# Present and future perspectives of photodynamic therapy for cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (SCC) is the second most common skin cancer. Surgery remains the main stay of treatment, but some patients are not eligible for surgery and, more importantly, lesions at critical sites need nonsurgical approaches for tissue preservation. In this context, photodynamic therapy (PDT) has been extensively studied as noninvasive or minimally invasive treatment, and studies have shown promising results in terms of safety, efficacy, and cosmetic outcome. Also, studies have proposed different mechanism for its efficacy. However, human studies demonstrating its efficacy are limited in terms of sample size and tumor depth of invasion. Good results are mainly seen in case reports of microinvasive SCC, which is defined as SCC limited to papillary dermis. This inadequacy is due to inadequate penetration of topically applied photosensitizers through keratinized tumor surfaces. To overcome these hurdles, pretreatment with lasers or microneedles and encapsulation of photosensitizers into nanoparticles have been tried. Hence, the present article will discuss studies that have demonstrated the efficacy and safety of PDT for cutaneous SCC, studies that have postulated the mechanism of action of PDT, agents that have been used as PDT enhancers, and finally, the recent use of adjuvant therapy in combination with PDT. ( J Am Acad Dermatol 2019;80:765-73.)

***Key words:*** cutaneous squamous cell carcinoma; mechanism of action; nanomedicine; photodynamic therapy; surgery.

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer after basal cell carcinoma, and the annual incidence is increasing. In the United States, the annual incidence is ~200,000-700,000 cases.[1,2] Surgery remains the main stay of treatment, but elderly patients with comorbid conditions and those on immunosuppressants or anticoagulants might be ineligible for surgery. Moreover, eyelid, lip, or ear lesions need maximum tissue preservation, and thus, surgery becomes inappropriate or might lead to poor cosmetic outcomes. Hence, nonsurgical approaches are required. Treatment with cryotherapy, imiquimod, and 5-fluorouracil are associated with poor outcome and high recurrence rate. Photodynamic therapy (PDT) is comparatively effective, provides a good cosmetic outcome, and has a low recurrence rate. In addition, it can be used as a field treatment for actinic keratosis lesions,[3] which are known precursors of cSCC. Hence, it is worth mentioning that PDT not only treats cSCC, but also can prevent cSCC.

*Abbreviations used:*

| | |
|---|---|
| 5-ALA: | 5-aminolevulinic acid |
| AFL: | ablative fractional laser |
| cSCC: | cutaneous squamous cell carcinoma |
| DAMPs: | damage-associated molecular patterns |
| hALA: | aminolaevulinic acid hexylester |
| MAL: | methyl aminolaevulinate |
| PBN: | plum blossom needle |
| PDT: | photodynamic therapy |
| PLGA: | poly lactic-co-glycolic acid |
| SCC: | squamous cell carcinoma |
| STAT3: | signal transducer and activator of transcription 3 |

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PDT works by activating a photosensitizer by visible light to produce reactive oxygen species within target cells, resulting in their destruction.[4] In dermatology, PDT is usually given with topical photosensitizers, most often 5-aminolaevulinic acid or methyl aminolevulinate (MAL). After an incubation period, an appropriate wavelength of light activates photosensitizer-promoting photodynamic reactions. For skin, PDT has the additional benefits of easy access and good cosmetic outcome. Regarding cSCC, PDT is recommended for in situ lesions, with studies reporting lesion clearance rates of 88%-100% 3 months after 1-2 cycles of MAL-PDT and no recurrence in 68%-89% of treated lesions at 17-50 months follow-up.[5-9] However, data supporting the efficacy of PDT for invasive SCC are currently limited. The main reason for the inadequacy of topical PDT might be inadequate penetration of topically applied photosensitizer through tumor tissue or insufficient local bioavailability and, hence, decreased cellular uptake. To facilitate drug delivery, clinicians have tried pretreatment with ablative fractional laser (AFL)[10] and plum blossom needle (PBN).[11] Also, to attain the maximum concentration of drug at the desired site, intralesional injection of photosensitizer has been tried in the clinic.[12] Moreover, researchers are using nanomedicine to encapsulate photosensitizer that can enhance PDT efficacy by increasing the amount of drug delivered and drug retention time at target sites.[13,14] More recently, the combined use of PDT with 5-fluorouracil or resveratrol and whether it improves clinical outcomes of SCC was investigated.[15,16]

**CAPSULE SUMMARY**

- Photodynamic therapy might benefit patients with cutaneous squamous cell carcinoma who are not eligible for surgery.
- The effects of photodynamic therapy can be enhanced with the use of microneedles, lasers, intralesional-injected photosensitizers, nanoparticles, or adjuncts.

