Efficacy and safety of ingenol disoxate gel in field treatment of actinic keratosis on full face, scalp or large area (250cm^2) on the chest: results of four phase 3 randomized controlled trials

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Abstract

Introduction: Actinic keratosis (AK) is a skin condition arising from chronic exposure to ultraviolet light and may lead to the development of malignancies. This trial aimed to evaluate efficacy and safety of ingenol disoxate gel (IngDsx, 0.018% for face/chest [FC]; 0.037% for scalp [S]), versus vehicle.

Methods: Four identical phase 3 trials in patients with AK on the full face/up to 250cm^2 of chest or full balding scalp, with an initial 8-week period and 12-month follow-up, were conducted. FC and S trials were pooled for analysis. The primary endpoint was complete clearance at Week 8.

Results: Across trials, 616 patients were randomized to FC and 626 to S, with 410 and 420 assigned to receive IngDsx, respectively. In the FC and S trials, 25.9% and 24.5% of patients in the IngDsx group, respectively, achieved the primary endpoint. IngDsx was relatively well tolerated. During extended follow-up, there were more identified non-melanoma skin malignancies in the IngDsx group than vehicle group; HR: 2.38 (95% CI: 1.28, 4.41).

Conclusion: Treatment with IngDsx was superior to vehicle on all clinical endpoints, patient-reported and cosmetic outcomes. During the 12-month follow-up, slightly increased skin malignancies in the treatment area were identified, potentially due to unintentional detection bias.

Keywords: ingenol disoxate, actinic keratosis, ingenol, cancer

Introduction

Actinic keratosis (AK) is a common skin condition arising as a result of chronic exposure to ultraviolet (UV) radiation that presents in the form of red, scaling, cutaneous lesions composed of proliferative, pre-cancerous keratinocytes [1-5]. As the surrounding field of skin has also been exposed to the same chronic UV light, a constant generation of subclinical and clinical AK and even the development of malignancies, such as squamous cell carcinoma (SCC) is observed [6]. Indeed, AK is a risk marker for malignancies, with stage 1 AK (AK with atypical basal cells) frequently represented in development of invasive SCC [7,8].

Although AK can progress to malignancies, there can also be variability in the lesions over time, with up to 21% of cases exhibiting spontaneous complete field regression [4,9,10-12]. However, data on relative changes in AK counts over time are rather heterogeneous [4] and new lesions can occur at the same time as regression of others [12]. Recurrence rates one year after regression range from 15–53% [4].

Actinic keratosis treatment can be lesion-directed or field-directed [13,14]. Lesion-directed methods target individual, visible lesions, but do not address any additional photodamage in the surrounding area; field-directed therapies treat the whole field of cancerization, including subclinical lesions [14,15].

Ingenol mebutate (Picato® LEO Pharma) is approved for the field-directed treatment of AK [16].

Ingenol disoxate (IngDsx) is a novel ingenol derivative [17-19] offering improved stability and biologic properties compared with ingenol mebutate [18]. Unlike ingenol mebutate, which requires refrigeration to prevent degradation, IngDsx can be stored at ambient temperatures, thus offering improved ease of handling for patients [18]. Preclinical data suggest that IngDsx has a dual mode of action similar to ingenol mebutate; IngDsx has direct cellular cytotoxicity with higher cytotoxic potency than the mebutate derivative and it induces pro-inflammatory mediators through activation of protein kinase C (PKC) isoforms [18].

During phase 1/2 trials, IngDsx gel was applied to areas of 25–250cm^2 on the face/chest (FC), scalp (S), and trunk/extremities in once-daily regimens for two or three consecutive days [17,19,20,21]. Ingenol disoxate was well tolerated as field therapy for AK and dosing for two or three consecutive days was comparable with respect to local skin responses (LSRs), [19,20,21]; preliminary efficacy at Week 8 in the larger 250cm^2 treatment area was encouraging [19-21].

The aim of the current analysis was to fully establish the efficacy, and further evaluate the safety, of IngDsx gel (0.018% for FC; 0.037% for S) versus vehicle, when applied once-daily for three consecutive days for AK on the full FC (~250cm^2) or full balding S (25–250 cm^2) utilizing pooled data from four phase 3 trials at 8 weeks with a 12-month follow-up period (NCT02547233 [FC233], NCT02549339 [FC339], NCT02547363 [S363] and NCT02549352 [S352]).

Methods

Trial design

Four identically designed, phase 3, international, multicenter, randomized, double-blind, vehicle-controlled trials in patients with AK on either the full FC (up to 250cm^2 on the chest), (FC233 and FC339) or full balding S (S352 and S363) were carried out. Trial NCT02547233 (FC) was carried out in centers in France, Spain, the United Kingdom (UK) and the United States (US); trial NCT02549339 (FC) in Canada, Germany, Italy, Spain and the US; trial NCT02547363 (S) in Canada, France, the UK and the US; and trial NCT02549352 (S) in Germany, Italy and the US. These trials consisted of a 3-day treatment period of once-daily IngDsx gel (0.018% for FC; 0.037% for S) or vehicle gel, followed by an initial 8-week follow-up period with clinical assessments at Weeks 1, 2, 4 and 8 (Part 1) and an additional 12-month follow-up (Part 2) with clinical assessments at Months 5, 8, 11 and 14. During the 12-month follow-up period, cryotherapy was allowed to treat remaining and recurrent lesions.

Standard protocol approvals, registration and patient consents

These trials were conducted in accordance with the Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects. All patients received written and verbal information concerning the clinical trial and were asked to consent that their personal data were recorded, collected, processed and could be transferred to European Union (EU) and non-EU countries in accordance with any national legislation regulating privacy and data protection.

Patients

Patients were ≥18 years of age with 5–20 clinically typical, visible and discrete AK within the selected treatment area of sun-damaged skin on either the full face (F), full balding S (>25cm^2 and up to ~250cm^2), or a contiguous area of ~250cm^2 on the chest (C). In addition, patients had a tracking area of 50cm^2, within the treatment area, containing a minimum of three clinically typical, visible and discrete AK. Individuals were excluded if the location of the treatment area was within 5cm of an incompletely healed wound, BCC or SCC. Patients were also excluded if lesions in the treatment area had an atypical clinical appearance (e.g., hypertrophic, hyperkeratotic or cutaneous horns) and/or recalcitrant disease or if they had been treated with ingenol mebutate within the last 12 months.

Patients were randomized in a 2:1 ratio (using a block size of 6) on Day 1 to receive either IngDsx gel once-daily for three consecutive days (0.018% for FC; 0.037% for S) or vehicle; treatment was administered

at home, after it was dispensed on Day 1. The randomization was stratified by site and managed with an interactive web response system. The packaging and labeling of the investigational medicinal products (IMPs; ingenol disoxate 0.018% [FC] or 0.037% [S] gel or vehicle gel) contained no evidence of the identity of the allocated treatment. It was not considered possible to differentiate between the IMPs solely by sensory evaluation. All staff involved in the conduct of the trial remained blind to treatment allocation and results of unblinded analyses for the entire duration of the trial. This principle was applied to the entire investigator staff and to the staff employed by the sponsor, except for those in the unblinded analysis group.

The first patient first visit was in November 2015 and the final patient final visit of all trials was in November 2017.

Objectives and endpoints

The primary objective of all studies was to confirm the efficacy of IngDsx (0.018% for FC; 0.037% for S) gel as field treatment in AK when applied topically to the FC or S, once-daily for three consecutive days. This was to be measured by the primary endpoint, which was 'complete clearance,' defined as no clinically visible AK in the treatment area (AKCLEAR100) at Week 8 through the investigator's assessment of AK count.

