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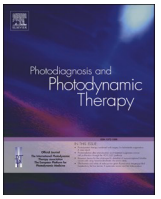

Ten-years follow-up of photodynamic therapy for non-melanoma skin cancer: Outcomes and prognostic factors

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ABSTRACT

Non-melanoma skin cancers (NMSCs), primarily basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are becoming increasingly prevalent. In 2021, the global incidence reached 6.64 million cases, with Australasia and North America exhibiting the highest rates and middle socio-demographic regions experiencing the fastest growth. Photodynamic therapy (PDT) presents a minimally invasive treatment alternative. This study assessed the 10-year long-term efficacy of PDT using methyl aminolevulinate (MAL) for superficial BCC (sBCC)and nodular BCC (nBCC), and 3 patinets with in-situ SCC, after the final PDT treatment. The original group included 64 patients (32 males and 32 female) with 82 histologically confirmed NMSC lesions were initially treated. After 2 months, PDT showed high efficacy: 94.8 % complete response in untreated nBCC, 100 % in recurrent nBCC with dermal invasion after surgery, 92.3 % in sBCC and 100 % in SCC in situ. In contrast, nBCC with perineural invasion after surgery showed no response. Based on these outcomes, 48 patients with 66 lesions were selected for long-term follow-up, and 45 of them completed full clinical follow-up to 10 years. These comprised 11 sBCC, 45 untreated nBCC, 4 nBCC with dermal invasion after surgery, 3 nBCC with perineural invasion after surgery, and 3 in situ SCC. No invasive squamous cell cancers were followed. For sBCC, 72.7 % of lesions achieved complete clearance after the last PDT session, with a 10-year tumor-free survival rate of 70 %. Previously untreated nBCC showed an 80 % complete response after the final PDT session (78 % survival at 5 years), while recurrent dermal-invasive nBCC demonstrated a 100 % complete response. However, PDT proved ineffective for nBCC with perineural invasion (66.7 % recurrence). In-situ SCC lesions achieved a 100 % complete response with sustained 10-year tumor-free survival. Multivariate Cox regression analysis identified age >65 years and tumor thickness >2 mm as factors significantly associated with increased recurrence risk, considering demographic data, lesion characteristics, and PDT treatment parameters. PDT demonstrates notable effectiveness for superficial and nodular BCC, especially with tailored protocols (4 sessions for nodular, 2 for superficial). However, SCC treated with PDT is not a standard therapy for invasive disease and should be undertaken in a clinical trial which requires vigilant long-term surveillance for potential recurrence. PDT is not advised for nodular BCC with perineural invasion. Age and tumor thickness are key prognostic indicators.

1. Introduction

Non-melanoma skin cancer (NMSC) is the most common human malignancy worldwide, and its incidence has been steadily increasing over recent decades [1]. Basal cell carcinoma (BCC) is the most frequent subtype, followed by squamous cell carcinoma (SCC), and together they account for approximately 99 % of all NMSC cases [2]. Global data indicate a significant rise in the age-standardized incidence rate of NMSC, from 54.08 per 100,000 individuals in 1990 to 79.10 per 100.000 in 2019, with an estimated annual percentage change of 1.78 % [3]. Factors contributing to the global increase include demographic changes, greater ultraviolet (UV) exposure, and improved detection. Despite its high incidence, epidemiological data for NMSC remain incomplete in many regions, largely due to underreporting [1]. In

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Ecuador, the National Tumor Registry reports skin cancer as the second most frequent cancer among men and the third among women. Ecuador's equatorial location and its high-altitude results in intense UV radiation exposure, significantly elevating skin cancer risk [4]. These factors underscore the urgent need for novel therapeutic strategies that maximize efficacy while minimizing adverse effects.

Photodynamic therapy (PDT), a clinically attractive cancer treatment, has gained significant attention due to its high selectivity, minimally invasive nature, and limited side effects [5]. PDT is an oxygen-dependent technique that selectively destroys sensitized tissues upon exposure to a light source with an appropriate wavelength. PDT treats various oncological, dermatological, ophthalmological, and infectious diseases [5,6].

One of the most used photosensitizers for treating BCC is the endogenous porphyrin precursor Protoporphyrin IX, specifically 5-aminolevulinic acid (ALA) and Methyl aminolevulinate (MAL). ALA administration enhances cellular heme biosynthesis, resulting in mitochondrial accumulation of protoporphyrin IX (PpIX) due to regulation at the ALA synthesis and conversion level [6]. The overproduction of PpIX following ALA administration is more pronounced in tumor cells than in surrounding healthy tissues, offering a degree of selectivity for neoplastic tissues due to their accelerated metabolism [7]. Similarly, MAL, a methyl-esterified derivative of ALA, enhances lipophilicity and tissue penetration. Studies have shown that MAL penetrates up to 2 mm into the tissue, whereas ALA reaches only 1 mm [8].

This study aims to evaluate the use of methyl aminolevulinate (MAL) as a photosensitizer for the treatment of both superficial and deep (>2 mm) basal cell carcinomas (BCC), using the LINCE® device. This device emits red light at 635 nm (100 mW/cm^2) for photodynamic therapy and blue light at 400 nm for PpIX fluorescence detection during diagnosis. For this study, based on histopathological findings assessing tumor thickness, lesions were classified as previously untreated nodular BCC, recurrent nodular BCC invading the dermis after surgery and invading perineural after surgery, and superficial BCC.

2. Patients and methods

This investigation was conducted in two sequential phases. Phase 1 (early outcome) included 62 patients with 82 histologically confirmed lesions—basal cell carcinoma (BCC) and in-situ squamous cell carcinoma (SCC)—treated with methyl-aminolevulinate photodynamic therapy (MAL-PDT) at two hospitals: 14 patients were managed at the Dermatological Hospital Gonzalo González (DHGG) and 48 patients at Hospital Carlos Andrade Marín (HCAM). Phase 2 (long-term follow-up) was necessarily limited to the 48 HCAM patients, because DHGG discontinued public services during the study period and could not support 10-year surveillance. This final cohort comprised 23 men (38 lesions) and 25 women (28 lesions), ranging in age from 36 to 92 years.

2.1. Treatment protocol for superficial BCCs

For superficial BCCs (up to 2 mm of tumor thickness), the lesion was first cleansed with saline solution to remove any surface debris. Subsequently, a thin layer of 20 % methyl aminolevulinate (MAL) was applied and covered with plastic film and aluminum foil. After an incubation period of 3 hours, fluorescence detection was performed using the LINCE® device, which features a 400 nm blue light source. This step allowed visualization of protoporphyrin IX (PpIX) accumulation within the tumor. The Institute of Physics at the University of São Paulo - São Carlos, Brazil, provided the device.

Following this diagnostic phase, red light at 635 nm and a power density of 100 mW/cm^2 delivered by the LINCE® system was applied to the lesion. This illumination induces the generation of reactive oxygen species (ROS), which cause oxidative damage to tumor cells, ultimately leading to cell death through apoptosis or necrosis. The red-light exposure lasted for 20 min. At the end of the session, PpIX fluorescence was reassessed to confirm its complete photobleaching during irradiation.

