ORIGINAL ARTICLE

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Efficacy of photodynamic therapy using ALA-HCl in gel with a lipid nanoemulsion and MAL-HCl in cream in superficial basal cell carcinoma

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Abstract

Introduction and Objective. Photodynamic therapy (PDT) is a therapeutic option for low-risk basal cell carcinoma (BCC). The aim of the study was to assess the efficacy of topical PDT in the treatment of superficial BCC (sBCC) using two different photosensitizers: aminolevulinic acid hydrochloride (ALA-HCl) in a gel formulation with a lipid nanoemulsion (ALA-HCl in gel) and ALA methyl ester hydrochloride (MAL-HCl) in a cream formulation (MAL-HCl in cream).

Materials and Method. 21 patients were treated twice with a one week interval between treatments. The formulations were applied onto lesions: 10 patients were treated with MAL-HCl in cream, and 11 with ALA-HCl in gel. After three hours of incubation and removing the preparations, fluorescence was assessed. The skin areas were then irradiated with red light 630 ± 5 nm.

Results. At the follow-up visit 12 weeks after the second treatment, complete clinical remission was found in 82% after ALA-HCl in gel and in 80% after MAL-HCl in cream. An excellent cosmetic result was found in 96% of patients after MAL-HCl in cream and in 100% after ALA-HCl in gel. Faster skin healing and less post-inflammatory hyperpigmentation during follow-up visits was observed after treatment with ALA-HCl in gel.

Conclusions. Both formulations – ALA-HCl in gel and MAL-HCl in cream – were highly effective photosensitisers for PDT. The advantage of ALA-HCl in a gel formulation with a lipid nanoemulsion was faster skin healing, resulting in better cosmetic results.

Key words

photodynamic therapy, nanoemulsion, formulations, basal cell carcinoma

INTRODUCTION

Basal cell carcinoma (BCC) remains the commonest type of skin cancer and represents about 80% of cancers originating from keratinocytes [1, 2]. People who work outdoors, especially farmers, stand a relatively elevated risk of experiencing BCC and relapse [3]. Significantly, the incidence of BCC is much higher in the Caucasian population [4], and the annual prevalence of the disease in Europe has increased at a rate of 5% annually during the last decade, in comparison to about 2% in the USA [5]. The prevalence of BCC rises sharply after the age of 40, although recently there has been an rise in occurrence among the population of younger people, especially women, as a direct consequence of increased exposure to UV radiation from the sun or artificial sources, and smoking [6].

BCCs are known to develop as a result of a complex interaction of both environmental factors and individual phenotype and genetic makeup. The main predisposing factors for BCC include exposure to UV radiation, male gender, advanced age (>60 years), fair skin type (Fitzpatrick I and II), prolonged immunosuppression, radiation therapy, family or personal history of BCC, and certain genetic syndromes [7]. It should be noted that BCC is one of the cancers with the highest burden of genetic mutations, mainly induced by ultraviolet radiation. In most cases, this is due to aberrant activation of the hedgehog (HH) signalling pathway (90% PTCH1 mutations and about 10% SMO). Along with the hedgehog pathway, other signaling pathways, including TP53 and RAS, may also be involved in the pathogenesis of BCC. In addition to the hedgehog pathway, several more signalling pathways, including TP53 and RAS, are also likely to be implicated in the pathogenesis of BCC [8].

The cellular aspect of BCC is worth emphasizing. The name of the tumour refers to its origin from cells with a histological resemblance to the epidermal keratinocytes. However, the cellular background of BCC remained obscure and extensively discussed over a long period of time [7]. Through molecular genetics studies, it has become clear that nodular BCCs appear to be formed from follicular stem cells, while superficial BCCs originate from the interfollicular epidermis, which is dependent on a specific type of signalling [9]. It

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has been shown that only oncogene-targeted interfollicular epidermal stem cells can transform into BCCs after HH signalling because of an enhanced capacity for self-renewal and refractory apoptosis facilitated by p53, leading to prompt clonal outgrowth and advancement to malignancy [10]. It is noteworthy that the tumour microenvironment is able to specifically regulate tumour cells, affecting the keratinocyte HH pathway and promoting BCC growth [11].

Although mortality from this cancer is minimal and the risk of recurrence or metastasis is low, BCC may be related to marked morbidity, particularly if not treated for a long time [12].

At present, approximately 26 different types of BCC are known, however, there are in general 4 distinct clinicopathological subtypes: nodular, superficial, morpheaform and fibroepithelioid [13, 14].