The secondary objectives were to evaluate the safety of IngDsx gel in AK when applied topically once-daily for three consecutive days as field treatment and evaluate the long-term efficacy of IngDsx gel (0.018% for FC; 0.037% for S) in AK over an extended 12-month follow-up period after initial 'complete clearance' at Week 8. Secondary endpoints were 'partial clearance' at Week 8, defined as ≥75% reduction in the number of clinically visible AK in the treatment area (AKCLEAR75), AKCLEAR75 at Week 4 and percent reduction in AK count in the treatment area at Week 8 versus baseline. Other endpoints investigated included AKCLEAR100 and percent reduction in AK count at Week 4, patient-reported outcomes (Skindex-16, Treatment Satisfaction Questionnaire for Medication [TSQM], cosmetic outcome) and global photo damage using a 7-point symmetric scale (range: +3 [marked improvement] through −3 [marked worsening]).

Safety endpoints were any adverse events (AEs) and serious adverse events (SAEs) recorded at all visits during the 8-week treatment follow-up. Local skin reactions were assessed by the investigator during the 8-week follow-up period at Visits 2–7 using the LSR grading scale (0–4, with 4 being the highest grade of severity). Local skin reactions were analyzed separately from other AEs [22]. During the extended 12-month follow-up period, AEs and SAEs in the treatment area were recorded.

Statistical methods

Based on the phase 1/2 results with IngDsx, the assumptions for AKCLEAR100 rate for a 3-day regimen were 25% for both IngDsx 0.018% (FC) and 0.037% (S) gel and 10% for vehicle 8 weeks after treatment completion. With a 2:1 randomization ratio, a total of 306 patients (204 IngDsx and 102 vehicle) was required in each of the four trials. With this sample size, testing at a two-sided significance level of 5%, a Pearson's chi-square test had 90% power to detect a difference in AKCLEAR100 rates. All significance tests were two-sided using the 5% significance level. All confidence intervals were presented with 95% degree of confidence. Missing values for AK counts were imputed using multiple imputation.

Results

Demographics

Across the two FC trials (NCT02547233 and NCT02549339), 820 patients were assessed for eligibility and 616 patients were randomized (410 to IngDsx 0.018% and 206 to vehicle), of which 611 patients were included in the full analysis set (FAS), (407 IngDsx 0.018% and 204 vehicle); 5 patients were excluded from analysis (three patients did not receive assigned treatment and two patients did not apply assigned treatment). The percentage of those patients in the two FC trials completing the 8-week follow-up period was 98.5% (404 patients) in the IngDsx group and 92.7% (191 patients) in the vehicle group. Across the two S trials (NCT02547363 and NCT02549352), 764 patients were assessed for

eligibility and 626 patients were randomized (420 to IngDsx 0.037% and 206 to vehicle), of which 623 patients were included in the FAS (418 IngDsx 0.037% and 205 vehicle); three patients were excluded from analysis as they did not receive the assigned treatment. The percentage of those completing the 8-week follow-up period was 98.6% (414 patients) in the IngDsx group and 92.2% (190 patients) in the vehicle group. Reasons for discontinuation from the FC and S trials can be found in the supplementary information (**Table S1**). The patient demographics and disease characteristics at baseline for the pooled analyses are shown in **Table 1**.

Efficacy

Significantly more patients in the IngDsx group achieved AKCLEAR100 at Week 8 compared with vehicle in the FC and S trials (25.9% versus 2.0% and 24.5% versus 1.5%, respectively, both P<0.001). Across all trials, the partial clearance rates (AKCLEAR75 at Week 8) were higher in the IngDsx group than the vehicle group and were statistically significant in both FC and S trials (58.8% versus 7.7% and 61.0% versus 6.0%, respectively, both P<0.001). AKCLEAR100 and AKCLEAR75 at Weeks 4 and 8 are shown in **Table 2** and **Figure 1**.

In the FC trials, 19.5% of patients in the IngDsx group achieved AKCLEAR100 at Week 4 compared with 2.1% of the vehicle group. Approximately 70% of those with AKCLEAR100 at Week 4 in the IngDsx group maintained this response to Week 8. In the S trials, 21.8% of patients achieved AKCLEAR100 at Week 4 compared with 2.1% of the vehicle group and 66% of those with AKCLEAR100 at Week 4 in the IngDsx group maintained this response to Week 8.

The mean percent reductions in AK count at Weeks 4 and 8 (**Table 2**) were higher in the IngDsx group than the vehicle group across all four trials and the differences were statistically significant. For most patients, the absolute AK count was reduced from baseline to Week 4 and then further reduced or sustained at Week 8. In the FC trials, the estimated mean percent reduction in AK count at Week 8 was 73.5% in the IngDsx group and 11.2% in the vehicle group (P<0.001). In the S trials, the estimated mean percent reduction in AK count at Week 8 was 73.4% in the IngDsx group and 6.0% in the vehicle group (P<0.001). The additional endpoint of percent reduction in lesion count at Week 4 was 74.4% in the IngDsx group in the FC trials versus 14.1% in the vehicle group, and 71.5% in the IngDsx group in the S trials versus 9.3% in the vehicle group. The rates were similar to those at Week 8 in all trials.

Patient-reported outcomes, including TSQM data, are shown in **Table 3**. In all trials, approximately 90% of patients treated with IngDsx reported improvement in the overall feel of the treatment area at Week 8. The most frequent outcome was 'much improved,' reported by 53.4% and 61.1% of patients in the IngDsx group in the FC and S trials,

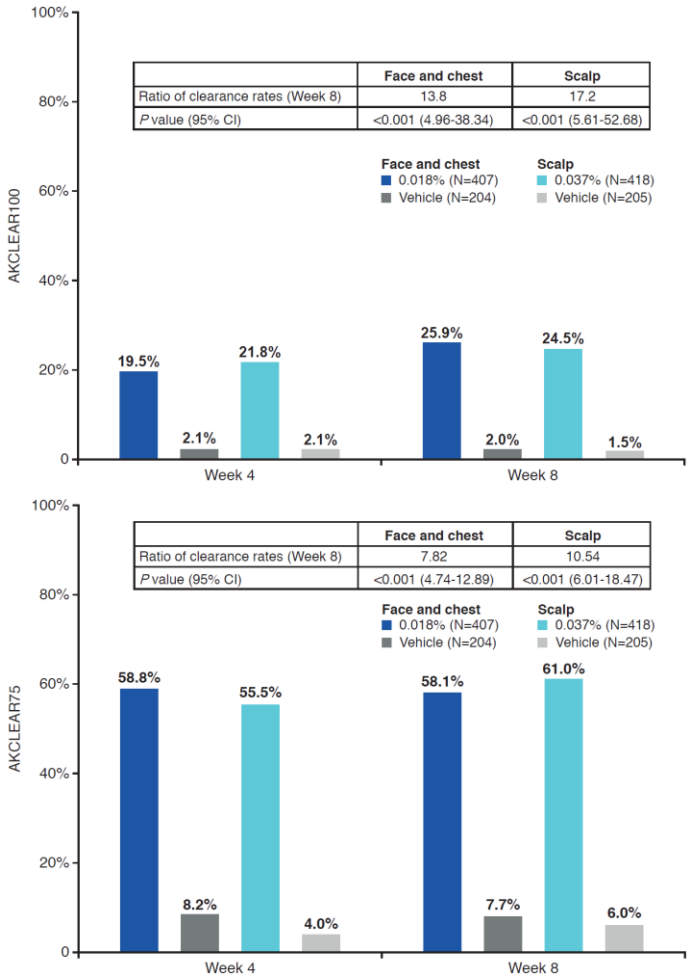

Figure 1. ***A)*** *AKCLEAR100 at Weeks 4 (other endpoint) and 8 (primary endpoint) for the face/chest (FC) and scalp (S) pooled analyses (full analysis set) and* ***B)*** *AKCLEAR75 at Weeks 4 and 8 (secondary endpoints) for the face/chest (FC) and scalp (S) pooled analyses (full analysis set).*

