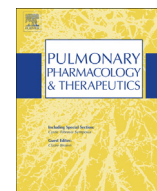




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journal homepage: www.elsevier.com/locate/ypuptEffects of JNJ-40929837, a leukotriene A₄ hydrolase inhibitor, in a bronchial allergen challenge model of asthmaW. Barchuk^{a,*}, J. Lambert^b, R. Fuhr^c, J.Z. Jiang^d, K. Bertelsen^e, A. Fourie^a, X. Liu^a, P.E. Silkoff^f, E.S. Barnathan^f, R. Thurmond^a^a Immunology, Janssen Research & Development, LLC, San Diego, CA, USA^b Early Phase Clinical Unit, PAREXEL International, Harrow, UK^c Early Phase Clinical Unit, PAREXEL International, Berlin, Germany^d Biostatistics, Janssen Research & Development, LLC, San Diego, CA, USA^e Clinical Pharmacology, Janssen Research & Development, LLC, Titusville, NJ, USA^f Immunology, Janssen Research & Development, LLC, Spring House, PA, USA

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ABSTRACT

Leukotriene B₄ (LTB₄) is a chemotactic mediator implicated in the pathogenesis of asthma. JNJ-40929837 is an oral inhibitor of LTA₄ hydrolase, which catalyzes LTB₄ production. We evaluated the effects of JNJ-40929837 in a human bronchial allergen challenge (BAC) model. In this double-blind, 3-period crossover study, 22 patients with mild, atopic asthma were randomized to one of three treatments per period: 100 mg/day JNJ-40929837 for 6 days followed by 50 mg/day on day 7; 10 mg/day montelukast for 6 days; and matched placebo. The BAC was performed on day 6 of each treatment period. Primary outcome was BAC-induced late asthmatic response (LAR) measured by maximal percent reduction in forced expiratory volume (FEV₁) in one second. Secondary outcomes included early asthmatic response (EAR) by maximal percent reduction in FEV₁, EAR and LAR evaluated by area under the FEV₁/time curve (AUC₀₋₂, AUC₃₋₁₀, respectively), change in baseline FEV₁ after 5-day treatment, safety, and correlation of JNJ-40929837 to the divalent cation ionophore A23187-stimulated whole blood LTB₄ levels and sputum basal LTB₄ levels. No significant differences were observed in the primary or secondary FEV₁ endpoints with JNJ-40929837 versus placebo. Compared with placebo ($n = 17$, LS mean = 27.7), there was no significant attenuation of the maximal percent reduction in the LAR FEV₁ with JNJ-40929837 ($n = 16$, LS mean = 28.6, $P = 0.63$) but montelukast ($n = 17$, LS mean = 22.6, $P = 0.01$) significantly attenuated the LAR. JNJ-40929837 substantially inhibited LTB₄ production in whole blood, decreased sputum LTB₄ levels and was well-tolerated. The number of adverse events leading to study withdrawal was the same in JNJ-40929837 and placebo groups. In conclusion, JNJ-40929837 demonstrated target engagement in blood and sputum. No significant impact in response to allergen inhalation was observed with JNJ-40929837 versus placebo.

Registration: This study is registered at ClinicalTrials.gov: NCT01241422.

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1. Introduction

Asthma is a chronic inflammatory disease characterized by variable airway obstruction, bronchial hyperresponsiveness, and airway inflammation [1,2]. In a pathway distinct from the cysteinyl leukotriene (LTC₄, LTD₄, LTE₄) synthetic pathway, the enzyme

leukotriene A₄ hydrolase (LTA₄H) catalyzes hydrolysis of LTA₄ to produce the pro-inflammatory mediator LTB₄. LTB₄ is a chemo-attractant and activator of leukocytes by binding to two different G protein-coupled receptors BLT1 and BLT2 [3,4]. Studies in mice lacking BLT1 receptors have shown that LTB₄ plays a role in eosinophil and effector T-cell recruitment, interleukin-13 production, goblet cell hyperplasia and mucus secretion, immunoglobulin E (IgE) production, and airway hyperresponsiveness to methacholine [5,6]. The importance of LTB₄ in development of airway hyperreactivity is supported by data in a primate model with the LTB₄ receptor antagonist CP-105,696, which reduced airway hyperresponsiveness (AHR) induced by multiple antigen challenges [7].

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Furthermore, the reduction of bronchial hyperresponsiveness in human asthma by the 5-lipoxygenase inhibitor, zileuton, has been attributed in part to its inhibition of LTB₄ synthesis [8].

In a murine mast cell-dependent model, a selective inhibitor of LTA₄H, JNJ-26993135, was shown to attenuate allergen-induced airway inflammation and airway hyperresponsiveness to methacholine [9]. Structurally identified as compound 12d in a previous report [10], JNJ-40929837 is an orally active, potent inhibitor of LTA₄H that has shown efficacy in several murine allergic airway inflammation models [unpublished data]. A previous single and multiple ascending-dose study demonstrated that doses of JNJ-40929837 100 mg and higher were associated with a 95% reduction in plasma LTB₄ levels over a 24 h period in an ex vivo stimulation assay; whereas 250 mg and higher doses of JNJ-40929837 were associated with moderate QTcF prolongation and moderate increases in heart rate [unpublished data]. In this proof-of-concept, phase 2a study, we hypothesized that JNJ-40929837 would attenuate the allergen inhalation challenge-induced late asthmatic response (LAR) in asthmatic patients.

2. Methods

2.1. Patients

Patients aged 18–55 years with stable, mild, atopic asthma requiring only occasional short-acting β_2 adrenergic receptor agonists (SABAs) ≤ 3 times/week and history of allergen-induced asthma symptoms were enrolled. Key inclusion criteria were forced expiratory volume in 1 s (FEV₁) $\geq 75\%$ predicted value, a positive skin prick test to grass, dust or cat allergen with a history of asthma symptoms upon exposure to indoor or outdoor allergens and, in response to bronchial allergen challenge (BAC) at the time of screening with one of these allergens, both an early asthmatic response (EAR) of $\geq 20\%$ fall in FEV₁ 0–2 h postBAC and a LAR of $\geq 15\%$ fall in FEV₁ 3–10 h postBAC. Methacholine (MCh) challenge was performed during screening but a minimum provocative concentration producing a 20% reduction in FEV₁ (PC₂₀) was not required for study entry. Key exclusion criteria were worsening of asthma or a respiratory tract infection within 6 weeks of screening, use of SABAs more than thrice per week within 4 weeks of screening, omalizumab, and allergen immunotherapy or use of long-acting beta-agonists within 6 months of screening, inhaled corticosteroids or systemic corticosteroids within 2 months of screening, anti-leukotriene agents and theophylline containing products within 2 weeks of screening. Inhaled SABAs were permitted except within 8 h before each clinic visit. Concomitant prescription/nonprescription medications, non-pharmacologic therapies, tobacco use, and caffeinated beverages were prohibited during the study.

