

Effect of IVL745, a VLA-4 antagonist, on allergen-induced bronchoconstriction in patients with asthma

Virginia Norris, MRCP,^a Lee Choong, BSc,^a Duyen Tran, BSc,^a Zoe Corden, BSc,^a Malcolm Boyce, FRCP,^a Hasan Arshad, MD,^b Stephen Holgate, MD,^b Brian O'Connor, MD,^c Sylvaine Millet, BSc,^d Barry Miller, MS,^d Shashank Rohatagi, PhD,^d and Stephane Kirkesseli, MD^d *London and Southampton, United Kingdom, and Bridgewater, NJ*

Background: Very late antigen (VLA-4) antagonists have been proposed as potential therapies for diseases in which cell recruitment and accumulation are causative. Asthma, which is characterized by airway inflammation involving the accumulation of eosinophils and mononuclear cells, is one such disease.

Objective: We sought to assess the effect of IVL745, a VLA-4 antagonist, on the early and late asthmatic response (LAR) and on markers of airway inflammation after allergen inhalation. **Methods:** The study was of a placebo-controlled, double-blind, randomized, 2-way crossover design. Sixteen subjects with mild-to-moderate asthma controlled with short-acting β_2 -agonists only and with a LAR to inhaled allergen participated in the study. At one treatment period they took 20 mg of IVL745 and one treatment period placebo. Both treatments were taken twice daily for 7 days, with a single dose on day 8. Treatments were separated by a washout period of at least 2 weeks. On day 7 of each treatment period, sputum was induced and collected, and exhaled nitric oxide (NO) was measured. On day 8, an inhaled bolus allergen challenge was performed, and blood was taken for pharmacokinetics. On day 9, exhaled NO was measured, and a methacholine challenge was done. On day 10, sputum was induced and collected. Adverse events, peak expiratory flow (PEF), use of short-acting

β_2 -agonists, and asthma symptoms were recorded daily throughout the study.

Results: There was no statistically significant difference between IVL745 and placebo in the effect on the LAR after allergen challenge, as measured by the area under the curve of the percentage change in FEV₁ from the prechallenge baseline (mean [SEM], -81.99 [18.80] after IVL745 and -72.58 [21.29] after placebo; 95% CI of difference, -36 to 16.8 ; $P = .46$) or by the maximum percentage change from the prechallenge baseline (mean [SEM], -23.44 [4.73] after IVL745 and -21.30 [5.17] after placebo; 95% CI of difference, -11 to 6.29 ; $P = .60$). There was a statistically significant decrease in the percentage of eosinophils in sputum on day 7 of treatment with IVL745 (mean [SEM], 7.32 [1.46]) compared with placebo (mean [SEM], 15.00 [1.92]; 95% CI of difference, -13 to -1.2 ; $P = .02$). There was no statistically significant difference between IVL745 and placebo with respect to the early asthmatic response, methacholine hyperresponsiveness, exhaled NO, postallergen sputum, symptoms, inhaled β_2 -agonist use, or PEF.

Conclusion: In patients with mild-to-moderate atopic asthma, IVL745 did not affect the early and late response to inhaled allergen or markers of airway inflammation, except for a modest reduction in sputum eosinophils. (*J Allergy Clin Immunol* 2005;116:761-7.)

From ^aHammersmith Medicines Research, Central Middlesex Hospital, London; ^bSouthampton General Hospital, Southampton; ^cGuy's, King's and St Thomas' School of Medicine, London; and ^dAventis Pharmaceuticals, Bridgewater.

Supported by Aventis Pharmaceuticals.

Disclosure of potential conflict of interest: B. O'Connor had had ad hoc consultancy arrangements with GlaxoSmithKline, AstraZeneca, Altana, Aventis, Celgene, Pfizer, Boehringer Ingelheim, and various small biotechnology companies and receives grant and research support from several pharmaceutical companies. As director of a phase 2 clinical research unit, he performs a number of studies similar to that reported in this article; Kings College receives the funding. He has spoken for AstraZeneca, GlaxoSmithKline, Pfizer, Boehringer Ingelheim, and Altana. B. Miller and S. Kirkesseli possess shares of stock and stock options in and are employees of Sanofi-Aventis (formerly Aventis). S. Rohatagi was an employee of Aventis at the time of this study.

Received for publication October 18, 2004; revised April 3, 2005; accepted for publication April 8, 2005.

Available online August 18, 2005.

