DOI: 10.1111/jdv.15998 JEADV

REVIEW ARTICLE

The more the better? An appraisal of combination therapies for actinic keratosis

T. Steeb,[1] A. Wessely,[1] U. Leiter,[2] L.E. French,[1] C. Berking,[1] M.V. Heppt[1,*]

[1]Department of Dermatology and Allergy, University Hospital, LMU Munich, Munich, Germany
[2]Department of Dermatology, Center for Dermatooncology, University Hospital Tübingen, Tübingen, Germany
*Correspondence: Markus V. Heppt. E-mail: Markus.Heppt@med.uni-muenchen.de

Abstract

Actinic keratoses (AK) are common precancerous lesions of the skin. Numerous interventions exist for the treatment of AK, including lesion- and field-directed approaches. In daily practice, different treatment modalities are often combined to maximize clearance rates. However, whether a combination therapy is preferable to monotherapy in terms of efficacy and safety has been subject of intense debate. In this review, we summarize the current knowledge on the efficacy and safety of local combination therapies for the treatment of patients with AK. Combination approaches of cryosurgery followed by photodynamic therapy (PDT), laser-assisted PDT, PDT in combination with topical interventions and microneedling-assisted PDT have shown slightly better efficacy results with similar tolerability compared to the respective monotherapy. However, the individual usage of combination therapies should be checked on a case-by-case basis and take into account individual patient- and lesion-specific aspects as more resources are needed and because the individual monotherapies are already highly effective.

Received: 17 June 2019; Accepted: 25 September 2019

Conflicts of Interest

CB has been a member of advisory boards for Almirall Hermal, Biofrontera, Galderma, ISDIN and Leo Pharma. CB has received speaker's honoraria by Almirall Hermal, Galderma and Leo Pharma. CB has received funding for clinical research by Leo Pharma. The remaining authors declare no conflict of interests.

Funding sources

None declared.

Introduction

Long-term exposure to ultraviolet (UV) radiation can lead to the formation of actinic keratoses (AK) in light-skinned individuals.[1,2] These precancerous lesions present as diffuse red and keratotic or scaling plaques with a rough, sand paper-like surface on chronically sun-exposed areas such as the face, ears, arms and dorsal hands.[2,3] Visible AK lesions are often surrounded by tissue that harbours significant UV-induced histologic and genetic alterations but appears clinically unaltered. This so-called field cancerization is a commonly observed phenomenon in chronically sun-damaged skin and requires appropriate treatment approaches.[4] Although the risk is presumably low for single lesions, AK can progress to cutaneous squamous cell carcinoma (cSCC).[5] The presence of multiple lesions and additional signs of chronic UV damage on the adjacent skin increases the risk for progression considerably.[6,7] As it is currently not possible to predict which AK will transform into invasive cSCC, early and consequent treatment of AK lesions is recommended by international treatment guidelines.[8]

Today, a variety of interventions is available for the effective treatment of AK. Selecting an appropriate therapy may pose a major challenge in daily practice. According to the mode of application, interventions are traditionally classified as either lesion- or field-directed.[9] Lesion-based approaches are suited for single or isolated AK, whereas field-directed treatments are preferable for multiple AK as they also address subclinical changes of an actinically damaged field. Both strategies can be combined for difficult-to-treat AK such as hyperkeratotic lesions or lesions on the dorsal hands. Common examples for the combination of a lesion-directed with a field-directed regimen are surgery or cryosurgery followed by a topical intervention or laser-assisted photodynamic therapy (PDT). However, ablative and non-ablative lasers may also be applied as field-directed modalities. Examples for the combination of two field-directed approaches include microneedling (MN)-assisted PDT and PDT followed by a topical intervention such as imiquimod, 5-fluorouracil (5-FU) or ingenol mebutate or the sequential treatment with PDT.

Combination therapies are commonly applied in the daily practice; however, it has been subject of debate if combination therapies should be preferred to monotherapies for the treatment of AK, as monotherapies already offer high clearance rates. In this narrative review, we aimed to summarize the current knowledge on the efficacy and safety of clinically relevant combination therapies for the treatment of patients with AK in order to provide a practical aid for clinical decision-making.

