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Analysis of tumoral, stromal and glycolitic markers in the response basal cell carcinoma and Bowen disease to photodynamic therapy in real life

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ABSTRACT

Background: Photodynamic therapy (PDT) is a widely-used non-surgical treatment for non-melanoma skin cancers, including basal cell carcinoma (BCC), actinic keratoses (AK), and Bowen's disease (BD). PDT has high success rates, but various factors, can influence treatment response. This study investigates the clinical, histological, and molecular factors that affect the efficacy of methyl aminolevulinate PDT (MAL-PDT) for BCC and BD.
Methods and Patients: Prospective observational multicentric study performed between May 2019 and January 2021 with 64 patients included. Clinical data such as tumor thickness, location, and histological subtype were recorded. Immunohistochemical analysis was performed on tumor samples to assess the expression of biomarkers including p53, β-catenin, and GLUT1.
Results: Tumor thickness was found to be a critical determinant of MAL-PDT response, with thicker nodular BCCs showing reduced response rates compared to thinner, superficial BCCs and BD lesions. Immunohistochemical analysis revealed that p53 positivity was associated with better treatment outcomes, while increased β-catenin and cytoplasmic GLUT1 expression correlated with resistance to PDT. On the other hand, the metabolic profile of the tumors indicated that tumors with higher glycolytic activity were less responsive to treatment, therefore, using metformin, a glycolytic inhibitor, as a potential adjuvant therapy to improve outcomes in resistant tumors should be considered.
Conclusion: This study emphasizes the importance of personalized approaches in the use of MAL-PDT, tailoring treatment according to tumor-specific characteristics. Biomarkers such as p53, β-catenin, and GLUT1 can serve as predictive tools for PDT response, helping clinicians identify patients who may benefit from alternative or combined treatments to enhance therapeutic efficacy.

1. Introduction

Photodynamic therapy (PDT) stands out as a popular non-surgical treatment option for non-melanoma skin cancers (NMSC). It has a high level of scientific evidence to treat not only actinic keratoses (AKs) and Bowen's disease (BD), but also basal cell carcinoma (BCC) [1].

Its key benefits include achieving high rates of response, with approximately 91 % of BCC cases showing complete resolution at three months and maintaining a 76 % response rate even up to five years post-treatment [2].

Furthermore, PDT offers the advantage of flexibility, allowing the possibility of repeating the treatment as needed, and the option to be combine with other therapies with synergic effect. This approach has an excellent cosmetic outcome, contributing to high levels of patient satisfaction [3].

However, it has several limitations, such as pain during illumination

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and, more importantly, the penetration capacity of the photosensitizer as well as the light. Numerous clinical factors have been associated with bad response and resistance to PDT, with tumor thickness being extensively studied, especially in cases of BCC. As per treatment guidelines, PDT is generally not recommended for nodular BCCs exceeding 2 mm in thickness [4].

Although response rates to PDT are high for nodular and superficial BCCs and BD, there are some patients who do not respond adequately. This problem has already been described by Sieron et al. and Perona and Sánchez et al. in their works on the emergence of resistance to oncological treatments. These neoplastic cells, due to their survival mechanisms, escape cell death [5,6].

Finding biomarkers are a good strategy to improve the efficacy of therapies. In BCC certain factors were linked to a less favorable response to treatment, including tumor location in the H area (high risk area: nasolabial fold, nasal, orbital and auricular areas), advanced age, darker skin type, and a high frequency of MAL-PDT sessions. Histologically, the absence of peritumoral lymphocytic inflammatory infiltrate was also associated with poorer outcomes. Additionally, negative p53 immunoreactivity and a β-catenin pattern characterized by peripheral reinforcement of basaloid cell islands were identified as indicators of tumor resistance to PDT. These molecular observations have been supported by in vitro experiments using BCC cell lines, confirming their relevance in understanding treatment resistance [7–9].

On the other hand, a larger tumor size and a higher mitosis count might be associated with a better response of Bowen disease to MAL-PDT. Furthermore, p53 expression was a marker of good response like for BCC, although intense expression of cyclin D1 and EGFR appear to be markers of poor MAL-PDT response of BD [10].

Only a limited number of studies got deeper into the underlying mechanisms responsible for poor tumor response to PDT, particularly in the context of non-melanoma skin cancers (NMSC). Nonetheless, this area has garnered increasing research attention [11].

In our own research, we observed resistance to MAL-PDT in cultures of murine keratinocytes (Pam-212), where we noted activation of the PI3K/Akt pathway. Similarly, activation of the PI3K/Akt and MAPK/ERK pathways has been identified in PDT-resistant cells derived from the human SCC cell line SCC-13, suggesting a potential association with the more aggressive behavior observed in invasive SCCs among patients treated with PDT [12].

So far, research has focused on tumor characteristics that are very important in determining tumor invasiveness in which there is growing evidence that the tumor microenvironment (TMA), consisting of the stroma, its host cells, vascularization and inflammatory infiltrates, play a key role. Within this TMA, the epithelial-mesenchymal transition (EMT) conditions, which includes, the loss of epithelial cell-cell adhesion, acquiring mesenchymal characteristics, increasing migration capacity and inducing overexpression of mesenchymal markers is a fundamental factor in tumor progression [4,13].

It is also being demonstrated in other therapies that tumor cell metabolism can provide an alternative for developing new therapeutic modalities to tackle the phenomenon of resistance, achieving tumor eradication. In addition, several studies have observed that the efficacy of PDT is enhanced when it is used in conjunction with glycolytic inhibitors, such as metformin [14].