Table 2. *Percent reduction in AK count at Week 4 (other endpoint) and Week 8 (secondary endpoint) in the face/chest (FC) and scalp (S) trials (full analysis set).*

Endpoint	Face/chest		Scalp	
Treatment group	**IngDsx**	**Vehicle**	**IngDsx**	**Vehicle**
Total no. patients	**407**	**204**	**418**	**205**
Reduction in AK Count, mean %				
Week 4[1,2]	74.4	14.1	71.5	9.3
Week 8[3,4]	73.5	11.2	73.4	6
Ratio of AK count reduction[5]	0.3		0.28	
P value (95% CI)[6]	<0.001 (0.26-0.34)		<0.001 (0.25-0.32)	

[1]Observed cases.
[2]Estimated by a negative binomial regression on AK count at Week 4 with the log baseline AK count as offset and treatment group, and pooled site as factors.
[3]Negative binomial regression with treatment group and pooled site as factors and log baseline counts as offset variable.
[4]Missing values imputed using multiple imputation.
[5]0.037% for FC or 0.018% for S relative to vehicle.
[6]*P*-values from the 3 secondary endpoint analyses adjusted for multiplicity using the Holm-Bonferroni method.
Abbreviations: AK, actinic keratosis; CI, confidence interval; FC, face/chest; S, scalp.

respectively, with only 6.2% and 4.4% of patients in the corresponding vehicle groups. In the Skindex-16 assessment, the changes from baseline were greater in the IngDsx group versus vehicle, with treatment differences in favor of IngDsx by Week 8. These were statistically significant in the symptom domain and the emotion domain, with an estimated treatment difference for symptoms of −8.5 (95% confidence interval (CI): −10.9, −6.1; P<0.001) in the FC analyses and −6.6 (95% CI: −9.2, −4.1; P<0.001) in the S analyses. Additionally, global photo damage outcomes showed improvement in the IngDsx group versus vehicle group at Week 8, with approximately 80% of patients in the IngDsx group across all trials showing improvement versus approximately 20% of the vehicle group in both pooled analyses (see supplementary information, Table S2).

Safety

Local skin reactions peaked at Day 4 for approximately 87% and 70% of patients and at Day 8 for nearly all remaining patients in the FC and S trials, respectively. In most cases, LSRs declined to mild levels within two weeks of treatment initiation and resolved within four weeks. Composite LSRs are shown in the supplementary information, Figure S1.

A summary of the safety data can be found in Table 4. At Week 8, 70.3% (N=286) and 78.5% (N=329) of patients in the IngDsx treatment group reported AEs in the FC and S trials, respectively versus 23.5% (N=48) and 37.3% (N=76) of those receiving vehicle. There were 42 (N=30, 7.4%) severe related AEs in the FC trials and 35 (N=28, 6.7%) in the S trials among those receiving IngDsx. Six serious related AEs were reported in the FC trials in those receiving IngDsx; none were reported in either group for the S trials. The most common AEs in the 8-week follow-up were administration site pain and pruritus for all trials and treatment groups (Table 5). At month 12, 17.5% (N=70) and 10.9% (N=45) of patients in the IngDsx group reported AEs for the FC and S trials, respectively versus 12.1% (N=12) and 9.0% (N=16) of those in the vehicle group. At this time point, application-site scar, SCC and Bowen's disease were among the most common AEs. AEs leading to discontinuation at Week 8 in the IngDsx group included application site pain (N=13 patients in the FC trial, 8 in the S trial) or pruritus (N=6 patients in FC trials) and headache (N=2 patients in FC trials). In the vehicle group, there was one discontinuation due to application site pain in the S trials.

During the extended follow-up period, there were more cases of identified SCC, BCC and Bowen disease in the treated areas in the IngDsx group than in the vehicle group (**Table 6**), with a hazard ratio of 2.38 (95% CI: 1.28–4.41).

Table 6. *Integrated analysis of skin malignancies from treatment group in all trials (safety analysis set).*

Endpoint	Face/chest		Scalp	
Treatment group	**IngDsx**	**Vehicle**	**IngDsx**	**Vehicle**
Total no. patients	**407**	**204**	**419**	**204**
Consolidated Preferred Term[1]	**E/N (%)**	**E/N(%)**	**E/N (%)**	**E/N(%)**
Basal cell carcinoma	24/20 (4.9)	3/3 (1.5)	2/2 (0.5)	1/1 (0.5)
Bowen's disease	19/15 (3.7)	6/4 (2.0)	8/8 (1.9)	0/0 (0.0)
Keratoacanthoma	1/1 (0.2)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)
Malignant melanoma	1/1 (0.2)	0/0 (0.0)	1/1 (0.2)	0/0 (0.0)
Squamous cell carcinoma of skin	16/14 (3.4)	5/4 (2.0)	5/5 (1.2)	2/1 (0.5)
All	61/48 (11.8)	14/10 (4.9)	16/16 (3.8)	3/2 (1.0)

[1]Number of events (E) and patients who have an event (N) from baseline to Month 14.
Abbreviations: E/N, events/number of patients; FC, face/chest; S, scalp.

Discussion

Ingenol disoxate gel was superior to vehicle for the primary and all secondary endpoints. For the primary endpoint, AKCLEAR100 at Week 8, similar results were seen in both per protocol and sensitivity analyses (data not shown). Complete clearance rates on the scalp observed at 8-weeks in this analysis are comparable with complete clearance rates observed at 8-weeks in two trials with ingenol mebutate [23]; following treatment of the scalp for three days with 0.015% ingenol mebutate, complete clearance was seen in 23% of patients (vs. 2% of patients treated with vehicle). For other efficacy endpoints, the results in all trials showed a similar pattern to the primary and secondary endpoints.

The standard methodology for conducting well-controlled studies in AK emphasizes complete clearance of all AK lesions in a treatment field within a defined time span [24]. This is however, not necessarily well-suited to a disease such as AK, which often has a fluctuating course [4,5,15,25]. In this respect, percent reduction in AK count may be a more clinically relevant endpoint and allow for comparison between studies, being independent of the starting number of AK lesions at baseline, unlike complete clearance. The use of imaging techniques such as confocal microscopy or optical coherence tomography may be beneficial during screening, treatment and follow-up, as they could provide a non-invasive, in-depth, consistent, comparable image of single lesions, including subclinical or residual disease, allowing continuous monitoring of healing events and response to therapy [26,27]. However, only a small area within a whole field can be visualized and advanced imaging techniques are not widely available in real-world clinical practice, meaning they are currently not possible to perform on a routine basis [28]. It should also be noted that such techniques cannot always distinguish between clinically apparent AK lesions or sub clinical lesions and SCC, in the context of field cancerization [26].