## HUMAN STUDIES

The largest human study in terms of sample size (45 patients) was a randomized clinical trial reported by Choi et al (Table I).[10] In their study, they compared 1 session of AFL-MAL-PDT (AFL performed using a 2940-nm erbium-doped yttrium aluminum garnet laser with 550—600-$\mu$m ablation depth, level-1 coagulation, 22% treatment density, and a single pulse) with 2 sessions of conventional MAL-PDT for the treatment of microinvasive SCC and found that the overall complete response rates 3 months after treatment were 84.2% and 52.4% with AFL-MAL-PDT and MAL-PDT respectively. The recurrence rate was significantly lower with AFL-MAL-PDT (12.5%) than with MAL-PDT (63.6%) at 24 months. Another large study that enrolled 25 patients from 5 centers in 3 countries was reported by Kubler et al.[17] This was a prospective, open-label, multicenter study carried out on patients with early SCC of the lips. These patients were treated with 0.15 mg/kg temoporfin intravenously, followed 4 days later by a single nonthermal illumination of the tumor. Complete response rate 3 months after treatment was 96%, and 2 patients had recurrence 4 and 18 months after PDT. Another 5 studies demonstrating the efficacy of PDT for SCC were case reports, all of which showed complete response after 3-12 months of treatment and no recurrence at 6-24 months.[11,12,18-20] Of these 5 SCC cases, the largest tumor was an invasive SCC measuring ~4 × 5 × 2 cm, which disappeared after 9 sessions of topical aminolevulinic acid (ALA) PDT pretreated with PBN.[11] In terms of maximum tumor depth, a 5.5-mm tumor achieved complete remission after 7 sessions of topical ALA-PDT.[19] Surprisingly, 1 case of infiltrative SCC responded to 1 session of ALA-PDT, but this study involved using Intralesional ALA rather than topical ALA like other studies.[12]

## MECHANISTIC STUDIES

Findings from mechanism-based studies have indicated that PDT induces apoptosis of SCC cells basically via generation of reactive oxygen species and induction of antitumor immune responses. However, complete disease clearance and long-term control was achieved by an indirect pathway that causes post-PDT hypoxia or anoxia of tumor cells as a consequence of vascular occlusion (Fig 1). ALA-PDT was seen to exert photodynamic activity in A431 cells by arresting cell proliferation at the G0-G1 phase of the cell cycle.[21] It induced apoptosis via activation of caspase 3. There was involvement of signal transducer and activator of transcription 3 (STAT3) and its target genes (*Bcl2* and *BAX*) in ALA-PDT—induced apoptosis of A431 cells. Further experimentation with A431 cells and COLO-6 cells revealed higher levels of expression of STAT3 and p-STAT3 in the cancer tissue than the corresponding adjacent tissue.[22] The p-STAT3 expression level in cancerous tissue showed a correlation with tumor size and tissue histopathologic differentiation. Knocking down of STAT3 could inhibit cell