An occlusive dressing was then applied to the treated area, and patients were advised to avoid sun exposure on the site for 7 days to prevent photosensitization of healthy skin due to residual PpIX. The same treatment protocol was repeated one week later.

Thirty days after the final PDT session, a biopsy of the treated area was performed. In addition, a dermatoscopic examination was carried out to identify any atypical regions exhibiting signs suggestive of residual BCC, such as telangiectasia or bluish or dark pigmentation.

2.2. Treatment protocol for nodular BCCs and SCC

For nBCC and SCC (with varying thickness), the same procedure described above for superficial lesions was followed, with one key modification: prior to applying 20 % MAL, a deep curettage was performed to remove as much tumoral tissue as possible. Thirty days after the second PDT cycle, a biopsy of the treated area was performed. If histopathological analysis revealed the presence of residual tumor cells, two additional PDT cycles were administered. If no tumor cells were detected, the patient was considered to have completed treatment.

Following completion of either two or four PDT cycles, a final biopsy and dermatoscopic evaluation were conducted to assess treatment outcomes. All patients were monitored at 45 days and 4 months post-treatment through clinical and dermatoscopic examination. Thereafter, long-term follow-up continued for up to 10 years for all BCC cases and SCC, regardless of subtype, depending on each patient's availability and clinical evolution.

During this period, regular evaluations were conducted to detect any signs of recurrence or new lesion development, ensuring comprehensive monitoring of treatment outcomes over time.

2.3. Statistical analysis

This study included 48 patients with 66 histologically confirmed BCC lesions, each treated with standardized PDT and followed prospectively for up to 10 years. All patients were included in the survival analysis after treatment completion. Follow-up duration ranged from 1 to 10 years, depending on patient availability and clinical evolution. For recurrence-free patients, follow-up was censored at their last recorded visit.

Descriptive statistics summarized patient demographics, tumor characteristics (type, thickness, location), number of PDT cycles, and treatment outcomes. Tumor-free survival was estimated using Kaplan-Meier (KM) survival curves, and survival distributions were compared using the log-rank test. Median RFS and recurrence rates at defined time points (e.g., 1, 3, 5 and years) were reported, following standard practice in long-term oncology outcomes research.

Univariate and multivariate Cox proportional hazard regression models were used to identify factors associated with recurrence risk, including covariates such as age, sex, BCC subtype (superficial vs nodular), lesion thickness, anatomical location, and number of PDT sessions.

All statistical analyses were conducted using R Studio with a p-value <0.05, considered statistically significant to compare the inferential results between the participants with different variables, and descriptive analyses were expressed as frequencies and percentages. The Pearson Chi-square analysis was then used to examine the data further. This test determines whether statistically significant differences exist between the expected and observed frequencies in different categories.

3. Results

A total of 62 patients with 82 histologically confirmed lesions entered the early-outcome phase of the study. The cohort comprised 30 men (48.4 %) and 32 women (51.6 %), aged 34–92 years (mean ± SD = 71.2 ± 4.0 years); 88 % had Fitzpatrick skin phototypes II–III (Table 1).

Table 1
Demographic data, lesion characteristics, PDT reactions in BCC and SCC.

			nBCC							
	sBCC		Without previous treatment		after surgery Inv. Dermis		after surgery Inv. Neural		SCC in situ	
	N = 13		*N* = 40		*N* = 4		*N* = 3		*N* = 4	
	n	%	n	%	n	%	n	%	n	%
Demographic data										
Sex										
Female	7	53,8	19	48	3	75	–	–	3	75
Male	6	46,2	21	52	1	25	3	100	1	25
Mean age (years), SD	68.14	12.4	74.12	10.6	70.2	0.29	67.3	28.6	76.6	5.3
Skin phototypes										
I	–	–	1	2.5						
II	8	61.5	17	42.5	2	50	1	33	1	25
III	4	30.8	20	50	2	50	2	67	2	50
IV	1	7.7	1	2.5					1	25
VI	–	–	1	2.5						
Lesion characteristics										
Mean lesion/field size (cm2), SD	3.39	4.53	1.31	1.69	7.75	7.27	8.45	2.19	1.96	1.79
Median tumor thickness (mm2), IQR	0.65	0.38–1.4	1.5	1.15–1.78	1.7	1.32–1.9	2.85	2.28–3.42	0.55	0.48–0.63
Location										
Head/neck	0	0	2	3.4	1	25				
Face	9	69.2	51	87.9	3	75	3	100	2	50
Back	3	23.1	2	3.4	–	–			–	–
Extremities	1	7.7	3	5.2	–	–			2	50
PDT treatment										
N session, SD	2.36	0.81	3.84	1.71	2.5	1	4.67	1.15	2.67	1.15
Median pain score, IQR	3	2–3	2	1–3	1	1–1.5	3	3–4	1	1–2.5

At the 2-month post-treatment biopsy, MAL-PDT showed high efficacy across tumor subtypes: previously untreated nodular BCC achieved a 94.8 % complete response (no tumor), while all recurrent nodular BCCs with dermal invasion responded fully. However, none of the perineural-invading recurrent nodular BCCs showed response. Among superficial BCCs, 92.3 % responded completely, and all in situ SCC lesions showed 100 % complete response. (Fig. 1) Because the Dermatological Hospital Gonzalo González discontinued public services, only the 48 patients (66 lesions) treated at Hospital Carlos Andrade Marín (HCAM) could be monitored beyond 3 months. This HCAM subset therefore constitutes the long-term cohort and provides the exclusive data source for all Kaplan–Meier survival curves and multivariable Cox regression analyses presented in this manuscript.

10 years follow-up cohort comprised 48 patients with 66 histologically confirmed skin lesions, primarily diagnosed as BCC. 11 lesions were classified as superficial BCC (sBCC) and 52 as nodular BCC (nBCC). nBCC was further subdivided into three groups based on treatment history. The first group consisted of untreated nBCC, with 45 nodular cases. The second group included recurrent nBCC after surgery that invaded the dermis (nBCC), accounting for 4 nodular lesions. The third group consisted of recurrent nBCC after surgery invaded perineural with 3 lesions. This categorization allowed for an in-depth understanding of the tumor's response to PDT based on previous treatment and invasion depth. Additionally, 3 lesions were identified in patients as in situ SCC, which were included as a separate subgroup due to their distinct histological behavior and treatment considerations.

