Received: 28 September 2020 | Revised: 13 December 2020 | Accepted: 13 December 2020

DOI: 10.1111/cts.12968

ARTICLE

Characterization of LY2775240, a selective phosphodiesterase-4 inhibitor, in nonclinical models and in healthy subjects

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Funding information
Eli Lilly and Co. funded this study.

Abstract

LY2775240 is a highly selective, potent and orally-administered inhibitor of phosphodiesterase 4 (PDE4), and is being investigated as a treatment option for inflammatory disorders, such as psoriasis. LY2775240 was investigated in rodent and rhesus monkey nonclinical models. Treatment with LY2775240 led to significant reductions in TNFα production, a marker of PDE4 engagement upon immune activation, in both nonclinical models. In the first part of a 2-part first-in-human randomized study, a wide dose range of LY2775240 was safely evaluated and found to be well-tolerated with common adverse events (AEs) of nausea, diarrhea, and headache. No serious AEs were reported. The pharmacokinetic profile of LY2775240 was well-characterized, with a half-life that can support once-a-day dosing. An ex vivo pharmacodynamic (PD) assay demonstrated dose-dependent PDE4 target engagement as assessed by reduction in TNFα production. A 20 mg dose of LY2775240 led to near-maximal TNFα inhibition in this PD assay in the first part of the study and was selected for comparison with the clinical dose of apremilast (30 mg) in the crossover, second part of this study. The 20 mg dose of LY2775240 demonstrated sustained maximal (50%–80%) inhibition of TNFα over all timepoints over the 24-h duration. The comparator apremilast achieved peak inhibition of ~ 50% at only 4 h postdose with a return to about 10% inhibition within 12 h of dosing. In summary, the nonclinical data and safety, tolerability, and PK/PD data in healthy subjects supports further investigation of LY2775240 in inflammatory indications.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Phosphodiesterase 4 (PDE4) inhibitors, such as apremilast, are currently approved to treat autoimmune disorders, such as psoriasis. LY2775240 is an oral PDE4 inhibitor being developed for treatment of a variety of inflammatory disorders. The degree of enzymatic inhibition achieved by PDE4 inhibitors clinically is poorly understood.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study investigated single ascending doses of LY2775240, a highly selective oral PDE4 inhibitor, in healthy subjects. LY2775240 was well-tolerated over the

dose range evaluated, and pharmacokinetic/pharmacodynamic (PD) profiles were well-characterized.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study evaluated different doses of LY2775240 and subsequently compared a selected LY2775240 dose with the clinical dose of apremilast with an ex vivo assay. This information builds a connection between target engagement and clinical efficacy.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This is the first report of an ex vivo PD assay that has been systematically implemented in a PDE4 inhibitor Phase 1 study. Early investigation of exposure-response relationships versus a comparator can support evaluation of clinically meaningful doses of investigational agents.

INTRODUCTION

There remains substantial unmet need for orally administered treatment options for autoimmune and inflammatory conditions. Psoriasis, in particular, has been treated with topical steroids and nonspecific immunomodulators, such as methotrexate, before monoclonal antibodies targeting tumor necrosis factor alpha (TNFα) became available. Monoclonal antibodies targeting the p40 subunit of interleukins 12 and 23 (IL-12/23p40), interleukin-17 (IL-17), and the p19 subunit of interleukin-23 (IL-23p19) have provided substantially improved safety and efficacy, however, they all need to be administered either intravenously or by subcutaneous injection.[1] Janus kinase inhibitors are orally available and approved for rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis, and they are currently being studied in atopic dermatitis and psoriasis.[2]

Phosphodiesterase-4 (PDE4) inhibitors are a class of drugs that have been investigated for the treatment of several respiratory and autoimmune diseases, including but not limited to ankylosing spondylitis, psoriasis, psoriatic arthritis, sarcoidosis, systemic lupus erythematosus, inflammatory bowel disease, atopic dermatitis, rheumatoid arthritis, and multiple sclerosis, among others.[3] PDE4 belongs to the 3′, 5′-cyclic nucleotide phosphodiesterase family of enzymes that are responsible for hydrolysis and subsequent inactivation of cyclic nucleotides.[3,4] The cyclic nucleotides play a critical role in transducing extracellular stimuli into intracellular signals, which then subsequently control downstream processes, allowing the cells to interact with their environment and regulate physiological processes, including those involved in inflammation.[5] Cyclic nucleotides, such as cyclic adenosine monophosphate (cAMP), modulate the expression of inflammatory cytokines, namely TNFα, IL-23, and IL-17, among others.[6] Phosphodiesterase enzymes are organized into at least 11 families, based on differences in their sequence, substrate specificity, and biochemical properties.[7] Further, the PDE4 family consists of 4 genes (PDE4A–D) and over 20 splice variants are expressed in inflammatory cells, endothelial cells, smooth muscle cells, and keratinocytes.[8] Inhibition of PDE4 results in higher cytoplasmic levels of cAMP and reduced secretion of pro-inflammatory cytokines.[9–11]

Three PDE4 inhibitors are currently approved in the United States and Europe. Apremilast is indicated for psoriatic arthritis, plaque psoriasis, and oral ulcers in Behcet's disease.[12–14] To reduce the risk of gastrointestinal symptoms, apremilast is titrated over a 5-day period to achieve a recommended dose of 30 mg tablets twice-a-day. Roflumilast, an orally administered drug indicated for chronic obstructive pulmonary disease, is administered as a 500 ug tablet once-a-day.[15] Last, crisaborole is indicated for atopic dermatitis, and it is administered as a 2% topical ointment twice daily.[16]

LY2775240 (Eli Lilly and Co., Indianapolis, IN)[17] is an investigational, orally bioavailable PDE4 inhibitor. In this report, we characterize LY2775240 in nonclinical mechanistic models followed by description of the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) results following administration of LY277520 in a single ascending dose study in healthy subjects. Subsequently, we directly compared LY2775240 with apremilast using an ex vivo PD assay. Based on these results, we believe LY277520 is a promising novel oral PDE4 inhibitor that warrants further clinical investigation.