Ethical approval for this study was obtained from ethics committees in Germany (Landesamt für Gesundheit und) and United Kingdom (NHS National Research Ethics Service, approval number 10/H0717/80). The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and applicable regulatory requirements. All patients provided written informed consent.

2.2. Study design

This was a randomized, double-blind, double-dummy, placebo-, and active-controlled, 3-period crossover study conducted at two centers (Germany and United Kingdom). Eligible patients were randomized to receive one of three different treatment sequences and each treatment was separated by a washout period of at least 14 days each (Fig. 1). The treatments were as follows: (1) 100 mg

JNJ-40929837 single dose on day 1 (morning), 50 mg bid on days 2–6 (each morning and evening, approximately 12 h apart), followed by only a morning dose of 50 mg on day 7; (2) active control, montelukast 10 mg bid on days 1–6 (evening, approximately 12 h after the morning dose); and (3) placebo bid (approximately 12 h apart) on days 1–6, followed by a morning dose on day 7. On day 1, morning dose was given in the clinic with ECG monitoring; doses on days 2–4 were taken by patients while at home and all dosing was done in the clinic on days 4–7. JNJ-40929837 dosing regimen was chosen to achieve maximal possible plasma concentrations while patients underwent ECG monitoring.

On day 1 of each treatment period, patients underwent fractional exhaled nitric oxide (FENO) measurement, spirometry (American Thoracic Society/European Respiratory Society guidelines) [11], and sputum induction (substudy only) followed by first study-drug dose, telemetry monitoring, and hourly electrocardiogram sampling through 4 h postdose. Patients took the study drug at home until returning to the clinic on day 5 for the remainder of the treatment period. On day 5 (~24 h preBAC), patients underwent FENO measurement spirometry, and sputum induction (substudy only). On day 6, FENO measurement, spirometry, and BAC (using the same cumulative allergen dose as screening, administered as bolus) were performed 2 h postdose, with FEV₁ measurements performed until 10 h postBAC to assess EAR and LAR; FENO testing was repeated at 11 h postBAC. On day 7 (~24 h postBAC), FENO measurement, spirometry, and MCh challenge were performed followed by sputum induction one hour after MCh challenge (substudy only). A follow-up visit was conducted approximately 14 days after the end of treatment period 3.

2.3. Randomization and allocation concealment

Patients were randomly assigned to 1 of 6 treatment sequences using randomly permuted blocks based on a computer-generated randomization schedule. To maintain blinding, an appropriate combination of JNJ-40929837 tablets, montelukast capsules and matching placebo tablets or capsules was used in each treatment period. Montelukast tablets were over-encapsulated because no placebo tablet was available to match the unique shape of the montelukast tablet.

2.4. Outcome measurements

The primary outcome was LAR, as measured by maximum percent decline in FEV₁ between 3 and 10 h postBAC in JNJ-40929837 group compared with placebo. Secondary outcomes were EAR, measured by maximum percent decline in FEV₁ between 0 and 2 h postBAC; EAR and LAR, measured by area under the FEV₁/time curve from 0 to 2 h postBAC (AUC_{0–2}) and from 3 to 10 h postBAC (AUC_{3–10}), respectively; change in FEV₁ from period 1

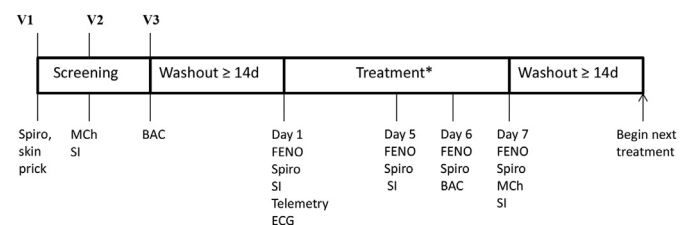


Fig. 1. Study design and assessment schedule. BAC: Bronchial allergen challenge, ECG: Electrocardiogram, FENO: Fractional exhaled nitric oxide measurement, MCh: Methacholine challenge, SI: Sputum induction, Spiro: Spirometry, V: visit. *Begin treatment with either JNJ-40929837 (day 1: 100 mg, days 2–6: 50 mg), montelukast (days 1–6: 10 mg) or placebo, according to assigned sequence.

pretreatment to day 5 of each treatment period; safety (treatment-emergent adverse events [TEAEs]); and pharmacokinetic/pharmacodynamic (PK/PD) relationship between plasma JNJ-40929837 and plasma LTB₄ levels. Exploratory outcomes included change in AHR to MCh challenge, sputum eosinophil and neutrophil counts (sputum substudy only) and FENO levels.

2.5. Laboratory procedures

2.5.1. Fractional exhaled nitric oxide (FENO) measurement

The FENO levels in exhaled air were obtained by NIOX analyzer (Aerocrine AB, Solna, Sweden). The mean of three readings was recorded.