The objective of this study was to consider the influence of different factors in the effectiveness of MAL PDT to treat BCC and in situ SCC in real life, taking into account variables depending on the patient and also those depending on the tumor characteristics, considering markers of the neoplastic cells and also the surrounding stroma, apart from glycolytic markers.

2. Material and methods

A prospective observational multicentric study was performed between May 2019 and January 2021, in the Dermatology Service at Miguel Servet University Hospital in Zaragoza and Barbastro Hospital, Spain. Adults older than 18 years with histological diagnosis of superficial or nodular basal cell carcinoma and Bowen's disease eligible for PDT treatment were included. Exclusion criteria: Metvix® sensitization, PDT contraindication, photodermatosis, used any treatment for basal cell carcinoma and Bowen's disease previous 12 weeks. All patients signed informed consent.

Patients were treated with PDT, a curettage of the lesion was performed, then MAL cream 160 mg/g (Metvix®) was used as a photosensitizer with an incubation period of 3 h in occlusive cure. It was illuminated with the Aktilite® lamp with a dose of 37 J/cm2. Two PDT sessions were performed separated by two weeks. Before the first PDT session, a biopsy of the lesion was performed using a 4-mm punch. Pain during illumination was measured on a scale of 0 to 10, with 10 representing the maximum pain perceived during treatment.

Anthropometric and clinical data were collected, such as age, weight, height, BMI (body mass index) gender, immunosuppression history, Fitzpatrick skin phototype (high III-VI and low I-II), size, location and basal cell carcinoma clinical subtype (superficial or nodular), habitual use of medications including metformin, vitamin D, lipid-lowering, antihypertensive. The serum levels of 25-hydroxyvitamin D (25-OHD), glucose, intact parathormone (PTHi), lipids, folic acid, vitamin B12, calcium, phosphorus, iron, ferritin, were measured. The histological variables of the tumors were collected, such as histological subtype, tumor thickness, presence of ulceration, peritumoral inflammatory infiltrate, cell pleomorphism, intratumoral necrosis, perineural invasion, solar elastosis. An immunohistochemical study of the tumor and tumor stroma was also performed, measuring beta catenin (Dako/Agilent), Cyclin D1 (Dako/Agilent), Ki67 (Dako/Agilent), P53(Dako/Agilent),CD31(Dako/Agilent),CD10(Dako/Agilent),vimentin (Dako/Agilent), and Glut 1 (Roche).

Finally, the association between these variables with the response to treatment at 3, 6 and 9 months was determined. The lesions that persisted in the follow-up visits were excised and histologically analyzed.

A descriptive statistical analysis was carried out. The qualitative variables were described as proportions and the quantitative variables were described, according to the Kolmogorov-Smirnov test, as measures of central tendency (mean or median) and dispersion (standard deviation or percentiles). ANOVA test was used for continuous variables and the PRE-POST longitudinal comparisons were carried out using the Student's *t*-test for paired data. In addition, regression models have been created at an exploratory level to evaluate the existence of a relationship between variables. All analysis were performed on the whole data set using all available information with intention to treat (ITT) criteria, correcting the information as specified in the section on data management. For all comparisons a statistical significance level of 0.05 bilateral was considered. Statistical analysis was performed using SAS (Statistical Analysis System) software, version 9.4 or later

The study was approved by the Clinical Research Ethics Committee of Aragon (CEICA) (EPA19/032).

3. Results

3.1. Clinical description of the sample

A total of 64 patients were included, 45.3 % male and 54.7 % female, with a mean age of 75.56 SD, 9.70 years. The mean BMI was 26.84 SD 4,37 kg. 57.2 % corresponded to high phototype and 42.9 % a low phototype. Dark brown-black eye color was observed with a frequency of 33.3 % followed by light brown 28.6 %, and brown was the most common hair color with a frequency of 58.7 %, followed by blonde 23.8 %. Twenty-three percent of the patients had a personal history of BCC, 9.4 % of squamous cell carcinoma and 62.5 % had no history of skin cancer. Three percent were immunosuppressed (Table 1).

The most common histological type was superficial BCC 36,33 %, followed by BD 33,33 % and nodular BCC 30,43 % (Table 1). The clinical

Table 1
Characteristics of the sample and statistically differences among the 3 types of tumors.

