Photodiagnosis and Photodynamic Therapy 27 (2019) 34–38

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Sequential daylight photodynamic therapy and ingenol mebutate versus 2 sessions of daylight photodynamic therapy for the treatment of actinic keratosis: An observational, prospective, comparative study

Tamara Gracia-Cazaña[a,*], Ana Julia García Malinis[b], Manuel Almagro-Sánchez[c], Dolores Planas Linares[b], Yolanda Gilaberte[d]

[a] Department of Dermatology, Hospital de Barbastro, Huesca, Spain
[b] Dermatology Department. Hospital San Jorge, Huesca, Spain
[c] Dermatology Department, Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain
[d] Dermatology Department, Hospital Universitario Miguel Servet, Zaragoza, Spain

ARTICLE INFO

Keywords:
Daylight-photodynamic therapy
Ingenol mebutate
Actinic keratosis

ABSTRACT

Background: Field-directed therapy, such as daylight-photodynamic therapy (DL-PDT) or ingenol mebutate (IM), is indicated for multiple actinic keratosis (AK) lesions located on contiguous areas of skin with significant sun damage.

Objective: To compare the efficacy of sequential DL-PDT and IM treatment with that of 2 sessions of DL-PDT in AK patients.

Material and methods: For this observational, multicenter, prospective study we recruited patients for whom DL-PDT was indicated for the treatment of AK lesions (grades I and II) located on the head. After 1 month of follow-up those who did not achieve a satisfactory clinical response received either a second session of DL-PDT or were treated with IM. Epidemiological and clinical data were collected and analyzed.

Results: Forty-three patients were enrolled (39 male, 4 female). The mean (standard deviation, SD) age was 78 (7.84) years, and the mean (SD) number of AK lesions per patient was 9.58 (1.16). After the first session of DL-PDT, 27 patients (62.8%) required further treatment: 13 (48.1%) received a second session of DL-PDT and 14 (51.9%) were treated with IM. After 1 year of follow-up, lesion clearance rates were higher in patients who received 2 sessions of DL-PDT than those treated with sequential DL-PDT plus IM (75.2%vs 54.6%, $p = 0.0013$). Local skin reactions were more frequent in the DL-PDT plus IM group than the group treated with 2 sessions of DL-PDT ($p = 0.0245$).

Conclusions: The combination of DL-PDT plus IM appears to have no synergistic effect in the treatment of field cancerization, and offers no benefits over 2 sessions of DL-PDT monotherapy, although both combinations produced high lesion clearance rates, a good safety profile, excellent cosmetic outcome, and good patient satisfaction.

1. Introduction

Actinic keratosis (AK) is a precancerous skin lesion that develops in sun-exposed areas of skin, and is the most common premalignant skin tumor [1]. Given the known risk of progression to squamous cell carcinoma (SCC) and the lack of prognostic markers of lesion progression, treatment of the entire affected skin area is warranted [2]. Field cancerization, i.e., the presence of genetic alterations in tissue chronically exposed to a carcinogen (mainly UV radiation), is an important component of the genesis of non-melanoma skin cancer (NMSC) [3]. These mutations are responsible for the development of subclinical and multifocal clinical cancer lesions, and are associated with an increased risk of multiple cancers in the affected area.

AK treatment is selected depending on lesion number and extension. AK is most often managed using non-invasive treatments, particularly in cases involving multiple lesions in the context of field cancerization. Field-directed regimens, such as daylight-photodynamic therapy (DL-PDT) and ingenol mebutate (IM) treatment, are indicated for multiple AK lesions on contiguous areas of skin and areas with significant sun damage. DL-PDT and IM are effective treatments for AK [4], with

* Corresponding author at: Department of Dermatology, Hospital de Barbastro, Av Pirineos nº 11 1ºA, P.O. Box: 22011 – Barbastro, Huesca, Spain.
E-mail address: tamgracaz@gmail.com (T. Gracia-Cazaña).

https://doi.org/10.1016/j.pdpdt.2019.05.030
Received 31 January 2019; Received in revised form 14 May 2019; Accepted 24 May 2019
Available online 26 May 2019
1572-1000/

similar clearance rates (72.4% and 73.6%, respectively) after 3 months, although IM causes more marked local skin reactions (LSRs) [5].