2.5.2. Bronchial allergen challenge (BAC)

All patients underwent skin prick testing with the following allergens (Soluprick®, ALK Abelló): 6 grass mix (ALK 299), *Dermaphagoides pteronyssinus* (ALK 503) and cat hair and dander (ALK 555). Beginning in January 2011, patients were also tested with 3 tree mix (ALK 197). Sodium chloride 0.9% solution (001) was used as a negative control and histamine dihydrochloride 10 mg/mL solution (001) as a positive control. The selection of allergens for the BAC was dependent on skin prick test results and the medical history of the patient. A 100,000 SQ (IU/mL) stock solution of allergen used for the challenge was made up of one of the freeze-dried allergens used for skin testing. Dilutions of 32,000, 16,000, 800, 4000, 2000, 1000, 500 and 250 SQ units/mL were produced from the stock solution by dilution with normal saline. The challenge was supervised by an experienced physician and two personnel trained in handling emergency cardiopulmonary events. The minimum FEV₁ required to proceed with the allergen challenge was at least 70% of predicted. Normal saline and allergen were administered by nebulization using a dosimeter (Mefar MB3, Brescar, Italy). At screening, normal saline followed by increasing concentrations of allergen were inhaled as five breaths beginning with a starting concentration of 250 SQ units/mL. The FEV₁ measurements were performed after each inhalation and the lowest FEV₁ value was chosen as patient's FEV₁ in response to challenge at a given concentration. Compared to the postsaline FEV₁, if the fall in FEV₁ at a given concentration was <10%, a 4-fold increment in allergen concentration was used for the next inhalation. If the fall in FEV₁ was between 10 and 15%, a two-fold increment in allergen concentration was administered. The challenge was halted if a patient had >15% fall in FEV₁ and repeated FEV₁ measurements were taken; if FEV₁ remained <20%, the challenge was repeated using the same allergen concentration. In patients experiencing a fall in FEV₁ >20% postchallenge, repeated FEV₁ measurements were taken at 30, 45, 60 and 90 min and then every 30 min through 10 h to determine whether an EAR and LAR had occurred. One hour after the end of procedure, following an FENO measurement, patients were treated with 200 µg of salbutamol via metered dose inhaler (MDI) with a spacing device (or 2.5 mg by nebulization, if clinically warranted) to reverse bronchoconstriction. During the treatment periods, the allergen challenge procedure was similar except that a single bolus dose of allergen (calculated by adding together all the doses of allergen used in the incremental challenge at screening) was administered at the cumulative allergen concentration that caused a fall in FEV₁ ≥20% at screening.

2.5.3. Methacholine challenge

Methacholine dilutions of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 6, 8, 16 and 32 mg/mL were prepared by the site pharmacist using Provo-kit® 0.33%; CAS Number 62-51-1. Saline and methacholine were administered as a nebulized solution using a dosimeter (Mefar MB3, Brescar, Italy). At screening and on day 7 of each treatment

period, normal saline followed by doubling concentration increases in methacholine were inhaled in five breaths and FEV₁ measurements were taken 30 and 60 s later until a ≥20% fall in FEV₁ (compared to postsaline values) was achieved. The interval between subsequent increments of inhaled methacholine was approximately 3 min. Patients achieving a 20% fall in FEV₁ were treated with 200 µg of salbutamol via MDI with a spacing device (or 2.5 mg by nebulization, if clinically warranted) to reverse bronchoconstriction.

2.5.4. Sputum substudy

Induced sputum from a minimum of 12 patients enrolled in the substudy was to be analyzed for eosinophil and neutrophil counts and sputum LTB₄ concentrations. Briefly, after baseline spirometry, patients were administered 200 µg of salbutamol via MDI with a spacing device. After 15 min, the best of 3 postbronchodilator FEV₁ measurements was used to calculate baseline FEV₁ for the induction procedure. A total of three 7 min inductions were to be performed in a stepwise fashion using 3%, 4% and 5% hypertonic saline using a high-output ultrasonic nebulizer (~2.5–5 mL/min). Sputum samples were obtained by having patients perform a deep chest cough when they felt a sample was available. FEV₁ measurements were taken after each induction period. Progression to the next saline concentration occurred if the fall in postinduction FEV₁ was <10%; the same saline concentration was used for the next induction for an intermediate fall of 10–20% and the induction procedure was terminated if a 20% fall in FEV₁ was noted. At the end of the procedure, patients were assessed to determine if a bronchodilator needed to be administered.

Sputum samples were processed without dithiothreitol (DTT) for LTB₄ measurements, or with DTT for cytospin slide preparation, using the ACCUFILTER™ Sputum Processing Kit (Cellometrics). After weighing, freshly expectorated sputum was poured into a 150 mm tissue culture dish, sputum fluid transferred to a 15 mL conical tube and an equal volume of Dulbecco's phosphate buffered saline added. After rocking and centrifuging at 600 × g for 10 min at 4 °C, 500 µL aliquots of supernatant were frozen at –70 °C. LTB₄ quantitation was performed on thawed aliquots using a qualified UPLC-MS/MS method (lower limit of quantification, LLOQ: 0.2 ng/mL) [12]. Selected squamous cell-free sputum plugs (0.1–0.3 g) were placed in the ACCUFILTER™ collection vial, and a dithiothreitol working solution equal to 4× the sputum weight was added to the collection vial, and the mixture was rocked for 15 min at room temperature. A volume of phosphate buffered saline (PBS) equal to 4× the sputum weight was added and the cell suspension was filtered by gravity into the ACCUFILTER™ receiving vial. The suspension was centrifuged at 600 × g for 4 min and the cell pellet resuspended in PBS to achieve a concentration of 1 × 10⁶ cells/mL for cytospin slide preparation.

2.6. Pharmacokinetic/pharmacodynamic measurements

Blood samples were collected at various time points pre- and postdose to measure plasma JNJ-40929837 concentrations using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (Janssen Research & Development, Raritan, NJ). Heparinized blood samples were collected at various time points pre- and postdose for ex vivo stimulation by 0.17 mg/mL divalent cationic ionophore A23187 (Sigma Aldrich, Berlin, Germany) and measurement of plasma LTB₄ production. Briefly, 0.5 mL each of whole blood and RPMI-1640 medium (Hyclone SH30096.01) were mixed and 45 µL of the ionophore solution (1.7 mg/mL in dimethylsulfoxide) was added. Samples were incubated (60 min at 37 °C) in a humidified incubator under 5% CO₂, centrifuged (10 min at 530 × g at 4 °C), and 500 µL supernatant aliquots were removed and stored frozen at –70 °C. LTB₄ was quantitated by a selective

ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS) method using leukotriene B₄-D₄ as an internal standard (LLOQ) of 0.2 ng/ml [13]. Serum samples were collected for exploratory analysis of serum IgE levels.

Spot urine samples were collected for urine LTE₄/creatinine ratio analysis. Urine samples (100 µl) were processed by a liquid–liquid extraction and LTE₄ was quantified by Reverse-phase high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS); LLOQ was 0.015 ng/ml for LTE₄.