**Table I.** Human studies demonstrating efficacy and safety of PDT for cSCC

| Study | No. patients, diagnosis | Treatment | Incubation period, hr, light | Treatment session | Result | Recurrence |
|---|---|---|---|---|---|---|
| AC Kubler[17] | 25, SCC of lip (Tis, T1, T2/N0/M0) | 0.15 mg/kg intravenous Foscan-PDT | 96, red light 20 J/cm$^2$, irradiance 100 mW/cm$^2$, wavelength 652 nm | 1 | CR after 12 wk was 96% (24/25) patients | Recurrence observed in 2 patients 4 and 18 months after PDT |
| Seung-Hwan Choi[10] | 45, microinvasive SCC of face, scalp, trunk or extremities | 21 patients received AFL-MAL-PDT and 24 patients received MAL-PDT only | 3, red light at 37 J/cm$^2$ | 1 session of AFL-MAL-PDT and 2 sessions of MAL-PDT at 1-wk intervals | CRs 12 wk after treatment were 84.2% with AFL-MAL-PDT and 52.4% with MAL-PDT | Recurrence rate significantly lower with AFL-PDT (12.5%) than with MAL-PDT (63.6%) at 24 mon |
| Peiru Wang[11] | 1, giant invasive SCC at preauricular region | Plum blossom needle assistedtopical ALA-PDT | 4, red light 126 J/cm$^2$, irradiance 100 mW/cm$^2$, wavelength 633 nm | 9 sessions at 2-wk intervals | CR achieved after 9 sessions of treatment | No recurrence at 18 mon follow-up |
| Eleni Sotiriou[12] | 1, infiltrative SCC on cheek | 10% of 0.8 mL intralesional ALA-PDT | 4, red light 100 J/cm$^2$, irradiance 100 mW/cm$^2$, wavelength 570-670 nm | 1 | CR achieved 12 wk after treatment | No recurrence 16 mon after PDT |
| MC Fargnoli[18] | 1, microinvasive SCC of lip | MAL-PDT | 3, red light 37 J/cm$^2$, 635 $\pm$ 18 nm for 7 min and 40 s at 8-cm distance | 2 sessions at 1-wk intervals | CR achieved after 2 sessions of treatment | No recurrence at 24-mon follow-up |
| Qiang Li[19] | 1, thick invasive SCC on scalp with a tumor depth of 5.5 mm | Topical 20% ALA-PDT | 6, red light total dose of 791 J/cm$^2$, 633 nm, 126 mW/cm$^2$ | 7 sessions at 1-wk intervals | CR achieved 12 mon after treatment | - |
| R Rossi[20] | 1, SCC of lower eyelid | Topical 20% ALA-PDT after frost suture to protect eyeball | 4, red light 80 J/cm$^2$, 633 $\pm$ 3 nm for 16 min and 40 s | 1 | CR achieved after 1 session of treatment | No recurrence at 6-mon follow-up |

*AFL*, Ablative fractional laser; *ALA*, aminolevulinic acid; *CR*, complete response; *MAL*, methyl aminolevulinic acid; *PDT*, photo dynamic therapy; *SCC*, squamous cell carcinoma.

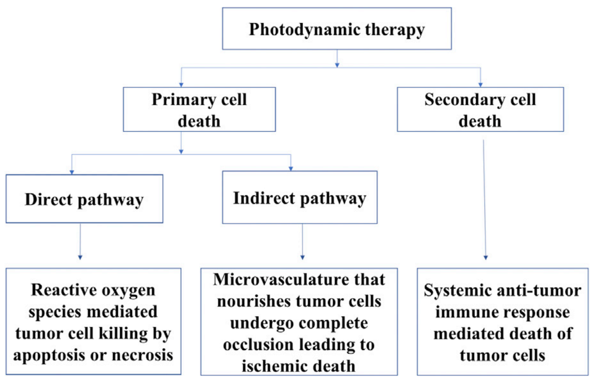


**Fig 1.** Distinct mode of action of photodynamic therapy.

proliferation and promote cell apoptosis, and over-expression of STAT3 could have the opposite effect. In addition, ALA-PDT could weaken the protein expression of STAT3 and Bcl-2 and strengthen the protein expression of BAX.

In experiments with the ultraviolet radiation−induced SCC mice model treated with multiple sessions of topical ALA-PDT, immunohistochemical analysis showed a significant increase in $CD4^+$ and $CD8^+$ cells, suggesting the elicitation of specific immunity against SCCs.[23] Possibly through secretion of tumor necrosis factor $\alpha$, $CD4^+$ cells might activate $CD8^+$ cells, which could, in turn, exert a cytotoxic action on SCC cells. Wang et al[24] evaluated in vitro and in vivo expression of 3 major damage-associated molecular patterns (DAMPs), calreticulin, heat shock protein 70, and high-mobility group box 1, in ALA-PDT−treated cells. The results showed that ALA-PDT could enhance the expression of calreticulin, heat shock protein 70, and high-mobility group box 1, and these DAMPs could further lead to maturation of dendritic cells, including phenotypic maturation (increase of surface expression of major histocompatibility II, CD80, and CD86) and functional maturation (enhanced capability to secrete interferon $\gamma$ and interleukin 12). Hence, the study concluded that ALA-PDT can increase DAMPs and enhance tumor immunogenicity.