Strategies used to optimize efficacy and improve tolerability include repeated treatment with DL-PDT or IM, or their combination with other treatment modalities [6]. In this study, we evaluated the efficacy of sequential treatment with DL-PDT plus IM versus 2 sessions of DL-PDT in AK patients.

2. Materials and methods

2.1. Study design and subjects

This observational, multicenter, prospective pilot study included patients with a clinical diagnosis of AK on the scalp or face [7]. Three hospitals participated in the study: Hospital San Jorge and Hospital Barbastro (Huesca, Spain) and Complejo Hospitalario Universitario A Coruña (A Coruña, Spain). Participants were recruited between February and June of 2017. All patients eligible for DL-PDT according to clinical guidelines [7] were invited to participate in the study, applying the following inclusion criterion: presence on the face or scalp of > 4 actinic keratosis lesions graded I or II according to Olsen et al. [7]. The following exclusion criteria were applied: pigmented AK; skin cancer in the treated area; treatment of AK during the preceding 3 months; hypersensitivity to IM or methyl-aminolevulinate (MAL). Demographic characteristics and skin cancer risk factors including patient history of treatment with immunosuppressive drugs and skin cancer were also recorded and analyzed.

2.2. Treatment protocol

Daylight PDT consisted of the application of organic sunscreen (Actinica® lotion, SPF 50+, Galderma, France) to all photoexposed parts of the body in which lesions were located. After allowing absorption for 15 min, scales and hyperkeratosis were removed from the affected skin by gentle rubbing with sandpaper or curettage. Following the standard procedure [8], MAL cream (160 mg/g; Metvix, Galderma, France) was applied to the affected skin and left uncovered. Thirty minutes after MAL application patients were exposed to daylight illumination for 2 continuous hours, between 11:00 and 14:00. Daylight PDT was conducted in full daylight in cloudy, cloudy-to-sunny, or sunny conditions. After treatment, patients were instructed to remain indoors for the rest of the day to avoid further light exposure [9].

Patients who did not show a satisfactory clinical response after 1 month of follow-up underwent another session of DL-PDT or received IM treatment in accordance with standard clinical guidelines. The latter consisted of the application by the patient themselves of 150 μg/g IM gel on the cancerization field of the remaining lesions once daily for 3 consecutive days, in accordance with the official protocol for AK treatment with IM gel (maximum total surface area, 25 cm^2) [10].

2.3. Efficacy endpoints

The primary efficacy endpoint was the complete lesion response rate 12 weeks after the first DL-PDT session. Lesions that responded completely were followed up at 6, 9, and 12 months. The clinical response of each patient was selected as a secondary endpoint.

2.4. Safety endpoints

The type and number of LSRs directly related to treatment were monitored, and any adverse events observed during the study were recorded.

2.5. Additional endpoints

Global patient satisfaction and cosmetic outcome were also evaluated. Patient quality of life was assessed using the validated Actinic Keratosis Quality of Life Questionnaire (AKQoL) [11].

2.6. Statistical analyses

Descriptive statistics were calculated for all variables. Continuous variables are presented as the number of valid cases (mean, standard deviation [SD], median, percentiles 25 and 75 [P25–P75], minimum and maximum). Categorical variables are represented as the frequencies of each category (absolute and relative to the total number of valid values [N]). A Student's *t*-test was used to compare the means of continuous variables between 2 groups, and associations between qualitative variables were assessed using the Chi-squared test or Fisher's exact test. Normal distribution of quantitative variables was evaluated using the Kolmogorov-Smirnov test. In all instances, the level of significance was set at $p < 0.05$.