The first step in the diagnosis of BCC is visual inspection, followed by dermoscopy / videodermoscopy with confirmation by biopsy and histopathology, when required [3, 15]. Of note, a systematic review and meta-analysis of the precision of dermoscopy for BCC reveal a pooled sensitivity of 89% – 91% and specificity of 95%. Other non-invasive diagnostic methods include reflectance confocal microscopy, optical coherence tomography, high-resolution ultrasonography, Raman spectroscopy or terahertz pulse imaging [14, 16]. The treatment of BCC is based on identifying low-risk ('easy-to-treat') and high-risk ('difficult-to-treat') cancers [4].

Photodynamic therapy (PDT) uses a source of visible light to activate a photosensitizing agent (usually aminolevulinic acid, ALA, or methyl aminolevulinate, MAL) applied to the lesion, leading to the release of reactive oxygen species and a tissue-destructive effect [17]. This method should be considered to treat low-risk BCC, i.e. superficial and small nodular lesions that are less than 2 mm thick, when surgical excision is not suitable or there are certain restrictions from the patient, such as age, comorbidities, medications used. In addition, PDT appears to be a good therapeutic option for recurrent small and large tumours. According to the European consensus, two photosensitizers are recommended: ALA and MAL [4]. Of note, the NCCN recommendations additionally include porfimer sodium [13]. Data from clinical trials indicate that complete remission rates in patients with BCC treated with PDT range from 60% – 100%. Furthermore, a number of randomized trials and meta-analyses have shown that good or excellent cosmetic rates for PDT were higher, compared to surgery. However, surgical excision appears to be associated with better disease control [13].

Many different photosensitizers have been used to date, with ALA and MAL pro-drugs being the most widely used. The differences between the two substances are clear, especially in terms of the stability of the active substance and the ability to penetrate the epidermal layers. ALA is characterized by susceptibility to degradation and low stability. MAL, on the other hand, shows greater resistance to degradation; however, it requires cleavage, which is related to the fact that after the same incubation period, it is ALA that induces more protoporphyrin IX (Pp IX) [18].

At the end of 2011, a modified form of photosensitizer, stable ALA-HCl in a gel formulation with a lipid nanoemulsion, was granted marketing authorization throughout the European Union. With this formulation, ALA-HCl remains stable for 24 months; moreover, its penetration through the stratum corneum has been shown to be increased [19].

OBJECTIVE

The aim of this study was to determine the effectiveness and tolerability of PDT using commercially available ALA-HCl in a gel formulation with a lipid nanoemulsion, or 5-aminolevulinic acid methyl ester hydrochloride (MAL-HCl) in cream in patients with superficial BCC.

MATERIALS AND METHOD

Study cohort. 21 Caucasian adults (II and III skin phototypes), aged between 27–92 years, with non-pigmented superficial BCCs (sBCCs) located on the face and/or trunk were considered in the retrospective study.

All members of the study group received two PDT treatments with one-week interval at the Outpatient Clinic of the Department of Dermatology at the Medical University of Lublin, Poland, between March 2019 – January 2023.

Criteria for inclusion in the study:
- superficial BCC confirmed with dermoscopy;
- either a previous history of histopathologically confirmed sBCC elsewhere on the skin, or a biopsy which confirmed the diagnosis of sBCC.

Exclusion criteria:
- other than a superficial type of BCC,
- receiving any BCC topical or systemic therapies at least three months prior to PDT.

Before inclusion in the study, written informed consent was provided by all patients. The study was approved by the local Ethics Committee (KE-0254/151/05/2023).

Study formulations. The photosensitizers used in the study were: 5-aminolevulinic acid methyl ester hydrochloride (MAL-HCl; methyl 5-amino-4-oxopentanoate, hydrochloride; $[H_3COOC–CH_2–CH_2–CO–CH_2–NH_3^+]Cl^-$) obtained from Arisun chempharm Co., Ltd., Xi'an, China, and commercially availabl/e ALA-HCl in gel with a lipid *nanoemulsion*. The gel contained 10% 5-aminolevulinic acid hydrochloride (ALA-HCl; 5-amino-4-oxopentanoic acid, hydrochloride; $[HOOC–CH_2–CH_2–CO–CH_2–NH_3^+]Cl^-$) (corresponding to 7.8% ALA) and excipients with known effect: sodium benzoate, soy phosphatidylcholine and propylene glycol. MAL-HCI photosensitizer was introduced into lipobaza cream using a pharmaceutical stirrer. The obtained formulation contained 12.5% of the MAL-HCl (corresponding to 10% of MAL).

