ATI-1777, a Topical Jak1/3 Inhibitor, May Benefit Atopic Dermatitis without Systemic Drug Exposure: Results from Preclinical Development and Phase 2a Randomized Control Study ATI-1777-AD-201

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Introduction: Atopic dermatitis, a chronic, pruritic skin disease, affects 10−30% of children and up to 14% of adults in developed countries. ATI-1777, a potent and selective Jak1/3 inhibitor, was designed with multiple sites of metabolism to deliver local efficacy in the skin and limit systemic exposure. In preclinical studies, ATI-1777 selectively inhibited Jak1/3 with limited systemic exposure and without any adverse effects. **Primary objective:** The primary goal of this study was to assess the preliminary clinical efficacy of ATI-1777 topical solution in adults with moderate or severe atopic dermatitis. **Design:** ATI-1777-AD-201, a phase 2a, first-in-human, randomized, double-blind, vehicle-controlled, parallel-group study, evaluated the efficacy, safety, tolerability, and pharmacokinetics of ATI-1777 topical solution in 48 participants with atopic dermatitis over 4 weeks. **Primary endpoint:** The primary endpoint was a reduction of a modified Eczema Area and Severity Index score from baseline. **Results:** Reduction was significantly greater in the ATI-1777−treated group on day 28 than in vehicle-treated group (percentage reduction from baseline = 74.45% [standard error = 6.455] and 41.43% [standard error = 6.189], respectively [$P < .001$]). Average plasma concentrations of ATI-1777 were <5% of the half-maximal inhibitory concentration of ATI-1777 for inhibiting Jak1/3. No deaths or serious adverse events were reported. **Conclusion:** Topical ATI-1777 does not lead to pharmacologically relevant systemic drug exposure and may reduce clinical signs of atopic dermatitis. **Trial Registration:** The study was registered at ClinicalTrials.gov with the number NCT04598269.

Keywords: Hematopoiesis, Metabolism, Porcine model, Screening, Skin

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Abbreviations: AD, atopic dermatitis; AE, adverse event; BSA, body surface area; IGA, Investigator's Global Assessment; IC_{50}, half-maximal inhibitory concentration; Jaki, Jak inhibitor; mEASI, modified Eczema Area and Severity Index; PK, pharmacokinetic; PP-NRS, Peak Pruritus Numerical Rating Scale; SE, standard error; STAT, signal transducer and activator of transcription; TEAE, treatment-emergent adverse event

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, pruritic, inflammatory skin disease involving inflammation and skin barrier defects in response to environmental stimuli. AD can cause significant morbidity and adversely affect QOL (Kapur et al, 2018; Tanei, 2020). Approximately, 10−30% of children and up to 14% of adults in developed countries are affected by AD (Kim et al, 2019; Montilla et al, 2019; Napolitano et al, 2020).

Topical corticosteroids, the most common AD treatments, are first-line treatments in patients who fail to respond to appropriate skin care and use of emollients. However, skin lesions commonly worsen after corticosteroid discontinuation, and corticosteroids can result in significant toxicity (Hengge et al, 2006). The first-line systemic AD treatment for moderate or severe AD in adults is dupilumab (Dupixent), a fully humanized mAb against the alpha subunit of IL-4 and IL-13 receptors. In addition, a few oral Jak inhibitors (Jakis)

have been approved for AD, each with black box warnings (see below). Because of the limitations of current therapeutic options for patients with AD, there is a role for additional efficacious treatments with acceptable safety profiles.

Lesional skin from patients with AD contains elevated levels of proinflammatory cytokines and T helper cell infiltration that propagate disease pathophysiology (Howell et al, 2019; Rodriques and Torres, 2020). The Jak family of signal transducers is an advantageous treatment target because a variety of cytokines exert their biological effects through the Jak–signal transducer and activator of transcription (STAT) pathway. AD is particularly driven by IL-4, IL-13, and IL-31, which are largely regulated by Jak1 or Jak3. Therefore, Jak1/3 inhibitors may aid in controlling AD-associated inflammation.

The efficacy of Jakis approved for use, such as orally administered baricitinib (OLUMIANT; Jak1/2 inhibitor) (Guttman-Yassky et al, 2019), upadacitinib (RINVOQ; Jak1 inhibitor) (Guttman-Yassky et al, 2020), and abrocitinib (CIBINQO; Jak1 inhibitor) (Gooderham et al, 2019) and topically administered delgocitinib (Corectim; pan-Jaki) (Dhillon, 2020) and tofacitinib (XELJANZ; Jak1/3) (Bissonnette et al, 2016) provides proof of concept for Jaki as treatments for AD. However, systemic Jakis have been associated with a risk of serious adverse events (AEs). United States Food and Drug Administration–approved Jakis carry black box warnings for serious infections, mortality, malignancy, major adverse cardiovascular events, and thrombosis (OPZELURA Prescribing Information, RINVOQ Prescribing Information, XELJANZ Prescribing Information, CIBINQO Prescribing Information). Although topical administration may lower the systemic exposure potentially responsible for these events, plasma drug levels described in the label for topical ruxolitinib (OPZELURA; OPZELURA Prescribing Information) approximate or exceed those required for blockade of IFN-γ (305 nM) and IL-4 (564 nM), suggesting that more limited systemic exposure may be needed to fully capture this benefit of topical application (unpublished data on human whole blood potency of ruxolitinib).

ATI-1777 is an adenosine triphosphate–competitive Jak1/3 inhibitor that blocks STAT phosphorylation and downstream effects on gene expression. ATI-1777 was designed to act locally in the skin and to be rapidly metabolized in the blood, resulting in subpharmacological systemic levels of the drug, an approach aimed to provide efficacy in dermatological diseases such as AD and minimize the risks of systemic toxicity. Once potency and stability of ATI-1777 were achieved, a unique pharmacological pig model was designed to evaluate the topical efficacy of a solution formulation. Finally, to evaluate the efficacy, safety, and tolerability of ATI-1777, a phase 2a, first-in-human study (ATI-1777-AD-201 ClinicalTrials.gov, NCT04598269) was conducted in participants with AD. We provide an overview of the path from preclinical discovery to clinical development of ATI-1777 as well as results from Study ATI-1777-AD-201.

RESULTS

Preclinical studies

ATI-1777 development. We incorporated metabolically labile substituents into known Jak pharmacophores (Figure 1). Substituting a pyrrolopyridine core into a Jak pharmacophore resulted in compound 1, and adding a metabolically labile ethyl ester resulted in compound 2, which demonstrated decreased potency versus compound 1, so the N-methyl was removed, providing compound 3. By design, compound 3 is stabilized in the desired active conformation by the intramolecular H-bond to the ester, which improved Jak potency and cell permeability. Removal of the piperidine-methyl in compound 3 led to ATI-1777 where unexpectantly, the methyl on the piperidine ring was not required for potency or high kinase selectivity. Overall, these efforts led to ATI-1777, a potent and selective Jak1/3 inhibitor with multiple sites of metabolism resulting in high clearance and limited systemic exposure.

Jak family member inhibition in vitro. The inhibitory activity of ATI-1777 was examined in vitro against the catalytic domain of each Jak family member expressed as a recombinant human protein. Under nonphysiological, Km adenosine triphosphate concentrations, ATI-1777 potently and differentially inhibited the catalytic activity of Jak family members with half-maximal inhibitory concentration (IC_{50}) values in the low nanomolar range (Table 1). To address the kinome selectivity of ATI-1777 for inhibition of Jak family members, 214 human protein kinases were assessed. Ten of the alternate kinases showed ≥50% inhibition by ATI-1777 at a concentration of 1 μM. On the basis of IC_{50} values, the selectivity of ATI-1777 for Jak1/3 relative to those of these alternate kinases ranged from 32 to 833 fold (Table 2).

To assess the selectivity of ATI-1777 against Jak family members under physiological adenosine triphosphate concentrations, inhibition of STAT phosphorylation was determined in isolated PBMCs. ATI-1777 was most potent for Jak1/3, with approximately 5-fold selectivity over Jak1/2 and approximately 42- to 47-fold selectivity over Jak2 (Table 3).

To determine the full potential of ATI-1777 kinase inhibition, the major ATI-1777 metabolite CDD-1913 was evaluated for its kinase inhibition ability. CDD-1913 is formed through esterase hydrolysis of ATI-1777 and is further metabolized by the liver. The only kinase to be inhibited

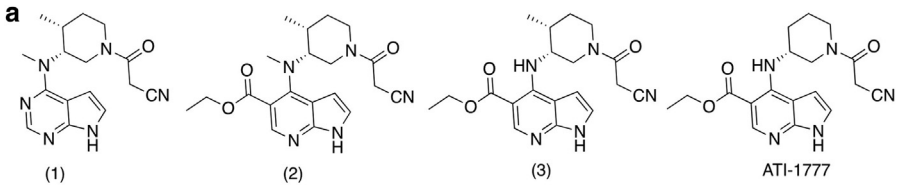

Figure 1. Development of ATI-1777. (**a**) ATI-1777 was developed using a series of steps to yield the final product. Compound 1 shows a Jak pharmacophore with pyrrolopyridine core substitution (1). Next, an ethyl ester was added to compound 1, resulting in compound 2 (2). An N-methyl was removed from compound 2, resulting in compound 3 (3). Finally, the piperidinemethyl was removed to yield ATI-1777.