$N = 64$	
Sex, n (%)	
Women	35 (54,7 %)
Men	29 (45,3 %)
Age, years	
Mean (SD)	75,56 (9,70)
Range (min, max)	(46, 91)
BMI kg/m2	
Mean (SD)	26,84 (4,37)
Range (min, max)	(19, 39)
Phototype n (%)	
II	27 (42,9 %)
III	35 (55,6 %)
IV	1 (1.6 %)
N missing	1
Eye Colour n (%)	
Blue-grey	11 (17,5 %)
Dark brown- Black	21 (33,3 %)
Light brown	18 (28,6 %)
Green	13 (20,6 %)
N missing	1
Hair Colour n (%)	
Brown	37 (58,7 %)
Black	11 (17,5 %)
Blonde	15 (23,8 %)
N missing	1
Prior skin cancer n (%)	
SCC	6 (9,4 %)
BCC	15 (23,4 %)
None	41 (62,5 %)
Immunodepression n (%)	
Yes	2 (3,1 %)
No	62 (96,9 %)
Chronic medication n (%)	
Oral Vitamin D	11 (17,2 %)
Simvastatin	30 (46,9 %)
Metformin	11 (17,2 %)
Hydrochlorothiazide	14 (21,9 %)
ACE Inhibitors	5 (7,8 %)
NSAIDs	1 (1,6 %)
Previous treatment n (%)	
Cryotherapy	2 (3,1 %)
Imiquimod	2 (31,1 %)
Other	1 (1,6 %)
None	59 (92,2 %)
Hystological type n (%)	
In situ SCC	20 (33,33 %)
Nodular BCC	14 (30,43 %)
Superficial BCC	30 (36,23 %)
Localization n (%)	
Head and Neck	14 (21,9 %)
Trunk	342 (50,0 %)
Upper and lower limb	18 (28,1 %)
Tumor size, mm	
Mean (SD)	18,16 (11,25)
Range (min, max)	(5,60)
Biochemical profile	
Vitamin D (ngr/ml)	28,89 (16,67)
Mean (SD)	(3,1, 98,8)
Range (min, max)	66,51 (32,01)
PTH (pg/mL)	(3,30, 208,0)
Mean (SD)	200,5 (47,31)
Range (min, max)	(121, 363)
Total cholesterol (mg/dl)	57,15 (15,26)
Mean (SD)	(20, 93)
Range (min, max)	117,73 (33,99)
HDL cholesterol (mg/dl)	(51, 242)
Mean (SD)	126,38 (45,49)
Range (min, max)	(51, 282)
LDL cholesterol (mg/dl)	108,13 (25,02)
Mean (SD)	(77,0, 182,0)
Range (min, max)	9,63 (0,42)
Triglycerides (mg/dl)	(8,6, 11,4)
Mean (SD)	3,28 (0,46)
Range (min, max)	(2,4, 4,7)
Glucose (mmol/L)	8,64 (4,69)
Mean (SD)	(2,3, 24,1)
Range (min, max)	351,78 (351,17)
Calcium (mg/dl)	(80,0, 1500,0)
Mean (SD)	78,30 (25,80)
Range (min, max)	(19,0, 136,0)
Phosphorus(mg/dl)	117,101 (103,73)
Mean (SD)	(8,10, 446,0)
Range (min, max)	
Folic acid (ng/mL)	
Mean (SD)	
Range (min, max)	
Vitamin B12(pg/ml)	
Mean (SD)	
Range (min, max)	
Iron (mcg/dl)	
Mean (SD)	
Range (min, max)	
Ferritin (ng/ml)	
Mean (SD)	
Range (min, max)	

(*continued on next page*)

Table 1 (*continued*)

mean tumor size was 18.02 (SD, 11.25) mm. The most common location of the lesions was the trunk (50 %), followed by the lower and upper extremities (28.1 %) and the head and neck (21.9 %). Ninety two percent did not previously receive any treatment for the tumor, 3.1 % had received previous cryotherapy and 3.1 % imiquimod at least 3 months before.

The most common chronic medications in our sample were statins (46.9 %), followed by hydrochlorothiazide (21.9 %), metformin (17.2 %), vitamin D (17.2 %) and ACE inhibitors (7.8 %) (Table 1).

The results of the serum levels parameters measured in the study are summarized in Table 1, being of all of them in the normal range.

Regarding the PDT procedure, the mean score of pain during illumination was 3.62 (SD 2.81) (VAS 0–10).

3.2. Histological characteristics of the sample

The mean tumor thickness was 0.53 mm (SD 0.61) (Table 2) being the nodular BCC 1.45 mm (SD 0.76) the highest. Twelve percent of the cases presented histological ulceration, more frequent in superficial BCCs (23.3 %, $p = 0.049$). Whereas solar elastosis was present in almost all the tumors (98.5 %), cellular pleomorphism or tumor necrosis were not observed.

3.3. Response to treatment

At the end of the follow-up, 73,4 % of the tumors showed a complete response as follows: 95 % in situ SCC, 66.7 % superficial BCC and nodular 57.1 % BCC ($p = 0.025$). Only 5 tumors had been previously treated with other therapies (4 in situ SCC treated and one superficial BCC), and all of them were cured after PDT.

The response to treatment was not statistically significant associated with any epidemiological and clinical variables such as age, BMI, gender, immunosuppression history, skin phototype, eye color, hair color, tumor size, and location.

Regarding chronic treatments, the use of ACE inhibitors was associated with PDT failure (OR 14.14, IC95 % 1.45, 137.76, $p = 0.025$) (Table 3). No association was found between the intake of oral VD or metformin and the response to PDT.

No-responder patients showed a trend to have lower serum levels of VD compared to the responders (26.89 (SD 17.14) vs 29.15 (SD 16.13) ngr/ml respectively) and high PTH (84.68 (SD 55.68) vs 64.85 (SD 25.93) pg/ml. No significant differences were observed in other serum parameters.

Table 2
Histological and immunohistochemical parameters.

