinded to treatment including the patient, investigator(s), study coordinator, pharmacist, and PDL's medical monitor and Clinical Research Associates (CRA). Sites were supplied with blinded kits that were assigned to each patient.

Spirometry was performed prior to dosing according to American Thoracic Society criteria to determine baseline FEV₁, forced vital capacity (FVC), FEV₁/FVC, and forced expiratory flow (FEF)_{25-75%}.^{E1}

Asthma symptoms, medication use, and peak flow recording were assessed using each patient's daily diary recording (by interactive voice response system). The daytime mean symptom scale used a range of response categories for each question from 0 to 6, indicating the least to the most asthma symptomatology. The nocturnal diary scale used

response categories ranging from 0 (indicating no awakening with asthma symptoms) to 3 (indicating awake all night). Daily daytime scale scores were computed as the mean total of the 4 questions on the daytime symptom scale. An overall diary score for the week was computed as the mean of at least 4 of 7 daily daytime scale scores. Weekly mean scores for the nocturnal diary scale were computed in a similar manner. A decrease in the weekly score for the daytime and nocturnal scales indicated an improvement in asthma symptoms. The change from baseline in the asthma scale scores was computed as the difference between the mean score from the last week of the pre-treatment run-in period (Days -7 to -1) and the last week of Treatment Period 1 (Days 77 through 83) and the last week of Treatment Period 2 (Days 134 to 140).

Asthma-free days were defined as those days in which both the diurnal and nocturnal diaries indicated no symptoms. The method by which this variable was analyzed is dependent on the distribution of missing diary entries. If there were few missing data, changes in mean asthma-free days were analyzed. If patients were inconsistent in reporting their asthma symptoms, then the proportion of patients who experienced asthma-free days was compared by treatment group.

Rescue use of β_2 -agonist metered dose inhaler (MDI) was recorded in the daytime and nocturnal symptom diaries. Patients were instructed to record the number of actuations of β_2 -agonist from an MDI that was used during the day in the daytime diary recording and the number of actuations of β_2 -agonist MDI that were used after going to sleep for the night in the nocturnal diary recording. The mean daily use of β_2 -agonist MDI was

computed for daily and nocturnal use for each week of diary recording. The change from baseline in the use of β_2 -agonist rescue medication was computed as the difference between the mean daily score from the week of the pretreatment run-in period (Days -7 to -1) and the last weeks of the Treatment Period 1 (Days 77 to 83) and the last week of Treatment Period 2 (Days 134 to 140).

Serial serum chemistry panel, hematology, and whole blood eosinophils were monitored. At each visit, serum daclizumab levels and circulating human anti-daclizumab antibody were measured. Daclizumab levels were determined using an enzyme-linked immunosorbent assay (ELISA)-based system. Serum samples were first screened for anti-daclizumab antibodies using a bridging ELISA format. Positive samples from the anti-daclizumab assay were further evaluated in confirmatory and neutralizing assays. Serum eosinophil cationic protein (ECP) levels were measured by Immulite 2000 Automatic Immunoassay Analyzer (Diagnostic Products Corporation, Los Angeles, CA). A central laboratory performed these analyses. Patients recorded asthma symptoms, nocturnal awakenings due to asthma, rescue medications, and peak expiratory flow twice daily using an IVRS diary through Day 140 (Treatment Periods 1 and 2). Patients were instructed to call the study center if they met criteria for exacerbation, and study sites recorded exacerbation history at each visit. Investigators assessed the need for systemic steroid rescue.

Statistical Methods

Within-group changes were evaluated by paired *t*-tests, and between-group significance by *t*-tests. Kaplan-Meier and log rank methods were used to assess time-to-event variables. Last-observation-carried-forward methods were used for patients who prematurely withdrew due to efficacy-related reasons. Available data were analyzed for patients who were premature non-efficacy-related terminations. Asthma-free days were defined as days in which both diurnal and nocturnal diaries indicated no symptoms. Asthma exacerbations that were recorded following contact with the study site were included in the analysis. Patients who withdrew prior to completing the treatment and follow-up interval were included in the analysis up to the time the patient withdrew from the protocol.

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