Combination of lesion- and field-directed regimens

Cryosurgery and topical interventions

Cryosurgery is a practicable, widely used and presumably lesion-directed approach for isolated lesions. During the procedure, liquid nitrogen is applied in one to three freeze–thaw cycles in order to destroy AK lesions. In patients with multiple lesions or field cancerization, cryosurgery can also be applied over a wider area as 'cryopeeling' (extensive cryosurgery), underlining that the mode of application but not the intervention *per se* determines whether a treatment is lesion-directed or field-directed.[10] Cryosurgery is highly recommended for single AK in pertinent treatment guidelines. However, in daily practice, isolated lesions without signs of actinic damage are rarely observed and the addition of a field-directed treatment can help to overcome the limitations of cryosurgery. A variety of topical agents for the treatment of AK is available with distinct mechanisms of actions, ranging from cytostatic effects to immune activation. The downsides are a longer duration of application and questionable efficacy in patients with thicker lesions who were commonly excluded in larger trials. This may be due to a poorer penetration of topical drugs through hyperkeratotic lesions. In contrast, cryosurgery showed high clearance rates, particularly in thicker AK which provides a solid rationale to combine this approach with field-directed drug treatment.[11]

We recently investigated in a systematic review and meta-analysis whether an upfront combination of cryosurgery with a topical intervention is superior to cryosurgery alone.[12] We identified nine randomized controlled trials (RCTs) with an overall sample size of $n = 1644$ participants. The majority investigated cryosurgery followed by imiquimod ($n = 4$). Two studies assessed cryosurgery followed by ingenol mebutate and the remaining three studies assessed 3% diclofenac in 2.5% hyaluronic acid, 5-FU 0.5% cream and photodynamic therapy with 5-aminolevulinic acid (5-ALA) after cryosurgery, respectively. The pooled results showed significantly higher participant complete clearance rates for cryosurgery in combination with a topical approach compared to monotherapy (RR 1.74, 95% CI 1.25–2.43. $I^2 = 73\%$). However, the quality of evidence for this result was estimated as low (GRADE ++--). This rating indicates that we have limited confidence in the effect estimate. The true effect may be substantially different from the estimate of the effect.[13] Besides, the proportion of patients who had at least 75% of their lesions cleared was not statistically different between the combination and monotherapy group (RR 1.64, 95% CI 0.88–3.03, $I^2 = 77\%$, quality of evidence: very low, GRADE +---). Hence, we have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.[13] Safety was defined as the number of patients who completed the study protocol and did not withdraw due to adverse events. The proportion was equally distributed in both groups (RR 0.98, 95% CI 0.95–1.01, $I^2 = 75\%$, GRADE +---). The evidence for this outcome was graded to be very low GRADE +---. We also conducted comparator-specific stratified analysis for the interventions imiquimod and ingenol mebutate. Cryosurgery followed by ingenol mebutate showed no significant differences regarding participant complete clearance rate (RR 3.51, 95% CI 0.22–56.53, $I^2 = 77\%$, GRADE +---) or partial clearance rate (RR 2.97, 95% CI 0.28–30.96, $I^2 = 83\%$, GRADE +---) in comparison with cryosurgery alone. The combination of cryosurgery with imiquimod revealed no significant difference of participant complete clearance in comparison with cryosurgery alone (RR 2.46, 95% CI 0.63–9.57, $I^2 = 87\%$, GRADE +---). In all studies, cryosurgery was performed upfront. In order to decrease the targeted area and thereby minimize commonly observed adverse effects such as hypopigmentation, starting with a topical drug treatment and subsequently performing cryosurgery for recalcitrant lesion may be another interesting approach which has not yet been evaluated in RCTs to our knowledge. The interpretation of the data available for cryosurgery plus topical treatment is difficult. While for the pooled analysis, a statistically significant superiority for the combination was observed, this difference was not consistent in the comparator-stratified specific analyses. This may be due to a small sample size for the specific comparators but can also indicate that the advantage for the combination does not exist for specific agents. Furthermore, there was high clinical and statistical heterogeneity which needs to be kept in mind in the interpretation of the pooled analysis. Nevertheless, based on these results and our clinical experience, we still propose a small advantage for the combination therapy, in particular if multiple lesions or field-cancerization are present.