Other aspects of AK treatment have also been investigated, including long-term risk of malignancies after treatment. A retrospective comparison of the topical field-based treatments 5-fluororacil and imiquimod, looked at whether they were able to reduce the risk of subsequent malignancies in the short and long term since a key goal of AK treatments is to prevent this progression. The trial found that there was no difference in the short- or long-term risk of subsequent site-specific malignancies [29]. In a case report, however, treating

an apparent AK with 5% 5-fluorouracil revealed a BCC, which was diagnosed by dermoscopy after the AK had been cleared, and was later destroyed by cryotherapy [30]. Criscione et al. also reported that 65% of primary SCCs and 36% of primary BCCs in their study cohort had previously been clinically diagnosed as AKs [5]. This points to AK being a biomarker for malignancy risk and highlights the difficulty in making clinical differential diagnosis in AK with overlying or colliding lesions with different characteristics without the aid of technology.

In the current trial, there were more identified cases of non-melanoma skin malignancies inside the treatment area in the active treatment arm than in the vehicle group. A possible explanation for this is investigator detection bias whereby clearing the actinically damaged field from multiple AKs may reveal malignancies that previously did not stand out among the AK lesions, but are later revealed as persistent or emerging suspicious lesions. Individual AK lesions in the tracking area were monitored and the use of cryotherapy was allowed in the extended follow-up period (from Week 8 to Month 14) after lesion counting; if cryotherapy was used without prior biopsy, this may also have introduced bias. Although use of cryotherapy was recorded, there were no records of the number of AK lesions removed by it. If investigators were less likely to take a biopsy of a suspicious AK lesion in the vehicle group versus the IngDsx group, there is potential for unintentional bias and under-reporting of skin malignancies; in addition, many skin malignancies could have been removed by local destructive therapy in the vehicle group without being diagnosed as such. Furthermore, if the investigator was unblinded to some extent by observation of LSRs, remaining lesions in IngDsx-treated patients could be perceived as suspicious because they were treatment-resistant, which may introduce bias towards taking biopsies from them (see supplementary information for more detailed consideration and an exploratory investigation of actual and cumulative AK counts in the tracking area (**Table S3**).

The increased incidence of identified cases of non-melanoma skin malignancies inside the treatment area during long-term follow-up in the IngDsx trials was not observed in long-term follow-up with ingenol mebutate [31] where the contiguous skin area treated was smaller than that in the IngDsx trials (25cm^2 versus. >25cm^2 up to 250cm^2). The differences and challenges of long-term follow-up should be considered when comparing the results of the 1-year follow-up with IngDsx and ingenol mebutate. All patients that completed the IngDsx trials, independent of clearance status were eligible for inclusion in the long-term follow-up trial, however long-term follow-up in the ingenol mebutate trials only included patients that achieved complete clearance in the phase 3 trial. Cryotherapy was allowed at the investigator's discretion in the long-term follow-up trial with ingenol mebutate, however very few patients from the vehicle group were included as only completely cleared patients were included in the long term follow up studies. Thus, the potential bias introduced by cryotherapy was only seen in the IngDsx trials since patients were followed up independent of clearance status and many more interventions of this kind were done in the vehicle arm than in the IngDsx arm (**Table S3**). The data observed with IngDsx are however considered relevant to the evaluation of the safety profile of ingenol mebutate. As of January 2020, safety concerns with ingenol mebutate have been raised following the completion of a 3-year safety study which showed a higher incidence of skin malignancy with ingenol mebutate than with the comparator imiquimod within the treatment area, but not outside the treatment area. An Article 20 procedure with the European Medicines Agency has now been triggered. While ingenol mebutate is under review, as a precaution, the Pharmacovigilance Risk Assessment Committee they have recommended that ingenol mebutate treatment is suspended in the EU.

Conclusion

In this pooled analysis of four IngDsx trials, treatment of AK with IngDsx on the FC (up to 250cm^2) or full balding S (>25cm^2 to ~250cm^2) was shown to be superior to vehicle for primary and all secondary endpoints. Treatment was well tolerated, with

administration site pain and pruritus as the most common adverse events in the IngDsx group and high treatment completion rates. However, during the 12-month follow-up, an imbalance in malignancies in the treatment area was seen, most likely due to an unintended observer bias.

Potential conflicts of interest

Brian Berman MD PhD: LEO Pharma US Speaker, Advisory Board and Investigator; personal fees from LEO Pharma US, during the conduct of the study; grants and personal fees from Biofrontera, personal fees from LEO Pharma US, Pierre-Fabre, Ortho Dermatologics and Sun, outside the submitted work. Michael Bukhalo MD: grants from LeoPharma, during the conduct of the study; grants and personal fees from Novartis, Boehringer Ingelheim, LeoPharma, Allergan and MedImmune; grants from Eli Lilly, DUSA Pharmaceuticals, Merck, Galderma, Centocor, Celgene and Athenex, outside the submitted work. C William Hanke MD MPH: Leo Pharma US Advisory Board and Investigator, during the conduct of the study and outside the submitted work. Mikala Fiig Jarner, MSc: Employee of Leo Pharma. Thomas Larsson, Dr Med Sci: Employee of Leo Pharma, personal fees from LEO Pharma A/S, during the conduct of the study; personal fees from LEO Pharma A/S, outside the submitted work. Daniel M Siegel MD, MS: LEO Pharma US Speaker, Advisory Board and Investigator; personal fees from LEO. Torsten Skov: Employee of Leo Pharma. Rolf-Markus Szeimies MD PhD: LEO Pharma Advisory Board and Investigator; grants, personal fees and non-financial support from LEO Pharma, Galderma International, grants and personal fees from Biofrontera and Almirall, during the conduct of the study, grants from Dr. Wolff-Group, Eli Lilly, photonamic and Galapagos, personal fees and non-financial support from Janssen, grants and personal fees from Novartis, outside the submitted work.

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References

1. Berman B, Cockerell CJ. Pathobiology of actinic keratosis: ultraviolet-dependent keratinocyte proliferation. *J Am Acad Dermatol*. 2013;68(1 Suppl 1):S10-9. [PMID: 23228301].
2. Rigel DS, Stein Gold LF. The importance of early diagnosis and treatment of actinic keratosis. *J Am Acad Dermatol*. 2013;68:S20-7. [PMID: 23228303].
3. Christensen SR. Recent advances in field cancerization and management of multiple cutaneous squamous cell carcinomas. *F1000Res*. 2018;7. [PMID: 29904586].
4. Werner RN, Sammain A, Erdmann R, Hartmann V, Stockfleth E, Nast A. The natural history of actinic keratosis: a systematic review. *Br J Dermatol*. 2013;169:502-18. [PMID: 23647091].
5. Criscione VD, Weinstock MA, Naylor MF, Luque C, Eide MJ, Bingham SF. Actinic keratoses: natural history and risk of malignant transformation in the Veterans Affairs Topical Tretinoin Chemoprevention Trial. *Cancer*. 2009;115:2523-30. [PMID: 19382202].
6. Lanoue J, Chen C, Goldenberg G. Actinic keratosis as a marker of field cancerization in excision specimens of cutaneous malignancies. *Cutis*. 2016;97:415-20. [PMID: 27416085].
7. Dreno B, Amici JM, Basset-Seguin N, Cribier B, Claudel JP, Richard MA. Management of actinic keratosis: a practical report and treatment algorithm from AKTeam expert clinicians. *J Eur Acad Dermatol Venereol*. 2014;28:1141-9. [PMID: 24612407]
8. Fernandez-Figueras MT, Carrato C, Saenz X, Puig L, Musulen E, Ferrandiz C, Ariza A. Actinic keratosis with atypical basal cells (AK I) is the most common lesion associated with invasive squamous cell carcinoma of the skin. *J Eur Acad Dermatol Venereol*. 2015;29:991-7. [PMID: 25428612].
9. Szeimies RM, Gerritsen MJ, Gupta G, Ortonne JP, Serresi S, Bichel J, Lee JH, Fox TL, Alomar A. Imiquimod 5% cream for the treatment of actinic keratosis: results from a phase III, randomized, double-blind, vehicle-controlled, clinical trial with histology. *J Am Acad Dermatol*. 2004;51:547-55. [PMID: 15389189].
10. Korman N, Moy R, Ling M, Matheson R, Smith S, McKane S, Lee JH. Dosing with 5% imiquimod cream three times per week for the

treatment of actinic keratosis: results of two phase 3, randomized, double-blind, parallel-group, vehicle-controlled trials. *Arch Dermatol*. 2005;141:467-73. [PMID: 15837864].