Juarez et al[25] reported a study using riboflavin tetraacetate as a photosensitizer in PDT in a human cancer cell line of squamous carcinoma (SCC-13). Cell viability, cell death, and generation of intracellular reactive oxygen species were analyzed after riboflavin tetraacetate PDT. The study indicated that primary cell death was via apoptosis by reactive oxygen species production, and cell death induced by riboflavin tetraacetate PDT involved activation of extracellular signal−related kinase (ERK) 1/2 protein. Sharma et al reported hypericin PDT−induced rapid necrotic death in human SCC cultures after multiple treatments.[26] Hypericin PDT−induced reactive oxygen species mediated cell death, and the mode of cell death was a necrotic caspase-independent mechanism. Although the researchers of these studies postulated mechanisms mediating PDT efficacy for SCC, Gilaberte et al proposed a mechanism involved in the resistance of SCC to PDT.[27] To search for potential factors implicated in SCC resistance to PDT, authors used the SCC-13 cell line (parental) and resistant SCC-13 cells, obtained by repeated MAL-PDT treatments (5th and 10th PDT generations). The study suggested that genomic imbalances related to cyclin D1, epidermal growth factor receptor, and particularly mitogen-activated protein kinase kinase kinase 1 (MAP3K1) might be involved in the development of resistance of SCC to PDT.

## NANOMEDICINE

Use of existing photosensitizers in conjunction with nanoparticles and a move to nanomedicine presents an intriguing possibility. Nanomedicine is the medical application of nanotechnology for diagnosis and treatment of human diseases. It uses precisely engineered materials, known as nanoparticles, which have unique physicochemical properties, such as small size, large surface-area-to-mass ratio, and high reactivity, which are used to overcome some limitations found in standard therapeutic agents.[28] Nanoencapsulation of photosensitizer is a promising approach that improves efficiency and can overcome many obstacles seen with classic PDT. Nano-sized drug carriers are known to accumulate at tumor sites by a so-called enhanced permeation and retention effect, resulting in their efficient passive accumulation in solid tumor tissues due to leaky vasculature and poor lymphatic drainage that is characteristic of tumors.[29]

A novel ALA delivery approach involving poly lactic-co-glycolic acid (PLGA) nanoparticles was reported in 1 study.[13] The characteristics, uptake, protoporphyrin IX fluorescence kinetics, and cytotoxicity of ALA-loaded PLGA nanoparticles toward a human skin SCC cell line were examined. The study found that ALA-loaded PLGA nanoparticles could be taken up by SCC cells and localized in the cytoplasm. The protoporphyrin IX fluorescence kinetics showed that ALA loaded in nanoparticles were more effective than free ALA of the same concentration. Further experiments were conducted in animal models to evaluate the effectiveness of nanoparticle assisted ALA delivery for topical PDT of cutaneous SCC.[14] Ultraviolet light−induced cSCCs on mice were treated with topical PDT with free ALA or

ALA-loaded nanoparticles; results demonstrated a better effect with ALA-loaded nanoparticles. Hence, these studies concluded that PLGA nanoparticles could provide a feasible ALA delivery system to improve the efficacy of ALA-PDT for the treatment of cSCC.

