Trial of thromboxane receptor inhibition with ifetroban: TP receptors regulate eicosanoid homeostasis in aspirin-exacerbated respiratory disease

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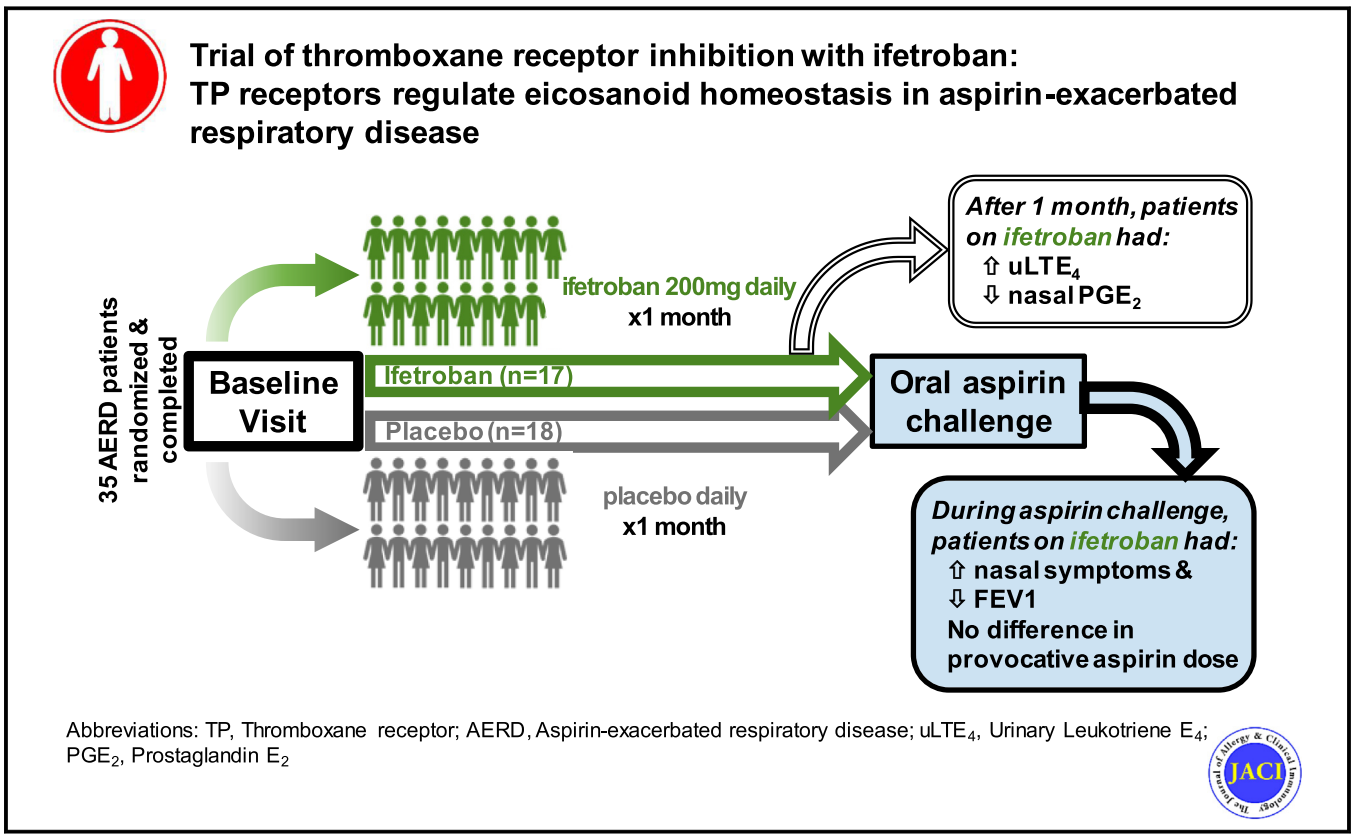
GRAPHICAL ABSTRACT



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Background: Aspirin-exacerbated respiratory disease (AERD) is the triad of asthma, nasal polyposis, and respiratory reactions to COX-1 inhibitors. Overproduction of cysteinyl leukotrienes and underproduction of prostaglandin E_2 (PGE_2) are hallmarks of AERD. A mouse model predicted a key role for the thromboxane-prostanoid (TP) receptor in AERD.

Objective: Our aim was to determine whether ifetroban, a TP receptor antagonist, attenuates aspirin-induced respiratory symptoms in patients with AERD.

Methods: A total of 35 patients with AERD completed a 4-week double-blinded, placebo-controlled trial of ifetroban and underwent an oral aspirin challenge. The primary outcome was change in the provocative dose of aspirin that caused a 2-point increase in Total Nasal Symptom Score. Changes in lung function, eicosanoid levels, and platelet and mast cell activation were assessed. Cultured human nasal fibroblasts were stimulated with or without the TP agonist U46619 and assayed for prostanoid production.

Results: Ifetroban was well tolerated in AERD and did not change the mean 2-point increase in Total Nasal Symptom Score ($P = .763$). Participants taking ifetroban had greater aspirin-induced nasal symptoms and a greater decline in FEV_1 value than did participants receiving placebo (–18.8% ± 3.6% with ifetroban vs –8.4% ± 2.1% with placebo [$P = .017$]). Four weeks of ifetroban significantly increased urinary leukotriene E_4 levels and decreased nasal PGE_2 levels compared with placebo. Peak aspirin-induced urinary thromboxane levels correlated with peak urinary leukotriene E_4 and prostaglandin D_2 metabolite levels in participants taking ifetroban. U46119 significantly potentiated the production of PGE_2 by cultured nasal fibroblasts from subjects with AERD but not by cultured nasal fibroblasts from controls without polypoid sinusitis.

Conclusion: Contrary to our hypothesis, TP receptor blockade worsened aspirin-induced reactions in AERD, possibly by exacerbating dysregulation of the eicosanoid system. TP signaling on stromal cells may be critical to maintaining PGE_2 production when COX-2 function is low. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)

Key words: *Aspirin-exacerbated respiratory disease, AERD, mast cell, ifetroban, platelet, thromboxane receptor, Samter triad*