Reprint requests: Virginia Norris, MRCP, Hammersmith Medicines Research, Central Middlesex Hospital, Acton Lane, London NW10 7NS, United Kingdom. E-mail: vnorris@hmlondon.com.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.04.045

Key words: Very late antigen (VLA-4, $\alpha 4\beta 1$) antagonist, inhaled allergen challenge, late asthmatic response, eosinophil

Very late antigen (VLA-4, $\alpha 4\beta 1$) is a heterodimeric cell-surface molecule found on all leukocytes except neutrophils, and its expression is upregulated by IL-4 and IL-13. VLA-4 is the receptor primarily responsible for mononuclear cell and eosinophil trafficking: binding of VLA-4 to vascular cell adhesion molecule 1 (VCAM-1) on vascular endothelium arrests cell rolling and promotes extravasation.^{1,2} VLA-4 also interacts with the extracellular matrix protein fibronectin, which is implicated in the regulation of eosinophil apoptosis.^{3,4} VLA-4 antagonists have therefore been proposed as a therapeutic target in diseases in which cell recruitment and accumulation are causative.^{5,6} Asthma, which is characterized by airway inflammation involving the accumulation of eosinophils and mononuclear cells, is one such disease.⁷ VLA-4 antagonists are effective in animal models of asthma.^{1,7,8} To date, no results of human studies have been published.

Abbreviations used

AUC:	Area under the curve
EAR:	Early asthmatic response
IC ₅₀ :	Inhibitory concentration of 50%
LAR:	Late asthmatic response
NO:	Nitric oxide
PC ₂₀ :	Provocative concentration causing a 20% decrease in FEV ₁
PEF:	Peak expiratory flow
VCAM-1:	Vascular cell adhesion molecule 1
VLA-4:	Very late antigen

IVL745, 3-[N-(3,4-dimethoxybenzyl)-2-{2-[3-methoxy-4-(3-*o*-tolylureido) phenyl] acetylamino} acetamido] propionic acid, is a VLA-4 antagonist that is effective in animal models of airway inflammation and asthma. IVL745 was assessed in the Brown Norway rat model of antigen-induced lung inflammation. IVL745, 3 mg/kg, significantly inhibited antigen-induced eosinophil and lymphocyte accumulation when lung tissue was examined 24 hours after antigen challenge. The effects of IVL745 on allergen (*Ascaris suum*)-induced bronchoconstrictor response in sheep was also assessed. A single dose of 1 mg/kg given 30 minutes before allergen challenge completely inhibited the late asthmatic response (LAR) and the development of hyperresponsiveness (24 hours after allergen challenge) to carbachol. IVL745 is safe and well tolerated in healthy volunteers and asthmatic subjects up to the maximum tested dose of 20 mg administered by means of inhalation twice daily. It therefore merited assessment as an anti-inflammatory agent in patients with asthma.

Inhaled allergen provocation in asthma has been used extensively to test anti-inflammatory agents of potential clinical benefit in asthma. In susceptible individuals there is an early asthmatic response (EAR) within the first 2 hours, followed by an LAR at 3 to 9 hours after inhalation of allergen. All anti-inflammatory drugs (eg, corticosteroids, theophylline, leukotriene receptor antagonists, cromones, and anti-IgE⁹⁻¹⁴) and functional antagonists (eg, long-acting β_2 -agonists¹⁵) that are effective in controlling asthma inhibit the LAR to inhaled allergen. The LAR is accompanied by hyperresponsiveness of the airways to direct and indirect bronchoconstriction stimuli¹⁶ and increased numbers of inflammatory cells, particularly eosinophils, in induced sputum.¹⁷ We therefore assessed the effects of IVL745, an inhaled VLA-4 antagonist, against the LAR, sputum eosinophil counts, exhaled nitric oxide (NO),¹⁸ asthma symptoms, inhaled β_2 -agonist use, and peak expiratory flow (PEF).

METHODS

The study was done at 3 centers (Southampton General Hospital, Hampshire; Kings Hospital, London; and HMR, Central Middlesex Hospital, London). The study was approved by each center's local research ethics committee, and all subjects gave fully informed, written consent. The study was performed in accordance with the International Conference of Harmonization Guideline for Good Clinical Practice.

Subjects

Sixteen adults (14 men and 2 women) aged 20 to 49 years (mean, 28.9 years) with mild-to-moderate atopic asthma were enrolled in the study. Subjects had the following: mild-to-moderate asthma requiring treatment with short-acting inhaled β_2 -agonists only; FEV₁ at least 70% of predicted normal value; a positive skin prick test to allergen; a provocative concentration of less than 8 mg/mL methacholine causing a 20% decrease in FEV₁ (PC₂₀); and a documented EAR and LAR to inhaled allergen. Subjects had to be nonsmokers for at least 12 months and not have used any inhaled corticosteroids for at least 2 months, leukotriene antagonists for at least 2 weeks, or long-acting β_2 -agonists for at least 48 hours before screening. Subjects were excluded if they had experienced a recent (within the last 6 weeks) respiratory infection, a medical condition other than asthma, were pregnant or lactating, or if of child-bearing potential and not using an adequate barrier method of contraception.