An intention-to-treat approach was used, with data from the last observation carried forward in cases of missing follow-up data.

2.7. Ethical considerations

The study protocol was approved by the Galicia Ethical Committee for Clinical Research (MAS-ING-2015-01) and the relevant competent authorities. This post-authorization study was registered with the Spanish Agency of Medicines and Health Products (AEMPS) (number 2016/032). The study was performed in accordance with national drug laws, Good Clinical Practice guidelines, and the Declaration of Helsinki.

3. Results

In total, 43 patients (39 male, 4 female) were enrolled (mean [SD] age, 78 [7.84] years), 27 (62.8%) of whom had received no previous treatment for AK. The mean (SD) number of AK lesions per patient was 9.58 (1.16). A total of 412 AK lesions were analyzed. The distribution of Olsen grades was as follows: grade I, 37.4% (n = 154); grade II, 55.8% (n = 230); grade III, 6.8% (n = 28). Lesions were located on the face (32.6%, n = 14), scalp (44.2%, n = 19), or both (23.3%, n = 10).

Previous immunosuppressive treatment was recorded for 4 (9.3%) patients. Analysis of phototype revealed a homogeneous distribution: phototype I-II, 51.2% (n = 22); phototype III-IV, 48.8% (n = 21). Nineteen patients (44.2%) had a history of previous non-melanoma skin cancer, of which basal cell carcinoma (BCC) was the most frequent form (50% of this cohort). Of the 43 enrolled patients, 28 had received previous treatment, the most common of which was cryotherapy (46.4%, n = 13), followed by MAL-PDT (14.3%, n = 4).

After the first session of DL-PDT, the mean (SD) number of AK lesions was 0.5 (0.89) in the group that responded to treatment, and 4.11 (1.76) in the group assigned a second treatment session.

After 1 year of follow-up, the number of AK lesions was significantly lower in patients treated with 1 versus 2 sessions of DL-PDT (1.13 [1.09] versus 4.07 [4.12]; p = 0.007).

Comparison of Olsen grades between groups (Table 1) revealed that 91.6% of AK lesions treated with a single session of DL-PDT showed a complete response at 3 months, as compared with 76.7% of AK lesions treated with either DL-PDT + IM or 2 sessions of DL-PDT ($p < 0.01$). After 1 year of follow-up, these percentages dropped to 87.4% and 65.6% respectively ($p < 0.01$). Thus, in both groups no increase in either thickness or Olsen grade was observed for the majority of unresolved AK lesions.

After 1 cycle of DL-PDT, 27 (62.8%) patients showed an incomplete response, and required further treatment: 13 (48.1%) received a second session of DL-PDT and 14 (51.9%) received IM treatment. After 3 months of follow-up, a complete response was observed for 80% (n = 116) of lesions in patients who received 2 DL-PDT sessions, and for 73.2% (n = 104) of lesions in patients who received DL-PDT plus IM (p = 0.0063). After 1 year of follow-up, the lesion response rate was

Table 1
Summary of the recovery rate according to the Olsen grade of AK lesions in patients that received a single session of DL-PDT (1 × DL-PDT) versus those who received either 2 sessions of DL-PDT (2 × DL-PDT) or 1 session of DL-PDT followed by IM treatment (DL-PDT + IM).