Table 1. Inhibition of Catalytic Domain Activity of Jak Family Members

Enzyme	ATI-1777 IC_{50} (nM)
Jak1	1.5, n = 1
Jak3[1]	3.6 ± 0.9[1]
Jak2	7.1, n = 1
TYK2	19.0, n = 1

Abbreviation: IC_{50}, half-maximal inhibitory concentration.
[1]Value represents mean ± SD determined from 8 different lots of ATI-1777.

>80% by CDD-1913 was Jak3 (94%), with a potency of 239 nM. In human PBMCs, CDD-1913 did not inhibit IL-2–mediated Jak1/3 activation (IC_{50} value > 20,000 nM), providing a selectivity index of over 1000 times, versus ATI-1777.

Metabolism and pharmacokinetics. Using the unified model for predicting human hepatic and metabolic clearance from human and animal microsomes (Riley et al, 2005), the half-life for loss of parent compound (ATI-1777) was ≤30 minutes (Table 4).

ATI-1777 stability was examined with recombinant human cytochrome P450 enzymes to identify specific isozymes responsible for ATI-1777 metabolism. In the presence of nicotinamide adenine dinucleotide phosphate, ATI-1777 was most extensively metabolized by CYP3A4 (0.56% remaining) and, to a lesser extent, by CYP2C8, CYP1A2, and CYP2C19 (41.2, 55.7, and 75.5% remaining, respectively). No appreciable metabolism of ATI-1777 was observed with CYP2D6, CYP2C9, or CYP2B6 (Table 5).

Activity in a porcine model. Previous studies assessing AD treatments have utilized murine models. Owing to the thinness of mouse skin, it was suspected that this could lead to systemic drug levels, which may confound any conclusions of topical activity. Therefore, a minipig model was developed to test ATI-1777 topical absorption and its effect on the Jak pathway in a model of cytokine-driven skin inflammation because pig skin is similar to human skin in general structure, thickness, hair follicle content, pigmentation, collagen, lipid composition, and immunology.

Multiple systems were attempted to identify a robust preclinical system that would respond to Jak-dependent cytokines in pig skin, allowing us to gauge the activity of topically applied ATI-1777 in various formulations. Although cytokines such as IL-4, IL-13, and IL-31 are thought to be more critical to AD, IL-15 was selected for the in vivo pig model because porcine IL-15 was commercially available, and signals induced by IL-13 and IL-31 were not readily detectable in the pig model (eg, phosphorylated STAT levels in skin lysates, data not shown). To select the most robust assay for this pharmacodynamic model, multiple sites on the back of a pig were injected intradermally with saline or porcine IL-15, and after 3 hours, the skin was harvested, and RNA was prepared. Fifteen differentially expressed genes (>4-fold) were identified relative to the naïve group by bulk RNA sequencing analysis (Figure 2a). Five genes in the IL-15–treated pigs were significantly upregulated compared with those in the vehicle-treated group (Figure 2b). Selected upregulated genes were verified by qPCR (Figure 2c). *CCL8* was a strongly induced gene and used to profile multiple formulations containing ATI-1777, for their ability to block Jak–induced gene expression. The formulation chosen for inclusion in the spray was the only one that was able to block this effect.

Table 2. Determination of ATI-1777 IC_{50} for Kinases of Potential Cross-Over

Kinase	Class	ATI-1777 IC_{50} (nM)	Fold Selectivity
Aurora A	Serine/threonine	641	427
KDR (VEGFR2)	Tyrosine	127	85
TXK	Tyrosine	351	234
HPK1 (MAP4K1)	Serine/threonine	285	190
BTK	Tyrosine	210	140
MARK1	Serine/threonine	1250	833
MKNK1/MNK1	Serine/threonine	584	389
RSK2	Serine/threonine	1050	700
FLT4 (VEGFR3)	Tyrosine	113	75
LRRK2	Serine/threonine	48	32
Jak1	Tyrosine	1.5	1

Abbreviation: IC_{50}, half-maximal inhibitory concentration.
n = 1 for each kinase.

Table 3. Efficacy of ATI-1777 in Human PBMCs

		ATI-1777	
Ligand	Kinase	IC_{50} nM Mean ± SD	Fold Jak1/3
IL-2	Jak1/3	19 ± 6, n = 12	1.0
IFN-γ	Jak1/2	97 ± 34, n = 12	5.1
GM-CSF	Jak2/2	794 ± 121, n = 5	41.8
IL-12	Jak2/TYK2	898 ± 178, n = 3	47.3

Abbreviations: IC_{50}, half-maximal inhibitory concentration; STAT, signal transducer and activator of transcription; TYK2 = tyrosine kinase 2.
Primary PBMCs were preincubated with ATI-1777 (final concentrations: 20 μM to 2×10^{-6} μM) at 37 °C for 1 hour, followed by 5–10-minute incubation with the cytokine of interest. Cells were lysed, and the phosphorylation state of the relevant STAT was determined by immunoassay.

Table 4. In Vitro Metabolic Stability of ATI-1777 in Liver Microsomes

Study Number	1		2	
Species	$T_{1/2}$ (min)	CL_{int} (ml/min/kg)	$T_{1/2}$(min)	CL_{int} (μl/min/mg)
Human	8.71	200	19	74
Rat	6.70	370	27	52
Mouse	1.66	3281	ND	ND
Dog	17.4	198	30	46
Monkey	2.16	939	5	264
Minipig	2.85	365	5	295

Abbreviations: CL_{int}, intrinsic clearance; ND, not determined; $T_{1/2}$, elimination half-life.
The differences in intrinsic clearance between the 2 studies are likely due to different scaling factors used in the calculations. n = 1.

Table 5. Metabolism of ATI-1777 by CYP Enzymes in the Presence of NADPH

	Percentage Remaining (%)						
Compound	**CYP1A2**	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
Reference	15.1	20.7	0.852	2.83	7.43	2.48	0.252
ATI-1777	55.7	93.9	41.2	91.3	75.5	87.9	0.560

Abbreviations: cytochrome P450; NADPH, nicotinamide adenine dinucleotide phosphate.
The following reference substrates were used as controls: phenacetin for CYP1A2, diclofenac for CYP2C9, S-mephenytoin for CYP2C19, bufuralol for CYP2D6, midazolam for CYP3A4, bupropion for CYP2B6, and paclitaxel for CYP2C8. n = 1.

The intended clinical formulation of ATI-1777 (2% solution) was applied to the backs of pigs, and 1 hour later, IL-15 was injected into the dermis. Three hours after IL-15 injection, sites were biopsied, and CCL8 expression was quantified using qPCR as a measure of IL-15 action and the ability of ATI-1777 to block this signaling. ATI-1777 application inhibited IL-15–induced CCL8 expression in pig skin by 83% compared with treatment with vehicle solution ($P = .0031$) (Figure 2d).

Porcine toxicology and tolerance. To support clinical trials in AD, dermal tolerability and toxicokinetics were measured