$N = 64$	
Tumour thickness (mm)	
Mean (SD)	0,56 (0,61)
Range (min, max)	(0,1,2,9)
Solar elastosis n (%)	
Yes	62 (98,4 %)
No	1 (1,6 %)
N missing	1
Ulceration n (%)	
Yes	8 (12,7 %)
No	58 (87,3 %)
N missing	
Celular pleomorfism	
Yes	0
No	63 (100 %)
N missing	1
Intratumoral necrosis n (%)	
Yes	0
No	63 (100 %)
N missing	1
Peritumoral infiltrate	
Intense	28 (44 %)
Mild	28 (44 %)
Absent	7 (11.1 %)
N missing	1
Ki 67	
Mean (SD)	8,73 (8,35)
Range (min, max)	(0,0,50,0)
P53	
Mean (SD)	24,28 (32,06)
Range (min, max)	(9,9,90)
CD 31	
Mean (SD)	16,10 (8,73)
Range (min, max)	(3,0,40,0)
Beta Catenin n (%)	
Pos/ +	5 (8,2 %)
Pos/ -	35 (57,4 %)
Neg	21 (34,4)
Cyclin D1	
Mean (SD)	15,29 (22,41)
Range (min, max)	(0,00, 80,0)
Glut 1 cytoplasm n(%)	
Only cytoplasm	7 (15,9 %)
Cytoplasm + membrane	14 (31,8 %)
Only membrane	23 (52,3 %)
N missing	20
Cd 10 intensity n(%)	
Mean (SD)	1,42 (1,00)
Range (min, max)	(0,0, 3,0)
Mild	17 (28,8 %)
Moderate	20 (33,9 %)
Severe	9 (15,3 %)
N missing	5
Cd 10 area n(%)	
Mean (SD)	1,71 (1,15)
Range (min, max)	(0,0, 3,0)
Mild	10 (16,9 %)
Moderate	17 (28,8 %)
Severe	19 (32,2 %)
N missing	5
Vimentin intensity n(%)	
Mean (SD)	2,81 (0,54)
Range (min, max)	(1,0, 3,0)
Mild	4 (6,9 %)
Moderate	3 (5,2 %)
Severe	51 (87,9 %)
N missing	6
Vimentin area n(%)	
Mean (SD)	2,88 (0,38)
Range (min, max)	(1,0, 3,0)
Mild	1 (1,7 %)
Moderate	5 (8,6 %)
Severe	52 (89,7 %)
N missing	6

Table 3
Association between the different variables and healing results after photodynamic therapy at 9–12 months.

$N = 69$	Cured	Not cured	P-valour
Sex, n (%)			
Total	47	17	0,1916
Women	28 (80 %)	7 (20 %)	
Men	19 (65,51 %)	10 (34,48 %)	
Age, years			
Mean (SD)	75,02 (8,51)	77,06 (12,62)	0,4626
Range (min, max)	(46, 91)	(46, 91)	
BMI kg/m2			
Mean (SD)	26,87 (4,39)	26,76 (4,45)	0,9315
Range (min, max)	(21, 39)	(19,0;38,0)	
Phototype n (%)			
II	19 (70,37 %)	8 (29,62 %)	0,1548
III	28 (80 %)	7 (20 %)	
IV	1	1 (100 %)	
N missing		1	
Eye Colour n (%)			
Blue-grey	7 (63,63 %)	4 (36,36 %)	0,6660
Dark brown- Black	15 (71,42 %)	6 (28,57 %)	
Light brown	15 (83,30 %)	3 (16,66 %)	
Green	10 (76,92 %)	3 (23,07 %)	
N missing		1	
Hair Colour n (%)			
Brown	29 (78,37 %)	8 (21,62 %)	0,6098
Black	7 (63,63 %)	4 (36,36 %)	
Blonde	11 (73,3 %)	4 (26,66 %)	
Prior skin cancer n (%)			
SCC	4 (66,66 %)	2 (33,33 %)	0,7741
BCC	12 (80 %)	3 (20 %)	
None	31 (72,09 %)	12(27,90 %)	
Immunodepression n (%)			
Yes	2 (100 %)	17 (27,41 %)	0,875
No	45 (72,5 %)		
Chronic medication n (%)			
Oral Vitamin D	8 (72,72 %)	3 (27,27 %)	0,9533
Simvastatin	21 (70 %)	9 (30 %)	0,5586
Metformin	7 (63,63 %)	4 (36,36 %)	0,4186
Hydrochlorothiazide	10 (71,14 %)	4 (28,57 %)	0,8473
ACE Inhibitors	1 (20 %)	4 (80 %)	0,0048
NSAIDs	1 (100 %)		0,5444
Previous treatment n (%)			
Cryotherapy	2 (100 %)	17 (28,81 %)	0,5804
Imiquimod	2 (100 %)		
Other	1 (100 %)		
None	42 (71,18 %)		
Localization n (%)			
Head and Neck	12 (85,71 %)	2 (14,28 %)	0,4535
Trunk	23 (71,87 %)	9 (28,12 %)	
Upper and lower limb	12 (66,66 %)	6 (33,3 %)	
Tumor size, mm			
Mean (SD)	18,17 (12,17)	18,12 (8,54)	0,9870
Range (min, max)	(5,60)	(8,40)	
Biochemical profile			
Vitamin D (ngr/ml)	29,87 (16,60)	25,64 (17,16)	0,4273
Mean (SD)	(8, 98,8)	(3,1, 62,7)	0,2643
Range (min, max)	63,69 (26,82)	75,20 (44,66)	0,6880
PTH (pg/mL)	(32,9; 138,6)	(3,3; 187,6)	0,6804
Mean (SD)	201,93 (50,66)	196,20	0,7549
Range (min, max)	(121,0; 363,0)	(36,61)	0,9342
Total cholesterol (mg/dl)	57,64 (15,74)	(121,0; 244,0)	0,7867
Mean (SD)	(20, 93)	55,73 (14,18)	0,3706
Range (min, max)	118,55 (36,71)	(31,0; 84,0)	0,4719
HDL cholesterol (mg/dl)	(51, 242)	115,33	0,9543
Mean (SD)	126,67 (50,36) (51,0,	(25,25)	0,3329
Range (min, max)	282,0)	(74,0; 158,0)	0,7059
LDL cholesterol (mg/dl)	108,64 (24,04)	125,53	0,5212
Mean (SD)	(77,0, 182,0)	(27,34)	
Range (min, max)	9,65 (0,28)	(81,0; 185,0)	
Triglycerides (mg/dl)	(9,1; 10,4)	106,60	
Mean (SD)	3,25 (0,48)	(28,61)	
Range (min, max)	(2,4, 4,7)	(79,0; 175,0)	
Glucose (mmol/L)	8,67 (4,29)	9,53 (0,74)	
Mean (SD)	(3,4; 24,1)	(8,6; 11,4)	