Design

The study was of a placebo-controlled, double-blind, randomized, 2-way crossover design. During each study period, subjects were treated with either IVL745 or matched placebo twice daily by inhalation for 7 days, with a single dose on day 8. Treatments were separated by a washout period of at least 2 weeks.

At screening, subjects underwent a methacholine challenge and a skin prick test to allergen.

At baseline, subjects underwent an inhaled incremental allergen challenge and then, 24 hours later, a methacholine challenge. Exhaled NO was measured before and 24 hours after the allergen challenge. There was at least a 2-week washout period between baseline and the start of the study.

IVL745 (20 mg) and placebo (100% lactose monohydrate) were administered twice daily through an Ultrahaler (Aventis, Bridgewater, NJ) metered-dose dry-powder inhaler¹⁹ as 10 consecutive actuations of the device. Day 1 morning, day 3 morning, day 5 morning, day 7 morning and evening, and day 8 morning doses were observed by site personnel for each treatment period. All other doses were taken by the subject without direct supervision. Morning doses were taken around 8 AM, and evening doses were taken around 8 PM. Adherence to treatment was checked by weighing the devices and by subjects' recording of the time of dosing in a diary.

At each treatment period, the subjects underwent the following tests. On day 7, they underwent induced sputum collection and exhaled NO measurement 1 hour after the morning dose. On day 8, they underwent an inhaled bolus allergen challenge 1 hour after the morning dose. On day 9, they underwent exhaled NO measurement, and a methacholine challenge was performed (approximately 24 hours after the allergen challenge). On day 10, they underwent induced sputum collection. Blood samples for pharmacokinetics were taken frequently for 24 hours after dosing on day 8 of each treatment period.

Adverse events, PEF, use of short-acting β_2 -agonists, and asthma symptoms were recorded daily throughout the study. Table 1 shows the overall study schedule.

Specific tests

Allergen skin test. One hundred microliters of 3 allergens (house dust mite, cat hair, and 6 grasses from ALK Abello, Ltd, Hørsholm, Denmark) plus positive (histamine concentration of 10 mg/mL) and negative controls were applied to the volar surface of the forearm at least 2 cm apart. The skin was then pricked, without causing bleeding, with a sterile lancet. The solutions were then wiped off the skin. After 15 minutes, the sites were observed for the development of a wheal and flare. A positive test result was one in which the wheal response was at least 2 mm greater than that produced by the negative control.

TABLE I. Overall study schedule

	Baseline		Washout	Treatment period 1										Washout	Treatment period 2									
Study day	1	2	14 d minimum	1	2	3	4	5	6	7	8	9	10	14 d minimum	1	2	3	4	5	6	7	8	9	10
Administration of study drug (or placebo) twice daily (single dose on day 8)				X	X	X	X	X	X	X	X				X	X	X	X	X	X	X			
Allergen challenge	X										X										X			
Induced sputum										X			X								X			X
Methacholine challenge		X											X										X	
Exhaled NO	X	X								X	X									X	X			
PEF	◀																							▶
Concomitant medication	◀																							▶
Adverse events	◀																							▶

Inhaled methacholine challenge. A prechallenge baseline FEV₁ (Vitalograph dry wedge spirometer, Buckingham, United Kingdom) to inhaled 0.9% saline was established. Subjects then inhaled doubling concentrations of methacholine, and FEV₁ was measured after each concentration. The concentration of methacholine was increased until a decrease in FEV₁ of at least 20% from the saline baseline value was achieved or the maximum concentration (32 mg/mL) was inhaled. All inhalations were delivered through a breath-activated dosimeter (Mefar, Bovezzo, Italy), with the subject inspiring slowly from functional residual capacity to total lung capacity over 1 second and then holding his or her breath for 6 seconds while wearing a nose clip. Five breaths were inhaled for each concentration. The PC₂₀ was calculated by means of interpolation on a logarithmic dose-response curve.²⁰