Fig. 2-b presents the outcomes of patients post-PDT, categorized by initial tumor type. The "no tumor" group represents patients where the treated lesion showed no evidence of cancer at the follow-up assessment. The "recurrence" group includes patients whose treated lesion initially responded to PDT but subsequently showed a reappearance of the tumor. Finally, the "gave up" group consists of patients who discontinued treatment after only one PDT session. The percentage of treated patients within each outcome category is also depicted in this

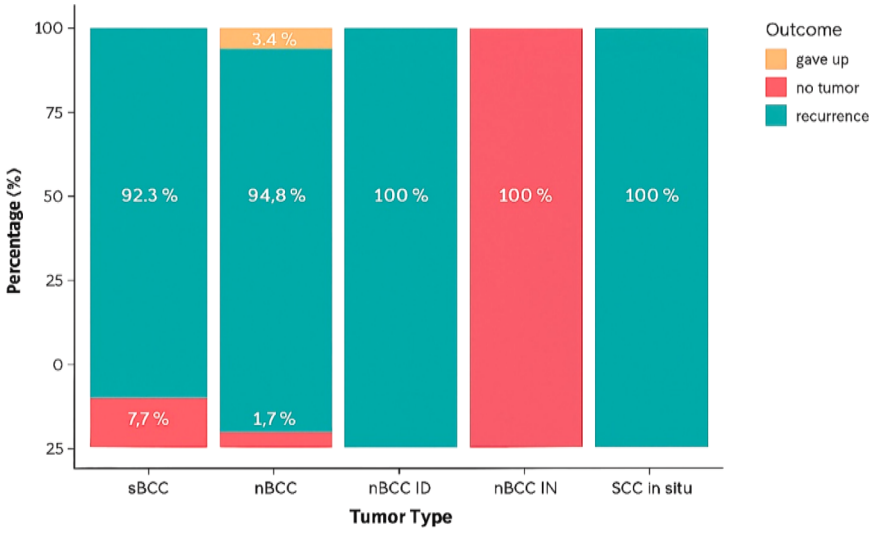

Fig. 1. Early (2-month) histological clearance in the full 62-patient cohort (HCAM and DHGG).

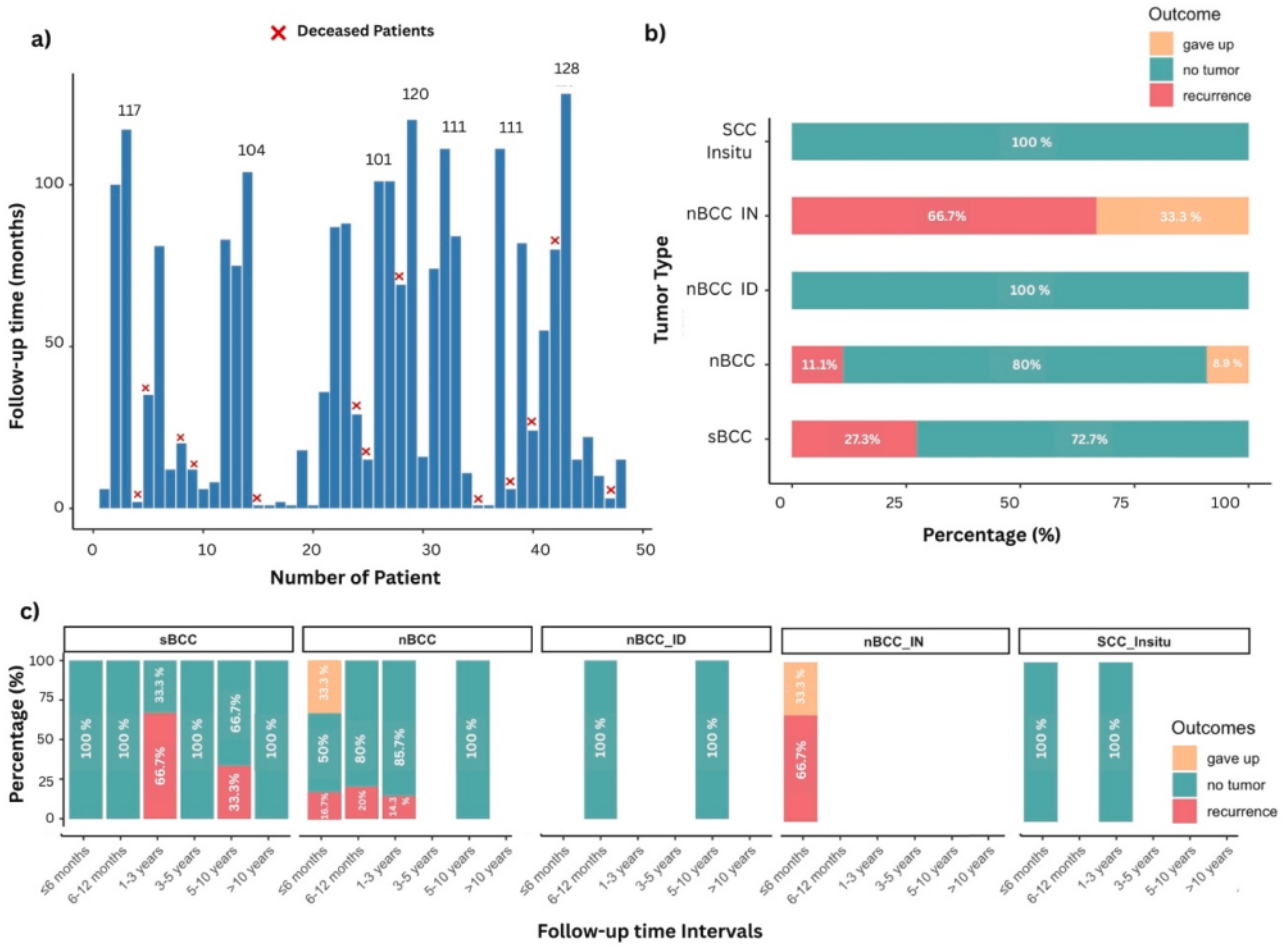

Fig. 2. a) Follow-up duration (months) for 45 patients. b) Treatment outcomes (%) by tumor type in the 45 patients selected for long-term follow-up. c) Follow-up intervals stratified by tumor type and treatment outcome (%).

figure.

Of the 11 sBCC-treated lesions, the most common tumor location was face (64 %) and back (27 %). The median histological tumor thickness was 1.5 mm, and the median lesion size ± SD was 3.39± 4.53 cm^2. The mean number of PDT sessions was 2.36 ± 0.81. The median pain score reported for maximal pain experienced during PDT was 3. (Table 1) A complete clinical response (no tumor) was observed in 72.7 % of the lesions, while 27.3 % experienced recurrence during follow-up. Importantly, all lesions remained tumor-free in the short term (≤6 months and 6–12 months). However, recurrences were observed between 1 and 3 years and again between 5 and 10 years after treatment, emphasizing the importance of long-term follow-up even in lesions that initially responded well. (Fig. 2-b). In Fig. 2-c, for sBCC, recurrences were distributed across three intervals: 1–3 years, 3–5 years, and 5–10 years. This suggests that although early recurrences are common, some tumors may remain dormant or minimally active for extended periods before reappearing. Therefore, even superficial tumors require continuous and extended monitoring to detect late recurrences.