Laser-assisted photodynamic therapy

Photodynamic therapy with 5-ALA or its ester methyl-aminolevulinate (MAL) is a highly effective treatment for multiple AK or field cancerization with an excellent cosmetic outcome.[14,15] Both photosensitizers penetrate the stratum corneum and selectively accumulate in dysplastic cells where they are converted to the photosensitizer protoporphyrin IX (PpIX).[16,17] However, one of the main side effects is local pain during illumination, which can limit treatment compliance and patient satisfaction.[18] Other limiting factors include the thickness of the individual lesions, as the photosensitizing agent poorly penetrates hyperkeratotic lesions, therefore requiring curettage or another physical

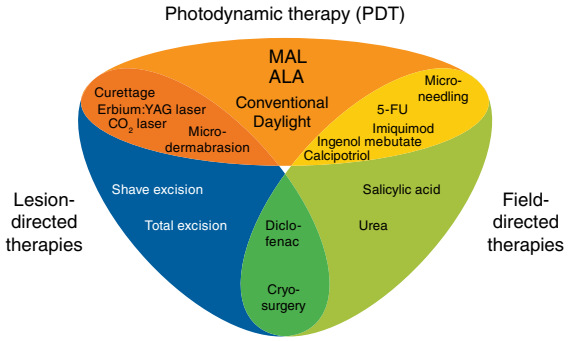

Figure 1 Venn diagram highlighting the central role of photodynamic therapy in the combination treatment of actinic keratoses. 5-FU, 5-fluorouracil; ALA, aminolevulinic acid; MAL, methyl aminolevulinate.

pretreatment prior to PDT. As field-directed approach, PDT may be combined with lesion-targeted pretreatment by ablative and non-ablative laser devices (Fig. 1). Ablative fractional lasers including Erbium:YAG or carbon dioxide (CO_2) lasers heat the treated tissue up to 100°C and thereby vaporize microscopic vertical channels into the skin that facilitate the penetration and enrichment of 5-ALA or MAL in dysplastic cells.[19] This concept has been denoted as laser-assisted drug delivery.[19] We recently performed a meta-analysis including seven RCTs which demonstrated that laser-assisted PDT is more efficient but not more painful than PDT or laser monotherapy for the treatment of patients with AK.[20] Six of the seven studies assessed MAL and one study 5-ALA as photosensitizer. Regarding the type of laser, three studies investigating an Erbium:YAG laser were included, whereas four studies assessed a carbon dioxide (CO_2) laser. However, the results of the meta-analysis were pooled irrespective of the individual photosensitizer or laser and therefore assumptions regarding the influence of the photosensitizer or the type of laser cannot be made. The clearance rates for laser-assisted PDT were significantly higher for the combination than for PDT monotherapy (RR 1.33, 95% CI 1.24–1.42, I^2 = 25%); however, the evidence for this outcome was graded as low (GRADE ++--). Besides, no difference in pain intensity between laser-assisted PDT and other interventions was observed (mean difference 0.31, 95% CI −0.12 to +0.74, I^2 = 0%, low quality of evidence, GRADE ++--).

In addition to the evidence presented here, the experience of the practitioner's individual experience regarding specific combinations must also be taken into account. The enrichment of PpIX in PDT was investigated comparatively after several physical pretreatments in an intra-individual trial.[21] It was highest with ablative fractional laser, followed by microdermabrasion, microneedling and curettage. However, the photosensitizer uptake does not necessarily reflect the effectiveness of PDT. Based on these results, the clinical efficacy of PDT was further evaluated after a tailored pretreatment with either ablative fractional laser (AFL) or microdermabrasion in a recent side-by-side trial.[22] Two large areas were randomized intra-individually to receive a single treatment with AFL + daylight PDT or