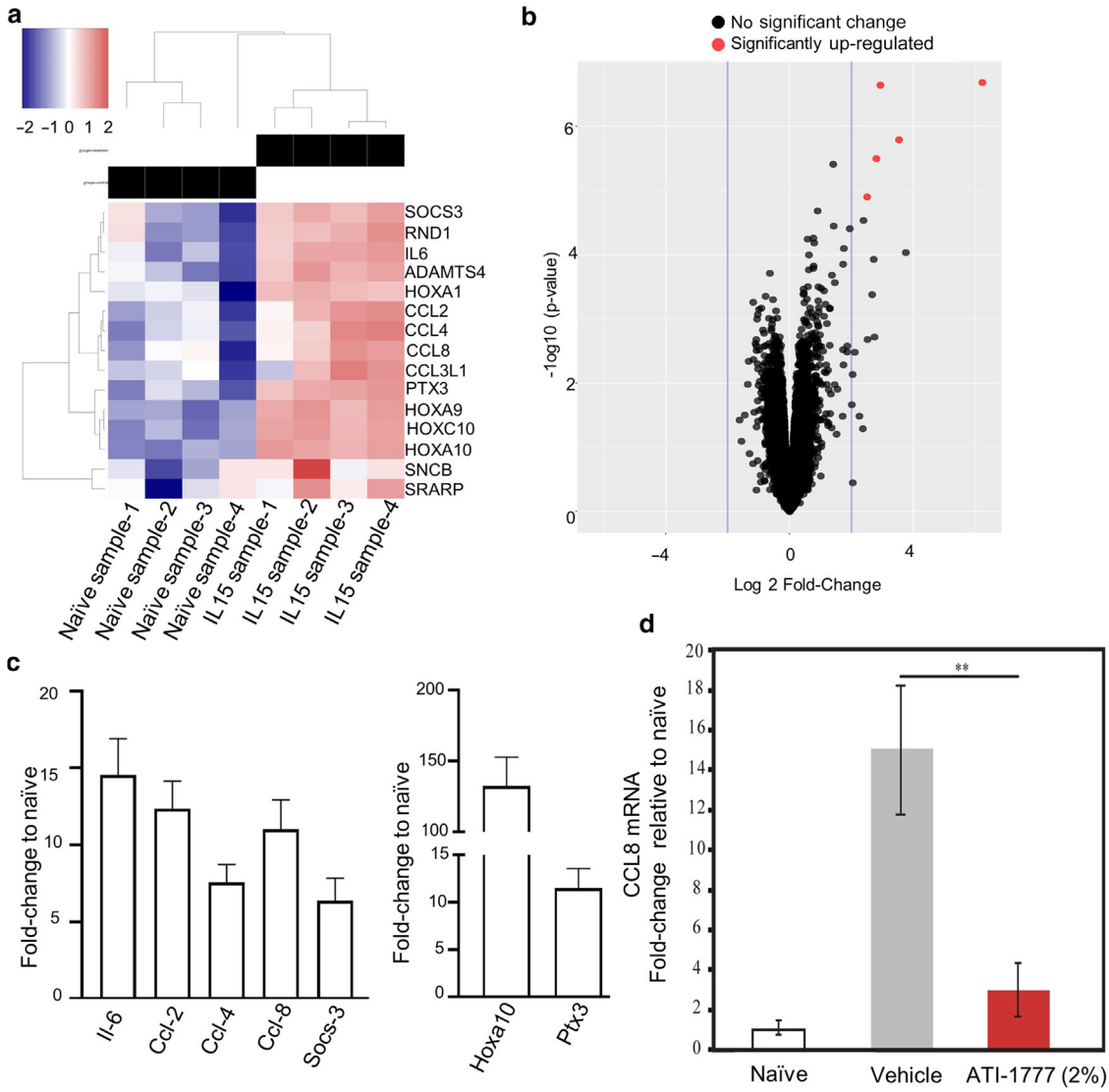

Figure 2. PD effects of ATI-1777 in a minipig model. (**a**–**c**) Locations on a minipig were treated intradermally with saline (naïve) or IL-15; n = 4. (**a**) Heat map of RNA sequencing showing differentially expressed genes. (**b**) Volcano plot showing $\log_2$ fold changes in gene expression. Red dots represent significantly upregulated genes *Hoxa10*, *Hoxc10*, *Ptx3*, *Adamts4* and *Hoxa9*. (**c**) qPCR validation of transcription of upregulated genes in IL-15–treated minipigs. n = 8. (**d**) Sites on a minipig were treated with vehicle or vehicle containing 2% ATI-1777. After 1 hour, sites were injected intradermally with IL-15. Three hours later, biopsies were harvested. *CCL8* mRNA was quantified by RT-PCR and expressed as fold change relative to naïve pig skin. n = 8. Significance was determined by a 2-sided *t*-test. Error bars represent SEM.

in 2 minipig studies: a 7-day tolerability and pharmacokinetic (PK) study and a 28-day repeat exposure study. The 28-day study was conducted in accordance with the United States Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies.

In the 7-day study, the intended clinical solution, 2% ATI-1777, was applied to the skin for 7 consecutive days. There were no adverse clinical observations or changes in clinical pathology parameters throughout the dosing period. PK analyses showed that systemic exposure to ATI-1777 was unquantifiable (lower limit of quantification [LLOQ] = 0.5 ng/ml) at most time points (Table 6).

During the 28-day study, the topical ATI-1777 solution was administered daily for 28 days at doses of 0, 40, 80, and 160 mg/day and remained on the skin for 20–22 hours each day (application site was wiped clean with a moist towel at end of each day). No ATI-1777–related effects were noted at any dose. Plasma concentrations of ATI-1777 were nonquantifiable in most pigs who received 40 and 80 mg/day of ATI-1777 on days 1 and 28, and when quantifiable, plasma concentrations were low (≤4.94 ng/ml) (Table 7). For the 160 mg group on day 28, quantifiable plasma concentrations were observed for all pigs, albeit still low (<6.26 ng/ml). These plasma concentrations were well below the IC_{50} of ATI-1777 for inhibiting Jak1/3 (45 ng/ml). CDD-1913 plasma concentrations were nonquantifiable on days 1 and 28 in all ATI-1777–treated pigs.

At the time of terminal sacrifice (day 28), pigs had high and sustained concentrations of ATI-1777 in the skin (≥4280 ng/g in the skin from males and ≥1960 ng/g in the skin from females) but no consistent pattern of systemic exposure across time points. Quantifiable concentrations of CDD-1913 were observed in some skin samples, but the levels were substantially lower than those of the parent compound (~4 orders of magnitude lower). These data demonstrated that administration of ATI-1777 topical solution on minipigs did not present any adverse effects, showed significant skin concentrations, demonstrated pharmacodynamic responses, and had limited systemic bioavailability.

Formulation selection for use in clinical trials. Cream, gel, ointment, foam, aqueous, and nonaqueous solutions were evaluated for topical delivery of ATI-1777 (data not shown), and a nonaqueous solution delivered in a spray bottle was chosen as the final dosage form. The solution formulation offered targeted epidermis/dermis delivery. The use levels of all excipients were equal to or below the maximum use levels present in the United States Food and Drug Administration's Inactive Ingredient Database for topical application.

Table 6. Mean Plasma Concentration (ng/ml) of 2% ATI-1777 after 7-Day Exposure in Minipigs

	Time after Dose (h)						
Dose (mg/day)	**0**	**1**	**2**	**4**	**8**	**12**	**24**
160	0.00	0.00	0.387	0.221	0.391	0.00	0.00

Abbreviation: BSA, body surface area.
n = 3 males.
Dose = 8 mls of 2% solution applied over 400 cm^2 (10% BSA).

Clinical trial

Disposition and baseline demographics. ATI-1777-AD-201 was a phase 2a, first-in-human, randomized, double-blind, vehicle-controlled, parallel-group study to evaluate the efficacy, safety, tolerability, and PK of 2% ATI-1777 topical solution applied twice daily for 4 weeks compared with those of vehicle, with a subsequent 2-week follow-up period. Participants could apply up to 8 ml of solution twice daily.

Overall, 50 participants with AD were randomized (Figure 3). All 50 randomized participants were treated, but 2 of these patients were lost to follow-up after day 1, and their dosing diary date and time were missing, so they were not included in the full analysis set. The primary reasons for screen failure were participants not willing to refrain from the use of moisturizers, emollients, and sunscreen on AD study treatment areas for the duration of protocol therapy (9 participants), participants who did not have at least a 6-month history of AD before the screening visit (9 participants), and participants with no significant AD flares for the 4 weeks before the screening visit (8 participants). Two participants in the ATI-1777–treated group and 7 participants in the vehicle-treated group discontinued treatment early. The reasons for discontinuation were withdrawal by participant (1 participant in the ATI-1777-treated group, 4 participants in the vehicle-treated group), AE (no participants in the ATI-1777-treated group and 3 participants in the vehicle-treated group), and loss to follow-up (1 participant in the ATI-1777-treated group, no participants in the vehicle-treated group) (Figure 4). The majority of participants were female (75.0%) and White (56.3%). The median age was 44 years, and the range was 18–65 years. The majority of participants (95.8%) had an Investigator's Global Assessment (IGA) of 3. To evaluate the extent and severity of AD, a modified Eczema Area and Severity Index (mEASI) was employed that excludes the evaluation of the scalp, face, palms of hands, soles of feet, groin and genitalia. Mean baseline mEASI scores and AD body surface area (BSA) affected were similar between treatment groups (Table 8).

Efficacy of ATI-1777. On day 28, the primary endpoint of percentage reduction from baseline in mEASI score was 74.45% (standard error [SE] = 6.455) in the ATI-1777–treated group, compared with 41.43% (SE = 6.189) in the vehicle-treated group ($P < .001$) (Figure 4a). Differences were observed as early as day 8, the first visit after beginning treatment. To further validate the results, a mixed model for repeated measures without imputation was used to analyze these data. Similar results were found, with a 78.46% (SE = 5.943) percentage reduction from baseline in mEASI score in the ATI-1777–treated group and a 51.03% (SE = 6.214) reduction in the vehicle-treated group.

More participants receiving ATI-1777 achieved a 50 or 75% reduction in mEASI (91.3 and 65.2%, respectively) than those receiving the vehicle (40 and 24%, respectively; $P = .003$) (Figure 4b and c). Overall, ~30% of participants (7 participants) in the ATI-1177–treated group and ~20% of participants (5 participants) in the vehicle-treated group achieved a 90% reduction in mEASI (Figure 4d). At the end of the treatment period, a nominally greater proportion of

Table 7. Mean (SD) Plasma Concentration (ng/ml) of 2% ATI-1777 after 28-Day Exposure in Minipigs

Dose Level (mg/Day)	Time after Dose (h)							
	0	0.5	1	2	4	8	12	24
40	0.340 (0.579)	0.143 (0.314)	0.0685 (0.217)	0.108 (0.342)	0.121 (0.383)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
80	0.118 (0.290)	0.647 (1.58)	0.0995 (0.244)	0.197 (0.482)	0.193 (0.474)	0.173 (0.425)	0.823 (2.02)	0.00 (0.00)
160	1.82 (1.74)	0.479 (1.03)	1.14 (1.40)	1.12 (0.921)	0.755 (1.03)	0.684 (1.20)	1.02 (2.02)	0.0569 (0.180)

n = 10 (5 males and 5 females).

participants in the ATI-1777–treated group (39.1%) achieved an IGA score of 0 or 1 with ≥2 point improvement than those in the vehicle-treated group (24%; $P = .131$) (Figure 4e).