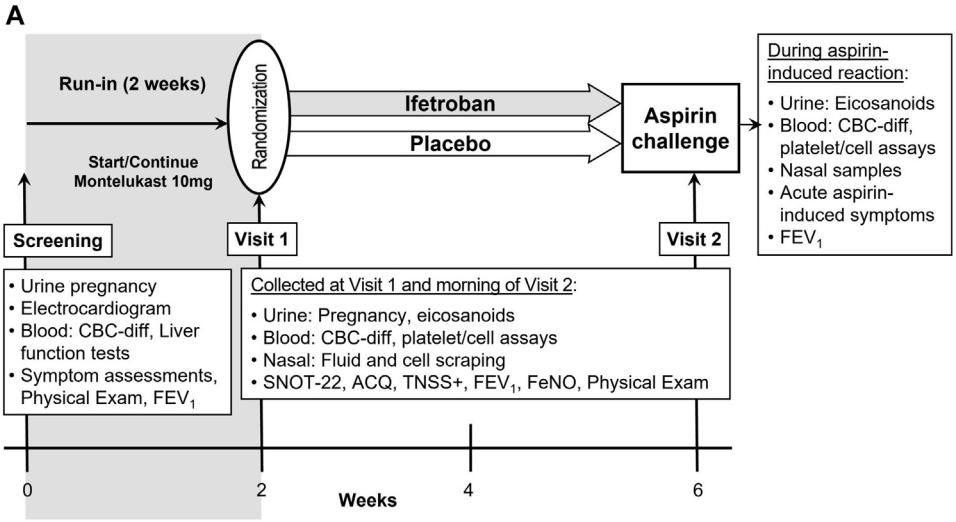
Abbreviations used

AERD:	Aspirin-exacerbated respiratory disease
CR:	Creatinine
CRSsNP:	Chronic rhinosinusitis without nasal polyposis
CysLT:	Cysteinyl leukotriene
EP_2:	E prostanoid 2
HMGB1:	High mobility group box 1
6-keto $PGF_{1\alpha}$:	6-Keto prostaglandin $F_{1\alpha}$
5-LO:	5-Lipoxygenase
LTE_4:	Leukotriene E_4
PD2:	2-Point increase in Total Nasal Symptom Score
PGD_2:	Prostaglandin D_2
PGE_2:	Prostaglandin E_2
PGI_2:	Prostaglandin I_2
PTGES:	Prostaglandin E synthase
PTGIS:	Prostaglandin I2 synthase
TBXA2R:	Thromboxane A2 receptor
TNSS+:	Extended version of the Total Nasal Symptom Score
TP:	Thromboxane-prostanoid
TXA_2:	Thromboxane A_2
TXB_2:	Thromboxane B_2

Aspirin-exacerbated respiratory disease (AERD) is a chronic inflammatory respiratory syndrome defined clinically by asthma, chronic rhinosinusitis with nasal polyposis, and respiratory reactions to medications that inhibit COX-1, affecting approximately 14% of adults with severe asthma.[1] Cysteinyl leukotriene (cysLT) overproduction is a hallmark of AERD, both at baseline and during the COX-1 inhibitor–induced reactions, which results in a further surge in cysLTs, accompanied by release of other mast cell activation products, including prostaglandin D_2 (PGD_2). CysLTs and PGD_2 are powerful bronchoconstrictors and likely contribute to vascular leak and tissue eosinophilia. Inhibition of 5-lipoxygenase (5-LO), an enzyme responsible for cysLT generation, improves basal symptoms and lung function in AERD while attenuating the severity of reactions to aspirin.[2,3] The overproduction of cysLTs and its surge during clinical reactions are both thought to reflect insufficient production of COX-derived prostaglandin E_2 (PGE_2),[4] which suppresses 5-LO activity in myeloid cells and inhibits mast cell activation[5,6] and to reflect diminished expression of Gs-linked E prostanoid 2 (EP_2) receptors, which are responsible for these effects. The mechanisms responsible for the impaired function of the COX-2/PGE_2/EP_2 receptor system in AERD are incompletely understood and fundamental to disease pathogenesis.

Thromboxane-prostanoid (TP) receptors recognize not only thromboxane A_2 (TXA_2), the major platelet-derived COX product, with high affinity, but also bind other COX products, including stable PGD_2 metabolites[7] and F-series isoprostanes that are nonenzymatic products of oxidative stress. TP signaling leads to constriction of human and rodent bronchi by both neurally mediated pathways[8,9] and direct smooth muscle stimulation.[10] Multiple prostaglandins, including PGD_2, prostaglandin F_2, and high-dose PGE_2, require TP receptors to contract human bronchi *ex vivo*; TP receptors are widely expressed on both stromal and immune cell types.[11,12] Specifically, platelet-derived TXA_2 autoamplifies platelet aggregation, can activate TP receptors on endothelial cells to drive adhesion receptor expressions that facilitate leukocyte recruitment,[13] and can also induce endothelial cells to upregulate COX-2 and generate both prostaglandin I_2 (PGI_2) and PGE_2.[14] We previously found high levels of activated platelets and platelet-adherent granulocytes in the blood and sinonasal tissues of patients with AERD.[15] Moreover, we demonstrated that TP receptor blockade inhibited all features of aspirin reactions, including mast cell activation and cysLT production, in a mouse model of AERD.[16] Collectively, these observations suggested a role for TP receptor signaling at multiple control points in AERD, as well as a potential utility for therapeutic TP antagonism in this context.[17]

This placebo-controlled trial aimed to test the hypothesis that ifetroban, an oral highly selective TP receptor antagonist, would attenuate the severity of sinonasal and respiratory symptoms induced during aspirin challenges in patients with AERD by blocking platelet activation, reducing cysLT generation, and reducing aspirin-induced bronchoconstriction. We found ifetroban to be well tolerated in patients with AERD; surprisingly, however, we found that TP antagonism significantly increased the severity of reactions to aspirin, likely by further perturbing the



B

	Baseline	Minutes after reaction onset						
		0	30	60	90	120	150	180
Vital Signs	✓				✓			✓
TNSS+	✓	✓	✓	✓	✓	✓	✓	✓
FEV_1	✓	✓			✓			✓
Nasal fluid	✓	✓		✓				
Urine	✓	✓			✓			✓
Blood draw	✓			✓				

FIG 1. Detailed trial schematic. **A,** Study schematic showing time line and schedule of assessments. **B,** Table of time points at which assessments were performed on the day of the aspirin challenge. *ACQ,* Asthma Control Questionnaire; *CBC-diff,* Complete blood count and differential; *SNOT-22,* 22-Item Sino-Nasal Outcome Test.

ability to maintain homeostatic synthesis of anti-inflammatory prostanoids. Our findings implicate TP-active prostaglandins in a hierarchy with PGE_2 and lend new insight into the "unbraking" phenomenon induced by COX-1 inhibition that is unique to AERD.

METHODS

Study design

Participants were enrolled in a 6-week, double-blinded, placebo-controlled trial of ifetroban. At the end of a 4-week treatment phase (ifetroban or placebo) each participant underwent a graded oral aspirin challenge and desensitization procedure (Fig 1, *A*) to initiate high-dose aspirin therapy.

Participants

The patients with AERD had a history of physician-diagnosed asthma, chronic rhinosinusitis with nasal polyposis, and at least 2 respiratory reactions to aspirin or another nonselective COX inhibitor, with features of upper and/or lower airway involvement. The patients had stable asthma, defined as a postbronchodilator FEV_1 value of 70% of predicted or better, no oral glucocorticoids for at least 2 weeks before the first study visit, and no history of hospitalization or emergency room visits due to asthma for at least 6 months before enrolling in the study.

Patients were excluded if they had current severe gastroesophageal reflux disease or a history of peptic ulcer disease or a gastrointestinal bleed. Patients receiving zileuton, daily oral corticosteroids, or any respiratory biologic therapy within 4 months of visit 1 were excluded. All participants were between the ages of 18 and 70 years, and none of them were pregnant, breast-feeding, or smoking while in the trial.

This was a single-site study conducted at the Asthma Research Center at Brigham and Women's Hospital. The institutional review board of Mass General Brigham approved this study, and all participants gave written informed consent. Participants were recruited from April 2018 through December 2021.

Clinical procedures

After a 2-week run-in period of stable asthma treatment and montelukast, 10 mg daily, participants were randomized to a morning premeal dose of either ifetroban, 200 mg daily by mouth, or matched placebo for 4 weeks, followed by a 4-dose aspirin challenge (40.5, 81, 162, and 325 mg) with 90-minute intervals.[18] The challenge was terminated when patients either reported at least a 2-point increase above baseline in the extended version of the Total Nasal Symptom Score instrument that includes 4 questions about extrasinus symptoms (TNSS+)[19] or experienced a decline in FEV_1 value of at least 15% from baseline or reached a dose of 325 mg of aspirin without evidence of reaction symptoms. Participants were observed for a 3-hour period following the onset of their reaction, with serial measurements of lung function and TNSS+ and collection of blood, urine, and nasal samples (Fig 1, *B*). Participants who wished to take daily aspirin therapy after desensitization continued to escalate aspirin to the target dose after the 3-hour observation period.