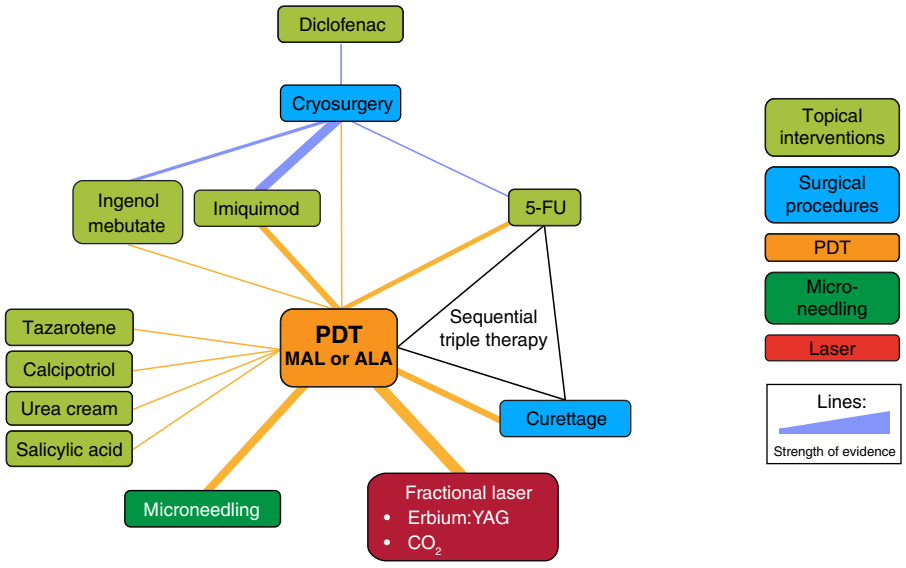

Figure 2 Network of combination therapies for actinic keratoses. 5-FU, 5-fluorouracil; ALA, aminolevulinic acid; CO_2, carbon dioxide; MAL, methyl aminolevulinate; PDT, photodynamic therapy.

microdermabrasion + daylight PDT. Interestingly, AFL was superior to microdermabrasion in terms of lesion clearance, but was also associated with a higher rate of local skin reactions.

Surgical procedures and topical interventions

Shave excision and complete excision Shave excision or complete excision as excisional biopsy in combination with topical interventions is clinically widely used for AK. They are either applied to remove hyperkeratotic AK in order to improve the permeation capability of topical drugs or to remove single lesions that were not cleared by previous topical therapies. However, high-quality evidence is lacking as no RCTs and only few case series have been published on shave and complete excision for AK.[23-25]

Curettage Another question in this context is whether curettage of lesions can be considered as true surgical treatment. In many cases, curettage is performed on hyperkeratotic lesions prior to PDT to improve the penetration of the photosensitizers and to remove crusts or keratotic components. Therefore, it is certainly correct to classify curettage prior to a treatment as a basic measure instead of speaking of sequential combination therapy. Physical pretreatment is currently recommended as standard measure in international PDT protocols.[26] In a randomized, intra-individual study (n = 22), Nissen *et al.*[23] investigated whether PpIX accumulation in AK lesions can be increased by different interventions in order to improve MAL-PDT efficacy on extremities. Four symmetrical areas on dorsal hands were selected for pretreatment with or without curettage and MAL application for either 3 h or 21 h prior to illumination. Extended MAL application for 21 h led to an increased accumulation of PpIX in the treated lesions, but did not result in better treatment outcomes. MAL application for 3 h without previous curettage achieved the lowest median total clearance rate (33.3%) due to insufficient PpIX accumulation, whereas all other interventions showed improved clearance rates (curettage + 3 h MAL and no curettage + 21 h MAL 55.0%, curettage + 21 h MAL 53.6%, statistically not significant). Additionally, PpIX accumulation was correlated with pain and erythema. These results indicate that PpIX accumulation in AK lesions on dorsal hands can be increased by curettage and/or extended MAL application prior to illumination but can also result in enhanced side effects and does not improve clearance rates. In another case series, Jambusaria-Pahlajani *et al.*[24] investigated the efficacy of curettage prior to PDT in four organ transplant recipients with multiple AK refractory to other treatments. Hyperkeratotic lesions were removed by light curettage, followed by a topical treatment with 5-FU 5% cream twice daily for 5 days and PDT (MAL-PDT: n = 1, ALA-PDT: n = 3). All patients had a complete or partial clearance of their AK and good cosmetic outcome, indicating that this sequential approach is effective. Gholam *et al.* retrospectively compared the efficacy of pretreatment of MAL-PDT with either curettage (n = 15), salicylic acid 10% (n = 15) or urea cream 40% (n = 14).[25] The combination with curettage achieved the highest response rates (68.5%), followed by salicylic acid (61.4%) and urea cream (60.8%), albeit without statistical significance. Patients pretreated with curettage experienced significantly less pain on a visual analogue scale than salicylic acid and urea-pretreated patients (curettage: 4.4 ± 2.1 vs. salicylic acid: 6.3 ± 2.7, P = 0.02; urea: 6.1 ± 1.8, P = 0.04) and had less pronounced local reactions compared to the other interventions. The patients' satisfaction and the cosmetic outcome evaluated four weeks after PDT were good to excellent with no significant differences between the groups.