At day 28, BSA affected by AD was reduced significantly more in the ATI-1777–treated group than in the vehicle-treated group (change from baseline −5.59 and −2.09%, respectively; $P < .001$) (Figure 4f). Change from baseline at day 15 was −3.33% in the ATI-1777–treated group versus −1.57% in the vehicle-treated group. Change from baseline at day 8 was −1.55% in the ATI-1777–treated group versus −0.37% in the vehicle-treated group. The least squared means change from baseline of Peak Pruritus Numerical Rating Scale (PP-NRS) scores was greater in the ATI-1777–treated group than in the vehicle-treated group (−2.92 [SE = 0.548] and −1.70 [SE = 0.536] in the ATI-1777–treated and vehicle-treated groups, respectively). The between-group difference in least squared means change from baseline trended toward but did not achieve statistical significance ($P = .060$) (Figure 4g). Together, these data indicate that ATI-1777 may reduce the clinical signs and symptoms of AD.

ATI-1777 PK. Approximately 86% of plasma samples in the ATI-1777–treated group had concentrations below the lower limit of quantification of 1 ng/ml, and all concentrations were less than half of the IC_{50} of ATI-1777 for inhibiting Jak1/3 (45 ng/ml) (Figure 5a). Mean drug levels at each timepoint were <5% of the IC_{50} of ATI-1777 for Jak1/3. Three participants had samples with concentrations >10% of the IC_{50} for Jak1/3, and only 2 samples from each of the 3 participants were above 3 ng/ml (Figure 5a). There was no correlation between clinical manifestation and plasma ATI-1777 concentration. Together, these data indicate that topical application of ATI-1777 over 28 days does not lead to pharmacologically relevant systemically active levels of ATI-1777 in the blood.

Safety of ATI-1777. No deaths or treatment-emergent AEs (TEAEs) of special interest (ie, application site AEs, serious infections, malignancies, thrombosis, cytopenia, liver enzyme elevation, elevations in lipid parameters) were reported. Most TEAEs were mild or moderate in severity and considered not related to the study treatment. AEs were reported for similar proportions in each treatment group (39.1% in the ATI-1777–treated group and 36.0% in the vehicle-treated group) (Table 9). Three TEAEs were reported in more than 1 participant in the study: urinary tract infection (1 participant in each group), blood creatinine phosphokinase increase (2 participants in the ATI-1777–treated group), and headache (2 participants in the ATI-1777–treated group). Both events of blood creatinine phosphokinase increase were transient and resolved without intervention. One event of blood creatinine phosphokinase increase was considered severe because it was medically significant; however, because the participant did not experience any clinical symptoms associated with the event, including no myalgias and no evidence of rhabdomyolysis, the event was not considered clinically significant. Both TEAEs of blood creatinine phosphokinase increase were assessed by the investigator not related to the study drug. A TEAE of atrial fibrillation in the ATI-1777–treated group was also considered severe and not related to the study drug. One related TEAE was reported in each treatment group: mild application site pruritus (ATI-1777–treated group) and moderate application site irritation (vehicle-treated group). No participants in the ATI-1777–treated group and 3 participants in the vehicle-treated group discontinued study participation owing to TEAEs.

DISCUSSION

ATI-1777 was developed to generate efficacy while addressing safety concerns associated with available systemic AD treatment by minimizing systemic exposure. The 2 key advances required to progress this concept were the

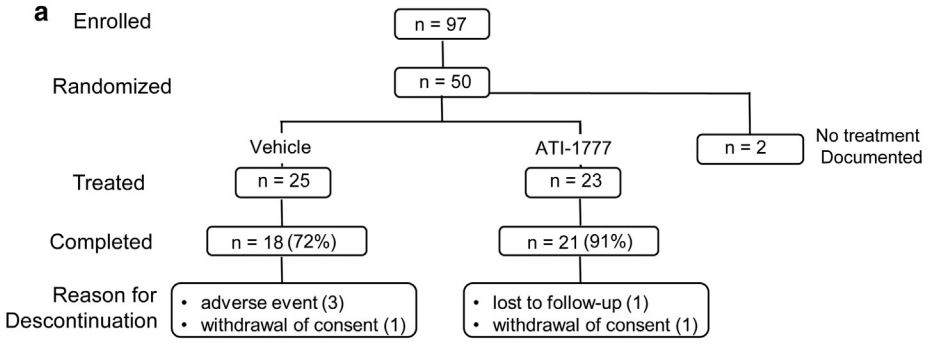

Figure 3. Disposition of participants enrolled in ATI-1777-AD-201. (**a**) Two participants randomized to the ATI-1777–treated group were lost to follow-up after day 1, and their dosing diary date and time were missing (ie, no treatment was documented).

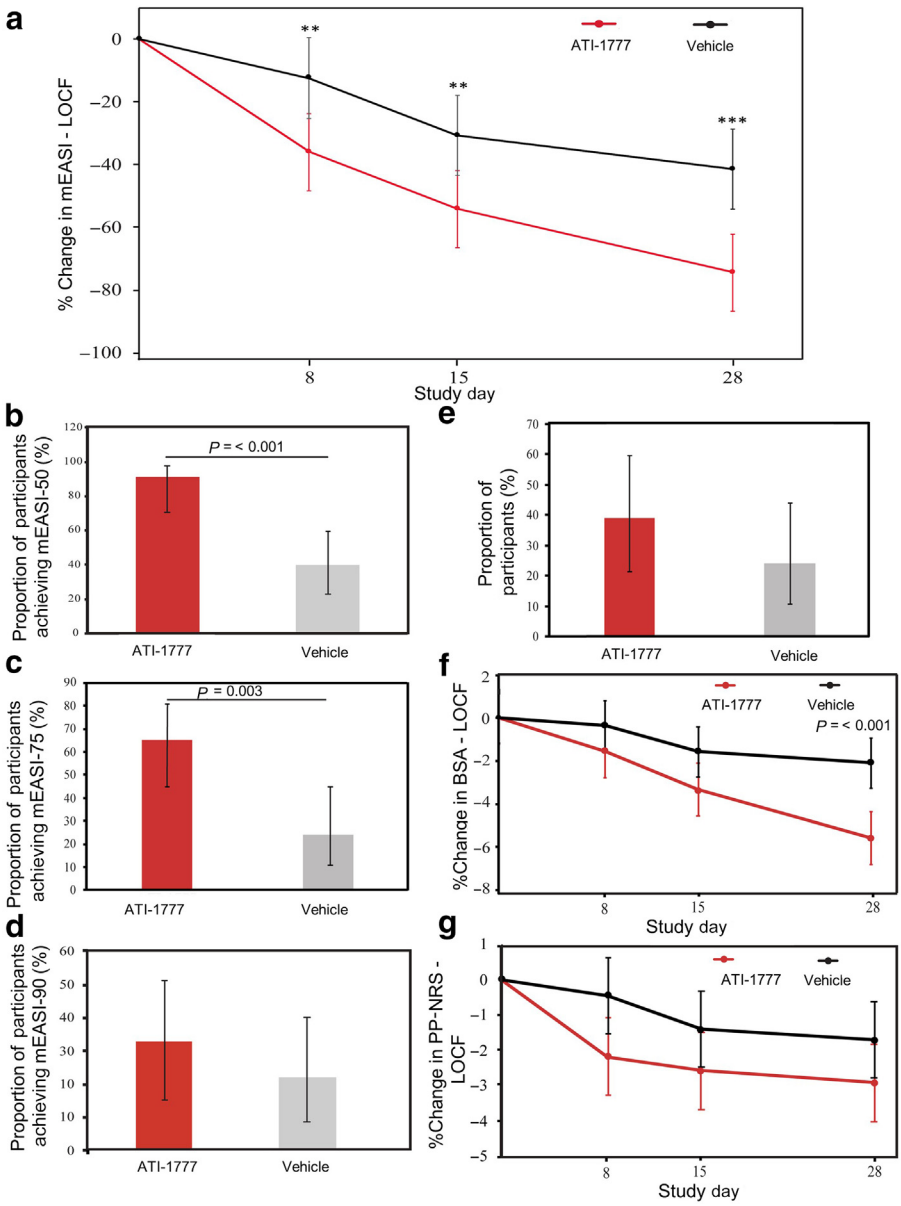

Figure 4. Measures of ATI-1777 clinical efficacy. (**a**) mEASI score change from baseline (after baseline −baseline) using the LOCF. This treatment comparison was made within the context of an MMRM analysis. The proportion of participants achieving (**b**) EASI-50, (**c**) EASI-75, and (**d**) EASI-90 on day 28. (**e**) The proportion of participants achieving IGA score of 0 or 1 with ≥2 point improvement at day 28. Change from baseline (after baseline −baseline) in (**f**) AD BSA and (**g**) PP-NRS score. The treatment comparisons were made within the context of an MMRM analysis. (**c, d**) *P*-values represent 1-sided *P*-value of responders in the treatment group at a 0.05 level of significance. Error bars represent 95% confidence intervals. ATI-777 group n = 23 and vehicle group n = 25. AD, atopic dermatitis; BSA, body surface area; IC_{50}, half-maximal inhibitory concentration; IGA, Investigator's Global Assessment; LOCF, last observation carried forward; mEASI, modified Eczema Area and Severity Index; MMRM, mixed model for repeated measure; PP-NRS, Peak Pruritus Numerical Rating Scale.

development of a potent and selective Jaki that rapidly disappeared from systemic circulation after topical application and the development of a translatable animal model of cytokine-mediated inflammation that predicted efficacy in human skin.