METHODS

Ethics

These studies were conducted in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki. All subjects provided written informed consent before beginning any study procedures. The protocols, amendments, and subject-informed consents received appropriate institutional review board approval prior to initiation.

Material synthesis

LY2775240 was synthesized as indicated[17] and stored as a 10 mM solution in dimethyl sulfoxide (DMSO; 100%) at −20°C. Apremilast was commercially sourced (Sequioia/Allchem) as well as prepared at Eli Lilly & Co.[18]

Phosphodiesterase enzyme selectivity assays

All 3′, 5′ cyclic nucleotide phosphodiesterase enzyme activities were measured with a radiometric enzyme assay based on scintillation proximity assay detection system or a filtration binding format. Compounds tested were diluted in pure DMSO using 10-point concentration response curves. Selectivity against PDE3A (catalytic domain), PDE4 (A, B, C, and D), PDE7B, and PDE8A were measured using [3H]-cAMP, whereas selectivity against PDE2A, PDE5A, PDE6AB, PDE9A, PDE10A, and PDE11A were measured using [3H]-cGMP, as described.[5]

Mouse PK/PD study

Using an Institutional Animal Care and Use Committee (IACUC)-approved method, female C57Bl/6 mice 8–10 weeks old were dosed at 0, 3, or 10 mg/kg orally in a vehicle of 1% hydroxyethylcellulose, 0.25% polysorbate 80, 0.05% antifoam in purified water at 18 h and again 2 h prior to single lipopolysaccharide (LPS) injection (3 mg/kg, intraperitoneally, dose volume of 200 µl, $n = 5$). Mice were euthanized at 1.5 h after LPS injection and plasma was collected. TNFα concentration was analyzed by enzyme-linked immunosorbent assay (ELISA; R&D Systems DuoSet ELISA Mouse TNFα).

Rhesus PK/PD study

The in-life phase of this study was conducted at ICOS Corporation (Bothell, WA) and SNBL (Shin Nippon Biomedical Laboratories, Everett, WA) using rhesus monkeys using an IACUC-approved method. Briefly, LY2775240 was administered orally (20 mg/kg, 2 ml/kg dose volume) via nasogastric gavage. Heparinized peripheral blood samples were collected predose and at 0.08, 0.25, 0.5, 1, 2, 4, 8, 12. and 24 h postdose by venipuncture and, within 30 min, blood (0.45 ml) was incubated with LPS (0.5 ml assay volume, 100 ng/ml for 8 h at 37°C). These plasma samples were analyzed for rhesus TNFα concentration by ELISA (KPC3011 and KPC3012; BioSource International, Camarillo, CA).

Subjects

Overtly healthy men or women aged 21–65 years, inclusive, and with a body mass index of 18.0–32.0 kg/m^2, inclusive, could participate. Female subjects were not of child-bearing potential and male subjects agreed to the use of contraception per protocol. Key exclusion criteria included consuming more than 10 cigarettes per day, or the equivalent, being unable or unwilling to refrain from nicotine use during clinical research unit (CRU) admissions, and intending to use over-the-counter or prescription medication within 7 and 14 days, respectively, before dosing (apart from vitamin/mineral supplements, occasional paracetamol/acetaminophen, or thyroid replacement therapy).

Phase 1 study design

This Phase 1 study was designed to evaluate safety, tolerability, and PKs of LY2775240 for the first time in humans. The study comprised of two parts. Part A was a subject- and investigator-blind, placebo-controlled, four-period crossover design with two alternating cohorts of healthy subjects. Part B was an open-label, two-period, crossover design in healthy subjects that included a single doses of either LY2775240 or apremilast in each period. These studies took place at the Lilly-NUS Centre for Clinical Pharmacology, Singapore.

Part A

Eight healthy subjects in each cohort were initially randomized in a 6-to-2, active-to-placebo ratio; over 4 periods, such that each subject would receive at maximum 3 doses of LY2775240 and 1 dose of placebo over the study duration. Only a single dose of either LY2775240 or placebo was administered during each period, and the washout period for each subject was ~ 2 weeks. LY2775240 dose levels of 0.1, 0.5, 1.5, 5, 10, 20, 30, and 40 mg given orally as a single dose were investigated in this part of the study. Subjects who withdrew from the study before completing all four periods in part A were replaced and replacement subjects assumed the treatment sequence of the original subject and completed the remaining study periods. Subjects were admitted to the CRU on day 1 or on the day of dose administration. Subjects were discharged from the CRU on day 3, at the discretion of the investigator, after the safety assessments and sample collections had been completed. Subjects were required to return on days 5 and 7 during each dosing period, for safety assessments and sample collections. The study follow-up visits took place ~ 2 weeks after the subjects' last dose of study drug.

Part B

Eleven healthy subjects were randomized to each received a single dose of either LY2775240 or apremilast (commercially sourced Otezla; Celgene Corporation, Summit, NJ) in a crossover study design, with at least 5-day washouts between doses. The dose of LY2775240 20 mg was selected based on all safety, tolerability, PK, and PD data from part A. Apremilast dose of 30 mg was selected for the comparison because it is the highest approved dose. Subjects were admitted to the CRU on day −1 or on the day of dose administration. Subjects were discharged from the CRU on day 3, at the discretion of the investigator, after the safety assessments and sample collections had been completed. The study follow-up visits took place ~ 1 week after the subjects' last dose of study drug.