Combination of field-directed regimens

Topical interventions combined with PDT

The combination of two or more field-directed treatments is less established than the combination of lesion- and field-directed regimens (Fig. 2). As a fixed combination of 5-FU 0.5% and 10% salicylic acid is available on the market, we did not consider this agent as sequential combination for the purpose of our review, although both topical field-directed regimens may also be applied as monotherapy. A sequential combination of two field-directed treatments may offer additive effects through different mechanisms of action. The results of a meta-analysis confirm this hypothesis and suggest that the combination of PDT with another topical drug intervention does improve AK clearance rates compared to either monotherapy alone.[27] Ten RCTs with an overall sample size of 277 participants were included. Four of these studies investigated a combination of PDT with imiquimod cream, three with 5-FU cream and one each with ingenol mebutate gel, tazarotene gel and calcipotriol ointment, respectively. The results showed that patients treated with a combination of PDT and a topical intervention showed higher participant complete (RR 1.63; 95% CI 1.15–2.33, I^2 = 3%) and partial clearance rates (RR 1.19; 95% CI 0.84–1.67, I^2 = 38%). However, the quality of evidence for these outcomes ranged from low (GRADE ++--) to very low (GRADE ++--) according to the authors judgement with the GRADE approach. Similarly, the lesion-specific clearance was higher for PDT plus topical intervention compared to monotherapy, though the certainty of the evidence was estimated as very low (RR 1.48; 95% CI 1.04–2.11, I^2 = 93%, GRADE ++--). The authors reported that PDT-induced pain and local skin reactions after treatment were poorly and inconsistently described in the identified RCTs. Hence, a general assumption about the effect of adding a topical intervention to PDT treatment cannot be made. To investigate the influence of the topical intervention, a subgroup analysis was performed for PDT combined with imiquimod. This analysis revealed an increased participant complete clearance rate

compared to monotherapy (RR 1.57, 95% CI 1.09–2.25, I^2 = 0%, GRADE +---). However, the significance of this effect remains questionable as the lower limit of the 95% confidence interval is close to the line of no effect. This meta-analysis highlights that the sequential application of two field-directed treatments can represent a suitable approach in patients with multiple AK and field cancerization; however, the true efficacy of this combination may deviate and depend on the clinical context.

Microneedling-assisted PDT

Pretreatment with microneedling (MN) represents another approach to augment the effects of PDT and topical drugs. MN can be achieved by a series of tiny needles either on a roller or a mechanical stamp which puncture the superficial epidermis in order to enhance drug delivery for an improved PDT efficacy. The combination of microneedling with PDT was initially developed to decrease the incubation time of the photosensitizer and to increase the efficacy of PDT by enhanced drug delivery through the perforated microchannels.