## PDT ENHANCERS

PDT is recommended for SCC in situ lesions, but currently, it is not recommended for invasive SCC. Therefore, to expand this narrow indication, certain agents were used in combination with PDT to enhance its efficacy. Agents like 5-fluorouracil,[15] resveratrol,[16] and vitamin D[30] were found effective as adjuvants for PDT to treat SCC (Table II).[31-33] In addition, mechanism-based studies were performed to identify the reasons of this enhanced effect. 5-fluorouracil and vitamin D led to tumor selective increases in protoporphyrin IX levels and enhanced cell death upon illumination.[15,30] 5-fluorouracil pretreated cancer cells (A431 and 4T1) showed a 3–6-fold induction of p53. The fact that A431 contains a mutant form of p53 and 4T1 completely lacks p53 did not prevent the development of the 5-fluorouracil effect. Hence, the study concluded that 5-fluorouracil enhances PDT of SCC via a p53-independent mechanism that increases protoporphyrin IX levels and promotes tumor cell death. Xin Zhang et al reported a study in which human HaCaT keratinocytes and human A431 epidermoid carcinoma cells treated with resveratrol and ALA-PDT showed increased expression of p-ERK, p-p38, p53, and caspase 3.[16] The authors further checked the involvement of the p38 pathway and found that inhibition of the p38 pathway could reduce the effect of resveratrol. Hence, it was concluded that resveratrol could enhance the effect of ALA-PDT against skin cancer cells through the p38-MAPK pathway. In contrast, Xinhong et al reported that inhibition of the MAPK pathway enhances the cytotoxic effect of ALA-PDT on cSCC.[31] Protoporphyrin IX is induced to a far greater extent by ALA-hexylester (hALA) than ALA in tumor cells.[34] So, Yanase et al in their study used hALA as a photosensitizer to treat SCC in nude mice.[33] The authors wanted to see if hyperthermia could enhance the anti-tumor effect of hALA-PDT. The mice were treated with hALA-PDT and hyperthermia, hALA-PDT alone, or hyperthermia alone. The results demonstrated a significant inhibitory effect on the tumor growth of SCC, showing a progression in the deep layer with the hALA-PDT and hyperthermia treatment compared with hALA-PDT or hyperthermia alone. Hence, it was concluded that hyperthermia enhances the antitumor effect of hALA-PDT in this SCC tumor model. Yet, another study demonstrated the enhanced effect of ALA-PDT by low dose methotrexate (~2 nM concentrations) in the human A431 SCC cell–implant mouse model.[32]

## DISCUSSION

In an attempt to expand the clinical application of PDT for cSCC, different methods have emerged. These attempts were made because noninvasive methods like imiquimod and 5-fluorouracil have failed to completely eradicate the disease. Likewise, superficial ablative techniques, such as electrodesiccation and curettage, cryotherapy, and $CO_2$ laser, do not allow histologic margin control and, thus, are generally inappropriate for treatment of invasive SCC. Chemotherapy and radiotherapy are associated with multiple adverse effects. Because of poor tissue selectivity, normal adjacent cells are also destroyed. The most acceptable and definitive treatment approach, surgery, is not applicable for all the patients.

Reasons for selecting PDT as an alternative treatment modality include its comparatively less invasiveness, high tissue selectivity, lack of serious adverse effects besides pain (which can be controlled with lidocaine injection and cold air), good cosmetic outcome, low recurrence rate, and, more importantly, safety for elderly patients and patients with comorbid conditions for which surgery is inappropriate. Despite all these advantages, PDT still needs some modifications so that it can be successfully used for invasive SCC. Inability of topically applied photosensitizers to reach deeper layers of skin renders it inadequate for the treatment of invasive SCC. Thus, studies have mainly focused on methods to increase drug permeation and achieve desired concentration at tumor site. PDT with intravenous injection of photosensitizer achieved complete response in 96% of treated patients,[17] but there is a risk for systemic skin photosensitivity. Patients should be advised to avoid sun exposure and remain indoors after intravenous photosensitizer injections, as was done in this study. But requiring such precaution might decrease patient compliance, and therefore, this concern needs to be addressed in the future whenever the intravenous route is chosen.

Pretreatment with AFL[10] and PBN[11] both provided excellent results, but there was a vast difference between the treatment sessions required. Treatment with AFL-MAL-PDT and PBN-ALA-PDT for microinvasive SCC and invasive SCC, respectively, provided complete remission in 2 and 9 sessions, respectively. At present, it is difficult to say that AFL requires less treatment sessions than PBN because other factors need to be considered as well, such as tumor depth and the photosensitizers

**Table II.** In vitro and in vivo studies demonstrating the enhancement of PDT when used with adjuvant to treat cutaneous SCC cells