Pharmacokinetics

In part A, LY2775240 PK samples were collected at predose, 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, 48, and 96 h postdose. In part B, LY2775240 PK samples were collected at predose, 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, and 48 h postdose, whereas PK samples for apremilast were collected at predose, 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 h postdose. Plasma samples obtained during this study were analyzed for LY2775240 using a validated liquid chromatography tandem-mass spectrometry (LC-MS/MS) method at Covance Bioanalytical Services located in Indianapolis, IN. Plasma samples obtained during this study were analyzed for apremilast using a validated LC-MS/MS method at Q2 Solutions in Ithaca, NY. The PK parameters for LY2775240 and apremilast were computed by standard noncompartmental methods of analysis using WinNonlin (version 7.0, Pharsight).

Pharmacodynamics

Venous blood (1 ml) was collected into LPS TruCulture Tubes (catalog number 782-001087; Myriad RBM, Austin, TX) containing 2 ml of media with 100 ng/ml LPS (final concentration). Blood samples were incubated for 24 h at 37°C, after which the cell-free supernatant was collected and stored frozen until shipment for analysis of TNFα concentrations. Percent change in TNFα cytokine concentrations was explored as a surrogate of PDE4 inhibition. The cell-free supernatant was assayed for TNFα concentrations using a validated Meso Scale Diagnostic (Meso Scale Diagnostics, Rockville, MD) Proinflammatory Panel I Assay method at BioAgilytix (Durham, NC). In part A, this assay was implemented at LY2775240 dose levels of 1.5 mg and higher. Blood samples were collected at the following timepoints for PD evaluation: part A - predose, 4, 12, and 24 h; and part B - predose, 1, 4, 8, 12, and 24 h.

Safety assessments

An adverse event (AE) was any untoward medical occurrence in a subject who had been administered pharmaceutical product, regardless of causal attribution. All AEs were graded mild, moderate, or severe in intensity by the investigator. A serious AE (SAE) was any AE that was fatal, life-threatening, required subject hospitalization, resulted in a congenital abnormality/birth defect, or resulted in persistent or significant disability/incapacity. All AEs and SAEs, regardless of attribution, were collected after the informed consent form was signed and were reported until 1 (part B) to 2 (part A) weeks after the last dose of LY2775240, apremilast, or placebo. A treatment emergent adverse event (TEAE) was an AE that occurred any time after the study drug was administered or that was present prior to dosing and became more severe any time after the study drug was administered. The investigator determined whether an AE had a reasonable possibility of being related to study treatment or a study procedure. The frequencies of TEAEs were summarized by intensity and relationship to LY2775240, apremilast, or placebo, for each cohort and overall, using the Medical Dictionary for Regulatory Activities (version 19.1) system organ class and preferred term.

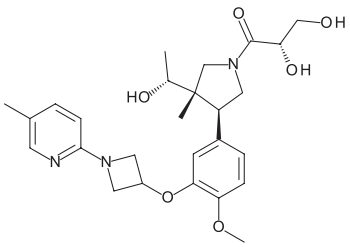
RESULTS

In vitro potency and selectivity

LY2775240 was evaluated for potency and selectivity within the phosphodiesterase family and displayed a high degree of selectivity for the PDE4 subfamily over the other phosphodiesterases, with at least a 100-fold margin (Figure 1). Within the PDE4 subfamily, LY2775240 was most potent against PDE4A, B, and D (0.09, 0.09, and 0.14 nM, respectively) while retaining activity against PDE4C (2.4 nM). We evaluated LY2775240 in a human whole blood assay[19] where the presence of PDE4 inhibition reduces the amount of TNFα secreted in response to the Toll-like receptor 4 agonist LPS. LY2775240 potently inhibits this functional response, with about an order of magnitude increased potency compared to the PDE4 inhibitor apremilast (Figure S1). When evaluated with a Phase-1 ready assay system using either an LPS stimulation or a media-only control, LY2775240 inhibited LPS-stimulated TNFα but had no effect with the media-only control (Figure S1). In a head to head comparison with apremilast using these assay tubes and blood from four healthy donors (Figure 2), LY2775240 was more potent than apremilast (half-maximal inhibitory concentration = 18 nM vs.

(a)

LY2775240



(b) Potency/selectivity values

phosphodiesterase	IC-50
PDE1A	>10,000 nM
PDE1B	>10,000 nM
PDE1C	>10,000 nM
PDE2A	>10,000 nM
PDE3A	>10,000 nM
PDE4A	0.09 nM
PDE4B	0.09 nM
PDE4C	2.4 nM
PDE4D	0.14 nM
PDE5A	>10,000 nM
PDE6A/B	>10,000 nM
PDE7B	>10,000 nM
PDE8A	>10,000 nM
PDE9A	>10,000 nM
PDE10A	440 nM
PDE11A	>10,000 nM

human whole blood assay	IC-50
LPS stim. TNFa	18 nM

FIGURE 1 PDE4 inhibitor LY2775240, (2S)-2,3-dihydroxy-1-[(3S,4S)-3-[(1R)-1-hydroxyethyl]-4-[4-methoxy-3-[1-(5-methyl-2-pyridyl) azetidin-3-yl]oxy-phenyl]-3-methyl-pyrrolidin-1-yl]propan-1-one. (a) Chemical structure. (b) Potency and selectivity values. IC_{50}, half-maximal inhibitory concentration; LPS, lipopolysaccharide; PDE4, phosphodiesterase 4; stim., stimulated

290 nM). LY2775240 was evaluated in the DiscoverX multicellular assay system, and the results are consistent with activity expected for a PDE4 inhibitor. The main comparator match for the results generated with LY2775240 was apremilast. In addition to the expected inhibition of TNFα, the expression of E-selectin, secreted IgG, plasminogen activator inhibitor-1, and secreted IL-10 were also affected (Figure S2).