Inhaled allergen challenge. At baseline, subjects underwent an inhaled incremental allergen challenge. The allergen to which the subject had been most responsive on skin prick testing was used. Freeze-dried allergen extract (ALK Abello, Ltd) was diluted in 0.9% saline to provide concentrations of 250 to 32,000 SQU/mL. Baseline FEV₁ was determined after inhalation of 0.9% saline, and then the subject inhaled incremental concentrations of allergen, starting with the lowest concentration. All inhalations were delivered through a breath-activated dosimeter (Mefar) by inspiring slowly from functional residual capacity to total lung capacity over 1 second and then holding breath for 6 seconds while wearing a nose clip. Five breaths were inhaled for each concentration. Inhalation of increasing concentrations of allergen continued until an EAR (a decrease of >20% of saline baseline value) was achieved. FEV₁ was then measured at the following times after allergen inhalation: 20, 30, 45, and 60 minutes and 2, 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, and 9 hours. The LAR was defined as a decrease in FEV₁ from saline baseline value of more than 15% between 3 and 7 hours after allergen challenge.

After dosing in each treatment period, subjects underwent an inhaled bolus allergen challenge. The concentration of allergen inhaled was equal to the sum of the incremental concentrations inhaled.²¹

Sputum. Induced sputum was collected and processed using a standard protocol.²² After bronchodilatation with salbutamol, subjects inhaled hypertonic (4.5%) saline by means of nebulization. Saline inhalation was performed in 3 periods of 7 minutes; the total time for nebulized saline inhalation was 21 minutes. After each 7-minute period, subjects blew their noses and rinsed their mouths to minimize salivary contamination before expectorating sputum.

For sputum processing, dithiothreitol (Sigma, Poole, United Kingdom) was added to the sputum. The sample was vortexed, fol-

lowed by gentle aspiration, and then left on a bench rocker for 15 minutes until the sputum was homogenized. Dulbecco's PBS (Sigma) was then added to the sample and vortexed to ensure adequate mixing. The mixture was filtered through a 48-μm nylon mesh filter and centrifuged at 3000 rpm for 4 minutes at 20°C.

The cell pellets from the sputum centrifugation were resuspended for total cell counts and cell viability (by using Trypan Blue exclusion) analysis with a hemacytometer. Slides were prepared by using a Cytospin instrument (Shandon, Runcorn, United Kingdom) and were stained with Wright's Giemsa Accustain (Sigma) for differential cell counts. Supernatant was stored at -80°C.

The following total and differential cell counts were analyzed in the sputum: epithelial cells, lymphocytes, eosinophils, basophils, macrophages, and neutrophils. Fluid-phase measurements included eosinophil cationic protein, IL-5, GM-CSF, TNF-α, tryptase, and albumin.

Exhaled NO. Exhaled NO was measured with a modified chemiluminescence analyzer (Model LR2500, Logan Research, Rochester, United Kingdom). Patients put on a nose clip and then took a deep breath in through the mouth. They then exhaled slowly for 30 seconds into the mouthpiece, using a biofeedback indicator to guide flow rate. Measured in this way, NO is representative of the lower airways because expiration against resistance closes the soft palate, ensuring no admixture from the nasopharynx.

Safety and tolerability

Adverse events were summarized by treatment, and no formal statistical tests were done.

Pharmacokinetic analysis

Plasma samples were analyzed for IVL745 by using a validated liquid chromatography tandem mass spectrometry method with a minimum quantifiable limit of 10 pg/mL.²³ Pharmacokinetic parameters were calculated by using noncompartmental methods.

Efficacy variables and analysis

The primary efficacy variables were as follows:

1. magnitude of LAR as measured by the area under the curve (AUC) of the percentage change in FEV₁ from the prechallenge baseline value for the time period 3 to 9 hours after allergen challenge;
2. magnitude of LAR as measured by the maximum percentage change in FEV₁ from the prechallenge baseline value in the time period 3 to 9 hours after allergen challenge.

Secondary efficacy variables were EAR (measured as the AUC and the maximum percentage change of FEV₁ from prechallenge

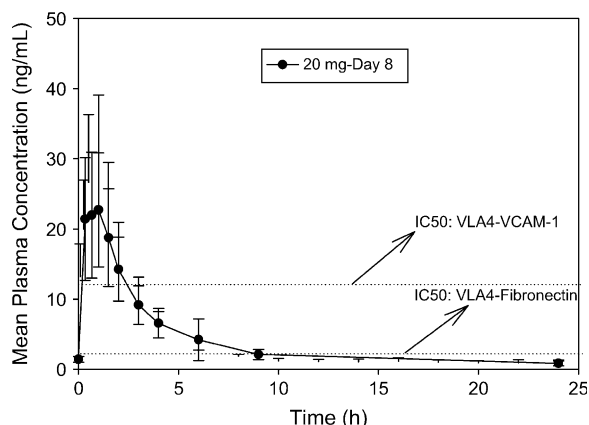


FIG 1. Pharmacokinetics of IVL745 (mean [SD]) on day 8, with IC₅₀ VLA-fibronectin and IC₅₀ VLA-VCAM-1 values shown.