Thus, several RCTs investigated microneedling-assisted PDT vs. PDT monotherapy. Torezan *et al.*[28] compared the efficacy of two MAL-PDT approaches in a randomized, split-face study (n = 10). AK were pretreated with curettage on one side, and MN was conducted after MAL application on the other side of the face. Patients were illuminated with a red light-emitting diode afterwards for 90 min. Both interventions led to improved cosmetic outcomes and a clearance rate of 88.3% with no significant differences. However, adverse effects as erythema, oedema, crusting and pain were more severe and more commonly reported on the MN-treated side. Another study by Spencer *et al.* investigated whether MN and subsequent ALA-PDT is more efficacious than ALA-PDT monotherapy for clearing AK located on the face of 20 patients.[29] MN pretreatment led to a significantly better mean AK lesion reduction than PDT monotherapy (89.3% vs. 69.5%). Besides, 75% of the pretreated sides achieved complete clearance in comparison with 30% of the monotherapy side. The Microneedle Photodynamic Therapy II (MNPDT-II) study had a randomized, single-blinded, split-face controlled, 2 × 2 factorial study design (n = 33).[30] Participants were randomized to receive either 5-ALA application for 10 min or 20 min prior to illumination after a pretreatment with a microneedle roller or a sham roller. For the 20-min incubation arm, a statistically significantly different average AK clearance of 76% was achieved on the MN side vs. 58% on the sham side, including three patients with complete clearance. However, the latter result was not statistically significant. Pain during illumination was not significantly different between both pretreatment groups. MAL incubation for 10 min resulted in lower AK clearance rates of 43% after MN pretreatment and 38% on the sham side with no significant difference. Pain during illumination was also not significantly different between MN and sham pretreatment. Lev-Tov *et al.*[31] undertook a trial with 51 participants in which MN and sham pretreatments were randomized to the right and left foreheads and the sham-treated sides were incubated with 5-ALA for 60 min. Subsequently, MN-pretreated sides were further randomized to 20, 40 or 60 min 5-ALA incubation. The lesion response rate for the 20, 40 and 60 min MN incubation times vs. the corresponding sham MN treatment with 60 min 5-ALA incubation were 71.4% and 68.3%, 81.1% and 79.9%, and 72.1% and 74.2%, respectively. The differences in efficacy between the MN and sham pretreatments were not significant. Statistical significant differences in pain scores between MN and sham pretreatment were reported, but these were relatively low. No adverse events were reported. Besides this, unpublished results were available for a registered phase-2 trial (NCT02632110) comparing the effect of MN, incubation time and light power density on ALA-PDT for the field treatment of AK on the face among 137 participants.[32] MN pretreatment resulted in a better mean lesion clearance as well as participant complete clearance than ALA-PDT monotherapy.

Overall, the studies described here suggest that MN pretreatment may enhance the efficacy of PDT, although the data from these RCTs are heterogeneous. The study by Torezan *et al.* reported more adverse events with the combination and the microneedling pretreatment itself can cause painful sensations apart from the illumination which was not consistently reported. Nevertheless, this combination can be beneficial for therapy-resistant or difficult-to-treat lesions. However, the procedure of the MN needs to be standardized for the use in daily practice.

Concluding remarks

As already a variety of effective lesion- and field-directed procedures exist, sequential combinations further increase the number of possible approaches. Large clinical heterogeneity results from differences in timing and the type of application, which are not standardized particularly for physical or ablative therapies such as cryosurgery, curettage or laser application. Also, it is currently difficult to assess which treatment combination is the most effective. Interestingly, the majority of studies investigating combination therapies covered combination therapies with PDT, which does not necessarily mean that PDT is the best option for a combination treatment. As we did not perform a systematic literature search for the identification of studies, we cannot exclude that the combination approaches presented here are biased by our own clinical experience.