The KM survival curve for sBCC is shown in Fig. 3-a and reveals an initial 100 % tumor-free survival probability within the first year. A gradual decline began after the 12-month mark, corresponding with observed recurrences. By the 10th year, the survival probability decreased to approximately 70 %, consistent with the documented recurrence rate. The risk table, which presents the number of patients still under observation (at risk) at different time points, shows a rapid decrease in cases beyond the 5-year mark. This decline indicates that fewer patients remained in follow-up after this period, which may affect the reliability and accuracy of the survival curve estimates in the later years. These findings support the effectiveness of PDT for sBCC in the short term but highlight the potential for recurrence in a subset of cases [9,10].

A total of 45 nBCC lesions without previous treatment were managed with PDT. The most common lesion site was the face (87 %). The median histological tumor thickness was 1.5 mm, and the median lesion size was 1.31± 1.6. The mean number was 3.84±1.7, with a median pain score of 2. (Table 1)

A complete clinical response after PDT sessions was observed in 80 % of cases, while 11.1 % of patients experienced recurrence and 8.9 % discontinued treatment (gave up). The stratified analysis revealed that lesions with tumor thickness ≤ 2 mm responded favorably to 2 sessions, whereas those with thickness ≥ 2 mm required 4 sessions or more to achieve clearance. (Table 1) Even patients undergoing extended PDT (8–12 sessions) due to larger tumors showed a complete response. This finding highlights the flexibility and effectiveness of PDT in treating lesions with different tumor burdens, demonstrating that the therapy can be successfully adapted in terms of session number according to tumor size and severity [9,10].

Most recurrences occurred between 1 and 3 years, with many tumor-free outcomes during the first 12 months (Fig. 2-c). KM survival analysis showed a progressive decrease in tumor-free survival beginning after the first year, aligning with the recurrence window. At 5 years, the survival rate was approximately 78 %, consistent with the clinical recurrence data [10]. (Fig. 3-a)

Four lesions with recurrent post-surgical nodular BCC (nBCC ID) exhibiting dermal invasion were treated. Dermal invasion was defined as histological extension beyond the papillary dermis into the reticular

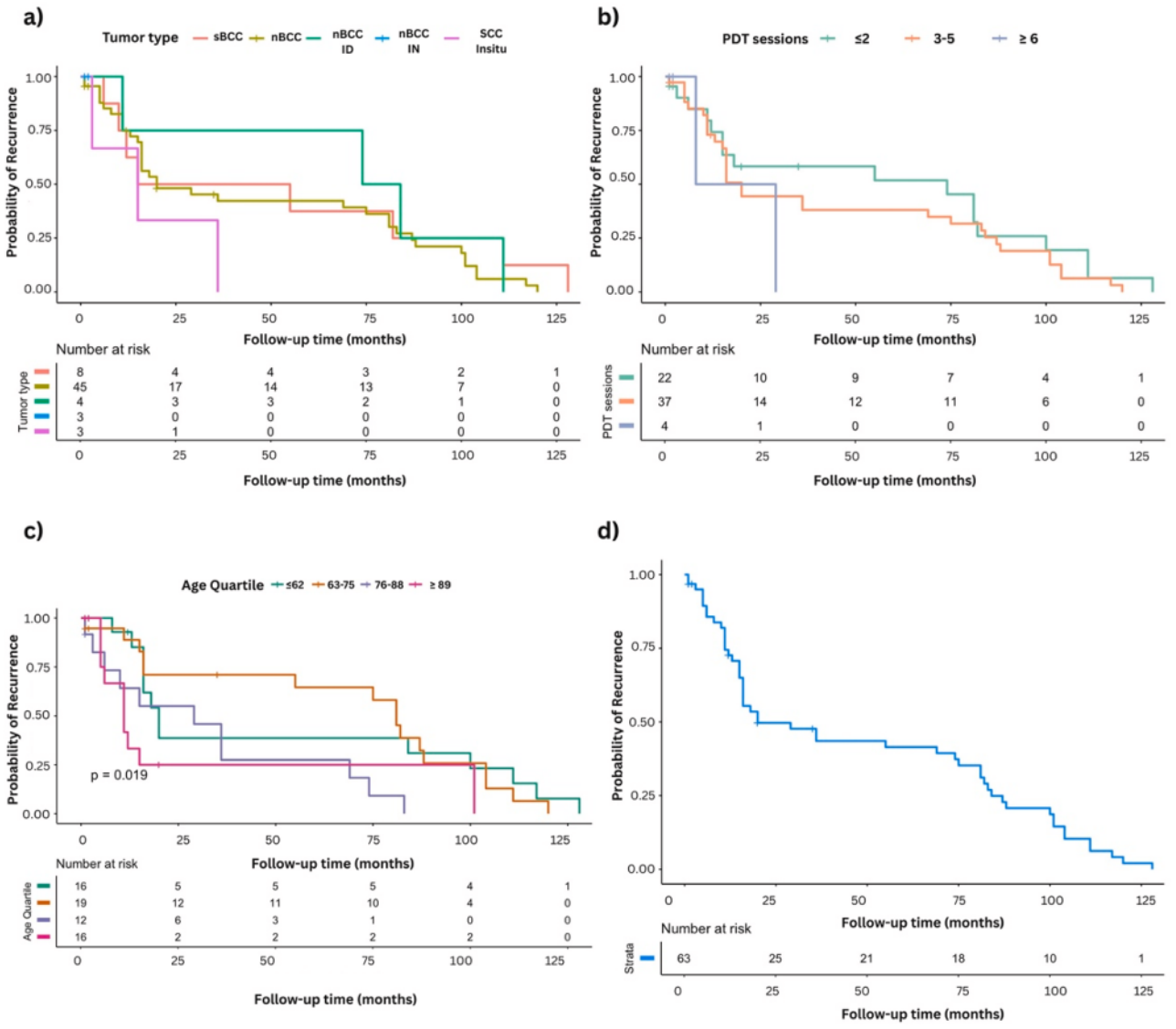

Fig. 3. Kaplan-Meier survival curves over a 10-year follow-up period based on: a) tumor type, b) number of PDT sessions, c) age quartiles, d) Overall Kaplan-Meier survival curve for all patients over 10 years.

dermis without perineural involvement. All nodular BCCs in this study, by definition, invaded at least the superficial dermis; the 'dermal invading' category therefore refers to lesions that extended deeper than 2 mm but showed no nerve sheath infiltration. This distinction allowed us to compare true deep-dermal disease with the perineural-invading subgroup, which represents a higher biological risk

The face was the predominant site (75 %) in nBCC ID. The median histological tumor thickness was 1.7 mm, and the median lesion size was 7.75 cm^2. The mean number of PDT sessions was 2.5 ± 1, and the median pain score was 1. (Table 1) Despite the invasive histology, all patients (100 %) responded completely to PDT, suggesting that PDT remains a viable option in cases of dermal invasion without deeper structure involved. The Kaplan-Meier curve maintained full survival probability for this group throughout follow-up, although the small sample size may limit generalization. (Fig. 3-a)

Conversely, three lesions of perineural invasion post-surgery were treated, all located on the face. These tumors had a significantly higher median thickness of 2.85 mm, and the mean lesion size was 8.45 ± 2.19 cm^2. The mean number of PDT sessions was 4.67±1.15, and the median pain score was 3, with one case reporting very high discomfort (Table 1). Unfortunately, PDT proved ineffective in this subgroup: 66.7 % of patients experienced recurrence, while 33.3 % discontinued treatment (Fig. 2-b). These results highlight the limitations of PDT in treating aggressive nBCC subtypes with perineural involvement. The KM curve for this group showed a steep drop in survival probability within the first two years, consistent with rapid recurrence or dropout. In Fig. 3-c, all outcomes for this group occurred within the first 6 months, highlighting the critical importance of early monitoring and intervention. Specifically, 66.7 % of lesions presented recurrence, and 33.3 % represented patients who gave up on the treatment, possibly due to side effects, lack of perceived efficacy, or the burden of adhering to the treatment regimen.