Treatment protocol. In each patient, prior to PDT, a qualifying physical examination and videodermoscopy using a FotoFinderDermoscope 800 were performed. Non-pigmented superficial BCC (sBCC) was confirmed in all patients. Only patients who presented the typical dermoscopic features of sBCC, i.e. short-fine, microarborizing vessels, were qualified for the study.

Application of Study Formulations. After careful debridgement, a photosensitizing substance, MAL-HCl or ALA-HCl, was applied directly on the lesion. 10 patients were treated with MAL-HCl in cream, and 11 with ALA-HCl in gel. In the case of the gel, 10 minutes were awaited until it had

partially dried. A plastic occlusive dressing and aluminum foil were then applied. After three hours, the dressing was removed, and the residual preparations were wiped away using 0.9% sodium chloride solution

Evaluation of skin fluorescence after application of tested preparations. The intensity of fluorescence was qualitatively assessed using a FotoFinderDermoscope 800 attachment with white and violet LEDs. The observed fluorescence was evaluated as high, medium or low, which corresponded to the numbers 3, 2, 1, respectively.

Irradiation with red light 630 ± 5 nm. During the irradiation, all participants wore protective goggles. For the exposure, the Red Beam Pro+, Model APRO (MedLight GmbH, Herford, Germany) was used.

The Red Beam Pro+ lamp contains 78 diodes emitting red light 630 ± 5 nm. A dose of 50 J/cm2 was used during a single session, light intensity – 68 mW/cm2. The lamp distance from the skin surface was 8 cm. Patients were advised to avoid UV light exposure for 48 hours after treatment.

Evaluation of effectiveness. Lesion clearance was assessed visually 12 weeks (checkpoint visit) after the second treatment. The overall percentage of complete responses in patients 12 weeks after the second treatment, defined as complete clinical remission (lesion clearance), was considered primary efficacy. During the checkpoint visit, in case of clinical and dermoscopic doubts, patients were given photodynamic diagnostics. The same photosensitizer was applied to the lesion, and fluorescence was evaluated after three hours; if present, the patients were qualified for another treatment.

Cosmetic outcome. Twelve weeks after the second treatment, cosmetic results were evaluated using a four-point scale (Tab. 1). The skin lesions were photographed.

Table 1. Evaluation of cosmetic effect

Grade	Definition
Poor	extensive occurrence of scarring, atrophy, or induration
Fair	slight to moderate occurrence of scarring, atrophy, or induration
Good	no scarring, atrophy, or induration, moderate redness or increase in pigmentation compared with adjacent skin
Excellent	no scarring, atrophy, or induration, slight or no redness or change in pigmentation compared with adjacent skin

RESULTS

Study group characteristics. Patient characteristics of sBCC and lesions are shown in Table 2.

Table 2. Characteristics of the study group

Variable	Category	Parameter	Estimate
Age	Years	Min-max	27–92
Gender	Male Female	N(%)	9 (43%) 12 (57%)
Localisations of lesions	Head Trunk	N(%)	10 (48%) 11 (52%)

Evaluation of fluorescence after application of test preparations. Fluorescence was evaluated for all patients prior to red light irradiation. In the majority of patients (72%) after the application of ALA-HCl in gel, intense and focused PpIX fluorescence was observed. In contrast, after MAL-HCl in cream application, PpIX fluorescence tended to be moderate and dispersed (60%) (Tab. 3; Fig. 1 and 2). It is worth noting that very intense PpIX fluorescence was observed in one of the patients with seborrheic skin, which was probably related to the increased penetration of nanoparticles through the dilated pores characteristic of seborrheic skin (Fig. 2).

Photosensitizer / Fluorescence intensity	ALA-HCl	MAL-HCl
Intense fluorescence	8 patients (72%)	4 patients (40%)
Moderate fluorescence	3 patients (28%)	6 patients (60%)
Low fluorescence	0 patients (0%)	0 patients (0%)