Overall, the results on both the individual study and the meta-analysis level allow the interpretation that a small increase in effectiveness can be achieved through a combination of several therapy procedures. The tolerability seems to be similarly good, and the occurrence of side effects is not massively increased. Especially, a combination of lesion-directed and field-directed approaches might be particularly helpful for

hyperkeratotic AK in order to improve permeation of the topical intervention. However, we consider the strength of the additional effect of a combination therapy to be rather low. This is probably due to the fact that most interventions as monotherapies have a good effectiveness with a high rate of lesion clearance. Especially for lesions that are not pretreated, monotherapy is widely sufficient for disease control. Here, a primary combination of several procedures may represent overtreatment and waste resources of the healthcare system.

Acknowledgements

TS is supported by the German Cancer Aid, Grant No. 70112351 to CB.

References

1 Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. *J Am Acad Dermatol* 2000; **42**: 4–7.
2 Moy RL. Clinical presentation of actinic keratoses and squamous cell carcinoma. *J Am Acad Dermatol* 2000; **42**: 8–10.
3 Rowert-Huber J, Patel MJ, Forschner T *et al.* Actinic keratosis is an early in situ squamous cell carcinoma: a proposal for reclassification. *Br J Dermatol* 2007; **156**(Suppl 3): 8–12.
4 Figueras Nart I, Cerio R, Dirschka T *et al.* Defining the actinic keratosis field: a literature review and discussion. *J Eur Acad Dermatol Venereol* 2018; **32**: 544–563.
5 Criscione VD, Weinstock MA, Naylor MF *et al.* Actinic keratoses: Natural history and risk of malignant transformation in the Veterans Affairs Topical Tretinoin Chemoprevention Trial. *Cancer* 2009; **115**: 2523–2530.
6 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**: 502–518.
7 Cerio R, Dirschka T, Dreno B *et al.* Actinic keratosis, a chronic, progressive disease: understanding clinical gaps to optimise patient management. *Acta Derm Venereol* 2017; **97**: 997–998.
8 Werner RN, Stockfleth E, Connolly SM *et al.* 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**: 2069–2079.
9 Arenberger P, Arenbergerova M. New and current preventive treatment options in actinic keratosis. *J Eur Acad Dermatol Venereol* 2017; **31**(Suppl 5): 13–17.
10 Chiarello SE. Cryopeeling (extensive cryosurgery) for treatment of actinic keratoses: an update and comparison. *Dermatol Surg* 2000; **26**: 728–732.
11 Zane C, Facchinetti E, Rossi MT, Specchia C, Ortel B, Calzavara-Pinton P. Cryotherapy is preferable to ablative CO_2 laser for the treatment of isolated actinic keratoses of the face and scalp: a randomized clinical trial. *Br J Dermatol* 2014; **170**: 1114–1121.
12 Heppt MV, Steeb T, Ruzicka T, Berking C. Cryosurgery combined with topical interventions for actinic keratosis: a systematic review and meta-analysis. *Br J Dermatol* 2019; **180**: 740–748.
13 Balshem H, Helfand M, Schunemann HJ *et al.* GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol* 2011; **64**: 401–406.
14 Braathen LR, Szeimies RM, Basset-Seguin N *et al.* Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus. International Society for Photodynamic Therapy in Dermatology, 2005. *J Am Acad Dermatol* 2007; **56**: 125–143.
15 Morton CA, McKenna KE, Rhodes LE. Guidelines for topical photodynamic therapy: Update. *Br J Dermatol* 2008; **159**: 1245–1266.
16 Peng Q, Berg K, Moan J, Kongshaug M, Nesland JM. 5-Aminolevulinic acid-based photodynamic therapy: principles and experimental research. *Photochem Photobiol* 1997; **65**: 235–251.