Specimen procurement and laboratory procedures

For the aspirin challenge visit, blood was drawn the morning before administration of aspirin and 1 hour after the onset of the aspirin-induced reaction. Platelet-rich plasma was obtained from the top layer of blood samples after a 20-minute centrifuge at 200 *g*. Monitoring of platelet activation in platelet-rich plasma and quantifying of platelet-leukocyte aggregates in whole blood was performed as previously described,[20] by flow cytometry with antibodies specific for CD61, CD62P, CD45, CD14, and CCR3 or isotype controls. Analyses were completed with FlowJo software, version 10 (TreeStar, Ashland, Ore). $CD45^+$ leukocytes were classified as eosinophils ($CCR3^+$ granulocytes), neutrophils ($CCR3^-$ granulocytes), monocytes ($CD14^+$), and lymphocytes. The presence of adherent platelets was determined by the relative expression of CD61 on each cell type.

For the aspirin challenge visit, urine samples were collected before the administration of aspirin, again at the onset of the aspirin-induced reaction, and 90 and 180 minutes later. Nasal fluid was collected before aspirin administration, at the onset of the reaction, and 60 minutes later. Urinary and nasal eicosanoids were measured by mass spectrometry at Vanderbilt University (urine) and the LIPID MAPS Lipidomics Core at the University of California, San Diego (nasal fluid), as previously described.[20,21]

Serum was assayed by ELISA for P-selectin (R&D Systems, Minneapolis, Minn), high mobility group box 1 (HMGB1) (MyBioSource, San Diego, Calif), and CXCL4 and CXCL7 (Abcam, Waltham, Mass), and serum and nasal fluid were assayed for soluble IL-1 receptor-like 1 (ST2) (R&D Systems).

In a separate set of experiments, patients with AERD or chronic rhinosinusitis without nasal polyposis (CRSsNP) who did not participate in the clinical trial but were undergoing elective sinus surgery had sinus tissue collected during surgery (samples collected from August 2018 to July 2022), from which nasal fibroblasts were cultured. The nasal polyp or sinus tissue excised at surgery was minced and incubated in RPMI medium with 10% FBS and 1× trypsin at 37°C for 30 minutes. The cell suspension was washed and centrifuged, and the cell pellet was resuspended at a concentration of 3×10^6 cells/mL and snap-frozen for storage. Cells were thawed for experiments in a 37° water bath and resuspended in 10 mL of RPMI medium with 10% FBS and 1% penicillin-streptomycin and transferred to a 25-mL flask. Cells were passaged when they reached confluency and were used for assays between passages 3 and 5. Fibroblasts were starved for 24 hours in RPMI medium without FBS before stimulation and were stimulated 24 hours after seeding at a concentration of 2×10^5 cells/mL with IL-1β (Abcam) and/or the TP agonist U46619 (Cayman Chemical, Ann Arbor, Mich). Supernatants were assayed by ELISA for PGE_2 and the PGI_2 metabolite 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) (both from Cayman Chemical).

Western blotting was performed on whole cell lysates, with 2×10^4 cells loaded per lane, by using a polyclonal antibody for detection of COX-2 (Cayman Chemical). RNA was extracted from the fibroblast cultures with TRI Reagent (Qiagen, Hilden, Germany) and converted to cDNA by using the RT2 First Strand Kit (Qiagen). Expression of thromboxane A2 receptor (*TBXA2R*) transcript was examined by using RT2 SYBR Green qPCR Master Mix (Qiagen) and normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (primers from Qiagen).

Outcomes

The primary outcome compared the effect of 4 weeks of ifetroban versus placebo on the provocative dose that caused a 2-point increase in TNSS+ (PD2) during an aspirin challenge. PD2 was calculated as follows:

$$inverse\ log_{10}\left(\frac{(2-(PrevTNSS-BaselineTNSS))\times(log_{10}\,ProvocDose-log_{10}\,PrevDose)}{(MaxTNSS-BaselinTNSS)-(PrevTNSS-BaselinTNSS)}+(log_{10}\,PrevDose)\right)$$

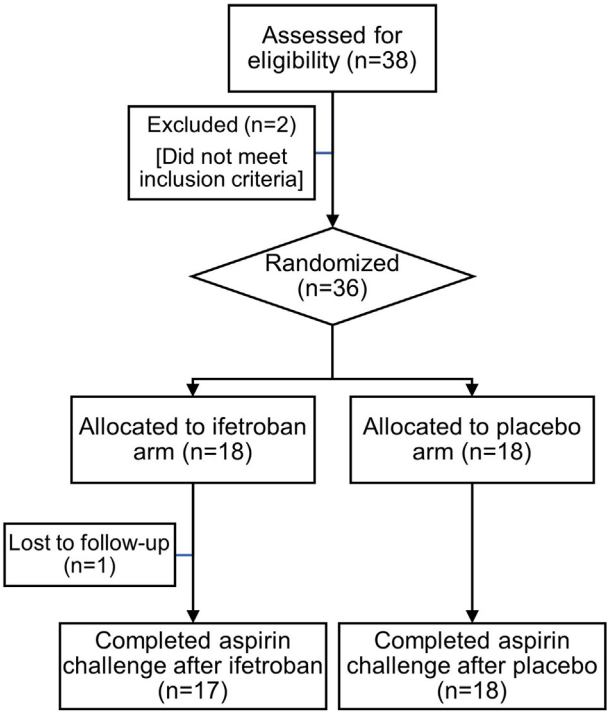


FIG 2. Patient flow through the study.

Participants who did not develop any clinically apparent reaction to aspirin during an aspirin challenge were assigned a PD2 of 650, which is double the maximum dose of aspirin given during the challenge.

Secondary outcomes included differences in the severity of bronchoconstriction and change in urinary and nasal eicosanoids during the aspirin-induced reaction while receiving ifetroban or placebo, as well as the effect of 4 weeks of ifetroban on lung function, asthma control determined by the Asthma Control Questionnaire 6, sinonasal symptom control determined by the Sino-Nasal Outcome Test (SNOT-22), and fractional exhaled nitric oxide. Additional predefined outcomes included changes in platelet activation and nasal and serum tryptase levels.

Randomization

Randomization consisted of blocks of 4 patients randomly assigned (allocation ratio 1:1) to ifetroban or placebo. Matching capsules of ifetroban and placebo were supplied by Cumberland Pharmaceuticals (Nashville, Tenn) and provided to the Brigham and Women's Hospital Investigational Drugs Service pharmacy for storage and distribution. The Investigational Drugs Service conducted the randomization and kept records to maintain study blinding.

Blinding

All participants, clinical care providers, and research staff were kept blinded as to intervention, and all clinical trial biologic analyses were performed in a blinded manner.