Nonclinical in vivo efficacy

LY2775240 was evaluated with two nonclinical mechanistic models. In the first, LY2775240 was dosed orally to mice, followed 1 h later with an LPS challenge, and the amount of TNFα in the plasma was measured an additional 2 h later (Figure 2). Significant reductions in measured TNFα were observed with both the 3 and 10 mg/kg dose levels of LY2775240, with reduction to 53% and 35% of vehicle control, respectively. Similarly, in rhesus monkeys dosed with LY2775240 (20 mg/kg), increasing plasma concentrations of LY2775240 were associated with a marked reduction in TNFα levels (Figure 2).

Subjects and disposition

A total of 34 healthy subjects, 32 men and 2 women, between the ages of 28 and 61 years, participated in this 2-part Phase 1 study (Figures 3, 4). Subject demographics are presented in Table 1. All 34 subjects who entered the study were randomly assigned to treatment and received at least 1 dose of study drug. Twenty-seven subjects completed the study. Reasons for discontinuation included scheduling conflicts (5 subjects), starting a new job (1 subject), and lost to follow-up (1 subject).

Safety

There were no deaths, SAEs, or discontinuations due to AEs. TEAEs considered by the investigator to be related to the study treatment are listed in Table 2 for parts A and B, respectively. The most common AEs assessed as related to LY2775240 were nausea, diarrhea, and headache. These occurred in 21 of the 23 treated subjects. Nausea occurred in 14 of 34 (41%), diarrhea occurred in 9 of 34 (26%), and headache occurred in 8 of 34 (24%) subjects. These AEs were consistent with those reported with other oral PDE4 inhibitors. They did occur more commonly at doses greater than or equal to 20 mg. The most common AEs assessed as related to apremilast (each reported only once) included eructation (belching), abdominal distension, flatulence, and somnolence. These occurred in 2 of 11 subjects. Overall, all AEs were assessed to be mild by the investigator. There were no changes in laboratory values, including markers of inflammation, or vital signs that were assessed to be clinically significant or reported as an AE. There were no clinically significant changes in electrocardiogram parameters.