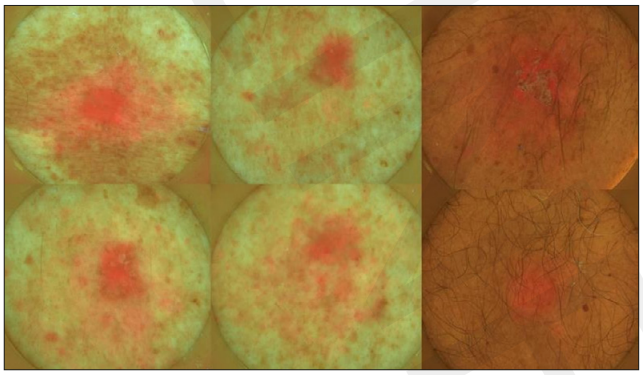

Figure 1. Images showing the results of UV lamp examination of BCC lesions overlaid with MAL-HCl. It was noted that PpIX fluorescence tended to be moderate and dispersed

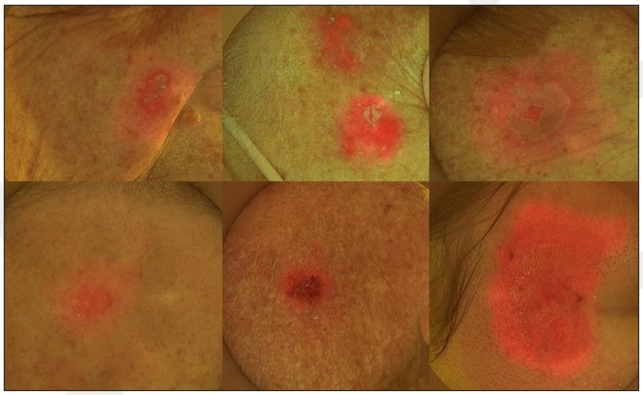

Figure 2. Images showing the results of UV lamp examination of BCC lesions with ALA-HCl applied. Intense and focused PpIX fluorescence was observed.

Efficacy of PDT with study formulations. Twelve weeks after the last PDT treatment, the rate of complete remission was shown to be 82% (n = 9) in the ALA-HCl in gel group and 80% (n = 8) in the MAL-HCl in cream group. Patients without complete clinical remission, based on the result of photodynamic diagnosis, were qualified for subsequent PDT procedures (n=2 for ALA-HCl in gel and n=2 in MAL-HCl in cream group) (Fig. 3 and 4).

Cosmetic Outcome. In the majority of patients, the cosmetic result was determined to be excellent – 96% for MAL-HCl in

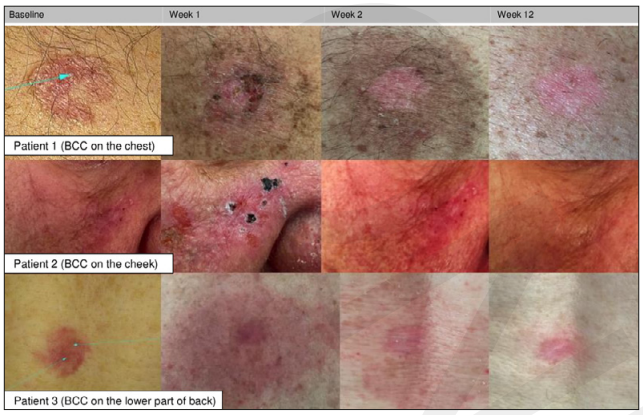

Figure 3. Selected results of PDT with MAL-HCl in cream. The blue arrow indicates the site selected for dermoscopic evaluation

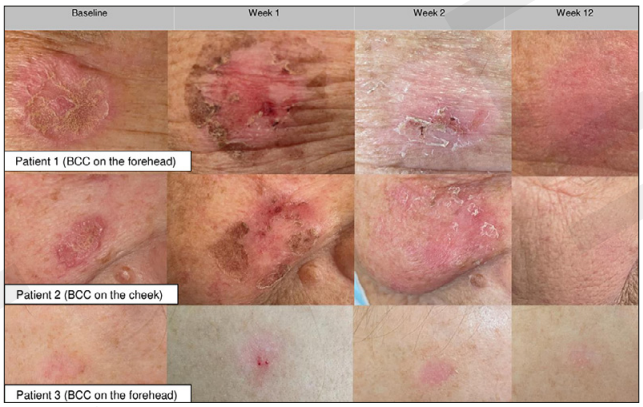

Figure 4. Selected results of PDT with ALA-HCl in gel

cream and 100% for ALA-HCl in gel. Faster skin healing and less post-inflammatory hyperpigmentation during follow-up visits was observed after treatment with ALA-HCl in a gel formulation with a lipid nanoemulsion.