| Study | Subject | Adjuvant | Photosensitizer | Light | Outcome | Proposed mechanism |
|---|---|---|---|---|---|---|
| Sanjaya Anand[15] | UVB-induced and human A431 cells implanted SCC mouse model | 2% 5-FU topical solution in UVB-induced and 300 mg/kg 5-FU intraperitoneal injection in implanted mouse model daily for 3 d | 5-ALA incubated for 4h | 100 $J/cm^2$ of 633 nm light | 5-FU pretreated cells led to 2.2—4-fold increase in PpIX levels, up to 5-fold increase in cell death | 5-FU pretreatment inhibits proliferation, enhances differentiation, and alters expression of key enzymes in heme biosynthetic pathway via p53-independent mechanism |
| Kishore R. Rollakanti[30] | UV-induced SCC mouse model | 3 $\mu$g/g vitamin D ointment daily for 3 d | 20% topical ALA solution for 4 h | - | Large and significant increase in PpIX signal intensity was seen in the vitamin D—preconditioned tumors | - |
| Xin Zhang[16] | Human A431 SCC cells | Resveratrol 0-100 mg/L | ALA | - | Res pretreatment resulted in significant inhibitory effect on A431 cell viability. A431 cell viability was inversely proportional to Res concentration. | Res could enhance the effect of ALA-PDT against SCC cells through p38/MAPK pathway |
| Xinhong GE[31] | human SCC cell line, SCL-1 | Inhibitors of MAPK signaling components (ERK, P38, and JNK) | 40 $\mu$g/mL ALA solution | Narrow-spectrum red light meter with wavelength 632 nm and power density 23 $mW/cm^2$ | Addition of inhibitors for ERK, p38, and JNK led to a more dramatic decrease in SCL-1 cell viability than seen with ALA-PDT alone | Enhanced effect of ALA-PDT on SCL-1 cells might occur by inhibition of ERK, p38, and JNK pathways |
| Shigeaki Yanase[33] | Implanted SCC mouse model | Hyperthermia with light dose of 437.5 $J/cm^2$ | 250 mg/kg hALA | Near infrared irradiator at a light dose of 50 $J/cm^2$ | Tumor growth rates on day 12 were 97.10% in hyperthermia-treated, 67.55% in hALA-PDT—treated, and 33.90% in PDT + hyperthermia—treated cells | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sanjay Anand[32] | Implanted human A431 cell mouse model | 2 mg/kg intramuscular MTX daily for 1 or 3 d | 75 mg/kg ALA intramuscular | Light-emitting diodes with a peak output at 395 nm and bandwidth of 14 nm | SCC cells accumulated PpIX to higher (10–12-fold) relative levels than NHEK at MTX concentration of 0.01 mg/L | PpIX enhancement is mediated by upregulation of coproporphyrinogen oxidase and stable or slightly decreased expression of ferrochelatase |

*5-ALA*, 5-Aminolevulinic acid; *5-FU*, 5-fluorouracil; *ALA*, aminolevulinic acid; *ERK*, extracellular signal–regulated kinase; *hALA*, aminolevulinic acid hexylester; *HT*, hyperthermia; *JNK*, c-Jun N-terminal kinase; *MAPK*, mitogen-activated protein kinase; *MTX*, methotrexate; *NHEK*, normal human epidermal keratinocytes; *PDT*, photodynamic therapy; *PpIX*, protoporphyrin IX; *Res*, Resveratrol; *SCC*, squamous cell carcinoma; *SCL*, squamous carcinoma cell line; *UV*, ultraviolet.

used. Hence, further studies comparing the effect of AFL with PBN are needed. Clinicians do not randomly choose AFL or PBN because PBN has an advantage over AFL in terms of cost and experience. PBN is far cheaper than AFL and easy to use. PBN does not require an experienced handler, whereas AFL requires skilled personnel to use and adjust laser parameters. Another important aspect that needs to be considered in future studies is if pretreatment with AFL or PBN can be avoided with intralesional photosensitizers. The study showing complete clearance of infiltrative SCC by 1 session of intralesional ALA-PDT is quite interesting. However, whether PBN can be replaced by intralesional photosensitizers to decrease treatment session numbers or not needs to be answered by future studies.

Nanotechnology is widely used in the field of medicine, including for the prevention of skin cancers with sunscreen production and the treatment of skin cancers. However, the study reporting its use for cSCC is limited. It would be inappropriate to draw any conclusions with these limited studies. Moreover, these studies are limited to in vitro and in vivo studies. Nanomedicine seems to have a long way to go to bring PDT into clinical application for the treatment of cSCC. Nonetheless, adjuvant therapy in combination with PDT might expand the clinical application of PDT in the near future. A number of agents, such as 5-fluorouracil, resveratrol, vitamin D, and methotrexate, have enhanced the efficacy of PDT for SCC cells in vitro and in vivo.[15,16,30,32] Mechanism-based studies have proposed quite logical reasons for this enhancement, providing enough evidence and satisfaction to start studies in humans. Moreover, these agents and PDT are already in use in clinics. In addition, studies on animals have shown that these agents are safe to be used in combination with PDT for SCC. Hence, human studies of these agents in combination with PDT are expected in the near future. Also, long-term studies with close follow-up are required to determine its safety.