After its discovery, ATI-1777 and its major metabolite CDD-1913 were determined to have selectivity for Jak1/3 over other kinases within the Jak family and across the human kinome. In addition, ATI-1777 demonstrated low nanomolar potency for inhibition of Jak1/3 catalytic activity. A highly selective Jak1/3 inhibitor may minimize hematopoietic effects, such as increased platelet counts associated with systemic Jak2 inhibition (Kim et al, 2020). In vitro metabolism of ATI-1777 predicted rapid clearance of ATI-1777 in vivo, which was supported in 7- and 28-day porcine studies that found sustained concentrations of ATI-1777 in the skin, with little or no systemic exposure. Furthermore, in the first-in-human study of ATI-1777, plasma ATI-1777 concentrations were substantially below the IC_{50} of ATI-1777 for inhibiting Jak1/3, and ∼85% of treated participants had exposures below the lower limit of quantification. Therefore, ATI-1777 is not likely to have systemic clinical/toxicological significance. In contrast, other topically applied Jaki may reach systemic levels in excess of the Jak2/2 IC_{50} (OPZELURA Prescribing Information). Minimizing systemic exposure may decrease the risk of toxicities that result from chronic, long-term systemic Jaki exposure. Indeed, during the study ATI-1777-AD-201, ATI-1777 topical solution was well-tolerated over 4 weeks. No deaths or serious AEs were reported, and most TEAEs were mild or moderate and considered not related to study treatment.

To demonstrate inhibition of Jak1/3 signaling in the skin by topically applied ATI-1777, a porcine model was developed because topical application on mice may result in high levels of systemic drug exposure. IL-15−induced CCL8 expression was inhibited by ATI-1777. IL-15, a Jak1/Jak3-dependent

Table 8. Participant Demographics and Baseline Characteristics

Characteristic	ATI-1777 (n = 23)	Vehicle (n = 25)	Total (n = 48)
Female, n (%)	16 (69.6)	20 (80.0)	36 (75.0)
Age (y)			
Median	46.0	42.0	44.0
Q1, Q3	35.0, 52.0	29.0, 53.0	29.5, 53.0
Race, n (%)			
White	15 (65.2)	12 (48.0)	27 (56.3)
Black or African American	7 (30.4)	10 (40.0)	17 (35.4)
Asian	1 (4.3)	0	1 (2.1)
Ethnicity, n (%)			
Hispanic or Latino	5 (21.7)	5 (20.0)	10 (20.8)
Female of childbearing potential, n (%)	8 (34.8)	14 (56.0)	22 (45.8)
BMI, mean (SD)	32.178 (12.3)	32.089 (6.9)	32.131 (9.8)
Baseline AD severity, n (%)			
Moderate	22 (95.7)	24 (96.0)	46 (95.8)
Severe	1 (4.3)	1 (4.0)	2 (4.2)
Duration of disease, y			
Median	18.0	29.0	21.0
Q1, Q3	8.0, 35.0	13.0, 37.0	10.0, 37.0
mEASI score, mean (SD)	8.63 (3.8)	7.68 (3.7)	N/A
AD BSA, mean (SD)	9.61 (5.4)	6.96 (4.3).	N/A

Abbreviations: AD, atopic dermatitis; BMI, body mass index; BSA, body surface area; mEASI, modified Eczema Area and Severity Index; N/A, not available; Q1, first quartile; Q3, third quartile.

cytokine, acted as a surrogate for inhibition of IL-4, IL-13, and IL-31.

Clinical efficacy in the ATI-1777-AD-201 clinical trial found that the ATI-1777—treated group showed meaningful improvement over 4 weeks of treatment, as demonstrated by a reduction in mEASI. Reductions in mEASI were also observed in the vehicle-treated group, which may be attributed to the emollient present in the vehicle solution.

The ATI-1777—treated group improved at a faster rate than the vehicle-treated group, with differentiation between the groups starting at 1 week. Although not statistically significant for all measures because this was a small proof-of-concept study powered only for the primary endpoint, positive trends in favor of ATI-1777 were observed in key secondary measures, including mEASI-50/75/90 responders, IGA responders, and AD BSA change from baseline. Although oral Jakis have a high efficacy rate in reducing pruritus, topical ATI-1777 demonstrated a moderate but not statistically significant reduction in PP-NRS compared with the vehicle at day 28 ($P = .06$). This may be explained by the high clearance characteristics of ATI-1777. In addition, this clinical trial did not require a minimum PP-NRS score for enrollment, which may have resulted in enrollment of participants with low PP-NRS scores. These results are consistent with the previously reported efficacy of topically applied Jakis to treat AD (Bissonnette et al, 2016; Dhillon, 2020). Importantly, clinical efficacy was demonstrated while 86% of plasma samples were below the lower limit of quantification, and no quantifiable levels were above the ~IC_{25} of ATI-1777 for inhibiting Jak1/3. With respect to the concerns of systemic Jak2 inhibition, all plasma concentrations of ATI-1777 were below IC_{10} of ATI-1777 for inhibiting Jak1/2.

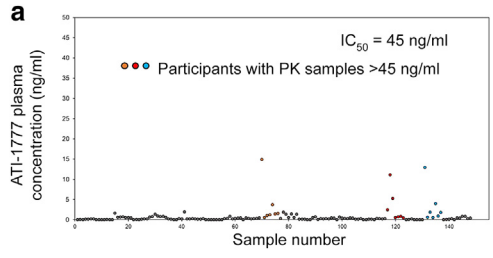

Figure 5. ATI-1777 systemic absorption. (**a**) A total of 8 PK samples were collected from each participant (2 samples per visit on days 0, 8, 15, and 28). Each circle represents 1 plasma sample (ng/ml). All samples from the 3 participants with concentrations >10% of the IC_{50} of ATI-1777 for inhibiting Jak1/3 are indicated in orange, red, and blue with each color representing a different participant. n = 25 participants who received ATI-1777. $^{**}P \leq .01$ and $^{***}P \leq .001$. IC_{50}, half-maximal inhibitory concentration; PK, pharmacokinetic.

Some limitations should be considered when interpreting these results. The clinical study had a relatively small sample size (23 participants received ATI-1777) and a relatively short duration of 4 weeks. The short duration may have adversely affected the significance of the outcomes, such as a reduction in pruritus, and not have provided adequate time to see clinically meaningful TEAEs or effects on immune cells present in the skin.

Currently approved treatments for AD either have limited efficacy or are associated with toxicities; new safe and effective treatments are needed. Overall, ATI-1777 is a highly selective Jak1/3 inhibitor that is rapidly cleared and metabolically labile. Clinical efficacy and lack of systemic exposure demonstrated in the phase 2a AD trial serve as proof of concept and support continued evaluation of ATI-1777 as a potential best-in-class Jaki that meets the needs for treatment of mild-to-moderate AD. These results are currently being expanded upon in an ongoing phase 2b study in participants with AD (NCT05432596).

MATERIALS AND METHODS

Preparation of ethyl (R)-4-((1-(2-cyanoacetyl)piperidin-3-yl) amino)-1H-pyrrolo[2,3-b]pyridine-5-carboxylate (ATI-1777)

To a solution of ethyl 4-chloro-1H-pyrrolo[2,3-b]pyridine-5-carboxylate (5.00 g, 22.3 mmol) in N-methyl-2-pyrrolidone (35 ml) was added (R)-1-benzylpiperidin-3-amine (8.48 g, 44.6 mmol) and triethylamine (1 ml) in a sealed tube. The mixture was heated at 170 °C for 16 hours. After cooling to room temperature, the reaction mixture was quenched with water to precipitate a solid, which was filtered and washed with water, dried, and concentrated in vacuo to provide ethyl (R)-4-((1-benzylpiperidin-3-yl)amino)-1H-pyrrolo[2,3-b]pyridine-5-carboxylate as a brown solid (8.00 g, 95% yield): MS (ES) m/z 379.1 (M+H).

To a solution of ethyl (R)-4-((1-benzylpiperidin-3-yl)amino)1H-pyrrolo[2,3-b]pyridine-5-carboxylate (0.25 g, 0.66 mmol) in methanol (5 ml) was added 10% palladium on carbon (0.3 g, 50% wet w/w), and the mixture was stirred under a hydrogen atmosphere at ambient temperature for 12 hours. The reaction mixture was diluted with 50% methanol/ethyl acetate and filtered through celite. The

Table 9. Treatment-Emergent AEs by SOC and PT (Full Analysis Set)