Variable	AK (Olsen grade)	Total	2 × DL-PDT or DL-PDT + IM	1 × DL-PDT	p-value
First visit	Total	412 (100%)	260 (100%)	152 (100%)	0.0420
	Grade 1	154 (37.4%)	86 (33.1%)	68 (44.7%)	
	Grade 2	230 (55.8%)	153 (58.8%)	77 (50.7%)	
	Grade 3	28 (6.8%)	21 (8.1%)	7 (4.6%)	
	N missing	58	57	1	
2nd visit (1 month)	Total	424 (100%)	284 (100%)	140 (100%)	< 0.0001
	Grade 1	114 (26.9%)	106 (37.3%)	8 (5.7%)	
	Grade 2	29 (6.8%)	28 (9.9%)	1 (0.7%)	
	Grade 3	1 (0.2%)	1 (0.4%)	0 (0%)	
	Recovery	280 (66.0%)	149 (52.5%)	131 (93.6%)	
	N missing	46	33	13	
3rd visit (3 months)	Total	430 (100%)	287 (100%)	143 (100%)	0.0004
	Grade 1	45 (10.5%)	35 (12.2%)	10 (7%)	
	Grade 2	32 (7.4%)	31 (10.8%)	1 (0.7%)	
	Grade 3	2 (0.5%)	1 (0.3%)	1 (0.7%)	
	Recovery	351 (81.6%)	220 (76.7%)	131 (91.6%)	
	N missing	40	30	10	
4th visit (6 months)	Total	428 (100%)	285 (100%)	143 (100%)	< 0.0001
	Grade 1	64 (15%)	56 (19.6%)	8 (5.6%)	
	Grade 2	25 (5.8%)	25 (8.8%)	0 (0%)	
	Grade 3	3 (0.7%)	3 (1.1%)	0 (0%)	
	Recovery	336 (78.5%)	201 (70.5%)	135 (94.4%)	
	N missing	42	32	10	
5th visit (1 year)	Total	399 (100%)	256 (100%)	143 (100%)	< 0.0001
	Grade 1	54 (13.5%)	42 (16.4%)	12 (8.4%)	
	Grade 2	48 (12%)	44 (17.2%)	4 (2.8%)	
	Grade 3	4 (1%)	2 (0.8%)	2 (1.4%)	
	Recovery	293 (73.4%)	168 (65.6%)	125 (87.4%)	
	N missing	71	61	10	

75.2% (n = 103) in patients who received 2 DL-PDT sessions versus 54.6% (65) in patients who received DL-PDT plus IM (p = 0.0007).

Fig. 1 and Table S1 shows the response rate of AK lesions to treatment, stratified according to Olsen grade, at each follow-up visit. A higher response rate was observed for the group that received 2 DL-PDT sessions. After 1 year of follow up, grade-I AK lesions (n = 19) predominated in the group that received 2 DL-PDT sessions, while grade-II AK lesions predominated in the DL-PDT plus IM group (n = 31).