17 Juzeniene A, Juzenas P, Ma LW, Iani V, Moan J. Topical application of 5-aminolaevulinic acid, methyl 5-aminolaevulinate and hexyl 5-aminolaevulinate on normal human skin. *Br J Dermatol* 2006; **155**: 791–799.
18 Stritt A, Merk HF, Braathen LR, von Felbert V. Photodynamic therapy in the treatment of actinic keratosis. *Photochem Photobiol* 2008; **84**: 388–398.
19 Waibel JS, Rudnick A, Shagalov DR, Nicolazzo DM. Update of ablative fractionated lasers to enhance cutaneous topical drug delivery. *Adv Ther* 2017; **34**: 1840–1849.
20 Steeb T, Schlager JG, Kohl C, Ruzicka T, Heppt MV, Berking C. Laser-assisted photodynamic therapy for actinic keratosis: A systematic review and meta-analysis. *J Am Acad Dermatol* 2019; **80**: 947–956.
21 Bay C, Lerche CM, Ferrick B, Philipsen PA, Togsverd-Bo K, Haedersdal M. Comparison of physical pretreatment regimens to enhance protoporphyrin IX uptake in photodynamic therapy: a randomized clinical trial. *JAMA Dermatol* 2017; **153**: 270–278.
22 Wenande E, Phothong W, Bay C, Karmisholt KE, Haedersdal M, Togsverd-Bo K. Efficacy and safety of daylight photodynamic therapy after tailored pretreatment with ablative fractional laser or microdermabrasion: a randomized, side-by-side, single-blind trial in patients with actinic keratosis and large-area field cancerization. *Br J Dermatol* 2019; **180**: 756–764.
23 Nissen CV, Heerfordt IM, Wiegell SR, Mikkelsen CS, Wulf HC. Increased protoporphyrin IX accumulation does not improve the effect of photodynamic therapy for actinic keratosis: a randomized controlled trial. *Br J Dermatol* 2017; **176**: 1241–1246.
24 Jambusaria-Pahlajani A, Ortman S, Schmults CD, Liang C. Sequential curettage, 5-fluorouracil, and photodynamic therapy for field cancerization of the scalp and face in solid organ transplant recipients. *Dermatol Surg* 2016; **42**(Suppl 1): S66–S72.
25 Gholam P, Fink C, Bosselmann I, Enk AH. Retrospective analysis evaluating the effect of a keratolytic and physical pretreatment with salicylic acid, urea and curettage on the efficacy and safety of photodynamic therapy of actinic keratoses with methylaminolaevulinate. *J Eur Acad Dermatol Venereol* 2016; **30**: 619–623.
26 Morton CA, Wulf HC, Szeimies RM *et al.* Practical approach to the use of daylight photodynamic therapy with topical methyl aminolevulinate for actinic keratosis: a European consensus. *J Eur Acad Dermatol Venereol* 2015; **29**: 1718–1723.
27 Heppt MV, Steeb T, Leiter U, Berking C. Efficacy of photodynamic therapy combined with topical interventions for the treatment of actinic keratosis: a meta-analysis. *J Eur Acad Dermatol Venereol* 2019; **33**: 863-873.
28 Torezan L, Chaves Y, Niwa A, Sanches JA Jr, Festa-Neto C, Szeimies RM. A pilot split-face study comparing conventional methyl aminolevulinate-photodynamic therapy (PDT) with microneedling-assisted PDT on actinically damaged skin. *Dermatol Surg* 2013; **39**: 1197–1201.
29 Spencer JM, Freeman SA. Microneedling prior to Levulan PDT for the treatment of actinic keratoses: A split-face, blinded trial. *J Drugs Dermatol* 2016; **15**: 1072–1074.
30 Petukhova TA, Hassoun LA, Foolad N, Barath M, Sivamani RK. Effect of expedited microneedle-assisted photodynamic therapy for field treatment of actinic keratoses: A randomized clinical trial. *JAMA Dermatol* 2017; **153**: 637–643.
31 Lev-Tov H, Larsen L, Zackria R, Chahal H, Eisen DB, Sivamani RK. Microneedle-assisted incubation during aminolaevulinic acid photodynamic therapy of actinic keratoses: a randomized controlled evaluator-blind trial. *Br J Dermatol* 2017; **176**: 543–545.
32 NCT02632110. Microneedle Lesion Preparation Prior to Aminolevulinic Acid Photodynamic Therapy (ALA-PDT) for AK on Face. Available at: https://clinicaltrials.gov/ct2/show/NCT02632110 (last accessed 23 May 2019).