2.6.1. PK/PD analysis

For analysis of all dose and exposure response relationships, the Hill equation [14] was to be applied to the ex vivo LTB₄ stimulation assay data using the following equation:

$$E = E_{\max} * [1 - (C / (C + EC_{50}))],$$

where $E_{\max}$ (maximal response), EC_{50} (plasma drug concentration resulting in 50% of maximum response) were the parameters to be estimated, and C was the plasma concentration of JNJ-40929837. Weighted ($1/y^{\text{hat}}$) values were used to further optimize the model fit via visual inspection and to limit the variations around fitted parameters (WinNonlin, Version 5.2). To assess the inhibition of LTB₄ production, blood was stimulated ex vivo with ionophore and the plasma levels of LTB₄ were quantitated.

2.7. Statistical analysis

Approximately 18 patients (3 per sequence) were planned to ensure ≥ 14 patients complete the study, which would provide 80% power to detect a 10% difference in LAR between JNJ-40929837 (mean, standard deviation [SD]: 7.8 [9.9]), as in an earlier study with montelukast and placebo (17.8 [13.8]), at a significance level of 0.05 (2-sided) using a paired t -test [15]. No formal calculations were done for the sputum substudy sample size.

Efficacy analyses included data from all patients during all treatment periods during which a patient received at least one study-drug dose and had ≤ 1 calculable value. The primary endpoint was compared between JNJ-40929837/montelukast and placebo using a mixed-effect analysis of covariance (ANCOVA) model that included treatment, sequence, and period as fixed factors; patients nested within sequence as a random factor; and the LAR at screening as a baseline covariate. Analyses for MCh provocative concentration producing at least a 20% reduction in FEV₁ (PC_{20}), sputum eosinophil/neutrophil counts, and FENO were based on log-transformed data; percent change in geometric means (GM) was expressed in original scale via antilogarithm. Efficacy data were presented as least square (LS) mean $\pm$ standard error (SE).

3. Results

A total of 22 patients were enrolled from October 2010 to June 2011 at 2 sites in the United Kingdom and Germany (JNJ-40929837 [$n = 17$]; montelukast [$n = 18$] and placebo [$n = 19$]; Table 1). Seven patients were withdrawn from the study (withdrawal of consent: 1, study protocol violation [positive alcohol screen]: 1, adverse event during a treatment period: 5). Fifteen patients completed all three treatment periods. Study enrollment took longer than expected and necessitated conducting the study partly during the tree allergy season, which resulted in a protocol amendment being issued in January 2011 to allow skin prick testing in order to exclude tree-sensitive patients.

Table 1
Patient demographics.

	Placebo ($n = 19$)	JNJ-40929837 ($n = 17$)	Montelukast ($n = 18$)
Sex, n (%)			
Men	8 (42)	8 (47)	9 (50)
Women	11 (58)	9 (53)	9 (50)
Race, n (%)			
White	15 (79)	14 (82)	15 (83)
Multiple	2 (11)	2 (12)	2 (11)
Black or African American	1 (5)	1 (6)	1 (6)
Asian	1 (5)	0	0
Age (years), mean (SD)	28.2 (8.42)	28.5 (8.59)	28.7 (8.44)
Weight (kg), mean (SD)	71 (13.5)	73 (11.5)	76 (14.1)
Allergen used, n (%)			
Cat dander	8 (42)	6 (35)	7 (39)
Grass mix	5 (26)	5 (29)	6 (33)
House dust mite	6 (32)	6 (35)	5 (28)
Baseline FEV₁, % predicted	86.97	82.96	85.60
PC₂₀ (mg/mL)^a	2.01 (25.8)	2.22 (27.9)	2.07 (25.0)
FENO (ppb)	41.9 (15.7)	40.7 (15.2)	38.5 (15.3)
Sputum cell counts^a			
Neutrophils			
Absolute ($\times 10^6$ /g)	0.04 (50.5)	0.04 (39.2)	0.03 (57.1)
Percent (%)	2.21 (44.9)	2.64 (30.5)	2.08 (50.2)
Eosinophils			
Absolute ($\times 10^6$ /g)	0.02 (91.0)	0.02 (85.0)	0.03 (95.7)
Percent (%)	1.25 (82.8)	1.46 (77.3)	1.62 (87.4)

FENO: Fractional exhaled nitric oxide measurement, FEV₁: Forced forced expiratory volume in 1 s, ppb: parts per billion, PC₂₀: Provocative concentration producing at least a 20% reduction in FEV₁.

^a Data presented as geometric mean (CV).

3.1. Allergen-induced asthmatic responses

Fig. 2 presents the percent change in FEV₁ at each time point for each treatment.

3.1.1. Primary outcome

3.1.1.1. LAR. The LS mean (SE) of JNJ-40929837 did not demonstrate a significant ($P = 0.630$) difference in LAR versus placebo (Table 2). The LAR was significantly ($P = 0.014$) attenuated with montelukast versus placebo.

3.1.2. Secondary outcomes

3.1.2.1. EAR. The EAR with JNJ-40929837 was not significantly ($P = 0.963$) different versus placebo, but was significantly ($P < 0.001$) attenuated by montelukast versus placebo (Table 2).

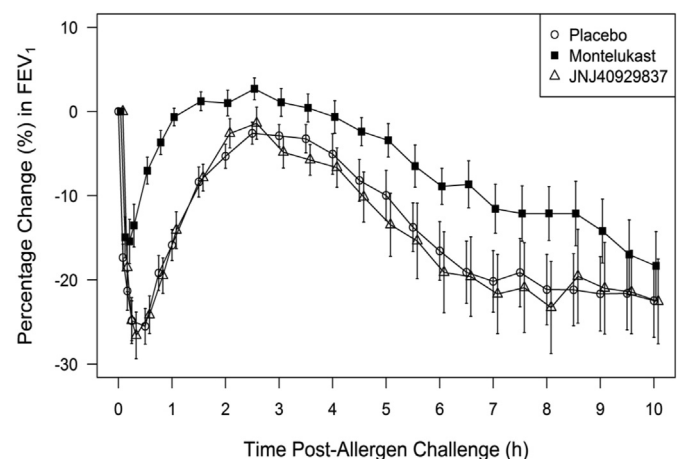


Fig. 2. Time course of the percentage change (mean $\pm$ standard error) in FEV₁ after allergen challenge in each treatment period. FEV₁: Forced expiratory volume in 1 s.

Table 2

Primary and secondary efficacy analyses results.