baseline value 0 to 2 hours after allergen challenge), PEF (morning and evening) before dosing, asthma symptom score, number of nighttime awakenings, β_2 -agonist use, cell and cytokine profiles in induced sputum (comparison between placebo and IVL745 was done for days 7 and 10), FEV₁, forced vital capacity, methacholine PC₂₀ 24 hours after allergen challenge (placebo and IVL745 compared), and NO measurements.

The AUC was calculated by using the trapezoid method. Differences between groups were analyzed by using ANOVA models appropriate to the 2-way crossover design. Necessary assumption checks (to test normality and equality of variance) were verified for the primary efficacy variables. The Wilcoxon rank sum test was used if assumptions were seriously violated. Hypothesis testing was done at the 5% 2-sided significance level for primary and secondary efficacy variables.

Sample size

The sample size for this study was based on the simplified assumption of a comparison of the 2 treatments by using a paired *t* test. A 30% mean difference in the magnitude of the LAR between treatments would be considered clinically important, so that value was used to compute the sample size. Given that estimate, the sample size required to detect a 30% difference in the magnitude of the LAR would be 12, assuming a type I error rate of 0.05, a type II error rate of 0.10 (ie, 90% power), and a within-patient SD of 25%.

RESULTS

Safety and tolerability

Safety and tolerability were very good. Adverse events were similar in nature and incidence with IVL745 and placebo. Thirty-one all-causality adverse events were reported during the study. Sixteen (all mild) were reported with placebo, and 15 (14 mild and 1 moderate [muscular skeletal shoulder pain]) were reported with IVL745. The most common adverse events were headache (6 with placebo and 6 with IVL745), nasopharyngitis (2 with IVL745 and 1 with placebo), diarrhea (1 with placebo and 1 with IVL745 [same subject]), nausea (1 with placebo and 1 with IVL745), and hiccups (2 with placebo).

TABLE II. Magnitude of LAR as measured by AUC of the percentage change in FEV₁ from the prechallenge baseline value for the time period 3 to 9 hours after allergen challenge

	N	Mean	SEM	Median	95% CI of difference	P value
IVL745	16	-81.99	18.80	-71.74	-36 to 16.8	.46
Placebo	16	-72.58	21.29	-50.09		

Pharmacokinetics

Fig 1 shows the pharmacokinetic parameters on day 8. IVL745 was rapidly absorbed, with a median time of peak concentration of 1 hour, and the plasma concentrations followed a biexponential decrease after reaching peak concentrations. The plasma AUC₀₋₂₄ and peak concentration values for IVL745 at 20 mg were 97.98 ng.h/mL and 25.88 ng/mL. The mean terminal half-life was 7.19 hours.

Efficacy

There was no statistically significant difference between IVL745 and placebo in the effect on the LAR 3 to 9 hours after allergen challenge, as measured by the AUC of the percentage change in FEV₁ from the prechallenge baseline value (Table II and Fig 2) or by the maximum percentage change from the prechallenge baseline value (Table III).

With the exception of the day 7 sputum eosinophil counts and day 7 neutrophil counts (Fig 3), secondary efficacy variables did not differ significantly between treatments. The median eosinophil count on day 7 was 0.048×10^6 /mL after treatment with IVL745 and 0.206×10^6 /mL after placebo. The median neutrophil count on day 7 was 0.495×10^6 /mL after IVL745 and 0.296×10^6 /mL after placebo. There was a statistically significant decrease in the percentage of eosinophils in sputum on day 7 of treatment with IVL745 (mean, 7.32; SEM, 1.46) compared with placebo (mean, 15.00; SEM, 1.92) (95% CI of difference, [-13, -1.2]; *P* = .02). There was a statistically significant increase in the percentage of neutrophils in sputum on day 7 of treatment with IVL745 (mean, 40.67; SEM, 3.65) compared with placebo (mean, 21.49; SEM, 4.80) (95% CI of difference, [4.82, 33.5]; *P* = .02). No other sputum variables differed significantly between treatments.