(*continued on next page*)

Table 3 (*continued*)

N = 69	Cured	Not cured	P-valour
Range (min, max)	354,73 (311,60)	3,37 (0,39)	
Calcium (mg/dl)	(80,0, 1494,0)	(2,8; 4,0)	
Mean (SD)	79,08 (26,27)	8,58 (5,90)	
Range (min, max)	(24,0; 136,0)	(2,3; 23,1)	
Phosphorus(mg/dl)	1112,16 (88,84) (8,1;	461,00	
Mean (SD)	359,3)	(452,16)	
Range (min, max)		(161,0;	
Folic acid (ng/mL)		1500,0)	
Mean (SD)		75,92 (25,14)	
Range (min, max)		(19,0; 103,0)	
Vitamin B12(pg/ml)		133,42	
Mean (SD)		(146,79)	
Range (min, max)		(14,6; 446,0)	
Iron (mcg/dl)			
Mean (SD)			
Range (min, max)			
Ferritin (ng/ml)			
Mean (SD)			
Range (min, max)			
Tumour thickness (mm)			
Mean (SD)	0,46 (0,48)	0,86 (0,87)	**0,0263**
Range (min, max)	(0,1; 2,1)	(0,1; 2,9)	
Solar elastosis n (%)			
Yes	46 (97,9 %)	16 (100,0 %)	0,5564
No	1 (2,1 %)		
N missing			
Ulceration n (%)			0,3698
Yes	7 (14,9 %)	1 (6,3 %)	
No	40 (85,1 %)	15 (93,8 %)	
Intratumoral necrosis n			
(%)	0 (0 %)	0 (0 %)	
Yes	47 (74,06 %)	16 (25,39 %)	
No			
Ki 67			0,4368
Mean (SD)	9,23 (8,90)	7,27 (6,47)	
Range (min, max)	(0,0,50,0)	(1,0; 20,0)	
P53			**0,0222**
Mean (SD)	29,59 (34,26)	8,00 (15,98)	
Range (min, max)	(0,0; 90,0)	(0,0; 60,0)	
Cd 31			0,2431
Mean (SD)	15,35 (8,89)	18,40 (8,07)	
Range (min, max)	(3,0,40,0)	(4,0; 30,0)	
Beta Catenin n (%)			
Positive	26 (65,00 %)	14 (35,00 %)	**0,0113**
Negative	20 (95,23 %)	1 (4,76 %)	
Ciclin			0,6501
Mean (SD)	15,80 (22,86)	5,00 (,)	
Range (min, max)	(0,00, 80,0)	(5,0; 5,0)	
Glut 1 tumoral positivity n			**0,0318**
(%)	3 (42,85 %)	4 (57,14 %)	
Only cytoplasm	12 (85,71 %)	2 (14,28 %)	
Cytoplasm + membrane	20 (86,95 %)	3 (13,04 %)	
Only membrane	12	8	
N missing			
Cd 10 intensity			
Mean (SD)	1,45 (1,07)	1,33 (0,82)	0,6899
Range (min, max)	(0,0, 3,0)	(0,0; 3,0)	
Cd 10 area			
Mean (SD)	1,66 (1,18)	1,87 (1,06)	0,5491
Range (min, max)	(0,0, 3,0)	(0,0; 3,0)	
Vimentin intensity			
Mean (SD)	2,77 (0,61)	2,93 (0,26)	0,3141
Range (min, max)	(1,0, 3,0)	(2,0; 3,0)	
Vimentin area			
Mean (SD)	2,84 (0,43)	3,00 (0,00)	0,1529
Range (min, max)	(1,0, 3,0)	(3,0; 3,0)	

Regarding the histological variables, only tumor thickness (OR=0.38, 95 %CI 0.15, 0.96, $p = 0.041$) was associated with the response to PDT. The inflammatory infiltrate was intense in 91,5 % of the responsive tumors to PDT, whereas this response was lower in those without inflammatory infiltrate (57 %), although the difference was not statistically significant.

Immunohistochemical expression of P53 was associated with a good outcome (OR 1.03, 95 %CI 1.00, 1.07, $p = 0.005$) (Fig. 1), whereas positive immunostaining for b-catenin (OR 0.09, 95 %CI 0.01, 0.77, $p = 0.011$) (Fig. 2) and cytoplasmic Glut1 immunostaining vs membrane in the tumoral cells (0R 0.11, 95 %CI 0.02, 0.77) (Fig. 3) were associated to lack of response to PDT ($p = 0.006$).