For SCC in situ, three lesions were treated with PDT. The location of lesions was the face (100 %), and the median histological tumor thickness was 0.55 mm. The mean lesion area was 1.96 ± 1.79 cm^2. Patients underwent an average of 2.67±1.15 PDT sessions, and the median pain score during treatment was 1, indicating low treatment-associated discomfort. A complete response was achieved in all lesions (100 %), as no recurrence or treatment discontinuation was observed after the last PDT session (Fig. 2-b). According to follow-up interval outcomes, 100 % of the SCC lesions remained tumor-free during all time intervals from 0–6 months, through 1–3 years, up to more than 10 years, showing

long-term sustained response to PDT. (Fig. 2-c). During follow-up, no recurrences were documented; however, two lesions were censored before 30 months because their carriers were lost to follow-up, leaving a single lesion observed through the end of the study. This early censoring is reflected in the Kaplan–Meier curve (Fig. 3-a, magenta line), which shows step-downs driven by the shrinking number at risk rather than by tumor relapse. Given this very small sample, the SCC-is curve is presented only to illustrate long-term durability in appropriately selected cases and is not included in statistical comparisons with the larger BCC cohorts.

Table 2 provides a statistical comparison between the initial tumor type and the corresponding long-term outcomes observed in the study. Chi-square (x^2) analysis showed no statistically significant difference in event-free survival between tumor types ($x^2 = 3.5$, df = 4, $p = 0.5$). This suggests that the different tumor type (specifically BCC and SCC and their subtypes) did not have demonstrably different rates of recurrence or progression within the study's timeframe and sample size. The chi-square statistic of 3.5 with 4 degrees of freedom yields a p-value of 0.5, which is well above the conventional significance level of 0.05, thus failing to reject the null hypothesis of no association between tumor type and event-free survival.

Despite the lack of statistical difference, clinically relevant patterns were observed in the log-rank analysis (Table 2). For sBCC group, we observed 11 events of either no tumor, recurrence, or treatment gave up, compared to an expected 9.98 events. This group contributed moderately to the overall chi-square statistic ($(O–E)^2/E = 0.40$), suggesting a slightly lower-than-expected event rate (recurrence or treatment dropout). Although the group size was small ($n = 11$), the survival trend for sBCC was better than expected, but the deviation was not statistically significant.

For nBCC ($N = 45$), 36 events were observed compared to 34.57 expected. This group closely followed the overall survival pattern ($(O–E)^2/E = 0.059$), contributing negligibly to the total chi-square value. In contrast, for nBCC with dermal invasion post-surgery, 4 events were observed versus 5.09 expected; for nBCC with perineural invasion post-surgery, 0 events were observed versus 0.095 expected. The chi-square contribution for these cases was 0.23 and 0.095, respectively, with small values indicating no significant survival difference. In SCC in situ ($N = 3$), 3 events occurred compared to only 1.27 expected. This group had the most significant contribution to the chi-square ($(O–E)^2/E = 2.36$), reflecting a higher-than-expected recurrence/dropout rate, although not statistically significant due to the small sample size. (Table 2).

The overall KM survival curve described in Fig. 3-d reflects the cumulative tumor-free survival of all 48 patients with 66 lesions treated with PDT. Despite the absence of statistically significant differences in survival probability based on the number of PDT sessions (Fig. 3-b) (log-rank $x^2 = 1.9$, $p > 0.5$), the global curve reveals that most recurrences occur between 1- and 5 years post-treatment, underscoring the importance of long-term clinical follow-up. The curve plateaus after year 10, suggesting durable responses for lesions that remained tumor-free beyond this threshold.

When comparing the global curve to KM survival curves stratified by tumor type (Fig. 3-a), distinct patterns emerge. SCC In situ and nBCC with dermal invasion demonstrated 100 % long-term tumor-free survival, with no recurrences observed during the follow-up period. In contrast, sBCC and nBCC without previous treatment displayed progressive declines in survival probability, particularly after the first year, corresponding to recurrence clusters observed between the 1–3 year and 5–10-year intervals (Fig. 2-c). Notably, nBCC with perineural invasion exhibited the poorest outcome, with no sustained responses< 66.7 % of lesions recurred and 33.3 % of patients discontinued treatment.

These comparative results are further supported by the log-rank analysis across tumor types (log-rank $x^2 = 3.5$, $p > 0.5$), which, although not statistically significant, highlight clinically meaningful survival differences-especially in high-risk subtypes. This justifies the inclusion of tumor type in the subsequent Cox proportional hazards regression model.

The multivariate Cox regression model (Table 3) assessed the influence of demographic, tumor and treatment-related factors on the risk of tumor recurrence over time. The overall model was statistically significant (Likelihood ratio test = 31.34, $p = 0.002$), indicating that the included variables, demographic data, lesion characteristics, and treatment protocol explained a significant portion of the variability in survival. Table 3 describes the multivariate Cox regression for a factor associated with clear outcomes after 10 years of follow-up. The Hazard Ratio (HR) indicates relative event risk between groups for each factor. HR > 1 means increased risk; HR < 1 decreased risk. The 95 % Confidence Interval (95 % CI) estimates the true HR range ('Inferior' and 'Superior' bounds). If the 95 % CI excludes 1, the factor's association with the outcome is statistically significant (p-value < 0.05).

Sex (female) was not statistically significant ($p = 0.119$); however, women exhibited a higher hazard ratio (HR = 2.84), suggesting a possible trend, although the wide confidence intervals reduce its reliability (Table 3). This elevated HR in women may be attributed to hormonal influences, variations in sun exposure, or differences in healthcare access and utilization [11]. Age, conversely, was significantly associated with increased recurrence risk (HR = 1.08; 95 % CI:1.03=1.13, $p = 0.0048$) (Table 3). This suggests that each additional year of age increases the hazard of recurrence by approximately 8 %, consistent with prior research demonstrating age-related declines in skin immune surveillance and repair [10]. Comparing these findings with KM survival of 34-c. Kaplan-Meier analysis showed a visible trend of decreasing recurrence-free survival with increasing age (log-rank $p = 0.019$). The oldest group (≥88 years) had the poorest survival outcomes,

Table 2
Statistical Comparison of Tumor Type Outcomes.