11. Alirezai M, Dupuy P, Amblard P, Kalis B, Souteyrand P, Frappaz A, Sendagorta E. Clinical evaluation of topical isotretinoin in the treatment of actinic keratoses. *J Am Acad Dermatol*. 1994;30:447-51. [PMID: 8113458].
12. Marks R, Foley P, Goodman G, Hage BH, Selwood TS. Spontaneous remission of solar keratoses: the case for conservative management. *Br J Dermatol*. 1986;115:649-55. [PMID: 3801305]
13. Ulrich M, Drecoll U, Stockfleth E. Emerging drugs for actinic keratosis. *Expert Opin Emerg Drugs*. 2010;15:545-55. [PMID: 20670178].
14. Jetter N, Chandan N, Wang S, Tsoukas M. Field Cancerization Therapies for Management of Actinic Keratosis: A Narrative Review. *Am J Clin Dermatol*. 2018;19:543-57. [PMID: 29582369].
15. Werner RN, Stockfleth E, Connolly SM, Correia O, Erdmann R, Foley P, Gupta AK, Jacobs A, Kerl H, Lim HW, Martin G, Paquet M, Pariser DM, Rosumeck S, Rowert-Huber HJ, Sahota A, Sangueza OP, Shumack S, Sporbeck B, Swanson NA, Torezan L, Nast A, International League of Dermatological S, European Dermatology F. Evidence- and consensus-based (S3) Guidelines for the Treatment of Actinic Keratosis - International League of Dermatological Societies in cooperation with the European Dermatology Forum - Short version. *J Eur Acad Dermatol Venereol*. 2015;29:2069-79. [PMID: 26370093].
16. EMC. Picato® Summary of Product Characteristics 2019. Available from: https://www.medicines.org.uk/emc/product/2888/smpc. Accessed on October 7, 2020.
17. Berman B, Tyring S, Nahm WK, Osterdal ML, Petersen AH, Siegel DM. Three-Day Field Treatment with Ingenol Disoxate (LEO 43204) for Actinic Keratosis: Cosmetic Outcomes and Patient Satisfaction from a Phase II Trial. *J Clin Aesthet Dermatol*. 2017;10:26-32. [PMID: 29399258].
18. Bertelsen M, Stahlhut M, Grue-Sorensen G, Liang X, Christensen GB, Skak K, Engell KM, Hogberg T. Ingenol disoxate: A novel 4-Isoxazolecarboxylate ester of ingenol with improved properties for treatment of actinic keratosis and other non-melanoma skin cancers. *Dermatol Ther (Heidelb)*. 2016;6:599-626. [PMID: 27503482].
19. Weiss J, Ulrich M, Bukhalo M, Osterdal ML, Bang B, Hanke CW. A seamless phase I/II dose-finding trial assessing ingenol disoxate (LEO 43204) for field treatment of actinic keratosis on the scalp. *Br J Dermatol*. 2017;176:1456–1464. [PMID: 28078678].
20. Siegel DM, Tyring S, Nahm WK, Osterdal ML, Petersen AH, Berman B. Three-day Field Treatment with Ingenol Disoxate (LEO 43204) for Actinic Keratosis: A Phase II Trial. *J Clin Aesthet Dermatol*. 2017;10:19-26. [PMID: 29399263].
21. Bourcier M, Stein Gold L, Guenther L, Andreassen CM, Selmer J, Goldenberg G. A dose-finding trial with a novel ingenol derivative (ingenol disoxate: LEO 43204) for field treatment of actinic keratosis on full face or 250 cm^2 on the chest. *J Dermatolog Treat*. 2017;[Epub ahead of print]:ssApr 4. doi: 10.1080/09546634.2017.1303568. [PMID: 28264612].
22. Rosen R, Marmur E, Anderson L, Welburn P, Katsamas J. A new, objective, quantitative scale for measuring local skin responses following topical actinic keratosis therapy with ingenol mebutate. *Dermatol Ther (Heidelb)*. 2014;4:207-19. [PMID: 25073700].
23. Lebwohl M, Swanson N, Anderson LL, Melgaard A, Xu Z, Berman B. Ingenol mebutate gel for actinic keratosis.*N Engl J Med* 2012;366:1010-9. [doi: 10.1056/NEJMoa1111170].
24. Skov T, Stockfleth E, Szeimies RM, Berman B. Efficacy Endpoints in Clinical Trials in Actinic Keratosis. *Dermatol Ther (Heidelb)*. 2018;8:425-33. [PMID: 29916197].
25. Steeb T, Heppt MV, Becker L, Kohl C, French LE, Berking C. Long-term efficacy of interventions for actinic keratosis: protocol for a systematic review and network meta-analysis. *Syst Rev*. 2019;8:237. [doi: 10.1186/s13643-019-1156-8].
26. Casari A, Chester J, Pellacani G. Actinic Keratosis and Non-Invasive Diagnostic Techniques: An Update. *Biomedicines*. 2018;6:8. [PMID: 29316678].
27. Ruini C, Hartmann D, Bastian M, Ruzicka T, French LE, Berking C, von Braunmühl T. Non-invasive monitoring of subclinical and clinical actinic keratosis of face and scalp under topical treatment with ingenol mebutate gel 150 mcg/g by means of reflectance confocal microscopy and optical coherence tomography: New perspectives and comparison of diagnostic techniques. *J Biophotonics*. 2019;12:e201800391. [PMID: 30653833].
28. Fernandez Figueras MT. From actinic keratosis to squamous cell carcinoma: pathophysiology revisited. *J Eur Acad Dermatol Venereol*. 2017;31 Suppl 2:5-7. [PMID: 28263020].
29. Neugebauer R, Su KA, Zhu Z, Sokil M, Chren MM, Friedman GD, Asgari MM. Comparative effectiveness of treatment of actinic keratosis with topical fluorouracil and imiquimod in the prevention of keratinocyte carcinoma: a cohort study. *J Am Acad Dermatol*. 2019;80:998-1005. [PMID: 30458208].
30. Balcere A, Karls R, Čēma I, Rone Kupfere M, Vīksna L, Krūmiņa A. Treatment of Actinic Keratoses Facilitates Dermatoscopic Diagnosis of Early Basal Cell Carcinoma: A Case Report and Review. *Case Rep Dermatol*. 2019;11:16-22. [doi: 10.1159/000496329].
31. Lebwohl M, Shumack S, Stein Gold L, Melgaard A, Larsson T, Tyring SK. Long-term follow-up study of ingenol mebutate gel for the treatment of actinic keratoses. *JAMA Dermatol*. 2013;149:666-70. [PMID: 23553119].