DISCUSSION

PDT was first used to treat BCCs in 1990 [20]. Since then, this group of cancers has been continuously treated with PDT due to the high global incidence of BCC as well as the easy availability of the tumour for both observation and treatment. In addition, it is emphasized that using sBCC as a research model for PDT treatment has an advantage in that it allows focus on some of the limitations of PDT treatment, such as PDT resistance and disease recurrence. In addition, attention is drawn to the alarming increase in the incidence of sBCC in young people, especially women. PDT, as a cosmetically acceptable method, appears to be of particular interest in this group of patients [21].

The main photosensitizer used in PDT therapy is PpIX. The process of its production is endogenous and results from the transformation of a pro-drug selectively captured by cancer cells, such as ALA-HCl or its ester, MAL-HCl. At the cell level, ALA-HCl undergoes enzymatic conversion via the heme biosynthesis pathway to PpIX. PpIX molecules absorb light in the process of initiating photosensitization, which leads to the promotion of electrons from the basal state to produce a triplet excited state. Subsequently, the excited PpIX produces reactive oxygen species, which exhibit destructive effects on BCC cells [22]. In addition to the destructive effects on BCC cells, it is notable that PDT-induced immune modifications contribute to therapeutic efficacy [23]. Furthermore, there was a study in which thermographic and fluorescence images were analyzed to evaluate the temperature pattern during treatment of BCC with PDT. It was shown that the vast majority of energy conversion was due to photodynamic rather than thermal effects, highlighting the mechanism of destructive action against cancer cells [24].

To date, nine randomized controlled trials have been published in which MAL-PDT was used to treat sBCC. Most of the publications were comparative in nature and involved evaluating the efficacy of MAL in relation to other therapeutic modalities, such as imiquimod, 5-fluorouracil, cryotherapy, ALA-HCl, hexyl aminolevulinate (HAL) and surgical excision [25–31]. In three studies, MAL-PDT was compared with an ALA-HCl nanoemulsion (BF-200). Salmivuori et al., additionally compared histological clearance, tolerability and cosmetic effects with low concentrations of HAL. It was found that histologically-confirmed lesion clearance was 93.8%, 90.9% and 87.9% for MAL, BF-200 ALA and HAL, respectively, with no statistical differences ($P = 0.84$). In addition, there were no differences between preparations with regard to pain, post-treatment responses or cosmetic effects [32]. A particularly relevant study by Morton et al. demonstrated that BF-200 ALA is not inferior to MAL. The study showed that complete clinical remission occurred in 93.4% of patients in the BF-200 ALA group, versus 91.8% in the MAL group. Moreover, the recurrence 12 months following the final treatment of the lesion topped out at ≤ 10% [33]. Alique-García et al. in a group of 22 patients diagnosed with sBCC receiving PDT showed that after one year, 93.75% of foci resolved after ALA-HCl (BF-200) and 50% after MAL-HCl. There were no differences in the aspect of tolerability, which was defined as good or regular [34].

It is of particular note that all the cited studies used MAL-HCl in the form of conventionally available Metvix at a concentration of 16%, which is higher than used in the current study. This is undoubtedly noteworthy since the photosensitizer concentration in ALA-HCl in Gel with a lipid *nanoemulsion* was 7.8%, while in the studied MAL-HCl in cream it was 10%. This indicates that although the photosensitizer concentration was about 20% lower in the studied gel, the observed fluorescent effects were more intense and the treatment was associated with the faster and better cosmetic results. Therefore, both MAL-PDT in cream, as well as ALA-PDT in gel (in nanoemulsion form), are highly effective photosensitizers in terms of clinical improvement, which is consistent with the results of the current study.

However, there is a lack of comparative studies assessing long-term prevention of sBCC recurrence in patients. Based

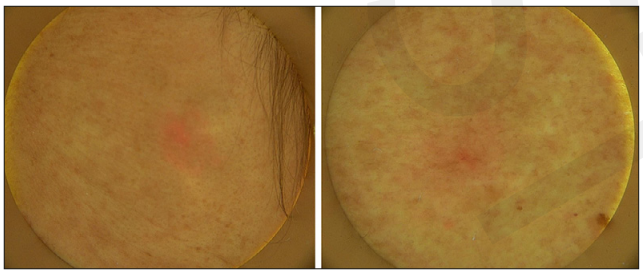

Figure 5. Results of photodynamic diagnostics performed during a visit at 12 weeks after the last PDT treatment

on photodynamic diagnostics performed at a visit 12 weeks following the final PDT treatment, in the current study two patients in both of the two groups required an additional PDT session. It should be emphasized that the use of this diagnostic too objectively allows for determining indications for continuing PDT [35] (Fig. 5).