	ATI-1777 (n = 23)		Vehicle (n = 25)		Total (n = 48)	
System Organ Class Preferred Term	Number of Subjects, n (%)	Number of Events	Number of Subjects, n (%)	Number of Events	Number of Subjects, n (%)	Number of Events
Any AE	9 (39.1)	16	9 (36.0)	10	18 (37.5)	26
Infections and infestations	3 (13.0)	4	3 (12.0)	3	6 (12.5)	7
Urinary tract infection	1 (4.3)	1	1 (4.0)	1	2 (4.2)	2
COVID-19	0	0	1 (4.0)	1	1 (2.1)	1
Candida infection	1 (4.3)	1	0	0	1 (2.1)	1
Folliculitis	1 (4.3)	1	0	0	1 (2.1)	1
Pharyngitis streptococcal	0	0	1 (4.0)	1	1 (2.1)	1
Tinea infection	1 (4.3)	1	0	0	1 (2.1)	1
Investigations	3 (13.0)	5	1 (4.0)	1	4 (8.3)	6
Blood CPK increased	2 (8.7)	2	0	0	2 (4.2)	2
Amylase increased	1 (4.3)	1	0	0	1 (2.1)	1
Lipase increased	1 (4.3)1	1	0	0	1 (2.1)	1
SARS-CoV-2 test positive	0	0	1 (4.0)	1	1 (2.1)	1
Transaminases increased	1 (4.3)	1	0	0	1 (2.1)	1
Nervous system disorders	3 (13.0)	3	1 (4.0)	1	4 (8.3)	4
Headache	2 (8.7)	2	0	0	2 (4.2)	2
Dizziness	1 (4.3)	1	0	0	1 (2.1)	1
Somnolence	0	0	1 (4.0)	1	1 (2.1)	1
General disorders and administration site conditions	1 (4.3)	1	1 (4.0)	1	2 (4.2)	2
Application site irritation	0	0	1 (4.0)	1	1 (2.1)	1
Application site pruritus	1 (4.3)	1	0	0	1 (2.1)	1
Respiratory, thoracic, and mediastinal disorders	0	0	2 (8.0)	2	2 (4.2)	2
Oropharyngeal pain	0	0	1 (4.0)	1	1 (2.1)	1
Sinus congestion	0	0	1 (4.0)	1	1 (2.1)	1
Skin and subcutaneous tissue disorders	0	0	2 (8.0)	2	2 (4.2)	2
Rash	0	0	1 (4.0)	1	1 (2.1)	1
Skin fragility	0	0	1 (4.0)	1	1 (2.1)	1
Cardiac disorders	1 (4.3)	1	0	0	1 (2.1)	1
Atrial fibrillation	1 (4.3)	1	0	0	1 (2.1)	1
Gastrointestinal disorders	1 (4.3)	1	0	0	1 (2.1)	1
Food poisoning	1 (4.3)	1	0	0	1 (2.1)	1
Neoplasms benign, malignant, and unspecified (including cysts and polyps)	1 (4.3)	1	0	0	1 (2.1)	1
Acrochordon	1 (4.3)	1	0	0	1 (2.1)	1

Abbreviations: AE, adverse event; CPK, creatine phosphokinase; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term; SOC, system organ class.

Note: AEs were coded using MedDRA, version 23.1. For each SOC and PT, subjects are included only once, even if they experienced multiple events in that SOC or PT. Treatment-emergent AE is defined as any AE with an onset date on or after the first administration of study medication and before the date of post-treatment follow-up.

filtrate was concentrated in vacuo to provide ethyl (R)-4-(piperidin-3-ylamino)-1H-pyrrolo[2,3-b]pyridine-5-carboxylate as an off-white solid (0.1 g, 90% yield): MS (ES) m/z 289.3 (M+H).

To a solution of ethyl (R)-4-(piperidin-3-ylamino)-1H-pyrrolo[2,3-b] pyridine-5-carboxylate (9.00 g, 31.3 mmol) in dichloromethane (50 ml) was added N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrogen chloride (9.70 g, 62.5 mmol); 1-hydroxybenzotriazole (8.43 g, 62.5 mmol); N,N-diisopropylethylamine (11.3 ml, 62.5 mmol); and cyanoacetic acid (0.76 g, 9.0 mmol). The mixture was stirred at ambient temperature for 18 hours. The reaction mixture was quenched with water and extracted with dichloromethane. The organic layer was washed with water and brine and dried over anhydrous sodium sulfate. The solution was filtered and concentrated in vacuo. The crude material was purified using flash chromatography (5% methanol/dichloromethane) to provide ethyl (R)-4-((1-(2-cyanoacetyl)piperidin-3-yl)amino)-1H-pyrrolo[2, 3-b]pyridine-5-carboxylate (7.5 g, 68% yield): proton nuclear magnetic resonance (400 MHz, DMSO-d_6) δ 11.68 (br s, 1 H), 8.86-8.79 (m, 1 H), 8.54 (s, 1 H), 7.20 (s, 1 H), 6.67 (s, 1H), 4.17−4.44 (m, 3 H), 3.70−4.05 (m, 4 H), 3.45−3.48 (m, 1 H), 3.11−3.24 (m, 1 H), 2.06 (br s, 1 H), 1.57−1.72 (m, 3 H), and 1.28−1.32 (t, J = 7.2 Hz, 3 H); MS (ES) m/z 356.1 (M+H).

Preparation of compounds

Compounds, CDD-001777 (ATI-1777) and CDD-001913, were synthesized by Aclaris Therapeutics and provided as solid powders

by the Medicinal Chemistry Department. Ibrutinib (CDD-000959) was received from Selleckchem (Houston, TX) and reconstituted in DMSO to a concentration of 20 mM.

Enzyme inhibition assays

The potency of CDD-001777 (ATI-1777) and the CDD-001777 metabolite CDD-001913 against the activity of Jak3 (amino acid 781−1124, Thermo Fisher Scientific, Waltham, MA) was quantified by measuring the phosphorylation of SRCtide (FAM-GEE-PLYWSFPAKKK-NH2). Kinase reactions were run in a 384-well Greiner plate (Greiner Bio-One, Monroe, NC) with 2% final DMSO (JT Baker, Radnor, PA) concentration under the buffer conditions of 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich, St. Louis, MO), pH 7.5, 10 mM magnesium chloride (VWR International, Radnor, PA), 0.01% BSA (Fraction V, Calbiochem, San Diego, CA), and 0.0005% Tween-20 (Thermo Fisher Scientific). The kinase reaction components were 2.5 nM Jak3 (Thermo Fisher Scientific), 1 μM SRCtide peptide (Anaspec, Fremont, CA), and 1 μM adenosine triphosphate (Sigma-Aldrich). CDD-001777 was tested in a dose−response starting at 2 μM (11 concentrations, 3-fold serial dilution, duplicate reactions). The reactions were incubated at room temperature for 40 minutes and then stopped by adding a 1:1 volume of 30 mM EDTA (VWR International) in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich), pH 7.5 (15 mM EDTA final). After the reaction was stopped, the phosphorylated and unphosphorylated peptides were separated and quantified using a Caliper LC3000/EZ-Reader system and HTS Well Analyzer Software (Caliper, a PerkinElmer Company, Hopkinton, MA). GraFit (Erithacus Software, Horley, United Kingdom) was used to calculate inhibitor potency by fitting dose−response data to the 4-parameter logistical IC_{50} equation. n = 1 for Jak1, Jak2, and TYK2, and therefore, no mean, median, or SD was calculated.

The potency of CDD-001777 and the CDD-001777 metabolite CDD-001913 against Jak1, Jak2, and TYK2 was performed at Thermo Fisher Scientific using their proprietary Z′-LYTE biochemical assay, following the manufacturer's instructions. The following controls were used for each kinase and were run on the same plate with the compound: 0% phosphorylation control, 100% phosphorylation control, 0% inhibition assay, and 100% inhibition control. The following controls were prepared for each concentration of test compound: development reaction interference and test compound fluorescence interference.

Metabolism in liver microsomes

Dog, human, monkey, and rat liver microsomes were obtained from Life Technologies (Carlsbad, CA) and Corning (Corning, NY). Minipig liver microsomes were obtained from Sekisui Xenotech (Kansas City, KS). ATI-1777 was prepared in DMSO (Leonid Chemical, Bangalore, India) to a final concentration of 1 mM. ATI-1777 or control was incubated with liver microsomes for 5 minutes before the addition of 10 mM of nicotinamide adenine dinucleotide phosphate (10 mM, Sisco Research Laboratories, Mumbai, India). At the end of the incubation period (0, 5, 15, and 30 minutes) of respective tubes, an aliquot of 200 μl of 0.05% formic acid (Spectrochem, Mumbai, India) in acetonitrile (JT Baker) with Loperamide (Internal standard, Sigma-Aldrich) was added to each tube to stop the reaction. Supernatants from each reaction tube were then taken for liquid chromatography with tandem mass spectrometry analysis. n = 1 for each liver microsome tested, and therefore, no mean, median, or SD was calculated.

Cytochrome P450 phenotyping

Recombinant human enzymes from Cypex (Scotland, United Kingdom) were prepared at the following concentrations: 200 nM for CYP1A2, 250 nM for CYP2B6/2C8, 100 nM for CYP2C9/3A4, 350 nM for CYP2C19, and 50 nM for CYP2D6. ATI-1777 was brought to a concentration of 2 μM in DMSO. A total of 20 μl of 2 μM dosing solution and 10 μl of blank buffer were added to all wells on ice. The plate was then prewarmed, and 10 μl of 8 mM nicotinamide adenine dinucleotide phosphate (MedChemExpress, Monmouth Junction, NJ) solution or blank buffer was added to the designated wells containing dosing solution for 60 minutes (for CYP1A2, CYP2C19, CYP2B6, and CYP2C8) or 30 minutes (for CYP2D6, CYP3A4, and CYP2C9). At the end of the incubation, 80 μl of acetonitrile containing internal standard was added to all wells. The plate was shaken for 10 minutes and then centrifuged. The supernatant was taken from each well and analyzed by liquid chromatography with tandem mass spectrometry. n = 1 for each cytochrome P450 tested, and therefore, no mean, median, or SD was calculated.

PBMC isolation

Human PBMCs were separated using Histopaque-1077 Ficoll (Sigma-Aldrich) through density centrifugation following the manufacturer's instructions. Cells were suspended in PBMC freeze media comprising 90% complete PBMC media (98% DMEM, 10% fetal bovine serum, and 1% penicillin/streptomycin; Gibco, Waltham, MA) and 10% DMSO (Sigma-Aldrich) at 5×10^7 cells/ml. A total of 1 ml of cell suspension was added to cryogenic vials and placed in a cryogenic freezing chamber at −80 °C overnight before transfer to liquid nitrogen storage.