Table 1. *Patient demographics and disease characteristics at baseline for the pooled analyses (full analysis set).*

Endpoint	Face/chest		Scalp	
Treatment group	**IngDsx (0.018%)**	**Vehicle**	**IngDsx (0.037%)**	**Vehicle**
Total no. patients	**407**	**204**	**418**	**205**
Age (years) Mean (SD)	68.6 (9.0)	66.8 (9.5)	70.3 (9.1)	70.4 (9.3)
Age group				
<65 years, n (%)	136 (33.4)	72 (35.3)	105 (25.1)	45 (22.0
65–74 years, n (%)	153 (37.6)	86 (42.2)	173 (41.4)	89 (43.4)
≥75 years, n (%)	118 (29.0)	46 (22.5)	140 (33.5)	71 (34.6)
Sex				
Male, n (%)	275 (67.6)	125 (61.3)	417 (99.8)	205 (100.0)
Race*				
White, n (%)	406 (99.8)	204 (100)	415 (100.0)	202 (100.0)
Other, n (%)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity*				
Hispanic or Latino, n (%)	23 (5.7)	6 (2.9)	16 (3.9)	6 (3.0)
Not Hispanic or Latino, n (%)	384 (94.3)	198 (97.1)	399 (96.1)	196 (97.0)
Fitzpatrick skin type				
Type I, n (%)	64 (15.7)	28 (13.7)	42 (10.0)	25 (12.2)
Type II, n (%)	232 (57.0)	130 (63.7)	229 (54.8)	106 (51.7)
Type III, n (%)	102 (25.1)	40 (19.6)	125 (29.9)	61 (29.8)
Type IV, n (%)	7 (1.7%)	5 (2.5)	19 (4.5	12 (5.9)
Type V, n (%)	2 (0.5)	1 (0.5)	3 (0.7)	1 (0.5)
AK count group, n (%)				
5–10 AKs	177 (43.5)	90 (44.1)	176 (42.1)	64 (31.2)
≥11 AKs	230 (56.5)	114 (55.9)	242 (57.9)	141 (68.8)
Skin cancer history, n (%)				
Yes	210 (51.6)	112 (54.9)	195 (46.7)	107 (52.2)
No	197 (48.4)	92 (45.1)	223 (53.3)	98 (47.8)
Type and size of treatment area, n (%)				
Face, full beard	22 (5.4)	14 (6.9)	-	-
Face, no full beard	313 (76.9)	145 (71.1)	-	-
Chest, 250 cm^2	72 (17.7)	45 (22.1)	-	-
Size of treatment area, n (%)				
25–74 cm^2	-	-	38 (9.1)	18(8.8)
75–124 cm^2	-	-	45 (10.8)	19 (9.3)
125–199 cm^2	-	-	92 (22.0)	46 (22.4)
200–250 cm^2	-	-	243 (58.1)	122 (59.5)
Duration of AK group, n (%)				
0–7 years	174 (42.8)	85 (41.7)	233 (55.7)	110 (53.7)
≥8 years	233 (57.2)	119 (58.3)	185 (44.3)	95 (46.3)

*Percentages are based on observed cases.
Abbreviations: AK, actinic keratosis; FC, Face/chest; S, scalp; SD, standard deviation.

Table 3. *Patient-reported outcomes—cosmetic, Skindex-16 and Treatment Satisfaction Questionnaire for Medication for pooled analyses (observed cases) for face/chest (FC) and scalp (S) trials (full analysis set).*

Endpoint	Face/chest		Scalp	
Treatment group	**IngDsx**	**Vehicle**	**IngDsx**	**Vehicle**
Total no. patients	**407**	**204**	**418**	**205**
Cosmetic outcome at Week 8				
Overall feel of the treatment area, n (%)				
Much improved	207 (53.4)	11 (6.2)	242 (61.1)	8 (4.4)
Somewhat improved	145 (37.4)	31 (17.4)	105 (26.5)	42 (23.1)
No change	28 (7.2)	125 (70.2)	45 (11.4)	120 (65.9)
Somewhat worsened	8 (2.1)	9 (5.1)	3 (0.8)	10 (5.5)
Much worsened	0 (0.0)	2 (1.1)	1 (0.3)	2 (1.1)
Overall appearance of the treatment area, n (%)				
Much improved	216 (55.7)	11 (6.2)	257 (64.9)	10 (5.5)
Somewhat improved	147 (37.9)	36 (20.2)	106 (26.8)	38 (20.9)
No change	19 (4.9)	117 (65.7)	28 (7.1)	121 (66.5)
Somewhat worsened	5 (1.3)	12 (6.7)	3 (0.8)	12 (6.6)
Much worsened	1 (0.3)	2 (1.1)	2 (0.5)	1 (0.5)
TSQM domain score at Week 8[1]				
Effectiveness				
Mean	74.1	38.5	73.2	35.3
SD	19.1	28.6	20.5	28.0
Number	397	186	405	188
Side Effects				
Mean	92.6	99.8	93.3	99.2
SD	18.0	1.7	17.7	6.1
Number	397	185	404	187
Convenience				
Mean	79.8	78.9	78.8	76.2
SD	16.1	17.0	17.1	20.3
Number	397	186	403	185
Global Satisfaction				
Mean	73.0	39.1	71.8	35.3
SD	20.9	30.1	22.6	28.2
Number	390	179	395	183
Estimated treatment difference[2]		34.22		36.48
Lower 95% CI		29.99		32.19
Upper 95% CI		38.45		40.78
P value		<0.001		<0.001
Skindex-16 Symptom Scores by visit				
Day 1				
Mean	18.7	19.4	17.7	16.4
SD	20.9	21.6	19.9	20.3
Number	402	202	410	203
Day 8				

Mean	60.5	11.4	57.1	11.8
SD	26.4	16.7	26.5	18.4
Number	394	197	400	192
Week 8				
Mean	7.6	16.1	8.4	14.4
SD	12.5	20.9	14.5	19.6
Number	402	188	404	187

[1]Score ranges from 0 to 100 where high values mean more effective, fewer side effects, more convenient or higher satisfaction.
[2]Analysis of variance of difference in global satisfaction with treatment group and pooled site as factors.
Abbreviations: CI, confidence interval; FC, face/chest; S, scalp; SD, standard deviation; TSQM, Treatment Satisfaction Questionnaire for Medication.

Table 4. *Incidence of adverse events at Week 8 and after 12-month extended follow-up period (safety analysis set).*

Endpoint	Face/chest				Scalp			
Treatment group	**IngDsx**		**Vehicle**		**IngDsx**		**Vehicle**	
Total no. patients	**407**		**204**		**419**		**204**	
Week 8 (safety analysis set)								
Adverse event category	**AE[1]**	**n (%)**	**AE[1]**	**n (%)**	**AE[1]**	**n (%)**	**AE[1]**	**n (%)**
All adverse events	633	286 (70.3)	74	48 (23.5)	752	329 (78.5)	116	76 (37.3)
Related adverse events	507	260 (63.9)	18	15 (7.4)	594	307 (73.3)	34	27 (13.2)
Related administration site reactions inside treatment area	401	246 (60.4)	16	13 (6.4)	401	276 (65.9)	17	16 (7.8)
Severe adverse events	46	34 (8.4)	0	0 (0.0)	41	32 (7.6)	5	5 (2.5)
Severe, related adverse events	42	30 (7.4)	0	0 (0.0)	35	28 (6.7)	1	1 (0.5)
Serious adverse events	7	2 (0.5)	0	0 (0.0)	8	7 (1.7)	5	5 (2.5)
Serious, related adverse events	6	1 (0.2)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
Adverse events leading to discontinuation of treatment	28	15 (3.7)	0	0 (0.0)	9	9 (2.1)	4	2 (1.0)
Adverse events leading to withdrawal from trial	1	1 (0.2)	0	0 (0.0)	0	0 (0.0)	2	2 (1.0)
After 12-month follow-up period (follow up analysis set)								
Total no. patients	**400**		**182**		**412**		**178**	
All adverse events	90	70 (17.5)	26	22 (12.1)	55	45 (10.9)	21	16 (9.0)
Related adverse events	9	9 (2.3)	4	4 (2.2)	7	7 (1.7)	0	0 (0.0)
Related administration site reactions inside treatment area	2	2 (0.5)	4	4 (2.2)	3	3 (0.7)	0	0 (0.0)
Severe adverse events	0	0 (0.0)	0	0 (0.0)	2	2 (0.5)	0	0 (0.0)
Severe, related adverse events	0	0 (0.0)	0	0 (0.0)	2	2 (0.5)	0	0 (0.0)
Serious adverse events	1	1 (0.3)	0	0 (0.0)	1	1 (0.2)	0	0 (0.0)
Serious, related adverse events	0	0 (0.0)	0	0 (0.0)	1	1 (0.2)	0	0 (0.0)