	Placebo	JNJ-40929837	P-value (vs. placebo)	Montelukast	P-value (vs. placebo)
	LS mean (SE)	LS mean (SE)		LS mean (SE)	
LAR	27.7 (3.08)	28.6 (3.10)	0.630	22.6 (3.07)	0.014
EAR	29.6 (2.39)	29.7 (2.40)	0.963	17.8 (2.42)	<0.001
AUC ₀₋₂	31.1 (2.77)	28.1 (2.82)	0.371	7.53 (2.74)	<0.001
AUC ₃₋₁₀	103.0 (17.28)	98.4 (17.72)	0.749	61.21 (17.16)	0.006
FEV ₁ (day1–day5)	1.99 (1.87)	3.59 (1.88)	0.38	5.08 (1.90)	0.09

AUC: Area under the curve; EAR: Early asthmatic response; FEV₁: Forced forced expiratory volume in 1 s; LAR: Late asthmatic response; LS: least square; SE: standard error.

3.1.2.2. Area under the curve (AUC) analyses. No attenuation of the EAR was observed with JNJ-40929837 compared with placebo ($P = 0.371$), in contrast to the AUC₀₋₂ for montelukast, which was significantly smaller compared with placebo ($P < 0.001$) (Table 2). Treatment with JNJ-40929837 led to no significant difference in AUC₃₋₁₀ versus placebo ($P = 0.749$) representing no attenuation of LAR, while in contrast, montelukast treatment led to significant ($P = 0.006$) decrease in AUC₃₋₁₀ compared to placebo.

3.1.2.3. Change in FEV₁ from day 1 to day 5. No significant changes were observed in percent predicted FEV₁ from period 1 day 1 predose to day 5 (24 h preBAC) of each treatment period for JNJ-40929837 versus placebo ($P = 0.38$) or montelukast versus placebo ($P = 0.09$) (Table 2).

3.1.3. Exploratory analyses

3.1.3.1. Airway reactivity to methacholine. Compared with the PC₂₀ obtained during screening, airway reactivity worsened in all treatment groups after BAC. The PC₂₀ expressed as GM (range) assessed 24 h postBAC decreased from the screening baseline for placebo, JNJ-40929837, and montelukast (Table 3). The decrease in methacholine PC₂₀ was not significantly different between JNJ-40929837 and placebo ($P = 0.89$) but was significantly different between montelukast and placebo ($P = 0.04$) showing that montelukast attenuated the worsening of airway reactivity seen postBAC.

3.1.3.2. Exhaled nitric oxide (NO). Neither JNJ-40929837 nor montelukast demonstrated a significant improvement in FENO (expressed as GM) from just before the allergen challenge on day 6–11 h postBAC (JNJ-40929837 vs. placebo $P = 0.30$, montelukast vs. placebo $P = 0.49$) or just before BAC to 24 h postBAC on day 7 (JNJ-40929837 vs. placebo $P = 0.64$, montelukast vs. placebo $P = 0.58$) (Table 3). Also, JNJ-40929837 and montelukast treatment were associated with non-significant increases in GM FENO levels from day 1 predose to day 5 (24 h preBAC) vs. placebo (JNJ-

40929837 vs. placebo $P = 0.14$, montelukast vs. placebo $P = 0.42$) (Table 3).

3.1.3.3. Induced sputum. Of the 13 patients enrolled in the sputum substudy, patient samples available were as follows– 1. Both 24 h pre- and postBAC: placebo (10), JNJ-40929837 (7), and montelukast (5) and, 2. A pretreatment and a day 5 sample: placebo (10), JNJ-40929837 (10), and montelukast (9). JNJ-40929837 was associated with a slight numerical decrease in absolute eosinophil and neutrophil counts from day 5 (24 h preBAC) to day 7 (24 h postBAC) and in absolute eosinophil counts from day 1 predose to day 5 (24 h preBAC). Montelukast showed a numerical decrease in absolute eosinophil and neutrophil counts from day 5 (24 h preBAC) to day 7 (24 h postBAC) but no change was observed in either from day 1 predose to day 5 (24 h preBAC). However, compared with placebo, neither treatment demonstrated a significant reduction in sputum eosinophil nor neutrophil counts (Table 4).

3.1.3.4. PK/PD analyses. Following JNJ-40929837 administration, ex vivo A23187-stimulated plasma LTB₄ concentrations decreased from 20.8 ± 19.13 ng/mL at baseline to 0.4 ± 0.29 ng/mL at 2 h postdose, and remained suppressed throughout the 7-day treatment period (ranging from 0.5 to 1.7, Fig. 3A). Treatment with JNJ-40929837 led to significant difference from baseline in LTB₄ at 2 h postdose (treatment difference, -15.5 ± 3.33 ; $P < 0.001$) compared with placebo. Significant differences were observed at all time points throughout day 7.

The observed mean (SD) plasma JNJ-40929837 concentrations 2-h post-dose after oral administration of JNJ-40929837 were as: day 1, 318 (58.9); day 5, 234 (76.1); day 6, 219 (41.3) and day 7, 251 (82.7) ng/mL (Fig. 3B). Analysis of exposure–response relationships between individual ex vivo LTB₄ plasma concentration versus JNJ-40929837 plasma concentration data resulted in an estimated EC₅₀ of 4.2 ng/mL (Fig. 3C).

For patients in the sputum substudy, significant decreases in the GM of sputum LTB₄ concentrations were noted from day 1 (predose) to day 5 (24 h preBAC), 92% ($P < 1.6 \times 10^{-6}$) and from day 1

Table 3

Exploratory analyses results.

	Placebo	JNJ-40929837	P-value (vs. placebo)	Montelukast	P-value (vs. placebo)
	GM (range)	GM (range)		GM (range)	
MCh PC ₂₀					
Baseline	2.01 (0.47, 14.4)	2.22 (0.47; 14.4)		2.07 (0.47; 14.4)	
24 h postBAC	0.85 (0.12; 16.1)	0.87 (0.16; 16.6)	0.89	1.36 (0.35; 6.47)	0.04
FENO					
PreBAC on day 6	44 (12; 177)	48 (18; 194)		39 (14; 141)	
11 h postBAC	45 (13; 156)	49 (19; 170)	0.30	41 (13; 130)	0.49
24 h postBAC on day 7	65 (17; 192)	72 (19; 224)	0.64	59 (17; 193)	0.58
Predose on day 1	42 (13; 151)	41 (13; 113)		38 (13; 113)	
24 h preBAC on day 5	41 (11; 178)	44 (18; 199)	0.14	40 (16; 150)	0.42

FENO: Fractional exhaled nitric oxide measurement; BAC: Bronchial allergen challenge; GM: geometric mean; MCh: Methacholine challenge; PC₂₀: Provocative concentration producing at least a 20% reduction in FEV₁.