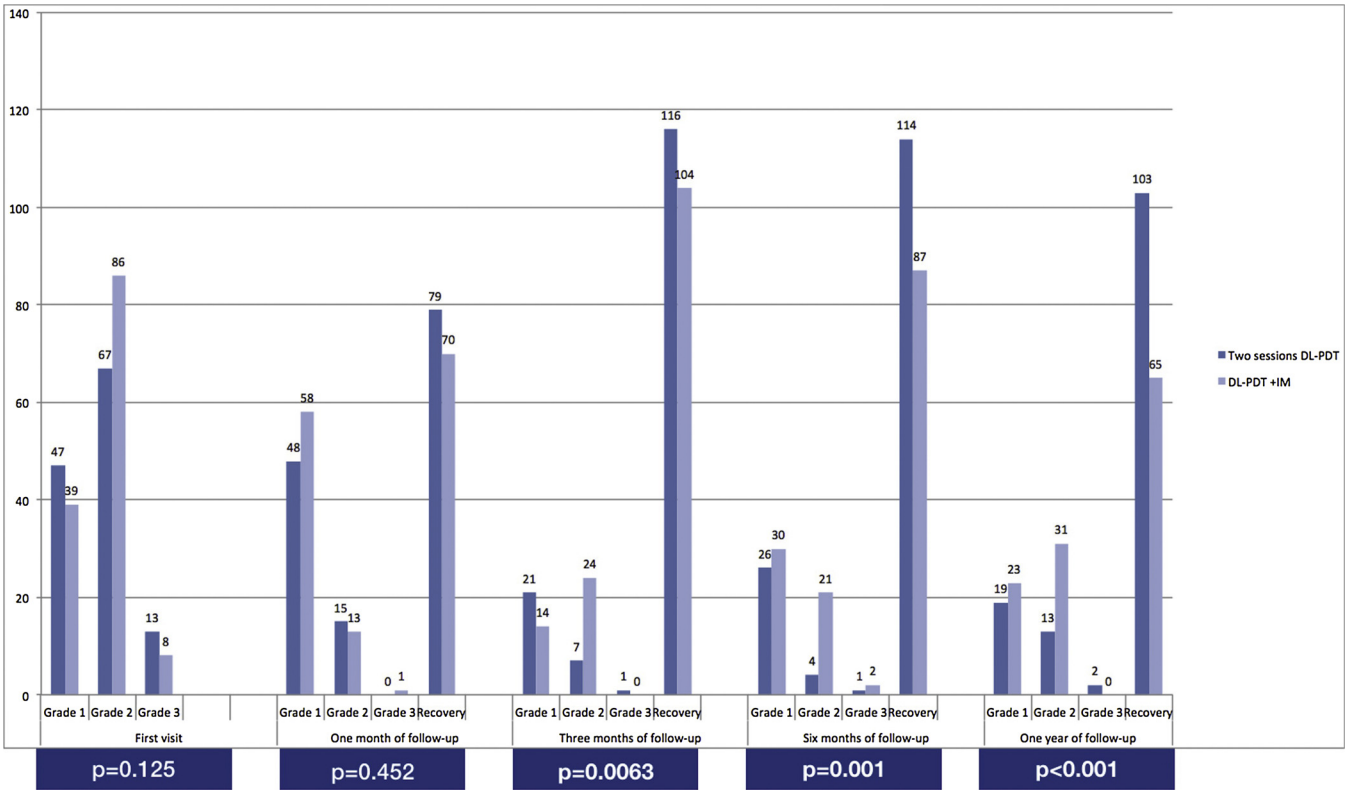

Fig. 1. Bar chart illustrating recovery rate according to Olsen grade. The number of AK lesions is indicated above each bar.

Table 2
Clinical data for enrolled patients and local skin reaction score following 2 sessions of DL-PDT or 1 session of DL-PDT followed by IM treatment (DL-PDT + IM).

		2 × DL-PDT 13 (100.0%)	DL-PDT + IM 14 (100.0%)	P
Patient's level of satisfaction	High	10 (76.9%)	8 (61.5%)	0.3954
	Medium	3 (23.1%)	5 (38.5%)	
	N Missing	0	1	
Cosmetic result	Very good	6 (46.2%)	3 (23.1%)	0.3540
	Good	6 (46.2%)	7 (53.8%)	
	Regular	1 (7.7%)	3 (23.1%)	
	N Missing	0	1	
AKQoL	Average (SD)	5.31 (4.71)	5.23 (4.73)	0.9672
	Median	5	5	
	(P25; P75)	(2;6)	(0;8)	
Local Skin Reactions				
	Yes	10 (76.9%)	12 (100%)	0.0761
Erythema	No	3 (23.1%)		
	N missing	0	2	
Desquamation	Yes	6 (46.2%)	8 (66.7%)	0.3019
	No	7 (53.8%)	4 (33.3%)	
	N missing	0	2	
Crust	Yes	5 (38.5%)	7 (58.3%)	0.3204
	No	8 (61.5%)	5 (41.7%)	
	N missing	0	2	
Edema	Yes	1 (7.7%)	5 (41.7%)	0.0469
	No	12 (92.3%)	7 (58.3%)	
	N missing	0	2	
Pustules	Yes	1 (7.7%)	3 (25%)	0.2383
	No	12 (92.3%)	9 (75%)	
	N missing	0	2	
Erosion and Ulceration	Yes	1 (7.7%)		0.3268
	No	12 (92.3%)	12 (100%)	
	N missing	0	2	

No differences were observed between the 2 groups after accounting for age, sex, phototype, and prior immunosuppressive treatment ($p > 0.05$).