Undoubtedly, the cosmetic effect after PDT that is satisfactory for patients is of particular importance, which is an undeniable advantage considering the tendency for sBCC to occur in young people. In this study, faster skin healing was observed after the use of ALA-HCl in gel formulation with a lipid nanoemulsion, which may be related to the preferential accumulation of the substance in the form of nanoparticles in cancer cells, better course of the procedure, and faster recovery. These observations were probably due to the advantages of nanoemulsion as a form of drug delivery to cells.

The studies regarding cancer therapy demonstrate that active ingredients in the forms of nanoemulsions are efficiently taken up by cancer cells and eliminate toxicity in surrounding tissue [36]. Moreover, the ALA-HCl in gel formulation with a lipid nanoemulsion contains excipients, i.e. soy phosphatidylcholine and propylene glycol, which increase the affinity of ALA to the epidermal tissue [37]. The higher precision of the nanoemulsion is confirmed by the higher PpIX fluorescence intensity after application of ALA-HCl in gel, compared to MAL-HCl in cream.

The limitations of the study included the limited number of patients, its single-centre nature, and the lack of a control group. However, based on the promising results, the authors would like to expand the study in the future.

CONCLUSIONS

1) ALA-HCl in gel with a lipid *nanoemulsion* and MAL-HCl in cream appeared to be similarly effective in patients with sBCC.
2) The advantage of ALA-HCl in gel with a lipid *nanoemulsion* promoted faster skin healing, resulting in better cosmetic outcomes compared to MAL-HCl in cream.