DISCUSSION

The effect of IVL745, a VLA-4 antagonist, on the early and late response to inhaled allergen and markers of airway inflammation in patients with mild-to-moderate atopic asthma was assessed in this study. The dose administered was 20 mg twice daily via the Ultrahaler metered-dose dry-powder inhaler. This was considered the maximum feasible dose given the number of actuations required for dosing by using the formulation (10 actuations

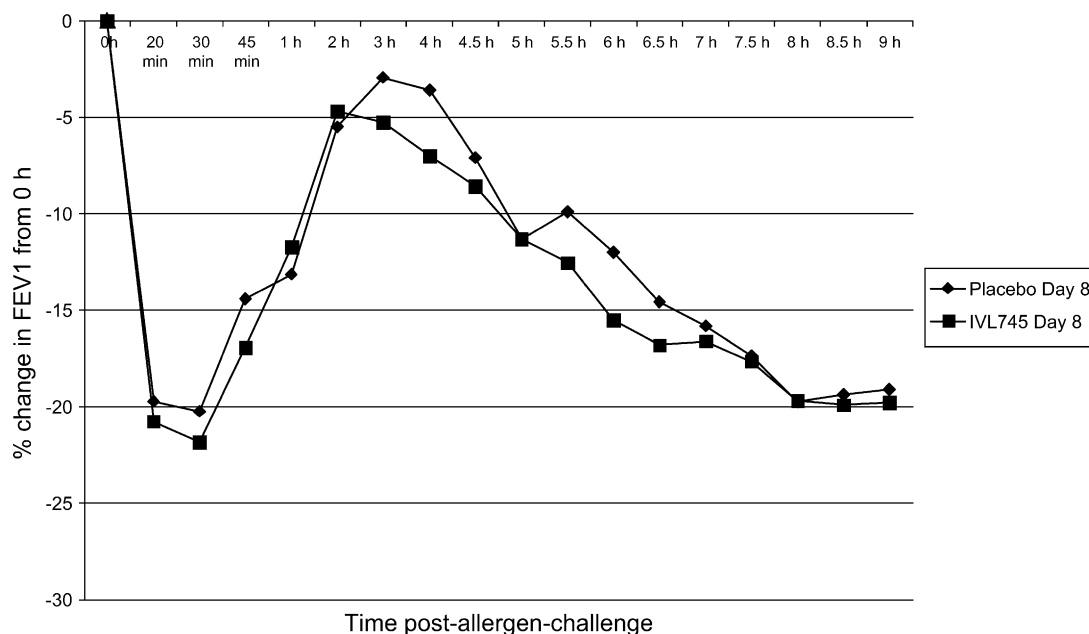


FIG 2. Mean change (percentage) in FEV₁ from prechallenge value in the 9 hours after allergen inhalation on day 8 for placebo and IVL745.

TABLE III. Magnitude of LAR as measured by the maximum percentage change in FEV₁ from the prechallenge baseline in the 3 to 9 hours after allergen challenge

	N	Mean	SEM	Median	95% CI of difference	P value
IVL745	16	-23.44	4.73	-21.86	-11 to 6.29	.60
Placebo	16	-21.30	5.17	-18.05		

for 20 mg). There was no effect of IVL745 on the LAR, which was the primary efficacy variable. Except for a modest reduction in sputum eosinophils on day 7 (before allergen challenge), there was no effect of IVL745 on any other secondary efficacy variable measured.

IVL745 has a favorable profile for an asthma therapy: it can be delivered by means of inhalation, has low oral bioavailability, and yields low systemic exposure.¹⁹ In preclinical experiments single doses of IVL745 reduced the airway inflammatory response of sensitized rats (3 mg/kg intratracheally: significant inhibition of antigen-induced accumulation of eosinophils and lymphocytes) and sheep (1 mg/kg nebulized: significant inhibition of the early-phase bronchoconstriction and total inhibition of the late-phase bronchoconstrictor response to inhaled allergen). That effect was observed both immediately and for at least 6 hours after a single dose. In the present study a lower dose (0.19-0.36 mg/kg) was administered to human subjects compared with the effective dose in animal models on a milligram per kilogram basis, and that might account for the negative results. However, the animal

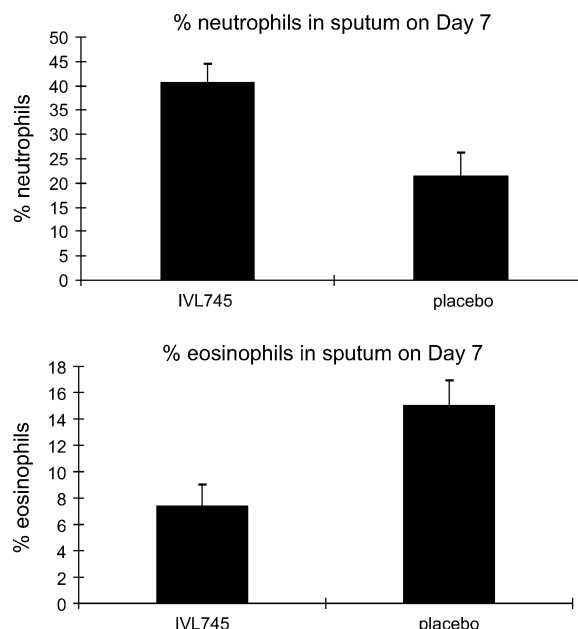


FIG 3. Percentage of eosinophils and neutrophils in sputum on day 7 (error bars indicate SEM).