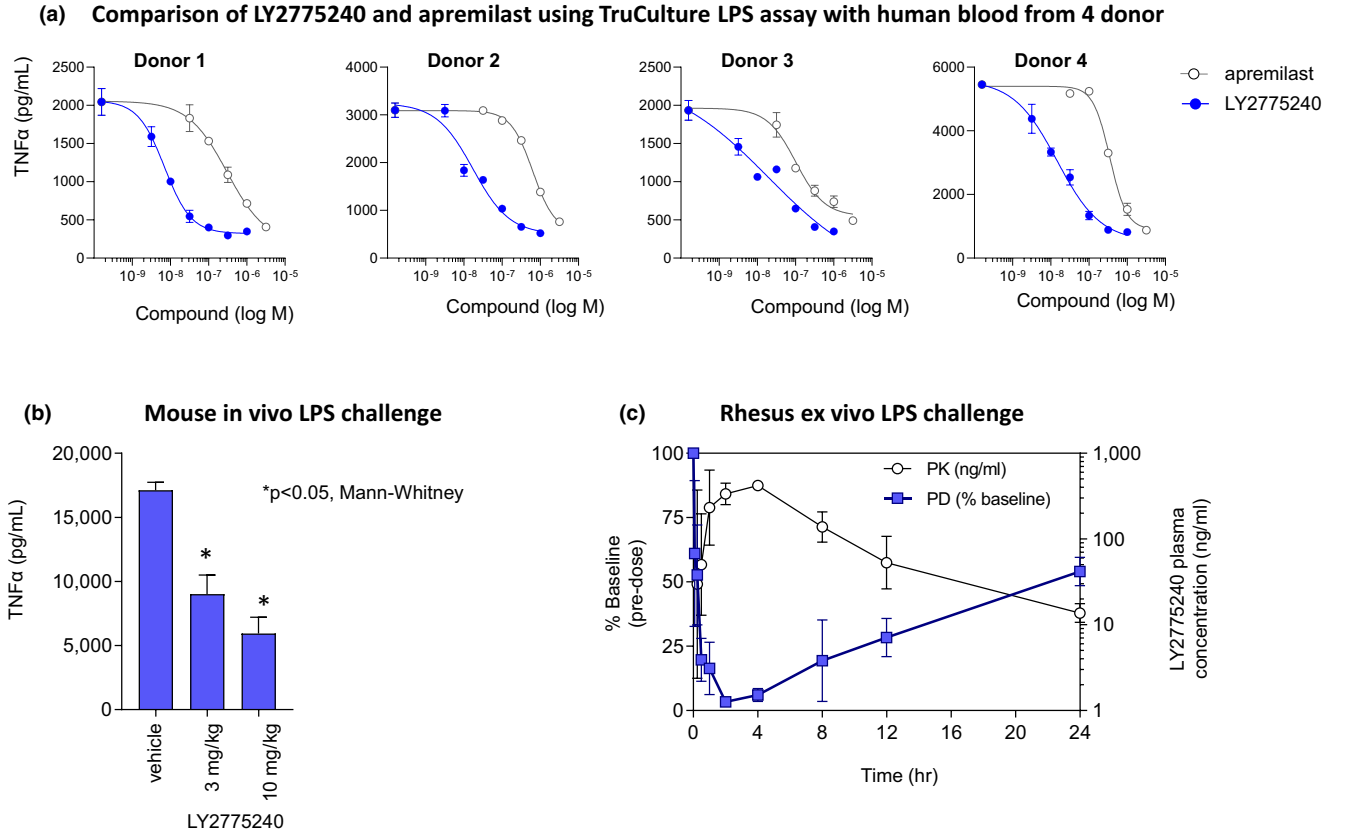


FIGURE 2 Characterization of LY2775240 in vitro in human whole blood assays and in vivo with preclinical assays. (a) Comparison of LY2775240 with the approved PDE4 inhibitor apremilast in a whole blood assay using the Phase-1 ready TruCulture assay, where addition of LY2775240 or apremilast reduced the subsequent secretion of TNFα upon stimulation with the bacterial endotoxin LPS using blood from four healthy donors ($n = 4$, mean ±SE). (b) Mice ($n = 5$ per group) were dosed orally with LY2775240 (3 or 10 mg/kg) or vehicle (1% HEC, 10 ml/kg dose volume) and were challenged with an intraperitoneal injection of LPS (10 mg/kg, 1 ml dose volume) 1 h later. Plasma samples were collected at 3 h for measurement of the cytokine TNFα by ELISA. (c) Rhesus monkeys ($n = 3$) were dosed with LY2775240 (20 mg/kg, 2 ml/kg dose volume) by oral gavage and blood samples were collected at the indicated times for pharmacokinetic measurements and an ex vivo pharmacodynamic assay of PDE4 function. The left *y*-axis indicates the percent baseline of the LPS-stimulated TNFα secretion, while the right *y*-axis indicates the plasma concentration of LY2775240. ELISA, enzyme-linked immunosorbent assay; HEC, hydroxyethylcellulose; LPS, lipopolysaccharide; PD, pharmacodynamic; PK, pharmacokinetic; PDE4, phosphodiesterase 4

Pharmacokinetics of LY2775240

The concentration versus time profile of LY2775240 following oral administration in part A is shown in Figure 3 and PK parameters are presented in Table 3. The maximum LY2775240 concentration occurred within 1–4 h after dosing. The mean half-life was ~ 12 h (Table 3). Mean apparent oral clearance across dose levels ranged from 1.69 to 4.90 L/h. The ratios of dose-normalized geometric means for maximum plasma concentration (C_{max}) and area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$) were 0.215 and 0.349, respectively. The 90% confidence intervals of the ratio of dose-normalized geometric mean for C_{max} (0.178, 0.261) and $AUC_{0-\infty}$ (0.289, 0.422) suggested notable subproportional increase in exposure with increasing dose levels. The intersubject variability for C_{max} ranged from 22% to 51%; the intersubject variability for $AUC_{0-\infty}$ ranged from 23% to 70%.

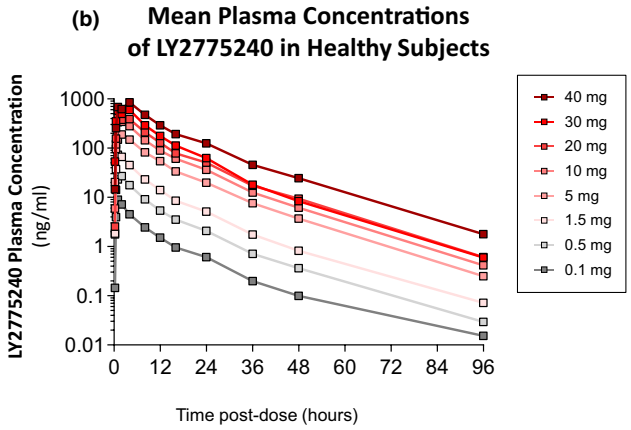
A dose of 20 mg LY2775240 was selected for the crossover comparison with apremilast in part B of the study. The PK parameters of 20 mg LY2775240 in part B (Figure 4) were comparable with corresponding parameters from part A. Summary of mean apremilast and LY2775240 PK parameters from part B are presented in Table S1.

Pharmacodynamics of LY2775240

A marked reduction in TNFα production was measured in a dose-dependent manner, consistent with increasing plasma concentration of LY2775240 (Figure 3). In these analyses, all data were normalized to the subjects' predose reading, which was set to 100%. The median placebo response was consistent across the time course, remaining near 100% (Figure 3). The LY2775240 1.5 mg dose resulted in a 25%–50% drop in signal over the 24-h time course, whereas the 5 mg dose

(a) **Phase 1, Part A Study Design**

	Treatment Period 1		Treatment Period 2		Treatment Period 3		Treatment Period 4		Follow up
Cohort 1	0.1 mg (n=6) Placebo (n=2)		1.5 mg (n=6) Placebo (n=2)		10 mg (n=6) Placebo (n=2)		30 mg (n=6) Placebo (n=2)		
Cohort 2		0.5 mg (n=6) Placebo (n=2)		5 mg (n=6) Placebo (n=2)		20 mg (n=6) Placebo (n=2)		40 mg (n=6) Placebo (n=2)	



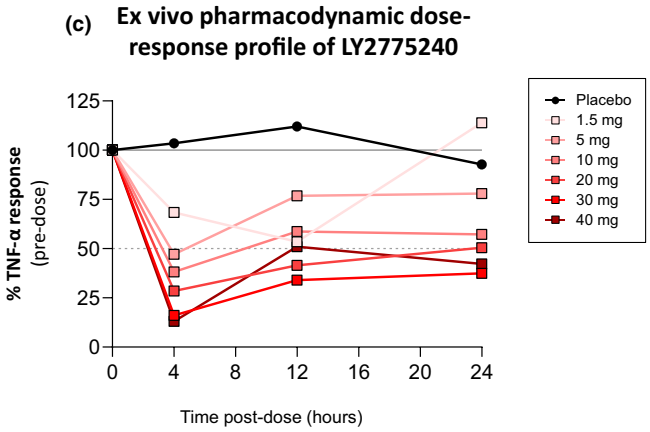


FIGURE 3 Clinical PK and PD evaluation of LY2775240. (a) Phase 1 study scheme. In part A, healthy subjects in two cohorts received single ascending doses of either LY2775240 or placebo in each treatment period, followed by 2-week washout period. (b) Plasma concentrations in healthy subjects following single ascending doses from 0.1 mg to 40 mg LY2775240. PK sampling occurred up to 96 h postdose. Each data point represents mean values ($n = 6$ subjects per cohort, except dose level 0.1 mg, time point = 96 h is based on $n = 2$ with quantifiable concentrations). (c) PD responses of PDE4 inhibition using the TruCulture LPS assay system over 24 h. Each data point represents the median ($n = 6$ active-treated subjects per cohort and $n = 14$ for placebo-treated subjects). PD, pharmacodynamic; PK, pharmacokinetic; PDE4, phosphodiesterase 4