4. Discussion

In this study we prospectively evaluated the effects of different clinical, histological and molecular characteristics of BCC and BD on the response to MAL-PDT. Analysis of the clinical variables revealed that the response of nBCC was poorer than sBCC and these worse than BD. Other factor associated with a poorer response was the use of ACE inhibitors. The only histological variable associated with a poor response was the tumoral thickness. Finally, we found that whereas positive p53 immunoreactivity was predictor of good response, cytoplasmic Glut1 and b-catenin immunostaining in the tumoral cells were associated with tumor resistance to MAL-PDT.

The response rate to PDT varied depending on the histological subtype of BCC, with sBCC showing a response rate of 66.7 % compared to 57.1 % for nBCC, and being the highest response rate for Bowen's disease (95 %). Prior research has consistently shown higher cure rates for BD and sBCC (ranging from 82 % to 100 %) compared to nBCC (ranging from 33 % to 100 %) following PDT [7–9,15–17]. This discrepancy could be linked to additional factors like tumor thickness. Morton et al. and subsequently other investigators suggested that lesion thickness plays a crucial role in determining the effectiveness of topical PDT, proposing a thickness threshold of 2 mm [9,18]. In fact, tumoral thickness was the only histological factor associated with the response in our sample.

Previous studies have shown the synergistic action of common chronic drugs, such as verapamil and lovastatin with photofrin enhancing its phototoxic effect [19]. In our study, only the intake of ACE inhibitors among all the drugs considered was statistically significant associated with a no response to PDT. One potential reason for this interference is that ACE inhibitors can affect the vasculature and blood flow, which may impact the delivery of the photosensitizing agent or the oxygen supply to the target tissues during the PDT session. Furthermore, it has been evaluated the potential of 5-aminolevulinic acid hydrochloride (ALA) in suppressing ACE2 expression and the production of intracellular porphyrins. ALA suppressed the ACE2 expression and increase the intracellular heme level. ACE inhibitors could influence ACE2 expression impairing the ALA induction of intracellular PpIX [20].

We confirmed that negative p53 immunoreactivity was associated with tumor resistance to PDT. These molecular findings corroborate our previous clinical and in vitro studies, in which a better response to PDT was observed in the p53-positive (ASZ) than the p53-negative (BSZ) BCC cell line [7]. We previously reported similar findings in Bowen's disease patients treated with MAL-PDT and in the SCC, cell lines SCC-13 and A-431 [10]. Furthermore, multiple in vivo studies suggest that p53 may play a role in the observed increase in PpIX levels and subsequent cell death with increased selective accumulation [21–24].

We found that redistribution immunostaining for b-catenin from the membrane to the nucleus and the cytoplasm was associated with good response to PDT. These results agree with our previous studies in BCC where tumors with increased peripheral palisading immunostaining with β-catenin responded poorly to PDT and also more recently with the work of Mork E, *et al.* These authors found that stromal and deep edge b-catenin expression were most prominent in BCCs that recurred after PDT [25].

Our group demonstrated in vitro that BCC cells resistant to PDT shown aerobic glycolysis metabolism and metformin, which inhibits the AMP-activated protein kinase pathway, improved the cytotoxic effect of PDT on parental and resistant cells [14]. Therefore, we investigate the possible role of Glut 1 as potential glycolytic immunohistological