TABLE I. Patients' baseline demographics and characteristics

Characteristic	Ifetroban (n = 18)	Placebo (n = 18)	*P* value
Age (y), mean ± SD	41 ± 12	48 ± 12	.13
Sex (female), no. (%)	11 (61%)	7 (39%)	.18
Race, no. (%)			
White	16 (89%)	16 (89%)	1*
Black	1 (6%)	1 (6%)	
Asian	0	1 (6%)	
Other	1 (6%)	0	
Ethnicity, no. (%)			
Hispanic	2 (11%)	2 (11%)	1*
FEV_1 value (L), mean ± SD	3.38 ± 0.89	3.47 ± 0.97	.77
FEV_1 predicted value (%), mean ± SD	96.27 ± 10.86	95.13 ± 12.58	.77
FVC (L), mean ± SD	4.38 ± 1.18	4.61 ± 1.17	.56
FENO level (ppb), mean ± SD	41.06 ± 49.78	36.78 ± 27.17	.75
Peripheral eosinophil level (cells/μL), mean ± SD	306 ± 283	415 ± 276	.25
Peripheral basophil level (cells/μL), mean ± SD	60 ± 28	90 ± 14	.37
ACQ-6 score, mean ± SD	0.44 ± 0.50	0.52 ± 0.68	.71
SNOT22 score, mean ± SD	18.39 ±15.21	20.28 ± 19.73	.75
TNSS+, mean ± SD	4.42 ±5.72	5.5 ± 4.64	.54
Any ICS, no. (%)	16 (89%)	16 (89%)	1
Low-dose ICS (≤ 200 μg), no. (%)	2 (12.5%)	2 (12.5%)	.93 (chi-squared)
Medium-dose ICS (201-500 μg), no. (%)	7 (43.8%)	6 (37.5%)	
High-dose ICS (>500 μg), no. (%)	7 (43.8%)	8 (50%)	
Long-acting β-agonist, no. (%)	12 (66.7%)	12 (66.7%)	1
Long-acting muscarinic antagonist	0	0	1

FENO, Fractional exhaled nitric oxide; *FVC*, Forced vital capacity; *ICS*, inhaled corticosteroid.
*Fisher exact test.

Statistical methods

The study was powered to detect an effect of ifetroban on the PD2 during aspirin challenge with a planned sample size of 40 (which provided 83% power to detect a 3-fold shift in the PD2 between placebo and ifetroban at a .05 level of significance). Because of difficulty recruiting patients for a clinical trial that required respiratory procedures during the COVID-19 pandemic, the trial was terminated early and our final sample size was 35, with which we had 78.6% power to detect the prespecified effect size.

As prespecified, to account for the parallel (noncrossover) design, we used a 2-sided 2-sample *t* test for mean difference with unequal variance, comparing the difference in PD2 between treatment with ifetroban and placebo. For secondary outcomes, a 2-sided 2-sample *t* test was also used to compare change in outcomes during the reaction, as well as change in outcomes versus at baseline for placebo and ifetroban.

RESULTS

Patients

A total of 38 potential participants were screened, 36 of whom underwent randomization per protocol and 35 of whom completed the trial (April 2018 to December 2021) and were analyzed (Fig 2). The baseline characteristics of the patients who were randomized are summarized in Table I.

Primary outcome

There was no difference between the mean PD2 for patients in the ifetroban arm and those in the placebo arm (mean PD2 of 121 ± 48 in the ifetroban arm vs mean PD2 of 142 ± 46 in the placebo arm [P =.763]) (Fig 3, *A*). Of the 35 patients with AERD who completed the trial, 4 did not develop any clinically apparent reaction to aspirin; of those 4 patients, 2 were receiving ifetroban and 2 were receiving placebo, and they were assigned a PD2 of 650.

Secondary clinical outcomes

After 4 weeks of ifetroban or placebo, there were no significant between-arm differences in change in FEV_1 value (–0.5% ± 1.2 with placebo vs –0.2% ± 1.3 with ifetroban [P = .840]), Asthma Control Questionnaire 6 score (–0.03 ± 0.11 with placebo vs +0.17 ± 0.16 with ifetroban [P = .307]), 22-Item Sino-Nasal Outcome Test score (–2.0 ± 2.3 with placebo vs +1.5 ± 3.0 with ifetroban [P = .353]), or fractional exhaled nitric oxide level (–3.4 ± 3.0 with placebo vs +7.9 ± 9.0 with ifetroban [P = .246]) from visit 1 (baseline) to visit 2 (after 4 weeks of placebo or ifetroban).

During their aspirin challenge reactions at visit 2, the participants in the ifetroban arm had a significantly greater aspirin-induced increase in TNSS+ (+9.5 ± 1.4 with placebo vs +14.3 ± 1.9 with ifetroban [P = .048]) and a greater aspirin-induced decrease in FEV_1 value (–8.4% ± 2.1% with placebo vs –18.8% ± 3.6% with ifetroban [P = .017]) than did the participants in the placebo arm (Fig 3, *B* and *C*). There was no difference between the doses of aspirin that provoked reactions for patients in the ifetroban arm and those that provoked reactions for patients in the placebo arm.

Effect of ifetroban on mechanistic outcomes

After 4 weeks of ifetroban, participants had significantly increased urinary leukotriene E_4 (LTE_4) levels (+0.06 ± 0.04 ng/mg of creatinine [Cr] with placebo vs +0.70 ± 0.27 ng/mg of Cr with ifetroban [P = .029]) (Fig 4, *A*) and decreased

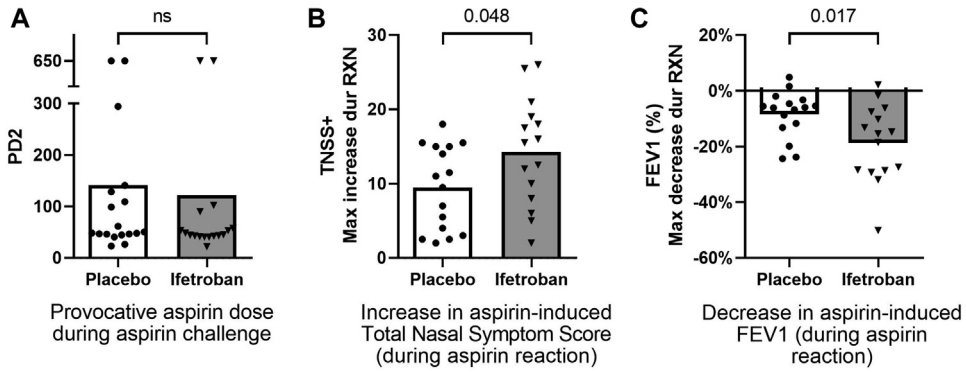


FIG 3. Effect of ifetroban on PD2, TNSS+, and FEV_1 value during reaction to aspirin. The primary outcome, change in the provocative dose of aspirin that would elicit a PD2 **(A)**, along with the maximum increase in TNSS+ **(B)** and maximum percentage of decrease in FEV_1 **(C)** value during aspirin-induced reactions are shown for participants in the placebo and ifetroban groups.