From the studies thus far, it can be concluded that either PDT with intralesional photosensitizers or topical PDT after pretreatment with PBN or AFL can offer an acceptable alternative to surgery in selected patients. Because the European Dermatology Forum guidelines does not currently recommend PDT for invasive SCC,[35] PDT should not be chosen as a first-line treatment. Hence, optimization of treatment protocols and larger studies that justify the results are highly desirable.

## REFERENCES


1. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. *J Am Acad Dermatol*. 2013;68:957-966.
2. Rogers HW, Weinstock MA, Harris AR, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol*. 2010;146:283-287.
3. Morton C, Szeimies RM, Sidoroff A, et al. European Dermatology Forum guidelines on topical photodynamic therapy. *Eur J Dermatol*. 2015;25(4):296-311.
4. Henderson BW, Dougherty TJ. How does photodynamic therapy work? *Photochem Photobiol*. 1992;55:145-157.
5. Morton CA, Horn M, Leman J, et al. A randomized, placebo-controlled, European study comparing MAL-PDT with cryotherapy and 5-fluorouracil in subjects with Bowen's disease. *Arch Dermatol*. 2006;142:729-735.
6. Lehmann P. Methyl aminolaevulinate-photodynamic therapy: a review of clinical trials in the treatment of actinic keratosis and nonmelanoma skin cancer. *Br J Dermatol*. 2007;156:793-801.
7. Calzavara-Pinton PG, Venturini M, Sala R, et al. Methylaminolaevulinate- based photodynamic therapy of Bowen's disease and squamous cell carcinoma. *Br J Dermatol*. 2008;159:137-144.
8. Truchuelo M, Fernandez-Guarino M, Fleta B, et al. Effectiveness of photodynamic therapy in Bowen's disease: an observational and descriptive study in 51 lesions. *J Eur Acad Dermatol Venereol*. 2012;26:868-874.
9. Cavicchini S, Serini SM, Fiorani R, et al. Long-term follow-up of metil aminolevulinate (MAL)-PDT in difficult-to-treat cutaneous Bowen's disease. *Int J Dermatol*. 2011;50:1002-1005.
10. Choi SH, Kim KH, Song KH. Effect of methyl aminolevulinate photodynamic therapy with and without ablative fractional laser treatment in patients with microinvasive squamous cell carcinoma: a randomized clinical trial. *JAMA Dermatol*. 2017;153(3):289-295.
11. Wang P, Zhang L, Zhang G, et al. Successful treatment of giant invasive cutaneous squamous cell carcinoma by plum-blossomneedle assisted photodynamic therapy sequential with imiquimod: case experience. *Photodiagnosis Photodyn Ther*. 2018;21:393-395.
12. Sotiriou E, Apalla Z, Ioannides D. Complete resolution of a squamous cell carcinoma of the skin using intralesional 5-aminolevulinic acid photodynamic therapy intralesional PDT for SCC. *Photodermatol Photoimmunol Photomed*. 2010;26:269-271.
13. Wang X, Shi L, Tu Q, et al. Treating cutaneous squamous cell carcinoma using 5-aminolevulinic acid polylactic-co-glycolic acid nanoparticle-mediated photodynamic therapy in a mouse model. *Int J Nanomedicine*. 2015;10:347-355.
14. Shi L, Wang XL, Zhao F, et al. In vitro evaluation of 5-aminolevulinic acid (ALA) loaded PLGA nanoparticles. *Int J Nanomed*. 2013;8(1):2669-2676.
15. Anand S, Rollakanti KR, Brankov N, Brash DE, Hasan T, Maytin EV. Fluorouracil enhances photodynamic therapy of squamous cell carcinoma via a p53-independent mechanism that increases protoporphyrin IX levels and tumor cell death. *Mol Cancer Ther*. 2017;16(6):1092-1101.
16. Zhang X, Liu X, Kang S, Liu C, Hao Y. Resveratrol enhances the effects of ALA-PDT on skin squamous cells A431 through p38/MAPKsignaling pathway. *Cancer Biomark*. 2018;21(4):797-803.