achieved 50% inhibition at 4 h postdose before returning to near the baseline value at 24 h. The 10 mg dose achieved ~ 60% inhibition at 4 h and the inhibition remained just below 50% over the 24-h duration. The 20 mg dose of LY2775240 displayed about 70% inhibition at 4 h and the inhibition remained greater than 50% over the course of the treatment period. The 30 and 40 mg dose levels did not lead to notable increases in mean TNFα inhibition compared to the 20 mg dose, and increased numbers of AEs were reported at the 30 and 40 mg dose levels. Hence, the 20 mg dose was chosen for further evaluation in part B because it was the lowest LY2775240 dose that maintained greater than 50% inhibition over 24 h in the PD assay. Overall, the results from ex vivo stimulation with LPS indicate that LY2775240 demonstrates sustained inhibition of the TNFα production at doses of 20 mg and higher.

The ex vivo stimulation assay was implemented in part B to compare the extent of PDE4 related PD effect observed following administration of a single marketed dose of apremilast (30 mg) versus a single 20-mg dose of LY2775240 (Figure 4). Two additional sample collection times were added in part B compared to part A, specifically at 1 and 8 h, to complement the predose, 4, 12- and 24-h sample collections used in part A. A single 20 mg dose of LY2775240 resulted in peak inhibition of PDE4-mediated TNFα release at 1–4 h postdose, achieving ~ 80% inhibition. Additionally, TNFα inhibition of 50% or greater was maintained throughout the 24-h postdose assessment. In contrast, the maximum inhibition of TNFα release following administration of 30 mg apremilast was ~ 45% at 4 h postdose (~ 20% inhibition at 1 h), and diminished thereafter during the 24-h assessment.

DISCUSSION

LY2775240 is a potent, selective, orally administered inhibitor of PDE4 (Figure 1). As the predominant phosphodiesterase expressed by monocyte/macrophage cells, inhibition of PDE4 can suppress the production of TNFα by these key cells.[8] The oral PDE4 inhibitor apremilast is approved to treat psoriasis and other inflammatory conditions, and is given as a twice daily 30 mg dose. Although there are reported values of PDE4 inhibition following apremilast treatment[20] the time course of PDE4 inhibition over the course of dosing duration is not well understood. In this study, we utilized a novel PD assay to directly compare the PDE4 inhibition achieved by LY2775240 with that achieved by apremilast, over the