[1]Different adverse events within the same preferred term and system organ class and involving the same subject have been counted as one.
Abbreviations: CI, confidence interval; FC, face/chest; S, scalp; SD, standard deviation; TSQM, Treatment Satisfaction Questionnaire for Medication.

Table 5. *Most frequent related AEs at Week 8 and 12-month extended follow-up period (safety analysis set).*

Endpoint	Face/chest		Scalp	
Treatment group	**IngDsx**	**Vehicle**	**IngDsx**	**Vehicle**
Total no. patients	**407**	**204**	**419**	**204**
Week 8				
SOC Preferred Term[1]	**n (%)**	**n (%)**	**n (%)**	**n (%)**
General disorders and administration site conditions				
Application site pain	233 (57.2)	6 (2.9)	245 (58.5)	6 (2.9)
Application site pruritus	134 (32.9)	5 (2.5)	131 (31.3)	8 (3.9)
Application site discomfort	15 (3.7)	3 (1.5)	9 (2.1)	1 (0.5)
Eye disorders				
Periorbital edema	15 (3.7)	0 (0.0)	23 (5.5)	0 (0.0)
Eyelid edema	1 (0.2)	0 (0.0)	12 (2.9)	0 (0.0)
Nervous system disorders				
Headache	19 (4.7)	0 (0.0)	32 (7.6)	3 (1.5)
Psychiatric disorders				
Insomnia	13 (3.2)	0 (0.0)	20 (4.8)	0 (0.0)
After 12-month follow-up period (follow-up analysis set)				
Total no. patients	**400**	**182**	**412**	**178**
Neoplasms benign, malignant and unspecified (incl cysts and polyps)				
Squamous cell carcinoma of skin	3 (0.8)	0 (0.0)	1 (0.2)	0 (0.0)
Basal cell carcinoma	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Bowen's disease	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Malignant melanoma	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
SOC total	5 (1.3)	0 (0.0)	2 (0.5)	0 (0.0)
General disorders and administration site conditions				
Application site scar	2 (0.5)	3 (1.6)	1 (0.2)	0 (0.0)
Application site macule	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Application site scab	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Application site pruritus	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
SOC total	2 (0.5)	4 (2.2)	3 (0.7)	0 (0.0)
Skin and subcutaneous tissue disorders				
Alopecia	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Pemphigoid	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Post inflammatory pigmentation change	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
SOC total	1 (0.3)	0 (0.0)	2 (0.5)	0 (0.0)
Injury, poisoning and procedural complications				
Scar	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
SOC total	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Total number of adverse events[2]	9	4	7	0
Total number of patients	9 (2.3)	4 (2.2)	7 (1.7)	0 (0.0)

[1]Classification according to MedDRA version 18.1.
[2]Different adverse events within the same preferred term and system organ class and involving the same subject have been counted as one.
Abbreviations: FC, face/chest; n, number of patients; S, scalp; SOC, system organ class.

Supplementary Information

Methods

A protocol amendment was issued on March 04, 2016. The protocol was updated to specify the method (interactive web response system) for ensuring that the face/chest (FC) trials enrolled a minimum of 15% and a maximum of 25% of patients in the chest groups. The amendment also clarified which medications were permitted and prohibited during the extended follow-up period: lesion-directed laser treatment was added to the allowed medications, and fluorouracil (Actikerall®), even as lesion-directed treatment, and laser treatment as field treatment, were prohibited.

Results:

Reduction of AK count

Longer-term efficacy was also assessed in terms of reduction in Actinic keratosis (AK) count in the tracking area, as shown in Table S2. The AK count in the tracking area was used to distinguish between AK lesions present at baseline and new AK lesions that emerged during follow-up. Thus, the endpoints related to AK count in the tracking area enabled the clearance rate of AK lesions present at baseline to be distinguished from occurrence of new AK lesions.

AKCLEAR100 and percent reduction were evaluated at Week 8 in a 50 cm^2 tracking area located within the larger treatment area, providing indirect evidence on the effect on subclinical AK. The addition of percent reduction in AK count as an endpoint provided further supportive information on the efficacy of IngDsx. The percent reduction in AK count is also less sensitive to varying AK count at randomization than AKCLEAR100 [1].

Consideration of observed discrepancy in skin malignancies between IngDsx and vehicle groups

We conducted an exploratory investigation to understand the observation of a higher incidence of skin malignancies inside the treatment area in the IngDsx group compared with the vehicle group. In these trials, the AK lesions in the tracking area were assessed, where each individual AK lesion could be followed. Once a lesion appeared, it was included in the cumulative count whether it was present at the next visit or not. Use of cryotherapy was allowed in the extended follow-up period (from Week 8 to Month 14) after the AK lesions had been counted. This use of cryotherapy was recorded in the trials, but there were no records of the number of AK lesions removed by cryotherapy.

The cumulative number of AK lesions in the tracking area increased from Week 8 to Month 14 in both treatment groups. In contrast, the actual AK count decreased in the vehicle group from Week 8 to Month 14 whereas it remained constant in the IngDsx group (Table S3). In the vehicle group, there was no overall tendency towards a reduction in AK count over the initial 8-week follow-up period. The difference between the mean cumulative count and the mean actual count in each treatment group represents the mean number of AK lesions that were observed at one of the visits during the 12-month follow-up, but that were not present at the final visit. Some of these AK lesions may have resolved spontaneously, but it can be assumed that most of the AK lesions were removed by cryotherapy (and other local destructive therapy).

The difference in the mean number of removed AK lesions between the treatment groups at Month 14 was 2.1, ie, in a vehicle-treated patient, 2.1 more AK lesions were removed by local therapies in the tracking area than in those receiving IngDsx. Taking into account around 400 vehicle-treated patients, the estimated number of AK lesions removed in the treatment area of these patients would be around 2 x 4.5 x 400=3,600 AK lesions (removed by destructive therapies). The factor of "2" reflects the relative number of AK lesions in the treatment area (mean IngDsx: 3.6 and vehicle 11.9) versus the tracking area (mean IngDsx: 1.6 and vehicle: 5.3) at Week 8. With around 800 patients in the IngDsx groups, the estimated number of AK lesions removed in the treatment area would be around 2 x 2.4 x 800=3,840. Biopsies were performed in 23 (5.6%) and 68 (8.2%) vehicle- and IngDsx-treated patients, respectively. The biopsies showed skin neoplasms in 14 and 58 patients, respectively. If biopsies had been performed on the 3,600 AK lesions removed in the vehicle-treated patients, 4.4% (based on observations in a previous trial) would be expected to have BCC, SCC or Bowen's disease, ie, 160 skin malignancies may have gone unnoticed in this group. This indicates that many skin malignancies could have been removed by local destructive therapy in the vehicle group without being diagnosed as such. If investigators were less likely to take a biopsy of a suspicious AK lesion in the vehicle group versus the IngDsx group, there is potential for unintentional bias in the reporting of skin malignancies. The majority of patients treated with IngDsx experienced some level of local skin response. If the investigator was unblinded to some extent by observation of local skin responses (LSRs), remaining lesions in IngDsx-treated patients would be perceived as suspicious because they had been treatment resistant. This may introduce investigator bias towards performing

biopsies from lesions in the IngDsx group and lead to a higher frequency of skin malignancies being diagnosed in this group. The most plausible explanation of the increased incidence of skin malignancies observed in the IngDsx treatment groups is therefore related to detection bias.