	N	Observed	Expected	(0-E)^2/E	(0-E)^2/V
sBCC	11	8	9.9840	0.3943	0.592
nBCC	45	36	34.5607	0.0599	0.211
nBCC ID	4	4	5.0917	0.2341	0.282
nBCC IN	3	0	0.0952	0.0952	0.102
SCC Insitu	3	3	1.2684	2.3641	2.583

Chi-square = 3.5 on 4 degrees of freedom (df), $p = 0.5$.

Table 3
Multivariate Cox regression for a factor associated with clear outcomes after 10 years Follow-up.

	HR (exp (coef))	95 % IC Inferior	95 % IC Superior	p-value
Demographic data				
Age	1.08	1.13	1.03	0.0048
Sex (Female)	2.84	1.26	0.133	0.119
Lesion characteristics				
Tumor Type				
nBCC	2.45	14.1	0.48	0.335
nBCC ID	0.17	1.15	0.00805	0.0521
nBCC IN	NA	NA	NA	NA
SCC In situ	19.8	232.	1.74	0.015
Tumor Location				
Extremities	3.11e+7	Inf	0	0.998
Face	7.20e+7	Inf	0	0.998
Head/Neck	3.76e+8	Inf	0	0.998
Median lesion (cm^2)	1.31	1.73	0.996	0.0531
Tumor Thickness (≤2 mm)	0.176	0.52	0.0597	0.00167
Treatment protocol				
PDT sessions				
3–5 sessions	0.445	1.82	0.109	0.259
≥ 6 sessions	2.84	22.6	0.356	0.325

Likelihood ratio test= 31.34 on 12 df, $p = 0.002$.
Score (log-rank) test = 29.38 on 12 df, $p = 0.003$.

aligning with the Cox regression results, where age was a significant independent predictor of recurrence. These results suggest that older age is associated with a higher risk of recurrence after PDT sessions.

In the case of lesion characteristics, Tumor type was a significant predictor of recurrence. In the comparison of sBCC, patients with SCC In situ demonstrated a markedly increased risk of recurrence, with an HR of 19.8 (95 % CI: 1.69–0.32: $p = 0.0174$). This finding reflects the markedly lower recurrence-free survival observed in SCC In situ cases on KM curves (Fig. 3-a), indicating a substantially worse prognosis. Although not statistically significant, nBCC without previous surgery and nBCC with dermis invasion (nBCC ID) revealed contrasting trends. nBCC showed a higher HR (2.41), while nBCC ID had a notably low HR of 0.10 ($p = 0.0621$), hinting at a potentially reduced recurrence risk in the dermis-invasive subtype. However, the wide confidence intervals (0.009–1.12) and borderline p-value suggest that this result should be interpreted cautiously. The nBCC with perineural invasion (nBCC IN) subgroup was excluded due to insufficient data, limiting further interpretation for this high-risk group. (Table 3)

Regarding lesion dimensions, tumor thickness $\leq$2 mm was significantly associated with a reduced hazard of recurrence (HR= 0.176: 95 % CI: 0.0597–0.52: $p = 0.00167$). This protective effect reinforces the KM results, showing superior recurrence-free survival among patients with thinner lesions. In contrast, lesion size was measured as the median surface area approached but did not reach statistical significance (HR =1.31: $p = 0.0531$). Nevertheless, this trend suggests that more extensive lesions may carry an elevated risk of recurrence, warranting consideration in clinical assessment. (Table 3)

Tumor location-whether on the face, extremities, or head/neck did not significantly impact recurrence risk ($p \approx 0.998$ for all). Similarly, the number of PDT sessions did not demonstrate a significant association with recurrence. Patients receiving 3–5 sessions had an HR of 0.445, while those undergoing six or more sessions had a higher HR of 2.84. Although these differences were not statistically significant ($p \approx 0.3$), the absence of a consistent dose-response relationship is notable. (Table 3) The KM curve showed overlapping survival curves across session groups, further supporting the conclusion that increasing the number of PDT sessions did not yield improved outcomes in terms of recurrence prevention.

PDT demonstrated high efficacy in treating BCC in this 10-year follow-up cohort, particularly for superficial and moderately invasive lesions. A representative case is illustrated in Fig. 4, showing a patient with two lesions, one nBCC on the left frontal region and another lesion on the left eyebrow. Pretreatment biopsy of Lesion 1 (nBCC) showed a thickness of 2.9 mm and was treated with 4 cycles of PDT. The lesion exhibited moderate inflammation and crust formation during treatment, and a biopsy 45-day post-treatment revealed fibroblast proliferation and scar tissue, which indicated successful tissue repair [12]. Lesion 2 (SCC), with 0.7 mm of thickness, was treated with 2 PDT sessions and showed inflammation during treatment and post-treatment scarring at 30 days. A biopsy conducted 30 days post-PDT showed no evidence of tumor, only characteristic scar tissue with high percentage of complete responsive after 2 months (Fig. 1). Furthermore, long-term follow-up over 10 years revealed no tumor recurrence, as illustrated in Fig. 3, confirming a good clinical response. These outcomes highlight the effectiveness of PDT in treating BCC, mainly when an adequate number of sessions is used depending on the lesion's thickness and type.