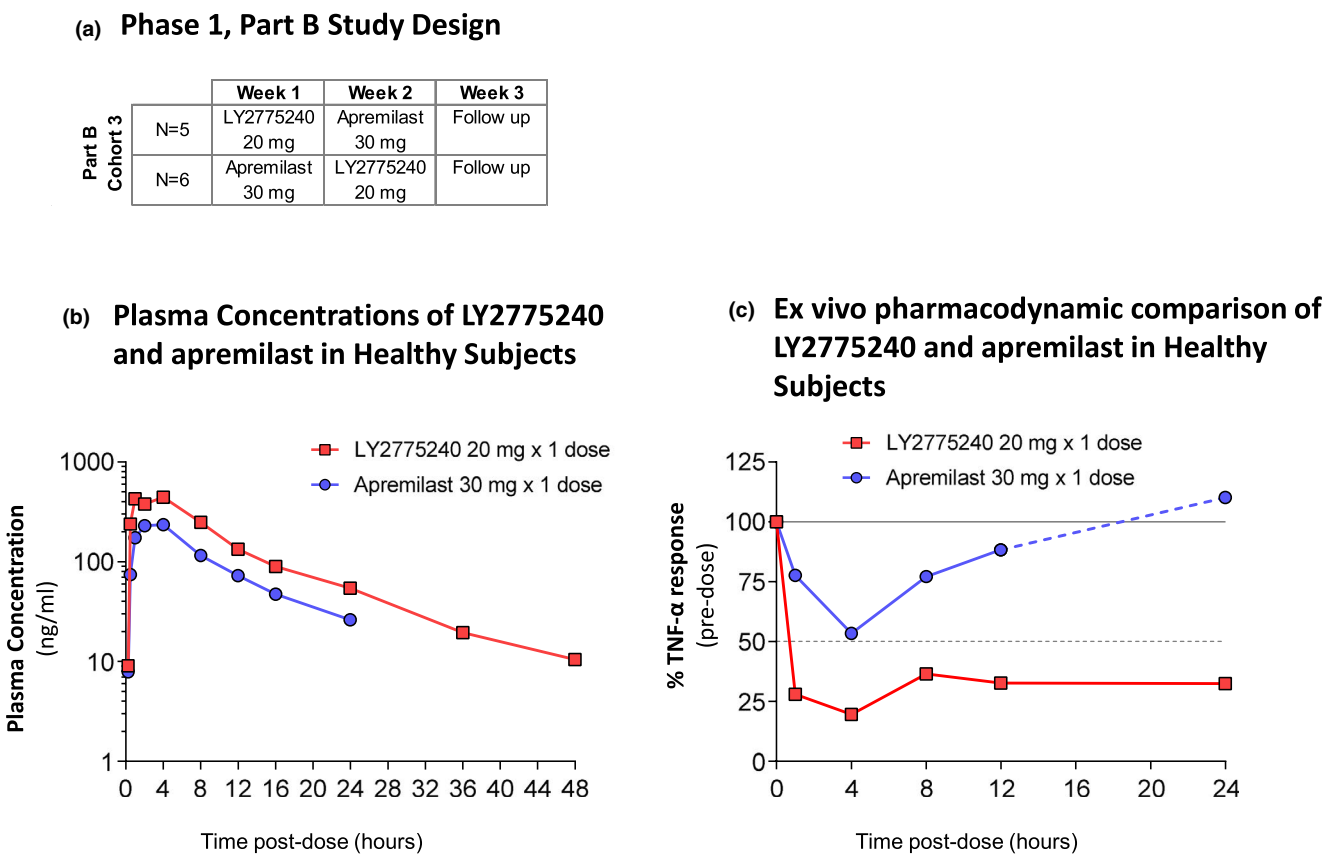


FIGURE 4 Pharmacokinetic and PD comparison of LY2555240 (20 mg) and apremilast (30 mg) following a single dose in healthy subjects. (a) Phase 1 study scheme. In part B, healthy subjects received single doses of either LY2775240 (20 mg) or apremilast (30 mg) in week 1. After a washout period of at least 5 days, subjects who received LY2775240 in week 1 received apremilast in week 2. Subjects who received apremilast in week 1 received LY2775240 in week 2. (b) Plasma concentrations following single administration. Each data point represents the mean plasma concentration ($n = 11$ subjects each). (c) PD responses of PDE4 inhibition using the TruCulture LPS assay system over 24 h. Clinically, apremilast is administered twice daily, for this reason the line after 12 h is dashed. Each data point represents the median ($n = 11$ subjects). LPS, lipopolysaccharide; PD, pharmacodynamic; PDE4, phosphodiesterase 4

course of a 24-h duration. The objectives of this study were to explore for the first time the safety, tolerability, PK, and PD profile of LY2775240 in a two-part, single-ascending dose study in healthy subjects.

The in vitro and in vivo characterization identified LY2775240 as a potential best-in-class PDE4 inhibitor. Using a human whole blood functional assay of PDE4 inhibition, we measured a potency of 18 nM for LY2775240; in contrast, apremilast was consistently an order of magnitude less potent (Figure 2). Whereas greater than 80% inhibition was measured in these assay, studies with lung explants and roflumilast interestingly measured ~ 60% inhibition.[21] Profiling using a multicellular assay system indicated LY2775240 had activity consistent with PDE4 inhibitors (Figure S2). In two preclinical models of LPS-stimulated TNFα production, one in mice and one in rhesus monkeys, oral dosing with LY2775240 dose-dependently inhibited PDE4-mediated responses (Figure 2). These data supported further evaluation of LY2775240 in a Phase 1 clinical trial.

TABLE 1 Baseline demographics and characteristics

	Cohort 1, part A	Cohort 2, part A	Cohort 3, part B
	$N = 11$	$N = 12$	$N = 11$
Age, mean ± SD	42.2 ± 9.4	40.9 ± 10.2	36.8 ± 6.6
% male, mean	90.9	91.7	100
Weight, kg ± SD	73.5 ± 11.4	74.6 ± 10.8	73.2 ± 9.96
BMI, kg/m^2 ± SD	25.0 ± 3.04	25.1 ± 2.78	24.8 ± 2.93
Asian sub-race			
Chinese	10	7	4
Indian	1	0	0
Sri Lankan	0	1	0
Pakistani	0	1	0
Indonesian	0	0	1
N/A	0	3	6

Abbreviations: BMI, body mass index; N/A, not applicable.

TABLE 2 TEAEs related to study treatment

	Part A Placebo ($N = 16$)	Part A 0.1 mg ($N = 6$)	Part A 0.5 mg ($N = 6$)	Part A 1.5 mg ($N = 6$)	Part A 5 mg ($N = 6$)	Part A 10 mg ($N = 6$)	Part A 20 mg ($N = 6$)	Part A 30 mg ($N = 6$)	Part A 40 mg ($N = 6$)	Part B 20 mg LY2775240 ($N = 11$)	Part B 30 mg Apremilast ($N = 11$)
Nausea						2		4	5	3	
Diarrhea					2		1	5	1		
Headache	3				1		1		3	3	
Vomiting						1		4	2	2	
Dizziness									2	1	
Increased appetite				2							
Epigastric discomfort	2										
Abdominal distension								1			1
Chest pain						1					
Cold sweat									1		
Cough	1										
Decreased appetite						1					
Oropharyngeal pain	1										
Palpitations						1					
Pyrexia	1										
Rash pruritic					1			1			
Rhinorrhea											
Eructation										2	1
Constipation										1	
Flatulence											1
Somnolence											1

Abbreviation: TEAEs, treatment emergent adverse events.

TABLE 3 Pharmacokinetics of LY2775240 (Phase 1, part A)

	LY2775240 0.1 mg N = 6	LY2775240 0.5 mg N = 6	LY2775240 1.5 mg N = 6	LY2775240 5 mg N = 6	LY2775240 10 mg N = 6	LY2775240 20 mg N = 6	LY2775240 30 mg N = 6	LY2775240 40 mg N = 6
C_{max}, ng/ml	8.80 (27)	30.0 (22)	76.5 (43)	213 (31)	319 (51)	410 (45)	669 (35)	799 (46)
T_{max}, h[a]	1.00 (1.00, 2.00)	1.50 (0.50, 2.00)	1.00 (1.00, 2.00)	1.50 (0.50, 2.02)	2.00 (1.00, 3.88)	1.50 (1.00, 3.88)	3.88 (1.00, 3.88)	3.88 (0.50, 3.88)
AUC_{0-24}, ng h/ml	52.7 (39)	188 (37)	467 (55)	1590 (36)	2750 (51)	3530 (63)	5620 (23)	7650 (62)
AUC_{inf}, ng h/ml	59.1 (45)	211 (42)	524 (58)	1850 (40)	3160 (55)	4090 (67)	6330 (23)	9000 (70)
$t_{1/2}$, h[b]	9.74 (6.47, 15.5)	11.9 (6.99, 15.7)	13.5 (12.0, 15.5)	11.9 (10.8, 12.8)	12.6 (11.5, 13.9)	12.2 (11.8, 12.8)	12.3 (11.8, 13.2)	12.1 (10.7, 14)
CL/F (L/h)	1.69 (45)	2.36 (42)	2.86 (58)	2.71 (40)	3.17 (55)	4.90 (67)	4.74 (23)	4.45 (70)
Vz/F (L)	23.8 (29)	40.7 (58)	55.7 (70)	46.4 (42)	57.6 (63)	86.3 (66)	84.3 (25)	77.7 (60)