LSRs were more frequent in the DL-PDT plus IM group than the group that received 2 DL-PDT sessions ($p = 0.07$). No differences in patient satisfaction, cosmetic outcome, or AKQoL were observed between groups (Table 2).

4. Discussion

In this study, we found that sequential administration of MAL-DL-PDT and 0.015% IM gel was less effective than 2 sessions of MAL-DL-PDT in achieving a complete lesion response after 3 months and 1 year of follow-up, irrespective of Olsen grade. Highlighting the fact that it takes about three months to obtain maximal effect on AKs and after one month the thick AKs are transformed into thinner AKs, especially in the cases treated with two sessions of DL-PDT; data not shown until now in the literature, despite the limited number of patients.

While previous studies have compared the use of IM and DL-PDT for the treatment of grade-I and II AK lesions [5,12], the effect of sequential treatment has not been investigated. An intra-individual comparative analysis of MAL-DL-PDT versus IM [5] reported similar efficacy after 3 months (DL-PDT, 72.4%; IM 73.6%; $p = 0.74$). The authors reported a lower clearance rate for grade II than grade I AK lesions in patients that received DL-PDT, but no difference in clearance rates between lesion grades in patients treated with IM. Moreover, fewer local skin reactions were observed in the DL-PDT than the IM group [5]. In their randomized split-face clinical trial comparing MAL-DL-PDT with IM for the treatment of AK of the face and scalp, Moggio et al. [12] reported similar efficacy for both grade I and II AK lesions. However, DL-PDT was associated with lower pain and inflammation scores, a better cosmetic outcome, and a greater patient preference.

One appealing therapeutic strategy for AK is to combine treatments in order to obtain synergistic effects that improve efficacy and reduce unwanted side effects. Most studies evaluating the potential synergistic effects of sequential treatment have been performed with conventional PDT. In AK patients, sequential treatment with MAL-PDT and imiquimod appears to produce a significantly better clinical and histologic response than either MAL-PDT or imiquimod alone [13], and less intense local reactions and better tolerance and satisfaction than imiquimod monotherapy. Our results obtained in patients treated with PDT followed by IM are in good agreement with those of Berman et al. [14], who evaluated the efficacy and tolerability of conventional ALA-PDT or IM with or without prior treatment with ALA-PDT in 24 healthy subjects with 4–8 clinically visible AK lesions in a discrete facial area of 25 cm^2 (follow-up period, 71 days). In the group that received 2 consecutive cycles of ALA-PDT ($n = 8$), the authors observed a trend towards increased efficacy and a significantly lower mean composite LSR score than the groups that received IM alone ($n = 8$) or sequentially after 1 cycle of ALA-PDT ($n = 8$) (ALA-PDT, 97.5%; IM, 91.7%; ALA-PDT + IM, 86.7%).

Nissen and coworkers reported that pretreatment with 5% fluorouracil cream for 7 days enhances the efficacy of DL-PDT in patients with acral AK lesions [6]. They observed a significantly higher overall lesion response rate in patients treated with 5-FU and daylight-PDT (62.7%) than those treated with daylight-PDT alone (51.8%) ($p = 0.001$). The application of diclofenac plus hyaluronic acid gel 30 days before or after MAL-DL-PDT also reduces the inflammation and number of DL-PDT sessions required, increasing patient compliance and consequent quality of life without improving efficacy [15].