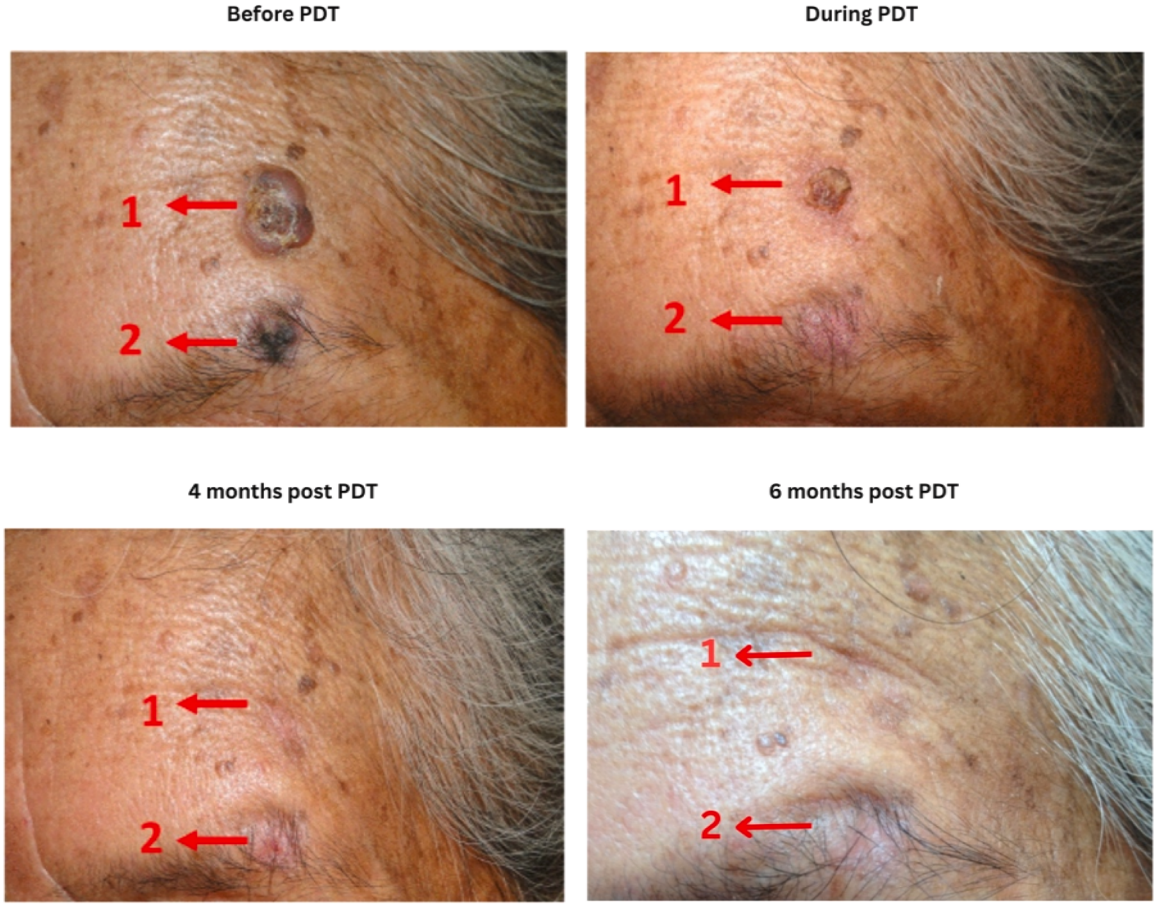

Fig. 4. Clinical progression of a patient with two lesions treated with PDT: Lesion 1 – Nodular BCC (2.9 mm thickness, 1.7 cm^2) and Lesion 2 – SCC (0.7 mm thickness, 0.64 cm^2). Images captured before PDT, during treatment, 4 months post-treatment, and 6 months post-treatment.

4. Discussions

Before interpreting our treatment recommendations, it is important to acknowledge a key limitation of this study: long-term (10-year) surveillance was feasible only for the 48 patients treated at Hospital Carlos Andrade Marín (HCAM). The 14 patients managed at Dermatological Hospital Gonzalo González (DHGG) could not be followed beyond three months because that institution discontinued public services. We nevertheless retained their short-term biopsy data in the early-outcome analysis to preserve the multicenter character of the study and to provide a more representative snapshot of MAL-PDT effectiveness at baseline. Importantly, all survival curves and Cox models were generated exclusively from the HCAM cohort; thus, the absence of DHGG follow-up does not affect the durability estimates presented here.

Considering our findings over a 10-year follow-up period, a standardized protocol of 4 PDT sessions appears advisable for nodular BCCs, regardless of the biopsy-reported thickness, unless perineural invasion is present, in which case PDT is generally considered less effective [13]. This recommendation aligns with several studies that have explored the efficacy of multiple PDT sessions for thicker or more aggressive BCC subtypes. For instance, a study by Roozeboom et al. [14] reported improved long-term clearance rates in nBCC treated with multiple PDT sessions compared to single sessions. They suggested that the increased number of treatments allows for deeper and more complete tumor destruction, particularly in lesions extending beyond the superficial dermis.

Conversely, for superficial BCCs 2 sessions of PDT remain sufficient to achieve a complete response. This is consistent with established literature, where numerous studies have demonstrated the effectiveness of 1–2 PDT sessions for sBCC. For instance, a meta-analysis by Szeimies et al. [15] and Sjöholm et al. [16], concluded that 2–3 sessions of PDT are sufficient for achieving excellent clearance rates in sBCC, with long-term outcomes compared with other modalities like cryotherapy or topical treatments. The rationale behind fewer sessions in sBCC lies in their limited thickness and the ability of PDT to target the tumor within the superficial skin layers.

Cox regression analysis further substantiates these clinical observations regarding the effectiveness of PDT protocols for different BCC subtypes (Table 3). This statistical modeling identified three variables significantly associated with the risk of recurrence following PDT.

Firstly, age at treatment demonstrated a statistically significant HR of 1.08 ($p = 0.0048$). This indicates that the hazard of tumor recurrence increased by 8 % for each additional year of age. This finding may be attributed to several age-related physiological changes that could impact the efficacy of PDT. For instance, impaired tissue healing in older individuals might hinder the complete resolution of treated areas and increase susceptibility to recurrence [17]. Furthermore, immune senescence, a gradual decline in immune function with age, could lead to a less robust anti-tumor response following PDT [16,17]. Finally, the inflammatory response, a crucial component of PDT's mechanism of action, may be delayed or less effective in elderly patients [18]. Supporting this, a study by Wang et al. [19] investigated the influence of age on PDT outcomes for superficial skin cancers and found a trend towards higher recurrence rates in older patients, potentially linked to diminished immune surveillance.

Secondly, our analysis revealed that a tumor thickness of $\leq$ 2 mm was associated with a significantly lower risk of recurrence (HR = 1.176, $p = 0.00167$). This highlights the importance of tumor thickness as a prognostic factor in PDT for skin cancer. Thinner tumors, confined to more superficial skin layers, respond more favorably to PDT and exhibit a reduced propensity for recurrence. This observation aligns with Szeimies et al. [10] and Collier et al. [17], demonstrating that superficial BCCs treated with PDT had significantly lower recurrence rates than thicker nodular BCCs. PDT is efficacy largely depends on light penetration and the subsequent generation of cytotoxic reactive oxygen species within the tumor tissue. This process is more uniform and complete in thinner lesions, leading to better long-term control. Moreover, thinner tumors may have a lower tumor burden, making them more susceptible to eradication by PDT-induced mechanisms.

Regarding histological subtype, SCC in situ showed a high point estimate for recurrence in the multivariable model (HR = 19.8, $p = 0.015$). This value must be interpreted with caution: the 95 % confidence interval is very wide because it is based on only confidence interval is three lesions and zero observed recurrences, so the estimate is highly unstable and susceptible to sparse-event bias. Nevertheless, similar studies have reported greater long-term failure rates for SCC in situ than for superficial BCC after PDT [20,21]. Including Lehmann et al. [11], who attributed the difference to distinct tumor biology and growth patterns. This emphasizes the importance of considering histological subtypes when selecting PDT as a treatment option and highlights the need for careful long-term surveillance in patients with SCC in situ treated with PDT.

Finally, sex showed a non-significant trend toward higher recurrence in women (HR = 2.84, $p = 0.12$). Although not statistically decisive in our cohort, sex-based differences have been reported elsewhere—potentially linked to hormonal milieu, behavioral sun-exposure patterns, or healthcare-seeking behavior [20]. For instance, some research suggests that estrogen might influence the progression of certain skin cancers, although the exact mechanisms of skin cancer and PDT response are still being investigated [22]. A study by Paolino et al. [9], explored potential sex-based differences in NMSC treatment outcomes and suggested that hormonal factors or behavioral differences in sun protection might play a role. Variations in healthcare-seeking behavior and adherence to follow-up appointments could also contribute to observed differences in recurrence rates. Further research is warranted to elucidate the specific factors contributing to the higher recurrence risk observed in female patients in our cohort.