Table 4

Absolute and percent total sputum eosinophil and neutrophil counts from pretreatment to day 5 and preallergen to day 7.

	Placebo	JNJ-40929837		Montelukast	
	GM (range)	GM (range)	P-value (vs. Placebo)	GM (range)	P-value (vs. Placebo)
Eosinophils					
<i>Baseline</i>					
Absolute number of cells	0.02 (0; 0.63)	0.02 (0; 0.39)		0.02 (0; 0.42)	
% Total	1.19 (0.11; 16.3)	1.29 (0.12; 22.8)		1.78 (0.12; 28.7)	
<i>Day 5 (preBAC)</i>					
Absolute number of cells	0.04 (0; 0.80)	0.01 (0; 0.06)	0.44	0.02 (0; 0.15)	0.67
% Total	2.03 (0.11; 86.5)	0.93 (0.11; 7.35)	0.57	0.98 (0.12; 4.92)	0.69
<i>Day 7 (postBAC)</i>					
Absolute number of cells	0.21 (0; 6.86)	0.07 (0; 0.60)	0.70	0.03 (0; 0.61)	0.39
% Total	7.26 (0.12; 54.5)	3.11 (0.21; 17.7)	0.86	2.88 (0.72; 16.5)	0.10
Neutrophils					
<i>Baseline</i>					
Absolute number of cells	0.04 (0; 0.19)	0.02 (0; 0.19)		0.02 (0; 0.27)	
% Total	2.63 (0.12; 14.16)	1.28 (0.12; 15.5)		2.20 (0.12; 13.32)	
<i>Day 5 (preBAC)</i>					
Absolute number of cells	0.05 (0; 0.19)	0.02 (0; 0.15)	0.56	0.02 (0; 49.4)	0.86
% Total	2.47 (0.12; 10.6)	1.34 (0.12; 13.3)	0.40	1.18 (0.12; 82.3)	0.66
<i>Day 7 (postBAC)</i>					
Absolute number of cells	0.08 (0.01; 1.08)	0.07 (0.00; 1.28)	0.603	0.05 (0.01; 0.39)	0.40
% Total	2.82 (0.12; 14.4)	3.39 (0.24; 27.4)	0.510	4.30 (0.75; 17.3)	0.78

Absolute number of cells ($\times 10^6/g$); BAC: Bronchial allergen challenge; GM: Geometric mean; P-values are for comparisons of % change from day 1 to day 5 and from day 5 to day 7 between placebo and active drugs.

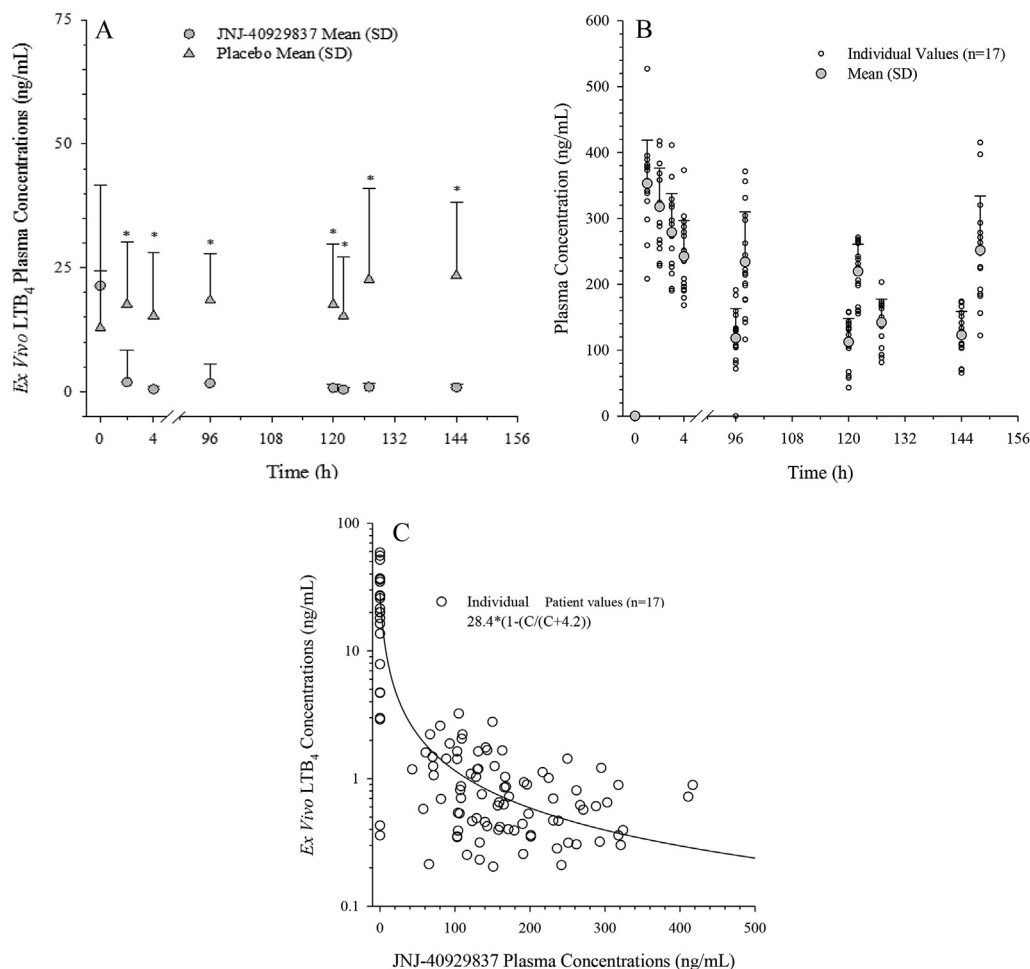


Fig. 3. PK/PD modeling of the effect on plasma LTB₄ concentrations following study treatment. (A) Ex vivo plasma LTB₄ concentrations (X-axis represents time after first dose; * $P < 0.001$). (B) JNJ-40929837 plasma concentrations (X-axis represents time after first dose). (C) Exposure–response relationship between ex vivo plasma LTB₄ concentrations and JNJ-40929837 concentrations.