REFERENCES

1. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the US population, 2012. JAMA Dermatol. 2015;151:1081. doi:10.1001/jamadermatol.2015.1187
2. Nagarajan P, Asgari MM, Green AC, Guhan SM, Arron ST, Proby CM, Rollison DE, Harwood CA, Toland AE. Keratinocyte Carcinomas: Current Concepts and Future Research Priorities. Clin Cancer Res. 2019;25:2379–2391. doi:10.1158/1078-0432.CCR-18-1122
3. Szewczyk M, Pazdrowski J, Golusiński P, Dańczak-Pazdrowska A, Łuczewski Ł, Marszałek S, Majchrzak E, Golusiński W. Basal cell carcinoma in farmers: an occupation group at high risk. Int Arch Occup Environ Health. 2016;89:497–501.
4. Cameron MC, Lee E, Hibler BP, Barker CA, Mori S, Cordova M, Nehal KS, Rossi AM. Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and disease associations. J Am Acad Dermatol. 2019;2:303–317. doi:10.1016/j.jaad.2018.03.060
5. Naik PP, Desai MB. Basal Cell Carcinoma: A Narrative Review on Contemporary Diagnosis and Management. Oncol Ther. 2022;2:317–335. doi:10.1007/s40487-022-00201-8
6. Christenson LJ, Borrowman TA, Vachon CM, Tollefson MM, Otley CC, Weaver AL, Roenigk RK. Incidence of basal cell and squamous cell carcinomas in a population younger than 40 years. JAMA 2005;294:681–90. doi:10.1001/jama.294.6.681
7. Fania L, Didona D, Morese R, Campana I, Coco V, Di Pietro FR, Ricci F, Pallotta S, Candi E, Abeni D, Dellambra, E. Basal Cell Carcinoma: From Pathophysiology to Novel Therapeutic Approaches. Biomedicines 2020;8:449. doi:10.3390/biomedicines8110449
8. Seidl-Philipp M, Frischhut N, Höllweger N, Schmuth M, Nguyen VA. Known and new facts on basal cell carcinoma. J Dtsch Dermatol Ges. 2021;19:1021–1041. doi:10.1111/ddg.14580
9. Grachtchouk M, Pero J, Yang SH, Ermilov AN, Michael LE, Wang A, Wilbert D, Patel RM, Ferris J, Diener J, Allen M, Lim S, Syu LJ, Verhaegen M, Dlugosz AA. Basal cell carcinomas in mice arise from hair follicle stem cells and multiple epithelial progenitor populations. J Clin Invest. 2011;121:1768–1781. doi:10.1172/JCI46307
10. Sánchez-Danés A, Hannezo E, Larsimont JC, Liagre M, Youssef KK, Simons BD, Blanpain C. Defining the clonal dynamics leading to mouse skin tumour initiation. Nature 2016;536:298–303. doi:10.1038/nature19069
11. Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nat Med. 2014;20:847–856. doi:10.1038/nm.3643
12. Miller DL, Weinstock MA. Nonmelanoma skin cancer in the United States: incidence. J Am Acad Dermatol. 1994;30:774–778. doi:10.1016/s0190-9622(08)81509-5
13. NCCN. Basal Cell Skin Cancer (Version 1.2022). Available at: https://www.nccn.org/professionals/physician_gls/pdf/nmsc.pdf Accessed May 3, 2023.
14. Heath MS, Bar A. Basal Cell Carcinoma. Dermatol Clin. 2023;41:13–21. doi:10.1016/j.det.2022.07.005
15. Naik PP, Desai MB, Basal Cell Carcinoma: A Narrative Review on Contemporary Diagnosis and Management. Oncol Ther. 2022;10:317–335. doi:10.1007/s40487-022-00201-8
16. Niculet E, Craescu M, Rebegea L, Bobeica C, Nastase F, Lupasteanu G, Stan DJ, Chioncel V, Anghel L, Lungu M, Tatu AL. Basal cell carcinoma: Comprehensive clinical and histopathological aspects, novel imaging tools and therapeutic approaches (Review). Exp Ther Med. 2022;23:60. doi:10.3892/etm.2021.10982
17. Ou-Yang Y, Zheng Y, Mills KE. Photodynamic therapy for skin carcinomas: A systematic review and meta-analysis. Front Med. (Lausanne) 2023;19(10):1089361. doi:10.3389/fmed.2023.1089361
18. Schmitz L, Novak B, Hoeh AK, Luebbert H, Dirschka T. Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin. Photodiagnosis Photodyn Ther. 2016;14:40–46. doi:10.1016/j.pdpdt.2015.11.004
19. Zeitouni NC, Bhatia N, Ceilley RI, Cohen JL, Del Rosso JQ, Moore AY, Munavalli G, Pariser DM, Schlesinger T, Siegel DM, Willey A, Goldman MP. Photodynamic Therapy with 5-aminolevulinic Acid 10% Gel and Red Light for the Treatment of Actinic Keratosis, Nonmelanoma Skin Cancers, and Acne: Current Evidence and Best Practices. J Clin Aesthet Dermatol. 2021;14:E53-E65.
20. Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous protoporphyrin IX: Basic principles and present clinical experience. J Photochem Photobiol B Biol. 1990;6:143–148. https://doi.org/10.1016/1011-1344(90)85083-9
21. Collier NJ, Rhodes LE. Photodynamic Therapy for Basal Cell Carcinoma: The Clinical Context for Future Research Priorities. Molecules 2020;25:5398. doi:10.3390/molecules25225398
22. Kou J, Dou D, Yang L. Porphyrin photosensitizers in photodynamic therapy and its applications. Oncotarget. 2017;8:81591–81603. doi:10.18632/oncotarget.20189
23. Adamek M, Kawczyk-Krupka A, Mostowy A, Czuba Z, Krol W, Kasperczyk S, Jakobisiak M, Golab J, Sieron A. Topical ALA-PDT modifies neutrophils' chemiluminescence, lymphocytes' interleukin-1beta secretion and serum level of transforming growth factor beta1 in patients with nonmelanoma skin malignancies. A clinical study. Photodiagnosis Photodyn Ther. 2005;2:65–72. doi:10.1016/S1572-1000(05)00004-9
24. Stringasci MD, Salvio AG, Moriyama LT, Vollet-Filho JD, Fortunato TC, Bagnato VS, Kurachi C. Energy analysis of PDT using thermography during the treatment of basal cell carcinoma. Photodiagnosis Photodyn Ther. 2020;29:101586. doi:10.1016/j.pdpdt.2019.101586
25. Arits AH, Mosterd K, Essers BA, Spoorenberg E, Sommer A, De Rooij MJ, van Pelt HP, Quaedvlieg PJ, Krekels GA, van Neer PA, Rijzewijk JJ, van Geest AJ, Steijlen PM, Nelemans PJ, Kelleners-Smeets NW. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol. 2013;14:647–654. doi:10.1016/S1470-2045(13)70143-8
26. Jansen MHE, Koekelkoren FHJ, Nelemans PJ, Arits AHMM, Roozeboom MH, Kelleners-Smeets NWJ, Mosterd K. Comparison of