To our knowledge, ours is the first study to investigate the efficacy of the combination of DL-PDT plus IM. As expected, we observed more severe LSRs and inflammatory reactions in skin areas exposed to IM. Prior DL-PDT did not significantly reduce LSRs caused by IM, in comparison to those reported in IM clinical trials: local edema, 41.7% vs 53.8%; erythema, 100% vs 69.2%; crusting, 58.3% vs 53.8%; scaling, 66.7% vs 61.5% [16].

The absence of a synergistic therapeutic effect of DL-PDT plus IM on AK lesions may be due to the mechanism of action of IM, which induces primary necrosis in tumor cells and markedly reduces the percentage of p53-mutated versus non-p53-mutated cell clones [17,18]. Effective MAL-PDT has also been associated with reduced p53 expression [19]. For this reason, IM may be less efficacious in AK lesions with fewer p53-positive cell clones.

Limitations of the present study include the non-blinded evaluation and the small number of patients. The evaluation of patient satisfaction would also have been more reliable if an anonymous questionnaire had been used. Another limitation is the inclusion in our analysis of grade-III AK lesions. This may have decreased the rate of response per patient. However, we include these lesions in our analysis in order to better replicate the type of scenario typically encountered in clinical practice.

To the best of our knowledge, this is the first study to compare the efficacy of AK treatment with sequential MAL-DL-PDT plus IM versus 2 sessions of MAL-DL-PDT in monotherapy. A key strength of this study is the 1-year follow-up period.

In conclusion, although both combinations (2 sessions of MAL-DL-PDT or MAL-DL-PDT + IM) resulted in good lesion clearance rates in the short term, a good safety profile, and an excellent cosmetic outcome, response rates after 1 year of follow-up were greater for the group that received 2 sessions of MAL-DL-PDT.

Conflict of interest to declare

The authors thank Leo Pharma for assistance with data storage and statistical analyses.

Acknowledgments

The authors thank Owen Howard for English-language editing and

Leo Pharma for assistance with data storage and statistical analyses. This project received support (European FEDER Funding) from the B18-17D “Dermatology and Photobiology” Research Group, as recognized by the Government of Aragon.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pdpdt.2019.05.030.