(predose) to day 7 (24 h postBAC), 92% ($P < 0.00012$), after treatment with JNJ-40929837. In contrast, no significant changes in sputum LTB₄ concentrations were observed after treatment with montelukast or placebo (Fig. 4A, B).

The urinary excretion of LTE₄ is a marker of cysteinyl leukotriene production. Compared with placebo, where GMs of urinary LTE₄/creatinine ratios (Fig. 5) did not change significantly from day 1 to day 5 (24 h preBAC), +23%, $P = 0.063$; and day 6 (just before BAC), and -2%, $P = 0.81$, treatment with JNJ-40929837 showed significant increases at both time points (+87%, $P = 0.0031$ and +58%, $P = 0.0024$, respectively). Treatment with montelukast showed a trend for a decrease from day 1 to day 5 (-12%, $P = 0.27$) and a significant decrease from day 1 to day 6 (-37%, $P = 0.038$). After BAC on day 6, the LTE₄/creatinine ratio increased for all groups and there was no significant difference between the groups. However, at later time points the ratios in patients receiving placebo or montelukast quickly dropped to the level prior to BAC, but a significant delay was seen in patients receiving JNJ-40929837.

From day 1 (predose) to day 7 (24 h postBAC), mean baseline serum IgE levels decreased by 0.21 nmol/L from 2.20 nmol/L for JNJ-

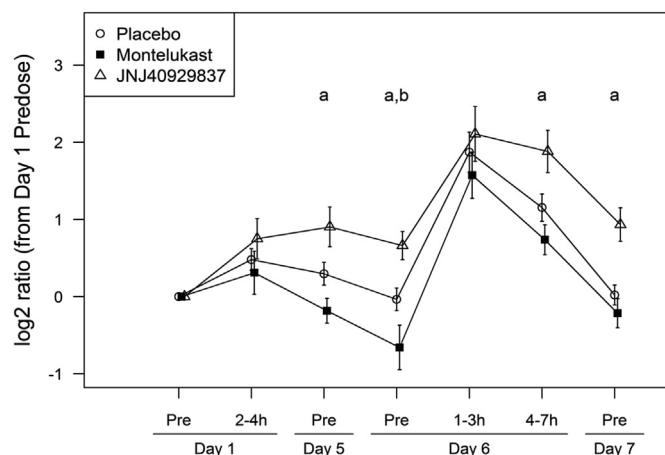


Fig. 5. Change in urinary LTE₄/creatinine levels following treatment. Change in urinary LTE₄/creatinine levels following treatment. At each time point (after day 1 predose), effect of active treatments (JNJ40929837 or montelukast) against the placebo were evaluated using linear mixed effect model, where treatment effects within each patients were considered as fixed effects and variations among patients were considered as a random effect. P value less than 0.05 was indicated as (a) for effect of JNJ40929837, and (b) for montelukast.

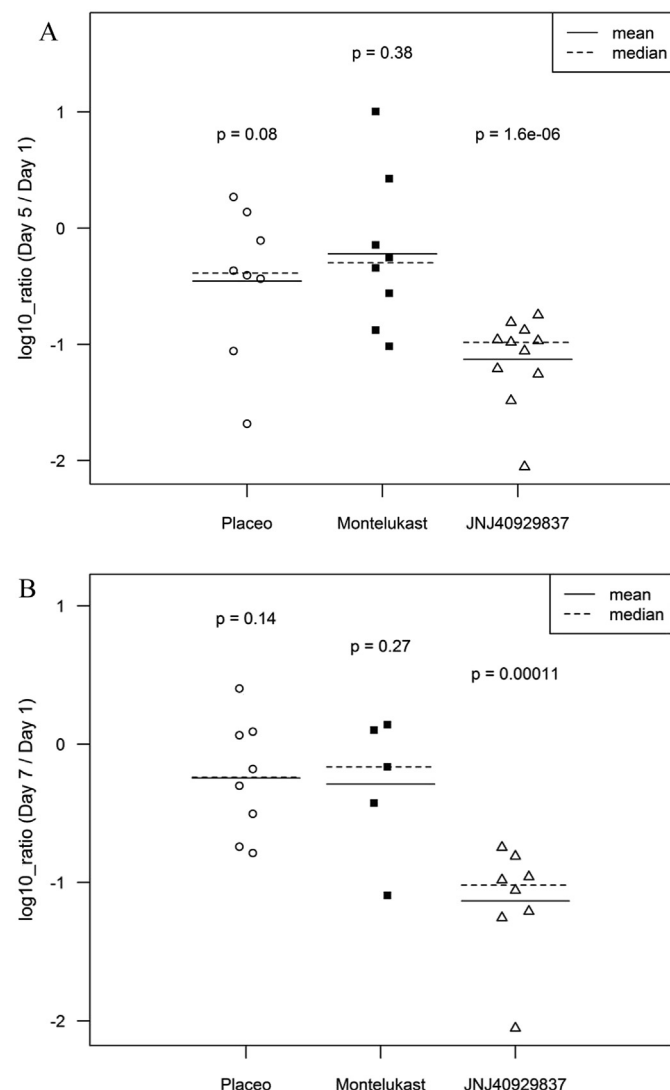


Fig. 4. Change in sputum LTB₄ concentration following treatment (A) from day 1 to day 5, and (B) from day 1 to day 7. For each treatment arm, a P value is calculated based on paired t test between day 5 and day 1 (A), and between day 7 and day 1 (B).

40929837, and 0.19 nmol/L from 2.24 nmol/L for montelukast, compared with 0.08 nmol/L from 2.27 nmol/L for placebo. Compared with placebo, the decreases in IgE levels were not significant in either the JNJ-40929837 or montelukast groups.

3.2. Safety

Adverse events leading to study withdrawal included clinically unacceptable large decreases in FEV₁ during the EAR in 2 placebo-treated patients and 1 JNJ-40929837-treated patient, a clinically unacceptable large decrease in FEV₁ during the LAR in 1 JNJ-40929837-treated patients and one event of tonsillitis in a montelukast-treated patient. Overall, adverse events were reported in 12 (71%) patients with JNJ-40929837, 10 (56%) patients with montelukast, and 11 (58%) patients with placebo; the number of individual adverse events was similar between the JNJ-40929837 and placebo groups (Table 5). Most adverse events were short-lived and were of mild or moderate severity except for the large decrease in FEV₁ during the EAR in the placebo group that led to study withdrawal, which was considered severe. No serious adverse events or deaths occurred. No clinically significant changes were observed in lab values, vital signs or physical examination abnormalities across treatment groups. Treatment with JNJ-40929837 100 mg loading dose on day 1 led to clinically significant increase in heart rate about 11 beats/minute around time of maximum plasma JNJ-40929837 concentrations, although this effect was not seen at steady-state on day 5; there was no apparent effect on the QTcF interval.