long-term cosmetic outcomes for different treatments of superficial basal cell carcinoma. J Am Acad Dermatol. 2018;79:961–964. doi:10.1016/j.jaad.2018.04.053
27. Roozeboom MH, Nelemans PJ, Mosterd K, Steijlen PM, Arits AH, Kelleners-Smeets NW. Photodynamic therapy vs. topical imiquimod for treatment of superficial basal cell carcinoma: a subgroup analysis within a noninferiority randomized controlled trial. Br J Dermatol. 2015;172:739–745. doi:10.1111/bjd.13299
28. Roozeboom MH, Arits AHMM, Mosterd K, Sommer A, Essers BAB, de Rooij MJM, Quaedvlieg PJF, Steijlen PM, Nelemans PJ, Kelleners-Smeets NWJ. Three-Year Follow-Up Results of Photodynamic Therapy vs. Imiquimod vs. Fluorouracil for Treatment of Superficial Basal Cell Carcinoma: A Single-Blind, Noninferiority, Randomized Controlled Trial. J Invest Dermatol. 2016;136:1568–1574. doi:10.1016/j.jid.2016.03.043
29. Basset-Seguin N, Ibbotson SH, Emtestam L, Tarstedt M, Morton C, Maroti M, Calzavara-Pinton P, Varma S, Roelandts R, Wolf P. Topical methyl aminolaevulinate photodynamic therapy versus cryotherapy for superficial basal cell carcinoma: a 5 year randomized trial. Eur J Dermatol. 2008;18:547–553. doi:10.1684/ejd.2008.0472
30. Kessels JPHM, Kreukels H, Nelemans PJ, Roozeboom MH, van Pelt H, Mosterd K, de Haas ERM, Kelleners-Smeets NWJ. Treatment of superficial basal cell carcinoma by topical photodynamic therapy with fractionated 5-aminolaevulinic acid 20% vs. two-stage topical methyl aminolaevulinate: results of a randomized controlled trial. Br J Dermatol. 2018;178:1056–1063. doi:10.1111/bjd.15967
31. Szeimies RM, Ibbotson S, Murrell DF, Rubel D, Frambach Y, de Berker D, Dummer R, Kerrouche N, Villemagne H, Excilight Study Group. A clinical study comparing methyl aminolevulinate photodynamic therapy and surgery in small superficial basal cell carcinoma (8–20 mm), with a 12-month follow-up. J Eur Acad Dermatol Venereol. 2008;22:1302–1311. doi:10.1111/j.1468-3083.2008.02803.x
32. Salmivuori M, Grönroos M, Tani T, Pölönen I, Räsänen J, Annala L, et al. Hexyl aminolevulinate, 5-aminolevulinic acid nanoemulsion and methyl aminolevulinate in photodynamic therapy of non-aggressive basal cell carcinomas: A non-sponsored, randomized, prospective and double-blinded trial. J Eur Acad Dermatol Venereol. 2020;34:2781–2788. doi:10.1111/jdv.16357
33. Morton CA, Dominicus R, Radny P, Dirschka T, Hauschild A, Reinhold U, et al. A randomized, multinational, noninferiority, phase III trial to evaluate the safety and efficacy of BF-200 aminolaevulinic acid gel vs. methyl aminolaevulinate cream in the treatment of nonaggressive basal cell carcinoma with photodynamic therapy. Br J Dermatol. 2018;179:309–319. doi:10.1111/bjd.16441
34. Alique-García S, Company-Quiroga J, Sánchez Campos A, Hernández Núñez A, Borbujo J. Treatment of superficial basal cell carcinoma with photodynamic therapy. Observational study in 22 patients with 5-aminolaevulinic acid and methyl aminolaevulinate. Photodiagnosis Photodyn Ther. 2019;26:190–192. doi:10.1016/j.pdpdt.2019.03.018
35. Redondo P, Marquina M, Pretel M, Aguado L, Iglesias ME. Methyl-ALA-induced fluorescence in photodynamic diagnosis of basal cell carcinoma prior to Mohs micrographic surgery. Arch Dermatol. 2008;144:115–117. doi:10.1001/archdermatol.2007.3
36. Sánchez-López E, Guerra M, Dias-Ferreira J, Lopez-Machado A, Ettcheto M, Cano A, et al. Current Applications of Nanoemulsions in Cancer Therapeutics. Nanomaterials 2019;9:821. doi:10.3390/nano9060821
37. Benaouda S, Jones A, Martin GP, Brown MB. Localized Epidermal Drug Delivery Induced by Supramolecular Solvent Structuring. Mol Pharm. 2016;13:65–72. doi:10.1021/acs.molpharmaceut.5b00499