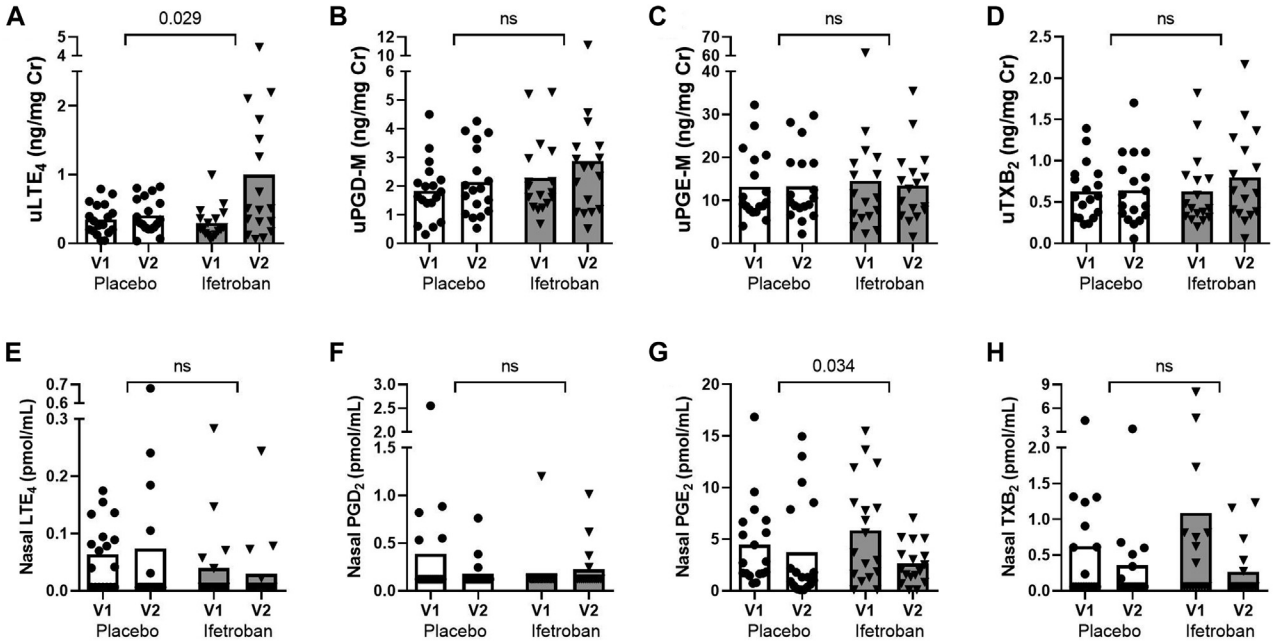


FIG 4. Urinary and nasal eicosanoid levels before and after 1 month of treatment. Pretreatment (before ifetroban or placebo at visit 1) and posttreatment (visit 2, before the start of aspirin challenge) levels of urinary (*top row*) and nasal (*bottom row*) eicosanoids [LTE4 **(A, E)**, PGD2 **(B, F)**, PGE2 **(C, G)**, and TXB2 **(D, H)**] analyzed by mass spectrometry are shown. *P* values compare delta between the placebo and ifetroban treatment arms.

nasal PGE_2 levels (–0.74 ± 0.77 pmol/mL with placebo vs –3.48 ± 0.97 pmol/mL with ifetroban [P = .034]) (Fig 4, *G*) from visit 1 to visit 2 compared with the levels in the participants who received placebo. Nasal LTE_4 levels (Fig 4, *E*) and urinary levels of the PGE_2 metabolite (Fig 4, *C*) did not change. Neither nasal PGD_2 nor urinary PGD_2 metabolite (Fig 4, *B* and *F*) nor nasal or urinary levels of thromboxane B_2 (TXB_2), the stable metabolite of TXA_2, (Fig 4, *D* and *H*) changed from visit 1 to visit 2 regardless of whether the participants were receiving the study drug or placebo.

During their aspirin challenge reactions at visit 2, the participants taking ifetroban had significantly higher aspirin-induced increases in urinary TXB_2 level (–0.07 ± 0.14 ng/mg of Cr with placebo vs +0.55 ± 0.25 ng/mg of Cr with ifetroban [P = .035]) and nasal TXB_2 level (–0.15 ± 0.18 pmol/mL with placebo vs +0.49 ± 0.24 pmol/mL with ifetroban [P = .040]) (Fig 5, *D* and *H*) and trended toward a higher aspirin-induced increase in urinary LTE_4 level (+0.15 ± 0.10 ng/mg of Cr with placebo vs +0.72 ± 0.30 ng/mg of Cr with ifetroban [P = .091]) (Fig 5, *A*), nasal LTE_4 level (Fig 5, *E*), and urinary PGD_2 metabolite level (Fig 5, *B*) than did those participants who received placebo. The changes in urinary PGE_2 metabolite and nasal PGE_2 levels during reactions were no different between the placebo and ifetroban arms (Fig 5, *C* and *G*). During the aspirin reactions, the peak aspirin-induced level of urinary TXB_2 was correlated with the peak level of urinary LTE_4 (but only for participants in the ifetroban arm [r = 0.578; P = .033]); it was not significantly correlated for the placebo

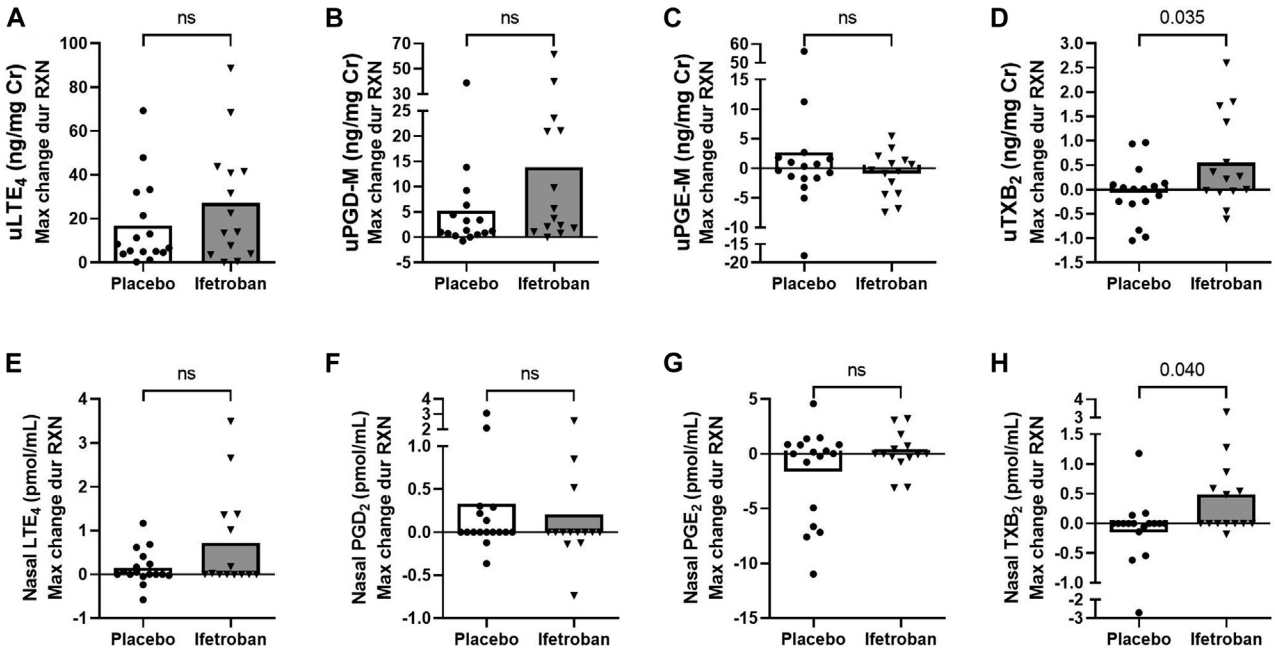


FIG 5. Effect of ifetroban on urinary and nasal eicosanoid levels during reaction to aspirin. Aspirin-induced maximum change from pre-aspirin baseline in urinary (*top row*) and nasal (*bottom row*) eicosanoid levels [LTE4 **(A, E)**, PGD2 **(B, F)**, PGE2 **(C, G)**, and TXB2 **(D, H)**] during aspirin-induced reactions are shown for participants in the placebo and ifetroban treatment arms. *P* values compare delta between the placebo and ifetroban treatment arms.

arm (Fig 6, *A* and *B*). Similarly, the peak aspirin-induced level of urinary TXB_2 was strongly correlated with the peak level of urinary PGD-M for participants taking ifetroban ($r = 0.833$; $P = .0004$) but only weakly correlated for participants in the placebo arm (Fig 6, *C* and *D*).