4. Discussion

In this study, JNJ-40929837 was clearly shown to engage its target, and reduced ionophore stimulated plasma LTB₄ levels by at least 80% throughout the treatment period, and sputum LTB₄ levels by at least 90% prior to the BAC. However, JNJ-40929837 failed to attenuate the LAR or EAR following BAC. Preclinical studies of an LTA₄H inhibitor in a murine model of asthma suggested that reducing LTB₄ production might be effective in treating human asthma because dose-dependent inhibition of airway inflammation and airway hyperreactivity were demonstrated [9]. JNJ-40929837

Table 5

Most common treatment emergent adverse events in ≥ 2 patients in any treatment group (Safety analysis set).

	Placebo (n = 19) n (%)	JNJ-40929837 (n = 17) n (%)	Montelukast (n = 18) n (%)
Headache	4 (21.1)	4 (23.5)	3 (16.7)
Nasopharyngitis	3 (15.8)	2 (11.8)	2 (11.1)
Diarrhea	2 (10.5)	2 (11.8)	1 (5.6)
Dizziness postural	2 (10.5)	1 (5.9)	0
Nausea	1 (5.3)	2 (11.8)	1 (5.6)
Dyspnea ^a	1 (5.3)	2 (11.8)	0
Oropharyngeal pain	1 (5.3)	2 (11.8)	0
Asthma ^a	0	2 (11.8)	0

^a One event in the JNJ-40929837 arm led to study withdrawal.

did not significantly attenuate eosinophilic and neutrophilic inflammation but interpretation of this data is limited, since relatively few patients were able to produce adequate sputum samples in this study. Moreover, JNJ-40929837 did not attenuate postBAC worsening of airway reactivity. Although specialized techniques such as barometric whole-body plethysmography and increases in enhanced pause [16], low-frequency forced oscillation [17] and respiratory input impedance [18] were not used to study allergen-induced bronchoconstriction in our murine models of LTB₄ inhibition, the failure of reduced LTB₄ production to attenuate the EAR and LAR following human BAC due to the absence of significant effects of JNJ-40929837 on sputum cell counts and airway reactivity, is perhaps not unexpected.

Treatment with JNJ-40929837 in this study led to elevation in urinary LTE₄ levels prior to the BAC, although the magnitude of this effect did not worsen after the challenge. This was likely the result of shunting of LTA₄ into the cysteinyl leukotriene synthesis pathway, due to inhibition of LTA₄ hydrolase by JNJ-40929837. This increase in cysteinyl leukotriene production may have contributed to the lack of efficacy seen in this model. Montelukast treatment was associated with a decrease in urinary LTE₄ levels in this study, an effect that has been seen in several other studies [19,20]. A decrease in urinary LTE₄ levels with montelukast would in principle be unexpected since montelukast is a cysteinyl leukotriene receptor antagonist and should not affect LTE₄ synthesis. This phenomenon may represent a more general anti-inflammatory effect of the molecule or a feedback mechanism.

The safety profile of JNJ-40929837 in this study was favorable. The number of individual adverse events was similar in JNJ-40929837 and placebo arms, and the number of adverse events (large falls in FEV₁ during the BAC procedure) leading to study withdrawal in the two groups was similar. While tachycardia was seen after loading dose administration, this effect was not sustained at steady state. Given the QT prolongation seen in phase I studies and the tachycardia seen in this study, the ECG effects of JNJ-40929837 should be further characterized.

Previously, a clinical study with a LTB₄ receptor antagonist, LY293111, did not demonstrate any effects on the EAR and LAR in a BAC model, although a significant decrease in neutrophils in the BAL was observed [21]. These EAR and LAR results are consistent with our results. LY293111 was synthesized before the discovery of an immunologically important second LTB₄ receptor in 2000 [22], and it is unlikely that the effects of LTB₄ at both receptors were completely abrogated by that molecule. This study of an LTA₄ hydrolase inhibitor, which, in contrast to receptor blockade, inhibits LTB₄ production, provides clearer evidence that targeting LTB₄ is not effective in a human model of allergen-induced bronchoconstriction.

Attenuation of the EAR and LAR in the BAC model has been demonstrated with the cysteinyl leukotriene receptor inhibitor

montelukast [15,23–25] as well as with the 5-lipoxygenase activating protein (FLAP) inhibitors BAYx-1005 and MK-886 [26,27], which inhibit both cysteinyl leukotriene and LTB₄ production. The findings of the montelukast BAC studies taken together with those of our study of a pure LTB₄ production inhibitor, suggest that the effects of FLAP inhibitors in the BAC model are largely due to their inhibition of the cysteinyl leukotriene pathway.

Although targeting LTB₄ in this BAC study was not successful, a negative BAC result should not rule out further study of JNJ-40929837 in chronic asthma as target engagement in blood and sputum, and reductions in sputum neutrophil counts, albeit not significant, were observed. The role of LTA₄ hydrolase inhibitors in severe asthma and perhaps other respiratory diseases characterized by sputum neutrophilia, such as chronic obstructive pulmonary disease and cystic fibrosis, should be explored [28–30]. This is supported by the fact that the 5-lipoxygenase inhibitor zileuton is an option for the treatment of severe asthma that is aspirin-sensitive [31,32], though data are lacking in the non-aspirin-sensitive severe asthma population. Caution should be exercised, however, since recent data in mice have shown that LTA₄H plays a role in degradation of the neutrophil chemoattractant, proline-glycine-proline (PGP), in acute pulmonary inflammation [33], although the contribution of PGP to chronic human neutrophilic asthma, and its potential degradation by peptidases other than LTA₄H, is currently unclear. Consideration should be given to combining JNJ-40929837 with a cysteinyl leukotriene receptor antagonist in future studies, to protect against leukotriene shunting along the cysteinyl leukotriene pathway, which could potentially exacerbate asthma.

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Previous presentations

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