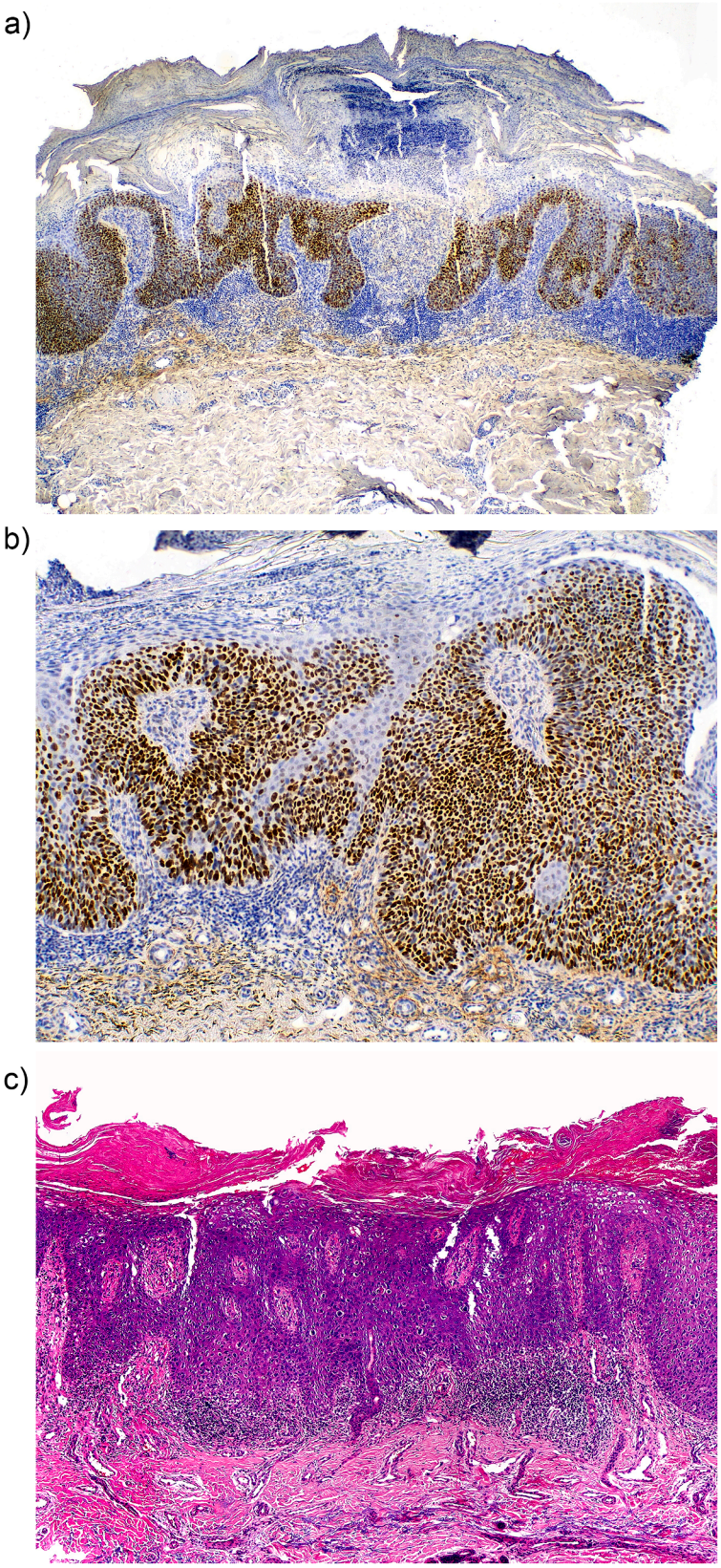

Fig. 1. A. Histopathological photograph of immunohistochemical expression of p53 in Bowen's disease. in Bowen's disease showing intense nuclear positivity in almost the entire thickness of the tumour epidermis (x40). B. Aproach of immunohistochemical expression of p53 in Bowen's disease showing intense nuclear positivity in almost the entire thickness of the tumour epidermis (x400). C. Histopathological photograph of Bowen's disease. Hyperkeratosis is seen on an epidermis with psoriasiform hyperplasia, with high-grade dysplasia affecting the entire thickness of the epidermis and a lichenoid inflammatory infiltrate in the superficial dermis (haematoxylin-eosin x 200). B.