One month of ifetroban treatment did not significantly affect baseline or aspirin-induced changes in nasal or serum tryptase levels, circulating platelet-leukocyte aggregates, peripheral blood platelet expression of CD62P, blood eosinophil or basophil counts, nasal HMGB1 levels, nasal or serum soluble ST2 levels, or serum CXCL4 or CXCL7 levels (data not shown). During their aspirin challenge reactions at visit 2, the participants taking ifetroban displayed an aspirin-induced increase in serum P-selectin levels from baseline, whereas the participants in the placebo group did not (–6.1 ± 4.2 ng/mL with placebo vs +4.8 ± 3.0 ng/mL with ifetroban [$P = .045$]) (see Fig E1 in the Online Repository at www.jacionline.org).

Prostanoid production from cultured nasal fibroblasts

When stimulated for 24 hours with IL-1β alone, cultured human nasal fibroblasts from patients with AERD produced less PGE_2 than did those from patients with CRSsNP (16 ± 68 ng/mL and 93 ± 32 ng/mL, respectively [$P = .048$]). However, the AERD-derived fibroblasts showed a dose-dependent increase in PGE_2 production following the addition of U46619, a TP receptor agonist, whereas addition of U46619 to the CRSsNP-derived fibroblasts did not further increase their IL-1β–induced PGE_2 production (Fig 7, *A*). AERD-derived fibroblasts also produced less PGI_2 (as reflected by the PGI_2 metabolite 6-keto $PGF_{1\alpha}$) than did those from patients with CRSsNP when stimulated for 24 hours with IL-1β alone (3.3 ± 1.3 ng/mL and 18.1 ± 4.9 ng/mL, respectively [$P = .019$]). Both sets of fibroblasts showed a dose-dependent increase in 6-keto $PGF_{1\alpha}$ production following the addition of U46619 (Fig 7, *B* and *D*). AERD-derived fibroblasts demonstrated a dose-dependent U46619-induced increased in COX-2 protein when assessed by Western blot (see Fig E2 in the Online Repository at www.jacionline.org). TP receptor mRNA expression levels were similar in the fibroblasts from the 2 groups (0.00089 ± 0.00034 in AERD-derived fibroblasts [n = 6 samples] and 0.00065 ± 0.00007 in CRSsNP-derived fibroblasts [n = 4 samples], corrected for *GAPDH* [mean ± SE]).

Safety

Adverse events were categorized according to the Common Terminology Criteria for Adverse Events, version 5.0. Systemic reaction to aspirin challenge with extrapulmonary symptoms was categorized as such according to the definition outlined in the study protocol. The number of total and severe adverse events was similar between the placebo arm and the ifetroban arm, with no significant differences (see Table E1 in the Online Repository at www.jacionline.org). No patients withdrew their participation owing to any ifetroban-related adverse events.

DISCUSSION

The steady-state overproduction of cysLTs and the "unbraking" phenomenon induced by aspirin are features unique to AERD. Both are thought to reflect impaired production of COX-2–derived PGE_2 by stromal cells, which renders patients with AERD susceptible to depletion of PGE_2 in response to a

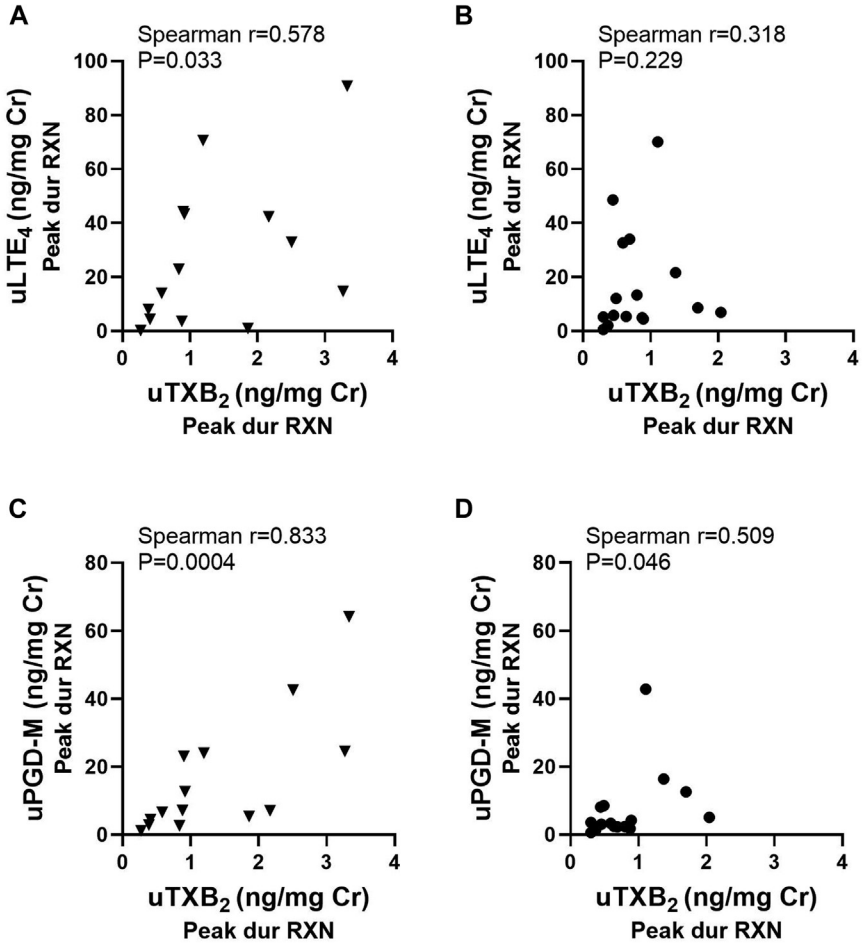


FIG 6. Relationship between urinary eicosanoid levels during aspirin-induced reactions. Correlation between peak urinary TXB_2 and peak urinary LTE_4 and urinary PGD metabolite levels during the aspirin-induced reaction are shown for participants in the ifetroban (**A** and **C** [t*riangles*]) and placebo (**B** and **D** [*circles*]) arms.

low (COX-1–selective) aspirin dose. Deletion of microsomal PGE_2 synthase 1, the dominant enzyme that converts COX-2–derived PGH_2 to PGE_2, in a murine model decreases PGE_2 levels in the lung by approximately 80% and reproduces many features of AERD, including high levels of activated platelets, aspirin-induced changes in airway resistance, activation of mast cells, and increases in both cysLTs and PGD_2 levels, supporting the pathogenetic importance of PGE_2 in controlling multiple effector pathways. The involvement of TP receptors in PGD_2-induced bronchoconstriction[22] and activations of both platelets and endothelial cells,[14] combined with the profoundly inhibitory effect of TP antagonism with ifetroban in our preclinical murine model,[16] prompted this proof-of-concept study in a cohort of carefully phenotyped patients with AERD.