Abbreviations: $AUC_{0-\infty}$, area under the concentration time curve from time 0 extrapolated to infinity; AUC_{0-24}, area under the concentration time curve from time 0 to 24 hours; CL/F, apparent total body clearance of drug following oral administration; C_{max}, maximum observed drug concentration; T_{max}, time of C_{max}; $t_{1/2}$, half-life associated with the terminal rate constant in non-compartmental analysis; Vz/F, apparent volume of distribution of drug during terminal phase following oral administration.

Data are geometric mean (coefficient of variability [CV%]), unless otherwise noted.

[a]Median (minimum, maximum).

[b]Geometric mean (minimum, maximum).

When administered as a single dose in healthy subjects, LY2775240 was well-tolerated with an adverse event profile that was generally consistent with other oral PDE4 inhibitors (Tables 1, 2). Like other PDE4 inhibitors, the most common AEs were related to the gastrointestinal system.[22,23] The majority of the gastrointestinal AEs with LY2775240 occurred at doses at or above 20 mg. Following oral administration, LY2775240 was rapidly absorbed, with a median time of maximum plasma concentration occurring at 1–4 h postdose; the mean terminal half-life was ~ 9.7–13.5 h across all dose levels (Table 3). Systemic exposure, based on AUC and C_{max}, appeared to increase subproportionally; the reason for this is not completely understood and was not observed in monkey studies (data on file) and will need future investigation. At this time, we cannot rule out nonlinearity in drug bioavailability or elimination, or a combination of both, with increasing doses of LY2775240. The PK profile supports a once-daily dosing regimen.

We incorporated an ex vivo assay measuring PDE4 inhibition as part of this clinical study and measured increasing inhibition of TNFα production with increasing dose levels of LY2775240 (Figure 3). At all time points postdose, the 20 mg dose of LY2775240 achieved between 50% and 80% inhibition in this functional assay, and this profile was consistent in both parts of the study, correlating well with the increasing plasma concentrations as indicated by the PK profile. When we directly compared a single 20 mg dose of LY2775240 with the clinical dose of apremilast (30 mg), we found that LY2775240 compared quite favorably (Figure 4). Apremilast achieved between 10% and 50% inhibition during the first 12 h postdose. As the apremilast clinical dose is 30 mg twice daily, the inhibition observed after 12 h in our study is unlikely to reflect the PD effect under the approved clinical dosing regimen. Overall, these results for apremilast are aligned with reported values from a prior study,[24] in which an inhibitory effect was reported at 2 h postdose on days 1 and 29 during treatment with 20 mg once-daily apremilast; however, information on time course of inhibition is lacking in these literature reports.

To our knowledge, this is one of the first instances where a functional ex vivo assay measuring the full time course of PDE4-mediated TNFα inhibition has been carefully implemented in a clinical study. Ex vivo assays are difficult to implement to evaluate a time course of PD effect within a clinical setting, and this is partly due to the technical factors, as assay results can be highly variable if bench processing and other steps are not done correctly. Before using this assay in the clinical study, we first evaluated LY2775240 both in vitro and with two nonclinical models, and these experiences enabled us to refine our methods prior to implementation of this assay in the clinical study. The results of the ex vivo PD assay demonstrated potent and sustained inhibition of PDE4 function with a 20 mg dose of LY2775240 and, in fact, a 5 mg

dose of LY2775240 was shown to result in similar levels of PDE4 inhibition as the clinical dose of apremilast. The clinical hypothesis is that increased inhibition of PDE4 will result in greater clinical benefit, and this information helps build a connection between target engagement and clinical efficacy. Together with the safety, tolerability, and PK data, this PD information can help guide the dose selection of LY2775240 in subsequent clinical studies. Overall, the safety, tolerability, and PK/PD profiles of LY2775240 reported here support further development of LY2775240 for psoriasis and other inflammatory disorders.

ACKNOWLEDGMENTS

The authors would like to thank the many dedicated Lilly employees who contributed as well as the subjects who participated in the study. Specifically, we thank (in alphabetical order):

Garrett Adams, Robert Benschop, Henry Bryant, Thomas Bumol, Veavi Chang, Shane Pih Chua, Kenneth Custer, Jeffrey Dodge, Parag Garhyan, Ronan Kelly, Paul Klekotka, Robert Konrad, Ajay Nirula, Lance Pfeiffer, Timothy Richardson, Jochen Schmitz, Amy Schramm, Kannan Thirunavukkarasu, Choayu Xie, and Lian Zhou.

CONFLICT OF INTEREST

All authors were employees of Eli Lilly and Co. during study duration.

AUTHOR CONTRIBUTIONS

D.R.P., S.U., S.H., K.C., and D.J.D., wrote the manuscript. D.R.P., S.U., D.P., K.C., and D.J.D. designed the research. S.H., C.J.B., Y.M., S.E.S., and K.C. performed research. S.U., J.L., K.C., and D.J.D. analyzed the data. M.S.Z. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Patel DR, Urva S, Ho S, et al. Characterization of LY2775240, a selective phosphodiesterase-4 inhibitor, in nonclinical models and in healthy subjects. *Clin Transl Sci*. 2021;14:1037–1048. https://doi.org/10.1111/cts.12968