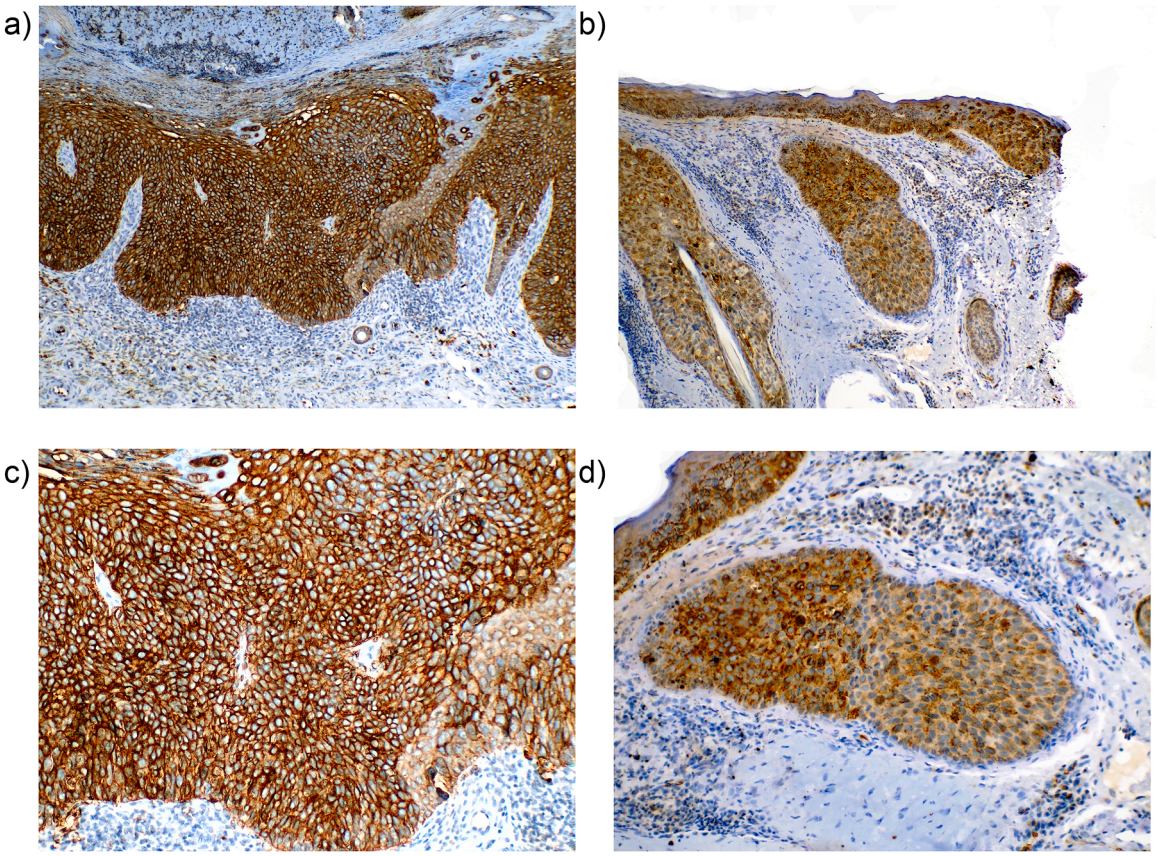

Fig. 2. A Histopathological photograph of immunohistochemical expression of Glut1 in Bowen's disease showing intense membrane positivity throughout the thickness of the lesional epidermis (x200). B. Immunohistochemical expression of Glut1 in Bowen's disease showing intense cytoplasmic positivity in tumour keratinocytes in the full thickness of the epidermis and affected adnexa (x200). C. Immunohistochemical expression of beta-catenin in a basal cell carcinoma showing cytoplasmic positivity, more intense in the peripheral row of tumour islets (x200). D. immunohistochemical expression of Glut1 in Bowen's disease showing intense membrane positivity throughout the thickness of the lesional epidermis (x400).

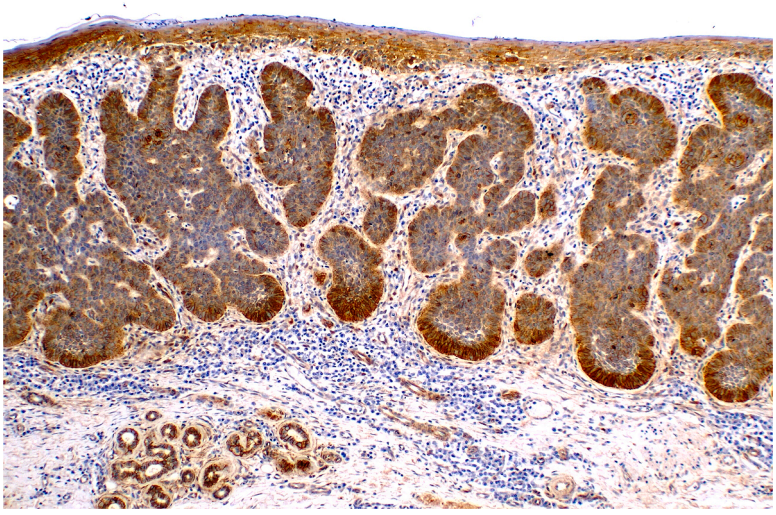

Fig. 3. Histopathological photograph of immunohistochemical expression of Glut1 in Bowen's disease showing intense cytoplasmic positivity in tumour keratinocytes in the full thickness of the epidermis and affected adnexa (x200).

biomarker [26]. Glucose serves as a vital fuel source for cells, particularly in the production of ATP. It enters into the cells through specialized transporter proteins known as GLUT. Notably, even under aerobic conditions, cancer cells consume large amounts of glucose that is utilized for glycolysis rather than for the oxidative phosphorylation of mitochondria for ATP production, through a phenomenon known as the

Warburg effect [27,28]. This reliance on glycolysis has led to proteins involved in glucose metabolism becoming potential targets for cancer therapy. Several inhibitors aimed at these proteins are currently in clinical development as promising anti-cancer agents. GLUT1 overexpression is commonly observed in cancer cells, making it a focal point for potential molecular targeting in cancer treatment. The significance of cytoplasmic GLUT-1 expression in several tumors is unknown. GLUT1 expression levels in basal cell carcinoma may correlate with clinicopathological features and patient outcomes [29]. Higher levels of GLUT1 expression have been associated with more aggressive tumor behavior and poorer prognosis in certain cases [29]. On the other hand, it has been developed a glucose-I_2BODIPY conjugate, which targets GLUT1 in cancer cells for molecule-targeted PDT. Miura et al. observed that this compound deactivated GLUT1 protein in cancer cells without affecting essential housekeeping proteins like α-tubulin and PCNA. This treatment induced significant light-triggered cytotoxicity by modulating the EGFR/MAPK signaling pathway [30].

The main limitation of the study is the small sample which also include 3 different histological tumors that could bias the real influence of the investigated variables. Due to a lack of statistical power, it was not possible to build multivariate models. Perhaps with a larger sample size it would be possible to retain the significance of the univariate associations also when creating the multivariate associations. As a fortress, this was a prospective study, all the patients followed the same protocol which makes the results more robust.

In conclusion, our study provides sheds light on the diverse clinical and molecular factors influencing the efficacy of MAL-PDT in the treatment of BCC and BD. These findings underscore the importance of personalized treatment strategies based not only on the histological subtype and thickness but also on molecular characteristics, such as p53, b-catenin and GLUT1 expression. Our study not only confirms the importance of p53 as a tumoral biomarker to predict the response of keratinocyte carcinomas to MAL-PDT, but also supports the relevance of other molecular pathways, such as tumoral metabolism, GLUT1, and cellular adhesion, b-catenin, in the response of BCC and BD to PDT. This would help to guide treatment decisions improving efficiency and decreasing resistances.

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CRediT authorship contribution statement

L Bernal-Masferrer: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Data curation. **T Gracia-Cazaña:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **L Najera-Botello:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **MC Gomez-Mateo:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **P Cerro:** Writing – review & editing, Resources, Funding acquisition, Data curation. **MC Matei:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **M Gallego-Rentero:** Writing – review & editing, Formal analysis, Conceptualization. **S González:** Writing – review & editing, Supervision, Conceptualization. **A Juarranz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Y Gilaberte:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

Conflicts of Interest

The authors declare no conflict of interest.

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