studies were done with single doses, and repeated twice daily dosing (as in the present study) would be expected to be more effective than a single dose of the same milligram per kilogram value given that current anti-inflammatory drugs for asthma (eg, steroids) are more effective on repeated dosing than after a single dose.

The inhibitory concentration of 50% (IC₅₀) values for IVL745 for adhesion to VCAM-1 and fibronectin are 20 nM (11.85 ng/mL) and 2 nM (1.185 ng/mL), respectively. In human subjects systemic exposure was less than that achieved in animal models; however, blood concentrations achieved after inhaled administration of IVL745 (peak concentration) were higher (2-fold and 20-fold, respectively) than the IC₅₀ values for VCAM-1 and fibronectin (Fig 1). It is unclear how systemic exposure relates to efficacy of VLA-4 antagonists in asthma. In a number of animal models with different VLA-4 antagonists, local delivery to the lung was efficacious, whereas administration through other routes that achieved far higher systemic exposure were not efficacious.⁵ The oral bioavailability of IVL745 is negligible, and therefore in our study the systemic exposure reflects the amount of drug deposited in the lungs rather than being absorbed through the gastrointestinal tract. Therefore because the drug was deposited directly to the organ of interest and also achieved systemic exposures in excess of the IC₅₀, it is likely that the local exposure in lungs was adequate. However, we cannot totally rule out that efficacy could have been achieved if higher doses could have been given.

Lack of compliance with the dosing regimen is not likely to account for the negative results. Forty percent of the doses were administered by site personnel; other doses were recorded in a diary, and the devices were weighed. Those records showed that compliance with the dosing regimen was excellent.

The only marker of airways inflammation to show a significant difference between IVL745 and placebo was the sputum eosinophil count after 7 days' treatment. Eosinophils accumulate in the lungs in large numbers in patients with asthma. Increases in sputum eosinophils predict loss of control of asthma,²⁴ and antiasthma treatments that are effective anti-inflammatory agents do reduce lung eosinophilia. However, drugs that selectively reduce eosinophils, such as recombinant human IL-12 and IL-5 blocking mAbs, have failed to affect the disease process, and the role of eosinophils in the pathogenesis of asthma has therefore been questioned.²⁵⁻²⁷ A VLA-4 antagonist, developed to prevent eosinophil extravasation, has been shown in this study to reduce eosinophil numbers in sputum, if only moderately. However, as with other drugs that reduce eosinophil numbers, they do not reduce the LAR. Therefore this study further supports the questioning of a central role of eosinophils in the LAR. It might be because therapies targeting eosinophils only partially suppress lung eosinophils and that complete suppression is needed for efficacy or redundant pathways might maintain accumulation of eosinophils in the lung. Studies with VLA-4 antagonists have shown positive results in multiple sclerosis and Crohn's disease,^{28,29} and results of other trials in asthma are awaited.

In conclusion, in patients with mild-to-moderate atopic asthma, IVL745, an inhaled VLA-4 antagonist, did not affect the early and late response to inhaled allergen or markers of airway inflammation, except for a modest reduction in sputum eosinophils after 7 days' treatment.