Cell culture and plating

Human PBMCs were slowly thawed in a water bath and then transferred to assay media (90% RPMI 1640, 10% heat-inactivated fetal bovine serum, 10 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, 1% penicillin/streptomycin/glutamine, 12.2 mM 2-mercaptoethanol [Gibco]). The PBMCs were plated at 200,000 cells per well in 120 μl in a V-bottom polystyrene 96-well plate. The plates were placed in a 37 °C incubator until use. PBMCs and Jurkat cells were cultured at 37 °C with 5% carbon dioxide unless otherwise stated.

Jak inhibition in PBMCs

Serially diluted ATI-1777, CDD-1913, or vehicle (0.1% DMSO) was added to plated human PBMCs in 15 μl aliquots for final concentrations of 20 μM to 2×10^{-6} μM. After a 1-hour incubation while shaking, 1 of the following cytokines was added to its respective concentration for 5 minutes: 25 ng/ml of IL-2, 25 ng/ml of IL-12, 10 ng/ml of IFNγ, or 5 ng/ml of granulocyte-macrophage stimulating factor (R&D Systems, Minneapolis, MN). Cells were pelleted and lysed in MSD Tris Lysis buffer containing protease and phosphatase inhibitor cocktails (MSD, Rockville, MD). Cells were incubated while shaking at 4 °C for 30 minutes, and then using MSD, they were evaluated for either STAT5 phosphorylation by the Phospho/Total STAT5a,b Assay Kit or the Phospho-STAT5a,b Assay Kit or STAT4 phosphorylation by the Total Stat4 Assay Kit and/or the Phostpoh-STAT4 Assay Kit. STAT1 phosphorylation was measured by the PathScan Phospho-STAT1 ELISA kit (Cell Signaling Technology, Danvers, MA).

Minipig tolerability and toxicology

Male and female Göttingen Minipigs (23 animals per sex) were received from Marshall BioResources (North Rose, NY). Pigs were acclimated to the test facility for at least 13 days before initiation. Pigs ranged in age from approximately 3 to 5 months. Minipig studies were conducted by NAMSA (Medical Device Contract Research Organization), which is approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. Association for Assessment and Accreditation of Laboratory Animal Care International follows the standards of animal care described here: https://www.aaalac.org/resources/the-three-primary-standards/.

The 7-day tolerability and toxicology. Each pig was administered the assigned ATI-1777 and corresponding placebo formulation once daily to designated areas on 1 male and 1 female minipig. The application area on each pig represented approximately 400 cm^2, of which 200 cm^2 was designated for application of the appropriate ATI-1777 formulation, and 200 cm^2 was designated for application of the appropriate placebo formulation. The dosing volume for the ATI-1777 solution formulation was approximately 20 µl/cm^2 for a total dose of 80 mg per pig per day. Placebo formulations were applied in a similar manner to the designated area on each minipig. The test and placebo formulations were applied and remained on the application site for approximately 20 hours each day. Prior to the next daily application, the skin was wiped with a mild detergent (approximately 1 hour prior to the next application). Each pig was observed daily for the occurrence of clinical signs, and the application sites were evaluated for dermal irritation using a modified Draize procedure.

The 28-day tolerability and toxicology. Forty-six minipigs were assigned to 5 treatment groups, 3−5 pigs per group, with an equal number of males and females per group. The groups were sham, vehicle, 40 mg/day ATI-1777, 80 mg/day ATI-1777, and 160 mg/day ATI-1777. ATI-1777 was administered in a topical formulation containing either 0.5% or 2% ATI-1777, as described earlier. Blood samples (approximately 1.0 ml) were collected from pigs through a jugular vein on days 1 and 28 of the dosing phase. Samples were collected before dose and approximately 30 minutes and 1-, 2-, 4-, 8-, 12-, and 22-hour post-test article application completion. Pigs were nonfasted at scheduled collections unless fasted for other study-related procedures. On day 1, the 4-hour postdose collections were taken prior to anesthesia for electrocardiography. Prior to blood collections, pigs were administered acepromazine intramuscularly at a dose of 0.2 to 0.4 mg/kg. Blood was collected into tubes containing potassium (K2) EDTA as the anticoagulant. Plasma samples were analyzed by Covance-Salt Lake City for test article content using a validated liquid chromatography and tandem mass spectrometry method. The toxicokinetic analysis was performed by Covance.

RNA sequencing and analysis

RNA samples were extracted from the minipig skin and sequenced on an Illumina HiSeq. Sequencing performance was assessed for the total number of aligned reads, total number of uniquely aligned reads, and features detected. All gene counts were then imported into the R/Bioconductor package EdgeR (Robinson et al, 2010), and trimmed Mean of M-values normalization size factors were calculated to adjust for samples for differences in library size. The performance of all genes was assessed with plots of the residual SD of every gene to their average log count with a robustly fitted trend line of the residuals. For each contrast extracted with Limma, global perturbations in known Gene Ontology terms, Molecular Signatures Database, and Kyoto Encyclopedia of Genes and Genomes pathways were detected using the R/Bioconductor package GAGE (Luo et al, 2009) to test for changes in the expression of the reported $\log_2$ fold changes reported by Limma in each term versus the background $\log_2$ fold changes of all genes found outside the respective term. The R/Bioconductor package heatmap (Zhao et al, 2014) was used to display heatmaps across groups of samples for each Gene Ontology or Molecular Signatures Database term with a Benjamini−Hochberg false discovery rate adjusted *P*-value less than or equal to .05. RNA sequencing data are available in the Gene Expression Omnibus (accession number GSE244201; https://www.ncbi.nlm.nih.gov/geo/).

Activity in porcine model. ATI-1777 was formulated in solution at 2% weight per volume. Solution with or without ATI-1777 was applied to the back of a pig, and after 1 hour, 100 ng porcine IL-15 was injected intradermally. After an additional 3 hours, sites were biopsied, and tissue was analyzed for changes in levels of mRNA for *CCL8*. The tissue was cut into small pieces and treated with TRIzol (Invitrogen, Waltham, MA). RNA was extracted with the Qiagen (Germantown, MD) RNeasy Mini Kit following the manufacturer's instructions. RNA concentration was read by NanoDrop. Reverse transcription and subsequent qRT-PCR were completed using Applied Biosystems (Foster City, CA) high-capacity cDNA RT kit and TaqMan Universal Master Mix II and pig probes (ACTB identification number Ss03376563_uH, CCL8 identification number Ss04245586_m1) (Thermo Fisher Scientific).

Analysis software

Discovery Workbench (Meso Scale Discovery Technologies); Odyssey Imaging Software (LiCor); Excel 2010 (Microsoft); GraFit, version 5 or 7 (Erithacus); and SAS were used for data analysis. IC50 was determined after iterating to the best least-squares fit.

Instrumentation. Plates for the total STAT4, phosphorylated STAT4, and IFNγ assay were analyzed using the Sector S 600 (Meso Scale Discovery Technologies). Plates for the phosphorylated STAT2 Assay were analyzed using the SpectraMax-M2 plate reader. Dot blots for the phosphorylated PLCγ1 assay were analyzed using the Odyssey Imager (LiCor). qRT-PCR was performed on the Applied Biosystems QuantStudio 6 Flex RT-PCR system. Mass spectrometry was conducted on the API-4000 (Sciex, Framingham, MA). Liquid chromatography was assessed on a Shimadzu Scientific Instruments (Columbia, MD) SIL-HTc.

ATI-1777-AD-201

ATI-1777-AD-201 was conducted in compliance with Good Clinical Practice Guidelines established by the International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and the Declaration of Helsinki. A central institutional review board (Advarra, Columbia, MD) and independent ethics committees of the investigational centers reviewed and approved the final protocol, amendments, and informed consent documentation. Written, informed consent was obtained for all patients.

Study design. ATI-1777-AD-201 was a randomized, double-blind, parallel-group, multicenter, vehicle-controlled study to evaluate the efficacy, safety, tolerability, and PK of ATI-1777 solution after twice-daily applications to target areas of male and female

participants, aged 18–65 years, inclusive, with moderate-or-severe AD (clinicaltrials.gov, NCT04598269). All AD lesions in protocol-allowed areas were to be treated. The study consisted of a screening period of up to 30 days, a 4-week treatment period, and a 2-week post-treatment follow-up period. The maximum total duration of the study for participant remaining in the study until their final follow-up assessment was 72 days. The study protocol was amended 3 times. The first 2 amendments clarified study assessments, and the third amendment increased enrollment to 50 subjects to achieve 34 subjects who completed the study. The first participant was enrolled on October 19, 2020, and the last participant completed the study on April 22, 2021. The study was stopped after the last participant had completed the study.

Participants. Eligible patients were aged 18–65 years (inclusive) with moderate or severe AD. Inclusion criteria included diagnosis of AD fulfilling the specified diagnostic criteria of Hanifin and Rajka (1980), at least a 6-month history of AD prior to the screening visit and no significant AD flares for the 4 weeks prior to the screening visit, at least 1 lesion that measures at least 3 cm^2 at the screening visit and on day 1 prior to the first dose of study medication (this lesion must have been representative of the subject's disease state but not located on the hands, feet, or genitalia), a stable diagnosis of moderate or severe (IGA score 3 or 4) AD at the screening visit, and AD affecting 3–20% BSA (not including head [neck, scalp, face], palms of hands, soles of feet, groin, and genitalia) at the screening visit. Participants were enrolled at 14 sites across the United States.