References

1. Skov T, Stockfleth E, Szeimies RM, Berman B. Efficacy Endpoints in Clinical Trials in Actinic Keratosis. *Dermatol Ther (Heidelb)*. 2018;8:425-33. [PMID: 29916197].

Table S1. *Reasons for discontinuation (up to week 8), (randomized patients).*

	Face/Chest		Scalp	
	IngDsx, n (%)	Vehicle, n (%)	IngDsx, n (%)	Vehicle, n (%)
Total number of patients randomized	410	206	420	206
Number of patients withdrawn up to Week 8	6 (1.5)	15 (7.3)	6 (1.4)	16 (7.8)
Reason for withdrawal				
Adverse event	1 (0.2)	0	0	2 (1.0)
Unacceptable LSR	1 (0.2)	0	0	0
Withdrawal by subject	1 (0.2)	12 (5.8)	4 (1.0)	10 (4.9)
Lost to follow-up	0	3 (1.5)	1 (0.2)	1 (0.5)
Protocol deviation	2 (0.5)	0	0	0
Other	1 (0.2)	0	1 (0.2)	3 (1.5)

Abbreviations: LSR, local skin response.

Table S2. *Global photo damage outcomes for pooled analyses at week 8* for face/chest and scalp.*

Endpoint	Face/Chest		Scalp	
Treatment group	IngDsx	Vehicle	IngDsx	Vehicle
Total no. patients	407	204	418	205
Marked improvement, n (%)	69 (17.1)	0 (0.0)	128 (31.2)	4 (2.1)
Moderate improvement, n (%)	133 (32.9)	9 (4.8)	92 (22.4)	12 (6.3)
Minor improvement, n (%)	118 (29.2)	33 (17.5)	114 (27.8)	24 (12.7)
No change, n (%)	81 (20.0)	133 (70.4)	73 (17.8)	127 (67.2)
Minor worsening, n (%)	3 (0.7)	10 (5.3)	3 (0.7)	19 (10.1)
Moderate worsening, n (%)	0 (0.0)	3 (1.6)	0 (0.0)	1 (0.5)
Marked worsening, n (%)	0 (0.0)	1 (0.5)	0 (0.0)	2 (1.1)

*Percentages are based on observed cases.

Table S3. *Actual and cumulative actinic keratosis count in tracking area in all trials.*

Visit	Mean cumulative AK count, IngDsx	Mean cumulative AK count, vehicle	Mean actual AK count, IngDsx	Mean actual AK count, vehicle	Difference between mean cumulative count and mean actual count, IngDsx	Difference between mean cumulative count and mean actual count, vehicle
Week 8	1.6	5.3	1.6	5.3	0.0	0.0
Month 14	4.1	7.8	1.7	3.3	2.4	4.5

Abbreviations: AK, actinic keratosis.

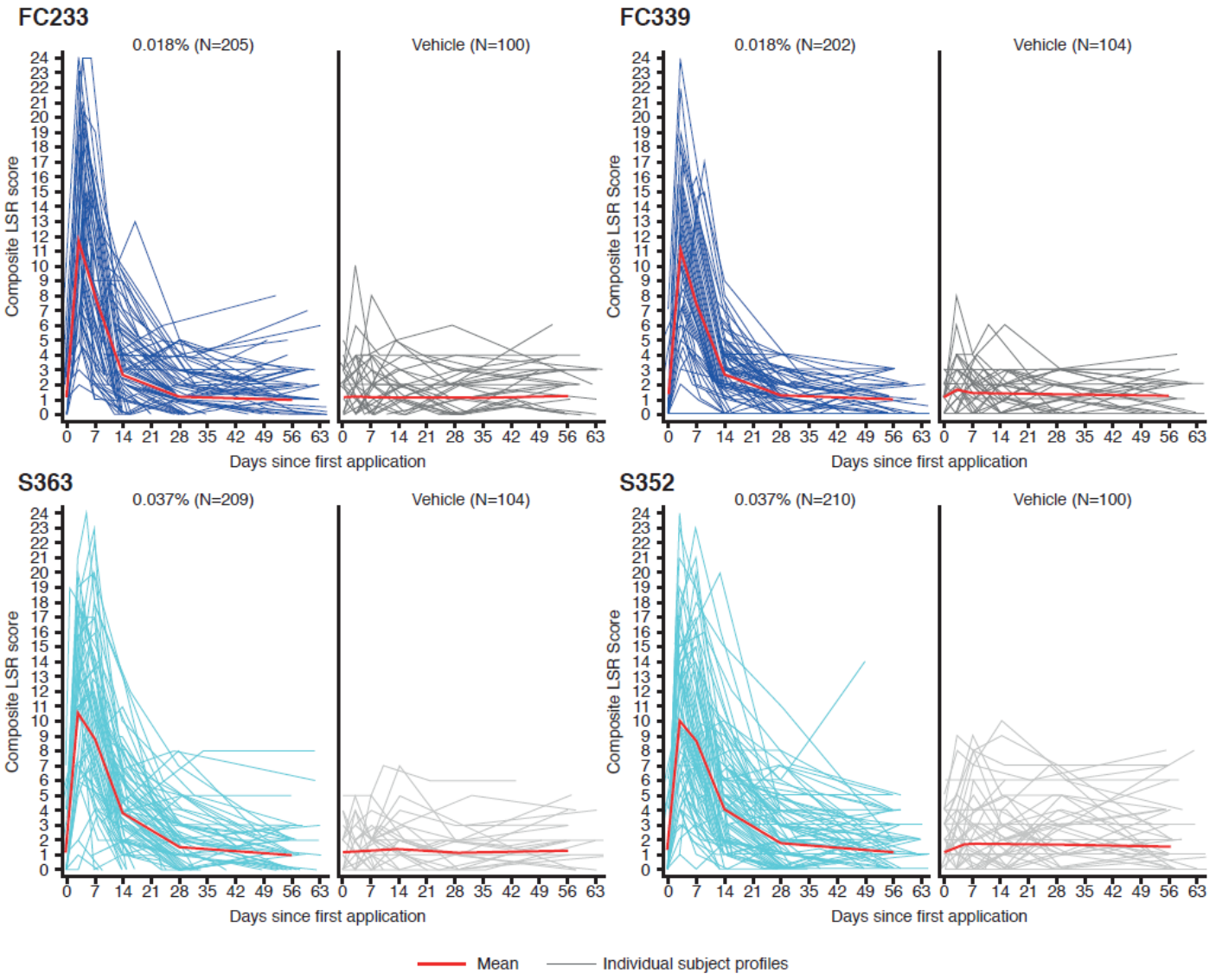

Figure S1. *Composite local skin reaction profiles.*