17. Kubler AC, de Carpentier J, Hopper C, Leonard AG, Putnam G. Treatment of squamous cell carcinoma of the lip using Foscan-mediated photodynamic therapy. *Int J Oral Maxillofac Surg*. 2001;30:504-509.
18. Fargnoli MC, Kostaki D, Piccioni A, Di Stefani A, Peris K. Photodynamic therapy for the treatment of microinvasive squamous cell carcinoma of the lowerlip: a case report. *G Ital Dermatol Venereol*. 2015;150(3):331-335.
19. Li Q, Gao T, Long HA, Ujiie H. Clearance of a thick invasive squamous cell carcinoma after multiple treatments with topical photodynamic therapy. *Photomed Laser Surg*. 2010;28(5):703-706.
20. Rossi R, Puccioni M, Mavilia L, et al. Squamous cell carcinoma of the eyelid treated with photodynamic therapy. *J Chemother*. 2004;16(3):306-309.
21. Qiao L, Xu C, Li Q, et al. Photodynamic therapy activated STAT3 associated pathways: targeting intrinsic apoptotic pathways to increase PDT efficacy in human squamous carcinoma cells. *Photodiagnosis Photodyn Ther*. 2016;14:119-127.
22. Qiao L, Mei Z, Yang Z, Li X, Cai H, Liu W. ALA-PDT inhibits proliferation and promotes apoptosis of SCC cells through STAT3 signal pathway. *Photodiagnosis Photodyn Ther*. 2016;14:66-73.
23. Wang H, Li J, Lv T, Tu Q, Huang Z, Wang X. Therapeutic and immune effects of 5-aminolevulinic acid photodynamic therapy on UVB-induced squamous cell carcinoma in hairless mice. *Exp Dermatol*. 2013;22(5):362-363.
24. Wang X, Ji J, Zhang H, et al. Stimulation of dendritic cells by DAMPs in ALA-PDT treated SCC tumor cells. *Oncotarget*. 2015;6(42):44688-44702.
25. Juarez AV, Sosa Ldel V, De Paul AL, et al. Riboflavin acetate induces apoptosis in squamous carcinoma cells after photodynamic therapy. *J Photochem Photobiol B*. 2015;153:445-454.
26. Sharma KV, Davids LM. Hypericin-PDT-induced rapid necrotic death in human squamous cell carcinoma cultures after-multiple treatment. *Cell Biol Int*. 2012;36(12):1261-1266.
27. Gilaberte Y, Milla L, Salazar N, et al. Cellular intrinsic factors involved in the resistance of squamous cell carcinoma to photodynamic therapy. *J Invest Dermatol*. 2014;134(9):2428-2437.
28. Li Q, Gan L, Tao H, et al. The synthesis and application of heparin-based smart drug carrier. *Carbohydr Polym*. 2016;140:260-268.
29. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Adv Drug Deliv Rev*. 2013;65:71-79.
30. Rollakanti KR, Anand S, Davis SC, Pogue BW, Maytin EV. Noninvasive optical imaging of UV-induced squamous cell carcinoma in murine skin: studies of early tumor development and vitamin D enhancement of protoporphyrin IX production. *Photochem Photobiol*. 2015;91(6):1469-1478.
31. Ge X, Liu J, Shi Z, et al. Inhibition of MAPK signaling pathways enhances cell death induced by 5-aminolevulinic acid-photodynamic therapy in skin squamous carcinoma cells. *Eur J Dermatol*. 2016;26(2):164-172.
32. Anand S, Honari G, Hasan T, Elson P, Maytin EV. Low-dose methotrexate enhances aminolevulinate-based photodynamic therapy in skin carcinoma cells in-vitro and in vivo. *Clin Cancer Res*. 2009;15(10):3333-3343.
33. Yanase S, Nomura J, Matsumura Y, Kato H, Tagawa T. Hyperthermia enhances the antitumor effect of photodynamic therapy with ALA hexyl ester in a squamous cell carcinoma tumor model. *Photodiagnosis Photodyn Ther*. 2012;9(4):369-375.

34. Yanase S, Nomura J, Matsumura Y, et al. Enhancement of the effect of 5-aminolevulinic acid-based photodynamic therapy by simultaneous hyperthermia. *Int J Oncol*. 2005;27:193-201.

35. Morton C, Szeimies RM, Sidoroff A, et al. European Dermatology Forum. European Dermatology Forum guidelines on topical photodynamic therapy. *Eur J Dermatol*. 2015;25(4):296-311.