Interventions. During the treatment period, participants applied the study drug (up to 8 ml ATI-1777 topical solution 2.0% w/w or vehicle) twice daily for 28 days to target areas affected by AD.

Objectives. The primary objective of the study was to assess the preliminary clinical efficacy of ATI-1777 topical solution in adults with moderate or severe AD. The primary endpoint was a change from baseline in mEASI score at week 4. Secondary endpoints were percentage change from baseline in mEASI score at each visit (days 8, 15, and 28), proportions of participants who achieve mEASI-50/75/90 within 4 weeks of the start of treatment, change from baseline in IGA score at each study visit, change from baseline in AD BSA value at each study visit, and change from baseline in PP-NRS score over time. The secondary objective was to assess the safety and tolerability of ATI-1777 topical solution twice daily for 4 weeks in adults with moderate or severe AD. Secondary endpoints were TEAEs; serious AEs; and AEs of special interest, laboratory values, vital signs, physical examination results, and 12-lead electrocardiogram results.

Sample size determination. A total of 50 participants were enrolled and randomized to achieve 34 subjects who completed the study. A 1-sided hypothesis test was selected for this early proof-of-concept study to determine whether ATI-1777 was superior to placebo. A protocol amendment that occurred before unblinding of the data updated the analysis from a 2-sided test powered at 90% to a 1-sided test. The sample size of 34 subjects did not change and resulted in 95.6% power to detect a statistically significant difference between the treatment groups in the primary endpoint (percentage change from baseline in mEASI scores). This power calculation was based upon a 1-sided treatment contrast within a 1-way ANOVA model and assumed group means of 65 and 20% for ATI-1777 and vehicle, respectively. The group means were based upon slightly more conservative estimates than were observed in a phase 2 study of topical ruxolitinib in AD (Kim et al, 2020). The SD for the percentage change from baseline in mEASI was assumed to be 38.3%. This variance assumption was based on data observed in a pilot AD study for the topical compound ATI-502 (ATI-502-AD-201).

The sponsor and ProPharma safety physician met regularly (every 4 weeks) and assessed the ongoing safety of the study. Attention was paid to the number of severe AEs and serious AEs, with particular focus paid to the number of participants who met individual participant stopping rules. PK data were used in the assessment: events associated with high plasma levels were considered of high significance.

Randomization. Participants who met all the entry criteria were randomized on day 1 through an interactive voice/web response system (IXRS) to ATI-1777 or vehicle treatment in a 1:1 ratio. Participants were stratified at randomization by severity of AD (moderate vs severe). Each participant was assigned a unique patient number, which was obtained from the IXRS after the patient provided informed consent. The investigator kept a record (the screening log) of patients who entered screening. Randomization was performed through a centralized IXRS. Participants were allocated to treatment according to the randomization code.

Blinding. The study was double blinded; therefore, the sponsor, investigators, study personnel, and the study participants were blinded to treatment allocation. ATI-1777 and vehicle were coded and labeled in a manner that protected blinding. The IXRS permitted rapid identification of the product (in case of medical emergencies) that did not permit detectable breaking of the blind. Breaking of the blind was only allowed in the case of an emergency when knowledge of the study medication was essential for the clinical management of the patient and was organized through IXRS.

Statistical methods. All efficacy and safety analyses were completed for the full analysis set (all participants who received at least 1 dose of study medication; 23 participants in the ATI-1777 group and 25 participants in the vehicle group). As described in sample size determination, all *P*-values for efficacy were based on a 1-sided hypothesis test of the superiority of ATI-1777 to vehicle. The primary efficacy analysis was the treatment comparison between ATI-1777 and vehicle for the percentage change from baseline in mEASI scores at week 4. This treatment comparison was made within the context of a global Mixed Model Repeated Measures analysis where the mEASI scores over time were treated as repeated measures within a given participant. Treatment group, time (study visit), and treatment by time interaction were entered into the model as categorical factors, baseline mEASI score and/or baseline severity of AD were included as a continuous covariate, and participant identification entered the model as a random effect. For the primary efficacy analysis on the full analysis set population, missing data were imputed using the last observation carried forward. Missing data were not imputed for the analyses that were conducted on the per-protocol population.

Treatment comparisons between ATI-1777 and vehicle for each of the continuous efficacy endpoints that were conducted over time (change in IGA, BSA, and PP-NRS) were analyzed using a similar model as described for the primary endpoint. Treatment group comparisons for categorical efficacy endpoints (IGA response, EASI-50, EASI-75, and EASI-90) that were conducted over time employed a logistic regression model fit at each scheduled visit separately,

where appropriate. The logistic regression model included treatment group as a factor and the baseline value and/or baseline severity of AD as a covariate.

Efficacy analyses. This study employed an mEASI that excluded evaluation of the head (neck, scalp, face), palms of hands, soles of feet, groin, and genitalia. The mEASI evaluated AD in each of 3 body regions (trunk, upper extremities, and lower extremities). Each respective body region received a score between 0 and 6 on the basis of the percentage involvement in that region. The total percentage of the participant's AD-affected BSA was estimated by the investigator or designee using the handprint method, which estimates that the area of a participant's full handprint constituted 1% of their total BSA.

The IGA was the investigator's assessment of the overall appearance of the lesions at a particular point in time. The IGA scoring ranged from 0 (clear) to 4 (severe) and, whenever possible, was assessed by the same individual during each visit. The PP-NRS (Yosipovitch et al, 2019) is a single patient-reported item designed to measure peak pruritus or worst itch over the previous 24 hours on the basis of the following question: On a scale of 0 to 10, with 0 being "no itch" and 10 being "worst itch imaginable," how would you rate your itch at the worst moment during the previous 24 hours? Participants recorded their completed assessments in their diaries each morning before applying study medication during the treatment period.

PK sampling. The PK of ATI-1777 was preliminarily evaluated by measurement of ATI-1777 concentrations in plasma samples obtained using a sparse sampling schedule. A sparse sampling approach was employed because it was not anticipated that clinically relevant plasma concentrations would be observed. A total of 8 PK samples were collected from each participant. One sample was collected before the study medication application, and 1 sample was collected approximately 2 hours after application on each of days 1, 8, and 15.

Safety analysis. Investigators were responsible for evaluating an AE's potential relationship to the study drug. Participants were monitored for AEs associated with Jak inhibition, including malignancies, thromboses, and cytopenias. TEAEs were defined as AEs with onset dates on or after the date of the first administration of study medication and before the last administration ±14 days. Participants with multiple AEs were counted only once within each preferred term and system organ class. The full analysis set was used for the analysis of safety data. AEs were coded with the Medical Dictionary for Regulatory Activities. Severity grading for every AE was performed by the investigator on the basis of his/her best medical judgment as follows: for mild, asymptomatic, or mild symptoms, clinical or diagnostic observations only, intervention not indicated; for moderate, minimal, local, or noninvasive intervention indicated, limited age-appropriate instrumental activities of daily living; and for severe, medically significant, disabling, or limiting self-care activities of daily living.

Ethics statement

Minipig studies were conducted by NAMSA (Medical Device Contract Research Organization), which is approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. Association for Assessment and Accreditation of Laboratory Animal Care International follows the standards of animal care described here: https://www.aaalac.org/resources/the-three-primary-standards/. ATI-1777-AD-201 was conducted in compliance with Good Clinical Practice Guidelines established by the International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and the Declaration of Helsinki. A central institutional review board (Advarra) and independent ethics committees of the investigational centers reviewed and approved the final protocol, amendments, and informed consent documentation. Written, informed consent was obtained for all patients.

Data availability statement

Owing to the nature of this research, the data that support the findings of this study are available to qualified investigators on request to the corresponding author PC. RNA-sequencing data from the pig studies are available in the Gene Expression Omnibus (accession number GSE244201; https://www.ncbi.nlm.nih.gov/geo/).

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CONFLICT OF INTEREST

PC, CX, SM, HH, KLK, JH, DRA, JB, SH, WS, JM, DB, BB, NW, JS, ST, JJ, and DG are shareholders of Aclaris Therapeutics. DG is a shareholder of Johnson & Johnson. DG is the Head of Clinical Development and immunology at Johnson & Johnson.

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Conceptualization: WS, ED, JM, NW, DA, JB, SH, JJ, ST, JS, PC, DG; Data Curation: DB, ST; Formal Analysis: DB; Funding Acquisition: JM, WS, PC, HH, JJ, ST; Investigation: CX, SM, HH, KK, JH, C-YL, JB, SH, JJ, PC, TR, SF, BB; Methodology: PC, CX, SM, HH, KK, JH, C-YL, ST, JS, PC, DG, DB, BB; Project Administration: WS, JM, JJ, ST, JS, PC, DG; Resources: JM, JJ, HH, ST, JS, TR, SF, DB, PC, DG; Supervision: JJ, WS, JM, HH, ST, JS, PC, DG; Validation: ST, DB, JS; Visualization: HH, JH, PC, DB; Writing — Original Draft Preparation:

ST, JS, TR, SF, DB, PC, DG; Writing – Review and Editing: BB, ST, JM, JJ, JS, TR, SF, DB, PC, DG

Disclaimer

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