Reactions to COX-1 inhibition in patients with AERD can include both sinonasal symptoms (congestion, sneezing, ocular discharge, and itching) and lower airway obstruction, all of which involve cysLTs and mast cell activation.[2] These reactions typically occur at a provocative dose of 160 mg of aspirin or less (a dose predicted to deplete COX-1–derived prostaglandins). We used PD2, a parameter that measures the threshold dose at which a reaction to aspirin would occur, as our primary outcome in participants treated for 4 weeks with ifetroban vs placebo before standardized challenge with oral aspirin. Although we were not able to reach our planned sample size of 40, our final sample size of 35 allowed our analyses to be sufficiently robust to make us confident that the primary outcome would not have been different had the full sample size of 40 participants completed the study. Ifetroban was safe and well tolerated, and it did not change baseline (pre-aspirin) lung function or sinonasal symptoms. Although treatment with ifetroban did not change PD2, patients treated with ifetroban unexpectedly displayed more severe aspirin-induced reactions, as evidenced by both significantly greater increments in TNSS+ and greater decreases in FEV_1 value (Fig 3). These findings prompted us to examine potential ifetroban-induced changes in lipid mediator homeostasis.

Previous studies demonstrated that steady-state levels of urinary LTE_4, the stable metabolite of the cysLTs, are correlated with the severity of clinical reactions to aspirin challenges in patients with AERD.[17,23,24] Accordingly, we found that urinary LTE_4 levels rose significantly from visit 1 to visit 2 in participants treated with ifetroban but did not change in the placebo group (Fig 4, *A*). Steady-state LTE_4 overproduction in AERD may

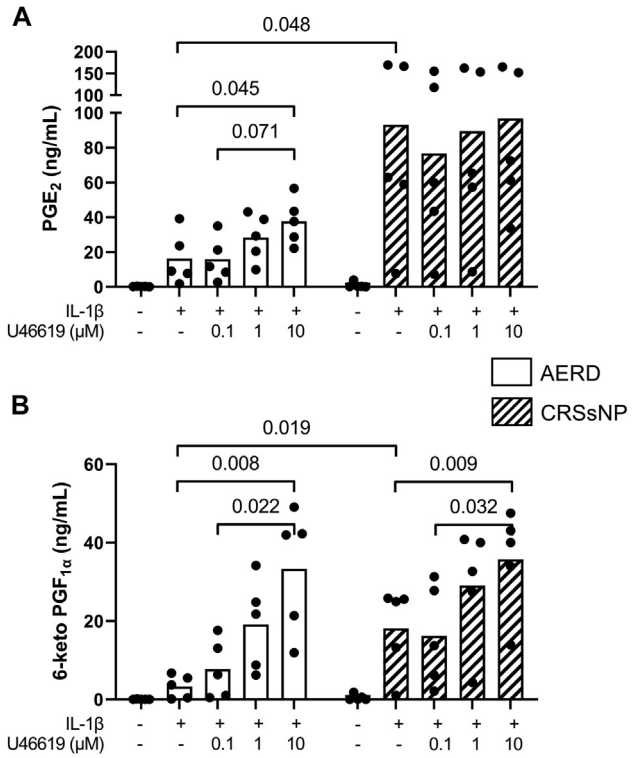


FIG 7. Prostanoid production by cultured nasal fibroblasts. Fibroblasts cultured from surgically excised nasal tissue from patients with AERD (n = 5 [*white columns*]) or CRSsNP (n = 5 [*lined columns*]) were stimulated with or without IL-1β and the TP receptor agonist U46619 for 24 hours. Levels of PGE_2 (**A**) and the PGI_2 metabolite 6-keto PGF1α (**B**) were measured by ELISA in the supernatants.

reflect insufficient PGE_2-induced signaling by EP_2 receptors expressed by several cysLT-producing cell types (eg, mast cells, eosinophils, platelet-adherent granulocytes).[15] Remarkably, nasal fluid levels of PGE_2 declined significantly (by ~60%) in response to a month of ifetroban therapy (Fig 4, *G*) without a corresponding change in urinary PGE_2 metabolite level. Collectively, these findings suggest that TP signaling may play an unanticipated regulatory role in maintaining PGE_2 production in the respiratory tract in AERD but not in nonrespiratory locations (such as the kidney or urinary bladder, which have high expression of PGE synthase) that contribute substantially to the urinary measurement.

Mast cell activation plays a major role in driving both changes in airway physiology and lipid mediator levels during reactions to aspirin in AERD, particularly levels of cysLTs and PGD_2, the major mast cell–derived COX pathway product. Importantly, COX-2 mRNA expression is downregulated in multiple PGE_2-generating stromal cell types in nasal polyps and likely accounts for the abnormally low levels of PGE_2 (which is predominantly COX-2–dependent and derived from stromal cells) found in the respiratory tract of patients with AERD.[4] In contrast, COX-2 expression by mast cells is upregulated in nasal polyps,[25] potentially accounting for the lack of suppression of PGD_2 production at the lower COX-1–specific aspirin doses that induce reactions in AERD. Moreover, mast cells in nasal polyp tissue, regardless of aspirin tolerance, strongly express thromboxane synthase transcripts,[25] suggesting that through metabolism of their COX-2–derived PGH_2 intermediate, they are likely to be a source of TXA_2, which may resist suppression during reactions to aspirin. Nasal and urinary levels of TXB_2 rose in participants treated with ifetroban (Fig 5, *D* and *H*), but not in those treated with placebo; 1 explanation for this difference is that in the ifetroban arm the reduction of TP-dependent production of PGE_2 and PGI_2 may have led those respiratory mast cells toward a more activated state. The fact that the peak levels of urinary TXB_2 were correlated with peak urinary LTE_4 and PGD-M levels (Fig 6), both of which tended to be higher in the ifetroban group (Fig 5, *A* and *B*), suggests that mast cells are the major sources of all 3 mediators and are highly susceptible to removal of the residual PGE_2 "brake" during aspirin challenge. The increase in serum soluble P-selectin levels that was observed during reactions in the ifetroban group in our study (see Fig E1) could reflect an impact of the mast cell–derived cysLTs at type 2 cysLT receptors, which are not blocked by montelukast and can drive P-selectin expression by endothelial cells.[26]

When stimulated with IL-1β, sinonasal fibroblasts cultured from AERD tissues display weak induction of COX-2 expression and blunted production of PGE_2 compared with cells from control tissues.[4,27] This induction pathway requires autocrine amplification of COX-2 expression by PGE_2 signaling through EP_2 receptors. The diminished expression of EP_2 receptors by fibroblasts from patients with AERD impairs the function of this pathway,[27] revealing substantial differences between sinonasal fibroblasts from subjects with and without AERD. Transgenic overexpression of EP_2 receptors corrects COX-2 expression and PGE_2 production in AERD fibroblasts, implying a pivotal role of EP_2 receptors in maintaining PGE_2 production.[28] Previously, platelets were shown to elicit COX-2 expression and the production of PGE_2 and PGI_2 by human endothelial cells *ex vivo* by a pathway requiring platelet-derived TXA_2 and endothelial TP receptors.[14] Notably, a review of our single-cell RNA sequencing data from nasal tissue revealed that endothelial cells and fibroblasts were the principal cell types that express *TBXA2R,* encoding TP receptors, as well as prostaglandin E synthase *(PTGES*) and prostaglandin I2 synthase (*PTGIS*).[25] Additionally, a lipidomic analysis supernatants from IL-1β–stimulated fibroblasts from patients with CRSsNP revealed both PGE_2 and PGI_2 metabolites but no thromboxane metabolites (data not shown). We found that, consistent with the literature,[28] cultured polyp fibroblasts from patients with AERD generated significantly less PGE_2 than did control fibroblasts from individuals with CRSsNP when activated by IL-1β (Fig 7, *A*). Remarkably, however, costimulation with U46119, a selective TP receptor agonist that is blocked by ifetroban,[29] markedly potentiated the IL-1β–induced production of PGE_2 by fibroblasts from patients with AERD but not by control cells from patients with CRSsNP, which may have reached their maximum plateau with IL-1β alone (Fig 7, *A*). Additionally, TP receptor stimulation dramatically (~10-fold) potentiated the production of PGI_2 by fibroblasts from patients with AERD while also doubling PGI_2 generation by control fibroblasts (Fig 7, *B*). Whereas PGE_2 stabilizes mast cells and attenuates 5-LO activation, PGI_2 suppresses platelet activation and blocks cytokine generation and expansion of group 2 innate lymphoid cells.[30] PGI_2 is highly unstable and challenging to measure, and although the levels of its metabolites were below the limits of detection in nasal fluids, it is tempting to speculate that low levels of COX-2–dependent PGI_2 production may be another factor