The Kaplan-Meier survival curves further reinforce these findings. Stratification by age revealed lower recurrence-free survival in patients aged $\geq$ 88 years, consistent with the increases in HR (1.08) for age. Similarly, patients with tumors $\leq$2 mm tick had significantly higher survival probabilities than deeper lesions [18]. These trends validate the importance of age and tumor thickness as predictors of treatment success.

When considered in the context of the clinical case present in Fig. 4, Lesion 1, a nBCC, with a 2 mm thickness treated with 4 PDT sessions, and Lesion 2, and SCC in situ with a 0.7 mm thickness treated with 2 PDT sessions, effectively illustrate a successful response strategy that is tailored to individual lesion characteristics. The favorable outcomes observed in Lesion 1 reinforce our recommendation for a more intensive PDT regimen (4 sessions) for nodular BCCs, particularly those with a measured thickness beyond the superficial dermis. The increased number of sessions likely contributed to a more complete eradication of the tumor cells, aligning with the earlier findings regarding the benefit of multiple sessions for thicker BCCs. Conversely, the successful treatment of Lesion 2, a thin SCC in situ, with 2 PDT sessions highlights the potential for a less intensive approach in specific superficial lesions. While our overall analysis indicated a higher recurrence risk for SCC in situ compared to BCCs, this specific case suggests that very thin SCC in situ lesions might respond favorably to a limited number of PDT sessions. This underscores the importance of considering the histological type and the specific characteristics of the lesion, such as its thickness, when determining the optimal PDT protocol.

Similar studies have also advocated for tailoring PDT protocols based on tumor characteristics. For example, Lucena et al. [23], investigated the efficacy of varying PDT regimens for different subtypes and thicknesses of NMSCs and concluded that a more aggressive approach (e.g., higher light doses or multiple sessions) was often necessary for thicker or more aggressive lesions to achieve comparable outcomes to those seen in thinner, less aggressive tumors treated with less intensive PDT [15,16,22]. Another study by Dushkin et al. [24], specifically examined the role of lesion thickness in predicting PDT response in superficial skin cancers

and found that thinner lesions generally exhibited higher initial clearance rates and potentially lower recurrence rates with fewer treatment sessions. These findings support the rationale for a personalized approach to PDT, where the number of treatment sessions and potentially other parameters are adjusted based on factors such as histological subtype and tumor thickness to optimize treatment outcomes and minimize the risk of recurrence.

5. Conclusion

Over the course of this multicenter study, we first assessed the early cytological response of 82 histologically confirmed lesions—baseline-untreated nBCC, recurrent nBCC with dermal or perineural invasion, sBCC and SCC-is—to a standard MAL-PDT protocol. Two months after therapy the procedure proved uniformly effective, yielding complete-response rates of 94.8 % in de-novo nBCC, 92.3 % in sBCC and 100 % in both dermal-invading nBCC and SCC-is. These results confirmed PDT as an excellent tissue-sparing option for immediate tumor clearance across the non-melanoma spectrum.

10 years follow up in the 48-patient HCAM cohort (66 lesions) demonstrated that durability depends strongly on histology and thickness. Superficial BCC preserved a 72.7 % complete clinical response at ten years (≈ 70 % tumor-free survival), whereas de-novo nBCC maintained an 80 % five-year control rate when four treatment sessions were delivered. Dermal-invading, surgery-recurrent nBCC remained tumor-free throughout follow-up, but the perineural-invading variant fared poorly, with a two-thirds recurrence rate and one-third treatment abandonment. All three SCC-is lesions stayed clear of disease, mirroring the excellent short-term response yet offering too little statistical power for firm comparative statements.

Multivariate Cox regression analysis identified several significant factors influencing the risk of recurrence after PDT. Increasing age was significantly associated with a higher risk of recurrence (HR = 1.08, $p = 0.0048$), indicating an 8 % increase in recurrence risk for each additional year. Conversely, tumor thickness $\leq$ 2 mm was a significant protective factor associated with a reduced hazard of recurrence (HR = 0.176, $p = 0.00167$). Histological type was also a significant predictor, with SCC in situ demonstrating a markedly higher risk of recurrence than sBCC (HR = 19.8, $p = 0.015$). The Kaplan-Meier (KM) survival curves corroborated the findings from the Cox regression. sBCC and nBCC without prior treatment showed a gradual decline in tumor-free survival, aligning with the observed recurrence rates. SCC in situ and nBCC with dermal invasion exhibited excellent long-term survival with no recurrences. The poorest survival outcomes were observed in nBCC with perineural invasion, characterized by rapid recurrence or treatment discontinuation. The global KM curve highlighted a critical recurrence window between 1- and 5 years post-treatment, emphasizing the need for prolonged follow-up. Stratification by age in KM analysis also supported the Cox regression results, with older patients showing lower recurrence-free survival. Similarly, thinner lesions ($\leq$ 2 mm) demonstrated superior survival probabilities than thicker ones in the KM curves

These conclusions underscore the variable efficacy of PDT based on tumor type and characteristics. While PDT is a valuable treatment modality for superficial and nodular BCCs, particularly when tailored protocols are employed, its effectiveness is limited in aggressive subtypes like nBCC with perineural invasion. The excellent long-term outcomes observed for SCC in situ in this cohort warrant further investigation in more extensive studies. Age and tumor thickness are critical factors when predicting PDT outcomes and planning treatment strategies.

6. Recommendation

Based on our 10-year follow-up of PDT in NMSC patients, we recommend a tailored treatment approach: 4 PDT sessions are advisable for nodular BCCs regardless of initial biopsy thickness (unless perineural invasion is present, where PDT is likely ineffective). In comparison, 2 sessions appear sufficient for superficial BCCs. Given the significantly higher recurrence risk observed in SCC in situ compared to BCCs despite initial complete responses, PDT for this histological subtype should be approached cautiously and necessitates rigorous, long-term surveillance. Furthermore, patient age and tumor thickness are critical prognostic factors; older patients and those with thicker lesions (especially > 2 mm) require closer monitoring and potentially more aggressive initial management strategies. While PDT demonstrates excellent outcomes for dermal-invasive recurrent nBCC without perineural involvement, it is not recommended for cases with perineural invasion. Future research should refine PDT light-dose and session number for thick or high-risk BCC, explore combination regimens for perineural disease, and expand cohorts to clarify the influence of patient sex and to validate the promising treatment in SCC.

CRediT authorship contribution statement

María Paulina Romero: Writing – original draft, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Myriam González:** Writing – original draft, Formal analysis, Data curation. **David Giancarlo García:** Data curation. **Natalia Inada:** Validation. **Vanderlei Bagnato:** Project administration. **Franklin Cabrera:** Supervision, Investigation, Data curation, Conceptualization.

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