REFERENCES

- Ulbrich H, Eriksson E, Lindbom L. Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacol Sci* 2003;24:640-7.
- Busse W, Lemanske R. Asthma. *N Engl J Med* 2001;344:350-62.
- Anwar A, Moqbel R, Walsh G, Kay A, Wardlaw A. Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med* 1993;177:839-43.
- Anwar A, Walsh G, Cromwell O, Kay B, Wardlaw A. Adhesion to fibronectin primes eosinophils via α -4, β -1. *Immunology* 1994;82:222-8.
- Lobb R, Adams S. Small molecule antagonists of α 4 integrins: novel drugs for asthma. *Exp Opin Invest Drugs* 1999;8:935-45.
- Lin K, Castro A. Very late antigen (VLA-4) antagonists as anti-inflammatory agents. *Curr Opin Chem Biol* 1998;2:453-7.
- Abraham W, Ahmed A, Szelczak M, Narita M, Arrhenius T, Elices M. Blockade of the late-phase airways responses and airway hyper-responsiveness in allergic sheep with a small-molecule peptide inhibitor of VLA-4. *Am J Respir Crit Care Med* 1997;156:696-703.
- Koo G, Shah K, Ding G, Xiao J, Wnek R, Doherty G, et al. A small molecule very late antigen-4 antagonist can inhibit ovalbumin-induced lung inflammation. *Am J Respir Crit Care Med* 2003;167:1400-9.
- Kelly EA, Busse WW, Jarjour NN. Inhaled budesonide decreases airway inflammatory response to allergen. *Am J Respir Crit Care Med* 2000;162:883-90.
- Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma [published erratum appears in *Lancet* 1994;343:1512]. *Lancet* 1994;343:1006-8.
- Diamant Z, Grootendorst DC, Veselic-Charvat M, Timmers MC, De Smet M, Leff JA, et al. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. *Clin Exp Allergy* 1999;29:42-51.
- Rasmussen JB, Eriksson LO, Margolskee DJ, Tagari P, Williams VC, Andersson KE. Leukotriene D4 receptor blockade inhibits the immediate and late bronchoconstrictor responses to inhaled antigen in patients with asthma. *J Allergy Clin Immunol* 1992;90:193-201.
- Crimi E, Brusasco V, Crimi P. Effect of nedocromil sodium on the late asthmatic reaction to bronchial antigen challenge. *J Allergy Clin Immunol* 1989;83:985-90.
- Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am J Respir Crit Care Med* 1997;155:1835-40.
- Twentyman OP, Finnerty JP, Harris A, Palmer J, Holgate ST. Protection against allergen induced asthma by salmeterol. *Lancet* 1990;336:338-42.
- Cartier A, Thomson N, Frith P, Roberts R, Hargreave F. Allergen induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982;70:170-7.
- Fahy J, Liu J, Wong H, Boushey H. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. *J Allergy Clin Immunol* 1994;93:1031-9.
- Kharitonov S, O'Connor B, Evans D, Barnes P. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;151:1894-9.
- Rohatigi S, Chapel S, Kirkesseli S, Newman S, Zhang J, Paccaly D, et al. Pharmacoscintigraphic comparison of HMR1031, a VLA-4 antagonist, in healthy volunteers following delivery via a nebulizer and a dry powder inhaler. *Am J Ther* 2004;11:103-13.
- Sterk P, Fabbri L, Quanjer H, Cockcroft D, O'Bryne P, Anderson S, et al. Responsiveness Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J Suppl* 1993;16:53-83.
- Taylor D, Harris J, O'Connor B. Comparison of incremental and bolus dose inhaled allergen challenge in asthmatic people. *Clin Exp Allergy* 2000;30:56-63.
- Pizzichini E, Pizzichini MMM, Efthimiadis A. Indices of airway inflammation in induced sputum; reproducibility and validity of cell and fluid-phase movements. *Am J Respir Crit Care Med* 1996;154:308-17.

23. Rohatagi S, Shah B, Zhang J, Zeng Z, Kirkesseli S, Jensen BK. Estimating mass balance for inhaled drugs in humans: an example with a VLA-4 antagonist, IVL745. *J Clin Pharmacol* 2004;44:348-58.
24. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med* 2000;161:64-72.
25. Leckie MJ, Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness and the late asthmatic response. *Lancet* 2000;356:2144-8.
26. Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyperreactivity and the late asthmatic response. *Lancet* 2000;356:2149-52.
27. Bochner BS. Verdict in the case of therapies versus eosinophils: the jury is still out. *J Allergy Clin Immunol* 2004;113:3-9.
28. Kumfil T, Heydari N, Hohfeld K. Antegren (natalizumab). A promising new approach to therapy of multiple sclerosis. *Nervenarzt* 2002;73:552-5.
29. Elias MJ. Natalizumab. *Curr Opin Investig Drugs* 2003;4:1354-62.

Correction

With regard to the February 2005 article entitled "Association of defensin β -1 gene polymorphisms with asthma" (2005;115:252-8): In Table IV, the heading for columns 5, 7, and 9 appears incorrectly as *T:U ratio*. The heading for these columns should have appeared as *S:E(S) ratio*, referring to the observed and expected scores for the FBAT test statistics. While in the original publication the authors did incorrectly state that the values referred to the ratio of transmitted to untransmitted minor alleles for each locus, both the direction of association and the levels of significance for each analysis were reported correctly, and this error does not in any way alter the authors' interpretation of their findings or their conclusions.