permitting excessive inflammation in AERD. Moreover, our data suggest that TP receptor signaling provides an accessory pathway that can partially compensate for the impairment of the IL-1β/PGE_2/EP_2 amplification loop for COX-2 induction in stromal cells that may underlie AERD. Moreover, the TP pathway likely requires paracrine signaling because fibroblasts do not generate TXA_2. Blockade of this accessory pathway likely accounts for the unexpected effects of TP antagonism in our study and could be an additional factor (by blocking platelet COX-1 and TXA_2 production by other cells) contributing to the unbraking phenomenon during aspirin-induced reactions in patients with AERD.

Interestingly, as in our previous study using the antiplatelet agent prasugrel (which blocks the adenosine diphosphate-specific $P2Y_{12}$ receptor), ifetroban had no impact on indices of platelet activation.[20] Prasugrel, unlike ifetroban, had no impact on lipid mediators, likely because $P2Y_{12}$ receptors (unlike TP receptors) cannot affect COX induction pathways in stromal cells. Although $P2Y_{12}$ and TP receptor signaling pathways both amplify platelet activation in response to a wide array of stimuli, they do not affect the aberrantly high levels of platelet activation in AERD, the basis of which remains to be determined.[15,31]

It is important to note that the effects of blocking TP receptors in AERD are entirely different from the protective effects predicted from our mouse model.[16] Although species differences are always possible explanations, we suspect that the use of a mouse with a deletion in the terminal enzyme needed to convert COX-2–derived PGH_2 to PGE_2 eliminates the potential reliance on TP-driven COX-2 expression and potentiation of protective PGE_2 by stromal cells. Therefore, the important contribution of TP-dependent PGE_2 production noted in our human nasal fibroblast experiments (and uncovered by ifetroban treatment in our participants with AERD) was absent in our preclinical murine model of AERD, as PGE_2 production is already nearly fully suppressed in the mice. Further, as pretreatment with montelukast (an inhibitor of the type 1 cysLT receptor) is considered a safety requirement before aspirin challenge in patients with AERD, all of our participants were taking montelukast daily throughout the study. The combined inhibition of TP receptors and the type 1 cysLT receptor may have had unanticipated effects[32] in our participants that were not elucidated with the mouse model. Although animal models are useful tools for discovering potential immunologic disease mechanisms, it is essential to study the native disease in humans, in whom the pathogenic perturbations are both more subtle and more complex than they are in a gene-deleted animal.

Clinical implications: TP inhibition with ifetroban is well tolerated in patients with AERD but may worsen their aspirin-induced reactions, likely owing to ifetroban's inhibition of PGE_2 and increase in cysteinyl leukotriene level.

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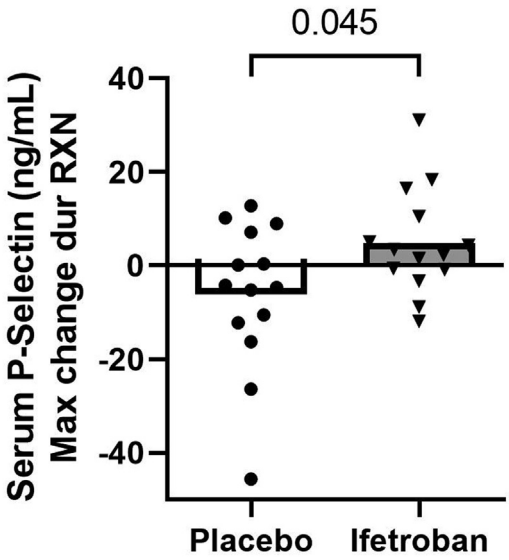

FIG E1. Serum P-selectin levels during reaction. Aspirin-induced change in serum P-selectin levels from pre-aspirin baseline, as measured by ELISA, are shown for participants receiving placebo and ifetroban. *P* values compare Δ between the placebo and ifetroban treatment arms.

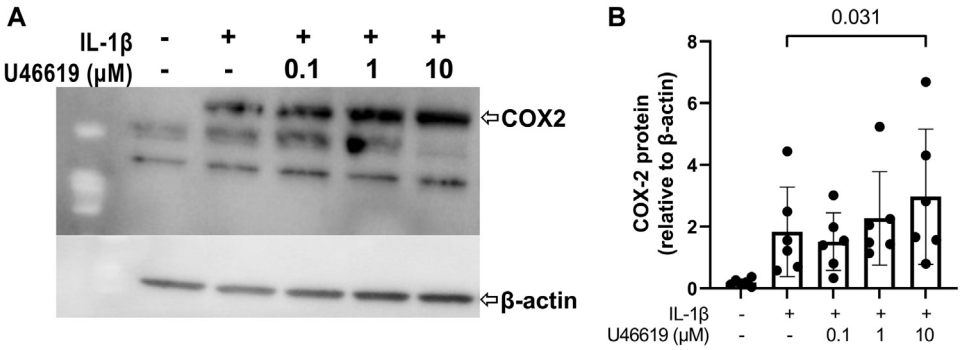

FIG E2. COX-2 protein in cultured nasal fibroblasts. Fibroblasts cultured from surgically excised nasal tissue from patients with AERD were stimulated with or without IL-1β and the TP receptor agonist U46619 for 24 hours, after which levels of COX-2 protein were assessed by Western blot. A representative blot from a patient with AERD is shown (**A**), with summarized data from 6 patients with AERD shown as quantified by ImageJ software (**B**).

TABLE E1. Adverse events

Adverse event	Ifetroban	Placebo
Before aspirin challenge (screening through the morning of visit 2), no.		
Total adverse events reported	6	5
Bruising, grade 1	1	0
Constipation, grade 1	1	0
Ear pain, grade 1	0	1
Eye disorder: stye, grade 1	1	0
Nasal congestion, grade 1	0	1
Otitis media, grade 2	1	0
Rash maculopapular, grade 1	0	1
Sinus disorder (bloody nose), grade 1	1	0
Upper respiratory infection, grade 2	0	2
Vomiting, grade 1	1	0
During/after aspirin challenge (visit 2 and follow-up), no.		
Total adverse events reported	6	6
Lower gastrointestinal hemorrhage, grade 1	0	1
Lung infection (influenza B–related pneumonia), grade 2	1	0
Pregnancy in participant partner, grade 1	0	1
Rash, maculopapular, grade 1	0	1
Stomach pain, grade 1	0	1
Systemic reaction to aspirin challenge with extrapulmonary symptoms	3	2
Wheezing, grade 1	2	0

All events that occurred after randomization through 2 weeks after the aspirin challenge/desensitization (follow-up period) were included. Data listed are numbers of participants reporting each type of adverse event.