References

[1] R.A. Schwartz, The actinic keratosis. A perspective and update, Dermatol. Surg. 23 (1997) 1009–1019 quiz 1020-1.
[2] J. Röwert-Huber, M.J. Patel, T. Forschner, C. Ulrich, J. Eberle, H. Kerl, et al., Actinic keratosis is an early in situ squamous cell carcinoma: a proposal for reclassification, Br. J. Dermatol. 156 (Suppl. 3) (2007) 8–12.
[3] B.J.M. Braakhuis, M.P. Tabor, J.A. Kummer, C.R. Leemans, R.H. Brakenhoff, A genetic explanation of Slaughter’s concept of field cancerization: evidence and clinical implications, Cancer Res. 63 (2003) 1727–1730.
[4] E. Stockfleth, C. Ferrandiz, J.J. Grob, I. Leigh, H. Pehamberger, H. Kerl, et al., Development of a treatment algorithm for actinic keratoses: a European Consensus, Eur. J. Dermatol. 18 (2019) 651–659 s. f..
[5] G. Genovese, D. Fai, C. Fai, L. Mavilia, S.R. Mercuri, Daylight methyl-aminolevulinate photodynamic therapy versus ingenol mebutate for the treatment of actinic keratoses: an intraindividual comparative analysis, Dermatol. Ther. 29 (2016) 191–196.
[6] C.V. Nissen, I.M. Heerfordt, S.R. Wiegell, C.S. Mikkelsen, H.C. Wulf, Pretreatment with 5-Fluorouracil cream enhances the efficacy of daylight-mediated photodynamic therapy for actinic keratosis, Acta Derm. Venereol. 97 (2017) 617–621.
[7] E.A. Olsen, M.L. Abernethy, C. Kulp-Shorten, J.P. Callen, S.D. Glazer, A. Huntley, et al., A double-blind, vehicle-controlled study evaluating masoprocol cream in the treatment of actinic keratoses on the head and neck, J. Am. Acad. Dermatol. 24 (1991) 738–743.
[8] Y. Gilaberte, M. Aguilar, M. Almagro, O. Correia, C. Guillén, A. Harto, et al., Documento de consenso hispano-portugués para el uso de la terapia fotodinámica con metil aminolevulinato y luz de día en el tratamiento de las queratosis actínicas, Actas Dermosifiliogr. 106 (2015) 623–631.
[9] S.R. Wiegell, S. Fabricius, M. Gniadecka, I.M. Stender, B. Berne, S. Kroon, et al., Daylight-mediated photodynamic therapy of moderate to thick actinic keratoses of the face and scalp: a randomized multicentre study, Br. J. Dermatol. 166 (2012) 1327–1332.
[10] M. Lebwohl, N. Swanson, L.L. Anderson, A. Melgaard, Z. Xu, B. Berman, Ingenol mebutate gel for actinic keratosis, N. Engl. J. Med. 366 (2012) 1010–1019.
[11] I. Longo Imedio, C. Serra-Guillén, Adaptación y validación de la versión española del cuestionario Actinic Keratosis Quality of Life (AKQoL), Actas Dermosifiliogr. 107 (2016) 474–481.
[12] E. Moggio, M. Arisi, C. Zane, I. Calzavara-Pinton, P. Calzavara-Pinton, A randomized split-face clinical trial analyzing daylight photodynamic therapy with methyl aminolaevulinate vs ingenol mebutate gel for the treatment of multiple actinic keratoses of the face and the scalp, Photodiagnosis Photodyn. Ther. 16 (2016) 161–165.
[13] C. Serra-Guillén, E. Nagore, L. Hueso, V. Traves, F. Messeguer, O. Sanmartín, et al., A randomized pilot comparative study of topical methyl aminolevulinate photodynamic therapy versus imiquimod 5% versus sequential application of both therapies in immunocompetent patients with actinic keratosis: clinical and histologic outcomes, J. Am. Acad. Dermatol. 66 (2012) e131–7.
[14] B. Berman, M.S. Nestor, J. Newburger, H. Park, N. Swenson, Treatment of facial actinic keratoses with aminolevulinic acid photodynamic therapy (ALA-PDT) or ingenol mebutate 0.015% gel with and without prior treatment with ALA-PDT, J. Drugs Dermatol. 13 (2014) 1353–1356.
[15] C. Cantisani, G. Paolino, M. Scarnò, D. Didona, M. Tallarico, E. Moliterni, et al., Sequential methyl-aminolevulinate daylight photodynamic therapy and diclofenac plus hyaluronic acid gel treatment for multiple actinic keratosis evaluation, Dermatol. Ther. (2018) e12710.
[16] M.I.R. Saraiva, L.K.L. Portocarrero, Vieira MAHB, B.C.C. Swiczar, A.T. Westin, Ingenol mebutate in the treatment of actinic keratoses: clearance rate and adverse effects, An. Bras. Dermatol. 93 (2018) 529–534.
[17] S.-J. Cozzi, S.M. Ogbourne, C. James, H.G. Rebel, F.R. de Gruijl, B. Ferguson, et al., Ingenol mebutate field-directed treatment of UVB-damaged skin reduces lesion formation and removes mutant p53 patches, J. Invest. Dermatol. 132 (2012) 1263–1271.
[18] S.M. Ogbourne, P.G. Parsons, The value of nature’s natural product library for the discovery of New Chemical Entities: the discovery of ingenol mebutate, Fitoterapia 98 (2014) 36–44.
[19] L. Bagazgoitia, J. Cuevas Santos, Ú Juarranz, P. Jaén, Photodynamic therapy reduces the histological features of actinic damage and the expression of early oncogenic markers, Br. J. Dermatol. 165 (2011) 144–151.